LSR Quick Notes

Initial Set-up:

- Turn on the LSRII cytometer allowing ample laser warm-up time.
- Fill sheath fluid tin and empty the waste can if needed.
- Clear any air bubbles in the sheath fluid line.
- Log in to Windows using your standard hospital login and open the DIVA software.

Special Note: If you are using either the Behemoth or the Octopus raise the cytometer lid GENTLY.

Quality Control:

- Log on as "Quality Control". Password=qc.
- Scroll to last QC dated experiment and duplicate without data.
- If you get a warning window that says "CST Mismatch" check the "Don't show this message again for the current login session" and then choose "Keep BD FACSDiva Settings."
- Rename experiment with current date and delete the numerical suffix.
- Add 1 drop of Spherotech Rainbow beads (vortexed thoroughly) to approx. 400uL sheath fluid.
- Run beads on "Low" observing histograms, looking for stable, sharp, symmetrical peaks>Record.

Experiment set-up:

Set Up an Experiment from Scratch

- Log in under lab PI name and find personal folder.
- Create and name a new experiment.
- Add a needle icon and rename it "Voltages".
- Rename the sample tube under "Voltages" to "Unstained Cells".
- Go to the "Instrument" window and click on "Parameters".
- Delete detectors that are not needed.
- Create a FSC/SSC dot plot on the standard worksheet of the graphics screen.
- Run "Unstained Cells" and adjust FSC/SSC voltages in order to see cells on the dot plot.
- Gate on your cells on the dot plot.
- Create a histogram for each color and designate its data source as the P1 gate from the dot plot.
- Check the "Mark Grid" checkbox in the Inspector for each histogram.
- Run unstained sample and adjust voltages for colors to set background > Record.

Set Up an Experiment via "Duplicate Without Data"

- Log in under lab PI name and find experiment that you wish to duplicate.
- Open and highlight the experiment name>right click> "Duplicate Without Data".
- In the duplicated experiment **"Delete" and "Unlink" the previous compensation set-up as well as all experiment sample tubes but the first.**
- Activate the "Unstained Cells" tube under the "Voltages" needle icon and open the voltage graphs on the "Standard Worksheet".
- Run unstained sample and adjust voltages for scatter and colors as needed > Record.

Compensation: You must **ALWAYS** create a new compensation panel if using multiple fluorochromes.

- Go to "Experiment" at the main menu bar >Compensation Set-up>Create Compensation Controls.
- Activate the "Unstained Control" sample in the compensation panel.
- Place unstained cells on the SIP running on high and "Record".
- Repeat for each of the single stain compensation samples listed on the compensation panel.
- Adjust the P1 gate on the scatter graph around the population of interest. Activate the P1 gate, right click and "Apply to all Compensation Controls".
- Set interval gates for each single color sample in order to define positive staining for that color.
- After setting all gates go to "Experiment" >Calculate Compensation>Link + Save.

Running Samples:

Run Samples from an Experiment Set Up from Scratch

- Create a new needle icon (experiment group) for your samples and name it.
- Create antigen and fluorophore labels for each detector in the "Inspector".
- Switch to and create a "Global" worksheet on the graphics screen.
- Create a dot plot for FSC/SSC with a polygon gate for the population of interest.
- Add dot plots and/or histograms specific to your experiment.
- Set "events to record" at the acquisition control window to the number desired.
- Run first sample and record.
- After 1st sample, click "Next" on the acquisition control window or "New Tube" at the top of the browser to create the next sample tube.
- Rename second sample, acquire and record and so on.

Run Samples from an Experiment Set Up Via "Duplicate Without Data"

- Click on the needle icon that you named previously for your samples and rename it if necessary.
- On the graphics screen switch to the "Global" worksheet revealing the graphs that were made in the previous experiment.
- Set "events to record" at the acquisition control window to the number desired.
- Run first sample and record.
- After 1st sample, click "Next" on the acquisition control window or "New Tube" at the top of the browser to create the next sample tube
- Rename second sample, acquire and record and so on.

Export Your Data:

- Highlight the experiment name that is open.
- File>Export>FCS files.
- Export as FCS 3.0 files to your folder on your U drive or other network drive that is yours.

Shut down:

- While exporting data create a new needle icon and label "Cleaning", labeling the first sample, "Bleach".
- Clean the machine by putting 10% bleach on the SIP with the arm in the open position for 1 minute followed by 5 minutes with the SIP arm closed running on High while recording..
- Repeat with DI water, creating a second sample tube named "Water" and recording.
- Close experiment and log out of DIVA.
- If someone is to follow, leave the computer and the cytometer on, otherwise turn the cytometer off but leave the computer on.

Special Notes:

If you are using either the Behemoth or the Octopus close the lids **VERY GENTLY** if shutting down.

ABSOLUTELY NO INTERNET SURFING OR E-MAIL USAGE ON THE CYTOMETER COMPUTERS.