# SRL NEWSLETTER

## INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY (ISAC)

**Newsletter Volume 2 Issue 4** **January 2022**

## Gentle Sorting and Sorter Induced Cell Stress (SICS): What to Do About It

Alan Saluk

Peter Lopez

**We recently had the opportunity to "sit down" with Peter Lopez and Alan Saluk to discuss the impact of cell sorting on cellular health and function. Both Peter and Alan have done extensive work to evaluate the impacts of sorting on cells and we want to thank them for taking the time to discuss this critical subject.**

***Q: Can you tell us a little about yourself and your background?***

**Peter:** *Since graduating from Upstate Medical Center (Syracuse NY) in 1977 my entire career has been devoted to flow cytometry. I studied cytopathology so I'm pretty good with a microscope which I think enhances my skills as a flow cytometrist. The slit-scan flow cytometry project that Leon Wheeless was working on in Rochester was my first job after college. It was very exciting to me since it combined cytopathology with the basic components of flow cytometry—lasers, fluidics, optics, electronics and computers, all things that I already had interest in, and was dabbling with myself as hobbies. After Leon hired me, I quickly realized I had found my niche. I was recruited to every flow cytometry job I've ever had (even my first job with Leon) and I've been lucky to have had the opportunity to work with many seminal contributors to the biomedical sciences.*

### Inside this Issue



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**Alan:** *I originally encountered Flow Cytometry as a summer college intern with Coulter (aka Beckman-Coulter aka Beckman) working with the Applied Research Group in 1990. I bounced around different projects as needed. I was trained initially with the EPICS Profile, graduated up to the EPICS Elite Cell Sorter, and that initial introduction to an open architecture cell sorter captured my imagination. From there I had the opportunity to move from Miami in the Southeast corner of the US to San Diego in the Southwest corner in 1998 to work at The Scripps Research Institute under Joe Trotter. When Joe left for Becton-Dickinson in 2000, I became interim Director and in 2001 became Director of the Flow Cytometry Core until 2019 where I was retitled Senior Scientific Director. During that time, the TSRI flow core has strived to be on the cutting edge of cell sorting and advanced analysis and we have worked with most major instrumentation manufacturers.*

***Q: What have you experienced with cell sorting and its impact on cellular function post sort?***

**Peter:** *I believe quality cell sorting is one of the primary functions of an academic core cytometry laboratory. Cell sorters have evolved with time but the base technology of droplet sorting has remained constant. When I worked at Cytomation with the MoFlo (the first commercial high-speed cell sorter), people would tell us you can't sort cells as fast as we described because they wouldn't survive, without any data in hand. We already knew this wasn't the case for lymphocytes, but on the other hand, core labs don't only sort lymphocytes. I credit Rich Cross at St. Jude, an early MoFlo user, for alerting me to the fact that activated T-cells seemed a bit tweaked after sorting with a 70um nozzle on the MoFlo, which got me wondering about how cell sorting might affect other cell types. 25 years later we know of many anecdotes describing problematic cell types, and we are now aware that the basic droplet cell sorting technology can cause a variety of post-sort issues which we call Sorter Induced Cellular Stress (SICS).*

**Alan:** *It's an interesting situation being a core facility utilizing staff operated cell sorters, as ultimately, any unsuccessful sort is typically assigned to the cell sorter itself and the burden is placed on the core staff to identify that actual cause. One of the first cases of this nature, that I recall in my early years, was a PI sorting a human cancer cell line that after every sort just seemed to die out, yet other cell types from the same lab seemed fine. Upon closer examination I learned he was using an IgM to stain for CD3 and was in such excess that he was essentially crosslinking the CD3 receptors and sending an activation signal that that specific cell line didn't do well with. Two lessons came from that: There are numerous factors impacting the success of an experiment that occur before the cells even arrive to be sorted and getting all the relevant information from researchers you are performing sorts for are key to unraveling unhappy sorts.*

***Q: In your experience, what cell types or functions seem to be most impacted?***

**Peter:** *This is where I feel things become a bit complicated, since there are so many cell types with so many unique functions being studied after cell sorting, that there are many answers. If you talk about gene expression, the impact there has been shown to be negligible. If representative cell function is the desire from our sorted cells, that's where we need to investigate the specific function post sort. The one thing we believe, coming from our work and that of others, is that metabolite assessment post sort is where you will reliably see an impact, since cellular metabolites seem to be depleted across the board in droplet sorters, regardless of cell type. Since metabolites are depleted, cells will need to re-establish their metabolome post sort, and the recovery mechanism may have impact on function or fitness of the sorted cells, so this is a discussion we should have with investigators using cell sorting.*

**Alan:** *Pinning it down to specific cell types misses the point, some cell types are inherently fragile*

*and require extra care even if they are not sorted. I tend to view it as a continuum going from a resting lymphocyte to activated neuronal cells. The resting lymphocyte is a simple small spheroid cell that is unlikely to be perturbed by sheer forces or minor changes in environmental conditions, whereas a microglia is dendritic and requires specialized conditions to proliferate and retain function. One has to take these factors into consideration as they discuss the variables in a sort experimental design with the core staff. One thing I want to expand on here is the nomenclature of high-speed versus low-speed sorting.*

***Q: What are the factors that impact the cell health i.e., pressure, nozzle, temperature (both sample and collection) and/or flow rate? Can you explain why the speed (cell/second) is not the most reliable way to predict the impact on the cells?***

**Peter:** *All of these items can have an effect but they would need to be evaluated individually. We're just now starting to investigate the specific contributors to SICS and our focus is to use metabolomics as the readout while we change other parts of the system, like pressure for instance. Our goal is to find the contributor(s) to SICS that are inherent to the conventional cell sorting process, and see if we can improve the sorting process by minimizing or changing these contributors. Regarding speed (as cells/second), I hear this questioned while people are already sorting with a certain nozzle size, and they ask us to slow down the cells/second during the sort to be less harsh on the cells. Slowing down the event rate is obtained by a slightly lower differential pressure, but the system overall is still operating at 60psi, for example. What they really want is an overall system-pressure decrease (both sheath and sample pressures), that you can only obtain by using a larger-orifice nozzle. I don't think anyone has looked at cell-function effects of running a sort using a 100 um nozzle at 5,000 vs. 15,000 cells/second, but my guess is that the effect would be minimal.*

**Alan:** *A high-speed sort will typically have a 70um nozzle and a pressure anywhere from 30-70psi depending on instruments. A low-speed sort is typically a 100um or 120um nozzle around 10-20psi. The throughput of cells correlates to the rate of droplet formation, which is typically higher with a smaller nozzle at higher pressures with sample rate optimized for recovery. Sample differential does not define the speed of the sort, so cranking your differential for a 100um sort so you're getting 30,000 events/second is not a high-speed sort, it's just a low speed sort with higher throughput and lower recoveries.*

***Q: Have you compared cellular function after sorting on different platforms?***

**Peter:** *We've learned that sorting using microfluidic platforms instead of conventional droplet sorters can have an advantage on minimizing SICS and preserving cell function after sorting. Regarding function of cells using different manufacturers of conventional droplet sorters, that's a loaded question. While individual scenarios should be evaluated in labs that have different brands of conventional cell sorters, my wish is that manufacturers adopt the "SICS Score" described in our paper and do the assessment themselves.*

**Alan:** *The ability to properly compare sorting platforms is difficult for a host of reasons. Usually, a researcher with access to different platforms will have an issue on a given instrument and then gravitate towards the one where their sort worked without ever really trying to determine why there was any difference. An informative comparison occurred in my lab back in the early 2000s when the BD FACSAria first came out with its hybrid cuvette nozzle. We had a researcher who was transplanting sorted mouse lymphocytes into a different host background after labeling the cells with CFSE to track proliferation. He had successfully been performing these experiments on the BD FACSVantage and when we moved to the Aria he was no longer seeing the proliferation. We tried it again on a Beckman Digital MoFlo and the cells successfully proliferated. The obvious difference was jet-in-air*

*versus the new hybrid cuvette somehow impacted the cells. We then compared the Vantage to two Aria's and two MoFlo's, all using the same sample at the same time, and the results were striking. All systems showed good viability post-sort using trypan blue, but it wasn't until the transplanted cells were recovered that there was an issue with the early hybrid cuvette nozzle. With all systems being run with a 70um nozzle at 35psi it was clear that the cells were behaving differently based on instrumentation. Sadly, when I pointed these issues out to BD they didn't like it very much and deemed me persona non grata. They quietly modified their cuvetted flow cell geometry and never acknowledged the issue, but it was clear as day. Now mind you the sort could be performed on the Aria at a lower psi, but the idea that all sorters treat sorted cells the same was dispelled for us.*

***Q: What makes a sorter a "gentle sorter", is there really such a thing?***

**Peter:** *I think the term "gentle sorting" has been used primarily when talking about alternative sorting technologies such as microfluidic sorters. The goal is that the sorted cells function representatively. We published a study where sorted iPSCs were used downstream in the blastocyst complementation assay, and cells sorted using conventional jet-in-air sorters would not function well in this assay. We were able to get the assay to work reliably when we moved to the On-Chip microfluidic sorter. So that's an example of what I would call gentle sorting.*

**Alan:** *I don't believe the hydrodynamics of a droplet-based sorter can get much simpler than the classic jet-in-air design. But it's clear that the increased sensitivity of the hybrid-cuvette designs is of significant value. So I believe it is a series of tradeoffs in that you can always go slower on a hybrid cuvette instrument and retain cell functionality that would be lost at higher pressure and nozzle combination. But if you need the highest throughput feasible to recover a very rare population, the classic jet-in-air design has worked better in our hands.*

***Q: Peter, your recent Brief Report in Cytometry on sheath fluid impacts on cellular metabolites in cells afflicted by sorting induced cellular stress was fascinating. Can you give us some practical advice for what sheath to use? Should we all be sorting with media instead?***

**Peter:** *We really struggled with the title of that paper, which highlights sheath fluid. The primary finding is the metabolite profile is depleted after sorting, but the authors decided to go with the sheath fluid title, even though that was an incidental finding based on a hunch I had. We found that complete media as sheath (CMAS) can spare cells of the SICS metabolic phenotype, but we don't know why. We're certainly not suggesting people use CMAS on a regular basis. It's a total pain, although we did not see any issues with foaming in the waste catcher or the collection tubes. However, if you don't have a microfluidic sorter and you have users who want to sort cells for metabolomic profiling, CMAS is an option that can be considered.*

***Q: How can a researcher determine if sorting is altering cell function without performing a metabolic panel assay? What are the warning signs there is an issue?***

**Peter:** You *need to discuss with users what they are going to do with their cells after the sort, and note if there's a functional assay in mind. Then you need to consider, but ideally first test to see if that function might be impacted by the sorting process. If sorted cell counts after sorting are much less than indicated by the machine, this can be a warning sign. While we expect a small portion of cells might die after sorting, sometimes the starting population had a high proportion of dead or dying cells in the first place and this can be a warning sign of downstream issues. Of course, a post-sort analysis should be done whenever possible, and this can also be informative.*

**Alan:** *That is such a tricky question in that not every sorting experiment has metabolic func-*

*tion or a gene array readout to determine if cells are behaving as expected. Even good viability is not always indicative of good functionality. Ultimately the pattern recognition in the researcher comes into play; are the cells physiologically looking like I expect them to if I returned them to culture, are they growing in similar ways compared to the cells that weren't sorted? This is ultimately one of the weak links in the experimental design follow through with sorted cells. One of the cautionary approaches we take is when the researcher isn't sure about how these factors impact their downstream experiment, we start them off slower with a larger nozzle. If they need to significantly increase their throughput, we have a baseline to assess the next experiment at higher speeds.*

***Q: It seems cells that have downstream cellular functional assays would be the most impacted by SICS, what impacts could be possible on cells that go directly into studies such as RNASeq or proteomics?***

**Peter:** *I think the biggest impact on these molecular assays come from cells being dead or not after sorting. Another potential impact is the amount of cellular debris that is included around with sorted cells, and if this has any impact on these other technologies.*

**Alan:** *In some of the studies we did in conjunction with an ABRF paper (see below Box et al) were able to see murine B-cells sorted at high-speed and then cultured for various times post-sort were shown to express some heat shock proteins suggesting SICS was in play. The conjecture was that the cells were stressing and that it took a bit for the genes to turn on post sort. If your researchers are sorting single cells into plates straight into a lysis buffer, I don't see the downstream readout being affected. Now if your cells are being processed or cultured after, or if there is some other downstream manipulation prior to sequencing, then gene activation might impact some of the readouts. Maybe looking forward we can collectively assemble a set of cell-type specific genes that should be monitored if the cells in a sequencing assay are being sorted prior to the readout.*

***Q: Have you implemented any of your findings in your facility or purchasing decisions? What advice do you have when SRL's looking at a new sorter and need to weigh their options for choosing based on manufacture data and claims?***

**Peter:** I *think the best advice is to actually demo the instrument, keeping in mind the utility and requirements of the sorted material downstream. More often than not I think people are more interested in seeing if the instrument sorts well by accessing sorted cell purity, viability, instrument efficiency and sensitivity—all very important considerations, but I don't think people typically take sorted cells from a demo and use them in the subsequent applications.*

**Alan:** *I think I recently made the analogy of the Ferrari and the Range Rover in terms of how you consider your new instrumentation in a discussion with some colleagues. The Ferrari is analogous to the high-dimensional high-sensitivity sorter that appeals to some groups whereas the Range Rover is the more utilitarian sorter that covers many of the functionalities such as biosafety integration, sample handling automation and advanced plate sorting features. Ultimately, anybody considering such a large purchase should be diligent; ask for a demo and perform the experiment most representative of what you will be doing back in your facility. Talk to labs who have purchased an instrument you are interested in, and push to see what manufacturer is most supportive and willing to work with you in terms of service support and pricing. We are in a unique time with substantive innovations and market competition unlike anything I have witnessed in my career, and I think there is something for everybody out there in our current sorter marketplace.*

**Wow, thank you Peter and Alan for all the educational information! Definitely a lot to consider to ensure that cells post sort are viable, metabolically intact, and able to move into down-stream assays without compromising those results. One important take away is that SRL's need to ask the researchers who come to their facility to sort live cells, what they are planning to do next with the cells, and guide them on the best ways to minimize SICS.**

**REFERENCES**


Ryan, K, Rose, RE, Jones, DR, Lopez, PA. Sheath fluid impacts the depletion of cellular metabolites in cells afflicted by sorting induced cellular stress (SICS). *Cytometry*. 2021; 99: 921– 929. https://doi.org/10.1002/cyto.a.24361

Monica DeLay, Peter Lopez, Alan Bergeron, Andrew Box, Kathy Brundage, Sridar Chittur, Matt Cochran, E. Michael Meyer, Scott Tighe, Alan Saluk Evaluating Effects of Cell Sorting on Cellular Integrity and Gene Expression *ABRF poster session 2014* https://www.abrf.org/assets/docs/fcrg_poster_2014.pdf

Box, A, DeLay, M, Tighe,S, Chittur, SV, Bergeron, A, Cochran, M, Lopez, P, Meyer EM, Saluk, A, Thornton, S, Brundage K. Evaluating the effects of cell sorting on gene expression. J. Biomol Tech. 2020; 31(2):100-111. https://doi.org/ 10.7171/jbt.20-3103-004


## ISAC SRL Newsletter Survey:


We hope you have enjoyed reading the newsletter over the last year. We would love to get some feedback on what you like, what you might like to see in it, and how can we improve it. The survey should take approximately 5 minutes. The link is below:

**https://forms.gle/qZKSEsnGPcfhPqmp8**

# Highlighted SOPs in the ISAC SRL SOP Repository

This quarter, we would like to highlight two SOPs – both for sorting in a BSL-2 environment. To sort BSL-2 classified material requires extra precaution, the highlighted SOPs describes many of these safety measures. If you have to start sorting BSL-2 classified material, read these SOPs – you do not have to start from scratch, lots of help can be found here.

From the University of Utah Flow Cytometry Core Facility "BSL2 w/ enhanced precautions BD FACSAria Sort" written by James Marvin is highlighted and from the University of California Merced Stem Cell Instrumentation Foundry "SOP for BSL-2 samples on Aria 3" written by David Gravano is highlighted.

In addition, several great SOPs for preparing sorters for sorting and unclogging nozzles can be found in the SRL SOP repository

https://archive.org/details/@isac_srl

Help us grow the SOP Repository by submitting your lab's favorite SOP here

https://tinyurl.com/SOPRepositoryform

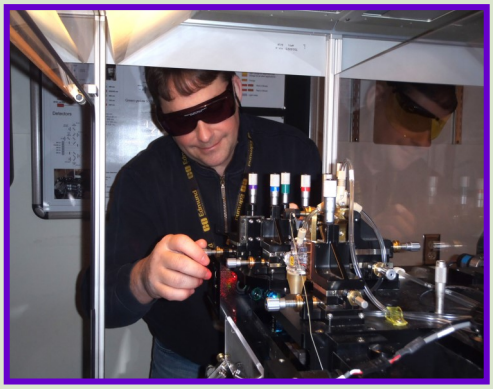
# Council's Corner:

# A Quarterly update from ISAC Council about what Council has been up to

Over the last quarter, Council has been planning the new membership tiers, which very recently passed an electronic vote, with 83.3% of electors selecting the new structure. The new membership tiers will be launched in January. We have also been planning for CYTO 2022 in Philadelphia and have a great line-up of speakers. Mark your calendars and we hope to see you there!

**Aja Rieger, SRL Committee Council Liaison, 2020-2022**

# FLOW STAR:
# William Telford, Ph.D.



**1. What do you think is your biggest contribution to the field of cytometry?**

My biggest contribution to cytometry is probably the introduction of new laser wavelengths, allowing expansion of both the number and variety of fluorochromes and markers we can detect. I was fortunate that the explosion of laser capabilities and my management of shared resource labs occurred at the same time – we had a lot of fluorescent probes we needed to analyse but could not due to excitation limitations, so this led us to test new wavelengths such as the yellow. We're still working in this area, using non-visible deep ultraviolet and near infrared lasers to improve both high-dimensional flow and gain access to new probes and techniques.

**2. What is your favourite memory from CYTOs so far?**

I love the training and technology workshops, such as the Annual Course and the Asilomar (now Scintillon) technology development meetings. You get both the most experienced scientists and the new ones coming up together for training and brainstorming. I've been lucky enough to participate both as a student and as faculty in both.


**3. What do you like to do when you are not in the lab, the most favourite pastime?**

I enjoy both backpacking and cycle touring, although my bad knees are slowing me down for both!

**4. Who has been your favourite mentor?**

My first real mentor in flow cytometry was Louis King, the manager of the Michigan State University flow lab. He taught me the nuts and bolts of instruments. While I had worked in flow before, he taught me how the instruments really worked and what you could do with them. I was also fortunate enough to connect with some of the most influential investigators in cytometry, such as Howard Shapiro, Scott Cram, Zbigniew Darzynkiewicz, and Awtar Krishan all are very generous with their expertise, and they helped me at critical times in my professional development.

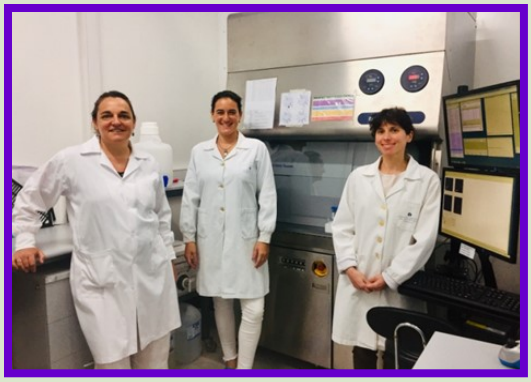
**5. Any one message for the present day cytometrist.**

Understand the technology, even if you are a biologist! Learning how instruments work "under the hood" has proven incredibly useful to me again and again. And it is obvious, but network with people who are strong in the areas you are not. I am an immunologist, but some of the most interesting work I have done is in technology and engineering, thanks to colleagues in these areas who took the time to educate me.

**6. Any advice to those who might want to establish an SRL.**

I think of my SRL like a small business, working hard to serve my customers. I also want to give my users an edge over other investigators, providing expertise and services they can't get anywhere else. Our laser work stemmed from this – our people could analyse things no one else could.

# *Highlighted Flow Cytometry Shared Resource Facility*
# *Cell Biology Unit (CBU)*
# *Institut Pasteur de Montevideo*



**1. Location**
Montevideo, Uruguay
http://pasteur.uy/investigacion/unidades/biologia-celular/

**2. Meet the staff**
**Mariela Bollati-Fogolín —** Head of the CBU since 2006.
Mariela has been involved in the flow cytometry field for over 20 years now. She is currently the Chair of ISAC CYTO Women Taskforce, participates in different ISAC's Committees. In addition, she is member of the Executive Committee of the Grupo Rioplatense de Citometría de Flujo (Uruguayan-Argentine Flow Cytometry Society), the Advisory Committee of CABBIO (Latin-American Center for Biotechnology) and the LatinFlow.
**María Paula Céspedes —** an Associate Technician of the CBU since 2018**.**
Paula is a Biochemist and has a Master degree in molecular and cellular biology. She actively participates in the CBU both in supporting flow cytometer users as well as trainings new ones. Her commitment to research and her flow cytometry expertise is supported by numerous collaborations with other research groups.
**Karen Perelmuter —** a Research Technician in the CBU since 2009.
She has a degree in Biology and a Master in Biotechnology. She has excellent skills in cell technology, quality control and is truly committed with our quality management system.

**3. Instrumentation in the facility**
Attune™ NxT (4 lasers)
Accuri™ C6 (4 lasers)
CyAn™ ADP (4 lasers)
FACSAria™ Fusion (4 lasers) in biosafety cabinet

**4. What recent accomplishment in your lab are you most proud of?**
One of the main challenges during the pandemic was to manage the unit workload and the training program while following safety protocols regarding occupancy rates and rotation of unit staff and flow lab users. As a result, we developed online training program with virtual practical tutorials in order to give users an insight on basic aspects of flow cytometry, and practical instrument-specific features.
Another goal achieved of which we are proud of was to solve problems with the flow cytometers while field service engineers, who come from other countries, could not enter our country due to the closed borders because of the sanitary situation. We have accomplished it with our creativity, virtual guidance and the support of the institutional maintenance staff.

**5. What is the most unique/odd sample have you analyzed in the facility?**
Blood and peritoneal lavage from Russian sturgeons (*Acipenser gueldenstaedtii*) in collaboration with the Immunology group from the Sciences Faculty at Universidad de la República (Uruguay). It is worth to mention that Uruguay is the largest exporter of caviar in Latin America and in this context we were looking at inflammation triggered by a bacterial infection in the presence of chronic heat-induced stress on sturgeons which finally impairs the caviar production. It was a challenging project, since there are very limited published information and not many reagents available for this specie. Our motivation has been that we will celebrate with caviar *ad libitum* when those results are published.

# Ask Old Dr. FITC

***Dear Dr. FITC,***
***I'm new to a lab and one of my postdocs is talking about sorting our cells. I am so excited, I hope my cells are Gryffindor, or maybe Ravenclaw… not Slytherin, definitely NOT Slytherin. How does the sorting instrument decide what to sort cells into?***
***-Harry P.***

Hi Harry,
Let me try to explain. First of all, Slytherin is a highly respected house with a lot of heroes. Don't let a few bad ones ruin your opinion of the whole house. I myself have a definite affinity for *Green*. But I will move on to your Sorting question. There are several types of sorters which employ microfluidics, buoyancy, centrifugation or filtration to sort cells, but the two most common are MACS (Magnetic-Activated Cell Sorting) and flow sorting. With MACS, magnetic particles bind to cells through ligands and antibodies that can bind markers on the cell surface. Then the targeted cells can be magnetically isolated from a sample. Usually with flow sorting, fluorescent markers are associated with a cell intracellularly or extracellularly through a variety of techniques including esterification of dyes, antibody binding, or expression of fluorescent markers. As an aside, some researchers mistakenly will use the term FACS™ (Fluorescent Activated Cell Sorting) to describe flow sorting or analysis. The term FACS™ was coined by some of the forerunners in the field, Leonard and Leonore Herzenberg at Stanford University and trademarked by Becton Dickinson (BD). When fluorescent sorting, the cells are identified one at a time by a laser and sorted based on the measured properties. After identification the instrument introduces a positive or negative electrical charge which is directed by the electric field into the tube. Imagine if you will, the sorting hat actually electroshocked the students and brought them via large magnets to their appropriate house table as they stepped off the stage. (That would have been a very different type of movie.) Enjoy your sorting!

## Complaint Department: Holiday Deadlines and Unrealistic Expectations

*Why is it that after 2 weeks in a row in November where 3 of the instruments sat idle, December 1st arrives, and you get three new labs that have never spoken to the flow cytometry core before in their existence and they each have flow cytometry which **MUST** be done before holiday shutdowns?*

*I know it's the same phenomenon that happens whenever the core director plans a vacation. Is it just the same strange coincidence of scarcity that makes baseball cards and beanie babies rare? At any rate, it's a short complaint department this newsletter because sometimes we're too busy to complain. And really, as a core director or staff you would complain a lot more about not enough work than too much.*

If you have a user, instrument, or management anecdote that you would like to get off your chest please send an email describing it to **isacsrl.outreach@gmail.com**. One complaint will be "highlighted" each issue of the newsletter.

# ISAC SRL related Events and Resource Links

If you, have a meeting/webinar that you would like to have included in the next newsletter please send the information to the Outreach Task Force at isacsrl.outreach@gmail.com.

| Upcoming Meetings and Webinars | | |
|---|---|---|
| **Meeting** | **Dates** | **Link/Webinar** |
| **The Nordic Flow Cytometry Meeting—Oslo, Norway** | **Feb 10 –11, 2022** | https://www.flowcytometri.no/oslo-2022/ |
| **Iberian Cytometry Society Workshop at the XLVII Annual Meeting of the Portuguese Society of Immunology** | **April 20-22, 2022** | https://tinyurl.com/bdhwxfnv |
| **The Association of Biomolecular Resources Facilities (ABRF) Annual meeting — Palm Springs, CA** | **March 27-30, 2022** | www.abrf.org |
| **VI National Congress of ISCCA—Societa Italiana per l'Analisi Citometrica Cellulare—Catania, Italy** | **May 25 –27, 2022** | https://www.iscca.eu/Meeting.aspx |
| **XXXVth InternationalSymposium on Technical Innovations in Laboratory Hematology—Bologna, Italy** | **May 25 –27, 2022** | https://www.islh.org/2022/ |
| **CYTO 2022<br>Moving Forward: Empowering Scientist. Advancing Cytometry** | **June 3—7, 2022** | isac-net.org/page/CYTO |

| Recorded CYTO U Webinars—Recent Additions<br>https://learning.isac-net.org/ | |
|---|---|
| **Topic** | **Presenter** |
| **Creative Funding Options for SRLs: From Crowd Funding to Philanthropy** | **Kylie Price** (Senior Scientist and Head of Research technology at Malaghan Institute Medical Research, New Zealand )<br>**Dagna Sheerar (**Flow Cytometry Technical Director at University of Wisconsin, USA) |
| **Changing Mindsets: Benchmarking and quality assurance** | **Marta Monteiro, Ph.D.** (Head of Flow Cytometry & Antibodies Core Facility at Instituto Gulbenkian de Ciência, Portugal)<br>**Julie Auger** (Executive Director of Research Core Facilities Program at University of California Davis, USA) |