BD FACS LSR Fortessa User Manual

Flow Cytometry Unit – Biomedical Core facility

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A. Starting Up

1. Turn on the instrument:
    a. LSR Fortessa - red switch on the wall behind the monitors (Fig. A1).
    b. Computer – blue button on the front of the computer.
    c. Monitors – blue buttons on the front of the monitors.
2. Wait at least 10 min for the lasers to warm up. While waiting, proceed to sections 3-9.
3. Drain bubbles:
    a. Tap the sheath filter (located on the front panel of the plenum) to release trapped bubbles, then connect the drain line to the orange connector (Fig. A2). Drain about 1ml of sheath to a beaker. Disconnect the drain line.
    b. Roll down the roller clamp (located in the sheath line between the plenum and the cytometer) to drain about 1ml of sheath. Roll up the roller clamp (Fig. A3).
4. Load a FACS tube marked H_2O (or DDW).
5. Press Prime on the control panel (Fig. A4). Repeat. Make sure that air bubbles come out of the SIP.
6. Run a tube of H_2O for 10 minutes.
7. Press Standby on the control panel (Fig. A4).
8. The instrument is now ready for use.
9. Run BD FACSDiva software by double-clicking the software icon on the desktop (Fig. A5).

Figure A1 Power panel

Figure A2 Sheath filter

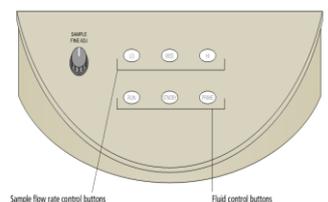**Figure A3** Roller clamp

Figure A5 FACSDiva icon

Figure A4 Control panel

B. Creating a new experiment

1. Click the buttons on the Workspace toolbar to display the following windows as needed:
   - Browser
   - Cytometer
   - Inspector
   - Worksheet
   - Acquisition Dashboard
2. In the browser window navigate to the folder of the current year, month, and head of lab.
3. If you do not see the lab folder, create a new one.
4. In the browser window, create a new experiment by clicking Experiment -> New Experiment.
5. Use the Browser toolbar to add Specimen and Tube elements to your experiment (Fig. B1).
6. To specify the parameters for the new experiment select Cytometer Settings in the Browser. The cytometer settings will appear in the Inspector under the Parameters tab (Fig. B2).
   a. Check the boxes of W and H for the FSC and SSC parameters (later used for doublets discrimination).
   b. Make sure the parameters you need appear on the Parameters tab. Select the desired parameter from the menu by clicking the parameter name and displaying available fluorochromes in the list.
   c. Delete any unnecessary parameter by clicking the point on the left of the parameter's name and then clicking delete (Fig. B1).
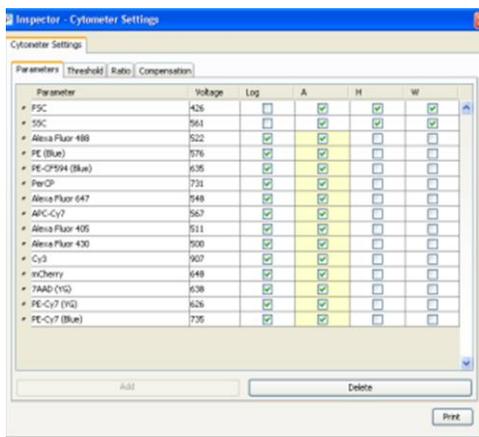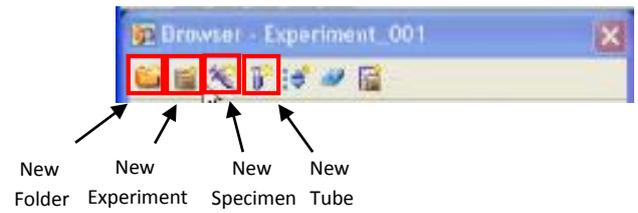

Figure B2 Cytometer settings
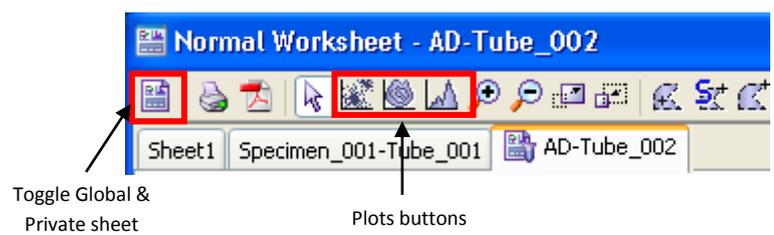

Figure B1 Browser toolbar

Figure B3 Normal Worksheet toolbar

7. For multi-colored samples create compensation controls by selecting Experiment -> Compensation Setup -> Create Compensation Controls.
   When done, switch to a Global sheet by clicking the Global sheet icon (Fig. B3).
8. Create the plots and gates needed for recording using the Worksheet toolbar (Fig. B3).
   a. FSC-A vs. SSC-A to show the physical cell parameters corresponding to size and granularity.
   b. FSC-A vs. FSC-H for doublet discrimination (optional: SSC-A vs. SSC-H for another doublet discrimination step).
   c. Fluorescent parameter 1 vs. Fluorescent parameter 2 (if you have only one fluorescent parameter, plot it vs. SSC-A or FSC-A).

   d.   Use histograms only when it is necessary (prefer dot plots).

9.   For each fluorescent plot, select the plot and check the Biexponential boxes in the Inspector window.

10. Right-click a plot and select the items from the menu to show Statistics View and Population Hierarchy (or Ctrl+R / Ctrl+G).

11. Adjust voltages on all parameters by using the Parameters tab in the Cytometer window or the Cytometer Settings section in the Inspector (Fig. B1).

- Load the unstained control tube onto the cytometer.
- Optimize the FSC-A and SSC-A voltages to place the population of interest on scale.
- Optimize the FSC-A threshold value to eliminate debris.
- Unload the unstained control tube from the cytometer.
- Load the multicolor sample onto the cytometer (or each of the single stain controls).
- Adjust voltages such that populations are on scale and most events are >0 and <10^5.
- Adjust voltages to best resolve all populations of interest (best resolution with low CV).
- Unload the multicolor sample from the cytometer.

12. Record data for the compensation control tubes.

   a.   In the Browser window select the specimen Compensation Controls and a Tube in it.

   b.   Load the relevant tube and read 5,000 events (the default).

   c.   Adjust the FSC-A / SSC-A gate to include all singlet events (may vary between beads and cells).

13. When done select Experiment -> Compensation Setup -> Calculate Compensation. In the results window click Save and Link.

14. For adding a short description of each color (recommended) select Experiment > Experiment Layout. Use the labels tab (Fig. B4).
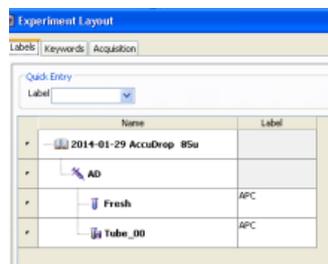

Figure B4 Experiment Layout

15. Use gating tools to define the population(s) of interest.

16. Load the sample:

   a.   Gently move the lever right or left (does not matter).

   b.   Push up the sample tube onto the SIP (sample injection port). Hold on to the tube

   c.   Close the lever and release the tube.

       The SIP should not touch the bottom of the tube.

17. Adjust the flow rate in the control panel (Fig. A3).

18. Verify that the current tube pointer is indicating the appropriate tube in the Browser and click Acquire.

19. Click Record Data to save data for the tube.

C. Shutting Down

1. Press Run + High on the control panel.
2. Load a FACS tube marked FACS clean (or 'C')' leave the lever open and click Acquire, after 1 min close the lever and acquire for an additional 5 min.
3. Load a FACS tube marked FACS rinse (or 'R')' leave the lever open and click Acquire, after 1 min close the lever and acquire for an additional 5 min.
4. Load a FACS tube marked H_2O leave the lever open and click Acquire, after 1 min close the lever and acquire for an additional 10 min.
5. Turn off the cytometer from the red switch on the wall (Fig. A1).
6. Exit Diva software by selecting the menu item File -> Quit.
7. Lock the computer (Ctrl+Alt+Delete). On weekends turn the computer off.

D. Troubleshooting

Problem: Can't see events on my plots.

Possible reason 1: System clog.

Solutions:

1. Click Prime in the control panel. Repeat 3 to 5 times as needed.
2. Run 5 minutes of Clean, 5 minutes of Rinse and 5 minutes of Water.

Possible reason 2: Threshold too high.

Solutions:

1. Reduce Threshold in the Cytometer window in the Threshold tab. Use initial values between 200 and 5,000.

Possible reason 3: Events are on the axis in FSA-A and / or SSC-A.

Solutions:

1. Reduce FSC-A voltage. Use initial values between 150 and 250.
2. Reduce SSC-A voltage. Use initial values between 100 and 350.

Problem: Fluidics cart is beeping.

Possible reason: Sheath fluid ran out.

Solutions:

1. Click Alarm button on the fluidics cart to silence the alarm.
2. Take X1 PBS from the big tank in the cold room and fill 20 liters into the sheath fluid box.
3. Decant the waste tank box into the sink. Add 0.5 liter of chlorine (bleach / אקנומיקה, found under the sink).
4. Make sure to screw in caps on both boxes.