REPORT

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ISAC INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

Cyt-Geist: Current and Future Challenges in Cytometry: Reports of the CYTO 2019 Conference Workshops

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

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Contents

In May 2018, we initiated a new, unique project: the CYTO Conference workshop summary report. The main motivation was to present to the reader key findings from the live discussions, indicate the trends, and discuss identified challenges and proposed solutions or guidelines in specific fields of cytometry. CYTO workshops are well-known dynamic and open forums for free discussions and experience exchange at different levels and among various scientific specialties. The allocated time for this format however is limited, so workshops must be run in parallel. That format unfortunately does not allow one to attend all workshops of a CYTO conference. Furthermore, not all International Society for Advancement of Cytometry (ISAC) members can join every annual CYTO meeting thus they cannot benefit from any of the workshops.

Publication of the workshop summaries and making it thereby available for a global audience not only captures the live discussions but may seed new initiatives to support hot topics or new trends. Good examples are last year's CYTO Lab Hacks initiative; or this year's workshop African Working Group under the umbrella of ISAC and announcing its Instrument4Science Task Force [I4S] initiative.

The joint effort of the 73 scientists facilitating CYTO2018 workshops was a successful endeavor and the final report summary was published in June this year (1). The download numbers of full-text manuscript from the journal website were 1,140 within the first four months. Encouraged by the impressive interest, we decided to continue the project and publish joint manuscript from this year's CYTO2019 workshops, held in Vancouver. We received summaries from all 11 workshops and grouped them into three chapters: 1. Trends; 2. Shared Resource Laboratory (SRL) Best Practices; and 3. Quality Assurance and Reproducibility.

Some topics that gathered the attention of last year's workshop participants were further discussed also this year. Good examples here could be mass cytometry presented as one of the trends last year and discussed this year in more detail in the context of clinical trials (WS05). Automated algorithm-based data analysis of multisite clinical studies data received well-deserved attention in one of the workshops included in this manuscript (WS10).

We hope that this short summary will serve the scientific community as a useful source of information and will help to connect scientists and initiate new fascinating projects and even more exciting workshops.

Chapter 1: Trends

This chapter consists of reports from three workshops that focus on the current trends in cytometry.

The main trend identified at CYTO2019 was related to information sharing and promoting interdisciplinary dialogue. As already seen last year, there is a strong ISAC community request to establish cytometry-related databases as reported by the authors of WS01 and WS11. There is a clear and sound demand for open access to data, protocols, and guidelines that would help to drive the research and in consequence, move the innovation forward. WS01 proposes the launch of a cell sorting data and guidelines repository for different cell types. Even though such initiative was welcomed with great enthusiasm by the society and many workshop participants volunteered to help with such a database creation, there are some obstacles like source of financing, management, or location, which need to be overcome first. WS11 is intended to serve the community as open forum for driving innovation in cytometry. The above-mentioned databases could be of great help for the third initiative in this chapter, the African Working Group of ISAC Instrument4Science Task Force (I4S) (WS03). The African Working group needs substantial support of reagent and instrumentation vendors to help progress African cytometry. I4S is initiated by the ISAC for consolidating solitary efforts by individuals or individual companies in Africa that may be expandable to many resource poor countries on other continents.

WS01: Creating a Repository of Best Practice Guidelines with Successful Cell Sorting Outcomes from Different Cell Types

Gelo Victoriano Dela Cruz, Anna Fossum, Peter Lopez.

Introduction and Aims

Flow Cytometric Cell Sorting is an essential technology used in the biomedical sciences. This technology allows for the rapid purification of specific populations from a heterogeneous cell suspension based on user-defined light scatter and/or fluorescence properties. In a typical cell sorting experiment, samples are first dissociated into a single-cell suspension, washed to remove debris, and then concentrated using centrifugation. In addition to getting samples into a single-cell suspension, samples may be subjected to additional steps including antibody staining before analyzing and sorting.

The research goals dictate the success of a cell sorting experiment. This is often measured by the viability and yield of cells after sorting and the quality of isolated macromolecules, among others. Performance characteristics related to the mechanical isolation of cells can vary due to instrument setup or cell type. Riddell et al. (2) established an approach that utilizes fluorescent beads to optimize and document electrostatic sorter performance. While the mechanical optimization of the cell sorting process can be achieved using beads, cells may add another variable due to size or condition and contribute to an unintended outcome, as shown by Osborne (3). Also, electrostatic cell sorting may cause any number of perturbations to the sorted cells, recently described as sorter-induced cellular stress (SICS) (4).

Due to the myriad of cell types seen in a shared resource laboratory for cell sorting, as well as the variety of cell sorters and cell sorter configurations, a database or repository containing contributed recommendations on how to obtain the best results for successful sorting of various cell types and various cell sorters would be very beneficial to those performing cell sorting experiments.

Currently, there is a wealth of both published and unpublished cell sorting data. Published data can be found in methodologies and supplementary information in research studies utilizing cell sorting. The MIFlowCyt standard was adopted in 2008 for reporting of information about flow cytometry (including cell sorting) experiments. However, the MIFlowCyt information is not in a searchable database format that can be easily accessed.

This workshop surveyed the workshop participants and the greater cytometry community via an online survey as to what data are needed in a database for cell-sorting information. In addition, suggestions on where the database can be hosted or located and how the database can be curated and maintained were discussed. The online survey was conducted before CYTO Conference 2019, and the results discussed during the workshop.

Methods

An online survey was sent out to the greater cytometry community before the CYTO Conference 2019 through the Purdue Cytometry List. The survey contained questions pertaining to cell-sorting information needed in an online database in an open-ended format.

The workshop started with a brief introduction about cell sorting, the current status of cell-sorting information resources and SICS. The FPbase (www.fpbase.org) website was shown as an example of a research resource that a cell-sorting repository can be patterned after. The Fpbase is a database of fluorescent proteins and their properties that is free and open-source, community-editable but curated, and most importantly, with searchable data.

The results of the pre-congress survey (see Supporting Information 1, WS01_SI1) were shown and discussed, and attendees were then polled with the following two key questions using live polling with Slido (www.slido.com).

Results

The workshop had at least 147 participants. Live polling contributions to the first key question had natural groupings of slightly different responses which allowed for the results to be binned into several categories (Fig. 1 and Supporting Information 2, WS01_SI2). Majority of the participants recommended to have this repository as an ISAC-provided resource (Fig. 2 and Supporting Information 3, WS01_SI3).

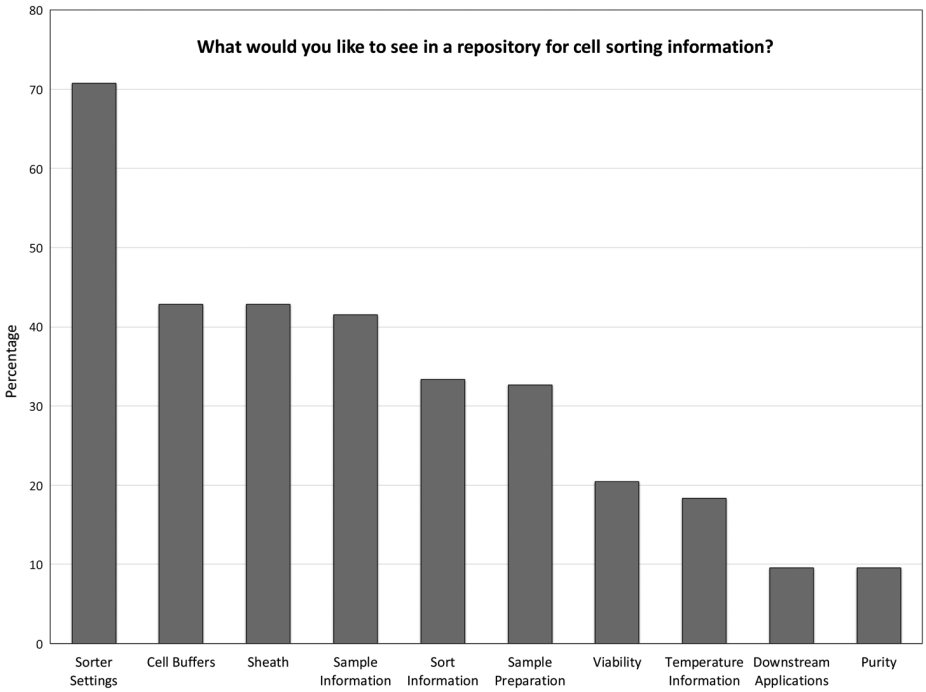

Figure 1. Summary of responses for Q1 "What would you like to see in a repository for cell sorting information?" Sorter Settings includes instrument name, information on nozzle size, and sample and sheath pressure. Cell buffers refer to buffers used in the resuspension of cells for sorting, as well as the buffers used for collection. Sheath refers to the type of sheath buffer used and Sample Information to sample source and type (tissue culture or primary). Sample preparation refers to the sample preparation protocol used. Viability includes pre- and post-sort viability of the cells. Temperature information refers to the temperature settings in the instrument as well as any specific temperature handling conditions.

Discussion

During the workshop, it was clear that there is a need for a cell sorting information resource as evidenced by the lively and enthusiastic discussion. The pre-congress survey had 28 respondents and showed that information on nozzle size, sheath pressure, sample preparation, and temperature conditions, among others, rated highly as important information that should be reported about cell sorting experiments. The live polling from the workshop showed similar results. Even though both pre-congress survey and online polling results show that there are a myriad of variables that can be reported, a consensus was still established.

From the live polling data, the replies were classified into several general categories (Fig. 1). Sorter settings include nozzle size, and sample and sheath pressure. Cell buffers include buffers used for sample resuspension for sorting and for collection. Sheath refers to the type of sheath fluid used for the sort. Sample information includes cell type and source (species and whether it is a cell line or are primary cells). Sort information refers to threshold rate, the sort mask, gating strategy, and sort efficiency. Sample preparation refers to the sample preparation protocol for the specific cells, while viability refers to the pre- and post-sort viability of the cells. Temperature information includes instrument (sample and collection chamber) temperature settings as well as any specific temperature conditions involved in the experiment.

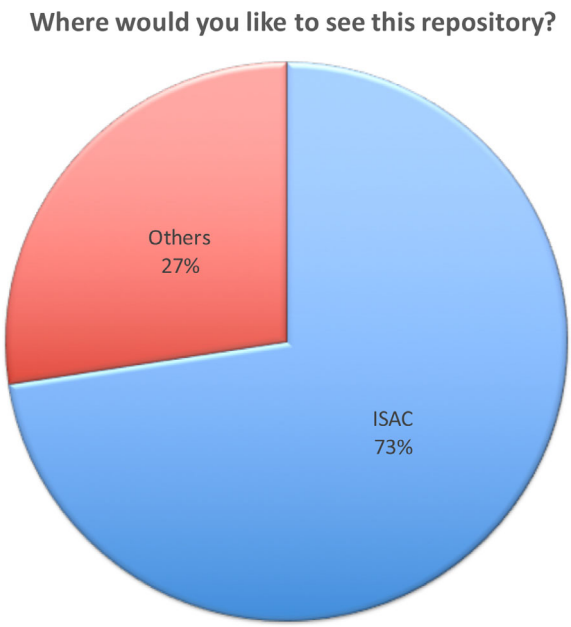

Figure 2. Summary of responses for Q2 "Where would you like to see this repository?" ISAC includes responses saying it should be ISAC-sponsored, ISAC-hosted and ISAC-related (*Cytometry Part A* or Cyto U). [Color figure can be viewed at wileyonlinelibrary.com]

There is a lot of information that workshop participants deemed important for a cell-sorting repository. It is important to note that these variables are related (i.e., nozzle size to sheath pressure, threshold rate to flow rate to sample concentration) and changing one variable might change others (i.e., going from 100 μm nozzle to 70 μm nozzle will change the sheath pressure). Furthermore, the discussions during the workshop indicated that not only successful cell-sorting outcomes be included in the repository but also information on cell-sorting experiments that failed.

As mentioned earlier, there was a lot of enthusiasm about the possibility of creating a repository for cell-sorting data. After the workshop, several attendees approached the facilitators and volunteered to help with creating and establishing this repository. However, in order to set this up, we need more than volunteers.

The creation of a repository with cell sorting information can be beneficial to the cytometry community. It will provide researchers with an easily searchable resource where they can search for the best conditions for cell sorting for their particular cell type, instrumentation, and downstream applications of the sorted material. The repository can be a good starting point for researchers trying out new cell types and can provide them with information on what not to do for these sorts. This will allow them to save on time and resources and hopefully help with the success of their sorting experiments.

On the down side, creating a repository such as this will require resources including funding and manpower. Funds will be needed to acquire server and hosting services, and possibly, hiring programmers to create the repository if our volunteers are not able to in a timely manner. Also, the funding has to be continuous, as the cost of maintaining a server and hosting services for this repository is not a one-time expense. Majority of the attendees suggested that this be funded by ISAC (Fig. 2) as a resource available to members, but it was also suggested that it be an ISAC-related resource that has independent funding through sponsorships by companies, a model of which will have to be devised to make the funding sustainable.

In addition to funding, manpower is also needed. To establish the repository, programmers are needed to create the repository to specifications. Once created, administrators will be needed in order to curate entries and manage the repository. Although we have volunteers from the cytometry community, we might need to hire the services of programmers to create the repository. This will be possible if we get enough financial support from ISAC or from other funding sources.

The repository can be a stand-alone resource that is funded by either private money (sponsorships from companies), or one that is under the auspices of ISAC. Regardless of funding source, the repository should be a community-led effort that is curated and open to all.

Perspectives

Establishing a repository for cell-sorting information is a challenging endeavor that will be extremely beneficial to the cytometry community. In order to establish, build, and maintain the repository, a sustainable model for funding should be devised and implemented. Also, the project should harness the skills and talents of eager volunteers from the community in order to maximize resources and ensure success.

Conflicts of Interest

The authors declare no conflicts of interest.

WS03: The State of Flow Cytometry in Africa

Lydia Tesfa, Iyadh Douagi, William G. Telford, J. Paul Robinson, Howard Shapiro, Karen Hogg, Paul Wheeler, Daniel Bitoun, Samuel Tony Boova, Ines Nasdala, Alfonso Blanco Fernández.

Introduction and Aims

Instrument access, reagent and assay availability, training, and education are all paramount in supporting scientists and clinicians in advancing their scientific studies in Africa. Nemes et al. (5) have indicated the need for training scientists in basic and advanced cytometry, assay developments, and operational theories of cytometry instrumentation. To achieve these goals, collaborative support and dedication from cytometry researchers, shared resource managers and reagent and instrumentation vendors is critical.

The first ISAC Cytometry in Africa Workshop was organized at CYTO 2019 to get a clearer picture about the status of cytometry in Africa. It sought to identify both strengths and needs in the African flow cytometry community and highlight examples of the different actions and initiatives as well as share obstacles and outcomes. The workshop attempted to strategize mechanisms for enhancing the efficiency and impact of the limited economic and human resources available in the African countries.

The specific aims of the workshop were to review results from a recent survey of African cytometry resources and personnel, showcase existing collaborative efforts, and discuss both current and new strategies for improving collaborative support and science. A further aim of the workshops was to increase awareness of the recently launched ISAC Task Force Instrument4Science (IS4S) that aims to promote and advance Science in Africa, Latin American and Caribbean countries, Eastern European countries, the Middle East, and Asian countries with economic and/or political difficulties by facilitating the donation of instruments and/or improve collaborative efforts with the local communities of these areas. This workshop constituted an excellent opportunity to collect feedback from participants and attendees on how to improve collaborative efforts in Africa.

Method

The State of Flow Cytometry in Africa CYTO2019 Workshop comprised of three main blocks: on-site presentation of the results of the survey, support efforts by both academic and industry scientists, discussion of current and proposed actions and plans for future activities. This is further discussed in Figure 3 and Supporting Information, see WS03_SI Highlights_Presentation Final. The first block was the

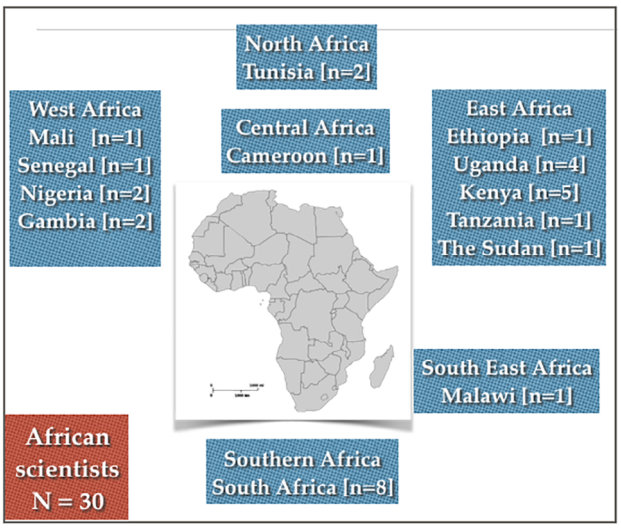

Figure 3. The state of flow cytometry in Africa. See presentation highlights in the Supporting Information (WS03_SI Highlights_Presentation Final). [Color figure can be viewed at wileyonlinelibrary.com]

introduction of pre-workshop survey results targeting the broad spectrum of clinical and research laboratories in Africa. This survey was broadly distributed to investigators throughout the African continent, as well as non-African scientists with collaborative links to African research institutions. Current and proposed efforts were presented in the second block, including donations, education, research efforts and other initiatives by both academic and industry investigators. The floor was then opened for discussion, where several significant African collaborative projects not included in the second section were described and discussed. At the end, a preliminary plan was put into place to align these efforts with the I4S, the ISAC Live Education Task Force, and the ISAC Education Committee.

The workshop was pleased and honored to have the participation of Howard Shapiro (via Skype), who has promoted many appropriate technologies for improved flow cytometric analysis in the developing world.

Results

Survey Results. The results of this survey were presented at the workshop and are briefly summarized below (the complete results are attached as Supporting Information see WS03_SI1 Survey_Results).

Thirty-seven survey questions were sent out to flow cytometry users in Africa. The respondents (n = 42) were from all regions of Africa, the vast majority of them researchers (83.3%). Over 90% of the surveyed had a cytometer, which over 30% having multiple instruments. The survey covered satisfaction of the investigators with their resources, user and instrument operator experience, level of and access to training, access to service and service contracts and maintenance costs.

The results of this survey reveal a movement in the research community toward sophisticated research studies, indicating for better access to more advanced equipment. Inquiries about applications showed immunophenotyping and tumor diagnosis to be the unsurprising dominant techniques, and cell cycle analysis, cell proliferation showed frequent usage. Nonbiomedical applications (11.9%) including microbial ecology, plant biology, and biotechnology were also identified as important.

Overall, the survey showed some interesting trends. It demonstrated considerable resources both in equipment and in personnel but with uneven distribution across countries and institutions. It also showed the increased emphasis on biomedical research and advanced clinical applications, in addition to more routine clinical analysis. The variety of scientific research described (both biomedical and biotechnological, including drug discovery and agriculture) was considerable. It also showed that, despite the best efforts of both foreign biomedical scientists and vendor community, access to instrument support, reagents and assays, training and education all remain challenging and need improvement.

Academic and Corporate Initiatives

International Cytometer Donations. One way to improve cytometry access is to donate refurbished instruments to collaborating laboratories. The National Cancer Center Institute (NCCI) program under Dr. William Telford has been operating a cytometer donation program since 2002. This program has arranged the donation of over 16 flow cytometers to laboratories all over the world. This donation program was described together with the challenges of the process: gifting to the recipient instrument, restoration and shipping, navigating international customs, and supporting equipment in often remote parts of the world. Training and educational support provided after the donation is critical for success of these efforts; this is an area that ISAC traditionally excels in, and the efforts and experience of the Live Education Task Force and the ISAC Education Committee have been leveraged toward this project. One focus of the Instruments 4 Sciences Task Force will be to facilitate such equipment donations.

The ethical challenges of used equipment donations for clinical use were also discussed. Clinical analysis typically demands new instrumentation still supported by the manufacturer. Dr. Alfonso Blanco (UCD Conway Institute, Ireland) discussed his clinical and educational project in Sudan as model for collaborative sites and building up the local knowledge.

The donation of second-hand instruments constitutes both legal and ethical challenges for the corporates due to international corporate policies and restrictions on importing used equipment especially in the clinical diagnostic field. However, companies are supportive of these efforts in principle, and ways that vendors can participate in this process were discussed. More interest was expressed in the donation of new equipment, particularly for clinical applications.

Sustainable Cytometry for Africa. Instruments are important but can the hosting labs use them? Support infrastructure, including good lab space, access to clean water and stable electrical system is critical to maintaining instrumentation in a useful state. High-end cytometers often place excessive demands on laboratory resources. Therefore, alternative systems appropriate to the region must be considered for a correct and fast diagnosis of different diseases such as malaria. Howard Shapiro presented his theory and designs for building appropriate cytometry technology for laboratory setting with limited infrastructure. In Howard Shapiro's own words: "In the 1960s, when I was in medical school, the light microscope was the primary tool for students, who did all the counts for the labs. Of red and white blood cells, segmented or stabs. We learned to use Giemsa's dye for blood smear staining but didn't see much of malaria while training. We also used Ziehl-Neelsen (Z-N) stain for tuberculosis; Though red bugs on blue can be easy to see, a slide may yield nothing but eyestrain and tedium, while colonies grow some weeks later in medium. Let Giemsa and Z-N take bows for their ages and put brighter talent on microscope stages! A multiparameter low-magnification wide-field imager (conceptually a 'flowless flow cytometer') applicable to a wide range of tasks including the diagnosis of Malaria/Tuberculosis could be affordable (production cost <US$1,000) and sustainable for resource-poor countries in Africa and elsewhere. This is increasingly becoming a multinational effort pointing toward an open-source design."

Several cytometer manufacturers have also produced low-cost instrumentation aimed at simple clinical analysis in regions with limited resources. Many of these systems, point-of-care (POC), cytometers with simple maintenance needs but limited analytical capacity. However, recent technological advances in photonics have also provided high-capability instrumentation in smaller packages, allowing easier installation and operation in remote areas. The companies participating in this workshop presented their systems, as well as their overall strategies for providing reagent and technical support.

Cytometry Education in Africa. Training and education are both critical for any research program. However, training and education are not always synonymous. While education programs should teach up to the highest levels of the field, training is often more specific to the instrumentation and analytical needs of the institution. Dr. Paul Robinson from Purdue University emphasized this point in his description of his workshops in Africa, including an upcoming conference in Nairobi Kenya. He also encouraged the instrument and reagent manufacturers to provide support for these efforts. Dr. Blanco also described his training programs for his cytometry project in Sudan.

Each one of the four participating companies have provided a huge variety of training and education programs that they sponsor and/or organize, including a Good Laboratory Practices (GLP) program for clinical diagnostic laboratories, regional Round Tables, Hematology Standardization for Leukemia/Lymphoma or the Flow Cytometry Continuous Educational and Regional Immunology Workshop with ISAC. The companies also provide basic instrument training; BD Biosciences, for example, is opening new training centers in the key geographic regions of Africa to provide local training without the requirement for long distance travel.

Education has been a traditional strength of ISAC, and the ISAC Education Committee, ISAC Live Education Task Force (LETF) and CYTO U all highlight this emphasis on both training and education. Close coordination between any African working group, companies and these existing ISAC committees will be critical to leverage existing experience in cytometry training.

Partnerships to advance clinical research and clinical trials capacity. Different examples of global health initiative emphasizing partnerships and alliances between researchers, clinicians, corporates and academics were presented, such as the CARES Program from Beckman Coulter (6). Drs. Karen Hogg summarized a project lead by Professor Paul Kaye (University of York, UK), a consortium of European partners from European and African partners, funded by European and Developing Countries Clinical Trials Program, to study the immunopathology of visceral leishmaniasis across four leishmaniasis endemic countries of East Africa and a vaccine trial in Sudan. The cytometers purchased for the project and the training provided will significantly enhance research capabilities of all sites involved.

Open Discussion. The floor was then opened for discussion. Several attendees described their own African programs and experiences. Dr. Huw (Zip) Kruger Gray shared his experiences working on a POC cytometry project in Nigeria. Logistic and bureaucratic difficulties were discussed, although it was emphasized that these issues could not be generalized to all countries. Working closely with the local governments and research communities was essential to dealing with bureaucratic obstacles. The importance of working both with the national and international societies (such as the African Academy of Sciences), international agencies (such as WHO) and established international research organizations and foundations (Wellcome Trust, USAID, Gates, Pasteur, etc.) was strongly emphasized.

Since cultural and political situations vary dramatically between different counties, it was suggested that the establishment of working groups for defined regions might help in dealing with these obstacles.

Discussions

The survey of African cytometry resources yielded several other interesting findings. There was a lack of knowledge about the online resources available such as the Cytometry Purdue Mailing list, the existence of the ISAC or the Pan African Cytometry Association. The participating manufacturers all suggested that they could direct their customers to these valuable and free resources.

A significant finding from this workshop is that many African Scientists are involved in projects and collaborations

with ISAC-associated scientists. The researchers hope that the workshop will yield fruitful partnerships that will spur substantial ISAC support for African investigators.

Education, service, and post-installation support, adequate infrastructures and both local and national politics are all critical issues for managing cytometry facilities in Africa. Although these problems are often difficult to solve, a team effort between academia, manufacturers, funding bodies, local authorities and the local scientific community can produce surprisingly good results.

A key outcome of the workshop was the establishment of an African Working Group that would connect both African and foreign investigators working on joint projects. A positive observation of the workshop was the large number of existing projects involving ISAC investigators and African scientists, many of which are not widely publicized. A working group will provide a clearinghouse for such efforts and will encourage future project in this area.

Another key outcome of the workshop was the official launch of the ISAC Task Force "Instruments 4 Science." This initiative is designed to promote the provision of instruments (new or refurbished) flow cytometers to institutions and laboratories not yet able to purchase their own equipment. I4S will connect donors with recipients and will help and advice in the donation process, including legal transfer of ownership, instrument refurbishment and shipping, and importation into the recipient country. I4S will be focus on providing support not just in African groups, also to Latin-American and Caribbean countries, Eastern European countries, the Middle East, and Asian countries with economic and/or political difficulties. I4S will work closely with the Education Committee, the Live Education Task Force, and other training programs within ISAC to support and train donation recipients to help them get the most out of their equipment. I4S will also attempt to establish relationships between donors and instrument manufacturers to provide support for instrument donations and will encourage efforts to place new equipment in recipient laboratories as part of international research collaborations. I4S will also work closely with ISAC Finance Committee and Funding Task Force to explore different mechanisms for making this effort financially possible. It will also cooperate with the ISAC Education Committee and Live Education Task Force to provide cytometry training and education. Instruments 4 Science is still in the exploratory stage, and eager for participants who have experience or an interest in instrument donations and working with recipients in the developing world. Dr. Blanco and Dr. Telford will spearhead I4S as co-chairs.

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Conflicts of Interest

The authors declare no conflicts of interest.

WS11: CYTO Lab Hacks: A Platform for the Exchange of Innovations in Cytometry

Cláudia Bispo, Bunny Cotleur, Christopher Hall, Virginia Litwin, Jakub Nedbal, Betsy M. Ohlsson-Wilhelm.

Introduction and Aims

Publishing in peer-reviewed journals and books or through meeting proceedings and presentations remain the main tools for disseminating scientific findings. These communication platforms have served the scientific community successfully for centuries. However, they introduce practical and administrative delays and barriers to the knowledge spread from the scientists to their peers and the wider public. These barriers intrinsically promote groundbreaking discoveries over incremental, unfounded, or poorly communicated ones. Inadvertently, they also sustain a culture of scientific competition, confidentiality, and reticence to the disclosure of details. In the past decades, the emergence of rapid, low-cost, and content-rich communication over the Internet has started to challenge this traditional model and created opportunities to build on it and improve it (7). Consequently, social media (8) and preprint archives (9) are increasingly used by scientists, funders, and publishers to improve and accelerate knowledge exchange and peer-review process. Beyond publishing; numerous scientific, political, and funding initiatives have emerged to improve transparency and rate of data, information, and knowledge exchange. ISAC has contributed to this development in the area of cytometry by introducing the MIFlowCyt (10) standard, FlowRepository (11), and the FCS data file standard (1,12,13).

The CYTO Lab Hacks website is being developed to become a directory of cytometry innovations. By innovations we mean: new or improved protocols, instruments, methods, procedures, teaching materials, and/or software. These innovations are already being regularly shared by scientists through publications, content-sharing repositories, personal or institutional websites, forum entries, blogs, videos, social media, and their combinations. Distributing these innovations through this broad range of media creates a barrier to discovery and utilization by peers. Consequently, their impact tends to be diminished. CYTO Lab Hacks aims to solve this problem by providing a centralized directory and a database of cytometry-related innovations. These innovations could be

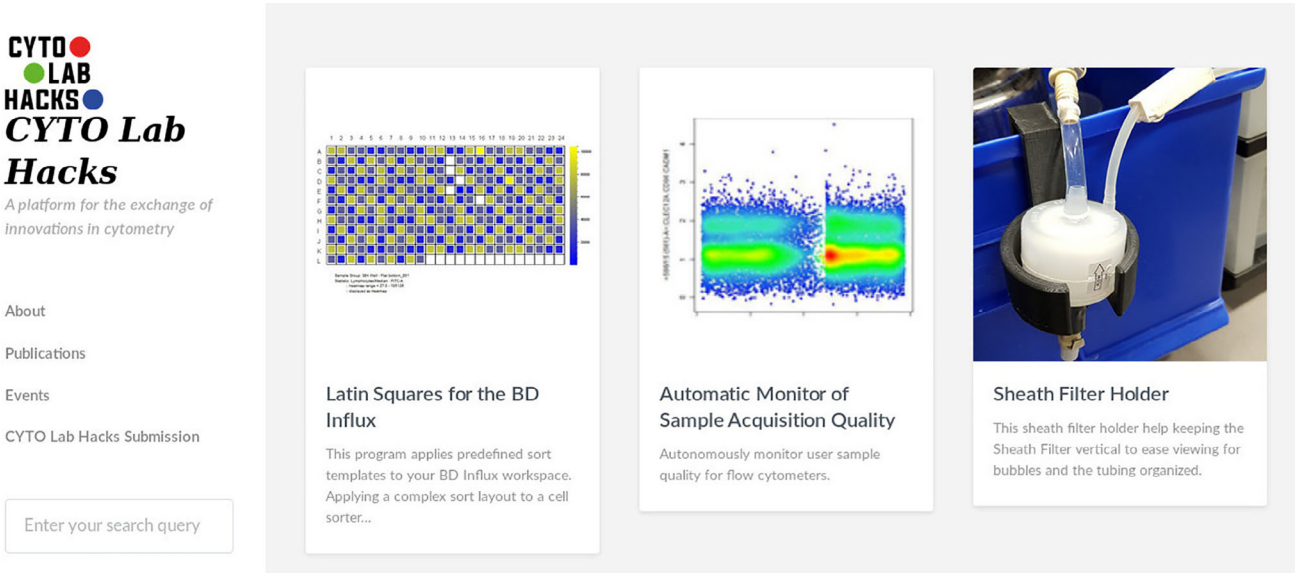

Figure 4. Snapshot of the CYTO Lab Hacks website. The website features a navigation panel with four sections. The entries lead to website sub-sections: 1. "About" the CYTO Lab Hacks project; 2. "Publications" related to the project; 3. scheduled "Events"; and 4. "CYTO Lab Hacks Submission" button to post new innovation. The main window to the right shows three innovations currently posted on the website. The image is current as of October 2019. [Color figure can be viewed at wileyonlinelibrary.com]

published in any form anywhere across the Internet. The purpose of CYTO Lab Hacks is to bring them all into one clear, concise, categorized, and searchable directory pointing to the relevant repositories containing further details about the submitted innovations. CYTO Lab Hacks should deliver advantage to both the contributors and visitors. The contributors would gain a much larger audience for their innovations in exchange for a small amount of effort when submitting a summary and a link to the innovation. The visitors would have one place to look for a broad range of cytometry-related innovations without needing to trawl a range of online resources.

The concept of CYTO Lab Hacks was first presented at a workshop during CYTO 2018. We collected feedback from the participants, recruited volunteers, and collectively devised a development strategy for this initiative (1). Subsequently, we organized a group of volunteers. The group agreed on priorities for the development of CYTO Lab Hacks. It started developing a prototype website to verify the concept. At the CYTO 2019 workshop, we introduced the website with example content to draw experience and feedback from the workshop participants. The conclusions are reported in this manuscript.

Methods

The workshop was structured into three parts (Supporting Information 1, see WS11_SI1_Presentation). We first introduced CYTO Lab Hacks and its prototype website (http://bit.ly/CytoLabHacks, Fig. 4). We presented the structure of the workshop and three examples of innovations submitted to the CYTO Lab Hacks website. We then introduced three questions to be discussed for 20 min in three separate breakout groups. The three discussion questions were:

- What makes an innovation generate interest and enthusiasm in the cytometry community?
- How to organize the CYTO Lab Hacks group to work efficiently toward its goals?
- How should CYTO Lab Hacks website look like? What should each submission include?

Each group was led by one leader with the responsibility for moderating the discussion and recording the findings into written notes or audio recording. Finally, each leader briefly summarized the conclusion of their group discussion to all workshop participants. At the very end, volunteering participants came forward to briefly present their own innovations, which they could contribute to CYTO Lab Hacks. The workshop was highly interactive and rapidly paced. The choice of three topics and the small size of each breakout group (< 20) allowed all participants to get involved in their preferred subject and have their opinion heard.

Outcome

In the following paragraphs, we summarize the discussion outcomes from the workshop breakout groups. Each breakout group had one question to address.

What makes an innovation generate interest and enthusiasm in the cytometry community? This was an open-ended question designed to solicit an opinion on what the cytometry community would like to share and what would encourage them to use the website. The lively

discussion singled out training and documentation as the overriding themes. Participants wanted examples of standard operating procedures (SOP) for user and instrument training, techniques, reagent composition, DIY devices, and protocols. It was felt that many laboratories were needlessly reproducing the same documentation, wasting valuable time and resources. There was interest in physical hacking of instruments, with access to 3D printer schematics for tube holders and chilling units for sorters as examples. Software resources such as tools to simplify routine tasks including automatic search for customer publications, automating quality control (QC) of instruments, and scripts to simplify data backup. A database of software versions and instrument compatibility was mentioned as being a useful and currently missing resource for the community. Databases for antibody panel designs, online resources, for example,. spectrum viewers, educational resources, fluorophores, and of manufacturer instructions/best practices were other listed examples. An overarching theme of the discussion was the need to find ways to save money and time. The CYTO Lab Hacks resource was seen as a promising solution by saving time through offering a central store of useful resources and in offering a way to easily search technical solutions that could circumvent costly replacement parts or entire instruments by manufacturing your own parts or implementing software upgrades.

How should CYTO Lab Hacks website look like? What should each submission include? The prototype website and a video demonstrating the envisioned submission process (see Supporting Information Video S1, WS11_SV1) were presented to the group. The group responded to both overwhelmingly positively, finding the concept genuinely useful to their workflows. None of the participant was aware of any existing alternatives. The group emphasized the need to develop the website as a directory for innovations rather than an all-purpose data repository. Suggestions for practical features arose from the discussion. The group emphasized the need to provide powerful search and categorizing tools for the website, including subscription for automatic notifications. The user experience (UX) should be simplified by avoiding one-fits-all submission process, but rather offering conditional questions leading category-specific submission workflows. Forms should include predefined default checkboxes and selections to simplify submission process. An edited list of tags (keywords) should be prepared to prevent the use of multiple similar keywords. Submissions should additionally include a free text description area spelling out presumed applications and uses for the innovation. Comments section needs to follow each submission to serve as peer-review. An effortless scoring and basic peer-review should be offered through like/dislike, star-rating and "used it" buttons. The website should offer a forum and an area to post questions and requests for help in accomplishing tasks. Finally, the website should be automatically scoring innovations according to popularity and prioritize displaying valued innovations over old and rarely visited ones. Beyond the website and the submission process, the group raised the point of needing to keep the operation streamlined and low cost in terms of money and labor. A contingency plan should be included for anticipated failures and breaking points.

How to organize the CYTO Lab Hacks group to work efficiently toward its goals? This discussion was centered about the professional resources required for the successful delivery of the website. The top role everyone mentioned was an IT professional involved in the organization, development, and maintenance of the website. The community input is critical in the feature selection process and the donation of innovation submissions to the website. The next role centered on communications to maintain regular progress communications. There should be a general communication to the users of the website and an ISAC-centered communication to drive traffic specifically from ISAC members. The third role was for a "curator," one looking for potential submissions and inviting their authors. The group expected early adopters would volunteer to supply material, but the curator would continuously procure new material. This role would work with the communications contact to request material from ISAC members; but then go beyond and actually look outside membership for relevant hacks. The curator would also maintain and add relevant tags (keywords) and categories. The group agreed on the need for a project lead, while retaining the committee approach to build resilience, institutional memory, and legacy and avoid overbearing the project lead. The project lead would encourage other volunteers to remain on track and inform everyone of deadlines and progress. There should be quarterly video conference calls to check progress. The group also discussed the question of ownership of the content and licensing and question of transfer of ownership from the employer to the website. However, this discussion did not conclude with specific suggestions.

Conclusions and Perspectives

This workshop was successful and demonstrated the virtue of crowdsourcing ideas for this community-led project. With only 60 min available, we divided the participants into breakout groups small enough for everybody to be heard. This maintained high level of authentic engagement within each group, while allowing us to cover three discussion topics in parallel.

The three discussion groups returned a wealth of actionable ideas. As a result, we will reorganize the group of volunteers into a more distributed leadership model. This should allow agile and robust governance and higher productivity in delivering the CYTO Lab Hacks project and website. The crowdsourced evidence for the most desired innovations will guide the future "curator" to prioritize submissions based on a representative opinion rather than personal preference. The development of the website functions, user experience, and interface will be driven by the feedback from this workshop. Preparing a prototype website and a case study video for the purpose of this workshop turned out to be very important (Supporting Information Video S1, WS11_SV1). It effectively engaged the participants minds and allowed them to provide

constructive feedback. Moreover, the workshop served the important purpose of confirming the interest of the cytometry community in the CYTO Lab Hacks concept. It was an important reality check ensuring the project represents the opinions of the community not just the limited group of volunteers. Finally, the workshop was an excellent advertising opportunity informing ISAC members about the progress of this initiative.

Following this workshop, we will restructure the volunteer group to drive the project toward completion and roll out at CYTO 2020. We will seek funding support from within and outside ISAC to cover the development cost of the website. We expect a significant volunteer effort being required during the development and roll-out phases. We anticipate gradual growth of interest from the cytometry community, developing a self-moderating and self-reviewing process. This will eventually lower the required volunteer effort to editorial and communication roles. We anticipate CYTO Lab Hacks integrating well with educational and innovation related activities of ISAC. It would provide resource of state-of-the-art content for these activities and a communication platform with significant outreach to the ISAC members and the wider cytometry community. We encourage anyone interested in CYTO Lab Hacks to visit its prototype website (http://bit.ly/CytoLabHacks) and contact the authors of this manuscript.

Acknowledgments
We would like to thank ISAC for facilitating this workshop, the participants for their valuable contributions and Pansy Aung for helpful discussions and recording the supplementary video.

Conflicts of Interest
The authors declare no conflicts of interest.

Chapter 1: Trends Conclusion

The three workshops of this chapter revealed several outcomes, this includes identifying the need for creating a sorting data repository for different cell types, launching the African Work group within the I4S and initiating the dialogue between instruments and reagents suppliers for more directed and efficient aid for clinicians and cytometrists in Africa. The updates on the work on CYTO Lab Hacks online directory clearly show that there is a good progress and the future directions are well set as also confirmed by the workshop participants.

Currently in the era of Internet, we are daily flooded with information of often unclear quality, in some cases contradicting statements, but sometimes the answer to our questions can be found nowhere. What if there was a directory that resembles the concept of the Internet of Everything, combining cytometry-related material developed by the global scientific community in form of concise summaries, links to repositories with experimental protocols, descriptions of hardware changes, document templates, software code, in the end providing knowledge that could be applied for new innovations. The information that are to be used for further research are finally easily accessible, systematized, verified, and supported with solid data. There would be no more need for reinventing the wheel, no time wasted and no scattered approach in research and development only directed and rapid advancement of cytometry. This goal is emphasized by the above initiatives and is worth going for.

Chapter 2: Shared Resource Laboratory (SRL) Best Practices

The four workshops compiled in this chapter address burning topics related to SRLs. As in the related 2018 Workshop summary chapter many challenges and problems associated with running SRLs were discussed. The participants attempted to address the questions on how to handle difficult situation with costumers (WS02), which career path options SRL members can choose from (WS08), what are the best practices in training of users in cell sorting and how this can be beneficial to the SRLs (WS06) and does the best SRLs practices successful implementation affected by the number of SRL staff (WS09).

WS02: Strategies for Successful Conflict Resolution: You Can't Hide in Your Office and Hope the Problem Goes Away

Jessica B. Back, Ann Marie Des Lauriers-Cox.

Introduction and Aims
Workplace conflict can be pervasive and lead to wasted time, poor decision-making, and employee attrition (14). The unique service-based and collaborative environment of SRL combined with the distinctive ways that scientists tend to interact and communicate with others is an excellent recipe for conflict.

This workshop sought to address three key components toward creating effective working relationships with disgruntled or difficult colleagues. First, in order to effectively address conflict, one must work toward a resolution that is beneficial and acceptable to all parties. Second, a necessary requirement for establishing meaningful conflict resolution is to balance help and support while maintaining self-worth and respect. Third, to empower SRL staff to address challenging situations independently. The goal was for participants to leave the workshop with a toolbox of strategies and techniques for resolving conflict. Specific emphasis was placed on resolving issues in the context of a service-oriented environment.

Methods
A combination of a pre-workshop survey, on-site presentations, live polling, and break-out discussion sessions was utilized to examine methods for successful conflict resolution within an SRL. Break-out discussions were performed in four groups of attendees that were each assigned real-world case

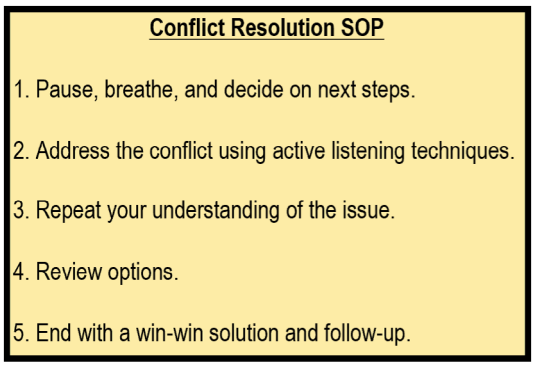

Figure 5. Five-step SOP for conflict resolution. [Color figure can be viewed at wileyonlinelibrary.com]

studies to discuss, and feedback from each group was reported to the attendees.

Results

Survey Results. In order to gain real-world insight into common conflicts experienced by SRL staff, the workshop organizers solicited input from the cytometry community through a pre-conference SurveyMonkey questionnaire. The questionnaire was shared with the community through the Purdue Cytometry List and the ISAC website Social Link (See Supporting Information S1, WS02_SI1). These real-world examples of conflict were presented as case studies for break-out group discussion during the workshop as exemplified in Figure 5.

Workshop Presentation Results

The workshop presentations aimed to provide attendees with specific strategies for effective conflict resolution in the form of a five-step SOP. In addition to the five-step SOP, strategies for how to proceed when the process breaks down or comes to a stand-still as well as when and how to say "No" were discussed.

Pause, breathe, and decide on next steps. Self-awareness is a critical first step toward effective conflict resolution. Each party must be able to identify how they are feeling and become aware of their own personality traits. This self-awareness helps each party anticipate their own behavior, what they are likely to do next, and whether that behavior is appropriate for the situation. To this end, attendees were asked to participate in a self-assessment exercise (15) using Sli.do polling and identify their reactions to five different scenarios as 1. Attack, 2. Retreat, 3. Verbal, 4. Nonverbal, 5. Internalize, or 6. Externalize. Nineteen attendees participated in the poll and the results were presented in real-time (Supporting Information S2, WS02_SI2).

Address the conflict using active listening techniques. It is important to be prepared for any discussion of conflict with the other party. This preparation includes gathering facts and identifying the interests, aspirations, and needs of all parties involved. The goal of any discussion should be to focus on the problem rather than the personalities involved. Storytelling can be an effective way to illustrate perspective, but it is also important to create space for the other party to process the information presented and respond accordingly. In every case, assume positive intent and be mindful of nonverbal cues (body language).

Repeat your understanding of the issue. It is also important to make sure each party understands the other's point of view. Four listening tools can be used to make others feel safe to speak frankly: 1. show a genuine interest by inviting others to express themselves and share their thoughts and views; 2. create a safe space for discussion by acknowledging the perceived issues, especially if the other party appears reluctant to talk; 3. paraphrase what you have heard to acknowledge and verify the story; and 4. offer a best guess of what you perceive the other party may be thinking or feeling if they are still not sharing their point of view.

Review options. Stay focused on the problem and improving the situation. In order to negotiate an effective compromise, it is necessary to be both accommodating and assertive. Negotiations must be done in good faith through a series of honest efforts by both parties to reach an agreement.

End with a win–win solution and follow up. Once an acceptable solution is achieved take care to define expectations and review next steps to take. Follow-up with a written summary of the process making sure no questions linger and that what was agreed upon is accomplished.

Break-out Discussion Session Results

The ~50 workshop participants were divided into four groups and assigned a case-study created from the pre-conference survey to discuss. Each group was asked to identify a spokesperson who would take notes and present the group's discussion to all of the workshop participants.

Case Study 1. In a growing SRL with two staff (Director and Assistant Director) the original Director (Dir 1) has retired. A new Director (Dir 2) has been put in place. The Assistant Director (AD) has stayed in place through the transition. Under Dir 1, the AD had lots of intellectual and research freedom and support, which led to development of some unique skills and expertise in the field. With Dir 2 in place, the AD feels these skills/expertise are being exploited to help "pad" Dir 2's CV and build a reputation for Dir 2 whose own research has not been getting consistently funded. AD doesn't want to leave the position because they like the work, location, etc. but also doesn't want to continue writing papers and building a reputation for Dir 2 and essentially serving as Dir 2's "Golden Goose." When AD has tried to raise some of these issues/concerns with Dir 2 (i.e., Defining specific roles in papers), they have been dismissed or waved-off as premature. What should AD do? How would you start a meaningful

conversation with Dir 2? What compromises can AD make? Or has AD already compromised enough?

The recommended resolution for this Case Study was to first have the AD establish a timeline and provide a detailed written summary of the initial conversations with Director 2. Next, the AD would meet with Director 2 to define their specific goals and interests, noting their preference for acknowledgment in scholarly products. The AD would also ask Director 2 to define their expectations for the AD. After this discussion, the AD would follow up with a meeting summary by email showing responsibilities and commitments of both parties. In the event, the AD needed to move their concerns up the Institutional chain of command, these written summaries would provide documentation to support AD's claims.

Case Study 2. A new technician has been hired in a core. The Manager has been working alone for almost a year and starts dividing up work and assigning projects to the technician. The first project the technician works on is with a Post-Doc looking for a rare stem cell population in Rat Intestines. The Manager has had consistent non-specific binding issues with these samples and has been unable to make any headway with the Post-Doc. After much digging and troubleshooting, the new Technician determines the Antibody the Post-Doc was using was a Rat-αHuman antibody. When the technician informs the Post-Doc of the antibody issue the Post-Doc is embarrassed about making such a "basic" mistake but also fearful of telling their PI as they have wasted 1 year and countless animals and resources on this project. At this point, the technician feels the issue is resolved and moves on to help other clients. Approximately, 9 months later, the technician receives an e-mail from the Post-Doc stating that one of the Reviewers for the paper they submitted on this research is asking to see the Isotype Controls for this data and they need to get it from the core to respond to the Reviewer. The technician knows that the data are unusable because the wrong antibody was used. What would you do? Who do you inform? How do you handle this seemingly obvious disregard for research integrity?

The recommended resolution for this Case Study was to first request a copy of the submitted manuscript from the researcher to acquire specific information about the data set used. This would allow the SRL staff to verify whether the error was noted or if a false assumption was made by the SRL staff. Next, the SRL staff would request a meeting with the Post-doc to discuss the results in detail and reiterate their concerns about the validity of the data. If the Post-Doc still omitted this error; it was recommended the SRL staff elevate their concerns to the PI and the Institutional Office of Research Integrity.

Case Study 3. A SRL Director is also Junior faculty in an academic department. Because of these "two hats" they also have two different supervisors—the SRL Director reports to the VP for Research and the Faculty Member reports to the Department Chair. Coincidentally, the SRL is located within Director's Academic Department's space. The Academic Chair routinely asks the Director to lie about SRL business to the VP of Research: Although the SRL chargeback rates were lower than most academic institutions, the Departmental Chair would constantly ask for departmental discounts. When presented with the NIH guidelines which state that differential rates may not be charged, the Academic Chair appears to retaliate by telling the VP of Research that the Director was doing a bad job but would not tell the VP or the Director what they were unhappy about. The Director quickly realized that their academic promotion would be tied to their disregarding NIH regulations on pricing along with lying about core activities to the VP of Research. What would you do? How would you handle this conflict of interest from a supervisor?

The participants in this group first discussed the option of leaving the job as it was possible this delicate situation could lead to termination of employment. A second recommendation was for the SRL Director to meet with the chair to address their integrity concerns. This would be followed by examining different management structure options. Next, the SRL Director should take their concerns up the Institutional chain of command to the appropriate Dean. Additionally, they could take their concerns to the Human Resources Department and request an appeal for proper time charges to show correct usage.

Case Study 4. At a busy SRL with several locations, technicians staff each location and often operate outside of the direct oversight of the director. All of these technicians have similar duties and responsibilities within the SRL. One of the technicians is viewed by customers as messy, distracted, and abrasive. Customers feel this technician rushes to get their samples done and does not take enough time to listen to their requests or concerns. Because of this, customers are avoiding working with this technician causing their workload to fall on the other technicians in the facility. The other technicians are getting frustrated and overwhelmed by the imbalance of work and with continuously cleaning up after this technician. What would you do? How would you handle this conflict between staff? Between the technician and customers?

The recommendation from this group was for the SRL staff to take a direct approach and discuss the situation with the technician giving specific examples of their complaints. If no change in behavior is resulted from this discussion the SRL staff would need to inform their director. The director would then make his/her own impartial evaluation of the situation and identify the needs and interests of all involved parties. It would then be the responsibility of the director to establish a clear and concrete action plan with appropriate follow-up.

Conclusions

Conflict is inevitable when people interact; therefore, it is important to develop a toolbox of techniques for addressing conflict to create an effective environment for collaboration. Workshop attendees were provided with a SOP for addressing

conflict and given an opportunity to apply this tool to real-word case studies during a break-out session. For SRLs to be effective research partners, it is imperative they create a culture that encourages feedback, honest dialogue, and transparency.

Conflicts of Interest

The authors declare no conflicts of interest.

WS08: SRL Careers: What Can ISAC Do To Help Us?

Yasmin Haque, Rachael Walker, Aja M. Rieger, Julfa Begum, Kylie M. Price.

Introduction and Aims

A SRL has been defined as a core resource that provides highly skilled technology scientists and advanced instrumentation to enhance the scope and quality of biomedical research (16). Staff at different stages of their careers can often be found within an SRL and each will have differing needs in terms of support, education, mentoring and coaching. This was highlighted within the paper by Barsky et al. (17), which listed continuing education of SRL staff as an SRL Best Practice. However, in practice, many SRL staff can struggle to find relevant support for continued education especially from their Institutional workplace. Within many SRLs, there is a career track that leads toward becoming a core manager/director. Unfortunately, this track does not exist in all Institutes. Therefore, junior SRL members are often uncertain of how to get onto a rung of the career ladder. A key aim of this workshop was to discuss how to support junior SRL staff or newly appointed SRL managers. In addition to this, this workshop aimed to cover what the next career steps for an established core manager could be. This included discussions on studying for further qualifications such as an MBA, obtaining certified professional recognition, aiming to become a director of several core facilities, taking on administrative roles within your organization, or transitioning into industry.

This workshop sought to investigate whether there was a need from SRL members of all levels for support from an Institutional level, a local level, and whether ISAC could do more to support career development. Another goal was to be able to access one or two key ideas for potential program development or future workshops that would benefit the SRL community.

Method

Prior to CYTO 2019, a pre-congress workshop questionnaire was sent out to the flow cytometry community using a Google Survey (see Supporting Information, WS08_SI1). This survey consisted of 19 questions and was sent to the Purdue Cytometry List, London Flow Club mailing list and Twitter account, *flowcytometry*UK mailing list and posted on LinkedIn. The survey was designed to determine the current level of SRL support in the cytometry community across all sectors and career stages. It also aimed to address what people thought were key challenges of running a core facility. In short, the challenge areas identified were "Setting Expectations," "Other Management Skills," "Education," and "Facility Direction." The results were collated, summarized. and presented at the end of the CYTO 2019 workshop. The survey and workshop results can be found in the Supporting Information.

The 29 delegates who attended the workshop were asked to actively participate and share their experiences, Figure 6 gives the delegate responses. Delegates were randomly assigned to groups, to ensure there was a mix of experienced and junior SRL members in each group. Each group looked at the needs of one of the following distinct career stages: new to an SRL, new SRL manager or established SRL manager/director, regarding:

1. What internal/local support each group has
2. What ISAC could do to support career development
3. Helpful tips for career development

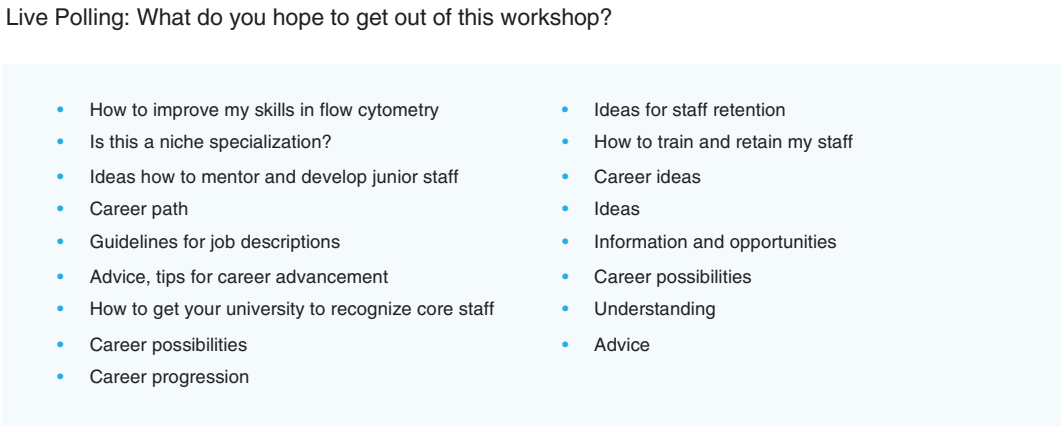

Figure 6. Workshop attendees were asked to give their reasons for attending the workshop and what they hoped to get out of the workshop. All delegate responses are listed. [Color figure can be viewed at wileyonlinelibrary.com]

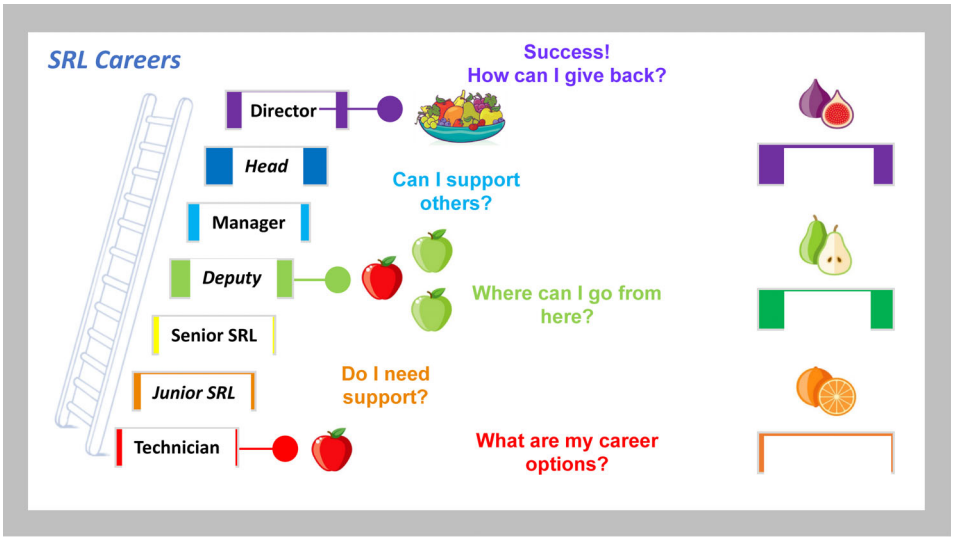

Figure 7. Some key considerations for a SRL career pathway are highlighted. [Color figure can be viewed at wileyonlinelibrary.com]

Survey Results

The survey was completed by 100 respondents, the majority of whom identify themselves as an SRL manager or SRL staff (Director = 16% SRL Manager = 40%); SRL staff (Senior = 19% Junior = 10%). Eight-two percent were working in an academic setting, with 50% of respondents having less than 10 years' experience in an SRL. All career stages were represented which reflected a good proportion of the cytometry community (although, no clinicians replied to the survey).

When surveyed regarding what the greatest challenges in an SRL are, the respondents put near equal importance on setting up a reputable respected facility, juggling managerial tasks (such as managing a team, managing users, time management, troubleshooting and finances) and the educational components of running a core, including not only user and SRL staff education but also their own continuing education and development. Furthermore, in the "Setting Expectations" challenge area, establishing a reputable core facility was identified as a key factor and scored the highest (See Supporting Information Fig. 3B, WS08_SI2).

The importance of mentoring was also explored. Such a program is needed to give advice on careers, personal development, job opportunities and self-driven career development. The online survey determined that only 9% of all respondents receive official mentorship, either through ISAC, the Association of Biomolecular Research Facilities (ABRF), International Clinical Cytometry Society (ICCS) or other internal organizational programs for staff development (See Supporting Information Fig. 7C, WS08_SI2). In addition, those surveyed were asked questions about whether they would be interested in participating in a mentoring program either as a mentor, a mentee, or as an organizer/advertiser to help set up a program. Mentoring at a local level (regional flow clubs) by ISAC Affiliated Societies or ISAC scored highly (58%, 52%, and 59%, respectively). Ninety-three percent felt that this mentorship program should come at no cost. The ability to discuss facility issues with a mentor was highlighted as important. It was also noted that other staff members of the respondent's SRLs would also benefit from career support and potential mentorship. Fifty percent answered that there was some form of support at their current workspace. Of the 25% of respondents that already receive mentorship, 16% stated they receive mentorship informally from Facility members, work colleagues, or from within their local communities.

Workshop Results

During the workshop, attendees were polled using Sli.do. The initial live questioning was designed to consolidate the need for careers guidance and gauge the existing avenues that individuals receive support (results in the Supporting Information). Of the 29 attendees, 10 were managers/directors and 10 were SRL staff (senior/junior). Fifty percent of workshop attendees had under 5 years' experience in cytometry. Attendee's expectations were to gain knowledge regarding: how to mentor and develop junior SRL members; careers advice and tips for career advancement; ideas for staff retention and how to get Institutional recognition of SRL careers. Due to time constraints, the pre-congress survey was not discussed in any detail as it was felt that the discussions should not be influenced by the survey results.

Outcomes from the discussion groups showed that SRL members at each stage require support at the local and international level. It was suggested that this support could come from other local cytometrists, a regional or national cytometry society and from ISAC. However, each career stage had differing needs. For example, those new to an SRL require help with learning more about the technology, advice on working in an SRL, careers advice, and mentorship to progress. New managers require help and guidance from other SRL managers, advice for setting up a core, budgeting, and staff management training. For established managers, formal

Table 1. Summary of all the suggestions gathered during the workshop group discussions

NEW TO AN SRL	NEW SRL MANAGER	ESTABLISHED SRL MANAGER/DIRECTOR
	What internal/local support does this group have	
Get trained by more senior people at your workplace or within the local community	Meet with other core facility managers at your workplace (if they exist), ask them about their processes and replicate what will work for you	Meet with other well-established core facility managers at your workplace. Freely pick their brains, share and discuss issues/topics, presumably you share many of the same clients
Do a lab-rotation (1–2 weeks in a neighboring core facility)	Arrange a secondment to a higher role with more responsibility (initiatives sometimes exist within a university as part of succession planning activity for departments)	Many SRL staff find there is no established career track within their institute and therefore often lack both career and technical support
Attend local meetings and conferences	Attend local meetings and conferences	There can be a barrier to becoming a core manager/director as many advertised positions require a PhD or proven management experience
Join local conference organizing committee(s)	Join local cytometry groups	
Hold monthly less formal meetings with cytometrists from the town, city, region, and so on		
Find a local level mentor (not your manager and preferably not someone within your own workplace, best to be external)	Find a local mentor	
Try to get education perks from your workplace. Will they pay for relevant studies (e.g. MBA, immunology papers, professional development or training courses)?	Go on staff development courses and formalize skills	
Apply for internal or local awards if available. If they do not exist try to suggest these are created and increase your profile in the process		
	What ISAC could do to support career development at this stage of your career	
Mentorship program open to all ISAC members (not just the Emerging Leaders or Scholars)	Mentorship program. Ideally a mentor should not be in the same geographical area (not a competitor for users). Mentor SRL should have a similar structure and size (expertise/experience is more important than location)	A mentorship program was determined to be not as necessary for an established manager
Senior ISAC members to publish their career pathways, to provide different career models. This information could be on the societ's website	Information about what other careers look like and examples of career progression	A peer support database to cover specific SRL issues such as maternity leave or dealing with a difficult member of staff
Provide travel grants for Junior SRL staff in particular	Early career opportunities to attend CYTO congress	A coaching program (methods to coach and develop junior staff). Information in a webinar, workshop, or on the website
Include leadership development talks/ session for junior SRLs in the conference program	Read International Society for Advancement of Cytometry (ISAC) flow cytometry shared resource laboratory (SRL) best practices publication in	Further education about skills necessary for a core manager

(Continues)

Table 1. Continued

	Cytometry A: https://doi.org/10.1002/cyto.a.23016	
Junior SRL session could include a mechanism for creating a buddy system to help extend Junior SRL networks (slightly different to first time attendee as you could be at any career stage and a first time attendee)		Documentation and advice for the grant application process
Website pages about how to get involved in ISAC or have a junior SRL-specific page		
Emphasize how cheap junior-SRL conference rates are. Detail actual rates in a conference advertising e-mail campaign		
Encourage managers to consider sending junior staff to CYTO		
Guidelines for managers on what they need to let their junior SRL staff know about (e.g., ISAC, Purdue list, ISAC forums, local conferences and societies, funding award opportunities)		
Helpful tips regarding how to develop this stage of your career		
Encourage SRL management to provide you with some career pathway guidelines (ask them to write some if there aren't any)		Arrange a sabbatical to another core facility that represents the future direction of your own SRL (size, instrumentation, research programs/outputs, etc.)
Actively set expectations and discuss how to progress upwards	Attend CYTO congress	Attend both academic and industry-related conferences
Seek management experience, ask for small tasks, for example, creating monthly user-hour reports	Find local supports to aid in career progression	Host the local or national cytometry society conference(s) in your city
Practice report writing, ask if you can provide your manager with a monthly report detailing activities (should be a win–win)	Become involved with local cytometry groups	Be actively involved in ISAC at various levels
Come up with small ideas for core facility publications, ask for a small amount of time to work toward group papers	Become involved with ISAC or affiliated societies	Collaborate with pharma or manufacturers, get involved in areas of innovation, research and development
Ask to get involved in your local cytometry society or group	Think about publishing (meeting reports, reviews and papers)	Publish (relevant reviews, letters, and competitive scientific research papers)
Get involved with teaching/training at your institution	If at a university, get more involved with teaching and training courses. This could be both formal and informal teaching	Formalize your teaching/training skills
Ask to join departmental committees (e.g., post-doc, equipment, social committees) to gain experience	Take on other administrative roles within your department	Take on other administrative roles within your department/institute

mentorship was not deemed as important; however, a peer support network for specific issues was flagged as an idea. This peer support network would be a database of other SRL managers with experience in specific areas such as, establishing a healthy work-life balance, taking parental leave, managing staff, or growing a facility.

The discussion also raised the possibility that ISAC could specifically support career development for both established professionals and those new to an SRL. This could be in the form of digital resources, including member profiles with career pathways, guidelines for managers, advice for junior SRL, documentation and advice for the grant application process, and information on how to get involved with ISAC. The results of the discussion were summarized by the facilitators at the end of the workshop (see Fig. 7 and Table 1).

Conclusion

Many ideas were presented during the survey and workshop; however, one consistent message was the need for a more structured ISAC SRL Careers and Mentorship Program, available to all ISAC members. More experienced SRL members who have attended several CYTOs commented on how the SRL track of workshops was immensely helpful for them, while junior SRL members stated that they would like more SRL tutorials/workshops at CYTO meeting. The discussions identified a large market for junior SRLs as a pivotal group in need of support. The survey highlighted that this was the main group that would benefit from formal mentoring. The discussions also identified several other resources that ISAC could create and offer to cytometrists, including specific workshops at CYTO 2020 and web-based resources. These could include training and advice on SRL careers, further education and mentorship.

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Conflicts of Interest

The authors declare no conflicts of interest.

WS06: User Training in Cell Sorting in Core Facilities—Eliminating the Staff to Instrument Bottleneck

Derek Davies, Claudia Dumrese, Christina Ewald.

Introduction and Aims

An issue many SRLs offering cell-sorting services face is staff availability limiting access to cell sorters for untrained researchers. Instrument time, flexibility of service hours, as well as financial aspects limit the scope of sorting experiments. Because the idea behind SRLs is to allow access to high-end technologies and make these available for researchers at reasonable cost, we consider this issue a key challenge for any SRL lab. One way to circumvent this bottleneck is to train end users. Our workshop was aimed at SRL and core facility staff, researchers from institutions running group allocated cell sorters, and company representatives designing cell sorters and associated training programs for the community. We aimed to gather the community's experience with different models of cell-sorting service and training to gauge whether user training in cell sorting is feasible for the majority of SRLs and to develop and document consensus strategies for successful cell sorting training. For the future, we envisage the development of a Best Practice consensus in user training, including development of SOPs and metrics for success, to provide the community with detailed guidance for the successful implementation of a sorter training program. By providing guidelines and data on the training effort, staff resources, instrumentation, and infrastructure required, we hope to encourage the community to tackle the limitations associated with dedicated service sort operators.

Methods

We combined results from a pre-workshop survey with on-site presentations, group work, and discussion. Group work was performed in three taskforces focusing on individual aspects of user training. Results and feedback from the groups were reported to the audience for discussion and documentation of consensus.

Survey Results

Complete survey data are given in the Supporting Information, see WS06_SI1_SurveyResults. The survey was submitted to Purdue list and numerous other international networks on May 10, 2019 and closed on June 20, 2019. Of 105 participants distributed internationally, with the majority located in North America and Europe, most (87%) work in a SRL environment and operate a cell sorter independently (81%) on a daily or weekly basis, see Table 2. Results showed that efforts to accommodate user training needs have already begun in the community with a considerable number of SRLs offering a combination of self-service and operator-based service (42%). However, 36% still have all sorts performed by dedicated staff. Access to a training program was available to 60% of participants. Training resources varied with 52% having access to a training program for users organized by SRLs. The main limitations indicated for cell sorting were inflexibility of service hours, long waiting times, and financial constraints.

A majority of survey participants who were users of a cell sorting service indicated that they appreciate the support of experienced staff (61%), 32% felt that sorters are too complex to be operated by users and 37% feared quality would suffer without experienced staff operators. Encouragingly, 26% indicated that they would like to operate cell sorters independently. Nobody showed concern that training costs would be too high and only 4% had considered buying their own cell sorter (MAA). When asked about the biggest challenges in training users in cell sorting a wide range of issues

Table 2. Overview of selected survey results. Questions with multiple answers allowed (MAA) are marked with an asterisk

Location (%)	
North America	58
Europe	34
Oceania	3
Asia	2
South America	2
Africa	1
Frequency of sorting (%)	
Daily	67
Weekly	18
Monthly	7
Less frequently	6
Never	2
Sorting service model (%)	
Combination of self-service and staff service	42
All sorts performed by dedicated staff	36
Certain machines open for self-service	19
All sorts as self-service	3
Training type available (%)*	
User training by SRL	52
Training by manufacturer	14
Instrument manual	9
Introduction by colleague	7

was brought forward. The most prominent categories listed were instrument complexity, ensuring quality of sorts, teaching technical troubleshooting, and sort optimization. Further concerns were the trainability of users, their dedication to complete a training program, and the challenges of trusting users to operate instruments in a responsible manner. Additionally, time invested by staff and users, the need for routine and high frequency of sorts, and infrastructure limitations were of considerable concern. Furthermore, the cost-efficiency of staff operated sorts for users, biosafety, supervision, and support outside of service hours, and the need for objective metrics to assess training success were of interest to survey participants. In conclusion, the survey results confirmed that there is a clear need for user training programs in SRLs, but numerous challenges must be overcome to ensure high sort quality and that now is a suitable moment to initiate a Best Practice consensus in training standards in this developing field.

Workshop Presentation Results

The workshop presentations aimed to provide an overview of the challenge at hand and to introduce the sorter training programs at the Francis Crick Institute (FCI) in London and the Cytometry Facility at the University of Zürich. We presented the current staff and instrument numbers at each facility and the user cell sorting training programs in place since 2017 and 2012 at FCI and CFZ, respectively (See Supporting Information, WS06_SI2.)

The motivation to train users in cell sorting at both institutions stemmed from a desire to reduce waiting times for users, free up staff time and to educate and empower users. Initial concerns such as an unprofitable cost benefit ratio, increased repair costs due to user damage or service staff becoming redundant were not realized over the course of several years in both institutions.

Each SRL runs multiple analyzers and sorters and employs more than five full-time equivalent. Both offer training, cytometer operation, consultancy, and development support and run analyzers primarily as self-service. The biggest differences are the number of laboratory sites (FCI 1, CFZ 5) and the number of sorting hours by independent users. Approximately, 90% of sorts at the FCI are staff operated, whereas only 29% of sorter hours are staff operated at CFZ. Since 2017, roughly 60 users have been trained on the Avalon and Aria sorters at the FCI (in the future users may also be trained on the MoFloXDP if required). Comparatively, CFZ has accepted 118 trainees for Aria training over the past 4 years; 64% have successfully completed the program, 25% are still in the supervised training phase and only 11% have dropped out due to project changes. At both facilities, trainees are selected based on previous experience in flow cytometry and a proven need for regular sorts or sorting outside of service hours. Careful project assessment aims to ensure user trainability and avoid training users who will not practically apply their new knowledge in the future. The FCI is in the process of monitoring usage figures as the number of independent users increases. At CFZ, three staff (2.2 FTEs) are regularly involved in sorting services, of these only two staff (1.3 FTEs) are involved in sorter training. Since 2012, the number of total sorting hours has more than tripled, hours booked by independent users have risen more than fivefold, while only 1.6 times the staff hours have been invested.

At both facilities, training consists of an initial block of three introductory session combining theory and hands-on technical training at the instrument without user samples (individually or in small groups totaling ca. 8 h). Trainees then sort their own samples in several supervised sessions during service hours. So far, no formalized examination is required to complete the training at the FCI. At CFZ, the training is concluded by a 2 h practical and theoretical exam that also assesses routine troubleshooting skills.

Consensus and best practice proposal. The 82 workshop participants were divided into three taskforces moderated and documented by the workshop organizers and facilitators.

Is a self-service model financially viable? The first taskforce investigated the financial aspects of training users in cell sorting. The majority of participants in the group were training users already and indicated that they offer reduced rates for independent users compared to full service fees. Reduced rates were generally based on estimates, rather than actual data on reduced staff costs. Further financial gains for users can be achieved by increased usage spreading base costs across more users, thereby making the instrument more

affordable per hour. While, half the participants believed that more than one sorter is required to train users, the other half was open to train users in an environment providing only one sorter. Participants agreed that extra staff hours are required to train users; however, they supported the idea that experiments should be prioritized over training times. A majority of participants considered repair and maintenance costs due to user damage an important issue, a fact that most addressed with a service contract, adding that both the Aria and Sony sorters have proven to be very robust in this context. To maximize return on investment without sacrificing quality, teaching should ideally occur in groups no larger than three. It was noted that with efficient and effective teaching, costs can be kept at an acceptable level, and that participants expected or observed positive effects on instrument usage hours and income through independent users. Participants agreed that balancing training costs for slower trainees against high scientific quality may be a challenge. Cost of training should therefore be harmonized for the core curriculum to avoid fee disparities between different trainees. Follow-up hands-on training covering project specific content during user sample sorts or refresher lessons should be charged at an hourly rate to ensure the required teaching effort provided by SRL staff reflects the individual user's trainability and learning curve. It was difficult to define a common teaching cost standard per user due to fundamentally different financing models of different SRLs. The same is true for a common definition of cost to benefit ratio, since not all SRLs motivation is financial gain. Metrics that may be applicable for many SRLs can be the ratio of staff hours invested in service and training to total sorter hours before and after introducing user training. The authors suggest monitoring sorter usage hours for service and independent use as well as staff hours invested in service and training as crucial data required to assess the financial benefits of training. In conclusion, the financial taskforce agreed that training users in cell sorting are expected to be financially viable and beneficial for the majority of SRLs.

What should a training look like? The majority of participants either already ran a training program or were considering it. Participants supported requiring trainees to have previous experience in flow cytometry and a minimum number of sorts on a regular basis or the need to sort outside service hours. The level of technical detail to be taught was deemed to vary depending on the instrument. However, in all cases, training should involve troubleshooting skills and biosafety procedures. Participants agreed that even recently introduced user-friendly sorters such as the BD Melody, the Sony SH800 and the Bio-Rad S3 require users to have knowledge of the sorting process. Ideally, the training procedure should be tailored to individual project needs. Participants supported a unified core curriculum followed by individual training adapted to cell type and down-stream processing of sorted cells. All training options discussed included a theory session before hands-on training. The length of training could vary but should include continuous assessment of user performance. An end of training quiz or even a post theory quiz before starting practical training was supported. First sorts after training should be conducted during work hours with staff available before allowing independent use. Users lacking practical troubleshooting experience due to uncomplicated samples should attend a dedicated troubleshooting session simulating a range of different issues. In addition, detailed troubleshooting SOPs guiding users through technical and sample-based issues can help fill the support gap outside service hours. Participants stressed the importance of clear communication between staff and users through suitable channels. Manufacturers' courses and other external courses can offer a valuable alternative for users to get a basic level of instrument understanding in a relatively short time. However, SRLs are in the best position to offer support using the end-users' own samples in their own environment while being able to provide feedback on sort optimization and sort quality. When training in a setting close to the trainees' workplace, it is important to ensure that trainees can focus on the training without being distracted by other duties to ensure quality of results. In conclusion, the taskforce supported a concise training schedule covering theoretical knowledge and practical instrument handling as well as biosafety procedures for cell sorting. This part of the training should be the same for all trainees, hence resulting in a unified cost of base training for all users. Afterward, trainees gather experience during supervised sorts based on their individual experimental needs and performance. This strategy will provide a solid base of theoretical knowledge, while giving trainees longer periods of hands-on training to master the technique and gather troubleshooting experience according to their individual trainability. The frequency of training offered will vary depending on staff resources of the individual SRL but could either be on-demand or offered at set times during the year.

How to ensure and assess the success of a training program. The third taskforce discussed necessary considerations when introducing a new or evaluating an existing training program. The majority of participants already trained users in cell sorting. To balance training accessibility and sustainability of the training program, the group supported selecting trainees based on inclusion and exclusion criteria. It was noted, however, that in a situation of training being offered as a service for a fee, it is an option to offer training to any interested party as long as it does not negatively affect other tasks of staff. To ensure that as many users as possible will complete the training and employ their skills in the context of their research, user and staff turnover, as well as user diversity and trainability should be considered carefully. Furthermore, the taskforce acknowledged the importance of proactive communication, conflict and expectation management between staff and trainees before, during, and after the training. To encourage good performance and responsible behavior, the options of an exam and a penalty point system were brought forward. Regarding infrastructure requirements, the group agreed that ideally instruments should be as user friendly as possible and that a minimum of two sorters increases flexibility in case of instrument issues. Instrument

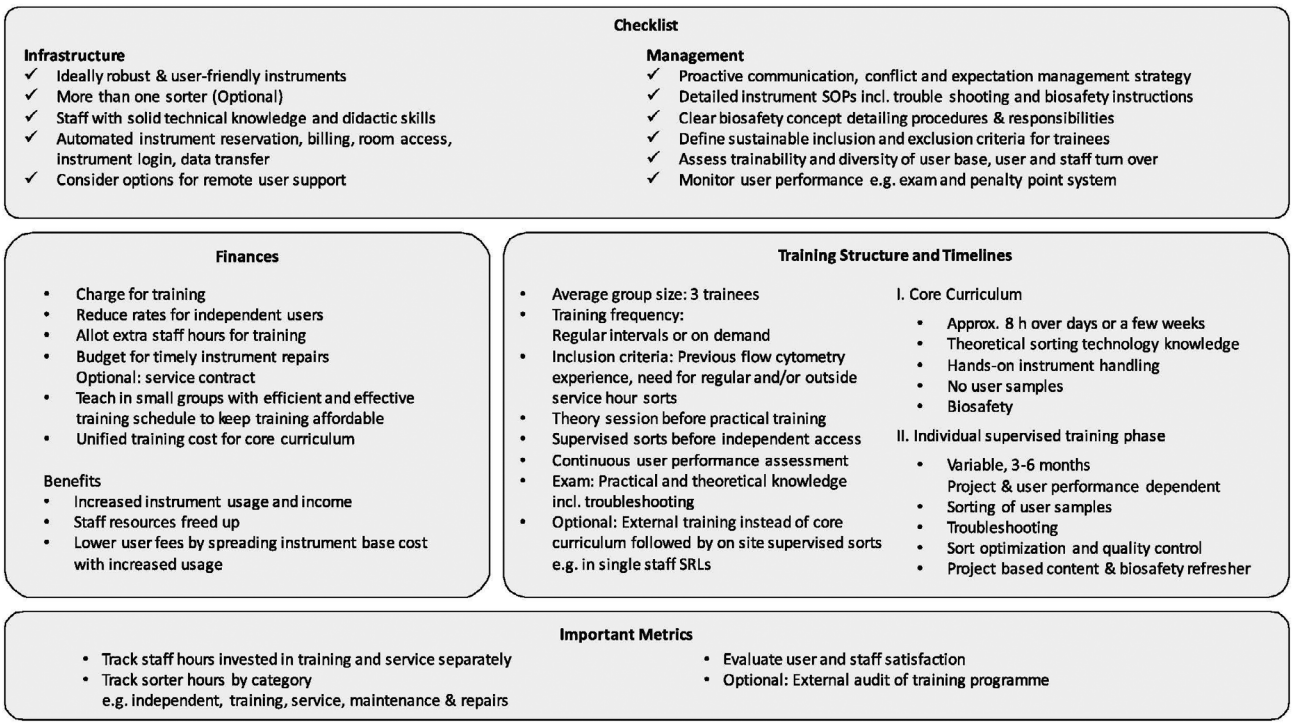

Figure 8. Roadmap to success—Checklist and Best Practice Guidelines: What, how and whom to train, what to invest and expect in return.

reservation, billing, room access, instrument login, data transfer, and potential remote support through staff using desktop sharing software should be as automated as possible. Staff should be qualified at a high enough level to transfer technical knowledge as well as guiding trainees didactically through the training process to create a positive learning environment. Ideally, training should not be the responsibility of a single staff member; however, the group agreed that single staff SRLs can tackle user training as long as they manage their resources carefully. The authors suggest monitoring instrument hours, staff hours invested in training as well as staff and user satisfaction to assess the success of a training program. Regular internal evaluation will help optimize the training strategy, ideally at times in combination with an external party reviewing the program.

Discussion

One of the key challenges SRLs face today is to successfully balance economic principles, research, and teaching in a fast paced and knowledge-driven environment. SRLs have to rationalize staff hours against difficult to track scientific output and streamline training strategies for different user bases and technologies, while ensuring high scientific quality. This workshop aimed to support the community in this challenge by providing data and guidelines in the context of user training in cell sorting. After assessing the consensus in the community, we have proposed best practice guidelines for the financial aspects of training, training content, structure, and time frames, as well as a checklist to be used by any interested party to evaluate an existing or proposed training program, see Figure 8. Requirements of SRLs operating instruments certified for diagnostic purposes remain to be addressed, an aspect that may become more relevant in the light of the trend to employ standardization procedures and quality control in flow cytometry on a regular basis. As the next step, we propose to establish a detailed SOP for training content listing all relevant points to be included in a sorter training curriculum and detailing exam content. We further suggest to investigate options of readily available pre-training resources to optimally prepare users for training. Discussions highlighted that while working toward a common teaching standard, more objective data across a larger number of SRLs is needed to assess the financial and educational benefits of sorter training. We encourage the community to track staff hours and instrument use in more detailed categories to lay the foundation for future assessments.

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Conflicts of interest

The authors have no conflicts of interest to declare.

WS09: When the Shoe Doesn't Fit: Defining and Overcoming Barriers to Achieving Best Practices for Solo-Staff SRLs

Laura J. Lewis-Tuffin, Roxana Del Rio-Guerra.

Introduction and Aims

The SRL is a common model for providing high quality, specialized, technology-oriented services that enhance the scope of biomedical research. Several factors contribute to SRL staff levels, including number of investigators served, size of the institution, resources (financial, equipment, and space), types and utilization of services, and the "culture" of the institution. Many SRLs operate with a single staff member who provides all services (solo-staff SRLs). Based on the 2014 ISAC SRL survey (18), roughly 20% of SRLs have one employee, and approximately 44% of SRLs have no more than two employees. In these SRLs, specific challenges due to the low staff number can arise.

In 2016, a group of ISAC members from a cross section of flow cytometry (FC) SRLs published a comprehensive document to establish standards and provide guidance for best practices in FC SRLs (17). Subsequently, a webinar series was developed for CYTO University, elaborating on the seven areas: SOPs, Training and Education, Quality Assurance, Laboratory Safety, Data Management, Staffing, and Operations (19). With the increasing attention paid to unreproducible research and discussion in the scientific community on how to address this problem, the definition of best practices for the operation of SRLs is critical. Moreover, these best practices are being used to develop an ISAC SRL Recognition Program to promote excellence in SRLs. However, while the best practices were intended to be achievable by any SRL, solo-staff SRLs face barriers (some of which are also faced by multistaff SRLs) that make them only partly achievable. This may prevent solo-staff SRLs from participating in the ISAC SRL Recognition Program. Therefore, the questions we wanted to address in this workshop were 1. Which of the seven best practices are easy and which are difficult for solo-staff SRLs to implement? 2. What are common barriers to implementation of best practices in solo-staff SRLs? 3. What strategies are solo-staff SRLs using to breach some of the barriers?

Methods

Prior to the 2019 meeting, we posted a survey on the Purdue Cytometry discussion list regarding SRL size and progress made on achieving the seven best practices (See Supporting Information, WS09_Sl1 and WS09_Sl2). We gathered demographics on the respondent's facility, asked whether or not they have achieved each of the best practices, and if not, what prevents achievement. Key survey results were presented at the beginning of the workshop (Table 3). The 23 workshop attendees were a roughly even mix of solo-staff and multistaff SRLs, from both academic and industry SRLs. Workshop attendees were split into seven groups, one for each best practice area. The discussion considered three main questions: What barriers prevent full implementation? What strategies promote successful implementation? and If the practice cannot be implemented, can it be modified it to reflect solo-staff SRL reality? At the end of the workshop, each group summarized and presented their findings to all participants.

Results

The pre-meeting survey had 59 responses and generated a large volume of data comparing solo-staff to multistaff SRLs in each of the best practice areas. A full analysis of the data is beyond the scope of this workshop report, but responses to the question "Do you have complete, well-defined procedures?" are presented in Table 3 and discussed here. For the purpose of this brief report, SRLs were defined as solo-staff if they had only one full-time staff member, regardless of the number of part-time staff in the SRL. Of the 27 solo-staff SRL responses, 14 had zero part-time staff, 10 had one part-time staff member, 1 had two, and 2 had more than two part-time staff. The survey results and workshop discussion confirmed that solo-staff SRLs indeed struggle to meet all seven best practices, particularly in comparison to multistaff SRLs.

Best Practice Areas of Success

The two best practice areas with the highest number of positive responses in the pre-meeting survey, regardless of SRL staff number, were Laboratory Safety and Quality Assurance (QA): more than 80% of respondents answered "somewhat" or "yes" to the question of do they have complete procedures. Still, workshop participants noted several barriers to full implementation of both best practices. The biggest issue for solo-staff SRLs is lack of time. For Laboratory Safety, the issue of biosafety, especially in the sorting context, is a particular concern. Many solo-staff SRLs rely almost exclusively on their institutional biosafety officers, who frequently lack time or understanding of sorter biosafety issues, leaving such decisions to SRL staff. Good sorter biosafety resources are available (20), but the amount of information can be overwhelming and the time needed to consider specific issues is a serious constraint for the solo-staff SRL.

For QA, one barrier to implementation is a lack of practical knowledge about the full range of required QA and QC procedures. This is a by-product of the lack of time for professional development. When a solo-staffer attends meetings or courses, the majority of their SRL services are shutdown. Often, such absences are discouraged by their institutions. Additional barriers included difficulty accessing heavily used equipment to perform regular QA/QC, equipment without built-in QA/QC routines, and the need for solo-staff SRLs to rely on end-users to help with QA/QC or to alert SRL staff to emerging instrument issues. While some of these barriers are not unique to solo-staff SRLs, they are worsened by low staff number.

Despite these barriers, workshop participants agreed on the importance of these two best practices. Practical SOPs would help with the biosafety issues. For QA, establishing consistent timing of QA/QC procedures can help with accessing equipment. Enlisting end-users in the process is

Table 3. Selected questions and responses from the pre-meeting survey for this workshop

QUESTION	RESPONSE			
How many full time staff work in the SRL?	45% One 15% Two 40% More than 2			
At what type of institution do you work?	59% Academic/University 19% Nonprofit research 17% Academic/Medical Center 5% Others			
Where are you located (geographically)?	64% USA or Canada 31% Europe 3.5% Asia 1.5% Australia			
In what type of SRL do you work?	90% Flow Cytometry 8.5% Combined flow cytometry/ imaging 1.5% Imaging/Microscopy			
For this Best Practice Area, do you have complete, well-defined procedures?	# Staff	No	Somewhat	Yes
Laboratory safety	One	15.4%	34.6%	50.0%
	Two	11.1%	22.2%	66.7%
	>Two	8.7%	13.0%	78.3%
Quality assurance	One	19.2%	38.5%	42.3%
	Two	11.1%	55.6%	33.3%
	>Two	0.0%	39.1%	60.9%
Training and education	One	30.8%	42.3%	26.9%
	Two	11.1%	55.6%	33.3%
	>Two	8.7%	43.5%	47.8%
SOPs	One	30.8%	53.9%	15.4%
	Two	11.1%	33.3%	55.6%
	>Two	8.7%	39.1%	52.2%
Data management	One	42.3%	42.3%	15.4%
	Two	33.3%	44.4%	22.2%
	>Two	21.7%	39.1%	39.1%
Operations	One	42.3%	38.5%	19.2%
	Two	22.2%	55.6%	22.2%
	>Two	8.7%	43.5%	47.8%
Staffing	One	69.2%	19.2%	11.5%
	Two	44.4%	0.0%	55.6%
	>Two	21.7%	43.5%	34.8%

another important strategy. This can be done by recruiting power users to help or by defining daily instrument QC as part of the expected responsibilities during new user training on equipment. For equipment without built-in QA/QC routines, it is possible to create a QC sample and define a QC routine using readily available fluorescent beads. This sample and routine are given to end-users to record as part of their normal instrument workflow, thus establishing a history of instrument performance.

Best Practice Areas Needing Work

Four best practice areas revealed differences between solo-staff and multi-staff SRLs in the pre-meeting survey: Training and Education, Standard Operating Procedures, Data Management, and Operations. For all of these, multistaff SRLs were more likely to respond yes (>40% of multi-staff vs. <27% of solo-staff) and solo-staff SRLs were more likely to respond no (>30% of solo-staff vs. <22% of multistaff) to the questions about having complete procedures (Table 3).

For Training and Education, participants noted several barriers which SRL staff may not control: 1. end-user motivation to run/sort their own samples, 2. the type of equipment available (some analyzers and sorters are easier to learn than others), and 3. the amount of staff time available for training. With respect to available staff time, workshop participants prefer to provide training in both FC theory and practice, to

have enough training time to ensure end-users are fully competent on the instruments, and to have enough time to provide both beginner/intermediate and advanced levels of training. However, successful implementation of all of that can require tradeoffs, particularly in a solo-staff environment. Some simply don't train their end-users in cell sorting and determine equipment training by sample type. Several workshop attendees limit how many times a year they offer theoretical and introductory training. Having well-defined training SOPs is an important step that may not be completed in solo-staff SRLs. Finally, equipment availability relative to staff training time can limit the type of training provided.

The main concern about SOPs was that the number of required SOP documents was overwhelming for solo-staff SRLs already lacking in time. There was also concern about writing SOPs for the correct biosafety level, especially when modifying published SOPs that don't match local needs, as this may require additional literature searches and consultations. All SRLs, regardless of staff size, could increase their implementation of this best practice if an easy mechanism for sharing SOPs were established. This could include a library of easily modified, generic SOPs. It could also include instrument-, task-, or biosafety-specific SOPs. This is a beneficial service that ISAC is uniquely positioned to provide.

Despite difficulties in achieving these two areas, solo-staff SRLs attending the workshop remain supportive of the Training and Education and SOPs best practice areas. In contrast, Data Management and Operations best practices were largely seen as out of reach for solo-staff SRLs, except possibly in industry environments, which have a specific culture around record keeping and may have a more accessible operations hierarchy. Again, the main problem cited was lack of staff time to work on these best practices due to higher priority tasks.

For Data Management, as with QA, an additional barrier is difficulty accessing heavily used equipment to perform data backups, particularly when one's primary responsibilities leave little flexibility to interact with other equipment. Several strategies can help in implementation of this best practice, such as establishing consistent timing for data backup procedures, and enlisting local information technology (IT) personnel to create automated backup routines and to streamline computer usage and maintenance procedures. However, in solo-staff SRLs, data backup and data reproducibility largely falls on SRL users. This makes it necessary to educate end-users about the importance of data backup procedures, data collection guidelines (MIFlowCyt (10)), and data documentation and description. All of this must be explicitly considered when designing end-user training curricula. Written SOP guidelines for data backup and data reproducibility can help reinforce a commitment to data management for users and staff. Such guidelines exist and are enforced in private companies but may not be easily implemented at public institutions.

For Operations, additional barriers include the lack of relevant management experience or business skills, lack of access to the appropriate people in Research Administration, and local policies outside of SRL staff control. SRL staff education in the form of online business skills and management training classes could be helpful for this practice area. However, this best practice is particularly dependent on local institutional culture, to which solo-staff SRLs are especially vulnerable.

Staffing had the highest level of negative responses in the pre-meeting survey: 70% of solo-staff and 22% of multistaff SRLs said they do not have complete procedures, and only 35% of multi-staff SRLs said they do (Table 3). At its simplest, correctly staffing an SRL means "matching the staffing level, expertise and capabilities to the strategic goals of the facility" (17). Three main barriers to achieving proper staffing levels were identified in this workshop. These are issues which SRL staff, particularly solo-staff, may have little control over. Specifically: 1. SRL staff may not have decision-making power regarding when to change staffing levels, or even what one's strategic goals are; 2. the need to change staffing levels may require buy-in from administrators who don't understand SRL operations, who may not understand the different time investments required for tasks such as end-user training versus performing cell sorts, or who are unresponsive to end-user needs; and 3. requests to change staffing levels may be held up by administrators who hesitate to hire in case the financial picture of the SRL declines. No specific strategies were identified by workshop participants to help with implementation of this best practice area. The authors of this work note that administrators may respond to quantifiable demonstrations of staff limitations. We suggest tracking as much SRL data as possible (equipment use hours, user/project numbers, staff hours dedicated to best practice areas as well as to tasks like routine repair/maintenance, user communication, IT trouble shooting, overtime, etc.), to provide a numerical picture of SRL operations. In addition, it would be helpful for the ISAC community to develop guidance on ideal staff-to-instrument and staff-to-service ratios.

Discussion

This workshop was originally conceived as a way to help solo-staff SRLs improve their ability to achieve the seven best practice areas. Both the pre-meeting survey and workshop discussion revealed that SRLs of all sizes struggle to meet them. The best practices with highest implementation rates were Laboratory Safety, Quality Assurance, and Training and Education. This is not surprising, as these intersect directly with the mandate of the typical FC SRL. The remaining four best practice areas had lower implementation rates. There are several ways that ISAC could help SRLs of all sizes. With respect to staffing, published guidance on ideal staff-to-instrument and staff-to-service ratios would be useful. Such guidelines would not fit the needs of all institutions, but sometimes just having a benchmark can jump-start a conversation. ISAC could also help by providing information-repository infrastructure and training for QA/QC protocols and repository infrastructure for SOPs and training materials. Lack of time to create de novo solutions was cited repeatedly as a reason for not implementing a best practice. A platform

that enables easy exchange of this type of information would benefit all of us.

The biggest challenges for solo-staff SRLs are limited time together with a lack of institutional support. These directly impact solo-staff ability to implement the best practices. When institutional administration is unwilling, or use of instruments or services does not justify the addition of SRL personnel, the solo-staff employee does it all: attending meetings, training users, instrument maintenance, monthly billing and reports. Faced with limited time, solo-staff typically choose to provide high-quality service to the users, at the expense of important but lower priority tasks that are also expected from SRLs (16). For example, these SRLs may advise users on the proper identification and collection of flow cytometric data, but they do not have time to verify that labeling and acquisition was appropriate or that users backed up their FCS files. Those responsibilities must fall on the users.

During our workshop discussion, setting aside time for specific tasks (such as QA/QC and data management) was frequently proposed as a strategy to enable best practice implementation. Such strategies are reasonable when considered independently of each other. They are less workable for the solo-staff SRL when taken together in the context of a 40 hour work week and the need to devote most of one's time to the main priorities of end-user training, sorting, and so on. Despite being a worthy goal, achieving all seven best practices appears to be unrealistic for most solo-staff SRLs and many multistaff SRLs.

Conclusions

With the results of this workshop in mind, we would like to suggest that the human resources being put into developing an ISAC SRL Recognition Program might be put to better use. The proposed program asks SRLs to submit documentation on how they implement the best practices, for review by ISAC members. Development and implementation of this program use ISAC membership time and energy that could be used for other purposes, such as establishing SOP and training material repositories. Further, it will reward SRLs that are already established, well staffed, and don't need additional help. Wouldn't it be better to improve the use of cytometry technology and elevate the quality of everyone's research, by putting that human time and energy toward developing resources that help all SRLs follow the ISAC best practices, regardless of their size, age, or experience? We think so.

Acknowledgments

The workshop organizers would like to thank all those who responded to the pre-workshop survey as well as the enthusiastic and insightful attendees at this workshop.

Conflicts of Interest

The authors declare no conflicts of interest.

Chapter 2: Shared Resource Laboratory (SRL) Best Practices Conclusion

The reports presented in this chapter provide a treasure trove of information for community members and propose solutions to challenges that SRL members encounter in their day-to-day operations. The key point of all workshop discussions is the need for knowledge sharing including exchange of written procedures and guidances between ISAC communities. WS02 proposes the creation of a toolbox of strategies and techniques for conflict workplace settlement that could be used by the SRL staff in needed as a handbook. Human interactions as complex as they are must be managed with care to shape healthy work culture based on respect and mutual understanding. Even though each situation is different, there is still possibility to profit from the experiences of others and resolve the next conflict better and faster. From the feedback provided already at last year's CYTO, it became evident that SRLs members are overloaded with work. As a consequence, following SRLs best practices or continue education may become challenging to them. That is especially visible for solo staff SRLs. One solution to that would be to hire more operators but how to justify the new staffing requests? The participants of WS06 proposed that ideal staff-to-instrument and staff-to-service ratios should be monitored and next used for the development of SRL related operational guidances by the ISAC community (WS06 and WS09). SRL staff hands and heads can be freed from servicing customers in technologies such as cell sorting to perform other tasks by moving toward a self-service model. Such solution was presented in WS06; however, this requires special measures such as proper training to assure the quality of the results as well as the instruments continuous operability to avoid generating associated servicing/repair costs and complaints from PIs. Training of the users requires structured and harmonized community approach. The guidances, written standard procedures, including checklists and trainee skills assessment and their regular monitoring should be developed by the ISAC community. Another important topic for SRL staff is clear career path. As discussed at WS08, in many SRLs, the next steps for staff in professional development are not established. In view of the workshop participants, there is also no sufficient support from ISAC SRL careers mentorship program.

Chapter 3: Quality Assurance and Reproducibility

Quality assurance and reproducibility are the paramount in flow cytometry-based assays especially in context of clinical trials and always induce vivid discussions among cytometrists. Here, we compiled for workshop reports around the catchphrase of the last few years: Reproducible Science. Growing interest in the study of ever smaller particles (WS04), complexity of mass cytometry-based clinical trials (WS05), the necessity to process vulnerable and unique cells (WS07), or the need for robust and automated analysis of big data sets

(WS10) are in the focus of the here compiled four reports. All these questions cause issues for cytometrists, have been already discussed at last year's workshops and some of the reports below can be seen as follow-ups.

WS04: Applying Scatter and Fluorescence Standardization to Flow Cytometric Data of Small Particles

Joshua A. Welsh, Vera A. Tang.

Introduction and Aims

Methods for standardization are not well utilized in small particle flow cytometry. To facilitate standardized data reporting, fluorescence and scatter calibration were demonstrated in this workshop with the use of MESF beads and modeling software, FCM_{PASS}. FCM_{PASS} software was made available online for workshop attendees with an example small particle dataset shared on FlowRepository to encourage participation during the session. Post-workshop discussion and survey responses indicated that attendees found the workshop to be useful. Seventy-seven percent of those who responded to the survey felt they could now apply light scatter calibration to their small particle work and 66% responded similarly with regards to fluorescence calibration. Future improvements to promote hands-on participation include having smaller workshops in a computer laboratory environment with step-by-step guidance for software use and data analysis.

Standardization methods are not well used and often not reported for small particle analysis. This is critical because current flow cytometry instrumentation is working at their detection limit, which need to be quantified to allow for comparison and validation. There is as yet no consensus method for standardization of small particle analysis. The goal of this workshop was to go over some proposed methods for scatter and fluorescence standardization using scatter modeling software as well as MESF beads using FCM_{PASS} software (21).

Workshop Methods

In order to engage the audience, a link to the FCM_{PASS} software (https://nanopass.ccr.cancer.gov) was included with the workshop abstract, so that attendees would have an opportunity to familiarize themselves with the software, and also get a chance to test it with their own data. A sample data set including MESF beads (PE QuantiBrite, Becton Dickinson, San Jose, CA), polystyrene and silica NIST-traceable scatter calibration beads (ThermoFisher Scientific, Waltham, MA), and a fluorescent virus test sample (ViroFlow Technologies, Inc, Canada) was uploaded onto FlowRepository (https://flowrepository.org/id/FR-FCM-Z24E) prior to the workshop for attendees who currently do not conduct small particle research (11,22). The presentation demonstrated using the FCM_{PASS} software how to perform fluorescence and light scatter calibration on the sample data set provided on FlowRepository (Figure 9). The audience was encouraged to follow along if they had downloaded the software prior to the workshop. Workshop slides were shared using the Figshare platform upon completion of the workshop (23).

Summary of Discussion with Attendees

Questions were raised regarding the following topics:

1. Ability to correlate size of particle with scatter intensity information
2. General information (where to purchase, specifications, etc.) on the reference particles used for the demonstration: scatter calibration beads, MESF beads for fluorescence calibration, biological test material (fluorescently labeled virus)
3. Other reference particles currently on the market and the opinion of the presenters on their usefulness for calibration (MegaMix beads)

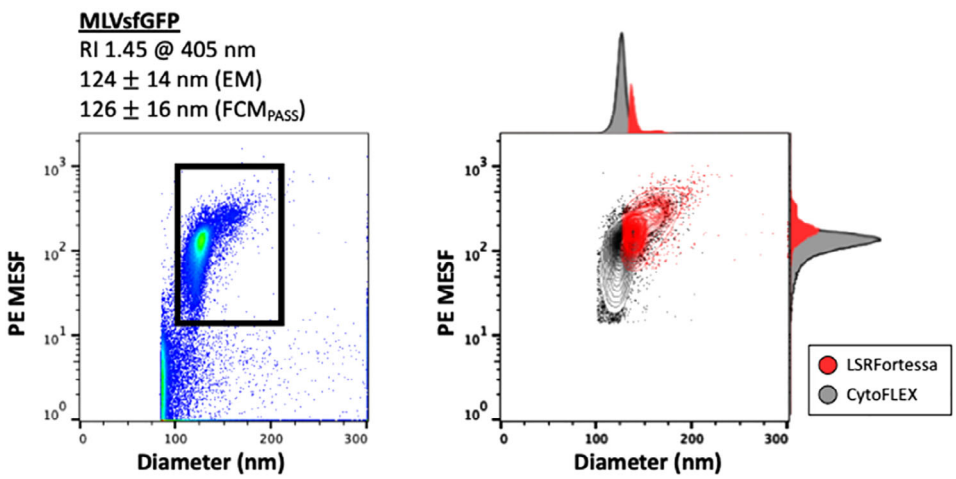

Figure 9. Fluorescence and light scatter calibration. [Color figure can be viewed at wileyonlinelibrary.com]

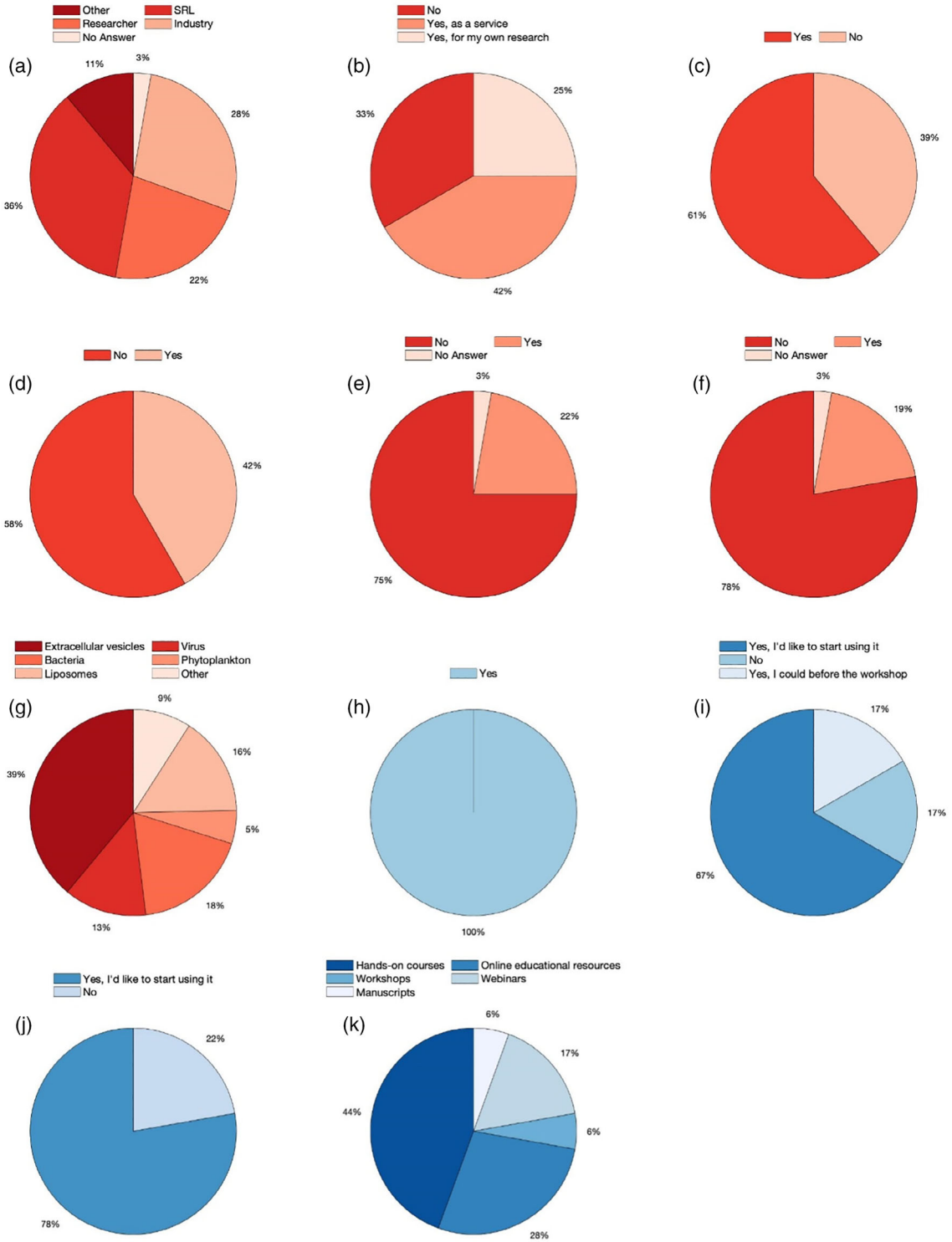

Figure 10. Summary of responses for pre-workshop (red) and post-workshop (blue) survey results for the following questions: (a) What is your current position? (b) Do you analyze small particles (<500 nm)? (c) Have you previously performed fluorescence calibration? (d) Do you use fluorescence calibration for your small particle analysis? (e) Have you previously performed light scatter calibration for your small particle work? (f) Do you use light scatter calibration for your small particle analysis? (g) Which small particles are you interested in analyzing? (h) Do you feel that this workshop was helpful? (i) Do you feel you can now perform fluorescence calibration in your future work? (j) Do you feel you can now perform light scatter calibration in your future work? and (k) What learning tools do you think are required to help researchers and SRLs analyze small particles? Select all that are relevant. [Color figure can be viewed at wileyonlinelibrary.com]

4. Accuracy of fluorescence calibration using currently available materials—Accuracy versus Precision

Poll/Survey Questions and Responses

Attendees were prompted to answer poll questions at both the start and end of the workshop (summarized in Fig. 10, raw data in the Supporting Information, WS04_SI1 and WS04_SI2). While the workshop was well attended, with 228 attendees (~10% of CYTO2019), the pre-workshop poll was answered by 36 participants and the post-workshop poll answered by just 18 participants. Due to the feedback being such a minority of the attendance, little can be concluded about the audience background; 57% of those that replied had previously performed fluorescence calibration, 45% had used fluorescence calibration for small particles, with just 19% having previously performed light scatter calibration. The post-workshop poll found that 100% of responders found the workshop helpful, 66% of responders felt they could now perform fluorescence calibration in their future work, 17% already could perform calibration, and 17% did not feel they could. Seventy-seven percent of responses felt they could now apply light scatter calibration to their small particle work, and 33% felt they still could not.

General Conclusions and Future Improvements

There is a growing interest in small particle analysis within the cytometry community. Many of those conducting small particle analysis appear to be unsure of how to calibrate their instrument. Prior to the workshop, many were unaware that analyzing beads of different diameters without modeling was not light scatter calibration, but quality control, which may have affected the pre-workshop poll results. It is clear from the attendees' responses and discussions at the workshop, that there is a desire by the community for more education.

Only one attendee downloaded the software ahead of the workshop. No one brought a downloaded version of their own data to try on the software. We were unable to foster the interactive hands-on environment, we had hoped for to teach how to use software. This was partly due to the unexpectedly large attendance. Future workshops may benefit from having smaller groups and a longer duration to allow for more interaction. Holding this type of workshop at a location such as a computer laboratory would also facilitate hands-on participation.

Due to the time constraints of presenting the background to fluorescence and light scatter calibration as well as a live demo for the first time, the polls were advertised on the first slide of the workshop, while attendees were arriving and were purposely kept short, with less than six questions, so as to encourage compliance. Despite this poll, responses were poor. Future workshops may benefit from integrating individual interactive polls into the presentation itself rather than allowing offline completion post-workshop. This may also foster better discussion within the workshop between participants.

Conflict of Interest

JAW is an inventor on patents and patent applications related to EV flow cytometry. VAT is CSO of ViroFlow Technologies, Inc.

WS05: How to Ensure Robustness and Reproducibility When Using Mass Cytometry for Clinical Trials?

Diana L. Bonilla Escobar, Jose Estevam, Michael Leipold, Adeeb H. Rahman, Jonathan Irish, James Lederer, Brice Gaudillière, Nima Aghaeepour, Elena W.Y. Hsieh, Greg Behbehani, Christopher O. Ciccolella, Radhika Rayanki, Christopher Groves.

Introduction and Aims

Mass cytometry is a cutting-edge technology that merges the high-throughput single cell analysis with the specificity and resolution of mass spectrometry. It allows over forty parameters to be measured simultaneously on single cells, allowing deeper profiling of samples. (24,25) While mass cytometry has highly contributed to basic scientific discovery, some of the major challenges come when trying to implement this technology in clinical studies. (26–28) The addition of mass cytometry to the pool of technologies in clinical studies requires better standardization and quality control. (29) Discussion on the validation of mass cytometry assays for clinical use including application validation, reagents and protocols, instrument operation, quality control, and data analysis is needed. We gathered experts in the field to learn from their experience on how to overcome these challenges. The intent of the workshop was to address the challenges in implementing mass cytometry in clinical studies and discuss solutions adapted by experts in the field. By the conclusion of the workshop, the audience had a better understanding of the key points that require optimization to ensure high quality, reproducible data. Consensus was provided by the expert panel for successful implementation of mass cytometry in clinical studies.

The goals of the workshop were to

1. To conduct an extensive pre-meeting survey to better understand the various approaches to develop, validate and implement mass cytometry panels in clinical studies.
2. To understand the key areas that need to be optimized to ensure high quality, reproducible mass cytometry data.

The workshop began with an introduction to the top three problem areas identified as part of the extensive pre-meeting survey, see Figure 11. Each problem area was addressed separately with a panel of mass cytometry experts with expertise in that particular component of the mass cytometry workflow. Each participant provided suggestions for addressing the problem area and then ample time followed each section for audience participation. At the end of each section, a brief summary of potential solutions was discussed with the panel and audience.

Methods

The workshop was directed to anyone interested in generating high quality, reproducible mass cytometry data for clinical trials: assay and reagent manufacturers, pharmaceutical scientists, regulatory agencies, clinical laboratories, contract

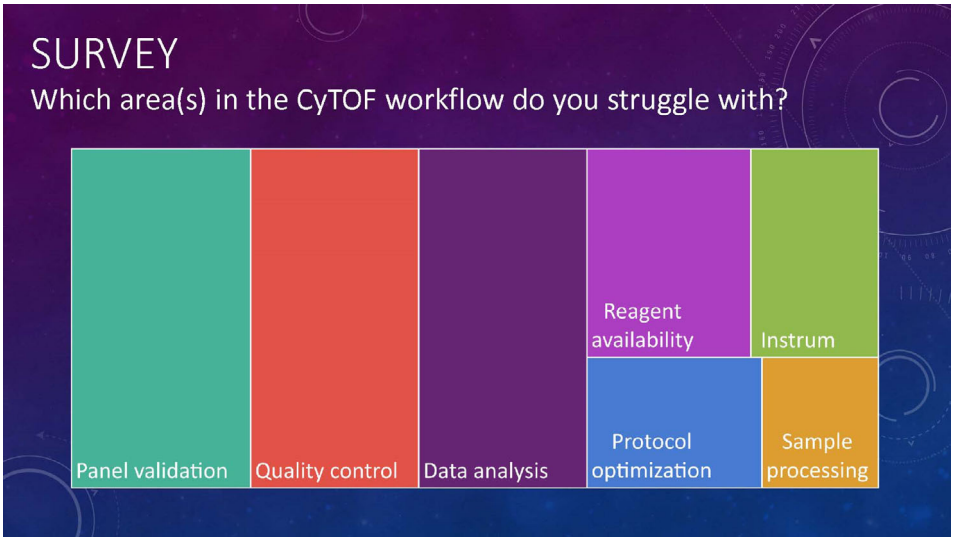

Figure 11. Which areas do you struggle with? [Color figure can be viewed at wileyonlinelibrary.com]

research organizations, or research scientists. We invited a group of eleven facilitators, to contribute to the workshop discussion through their experience and expertise with using mass cytometry: Michael Leipold, CyTOF Manager at the Human Immune Monitoring Center in Stanford University, Adeeb Rahman, an Associate Professor and Director of Technology Development at the Human Monitoring Center from Icahn School of Medicine at Mount Sinai, Jonathan Irish, Professor and Scientific Director of Cancer & Immunology Core (CIC) and Mass Cytometry Center of Excellence (MCCE) at Vanderbilt University, James Lederer, Professor at Harvard Medical School, Brice Gaudillière, Assistant Professor at Stanford University, Nima Aghaeepour, Assistant Professor at Stanford University, Elena Hsieh, Professor at University of Colorado Denver, Greg Behbehani, Assistant Professor at Ohio State University Wexner Medical Center, Christopher Ciccolella, Omiq Co-Founder and CEO and Radhika Rayanki and Christopher Groves, Associate Scientists at AstraZeneca.

The workshop agenda included 5 min for setup and facilitator introductions, 5 min for presentation of main survey results, 45 min for discussion of the three main challenges with the expert panel identified through the pre-workshop survey, highlighting current thoughts on the most voted/relevant survey questions, and five minutes for wrap up. Each presentation was interactive with discussions between facilitators and the audience. A total of 120 participants attended the workshop. An extensive pre-workshop survey of 16 questions was conducted and 33 answers were collected from mass cytometry users in academia and pharmaceutical industry (see Supporting Information, WS05_SI1 and WS05_SI2). The pre-meeting survey covered the following areas: Reagent and Protocol Optimization, Panel Validation, Assay Validation, Instrument Validation and Quality Control, Data Analysis and Statistical Evaluation, Quality Control, and Regulation. The questionnaire was elaborated using feedback from facilitators and distributed to the users through user forums, cytometry societies and social networks. Facilitators were asked to describe major challenges encountered when implementing mass cytometry in clinical studies and to give a brief description on how the challenges were solved, especially if any new tools or protocols were developed.

Results

Out of 16 survey questions, only eight were discussed during the workshop because of time limitations. The focus of the workshop discussion was around the three most voted topics, which included study design, panel validation, and data analysis of large datasets.

The first question in the survey was if the participants were using mass cytometry: 79% of the responders were currently using this technology platform but only 21% in clinical trials. The reasons for people not using mass cytometry included: using other single cell discovery platforms, lack of instrument or reagents, lack of knowledge or expertise, challenges of infrastructure, high cost or issues with workflow, analysis complexity, reproducibility, scalability, and time-consumption.

We then asked whether mass cytometry was mature enough to be used in clinical trials, most responders (67%) indicated "No." This is part of the reason the technology has not been widely implemented in the field and included in this observation are inherent challenges associated with the use of mass cytometry that were discussed during the workshop.

The next question was which segment in the mass cytometry workflow the users struggle with the most, including experimental design, reagent availability, protocol optimization, panel design and validation, instrumentation, sample processing, quality control, software/data analysis and statistical evaluation and regulation. Out of the 72 answers, the most voted on options were panel validation (22%), quality control and study design (20%), and data analysis (20%), as the main

challenges in implementing this technology in clinical studies. These three areas were selected for further discussion during the workshop, having 15 min per topic. For each topic, a group of three to four facilitators provided a brief introduction based on their expertise, followed by an open discussion with the participants.

The first 15 min discussion on study design focused on the description of the main challenges reported by the participants, such as scalability of mass cytometry, lack of a statistician/power calculation up front, limited sample size, complexity of longitudinal studies, complexity of multisite studies, unclear outcomes, confounding factors, too many patient subgroups, lack of training and lack of guidance for troubleshooting. The facilitators for this discussion were James Lederer, Brice Gaudillière, Nima Aghaeepour, and Christopher Groves and the specific discussion topics (most voted ones) were complexity of multisite and longitudinal studies (38%) and mass cytometry scalability (17%). The key points from this discussion were controls are very important to make a study less prone to errors and automation of reagents, sample preparation, and sample acquisition helps to reduce variation and improve scalability. The facilitators emphasized the importance of using the same lot of antibodies within a study, running samples within the same interval of time, maintaining quality control of the CyTOF instrument, especially in multisite studies, and validating instruments using similar metrics to ensure comparable performance. An important piece of advice provided during this section includes the various sample collection timepoints from the same patient/healthy donor in the same batch to avoid variability associated with the mass cytometry instrument. Additionally, if many samples are run together over a time period, run QC controls to quantify variability and decide whether it is acceptable to combine the data together.

The second 15 min discussion on panel validation focused on description of the main challenges reported by the participants such as availability of tools/support to design a new panel, adding/validating a new target, tailoring a panel for a new sample type, selecting appropriate positive/negative controls for rare cell subtypes, lack of cell standards for validation, differences in signal intensity between positive controls and the real sample, clinically relevant markers below the limit of detection with mass cytometry, panel quality control, issues with antibody titration, validation of custom-made reagents, lack of training, or guidance for troubleshooting. The facilitators for this discussion were Michael Leipold, Adeeb Rahman, Greg Behbehani and Radhika Rayanki, and the specific discussion topics (most voted ones) were differences in signal intensity between positive controls (15%) and the real sample and clinically relevant markers being below the limit of detection with mass cytometry (12%). The key points from this discussion were as soon as a patient sample is received, generate multiple sample aliquots ensure sufficient material is available for retesting and to have cells available for generating positive controls to evaluate the markers in the panel. The strategies discussed for markers below limit of detection were 1. panel redesign: reposition the marker with low expression, moving to a different metal according to ion detector sensitivity; 2. amplify the signal by using different antibody clones with different specificities for the same marker; 3. account for the background; and 4. use high-yield polymers that boost metal signal, such as cadmium-containing Qdots or a silver nanoparticles, being careful with the background in other channels because of their natural abundance. It was also mentioned that differences in intensity between controls and samples are likely due to biology and therefore controls should only be used to detect a positive signal and demonstrate that the assay works well. The facilitators highlighted the importance of validating a panel using more than one healthy donor, because of biological variation that exists between healthy donors and to accommodate for expression levels or changes in population frequency, as well as including reagent processing controls that resemble your sample type as much as possible. It was also described that the use of commercial controls (vericells or cytotrol cells for example) can be helpful as a staining control to confirm the antibodies were added, but since these controls contain distinct cell types and likely do not have similar expression levels as the test samples, these commercial controls cannot be used for normalization. It is important to include healthy control samples in each barcoded set or with each sample. The controls should be processed at the same as the test samples to verify thawing, processing, and staining were done in a consistent manner according to established procedures. To track and minimize technical variation with antibody staining, facilitators have developed lyophilized antibody panels to minimize variability in preparing cocktails and thus streamlining the sample staining workflow. Additionally, there was also a discussion regarding preparing a master antibody cocktail mix that can be aliquoted and stored at –80°C and thawed when needed (30).

The third 15 min discussion on data analysis focused on the description of the main challenges reported by the participants such as analysis algorithm complexity, analysis algorithm availability, batch analysis, need for compensation, not having access to a bioinformatician, lack of understanding of the proper use of algorithms, high level of publications/methods/tools available, lack of agreement of clean-up gating protocol and lack of reproducibility. The facilitators for this discussion were Jonathan Irish, Elena Hsieh and Chris Ciccolella, and the specific discussion topics (most voted ones) were analysis algorithm complexity and lack of understanding of the proper use of algorithms (36%) and batch analysis (17%). The key points from this discussion were emphasis on the importance of having a normalization process to compare signal intensity for all channels across all samples when processing batches of samples in prospective studies. A master antibody cocktail can be created, and master reference samples spiked into every barcode can be included to help with the normalization process. Of additional importance is to include bioinformatics input from the point of experimental design to analysis and to keep bioinformaticians involved throughout the entire study, or if this is not feasible, to reach out to this expertise in user forums.

Mass cytometry users should be focused on learning the principles of data analysis more than trying to learn every single algorithm. In terms of batch effects, there was no clear consensus on how to proceed but a series of recommendations were provided. To reduce batch effects: maintain similar samples or tissues together, process samples at the same time, maintain consistency with sample collection, preparation, processing, acquisition and generate a master mix to lyophilize or freeze down, assign all samples or all time points for one patient/healthy donor into the same barcoding group for longitudinal studies and add reference controls into every barcode. Mass cytometry user should be careful since some computational tools are more susceptible to batch effects than others and this is important to consider when defining a data analysis strategy. Also of importance is to remember that advanced tools are need required to compare batches. All one needs to do is to simply overlay histograms and look for systematic shifts across the markers of interest and deal with it by, for example, analyzing each batch separately if there are issues. The dominant strategy for handling batch effects seems to be to analyze data in batches and then report cell population percentages for each sample. This reportable is then used to draw conclusions about differences between samples. The main problem with this strategy however is that it does not scale. It demands individual processing of small groups of samples and this limits the available options for larger scale data visualization and mining on the complete data set. Some algorithms to deal with batch issues were mentioned. The first type of strategy that can be used for scaling/normalization is to draw from the various types of scaling methods that operate on a distribution of data only with respect to itself. The problem with these methods is that they require a high degree of supervision by the user applying them, and certain fundamental assumptions to be made or known about the distributions ahead of time. Another layer of complexity in the application of such methods comes from the fact that there are true biological differences between samples nested within the batch effects. The presence or absence of such differences can impact the utility of the scaling methods being used. In summary, the use of these analysis methods can solve certain aspects of batch effects, but their application can be a time-consuming and currently lacks well-understood principles for correct application.

Another strategy that can be used for scaling/normalization is to correct a sample distribution by using a separate control distribution. This strategy currently has very little consensus or precedent for how to apply properly and likely should also require the use of domain-specific logic for CyTOF data. The foundation for being able to do this type of normalization is to include some form of barcoded control in all samples acquired on a CyTOF, where the control is ideally cells of a tissue (or tissues) similar to the sample being studied, and that has been processed identically or alongside the sample being acquired. Algorithms can then be used to align the control sample (reference) distributions to each other, generating a specific scaling (normalization/correction) factor per channel per barcode (batch), which would then be applied to every one of the samples within the specific barcode. As mentioned, consensus does not currently exist for which alignment methodology to apply, but this is an area of active research and reliable methods will emerge, and some existing normalization methods for other technological platforms (i.e., RNA sequencing) could be applied. At the very least, including such controls allows certain conclusions to be made manually about differences between samples that cannot be made without their presence, such as if a marker is truly increasing or decreasing between batches. For example, quantile normalization, method commonly used in RNA sequencing could be applied, but one should carefully inspect the post-normalized data because adjustments could be too dramatic and lead to artifacts when looking at the data in bivariate way. The median signal intensity and cellular population frequencies should be stable in your reference sample and can be used to decide how to best normalize the data. A correction coefficient can be created using reference samples for every barcode set to normalize, either with a scaling/mapping function or using the mean of the median signal intensity per channel for every reference sample across barcodes. A variance coefficient (i.e., adjustment factor, scaling factor) is obtained and adjusted in every channel and every sample and then new fcs files are generated for each sample that has that correction. The reference sample will be the "ground truth." It would be even better/more accurate to have a second set of reference samples that are evaluated post normalization after application of the variance coefficient (i.e., adjustment factor, scaling factor) obtained from analysis of the first set of reference samples. However, this approach poses extra cost and analytical time. Questions were raised as to how to judge the post normalized data—how does one know if the correction made the data more consistent across batches? Advice was given to assess whether when applying an adjustment strategy, the signal variability or total variance across reference samples (signal intensity and population frequencies) is decreased, which should be statistically assessed and the samples will be adjusted based on the references, marker by marker. This method can also be adjusted to use a correction function for nonuniform corrections within a channel distribution. Finally, it is critical to check the accuracy of your validation using your biological and scientific knowledge. Recently, *Frontiers Immunology* describes an R-package implementation of the multiple strategies described above for batch normalization, with user-defined options of batch adjustment (quantile normalization vs. percentile scaling/mapping vs. medians), and an optional output with pre- and post-adjustment signal intensity histograms per channel, scaling factors per channel per barcode, and statistical measure of variance/variability pre- and post-adjustment (31).

Conclusions

The high number of cell types that can be measured using mass cytometry can be extremely useful to support drug development. Mass cytometry can be used to for the characterization of various disorders and monitoring the

pharmacodynamic effects of therapeutic interventions. However, many challenges associated with various aspects of the mass cytometry workflow still exist and therefore need to be addressed before the technology can be more widely adopted for clinical sample testing.

The workshop reviewed the most common problem areas when processing and analyzing samples on the CyTOF instrument for research and clinical studies. The component requiring the most attention was discussed with a panel of mass cytometry experts and included intensive audience participation. There was general agreement among the facilitators on how best to approach each of the problem areas.

The top three key problem areas discussed were study design, panel validation and data analysis:

1. The key points on study design were that assay controls need to be included to control for experimental errors. Furthermore, automation of reagent preparation, sample preparation, and sample acquisition reduces the technical variability and allows more samples to be included in each batch.
2. The key points on panel validation were to generate enough samples in the event retesting is needed and to include positive control samples to verify the presence of each of the markers in the panel. There was a focused discussion on how to deal with markers below the limit of detection with advice from the expert panel, which included several strategies for managing this issue. The facilitators also highlighted the importance of using multiple donors to validate the panel because of the inherent biological variation that exists between donors/patients as well as including reagent processing controls as close as possible to your test sample.
3. The key points on data analysis were to a. perform a normalization process to compare signal intensity for all channels across all samples, b. involve bioinformatics support early in the process of designing a panel through validation and during clinical study conduct to increase the likelihood of a successful data analysis plan, and c. in regards to batch effects, there was no clear consensus on how to proceed, but several strategies were more popular than others.

The workshop helped assess current best practices with various aspects of the mass cytometry workflow and contributed to our understanding of the issues faced by mass cytometry researchers, illustrating that with the appropriate strategies, high-quality, reproducible mass cytometry data can be produced in a clinical trial setting.

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Conflicts of Interest

The authors declare no conflicts of interest.

WS07: Finding Solutions to Difficult Developmental Biology and Stem Cell Flow Cytometry Issues

Gelo Victoriano Dela Cruz.

Introduction and Aims

In developmental biology, flow cytometry is used as a tool to isolate and study populations of cells that arise from progenitors found in the germ layers of the embryo. Hematopoietic stem cells (HSCs) are one of the most characterized stem cells in the developing embryo. However, unlike HSCs, there are other embryonic populations that are challenging to isolate and are very limited in number. In mouse pancreatic development, researchers look at very early embryonic stages when the pancreatic bud starts to form and by E12.5, only 10,000 cells are present (Yung Hae Kim, personal communication, October 30, 2018).

Isolated pluripotent stem cells (PSCs) derived from the inner cell mass of the blastocyst (32) are used to recapitulate developmental steps *in vitro*. In order to mimic these steps, PSCs are grown on feeder cells and/or extracellular matrix-coated surfaces and stimulated with different factors over a period of time with developmental time points usually marked by the expression of fluorescent protein (FP) reporters.

In order to study stem cells and developing cells using flow cytometry, they have to be isolated from the developing embryo or the feeders/extracellular matrix they are propagated in. Improper isolation of these cells can lead to cell death (33). Furthermore, in *in vitro* differentiation assays, it is difficult to determine what can be used as proper experimental controls since the autofluorescence of cells vary during differentiation (34).

This workshop was conducted to solicit ideas from attendees on how to deal with challenges in the flow cytometry of developmental biology and in *in vitro* stem cell differentiation studies.

Methods

This workshop focused on four questions:

1. How do we get around not having enough cells for flow cytometry?
2. How do we maintain sample quality?
3. What controls should be used for the different stages of differentiation? (see Figure 12)
4. When isotype controls cannot be obtained, what kind of controls can we use?

Attendees to the workshop were presented with scenarios (See Supporting Information, WS07_SI1) and then polled

with these questions using Slido (www.slido.com). Their responses were recorded and discussed with moderation by the workshop facilitator.

Results

Workshop 7 had at least 40 participants based on live polling. Live polling responses were collected (See Supporting Information 2, WS07_SI2) and the most discussed responses are summarized in Table 4.

Discussion

During the workshop, two scenarios were given to illustrate the questions presented. The first scenario was in pancreatic development in the mouse. Due to the nature of the sample source, only a limited amount of cells are isolated. This led to the question on how to get around not having enough cells for experiments. The discussion on this question emphasized the optimization of the dissection and dissociation protocols for the specific samples and tissues of origin. In very small tissues, this would help greatly in minimizing cell loss due to suboptimal preparation conditions.

To deal with the low sample number for analysis, attendees suggested to spike in cells from a wild-type source or from other cell sources. If the cells of interest are labeled with a fluorescent protein, wild-type cells with no or a different FP can be used to bulk up the cell number. This will allow for the discrimination of the cells of interest from the rest of the sample, and minimize cell loss during analysis. It should be noted that the population percentages for the cells of interest might be skewed due to the addition of the spike in cells. In addition, attendees also suggested that similar samples can be pooled together. This can help with the cell number, however, it is not always possible especially with small litter sizes.

It was also suggested to use cell lines for setting up the experiment. In some investigations where cell lines derived from the cells of interest are available, these can be used in lieu of the cells of interest for setting up voltages and gatings. This eases the burden of using the limited sample for setting up the experiment.

Unlike blood, primary cells from developing organs have to go through several dissociation steps involving both mechanical and enzymatic means before they can be used for flow cytometric analysis. Due to these dissociation steps, cellular integrity can be compromised. To maintain sample quality, it was again emphasized that dissection and dissociation protocols be optimized. In addition to optimizing the dissociation procedure, the temperature conditions used throughout the experiments should also be optimized. It is recommended that the cells of interest should be used to optimize these conditions, or at the very least, cells that are similar to the cells of interest (i.e., derived cell lines). Optimizing the conditions and protocol will allow for better isolation and dissociation of cells and also minimize work time with the cells, thus maintaining cellular integrity.

To keep cells "happy" and alive during the preparation steps, workshop attendees recommended to use serum-free media instead of the regularly used phosphate-buffered saline (PBS). It should be ensured that the media used not have phenol-red that may interfere with analysis. Also, the buffering system used for the media should be considered. HEPES-buffered media maintains the pH better at room conditions over carbonate-buffered media, making it a favorable choice for flow cytometry sample preparation.

The second scenario presented dealt with *in vitro* differentiation of PSCs. Most researchers use the expression of one or more FP reporters to mark the specific development points they are interested in. However, determining which proper negative control to use can be difficult as the cells at the beginning are not the same as the cells obtained later in the differentiation, and they can have different autofluorescence.

To use as a negative and autofluorescence control, attendees suggested that a parallel differentiation using non-fluorescent PSCs be done. It will however, be difficult to guarantee that the parallel differentiations are at the same developmental time point. A possible solution is to differentiate the transgenic cells together with nontransgenic cells and use the non-transgenic cells as an internal negative control in the sample. This can be done the same way with barcoded cells to verify that the control cells are not expressing the fluorescent reporter.

Table 4. Summary of most discussed live polling responses

Q1	How do we get around not having enough cells for flow cytometry?
	• improve/optimize tissue dissection and dissociation protocols
	• spike in using other cells/wild-type cells to increase cell number
	• use cell lines for set-up
	• pool samples if possible
Q2	How do we maintain sample quality?
	• improve/optimize dissection and dissociation protocols
	• optimize temperature conditions
	• optimize conditions throughout the experiment using non-essential but similar cells
	• use serum-free media instead of PBS
	• minimize work time with cells
Q3	What controls do we use for the different stages of differentiation?
	• differentiate a parallel flask of non-transgenic cells to use as control
	• use reference beads
	• barcode transgenic and non-transgenic PSCs and differentiate together
	• add blocking agent to reporter
Q4	For antibody staining where isotype controls cannot be obtained, what kind of controls can we use?
	• blocking before antibody staining
	• use fluorescence-minus-one controls

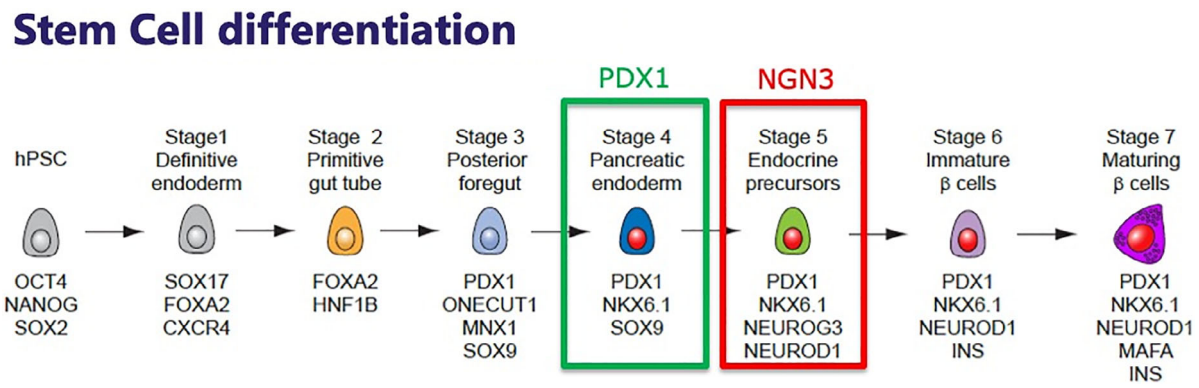

Figure 12. What controls can be used for the different stages of differentiation? [Color figure can be viewed at wileyonlinelibrary.com]

Reporter expression can also be quenched by the addition of or exposure to a blocking agent. One method mentioned in the discussions is the use of photoactivatable FPs as a reporter for gene expression in in vitro differentiation experiments. This ensures that the cells being analyzed are all at the same developmental time point. An aliquot of the sample can then have the fluorescence deactivated and be used as a negative control for the fluorescence.

In experiments requiring antibody staining, it was recommended to block the cells with serum or an Fc blocking agent, instead of using isotype controls. Keeney et al. (35) outlined several alternatives to isotype controls including isoclonic controls, unstained cells and the above-mentioned serum-blocking. In addition, Andersen et al. (36) mentioned the use of purified human IgG or the commercially available Fc-block as effective against non-specific monoclonal antibody binding.

It was very informative to solicit the attendees' suggestions and ideas on dealing with the challenges encountered when doing these studies, despite their unfamiliarity with these samples. In the discussion on controls for differentiation protocols, one attendee pointed out the need to rely on what is available, implying that it is possible that the perfect control does not exist yet. Hughes et al. (37) looked at antibody staining in stem cell differentiations and concluded that finding appropriate isotype controls may not be possible and that it may be necessary to rely on unstained cells as an imperfect substitute.

The workshop shows that flow cytometry with early embryonic and in vitro stem cell differentiation samples is a niche area. This was evident in the participants' lack of direct knowledge and experience with these samples. However, it can be noted that workshop attendees gave suggestions on solutions that have worked with other cell types that can possibly work in these investigations. In addition, recent developments like barcoding may also prove useful in studying these cell types.

We are hoping that the results from this workshop will be used to develop tools and protocols that can help overcome challenges in working with these difficult samples.

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Conflicts of Interest

The author declares no conflict of interest.

WS10: Flow Cytometry Data for Clinical Trials, Manual, or Automated Analysis?

Yongliang Steve Sun, Cherie Green, Ryan Remy Brinkman, Michael Nathan Hedrick.

Introduction and Aims

FCM data analysis, including applying appropriate cell population identification strategies, is the key final step to ensure high quality and reproducible FCM-based experiments. Manual analysis remains the standard of practice; however, this approach is not only a rate-limiting step but also one of the primary sources of variation in FCM-based assays (38–40). As FCM panels increase in complexity due to the ever-expanding development of the technology and high volume of samples from clinical trials, using manual analysis also poses challenges for increasing capacity and ensuring scientific rigor (41,42). Cytometry bioinformatics approaches have matured over the last few years leading to opportunities to solve the challenges of manual analysis approaches. Application of algorithm-based gating is of particular interest in clinical trials where large FCM data sets are generated to understand disease biology and demonstrate mechanism of action and efficacy of novel therapeutics (38,40,42). This workshop was intended for flow cytometrists who want to generate high quality and reproducible FCM data using emerging automated analysis tools. While the workshop was intended for all scientists in the field of cytometry, the focus was on translational and clinical research from biotechnology and pharmaceutical companies, clinical laboratories, and contract research organizations generating FCM data to support clinical trials in drug development. The main objective of the

workshop was to open a dialog and generate ideas for how to use algorithm-based analysis approaches. Our secondary goal was to discuss the challenges and opportunities resulting from automated analysis. The workshop focused on gathering information on the current landscape of use of automated analysis approaches with an emphasis on understanding the obstacles for implementation. For those researchers currently using algorithm-based approaches, we set out to understand the primary purpose of their analyses and how these approaches are used including challenges and opportunities for further adoption.

Method

The workshop was well attended with around 150 participants. Live polls were conducted during the workshop to gather information and engage attendees in discussion of the results. Three case studies were presented to further elicit questions and deeper discussions. The case studies covered: 1. the application of automated analysis to analyze several multiparameter immunophenotyping panels for large-volume of clinical samples, 2. the standardization of automated analysis across diseases, instruments, and laboratories, and 3. automated analysis for quality control and detection of immune checkpoint marker PD-L1 in hematologic malignancies. The workshop concluded with a summary of the interactive discussions and a proposal for next steps.

Results

There were 60–147 respondents for each question of the interactive survey (See Supporting Information, WS10_SI1 Reults for graphs and table of the poll results). The audience consisted of those who self-identified as coming from academia (35%), biotech (24%), pharma (21%), CRO (9%), diagnostic lab (2%), regulatory agency (1%), and other (8%). Out of 132 respondents that are working on FCM, 46% used it for clinical trials, 31% for preclinical research, 18% for basic research and 5% for diagnostic applications. Sixty percent (88/147) of attendees exclusively use manual analysis. When we surveyed the primary reason why automated analysis was not employed, 43% of respondents chose lack of trust/understanding, 27% lack of resources, 12% keeping consistency with previous results, including literature, and 8% of respondents tried to validate the automated analysis but were unsuccessful. The primary driver for the decision to use automated analysis was high-dimensional panels (45%), large sample volume (19%), consistency (19%), efficiency (6%) and other (11%). When asked what the primary purpose of automated FCM analysis was, the majority of participants selected defined reportable results that mimic manual cell population identification or some combination of defined reportable results, biomarker discovery and visualization. The most commonly used approaches were dimensionality reduction tools such as t-distributed stochastic neighbor embedding (t-SNE), unsupervised clustering algorithms such as flowSOM, or a combination of such methods with manual analysis. To validate automated analysis, the majority of respondents compared to results from manual cell population identification and correlated to clinical readouts. Additionally, some (18%) used data from other technologies such as genomic cytometry for confirmation.

The three case studies, including one published in peer-reviewed literature (43), illustrated the automated analysis workflow and validation process, demonstrating high correlations between automated and manual cell population identification. The survey indicated that the biggest challenge resulting from the use of automated analysis was higher requirements for standardization (compared to manual analysis, 67%), followed by implementation and monitoring for clinical trials (22%), metadata accuracy (8%) and consistent labeling of fluorophores (3%). The biggest opportunity resulting from the use of automated analysis was consistency (45%), followed by high throughput (31%), comprehensive analysis (16%), and efficiency (6%). At the end of the workshop, a working group was proposed to establish best practices for the use and validation of automated/algorithm-based analysis in clinical trials using FCM.

Discussion

The workshop had lively interactions and discussions. The survey showed that the main reason for not adopting automated/algorithm-based analysis approaches in FCM was lack of trust/understanding (43% of the participants). We found this surprising as many novel and robust computational methodologies for analyzing complex FCM have been published in the last few years (40,44,45). The workshop panelists demonstrated further evidence of the value of using algorithm-based approaches to analyze clinical trial data. All three case studies presented during the workshop compared automated to manual analysis for pre-defined reportable results, demonstrating a high degree of correlation between them (43). In this regard, automated analysis has the advantages of consistency, high-throughput and efficiency. There is an urgent need for close collaborations between scientists in both the bioinformatics and cytometry fields, so that the latter have a better understanding of capabilities and advantages that automated analysis can offer (46). Lack of resources (27%) was also identified as an obstacle for adoption of automated tools. The group discussion further elucidated this topic as a general lack of availability of bioinformatics or computational scientists within organizations generating FCM data, limiting the resources for development of automated analysis even though many top performing approaches are publicly available. User-friendly interfaces will also help users understand these tools. In addition, we expect that the validation of automated analysis methods (e.g., comparison of manual to automated) will help promote adoption of these newly developed algorithm-based approaches (47–50). Many respondents (45%) ranked high-dimensional panels as the primary driver for automated analysis, followed by large sample volume (19%) and then consistency (19%). This is reflective of the increasing size and complexity of clinical FCM data sets following advancements in the technology and instrumentation, as well as the growing use of FCM in clinical trials.

Currently, defined reportable results that mimic manual cell population identification or its combination with biomarker discovery or visualization are the primary goals of automated analysis of clinical trial data. Alternatively, comprehensible visualization is essential to validate and discover novel cell populations. Dimensionality reduction tools such as t-SNE, unsupervised clustering such as flowSOM, or the combination of the two are being applied to try to realize unbiased comprehensive analysis of high-dimensional FCM data. The challenges of validating biomarker discovery such as a novel cell population might be the cause of the skepticism expressed in the workshop. Correlations with clinical outcomes in clinical trials might increase the confidence in the field.

The majority (67%) of respondents listed a higher requirement of standardization when performing automated analysis compared to manual cell population identification as the biggest challenge for automated analysis. This included a need for uniform sample quality, instrument settings, reagents, staining procedures and acquisition. Implementations such as FCS data transfer, data reporting and visualization need an automated workflow as much as possible. Consistent metadata format and fluorophore labeling are required. Moreover, automated sample metadata entry was preferred to manual entry to minimize error rates.

As for the biggest opportunities of using automated analysis, the majority of attendees voted consistency (45%) or high-throughput (31%); the rest selected comprehensive analysis and efficiency. By using machine-based defined criteria, automated analysis improves data consistency, as it does not have the human subjectivity that manual analysis has, especially for poorly resolved cell populations (43,44,49). Once the automated analysis pipeline is established, it can efficiently analyze large clinical trial datasets, whereas manual analysis is labor intensive. Furthermore, the conventional bivariate dot plot manual cell population identification is limited in its capacity to decipher the full potential of high-dimensional complex FCM data. When research objectives are carefully defined and matched with appropriate algorithms, automated analysis can provide more robust and timely results than manual approaches.

Conclusions and Perspectives

The workshop had a high participation rate and lively discussions leading to unique insights into the future opportunities and challenges of cytometry bioinformatics for clinical trials, indicating the goal of the workshop was reached. It highlighted both the hesitation and the desire among attendees to migrate to automated analysis for clinical trial data. Some respondents (8%) unable to validate automated analysis present an opportunity for further efforts in education, training and reports of successful adoption to support the community in moving toward this goal. Moreover, close interdisciplinary collaborations between bioinformatics and flow cytometry are urgently needed to fully utilize and improve automated analysis methodologies for answering research questions. These computational tools should evolve to have more user-friendly interfaces. Flow cytometrists require some bioinformatics knowledge to extract the most information from massive and high-dimensional complex FCM data sets. Computational FCM analysis holds the key to our deeper understanding of the immune system, complex disease states and the development of novel therapeutics to improve health care in the coming years.

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Conflicts of Interest

The authors declare no conflicts of interest.

Chapter 3: Quality Assurance and Reproducibility Conclusion

This chapter focuses on the quality assurance and reproducibility in cytometry. As already discussed in last year workshops reports (1) also in this chapter, many aspects of cytometry-based clinical trials were identified that still require improvement. The good news is that now procedures have been developed to successfully support proper scatter and fluorescence standardization in flow cytometry of small particles (WS04). However, there are still many issues. In that line, challenges in mass cytometry-based clinical studies (as pin-pointed by the workshop participants) are associated predominantly with panel validation, study design, and quality control (WS05). These problems, however, do not seem to differ significantly from those that flow cytometry-based clinical trials are facing. It can be that certain biological obstacles simply cannot be overcome at this time. This is also most likely the case in studies of embryonic cells developmental stages where to date neither established procedures for handling of the vulnerable cells nor appropriate controls to monitor their quality or their stages of differentiation exist (WS07).

This year a lot of attention has been drawn to data analysis in clinical studies. The need to introduce automated cytometric data processing was discussed in detail as this approach ensures consistency of results in clinical trials by among others reducing or even getting rid of the subjective human factor. But even though many cytometrists declare interest in algorithm-based data analysis, still the majority of the laboratories gate their big data sets manually. The main reason behind, as admitted by workshop participants (WS10) is the lack of trust in the algorithm-based analysis that emerges from poor or no understanding of the process/method. Interestingly, the same explanation is given by the mass spectrometrists for their method choice for clinical data analysis (WS05).

Final Conclusions

Trends

As with last year, the trend of initiation and maintenance of dialogues between various ISAC community members have

gained more popularity also at CYTO2019. It is evident that community members want to put their plans into action. This is reflected in establishing thematic task forces or subgroups to address the problem with collective, systematic and multi-faceted approach. Noteworthy here is the continued effort of community members gathered around Lab Hacks directory that easily can be called cytometric Internet of Everything or Internet of Cytometry.

Shared Research Laboratory (SRL) Best Practices

SRLs are providers of high-quality cytometry services, which include but is not limited to sample processing, quality assurance, data analysis, and reporting and possibly interpretation. As cytometry attracts more and more attention in scientific projects, it is not surprising that SRL facilities are fully booked and SRL staff is exceptionally busy handling cells and more importantly dealing with people and in not infrequent occasions are managing conflicts. Due to all that complexity, there is a sound request from SRL community to provide guidances, procedures, work instructions or even strategies that would facilitate their day-to-day operations, free up their hands to focus on quality service, science and foster their careers.

Quality Assurance and Reproducibility

Assuring quality and reproducibility in scientific research requires in depth knowledge of the materials used and experimental environment being aware of its known limitations. The reality, however, is that not all can be controlled or predicted because often the control material does not fit for the purpose used, models do not work as expected or there is no clear understanding on what and how monitoring should be done in given experimental setup. Clinical trials consist of many steps that require careful observation and control. The main challenge here lies in their high complexity, physiological dependencies that we do not entirely understand, and technical and/or biological limitations that are yet to be overcome. Big multi-sites studies produce enormous number of data that should be processed within reasonable time, appropriate care and systematic approach. To fulfil all these requirements, automated algorithm-based data analysis maybe a key and should be employed.

Final Remarks

This second joint manuscript summarizing CYTO2019 workshops intends to convey a strong message to the entire ISAC community and beyond: We, the scientists must talk! Exchanging experiences, formulating and addressing questions, thinking outside the box, continuous education, identifying areas for improvements and gathering around in task forces or thematic groups to jointly attempt advance cytometry should be dynamic and living ISAC community members approach.

As short in form, this report contains only key messages or findings from the workshops and should be considered as a guide that can facilitate understanding ISAC community research status quo. Voice or video recordings are this time available for some workshops (as stated by the authors in individual reports).

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Glossary

AAPS	American Association of Pharmaceutical Scientists
ABRF	Association of Biomolecular Research Facilities
AD	Assistant Director
CFZ	Cytometry Facility Zurich
CIC	Cancer & Immunology Core
CYTOU	CYTOUniversity
FC	Flow Cytometry
FCI	Francis Crick Institute
FP	fluorescent protein
FTE	full-time equivalent
GLP	Good Laboratory Practices
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HSCs	hematopoietic stem cells
I4S	Instruments 4 Science
ICCS	International Clinical Cytometry Society
ISAC	International Society for Advancement of Cytometry
IT	Information Technology
LETF	Live Education Task Force
MAA	Multiple Answers Allowed
MCCE	Mass Cytometry Center of Excellence
MIFlowCyt	Minimum Information about a Flow Cytometry experiment
NCCI	National Cancer Center Institute
PACA	PanAfrican Cytometry Association
PBS	phosphate-buffered saline
POC	point-of-care
PSCs	pluripotent stem cells
QA	quality assurance
QC	quality control
SICS	sorter induced cellular stress
SOP	standard operating procedure
SRL	shared resource laboratory
t-SNE	t-distributed stochastic neighbor embedding
UX	User Experience
Z-N	Ziehl-Neelsen

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