ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, July-September 2012

What's new?

- **Reminder**: as announced in last quarter's newsletter, in order to improve the performance of the DiVA software for everyone, **we have now started implementing a weekly purge of the flow cytometry workstations DiVa browser experiments and D: drives**. It is the individual responsibility of each user to assure that their data and experiments are transferred from the DiVa browser and D:drives before the purge which will take place every weekend. **Note that in order to recover the instrument settings for a specific instrument you'll need to save/ export the associated 'experiment', not just the FCS files, to your assigned PI folder on the X9000. Use the Import Experiment option within DiVa to replace the experiments you need.** A directory structure has been created on the Institute's X9000 server according to all our known PI's. **Each PI has ownership privileges (owner of the folder)** 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 **ownership** privileges to assign (and thus limit) accessibility of their assigned directory to lab members and collaborators. For more information see last quarter's newsletter application notes. Instructions will also be posted on our website (www.rpciflow.org).
- In response to an audit of the Institute's Core Facilities management **it will soon be required that all core facilities gather grant information before services are provided.** We will be working closely with the Institute's Core Facility Management how to best implement these changes. Options currently being evaluated are required 'grant information' fields for calendar use and instrument logon and requisition forms for services provided.
- **The ImageStreamX-MKII has arrived! The ISX-MKII is the third generation of the ImageStream platform.** We are currently beta-testing the ISX-MKII so the 'old' ISX is still available. Once the stability and performance of the ISX-MKII has been verified it will replace the ISX. Notable changes of the ISX-MKII are the ability to perform live gating, a simplified Inspire user interface, the ability to increase the effective use of a sample from 30 to 95%, the replacement of the 658nm laser with a 642nm laser and the addition of a 592nm laser. In addition there are many changes 'under the hood' that the user may not immediately be aware of. The opportunity to beta test the MKII ensures we stay at the cutting edge of this exciting imaging flow cytometry platform.
- **Our new website is now online,** check it out at www.rpciflow.org . It is still a work in progress but we believe it has much improved over the previous website. As always, we look forward to any comments and suggestions that you may to improve the website and our services.

- There's a new face in the facility, **Jennifer Piraino** has joined the clinical lab where she will be contributing to our clinical diagnostic and support efforts. Jennifer previously worked at Cleveland BioLabs where she worked in a research support setting.

Recent Publications / Grant Funding

- **Minderman H, Humphrey K, Arcadi JK, Wierzbicki A, Maguire O,** Wang ES, Block AW, Sait SNJ, George TC, **Wallace PK**. Image Cytometry Based Detection of Aneuploidy by Fluorescence *In situ* Hybridization in Suspension. Cytometry A, in press, June 2012.
- **Paul Wallace** and **Hans Minderman** have been invited to join the **Center for Transplantation and Immunology Research (CTIR) initiative at the University at Buffalo and ECMC**. The intent of the CTIR is to provide a setting for investigators at all of the teaching and research institutions in Buffalo to interact and coordinate efforts in both basic and applied immunological sciences. The mission of the CTIR will be to harness unique capabilities in order to: develop new diagnostic and therapeutic tools for the treatment of transplantation and immunological disorders, to promote scientific collaborations exploring novel research areas to better understand the immunological barriers to transplant tolerance and to provide a structure to conduct original clinical trials bringing innovative therapies to patients in Buffalo.

Courses / Presentations /Meetings

On April 25 and 26, **Hans Minderman** presented on **Preclinical and Clinical Applications of ImageStream Cytometry** at MD Anderson Medical Center (Houston , TX) and the FlowTex cytometry users group (Houston, TX).

On May 29 and 30, **Paul Wallace** presented on **How to Diagnose PNH: a Progressive, Life-Threatening Disease** at the Providence Medical Center (Portland OR) and the Flow Cytometry Kaiser Regional Laboratory (Berkely, CA)

The **21st annual meeting** of the Great Lakes International Imaging and Flow Cytometry Association (**GLIIFCA**) will be held **September 28-30** in our own backyard at the **Buffalo Niagara Convention Center**.
Paul Wallace will be hosting the event and which will feature plenary session presentations by **Sharon Evans**, **Hans Minderman** and **Orla Maguire**. Please see the GLIIFCA website for registration and information.

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.

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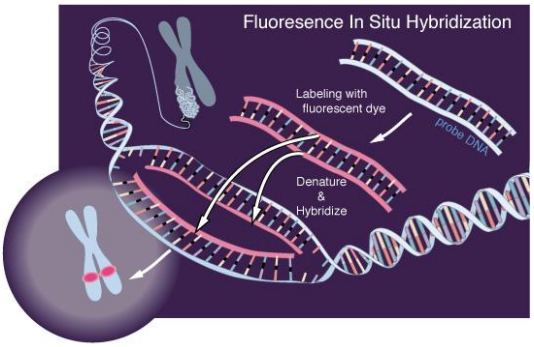
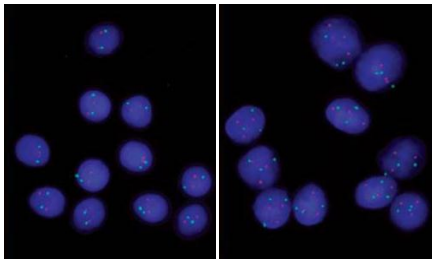
Service / Application High Lights

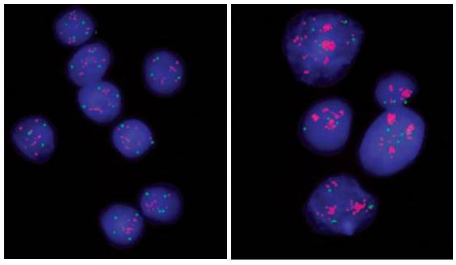
FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN SUSPENSION



Background:
FISH is a technique by which the presence of specific nucleic acid sequences can be detected following in situ hybridization with fluorescently-labeled probes that contain complementary sequences to the nucleic acid sequence of interest. This method is commonly applied to detect, for example, chromosomal abnormalities that lead to loss or gain of sequences of interest. The assay is conventionally evaluated by fluorescence wide-field microscopy (see examples below)



Example of normal disomy 17 (left, aqua probe) versus trisomy 17 (right)

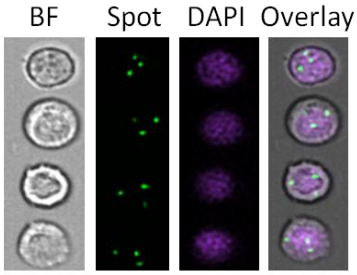


Example of intermediate Her-2/neu gene amplification (left, red probe) versus high amplification (right)

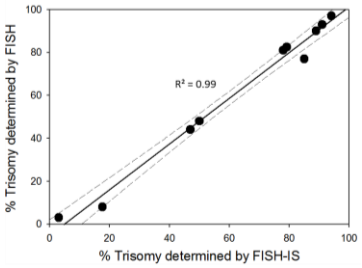
Image Source: Espinosa AB, et al: Am J Clin Pathol. 2003 Dec;120(6):917-27

Fluorescence in situ hybridization in suspension (FISH-IS):

Our facility, in collaboration with Amnis Corp., Dr Eunice Wang from the RPCI Leukemia Service, and the RPCI clinical cytogenetics lab (Drs Sheila Sait and AnneMarie Block), have adapted the FISH technique to be applied to cells in suspension and to be analyzed with the ImageStream platform. The images to the right are examples of ISX imagery from an AML patient sample diagnosed with trisomy 8 and probed with a spectrum green-conjugated CEP-8 probe.



The method and its verification will be published in the upcoming issue of Cytometry A. The advantage of FISH-IS over conventional slide-based FISH is that the number of cells analyzed can be increased by several magnitudes (100's of thousands of cells compared to a few hundred cells) thus enabling the detection in relatively rare cell populations such as may be present at minimal residual disease stages. The figure to the right shows the strong correlation between clinical samples (AML with trisomy 8) that were analyzed by conventional FISH and FISH-IS. The intercept of the correlation line with the x-axis (FISH-IS) indicate the higher sensitivity of FISH-IS to detect relatively rare cells.



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