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

Aurora CS (Cell Sorter) Training Guide

Contents

Aurora CS Startup

Check fluidics tanks

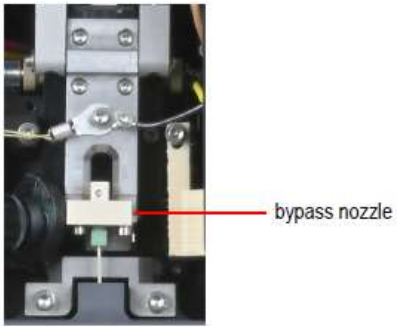
- Empty the Waste tank, add approx. 2 cups of bleach.
- Fill the Sheath tank.

Turn ON all needed equipment:

- Computer (choose the Operator account).
- The **Air valve** (located on the right side of the Biosafety cabinet)
- Instrument (push the button on the left panel)
- The blower of the biosafety cabinet, if necessary.

Start SpectroFlo

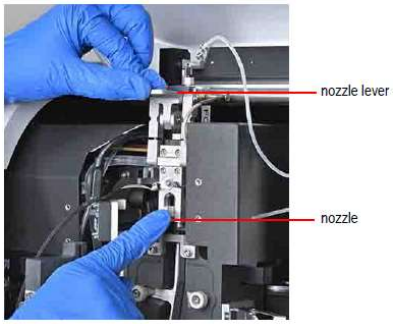
- Log in **SpectroFlo** software and confirm that the cytometer is connected.
  - Once connected, the sorter will **Warm-up** for 30 minutes: QC cannot be run.
- Click **Acquisition** from the **Get Started** page.
- **Make sure the bypass nozzle is installed.** Place the long tube inside one of the two waste buckets.
- Click **Fluidics Startup** icon from menu on the left and ensure all the steps listed on the screen have been completed.
- Click **Start.** (Fluidics Startup takes approximately two minutes.)
- Click **Done** when fluidics startup is complete.



Install the nozzle

- Open the **sort chamber** door.
- Lower the **nozzle lever** and **remove the bypass nozzle**.
- Use a moist paper towel to **wipe the deflection plates**.
- Check that the black **O-ring** in the nozzle is present.
- With the nozzle lever still in the down position, **insert the sorting nozzle** by pushing it all the way until it stops, then **lift the nozzle lever** to secure it in place.

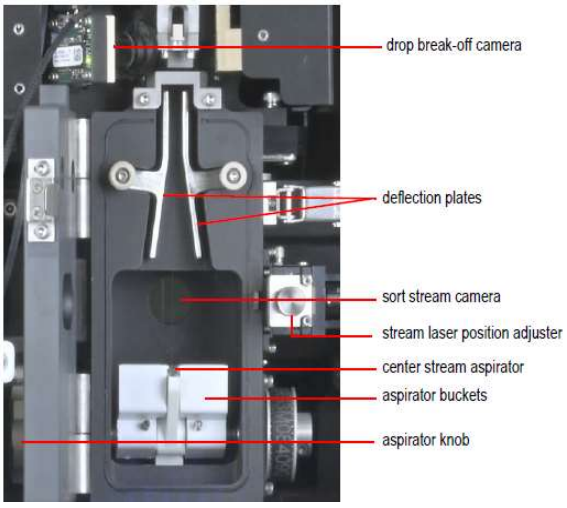
Start the stream – Stream Control Window



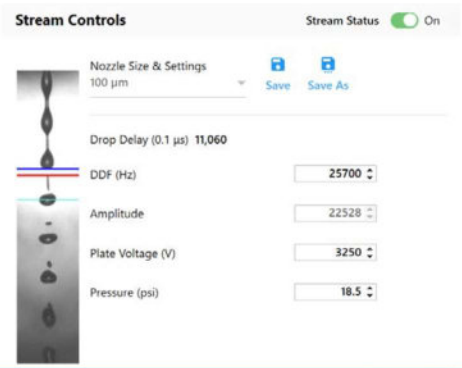
- Create a Default experiment or open a previously saved experiment.
- Open the **Sorter Control** window by clicking **Sorter Control**, at the bottom of the experiment control panel.
- Choose the installed nozzle size under **Nozzle Size & Settings.**
- Change the **Stream Status** to **On**. **Confirm that the stream is aligned with the waste tray.** If it isn't, using the screwdriver, loosen the marked large screws at both sides of the sort block and shift it by hand until the stream is centered. Retighten the screws.
- **Close door to stream access** and **the top panel.**
- Wait for the drops to stabilize (it can take 5 to 10 minutes).

Set the breakoff

- Change the Drop Drive Frequency (**DDF**) so that the last attached drop is as high as possible while still being able to see at least one pair of connected droplets.
  (The range of suggested values is in **Nozzle Settings Table**)
- Then adjust the **Amplitude** until the Drop interval actual values match the recommended value for the nozzle.



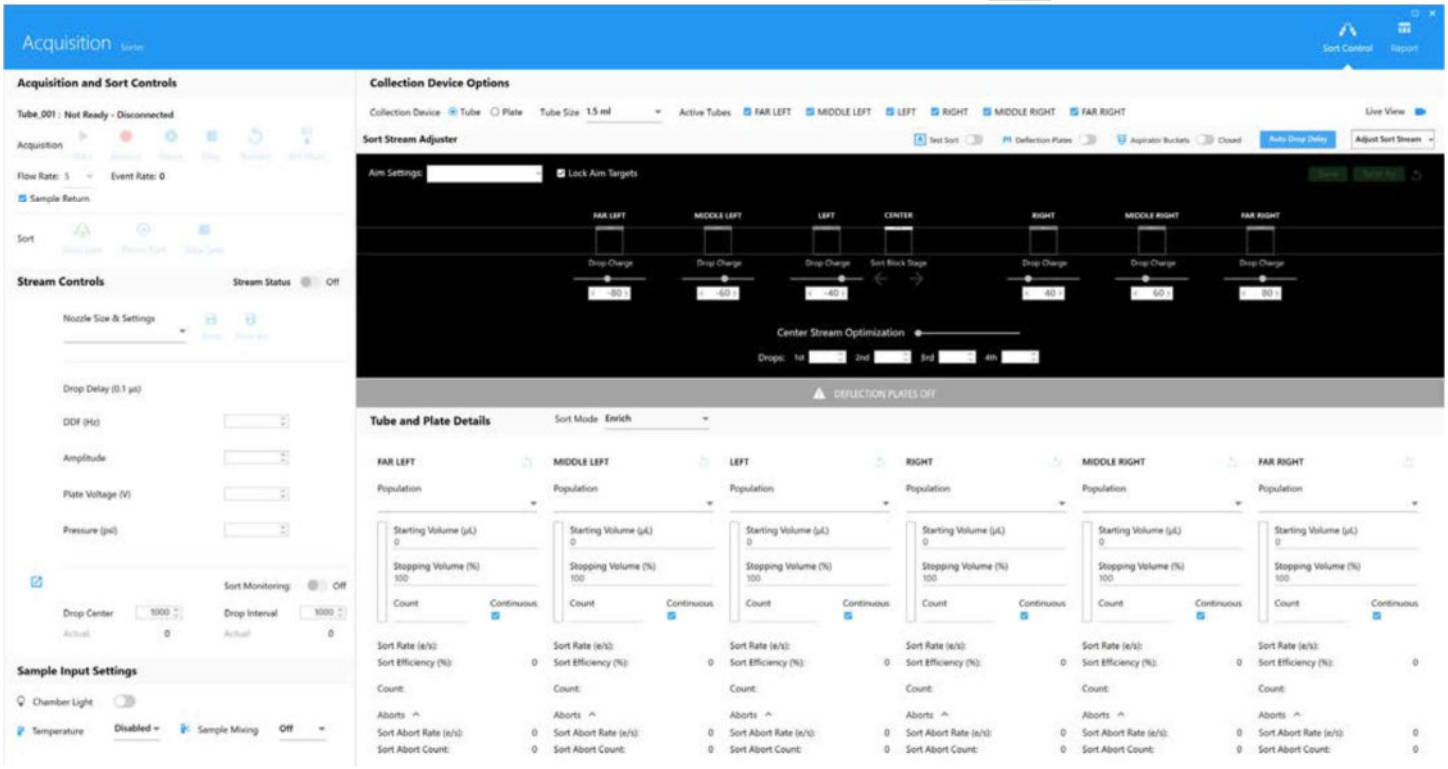
- Check that the actual **Drop Interval** value is stable.
- **Type** the **actual Drop Center** into the target box.
- Turn on the **Sort Monitoring** button. The software will automatically adjust the Amplitude and maintain the stability of the breakoff point.



Run Quality Control (QC) test

QC routine is run once a day by staff, as for the Aurora analyzers.

**Important:* During this step, a sorting nozzle must be installed.



Drop Delay setup

Perform Auto Drop Delay

- In the Sort Stream Adjuster window, click on **Auto Drop Delay.**
- Load a tube of Accudrop beads into the sample loading station. (2 drop beads to 500ul diH20)
- Click Start in the Auto Drop Delay Window.
- Adjust flow rate to get an acquisition of more than 3000 evts/sec.
- Click **OK** when the Auto Drop calculation completes successfully.

Adjust Drop Delay

- To check the drop delay manually, in the **Sort Stream Adjuster** window, click on the triangle in the "**Adjust sort streams**" button and select "**Adjust drop delay**".
- Load a tube with Accudrop beads.
- Click Start and adjust the flow rate to be around 3,000 EPS.
- Adjust the FSC and SSC gains so that the population of beads is on scale.
- Watch the streams and adjust the position of the box to overlap the stream deflected to the right.
- A well-defined spot to the right and no beads in the center indicates that the current drop delay is in the correct range.
- Click again on the triangle and select Adjust Sort Stream to continue with sorting the samples.

SpectroFlo Experiment Setup

- Click **New** in the Acquisition Experiments window.
- Enter an experiment name.
- Select all the fluorescent tags used in the experiment. Click ***Next***.
- Select **+ Reference Group**. (Must have an unstained cell control for autofluorescence subtraction!)
    - Select beads or cells for each control and assign negative if needed
    - Type Labels for each of the Fluorochromes. (optional)
    - Click **Save**
    - Under ***Acquisition*** tab, make sure the Default Raw Worksheet (Raw) is selected for reference controls.
    - Select the stopping gate (P1), events to record, stopping time, and stopping volume. It will stop at whichever one it reaches first.
- Click **Save and Open** to open the new experiment.
- Load the **Unstained Control** in the sample holder
- Click **Start** to run sample. Choose **CytekAssaySettings** in the Instrument Control window. Adjust FSC and SSC gains to get the sample on scale. ** It may be necessary to adjust the gains for the fluorescence detectors if signals are out of scale.*
- Load and Record each control in the corresponding tube.

Unmixing

- Click on the Unmix button once all Reference Controls are recorded.
- The first window that appears allows you to choose controls from the library.
- If you want to subtract autofluorescence check the box on the bottom left of the window.
- Adjust:
    - The **gate on the FSC-SSC** plot;
    - The **positive** and **negative gates** on each Fluorochrome histogram
    - The **location of the pair of gates on the brightest peak** on the spectral plot.
- Click "**Live Unmixing**" in the bottom right corner.
  (DO NOT select "Unmix, Save and Open" – this option is for post-acquisition analysis.)

Adjust the side streams

- Select the sort device (tube or plate) in the **Collection Device Options** section.
- **To aim the streams into tubes:**
    - Uncheck the unused streams.
    - Place sort device with Aiming tubes into the sort chamber.
    - Close the sort door and turn on the **Live View.** (Turn off the chamber light if necessary)
    - Click the **Test Sort** button. This turns on the deflection plates.
    - Open the **Aspirator Buckets** so the streams can be seen entering the Aiming tubes.
    - Use the slider in the **Sort Stream Adjuster** window to adjust the position of each stream so that it is aimed in the center of each tube .
    - Move the boxes in the **Sort Stream Adjuster** window to cover the streams and click **Lock Aim Targets.**
    - **To stop:** click **Test Sort** and **Aspirator Buckets** buttons and close the **Live View** window.
- **To aim the stream into a well of a plate:**
    - Change the Collection Device from Tube to Plate.
    - Select plate template from the Plate Selection dropdown menu.
    - Set Far Right Drop Charge to 100 in the Sort Stream Adjuster window
    - Press the green "Adjust Aiming" button in the sort layout window. Turn on **Enable aiming**.
        - With a plate and lid installed, verify the accuracy of A1 using the "Drop Burst" button
        - To adjust Top-to-Bottom accuracy, use the blue arrows to change the plate position. DO NOT CHANGE THE Y VALUE. It must remain Y=0
        - Press "Save" and "Drop Burst" to test the updated coordinates.
        - To adjust Left-to-Right accuracy, adjust the Drop Charge value in the Sort Stream Adjuster window
        - Turn off **Enable aiming**.

Sorting

- In the Sort window under collection device options:
  - Select a **Collection Device** (Tube or Plate)
  - Select **Tube or Plate size.**
  - Uncheck **inactive Tubes.**
- **When sorting into plates:**
  - Select wells to sort into
  - Press “Add Group” folder icon to choose a population to sort.
  - Choose “Single” sort mode for single cells per well.
  - Select a serpentine Sort Direction for a quicker sort
- **When sorting into tubes:**
  - Select **Sort Mode** = Purity Multiway
  - Choose a **Population** for each tubeChoose Starting/Stopping Volume, Count or check Continuous.
  - **Add population(s)** to be sorted.
- *Optional:* Choose Temperature and Sample mixing speed.
- **Install collection tubes/plate** in the sorting device and place in the DDU chamber.
- Load the sample tube and Click **Start** in Acquisition window
- Click **Start Sort** in the Sort window.

Monitoring the sort

The Sort layout window will display the number of events sorted into each sort location, the sort rate, sort conflict rate and sort efficiency.

Replacing the Collection Tubes

1. To stop a sort while it is running, click the **Pause** Sort button in the Sort window. This will pause sample flow.
2. Remove the collection tube holder by pulling it straight towards you
3. Replace the collection tubes as needed.
4. Reinstall the tube holder.
5. Click the Resume button in the Sort window to resume sample flow and continue sorting.
6. The sort counters resume from the value where they stopped. The threshold counter restarts. However, the value is accumulated and the total count is saved in the final sort report.
7. The **Pause/Resume** function allows you to temporarily pause the sort, and retain the sort counter values. This is useful when you need to make adjustments during a sort.

Aurora CS Shutdown

A. Fast Shutdown

1. Clean the work area
   - Remove all your tubes, wipe the biosafety cabinet work area with isopropanol 70%.
2. Turn off stream
3. Install bypass nozzle
4. Clean flow cell
   - Select clean flow cell under Cytometer in the left side menu. Follow the wizard.
5. Sign out of SpectroFlo Software and shutdown computer
6. Close air valve
7. Turn off instrument
8. Close the sash of the Biosafety cabinet to turn off the blower

B. Complete Shutdown

(performed by staff on a weekly basis, takes about 10 minutes)

1. Under Cytometer icon (left toolbar) choose Fluidics Shutdown and follow wizard
   - Disconnect tubes from sheath tank and connect to filled tank of DI water
   - Fill a FACS tube with 3ml 10% bleach (FACSClean). Click Continue.
   - Fill a FACS tube with 3ml DI water. Click Continue.
   - Fill a FACS tube with 3ml Contrad. Click Continue.
   - Fill a FACS tube with 3ml DI water. Click Continue.
   - Click Done.
2. Sign out of SpectroFlo Software and shutdown computer

Aurora CS Cytek Nozzle Settings Table

Parameter	70 µm	85 µm	100 µm	130 µm	Bypass (90 µm)
Pressure (PSI)	63	34	18.5	8	21
DDF (drops/s / Hz)	70,000–80,000	40,000–60,000	22,000–35,000	16,000–20,000	N/A
Amplitude (max value)	40,000	40,000	40,000	40,000	40,000
Drop center	~170	~210	~260	~260	N/A
Drop interval	10	10	20	30	N/A
Plate voltage (V)	5,000	4,000	3,000	2,000	N/A
Drop compensation (Drop 1,2,3,4)	20, 10, 5, 2	20, 10, 5, 2	20, 10, 5, 2	20, 10, 5, 2	N/A

Aurora CS Emergency Aerosol Evacuation

The Cytek Aurora sorter provides three emergency aerosol evacuation solutions:

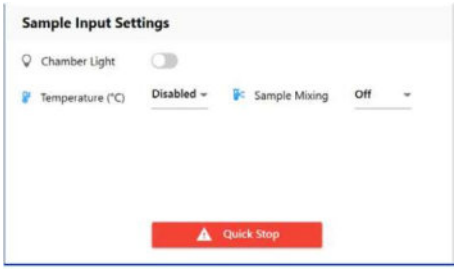
- Automatically through **clog detection** (when Sort Monitoring is enabled)
- When the **software Quick Stop button** in the Sorter Control window is pressed
- When the **Hardware Quick Stop button** on the instrument is pressed

When one of these instances occurs, the following happens:

- Aspirator buckets close
- Deflection plates turn off
- Drop charge turns off
- Software message appears, explaining how to proceed
- Stream is turned off
- Sample pressure cylinder depressurizes and raises
- If the sort was into a plate, plate movement is stopped
- The aerosol evacuation system is activated and the chamber doors lock

Software Quick Stop

- The software provides two ways for activating the aerosol evacuation system—when a clog is detected via Sort Monitoring and manually when you press the Quick Stop button in the Sort Control window. The software Quick Stop is similar to the hardware Quick Stop button. When Sort Monitoring cannot maintain a consistent droplet interval and detects a clog or when you press the Quick Stop software button, the aerosol evacuation system is activated and the software automatically stops all sorting operations.



Hardware Quick Stop Button

- If you encounter a situation that requires an immediate halt of all sorting operations, press the red Quick Stop button located on the right side of the instrument. Once pressed, wait until the aerosol evacuation system has cleared any aerosols and the chamber doors unlock. At this point you can continue operation.

Aurora CS: Standard Procedure for Removing a Clog

Typically, nozzle clogs are detected by the software without user input.

1. **The following events will be automatically executed when a clog is detected**:
    1) Aspirator buckets close
    2) Defection plates turn off
    3) Drop charge turns off
    4) Software message appears, explaining how to proceed
    5) Stream is turned off
    6) Sample pressure cylinder depressurizes and raises
    7) If the sort was into a plate, plate movement is stopped
    8) The aerosol evacuation system is activated and the chamber doors lock.

If the stream does not stop automatically but **the image of the stream is distorted,** indicating that a clog is obstructing the nozzle, **turn off the stream** by clicking the **Stream Status OFF.**

If the software is unresponsive **press the Quick Stop button**
found on the bottom right of the Instrument.

Steps 2 and 3 (below) must be performed by all users:

2. **Wait for the AMS to finish venting.**
   Doors will unlock when completed. Do not use the biosafety cabinet during this time!

3. **Remove the sample and collection tubes**.

Semi-assisted users should contact CHOP Flow staff to remove the clog at this point.

Unassisted users will proceed with the following steps:

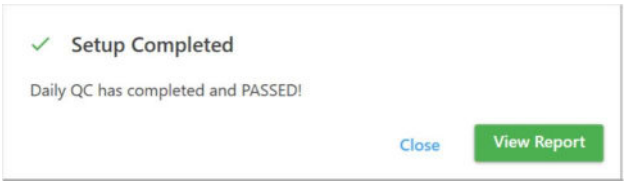
4. **Remove and sonicate the nozzle** (~ 1 minute).
5. **Reinstall the nozzle.**
6. **Clean the sort block.**
7. **Restart the stream** and **continue with the normal sort setup**, including Drop Delay.
8. **Resume sorting.**

All users should refilter the samples to prevent further clogging.

Aurora CS Daily QC

Daily quality control (QC) is part of instrument setup. The daily QC cannot be performed before the sorter is fully warmed up. The status indicators at the bottom of the screen provide the status of the warm-up time.

1. Select **QC & Setup** from the Get Started menu, or click the **QC & Setup tab**.
2. Prepare SpectroFlo QC beads (1 drop of beads in 0.5 mL of sheath solution in a 12 x 75-mm tube).
3. Select the current bead lot from the Bead Lot menu.
   (*For Flow Lab Staff only:* Each vial of SpectroFlo QC beds has a bead lot ID file that has to be imported into the library. Bead lot files can be downloaded from the Resources section at http://www.cytekbio.com.)
4. Ensure the stream is turned on.
5. Load the beads on the instrument and click **Start**.
6. As the instrument begins acquiring the QC beads, they appear in the scatter plot. The laser delays are initially set to 0, then optimized thereafter. The performance measurements are established and compared to the pass/fail criteria (see "Pass/Fail Criteria" on page 43). The procedure takes approximately 3 to 5 minutes to complete. Once acquisition is complete, two SIT Flushes are automatically performed to clear the beads from the sample line. A message is displayed when Daily QC passes.



7. To view the QC report, click View Report. If QC fails, remove the tube and follow the guidelines that appear. The recommended solution will vary depending on the reason for the failed test.

QC Report

At the completion of Daily QC, a QC report is generated. The report includes the following sections:

The header section contains the Pass/Fail status of the run, name of the instrument, instrument configuration, date the Daily QC was run, user who ran the Daily QC, instrument serial number, nozzle size, sheath pressure, and SpectroFlo QC bead lot and expiration date.

The results section contains the gain, gain change, median fluorescent intensity of the QC bead, %rCV, and a pass/fail indicator for each detector channel. The center wavelength of the detector is shown in parentheses next to the detector name.

The Laser Settings section contains the laser delays for all non-primary lasers, and area scaling factors for all lasers and the FSC detector.

Pass/Fail Criteria

The pass/fail criteria are the following:

- %rCV must not exceed 6% for the FSC channel
- %rCV must not exceed 8% for the SSC-B channel
- %rCV must not exceed 6% for the third channel of each laser (V3, B3, R3, YG3, and UV3)
- % delta gain change for all channels must not exceed 100% from the last Daily QC run performed by Cytek Service personnel.

QC reports are automatically exported as CSV files to the Setup folder (C:\CytekbioExport\Setup).

Aurora CS Sample Flow Rates

Ten preset settings measured by the flow meter, correspond to the following approximate flow rates:
1 – 10 µL/min
2 – 17 µL/min
3 – 24 µL/min
4 – 31 µL/min
5 – 38 µL/min
6 – 45 µL/min
7 – 52 µL/min
8 – 59 µL/min
9 – 66 µL/min
10 – 73 µL/min

Maximum data acquisition rate: 25,000 events/s