Children's Hospital of Philadelphia
RESEARCH INSTITUTE
Flow Cytometry Core

3615 Civic Center Boulevard
Abramson Research Building, room 1207
Philadelphia, PA 19104
Phone: 215-590-3402
email: flowcytometry@chop.edu

Rev. 20JAN2026

FACSJazz Training Guide

Contents

Startup

Check fluidics tanks

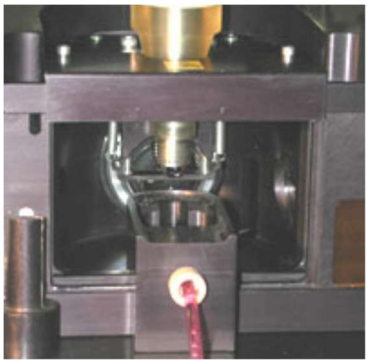
- Empty waste tank and add ~1 cup bleach.
    - Turn on vacuum to pressurize the waste tank.
- Fill Sheath tank up to the weld line.
    - Pressurize sheath tank by switching on the **AIR** switch (right side of the cytometer near the sample station).
    - The gauge on the tank should read ~25.

Turn on all needed equipment

- **Main power** (green switch on the large "cube") to start electronics.
- Computer, log into Windows.
- Chiller (optional)
  (Power button, then center button to run)

Start the Stream

- Start the software modules by double-clicking the BD FACS Sortware icon and click "Connect".
  (It may be necessary to connect twice to get stage centered. The **Sortview** and **Pressure Console** modules start at the same time.)
- Install a sort device in the sorting chamber.
  Sort devices are available for 5 ml tubes, 15 ml tubes, multiwell plates and microscope slides.
- **Restore "Align UR" workspace** (click File/Restore/Workspace/AlignUR; deselect Data Sources; select restore laser delays)
- **Rinse and Backflush**: remove the pipe from under the nozzle and click **Rinse** and **Backflush** in the Pressure Console window. (Remove any tube from the sample tube holder).
    - Tap the blue in-line filter on the left of the biosafety cabinet to release any trapped bubbles.
    - After about 1-2 minutes click **Rinse** in the Pressure Console window to stop both rinsing and back flushing.



- **Purge:**
    - If air bubbles are seen below the green line in the tubing above the nozzle, click **Purge** to allow air to run through the tubing for ~1 minute
    - Click **Purge** again to stop taking air through the tubing
    - Place the pipe below the nozzle and fill with PBS/Isoflow. Click **Purge** and wait for the Isoflow to run through the tubing past the "Y"-shaped connector.
    - If no bubbles appear in the tubing, click **Stream** on the Pressure Console window.

  Important: If any bubbles are still visible in the flow cell or in the tubing, repeat the **Purge** step, as described above, as many times as necessary to remove all air bubbles from the flow cell and from the tubing.

Align the stream and lasers

Rough alignment

- Remove the Flush Bucket and Pipe from under the nozzle.
- Open the access door by unscrewing the screw on the right side.
- Close the deflection plates and install the access door (install screw on the right side).
- Turn on the Plates and Illum buttons to illuminate the stream above the waste drain (AccuDrop laser)
- Adjust using brass knobs to align the stream over the pin holes (top camera of Sortview window)
- Adjust using black knobs to align the bottom of the stream to the waste drain (bottom camera of Sortview window)

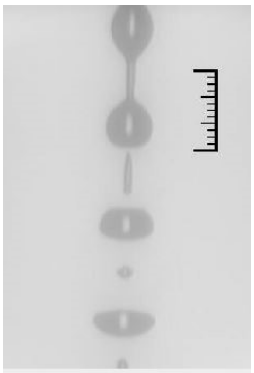
(ensure the light above the waste is at its brightest – this is essential for testing the Drop Delay later)

- If the stream is out of focus in the pinhole view, adjust using the brass knobs
- Close the nozzle access door and turn on lasers by covering the shutter pinhole by the access door
- Press Test Stream in the Sort Settings window to check that the AccuDrop laser is aligned with the side stream
  If needed, turn the front black knob to make the side spot brighter

Fine alignment (use Ultra Rainbow beads) and QC

- In the Recording Settings Window in the Default Display Count box choose 100, for Path create folder for user, and under Recording Rule/ Event Limit choose 5,000.
- Load a tube with UR beads, hold down Boost until events are seen in the dot plots. Adjust the PSI until the event rate is ~100-200 events/second (EPS)
- Check that the laser delays match the posted values (written on the "cube").
- Fine-adjust the position of the stream using brass knobs until the beads are well-focused on all plots. *RCV values for the Blue channels should be below 3% and the Yellow and Red channels below 5%.
- By adjusting voltages, move focused bead clusters between 20 and 40k on the FSC/SSC plot and between 40k and 50k on the fluorescence plots.
- Record 5,000 events.

AccuDrop Setup (drop delay)



- Watch the drops for a minute or two to confirm that they are stable.
- Check that satellite drops fuse with the large drops from above (using the cameral dial).
- Phase charge timing: click Test streams, Flash charge, Short flash (adjust piezo amplitude so that the side stream stays in the same place for each phase).
- In the Sort Layout Window, choose AccuDrop Setup from the Device dropdown menu. Select "Singlets" as the sort population. (Make sure Enrich mode is selected).
- Click the **AccuDrop** button in the Pressure Console window (stream camera view will darken – this is normal).
- Load a tube with AccuDrop beads and acquire events (start the sample, click acquire).
- Boost until events are seen. Adjust the sample pressure until the event rate is ~2,000 EPS.
- A fluorescent spot of AccuDrop beads should be visible in the AccuDrop camera view.
- Click **Start** in the Sort Layout window and then adjust the **Drop Delay** until the bright spot on the left has maximum brightness and the middle spot is invisible or barely visible. (Important: the "Singlets" gate must include all events!)

Aim the side streams

- Install an aiming slide on the sort device.
- Click Sort Ready and click Test Streams (a quick on/off) to deflect the stream.
- Adjust Side Stream values to center the side stream drops on the sort device.
- Note: When in the "Sort Ready" position, the coordinates of the Tray should read "X: 76 Y: 5" in the Tray Control Tab

Experiment Setup

Option I. **Use an existing template to create a new experiment**. (In this case, the cytometer settings, compensation, and the analysis template (plots, histograms, statistics etc.) will be restored from the experiment template.)

- Click File/Restore/Workspace/3 Laser/Choose experiment; deselect Data Sources; deselect restore Fluidics Startup.

Option II. **Create a brand new experiment**

- Open a new Workspace
  - Click File/New Workspace; deselect Data Sources; deselect Restore Fluidics Startup.
  - Turn on the necessary lasers and check the Log box for fluorochromes.
  - Add antigen names for fluorochromes (Optional).
- **Compensation**
  - Create an FSC/SSC dot plot and a dot plot for each fluorochrome using the ACD parameter.
    - Gate the population of interest in the FSC/SSC plot.
    - Apply this parent gate to each single-color control plot.
  - Run and acquire each compensation control and adjust the PMT gains to ensure each fluorochrome is brightest in its own channel. DO NOT RECORD ANYTHING YET!
  - When all PMT gains are optimized, record 5000 events for each compensation control**.**

    (All compensation controls must have the same gains.)
  - Perform the following steps for each compensation control recorded:
    - Click on a compensation control file.
    - Click on the dot plot that matches the fluorochrome of the control.
    - In the Cytometer window, under Source choose the matching fluorochrome file in the dropdown menu.
    - In the Gate Hierarchy convert the parent gate to "Local" by right-clicking on the gate name.
    - Create and name a positive and negative gate for the chosen dot plot, ensuring both gates are "Local".

      (If single-color control does not have a negative population, an unstained sample must be recorded.)
    - Open the Compensation Tab:
      - Click the Manage Parameters Tab and select the single-color controls.
    - Open the Matrix Tab:
      - Choose the negative and positive for each fluorochrome.

        (If an Unstained sample was collected, choose the FSC/SSC gate for the negative.)
    - Click Calculate.
- **Experiment**
  - Add a worksheet.
  - Click on "Cytometer" in the File window.
  - Create plots and choose *DSP parameters (*compensated).
  - Load the first sample and adjust FSC-H and SSC-H if needed.
  - **Add gates**. **Show Population Hierarchy** and **Show Statistics.**

Sorting

- In the Sort window, choose:
  - Select a Device (2 Tube, Plate.)
  - Select Mode = 1.0 drops, pure
  - Enter Target Events = how many events to collect. Continuous = collection must be stopped manually.

    - Add population(s) to be sorted.
    - Click “Sort Ready” to get the Collection Device into the sorting position. (X: 76, Y: 5)
- Click “Sample”, “Acquire”, and “Start Sort”.

Monitoring the Sort

- **The position of the last attached drop must not change for the duration of the whole sort.**
- Adjust the piezo amplitude in order to keep the last attached drop in a fixed position.
- The frequency may be adjusted minimally.
- **If the drops change position or shape**: Stop the sort, reset the drops, and re-determine the drop delay.
- The EPS must stay below 10,000 events/second while sorting.
- When sorting in “pure” mode, the sorting efficiency may be improved by decreasing the EPS rate, diluting the sample, and by filtering the sample. (Sorting efficiency is assumed 100% when sorting in “enrich” mode.)
- **Never allow a tube to run dry** otherwise air will be drawn into the nozzle and will require de-bubbling, realignment, and re-setting the drop delay (…this can take about 30 minutes).
- Do not allow the collection tube to overflow.
- A sort report is automatically saved.

Shutdown

- Load a tube with 10% bleach and run it for 2 - 3 minutes.
- Load a tube with DI water and run it for 2 - 3 minutes.
- **Save** the workspace and pdf files.
- Leave the tube with DI water on the sample holder.
- Turn off “everything”.
    - Select Cytometer > Shutdown Cytometer, then click OK. Wait until blue text appears in camera views! (The cytometer interface is now disconnected from the instrument electronics.)
    - Turn off the main power on the cube (green switch).
    - Exit BD FACS Sortware software.
    - PC
    - Chiller
    - Air (Toggle switch on the right side of the cytometer)
    - Vacuum (Red arm on the line to the waste tank)
    - Blower (biosafety cabinet)
- Install the pipe
    - Place **the flush bucket under the nozzle**.
    - Fill the pipe with DI water and **submerge the nozzle in water**.
- Open the deflection plates.
- Take care of fluidics
    - Empty the waste tank in the sink and rinse it with water. Never leave the waste tank full because it contains bleach which corrodes the steel.
- Clean-up
    - Discard any remaining samples in the biohazard container.
    - Place the UR beads and AccuDrop beads back in the fridge.

Troubleshooting

- Tray not centered upon connecting the software:
    - Close the software and connect a second time.
    - The tray should center with a proper connection.
      (If not, wait a minute or two for before trying again)

- Droplets are unstable:
    - Check the pressure gauges on sheath and waste tanks.
        - Sheath pressure should be ~25 and Waste should be over 5.
    - Run water on 1 PSI and boost aggressively to potentially clear any bubbles or clogs present.
    - Clear air in the system:
        - Stop the stream
        - Return to the Purge step in the Start the Stream section and repeat until all bubbles are clear.

- Tray at incorrect “Sort Ready” position (not at X=76, Y=5):
    - In the Sort window, choose “AccuDrop Setup” in the Device dropdown menu.
    - Select any population to sort.
    - Without running any sample, Acquire and Sort for a few seconds.
    - Return settings back to what they were previously (e.g. 2 Tube, 1.0 Drop Pure)
    - Click “Sort Ready” and the tray should return to the correct coordinates (X=76, Y=5).

- Efficiency not displayed for 1.0 Drop Pure during sort:
    - Refer to the steps above for correcting an incorrect tray position.

- Poor Efficiency when sorting (<70%):
    - Lower the event rate (EPS)
    - Dilute the sample
    - Filter the sample
    - Evaluate gating:
        - Are there any events (cells of interest) off scale?
        - Are there overlapping gates?
        - Is the population extremely rare (<1% all events)?
        - Is a gate bisecting a dense population
    - Check UR Alignment