ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, April - June 2014

What's New?

- If you hadn't noticed yet, the **USB ports of the flow cytometer work stations have now been locked** to prevent spreading of viruses and worms through the use of infected USB drives which had become an issue of late. The data can be transferred from the workstation directly to your PI's assigned folder on the Institute server (X9000, soon to be replaced by 3PAR). Informational leaflets on how your PI can grant you access to the PI folder are available in the magazine rack in the user room or you can always ask for help from one of the flow personnel.
- In consideration of the current funding climate, **there will not be any pricing changes** for the majority of the flow and image cytometry services **for fiscal year 2015**. The only **exceptions are the sorting facility charges** that use the **ARIA sorters** for which a **3% increase** will be implemented at the start of FY2015.
- The facility will work with DVS Sciences to perform the **first CYTOF experiments originating from RPCI**. CYTOF is a **mass-spectrometry** based system that uses antibodies conjugated to rare earth elements for immunophenotyping rather than fluorescent tags commonly used in flow cytometry. The advantage of this approach is that the number of **analytes** that can be assessed simultaneously **far outnumber (>30)** what is possible by **conventional flow cytometry**. The long term plan is to use the preliminary data to support a shared instrument grant application (for FY 2016) to obtain a CYTOF for RPCI. In the meantime, RPCI researchers have access to the CYTOF technology through a collaboration with the University of Rochester.
- Our masters student **Kah Teong Soh** has been selected to present his work on **RNA in situ hybridization** using flow cytometry as an **oral presentation** at this year's **CYTO** meeting. Nice work Kah! For more info see this edition's application highlights.
- Our **student**, **Hillary Figler** (University of Rochester), who participated in last year's **RPCI summer research program** was accepted to present her work that she performed at RPCI at the 2014 **National Conference of Undergraduate Research** (NCUR) in Kentucky (April 3-5). Congratulations Hillary!
- **REMINDER:** the **only reagent tubes** that should be used on the flow cytometers are **the BD Falcon cat # 352052 5ml 12x75 mm style tubes**. Similar tubes from **other vendors** may look the same but the rings at top of the tubes that ensure an airtight fit to the sampling probe are just sufficiently different from the BD tubes that they **cause damage to rubber seal** that engages these rings. If you use the other tubes, you have to exert excessive force to put the tubes on and although you may successfully complete your acquisition, the damage done to the rubber seal will affect the users who use the machine after you.
- Having participated in many of the excellent **Buffalo Immunology Conferences** and considering the many flow cytometry-based presentations, **starting with the 2015** meeting the **Flow and Image Cytometry** Facility will **sponsor an award** for the student presentation (poster or oral) with the **best innovative flow or image cytometry applications**.

Recent Publications / Grant Funding

- Zhao W, **Minderman H**, Russell MW. Identification and characterization of intestinal antigen-presenting cells involved in uptake and processing of a nontoxic recombinant chimeric mucosal immunogen based on cholera toxin using imaging flow cytometry. Clin Vaccine Immunol. 2014 Jan;21(1):74-84. doi: 10.1128/CVI.00452-13. Epub 2013 Nov 6.
- Belyea BC, Xu F, Pentz ES, Medrano S, Li M, Hu Y, Turner S, Legallo R, **Jones CA**, **Tario JD**, Liang P, Gross KW, Sequeira-Lopez ML, Gomez RA. Identification of renin progenitors in the mouse bone marrow that give rise to B-cell leukaemia. Nat Commun. 2014 Feb 18;5:3273. doi: 10.1038/ncomms4273.
- Glenn ST, **Jones CA**, Sexton S, Levea CM, Caraker SM, Hajduczok G, Gross KW. Conditional deletion of p53 and Rb in the renin-expressing compartment of the pancreas leads to a highly penetrant metastatic pancreatic neuroendocrine carcinoma. Oncogene. 2013 Dec 2. doi: 10.1038/onc.2013.514. [Epub ahead of print]
- **Maguire O**, **Tario JD**, Shanahan T, **Wallace PK**, **Minderman H**. Flow cytometry and solid organ transplantation: a perfect match. Immun Invest 2014, in press.
- Berenson C, Kruzel RL, Eberhardt E, **Dolnick R**, **Minderman H**, **Wallace PK**, Sethi S. Impaired innate immune alveolar macrophage response and the predilection for COPD exacerbations. Thorax March 2014, in press.

Courses / Presentations / Meetings

- **29th annual** Congress of the International Society for Advancement of Cytometry (**CYTO 2014**). Fort Lauderdale May17-21, 2014. For more info see:**www.cytoconference.org**
- **6th annual ImageStream Interest Group Meeting.** June 2nd, 2014, University of Rochester Medical Center, Class of '62 Auditorium. This is a 2 day **free** annual meeting which rotates between RPCI, URMC and Cincinnati Childrens Hospital. The first day consists of presentations by researchers using the ImageStream technology. During the second day interested parties can schedule one-on-one time with ImageStream application specialists to go over specific analysis approaches / questions relevant to their own generated data files. For more information see: www.amnis.com or contact Hans at x1162 or hans.minderman@roswellpark.org
- **23rd annual meeting** of the Great Lakes International Imaging and Flow Cytometry Association (**GLIIFCA**) Sept 19-21, 2014, Oconomowoc, WI. For more information see: www.gliifca.org
- **10th annual Western New York Flow Cytometry User Group Meeting.** University of Rochester Medical Center, Class of '62 Auditorium. For more information see: http://www.urmc.rochester.edu/flow-core/wnyfug/

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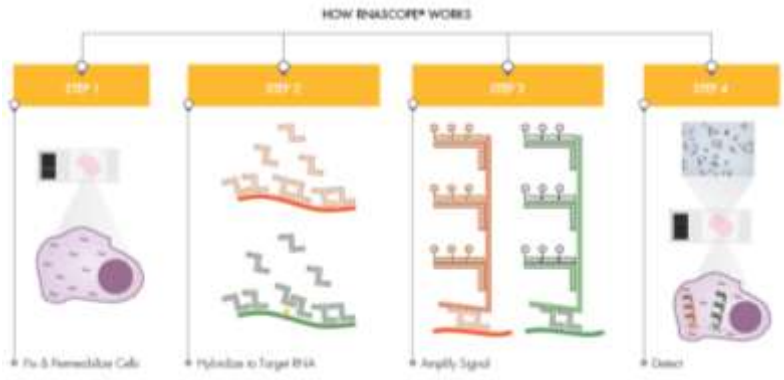
Service / Application Highlights

RNA IN SITU HYBRIDIZATION ANALYZED BY FLOW CYTOMETRY

Background:

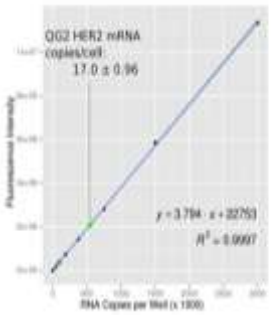
- *In situ* signal amplification method – RNAScope - for the analysis of intracellular mRNAs in single cells by flow cytometry
- Uses a DNA branching technique to amplify signal
- Specificity of amplification is achieved by the design of paired 'Z' probes which are designed to hybridize to flanking sequences. Only the matched pairs of Z-probes will be amplified.
- Subsequent detection by flow cytometry
- Sensitivity: a single RNA transcript
- Investigators
  - Kah Teong Soh
  - Paul Wallace
  - Emily Park (BD Sciences)
  - Mary Beth Hanley (BD Sciences)

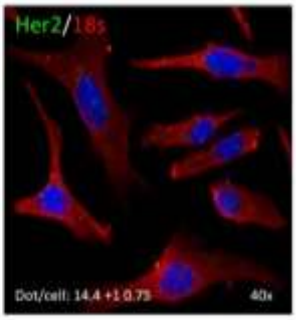


http://info.acdbio.com

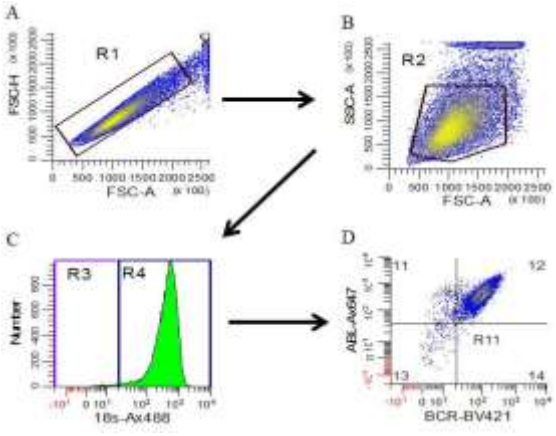
Linearity and Quantification

The plot to the right demonstrates the linearity between the fluorescence intensity signal following in situ hybridization and amplification using a Her2 specific probe set in RNAScope and the number of mRNA copies/cell (from Wang et al. J Mol Diag 14(1)22-29, 2012). In the fluorescence image, individual copies of mRNA are shown as green dots.





Flow Cytometric quantitation of total BCR and ABL mRNA expression



The histograms to the left show the hierarchical gating strategy for RNAScope analysis of K562 cells hybridized with 18s ribosomal RNA (labeled with AlexaFluor 488), BCR (labeled with Brilliant Violet 421) and ABL (labeled with AlexaFluor 647) RNA probes. This strategy was designed in the context of studies aimed at quantifying total BCR and ABL transcription activity during the transition of MDS to AML.

For more information:

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