ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, APRIL 2010

What's new?

- **This news letter!** Flow and Image Cytometry is a lively field with ongoing development of new instrumentation, applications and hardware and software availability. In an effort to keep our users up to date with these developments we are issuing this newsletter on a quarterly basis. In addition to a brief overview of what is new in our facility this news letter will also high light a specific technology or application. For additional information on any of the topics featured in this news letter please stop by the facility (Cancer Cell Center Rm 311 or contact us at 845-8471.
- **A second-shift for sorting** has been implemented and the available sort times can now be viewed on our on-line calendar.
- **The confocal microscope** can now be reserved for *user-operated* use (after receiving the appropriate training by our staff)
- We are working with the IT department to migrate the flow cytometry data access to the institute server, this will allow users who have a Lawson account (linked to the RPCI email address) direct access to their data from their own computer and will ensure data back up according to Institute policies. Once implemented **access to the user equipment and data files will be limited to registered users only** who will be able to log into the system by using their Roswell ID. If you are an outside user and do not have a Roswell Park ID, in order to be recognized as a registered user we will need the following information: name, PI, contact phone #, RPCI email address, last 4 digits of social security **#** and birth date. If you haven't provided that information to us yet (in response to an email that was sent out several months ago), please contact Earl Timm at x8416
- **Paul Wallace** was elected treasurer of the International Society of Analytical Cytometry (ISAC)
- **Hans Minderman** has been invited by the NIH to present at the Southern Biomedical Engineering Conference (SBEC) in Baltimore, MD on the topic of clinical applications of multispectral image cytometry.

Recent Publications from the Facility

- **Joseph Tario, Dalin Pan** and **Paul Wallace** co-authored a chapter on tracking immune cell proliferation and cytotoxic potential using flow cytometry for the 3rd edition of Flow Cytometry Protocols (Hawley & Hawley eds, Humana Press)
- Battiwalla, M., Hepgur, M., **Pan, D**., McCarthy, P.L., Ahluwalia, M.S., **Camacho, S.H.**, Starostik, P., and **Wallace, P.K.** (2010). Multiparameter flow cytometry for the diagnosis and monitoring of small GPI-deficient cellular populations Cytometry B Clin Cytom *(in press)*.
- Segal BH, Han W, Bushey JJ, Joo M, Bhatti Z, Feminella J, Dennis CG, Vethanayagam RR, Yull FE, Capitano M, **Wallace PK**, **Minderman** H, Christman JW, Sporn MB, Chan J, Vinh DC, Holland SM, Romani LR, Gaffen SL, Freeman ML, Blackwell TS. NADPH oxidase limits innate immune responses in the lungs in mice. PLoS One.16;5(3):e9631, 2010.

Courses / Presentations /Meetings

Our facility organizes several courses, **free of charge**. Sign-up sheets for these courses are posted outside of the user room on the 3rdh floor of the CCC building. The following courses are offered:

- Introduction to flow cytometry
- Compensation in flow cytometry
- Immunophenotyping in flow cytometry
- DNA analysis in flow cytometry
- Introduction to confocal and imagestream
- Imagestream data analysis

Note: hands-on, one-on–one instruction on data acquisition and analysis by flow cytometry, confocal microscopy and ImageStream is always available by appointment.

Paul Wallace will be part of the faculty presenting at the following courses:
Bowdoin College (Flow Cytometry Research Course) 6/20-6/25
Univ of Pittsburgh (Clinical Flow Cytometry) 9/20-9/23
Contact Paul Wallace for more information or check www.vsh.com/courses

Our facility will be well-represented at this years ISAC meeting in Seattle, WA. **Hans Minderman** is the co-organizer of a workshop on the topic of "Revisiting Analytical and Statistical Approaches to Cytometry in the Context of Imaging" , will present an oral presentation on the "Feasibility of ImageStream-based high-throughput analysis of numerical chromosome abnormalities" and a poster presentation on "Correlating a quantitative parameter for nuclear NF-kappaB translocation with in vivo immunosuppression". **Joseph Tario** will present a poster presentation on "Assessing Cytotoxic Potential Using Flow Cytometry".

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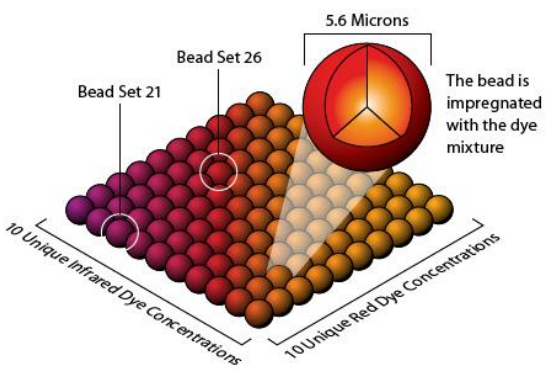
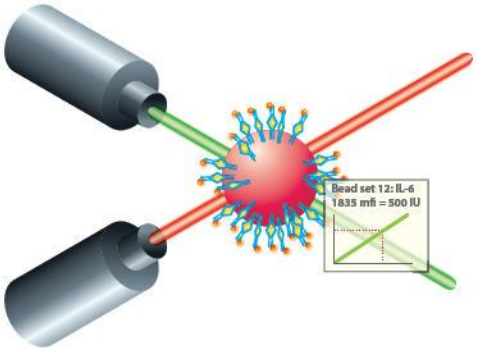
Service / Application High Lights

For this inaugural issue of the news letter we will give a brief overview of the Luminex research services in the facility.

- Luminex may be a misleading term The actual assay refers to a soluble bead array-based multiplexing technique which in large part was developed right here at RPCI under the leadership of Carleton Stewart. The Luminex Corp. is the leading company that has developed and commercialized various applications of this technique.

- The basis of the Luminex assay is the xMAP technology which color-codes microspheres with 100 different combinations of shades of 2 dyes creating 100 bead sets that can be discriminated by flow cytometry. Each bead set can then be coated with an analyte specific capture reagent (antibody) and binding of the reagent can then be quantified using reporter antibodies, in the same manner as ELISA assays. Thus, xMAP technology allows multiplexing of up to 100 unique assays within a single sample. It detects 3 colors: 2 colors for the identification of the unique bead and one color to quantify the analyte present on the bead.





Images from www. panomics.com

In our facility, the assay is performed in a 96-well plate format of which commonly 82 wells are available for sample analysis (14 wells are used for internal standards). Thus in principle, 100 analytes can be assayed on 82 samples in a single experiment.

- Common applications: protein expression profiling (detection of cytokines, chemokines and phospho-proteins in suspension eg. serum, cell culture supernatant, cell lysate). For a full repertoire of available analytes please see the websites of our preferred vendors: www.invitrogen.com or www.millipore.com and search for “luminex”.

- Due to re-organization, the Luminex services were temporarily suspended but were re-started in November of last year. Since the re-organization, the average turn-around time for sample processing and reporting is 2 weeks. Please contact the facility for details on how the samples should be presented. Actual costs for the assay are dependent on a number of variables including the number of samples and the number and specific characteristics of the analytes you are interested in. For a detailed quote on your specific study, please call the facility.

- New applications in the pipeline: xTAG and FlexmiR. The latest applications of the soluble bead array technology are molecular-based assays in which the 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). We are currently in the process of evaluating the potential interest in these applications amongst our users. If you have specific interest in having these applications available we encourage you to contact the facility.

- Luminex facility contacts:

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Mike Rickert

Ree Dolnick