SOP Number 5

SOP Title BD JAZZ Fluorescence Activated Cell Sorter Training and Operation

	NAME	TITLE	DATE
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1. PURPOSE

This procedure outlines the steps to be used in training users for operating the BD FACS JAZZ system.

2. SCOPE

This procedure applies to all students, staff and researchers who use the BD FACS JAZZ system.

3. DEFINITIONS

The BD FACS JAZZ system is used to separate, count, analyse and sort cells. The system (now referred to as the JAZZ) includes lasers, dichroic mirrors, optical filters, lenses, photomultiplier tube detectors, HP computer, and BD Sortware software for control and operation. The JAZZ is used primarily for cells expressing fluorescent proteins and/or stained with fluorescent dyes and antibodies.

4. RESPONSIBILITIES

4.1 It is the responsibility of the Microscopy Technician to organize regular training sessions for the safe use and upkeep of the JAZZ.

4.2 It is the responsibility of all Users to ensure they receive proper instrument and procedural training prior to using the JAZZ.

4.3 It is the responsibility of all Users to operate the JAZZ according to the procedures enclosed in this document.

4.4 It is the responsibility of all Users to immediately report any damage or malfunction of the JAZZ to the Microscopy Technician.

4.5 It is the responsibility of all Users to undertake a pre-use inspection of the instrument before operation.

4.6 It is the responsibility of all Users to undertake a post-use inspection of the JAZZ after operation making sure that the instrument is clean and ready for the next user

5. SPECIFIC PROCEDURE

5.1 Training overview

To begin, new users are questioned about their experience with cytometry. The basics of fluorescence can be explained as well as how a photomultiplier tube (PMT) works.

Next is a review of hardware. The specifications of the system can be pointed out at the same time. Powering up the system is also shown. The demonstration of loading a sample is now done. An explanation of the concept of channels is made and how they are set up for different fluorescent dyes. Acquiring and recording data is shown.

Optimization of PMT voltage is demonstrated and also the use of fluorescent beads to align the stream and calibrate the drop delay. Testing the sort stream, drawing gates, sorting and analyses are all part of the training.

Saved data can be viewed and a table of statistics can be shown. The training session is finished with instruction on how to clean up and power down.

5.2 Pretraining discussion.

Before training it is important to ascertain a new user's experience using cytometers. This includes use of fluorescent dyes, digital detectors (scatter detectors and fluorescent intensity detectors), acquisition software, and anything else that is relevant. As much as possible, training should be personalized for the new user.

A brief discussion of the properties of fluorescence may be necessary using diagrams online showing excitation and emission spectra of commonly used dyes (BD has a good spectrum viewer website (http://www.bdbiosciences.com/ca/research/multicolor/spectrum_viewer/index.jsp).

5.3 Purpose of Flow Cytometry

The purpose of flow cytometry is to arrange cells single file in a stream and pass them by detectors for the purpose of determining their size, shape and specific expression of antigens as indicated by dyes and/or fluorescently labelled antibodies.

5.4 Instrument Specifications

Solid state lasers:

50 mW 488nm .

50 mW 640nm.

Forward scatter and side scatter detectors which use the 488nm laser as a light source.

Filters and potential fluorophores:

530/40 FITC/GFP (488nm laser)

585/29 PE/PI (488nm laser)

692/40 Percp 5.5 (488nm laser)

750/LP PE-Cy7 (488nm laser)

670/30 APC (640nm laser)

750/LP APC-Cy7 (640nm laser)

The acquisition software is BD Sortware which runs on a HP Windows 7 Pro computer.

5.5 Hardware Review

The cytometer, electronics box, waste container, vacuum pump, PBS supply and compressor are shown in Figure 1.

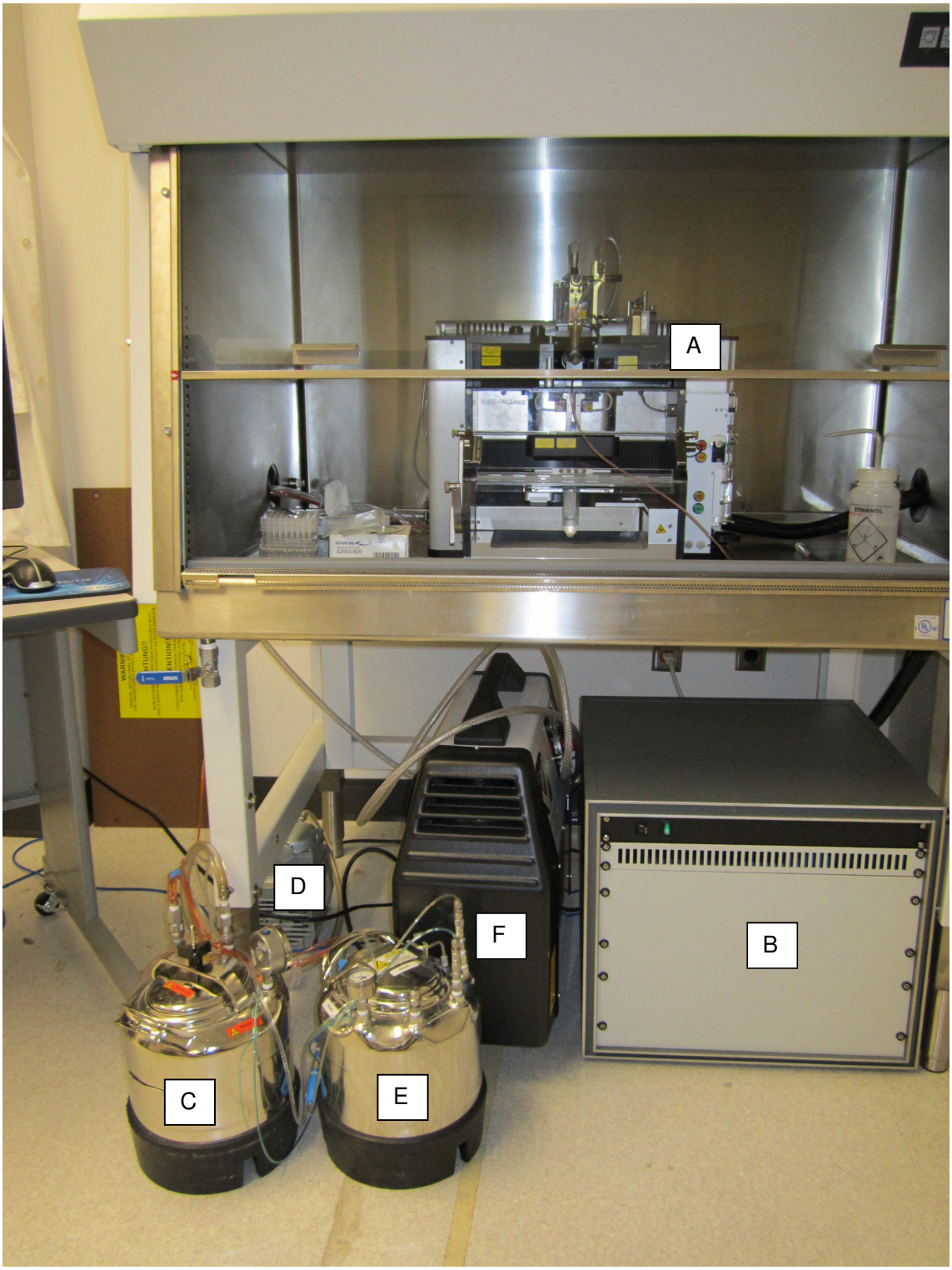

Figure 1. Cytometer (A), electronics box (B), waster container (C), vacuum pump (D), PBS supply (E), and compressor (F).

The HP desktop computer is shown in Figure 2.

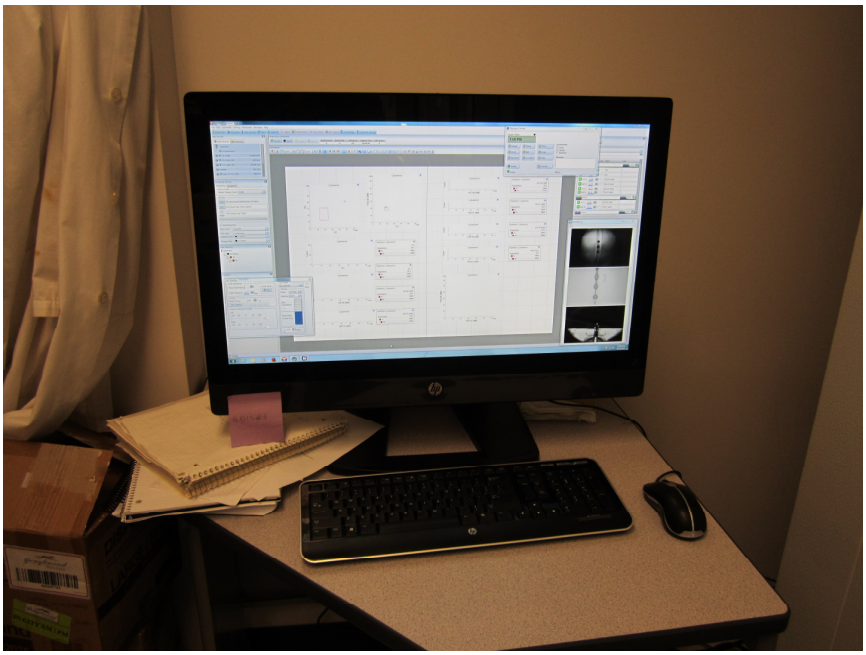

Figure 2. The HP desktop computer with BD Sortware (version 1.2.0.142).

5.6 Power up

Before starting up, check the Sheath tank (Figure 1E) and fill to ¾ full with PBS if needed. If the Sheath tank is filled up, detach the Waste tank (Figure 1C), unseal and remove the lid, add bleach for a few minutes then empty, reseal and attach to the system.

5.6.1 The power switch for the electronics box is on the front top right (Figure 1B).

5.6.2 The vacuum pump has a separate power switch (Figure 1D).

5.6.3 The cytometer has a switch on the bottom right side (Figure 1A).

5.6.4 The compressor has a power switch on the right side (Figure 1F). The computer's power switch is on the top right side and the Windows 7 password is 'BDIS#1'. The operating software is the BD Sortware. Verify that the BD FACS Sortware workspace, the SortView pane, and the Pressure Control panel have opened.

5.7 Initiating the stream, debubbling and stream alignment

5.7.1 If starting from a Dry Shutdown or preparing to sort, sonicate the nozzle in 70% ethanol in a small beaker or capped tube. If preparing to sort, remove the charge plates and sonicate them at the same time (Figure 3).

Figure 3. The charge plates in the sonicator.

Replace the nozzle in the screw cap and attach to the flow cell. Place the flush bucket under the nozzle and place the debubble resevoir in between the flush bucket and the nozzle (Figure 4).

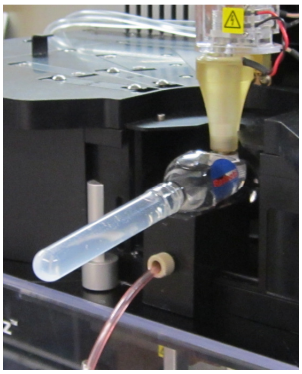

Figure 4. The flush bucket and the debubble resevoir in location below the nozzle.

Fill the debubble reservoir with 70% ethanol and click Purge on the Pressure Control panel to fill the nozzle. After the 70% ethanol appears above the flow cell assembly, click Pulse to help dislodge bubbles. Stop the purge and refill the debubble reservoir with more 70% ethanol as needed. If bubbles are still present after pulsing use the "nozzle prime" technique to dislodge bubbles. While in PURGE mode, remove the debubble reservoir to introduce a small amount of air into the nozzle. Then, refill the debubble reservoir with 70% ethanol and re-submerge the nozzle. Click Purge to remove air from the nozzle and repeat the Pulse. This step may need to be repeated several times. Once no more bubbles are observed, click the Stream button and remove the debubble reservoir and flush bucket.

5.7.2 If starting from a Wet Shutdown, open the deflection plates if they are not open (Figure 5), click the Stream button then remove the debubble reservoir and the flush bucket under the nozzle assembly.

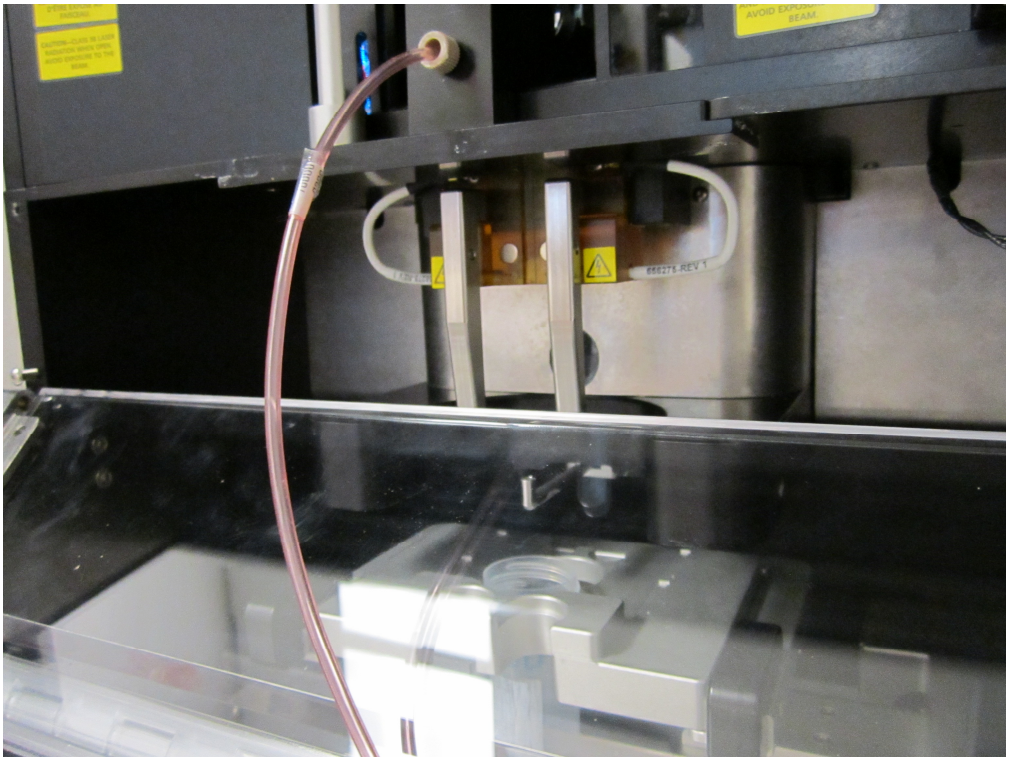

Figure 5. The deflection plates in the open position.

Remove any excess fluid from the nozzle tip using a tissue or cotton applicator and turn on the illumination laser (the on button is labelled 'Illumination' and found on the front right of the cytometer) to view the stream at the bottom (Figure 6, bottom panel).

5.7.3 Align the stream to the pinholes (Figure 6, top panel) using the silver knobs and align the bottom of the stream to the stream drain using the black knobs all found on the top of the flow cell assembly (Figure 7).

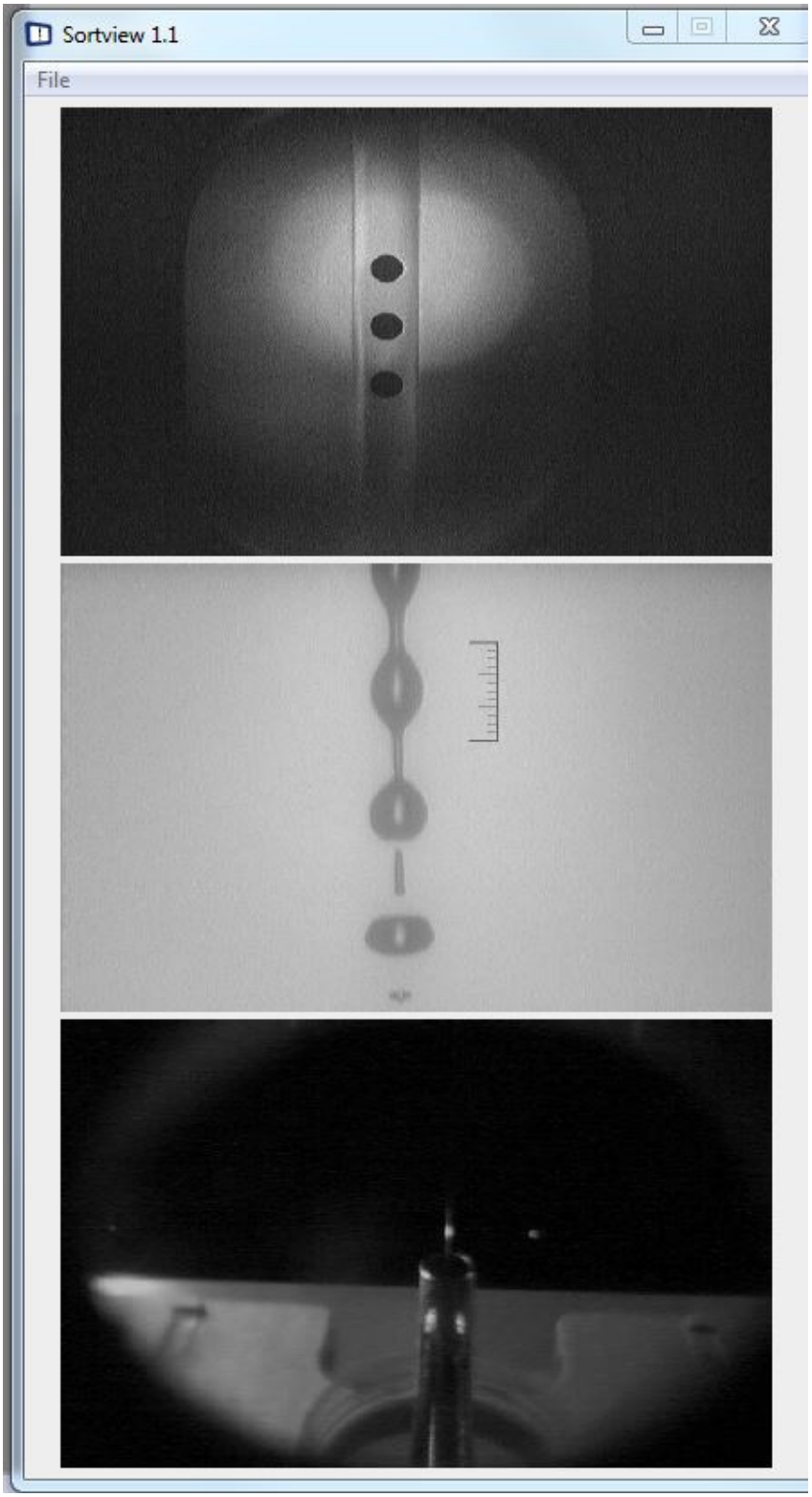

Figure 6. The sortview panel. The pinholes are visible in the top panel. The drop breakoff point is visible in the middle panel and the stream position at the stream drain is visible in the bottom panel.

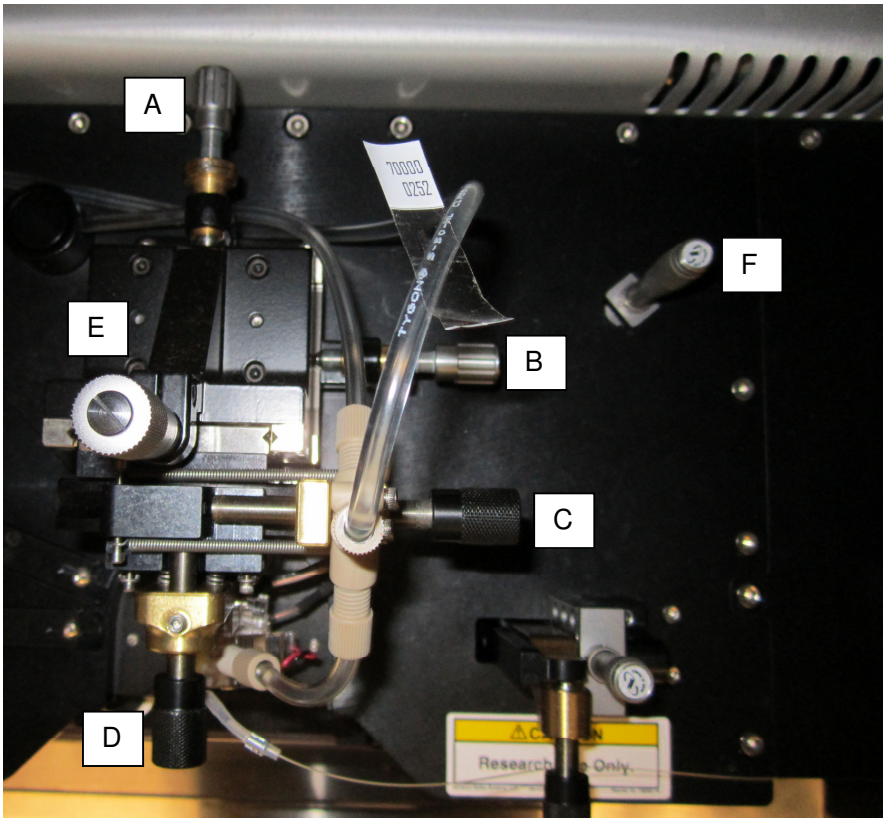

Figure 7. A view of the top of the flow cell assembly from above. The silver knobs (A and B) for adjusting the top of the stream and the black knobs (C and D) for adjusting the bottom of the stream. The knob for adjusting the nozzle height is also in view (E) as well as the height for camera view of the drop breakoff (F)

5.7.4 The stream should be centered on the pinholes with crisp edges on the sides of the stream. The nozzle tip, which is adjusted with the knob shown in Figure 7E, should be about one pinhole distance above the first pinhole (Figure 6).

Repeat these steps until the stream is centered and in focus over the pinholes and centered in the stream drain and the illumination laser beam.

Close the sliding nozzle access door. Open the shutters for both lasers and check that they are aligned with the appropriate pinhole. Set the laser delay for the 640nm laser to 10.9 in the 'Cytometer Settings' pane of the Sortware (Figure 8).

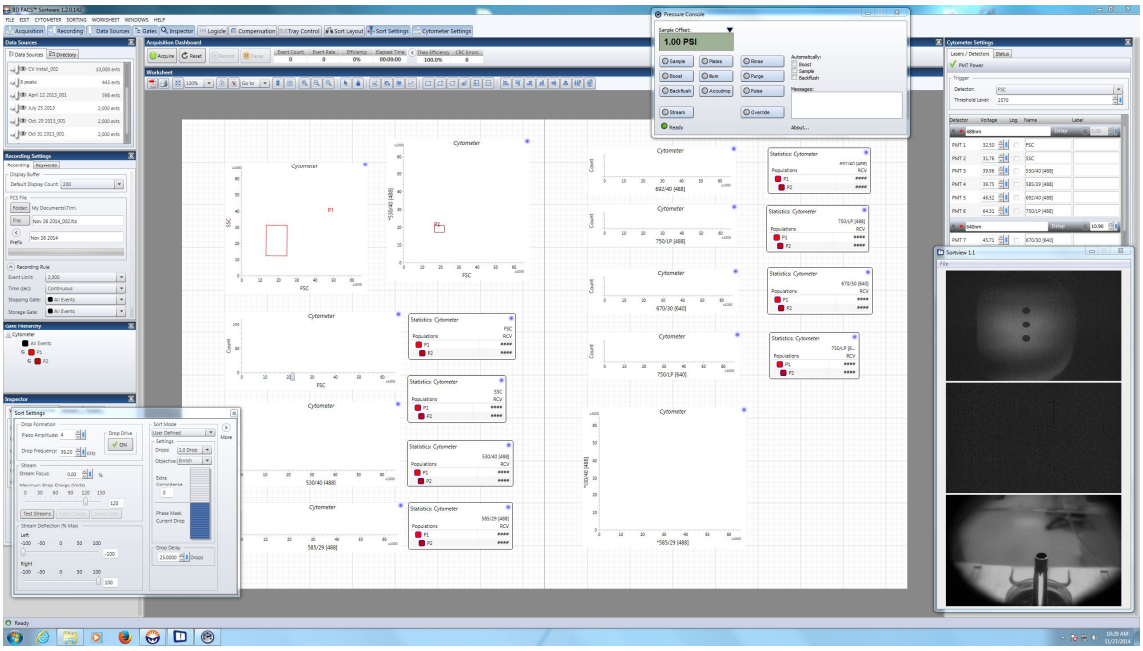

Figure 8. The Sortware with the Ultra Sphero Rainbow workspace loaded.

5.7.5 Turn on the drop drive in the 'Sort Settings' pane of the Sortware and set the Piezo amplitude to 4.0. Under the File pull down menu, select 'Restore Workspace'. In the dialogue box that appears, navigate to 'Tim' and select Ultra Sphero Rainbow from the list. Deselect the Fluidics Setup checkbox to prevent overwriting the optimized daily sort settings. The Sortware should now look like Figure 8. At this point the stream should be run for at least ½ hour. If sorting, the stream should be run for an hour before starting calibrations.

5.7.6 Obtain the tube of Ultra Sphero Rainbow beads from the refrigerator (labelled SPHERO), vortex briefly and load onto the sample line found on the bottom front right of the cytometer (Figure 9). Please note, all samples must be contained in a 5ml polypropylene tube.

Figure 9. The sample line loaded with a sample.

Set the 'Default Display Count' in the Recording Settings pane to 200. Click the 'Sample' button on the Pressure Console. Click 'Acquire' on the Acquisition Dashboard. Once the signal from the beads is observed on the plots and histograms, use the silver knobs on the flow cell assembly (Figure 7A and 7B) to get maximum signal and sharpest peaks. The 530/40 [488] channel is good for observing sharp peaks (Figure 10).

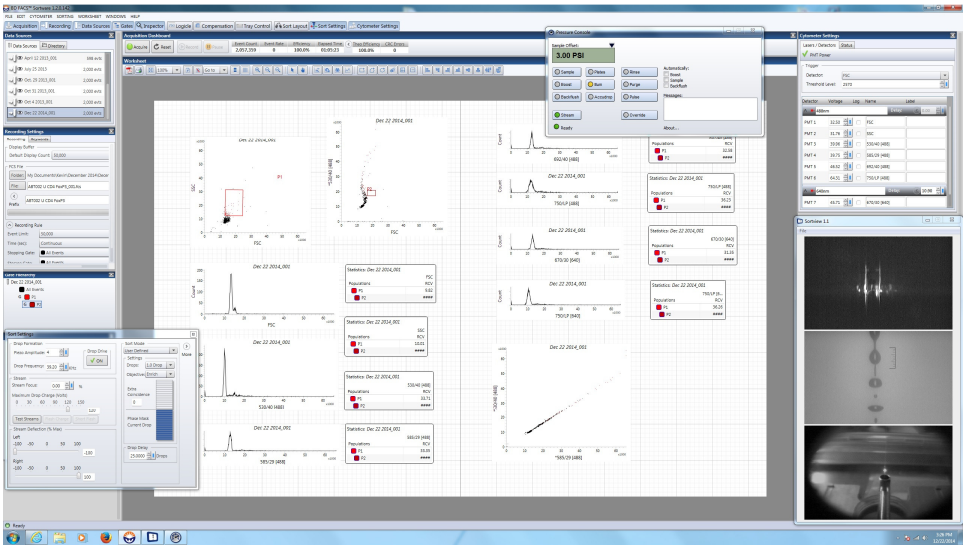

Figure 10. The BD Sortware loaded with a workspace for observing Ultra Sphero Rainbow beads. The 530 /40 [488] histogram shows a sharp peak after aligning the stream.

5.8 Compensation, analysis and sorting

At this point, old workspaces can be reloaded, or new workspaces created as required and new samples run and recorded.

5.8.1 To start building a compensation matrix, run unstained and single stained samples adjusting PMT voltages as needed. Record a minimum of five thousand events for each of these after voltages are established. Open the Compensation matrix and under Manage Parameters, select the channels to be compensated (Figure 11)

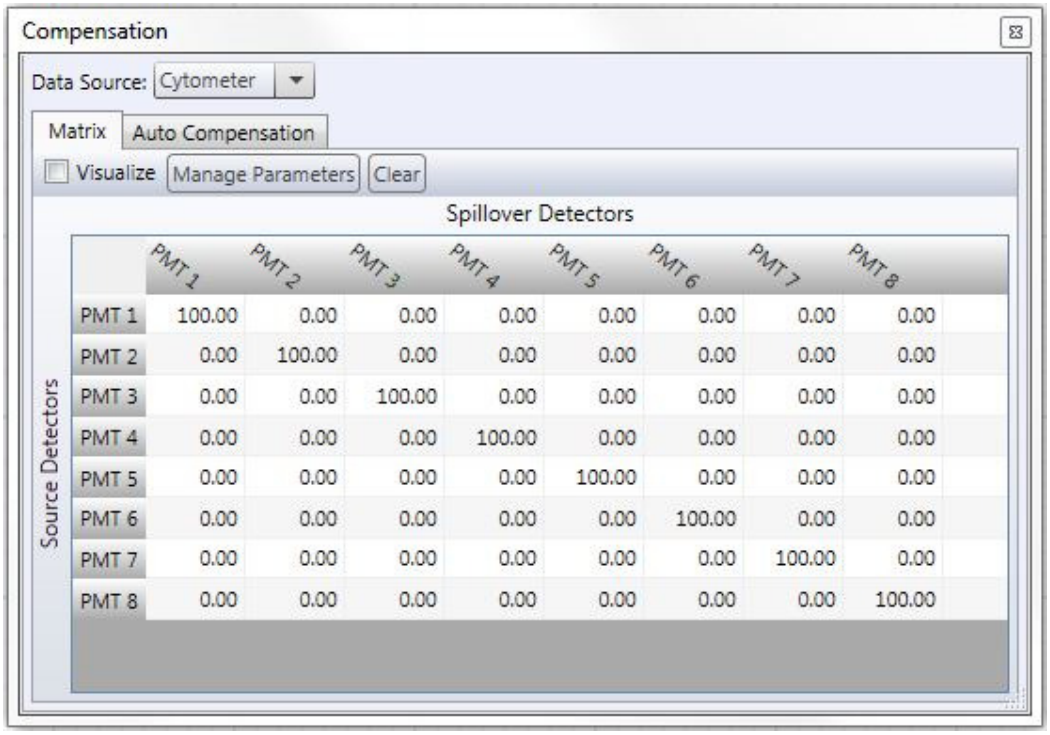

Figure 11. The compensation matrix

Set all histograms for the single stains with digital detectors (DSPs). In the recorded Unstained file, draw a gate on a FSC/SSC plot around the desired cell population. Load each single stain recording and draw interval gates on all the individual single stain histograms. Make sure that the single stain interval gates are children of the Unstained gate drawn first. This done in the Gate Hierarchy pane of the Sortware. Under the Auto Compensation tab of the Compensation pane (Figure 11), enter the gates for positive and negative in each column. Click calculate and check the compensation matrix. Check the Visualize box and the compensation matrix becomes active.

5.8.2 To prepare for sorting, clean the deflection plates and reinsert them in place with the magnets flush to the magnets on the cytometer. Close the plates as shown in Figure 12.

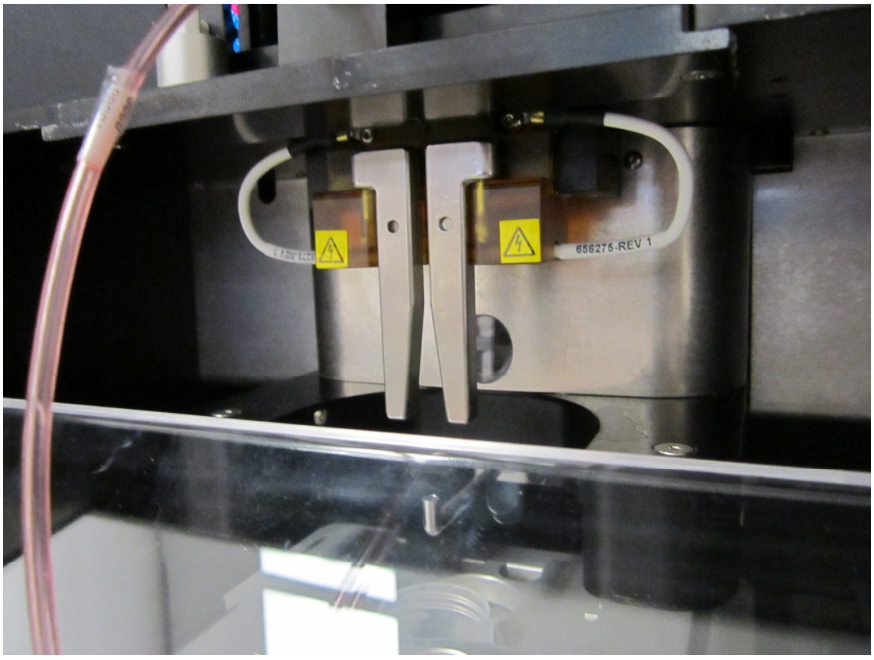

Figure 12. The deflection plates in the closed position.

Turn on the deflection plates and check to make sure that the sort streams are visible against the illumination laser by turning on the Test Stream in the Sort Settings pane (Figure 13). Please note that if you need to adjust the stream to see the Test Streams, the alignment of the stream on the pinholes will need to be readjusted as in 5.7.6.. Adjust the camera height using the vertical silver knob to the right of the flow cell assembly on top of the cytometer (Figure 7F) to make the drop breakoff within the bars of the middle panel of the Sortview pane (Figure 6, middle panel).

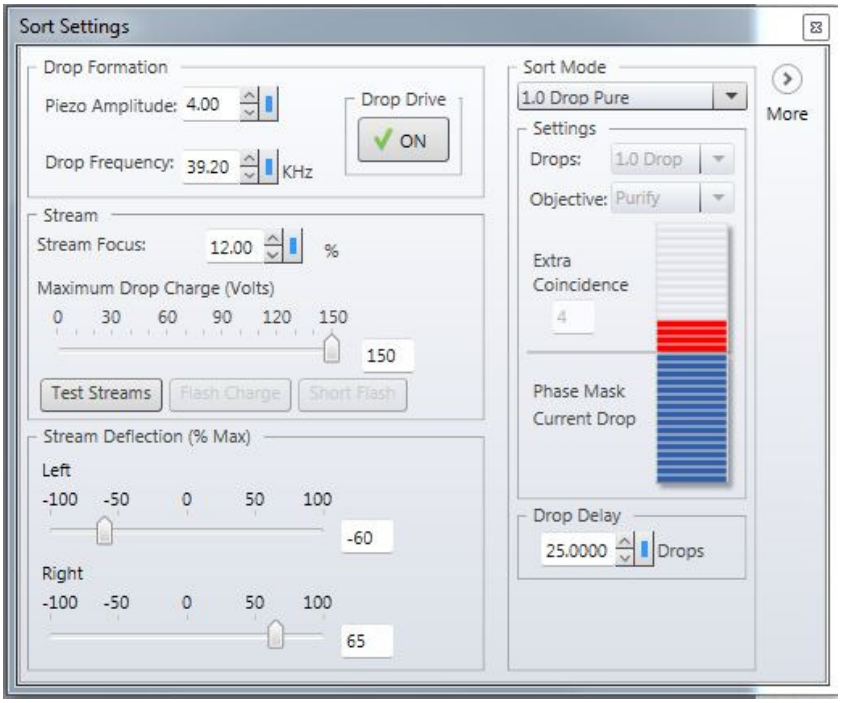

Figure 13. The Sort Settings pane.

Set the Stream Deflection to -60 on the left and 65 on the right in the Sort Settings pane (Figure 13). Set the Stream focus to 12.0 and the Maximum Drop Charge to 150. Adjust the Piezo amplitude until both sort streams are clearly visible and have the maximum deflection. Click the Flash Charge and adjust the Piezo Amplitude if needed. Click the Short Flash and again adjust the Piezo Amplitude if needed to get maximum deflection.

5.8.3 Before sorting can be started the Drop Delay needs calibrating with Accudrop beads. Load the Accudrop workspace under Tim. Load the accudrop beads on the sample line and start acquiring signal. On the Pressure Consol, click Accudrop to insert the optical filter in front of the Illumination laser. In the Sort Layout pane (Figure 14), select Accudrop in the Sort Device menu, and start the sort.

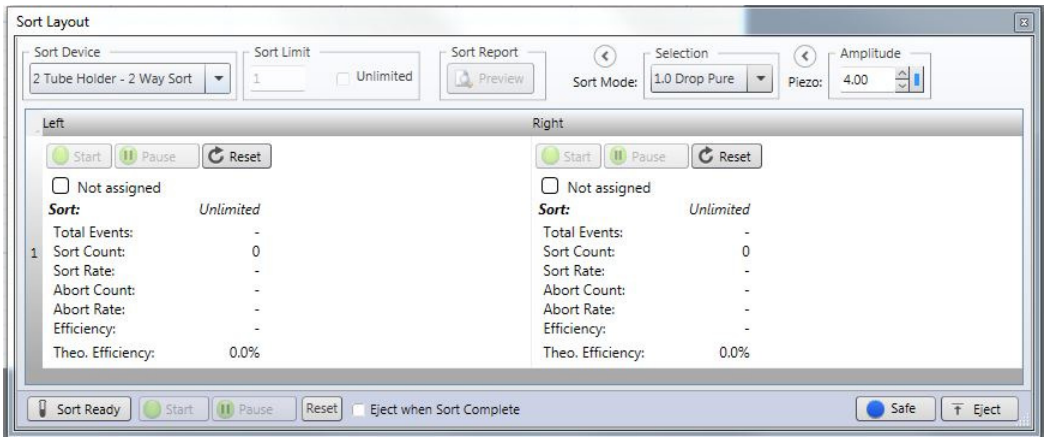

Figure 14. The Sort Layout pane

Adjust the Sample Offset pressure in the Pressure console of the sortware until the event rate is 3000/sec. Adjust the Drop Delay on the Sort Settings pane until all the visible fluorescence from the illumination laser is in the sort stream and not the waste stream.

5.8.4 Sorting samples. A typical sort workspace is shown in Figure 15.

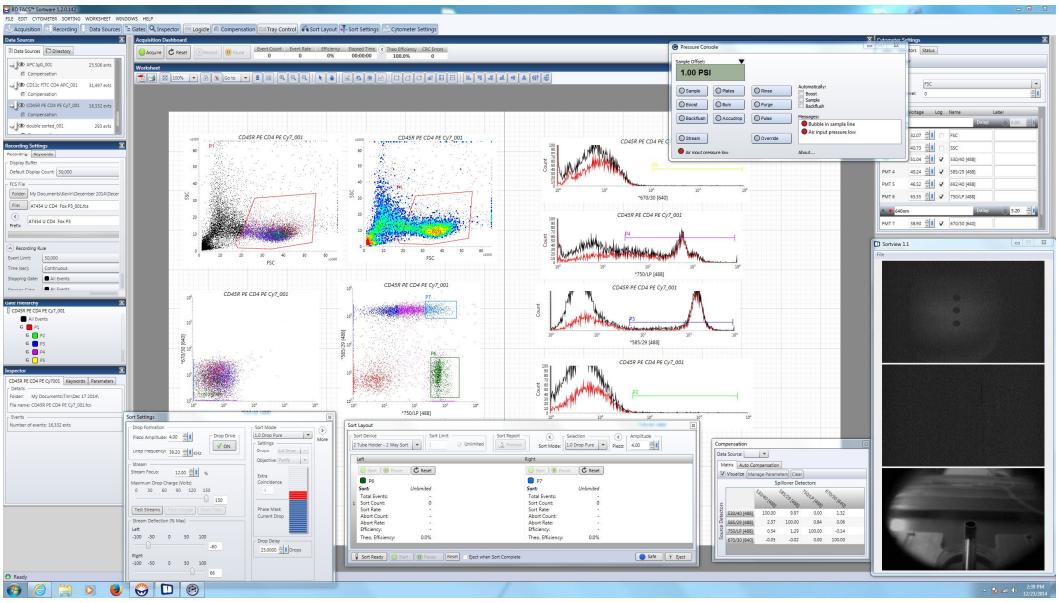

Figure 15. A sort workspace.

After calibrations and compensation are established. The desired Sort Device is selected in the Sort Layout (Figure 14). Sorting cannot procede until gates are entered in any sort device used (Figure 15). The gates used for sorting must be drawn in DSP parameters. Once this is setup, click Sort Ready on the Sort Layout (Figure 14) to move the collection devices into the proper location. Start the sample on the Pressure Console and start the sort on the Sort Layout. Adjust the Sample offset pressure so that the event rate is less than 4000. Please note, the event rate can be pushed higher if desired but the efficiency of the sort will decrease. Observe the drop shape and location. If the breakoff point changes dramatically, the Drop Delay will need to be recalibrated as in 5.8.3.. The Piezo Amplitude in the Sort Settings pane will need adjusting over time to keep the sort streams working properly.

5.8.5 After sorting is complete, a sort report can be generated by clicking Sort Report on the Sort Layout pane.

Use a small amount of sorted sample to check the collected material for purity and viability and record this data. Click the Safe button on the Sort Layout to move the large collection tube under the drain.

5.9 Clean up and power down

Clean the sample line by loading and running 10% bleach for 5 minutes. Backflush and load a sample tube with distilled water.

Perform a Wet Shutdown. Place the flush bucket under the nozzle. Place the debubble reservoir between the nozzle and flush bucket. Fill the debubble reservoir with 70% ethanol then turn off the stream. Under Cytometer on the pull down menu, click Shutdown Cytometer. Wait until the Sortview panel stops and then power off the compressor, cytometer, electronics box and vacuum pump. Release the pressure on the Sheath tank and turn off the computer.

5.10 Signature Page

Please read the SOP, particularly section 4 (Responsibilities).

I,_____, have read the SOP.

Date:

Signature:

6. CHANGE HISTORY

SOP no.	Effective Date	Significant Changes	Previous SOP no.

7. APPENDIX

The BD Sortware Users Manual can be found on the Desktop of the computer.