ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, April-June 2011

What's new?

- The **new Fortessa and LSRII cytometers have arrived**. See our service/application highlights this quarter for more details.

- **Reminder**: 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.

- Unfortunately, we are still experiencing problems with users over-reserving time on the cytometers in excess of 50% differences between reserved times and actual used time. We would like to use this format to remind you of the following **rules for using the equipment in our facility**:
    1. There is a 30 min grace period for reserving the equipment. If a user does not show up within 30 minutes of their reserved time on the calendar without notifying our facility, the reserved time is forfeited and others can use it on a first come first serve basis.
    2. If you are running over your reserved time and someone signed up immediately after you, you need to call the next user and ask for permission to run late. The next user has the right to claim their reserved time.
    3. At the end of your run on any cytometer, please remember to run bleach for 5 min, then PBS for 5 min and finally place instrument in standby mode.
    4. After 4:00 pm, if you are the last user signed up it is your responsibility to shut down the equipment. For the flow cytometers this means running bleach and PBS as you normally would at the end of an experiment then leaving in standby for 5 min standby (to cool off laser) and then shut down both the instrument and computer.
    5. DIVA users need to export their experiment at the end of their run and delete it from the browser. Experiments left in the browser will be deleted by staff.

- In accordance with the Institute's Core Facility Management, a **3% price increase** to the Flow and Image Cytometry services will be in effect on April 1, 2011 to compensate for the annual indexed increase of operational costs. (examples: hourly flow cytometry and confocal microscopy rates for CCSG members will increase from $36.05/h to $37.13/h)

Recent Publications / Grant Funding

- Melchjorsen J, Risør MW, Søgaard OS, **O'Loughlin KL**, Chow S, Paludan SR, Ellermann-Eriksen S, Hedley DW, **Minderman H**, Østergaard L, Tolstrup M. Tenofovir selectively regulates production of inflammatory cytokines and shifts the IL-12/ IL-10 balance in human primary cells. J. AIDS, in press Feb 2011.
- **Maguire O, Collins C, O'Loughlin K**, Miecznikowski J, **Minderman H**. Quantifying Nuclear p65 as a Parameter for NF-κB Activation: Correlation Between ImageStream Cytometry, Microscopy and Western Blot., Cytometry A, in press March 2011
- **Joseph Tario, Jr.,** Katharine Muirhead, **Paul Wallace**. Simultaneous Measurement of Treg and Teff Cell Proliferation using Cell Tracking Dyes. (CYTO 2011, Baltmore, MD)
- **Maguire O, O'Loughlin K, Minderman H.** Time Kinetic study of p65 phosphorylation events in relation to NF-κB nuclear translocation (CYTO 2011, Baltmore, MD)

Courses / Presentations /Meetings

- Our facility will host the **22nd Clinical Course on Flow & Image Cytometry.** This course takes a practical approach to training participants in clinical flow cytometry using hands-on laboratory and computer work in small groups to supplement lectures. The emphasis of the course will be divided between basic flow theory, current practice in the clinical laboratory, flow cytometric diagnosis of leukemia & lymphoma, and frontiers for the future. The **Course will be limited to approximately 45 participants**. For information please check **www.roswellpark.org/FlowCytometry2011**. To register or to be placed on a mailing for additional information please contact: Michelle Pelletier
- Our facility will be represented at CYTO 2011 by Paul Wallace and Hans Minderman and poster presentations by Orla Maguire and Joe Tario. Come and see us if you go to the meeting!
- Our facility will be hosting an **on-site demo for the LEICA MULTIPHOTON MICROSCOPY system** from **April 25-April 27, 2011**. Leica has agreed to install their system on-site which will give our users an excellent opportunity to test the applicability of multiphoton microscopy to their specific research models. Major advantages of multiphoton (MP) vs single photon microscopy are the extended imaging depth (400/500 micron vs ~70 micron) and strongly reduced phototoxicity to live tissues out of the imaging plane of focus. If your research involves deep tissue imaging and intravital imaging we strongly encourage you to take advantage of this opportunity. Please contact Hans Minderman (x1162) if you are interested in analyzing your samples on the MP microscope so that we can optimize the scheduling during the demo.

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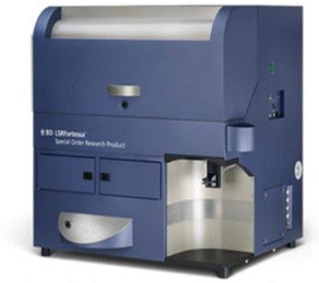
Service / Application High Lights

THE NEW ARRIVALS:



LSR Fortessa SORP (13 parameters):
DiVa version 6.3
(HTS ready, has internal components allowing a HTS(96 well plate sipper) to be connected, HTS software option enabled)

Technical Specifications:
3 laser, separate interrogation of sample for each laser
11 color detection + 2 scatter parameters (from 488nm)
Hydrodynamically focused sample interrogated within a quartz cuvette flow cell
Sample aspiration rates at 12ul, 35ul, 60ul per minute
262,144 channel (18 bit) dynamic range on all parameters, Windows XP-Pro OS, Diva software acquisition (software/ instrument maximum of 20 parameters processed)

Excitation Lasers: 488nm (50mw), 640nm (40mw); 405nm (50mw)

Emissions detected:

405nm Violet Laser:
450/50 BD Horizon V450/Pacific Blue/Marina Blue/Alexa Fluor 405
525/50 BD Horizon V500/AmCyan/Qdot 525
605/12 Qdot 605

488nm Blue Laser:
488/10 FSC
488/10 SSC
530/30 FITC/Alexa 488
575/26 PE
610/20 PE-Texas Red/PI
695/40 PerCP-Cy5.5/PE-Cy5/PerCP
780/60 PE-Cy7

640nm Red Laser:
670/14 APC
730/45 Alexa Fluor 700
780/60 APC-Cy7/APC-H7

LSRII-B SORP Instrument (18 parameters):
DiVa version 6.3

Technical Specifications:
4 laser, separate interrogation of sample for each laser
16 color detection + 2 scatter parameters (from 488nm)
Hydrodynamically focused sample interrogated within a quartz cuvette flow cell
Sample aspiration rates at 12ul, 35ul, 60ul per minute
262,144 channel (18 bit) dynamic range on all parameters, Windows XP-Pro OS, Diva software acquisition
Note: The 'old LSRII' will now be designated LSRII-A. The configurations of LSRII-A and LSRII-B will be identical except for the UV laser excitation source and corresponding detectors (LSRII-A only) and the higher power 405 nm laser on the LSRII-B (100 mw vs 50 mw)

Excitation Lasers: 488nm (20mw); 640nm (40mw); 405nm (100mw); 561nm (50mw)

Emissions detected:

405nm Violet Laser intercept:
780/60 QDot-800
710/50 QDot-705
660/20 QDot-655
610/20 QDot605
525/50 AmCyan/ BD Horizon V500
450/50 Pac Blue/ BD Horizon V450

488nm Blue Laser intercept:
488/10 FSC
488/10 SSC
525/50 FITC/Alexa 488
695/40 PerCP, PerCP-Cy5.5

561nm (Yellow/ Green) intercept:
780/60 PE Cy7
710/50 PE-Cy5.5
670/30 PE-Cy5
610/20 PE
TxRd/mCherry
582/15 PE

640nm Red Laser intercept:
730/45 Alexa-700
670/50 APC
780/60 APC CY7

For more information please call:

Earl Timm	x8235
Ed Podniesinski	x3470
Hans Minderman	x1162
Paul Wallace	x8471

Earl Timm

Ed Podniesinski