DiVa Software Guide for Acquiring Data

Login to computer using Healthcare or Iowa domain credentials (Healthcare ID or HawkID)

Double click on the FACSDiVa icon

Click on pull-down menu and choose principal investigator

Password is principal investigator’s first name (lower case)

Click on “OK” or hit the “enter” key
If prompted, click on “Use CST Settings”

Click on “Experiment” pull-down menu

Click on “New Experiment”

Choose appropriate template

Click “OK”
Find the Browser window

Right click on experiment name

Click on “Rename”

The general naming scheme for each experiment should include the PI’s last name plus an experiment description plus the date, e.g. “Berg FITC/PE/PerCP/PE-Cy7/APC 170404”

Change “blank” to today’s date by yymmdd, e.g. 170404 is April 4, 2017
Right click on specimen name

Click on "Rename" and change to today's date by yymmdd, e.g. 170404 is April 4, 2017

Click on the "+" to expand the tree and expose the sample line
With a sample tube loaded on the instrument and the fluidics control on “run,” events will appear in the plots on the computer monitor when the software is in acquisition mode. The record button saves a data file.

Click on the pointer to start acquisition

Clicking on either of these will toggle acquisition on and off.

When the pointer is yellow and “Acquire” box is green, the software is in acquisition mode.

To save data to a FCS (Flow Cytometry Standard) file, click on the “Record Data” button.

Creates next data file

Puts instrument in acquisition mode (events show up on the computer monitor)

Records data file

Sets stop criteria

Always set the “Storage Gate” to “All Events”

Sets number of events to record that satisfy the stop criteria

Sets number of events that will be displayed on monitor

0 = ∞
Adding or Deleting Channels or Changing Fluorochrome Name Assigned to a Channel

Click on “Cytometer Settings” under experiment name.

Go to the inspector window.

Click on “Parameters” tab.

Click on parameter that needs changed.

Choose new parameter from list of fluors.

Click on the “Add” button to add another parameter, e.g. add an eighth color to a seven color experiment. To delete an unwanted parameter, click on the parameter name and then click the ”Delete” button.

Be sure that “W” (pulse width) is checked for “FSC” (forward scatter).
Creating Compensation Controls

If your experiment requires compensation and the protocol you are using doesn’t have compensation controls, use the following steps to create them.

1. Click on “Experiment” pull-down menu.
2. Scroll down to “Compensation Setup”.
3. Click on “Create Compensation Controls”.

In the “Create Compensation Controls” window:
- Fluors must match your stains exactly.
- Delete controls that have an antigen label (unless using multiple comp controls of the same tandem fluorochrome).

Click on “OK”.
Compensation controls will appear here.

Click on the “+” to reveal the individual comp controls.

After running comp tubes, center P2 gate over the positive population for each comp tube.

If using beads, add a P3 gate around the negative population for each comp tube.
Click on “Experiment” pull-down menu

Scroll down to “Compensation Setup”

Click on “Calculate Compensation”

Click on “Apply Only”
To view compensation values, find the Browser window and click on “Cytometer Settings”.

Then find the Inspector window and click on the “Compensation” tab.

Or find the Cytometer window and click on the “Compensation” tab.
Creating a Template

1. Right click on the experiment name.
2. Click on “Duplicate Without Data”.
3. Right click on duplicated experiment name.
4. Click on “Rename”.
5. Remove the date information and replace it with the word “blank.”
Hold down the shift key and highlight all of the tubes except the first tube.

Right click and choose “Delete”
Right click on duplicated experiment

Scroll down to “Export”

Click on “Experiment Template”

Click on “Finish”
Right click on experiment name (acquisition must first be in “stop” mode).

Scroll down to “Export”

Click on “FCS files”
Leave default setting (FCS3.0) or choose FCS3.1

Click “OK”

Set to “c:\export”

Click “Save”
Click on the “File” pull-down menu

Scroll down to “Quit”

On the desktop, double click on the “Export Shortcut”

Delete the information in the red area of the file name, but leave the date
Add a description if you want more than the date to help track experiments.

Drag folder to server shortcut (which is set up to put files in the correct principal investigator and instrument folder).

Drag from Export folder into Recycle Bin and empty the bin.
Log off computer