REVIEW ARTICLE

CYTOMETRY
Journal of Quantitative Cell Science PART A

MiSet RFC Standards: Defining a Universal Minimum Set of Standards Required for Reproducibility and Rigor in Research Flow Cytometry Experiments

Fabienne Lucas,[1*] Jesus Gil-Pulido,[2] John LaMacchia,[1] Fred Preffer,[3] Paul K. Wallace,[4] Peter Lopez[5]

[1]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

[2]Institute of Molecular Biology, Mainz, Germany

[3]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

[4]Roswell Park Comprehensive Cancer Center, Buffalo, New York

[5]New York University School of Medicine, New York, New York

Received 28 August 2019; Revised 30 October 2019; Accepted 11 November 2019

*Correspondence to: Fabienne Lucas, Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA Email: flucas@bwh.harvard.edu

Published online 26 November 2019 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.23940

Abstract

Poor adherence to best practices, insufficient training, and pressure to produce data quickly may lead to publications of suboptimal biomedical research flow cytometry data, which contributes to the body of irreproducible research findings. In addition, documentation of compliance with best flow cytometry practices for submission, visualization, and publication of flow cytometry data is currently endorsed by very few scientific journals, which is particularly concerning as numerous peer-reviewed flow cytometry publications emphasize instrumentation, experimental design, and data analysis as important sources of variability. Guidelines and resources for adequate reporting, annotation and deposition of flow cytometry experiments are provided by MIFlowCyt and the FlowRepository database, and comprehensive expert recommendations covering principles and techniques of field-specific flow cytometry applications have been published. To facilitate the integration of quality-defining parameters into manuscript and grant submission and publication requirements across biomedical fields that rely on the use of flow-cytometry-based techniques, a single comprehensive yet easily and universally applicable document is needed. To produce such a list of gold-standard parameters that assess whether a research flow cytometry experiment has been planned, conducted, interpreted, and reported at the highest standard, a new initiative defining the minimum set of standards a robust and rigorous research flow experiment must fulfill (MiSet RFC Standards) was proposed at CYTO 2019. MiSet RFC Standards will integrate and simplify existing resources to provide a universal benchmark a flow cytometry experiment can easily be measured against. The goal of MiSET RFC Standards is its integration into peer-review and publication procedures through partnership with stakeholders, journals and publishers in biomedical and translational research. This article introduces the aims and anticipated timeline and discusses strategies for interdisciplinary consensus and implementation. A single-resource broadly applicable guideline will harmonize standards across different fields of biomedical research and lead to publication of more robust research findings. © 2019 International Society for Advancement of Cytometry

Key terms

rigor; reproducibility; data standards; research; guideline

Over the past decades, the scientific community has witnessed an unprecedented speed of discovery and multiplication of knowledge that is tainted by abundant reports of irreproducible research findings (1–3). In 2015, it was estimated that irreproducibility of published scientific articles ranges from 50% to 89%, with an economic impact of around $28B/year in the United States alone (4). Institutions such as the National Academies of Sciences, Engineering, and Medicine, as well as expert consortiums such as the Committee on Responsible Science, have provided independent and objective guidance for more than 25 years to ensure responsibility

and integrity of scientific research (5,6). Regardless, the "reproducibility crisis" is widely considered a product of a multitude of challenges that are further complicated by specific requirements of a particular research setting or discipline (7,8).

While the number of published flow cytometry studies has rapidly grown over the last decades, both clinical and research flow cytometry experiments generally face substantial variability. A 2014 survey among 276 flow cytometrists suggests that this is mainly caused by aspects of data analysis, instrumentation, sample preparation and reagents (9). Moreover, especially research flow cytometry studies may often be conducted in the context of lack of expert education and/or limited access to shared resources laboratories (SRLs). Over the last years, SRLs have become an essential component of the biomedical research team by providing access to highly specialized technologies, trained staff, and adherence to rigorous quality standards (10,11). In the clinical flow cytometry setting, harmonization and standardization efforts are sustained by regulating agencies (e.g., Food and Drug Administration (FDA), Clinical Laboratory Improvement Amendments (CLIA) in the United States, European Medicines Agency (EMA) in Europe), method validation protocols (12), and the International Council for Standardization of Hematology (ICSH) and the International Clinical Cytometry Society (ICCS) working group practice guidelines (13–17). Clinical flow cytometry is also subject to constant calibration and optimization to ensure it fulfills the requirements for a clinical test due to immediate implications in patient care. As research flow cytometry is a commonly used tool in translational settings to inform future clinical strategies, similar harmonization and standardization efforts to ensure precision and accuracy should be sought.

The International Society for the Advancement of Cytometry (ISAC) is an expert flow cytometry community that provides consensus recommendations to accelerate flow cytometry research in the setting of cross-disciplinary collaboration. ISAC endorses several resources that help to define, annotate, and report flow cytometry experiments (e.g., optimized multicolor immunofluorescence panels (OMIPs) (18) and MIFlowCyt (19)), as well as recommendations issued for specific settings of immunology research and SRLs (11,20–22). However, these resources have not found widespread adaption, and very few scientific journals and publishers currently require adherence to MIFlowCyt standards or deposition of experimental data and methodology. To produce a single comprehensive yet broadly applicable consensus document that will achieve widespread adoption across different biomedical societies, scientific journals and publishers, and grant application procedures, a new ISAC initiative called MiSET RFC Standards was proposed. MiSet RFC Standards will integrate and simplify existing resources to define the minimum set of common standards a robust and rigorous research flow experiment must fulfill. This will provide a single easy to follow, and relevant resource that will help assess the overall quality of a biomedical research flow cytometry experiment. This article gives an overview of peer-reviewed publications on best flow cytometry practices and currently available major flow cytometry data reporting tools, introduces the mission, aims and potential gold-standard parameters, and discusses strategies for consensus and implementation in both the expert flow cytometry and collaborating communities.

Existing Flow Cytometry Resources and Guidelines on Preparing and Reporting a Research Flow Cytometry Experiment

Research studies conducted by the flow cytometry community have driven innovation in multiple diverse areas. These range from instrument, assay and reagent development to complex data analysis algorithms, as well as translational applications such as biomarker discovery in cancer and autoimmune diseases (23). To enable training and continuing education in flow cytometry and to provide a framework for successful planning and interpretation of flow cytometry experiments, various learning resources and interactive in-person meetings and online platforms have been created (e.g., CYTO conference, local/regional/national/international flow cytometry meetings and educational activities, SRL websites, Cyto University (Cyto U) (24), Purdue Cytometry Discussion List (25), Expert Cytometry (ExCyte) (26)). Since 2017, credentialing as "Specialist in Cytometry" (SCYM) or "International Specialist in Cytometry" (SCYM^{I}) is offered by the American Society of Clinical Pathologists (ASCP) to cytometrists with a relevant degree and documented experience in flow cytometry applications, cytometric analysis, and quality assurance after successfully passing the certification examination, which is an essential step to harmonizing education and training. Similarly, the European Society for Clinical Cell Analysis (ESCCA) offers the European Certificate for Cytometry Operators and the European Certificate for Cytometry Specialists.

Simultaneously, substantial efforts were made to communicate best flow cytometry practices through technical publications on standards of instrumentation, experimental design, and data analysis. As a result, critical methods of instrument standardization and calibration (27–31), panel and experimental design (32–36), sample preparation and controls (37–39), spillover correction (40), and data presentation and publication (41,42) are emphasized in various seminal publications. Moreover, more than 40 OMIPs have been published to date, providing a peer-reviewed collection of optimized multicolor panels that can be used in a variety of flow cytometry experiments (18), therefore addressing one of the major sources of variability (9). ISAC recommendations on specific criteria for recording and reporting information about a flow cytometry experiment were first published more than 10 years ago as MIFlowCyt, the "Minimum Information about a Flow Cytometry Experiment" (19). MIFlowCyt requirements included a detailed description of experiment overview, samples, instrumentation and data analysis with the aim to provide the basis for consistent data annotation and data sharing. This was complemented by FlowRepository, a database

containing predominantly published and peer-reviewed flow cytometry experiments allowing query and download of deposited data (43). Further support was provided by publications of protocols outlining the steps involved in data deposition, sharing, and annotation (44) as well as by the development of software packages that facilitate data exchange and integration between computational systems and enable cross-platform import, export, and sharing of gated cytometry data (45). More recently, it was proposed that flow cytometry data produced in clinical studies should also be deposited in FlowRepository, but issues such as compliance with data privacy policies hamper such considerations (46). While these recommendations are jointly supported by ISAC, the International Clinical Cytometry Society (ICCS) and ESCCA and have been endorsed by the Data Interoperability Steering Committee of the Division of Allergy, Immunology, and Transplantation within the National Institute of Allergy and Infectious Diseases (NIAID) and are included in the Minimum Information for Biological and Biomedical Investigations (MIBBI), adoption across the scientific community has been sporadic. Moreover, flow cytometry data reporting according to the MIFlowCyt standard and deposition of source data is required by very few scientific journals. This is in stark contrast to the requirements for sequencing, microarray or proteomics data, where data deposition and annotation is generally considered a requirement for manuscript submission and peer-review. Regardless, taken together, a multitude of resources are currently available to provide guidance on designing, conducting, and reporting of a research flow cytometry experiment (Fig. 1).

Ensuring Published Data Quality by Defining Complete Standards of a Research Flow Cytometry Experiment: Mission and Aims of the MiSet RFC Standards Initiative

Despite the multiple resources available to provide guidance on designing, conducting and reporting flow cytometry experiments, there is currently no single resource that is actively used and endorsed in day-to-day practice to fully capture the overall quality of such experiments. Creating and widely implementing such a reference document is essential to ensure that flow cytometry data are published, deposited and made publicly available at the highest possible standard. The mission of the MiSet RFC Standards initiative is to implement a single comprehensive yet easily and universally applicable checklist of quality-defining parameters into the manuscript and grant submission and publication guidelines across biomedical fields that rely on the use of flow-cytometry-based techniques. Our aims are to (1) provide a data-driven consensus document based on review, discussion and integration of existing standards and resources (including technical best-practice publications, OMIPs, MIFlowCyt/FlowRepository, etc.), (2) create impactful and lasting partnership with and engagement of collaborators and stakeholders, and (3) advocate for integration of and adherence to these standards by the scientific community, funding organizations and scientific journals (Fig. 2).

Proposed Action Steps and Timeline for MiSet RFC Standards Initiative

The aims and mission of the MiSet RFC Standards initiative were proposed to ISAC leadership at CYTO 2019. The following action steps will be taken, all to be achieved within a 3-year timeline:

Assembly of a Multidisciplinary Expert Working Panel

The proposed composition of the working panel includes lead authors of seminal best-practice publications, MIFlowCyt and FlowRepository, members of the Cytometry A and B editorial boards, ISAC Marylou Ingram Scholars and SRL Emerging Leaders, interested ISAC members and colleagues from research laboratories and SRLs, representatives from the fields of hematology, oncology, immunology, and translational medicine as well as stakeholders in publishing, regulatory and standards initiatives. The goals of the initial working panel will be to

1. assemble a steering committee to provide overall direction and representation of collaborators and stakeholders;
2. discuss and confirm the scope of the project with focus on immunophenotyping;
3. refine and focus working group aims and mission;
4. agree on and commit to action steps and timeline;

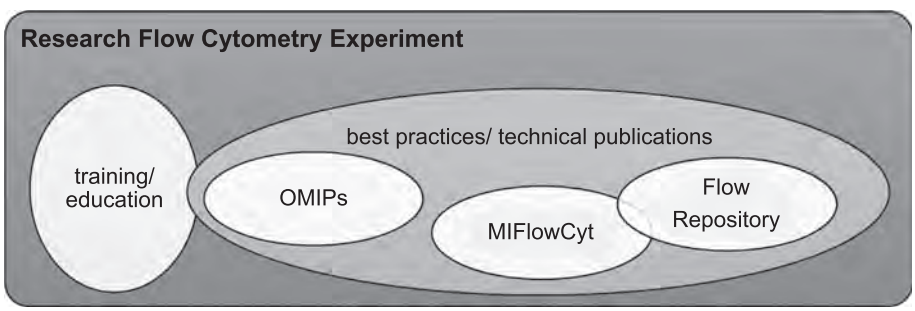

Figure 1. Overview of integration of currently available resources and peer-reviewed guidelines for research flow cytometry experiments. Abbreviation: OMIP = optimized multicolor immunofluorescence panels (16).

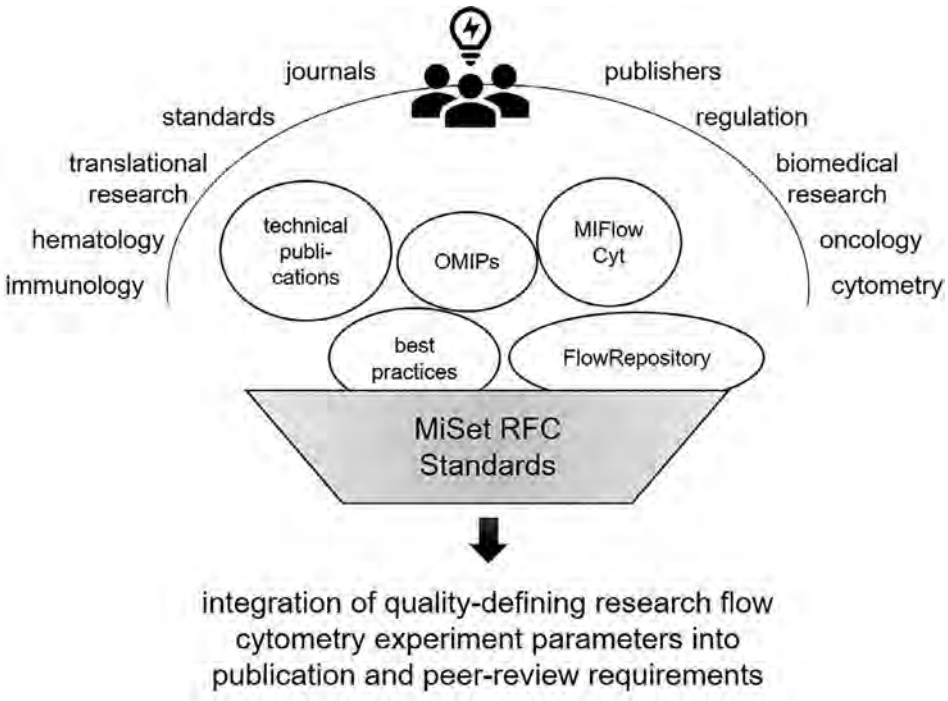

Figure 2. Mission of MiSet RFC Standards initiative.

5. obtain full ISAC task force status and endorsement;
6. identify and recruit additional expert members and consultants as needed;
7. engage collaborators and partners in but not limited to the following:
   - ICCS, ICSH, ESCCA
   - Association of Biomolecular Resource Facilities (ABRF)
   - Federation of American Societies for Experimental Biology (FASEB)
   - American Society of Hematology (ASH), European Hematology Association (EHA), American Society of Clinical Oncology (ASCO), American Association for Cancer Research (AACR)
   - IUIS (International Union of Immunological Societies)
   - National Institute of Standards and Technology (NIST)
8. identify potential funding sources.

Engagement of the Flow Cytometry Community and Outreach to Collaborating Biomedical Societies and Stakeholders

Once official ISAC task force status is achieved, engagement with the flow cytometry community, collaborators and stakeholders will be sought to:

1. survey and engage the expert cytometry community via ISAC communication channels and educational resources and newsletters;
2. inquire about existing discipline-specific procedures to assess the quality of research flow cytometry experiments;
3. become informed about current publication standards applied to flow cytometry experiments by official journals of hematology, oncology, immunology, and translational medicine societies and major publishers and journal families.

Refinement and Discussion of Criteria Suitable to Be Included into MiSet RFC Standards

As soon as initial data on current practices and needs of the expert and nonexpert flow cytometry communities is achieved, the suitability of a variety of parameters to become a broadly applicable minimum standard criterion will be discussed. A preliminary, albeit not complete, list of parameters is provided below. These will be further refined via surveys and expert-reviews. Extensive interdisciplinary input will be sought to ensure a balanced, objective and relevant discussion and to fulfill the mission to have universally applicable character. This will be complemented by integration with ISAC MIFlowCyt, FlowRepository, and Data Standards expert panels and standardization/harmonization consortiums to accelerate the adoption of minimum standards.

Identification of Special Circumstances and Needs

Survey data and expert review/evaluation will be used to create a preliminary set of minimum standards. We will re-engage with collaborators and stakeholders to assess the suitability of these preliminary minimum standards for different research settings pursuing a beta-testing/user acceptance testing approach. This will enable us to make relevant revisions

to MiSET RFC Standards and tailor implementation strategies to "real-world" needs.

Composition and Implementation of MiSet RFC Standards

Surveys, expert evaluations, and testing phases will produce data-driven progress reports that will be submitted to CYTO and other suitable meetings during the first 18 months of the initiative. We anticipate that a final consensus document will be written, and the manuscript submitted for rigorous peer-review within 2 years. The final set of minimum standards will then be implemented via publication of peer-reviewed guidelines, and direct presentation to collaborators and stakeholders by 2023.

Progress Updates and Monitoring of Suitability of MiSet RFC Standards

We envision the entire composition and implementation of MiSET RFC Standards to be a dynamic interactive process that is driven by data, exchange, and collaboration. Throughout the entire process, ISAC leadership and all collaborating parties will be updated yearly. It is the task of the multidisciplinary Steering Committee to hold the group accountable for meeting milestones. After publication of ISAC minimum standards by 2023 at the latest, the task force will continue to regularly monitor the suitability of the guidelines in the context of latest development and changing technologies through integration into AI-based reagent and experiment search databases, and via surveys and quality assessment experiments.

Suggested Parameters to Be Included into MiSet RFC Standards

The following section provides an outline of the parameters that are common causes of variability in a research flow cytometry experiment, as identified by the various resources currently available described above. A thorough review and side-by-side comparison of these resources will be the first step to provide the basis for the discussion of the minimum set of standards an entire research flow cytometry experiment must fulfill to ensure the highest quality of data. All parameters will be assigned to specific experimental phases (i.e., "plan," "conduct," "analyze," "interpret," and "report"). Common parameters including but not limited to those listed in Table 1 and summarized in Figure 3 will be transformed into a survey to the broad flow cytometry community. This survey will establish the importance and ranking of published flow cytometry standards and provide valuable data on the perceived importance and support of flow cytometry guidelines, existing knowledge and weighing of quality criteria, and invite suggested additions. Survey analysis results will then be reviewed and discussed by experts and the Steering Committee to produce a preliminary consensus MiSET RFC Standards document. This will be further refined after user acceptance testing utilizing resources and pipelines provided by stakeholders in industry and collaborating partners.

Table 1. Common parameters defining the quality of flow cytometry experiments, to be further refined in MiSet RFC Standards consensus document

EXPERIMENTAL PLANNING STAGE
Parameters relevant to a priori experiment planning, biostatical considerations, and sample requirements • Written experimental plan, to include background, rationale, a priory definition of hypotheses and aims, expected outcomes, sample size calculation, number of repetitions or replicates, anticipated data analysis pipeline, statistical analyses • Sample handling and processing prior to flow cytometry preparation, minimal accepted viability • Controls (isotypes, fluorescent minus one (FMO), biological controls) *Strategies for* • Panel design and steps for optimization, or rationale for selected OMIPs • Reagent selection and titration • Instrument quality control (QC) to ensure data consistency and robustness of panel in longitudinal or multicenter studies
STAGE WHEN EXPERIMENT IS CONDUCTED
• Steps for instrumentation optimization, calibration, and performance tracking • Collaboration with SRLs • Controls and standards • Minimum data acquisition during measurement to ensure robust statistics • Spillover correction and annotation
EXPERIMENTAL ANALYSIS STAGE
• Choice of analysis software/platform/pipeline • Data visualization • Normalization and standardization of data, accounting for variance • Statistical methods • Documentation
EXPERIMENTAL INTERPRETATION STAGE
• Data visualization • Sources of variability and heterogeneity • Statistical interpretation • Data supported conclusions
EXPERIMENTAL REPORTING STAGE
• Disclosure of gating strategies • Harmonization of nomenclature • Axes and data labeling of plots and graphs • Rationale for choice of "representative" samples/plots, reporting of variability, and sources of variation • Deposition of used reagents and instrumentation information • Descriptions of methods • Data deposition, including reagents, methods, and raw flow cytometry data

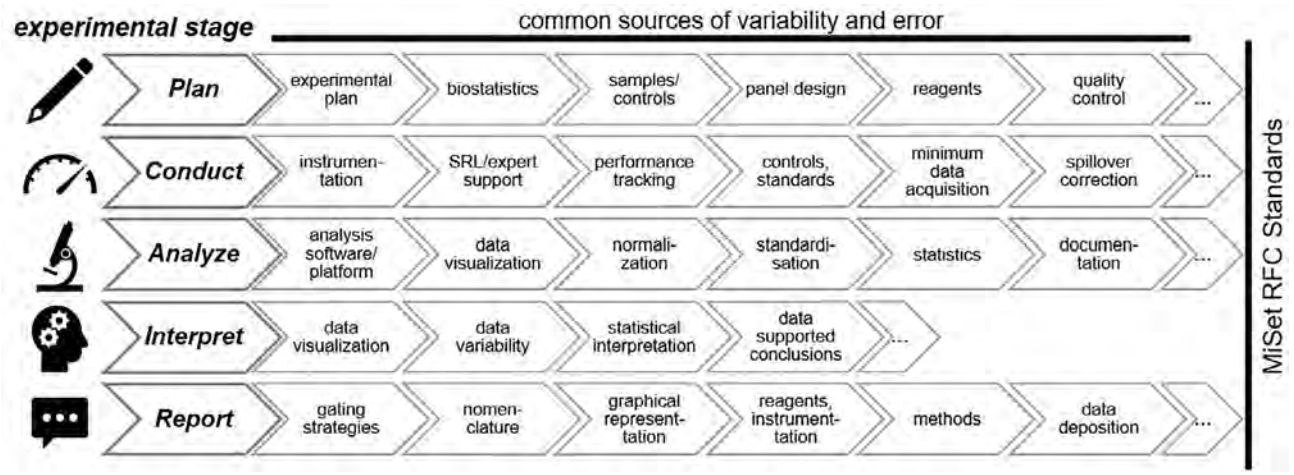

Figure 3. Overview of parameters during different experimental phases that are common sources of variability in flow cytometry experiments, to be further refined in MiSet RFC Standards consensus document.

Expected Benefit from and Vision for Integration of MiSet RFC Standards

A single comprehensive consensus document defining the minimum standards for an entire research flow cytometry experiment will provide a valuable and universally applicable benchmark against which this type of experiment can be measured. Initially MiSet RFC Standards will embrace biomedical and translational research experiments, with a natural outgrowth to also encompass standards for cell sorting and other biological research areas. MiSet RFC Standards are envisioned to be a clearly defined, data-driven set of minimum standards guiding each step of the preanalytical, data production, interpretation, and publication experimental phase. This will also provide an extremely valuable resource for the scientific peer-review process, where integration of flow cytometry standards and deposition of source data are currently mostly optional. In addition, the adequate interpretation of flow cytometry data often largely depends on the expertise of specific reviewers, and the input of expert flow cytometry reviewers is rarely sought during the review process of a manuscript or proposal containing flow cytometry data. MiSet RFC Standards will provide an objective reference that will guide the assessment of quality and validity of a research flow cytometry experiment. The integration with MIFlowCyt and FlowRepository will ensure a complete process that includes data production, reagent, methods, and experiment annotation and deposition. Through the integration with key immunology, hematology, oncology, and translational medicine societies, scientific journals and publishers, and stakeholders in industry and regulations, the foundation will be laid for the widespread adoption of MiSet RFC Standards. Our major goal is that MiSet RFC Standards will become an established feature of manuscript submission and publication guidelines of scientific journals, in conjunction with the requirements to annotate and deposit data following the MIFlowCyt and FlowRepository standards. Following these standards will result in the production and publication of consistent and transparent flow cytometry data and methods that can be externally validated and reproduced. Moreover, rigorously produced flow cytometry data will facilitate all subsequent data analyses and data mining, especially if complex analysis platforms and automated single-cell analysis algorithms are used. It is anticipated that automated analytical techniques will become a cornerstone in the quest to increase reproducibility in cytometric data analysis, as discussed by Brinkman and colleagues in this special issue of Cytometry A. The standardization of data production will therefore become even more important as new analytical tools continue to emerge and improve.

Discussion

Efforts to define the minimal standards to support the evaluation and interpretation of experiments have been made in various areas of biomedical research, such as microbiology (47–49), molecular studies (50), extracellular vesicle studies (51–53), immunopeptidomics (54), HIV research (55,56), and biobanking (57). Standard definitions have also been shown to significantly improve the reproducibility of clinical flow cytometry experiments. For example, the EuroFLOW consortium has developed a standardized procedure detailing the entire process from instrument settings to data analysis, including tools to process large data files in diagnostic laboratories (58,59). Similar to the MiSet RFC Standards initiative, EuroFLOW was created to address the main sources of variability affecting the validity and reproducibility of clinical flow cytometry experiments (60). These included issues related to operator/center expertise and training, panel design, data analysis and interpretation of results. EuroFLOW standards are commonly considered a robust approach (61,62). Minimum standards have also been defined in the setting of flow cytometry SRLs, providing guidance for best practices in instrumentation, training, experimental design, biosafety standards, and sample handling (11,20,21). For the specific needs

of the immunology community, a comprehensive guide covering the principles, techniques and applications of immunology flow cytometry has been published by the European Journal of Immunology (22).

These substantial efforts emphasize the need for standard definitions to create robust and reproducible results in the setting of specialized experimental settings, research questions, sample types, and access to instrumentation or the expertise of SRLs/flow cytometry collaborators. MiSet RFC Standards will build on these extensive resources by attempting to identify those criteria that are generally applicable to flow cytometry conducted in the research setting, thereby simplifying the best-practice guidelines and making them accessible as a single and streamlined document that is applicable to the variety of parties. Altogether, with a current focus on immunophenotyping experiments, MiSet RFC Standards will be an essential step in addressing issues of reproducibility, promote training, education, and adherence to best practices. As a result, MiSet RFC Standards will serve as a template for minimum standard for more broad-based cytometric applications, including cell signaling, cell cycle, cell activation, and proliferation.

Acknowledgments

We would like to acknowledge Dr. Tim Bushnell. Dr. Maurice Shen (BenchSci), Dr. Jonnie Moore and GLIIFCA for providing intellectual and strategic input and feedback.

Disclosure of Conflict of Interest

F.L. is an ISAC Marylou Ingram Scholar (2019–2024).

Literature Cited

1. Begley CG. Six red flags for suspect work. Nature 2013;497:433–434.
2. Begley CG, Ellis LM. Drug development: Raise standards for preclinical cancer research. Nature 2012;483:531–533.
3. Begley CG, Ioannidis JPA. Reproducibility in science: Improving the standard for basic and preclinical research. Circ Res 2015;116:116–126.
4. Freedman LP, Cockburn IM, Simcoe TS. The economics of reproducibility in preclinical research. PLoS Biol 2015;13:e1002165.
5. National Academies of Sciences, Engineering, and Medicine, Policy and Global Affairs, Committee on Science, Engineering, Medicine, and Public Policy, Committee on Responsible Science. Fostering Integrity in Research. Washington, DC: National Academies Press, 2017 Available at: http://www.ncbi.nlm.nih.gov/books/NBK475953/. Accessed July 20, 2019.
6. National Academy of Sciences (US), National Academy of Engineering (US), Institute of Medicine (US) Panel on Scientific Responsibility and the Conduct of Research. Responsible Science: Ensuring the Integrity of the Research Process: Volume II. Washington, DC: National Academies Press, 1993 Available at: http://www.ncbi.nlm.nih.gov/books/NBK236203/. Accessed July 20, 2019.
7. Perezgonzalez JD. Three more steps toward better science. F1000Res 2018;7:1728.
8. Fanelli D. Opinion: Is science really facing a reproducibility crisis, and do we need it to? Proc Natl Acad Sci U S A 2018;115:2628–2631.
9. Hulspas R. Reliable Cell Product Characterization by Flow Cytometry. 2017. Available at: https://biopharma-asia.com/magazine-articles/reliable-cell-product-characterization-flow-cytometry/. Accessed October 7, 2019.
10. Moore J, Roederer M. The flow cytometry shared resource laboratory: Best practices to assure a high-quality, cost-effective partnership with biomedical research laboratories. Cytometry Part A 2009;75A:643–649.
11. Barsky LW, Black M, Cochran M, Daniel BJ, Davies D, DeLay M, Gardner R, Gregory M, Kunkel D, Lannigan J, et al. International Society for Advancement of Cytometry (ISAC) flow cytometry shared resource laboratory (SRL) best practices. Cytometry Part A 2016;89A:1017–1030.
12. Selliah N, Eck S, Green C, Oldaker T, Stewart J, Vitaliti A, Litwin V. Flow Cytometry method validation protocols. Curr Protoc Cytom 2019;87:e53.
13. Davis BH, Dasgupta A, Kussick S, Han J-Y, Estrellado A, ICSH/ICCS Working Group. Validation of cell-based fluorescence assays: Practice guidelines from the ICSH and ICCS—Part II—Preanalytical issues. Cytometry Part B 2013;84B:286–290.
14. Davis BH, Wood B, Oldaker T, Barnett D. Validation of cell-based fluorescence assays: Practice guidelines from the ICSH and ICCS—Part I—Rationale and aims. Cytometry Part B 2013;84:282–285.
15. Wood B, Jevremovic D, Béné MC, Yan M, Jacobs P, Litwin V, ICSH/ICCS Working Group. Validation of cell-based fluorescence assays: Practice guidelines from the ICSH and ICCS—Part V—Assay performance criteria. Cytometry Part B 2013;84B:315–323.
16. Barnett D, Louzao R, Gambell P, De J, Oldaker T, Hanson CA, ICSH/ICCS Working Group. Validation of cell-based fluorescence assays: Practice guidelines from the ICSH and ICCS—Part IV—Postanalytic considerations. Cytometry Part B 2013;84B:309–314.
17. Tanqri S, Vall H, Kaplan D, Hoffman B, Purvis N, Porwit A, Hunsberger B, Shankey TV, ICSH/ICCS Working Group. Validation of cell-based fluorescence assays: Practice guidelines from the ICSH and ICCS—Part III—Analytical issues. Cytometry Part B 2013;84B:291–308.
18. Mahnke Y, Chattopadhyay P, Roederer M. Publication of optimized multicolor immunofluorescence panels. Cytometry Part A 2010;77A:814–818.
19. Lee JA, Spidlen J, Boyce K, Cai J, Crosbie N, Dalphin M, Furlong J, Gasparetto M, Goldberg M, Goralczyk EM, et al. MIFlowCyt: The minimum information about a flow cytometry experiment. Cytometry Part A 2008;73A:926–930.
20. Holmes KL, Fontes B, Hogarth P, Konz R, Monard S, Pletcher CH, Wadley RB, Schmid I, Perfetto SP. International Society for the Advancement of Cytometry cell sorter biosafety standards. Cytometry Part A 2014;85A:434–453.
21. Schmid I, Lambert C, Ambrozak D, Marti GE, Moss DM, Perfetto SP, International Society of Analytical Cytology. International Society for Analytical Cytology biosafety standard for sorting of unfixed cells. Cytometry Part A 2007;71A:414–437.
22. Cossarizza A, Chang H-D, Radbruch A, Acs A, Adam D, Adam-Klages S, Agace WW, Aghaeepour N, Akdis M, Allez M, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). Eur J Immunol 2019;49:1457–1973.
23. Czechowska K, Lannigan J, Wang L, Arcidiacono J, Ashhurst TM, Barnard RM, Bauer S, Bispo C, Bonilla DL, Brinkman RR, et al. Cyt-Geist: Current and future challenges in cytometry: Reports of the CYTO 2018 conference workshops. Cytometry Part A 2019;95A:598–644.
24. Anon. CYTO University Online Learning by CYTOU—CYTO University. Available at: http://www.cytou.org/store/provider/provider09.php. Accessed July 21, 2019.
25. Anon. Purdue Cytometry Discussion List | Purdue University Cytometry Laboratories. Available at: http://www.cyto.purdue.edu/hmarchiv/index.htm. Accessed July 21, 2019.
26. Anon. Get Advanced Flow Training. Expert Cytometry. Available at: https://expertcytometry.com/. Accessed July 21, 2019.
27. Hurley AA. Quality control in phenotypic analysis by flow cytometry. Curr Protoc Cytom 2001;Chapter 6:Unit 6.1.
28. Perfetto SP, Ambrozak D, Nguyen R, Chattopadhyay P, Roederer M. Quality assurance for polychromatic flow cytometry. Nat Protoc 2006;1:1522–1530.
29. Perfetto SP, Ambrozak D, Nguyen R, Chattopadhyay PK, Roederer M. Quality assurance for polychromatic flow cytometry using a suite of calibration beads. Nat Protoc 2012;7:2067–2079.
30. Mittag A, Tárnok A. Basics of standardization and calibration in cytometry—A review. J Biophotonics 2009;2:470–481.
31. Wang L, Hoffman RA. Standardization, calibration, and control in flow Cytometry. Curr Protoc Cytom 2017;79:1.3.1–1.3.27.
32. Baumgarth N, Roederer M. A practical approach to multicolor flow cytometry for immunophenotyping. J Immunol Methods 2000;243:77–97.
33. Maecker HT, Frey T, Nomura LE, Trotter J. Selecting fluorochrome conjugates for maximum sensitivity. Cytometry A 2004;62:169–173.
34. Mahnke YD, Roederer M. Optimizing a multicolor immunophenotyping assay. Clin Lab Med 2007;27:469–485.v.
35. Maciorowski Z, Chattopadhyay PK, Jain P. Basic multicolor flow cytometry. Curr Protoc Immunol 2017;117:5.4.1–5.4.38.
36. Herzenberg LA, Tung J, Moore WA, Herzenberg LA, Parks DR. Interpreting flow cytometry data: A guide for the perplexed. Nat Immunol 2006;7:681–685.
37. Maecker HT, Trotter J. Flow cytometry controls, instrument setup, and the determination of positivity. Cytometry Part A 2006;69A:1037–1042.
38. Andersen MN, Al-Karradi SNH, Kragstrup TW, Hokland M. Elimination of erroneous results in flow cytometry caused by antibody binding to Fc receptors on human monocytes and macrophages. Cytometry Part A 2016;89A:1001–1009.
39. Roederer M. How many events is enough? Are you positive? Cytometry Part A 2008;73A:384–385.
40. Roederer M. Compensation in flow cytometry. Curr Protoc Cytom 2002;Chapter 1:Unit 1.14.
41. Roederer M, Darzynkiewicz Z, Parks DR. Guidelines for the presentation of flow cytometric data. Methods Cell Biol 2004;75:241–256.
42. Alvarez DF, Helm K, Degregori J, Roederer M, Majka S. Publishing flow cytometry data. Am J Physiol Lung Cell Mol Physiol 2010;298:L127–L130.
43. Spidlen J, Breuer K, Rosenberg C, Kotecha N, Brinkman RR. FlowRepository: A resource of annotated flow cytometry datasets associated with peer-reviewed publications. Cytometry Part A 2012;81A:727–731.
44. Spidlen J, Breuer K, Brinkman R. Preparing a Minimum Information about a Flow Cytometry Experiment (MIFlowCyt) compliant manuscript using the International Society for Advancement of Cytometry (ISAC) FCS file repository (FlowRepository.org). Curr Protoc Cytom 2012;Chapter 10:Unit 10.18.
45. Finak G, Jiang W, Gottardo R. CytoML for cross-platform cytometry data sharing. Cytometry Part A 2018;93A:1189–1196.

46. Spidlen J, Brinkman RR. Use FlowRepository to share your clinical data upon study publication. Cytometry Part B 2018;94B:196–198.
47. Lourenço A, Coenye T, Goeres DM, Donelli G, Azevedo AS, Ceri H, Coelho FL, Flemming H-C, Juhna T, Lopes SP, et al. Minimum information about a biofilm experiment (MIABiE): Standards for reporting experiments and data on sessile microbial communities living at interfaces. Pathog Dis 2014;70:250–256.
48. Roux S, Adriaenssens EM, Dutilh BE, Koonin EV, Kropinski AM, Krupovic M, Kuhn JH, Lavigne R, Brister JR, Varsani A, et al. Minimum information about an uncultivated virus genome (MIUViG). Nat Biotechnol 2019;37:29–37.
49. Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, TBK R, Schulz F, Jarett J, Rivers AR, Eloe-Fadrosh EA, et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol 2017;35:725–731.
50. Huang J, Mirel D, Pugh E, Xing C, Robinson PN, Pertsemlidis A, Ding L, Kozlitina J, Maher J, Rios J, et al. Minimum information about a genotyping experiment (MIGEN). Stand Genomic Sci 2011;5:224–229.
51. Witwer KW, Soekmadji C, Hill AF, Wauben MH, Buzás EI, Di Vizio D, Falcon-Perez JM, Gardiner C, Hochberg F, Kurochkin IV, et al. Updating the MISEV minimal requirements for extracellular vesicle studies: Building bridges to reproducibility. J Extracell Vesicles 2017;6:1396823.
52. Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, Quesenberry P, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: A position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles 2014;3:26913.
53. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018;7:1535750.
54. Lill JR, van Veelen PA, Tenzer S, Admon A, Caron E, Elias JE, Heck AJR, Marcilla M, Marino F, Müller M, et al. Minimal information about an immunopeptidomics experiment (MIAIPE). Proteomics 2018;18:e1800110.
55. Paxton H, Kidd P, Landay A, Giorgi J, Flomenberg N, Walker E, Valentine F, Fahey J, Gelman R. Results of the flow cytometry ACTG quality control program: Analysis and findings. Clin Immunol Immunopathol 1989;52:68–84.
56. Mandy F, Bergeron M, Houle G, Bradley J, Fahey J. Impact of the international program for quality assessment and standardization for immunological measures relevant to HIV/AIDS: QASI. Cytometry 2002;50:111–116.
57. Campbell LD, Astrin JJ, DeSouza Y, Giri J, Patel AA, Rawley-Payne M, Rush A, Sieffert N. The 2018 revision of the ISBER best practices: Summary of changes and the editorial team's development process. Biopreserv Biobank 2018;16:3–6.
58. Pedreira CE, da Costa ES, Lecrevise Q, Grigore G, Fluxa R, Verde J, Hernandez J, van Dongen JJM, Orfao A, EuroFlow. From big flow cytometry datasets to smart diagnostic strategies: The EuroFlow approach. J Immunol Methods 2019;112631. https://doi.org/10.1016/j.jim.2019.07.003
59. Kalina T, Flores-Montero J, van der Velden VHJ, Martin-Ayuso M, Böttcher S, Ritgen M, Almeida J, Lhermitte L, Asnafi V, Mendonça A, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 2012;26:1986–2010.
60. van Dongen JJM, Orfao A. EuroFlow: Resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics and personalized medicine. Leukemia 2012;26:1899–1907.
61. Kalina T, Brdickova N, Glier H, Fernandez P, Bitter M, Flores-Montero J, van Dongen JJM, Orfao A. Frequent issues and lessons learned from EuroFlow QA. J Immunol Methods 2018:112520. https://doi.org/10.1016/j.jim.2018.09.008.
62. Solly F, Angelot-Delettre F, Ticchioni M, Geneviève F, Rambaud H, Baseggio L, Plesa A, Debliquis A, Garnache-Ottou F, Roggy A, et al. Standardization of flow cytometric immunophenotyping for hematological malignancies: The FranceFlow group experience. Cytometry Part A 2019;95A:1008–1018.