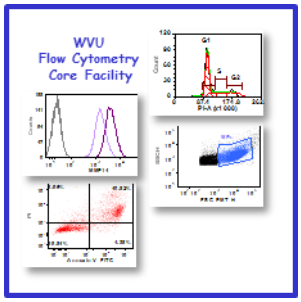
WVU FLOW CYTOMETRY CORE FACILITY



Newsletter Volume 1, issue 1 July 2014

Assay highlight: Cell sorting

In our inaugural issue we have chosen to highlight one of our exceptional services: **cell sorting**. Many of you are familiar with this technique; for those who aren't, it is the method for aseptic isolation of one or more desired population(s) of cells from a mixture, based on the expression of one or more markers detected by fluorescently labeled reagents. Typical isolations yield greater than 95% purity.

Why would you want to sort?

Aseptic cell sorting facilitates many downstream applications such as sterile cell culture, analysis of *in vitro* responses as well as RNA, DNA and protein isolation from highly enriched cell populations. Cell sorting based on cell surface markers detected by fluorescently tagged reagents has been around for many years and is a common application in our facility. We also routinely isolate rare cell populations such as normal and cancer stem cells from tumors or tissues, using non-traditional dyes such as Hoechst and Aldefluor.

In recent years, advances in the ease of tagging cells with fluorescent proteins via transfection of genes has popularized the use of cell sorting as a replacement or an adjunct to traditional methods of selection (via antibiotics). This has greatly enhanced the quality and speed of recovery of target populations and is a very popular application with a large portion of our user base (and at facilities such as ours across the country).

Our facility is equipped with a BD FACSAria high speed sorter. This instrument uses 3 lasers (407nm, 488nm and 633 nm) to detect up to 10 different fluorochromes as one time. In its current configuration, up to 4 distinct cell populations can be simultaneously sorted and collected into tubes or multi-well (6, 12, 24 or 96-well) plates, in a temperature controlled environment. The cell sorter is housed in a biosafety cabinet equipped with an aerosol management system and meets the BSL2 criteria. This facilitates the sorting of live prokaryotic (bacterial) and eukaryotic cells (including human cells) approved under BSL2 guidelines. Due to the complexity of instrument operation, all sorting is performed by the facility staff.

If the idea of sorting interests you please feel free to stop by the facility or email the Technical Director for more information.

Inside this Issue

Facility Location:
2160 HSCN
Phone:304-293-6273
e-mail: flowcore@hsc.wvu.edu

Hours of operation:
9:30 am to 5:00 pm, M-F

After hours access for experienced users by prior approval from Kathy Brundage

Instrument Highlight: LSRFortessa

In Sept 2013, our facility acquired the analyzing flow cytometer BD LSRFortessa with an S10 equipment grant from NIH. This instrument enhances and expands the types of flow cytometric analyses our facility can offer. It is easy to use and maintain, and has quickly become quite popular with our regular users.

The LSRFortessa is powered by four solid state lasers; 405 nm (violet), 488 nm (blue), 561 nm (yellow-green) and 628 nm (red). In its current configuration, the instrument has a capacity for the simultaneous detection and resolution of 12 distinct fluorescent dyes. This gives the user a wide array of dyes to choose from (See table on our website). Dyes with emission spectra outside of the current configuration can also be accommodated by substituting appropriate band-pass filters. The LSRFortessa is controlled by FACSDiva software (currently version 8) which is quite user friendly. Files from a previous experiment can be duplicated without duplicating associated data, thereby permitting consistency of instrument settings and worksheets from one experiment to another.

The 561 nm laser facilitates enhanced detection of PE and PE-tandem dyes which were excited by the 488 nm laser in the FACSCalibur. Additionally, it expands the range to include dyes such as **mCherry** and **mTomato** (and other fruits and vegetables) as well as the **Alexa** series of dyes that excite optimally in 500-600 nm range.

The instrument is also equipped with a Forward Scatter photomultiplier tube (FSC PMT) which enables resolution in the 250 to 1000 nm range (and even smaller particles when combined with appropriate dyes). The FSC PMT allows for enhanced detection and analysis of such microparticles as exosomes, microvesicles, mitochondria and bacteria.

Acquisition on LSRFortessa is accomplished either by the old fashioned 'one sample at a time' way (for small experiments) or, employing a high throughput system (HTS) where samples are injected from 96 or 384 well plates. While the initial setup of an experiment using the HTS is a bit involved, the speed at which the acquisition is completed more than makes up for it.

Training for user operated acquisition on LSRFortessa (with or without HTS) and the associated software is available and encouraged. Please contact us to schedule training on this new instrument.

To log in and reserve a flow cytometer, AutoMACS, MSD or analysis computer, please point your browser to the following URL →

CORES
Core Ordering & Reporting Enterprise System
https://cores-wvu.mis.vanderbilt.edu/login.cfm

Other Instruments in this facility

FACSAria Cell Sorter/Analyser
Operator: Facility Staff
Lasers: 488 nm Sapphire(SS)
633 nm HeNe
407 nm Violet(SS)
Detection Parameters:
Forward Scatter, Side Scatter, simultaneous detection of up to 11 fluorochromes
Applications:
Cell Sorting (Aseptic)
Cell phenotyping
Cell Viability
FISH, FRET, SPA

FACSCaliber Analyser
Operator: User
Lasers: 488 nm Argon
633 nm Red Diode
Detection Parameters:
Forward Scatter, Side Scatter, simultaneous detection of up to 4 fuorochromes
Applications:
Cell phenotyping
Cell cycle analysis

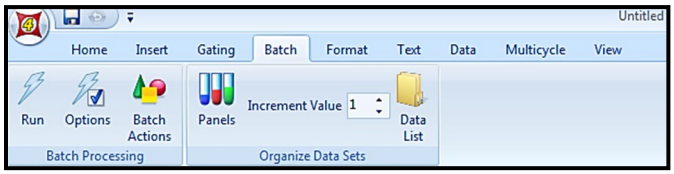
AutoMACS Magnetic Bead separator
Operator: User
Application:
single marker (extracellular) sorting, depletion sorting

MSD Multi-Array Platform
Operator: User
Applications:
Detection of cytokines, cell signaling proteins
multiplexed assay (up to 10)
Detection range:
1 – 10,000 pg/ml
Sample volumes:
25 µl or less

Analysis Tips

Batch Analysis with FCS Express 4 Part I

A useful feature of the FCS Express 4 software is the ability to quickly perform analysis of your FCS files using the **BATCH** feature available under a separate tab on the menu bar (see below).



With the BATCH feature you can print, export files to powerpoint or as pdf as well as export statistics to excel. This feature is easy to use, quick and requires you to create only a single set of graphs.

In this issue we will discuss how to BATCH your files for printing and exporting to powerpoint and as a pdf. In the next issue, we will discuss BATCH exporting of statistics to excel.

Batching Files for Export

Setting up the analysis page(s):

1. On one or more pages, using a single file, create a template that contains a set of plots you wish to use for all your files.
2. Create and adjust all the gates and markers as needed.
3. Check the appropriateness of gates/markers by opening all relevant control and a few experimental files in this set of plots.
4. Format the plot axes, dot numbers and colors, line widths, and fonts so they look the way you want them to.
5. Make sure the statistics box(es) contain(s) all the parameters you want for your analysis.

Running the batch process:

1. Click on the BATCH tab → Data List (icon to the far right; see picture).
2. In the box that opens, check and make sure all the files you want to run in this BATCH are present and that the file displayed in all your graphs on your analysis page is the first (top) file in the Data List.
3. Use the **X** or **+** to remove or add files to the list.
4. Close the Data List box.
5. Click on the Batch Actions icon (see picture)
6. Click and choose item(s) from the dropdown list of action(s) you would like to perform.
7. If you choose to save to powerpoint or pdf a box will open giving you several options including a place to name the new file and a location to save the file.
8. Close the box and click on the Options icon (see picture).
9. Check to ensure that it lists the correct number of files to be processed. Close the box.
10. Click the RUN icon (see picture). This starts the action you have chosen to perform. If saving to powerpoint or pdf you will see these programs open and when the software is done it closes the resulting file but leaves the software open.

If you are interested in using this feature and would like some assistance going through it the first few times please ask the staff of the facility for help.

Note to users:

Please acknowledge the WVU Flow Cytometry Core Facility when reporting your flow cytometry data, using the appropriate phrase(s):

FACSAria users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry Core Facility, which is supported by the National Institutes of Health equipment grant number RR020866 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

LSRFortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry Core Facility, which is supported by the National Institutes of Health equipment grant number S10OD016165 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

Frequently Asked Questions

1. Do I need to bring no stain and single stain controls every time? If so, Why?

   ANSWER: YES! No stain (negative) and single stain (positive) controls are required for each experiment. Small (tiny) variations in cell staining from one day to another is unavoidable, due to small differences in culture conditions or cell densities or other not so obvious reasons. Our instruments are highly sensitive and can detect these tiny variations in staining quite readily. Additionally, humidity, room temperature and laser age as well as alignment (which can drift over time) all affect signal detection by the instruments. Also, please keep in mind that every time the instrument is serviced (normally, at least two preventive maintenance visits per year) the lasers are re-aligned and optics are adjusted, resulting in minor changes in voltage settings for optimal laser performance. So, to correct your data for these minor variations, each set of experiments must be accompanied with controls built in and generated the same day. This helps fine tune the instrument voltages for each experiment and correct normalization of data.

2. What should I do if I go to sign up for an appointment and I see the calendar but it does not let me make an appointment?

   ANSWER: Check with your PI to make sure he has associated you with a funding string since you cannot make an appointment if you have no funding string associated with your name. It is also possible that the funding string you have been associated with has expired and a new one needs to be added to the system and/or linked to you. If this is the case please have the PI send a new funding string to kbrundage@hsc.wvu.edu so that the new funding string can be entered into the CORES scheduling/billing system.

Please email us with questions you would like to see answered here in future issues!

Upcoming Events:

Friday July 4	**Independence Day**	**Facility closed**
Monday September 1	**Labor Day**	
Tuesday August 11	**EMD Millipore Seminar Smart Flare and other Flow Cytometry applications**	**Rm 2157, 2:00 pm**

Fee Schedule (2014-2015 hourly rates)

	WVU user	Non-WVU user
Data acquisition by User	$33.00	$48.84
Facility assistance for data acquisition	$17.00	$25.16
Data acquisition by Facility Staff	$50.00	$74.00
Sorting	$74.00	$109.52
Data Analysis (unassisted)	0.00	0.00
Data Analysis by Facility Staff	$50.00	$74.00

New User Guide

Hands-on training for FACSCaliber and LSRFortessa is **mandatory** for all new users and must be scheduled by consultation with facility director.

Sorting as well as data acquisition on FACSAria is by facility staff only.

Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own.