Great Lakes International Imaging and Flow Cytometry Association

OLYMPIA RESORT
AND CONFERENCE CENTER
FRIDAY, SEPTEMBER 19 – SUNDAY, SEPTEMBER 21, 2014
Oconomowoc, WI

GLIIFCA 23

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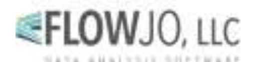

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ABOUT GLIIFCA

GREAT LAKES INTERNATIONAL IMAGING AND FLOW CYTOMETRY ASSOCIATION, INC.

EIN# 16-1545169

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

THE HISTORY OF GLIIFCA - BY CARLTON C. STEWART

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

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

GENERAL INFORMATION

(WHAT YOU ALWAYS WANTED TO KNOW ABOUT GLIIFCA 23 BUT WERE AFRAID TO ASK!)

CONFERENCE REGISTRATION DESK

- The registration desk is located at Olympia Center (**OLY**) Lobby:
    - Friday, Sep. 19 – 5:30pm to 10:00pm
    - Saturday, Sep. 20 – 8:00am to 11:00pm
    - Sunday, Sep. 21 – 9:00am to 12:30pm
- The conference registration fee includes Friday reception, Sat/Sun breakfast, Sat. lunch, evening reception, banquet and coffee breaks.

POSTERS

- Posters set up: Friday, September 19 after 5:00pm in **Olympia Center (OLY) AB**
- 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 **VELCRO** only
- Poster viewing: from Friday 6:30pm to Sunday 10:30am
- Poster presentation and judging: Saturday 5:30pm to 7:00pm

EXHIBITS

- Scheduled vendors will have booths in the Exhibit/Poster area (**OLY Center AB**)
- **Please Note: Booth set-up for OLY Center AB starting at noon on Sept. 19 with opening of OLY Center AB at 5:30pm. Break down starting at 12:30pm on Sunday, Sept. 21**
- All activities other than the plenary sessions, roundtable luncheon, Steering Committee meeting and banquet will be located in the Exhibit/Poster area (**OLY Center AB**)
- **Please frequent the booths and show our appreciation for the generous financial support provided by the vendors who substantially help "pay the freight" for this meeting**

LOCATION OF ALL ORAL PRESENTATIONS (SAT/SUN): OLY CENTER AB

BREAKFASTS

Free continental breakfast will be provided for all the registrants in the Exhibit/Poster area (**OLY Center AB**) on Saturday from 7:15am to 8:15am, and on Sunday from 7:30am to 8:30am

COFFEE BREAKS

- The refreshments will be served in **OLY Center AB**:
    - Saturday – 9:45am to 10:15am, and 3:30pm to 4:00pm
    - Sunday – 10:00am to 10:30am

INDUSTRIAL SCIENCE SYMPOSIUM

- Industrial Science Symposium presentations: 6.25pm to 10:00pm, **Olympia Center (OLY) C**.

SOCIAL ACTIVITIES

OPENING WINE AND CHEESE RECEPTION

- 5:30pm to 7:00pm, **OLY Center AB**
- Use 3 drink tickets for wine and beer

SATURDAY LUNCHEON ROUNDTABLES (12 NOON TO 1:15PM)

- Free lunch/pop; 2/3 roast beef/turkey and 1/3 veggie wrap in **Illinois A** (Tables 1-3), **Illinois B** (Tab 4-6), **Illinois C** (Tab. 7-9); **Wisconsin A** (Tab. 10-12); **Wisconsin B** (for non-participants),
- Pick up box lunch (roast beef, turkey or veggie wrap) and soft drink displayed in each room and move to roundtable of 10 labeled with the title of discussion topic. The attendance at each table is determined from the **sign-up sheet** at GLIIFCA registration desk. Non-participants can lunch in the **Wisconsin B**.

SATURDAY WINE AND CHEESE HAPPY HOUR:

- 5:30pm to 8pm in the Exhibit/Poster area (**OLY Center AB)** with cheese and fresh fruit trays (you can use your drink tickets).

GLIIFCA BANQUET (MAD SCIENTIST PARTY!)

- Free to registrants and paid guests
- Commences at 8:00pm, **Illinois Room**
- 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 9:00pm – 11:00pm; requests encouraged (get up and have fun!)

DRINKS

- Full service bar will be located in the Exhibit/Poster area **(OLY Center AB)** for Friday opening reception and Saturday afternoon Wine & Cheese reception, and in **Illinois Room** for banquet on 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

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

Other Information

GLIIFCA Website

- http://www.gliifca.org

CMLE Credits

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

Name Tags And Evaluation Forms:

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

Additional Enquiries, comments, suggestions

- Contact Dr. Alexander Nakeff (e-mail: caralex3@comcast.net, cell: (313) 820-6227) or leave message at the GLIIFCA registration desk
- Comments and suggestions: e-mail Dr. Bartek Rajwa at president@gliifca.org

Steering Committee Breakfast Meeting

- Sunday morning (7:30 to 8:30am) in **Crown Room**

CONFERENCE SPONSORS

The 2014 GLIIFCA meeting is kindly supported by:

- Beckman-Coulter – support for the Wine and Cheese Happy Hour and coffee breaks
- BD Bioscience – support for the Best Poster Award, and GLIIFCA Travel Awards
- Biolegend
- Bio-Rad – support for the conference coffee breaks
- Biotechne – support for the Best Poster Award, and GLIIFCA Travel Awards
- Cell Signaling Tech
- Cytek – support for the Wine and Cheese Happy Hour
- Cytobank
- eBioscience – support for the Wine and Cheese Happy Hour
- EMD Millipore – support for the GLIIFCA Mad Scientist Ball
- Enzo Life Sciences
- FlowJo LLC
- Handyem Inc.
- ImmuDex – suport for the ImmuDex Translational Cytometry Lecture
- Intellicyt
- Leinco
- Life Technologies (Thermo Fisher Scientific) – support for GLIIFCA Travel Awards
- Miltenyi Biotec
- Propel Labs LLC
- Sony Biotechnology – support for the GLIIFCA Mad Scientist Ball
- Spherotech – support for Jeffrey Wang Memorial Achievement Award and coffee breaks
- Stratedigm – support for the Wine and Cheese Happy Hour
- Sysmex
- Tonbo
- Verity
- ViroCyt

CONFERENCE PROGRAM

FRIDAY

OPENING RECEPTION

The Wine and Cheese Opening Reception will start on 5:30 PM following the Core Facility Managers' Workshop.

INDUSTRIAL SCIENCE SYMPOSIUM

The Industrial Science Symposium will feature presentations from the cytometry vendors. The symposium will take place from 6:25 PM to 10:00 PM. The ISS Program can be found on page 12.

SATURDAY

8:00 AM – 8:15 AM	*Welcome address*, **Bartek Rajwa** (GLIIFCA President)

SESSION 1: CYTOMETRY IN CELL-BASED SCREENING (CHAIRED BY DR. BARTEK RAJWA)

8:15 AM – 9:00 AM	*Multi-color, multi-parameter high-throughput flow cytometry for compound screening using correlated cell physiology signatures*, **T. Vincent Shankey** (AsedaScience AG, University of Miami)
9:00 AM – 9:45 AM	*Multiplexing high throughput flow cytometry platform for drug discovery*, **Yang Wu** (University of New Mexico)
9:45 AM – 10:15 AM	Coffee break sponsored by Spherotech
10:15 AM – 11:00 AM	*Data for decision making – critical steps to improve the translatability of flow cytometric data*, **Virginia Litwin** (Covance)

THE 2014 CARLTON AND SIGRID STEWART KEYNOTE LECTURE

11:00 AM – 11:05 AM	*Introduction,* **J. Paul Robinson** (Purdue University)
11:05 AM – 12:00 noon	*Constitutive level of the ROS/DNA damage- and mTOR/S6K1-signaling. The Biomarkers of aging and senescence, assessed by cytometry*, **Zbigniew Darzynkiewicz** (Brander Cancer Research Institute, New York Medical College).

LUNCHTIME ROUNDTABLE WORKSHOPS

12:00 noon – 1:15 PM	Roundtable workshops

SESSION 2: CYTOMETRY DATA ANALYSIS (CHAIRED BY DR. WILLIAM C. EADES, JR.)

1:15 PM – 2:00 PM	*Development of a flow cytometry computational pipeline*, **Wade T. Rogers** (University of Pennsylvania)
2:00 PM – 2:45 PM	*A non-parametric Bayesian model for joint cell clustering and cluster matching under random effects*, **M. Murat Dundar** (Indiana University - Purdue University, Indianapolis)

2:45 PM – 3:30 PM	*Deriving information from high-dimensional data*, **Daniel Normolle** (University of Pittsburgh)
3:30 PM – 4:00 PM	Coffee break sponsored by Beckman-Coulter

Session 3A: Clinical And Translational Cytometry (Chaired by Dr. Jyoti Watters)

4:00 PM – 4:45 PM	*A primer on microparticology and implications for translational biomedical science*, **Michael Larson** (Medical College of Wisconsin) – Young Investigator Presentation
4: 45 PM – 5:30 PM	*Monitoring immune and anti-tumor responses prostate cancer patients following DNA immunization using multi-parameter flow cytometry*, **Brian Olson** (University of Wisconsin) – Young Investigator Presentation

Poster session

5:30 PM – 7:00 PM	*Poster session.* Sponsored by BD Bioscience
6:00 PM – 7:00 PM	*Wine and Cheese Happy Hour.* Sponsored by Cytek, Beckman-Coulter, eBioscience, and Stratedigm

Social activities

8:00 PM – 11:30 PM	Mad Scientist Costume Ball! Co-sponsored by EMD Millipore and Sony Biotechnology
11:30 PM – 2:30 AM	Scientific networking at Club Indigo

Sunday

Session 3B: Clinical and translational cytometry (Chaired by Dr. Phil Hexley)

8:30 AM – 9:15 AM	*Use of multi-parameter flow cytometry to determine immune phenotypes associated with decreased respiratory illnesses and allergic disease*, **Christine Seroogy** (University of Wisconsin)
9:15 AM – 10:00 AM	*A mine is a terrible thing to waste: the value of high dimension, single cell technologies in cytometry*, **Pratip Chattopadhyay** (NIH). Sponsored by Immudex
10:00 AM – 10:30 AM	Coffee break sponsored by Bio-Rad
10:30 AM – 11:15 AM	*Advances in microvesicle detection by flow cytometry for research and clinical applications*, **Nancy Fisher** (University of North Carolina)
11:15 AM – 12:00 PM	*Flow cytometric detection of minimal residual disease in multiple myeloma*, **Paul Wallace** (Roswell Park Institute)

ROUNDTABLE LUNCH WORKSHOPS

The round-table lunch workshops will cover the following topics:

R1 – *ONE AT A TIME: DEVELOPMENT AND APPLICATION OF SINGLE CELL SORTING.*

HOSTED BY LARA KREBS (ELI LILLY) AND PRATIP CHATTOPADHYAY (NIH)

This roundtable discussion will cover a) the need for single cell sorting, b) laboratory and statistical methods for ensuring single cells are sorted, c) strategies for combining cell sorting with microscopic fluorescent cell imaging, d) index sorting, e) downstream transcriptomics applications (and their potential value in confirming single cell deposition).

R2 – *HIGH-CONTENT AND HIGH-THROUGHPUT FLOW CYTOMETRY. ARE WE THERE YET?*

HOSTED BY T. VINCE SHANKEY (ASEDASCIENCES AG, UNIVERSITY OF MIAMI)

The cytometry community criticizes high-throughput screening (HTS) for poor content and lack of single-cell information. The image-based high-content screening (HCS), and high-throughput flow cytometry (HTFC) are portrayed as promising alternatives. However, in contrast to uniparametric HTS, the data processing and data reduction remain a big challenge for high-throughput single-cell analysis methods severely limiting their practical applicability. Is cytometry really well-suited for screening? Let's discuss! Suggested reading: S. Singh et al. (2014). *Increasing the Content of High-Content Screening - An Overview*. J Biomol Screen 19, 640–650. doi:10.1177/1087057114528537

R3 – *AUTOMATED DATA ANALYSIS IN CYTOMETRY*

HOSTED BY WADE T. ROGERS (UNIVERSITY OF PENSYLVANIA)

There's been a lot of hand-wringing about analysis of flow cytometry data. We all know how labor intensive this is, how hard it is to know we've done all we can, and how frustrating it is to have to go back and do it repeatedly until we get it right (whatever that is). Automated computational analysis may seem like a panacea. But is it, really? We'll compare the attributes of human versus automated analysis, and try to come up with guidelines for how to choose between them.

R4 – *FLOW CYTOMETRY OF MICROPARTICLES*

HOSTED BY MICHAEL LARSON (MEDICAL COLLEGE OF WISCONSIN)

Anthropologists can examine garbage to learn a lot about the subject of their study. Have you ever stopped to consider that the debris or "cellular garbage" your cells shed might actually contain useful information? This roundtable lunch session will host a discussion on some why's and how's of looking at what some considered cellular debris.

R5 – *QUALITY CONTROL IN CYTOMETRY*

HOSTED BY RYAN DUGGAN (UNIVERSITY OF CHICAGO)

Quality Control and Quality Assurance (QC/QA) is likely one of the most mundane tasks of managing a cytometer, and yet is absolutely critical for the long term success of applications being run. In this roundtable discussion, we'll share strategies, techniques, and principles for the performance, analysis, and interpretation of QA/QC protocols.

R6 – *Emerging technologies in cytometry (multispectral, scanning, and Raman-based cytometry)*

HOSTED BY J. PAUL ROBINSON (PURDUE UNIVERSITY)

Multispectral cytometry is now entering the mainstream of cytometry. This table will focus on advantages, disadvantages, opportunities and applications of spectral cytometry systems. We will compare multispectral cytometry to traditional polychromatic instruments.

R7 – *Imaging flow cytometry applications*

HOSTED BY MONICA DELAY (CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER)

Imaging flow cytometry is a powerful tool that can provide a new perspective of cellular analysis. This roundtable will highlight applications of imaging flow cytometry for cellular analysis, as well as provide a forum for user exchange of strategies and/or discussion of issues involving the technology.

R8 – *Training strategies in flow cytometry service laboratories*

HOSTED BY DAVID LECLERC (UNIVERSITY OF CHICAGO)

One of the critical mission of a flow cytometry service laboratory (FCSL) is the training of it's users. Most FCSLs offer some kind of training course to help their users understand the technology and generate insightful data. However, these courses are often prepared in-house and there are very few tools available to evaluate the adequacy of their content. At this roundtable, we will present and discuss the different training programs offered by each participants and attempt to identify the best practices that characterizes successful courses. This informal discussion will cover the topics of course length, course content, methods of presentation, etc.

R9 – *Flow cytometry biosafety*

HOSTED HANK PLETCHER (UNIVERSITY OF PENNSYLVANIA)

The host of this roundtable will provide a review of the latest ISAC Cell Sorter Biosafety Standards. The participants will have a chance to learn what's new in this document. Some case studies will be discussed.

R10 – *Grants and funding opportunities for cytometry research*

HOSTED BY BETSY OLHSSON-WILHELM (SCIGRO, INC.)

This roundtable discussion will focus on funding opportunities and ways to foster interactions between academic researchers and industry. Industrial and foundation support as well as federal opportunities will be reviewed.

R11 – *RNA in Cellular Context: Enabling Translational Research*

HOSTED BY MATTHEW CATO (EBIOSCIENCE)

Flow cytometry is well established as the most powerful tool for single cell analysis of complex heterogeneous samples. The advent of advanced intracellular staining techniques enabling the simultaneous measurement of surface antigens together with cytoplasmic and nuclear proteins as well as post-translational modifications has revolutionized the way researchers approach scientific questions. The most recent advancement in intracellular flow cytometry is the ability to directly detect nucleic acids within individual cells. By coupling multicolor detection of both surface and intracellular protein targets together with the

direct measurement of RNA by flow cytometry it is now possible to measure transcription of multiple targets in heterogeneous populations without sorting, evaluate both transcriptional and translational regulation within the same cell, identify virally infected cells and overcome the lack of available antibodies to novel targets and unique species. Please join us to discuss how the use of bDNA signal amplification is changing the way scientists approach gene expression studies and how advancements in the FlowRNA approach are leading to new biologic insights that have never before been possible.

Industrial Science Symposium

The industrial science symposium chaired by Karen Domenico and Louis King and will feature presentations from the cytometry vendors. The symposium will take place from 6:25 PM to 10:00 PM on Friday, Sep. 19th.

6:25 PM - 6:30 PM	*Introduction*, **Karen Domenico** & **Louis King**
6:30 PM - 6:50 PM	**ViroCyt**, *Virus Counter 3100*, Mark Rehse
6:50 PM - 7:10 PM	**Miltenyi Biotec**, *(1) Flow Sorting Without Droplets: Imagine a Sterile, Aerosol Free, High Speed Sorter. (2) Recombinantly Engineered Antibodies (REAfinity) and VioBright Dyes – Smart Antbodies for Smart Cytometr*, Matthew Drew
7:10 PM - 7:30 PM	**BD Bioscience**, *Biosafety and the BD FACSAria™ Fusion*, Gil Reinin
7:30 PM - 7:50 PM	**Verity**, *Effective Use of Batch Processing in ModFit LT*, Mark Munson
7:50 PM - 8:10 PM	**Beckman-Coulter**, *The Power of Choice: Introducing the CytoFLEX Research Cytometer*, Dom Fenoglio
8:10 PM - 8:30 PM	**EMD Millipore**, *Improved Fluorescent Detection on the New Guava easyCyte 12 and 12 HT*, Jerry Aultz
8:30 PM - 8:50 PM	**Sony Biotechnology**, *Sony Flow Cytometry: Technology for Your Science*, David Buschke
8:50 PM - 9:10 PM	**eBioscience**, *RNA in Cellular Context: Enabling Translational Research*, Matthew Cato
9:10 PM - 9:30 PM	**Life Technologies**, *The Next Generation In Acoustic Cytometry: Attune® NxT Acoustic Focusing Cytometer*, Brian Wortham
9:30 PM - 9:50 PM	**Amnis/EMD Millipore**, *Imaging Flow Cytometry: evidence that it is becoming a need to have technology*, Robert Thacker

PRESENTATION ABSTRACTS

A MINE IS A TERRIBLE THING TO WASTE: THE VALUE OF HIGH DIMENSION, SINGLE CELL TECHNOLOGIES IN CYTOMETRY

PRATIP K. CHATTOPADHYAY (NIH)

The complexity of cells is remarkable, both in terms of protein and gene expression. In the past 10-15 years, there has been a revolution in single cell technology, with the advent of new technological platforms that appreciate this complexity. In this talk, I'll review the advantages and limitations of multiparametric technologies, in the framework of biological and disease settings. In particular, I'll discuss how we have integrated polychromatic flow cytometry, single cell transcriptomics, and advanced bioinformatics to reveal previously unappreciated correlates of disease and vaccine-induced immunity. Finally, I'll demonstrate how our work - pursuing the most complex cellular phenotypes - can be used to reveal simplest, most clinically-relevant phenotypes, which can then be validated in larger studies with simpler, more readily available technology.

CONSTITUTIVE LEVEL OF THE ROS/DNA DAMAGE- AND MTOR/S6K1- SIGNALING, THE BIOMARKERS OF AGING AND SENESCENCE, ASSESSED BY CYTOMETRY

ZBIGNIEW DARZYNKIEWICZ (BRANDER CANCER RESEARCH INSTITUTE, NEW YORK MEDICAL COLLEGE)

Presented will be the evidence that constitutive level of signaling throughout the IGF-1/mTOR/S6K1 pathways is the primary factor contributing to aging and cellular senescence. Also outlined will be specific interactions between mTOR/S6K1 and ROS-DNA damage signaling pathways. Particular sites along these pathways, including autophagy, provide rational targets for potential antiaging (gero-suppressive) and/or chemopreventive intervention. Presented are applications of flow- and laser scanning- cytometry utilizing phospho-specific Abs, to monitor activation along these pathways in response to the reported anti-aging drugs. Specifically, the effectiveness of rapamycin, metformin, berberine, resveratrol, vitamin D3, 2-deoxyglucose, and acetylsalicylicacid (aspirin) to attenuate the level of constitutive mTOR signaling was tested by cytometry by measuring phosphorylation of the mTOR-downstream targets including ribosomal protein S6; the data were confirmed by western blotting. The results show that the ratiometric analysis of phosphorylated to total signaling protein along the mTOR pathway offers a useful parameter reporting the effects of gero-suppressive agents. The ability of the studied gero-suppressive agents to suppress the level of constitutive DNA damage signaling induced by endogenous ROS was also measured. Altogether, the data obtained by flow and laser-scanning cytometry, on several human cancer cell lines as well as on WI-38 fibroblasts and normal lymphocytes, indicate that while the primary target of each of these agents may be different, there is a common downstream mechanism in which the decline in mTOR/S6K1 signaling and translation rate is coupled with a reduction of oxidative phosphorylation and abundance of ROS that leads to decreased oxidative DNA damage. The combined assessment of constitutive γH2AX expression, mitochondrial activity (ROS, ΔΨm), and mTOR signaling by cytometry provides an adequate gamut of cell responses to test effectiveness of gero-suppressive agents. Described will be also an in vitro model of induction of cellular senescence by persistent replication stress, its quantitative analysis by laser scanning cytometry, and application to detect the effectiveness of the studied agents to attenuate the induction of the senescence phenotype.

A Non-parametric Bayesian Model for Joint Cell Clustering and Cluster Matching under Random Effects

M. Murat Dundar (IUPUI)

Most of the methodologies proposed for automated analysis of FC data attempts to perform clustering one sample at a time, ignoring the fact that multiple samples can be considered as different realizations of a single underlying model reflecting the biological reality. For samples containing abundant and well-separated biological populations this limitation is of no practical consequence. The individual samples may be clustered, and the biological populations present in multiple samples can be aligned and matched post-clustering in order to perform a secondary analysis. However, this conventional approach may fail if some of the cellular classes are represented by a low number of cells, if the population locations significantly vary from sample to sample, or if populations disappear or appear between samples. In this talk, Dr. Dundar presents a non-parametric Bayesian algorithm called ASPIRE (anomalous sample phenotype identification with random effects) that identifies biologically significant phenotypes across a batch of samples in the presence of random effects. This approach does not assume a priori the number of cell types (global clusters or meta-clusters) present in the biological samples analyzed, whether they are normal or anomalous. However, it takes advantage of the fact that different samples share common characteristics, as they represent snapshots of the same underlying biological phenomenon. Therefore, the proposed methodology assumes that certain cell types would occur in multiple samples, forming noisy realizations of global clusters. The goal is to discover local clusters across samples and then recover global clusters identifying different cell populations.

A Primer On Microparticology And Implications For Translational Biomedical Science

Michael Larson (Medical College of Wisconsin)

Microparticles or microvesicles (MPs) are subcellular-sized vesicles that are shed from virtually every mammalian cell in response to a myriad of stimuli. Once considered inert debris or cellular dust, the roles of MPs in human biology are progressively becoming clearer with advances in flow cytometry. While some MPs may be biologically beneficial, MPs are generally elevated in diseased states and are, thus, considered to play largely pathological roles. Changes in MP concentrations are more rapid and dynamic relative to changes in circulating cell characteristics or concentrations. Accordingly, MP levels can serve as an adjunct for rapid clinical diagnosis, prognosis, and as a marker of treatment efficacy. However, detection of MPs from biological fluids is not trivial. Multiple factors influence the potential difference in concentration and characteristics of MPs detected by a cytometer as compared to what truly is in the vasculature. These include methods around the collection and preparation of the sample fluid, selection of molecular markers, and flow cytometer variables. Systematic studies of RBC-derived MPs accentuate the difficulty in minimizing such variability. Researchers investigating MPs must consider the method of sample collection from the body to the bench, (needle diameter, time delay, etc.), vesicle isolation (centrifugation, buffers), molecular markers, and cytometer settings. Standardization of protocols is desperately needed before cytometric analysis of MPs moves from experimental to clinical realms.

Data for Decision Making – Critical Steps to Improve the Translatability of Flow Cytometric Data

Virginia Litwin (Hematology Dept. Covance Central Laboratory Services)

Since the advent of Translational Science, circa 2000, the interplay among academia, regulatory agencies, and industry has increased dramatically. The strategic goal of Translational Science is to accelerate the

implementation of discoveries from 'bench to bedside'. More specifically, Translational Science aims to catalyze the generation of innovative methods and technologies that will enhance the development, testing, and implementation of diagnostics and therapeutics across a wide range of diseases and conditions. After almost 15 years, the success of the Translational Science approach in the approval of new molecular entities (NME) is debatable, thus, process improvements such as the need for robust assessment tools as well as better methodology and validation are being highlighted. Flow cytometry has become a valuable technology in Translational Science, and is routinely used in each phase of the life-cycle of an NME: target discovery, compound screening, lead candidate characterization, and the evaluation of clinical responses. During the drug development process, information and analytical methods are transitioned from non-regulated laboratories to regulated environments. With the understanding that the intended use of the data dictates the validation requirements of the method, non-regulated laboratories do not need to engage in full method validation, but must take steps to ensure that data are reliable and suitable for decision making. In this presentation, the current best practices for instrument and method validation in flow cytometry will be reviewed. Recommendations regarding the minimum requirements for instrument monitoring, assay development, and method validation in a non-regulated environment aimed at maximizing assay translatability will be reviewed.

Deriving Information From High-Dimensional Data

Daniel Normolle (University of Pittsburgh)

Contemporary assay platforms report markers whose number is essentially unbounded and which have a complicated correlational structure. An appropriate analysis strategy depends on the intent of the study and must address threats to the validity of high-dimensional data-derived models. Analysis methods, such as t-tests, designed for bivariate data, are ill suited for generalization to the analysis of high-dimensional data, and over-fitting (in prediction modeling) and control of the family-wise error rate (in hypothesis testing) cannot be addressed in a post manner. Contemporary machine learning methods can address some of these issues, but there are many of them, and limited guidance for choosing between them. A general modeling framework that addresses these issues will be presented, along with an example that demonstrates issues and potential solutions.

Monitoring Immune and Anti-Tumor Responses Prostate Cancer Patients Following DNA Immunization Using Multi-Parameter Flow Cytometry

Brian Olson (University of Wisconsin)

Despite several new agents recently approved for the use of advanced prostate cancer, it remains the most commonly diagnosed malignancy in the United States and second leading cause of cancer related death. However, the relatively long period of time from disease recurrence to mortality, along with a predisposition to inflammation and other factors, has led to great interest in the development of immunotherapeutic approaches to treat patients with recurrent disease. This is most clearly evidenced by the approval of Sipuleucel-T for men with advanced prostate cancer, an immunotherapy that targets the tumor antigen prostatic acid phosphatase (PAP). Our group has similarly focused on targeting PAP, using plasmid DNA as a means of antigen delivery. We have previously shown by standard immunological assays that this vaccine can augment immune responses in patients, with these responses being associated with favorable changes in serum markers of disease. Using a multi-parameter flow cytometry assay to interrogate cytokine expression by various T cell subsets, it is possible to interrogate in more detail the nature of these immune responses. Additionally, using a novel flow-based assay, we have been able to detect and characterize an antigen-specific regulatory T cell population that can mask the presence of effector responses following immunization. Finally,

using a separate flow-based assay to identify circulating tumor cells, it may be possible to identify mechanisms tumor use to evade anti-tumor immune responses.

Development of a Flow Cytometry Computational Pipeline

Wade T. Rogers (University of Pennsylvania)

Single cell analyses are becoming major players in life science markets. However, the increasing dimensionality, complexity and volume of data produced by flow cytometry pose a serious challenge that must be overcome in order to realize the full clinical potential of the method. High dimensional cell-based measurements, coupled with sophisticated computational analysis (collectively termed "cytomics") may yield phenotypic or functional patterns that can provide informative biomarkers for discovery and clinical use. We envision an industrial-scale computational framework that will support the development and deployment of a data analysis pipeline that (a) is robust, hardened, and fully automated, eliminating analysis subjectivity and facilitating regulatory filing, (b), can be deployed in a centralized application-as-service business model for prospective, on-demand analysis of laboratory data and (c) enables retrospective datamining of cytomic data for analysis and discovery of new biomarkers.

Use of Multi-parameter Flow Cytometry to Determine Immune Phenotypes Associated with Decreased Respiratory Illnesses and Allergic Disease

Christine Seroogy (University of Wisconsin)

Wheezing illnesses in early childhood are a major source of morbidity, and are the leading cause of hospitalization during infancy. Recurrent wheezing may be the first sign of asthma, especially in children who also develop allergic sensitization. Risk factors for wheezing illnesses include immunologic immaturity, particularly related to antiviral defenses. There is accumulating evidence that immune maturation may be affected by environmental exposures beginning in the prenatal period. For example, farming environments stimulate innate pathways via Toll-like receptors (TLRs) to increase the production of proinflammatory cytokines (i.e. IL-6 and TNF-a). To date, studies have not been undertaken to fully characterize, at the single cell level, the cellular source(s) for these cytokines and its relationship to antiviral responses. Our group has established a birth cohort of infants born into farming and non-farming environments, called the Wisconsin Infant Immune Surveillance Cohort or WISC study, to investigate effects of farm exposures on early immune maturation and respiratory health. Epidemiologic data from the WISC study area have demonstrated that infants raised on farming environments have a significant decrease in allergic diseases and uniquely, also have a marked reduction in respiratory illnesses during the first two years of life. Optimization of a multi-parameter flow cytometry assay to define innate cell responses to varied TLR agonists and use of this assay in our ongoing WISC study will be discussed. (Funding Sources: U19 AI 104317; U54 OH010170)

Multi-color, Multi-parameter High-throughput Flow Cytometry for Assessing the Phenotypic Impact of Pharmaceutical Compounds

T. Vincent Shankey (AsedaScience AG, University of Miami)

Pharmaceutical research organizations use phenotypic analysis to develop, screen, validate, and study the mechanisms of action of candidate compounds. High-throughput flow cytometry (HTFC) is an emerging method for cell-based compound screening.The recent progress in HTFC platforms, including automated samplers and laboratory robotics, allows flow cytometry to perform rapid sequential analysis of large numbers of samples exposed to test compounds. However, the major remaining difficulty is design of a platform/method combination providing robustness, reproducibility and multiplexed data readout for heterogeneous biological models, including mixtures of cells in different stages of the cell cycle. Additionally,

a consequence of the complexity of detected phenotypes is the increasing complexity of the collected data. Thus, feature extraction, data reduction, analysis, and mining represent a real and significant problem, limiting our ability to utilize HTFC for routine compound screening. We utilize an IntelliCyt HyperCyt autosampler integrated with a Beckman Coulter Cyan instrument, and Beckman Coulter robotic liquid handling systems. This hardware combination allows the analysis of a 384-well plate in less than 30 minutes. Our phenotypic profiles include glutathione content, multiple viability markers, reactive oxygen species, and mitochondrial membrane potential (MMP) performed simultaneously with live cell cycle analysis. Our multiplexed data collection scheme allows measurement of various physiological parameters for each cell cycle-defined subpopulation. Consequently, the results form a multi-dimensional array of numerical values describing dissimilarities between controls and measured samples in the biological feature space. These values are interconnected and statistically dependent owing to correlations between the measured physiologies. This presentation will focus on important technical details for these types of assays, including validation of lab robotics, establishing measures of reproducibility, and tracking time-dependent drift in cellular responsiveness. We also demonstrate a dedicated data analysis approach. Although traditional screening experiments usually report logEC50 values (logarithm of half maximal effective concentration) for individual phenotypic markers, a more informative approach involves presentation, visualization, and comparison of phenotypic "signatures" or profiles based on multidimensional dissimilarity between the samples and relevant controls. We will demonstrate methods for profile compression and comparisons based on polyadictensor decompositions and measurement of distances between profile curves. We conclude by discussing a case study showing the use of our approach to identify compounds which cause cell-cycle dependent mitochondrial toxicity.

Flow Cytometric Detection of Minimal Residual Disease in Multiple Myeloma

Paul K. Wallace (Department of Flow & Image Cytometry, Roswell Park Cancer Institute. Buffalo, NY)

Current therapeutic approaches for plasma cell myeloma attain an overall survival of more than six years for the majority of newly diagnosed patients. Novel effective treatments are being developed at an unprecedented rate for these patients but are becoming available at a very slow rate. This is because randomized phase 3 clinical trials take years to show a benefit using progression free survival (PFS) and overall survival (OS) as study endpoints. PFS and OS are currently the only accepted FDA endpoints for demonstrating drug efficacy. Recognizing the increasing gap between drug development and approval regulatory bodies are now investigating whether biomarker evaluation of response, such as measurement of minimal residual disease (MRD) as assessed by flow cytometry, can provide an early, robust prediction of survival and so improve the pace and cost of the drug approval process. Over the past decade, several publications have emerged demonstrating enhanced prediction of outcome using flow MRD testing for multiple myeloma over conventional response assessments. In these studies, flow cytometry demonstrated independent prediction of progression-free and overall survival, One recent large study evaluated over 1,000 patients divided among three treatment arms using a 6-color antibody panel testing for CD19, CD56, CD38, CD138, CD45, and CD27 to distinguish abnormal from normal plasma cells (Rawstron et. al. J Clin Oncol 31:2540-2547, 2013). They counted 500,000 bone marrow cells and required greater than 50 abnormal cells as a cutoff for MRD positivity. Taking this approach, they had a maximum detection sensitivity of 0.01% and found achievement of MRD negativity by flow cytometry was a powerful predictor of favorable PFS and OS outcomes. To be clinically useful and acceptable as a surrogate for PFS and OS by the FDA a standardized, reproducible assay that can be performed in multiple laboratories is required. A recently published survey of labs performing MRD testing for myeloma revealed a striking heterogeneity in practices with major

differences in antibody panels, gating strategies and event counts (Roschewski et. al. J Clin Oncol 32:1-2, 2014). For the FDA to accept flow-MRD as a surrogate for PFS and OS a standardized approach in which different labs achieve comparable results is necessary. Recently a group with participants from the ICCS, NIH and FDA has been working on consensus guidelines for flow-MRD in multiple myeloma. This working group, building on the published studies showing correlation with outcomes, has established a standardized methodology, 8 color antibody panel with a minimum sensitivity of 0.01% and an upper sensitivity of 0.001%. This presentation will review the treatment options for multiple myeloma, current standards for measuring treatment responses, the application of flow cytometry to this problem and the development of a consensus for a harmonized procedure to evaluate MRD by flow.

Multiplexing High Throughput Flow Cytometry Platform for Drug Discovery

Yang Wu (University of New Mexico)

G-protein coupled receptors (GPCRs) are the largest targeted family in drug discovery, representing 19% of all drug targets. Approximately 36% of current drugs target 35 of the 376 potential drug targets in the GPCR family. The huge gap between current and potential targets left the door wide open to the discovery of new ligands for druggable GPCRs. However, the search for ligands for the current none-drug targets, new drugs for current targets that regulate novel signaling pathways or serving a new role in a different disease both remained challenging. Generalized approaches for small molecule discovery for non-canonical ligands of GPCRs as well as ligands for orphan receptors are relatively unavailable to the research community. We developed a robust and highly adaptive high-throughput flow cytometry platform based on fluorogen activating protein (FAP) technology for the discovery of ligands of GPCRs and other cell surface proteins in a pathway-independent fashion. We have used this novel platform to identify unique small molecule ligands for human beta-2 adrenergic receptor from large combinatorial libraries, and have expanded the technology by screening multiple cell lines in the same well, and multiple proteins expressed in the same cell. Current data shows that a total of 25-30 cell lines can be bar-coded with two different fluorescent dyes for functional studies. In a series of pilot multiplexing screens, 3 FAP-tagged GPCRs are expressed in three cell lines with their specific bar-codes, and receptor-specific as well as non-specific ligands have be identified from the screen. Taken together, we have developed a powerful FAP based HT multiplexing platform to identify regulators of GPCRs and other surface sensory proteins. The scalable platform can be used to identify compounds that induce receptor internalization independently of the signaling pathway, to distinguish receptor specific ligands in the multiplexing screen, and to accelerate the discovery of new targets from the druggable genome.

Industrial Science Symposium Abstracts

Amnis – EMD Millipore Company

Imaging Flow Cytometry: evidence that it is becoming a need to have technology

Robert Thacker

The Amnis cytometry systems, the ImageStreamX-Mark II and the FlowSight, combine high-speed image capture with image quantification to create a unique platform that merges the statistical power of flow cytometry with microscopy. These systems enable robust discrimination of both single cells and entire cell populations based on their appearance and subcellular distribution of fluorescence. As such, these platforms facilitate novel approaches to, and improve upon, traditional flow and imaging based applications. As we gain insight into the deep complexity of biological systems, imaging flow cytometry is emerging as an accepted and often times expected approach to answering these difficult questions. A brief description of the technology and data from several emerging applications demonstrate how imaging flow cytometry is becoming a preferred and often necessary approach to move research forward faster.

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BD Biosciences

Biosafety and the BD FACSAria™ Fusion

Gil Reinin

In the last few years there has been an increased customer awareness of the biosafety risk from aerosols produced in a clog situation while cell sorting, especially when sorting unfixed human cells. This concern was validated by aerosol generation studies where the data demonstrated that aerosol droplets produced from a stream clog could potentially be a safety risk if the proper biosafety measures were not taken. Biosafety measures include a proper risk assessment of samples being run, wearing proper PPE, always using an aerosol management system, and if required, the use of a BSC that can pass international biosafety standards such as the NSF-49 standard for product, personnel, and environmental protection. As a result of these guidelines a greater percentage of sorters are being purchased with biosafety cabinets. In this talk we will review recent biosafety guidelines for sorting and discuss how the BD FACSAria™ Fusion addresses these guidelines.

Beckman Coulter Life Sciences, Inc.

The Power of Choice: Introducing the CytoFLEX Research Cytometer

Dom Fenoglio

The CytoFLEX flow cytometer is the latest addition to Beckman Coulter's rapidly expanding cytometry portfolio. The innovative design of the bench-top CytoFLEX flow cytometer offers built-in system flexibility and superior sensitivity and resolution for your multicolor analysis. Beckman Coulter Life Sciences acquired Xitogen in June 2014 and the CytoFLEX is the first cytometer to be commercialized from this recent acquisition. Dr. Yong Chen, PhD, founder of Xitogen, now Chief Technology Officer for Beckman Coulter Life Sciences, believes that flow cytometers should be easy to use, able to grow with the technical needs of the users, and provide the highest quality performance. This presentation will describe the technology and introduce the instrument that will revolutionize your flow cytometry.

Contact: Dom Fenoglio at edfenoglio@beckman.com

eBioscience, an Affymetrix Company

RNA in Cellular Context: Enabling Translational Research

Matthew H. Cato

Quantify and visualize gene expression in tissue lysates, paraffin-embedded tissues, or fixed cells using the power of branched DNA technology, providing signal amplification at the core of the QuantiGene® assay portfolio. Detection of multiple probes within the same sample on a Luminex® platform provides a simplified means to screen relevant RNA transcript(s) and isolate RNA presence within particular cell populations. Confirm data by measuring protein expression levels using advanced immunochemistry or flow cytometry techniques to develop a powerful, translational workflow approach that offers pertinent data in limited sample and shortened project time.

EMD Millipore

Improved Fluorescent Detection on the New Guava easyCyte 12 and 12 HT

Jerry Aultz

Multi-laser flow cytometers can dramatically increase the capability of cell analysis. Here we present data from a new high performance flow cytometer which provides multi-dimensional information at a cellular level. The guava easyCyte™ 12 platform is a microcapillary flow cytometer which enables absolute cells counts, utilizes low sample volumes, and is easy to operate and maintain. With three lasers, the easyCyte™ 12 provides up to ten fluorescent and two scatter detection channels, thereby increasing the amount of information that can be obtained from each sample. The platform also includes the guava InCyte acquisition and analysis software, which provides a simple and intuitive tool for answering complex questions from both tube- and 96-well plate-based assays. Data will include 8-peak Rainbow bead results, Human CD4+ T-Helper cells in 10 colors, and 8-color immunophenotyping of human lymphocytes.

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Life Technologies

The Next Generation In Acoustic Cytometry: Attune® NxT Acoustic Focusing Cytometer

Brian Wortham

Presenting the latest addition to the Attune® acoustic focusing cytometer family. Designed by the Molecular Probes®, the affordable Attune® NxT acoustic focusing cytometer is the only high performance, multidimensional analyzer that makes the detection of rare events easier and faster with a No Wash/No Lyse assay for whole blood and unprecedented sample speed of up to 1mL/min without compromising sensitivity and resolution. The Attune® NxT system can be configured up to 4 lasers, enabling the simultaneous detection of 14 fluorescent parameters, providing more flexibility in reagent selection for panel design or fluorescent protein detection. In this tutorial, we will show examples including: identification of cellular subsets using a >10 parameters in circulating whole blood and mouse splenocytes, fluorescent protein detection, and Molecular Probes® Reagents including Click-IT® Plus for cell proliferation, mitochondrial membrane potential using TMRM off of the yellow laser, and bacteria detection using BacLight™ Bacterial Counting Kit . Attune® NxT modularity allows the system to be defined the time of purchase and can be easily modified or easily upgraded in the future to meet your changing research needs. The Attune® NxT software is designed to make understanding your research simpler through it’s intuitive design, streamlined workflow, top notch analysis speed and complete suite of acquisition and analysis tools. This talk will also introduce CellTrace™ Far Red stain for dye dilution applications off of the red laser.

Contact: Gayle Buller at gayle.buller@thermofisher.com

MILTENYI BIOTEC

(1) FLOW SORTING WITHOUT DROPLETS: IMAGINE A STERILE, AEROSOL FREE, HIGH SPEED SORTER. (2) RECOMBINANTLY ENGINEERED ANTIBODIES (REAFINITY) AND VIOBRIGHT DYES – SMART ANTBODIES FOR SMART CYTOMETRY.

MATTHEW DREW

Flow Sorting Without Droplets: Imagine a Sterile, Aerosol Free, High Speed Sorter As researchers face growing demands sorting cells, reducing operator risk, maintaining sample sterility and increasing the ease-of-use have become additional values sought by researchers. Miltenyi Biotec has in partnership with OWL Biomedical developed the all new MACSQuant Tyto, the first closed sorter system on the market. By using a sterile, one-way cartridge system that enables droplet and aerosol free measurements, the risk of contamination of the sample or the operator is successfully eliminated. The MACSQuant Tyto sorts the sample in a disposable cartridge that incorporates a patented microchip (MEMS) and the world's fastest valve using 3 lasers and 8 fluorescent parameters. After running the sample the positive and negative cell populations can be easily recovered for subsequent analysis or processing for downstream applications, e.g. cell processing using the CliniMACS® Prodigy. The system is fully featured with an on board temperature control using Peltier solid state system, and a convenient, compact space saving design. The MACSQuant Tyto is designed to accommodate everyday experiments amongst cell biologist with minimal training and ideal for all core facilities, research labs and production facilities.

REAfinity Antibodies are the next generation of flow antibodies and are the superior choice over mouse or rat monoclonals. Clones with the best binding affinity and specificity are recombinantly engineered to provide enhanced performance. Purity, lot to lot dependability, no need to Fc receptor block and a universal isotype control make these antibodies novel. When coupled with Miltenyi's VioDyes, including new VioBright dyes, the portfolio offers significant advantages over other commercially available antibodies. These dyes are engineered to produce high fluorescence intensities, low spillover, stability during fixation, and the ability to withstand photo degradation. These two portfolios will allow researchers to spend more time on research and less time worrying about the quality and performance of their antibodies.

The MACSQuant is a powerful 3 laser, 10 parameter flow cytometer with built in automation capabilities, like the ability to run plates or up to 24 tubes with the push of a button, but did you know it can automatically process AND analyze a 12-plex cytokine array? The MACSQuant uses integrated Express Modes which include automated protocols for processing samples along with automated analysis templates for analyzing data. Kits range from multiplexed cytokine detection to CD4 RTE enumeration and these kits are optimized to make set up, acquisition, and analysis, easy and reproducible. This seminar will provide a detailed walk through of our MACSPlex Cytokine Kit and insight into the design and development of an Express Mode.

SONY BIOTECHNOLOGY

SONY FLOW CYTOMETRY: TECHNOLOGY FOR YOUR SCIENCE

DAVID BUSCHKE

Sony Biotechnology Inc. is a manufacturer of innovative solutions for the flow cytometry market. Using intellectual property originally developed for the consumer entertainment industry, Sony Biotechnology has developed a new line of cell sorters and analyzers designed to remove many of the barriers preventing flow cytometry from being a more common tool in the research lab. High quality lasers and optics, patented laser alignment techniques, and high speed signal processing used in both Blu-ray players and Playstation 4

electronics have now found their way into Sony's new line of benchtop cell sorters and spectral analyzers. The SH800Z Cell Sorter is the first flow cytometer to leverage a disposable microfluidic chip to simplify the fluidic path and automate the more challenging functions with an electrostatic droplet sorter. This benchtop cell sorter is designed to address close to 70% of the cell sorting applications routinely run today. The SH800 features include

- Up to 4 excitation laser options (405, 488, 561 and 638nm)
- Up to six fluorescence detectors with a flexible filter set to detect a broad range of fluorochrome combinations
- Three nozzle sizes(100um standard, 130um, and, coming soon, the 70um)
- Replaceable sample path
- Sort Deposition System, SDS, for sorting into tubes or plates
- Easy to use software including
  - Automated routines for laser alignment and sort calibration
  - Index sorting for single cell applications

Combining techniques originally proven in imaging microscopy and spectroscopy with state of the art optics and electronics, the SP6800 Spectral Analyzer fundamentally expands the way cell and biomarker analysis is done today. This new technology eliminates the complexities surrounding high content, multi-color analysis by eliminating the need for matching filters to fluorochromes. Developing a multicolor panel has never been easier. In addition, the SP6800 Spectral Analyzer offers a means of capturing the autofluorescent fingerprint of a cell or the media surrounding the cell. The researcher can then use this data as an added parameter to identify cell populations or subtract it from signal to reduce background and provide improved resolution. Please stop by our table to learn more about Sony Biotechnology.

Contact: David Buschke at david.buschke@sonybiotechnology.com

Verity Software

Effective Use of Batch Processing in ModFit LT

Mark E. Munson

ModFit and its successor, ModFit LT, pioneered the application of mathematics to biological processes – a technique we call modeling. There are two primary advantages to using modeling for flow cytometry data; the first is objective results, and the second is automated analysis. Objective analysis yields reproducible results, due in part to the elimination of manual gating, and automation yields workflow efficiency and more effective use of your valuable time. The next advance is to use the batch processing capability of ModFit LT to automatically process about 90% of routine samples, which then allows you to use your expertise on the 10% of files that are problematic and really need your assistance. This presentation will show you how to do that by using a few simple options that are ModFit LT's "best-kept secrets."

Contact: Mark Munson at mem@vsh.com

ViroCyt

Virus Counter 3100

Mark Rehse

ViroCyt, LLC is the leader in the field of rapid virus quantification. Our goal is to provide the tools that deliver meaningful improvements to mission critical processes, such as vaccine manufacturing, protein expression,

antiviral development and other settings where viruses play a significant role. Our flagship product, the Virus Counter 3100, is designed to accurately and precisely determine Total Virus Particle concentration (tvp/mL) in minutes instead of days required for traditional plaque titer assays. The technology behind the Virus Counter is based on a specialized flow cytometer developed specifically for use with nanometer-sized particles. When using the Virus Counter, each sample is stained with two different fluorescent dyes: one specific for nucleic acids and the other specific for envelope protein. Following a thirty minute incubation, the stained sample is analyzed on the Virus Counter which scores viral particles based on simultaneous protein and nucleic acid signals. Scored events are counted and combined with an extremely accurate flow rate measurement to arrive at a volumetric virus particle concentration. There are many advantages to the Virus Counter, chief among them is the time to result. By reducing a measurement time which previously took hours or days into a process which takes less than an hour, the labor and time involved in quantifying viruses is dramatically reduced. The Virus Counter has been shown to accurately measure many different varieties of virus using the same procedure, thus eliminating virus-specific procedures and reagents. Dramatic time and cost savings, combined with excellent reproducibility and a universal assay, make the Virus Counter 3100 a potent tool for monitoring in-process virus production. The first and only automated technology specifically developed to rapidly quantify intact virus particles

- Results in minutes, not days or weeks required for other methods
- Multiple detection systems available
- Use in standard mode or add a 96-well plate autosampler for overnight processing
- Rugged design for industrial settings, including 21 CFR Part 11-capable software for GMP environments

The ViroCyt Virus Counter is now being used by the leading companies, regulatory agencies and research institutes around the world. Why? Infectivity assays alone don't provide enough information about viral preparations.

- The Virus Counter 3100 is a breakthrough work-flow solution for the challenges of virus quantification
- Combination of purpose-built hardware, software and reagents ensure accurate and reproducible results are available in minutes
- Provides immediate insight and the ability to make critical decisions in real-time
- The Virus Counter 3100 will provide a cost-effective, rapid and precise method to quantify viral constructs
- The optional 96-well plate autoloader will provide your facility with walk-away automation

Contact: Mark Rehse at mrehse@virocyt.com

POSTER ABSTRACTS

SORTING ESCHERICHIA COLI PERSISTER CELLS

GRACE ALTIMUS, ALI ADEM BAHAR, DACHENG REN

SYRACUSE UNIVERSITY, DEPARTMENT OF BIOMEDICAL AND CHEMICAL ENGINEERING

Persister cells are phenotypic variants that can be found in virtually any bacterial population. It is well documented that that persister cells reside in a dormant state with high level tolerance to many antibiotics. After antibiotic treatment, surviving persister cells can, through mechanisms largely unknown, awaken and regenerate the bacterial population, allowing for the propagation of chronic infection. Traditional methods to study persister cells are limited, and the field would greatly benefit from a protocol that allows for rapid, accurate isolation of persisters from the normal bacterial cell population. Building on methods described by Shah *et. al. (BMC Microbiology,* **6:** 53*,* 2006)*,* we have devised a FACS-based method for isolating persister cells of *Escherichia coli* strain ASV. Using a FACS Aria II (Becton, Dickinson & Company, San Jose, CA, USA), a population of *E. coli* ASV was analyzed and separated into two distinct populations based on green fluorescence; bright fluorescence indicates normal bacterial cells, while dim fluorescence indicates persister cells. The bacterial population was visualized using the forward scatter PMT available on the Aria II, an addition that allows for more accurate separation of bacterial cells from the background and electronic noise inherent in the system. With better small cell resolution and the ability to effectively distinguish between bright and dim populations with green fluorescence, these populations were able to be successfully sorted, collected, and later analyzed. Subsequent testing revealed that these populations did correspond to normal and persister cells, respectively. The use of the forward scatter PMT and the ability of the Aria II to discriminate between the bright and dim population provides not only a useful protocol for isolating persister cells, but also for the accurate separation of small and dim populations from mixed samples using the Aria II system enhanced with the forward scatter PMT.

REVEALING THE IMMUNE POPULATION HIERARCHY USING NOVEL INTERACTIVE VISUALIZATION TOOLS, A COMPARISON OF METHODS: SUNBURST, VISNE, AND SPADE ON MASS CYTOMETRY DATA

TIFFANY J. CHEN, PH.D.[1], GEOFFREY KRAKER[1], AND NIKESH KOTECHA, PH.D.[1]

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The development of new technologies for high-parameter data has resulted in a critical bottleneck: identification of immune subsets is restricted to expert-based analysis, focusing on post-acquisition characterization of cell populations. Identification of cell subsets in flow cytometry has primarily focused on manual analysis, despite the fact that computational tools have proven useful for high-parameter and cross-sample comparisons. Sharing well-annotated data improves transparency and facilitates vital reproduction of results by external groups. Adoption of these new tools for immune subset discovery requires thorough collaborative investigation and validation of identified cell populations. To this end, in this study we compare the ease of discovery of immune subsets by comparing analysis through the use of three visualization tools: the sunburst hierarchy, the SPADE tree, and dimensionality reduction using viSNE. The sunburst hierarchy is a visual and interactive representation of traditional manual gating, whereas the SPADE tree is a semi-automated clustering and visualization tool for identification of cell subsets. viSNE allows interaction with high parameter data in the context of two-dimensional space where gating can be accomplished. In this study, we demonstrate the ability to automatically elucidate many immune subsets using Cytobank via an iterative

analytic approach, combining computational tools (viSNE and SPADE) to recapitulate manually derived cell subsets.

Global Gene Expression Analysis of Mitotically Quiescent and Primed Human Bone Marrow derived Hematopoietic Stem Cells Reveals the Important Role of Dmtf1

Brahmananda R. Chitteti, Michihiro Kobayashi, Edward F. Srour
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In adults, hematopoietic stem cells (HSC) reside in deep dormancy within the bone marrow niches thereby sustaining lifelong maintenance of hematopoiesis. Recent studies demonstrate that cancers also utilize the same mechanisms as normal stem cells to preserve the cancer stem cell pool. Key transcription factors (TFs) or molecular pathways that regulate HSC quiescence are not yet completely known. In order to identify putative regulators of quiescence, we used global gene expression profiles of human bone marrow derived $CD34^{+}$ cells that are in quiescent (G_0) or active (G_1) phases of cell cycle, and identified several differentially expressed genes and TFs that could potentially regulate quiescence. We found 6 cell cycle related transcription factors - DMTF1, ERF, AHR, STAT1, NFYC, and E2F4 upregulated in quiescent cells. Differential expression of Dmtf1 is found high compared to the other five TFs (Fold change: 4.93, $p < 0.002$). We validated the role of Dmtf1 in HSC quiescence, and found that proliferation and other hematopoietic parameters were accelerated when Dmtf1 was functionally disrupted in human umbilical cord blood derived $CD34^{+}$ cells. We also confirmed that human Dmtf1 can modulate cell cycle progression via Arf-p53 independent mechanism.

Daunorubicin Uptake by Circulating Blast Cells and the Early Damage Response During Induction Chemotherapy for Acute Myeloid Leukemia

Sue Chow, Mark Minden, and David Hedley
Ontario Cancer Institute/Princess Margaret Hospital, University of Toronto.

Background: Combined treatment with daunorubicin and cytosine arabinoside causes the rapid disappearance of blast cells from the circulation, and cures a minority of patients with AML. The acute responses that determine whether chemotherapy-induced DNA damage results in cell death, or elicit repair (hence treatment resistance) have been extensively studied in vitro and are critical to patient outcome; but surprisingly little work has been done studying these responses in the actual patient. **Methods:** We obtained IRB approval to draw blood samples at intervals during the first 48 hours of induction chemotherapy of AML patients. We also treated 3 AML cell lines with daunorubicin in vitro, and identified 14 different candidate damage response markers that we then tested in serial blood samples obtained from 15 patients. Finally, we tested a validation cohort of patients for damage response 3 hours after the first dose of daunorubicin. **Results:** We observed increased fluorescence in blood cells at 488nm excitation shortly after the first dose of daunorubicin. In collaboration with John Nolan (La Jolla Bioengineering Institute), spectral flow cytometry was used to show that the emission spectrum of daunorubicin-treated blast cells was similar to that previously reported for daunorubicin. Using confocal microscopy, we also found that it was co-localized to DAPI, which is consistent with this drug forming a stable ternary complex with DNA and topoisomerase II. Of the damage response markers identified in vitro, only γH2AX, Chk-2 T68, and p53 S37 showed consistent increases in the patient blood samples, and in most cases the response was maximal by 3 hours following the first dose. We then studied an expansion cohort to give a total of 19 newly-diagnosed AML patients receiving daunorubicin-based induction chemotherapy, with samples obtained pre-treatment and after 3 hours. The

flow cytometry protocol was based on: γH2AX-FITC + daunorubicin at 650nm emission (488nm laser); p53-S37-A647 (635nm); and CD34/117-Brilliant Violet + CD45-Krome Orange (405nm). We observed a wide range in daunorubicin fluorescence between patients, with the blast cell and lymphocyte levels closely correlated. Activation of the initial DNA damage marker γH2AX showed a close correlation with daunorubicin fluorescence in both cell populations. However, there was a striking range in the levels of p53 activation, as determined by serine-37 phosphorylation, between individual patient blast cells. This was not correlated with the daunorubicin content, nor was it observed in lymphocyte populations. Furthermore, the extent of p53 activation was closely correlated with Chk-2 activation in the blast cells, suggesting that the magnitude of p53 response is determined upstream of Chk-2 (e.g. ATM/ATR). **Conclusions:** AML patients show a striking range in the cellular uptake of daunorubicin, and the levels are closely correlated with the initial damage response marker γH2AX, but not with p53 activation. A larger patient cohort is needed to determine if these responses are predictive of patient outcome. These findings are novel, and point to a potentially important role for flow cytometry monitoring the early treatment responses during induction chemotherapy, in addition to its established clinical applications.

Use Of Imaging Flow Cytometry For Profiling Mammalian Spermatozoa

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Most methods used to evaluate semen quality do not correlate highly with fertilizing capacity. Motility, morphology and functional tests have shown their importance but the correlation between the different parameters or with fertility has been weak. Morphological characterization by brightfield microscopy remains the reference for identifying cellular defects. Although microscopic evaluation provides important information the analysis is time consuming and relatively few cells are scored per sample. This approach lacks objectivity and reproducibility needed to identify subtle changes in large populations. The application of dye-conjugated biomarkers in conjunction with flow cytometry (FC) allows for evaluation of a large number of spermatozoa and, therefore, a more objective approach to semen assessment. Measuring various parameters via FC influencing sperm function such as DNA integrity, membrane fluidity, or acrosome status is important to understand semen quality. Albeit, the FC analysis is not able to address sperm morphology and so the microscope will always be a key factor for semen evaluation. The recent combination of quantitative image analysis and FC in a single platform (Amnis, ImageStream Mark II) overcomes the aforementioned issues and creates an amazing new perspective on semen evaluation. With the quantitative imaging FC a novel method is presented for accurate quantification of cell functionality and defects. In this work, we have evaluated the ImageStream Mark II ability to assess sperm morphology relative to microscopic gross morphology determined using a computer-assisted semen analyzer, or detailed morphology using fixed/stained sperm on a microscope slide. In addition, sperm characteristics like DNA integrity, acrosome status and mitochondrial index and were evaluated in single and multiplexed samples. A large number of images collected by the ImageStream II multispectral imaging cytometer were quantified and processed using the IDEAS software program allowing for categorization into morphology-based algorithms. Acquiring a plurality of different images facilitates the determination of a more complete and robust evaluation of different morphological and functional sperm cell parameters. The imaging FC combines the statistical power and fluorescence sensitivity of standard FC with the spatial resolution and quantitative morphology of digital microscopy. The technology creates a link between morphological and functional sperm status.

Neuronal and Neural Stem Cell Marker Expression in Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) are multipotent cells capable of differentiating into mesodermal derivatives namely osteoblasts, chondrocytes, adipocytes, myoblasts, and fibroblasts, to generate bone, heart cells, fat cells, muscle, and tendons/ligaments/connective tissue, respectively. However MSCs can also differentiate into ectodermal derivatives namely neurons and glial cells, but results found through the literature have been inconsistent. To further understand their plasticity, MSCs derived from bone marrow of wild-type mice and R6/2 transgenic mouse model of Huntington's disease (HD) were isolated via plastic adherence. Flow cytometry and immunocytochemistry were done to characterize MSCs expression including neuronal, glial and neural stem cell markers using the following panel of antibodies: NeuN, Sox2, GFAP, Sca-1 (Ly-6A/E), CD90, CD105, etc. Furthermore, to determine the multipotentiality of the MSCs, specific induction media were used to differentiate the cells towards osteogenesis, adipogenesis and chondrogenesis. mRNA extraction for RT-PCR was carried out to study RNA expression levels of mesenchymal and neuronal genes of interest. FACS analysis show that MSCs express characteristic mesenchymal markers as well as neuronal, glial and neural stem cell markers at varying levels, dependent on the number of culture passage. NeuN, GFAP, Sox2 expression in naïve MSCs positive for Sca-1(Ly-6A/E), CD90, CD105 may reflect that MSCs are readily capable of neuronal differentiation *in vitro* given the specific culture conditions. Further FACS analyses are needed to fully understand the neuronal marker expression of MSCs and the possible effect of culture medium on their neuronal differentiation.

CD166 Directs Multiple Myeloma Cell Homing To The Bone Marrow Microenvironment And Promotes Disease Progression

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Multiple myeloma (MM) is a plasma cell malignancy characterized by multiple lytic lesions throughout the skeleton, suggesting that trafficking of MM cells from the bone marrow (BM) and lodgment of these cells at secondary sites is important in disease progression. CD38+CD138-MM cells were previously characterized as putative MM stem cells (MMSC, Cancer Res. 2008; 68(1):190-7.). With flow cytometry, we analyzed CD38+CD138-cells contained within the MM cell line H929 and determined that a fraction of these cells (29.9%±1.4%) expresses CD166. CD166 is a member of the immunoglobulin superfamily capable of mediating both homophilic and heterophilic (CD6) interactions and has been shown to enhance metastasis

and invasion in several tumors including breast cancer and melanoma. Studies from our laboratory suggest that CD38+CD138-CD166+ MM cells possess many functional properties commonly associated with MMSC including cell cycle quiescence, maintenance and propagation of daughter cells on a stromal substrate and gene expression profile. We hypothesized that CD166 promotes MM cell trafficking to the BM and is critical for disease progression. To test this hypothesis, H929-GFP myeloma cells were injected intravenously into NSG mice and GFP cells were recovered from the BM 14hr later. Flow analysis showed that while only 3.3%±1.5% of total H929-GFP cells express the CD38+CD138-phenotype, the frequency of CD38+CD138-cells contained in BM-homed H929-GFP cells was significantly higher (53.4%±3.7%, n=3, p<0.01), suggesting a preferential homing of MMSC to the marrow microenvironment. Interestingly, whereas only 29.9%±1.4% of CD38+CD138-cells expressed CD166 prior to injection, 84.1%±10.8% of BM-homed H929-GFP CD38+CD138-cells expressed CD166 (n=3, p<0.01), suggesting that CD166 plays a critical role in directing homing of MM cells to the BM. Next, CD166 expression on H929-GFP cells was knocked down (KD) with shRNA in order to examine if reduced CD166 expression inhibit the homing of MM cells to the BM. The number of BM-homed GFP cells was significantly decreased for CD166KD cells (5658±904, n=6) compared to mock control (8551±848, n=6; p<0.05). To examine the potential role of CD166 in osteolytic lesions, we used a novel Ex Vivo Organ Culture Assay (EVOCA) in which MM cells are co-cultured over calvariae from 10d-old pups for 7 days creating an in vitro 3D system for the interaction of MM cells with the bone microenvironment. EVOCA data from two MM cells lines, H929 and OPM2 as well as from three primary MM patients' CD138+ BM cells demonstrated that bone osteolytic lesions were significantly reduced when CD166 was absent on either MM cells (sorted CD166-fraction) or osteoblast lineage cells (calvariae from CD166 -/-mice). Osteolytic lesions can be caused by decreased osteoblastogenesis or increased osteoclastogenesis. Co-culturing BM stromal cells (BMSC) from WT or CD166-/-mice with mock or CD166KD H929 cells in osteoblastogenic media revealed that loss of CD166 on either MM cells or BMSC released the inhibition of mRNA expression of RUNX2, a critical transcriptional factor for early osteoblast differentiation. Gene analysis of calvariae co-cultured with mock or CD166KD H929 cells revealed that the ratios of mRNA levels of RANKL to that of OPG, an important inhibitor of osteoclastogenesis, were elevated in cultures containing a CD166+ component suggesting that CD166 enhances osteoclastogenesis. Furthermore, cocultures of BM monocytes (BMM) from WT or CD166-/-mice with control or CD166KD H929 cells in the presence of RANKL and MCSF confirmed that the absence of CD166 on either BMM or MM cells results in significantly reduced osteoclast differentiation as evidenced by TRAP staining.

CD34+/CD45-DIM STEM CELL MOBILIZATION BY HYPERBARIC OXYGEN - CHANGES WITH OXYGEN DOSAGE

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Because hyperbaric oxygen treatment mobilizes bone marrow derived-stem/progenitor cells by a free radical mediated mechanism, we hypothesized that there may be differences in mobilization efficiency based on exposure to different oxygen partial pressures. Blood from twenty consecutive patients was obtained before and after the 1st, 10th and 20th treatment at two clinical centers using protocols involving exposures to oxygen at either 2.0 or 2.5 atmospheres absolute (ATA). Post-treatment values of CD34 +, CD45-dim leukocytes were always 2-fold greater than the pre-treatment values for both protocols. Values for those treated at 2.5 ATA were significantly greater than those treated at 2.0 ATA by factors of 1.9 to 3-fold after the 10th and before and after the 20th treatments. Intracellular content of hypoxia inducible factors − 1, − 2, and − 3, thioredoxin-1 and poly-ADP-ribose polymerase assessed in permeabilized CD34 + cells with fluorophore-conjugated antibodies were twice as high in all post- *versus* pre-treatment samples with no significant

differences between 2.0 and 2.5 ATA protocols. We conclude that putative progenitor cell mobilization is higher with 2.5 *versus* 2.0 ATA treatments, and all newly mobilized cells exhibit higher concentrations of an array of regulatory proteins.

IDENTIFYING ACUTE MYELOID LEUKEMIA SAMPLES USING FLOWCYTOMETRY DATA, WITH FLOWMATCH, A TEMPLATE-BASED CLASSIFICATION METHOD

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Background. Classifying large collections of flow cytometry files on the basis of their phenotypic similarity is a major challenge for bioinformatics. Yet, computing such classifications quickly and organizing them in a hierarchy are crucial for effective and robust cytometry data mining and management of data repositories. Here we discuss how we use flowMatch, an algorithmic pipeline for automated mining and organization of flow cytometry data collections, to classify samples into AML and healthy classes, and further classify the AML samples into three major classes, AML with Monocytic Differentiation, Granulocytic Differentiation, and without Maturation, respectively. **Methods.** The algorithms in flowMatch summarize a set of samples belonging to a biological class or category with a statistically-derived template for the class. Whereas individual samples are represented in terms of their cell populations (clusters), a template consists of generic meta-populations (group of homogeneous cell populations obtained from the samples in a class) that describe key phenotypes shared among all those samples. We organize the samples into a template tree in order to facilitate fast classification, and to permit template updating in dynamic classification. An innovative combinatorial measure for the dissimilarity of samples or templates, computed by means of a mixed edge cover in a graph model, is at the heart of this approach. The algorithms were prototyped in R language for statistical computing. **Results.** This template-based classification and organization scheme has several advantages over techniques that directly compare pairs of samples, such as nearest-neighbor classifiers. It is more efficient since one compares a sample with a few templates only, rather than with all other samples; it is more robust since a template describes the features common to cell populations in several samples, while ignoring noise and small sample-specific variations. Utilizing the measure of dissimilarity we have designed, template-based classification also helps to identify subclasses of a class, resulting in a tree-like hierarchy of phenotypic similarities. These advantages of flowMatch are demonstrated on AML data from the Roswell Park Cancer Center. This data set is expected to be challenging for template-based classification methods due tothe phenotypic heterogeneity of AML. However, we we were able to classify AML from healthy samples, and also classify AML samples into three major classes based on their cell's specificity. We have compared flowMatch with another package called flowPeaks that employs a different approach, and we report results from both. **Conclusions.** The presented system allows efficient and robust hierarchical classification of large FC data repositories and collections. The flowMatch package is available at www.bioconductor.org.

CHANGE IN FOCUS OF A CORE FACILITY OPERATION

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The UNMC Flow Cytometry Research Facility has changed focus from a full valet service, wherein samples were dropped off by clients and the core staff acquired and analyzed all data. All the instruments were run solely by the staff members of the core. This method of operation became unsustainable, and a change need to occur. The facility needed to initiate a shift in services offered at the facility, focusing on the end users

becoming independent operators and analyzing their own data. In order to facilitate this change, we had three key steps. The first step was to offer an introductory class in flow cytometry. After the users took the class, we trained them to make their own appointments, using an in-house software. We then offered one-on-one instrument training. We encouraged the users to opt into this new system by offering free training and education during a transition period. The prices for assisted acquisition would increase and the unassisted price would decrease at the end of the transition period or the full-switch. This would financially encourage the users to run on their own. We were able to go from 100% of the analyzer revenue being generated by the core staff down to 40% at the time of the full switch. We did a survey at the beginning of the full switch and were pleased to see that at least 90% of the respondents viewed the changes favorably.

SPEAKERS

PRATIP CHATTOPADHYAY

Pratip is a Johns Hopkins and NIH-trained researcher, a leader in the development and application of polychromatic flow cytometry, immunoassays, and emerging single cell technologies (such as high parameter single cell gene expression assays). He is an internationally recognized lecturer and teacher and an ISAC Scholar.

ZBIGNIEW DARZYNKIEWICZ

Zbigniew Darzynkiewicz, MD, PhD is the Director of the Brander Cancer Research Institute and Professor of Pathology, Medicine and Microbiology/Immunology at the New York Medical College. Formerly (1980-90) Dr. Darzynkiewicz was a member of the Sloan-Kettering Institute for Cancer Research, New York, N.Y. and a professor of Cell Biology and Genetics at Cornell University Medical School. Dr. Darzynkiewicz's research concentrates on cell biology with a focus on molecular mechanisms associated with cell proliferation, apoptosis, and sensitivity to anti-cancer drugs. He developed several analytical methodologies to analyze cell cycle kinetics, tumor progression, apoptosis and cell senescence that have world-wide application. He is a past President of the Cell Kinetics Society (1986-87) and past President of the International Society for Analytical Cytology (ISAC; 1993-1994).

M. MURAT DUNDAR

Dr. Dundar received his BS degree from Bogazici University, Istanbul, Turkey, in 1997 and MS and PhD degrees from Purdue University, West Lafayette, IN, USA, in 1999 and 2003 respectively, all in Electrical Engineering. He is currently an Associate Professor of Computer Science at IUPUI. Before joining IUPUI he was with the CAD and Knowledge Solutions group of Siemens Health, where he was involved in the development of a broad spectrum of computer aided diagnosis/detection applications including FDA-approved Lung and Colon CAD products. He is a co-author on over 40 peer-reviewed publications and a co-inventor in 5 patents. He and his colleagues at Siemens Health received the Data Mining Practice Prize Award for their work on medical image mining by ACM SIGKDD in 2009. Dr. Dundar is also the main author of the paper that received the best scientific paper award in the Bioinformatics and Biomedical Applications track at the 20th International Conference on Pattern Recognition (ICPR'10). Recently he has received the 2013 NSF Early Faculty Career Development (CAREER) Award.

NANCY FISHER

Dr. Nancy Fisher is the Director of the Flow Facility Core at the University of North Carolina, Chapel Hill. Dr. Fisher was a graduate student at UNC. Following graduate school Dr. Fisher went on to post-doctoral studies at the University of Vermont (UVM). She first began studying T-cell responses to Coxsackie virus-induced myocarditis in the Department of Pathology. She later went on to study in Ralph Budd's lab (Dept. of Medicine, UVM), where she focused on the Lupus LPR mouse model and T-cell apoptosis. While in Budd's lab, she became an early investigator in T-cells of the intestinal mucosa. Dr. Fisher was then hired by the Univ. of Texas Medical Branch (UTMB) in Galveston at the Child Health Research Center (CHRC) as an Assistant Professor, and later an Associate Professor where her research focused on intestinal lymphocytes and oral tolerance. Her work was funded by NIH and the Crohn's and Colitis Foundation. While at UTMB, she also taught graduate students in cell biology and immunology classes for the Microbiology and Immunology department. In 1999, Dr. Fisher moved back to Chapel Hill and to UNC in the Department of Medicine as an Associate Professor in the Division of Gastroenterology where she continued her studies in intestinal immunobiology. From 2003 to 2011, she worked at Becton Dickinson Technologies in the department of Cell and Tissue Technologies where she led the early research and development into a regulatory T-cell therapy

product. She also developed a six-hour flow cytometry based regulatory T-cell functional assay that measures a T-cell's ability to suppress effector T-cell function.

Michael Larson

Michael "Mike" Larson is currently a 7th year MSTP trainee/senior medical student at the Medical College of Wisconsin. Mike completed his undergraduate education in applied physics with an emphasis in electrical engineering in 2008 at Utah State University, where he minored in chemistry and biology. Later that year, he began medical school as part of the MSTP (combined MD/PhD program) at MCW. He finished his PhD in biophysics earlier this year (Jan 2014). Mike's PhD work centered on the use of a phospholipid binding peptide as a novel approach to identifying damaged cells and subcellular-sized vesicles in hemolysis and hemolytic anemias. Mike is interested in developing point-of-care tools for improved patient care, including bedside cytometry and spectroscopy.

Virginia Litwin

Since 2010, Virginia Litwin has been a Principal Scientist in Hematology at Covance Central Laboratory Services. She is the global scientific lead for all flow cytometry method development and validation. She was the recipient of the 2014 Covance Science and Technology Award. Virginia obtained a Ph.D. in Virology/Immunology from the University of Iowa. As a graduate student, she was a pioneer in the application of flow cytometry to the characterization of virally infected cells, and the function of viral glycoproteins. During her post-doctoral fellowship at DNAX Institute for Cellular and Molecular Biology, she identified one of the first KIR receptors (KIR3DL1) on human NK cells. This was followed by research on HIV viral entry and fusion at Progenics Pharmaceuticals. Since 1999 when she joined the clinical pharmacology/experimental medicine department at Bristol-Myers Squibb, she has focused primarily on the development and validation of flow cytometry-based biomarker methods for clinical trials. She founded the Flow Cytometry Action Program Committee of the AAPS Ligand Binding Assay Bioanalytical Focus Group, and is an active contributor to the International Clinical Cytometry Society (ICCS), and the International Society for Analytical Cytology (ISAC). She is an editor for Clinical and Vaccine Immunology, and serves on the editorial board of Cytometry Part B, Clinical Cytometry. She recently edited the book, Flow Cytometry in Drug Discovery and Development (John Wiley & Sons), and the Special Issue of the Journal of Immunological Methods on Flow Cytometry Biomarkers and Translational Medicine.

Daniel Normolle

Daniel Normolle, PhD is an Associate Professor of Biostatistics at the University of Pittsburgh and the Director of the University of Pittsburgh Cancer Institute Biostatistics Facility. He earned his doctorate in Computational Statistics in 1989 that considered early machine learning methods, and has maintained an interest in the application of machine learning to laboratory data since then. His primary research is in Translational Oncology, specifically, methods for the explicit application of pre-clinical data to the design of clinical research. He has been the designated statistician on over 80 clinical trials opened since 1994, and is an internationally recognized authority on the design of dose-escalation trials. He is a co-author on 128 peer-reviewed papers. In his spare time, he builds stereo amplifiers out of vacuum transmitting tubes run at high voltages, and has so far not been electrocuted.

Brian Olson

Dr. Brian Olson PhD is an assistant scientist with an appointment at the UW Carbone Cancer Center (UWCCC) in the McNeel lab. Brian attended the University of Wisconsin-Madison for his undergraduate education, obtaining a Bachelor of Science in Biochemistry in 2004. During this time, he worked in the laboratory of Dr. Richard Burgess, the James Watson Professor Emeritus of Oncology, conducting research into antibody production, protein purification, and subunit interactions of bacterial RNA polymerase. He then attended

graduate school at the University of Wisconsin-Madison, working in the McNeel lab where his research focused on the pre-clinical development of a DNA vaccine targeting the androgen receptor. During this time, he received a pre-doctoral training award from the Department of Defense Prostate Cancer Research Program (DOD PCRP) which funded his graduate education. After obtaining his PhD in Cancer Biology, he then received a post-doctoral training award from the DOD PCRP, which funded his post-doctoral training in the laboratories of Dr. William Burlingham (UW Hospital and Clinics, Department of Surgery) and Dr. McNeel, which focused on evaluating mechanisms of antigen-specific immune tolerance and the role of pre-existing immunity on vaccine efficacy. As an assistant scientist in the McNeel lab, Dr. Olson has received a Investigator Initiated Trial award from the UWCCC, funding his research focused on the clinical development of a DNA vaccine targeting the androgen receptor in combination with androgen deprivation therapy to prevent the generation of castrate-resistant prostate cancer. He is also interested in studying mechanisms of resistance to these therapies, and to design therapeutic strategies to overcome tumor escape variants that avoid primary therapies.

WADE ROGERS

Dr. Wade Rogers is Director of Computational Biology and Research Informatics at the Perelman School of Medicine at the University of Pennsylvania. He has more than 30 years of experience as a scientist, educator and innovator in industry and in academia having worked in various disciplines including oil exploration, neuroanatomy, geology and computational biology. Dr. Rogers started his career at the National Bureau of Standards (now NIST) before joining the DuPont Company in the early 1980s. He has held positions at DuPont Pharmaceuticals, Bristol-Myers Squibb and Cira Discovery Sciences. Today, at the University of Pennsylvania, Dr. Rogers' interests are focused on developing computational algorithms for finding patterns in high dimensional biology, especially patterns in flow cytometric data.

VINCENT T. SHANKEY

Dr. T. Vincent Shankey received his PhD degree in immunology and medical microbiology from the University of Florida School of Medicine (1977), followed by a postdoctoral fellowship in the Department of Pathology at the University of Pennsylvania from 1977 to 1981. Dr. Shankey has used flow and image cytometry in his research for over thirty years, working in clinical flow cytometry for much of that time. From 1987 through 2001, Dr. Shankey was director of research for the Urology Department and scientific director of the Clinical Flow Cytometry laboratory at Loyola University Medical Center, Chicago. In 2001, he was recruited to join the Advanced Technology Center/Systems Research Group at Beckman Coulter. During this period, the focus of his research was the development of novel approaches to measuring signal transduction pathways and their alterations in human disease. Dr. Shankey has over 60 publications focusing on different aspects of cancer cell biology and immunology. Since late 2013 he serves as a Vice-President for Research at AsedaSciences AG.

PAUL WALLACE

Dr. Paul K. Wallace joined the staff of Roswell Park Cancer Institute (RPCI) in 2003, as Director of the Flow and Image Cytometry Resource. He is an Associate Professor in the Department of Cancer Prevention and Pathology and a Member of the Immunology Program. He is also an Associate Professor in the Departments of Pathology and of Biotechnical and Clinical Laboratory Sciences, University at Buffalo (SUNY). He earned his doctoral degree in Immunology at the Medical College of Pennsylvania (Drexel University), Philadelphia, PA, and completed a postdoctoral fellowship in Immunology at Dartmouth Medical School, Hanover, NH in 1996 and remained at Dartmouth as an Assistant Professor until joining Roswell Park. Dr. Wallace is a President-Elect of the International Society for Advancement of Cytometry (ISAC). He is also an active member of the International Clinical Cytometry Society (ICCS).

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