Great Lakes International Imaging and Flow Cytometry Association

GLIIFCA 19

September 24th- September 26th, 2010
Detroit, MI, USA
www.gliifca.org

2010 Program Chairs:	**Vera Donnenberg, President**
	Keith Shults
	Joanne Lannigan
Site Organizer:	**Alexander Nakeff**

Corporate Sponsors & Members:

GLIIFCA 19

Program

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GLIIFCA 19 GENERAL INFORMATION

(What You Always Wanted To Know About GLIIFCA 19 But Were Afraid To Ask!)

CONFERENCE REGISTRATION DESK: Foyer Mackinac, Level 5

Conference registration fee ($80 early registration, $95 on-site) includes Friday reception, Sat/Sun breakfast, Sat. Lunch, evening reception, banquet and coffee breaks.

Friday, Sept 24 6:00 to 11:00pm
Saturday, Sept 25 8am to 11pm
Sunday, Sept 26 9am to 12:00pm

POSTERS:

***SET UP**:* Friday, September 24 after 5pm Mackinac Ballroom, Level5
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 using only ***VELCRO*** only
Viewing on Friday to Sunday 10:30am
Presentation and Judging: Saturday 5:00 to 7pm

EXHIBITS:

Scheduled exhibitors will have booths in the Exhibit/Poster area (Mackinac Level 5)
Booths will be open from 6pm Friday to the end of the meeting
All activities other than the plenary sessions, roundtable luncheon, Steering Committee meeting and banquet
will be located in the Exhibit/Poster area (Mackinac Level 5)

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.

BREAKFASTS:

Free continental breakfast provided for all registrants in the Exhibit/Poster area (Mackinac Level 5) on:

Saturday: 7 to 8am
Sunday: 8 to 9am

Steering Committee breakfast meeting, Sunday morning (7:30 to 9am) in La Salle A+B

COFFEE BREAKS:

Snacks and drinks available in the Exhibit/Poster area (Mackinac Ballroom Level 5) - no need to line up!

INDUSTRIAL SCIENCE SYMPOSIUM & FRIDAY RECEPTION:

Industrial Science Symposium presentations: 7 to 10pm, Marquette A+B, Level 5

Reception: 5:30 to 7:30pm and 10 to 11pm, Mackinac Level 5
Use 3 drink tickets for wine and beer

SYMPOSIA LOCATION: Marquette A+B

SATURDAY LUNCHEON ROUNDTABLES (12 to 1:30pm):
Free lunch/pop; 2/3 ham/turkey and 1/3 veggie wrap in Diluth A/B (Tables 1-4), LaSalle A (Tab 5-8), LaSalle B (Tab. 9-12), Brule B (3 tables for non-participants in workshops), Level 5
Pick up wrapped sandwiches (roast beef, turkey and veggie wrap, condiments, chips, cookie, fruit and soft drink and move to roundtable of 10 labeled with the title of discussion topic - attendance at each table determined from signup sheet at GLIIFCA registration desk. Non-participants can lunch in Brule B.

SATURDAY WINE AND CHEESE HAPPY HOUR:
5:00 to 8pm in the Exhibit/Poster area (Mackinac Level 5) with cheese and fresh fruit trays (use drink tickets).

BANQUET:
Free to registrants and paid guests
Commences at 8pm, Cabot Ballroom, Level 4.
Numerous food stations (salad, entrée and dessert) provided to minimize waiting time
Full service bar available for drinks (use drink tickets or pay cash)
DJ with dance music until 11pm; requests encouraged (get up and have fun!)

DRINKS:
Full service bar located in the Exhibit/Poster area (Mackinac Ballroom, Level 5) for Friday reception and Saturday afternoon for Wine & Cheese reception and Cabot Ballroom, Level 4 for banquet Saturday evening
Three free drink tickets/registrant ***for beer and wine only*** – beer in bottles/cans
Mixed drinks – cash bar (your cost).
All pop in bottles/cans is free

FACILITIES/SERVICES:
PARKING: Use Beaubien Place Parking, self-parking [$12/day & $8/day (Sat/Sun) with NO in-and-out privileges] or valet service
Message Board: on easel next to the GLIIFCA Registration Desk, Mackinac Ballroom Foyer, Level 5
Xerox copying, faxing, etc: Ask at Marriott Hotel Registration (Level 3)

CMLE CREDITS:
To receive CMLE credit, sign application form at the GLIIFCA registration desk

NAME TAGS AND EVALUATION FORMS: Before leaving….

Fill out evaluation form and leave at Registration/Check Out Desk with your name tag

ADDITIONAL ENQUIRIES: Contact Dr. Alexander Nakeff (Email: caralex3@comcast.net; Cell: (313) 820-6227) or leave message for him at the GLIIFCA registration desk.

GENERAL MEETING SCHEDULE:

All symposia to be held in the Marquette A+B, Level 5. Poster viewing, Vendor Exhibits, breakfasts and breaks will all be held in Mackinac, Level 5.

Friday, September 24

10a – 3p **Resource Managers Workshop**
Sponsored by: GLIIFCA & ISAC

4p – 10p **Registration**
Foyer, Mackinac Level 5

5:30p – 11p **Opening Reception**
Mackinac Level 5
Sponsored by: iCyt

7p – 10p **Industrial Science Symposium**
Marquette A+B, Level 5

Saturday, September 25

7a – 8a **Continental Breakfast**

8a – 11a **Symposium I**
Clinical Cytometry
Phil Marder Memorial Lecture

9:30 – 10a **Coffee Break**
Sponsored by: Spherotech

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

12:00-1:30p **Luncheon Roundtables**
See page 3 for locations

1:30p – 5:15p **Symposium II**
Cutting Edge Cytometry

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

5:00p – 8p **Wine & Cheese Reception**
Sponsored by: Imgenex

5:00p – 7p **Poster Presentations and Judging**

8p – 11p **GLIIFCA Annual Banquet**
Sponsored by: Accuri, Becton-Dickinson
Cabot Ballroom, Level 4

Party Theme: Star Wars!
Costume Prize Sponsored by TreeStar

Sunday, September 26

8a – 9a **Continental Breakfast**

7:30a – 9a **Steering Comm. Meeting**
LaSalle A+B

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

10:30a – 11a **Coffee Break**
Sponsored by: Beckman Coulter

11:45a–12p **Closing Remarks/Awards**

Chao-Huei Jeffrey Wang Memorial Achievement Award
Sponsored by: Spherotech

Alex Nakeff Young Investigator Award

Poster Awards
Sponsored by:
Jackson Immunoresearch (2), Cell Signaling Technologies (1), Tree Star (1),Imgenex (1), Millipore (1), R&D (2), Cytek (1)

Travel Stipend Awards
Sponsored by:
Tree Star (1), R&D (3)

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

Speaker Schedule – At a Glance

Resource Managers' Workshop
Friday, September 24th, 10:00a – 3:00p
Convener: Sally Quataert

Industrial Science Symposium
Friday. September 24th, 7:00p – 10:00p
Convener: Karen Domenico

7:00-7:20 *Joe Trotter, Becton Dickinson*, New Influx Software: Fundamentally Changing How Software Supports Cell Sorting.

7:20-7:40 *Matthew Shallice, Life Technologies*, Breakthrough Technology for Cellular Analysis: The Applied Biosystems Attune Acoustic Focusing Cytometer, Novel Cytometry Reagents from Life Technologies-Molecular Probes, and New Applications.

7:40-8:00 *Maria Dinkelmann, Accuri Cytometers*, Simplifying Flow Cytometric Analysis by Standardizing the Fluorescence Detection Scale.

8:00-8:20 *Nikesh Kotecha, Cytobank*, Cytobank: A Web-based Approach for Analysis and Publication of Flow Cytometry Experiments.

8:20-8:40 *Jeffrey Cobb, Beckman Coulter*, A Novel Orange-emitting Dye for use in Multicolor Flow Cytometry Panels using Violet Diode Laser Excitation.

8:40-9:00 *Nicholas Ostrout, Treestar, Inc.*, Antibody Panel Design--Revolutionized.

9:00-9:20 *Young Song, iCyt Mission Technologies,* Eclipse: A Multi-laser, Versatile Affordable Flow Cytometry Analyzer with Outstanding Sensitivity, Resolution, and Throughput.

9:20-9:40 *Ashley Weant, Miltenyi Biotec, Inc.*, Enumeration of Circulating Endothelial Progenitor Cells by utilizing the MACS Enrichment Unit and Flow Analysis by the MACSQuant Analyzer.

9:40-10:00 *Nathan Portier, Union Biometrica.* Flow Cytometry for Analysis and Sorting of Large Objects Using Union Biometrica's New BioSorter Instrument.

Symposium I – Clinical Cytometry, Special Lecture
Saturday, September 25th, 8:00 am – 11:00 am
Convener: Vera Donnenberg

8:00-8:45 *Joseph Tario, Jr., RPCI*, Assessing Cytotoxic Potential Using Flow Cytometry

8:45-9:30 *J. Paul Robinson, Purdue University,* Everest, Death & AIDS: Three Relatives.

9:30-10:00 Coffee Break

10:00-10:45 Phil Marder Memorial Lecture *Lisa Green, Covance Laboratories*, Flow Cytometry as an Essential Tool in Pharmaceutical Discovery and Development—A Tribute to the Live and Groundbreaking Career of Philip Marder (1948-2010).

11a-12p The Carleton and Sigrid Stewart Keynote Lecture. *Katharine A. (Kathy) Muirhead, COO, SciGro, Inc.* Cell Tracking: Past, Present and Future.

Luncheon Roundtables
12:00p – 1:30p
Conveners: Joanne Lannigan, Paul Champoux

Symposium II – Cutting Edge Cytometry
Saturday, Sept. 26th, 1:30 pm – 5:00 pm
Conveners: Vera Donnenberg, Joanne Lannigan

1:30 – 1:45 *Ryan Wetzel, U Pittsburgh*, Targeting Embryonic Pathways in Cancer

1:45-2:15 *Robert F. Murphy, Carnegie Mellon*, Proteome-Scale Analysis and Modeling of Subcellular Patterns

2:15-3:00 *Lisa Nichols, U of Virginia,* A model system for therapeutic vaccination for HIV-associated malignancies—tracking down persistent KSHV infection in vivo.

3:00- 3:30 *William Buchser, U Pittsburgh,* Imaging the Interaction of Tumor and Immune Cells- in vitro and in vivo High Content Analysis

3:30-3:45 Coffee Break

3:45 – 4:30 *Jonathan Kipnis, U Virginia*, Mind-Body Connection—T Lymphocytes Control Cognitive Function

4:30 – 5:15 Eric Chase, Cytek Development, Using Q and B Values to Predict the Resolution of Dimly Stained Cells from Instrument Noise

5:00p – 7:00p Poster Presentation and Judging

Symposium III – Technical Innovations
Sunday, September 26th, 9:00 am – 11:45 am
Convener: Keith Shults

9:00 – 9:45 *Michael Meyer, U Pittsburgh*, An Enhancement to Forward Light Scatter Collection Improves Resolution of Red Blood Cell Derived Microvesicles.

9:45 – 10:30 Giacomo Vacca, Abbott Laboratories, Laser Rastering: Technology and Applications.

10:30 – 11:00 Coffee Break

11:00 – 11:45 Todd Covey, Nodality, Inc. Single Cell Network Profiling (SCNP) by Flow Cytometry as a Tool toMeasure Potency, and Selectivity of JAK/STAT Inhibitors in PBMC and Whole Blood Discrete Cell Subsets

Industrial Science Symposium

Friday, September 24th
7pm – 10pm, Marquette A+B, Level 5

Convener: Karen Domenico, University of Toledo Medical Center

7:00-7:20 New Influx Software: Fundamentally Changing How Software Supports Cell Sorting.
Joe Trotter, Becton Dickinson.

7:20-7:40 Breakthrough Technology for Cellular Analysis: The Applied Biosystems Attune Acoustic Focusing Cytometer, Novel Cytometry Reagents from Life Technologies-Molecular Probes, and New Applications.
Matthew Shallice, Life Technologies.

7:40-8:00 Simplifying Flow Cytometric Analysis by Standardizing the Fluorescence Detection Scale.
Maria Dinkelmann, Accuri Cytometers.

8:00-8:20 Cytobank: A Web-based Approach for Analysis and Publication of Flow Cytometry Experiments.
Nikesh Kotecha, Cytobank.

8:20-8:40 A Novel Orange-emitting Dye for use in Multicolor Flow Cytometry Panels using Violet Diode Laser Excitation.
Jeffrey Cobb, Beckman-Coulter.

8:40-9:00 Antibody Panel Design--Revolutionized.
Nicholas Ostrout, Treestar, Inc.

9:00-9:20 Eclipse: A Multi-laser, Versatile Affordable Flow Cytometry Analyzer with Outstanding Sensitivity, Resolution and Throughput.
Yong Song, iCyt Mission Technologies.

9:20-9:40 Enumeration of Circulating Endothelial Progenitor Cells by utilizing the MACS Enrichment Unit and Flow Analysis by the MACSQuant Analyzer.
Ashley Weant, Miltenyi Biotec, Inc.

9:40- 10:00 Flow Cytometry for Analysis and Sorting of Large Objects Using Union Biometrica's New BioSorter Instrument.
Nathan Portier, Union Biometrica.

Abstracts:

New Influx Software: Fundamentally changing how software supports cell sorting.

Joe Trotter, Director of Cytometry within the Advanced Technology Group, BD Biosciences

New software for the BD Influx cell sorter will be presented that makes use of 1: Proven web based Representational State Transfer (REST) technology and 2: Many of the new Microsoft components native to Windows 7 and .Net. These two components provide a client-server separation of responsibilities and tasks that not only utilizes the best available technologies, but provides a new way to efficiently interact with a state of the art cell sorter. The new server software component that uses a RESTful approach for the BD Influx will be discussed and demonstrated along with a completely new user interface created using Microsoft .Net and the Windows Presentation Foundation framework . Now, not only can time, light scatter and fluorescence data can be saved for each event, but all the other relevant processing information as well, making features such index sorting an intrinsic powerful part of the new system.

Breakthrough Technology for Cellular Analysis: The Applied Biosystems Attune Acoustic Focusing Cytometer, Novel Cytometry Reagents from Life Technologies-Molecular Probes, and New Applications.

Matthew Shallice, Life Technologies.

The Attune ™ Acoustic Focusing Cytometer is the first cytometer on the market to use sound waves to focus cells for single cell analysis which gives significant performance advantages compared to traditional flow cytometers. This technology allows users the ability to quickly process dilute samples and to control transit times in the laser beam, yielding increased precision and improved resolution at higher throughput. To demonstrate the capabilities of the Attune™ Acoustic Focusing Cytometer, this talk will highlight some novel reagents including CellTrace™ Violet Cell Proliferation Kit, a CFSE alternative for the violet laser and a dual pulse labeling technique using the Click IT® Cell Proliferation Assays. Besides new reagent technologies, a number of novel applications will be highlighted including resolving PBMC populations in dilute whole blood for rare event detection and new opportunities to circumvent spectral overlap without the need for compensation.

Simplifying Flow Cytometric Analysis by Standardizing the Fluorescence Detection Scale

Maria Dinkelmann, Ph.D., Accuri Cytometers, Inc. Ann Arbor, MI

The design concept of the Accuri® C6 Flow Cytometer® System is to standardize fluorescence detection so that each C6 produced has predictable, comparable performance. This standardization is performed at the time of manufacture using industry-standard beads (the Spherotech Rainbow Fluorescent Particles) to optimize both the optical alignment of lasers and detectors and the voltage settings of the light scatter and fluorescence detectors. The end-user has no need to make adjustments to optical alignment or voltage settings. We report findings on the performance of this flow cytometer which show 1) 34 instruments in active use in the field for 6 to 24 months maintained alignment and fluorescence performance as indicated

by Spherotech Rainbow Bead data, 2)comparison of fluorescence detection on ten C6 instruments using 2 other well-known bead standards (Thermo Scientific Cyto-Cal and BD C,S & T) showed no significant difference in fluorescence detection between instruments, and 3) comparison of 4 different CD4-fluorophore conjugates from 3 different manufacturers were found to give highly reproducible, predictable fluorescence intensity values on the C6 fluorescence scale, regardless of blood donor, C6 instrument used, or variations in the level of non-specific, background staining of the non-CD4 expressing lymphocytes. The practical implications of standardized fluorescence detection include simplification of flow cytometric assays through the use of standard templates and the removal of voltage changes by operators as a source of variation, yielding consistent results over time, and between different C6 instruments.

Cytobank: A Web-based Approach for Analysis and Publication of Flow Cytometry Experiments

Nikesh Kotecha, PhD, Cytobank Inc.

Advances in cytometry have enabled routine generation of multi-color flow cytometry experiments designed to measure large numbers of samples across tens of markers and generating a large amount of raw data and make it difficult to manage, analyze and collaborate with these experiments. Cytobank (www.cytobank.org) is a web-based platform to manage, share and analyze your flow cytometry data from anywhere. The approach grew out of work in Dr. Garry Nolan's lab at Stanford to handle large flow experiments, provide various levels of experiment access (from analyzed and gated results to raw data) and enable a platform for novel visualizations and analyses of flow cytometry data. Cytobank is available to the community at www.cytobank.org

A Novel Orange-Emitting Dye for Use in Multicolor Flow Cytometry Panels Using Violet Diode Laser Excitation

Jeffrey Cobb, Beckman Coulter, Inc., [1]Miami, FL, USA

Background. Violet diode lasers have become common excitation sources on flow cytometers, but the advantages of this laser are constrained by limited choices for organic, violet-excited fluorochromes. These fluorochromes include several dyes emitting around 450 nm, and two (Horizon™ V500 and Pacific Orange™ dyes) emitting at longer wavelengths. These longer wavelength fluorochromes are plagued by relatively low fluorescence signals in relation to background, and generally must be used with densely expressed antigens. We have developed a novel violet-excited organic dye, given the development name "UV3," which has an emission spectrum closely matching that of Pacific Orange dye. It has excitation and emission maxima of 398 nm and 528 nm, respectively.
Methods. Antibodies were conjugated with UV3 dye via N-hydroxysuccinimide esters. Human blood was stained with antibody conjugates and analyzed on a Gallios™ flow cytometer equipped with violet, blue, and red excitation.
Results. The UV3 fluorochrome shows optimal performance with violet laser excitation and a 550/40 bandpass filter (the standard FL10 channel on Gallios and Navios™ flow cytometer systems), while no excitation is detected using a 488 nm laser. UV3 dye is at least as bright as V500 dye and can provide more than twice the population separation obtained with Pacific Orange dye conjugates, with little compensation versus Pacific Blue™ dye. Therefore, common gating markers can be easily transferred to this fluorochrome, freeing other valuable fluorochromes for use with additional markers.

Conclusions. UV3 dye conjugates can be used on any flow cytometer equipped with violet excitation (approximate wavelength range 400 to 410 nm) and appropriate emission filters. Data is shown using UV3 conjugates of anti-human CD4, CD19 and CD45 in multicolor applications, including side scatter/CD45 gating and a 6-plus color stain. As a result, UV3 dye provides an optimized second organic fluorochrome for violet excitation, enabling 10-color applications on the Gallios / Navios flow cytometer systems.

Antibody Panel Design--Revolutionized.

Nicholas Ostrout,PhD, Treestar, Inc.

The current process of selecting and ordering antibodies for flow cytometry panels is antiquated and cumbersome. Sifting through the thousands of targets, formats and clones from a number of major manufacturers is time consuming and inhibits effective comparison, thereby reducing optimal panel design. Furthermore, properly matching the fluorochromes to the instrument is often difficult for novice and even advanced users considering the complexity of most systems today. FlowJo has designed and created a new tool, the Antibody Panel Development Wizard. The wizard is designed to take users through a step wise process to develop optimal antibody panels for their experiments based on the configuration of the particular instrument selected. Optimal fluorochromes are automatically identified based on the instrumental set up. Excitation and emission wavelengths of all fluorochromes have been entered into the database, so as users select specific formats, results refine to limit spectral overlap and prevent the selection of fluorochromes with identical emission profiles. The database currently features reagents from several leading antibody manufacturer, allowing the customer the convenience of many product options and comparisons in one location. The Antibody Panel Development Wizard promises to be an innovative and informative new tool to help keep pace with new trends in flow cytometry.

Eclipse™: A Multi Laser, Versatile Affordable Flow Cytometry Analyzer with Outstanding Sensitivity, Resolution and Throughput

Yong Song, Ph.D Product Manager iCyt Mission Technology, Champaign, IL

The iCyt® Eclipse analyzer is a compact bench top flow cytometry analyzer with an autoloader that supports a wide variety of multi-well plate and test tube formats. With up to four lasers (405, 488, 561, 642 nm) and user-configurable fluorescent detectors, the Eclipse is compatible with an immense selection of fluorochromes and fluorescent proteins. The system provides simultaneous detection of 5-color, forward scatter, side scatter and electronic volume (EV) with excellent sensitivity and resolution. It can process a 96-well plate in less than 60 minutes. The Eclipse provides a powerful analysis tool for most flow cytometry applications, including immunophenotyping, absolute cell counting, viability, apoptosis, particle sizing and cell cycle analysis. The EV parameter provides accurate cell volume (size) measurement. Furthermore, with accurate cell volume measurement, fluorescence concentration or surface density can be determined allowing for normalization of fluorescent intensity on a per volume or surface area basis. The intuitive Eclipse software simplifies operation and enables data analysis and easy database management. The highly versatile Eclipse offers an affordable solution that will boost productivity in any busy cell biology, immunology, oncology or microbiology research laboratory. *For research use only. Not for use in diagnostic procedures.*
iCyt is a Sony group company.

Enumeration of Circulating Endothelial Progenitor Cells by Utilizing the MACS® Enrichment Unit and Flow Analysis by the MACSQuant® Analyzer

Ashley Weant, PhD Flow Cytometry Specialist, Miltenyi Biotec Inc.

Circulating Endothelial Progenitor Cells (EPCs), first described by Asahara et al, are found to be among a subset of CD34+ cells in peripheral blood (1). EPCs are defined by co-expression of the markers CD34, CD309 (VEGFR- 2/KDR), and CD133. CD133 expression seems to be restricted to the most primitive EPCs and is lost during maturation to endothelial cells (ECs). Enumeration of EPCs is considered to be a measurable parameter in the assessment of risk factors for various cardiovascular diseases (2-4).

Since EPCs are rare in peripheral blood (frequency of 0.001% in PBMC), EPC enumeration protocols are rather extensive and laborious. To obtain reliable enumeration results for these rare cells, the sensitivity of flow cytometric analysis needs to be increased. With such rare populations, magnetic pre-enrichment improves the detection and enumeration of these rare cells. The EPC Enrichment and Enumeration Kit in combination with the MACSQuant® Analyzer is designed to simplify the enumeration of circulating EPCs from peripheral blood, cord blood, bone marrow or leukapheresis products. This is achieved by enrichment of CD34+ cells, which reduces the number of events that have to be subsequently analyzed. Based on the preparation of the sample with the enrichment and staining cocktail, the MACSQuant Analyzer performs a pre-enrichment with the MACSQuant Column, followed by optimized washing of the cells and four-color flow cytometric analysis.

The EPC Enrichment and Enumeration Kit combined with the MACSQuant Analyzer have drastically reduced the time and difficulty of such a protocol by allowing the magnetic enrichment and flow cytometric analysis in one constant experiment. Similar enrichment strategies can be applied for analysis or enumeration of other rare cells, such as antigen-specific T cells and perhaps circulating tumor cells.

1. Asahara, T et al. Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. Science. 1997. 275:964-967.
2. Urbich, C and Dimmeler, S. Risk factors for coronary artery disease, circulating endothelial progenitor cells, and the role of HMG-CoA reductase inhibitors. Kidney Int. 2005. 67:1672-1676.
3. Werner, N and Nickenig, G. Influence of cardiovascular risk factors on endothelial progenitor cells. Arterioscler Thromb Vasc Biol. 2006. 26:257-266.
4. Vasa, M et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res. 2001. 89:E1-E7.

Flow Cytometry for Analysis and Sorting of Large Objects Using Union Biometrica's New BioSorter® Instrument

Nathan Portier, PhD, Application Scientist, Union Biometrica

Many objects are too large or fragile for conventional flow cytometry. Union Biometrica, known for the COPAS instruments, introduces the BioSorter instrument for the automated analysis and sorting of large (10-1,500 micron) objects at rates of 10-500 objects/second. Using object size, optical density and fluorescence intensity as sorting criteria, selected objects can be dispensed in multi-well plates or bulk receptacles. A gentle pneumatic sorting mechanism avoids harming sensitive objects thereby making the instrument suitable for live biological materials or sensitive chemistries. This includes Fragile Large Cells & Cell Clusters such as hepatocytes, adipocytes, cardiomyocytes, kidney tubular cells, embryoid bodies, pancreatic islets. Other suitable samples are small model organisms such as all stages of *C.elegans*, *Drosophila* eggs, embryos and larvae, and Zebrafish eggs, embryos and the early

larval stages. Finally, small plant seeds, like *Arabidopsis,* and plant pollen, as well as beads and microcarriers of various sizes and shapes are all acceptable samples for analysis and sorting. The BioSorter can be configured to accommodate the full 10 to 1500 micron range in four steps, each with a specially engineered Fluidics and Optics Core Assembly (FOCA) optimized for a particular size range. An operator can easily switch between these different modules to maximize sensitivity and speed for any given sample type. BioSorter instruments are available with up to four excitation lasers and detection optics to accommodate a variety of fluorescence excitation and emission requirements. Custom optics and low dispersion lenses allow these instruments to precisely focus the excitation lasers across all flow cell sizes. Instruments have an extinction detector for measuring the optical density and relative size of the analyzed objects and three PMT (photo multiplier tube) fluorescence detectors which can be used to detect fluorescence emissions in the different regions of the visible spectrum. Interchangeable PMT filters and dichroic mirrors enable specific selection of fluorescent emission wavelengths. The standard filter set detects fluorescence in the green, yellow and red regions of the spectrum. A unique feature available with these instruments that comes as an add-on module is Profiler II™. With the use of Profiler II™ multiple sequential measurements of extinction and the three colors of fluorescence can be collected and plotted to generate two dimensional representations of each interrogated object. These axial profiles can be analyzed and used as sort criteria based on the localization of optical density and fluorescence within each object, including peak height, peak width, peak counting and relative peak position. The electronic cross section of each object enables users to optimize sort criteria and sample analysis in real time while utilizing our advanced FlowPilot-Pro™ software.

Non-Presenting Vendor Abstracts:

A Compact Bench-Top Analyzer for Precise, Small Particle Analysis.

David Coder and Jeff Harvey, Bay Bioscience, USA

Flow cytometry of microbes and small particles has always been a challenge. Samples ranging from small parasites to bacteria to viruses as well as platelets and platelet-derived microparticles have been difficult to resolve from the background noise in light scatter. While fluorescent probes can make it easier to resolve small organisms or microparticles from debris, the small amount of material available for labeling with a fluorescent probe often makes for a very dim signal. Steen and co-workers (1979) developed a sensitive arc-lamp-based instrument designed specifically for work with bacteria. The instrument was later sold as the Skatron Argus flow cytometer. An updated version of this machine was developed at Los Alamos National Laboratory for DoD in the 1990's that became the Bio-Rad Bryte HS, but it was withdrawn from the marketplace. In 2000, Apogee Flow Systems in the UK took over production and produced a miniaturized and improved version that was sold mostly in Europe as the A40/50 micro series. As of 2010, the instrument is now available in the US. Key features include: high sensitivity narrow- and wide-angle darkfield light scatter with PMT detection, 20nm scatter resolution, 4 color fluorescence, choice of lasers from 375nm to 635nm, 20K counts/second acquisition time, volumetric measurement for cell concentration. Example data show clear resolution of various bacterial species (*Lactobacillus* and *Pediococcus*), cyanobacteria from marine samples (*Prochlorococcus* and *Synechococcus)*, as well as yeasts and *Toxoplasma*. Platelet-derived microparticles are easily resolved from background debris using CD41-PerCP.

Symposium I

Saturday, September 25th
8am – 11 am
***Clincal Cytometry, Special Lecture,* Marquette A+B, Level 5**
Convener: Vera Donnenberg, University of Pittsburgh

8:00 – 8:45 Assessing Cytotoxic Potential Using Flow Cytometry
Joseph D. Tario, Jr., Roswell Park Cancer Institute

8:45 – 9:30 Everest, Death & AIDS: Three Relatives
J. Paul Robinson, Purdue University

9:30 – 10:00 Coffee Break – Poster/Exhibit Viewing
Coffee break sponsored by Spherotech

10:00 –10:45 *Philip Marder Memorial Lecture:*

Flow Cytometry as an Essential Tool in Pharmaceutical Discovery and Development—A tribute to the life and groundbreaking career of Philip Marder (1948 – 2010)
Lisa J. Green, MS, Covance Laboratories

Abstracts:

Assessing Cytotoxic Potential Using Flow Cytometry

Joseph D. Tario, Jr., Roswell Park Cancer Institute

Background: The chromium (^{51}Cr)-release assay has traditionally been considered the gold standard for determining the cytolytic potential of effector cells. Although the assay is reliable, it exhibits a number of functional limitations that render it impractical for use in some laboratories. Aside from the potential hazards associated with radioactivity, additional disadvantages include difficulty in labeling targets with ^{51}Cr, and the spontaneous release of the isotope from targets, causing extremely high background levels. This noise makes resolution of effector-mediated lysis difficult, especially in longer assays such as the detection of low frequency effectors or in measurements of antibody dependent cytotoxicity. The use of flow cytometry and cell tracking dyes to assess cytotoxicity does not require radioactivity and has the distinct advantage of being able to measure killing at the single cell level even when targets and effectors cannot be distinguished on the basis of light scatter. The multicolor fluorescence-based assay described here uses killing of a cultured cell line (K562) by lymphokine (IL-2) activated killer (LAK) cells as a model system, but the principles and general procedures are applicable to virtually any effector-target combination.
Methods: In addition to illustrating that a new far-red cell tracking dye (CellVue Claret) does not alter LAK functionality, we demonstrate two different methods for measuring target cell death: (i) on a relative basis by determining percentage of targets deemed dead based on their inability to exclude 7-AAD, and (ii) on an absolute basis by using counting beads to enumerate the number of viable target cells that remain when effectors are present vs. when they are absent. The latter method is unaffected by cells lost due to complete lysis, and is therefore particularly useful for longer term cytotoxicity assays.
Results: We demonstrate that CellVue Claret staining does not affect the cytolytic potential of LAK cells. The use of 7-AAD to quantify dead target cells (Method 1) is less sensitive than the use of enumeration beads to count viable target cells remaining (Method 2).

Discussion: In order to enumerate dead cells with a viability dye, Method 1 requires that cells remain intact enough to contain a nucleus. It has been demonstrated in longer-term assays that killed cells may fragment, enucleate and thus become uncountable by this method. Our data suggest that even in a relatively short term assay, Method 2 - which relies on the enumeration of live cells remaining in the sample - is inherently a more accurate way to quantify cytotoxicity. We performed a number of analyses with modified region placement in attempts to explain this result, but found that in all cases we obtained decreased values for percent cytotoxicity, due to increased levels of noise in the live K562 region. We thus concluded that the decreased sensitivity of Method 1 was due to systematic loss of dead cells from the assay.

Everest, Death & AIDS: Three Relatives

J. Paul Robinson, SVM Professor of Cytomics, Professor of Biomedical Engineering, Purdue University

More than 25 million people have died from AIDS since 1981. About 2 million die every year. Outside of the Western world, AIDS and death are closely related terms. The real issue for us as scientists is what can we do to change the status of this disease. Many will say that they can do little, and perhaps they are right. However, if we only attack problems or challenges that are easy, we will never solve the hard problems. In 2008 I decided that my attempts to bring low cost CD4 testing to resource limited countries were getting a failing grade. The challenge was so big, I could not see a way around it. At a time when I felt the situation was impossible to solve, I said to myself "Surely it would not be easier to climb the highest mountain in the world, than solve this technology problem". I had absolutely no experience or knowledge on mountain climbing. It turns out, it was easier to climb Everest. Despite that, several people died during my climb – but I returned with a stronger desire to meet the challenge of low cost CD4. Low cost, means really cheap instruments – less than $5000. It means battery operated instruments, that don't require computers and software to get that elusive CD4 measurement. It means someone being able to perform a CD4 without a technical background. It means us giving away our secrets that keep this technology complex and expensive. It means that the many companies that are purporting to create solutions stop advertizing false claims, and it means companies stop taking funds by using Africa as an 'excuse' to get them, and then do nothing to impact Africa. It's time to take the gloves off on CD4 and AIDS. There are many companies that make a very large amount of money from AIDS in Africa. It's time to ensure that real solutions are produced and real solutions are delivered in a way that the people who need CD4 and therapeutics can actually afford them. If an old professor who has never climbed a mountain in his life, can go summit Mt. Everest, surely we, who know cytometry the best, can help to deliver really low cost CD4 solutions to the many Africans who need them to qualify for therapeutics.

Flow Cytometry as an Essential Tool in Pharmaceutical Discovery and Development—A Tribute to the Life and Groundbreaking Career of Philip Marder (1948 – 2010)

Lisa J. Green, MS
Covance Laboratories

In a 35 year industry career Phil Marder was supremely successful in applying his passion for flow cytometry to the challenging and diverse science of drug development. Combining profound technical expertise in both cytometry and cell biology with a collegial and practical approach, Phil uniquely positioned his laboratory to fulfill a broad spectrum of research and development needs. In this seminar we will review Phil Marder's many contributions to drug development and the Flow community.

The Carleton and Sigrid Stewart Keynote Lecture

Saturday, September 25th, 2010
11:00 am – 12:00 pm
Marquette A+B, Level 5

CELL TRACKING: PAST, PRESENT AND FUTURE

Katharine A. (Kathy) Muirhead, Ph.D., Chief Operating Officer, SciGro, Inc

The ability to tag selected cells and track their functions and interactions is critical to understanding how molecular differences at the genomic and proteomic level are translated into differences in biological behavior at the system level. The range of tools available for cell labeling and tracking has expanded dramatically in the last quarter century, with an emphasis on fluorescence-based methods. This presentation will provide an overview of cell tracking reagents, methods and applications in the general context of cell-based immunotherapy. Following the example set by Carl and Sigie, critical questions/controls and lessons learned the hard way will be highlighted as well as areas for further exploration (e.g., cytoferroequinobiology).

Luncheon Roundtables

Saturday, September 25th, 2010, 12:00-1:30PM

Conveners: Joanne Lannigan, University of Virgina
Paul Champoux, University of Minnesota

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.

Locations: Free lunch/pop; in **Diluth A/B (Tables 1-4), LaSalle A (Tab 5-8), LaSalle B (Tab. 9-12), Brule B (3 tables for non-patricpants in workshops), Level 5.** Pick up wrapped sandwiches (roast beef, turkey and veggie wrap, condiments, chips, cookie, fruit and soft drink and move to roundtable of 10 labeled with the title of discussion topic - attendance at each table determined from sign up sheet at GLIIFCA registration desk. **Non-participants can lunch in Brule B.**

R1—Soluble bead array (SBA)/Luminex roundtable: Whether you use or are thinking about using the Luminex, Cytokine Bead Array or some other technology for the multiplexed detection of soluble analytes, this roundtable will be of interest to you. We will discuss the advantages and challenges of multiplexed technologies, learning from each other's experiences and consider its future beyond cytokines. Topics will include: (i) comparisons with ELISA, (ii) reproducibility and sensitivity issues, (ii) experiences with kits from different manufacturers as well as "home brew" reagents, (iii) automating the process with robots and different software packages, (iv) setting a fair price, and (v) moving beyond cytokines and chemokines and into the world of detecting signaling proteins, miRNA, and gene products. Participants will be encouraged to bring sample data and their questions. *Moderator: Ree Dolnick, Roswell Park Cancer Institute*

R2— Violet Lasers are for more than QDots: Violet lasers are now becoming as standard as red and blue lasers on most new flow cytometers. Previously, the use of QDots expanded the use of the violet laser beyond CFP and Pacific Blue. Now an investigator has many more choices for not only immunophenotyping, but also a wide variety of other applications. This roundtable will review the proliferation dyes, viability dyes, cell cycle dyes and new immunophenotypic fluorochromes that have now set the standard for use of a violet laser. *Moderator: Drew Bantly, University of Pennslyvania*

R3—Microparticles: Microparticle enumeration and classification has become an increasingly popular flow cytometric application. The downside of the growth in popularity of flow cytometric analysis is that fields of research with historically very little reliance on flow are now getting involved without a proper understanding of the experimental controls and limitations of instrumentation. This has most likely produced a body of literature that is irreproducible from lab to lab and is often times down right misleading in its interpretation. We will hopefully have a lively conversation regarding a few key controls that should accompany all MP or small particle analysis along with some technical jargon that is useful in the ongoing field of MP analysis. *Moderator: James Marvin, Northwestern University*

R4—Fluorescent Proteins: As the fluorescent protein pallet increases, the challenges facing the cytometrist follow suit. The plight of the manic cytometrist has now included trying to understand more biology of expression vectors while juggling the basic excitation and emission characteristics of these proteins. We will discuss in general; some fluorescent protein constructs, delivery, and what that means for the cytometrist. We will also look at instrument requirements and hopefully address some of the advantages and disadvantages of a few new laser lines that have recently hit the market. If time permits and there is sufficient interest, we can touch on the world of scientific applications and their logistics; such as expressing multiple fluorescent proteins simultaneously, sorting, and/or Fluorescence Resonance Energy Transfer (FRET). *Moderator: Mike Meyer, University of Pittsburgh.*

R5—Knowing your limitations: Techniques for testing fluorescence sensitivity: A discussion of various techniques and methods that have been employed to test the ability of an instrument to detect low levels of fluorescence above background. Pros/Cons, and potential pitfalls of the most common techniques will be highlighted. *Moderator: Ryan Duggan, University of Chicago*

R6—Methods for Automated Analysis of Subcellular Patterns: The focus of this roundtable will be on various methods for automating the analysis of subcellular patterns in microscope images. Potential topics will include whether and when segmentation of images into single cell regions is needed, types of features for capturing pattern information, types of machine learning classifiers, and when to do comparison, regression, classification, or clustering. *Moderator: Bob Murphy, Lane Center for Computational Biology, Carnegie Mellon University*

R7—Clinical Cytometry: Discussion intended to cover the various challenges and rewards of working in clinical flow cytometry. Topics covered will include but not be limited to, maintaining technical proficiency in an ever-evolving discipline, adapting to emerging applications in the era of personalized medicine, providing appropriate continuing education for staff and operating a state-of-the-art laboratory in tight-budget times. Participants are encouraged to bring concerns particular to their laboratory for the purposes of general group discussion/education. *Moderators: Mary Paniagua and Laura Marszalek, Northwestern Memorial Hospital*

R8—DNA Analysis: Sample prep, enzymatic digests, and fixation all lead to one goal - making sense of the DNA histogram patterns. With all our wonderful technology, however, let's not forget about the underlying biology. For an analysis to make sense, the biology has to make sense. *Moderator: Mark Munson, Verity Software House, Inc.*

R9—Cell Signaling Concepts and Applications*:* The use of cell signaling offers great promise in the elucidation of the cellular processes controlling proliferation, differentiation and cell death. As with all cytometry based technologies, many pitfalls may be avoided if the researcher takes the necessary steps to ensure the collected data is a reflection of the system being tested and not a random number generator. This roundtable discussion will revolve around antibody QC techniques, instrument standardization, fixation techniques, experimental design and informatics challenges. *Moderator: Keith Shults, Nodality, Inc.*

R10—The Amnis ImageStream-Applications and experiences in a core facility: The ImageStream imaging flow cytometer allows users to perform multi-color flow cytometry and quantitative image analysis simultaneously on a large number of cells. This discussion will cover a general introduction to and advantages of the ImageStream technology, common applications, and a general description of data analysis. We will also discuss experiences and ideas on how the ImageStream can be integrated into a core facility. *Moderators: Monica Delay, Tristan Bourdeau, Cincinnati Children's Hospital*

R11—Informatics Approaches for flow cytometry data management and analysis: Advances in cytometry have enabled routine generation of large multi-color flow cytometry experiments designed to measure large number of samples across tens of markers and highlighting the need for novel analysis approaches for identifying "interesting" cell populations and comparing them across samples. In addition, these experiments generate a large amount of (hundreds of megabytes to gigabytes) raw data making it difficult to manage, analyze and collaborate with these experiments. The goal of this roundtable is to discuss approaches to address these challenges of flow analysis and data management. The discussion will start by summarizing activities from a recent workshop at CYTO 2010 where we outlined the general classes of questions investigators ask of their data, identified informatics papers and posters at CYTO 2010 and mapped them to the questions they answered. *Moderator: Nikesh Kotech, Cytobank ,Inc*

Symposium II

Saturday, September 25th, 2010
1:30 pm – 5:15 pm
Cutting Edge Cytometry
Marquette A+B, Level 5

Conveners: Vera Donnenberg, Univeristy of Pittsburgh
Joanne Lannigan, University of Virginia

1:30 – 1:45 **Targeting Embryonic Pathways in Cancer**
Ryan A. Wetzel, University of Pittsburgh School of Medicine

1:45 -2:15 **Proteome-Scale Analysis and Modeling of Subcellular Patterns**
Robert Murphy, Carnegie Mellon University

2:15-3:00 **A Model System for Therapeutic Vaccination for HIV-Associated Malignancies - Tracking Down Persistent KSHV Infection In Vivo.**
Lisa Nichols, University of Virginia

3:00 – 3:30 **Imaging the Interaction of Tumor and Immune Cells - in vitro & in vivo High Content Analysis**
William Buchser, University of Pittsburgh Cancer Institute.

3:30 – 3:45 **Coffee Break and Poster/Exhibit Viewing**
Coffee break sponsored by Millipore

3:45 – 4:30 **Mind-body connection - T lymphocytes Control Cognitive Function**
Jonathan Kipnis, PhD, University of Virginia

4:30 – 5:15 **Using Q and b Values to Predict the Resolution of Dimly Stained Cells from Instrument Noise**
Eric Chase, Cytek Development

Abstracts:

Targeting Embryonic Pathways in Cancer

*Ryan A. Wetzel, Albert D. Donnenberg, William Buchser, * Vera S. Donnenberg*
University of Pittsburgh School of Medicine. Pittsburgh, PA USA

Cancer dormancy is particularly important in breast cancer, where cancer recurrence may occur years after apparently successful therapy. The cancer stem cell paradigm explains, in part, tumor heterogeneity and has led to the hypothesis that therapy resistance originates in the mechanisms by which normal tissue stem cells protect themselves from toxic insults. The hypothesis underlying this work is that the Hh pathway plays a critical role in tumor self-protection, compared to normal hematopoietic and mesenchymal progenitor cells, which are less dependent on hedgehog for the activation of their protective pathways. This predicts that in breast cancer inhibition of the Hh pathway should have multiple downstream effects, resulting in increased sensitivity of breast cancer to antineoplastic agents and increased differentiation leading to increased

sensitivity to hormonal therapy. The effects of inhibition of Hh signaling in breast cancer cell lines (MCF7, BT474 and MDAMB231), as compared to mesenchymal progenitor cells (specificity control) were measure using immunohistochemical detection of cytokeratin, CD44, Ki67, estrogen receptor, sonic Hh (SHh) and Gli. The addition of exogenous sonic hedgehog (C24II, 200 ng/mL, 48h) to the breast cancer cell line MCF7 resulted in increased cytoplasmic and nuclear Gli-1, higher proportion of cells in cycle (Ki67), and an unexpected increase in ER expression, without any notable increase in CD44, an adhesion molecule reported on breast cancer stem cells, or any significant decrease in cytokeratin expression. In normal bone marrow derived mesenchymal cells, positive for SHh but negative for smoothened (SMO) or Gli, addition of exogenous SHh had no effect on proliferation or differentiation of these cells. The addition of the PTCH inhibitor cyclopamine (5 μM) in MCF7 blocked SHh increased proliferation (Ki67) and ER upregulation. No effects of cyclopamine were detected in mesenchymal progenitor cells. Taken together, these preliminary findings provide early evidence for selective effects of Hh pathway inhibition of tumor cells.

Proteome-Scale Analysis and Modeling of Subcellular Patterns

Robert F. Murphy, Lane Center for Computational Biology, Carnegie Mellon University

An important challenge in the post-genomic era is to identify subcellular location on a proteome-wide basis, and learn how protein locations change under various conditions (such as in the presence of potential pharmaceuticals). A major source of information for this task is imaging of tagged proteins using light microscopy. We have previously developed automated systems to interpret the images resulting from such experiments and demonstrated that they can perform as well or better than visual inspection. We have recently applied these methods to large collections of images from yeast, human tissues, and GFP-tagged mouse 3T3 cell lines. A distinct but related task is learning from images what location patterns exist (rather than classifying them into pre-specified patterns). We have demonstrated the ability to unmix location patterns that consist of combinations of fundamental patterns, so that each protein can be represented by the amounts that are found in each distinct organelle or structure. This unmixing approach is particularly useful for analyzing the effects of perturbagens (e.g., drug candidates, inhibitory RNAs) on subcellular patterns.

A Model System for Therapeutic Vaccination for HIV-Associated Malignancies - Tracking Down Persistent KSHV Infection In Vivo.

Lisa A Nichols,[1] Drew Roberts,[2] Timothy NJ Bullock,[2] and Dean H Kedes[1,3]
Depts. of Microbiology,[1] Pathology,[2] and Medicine,[3] University of Virginia, Charlottesville, VA 22908

The AIDS-defining cancers Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma make up more than half of malignancy-induced deaths in developed countries. For KS, the single most common AIDS-associated malignancy, the viral culprit is KS-associated herpesvirus (KSHV), which also causes primary effusion lymphoma and multicentric Castleman's disease. One of the central challenges in developing therapeutic strategies against KS and all AIDS-associated malignancies with a viral etiology is finding a way to circumvent the poor vaccine response that so frequently occurs in immunocompromised patients—the most critical target population. Our goal is to assess both KSHV-specific immune

responses and establish vaccination strategies for CD4 T cell deficient hosts. In patients, KSHV infection is primarily latent (non productive) and is present at low levels in patients. As a result, it is technically challenging to detect infected cells and quantify infection levels. We present here a tractable model system that allows both evaluation of cellular immune responses as well as detection and characterization of infected host cell populations using both traditional and imaging flow cytometry.

Imaging the Interaction of Tumor and Immune Cells - in vitro & in vivo High Content Analysis

William Buchser. Michael Lotze, D.A.M.P. Laboratory. University of Pittsburgh Cancer Institute.

Imaging techniques applied to *in vitro* and *in vivo* experiments are one few ways to study interactions between cells. In Cancer Immunology, for example, the interaction between the tissue and the motile lymphocytes can define the inflammatory state. A damaged epithelium can be killed by infiltrating lymphocytes (if infected), or survival (if wound healing) based on the type of infiltrate and the set of "patterns" (PAMPs and DAMPs) present at the site. Lymphocytes, such as NK cells, and monocytes, such as Dendritic cells have direct interaction both with each other and the stressed epithelia. To study this interaction, we use either simple culture systems or tissue slices. Imaging cytometry (specifically Cellomics Arrayscan) is used to capture the physical arrangement and the proximity of each cell being studied. Population analysis can then be used to extract information about cell interactions and local field effects. We have shown that NK cells can induce survival (through autophagy) in tumor cells when appropriately activated with IL-2.

Mind-Body Connection - T Lymphocytes Control Cognitive Function

Jonathan Kipnis, PhD, Assistant Professor, Department of Neuroscience, University of Virginia

T cells were recently shown to support learning and memory, albeit the underlying cellular and molecular mechanisms are yet unknown. The first step in the cascade of neuro-immune interactions that influence learning and memory takes place in the meninges, where T cells regulate the phenotype of meningeal myeloid cells. Cognitive task performance leads to accumulation of IL-4 producing T cells, as well as activation of myeloid cells in the meningeal spaces. Acute lymphopenia or blockade of T cell migration into the meninges result in impairment of learning and memory, and a skewed pro-inflammatory phenotype of meningeal myeloid cells. The role of IL-4 is critical, as IL-4 knockout mice also exhibit cognitive impairment, along with a similarly skewed pro-inflammatory meningeal myeloid cell phenotype. Our results point to a critical role for meningeal T cells, and for IL-4 in particular, in the regulation of meningeal myeloid cells, and concomitant cognitive function. These results might lead to development of new immune-based therapies for cognitive conditions associated with immune decline, such as HIV- and age-associated dementia.

Using Q and b Values to Predict the Resolution of Dimly Stained Cells from Instrument Noise

Eric Chase, Cytek Development

The fluorescence sensitivity of flow cytometers is traditionally quoted as less than a certain number of MESF (mean equivalent soluble fluorophores) in a given channel. This number does not predict the ability of a flow cytometer to resolve dimly stained cells from background. In flow cytometers with area processing, this number could be 0, or even negative, resulting in a meaningless negative sensitivity value.

The development of the Q and b measurements (1) provides an alternative measurement of fluorescence sensitivity. The Q and b values for each channel can be measured (2), and a resolution parameter can be calculated from the measured Q and b values. The resolution parameter R is the mean intensity of a population (in MEF) that is separated from instrument noise intensity by 2 standard deviations of the instrument noise plus 2 standard deviations of the sample intensity. Thus a sample with an MEF equal to R will be nearly completely separated from instrument noise. R will be somewhat larger than the older definition of fluorescence sensitivity, but is more useful for estimating the ability of an instrument to resolve a stained population from noise.

References:

1. ES Chase and RA Hoffman: Resolution of dim fluorescence particles-a practical measure of fluorescence sensitivity. Cytometry 33:267-279 (1998).
2. Alan Stall: Qr and Br in BD FACSDiva v6 Software-Parameters for Characterizing Detector Performance. BD Bioscience Application Note (2008).

Symposium III

Sunday, September 26th, 2010
9:00 am – 11:45am
Technical Innovations in Cytometry
Marquette A+B, Level 5

Convener: Keith Shults, Nodality, Inc.

9:00 - 9:45 An Enhancement to Forward Light Scatter Collection Improves Resolution of Red Blood Cell Derived Microvesicles
E. Michael Meyer, University of Pittsburgh

9:45 – 10:30 Laser Rastering: Technology and Applications
Giacomo Vacca, Ph.D., Abbott Laboratories

10:30 – 11:00 Coffee Break – Poster/Exhibit Viewing
Coffee break sponsored by Beckman Coulter

11:00 – 11:45 Single Cell Network Profiling (SCNP) by Flow Cytometry as a Tool to Measure Potency, and Selectivity of JAK/STAT Inhibitors in PBMC and Whole Blood Discrete Cell Subsets.
Todd M Covey, Nodality, Inc.

Abstracts:

An Enhancement to Forward Light Scatter Collection Improves Resolution of Red Blood Cell Derived Microvesicles

E. Michael Meyer[1], Chenell L. Donadee[2], Mark T. Gladwin[2], Albert D. Donnenberg[1,3]
University of Pittsburgh Cancer Center Flow Cytometry Facility, University of Pittsburgh, Pittsburgh, PA[1] Division of Pulmonary Allergy and Critical Care Medicine University of Pittsburgh Medical Center, Pittsburgh PA[2] University of Pittsburgh School of Medicine, Department of Medicine, Pittsburgh PA[3]

Detection of red blood cell (RBC) derived microvesicles is an area of intense interest because they hold promise as a biomarker of disease activity in sickle cell anemia, thalassaemia intermedia and cardiovascular disease. Their quantification in stored blood products may also serve as an objective quality indicator. Flow cytometry based assays have been devised to quantify RBCMV in blood on the basis of conventional forward light scatter (FSC) and annexin V binding. In such assays, RBCMV are detected as a discrete population of FSC low cells, the majority of which bind annexin V, indicating loss of phospholipid asymmetry. Intact RBC are largely annexin negative, but form one continuous population with transitional cells having slightly lower FSC and higher annexin binding. Presumably this transitional population is intermediate to the formation of RBCMV. The newly released Beckman-Coulter Gallios cytometer has the capability to detect FSC light in a mode that preferentially amplifies signals collected between 8-19º. This mode, termed Enhanced Wide Angle Forward Scatter, is particularly useful for resolving size difference among small particles (micron to submicron range). EWA FSC offers better signal to noise for small particle detection compared to standard low angle or wide angle FSC where gain must be increased over the entire angle of detection.

In this study, we demonstrate that EWA FSC is able to resolve RBCMV from both the transitional population and from intact RBC in blood bank whole blood products that had been aged 3 and 21 days. Intact RBC and fragments were detected as Glycophorin-A (Gly-A, CD235a) (PE-Cy5) positive events. Annexin V binding was measured on each Gly-A+ population. With EWA FSC, intact RBCs were detected as a homogenous scatter population with a mean half-peak CV of 10.8%. This population was easily distinguished from the lower scatter transitional population and from microvesicles themselves. In contrast, using conventional FSC, RBCs formed a diffuse population (mean half-peak CV = 17.7%) that was continuous with transitional vesicles. The ability to resolve RBC populations by EWA FSC enables further time course studies to determine whether the emergence of a transitional population predicts the subsequent degradation of RBC into RBCMV.

Laser Rastering: Technology and Applications

Giacomo Vacca, Ph.D., Abbott Laboratories

Laser Rastering is a technological advance in flow cytometry that allows far more rapid cellular analysis than was previously possible. It is based on scanning a tight laser beam spot across a broad core stream: The large core cross section gives higher potential throughput, while scanning enables that potential. The invention relies on powerful digital hardware and software, particularly a field-programmable gate array that crunches the raw digitized data on the fly and extracts cellular information for higher-level processing. In our development program, we have focused on building a clinical hematology analyzer based on this technology; results to date include counting rates routinely in excess of 300,000 cells per second and excellent correlation with established reference methods. As we test the boundaries of capability of Laser Rastering in fields beyond hematology, we are investigating the tradeoffs between throughput and fluorescence sensitivity, as well as those between routine counting and rare-event analysis. I will give an overview of the technology, as well as present key results to date and next steps.

Single Cell Network Profiling (SCNP) by Flow Cytometry as a tool to measure Potency, and Selectivity of JAK/STAT Inhibitors in PBMC and whole blood discrete cell subsets

Todd M Covey, Santosh Putta, Michael Gulrajani, Aileen Cohen, and Alessandra Cesan, Nodality, Inc.

Background: With the development of targeted therapeutics aimed at modulating signal transduction networks, there is a need for measuring the pharmacodynamic impact of these agents in relevant complex tissues at the single cell level. The ability to assess target coverage and selectivity is paramount – first, for selection of candidate molecules to progress through the drug development process and second, once a candidate drug moves into clinical testing, for guiding dose and schedule selection.
Objectives: This study tested the ability to use single cell network profiling (SCNP), in which cells are perturbed with extracellular modulators and their response ascertained by multiparametric flow cytometry, to 1) measure the potency and selectivity of a panel of compounds with reported inhibitory effects on JAK/STAT signaling in whole blood versus fractionated peripheral blood mononuclear cells (PBMC) and 2) test the ability of SCNP to capture rare cells amid a heterogeneous population of cells.
Methods: We measured modulation of JAK/STAT, MAPK and NFkB signaling pathways in cell subsets after *in vitro* exposure of whole blood and PBMC to GM-CSF (monocytes/neutrophils), IL-2 (T-cells), and CD40L (B-cells) in the presence and absence of known JAK inhibitors. For evaluation of SCNP in

rare cells, phosphorylation of STAT proteins in response to IL-27 was examined on CD34+ CD11b- CD33- progenitors in healthy PBMCs.
Results: The relative selectivity of JAK3 versus JAK2 inhibition was simultaneously measured by plotting p-Stat-5 inhibition curves from gated T cells (JAK3 mediated) and monocytes (JAK2 mediated). Notably, CP-690550, currently in clinical trials for a variety of autoimmune diseases, was 36-fold more potent at inhibiting JAK3 compared to JAK2 activity, which is consistent with published reports. In addition, off-target signaling effects outside the JAK/STAT pathway were observed (eg, Cucurbitacin I induced a dose-dependent increase in p-ERK in the monocyte subset and "Stat3 Inhibitor VII" blocked CD40L stimulated p-ERK and pNFkB p65 in B cells). Importantly, a significant decrease in potency in all compounds except CP-690550 was observed when studies were performed in whole blood, the clinically relevant tissue, versus fractionated PBMC. Notably, SCNP technology captured IL-27 induced p-Stat-1 and p-Stat-3 responses and inhibition by Pyridone 6 in CD34+ CD11b- CD33- progenitor cells, a very rare (<40 cells; 0.11% of the total live cells) cell population in PBMC.
Conclusions: SCNP technology allowed for the evaluation of 1) compound activity in primary cells in a physiologically relevant environment (whole blood), 2) signal transduction target and off-target effects in relevant cell populations, and 3) robust IC50 determination from rare subpopulations (<100 cells). Therefore, this technology is well suited for application in oncology drug development where assessment of candidate drug effects on rare cell populations (eg, cancer stem cells, minimal residual disease, and circulating tumor cells) is crucial and permits the rational design of clinical trials based on biologically active dose rather than the traditional maximum tolerated dose (MTD) design, which is better suited for cytotoxic, non-targeted drugs.

POSTER ABSTRACTS

Abstract number corresponds to poster board number.
All posters displayed in Mackinac, Level 5

1. Myosin Light Chain Kinase Inhibitor (ML-7): An Inducer of Apoptosis in Human Cells in Vitro

Madeline Fry[1], Huijun Yu[1], Shruthi Bharadwaj[1], Balaji Ganesh[2], Primal de Lanerolle[1]
Department of Physiology and Biophysics, University of Illinois at Chicago[1]
Research Resources Center Flow Cytometry Service, University of Illinois at Chicago[2]

MLCK is a calcium-calmodulin dependent enzyme that regulates the actin-myosin II interaction by phosphorylating ser 19 on the regulatory light chain of smooth muscle and non- muscle myosin II. MLCK is potentially involved in regulating all energy-dependent processes in cells, including cell motility and cell division. Because of this, it is reasonable to predict that MLCK is an important regulator of tumor growth and metastatic colonization. Through concurrent studies in our lab, we have shown that cancer cells have less total MLCK than their primary counterparts. This suggested that cancer cells might be more sensitive to pharmacological inhibition of MLCK by ML-7. ML-7 is a cell permeable, potent, and selective inhibitor of MLCK. In studies comparing the induction of apoptosis by ML-7 in HeLa (cervical cancer) cells and human uterine fibroblasts (HUF)(primary cells), we found that apoptosis is induced in HeLa cells both more quickly and at a lower dosage of ML-7 than in HUF cells, suggesting that ML-7 has potential as a chemotherapeutic agent. We also found that ML-7 stimulates the ability of etoposide, doxorubicin, and 5-FU, to induce apoptosis in cervical cancer, prostate adenocarcinoma and colorectal carcinoma cells, respectively. Our results provide preliminary evidence that ML-7 is effective in the treatment of some human cancers and that it should be further investigated for its chemotherapeutic efficacy.

2. An Effective Bio-Safety Cabinet for Modern Cell Sorters

Edward Podniesinski*, Earl A. Timm Jr.*, Andrej Wierzbicki*, Jan Hendrikx**, Jennifer Wilshire**, Michael Funk*** Paul Wallace*
** Roswell Park Cancer Institute, Buffalo NY14263 ** Sloan-Kettering Institute, New York NY 10065*
****Bio-Bubble Inc., Fort Collins Utah 80626*

Modern cell sorters already provide optional aerosol management within the immediate proximity of the sort chamber where sort fractions and waste decisions are made. A Bio Safety level facility must provide protection against the aerosolization of live samples that possibly contain blood borne pathogens such as hepatitis and HIV for example.

A cost effective, primary containment barrier, the Bio-Bubble, was designed and built around a modern sorter with total exhausted HEPA filtered air. The negative pressure enclosure protects the operator and environment not only from aerosols generated within the sort chamber but also from the sample introduction area. User customized window access patches add versatility to specific experimental needs. The enclosure allows generous access for service due to its simplistic design.

We demonstrate the effectiveness of aerosol containment under different operational scenarios using particles mimicking air borne pathogens. Sorter instrument internal temperature risk exposure, noise and air change data will be presented.

3. Single Cell Network Profiling (SCNP) by Flow Cytometry as a Tool to Measure Potency, and Selectivity of JAK/STAT Inhibitors in PBMC and Whole Blood Discrete Cell Subsets

Todd M Covey, Santosh Putta, Michael Gulrajani, Aileen Cohen, and Alessandra Cesano, Nodality, Inc.

Background: With the development of targeted therapeutics aimed at modulating signal transduction networks, there is a need for measuring the pharmacodynamic impact of these agents in relevant complex tissues at the single cell level. The ability to assess target coverage and selectivity is paramount – first, for selection of candidate molecules to progress through the drug development process and second, once a candidate drug moves into clinical testing, for guiding dose and schedule selection. ***Objectives:*** This study tested the ability to use single cell network profiling (SCNP), in which cells are perturbed with extracellular modulators and their response ascertained by multiparametric flow cytometry, to 1) measure the potency and selectivity of a panel of compounds with reported inhibitory effects on JAK/STAT signaling in whole blood versus fractionated peripheral blood mononuclear cells (PBMC) and 2) test the ability of SCNP to capture rare cells amid a heterogeneous population of cells.
Methods: We measured modulation of JAK/STAT, MAPK and NFkB signaling pathways in cell subsets after *in vitro* exposure of whole blood and PBMC to GM-CSF (monocytes/neutrophils), IL-2 (T-cells), and CD40L (B-cells) in the presence and absence of known JAK inhibitors. For evaluation of SCNP in rare cells, phosphorylation of STAT proteins in response to IL-27 was examined on CD34+ CD11b- CD33- progenitors in healthy PBMCs.
Results: The relative selectivity of JAK3 versus JAK2 inhibition was simultaneously measured by plotting p-Stat-5 inhibition curves from gated T cells (JAK3 mediated) and monocytes (JAK2 mediated). Notably, CP-690550, currently in clinical trials for a variety of autoimmune diseases, was 36-fold more potent at inhibiting JAK3 compared to JAK2 activity, which is consistent with published reports. In addition, off-target signaling effects outside the JAK/STAT pathway were observed (eg, Cucurbitacin I induced a dose-dependent increase in p-ERK in the monocyte subset and "Stat3 Inhibitor VII" blocked CD40L stimulated p-ERK and pNFkB p65 in B cells). Importantly, a significant decrease in potency in all compounds except CP-690550 was observed when studies were performed in whole blood, the clinically relevant tissue, versus fractionated PBMC. Notably, SCNP technology captured IL-27 induced p-Stat-1 and p-Stat-3 responses and inhibition by Pyridone 6 in CD34+ CD11b- CD33- progenitor cells, a very rare (<40 cells; 0.11% of the total live cells) cell population in PBMC.
Conclusions: SCNP technology allowed for the evaluation of 1) compound activity in primary cells in a physiologically relevant environment (whole blood), 2) signal transduction target and off-target effects in relevant cell populations, and 3) robust IC50 determination from rare subpopulations (<100 cells). Therefore, this technology is well suited for application in oncology drug development where assessment of candidate drug effects on rare cell populations (eg, cancer stem cells, minimal residual disease, and circulating tumor cells) is crucial and permits the rational design of clinical trials based on biologically active dose rather than the

traditional maximum tolerated dose (MTD) design, which is better suited for cytotoxic, non-targeted drugs.

4. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo in streptozocin-induced diabetes mellitus type 1 in mice

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We hypothesized that oxidative stress from hyperbaric oxygen (HBO_2, 2.8 ATA for 90 min daily) exerts a trophic effect on vasculogenic stem cells n streptozocin-induced diabetes mellitus type 1 mouse model via NO-dependent mechanism. Circulating stem/progenitor cell (SPC) recruitment and differentiation in subcutaneous Matrigel in STZ-mice were stimulated by HBO_2 and by a physiological oxidative stressor, lactate. In combination, HBO_2 and lactate had additive effects. Vascular channels lined by $CD34^+$ SPCs were identified and they were significantly reduced in diabetic animals. HBO_2 and lactate accelerated channel development, cell differentiation based on surface marker expression, and cell cycle entry. $CD34^+$ SPCs of diabetic animals in blood and bone marrow defined down regulated thioredoxin-1 (Trx1), Trx reductase, hypoxia-inducible factors (HIF)-1, -2, and -3, phosphorylated mitogen-activated protein kinases, vascular endothelial growth factor, and stromal cell-derived factor-1 in. Cell recruitment to Matrigel and protein synthesis responses was abrogated in STZ-mice. By causing an oxidative stress, HBO_2 activates a physiological redox-active autocrine loop in SPCs that stimulates vasculogenesis. Thioredoxin system activation leads to elevations in HIF-1 and -2, followed by synthesis of HIF-dependent growth factors. HIF-3 has a negative impact on SPCs.

5. Normal bone marrow signal transduction profiles: A requisite for enhanced detection of signaling dysregulations in AML

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Molecular and cytogenetic alterations are involved in virtually every facet of acute myeloid leukemia (AML) cell transformation including dysregulation of major signal transduction pathways. The present study examines five phospho-proteins pErk, pAkt, pS6, pStat3 and pStat5 in response to five cytokine/GF's (SCF, Flt3-L, GM-CSF, IL-3, G-CSF) within seven immunophenotypically defined populations, spanning progenitor to mature myeloid/myelomonocytic cells in 11 normal adult bone marrows with further comparison to 14 AML samples. The data from the normal cohort showed pathway specific responses related to lineage, maturation and stimulus. Heterogeneous signaling responses were seen in homogeneous immunophenotypic subsets emphasizing the additive information of signaling. These profiles provided a critical baseline for detection of dysregulated signaling in AML falling into four broad categories viz. lack of response (complete/partial), increased activation, altered constitutive expression and dysregulated response kinetics; easily identified in 12 of 14 AML's. These studies clearly show robust and reproducible flow cytometry phospho-protein analyses capable of easily detecting abnormal signal transduction responses in AML potentially contributing to more definitive reliable identification of abnormal cells. As functional correlates of

underlying genetic abnormalities, signal transduction abnormalities may provide more stable indicators of abnormal cells than immunophenotyping which frequently changes post-therapy and with disease recurrence.

6. Drug characterization, comparison and evaluation by changes in mitochondrial membrane potential using HTS on HyperCyt and CyAn flow cytometer

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Mitochondria in the kidney experience periodic changes from metabolic demand and xenobiotic metabolism resulting in persistent damage to mitochondria. Damaged mitochondria accumulation makes the kidney more susceptible to failure when stressed by disease or xenobiotic exposure. To asses mitochondrial morphological and functional changes as a result of xenobiotic exposure, flow cytometry techniques have been developed to quantify features of mitochondria function related to changes in mitochondrial membrane potential, apoptosis, and mtDNA content.
Traditional flow cytometry assays are developed for a system focused on single tube design. To create a viable high throughput flow cytometry screen, a comprehensive system of robotic components must be integrated, which allows faster data processing as well as faster sample through put.
In this presentation, we show the drug characterization and comparison of toxicity values obtained by two different systems for the analysis of assorted drugs in HepG2 cells. Both systems were used as implemented via: Laser Scanning Cytometry (LSC) and with the HyperCyt™ instrument matched with a CyAn™ cytometer. We present some new ideas regarding methods for quantification of results.
Acquisition of several parameters of cellular responses to compounds enabled the characterization and comparison of toxicity mechanisms involved. Data were obtained using a set of four fluorescent biomarkers, enabling characterization of mitochondrial membrane potential (MMP), plasma membrane permeability, ROS generation, DNA content, nuclear circularity and area. The effect of glucose deprivation was also analyzed.
A.J.G. *thanks the Spanish Ministry of Science for the Postdoctoral Research Mobility Grant (MICINN/Fulbright-EX2008-0452) for USA.*

7. A novel orange-emitting dye for use in multicolor flow cytometry panels using violet diode laser excitation

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Background. Violet diode lasers have become common excitation sources on flow cytometers, but the advantages of this laser are constrained by limited choices for organic, violet-excited

fluorochromes. These fluorochromes include several dyes emitting around 450 nm, and two (Horizon™ V500 and Pacific Orange™ dyes) emitting at longer wavelengths. These longer wavelength fluorochromes are plagued by relatively low fluorescence signals in relation to background, and generally must be used with densely expressed antigens. We have developed a novel violet-excited organic dye, given the development name "UV3," which has an emission spectrum closely matching that of Pacific Orange dye. It has excitation and emission maxima of 398 nm and 528 nm, respectively.
Methods. Antibodies were conjugated with UV3 dye via N-hydroxysuccinimide esters. Human blood was stained with antibody conjugates and analyzed on a Gallios™ flow cytometer equipped with violet, blue, and red excitation.
Results. The UV3 fluorochrome shows optimal performance with violet laser excitation and a 550/40 bandpass filter (the standard FL10 channel on Gallios and Navios™ flow cytometer systems), while no excitation is detected using a 488 nm laser. UV3 dye is at least as bright as V500 dye and can provide more than twice the population separation obtained with Pacific Orange dye conjugates, with little compensation versus Pacific Blue™ dye. Therefore, common gating markers can be easily transferred to this fluorochrome, freeing other valuable fluorochromes for use with additional markers.
Conclusions. UV3 dye conjugates can be used on any flow cytometer equipped with violet excitation (approximate wavelength range 400 to 410 nm) and appropriate emission filters. Data is shown using UV3 conjugates of anti-human CD4, CD19 and CD45 in multicolor applications, including side scatter/CD45 gating and a 6-plus color stain. As a result, UV3 dye provides an optimized second organic fluorochrome for violet excitation, enabling 10-color applications on the Gallios / Navios flow cytometer systems.

8. An Edu®-Based Flow Cytometry Assay to Measure Lymphocyte Proliferation in the Clinical Laboratory: Replacement Of The "Gold Standard" (3H) thymidine Method

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Proliferative responses to mitogens and antigens are used as a measurement of lymphocyte function and immune competence in several clinical contexts. The current "gold standard" assay for measurement of lymphocyte proliferative responses uses tritiated (^{3}H) thymidine, a radioactive nucleoside. We developed a clinically validated test method for lymphocyte proliferation on a flow cytometric platform utilizing EdU® (thymidine analog). This new method allows for the identification of specific lymphocyte subsets, a visual interpretation of proliferation, and the ability to measure cell viability, death and apoptosis. Lymphopenic patients can show a dilution effect when proliferation is measured as total PBMC response, therefore T cell competence cannot be accurately deduced in these patients by the current method. The new flow cytometric method allows for measurement of T cell-specific proliferation, even in the context of significant T cell lymphopenia thereby eliminating the previously observed dilution effect. To verify accuracy of the new method, ten normal adult donors and two patients were tested by both methods and clinical interpretations of results were compared based on the standard reporting format. PBMCs were cultured *in vitro* with phytohemagglutinin (PHA), pokeweed mitogen (PWM), *Candida albicans* (CA) and Tetanus

Toxoid (TT). Cells were evaluated after 4 (mitogen) or 7 (antigen) days of culture followed by pulse-labeling with (^{3}H) thymidine or EdU®. Proliferation using the current method was compared to flow-based proliferation as percent of total CD45+ lymphocytes that were EdU+. All clinical interpretations of mitogen and antigen proliferative responses were concordant between the current and proposed methods for the adult normal donors. For one patient sample, response to CA stimulant showed normal proliferation by (^{3}H) thymidine, but abnormal results by flow. However, the clinical history of the patient correlated with the flow results. Therefore, the new method is an accurate and comparable method of determining lymphocyte proliferative capacity to mitogen and antigen stimulation.

9. Evaluation of Commercial Kappa/Lambda Reagents for Flow Cytometric Analysis of Normal and Abnormal Specimens

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Abstract: The evaluation of cell surface Kappa/Lambda expression can identify clonally restricted B lymphocyte populations and thus can aide in the diagnosis of hematologic malignancy. Several B cell disorders are associated with decreased levels of Kappa/Lambda at the cell surface. In these cases, it is important to utilize Kappa/Lambda reagents that can optimally identify Kappa/Lambda cell surface expression even when decreased levels are present. In this study, flow cytometric analysis was used to evaluate the performance of Kappa/Lambda reagents from two different commercial vendors (Becton Dickinson, and Dako). Normal specimens and abnormal specimens with decreased levels of Kappa/Lambda cell surface expression were interrogated in the study. Mean channel fluorescence (MCF) and Signal to noise ratios (S:N) of Kappa and Lambda expression for all reagents were collected and compared. The Dako reagents demonstrated an increased MCF and S:N of both Kappa and Lambda expression compared with the Becton Dickinson reagents when normal peripheral blood specimens were analyzed ($P < 0.05$). Abnormal specimens with decreased levels of Kappa at the cell surface were also analyzed. Again, The Dako reagents demonstrated an increased MCF and S:N compared with the Becton Dickinson reagents ($P < 0.05$). Dako Kappa and Lambda reagents have beneficial features over commensurate Becton-Dickinson reagents in detecting cell surface expressed Kappa and Lambda especially when decreased levels are present.

10. Novel Method for Evaluating Multiple Tandem-Conjugates in a Complex Panel

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There has been much discussion regarding the stability, Myeloid non-specific staining and variable energy transfer between batches for all of the cyanine dye tandem reagents. Here we focus on a single reagent, Pe-Cy7 (PC7) and try to elucidate some of these concerns.

Our initial focus is on the variations in energy transfer (effecting compensation) between 9 different antibody conjugates from 3 vendors. We wanted to have a consistent intensity of Pe-Cy7 staining for all 9 reagents, and to determine each one's effect on measuring dim positive signals in the spill over channels. To this effect we developed a "two color" antibody capture bead staining protocol.

Comparing spill matrices for 9 reagents was cumbersome, in addition this method did not illuminate how much variation was unacceptable. Arrays of dot plots were found to be less than useful to display small differences. To avoid these issues we concatenated the data files, so that we could show the results in a single plot. Also, Pe-Cy7 stability was tested at 1, 22 and 46 hours post staining at ambient light and temperature.

The spill of Pe-Cy7 into PE was consistent between 8 of the reagents, possibly allowing the use of a single compensation setting for well-resolved markers, the 9th reagent clearly was not. Pe-Cy7 spill into APC-Cy7 was more inconsistent and would be more problematic. Also, Pe-Cy7's spill into various channels could not be predicted by looking at a single channel, suggesting that the spill was coming from more than one source. By using antibodies that exclusively stain lymphocytes we were able to demonstrate differences in Myeloid non-specific staining not only between vendors, but within a single vendor. Pe-Cy7 stability was well maintained for one day, but there was a noticeable degradation at 46 hours.

11. Post Sort Product Validation: Ensuring Sort Counter Accuracy in Addition to Purity Checks as a Part of Sorting QC/QA Program

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The quality control of the products of cell sorting has been limited. The current practice in our lab is restricted to doing a "purity check", simply analyzing a small aliquot of the sorted product back through the gate hierarchy that was used to establish the original sort parameters. However, other factors may influence the quality of the sorted product, such as viability and the number of cells actually recovered.
Here we will address the recovery of sorted cells vis-à-vis the sort counters. To that end we will evaluate several methods to count cells: the Beckman Coulter ViCell XR, the Millipore Scepter and the use of absolute counting beads by flow cytometry.
Additionally, a standard curve was performed to determine if the fluidic path affects the accuracy of the bead counts. The following BD instruments were tested: a BD FACSCalibur, BD FACSCanto, BD FACSVantage DiVa and BD FACSAria II. All instruments compare favorably with the worst R^2 at 0.996.
The Scepter produced consistently low results, while the other methods tended to trend slightly higher than the sorter cell counters. The counting beads gave the most consistent results.

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Applied Cytometry Sys., Sacramento, CA
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