

# GREAT LAKES INTERNATIONAL IMAGING AND FLOW CYTOMETRY ASSOCIATION, INC.

GLI<sup>2</sup>FCA<sub>NINE</sub>

THE NINTH ANNUAL FLOWGADOWN

“The 9 Lives of Flow”

HOTEL ST. REGIS  
DETROIT, MICHIGAN  
OCTOBER 13 - 15, 2000



Site Organizer: Alex Nakeff  
Program Chair: Phil Marder

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**GLIIFCA NINE – OCTOBER 13 – 15, 2000 HOTEL ST REGIS, DETROIT, MI – PROGRAM**

This continuing medical laboratory education activity is recognized by the American Society of Clinical Pathologists as meeting the criteria for 12 hours of CMLE credit.

**The theme for the Flowdown will be “9 Lives of Flow, FlowgaDown”; dress for a cat toga party.**

**FRIDAY Evening - October 13**

**Registration** 5PM –7 PM  
**Reception/Exhibitor Session/Poster Set-Up** 7PM – 11 PM  
Open bar from 7 – 9 PM only, use your drink tickets from 9 – 11 PM

**SPONSORED BY BECKMAN COULTER**

**SATURDAY - October 14**

**Breakfast** 7:30 – 8:30 AM

**PLENARY SESSION I**

(times include 5 minutes for questions)

**“New Applications in Multiplexed Cytometry”**

**Chair –Carleton Stewart, Welcoming Remarks:** 8:30 – 8:35 AM

**Featured Speaker: Marie Iannone**, Research Triangle Park, NC,  
“Multiplexed Molecular Analyses Using Fluorescent Microspheres”. 8:35 – 9:10 AM

**Plenary Talks:**

“A History of Multiplexing Analyte Assays by Flow Cytometry”, **Frank Mandy**, Ottawa, Ontario 9:10 – 9:35 AM

“Extracellular Receptor and Intracellular Cytokine Analysis by Flow Cytometry”, **Carl Stewart**, Buffalo, NY 9:35 – 10:00 AM

“Microarrays in Drug Discovery”, **Alex Nakeff**, Detroit, MI 10:00 – 10:25 AM

**Coffee Break**

**SPONSORED BY CALTAG**

10:25 – 10:40 AM

“DNA Microarrays to Study Cancer – Possibilities and Pitfalls”, **David I. Smith**, Mayo, Rochester, MN 10:40 – 11:15 AM

**Platform Presentation Award Nominee:** “Diphenyliodonium Induces Superoxide Production but Inhibits Hydrogen Peroxide Production in Mitochondria”, **Nianyu Li**, West Lafayette, IN. 11:15 – 11:30 AM

**Luncheon Roundtables**

11:30 – 1:30 PM

**Chair- Julie Auger**, Chicago, IL

A variety of round table discussions will be offered covering a wide range of flow cytometry and image topics. Some topics will include cytokine networks, leukemia/lymphoma phenotyping, cell cycle and cell kinetics, cell sorting, fluorochromes, bead assays, image analysis, etc. Someone experienced in the field will lead each discussion. A sign-up sheet will be available Friday night for roundtable selection. There is no charge.

**Keynote Address:**

1:30–2:15 PM

**Howard Shapiro**, Boston, MA. “You ain’t seen nothin yet...!”

**PLENARY SESSION II**

(times include 5 minutes for questions)

**"Novel Imaging Applications"**

**Chair – Michail Esterman,**

**Featured Speaker: - Tom Large**, Indianapolis, IN, “High Content Image Based Assays” 2:15 – 2:45 PM

**Plenary Talks:**

“Strategies for Sampling”, **Cindy Fishman**, Indianapolis, IN 2:45 – 3:05 PM

“New approaches”, **Wade Schuette**, Ann Arbor, MI 3:05 – 3:30 PM

“Image Management”, **Jeff Hanson** Indianapolis, IN 3:30 – 3:55 PM

**Coffee Break**

**SPONSORED BY SPHEROTECH**

3:55 – 4:15 PM

**Platform Presentation Award Nominee:** “Effects of Bacterial Culture Supernatant Fluids on Neutrophil Apoptosis”, **Elizabeth Frede**, Cincinnati, OH 4:15 – 4:30 PM

**Platform Presentation Award Nominee:** “Dysregulation of Lymphocyte Interleukin-12 Receptor Expression in Sezary Syndrome”, **Mohamed H. Zaki**, Philadelphia, PA 4:30 – 4:45 PM

Wine and Cheese Happy Hours  
& Poster Session in Exhibit Area

**SPONSORED BY CELLOMICS**

5:15 – 7:30 PM

**“9 LIVES OF FLOW FLOWGADOWN”**

**SPONSORED BY BECTON DICKINSON**

8 - 12 PM

**SUNDAY - October 15**

Steering Committee Meeting (Lothrop Room)  
Breakfast

7:30 – 9:00 AM  
8:00 – 9:00 AM

**PLENARY SESSION III**

**New Cytometry Products on the Horizon**

**Chair, Phil Marder, Indianapolis, IN**

**The following vendors will each give a fifteen minute talk (including 5 min. for questions) on their new products:**

Beckman Coulter - Grant Howes  
BD Biosciences - Todd Christian  
BIOErgonomics – Dan Collins  
Caltag – Bob Johnson  
Cytomation – Matt Ottenberg

9:00 - 9:15 AM  
9:15 - 9:30 AM  
9:30 - 9:45 AM  
9:45 - 10:00 AM  
10:00 - 10:15 AM

**Coffee Break**

**SPONSORED BY VERITY SOFTWARE HOUSE**

10:15 – 10:45 AM

Flow-Amp- David Kaplan  
Luminex – Kerry Oliver  
R&D Systems – Frank Mortari  
Verity Software House – Mark Munson

10:45 - 11:00 AM  
11:00 - 11:15 AM  
11:15 - 11:30 AM  
11:30 - 11:45 AM

**PRESENTATIONS OF PLENARY AWARD & TRAVEL STIPENDS**

11:45 AM - noon

**Platform Talk Award**

A \$150 award will be given to the platform speaker who presents the best talk. Speakers will be selected from clinical and research abstracts submitted by technicians, graduate students and post-docs (or equivalent). Selected speakers will be notified prior to the meeting. Talks will be limited to 10 minutes plus discussion. Judging criteria can be obtained from our web page or from Julie Auger, Education Chair (jauger@flowcity.bsd.uchicago.edu). Indicate clearly on your submitted abstract that you want to compete for the Platform Speaker Award.

**Travel Stipends**

To qualify for a travel stipend (\$100.00) you must submit an abstract; a statement of need signed by your laboratory director and **bring a poster** to the meeting. Not available to principal investigators, faculty or lab directors. One stipend per laboratory.

## **SPEAKER ABSTRACTS**

### **MULTIPLEXED MOLECULAR ANALYSES USING FLUORESCENT MICROSPHERES**

**Marie A. Iannone**

Department of Molecular Sciences  
Glaxo Wellcome Research Labs  
Research Triangle Park, North Carolina

Through the use of fluorescently distinct microsphere populations, flow cytometry is being increasingly recognized as a powerful high throughput technology. Analysis of binding reactions on the surface of microspheres expands the use of cytometry from cellular analysis into the realm of molecular analysis. This presentation will describe two separate applications that make use of multiplexed analysis of fluorescent microspheres: single nucleotide polymorphism (SNP) genotyping and nuclear receptor binding interactions.

### **A HISTORY OF MULTIPLEXED ANALYTE ASSAYS BY FLOW CYTOMETRY: SYNERGISTIC MULTI-ANALYTE PROFILING USING SUSPENSION ARRAY TECHNOLOGY (SAT)**

**Frank Mandy**

National Laboratory for HIV Immunology, Bureau of HIV/AIDS, STD and TB,  
Centre for Communicable Diseases Prevention, Population and Public Health, Branch, Health Canada, Ottawa,  
Ontario, Canada

In clinical immunology, flow cytometry has been most frequently used to study ligand-receptor interactions on leukocytes. In hospitals, the once only research instrument became the workhorse of clinical laboratories monitoring HIV disease progression. Over the past decade immunophenotyping in general, CD4<sup>+</sup> T-cell enumeration more specifically, has provided the opportunity to bring, the flow cytometer into the hospital immunology/hematology/oncology laboratories in significant numbers. The challenge is how to expand the productiveness beyond leukemia and HIV of these instruments in the immunology and oncology laboratories of the future.

A shift of cytokine secretion of the Th<sub>1</sub> and Th<sub>2</sub> type may be a marker of immunologic response. A sensitive and reproducible cytokine panel will be of value in monitoring therapies. To date secreted cytokines from peripheral blood are measured by ELISA from activated/cultured lymphocytes. A more sensitive assay system would permit the measurement of secreted cytokines directly from whole blood. The suspension array technology (SAT) is a promising candidate. In 1982 and again in 1984 attempts were made to integrate microspheres into blood cell suspension as an internal reference marker for absolute cell counting by Stewart and Valet respectively. These attempts were ahead of their time.

Throughout the presentation, the acronym SAT will be used. The potential of this technology is based on its unique capacity for quantitative measurement of liquid-to-solid phase molecular interactions. There are two options: an instrument system that has the capacity to analyze both cells and microspheres, or develop dedicated flow cytometers for microspheres alone. In the bio-medical field, the combinatorial technology is making rapid in-roads. Can flow cytometry based technology progress fast enough to meet the requirements of medical diagnostics of the next decade? Advanced assay platforms must combine high throughput with sensitivity, specificity and yet remain cost-effective. In this presentation, technologies, which are applicable to both existing and to future multi-color instruments, will be discussed.

The fluorosphere based SAT can be tuned for various types of reactions. (1) Sandwich type immunoassays where microspheres with different spectral address carry different ligands. (2) Oligonucleotide hybridization assays where microspheres with different spectral address carry different DNA sequence segments, to hybridize various specific nucleic acid fragments such as viral segments. (3) Single Nucleotide Polymorphism identification assays (SNP's) for a variety of samples through DNA hybridization on the surface of the fluorospheres. (4) An oligonucleotide-encoded synthetic peptide library can be assembled on

fluorospheres with corresponding and different spectral addresses to screen for unique peptide sequences. This latter screening assay is based again on ligand binding. Commercial analytical systems such as FlowMetrix™ and Luminex 100™ by Luminex Corp. will be reviewed. These systems permit the simultaneous measurement of multiple analytes from small samples. The multiplexed assay system offers the capacity to perform simultaneously, multiple, discrete, homogeneous assays from a single small sample. The effective application of multi-lasers configuration will also be described.

## **EXTRACELLULAR RECEPTOR AND INTRACELLULAR CYTOKINE ANALYSIS BY FLOW CYTOMETRY**

**Carleton C. Stewart**

Laboratory of Flow Cytometry  
Roswell Park Cancer Institute  
Buffalo, New York

Immunophenotyping by flow cytometry has become the method of choice for resolving complex populations of cells using antibodies to proteins expressed on the extracellular membrane. While phenotyping has been used to identify cells in complex populations, it is now clear their functional state can also be evaluated by determining their cytokine receptor repertoire, which can be thought of as the “ears” of cells and their intracellular cytokine repertoire, which are their “words”. Cellular receptors can be measured using antibodies to them or cytokines conjugated with fluorochromes. Our results do not support the use of conjugated cytokines. While the most extensive application of intracellular cytokine evaluation has been to identify TH1 and TH2 T cells, we will describe intracellular expression of several other cytokines in T cells, NK cells and monocytes. The greatest impediment to intracellular protein detection is the quality of the detection antibodies. In some instances the antibodies are so bad to both extracellular receptors and to intracellular cytokines that the data is completely artifactual. A simple method for determining antibody or fluorochrome conjugated cytokine quality will be described so that good data is assured. Since fixation can dramatically alter epitope availability, antibody binding to fixed permeabilized cells must also be confirmed. Utilizing the proper procedures with high quality antibodies will result in new insights in the biology of processes in specific cell subsets by combining membrane with intracellular immunophenotyping. This work was supported by NIH grant #5R01CA6020006.

## **MICROARRAYS IN DRUG DISCOVERY**

**Alexander Nakeff**<sup>¶</sup>, Nisha Sahay<sup>‡</sup>, Balnehr Subramanian<sup>¶</sup>

<sup>¶</sup>Josephine Ford Cancer Center, Henry Ford Health System, Detroit, MI 48202

<sup>‡</sup>Genomic Solutions, Inc., Ann Arbor, MI 48108

XK469 is an investigational anticancer agent that exhibits anti-proliferative activity *in vivo* and is presently in NCI pre-clinical development. We examined the efficacy of this drug to alter the expression of 1,152 cancer-related genes in H116 human colon adenocarcinoma cells. Analysis of gene expression in these target cells utilized a unique DNA microarray (GeneMAP™ Cancerarray) following a 24h-exposure to a drug dose that killed 99% of H116 clonogenic cells. Analysis of the functional gene cluster profile (FGCP) of those 71/1152 genes that displayed a >2-fold increase or decrease in expression (over untreated control) identified the MAPK molecular pathway and specifically MEK inhibition as a potential target responsible for the anti-proliferative activity of XK469. Changes in gene expression not only account for the anti-proliferative efficacy of XK469 on H116 cells *in vitro* but also its *in vivo* activity against H116 human tumors in SCID xenografts and hypothesized drug-induced toxicity in the murine hosts. These data provide evidence that links the human solid tumor anti-proliferative activity of XK469 to the suppression of MEK gene expression.

### **GENERATING A MOLECULAR SIGNATURE FOR OVARIAN CANCER**

**David I Smith**<sup>1</sup>, Viji Shridhar<sup>1</sup>, Julie Staub<sup>1</sup>, Rajeswari Avula<sup>1</sup>, Jim Lillie<sup>2</sup>,  
John Lee<sup>2</sup>, Fergus Couch<sup>1</sup>, Kim Kalli<sup>3</sup>, and Lynn Hartmann<sup>4</sup>.

<sup>1</sup>Division of Experimental Pathology, Mayo Foundation; <sup>2</sup>Millennium Predictive Medicine, Cambridge, MA;

<sup>3</sup>Division of Endocrinology, Mayo Foundation; and

<sup>4</sup>Division of Oncology, Mayo Foundation, Rochester, MN

Our goal is to generate a molecular profile of ovarian cancer by utilizing state-of-the-art technologies to identify clinically relevant molecular markers. The hypothesis to be tested is that variations in the gene expression patterns truly reflect the variation in the clinical behavior of the cells. In our primary screen we utilized a 25K cDNA array produced by our collaborators at MPMx (Millennium Predictive Medicine, Cambridge, MA). We compared the expression profile of pooled normal ovarian epithelial cell brushings with 8 stage I/II tumors and 12 stage III/IV tumors. Of the 12 stage III tumors, 6 tumors are from patients with good response to chemotherapy and good prognosis (long-term survival of 5-7 years) and 6 stage III tumors from patients with poor prognosis (less than two-year survival) since the time of diagnosis. We have also constructed a number of subtraction suppression hybridization cDNA libraries to identify additional genes not present on the 25K cDNA arrays. Several thousand clones from each library were sequenced. We have performed semi-quantitative PCR analysis on 30 of the clones identified as being differentially expressed and find that only 70% of the clones are truly aberrantly expressed. We have been carrying out complementary cytogenetic analysis on the same ovarian tumors that were transcriptionally profiled. This includes comparative genomic hybridization and loss of heterozygosity studies with microsatellite markers. These studies have delineated several chromosomal regions as being frequently deleted in ovarian tumors, as well as several regions of DNA amplification. Our next step will be to take the genes identified as being potentially differentially expressed from the 25K cDNA arrays, as well as genes identified from the subtraction cDNA libraries, and test these genes against a larger number of ovarian tumor specimens. We will also include several hundred EST fragments that we have generated by differential display screening of ovarian tumor cell lines vs. a normal ovarian epithelial cell line, and also between stage I and stage III primary tumors. The secondary microarrays will then be used to interrogate many ovarian tumor specimens in attempts to generate a comprehensive molecular profile of ovarian tumors.

### **DIPHENYLIODONIUM INDUCES SUPEROXIDE PRODUCTION BUT INHIBITS HYDROGEN PEROXIDE PRODUCTION IN MITOCHONDRIA**

**Nianyu Li**, Kathy Ragheb, Gretchen Lawler, Bartek Rajwa, Jennie Sturgis, Elikplimi K. Asem,  
J. Paul Robinson

Cytometry laboratories, Purdue University  
West Lafayette, IN 47907-124

POSTER #8

Diphenyliodonium (IDP) is known to inhibit both NAD(P)H oxidase and mitochondria NADH reductase (complex I). Recent studies showed that mitochondrial complex I inhibitors could affect mitochondria reactive oxygen species (ROS) production. However, the relationship between respiration inhibition and ROS production has not been well characterized. We therefore investigated the effect of iodonium compounds on mitochondria ROS production. Hydroethidine was used to measure superoxide production and 2',7'-dichlorodihydro-fluorescein diacetate (DCFH-DA) was used to measure hydrogen peroxide production. Mitochondria function was estimated by studying the mitochondrial membrane potential with 5,5',6,6'-tetrachloro-1,1',3,3' tetraethylbenzimidazolylcarbocyanine iodide (JC-1). Our results demonstrated that both IDP and rotenone could induce mitochondrial superoxide production while inhibiting mitochondria hydrogen peroxide production. In addition, both IDP and rotenone were able to reduce mitochondria membrane potential. The decrease of mitochondrial membrane potential by these mitochondrial complex I inhibitors was closely correlated to their effects on mitochondrial ROS production. These observations suggested that complex I inhibitors IDP and rotenone have a differential effect on mitochondria derived ROS. Both appear to induce superoxide production with a concomitant reduction in hydrogen peroxide production.

**KEYNOTE ADDRESS**

**YOU AIN'T SEEN NOTHIN' YET!**

**Howard Shapiro M.D., P.C.**

283 Highland Ave., West Newton, MA

Cytometer developers strive to maintain or improve performance while making apparatus smaller, more energy-efficient and user-friendly, and cheaper. Improvements have come from several directions. New diode and solid-state laser sources and new fluorescent probes can lead to better scatter and fluorescence measurement sensitivity, which may be further increased by detectors now in development. Digital signal processing electronics are replacing analog log amps and fluorescence compensation circuitry; this improves accuracy and may also lower instrument costs. Micromachined fluidics in new, nonstandard flow cytometers permit high-sensitivity and kinetic measurement and sorting to be extended down to the molecular level, and also facilitate complex sample preparation and treatment procedures, making a broad range of new applications practical.

**AUTOMATED IMAGING AND THE PROMISE OF PHENOTYPIC SCREENING FOR DRUG DISCOVERY**

Oscar J. Trask and **Thomas H. Large**

Sphinx Pharmaceuticals, 20 TW Alexander Drive, Research Triangle Park, NC 27709

There is a growing appreciation of the power of sub-cellular imaging to contribute to drug development by providing richer information regarding compound effects on target function. High-throughput screening (HTS) is established as a critical element in drug discovery and functional, cell-based assays are becoming the predominant HTS format. For follow-up secondary efforts, cell-based assays also can provide richer information on compound hits regarding target selectivity, mechanism of activity and additional cellular effects such as toxicity. Historically, sub-cellular imaging has not been applied to these early stages of drug discovery, in part, because of the lack of microscopes and data analysis tools with sufficient throughput to satisfy the demands of an HTS environment. However, the recent development of high-throughput imaging instrumentation and software has made it feasible to begin applying these tools to lead generation. In this era of functional genomics, automated image analysis also is likely to find important applications in drug target identification and validation. Finally, a novel and potentially valuable additional application is termed phenotypic screening, the identification of lead compounds that modulate cellular functions, e.g. mitosis, rather than the function of a defined target protein.

**QUANTITATIVE IMAGE ANALYSIS APPLIED TO A MOUSE MODEL OF ALZHEIMER'S  
DISEASE NEUROPATHOLOGY**

**Cindy Fishman**<sup>1</sup>, Kelly Bales<sup>2</sup>, Cynthia DeLong<sup>2</sup>, William Jordan<sup>3</sup>, Sandy White<sup>3</sup>, Michael Esterman<sup>4</sup>,  
Jeffrey Hanson<sup>4</sup>, David Cummins<sup>5</sup> and Steven Paul<sup>2</sup>.

1) Division of Veterinary Pathobiology, Purdue University, West Lafayette, Indiana. 2) Division of Neuroscience, 3) Division of Toxicologic Pathology, 4) Division of Information Technology Discovery and 5) Division of Statistical and Mathematical Sciences, Lilly Research Laboratories, Indianapolis, Indiana.

Quantitative image analysis adds dimension to the use of histopathology in biomedical research. Sampling strategies, including numbers of animals, portions of organs and percent of tissue sections evaluated, are some of the most important considerations in designing a study using image analysis of microscopic tissue lesions. This is particularly true in the brain, where structures and lesions are distributed non-uniformly. Knowledge of lesion distribution between and within animals of a given model is important in experimental design. Other important considerations include uniformity in tissue processing, tissue stains, which specifically demonstrate the lesion of interest and the selection of appropriate controls. Although many potentially valid strategies for the morphologic evaluation of spontaneous and experimentally- induced Alzheimer's disease (AD) neuropathology exist, published methods are often vague. Here, one multidisciplinary approach to experiments using a transgenic mouse model of AD neuropathology and Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) will be described.

**NEW APPROACHES**

**Wade Schuette**

University of Michigan Medical Center, Information Technologies, Ann Arbor, MI

Reliable, reproducible quantitative scoring of images of Immunohistochemically stained specimens has been difficult, due in part to the fact that the stains and counter stains in common use have overlapping spectra. The published techniques used to attempt to do this are briefly reviewed, and two new techniques presented that have a solid mathematical basis, along with slides showing the actual results of use of these new techniques. One of the new techniques is hardware based, using an electronically tunable filter, and the other is software based and works with standard RGB color images.

**IMAGE DATA MANAGEMENT**

**Jeffrey Hanson** and Michail Esterman  
Lilly Research Labs, Indianapolis, IN

Sometimes, it is easier to re-run an experiment than to find the historical image and its associated data. Because of this, we wanted to adopt an image data management strategy that would encompass multiple labs with different types of images. Many different tools are available for image data storage - we adopted Scimagix's SIMS product. We defined the data associated with each kind of image in a way to facilitate searching across different kinds of studies. One of the more tedious steps is loading the text data with the image into the database. We created a user interface to record the annotation data at the time of image acquisition in a format that streamlines data loading. As a result, images and the complex data associated with them can be easily collected and deposited in a single tool. This strategy has the potential to enable more sophisticated data searching as it is adopted across multiple labs.

**EFFECTS OF BACTERIAL CULTURE SUPERNATANT FLUIDS ON NEUTROPHIL APOPTOSIS**

**E.A. Frede**, I.A. Holder, A. Neely, G.F. Babcock  
Shriners Hospitals for Children & U. of Cincinnati

POSTER #9

We have shown that cell-free supernatant fluids (supes) from a variety of microorganisms induce apoptosis in human neutrophils (PMNs). We have focused on the supes from *Pseudomonas aeruginosa* (*P.a.*) and several mutant strains. Six *P.a.* strains were cultured for 6 hrs, centrifuged and filter sterilized. The supes were produced from a standardized bacterial count and were incubated with PMNs for .5, 1.0, and 1.5 hrs. Apoptosis was determined by flow cytometry using FITC-Annexin V and 7-aminoactinomycin D. In some experiments, the supes were treated to remove LPS and *P.a.* exotoxin, studied for heat inactivation, treated with proteinase K, and sized by membrane filtration. Bax and Caspase 3 were also examined by flow cytometry. *P.a.* strain SBI-N induced apoptosis in PMNs to 200% of the control. Supes from invasion deficient strains demonstrated similar properties. The removal of LPS did not alter apoptosis. Characterization of the supes indicates that the active component(s) are heat stable, resistant to proteinase K treatment, and have a molecular weight less than 3500. These data suggest that various micro-organisms produce substances that are capable of inducing apoptosis in PMNs. This would be a selective advantage for pathogens, potentially increasing invasiveness. The nature of these substances is unknown. However, it appears that in the case of *P.a.*, it is neither LPS nor a protein, and is small in molecular weight.

**DYSREGULATION OF LYMPHOCYTE INTERLEUKIN-12 RECEPTOR EXPRESSION IN SEZARY SYNDROME<sup>1</sup>**

**Mohamed H. Zaki<sup>1</sup>**, Ryan B. Shane<sup>1</sup>, Yuemei Geng<sup>1</sup>, Louise C. Showe<sup>3</sup>, David H. Presky<sup>4</sup>, Jonni S. Moore<sup>2</sup>,  
Alain H. Rook<sup>1</sup>

University of Pennsylvania, Philadelphia, PA

POSTER #10

Initial phase I and II clinical trials with recombinant human IL-12 (rhIL-12) have demonstrated the therapeutic efficacy of this cytokine in early stage cutaneous T-cell lymphoma (CTCL) as compared to more advanced stages such as the leukemic Sezary syndrome. In an effort to optimize the use of rhIL-12, we studied by flow cytometry the regulation of the IL-12 receptor  $\beta$ 1 (high affinity chain) and  $\beta$ 2 (chain necessary for IL-12 signal transduction) on CD4+ and CD8+ T-cells in normals and patients with different degrees of leukemic involvement with Sezary syndrome. The  $\beta$ 1 &  $\beta$ 2 chains was not readily detectable on resting normal and T-cells from Sezary patients, but expression was induced following T-cell activation with PHA, IFN $\gamma$ , IL12 and IL-10 on normal controls only. CD8+ T-cells routinely exhibited a greater expression of  $\beta$ 2 than did CD4+ T-cells. Investigation of patients with Sezary syndrome and a low tumor cell burden (<15% circulating Sezary T-cells) revealed a pattern of  $\beta$ 2 expression that was intermediate between advanced Sezary syndrome and normal volunteers. These results indicate that IFN $\gamma$  and rhIL-12 potently upregulates  $\beta$ 2 chain expression on T-cells from normal volunteers, while a similar, but less marked effect occurs on T-cells from Sezary syndrome patients and a low circulating tumor cell burden. In contrast, the  $\beta$ 2 chain appears to be suppressed on both T-cells from Sezary patients with a high tumor cell burden. Therefore, rhIL-12 is likely to be most effective for early stage CTCL due to a greater display of  $\beta$ 2 receptors on responding CD8+ antitumor cytotoxic T-cells.

**VENDOR PRESENTATIONS**

**Beckman Coulter**  
**PROGRESSIVE AUTOMATION – THE PREPPLUS WORKSTATION**  
**Grant Howes**

Cytometry, Beckman Coulter, Inc., Marketing Manager

One of the most time consuming and variable aspects of flow cytometry is sample preparation. The new PrepPlus system from Beckman Coulter automatically pipettes reagents, samples, controls and fluorosphere reagents directly into daughter tubes, at a rate of up to 90 tubes per hour. To ensure safety is maintained, the PrepPlus provides Close Tube Sampling from a variety of primary sample tubes. At all stages of the preparation process, from the addition of blood to antibody followed by cell lysis, sample tubes remain in the same carousel, eliminating repetitive transfer steps. Using software provided by Beckman Coulter, common requirements such as CD4 analysis with the tetraONE system are easily programmed, but the PrepPlus is flexible enough to be customized for other applications in the flow cytometry laboratory.

**Becton Dickinson Biosciences**  
**TECHNOLOGICAL ADVANCES IN FLOW CYTOMETRY**  
**Todd P. Christian**

BD Biosciences, Marketing Manager

Recently, there has been significant activity to develop and bring to market new instrumentation tools for flow cytometry. Some of our most recent advances include the FACSDiVa and BD FACS AccuDrop Options for the FACSVantage SE, the two- and three-laser BD LSR, the BD Multiwell AutoSampler for the FACSCalibur. Whether you're working in a clinical diagnostic, basic research, or biotechnology and pharmaceutical environment, we have been working to develop tools to expand your capabilities with the same quality and reliability you've come to expect from BD.

**BioErgonomics**  
**IMPORTANT CONSIDERATIONS IN THE DEVELOPMENT OF *IN VITRO* MODELS OF IMMUNE  
ACTIVATION: EFFECTS ON CYTOKINE SECRETION AND RECEPTOR EXPRESSION.**

**Daniel P. Collins**  
BioErgonomics, Inc., 4280 Centerville Road, St. Paul, MN 55127

Alterations of immune function are often qualified by changes in *in vitro* responses to non-specific polyclonal activators, like anti-CD3 antibody and mitogens. This study reviews the most commonly used methods of *in vitro* activation by non-specific polyclonal activators on isolated T-cells and mononuclear cells. The resultant biological activity was assessed by measuring internal expression of cytokines, functional cytokine receptor expression, and secretion of cytokines by flow cytometric analysis. Secretion of cytokines was quantitated by single (ImmunoFlow™) and multiplexed (MultiFlow™) flow cytometry-based IFA. Results demonstrated differential cytokine secretion dependant upon method of activation and cell population making up the responding cells. Results also demonstrated the importance of coincidental quantitation of secretion with identification of cytokine-producing cells.

**Caltag**  
**CY7 TANDEM CONJUGATES**  
**Bob Johnson**, Caltag, Burlingame, CA

A number of fluorescence resonance energy transfer (FRET) dyes are used in flow cytometry. Two of these dyes, PE-Cy7 and APC-Cy7, are tandems of the phycobiliproteins, phycoerythrin (PE) and allophycocyanin (APC), linked to the indocyanin dye, Cy7. The introduction of these dyes facilitated the expansion of the number of parameters that can be used simultaneously in flow cytometry to 6 or 7 depending upon the instrument used. Until recently, the number of commercially available antibodies linked to these dyes has been limited. In addition, because these reagents are relatively new, little formal information concerning their use exists. Therefore, to address this issue, this tutorial will cover some of the more fundamental and basic questions related to the use of these dyes on commercial cell sorters, as well as benchtop analyzers. Topics will include the appropriate optical configuration as well as set-up and compensation for using these dyes. In addition, flow cytometry data will be presented and discussed using several commercially available reagents directed to several antigens expressed by thymocytes and peripheral blood lymphocytes.

**Cytomation**  
**Matt Ottenberg**, Vice President of Product Development  
Cytomation, Inc., 4850 Innovation Drive, Fort Collins, CO 80525

A flow spectra-radiometer system has been developed to enable complete characterization of the spectral distribution of signals from the MoFlo® cytometer system. This system is useful for development of custom fluorescent dye compounds and for configuring complex multi-color flow experiments.

A system developed for automatic, real-time tracking of sub-populations has been demonstrated for non-human mammalian sperm sorting. The system, called CyTrak™, uses the on-board Digital Signal Processing system of the MoFlo® to continuously adjust sort parameters in response to variations in the target population. It has been demonstrated to operate autonomously over 3-4 hour periods while maintaining sorting purity, recovery and yield.

A rapid sample input system, called MoSkeeto™, has been developed with the University of New Mexico. Designed for high-throughput screening, it aspirates directly from microtitre plates.

**Flow-Amp**  
**David Kaplan**, Flow-Amp Systems, Cleveland OH

Flow cytometric analysis is a powerful technique for the single cell assessment of cell surface expression of selected molecules. The major deficiency of flow cytometry has been its relative insensitivity. Only molecules expressed in abundance have been readily observed. Consequently, we have developed an enzymatic amplification procedure for the analysis of cell surface molecules by flow cytometry. Transformed and non-transformed cells expressing MHC class I, CD5, CD3, CD4, CD6, CD7, CD34, CD45, MHC class II, Fas ligand, and phosphatidylserine were assessed. Our enzymatic amplification technology increased the fluorescence signal between 10 and 100 fold for all surface molecules tested. Enzymatic amplification staining (EAS) produces a significant enhancement in the resolving power of flow cytometric analysis of cell surface molecules. Using this technique we have been able to detect the presence of molecules that could not be observed by the standard procedure.

**Luminex Corporation**  
**The Luminex LabMAP™ System: A High Throughput Multiplexed Bioassay System.**  
**Kerry Oliver**, Luminex Corporation, Austin, Tx

Luminex has combined fluorescence-based assays with the ability to carry out parallel reactions to create LabMAP, a multiplexing technology capable of rapid analysis of 100 different analytes/reactions in a single sample. Assays are developed in a homogeneous format on unique sets of “fluorescently bar-coded” microspheres and quantified by the fluorescence intensity of a reporter ligand. Most assays formatted for microtiter-based assays are amenable to miniaturization and rapidly transfer to the LabMAP system, including immunoassays, enzymatic assays, transcriptional profiling, and receptor binding assays.

A multi-channel microfluidic system has been integrated with the original Luminex 100 system to form the Luminex HTS. The Luminex HTS has been designed for the high throughput requirements of pharmaceutical drug discovery laboratories and high capacity clinical laboratories and currently is capable of generating nearly 400,000 assay results per day.

**R and D Systems**  
**Frank Mortari**  
Minneapolis, MN

Investigations in the field of immunology require the necessary tools to follow the cellular communications that take place between the various players of the immune system. Chemokine and their receptors, traditionally thought to be exclusively involved in inflammatory responses, have more recently been appreciated for their role in hematopoiesis, angiogenesis, oncogenesis and HIV pathogenesis. A brief review of chemokine receptors and their ligands will be presented in order to familiarize those new to this field to the complex nomenclature and to the reagents commercially available for this field of research.

**Verity Software House**  
**Mark Munson**  
Topsham, Maine

Verity Software House will review nine features of flow cytometry and show software designed for rapid, powerful and simple analysis for each. ModFit LT, WinList and QuantiCALC each addresses specific needs of a busy flow laboratory, in clinical and/or core facility applications.

**ABSTRACTS FOR POSTER PRESENTATIONS**



POSTER #2

**Comparison of a Traditional Acid/Heat DNA Denaturation Method with a Commercially Available Enzymatic Kit for the Immunocytochemical Detection of BrdU Incorporation.**

**L.J. Ostruszka** and M.A. KuKuruga  
University of Michigan Medical Center.

We use an optimized acid/heat denaturation method to reveal incorporated BrdU in proliferating cells. Exponentially growing SW620 cells expressing HSV-TK were irradiated with 7.5 Gy of ionizing radiation or treated with hydroxyurea (IC<sub>50</sub>). After 24 hr, cells were pulse labeled with 30  $\mu$ M BrdU for 15 min. DNA was denatured using 0.1 N HCl followed by heating at 95°C. Incorporated BrdU was detected using indirect immunofluorescence and flow cytometry. We compared this method with cells labeled using Pharmingen's BrdU Flow Kit (2354KK), which uses DNase at 25°C and direct BrdU labeling. Both methods produced similar cell cycle histograms in untreated and treated cells. Acid/heat and enzymatic denaturation of DNA were effective in revealing incorporated BrdU (48.6% vs. 44.9% BrdU positive), however the corrected mean fluorescence in acid/heat denatured samples was ~15 times greater. If DNase denaturation was combined with indirect BrdU labeling, mean fluorescence increased, but to only 22% of the acid/heat method. While the acid/heat method proved far more sensitive, Pharmingen's Flow BrdU kit showed a remarkable advantage in cell recovery.

**An Intracellular Cytokine Assay for Quantitative Detection of TNF $\alpha$  in Human Monocytes**

**Lisa Green**, Tai Hunte, and Philip Marder  
Lilly Research Laboratories, Indianapolis, IN

Cytokines are key mediators of the inflammatory response. Utilizing antibodies to cell surface antigens, a secretion inhibitor, and cell permeabilizing reagents, flow cytometric analysis can reveal cytokine expression patterns in defined cell populations. In this study we evaluated TNF $\alpha$  production by LPS-stimulated human monocytes in whole blood and PBMC preparations. We found that the LPS stimulated CD14+ cells reliably produced a robust TNF $\alpha$  signal. The mean phycoerythrin (PE) fluorescence intensity of CD14 + cells stained with PE-labeled anti-TNF $\alpha$  increased 20 to 40-fold over background following a 4 hour LPS treatment. The TNF $\alpha$  signal was dependant on the concentration of LPS used. Dexamethasone inhibited TNF $\alpha$  production in a concentration dependant manner. Our studies indicate that this technique provides an excellent method for quantitative evaluation of anti-inflammatory and immunoregulatory molecules.

POSTER #3

**Lymphocyte Subset Proliferative Responses of *Mycobacterium Bovis*-Infected Cattle:  $\gamma\delta$  TCR<sup>+</sup> and CD4<sup>+</sup> T Cells are the Predominant Cells Responding.**

W.R. Waters<sup>1</sup>, M.V. Palmer<sup>1</sup>, **B.A. Pesch<sup>1</sup>**, S.C. Olsen<sup>1</sup>, M.J. Wannemuehler<sup>2</sup>, and D.L. Whipple<sup>1</sup>.  
USDA-National Animal Disease Center<sup>1</sup>, and Iowa State University<sup>2</sup>, Ames, IA

A flow cytometric-based, proliferation assay using PKH67 was used to determine the relative contribution of individual lymphocyte subsets in the response to *Mycobacterium bovis* infection and/or sensitization with mycobacterial purified protein derivative (PPD). Peripheral blood mononuclear cells (PBMC) from *M. bovis*-infected cattle proliferated in response to in vitro stimulation with *M. bovis* PPD. CD4<sup>+</sup> T cells and  $\gamma\delta$  TCR<sup>+</sup> cells were the predominate subsets responding. Intradermal injection of PPD for comparative cervical testing (CCT) induced a boost in the in vitro proliferative response of CD4<sup>+</sup> but not  $\gamma\delta$  TCR<sup>+</sup> cells of infected cattle. These data indicate that CD4<sup>+</sup> and  $\gamma\delta$  TCR<sup>+</sup> cells of *M. bovis*-infected cattle proliferate in a recall response to *M. bovis* PPD.

POSTER #4

**Comparative Expression of Homing Receptors on Blood and Milk Leukocytes of Dairy Cattle.**

**B.A. Pesch**, J. A. Harp.

USDA-National Animal Disease Center, Ames, IA.

Four receptors linked with leukocyte homing were compared in blood and milk samples from dairy cattle around the time of parturition. Homing accessory molecules CD11a and CD44, the peripheral homing receptor CD62L, and the mucosal homing receptor  $\alpha 4\beta 7$ , were compared in the T cell subsets CD4, CD8, and  $\gamma\delta$ , and granulocytes. Milk T cell subsets uniformly expressed higher amounts of CD11a, CD62L, and  $\alpha 4\beta 7$  than did blood T cells. There was no significant difference in CD44 expression between milk and blood derived T cells. The percent of granulocytes expressing CD44 was higher in blood than in milk, while  $\alpha 4\beta 7$  expression was higher in milk than in blood. CD11a and CD62L expression was slightly lower in milk granulocytes than in blood. These findings have implications for strategies to reduce inflammatory damage caused by mastitis.

POSTER #5

**New Far Red and Near Infrared Dyes for Cell Tracking.**

**Part I. Flow Cytometric Applications.**

R. Schretzenmair<sup>1</sup>, C. Pletcher<sup>1</sup>, B. Gray<sup>2</sup>, E. Breslin<sup>2</sup>,  
K. Muirhead<sup>3</sup>, B. Ohlsson-Wilhelm<sup>3</sup> and J. Moore<sup>1</sup>.  
<sup>1</sup>Univ. of Pennsylvania, <sup>2</sup>PTI Research, Inc.,  
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Cell tracking agents like PKH26 are used to evaluate immune responses to antigenic stimulation. Both numbers of cells proliferating and extent of proliferation are assessed by monitoring dye dilution. However, longer wavelength tracking dyes are desirable for correlated multiparametric immunophenotyping on clinical analyzers.

We have synthesized and begun to evaluate the cell tracking abilities of PTIR 271-274, new membrane intercalating dyes which fluoresce in the far red or near IR. PTIR 271 and 271.5 are suitable for use with 4 color clinical analyzers. The spectra of PTIR 272-4 require an instrument capable of accommodating longer wavelength light sources and filters. All dyes are quite bright, well-retained by cells, and have no effect on cell viability or growth, making them good candidates to monitor multiple generations following antigen-induced division. Initial studies indicate that PTIR 271, 271.5 and 272 are optically compatible with FITC and PE, requiring little if any compensation.

POSTER #6

**New Far Red and Near Infrared Dyes for Cell Tracking. Part II: Imaging Applications**

A. Al-Mehdi, M. Meuler, \*B. Gray, \*E. Breslin, J. Sanzo, S. Margulies, †B. Ohlsson-Wilhelm, & †K. Muirhead. Univ. of Pennsylvania, Philadelphia, PA 19104, \*PTI Research, Exton, PA 19340, & †SciGro, Inc., Malvern, PA 19355

Objective: Determine whether stable membrane dyes that fluoresce in the far red/near infrared allow improved visualization of metastatic tumor cell trafficking in intact lung preparations.

Methods: A series of long red fluorescing membrane dyes was synthesized. Viability, growth, and dye retention were assessed *in vitro* using tumor cell lines YAC1 and MDA-MB-435s (MDA). MDA trafficking in microvessels of intact perfused rat lungs was imaged as previously described (*Nature Med.* 2000;6:100).

Results & Conclusions: PTIR271, PTIR273 and PTIR274 (respective emission maxima: 670nm, 790nm, and 820nm) had no effect on YAC1 viability or growth at dye concentrations up to 5  $\mu$ M. MDA cells labeled with PTIR271 were readily detected in subpleural vessels using a BioRad Radiance 2000 confocal microscope. Comparative studies with PTIR273 and 274 are in progress.

POSTER #7

**Characteristics of Tcr-Zeta Chain Expression In Lymphocyte Subsets Of Patients With Androgen-Independent Prostate Cancer In A Phase II Clinical Trial With The Vitamin D Analog, 1-Alpha-Hydroxyvitamin D2**

K Schell, A Dresen, M Pomplun, K Tutsch, G Wilding, and H Bailey  
University of Wisconsin Comprehensive Cancer Center, Madison, Wisconsin

Androgen-independence will eventually develop in most cases of metastatic prostate cancer. Because most conventional therapies have shown little effect on morbidity or mortality, there is a need for different treatment modalities. This study examines the clinical effect of 1<sub>α</sub>-OH-D2. Because immuno-suppression is a product of metastatic disease and because a decrease in the expression of the signal-transducing zeta chain has been associated with metastatic disease, patients were monitored for expression in T-cells and in NK cells as a possible surrogate for clinical effect. Using three-color flow cytometry, we have not observed any decrease in the levels of TCR zeta chain expression in T-cells or NK cells from the prostate cancer patients entered on study to date. However, all patients prior to 1<sub>α</sub>-OH-D2 therapy exhibited increased number of "cytotoxic" cells over normal donors. These cytotoxic cells were either traditional NK cells with a CD3<sup>-</sup>, CD56<sup>+</sup> phenotype or a cytotoxic T-cell with a CD3<sup>+</sup>, CD56<sup>+</sup> phenotype. One patient demonstrated a high percentage of CD3<sup>+</sup>, CD56<sup>+</sup> cells which also showed an increase in Fc receptors (CD16) as well as a CD4<sup>+</sup>, CD8<sup>+</sup> double phenotype. In some patients 1<sub>α</sub>-OH-D2 therapy induced a decrease in the TCR zeta chain expression in all subpopulations but levels returned to baseline or higher when doses were decreased (<12.5 μg/day 1<sub>α</sub>-OH-D2). In others only an increase in the fluorescence of the TCR zeta chain was noted. These preliminary data suggest a relationship between prostate cancer and/or therapy for prostate cancer and specific immune effector cell populations.

**GLIIFCA NINE – OCTOBER 13 – 15, 2000 HOTEL ST REGIS, DETROIT, MI – PROGRAM**

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