

Date: 9/13/11

Subject: Core Update

Latest news from the Ragon Imaging Core (Flow Cytometry):

1. Flowjo deal
2. Biacore meetings
3. User guide updated
4. Next Intro to Flow class
5. Immunology talk in Worcester, data from Fluidigm and Cytof
6. NECyto annual meeting

1. Thursday is the deadline for placing an order to take advantage of the buy 2 get 1 free promo, which would get you a dongle to run the Flowjo software on your own computer for \$1000 instead of the usual price of \$1500, please let me know asap if you are interested and what fund it should go on. We still need one more to make the order a multiple of 3.

2. There are a couple of Biacore educational opportunities coming up soon: an advanced Kinetic analysis training session in Boston November 16th-17th, http://www.biacore.com/lifesciences/training/courses/usa_canada/kinetic/index.html and the "Developments in Protein Interaction Analysis Conference" (DIPIA) will be held in Boston November 12-15th. www.gelifesciences.com/dipia

3. I've made a bunch of updates to the User Guide for the LSR 2 and Fortessa, you can download them from the website by clicking on the "core documents" page and selecting "LSR and Fortessa" User Guide. HTS instructions are now a section of the user guide and the hard copy can be found in the binders near the 3 and 5 laser LSRs. I have taken out a few sections that were in the old version, which will be part of a "New User Guide" that I will be posting soon.

4. The next Intro to Flow Class will be held on September 30, 2011. Please see the website for details:

http://www2.massgeneral.org/aids/flow_cytometry/training.htm

The current class list is:

Igor Theurl
Nienke Teijlingen
Dongfang Liu

5. On Sept 21st, Rich Konz of Umass Medical School in Worcester is hosting a talk by Pratip K. Chattopadhyay from the Vaccine Research Center of the NIH, titled "The Next Generation of Approaches for Assessment of Human Immunity."

Abstract: The immune system is remarkably complex, consisting of a variety of cell types, in various differentiation states, capable of diverse functions. Our efforts to understand disease pathogenesis, or identify early predictors of vaccine efficacy, require careful consideration and interrogation of this complexity. Historically, immune analysis has relied on simple enumeration of bulk cell populations or measurement of a single "representative" function (e.g., IFN γ secretion). However, with the development of multiparameter flow cytometry, the limits of historical approaches have become apparent as new, immunologically important cell types are defined (e.g., central memory T-cells or polyfunctional T-cells). Still, the relationship between the frequency of these cells and effective immunity remains weak; thus, the identities of key cell subsets and functions remain elusive. Identification of these would greatly aid vaccine efforts (by weeding out ineffective vaccines at early, preclinical stages) and drug development (by identifying high value targets). Against this backdrop, I will 1) review our recent work to make better use of the multiparameter flow cytometry data we generate, and 2) describe our experience (promises and pitfalls) with two new, highly multiplexed technologies for immune assessment. The first technology, Fluidigm, can interrogate 96-500 different gene transcripts at the single cell level, providing unprecedented resolution of the transcriptome. The second, CyTOF, allows the measurement of nearly 40 cellular parameters simultaneously, with exquisite sensitivity. Finally, I will present an integrated approach (or "pipeline") for the use of all these technologies in studies of human immunity.

Let me know if you want more details.

6. The New England Cytometry group will be having their annual meeting on November 1, at the Starr Center at the Schepens Eye Research Institute over on the MGH Main Campus:

<http://www.newenglandcytometry.com>

Thanks!
Mike Waring

Date: 9/7/11

Subject: Core Update

Hello flow core users. Some updates from the Ragon Imaging Core:

1. Instrument operation tip
2. NECyto website and meeting info
3. Ragon flow blog
4. Intro to Flow class Sept 30
5. Free Flow analysis software listing
6. Flowjo offer--buy 2 get 1 free

1. Instrument operation-- the low/med/hi buttons on the LSRs and calibur only matter when the instrument is on "run", and have no effect when in standby or while priming.
2. New England Cytometry has a new website, www.newenglandcytometry.com where you can register online for the upcoming meeting at the Starr Center (Schepens Eye Institute) being held November 1. See the bottom of this message for the list of speakers.

3. I'm making an effort to resurrect, or maybe more accurately, "start", a flow blog for various topics that I think are worth talking about. You can find it at ragonfacs.blogspot.com. The ragonfacs gmail account is no longer used for core business, so emails should still go to parcfacs@partners.org.

4. The next session of the Introduction to Flow Cytometry will be held on Friday, September 30, 2011 at 1 pm in room 5216. See our training page for details, and let me know if you would like to attend.
http://www2.massgeneral.org/aids/flow_cytometry/training.htm

5. For those interested, here is a page listing free Flow analysis software:

<http://flowcyt.cyto.purdue.edu/flowcyt/software/Catalog.htm>

The other option for analysis is to borrow a flowjo dongle (thumb drive that allows you to run the software) from the core, see me for details.

6. If you want your own Flowjo license, they are having a sale until September 15th, buy 2 and get 1 free. If you are interested, let me know and I can put 3 users in contact so you can all benefit from this sale. Regular price is \$1495, but with this deal you can get it for \$1000.

Mike

NECyto announcement from Rich Konz:

Greetings everyone:

New England Cytometry is pleased to announce the date for our annual Fall meeting. It will be on November 1st, 2011 from 8:00 am to 5 pm at the beautiful Starr Center at the Schepens Eye Research Institute in Boston.

http://www.schepens.harvard.edu/starr_center

We have an **excellent** line up of speakers again this year:

Dale Greiner, Ph.D.

Michael Brehm, Ph.D.

UMASS Medical School

<http://www.umassmed.edu/pmm/faculty/greiner.cfm>

<http://www.umassmed.edu/pmm/faculty/brehm.cfm>

Sue Reynolds

BD Biosciences

<http://www.bdbiosciences.com>

TBA

Fluidigm

<http://www.fluidigm.com>

Alan Waggoner, Ph.D.

Carnegie Mellon University

<http://www.cmu.edu/bio/faculty/waggoner.html>

Charles Goolsby, Ph.D.

Northwestern University

<http://www.pathology.northwestern.edu/Faculty/FacultyBio.cfm?ID=11>

Vincent Shankey, Ph.D.

Beckman Coulter

<http://russiancytometryschool.org/shankey.html>

Howard Shapiro, M.D., PC.

<http://www.shapirolab.com/>

David Basiji, Ph.D.

Amnis Corporation

<http://www.amnis.com>

Eric Rosenberg, M.D.

MGH

<http://www.massgeneral.org/transplant/doctors/doctor.aspx?ID=17081>

Mike Evans

Sexing Technologies

<http://www.sexingtechnologies.com>

William Hyun, Ph.D.
UCSF

http://cancer.ucsf.edu/people/hyun_william.php

We will look forward to see you on the 1st of November.

Please check our web site for meeting updates <http://www.newenglandcytometry.com>

BIG NEWS!!!

We finally have online credit card registration. Many thanks to Steve and Carol Benoit and Glenn Paradis for all their hard work to get secure online credit card registration up and running.

To register go to:

<http://www.regonline.com/Register/Checkin.aspx?EventID=987068>

and register as a participant. [\$75.00]

Registration is due no later than 21 October, 2011. If you cannot register with a credit card, please contact me directly.

This promises to be a great NECyto meeting with a terrific line up of speakers at an excellent venue.

Best regards and looking forward to see you on the 1st of November!

-Rich

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The sea, the great unifier, is man's only hope. Now, as never before, the old phrase has a literal meaning: We are all in the same boat.

Jacques Yves Cousteau

Date: 8/29/11

Subject: Core Update

1. Core printer use
2. Departed users accounts
3. Exporting experiments from Diva
4. Flowjo Deal--buy 2 get 1 free
5. Transferring files from the cytometer workstations
6. Filter replacement on 3L

1. The printer in the analysis area of the core is for core-related use only, please refrain from printing papers, manuscripts, etc.

2. Do not use accounts of departed users. If you are logging in with someone else's account, and they leave, be sure to let us know and we'll make an account for you. When a user leaves, their account is deleted, and you will not have access to the instrument anymore. You should have your own account, but there are exceptions that have been made in the past that this applies to.

3. Exporting experiments-- there is more about exporting files in the userguide explaining FCS vs Experiment exporting, but the best strategy that I have come up with to minimize file size while keeping all of your workspace elements etc is to export as fcs files, then duplicate without data and export the empty experiment as an experiment. Once this is all deleted from diva, you can import the empty version of the experiment layout, and then import the fcs files into this existing experiment, and it will have the same result as exporting as FCS and as an experiment, without having to double your memory usage.

4. Flowjo deal--until September 15th, buy 2 and get 1 free. If anyone is interested in getting flowjo let me know, this is the best pricing you will likely get! When I get a group of 3, I'll put you in touch with each other and you can take advantage of the deal.

5. File Transfer methods--there are a few ways to transfer files from the cytometer workstations. You are limited to 3 GB of file storage on the D drive of the workstations, but please try to minimize this as excess data makes it take longer to defragment and scan for viruses:

--You can create an account and then log onto transfer.research.partners.org. This service will allow you to upload a folder of up to 30GB to a server, and an email will be sent with a link to retrieve the files from any computer.

--You can use internet explorer to log onto portal.partners.org, and in the window that pops up you would enter partners\abc12 (your user code) and then your password for logging into partners, and this will open your H drive on the "Network places" on that computer (which is accessible when you log onto the partners network on a PC) and allow you to drag and drop your files.

--We have an account on a MAD storage system that is web-based, so you can upload files there and then access them from any computer. This requires a user account so contact me if you would like to use it.

--you can also burn cd's/dvds or transfer the files to external storage (flashdrives, portable hard drives)-- be aware that partners is requiring encryption of portable storage devices.

6. We had to replace a bandpass filter on the 3 Laser LSR, which was for the Q800 detector. It is now a 780/60 like the rest of the instruments, and if you have tried Q800 in the past and it didn't work on this instrument, it should work with this new filter. Let me know if you have any questions.

Mike

Date: 8/22/11

Subject: Core Update

I apologize for sending out messages on consecutive days, but the first item is time-sensitive so I wanted to get it out asap...

1. Flowjo Deal!
2. NECyto meeting November 1

1. Through Sept 15th, Flowjo is having their buy 2 get 1 free offer, let me know if you are interested so I can get enough people in contact with each other to take advantage of this great offer. Academic list price is \$1495 each, so with this deal you'd only end up paying \$1000 each.

<http://www.flowjo.com/index.php>

2. Mark your calendars: The New England Cytometry Users Group (NECyto) meeting is going to be held on November 1st at the Starr Center at the Schepens Eye Research Institute (185 Cambridge Street, on the MGH Main Campus next to Simches), and will have another great lineup of speakers this year, save the date and watch your updates for more information about registration and speaker details.

http://www.schepens.harvard.edu/starr_center

Date: 8/16/11

Subject: Sorting Rules

Hello all, just a quick update on the sorting situation. The Biosafety office wants us to wear N95 masks when working with human samples, they are on the shelf in the sorter room, make sure you use the size you were fit-tested for.

Once cleared to run by yourself, I will give you access to book the sorter, please use the 4:30 to 4:45 slot or the 4:45 to 5 pm slot to enter your name, and then put the hours you want to book in the 'notes" field, and work together to plan conflicts/overlap. If you need an arbitrator, or think this will lead to problems, let me know and we'll do it differently.

The o-rings on the 70 um nozzles have been damaged, so we have to manually insert a new oring, just like for the closed loop nozzle. The process is the same, and it is important to visually confirm that the oring is installed correctly on the bottom of the flow cell.

Also, we've been having issues with the closed loop nozzle oring not being inserted correctly, which will lead to leaking and then the flow cell will become crusty and will degrade the signals, so please make sure that you visually confirm the oring is installed correctly (see me for a refresher if you need to be reminded). Also, if you lose an o-ring when removing the nozzle (so that it may be in the nozzle socket), it is really important to make sure you look carefully for it to make sure it is not in there. Adam has pulled out the closed loop in the morning and it has come out with 2 orings stuck to it. An extra o-ring in there will prevent the nozzle from seating properly, again leading to leaking. Another danger is if the o-ring falls into the waste aspirator, it may be sucked into the tubing and then block the waste line, so please be sure to look there if you drop one.

I'm also working on a new form for "after hours" sorting--since we don't really have to know what you are sorting, the new form will instead have a checklist of what you have to do for shut down, so that you can confirm that each step was performed and then you can initial/sign the bottom and keep up with my changes to the shutdown process.

If you have any suggestions on how to improve core signup/usage (especially for the sorting bit) please let me know, we are here to facilitate research not inhibit it!

Mike

Date: 8/16/11

Subject: Core update

A quick update from the Ragon Flow Core:

1. New Reagent rep for BD
2. Next Intro to Flow Class

1. Stephanie Ventullo, who has been our BDBiosciences reagent rep for several years, is moving on to instrumentation, and our new reagent rep for MGH is Nick Confuorto, nicholas_confuorto@bd.com, feel free to contact him with any reagent questions.

2. The next Ragon Institute Introduction to Flow Cytometry class will be held on Thursday, August 25th at 1 pm. Please visit our training page for details:

http://www2.massgeneral.org/aids/flow_cytometry/training.htm

The current class list is:

Bjorn Khul

Yan Liu

Nicole Lai

Theodorus De Groot

Elena Stampouloglou

Francesca Bersani

Let me know if you have any questions.

Mike Waring

Date: 8/4/11

Subject: Core update

1. Next Intro to Flow Class
2. It's a girl
3. Providing Information for new users
4. Booking sorts
5. Using the LSRs ****ALL USERS PLEASE READ****

1. The next Intro to Flow class will be held on Thursday, Aug 25th, at 1 pm in room 5216. Please visit the website "Training" page for details:

http://www2.massgeneral.org/aids/flow_cytometry/training.htm

2. Caterina Page Waring was born July 18th, everyone is doing fine! I'm back in the office almost full time, so assisted flow sessions and training etc can be scheduled, just let me know.

3. When directing new users to our core, please send them to our website for information about what the core offers.

http://www2.massgeneral.org/aids/flow_cytometry.html is the main page, with a menu on the left for the different instruments.

New users, please be sure to provide a fund number for charging core fees, along with the PI on the fund (not necessarily your PI but the person the fund was awarded to).

You can also find our page by going to www.ragoninstitute.org and clicking on the "flow cytometry" link to the right.

4. Just a clarification, if a user is ONLY sorting with us and does not need to book time independently, we will book the time on schedulebook as "Guest" and put the user's name as a "note" on the reservation. Times booked as "Guest" are already reserved.

5. On the various LSRs we have, we've been having some issues with air in the sheath filters lately, so I just want to reiterate/remind that even if the tanks do not need to be filled, you should vent the filter to make sure there is no air in the system. If you do find significant air, please let me know so that I can follow up with the prior user to make sure the tanks are being filled correctly. It is for your own protection as well to make sure there is no air present when you are running your samples. If you do not know what I am talking about, please schedule a time for a training session so that I can show you the proper way to fill the sheath tanks.

If you would like to be removed from our distribution list for the Ragon Institute Imaging Core (Flow Cytometry) please let me know and I will delete your account.

Mike Waring

Date: 7/15/11

Subject: Core update

This is an update from the Ragon Institute Imaging core, flow cytometry facility. If you feel you have received this message in error please let me know and I will remove you from our list.

1. Resources on schedulebook
2. Sort requests, email Adam
3. Viability staining for sorting
4. 4L violet laser replaced
5. Biacore fixed
6. HTS units both operational
7. MGH Core listing
8. Time off

1. For gaining access to resources on Schedulebook, I am the contact person. For use and training on the instruments, the contact person is listed in the "Resource Notes" which can be opened by clicking the "N" next to the name of the device on the calendar. You can log in as a guest to view the calendar by using the username flowguest and password flow.

2. For sorting, the parcfacs@partners.org account cannot be accessed off of the partners network, so for the next month or so while I am out (see #8), you should cc Adam's account on sort requests (atchicoine@partners.org).

3. If you are using a viability dye for sorting, you may want to consider using either PI or 7AAD or something similar, rather than the live/dead fixable dyes for viability--the intent of these amine-reactive dyes is to allow you to fix the cells and know what cells were alive at the time of fixation. If you are not fixing them, you may get additional cell death by the time your cells are sorted, so it will not accurately reflect your sample's viability. Also, you are coating your cell with dye, which may impact your cell's health (we have not seen an issue, but some cell types might be sensitive).

4. The violet laser on the 4L cytometer has been replaced, and the replacement is higher power so you will have an increase in signal-- our QC particles gave the same MFI with a voltage setting 20 volts lower than previously.

5. The Biacore is fixed. If you are interested in using it, please let me know.

6. One HTS unit was fixed, and the replacement has arrived for the second unit. They are interchangeable, only the cover is different (the one with the black plastic on the back of the cover is used with the fortessa/5L with either base unit).

7. We are now listed on the official MGH Cores directory:

http://www.partners.org/researchcores/cytometry/ragon_cytometry_MGH.asp

8. I will be going on paternity leave any day now, and Adam will be here by himself. I will be checking my email intermittently. Please contact him with any core issues, or use the parcfacs@partners.org address. We will be reducing the available sorting hours so that Adam will have time to perform other essential core tasks.

Thank you!
Mike Waring

Date: 6/22/11

Subject: Core update

1. Work related emails
2. Flow class
3. Flash drives must be encrypted
4. Alternative file transfer options
5. Compensation meeting presentations online
6. Schedulebook accounts

1. I have a lot of users that communicate with an email account other than their partners one, just an FYI this is against hospital policy:

<http://library.partners.org/PartProd/trove.asp?FI=Information+Systems&DI=Policy%3a+Partners+Acceptable+Use+%28PH-502%29&HU=EmptyURL>

You may have to log in with your Partners login to access the page.

The important bit:

"Prohibited Activities

Prohibited activities include but are not limited to:

- Use of personal email accounts (e.g. Gmail, Yahoo, AOL, Hotmail, etc) for business related communications instead of their partners.org email account.
- Auto-forwarding of partners.org emails to an outside account"

For new users that may not know this yet, if you want to access your outlook account from the web, you can log on through www.partners.org/email

2. The next Intro to Flow class will be June 30, see the "Training" page of our website for details: (http://www2.massgeneral.org/aids/flow_cytometry/training.htm)

If you would like to attend, please let me know--the following people have signed up already:

Chris Ecker, Batur Ercan, Qian Chen, Jerome Rogich, Keith Rands, Matthew Brenn, Kailan Sierra-Davidson, Patrice Starck, Bjorn Kuhl, Christian Koerner

3. As announced in a recent hospital message, flash drives must now be encrypted:

"Partners HealthCare has implemented a *USB Portable Device Encryption* policy. The policy describes mandatory encryption requirements for USB portable drives (also known as jump drives, thumb drives, flash drives or memory sticks) used for work-related purposes. This is a system-wide policy, which applies to all Partners HealthCare entities, employees, and agents.

Under the policy, all USB portable drives used to store Confidential Data must be encrypted in accordance with the standard described in the document. The encryption requirements are required by federal and state law, and are intended to secure USB portable drives from unauthorized access when they are lost or stolen.

The policy is available at:

<http://library.partners.org/PartProd/trove.asp?SC=portable%20usb%20drive%20encryption&FI=Information+Systems&DI=Policy%3a+Portable+USB+Drive+Encryption&HU=EmptyURL>

You are required to read and abide by the policy. Please make sure you understand how the policy applies to you, and ask your manager or your site's security officer if you have any questions:

http://intranet.partners.org/finance/hipaa/Security_3.asp"

4. There are a few options for file transfer if you don't want to use flash drives.

--Research Computing offers a large file transfer service, transfer.research.partners.org, you have to create an account but you can then zip folders of data and upload them to a server, the service sends a link to the specified email address for you to retrieve them.

--portal.partners.org allows you to log onto the partners server from non-partners workstations, allowing you to transfer files to your H drive or other drives that you have been given access to in the Partners system. In the dialog box that opens the domain is "partners\abc123" (your partners ID) and the password is your password, however it only seems to work in Internet Explorer, I have not been able to get it to work in Firefox.

--We now have access to a MAD server, which is accessed via a web browser and you can transfer files that way. Info about the project:

http://www.research.partners.org/wiki/index.php/The_MAD_project

Let me know if you are interested and I'll send you the info.

5. There was a recent meeting in the Longwood Medical Area about compensation, and the presentations have been posted online for those that were not able to attend. Here is the link: <http://www.flowimagingcytometry.org/eve.html>.

6. Just a reminder/notification that the schedulebook calendar system is used to reserve many of the Ragon resources (PCR machines, etc). Creating accounts or adding reservation rights can only be done by myself, Thomas Diefenbach (head of the microscope half of the Imaging Core), or Adam Chicoine (my technician). Other researchers are in charge of the specific instruments, so for questions relating to instrument use you should contact them. Clicking on the green "N" next to the name of the instrument on the calendar will bring up the resource note, where you will find out who is in charge of that instrument.

Date: Tue, May 24, 2011

Subject: Core update

Visit our newly-revised website, http://www2.massgeneral.org/aids/flow_cytometry.html, if you find any bad links let me know!

1. new email address for Sorting requests
2. viewing information about your fcs files
3. "Broken link" data files from diva
4. Next Intro to Flow Cytometry
5. Core updates

1. Due to hospital policy regarding work-related emails, we will have to discontinue use of the ragonfacs@gmail.com account for booking sorts. Requests and sort inquiries should now be sent to parcfacs@partners.org which is the OLD flow core email account back when it was the Partners AIDS Research Center... I will be updating the website and documents with the new address.

2. Viewing FCS file information: It is possible to open an fcs file as a text file (Wordpad seems to work best) in order to view information about it such as the user account that was used to acquire it, the tube name, experiment name, export time, etc. I've attached a screen shot of a typical file and highlighted some text to show where some of these fields can be found. This can be useful to identify fcs files that are not associated with an experiment or only have a number for a name (which happens when you export as an experiment).



fcswordpad.JPG
(42 KB)

3. Diva "Broken Link" Data: sometimes when an experiment is deleted from Diva, some of the fcs files may get left behind. I have not figured out what causes this, or if it is an indication that the export failed for that file or not, but what ends up happening is that the files get left behind in the diva database with no way to delete them except by brute force (they cannot be found within diva, so it is necessary to delete them from the database folder on the hard drive). We typically just move them to a new folder "BD Broken Links", and using the above method (#2) it is possible to identify the user that they belong to. So if you see a folder appear in your D drive folder called "Broken Link data", these are files that we have identified as yours, and you can either check the experiment they came from to make sure you have them and then back them up elsewhere before deleting them, or just delete them from your folder.

4. The next Intro to Flow Cytometry class will be THIS Friday, May 27, 2011 at 12 pm in room 5216. View the "Training" page of our website for details, as well as links to online tutorials and a copy of the presentation.

http://www2.massgeneral.org/aids/flow_cytometry/training.htm The current class list is:
Adrienne Gladden

Katherine Yang
William Gostic
Yelena Leitman
Gregory Kranias
Anna Khachatryan
Matthew Stein
Samy Hakrrouch
Lisa Buvall
Shannon Wilton

Let me know if you would like to be added or removed...

5. All of my prior core updates will shortly be available on the "Core Documents" page of our website, http://www2.massgeneral.org/aids/flow_cytometry/core_Documents.htm.

Date: Tue, May 17, 2011 at 1:48 PM

Subject: Core update

Greetings from the Ragon Institute Imaging Core, Flow Cytometry group.

1. Next Intro to Flow Cytometry class May 27th.
2. Mid-range Rainbow beads for tracking settings available through the core
3. Schedulebook for reserving analysis stations.
4. File transfer options

1. The next Intro to Flow class will be held on Friday, May 27, at 12 pm in CNY149 room 5216. Let me know if you would like to attend. There is a \$95 fee for attending the class, so I will need to be provided with a fund number to cover the fee, and please be sure to include the PI on the fund, the fund NAME, and if it is an NIH fund I need the NIH number as well. Before the class I recommend going to our "Training" page (linked from the main page http://www2.massgeneral.org/aids/flow_cytometry.html) and viewing the online tutorials linked there. You will also find a copy of the presentation there, which you can look through beforehand to get a preview.

2. The core now has a supply of aliquots of mid-range rainbow beads for tracking your settings, which is CRITICAL to data quality when comparing samples collected on different days, especially for MFI comparisons. Please read my write up found on the "Core Documents" page of the core website linked above, or the direct link is:
http://www2.massgeneral.org/aids/flow_cytometry/documents/rainbowtracksettings.pdf

The beads have been aliquotted into small plastic tubes and are in a labeled white freezer box in the top refrigerator in the sorter anteroom, CNY149 room 5555. You can take one of the tubes, add a little sheath, and drop the tube directly into the 12x75mm polystyrene FACS tubes and load it on the cytometer. Let me know when they start to run low. Feel free to email me any questions, or stop by my desk in "room" 5233.

3. We are now following the analysis station calendars on Schedulebook, so if you want to use the analysis stations please let me know if you need access. There is an Image analysis PC, a flow analysis Mac, and a flow analysis PC. The image analysis pc has Flowjo as well as a bunch of imaging software (see Thomas for details about the latter), the Flow PC has Modfit and Diva, and the Mac has Flowjo and Cellquest Pro. I have created an "Analysis" sign up account, username "data" password "data", if you don't have a schedulebook account you can create a reservation with this account and just put your name in the "Notes" field so we know who it is. Do not delete other users's reservations, I get a report of all reservation events, so any conflict of reservation can be figured

out, and any abuse of the system will result in a loss of privileges. We also provide loans of flowjo and diva dongles for doing analysis on your own computers, let me know if you are interested.

4. Due to the high volume of data that is generated in the core, we are unable to offer long-term storage on the instrument workstations. Users are limited to 3 GB of data in their personal folders on the instruments. In order to move the data, you can either use a flash drive, burn cds or dvds, or transfer over the network.

*For flash drives, please be sure you wipe them before using them to eliminate any virus they may be harboring.

*There are 3 options for file transfer over the network

--Using Internet Explorer (hasn't worked for me in Firefox), go to portal.partners.org and log on with your partners username (abc12) and password. The drives that you have mapped to in the partners network (h drive, etc) will show up in your "Network Places" and you can drag-and-drop files to them.

--Use the Partners large file transfer service--transfer.research.partners.org and you will have to create an account. You log in with your partners email address and can upload entire folders up to 30 GB. The service emails a link to the account that you indicate for where to retrieve the files.

--We have set up an account on a MAD server (Massive Array of Disks project) that can be accessed from the internet. Contact me if you would like to use this, which requires a username and password to be provided.

Date: Fri, Apr 22, 2011 at 12:23 PM

Subject: Core update

1. New Fluorochrome!
2. New filters have been installed
3. Papers with data generated in the core
4. MAD storage
5. Silver earring found by the 3 Laser

1. We will be hosting a seminar from Biolegend about a new fluorochrome, BV421, which is detected in the same channel as Pacific Blue and is as bright as PE, so the separation is maybe a log higher than Pacific Blue--we've tested out a few markers and it looks really good. They have a wide selection already available, which can be found on their website. Please see the attached flyer for details on the seminar, and let me know if you want to attend. Breakfast will be provided.

<http://www.biolegend.com/brilliantviolet>

Current product list:

http://www.biolegend.com/index.php?page=pro_sub_cat&action=search&criteria=brilliant+violet

<<Biolegend_MGH.pdf>>

2. We have swapped out some of the filters for the new 4 Laser to improve the signals in those channels. The new filter setup is now "default" and the filters that it was shipped with that we had been using is called "factory". We did find that the filter that we chose for Q655 (660/40), while it worked fine on the 3 laser LSR, gave poor resolution of positive and negative beads and very high signal for the unstained beads, so we changed it to 660/20 and that works much better. I don't think anyone has set up their panel using the 660/40, but I just wanted to make users aware of the change. Printed versions of both configurations are available at the instrument for reference (please do not remove!), and will be posted on the website.

3. I would like to request anyone that has a paper either accepted or under review that has used the instruments of the Ragon Imaging core to please send me the information about the paper (Title, authors, journal) so we can keep track of the impact the core has had on the MGH Research community. Your help would be greatly appreciated.

4. We have set up an account for a web-based server for transferring files from the cytometers and microscopes. This will avoid the need for flash drives or burning cds, and may be a better option than the large file transfer service or the partners portal login (which requires you to be on a computer in the partners network). If you are interested, let me know and I can give you the details.

5. A silver earring was found near the 3 Laser LSR, if you have lost it I have left it near the instrument, on the wall. Let me know, or come and claim it.

Date: Tue, Apr 19, 2011 at 4:56 PM

Subject: Ragon flow Core update

1. If you use Compensation beads, please read this!
 2. New filters on new 4 Laser LSR
 3. New User Guide on the website
 4. New Resources on schedulebook
-

1. Compensation bead update--As I have broadcast in the past, not all comp beads are created equal--different batches of plastic will have different optical properties and you must make sure your binding bead and non-binding bead match to get proper compensation.

The way to tell if your beads don't match is either put the binding bead and non-binding bead in the same tube WITHOUT ANTIBODY and run them--if you see 2 peaks in a pacific blue histogram, they do NOT match and CANNOT be used together! If you've already mixed them with antibody, then APC or FITC or PE should NOT spill over into Pacific Blue, so you can view a dot plot of one of them vs. pacific blue, and they should have the same pacific blue signal (or again a histogram of pacific blue and look for 2 peaks which would indicate a mismatch). If there is a shift, this is bead mismatch and NOT spectral overlap.

You should try to match the lots (keep the kit together!), or failing that you should match the expiration dates. If you cannot find a matching negative, just use a drop of the binding bead and keep it in a separate tube to run as unstained. Rat and mouse kits may come from different batches, so you may not be able to use the same unstained for all tubes. See me if you run into this issue.

Most recent batch of Polystyrene, WD1B1B, covers expiration dates from Aug 31 2013 thru Feb 2014, and possibly earlier or later but those are the known timepoints. Dec 31 2012 is still Z02 and is INCOMPATIBLE with the newer batch. I'm not sure what the exact cutoff is, I've asked BD and will share the reply. If you have beads that expire between 12/31/12 and 8/31/13, let me know and we can compare them to find out. IF you have older beads I have most of that info so let me know and we can figure it out.

2. New Filters will be installed on the new 4 laser SLR2 ("4R") Wednesday, the filter assignments will be printed and left by the cytometer and will be on the website, if you want to use the filters that were shipped with the instrument (to keep signals consistent for an experiment/study in progress), refer to the "factory" layout to see which ones to change. See me with any questions.

3. New User Guide has been posted to the website, it combines all of our LSR instruments in one guide, so there are some instrument-specific sections, as well as all of the default configurations for the instruments in the one document. Let me know if you find anything that needs clarification or correcting.

4 New resources added to schedulebook, they will by default appear in your display view, but you will not be able to reserve them until we have given you access to them. If you wish to hide them from view, you can go to Tools>personal settings, and then hold down <control> while you click on them to de-select them from the list of "visible resources" (on a mac, hold the apple key), which will prevent the resources you have already selected to view from being de-selected as well. See the calendar instructions for more tips on how to use them:

http://www2.massgeneral.org/aids/flow_cytometry/documents/schedulebook.pdf

If you do not wish to use the Ragon Institute Flow core or receive these updates, let me know and I will delete your account.

Date: Fri, Apr 8, 2011 at 1:57 PM

Subject: Core update--compensation, filters, and classes

Latest from the Ragon Institute Imaging Core, Flow Cytometry wing:

1. Make sure your compensation particles match!
2. Filter changes on the new 4 laser
3. Next Intro to Flow

1. Compensation mismatches

An issue that originally came up in May 2009 has recently resurfaced, so I want to remind everyone that it is really important to make sure your stained particles match the unstained particles you are comparing them to when doing compensation. Different batches of the BD Compbeads (or any beads, in general) will have different levels of autofluorescence, we have seen signal shifts of up to half a log between different batches. The batch of plastic used is not indicated on the vial, but you should be able to match the expiration dates if there is doubt. Vials with the same expiration date should have been made with the same plastic batch, but even a 2 month difference may mean it is a new batch (cutoffs I know of are 4/30/10, 10/31/10, 3/30/11; all BD Compbeads with exp date 5/30/11 thru 9/30/11 are the same lot, Z01. I do not have info on batches later than that). If there is a difference, you will either under- or over-compensate for that color as the software will attribute the shift to spectral overlap. I am posting a longer writeup on the Purdue list and on the mailing list maintained by Tufts, if you are interested... you can always use a tube of the binding bead without any antibody as the negative to get an exact match, but as long as you have the non-binding bead of the same batch you don't need to do that.

Keep in mind this applies to other particles as well: if you are using pre-treated cells (fixed a large batch and stored them in aliquots for viability dye staining), be sure the negative and positive come from the same batch, if not the same tube--the longer they are kept, the higher the autofluorescence will become and comparing them to a fresher cell will result in compensation errors. or there is a product from Invitrogen called Arc Beads that are amine-coated beads that will bind the viability dye. This bead would have its own negative bead, as well. You would not be able to compare it to the unstained BD Compbead...

2. We have some new filters for the 4 Laser Rental LSR, to improve detection in a few channels. The filter swap will take place Monday next week, if you wish to keep using the old filters and are happy with the signals they give, they will be kept in a box in the drawer on the instrument table and I can show you how to swap them in.
3. The next Intro to Flow Class will be Wed, April 13th, for more details please go to the Training page of our website:

http://www2.massgeneral.org/aids/flow_cytometry.html and click on "Training"
Current class list is below, let me know if any additions/subtractions are required.

Charlie Lee

Kristina Todorova

Sergia Velho
Yajie Wang
Elizabeth Tiglao
Jens Dinter
Shohei Shinozaki
Rachel Ingraham
Clara Degos
Yasuyo Sano

Date: Mon, Mar 21, 2011 at 3:53 PM

Subject: Ragon flow Core update

Its been a while so there are a lot of items to update about for the Ragon Institute Flow Cytometry core:

1. Next Ragon Institute Intro to Flow Cytometry class
2. Cell straining options
3. New User Guides for LSRs
4. Sorter Contamination resolved
5. Default filter change on new 4 Laser LSR ("4R")
6. Connecting over network for data transfer
7. Phosphoflow seminar in the Longwood area April 7th
8. Research Course in Flow Cytometry information

1. The next Intro to Flow Class will be Wednesday, April 13, 2011 at 11 am in room 5216. Please visit our website for details:

http://www2.massgeneral.org/aids/flow_cytometry.html and click on the "Training" link to the left.

2. Users have asked about cell strainer options. The sorting core uses the BD Falcon tubes. Mesh size should be based on your cells. The following are options posted to the Purdue message board recently:

BD Falcon tubes with cell strainer caps (\$493 for case of 500), Fisher # 08-771-23

Partec Celltrics http://www.partec.com/cms/front_content.php?idcat=139

Small parts nylon mesh http://www.smallparts.com/plastic-mesh/s/16414371/ref=sp_pi_16414371

If anyone has alternative products that you like, please let me know and I will share the info.

3. I'm almost done assembling and updating the new LSR user guide combining all of the instruments into one document, it will be posted to the website and printed versions will be by each instrument. I'll also be posting a new user guide with beginner info on sample prep, antibody usage, etc.

4. Contamination: we had a bout of contamination on the sorter, and have changed our shutdown procedure to avoid a relapse. As a result, morning setup and evening shutdown may take longer, but we should still be able to book sorts from 10 to 4:30.

5. We will be changing the default filters on the new 4 Laser LSR, if you have a panel that has been using the current filters and want to keep using that setup, we will still have the filters available for you to swap them back in. I'm happy to discuss with you if you have concerns.

6. All of the cytometers are on the network now, so there are 2 other options for transferring files instead of having to use USB storage devices or burning the data.

A. File transfer: Using Internet Explorer, go to portal.partners.org and type `partners\abc12` (your user ID) and your password. The drives mapped to your profile will appear in either "Network Places" or in "My computer". Can drag and drop, but be

aware of storage limits, and when you close Explorer it will close the connection, so leave Explorer open while transferring.

B. Partners also offers a large file transfer service: go to <https://transfer.research.partners.org>, you will need to create an account. The mail program zips the folder or files for you and you can send it to yourself, it Emails you a link to download the files from the network.

7. There will be a Phosphoflow seminar at Harvard Medical School on Longwood Ave on April 7

http://www.floocyte.org/FRTP/Current%20Courses/Boston_ADV_2011.html

8. This summer is the 34th Annual Research Course in Flow Cytometry-- "A research methods course in flow cytometry will be offered with emphasis on applications in cell biology, immunology, cell labeling, fluorescent protein analysis, bead-based assays, screening and sorting. The faculty consists of over 20 distinguished members of the flow cytometry community."

To find more information and to register for the course, go to the website for the Center for Biomedical Engineering

(<http://cbme.unm.edu/>) and click the "Upcoming Courses" tab under the "Events" tab.

Date: Fri, Mar 11, 2011 at 9:54 AM

Subject: Ragon flow core--Virus on new 4 laser

Once again, the new 4 laser has become infected with a computer virus, infection was detected on Monday and then again today. ANYONE that has used portable storage on that computer this week, please let me know, and scan your drives for possible infections. I'm going to try to figure out where it is coming from. Please make sure that any computer you are using your flash drives on is scanned for viruses, and check your flash drives! I don't want to ban flash drives and make everyone burn cd's but if users are not more careful with their file transfers, this may become necessary. There are many other cores that have such a policy.

Date: Tue, Mar 8, 2011 at 12:42 PM

Subject: Intro to Flow Cytometry class and core update

Hello users of the Ragon Institute Flow Cytometry Core. If you feel you have received this email in error, or no longer wish to be on this distribution list please let me know and I will remove you.

1. Intro to Flow class TOMORROW
2. realignment of the 4 Laser LSR
3. sorter contamination

1. Below please find the class list for tomorrow, Wed, March 9th at 11 am in CNY149 room 5216. Visit our website for details:

Www2.massgeneral.org/aids/flow_cytometry.html and click on "Training".

I recommend going through the introductory tutorial linked from that page to prepare for the class. If you print the presentation, it is 90 slides so you may want to do multiple slides per page.

2. We just had the 4 laser LSR in 5555 realigned and a focusing lens added to the UV laser, so you may see shifts in signals.
3. We have been dealing with a recurring issue of contamination on the sorter, and have changed our protocols for cleaning and shutdowns to try to prevent a return. Always please let me know of any issues in the core, whether it is contamination in a sort, or instrument issues, or something running in an unusual manner.

The class list for tomorrow is below. If you are on it or missing in error, please let me know and I'll fix it.

Matt Zubrowski

Himashinie Diyabalanage

Anna Kaliszewska

Adrian Veres

Erin Groden

Georgio Kourjian

Asheley Kern

Madeline Lindqvist

Amol Kavishwar

Emily Ricq

Sarah Javaid

Adam Greenblat

Julian He

Jicole Wilson

Raveshni Durgiah

Christine Thoens

The following are on my list for the next class in early April:

Charlie Lee
Kristina Todorova
Sergia Velho
Yajie Wang

Date: Wed, Mar 2, 2011 at 2:02 PM

Subject: Flow Cytometry Core update

The next intro to flow class will be held on March 9th. The class list is below. If you are interested in attending but are not on the list, let me know and I will add you. Details can be found at www2.massgeneral.org/aids/flow_cytometry.html and click on "Training".

The following are signed up:

Sergia Velho

Himashinie Diyabalanage

Anna Kaliszewska

Adrian Veres

Erin Groden

Georgio Kourjian

Ashley Kern

Madelene Lindqvist

Amol Kavishwar

Emily Ricq

Sarah Javaid

Date: Fri, Feb 18, 2011 at 4:10 PM

Subject: Ragon Flow Core update

I will be on vacation next week, returning 2/28, for core issues in the meantime contact Adam Chicoine (atchicoine@partners.org or, preferably, ragonfacs@gmail.com)

1. Same day booking may exceed 2 hour limit
2. Biacore 3000 training
3. New Spectraviewer tool available
4. Intro to Flow Cytometry class March 9th

-
-
1. Booking clarification--usage limits may be exceeded if the reservation is made on the same day as the time slot.
 2. Biacore training will commence in March, if you are interested and have not contacted me yet, please do so. I will set up a separate distribution list for Biacore related matters.
 3. There is a new spectraviewer hosted by Biolegend, linked from our "web links" page.
 4. The next Intro to Flow class will be held on Wednesday, March 9, 2011, at 11 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/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 \$95 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.

Date: Mon, Feb 7, 2011 at 2:53 PM

Subject: Computer virus on new 4 Laser LSR, Ragon Imaging Core

A computer virus was detected on the new 4 Laser LSR this morning. It manifests upon logging into windows after starting up with a message saying that "folder C://Documents could not be found", and Malwarebytes finds 8 malicious items upon doing a scan (we've seen this one before).

I have contacted the users that ran Diva in the past few days, and 2 of them have the infection on their flash drives (at least the later one probably picking it up from the LSR), but I have no way to know if someone went and transferred files without logging into diva, so if you have used a flash drive or external hard drive on the new 4 laser LSR in the past few days without logging into diva, let me know so that I know who has been on it and we can pinpoint the source and who might be at risk so it does not spread.

I am tracing back the infections I have found, it seems that it shows up as "autorun.inf" and "system.exe" which are generic names that are often harmless (autorun is usually on flash drives to launch the windows browser and show what is on it) but I think these are suspicious in that the "created" date is 2/4 and 2/5 (on diff't drives), and I am going to see what other devices they have been used on. Hopefully the spread is limited, and as one user asked: yes if you use a mac you will not be affected (a good way to look for them is to attach the drive to a mac, so that if the virus is present you wont infect the computer), but if you use an infected flash drive on the cytometer computers you will spread the virus to other users. SO please be careful! I'd prefer if you had a flash drive that you could wipe clean before copying files from the cytometers, then transfer them to your workstation and wipe the drive again before retrieving more files.

Date: Tue, Feb 1, 2011 at 1:38 PM

Subject: Ragon Flow Core update

1. Core Rate Changes

2. "Restricted time" hours change-PLEASE READ!!

3. Data storage reminder

1. As an official MGH Core Facility, the Ragon Imaging Core will periodically reassess our user fees and make adjustments to the rates. New rates go in effect today, and will apply to February 2011 usage of the core. Thomas has sent out the Microscopy rates to his users, but the new flow core rates are:

Calibur, 3L, 4L, rental 4L: \$40 per hour (no change)

5L: \$50 per hour (lower)

Sorting: \$100 per hour (same)

Training: \$95 Intro to flow class; instrument orientation is billed at usage rate for instrument.

Biacore: will be billed at \$15 per hour, if you are interested in measuring protein interactions on this instrument let me know!

2. Core rule change-- we will be shifting the restricted hours so that the 2 hour limit applies from 12pm to 9pm (instead of 9 am to 6 pm), and as before there are no more than 2 reservations per week between 2 and 6pm. This will be enforced starting next week-- for this week (since reservations have all been made already) the old 9 to 6 time window will apply. In the past and moving forward, this is total reservations for ALL analysis cytometers, not per instrument, so you may NOT book 2 hours on one instrument and an hour on a different one in the same day within the restricted time. If the time is still available on the morning of the opening, you may exceed these limits. In addition, the limit of 2 bookings between 2 and 6 pm is again for the whole core, not per instrument.

3. Data storage on the instrument workstations is limited to 3 GB, and if you are over this you will be posted on the dry erase board near the instrument. Chronic offenders will be given a warning by email, and repeat warnings will result in loss of access to the instruments until it is fixed. Please see me if you have any questions.

Date: Fri, Jan 21, 2011 at 2:04 PM

Subject: Core update

Visit the Ragon Flow Core website! [Www2.massgeneral.org/aids/flow_cytometry.html](http://www2.massgeneral.org/aids/flow_cytometry.html)

1. New instrument! GE Biacore 3000 added to the core
2. Flowjo 3-for-2 deal
3. Next flow class

1. The Ragon Imaging Core is proud to announce the addition of a GE Biacore 3000 to our repertoire. This instrument allows you to characterize and identify binding partners. You are able to perform Kinetic/affinity characterization, screening, and profiling; measure concentrations; LMW interaction analysis; and also recover analytes that bind to your substrate. Select from the tabs across the top of the frame of the below website for more information:

http://www.biacore.com/lifesciences/products/systems_overview/3000/system_information/index.html

I will be setting up a user list for interested researchers, and we will arrange to have the applications specialist for our area come in and speak with users to help optimize their experiments. Let me know if you are interested-- please use the subject "Biacore" and don't just reply to this message, and include the applications you hope to use it for.

2. Flowjo is having a deal for January, buy 2 get 1 free, if you are interested let me know and I'll get you matched up to take advantage of this great deal! (\$1000 per license instead of \$1500).

3. The next intro to flow class will be Thursday, Jan 27th, 2011 at 2 pm in 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. My current class list is below, please let me know corrections (additions/subtractions) as appropriate.

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.

Katerina Mantzavinou

Anh Hoang

Ajay Shah

Teuta Zoto

Sinan Akdeniz

Tim Dudek

Jordan Ciciliano

Shizuko Kosugi

Eitan Shimko
Uriel Nieves
Morgane Griesbeck
Ying Chan
Morris Ling
Prince Yalley

Date: Thu, Jan 13, 2011 at 11:25 AM

Subject: Core update

A lot of items to update in the flow core, please read through the list to see if any of it is of interest to you! If you would like to be removed from this distribution list, let me know and I will delete your account.

1. Publications/acknowledgements of the core
2. Reservations: book only the time you need, and let me know if you are booking an instrument for the first time.
3. Phosflow seminar next week!
4. Flowjo deal--3 for 2!
5. Intro to Flow class Jan 27th
6. Filter change on new 4 Laser LSR, ready for use!
 1. If you haven't already, it would be greatly appreciated if you could send me any citations for papers that made use of the Ragon Imaging core (Microscopes or flow cytometry) for our records.
 2. A reminder to be considerate of the other users, and make sure you only book the amount of time that you need. There are a lot of long reservations lately which are all within the rules of booking time but are making it difficult for some to get time on the instrument especially the 4 laser LSR. I'll be following up and making sure that users are not overbooking time (so reserving 2 hours and then only using for 15 minutes) so please be aware.

Also, you do NOT automatically have access/accounts on all core instruments, so if you are planning to use a different instrument than usual, be sure to check with me first to make sure there are no quirks you need to know about, and so I can create an account on that instrument for you and grant privileges on schedulebook.

3. There will be a seminar about BD's Phosflow technology on Tuesday, 1/19, from 11 to 1, please let me know if you are interested in attending. The abstract is:

Activation State Analysis in Single Cells: Techniques and Applications for Analyzing Phosphoproteins Using Flow Cytometry

Intracellular assays of signaling systems have been limited by the inability to correlate functional subsets of cells in complex populations on the basis of active protein states within the native context of the cell. We demonstrate the ability to simultaneously monitor active protein states via phospho-epitope recognition in subpopulations of complex cell populations by multiparametric flow-cytometric analysis. Multi-dimensional assessment of active protein states, in combination with surface markers and other flow cytometric detectable parameters (ie: cytokines, apoptosis), can provide functional assessment on a single cell level that may have utility in clinical diagnostics and/or disease progression. Furthermore, the ability to profile both activating and inhibiting conditions of multiple protein states simultaneously within the cell in a rapid and parallel manner may be extended to pharmaceutical screening of compounds. Detailed examples for the utility of assessing the signaling pathways using flow cytometry in various model systems will be discussed.

The talk will consist of a 30 minute seminar, followed by a "virtual workshop" where specific experiments can be discussed, and some tips and tricks for successful phosflow staining will be shared.

4. Flowjo is having a deal for January, buy 2 get 1 free, if you are interested let me know and I'll get you matched up to take advantage of this great deal! (\$1000 per license instead of \$1500).

5. The next intro to flow class will be Thursday, Jan 27th, 2011 at 2 pm in 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. My current class list is at the end of this email, please make corrections (additions/subtractions) as appropriate.

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.

6. We were seeing high background in the APC channel on the new 4 Laser LSR ("Rental"), which I was able to eliminate by choosing a bandpass filter of a slightly longer wavelength and avoiding the background laserlight that was finding its way into the detector with the typical 660/20 filter that was in place. It now has a 670/14, but we can probably get better signal with a wider bandpass so I will work on purchasing a replacement to hopefully get even more signal from that channel. People are slowly starting to switch over to this new instrument, so let me know if you want an account and I will get you set up.

Mike

The next flow class list so far:

Katerina Mantzavinou

Anh Hoang

Ajay Shah

Teuta Zoto

Sinan Akdeniz

Tim Dudek

Jordan Ciciliano

Shizuko Kosugi

Date: Tue, Jan 4, 2011 at 11:26 AM

Subject: First core update of 2011!

1. 5 Laser update, New 4 laser LSR
2. Booking time: "after hours"
3. Website address
4. Product Wiki for cytometers
5. Core usage rules and penalties.

1. The blue laser has been replaced on the 5 Laser LSR Fortessa, so everything should be working. The new 4 Laser LSR ("Rental") is set up, we're having some issues with bubbles in the flow cell, a service call is open and I'll update when that has been resolved. We are also investigating why unstained comp beads have a high signal in the APC channel so be sure to determine your settings with unstained cells or match MFIs of rainbows from another instrument--it should be fine but negative beads will have high signals in APC. Let me know if you have any questions, the configuration is on the website.

2. With regards to booking time on the cytometers and user responsibility to turn them off, "After hours" is after 5 pm, which is when Adam and I leave for the day. Anyone signed up after 5PM becomes the responsible party to make sure the instruments are turned off at the end of the day. Failure to do so will result in logging a "violation", repeat offenders will be subject to penalties.

3. To find our website, go to http://www2.massgeneral.org/aids/flow_cytometry.html or go to www.ragoninstitute.org and click on "Flow Cytometry" on the right side.

4. Product wiki reviewing flow cytometers:
<http://www.productwiki.com/flow-cytometers/>

5. Rules reminder:

I am going to begin tracking warnings and violations relating to core usage. Normal operating procedures are laid out in the "using the calendars" document on our website's "core documents" page and in the user guides, and summarized here for your convenience. Everyone begins with a clean slate, and if you have had any violations since tracking started you have been notified. Future violations will be tracked with applicable penalties applying on repeated offenses.

a. Calendar bookings are limited to 2 hours in length between 9 am and 6 pm, and no more than 2 bookings per week between 2 and 6 pm. Non-Ragon users may not book time more than 1 week in advance, and Tues, Wed, and Thurs afternoons from 3 to 7 are reserved for Ragon users until the day before, when unclaimed time can be booked by all.

Violations of these limits will result in warnings for the first 2 occurrences, and a 1 week ban from reserving instrument time on a third offence.

b. data storage is limited to 2 experiments in Diva and nothing older than 2 weeks, and no more than 3 GB in the D drive folders. To avoid excess data in Diva, the core exports data as an experiment into users' D drive folders, into a new folder labeled

"experiments mmddy". users are responsible for checking these folders to see if they need the data and storing or deleting as appropriate. These folders should be removed, and storage on the d drive should be minimized.

You will be warned when your folder has exceeded the limits, on the 3rd warning your access will be blocked until it is reduced.

c. If you are the last user, it is your responsibility to make sure the instrument is turned off at the end of the day. If you cancel on the day of your reservation and were the last user, you must make sure that the user before you knows that they need to turn it off, and all users should check the calendars when they are done to verify there is a reservation after them. I

receive notices when events are booked or cancelled so I can verify the timing of any changes.

d. All users are responsible for making sure the tank levels are adequate for use before and after your time, and that instruments are kept in good condition (no broken parts, etc). Any breakage found or caused should be brought to the attention of core personnel.

****Any misuse of the instruments will result in a warning for a first offense, a 1 week ban for a second offense, or a 1 MONTH ban for a third offense.****

I appreciate your cooperation in keeping the core running smoothly!