WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY
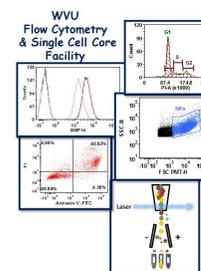
Helpful Hints for Proper Gating of Flow Cytometry Data
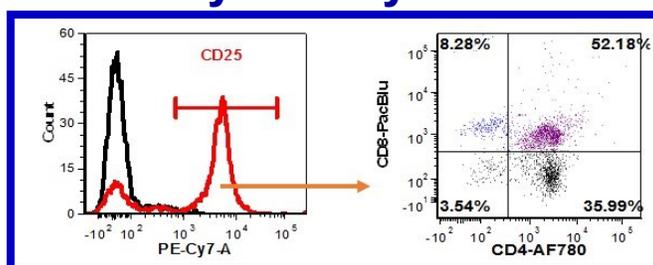
In flow cytometry, gating is the means by which we group cell populations based on similar characteristics. Whether you are new to flow cytometry or an old pro gating can be one of the biggest challenges in analyzing flow cytometry data. Trying to identify cell populations when they are similar in size or have similar expression levels can be difficult. There are some general rules which can make the gating process easier. In this article some useful gating rules and recommendations for analyzing flow cytometry data is discussed.

Pre-Assay Recommendations

Prior to actually performing the assay, there are several things to consider during the experimental planning phase that will make gating easier during data analysis.

1. *Have a clear understanding of the markers that will define the population(s) of interest.* Check the literature for information on the population(s) of interest. Make sure to use the most current information when choosing markers to identify the population(s).

2. *If working with a mixed cell population make sure you have an idea of their sizes and consider using a live/dead dye to gate out dead cells and debris.* Remember, dead and dying cells can pick up antibodies and other dyes non-specifically.

3. *Have a good idea of the level of expression of each marker.* Also, know if treatment or disease state changes the level of expression of each marker.

4. *Determine what the controls should be for the assay.* Unstained and single stained controls are always required. Even though the instruments are pretty stable, temperature in the room, the staining process, instrument maintenance and the age of the lasers all can affect the data.

5. *Consider using FMO (Fluorescence Minus One) controls especially if using more than 4 fluorochromes.* FMO controls are additional controls that aid in the identification and gating cells when there is data spreading due to the multiple fluorochromes.

Inside this Issue

Facility Location:
2160 HSCN

Phone:
304-293-6273

email:
flowcore@hsc.wvu.edu

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

After hours access is available for experienced users with prior approval from Dr. Kathy Brundage

Contact Dr. Brundage at:
kbrundage@hsc.wvu.edu

Post-Acquisition Recommendations

There are several different gating strategies that are commonly used when defining populations of cells.

1. *Forward Scatter (FSC) and Side Scatter (SSC) gating*

   This gating strategy uses size and granularity to identify cells. It is usually the first type of gating done and allows for the removal of dead cells/debris. Dead cells/debris tend to have less FSC (smaller) and more SSC then live cells. To ensure complete exclusion of dead cells, a live/dead stain should be used as well.

2. *Gating using a single parameter*

   This gating is usually done after the FSC/SSC gating. It allows you to identify cell populations based on how much of a particular protein a cell is expressing. A simple example is shown in the figure at the beginning of this article (graph on left side).

3. *Gating using multiple parameters*

   For this gating you will need to use a two dimensional plot. The parameters on the x-axis and y-axis can be a particular protein/fluorochrome, FSC, SSC or a live/dead stain. By dividing the plot into 4 quadrants you can identify 4 populations. These populations would be:

   > 1. Parameter 1 (+) only
   > 2. Parameter 1 (+) and 2 (+)
   > 3. Parameter 1 (-) and 2 (-)
   > 4. Parameter 2(+) only

   An example of this type of gating is shown at the beginning of this article (graph on right side).

4. *Backgating*

   This is one of the most useful strategies to check your gating, particularly when you have a mixture of cell types with different FSC and SSC characteristics. For this strategy, coloring the gated cell populations with different colors on one graph allows you to visualize the gated populations on other graphs with different parameters.

5. *Gating based on pulse area*

   This gating strategy is useful to identify and remove doublets/clusters of cells from your analysis. With this gating, the width (W) for one parameter such as FSC is plotted vs the area (A) or height (H) of the same parameter (FSC). The W parameter can be thought of as a measurement of time of flight. Single cells will have similar W values and cluster tightly together while clusters of two or more cells are larger and will take longer to go through the laser sensing area . This means that cells in clumps will have larger W values then single cells. For this strategy, the gate is put around the cluster of single cells thus excluding the ones with larger W values. An example is shown below:
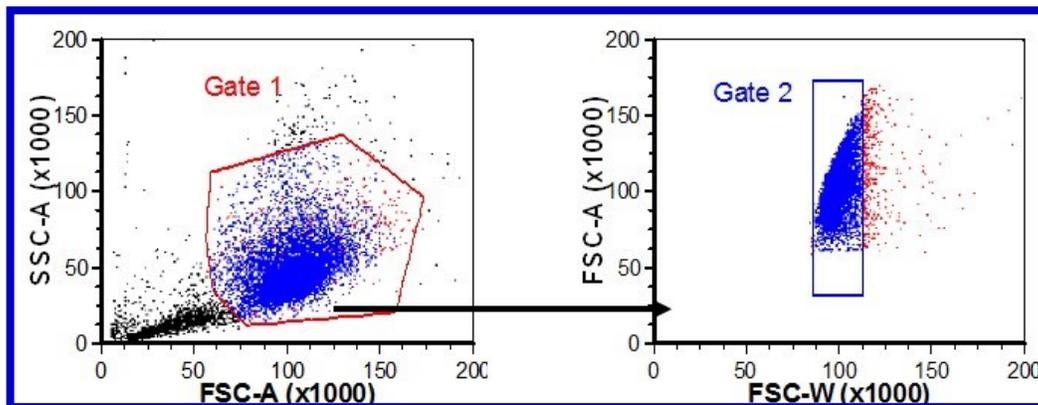
Using the gating strategies discussed above will improve your data analysis and help to generate more consistent and reproducible data. If you have any questions or would like to discuss the gating strategies presented in this article please contact the WVU FCSCCF.

Flow Cytometers in the Facility

FACSAria III Cell Sorter

Operator: Facility Staff

Lasers:

    488 nm solid state

    561 nm solid state

    633 nm solid state

    407 nm solid state

Detection Parameters:

    Forward Scatter

    Side Scatter

    Simultaneous detection
        of 13 fluorochromes

Applications:

    Cell Sorting (Aseptic)

    Cell phenotyping

    Cell Viability

    FISH, FRET, SPA

LSR Fortessa

Operator: User of Facility Staff

Lasers:

    405 nm solid state

    488 nm solid state

    561 nm solid state

    628 nm solid state

Detection Parameters:

    Forward Scatter

    Side Scatter

    Simultaneous detection
        of 12 fluorochromes

Applications:

    Cell phenotyping

    Cell Viability

    Cell Cycle analysis

    FISH, FRET, SPA

Other Instrumentation Available in the Facility

AutoMACS Magnetic Bead separator

Operator: User

Application:

- Single extracellular marker cell sorting
- Depletion/negative cell sorting

gentleMACS Octo Dissociator with Heaters

Operator: User

Application:

- Dissociation of tissues into single cell suspension for culture or flow cytometry assays
- Homogenizes tissues for downstream molecular biology applications

C1 Single Cell Auto Prep System

Operator: User or Staff

Application:

Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for downstream genomic applications.

Downstream applications:

- RNA seq
- DNA seq
- PCR

Format: 96 or 384 chambers per chip

MSD Multi-Array Platform

Operator: User

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
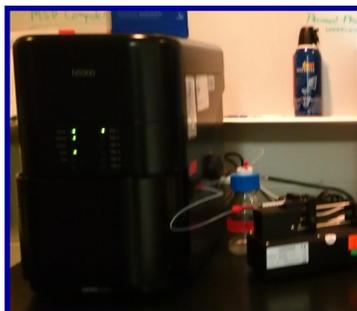- Assay Time: 4—6 hours depending on analytes being detected

Nanosight NS 300

Operator: User or Staff

Application:

- Determines the size and concentration of particles 10 nm to 1 microns in size
- Equipped with 4 lasers (405 nm, 488 nm, 532 and 642) to detect fluorescently labeled particles

Zetasizer Nano Z

Operator: User or Staff

Application:

- Measures the zeta potential of particles in a solution using laser Doppler micro-electrophoresis

Fee Schedule

Instrument	Operator	For WVU & NIOSH Users	For Non-WVU Users
AutoMACS	Facility Staff or User	$14.70/h	$22.05/h
C1 Single Cell Auto Prep System	Facility Staff	$210/plate	$315/plate
	User	No Cost	$112.50/plate
FACSAria III	Analysis: Facility Staff	$52.50/h	$78.75/h
	Analysis: User	$34.65/h	$51/98/h
	Sorting	$77.70/h	$116.55/h
	Sorting Setup	$19.43/sort	$29.15/sort
gentleMACS	Facility Staff or User	$10.50/sample	$15.75/sample
LSRFortessa	Facility Staff	$52.50/h	$78.75/h
	User	$34.65/h	$51.98/h
MSD Sector Imager	Facility Staff or User	$10.50/h	$15.75/h
NanoSight NS300	Facility Staff	$61.00/h	$91.50/h
	User	$42.50/h	$63.75/h
Zetasizer Nano Z	Facility Staff	$25/sample + $52.50/h	$38/sample + $78.75/h
	User	$25/sample	$38/sample + $15/h

Agilent CrossLab
From Insight to Outcome

Internal WVU user :
Click here to login or register using your institute login and password.

Not a WVU user?
Login using iLab credentials ☐
If you don't have an account, please register for an iLab account.

The facility uses iLAB scheduling/billing software from Agilent to manage the use of the facility's instrumentation. If you would like to use the instruments housed in the facility please use the URL shown below to register as a WVU User and to login to reserve an instrument.

https://wvu.corefacilities.org/account/login

FCS Express 6: Spreadsheets

In FCS Express 6, Excel style spreadsheets can be inserted into a layout. These spreadsheets function just like typical Excel spreadsheets. These spreadsheets allow you to add statistics directly into them as well as adding statistics from data files in the data list. To insert a spreadsheet into a layout:

1. Go to the "Insert" tab in the software and select "Spreadsheet" then "New"
2. If you click on the spreadsheet to select it, two new tabs appear "Spreadsheet-Layout" and Spreadsheet—Formula"
3. Now you are ready to add any statistics and formulas to the spreadsheet
4. To add statistics from data files to the spreadsheet, drag and drop the files either individually or in a group onto the spreadsheet. You do not have to open each file to add the data.
5. Spreadsheets created in FCS Express can be exported in standard Excel formats such as .xls, .cvs, .xml and .txt.

Check the De Novo Software website for more details and tutorials.

Upcoming Holidays & Events

January 15	Martin Luther King's Day	Facility Closed
January 16	FCS Express6 Seminar: Clustering with tSNE & SPADE	2:30 pm—4 pm, HSCN 2094
February 13	FCS Express6 Seminar: Cell Cycle & Proliferation	2:30 pm—4 pm HSCN 2094
March 30	Spring Break Holiday	Facility Closed

New User Guide

Hands-on training for FACSCaliber, LSRFortessa, C1 Single Cell Auto Prep System, NanoSight NS300 and Zetasizer Nano Z is **mandatory** for all new users and must be scheduled by consultation with the facility director.

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. Sorting on the FACSAria is by facility staff only.