ROSWELL PARK CANCER INSTITUTE
FLOW AND IMAGE CYTOMETRY FACILITY
NEWSLETTER, October-December 2013

What's New?

- the **autoMACS** is now on our calendar system and can be accessed through http://rpciflow.calendarhost.com/cgi-bin/calweb/calweb.cgi, select "Miltenyi autoMACS" from the menu. **Please use this to sign up and to check availability**. You will not be charged for the calendar reserved time for the autoMACS.
- A change has been implemented in the **billing for the autoMACS**. To accommodate users who run many autoMACS separations each week, the **maximum charge will now be 2 daily fees per each 14-day cycle**. A cycle is started when a column is installed. Each daily use beyond the two will be booked as "non-billable" and will appear as such on your bill. This change is effective immediately, with the October billing cycle.
- **Shared Resources Survey.** Many of you will have received an invitation to participate in an on-line survey regarding the Shared Resources at RPCI. As one of the Resource Facilities we encourage you to participate in the survey as it will help us to understand how we may improve our services. Survey participants are entered into a drawing to be one of 5 winners of a $100 gift certificate from Wegman's, Target, Wal-Mart, Noco, or Tops.
- The **network version of WinList has been moved to a new location**. The old share \\F823HG1 no longer exists.The path is now located on the X9000:\\flowcyto\flowresearch$\VeritySoftwareHouse\Winlist 7.1.1 In order to access the new location delete your old shortcut by dragging it to the Recycle bin. You may need to log out of your computer and log back in to pick up folder group membership. Once within the above directory, right click on the WinList3D.exe file and use "Send to" Desktop (Create Shortcut) in the menu display. Alternatively, there is a script called *WinList3D_NetworkSetup* in the same directory that automatically creates the shortcut for you and verifies the required DLL files are up to date but this requires Roswell Admin rights to run (only needs to be run once).To date there are **40 network licenses available for Flow Cytometric data analysis**: 10 for FCSExpress version 3, 10 for FCSExpress version 4, and 20 for WinList version 7.1.1. All are located on the X9000 network share and should eliminate resource shortages experienced on the former \\F823HG1
- **Paul Wallace** presented at the November meeting of the **New England Flow Cytometry User Group** on "Monitoring Immune Responses with MHC Class I Multimers: Focus on CMV Immunity in Patients Following Allogeneic Hematopoietic Stem Cell Transplant."
- Oh no, **AnnMarie Eckel**, our administrative assistant, changed jobs! AnnMarie was offered a great job opportunity in the new Gates Vascular Institute. We'll miss her dearly and wish her all the best in this new exciting opportunity. Please bear with us while we are searching for a candidate to take over AnnMarie's responsibilities!
- In October, **Paul Wallace** presented at **Strong Memorial Hospital** in Rochester on "How to Diagnose PNH: a Progressive Life-Threatening Disease."
- As per the **Institute's Shared Resources** policy we are required to collect information on **grant account numbers** for services you are requesting. According to the policy you will be given the opportunity to correct or dispute any errors on the invoices that you will receive, including account #'s, but it is **essential that correct account information**, to the best of your knowledge, is given at time of work request. Any changes (eg due to incorrect account numbers) requires re-invoicing and a preventable duplication of work on the part of the Shared Resources Administration. For laboratories that have **multiple grant accounts** to which different services should be charged we offer the option of creating **different user accounts** associated with different grant account #'s. That way you will have the flexibility to instruct your personnel to us the correct logins when services are requested.
- **Paul Wallace** presented on 'Implementing a Flow Cytometric Assay for Minimal Residual Myeloma' during the October meeting of the **International Clinical Cytometry Society (ICCS)**. At the meeting, Paul was also was **appointed to a Counselor** position of the ICCS.
- **Reminder: The data from the flow cytometry workstations are purged monthly**. It is the **users' responsibility** to make sure the data is transferred off the workstation before the monthly purge. Note that the **new path to the Roswell X9000 data storage is \\flowcyto\flowResearch$\PI<your PI Name>**. If you need help accessing this central data storage please contact Ed Podniesinski or any of the personnel assigned to the user room for the day.

Recent Publications / Grant Funding

- **Maguire O**, Tornatore KM, **O'Loughlin KL**, Venuto RC, **Minderman H**. Nuclear translocation of nuclear factor of activated T cells (NFAT) as a quantitative pharmacodynamic parameter for tacrolimus. Cytometry A. 2013 Oct 17. doi: 10.1002/cyto.a.22401. [Epub ahead of print]
- Valerio MS, **Minderman H**, Mace T, Awad AB. β-Sitosterol modulates TLR4 receptor expression and intracellular MyD88-dependent pathway activation in J774A.1 murine macrophages. Cell Immunol. 2013 Sep 7;285(1-2):76-83. doi: 10.1016/j.cellimm.2013.08.007. [Epub ahead of print]
- Baysal BE, De Jong K, Liu B, Wang J, Patnaik SK, **Wallace PK**, Taggart RT. Hypoxia-inducible C-to-U coding RNA editing downregulates SDHB in monocytes. PeerJ. 2013 Sep 10;1:e152. doi: 10.7717/peerj.152.

Courses / Presentations / Meetings

- **November 21**, 2013 will be the **Shared Resources Awareness Day**. Staff, Postdocs and Students are invited to the Gaylord-Cary Conference room from 11:00 am-1:00 pm (Lunch to be Provided). There will be a Faculty Reception from 4:00 pm-6:00 pm. Come and learn what NEW ADVANCEMENTS and SERVICES our Shared Resources have to offer you!

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Service / Application Highlights

NEW LUMINEX-200 MILLIPLEX CYTOMETER

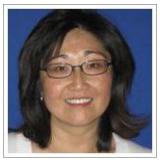
A new Luminex-200 cytometer has arrived.
This instrument was purchased to replace an old Luminex 100 which could not be upgraded to use the new xPONENTsoftware required to analyze magnetic bead-based assays. The Luminex 200 features improved acquisition time and design changes making instrument setup easier but it still uses the same basic principle of the luminex bead-array assay. The addition of the Luminex 200 increased our capacity and range of assays. In that regard, in response to the increased demand for the luminex analysis, Kitty de Jong is now also trained to perform the data acquisition and analysis and will share this responsibility with Ree Dolnick.

Configuration:
The instrument setup will remain the same as our contemporary and currently operational Luminex 100. The capacity for multiplexing will remain up to 100 analytes per sample. xPONENT version 3.1software will provide the user interface to control Luminex 200/100.

Applications:
Bead based multiplex assays including

- Proteins (*i.e. Immunology , cancer biomarkers, bone metabolism, neuroscience, cardiovascular)*
- Nucleic Acids (mi RNA, mRNA, DNA)
- Signaling Pathways and Phosphoprotein Detection

For more information visit http://www.rpciflow.org/services/multiplex.html or call Ree Dolnick (x8235) Kitty de Jong (x8416)



NEW UV EXCITABLE DYE

There's a **new UV excitable dye** that has recently come to the market. Please see the manufacturer's information to the right. This is an exciting development for multicolor panel design because it expands the utility of the relatively underused 355 UV laser line. The BUV395 is the first member of the so-called BUV dyes that is commercially available. Development and release of additional members of the BUV family are in the works. At the recent

Features

- UV (355 nm)–excitable dye
- Provides great population resolution
- Virtually no spillover into any other detector
- More choice and flexibility for multicolor panel design

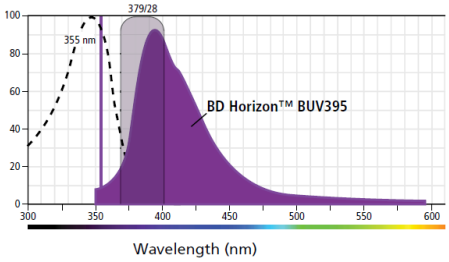


Figure 1. Excitation and emission profile of BUV395.
Recommended filter: 379/28.

	Stain Index	
Specificity	BUV395	FITC
Human CD4	223	52
Human CD56	21	10

Table 1. Stain index comparison of CD4 and CD56 stained with BUV395 and FITC reagents.
Relative stain index values are dependent on instrument configuration including lasers, filters, and laser power.

BD Horizon™ Brilliant Ultraviolet™ 395 (BUV395) is a UV-excitable dye that has been developed exclusively by BD Biosciences to expand the multicolor capabilities of flow cytometers equipped with a 355-nm laser. Not only does this dye provide an additional color, it is an optimal dye for multicolor flow cytometry because it has little to no spillover into any other detector.

A bright dye for the UV laser
Currently available UV-excitable fluorochromes are so dim that they are not practical for immunophenotyping applications. However, BUV395 is bright, providing great resolution for bright markers such as CD4 as well as dimmer markers such as CD56 (Figure 2). In many cases, BUV395 reagents are brighter than FITC reagents (Table 1).

With an excitation max of 348 nm and an emission max of 395 nm, BUV395 can be excited by the 355-nm laser and detected with a 379/28 filter (Figure 1). This dye is not recommended for instruments equipped with a 375-nm laser.

Virtually no compensation requirements
BUV395 is an optimal dye for multicolor flow cytometry because it has virtually no spillover into any other detector (Table 2). Additionally, other fluorochromes have little to no spillover into the BUV395 detector. BUV395 allows you to add an additional color to a panel without increasing the complexity of compensation requirements.

More choice and flexibility for multicolor panel design
BUV395 provides more choices for multicolor flow cytometry, making multicolor panel design easier and more accessible. Using BUV395 with other fluorochromes offered by BD Biosciences allows you to detect 15 fluorescence parameters from a single sample.

Managing spillover between reagents can be one of the more difficult elements of multicolor panel design. By spreading markers over multiple lasers, the overall compensation requirements of a panel can be reduced. For example, by assigning one marker to each laser, a 5-color panel with minimal compensation requirements can be run on an instrument equipped with UV, violet, blue, red, and yellow-green lasers. The availability of UV-excitable reagents makes it easier to design panels with less spillover. This diminishes one of the most difficult elements of multicolor panel design.