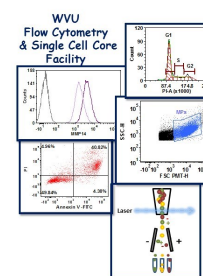


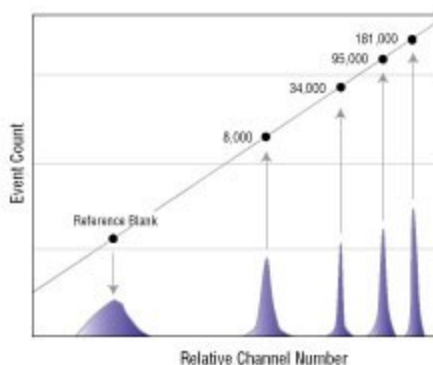
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 3, issue 1

July 2016

MESF: What is it and why would you want to use it?



What is MESF?

MESF stands for the molecules of equivalent soluble fluorochrome and is a standard fluorescence unit of intensity used in flow cytometry. It is based on the concept that a sample labeled with a fluorochrome will have the same fluorescence intensity as an equivalent number of molecules of the same fluorochrome in a solution if they are measured under identical conditions. MESF is a fluorescence unit that provides users with a tool to compare flow cytometry data in a quantitative manner over time and across instruments. What MESF won't do is give an actual number of fluorochrome molecules on the cells.

How do I determine MESF?

Microbeads standards are used to determine the MESF in an experiment. Several companies including Bangs Laboratories (Quantum™ MESF and Quantum™ Simply Cellular) and Sigma-Aldrich (Quantum Fluorescence Kits for MESF Units) sell microbeads that can be used to determine MESF. There are two types of microbeads commonly used to determine the MESF.

One type of microbead is labeled with a known amount of a single fluorochrome such as Alexa Fluor®488, APC or PE-Cy™5. They usually come as a mixture of 5 bead populations each labeled with a different amount of the same fluorochrome. If you use these beads you must use a bead mixture for each fluorochrome you are measuring in your assay.

The second type of microbead is labeled with a known amount of an antibody to either mouse IgG, human IgG or rat IgG. Again, they are sold as a mixture of 5 beads populations each labeled with a

Inside this Issue

- | | |
|----------|---|
| 1 | MESF: What is it and why would you want to use it? |
| 2 | Flow Cytometers in the Facility |
| 3 | Other Instrumentation Available in the Facility |
| 3 | New Users Guide |
| 3 | Fee Schedule |
| 4 | FCS Express 5: Histogram Normalization |
| 4 | Upcoming Holidays & Events |

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

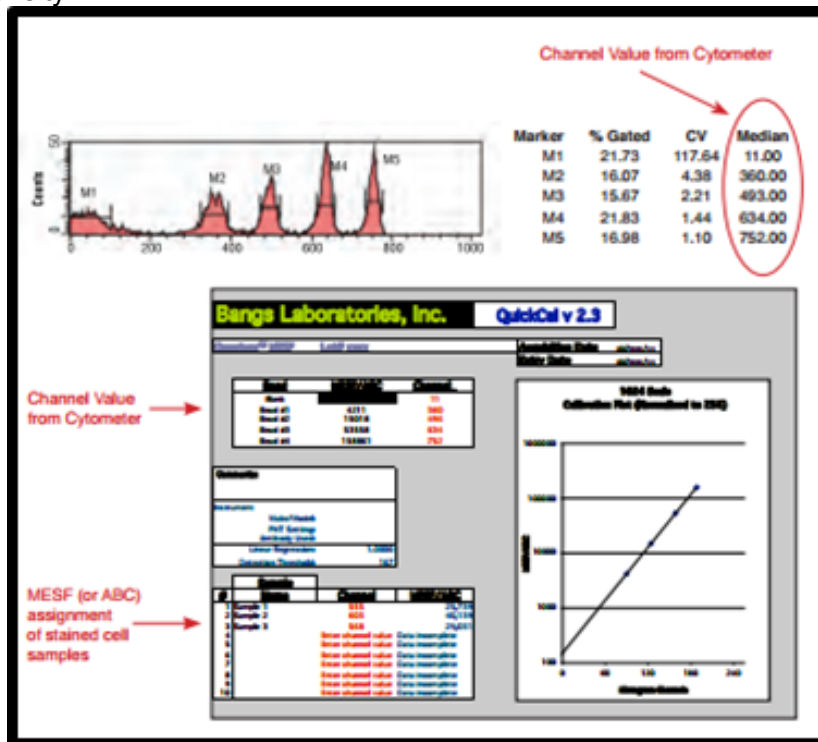
After hours access for experienced users by prior approval from Kathy Brundage

Contact Kathy at:

kbrundage@hsc.wvu.edu

different amount of the antibody. When using this type of microbead, it is important to remember to use the beads that will bind the constant region of the antibody you want to measure. For example, if you are staining human cells with a mouse monoclonal antibody you must use the microbeads labeled with an antibody that recognizes mouse IgG. Unlike the other type of microbeads, this type requires that you incubate the microbeads with your antibody. Each antibody you want to determine the MESF for has to be incubated by itself with the beads. So if you are using 3 antibodies in your experiment, you will have 3 tubes of microbeads each stained with one of the antibodies.

Regardless of which type of microbeads you use, they must be analyzed at the same time with the same instrument setting as the rest of your samples. Once you have run the samples and MESF beads, the peak channel for the each bead population is plotted vs MESF/ABC (antibody binding capacity). Using this standard curve, you can determine the MESF for your samples. Many companies such as Bangs Laboratories Inc. provide a free program download that will do the calculations for you (see Figure below). All you have to do is enter the peak channel values you obtain from the beads to generate a calibration curve then enter the values for your samples. MESF can be presented in a manner similar to MFI (mean/median fluorescent intensity).



If you are interested in determining the MESF in your experiments or would like more information contact Kathy Brundage (Director of the FCSCCF).

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 up to 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 up to 12 fluorochromes

Applications:

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



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 in this facility

<p><u>AutoMACS Magnetic Bead separator</u></p> <p>Operator: User</p> <p>Application:</p> <ul style="list-style-type: none"> Single marker (extracellular) sorting Depletion sorting 	<p><u>gentleMACS Octo Dissociator with Heaters</u></p> <p>Operator: User</p> <p>Application:</p> <ul style="list-style-type: none"> Dissociation of tissues into single cell suspension for culture or flow cytometry assays Homogenizes tissues for downstream molecular biology applications
<p><u>C1 Single Cell Auto Prep System</u></p> <p>Operator: User or Staff</p> <p>Application:</p> <p>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:</p> <ul style="list-style-type: none"> RNA seq DNA seq PCR <p>Format: 96 or 384 chambers per chip</p> 	<p><u>MSD Multi-Array Platform</u></p> <p>Operator: User</p> <p>Applications:</p> <ul style="list-style-type: none"> 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 

Fee Schedule (2015-2016)

	User Operated Analyzer	Facility Operated Analyzer	FACSAria Sorting	AutoMACS	MSD Sector Imager 2400	User Operated C1	Facility Operated C1	gentleMACS Octo Dissociator
WVU User	\$33/h	\$50/h	\$74/h	\$14/use	\$10/use	No Cost	No Cost	\$10/sample
Non-WVU User	\$49.50/h	\$75/h	\$111/h	\$21/use	\$15/use	\$75/plate	\$225/plate	\$15/sample

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 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.

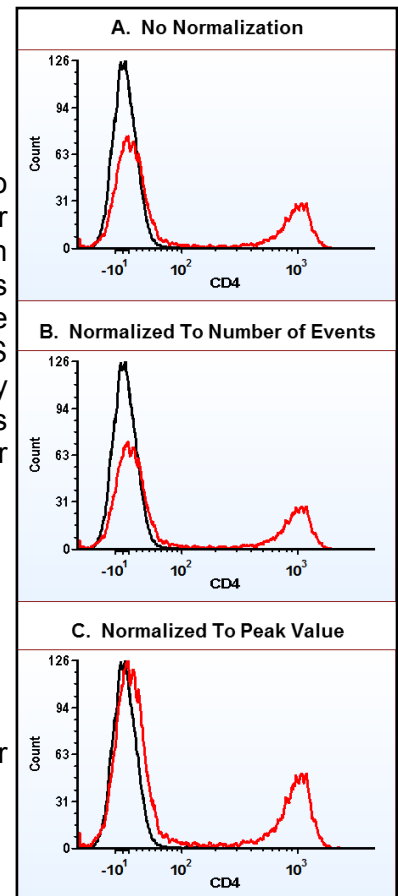
FCS Express 5 Software:

Normalization of Histograms

Some times you may want to plot multiple files on a single histogram to demonstrate the increase/decrease in expression of a particular marker. This is done by adding overlays to an existing histogram. In many cases, one file may have a much larger peak than the others (see Figure A). Sometimes the difference in peak sizes results in one peak being so small you can not see it well on the histogram. The FCS Express software allows the normalization of the histograms either by number of events (see Figure B) or by Peak Value (see Figure C). This normalization does not change the statistics it just allows for a better representation of the data.

To normalize histograms:

1. Right click on histogram
2. On the drop down menu click on "FORMAT"
3. In the Format box, click the "+ HISTOGRAM" followed by clicking on "HISTOGRAM SPECIFIC OPTIONS"
4. In the box, select if you want to normalize by either the number of events or peak value.
5. Select the file that the data will be normalized to
6. Click "OKAY"



Upcoming Holidays & Events

July 21 –22, 2016	Kathy out of Lab	No sorting Fortessa available for experienced user only
September 5, 2016	Labor Day 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).