LSRII Training Quick Reference- Acquisition

Preparing the instrument: (*this should be done for you between the hours of 8am - 8pm but always check!*)

1. Turn on cytometer with green power switch
2. Check sheath container to make sure there is enough fluid for acquisition
3. Check waste container to make sure it is not over half way full
4. Let instrument warm up 30 minutes after powering on

Setting up your work space

1. Log in to computer with network password
2. Launch FACSDiVa software and when prompted use assigned password to log into the diva software (password assigned by flow core administrator at time of training).
3. Check the Instrument Window to make sure instrument is connected.
   The lower right of the dashboard will have a yellow dot that it is connecting.
   Should this dot be black quit the DIVA program and restart the computer.
   The acquisition dashboard will open when instrument connects.
4. Choose to use CST settings if prompted. **DO NOT use current settings**
5. Create browser elements
   - Create experiment in Browser either by clicking on New Experiment button in the Browser toolbar or by selecting Experiment → New Experiment and using a saved template/blank template
   - Rename the experiment using a distinctive name (i.e.- user initials/date)
   - If running tubes, click the specimen button (icon looks like a syringe) in the Browser toolbar
   - If running a plate, click the plate icon in the Browser and choose the correct plate type
   - Rename the plate or specimen in the browser (optional).

6. Specify parameters in the Cytometer window
   - Open specimen in the Browser using the plus sign symbol to the left and click on acquisition pointer to the left of the first tube
   - In the Cytometer window, highlight and delete unnecessary parameters using the Delete button within the Cytometer window
   - Add parameters that you will need by using the Add button and change parameters by using the drop-down menu that appears when you click on the arrow next to the parameter.

Compensation - Perform Auto compensation when using 2 or more colors

1. Use the auto compensation feature to automatically calculate compensation
2. Go to Experiment → compensation setup → Create Compensation Controls
3. A dialog box will appear – add labels, change the order of tubes and add or delete parameters as needed. Click OK
4. Toggle to the worksheet to view the plots for compensation (upper left hand button on right screen)
5. Point acquisition pointer at the UNSTAINED CONTROL tube
6. Press Run on the cytometer and put the unstained/negative control on the SIP
7. Click Acquire in the Acquisition Dashboard
8. Run the negative setup control to optimize instrument settings (FSC, SSC, and baseline voltages)
9. Adjust the P1 gate on the FSC/SSC around the population of interest.
10. Right click on the P1 gate and click “apply to all compensation tubes”. This will apply your P1 gate to all compensation tubes for your controls.
11. If using very bright fluorochrome/antibody combinations run single positive controls to make sure they are not off scale. You may have to adjust baseline settings specific to the experiment.
12. Record negative control when adjustments have been made.
13. Click Next Tube in the Acquisition Dashboard when all 5000 events are collected. The green acquisition pointer in the browser will move to the next tube and the next worksheet will open.
14. Take off unstained control and place on the first positive control. Click acquire, then record and continue until all positive controls have been run and recorded

15. When all control tubes have been recorded, put cytometer into standby and use interval gates to gate the positive peak in each of the histograms
16. Choose Experiment → compensation setup → Calculate Compensation
17. When prompted, name the compensation and click Apply Compensation or Link and Save.
18. Toggle to the Global Worksheet using the button in the upper left hand corner of the right screen.

Recording Data

1. Add plots to the global sheet by clicking on icon in the global Worksheet toolbar and clicking in the white space of the global sheet.
2. Change parameters of the plot by clicking on x or y axis labels and selecting from drop down menu
3. Click once on the acquisition pointer to the left of the first sample tube in the browser (if you double click, it will start to acquire).
4. Rename tube (optional) in the Inspector under the Tube tab and hit enter. Label your parameters (optional) in the Inspector under the Label tab.
5. Change the number of events to be recorded in the Acquisition Dashboard with drop down menu or by typing in the number of events.
6. Select a storage gate and/or a stopping gate (optional) by selecting gates from the drop down menu in the acquisition dashboard (requires that gates be set first).
7. Press run on the cytometer and put the first sample tube on the SIP
8. Click acquire in the Acquisition Dashboard and optimize settings if necessary using negative control (FSC, SSC and baseline settings).
9. When sample is optimized, click Record data in Acquisition Dashboard.
10. Click Next Tube in the Acquisition Dashboard and proceed to second tube.
11. Continue until all sample tubes have been recorded.

Exporting and Saving Data

1. To save experiments go to File → Save
2. **Save an Experiment Template by right clicking on your experiment in the Browser → Export → Experiment Template**
3. Right click on Experiment in Browser → Export → Export FCS -or- Experiment
4. Choose FCS 2.0 or 3.0 if exporting as FCS
5. Browse for the correct folder to place your files in
6. Select folder and click export or OK
7. **Delete files from Diva software**.
8. If using Cellquest, or Cellquest pro, files need to be exported as FCS 2.0 files and converted using FACSconvert software located on all Mac workstations
9. If using WinMDI, export files as FCS 2.0 files and open files directly up in software
10. If using FACSDiVa or FlowJo, export files as Experiments or FCS 3.0 files

Cleaning and shutting down the instrument

1. Instrument must be cleaned with Facsclean (bleach 10%); Facsrinse and DI water for 6 minutes each on high.
2. After cleaning, if no one is signed up after you, shut instrument off using green power switch and log off of computer.

Note: Every month files will be deleted. Files from the current month will remain. If you have files that you are using as templates please either re-name the files as "template" or create a template (see above).
You are responsible for your own data; the database is not backed up.