



# Great Lakes International Imaging and Flow Cytometry Association

## GLIIFCA 16

September 28 – September 30, 2007

Hilton Windsor

Windsor, Ontario, Canada

[www.gliifca.org](http://www.gliifca.org)

2006 Program Chairs:

Tim Bushnell, President

Paul Wallace

Mike Sramkoski

Site Organizer:

Alexander Nakeff

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# GLIIFCA 16

## Program

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## **GLIIFCA 16 INFORMATION**

*(What You Always Wanted To Know About GLIIFCA 16 But Were Afraid To Ask!)*

**REGISTRATION DESK:** Friday 4PM to end of the meeting outside the Hilton Great Lakes Ballroom.

### **FRIDAY RECEPTION/INDUSTRIAL SCIENCE SYMPOSIUM/POSTER SETUP:**

Reception, Exhibit/Poster area (Radisson Riverfront Club), 5:30 to 11PM (use 4 drink tickets for wine/beer)

Industrial Science Symposium ORAL presentations: 7:00-10:00PM, Hilton Great Lakes Ballroom

**SYMPOSIA SESSIONS:** Erie/Huron Rooms in Hilton Great Lakes Ballroom

### **BREAKFASTS:**

Free continental breakfast provided for all registrants in the Exhibit/Poster area (Riverfront Club) on:

Saturday: 7:00 to 8:00AM

Sunday: 8:00 to 9:00AM

**Steering Committee breakfast meeting, Sunday morning (7:30 to 9:00AM) in Macdonald/Cartier Room.**

### **COFFEE BREAKS:**

Snacks and drinks available in the Exhibit/Poster area (Radisson Riverfront Club) - no need to line up!

### **SATURDAY LUNCHEON ROUNDTABLES (12:00 – 1:30PM):**

Free “box” lunch/pop; 2/3 roast beef and 1/3 veggie (egg and salmon salad) in Hilton Ontario Room (Great Lakes Ballroom) & Park Terrace Lounge. Sandwiches, chips and fruit at each roundtable of 10 – just pick up pop of choice and move to your roundtable!

### **SATURDAY WINE AND CHEESE HAPPY HOUR:**

5:15 to 7:30PM in the Exhibit/Poster area (Radisson Riverfront Club) with cheese and fresh fruit trays (use drink tickets).

### **BANQUET:**

Free to registrants and paid guests

Commences at 8PM, Hilton Great Lakes Ballroom

Numerous food stations (salad, entrée and dessert) provided to minimize waiting time

Bar available for drinks (use drink tickets or pay cash)

DJ (Frank’s) with dance music until 12:00AM; requests encouraged (get up and have fun!)

## **GLIIFCA 16 INFORMATION, Cont.**

### **DRINKS:**

Bar located in the Exhibit/Poster area (Radisson Riverfront Club) and Great Lakes Ballroom (banquet only)  
4 free drink tickets/registrant for beer and wine only  
Beer: Coors Lite & Walkerville; Wine: Pelee Island Cardonnyay & Merlot (glass)  
Mixed drinks – cash bar (your cost).  
All pop is free (ad libitum!) and all beer and pop in bottles/cans

### **EXHIBITS:**

Scheduled exhibitors will have booths in the Exhibit/Poster area (Radisson Riverfront Club)  
Booths will be open from 6PM Friday to the end of the meeting  
All activities other than the plenary sessions, roundtable luncheon, business meeting and banquet will be located in the Exhibit/Poster area (Radisson Riverfront Club)

**Please frequent the booths and show our appreciation for the generous financial support provided by the exhibitors who substantially help "pay the freight" for this meeting.**

### **POSTERS:**

Poster boards located in the Exhibit/Poster area (Radisson Riverfront Club)  
Numbers on posters correspond to poster abstract numbers in the program  
Poster board size= 3 ft wide and 4 ft high  
Please mount one poster on **each** side of a poster board  
Posters may be put up anytime on Friday and should be kept up until the end of the meeting  
Viewing on Friday 5:30PM to Sunday 10:30AM

### **FACILITIES/SERVICES:**

Parking: use valet service (your cost = \$14/day)  
Message boards: on easels next to the GLIIFCA Registration Desk  
Xerox copying, faxing, etc: Ask at Radisson/Hilton registration desks

### **EXTRACURRICULAR ACTIVITIES:**

Hotel brunch available on Sunday from 9AM – 2PM (your cost)

### **NAME TAGS AND EVALUATION FORMS:**

**BEFORE LEAVING:** Please fill out forms and leave at Registration/Check Out Desk with your name tags

**ADDITIONAL INQUIRIES: CONTACT DR. ALEXANDER NAKEFF**

## GENERAL MEETING SCHEDULE:

All symposia to be held in the Erie/Huron Rooms in the Hilton Great Lakes Ballroom. Poster viewing, Vendor Exhibits, breakfasts and breaks will all be held in the Radisson Riverfront Club.

### Friday, September 29

10a – 4p **Resource Managers Workshop**  
Sponsored by: ISAC and GLIIFCA

4p – 10p **Registration**  
Outside Hilton Great Lakes Ballroom

5:30p – 11:00p **Opening Reception**  
Radisson Riverfront Club  
Sponsored by: Beckman-Coulter

7p – 10p **Industrial Science Symposium**  
Hilton Great Lakes Ballroom

### Saturday, September 30

7:00a – 8:00a **Continental Breakfast**

8:00a – 11:00a **Symposium I**  
Clinical Cytometry

9:30 – 10:00a **Coffee Break**  
Sponsored by: iCyt

11a – 12p **The Carleton and Sigrid Stewart Plenary Lecture**

12:00-1:30p **Luncheon Roundtables**  
Hilton Ontario Rm & Park Terrace Lounge

1:30p – 3:30p **Symposium II**  
Cutting Edge Cytometry  
Data Analysis Symposium

3:30p – 3:45p **Coffee Break**  
Sponsored by: Spherotech

3:45p – 5:15p **Symposium III**  
Imaging Science

5:15p – 7:30p **Wine & Cheese Reception**

Sponsored by: Verity Software House

5:15p – 7:30p **Poster Presentations and Judging**

8p – 12am **GLIIFCA Annual Banquet**  
Banquet Sponsored by: Becton-Dickinson  
Hilton Great Lakes Ballroom

Party Theme: 16 Colors  
Costume Prize Sponsored by TreeStar

### Sunday, October 1

8a – 9a **Continental Breakfast**

7:30a – 9a **Steering Comm. Meeting**  
Macdonald/Cartier Room

9a – 11:45a **Symposium IV**  
Technical Innovations in Cytometry

10:30a – 11:00a **Coffee Break**  
Sponsored by: Amnis

11:45a–12:00p **Poster Awards**  
Sponsored by: (1) DeNovo Software, (1) Cytek, (1) Cedarlane

**Travel Stipend Awards**  
Sponsored by: (1) DeNovo Software, (3) Cell Signaling Technologies

12:00p **Closing Remarks**

**BEFORE LEAVING:** Please return evaluation form and name tag holder to Registration/Check out Desk. Thank you!

## Speaker Schedule – At a Glance

### Resource (Core) Managers' Workshop

Friday, September 28, 10:00a – 4:00p  
Conveners: Jonni Moore, Julie Auger

### Industrial Science Symposium

Friday, September 28 7:00p – 10:00p  
Conveners: Tom Sawyer, Karen Domenico

- 7:00-7:20 *Roy Edward, Biostatus Ltd*  
Anthraquinone Dyes for Problem-Solving in Live Cell Flow Cytometry and Imaging
- 7:20-7:40 *Jolene Bradford, Invitrogen,*  
Cell Proliferation Assay using Click Chemistry: A Novel Alternative to using BrdU Antibodies
- 7:40-8:00 *David Novo, De Novo Software*  
FCS Express and FCS Express Reader: New publishing capabilities
- 8:00-8:20 *Karen Helm, Univ. of Colorado*  
The Next Generation High Speed Sorting Platform: The New MoFlo XDP
- 8:20-8:40 *Scott Baldwin, CompuCyte Corp.*  
Quantitative Imaging Cytometry: A multi-faceted approach to sample analysis
- 8:40-9:00 *Paul Scibelli, Beckman Coulter*  
Exploring Plate Based On-Board Sample Preparation and Subsequent Mass Data Analysis Utilizing the Cell Lab Quanta SC MPL Flow Cytometer
- 9:00-9:20 *Larry Duckett, Becton Dickinson*  
New Solid State Laser Lines and Improvements in Data Resolution
- 9:20-9:40 *Kristi Harkins, Harkins Consulting*  
Device and Process Development to Support GMP Cell Sorting Applications on the iCyt Reflection™ BSC Platform

### Symposium I – Clinical Cytometry

Saturday, September 29 8:00 am – 11:00 am  
Convener: Paul Wallace

- 8p-8:45 *Ramsay Fuleihan, Northwestern Univ*  
Flow Cytometry in the Investigation and Diagnosis of Primary Immunodeficiency Diseases
- 8:45 – 9:30 *Charles Goolsby, Northwestern Univ*  
Flow Cytometric Analysis of T Cell Lymphoma and Leukemia
- 9:30 – 10:00 Coffee Break**
- 10:00 – 10:45 *Brian Grimberg, Case Western Reserve University*  
Combination of Hoechst and Thiazole Orange stains to monitor malaria life-cycle progression using flow cytometry

11a-12p **The Carleton and Sigrid Stewart Plenary Lecture**

*Frank Mandy, Chief, National Laboratory for Analytical Cytology Canada*  
Immunophenotyping Leukocytes and the Stewarts' Barbeque Theory.

### Luncheon Roundtables

12:00p – 1:30p  
Convener: Mary Paniagua

### Symposium II – Cutting Edge Cytometry/ Data Analysis Symposium

- 1:30 pm – 3:30 pm  
Convener: Mike Sramkoski
- 1:30 – 2:15 *Alan Moser, Cira Discovery Sciences*  
Cytometric Fingerprinting: Quantitative Characterization of Multivariate Distributions
- 2:45 – 3:30 *C. Bruce Bagwell, Verity Software House*  
A new paradigm for cytometry analysis
- 3:00 – 3:30 Discussion – Data Analysis

**3:30-3:45 Coffee Break**

### Symposium III – Imaging Science

- Saturday, Sept 30 3:45 pm – 5:15 pm  
Convener: Mike Sramkoski
- 3:45 – 4:30 *Dan Fisher, Roswell Park Cancer Institute (RPCI)*  
Thermal Therapy: A potential modality to overcome restrictions on lymphocyte infiltration of the tumor microenvironment
- 4:30 – 5:15 *Hans Minderman, RPCI*  
Potential and challenges for clinical application of IS100 image cytometry

**5:15p – 7:30p Poster Presentation and Judging**

### Symposium IV – Technical Innovations in Cytometry

- Sunday, September 30 9:00 am – 11:45 am  
Convener: Tim Bushnell
- 9:00 – 9:45 *James Kobie, Univ of Rochester*  
ExploraSpot-exploiting the FCS file format for automated analysis of fluorescent assays.
- 9:45 – 10:30 *Kathleen McGrath, Univ of Rochester*  
Delineating Erythropoiesis: Where flow meets Morphology
- 10:30 – 11:00 Coffee Break**
- 11:00 – 11:45 *Michael Betts, Univ of Pennsylvania*  
Polyfunctional assessment of T cell responses in humans

# Industrial Science Symposium

Friday, Sept 28<sup>th</sup>

7pm – 10pm

Hilton Great Lakes Ballroom

Conveners: Tom Sawyer, Karen Domenico

- 7:00-7:20 Anthraquinone Dyes for Problem-Solving in Live Cell Flow Cytometry and Imaging**  
*Roy Edward, Biostatus Ltd.*
- 7:20-7:40 Cell Proliferation Assay using Click Chemistry: A Novel Alternative to using BrdU Antibodies**  
*Jolene Bradford, Invitrogen.*
- 7:40-8:00 FCS Express and FCS Express Reader: New publishing capabilities.**  
*David Novo, De Novo Software.*
- 8:00-8:20 The Next Generation High Speed Sorting Platform: The New MoFlo XDP**  
*Karen Helm, Univ. of Colorado*
- 8:20-8:40 Quantitative Imaging Cytometry: A multi-faceted approach to sample analysis**  
*Scott Baldwin, CompuCyte Corp*
- 8:40-9:00 Exploring Plate Based On-Board Sample Preparation and Subsequent Mass Data Analysis Utilizing the Cell Lab Quanta SC MPL Flow Cytometer**  
*Paul Scibelli, Beckman Coulter*
- 9:00-9:20 New Solid State Laser lines and improvements in data resolution**  
*Larry Duckett, Becton Dickinson*
- 9:20-9:40 Device and Process Development to Support GMP Cell Sorting Applications on the iCyt Reflection™ BSC Platform**  
*Kristi Harkins, Harkins Strategic Consulting, LLC*

## Abstracts:

### **Antraquinone Dyes for Problem-Solving in Live Cell Flow Cytometry and Imaging**

Roy Edward, *Biostatus Ltd.*

Fluorescent live cell-permeant DNA binding anthraquinone dyes such as DRAQ5™ offer new routes to experimental design in imaging and flow cytometry. At their most fundamental they provide a convenient fluorescent emission signature which is spectrally separated from the commonly used marker protein (eGFP) and fluorescent tags such as Alexafluor 488, fluorescein and Cy2. Additionally, they do not excite in the UV and thus avoid the complications of compound UV-autofluorescence in drug discovery. They provide a convenient means of stoichiometrically labeling

cell nuclei in live cells without the aid of DMSO and can equally be used for fixed cells. The recent development of CyTRAK Orange™ allows simultaneous and differential labeling of both nuclear and cytoplasmic compartments in live or fixed cells to render the cell boundaries which may be beneficial for quantitative expression measurements and in cell-cell interactions. Recent examples of the application of these anthraquinone dyes will be described in the context of DNA content analysis in haematological malignancies, flow analysis of otherwise intractable adherent cell cultures and solid tissues and a variety of imaging procedures.

**Correspondence:** roy@biostatus.com, www.biostatus.com

## **Cell Proliferation Assay using Click Chemistry: A Novel Alternative to using BrdU Antibodies**

Jolene Bradford, *Molecular Probes/Invitrogen*

The measurement of cellular proliferation is fundamental to the assessment of cell health, genotoxicity, and in the evaluation of anticancer drugs. The most accurate method of doing this is by directly measuring DNA synthesis. Initially this was performed by incorporation of the radioactive nucleoside <sup>3</sup>H-Thymidine, which has largely been replaced by antibody-based detection of the nucleoside analog BrdU (5-bromo-2'-deoxyuridine). The Click-iT™ EdU Cell Proliferation Assay is an innovative alternative to the BrdU assay. EdU (5-ethynyl-2'-deoxyuridine) is a nucleoside analog to thymidine and is incorporated into DNA during active DNA synthesis. Detection is based on a click reaction, where copper catalyzes a covalent reaction between the incorporated EdU alkyne and a fluorochrome-conjugated azide. Standard methods of fixation and permeabilization can then be used in conjunction with Click-iT labeling and detection with no need for harsh DNA denaturation steps. An overview of click chemistry and how it applies to this application will be presented. Comparison of the Click-iT EdU method and the BrdU antibody method will be discussed. Examples of flow cytometry and imaging data using the Click-iT method will be shown.

**Correspondence:** Jolene.bradford@invitrogen.com

## **FCS Express and FCS Express Reader: New Publishing Capabilities**

David Novo, *De Novo Software*

FCS Express is a state-of-the-art flow Cytometry data analysis software used by thousands of researchers around the world. FCS Express is an integrated data analysis and presentation software, that allows scientists to perform advanced flow cytometric analysis in a modern Microsoft Office-style application. De Novo Software has recently introduced an entirely new suite of functionality to allow users to share analysis with each other, even if they have not purchased FCS Express. Any FCS Express user can simply “publish” their analysis to the internet, and anyone else in the world can open this analysis using the free FCS Express Reader. The FCS Express Reader allows you to modify any aspect of the published analysis, including changing gates, compensation settings, modifying plots and much more. This functionality is perfect for collaborators, teachers and companies wishing to showcase and share their analysis. We will demonstrate an overview of FCS Express, primarily showcasing the new publishing capabilities.

**Correspondence:** david.novo@denovosoftware.com

## **The Next Generation High Speed Sorting Platform: The New MoFlo XDP**

Karen Helm, *University of Colorado*

Highlights of the new cell sorter, by Dako, using new technology to achieve both the fastest and highest purity sort.

Changes in Electronics:

1. The first true high resolution five decade multi-channel digital acquisition system with 32 bit data processing of the history of flow cytometry.
2. Identification of side population cells (SP) based on the efflux of Hoechst 33342 and other rare cell populations can be studied using the MoFlo XDP. Excited by a UV laser, Hoechst 33342 blue and red fluorescence is captured and results in a small tail trailing off the main population. This side population of cells is quite rare and can easily be separated at high speeds on the MoFlo XDP thus decreasing the sorting time required.
3. High sample rates exceeding 100,000 events per second with excellent characterization of narrow pulses providing high speed small particle analysis and accurate detection of peak and area.
4. Flexible extraction of all parameters.
5. Digital triggering on any multi-laser parameter.
6. Unprecedented true Dynamic Range of 5 decades leading to a wide range of event detection with low noise, allowing broad range data acquisition and excellent dim particle analysis.

The new generation software system including the following features:

1. Height, Area, Width, Log, Log-Area SIMULTANEOUSLY for all parameters.
2. Reliably acquire and sort without dropping any events, while at the same time allowing the operator to run other applications.
3. Full Status and control of PMTs, sort parameters.
4. New Unique touch screen monitor for all function and parameter control.
5. More Powerful Sorting.
6. Summit can now save over 1,000,000,000 (one billion) events to an FCS file.

**Correspondence:** Karen.helm@uschsc.edu

## **Quantitative Imaging Cytometry: A Multi-Faceted Approach to Sample Analysis**

Scott Baldwin, *CompuCyt Corporation*

The interdisciplinary direction of life science research has required flow cytometry facilities to deal with increasingly varied sample types, coupled with a growing demand for improved visualization capabilities to support and enhance cytometric data. As a result, a versatile instrument platform combining imaging with traditional quantitative analysis would be a valuable addition to any core facility. CompuCyt's iGeneration family of Laser Scanning Cytometers (LSC) provides such versatility with several key strengths: (1) LSC enables total signal quantification for both fluorescent and chromatic dyes, yielding multiple quantitative features as well as sub-cellular localization of the bio-marker(s) of interest. (2) In addition to cell surface and intracellular markers, LSC also allows for quantification of extracellular markers in tissue. (3) Quantification is coupled with fluorescence, absorption, and light scatter imaging, enabling visualization of cellular and tissue morphology, features previously unattainable with traditional cytometry platforms. Recent advances in LSC technology offer confocal imaging capabilities as well, combining high spatial-resolution imaging and LSC quantification and imaging features on a single platform. LSC also offers extensive flexibility in allowable sample types, including suspended and adherent cells, tissue sections, tissue

microarrays, etc.; flexibility in carrier choices such as microscope slides, microtiter plates, and petri dishes; and flexibility in protocol development. This versatility makes the iGeneration systems ideally suited for both cellular and tissue-based analysis in key applications such as cell cycle and DNA content analysis, antigen expression, and immunophenotyping CompuCyte's products provide life scientists, pharmaceutical researchers, and clinical laboratory professionals with unique high-content analysis and reporting capabilities for discovery and objective characterization of biochemical cellular processes that define both normal and disease states.

**Correspondence:** sbaldwin@compucyte.com

## **Exploring Plate Based On-Board Sample Preparation and Subsequent Mass Data Analysis Utilizing the Cell Lab Quanta SC MPL Flow Cytometer**

Mark Cheetham, *Beckman-Coulter* (Presented by Paul Scibelli)

The ability to do "on the fly" sample prep is a new paradigm for flow cytometry, being able to prep your samples and acquire the sample data will broaden the application of flow into HTS, high throughput screening, areas. However generating data from up to 384 well plates presents its own problems, analysis using an SQL database allows an almost instantaneous reply of a plate of data allowing rapid data extraction into statistical analysis packages for result reporting.

**Correspondence:** mark.cheetham@coulter.com

## **New Solid State Laser Lines and Improvements in Data Resolution**

Larry Duckett, *Becton-Dickinson*

New solid-state lasers currently offer a wide variety of wavelengths and power. The number of solid-state lasers surpasses the number of ion laser that were available in the past. With the new wavelengths and the projected road map from our partners in the laser industry of greater power in the near future what we thought was the best combination of lasers and filters for many of the common fluorochromes and dyes are changing. We will look at some new solid-state laser lines (445nm, 532nm, 561nm, 594nm and 785nm) that have not been commonly used in the past on compact BD analyzers (BD LSR II) and sorters (BD FACSAria) and look at the improvements in data resolution over more conventional wavelengths.

**Correspondence:** Larry\_Duckett@bd.com

## **Device and Process Development to Support GMP Cell Sorting Applications on the iCyt Reflection™ BSC Platform**

Fredrick Molnar, *iCyt Mission Technology, Inc.* (Presented by Kristi Harkins)

The recent publication of standard safety practices for cell sorting, written and approved by the ISAC Biosafety Committee, has highlighted the need for cell sorting devices that provide improved operator and product protection. The Reflection BSC cell sorting system integrates multiple high-speed cell sorters into a standard Baker SterliGARD III® Advance, Class II biosafety cabinet. This allows cell sorting and sample handling to be performed in an environment that is fully compliant with both the new ISAC biosafety standards and NSF 49 minimum standards for a Class II, Type A2 biosafety containment. Efforts are currently underway to further develop this system into a cell sorting device suitable for GMP applications. Such a device must be compliant with the guidelines and oversight mechanisms established by the Center for Biologics Evaluation and Research (CBER) within the

FDA. Details of challenges associated with this work and development progress toward this goal will be presented. These include integration of disposable items such as sterile nozzles and flow bodies, sterile sample probes and tubing, pre-packaged sterile sheath fluid, and a novel sheath delivery system. Other essential design considerations will be presented such as high throughput parallel sorting, redundancy across all systems, reliability of components, and remote operation of the cell sorters.

**Correspondence:** molnar@i-cyt.com

## Symposium I

Saturday, Sept 29<sup>th</sup>

8am – 10:45 am

*Clinical Cytometry*

Erie/Huron in Hilton Great Lakes Ballroom

**Convener: Paul Wallace, Roswell Park Cancer Institute**

**8:00 – 8:45 Flow Cytometry in the Investigation and Diagnosis of Primary Immunodeficiency Diseases**  
*Ramsay Fuleihan, Northwestern University Feinberg School of Medicine, Children's Memorial Hospital*

**8:45 – 9:30 Flow Cytometric Analysis of T Cell Lymphoma and Leukemia**  
*Charles Goolsby, Northwestern University*

**9:30 – 10:00 Coffee Break – Posters and Exhibits**  
*Radisson Riverfront Club*

**10:15 – 11:00 Combination of Hoechst and Thiazole Orange to Monitor Malaria Life-Cycle Progression using Flow Cytometry**  
*Brian T. Grimberg, Case Western Reserve University*

### Abstracts:

#### **Flow Cytometry in the Investigation and Diagnosis of Primary Immunodeficiency Diseases**

*Ramsay Fuleihan, Northwestern University Feinberg School of Medicine and Children's Memorial Hospital*

Abstract not available at press time.

**Correspondence:** r-fuleihan@northwestern.edu

#### **Flow Cytometric Analysis of T Cell Lymphoma and Leukemia**

*Charles Goolsby, Northwestern University Feinberg School of Medicine*

T cell lymphoma and leukemia are a diverse group of malignancies having varied immunophenotypic features, pathology, and clinical course. Immunophenotypic features that can be seen include loss, or altered expression, of pan T cell antigens; predominance of one T cell subset or another (helper or suppressor cell immunophenotype); lack of expression of either CD4 or CD8; or aberrant expression of antigens such as CD10 in angioimmunoblastic T cell lymphoma as well as altered patterns of expression for combinations of antigens. However, the diagnostic sub-classification can be difficult. Even within a specific entity, immunophenotype, and to some extent pathology, can be variable and clinical features and presentation are currently key to sub-classification. In addition, there can be significant overlap in immunophenotype with reactive T cell processes. In this presentation, the immunophenotype and use of flow cytometric analysis techniques in the diagnosis of T cell lymphoma and leukemia will be discussed.

**Correspondence:** c-goolsby@northwestern.edu

## **Combination of Hoescht and thiazole orange stains to monitor malaria life-cycle progression using flow cytometry**

Brian T. Grimberg, *Case Western Reserve University*

The complex life cycle of the malaria parasite, *Plasmodium falciparum*, contributes to challenges in understanding how to limit infections and reduce the risk of severe malaria. Improved understanding of *P. falciparum* blood-stage growth and development would provide new opportunities to evaluate and interrupt the parasite's life cycle. An assay was developed to monitor the number of parasite infected cells and observed parasite development within infected erythrocytes using Hoechst 33342 (DNA stain) and thiazole orange (RNA stain). Simultaneous application of these fluorochromes to *P. falciparum* cultures was evaluated using a Becton-Dickinson LSR II flow cytometer. Control experiments showed that *P. falciparum* cultures can be exposed to Hoechst and thiazole orange simultaneously to achieve maximal staining of DNA and RNA. This data provides insight into the DNA and RNA content of different *P. falciparum* erythrocyte developmental stages. Staining of the *P. falciparum* nucleic acids allows for high fidelity examination of parasite development in the absence of fixatives, lysis, or radioactive isotopes. This method allows for examination of an erythrocyte from the moment of parasite invasion until it ruptures using sensitive and rapid assay procedures. Investigation of the mechanisms by which anti-malarial drugs and antibodies act against the different *P. falciparum* lifecycle stages will be aided by this cytometric strategy.

**Correspondence:** brian.grimberg@case.edu

## **The Carleton and Sigrid Stewart Plenary Lecture**

**Saturday, September 29**

**11:00 am – 12:00 pm**

**Frank Mandy, B.Sc.**

*Chief, National Laboratory for Analytical Cytology Canada*

*Education Director, ISAC*

## **Immunophenotyping Leukocytes and the Stewarts' Barbeque Theory**

AIDS had profound impact on the evolution of clinical flow cytometry. However, how to interpret the complex multi dimensional listmode files was a significant challenge in the early days of flow cytometry. Los Alamos scientists who were known for their scientific wizardry to make the world largest mushrooms also began to tackle the essential issues related to flow cytometric application in biology. Without the Stewarts barbeque theory the development of immunophenotyping would have been much slower and certainly a lot less entertaining. In this presentation I will make a feeble attempt to illustrate how some of their ideas were helpful to gain profound insight in cell based study of biology as it related to immune status monitoring of individuals living with HIV.

**Correspondence:** fmandy@rogers.com

**Luncheon Roundtables**  
**Hilton Ontario Room & Park Terrace Lounge**  
**Saturday, Sept 29 12:00-1:30PM**  
**Convener: Mary Paniagua, University of Chicago**  
**Free box lunch available at each table – drinks available at side tables.**

*The primary aim of these discussions is to provide a forum for participants to address their interests in a variety of cytometry and image topics.*

- 1) Intracellular Phosphoprotein Measurements Using Flow Cytometry: Discussion of the technical challenges, reagent availability and expanding applications for intracellular phosphoprotein measurements. *Moderators: Lisa Green, Lilly Research Labs and Sue Chow, Princess Margaret Hospital*
- 2) Cytometry and Global Health: The cytometry community can play a central role in helping to deal with health-care issues in countries faced by disabling diseases such as AIDS, malaria and TB. How can our community effectively contribute? Is it education, training, technology development or something else? The purpose of this roundtable will be to see how we can move as a community to participate in helping to solve some of these complex and distressing issues. *Moderator: J. Paul Robinson, Purdue University*
- 3) Regulatory Issues in the Clinical Flow Cytometry Laboratory: Discussion of how best to meet the requirements of the various regulatory agencies, CAP, CLIA, etc. *Moderator: Marybeth Dell, Roswell Park Cancer Institute*
- 4) Cytometry Education: General discussion to include the review of educational initiatives originating from ISAC's Education Committee and issues relating to challenges in cytometry education overall. *Moderators: Jonni Moore, University of Pennsylvania and Paul Wallace, Roswell Park Cancer Institute.*
- 5) Flow Cytometry for Microbial/Small Particle Analysis: Forum to discuss the challenges and various approaches to successfully analyze bacteria and small particles by flow cytometry. *Moderator: Dave Novo, Denovo Software.*
- 6) Image Cytometry: Discussion of the emerging clinical applications for use of the ImageStream and associated challenges. *Moderator: Hans Minderman, Roswell Park Cancer Institute*
- 7) Cell Sorting Basics: From instrumentation to sample prep, an opportunity to discuss the art of cell sorting. *Moderator: Tim Bushnell, University of Rochester*
- 8) Grantsmanship: The search to secure funds for your research—review of options available and issues critical for success. *Moderators: Kathy Muirhead and Betsy Ohlsson-Wilhem, SciGro*
- 9) Rare event detection and analysis: Discussion of the technical considerations, from sample preparation through data analysis, necessary to ensure robust detection and decision-making. *Moderator: Vera Donnenberg, University of Pittsburg*
- 10) Compensation Challenges in Multiparameter Flow Cytometry: Focus on the methods/techniques needed to ensure your multicolor data is correctly interpreted. *Moderator: Dave Allman, University of Pennsylvania*
- 11) Designing a Polychromatic Flow Cytometry Experiment: With the advent of 8, 11 or 18(!) color instruments, designing an experiment is more than just grabbing what reagent is conveniently available. Isotype controls, FMO's, biological gating controls? What's all the fuss with controls and why they are critical in proper interpretation of your data. *Moderator: Sally Quataert, University of Rochester Medical Center*

## Symposium II

Saturday, Sept 29

1:30 pm – 3:30 pm

*Cutting Edge Cytometry/Data Analysis Symposium*

Erie/Huron in Hilton Great Lakes Ballroom

Convener: Mike Sramkoski, Case Western Reserve University

**1:30 – 2:15 Cytometric Fingerprinting: Quantitative Characterization of Multivariate Distributions**

*Alan Moser, Cira Discovery Sciences, Inc*

**2:15 – 3:00 A new paradigm for cytometry analysis**

*C. Bruce Bagwell, Verity Software House*

**3:00 – 3:30 Extended Discussion- Data Analysis**

**3:30 – 3:45 Coffee Break**

### Abstracts:

#### **Cytometric Fingerprinting: Quantitative Characterization of Multivariate Distributions**

*Alan Moser, Cira Discovery Sciences, Inc.*

We describe a new method of analysis for high-dimensionality flow cytometric data which we call Cytometric Fingerprinting. Cytometric Fingerprints capture and encode the full multivariate correlation of complex, high-dimensional, flow cytometric data. As the name implies, this compact representation casts flow cytometric data in a form that enables the quantitative characterization and comparison of samples. This representation is particularly useful when cell populations are not clearly delineated by optimized assays and the distribution of events in the multi-parameter space is not bimodal.

The construction of Cytometric Fingerprints starts with listmode data where each event is represented by a point in a space whose dimensionality is determined by the number of scattering and fluorescence channels. A training set is selected, which may consist of one or more listmode files representing a sample or set of samples to be regarded as a template. Using this training dataset, the multidimensional space is partitioned into sub-regions (bins). Our method of binning is similar to Probability Binning [Roederer et al., *Cytometry* 45:47–55, 2001] in that each bin of the training data contains an equal number of events, however, it differs in two key ways: 1) Bins are formed by recursively splitting the data at the median value along the direction of maximum variance rather than along any of the original coordinate axes. This involves first determining the direction of maximum variance using Principle Components Analysis and then rotating the data space such that one of the transformed coordinates axes lies in this direction. (2) Rather than retaining bin information at only the last subdivision of the space, we keep the bin descriptions at each recursive step. Thus, the result is a hierarchical, multi-resolution representation of the data subdivided into regions defined by sets of oblique intersecting hyper-planes (polytopes). We refer to this method of binning as Oblique Recursive Subdivision. Cytometric Fingerprints are generated by projecting each individual sample onto this set of bins. Since the event counts for each bin can be enumerated as a one-dimensional list, each sample is represented by a one-dimensional fingerprint. These raw event-count fingerprints can be used directly or they can be transformed by resolution-dependent normalization to create density

fingerprints. Alternatively, we can employ Poisson statistics to create p-value fingerprints which represent the probability that a given bin differs significantly from the probability distribution function of the training set.

The utility of Cytometric Fingerprints comes from their ability to characterize how individual samples vary with respect to a given population. For example, a set of normal samples may be used as the training set. Unknown samples can then be classified by forming fingerprints with respect to these template bins and using conventional classification algorithms. Cytometric Fingerprints can also be used for quality control by comparing fingerprints formed from parameters common across multiple tubes for a sample.

In this talk, we describe our algorithm for forming Cytometric Fingerprints and demonstrate their use with two examples. In the first example, a sorted population of cells was spiked into a background population in varying concentrations. We compare the result of this analysis with conventional gating methods. In the second example, we use a synthetic data set to explore how Cytometric Fingerprints perform for rare event detection in the presence of simulated biological variability.

**Correspondence:** [allan.moser@ciradiscovery.com](mailto:allan.moser@ciradiscovery.com)

## **A New Paradigm for Cytometry Analysis**

*C. Bruce Bagwell, Verity Software House*

Conventional cytometry analysis uses one-parameter, two-parameter, and sometimes three-parameter histograms to represent parameter correlations in a listmode file. The data are generally classified into relevant populations by using a set of hierarchical gates defined on these histograms. Statistical analyses from these gated events along with a set of selected histograms are generally the ultimate output of cytometry analysis.

The major limitations of this approach are that it does not scale well with number of parameters, gates are often subjective in their placement and sensitive to population overlap, and the resultant reports are often filled with voluminous graphs and results that often confuse rather than illuminate.

Probability State Models (PSM's) either mitigate or eliminate these major problems. They scale well with number of parameters and account for population overlap. The graphical output is compact and easy-to-understand, even with a relatively large number of parameters. PSM's can often incorporate listmode data from multiple samples into one model and present a single coherent picture of a specimen's status.

The purpose of this presentation is to describe the principles of PSM and provide a number of examples of its use for typical cytometry applications.

**Correspondence:** [cbb@vsh.com](mailto:cbb@vsh.com)

## Symposium III

Saturday, Sept 29

3:45 pm – 5:15 pm

*Imaging Science*

Erie/Huron in Hilton Great Lakes Ballroom

Convener: Mike Sramkoski, Case Western Reserve University

**3:45– 4:30 Thermal Therapy: A Potential Modality to Overcome Restrictions on Lymphocyte Infiltration of the Tumor Microenvironment**

*Dan Fisher, Roswell Park Cancer Institute*

**4:45 – 5:30 Potential and Challenges for Clinical Application of IS100 Image Cytometry**

*Hans Minderman, Roswell Park Cancer Institute*

### Abstracts:

**Thermal Therapy: A Potential Modality to Overcome Restrictions on Lymphocyte Infiltration of the Tumor Microenvironment**

*Dan Fisher, Roswell Park Cancer Institute*

An often overlooked consideration in the development of T cell-based cancer immunotherapy is that effector CD8 T cells must efficiently traffic to the tumor microenvironment in order to control malignant progression. Trafficking of blood-borne T cells to target tissues is orchestrated by dynamic interactions with endothelial cells lining blood vessels which involve a four-step adhesion cascade: (1) initial tethering and rolling along endothelial surfaces, (2) activation of G-protein–linked chemokine receptors on T cells, (3) firm arrest on vessel walls, and (4) extravasation into the underlying tissue. Here, we used intravital microscopy to address two basic questions: (1) Are adhesive interactions between CD8 T cells and tumor vessels limited? (2) Can CD8 T cell trafficking across tumor microvessels be augmented by local inflammatory cues provided by systemic thermal therapy (STT)? These studies compared interactions between CD8 T cells with an effector phenotype (*i.e.*, L-selectin<sup>lo</sup>, CCR7<sup>lo</sup>, PSGL1<sup>+</sup> CD44<sup>hi</sup>, CXCR3<sup>+</sup>,  $\alpha$ 4 integrin<sup>hi</sup>, LFA-1<sup>hi</sup>) and vessels in colon 26 tumors implanted at subcutaneous sites. Interactions were also examined in normal subcutaneous vessels that are poor sites of recruitment under homeostatic conditions. CD8 T cells interactions with tumor vessels occurred at low frequency and were indistinguishable from interactions with normal vessels where < 10% of cells undergo tethering and rolling interactions. Investigation of the effects of fever-range STT (core temperature elevated to  $39.5 \pm 0.5^\circ\text{C}$ ) on adhesion in normal and tumor vessels was prompted by recent observations that STT enhances the trafficking of T lymphocytes across specialized high endothelial venules in lymph nodes by a mechanism dependent on upregulation of the gatekeeper adhesion molecule, intercellular adhesion molecule-1 (ICAM-1) (*Chen et al. Nature Immunol. 7:1299, 2006*). Normal vessels did not respond to thermal therapy with respect to changes in CD8 T cell interactions or intravascular ICAM-1 density. In contrast, STT caused an ~2-fold increase in E/P-selectin–dependent rolling interactions and an ~5-fold increase in ICAM-1-dependent firm arrest in intratumoral vessels. These findings correlated with strong induction of intravascular ICAM-1 in tumor vessels during thermal therapy. An absolute requirement for G-protein coupled chemokine-receptor activation in this process was demonstrated by evidence that the transition from E/P-selectin–dependent slow rolling to ICAM-1–dependent firm sticking was blocked by treatment of CD8 T cells with pertussis toxin. Taken together, these findings suggest that thermal therapy can overcome limitations in trafficking of tumor-reactive CD8 effector T cells to the tumor microenvironment during immunotherapy by targeting lymphocyte-endothelial interactions in tumor vessels. (Supported by NIH grants CA79765, CA094045, and DOD grant W81XWH-04-1-0354)

**Correspondence:** dtfisher@buffalo.edu

## **Potential and Challenges for Clinical Application of IS100 Image Cytometry**

Hans Minderman, *Roswell Park Cancer Institute*

The ImageStream multispectral imaging flow cytometer (IS100) has been developed to produce high resolution brightfield, darkfield, and fluorescence images of cells prepared in suspension at rates up to 100 cells per second. Its analysis software quantifies over 200 morphometric and photometric parameters for each cell. The potential for clinical application of this technology will be discussed in two clinical settings. First in context of evaluating determinants of response to novel therapeutics that target aberrant signal transduction commonly observed in cancer. Specifically, the NF- $\kappa$ B pathway as a target for the treatment of AML will be discussed and establishment of an in vitro model system for NF- $\kappa$ B activation in AML cells and the quantitative assessment of NF- $\kappa$ B translocation by IS100 will be demonstrated. Second, in context of leukemia cell karyotyping which affects the classification, treatment stratification and prognostication of AML. The ability to detect minimal (residual) disease is of great clinical significance for diagnosis, prognosis and follow up of these and other malignancies but because the reliability/sensitivity of conventional cytogenetics and Fluorescent In Situ Hybridization (FISH) is strongly limited by the abundance of the leukemic cells in the samples analyzed, karyotyping is not applicable to minimal disease stages. The IS100 has the potential for high throughput analysis of FISH and a method was established to perform FISH in suspension (FISH-IS) and an extended depth of focus (EDF) modification, necessary to accurately detect the relatively small FISH signals, was designed to enable image analysis of multiple focal planes within a 16  $\mu$ M range. The feasibility to perform high throughput FISH-IS will be discussed. To achieve the ultimate objective of using the IS100 clinically, it is important to verify that biological variations detected by the ImageStream are truly associated with (treatment of) the disease rather than artifacts induced by sample collection/preparation procedures. Therefore, a major challenge faced is to minimize the biological effects of sample preparation since, for example, many signal transduction pathways, including NF- $\kappa$ B, are activated or de-activated in response to cell stress. Our experience thus far at the Roswell Park Cancer Institute will be discussed.

**Correspondence:** [Hans.minderman@roswellpark.org](mailto:Hans.minderman@roswellpark.org)

## Symposium IV

Sunday, September 30

9:00 am – 11:45am

*Technical Innovations in Cytometry*  
Erie/Huron in Hilton Great Lakes Ballroom

Convener: **Tim Bushnell, University of Rochester**

**9:00 - 9:45 ExploraSpot—Exploiting the FCS file Format for Automated Analysis of Fluorescent Assays**

*James Kobie, University of Rochester*

**9:45 – 10:30 Delineating Erythropoiesis: Where Flow Meets Morphology**

*Kathleen McGrath, University of Rochester*

**10:30 – 11:00 Coffee Break**

**11:00 – 11:45 Polyfunctional Assessment of T Cell Responses in Humans**

*Michael Betts, University of Pennsylvania*

### Abstracts:

#### **ExploraSpot-exploiting the FCS file format for automated analysis of fluorescent assays**

James Kobie, *University of Rochester*

Abstract not available at press time

**Correspondence:** James\_Kobie@urmc.rochester.edu

#### **Delineating Erythropoiesis: Where Flow Meets Morphology**

Kathleen E. McGrath, *University of Rochester*

The study of erythropoiesis, the differentiation of red blood cells, has a long history. Thus its foundational definitions are based on classic technologies such as microscopic morphology. A challenge faced by this field is how to utilize the analytical tools and quantitative power of flow cytometry in a way that can be correlated to the extant knowledge based primarily on histology. Unfortunately, there are few flow cytometry markers available for the study of erythroid cells. The two most common for the murine system are Ter119 (recognizing a carbohydrate moiety associated with glycophorin A), an erythroid specific marker present at constant levels during most erythroblast stages, and CD71 (recognizing transferrin receptor), which decreases during erythroblast maturation, but without precise correlation to morphology of cell stage. We have recently used a new flow technology, the Amnis Corporation's ImageStream, that captures a bright field as well as multiple fluorescent images of individual cells. The images can then be analyzed for levels of fluorescence intensity in multiple ways (i.e. peak, minimum, mean) as well as the shape and size of the area of fluorescence. Combinatorial measurements can also be defined to compare levels and spatial

associations of multiple fluorescent channels. We are now applying this technology to distinguish the six stages of erythroblast development by changes in Ter119 peak intensity and area, DNA (Draq5 stain) peak intensity and area, and RNA content (thiazole orange stain). The proportions of erythroblasts in each stage of differentiation as determined by Imagestream analysis are consistent with proportions derived by classic morphological analyses. Furthermore, we can now use this technology to test the efficacy of other antibodies to characterize erythropoiesis for use in more classical flow cytometry approaches. Finally, the Imagestream technology allows us to measure cell characteristics not classically used in cytometry such as unevenness of surface, asymmetry of nuclei and unusually high contrast in bright field images. Combined with fluorescent criteria, we now can identify different forms of erythropoiesis even when a very rare population within other erythropoietic intermediates.

**Correspondence:** mckat1959@gmail.com

## **Polyfunctional Assessment of T Cell Responses in Humans**

Michael R. Betts, *University of Pennsylvania*

Virus-specific T cell responses in humans are multi-factorial and highly variable depending on the nature of the antigen being recognized. Optimal monitoring of virus-specific therefore requires the measurement of multiple T cell functions and memory phenotypes to fully account for the inherent variability of these responses. Using polychromatic flow cytometry, we have developed methods to allow the simultaneous measurement of up to six independent T cell functions and 3 T cell memory phenotype markers. These procedures have allowed us to identify differences in the functional and memory phenotype profile amongst T cells specific for HIV, CMV, EBV, Influenza and Vaccinia virus. Our results highlight the inherent variability of the human immune responses, and underscore the need for advanced immunologic monitoring techniques afforded only by the use of polychromatic flow cytometry.

**Correspondence:** betts@mail.med.upenn.edu

## POSTER ABSTRACTS

Abstract number corresponds to poster board number.

All posters displayed in Radisson Riverfront Club

### **#1. Development of a high-throughput assay for radiation biological dosimetry.**

*R.C. Wilkins, B.C. Kutzner, C.L. Ferrarotto, S. Dertinger, J.P. McNamee.  
Health Canada, Ottawa, Ontario*

In a mass casualty radiological event, it is imperative to quickly identify exposed individuals for the purpose of medical intervention and first responders who must be restricted from further exposure. The dicentric chromosome assay, the internationally recognized standard for radiation biological dosimetry, is sensitive and radiation specific but is extremely time consuming and labour intensive. Therefore, it is imperative to develop alternative techniques to increase the throughput of biological dosimetry in mass casualty events. To address this gap, we are adapting a flow cytometry-based assay that enumerates micronuclei in cultures of immortalized cells for use with primary human lymphocytes. In this assay, lymphocytes are isolated from whole blood and stimulated with PHA for 48 h. Cells are then incubated with the nucleic acid dye ethidium monoazide (EMA). Healthy cells exclude EMA whereas the compromised membranes of necrotic and apoptotic cells permit labelling. Subsequent to a photoactivation step that covalently binds EMA to chromatin of dead and dying cells, cytoplasmic membranes are lysed to release intact nuclei and sub-2n particles. Differential staining with SYTOX (a pan-DNA marker) and DiA (membrane marker) is applied to differentiate between nuclei/micronuclei, free metaphase chromosomes and other cellular debris. The isolated nuclei are then analysed by flow cytometry and identified as either whole or micronuclei. Preliminary data have demonstrated that this high-throughput technique displays a highly reproducible dose-response relationship in the 0 - 4 Gy range, with a sensitivity limit of at least 1 Gy. This work is now being validated using microscope scoring of the micronuclei. Once fully validated, this assay has the potential to greatly increase throughput for analysis of individual radiation exposures, which would be particularly beneficial for emergency triage purposes.

### **#2. Breakthrough Cell Proliferation Detection: Click Chemistry Based Labeling of Nucleic Acid Analog**

*Scott T. Clarke<sup>1</sup>, Jolene A. Bradford<sup>1</sup>, Suzanne B. Buck<sup>1</sup>, Kyle R. Gee<sup>1</sup>, Brian Agnew<sup>1</sup>, and Adrian Salic<sup>2</sup>*

<sup>1</sup>Molecular Probes® - Invitrogen Detection Technology • 29851 Willow Creek Rd • Eugene, Oregon 97402 • USA <sup>2</sup>Harvard Medical School • Boston MA • USA

Changes in cell proliferation as measured by the incorporation of nucleoside analog into actively synthesizing DNA have been the basis for assessing treatments which alter or block phases of the cell cycle. Initially radioactive thymidine was used and later replaced by bromo-deoxyuridine (BrdU) incorporation. A new method for a direct measurement of the S-phase fraction through the use of click chemistry is introduced. The assay uses the incorporation of an alkyne modified nucleoside, ethynyl-deoxyuridine (EdU). Cells are then fixed, permeabilized, and reacted with a dye labeled azide, catalyzed by copper(I). A covalent bond is formed between the dye and the incorporated nucleotide, and the fluorescent signal can then be measured. Cell cycle arrest drugs can be screened using this protocol, compatible with flow cytometry, imaging, and high content screening platforms.

### **#3. Discrimination of Necrotic Cells in Flow Cytometric Analysis of Fixed and Frozen Samples**

*Laura M. Storck, Sara M. DiNardo, Jennifer M. Nothstein, Toluwalope K. Disu, David C. McFarland and Kendall S. Frazier*  
Safety Assessment, GlaxoSmithKline, King of Prussia, PA

An advantage of Live/Dead® Fixable Dead Cell Stain Kits is that they utilize amine-reactive dyes that fluoresce in five discreet wavelengths and use only one channel each. Other channels are available for multicolor experiments that could also provide great flexibility in panel design. A method that allows dead cell discrimination by flow cytometry that is compatible with fixation and/or freezing and short-term storage is highly desirable as this adds flexibility in study design and would allow viability as an endpoint to be measured in samples received from remote locations. Five Live/Dead® Fixable Dead Cell Stain Kits (green, red, blue, aqua, violet) were evaluated using three cell types (HL-60, A549, lysed whole blood) and two preservation and storage procedures: 1) fixation with 0.2% paraformaldehyde (PFA) and storage at 4°C; and 2) fresh-frozen at -80°C. A short time course (0, 1, 7, 14 days) was performed to investigate stability over time and the impact of the preservation method. Results from fresh samples (Day 0) were compared to results of fixed and frozen samples after short-term storage (Days 1, 7, 14). Results from staining reagents were compared amongst themselves and against fresh samples (Day 0) stained with the exclusion dye ASBMS. Dunnett non-parametric and correlation statistical analyses were performed. Results indicate that statistically significant differences exist between the amine-reactive dyes and ASBMS, as well as between the two preservation methods. For all evaluated cell types, freshly stained cell populations demonstrated the highest percentages of dead cells, followed by fixed, and then frozen preparations. The percentage of dead cells reported with amine-reactive dyes was often lower than that of fresh samples stained with ASBMS. The performance of Live/Dead® Fixable Dead Cell Stain Kits may vary depending on cell type, color used, preservation technique and time of storage.

### **#4. QTube™: Automated quality assessment of flow cytometric data based on Cytometric Fingerprinting**

*Wade Rogers<sup>1</sup>, Herbert Holyst<sup>1</sup>, Jonni Moore<sup>2</sup>, Rich Schretzenmair<sup>2</sup>, Allan Moser<sup>1</sup>*

<sup>1</sup>Cira Discovery Sciences, Inc., Philadelphia, PA USA

<sup>2</sup>University of Pennsylvania, Abramson Cancer Center and Pathology and Laboratory Medicine, Philadelphia, PA

Flow cytometry is a complex technology that is increasingly being used by clinicians to improve the quality of health care. In order to minimize artifacts and assure accuracy, rapid and simple quality control methods are critical for effective use of the technology in the clinical setting. Most clinical flow cytometric assays involve the acquisition of data from several tubes in order to accommodate many markers or stimulation conditions. A common method of analysis involves the establishment of gates, based on parameters common to all tubes that can be uniformly applied across the entire panel, thus providing a basis for consistent population identification and minimizing time and effort in the data analysis process. The underlying assumption is that events are distributed the same way for each tube across the gating parameters. Violations of this assumption due to unanticipated and undetected shifts or other acquisition irregularities result in inaccurate phenotyping, potentially leading to inaccurate diagnosis.

We present an automated fingerprint-based method to quantitatively assess the consistency of gating data across a panel of tubes. The method, which we term QTube™, produces a numerical as well as a graphical assessment of gate data consistency. The algorithm operates directly on list-mode FCS data and requires no operator intervention. The method is scalable to arbitrarily large panels. We

demonstrate the applicability of QTube in small (5-10 tube) panels and in larger panels acquired in an automated 96-well plate stimulation protocol. The ability of QTube to immediately and automatically indicate acquisition abnormalities: (a) reduces the burden on operators to detect data defects via tedious visual inspection, (b) quantifies instrument performance in real time, and (c) detects performance deterioration before data integrity is compromised.

## **#5. Investigation of expression of CD32 (Fc gamma RIIB) in young calves: Probing the mystery of maternal suppression of active immunity.**

*BA McBey, KS Chattha, MA Firth, DC Hodgins, PE Shewen  
University of Guelph, Ontario Veterinary College, Department of Pathobiology, Guelph,  
Ontario*

Inhibition of neonatal (active) antibody responses by maternal antibodies is well documented in many mammalian species. Complexes of antigen and maternal antibodies crosslink membrane IgM with Fc gamma receptor IIB (CD32) on B cells, leading to inhibition of B cell activation. Expression of CD32 by neonatal B cells has not been well characterized in non-rodent species. Different isoforms of Fc gamma RII have been documented in humans (including some activating forms); these have not been investigated in cattle.

Blood was collected from 41 calves (<90 days of age) and 12 cows. The percentage, absolute number and mean fluorescence intensity of CD32<sup>+</sup> lymphocytes was determined using fluorochrome labelled monoclonal antibodies and flow cytometry (FACS Aria). The absolute number of CD32<sup>+</sup> lymphocytes increased with age. Mean fluorescence intensity, an indicator of the number of CD32 receptors per lymphocyte, was found to be significantly higher in adults. Potential isoforms of Fc receptors were investigated by sorting various cell populations (B cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and monocytes) from peripheral blood mononuclear cells (PBMCs), extracting RNA, and performing reverse-transcriptase – polymerase chain reactions (RT-PCR). We report here three isoforms of Fc receptors previously unrecognized in cattle: a homolog of human Fc gamma RIIB1 (a splice variant), soluble Fc gamma binding factor (lacking a transmembrane region) and Locus 782652, which has high sequence homology in the extracellular region with bovine Fc gamma RIIB2 (an inhibitory receptor) and sequence homology in the intracellular region with CD16 (bovine Fc gamma RIII, an activating receptor). Because inhibitory and activating isoforms of Fc gamma receptors share extracellular domains, detection of these receptors by FACS using monoclonal antibodies specific for the common extracellular domain will not clarify the functional state of the target cell. Studies are in progress to evaluate expression of these different isoforms by subsets of lymphocytes and to assess variation with age. Insights gleaned from this work should improve our understanding of the role of CD32 in the inhibition of neonatal antibody responses by maternal antibodies with a view to improving vaccination strategies in these animals.

## **#6. Zinc physiology of Plasmodium falciparum: Controlling metal availability to inhibit parasite growth**

*Rebecca Copeland, Thomas O'Halloran  
Northwestern University*

Zinc is an integral component within the cell with functions ranging from structural stabilizers to catalytic cofactors within metalloenzymes. The studies presented here focus on the unusual inorganic physiology of the malaria parasite. We and others have found that *P. falciparum* has the striking ability to concentrate zinc within the erythrocyte host to levels reaching twice the original uninfected erythrocyte concentrations. This work begins to examine the physiology of the 'free' or chelator

accessible portion of total zinc within the parasite. The life-stage dependence of free zinc accumulation has been examined as well as a preliminary glimpse into the origin of the acquired metal. In this work, zinc-binding fluorescent probes were used to compare labile zinc accumulation within a parasite-infected erythrocyte containing half its cytosolic proteins. It was found that despite the absence of many host proteins in the ghost, parasites were able to obtain their zinc stores. The inhibitory effects of several zinc-chelating agents suggest labile zinc stores are essential components within the asexual replication stages of the parasite.

## **#7. A Fluorogenic Method To Evaluate Natural Killer Cell Cytotoxicity**

*Anne Prada, Joyce Villanueva, Virgil Villanueva, Barbara Wanstrath, Daniel Marmer, Alexandra Filipovich, MD and Jacob Bleasing, MD, PhD.*

*Diagnostic Immunology Laboratory, Division of Hematology / Oncology, Cincinnati Children's Hospital Medical Center*

The innate branch of the immune system relies on the ability of Natural Killer (NK) cells to recognize and kill intracellular pathogens and tumor cells. NK cells perform this task using the Perforin (Prf) and Granzyme B (GrB) pathway. The process involves mobilization of intracellular granules, containing Prf and Grb, fusing of these granules with the NK-cell cell membrane and transfer to the target cell to initiate the cell death pathway, which ultimately leads to cell lysis. Defects in the Prf/GrB pathway are associated with distinct human disease states that are referred to as familial hemophagocytic lymphohistiocytic disorders, as well as with defined disorders of granules, including Chediak-Higashi syndrome and Griscelli syndrome.

The functional assay that is used to determine the relevance and significance of defects in the Prf/GrB-associated pathway is the NK-cell function assay. The clinical standard for determining NK-cell function has been the <sup>51</sup>Cr release assay (CRA). Target cells (K562 cells) are labeled with Na <sup>51</sup>CrO and upon incubation with patient peripheral blood mononuclear cells (PBMC), target cells are lysed and <sup>51</sup>Cr released. We set out to establish a flow cytometry based assay which would correlate with reported clinical results obtained with the CRA. The GranToxilux® kit from OncoImmunin was utilized for its ease of use and total assay time, as well as the fact that it could provide additional use through its use of GrB transfer. The assay uses a FL4 surface dye for the target cell label and a cell permeable Granzyme B substrate coupled to a FL1 fluorochrome to detect intracellular Granzyme B in target as well as patient effector cells (NK cells). We compared results for 26 control and 55 pediatric patient assays. We achieved good correlation between CRA and Grantoxilux® control assays.

We have established a validated clinical method to evaluate NK function which utilizes flow cytometry and correlates with the standard CRA. The use of a Granzyme B substrate in this assay also allows for the examination of patient effector (NK) cell dysfunction. Patients with defective Prf/GrB mobilization were clearly indicated by the retention of Granzyme B in their effector cells.

## **#8. Novel Lipophilic Tracking Dyes for Monitoring Cell Proliferation.**

*Joseph D. Tario, Jr.<sup>1</sup>, Brian D. Gray<sup>2#</sup>, Stephen S. Wallace<sup>1</sup>, Katharine A. Muirhead<sup>3</sup>, Betsy M. Ohlsson-Wilhelm<sup>4</sup>, and Paul K. Wallace<sup>1</sup>*

<sup>1</sup> Department of Flow and Image Cytometry, Roswell Park Cancer Institute, Buffalo, New York

<sup>2</sup> PTI Research Inc., Exton, Pennsylvania

<sup>3</sup> SciGro Inc. / Midwest Office, Madison, Wisconsin

<sup>4</sup> SciGro Inc. / Northeast Office, Cambridge, Massachusetts

<sup>#</sup> Current address: Molecular Targeting Technologies, Inc., West Chester, Pennsylvania

The advent of contemporary digital instrumentation has enhanced the potential and complexity of flow cytometric experiments, allowing for the detailed investigation of immunological composition and activity. The use of cell tracking labels such as PKH26 and CFSE have been important in observing such cellular features, but their emission characteristics have limited the design of such analyses. As the demand for multiparametric flow cytometry intensifies, it will become increasingly important to utilize a broader range of cell tracking reagents to optimize the measurement of fluorescent signals and to provide flexibility in the use of the limited number of fluorochrome and antibody combinations. We report on the development of three lipophilic membrane dyes, CellVue® Lavender, CellVue® Plum and CellVue® NIR780; with fluorescence emissions in the violet, far-red and near infrared wavelength regions respectively. These reagents demonstrate equivalence to pre-existent lipophilic dyes such as PKH26 in terms of staining procedure, membrane stability, optimal concentration, and effect on cellular proliferation. The CellVue dyes however, exhibit different spectral characteristics than conventional tracking compounds, and capitalize upon the increased number of lasers incorporated into commercially available instrumentation; thus permitting measurement of labeled populations in underexploited regions of the spectrum.

## **#9. Improved Cytometer Florescence Detection Response Utilizing an APD**

*Edward Podniesinski\**, *William G. Lawrence\*\**, and *Paul K. Wallace\**

\*Laboratory of Flow Cytometry, Roswell Park Cancer Institute

\*\* Department of Research, Radiation Monitoring Devices Inc,

Photo Multiplier Tubes (PMTs) are typically used as the most common photon/ amplifier detection device in flow cytometer instruments. Their useful range spans from 200nm up to 800nm but they tend to perform poorly at the upper florescence spectra. Avalanche Photodiodes (APDs) are an alternative device which exhibits the same wavelength detection bandwidth as a PMT but out performs the PMT at longer wavelengths. The APD detector has a better signal to noise performance than the tested PMTs over the entire 500 nm to 1100 nm spectral range. The improvement in signal to noise is small at shorter wavelengths, but becomes substantial in the red and near infrared. Additional data will be presented comparing a commonly used PMT and an APD, highlighting the advantages of using an APD as a florescence detector.

## **#10. Bone Marrow is a Preferential Homing site for Autoreactive T cells in type 1 diabetes**

*Nicolas Perez, Ruobing Li, Subha Karumuthil-Melethil, Karen Hagen and Chenthamarakshan Vasu.*

*University of Illinois at Chicago*

Pancreatic microenvironment is considered as the primary location of autoreactive T cells in type 1 diabetes (T1D). Diabetogenic T cells have also been detected in the spleen of NOD mice. However, it is not known whether the Bone marrow (BM) also contains T cells specific for self-antigen in hosts with autoimmunity. In this study, we investigated the presence of autoreactive diabetogenic T cells in the BM of T1D model NOD mice. Purified T cells from the BM and spleens of female NOD mice of

different ages were tested for their cytokine and proliferative responses by ELISPOT and CFSE dilution methods respectively. The BM T cells from both hyperglycemic and young euglycemic mice have shown profoundly higher T cell proliferation and cytokine production in response to stimulation with pancreatic beta-cell antigens as compared to T cells from spleen. The diabetogenic nature and homing properties of BM and spleen T cells sorted by flow cytometry were compared in adoptive transfer experiments. NOD-Scid mice that received BM T cells showed hyperglycemia earlier than those received splenic T cells. Flow cytometry analysis and histochemical staining of various tissues from T cell recipient NOD-Scid mice revealed rapid expansion and aggressive infiltration of BM T cells into pancreatic islets. Adoptive transfer of CFSE labeled BM T cells into wild-type mice resulted in their homing predominantly to the BM and pancreatic LNs suggesting their pancreatic antigen specificity. Overall, our study shows that a large number of diabetogenic T cells are present in the BM of female NOD mice and these autoreactive T cells can be detected much before the clinical onset of the disease.

### **#11. GM-CSF expanded CD11c+CD8a- DCs suppress autoimmune thyroiditis through the induction of Foxp3+ IL-10+ T regulatory Cells**

*Balaji B Ganesh, Karen Hagen\* and Bellur S Prabhakar  
University of Illinois at Chicago*

Experimental Autoimmune Thyroiditis (EAT) is a well-characterized mouse model for Hashimoto's thyroiditis. We have shown that treatment of CBA/j mice with Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) selectively expands CD11c+CD8a- myeloid dendritic cells (DCs) and that this expansion was associated with an increase in CD4+CD25+ T cells and suppression of autoimmune thyroiditis in an EAT model. In this study, we investigated the significance of the expansion of CD8a- myeloid DCs by GM-CSF and its direct role in inducing CD4+CD25+Foxp3+ T regulatory cells (Tregs). We show that co-culture of sorted CD4+ T cells with sorted CD8a- DCs from GM-CSF treated mice in the presence of mouse thyroglobulin (mTg) induced an increase in the number of CD4+CD25+Foxp3+ T cells that produced high levels of IL-10 as determined by Flow cytometry. These Tregs could suppress the mTg-specific proliferation of effector CD4+CD25- T cells and this suppression was reversed upon addition of anti-IL10R antibodies. In contrast, purified CD8a+ lymphoid DCs from GM-CSF treated mice failed to show a significant suppression of mTg specific T cell proliferation. In vivo experiments revealed that GM-CSF could induce increased numbers of Foxp3 and IL-10 expressing CD4+ T cells in SCID mice adoptively transferred with lymphocytes from wild type CBA/j mice. Moreover, adoptive transfer of purified CD8a- DCs from GM-CSF treated, but not untreated, SCID mice into CBA/J mice conferred protection against the induction of EAT. These results indicate that GM-CSF acts directly on CD8a- myeloid DCs and renders them tolerogenic in that they produce little or no pro-inflammatory cytokines. Antigen presentation by these cells results in the expansion of CD4+Foxp3+ Tregs and suppression of EAT.

### **#12 Use of DNA specific anthraquinone dyes to directly reveal cytoplasmic and nuclear boundaries in live and fixed cells**

*Roy Edward, Biostatus Ltd.*

Image-based high-content screening assays, demand solutions for image segmentation and cellular compartment encoding to track critical events – for example those presented by GFP reporters within cell cycle tracking and GPCR translocation assays. We have designed nuclear and cytoplasm discriminator CyTRAK™ probes - spectrally compatible with all XFP reporter variants offering new solutions in cytometry.

At their most fundamental they provide a convenient fluorescent emission signature which is spectrally separated from the commonly used reporter proteins (e.g. eGFP, YFP, mRFP) and fluorescent tags such as Alexafluor 488, fluorescein and Cy2. Additionally, they do not excite in the UV and thus avoid the complications of compound UV-autofluorescence in drug discovery whilst limiting the impact of background sample autofluorescence. They provide a convenient means of stoichiometrically labeling cell nuclei in live cells without the aid of DMSO and can equally be used for fixed cells. Further developments have permitted the simultaneous and differential labeling of both nuclear and cytoplasmic compartments in live or fixed cells to render the cell boundaries which may be beneficial for quantitative expression measurements and in cell-cell interactions.

Examples of CyTRAK™ probes will be shown on the context of HCS imaging platforms, confocal and epifluorescent microscopy and flow cytometry.

### **#13 Remote-Controlled Flow Cytometry: The use of remote access communication tools for distance learning.**

*David Leclerc, Micael Olson, and Ryan Duggan  
The University of Chicago Flow Cytometry Facility*

Modern, commercially available flow cytometers are more integrated with the PC than ever before. Most every control of the flow cytometer (High Voltage, Compensation, Threshold, Sample Acquisition, Laser selectors, etc...) can be controlled through software. Therefore, it is possible to exploit software tools for remote access to such controls. Presented here are freeware tools used by the University of Chicago Flow Cytometry Facility (UCFlow) for remote control of satellite facility instruments. UCFlow currently has 4 locations on the Campus of the University of Chicago. Using a freeware web application, LogMeIn (logmein.com), technologists sitting at their desks or at the cell sorter can view and control the software running an instrument across campus. Since the user interface is a web browser, the instrument could be operated from anywhere a network connection is available. The main use of these technologies is to assist users in remote locations with the setup, optimization, and gating strategies used for acquiring their data. Additionally, using instant message “chatting” via text, audio and/or video, allows for a robust and dynamic distance learning experience.

## GLIIFCA STEERING COMMITTEE - 2007

### *Contact info*

#### **Canada**

David Hedley david\_hedley@pmh.toronto.on.ca  
Ruth Wilkins Ruth\_Wilkins@hc-sc.gc.ca

#### **Illinois**

Mary Paniagua mpaniagu@bsd.uchicago.edu Executive Secretary  
Charles Goolsby c-goolsby@northwestern.edu  
Maurice R. G. O’Gorman mogorman@northwestern.edu

#### **Indiana**

Lisa Green lisa.green@lilly.com  
Philip Marder phil.marder@lilly.com  
Bartek Rajwa rajwa@flowcyt.cyto.purdue.edu

#### **Iowa**

Bruce Pesch bpesch@nadc.ars.usda.gov

#### **Massachusetts**

Brian DuChateau bkduchateau@bcsew.edu Assistant Treasurer  
Betsy Ohlsson-Wilhelm bmow@scigro.com

#### **Michigan**

Alexander Nakeff anakeff1@hfhs.org Treasurer, Site Committee  
Chair, Vendor Contact  
Louis King kingl@msu.edu

#### **Minnesota**

Waclaw Jaszcz jaszcz001@maroon.tc.umn.edu  
Wendy Walters waltew@parknicollet.com  
Paul Champoux champ004@umn.edu

#### **New York**

Tim Bushnell Tim\_Bushnell@URMA.Rochester.edu Webmaster, 2007 President &  
Program Chair  
Carleton & Sigrid. Stewart ccs2sjs@earthlink.net Historians  
Paul Wallace Paul.wallace@roswellpark.org 2008 President and Program  
Chair

#### **Ohio**

Karen Domenico kdomenico@mco.edu Vendor Contact  
R. Michael Sramkoski rms19@po.cwru.edu  
Tom Sawyer tsawyer@mco.edu Vendor Contact

#### **Pennsylvania**

Vera Donnenberg donnenbergvs@upmc.edu  
Jonni Moore moorej@mail.med.upenn.edu

#### **Wisconsin**

Katharine Muirhead kmuirhead@scigro.com  
Kathleen Schell kschell@facstaff.wisc.edu  
Matthew Hanson hansonm@surgery.wisc.edu

## GLIFCA 16 Registered Attendees

First_Name	Last_Name	Email_address	Institution
Lina	Alam	lalam@uhnres.utoronto.ca	Princess Margaret Hospital
Dave	Allman	dallman@mail.med.upenn.edu	University of Pennsylvania
Larry	Arnold	lwarma@med.unc.edu	Univ. of North Carolina - Chapel Hill
Cheryl	Aslakson	cheryl.aslakson@stjohn.org	St. John Hospital & Medical Center
Julie	Auger	jauger@bsd.uchicago.edu	Univ of Chicago
C. Bruce	Bagwell	cbb@vsh.com	Verity Software House
Andrew	Bantly	abantly@mail.med.upenn.edu	University of Pennsylvania
Eyayu	Belay	eyayu.belay@med.KUL.be	University Leuven
Michael	Betts	betts@mail.med.upenn.edu	University of Pennsylvania
Rita	Bowers	bowers_rita_k@lilly.com	Eli Lilly and Company
Timothy	Bushnell	tim_bushnell@urmc.rochester.edu	URMC
John	Casper	jasper@assaydesigns.com	Assay Designs Inc.
Kristin	Chadwick	kchadwick@robarts.ca	Robarts Research Institute
Paul	Champoux	champ004@umn.edu	University of Minnesota
Sue	Chow	sue.chow@uhn.on.ca	Princess Margaret Hospital
Matt	Cochran	matthew_cochran@urmc.rochester.edu	University of Rochester
Rebecca	Copeland	r-copeland@northwestern.edu	Northwestern University
BUNNY	COTLEUR	bunny@cotleur.com	CLEVELAND CLINIC, LERNER RESEARCH INSTITUTE
Jennifer	Cox	jcox@assaydesigns.com	Assay Designs Inc.
Mary	Dell	mary.dell@roswellpark.org	Roswell Park Cancer Institute
Karen	Domenico	Karen.Domenico@utoledo.edu	University of Toledo Medical Center
Albert	Donnenberg	donnenbergad@upmc.edu	University of Pittsburg
Vera	Donnenberg	donnenbergvc@upmc.edu	University of Pittsburg
Brian	DuChateau	bduchateau@convergedx.com	Converge Diagnostic Services
Ryan	Duggan	rduggan@bsd.uchicago.edu	University of Chicago
Dan	Fisher	Daniel.Fisher@RoswellPark.org	Roswell Park Cancer Institute
Ramsey	Fuleihan	r-fuleihan@northwestern.edu	Children's Memorial Hospital
Balaji	Ganesh	bganesh@uic.edu	University of Illinois at Chicago
Charles	Goolsby	c-goolsby@northwestern.edu	Northwestern University
Lisa	Green	Lisa.Green@Lilly.com	Eli Lilly & Co.
Brian	Grimberg	brian.grimberg@case.edu	Case Western Reserve University
Karen	Hagen	khagen@uic.edu	University of Illinois at Chicago
Paul	Hallberg	hallberg@email.chop.edu	Children's Hospital of Philadelphia
Kristi	Harkins	kristiharkins@hscteam.com	Harkins Strategic Consulting
Karen	Helm	Karen.helm@uchsc.edu	University of Colorado
Jamie K	Henderson	jkhender@wisc.edu	UW- Flow Cytometry Facility
Lindsay	Hendey	HendeyL@battelle.org	Battelle
Rebecca	Herman	HermanRK@battelle.org	Battelle
Douglas	Hodgins	dhodgins@uoguelph.ca	University of Guelph
Cheryl	Holdman	cheryl@flowcyt.cyto.purdue.edu	Purdue University
Bryan	Hughes	bryan.hughes@mirusbio.com	Mirus Bio Corp
Shilpa	Kalavapudi	KalavapudiS@battelle.org	Battelle
Adela	Khoong	akhoong@nmh.org	Northwestern Memorial Hospital
Jung-Sun	Kim	jkim@med.wayne.edu	Wayne State University
Louis	King	kingl@mail.msu.edu	Michigan State University
James	Kobie		University of Rochester
Barbara	Kutzner	Barbara_Kutzner@hc-sc.gc.ca	Health Canada
Joanne	Lannigan	jl7fj@virginia.edu	University of Virginia

Anthony	Lauer	anthony.lauer@mirusbio.com	Mirus Bio Corp
DAVID	LECLERC	DLECLERC@BSD.UCHICAGO.EDU	UNIVERSITY OF CHICAGO
Laurence	Lejeune	llejeune@ldi.jgh.mcgill.ca	Lady Davis Institute
Sally	Madsen-Bouterse	smadsenb@med.wayne.edu	Perinatology Research Branch, NICHD/NIH
Frances	Mandy	fmandy@rogers.com	National Laboratory for Analytical Cytology
Larry	Mann	mann_larry_1@lilly.com	Eli Lilly and Co.
Philip	Marder	phil.marder@lilly.com	Lilly Research Labs
Laura	Marszalek	lmarszal@nmh.org	Northwestern Memorial Hospital
James	Marvin	j-marvin@northwestern.edu	Northwestern University
Betty-Anne	McBey	bmbey@uoguelph.ca	University of Guelph
John	McCullough	john.mccullough@invitrogen.com	Invitrogen Molecular Probes
Bryan	McElwain	bryan.mcelwain@osumc.edu	Ohio State University
Nicole	McFarlane	mcfarln@mcmaster.ca	McMaster University
Kathleen	McGrath	mckat1959@gmail.com	University of Rochester
Janet	McLaughlin	jmclaugh@nmh.org	Northwestern Memorial Hospital
Hans	Minderman	hans.minderman@roswellpark.org	Roswell Park Cancer Institute
Christina	Moeller	christina.moeller@stjohn.org	St. John Hospital
Jonni	Moore	moorej@mail.med.upenn.edu	University of Pennsylvania
Brenda	Morse	bmorse@unmc.edu	University of Nebraska Medical Center
Alan	Moser	allan.moser@ciradiscovery.com	Cira Discovery Sciences, Inc.
Kathy	Muirhead	kmuirhead@scigro.com	SciGro, Inc/MidWest Office
Bill	Murphy	murphyw@mail.med.upenn.edu	University of Pennsylvania
Rakesh	Nayyar	rnayyar@uhnres.utoronto.ca	Princess Margaret Hospital
Maurice	O'Gorman	mogorman@childrensmemorial.org	Children's Memorial Hospital
Betsy	Ohlsson-Wilhelm	bmow@scigro.com	SciGro, Inc./NorthEast Office
Paul	Oleynik	poleynik@mail.mcgill.ca	Ontario Health Research Institute
Mike	Olson	mikeolson540@yahoo.com	University of Chicago
Carolina	Ostigui	costigui@nmh.org	Northwestern Memorial Hospital
Nicholas	Ostrout	ndo2@case.edu	Case Western Reserve University
Brenda	Paige	bpaige1@uic.edu	University of Illinois-Chicago
Mary	Paniagua	mpaniagu@bsd.uchicago.edu	University of Chicago
Nicolas	Perez	nperez3@uic.edu	University of Illinois at Chicago
Hank	Pletcher	pletcher@mail.med.upenn.edu	University of Pennsylvania
Edward	Podniesinski	ed.podniesinski@roswellpark.org	Roswell Park Cancer Institute
Jennifer	Powers	powersj@ccf.org	Cleveland Clinic
Anne	Prada	aeprada@comcast.net	Cincinnati Children's Hospital
Joel	Puchalski	jrpuhalski@wisc.edu	University of Wisconsin-Madison
Erik	Puffer	EBPUFFER@WISC.EDU	University of Wisconsin
Sally	Quataert	Sally_Quataert@urmc.rochester.edu	University of Rochester Medical Center
Kathy	Ragheb	kathy@flowcyt.cyto.purdue.edu	Purdue University
Bartlomiej	Rajwa	brajwa@purdue.edu	Purdue University
Lova	Rakotomalala	lova@flowcyt.cyto.purdue.edu	Purdue U.
Shawn	Rigby	rigbysm@iastate.edu	Iowa State University
J. Paul	Robinson	jpr@flowcyt.cyto.purdue.edu	Purdue University
Alexander	Rodriguez	arr11@case.edu	Case Western Reserve University
Wade	Rogers	wade.rogers@ciradiscovery.com	Cira Discovery Sciences, Inc.
Tom	Sawyer	Tom.Sawyer@utoledo.edu	University of Toledo Medical Center
Kathleen	Schell	kschell@wisc.edu	University of Wisconsin
Rick	Schretzenmair	rds@mail.med.upenn.edu	University of Pennsylvania
Joel	Sederstrom	sederstr@bcm.edu	Baylor College of Medicine
Beth	Seidler	Elizabeth.Seidler@stjohn.org	St. John Hospital

T Vincent	Shankey	Vincent.Shankey@Coulter.com	Beckman Coulter, Inc
Dagna	Sheerar	dsheerar@wisc.edu	University of Wisconsin - Madison
Cathy	Shemo	shemoc@ccf.org	Cleveland Clinic
Patricia	Simms	psimms@lumc.edu	Loyola University Medical Center
Michael	Sramkoski	r.sramkoski@case.edu	Case Western Reserve University
Laura	Storck	Laura.M.Storck@gsk.com	GlaxoSmithKline
Jennifer	Sturgis	jennie@flowcyt.cyto.purdue.edu	Purdue University
Suchitra	Swaminathan	suchitra-swaminathan@northwestern.edu	Northwestern University
Gabriella	Szekely-Klepser	gszekelyklepser@assaydesigns.com	Assay Designs Inc.
Joseph	Tario, Jr.	joseph.tario@roswellpark.org	Roswell Park Cancer Institute
Frances	Tong	ftong01@yahoo.ca	Princess Margaret Hospital
Gregory	Veltri	greg.veltri@cancer.org.uk	CRUK Cambridge Research Institute
Paul	Wallace	pkw@rpciflow.org	Roswell Park Cancer Institute
Dionne	White	dionne.white@utoronto.ca	University of Toronto
Nicole	White	white.1140@osu.edu	Ohio State University
Linda	Wilkie	lwilkie@unmc.edu	University of Nebraska Medical Center
Sharlene	Wright	sharlene.wright@coulter.com	Beckman Coulter
Ryan	Wychowanec	rdw@mail.med.upenn.edu	University of Pennsylvania
Robert	Zigon	robert.zigon@beckman.com	Beckman Coulter

## Registered Vendor Representatives

**Accuri Cytometers, Inc.**, Ann Arbor, MI

[www.accuricytometers.com](http://www.accuricytometers.com)

Jack Ball

Leo Ostruszka

**Amnis Corporation**, Seattle, WA

[www.amnis.com](http://www.amnis.com)

Scott Levy

Rick Demarco

**Applied Cytometry Sys.**, Sacramento, CA

[www.appliedcytometry.com](http://www.appliedcytometry.com)

Tracey Long

Philip Poemoceah

**BD Biosciences**, San Jose, CA

[www.bdbiosciences.com](http://www.bdbiosciences.com)

Bill Gunderman

Andy Oberszyn

Dave Ehman

Larry Duckett

David Matsuyama

Daniel Sirk

Lori Krueger

Andre Lubarsky

**Beckman Coulter**, Miami, FL

[www.beckmancoulter.com](http://www.beckmancoulter.com)

Dave McFarlane

Carole MacDougall

Paul Scibelli

**BioLegend**, San Diego, CA

[www.biolegend.com](http://www.biolegend.com)

Xifeng Yang

**Biostatus**, UK

[www.biostatus.com](http://www.biostatus.com)

Roy Edward

**Cedarlane Laboratories**, Hornby, ON

[www.cedarlanelabs.com](http://www.cedarlanelabs.com)

Tahir Maqbool

Moyo Williams

**Cell Signaling Technology**, Beverly, MA

[www.cellsignal.com](http://www.cellsignal.com)

Carrie Ann Brown

**CompuCyte**, Cambridge, MA

[www.compucyte.com](http://www.compucyte.com)

Scott Baldwin

**Cytek Development**, Gordonsville, VA

[www.cytek.com](http://www.cytek.com)

Ray Lannigan

Tim Johnson

Eric Chase

**Dako of Colorado**, Fort Collins, CO

[www.dako.com](http://www.dako.com)

Anita Jaichandra

Karen Helm

Jeff Louie

**De Novo Software**, Thornhill, Ontario

[www.denovosoftware.com](http://www.denovosoftware.com)

David Novo

Daniel Zimmerman

**eBioscience**, San Diego, CA

[www.ebioscience.com](http://www.ebioscience.com)

Edwin Chau

**GCAT, Inc.**, Fort Collins, CO

[www.gcatinc.com](http://www.gcatinc.com)

Roy Overton

**Guava Technologies**, Hayward, CA

[www.guava.com](http://www.guava.com)

Rich Doda

Dan Freeck

**iCyt Visionary Bioscience**, Champaign, IL

[www.i-cyt.com](http://www.i-cyt.com)

Fred Molnar

**Invitrogen**, Carlsbad, CA

[www.invitrogen.com](http://www.invitrogen.com)

Jolene Bradford

Kelly Lundsten

Hether Ide

Sara Staggs

**Perkin-Elmer**, Waltham, MA

<http://www.perkinelmer.com>

Rich Belcher

Ryan Luther

**Spherotech, Inc.**, Libertyville, IL

[www.spherotech.com](http://www.spherotech.com)

Brain Shah

**Stem Cell Technologies**, Vancouver, BC

[www.stemcell.com](http://www.stemcell.com)

**TreeStar, Inc.**, Ashland, OR

[www.treestar.com](http://www.treestar.com)

John Quinn

Susan Alderson

**Union Biometrika, Inc.**, Somerville, MA

[www.unionbiometrika.com](http://www.unionbiometrika.com)

Kathleen Barnhart

Weon Bae

**Verity Software House**, Topsham, ME

[www.vsh.com](http://www.vsh.com)

Mark Munson

Bruce Bagwell

## **GLIIFCA 16 Anthem**

***Lyrics by Tim Bushnell***

(Sung to the tune of 16 Candles – originally by The Crests)

Happy Birthday, Happy birthday GLIIFCA...

I love you so

Sixteen colors make a lovely light  
But not as bright as your FITC right now (as your FITC right now)  
Gate your pop-u-laaa-tions, make them all sort right  
For I'll be wishing... a first author paper, too (first author paper too)

You're only sixteen (sixteen)  
But you're my ace flow machine (ace flow machine)  
You're the most colorful, loveliest machine  
I've ever seen (I've ever seen) (Oh!)

Sixteen colors in my mind will glow  
For ever and ever I'll sort with you (I'll sort with you)

You're only sixteen (sixteen)  
But you're my ace flow machine (ace flow machine)  
You're the most colorful, loveliest machine  
I've ever seen (I've ever seen) (Oh!)

Sixteen colors in my mind will glow  
For ever and ever I'll sort with you (I'll sort with you)  
For I love to sort!!!

**NOTES:**

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