

## DIVA Tutorial

Log in using your PI as username. No password is required.

When you open DIVA, you will see the following five windows:

1. Browser

From this window you can:

- a. Open a new or existing experiment.
- b. Duplicate an experiment without data.
- c. Store a Template.
- d. Export data.

2. Cytometer

- a. Add or delete fluorophores (Parameter Tab).
- b. Set voltages (Parameter Tab).
- c. Choose fluorophore parameters (Log/Linear Scale, Area, Width, Height) - (Parameter Tab).
- d. View and/or edit compensation (Compensation Tab).

3. Acquisition Dashboard

- a. Acquire and record data.
- b. Set up number of events to record.
- c. Tell machine what gate to count from when recording.
- d. Monitor threshold and number of events acquired.

4. Inspector

- a. Provides information and options to make changes on whatever it is you are clicked on.
- b. If clicked on a worksheet/graph:
  - i. Label graphs.
  - ii. Assign a population to be viewed in the graph.
  - iii. Change axis to bi-exponential.
- c. If clicked on a tube:
  - i. Label tubes.
  - ii. Number of events collected for this tube.
- d. If clicked on an experiment:
  - i. View fluorophores and voltages used.
  - ii. View compensation.
  - iii. Print out parameters.

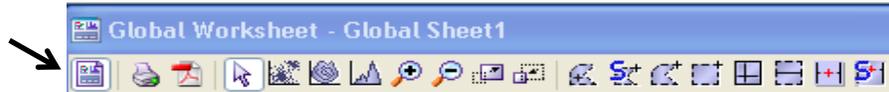
5. Worksheet

- a. Setup analysis/acquisition template.
- b. Add plots and graphs.
- c. Add Statistics.

## Experiment Set-up

### The Browser Icons/Commands Overview

- a. Experiment 
  - i. The icon is a notebook, when the experiment is closed, the book is closed.  When the experiment is open, the book is open. 
  - ii. The experiment must be open in order to add/delete tubes, make a worksheet, add fluorophores, or view data.
  - iii. Open an existing experiment in the browser by double clicking on the icon or by right clicking on it and choose the *Open Experiment* option.
- b. Worksheets  (Normal vs. Global)
  - i. Every new experiment will automatically provide you with one global worksheet.
  - ii. To add additional worksheets, right click the Global Worksheet icon and choose *New Global Worksheet*.
  - iii. Global worksheet (default worksheet used for samples): All tubes will have the same gating strategy, no changes from tube to tube. If you move the gate it will be applied to all samples.
  - iv. Normal Worksheet (used for Compensation Controls): Specific to each tube. Every tube will have a separate worksheet that allows for separate gating strategies per sample tube.
  - v. Toggle between Normal and Global worksheets by clicking on the far left icon located on the Worksheet toolbar.

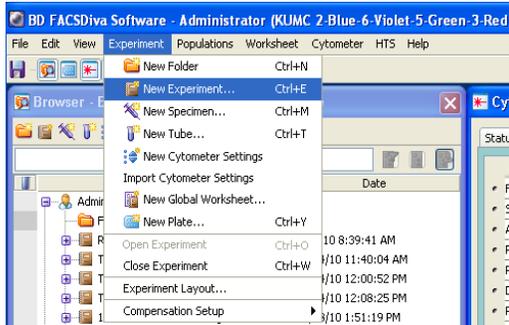


- c. Specimen 
- d. Tubes 
  - i. These are your samples that will be run on the flow cytometer.
  - ii. Tubes are added underneath the Specimen Icon in an experiment.
  - iii. Add tubes by clicking on the Tube icon, right clicking on an existing tube and choose *Duplicate Without Data*, or by clicking *Next Tube* in the Acquisition Dashboard.
- e. Cytometer Settings 
  - i. Clicking on the cytometer settings will allow you to view your parameters (voltages, fluorochromes, compensation) in the inspector window.
  - ii. View whether or not you have a compensation linked to the experiment: 
  - iii. Add analysis templates and/or application settings.
- f. Duplicate without Data
  - i. This function allows you to duplicate an entire experiment, specimen or tube.
  - ii. All of the labels, parameters, set-up will be carried over when using this tool.

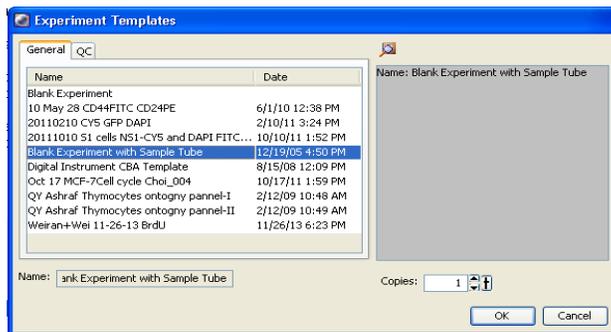
- iii. Right click on Experiment, Specimen, or Tube to locate this feature.

## Creating a New Experiment

1. Go to *Experiment* on the Tool Bar Menu and choose *New Experiment*



2. Select *Blank Experiment with Sample Tube*, Click OK



3. A new Experiment icon will appear in the browser:

 Blank Experiment with Sample Tube

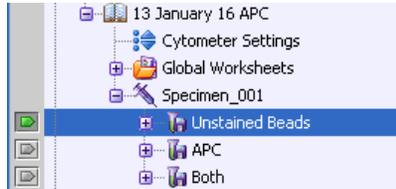
- a. Right Click on the experiment to rename.
4. Click on the “+” icon to the left of the Experiment to expand.
5. You’ll see the following sections are included in your experiment: Cytometer Settings, Global Worksheets, and Specimen.
  - a. You can expand each section by clicking on the “+” icon to the left.



## Specimens and Tubes

6. Add your tubes/specimens and label
  - a. Right click on the *Specimen* and choose *New Tube* or highlight *Specimen* and click on the tube icon.
  - b. Label tubes and specimens.

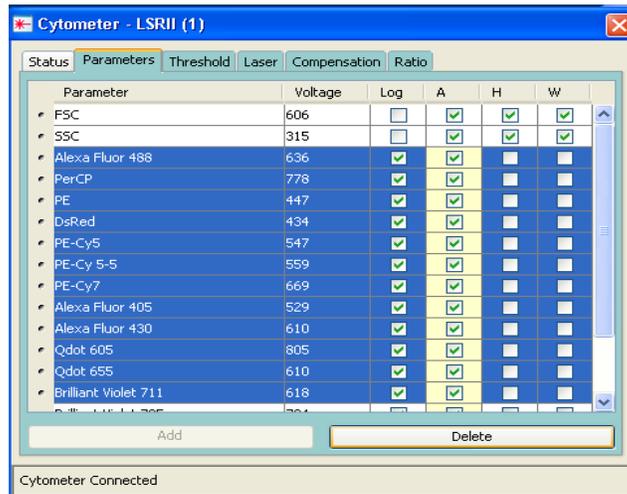
- c. Take a moment to look at the Cytometer and Acquisition Dashboard Windows – Notice that you only have one tab available in the Cytometer window and the Acquisition functions are grayed out.
- d. To the left of each tube is an arrow icon, click on the arrow to activate the tube – the arrow will turn green (alternatively you can double click the Tube icon).



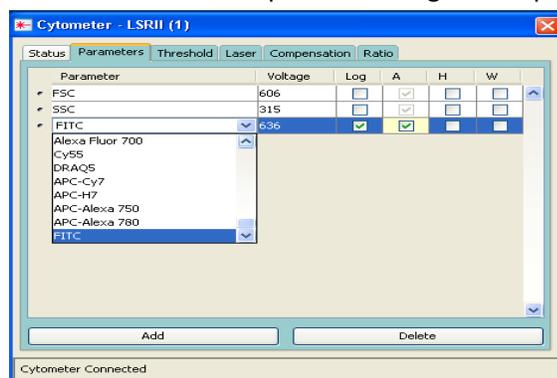
- e. Notice that you now have more options available in the Cytometer and Acquisition windows now that a Tube is “active”.
- f. **THE ARROW TO THE LEFT OF A TUBE MUST BE GREEN IN ORDER TO ACQUIRE OR SET UP PARAMETERS. IF THE TUBE IS NOT ACTIVE THEN YOU WILL NOT BE ABLE TO SETUP YOUR EXPERIMENT OR COLLECT EVENTS.**

## Fluorophores

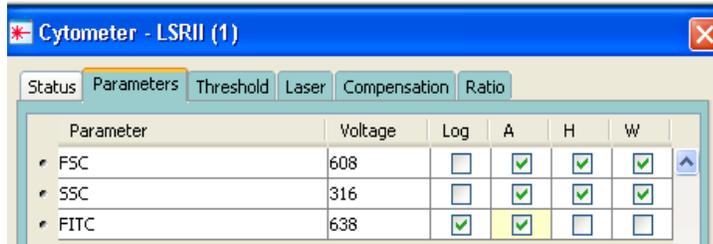
7. Add Fluorophores
  - a. Click on a Tube to make it “active”.
  - b. Click on the *Parameters* tab in the Cytometer Window.
  - c. Delete all of the fluorophores, but leave the FSC and SSC (First two listed).
    - i. Click on the “dot” to the left of a fluorophore to select. Hold down the Shift key to select multiple fluorophores and click the *Delete* button.



- d. Click on the *Add* button.
- e. Click within the fluorophore box to get a drop-down menu of available options.



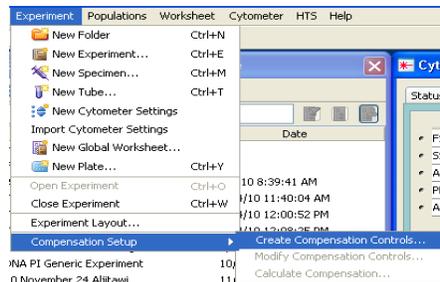
- f. Choose the fluorophore you need.
- g. Repeat above steps to add additional fluorophores.
- h. Verify that the FSC and SSC have the Log function unchecked and A, H and W checked.



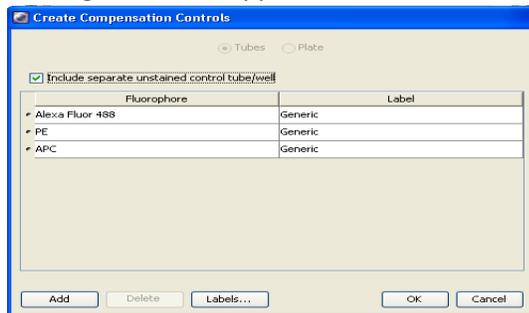
- i. Make sure that each fluorophore has the “A” function checked.
- j. A = area is the sum of all pulse heights, H = maximum height value for the pulse, and W = width of the pulse (calculated by the instrument’s electronics).

## Compensation Controls

8. Create Compensation Controls (if applicable).
  - a. Click on *Experiment* in the Tool Bar Menu.
  - b. Select *Compensation Setup* and choose *Create Compensation Controls*



- c. A dialogue box will appear:



- i. Verify that all your fluorophores are listed, “include separate unstained control tube” is checked, and the label for each fluorophore is “Generic”.
- ii. If you have labels associated with a tube, simply delete the ones with a label and leave the generic ones.
- iii. Click *OK*.

- d. A “Compensation Controls” Specimen will be in your experiment
  - i. Expand the Specimen and you will see that Tubes and Normal Worksheets have been created for each Fluorophore in your experiment.

**Worksheet**

9. Create Worksheet

- a. When acquiring on the LSR, it is best to keep the worksheet simple. You can analyze and make more graphs/plots during analysis using one of the Flow Core workstations (FlowJo and DIVA).
- b. Once data is acquired you can add and delete plots/gates as much as you want using the FSC files. VOLTAGES CANNOT BE CHANGED on FSC files. Voltages need to be determined during acquisition.
- c. The Tool Bar on the Worksheet window contains icons for graphs and gates. Hold the mouse over the icon in the tool bar to view what type of gate or graph you are choosing.



i. Graphs

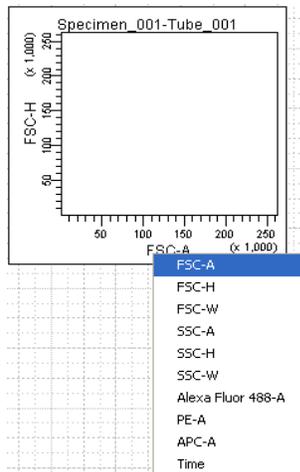
- 1. Dot Plot 
- 2. Contour Plot 
- 3. Histogram 

ii. Gates

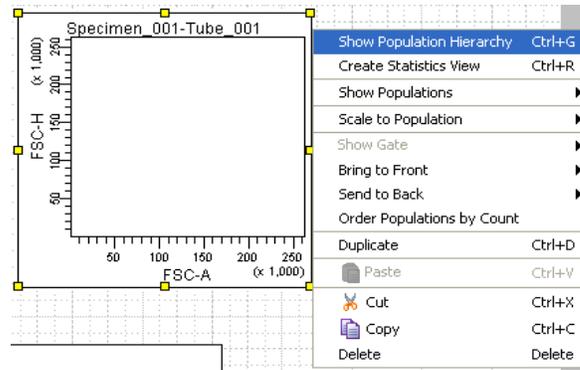
- 1. Polygon 
- 2. Rectangle 
- 3. Interval 
- 4. Quadrant 

**Plots**

- d. To add a plot to the workspace, click on the plot type that you want and then click within the worksheet space.
- e. Once you have chosen the appropriate plots, click on the axis to choose the fluorochrome of interest that you want displayed on the plot.



- f. Right click on a graph and choose *Show Population Hierarchy*
  - i. This allows you to track what is being shown in each plot.
  - ii. You will also be able to get the % of each population.



## Gates

- g. Click on the gate type (polygon, rectangle, interval, or quad) and then click back in the plot of interest. The gate will appear. You will also see that it has been added to your Population Hierarchy with a color (red) and name (P1) assigned.

Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	0	###	###
■ P1	0	###	###

- h. To add a gate based on your P1 data:
  - i. Click on the “P1” gate in the Population Hierarchy box.
  - ii. Click on a gate icon.
  - iii. Click on the appropriate plot. You’ll see the gate appear with a P2 label, you’ll also see that it has been added to the population hierarchy with a color (green) and label (P2) assigned.

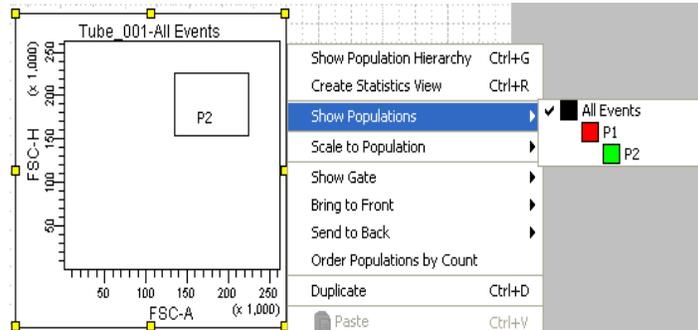
Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	0	###	###
■ P1	0	###	###
■ P2	0	###	###

- iv. Continue with the above process to add the appropriate gates to your workspace.

## Plot Populations and Options

i. Next you want to tell the software what populations to show in each plot

i. Right click on a plot



ii. Select “Show Populations”

iii. Pick the population you want the plot to depict (This particular plot is showing “All Events”)

j. Rename Populations

i. Double click on a population in the Population Hierarchy box

ii. Type name

k. Assign/Change color of a population

i. Right click on the color box located to the left of the population in the Hierarchy box

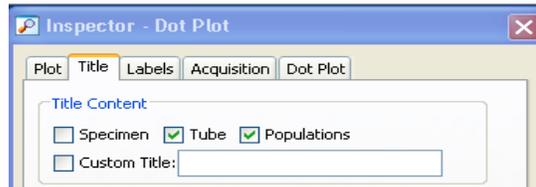
ii. A drop down menu of colors will appear, click on the color you want

l. Label your plots so that you know what population each one is showing

i. Select all the plots by holding down the shift key and clicking on all the plots

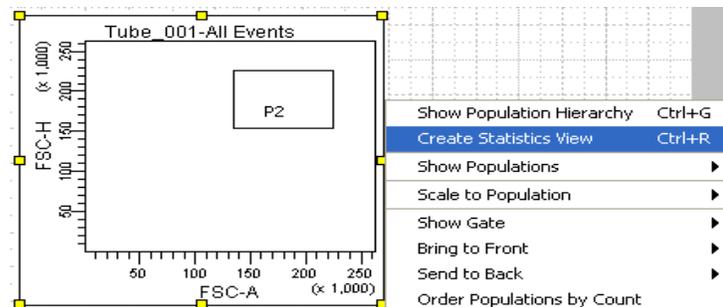
ii. Go to the Inspector Window and choose the *Title* tab

iii. Select the *Tube* and *Populations* boxes

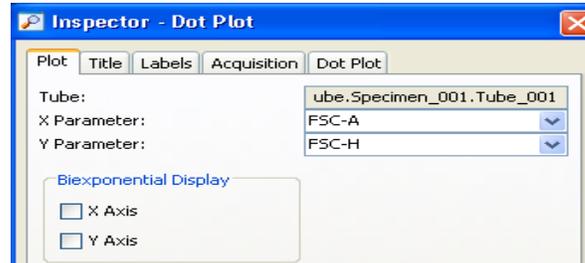


m. Add statistics

i. Right click on a plot



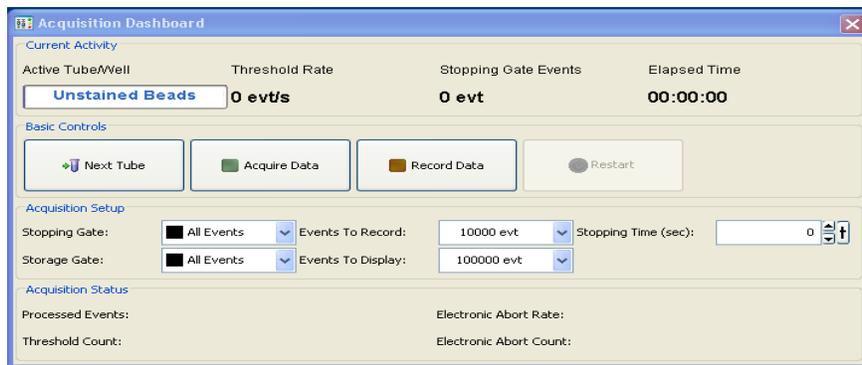
- ii. Choose *Create Statistics View*
  - iii. A statistics box will appear on the workspace
  - iv. Right click on the statistics box and choose “*Edit Statistics*”
  - v. Pick the parameters you are interested in: Mean, Median, Etc.
- n. Change an axis to Bi-exponential
- i. Click on the plot
  - ii. Go to the Inspector Window
  - iii. Select on the Plot tab
  - iv. Under the Bi-exponential Display section, check mark the appropriate axis



## Acquisition

### 10. Setup Acquisition Dashboard

- a. Go to Acquisition Dashboard Window
- b. Set the number of *Events to Record*
- c. Choose the *Stopping Gate*
  - i. This is the gate the machine will use to count the number of events
- d. Acquire Data Button: Acquires data points that you can see in the worksheet
- e. Record Data Button: Collects data for the FCS files
- f. Storage Gate: Leave this set on All Events

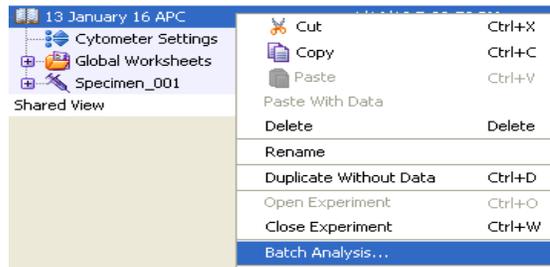


## Data Management

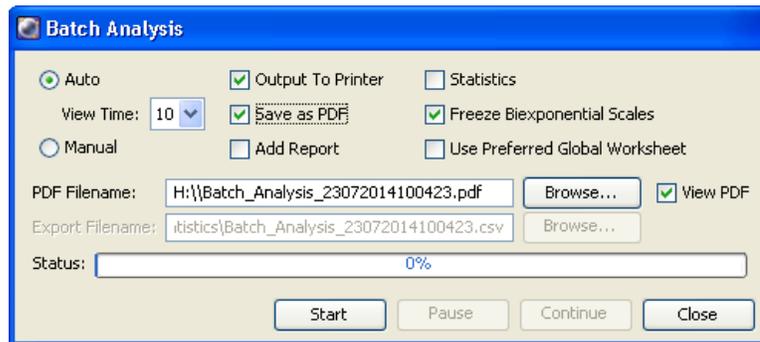
### 11. Export Data

- a. Batch Analysis: Provides a PDF. You cannot move gates or make any changes to a Batch Analysis file.
  - i. Right Click on either the Experiment icon or Specimen icon to batch the report
  - ii. Clicking on the Experiment icon will give you all of the analysis worksheets for every tube in the experiment.

- iii. Clicking on the Specimen icon will batch only the tubes associated with the specimen
- iv. Select Batch Analysis (In this case we are batching the entire experiment)



- v. A dialogue box will appear



- vi. Check the *Output to Printer* and *Save as PDF* options
  - vii. Click on *Browse* and navigate to your Jump Drive
  - viii. Click *Start*
  - ix. Click OK to the pop- up windows
  - x. Print
- b. Export FCS Files: Exports FCS files that can be imported into DIVA, FlowJo or other flow analysis software. You can change gates and plots, add statistics and create PDF files.
- i. Right click on the *Experiment*.
  - ii. Choose *Export*.
  - iii. Click on *Experiment or FCS File (v 3.0)*
    - a. Experiment: You will save all worksheets associated with the experiment and the parameters.
    - b. FCS Files: Saves data files only. No worksheets will be carried over when you open the files.
  - iv. Navigate to your Jump Drive and Save.