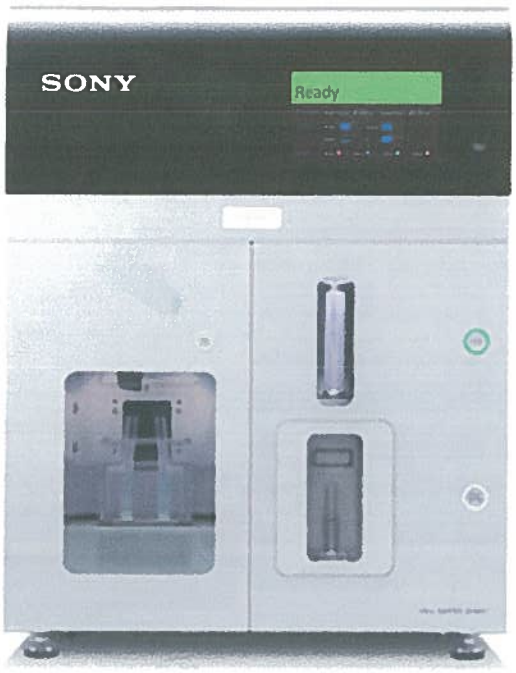


SONY SH800 Basic Training

Table of Contents

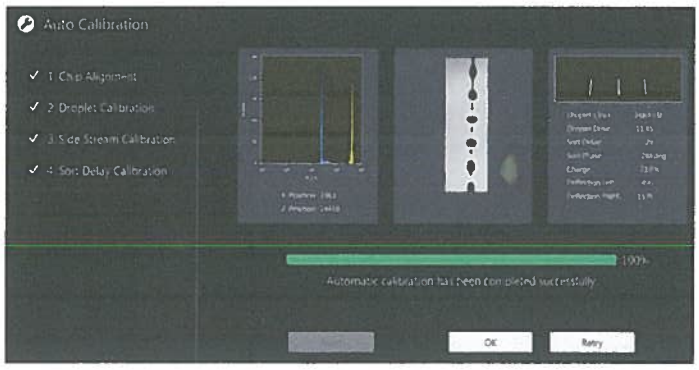
General Operation

1. Power Up

a. Fill the sterile sheath tank (if needed)
b. Empty the waste tank (if needed)
c. Fill the sterile water tank (if needed)
d. Turn on the air compressor (or house air supply)
e. Power up the SH800
f. Power up the computer (if not already on)
g. Log into Windows 8
h. Launch the SH800 software
i. Log in with user name and password

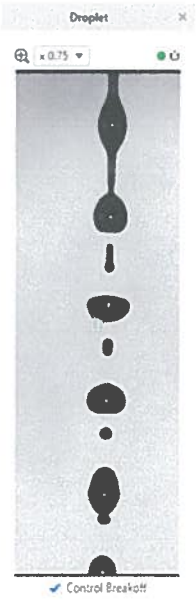
2. Automatic Alignment and Sort Setup

a. Scan the QR code on the sorting chip package
b. Follow the software prompts for Automatic Setup
   - If you do not plan to use the sorting feature, Check the box for Analyzer Mode
   - To perform the complete Automatic Setup, with Sort Calibration, leave the box unchecked
c. When prompted, mount a sample tube containing SONY Automatic Setup Beads (P/N LE-B3001)
   - The sample tube should contain at least 0.5 mL (10 drops) of bead suspension.
   - Shake the bottle vigorously before dispensing
   - Never dilute the bead suspension
   - Each box contains three bottles of bead suspension. Use one bottle completely before opening the next bottle.
   - Store Automatic Setup Beads in the dark at 4° C.
d. Click on OK to initiate the Automatic Setup processes:
   - Chip Alignment
   - Droplet Calibration
   - Side Stream Calibration
   - Sort Delay Calibration



3. Automatic Droplet Breakoff Control

a. The software stores an image of the droplet formation right after sort delay calibration.
b. The software constantly monitors the live image of the droplet formation throughout the day.
c. With Control Breakoff active (box checked) the software automatically adjusts the droplet drive amplitude (intensity) to maintain the droplet formation exactly as it was right after sort delay calibration was performed.
   - If the length of the first satellite droplet decreases, the software automatically decreases the droplet drive amplitude to compensate.
   - If the length of the first satellite droplet increases, the software automatically increases the droplet drive amplitude to compensate.
d. Control Breakoff should never be de-activated by the user during normal operation.
e. A green ball in the up right corner of the Droplet Viewer indicates the status of the breakoff control
   - A solid green ball indicates that the droplet formation is being controlled properly and the sort delay calibration is valid. The system will sort correctly when the green ball is solid.
   - A flashing green ball indicates that the software is working to return the system to the calibrated state.
   - A grey ball indicates that the Control Breakoff has been temporarily deactivated by the software while a cleaning procedure (Probe Wash Cycle for example) is in progress.
   - It is normal to see the flashing green ball for a few seconds after a Probe Wash cycle is complete.



f. An error message will be displayed if the droplet drive amplitude changes too much. The error message recommends that the user run the Sort Calibration again. It is ok to continue sorting despite the error message, but the Sort Calibration should be run before initiating the next sort.

4. Shutdown with Daily Cleaning

a. Go to the Cytometer Tab and click on Hardware and Software Shutdown button
b. Click on Start and follow the software prompts for Normal Cleaning
   - Provide 10 mL of 10% bleach in a 15 mL tube
   - Provide 12 mL of sterile DI water in a 15 mL tube
c. When prompted, click on Shutdown and OK. The system will power off and the software will close.
d. Shut down the computer (optional)
e. Turn off the air compressor (or house air supply)

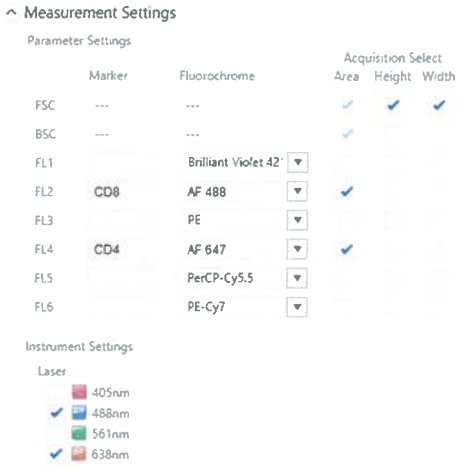
Training Exercise A: Simple 2-color Sort

1. Experiment Setup

a. Go to the Experiment Tab
b. Click on the New button
c. Template Options
   - Public Templates (select the Blank template)
   - My Templates
   - Recent Experiments
d. Experiment Manager
e. Experiment Information (overwrite the default name)
f. Sample Group Information

2. Measurement Settings

a. Uncheck the boxes for FL1, FL3, FL5, and FL6
b. Change the fluorochrome names for FL2 and FL4:
   - Change FITC to AF 488
   - Change APC to AF 647
c. Enter the marker names for FL2 and FL4:
   - For FL2 enter "CD8"
   - For FL4 enter "CD4"
d. Check the boxes to activate the 488 and 638 lasers

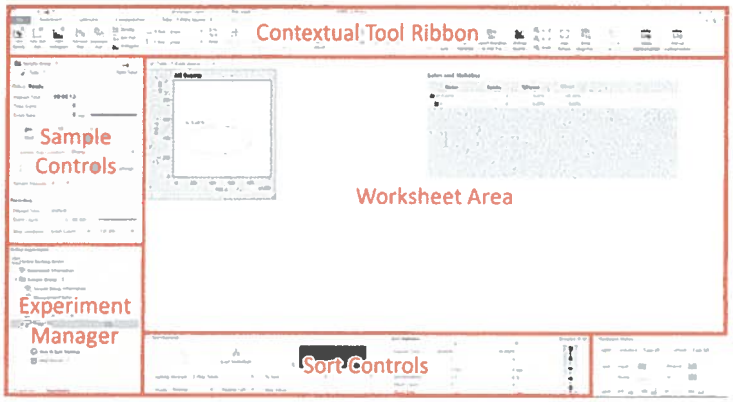


3. Create the Experiment

a. Click on the Create New Experiment button
b. The New Experiment Startup Procedure dialog box will appear, select the top option to go directly to Tube 1.

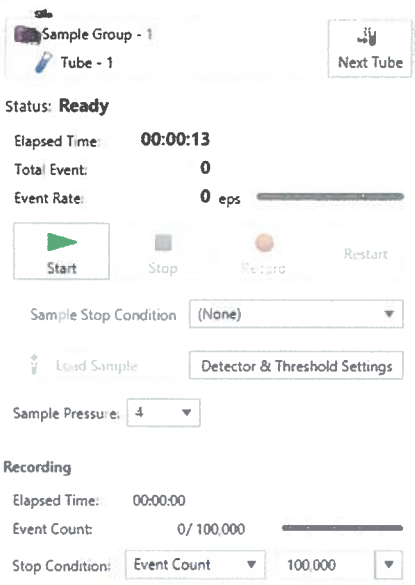
4. Main Screen Layout

a. Sample Controls
b. Experiment Manager
c. Sort Controls
d. Worksheet Area
e. Contextual Tool Ribbon



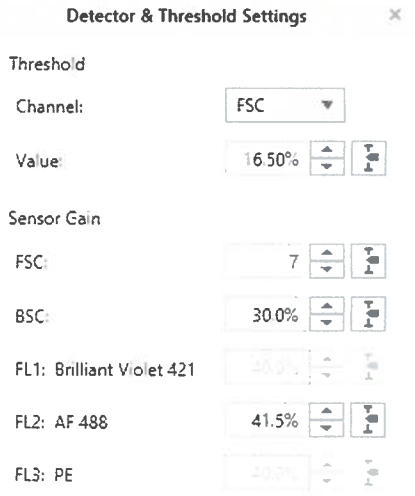
5. Run the 2-color Sample

a. Mount the sample tube
b. Click on the Start button
c. The sample tube is lifted into the sample chamber
d. Maximum sample pressure (the boost) is applied for a few seconds to force the sample fluid quickly through the sample line and into the sorting chip.
e. The sample pressure is then reduced to the selected level.
f. Sample flow is stable after 25 seconds.
g. Data recording or cell sorting should not be initiated until stable sample flow is achieved.



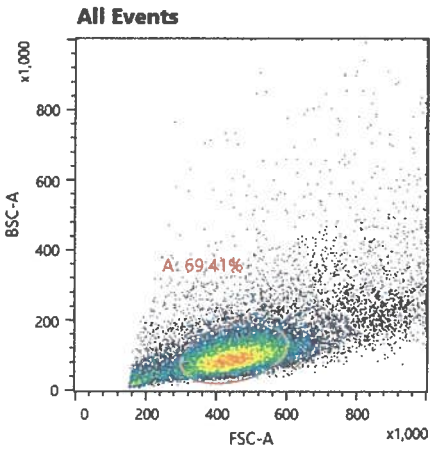
6. Adjust Detectors and Threshold

a. Click on Detectors & Threshold Settings
b. Adjust the FSC gain (1-16) to move the cells of interest to the middle of the x-axis scale on Plot 1
c. Adjust the BSC gain (0-100%) as needed
d. Adjust the Threshold value (0-100%) to eliminate excess debris (small particles of no interest or concern)
e. Click on Restart as needed to clear old data

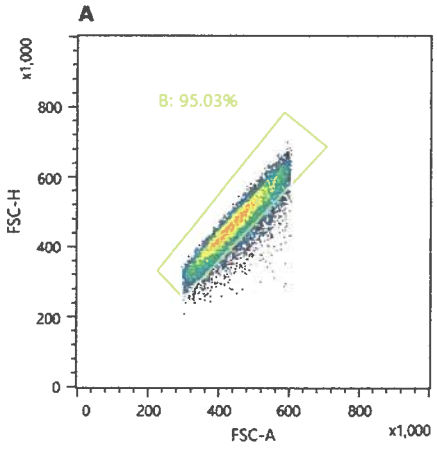


7. Worksheet Setup

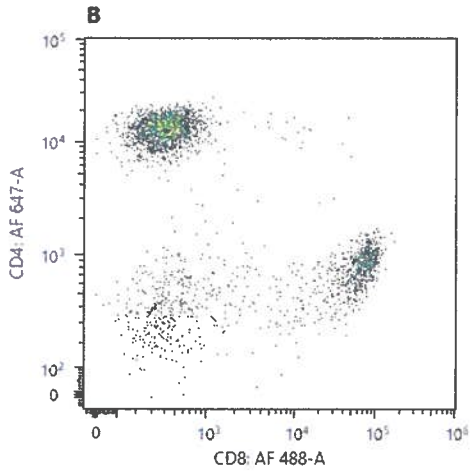
a. Plot 1 – Light Scatter
- Position Gate A around the cells of interest
- Re-size the gate as needed
- Rotate the gate as needed
- Convert Gate A to another shape:
  - Right-click on Gate A and select Convert to:
  - Choose Polygon
  - Drag any vertex (black dot)
  - Right click on any line and select Add Vertex
- Click on Undo as needed to restore Gate A
- Double click inside Gate A to create child plot gated on A, this will be Plot 2.



b. Plot 2 – Doublet Elimination
- Change the Y-axis of Plot 2 to FSC-H
  - FSC-A is the total forward light scatter signal produced as the cell passed through the laser.
  - FSC-H is the maximum intensity of the forward light scatter produced as the cell passed through the laser.
  - For single cells, the ratio of FSC-A to FSC-H is 1.
  - For cell aggregates (e.g. doublets) the ratio of FSC-A to FSC-H is less than 1.
- Draw a polygon gate around the single cells.
- Double click inside Gate B to create a child plot gated on B, this will be Plot 3.



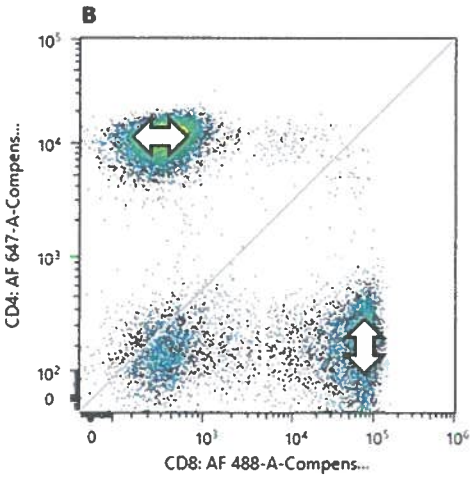
c. Plot 3 – Fluorescence
- Change X-axis and Y-axis to display the CD8 AF 488 and CD4 AF 647 parameters.
- Right-click on the plot and select Properties
- For both the X-axis and Y-axis:
  - Change the axis type to Biexponential
  - Set the max value to 100,000 or 1,000,000
  - Set the negative area to -500
  - Close the properties dialog box
- Adjust the FL2 and FL4 PMT values as needed to maximize the separation of positive cells from negative cells.



d. Accumulate some data in the display buffer
- DO NOT record data at this time
- Click on Restart to clear out the old data
- Let the data accumulate until populations are clearly displayed

e. Click on Pause to conserve sample.

f. Adjust Color Compensation
- Go to the Compensation Tab and click on Manual Compensation
- On Plot 3, click on the AF 488 positive population and drag it to the proper position
- Click on Manual Compensation again to de-activate this function.



8. Sort Setup

a. Create Sort Gates
   - Draw Rectangular gates around the CD4+ and CD8+ populations.
   - Right-click on each gate and select Properties from the menu.
   - Change the name of each gate to a meaningful name like "T4" or "CD4+"

b. Load Collection Tubes
   - Mount collection tubes in the appropriate tube holder.
   - Put at least 200 µL of PBS in each tube.
   - Mount the tube holder on the collection stage.
   - Go to the Sort Controls Click on Load Collection

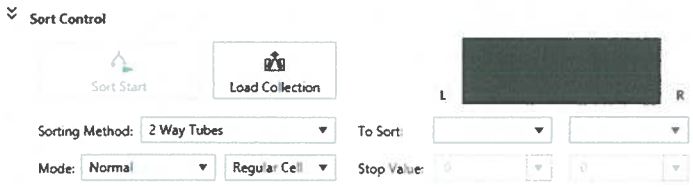
c. Select the Sorting Method:
   - 2 way Tubes

d. Select the Sorting Mode:
   - Ultra Purity (3 drop coincidence window)
   - Purity (2 drop coincidence window)
   - Semi-Purity (1.5 drop coincidence window)
   - Normal (1 drop coincidence window)
   - Semi-Yield (1 or 2 drop extended sort)
   - Yield (2 drop extended sort)
   - Ultra-Yield (3 drop extended sort)

e. Select Regular Cell or Large Cell
   - Use Regular cell setting if FSC gain is greater than 5
   - Use Large cell setting if FSC gain is 5 or less

f. Program the gates To Sort:
   - Select CD4+ from the left (L) tube pick list
   - Select CD8+ from the right (R) tube pick list

d. Program the Stop Value
   - A value of zero disables the automatic stop.
   - If desired, select a value from the pick list.
   - Alternatively, type in the desired value.

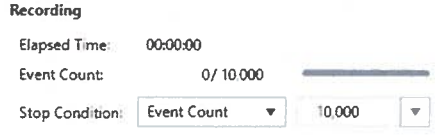


9. Initiate the Sort

a. Click on Resume to get the sample running again.
b. Click on Restart to clear out the old data.
c. Inspect the gate positions to ensure desired results.
d. Look at the Droplet Viewer to confirm that the droplet breakoff point is being maintained properly (solid green ball) and the sort calibration is valid.
e. Click on Start Sort

10. Record Data

a. Select the Stop Condition (Event Count)
b. Set Stop Condition Value (10,000)
c. Click on Record



11. Sort Statistics

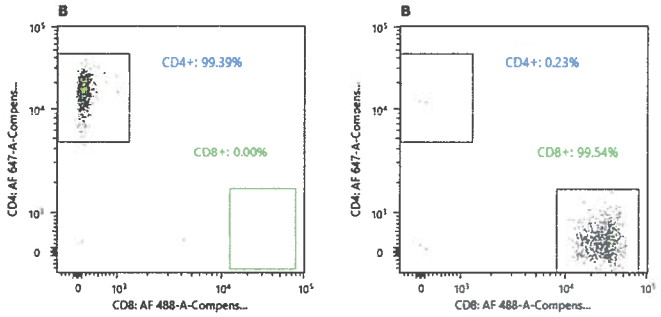
a. Elapsed Time
b. Sort Count
c. Sort Rate
d. Sort Efficiency
e. Abort Count
f. Abort Rate

12. Stop the Sort

a. Look at the Sort Count for the Left and Right tubes
b. 50,000 cells is enough for a good post sort analysis
c. Click on the Stop button

13. Post Sort Analysis

a. Remove the collection tube carrier from the sort collection area.
b. Click on Next Tube to add a tube to the experiment
   - Note that a second tab has been created in the Worksheet Area
   - The new worksheet tab is an empty template ready to receive data
   - The worksheet tab for Tube 1 can be viewed by clicking on that tab
c. Run water to ensure that the system is clean before doing post sort analysis
   - Mount a 15 mL tube with 10 mL of sterile deionized water on sample station
   - Click on Start
   - Increase the Sample Pressure to 10
   - After 30 seconds the Event Rate should be less than 3 events per second (eps)
   - Decrease the Sample Pressure to 4
   - Click on Stop
   - Because no data was recorded, the experiment tube can still be used. No need to create another tube.
d. Run one of the collection tubes
   - Mount one of the collection tubes on the sample station
   - Click start
   - Wait for at least 25 seconds for the sample flow to stabilize
   - Click on Record
   - 100 to 500 events is usually enough to evaluated the purity
   - Click on Stop
e. Create another experiment Tube (click on Next Tube)
f. Run water again (repeat section 13c above)
g. Run the other collection tube (repeat section 13d)



Training Exercise B: Multi-color Analysis Setup

1. Experiment Setup

a. Go the Experiment Tab
b. Click on New
c. Select the Blank Template from Public Templates
d. Overwrite the Experiment Name under Experiment Information

2. Measurement Settings

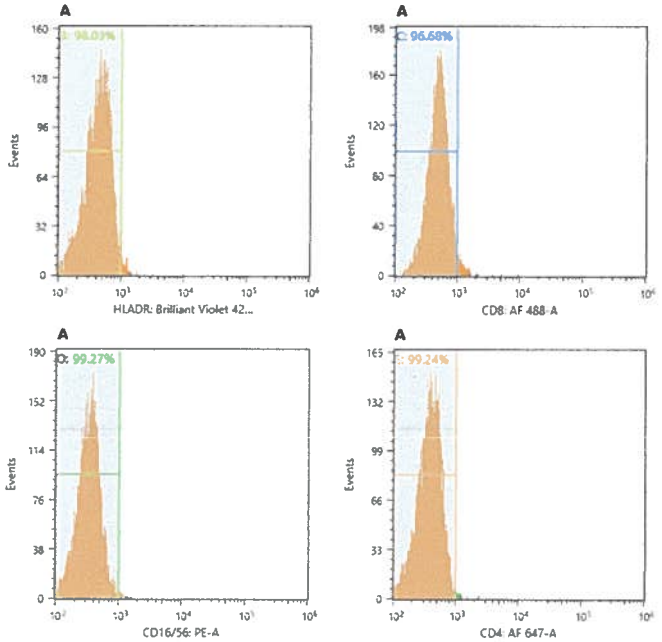
a. Check the boxes to activate FL1, FL2, FL3, and FL4
b. Uncheck the boxes to de-activate FL5 and FL6
c. Change the fluorochrome names:
   - Keep Brilliant Violet 421
   - Change FITC to AF 488
   - Keep PE
   - Change APC to AF 647
d. Enter the marker names:
   - For FL1 enter "HLA-DR"
   - For FL2 enter "CD8"
   - For FL3 enter "CD16/56"
   - For FL4 enter "CD4"
e. Check boxes to activate 405, 488, 561, and 638 lasers.

3. Create the Experiment

a. Click on Create New Experiment
b. The New Experiment Startup Procedure dialog box will appear.
   - Select the Start Compensation Wizard option
   - Click on OK.

4. Compensation Wizard

a. Follow the Compensation Wizard software prompts
b. Calculate the Color Compensation Matrix
c. Upon completion of the Compensation Wizard, the software will display the blank worksheet for Tube 1.



5. Run the Multi-color Stained Sample

a. Plot 1 – Light Scatter
   - Gate on the cells of interest
   - Double-click within the gate to create Plot 2
b. Plot 2 – Doublet Elimination
   - Change the Y-axis to FSC-H
   - Create a polygon gate around the single cells
   - Double-click within the gate to create Plot 3
c. Plot 3 – Fluorescence
   - Change the X-axis to CD8 AF 488
   - Change the y-axis to CD4 AF 647
   - Right-click on the plot
   - Select Duplicate to create Plot 4
d. Plot 4 – Fluorescence
   - Change the X-axis to HLA-DR BV421
   - Change the Y-axis to CD16/56 PE

6. Change the Axis Scaling

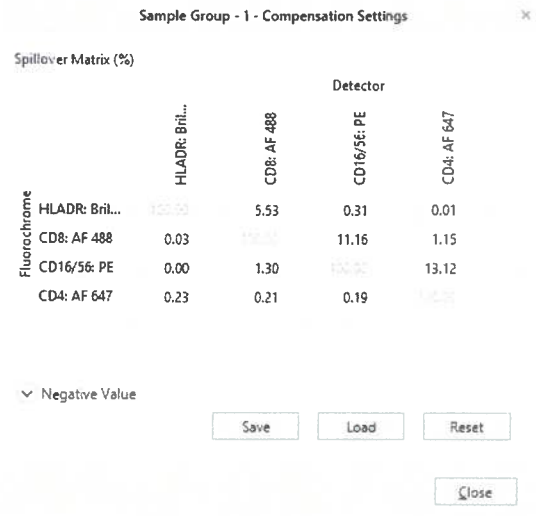
a. Right-click on the Plot 3 and select Properties from the menu
b. For both the X-axis and Y-axis:
   - Change the scaling to Biexponential
   - Change the maximum value to 100,000 or 1,000,000
   - Change the negative area to -500
c. Without closing the Properties Box, click on Plot 4 and make the same changes as above

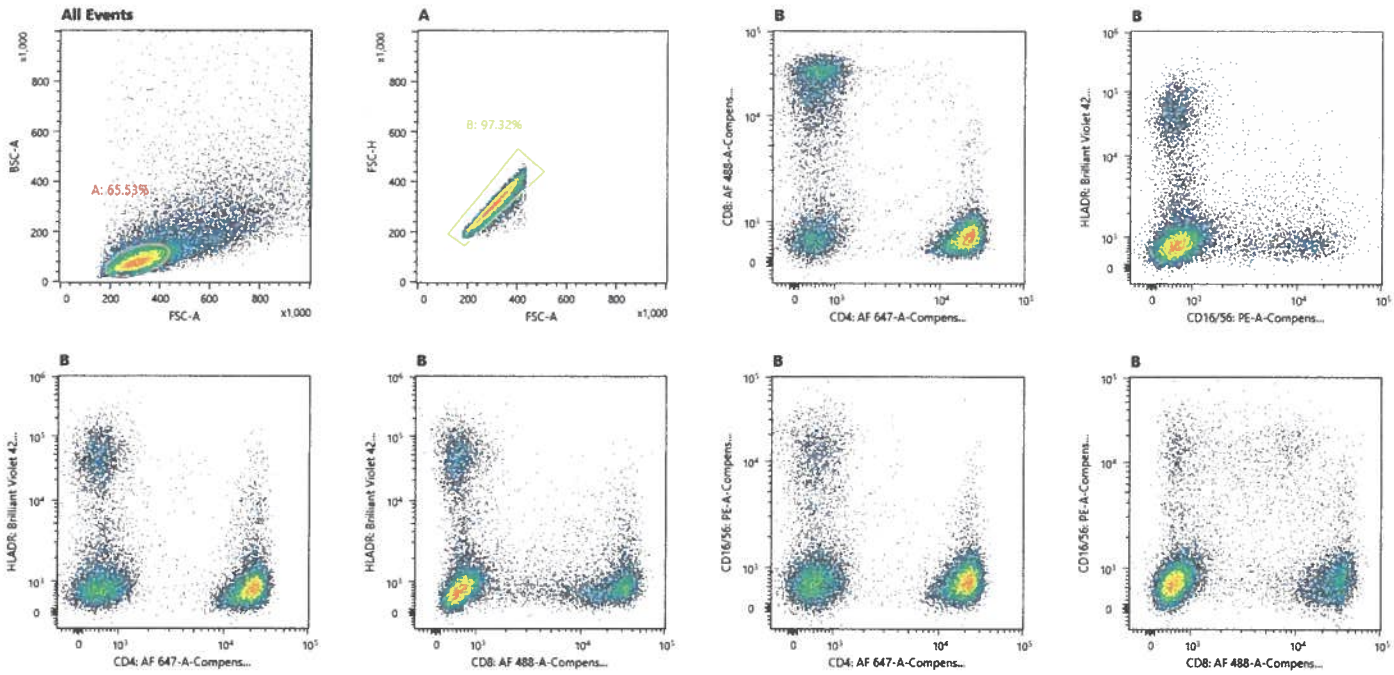
7. Create More Plots

a. Right-click on Plot 3 and select Duplicate. Repeat this 3 more times to create 4 new plots
b. Change the parameter of each new plot to make the remaining combinations:
   - CD4 AF 488 vs. HLA-DR BV421
   - CD8 AF 488 vs. HLA-DR BV421
   - CD4 AF 647 vs. CD16/56 PE
   - CD8 AF 647 vs. CD16/56 PE

8. Manual Color Compensation Adjustment

a. Go to the Compensation Tab and click on Manual Compensation.
   - Grab and drag populations.
   - The modified color compensation matrix is automatically applied to all tubes in the Sample Group.
b. Click on Manual Compensation again to de-activate this function.



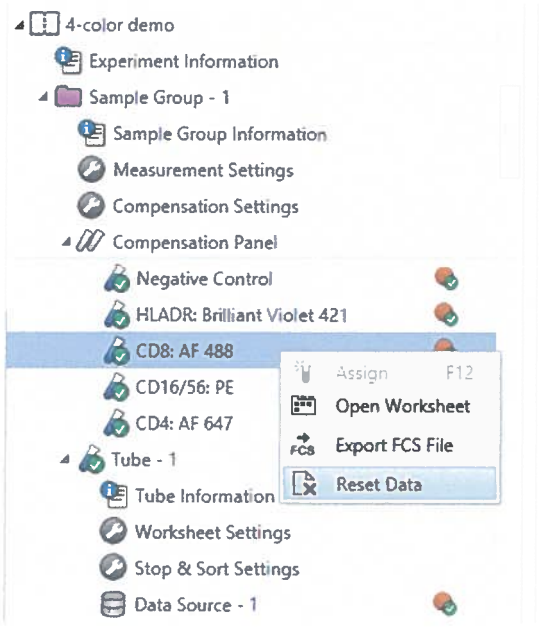


9. Re-calculate the Color Compensation Matrix

a. Go to the Compensation Tab and click on Calculate Matrix
b. The color compensation matrix is re-calculated based on the compensation control data stored in the experiment
c. The color compensation matrix is automatically applied to all tubes in the Sample Group.

10. Replace a Compensation Control

a. Go to the Experiment Manager
b. Expand the Compensation Panel
c. Right-click on the control you want to replace
d. Select Reset Data from the menu
e. Right-click again and select Assign to re-activate the tube for data acquisition
f. Mount the sample tube for the control
g. Click on Start
h. Record data
i. Go to the Compensation Tab and click on Calculate Matrix



11. Statistical Analysis

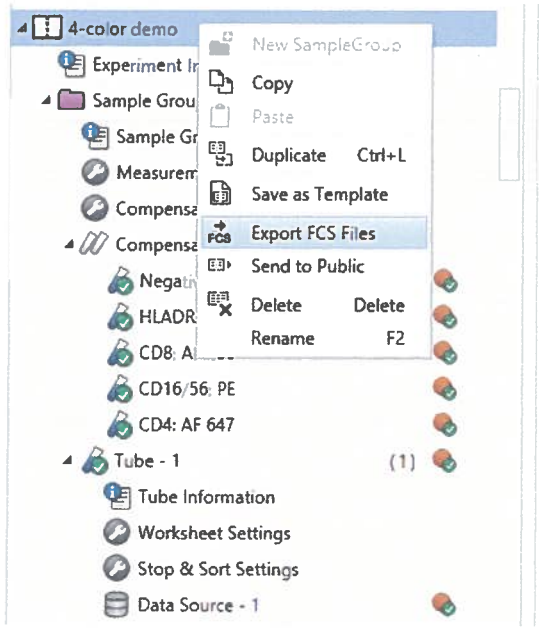
a. Create Quadrant gates on plots as desired
b. Edit the statistics table
c. Export the statistics to .csv

12. Reporting Data

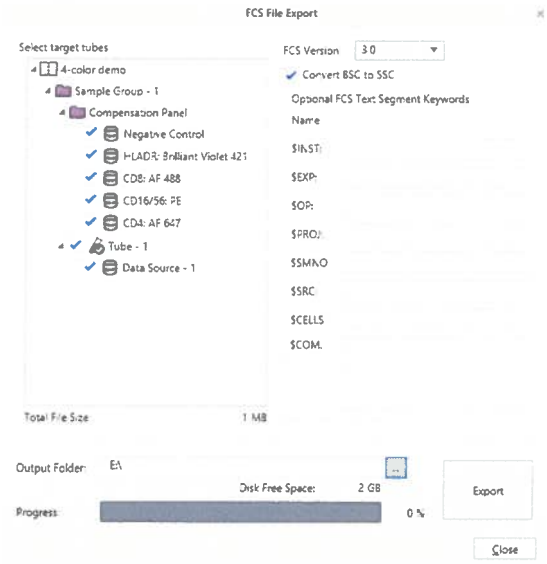
a. Print the pre-formatted report
   - Right-click on Worksheet
   - Select Print
b. Copy and paste a plot image
   - Right-click on a plot
   - Select Copy Plot Image
   - Paste into Paint or PowerPoint
c. Copy and paste a worksheet image
   - Right-click on the worksheet area
   - Select Copy Worksheet Image
   - Paste into Paint or PowerPoint

13. Export Data to .FCS Files

a. Go to the Experiment Manager
b. Right click on the name of the experiment you want to export
c. Select Export FCS File from the menu

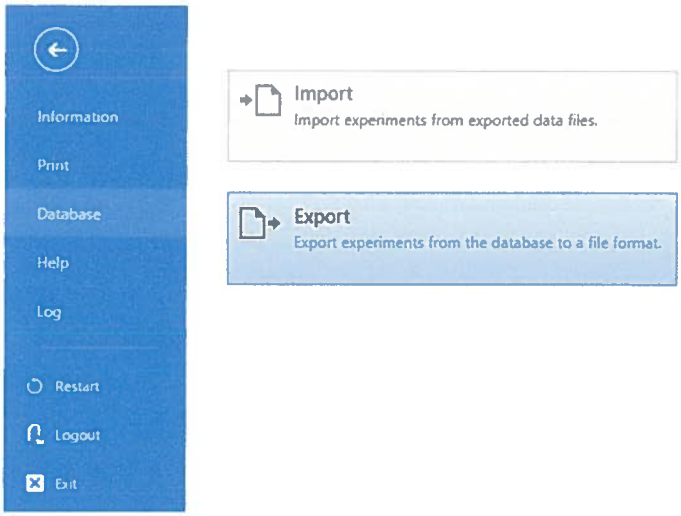


d. The FCS File Export dialog box will open
e. Check the data sources you want to export as FCS files
f. Navigate to the desired Output Folder
g. Click on Export

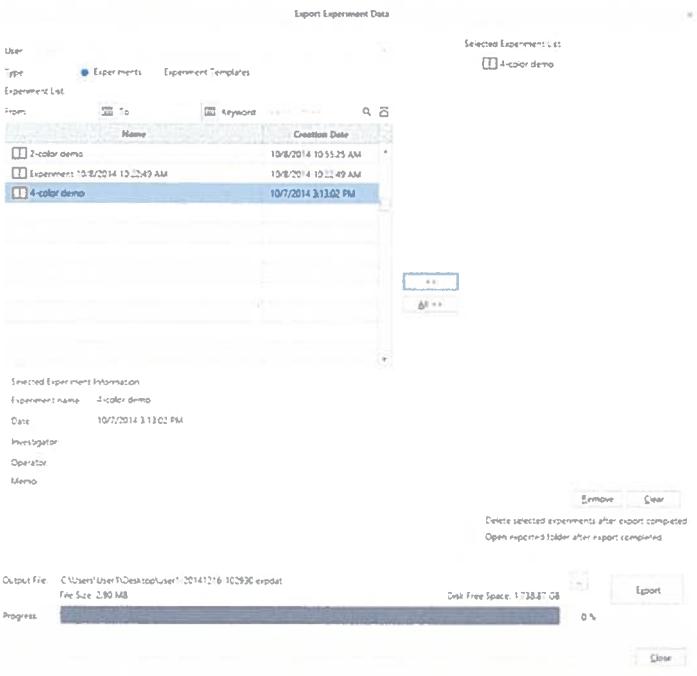


14. Export Data to a .expdat Database File

a. Go to the File menu
b. Click on Database
c. Click on the Export button



d. The Export Experiment Data dialog box will open
e. Highlight the experiments you want to export
f. Click on the arrow button to add to the Selected Experiment List
g. Navigate to the desired Output Folder
h. Click on Export



Training Exercise C: Plate Sorting

1. Mount a 96-well Plate

a. Attach the Plate Support Arm to the collection stage
b. Mount a 96-well plate into the Flat Plate Holder
   - The metal springs are in the back end and right side of the holder
   - Ensure that plate position A1 is in the front left corner of the holder (away from the springs)
c. Mount the Flat Plate Holder onto the support arm.
   - Ensure that all pins are aligned correctly
d. Leave the lid on the plate during plate position calibration.

2. Experiment Setup

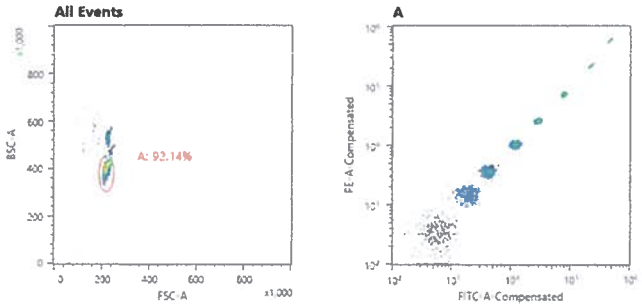
a. Go the Experiment Tab
b. Click on New
c. Select the Blank Template from Public Templates
d. Overwrite the experiment name under Experiment Information

3. Measurement Settings

a. Check boxes to activate FL2 and FL3 (FITC and PE)
b. Un-check boxes to de-activate FL1, FL4, FL5, and FL6
c. Check box to activate 488 laser

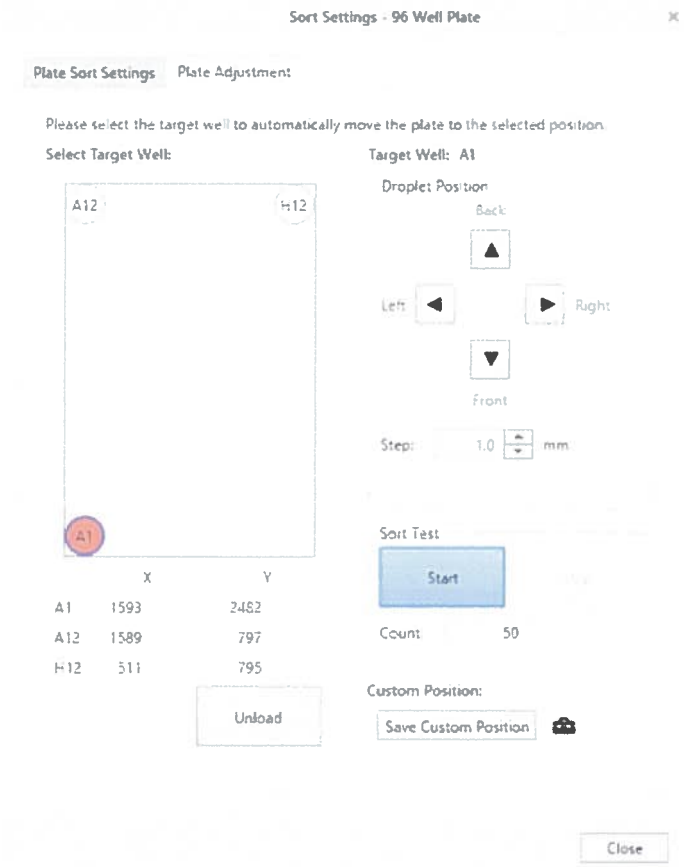
4. Run the 8-peak Bead Sample

a. Add 2 drops of 8-peak Beads to 500 µL water in a 5 mL tube
b. Mount sample on the sample station
c. Click on Start
d. Adjust FSC and BSC as needed
e. Gate on the beads
f. Double-click to create daughter plot (Plot 2)
g. Change the axis of Plot 2 to FITC and PE
h. Adjust PMTs as needed to resolve all 8 bead populations
i. Click on Pause



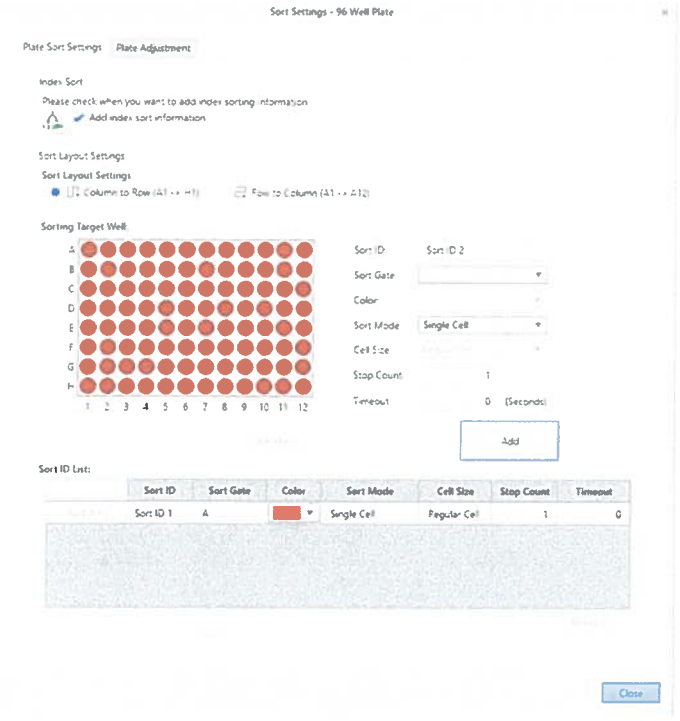
5. Plate Position Calibration

a. Go to the Sort Control and change the Sorting Method to 96 Well Plate
b. Click on the Sort Settings button
c. The Sort Settings – 96 Well Plate dialog box will appear
d. Click on the Plate Adjustment Tab
e. Calibration is performed on 3 test positions A1, A12, and H12
   - Click on A1, then click on Sort Test Start to deposit test droplets (50)
   - Click on A12, then click on Sort Test Start
   - Click on H12, then click on Sort Test Start
f. Remove the plate holder. Keep the plate mounted in the holder
g. Examine the position of each test puddle.
h. Make position adjustments as needed
   - Click on A1, then click the appropriate Droplet Position arrow buttons to change the position of droplet deposition. The default 1.0 mm step is good for 96 well plates
   - Click on A12, then click the appropriate Droplet Position arrow buttons to change the position of droplet deposition.
   - Click on H12, then click the appropriate Droplet Position arrow buttons to change the position of droplet deposition.
i. Re-mount the plate holder. Keep the plate mounted in the holder with the lid on.
j. Perform the calibration test again (Section 4i-k above)
k. Make more adjustments as needed (Section 4l above)
l. When finished with Plate Position Calibration, click on the Plate Sort Settings tab



6. Setup a Simple Index Sort

a. On the Plate Sort Settings tab, go to the Index Sort section and check the box to activate Index Sorting
b. Go to the Sort Layout Settings section and select the Columns to Row option
c. Go to the Sorting Target Wells section and drag the cursor over the plate to select all wells
d. Select Sort Gate: A. This gate contains all beads.
e. Color may remain red or choose another dark color.
f. Select Sort Mode: Single Cell.
   - Only use the Single Cell Sorting mode when sorting fewer than 5 cells per well.
   - Use the Purity mode when sort more than 5 cells per well
g. Change the Stop Count value to 1. This is one cell per well
h. Leave the Timeout value set at 0 seconds (disabled)
i. Click on the Add button to add this sorting job to the Sort ID List.
j. Click on the Close button



7. Initiate the Index Sort

a. Click on Resume to get the sample running again.
b. Click on Restart to clear out the old data.
c. Inspect the gate positions to ensure desired results.
d. Look at the Droplet Viewer to confirm that the sort calibration is valid (solid green ball in upper right corner)
e. Click on Index Sort Start

8. Monitor the Index Sort

a. The plate progress can be monitored by watching the diagram at the bottom center of the software screen

9. Plate Completion

a. Click on Finish if you do not want to perform the same sort on another plate
b. Click on Continue if you would like to perform the same sort on another plate
   - Exchange the plate
   - Click on Resume to get the sample running again
   - Click on Index Sort Start

9. Analyzing Index Sort Data

a. Load an Index Data Source
   - Go to the Experiment Manager
   - Double-click on the desired [Index 96 Well] Data Source
   - Click anywhere on the Worksheet to activate the Worksheet Tools Ribbon
   - Click on the Analyze Index Data button on the tool ribbon
   - The Index Analysis dialog box will open.
b. Well Select Mode
   - Click on any well.
   - A colored dot will appear on the data plots indicating light scatter and fluorescent intensities of the single bead sorted into the selected well.
   - Drag the cursor over the entire plate to select all wells. All sorted beads will be represented by colored dots in the data plots.
   - Click on a single well again. Then check the box for Show Cross Lines. The intensities can be read on the plot axis.
c. Gate Select Mode
   - Change the Analysis Mode to Gate Select Mode
   - Create a gate around any population on the FITC vs PE plot.
   - Select the new gate from the Selected Gate pick list.
   - The plate location is displayed for every sorted event that meets the criteria of the newly created gate.
   - Move the gate to a different population to display a different set of plate locations
   - Check the box for Overlay Index Events to display the each sorted event on the data plots

10. Export Index Sort Data to a CSV File

a. Go to the tool ribbon and click on the Export Data to CSV File button
b. Choose a path where the file can be saved (Desktop)
c. Click on Export
d. Go to the file location and open the file using Excel
e. The right-most column contains the well location value for each sorted event
f. The left-most column contains the timestamp for each sorted event
g. Each of the other columns contain the intensity values for every active parameter for each sorted event

11. Setup and run another 96 well plate sort

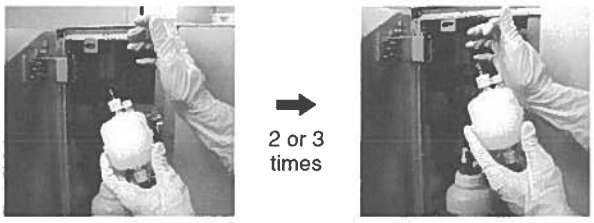
a. Create multiple sort jobs on the same plate.
b. Vary the gates, sort modes, and number sorted into each well.

Troubleshooting

1. Sheath De-bubble

Most problems are caused by air bubbles somewhere in the fluidic system. The Sheath De-bubble routine is the best way to removed air bubbles from the entire fluidic system.

a. Go to the Cytometer tab and Click on Sheath De-bubble
b. Confirm to continue
c. Open the instrument's left side panel
d. Remove the sheath filter from its holder
e. Strike the sheath filter with the heel of your hand



f. Hold the sheath filter so the upper vent cap is at very top.
g. Slowly open the vent cap to release trapped air.
h. When a small amount of liquid has escaped, close the vent.
i. Replace sheath filter and close the instrument panel.

2. Automatic Setup Failure

a. If the Automatic Setup fails, check the following items and then perform Sort Calibration again.
   - Confirm that the collection optics are in the default configuration
   - Check the sheath tank lid seal and release valve to make sure there are no air leaks.
   - Purge air bubbles from the fluidics system by performing the Sheath De-bubble routine (see Section 1 above).
   - Check the deflection plates and clean if needed.
   - Check the stream illumination laser windows and clean if needed.
   - Go to the Cytometer tab and click on the Sort Calibration button.
   - Follow the software prompts for Sort Calibration
   - If Sort Calibration fails, then reset the current chip.

b. Reset the Current Chip
   - Go to the Cytometer tab and click on Exchange Chip
   - Follow the software prompts for Exchange Chip
   - Swipe the QR code on the current chip package
   - Remove and reinsert the current chip
   - Proceed with the Automatic Setup
   - If the Automatic Setup fails, replace the current chip with a new chip

c. Replace the Current Chip with a Known Good Chip
   - Go to the Cytometer tab and click on Exchange Chip
   - Follow the software prompts for Exchange Chip
   - Swipe the QR code on the current chip package
   - Remove the current chip and insert the previously used chip that is known to work.
   - Proceed with the Automatic Setup
   - If the Automatic Setup fails again contact Sony Biotechnology technical support at 800-275-5963

3. Sample Path Clogs

If the data rate decreases to near zero, but the droplet formation is normal (as seen in the Droplet Viewer), then the sample path is clogged. This is easily remedied:

a. DO NOT click on Stop.
b. Click on Pause. This will pinch the sample line and stop the sample flow.
c. Click on Unload Sample
d. Go to the Cytometer tab and click on Probe Wash
e. Watch the sheath fluid drip from the sample probe. If need, repeat the Probe Wash until the sheath fluid drips at a normal rate, indicating that the sample path is clear.
f. Inspect the sample in the sample tube. If needed, filter the sample to remove clumps.
g. Re-mount the sample tube
h. Click on Load Sample
i. Click on Resume
j. Data should appear again within 90 seconds. Sorting will resume automatically.

4. Nozzle Clogs

If the data rate decreases to near zero and the droplet formation is disrupted (as seen in the Droplet Viewer), then the chip nozzle is clogged. Nozzle clogs are extremely rare, but are easily remedied:

a. The software should have already detected the problem and automatically stopped the sort. If not, click on Stop.
b. Perform the Sheath De-bubble routine (see section 1 above)
c. After the Sheath De-bubble is complete and the pressure has returned to normal, look at the droplet formation image in the Droplet Viewer. If needed, repeat the Sheath De-bubble until the droplet formation appears normal.
d. Go to the Cytometer tab and click on Sort Calibration. Follow the software prompts.
e. Return to your experiment, click on next tube, and reinitiate the sort.

5. What to Do If a Sample Runs Dry

a. The software should have already detected the problem and automatically stopped the sort. If not, click on Stop.
b. Perform the Sheath De-bubble routine (see section 1 above)
c. After the Sheath De-bubble is complete and the pressure has returned to normal, look at the droplet formation image in the Droplet Viewer. If needed, repeat the Sheath De-bubble until the droplet formation appears normal.
d. The system will automatically return to the Calibrated state. However, you may want to run the Sort Calibration to ensure accurate sorting.

Best Practices

1. Operate at the Lowest Possible Sample Pressure

a. Settings 1 to 5 are best
b. Settings 6 to 7 are ok
c. Settings 8 to 9 should be avoided
d. Setting 10 is for cleaning procedures only

2. Make Sort Samples as Concentrated as Possible

a. 5 million cells per mL is good
b. Some cells form clumps at high concentration

3. Filter Sort Samples to Remove Cell Clumps

a. 35 µm nylon mesh is recommended
b. Falcon™ Tube with Cell Strainer Cap (Corning 352235)
c. The sample may need to be re-filtered if sample path clogging if frequent (see Part E Section 2)

4. How to Run a Very Small Volume Sort Sample

a. Mount a sample tube containing only PBS or the buffer used for the sample cell suspension
b. Click on Start
c. Let the buffer run for 30 seconds (the boost)
d. Click on Pause
e. Click on Unload Sample
f. Replace the buffer tube with the sort sample tube
g. Click on Load Sample
h. Click on Resume
i. The sample will be delivered to the sorting chip under the selected sample pressure (no boost). It may take up to 60 seconds for data to appear.
j. Adjust sort gates as needed and initiate the sort

5. How to Run a Very Small Volume Post-sort Analysis

a. Mount a sample tube containing only PBS or the buffer used for sort collection
b. Click on Start
c. Let the buffer run for 30 seconds (the boost)
d. Click on Pause
e. Click on Unload Sample
f. Replace the buffer tube with the sample tube
g. Click on Load Sample
h. Click on Resume
i. The sample will be delivered to the sorting chip under the selected sample pressure (no boost). It may take up to 60 seconds for data to appear.
j. As soon as data begins to appear, click on Pause
k. Click on Unload Sample
l. Replace the sample tube with the buffer tube
m. Click on Load Sample
n. Click on Resume
o. Click on Record
p. Record data for until the data rate diminishes
q. Click on Stop

6. Stop the Sort Before the Sample Runs Dry

a. Refer to the table below. It provides the approximate sample consumption rate in µL per hour at each sample pressure setting.
b. The table also provides the total time (hours:minutes) to process specific volume of sample at each sample pressure.

Sample Pressure	Sample Rate	250 µL	500 µL	750 µL	1,000 µL	1,500 µL	2,000 µL	2,500 µL	3,000 µL	3,500 µL	4,000 µL
1	150 µL/h	1:40	3:20	5:00	6:40	10:00	13:20	16:40	20:00	23:20	26:40
2	400 µL/h	0:38	1:15	1:53	2:30	3:45	5:00	6:15	7:30	8:45	10:00
3	750 µL/h	0:20	0:40	1:00	1:20	2:00	2:40	3:20	4:00	4:40	5:20
4	1,000 µL/h	0:15	0:30	0:45	1:00	1:30	2:00	2:30	3:00	3:30	4:00
5	1,500 µL/h	0:10	0:20	0:30	0:40	1:00	1:20	1:40	2:00	2:20	2:40
6	2,000 µL/h	0:08	0:15	0:23	0:30	0:45	1:00	1:15	1:30	1:45	2:00
7	2,500 µL/h	0:06	0:12	0:18	0:24	0:36	0:48	1:00	1:12	1:24	1:36
8	3,000 µL/h	0:05	0:10	0:15	0:20	0:30	0:40	0:50	1:00	1:10	1:20

Cleaning for Aseptic Sorting

1. Sanitize the DI Water Supply

a. Remove the DI water filter and install the bypass line (green).
b. Remove the DI water tank and discard the water.
c. Fill the DI water tank 1/3 full will 70% ethanol.
d. Re-install the cap and probe assembly.
e. Swish the ethanol around in the DI water tank to wash the inner surfaces.
f. Re-install the DI water tank.
g. In the SH800 software, go to the Cytometer Tab.
h. Run 10 Probe Washes to flush ethanol through the DI water supply line.
i. Remove the DI water tank.
j. Under aseptic conditions, discard the remaining ethanol.
k. Under aseptic conditions, fill the DI water tank with sterile DI water.
l. Re-install the cap and probe assembly.
m. Re-install the DI water tank.
n. Run 5 Probe Washes to flush residual ethanol out of the DI water supply line.
o. Remove the bypass line and re-install the DI water filter.

2. Sanitize the Sample Line

a. Go to the Cytometer Tab and click on the Bleach Cleaning button.
b. Follow the software instructions for the Bleach Cleaning.
c. Go to the Cytometer Tab and click on the Shutdown Rinse button.
d. Follow the software instructions for the Shutdown Rinse.
e. DO NOT shut down the instrument.
f. Proceed to the next section.

3. Perform the Ethanol Cleaning

a. Go to the Cytometer Tab and click on the Ethanol Cleaning button.
b. Follow the software instructions for the Ethanol Cleaning.
c. The instrument will shut down automatically after the ethanol cleaning cycle.

4. Sanitize the Sheath Tank and Supply Line

a. Leave the instrument OFF.
b. Keep the air compressor ON.
c. Disconnect the sheath tank and dump the sheath fluid.
d. Add one liter of 70% ethanol and re-install the sheath tank lid.
e. Tumble the sheath tank to wash the inner surfaces.
f. Disconnect the waste line (red) from instrument's back panel.
g. Connect the waste line to sheath filter lower connecter.
h. Connect the sheath tank to sheath supply line (blue) and pressure line (clear).
i. Make sure the air pressure supply is ON.
j. Pump the ethanol out of the sheath tank, through the sheath supply line, and into waste tank.
k. Continue pumping until all the ethanol has been pumped out of the sheath tank.
l. If you have time, let the air flow through the sheath tank and supply line for about one hour to evaporate all the residual ethanol, then proceed directly to Section 6 below, otherwise perform Section 5 below.

5. Rinse the Sheath Tank to Remove Residual Ethanol

a. Skip this section if you completed the ethanol evaporation procedure in Section 4 Step l.
b. Disconnect the sheath tank.
c. Under aseptic conditions, add 0.5 liters of sterile sheath fluid to the sheath tank.
d. Re-install the sheath tank lid.
e. Swirl the fluid in the sheath tank to wash inner the surfaces.
f. Connect the sheath tank to sheath supply line (blue) and pressure line (clear).
g. Pump the fluid out of the sheath tank, through the sheath supply line, and into waste tank.
h. Continue pumping until all rinse fluid has been pumped out of the sheath tank.

6. Fill the Sheath Tank with Sterile Sheath Fluid

a. Disconnect the sheath tank.
b. Under aseptic conditions, add 5 to 10 liters of sterile sheath fluid to the sheath tank.
c. Re-install the sheath tank lid.
d. Connect the sheath tank to sheath supply line (blue) and pressure line (clear).
e. Pump sheath fluid out of the sheath tank, through sheath supply line, and into waste tank.
f. When sheath fluid begins to flow into the waste tank, disconnect the waste line (red) from the sheath filter lower connector.
g. Reconnect the waste line to the instrument's back panel for normal operation.
h. Reconnect the sheath filter to the lower connector.
i. Turn the air compressor OFF.

Software Review

1. File Tab
2. Experiment Tab
3. Cytometer Tab
4. Compensation Tab
5. Worksheet Tools Tab
6. Plot Tools Tab
7. Gate Tools Tab
8. Exporting FCS files

IT Considerations

1. Internet Connectivity
   a. Guest wireless network is usually sufficient
   b. Facilitates remote support via GoTo Assist and/or WebEx
2. Printing
   a. PDF print driver is recommended
   b. Network printers may require network connectivity
3. Backing up the database