BD FACSJazz Users Guide
Satchmo
Stanford Shared FACS Facility

PPE must be worn at all times in our BSL-2 environment
*Lab coat, gloves, and closed toed shoes are mandatory; respirator and goggles are available*

An Instrument Configuration is located on the website in the Instrument Overview area
*The configuration lists the lasers and filters for each machine*

All samples must be in POLYPROPYLENE round bottom tubes.

What samples do I need to bring?
- All users **ALWAYS** need to bring single stained compensation controls
  - If you have a 4 color panel you should bring: 5 compensation controls
    - One tube stained with each individual antibody
    - One unstained tube
    - No PI or viability dye in the single color controls

Don’t have enough cells for compensation?
User can purchase compensation beads from many different suppliers
- eBioscience UltraComp Beads 01-2222-42 *(for panels including UV and Violet dyes)*
- eBioscience OneComp Beads 01-1111-42 *(for panels that do NOT include any UV or Violet dyes)*
- BD CompBeads 552845 ‘Anti-Rat/Hamster’
- BD CompBeads 552843 ‘Anti-Mouse’
- ThermoFisher Amine Reactive Compensation Beads A10628 *(for LIVE/DEAD Fixable stains)*
Before you begin:
1. Make sure the plates are clean. You may choose to take them out and clean them.
2. Close the FACSSoftware.
3. Close the Pressure Controls choosing Just Quit
4. Log out of Windows (please make sure you sign out)
5. Log in to Windows with Your SUNet ID
6. Open FACS Software and push CONNECT

Stream Start up

*Note: there is an image of the knobs on the at the end of this section to guide you in which knob is which.*
1. Restore workspace—SSFF template “most recent date”
2. In the sort settings increase the piezo amplitude to between 3 and 6.
3. Press the ILLUM button.
4. Look at the third window of the stream camera.
5. Use the left/right and the pitch black knobs to adjust the stream so that it is falls into the aspirator and that it is bright and tight.

*like the image below*

6. Adjust the stream so that the stream is centered over the pinholes and in focus. Using the black left/right and the focus adjustment knobs.

*like the image below*

7. You may have to repeat 5 & 6 to get the adjustments correct.
8. Using the vertical adjustment knob, adjust the nozzle so that it is sitting at the top edge of the panel.
9. Adjust the piezo amplitude such that there is a proper gap and break of point. This can be monitored in the second camera view panel. The stream should look similar to this.
10. Click the **PLATEST** button to turn on the plates.
11. Close the shutter so that the plates will be activated.
12. Click **TEST STREAM** in the sort settings window. (*You should see a deflected drop to the left of your stream in the 3rd window of the streams.*)
13. Click **FLASH CHARGE** and adjust the piezo amplitude till the deflected drop stays out to the left.
14. Click **SHORT FLASH** and adjust the piezo amplitude till the deflected drop stays out to the left.

*like the image below*

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**Please note the **ILLUM** button and the **PLATES** button**

**Please note the diagram of the nozzle assembly with the labels of each knob.**
Bead Set up

197 Beads

1. Close the laser shutter, activate the shutter and make sure the lasers are on.
2. Make sure you have already restored the proper workspace.
3. Vortex and load beads onto sample port and press SAMPLE and press BOOST to get them sample running.
4. Press ACQUIRE in the acquisition bar.
5. Adjust the event rate to about 200 events/second by adjusting the offset to about .25 PSI.
6. In the recording settings adjust the events to display to 200.
7. Align the beads into their target boxes.
   a. Using the color adjustments knobs
   b. Using the focus adjustment knob
8. Press SAMPLE to stop the beads from running.
   And STOP ACQUIRING in the software.
9. Remove the beads from the sample port.
10. Press BACKFLUSH, to remove the beads from the sample line.
**Accudrop Beads**

1. Adjust the piezo amplitude such that there is a proper gap and break of point. This can be monitored in the third panel. The stream should look similar to this.
2. Click on the **PLATES** button to turn on the plates.
3. Click on **TEST STREAM** in the sort settings window. *(You should see a 2\(^{nd}\) drop to the left of your stream in the 3\(^{rd}\) window of the streams.)*
4. Click **FLASH CHARGE** and adjust the piezo amplitude till the second drop stays out to the left.
5. Click **SHORT FLASH** and adjust the piezo amplitude till the deflected drop stays out to the left. *(like the image below)*

6. Vortex and load beads on to sample port and press **SAMPLE** and press **BOOST** to get them sample running.
7. Press **ACQUIRE** in the acquisition bar.
8. Adjust the event rate to between 1000 events/second and 1500 events/second by adjusting the offset to about 1.5 PSI.
9. In the Sort Layout window, make sure the sort device says Accudrop Setup, and it is sorting P1 to the left.
10. Make sure the sort mode is **1.0 drop pure**.
11. Click **ACCUDROP** in the Pressure Console to turn on the accudrop filter.
12. Click **START** in the sort layout window.
13. Adjust the drop delay value until you only see the left stream visible and the center stream disappears. *(Should look like the images below)*

14. Click **ACCUDROP** in the control window to turn off the accudrop filter.
15. Click **STOP** to stop the sort.
16. Press **SAMPLE** to stop the beads from running.
17. Remove the beads from the sample port.
18. Press **BACKFLUSH**, to remove the beads from the sample line.
Sort Set up

1. Turn on the chiller if you would like to use it.
2. In the sort layout window, under sort devices choose the device you would like to sort into.
3. Place the appropriate tube holder in the sorting arm with the appropriate dummy tubes.
4. Click **SORT READY** in the sort layout window.
5. Click **TEST STREAM**.
6. Adjust the stream deflections till the streams hit the center of the tubes.
7. Click **TEST STREAM** to turn off your test streams.
8. In the inspector choose the path to save your data. (C:\Influx\your name) 
9. Change the Prefix to the name of your sample. *Make sure you are choosing Prefix and not FileName.*
10. Adjust the events to display to 10,000.
11. Vortex and load your on to sample port and press **SAMPLE**.
12. Press **ACQUIRE** in the acquisition bar.
13. Adjust the event rate to less than 10,000 events/second.
14. Press **SAMPLE** to stop running your sample.
15. Press **STOP** in the acquisition bar to stop acquiring.
17. Add your sorting gates to the sort layout.
18. Make sure you change the sort mode to **1.0 Drop Pure**.
19. Load your collection tubes.
20. Press **SAMPLE** to start running your sample.
21. Press **ACQUIRE** in the acquisition bar.
22. Press **START** to sort your sample.
23. Choose the number of events you would like to record.
24. Press **RECORD**.

**While your sample is running:**
- Safe a PDF of your worksheet by selecting the PDF icon and saving it to C:\Influx\your name.
- Monitor your stream and gap.
- Monitor your collection tubes so they don’t over fill.
- Monitor the sort streams.
- Keep your event rate less than 10,000 events per second.
- Do NOT increase your offset above 5.0 PSI, however idly not above 2.0 PSI.

24. Press **STOP** in the sort window to stop the sort.
25. Press **STOP RECORDING** in the acquisition bar to stop recording.
26. Safe a PDF of your sort counts by selecting **SORT REPORT PREVIEW** button on the sort layout window. Safe the file to C:\Influx\your name.
27. Press **BACKFLUSH**, to remove your cells from the sample line.
28. Repeat acquisition and sorting steps for as many samples as necessary.
Clean up

1. Turn off chiller if you were using it.
2. Run a tube of **BLEACH** at a PSI of 5, for 5 minutes.
3. Run a tube of **WATER** at a PSI of 5, for 5 minutes.
4. Turn off the **PLATES** and **ILLUM**
5. Exit the software.
6. Close the Pressure Controls choosing **Just Quit**.
7. Log out of Windows (please make sure you sign out)
8. Make sure to check in your data.

**IF you ran BSL-2 samples follow the additional cleaning steps below:**
1. Spray cavicide on all surfaces this includes your collection tube holder, the collection chamber, keyboard and mouse. Contact time for cavicide is 5 minutes unless your APB requires a longer period. *Please let staff know if different contact times are needed.*
2. After your collection tubes have sat with cavicide for 5 minutes, bring them over to sink and rinse them with hot water, then spray them with alcohol. Leave them on a clean diaper to dry by the sink.
3. Fill-out the biosafety clipboard next to the machine. List the biohazard, user, lab and clean up procedure.
4. Clean up and dispose of anything that came in contact with your samples in the biohazard waste container.

- **IF you are the last user of the day continue to the daily shut down.**
- **IF you are NOT the last user of the day, log out of windows and log back into windows as Operator for the next user.**

**Daily Shut down**

*Please let staff know when you are done with the cleaning if you are the last user on Friday.*

1. Push **STREAM** to stop the stream.  
2. Fill the de-bubbler with **10% BLEACH**.  
3. Place the de-bubbler back and the sink under the nozzle with **10% BLEACH**. Lower the nozzle so that it is sitting in the **BLEACH** press **PURGE** and then **PULSE** in the software.  
4. Remove the de-bubbler from under the nozzle and dump out any leftover fluid.  
5. Fill the de-bubbler with **WATER**.  
6. Place the de-bubbler back under the nozzle with **WATER**. Lower the nozzle so that it is sitting in the **WATER** press **PURGE** and then **PULSE** in the software.  
7. Leave the nozzle in the de-bubbler.  
8. Quit the BD FACSSortware.  
9. Turn the air off on the side of the machine.  
10. Flip the **GREEN** switch on instrument box.  
11. Turn the vacuum off to the instrument.  
12. Make sure you have Checked In your data.  
13. Log out of windows.
Troubleshooting

The Sheath Tank is not pressurizing
1. Ensure that the pressure release valve is closed.
2. Reinstall the sheath tank cover, feel and listen to be sure it is seated properly.
3. The large black gasket may need to be re-lubed, please ask for help.

Removing Bubbles from the System Lines
1. Check to make sure the sheath line filter is still in working condition.
2. Try purging and pulsing with ALCOHOL in the de-bubbler. Make sure to rinse the nozzle with WATER after the alcohol.

I got a clog and I have BSL-1 Samples—You may need to get staff for help
1. Push STREAM to stop the stream.
2. Remove your sample from the sample port.
3. Remove your collection tubes/plate from the collection area.
4. Remove the nozzle and place it in a tube of water.
5. Sonicate the nozzle.
6. Screw the nozzle back into the nozzle assembly.
7. Place the sink under the nozzle assembly.
8. Put a tube of ALCOHOL the instrument a press the OVERRIDE button. Wait for the alcohol to start to drop from the nozzle.
9. Fill the de-bubbler with ALCOHOL.
10. Place the de-bubbler back under the nozzle with ALCOHOL. Lower the nozzle so that it is sitting in the ALCOHOL press PURGE and then PULSE in the software.
11. Remove the de-bubbler from under the nozzle and dump out any leftover fluid.
12. Place a tube of WATER on the instrument and press OVERRIDE button and wait for the water to drip from the nozzle.
13. Fill the de-bubbler with WATER.
14. Place the de-bubbler back under the nozzle with WATER. Lower the nozzle so that it is sitting in the WATER press PURGE and then PULSE in the software. Do this until you no longer see bubbles coming from the nozzle assembly.
15. Remove the sink from under the nozzle assembly.
16. Push the STREAM button in the software to start the stream.
17. Push the BACKFLUSH button and allow the system to backflush for 10-15 secs.
18. Re-run the 197 beads and the Accudrop beads.
20. Filter your sample again, run your sample and check your gates.
21. Start sorting again.

I got a clog and I have BSL-2 Samples—You may need to get staff for help
1. Do NOT touch your sample or collection tubes
2. Turn the ON the Aerosol Management, and increase the suction to 100%, Wait 5 minutes allowing it to remove any potentially hazardous materials.
3. Follow steps 1-21 of “I got a clog and I have BSL-1 Samples”
Notes on Data

- Check in your data to protect it
- Data is deleted monthly. If it is not checked in it is gone.
- USB and personal back up devices are not allowed on our machines.
- Currently we are recommending FlowJo version 10.2
- You will be prompted for Username: ‘flowjo’ Password: ‘314159’.

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