

## How to run an experiment on the LSR II

1. Make sure that the lasers have warmed up for at least 30 minutes and the daily QC has been performed
2. Log onto BD FACSDiva using the username and password previously set up by the administrator
3. Make sure there is enough sheath fluid in the tank. Refill if necessary.
4. If the popup box appears that says “CST Mismatch,” click “Use CST Settings.” If you click “Keep Current Settings,” It will be fine, but because of a program glitch, error messages will pop up.
5. Create your experiment in Diva
  - a. Click on the folder (if any) in which your experiment should appear
  - b. In the toolbar, click “Experiment,” and “New Experiment”
  - c. A popup window will appear with experiment templates
  - d. Select “Blank Experiment with Sample Tube”
  - e. Right-click on the experiment name and click “Rename” to change the name of your experiment
6. Add your compensation controls
  - a. Maximize “Specimen 001” to show “Tube\_001”
  - b. Click on the green arrow to the left of the tube
  - c. Settings will appear in the “Cytometer” box showing every color that is available and its corresponding voltage decided by the UC program, CS&T, at the beginning of the day
  - d. Delete the colors that will not be used by either:
    - i. Searching through the list and deleting each unused color, or
    - ii. Deleting the number of colors that won't be used and selecting your colors from the drop-down menus (this method can be used if you would like the compensation controls run in a particular order, although compensation controls do not need to be in any specific order.)
  - e. Change the voltages to your preset voltages if you have them or leave the voltages that the Q.C program has chosen. If you are going to set your voltages, wait until step 7e.
  - f. From the toolbar, select “Experiment,” “Compensation Setup,” and “Create Compensation Controls”
  - g. Compensation controls will appear beneath Specimen\_001
7. Add your tubes

- a. Rename any categories of tubes (Specimen\_001, Specimen\_002, etc.) by right-clicking and selecting "Rename"
  - b. To add more categories, either:
    - i. Click "Experiment" from the toolbar, and then "New Specimen," or
    - ii. Click the "Specimen" icon from the workspace toolbar (which looks like a syringe)
  - c. To add additional tubes to any category, click on the category and then either:
    - i. Click "Experiment" from the toolbar, and then "New Tube," or
    - ii. Click the "Tube" icon from the workspace toolbar (which looks like a tube with a yellow asterisk)
    - iii. With a tube selected (green arrow), click "next tube" in the Acquisition Dashboard. This will carry over any settings (such as label or number of events to record) from the previous tube
  - d. To rename the tubes, right-click on the tube and select "Rename"
8. Label your tubes and change the number of events to acquire
- a. From the toolbar, select "Experiment" and the "Experiment Layout"
  - b. A popup window will appear with three tabs, "Labels," "Keywords," and "Acquisition"
  - c. In the "Labels" tab, type in any appropriate labels for your tubes
  - d. In the "Acquisition" tab, change the "Events to Record" to the appropriate number and click "OK"
9. Calculate Compensation
- a. Maximize the "Compensation Controls" category and select "Unstained Control" by clicking on the green arrow
  - b. install the unstained control onto the SIP
  - c. Press "RUN" and "H1" on the cytometer fluid control panel
  - d. In the acquisition dashboard, click "Acquire Data"
  - e. if you are using this control to set your voltages, change the FSC and SSC voltages until the population is visible and select the population with the P1 gate. Change any other voltages to meet your needs, Most people will make sure that they can see most to all of the negative population in the first quadrant of the histogram.
  - f. Right-click on the P1. gate and select "Apply to All Compensation Controls"

DO NOT CHANGE THE PMT VOLTAGES AFTER THE FIRST COMPENSATION CONTROL HAS BEEN RECORDED. IN ORDER TO CALCULATE COMPENSATION, ALL CONTROLS MUST BE RECORDED WITH THE SAME PMT VOLTAGE Settings. if you need to adjust the PMT voltage for a subsequent compensation control, you must record all compensation controls again.