

ROSWELL PARK CANCER INSTITUTE

FLOW AND IMAGE CYTOMETRY FACILITY

NEWSLETTER, January-March 2011

What's new?

- On behalf of everyone at the Flow and Image Cytometry Facility: **Happy New Year !**
- Due to the enthusiastic embrace by our users of the advanced capabilities of our current LSR-II we have witnessed a greater than 90% capacity of this equipment that has led to many a scheduling conflict for our users. Approval has been granted to us for the acquisition of an **additional LSR-II** (488nm, 640nm, 405nm, 561nm excitation; 12-parameters) and a **Fortessa Cytometer** (488nm, 640nm, 405nm excitation; 13 parameters). As part of the contract regarding these new cytometers, a **50% discount** on the purchase of all **BD Immunocytometry products** (cat# typically start with a "3") and a **15% discount** on the purchase of **BD Pharmigen products** (cat# typically start with a "5") has been negotiated that is available **to all RPCI research**. For information how to receive this discount please contact Darcie Ryan at purchasing (x3353) or Michelle Pelletier or Pamela Schnell at x8471.
- The **Luminex facility** has acquired the hardware necessary to use **magnetic beads**. The magnetic approach improves the washing procedures and thereby the signal to noise ratio for the detection of many analytes. It is anticipated that commercial vendors will ultimately change their kits exclusively to the magnetic beads and thus make the filter plate approach for washing obsolete. For further information please contact Mike Rickert or Ree Dolnick.
- The Live Cell Imaging capabilities of our facility have been improved by **consolidating the capabilities of the two existing live cell imaging systems into one system**. The current live cell imaging system enables **'standard' RGB fluorescence, brightfield and FRET time lapse imaging within one system**. The consolidation has improved the maintenance, troubleshooting ability and overall usability of the live cell imaging platform. The live cell imaging system is featured in this newsletter's service/application highlights.
- **Paul Wallace and Joe Tarjo** have been actively involved in the establishment of the **Roswell Park Center for Cellular Immunotherapy**. In collaboration with Drs K. Odunsi and T. Schwaab, a protocol to manufacture dendritic cells (DC) was developed. Autologous monocyte derived DCs will be loaded with the cancer testis antigen NY-ESO-1 and injected intranodally as an immunological vaccine into patients whose tumor express this antigen. DCs from this IRB approved trial will be the first product produced by this newly constructed cGMP facility.

Recent Publications / Grant Funding

- A 2-year, \$ 250,000 collaborative R21 grant application between **Michael Russell** (UB) and **Hans Minderman** was awarded. Start date 12/1/2010. These studies will use the ImageStream technology to quantify antigen processing and cell-cell interactions.
- In response to inquiries by our users we are currently exploring the possibility of acquiring a **multi-photon confocal system** for our facility. The plan is to identify the user base and their specific research questions and to identify the system that would best fit these identified needs. If a sufficient need is identified that would justify a viable **Shared Instrumentation Grant (SIG) Application**, a SIG will be submitted by Paul Wallace and Hans Minderman. If you have specific research questions in mind that will require a multi-photon approach and we haven't contacted you yet, please contact Paul (x8471) or Hans (X1162).
- **Tarjo JD, Jr., Pan D, Muirhead KA and Wallace PK**. Tracking immune cell proliferation and cytotoxic potential using flow cytometry, Vol. 3. New York: Humana Press, 2010.

Courses / Presentations /Meetings

- A **travel stipend is available** for users who would like to present data that involve the ImageStream technology. For details, contact Sherri Friend at Amnis Corp. (sfriend@amnis.com) or Hans Minderman (x1162).
- Hans Minderman was invited to present at the Belgian-Luxembourgish Cytometry Meeting (November 18-19) in Luxemburg on the clinical applications of ImageStream cytometry.
- Our facility will be participating in **the City Honors School Science Research Program**. This program which was initiated in 1998 by Ed Mirand and Craig Johnson, is a scientific research experience that is carried out over the entire four years of high school, during which students dedicate an entire Friday weekly working with their mentors at RPCI or the Hauptmann Woodward Institute. For more information on this program see <http://www.cityhonors.org/page/science-research-program/>
- There are now **two webinars** available presented by Dr Tad George of the Amnis Corp. on the topics of ImageStream Cytometry (<https://www.amnis.com/news.html>). Both provide a nice overview of the basic principle of the ImageStream platform and its applications.

Service / Application High Lights

The Leica AF6000LX Live Cell Imaging Platform.

Features:

- Time-lapse video recording of live cells in a fully temperature-, humidity- and gas-controlled environment
- High speed image capture to ensure minimum exposure of sample
- Multi channel fluorescence acquisition
- Fully programmable multiplex 4D image acquisition w/r to number of X,Y,Z positions and time)
- Data output formats compatible with ImageJ, ImagePro
- Fluorescence excitation by filter-limited arc-lamp or by monochromator
- FRET (YFP/GFP) filter set and software
- B/G/R filter set



Applications:

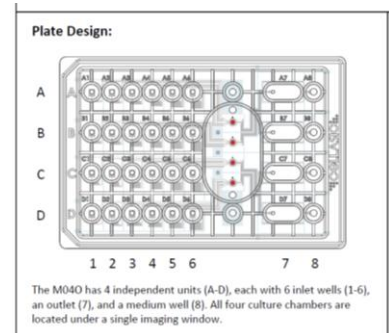
Examples of the various applications from our users are available on-line at <http://www.rpciflow.org/services.html> , on the right side of the page you will find links to the following movies:

- **Aspergillus:** maturation of GFP-labeled Aspergillus conidia in the presence of alveolar macrophages (courtesy of Dr Melissa Grimm, Dept of Medicine) - **download time ~15 sec.**
- **Kinetics:** time kinetics of heterogeneous GFP expression (Dr Venkatesh Natarajan, Dept of Cell Stress Biology) – download time ~ **4 min 30 sec**
- **Lymphocyte Subsets:** motility of differentially stained and primed lymphocyte subsets in the presence of tumor cells (courtesy of Dr Junko Matsuzaki, Dept of Gynecology) – download time ~ **25 sec**
- **Motility:** cell migration in response to a chemo-attractant (courtesy of Dr Alejandro Godoy, Dept of Urology) – download time ~ **1 min 30 sec**

The CellAsic ONIX multi-well microfluidic perfusion system.

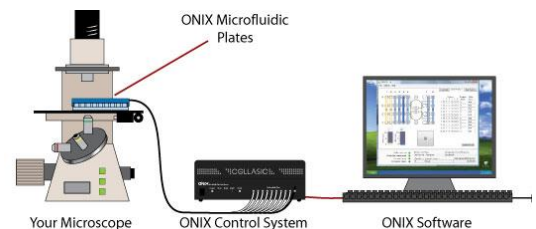
Features:

- 4 independently controlled culture chambers
- each culture chamber can be perfused according to controlled rates from 6 different source wells (e.g. drug 1, drug 2, wash medium, culture medium etc)
- exchange of culture / drug exposure environment without the need to remove the culture plate from the microscope stage enables tracking of individual cell behavior before, during and after drug exposure.
- For more information see <http://www.cellasic.com>



Applications:

- Our facility is currently beta-testing this platform, please contact the facility if you have specific applications in mind that you would like to test
- In combination with the Leica AF6000LX system, cell behavior can be studied in a temperature-, humidity- and gas-controlled environment.
- A movie of the controlled perfusion of trypan blue simulating a drug exposure and subsequent wash out of an observation chamber is available at <http://www.rpciflow.org/services.html> . Click on the **Onix Trypan Blue** link on the right side of the page.
- Since the culture plate is sealed gas-tight from its surrounding environment and the gas mixture within the culture plate to which the cells are exposed can be controlled, this set-up has the potential to study cells under low oxygen conditions.



For more information please call:

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