LSRII and LSRFortessa Training Guide

Topics to be Discussed

1. How samples should be run on the instruments
2. Instrument configurations
3. Instrument Startup
4. Instrument Operations
5. Data Collection Considerations
6. Instrument Cleaning and/or Shutdown

How samples should be run on the instruments

Sample Vessels: Falcon polystyrene tubes, 12mm X 75mm: 1) Cat#2052, non-sterile (without cap), 2) Cat #2054 (with cap), 3) Cat #2058, sterile and 4) Cat #2235, sterile with Blue capped mesh tubes. 96 well plates (Most vendors provide an appropriate footprint in the round bottom or flat bottom formats)

Concentration: Ideal range between 5 X 10^5 to 2 X 10^7 cells per mL resuspended in PBS containing 1% BSA. Avoid phenol red, an additive to media preps. Minimum sample volume is 500 μL. Do note that sample is taken up quickly.

Sample considerations: Prior to sampling (literally while in front of the instrument and not at the lab) filter each sample through filter top tubes. These sterile tubes have a 35 mm mesh filter in the cap.

If your samples contain cells with a propensity to form aggregates, you should treat the sample with an anti-agglutinating agent. There are reagents that can be added to your samples to reduce or eliminate the formation of aggregates:

1. DNAse
2. EDTA (1mM)
3. PBS without Ca^{2+} or Mg^{2+} containing 0.1% BSA or FCS.

Instrument configurations

BD LSRII and LSRFortessa: ***Review the on instrument laminated configurations.*** Note that these configurations are available on the website and on Fluorofinder.

http://pavir.org/means/cores/flow-cytometer-information/

https://app.fluorofinder.com/pavir

BD Fortessa (Storm)
5 laser (355,405,488,561,640)

Design Experiment

BD LSR (Night Crawler)
4 laser (405,488,561,633)

Design Experiment

LSRII (Night Crawler) and LSRFortessa (Storm) Start Up SOP

(Laminated copy on the instrument)

1. Check the Sheath Fluid
    a. Disconnect the vacuum line (green line) from the sheath tank.
    b. Vent the sheath tank and remove lid.
    c. Add NERL Diluent 2 (large boxes along the right side of the window) to a level matching the upper inside metal welding mark.
    d. Reseat the lid and reconnect vacuum line to the sheath tank.
2. Empty Waste Tank.
    a. Disconnect the waste line (orange line) and the alarm chord (black line).
    b. Unscrew the waste tank lid and pour liquid waste down the sink chasing the contents with water.
    c. Add 1L of concentrated bleach using dedicated graduated cylinder (both found under the sink).
    d. Place lid back on sheath container and reconnect waste and alarm lines.

When to Refill Sheath and Discard Waste on the analyzers:

- *If there is another user after you and...*
    i. If the weight registered on the scale holding the sheath tank is less than 17 pounds
    ii. If the liquid level is greater than the height of the holder housing the waste
    iii. If you plan to use the LSRs for longer than two hours, expect to have to refill or empty

Approximate use rate for Analyzer Fluids:

- *Sheath usage is approximately 1.1L per hour (18mL per min)*

- *Sample use is dependent on your flow rate:*
    - *LO is ~12uL/min (6uL to 24uL per min with fine adjustment)*
    - *MID is ~35uL/min (17 to 70uL per min with fine adjustment)*
    - *HI is ~60uL/min (30 to 120uL per min with fine adjustment)*

3. Startup computer: Log in with the Admin account and the password “BDIS#1”

4. Launch TeraTerm program. This is a terminal window app found on the desktop with a **“T”** icon.

5. Turn on the Flow Cytometer. Press the big green power button on the Flow Cytometer. TeraTerm will become active after 2-3 minutes, generating an IP address for the flow cytometer. Once this is complete proceed to step 6.

6. Launch the LabUsage App on the desktop to access the FACSDiva Software (Same user email and password as for the BookMyLabs Calender). At Diva

Login select the Administrator account using password “pass.” The cytometer will connect to the computer after about 2 minutes…

7. While allowing the lasers to warmup, cycle through 5 min of Bleach; 5 min of 70% EtOH; 5 min of MilliQ water with the Analyzer on RUN/HIGH. Switch to RUN/LOW once the cycle is complete.

8. Proceed with CST QC procedure. Navigate FACSDiva to the “Cytometer” dropdown menu and select CST. Load your CST beads on the Sampler. Once the CST App connects, select RUN and follow the onscreen prompts.

9. Should CST offer a passing grade, place the Analyzer on STANDBY and replace the CST beads with water on the Sampler. Close the CST window and select “Use CST Settings” when prompted when the instrument reconnects to FACSDiva.

10. Log out of the Administrator Account and log into your personal account in FACSDiva. You may now proceed with running samples

Instrument Operations in Diva

1. Each user has a personal account in FACSDiva. This will be where you store all your experimental templates and protocols.
2. Setting up your experiment
    a. Create a new Blank Experiment
    b. Rename the Experiment (right click>Rename) using a good descriptive term and maybe the date.
    c. Create a new Specimen (embedded will be your first sample tube).
3. Creating New Application settings (Optional: First time you run an experiment- this isn’t essential but can be helpful long term)

*** *Application settings are associated with a cytometer’s CST daily configurations. Each includes the parameters needed for the application, area scaling values, PMT voltages, and threshold values, but not compensation. Each time a CST performance check is run, the application settings associated with an experimental configuration are updated to account for changes in detector sensitivity. Using application settings provides an easy, consistent, and reproducible way to reuse cytometer settings for your commonly used applications****

    a. Select Cytometer Settings in the Experiment Browser (Also can be found in the top menu View>Cytometer>Parameters tab)
    b. Delete all parameters you will not be using:
        i. In Parameters tab, click on small button to left of parameter name.

ii. Click delete button (use control key and highlight for multiple deletions)
iii. Repeat for each parameter you are not using.

c. Click the H and W checkbox to select Height and Width for FSC and SSC to enable doublet discrimination
d. It can be helpful to add a target name to your parameter
    i. Highlight the first sample Tube under the Specimen.
    ii. Open the Inspector. The second Tab over is the Label Tab for your samples. You can add the target name next to the fluorochrome... Every tube generated thereafter will retain your label unless you change it for a specific tube...
e. Right click Cytometer Settings in the Browser, then select Application
f. Settings>Create Worksheet. A second global sheet is added with the plots created according to your selections in the Parameters tab.

****You will use the gray boxes and crosshairs on this worksheet to guide your optimization. This can be a helpful means of staying within the linear range of readings for each of your detector channels****

g. Load a fully stained sample tube on to the cytometer. Press RUN/LO on the LSR Fluidics control panel.
h. Optimize the FSC and SSC voltages to place the population of interest on scale.
i. Verify that the positive populations are on scale for each of the targets you wish to see. If a positive population is off scale, lower the PMT voltage for that parameter until the positive population is entirely on scale.
j. Adjust the Threshold to limit the collection of data points of no use.

****Check for noise and debris in the lower left section of your dot display. The default threshold trigger parameter is FSC at channel 5,000. Adjust higher if excessive debris and noise is present. You can also add a secondary parameter. In most cases, click "Add" and SSC parameter will be added. Make sure to check the correct button for "AND" instead of "OR" to and start at channel 5,000****

k. Unload the stained cells tube from the cytometer. Press STANDBY on the LSR Fluidics control panel.
l. You now have your Application settings. To save, Right click Cytometer Settings in the Browser, then select Application Settings>Save.
m. Name the Application Settings appropriately and Click OK. The application settings are saved to the catalog. Application settings do not include compensation settings.

4. Using previously created Application Settings (When you are doing the same experiment again)

a. In a newly created experiment, ensure that the current CST settings are applied. Then, right click the Cytometer Settings icon in the Browser and select Application Settings> Apply.
b. Select your correct previously created Application Settings from the catalog.
c. Click **OVERWRITE** in the dialog that appears.
d. If a message appears about area scaling, click **Yes** to accept all changes to cytometer settings.
e. The parameter list and PMT voltages are updated to match the application settings you previously created.

5. Compensation
    a. Ensure that you have the correct Application Settings applied, or if you are starting from scratch that you have the correct parameters and PMT voltages for your experiment.
    b. Select Experiment>Compensation Setup>Create Compensation Controls.
    c. Click OK to close the Create Compensation Controls dialog. A compensation controls specimen (Set of tubes) is added to the experiment, along with an unstained control tube, and a stained control tube for each parameter. Worksheets containing the appropriate plots are added for each compensation tube.
    d. Load the unstained control tube on to the sample line.
    e. Set the current tube pointer to the unstained control tube in the Browser.
    f. Press RUN/LO on the fluidics control panel.
    g. Move the P1 gate to fully incorporate the singlet population.
    h. Right click the P1 gate and select Apply to all Compensation Tubes.
    i. Click Record Data in the Dashboard to record the events from the unstained control tube. Usually 1,000 to 5,000 events is appropriate to the task.
    j. Unload the unstained control tube from the sample line. Press STANDBY on the fluidics control panel.

    Notice: Do not change the PMT voltages after the first compensation tube has been recorded. To calculate compensation, all tubes must be recorded with the same PMT

    k. Click Next Tube in the Dashboard.
    l. Acquire each compensation tube and record in this manner.
    m. Verify that the snap-to interval gates encompass the positive populations
    n. Select Experiment>Compensation Setup>Calculate Compensation. If the calculation is successful a dialog appears. Appropriately name the compensation setup. I usually select a Exp/Date nomenclature.
    o. Click Link & Save to close the dialog box and save the compensation setup and link it to the experiment's cytometer settings

6. Collecting data for each sample
    a. Return to your SPECIMEN and click on the first tube in your experiment. Or, generate a new SPECIMEN: If you have a previously created panel template, right click your experiment in the browser and choose New Specimen from the menu. Click the appropriate tab and select the panel template you created previously. Panel templates import worksheets into your experiment also.
    b. **(For Today)** Import a blank specimen and create worksheet elements you want to view for this experiment.
    c. Set the current tube pointer at the first tube, install the tube, press RUN/LO on the instruments fluidics control panel, and record in the FACSDiva Acquisition Dashboard.
    d. Sample will be saved automatically when it reaches the stop event count. If your sample runs out before enough events are collected, Press "Stop Acquisition" to save all the events collected to that point. All instrument settings, data and compensation settings are saved in the Experiment.

Important Consideration: Stop, Storage or Events to Record gates
On the Acquisition Dashboard Stopping Gate, Storage Gate, and Events to record parameters can be changed from the default values

- *The "Stopping Gate" defines the gate in which the number of events is to be acquired*
- *"Events to Record" can be set for a specific number of events to be acquired in the file. The defaults are "All Events" and 10,000. Frequently the "Stopping Gate" will use one of the gates that defines a population to guarantee a minimum number of the target population is collected.*

Instrument Cleaning and/or Shutdown

Review the Laminated SOP posted on each instrument

General Rules:
Proper Cleaning between users:

1. BSL-1 samples (less than 1 hour on the analyzer)
    - RUN mode, HI, 5 min with 70% ethanol + 5 min with ddH_2O
2. BSL-2 Samples or > 1 hour on the analyzer or you have problematic samples
    - RUN mode, HI, 5 min with 10% Bleach + 5 min with 70% Ethanol + 5 min with ddH_2O
    - Problematic samples: cells with a propensity to reaggregate; some tissue derived samples; Larger cell types (cells in the >20um range, i.e. Macrophage!!)

******Check to see if there are others after you. Communicate with each other if you have any questions (you're running late, you've encountered trouble, etc). If there is a break of more than three hours, power down the analyzer******