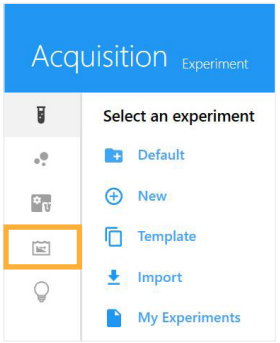
QUICK REFERENCE GUIDE

Cytek Aurora™ CS System

This guide provides basic instructions on startup, shutdown, daily QC, experiment setup, and sort setup using the SpectroFlo® CS software. For detailed information, refer to the Cytek Aurora CS User's Guide. Use this Quick Reference Guide only after you have become familiar with the procedures outlined in the User's Guide.

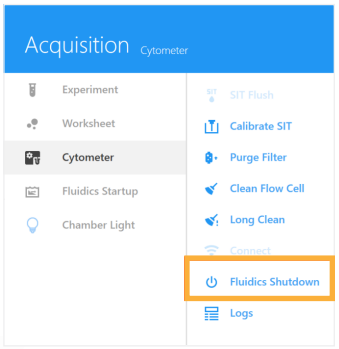
STARTUP

1. Ensure the sheath tank is full and the waste tank is empty.
2. Turn on the air supply. Ensure the air pressure is 80–85 PSI.
3. Turn on the Cytek Aurora CS system and workstation.
4. Launch the SpectroFlo CS software and log in.
5. Select **Acquisition** from the **Get Started** menu and ensure that the cytometer connects.
6. Select **Fluidics Startup** from the **Acquisition** module and follow the steps. Ensure bypass nozzle is inserted.
7. Click **Done** when the **Fluidics Startup** is complete.



SHUTDOWN

1. Select **Cytometer** from the **Acquisition** module and select **Fluidics Shutdown**.
2. Replace the nozzle with the bypass nozzle and follow the instructions.
3. Turn off the air supply to the sheath tank and click **Finish**.
4. Turn off the cytometer.
5. Close the SpectroFlo CS software and turn off the workstation.
6. Vent the sheath tank by pulling up on the bleed valve to release the pressure.
7. Fill the sheath tank and empty the waste tank.

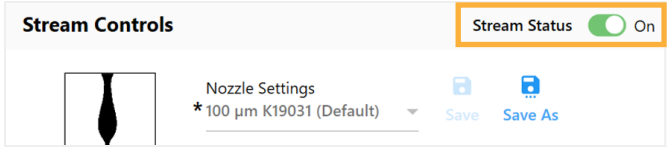


STREAM/BREAK-OFF OPTIMIZATION AND DAILY QC

1. Open an **Experiment**.
2. Click on the **Nozzle Settings** dropdown menu and select the settings to match the inserted nozzle size.
   NOTE: **Pressure, Drop Drive Frequency (DDF), Amplitude,** and **Plate Voltage** fields are populated once **Nozzle Settings** are selected.

Nozzle Size µm					
	70	85	100	130	Bypass
Pressure (PSI)	63	34	18.5	8	21
DDF (1,000 Drops/Sec)	70-80	40-60	22-35	16-20	NA
Amplitude	1-40,000	1-40,000	1-40,000	1-40,000	NA
Plate Voltage (V)	6,000	4,000	3,000	2,000	NA

   NOTE: The values above are provided as a reference; actual values may vary by instrument.
3. Install the selected nozzle and ensure that it is locked in place.
4. Click the **Stream Status** toggle to turn on stream.



5. Ensure the stream is centered between the deflection plates and the center stream aspirator. If adjustment is necessary, loosen the two set screws that hold the deflection chamber in place. Rotate the deflection chamber until the stream is centered between the deflection plates and directed into the center stream aspirator.

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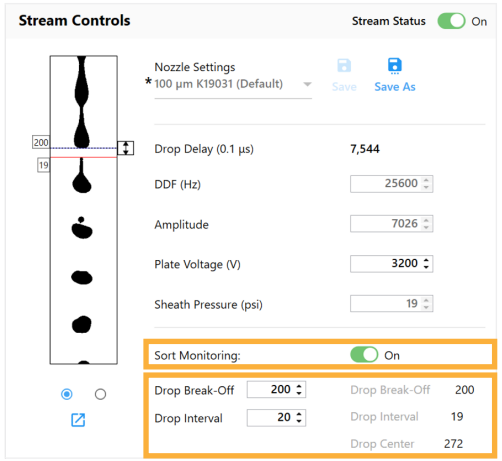
6. Adjust the **Amplitude** and, if needed, the **DDF** to achieve a stable break-off profile.
7. Enter the **Actual** values into the **Drop Break-Off** and **Drop Interval** boxes and turn on **Sort Monitoring**.
8. Run **Daily QC** in the **QC & Setup** module once the instrument warm up is complete.



   8a. Prepare SpectroFlo QC beads (1 drop of beads in 300 µL of sheath solution) and load the tube onto the sample loading station.

   8b. Select the current bead lot number from the **Bead Lot** menu and click **Start**.

   8c. Click **View Report** to see the **Daily QC** report. If QC fails, follow the guidelines in the **Daily QC Failed** dialog that appears.

EXPERIMENT SETUP

1. Within the **Acquisition Module**, open an existing experiment using the **My Experiments** option, or create a new experiment by selecting **New**.
2. Acquire reference controls and unmix if needed.
3. Open a **Worksheet Template** or **Create a Worksheet** with the appropriate plots/gates/stats to identify the populations to be sorted.
   NOTE: Confirm there are no conflicts in the gating strategy.
4. **Record** a **Pre-Sort Sample**, ensuring that enough events are collected to identify the populations to be sorted.

SORTING SETUP FOR TUBES

1. In the **Sorter Control** window, under **Collection Device Options**, select the **Collection Device**, **Tube Size**, and select the **Active Tubes**. Six-way sorting can be done with 1.5 mL tubes and 5 mL tubes.
2. Turn on **Test Sort**.
   WARNING: The deflection plates can be on, even when the sort door is open. A red LED illuminates and a red banner will display at the bottom of the **Sort Stream Adjuster** window to indicate that the deflection plates are on.
3. Use the **Center Stream Optimization** slider to achieve the tightest possible center stream profile. Use **Drops 1, 2, 3**, and **4** controls to fine-tune.
   NOTE: Each **Drop** number should be approximately half of the previous.
4. Place aiming tubes into the appropriate collection device and install it into the Droplet Deposition Unit (DDU) chamber.
5. Use existing **Aim Settings**:

   5a. Select the **Aim Settings** from the dropdown menu.
   NOTE: Ensure the correct **Collection Device** and **Tube Size** are selected.

   5b. Use **Drop Charge** adjusters to place the sort streams into the targets.

   5c. Turn on **Live View** to view the tubes.

   5d. Open the **Aspirator Buckets** to view the sort streams.

   5e. Ensure the sort streams are correctly aimed into the center of the tubes and use the **Drop Charge** adjusters to fine tune as needed.

OR

6. Create new **Aim Settings**:

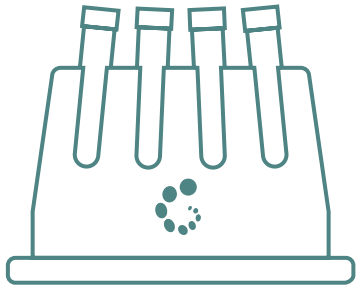
   6a. Follow steps 5c through 5e.

   6b. Adjust the aim targets to center around the appropriate side stream and click **Lock Aim Targets**.

   6c. Click **Save As** to name and save new settings.

   6d. Close the **Aspirator Buckets**.

   6e. Turn off **Test Sort**.



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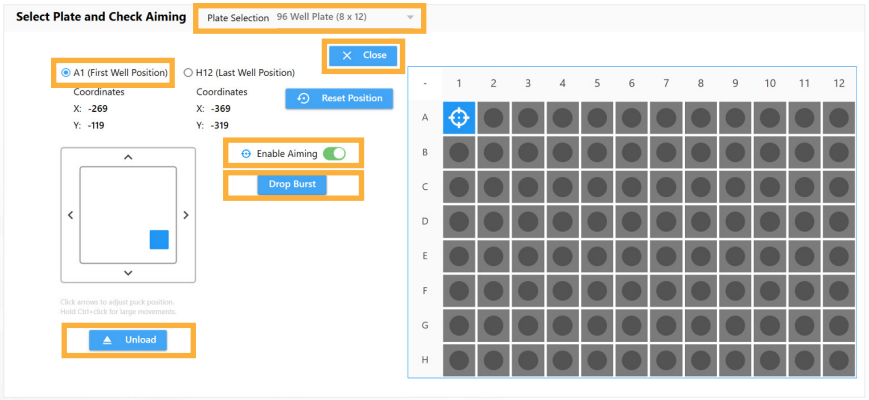
SORTING SETUP FOR PLATES

1. Select the **Plate** radio button from the **Collection Device Configuration**.
2. Select **Adjust Aiming** from the **Select Plate and Check Aiming** box.
3. From **Plate Selection**, choose the plate type: 96-well plate or 384-well plate.
4. Confirm that the **A1 (First Well Position)** radio button is selected.
5. Load a plate onto the puck and select **Load** to position the well plate for aiming into the A1 well.
6. Select **Enable Aiming**.
7. Select **Drop Burst**.
8. Confirm that the drop burst is centered. If it is not centered, adjust the drop charge to move the drop left or right. Adjust using the arrows to move the position of the puck up or down.
9. Select the **H12 (Last Well Position)** radio button to aim into the H12 well.
10. Select **Drop Burst**. Select multiple times if a droplet is not observed.
11. Confirm the drop burst is centered. If not, adjust the position of the puck using the arrows. Do not adjust the drop charge.
12. Select **Unload** and **Close** to exit.



AUTO DROP DELAY

1. Click **Auto Drop Delay**.
2. Prepare SpectroSort® beads and load them onto the sample loading station. To prepare the beads, vortex and add 1 drop of beads in 400 µL of Muse® System Check Diluent or Guava® Check Diluent.
3. Click **Start**.
4. A message is displayed that **Auto Drop Delay** was successfully calculated. Click **OK**, close the window, and the new drop delay value will be updated.
5. Close the **Auto Drop Delay** window.

SORTING

1. Choose a **Sort Mode** in **Tube and Plate Details**.
2. Select the sort gates for each collection tube from the **Population** dropdown menu.
   NOTE: The gates listed in the **Population** menus are the gates from the assigned worksheet.
3. Under **Starting Volume**, enter the amount of buffer in µL added to the tube or well you are sorting into. Under **Stopping Volume or Count**, add the number of cells or stopping volume you want to sort into each tube/well. Click the **Continuous** box if no stopping criteria specified.
   NOTE: Checking **Continuous** indicates that the sort will continue until the tubes (or wells) are full, which is determined from the **Starting Volume** and **Stopping Volume**. Alternatively, you can click **Stop Sort**.
4. Load the sort collection device with the collection tubes.
5. Load the sample tube onto the sample loading station. Click **Start** to begin acquisition.
6. Verify that all gates are placed correctly. Click **Start Sort** to begin sorting.
7. Once the sorting criteria are met, the sort will stop for that population.
   NOTE: To replace a full collection tube with a new one, select **Pause Sort** and click on the rewind icon next to the tube. Follow the instructions in the window, place a new tube with buffer into the appropriate location, and resume sorting.
8. Click **Report** to view the sort report once the sort is completed.

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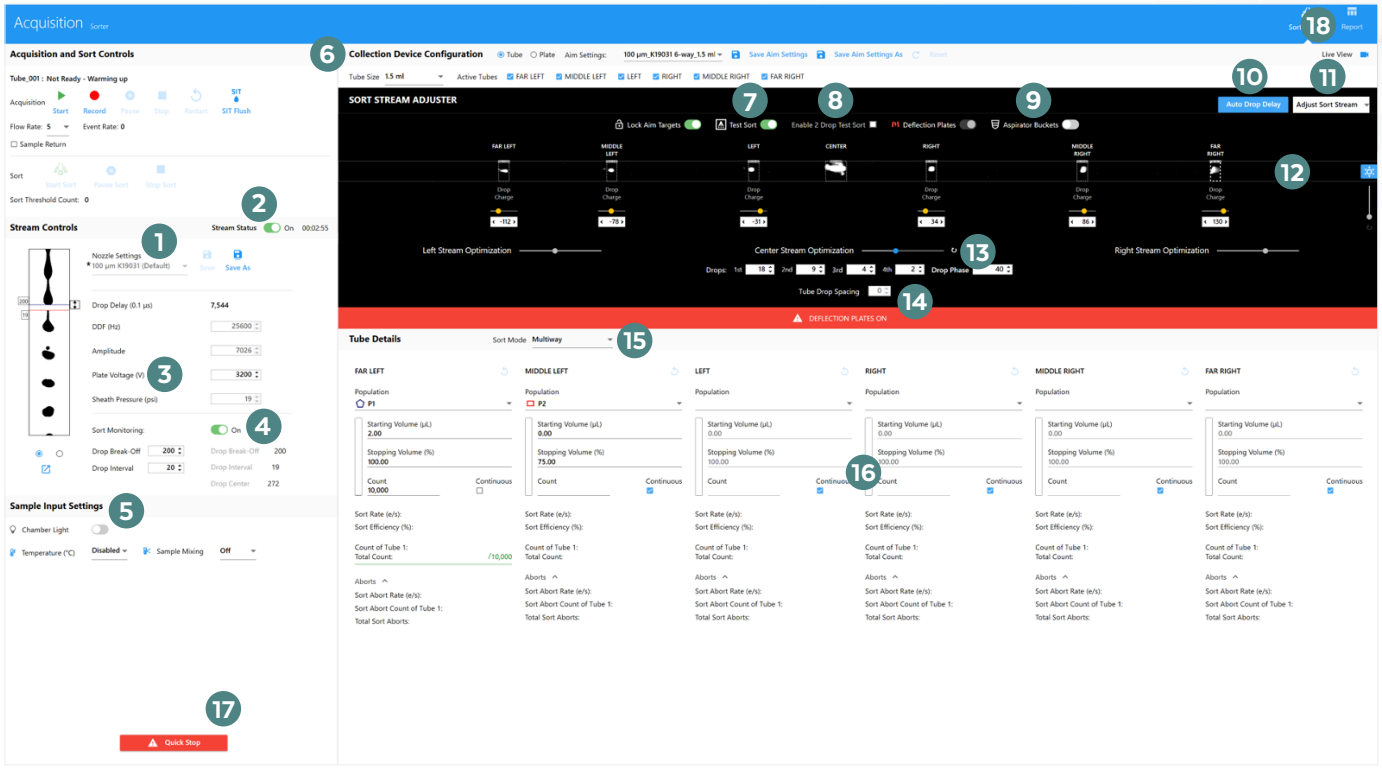
1. **Nozzle Settings:** Select settings that match the inserted nozzle size.
2. **Stream Status:** Toggle to turn on or off the stream.
3. **DDF, Amplitude, Plate Voltage, and Pressure:** These values are populated when nozzle settings are selected and can be adjusted as needed.
4. **Sort Monitoring:** Toggle to turn on. Sort monitoring will maintain a consistent drop interval once a stable break-off is established.
5. **Sample Input Settings:** Control sample chamber temperature and sample mixing.
6. **Collection Device Options:** Choose a collection device and tube size. Click to select the desired active tubes to sort into.
7. **Test Sort:** Toggle to turn on or off the side streams.
8. **Enable 2-Drop Test Sort:** Activate two drop test sort to adjust splitting side streams.
9. **Aspirator Buckets:** Toggle to open the aspirator buckets and verify trajectory of side streams.



10. **Auto Drop Delay:** Select to open the auto drop delay window. Follow the steps to automatically calculate the drop delay with SpectroSort beads.
11. **Live View:** Select to view a real-time image of the side streams as they enter the collection tubes.
12. **Brightness Adjuster:** Move the slider bar to adjust the brightness of the side stream camera to optimally visualize the side streams.
13. **Center Stream Optimization:** Use drops 1, 2, 3, and 4 controls to fine tune the center stream.
14. **Tube Drop Spacing:** Adjust this value to increase the number of non-charged targets between consecutively charged droplets from 0 to 8. A fixed value of 60 will be set for plates.
15. **Sort Mode:** Use dropdown menu to select sort mode.
16. **Tube and Plate Details:** Select populations to be sorted, set starting volume of buffer in the collection tubes and set sort stopping criteria.
17. **Quick Stop:** Click to activate the aerosol evacuation system in case of a clog or other stream perturbance that causes instability.
18. **Report:** Click report to view information from the completed sort.

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