3rd CYTOMETRY & MICROSCOPY SYMPOSIUM

3ième SYMPOSIUM DE CYTOMÉTRIE ET MICROSCOPIE

November 24th - 26th, 2011

24 au 26 Novembre, 2011

Delta Chelsea Hotel / Hôtel Delta Chelsea

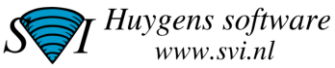
Toronto, Ontario

www.cytometry.ca

ASSOCIATION CANADIENNE DE CYTOMÉTRIE

CANADIAN CYTOMETRY ASSOCIATION

Our Sponsors



Welcome to the Canadian Cytometry Association's 3rd Cytometry & Microscopy Symposium. Visit the CCA's website (www.cytometry.ca) to find resources on microscopy and cytometry and to find out more about the CCA. We wish you an interesting and productive meeting.

Bienvenue au 3ième Symposium de Cytométrie et de Microscopie de l'Association Canadienne de Cytométrie. Visitez le site Web de l'ACC (www.cytometry.ca) pour trouver des informations sur la microscopie et la cytométrie et pour en savoir plus sur l'ACC. Nous vous souhaitons une conférence intéressante et productive.

The organizing committee / Le comité organisateur

Laurence Lejeune
President CCA / *Présidente de l'ACC*
Manager - Cytometry Facility, CHUM Research Centre-, St-Luc Hospital
Responsable - Service de Cytométrie, Centre de Recherche du CHUM, Hôpital Saint Luc

Claire Brown, PhD
Vice president CCA / *Vice-présidente ACC*
Director - Imaging Facility, Life Sciences Complex, McGill University
Directrice - Service d'Imagerie, Complexe des Sciences de la Vie, Université McGill

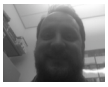
Guillaume Lesage, PhD
Treasurer CCA / *Trésorier ACC*
Administrator - Cell Imaging and Analysis Network, Dept. Biology, McGill University
Administrateur - Cell Imaging Analysis Network*, Dépt. Biologie, Université McGill*

Gabriel Lapointe
Research Assistant - Department of Biology, Concordia University
Assistant de Recherches - Département de Biologie, Université Concordia

Marie-Hélène Lacombe
Administrator CCA / *Administratrice ACC*
Immunophenotyping Platform, MUHC Research Institute, Montreal General Hospital
Plateforme d'immunophénotypage, Institut de recherche CUSM, Hôpital Général de Montréal

Gisele Knowles
Operational Director - Flow Cytometry and Scanning Microscopy, Sunnybrook Research Institute
Directrice des Opérations - Cytométrie de Flux et Microscopie à Balayage, Institut de Recherche Sunnybrook

Judith Lacoste, PhD
Microscopy Specialist - CIAN Dept. Biology, McGill University and MIA Cellavie Inc.
Spécialiste de Microscopie - CIAN, Dépt. Biologie, Université McGill et MIA Cellavie Inc.



Aleks Spurmanis
Microscopy Specialist - Imaging Facility, Life Sciences Complex, McGill University
Spécialiste de Microscopie - Service d'Imagerie, Complexe des Sciences de la Vie, Un. McGill

Nathalie Henley
Research Assistant - Maisonneuve Rosemont Hospital Research Centre
Assistante de Recherche - Centre de Recherche, Hôpital Maisonneuve Rosemont

TABLE OF CONTENTS

2011 CYTOMETRY AND MICROSCOPY SYMPOSIUM AT A GLANCE

	Thursday November 24 KEYNOTE LECTURE	Friday November 25 PLENARY SESSIONS	Saturday November 26 TUTORIALS				
08:00		Registration	Registration				08:00
09:00		Developmental Biology	Multicolor Applications	Live Cell Imaging Applications	Cell Cycle and Apoptosis	Instrument Standardization	09:00
10:00		Break - Posters – Exhibits	Break - Posters – Exhibits				10:00
11:00		Cell Biology	Cell Sorting Applications & Biosafety	Light Sources & Detectors	Advanced Techniques	Introduction to Image Processing and Analysis	11:00
12:00		Lunch - Posters - Exhibits	Lunch - Posters - Exhibits				12:00
13:00	Core Facility Manager meeting						13:00
14:00		Neurology	3D Imaging Techniques - *Part 1*	Data Analysis for Flow - *Part 1*	Measuring Protein-Protein Interactions - *Part 1*	Advanced Multicolor Applications for Flow - *Part 1*	14:00
15:00		Break - Posters - Exhibits	Break - Posters - Exhibits				15:00
16:00		Chronic Diseases	3D Imaging Techniques - *Part 2*	Data Analysis for Flow - *Part 2*	Measuring Protein-Protein Interactions - *Part 2*	Advanced Multicolor Applications for Flow - *Part 2*	16:00
17:00	Registration		Closing Remarks				17:00
18:00							18:00
19:00	**KEYNOTE ADDRESS** **D. Headley**						19:00
20:00	Opening Reception						20:00

SCHEDULE

Thursday November 24
Core Facility Manager Meeting

Sponsored by iLab Solutions

13:00-15:00 - Part I - *Scott Room*
Chair/Moderator: Claire Brown, McGill University

Association of Biomolecular Resource Facilities (ABRF)
Overview of the association, extended resources and ways the association helps core facilities in a wide range of scientific specialties.
Elke Küster-Schöck (McGill University/ABRF representative)

Funding problems and strategies
Overview of current funding situation.
Kevin Hamilton (Strategic research programs director, Sunnybrook Research Institute)

How can we as members of the ACC-CCA push for funding solutions in the future?

15:00-15:30 **Coffee Break -** Sponsored by Beckman Coulter

15:30-17:30 – Part II - *Scott Room*
Chair/Moderator: Laurence Lejeune, CHUM

Round Table Discussion

Maintenance contract or not? That is the question.
Service contracts – overview of issues. Role the ACC-CCA can play in negotiating reasonable contracts with companies.
Network software tools. Role the ACC-CCA can play in network software licenses.
Round Table Discussion of top topics voted from the survey.

Case scenarios
Presentation of "real life" case scenarios related to core facilities.

Keynote Address

19:00-20:00 Rediscovering cytometry in human cancer biology and advanced therapeutics.
David Hedley (Ontario Cancer Institute / Princess Margaret Hospital)
Churchill Ballroom

20:00-22:00 Opening Reception - sponsored by Molecular Devices
Churchill Courtyard

Friday November 25
Plenary Sessions

Churchill Ballroom

Developmental biology

Chair: Gisele Knowles, Sunnybrook Research Institute

8:30-9:00 **O1-** Human and mouse studies facilitated by phospho-flow and cytokine bead arrays.
Annie Bourdeau (Sunnybrook Research Institute)

9:00-9:30 **O2-** Profiling AML: A phospho-flow proteomics approach.
Julie Yuan (Hospital for Sick Children)

9:30-9:45 **O3-** Real-time and noninvasive optical imaging of tumor and vascular response to ionizing radiation *in vivo.*
Azusa Maeda (University of Toronto)

9:45-10:00 **O4-** PML nuclear bodies are juxtaposed to DNA-DSBs following IR-induced DNA damage.
Kenneth Chor Kin Tse (University of Toronto)

10:00-10:30 **Coffee Break – Exhibitor Hall** - Sponsored by OLYMPUS
Mountbatten Salon

Cell biology

Chair: Claire Brown, McGill University

10:30-11:00 **O5-** Imaging phagocytosis: receptors, signal transduction and the cytoskeleton.
Sergio Grinstein (Hospital for Sick Children Research Institute, University of Toronto)

11:00-11:30 **O6**- Effects of chlamydia infection on host cell division.
He Song Sun (Department of Cell and Systems Biology, University of Toronto at Scarborough)

11:30-11:45 **O7-** Role of anionic cell surface proteoglycans in PEI basedpolyplex attachment and internalization.
Laurence Delafosse (CNRC-IRB, Université de Montréal)

11:45-12:00 **O8-** Multiple approaches to study the trafficking of FCγ receptors upon endocytosis of immune complexes.
Christine Zhang (University of Toronto)

12:00-14:00 **Lunch – Exhibitor Hall** - sponsored by Molecular Devices
Mountbatten Salon
Poster session – Exhibitors

Lunch and Learn (12:30) – Sponsored by X-Cite® by LUMEN DYNAMICS
Kavita Aswani
Grab your lunch and hear about the latest
LED light source available for wide-field microscopy.

Neurology

Chair: Gabriel Lapointe, Concordia University

14:00-14:25 **O9-** Imaging brain matter.
Isabelle Aubert (Sunnybrook Research Institute, University of Toronto)

14:25-14:50 **O10-** Brain tumor initiating cells: why the cancer stem cell hypothesis matters to patients with brain tumors.
Sheila Singh (Stem Cell and Cancer Research Institute, McMaster University)

14:50-15:15 **O11-** A microprobe for in vivo single neuron electrophysiology and optogenetics.
Suzie Dufour (Centre de Recherche Université Laval Robert-Giffard, Université Laval)

15:15-15:30 **O12-** Calcium, calpain and calcineurin in low-frequency depression.
Lorelei Silverman (University of Toronto)

15:30-16:00 **Coffee Break – Exhibitor Hall -** Sponsored by TREE STAR
Mountbatten Salon

Chronic Diseases

Chair: Gisele Knowles, Sunnybrook Research Institute

16:00-16:25 **O13-** The immunology of HIV transmission.
Rupert Kaul (Departments of Medicine and Immunology, University of Toronto)

16:25-16:50 **O14-** Using flow cytometry to understand intra- and inter-tumoral heterogeneity in human cancer samples.
Laurie Ailles (Ontario Cancer Institute, University of Toronto)

16:50-17:15 **O15-** Immunogenetics of type I diabetes.
Janet Markle (Hospital for Sick Children, University of Toronto)

17:15-17:30 **O16-** Monitoring the emergence of newly formed plasma cells following long-term culture of human B lymphocytes.
Annie Roy (Héma -Québec)

17:30-18:00 **ACC-CCA General Assembly – All are Welcome!**

Free Evening

Saturday November 26
Morning Tutorials

Morning Concurrent Tutorial Sessions *– Part I (8:30 to 10:00)*

Multicolor Applications - *Rossetti Room*

8:30–9:15 **T1-** Introduction to Multicolor Flow Applications.
Laurence Lejeune (CHUM Research Centre-, St-Luc Hospital)

9:15-10:00 **T2-** Spectral Unmixing in Microscopy.
Ian Bates (Carl Zeiss Canada)

Live Cell Imaging Applications - *Wren Room*

8:30-8:55 **T3-** Live Cell Microscopy: Tips and Tools.
Judith Lacoste (McGill University)

8:55-9:20 **T4-** Live cell imaging - from beginners to technophiles.
Kevin Conway (Nikon Canada)

9:20-9:35 **T5-** Fluorescent Nuclear Markers in Live-Cell Imaging Experiments May Cause Significant Cytotoxicity.
Kimberly Young (McGill University)

9:35-10:00 **T6-** Confocal Imaging of Live Cells: Systems Optimized for High-Speed and Long Time-Course Studies.
George Sakellaropoulos (Olympus Canada and Andor Technology)

Cell Cycle and Apoptosis - *Scott Room*

8:30-9:00 **T7-** A brief history of cell cycle analysis.
Aleks Spurmanis (McGill University)

9:00-9:30 **T8-** Advantages of High Content Analysis for cell cycle, cell health and cytotoxicity applications.
Elizabeth Roquemore (GE Healthcare)

9:30-10:00 **T9-** Probability State Modeling of DNA Content S Phases.
Bruce Bagwell (Verity Software House)

Instrument Standardization - *Carlyle Room*

8:30-8:50 **T10-** Validating instrument performance in flow cytometry.
Marie-Helene Lacombe (McGill University)

8:50-9:15 **T11-** Standardizing multiple analyzers for uniform user results.
Julie Yuan (Hospital for Sick Children – UHN Flow Cytometry Facility)

9:15-9:30 **T12-** ABRF Light Microscopy Group Standardization Initiatives.
Claire Brown (McGill University)

9:30-9:55 **T13-** Over the rolling hills and into Flatland: Methods to measure and correct non-uniform fluorescence illumination.
John Oreopoulos (Spectral Applied Research)

9:55-10:10 **T14-** Confocal Resolution and Quality Control.
Tushare Jinadasa (McGill University)

10:10-10:30 **Coffee Break – Exhibitor Hall -** Sponsored by 

Mountbatten Salon

Morning Concurrent Tutorial Sessions *– Part II (10:30 to 12:00)*

Cell Sorting Applications & Biosafety - *Rossetti Room*

10:30–11:00 **T15-** Overview of specific cell sorting applications: How to sort cells that don't want to be sorted – neurons, tumor cells for starters.
Gisele Knowles (Sunnybrook Research Institute)

11:00-11:30 **T16-** Biosafety Challenges When Sorting Live Cells.
Dionne White (University of Toronto)

11:30-12:00 **T17-** Creating the model biosafety sorter: the development of the Beckman-Coulter Astrios.
Daniel Sirk (Beckman-Coulter)

Light Sources & Detectors - *Wren Room*

10:30-10:50 **T18-** Microscopy light sources.
Claire Brown (McGill University)

10:50-11:20 **T19-** CMOS, CCD and EMCCD cameras.
Vince Varallo (Olympus Canada and Photometrics)

11:20-11:40 **T20-** EMCCD cameras : new developments and applications.
Etienne Lareau (Nüvü Cameras)

11:40-12:10 **T21-** Microscopy detectors: noise and performance.
Judith Lacoste (McGill University)

Advanced Techniques - *Scott Room*

10:30-10:50 **T22-** A brief practical Introduction to fluorescent cell bar coding.
Carl Simard (Héma-Québec)

10:50-11:15 **T23-** Need to find a needle in a haystack? Use a magnet! Enhanced detection and analysis of rare cells with the MACSQuant® Family of analyzers.
Ashley Weant (Miltenyi Biotec)

11:15-12:00 **T24-** What the FRAP?! An introduction to advanced photobleaching and photoactivation microscopy.
James Jonkman (Advanced Optical Microscopy Facility (AOMF), University Health Network)

Introduction to Image Processing and Analysis - *Carlyle Room*

10:30-11:00 **T25-** Introduction to image processing and analysis.
Gabriel Lapointe (Concordia University)

11:00-11:30 **T26-** An introduction to multivariate image analysis Case study: an improved apoptosis assay.
Tony Collins (McMaster University)

11:30-12:00 **T27-** Reproducible and quantitative analysis of cell morphology and subcellular structures by controlling cell-surface adhesion.
Pauline Menager (CYTOO)

12:00-14:00 **Lunch – Exhibitor Hall** - sponsored by Molecular Devices
Mountbatten Salon
Poster session – Exhibitors

Lunch and Learn (12:30) – Sponsored by Science Exchange
Jay Connolly
Grab your lunch and hear about the easiest way to get experiments conducted by researchers in top core facilities and institutions.

Saturday November 26
Afternoon Tutorials

Afternoon Concurrent Advanced Tutorials *(14:00 to 17:30)*

3D Imaging Techniques - *Carlyle Room*

14:00-14:30 **T28-** Overview of available 3D imaging platforms.
Judith Lacoste (McGill University)

14:30-15:00 **T29-** Improving image resolution and signal to noise with deconvolution.
Vincent Schoonderwoert (Scientific Volume Imaging, - Huygens software)
by Video Conference

15:00-15:30 **T30-** 3-D image acquisition - advantages for observation and analysis.
Jeff Butler (Quorum Technologies)

15:30-16:00 **Coffee Break – Exhibitor Hall**
Mountbatten Salon

Sponsored by Molecular Devices OLYMPUS

**** Change of room - 3D Imaging Techniques - *Wren Room* ****

16:00-16:30 **T31-** Advances in Deep 3D Imaging with Multi-photon Excitation.
Andrew Millar (Olympus Canada)

16:30-17:00 **T32-** Advanced Techniques for 3D Image Visualization and Analysis.
Cory Glowinski (Bitplane Inc.)

17:00-17:30 **T33-** Intravital 3D Optical Imaging in Preclinical Cancer Research.
Ralph S. DaCosta (Ontario Cancer Institute, University Health Network)

Data Analysis for Flow Cytometry - *Rossetti Room*

14:00-15:00 **T34-** Gating is not enough: automated data analysis.
Ryan Brickman (British Columbia Cancer Research Centre)

15:00-15:30 **T35-** New concepts in sharing and analyzing data.
Laurence Lejeune (Collaboration with Cytobank)

15:30-16:00 **Coffee Break – Exhibitor Hall**
Mountbatten Salon

Sponsored by Molecular Devices OLYMPUS

16:00-17:00 **T36-** FlowJo Version 10 and The Fluorish Panel Wizard.
Isaiah Hankel (Tree Star, Inc)

17:00–17:30 **T37-** The Convergence of Flow and Image Cytometry Data Analysis.
David Novo (DeNovo Software)

Measuring Protein-Protein Interactions - *Scott Room*

14:00-14:30 **T38-** Co-localization Analysis…What can it tell you about protein-protein interactions?
Aleks Spurmanis (McGill University)

14:30-15:00 **T39-** Fluorescence Cross-Correlation Spectroscopy (FCCS).
Cecile Fradin (McMaster University)

15:00-15:30 **T40-** Raster Imaging Correlation Spectroscopy (RICS).
Claire Brown (McGill University)

15:30-16:00 **Coffee Break – Exhibitor Hall**
Mountbatten Salon

Sponsored by Molecular Devices OLYMPUS

16:00-16:30 **T41-** FRET Microscopy - Which technique should I use?
Claire Brown (McGill University)

16:30-17:00 **T42-** Practical FLIM-FRET for cell biologists.
Tony Collins (McMaster University)

Advanced Multicolor Applications for Flow - *Wren Room*

14:00-14:45 **T43-** Multi-color flow: the promise and the reality.
David Ehman (BD Biosciences)

14:45-15:15 **T44-** Needles in a haystack: finding dendritic cells and harvesting them.
Michele Anderson (Sunnybrook Research Institute, University of Toronto)

15:15-15:45 **T45-** Tracking human stem cell progenitors with FACS.
Sasan Zandi (UHN-Stem Cell Network)

15:45-16:15 **Coffee Break – Exhibitor Hall**
Mountbatten Salon

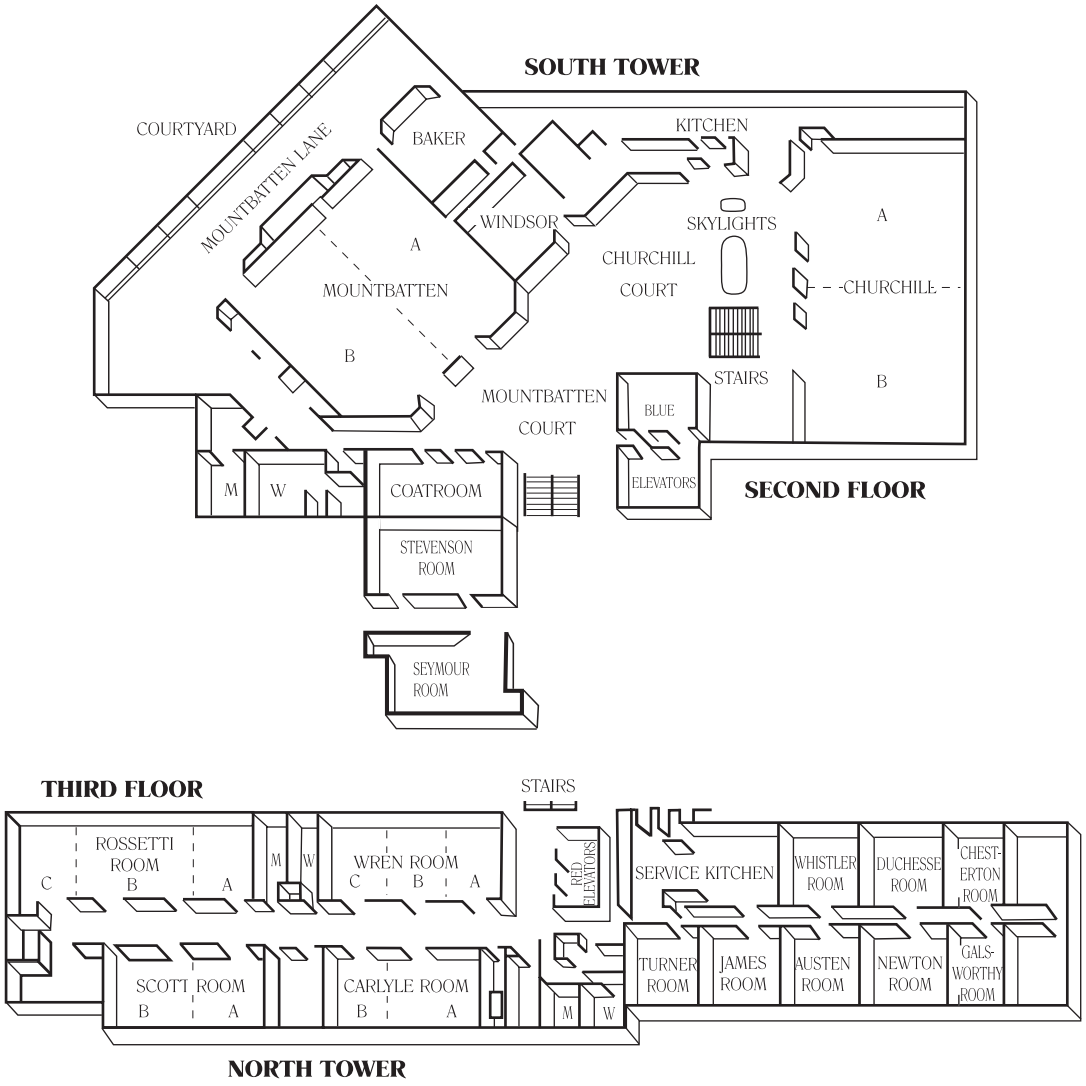
Sponsored by Molecular Devices OLYMPUS

**** Change of Room - Advanced Multicolor Applications for - *Carlyle Room* ****

16:15-16:45 **T46-** B cell signaling in Lupus.
Nan Chang (Toronto Western Research Institute)

16:45-17:30 **T47-** Building an Imaging System Using a Flow Cytometer.
Howard Shapiro (by video conference)

ROOM PLAN


SOUTH TOWER
COURTYARD
MOUNTBATTEN LANE
BAKER
KITCHEN
WINDSOR
SKYLIGHTS
A
CHURCHILL
COURT
MOUNTBATTEN
A
B
CHURCHILL
STAIRS
B
MOUNTBATTEN
COURT
BLUE
M
W
COATROOM
ELEVATORS
SECOND FLOOR
STEVENSON
ROOM
SEYMOUR
ROOM
THIRD FLOOR
STAIRS
ROSSETTI
ROOM
C
B
A
M
W
WREN ROOM
C
B
A
RED
ELEVATORS
SERVICE KITCHEN
WHISTLER
ROOM
DUCHESSE
ROOM
CHEST-
ERTON
ROOM
SCOTT ROOM
B
A
CARLYLE ROOM
B
A
M
W
TURNER
ROOM
JAMES
ROOM
AUSTEN
ROOM
NEWTON
ROOM
GALS-
WORTHY
ROOM
NORTH TOWER

TALKS: KEYNOTE ADDRESS (K), PLENARY LECTURES (O) AND TUTORIALS (T)

K - Rediscovering cytometry in human cancer biology and advanced therapeutics.

Davis Hedley

Ontario Cancer Institute, Princess Margaret Hospital,
Toronto.

O01 - Human and mouse studies facilitated by phospho-flow and cytokine bead.

Annie Bourdeau
Sunnybrook Research Institute, Toronto

O02 - Profiling AML: A phospho-flow proteomics approach

Julie Yuan (The SickKids – UHN Flow Cytometry Facility, Toronto, ON)

O03 - Real-time and non-invasive optical imaging of tumor and vascular response to ionizing radiation in vivo

Azusa Maeda1,2, Leigh Conroy1,2, Yonghong Chen1, Patricia E Lindsay3, Shani Mintzberg1,5, Carl Virtanen1,5, Julissa Tsao1,5, Neil Winegarden1,5,Yanchun Wang6, Lily Morikawa6, I Alex Vitkin1,2,3, David A Jaffray1,2,3,4, , Richard P Hill1,2,3, Ralph S DaCosta1,2,4
1 Ontario Cancer Institute, University Health Network, Toronto, ON,
2 Department of Medical Biophysics, University of Toronto, Toronto, ON, 3 Department of Radiation Oncology, University of Toronto, Toronto, ON, 4 STTARR Innovation Centre, Radiation Medicine Program, Toronto, ON, 5 University Health Network Microarray Centre, Toronto, ON,
6 Centre for Modelling of Human Disease, Mount Sinai Hospital, Toronto, ON

Over 50% of cancer patients receive radiation therapy (RT). Recent evidence suggests that while RT effectively kills tumor cells, the tumor vasculature also plays an important role in affecting tumor response to RT. However, experimental studies of radiobiological response of tumor cells and their vascular networks have been impeded by a lack of imaging techniques capable of visualizing changes microscopically in vivo. To address this need, we have developed an integrated system to monitor radiobiological response of multiple biological components.
Using a murine window chamber tumor (Me180 human cervical carinoma) model, our platform combines a small animal x-ray microirradiator with intravital confocal fluorescence microscopy and speckle variance optical coherence tomography (svOCT) to visualize and track radiation-induced changes in multiple tumor components simultaneously at a cellular level. Specifically, the data showed that a single fraction of 30 Gy delivered to the implanted tumor caused temporally-dynamic functional disruption in both large (>70 um) and capillary (<40 um) sized vessels, while leaving most of the large vessels structurally intact. In addition, we observed RT-induced platelet thrombosis as early as 1h post RT, as well as reduction in perivascular

cell coverage of tumor vasculature. These in vivo functional and morphological changes in the microvasculature were accompanied by changes in gene expression profile 4 days after RT.
Our results demonstrate the capability of this new preclinical experimental platform to enable quantitative, high-resolution and multiparametric intravital optical imaging of complex and dynamic radiobiological changes within the living tumor.

O04 - PML nuclear bodies are juxtaposed to DNA-DSBs following IR-induced DNA damage

Kenneth Chor Kin Tse, Shane Harding, Gaetano Zafarana, Kashif Ahmed, Graham Dellaire, David P. Bazett-Jones, Robert G. Bristow

The promyelocytic leukemia (PML) tumor suppressor protein aggregates in discrete PML nuclear bodies (PML-NBs) that are detectable by immunofluorescent microscopy. PML-NB number can change in response to cell cycle and various cell stresses, including DNA damage. Intra-nuclear gH2AX foci form following ionizing radiation (IR) as a function of dose and time and are used as an indicator of DNA double strand breaks (DNA-DSBs). However, whether PMl-NBs are associated with exogenous or endogenous (e.g. DNA replication associated) damage was unclear.

Using G0-G1 synchronized human fibroblast (GM05757) to exclude endogenous DNA damage, we examined the intra-nuclear locales of gH2AX and other DNA repair biomarkers with respect to PML-NBs following IR. PML +/+ and -/- MEFs did not vary in DNA-DSB repair based on comet assay. Consistent with this, PML-NBs did not respond to immediate DNA damage. However, 3D-confocal microscopy studies showed PML-NBs associated with the majority of residual gH2AX sites at later times (24h post-IR). Using quantitative microscopy, these associations were distinguishable as juxtaposition and not true co-localization (i.e., between gH2AX and DSB marker 53BP1). Continued presence of damage sensing and signaling proteins (MRE11, NBS1, MDC1 and 53BP1) and persistent kinase activity (DNA-PK, ATM, CHK2) was observed at these residual gH2AX sites. Absence of RAD51 and BRCA1 indicated that these were not replication-associated breaks and the exclusion of TRF2 suggested that these were not telomeric regions; rather PML-NBs associated specifically with unrepaired exogenous DSBs. Residual gH2AX sites were not enriched in euchromatin or heterochromatin. Interestingly, scanning electro imaging revealed a lower chromatin density near residual gH2AX foci, suggesting chromatin remodeling in the vicinity of unrepaired breaks. Live cell experiment using GFP-PML IV and mCherry-53BP1tudor domain suggested that PML-NB mobility facilitated the association with residual 53BP1 foci. Our data suggests that maintenance of genomic stability may depend, in part, on the association between PML-NBs and residual gH2AX foci.

O05 - Imaging phagocytosis: receptors, signal transduction and the cytoskeleton.

Sergio Grinstein.
Hospital for Sick Children Research Institute, University of Toronto

O06 - Chlamydia induces cancerous pathways by blocking normal mitotic patterns.

Hesong Sun, Department of Cell and Systems Biology, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, ON

Andrew Wilde, Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto, ON

Rene Harrison, Department of Cell and Systems Biology, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, ON

Chlamydia trachomatis, an obligate intracellular bacterium, is the most common cause of bacterial sexually transmitted diseases worldwide. Epidemiological studies have linked Chlamydia infections to increased risks of cervical cancer. Here we describe the first mechanism by which Chlamydia causes a cleavage furrow formation defect that consistently results in the formation of multinucleated host cells. Host signaling proteins essential for cleavage furrow initiation, ingression and stabilization are displaced from one of the prospective furrowing cortices after Chlamydia infection. This protein displacement leads to the formation of a unilateral cleavage furrow in infected human cells. The asymmetric distribution of signaling proteins is caused by the physical presence of the Chlamydia inclusion at the cell equator. By taking advantage of the protein displacing capability of the Chlamydia inclusion, we provide convincing evidence that astral microtubules deliver positive furrow-inducing signals to the plasma membrane during cell division. Internalized latex beads do not localize to the cell center as efficiently as Chlamydia inclusions, moreover, inhibition of bacterial protein synthesis with antibiotic reduces the frequency at which Chlamydia localizes to the cell equator. Together, these results suggest that Chlamydia effectors are involved in strategic positioning of the inclusion during cell division.

O07 - Role of anionic cell surface proteoglycans in PEI based-polyplex attachment and internalization

Laurence Delafosse (NRC-BRI and Université de Montréal Pavillon Roger-Gaudry Département de biochimie
2900, boul. Édouard-Montpetit, local D-360 Montréal, Québec H3T 1J4
Canada)
Yves Durocher (Project Leader in the Animal Cell Technology Group NRC-BRI)

Polyethylenimines (PEIs) are cationic polymers synthesized as either linear (lPEI) or branched (bPEI) forms and available over a wide range of molecular weights and polydispersities. PEIs associate with and condense DNA in solution, forming PEI:DNA complexes termed polyplexes. They offer an efficient vehicle for large-scale transfection applications, enabling the rapid production of recombinant proteins (r-proteins) and viral vectors. Because human embryonic kidney (HEK) 293 and Chinese hamster ovary (CHO) cells are the two dominant mammalian cell systems used for large-scale production of r-proteins, our goal is to further understand PEI-mediated transfection mechanisms in these cells. A first step in PEI-mediated gene transfer is the binding of polyplexes to the cell surface. The strong anionic charge present on the glycosaminoglycan (GAG) chains of cell surface heparan sulfate proteoglycans (HSPGs) makes them favorable binding sites for cationic polyplexes. In order to demonstrate the importance of cell surface heparan sulfate proteoglycans in the interaction of PEI:DNA polyplexes with the plasma membrane, we evaluated the impact of small chemical interfering with cellular sulfation. This was assessed using confocal microscopy and flow cytometry analysis of cells immunostained with an antibody recognizing heparan sulfate. Then, cellular attachment and endocytosis of the polyplexes formed either with bPEI and lPEI, as well as transfection efficacy, were quantified by flow cytometry.

O08 - Multiple Approaches to Study the Trafficking of Fcγ Receptors upon Endocytosis of Immune Complexes

Christine Y. Zhang and James W. Booth
Department of Immunology, University of Toronto, Toronto, Ontario, Canada, M5S 1A8

Fcγ receptors (FcγR) which recognize the Fc fraction of IgG play key roles in the modulation of a range of cellular responses as part of the host defense against foreign microbes and antigens. An important function of FcγR is to mediate internalization of soluble IgG-containing immune complexes via endocytosis. The mechanisms of internalization and intracellular transport of FcγR after internalization

are less clear. Utilizing fluorescence imaging, flow cytometry and biochemical approaches, we investigated the trafficking behaviours of human FcγRIIA and FcγRIIB2 upon clustering with immune complexes. We demonstrate that upon engagement by multivalent aggregated human IgG, FcγRIIA expressed in ts20 Chinese hamster fibroblasts is delivered along with its ligand to lysosomal compartments for degradation, while FcγRIIB2 dissociates from the ligand and is routed separately into the recycling pathway. FcγRIIA sorting to lysosomes requires receptor multimerization, but does not require either Src family kinase activity or ubiquitylation of receptor lysine residues. The sorting of FcγRIIB2 away from a degradative fate is not due to its lower affinity for IgG and occurs even upon persistent receptor aggregation. Upon co-engagement of FcγRIIA and FcγRIIB2, the receptors are sorted independently to distinct final fates after dissociation of co-clustering ligand. These results reveal fundamental differences in the trafficking behaviour of different Fcγ receptors.

O09 - Imaging brain matter.

Isabelle Aubert
Sunnybrook Research Institute, University of Toronto.

O10 - Brain tumour initiating cells: why the cancer stem cell hypothesis matters to patients with brain tumours.

Sheila Singh.
Department of Surgery, McMaster Children's Hospital,
McMaster University, Hamilton, ON, Canada.

O11 - A microprobe for in vivo single neuron electrophysiology and optogenetics.

Suzie Dufour.
Centre de Recherche Université Laval Robert-Giffard, Université Laval

O12 - Calcium, calpain, and calcineurin in low-frequency depression

Lorelei B. Silverman-Gavrila1, Moshe Praver2, Scott Medler3, Donald L. Mykles3, and Milton P. Charlton1*
1 Department of Physiology, Medical Sciences Building, Rm. 3308
University of Toronto
1 King's College Circle
Toronto, Ontario, Canada M5S 1A8
Email: milton.charlton@utoronto.ca
tel: (416) 978-6355
fax: (416) 978-4940
2 Columbia University School of Medicine, New York
3 SUNY Fredonia, Biology Department
4 Department of Biology, Colorado State University, Fort Collins, CO 80523

Low-frequency depression (LFD) of transmitter release at phasic synapses of crayfish leg extensor neuromuscular junctions (NMJ) occurs with stimulation at 0.2Hz in both dissected preparations and in free living animals. Since the fast Ca2+ chelator BAPTA-AM inhibits LFD but the slow chelator EGTA-AM does not, depression is not caused by widespread residual free calcium in the presynaptic terminals, but rather the Ca2+ sensor for LFD may be close to a Ca2+ source at active zones. LFD is regulated by

presynaptic Ca2+ activated phosphatase calcineurin (Silverman-Gavrila and Charlton, 2009) which can be activated by Ca2+-activated proteases calpain. A cDNA encoding a partial calcineurin A sequence was obtained with RT-PCR using degenerate primers to a highly-conserved sequence in homologous genes from other species. Immunostaining showed that both proteins are present at nerve terminals. Calpain inhibitors calpain inhibitor I, MDL-28170, and PD 150606, but not the control compound PD 145305, inhibit LFD both in the intact animal as shown by electromyograms and in dissected preparations as shown by intracellular recordings. These inhibitors probably act presynaptically as suggested by miniEPSP analysis. Calpain activity in CNS extract detected using a fluorimetric assay was modulated by calcium and calpain inhibitors. Western blot analysis with an antibody against calcineurin A showed that calpain-mediated proteolysis of calcineurin could occur in crayfish preparation in the presence of high Ca2+, but not in the presence of high Ca2+ and inhibitors of calpain. Inhibition of LFD by calpain inhibition causes rearrangement of the tubulin cytoskeleton at phasic terminals. High frequency depression (HFD) does not involve protein phosphorylation- or calpain-dependent mechanisms. In conclusion LFD might involve a specific pathway in which local Ca2+ signaling activates calpain and calcineurin at active zones and causes changes of tubulin cytoskeleton.

O13 - The immunology of HIV transmission.

Rupert Kaul
Departments of Medicine and Immunology, University of Toronto

O14 - Using flow cytometry to understand intra- and inter-tumoral heterogeneity in human cancer samples.

Laurie Ailles
Ontario Cancer Institute, University of Toronto.

O15 - Immunogenetics of Type I diabetes

Janet Markle (Hospital for Sick Children, University of Toronto)

O16 - Monitoring the emergence of newly formed plasma cells following long-term culture of human B lymphocytes

Annie Roy (Héma-Québec)

n vitro, CD40-CD154 interaction allows expanding human B lymphocytes and promoting their differentiation into antibody secreting cells. This culture system supports the emergence of cells expressing CD138, which is the hallmark for fully differentiated plasma cells. In vitro, long-term cultures of switched memory B lymphocytes result in emergence of about 50% CD138+ cells; whereas functional analysis indicated that the majority of these cultured cells were fully differentiated plasma cells. The question was thus whether the anti-CD138 used (B-A38) was able to detect all newly formed plasma cells. We have thus compared by flow cytometry the efficiency of five anti-CD138 mAbs: 1D4, B-A38, B-B4, DL-101 and MI15 to characterize RPMI-8226 (CD138high) and SKW6.4 (CD138dim) cell lines as well as in vitro generated plasma cells. All anti-CD138 antibodies tested were able to stain RPMI-8226 and SKW6.4 cell lines as well as in vitro generated plasma cells. B-A38, B-B4 and MI-15 appear to bind simultaneously, on RMPI-8226 cells suggesting that their respective epitope were distinct. However, the median fluorescence intensity for CD138 on RMPI-8226 and SKW6.4 cell lines as well as cultured plasma cells

greatly varies from one mAb to another. Among all, B-A38 gives the brightest fluorescence on cell lines, while DL-101 and B-A38 are the best to detect CD138 on in vitro generated plasma cells. Overall, these results suggest that CD138 epitope may differ on cell lines and normal human cells and highlight that a mix of B-A38 and DL-101 mAbs could be better to detect plasma cells in vitro.

T01 - Introduction to Multicolor Flow Applications

Laurence Lejeune
CHUM

T02 - Spectral Unmixing in Microscopy

Ian Bates (Carl Zeiss Canada Inc.)

T03 - Live Cell Microscopy: Tips and Tools

Judith Lacoste.
McGill University/ MIA Cellavie.

T04 - Live cell imaging - from beginners to technophiles

Kevin Conway (Nikon Canada, Inc.)

T05 - Fluorescent Nuclear Markers in Live-Cell Imaging Experiments May Cause Significant Cytotoxicity

Kimberly Young, Claire Brown, Department of Physiology, McGill University

Maintaining normally functioning cells poses one of the most significant challenges for performing live-cell imaging. Although fluorescent nuclear markers have recently been developed for live-cell experiments, many bind through DNA intercalation, which is known to be cytotoxic. We aim to determine the level of cytotoxicity of commonly used nuclear probes and to find alternatives or controls needed to validate live-cell experiments. We used a High Content Screening system to monitor live Chinese Hamster Ovary cells over the course of 72 hours. The cells contained a heterologously expressed adhesion adaptor protein, paxillin, fused to Green Fluorescent Protein. We tested several nuclear dyes including Hoescht 33258, Draq5, Red Nuclear Mask and Blue Nuclear Mask at a range of concentrations. We measured cell proliferation using a multi-wavelength cell-scoring algorithm from MetaXpress software in order to determine if the nuclear dyes were cytotoxic. Preliminary results suggest that the nuclear dye Hoescht 33258 causes decreased cell migration, halts cell division and creates abnormal nucleus morphology. Qualitative results indicate that these effects are less severe when the dyes are administered at lower concentrations. If basic cellular processes are disrupted to this magnitude in live-cell experiments, non-intercalating dyes such as histone markers should be considered. The extent to which nuclear dyes interfere with cell functions is still unclear, however with appropriate controls and concentrations, short-term experiments may not be severely affected.

T06 - Confocal Imaging of Live Cells: Systems Optimized for High-Speed and Long Time-Course Studies.

George Sakellaropoulos
Olympus Canada and Andor Technology

T07 - A brief history of cell cycle analysis

Aleks Spurmanis (McGill University)

From the "dark-ages" of auto-radiographic analyses to the development of multi-parametric cytometric methods, this tutorial provides a brief overview of key analytical methods developed over the course of the past 50 years that have enabled scientists to explore the kinetics of the cell cycle. More recent innovations will also be described including: the FUCCI probes, a FLIM-FRET based assay to monitor chromatin condensation and the use of a photo-switchable probe to visualize the formation of the nuclear envelope permeability barrier in living cells.

T08 - Advantages of High Content Analysis for cell cycle, cell health and cytotoxicity applications.

Elizabeth Roquemore (GE Healthcare)

T09 - Probability State Modeling of DNA Content S Phases

Bruce Bagwell
Verity Software House

Modeling DNA S-phases has traditionally been restricted to chi-square minimization of DNA content histograms using non-linear least-squares algorithms such as the Marquardt Compromise. Over the last thirty-five years, there have been numerous proposals for different mathematical constructs for these DNA models, but over time, there appears to be an unofficial consensus that the single broadened rectangle is optimal for modeling S-phases from complex solid tumors and multiple broadened trapezoids are optimal for tissue culture systems.

In 2006, Probability State Modeling (PSM) became available for modeling high-dimensional data such as bone marrow lineage progressions as well as peripheral blood T-cell Ag-dependent progressions. However, when PSM was applied to cell cycle data, the linear interpolation algorithms had insufficient flexibility to accurately model many DNA content S-phases. In order to solve this issue, a parabolic spline transition function (PS) is proposed for transitional phases such as DNA S-phases.

The tested hypothesis is that PS interpolation results in more accurate S phase estimates than either the PSM linear interpolation method or popular conventional histogram-based S-phase models. This new type of transition function not only allows for accurate fits for DNA S-phases but can be used to more accurately model any other type of cellular transition found in complex biologic processes.

T10 - Validating Instrument Performance in Flow Cytometry

Marie-Hélène Lacombe
McGill University

T11 - Standardizing multiple analyzers for uniform user results.

Julie Yuan (The SickKids – UHN Flow Cytometry Facility, Toronto, ON)

T12 - ABRF Light Microscopy Group Standardization Initiatives

Claire Brown (McGill University)

The Association of Biomolecular Resource Facilities (ABRF) is an international association that concentrates on core facility initiatives, instrument standards and technology development. The ABRF has a number of research groups that work on developing technology for instrument standardization and quality control. Currently there are no standards for light microscopy, so one of the central initiatives of the light microscopy research group (LMRG) is to develop standards for quality control. The first international study of the ABRF-LMRG looked at laser stability, detector co-registration and field uniformity of confocal laser scanning microscopes. Tests were developed to measure long-term and short-term laser stability. The long-term tests verify if the instrument is stable during a time period that would correspond to an experimental data set. This test ensures that comparisons between image intensities collected at the beginning and the end of an imaging session are accurate. In turn the short-term test ensures that the intensity measured within a z-stack of images or an image time series will be consistent. Detector co-registration is an important metric for co-localization studies. Finally, field uniformity is important for any quantitative analysis within images. The second international study is currently under way (120 participants, 24 countries) and is focusing on measuring objective lens quality, and spatial and spectral resolution of confocal laser scanning microscopes. An overview of the initiatives of the ABRF-LMRG, data from the international studies and future initiatives will be presented.

T13 - Over the rolling hills and into Flatland: Methods to measure and correct non-uniform fluorescence illumination.

John Oreopoulos (Spectral Applied Research, Inc.)

T14 - Confocal Resolution and Optics Quality.

Tushare Jinadasa (McGill University)

The collection of diffraction limited fluorescence images from LASER scanning microscopes now spans the life sciences and much of the physical sciences. However, the results from this powerful tool are only accurate if the optical components of the microscope can be validated. Some of these components can be verified through collecting and analyzing point spread functions (PSFs) from sub-resolution point sources. Therefore, in this protocol we describe how to prepare fluorescent microsphere samples, set up a confocal microscope to properly collect the images and perform measurements on microsphere samples. These measurements require collecting a 3D confocal image volume of the fluorescent microspheres in

order to accurately calculate the microscope PSF. The analysis of this PSF is used to determine the maximal resolvable spatial features and to identify any problems with the quality of the microscope's images. These include issues with the objective lens, scan components and other relay optics. Additionally, possible causes and remedies are provided for PSF data that do not conform to expected results. The preparation of microsphere samples requires two to three hours and an overnight drying period. The microscope setup takes about one hour, while collecting and analyzing the PSF images takes two to three hours.

T15 - Overview of specific cell sorting applications: How to sort cells that don't want to be sorted - neurons, tumor cells for starters.

Gisele Knowles
Sunnybrook Research Institute

T16 - Biosafety Challenges When Sorting Live Cells.

Dionne White
University of Toronto

T17 - Creating the model biosafety sorter: the development of the Beckman-Coulter Astrios.

Daniel Sirk (Beckman Coulter)

T18 - Microscopy Light Sources.

Claire Brown (McGill University)

There are many different types of fluorescence excitation light sources and for people who are not experts in the field it can be a daunting task to understand and choose the appropriate light source for their application. Traditional light sources like tungsten, mercury HBO and xenon lamps are still widely used and are satisfactory white light sources for transmitted light and fluorescence applications. Care must be taken if these sources are used for live cell imaging as they may result in exposure of living samples to harmful ultra-violet light (mercury HBO) or infra-red light that can cause heating (xenon). Filters can be added to the system to block these potentially damaging wavelengths. Newer metal-halide light sources have much longer bulb lifetimes and a more uniform excitation. Light emitting diode (LED) sources are poised to replace other light sources. They have a higher upfront cost but require no alignment, they last up to 10,000 hours (versus 200 for a mercury HBO source), have discrete colour bands, give uniform illumination and the light intensity does not decay over time. Lasers are still the source of choice for confocal microscopes and offer single excitation wavelength specificity, high power levels for photo-bleaching and photo-activation and they can be scanned across the sample in laser scanning applications. Various light sources on the market, old and new, will be presented as well as the pros and cons of each source for various applications including sensitivity, flexibility, live-cell and high speed applications.

T19 - CMOS, CCD and EMCCD cameras.

Vince Varallo (Olympus Canada and Photometrics)

T20 - EMCCD cameras: New developments and applications.

Etienne Lareau (Nüvü Cameras)

T21 - Microscopy detectors: noise and performance.

Judith Lacoste.
McGill University/ MIA Cellavie.

T22 - A brief practical Introduction to fluorescent cell bar coding.

Carl Simard (Héma-Québec)

T23 - Need to find a needle in a haystack? Use a magnet! Enhanced detection and analysis of rare cells with the MACSQuant® Family of analyzers

Ashley Weant, PhD
Flow Cytometry Specialist
Miltenyi Biotec, Inc

Monitoring human blood for changes in such rare cells as circulating tumor cells (CTC), circulating endothelial cells (cEC) or antigen-specific T cells can help researchers understand the body's response to treatments for certain diseases or trauma. A major obstacle to detecting these cells in human blood is their frequency. Typically, in flow cytometry the detection level for rare cells is about 1 in 1000 to 1 in 10,000, corresponding to the frequency of hematopoietic stem cells. Other cell types, such as cEC or CTC may occur at considerably lower frequencies. Detection of these rare cells requires acquisition and analysis of large cell numbers, which can be time consuming and limited by data file size.
Pre-enrichment, by MACS® Technology, of rare cell populations prior to flow analysis can facilitate their detection by increasing the ratio of the cell of interest to non-target cells. The MACSQuant® Family of analyzers has integrated this enrichment technology into its fluidics system enabling a seamless workflow from pre-enrichment to analysis. Additionally, with its volumetric measurement capabilities, the MACSQuant® Analyzer can enable to quantification of the target cells.
This presentation will highlight the use of MACS® Technology and reagents as well as the MACSQuant® Analyzer for detection and analysis of such rare cells as CTC, cEC, endothelial progenitor cells (EPC) and more.

T24 - What the FRAP?! An introduction to advanced photobleaching and photoactivation microscopy.

James Jonkman
Advanced Optical Microscopy Facility, University Health Network.

Fluorescence Recovery After Photobleaching (FRAP) is a relatively well-known technique for measuring protein mobility in living cells. Nevertheless, I'm quite surprised by how few people are making use of this tool! After tagging the protein of interest with a fluorescent protein (such as GFP), many confocal microscopes will facilitate photobleaching a small region in the cell and monitoring the recovery of fluorescence in this region as bleached molecules exchange with unbleached ones from elsewhere in the cell. A qualitative assessment can already tell you whether the mobility is fast or slow; but a more quantitative analysis can help to determine whether binding interactions are present, the number of binding states, and whether there is a proportion of immobilized protein that doesn't participate in the exchange. I'll show you some FRAP data taken on a spinning-disk confocal which has been optimized for straight-forward (yet powerful) acquisition and for simple (yet rigorous) processing and analysis. I'll also discuss photoactivation microscopy, including some of its applications and in general terms how the acquisition and analysis differs from FRAP data.

T25 - Introduction to image processing and analysis

Gabriel Lapointe
Concordia University

T26 - An introduction to multivariate image analysis case study: an improved apoptosis assay for high content screening.

Tony Collins
McMaster University

T27 - Reproducible and quantitative analysis of cell morphology and subcellular structures by controlling cell-surface adhesion.

Pauline Menager

Cells in most tissues have the same polarity and positioning of intracellular compartments. This internal organization is preserved during tissue growth, with cell divisions being properly oriented to produce daughter cells which are able to insert in the tissue without impairing cohesive integrity and function. On the contrary, in cultured cells, all collective properties are lost. In addition, cell migration introduces a large variance in cell shape and internal organization. Using concave adhesive micropatterns, it is possible to grow cells presenting a highly reproducible response to the adhesion, as judged by the polarized and highly reproducible organization of their internal compartments and the orientation of their divisions. These individual cells on micropatterns are similar to those found in tissue, wherein cell shape and migration are restricted by neighboring cells. The behavioral reproducibility of cells on micropatterns allows powerful quantification of the spatial distribution of cell compartments and individual proteins. Importantly, averaging the distribution observed across a limited number of cells leads to the definition of a Reference Cell™ which can be used to assess the significance of any perturbation introduced in and across large scale screening experiments. The ability of CYTOO's micropatterns to normalize a cell's internal organization and polarity up to mitosis and cell division can have a wide range of applications in HCA, from the large scale analysis of cell response to drugs, to the dissection of most cell functions and pathways by RNAi.

T28 - Overview of available 3D imaging platforms.

Judith Lacoste.
McGill University/ MIA Cellavie.

T29 - Improving image resolution and signal to noise with deconvolution.

Vincent Schoonderwoert (Scientific Volume Imaging)

T30 - 3-D Image Acquisition - Advantages for Observation and Analysis

Jeff Butler
Quorum Technologies, Inc.

T31 - Advances in deep 3D imaging with multi-photon excitation.

Andrew Millar
Olympus Canada, Inc.

T32 - Advanced techniques for 3D image visualization and analysis.

Cory Glowinski
Bitplane, Inc.

T33 - Intravital 3D Optical Imaging in Preclinical Cancer Research.

Ralph DaCosta
Ontario Cancer Institute

Dr. DaCosta will present an overview of preclinical multimodal microscopic imaging technologies and intravital animal models that are currently being developed and applied to cancer research in his lab at the Ontario Cancer Institute, University Health Network. Specific topics will include: in vivo multiphoton fluorescence microscopy, optical coherence tomography, bioluminescence, photoacoustic imaging, ex vivo gene-expression analysis, vascular and perivascular imaging, hypoxia imaging, small animal x-ray microirradiation and new intravital animal models.

T34 - Gating is not enough: Automate and let go.

Ryan Brickman (British Columbia Cancer Research Centre)

T35 - New Concepts in Sharing and Analyzing Data

Laurence Lejeune
CHUM

T36 - Flow Jo Version 10 and the Fluorish Panel Wizard

Isaiah Hankel (Tree Star, Inc.)

T37 - The convergence of Flow and Image Cytometry Data Analysis

David Novo (DeNovo Software)

This talk will highlight the similarities and differences between flow and image cytometry data analysis. The talk will demonstrate how flow cytometry data analysis techniques have been applied to image cytometry data.

T38 - Co-localization Analysis…What can it tell you about protein-protein interactions?

Aleks Spurmanis (McGill University)

When performed correctly, co-localization analysis can provide an investigator with useful information about the relative distribution of two or more biomarkers. With the advent of super-resolution imaging techniques, just how close are we getting to being able to visualize single molecule interactions? This tutorial will explore this idea from the perspective of light resolution. Some of the common analytical tools and methods used to analyze co-localization will also be discussed along with associated caveats. Guidelines on good image acquisition will also be presented.

T39 - Fluorescence Cross-Correlation Spectroscopy (FCCS)

Cecile Fradin

The concept of biomolecular interaction detection by fluorescence cross-correlation spectroscopy (FCSS) will be presented, followed by practical tips on how to implement this technique. Finally, an example of application of FCCS to the detection of protein interactions with lipid membranes will be briefly described.

T40 - Raster Image Correlation Spectroscopy (RICS)

Claire Brown (McGill University)

Raster image correlation spectroscopy (RICS) is a technique that can extract information about molecular dynamics and concentrations from fluorescence images of live-cells taken on confocal laser scanning microscope (CLSM) systems. RICS is ideally suited for live cell imaging because unlike photo-bleaching or photo-activation techniques it does not require the use of high energy laser light to perturb the system in order to measure molecular dynamics. RICS can generate spatial maps with ~1–2 μm resolution of sub-microscopic molecular movements of cytosolic proteins. RICS relies on a statistical spatial autocorrelation

analysis of intensity fluctuations within confocal images. The shape and amplitude of the resulting autocorrelation function (ACF) provides information about the molecular dynamics and concentration for the region of the cell under study. Two-color RICS cross-correlation (ccRICS) involves the calculation of a cross-correlation function (CCF) from the spatial intensity fluctuations within two images from different molecules, labelled with distinct fluorescent dyes. If the two molecules are moving together as part of a complex then the CCF will measure the dynamics and concentration of that complex. However, if no CCF is measured then there is no complex containing the two molecules. ccRICS does not report false negative results which can be a problem with Förster resonance energy transfer (FRET) experiments. The ccRICS technique provides a lot more information than the more traditional biochemical co-immunoprecipitation experiments giving details of the time and location of protein-protein interaction in intact cells. The RICS technology will be explained and examples of measurements on live-cells will be provided.

T41 - FRET microscopy - Which technique should I use

Claire Brown (McGill University)

Förester resonance energy transfer (FRET) microscopy is a technique that can be used in combination with fluorescence protein fusions in order to measure protein-protein interactions in the cell. Typically, a donor fluorescent molecule is excited and if an acceptor molecule is within ~2-8 nm the excitation energy can be transferred from the donor to the acceptor and the acceptor molecule will give off fluorescence. The amount of acceptor fluorescence is directly related to the FRET efficiency or the proximity of the two fluorescent molecules to one another. Sensitized emission FRET uses a ratio image of donor versus acceptor fluorescence due to FRET. Sensitized emission is ideal for rapid live-cell imaging but it requires a series of precise control experiments and it can be difficult to pick up subtle changes in small FRET signals (10-20%). Acceptor photo-bleaching relies on bleaching out the acceptor fluorescence and looking to see if the acceptor fluorescence increases due to the lack of FRET after bleaching. Anisotropy based FRET ideal for high throughput applications because it is fast. Fluorescence lifetime imaging microscopy (FLIM) relies on the fact that the average time the donor molecules stay in the excited state (fluorescence lifetime) is dependent on the fraction of molecules that undergo FRET. FLIM is very sensitive, and does not depend on protein concentration but it is very slow. This presentation will provide an overview of the pitfalls of FRET, the various ways to measure FRET, and the pros and cons of each technology.

T42 - Practical FLIM-FRET for cell biologists.

Tony Collins
McMaster University

T43 - Multi-color flow: the promises and the reality

David Eheman (BD Biosciences)

T44 - Needles in a haystack: finding dendritic cells and harvesting them.

Michele Anderson (Sunnybrook Research Institute,University of Toronto)

T45 - **Tracking human stem cell progenitors with FACS.**

Sasan Zandi (UHN - Stem Cell Network)

T46 - **B cell signalling in Lupus**

Nan Chang (Toronto Western Research Institute)

T47 - **Building an Imaging System using a Flow Cytometer**

Howard Shapiro

ABSTRACTS – POSTERS

P01 - Tumour vasculature effects of oncolytic vaccinia virus infection in a window-chamber tumour model

Fernando A. Angarita (1,2), Ralph DaCosta (3), Sergio A. Acuna (1), Emily Chen (3), Nan Tang (1), John C. Bell (4), J. Andrea McCart (1,2,5)

1. Division of Experimental Therapeutics, Toronto General Research Institute, University Health Network, Toronto, ON
2. Institute of Medical Science, University of Toronto, Toronto, ON
3. Ontario Cancer Institute, University Health Network, Toronto, ON
4. Ottawa Health Research Institute, Ottawa, ON
5. Department of Surgery, Mount Sinai Hospital and University of Toronto, Toronto, ON

Background: Oncolytic virotherapy utilizes attenuated viruses that specifically replicate within tumour cells. Recent evidence suggests that oncolytic viruses (OVs) can also interact with tumour vasculature leading to profound anti-angiogenic effects. We aim to understand the interactions between OVs and cancer-induced angiogenesis. Methods: A dorsal skin-fold window chamber was developed in C57BL/6 mice, which were inoculated with MC38 colon cancer cells expressing green fluorescent protein. A separate skin wound acted as a control for neo-angiogenesis. Mice received a single dose of vaccinia virus (VV) expressing red fluorescent protein (RFP), 5d after cancer cell injection. Tumour and blood vessel distribution was periodically assessed by confocal fluorescent microscopy (CFM) over 7 days. Tumours, normal tissues, and wounds were harvested 3 days post infection. Immunohistochemistry studies for VV and CD31 were performed. Results: RFP signal was detected 24h post-injection, increasing thereafter. Co-localization of viral RFP and endothelial CD31 signal was apparent 48h post-virus injection. Quantification of co-staining for CD31/VV infection revealed that 25% of tumour vessels ($p<0.05$) had co-localization of VV infection. Lung, liver, and kidney normal vasculature were not infected by virus ($p<0.05$), but 17% of neovasculature in the skin wounds was VV positive. Conclusion: vvDD-RFP exerts anti-tumour effects both by direct cell lysis and infection of associated tumour angiogenesis. The mechanisms behind this are under investigation. The window chamber/CFM is an excellent model system to address the selective interaction of OVs with tumour vasculature as it allows for repeated real-time imaging of tumour growth, tumour-induced angiogenesis, and viral infection over time.

P02 - Acute Damage Responses In Circulating Blast Cells During Induction Chemotherapy For Acute Myeloid Leukaemia

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

Using our rapid whole blood fixation/lysis protocol and combined labelling using phosphospecific antibodies to ERK, Akt, S6 and STAT5, we have found constitutive activation of at least one major signaling pathway in the majority of AML patients. Since these pathways can enhance cell survival, we hypothesize that individual cells showing high levels of pathway activation are relatively resistant to chemotherapy. Standard chemotherapy consists of daunorubicin and Ara-C, and can achieve rapid clearance of circulating blast cells with long term cures in a significant minority of AML patients. Chemotherapy-induced DNA damage triggers a number of pathways that cause cell cycle arrest and elicit repair, but irreparable damage typically results in apoptosis. To study these processes in patients, we first exposed OCI-AML2 blasts to daunorubicin in vitro, and then stained for the following markers by flow cytometry: γH2AX [detects DNA breaks], total p53, p53P-Ser15, p53Ac-Lys382, P-p38, P-SAPK/JNK [stress response

pathways], P-chk-2 [damage response checkpoint], cleaved PARP, and cleaved caspase-3 [apoptosis execution]. All of these markers showed some degree of activation that was generally proportional to drug dose and exposure time, with γH2AX and p53P-Ser15 giving the strongest signals. Next we obtained peripheral blood samples from a group of 10 newly-diagnosed acute leukemia patients prior to, and at 24 and 48 hours following the start of daunorubicin/Ara-C induction chemotherapy. Samples were processed rapidly using a whole blood protocol optimized for cell signaling, and aliquots frozen in FBS/glycerol for assay development. A preliminary assessment of the patient samples shows that several of the damage markers are activated during the early phase of chemotherapy, similar to effects in the daunorubicin-treated AML cell line. Ongoing work will optimize the flow cytometry protocols, including incorporation of phosphospecific antibodies and surface markers, and a prospective clinical study to ask if the responses are predictive of patient outcome.

P03 - Science Exchange: an innovative new platform for experiment outsourcing

Jay Connolly (1,2), Ryan Abbott (1,3), Dan Knox (1,4), Elizabeth Iorns, PhD (1,5)

1- Science Exchange, Palo Alto; 2- Harvard College, Cambridge; 3- Michigan State University, East Lansing; 4- MIT Sloan School of Management, Cambridge; 5- University of Miami Miller School of Medicine, Miami

Introduction: Science is becoming increasingly specialized, necessitating more collaboration. Many research institutions have established core facilities to help reduce reliance on 'bartering' to access specialized equipment and expertise. However, the core facility system is fragmented: researchers have varying degrees of access, evaluation of quality is hard and pricing is not transparent. Researchers seeking to collaborate with outside providers face barriers to finding and evaluating providers, coordinating logistics and paying for work. Core facilities lack a robust mechanism for marketing their expertise and services to attract outside business.
Methods: Science Exchange, an online marketplace accessible at www.ScienceExchange.com, was launched in August 2011 to address these issues. Science Exchange works by signing up core facilities as "providers" of different experiment types. Researchers can search for an experiment type and choose a facility to perform the work, or post an open project to receive bids from qualified facilities. Science Exchange is a centralized hub for provider information and reviews, and assists with project management, including payment. The goal of Science Exchange is to create a vibrant and accessible marketplace for experimental services.
Results: As of October 2011 more than 3,000 scientists from over 500 research institutions had registered. On average, experiments outsourced through Science Exchange have saved users 54% ($4,664 per experiment).
Conclusions: Science Exchange addresses the barriers to effective experiment outsourcing. As with any marketplace, awareness and participation by key players is essential. Our team is committed to responsiveness and making the platform as useful for researchers and providers as possible.

P04 - Microscope Maintenance

Brady Eason, Aleks Spurmanis and Claire Brown
Life Sciences Complex Imaging Facility, McGill University.

As light microscopes have evolved to become more powerful research tools, so has their complexity and demand for proper maintenance. The goal is to keep the microscope performing optimally and consistently at all times. To do this, the components should be inspected and cleaned on a monthly basis. For quality control, there are a number of standardized performance tests that can be carried out. For example, for quantitative intensity comparisons laser excitation powers must be accurate on three time scales, 1) seconds to minutes for individual data sets (e.g. 3D image stacks), 2) minutes to hours for samples within an experimental data set (e.g. control and treated samples), and 3) days to weeks for

samples from multiple experiments. Sample data, the tests for measuring laser powers, as well as potential sources of laser power fluctuations will be presented.
In addition to maintaining consistent laser powers, it is important to test the microscopes alignment. For multi-wavelength imaging using different detectors, co-registration of the signals detected on each detector is necessary for accurately measuring co-incidence (co-localization) between multi-probes within the sample. Lastly, a field uniformity test is necessary to ensure that the laser and the pinhole are properly aligned. If they are then intensity across the field of view should be relatively constant. Deviations in the intensity measurements within an image should be representative of the sample, not systematic error. Performing these tests will ensure that your confocal microscope will generate quantitative high quality fluorescence images.

P05 - High dimensional flow cytometry for comprehensive immune monitoring in clinical trials.

Dominic Gagnon, Yoav Peretz, Marylène Fortin, Claire Landry,
and David Favre
ImmuneCarta Services, 2901 Rachel Est, Suite 22, Montréal, QC, Canada, H1W 4A4

The immune monitoring of phase I to phase III clinical trials aims to design, perform and interpret immunological assays that enable institutions and companies to move vaccines, immunotherapeutics and drug candidates through the regulatory process (FDA, EMEA etc.). Mutliparametric flow cytometry allows the assessment of phenotypic and functional markers, the characterization of cell subset lineages, activation states and signalling molecules as well as the quantitative analysis of vaccine-, pathogen- or drug-specific responses related to antibody signatures, cytokine and chemokine profiles and signalling pathways.
Here, we will describe and discuss our experience as a contract research organization providing services to the biopharmaceutical industry, in the execution of high-throughput multiparametric flow cytometry and data analysis of subjects enrolled in phase I/II clinical trials. Over the past 7 years, we have implemented assays and high throughput analytical methods using 10 to 18-parameter flow cytometry on fresh or cryopreserved human peripheral blood samples in the overall setting of study planning, assay validation, specimen handling, assay execution, monitoring, reporting, and quality review performed as per applicable GLP regulations and GCLP guidelines governed by quality systems and standard operating procedures.
Overall, immune assays for diagnostic, research or biomarker discovery may impact on all aspects and stages of immune system testing, vaccine and immunotherapeutic design and development as well as drug screening. They are enablers, permitting GO/NO-GO decision-making, thus saving both time and money, enhancing safety and providing surrogate markers of clinical efficacy.

P06 - Confocal Microscopy Optics: Measuring and Interpreting Point Spread Functions

Richard W. Cole1, Tushrae Jinadasa2, and Claire M. Brown2,3*

1 Wadsworth Center, New York State Department of Health, Albany, N.Y., 12201 rcole@wadsworth.org
2 McGill University, Department of Physiology, Montreal, Quebec, Tushrae.Jinadasa@mcgill.ca
3 McGill University, Life Sciences Complex Imaging Facility, Montreal, Quebec, claire.brown@mcgill.ca

The collection of diffraction limited fluorescence images from LASER scanning microscopes now spans the life sciences and much of the physical sciences. However, the results from this powerful tool are only accurate if the optical components of the microscope can be validated. Some of these components can be verified through collecting and analyzing point spread functions (PSFs) from sub-resolution point sources. Therefore, in this protocol we describe how to prepare fluorescent microsphere samples, set up a confocal microscope to properly collect the images and perform measurements on microsphere samples.

These measurements require collecting a 3D confocal image volume of the fluorescent microspheres in order to accurately calculate the microscope PSF. The analysis of this PSF is used to determine the maximal resolvable spatial features and to identify any problems with the quality of the microscope's images. These include issues with the objective lens, scan components and other relay optics. Additionally, possible causes and remedies are provided for PSF data that do not conform to expected results. The preparation of microsphere samples requires two to three hours and an overnight drying period. The microscope setup takes about one hour, while collecting and analyzing the PSF images takes two to three hours.

P07 - Stress-induced localization of HSP70 proteins to centrosomes (Microtubule Organizing Centers-MTOCs) in cultured human neuronal cells

Sam Khalouei, Ari M. Chow, Ian R. Brown
Centre for the Neurobiology of Stress, Department of Biological Sciences,
University of Toronto Scarborough, Toronto, Ontario M1C 1A4

The centrosome is a cellular organelle that serves as the main Microtubule Organizing Center (MTOC) in animal cells. In neurons, centrosomes play key roles in the organization of the cytoskeleton and cellular polarity that is required for neurite outgrowth. Given that it is essential to preserve centrosome structure and function during periods of stress, we examined whether proteins of the HSP70 gene family localize to centrosomes in differentiated human neurons grown in tissue culture. Hsps are known to be induced by stressful stimuli and play important roles in cellular repair and protective mechanisms. Stable lines of human SH-SY5Y neuronal cells were established that expressed YFP-tagged protein products of two human inducible HSP70 genes (HSPA1A and HSPA6) and a constitutively expressed member (HSPA8). Following heat shock at 43 degree Celsius for 20 minutes, fluorescence microscopy demonstrated that the two YFP-tagged inducible HSP70 proteins rapidly moved into centrosomes and co-localized with the MTOC marker protein, gamma tubulin. The two inducible HSP70 proteins were detected in centrosomes immediately after the heat shock period and strong co-localization signals persisted up to 2 hours post-heat shock and were not detected by 6 hours. In contrast, the protein derived from the constitutively expressed HSPA8 gene did not localize to centrosomes at any time point. The transient association of stress-inducible members of the HSP70 gene family with centrosomes in differentiated human neurons suggests that these proteins may be involved in repair and protection mechanisms that preserve microtubule organizing centers (MTOCs) in neurons during periods of cellular perturbation.

P08 - Cell Imaging and Analysis Network (CIAN)

Bunnel, S., Küster-Schöck, E., Lacoste, J. and Lesage, G.
CIAN, Dept. Biology McGill University, Montreal, QC

The Cell Imaging and Analysis Network (CIAN) provides services and tools to researchers from within or outside the McGill community. CIAN is composed of six scientific platforms: Cell Imaging (confocal and fluorescence microscopy), Proteomics (2D, DiGE, related protein analyses), Automation/High throughput screening (pinning robot and liquid handler), Protein expression and antibody production, Genomics (real-time PCR), and Data storage/analysis (cluster, server and workstations). Users submit project proposals and can obtain training in any aspect of the facility. Since its opening in 2006, CIAN has served 305 users from 103 labs distributed over 40 affiliations. CIAN is designed to facilitate training, enhance interactions, share resources and expertise.

P09 - Utilizing Fucci Cells and FACS to Identify Herpesvirus Proteins that Promote G1/S Arrest

Patrick Paladino and Lori Frappier
Department of Molecular Genetics, University of Toronto

During lytic infection, herpesviruses arrest cells at the G1/S transition to promote viral but not cellular DNA synthesis. This activity appears to be a function of specific herpesvirus proteins, although very few have been examined for such effects. In this study, we screened a library of over 200 FLAG-tagged proteins encoded by Epstein-Barr virus (EBV), herpes simplex type 1 (HSV-1) and human cytomegalovirus (HCMV) for cell cycle perturbations at the G1/S interphase. Our initial screen used HeLa-Fucci cells, which are engineered to change colour at different stages of the cell cycle due to the transient expression of two fluorescently tagged proteins; resulting in red fluorescence in G1, green fluorescence in S, G2 and M, and yellow fluorescence at the G1/S interface. These cells were transfected with plasmids and FACS-sorted based on FLAG expression, then subsequently sorted for cell populations expressing red, green and yellow fluorescence. We identified 29 of 57 EBV, 11 of 47 HSV-1, and 18 of 105 HCMV proteins showing at least a 1.8-fold increase in G1/S cells. Most of these proteins are capsid, tegument components or immediate-early gene products. We also found 9 and 3 proteins that caused at least a 1.5-fold accumulation in G1 or in S/G2/M, respectively. We also identified previously known cell cycle regulators such as BRLF1 (EBV), ICP27 (HSV-1), and UL69 (HCMV) as well as several proteins that have not been functionally characterized. Details of our screen as well as experiments to validate and characterize these cell cycle effects will be presented.

P10 - Monitoring the emergence of newly formed plasma cells following long-term culture of human B lymphocytes

Annie Roy (1), Rayelle Itoua Maïga (1,2), Carl Simard (1), Sonia Néron (1,2)
(1)Hema-Quebec, R&D
(2) Departement de biochimie, Universite Laval

In vitro, CD40-CD154 interaction allows expanding human B lymphocytes and promoting their differentiation into antibody secreting cells. This culture system supports the emergence of cells expressing CD138, which is the hallmark for fully differentiated plasma cells. In vitro, long-term cultures of switched memory B lymphocytes result in emergence of about 50% CD138+ cells; whereas functional analysis indicated that the majority of these cultured cells were fully differentiated plasma cells. The question was thus whether the anti-CD138 used (B-A38) was able to detect all newly formed plasma cells. We have thus compared by flow cytometry the efficiency of five anti-CD138 mAbs: 1D4, B-A38, B-B4, DL-101 and MI15 to characterize RPMI-8226 (CD138high) and SKW6.4 (CD138dim) cell lines as well as in vitro generated plasma cells. All anti-CD138 antibodies tested were able to stain RPMI-8226 and SKW6.4 cell lines as well as in vitro generated plasma cells. B-A38, B-B4 and MI-15 appear to bind simultaneously, on RMPI-8226 cells suggesting that their respective epitope were distinct. However, the median fluorescence intensity for CD138 on RMPI-8226 and SKW6.4 cell lines as well as cultured plasma cells greatly varies from one mAb to another. Among all, B-A38 gives the brightest fluorescence on cell lines, while DL-101 and B-A38 are the best to detect CD138 on in vitro generated plasma cells. Overall, these results suggest that CD138 epitope may differ on cell lines and normal human cells and highlight that a mix of B-A38 and DL-101 mAbs could be better to detect plasma cells in vitro.

P11 - Analysis of cell division defects during the vascular response to injury

Rosalind Silverman-Gavrila1, Lorelei Silverman-Gavrila2, and Michelle Bendeck1*
1Department of Laboratory Medicine and Pathobiology, University of Toronto

2Department of Physiology, University of Toronto

Smooth muscle cell (SMC) proliferation in atherogenesis leads to neointimal thickening. We analyzed the fidelity of cell division and key regulators of the process during the vascular response to injury. Following balloon injury of the rat carotid artery we used en face confocal microscopy to image the full thickness of the vessel wall and measure SMC division and the frequency of defects in karyokinesis and cytokinesis. SMCs newly migrated to the neointima, and SMCs in the media immediately subjacent to these neointimal cells, had higher proliferation rates and division defects compared to medial SMCs distant from the site of neointimal formation, and in uninjured carotid arteries. The frequency of division defects was highest in the neointimal cells. Cell culture experiments identified PKC and RHAMM as critical regulators of the fidelity of SMC division, and the centrosomal targeting sequence of RHAMM was required for RHAMM localization and spindle pole organization.Neointimal SMCs from injured rat carotid arteries and underlying medial SMCs exhibit anelevated rate of mitosis and numerous spindle organization and cell division defects. PKC and RHAMM regulate centrosome-based division events in neointimal SMCs.

P12 - Fluorescent cell bar coding: A simple method to help in multiple myeloma diagnostics and prognostics

Carl Simard et Sonia Néron

Héma-Québec, 1070 des Sciences-de-la-Vie, Québec, Canada, G1V 5C3

Multiple myeloma (MM) is a complex disease defined as malignant growth of plasma cells in the bone marrow, leading to diverse clinical symptoms. Unfortunately the disease remains incurable and a survival of 3 to 7 years is expected for treated patients. Currently, MM diagnosis is mainly based on examination of bone marrow biopsy along with the presence of monoclonal immunoglobulin and diseases-related organ dysfunction. The clinical criteria for diagnosis and prognosis are however relatively subjective. To help better characterize MM, we propose here a flow cytometry approach, using the frozen bone marrow aspirates from patients to probe the physiological responses of primary MM plasma cells to cytokines. We have been using the fluorescent cell barcoding (FCB) methods as reported by Krutzik and Nolan (Nature Methods, 2006) and combined phospho-specific flow cytometry to extracellular phenotyping using antibody specific to CD38 and CD138. Flow cytometry analyses were done on seven human cell lines, mononuclear cells from healthy participant as well as one MM bone marrow aspirate. Our observations demonstrated that it is possible to analyze the specific response of plasma cells to a cytokine-panel without further purification or culture of the cells. This information may then be used to define an activation profile of the plasma cells, which could help clinicians to provide a more precise diagnosis or prognostic. Such method could also be of interest for researchers in the MM field, to study the cell physiology of primary MM cells even with a relatively limited amount of material, and without culturing.

P13 - Gain Independent Spillover Values for Multicolor Cytometer Compensation

Ming Yan,1 Zhenxiang Gong,1 Neha Pathak,1 Alex Zhong,1 and Alan Stall 2
1BD Biosciences, San Jose, CA, USA; 2BD Biosciences, San Diego, CA, USA

In this presentation, we define a new gain independent normalized spillover value (NSOV) based on single-color staining and a stable fluorescence calibrator. The NSOV is the spillover percentage in fluorescence units and can be used for establishing SOVs at different gain settings when used with a broadband fluorescence calibrator. Hard dyed multicolor beads, such as BD™ Cytometer Setup and Tracking (CS&T) beads, are routinely used to characterize and set up cytometers. After proper calibration to single stained CD4 antibodies bound per cell, a traceable fluorescence unit was assigned to a multicolor bead for a given optical filter, ie, the ABD unit assigned by BD. With this fluorescence calibrator, we can

convert median fluorescence intensity (MFI) to fluorescence ABD units for any given gain setting. In this case, we can transform the MFI-based SOV to NSOV in ABD units by using the measured MFI to ABD ratio of the hard dyed bead for the gain setting where SOV was measured. The NSOV, expressed in ABD units, is therefore gain independent as long as fluorescence was measured within the linear range. The NSOV provides a true measure of the spectral overlap in terms of a defined fluorescence unit and thus can be used to calculate the SOV for multicolor compensation at any cytometer gain setting as long as the MFI to ABD ratio of the multicolor fluorescence bead is known. This novel approach significantly reduces the time and reagents needed for controlling spillover during routine cytometer setup. A multicolor single particle with known ABD values can be used to determine the MFI measurements (gain settings) on a daily basis using a predetermined MFI to ABD ratio. Then, SOV values can be calculated using predetermined instrument specific NSOVs without the need to run compensation controls. The fluorescence sensitivity of a cytometer determines its capability to resolve dim populations from unstained cells in an immunofluorescence assay. One simple measure of sensitivity is the difference in fluorescence intensity of a specific positive cell and unstained population divided by twice the standard deviation (SD) of the unstained population. This normalized signal- to-background approach is best used to either compare sensitivity for a specific reagent among cytometers or to determine which dye color might be optimal for any specific marker when planning multicolor reagent panels. However, it does not directly address the underlying contributions that ultimately determine the fluorescence sensitivity of the cytometer itself, ie, optics, fluidics, and electronics. In a multicolor assay, the ability to accurately detect spectral spillover from other fluorescent dyes is also an important factor.

SYMPOSIUM ATTENDEES

Ailles Laurie
lailles@uhnresearch.ca
Ontario Cancer Institute

Anderson Michele
manderso@sri.utoronto.ca
University of Toronto

Angarita Fernando
fernando.angarita@utoronto.ca
Division of Experimental Therapeutics
Toronto General Research Institute - UHN
Toronto

Aswani Kavita
kavita.aswani@ldgi.com
Lumen Dynamics
Mississauga

Atagu Willis
willis.atagu@ldgi.com
Lumen Dynamics
Mississauga

Aubert Isabelle
isabelle.aubert@sri.utoronto.ca
Sunnybrook Research Institute

Bagwell Bruce
cbb@vsh.com
Verity Software House
Topsham

Bang Kyong Won Annie
abang@mtsinai.on.ca
Flow Cytometry Facilities
Samuel Lunenfeld Research Institute
Toronto

Barker Stephen
barkers@smh.ca
St. Michael's Hospital

Bates Ian
ibates@zeiss.com
Carl Zeiss Canada
Toronto

Bean Darla
dbean@inter-medico.com
Inter Medico
Markham

Bergeron Marc
marberge@sympatico.ca
Hematology
Enfant Jesus Hospital
Quebec

Bourbonniere Martin
Martin.Bourbonniere@ebioscience.com
eBioscience
San Diego

Bourdeau Annie
annie.bourdeau@sri.utoronto.ca
Sunnybrook Research Institute

Bradley Grace
grace.bradley@dentistry.utoronto.ca
Faculty of Dentistry
University of Toronto
Toronto

Brickman Ryan
rbrinkman@bccrc.ca
British Columbia Cancer Research Centre

Brown Claire
claire.brown@mcgill.ca
Physiology
McGill University
Montreal

Brown Ian
ibrown@utsc.utoronto.ca
Biological Sciences
University of Toronto
Toronto

Butler Jeff
jeff@quorumtechnologies.com
Quorum Technologies
Guelph

Cathcart Judy
judy@aomf.ca
Advanced Optical Microscopy Facility (AOMF)
University Health Network
Toronto

Chadwick Kristin
kchadwick@robarts.ca
Robarts Research Institute
UWO
London

Chang Nan-Hua
nchang@uhnres.utoronto.ca
Genetic and Development
Toronto Western Research Institute
Toronto

Chau Edwin
Edwin.Chau@ebioscience.com
eBioscience
San Diego

Chikh Ghania
ghania.chikh@pfizer.com
R&D
Pfizer Inc.
Ottawa

Chivers Sara
schivers@nikon.ca
Nikon Canada Inc. Instruments Division
Mississauga

Chow Ari
ari.chow@utoronto.ca
Biological Sciences
University of Toronto Scarborough
Toronto

Chow Sue
sue.chow@uhn.ca
Research
Princess Margaret Hospital
Toronto

Chue Bruno
bruno.chue@utsc.utoronto.ca
Biological Sciences
University of Toronto Scarborough Campus
Scarborough

Collins Tony
tjc@mcmaster.ca
Biochemistry
McMaster university
Hamilton

Connolly Jay
jsconnolly@post.harvard.edu
Community Relations
Science Exchange
Palo Alto

Conway Kevin
kconway@nikon.ca
Nikon Canada Inc. Instruments Division
Mississauga

DaCosta Ralph
rdacosta@uhnres.utoronto.ca
Ontario Cancer Institute

Daigle Olivier
odaigle@nuvucameras.com
Nüvü Caméras
Montréal

Deabreu-Breen Roxanne
roxanne.deabreu@olympus.com
Olympus Canada Inc.
Richmond Hill

Delafosse Laurence
laurence.delafosse@gmail.com
Biochimie / Bioprocédés
UdeM / CNRC-IRB
Montréal

Dufour Suzie
suzie.dufour@gmail.com
Centre de Recherche Université Laval Robert-Giffard
Université Laval

Dulude Gaël
gaeldulude@yahoo.ca
IRIC
Université de Montréal
Montreal

Eason Brady
brady.eason@mail.mcgill.ca
Physiology
McGill University
Montreal

Ehman David
david_ehman@bd.com
BD - Canada
Mississauga

Fayek Reda
rfayek@huron-technologies.com
Huron Technologies
Waterloo

Fitzgerald Fiona
fiona.fitzgerald@ge.com
GE Healthcare
Baie d'Urfé

Fradin Cecile
fradin@physics.mcmaster.ca
Physics
McMaster University
Hamilton

Gagnon Dominic
dgagnon@immunecarta.com
Cytométrie
ImmuneCarta Services
Montréal

Gimmig Sylvain
sylvain_gimmig@bd.com
BD - Canada
Mississauga

Glowinski Cory
cory@bitplane.com
Bitplane
South Windsor

Gosselin Annie
annie.gosselin.chum@gmail.com
Recherche
CR-CHUM Hôpital St-Luc
Montréal

Grinstein Sergio
sga@sickkids.ca
Hospital for Sick Children
University of Toronto

Hagen Andreas
andreas.hagen@ilabsolutions.com
iLab Solutions
Boston

Hankel Isaiah
isaiah@treestar.com
Tree Star, Inc.
Ashland

Harrison Rene
harrison@utsc.utoronto.ca
Dept. of Biological Sciences
University of Toronto Scarborough
Toronto

Hayworth Jacqueline
jhayworth@biolegend.com
Sales
BioLegend
San Diego

Hedley David
David.Hedley@uhn.on.ca
Princess Margaret Hospital
Ontario Cancer Institute

Henley Nathalie
nathaliehenley@gmail.com
Centre de Recherche
Hôpital Maisonneuve-Rosemont
Montréal

Hong Henry
henry.hong@utoronto.ca
Cell & Systems Biology
University of Toronto
Toronto

Iorns Elizabeth
elizabeth@scienceexchange.com
Science Exchange
Palo Alto

Jinadasa Tusahre
tushare.jinadasa@gmail.com
Physiology
McGill University
Montreal

Johnson Eddie
eddie.johnson@cedarlanelabs.com
Cedarlane
Burlington

Jonkman James
james@aomf.ca
Advanced Optical Microscopy Facility (AOMF)
University Health Network
Toronto

Kaul Ruppert
rupert.kaul@utoronto.ca
Departments of Medicine and Immunology
University of Toronto

Khalouei Sam
sam.khalouei@utoronto.ca
Biological Sciences
University of Toronto Scarborough
Toronto

Kianizad Korosh
koroshk@sri.utoronto.ca
Biological Sciences
Sunnybrook Research Institute
Toronto

Knowles Gisele
gknowles@sri.utoronto.ca
Flow Cytometry and Scanning Microscopy
Sunnybrook Health Sciences Research Institute
TORONTO

Kooistra Joel
Joel.Kooistra@cedarlanelabs.com
Cedarlane
Burlington

Küster-Schöck Elke
elke.kuster-schock@mcgill.ca
CIAN - Dept. of Biology
McGill University

Labonté Isabelle
isabelle.labonte@crulrg.ulaval.ca
Neurophotonic
Centre de recherche Université Laval Robert-Giffard
Québec

Lacombe Marie-Helene
flow.muhc.ri@gmail.com
Immunophenotyping Platform
MUHC Research Institute
Montreal

Lacoste Judith
judith.lacoste@mcgill.ca
CIAN - Dept. of Biology
McGill University and MIA Cellavie

Lapointe Gabriel
gabriel.lapointe2@gmail.com
Biology
Concordia University
Montreal

Lareau Étienne
elareau@nuvucameras.com
Biomedical
Nüvü Caméras
Montréal

Lejeune Laurence
cytometrie.chum@gmail.com
Service de Cytométrie
CRCHUM
Montréal

Lesage Guillaume
guillaume.lesage@mcgill.ca
Biology
McGill University
Montreal

Levadoux-Martin Marilyne
levadou@mcmaster.ca
Stem Cell and Cancer Research Institute
SCCRI-McMaster University
Hamilton

Liang Hong
liangh@mcmaster.ca
Flow cytometry Facility
McMaster University
Hamilton

Maeda Azusa
amaeda@uhnres.utoronto.ca
Medical Biophysics
University of Toronto
Toronto

Mandalfino Christina
Christina.Mandalfino@GE.com
GE Healthcare
Baie d'Urfé

Markle Janet
janet.markle@utoronto.ca
Genetics and Genome Biology
Hospital for Sick Children
Toronto

McBey Betty-Anne
bmcbey@uoguelph.ca
Pathobiology
University of Guelph
Guelph

McDonald Ken
ken.mcdonald@mcgill.ca
Physiology
McGill University
Montreal

McFarlane Dave
dmcfarlane@beckman.com
Beckman Coulter Canada
Mississauga

McIntosh Courtney
cmcintos@gmail.com
Cytometry
Sunnybrook
Toronto

Menager Pauline
pmenager@cytoo.com
CYTOO
Grenoble

Merchent Stepehen
stephen.marchant@stemcell.com
STEMCELL Technologies, Inc.
Vancouver

Millar Andrew
andrew.millar@olympus.com
Scientific Equipment Group
Olympus Canada Inc.
Richmond Hill

Moodley Serisha
serisha.moodley@utoronto.ca
Institute of Medical Science
University of Toronto
Mississauga

Morley Karen
kmorley@robarts.ca
Robarts Research Institute
UWO
London

Novo David
david.novo@denovosoftware.com
De Novo Software
Los Angeles

Nowensky Ada
ada.nowensky@ldgi.com
Lumen Dynamics
Mississauga

Oleynik Paul
poleynik@ohri.ca
StemCore
OHRI
Ottawa

Oreopoulos John
oreopoulous@spectral.ca
Spectral Appled Research
Richmond Hill

Paladino Patrick
patrick.paladino@utoronto.ca
Molecular Genetics
University of Toronto
Toronto

Parsons Michael
parsons@lunenfeld.ca
SLRI
Mount Sinai Hospital
Toronto

Patel Shobhna
shobhna.patel@pfizer.com
Preclinical
Pfizer Vaccines Research
Ottawa

Qiu George
george.qiu@ldgi.com
Lumen Dynamics
Mississauga

Ramsubir Shobha
shobha.ramsubir@stemcell.com
STEMCELL Technologies, Inc.
Vancouver

Roquemore Elizabeth
elizabeth.roquemore@ge.com
GE Healthcare

Roy Annie
annie.roy@hema-quebec.qc.ca
R&D
Hema-Quebec
Quebec

Rutter Martin
martin.rutter@miltenyibiotec.com
Miltenyi Biotec
Auburn

Sabra Sally
sabra@lunenfeld.ca
Samuel Lunenfeld Research Institute
Mount Sinai Hospital
TORONTO

Sakellaropoulos George
George.sakellaropoulos@olympus.com
Andor Technology
Olympus Canada Inc

Schoonderwoert Vincent
vincent@svi.nl
Scientific Volume Imaging

Shackleton Michele
mshackleton@inter-medico.com
Inter Medico
Markham

Shao Mei
mei.shao@ldgi.com
Lumen Dynamics
Mississauga

Shapiro Howard
hms@shapirolab.com
University of Toronto

Shen-Tu Grace
gshentu@gmail.com
Thoracic Research
Toronto General Hospital
Toronto

Silverman Lorelei
lorelei.silverman.gavrila@utoronto.ca
Physiology
Uof Toronto
Toronto

Silverman-Gavrila Rosalind
rosilverman@gmail.com
Laboratory Medicine and Pathobiology
University of Toronto
Toronto

Simard Carl
Carl.Simard@hema-quebec.qc.ca
R&D
Héma-Québec
Québec

Singh Sheila
ssingh@mcmaster.ca
Department of Surgery
McMaster Children's Hospital

Sirk Daniel
dpsirk@beckman.com
Beckman Coulter Canada
Mississauga

Spring Christopher
springc@smh.ca
Research Core Facilities
St. Michael's Hospital
Toronto

Spurmanis Aleks
aleksandrs.spurmanis@mcgill.ca
Physiology
McGill University
Montreal

Sun Hesong
ey119@msn.com
Cell and Systems Biology
University of Toronto at Scarborough
Scarborough

Temkin Robert
btemkin@utsc.utoronto.ca
Biological Sciences
University of Toronto Scarborough
Toronto

Thanabalasuriar Ajitha
ajitha.thanabalasuriar@mail.mcgill.ca
Microbiology and Immunology
McGill University
Montreal

Thiel Jeremy
jthiel@huron-technologies.com
Huron Technologies
Waterloo

Travis Erin
erin_travis@bd.com
BD - Canada
Mississauga

Trogadis Judy
trogadisj@smh.ca
Research Facilities
St. Michael's Hospital
Torotno

Tse Kenneth Chor Kin
kcktse@gmail.com
Medical Biophysics
University of Toronto
Toronto

Varallo Vince
vince.varallo@olympus.com
Olympus Canada

Weant Ashley
ashley.weant@miltenyibiotec.com
Miltenyi Biotec
Auburn

White Dionne
dionne.white@utoronto.ca
Immunology
University of Toronto
Toronto

Young Kimberly
kimberly.young@mail.mcgill.ca
Physiology
McGill University
Montreal

Yuan Julie
julie.yuan@sickkids.ca
Developmental & Stem Cell Biology
Hospital for Sick Children
Toronto

Zandi Sasan
szandi@uhnresearch.ca
UHN - Stem Cell Network

Zhang Christine
christine.zhang.a@gmail.com
Department of Immunology
University of Toronto
Richmond Hill

Zhang Jianhong
jhz.zhang@utoronto.ca
Samuel Lunenfeld Research Institute
Mount Sinai Hospital
TORONTO


BD
BD Biosciences


SPECTRAL
APPLIED RESEARCH


BD
BD Biosciences


SPECTRAL
APPLIED RESEARCH


BD
BD Biosciences


SPECTRAL
APPLIED RESEARCH


BD
BD Biosciences


SPECTRAL
APPLIED RESEARCH


BD
BD Biosciences


SPECTRAL
APPLIED RESEARCH

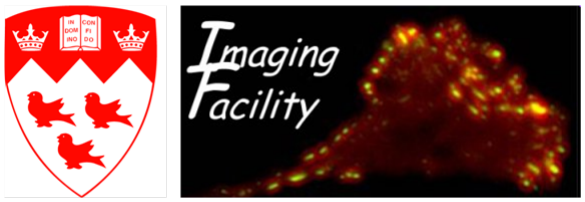

BD
BD Biosciences


SPECTRAL
APPLIED RESEARCH


BD
BD Biosciences

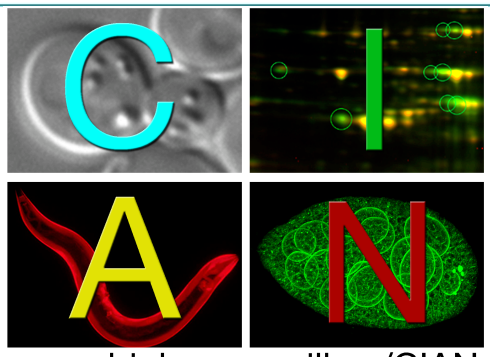

SPECTRAL
APPLIED RESEARCH

PARTICIPATING CORE FACILITIES



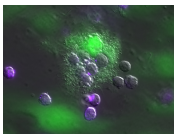
McGill University
Life Sciences Complex Imaging Facility

CHUM
CENTRE DE RECHERCHE
Service de Cytométrie



www.biology.mcgill.ca/CIAN

Sunnybrook
RESEARCH INSTITUTE



CENTRE FOR CYTOMETRY AND MICROSCOPY

CMAC
Center for Microscopy at Concordia
Centre de Microscopie à Concordia

L'Institut de recherche
du Centre universitaire de santé McGill
The Research Institute
of the McGill University Health Centre

Is your HCS system giving you the whole picture?

Experience the art and science of high content screening with ImageXpress Micro XL Widefield HCS System

See *your* bigger picture with ImageXpress® Micro XL System, capturing research-quality images that span up to one 384 well with a single image. Combined with the broadest range of objectives, the ImageXpress Micro XL System delivers the right resolution to map large objects and image twice the sample area, ensuring all your scientific breakthroughs are picture-perfect.

Looking for a better view? Find out more at highthroughputimaging.com/research/

Scan the QR code with your smartphone to widen your HCS view.

Molecular Devices

www.MolecularDevices.com