ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, October-December 2010

What's new?

- **The LSR-II and the Aria sorter were upgraded with a 561 nm laser excitation source**. The 561 nm laser is a more efficient excitation source for PE and PE-conjugate labels and will also allow excitation of the so-called 'fruit-dyes' (eg MCherry). For the LSR-II user cytometer, these upgrades necessitated various hardware reconfigurations. In practice, you will immediately notice the following
  - PE and PE-conjugates are no longer excited by the 488nm laser but are now excited by the 561 laser
  - The number of emission detectors registering excitation by the 405nm laser have expanded which allows detection of a larger range of Q-dot labels
  - The configuration files have changed to reflect the necessary hardware changes. A new convention for naming parameters has been adopted. The parameter names no longer reflect the 'most popular' labels for a given emission wavelength but are now given more generally applicable names.

There are many more 'behind the scene' changes and since these affect a large number of our user base, this quarter's Service/Application high light is dedicated to explaining these upgrades in more detail. **We are committed to make the transition from the old to the new configuration as seamless as possible for our users. Please don't hesitate contacting the individuals listed in the Service/Application high lights if you have any questions, concerns or need help configuring your new set-up templates.**

- The image facility is beta-testing the **ONIX Micro Fluidic Perfusion system** from CellASIC Corp. This system allows controlled perfusion of (drug-containing) media over a cell culture during observation with the live cell imaging system. Up to 4 cultures can be followed simultaneously over time with the option to have up to 4 different perfusion sources / rates/ culture. For more information please see www.cellasic.com or contact Ree Dolnick or Hans Minderman.

Recent Publications / Grant Funding

- **Paul Wallace** was awarded a $ 120,000 beta-test contract with Becton Dickinson to test their CD34 IVD kit.
- **Hans Minderman** was awarded a $35,000 one-year extension of a sponsored-research agreement with the Amnis Corporation to study clinical applications of the ImageStream platform.
- *Down-Regulation of Signal Transducer and Activator of Transcription 3 Enhances Acute Myeloid Leukemia-Derived Dendritic Cell Function* Michael T. Brady, PhD[1], Austin Miller, PhD[2], Sheila Sait, PhD[3], Laurie Ann Ford, BS, CCRC[1], **Hans Minderman**, PhD[4], Eunice S. Wang, MD[1], Kelvin P. Lee, MD[5], Heinz Baumann, PhD[6], and Meir Wetzler, MD[1]. Accepted for presentation at ASH, 2010
- *NFkB translocation as a quantitative parameter for clinical immunosuppression.* **Hans Minderman**, Oleh Pankewycz, **Kieran O'Loughlin**, Lin Feng, Mark Laftavi, **Paul Wallace**. Accepted for oral presentation during the 10th Euroconference on Clinical Cell Analysis (ESCCA).

Courses / Presentations /Meetings

- The American Society for Clinical Pathology **(ASCP) Global Outreach** partnered with U.S. Centers for Disease Control and Prevention (CDC) to provide laboratory training and implement laboratory quality improvement initiatives. These programs enhance the prevention, diagnosis, treatment, and monitoring of HIV/AIDS patients in resource-constrained countries. As part of this program, **Paul Wallace** served as a consultant and trainer for the following courses held in Vietnam: *CD4 External Quality Assurance Conference*, Hanoi: August 17 - 18, 2010 and *CD4 Training of Trainers*, Hanoi: August 19 - 27, 2010
- **Paul Wallace** served as faculty at the Clinical Flow Cytometry Course at the Hillman Cancer Center of the University of Pittburgh (sept 19-23, 2010)
- **Hans Minderman** served as faculty at the Integrated Cytomics Workshop at 6th European Course of Clinical Cytometry and presented an oral presentation on *NFkB translocation as a quantitative parameter for clinical immunosuppression* during the 10th Euroconference on Clinical Cell Analysis (ESCCA). Both meetings were held in Valencia, Spain.
- **Joseph Tario** was awarded **the Alexander Nakeff Young Investigator award** at the recent GLIIFCA meeting in Detroit. This honor is awarded at each year's GLIIFCA meeting to the technician, undergraduate, graduate or post doctoral student who gives the best presentation at the annual meeting.
- **Kristin Wright** and **Ed Podniesinski** both won awards for their posters presented at this year's GLIIFCA meeting. Kristin presented on the work flow in the clinical lab and Ed on the bio-bubble design for the Aria. Both posters can be viewed in the hallways on the 3rd floor of the CCC building.
- Due to a production delay in the FLEXMIR kits, the **workshop in collaboration with the Luminex Corp.,** that was planned to be held at Roswell Park Cancer Institute in the 3rd or 4th quarter of 2010 has been **postponed** to the 1st quarter of 2011. By including presentations from invited speakers as well as training sessions on software and hardware, the intent of the workshop is to (re-)introduce people not familiar with the multiplexing platform to old and new (FLEXMIR) applications. We hope to be able to provide you with the rescheduled date in the next newsletter.

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Service / Application High Lights

The 561nm laser excitation source upgrade and reconfiguration of the LSR-II and Aria cell sorter .

- **Advantages of the 561nm excitation source:**
  - Excellent excitation source for RFP (dsRed) variants
  - Improved S/N ratio's for PE and PE tandems without impacting FITC or GFP
  - Virtually no compensation with FITC or GFP
  - Excellent excitation source for the so-called "fruit dyes" (eg mCherry, mPlum. mRaspberry, mOrange, mBanana and td Tomato). For more information go to www.clonetech.com.

- **Associated hardware changes on the LSR-II**
  - The violet trigon detector array is changed to an octagon array which will expand the number of detectors for Q-dots off the violet laser from 3 to 8. For more information on Q-dots go to www.invitrogen.com.
  - The blue octagon detector array is changed for a trigon array (since PE dyes are now excited by the 561 laser)
  - A yellow-green octagon detector array is added for the 561 laser
  - The 633nm 20 mw laser is replaced by a 640nm 40 mw laser
  - Please see the tables below for the mirrors and filters associated with each detector

- **Associated software/configuration changes on the LSR-II**
  The old and new configurations are summarized below:

4 Laser LSR Configuration

Laser Name	Wavelength	Detector	Mirror	Filter	Parameter
Blue	488	A	755 LP	780/60 BP	PE-Cy7
		B	690 LP	710/50 BP	P5-5
		C	675 LP	685/35 BP	FL3, PC5, PCP
		D	600 LP	610/20 BP	PE-Texas Red
		E	550 LP	575/26 BP	PE, PI
		F	505 LP	530/30 BP	Alexa Fluor 488, FITC, GFP
		G		488/10 BP	SSC
355 UV	355	A	502 LP	530/30 BP	Hoechst Red, Indo-1 (Blue)
		B		450/50 BP	Hoescht Blue, Indo-1 (Violet)
Violet	405	A	595 LP	645 LP	QDOT605, SPR
		B	505 LP	525/50 BP	AMCY
		C		450/50 BP	AX405, PB, SPB,VLD
Red	633	A	755 LP	780/60 BP	AC7, AH7, AX750
		B	685 LP	730/45 BP	AX700
		C		660/20 BP	APC, Alexa Fluor 647

5 Laser LSR Configuration

Laser	Wavelength	Detector	Mirror	Filter	Parameter Name	Common Colors
Blue	488 nm	A	none	B670 LP	B1	PcP, Pcp-Cy5.5
		B	505 LP	B530/30 BP	B2	Fitc, GFP
		C			B3	SSC
Yellow-Green	561 nm	A	755 LP	Y780/60 BP	Y1	PE-Cy7
		B	690 LP	Y710/50 BP	Y2	PE-Cy5.5
		C	675 LP	Y685/35 BP	Y3	PE-Cy5
		D	600 LP	Y610/20 BP	Y4	PE-TxRed, mCherry
		E	550 LP	Y575/26 BP	Y5	PE
UV	355 nm	A	none	U670 LP or U530/30	U1	SP Red
		B	502 LP	U450/50 BP	U2	Hoescht, SP Blue
		C			U3	
Violet	405 nm	A	750 LP	V780/60 BP	V1	Qdot 800
		B	690 LP	V720/20 BP	V2	Qdot 705, SP Red
		C	640 LP	V655/20 BP	V3	Qdot 655
		D	570 LP	V605/20 BP	V4	Qdot 605
		E			V5	Qdot 585
		F	555 LP	V565/20 BP	V6	Qdot 565
		G	505 LP	V525/50 BP	V7	AmCyan
		H	none	V450/50 BP	V8	PacBlue, SP Blue
Red	640 nm	A	755 LP	R780/60 BP	R1	APC-Cy7, APC-H7
		B	685 LP	R730/45 BP	R2	Alexa 700
		C	none	R660/20 BP	R3	APC

Note that in the new configuration, the parameter name that used to represent the most common fluorochromes detected for a given wavelength range has now been replaced with a more generic parameter name. For detailed interactive information on excitation and emission characteristics of popular fluorochromes please see
http://www.bdbiosciences.com/external_files/media/spectrumviewer/index.jsp

- DiVa software and hardware can only process a maximum of 20 parameters total. In order to allow 22 parameters, four colors (fluorescence parameters) are affected by two switch boxes located under the LSRII instrument table. From these four parameters, only two are in circuit to acquire data at a time. The user decides which two of the four they want to use. Within DiVa software under "Parameter "Tab either locate parameter or "Add" the parameter switch box is set to Y/G-D or Violet- A
- In order to have our sorting capability be in line with the expanded 561nm excitation source, the ARIA-I has also been upgraded with a 561nm laser source.

For questions regarding these upgrades contact:

Earl Timm x4163
Ed Podniesinski x4579
Hans Minderman x1162
Paul Wallace: x8471

Earl Timm

Ed Podniesinski