

## Biomedical Sciences – Flow Cytometry Core

### LSR-II Operation with DiVa 6.0

1. Get a new experiment from top of Browser window.
2. Choose Parameters
  - a) Highlight “Cytometer Settings” under Experiment name in Browser window.
  - b) In the Inspector window, delete parameters not in use.
3. Create a new specimen and activate Tube\_001.
4. Set up Global Sheet with desired dot plots and histograms.
5. Set up Automatic compensation
  - a) From Toolbar select Experiment→Compensation Setup→Create Compensation controls
  - b) Verify the parameters you are using with the controls you have
  - c) Click “OK”
6. Unstained control
  - a) Activate unstained control in Browser
  - b) Show Grid on all histograms in Unstained control worksheet. (Ctrl+A)
  - c) Place Unstained on instrument and run in LOW
  - d) From the Parameters tab in the Cytometer window Set FCS and SSC voltages until population of interest is centered in dot plot.
  - e) Set all FL# voltages until autofluorescence is within first grid quadrant.
  - f) Record data file.
7. Single stained controls
  - a) Click “Next Tube”
  - b) Run single stained tubes and record data files.
8. Run Auto Comp
  - a) From Toolbar select Experiment→Compensation Setup→Calculate Compensation
  - b) If successful, leave the numbers and click "Link and Save"
  - c) Click on the “page” icon to go back to your Global Sheet.
9. Return to original specimen
  - a) Activate Tube\_001.
  - b) Check compensation by selecting Compensation in the Cytometer window.
10. Run Samples
  - a) Rename Specimen (Data file prefix)
  - b) Rename Tube (Data file suffix)
  - c) Set Labels
  - d) Set number of Stopping events to record.
  - e) Install tube on cytometer in Run
  - f) Click “Record”
  - g) Click “Next Tube” and repeat t-y
11. Export FCS files
  - a) Right click on Experiment name and select Export→FCS Files
  - b) Select 3.0
  - c) Choose directory
  - d) *You can also export your Experiment if you would like to keep it backed up.*
12. Delete Old Experiments and data files when data is safely on another computer.