# BD FACSDiva Software Quick Reference Guide for BD FACSAria Cell Sorters

## Workflow Overview

The following figure shows the daily flow cytometry workflow when using BD FACSDiva software.

Start Up System ▶ Check Performance ▶ Set Up Experiment ▶ Sort ▶ Analyze Data ▶ Cleaning and Shut Down System

To log into the PC: **Username** (BDAdmin) and **Password** (BDIS)

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## Starting Up the System

Flow Core staff responsibility – Mon-Fri

First user of the day responsibility – weekends/holidays

1. Unlock the screen with your PPMS account (UTSW credentials).
2. Launch Tera Term
3. Click on **Serial,** make sure the port is on **COM1**, and click OK.


4. Turn on the cytometer main power (green button on the side).
5. Open the software BD Coherent and make sure all the lasers are on. The UV laser needs to be turned on and the voltage set to 60mW. For the UV laser, do the following:
   a. Click on Laser STOP;
   b. Click on Laser START;
   c. Set to 60mW;
   d. Wait until the pointer goes to 60mW.



6. Start BD FACSDiva software, and log in.
7. Remove the closed-loop nozzle (metal one) from the flow cell.
8. Perform cleaning:
   a. Install a tube of 10% bleach and select **Cytometer → Cleaning Modes → Clean Flow Cell.** Repeat this process 2 times.
   b. Let the bleach set in the fluid line for 5 minutes.
   c. Turn on the stream for 2 minutes and turn it off when done.
   d. Repeat steps (a-c) with a tube of contrad.
   e. Open the flow cell access door and dry the deflection plates with kim wipes, and then close it (figure 1).

9. Sonicate the nozzle:

   a. Fill a 15mL tube with DI water, and place the nozzle inside;

   b. Put the tube in the sonicator for 2 minutes;

   c. Dry the nozzle with a kim wipe;

   d. Insert the nozzle below the flow cell (figure 2);

   e. Close the sorter door.

Figure 1

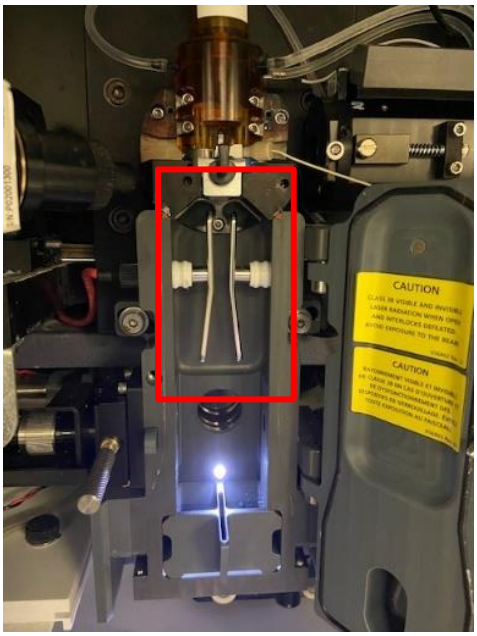
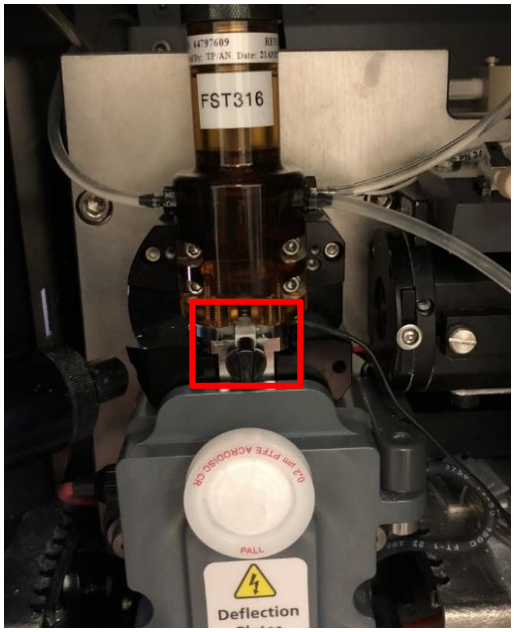


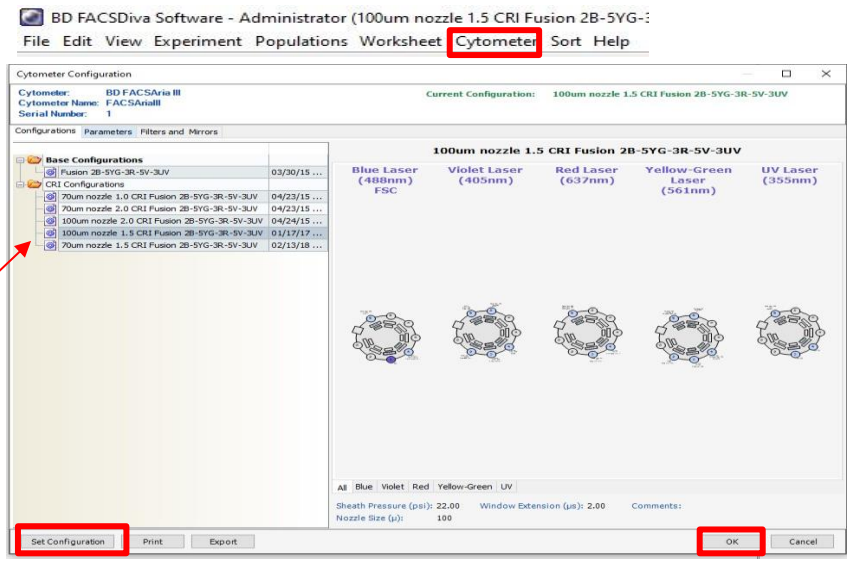

Figure 2



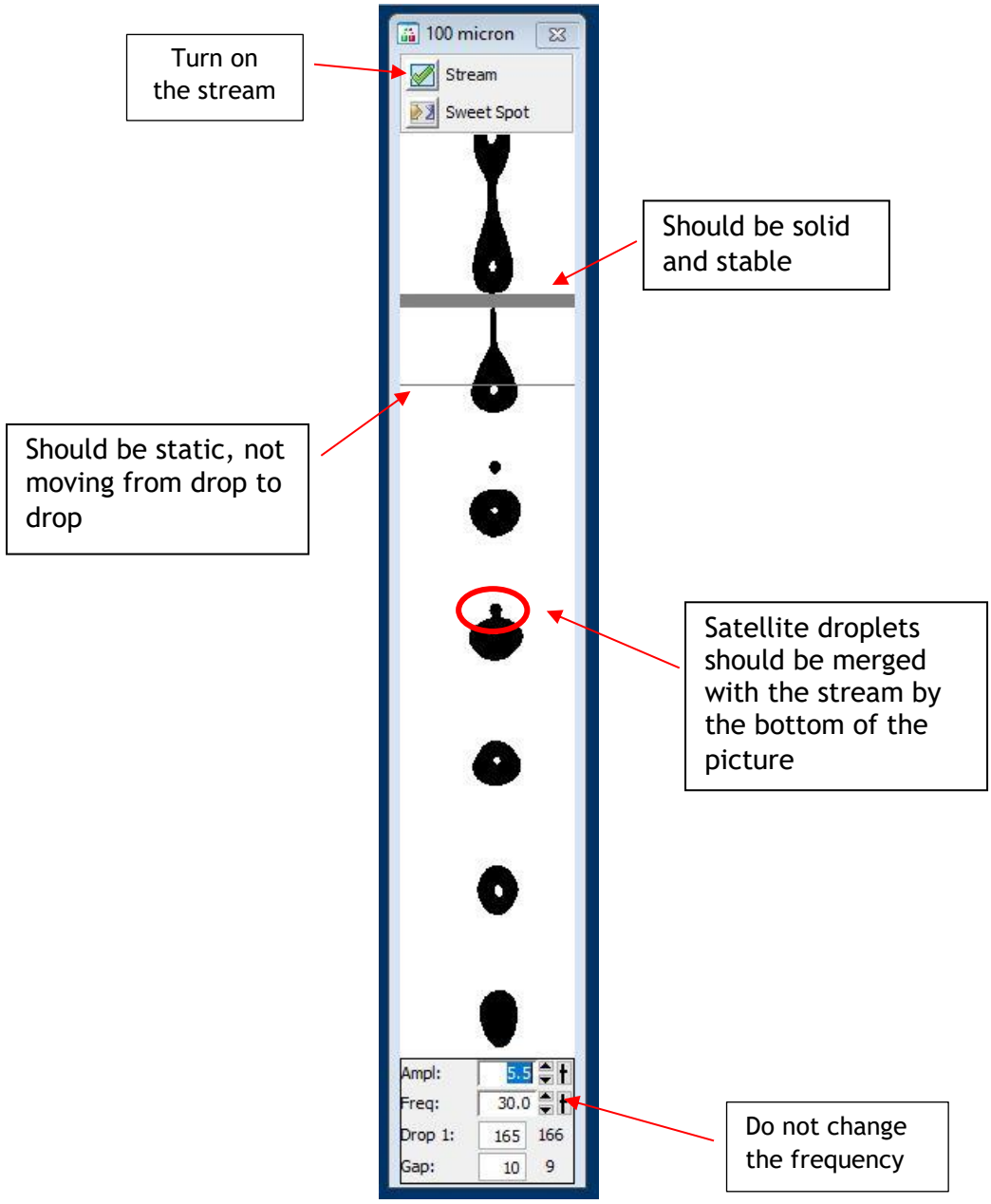

## Verifying Cytometer Configuration

1. Select Cytometer → View Configuration → Set Configuration → OK.

Select the nozzle you are using



2. Turn on the stream and make the adjustments. Adjust the **amplitude** to create a stream as seen here:

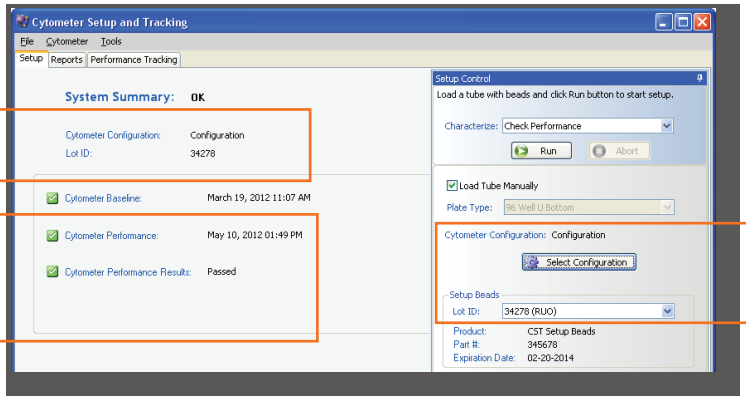


# Checking Cytometer Performance

Flow Core staff responsibility – Mon-Fri

First user of the day responsibility – weekends/holidays

1. Select Cytometer > CST.




Verify the bead Lot ID with CST vial

Verify if Cytometer Performance is not expired.

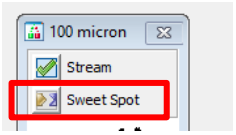
If failed or expired continue to step 2.

If needed, select a different bead Lot ID.

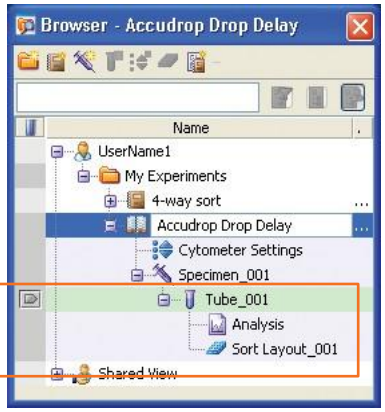
2. Load the CST tube - two drops of CST (blue vial) + ~500uL of FACSFlow.
    a. For Symphony S6 use the red vial!
3. Setup Control must be in "Check Performance" → Click Run
4. CST should pass with or without warnings.

    Failed CST it not acceptable.
5. Close the Cytometer Setup and Tracking window.

## Accudrop



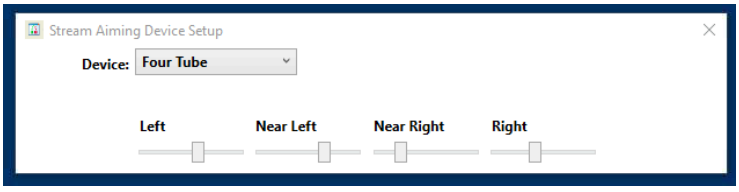

1. Turn on the Sweet Spot
2. Click on Experiment → New experiment → Accudrop Drop Delay template.
3. Expand the Specimen and expand the tube.




Bring in the provided experiment template.

4. Select Tube_001 on the pointer, open the sort layout ( ) by double-clicking it.
5. On the sort device settings, the setting should be 4-tube.
    a. Open the Sort Device Control software and change the device to Four Tube.
    b. 6-tube for the Symphony S6, located on the side stream window.

Sort Device Control Software icon



6. Turn on the voltage and select **Test Sort** to adjust the near left stream until it is in-between the tube lines (Figure 3). After that, de-select the test sort.

7. Get a prepared accudrop tube inside the fridge, vortex and **load** the tube into the sample chamber.

8. Adjust the flow rate to achieve these values of events/second:

   a. 70um = 1,000 to 3,000

   b. 85um = 800 to 2,000

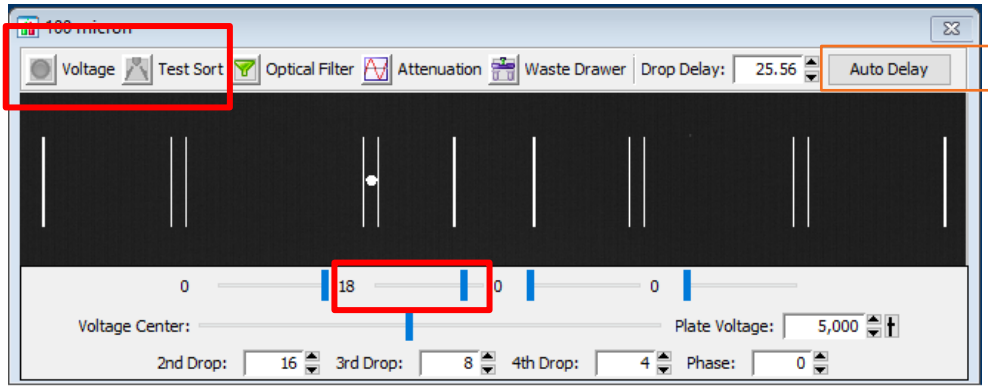
   c. 100um = 600 to 1,500

   d. 130um = 400 to 1,200


Adjust the flow rate until the threshold rate is appropriate for your nozzle size.

9. Select sort on the Sort Layout window, then "cancel" to keep the tubes covered.

Figure 3:

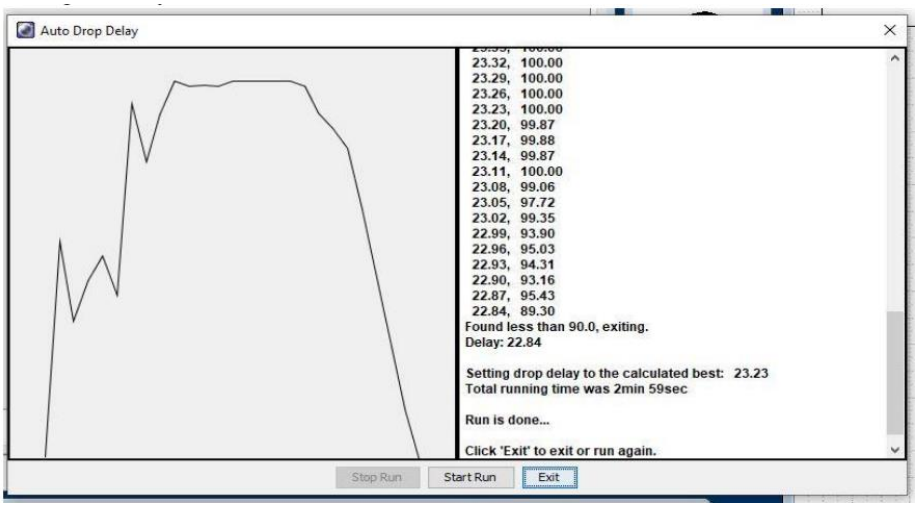



Sort beads, then click Auto Delay.

10. Click on the Auto Delay in the side stream window → Select Start Run → monitor the progress until the process is complete. Click Exit.

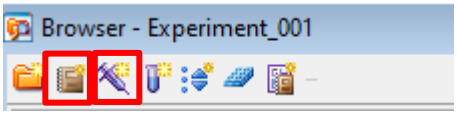
11. Auto-delay should yield a curve as seen in Figure 4.

Figure 4



# Setting Up the Experiment

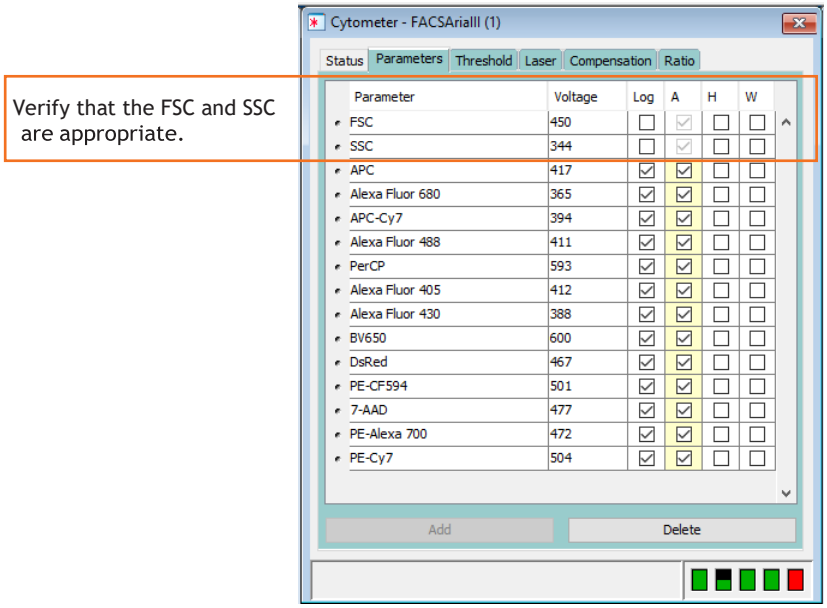
1. Create an experiment in the Browser.



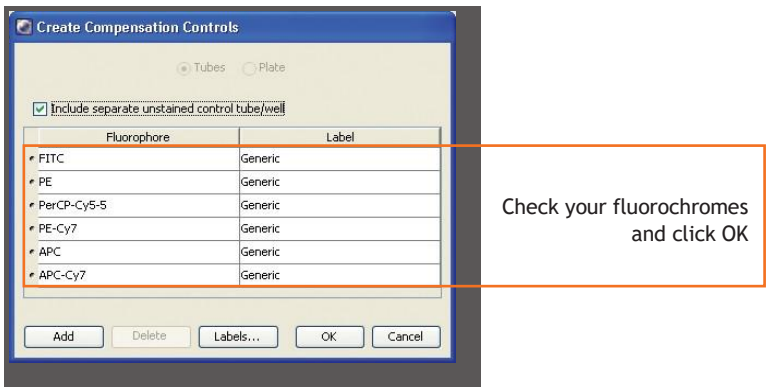

2. Create a new Specimen (syringe icon), click on the plus sign (+) to expand the specimen and place the acquisition pointer on Tube_001.


3. Click on the Parameters tab in the Cytometer window. Delete all the fluorochromes and add the ones you will be working with. Do not delete FSC and SSC.

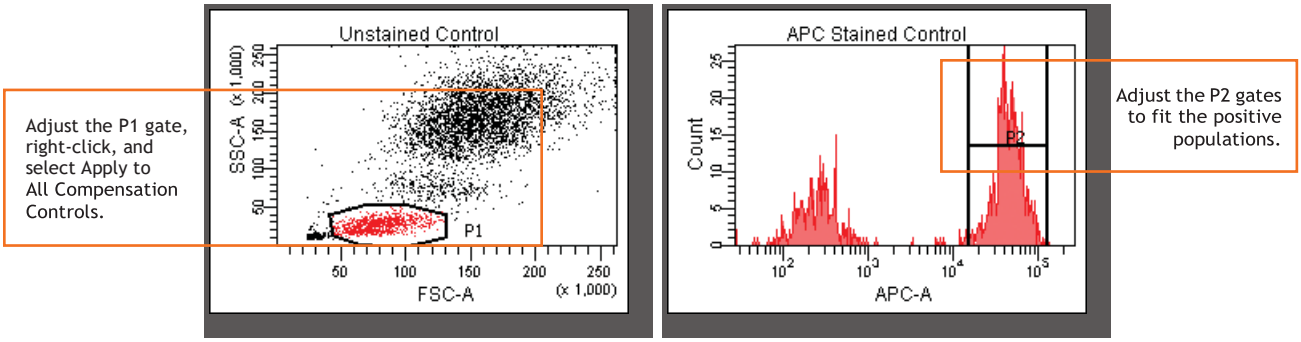
4. Select the H and W checkboxes in the Parameters tab to select height and width for FSC and SSC.



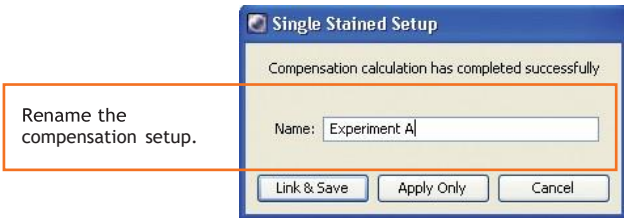

5. If you need Compensation, select: Experiment → Compensation Setup → Create Compensation Controls.



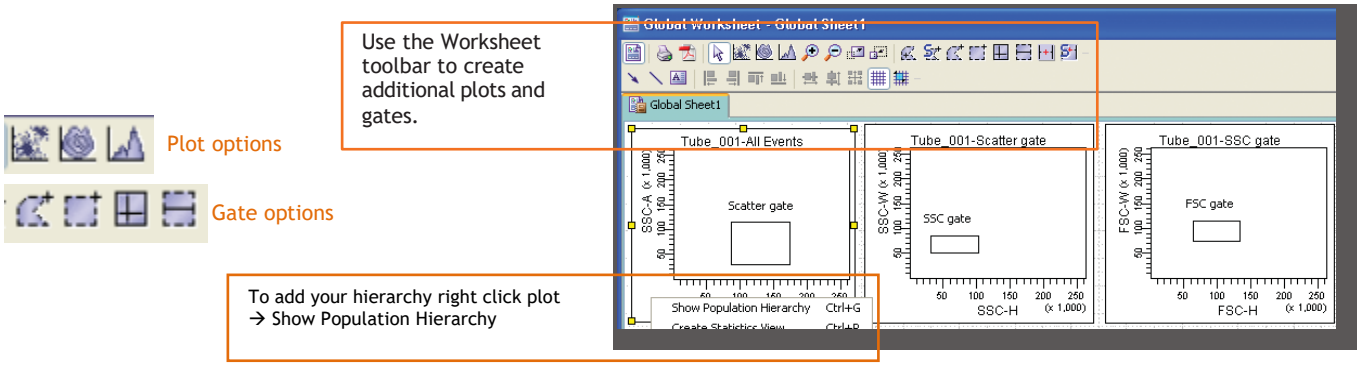
6. A specimen “Compensation Controls” has been created. Expand it and select unstained control on the pointer; “Normal Worksheet” have been created for each compensation tubes.
7. Load the tube and it will start acquiring.
8. Adjust the voltages for FSC, SSC and the fluorochromes to put the cells on scale. Make the adjustments for all your fluorochromes before recording.
9. Record data for the compensation control tube. After the acquisition is finished, click on Unload Tube on the Acquisition Dashboard and then load the next tube. Repeat the process for all your control tubes.

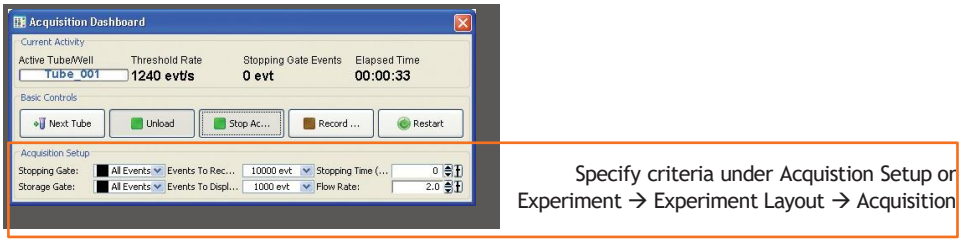


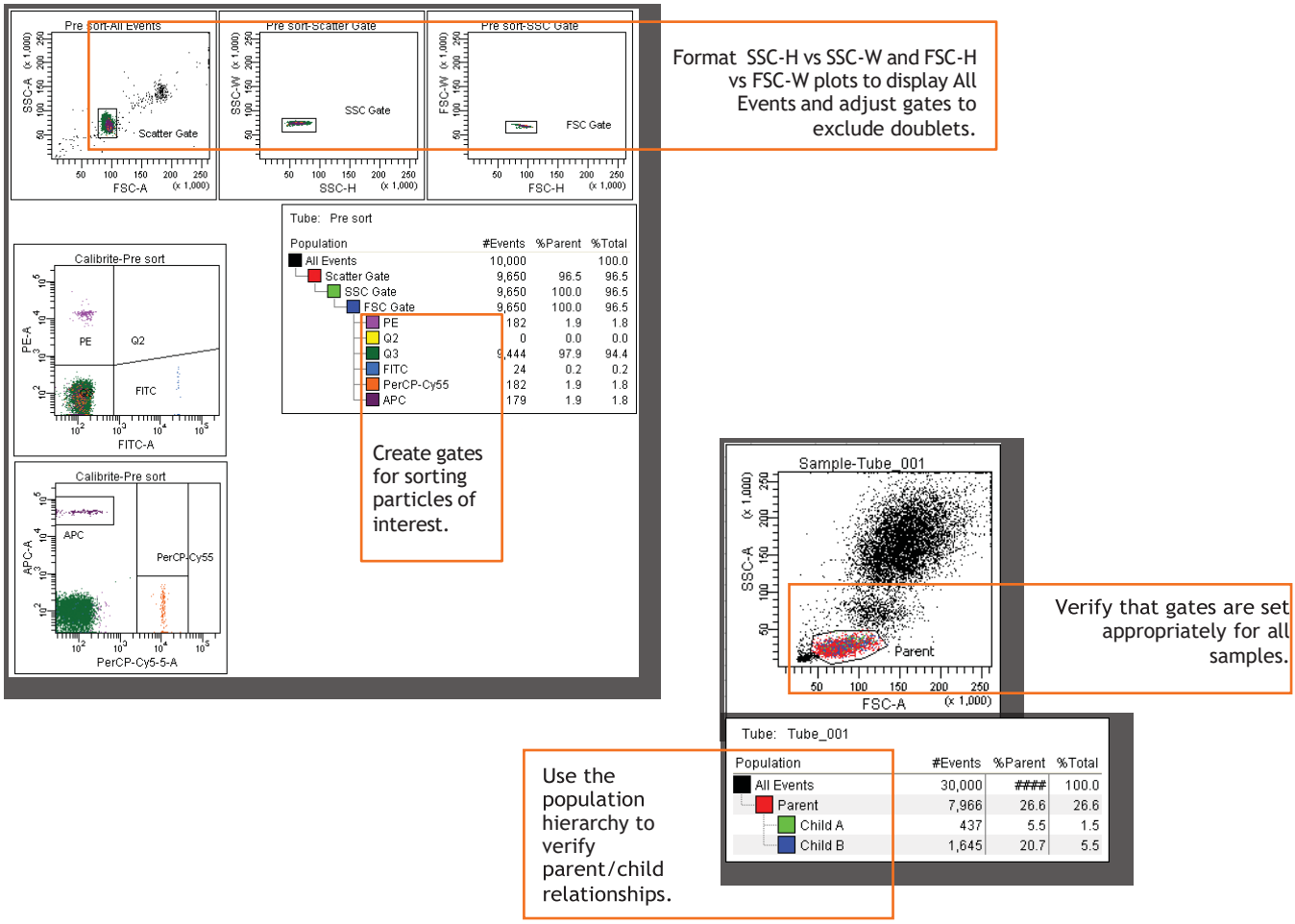

10. Select Experiment → Compensation Setup → Calculate Compensation → Apply Only.




11. On the Global Worsheet, create sorting plots and gates, then record pre-sort data.






## Sorting

1. Create a new sort layout ( ) → open the sort device app ( ) and choose your device, also select the device on the sort layout window on FACS Diva.

    a. For the Symphony S6 the device can be chosen in the side stream window.

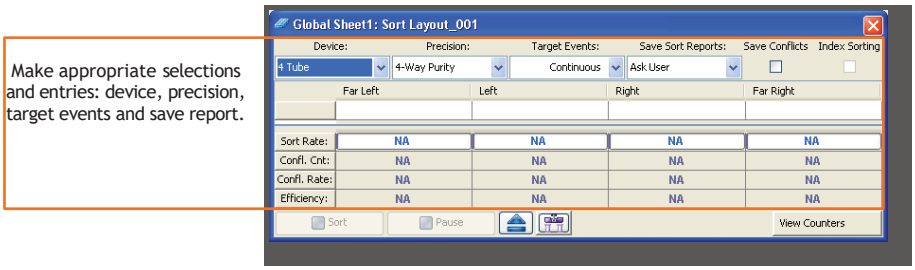
    b. Devices available: Four Tube (FACS tubes), Two 15mL Tube, Four 1.5mL Tube and ACDU (plate sorting). For the S6 the Six Tube option is available.

2. Change the Precision mode:

    a. Purity: two tubes

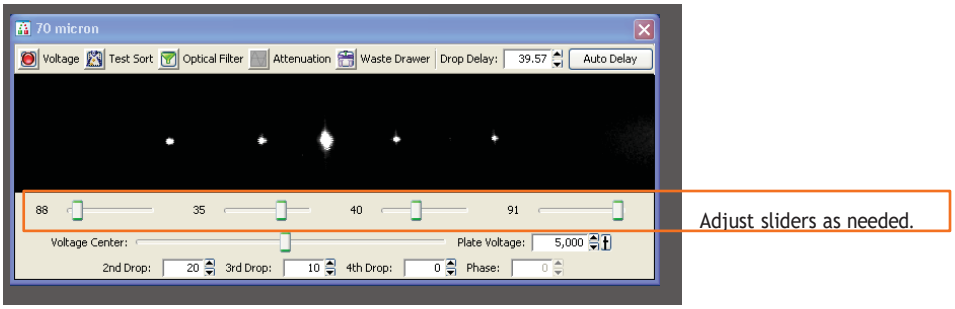
    b. 4-way purity: four tubes

    c. Single cell: plates



3. Install the collection tubes, turn on the voltage, click on Test Sort and optimize the side streams, depending on how many populations you are sorting.

    a. Ex.: 2 populations, calibrate 2 side streams. Start inward and move outward.




4. Insert your tube of cells → Load → Sort → Click "Ok" to open the waste drawer.

5. Stop the sort if needed, and save a copy of the sort report.

6. **Additional steps to do plate sorting:**

    a. Put the ACDU into the designated slot on the sort block;

    b. Change device in the sort device app to ACDU;

    c. When adjusting the side stream, slide the **far left** stream appropriately;

    d. In the sort layout window press the eject button so that the platform comes all the way forward;

    e. Place your plate **with the lid** on onto the platform (make sure A1 is placed correctly, bottom left corner of the plate platform);

    f. Click on Sort → Home Device → select the correct plate → press Go Home;

    g. Turn on the voltage and double click Test Sort;

    h. Make sure the drop lands on the A1 well, if its off center make adjustments by moving platform with home device arrows;

    i. When adjustments have been made, retest test sort to make sure the drop lands on A1.

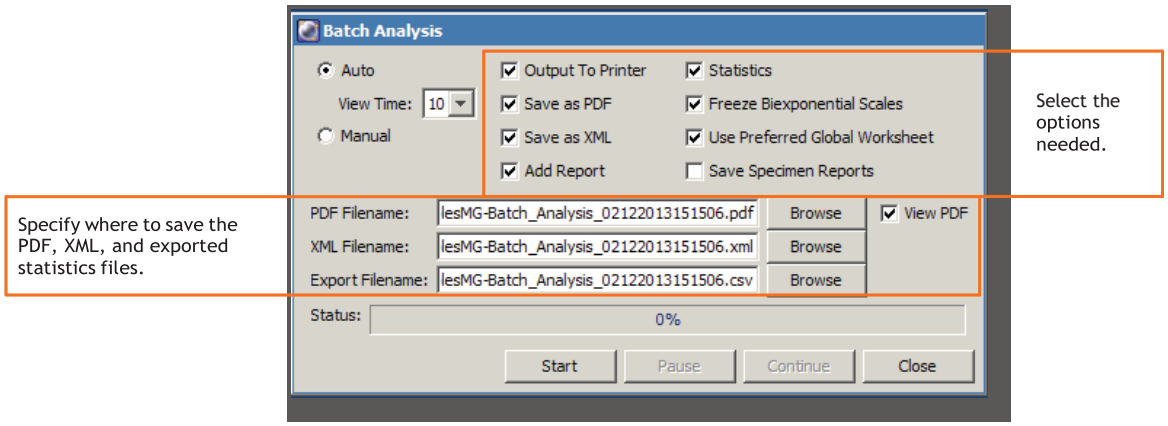
    j. Set Home → Apply.

    k. Now you can remove the lid and do the sort.

## Analyzing Data

1. Record post-sort data.
2. Check the sort purity.
3. To print or export the results:

- Right click your Experiment → Export → FCS Files (Folder: Documents - Backup - Year - Month - Your folder).
- To create a PDF, right-click a specimen or experiment and select Batch Analysis (using a global worksheet).




## Cleaning and Shutting Down the System:

1. Turn off the stream, remove the nozzle and sonicate it for 2 minutes.
2. Place the closed loop nozzle, clean with windex and shut down solution doing the following procedure:
    a. Load the windex tube.
    b. Cytometer → Cleaning Modes → Clean Flow Cell → do it 2x
    c. Load the shutdown solution tube and repeat step b.

**If you are the last user of the day:**

3. Turn off the sorter main power - green button.
4. Turn off chiller; switch on back of head unit.