31st Annual Meeting

Great Lakes International Imaging and Flow Cytometry Association

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Friday, September 16 – Sunday, September 18, 2022

Indianapolis, Indiana

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ABOUT GLIIFCA

GREAT LAKES INTERNATIONAL IMAGING AND FLOW CYTOMETRY ASSOCIATION, INC.

EIN# 16-1545169

The Great Lakes International Imaging and Flow Cytometry Association (GLIIFCA) was started in 1992 by Carleton and Sigrid Stewart and Alex Nakeff, and fosters the interaction of professionals from Great Lakes region (U.S. states of Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania and Wisconsin as well as the Canadian province of Ontario) with interests in flow and image cytometry. GLIIFCA organizes affordable annual meetings allowing the Great Lakes region cytometrists to learn about the latest developments in cytometry and related fields, network with colleagues, and share the excitement about their research.

THE HISTORY OF GLIIFCA - WRITTEN BY CARLETON C. STEWART

In 1992 GLIIFCA began as... GLIFCA (the Great Lakes International Flow Cytometry Association - the word Imaging was added in 1993) and was first part of an outreach program for a Clinical Cancer Resource Grant that I was writing at Roswell Park Cancer Institute in Buffalo, NY. I had been thinking and discussing forming an organization that would primarily focus on young investigators and technologists doing research in cancer using imaging and flow cytometry. The established leaders in each of these fields were invited to form the first Steering Committee. Carleton C. Stewart was elected first President, Alexander Nakeff first Vice President, Sigrid Stewart, first Secretary/Treasurer and James Jacobberger first Educational Officer. Alan Landay and Maurice O'Gorman were to be the Scientific Chairs for the next meeting which was held October 1 - 3, 1993 at the Hotel St. Regis in Detroit, MI. Other members of the Steering Committee were Paul Robinson, Waclaw Jaszcz, David Hedley, Betsy Ohlsson-Wilhelm and James Leary. This Steering Committee was established to help determine the policies of the organization, maintain a budget, and create the program for the annual meeting. In addition to funding for this organization by the grant, I envisioned participation by the vendors as equal members of GLIIFCA. The reps from each company were instrumental in passing the word to their customers throughout the capture area. And you know the rest of the story...

Our first meeting of the Great Lakes International Flow Cytometry Association was held September 25 - 27, 1992 at the Hotel St. Regis in Detroit, MI: The meeting was an overwhelming success. The scientific agenda included sessions on Immunophenotyping, tumor biology and instrumentation. Over 130 charter members, some of which had never attended a flow meeting before, were able to attend because the total cost per person amounted to under $150 including registration and accommodations. Because of the central location of Detroit to the Great Lakes Region most people were able to carpool. The meeting started with a well-attended reception on Friday evening with food and an open bar sponsored by Becton Dickinson Immunocytometry Systems. On Saturday the Flowdown, sponsored by Coulter Cytometry, was thoroughly enjoyed by all. Most participants stayed until the very end. The greatest single state/province represented was Ontario, Canada. US charter members were from Michigan, Indiana, Minnesota, Illinois, Ohio, New York, Pennsylvania, Wisconsin and New Jersey. We even had two guests from Russia, which truly made this an international event. While our main region of emphasis is the Great Lakes, we welcome our colleagues from across the land to participate.

GLIIFCA 31 SPONSORS

The GLIIFCA 31 meeting is kindly supported by:

- Agilent Technologies – Support for the Wine & Cheese Party
- Akadeum Life Sciences – Video sponsorship
- Applied Cells, Inc.
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- Beckman-Coulter Life Sciences
- BD Biosciences – Support for Cutting Edge Cytometry Applications Session, Flash Talk Awards, and Vendor Choice Award, Video sponsorship
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- Particle Metrix Inc.
- Proteintech
- Slingshot Biosciences, Inc.
- Sony Biotechnology Inc. – Support for Flow Cytometry & Omics Session
- Spherotech Inc. – Support for Coffee Break
- Standard BioTools – Video sponsorship
- Thermo Fisher Scientific – Support for Wine & Cheese Party
- Union Biometrica, Inc.
- Verity Software House

CONFERENCE PROGRAM

FRIDAY

GLIIFCA 31 CORE FACILITY MANAGERS' WORKSHOP (SALONS A-C)

12:00 PM – 5:00 PM

This workshop offers perspectives on developing marketing strategies for core facilities as well as addressing public relations and conflict resolution. Core managers and staff can network with colleagues facing similar challenges marketing and running flow core facilities. We will have a discussion regarding the challenges of educating our users on Current Best Practices in flow cytometry to build rigor and reproducibility into research. In addition, attendees are encouraged to bring their own issues for a general networking session. Come prepared to actively participate in this workshop. We look forward to learning from everyone's experiences.

12:00 PM - 1 PM	*Opening Networking Luncheon: Welcome and Introductions*, **Matt Cochran, Dagna Sheerar, Vicki Smith, Ann Marie DesLauriers-Cox**, **Celine Silva-Lages** Organizing Committee – lunch can be taken to eat elsewhere if preferred.
1 PM – 2 PM	Not So Conventional Flow *High-Dimensional Cytometry in a Shared Resource Setting: Standardization and Applications for Clinical Research,* **Caroline Roe, MLI**, Managing Director, Cancer & Immunology Core at Vanderbilt University *Seeing is Believing: Applications of Imaging Flow Cytometry to Study Erythropoiesis,* **Katie Giger Seu, PhD**, Research Associate, Cancer and Blood Diseases Institute at Cincinnati Childrens Hospital Medical Center.
2 PM – 2:45 PM	*Small Group Discussions* – Technical – These will be break out group sessions to discuss a variety of technical topics.
2:45 PM – 3 PM	*Coffee and Refreshments*
3:00 PM – 3:45 PM	*Small Group Discussions* – Management – These will be break out group sessions to discuss a variety of management topics
3:45 PM – 4:45 PM	*Burnout, cool on the track, not in the lab,* **Ann Marie DesLauriers-Cox**, University of Michigan, Ann Arbor, MI;
4:45 PM - 5:00 PM	*Closing Thoughts and Ideas* Thank you to GLIIFCA for generous sponsorship of this workshop Organizing Committee: **Matthew Cochran, Vicki Smith, Sally Quataert, Dagna Sheerar, Ann Marie DesLauriers-Cox, Celine Silva-Lages**

OPENING RECEPTION (SALONS 1-5)

The Opening Reception will take place in the **Salons 1-5** from **6:00 PM** to **9:00 PM**. Come and interact with the Vendors and fellow GLIIFCA conference participants!

MEET-AND-GREET (SALONS 1-5)

Come meet other attendees!

EXHIBITOR TECH TALKS PART 1 (SALONS 6-8)

Session Chairs: Christiane Hassel (Indiana University),
Karen Domenico (University of Cincinnati College of Medicine)

7:00 PM – 7:05 PM	**Exhibitor Tech Talk Introduction**
7:05 PM – 7:15 PM	Shibabrata Mukherjee, BD Biosciences
7:15 PM – 7:25 PM	Mark Barnes, Miltenyi Biotec
7:25 PM – 7:35 PM	Melvin Lye, Curiox Biosystems
7:35 PM – 7:45 PM	Garret Guenther, Agilent Technologies
7:45 PM – 7:55 PM	Kim Cardenas, BioLegend
7:55 PM – 8:05 PM	Seth Meyers, NanoString Technologies

SATURDAY

Breakfast will be available in the Exhibit area in **Salons 1-5** from 7:00 AM to 8:00 AM
ALL OF THE ORAL PRESENTATIONS WILL BE DELIVERED IN **SALONS 6-8**

8:00 AM - 8:15 AM	*Welcome Address*: **Laura Johnston** (GLIIFCA President)

SESSION 1: IMAGING CYTOMETRY (SALONS 6-8)

Supported by: Luminex Corp

8:15 AM - 9:00 AM	*Regulation of Foamy Macrophage Differentiation and Survival in Atherosclerosis* **Jesse Williams**, University of Minnesota
9:00 AM - 9:45 AM	*Uniting spatial transcriptomics with fluorescent tissue cytometry to define neighborhoods of health and disease within the kidney*, **Ricardo Melo Ferreira**, Indiana University School of Medicine

THE 2022 CARLETON AND SIGRID STEWART KEYNOTE LECTURE (SALONS 6-8)

Introduction: Paul Wallace

9:45 AM - 10:30 AM	*Maladaptive Pleural Environment and Cancer Progression*, **Vera Donnenberg and Albert Donnenberg**, University of Pittsburgh
10:30 AM - 11:00 AM	*Coffee Break* sponsored by Spherotech Inc.

EXHIBITOR TECH TALKS PART 2 (SALONS 6-8)

Session Chairs: Christiane Hassel (Indiana University)
Karen Domenico (University of Cincinnati College of Medicine)

11:00 AM – 11:05 AM	**Exhibitor Tech Talk Introduction**
11:05 AM – 11:15 AM	Justin Meyers, Beckman Coulter
11:15 AM – 11:25 AM	Vinicius Motta, Standard BioTools
11:25 AM – 11:35 AM	Sean Daley, Slingshot Biosciences
11:35 AM – 11:45 AM	Sven Kreutel, Particle Metrix

LUNCHTIME 11:45AM-12:30PM

ROUNDTABLE WORKSHOPS (SALONS A-D)

Workshop Organizers: Dagna Sheerar (University of Wisconsin), and Sherry Thornton (Cincinnati Children's Hospital and University of Cincinnati)

12:30 PM - 1:40 PM	*Multiple parallel roundtable workshops.*

ABSTRACT FLASH TALKS (SALONS 6-8)

1:45 PM – 1:55 PM	*Hot Flow: Attune Plumbing for Radioactive Samples*, **Kathryn Fox**
1:55 PM – 2:05 PM	*TROUBLESHOOTERS WANTED: FlowRemedy Diagnostic Chart for Cytometer Users in the Shared Resource Laboratory*, **Jennifer Jakubowski**
2:05 PM– 2:15 PM	*Testing Aerosol Containment of Flow Cytometry Cell Sorters in a Shared Resource Laboratory*, **Alyssa Sproles**
2:15 PM– 2:25 PM	*Novel Application of Fluorescent Flow Cytometry for Assaying Aggregation of Human Disease Proteins such as Transthyretin*, **Jane Dorweiler**
2:25 PM– 2:35 PM	*Establishing reference ranges for flow cytometry tests*, **Nagako Akeno**
2:35 PM– 2:45 PM	*Lymphocyte Function Evaluation Using a Flow Cytometry-based Proliferation Assay*, **Li Yang**
2:45 PM - 3:15 PM	*Coffee Break*

SESSION 2: CUTTING EDGE CYTOMETRY APPLICATIONS (SALONS 6-8)

Supported by: BD Biosciences and Bio-Rad

3:15 PM - 4:00 PM	*Throughput bottlenecks in image-activated sorting*, **Amar Basu**, Wayne State University
4:00 PM - 4:45 PM	*Identification of T cell subtypes that contribute to intestinal inflammation*, **Matt Olson**, Purdue University
4:45 PM - 5:30 PM	*Immune and ionic mechanisms mediating the effect of dexamethasone in severe COVID-19*, **Ameet Chimote**, University of Cincinnati

POSTER SESSION (SALONS 1-5)

5:30 PM – 7:30 PM	*Digital Poster Session*
5:30 PM - 6:30 PM	*Wine and Cheese Happy Hour* sponsored by Agilent Technologies, Leinco Technologies, and Thermo Fisher Scientific

THE WINE AND CHEESE HAPPY HOUR IS SPONSORED BY

SOCIAL ACTIVITIES (SALONS A-E)

7:30 PM - 11:30 PM	GLIIFCA 31 Banquet
11:30 PM - 2:00 AM	Post-banquet Scientific Networking

SUNDAY

Breakfast will be available in the Exhibit area **Salons 1-5** from 8:00 AM to 9:00 AM

BOARD OF DIRECTORS MEETING (SALONS A-C)

8:00 AM – 8:50 AM	GLIIFCA Board of Directors Breakfast

SESSION 3: FLOW CYTOMETRY AND OMICS (SALONS 6-8)

Supported by: Sony Biotechnology Inc.

9:00 AM - 9:45 AM	*Maximizing the Minimum: Best practices for low cell number LC-MS metabolomics* **Sam Weinberg**, Northwestern University
9:45 AM - 10:30 AM	*Dissecting granulocyte phenotypic plasticity using integrated approaches*, **Sergejs Berdnikovs**, Northwestern University
10:30 AM – 11:15 AM	*Automated flow cytometry and machine learning for prediction of small molecule human safety risk: the 3RnD platform,* **Andrew Bieberich,** AsedaSciences, Inc.
11:15 AM - 11:45 AM	*Coffee Break*

EXPERT PANEL DISCUSSION: AUTOMATED DATA ANALYSIS (SALONS 6-8)

Panel moderated by: Ryan Duggan, AbbVie, Inc.

11:45 AM – 12:30 PM	*Expert Panel Discussion* • Geoff Kraker, OMIQ • David Novo, De Novo Software • Dagna Sheerar, University of Wisconsin-Madison
12:30 PM - 12:35 PM	*Closing Remarks*

GLIIFCA 31 ROUNDTABLE LUNCH WORKSHOPS

SECTION: FLOW CYTOMETRY FUNDAMENTALS

Topic 1. Basic Panel Design, *Sarah Croswell, CCHMC Research Flow Cytometry Core, Cincinnati, Ohio.*

Location: **Salon A**

Panel design is often a rushed process that's more about what's in the fridge than what's best for the experiment. However, a little planning can prevent a lot of future frustration. We'll discuss some tools that are available for panel design and some things that you'll want to take into consideration when planning your next experiment.

Topic 2. Cell Sorting – Covering the Basics, *David Adams, Grand Poobah, University of Michigan Flow Cytometry Core*

Location: **Salon A**

Cell sorting is a common procedure, but a sort is only as good as its setup. In this roundtable, we will discuss the basic types of cell sorters, tips and tricks for optimization, and biosafety concerns. Whether you are new to the technology or a veteran with knowledge to share, come join our discussion.

Topic 3. Cell Sorting – Sample Preparation for the Successful Cell Sort and Analysis, *Galina Petrova, Manager, Children's Research Institute Flow Cytometry Core, Medical College of Wisconsin. Crisha Barret, Applications Scientist, Akadeum Life Sciences.*

Location: **Salon A**

The samples preparation is one of the most important steps that will define successful cell sort outcome regardless of sorting cells independently (self-service) or asking assistance from the Flow Core staff. In this roundtable, we will discuss the best practices for the isolation of cells from different tissues and presort sample preparation steps for the freshly isolated, frozen, or cultured cells that will set you up for success. Examples of tech notes and protocols will be available.

SECTION: RIGOR AND REPRODUCIBILITY: BEST PRACTICES AND TOOLS FOR SUCCESS

Topic 4. Standardization of Instrumentation: Reasons and Resources, *Dagna Sheerar, Flow Cytometry Director, University of Wisconsin Carbone Cancer Center Flow Cytometry laboratory*

Location: **Salon B**

With the increasing complexity of measurements and plethora of available instrumentation available in the field of Cytometry it is ever more important to ensure the quality of data produced. This roundtable will focus on the reasons behind instrument standardization and resources available to improve Rigor and Reproducibility across several areas of interest in Cytometry including small particle measurement, immunophenotyping, and fluorescence intensity measurements, to name a few.

SECTION: HIGH-DIMENSIONAL FLOW CYTOMETRY

Topic 5. High-Dimensional Panel Design, *Derek Jones, Senior Technical Director, Penn Cytomics and Cell Sorting Resource Laboratory, University of Pennsylvania*

Location: **Salon B**

As newer flow cytometers support an expanding number of fluorescent parameters, the need for rational panel design becomes increasingly important. We will discuss strategies for the design of high-dimensional flow cytometry panels, including fluorochrome brightness, antigen density and co-expression, compensation, and spreading error.

Topic 6. High Parameter Experimental Design and Analysis: Failing to Plan is Planning to Fail, *Celine Silva Lages, Core Manager, Research Flow Cytometery Core, Cincinnati Children's Medical Center and Veronica Obregon-Perko, FlowJo Application Scientist, BD Life Sciences*

Location: **Salon B**

The advent of high parameter flow cytometry has facilitated complex biological discoveries but involves several considerations for experimental design and analysis prior to collecting data. Missteps in panel design, compensation, sample cleanup, and normalization can impede analysis and introduce false discoveries when using automated computational tools. Join us to discuss key aspects of planning a high parameter experiment, including considerations and controls for longitudinal studies, to ensure high quality results.

Topic 7. Mass Cytometry – Potential and Pitfalls, *Matthew Cochran, Technical Director, Flow Cytometry Resource, Center for Advanced Research Technologies, University of Rochester Medical Center*

Location: **Salon C**

Join us in this roundtable for a discussion of all things mass cytometry. One piece we'll emphasize are the current possibilities for this tool at this time of massive technological expansion. We'll also discuss the pains both in terms of competition and technological limitations.

SECTION: SHARED RESOURCE LABORATORY MANAGEMENT

Topic 8. Buying New Equipment: Pricing your needs, *David Leclerc, Technical Director, Cytometry Antibody Technologies Facility, University of Chicago*
Location: **Salon C**

Customers looking for flow cytometry equipment face a complex offer of multiple platforms equipped with a large array of features. This round table aims to explore the prices of various features such as the number of lasers and detectors, presence of a plate loader, or the availability of the unmixing algorithm, and help the research community make better purchasing decisions.

Topic 9. SRL Responsibilities for High Dimensional Data Analysis: What Can We Reasonably Provide?
Lauren Nettenstrom, Assistant Director, University of Wisconsin-Madison Carbone Cancer Center Flow Cytometry Laboratory, ISAC SRL Emerging Leader
Location: **Salon C**

We will discuss the role the SRL should play in providing expertise and training regarding high dimensional data analysis vs. what the SRL actually has the bandwidth to provide. Be prepared to discuss fundamental

changes in SRL structure, hiring practices and how to deal with financial challenges that face large and small SRLs when attempting to provide these services, as well as how we can overcome these challenges to move forward in a way that best benefits our customers.

Topic 10. To Train or Not To Train – Is User-Run Sorting Right for Your Facility?, *Jessica B. Back, Deputy Director, Microscopy, Imaging, and Cytometry Resources Core, Karmanos Cancer Institute, Wayne State University*

Location: **Salon D**

We will discuss the pros and cons of providing user-run sorting capabilities in your facility. Topics will include how your users can get the most out of being autonomous, important safety implications to keep in mind, instrumentation considerations, setting realistic expectations, and tips and tricks for training.

SECTION: CUTTING EDGE CYTOMETRY

Topic 11. High Gain and High Parameter Imaging Cytometry Data: A look into what you might be missing, *Robert Thacker, Amnis Sales Specialist, Luminex Corporation*
Location: **Salon D**

Imaging flow cytometry is a powerful tool that can provide a new perspective of cellular analysis. This roundtable will examine different applications for cell analysis using imaging cytometry, and discussion questions that may arise when determining if imaging cytometry is the right tool for your experiments.

Topic 12. Nanoscale Cytometry: Analysis, Sorting, and Best Practices related to Nanoparticles, Viruses, EVs, and Exosomes, *Daniel Vocelle, Manager, MSU Flow Cytometry Core and Rachael Sheridan, Manager, Van Andel Institute Flow Cytometry Core*

Location: **Salon D**
Are you interested in running submicron particles in your SRL or need to troubleshoot a difficult assay? We will discuss current methods employed at our institutions for the analysis and sorting of small particles. Topics will include; best practices, protocol optimization, instrument set up and controls, and general troubleshooting. Examples of tech notes, protocols, and surveys will be made available.

GLIIFCA CONFERENCE COFFEE BREAKS ARE SUPPORTED BY

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Let the Possibilities Flow...

GLIIFCA 31 Presentation Abstracts

Regulation of Foamy Macrophage Differentiation and Survival in Atherosclerosis

Jesse W. Williams (University of Minnesota Medical School)

Atherosclerotic plaque build-up in the arteries contributes to cardiovascular disease through restricted blood flow, vascular stiffening, and plaque rupture which can lead to myocardial infarction or stroke. Plaque formation is driven by the continued expansion of macrophages in the artery intima where they contribute to plaque pathophysiology. We sought to determine molecular mechanisms that regulate monocyte and macrophage contributions to foamy macrophage formation in lesions. Using scRNA-seq and macrophage fate-mapping approaches, we expand our understanding of macrophage heterogeneity within atherosclerotic lesions. We defined multiple macrophage subsets including inflammatory, foamy, and intima-resident in nascent and diseased arteries. Furthermore, using trajectory analysis, we predicted genes associated with foamy macrophage differentiation during progressing disease. Candidate genes were compared against a genome wide functional CRISPR inhibition screen for foamy macrophage formation, identifying new putative regulators, including Trem2. Loss of Trem2 led to reduced cholesterol uptake by macrophages in vitro and Trem2-deficient macrophages were less adept to form foamy macrophages when competed against WT macrophages. Macrophage-specific deletion of Trem2 resulted in a dramatic attenuation of atherosclerotic plaque progression in a mouse model. Mechanistically, we find Trem2-deficient foamy macrophages induce an ER stress response through CHOP and sXBP1 following treatment with cholesterol, which contributes to increased susceptibility to cell death and reduced proliferation. Overall, our study describes new complexity to macrophage heterogeneity within atherosclerotic lesions and identifies Trem2 as a putative target for therapeutic intervention to reduce atherosclerotic plaque burden.

Uniting spatial transcriptomics with fluorescent tissue cytometry to define neighborhoods of health and disease within the kidney

Ricardo Melo Ferreira (Indiana University School of Medicine)

The kidney is a complex organ with a diverse array of epithelial, endothelial, immune, and stromal cells, all arranged in close proximity. Acute kidney injury (AKI) and chronic kidney disease (CKD) disrupt the delicate balance held between the nephron's functional units. Single cell sequencing provide resolution to injury transcriptomic signature, but the lack of spatial context prevents the understanding of cell-cell communication and the definition of the environments involved in disease. Conversely, spatial transcriptomics and multiplexed immunofluorescence can provide deep transcriptomic and proteomic neighborhoods of cells in their spatial context in health and disease. Murine injury of the kidney are provided as an example of identification and colocalization between immune and injured epithelial cells. We then describe, in human kidney biopsies, the process of aligning injury neighborhoods between multiplexed 3DTC and ST in the analytical space. To that goal, we define cell neighborhoods associated with injury and map them onto histlogical context. Finally, an use case is provided which illustrates the mapping of injury signals within the papilla of individuals with and without nephrolithiasis. At the conclusion of this discussion, it is expected that individuals will have a greater understanding of how to link neighborhoods defined by tissue cytrometry and spatial transcriptomics in the analytical space and by direct co-registration.

2022 Carleton and Sigrid Stewart Lecture

Maladaptive Pleural Environment and Cancer Progression

Vera Donnenberg and Albert D. Donnenberg (University of Pittsburgh School of Medicine)

The pleural environment is a unique cavitary environment with its own pathobiology. Perturbation of the pleural environment by infection, cardiac insufficiency or malignancy, drives pleural polarization toward inflammation and wound healing, in a process that can become self-sustaining. This maladaptive environment is responsible for the dire prognosis associated with malignant pleural effusions. Even in benign pleural effusions associated with cardiac insufficiency, effusions often persist after cardiac function improves. Malignant pleural effusions (MPE) are the most serious, devastating and understudied sequalae of metastatic cancer. Even in cancers that are initially responsive to therapy, MPE are uniformly fatal and preceded by significantly diminished quality of life by dyspnea and discomfort. Malignant pleural effusions are common and occur in a wide variety of cancers, with a US incidence of more than 150,000 cases per year and a life expectancy measured in months. A 2020 study estimated that MPE account for more than 1.6 billion dollars per year in hospitalization charges alone. Despite significant clinical progress in immuno-oncology, there has been almost no change in survival or quality of life for patients with MPE.

Over the past decade, we have examined the cellular contents and secretomic profiles of malignant pleural effusions, benign effusions and normal physiological pleural fluids to characterize their pleural environments. and demonstrated the presence of immunocompetent polyfunctional pleural T cells. Normal non-effusate pleural fluid is replete with resting effector and memory T cells, B cells and macrophages, and dominated by IL-6Rα, CCL2, CXCL10, FGF2, TGFβ1 and CCL22. Effector cytokines (IFNα, IFNγ, CCL3, TNFα and TNFβ) and most stimulatory cytokines (GM-CSF, TGFα, G-CSF, IL-2, IL-5, IL-7, IL-9, IL-12p40, IL-12p70, IL-3) were absent in NPF.

Benign effusions, whether due to cardiac insufficiency or chronic inflammation (asbestosis without malignancy) resulted in a profound secretomic change, with statistically significant increases in IL-6, TGFβ1, GRO, IL-10 and IL-8, and decreases in FGF2 and IL-15.

All cytokines and chemokines present at elevated levels in benign effusions were also elevated in malignant effusions, with statistically significant increases in G-CSF, CX3CL1, GM-CSF, IFNγ, IL-1TNFα, IL1Rα, CCL4, VEGF, TNFβ, EGF, IFNα, IL-4 and IL-12p40, compared to benign pleural effusions. Effector cytokines exceeded the upper 95th percentile of benign effusions in a proportion of MPE samples: IFNα (94%), IFNγ (72%), CCL3/MIP1α (11%), TNFα (44%), TNFβ (21%). Paired analysis of pleural and plasma cytokine levels revealed significantly higher levels of CCL2/MCP1, CXCL10/IP10, IL-6 and IL-7 in MPE. Further we have provided evidence that immunocompetent pleural T cells are present in all MPE, even in cancers that are considered immunologically cold such as breast cancer. However, their effector capacity is neutralized by a maladaptive regulatory pleural environment that is dominated by IL-6/sIL-6Rα, promoting the epithelial to mesenchymal transition (EMT) and aggressive tumor behavior. CD4-/CD8- (double negative) regulatory T cells, NK cells and high side scatter pleural B cells also contribute to the immunosuppressive environment through constitutive IL-10 secretion. Upon ex vivo activation, pleural T cells from MPE are potently cytotoxic against autologous tumor. A very similar cavitary environment is associated with peritoneal ascites, but not peritoneal malignancies without ascites.

Taken together, our data suggest that therapeutic modulation of the pleural and peritoneal secretomes is essential to unmasking local anti-tumor responses. We envision treating the pleural and peritoneal cavities as bioreactors, utilizing immunotherapeutic drugs and ex vivo activated cavitary T cells. Once anti-tumor responses are established locally, they will propagate systemically through the draining lymphatics and peripheral circulation. Given the similarities between the pleural and peritoneal secretomes the conditioning strategies maybe applicable to all cancers metastatic to the pleura and peritoneum as well as to pleural conditioning for cardiovascular diseases.

THROUGHPUT BOTTLENECKS IN IMAGE-ACTIVATED SORTING

AMAR BASU (WAYNE STATE UNIVERSITY)

Imaging flow cytometry (IFC) and image-activated cell sorting (IACS) represent the next generation of cell sorting technology. IACS promises the ability to sort cells and objects by morphology, spatial profiles in gene expression, nucleic acid localization, and more. Even in its relative infancy, IACS systems have demonstrated numerous modalities including brightfield, fluorescence, phase imaging, frequency modulated imaging, and coded aperture. Beyond imaging, the design of an IACS system must consider multiple potential bottlenecks that may limit the overall throughput, including a) computational image processing, b) variable compute latency, and c) physical sorting speed. This talk introduces our lab's efforts at tackling these issues using graphical processing units (GPUs) and piezoelectric jet sorting. We will also discuss methods to make IACS more accessible by utilizing off the shelf GPU modules, and through rapid prototyping of microfluidic flow cells using high resolution resin 3D printers.

IDENTIFICATION OF T CELL SUBTYPES THAT CONTRIBUTE TO INTESTINAL INFLAMMATION

MATT OLSON (PURDUE UNIVERSITY)

CD4 T cells play a dual role in both suppressing and contributing to inflammation and disease. This is particularly true in the intestines where CD4 T cells promote immune homeostasis and proper gut function by dampening responses to the microbiota or food antigens. However, when these cells become dysregulated or mistargeted, they can produce high levels of inflammatory mediators that lead to intestinal damage and disease. My laboratory is interested in understanding how specific inflammatory signals and transcription factor networks within gut T cells regulate this balance between immunosuppression and intestinal inflammation. In our current work, we describe a novel population of intestinal CD4 T cells that produce the serine protease granzyme A (GrA) within the steady state intestine. These steady state GrA+ T cells are generated in a microbiota-dependent manner and, in turn, directly interact with and regulate intestinal microbes. In settings of disease, GrA-producing CD4 T cells become spatially and functionally dysregulated where they become major contributors to intestinal pathology during graft-versus-host disease and colitis. Intriguingly, GrA+ T cells are functionally and phenotypically distinct from other CD4 T helper cell subtypes (i.e. Th1, Th17) and require unique inflammatory signaling pathways and transcriptional machinery for their differentiation. We are currently using this knowledge to develop therapies that specifically target GrA-producing T cells to suppress intestinal inflammation.

IMMUNE AND IONIC MECHANISMS MEDIATING THE EFFECT OF DEXAMETHASONE IN SEVERE COVID-19

AMEET A CHIMOTE (UNIVERSITY OF CINCINNATI)

Severe COVID-19 is characterized by cytokine storm, which is an excessive production of proinflammatory cytokines that contributes to acute lung damage and death. Dexamethasone is a corticosteroid routinely used to treat patients with severe COVID-19 and has been shown to reduce mortality in these patients. However, the mechanisms underlying the beneficial effects of dexamethasone are poorly understood. Using the NanoString platform, we conducted transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) from COVID-19 patients with mild disease, and patients with severe COVID-19 with and without dexamethasone treatment. Dexamethasone treatment of severe COVID-19 patients inhibited pro-inflammatory and immune exhaustion pathways, circulating cytotoxic and Th1 cells, interferon (IFN) signaling and genes involved in cytokine storm (IL-1, IL-6, and IFN-g), which were altered in severe compared to mild disease. Correlation network analysis revealed that Ca^{2+} transport and signaling is associated with the effect of dexamethasone. Ca^{2+} influx is regulated by Kv1.3 potassium channels, but their role in COVID-19 disease pathogenesis remains elusive. An increase in Kv1.3 mRNA was observed in the PBMCs of severe COVID-19 patients, which was significantly reduced in the dexamethasone-treated group. In agreement with

these findings, in vitro treatment of healthy donor PBMCs with dexamethasone reduced Kv1.3 protein abundance in T cells and CD56^{dim}NK cells. Furthermore, functional studies showed that dexamethasone treatment of T cells significantly reduced Kv1.3 activity and IFN-g production. Our findings suggest that dexamethasone attenuates inflammatory cytokine release via Kv1.3 suppression, and this mechanism contributes to dexamethasone-mediated immunosuppression in severe COVID-19.

MAXIMIZING THE MINIMUM: BEST PRACTICES FOR LOW CELL NUMBER LC-MS METABOLOMICS

SAM WEINBERG (NORTHWESTERN UNIVERSITY)

Cellular metabolism plays a critical role in modulating cell fate and function. In the last decade, significant progress has been made in identifying the specific metabolic pathways and functional mechanisms by which metabolism dictates biological outcomes, largely due to advances in liquid chromatography coupled to mass spectrometry (LC-MS) based metabolomic approaches. However, despite the power of this approach, its use has been limited by a requirement for large cell numbers isolated primarily from *in vitro* systems. Thus, the metabolic underpinnings of rare immune cell function has remained unexplored. New methods to address this gap have combined droplet-based cell sorting with LC-MS, but those studies are limited due to cellular stress provoked by sorting and inflexible workflows. Microfluidic sorting technology represents a potential solution to these problems by reducing cell stress and maintaining the *in vivo* cellular metabolic state during isolation. New workflows incorporating microfluidic sorting into LC-MS-based metabolomic methods provide a route forward for further investigating cell metabolism in physiologic contexts.

DISSECTING GRANULOCYTE PHENOTYPIC PLASTICITY USING INTEGRATED APPROACHES

SERGEJS BERDNIKOVS (NORTHWESTERN UNIVERSITY))

Eosinophils and neutrophils are key effector cells and primary targets for therapeutic intervention in inflammatory diseases, especially type 2 mediated diseases in allergy and asthma. New evidence suggests that there are different subsets of these cells, which may dictate their differential function in health versus inflammation. The canonical eosinophil development from lineage-committed CD117(+)CD34(+)IL-5Rα(+) progenitors in bone marrow or by extramedullary *in situ* hematopoiesis may not be the only mechanism of establishing pathogenic eosinophils in the tissues. Through single cell RNA-seq and lineage tracing approaches, we show evidence that in mice Lin(+)Siglec-F(-)Ly6g(+)IL-5Rα(+) bone marrow and lung precursor neutrophils retain an overlooked multipotent potential to differentiate to cells of other myeloid lineages such as eosinophils and monocytes. Likewise, we found evidence of a CD66b(+)CD16(+)CCR3(+) subset of human blood neutrophils to support eosinophil trans-differentiation. Integrating single cell sequencing, flow cytometry characterization, lineage tracing, tissue proteomics and metabolomics, we demonstrate that tissue extracellular matrix and metabolic reprogramming are key drivers of plasticity and determinants of granulocyte phenotype. Identification of tissue neutrophil precursor plasticity is new and potentially paradigm-changing in our understanding of heterogeneous granulocyte subsets in diverse inflammatory disease scenarios.

AUTOMATED FLOW CYTOMETRY AND MACHINE LEARNING FOR PREDICTION OF SMALL MOLECULE HUMAN SAFETY RISK: THE 3RND PLATFORM

ANDREW A. BIEBERICH (ASEDASCIENCES, INC.)

Flow cytometry with large data sets is often, in practice, discussed in the context of large numbers of clinical subjects. This data type comes with certain assumptions about control types and gating, sometimes with practical limitations on sample sizes. AsedaSciences has developed an automated flow cytometry screen that predicts human safety risk for small molecules such as early hit-to-lead pharmaceutical candidates, ag/industrial compounds, etc. In this case, the cells are held constant by stringent QC practices, while the test compounds take the place of clinical subjects as the individual nodes within a data network. Gating is avoided entirely through conversion of raw flow cytometry signals into distance metrics relative to controls. Public domain pharmaceuticals (and other chemicals) enabled the creation of a 300-compound training set with which to train a supervised machine learning classifier. The classifier is now routinely used to provide safety risk estimates for customers' proprietary compounds, and a customer-facing suite of web tools, the 3RnD® Platform, enables exploration and interpretation of the results. We will present the basic workings of the screen/algorithm/platform and describe interesting findings regarding how such screens must be monitored to detect performance deviations

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GLIIFCA 31 FLASH TALK ABSTRACTS

CORE MANAGEMENT

1. HOT FLOW: ATTUNE PLUMBING FOR RADIOACTIVE SAMPLES

KATHRYN FOX, DAGNA SHEERAR, KYLE CHRISTIE, ALEX HENKEL, LAUREN NETTENSTROM, OFELIA LAPACEK, ZACH STENERSON, ASHLEY WEICHMANN, JUSTIN JEFFREY, JAMEY WEICHERT, MANISH PATANKAR

UNIVERSITY OF WISCONSIN – MADISON

Flow cytometry with radio-labeled samples generates waste that is both a biological and radiological hazard. While samples can be cryopreserved until radiolabels have decayed (Carlson et al, PMC8313010), simple modifications can be made to the waste output(s) of the cytometer to reduce risks and allow for more timely analysis. Here we illustrate the modifications we made to a ThermoFisher Attune NxT cytometer and CytKick autosampler to route liquid waste to a shielded carboy and provide a link to the full parts list and instructions for the modifications. Before making the cytometer available for researchers, we worked with our institution's Office of Radiation Safety to test residual radiation levels in the instrument and the liquid waste. Through this testing we determined that a Sanitize SIP function followed by a Quick Deep Clean brought radiation levels in the waste output down to background levels and also greatly reduced the residual radiation reading of the Sample Injection Port. For permit purposes, the cytometer is housed in the Small Animal Imaging and Radiotherapy Facility where experienced staff can oversee periodic radiation testing and waste disposal. Fresh waste carboys are pre-loaded with concentrated bleach and placed within a shielded container. The waste station sits on a scale, labeled with a "full" weight so the fill level can be assessed without removing shielding or putting unnecessary strain on connection points during repeated checks. Once full, carboys are sealed with a solid cap and moved to a separate shielded storage location. When practical, carboys are stored for at least 10.5 half-lives, surveyed to confirm emissions have reached background, and liquid is rinsed down the drain. For longer-lived isotopes, full carboys are picked up by the Office of Radiation Safety. Future work will involve finding a bleach substitute to use if any researchers need to run samples labeled with radioiodine isotopes; bleach is not appropriate because the oxidation could volatilize the radioiodine. This new ability to do "hot flow" opens up the opportunity for Carbone Cancer Center researchers to do experiments that would otherwise not be allowed at UW.

2. TROUBLESHOOTERS WANTED: FLOWREMEDY DIAGNOSTIC CHART FOR CYTOMETER USERS IN THE SHARED RESOURCE LABORATORY

JENNIFER JAKUBOWSKI, DEREK D. JONES, AND JONNI S. MOORE

UNIVERSITY OF PENNSYLVANIA

Instrument operation and training are the bread and butter for a shared resource laboratory (SRL), where supporting instrument users is paramount. In order to promote a more user-enabled SRL environment, communication between the SRL staff and its large pool of users is key. A considerable portion of SRL staff time is spent on assisting users with analyzer problems over the phone or through email. In our large facility (37+ instruments), many flow cytometers are often at nearby satellite locations, requiring additional staff time for offsite travel support. Overall, most issues are easily avoidable or could be remedied by the user directly with the proper guidance. In an effort to extend our instrument user support services, we have developed and implemented a troubleshooting flowchart (FlowRemedy) to aid SRL users in resolving basic

analyzer issues. Phone calls, emails, and our internal software messaging system were monitored for SRL user analyzer issues over the course of 6 weeks to identify the most frequent user issues. Each specific problem was categorized and quantified for all analyzers (n=21). Troubleshooting flowcharts addressing the most common analyzer issues were then created and designated as FlowRemedy. FlowRemedy was displayed in all rooms with analyzers, email blasted to cytometer users (n=433), and posted on our department's web and social media page. All the above measures were monitored again for the same duration of time to determine the impact of FlowRemedy as a resource communication tool. After FlowRemedy implementation, there was a significant decrease in phone calls, emails, and internal software messages regarding analyzer issues. Correspondingly, users were able to run and finish their experiments with minimal interruptions within and between different user sessions. These findings suggest that FlowRemedy implementation served as an effective reference tool for user adoption when encountering basic analyzer issues.

3. Testing Aerosol Containment of Flow Cytometry Cell Sorters in a Shared Resource Laboratory

Abbey M. McKee, **Alyssa Sproles**, Celine S. Lages, and Sherry Thornton

Cincinnati Children's Hospital Medical Center

Under the normal operation of a flow cytometer, aerosols (3 to 200μm in size) are created and can become dangerous if an infectious agent is present. Recommendations from the International Society for Advancement of Cytometry include testing the aerosol management system (AMS) of each instrument's biosafety cabinet after every change to the cell sorter and repeated every 1 to 3 months. The most prominent published AMS testing protocol, Glo-Germ, utilizes Dragon Green fluorescent beads. Our derivations from the Glo-Germ protocol have allowed for a more efficient image acquisition, and increased precision during analysis. Our aerosol management testing protocol can identify declining performance of our various cell sorters' biosafety cabinet's AMS that otherwise, would have gone undetected. Repetition in testing every 3 months and after biosafety hood certifications, has proven critical to ensure the safety of our cell sorter operators and our clients.

Research

4. Novel Application of Fluorescent Flow Cytometry for Assaying Aggregation of Human Disease Proteins such as Transthyretin

Jane E. Dorweiler, Claire M. Radtke, Anita L. Manogaran

Marquette University

Protein aggregation is associated with several age-related human diseases, such as Alzheimer's disease and Amyloidosis. Yet, analysis of protein aggregation has been limited to biochemical assays of cell populations, and subtle variations have been difficult to detect. A recently developed reporter system in yeast can assess the aggregation state of a specific protein in cells using a fluorescent output. Here, we have modified this yeast reporter system to monitor protein aggregation of the human transthyretin (TTR) protein, associated with the fatal disease transthyretin Amyloidosis. The TTR gene is fused to a transcriptional activation domain, which when expressed as a native protein, can activate the transcription of a fluorescent reporter gene. In contrast, when the TTR protein is aggregated, there is no activation of the fluorescent reporter. We present details of this yeast-based system as well as how this technique can distinguish subtle variation in protein aggregation.

5. Establishing reference ranges for flow cytometry tests

Nagako Akeno, Samuel Chiang, Rebecca Marsh

Cincinnati Children's Hospital Medical Center

Accurate reference ranges are crucial in clinical lab settings for discriminating abnormal sample results. A number of biological factors such as ethnicity, age and sex could affect results and must be considered during validation. We describe our experience establishing two flow based test reference ranges. First a SAP and XIAP expression panel without any noted biases and second a T cell phenotyping panel showing skewed naïve/memory cells with age. When needed, age brackets can be estimated by developmental stages, statistically or visually. Each bracket is then be evaluated for Gaussian distribution which determines the statistic used for limits calculation. For SAP and XIAP with no age bias an overall bracket using the 2.5th and 97.5th percentile was chosen. The T cell panel required multiple age brackets with limits also determined by percentiles. Though tedious, careful evaluation and stratification of reference range data is important for reporting valid clinical test results.

6. Lymphocyte Function Evaluation Using a Flow Cytometry-based Proliferation Assay

Li Yang, Sam Chiang, Rebecca Marsh

Cincinnati Children's Hospital Medical Center

Many SCID and immunodeficient syndromes manifest defective cell proliferation and can be conventionally diagnosed by the 3H thymidine test. Here we compare two alternate rapid and sensitive flow-based assays. Flow cytometry-based cell proliferation in activated whole blood (FASCIA) directly stimulates whole blood cells, skipping tedious PBMC isolation. Because patient plasma is not replaced, circulating drugs in the sample can affect cellular function. Lymphopenic samples could also show reduced counts. This accurately reflects in vivo conditions but sometimes difficult to correlate to the thymidine results. To overcome these shortcomings, isolated PBMC with a tracking dye can be used. Comparing the two flow-based results to the thymidine incorporation assay found excellent agreement in identifying abnormal response in patients. We conclude that flow cytometry-based cell proliferation tests are viable alternatives to the standard 3H thymidine incorporation assay.

2022 Tech Talk Abstracts

The Exhibitor Tech Talks will take place Friday evening, September 16th and Saturday morning, September 17th. These sessions are intended to provide our exhibitors, a forum to present new technology, instrumentation, etc. Each presentation lasts approximately 8 minutes.

BD Biosciences

The Future of Discovery: The BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology

Shibabrata Mukherjee (shibabrata.mukherjee@bd.com)

The BD FACSDiscover™ S8 Spectral Cell Sorter with BD CellView™ Image Technology was revealed at CYTO this year. The BD CellView™ Image Technology, featured on the January cover of Science, is a novel high-speed cell imaging technology that empowers scientists to answer previously out of reach biological questions by amplifying the power of cell sorting and analysis through real-time integration of image and flow cytometry data. In this tech talk, Dr. Shibabrata Mukherjee will discuss how the BD CellView™ Technology works, possible applications and biological questions it could resolve. We invite you to join the discussion and imagine where this high-speed imaging technology could take your research.

Miltenyi Biotec

Sort the Impossible with MACSQuant Tyto

Mark Barnes (markba@miltenyi.com)

MACSQuant Tyto couples safety and ease of use for both novice and experienced users. In addition to typical sort applications for both research and clinical uses that allow for the recovery of viable, functional cells, the Tyto is also well suited for optimizing difficult sort applications. Here we will review sort applications that have been historically challenging including iPSC sorting, neuronal cell sorting, and nuclei sorting.

Curiox Biosystems

Cell Sample Prep - A Variable That Challenges Standardization

Melvin Lye (melvin@curiox.com)

Limited innovation in automated cell and organelle sample preparation methodology limits the effectiveness of modern analytical methods, such as single-cell 'omics, flow and mass cytometry. These techniques traditionally rely on manual centrifugation-based protocols for cell washing and suspension preparation, hampering researchers' access to the reproducibility and scalability benefits of automation.

We have developed a suite of cell suspension preparation systems that enable automation of cell washing protocols. Laminar Wash™ technologies robustly, gently, and efficiently remove debris, dead cells, and unbound reagent using laminar flow and liquid handling robotics, rather than turbulent methods. To evaluate performance, murine and humanized mouse peripheral blood mononuclear cells

and tumor infiltrating lymphocytes were prepared and immunostained for flow cytometry analysis. Workflow improvements were assessed, as well as data quality by flow cytometry isolating unique cell types.

AGILENT TECHNOLOGIES

ADVANCED DETECTOR TECHNOLOGY IN AGILENT NOVOCYTE FLOW CYTOMETERS

Garret Guenther (garret.guenther@agilent.com)

There are four types of detectors used in flow cytometers, photodiodes (PD), avalanche photodiodes (APD), photomultiplier tubes (PMT), and the newly integrated silicon photomultipliers (SiPM). Previously used in PET scanners and LiDAR, SiPMs are solid-state, silicon-substrate-based, photon-level-sensitive semiconductor devices, with a 7.2 log dynamic range. Consisting of a compact array of APDs operating in unison, the SiPM is a compact detector with photon counting capability. The innovative optics designed into the Agilent NovoCyte Advanteon, NovoCyte Quanteon, and NovoCyte Penteon flow cytometers incorporate up to 30 independent SiPM detectors, which collect and process signals for each of the fluorescence channels. This technical overview describes the novel SiPM detector incorporated for use in flow cytometry and highlights examples where optimal signal resolution is essential for acquiring quality data.

BIOLEGEND

SPARK AND FIRE DYES: COVERING THE SPECTRUM FROM ULTRAVIOLET TO INFRARED

Kim Cardenas (kcardenas@biolegend.com)

Development of advanced flow cytometers has driven the necessity for a broader variety of fluorochromes with unique spectral properties to maximize multiplexing capabilities. BioLegend has been at the forefront of developing new fluorochrome-conjugated antibodies to meet this demand to expand researchers' capabilities for high-parameter cell analysis. Here, we will discuss BioLegend's new Spark™ and Fire™ dye-conjugated antibodies and their uses in spectral flow cytometry.

NANOSTRING TECHNOLOGIES

PIONEER THE NEW FRONTIER SPATIAL BIOLOGY: HIGH PLEX SPATIAL MULTIOMICS TO SINGLE-CELL SPATIAL IMAGING

Seth Meyers (smeyers@nanostring.com)

Discover what's new in spatial biology. Get the latest updates in NanoString's spatial technologies and take innovation to a new level. Explore the powerful GeoMx Digital Spatial Profiler (DSP) the brand new CosMx Spatial Molecular Imager (SMI). GeoMx DSP ignited the spatial biology revolution and is the leading spatial profiling technology offering high-multiplexed, high-sensitivity measurement of protein and RNA nucleic acids. CosMx SMI takes spatial biology one level deeper and enables you to profile RNA and protein at the single-cell and subcellular level. What novel discoveries can you make from spatial biology?

Beckman Coulter

The CytoFLEX That Sorts

Justin Meyers (JLMEYERS@beckman.com)

The CytoFLEX SRT Cell Sorter is a benchtop sorter capable of meeting requirements for a wide range of sorting needs. Like the CytoFLEX Platform, it includes innovative technologies that simplify instrument setup and operation, empowering investigators to focus on research questions. The CytoFLEX SRT Violet-Blue-Yellow Green-Red (V-B-Y-R) Series has 15 fluorescent detectors when fully activated and can be purchased with as few as five with an option to activate additional lasers and detectors with an activation key. It is capable of complex sort logic with different combinations of sort settings on each of the four streams, including the ability to catch aborts of one of the other streams. An optional Biosafety Cabinet including aerosol evacuation or a standalone Aerosol Evacuation Unit is available.

Standard BioTools

Breaking Barriers: Introducing Eight 40-plus Marker Panels for Deep Immune Profiling by Mass Cytometry

Vinicius Motta (vinicius.motta@standardbio.com)

The Maxpar® Direct™ Immune Profiling Assay™ is a dry-format, 30-marker antibody panel designed as a simple, single-tube workflow for immune profiling of human PBMC and blood. The assay is customizable with 18-plus open channels available for panel expansion, and the recent addition of ready-to-go drop-in panels means that eight 40-plus marker panels are now available for deep profiling of all major immune cell types including cytokine and other activation marker expression. Mass cytometry doesn't require single staining controls or compensation, and antibody panels are easy to build, modify and optimize. In the seminar, I will present detailed information on these pre-designed Expansion Panel kits for the Maxpar Direct Immune Profiling Assay and discuss the results of using a 47-parameter panel to investigate cell activation using 9 cytokines and a barcoding approach, where unstimulated and stimulated cells were stained in a single tube using the Maxpar Direct Immune Profiling Assay.

Slingshot Bio

Go Beyond Blood - Synthetic Cells to Advance Data Quality and Reproducibility in Flow Cytometry Assays

Sean Daley (sean.daley@slingshotbio.com)

Synthetic biology and artificial cell technology have advanced by leaps and bounds over the past decade, and this emerging technology is addressing long-standing needs in biology research, pharmaceutical development, and clinical science. One outcome of this innovation is the development of synthetic cells as ideal cellular mimic controls for many applications in cell analysis, especially for flow cytometry. At Slingshot, we have developed the FlowCytes platform technology. FlowCytes are granular, semi-transparent synthetic cells with an optical, biochemical, and physical equivalent to that of living human cells. This cutting-edge technology is being applied to solve the needs of flow cytometry research of the future such as spectral analysis, quantitative numeration, and solutions for rare cell phenotypes.

PARTICLE METRIX

IMPROVEMENTS IN FLUORESCENCE-NTA: RELIABLE CHARACTERIZATION OF EXTRACELLULAR VESICLES

Sven Kreutel (kreutel@particle-metrix.com)

During the last decade, Nanoparticle Tracking Analysis (NTA) has emerged as a vital and fast characterization technology for Extracellular Vesicles (EVs), including Exosomes and Microvesicles in the size range from 30 nm to 1 µm. While classic NTA scatter operation feeds back the size and total particle concentration, the user typically cannot discriminate whether the particle is a vesicle, protein aggregate, cellular trash or an inorganic precipitate. However, fluorescence detection enables the user to gain specific biochemical information for phenotyping of all kinds of biological nanoparticles. Here, we report on the latest improvements in size resolution and multi-channel fluorescence colocalization studies of standard material as well as selected biological vesicles and viruses.

GLIIFCA Digital Poster Abstracts

Core Management

1. A Growing Need to Develop Protocols for Running Extracellular Vesicles on Various Core Facility Instruments

Sarah Croswell, Alyssa Sproles, Celine S. Lages, and Sherry Thornton

Cincinnati Children's Hospital and Medical Center and University of Cincinnati

Cores are often approached for assistance with running and developing novel experiments. Work with extracellular vesicles (EVs) using flow cytometry has increased in popularity as we can also study markers on them. We tested several instruments available in our core for those read-outs by using beads in a variety of size ranges. The BD LSRFortessa can detect 200nm beads but requires extensive cleaning and careful adjustment of the instrument settings. The Cytek 5-laser Aurora makes detecting 100nm beads possible by using SSC off the violet laser and requires less cleaning. However, there's concern that a large portion of EVs is still being missed. The ImageStream has the potential of detecting and visualizing even smaller particles creating interest in using it for the detection of EVs and a need by the core to assist in developing a protocol for running EVs on this system.

2. Lowering the Sample Signal as an Alternative to Extrapolating Data Points

Jason Kish

Bangs Labs

We explored a technique for more accurate quantification of high cell surface expression. A standard curve is generated using a Quantum MESF kit, containing one unlabeled bead population, and a series of five fluorescent bead populations labeled with varying amounts of FITC. Some investigations are more sensitive to the quantitation limits within a standard curve. The covered fluorescence range and size are ideal for a commonly investigated cell's size and expression level, and are not optimized for smaller particles like exosomes. Many scientists are only left with extrapolation as a way to overcome this limitation. We focused on upper limit extrapolation and explored the feasibility of a technique that may provide more relevant values. This was done by exposing antibody binding beads to mixtures of FITC-labeled and unlabeled antibody at various ratios. The technique is expected to lower the overall signal intensity, and generate a value within the confines of the standard curve.

3. Optimizing User-Staff Communication in a Large Multi-Site Shared Resource Laboratory

William Murphy, Derek Jones, Jonni Moore

University of Pennsylvania

Introduction: The Penn Cytomics Shared Resource Lab is one of the largest academic cytometry labs in the world, with over 9 satellites, 37 instruments and serving over 1000 users. Maintaining timely and useful interactions with our users is critical to assuring investigators have the technology access they require to

perform successful studies. This can include real-time digital communications (ex. Slack, instant messaging, text messaging), instrument desktop access to troubleshooting, messaging capabilities to staff online, seminars, live meetings). Our goal was to identify the preferred and most effective communication tools for each task and to create a comprehensive communication plan for Penn Cytomics. The overall aim is to identify processes to support the efficient operation of a large, shared resource lab in a cost-effective manner. This plan will become a cornerstone for all business, technical and scientific processes to best establish and maintain easy and effective communications with our users at close and distant sites, and to integrate this plan with our daily operations.

Methods: There are 3 primary steps to implementing a communication plan into our operation: design, develop, apply. Each part has multiple steps. The information reported here focuses on the design aspect. To understand how users best receive information, we conducted a survey, via Survey Monkey, of 431 users who had been logged into our system over the last 60 days. We asked their opinions about how we are communicating with them currently, what works and what doesn't, and some possible new approaches that could be more effective. We will expand this information by doing live interviews with selected responders.

Results and Conclusions: Using this data as well as user interviews, our next step is to design and develop a plan and processes that will be easy to implement and test it, integrated into in our daily operations. This user-facility communication plan will serve as the basis for enhancing interactions with our large and disperse client base and to identify processes that not only can support direct communication with users, but also enhance our workflow by identifications of instrument and user issues in a more timely manner.

4. Instrument settings and panel design considerations for spectral experiments on the Bigfoot cell sorter

Kenneth Quayle, Celine S. Lages, Sherry Thornton

Cincinnati Children's Hospital

The Bigfoot is a jet-in-air sorter capable of spectral unmixing of data from up to 60 PMT detectors. A standardized approach to detector gain settings is crucial for experimental setup. The vendor's workflow involves setting the MFI of unstained cells to the same value in all detectors. Using this approach, loss of resolution in dim populations is observed even when panels are well-designed from a theoretical perspective and populations are observable on other instruments.

In this study, we characterize noise in each PMT and investigate 3 different approaches to setting spectral PMT voltages: A) vendor's workflow; B) 2-peak bead separation adjustment set during instrument QC; and C) rSD of unstained cells set to 2.5x the rSD of noise. We propose a ranking for the "brightness" of fluors that takes into account each detector's background noise and demonstrates how this value, indicated as the Bigfoot quality index (BQI), can improve high-parameter panel design for the instrument.

5. A Novel Method for Antibody Labeling Using Proteintech FlexAble Kits

Afrida Rahman-Enyart, Michael Metterlein, Larisa Yurlova, Hui Luo, Xinxing Wang, Longtao Wu, Jacob Culver, Andrea Buchfellner, Yanghua Lian, Felix Hartlepp, Deepa Shankar

Proteintech

Multiplex assays are powerful tools for the simultaneous detection of multiple antigens in a single sample using several different antibodies. One multiplex assay can generate a detailed snapshot of a complex biological system and/or a host response. However, multiplexing experiments can become lengthy, complicated, and expensive when attempting to incubate samples with multiple primary and secondary antibodies. Labeling primary antibodies eliminates the time required for secondary antibody incubation,

resulting in quicker and simpler workflows. Direct antibody labeling also reduces background from secondary antibodies and allows for the use of same species antibodies without cross reactivity, generating cleaner results. Proteintech has recently launched FlexAble, a novel antibody labeling kit that uses a high-affinity, non-covalent FlexLinker to conjugate fluorochromes, enzymes, and molecules in any buffer condition. What makes FlexAble unique is that it is compatible with primary antibodies from any supplier, regardless of antibody concentration and formulation. Additionally, labeled antibodies are ready to use within 10 minutes after following a 2-step protocol and up to 50 different antibodies can be labeled with one standard kit. Precious antibodies are not wasted with FlexAble since as little as 0.5µg of antibody can be labeled with no buffer exchange required. FlexAble kits have been validated for immunofluorescence, flow cytometry, and western blot workflows and are ideal for multiplexing with different and same species antibodies. Since FlexAble kits are available for labeling of rabbit IgG and mouse IgG1 antibodies with various conjugates, several targets can be analyzed in one sample, simplifying multiplex experiments. Additionally, FlexAble kits provide an easy way for same species multiplexing, as individually labeled antibodies can be utilized in the same experiment, regardless of isotype. Overall, the novel FlexAble technology allows for quick and high affinity labeling of antibodies for simplified and cost-effective multiplex workflows.

6. The Nx One: Offloading Shared Resource Laboratories (SRLs) Instrumentation By Offering User-Operated Routine Cell Sorting

David W Voehringer, Hany Nassef, Anand Kesavaraju, Karthik Balakrishnan

Nodexus Inc

Interest in single-cell analysis has seen a rapid increase in recent years within a variety of fields. Flow Cytometry and Fluorescence-Activated Cell Sorting (FACS) continue to be the industry standards for analytical determination of cell phenotype as well as sorting of cells for further analysis or generation of clonal populations; however, barriers for access as well as universal adoption of this technology still exist. Even for low-parameter, routine workflows (e.g. GFP+ single cell sorting into plates), high-end, overbooked sorters in SRLs are typically the only option. These types of tests are commonly 50-75% of runs an SRL sorter processes.

Here we present a commercialized platform, the Nodexus NX One, that is affordable, efficient, and generates results that match or improve on traditional flow cytometers/sorters for routine workflows. The NX One can be a powerful tool for both researchers to bring a “workhorse” cell sorter into their labs as well as SRL managers who wish to offload high-end infrastructure for the experiments that demand the full capabilities of their systems. The platform also introduces a new potential revenue stream for SRLs that do not currently offer user-operated cell sorting. The NX One also maximizes cell viability through low-pressure microfluidics and offers contamination-free operation through disposable cartridges.

Nodexus’ “one-click” NX-One hardware system provides a hands-free workflow to deliver enriched single cells of interest into collection tubes or microplates (up to and including 384 well compatible). In addition to “Plate Sort”, “Bulk Sort”, and “Analysis” capabilities, the NX One can also be used in “Liquid Dispensing” mode for automated pre- or post-filling of wells in a microplate with cell culture media or target reagents into the entire plate or specified wells. The instrument is fully integrated with on-board fluidics and comfortably fits in a standard tissue culture hood. No drop delay or alignment are needed, and the only external connection is through a power cable. The NX One is applicable for users that are looking for superior performance in delivery of single- or bulk-sorted cells with high cell viability from an automated, benchtop system while maintaining the lowest upfront as well as ongoing operational costs.

The NX One launches in Q4 2022. Please visit Nodexus.com to learn more or contact us at info@nodexus.com to join the waitlist!

RESEARCH

7. BUILD BIGGER BETTER MULTICOLOR FLOW CYTOMETRY PANELS WITH STARBRIGHT DYES

ANDREW COSGROVE, MICHAEL BLUNDELL

BIO-RAD, INC.

The recently launched fluorescent dyes from Bio-Rad, StarBright ("SB") Dyes, deliver tunable brightness and spectral properties, greater stability, improved lot-to-lot reproducibility, and spectral consistency. SB Dyes are bright, have narrow emission spectra, and resolve issues of signal resolution when constructing complex panels. Additionally, SB Dyes can be fixed in both PFA based and alcohol-based fixatives, with minimal spectral changes, and can be pre-mixed for up to 28 days.

We present our recently launched SB Violet and UV Dyes and a preview of our newest additions to the SB range, the SB Blue and SB Yellow Dyes in conventional and spectral flow cytometry. SB Blue Dyes are bright and allow an expansion of dyes to be used using the 488 laser, whereas SB Yellow Dyes are optimally excited by the 561 laser with reduced excitation from the 488 laser making large panel design using both the 488 and 561 lasers.

8. GENTLE AND EFFICIENT REMOVAL OF DEAD CELLS USING AKADEUM'S BACS™ MICROBUBBLE PLATFORM

JASON S ELLIS, EDWARD C GRIMLEY, JON ROUSSEY, NADIA PETLAKH-CO, CASEY WEGNER, AND BRANDON MCNAUGHTON

AKADEUM LIFE SCIENCES

Cell viability is vital for accurately studying cell behavior. Dead or damaged cells may be misidentified as viable or release products that interfere with the function of healthy cells, skewing downstream analysis. To address these challenges, Akadeum developed a Buoyancy Activated Cell Sorting (BACS) Microbubble approach to depleting dead cells from biological samples. BACS Microbubbles are gentle, quick, easy to use, requiring minimal sample manipulation, and not relying on extra equipment or consumables. Depletion of dead cells is achieved through selective capture of cells with exposed phosphatidylserine with Annexin V-conjugated BACS Microbubbles. Akadeum Dead Cell Removal Microbubbles capture dead cells and float them to the sample surface for removal, leaving behind healthy, untouched cells. Akadeum's Dead Cell Removal kit results in significant reduction in dead cells while providing high recovery of live cells, improving flow cytometry and single cell sequencing applications.

9. CHARACTERIZATION OF BIONANOPARTICLES WITH COLOCALIZATION-NTA (C-NTA)

CLEMENS HELMBRECHT, **SVEN KREUTEL**

PARTICLE METRIX, INC.

Nanoparticle Tracking Analysis (NTA) has been widely used for the quantification of size and concentration in the size range 50 – 1000 nm. When combined with fluorescence detection, F-NTA enables the user to perform bio-specific analysis based on fluorescent labelling. The new ZetaView® Colocalization-NTA technology from Particle Metrix now features the determination of biomarker colocalization scores of non-immobilized vesicles like EVs and viruses or phages and a variety of other bionanoparticles.

GLIIFCA 31 SPEAKERS

GLIIFCA 31 speaker information can be found on the GLIIFCA website:

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GLIIFCA RAFFLE PRIZES AND FIRST TIME ATTENDEE WECOME BAG ITEMS PROVIDED BY:

GLIIFCA 31 GENERAL INFORMATION

CONFERENCE REGISTRATION DESK

- The registration desk is located in the lobby of the Indianapolis Marriott East:
  - Friday, Sep. 16 – 5:00 pm to 8:00 pm
  - Saturday, Sep. 17 – 8:00 am to 10:00 am
- The conference registration fee includes all coffee breaks, the Friday Opening Reception, Saturday breakfast, lunch, Wine & Cheese reception, banquet, and Sunday breakfast.

POSTERS

- Digital posters can be found at **HTTPS://GLIIFCA.ORG/2022-DETAILED-PROGRAM-FOR-ATTENDEES/**

EXHIBITS

- Scheduled vendors will have booths in the Exhibit area **(Salons 1-5)**
- **Please Note: Exhibitor set-up for Salons 1-5 starting at 12 pm on Sept. 16 with formal opening of Salons 1-5 at 6:00 pm. Break down starting at 11:45 am on Sunday, Sept. 18**
- All activities other than the Core Managers' Workshops **(Salons A-C)**, Roundtable Luncheon **(Salons A-D)**, Steering Committee Meeting **(Salons A-C)** and Banquet **(Salons A-E)** will be located in the Exhibit area **(Salons 1-5)**
- **Please frequent the vendor booths, and show your appreciation for the generous financial support provided by the vendors who substantially help "pay the freight" for this meeting**

LOCATION OF ALL ORAL PRESENTATIONS (SAT/SUN): SALONS 6-8

BREAKFASTS

Free continental breakfast will be provided for all the registrants in the Exhibit area **(Salons 1-5)** on Saturday from 7:00 am to 8:00 am, and on Sunday from 8:00 am to 9:00 am.

COFFEE BREAKS

- The refreshments will be served in **Salons 1-5**:
    - Friday – 2:45 pm to 3:00 pm (Core Managers' Meeting; **Salons A-C**)
    - Saturday – 10:30 am to 11:00 am, and 2:45 pm to 3:15 pm
    - Sunday – 11:15 am to 11:45 am

EXHIBITOR TECH TALKS

- Tech Talk presentations, **Salons 6-8**
    - Friday – 7:05 pm to 8:05 pm
    - Saturday – 11:05 am to 11:45 am

SOCIAL ACTIVITIES

OPENING RECEPTION

- 6:00 pm to 9:00 pm, **Salons 1-5**
- Use 3 drink tickets for wine and beer (drink tickets can be used at either the Friday Reception, Saturday Happy Hour, or the Saturday Banquet)
- Any unused drink tickets can be donated to the Common GLIIFCA Drink Pool at the Opening Reception

SATURDAY LUNCH (11:45 AM TO 12:30 PM)

- Free box lunch; available in **Salon E**. Salons A-D will be available for seating, but there is no designated location for lunch.

ROUNDTABLES

- Join selected roundtable labeled with the title of discussion topic (Salons A-D). The attendance at each table is determined from the **sign-up sheet** at GLIIFCA registration desk and is limited to 10 registrants per table.

SATURDAY WINE & CHEESE HAPPY HOUR

- 5:30 pm to 6:30 pm in the Exhibit/Poster area **(Salons 1-5)** with cheese trays (you can use your drink tickets for beer and wine).

GLIIFCA BANQUET (THEME: LAB FAB)

- Free to registrants, available to paid guests.
- Commences at 7:30 pm **(Salons A-E**)
- Buffet style (salads, entrées, side dishes, and dessert).
- Full-service bar available. Use drink tickets for beer and wine, cash for mixed drinks.
- DJ with dance music 9:00 pm – 12:00 am; requests encouraged (get up and have fun!)
- Any unused drink tickets can be donated to the Common GLIIFCA Drink Pool at the GLIIFCA Banquet

DRINKS

- Full-service bar will be located in the Exhibit area **(Salons 1-5)** for the Friday opening reception and the Saturday Wine & Cheese reception, and also in **Salons A-E** for the banquet on Saturday evening
- Three free drink tickets per registrant ***for beer and wine only*** – beer in bottles/cans
- Mixed drinks – cash bar (your cost)
- All soda is free

FACILITIES/SERVICES

- Message Board: on easel next to the GLIIFCA Registration Desk
- Xerox copying, faxing, etc.: ask at the Registration Desk

OTHER INFORMATION

GLIIFCA WEBSITE

- `http://www.gliifca.org`

NAME TAGS AND EVALUATION FORMS:

- Remember! Before leaving, please fill out evaluation form and leave at Registration/Check Out Desk with your name tag

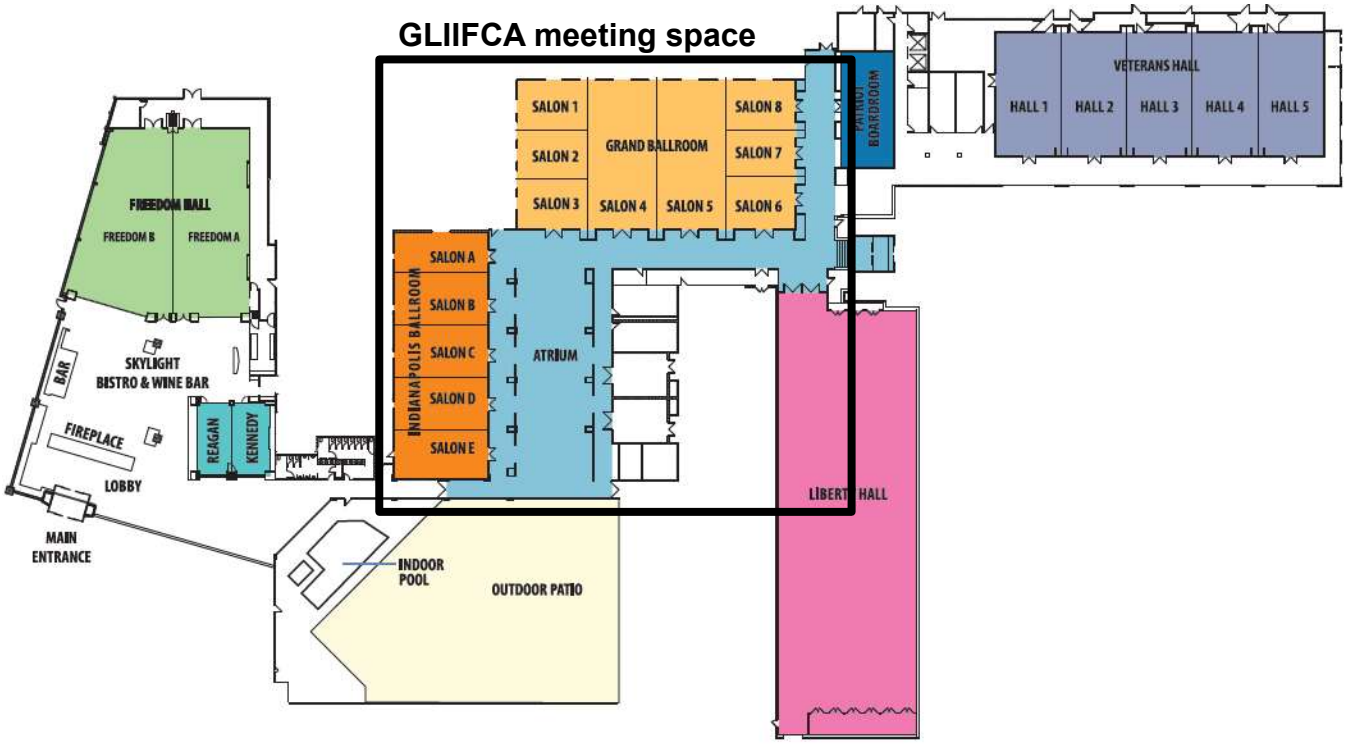
ADDITIONAL ENQUIRIES, COMMENTS, SUGGESTIONS

- Leave message at the GLIIFCA registration desk
- Comments and suggestions – e-mail Laura Johnston at president@gliifca.org

BOARD OF DIRECTORS BREAKFAST MEETING

- Sunday morning (8:00 am to 8:50 am) in **Salons A-C**

INDIANAPOLIS MARRIOTT EAST MAP



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