ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, January-March 2012

What's new?

- On behalf of everyone at the Flow and Image Cytometry Facility: **Happy New Year !**
- **Oh no, Earl Timm retired!** For many of our users, Earl has been the face of flow cytometry and the go-to person for instrument and experimental set-up questions but since Thanksgiving Day 2011 Earl is boldly going where no man has gone before to live long and prosper! Taking over Earl's responsibilities are the following lab members: **for questions / trouble shooting user room equipment**: Kieran O' Loughlin, Rose Furlage, Dalin Pan, Orla Maguire; **for sorting**: Craig Jones and Kitty DeJong (see below)
- **AnnMarie Eckel** has joined us as our **new secretary**. AnnMarie comes to us from the Pathology Lab and has been at RPCI for the past 6 years so she's knows the ropes which will help with a smooth transition.
- **Kitty DeJong** started 1/3/2011 as our **second sorter operator**. In addition to flow cytometry experience Kitty has extensive experience with Miltenyi's AutoMacs system.
- In collaboration with the Institute's IT department we have set up a **centrally accessible data storage** on the Institute's **new x9000 server** for all our users who have access to the Institute's intranet. Currently the allocated space can be used to **store flow cytometry data, imagestream data and confocal data**. A directory structure has been created according to all our known PI's. **Each PI has administrative privileges** to create subdirectories under their assigned directory according to any structure that works best for their lab (eg subdirectories according to lab members, research project, etc). Each PI also has administrative privileges to assign (and thus limit) accessibility of their assigned directory to lab members and collaborators. For questions how to access your server space please contact Ed Podniesinski. Our long range plan is to directly stream the data generated on the user machines to the properly identified sub directories on the X9000 which would be automatically assigned according to the log-in credentials of our users. The latter however will still need a bit of software development but is in the works. We'll keep you posted!
- Our facility participated in the **Luminex External Proficiency 1 (EP1) study** that was conducted by the External Quality Assurance Program Oversight Laboratory (**EQAPOL**), **an NIH, NIAID, DAIDS program**. There were 25 participating labs in this **international program** and each lab analyzed centrally standardized samples of 5 analytes in two different matrices (serum and supernatant). We are happy to report that our facility did very well with our data on average being at **95% of the consensus** concentrations. **Kudos to Mike and Ree for a job well-done!**

Recent Publications / Grant Funding

- McLoughlin RM, Calatroni A, Visness CM, **Wallace PK**, Cruikshank WW, Tuzova M, Ly NP, Ruiz-Perez B, Kattan M, Bloomberg GR, Lederman H, Gern JE, Gold DR. Longitudinal relationship of early life immunomodulatory T cell phenotype and function to development of allergic sensitization in an urban cohort. Clin Exp Allergy. 2011 Nov 9. [Epub ahead of print]
- Chanan-Khan AA, Chitta K, Ersing N, Paulus A, Masood A, Sher T, Swaika A, **Wallace PK**, Mashtare TL Jr, Wilding G, Lee K, Czuczman MS, Borrello I, Bangia N. Biological effects and clinical significance of lenalidomide-induced tumour flare reaction in patients with chronic lymphocytic leukaemia: in vivo evidence of immune activation and antitumour response. Br J Haematol. 2011 Nov;155(4):457-67.
- Wang ES, Zeidan A, Tan W, Wilding GE, Ford LA, **Wallace PK**, Hahn TE, Battiwalla M, McCarthy PL, Wetzler M. Cytoreduction with gemtuzumab ozogamicin and cytarabine prior to allogeneic stem cell transplant for relapsed/refractory acute myeloid leukemia. Leuk Lymphoma. 2011 Sep 6. [Epub ahead of print]
- Mace TA, Zhong L, Kilpatrick C, Zynda E, Lee CT, Capitano M, **Minderman H**, Repasky EA. Differentiation of CD8+ T cells into effector cells is enhanced by physiological range hyperthermia. J Leukoc Biol. 2011 Nov;90(5):951-62.
- **Hans Minderman,** Oleh Pankwycz, **Kieran O'Loughlin, Orla Maguire**, Lin Feng, Mark Laftavi, **Paul Wallace**. Development of a diagnostic test of response to NFkB-targeted therapy using multispectral imaging flow cytometry. NCI's principal investigators meeting for the innovative molecular analysis technologies (IMAT) program, Rockville, MD, 2011.
- KM Tornatore, **H. Minderman**, **O, Maguire, K. O'Loughlin**, G. Shan, G. Wilding, R Venuto. Nuclear localization of nuclear factor of activated T-cells as a pharmacodynamic marker for tacrolimus immunosuppression in renal transplant recipients. American Society of Transplantation- Scientific Exchange, December 2011

Courses / Presentations /Meetings

Sign up sheets for our **(free) courses in flow and image cytometry** are available on the bulletin board outside of the Grossberg conference room on the CCC 3rd floor. Currently the following courses are being offered: **Immunophenotyping, and introduction courses to flow cytometry and staining methods; DIVA software; ImageStream Cytometry and Confocal microscopy.**

StemCell technologies will be presenting a free webinar (Jan 24) on **aldehyde dehydrogenase (ALDH) as a biomarker in Leukemia research**. The ALDH assay is currently running in our facility (in a different context). To register for the webinar go to: https://www1.gotomeeting.com/register/571278209. For questions with regards to the ALDH assay in our facility please contact Dalin Pan or Rose Furlage (or Paul / Hans).

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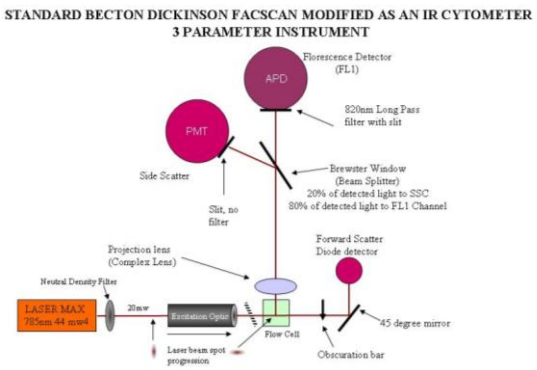
Service / Application High Lights

Far-red / Infra-red Flow Cytometry.

Background:
Reagents with far-red / infra-red excitation and emission profiles are commonly used in **intra-vital imaging** approaches where they can be used to trace labeled cells/molecules to specific organs / tumor sites. Additionally, many **photosensitizing drugs** have these high excitation/emission wavelength profiles. Several of our users have expressed their interest in **detecting the IR-dyes on a cellular level** however, due to limitations of excitation sources and sensitivity of the pmt detectors, this is not possible with our conventional flow cytometers or confocal microscope.

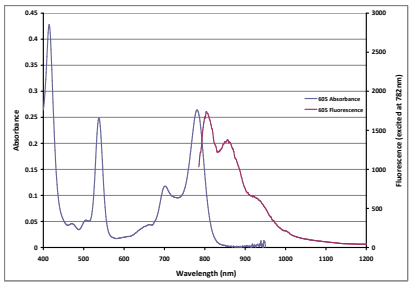


Thanks to our resident flow wizard Ed P we have a **modified FACScan** where the 488nm laser was replaced with a 785nm 40mw laser and an APD (Avalanche Photo Diode) which captures wavelengths up to 1100nm (through a 820LP emission filter). Scatter is detected at 785nm as well thus totaling **3 parameters that can be detected (far red fluorescence, FSC and SSC)**. So, although this instrument is currently limited to far red fluorescence only, Ed is constructing a DiVa platform (LSR skinned) instrument as a future replacement to the FACScan with additional excitation lasers. Stay tuned!

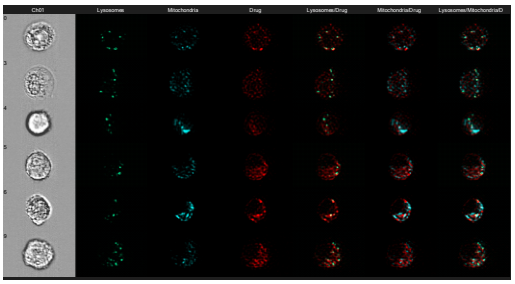
Depending on the specific excitation/emission profiles and brightness of the IR dye you are using you may be able to detect it on the **ImageStream (ISX) Cytometer**. The CCD detector of the Imagestream is more sensitive in the far red than the PMT's used in conventional flow. If your favorite IR dye is detectable on the ISX then you can perform that detection in a **multi-parameter setting** using the full repertoire of available lasers (405nm, 488nm, 561nm, 648nm).

Applications:

Example of an imagestream measurement of the photosensitizer 605-Me which was excited **at 405 nm** and emission captured from **740-800nm**, multiplexed with lyso- and mito-tracker stains. (*data courtesy of Dr Pandey)*)

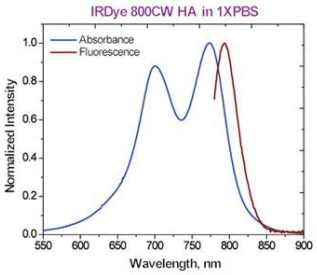


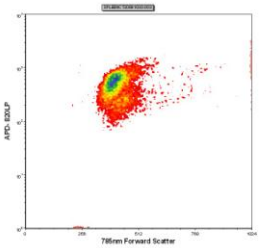
605-ME absorption and emission spectra



Imagestream analysis of a near-IR dye (605-Me).

Example of an IRdye (IRDye 800CW HA) that is more sensitively detected with the FACScan IR flow cytometer rather than the ISX. In this case, the closest laser line to the absorption peaks available on the ISX would be the 658 laser which only excites at ±30% efficiency whereas the modified FACScan can excite at 785nm at ±100% efficiency. The 2-parameter dot plot shows the IR-dye accumulation (y-axis) in mouse spleen cells as detected with the FACScan IR cytometer. In this same sample, the IRdye was undetectable by ISX (data not shown).





Ed Podniesinski **Kieran O'Loughlin**

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