Great Lakes International Imaging and Flow Cytometry Association

TROY MARRIOTT

FRIDAY, SEPTEMBER 27 – SUNDAY, SEPTEMBER 29, 2019

Troy, Michigan

GLIIFCA 28

TABLE OF CONTENTS

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... GLIIFCA (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 28 SPONSORS

The GLIIFCA 28 meeting is kindly supported by:

- ACEA Biosciences – Support for Coffee Break
- Beckman-Coulter – Support for the Nanoscale Cytometry Session and Wine & Cheese Party
- BD – Support for High Dimensional Flow Cytometry Session, Poster Awards, and Travel Grants
- BenchSci – Support for Wine & Cheese Party
- BioLegend – Support for Travel Grants
- Bio-Rad
- Bio-techne – Support for Poster Awards
- ChemoMetec
- Cytek Biosciences – Support for Poster Awards
- Cytobank
- De Novo Software
- FlowJo – Support for Travel Awards
- Fluidigm – Support for High Dimensional Flow Cytometry Session
- Immudex
- Jackson ImmunoResearch – Support for Poster Awards
- Leinco – Support for Wine & Cheese Party
- Luminex – Support for Travel Awards
- Miltenyi Biotec
- Namocell
- Nanocellect
- NanoString
- Nexcelom Biosciences
- Particle Metrix
- Propel labs
- Sony – Support for Opening Reception
- Spherotech – Support for Coffee Break
- Sysmex
- Thermofisher – Support for Opening Reception
- Verity

FRIDAY

GLIIFCA 28 CORE FACILITY MANAGERS' WORKSHOP (DENNISON 1-2-3)

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 - 12:15 PM	*Opening Networking Luncheon: Welcome and Introductions*, **Matt Cochran, Sally Quataert, Dagna Sheerar, Vicki Smith, Ann Marie DesLauriers-Cox**, GLIIFCA Organizing Committee
12:15 PM – 12:45 PM	*Branding Your Core Facility Services: How Marketing Support Can Help*, **Dagna Sheerar**, Manager, UWCCC Flow Lab, University of Wisconsin—Madison
12:45 PM – 1:30 PM	*The Ins and Outs of Developing Proper Marketing Plans and How to Apply Them in a Core Facility*. **David Leclerc**, Technical Director, University of Chicago
1:30 PM – 2:30 PM	*Conflict Resolution*, **Jessica Back**, Wayne State University; **Ann Marie DesLauriers-Cox**, University of Michigan
2:30 PM – 3:00 PM	*Coffee and Refreshments*
3:00 PM – 4:00 PM	*Marketing and Public Relations in the Shared Service Laboratory in Response to Manufacturer Marketing Myths,* Group discussion led by organizing committee
4:00 PM – 4:45 PM	*Identification and Dissemination of Best Practices for Research Labs,* **Fabienne Lucas**, Brigham and Women's Hospital, Boston, MA; **Matt Cochran**, University of Rochester, Rochester, NY
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**

OPENING RECEPTION (COVINGTON BALLROOM)

The Opening Reception will take place in the **Salon ABCDE** from **6:00 PM** to **9:30 PM**. Come and interact with the Vendors and fellow GLIIFCA conference participants!

GLIIFCA CONFERENCE OPENING RECEPTION SPONSORED BY

SONY ThermoFisher SCIENTIFIC

INDUSTRIAL SCIENCE SYMPOSIUM (SALON FGH)

Session Chairs: Christiane Hassel (Indiana University), Karen Domenico (Cincinnati Children's Hospital), Louis King (Michigan State University), and Fabienne Lucas (Brigham & Women's Hospital)

6:30 PM - 6:35 PM	**Industrial Science Symposium Introduction**
6:35 PM - 6:50PM	*Tools for VHH (Nanobody) Discovery and Application*, **Dr. David Fancy**, Jackson ImmunoResearch.
6:50PM - 7:05PM	*Namocell: The Fastest Way to a Single Cell*, **Soohee Cho**, Namocell
7:05PM - 7:20PM	*NTA: a complement to Flow Cytometry*, **Dr. Ray K. Eby**, Particle Metrix, Inc.
7:20 PM – 7:35 PM	*TotalSeq™ Antibodies Standardized Antibody-Oligo Conjugates for CITE-Seq™, or Single-cell Proteogenomics*, **Dr. Nathan Lucas**, BioLegend
7:35 PM – 7:50 PM	*The Antibody Crisis: Leveraging machine learning for AI-assisted antibody selection*, **Dr. Maurice Shen**, BenchSci
7:50PM - 8:05PM	*The Maxpar Direct Immune Profiling Assay for CyTOF systems: 30 markers. 1 tube. 5-minute data analysis*, **Clare Rogers**, Fluidigm
8:05PM - 8:20 PM	*High Sensitivity Flow Cytometry Enables Extracellular Vesicle Immunophenotyping*. **Dr. Haley Pugsley**, Luminex Corporation
8:20PM - 8:35PM	*Multiple modalities of NanoString GeoMx™ Digital Spatial Profiler allow for spatially resolved, multiplexed quantification of protein and mRNA distribution and abundance*, **Seth Meyers**, NanoString Technologies
8:35PM - 8:50PM	*Using the CytoFLEX Flow Cytometer to Enable Single Particle Analysis of Extracellular Vesicles*, **Kimmer Graham**, Beckman Coulter

SATURDAY

Breakfast will be available in the Exhibit/Poster area in Salon ABCDE from 7:00 AM to 8:00 AM

ALL OF THE ORAL PRESENTATIONS WILL BE DELIVERED IN **SALON FGH**

8:00 AM - 8:15 AM	*Welcome Address*: **Rachael Sheridan** (GLIIFCA President)

SESSION 1: YOUNG IMMUNOLOGISTS (SALON FGH)

Session Chairs: Dagna Sheerar (University of Wisconsin)
Michael Clemente (Western Michigan University School of Medicine)

8:15 AM - 9:00 AM	*Regulating How T Cells Recognize and Respond to Antigen,* **Adam Courtney**, Asst. Prof. Department of Pharmacology, University of Michigan, Ann Arbor, MI
9:00 AM - 9:45 AM	*Aging of B-1a Cell Derived Natural Antibodies*, **Nichol Holodick**, Asst. Prof. Biomedical Sciences, Western Michigan University School of Medicine, Kalamazoo, MI
9:45 AM - 10:30 AM	*New Insights into B Lymphocyte Function in Immunological and Reproductive Health*, **Kang Chen**, Asst. Prof. Obstetrics and Gynecology, Biochemistry Microbiology and Immunology, Oncology, Wayne State University, Detroit, MI

10:30 AM - 11:00 AM	*Coffee Break* sponsored by ACEA Biosciences

THE 2019 CARLETON AND SIGRID STEWART KEYNOTE LECTURE (SALON FGH)

Introduction: Joe Tario (Roswell Park)

11:00 AM - 11:45 AM	*High Risk Glioblastoma Cells Revealed by Machine Learning and Single Cell Signaling Profiles*, **Jonathan Irish**, Asst. Prof. Cell and Developmental Biology, Vanderbilt University, Nashville, TN

LUNCHTIME ROUNDTABLE WORKSHOPS (DENNISON 1-4, NILES 1-2, AND ATHENS)

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

11:45 AM - 1:30 PM	*Multiple parallel roundtable workshops*.

SESSION 2: HIGH DIMENSIONAL FLOW CYTOMETRY (SALON FGH)

Session Chairs: David Leclerc (University of Chicago)
Laura Johnston (University of Chicago)

Sponsored by: BD Biosciences and Fluidigm

1:30 PM - 2:15 PM	*Deep Ultraviolet and Near Infrared: Extending High Dimensional Flow Cytometry with Novel Laser Wavelengths*, **William Telford**, Head, ETIB Flow Cytometry Facility, NIH Bethesda, MD
2:15 PM - 3:00 PM	*Dissecting the Tumor Micro-Environment using High Dimensional Spectral Cytometry*, **Ryan Duggan**, Senior Scientist Immuno-Oncology / Cytometry, AbbVie, Chicago, IL
3:00 PM– 3:45 PM	*Visualizing High Dimensional Diversity of Immune Cells in Health and Disease with Computational Analysis Tools,* **Anna Belkina**, (ISAC SRL Emerging Leader) Asst. Prof. Pathology and Laboratory Medicine, Boston University, Boston, MA
3:45 PM - 4:15 PM	*Coffee Break* sponsored by Spherotech

SESSION 3: CLINICAL AND TRANSLATIONAL CYTOMETRY (SALON FGH)

Session Chairs: Sherry Thornton (Cincinnati Children's Hospital)
Matthew Bernard (Michigan State University)

4:15 PM - 5:00 PM	*Endothelial Cells in the Genesis of Asthma,* **Kewal Asosingh**, Associate Professor, Department of Inflammation and Immunity, Cleveland Clinic Lerner Research Institute, Cleveland OH
5:00 PM - 5:45 PM	*Detecting Signature Patient Responses to Immuno-oncology Therapy Using High-dimensional Flow Cytometry*, **Derek Jones**, (ISAC SRL Emerging Leader) Technical Director Flow Cytometry Core, University of Pennsylvania, Philadelphia, PA
5:45 PM - 6:30 PM	*Implementation of the OGATA Score in Screening for Myelodysplastic Syndrome*, **Mike Keeney,** President ICCS, Associate Scientist, Lawson Health Research Centre, Coordinator Flow Cytometry, London Health Sciences Centre, London, ON

POSTER SESSION (SALON ABCDE)

6:30 PM - 8:00 PM	*Poster Session*
6:30 PM - 7:30 PM	*Wine and Cheese Happy Hour* sponsored by Beckman Coulter, BenchSci, and Leinco

THE WINE AND CHEESE HAPPY HOUR IS SPONSORED BY

SOCIAL ACTIVITIES (MEDITERRANEAN ROOM)

8:00 PM - 11:30 PM	GLIIFCA 28 Banquet
11:30 PM - 2:00 AM	Post-banquet Scientific Networking

SUNDAY

Breakfast will be available in the Exhibit/Poster area Salon ABCDE from 8:00 AM to 9:00 AM

STEERING COMMITTEE MEETING (DENNISON 1-2-3)

8:00 AM – 8:50 AM	GLIIFCA Steering Committee Breakfast

SESSION 4: NANOSCALE CYTOMETRY (SALON FGH)

Session Chairs: David Adams (University of Michigan)
Galina Petrova (Medical College of Wisconsin)

Sponsored by: Beckman Coulter

9:00 AM - 9:45 AM	*Flow Virometry: Flow Cytometry for Virologists*, **Vera Tang**, (ISAC SRL Emerging Leader), Manager Flow Cytometry Core Facility University of Ottawa, Ottawa, ON
9:45 AM - 10:30 AM	*Microparticles in Kidney Injury,* **Begoña Campos-Naciff**, University of Cincinnati Department of Internal Medicine-Nephrology
10:30 AM - 11:00 AM	*Coffee Break*

SESSION 5: IMAGING CYTOMETRY (SALON FGH)

Session Chairs: Kathryn Fox (University of Wisconsin)
Jessica Back (Wayne State University)

11:00 AM - 11:45 AM	*Automated Identification and Quantification of Signals in Multichannel Immunofluorescence Images,* **Jonathan Hall**, Research Data Technician, Haab Lab, Van Andel Research Institute
11:45 AM - 12:30 PM	*"Cytometry au naturel": Classifying cells by label-free Imaging Flow Cytometry and Advanced Computer Vision,* **Andrew Filby**, Cytometry Core Platform, Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne
12:30 PM - 12:35 PM	*Closing Remarks*

GLIIFCA 28 ROUNDTABLE LUNCH WORKSHOPS

***For those not attending a Roundtable Luncheon Workshop, boxed lunches will be available in a designated area in **Athens**.

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

Topic 1. Antibody Rigor and Reproducibility, *Maurice Shen, Head of Academic Relations, BenchSci*
Location: **Dennison I**

Antibodies play an essential role in every flow cytometry experiment. However, sourcing reliable antibodies and achieving reproducibility has been documented as a major challenge due to various factors. In this roundtable, we will discuss what is known about the "Antibody Crisis" and established antibody validation methods. We'll then open the discussion to the audience to share their experience on the best practices for antibody selection for flow experiments and discuss potential solutions to this issue.

Topic 2. Instrument Standardization: Who, What, When, Where, Why, & How, *Dagna Sheerar, Manager, UWCCC Flow Lab*
Location: **Dennison I**

A lively and interactive discussion concerning the importance of instrument standardization in the context of rigor and reproducibility, including, but not limited to; examples of how to incorporate standardization techniques into your Quality Control routines, experimental data acquisition, user training and education, and the importance of standardization in the use of computational/machine learning data analysis algorithms.

Topic 3. Minimal (measurable) Residual Disease - Steps to Implementation, *Michael Keeney, Associate Scientist, Lawson Health Research Institute, London, Ontario, Canada*
Location: **Dennison I**

As new drug therapies are increasingly relying on MRD as a surrogate endpoint for treatment effectiveness, demands on clinical labs to introduce methodologies that were once performed only in research or highly specialized laboratories are increasing. Using MRD as an example, this roundtable discussion will focus on appropriate instrument set up, sample handling, and statistical considerations needed for the successful implementation of clinical and translational research. Particular focus will be placed on rare event analysis. This roundtable will provide a forum for participants to discuss issues in the design and implementation of these techniques.

Topic 4. MiSet RFC Standards: defining a universal minimum set of standards required for reproducibility and rigor in research flow cytometry experiments, *Fabienne Lucas, Clinical Pathology Resident Brigham and Women's Hospital Boston/ Clinical Fellow in Pathology Harvard Medical School, ISAC Marylou Ingram Scholar 2019-2023*
Location: **Dennison 2**

Important aspects of instrumentation, experimental design, data analysis and data reporting to produce valid flow cytometry data are widely available in the literature. Comprehensive guides covering the principles, techniques and applications of flow cytometry used in specific biomedical fields have also been published. However, there is currently no consensus on the minimum set of standards that overall define a rigorous and robust flow cytometry experiment in translational biomedical research. MISet RFC Standards (Minimum Set

of Research Flow Cytometry Standards) is a new interdisciplinary initiative that will build on, integrate and simplify existing resources, with the mission to provide a single-resource broadly applicable guideline to increase the rigor and reproducibility of translational research flow cytometry experiments. Join this round table discussion to learn more about the aims and anticipated timeline of this project, and to discuss strategies for interdisciplinary implementation.

Topic 5. Cell Sorting: Setting Yourself Up for Success, *Lauren Nettenstrom, Instrumentation Technologist, UWCCC Flow Cytometry Laboratory*
Location: **Dennison 2**

We will discuss the myriad steps you need to take well before you get to the instrument to set yourself up for a successful sort. Topics will include knowing your sorter, biosafety considerations, optimal panel design, and tips for sample prep.

SECTION: HIGH-DIMENSIONAL FLOW CYTOMETRY

Topic 6. Considerations for High-Dimensional Panel Design, *Derek Jones, Technical Director for Research and Development, University of Pennsylvania*
Location: **Dennison 2**

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 7. Data Analysis Workflows for Multiparameter Cytometry, *Anna Belkina, Assistant Professor of Pathology and Laboratory Medicine, Associate Director, Flow Cytometry Core Facility, Boston University School of Medicine*
Location: **Dennison 3-4**

When we have our cytometry experiments accurately recorded into a dataset, we are only halfway done (and some people may add – halfway at best). During this discussion session, we will talk all things data analysis – from getting datasets sufficiently annotated to choosing data analysis strategies and tools and presenting the data story to showcase the narrative of the study. Our discussion will emphasize, but not be limited to, the challenges of algorithmic high-dimensional data analysis and best solutions to address the most common tasks that comprise flow, mass and/or genomic cytometry data comprehension.

Topic 8. Machine Learning, *Jonathan Irish, Assistant Professor Cell and Developmental Biology, Vanderbilt University*
Location: **Dennison 3-4**

TBA

SECTION: EDUCATION AND PROFESSIONAL DEVELOPMENT

Topic 9. Education in Flow and Image Cytometry, *Alexis Conway, Graduate Course Director/Coordinator and Flow Cytometry Specialist, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
Location: **Dennison 3-4**

This round table is for engaging ideas for cytometry education. We will discuss development of materials for graduate students and core facility users; as well as outreach efforts for educating people in flow and image cytometry. Cyto Youth and other cytometry education initiatives will be discussed.

Topic 10. Young GLIIFCA: What Can GLIIFCA And ISAC Do For My Career? *Jessica Back, Associate Director, Microscopy, Imaging, and Cytometry Resources Core, Karmanos Cancer Institute, Wayne State University*
Location: **Niles 1-2**

There are many opportunities for professional development available through GLIIFCA and ISAC, but you may not know what these programs are or how to access them. This Roundtable will discuss the volunteer opportunities and travel/poster awards available through GLIIFCA; ISAC leadership development programs; ISAC taskforces and committees; CytoU offerings and opportunities for participation; and the SCYM Exam. Find out how you can get involved and what GLIIFCA and ISAC can do for you.

SECTION: IMAGING FLOW CYTOMETRY

Topic 11. Imaging Flow Cytometry: Emerging Applications and Analysis Tools, *Robert Thacker, Luminex*
Location: **Niles 1-2**

Come join in on a discussion that will cover some of the latest applications, get your questions answered about current applications, and learn about the latest features and masks in IDEAS designed to enhance the depth of your data analysis.

SECTION: NANOSCALE CYTOMETRY

Topic 12. Analysis of Viruses and Virus-like Particles by Flow Cytometry, *Vera A. Tang, Operations Manager & Adjunct Professor, uOttawa Flow Cytometry & Virometry Core Facility, Ottawa, ON, Canada*
Location: **Athens**

Advances in the technology of cytometers now allow for some commercial instruments to detect biological particles (such as viruses and EVs) down to the 100 nm diameter range with minor to no modifications to default instrument configurations. For virologists, this opens a new area of study where they can now perform phenotypic analysis on viruses and virus-like particles on a single-particle level. However, since these particles are very close to the limit of detection for current cytometers, and cytometers differ in optical configurations, it is very important that methods and reference materials are used for standardized data reporting. For this session, we will cover the following topics:

- Reference materials for QC and standardization for small particle FCM
- Methods for standardized data reporting for small particle FCM
- Fluorescence and scatter calibration
- Sample preparation and controls for small particle FCM analysis
- Challenges to distinguishing viruses, virus-like particles, extracellular vesicles

GLIIFCA 28 Presentation Abstracts

Endothelial Cells in the Genesis of Asthma

Kewal Asosingh (Lerner Research Institute, Cleveland Clinic)

Historical studies reported angiogenesis in the airway walls of asthma patients more than a century ago. However, current understanding of an endothelial contribution to the pathogenesis of asthma is still in its infancy. Recent clinical studies showed a strong correlation between new blood vessel formation and asthma severity. Allergen challenge studies in patients showed that an angiogenic response precedes airway inflammation and bronchoconstriction. Preclinical models recapitulated these findings and provided mechanistic insights into how endothelial cells sense allergens followed by a pro-Th2 angiogenic response. Allergen sensing by endothelial cells is a paradigm shift in the innate response of allergens and initiation of atopic asthma.

Visualizing High Dimensional Diversity of Immune Cells in Health and Disease with Computational Analysis Tools

Anna Belkina (Boston University)

Advances in both cytometer capabilities and breadth of reagent availability have led to the expansion of large flow and mass cytometry panels; however, despite this newer ease in generation of high-parameter flow data, the proper extraction of results from larger panels is currently bottlenecked due the limitations of available analysis tools that cannot properly analyze such large datasets. Computational analysis is imperative for the proper investigation of high-dimensional flow and mass cytometry datasets. In this talk, I will discuss several recent developments that enable more efficient and comprehensive computational analysis and visualization of cytometry datasets, including opt-SNE, our optimized adaptation of a classical t-SNE algorithm that can accommodate mega-scale datasets typical for flow cytometry but beyond reach for the traditional t-SNE implementations. We will look at how flow and mass cytometry human immunophenotyping data are visualized with cutting edge tools like opt-SNE, UMAP, and EmbedSOM, and discuss the benefits of each approach. To demonstrate the potency of various newer and adapted computational approaches, an assembly of methods will be presented that our lab uses to characterize the inhibitory receptor landscape of various immune subsets in HIV individuals and to propose specific inhibitory receptor and activation marker signatures to be investigated as potential biomarker readouts in HIV and aging.

Microparticles in Kidney Injury

Begoña Campos-Naciff (University of Cincinnati)

Background

Up to 1-in-3 hospitalizations can result in acute kidney injury (AKI), and in its severe form, it is associated with 30 – 50% hospital mortality. Kidney epithelial cell damage, the major cause of acute kidney injury (AKI), can occur due to ischemic (H202) or nephrotoxic insults (TNFα). Microparticles (MP) are extracellular microvesicles (1000 nm) released from plasma membranes of several cell types in response to stress or injury. MP are unique with respect to representing the cellular origin, activation state (e.g., apoptosis, activation, proliferation), antigenic composition, and functional properties. MP can be identified by specific antibodies to cell markers. MP could act as mediators of microvascular dysfunction and immune

dysregulation. Experimental models show therapeutic promise when applied within each injury paradigm, but present diagnostic markers cannot discriminate cause-specificity. Different types of MP could be able to characterize the pathogenesis and mechanism of kidney injury. It is also plausible that depending on origin or phase of activation of the parent cell type; MP could mediate organ cross-talk in the setting of AKI.

Objective

The objectives of this study are: 1) Characterize MP released in response to injury as a specific inductor of AKI in a model of Renal Proximal Tubular Epithelial Cells, 2) Evaluated whether these MP can discriminate IRI or NT in murine models and 3) Evaluate type, quantity and biological activity of MP in setting of kidney injury.

Methods

1) We used in vitro models of injury to human immortalized Renal Proximal Tubular Epithelial Cell line (RPTEC). Exposures of H202 and TNF-α were compared to controls. The presence of CD10, CD13, and CD146 proteins on the RPTEC cells was evaluated by western blot and confocal microscopy. Flow cytometric analysis was used to detect the release of MP containing CD10, CD13, and CD146. An analysis of MP was performed using FlowJo software. MP levels were expressed as mean and standard deviations times 105 and compared by unpaired t-tests.

2) In C57BL/6 mice, IRI was performed by clamping both renal pedicles for 30 minutes, followed by reperfusion and NT was induced by injecting intraperitoneal (IP) Cisplatin. Sham mice underwent surgery without a clamp in IRI and IP normal saline in NT. Mice were euthanized at 48 hrs and examined by standard techniques for renal histology. Citrate poor plasma was collected at 0, 12, 48 hrs. Blood urea nitrogen (BUN), neutrophil gelatinase-associated lipocalin (NGAL); and MP containing CD10, CD13 and CD146 were measured using flow cytometry and analyzed by flow jo software. MP were expressed as 105/ml, and compared within each model over time, and across models by Chi-square tests.

3) In a prospectively collected biological repository of 38 eligible stroke subjects (14 ischemic and 24 hemorrhagic) and 37 controls. Demographic, comorbid, and laboratory variables were collected at the time of admission. Kidney injury (KI) was defined as either admission creatinine > 1.2 mg/dl or development of AKI. Comparisons were made across Group I (Stroke) (Ia = no KI; Ib = KI) and Group II (no Stroke) (IIa = no KI; IIb = KI) Flow Cytometric analysis measured MPs in plasma for CD146 (endothelial cells), CD10, and CD13 (renal proximal tubular epithelial/RPTE markers). FlowJo software evaluated MP, and levels were expressed as 107 and compared by the Wilcoxon test (two-sided p-values).

Results

1) Western blot and confocal microscopy, in controls and treated cells, confirmed the presence of CD10, CD13, and CD146 proteins on RPTEC. Under both oxidative stress (H2O2) and inflammatory stress (TNF-α) cells showed morphological changes associated with apoptosis. Compared to controls MP were significantly increased in H202 treated cells: CD10 (0.456 vs 9.842; p = 0.013), CD13 (3.190 vs 53.882; p = 0.0001) and CD146 (5.991 v142.474; p = 0.0018). TNF-α treated cells at both concentrations also released CD10, CD13, and CD146 MP; however, only CD13 MP were statistically higher than controls at 100 ng/ml of TNF-α (26.67 vs. 56.911; p 0.02). CD146 MP showed a trend at both concentrations of TNF-α.

2) AKI was confirmed in IRI and NT samples by histology, BUN and NGAL levels at 48 hrs. In IRI (48 hrs), CD10 and CD13 MP increased by 1.77 and 1.5-fold respectively, but CD146 declined by 1.7-fold from baseline. In contrast, NT model showed a decline of MP quantity at 48 hrs relative to baseline in CD10, CD13, and CD146 by 1.5, 1.5 and 3.6-fold respectively. When examined across the models, the proportion of MP released

at 48 hrs. in IRI was 64% CD10, 6% CD13, and 30% CD146. Interestingly NT model showed distinctly different proportions: 6% CD10, 18% CD13 and 76% CD146 quantitatively. ($p<0.0001$ for comparison across models).

3) For Group I and II, CD146 MP were 48.8 vs 39.3 ($p = 0.0026$); whereas CD 10 MP's were 8.4 vs 15.4 ($p = 0.001$); and CD13 MP's were 19.4 vs 20.6 ($p = 0.21$) For Group II, RPTE MP were higher in IIb vs IIa: CD 10 levels were 22.5 vs 14.0 ($p = 0.048$), and CD 13 were 23.4 vs 19.9 ($p = 0.023$). Within Group I, RTPE MP and endothelial M were not different by KI status. When KI status was compared across Groups I and II (Ib vs IIb), CD146 levels were 50.0 vs 36.9 ($p = 0.002$); CD 10 were 8.9 vs 22.5 ($p = 0.0274$); CD 13 were 19.3 vs 23.4 ($p = 0.013$).

Conclusions

1) This is the first report of the detection of microparticles (CD10 and CD13) specific to and derived from human RPTEC. Furthermore, we demonstrate a significant increase in the level of MP derived from renal cells that is specific to oxidative and inflammatory stress. Additionally, the release of CD146 MP by stressed RPTEC could serve as a ligand for endothelial activation, suggesting renal origins of organ cross-talk. Thus, MP expressed by RPTEC could serve as putative biomarkers for AKI, and once released, may mediate organ cross-talk.

2) Despite similar biochemical, histological, and biomarker parameters, MP quantity changed over time differently in each model. More importantly, the pattern of MP proportions almost switched across IRI and NT models, allowing discrimination of ischemic and toxic cell injury. MP measured in plasma can phenotype AKI based on cause-specificity and can lead to the development of novel diagnostic or therapeutic strategies.

3) We also confirm that endothelial MP increase in Stroke. In non-Stroke patients, RPTE MP are higher associated with KI; however, this difference is not significant in Stroke subjects. Interestingly, when KI status was compared across Stroke vs. Controls, RPTE MP's were significantly decreased in Stroke patients. This suggests an interplay between the upregulation of endothelial MP and the downregulation of RPTE MP in the setting of dual organ injury. This is one of the first studies to detect RPTE MP's in plasma of patients with vital organ dysfunction.

New Insights into B Lymphocyte Function in Immunological and Reproductive Health

Kang Chen (Wayne State University)

B lymphocytes are both regulators and effectors of adaptive immunity. Besides presenting antigens to T lymphocytes, B cells are responsible for producing antibodies. A diversified and properly selected repertoire of antibodies is crucial for effective immune defense and the prevention of autoimmune diseases. In addition, B cells can control inflammation by exerting regulatory functions on a myriad of other immune and non-immune cells. The recent work in the Chen lab has revealed new insights into the control of antibody-producing and immunoregulatory functions of B cells. Translation of this new information would promote reproductive and immunological health.

Regulating how T cells Recognize and Respond to Antigen

Adam Courtney (University of Michigan)

Immunotherapies exploit the remarkable capacity of T cells to detect malignant cells and unleash an anti-tumor response. To harness the full therapeutic potential of T cells it is imperative that we understand how they sense and respond to antigen. When antigen is bound by the T cell antigen receptor (TCR), this recognition event is converted into an intracellular signal by the Src family kinase Lck. Because Lck phosphorylates the TCR complex, its activity is critical for T cell activation. The phosphatase CD45 activates Lck by opposing the negative regulatory kinase Csk. We have found that inhibition of Csk can sensitize T cells to respond to weaker or less abundant antigen. By titrating the activities of both Csk and CD45, we show that because CD45 facilitates a regulatable pool of active Lck, while also suppressing TCR signaling, the activating and suppressive roles of CD45 are interconnected and necessary for antigen discrimination. CD45 suppresses signaling events that are antigen-independent, or induced by low affinity antigen, whereas those initiated by high affinity antigen are not suppressed. Our findings reveal CD45 acts as a signaling 'gatekeeper' because it is required for graded signaling outputs while filtering weak or spurious signaling events. Overall, our findings illustrate how regulation of active Lck sets a critical threshold for T cell activation and that T cell antigen discrimination can be altered by manipulating the Csk/CD45 axis.

Dissecting the Tumor Micro-Environment using High Dimensional Spectral Cytometry

Ryan Duggan (AbbVie, Inc.)

The Tumor Immune Microenvironment (TIME) is a complex structure representing many cell types, each having various cell states. Understanding and phenotyping this heterogeneity can lead to new target identification as well as assessing which treatment might work best for a given patient. Single cell technologies are the ideal tool to dissect and phenotype the vast array of cells in a given TIME. Namely, Mass-, Fluorescence-, and Oligo-Cytometry developments over the past few years have brought forth a new level of interrogation and illumination of the intricate balance of cell phenotypes within these solid tumors. Spectral Cytometry retains all the positive attributes of conventional flow cytometry – ease of use, high throughput, large catalog of ready-to-use reagents – while expanding the number of simultaneous measurements per cell possible. Using spectral cytometry has allowed us to look deeper into key subsets of immune cells while using reagents on-hand and already developed data analysis pipelines. Example data showing immune cell augmentation and enhanced efficacy in the MC38 syngeneic mouse tumor model as a result of the combination treatment of anti-PD-1 and venetoclax (Bcl2 small molecule inhibitor) will highlight the utility of high dimensional spectral cytometry.

"Cytometry au naturel": Classifying cells by label-free Imaging Flow Cytometry and Advanced Computer Vision

Andrew Filby (Newcastle University)

Conventional flow cytometry (CFC) remains the "gold standard" technology for rapid, multi-parameter phenotyping of cells in heterogeneous biological systems. However, it relies heavily on labeling defined cellular targets with analyte-tagged antibodies and/or reagents that, based on the resulting staining profile, allow us to partition cells into different phenotypes, functional classes and transition states. While such directed measurements are powerful the associated reagents are expensive, add extra steps to protocols, and may even induce confounding changes to cell biology as antibody binding can lead to the internalization of receptors and signal transduction that alter the cells perceived identity. CFC is also a so-called "zero

resolution technology in that it doesn't provide morphological or spatial information. As cell phenotype and function is often linked to structure, we have been exploring the potential of Imaging Flow Cytometry (IFC) in combination with advanced computer vision techniques to determine whether there is sufficient information present in the label-free image channels (bright-field and dark-field) to classify cells without the need for labelling. We have successfully used this approach to partition both mammalian and yeast cells into the different phases of the cell cycle and have recently embarked on a high throughput screen to collect both directed fluorescent and label-free images on a per cell basis for over 380 human CD markers. Using the targeted fluorescent "ground truth" to train advanced computer vision networks based on machine and deep learning we have so far successfully identified several key immune cell subsets. Our comprehensive screen of label-free features that correlate with and potentially substitute for the expression of various CD markers moves us toward a library of "label-free CDs" that could be exploited for rapid "bench to bedside" diagnostic testing using a smaller, simpler imaging flow system.

Automated Identification and Quantification of Signals in Multichannel Immunofluorescence Images

Jonathan Hall (Van Andel Research Institute)

Multimarker fluorescence analysis of tissue specimens offers the opportunity to probe the expression levels and locations of multiple markers in a single sample. Software is needed to fully capitalize on the advantages of this technology for sensitive, quantitative, and multiplexed data collection. A major challenge has been the automated identification and quantification of signals. We report on the software SignalFinder, which meets that need. SignalFinder uses a newly developed algorithm called Segment-Fit Thresholding, which showed robust performance for automated signal identification in side-by-side comparisons with several current methods. Two utilities provided with SignalFinder enable downstream analyses. The first allows the quantification and mapping of relationships between an unlimited number of markers through user-defined sequences of AND, OR, and NOT operators. The second produces composite pictures of the signals or colocalization analysis on brightfield hematoxylin and eosin images, which is useful for understanding the morphologies and locations of cells meeting specific marker criteria. SignalFinder enables high-throughput, rigorous analyses of whole-slide, multimarker data, and it promises to open new possibilities in many research and clinical applications.

Aging of B-1a Cell Derived Natural Antibodies

Nichol Holodick (Western Michigan University Homer Stryker M.D. School of Medicine)

Streptococcus pneumoniae is the most common cause of pneumonia, which claims the lives of people over the age of 65 seven times more frequently than those aged 5-49. B-1a cells provide immediate and essential protection from *S. pneumoniae* through production of natural immunoglobulin, which is germline-like due to minimal insertion of N-region additions. We have previously demonstrated B-1a derived phosphorylcholine (PC)-specific and total IgM moves away from the germline with age as a result of selection. Consequently, the protective capacity of natural serum IgM against *S. pneumoniae* infection is significantly decreased in aged mice. Anti-phosphatidylcholine (PtC) antibodies, like anti-PC antibodies, contain few N-additions, and have been shown to be essential in protection from bacterial sepsis. Herein, we demonstrate the number of N-additions in PtC-specific B-1a cell antibodies do not change with age. Furthermore, the average percent of B-1a cells binding PtC-liposomes (13-15%) did not change in aged mice. Therefore, in aged mice the majority of natural IgM derives from non-PtC binding B-1a cells producing antibody that has moved away from the germline. In direct contrast, the PtC-binding B-1a cell population preserves its germline status. These results suggest a difference in selection pressures and/or self-renewal upon B cells expressing different specificities.

Elucidating the role of different B-1a cell progenitors and cues influencing B-1a cell circulation will be interesting and necessary lines of investigation to further understand how diversification and selection affect the maintenance of B-1a cells and the efficacy of the natural IgM they produce with increasing age.

2019 Carleton and Sigrid Stewart Lecture

High Risk Glioblastoma Cells Revealed by Machine Learning and Single Cell Signaling Profiles

Jonathan Irish (Vanderbilt University)

The goal of this study was to identify abnormal glioblastoma cells that stratify patient clinical risk using phospho-specific snapshot proteomics and single cell analysis of human tumors. A key data analysis goal was to go beyond cataloging cell subsets or revealing a heterogeneous phenotypic landscape by pinpointing specific tumor cell subsets that were closely linked to patient outcomes. To reveal signatures of phospho-protein signaling and abnormal co-expression of cell identity proteins, we created Risk Assessment Population IDentification (RAPID), a machine learning algorithm that uses single cell data to identify cells associated with differential clinical risk of death or other continuous variables. RAPID is unsupervised for both cell identification and patient clinical outcomes and uses Marker Enrichment Modeling (MEM) to quantify enriched features and label cytotypes. We applied RAPID to single cell mass cytometry data measuring 34 different phospho-proteins, transcription factors, and cell identity proteins in tumor tissue resected from patients bearing IDH wild-type glioblastomas. RAPID identified and characterized multiple biologically distinct tumor cell subsets that independently and continuously stratified patient outcome. Prognostic cells included glioblastoma cells with abnormal signaling and co-expression of stem cell and differentiated neural cell features. To facilitate clinical translation, the risk-stratifying cell signatures revealed here were validated using traditional cytometry analysis, as in standard clinical hematopathology, and are now undergoing validation with immunohistochemistry imaging, as in clinical neuropathology. Longer term, we believe RAPID will be broadly applicable for single cell studies where atypical cells may drive disease biology and treatment responses.

Detecting Signature Patient Responses to Immuno-Oncology Therapy Using High-Dimensional Flow Cytometry

Derek Jones (University of Pennsylvania)

Although many immunological observations have been made using conventional flow cytometry panels with 12-18 colors, new opportunities for discovery have arisen with the development of the next generation of high-parameter cytometers. In particular, the opportunity exists to perform immune monitoring and phenotyping at an unprecedented depth in patients undergoing active immunotherapy. Using highly optimized and standardized instrumentation, we developed a comprehensive 28-color T cell immunophenotyping panel for clinical studies, in collaboration with the Parker Institute for Cancer Immunotherapy. We focused initially on patients enrolled in an open-label, multicenter Phase 1b clinical study to evaluate the efficacy of a CD40 agonistic monoclonal antibody, administered with our without PD-1 blocking antibody, in patients with metastatic pancreatic adenocarcinoma. Using both existing and novel dimensionality reduction algorithms, we identified numerous previously unappreciated cellular populations and phenotypes at multiple time points during treatment. Furthermore, these populations in some cases corresponded directly with patient responsiveness, suggesting that the presence or absence of various cellular subsets could be used to predict patient outcome, prior to the onset of treatment. These data indicate that when paired with computational analytical approaches, high-dimensional flow cytometry can enable the

rapid and efficient identification of biomarkers of treatment response, and facilitate the isolation of cellular populations for further study in functional and genomic analyses.

Implementation of the OGATA score in screening for Myelodysplastic Syndrome

Michael Keeney (London Health Sciences Centre)

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell malignancies characterized by ineffective hematopoiesis in one or more cell lineages, peripheral cytopenias, and risk of transformation to acute myeloid leukemia. Traditional diagnosis is based on clinical history, peripheral blood counts, morphology and cytogenetics. Lower grade MDS may not show cytogenetic abnormalities and, by definition, have less than 5% blasts in the bone marrow leading to an increased reliance on morphology in approximately 50% of cases. However, there are many conditions that may lead to "dysplastic" changes in the bone marrow, including ethanol ingestion, antibiotic treatment and chemotherapeutic agents among others.

Flow cytometry provides a technology to identify aberrant marker expression on blood cells, which has led many groups to assess cell surface marker markers on myeloid and erythroid cells and their precursors, identifying changes in expected expression or intensity.

This presentation will review flow cytometric evaluation of MDS with a focus on a recently updated mini panel termed the OGATA Score and the experience of implementing it into the routine evaluation of unexplained cytopenias.

Flow Virometry: Flow Cytometry for Virologists

Vera Tang (University of Ottawa)

Recent developments in small particle flow cytometry have been driven by the burgeoning number of researchers interested in the study of extracellular vesicles. Several commercially available flow cytometers now have the ability to detect biological particles down to ~100nm diameter range. However, aside from extracellular vesicles, there are many submicron-sized particles that can be analyzed by flow cytometry including viruses, organelles, phytoplankton, and microplastics. This talk will focus on the use of flow cytometry to analyze viruses, delve into some of the challenges associated with this work, but more specifically, showcase how flow cytometry can give virologists new insight into the characterization of virus populations

Deep Ultraviolet and Near Infrared: Extending High Dimensional Flow Cytometry with Novel Laser Wavelengths

William Telford (NCI-National Institutes of Health)

High dimensional flow cytometry using traditional or spectral optics is about to exceed 30 simultaneous fluorescent parameters, with the mid-20s becoming routine. This capability has been achieved using a combination of five lasers (cyan 488, red 640, green 532 or yellow 561, violet 405 and UV 355 nm) that is now standard on high-end instruments. The visible spectrum has become almost as full as practically manageable using these excitation sources; to move beyond 30 colors, additional lasers and new fluorochromes will need to be employed. In this talk, we'll discuss recent advances in deep ultraviolet (DUV) lasers, with wavelengths below 320 nm. New fluorochromes are under development that can be excited at this formerly exotic wavelength range and should be compatible with existing fluorescent dyes. DUV lasers may therefore be the "sixth" laser wavelength that will enable analysis beyond 30 colors. We'll also go in the

other direction and discuss near infrared (NIR) lasers and the role they may play in adding additional analysis capabilities to high-dimensional flow cytometry.

THE GLIIFCA POSTER AWARDS ARE SUPPORTED BY

THE GLIIFCA TRAVEL AWARDS ARE SUPPORTED BY

GLIIFCA 28 POSTER ABSTRACTS

1. DEVELOPMENT OF BSTRONGXIMAB-9A (BSGX)-CY5.5 TO INTERROGATE TRA-1-60 STATUS IN PROSTATE CANCER

KATHERINE E. HEATH[1], JORDAN M. WHITE[2], MADISON N. WICKER[3], AGNES P. MALYSA[2], AND NERISSA T. VIOLA[2]

[1]BLOOMFIELD HILLS HIGH SCHOOL, BLOOMFIELD HILLS, MI, [2] KARMANOS CANCER INSTITUTE, DEPARTMENT OF ONCOLOGY, WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE, DETROIT, MI, [3]DEPARTMENT OF BIOLOGY, UNIVERSITY OF MICHIGAN – FLINT, FLINT, MI

Background:

Imaging technology for early relapse or recurrent prostate cancer is limited. TRA-1-60, a cell-surface antigen present on the transmembranous protein Podocalyxin (PODXL), has been characterized as a marker of pluripotency and as an aggressive phenotype in prostate cancer. A novel, therapeutic monoclonal IgG antibody, Bstrongximab-9A (BsgX, Curemeta, LLC) was developed to target TRA-1-60. Our goal was to develop an immunofluorescent imaging probe against TRA-1-60 by conjugating BsgX to the near-infrared emitting dye, Cy5.5-NHS Ester (BsgX-Cy5.5). We then compared the development of our BsgX-Cy5.5 to a commercially available anti-TRA-1-60 antibody conjugated with Alexa Fluor 488 (TRA-1-60-AF488) in vitro using confocal microscopy. We then used flow cytometry to examine our imaging probe in determining differential TRA-1-60 expression in high-expressing NCCIT and low-expressing PC-3, DU-145, and 22Rv1 cell lines.

Methods:

BsgX was conjugated to Cy5.5-NHS Ester (GE Healthcare). Briefly, BsgX was adjusted to pH = 9 with NaHCO3. After, various mole ratios (1:5, 1:7, and 1:10) of Cy5.5 were added and the reactions were incubated at room temperature for 1.5 h. Samples were then transferred to a 30 kDa MWCO spin column and spun 3 times at 3000 RPM for 15 minutes each to remove unbound Cy5.5. The concentration was checked at 280 nm using a NanoDrop UV-Vis spectrophotometer. anti-TRA-160-AF488 was purchased from Santa Cruz Biotech. The NCCIT embryonal carcinoma cell line (ATCC) served as a high TRA-1-60 expressing control. PC-3, DU-145, and 22Rv1 prostate cancer cell lines were used as low TRA-1-60 expressing controls. NCCIT, PC-3, DU-145 and 22Rv1 cells (2×104) were plated in a Millicell EZ 8-well microscope slide. After 24 h, the cells were washed with serum free media and incubated with either anti-TRA-160-AF488 (1:200), BsgX-Cy5.5 (1:100), or both in 1 mL of serum free media. Serum free media without fluorophore was added to the last well as a control. The cells were incubated for 1 h at 37 ˚C. Following incubation, the cells were fixed with 4% paraformaldehyde. The slides were mounted with prolong gold antifade with DAPI and imaged with a Zeiss LSM 780 confocal microscope at 63×. To compare BsgX-Cy5.5 to the commercially available anti-TRA-1-60-AF488 and the percent positive cells from the cell lines, 1×106 NCCIT, PC-3, DU-145, and 22Rv1 cells were plated in a 96 well plate and incubated for 45 min at 4 ˚C with anti-TRA-160-AF488, BsgX-Cy5.5, and efluor780 fixable viability dye. Fluorescence minus one (FMO) samples were incubated for 45 min at 4 ˚C with anti-TRA-1-60-AF488 or BsgX- Cy5.5 and the viability dye. Cells were examined on the BD LSRII flow

cytometer in the Microscopy, Imaging, and Cytometry Resources (MICR) Core. In vivo cell sorting of dissociated DU-145 tumors will be reported.

Results:

BsgX-Cy5.5 was conjugated successfully at a 1:5, 1:7, and 1:10 dilution. However, 1:7 was selected for the experiments for its clean results. In the NCCIT cell line, confocal microscopy results supported the expected binding of BsgX-Cy5.5 and anti-TRA-1-60-AF488 to TRA-1-60. In PC-3, DU-145 and 22Rv1, confocal microscopy results exhibited minimal binding of BsgX-Cy5.5 and anti-TRA-1-60-AF488.The flow cytometry results corroborated the confocal imaging with all cell lines. NCCIT cells exhibited the highest TRA-1-60 expression, 62.38% were stained double positive. PC-3, DU-145, and 22Rv1 all had significantly lower binding (3.31%, 3.09% and 0.11%, respectively).

Conclusion:

BsgX-Cy5.5 delineated high and low TRA-1-60 expression in the 4 cell lines in good agreement with the commercially available anti-TRA-1-60-AF488. BsgX-Cy5.5 will be useful for future studies including fluorescence activated cell sorting, from dissociated tumors, and in vivo studies including optical imaging.

2. Creating Better QC Tracking for the MoFlo Astrios Using R

Joshua L. Schipper, Rachael T.C. Sheridan

Van Andel Research Institute Flow Cytometry Core Facility, Grand Rapids, MI

While Summit software can create a CSV file containing the results from an Astrios QC run, the file itself is not easy to interpret visually, and Summit does not provide user-friendly methods for tracking QC results over time. I created a user-friendly program using R and the R package “Shiny” where the user can upload any number of desired QC data files generated from the software, and then output a human readable daily QC report highlighting any channels that failed QC, or a set of LJ plots for the tracking of CVs and PMT voltages for each channel. This program can handle a variety of laser and detector configurations without the need for a priori set up to increase ease of use across different instruments. GitHub link will be available at GLIIFCA.

3. Alternate Stream Calibration: A Method for Fine-Tuning Sorts

Steven Polter, Matthew Cochran, Timothy bushnell

Flow Cytometry Shared Resource Laboratory, University of Rochester Medical Center, Rochester, NY

By its nature modern Fluorescence Activated Cell Sorting is an operation layered with intricacy and nuance. With this complexity comes the opportunity to fine-tune the operating parameters impacting the output of the sorting process, including parameters that govern droplet formation.

The purpose of this investigation was to demonstrate that the modulation of droplet formation impacts the output of a given sort to improve cell yield and viability, as well as reducing collection volume, which is critical for downstream applications such as single-cell genomics.

Using the BD FACSAriaII, alternate stream calibrations were chosen to highlight the differences in output that can be achieved through selective modification of the instrument operating parameters. Sort recovery,

droplet volume, and total collection volume were measured using beads and compared to the standard sorting conditions to illustrate that sorting efficacy is not compromised by implementing Alternate Stream Calibration (ASC). Additionally, PBMCs were sorted using the chosen alternate calibrations to assess sort performance and viability of sorted cells. Results were compared between alternate and standard conditions. A recommended protocol for implementing ASC will be presented. All work conducted for the purpose of this study was supported by institutional funding from the University of Rochester Medical Center.

4. Developing a Model of the Relationship Between Autophagy and Cell Fate to Test Therapeutic Predictions for KRAS-driven Lung Cancer

Matthew D. Fountain, Katie R. Martin, Stephanie L. Celano, Jeffrey P. Mackeigan

Department of Obstetrics and Gynecology, Michigan State University College of Human Medicine, and Center for Cancer Cell Biology, Van Andel Research Institute, Grand Rapids, MI

KRAS, a GTPase that contributes to dysregulated cellular proliferation, is a feature of aggressive oncogenic mutation profiles in lung, colon, and pancreatic cancers. An important mechanism by which mutant KRAS signaling promotes cancer cell survival and tumor progression is through autophagy. Autophagy is a recycling process that cancer cells can leverage to withstand nutrient stressed tumor microenvironments during or after therapeutic treatment. Therefore, defining the dynamics between mutant KRAS signaling and autophagy could uncover molecular targets that disrupt this synergy, informing new therapeutic approaches for cancer. Here, we propose to develop a model to investigate the complex relationship between autophagy and cell fate in the context of KRAS-driven cancer. We will use a series of patient-derived tumor cell lines with varying KRAS mutations and flow cytometry to measure molecular signaling activation (MAPK, PI3K/AKT, and MTORC1 pathways) and tumor cell fate (i.e., cell death by TUNEL and proliferation by BrdU incorporation), as well as changes in autophagy. Time course experiments will be performed to temporally outline measurable inputs (molecular signaling readouts) relative to outputs (autophagy, survival, and death). The resulting datasets will then be integrated into a data-driven statistical model to identify meaningful correlations and aid our understanding of the role of autophagy in regulating cell survival and death signaling, and evaluate the therapeutic benefit of inhibiting autophagy in KRAS-driven lung cancer. In collaboration with Los Alamos National Laboratory, this project aims to make novel predictions regarding optimal autophagy inhibition, thereby accelerating the development of new cancer treatment strategies

5. High-throughput single cell deposition verification for monoclonality assurance using the Celigo Image Cytometer

Eric S. Zigon[1], Suzanne M. Purseglove[2], Vasilis Toxavidis[1], William Rice[2], John Tigges[1], and Leo Li-Ying Chan[2]

[1]Flow Cytometry Core, Beth Israel Deaconess Medical Center, Boston, MA
[2]Nexcelom Bioscience LLC, 360 Merrimack St. Building 9, Lawrence, MA

One of the major applications that is commonly performed on a fluorescence activated cell sorting (FACS) instrument is single cell sorting. Single cell sorting application is mainly used for research such as cell line development (CRISPR, RNAi) to ensure monoclonality for protein or antibody production. In addition, regenerative medicine often uses single cell sorting to study stem cell proliferation from single cell to single colony. Currently, single cell sorting is validated via light microscopy several days after initial sorting, where

the cells have grown into an observable colony. However, manual observation using microscopy is highly tedious and time-consuming. Therefore, there is a need for a high-throughput, practical, and accurate detection to validate and optimize single cell sorting of FACS. In this work, we demonstrate a novel high-throughput detection method to validate and optimize single cell sorting using the Celigo Image Cytometry (Nexcelom Bioscience, Lawrence, MA). The instrument was used to image the entire well of all 96 wells on a microplate to detect a single object sorted into the well in less than 4 min. Initially, the FACS (MoFlo Astrios EQ) was used to sort single green fluorescent bead into multiple 96-well microplates in two separate experiments. The microplates were used without any buffer; thus the number and location of beads can be accurately detected. Next, the results from the two experiments showing a sort efficiency of 82 and 90% were used to optimize the FACS to increase the efficiency closer to 100%. Finally, a practical experiment was performed where GFP expressing cells were sorted into multiple 96-well plates to determine the final efficiency of sorting actual cells. After imaging and analyzing the plate data, more cells can be sorted into wells without single cell. If more than one cell exists, then this allows the users to quickly disqualify those wells and minimize the reagents or time needed. The ability to rapidly detect single cell in multi-well microplates is highly important to both flow core laboratories to optimize their sorting instruments as well as to the users, who would like to confirm single cell in each well. The proposed method can highly improve the efficiency of workflow for both flow core managers and users.

2019 ISS ABSTRACTS

The Industrial Science Symposium will take place Friday evening, September 27th. This session is intended to provide our exhibitors, a forum to present new technology, instrumentation, etc. Each presentation lasts approximately 12 minutes.

JACKSON IMMUNORESEARCH

TOOLS FOR VHH (NANOBODY) DISCOVERY AND APPLICATION

Dr. David Fancy (dfancy@jacksonimmuno.com)

VHH antibodies (nanobodies), derived from Camelids such as Llamas and Alpacas, are rapidly being adopted in a variety of fields. Uses for these novel antibodies include various applications such as medical diagnostics, therapeutics, biosensors, crystallization partners, and detection of small molecules. Jackson ImmunoResearch has recently developed secondary antibodies that recognize Camelid antibody Fc and VHH domains with high specificity and affinity. These new tools, which come in a variety of conjugates, should aid researchers in developing and applying whole molecule or VHH domain Camelid antibodies in their fields of interest. This presentation will focus on the utility and performance of Jackson's Anti-Camelid antibodies with supporting data for Flow Cytometry, ICC, ELISA and Western Blot.

NAMOCELL

NAMOCELL: THE FASTEST WAY TO A SINGLE CELL

Soohee Cho (scho@namocell.com)

Namocell has combined the benefits of three key technologies: flow cytometry, microfluidics and liquid dispensing. The combination of these techniques is unique and enables users to accomplish single cell sorting and dispensing in one step, providing fast, efficient and gentle isolation of single cells.

Namocell Single Cell Dispensers are capable of isolating single cells in a high throughput manner that is still gentle on cells so that they can maintain their viability. It is easy to operate and requires no prior experience or training with flow cytometry.

Namocell's microfluidic cell cartridges ensure that there is no sample-to-sample cross contamination and keep the sample sterile. Gentle to the cell, effortless, compact, and cost-effective; it is designed to empower a variety of single cell applications including cell line development, CRISPR, monoclonal antibody development, and single cell genomics.

PARTICLE METRIX, INC.

NTA: A COMPLEMENT TO FLOW CYTOMETRY

Dr. Ray K. Eby (eby1@particle-metrix.com)

Nanoparticle Tracking Analysis captures the video-tracking of Brownian motion from sub-micron particles as they scatter laser light into a camera. NTA offers reliable measurement of nanoparticle size (10nm-1µm) and concentration and is routinely used on biological particles < 110 nm (EV, liposomes, viruses). Fluorescence-NTA measures a targeted group of labelled particles within a sample also measured for total concentration

with standard NTA. The ZetaView® NTA system uses automation and enhanced multi-point sampling for optimal concentration measures and fast sampling for low-bleach F-NTA. Choose from 2- or 4-laser systems (TWIN; QUATT) to provide maximum flexibility for F-NTA needs, ideal for core labs. Our multi-laser NTA systems uniquely measure EV biomarker populations within a single sample volume, increasing sample throughput while limiting the amount of precious EV sample required for analysis. Automated laser alignment and cut-off filter selection ensure rapid data collection.

BioLegend

TotalSeq™ Antibodies Standardized Antibody-Oligo Conjugates for CITE-Seq™, or Proteogenomics

Dr. Nathan Lucas (nlucas@biolegend.com)

High-throughput single cell RNA sequencing has transformed our understanding of complex cell populations and processes; however, it doesn't allow additional phenotypic analysis of the same cells. Researchers recently developed the method CITE-seq, which stands for the Cellular Indexing of Transcriptomes and Epitopes by sequencing. This method combines highly multiplexed protein detection with unbiased transcriptome profiling and is compatible with scRNA-seq platforms using a poly-A capture system. A collaboration between CITE-seq developers and BioLegend generated antibody-oligonucleotide conjugates under the brand name TotalSeq™. We demonstrate that our conjugates comply with BioLegend's quality standards, and the staining patterns are comparable with traditional flow cytometry. We also demonstrate the utility of CITE-seq using TotalSeq™ conjugates to improve cluster resolution as compared to samples analysed via RNA-seq alone and produce equivalent data when compared to CyTOF.

BenchSci

The Antibody Crisis: Leveraging Machine Learning for AI-Assisted Antibody Selection

Dr. Maurice Shen (maurice@benchsci.com)

The "reproducibility crisis" has generated much attention in the research community over the past years. While the issue is multifaceted at its core, rogue antibodies have been identified as one of the major culprits. It was estimated that 50% of commercial antibodies don't work as intended, and scientists often need to rely on published data to determine the suitability of antibodies.

To ensure scientists can find antibodies that have been proven to work repeatedly by peers, we developed an open access resource that uses a machine learning algorithm to screen the literature and identify which and how antibodies have been cited. The resulting peer-reviewed data are searchable by protein targets or product identifier and are filterable by experimental contexts as cited in papers, including technique, tissue, cell line, to help users pinpoint antibodies that have been published under experimental conditions matching their study interest.

FLUIDIGM

THE MAXPAR DIRECT IMMUNE PROFILING ASSAY FOR CYTOF SYSTEMS: 30 MARKERS. 1 TUBE. 5-MINUTE DATA ANALYSIS

Clare Rogers (clare.rogers@fluidigm.com)

The Maxpar® Direct™ Immune Profiling System is an innovative product for use on Helios™, a CyTOF® system, consisting of a 30-marker antibody panel dried in a single tube and 5-minute data analysis by Maxpar Pathsetter™ software. This system provides a sample-to-answer solution enabling enumeration of 37 immune cell populations from human PBMC and whole blood. In addition to details on workflow and software functionality, we'll present data on repeatability, precision, accuracy and site-to-site reproducibility obtained during product validation.

LUMINEX CORPORATION

SENSITIVITY FLOW CYTOMETRY ENABLES EXTRACELLULAR VESICLE IMMUNOPHENOTYPING

Dr. Haley Pugsley (hpugsley@luminexcorp.com)

The Amnis® CellStream® benchtop flow cytometry system from Luminex is a highly customizable and compact flow cytometer that is the first to use a camera for detection. Its unique optics system and design provide researchers with unparalleled sensitivity and flexibility when analyzing cells and submicron particles.

Only recently has the importance of extracellular vesicles as key mediators of intercellular communication been appreciated. Quantifying and characterizing extracellular vesicles in a reproducible and reliable manner has been difficult due to their small size (30 – 100 nm in diameter.)

Join us for this presentation to learn more about how the unique Amnis time delay integration and camera technology of the CellStream® System can enable immunophenotyping of extracellular vesicles

NANOSTRING TECHNOLOGIES

MULTIPLE MODALITIES OF NANOSTRING GEOMX™ DIGITAL SPATIAL PROFILER ALLOW FOR SPATIALLY RESOLVED, MULTIPLEXED QUANTIFICATION OF PROTEIN AND MRNA DISTRIBUTION AND ABUNDANCE

Seth Meyers (smeyers@nanostring.com)

Characterization of the spatial distribution and abundance of proteins and mRNAs with morphological context within tissues enables a better understanding of biological systems in many research areas, including immunooncology. However, it has proven difficult to perform such studies in a highly multiplexed manner. To address this, we have developed a novel optical-barcode based microscope and tissue-sampling platform designed to simultaneously analyze hundreds of proteins or mRNAs on a single FFPE section from distinct tissue spatial regions (GeoMx™ Digital Spatial Profiler, DSP). Using multiple modalities—Geometric, Segmentation, Contour, and Rare Cell—we spatially resolve protein and mRNA expression for over 30 immune targets on FFPE tissue sections from various organs, including colon and tonsil. We demonstrate multiplexed detection from discrete regions within a tumor (tumor center and immune invasive margin,) enabling systematic interrogation of immune activity in FFPE samples.

BECKMAN COULTER

USING THE CYTOFLEX FLOW CYTOMETER TO ENABLE SINGLE PARTICLE ANALYSIS OF EXTRACELLULAR VESICLES

Kimmer Graham (klghraham@beckman.com)

The ability to see very small particles including extracellular vesicles and viral particles has only recently been possible in flow cytometry due to advances in illumination and detection technologies not available in legacy instruments. We will briefly discuss how the CytoFLEX family of instruments allows researchers to see particles as small as 80nm using 405nm side scatter while simultaneously characterizing those particles.

GLIIFCA 28 SPEAKERS

KEWAL ASOSINGH, PHD

LERNER RESEARCH INSTITUTE – CLEVELAND CLINIC

Dr. Asosingh is a past ISAC Scholar, Chair of the Flow Cytometry Content Task Force and Associate Editor for the Cytometry Part A Journal. He has over 20 years of hands-on experience in flow cytometry. His initial training was in Clinical Flow Cytometry at the Academic Hospital of Brussels University. After his doctorate, he managed the Flow Cytometry Core in the Department of Hematology & Immunology. Dr. Asosingh has been teaching flow cytometry to undergraduate and graduate students, both in Brussels and the USA. Flow cytometry continues to be a vital tool in his research. Using cytometric techniques, his team has contributed to many basic, translational, and clinical research projects on numerous platforms. Currently, he is directing a flow cytometry-centric research laboratory investigating vascular mechanisms in lung diseases and serve as the Scientific Director of Flow Cytometry at the Cleveland Clinic Lerner Research Institute. Dr. Asosingh is also the director of two NIH-funded program project Cores on asthma models and an institutionally funded Core on pulmonary arterial hypertension models. He is also appointed as Associate Professor in Molecular Medicine at the Cleveland Clinic Lerner College of Medicine and was an ISAC Marylou Ingram Scholar (2011-2015.)

ANNA BELKINA, MD, PHD, ISAC SRL EMERGING LEADER

BOSTON UNIVERSITY

Dr. Belkina received her M.D. from Russian State Medical University and her Ph.D. in Molecular Medicine from BU School of Medicine investigating the epigenetic regulation of inflammatory responses driven by bromodomain proteins. Her breadth of knowledge of flow cytometric applications includes the development of intra- and extra-cellular staining protocols and panels for use in a wide variety of applications, including immunophenotyping of mouse and human cells, apoptosis assays, cell cycle profiling and stem cell isolation. At the FCCF, Dr. Belkina assists our users with experimental design, panel development, data analysis and many other facets of flow cytometry, and is a fully proficient BD FACSARIA operator. She also trains new users and is responsible for instrument maintenance.

Anna's research is focused on the intersection of immunology and computational biology, for her current research efforts include investigating the immune landscape of chronic inflammatory diseases and developing computational techniques to assess high-parameter single cell cytometry data. Anna is an active member of ISAC (International Society for the Advancement of Cytometry) and has been named 2015-2019 ISAC SRL Emerging Leader.

BEGOÑA CAMPOS-NACIFF, PHD

UNIVERSITY OF CINCINNATI

Dr. Campos received her PhD in Biochemistry from the National Polytechnic Institute School of Biological Science in Mexico. Her research at the University of Cincinnati College of Medicine has a strong focus on inflammation, oxidative stress, and endothelial dysfunction in the context of preeclampsia, dialysis vascular access dysfunction, stroke, and acute kidney injury. More recently Dr. Campos has been working in the evaluation of microparticles as a biomarkers of acute kidney injury, using in vitro and in vivo models.

KANG CHEN, PHD

WAYNE STATE UNIVERSITY

Dr. Chen studied in the National University of Singapore on a Singapore government scholarship. He initially majored in Computer Science and later graduated with First-Class Honors degree in biochemistry, studying tumor immunology and microbial pathogenesis. He did graduate research in immunology and microbial pathogenesis at Weill Cornell Medicine and Memorial Sloan-Kettering Cancer Center and postdoctoral research at Weill Cornell Medicine and Icahn School of Medicine at Mount Sinai. While in New York, he also worked with the United Nations Human Settlements Programme (UN-HABITAT) on initiatives of sustainable urbanization and public health and organized regular seminars to host world-renowned political, economic and scientific leaders in the UN Headquarters. He has been a member of the NIH/NIAID Mucosal Immunology Studies Team, the tumor biology and microenvironment program at Barbara Ann Karmanos Cancer Institute and the UN Committee on Human Settlements in consultative relationship with the UN Economic and Social Council (ECOSOC).

He has received many awards and authored many articles in top scientific journals. Currently, he is a Burroughs Wellcome Fund investigator in preterm birth, reviews manuscripts and grants for leading journals and funding agencies in the US, Europe and Asia, and co-chairs the Immunology Focus Group of Wayne State University, Barbara Ann Karmanos Cancer Institute and Henry Ford Health System.

ADAM COURTNEY, PHD

UNIVERSITY OF MICHIGAN

Dr. Courtney received his PhD in Biochemistry from the University of Wisconsin—Madison where he investigated how glycosylation can influence antigen recognition by B cells. Following his graduate studies, he investigated kinases involved in T cell signaling as a Robertson Foundation fellow of the Cancer Research Institute (CRI) at the University of California, San Francisco. These studies revealed critical regulatory features of the kinase Lck that influence antigen recognition by T cells. Dr. Courtney then joined the University of Michigan in 2019 as an Assistant Professor in the Department of Pharmacology. Currently, his research group aims to understand T cell signaling pathways important for antigen recognition and explore strategies to improve the capacity of T cells to target cancer.

RYAN DUGGAN

ABBVIE, INC.

Ryan Duggan is currently a Senior Scientist in Immuno-Oncology and team leader of the Flow Cytometry Core at AbbVie, Inc. in North Chicago, IL. Prior to this, he worked in the flow cytometry core facility at the University of Chicago for 16 years. His current interests revolve around using single-cell technologies to better understand the immune system's role in solid tumor cancers

ANDREW FILBY, PHD

NEWCASTLE UNIVERSITY

Dr. Filby graduated summa cum laude from the University of Huddersfield, achieving a 1st class honors in Biochemistry. He spent the third year of his undergraduate degree working for Syngenta in their central

toxicology laboratory developing a flow cytometry-based assay for measuring intracellular cytokine production. After graduating, he undertook a PhD at the National Institute for Medical Research (NIMR) in Mill Hill, London. He worked on the Src family kinases LCK and Fyn in adaptive immunity obtaining his PhD in molecular and cellular immunology in association with University College London (UCL). Dr Filby remained in the immunological field, working as a post-doctoral researcher in the laboratory of George Kassiotis, also at the NIMR, working on models of retroviral infection. He then worked for a short time in the commercial sector before taking up the deputy head role of the cytometry core at the London Research Institute (now the Francis Crick). Dr Filby is currently director of the Newcastle University Cytometry and Single Cell Core Technology Unit. He leads a dedicated team of cytometry specialists with the sole aim of developing and implementing comprehensive, cutting edge cytometry methods for the wider research community at Newcastle University and beyond. A significant part of his focus is the development of novel cytometry-based techniques that have underpinned several high-profile publications in journals including Science (2012, 2017 and 2018), Cell (2013) and Nature (2018). He also received the Cytometry Part A "paper of the year" accolade in 2011 for his work on debunking the myth of asymmetric division in adaptive immunity. He specializes in Imaging Flow Cytometry with a particular focus on "label-free" characterization of cell phenotypes and transition states using machine and deep learning approaches. Dr Filby is also an International Society for the Advancement of Cytometry (ISAC) council member for "technology development". Prior to this, he was one of the first people selected as part of the "Shared Resource Laboratory (SRL) Emerging Leaders" program. He serves on a number of ISAC committees and task forces and is heavily involved in a several educational initiatives for cytometry at both national and international levels. Dr Filby is driven by a passion to "measure all things of all cells in all biological systems".

JONATHAN HALL

VAN ANDEL RESEARCH INSTITUTE

Johnathan Hall is a Research Data Technician at the Van Andel Research Institute. He received his Bachelor of Science in Cell and Molecular Biology from Grand Valley State University in 2018 and is currently pursuing a Master of Science in Computer Science at Davenport University. His current project involves development of the SignalFinder-IF and -Microarray platforms, focusing on algorithm optimization and broadening applicability

NICHOL HOLODICK, PHD

WESTERN MICHIGAN UNIVERSITY HOMER STRYKER M.D. SCHOOL OF MEDICINE

Dr. Holodick performed her undergraduate work at Northeastern University in Boston, MA. She obtained her PhD in Immunology from Boston University in 2009, working on signaling and development of B-1 cells. Dr. Holodick expanded her work on B-1 cells during a postdoctoral fellowship at the Feinstein Institute for Medical Research in Manhasset, NY. During this time, she focused her research on the role of B-1 cells in infection during aging.

Recently, Dr. Holodick was appointed to Assistant Professor in the Center for Immunobiology and Department of Biomedical Sciences at WMed. In addition, Dr. Holodick is currently serving as Scientific Director for the Flow Cytometry Core facility.

JONATHAN IRISH, PHD

VANDERBILT UNIVERSITY

Jonathan Irish is Assistant Professor in the Department of Cell & Developmental Biology (CDB) at Vanderbilt University, School of Medicine. He holds a secondary appointment in Pathology, Microbiology & Immunology (PMI) and is Scientific Director of the Cancer & Immunology Core (CIC) and the Mass Cytometry Center of Excellence (MCCE).

Jonathan launched the Irish lab at Vanderbilt in 2012 after finishing his training with Garry Nolan (Ph.D.) and Ron Levy (Postdoc) at Stanford University.

DEREK JONES, PHD, ISAC SRL EMERGING LEADER

UNIVERSITY OF PENNSYLVANIA

Dr. Jones received his Ph.D. in Immunology and Infectious Diseases from the University at Albany and joined the Department of Pathology and Laboratory Medicine at the University of Pennsylvania as a postdoctoral fellow. He subsequently joined the Flow Cytometry and Cell Sorting Shared Resource Laboratory at the University of Pennsylvania, and currently serves as the Technical Director for Research and Development. He was selected as an ISAC SRL Emerging Leader (2019-2023) and is currently the President-elect of the Great Lakes International Imaging and Cytometry Association.

Dr. Jones has more than 10 years of experience in immunology and flow cytometry, with a focus on B cell and plasma cell development and function. His current research efforts include the development of high-dimensional assays for deep phenotyping on clinical and translational samples, in collaboration with the Parker Institute for Cancer Immunotherapy, and integrating novel technologies into the core facility to promote single-cell next-generation sequencing and proteomics within the cytometry community.

MICHAEL KEENEY, ART, FCSMLS

LONDON HEALTH SCIENCE CENTER

Mike Keeney is an associate Scientist at the Lawson Health Sciences Centre and Coordinator of Flow Cytometry at London Health Sciences Centre. His interests are mainly in malignant hematology and transplant. In collaboration with Rob Sutherland in Toronto, Mike developed the currently most widely used method for enumeration of CD34 cells known as the ISHAGE guidelines, for which Mike and Robb were awarded the Wallace H Coulter Distinguished Lecturer Award in 2006.

Mike has been a member of the CAP Diagnostic Immunology and Flow Cytometry Committee since 2001 and has introduced several new programs including most recently, MRD testing in B-ALL.

Mike is currently the president of the International Clinical Cytometry Society.

VERA TANG, PHD, ISAC SRL EMERGING LEADER

UNIVERSITY OF OTTAWA

Dr. Vera Tang is an immunologist and flow cytometry specialist with over 15 years of experience. Her science background is in immunological responses to viral infections. Dr. Tang is also an International Society for the Advancement of Cytometry (ISAC) Shared Resource Lab (SRL) Emerging Leader and the Co-President of the

Canadian Cytometry and Microscopy Association. She is currently the Operations Manager of the Flow Cytometry and Virometry Core Facility and an Adjunct Professor in the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa. Dr. Tang holds a Ph.D. in immunology from McMaster University and a B.Sc. from Queen's University.

WILLIAM TELFORD, PHD

NCI-NATIONAL INSTITUTES OF HEALTH

William Telford, Ph.D. received his Ph.D. in microbiology from Michigan State University in 1994, where his laboratory developed some of the earliest techniques for flow cytometric detection of apoptosis. He carried out his postdoctoral training in immunology at The University of Michigan Medical School, was appointed assistant scientist at the Hospital for Special Surgery – Weill Cornell University School of Medicine in New York City from 1997 to 1999. Dr. Telford was recruited as a Staff Scientist and manager of the ETIB Flow Cytometry Facility in 1999 and became an Associate Scientist in 2008.

Dr. Telford has over 20 years of experience in flow cytometry. He is extensively involved in both national and international cytometry education programs, including the National Flow Cytometry Resource Flow Cytometry Workshops (as a sustaining faculty member) and the International Society for Advancement of Cytometry, where he has taught numerous courses, tutorials and workshops over the last 10 years. He has over 100 publications in the fields of immunology and cytometry, with over half of them as primary, corresponding or originating author. He is the co-founder and coordinator of the NIH Flow Cytometry Interest Group and is a sustaining faculty member in the Indo-US Flow Cytometry Workshop program, teaching flow cytometry methods at many biomedical institutions in India. He maintains a small independent research and development program in the Facility aimed at both hardware and wetware development, particularly in the area of novel laser technology, and has published extensively in this area.

GLIIFCA 28 GENERAL INFORMATION

CONFERENCE REGISTRATION DESK

- The registration desk is located in the lobby of the Detroit Marriott Troy:
  - Friday, Sep. 27 – 4:00 pm to 8:00 pm
  - Saturday, Sep. 28 – 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

- Posters set up: Friday, September 27 after 12:00 pm in **Salon ABCDE**
- Numbers on posters correspond to poster abstract order in the program
- Poster board size = 3 ft. wide and 4 ft. high
- Please mount one poster on **each** side of a poster board using the provided **VELCRO** only
- Poster viewing: from Friday 6:30 pm to Sunday 10:30 am
- Poster presentation and judging: Saturday 6:30 pm to 8:00 pm

EXHIBITS

- Scheduled vendors will have booths in the Exhibit/Poster area **(Salon ABCDE)**
- **Please Note: Booth set-up for Salon ABCDE starting at 10 am on Sept. 27 with formal opening of Salon ABCDE at 6:00 pm. Break down starting at 11:30 am on Sunday, Sept. 29**
- All activities other than the Core Managers' Workshops **(Dennison 1-2-3)**, Roundtable Luncheon **(Dennison 1-4, Niles 1-2, Athens)**, Steering Committee Meeting **(Dennison 1-2-3)** and Banquet **(Mediterranean Room)** will be located in the Exhibit/Poster area **(Salon ABCDE)**
- **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): SALON FGH

BREAKFASTS

Free continental breakfast will be provided for all the registrants in the Exhibit/Poster area **(Salon ABCDE)** 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 **Salon ABCDE**:
    - Friday – 2:30 pm to 3:00 pm (Core Managers' Meeting; **Dennison 1-2-3**)
    - Saturday – 10:30 am to 11:00 am, and 3:45 pm to 4:15 pm
    - Sunday – 10:30 am to 11:00 am

INDUSTRIAL SCIENCE SYMPOSIUM

- Industrial Science Symposium presentations: 6:30 pm to 8:50 pm, **Salon FGH**.

SOCIAL ACTIVITIES

OPENING RECEPTION

- 6:00 pm to 9:30 pm, **Salon ABCDE**
- 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 LUNCHEON ROUNDTABLES (11:45 AM TO 1:30 PM)

- Free box lunch; available in **Dennison I** (Tables 1-3), **Dennison 2** (Tables 4-6), **Dennison 3-4** (Tables 7-9); **Niles 1-2** (Tables 10-12); **Athens** (for non-participants).
- Select a lunch (roast beef, turkey, or veggie wrap; fruit, chips) and beverage - and then move to selected roundtable labeled with the title of discussion topic. 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

- 6:30 pm to 7:30 pm in the Exhibit/Poster area **(Salon ABCDE)** with cheese trays (you can use your drink tickets for beer and wine).

GLIIFCA BANQUET (THEME: WIGGED OUT 'STACHE BASH)

- Free to registrants, available to paid guests.
- Commences at 8:00 pm **(Mediterranean Room**)
- 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/Poster area **(Salon ABCDE)** for the Friday opening reception and the Saturday Wine & Cheese reception, and also in the **Mediterranean Room** 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

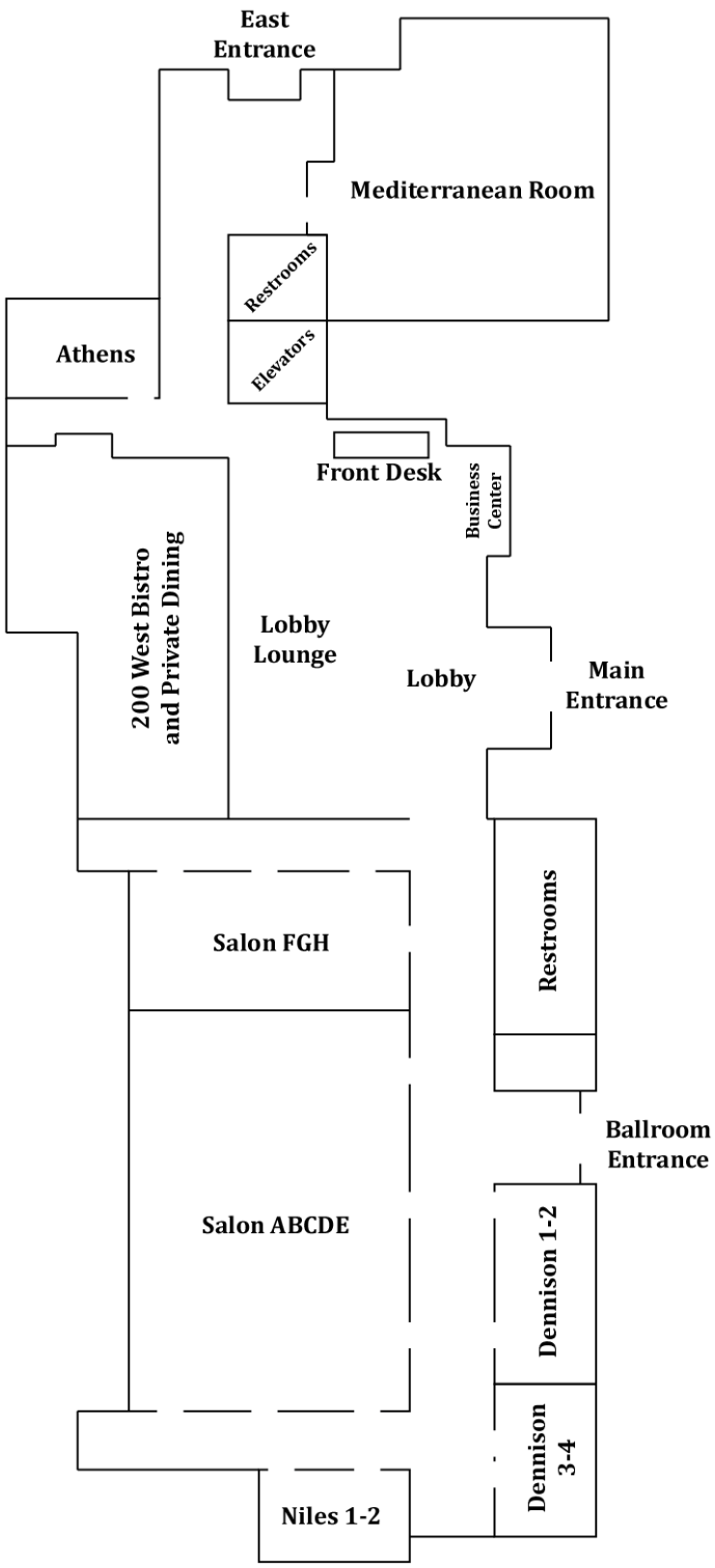
ADDITIONAL ENQUIRIES, COMMENTS, SUGGESTIONS

- Contact Dr. Alexander Nakeff (e-mail: caralex3@comcast.net, cell: (313) 820-6227) or leave message at the GLIIFCA registration desk
- Comments and suggestions – e-mail Dr. Rachael Sheridan at **RACHAEL.SHERIDAN@VAI.ORG** or Dr. Joseph Tario at president@gliifca.org

STEERING COMMITTEE BREAKFAST MEETING

- Sunday morning (8:00 am to 9:00 am) in **Dennison 1-2-3.**

DETROIT MARRIOTT TROY MAP



NOTES