Published in final edited form as:

Cytometry A. 2016 November ; 89(11): 971–974. doi:10.1002/cyto.a.23015.

Teaching Advanced Flow Cytometry in Africa: 10 Years of Lessons Learned

Elisa Nemes^{1,2,*†}, Wendy A. Burgers³, Catherine Riou³, Erica Andersen-Nissen⁴, Guido Ferrari⁵, Clive M. Gray², and Thomas Scriba^{1,2}

¹South African Tuberculosis Vaccine Initiative (SATVI), University of Cape Town, South Africa

²Division of Immunology, Department of Pathology and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa

³Division of Medical Virology, Department of Pathology and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa

⁴Cape Town HVTN Immunology Laboratory, Hutchinson Centre Research Institute of South Africa, Cape Town, South Africa

⁵Department of Surgery, Duke University, Durham, North Carolina

Key terms

flow cytometry; training; Africa; capacity building

Africa is particularly burdened by major infectious diseases and there are many groups throughout the continent whose research focuses on understanding cellular immunity to these pathogens(1). There is thus a continuous need for improving and further developing scientific skills in different regions that can enable the design, conduct and interpretation of data that support the immune monitoring of clinical research studies(2). To enhance technical and theoretical knowledge of African immunologists and in response to the paucity of training opportunities on the continent, the first Intracellular Cytokine Staining and Flow Cytometry Workshop was held in 2005 in South Africa. Since then the workshop has been held biennially, with the aim of training a new generation of African researchers so that cutting edge technology can be applied and human capital can be perpetuated locally by peer-training. We deemed it appropriate to relay our experiences to the international cytometry community and to promote discussion about access to flow cytometry training with those involved in similar educational efforts on the African continent.

Applicants based in Africa were selected for workshop attendance through a peer-review process based on: (I) the scientific merit of each submitted abstract; (II) flow cytometry experience; (III) training needs (at both individual and institutional levels); and (IV) potential impact on career advancement.

*Correspondence to: Elisa Nemes, PhD, Health Sciences Faculty, University of Cape Town, Room S2.02, Wernher Beit South Building, Anzio road, 7925 Observatory, Cape Town, South Africa. elisa.nemes@uct.ac.za.

[†]Elisa Nemes is an International Society for Advancement of Cytometry (ISAC) Marylou Ingram Scholar.

All selected participants received a full scholarship that covered all expenses, international and local transport, accommodation and meals. Funding was secured from a range of sources including educational grants, academic funding, private foundations and vendors (reported under Acknowledgements section).

Participant selection was highly competitive due to limited funding and the practical nature of the interactive workshop. On average, fewer than half of the applicants were selected (e.g., 28% in 2015), indicating the high demand for advanced flow cytometry training in Africa. Over the six workshops held between 2005–2015, 106 scientists working in 22 African countries attended (Fig. 1A), an average of 18 participants (range: 14–22) per workshop. Participants included junior faculty/postdoctoral fellows, postgraduate students, as well as laboratory managers and senior personnel. Such participant diversity translated into broad training demands specifically including optimization of instrument performance, appropriate design of new studies, analysis of existing datasets and training/supervision of others.

The 5-day flow cytometry workshop was designed to address such needs through a combination of lectures and hands-on tutorials focusing on the measurement of immune responses using multiparameter flow cytometry (3). The course program increased in complexity over time to keep pace with technology advances. In 2015 the workshop addressed: (I) basic principles of flow cytometry; instrument set-up, optimization and quality control (4); (II) panel design, antibody titrations and compensation (5,6); (III) experiment design and optimization (7,8); (IV) data visualization, analysis, interpretation and reporting (9–11); (V) controls, data validation and troubleshooting (12); and (VI) principles of assay standardization and validation for clinical trials according to Good Clinical Laboratory Practice guidelines (13,14) (Supporting Information tables 1 and 2). In these workshops, we progressed from demonstrating principles for 4 to >6 parameter instruments in the latest workshops. Hands-on tutorials initially included sample processing, but due to time limitations and broadening of the course scope, have recently been limited to those perceived as the most difficult and critical topics: instrument optimization and quality control, sample acquisition and data analysis. Most participants were extremely appreciative about the hands-on data analysis sessions, conducted using FlowJo software (FlowJo, LLC, Ashland Oregon), which is available free-of-charge to all scientists based in Africa.

Learning was evaluated by initial and final knowledge assessments (Supporting Information 3). Moreover, daily homework was assigned and discussed the following day during highly interactive sessions. Participants left the workshop with a better knowledge of current techniques. For example, an average two-fold increase in the scores achieved at workshop completion was observed when compared to the pre-workshop assessment in 2015 (Fig. 1B).

To ensure that the content and format of the workshop suited participants' needs over the years, anonymous feedback provided by each attendee at the end of the workshop was heeded to tailor and adjust the program of the next workshop, thus ensuring continuous improvement. Overall, the trainees expressed overwhelming enthusiasm, exemplified by this

Additional Supporting Information may be found in the online version of this article.

comment: *"It will help our whole team to improve the quality of our data, [and] prepare me for my dream, a PhD."* As this workshop was designed for more advanced flow cytometry users, some participants with limited experience regarded the program as too concentrated and suggested stretching the course over a longer period of time. This has not been possible so far due to resource limitations. English was the official language adopted for the workshops, and some participants coming from French-speaking countries perceived this as a barrier. French-speaking faculty members could assist when needed, but it's clear that many flow cytometry users would benefit from regional workshops conducted in languages other than English.

The course also provided a network of regional and international contacts, teaching materials and technical resources for participants to return home with and share with their peers. Post-training networking and resource sharing tools have been limited to date. We are working towards a more structured approach, building on existing platforms such as the Immunopaedia internet portal (immunopaedia.org.za).

From the start scientists within and outside Africa contributed to the organizing committee and faculty team. However, greater representation of faculty members affiliated to African institutions in 2013 and 2015 bears testament to the sustainable impact of the workshop (Fig. 1C). Notably, most of the recent African faculty members had attended the workshop as participants in the past.

The longer-term impact of the workshop was recently evaluated by a comprehensive survey, conducted in May/June 2016 (Supporting Information 4). Sixty-five participants who attended workshops from 2009 onwards were contacted via e-mail. Among the 34 respondents (52%), all were still working in Africa, 88% reported that attending the workshop had been important to achieve their career goals and thought the workshop provided good networking opportunities. All interviewees would recommend the workshop to their peers and expressed interest in further training through e-learning. Almost 90% of the respondents had trained peers at their home institution after the workshop (Fig. 2A), often adapting teaching materials shared during the workshop (Fig. 2B). Importantly, the majority of respondents reported paucity of local training opportunities (Fig. 2C) and that their home research organizations could not afford the cost for such training (Fig. 2D). We believe that "training the trainers" is the most effective and empowering approach, especially since access to advanced flow cytometry training on the African continent has improved only modestly over the past 10 years, according to respondents and our own observations.

In light of the increasing needs and efforts to conduct clinical research in Africa, we believe that these workshops serve not only to enhance local African scientific capacity, but also contribute to the global health agenda. In 2016 only 10 of 1,725 (0.6%) ISAC members held African affiliations (Michelle Butler, ISAC Executive Director, personal communication). There is a clear opportunity for the Society to expand and partner with African organizations and initiatives to further its reach on the African continent, to meaningfully contribute to capacity building and to broaden the diversity of its members.

In conclusion, there is further need for regional face-to-face flow cytometry training initiatives, ideally tailored to the participants' experience and needs, and offered free-of-charge. Some of this unmet need for flow cytometry training in Africa could be fulfilled by open-access e-learning-based courses, such as those offered by CYTO University (cytou.org).

Acknowledgments

The organizers thank all participants who attended the flow cytometry workshops, and in particular those who contributed to the 2016 survey.

Faculty 2005–2015: John Altman, Erica Andersen-Nissen, Omu Anzala, Michael Betts, Marty Bigos, Gaëlle Breton, Wendy Burgers, Stephen De Rosa, One Dintwe, Helene Dujardin, Michael Eller, Guido Ferrari, Deborah Glencross, Clive Gray, Willem Hanekom, Alexandre Harari, Jane Hughes, Kondwani Jambo, Claire Landry, Holden Maecker, John Mantovani, Agatha Masemola, Elisa Nemes, Janet Ottinger, Stephen Perfetto, John Quinn, Catherine Riou, Mario Roederer, Thomas Scriba, Rafick-Pierre Sekaly, Erica Smit, Andreia Soares, Sara Suliman, Kayla Treister, Claudio Vallan.

Logistical and Administrative Support: Dhuraiyah Abdullatief, Carina Kriel, Kathryn Norman.

Funders and Sponsors: African AIDS Vaccine Programme (AAVP)-World Health Organisation (WHO), Becton Dickinson (BD), Beckman Coulter, Biocom Biotech (South Africa), Canada-Africa Prevention Trials Network (CAPT), Center for AIDS Research (CFAR, Duke University Medical Center), Clinical Infectious Diseases Research Initiative (CIDRI, South Africa), Global Health Research Initiative (GHRI, Canada), HIV Vaccine Trial Network (HVTN, US), Institute of Infectious Disease and Molecular Medicine (IDM, South Africa), International Union of Immunological Societies (IUIS), National Institute for Communicable Diseases (NICD, South Africa), National Institute of Allergy and Infectious Disease (NIAID) and Office of AIDS Research (OAR), National Institute of Health (NIH, US), South African Immunology Society (SAIS), Scientific Group (South Africa), TreeStar/FlowJo, United Nations Programme on HIV/AIDS (UNAIDS).

Grant sponsor: NIH/OAR, Grant number: R13AI084562 (to G.F.); Grant sponsor: Wellcome Trust Strategic Award; Grant number: WT084323MA; Grant sponsor: Bill and Melinda Gates Foundation; Grant number: OPP1088952 (to J.M.); Grant sponsor: NIH/NIAID, Grant number: UM1 AI068618 (to J.M.)

Literature Cited

1. Elliott A, Nerima B, Bagaya B, Kambugu A, Joloba M, Cose S, Pantaleo G, Yazdanbakhsh M, Mabey D, Dunne D, et al. Capacity for science in sub-Saharan Africa. *Lancet*. 2015; 385:2435–2437. [PubMed: 26122054]
2. Cose S, Bagaya B, Nerima B, Joloba M, Kambugu A, Tweyongyere R, Dunne DW, Mbidde E, Kaleebu P, Elliott AM. Immunology in Africa. *Trop Med Int Health*. 2015; 20:1771–1777. [PubMed: 26391634]
3. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: Implications for vaccine design. *Nat Rev Immunol*. 2008; 8:247–258. [PubMed: 18323851]
4. 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. [PubMed: 23138348]
5. Mahnke YD, Roederer M. Optimizing a multicolor immunophenotyping assay. *Clin Lab Med*. 2007; 27:469–485. [PubMed: 17658403]
6. Nguyen R, Perfetto S, Mahnke YD, Chattopadhyay P, Roederer M. Quantifying spill-over spreading for comparing instrument performance and aiding in multicolor panel design. *Cytometry A*. 2013; 83(A):306–315. [PubMed: 23389989]
7. Lamoreaux L, Roederer M, Koup R. Intracellular cytokine optimization and standard operating procedure. *Nat Protoc*. 2006; 1:1507–1516. [PubMed: 17406442]
8. Smith SG, Smits K, Joosten SA, van Meijgaarden KE, Satti I. TBVI TB Biomarker Working, et al. Intracellular cytokine staining and flow cytometry: Considerations for application in clinical trials of novel tuberculosis vaccines. *PLoS ONE*. 2015; 10:e0138042. [PubMed: 26367374]

9. 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. [PubMed: 16785881]
10. Britten CM, Janetzki S, Butterfield LH, Ferrari G, Gouttefangeas C, et al. T cell assays and MIATA: The essential minimum for maximum impact. *Immunity.* 2012; 37:1–2. [PubMed: 22840835]
11. Roederer M, Nozzi JL, Nason MC. SPICE: Exploration and analysis of post-cytometric complex multivariate datasets. *Cytometry A.* 2011; 79A:167–174.
12. Maecker HT, Trotter J. Flow cytometry controls, instrument setup, and the determination of positivity. *Cytometry a.* 2006; 69A:1037–1042.
13. Horton H, Thomas EP, Stucky JA, Frank I, Moodie Z, et al. Optimization and validation of an 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T cells induced by vaccination. *J. Immunol. Methods.* 2007; 323:39–54. [PubMed: 17451739]
14. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, et al. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. *J Immunol Methods.* 2015; 417:22–33. [PubMed: 25523923]

Figure 1.

(A) Geographical distribution of institutional affiliations of flow cytometry workshop participants ($n = 106$ from 2005 to 2015). (B) Participant learning was evaluated by answering the same questions ($n = 14$) at the beginning (Day 1) and end (Day 5) of the workshop programme (Wilcoxon signed rank test). A fold change above 1 (dotted line) indicates improvement in questionnaire score (representative data are from the 2015 workshop). (C) Proportion of faculty members with African affiliation for each workshop.

A Since you attended the workshop, how many people have you trained in flow cytometry?

C Are there advanced training opportunities in flow cytometry in the country where you currently work?

B When training other people, did you use the teaching materials provided during the workshop?

D What cost would your current institute afford for someone to attend a 5-day flow cytometry workshop?

Figure 2.

Results from the 2016 survey. The survey was submitted to 65 participants who attended one of the workshops held between 2009 and 2015, and was completed by 34 participants (52%). Impact of the flow cytometry workshop was assessed in terms of capacity building: (A) spread of knowledge among peers and (B) utility of training materials provided. (C) Availability and (D) affordability of alternative flow cytometry training opportunities were also evaluated.