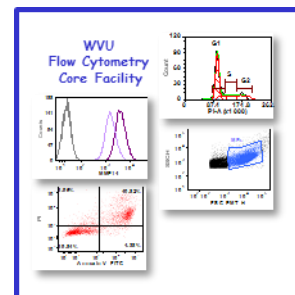
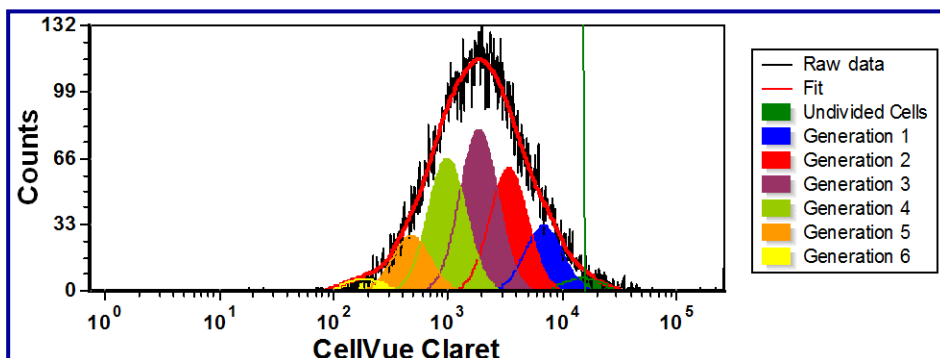


WVU FLOW CYTOMETRY CORE FACILITY



Assay Highlight: Cell Proliferation



The ability of cultured cells to proliferate and their rate of proliferation speaks volumes about their health. Over the years, scientists have used many different assays to measure cell proliferation. Most of these assays involve incorporation of ³H-thymidine or other thymidine analogs in newly synthesized DNA. The readout can be radionuclide measurement or detection of labeled DNA by antibodies and flow cytometry. Colorimetric assays employing the tetrazolium dye MTT to detect cellular oxidoreductase activity as a measure of cell viability and therefore changes in cell number, are also popular.

Flow cytometry has several advantages over the other methods for measuring cell proliferation. First, flow cytometry methods use non-radioactive reagents. Second, the assays are easy to perform and require no special equipment other than a flow cytometer. Finally, flow cytometry allows you to determine cell proliferation rate of different cell

populations in a mixed culture using fluorescently labeled antibodies.

There are a number of dyes and reagents that can be used for measuring DNA synthesis and cell division using flow

Table 1. List of Dyes Commonly Used in Proliferation Assays		
Reagent	Company	Method of Action
BrdU (5-Bromo-2'Deoxyuridine)	Abcam	Thymidine derivative; incorporates into DNA replacing the nucleotide thymidine
	Biolegend	
	Cell Signaling Technology	
	Life Technologies	
Click-IT®EdU (5-ethynyl-2'deoxyuridine)	Life Technologies	Thymidine derivative; incorporates into DNA replacing the nucleotide thymidine
	SigmaAldrich	
CellVue® Dyes	eBiosciences	Cell membrane labeling
CellTrace™ Dyes	Life Technologies	Binds to amine groups on proteins
CFSE (carboxyfluorescein succinimidyl ester)	Abcam	Fluorescence derivative; binds to amine groups on proteins
	Biolegend	
	eBioscience	
	Life Technologies	

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2160 HSCN
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9:30 am to 5:00 pm, M-F

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

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cytometry. Table 1 lists some of the most common reagents/dyes.

When using hydymidine derivatives like BrdU or EdU, cells are cultured in the presence of the thymidine derivatives for a period of time, typically 24 or 48 hours. During this incubation, as the cells duplicate their DNA, BrdU or EdU are incorporated into the DNA of the cells. The more the cells proliferate, the more BrdU or Edu is incorporated into the DNA. At the desirable time point, the cells are harvested and stained using antibodies that will detect either the BrdU or EdU. Due to the chemistry involved in the staining process, BrdU requires slight denaturing of the DNA in order for the antibody to bind whereas EdU does not. If in addition surface staining of cells is desired this should be completed prior to staining for BrdU and either before or after staining for EdU. Cells that have undergone proliferation will have higher levels of DNA-specific fluorescence and one can compare the mean/median fluorescent intensity (MFI) of cells cultured under different culture conditions or different cell types grown under the same conditions.

When using protein binding dyes, cells are labeled prior to placing them in culture for measurement of proliferation. With these dyes, cells are labeled in bulk by incubating them with the dye in the dark at 37°C for a short period of time (usually 30 minutes). After incubation, excess (unincorporated) dye is washed away and the cells are plated for the experiment. At this time a small aliquot of cells is removed and fixed. This sample represents the undivided cells. At different time points cells are collected and fixed for analysis. Similar to assays using thymidine derivatives, cells may be stained for surface markers expression prior to fixation. In this assay, as the cells divide, half of the dye is passed on to each daughter cell. So the more the cells divide the less intense their fluorescence. The MFI of the time 0 sample becomes the starting point for the analysis. Analysis of proliferation by this method requires a third party software such as FCS Express. Examples of the type of data obtained are shown in Box 1. For more information about the data analysis see page 3 of this newsletter or talk to the staff in the facility.

Box 1						
Division Index	Proliferation Index	% Divided	# of Fitted Cells	# of Original Cells	RMS Error	R-Chi Square
5.88	5.30	88.15	21275.00	3958.20	4.64	1.10

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



Core Ordering & Reporting Enterprise System

<https://cores-wvu.mis.vanderbilt.edu/login.cfm>

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

FACSCalibur Analyser

Operator: User

Lasers: 488 nm Argon
633 nm Red Diode

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 4 fluorochromes

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 OBIS LX
488 nm Sapphire (SS)
561 nm Sapphire (SS)
628 nm OEM

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 12 fluorochromes

Applications:

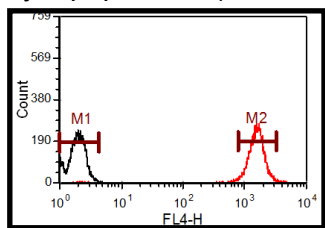
Cell phenotyping
Cell Viability
Cell Cycle analysis
FISH, FRET, SPA

Analysis Tips - Cell Proliferation Assay

In FCS Express 4, analysis of proliferation data uses curve fitting. Below is an outline of the steps required to analyze the data.

Setting up the Analysis page:

1. Open a new layout page.
2. Insert a Color Dot Plot (FSC-A vs SSC-A) using the unstained sample.
3. Draw a gate around the cell population to be analyzed and label it “Cells”
4. Insert a histogram showing the fluorescent dye the cells were stained with. Make sure the histogram is gated on the “Cells” gate.
5. Overlay on the histogram the Day 0 stained cells file.
6. Place one marker to denote the no stain population and a second marker to denote the starting Day 0 population (see below).



Proliferation Analysis:

7. Insert a proliferation histogram for the Day 0 sample. Gate it on the “Cells” gate and make sure it shows the correct fluorescent parameter.
8. Right click on the proliferation histogram, and select “Format” from the menu.
9. Click on the “Proliferation” tab on the left and under that click on “Fit Parameters”
10. Check “Use Fixed Starting Generation”
11. Click on the **T** and select “Statistics”

12. Under “Statistics select “Data Source” and select the Day 0 histogram

“DO NOT SELECT THE PROLIFERATION HISTOGRAM”

13. Click on “Statistics”
 - a. Select the marker that denotes the Day 0 population (M2 on the graph to the left)
 - b. Select “Gate 1” and “Geometric Mean”
 - c. Click “Okay”
14. Click on “Position Markers”
 - a. Select “Undivided Cell Peak”
 - b. Check “Show Position Marker on the Blot”
 - c. Select “Background”
 - d. Check “Show Position Marker on the Blot”
15. Right click on the proliferation histogram and select Format from the menu
16. Click on the “Overlay” tab on the left
 - a. Fit—keep as is
 - b. Noise - check off Visible
 - c. Fill histogram for generations
17. Click on the “Legend” tab on the left
 - a. Check Visible
18. Right click on the proliferation histogram and select Statistics from the menu to obtain proliferation statistics (Page 2 Box 1 and below) .

Generation #	# of Cells	# of Orig cells	% of Orig Cells
0.00	469.00	469.00	2.20
1.00	2395.00	1197.50	11.26
2.00	4604.00	1151.00	21.64
3.00	6071.00	758.88	28.54
4.00	4994.00	312.13	23.47
5.00	2016.00	63.00	9.48
6.00	429.00	6.70	2.02

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).

What's New

During the last 12-month period (July 2014 - June 2015), 19 peer reviewed publications from investigators at WVU reported using the WVU Flow cytometry facility for various application/analyses. The table below is a short list of the publications and the flow cytometry techniques utilized in some of these publications.

Reference	PI	Target cell(s)	Application / Assay
Jajosky et al. <i>Stem Cells Transl. Med.</i> 3(7): 836-848, 2014	Gibson	acute lymphocytic leukemia	Magnetic cell sorting phenotypic analysis proliferation assay
Thapa et al. <i>J. Mol. Cell. Cardiol.</i> 79: 212-223, 2015	Hollander	isolated mitochondria	mitochondria complexity membrane potential
Sharma et al. <i>Mol. Cell. Biol.</i> 34(22): 4143-4164, 2014	Ruppert	Breast cancer cell lines	Apoptosis assay
Luanpitpong et al. <i>Environ. Sci. Technol.</i> 48(21): 12912-12919, 2014	Rojanasakul	lung epithelium stem cell	stem cell sorting Cell proliferation Cell cycle analysis

Upcoming Events:

September 7, 2015	Labor Day	Facility Closed
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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.