

FACSMelody Training Guide

Startup

Check fluidics tanks

- Empty the waste tank then pour approx. one cup of bleach.
- Fill Sheath tank:
 - unscrew the black knob;
 - pull ring up to release pressure;
 - fill to top weld line.



Turn on all needed equipment

- Instrument (push the round button on the front of the instrument)
- Computer. Choose the BAdmin account (BDIS#1) (Connection works best if instrument is on before computer)
- Compressor (check the gauge on the sheath tank, it should read ~100PSI)
- Chiller (optional)

Start FACSChorus

- Log in FACSChorus software.
- Click **Run Daily Fluidics Startup**. Click **Continue**.
- Run **Flow Cell Clean twice** (use BD Detergent, then Water). Click **Continue**.

Install the nozzle

- Remove closed loop nozzle (turn black locking lever left); place closed loop nozzle in the holder.
- Insert the nozzle with the **red O-ring facing up** and turn the locking lever right.
- Open the sort block and use a moist paper towel to wipe the deflection plates.
- Close all access doors.
- Click **Continue**.

Cytometer Setup & Tracking (CS&T)

CS&T is optional

**Important: During this step, all doors and access panels must be shut.*

A. Run CS&T routine once a day (staff only) – as for the LSR analyzers.
Click **Continue** or **Skip**.

Accudrop

- *Important: During this step, all doors and access panels must be shut.*
- Load the AccuDrop beads and follow the prompts.

FACSChorus Experiment Setup

- Click **New Experiment** and provide the experiment's information. You can also select and duplicate an existing experiment from the experiment list.
 - Name the Experiment.
 - Select Fluorochromes and add Marker Labels.
- Click **View Data**
 - Load Sample and adjust Flow Rate.
 - Adjust Threshold, PMTs voltages, and gates.
 - Click **+** to add plots.

Update Compensation (optional)

Important:* For each fluorochrome you would like to update, you must select the uncompensated fluorochrome from the **Design Experiment tab (marked with an asterisk)

- Load controls and set voltages in **View Data** tab
- In the **View Data** tab under **Data Sources**, select **"Update Compensation"**
- Select the controls you would like to update and click Continue.
 - If you select the Unstained Control, the unstained control will be applied as the negative population
- Run, record and set gates for each single-color control. Click "finished".
- When compensation is updated, the FCS files for the single-color controls will be found in **Data Sources**.
- The updated compensation matrix can be viewed in the Sort Reports.

Sorting

- Turn AMS on for any samples that require biosafety containment level 2 (human samples, or whenever the aerosol management must be stringent).
- Click **Set Up Sort** tab
- In the Sort window choose:
 - Select a **Device** (2 Tube, 4 Tube etc.)
 - Select **Volume** of the Sort Device.
 - Enter **Target Events** = how many events you wish to collect. **Continuous** = you must stop collection
 - **Add population(s)** to be sorted.
- *Optional:* Connect the chiller tubes to the sorting device.
- **Install collection tubes** in the sorting device.
- Click **Start Sort**.

Shutdown

- 1) **Clean the work area.**
 - Remove all tubes, wipe the biosafety cabinet work area with isopropanol 70%.
- 2) Click **Cytometer** on the navigation bar.
- 3) Click **Daily Shutdown**.
- 4) **Turn off: computer, compressor, instrument, chiller.**
- 5) **Close the sash of the Biosafety cabinet**
 - Make sure AMS is turned off.
 - The blower will stop automatically.

Troubleshooting

The Drop Delay fails

- Verify that the Accudrop beads run at ~1,000 EPS at a Flow Rate of 1. The beads must be run in an experiment to see the event rate. Adjust the concentration of the beads to achieve the target event rate.
- Check that the drops are stable and the stream status shows a green check mark. Backflush or run a flow cell clean with DI water if there is any instability.
- Ensure that the stream is exactly centered in the waste trough. If it is not, adjust the sort block.
- Install the ND 1.5 filter in front of the FSC detector (staff only).

If the stream is unstable, if there is movement in the drops, or if the drops are asymmetrical:

- Run water on the highest flow rate setting
- Perform several backflushes
- Stop/start the stream
- Perform a Flow Cell clean with water
- Run 10% Bleach/FACS Clean at a medium flow rate, followed by water at a high flow rate.
- Sonicate the nozzle