

ROSWELL PARK CANCER INSTITUTE FLOW AND IMAGE CYTOMETRY FACILITY NEWSLETTER, October-December 2012

What's new?

- In response to requests by several users, the facility has purchased **6 licenses of FlowJo vX**. Each license is linked to a USB 'dongle'. Two of the dongles are connected to the 2 analysis workstations in the flow cytometry user room, making the software **freely available to all users on those workstations**. Two dongles are used for the facility's personnel which leaves two dongles for those who would like to have this software available in their own lab. **If you are interested in purchasing these dongles at considerably reduced cost** (our cost) please call **AnnMarie Eckel at x8471**. Requests will be honored on a first come first served basis.
- The facility will host a **FlowJo introduction course on December 8** presented by Dr Nicholas Ostrout, application specialist from FlowJo (See details under Courses). If you are planning to attend this course it is highly recommended to watch the **online tutorials on basic operation and 8 color PBMC experiments** which are available at <http://www.flowjo.com/home/tutorials/>
- **Orla Maguire** was awarded the prestigious **Alex Nakeff Young Investigator Award** at the recent meeting of the Great Lakes International Imaging and Flow Cytometry Association (**GLIFCA**) for her presentation on 'Image cytometry-based detection of aneuploidy by FISH-IS'. Congratulations, Orla!
- **OUCH!** Unfortunately the **63x objective on the confocal microscope** was **cracked beyond repair**. These objectives are very expensive (~\$8,000!) and it can sometimes take a few weeks before they can be replaced. These accidents happen when the objectives are switched without proper clearance from the stage or when the objective is moved up too close to the edge of the stage. **Please observe extreme caution when using the 63x objective on the confocal microscope**. In response to the positive feedback we have received and to keep your cost down, we would like to continue to make this equipment available to our users without mandatory operator assistance, however, we can only continue this practice in the absence of these types of preventable but expensive accidents. Please be considerate to the equipment and your fellow users and don't be afraid to ask for help if you are not sure how to operate this or any of our other equipment.
- The **CyTOF technology will soon be available** to interested users. CyTOF is a mass spectrometry-based cytometer that has the potential to analyze up to **100 isotope labels** in a single cell (**without compensation!**). An example of how this technology can be used in an immunophenotyping panel of 31 labeled antibodies was published in Science (Bendall SC et al, Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science 332(6030):687-689, 2011). The CyTOF technology will be available to our users through a **regional consortium with the University of Rochester**. As per the date of this publication, the instrument has arrived in Rochester and the final installation is awaiting the completion of the room where it will be located. If you are interested in learning more about this technology please visit the manufacturer's website www.dvsscience.com. If you are interested in using this new technology for your experiments, please contact our facility at x 8471 and we will help you make arrangements with the University of Rochester.

Recent Publications / Grant Funding

Doig CL, Singh PK, Dhiman VK, Thorne JL, Battaglia S, Sobolewski M, **Maguire O**, O'Neill LP, Turner BM, McCabe CJ, Smiraglia DJ, Campbell MJ. Recruitment of NCOR1 to VDR target genes is enhanced in prostate cancer cells and associates with altered DNA methylation patterns. Carcinogenesis. 2012 Oct 20. [Epub ahead of print]

Maguire O, Pollock C, Martin P, Owen A, Smyth T, Doherty D, Campbell MJ, McClean S, Thompson P. Regulation of CYP3A4 and CYP3A5 expression and modulation of "intracrine" metabolism of androgens in prostate cells by liganded vitamin D receptor. Mol Cell Endocrinol. 2012 Nov 25;364(1-2):54-64

Hampras SS, Nesline M, **Wallace PK**, Odunsi K, Furlani N, Davis W, Moysich KB. Predictors of immunosuppressive regulatory T lymphocytes in healthy women. J Cancer Epidemiol. 2012;2012:191090. Epub 2012 Aug 26

Courses / Presentations /Meetings

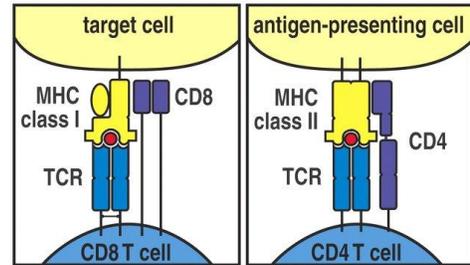
- **Orla Maguire** served as an international expert reviewer on imagestream cytometry for grant proposals submitted to the French "Fondation pour la Recherche Medicale".
- **Paul Wallace** served as reviewer on an NIH site visit of the Experimental Transplant Immunology Branch
- **Paul Wallace** spoke on the "Reconstitution of Immune Cell Subsets Following Bone Marrow Transplant" at the Scleroderma: Cyclophosphamide or Transplantation meeting in Potomac, MD.
- **Hans Minderman** has been invited to speak on ImageStream cytometry at the December meeting of the Research Triangle Cytometry Association, University of North Carolina at Chapel Hill
- **FlowJo Training Seminar, Thursday November 8**, Cancer Cell Center Rm ccc314, 10:30 am-1:30 pm. For more detailed information This free seminar will be taught by **Dr Nicholas Ostrout, application scientist, Tree Star Inc.** Lunch will be provided but if you plan to attend **please RSVP by Monday 11/5 to AnnMarie Eckel, x8574 or annmarie.eckel@roswellpark.org**.

Service / Application High Lights

DETECTION OF ANTIGEN SPECIFIC T CELLS USING DEXTRAMERS

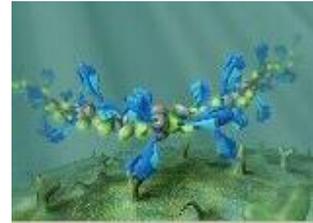
Background:

Antigen recognition and T cell responses are mediated through T cell receptor interaction with specific peptide-MHC molecule complexes presented on the surface of antigen-presenting cells or target cells. The peptides can be derived from tumor proteins, viral proteins, bacterial proteins, normal proteins, etc. This specific interaction between T cells and MHC-peptide complexes is the basis for the detection of antigen specific T cells using MHC multimers. Our facility, through Paul Wallace, has worked closely with the developer, Immudex, in optimizing this technology for specific flow cytometry applications.



MHC multimers:

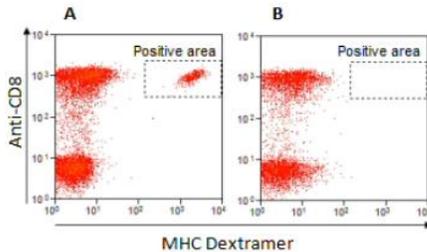
MHC multimers are fluorescently-labeled reagents consisting of a dextran backbone to which multiple MHC-peptide complexes are conjugated. Tetramers and dextramers are two different MHC-multimers that differ by size, the number of conjugated peptide-MHC complexes and their ability to detect low affinity antigen-specific T cells. Binding of the MHC multimers to corresponding antigen-specific T cells is commonly assessed by flow cytometry and is very useful to enumerate the presence of antigen-specific T cells.



Applications:

- Monitoring vaccine studies
- Monitoring effect of Immunotherapy
- Determination of immune status (e.g. in connection with transplantation and other immunodeficiency)
- Monitoring T-cell responses in infectious disease, cancer, autoimmunity and other malignancies
- Detection of antigen-specific T-cell responses in immunological research

Example: Detection of CMV-specific T cells

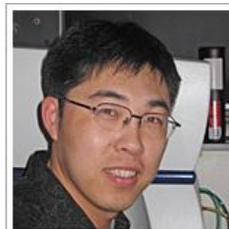


Whole blood from CMV+ and HLA-A*0101 positive donor were stained with the HLA-A*0101 (VTEHDILLY) Dextramer (A) and the negative control Dextramer (B). Live lymphocytes, CD3+ cells are shown. (From: www.immudex.com/CMVdextramer.htm)

For more information please call:

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