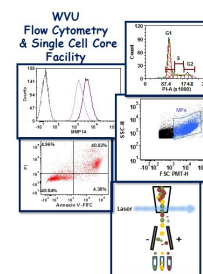


WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 3, issue 3

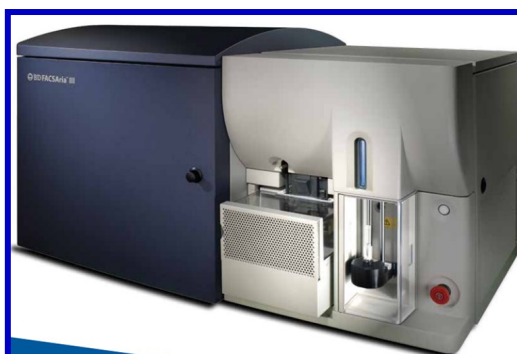
January 2017

What's New in the WVU FCSCCF for 2017

Happy 2017!! Hope everyone had a nice holiday break and are ready to get back in the lab. 2017 is going to be an exciting year in the facility. In the next few months we will be adding three new pieces of equipment to the facility thanks to Dr. Sally Hodder and the CTSI, Dr. James Simpkins and the Stroke CoBRE and the HSC Research Office.

New Sorter

The current sorter in the facility, FACSAria I, was purchased with an NIH S10 equipment grant in 2006. It has worked great over the years with minimal issues. Unfortunately, the manufacturer BD Biosciences will no longer service the instrument starting December 2017. Thus, we needed to purchase a new instrument. With funding from Dr. Hodder and the CTSI we have been able to purchase a new sorter, FACSAria III (see picture above). Although it looks almost identical to the current sorter it has some enhanced features. The most important one is a fourth laser. The new sorter will have violet, blue, yellow-green and red lasers. What this means to you the users is that since it will have the same laser and detector configuration as the LSRFortessa, the new sorter can be used for running samples for analysis when the LSRFortessa is busy. The new sorter will use the same software as the LSRFortessa as well. The one thing the new sorter will not have is the ability to sort into plates but that can be added at a later time if it is necessary. The expectation is that the new sorter will be installed at the end of January beginning of February.



Upgrade to FCS Express 5 Analysis Software

De Novo Software has developed a new version of FCS Express software (FCS Express 6). This new version has several new features with the most important one being that it will work on Macs as well as PCs. The facility is currently in the process of purchasing the new version. Once the purchase is complete, the facility will email all users the pertinent information for downloading the new version. In addition, a informational webinar on the new features of version 6 will be scheduled

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Facility Location:
2160 HSCN
Phone: 304-293-6273
e-mail: flow-core@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

Contact Kathy at:

kbrundage@hsc.wvu.edu

in the coming months. There will be no disruption of service for those who still want to use version 5 to complete on going studies.

Malvern NanoSight NS300

For all individuals who are interested in submicron particles, the facility will be getting a Malvern NanoSight NS300. This instrument will detect particles from 30 nm to 2 µm in size. It will determine the number of particles in the sample as well as their size. The model we are purchasing will come with 4 lasers (405 nm, 488 nm, 532 nm and 642 nm) and a 6 filter detection wheel so that the particles can be characterized using fluorescently labeled antibodies and dyes. The instrument should be in the facility no later than the end of March. The facility will email everyone once a delivery date is set.



Malvern Zetasizer Nano Z

Along with the NanoSight NS300 we are also purchasing a Zetasizer Nano Z. This instrument measures the zeta potential and electrophoretic mobility of particles in a solution using Laser Doppler Micro-Electrophoresis. For those not familiar with zeta potential, it is the measure of the electrical charge of particles suspended in a solution. It is often used in drug formulation and to explore protein aggregation and oligomerization. It should be in the facility by the end of March.



The two instruments from Malvern are being purchased with funds from the CTSI and the Stroke CoBRE grant.

Flow Cytometers in this facility

FACSAria Cell Sorter

Operator: Facility Staff

Lasers: 488 nm Sapphire
633 nm HeNe
407 nm Violet

Detection Parameters:

Forward Scatter

Side Scatter

Simultaneous detection of 11 fluorochromes

Applications:

Cell Sorting (Aseptic)

Cell phenotyping

Cell Viability

FISH, FRET, SPA

LSR Fortessa

Operator: User

Lasers: 405 nm OBIS LX
488 nm Sapphire
561 nm Sapphire
628 nm OEM

Detection Parameters:

Forward Scatter

Side Scatter

Simultaneous detection of 12 fluorochromes

Applications:

Cell phenotyping

Cell Viability

Cell Cycle analysis

FISH, FRET, SPA

New User Guide

Hands-on training for FACSCaliber, LSRFortessa and C1 Single Cell Auto Prep System is **mandatory** for all new users and must be scheduled by consultation with facility director.

Sorting 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.

To log in and reserve a flow cytometer, C1, 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 Instrumentation Available in the Facility

<p><u>AutoMACS Magnetic Bead separator</u></p> <p>Operator: User</p> <p>Application: Single extracellular marker cell sorting Depletion/negative cell sorting</p>	<p><u>gentleMACS Octo Dissociator with Heaters</u></p> <p>Operator: User</p> <p>Application: Dissociation of tissues into single cell suspension for culture or flow cytometry assays Homogenizes tissues for downstream molecular biology applications</p>
<p><u>C1 Single Cell Auto Prep System</u></p> <p>Operator: User or Staff</p> <p>Application: Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for downstream genomic applications.</p> <p>Downstream applications: RNA seq DNA seq PCR</p> <p>Format: 96 or 384 chambers per chip</p> 	<p><u>MSD Multi-Array Platform</u></p> <p>Operator: User</p> <p>Applications: Detection of cytokines, cell signaling proteins Multiplexed assay design: (1-10 analytes/plate) Detection range: 1 – 10,000 pg/ml Sample volumes: 25 µl or less Assay Time: 4—6 hours depending on analytes being detected</p> 

New Fee Schedule Starting February 1, 2017

	User Operated Analyzer	Facility Operated Analyzer	FACSAria Sorting	AutoMACS	MSD Sector Imager 2400	User Operated C1	Facility Operated C1	gentleMACS Octo Dissociator
WVU User	\$34.65/h	\$52.50/h	\$77.70/h	\$14.70/use	\$10.50/use	No Cost	\$210/h	\$10.50/sample*
Non-WVU User	\$51.98/h	\$78.75/h	\$116.55/h	\$22.05/use	\$15.75/use	\$112.50/plate	\$315/plate	\$15.75/sample*

***Only if facility tubes and filters are used. If you supply your own then there is no cost.**

FCS Express 5 Software

Loading Data According to Parameter Name

As you probably know from using the FCS Express software, the default for loading new data files into a graph is to match the parameters by number i.e. P1, P2, P3 and P4. This works fine when all the parameters are collected in the same order but if in one experiment you collected PE before PE-Cy5 and in the other you collected PE-Cy5 then PE this will be an issue if using the same template/layout to analyze the data. All you need to do to get around this issue is to make a simple change to the default settings. Outlined below is how to make the change:

1. Click on “**File**” tab in the tool bar
2. Select on “**Options**” (2nd from the bottom of the list)
3. A box opens
4. Select the 6th option in the right hand column “**In new layouts, remember parameters by**”
5. Change the numbers option to name
6. Click “**Okay**”

Now regardless of the order you collect the parameters they will load appropriately in the graphs. One recommendation is you change the default settings prior to making any graphs.

Upcoming Holidays & Events

January 1, 2017	New Year's Day	Facility Closed
January 16, 2017	Martin Luther King's Day	Facility Closed
March 24 –29, 2017	Kathy out of Lab	Facility opened; No sorting
April 14, 2017	Spring Holiday	Facility Closed

Note to users:

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

FACS Aria users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell 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).

LSR Fortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell 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).

C1 System users: Experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers U54GM104942 (CTR), P30GM103488 (CoBRE) and P20GM103434 (INBRE).