ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, January - March 2013

What's New?

- Since it is **mandatory** for users who are new to our facility to attend the **Introduction to Flow & Image Cytometry course** we are planning to offer this course on a monthly basis. The most recent edition of the course was January 14, 2013. The **other courses** will be scheduled **at least twice a year**. If the need arises to set up classes on a more frequent basis, we will make accommodations for that course. Please see the schedule for the upcoming courses below.
- A magazine rack with **"How do I …..?"** instructions for various procedures relevant to the use of the cytometers in our facility is placed at the wall on the left when you enter the flow cytometry user room. These instruction sheets address many of the most frequently asked questions; in particular with regards to how to access and manage the data and the software on the Institute's intranet.
- **Joe Tario** successfully defended his PhD thesis on phenotypic and functional studies of dendritic cell vaccines on December 11, 2012. So it's **Dr Joe** from here on. To celebrate Joe's achievement our Application Highlight this quarter features one of Joe's passions: cell tracking dyes!
- If you want to see **Dr Joe** (and **Paul**) in action, see this link to their recent contribution to the Journal of Visual Experimentation: http://www.jove.com/video/4287/optimized-staining-proliferation-modeling-methods-for-cell-division. Skip to the Conclusions…. (look at the camera Joe!)
- A **Beckman Coulter Gallios Flow Cytometer** is now available for research use. The Gallios has 3 lasers for excitation (violet, blue, red) andcan detect 10 colors. For scheduling and training please contact Kitty de Jong.
- **Ed Podniesinski** is a grandpa! Ed's daughter delivered a healthy baby girl on January 28. Ed is in the process of putting together her first flow cytometer with pink lasers from parts he found listed on Ebay!
- **Hans Minderman** will be chairing a workshop on exploring the interface between flow and imaging cytometry at the upcoming **CYTO2013**. The workshop is organized with collaborators from the London Research Institute, Cancer Research UK (Dr Andy Filby) and the National Institute of Standards and Technology (Drs Anne Plant and Michael Halter). **Paul Wallace** will be chairing a session at **CYTO2013** on Monitoring Cell Division.

Recent Publications / Grant Funding

- Optimized Staining and Proliferation Modeling Methods for Cell Division Monitoring using Cell Tracking Dyes. **Tario JD Jr, Humphrey K,** Bantly AD, Muirhead KA, Moore JS, **Wallace PK**. J Vis Exp. 2012 Dec 13;(70). doi:pii: 4287. 10.3791/4287.
- CD19 expression in acute leukemia is not restricted to the cytogenetically aberrant populations. Francis J, Dharmadhikari AV, Sait SN, **Deeb G, Wallace PK**, Thompson JE, Wang ES, Wetzler M. Leuk Lymphoma. 2013 Jan 3. [Epub ahead of print]
- The prolyl isomerase Pin1 targets stem-loop binding protein (SLBP) to dissociate the SLBP-histone mRNA complex linking histone mRNA decay with SLBP ubiquitination. Krishnan N, Lam TT, Fritz A, Rempinski D, **O'Loughlin K, Minderman H**, Berezney R, Marzluff WF, Thapar R. Mol Cell Biol. 2012 Nov;32(21):4306-22. doi: 10.1128/MCB.00382-12. Epub 2012 Aug 20.
- Neem oil limonoids induces p53-independent apoptosis and autophagy. Srivastava P, Yadav N, Lella R, Schneider A, Jones A, Marlowe T, Lovett G, **O'Loughlin K, Minderman H**, Gogada R, Chandra D. Carcinogenesis. 2012 Nov;33(11):2199-207. doi: 10.1093/carcin/bgs269. Epub 2012 Aug 21

Courses / Presentations / Meetings

- **ImageStream user group meeting**. Cincinnati Children's Hospital Medical Center , April 18, 2013. Registration (free) and program information will be available soon on www.amnis.com. This workshop has been organized annually between RPCI and the universities of Rochester and Pittsburgh. This will be the 5th annual edition of the workshop with Cincinnati now replacing Pittsburgh in the rotation. The workshop consists of data presentations and opportunities to work with Amnis' application specialist on specific data analysis questions.
- **CYTO 2013** May 19-22, San Diego, CA
- Title**: CompensationCourse**, location: Grossberg Library, Date & Time: January 31, 2013 1:00 pm – 4:00 pm
- Title: **Introductionto ImageStream**, location:RSC-400 computer lab, Date & Time: Wednesday, February 6, 2013 from 1:30 pm – 3:30 pm
- Title: **Introduction to Immunophenotyping**, location: Grossberg Library, Date & Time: Thursday, February 14, 2013 from 1:00 pm – 4:00 pm
- Title**: Introduction to Flow & Image Cytometry** – scheduled monthly, location: Grossberg Library, Date & Time: Monday, February 25, 2013 from 1:00 pm – 4:00 pm
- Title: **Practical Course in DNA Methods**, location: Grossberg Library, Date & Time: March 28, 2013 from 1:00 pm – 3:00 pm
- Title: **Introduction to Confocal Microscopy**, Location: Grossberg Library, Date & Time: tbd

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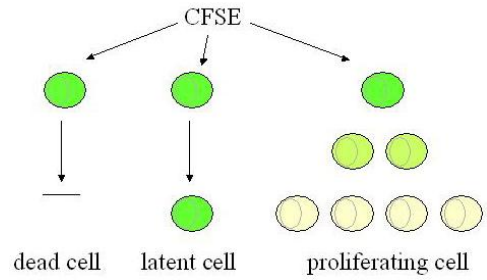
Service / Application Highlights

CELL TRACKING DYES

Background:
The use of so-called cell tracking dyes in flow cytometry allows the study of cell proliferation and/or the exchange of cell membrane material between cells. Cell proliferation is monitored based on the generational dilution of the signal intensity as a consequence of cellular material being divided between daughter cells. Exchange of cellular material between unrelated cells (trogocytosis) can be studied by detection of fluorescent-labeled membranes from a donor cell into a previously unlabeled acceptor cell. There are two basic classes of tracking dyes that differ by their mechanism of labeling. The membrane dyes (e.g. PKH26) are highly lipohilic dyes that partition into cell membranes while the protein dyes (e.g. CFSE) are amino-reactive dyes that form covalent bonds with cell proteins. For more information and tips and tricks please see the following online publication:
http://www.jove.com/video/4287/optimized-staining-proliferation-modeling-methods-for-cell-division

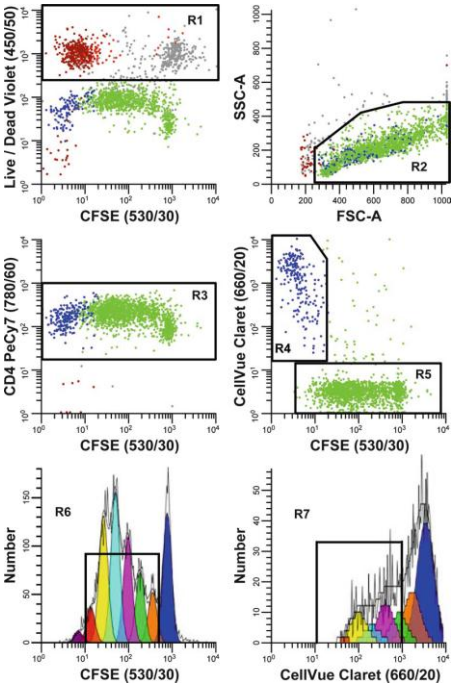


Commonly used cell tracking dyes:

Name:	Class:	Excitation Max	Emission Max
CellTrace Violet	Protein Dye	395 nm	455 nm
CFSE	Protein Dye	494 nm	521 nm
eFluor-670	Protein Dye	647 nm	670 nm
CellVue Lavender	Membrane Dye	422 nm	461 nm
PKH67	Membrane Dye	490 nm	502 nm
PKH26	Membrane Dye	551 nm	567 nm
CellVue Claret	Membrane Dye	650 nm	671 nm

Applications:
- stem and progenitor cell quiescence, proliferation and/or differentiation
- antigen-driven membrane transfer and/or precursor cell proliferation
- Immune regulatory and effector cell function

Example: From Tario J et al, Meth Mol Biol (699), 119-164, 2011.



Simultaneous analysis of Teff and Treg proliferation during an in vitro suppression assay. Teff cells were stained with CFSE and co-incubated for 4d with Treg stained with CellVue Claret in the presence of anti-CD3, anti-CD28, and irradiated accessory cells (Treg:Teff ratio of 0.25:1). LIVE/DEAD Fixable Violet reagent was used to exclude dead cells (R1, upper left plot; accessory cells = red-brown, nonviable Teff = grayand nonviable Treg = red) from all other data plots. CellVue Claret staining was used to distinguish viable Treg (R4, center right plot) from viable but highly proliferated Teff (R5, center right plot). A single parameter CFSE (530/30) proliferation profile for Teff (lower left plot) was generated by gating on cells that were CFSE + (R5), CD4+ (R3), viable (not R1), andhad lymphocyte scatter properties (R2). A single parameter CellVue Claret (660/20) proliferation profile for Treg was generated by gating on cells that were CellVue Claret + (R4), CD4+ (R3), viable (not R1), and had lymphocyte scatter properties (R2). Proliferative fractions, are shown as blue = parental generation, orange = first daughter generation, etc.

For more information please call:

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