

This document contains email updates and notices sent out to the Ragon Institute flow cytometry core facility distribution list, in reverse chronological order (most recent messages first).

From: Waring, Michael
Sent: Tuesday, December 15, 2009 11:54 AM
Subject: Flow update 12/15/09

1. The next Intro to Flow class will be Thursday January 7 2010 from 12-2 in room 5216.
2. Do not export to flash drives
3. Caps for tubes in the sorter room
4. on Vacation starting next week.
5. Compensating viability dyes with beads
6. Flowjo dongles AND diva dongles
7. Billing will be going out soon.

1. The next flow class will be Thursday January 7 2010 from 12-2pm in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flow_cytometry.html and click on the "Training" link in the menu on the left. It is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

2. When exporting from Diva, do not export directly to external storage (flash drive or external hard drive) as you may lose files in the process. This has been confirmed by BD, so be sure to export to the D drive into a folder for yourself with your full name, and then copy to other storage media to transfer to your computer.

3. All samples for sorting should be transported in SEALED tubes--if you use filter cap tubes, please replace the filter cap with a sealed cap before taking the tubes out of the hood. I have purchased a case of caps, so if you need some bring a beaker and you can have some. Or the part number from Fisher is 14-959-48, about \$200 for a case of 2000. You can also bring the filter cap tubes with you and transport your samples in eppendorf tubes or regular 12x75mm tubes, and we can filter the samples right before running, as we sometimes need to filter them again anyway.

4. I am not here from Sat Dec 19 thru Sun Jan 3, so any core issues and sort appointments should be directed to Adam (atchicoine@partners.org)

5. For compensation of amine reactive viability dyes (Live/Dead from Invitrogen, etc), Invitrogen now makes beads that will bind them (normal comp beads bind the antibody, these are coated with amine groups to bind the amine-reactive dyes). Product link here <http://products.invitrogen.com/ivgn/product/A10346?CID=Search-Product>
Or you can research other companies to see if they have started making a similar product. Let me know how they work for you.

6. A reminder that we have Flowjo dongles to loan out, and a new update that we also have several Diva dongles to loan if you would like to work with diva on your personal computer (PC

only, Diva does not run on mac), but you cannot set up your experiments on a separate workstation, as it will not have the same parameters as the instrument (in fact, the instrument has to be turned on as the computer gets that information from the instrument itself) but you can do analysis etc..

7. We hope to have the new flow/imaging core fund set up shortly, and billing for usage since July will soon follow. I apologize for the delay, and we will gladly work with you to resolve any issues with closed funds etc. If you need to use a separate fund for imaging vs. flow, please let me know so that we can keep them updated. I will be sending a separate notice to all PI's as well. You should receive a packet of invoices from July thru December for review, and will have 30 days to contact us to resolve issues (info will be on the invoice).

Let me know if you have any questions.
Mike Waring

From: Waring, Michael
Sent: Monday, December 07, 2009 3:19 PM
Subject: Ragon Flow Update 120709

1. Sealing sample tubes for transport
2. 4L Database broken file links
3. Exporting from diva
4. Exporting part 2: folders
5. Useful flow information on Invitrogen

1. For all sort users: A reminder that tubes should be SEALED for transport, so after filtering your samples you should swap out the filter cap for a solid cap, and transport them in a secondary container (either a tupperware type container or a bin).

2. In clearing out the database on the 4 laser this morning, we discovered that there were 6 GIGS of data in the database "Data" folder on the D drive that did not have a corresponding file in Diva--some kind of "broken links" or something. I cleaned them out and backed them up, but they do not have an experiment associated with them so we weren't sure who they belonged to. They do have the date of acquisition on them, so we are going back and looking up who was logged in at that time, and it seems they are caused when the software quits unexpectedly (which results in no "log out" time in the user log), we're going to look up who they belong to and make sure you are not missing any files. If any of the files were acquired under your profile, I will let you know. It is causing a disconnect with all of the files in the experiment, not just the one that was active when the software crashed.

3. Exporting on Diva: when you export as an experiment, a copy of your workspace is saved along with the data files, which are just numbered and if you use them in Flowjo, the tube name doesn't come up by default--the file name does so they are just numbered. Also H and W measurements for the same parameter (if you collected them) are switched. If you export as FCS, it names the file based on the tube name, and should copy to Flowjo correctly but none of your layout elements are preserved. I usually export as an experiment, and then export as fcs to the same target file (so exporting as experiment to "D>Mike Waring's folder" creates a folder "4 colors 120709" and copies the files to that folder, and then exporting as FCS to "Mike Waring's folder" will put the fcs export files in that same folder "4 colors 120709"). If you do fcs first and then experiment export, it will tell you the second time that an experiment by that name already exists (the folder for the fcs export), so do experiment first and then FCS.

4. Export to your own folder on ALL Instruments, do not use the default "BDEExport>Experiment" or "BDEExport>FCS" if they come up. If you don't have a folder on the D drive, please make one. After exporting, please check the size of the experiment you exported--go to your folder, right-click on the experiment folder, and select "properties". If it is more than 500MB please delete the experiment from Diva to free up space.

5. One of the options on the search engine for Invitrogen is to search their handbook: Probes.invitrogen.com, click search, and check "Molecular Probes Handbook", the last item in the right hand column of the list, for whatever topic you were interested in (Cell cycle, apoptosis, etc) and you will find tons of information and links to kits to be used in those assays. Hopefully you'll find it helpful.

Let me know if you have any questions.

Mike

From: Waring, Michael
Sent: Friday, December 04, 2009 10:07 AM
Subject: Flow update 12/4/09 VIRUS WARNING

1. "Autorun.inf" virus warning
2. CD and DVDs for flow data
2. floor cleaning
3. Sorter down time

1. ****IF YOU GET THE AUTORUN VIRUS FROM ONE OF THE CYTOMETER COMPUTERS PLEASE TELL ME!****

We have recently had a virus on the cytometer computers, which installs itself on autorun devices (flash drives, etc) and then gets installed on computers that it is subsequently used on. It will show up as a hidden file, "autorun.inf"-- if you go to "tools" and "folder options", to the "view" tab, you can choose to show hidden files. For a flash drive you can just delete it and it will be gone, the best way is to load it on a mac so that your PC doesn't get infected... if your pc is infected it will show up on all of your hard drives (usually C, plus the D drive on the cytometer computers). You should have "on access scan" enabled for your mcafee virus software that partners provides and this will block access to infected flash drives should you connect one to your computer. Make sure your antivirus is updated.

If you used a flash drive on that computer, please scan any PC's that you subsequently used that flash drive on. Partners build computers should already have antivirus installed on them, and Partners helpdesk provides antivirus software ***FOR HOME OR NON-STANDARD DEVICES ONLY*** if you don't have any on your computer, go to helpdesk.partners.org and then in "Useful Links" on the left side of the page, go to "virus information" and then follow the instructions. There are OTHER programs that use a file called "autorun" so don't just go through and delete all occurrences of that file on your computer.

2. I am going to order a bunch of CDs and DVDs for use in the core, please burn your data instead of using flash drives. If you **MUST** use a flash drive, you must erase it first using the iMac in the flow analysis area. We may purchase a few flash drives for the core that can be used to transfer files to your computer and then will be erased before using them on the cytometers again.

3. Next Tuesday, 12/8, the floors in the lab are scheduled to be cleaned, so the 3L, 5L and Calibur will be unavailable after 5 pm on that day. Please do not book any time on them until the next morning.

4. Next week we are getting an upgrade to the sorter, so it will be unavailable all week while the job is completed.

If you have any questions please let me know.
Mike

From: Waring, Michael
Sent: Wednesday, November 25, 2009 3:01 PM
Subject: Ragon Flow core update 112509

Happy Thanksgiving!

Adam and I will not be here on Friday, November 27th, and Adam will not be here on Monday, November 30, and I will be in meetings on Monday until lunch time, so our weekly Monday QC/cleaning will be done from 12-2 on all instruments. Plan accordingly.

I've added a document to the "Core Documents" portion of our website that collects all of my core updates from the previous year (back to March). Hopefully someone will find it useful.

I've also been working on updating the links etc on the website, some of which have become outdated (BD redid their whole site, so all of the BD links became dead). If you find any misdirects or dead links (or things like the link opening in a frame instead of a new window) please let me know! (and be specific about where the link occurs, there are some documents linked from multiple pages in our site)

The sorter upgrade will take place the week of December 7th, there will be NO SORTING DURING THAT WEEK while the upgrades are performed and then tested. Let me know if you have any questions.

Mike

-----Original Message-----

From: Waring, Michael
Sent: Thursday, November 19, 2009 3:58 PM
Subject: Flow update 111909

1. Human samples must be fixed, human sorts must be registered with Biosafety.
2. Schedulebook accounts deleted
3. Take bins and notebooks with you.
4. NO SAMPLES BY THE ANALYSIS STATIONS
5. BRDU proliferation assay alternative
6. next Flow class in January

1. Just a reminder, according to MGH biosafety policy, ALL Human samples must be fixed before running them on the LSRs or the Calibur! Either 2% PFA (paraformaldehyde) or 70% EtOH for at least 30 minutes. Let me know if you have any questions.
As I announced in a prior email, all sorts of human materials or of pathogens requires registration with COMS, if your sort request does not have a COMS number entered (new form on the website), it will be denied!

2. Schedulebook accounts--I am going to change the process for people that have "Read only" accounts on schedulebook, mostly those that only use the sorting services of the core. If you had a schedulebook account for checking the sort calendar, or if your account has been unused since I created them in May, they have been deleted. To check for sorting availability, use the "guest" account as described in the sorting guidelines on our website. If you need an account for reserving the other instruments, let me know and I will create one.

3. We are amassing quite a collection of sample transport bins and Notebooks in the core. Please remember to take them with you when you leave, and also put your name on them! I am happy to contact you to let you know they have been found, or return them to the proper TC room, but most don't have any identifying information in them.

4. PLEASE No samples in the analysis area! This is office space and not lab space. Someone left a box of samples in the entryway to the analysis stations. Take them back to your lab or dispose of them properly before using the analysis stations.

5. New product for cell cycle/proliferation assays: Brdu cell proliferation assay alternative www.invitrogen.com/clickit

6. Next flow class will be in early January. I will set the date soon, let me know if you are interested in attending so I can keep you informed.

Check our website for documents and updates,
www2.massgeneral.org/aids/flow_cytometry.html

Let me know if you have any questions.
Mike

From: Waring, Michael
Sent: Monday, November 09, 2009 3:46 PM
Subject: Sorting update--required reading

Sorry for the multiple flow core updates today, this message only applies to users of the sorter.

For all users of the Ragon Institute sorting services, failure to read the following announcements may impact our ability to perform future sorts for your project:

1. We will soon be upgrading the sorter with new fluidics and a new flow cell, during which time the sorter will be unavailable. We will be down for a whole week, I will send out a notice as soon as the date is set. Hopefully this will help us avoid future down times and improve the reliability of the sorter.

2. According to Boston and Cambridge city regulations, all work with human samples or any pathogens must be registered with an Institutional Biosafety Committee (IBC), which for MGH is the Committee on Microbiological Safety, or COMS, <http://www.hms.harvard.edu/orsp/coms/about.htm> for approval of the work and the protocols to

be performed. They have recently decided to register all cell sorters in the hospital, and require registration of any projects performing cell sorting on human samples.

--If you already have approval for cell sorting in your current application, your COMS number will have to be provided on the sort request forms that you fill out to schedule a sort with the Ragon Institute's sorting facility.

--If you or your PI is already COMS registered but sorting by flow cytometry was not covered in the original application, then an amendment will need to be submitted for approval to add this technique to your project

--If for some reason you are not registered then a new application is required.

Sorting of fixed cells or of non-human non-primate cells does not need to be registered. All unfixed human cells, including human cells transplanted into mice, would need to be registered.

All pathogens, animal or human, would also need to be registered.

I have posted a new sort request form and instructions on our website (www2.massgeneral.org/aids/flow_cytometry.html and select the "FACS Aria (sorting)" link in the menu to the left) and will post an updated "guidelines" which will explain it fully. If you are using human samples, you must include your COMS number in the space on the sort request form. For the Walker lab/Ragon Institute, this is Protocol 09-175. If you do not have or do not know your COMS #, please contact Anne Sallee, the MGH Biosafety Officer:

Ms. Anne Sallee
Environmental Health & Safety
West End House
16 Blossom Street
Boston, Massachusetts 02114
E-mail: ASallee@partners.org
Phone: 617-724-4579
Fax: 617-726-5126

Please contact me if you have any questions

Michael Waring

Ragon Institute of MGH, MIT, and Harvard

Flow cytometry core facility administrator

mwaring@partners.org

617-643-0891 (lab)

From: Waring, Michael
Sent: Monday, November 09, 2009 3:43 PM
Subject: Flow Core update

A reminder, the next Intro to flow class will be this Thursday at noon in 5216, see the website for details and let me know if you plan to attend.

www2.massgeneral.org/aids/flow_cytometry.html and click on "Training"

I just want to remind everyone using schedulebook that there is a 2 hour limit during working hours (9 to 6), and no more than 2 reservations between 2 and 6 per week. Schedulebook has a "reservation time limit" built in, I had set it to 4 hours so people using it later could book longer times, but I've changed it to 2 hours. If you need more than 2 hours, you will have to create a second event, but only if your time is after 6 pm or on weekends. Let me know if you have any questions.

The Fortessa ("5 laser LSR") is up and running, if you are interested in using it please let me know. I will be posting a 5 laser LSR page on the site soon.

Mike Waring

From: Waring, Michael
Sent: Wednesday, September 30, 2009 4:11 PM
Subject: Flow Updates Sept 30th.

1. I will not be here tomorrow or Friday, but I will be back on Monday. Adam will be around to help out with any instrument issues.
2. Sorter computer seems to have picked up a virus, where it installs an autorun file on flash drives--bring cd's if you want to copy your flow data after a sort.
3. Some of you may have noticed that we have not sent out any bills for July or August, due to some reorganization that we are undergoing. We hope to get those out in the next couple of weeks (along with Sept).
4. The topics to be covered at the New England Cytometry Group's fall meeting on Oct 22 are listed below, if you are interested in attending visit their website www.bostoncytometry.org. There is also a DNA analysis in Flow Cytometry class the day before (10/21), hosted by flocyte, go to their website for details www.flocyte.org and scroll over "training" and select "calendar".
The speakers for the meeting are:

Kamran Badizadegan, MD.

“Raindrops on Water: Quantitative Phase Microscopy for Analysis of Cell Structure and Dynamics”

Harvard Medical School / MGH

<http://hst.mit.edu/public/people/faculty/facultyBiosketch.jsp?key=Badizadegan>

Al Donnenberg, Ph.D.

“In Search of Optimized PMT Gain”

University of Pittsburg

<http://www.mirm.pitt.edu/people/bios/DonnenbergA1.asp>

Joonsoo Kang, Ph.D.

“The Molecular Signature of Innate Lymphocytes”

UMASS Medical School

<http://www.umassmed.edu/ivp/faculty/kang.cfm>

Peter Lopez

“Cytometry Shared Resource Management in a Changing Environment”

New York University

<http://pathology.med.nyu.edu/people/faculty/lopez-peter-a>

Phil McCoy, Ph.D.

“Mapping the Human Immunome : Small Steps and Giant Leaps”

NIH

<http://www.nhlbi.nih.gov/resources/chi/directors/mccoy.htm>

Jonathan Sexton, Ph.D.

“High Content Screening for Non-Classical Peroxisomal Proliferators to Treat Type II Diabetes Mellitus: Multivariate Analysis and Automated Cellular Classification”

North Carolina Central University
http://brite.nccu.edu/Dr_Sexton

Howard Shapiro, MD., PC

“TBA”

Howard Shapiro, MD., PC.
<http://www.shapirolab.com/>

Frank Traganos, Ph.D.

“Molecular Reporters of DNA Damage Detected by Cytometry”

New York Medical College

<http://www.nymc.edu/Research/CoreFacilitiesAndResources/FlowCytometry.html>

Please check our web site for meeting updates <http://www.bostoncytometry.org>

Let me know if you have any questions.

Mike Waring

Ragon Institute of MGH, MIT and Harvard

Flow Cytometry Core

From: Waring, Michael
Sent: Friday, October 30, 2009 3:46 PM
Subject: Flow core update

1. next flow class
2. next week's schedule--Adam not here
3. Fortessa issues
4. BD antibody selections

1. The next intro to flow class will be Thursday, November 12 at noon in 5216, let me know if you are interested and there will be more details to follow. Visit our training page (www2.massgeneral.org/aids/flow_cytometry.html and select "Training") for on line tutorials and resources.

2. Adam will not be here next week, so I will be a bit shorthanded. I will also not be around on Monday afternoon.

3. We've been having some issues with the Fortessa, currently the green laser has died and needs to be replaced, either Monday or Tuesday next week. The schedulebook calendar for it has a resource alert that will pop up if you try to book it, or a resource note that you can click on (look for the "N" next to the instrument name in the header for the column) where I will post updates.

4. BD has a useful PDF on their site for looking up what colors of the various markers they have. From the homepage www.bdbiosciences.com in the ad for V500, click on the "Buy 4 get 2 free" and on that page there are links towards the bottom for the documents for human, mouse, or non-human primate. You can also go to www.bdbiosciences.com/choice, or the direct links to the pdfs are:

http://www.bdbiosciences.com/documents/multicolor_human_antibody_reagents_23-10975-01.pdf

http://www.bdbiosciences.com/documents/multicolor_mouse_antibody_reagents_23-10974-01.pdf

http://www.bdbiosciences.com/documents/multicolor_nhp_antibody_reagents_23-10976-01.pdf

Let me know if you have any questions.
Mike Waring

From: Waring, Michael
Sent: Monday, September 21, 2009 4:42 PM
Subject: Flow update

1. new configuration on the 4 laser LSR
2. new arrival--5 laser LSR!
3. Flow meeting coming up Oct 22
4. Reservation limits--2 hours during working hours
5. Calibur usb hub

1. 4 laser default configuration is now "default" instead of "4 laser Lsr II", if you get an error message that your experiments configuration was 4 laser Lsr, you can just ignore/cancel/proceed (don't change the configuration from default) as they are both identical.

2. Our "5 Laser LSR II" is here! (Officially the BD LSR Fortessa). We'll start signing up people to use it this week. It should be much better with CFSE (reducing spectral overlap with the PE signals), and with red fluorescent proteins (better excitation with yellow/green laser) and PE Tandems will hopefully be brighter as well (better excitation). If you are interested in using it, let me know and I'll start getting people "cleared" to use it. It also has the HTS plate loading system like the 3 laser LSR, but in the Fortessa it is always installed, so there is no need to detach it completely to go back to tube loading. I'll set up the configurations etc so that you can select your fluorochromes. Keep in mind, PerCP is not in the default configuration and actually is not well-excited by the 561 laser, so it no longer takes the same channel as PE-Cy5 on that instrument.

3. There is a flow cytometry meeting coming up, October 22. Hosted by the New England Cytometry Group (www.bostoncytometry.org), it should be very informative. I've pasted the announcement and speaker list below. There is also a DNA instructional session the day before offered by flocyte, linked from the boston cytometry page.

4. Reminder, there is a 2 hour limit during working hours (9 to 6) for all reservations, as well as a limit of 2 reservations per week between 2 and 6 pm.

5. Has anyone had issues with calibur usb hub? I've been told that if you leave the flash drive plugged in while running your samples, the computer for some reason will shut down the drive and then re-open it and give you messages about it. It seems to be ok if you don't leave flash drives plugged in for the whole run, just use them at the end to copy files and then disconnect.

Mike Waring
Ragon Institute of MGH, MIT and Harvard
Flow cytometry core facility
Www2.massgeneral.org/aids/flow_cytometry.html

From: Waring, Michael
Sent: Friday, September 04, 2009 10:42 AM
Subject: Change in flow class and Flow update September

1. CHANGE IN FLOW CLASS TIME AND LOCATION!!!!
2. Flowjo sale
3. New sorting hours!
4. "CST Settings" on LSRs

1. Due to the high demand, I decided to change the location for the next Intro to Flow Cytometry class, which also required a change in date, hopefully this will not cause any conflicts with people's schedules. The class will now be held on FRIDAY, SEPT 11, at noon in room 2204. Please confirm whether you are able to attend. The class list is pasted below. Room 2204 is on the east side of the building, on the parking garage side of the building.

Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flow_cytometry.html and click on the "Training" link in the menu on the left. It is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

2. Treestar is having a sale on Flowjo dongles/licenses, \$500 off: <http://www.flowjo.com/home/sale.html> with our academic pricing of \$1495, that means \$995 per dongle. No need to organize a group order on this one, if you are interested just place the order directly.

3. Due to some scheduling changes, we are offering new hours for sorting. Adam will be doing QC and core upkeep in the mornings, and I'll be coming in a little later and then staying later. We'll be booking sort times from 12 to 5:30 on Mondays (Lab meeting in the mornings), then 10:30 am until 5:30 pm for the middle of the week, and then 10:30 to 4:30 on Fridays. These changes are reflected on the schedulebook calendar. Users that are only sorting can use the guest account to log in to view the calendar, see the directions on our website documents page.

4. When using the LSR's, and first logging in for the day, you may get a window that pops up asking if you want to use CST settings or keep current settings. The first button is "details", and if you click on that it will show you the current settings and the values determined by CST. Look at the "laser delay" settings, and if they are different, then when you run samples at your previous settings the signals will be different-- if the "CST" setting is higher, then your signals will be higher. You should always "Use CST settings" and not "keep current settings", as the current settings may not accurately reflect how the instrument is running. Just be aware you may have to adjust the voltages slightly to adjust for the scaling factor difference.

Let me know if there are any questions. Our website is www2.massgeneral.org/aids/flow_cytometry.html
Mike

From: Waring, Michael
Sent: Thursday, August 27, 2009 11:53 AM
Subject: Flow update 8/27/09

1. Starting with a new panel
2. Website updates
3. Schedulebook changes
4. HTS training (96 well plate loader for 3 laser lsr)

5. Next Intro to Flow Class 9/10/09

1. Just a reminder, it is a good idea for a new experiment to test your panel before undertaking a large experiment--make sure all of your labels work together, and only add a few new colors at a time, don't just pick 12 different reagents and dump them together and expect them to work! Make sure your combination works before committing to staining multiple samples.

2. We've been doing a lot of website updates, so if you encounter any dead links, please let me know, and please give me specifics ("The calendar link on the calibur page is not loading") as there are multiple pages that send you to the same place... The new page is http://www2.massgeneral.org/aids/flow_cytometry.html and all of the sub-pages are linked from the menu on the left.

3. There have been a few new resources added to schedulebook, if they have appeared in your view and you want to remove them, instructions can be found on our website on the "documents" page from the menu on the left, or the direct link to the document is http://www2.massgeneral.org/aids/flow_cytometry/documents/schedulebook.pdf If the date at the bottom is not 8/26/09, refresh the page to get the current version (Title line should be centered in the newest version).

All users should review the schedulebook instructions linked above, as a reminder of the booking restrictions in reserving the cytometers.

4. I will be scheduling a training class for the HTS system that allows you to run 96 well plates on the 3 laser LSR II. Please let me know if you are interested so that I can determine how many sessions I will have to plan (probably in groups of 3).

5. The next flow class will be on Thursday, September 10th from 12 to 1:30pm in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at http://www2.massgeneral.org/aids/flow_cytometry.html and then click on "Training" in the menu to the left. At a minimum, you should review the items in "For starters" (the introductory tutorials from the Invitrogen website and the drmr.com compensation quiz) before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

The current class list is below. Let me know if you would like to be added or removed.

Thanks!
Mike Waring

This message is an update on the Ragon Institute Flow Cytometry core facility, if you would like to be removed from my distribution list please let me know and I will delete your account.

From: Waring, Michael
Sent: Tuesday, August 18, 2009 9:21 AM
Subject: Flow core update

Our website has changed! You can now access it by going to www2.massgeneral.org/aids/flow_cytometry.html and all of the instrument links are in the menu on the left. Let me know what you think of the new look! The old address will no longer work.

My new technician has started, his name is Adam Chicoine and we hope to have him start sorting in a couple of weeks.

While we get Adam up to speed, sorting will be limited to afternoons only.

Reminder to notify me of any issues with the instruments in the core.

New resources have been added to schedulebook, if you do not want to view them in your login then follow the directions on our "documents" page on the website.

Starting in September, I'll be here until 6 so we will be extending our sorting hours to 5:30.

BD is holding a special promotion, buy 4 antibodies and get 2 free, any quantity! (So buy 8, get 4 free, etc).

<http://www.bdbiosciences.com/colors/specialoffer/>

Also they are now offering custom conjugation express, get custom conjugates in 3 weeks. Let me know if you want the list of available antibodies.

Mike

-----Original Message-----

From: Waring, Michael

Sent: Thursday, July 30, 2009 10:46 AM

Subject: Ragon Institute Flow Core update 7/30/09

1. "Borrowed" pipet
2. Time off
3. No Calibur usage for Fri, Mon, Tues, possibly Wed, 3 Laser relocated temporarily
4. Sorter schedule=my schedule
5. All human sorting must be COMS registered!
6. ALL sorts must have Sort request form at time of request.

1. It seems that the eppendorf P200 from the 3 laser LSR has gone missing since last week, if you walked off with it or have seen it, please return it. It has "Ragon Institute Flow Cytometry" written on the body in black Sharpie.

2. I am not here Mon, August 3, Thurs the 6, or Friday the 7th next week.

3. The Calibur will be out of service for the duration of the work (2-3 days), and the LSR we hope to set up in the Ragon Institute storage room in 5556, across from the autoclave room which is next to the door to 5228. There is a keyed door for access, if you will need to use the 3 Laser, let me know and I will tell you how to access it.

4. Since I don't have Andrew around to help anymore, I will just use the sorter schedule as my schedule for the coming weeks, until the new technician gets up to speed (he will start on Aug 10).

5. All projects using human samples or potential human pathogens for sorting require registration through COMS. The sort request forms have been updated with a place to enter your coms registration number. Please let me know if you need help getting this straightened out.

6. ALL sorts must have a sort request form at the time of the request. no sort will be booked without a sort request form first.

Let me know if you have any questions.
Mike

From: Waring, Michael
Sent: Friday, July 17, 2009 9:39 AM
Subject: Practical Flow Cytometry 4th edition

Someone borrowed my copy of Practical Flow Cytometry 4th edition by Howard Shaprio (orange textbook), please return it and in the future, all users please leave me a note when you borrow something from the core so that I know where to find it. I have a pdf of the book if you are interested I'll leave it on the PC analysis station in the flow office area, or you can find it for free online at <http://www.coulterflow.com/bciflow/research01.php>

I just found today that it is no longer available from Invitrogen (the link for it just sends you to the generic flow cytometry page).

And a reminder that today is Andrew's last day, so stop by and wish him good luck in Grad school!
Mike

From: Waring, Michael
Sent: Wednesday, July 15, 2009 4:23 PM
Subject: Flow core update 7/15/09

Andrew's last day is this Friday, so if you have worked with him over the last 2 years and wish to say goodbye, this is the last week to do so.

We are anticipating his replacement will be starting in mid-August, so for a few weeks we will be short handed and I will try to fit in as many sorts as possible (while still having time to take care of administrative duties) but I will not have time for training. The next flow class will be at the end of August.

I will not be here August 3, 6, and 7th, and will likely be busy with core duties (QC, waste and stocks, etc) on the 4th and 5th, so there will not be sorting available that week. I hope to coordinate the upgrade of the sorter for that week as well to avoid having two weeks of outage, I will keep you posted on the progress of that. Please plan accordingly.

The 3 laser/calibur space is going to be renovated to make room for a new instrument, and the work will take 3 days or so once started. During that time, the 3 laser LSR will be located in the store room (#5556, opposite the entrance to 5228 where the instrument is now) and should be available for use; the calibur will NOT be available. We hope this will happen next week, again I will post when it will actually occur.

Let me know if you have any concerns/questions.

Mike

From: Waring, Michael
Sent: Tuesday, July 07, 2009 11:07 AM
Subject: Core update 7/7/09

Sorry for the long update, but a lot is happening in the core!

1. Next flow class (new users)
2. Flow office relocation(all)
3. Flowjo for loan (flowjo users)
4. New LSR/interruption of usage for 3L and calibur
5. Schedulebook instructions typo
6. 3rd party analysis software
7. Tracking settings with rainbows (LSR users)
8. Registering sorter PIs (sort users)

1. The next Intro to Flow class will be held on Monday, July 13, at 12 pm in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply. The current class list is at the end of this email.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

2. We have relocated the flow core office space and analysis stations to 5233, the cubicle space across from the large lunch room 5237 near the central elevator bay. My new phone number is 617 643-7187, although any of the instrument numbers will reach me there also (Calibur/3 laser 617-724-2668, 4 laser 617-643-0889 and sorter room 617-643-0891).

3. The core now has several Flowjo dongles for loan if you want one for short term (1 week or less) use. Come see me in 5233 to borrow them. You will be responsible for replacing any lost dongle that you borrow.

4. We will be getting a new LSR in the next few months, and plan to put it in the bay where the 3 laser LSR and the calibur are currently kept. It will be necessary to temporarily move 3L and calibur while room is renovated, for 3 or 4 days until the work is done. We hope to keep the 3 Laser accessible but the calibur will be unavailable during this time unless we find a place to park it and plug it in. When the date for the work is set, I will update my distribution lists and the calendars.

5. It was brought to my attention that there was a typo in the schedulebook instructions from our web page, which I have corrected. The link should be www.schedulebook.com not schdeulebook.com... I apologize for any inconvenience this may have caused and thanks to CB for pointing it out (you know which one you are).

6. Is anyone interested in an alternate third-party flow analysis software? It is called FCsexpress, <http://www.denovosoftware.com/> and is based on the Powerpoint interface, so it uses all of the standard Microsoft Windows techniques and is supposed to be intuitive to use. If there is enough interest, we may purchase a copy for the core. If you would be interested in a

presentation/demo, let me know and I will try to plan something. A full list of features:
<http://www.denovosoftware.com/site/product2.shtml>

7. I have posted a document to help users in Tracking settings with rainbow beads (which also describes why you should):

<http://www2.massgeneral.org/aids/tracksettings.pdf>

8. It has recently come to light that we will have to start registering all users working with unfixed tissues on our instruments with the Committee on Microbiological Safety (COMS), which is the Institutional Biosafety Committee (IBC) for MGH. This of course includes all users that are sorting. I will provide details for those affected.

Let me know if you have any questions.

Mike

From: Waring, Michael
Sent: Tuesday, June 16, 2009 3:30 PM
Subject: Flow updates 6/12/09

Sorry for the long update, but I've numbered them below and indicated which groups need to read which items...

1. Data storage (LSR II users)
2. Distribution list consolidation (all users)
3. Human samples must be fixed! (all users)
4. Relocation (all users)
5. Flowjo order, 33% off! (Flowjo users)
6. Renovations--3L and calibur room (users of Calibur and 3 Laser LSR)
7. Andrew leaving July 17
8. Standby events in schedulebook (LSR users)
9. Next Intro to Flow class, June 26th! (New users)

1. Just a reminder on data storage on the LSR computers-- any folders that remain unmodified for 6 months will be removed. D drive folder limits remain at 3 GB, if you are in excess you will be notified but please keep track yourself! If you go over 10 GB, ALL of your data will be removed to an external drive and you will have to see me to retrieve it. Folders labeled "experiments mmddy" are folders that core personelle have created and contain experiment exports to clear excess data out of Diva (more than 2 experiments, and older than 2 weeks). You should go through these folders and either save experiments you have not backed up yet, or delete ones that you already have copies of. There should not be any folders in your D drive that are "experiments mmddy" that are more than a month old!

2. I've eliminated the separate "sorting" distribution list that I had, so those that were only on the sort list will now get the general emails. I'll try to indicate in the contents list at the top which items you need to read.

3. ALL HUMAN SAMPLES MUST BE FIXED in order to run them on the LSRs or on the Calibur. It is *MGH* policy, not only ours, that all samples of human origin are BL2, even uninfected cell lines, and must be worked with in a hood, transported with sealed caps (so filter caps should be replaced with solid caps after filtering), and fixed in 2% paraformaldehyde for 30 mins before running them on the cytometers. The exception of course is the sorter which is in its own hood, so unfixed samples can be run there. If you want to do viability to exclude dead cells, you can use invitrogen's live/dead dye, I will be putting together an information sheet about them. You should also be sure that all tube caps are securely affixed during transport.

4. We are reorganizing our office space, and in the next few weeks the flow core will be taking over the cubicle area across from the large 5th floor lunchroom, "room" 5233, just around the corner from the door to the 4 Laser LSR and the sorter. My desk, as well as all of our analysis stations for flow and one for microscopy, will be located there.

5. There is a special offer for Flowjo, buy 2 get 1 free, so I am gathering interested parties together so that a group order can be placed and each license/dongle will be at 33% off. Academic list price is \$1500, with the sale each will be \$1000. I have several interested already, and hope to get the order placed next week. If you are interested, let me know by Thursday and I will add you to the group.

Please also note that the Ragon Institute Flow Facility will have some dongles available for loan-- you will be required to sign them out, and be responsible for returning them or replacing them in the event of loss. See me for details.

6. Renovations--3L and calibur room-- we will be removing the bench and sink from the bay where the 3L LSR and the Calibur are currently set up, to make room for a new LSR2. I will notify all users when the date is set (within the next month), and we hope to have the instruments set up in another location for the 2-3 days that the work is in progress. I will keep you posted.

7. Andrew is leaving us for Graduate school in Chicago, his last day will be July 17th. We are in the process of finding a replacement for him, but there will be some decrease in capacity (sorting and otherwise) while the new person is brought up to speed.

8. When using Schedulebook, please note that any time with blue cells to the right of it is considered "reserved" and you should not book it, even if you are able to!

In order to reserve time for members of the Ragon Institute so that they get first dibs on access to their own instruments, we have blocked off time under "Ragon Institute" on Tues, Wed, and Thurs afternoons on the LSR2's. We are now trying allowing "standby" events for Ragon members to reserve this time--(you may have noticed the extra columns for each instrument). Ragon Institute users can create an event that overlaps with the reserved Ragon time, and when the ragon reservation is removed the day before, their event becomes the primary event and any unclaimed time can be taken by anyone. The calendar sets their entire event as standby, so if it extends beyond the "ragon" reservation of 3-7 pm, it is possible for someone else to book the "open" time--please consider any time that has blue cells in the skinny column next to it as "reserved"--I have a log of who reserved what and when, and I will delete anyone that claimjumps time. Thank you for your cooperation.

9. Next Flow class will be June 26th from noon to 2 (note time change) in CNY149, room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

Visit our website for info on our instruments, training, and useful flow links!
www2.massgeneral.org/aids/flow_index.asp

Mike W

From: Waring, Michael
Sent: Thursday, May 28, 2009 2:15 PM
Subject: Flow update 5/27/09

1. Track settings with rainbows
2. D drive storage limit, DATA REMOVAL POLICY
3. Reservation limits
4. Flowjo sale

1. I have posted the protocol for tracking cytometer settings using rainbow beads, please take a look and let me know if you have any questions on implementing it. ALL Ragon institute users should start using this, and I'll help you get it set up, just let me know when you want to go through it. It will also involve creating a template to use with your experiment so that you do not have to import an experiment full of data and then duplicate without data. I have a supply of rainbows so that all labs won't have to buy their own.
www2.massgeneral.org/aids/flowdocuments.asp

2. The storage limit on the D drive is 3 GB. When you exceed this amount, I will send you a reminder. If you go over 10 GB, I will remove all of your data from the D drive and you will have to see me to retrieve it.

3. Keep in mind that with the new calendars, the old reservation limits are still in effect.
--Anyone not in the Ragon Institute may NOT make a reservation more than 1 week in advance.
--Reservations are limited to 2 hours between the hours of 9 am and 6 pm.
--No user may have more than 2 reservations in a given week between 2 and 6 pm.
--On Tues, Wed, and Thurs the LSRs are reserved for Ragon Institute researchers from 3-7 until the day before, when these times are released if still unclaimed.
--The sorter is reserved for members of the Ragon institute on Tues and Thurs from 2:30 to 4:30 until the day before.

4. Flowjo is having their buy 2 get 1 free sale for the month of June, so a licence will be \$1000 per orderer. Let me know if you are interested and if we get enough I'll put you in touch so an order can be placed.

From: Waring, Michael
Sent: Tuesday, May 19, 2009 2:54 PM
Subject: Flow update: Comp beads and intro class and data storage

1. Comp beads--all users please read!
2. Intro to flow THIS FRIDAY
3. Data storage policy change

1. If you are using unstained beads to determine your settings for using the LSR's (which you shouldn't!), you should be aware that the newer lots of BD's compbeads have a higher fluorescence in the pacific blue channel, and possibly the other violet excited channels also (Pacific orange, amcyan, or Qdot). While my original message on the topic was restricted to compensation issues in using the newer lot mixed with the older lot, another ramification of this change has come to light: if you are adjusting your voltages based on the bead fluorescence, because the newer beads (expiring after Dec 2010) have brighter fluorescence, you may be inclined to set the voltage for Pacific blue (and other violet-excited dyes like Pacific Orange, Alexa 430, AmCyan, etc) much lower than previously, which would result in your CELLS, which are much dimmer in the violet excited channels, to be well within the noise of the instrument and possibly prevent you from seeing positive signals. So be aware, if you have been using beads

that expire before 12/2010 and were using unstained beads to set your voltages, the new beads at those same settings will be HALF A LOG HIGHER in the pacific blue channel, so DO NOT DROP YOUR VOLTAGE TO MATCH THE PREVIOUS MFI or your settings will be too low. I also know of at least 1 group that has to use an anti-rat bead for one of their colors, and this happens to be from the older lot, whereas the mouse reagents are all the newer lot, so they need to make sure to use an older negative bead mixed in with their positive rat bead, as the "universal" unstained comp tube used for the rest of their samples is inappropriate for the rat beads, so make sure if you have to mix your beads that they all are the same fluorescence when unstained! You can also include a drop of the appropriate "negative" non-binding bead in your single stain control, and set an interval gate on that population (extended all the way to the axis to select the entire negative population) so the software uses that matched "internal negative" for the comparison. See me if you have any questions. I will be writing up a protocol for using mid-range rainbow beads to track your settings, so that changes like this that come up will not affect your results when establishing your settings for the day.

2. The next Intro to Flow class will be held on Friday, May 22, at 10 am in room 5216 and lasts usually 90 minutes. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website linked from our training page, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply. Class list is included below, let me know if you are on the list but not coming, or not on the list and planning to come.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

3. Starting Monday, anyone with over 10 GB of data in their folder on the d drive of either LSR2 will have ALL of their data removed and you will have to retrieve it from me. The 3 GB limit is still in effect and should be observed. The "experiments MMDDYY" folders are what Andrew and I export from Diva to clear it out and should be deleted (either move/rename them if you need the files, or delete them if they are duplicates).

Mike

From: Waring, Michael
Sent: Wednesday, May 13, 2009 4:18 PM
Subject: Flow update May-compensation bead warning!

1. Next flow class May 22
2. 3L LSR sheath tanks
3. Comp bead warning

1. The next Intro to Flow class will be held on Friday, May 22, at 10:00 am in room 5216 and lasts about 90 minutes. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

2. The HTS unit for running 96 well plates on the 3 Laser LSR requires sheath fluid with surfactant. To avoid the time and hassle of swapping between regular sheath and surfactant sheath, we will run surfactant sheath all the time. The large tank is full at 56 lbs, and should be filled at 30 lbs, instructions for filling it are on the wipe board near the instrument, and will be added to the 3Laser User Guide with the next update.

3. It has come to my attention that BD must have changed the plastics they used for their antibody capture/compensation beads-- of the batches I tested so far, all of the ones that expire in 2010 have a lower fluorescence in the violet channels than those that expire in 2011. The latest "low" batch I tested expire 12/31/10 and earliest "high" batch I looked at from 2011 expire in April-if you have an expiration date of 1/2011 thru March 2011, I don't know which way they will go as we didn't have any from that time window. They are shipped as a pair, one vial of beads that bind antibody and another vial that do not that are used as a negative control. Make sure that your beads match, vials from the same kit seem to have sequential lot numbers (29849 and 29850 for example) although just matching the expiration dates should suffice as well. If you don't have to mix them, you can use unlabeled binding beads to be absolutely sure that they match your single stained controls (but there are times when you need to use both beads and cells in compensation for certain colors, so you may need the negative beads). If you have questions about this I'm happy to answer them, and I have some figures I can send you as well.

Mike W

-----Original Message-----

From: Waring, Michael

Sent: Wednesday, May 06, 2009 10:28 AM

Subject: flow update 5-6-09

--Compensation bead matching problem

--website update

--Yesterday I ran a sample on the sorter, where the positive (antibody binding) and negative (non-binding) compensation beads did not produce the same fluorescence, the unstained binding beads were half a log brighter in the Pacific blue channel than the "negative" beads. The result of this would be that signals would be overcompensated: e.g. the APC single stained control, which does not overlap into Pacific blue at all, would give a higher signal for Pac blue as compared to the negative beads. The software would see a higher Pac blue signal in the APC control (because in Pac blue the binding bead is more fluorescent than the negative bead) versus the negative bead, and compensate the signal out. The lot numbers were not matched on the 2 tubes, I'm not sure if that would have a bearing on the results, but you may want to just use a drop of the unlabeled capture beads to be sure your compensation particles match exactly, or just check your 2 beads to make sure they match.

--I've updated the website, so if you find any dead links or any problems please let me know. The main page is

www2.massgeneral.org/aids/flow_index.asp, or you can go to

www.ragoninstitute.org and click on the "flow cytometry" link on the

right. I have also posted instructions for using the schedulebook calendars on the "flow documents" page.

Mike

From: Waring, Michael
Sent: Monday, May 04, 2009 12:17 PM
Subject: Ragon Flow update 5.4.09

For the new schedulebook calendars, all of the usernames have been sent out, so the calsnets calendars should no longer be used for reserving instrument time. If you need a password let me know and I'll set up an account. I also set which instruments you can book, so let me know if you need one that has not been activated for you.

As issues come up related to making reservations I'll send out updates, and post the user guide to our website by the end of the week for the little tricks that may not be self-evident. The first one to come up is that the default settings for display limit the time to 8PM, you can go to tools>personal settings and adjust that to as late as midnight if you want/need to.

Flowjo will be having their buy 2 get 1 free deal in June, let me know if you are interested.

The 3 laser LSR computer has been replaced, The old one will be kept on the desk nearby for the next few weeks, please copy any data you need and then delete it so that I know that you have removed it.

Next Flow class will be Friday, May 22, 2009 at 10 am. Let me know if you are interested in attending. Details to follow.

Mike

From: Waring, Michael
Sent: Wednesday, April 29, 2009 3:19 PM
Subject: New calendars for Ragon flow facility, new computer for 3Laser LSR

--New Calendar system
--New 3 Laser computer

We are switching over to schedulebook for our flow cytometry signup calendars, and as they require a login to use them, I am in the process of creating accounts for everyone and you should receive an email from me by Friday noon. I am going by the past 6 months of usage to determine who needs access for what instrument-- if you need to sign up for something you have not used in 6 months, or if it doesn't show when you log in, please let me know so I can grant access to that calendar. We will go live starting on Monday, May 4. If you don't receive an email from me with your schedulebook username and password, please email me so that I can create your account-- If you are a technician running samples under your supervisor's account, and I don't send you a login, please let me know as I may have missed you since your name would not come up in the usage logs. All of the instruments that you have access to will show up on one page. I am also leaving out the analysis stations as the default, if you want access to the calendars for them please let me know. Each one will have its own calendar so let me know if you want iMac, PC, or G4 mac (not set up yet).

It seems that we have had a few instances of reservations on the calsnets calendars being deleted lately, either accidentally or purposefully. This will not be an issue with the new calendars. I

apologize to anyone whose reservations have been affected. Once the new calendars are live, I welcome any feedback you may have.

The microscopes will be hosted on the same server, so if you are using our imaging instruments, let Thomas know and he can grant you access to the microscope calendars.

I'll write up a user guide for them soon, but they are pretty self-explanatory.

The new computer on the 3 Laser LSR has been installed, the first time you use it please set up a folder for yourself on the D drive. The old computer has been set up on the bench nearby. If you still have data on the old computer, please make a copy of it for yourself and delete it from the old computer. If you have used the 3 laser in the past, just check the old computer to see if you have all of your files, and delete your folder on the d drive if you no longer need it. You should be able to copy over experiment templates to the new computer, let me know if you have any problems. It'll stay there for a week or two and then be set up as an imaging analysis station, so all information on it will be deleted to make room.

If you have any questions, let me know.
Mike

From: Waring, Michael
Sent: Friday, April 24, 2009 9:47 AM
Subject: Flow facility update 4/24/09

1. 3 Laser Computer upgrade
2. Next flow class Friday, May 22, 2009
3. Cytometric Bead Array demo

1. The computer on the 3 laser computer will be replaced Wednesday, April 29, in the morning. Please remove all data before then, as we will not be backing it up or copying it to the new computer. We will empty the database on Monday, and then again Wed morning to speed up the switch. If you use it on Monday or Tuesday next week, please export your experiments and copy them to a flash drive or dvd when you are done.

2. The next Intro to Flow class will be held on Friday, May 22, 2009, from 10 am to 11:30 am in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply. CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

3. We will have a demo of CBA assays from BD Biosciences on May 13th, if you are interested please let me know (Cytometric Bead Arrays, http://www.bdbiosciences.com/pharmingen/products/display_product.php?keyID=9)

A Cytometric Bead Array (CBA), commonly referred to as a multiplexed bead assay, is a series of spectrally discrete particles that can be used to capture and quantitate soluble analytes. The analyte is then measured by detection of a fluorescence-based emission and flow cytometric analysis.

The BD CBA allows you to quantitate soluble analytes in samples never previously considered due to sample volume limitations. In addition to the quantitative data, powerful visual images can

be generated revealing unique profiles or signatures that provide the user with additional information at a glance.

Visit our website for information about our instruments and useful links for flow cytometry related information,

www2.massgeneral.org/aids/flow_index.asp

Or go to www.ragoninstitute.org and click on the "Flow Cytometry" quick link on the right.

If you wish to be removed from this mailing list, please let me know and I will delete your account.

Mike Waring

From: Waring, Michael
Sent: Thursday, April 16, 2009 11:55 AM
Subject: Ragon Institute flow cytometry facility update 4/16/09

1. New computer for 3 Laser LSR
2. Gentamycin in sorter sheath fluid
3. Next Intro to Flow class TOMORROW
4. reporting problems with the instruments

1. We have just gotten the new computer for the 3 laser LSR, old files will NOT be transferred over, so if you have data on the 3 laser workstation, please make a copy of it before the switch. I will place a service call and schedule the upgrade for some day next week, and send another message when the date is finalized.

2. Starting this week we will be including Gentamycin in the sheath fluid for the sorter. Please let me know if you have any concerns.

3. The next Intro to Flow class will be held tomorrow, Friday April 17th from 10 am to noon in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at

www2.massgeneral.org/aids/flowtraining.asp

and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, plus any other materials that you have time to go over, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

The class list is included below my signature line below, if you are on the list but not attending let me know and I will remove you, or if you are not on the list let me know that you plan to attend.

4. A reminder that if you ever have any issues with the cytometers in our facility, please let me know so that they can be addressed/fixd. Email mwaring@partners.org.

Mike

From: Waring, Michael
Sent: Monday, April 13, 2009 1:42 PM
Subject: Flow updates

1. Limited access to PC analysis station
2. New Calendar for signup coming soon!

3. HTS use
 4. CBA Assays
 5. Diva export as experiment, using in Flowjo
 6. New computer for 3 Laser on order-copy your data!
 7. Intro to Flow Friday 4/17/09
-

1. For the next few weeks, the PC Flow Analysis station will only be available on a limited schedule (12-2pm daily, or after 5). Please use the calendar to reserve time and avoid conflicts (www.calsnet.net/cytometer_signup) and remember to indicate PC or Mac when booking the analysis stations, as we only have one calendar for both of them. There are no restrictions on the Mac.

2. We will be switching our reservation system to schedulebook instead of calsnnet. I will keep you informed as to the progress with getting it set up, and once it goes live I'll post a notice on the calsnnet calendars with the new address/etc. It will require a login so I will be sending everyone a username and password, and instructions for using the calendars, I'll start with people that have used it in the past 6 months and then if you haven't used it in a while you'll have to contact me before you book your time.

3. HTS is now ready for use, those interested in being trained so that you can run your samples from a 96 well plate on the 3 laser LSR, let me know and we'll schedule a time for training.

4. We will have a demo of CBA assays from BD Biosciences in May, if you are interested please let me know (Cytometric Bead Arrays, http://www.bdbiosciences.com/pharmingen/products/display_product.php?keyID=9)

5. When exporting from Diva as an experiment it is possible to use those files in Flowjo (they are named with a 5 or 6 digit number), but you have to "edit" the columns to show tube name, and then if you selected A, H and W for your parameters, H and W switched in the Flowjo labeling.

6. We will be getting a new computer for the 3 laser LSR, so all of your data that is on the old computer should be removed/copied before the switch. I will send out another message when the computer comes in but please make sure you have your data backed up as the old workstation will be rededicated as an imaging analysis station and all old data will be deleted.

7. The next Intro to Flow class will be held on this Friday April 17, from 10 am to noon in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp (>90 slides, if you print it do 4 per page) and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website before attending the class. It might also be a good idea to view the material on compensation. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

Mike W
Ragon Institute Flow Cytometry Facility

From: Waring, Michael
Sent: Thursday, April 02, 2009 1:39 PM
Subject: Core reminders

1. Last user turn off instruments!
2. Luminex seminar reminder
3. Calibur repair, and locations?

1. Once again, let me remind everyone that if you are the last one signed up on the cytometers they should be turned off when you are done. Check the calendar to make sure a later reservation wasn't deleted. And if you have the last reservation of the day and cancel your time, please notify the person before you, or just do a walk-by and make sure it was turned off, especially if you cancelled your time after the previous person finished!

2. The Luminex seminar by Dr. Luderer will be tomorrow at 10 am in 2204 (East elevators to 2, go to the left of the balcony, then first door on the right). Refreshments will be served. Let me know if you plan to attend (if you haven't already).

3. Field service will be in this afternoon to repair the laser on our FACSCalibur, so tomorrow it should be in working order.
For future reference, it might be useful to have a list of all of the caliburs in the building--if you know of others, please let me know, and when someone has an outage I can direct them to one they can use.

Mike

From: Waring, Michael
Sent: Wednesday, April 01, 2009 2:26 PM
Subject: Calibur out of service

The blue laser on the calibur is turning on and off (2 min on 1 min off), so the calibur is unusable at the moment. I have placed a service call and will post updates to the calendar. If you need to use a calibur, let me know and I am working on getting access to another instrument, otherwise I can help you run them on one of the LSRs or possibly the Aria. I apologize for the inconvenience.

Mike Waring
Ragon Institute Flow Cytometry Core
MGH

From: Waring, Michael
Sent: Wednesday, April 01, 2009 10:43 AM
Subject: Flow core usage reminders and announcements

Sorry for the recent flood of emails, but there has been a lot going on in the Flow Core! A few more items of interest:

1. Issues in Flowjo with "export>experiment" from Diva
2. Small cytometer demo
3. Calendar Signup
4. Changing configurations,
5. Tracking settings--rainbow bead part #
6. User Guides

1. It has come up on the Purdue list that when you export from diva as an EXPERIMENT, the H and W parameters get switched in Flowjo, so be careful of that especially if you are doing doublet gating, looking at H vs A will not be the correct plot (events on a 45 degree angle). Also, the data files are labeled with a number from the database, so to see what file it is, you can click on the "edit" box in the workspace in Flowjo and add "tube name" as a column to see what tube it is.... Or you can just export as FCS, and the H and W will be correctly assigned, and the file name will be specimen_tube.

2. If there is interest, I can arrange for a demo of the Accuri C6 cytometer, which is a small, inexpensive 4 color instrument with a plate loader option. Let me know if you would want to try it out.

http://www accuricytometers.com/products/c6_flow_cytometer/

<http://www accuricytometers.com/products/csampler/>

3. Just a reminder, when signing up on the Calendars, that your full name should be entered, along with contact info that has a good likelihood of actually getting in touch with you that day, and ALL NEW USERS MUST BE TRAINED BY ME before they can book usage in their name, and before they should be allowed to use the instrument independently. You should sign up with the name of the person that will actually run the instrument, and not the person being trained or the project that it is for. This will make sure that any information about the instrument that day reaches the relevant person.

4. I have put index cards on the front of the LSRs that should be used to indicate when configuration changes have been made. It should also serve as a reminder to switch the filters back, or notify the next person that they have been changed if they were not switched back. Please make use of them! When you change a filter, leave it on top of the detector array (with label facing down so filter is not lying flat) instead of putting them back in the box, to make it easier to find them again and know which have been switched.

5. Related to 4 above, I'll once again promote using rainbow beads to track your settings from day to day, as they also serve the secondary purpose of confirming the instrument is working correctly-- we had an issue a few months ago where the UV laser would die after a few hours, so that when we did QC in the morning it was fine, but in the afternoons people would get no signal. If you ran rainbow beads and saw no signal at your previous settings, you would immediately know there was a problem, rather than wondering if you used antibody, or if something else was happening. It will also usually indicate to you if a different filter is installed, as the signal would be different from the last time. We use a mid-range rainbow from Spherotech, part #RFP-30-5A, but any bead that gives signal and remains on scale at your sample settings can be used, just make sure you match the lot number each time. More about this in the User Guides for the instruments.

6. There are User Guides for the instruments, with configurations and other helpful info, linked from the website or hard copies are available for reference near the instruments.
www2.massgeneral.org/aids/flow_index.asp

Thanks
Mike Waring

From: Waring, Michael

Sent: Tuesday, March 31, 2009 2:09 PM

Subject: Ragon Institute Flow Core presents... Ragon Institute Imaging Core!

Hello Flow users. There have been inquiries in the past about using our new confocal microscope, and I am pleased to announce that we will be opening up access to our microscopes to other labs. Please see the message below from Thomas, who will be administering and training on our scopes, and contact him for more details if you are interested.

Mike Waring
Administrator
Ragon Institute Flow Cytometry Core (Formerly PARC)

From: Diefenbach, Thomas John
Sent: Tuesday, March 31, 2009 12:27 PM
To: Waring, Michael
Cc: Alter, Galit
Subject: Ragon Institute Imaging Core

The Ragon Institute Imaging Core

The mission of the Ragon Institute Imaging Core is to bring the latest imaging modalities to bear on fundamental molecular and cell biological questions pertaining to infectious diseases. The Imaging Core presently utilizes a Zeiss LSM 510 laser scanning confocal microscope in addition to an RPI/Zeiss high speed multi-channel fluorescence imaging system. The Imaging Core also serves as a resource for addressing the imaging needs of Ragon Institute Members.

The Imaging Core is located on the 5th floor of CNY 149 in the Charlestown Navy Yard, Room 5.228 (Infectious Disease Unit).

There are two prerequisites to using any of the Imaging Core's microscopes.

1. Attendance of one of the regularly scheduled microscopy fundamentals training classes.
2. Individual training provided on the microscope system you intend to use.

Once these prerequisites have been met, your account will be activated and you may begin booking time online to use the system.

Rates

The following services are provided to Ragon Institute Members:

Zeiss LSM 510 Confocal Microscope

- Unassisted Imaging is billed at \$90 per hour for all users.
- Assisted Imaging is billed at \$120 per hour.
- Microscopy Fundamentals class attendance is billed at the rate of

\$50 per person.
o Individual Training (2 hours) is \$70 per hour.

Zeiss Axio Observer Fluorescence Microscope

- o Unassisted Imaging is billed at \$50 per hour for all users.
- o Assisted Imaging is billed at \$90 per hour.
- o Unassisted imaging sessions over 3 continuous hours are billed at a rate of \$25 per hour.

Scheduling Instrument Time

Each imaging system has an online calendar to reserve time. Unassisted imaging may be scheduled through reservation of time using the online calendar. Assisted imaging time is scheduled by contacting Thomas Diefenbach at tdiefenbach@partners.org.

Additional Information

For additional information, consultation on imaging or experimental design, tips on sample preparation, or to register for the next training seminar, please contact:

=====
Thomas J. Diefenbach, Ph. D.
Associate Director, Imaging Core
Ragon Institute of MGH, MIT and Harvard
Staff Scientist, Massachusetts General Hospital
149 13th Street, Room 6.620
MGH - EAST
Charlestown, MA 02129
Tel: 617-724-7514
Fax: 617-726-4691
tdiefenbach@partners.org
=====

From: Waring, Michael
Sent: Wednesday, March 25, 2009 2:30 PM
Subject: Flow Core Website error..... And old mac files

It was pointed out that the link at the top of the General Guidelines document that I sent out yesterday was incorrect... it should be

www2.massgeneral.org/aids/flow_index.asp

I apologize for the error. The website has the corrected version uploaded already, feel free to download that one if you want a copy so as to avoid sending the file out again. I appreciate any feedback from users about such errors or any problems you have, and welcome feedback about how we can make it easier to use the core instruments.

Also, we have recently replaced the old mac analysis station with a new iMac, next week I will have the old mac set up again for people to retrieve any files you may need. Please make sure

to set time aside to retrieve files so you can make sure they are not lost. The long term plan is to set it up as a second analysis station when we get some additional space.

Laura Prickett runs the Aria and other instruments over in Simches, and they will be hosting a demo/training on the autoMACS cell separator. From Laura:

Miltenyi Biotec will give an information and training session on the AutoMACS at the HSCI-CRM Flow Core in the Simches building. Snacks are provided. It begins Monday, March 30, at 1:00 PM in Simches room 4820 (above Whole Foods) and will include a hands-on session. Using this machine

could really speed up your appointments on the FACSarias - better depletions

or enrichments of your samples mean shorter sort times. Plus - usage of the

AutoMACS is completely free! Please let me know if you're planning to attend.

(email laura at lprickett@partners.org)

And lastly, on the 3 laser LSR especially, you may notice that when you first load a tube on the instrument and click acquire, the populations will shift before stabilizing. For this reason I recommend that you don't simply click "record" from the start, but click "acquire" first, and once they have stabilized then click "record", otherwise you'll get funny artifacts at the beginning of your data files. You can actually view and gate on time as a parameter in a dot plot if you want/need to. Let me know if you need help with that.

Mike

From: Waring, Michael
Sent: Friday, March 20, 2009 10:31 AM
Subject: Flow core update

1. New users must contact me before using the instruments
2. Our Website
3. Website redux-- update and documents
4. Luminex seminar
5. Next Intro to Flow class April 17.

1. Reminder that ALL NEW USERS MUST SEE ME BEFORE USING THE INSTRUMENTS ON THEIR OWN OR SIGNING UP FOR INSTRUMENT TIME. There have been several names that I do not recognize or have in my user list popping up on the signup calendars. New techs must be trained by me, and not only by their lab.

2. Our website is www2.massgeneral.org/aids/flow_index.asp, and should be bookmarked on all of the core computers. If it is not, let me know and I'll fix that (on the PC analysis station, you are logging in under your profile, so it will not show up unless you bookmark it.) You can also find us by google searching for "PARC Flow Cytometry" or following the flow cytometry link from www.ragoninstitute.org

3. I'm in the process of a major update of the website, if you find anything that is not working (bad links, missing documents, etc) please let me know! All of our user guides and all of the sorting documents can be downloaded there.

4. We will be having a seminar by Dr. Jim Lederer on Friday, April 3, at 10 am in 2204 CNY149. He will be presenting their work on Luminex biomarker assay development for the BWH and HMS research community. They have generated some human and mouse cytokine and chemokine assays that are currently undergoing testing, and he will present an overview of the technology and the plans to make this into a Luminex consortium/user group, so the more participants the better it will be. If you are interested please let me know.

5. The next flow class will be Friday, April 17 at 10 am in 5216. Let me know if you are interested in attending. More details to follow, or you can visit the training page www2.massgeneral.org/aids/flowtraining.asp

From: Waring, Michael
Sent: Thursday, March 12, 2009 2:00 PM
Subject: core update/reminder

Flow update/reminder

1. new mac analysis station
2. HTS on 3 laser (and possible errors!)
3. Configurations on the LSRs
4. Next intro to flow class is tomorrow!

1. the Mac analysis station has been replaced, if you have any data on it that you need to retrieve, let me know and I can set it back up for you. We now have a new iMac for analysis using Flowjo or Cellquest Pro, located in 5246.

2. The HTS for running 96 well plates on the 3 Laser LSR has been installed, and we will begin training users in the next 2 weeks or so. If you are interested, let me know so we can work out a training schedule. While running in tube mode, Diva will give you errors that the plate reader is not connected (obviously!), you can just clear those from the status window and carry on.

3. If you need to change the configurations on the LSRs, please make note of the card on the front of the instrument that indicates whether the default configuration is installed or if there is a configuration change, and make sure it matches the current setup. Hopefully this will help in cases where someone forgets to switch the configuration back when their run is finished (but users must remember to switch the card at the beginning of their run for this to work...). Configuration diagrams and what filters should be installed are in the white "user guide" binders at each instrument. If you do switch filters, you can leave the "default" filters upside-down on top of the detector arrays to give a quick indicator that a change was made and make it easy to find the correct filters to change back to.

4. The next Intro to Flow class is tomorrow, Friday, March 13th, at 10:30 am in room 5216 (CNY149). This session is full so if you have not emailed me about attending, there may not be room for you.

Please look over the online tutorials on our training page, www2.massgeneral.org/aids/flowtraining.asp if you have the chance. You will also find a pdf of my presentation there.

Mike Waring

-----Original Message-----

From: Waring, Michael
Sent: Wednesday, March 11, 2009 2:15 PM
Subject: next flow class and updates 3-11-09

Flow core update:

1. HTS on 3 laser
2. Data storage
3. Referrals, direct them to website.
4. Flow Class Friday

1. We have just installed an HTS (High Throughput Sampler) on the 3 laser LSR, which will allow you to run samples from a 96 well plate instead of manually loading tubes. Andrew and I will spend the rest of this week writing up the usage protocol, if you are interested in learning how to use it please let me know.

2. A reminder about data storage on the LSR's: the limit in Diva is around 15 GB before it starts crashing, so it is necessary to minimize the number of experiments and amount of data kept in the database. No more than 2 experiments and nothing older than 2 weeks for any user. If you want to use a template of an experiment you have created, right click the experiment and "Duplicate without data", change the name to "4 color template" or something recognizable to you, make sure it is not linked to a compensation matrix (right click the instrument settings, and at the bottom it will either say "Unlink from xxxx" or "apply a matrix" or something like that. if unlink is an option, do it). Then export as an experiment into your folder on the d drive, and it will create a folder with the same name as the experiment, and will only contain the xml file (If you use rainbows to track settings, you can leave them in there so the baseline is kept with the experiments!). then when you need to use it, just import it back in, change the name, and go! do not store empty experiments in your diva profile, they will count towards your 2 experiment limit.

If you run an experiment that you KNOW is large (more than 20 tubes, more than 100k per tube), please export it at least within the week, so that you are not taking up hard drive space.

3. If you refer someone to our core, please direct them to our website www2.massgeneral.org/aids/flow_index.asp so that they can get access to our handouts etc and get information about what we have available.

4. *Please note the time change from our usual class schedule, 10:30 instead of 10 am*

The next Intro to Flow class will be held on Friday, March 13th, at 10:30 am in room 5216 (CNY149). Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, but also the material on Compensation if you have time, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply. My current class list is below, please let me know if you are on the list but do NOT plan to attend.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

-----Original Message-----

From: Waring, Michael

Sent: Friday, March 06, 2009 12:12 PM

Subject: Next flow class and other core updates

1. Config change on 3Laser LSR
2. *3L LSR UNAVAILABLE ON MONDAY*--upgrade install
3. Next flow class
4. Distribution list reorganization

1. After some issues with the APC Cy5-5 configuration on the 3 laser LSR, and needing to add another parameter to the listing, I've deleted the previous performance checks for that config which will only affect you if you are using the application settings for that config. If you are let me know and I'll help you resolve it.

2. Monday we will be installing an HTS system on the 3 laser LSR, which will allow you to run 96 well plates instead of tubes. I anticipate the install taking several hours, so I have blocked off the entire day up to 7 pm. it may be ready before then, but I can't make any guarantees. Once installed, Andrew and I will have to use it for a while to work out the proper protocol and then I will train interested users in how to operate it. we will be able to switch between using the HTS and tubes as needed.

Please note the time change from our usual class schedule, 10:30 instead of 10 am

3. The next Intro to Flow class will be held on Friday, March 13th, at 10:30 am in room 5216 (CNY149). Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website, but also the material on Compensation if you have time, before attending the class. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply. My current class list is below, please let me know if you are on the list but do NOT plan to attend.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

4. Due to the large number of users in the core, I've had to split up my distribution lists because they were exceeding the member limits, so if you get this email twice it means you are on 2 lists--I'll try to find those myself but let me know if it happens to you. If you want to be removed from future notices, let me know and I will delete your accounts.

Mike

-----Original Message-----

From: Waring, Michael
Sent: Tuesday, March 03, 2009 1:25 PM
Subject: Flow updates

The next Intro to Flow class will be held on Friday, March 13th, at 10:30 am in room 5216. Links to online training resources (and a copy of the presentation) can be found on our website at www2.massgeneral.org/aids/flowtraining.asp and it is advised that at a minimum, you review the introductory tutorials on the Invitrogen website before attending the class. After that, the compensation links are very helpful, and then any other materials that you have time to go over. There is a \$75 fee for attending the class. Let me know if you plan to attend, and please include a fund number for billing, the FULL NAME of the PI on the fund, and what group you belong to when you reply.

CNY149 room 5216 is on the 5th floor, if you take the central elevators to 5 and go through the door to the left of the balcony, head down the hallway to the right, it is the 6th door on the right, just before a bulletin board hanging on the wall.

Accuri Cytometers has offered to demo their C6 cytometer, a low-cost 4 color instrument.

<http://www accuricytometers.com/products/>

If you would be interested in seeing it, or even trying it out (they offered to leave one for a trial), let me know and if there is enough interest I will set it up.

3Laser LSR had some violet laser issues, was realigned and re-baselined so you may see some changes in voltages to get the same signals (20V or so with our beads...)

Mike