

ROSWELL PARK CANCER INSTITUTE FLOW AND IMAGE CYTOMETRY FACILITY NEWSLETTER, July-September 2011

What's new?

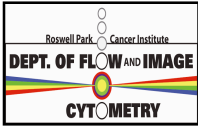
- **A new course** has been developed to help our users transition to the **Diva Flow Cytometry acquisition software**. If you are interested in attending this free course, the **sign up sheets for our free courses** are posted by the entrance to the flow cytometry users room.
- Information on all the **optical detector lay-outs** for the user flow cytometers and **corresponding filter characteristics** are now posted on the **poster board across from the flow cytometry user room**. Note that the filter characteristics represent actual scans performed by our engineer, Ed Podniesinski, rather than 'manufacturer's specifications'.
- **Dr Craig Jones has joined our group**. Craig comes to us from RPCI's Department of Cell and Molecular Biology where he worked for 23 years applying cellular, molecular, biochemical and imaging approaches to research. His specific flow cytometry experience include immunophenotyping mouse bone marrow and sorting. Craig will be working closely with Earl Timm for extensive advanced training and will take over Earl's responsibilities with regards to cell sorting and helping out our users upon Earl's retirement later this year.
- **Let us know how we are doing!** Please take our short survey: <http://www.surveymonkey.com/s/5D5PHSP> . Your anonymous feedback will help us help you!
- In preparation for submitting a **shared instrument grant for a multiphoton microscope in 2012**, a user group has been formed that is in the process of evaluating the specific needs / applications at our Institute and which of the current available technology would best fit those and future needs. An evaluation of Zeiss and Leica equipment has been completed by visiting and analyzing samples at the **Harvard Center for Biological Imaging** and an on-site demo at our facility, respectively. The following specialists have kindly offered their consultant services: **Dr Ammasi Periasamy, Director, WM Keck Center for Cellular Imaging, University of Virginia, Dr George McNamara, Analytical Imaging Core Facility, University of Miami and Dr Thorsten Mempel, Harvard Medical School**. Planned activities include training on specific intra-vital imaging approaches at Dr Thorsten Mempel's laboratory and Dr McNamara's facility and an on-site demo by TILL Photonics.
- Our facility hosted an advanced training on the ImageStream platform for **Dr. Debora Martorelli, C.R.O National Cancer Institute – Aviano, Italy and Dr.Miriam Ascagni, Advanced Light and Electron Microscopy Biolmaging Centre, Milano, Italy**.
- **Your confocal images may be worth an Amazon Gift card!** We would like to post a gallery of images obtained at our facility in our hallway. If you have any images that you are particularly proud of please submit them to Michelle Pelletier. If your picture(s) are selected for posting you will receive a **\$20 gift card for Amazon.com**
- Clinical applications of the ImageStream platform are expanding with collaborative studies initiated with **Kinex Pharmaceuticals, TetraLogic Pharmaceuticals** and the **Immune Tolerance Network**. In these studies, samples from patients treated with KX01, TL32711 (a Smac mimetic) and α -1-antitrypsin will be studied, respectively.

Recent Publications / Grant Funding

- Melchjorsen J, Risør MW, Søgaaard 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, 57(4):265-275, 2011**.
- **Maquire O, Collins C, O'Loughlin K**, Miecznikowski J, **Minderman H**. Quantifying Nuclear p65 as a Parameter for NF- κ B Activation: Correlation Between ImageStream Cytometry, Microscopy and Western Blot., **Cytometry A, 79(6):461-469, 2011**.

Courses / Presentations /Meetings

- Our facility will host the **22nd Clinical Course on Flow & Image Cytometry, Aug 1-4, 2011**. 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. This year's faculty include **Dr Fiona Craig, University of Pittsburgh School of Medicine; Dr Bruce Davis, Trillium Diagnostics, Bangor, ME, Dr Paul Wallace, RPCI, Dr Vince Shankey, Advanced Technology/Cellular Analysis Business Group, Beckman Coulter, Inc.; Dr Hans Minderman, RPCI, Dr George Deeb, RPCI; Dr Marc G. Golightly, Stony Brook University**. For further information please check www.roswellpark.org/FlowCytometry2011. To register please contact: Michelle Pelletier.
- The annual meeting of the **Western New York Flow Users Group** will be held on **Wednesday July 13, 2011**, at the University of Rochester Medical Center. For free registration and more info: <http://www.urmc.rochester.edu/flow-core/wnyfug/>
- **The Roswell Park Cancer Institute Science Retreat** will be held Friday July 15, 2011. Our facility will be represented by an oral presentation by Hans Minderman on the clinical, pre-clinical and translational applications of flow and image cytometry during the retreat and a poster presented at the Institute's core awareness presentations in the Gaylord / Cary room.



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Service / Application High Lights

The Cell Sorting Facility

Our facility offers cell sorting services to users from within and outside RPCI. The facility is equipped with two state of the art Flow Cytometry cell sorters, the FacsAria I and the FacsAria II. Each Instrument is capable of 1 - 4 way tube sorts, multiple well plate sorts and the ability to sort onto slides with sorting speeds of up to 15,000 cells/sec. Sorting can be performed under aseptic conditions so that the sorted cells can be used for subsequent culture, functional assays and/or animal studies. Cell sorting is available by appointment and a **“Sort and Acquisition Request Form” must be submitted for review before time is reserved for sorting.** The form can be found at our website www.rpciflow.org. Click the “Services” tab within the web page to find the form.

Instruments

The FacsAria I can detect up to 18 different colors along with Forward Scatter (FSC) and Side Scatter (SSC) parameters. This instrument has four emission lasers 488 nm (Blue), 635 nm (Red), 561 nm (Yellow-Green) and 405 nm (Violet). Since the FacsAria I is contained within a Bio-Bubble negative pressure chamber, the sort can optionally be performed with aerosol management (Bio Safety Level 2+).



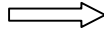
The FacsAria II can detect 11 different colors plus FSC and SSC. The Aria II has four emission lasers 488 (Blue), 635 (Red), 405 nm (Violet) and a 355 nm (UV).



Applications

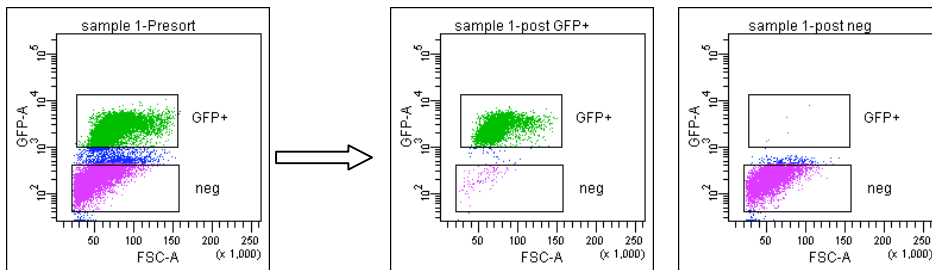
Many of our most recent sorting projects have involved sorting based on differential expression of fluorescent proteins (GFP, RFP, etc.), cell surface markers, DNA content (cell cycle) or retention of fluorescent substrates (stem cells). The examples below show the pre- and post-sort samples of yeast cells and human T-cell subsets.

PRE-SORT

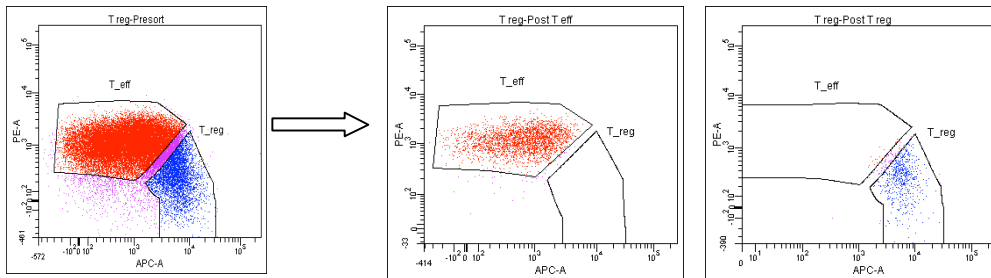


POST-SORT

Yeast: sorting based on differential expression of GFP



T-regulatory cells: sorting based on differential expression of CD25 and CD127



For more information please call:

- Earl Timm x8235
- Craig Jones x1745
- Hans Minderman x1162
- Paul Wallace x8471

Earl Timm



Craig Jones

