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

FACSAria Training Guide

This guide applies to the FACSAria Fusion and FACSAria II

Contents

FACSAria Startup

Training: Please note that all FACSAria training sessions must be scheduled on FACSAria II.

Check fluidics tanks

- Empty the waste tank then add approx. one cup of bleach.
- Fill Sheath tank (pull ring up to release pressure; fill to top weld line)

Turn on all needed equipment

There are differences between powering up FACSAria Fusion vs. FACSAria II.

- **Computer** (for Windows login, choose the Operator account)
- **Instrument** (push the green, square button)
    - *FACSAria Fusion:* button on right side
    - *FACSAria II*: button on left side
- Biosafety cabinet
    - *FACSAria Fusion*: Turn on biosafety cabinet using the button above the sash
    - *FACSAria II*: Turn on Biobubble using switch on power strip located next to chiller, close the large front flap but leave a smaller flap open for ventilation
- **Compressor**
    - *FACSAria Fusion:* Turn Air Valve to "70um" for the 70um nozzle, or ">70um" for any nozzle larger than 70um For 70um nozzle, you may need press "On" on the switch on the wall.
    - *FACSAria II:* The compressor encased in the fluidics cart starts automatically when the cytometer is powered on.
- **Chiller** (optional) (press power button, then center arrow button)

Start FACSDiva

- Log in to FACSDiva software.
- Click **Cytometer** Tab → **View Configuration** → **Select the correct configuration** for the installed nozzle.
- Click **Set Configuration** in left hand corner, then click **OK** in the popup filter check window.
- Click **OK** in right corner. Close the CST window. Choose **Use CST Settings** in popup window, if displayed.

Install the nozzle

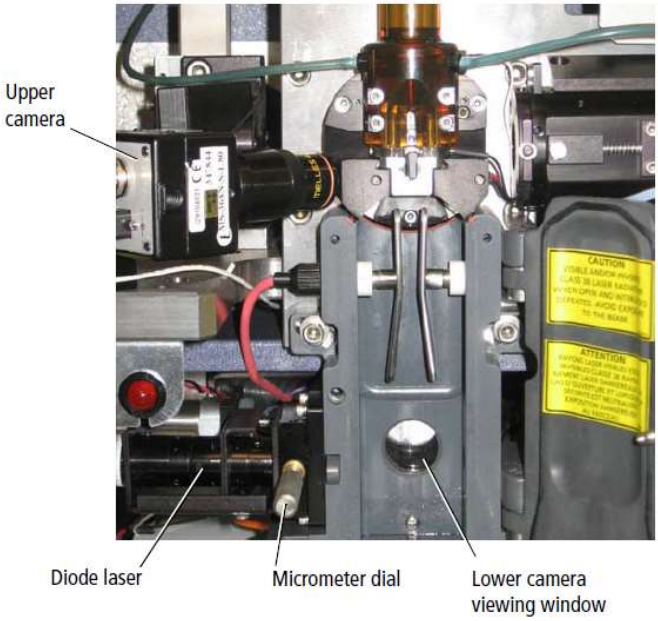
- **Load a tube with approx. 3ml Coulter Clenz detergent onto loading port**
- With the closed loop nozzle installed Click **Cytometer → Cleaning Modes → Clean Flow Cell→OK**
- Click **OK** when done.
- Remove closed loop nozzle (turn black locking lever left); place closed loop nozzle in the holder.
- Insert 70um or 100um nozzle with the **red O-ring facing up and in** and turn the locking lever right.
- Open the sort block and use a moist paper towel to wipe the deflection plates.

Start the stream

- **Click on the red X** in the Drops window to start the stream (be patient, it takes ~ 30 sec).
- **Check that the stream is aligned with the waste tray**
    - If it isn't aligned, adjust the sort block by loosening the screws on both sides of it, pushing the block into the correct position, and tightening the screws.
- **Close the sort block and secure it with the screw on the door.**

- If the stream is not centered between the center hash marks in the side stream window, you can adjust the camera behind the diode laser, to the left of the sort block, to center the stream (see picture below)

Figure 1-22 Stream-viewing optics



- **Close the upper door to engage the lasers.**
- Wait for the drops to stabilize (it can take ~ 5 minutes).

Cytometer Setup & Tracking (CS&T)

CS&T is optional.

Important:* **During this step, the top door must be shut to engage the lasers.

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

B. Quick check: Open the "CST" Experiment and run CS&T beads. MFIs values for the bright population should be approx. 10,000 on all plots.

Set the breakoff

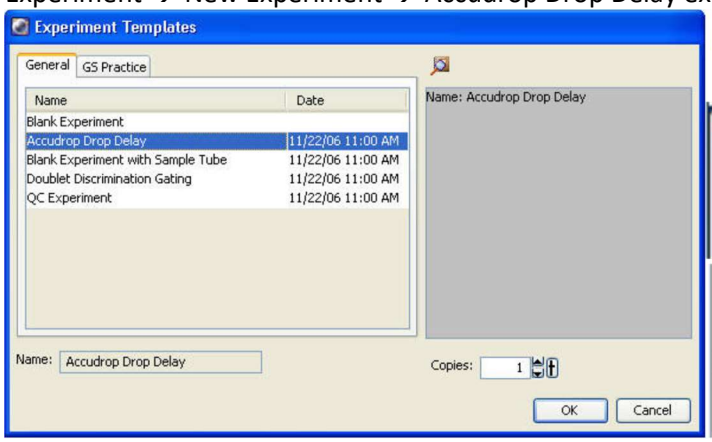
1) **Set the Freq. And Amp. to optimal value for the nozzle** (See table on page 7) in the Breakoff window
2) **Adjust the Freq and Ampl. until the following conditions are met:**
    a) Drop 1 actual value is between 125 and 250.
    b) The satellite drops should merge with the large drop ahead within 4 or 5 drops.
    c) Actual Gap value should be 10 ± 2 for 100um nozzle and 6 ±2 for the 70um nozzle
    d) The Gap values should be relatively stable when you stop changing the amplitude.
    e) Adjust frequency to set the Drop 1 position and adjust amplitude to set Gap value
3) **Type the actual Drop 1 and Gap values into their respective target boxes.**
4) **Click the "Sweet Spot" button to automatically adjust the Ampl. and maintain the stability of the breakoff point.** Write down the value of Ampl. If Ampl. changes less than ± 2V in the next 5 min, then the stream is sufficiently stable.

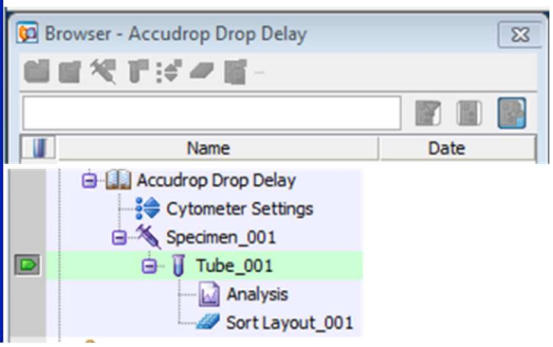
*"**Drop 1**" is the distance in pixels from the top of the image to the thin line that crosses the first detached drop.*
*"**Gap**" is the width in pixels of the thick line that indicates the separation between the stream and the first satellite drop.*

Accudrop setup

- **Important:* **During this step, the top door must be shut in order to engage the lasers.**
- **Side streams adjustment**
    - Click **Voltage** button in side stream window to charge the deflection plates.
    - Click the **Test sort** button to generate side streams, while the aspirator drawer is in WASTE position.
    - The streams should be **stable** and **focused.** If the center stream is not focused adjust the values for Drop 2, 3 and 4.
    - If streams are dim or not visible, adjust the Accudrop **micrometer dial** until visible and bright
    - **Click Voltage to Stop Test sort** when finished.

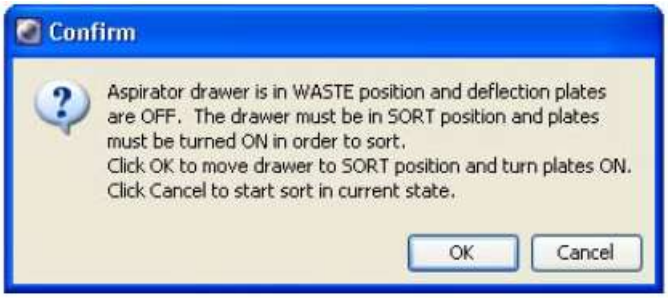
- **Accudrop Drop Delay experiment setup**
  - Experiment → New Experiment → Accudrop Drop Delay experiment → OK





  - Expand **Specimen_001** by clicking the "+"
  - Set the current tube pointer to **Tube_001**.
  - Expand **Tube_001** by clicking the "+"
  - **Double-click** on the **Sort Layout** to open it. (The sort layout window will pop up.)
    - **Device** should be "**2 Tube**"
    - **Precision** should be "**Fine Tune**"
    - **Target Events** should be set to "**Continuous**"
    - **The P1 gate** should be set on **Left stream**

- **Run Accudrop calibration wizard**
  - Load a tube of Accudrop beads onto loading port
  - Click **Load** in Acquisition Window to acquire events. **Set Flow Rate to ~1,000-1,500 evts/second**.
  - Click **Sort** in the Sort Layout Window to start sorting Accudrop beads.
  - Click **Cancel** in the popup window to keep the aspirator drawer in WASTE position (we do not need to collect the Accudrop beads).



  - Turn on **Voltage** and click the **Optical Filter** button in the Side Stream window to block the laser light reflected by the stream and let through only the fluorescent light from the Accudrop beads.
    - Make sure sorted stream is in the left box
  - Click **Auto Delay** → **Start Run**.
  - Click **Exit** after the Drop Delay is complete.

Align the Side Stream(s) to the hash marks in the Streams window

- *FACSAria Fusion:* Select Sort Device from drop down menu in the Side Stream window.

- *FACSAria II:* Click on the **Icon Sort Device** which is found in the Windows task bar in the bottom right corner to select the Sort Device.
- Click Voltage, then click on **Test Sort**.
- **Adjust the deflection of the streams** in the Side Stream window using the 4 respective sliders to place them between the hash marks.

FACSDiva 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.

Option II. **Create a brand new experiment** (click on the *New Experiment* icon in the *Browser* window).
Rename the experiment (right-click on the experiment name → Rename)

- Click on **Cytometer Settings → Parameters** tab and **Delete the parameters that are not needed**.
- **Set up Compensation Controls:**
  - In top menu bar, click **Experiment → Compensation Setup → OK.**
  - A **new specimen named "Compensation Controls"** will be created in the Browser window and the software will automatically switch to "**Normal Sheets**" mode. The "Compensation" specimen contains tubes for all compensation controls and one tube for unstained controls.
  - **Select and Activate the Unstained tube** to show the worksheet with histograms (one for each fluorescent parameter).
  - Load your compensation controls and run them one by one. **DO NOT RECORD ANYTHING YET!**
  - While acquiring, watch the histograms and adjust the voltages of the PMTs as necessary. There should be no events out of scale. Some researchers prefer to adjust the gains to avoid large spillover values.
- **Record compensation controls one by one.** *Do not change PMT voltages!*
- Click **Experiment → Compensation Setup → Calculate compensation.**
  (Typically, a message confirming that the compensation was calculated successfully is displayed.)
- **Click "Link and Save".** Now you are ready to record samples.

- Toggle back to **Global Sheets** by clicking the first icon in the top-left of the worksheet window.
- Create plots in Global Worksheet 1.
- Load your first sample and adjust FSC and SSC if needed.
- **Add gates**. **Show Population Hierarchy** and **Show Statistics** by right clicking on a plot.

Sorting

- *FACSAria Fusion*: Turn AMS on Low for any samples that require biosafety containment level 2 (human samples, or whenever the aerosol management must be stringent)
- Click on Sort in the top toolbar and choose **New Sort Layout**.
- In the Sort window choose:
  - Select a **Device** (2 Tube, 4 Tube etc.)
  - Select **Precision** = 4-way Purity
  - 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 **Acquire** in Acquisition window
- Click **Start Sort** in the Sort Layout. Click **OK.**

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.

- **NOTE.** When the Sweet Spot is on, sorting pauses automatically if the Gap value is out of range. This ensures that sorting occurs only under the proper breakoff conditions. However, the Sweet Spot does not monitor the Drop 1 value. If the Drop 1 value shifts by more than ±5 you should stop the sort and re-calculate Drop Delay.
- If a more severe problem such as a clog is detected, the stream shuts off and sorting stops. The deflection plates shut off, the aspirator drawer closes, and the sample tube is unloaded.

Replacing the Collection Tubes

1. To stop a sort while it is running, click the **Sort** button in the Sort Layout window.
2. **Click Stop Acquiring** in the Acquisition Dashboard to stop the sample flow.
3. **Turn off the deflection plates** by clicking the **Voltage** button in the **Side Stream window**.
4. **Remove the lower section of collection tube holder** by lifting up on the handle and pulling the lower section of the holder down and forward.
5. **Replace the collection tubes** as needed.
6. **Reinstall the tube holder** and pull down on the handle to secure it in place.
7. **Click Acquire Data** in the Acquisition Dashboard to restart the sample flow.
8. **Click the Resume button** in the Sort Layout window to continue sorting.
9. **Click OK** when you are prompted to open the aspirator drawer or turn on the deflection plates. 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.
10. 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. Be aware that **the sample continues to flow during a pause,** only the sorting is paused.

FACSAria Shutdown

1) Clean the work area.

- Remove all your tubes, wipe the work area with isopropanol 70%.

2) Clean the Flow Cell

- In the Droplets window click the green checkmark button to **Stop the stream** (wait about 15 seconds until the stream stops).
- **Remove the nozzle** from flow cell.
- **Install closed loop nozzle** into Flow Cell
- **Load a tube with approx. 3 ml Coulter Clenz detergent** onto loading port
- Click **Cytometer → Cleaning Modes → Clean Flow Cell→OK**
- Click **OK** when done.
- Exit FACSDiva (the sheath tank will depressurize immediately with a hissing sound)

3) Turn off: computer, instrument, chiller

4) *FACSAria Fusion:*

a. **Turn air valve to "OFF" position**

b. **Close the sash of the Biosafety cabinet**

- Make sure AMS is turned off.
- The blower will stop automatically.

FACSAria II: Turn off BioBubble

Troubleshooting

1. *Problem:* **Stream is unstable, there is movement in the drops, or the drops are asymmetrical**

   Most likely cause: air bubbles in the lines or in the flow cells
   Troubleshooting steps:
   1. Run water on the highest flow rate setting for a couple of minutes
   2. Perform several backflushes
   3. Stop/start the stream
   4. Perform a Flow Cell clean with DI water
   5. Run 10% Bleach/FACS Clean at a medium flow rate, followed by water at a high flow rate.
   6. Sonicate the nozzle

2. *Problem:* **Only the far-left stream appears when switching from plates to tubes**.

   Most likely cause: sorter stuck in plate mode (ACDU mode)
   Troubleshooting steps:
   1. Install the **ACDU adaptor** (if not already installed),
   2. In the **Sort menu**, click **Home Device**, choose **96 well plate - Falcon**, then click **Home**.
   3. Go to in the **Sort layout** window and click the **Eject** button to send device to back of instrument.
   4. R**emove the ACDU adaptor**.
   5. Test the streams again: all streams (two or four, depending on selection) should be visible on the stream view camera.

Removing a Clog

1. **Typically, nozzle clogs are detected by the software without user input.**
   The following events will be automatically executed when a clog is detected:
   - The stream and droplet generation will stop.
   - The waste drawer will cover the collection tube(s).
   - The high voltage on the deflection plates will turn off.
   - The sample will unload.

   A window stating that a clog has been detected will appear on the screen. Click OK.

   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 green check mark in the stream window and **turn the plate voltage off.**

   If the software is unresponsive **press the Emergency Stop button** found next to the laser switches.

   Steps 2 – 4 must be performed by all users:

2. *FACSAria Fusion*: **Turn the AMS on HIGH and wait for at least 2 minutes**. *Do not use the biosafety cabinet during this time!*
3. **Remove the sample and collection tubes**.
4. **Disengage the Emergency Stop button (if engaged)**.

The biosafety cabinet is equipped with an Aerosol Management System (AMS) which effectively removes the aerosols from the sorting block.

The AMS should be set on LOW while running samples under normal conditions.

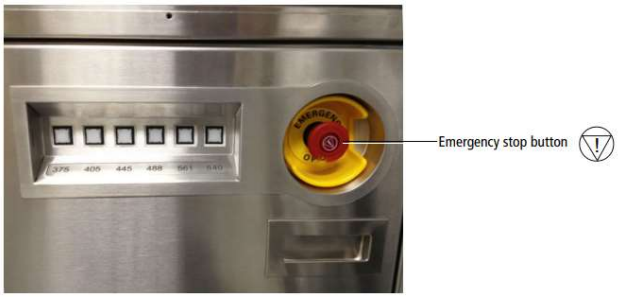
Semi-assisted users should stop at this point and contact CHOP Flow staff to remove the clog.
Unassisted users will proceed with the following steps:

5. **Remove and sonicate the nozzle** (~ 20 seconds).
6. **Reinstall the nozzle.**
7. **Clean and dry the deflection plates and sort block.**
8. *FACSAria Fusion*: **Return the AMS to Low.**
9. **Restart the stream** and **continue with the normal sort setup**, including Accudrop.
10. **Resume sorting.**

All users should filter the samples immediately prior to loading them on the sorter to prevent further clogging.

IMPORTANT SAFETY NOTES

- Cell sorters that use droplet generation methods, such as the BD FACSAria II and Fusion, can produce aerosols around the sample stream. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door closed during sorting. If you need to access the sort block and you are working with highly infectious samples, turn off the stream before opening the sort block door.
- The sample port and the ACDU (Automatic Cell Deposition Unit) are mechanical devices that can cause injury if not used properly. Fingers, hands and any other objects (except for the sample tube/plate) must be kept away from the sample port and the ACDU during sample loading and unloading. Never place any object underneath the loading port! Never initiate loading and unloading a sample without having the door of the sample port closed!
- **The emergency stop button** is a safety feature that can be used to stop the movement of the loading port and ACDU stage. The emergency stop button is located:
    - *FACSAria Fusion*: on the front of the optics drawer
    - *FACSAria II*: on the front of the sorter, on the right side.



- The button lights up when a tube is loading to remind you to keep your hands away from the loading port.

The following occur when this button is pushed:

- The tube is unloaded from the sample injection chamber.
- The ACDU stage (if in use) stops moving.
- The stream is turned off.
- The deflection plate voltage is turned off.
- The aspirator drawer (if open) closes to protect the sort collection tubes.
- A warning message is displayed on the screen.

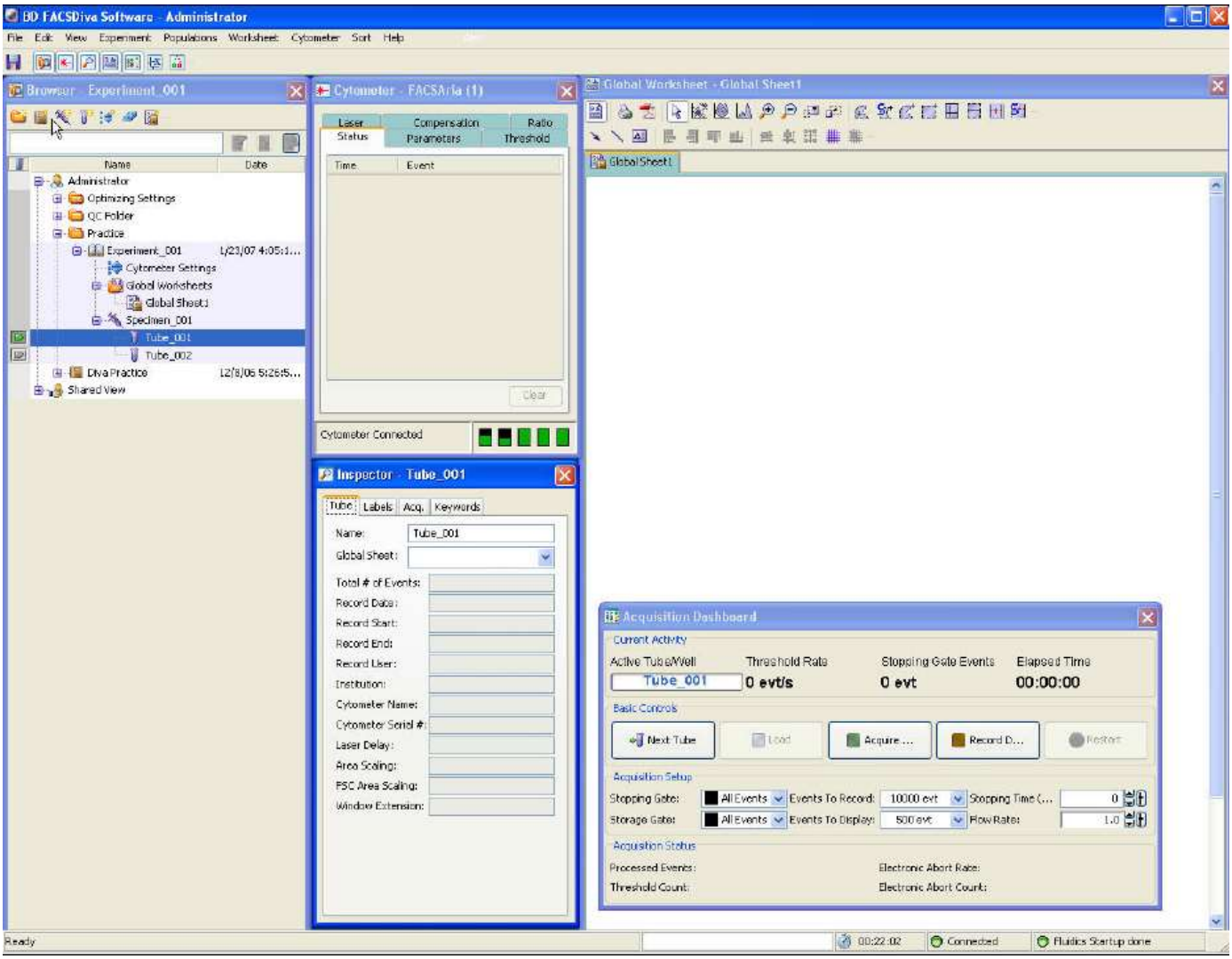
Do not reset the button until the message is displayed.

To reset the button, slowly turn the button clockwise until the light turns off and the button returns to its original position.

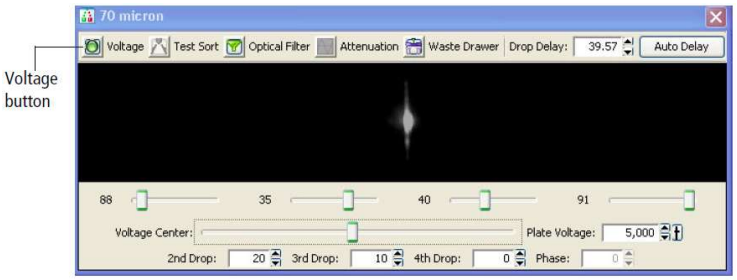
NOTE: The emergency stop button does not turn off the lasers or shut down the cytometer main power.

The FACSDiva Windows

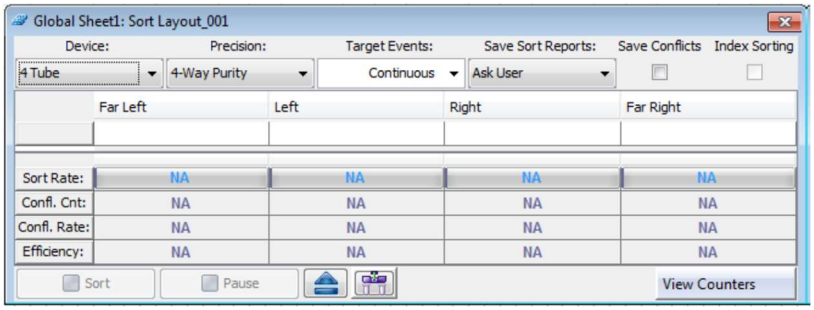
- Browser
- Cytometer
- Inspector
- Acquisition dashboard
- Worksheet



- **Streams window**



- **Sort Layout Window**



- **Breakoff Window**