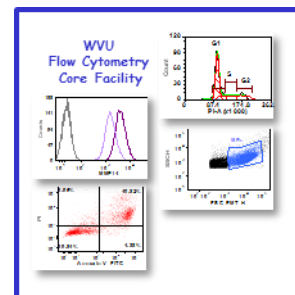# WVU FLOW CYTOMETRY CORE FACILITY

Issue highlight: High throughput System for LSRFortessa

In our inaugural issue, we discussed the capabilities of the newly acquired and highly popular analyzing flow cytometer, the LSRFortessa. One of the unique features of this instrument is that it can acquire samples either one tube at a time or from the wells of a microplate via a high throughput system (HTS, pictured below).
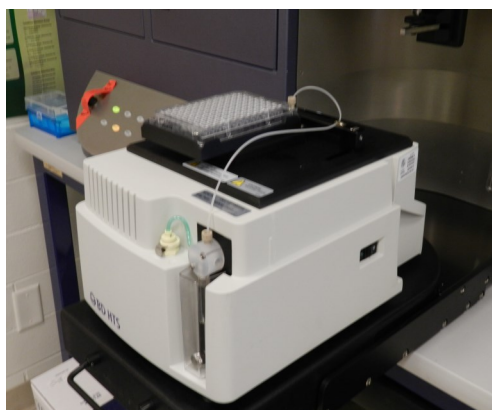
The HTS has multiple advantages. Cells can be stained directly in the wells of 96-well microplates which are loaded onto the HTS, thereby eliminating transfer of cells into tubes. The HTS can be set up to acquire test samples either manually, one at a time, or for automatic acquisition from single or multiple plates. The HTS will mix each sample for uniform re-suspension of contents prior to injection into the cytometer at 0.5 to 3.0 μl per sec up to 200 μl 'regular' mode or a fixed 22 μl per well in 'high throughput' mode. The regular mode is sufficient for most experiments. Since only a small amount of sample is loaded in the high throughput mode, it is particularly useful for antibody titrations, screening tests and any experiment requiring just a positive/negative (yes/no) determination. Thus, when using the HTS, acquisition proceeds at a pace that reduces user time in the facility.

While the number of cells stained for acquisition with tubes or with HTS remains unchanged, the cell density in microplates can be much higher. When setting up the microplates, unstained and single stained controls required for instrument setup and spectral overlap compensations are placed in the first row. The plate layout is entered by the user before the start of the experiment and can be saved as a template for future use. Compensation for spectral overlap between multiple fluorophores can be done before test sample acquisition or at the end of the experiment.

Initial setup of the experimental layout for HTS is somewhat complex and must be completed before starting an experiment. So if you are planning a large experiment and would like to try the HTS, we urge you to please contact us so we can help you optimize the setup.

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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

Contact Kathy at:

kbrundage@hsc.wvu.edu

Instrument Highlight: SECTOR Imager 2400

The SECTOR Imager 2400 by Meso Scale Discovery (Gaithersburg, MD) uses electrochemiluminescence to detect and quantitate proteins such as antibodies, cytokines, chemokines, growth factors, signaling molecules (including phospho-proteins), as well as markers associated with metabolic, cardiac, and vascular disease processes, in a multiplexing, high throughput, sandwich ELISA.

Plates with pre-spotted common panels are available. Capture antibodies against proteins of interest are pre-spotted at specific locations in the wells of a multiwell plate; with up to 10 distinct spots per well in a 96-well plate, and 4 spots per well in a 384-well plate. User-defined custom panels spotted either by the manufacturer or the user is also an option. The assay itself is similar to an ELISA assay. Plates are blocked and test samples such as serum, plasma, sputum, tissue culture supernatant or cell extract, containing target protein, are usually added undiluted, at as little as 25 μl per well. Antibodies tagged with an electrochemiluminescent tag are applied next, to detect the bound target protein. The plate is then washed and following the addition of a Read buffer, read in the SECTOR Imager 2400. Voltage applied to the plate causes any bound label to emit light at 620 nm; the intensity of emitted light gives a quantitative measure of the test sample. The assay can be completed within 4 to 6 hours; plate reading takes just a few minutes. Electrochemiluminescence generated at each spot is detected independent of its neighbors. Since no current runs through the side of the wells or the area between the spots, non-specific signals and background are at a minimum.

The greatest strength of this system lies in the large dynamic range of detection (>4 logs). This eliminates the need for multiple sample dilutions, which is particularly helpful when comparing samples from normal and diseased states. An added feature is that if an assay requires only part of a plate, the remainder of the plate can be sealed and stored at 4^0C for several months for future use.

The cost of the assay is similar to the cost of using the luminex system or cytometric bead array system. For example, a Human ultrasensitive Th1/Th2 7-Plex panel and all the necessary reagents and buffers, is approximately $1150/plate. For the cell signaling proteins, the cost is approximately $650/plate for a 2-plex that detects pAKT (pS473) and Total AKT. The cost for using the instrument is $10/appointment. You can run multiple plates per appointment.

The SECTOR Imager is housed next to the analysis computer in 2160 HSCN. If you are interested in using it or would like to see examples of data generated by WVU investigators using this system, please contact Kathy Brundage in the Flow Cytometry Core Facility. Additional details about the system and a list of available kits can be found at http://www.mesoscale.com/CatalogSystemWeb/WebRoot/

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

CⓞRES
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

LSR Fortessa
Operator: User
Lasers: 405 nm
         488 nm
         561 nm
         628 nm
Detection Parameters:
Forward Scatter, Side Scatter, simultaneous detection of up to 12 fluorochromes
Applications:
         Cell phenotyping
         Cell Viability
         Cell Cycle analysis
         FISH, FRET, SPA

Analysis Tips

Batch Analysis with FCS Express 4 Part II

In Batch Analysis Part II, we will discuss how to use the BATCH processing feature to export statistics to an Excel spreadsheet. This function can be performed in combination with running a batch analysis for generating a pdf or PowerPoint of your files (explained in **Batch Analysis with FCS express 4 Part I**)

Setting up the analysis page(s):

1. On one or more pages create a template that contains a single set of graphs that you want to analyze all the files with.

2. Create and adjust all the gates and markers on each graph.

3. Check to make sure the gates and markers are set appropriately by loading all the files one by one, making adjustments if necessary.

4. Format the graphs so they look the way you want them to with respect to axes, dot colors, line width and fonts.

Exporting Statistics to Excel:

1. Click on the BATCH tab → Data List

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 top (first) file in the Data List

3. Use the **X** or **+** to remove / add files to the list

4. Close the Data List box

5. Click on the Batch Action icon

6. In this box choose the "Export to Excel (Column Mode)" under the "Add Report" section on the left. A new box opens. Fill in the options under the "File Options" and "Worksheet Options"

7. To add keywords (used to identify each file) choose the "Keyword Token" under the "Add Report" section. A new box opens. Fill in the options under "Data Source", "Keyword" and "Field"

8. To add statistics choose the "Statistics Token" under the "Add Report" section. A new box opens. Fill in the options under "Data Source", "Statistic" and "Field". You will need to repeat this step for each statistic you want to export

9. Once all the statistics have been added and you are back to the "Batch Action" box, check that the correct number of files to be processed is listed under the "Options" icon.

10. Click on the RUN icon. This starts the action you have chosen to perform. At the end an excel file will open showing the statistics you exported. If you are also saving to PowerPoint or pdf (explained in **Batch Analysis of FCS Express 4 Part I**), these programs will open, create the appro[riate file(s) and then close the file(s). Note that these application(s) will remain open after saving the files.

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

Update on new techniques:

On Aug 11, 2014, the FCCF hosted a seminar by EMD Millipore. One of the products that the company representatives discussed was Smartflare, a novel fluorescence based assay for semi-quantitative detection of mRNA expression in eukaryotic cells. This assay capitalizes on the target cells' ability to uptake fluorescently labeled cDNA probe attached to gold particles via endocytosis and release the probe into the cytoplasm for hybridization with its target RNA. The beauty of the assay is that the probe is simply added to the media the cells are growing in, and within 16 hrs. the cells can be assayed without further processing. In addition to analyzing the cells by flow, the cells can be sorted based on RNA expression plus/minus surface marker and returned to culture.

One caveat to using this technology is that all eukaryotic cells do not endocytose the probe to the same extent. The company has an uptake control for testing efficiency of endocytosis. As a token for hosting the seminar, the company provided us with a free sample of the Cy5 labeled uptake control.

To date, we have tested it on two cell-lines derived from C57Bl/6 mice. In the figure at right, **panel A** shows uptake of 4, 8 and 12 μl of diluted uptake control per 50,000 cells in the mouse macrophage/monocyte line IC-21 after 20 hrs. **Panel B** shows a comparison of uptake in IC-21 versus a T-cell line EL4, and **panel C** shows the kinetics of endocytosis in both cell lines with 8 μl diluted uptake control in 50,000 cells over a period of 48 hrs. IC-21 cells show respectable endocytosis, starting uptake within 6 hrs, and are maximally labeled within 16 hrs. In contrast, EL4 cells fail to internalize appreciable amounts of the uptake control throughout the length of the experiment.
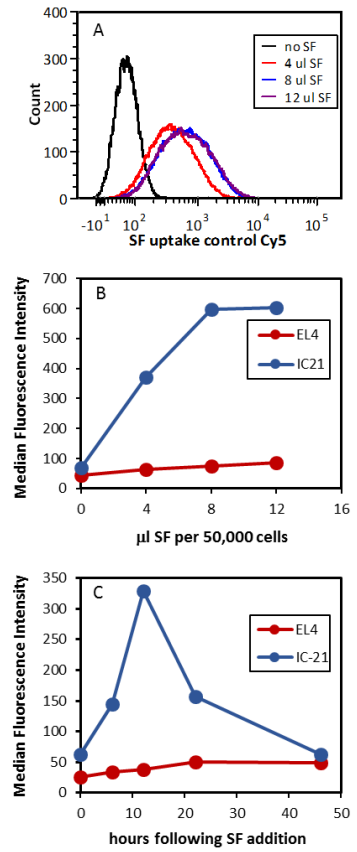
We have uptake control remaining and invite you to try it on cell lines of interest to you.

Email us with questions / topics you would like to see included here in future issues!

Upcoming Events:

October 8 – 25	Kathy out of town	Facility open to experienced users, sorting limited to Friday.
November 12	Seminar: MSD Data Analysis	Rm 2157 HSCN, 2:00—3:00 p.m.
November 26 - 28	Thanksgiving Break	Facility Closed
December 24 – 26	Winter Break	

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.