

# LSR Training Guide

*This training guide applies to LSRFortessa and LSR II.*

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## Startup

### Check fluidics tanks

- Empty waste tank and add ~1 cup bleach.
- Fill the Sheath Tank
  - **Open the sheath tank:** unscrew the black knob, lift the vent valve to depressurize the sheath tank, take the lid off
  - **Add sheath fluid** up to the weld line from the white carboys next to cytometer. Close the tank. Tighten the black knob to seal the tank.
- **Turn on Cytometer:** Press the large, green Power button on the side
- **Remove air bubbles** by opening the roller clamp on the tube attached to the sheath filter.
  - Use a beaker to collect 50 – 100 ml of sheath fluid.
- Remove the water tube from the SIP and run **Prime procedure** twice.

### Turn on the PC

- Log in Windows 7 as **Operator** (*no password is needed*)
- There is no need to restart the PC if it is already on.
- Open FACSDiva software and log on.



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## The Prime procedure

*The Prime procedure should be done after refilling the sheath tank or if an obstruction of the sample probe is suspected. During the prime procedure the flow cell is emptied, and the sample line is flushed with air. Then the flow cells will be refilled from bottom to top, to remove any air bubbles.*

This is how **Priming** should be performed:

- **Remove the FACS tube** from the SIP
- **Leave the arm to the side**
- **Push the Prime button** on the cytometer front panel and wait until the instrument goes in Standby mode
- **Push the Prime button** again and wait for the cytometer to go in Standby mode
- Now you may use the cytometer

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## FACSDiva Experiment Setup

- **Create a brand new experiment** (click on the *New Experiment* icon in the *Browser* window).  
Rename the experiment (right-click on the experiment name → Rename)
  - Click on **Cytometer Settings** → **Parameters** tab and **Delete the parameters that are not needed.**
  - In top menu bar, click **Experiment** → **Compensation Setup** → **OK.**
    - A **new specimen named "Compensation Controls"** will be created in the Browser window and the software will automatically switch to **"Normal Sheets"** mode. The "Compensation" specimen contains tubes for all compensation controls and one tube for unstained.
  - **Select and Activate the Unstained tube** by clicking on the bullet next to the tube name to show the worksheet with histograms (one for each **fluorescent parameter**).
  - Load your compensation controls and run them one by one by clicking **Acquire** in the Acquisition Dashboard. **DO NOT RECORD ANYTHING YET!**
  - While acquiring, watch the histograms and adjust the voltages of the PMTs as necessary.
  - **Record compensation controls one by one. Do not change PMT voltages!**
  - Click **Experiment** → **Compensation Setup** → **Calculate compensation.**  
(Typically, a message confirming that the compensation was calculated successfully is displayed.)
    - **Click "Link and Save".** Now you are ready to record samples.
- Toggle back to **Global Sheets** by clicking the icon in the top-left of the worksheet window.

- Add a **Specimen** and expand it by clicking on the + sign next to it.
  - Activate the tube by clicking on the bullet next to the tube name. This will then activate all the windows in Diva.
  - Create plots in Global Worksheet 1.
  - Load your first sample and adjust FSC-A and SSC-A if needed.
  - **Add plots and gates. Show Population Hierarchy and Show Statistics** by right clicking on a plot.
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## Exporting Data

- Right click on your experiment name and choose Export -> FCS Files.
  - A window with the FCS version Linear parameters pops up. Click OK
  - Click "Browse" in the next window.
  - Navigate to E:\ drive and your lab folder.
  - Click "Choose Directory".
  - Click "Save".
  - Drag data to a third party drop box such as OneDrive.
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## Clean Instrument when complete

1. Press RUN and HI on the cytometer fluid control panel.
2. Install a tube containing approx. 3 mL of a FACSClean solution (or bleach 10%) on the SIP.
3. Set the tube support arm to the side (vacuum on) and let it run for at least 30 seconds.
4. Move the tube support arm under the tube (vacuum off) and let it run for one minute.
5. Repeat steps 3 and 4 with a tube containing Coulter Clenz (detergent solution).
6. Repeat steps 3 and 4 with a tube containing deionized water.
7. Press the STNDBY button and leave the tube containing deionized water on the SIP.
8. If you are the last user of the day turn off the instrument by pressing the large, green, Power button.

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## Troubleshooting

### Very few events are detected / flow rate (*i.e. the number events-second*) is lower than expected

- Check that the Fine Tune knob is not turned all the way to the left (lowest setting).
- The **SIP may be clogged**:
  - Do the **Prime procedure** (see above)
  - **Run FACSClean** (10% Bleach) for 2 - 3 minutes followed by DI water for 1 minute.  
*When running bleach with default PMT voltages, the cytometer should detect >30 events/second. This is a good indication that the SIP is not clogged.*
  - Some samples tend to form cell aggregates. **Such samples should be filtered** through a 40 µm mesh.
  - **Dilute the samples.** (Very concentrate samples are often more likely to form cell aggregates that may clog the sample line).

### Data on the FSC vs. SSC plot suddenly appears abnormal

- **Refill the Sheath tank** if it is empty.
- **Bleed the sheath filter** to remove air bubbles

### No events are detected (zero events)

- Confirm that **the cytometer is connected to computer**. If not connected, try restarting the computer and the cytometer.
- **If the RUN button is orange** (not green):
  - Transfer the sample into an intact tube if **the tube is cracked**.
  - You may be using the **wrong type of tube**. The tubes used on the LSR instruments must be Falcon 12x75mm Tubes Cat# 352008. Replace tube if not the Falcon 12x75mm tube.

### The signals off the yellow-green and red lasers are not stable in time

- This problem usually indicates a fluidics instability which may have different causes. Please contact the staff. In the past this problem had to be diagnosed by a BD engineer.