ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, July - September 2010

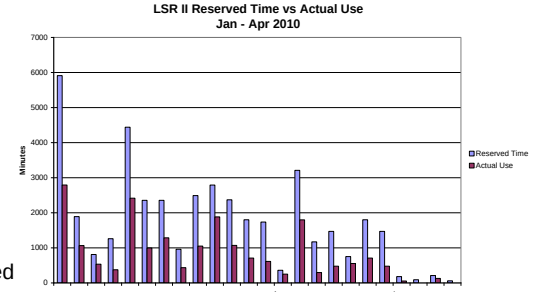
What's new?

- **The ImageStream-X** beta machine has now been replaced with the commercial edition. The major change that the users will notice is the outside appearance and the addition of a 96-well plate **auto sampler**. All the other options such as excitation sources, magnifications, EDF are identical to the former beta-machine.

- A custom-made aerosol containment barrier (BioBubble Inc, Fort Collins, CO) was placed around the **Aria I sorter** in compliance with **Bio Safety Level 2+** guidelines. For more information please visit http://home.rr.com/rpciflow

- The Luminex facility upgraded the software to **xPONENT 3.1** to accommodate the xTAG and FlexmiR molecular-based multiplexing assays in which the capture beads are coated with nucleic-acid capture sequences rather than antibodies. Applications of this multiplexing approach are in the field of **SNP detection** (xTAG) and **miRNA detection** (FlexmiR).

- **A friendly reminder**: unlike many other flow cytometry core facilities around the country, our current policy charges for actual usage time of the equipment rather than reserved time on the calendar. However, a recent review showed that for one of our heaviest used machines, **the LSR-II**, for the majority of users the **reserved time** on the calendar **exceeds the actual usage time by 40-50%** (see bar graph in which each pair of bars represents an individual user group)**.** Please be considerate to your fellow users and reconsider your sign-up procedures. If the excessive reserved time persists we will have to implement a charge based on reserved time or actual usage time, whichever is the longest.



- We are working on a **display** in the **3rd floor hallway outside the Grossberg** conference room of all our **available equipment with specifications**. Please take a look and let us know if you have any questions.

Recent Publications / Grant Funding

- Recurrent deletion of 9q34 in adult normal karyotype precursor B-cell acute lymphoblastic leukemia. Nowak NJ, Sait SN, Zeidan A, **Deeb G**, Gaile D, Liu S, Ford L, **Wallace PK**, Wang ES, Wetzler M. Cancer Genet Cytogenet. 199(1):15-20, 2010.

- **Hans Minderman** was awarded a **2-year R33 grant from the NIH** to evaluate clinical applications of the ImageStream technology

- **Paul Wallace** was awarded a **beta-test contract** with Becton Dickinson to evaluate a clinical CD34 kit for IVD use.

Courses / Presentations / Meetings

The facility, in collaboration with the **Luminex** Corp., is in the process of organizing a **workshop** to be held at Roswell Park Cancer Institute on **November 9th, 2010** in the Zebro conference room. This workshop will include presentations from invited speakers with the intent to (re-)introduce people not familiar with the multiplexing platform to old and new applications (**including miRNA and SNPs**). A detailed program will follow in the next newsletter.

Registration is now open for the 2010 edition of the **Western New York Flow Users group meeting**. The meeting will be held on 14 July, 2010 at the Kornberg Medical Research Building at the University of Rochester. The meeting will start at 9 am, with morning Vendor talks. These will be followed by the 4th annual presentation of the Leon Wheeless Award, being awarded to **Dr. Carleton Stewart**. The afternoon speakers include: J Thompson, M.D., Roswell Park Cancer Institute Arvin Rao, PhD., Carnegie Mellon University Leigh Samsel, NIH, NHLBI William Hyun, University of California, San Francisco Shannon Hilchey, University of Rochester. Registration is free and can be done electronically at: http://www.urmc.rochester.edu/flow-core/wnyfug/registration-form.cfm

ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, July - September 2010

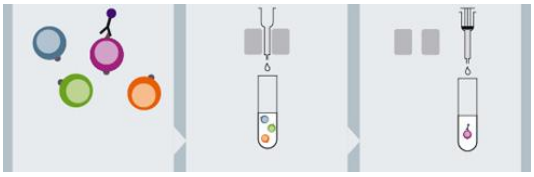
Service / Application High Lights

The autoMACS separator for immunomagnetic cell sorting .
(adapted from http:///www.miltenyibiotec.com)

- The autoMACS™ Separator is a benchtop instrument for high-speed automated cell sorting. Employing the MACS® Technology, the autoMACS Separator is designed for positive selection as well as depletion of magnetically labeled cells. The autoMACS Separator is operated with pre-set separation programs, thus, allowing optimization of cell sorting approaches according to cell abundance and the intensity of marker expression. The separated cells are immediately ready for experiments, cell analysis, or further subset sorting.

  • **Fast**—up to 4×10^9 magnetically labeled cells are sorted automatically within a few minutes.
  • **Efficient**—rare cells can be enriched up to 10,000-fold or unwanted cells may be depleted up to 99%.

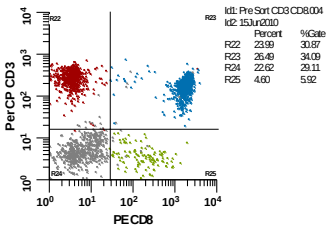
MACS® Cell Separation Technology is available for human, mouse, rat and non-human primate cell isolation. For any other cell type MicroBeads for indirect magnetic labeling can be used.



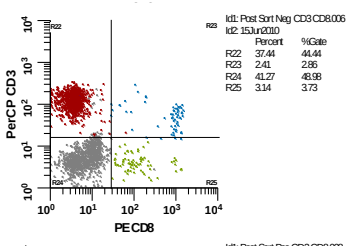
- **Principle of the technology**: MACS Cell Separation is based on MACS MicroBeads, specific monoclonal antibodies conjugated to superparamagnetic 50 nm particles. Labeled cells are retained in the magnetic field within a MACS Column. Separated cells—the positively labeled fraction as well as the non-labeled fraction—can directly be used for downstream applications, such as cell analysis, further expansion, or functional assays. The separated cells remain viable and their functionality is not impaired.

- **Example: PBMC separation of CD8+ cells**. Peripheral blood was collected by veinipuncture and subjected to Ficoll separation. Afterwards, cells were labeled with CD8+ microbeads as per the manufacturer's instructions. Aliquots were collected before and after sorting, and were stained with PE CD8 and PerCP CD3. ***(data provided by Dalin Pan, Joe Tario)***

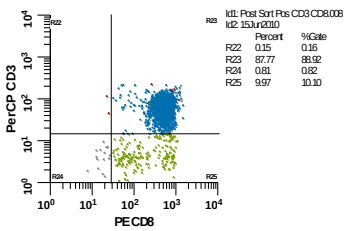
Pre-sort



Negative Sort Fraction



Positive Sort Fraction

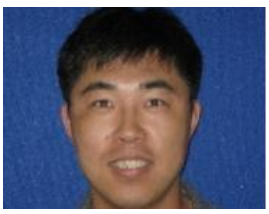


- **Common applications**: positive and negative separation of specific immunophenotypes. Enrichment of target cells before flow cytometric cell sorting to improve sorting efficiency. For a full repertoire of available analytes please see the Miltenyi Biotec website: www.miltenyibiotec.com.

- AutoMacs Application contacts:

Dalin Pan	x4163
Joe Tario	x4579
Hans Minderman	x1162
Paul Wallace:	x8471

Dalin Pan



Joe Tario