

Flow Cytometry Core Facility

What's New in Cytometry??

Happy 2017!! The FCCF has been busy!

Check out what's new in the FCCF!

- **NEW** Cell Signaling Luminex Assays
- **NEW** Data storage location
- **NEW** Data Analysis Software license
- Enhancements to CyTOF services
- Recruiting for additional staff
- **Coming soon** Self sorting services

The Flow Cytometry Core is starting the New Year with lots of new and exciting changes to enhance your flow cytometry research. Along with these changes will come lots of educational opportunities designed to reduce transition to new and improved ways to get to the answers you need for your research. You have spoken and we have listened. We are now putting into place many new tools and services to meet your flow cytometry needs.

Here's what we have been working on for the New Year. We are expanding our Luminex panels to include many of the cell signaling panels you have asked for. These will include assays for total and

phospho proteins. A Sample preparation workshop will be offered in February. We are also moving to a new data storage location; more reliable and more space, with automatic backup services to come soon. We will also be initiating a new site license for FCS Express, a new more powerful easy to use data analysis software package, for both Macs and PCs. We have implemented several new services to enhance our CyTOF services, which should make your access to this technology easier and more efficient. During the coming year we also plan to implement a program for those who are interested in doing their own cell sorting! Of



Your data will soon have a new home that is bigger and more reliable!

course all these efforts require additional support staff, so we are recruiting. Please see below for details on all these new programs and services.

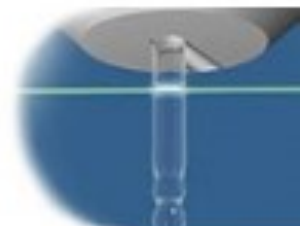
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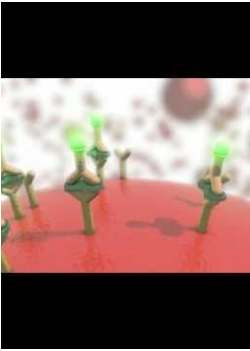
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Like Us on Facebook!

Want to keep up with new protocols, seminars, relevant publications and everything Flow??? Check us out and follow us on Facebook to see what we're up to and what's new in the Flow Cytometry world. www.facebook.com/uvaflow. This is a great way to get real time information about relevant webi-

nars, publications, new technologies, and core announcements. We also have lots of information on our website; visit us at www.med.virginia.edu/flow-cytometry-facility/





Look for a new array of cell signaling multiplex Luminex assays coming soon!

Cell Signaling Multiplex Assays

Beginning shortly, the FCCF will expand the choices of Luminex multiplex assays to include Cell Signaling Assays. These will include individual 2 plex assays (Total and Phosphoprotein) as well as several multiplex signaling panels (Multi-pathway; STAT; Akt/mTOR; etc.) panels. You will also be able to create your own custom panels (up to 8 plex) for any analytes that are compatible to be plexed. Most of the kits will

detect both human and mouse, and some will also detect rat as well.

As there are special considerations for sample preparation for these assays, the FCCF will be holding a wet workshop on February 22-23. Space will be limited, and will be on a first come first serve basis. We will be running a AkT (1-3) Phospho and Total assay. The workshop will include sample preparation

tips, seminars, and running the assay. Participants will receive actual results from their samples. No more than 2 samples per participant, unless we have less than full capacity of participants. Mark your calendars and watch for more details and a sign up sheet if interested in participating in this workshop

New Data Storage Server Location

On January 27th, SOM IT migrated flow cytometry data stored on our old server location to a new server managed through ITC.

In addition to increasing the data storage capacity, the server hardware is also newer and more reliable. The old server was full, at least 10 years old, and experiencing difficulties. Although there were some initial issues

during the transition, David Stewart of SOM IT (dls3kx@virginia.edu) has worked to resolve any access permissions issues. If there were errors in your files during the migration, you were sent a report with the list of files that were not copied due to these errors. The server is now secured through your eservices id and password, so at least it is one less password to remember. The new server

address is for **Macs:** `smb://sammas.storage.virginia.edu/som-fccf` and for **Windows:** `\\sammas.storage.virginia.edu/som-fccf`.

As always, it is prudent and recommended to always back up your data to a second storage device and to regularly remove older data which you no longer need regular access.

“The safest way to store data is in multiple places”



FCSExpress works in a Microsoft Office like environment, making data analysis and presentation possible in just one place!

FCCF initiates new data analysis software license

The FCCF has entered into a software license agreement with DeNovo software to offer site licenses for their FCSExpress6 and FCSExpressPlus data analysis software. After careful and thorough evaluation of this software, we feel the capabilities enabled by this software are far superior to those available through FlowJo. With the Mi-

crosoft office like environment, you can perform spreadsheet analysis, bar graphs, linear regression analyses, pie charts, heat maps and more, without ever having to leave the software. These graphical and data analysis tools also will live update as you change your analysis gates. The Plus version of the software can also analyze Im-

agestream data files and any image files created through microscopy cell segmentation software (such as ImageJ or CellProfiler).

Presentations are also a snap as the layout can be treated just like a powerpoint slide and be exported directly to powerpoint, pdf, excel, etc. In addition, there are tools available for doing high

FCCF initiates new data analysis software license (cont.)

dimensional data analysis, such as for CyTOF data; K means cluster analysis, PCA, tSNE/viSNE, and R integration.

Licenses will be available for purchase on a yearly basis and charges will only be accrued for those quarters in which you actually use or access the software.

The rate for the FCSEXPRESS6 version is \$240/yr. or less if you don't use it in any one quarter. This is less expensive than your

current FlowJo rate. For the FCSEXPRESSPlus the rate is \$564/yr. or less if you do not use it in any one quarter. The core will also host two licenses of FCSEXPRESSPlus, that will be available to use free of charge.

The FlowJo site license will remain in place until September 30th, 2017. After this time, if you wish to continue using FlowJo, you will need to purchase an individual license.

A demo seminar of the software was held on January 26th and another will be held on March 28th, so watch for further announcements for location and time.

You will soon be able to order your FCSEXPRESS licenses through iLab. For more information, please contact Joanne Lannigan, 4-0274 or jl7fj@virginia.edu.

Enhancements to CyTOF Services

If you are interested in using mass cytometry (CyTOF) in your research, the FCCF has added a number of resources to help get you started. Did you know we now have a Mass Cytometry Antibody bank? No need to go out and buy multiple bottles of metal tagged antibodies to develop your panel and see if mass cytometry is the technology for your research. Thanks to funding from the Jeffer-

son Trust and SOM, we have a fully stocked bank of both mouse and human antibodies which you can access for \$5 per aliquot or you can donate a single bottle of your favorite metal tagged antibody and get a credit equal to the number of tests for any other antibodies in the bank. In addition, if your specific antibodies of interest are not available commercially conjugated to your metal of choice, we offer metal tag conjugation services.

With the recent upgrade of the instrument to the Helios, acquisition rates are faster, sensitivity is greater, and sample efficiency is increased. With the addition of Brian Capaldo, Ph.D., we also now offer computational analysis services to help you with the high dimensional data analysis. We will be hosting a workshop and seminar series in March for those interested in getting started, so watch for more details coming soon.



With the use of heavy metal tagged antibodies and ICP mass spec, the CyTOF can evaluate >40 markers simultaneously on single cells.

Coming Soon! Self Cell Sorting Service

The FCCF is currently developing a cell sorting training program for those investigators who are interested in performing their own cell sorting. The FACSAria Fusion is a semi-automated, easy to use cell sorter that is fully integrated into a Class II biosafety cabinet for safe cell sorting. This cell sorter is capable of sorting up to 4 populations sim-

ultaneously, as well as single cell cloning. It is equipped with 5 lasers (375, 405, 488, 561, and 640nms) and can detect up to 15 fluorescent parameters.

Those seeking to become certified must have at least 3 years of flow cytometry experience, be regular users of the flow core, and complete the training course. The training course is currently

under development and will involve both theoretical and practical instruction. Certified individuals will have to pass both a written and practical exam before being allowed to schedule independent sorting. This service will not be available to any sorts requiring BSL2+ precautions. Stay tuned for more information.



FACSAria Fusion: Semi-automated, easy to use cell sorter, fully integrated into a Class II biosafety cabinet.

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We're Recruiting!!

Are you passionate about flow cytometry? Do you enjoy scientific research and working with people? Then you may be interested in a career in the Flow Cytometry Core. We are currently recruiting for a full time Flow Cytometry Core Specialist.. Check out our posting # 0620138.

"The incumbent supports the maintenance and quality control of flow cytometers, operating/troubleshooting of the flow cytometers, analyzing flow cytometry data, and providing oversight for core clients carrying out flow cytometry experiments."

Visit our website!
<https://med.virginia.edu/flow-cytometry-facility>

**Flow Cytometry Core Facility:
Your Partner in Single Cell
Analysis!**

Recent Publications from the FCCF

1: Smirnov A, **Solga MD**, **Lannigan, J**, Alison C. *High-Throughput Particle Uptake Analysis by Imaging Flow Cytometry*. Current protocols in cytometry. Forthcoming; 80 (11.22):1-17.

2: **Lannigan J**, Erdbruegger U. *Imaging flow cytometry for the characterization of extracellular vesicles*. Methods. 2017 Jan 1;112:55-67. doi: 10.1016/j.ymeth.2016.09.018. PubMed PMID: 27721015.

3: Barsky LW, Black M, Cochran M, Daniel BJ, Davies D, DeLay M, Gardner R, Gregory M, Kunkel D, **Lannigan J**, Marvin J, Salo-

mon R, Torres C, Walker R. *International Society for Advancement of Cytometry (ISAC) flow cytometry shared resource laboratory (SRL) best practices*. Cytometry A. 2016 Nov;89(11):1017-1030. doi: 10.1002/cyto.a.23016. PubMed PMID: 27813253.

4: Nascimento A, **Lannigan J**, Kashatus D. *High-throughput detection and quantification of mitochondrial fusion through imaging flow cytometry*. Cytometry A. 2016 Aug;89(8):708-19. doi: 10.1002/cyto.a.22891. PubMed PMID: 27387508.

5: **Lannigan J**, P Nolan J, Zucker R. *Measurement of extracellular vesicles and other submicron size particles by flow cytometry*. Cytometry A. 2016 Feb;89(2):109-10. doi: 10.1002/cyto.a.22814. PubMed PMID: 26889762.

6: Erdbrügger U, **Lannigan J**. *Analytical challenges of extracellular vesicle detection: A comparison of different techniques*. Cytometry A. 2016 Feb;89(2):123-34. doi: cyto.a.22795. Review. PubMed PMID: 26651033.

