SONY MA900 Cell Sorter Training Outline

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Biological Safety Cabinet (BSC) Option

1. Main Blower

a. Before you power up the MA900 Cell Sorter, make sure the BSC main blower is turned on. Otherwise the system will overheat and shut down.

2. Aerosol Management System (AMS)

a. If you are sorting hazardous material, make sure the AMS is operating on the LOW setting anytime that the sample is running.
   • Press the AMS blower button once to activate the LOW setting.

b. Before opening the Sort Collection Area Door (for example after sorting or to exchange plates), be sure to run the AMS on HIGH for 1 minute.
   • Press the AMS blower button again to activate the HIGH setting.

c. To turn the AMS off when it is not needed.
   • Hold the AMS blower button in until it goes off.

3. Cabinet Sashes

a. The front sash must be positioned at the red arrow stickers during normal operation.

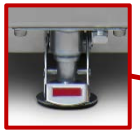
b. Some procedures (e.g. chip exchange or sample line exchange) require that the front sash be raised to its maximum height.
   • Silence the alarm by pressing the button.

c. Return the sash to the normal position after these procedures are completed.

d. The side sash on the left side of the BSC is used only by service personnel.

4. Fluidics Cart Lock

a. Note the location of the release lever and the red locking pedal



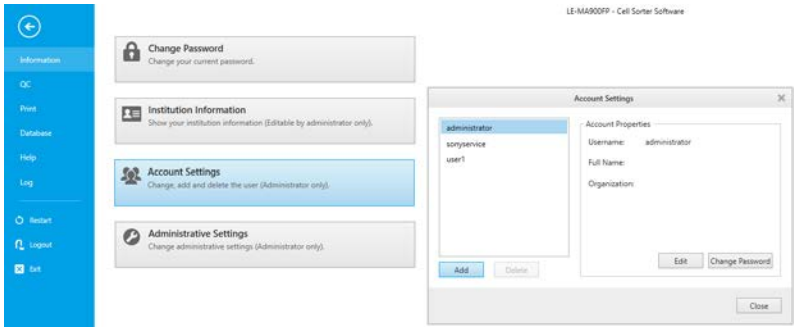
General Operation

1. Power Up

a. Make sure the main blower of the biosafety cabinet is turned on.
b. Step on the fluidics cart release lever and pull out the cart to access tanks.
c. Fill the sheath tank (if needed). Do not overfill the 10L line.
d. Empty the waste tank (if needed). Do not put bleach in the empty tank.
e. Push the fluidics cart back into place and step on the red locking pedal.
f. Wipe down the Sort Collection Chamber's surfaces and the deflection plates with water, followed by 70% ethanol, to remove static and PBS crystal buildup.
g. Turn on the air compressor. The switch is in the back on the upper right side. Then turn on the blue switch on the black air-line from the compressor to MA900.
h. Power up the MA900 cell sorter. The power button on the right side of the front.
i. Power up the computer (if needed). The power button is on the lower right edge.
j. Log into Windows 10 (username: fcm password: fcm).
k. Launch the Cell Sorter Software. Double-click on the desktop icon [MA].
i. Log in with user name and password. If new, see the next section to create a user.

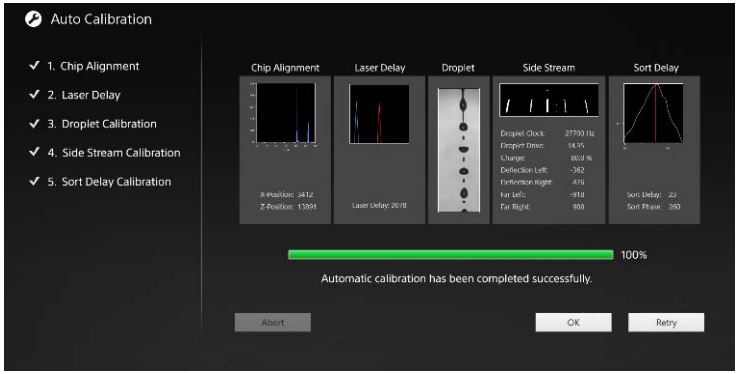
2. Create a New User (if needed)

a. Log in as the administrator (username: administrator password: administrator1)
b. Click on Information and then Account Settings
c. Click the Add button to add a new user.
d. Enter a Username and Password (must be 8-20 alphanumeric characters).
e. To finish, click on the Close button.
f. Click on Logout, then log in again with the username and password you created



3. Auto Calibration

a. Make sure that the Collection Tube Holder is NOT mounted in the Sort Collection Area.
b. Scan the QR code on a sorting chip package by holding it up to the camera on the computer.
c. Follow the software prompts for Auto Calibration.
d. Best Practice: During the fluidics check (first 10 min), click Sheath Filter De-bubble button and allow the procedure to complete.
e. When prompted, mount a sample tube containing at least 1 mL of SONY Automatic Setup Beads (P/N LE-B3001)
   - The sample tube should contain at least 1 mL (20 drops) of bead suspension.
   - Shake the dropper 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 to save for re-use for up to 1 week. Top off to about 1mL for another calibration.
     - Exception: For additional QC (called "Daily QC"), always use fresh beads
f. Click on OK to initiate the Auto Calibration process.



g. A confirmation screen will appear after Auto Calibration is complete.
h. Click on OK to continue.

4. Automatic Droplet Breakoff Control

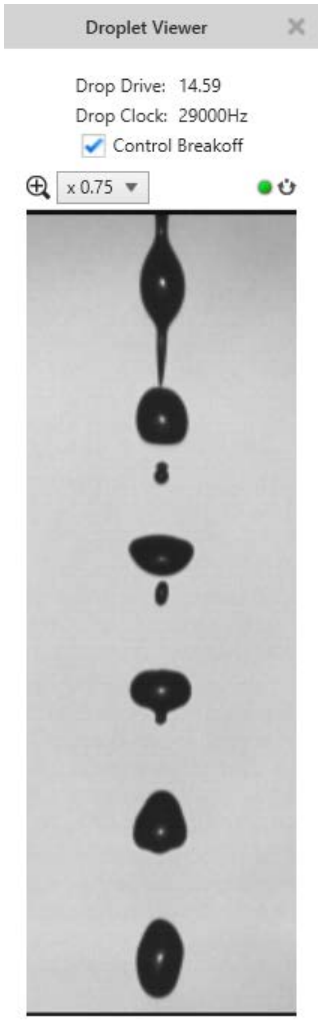
a. An image of the droplet formation is saved during sort delay calibration.

b. The software constantly monitors a live image of the droplet formation.

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 when the sort delay calibration was performed.

d. Control Breakoff check-box should never be de-activated by the operator.

e. A green ball in the upper 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 gray 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.

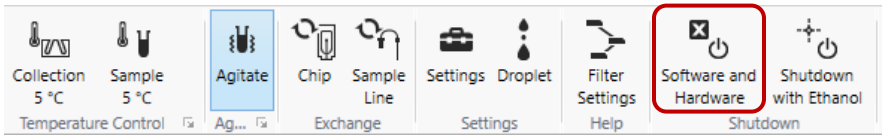


f. A warning message will be displayed if the droplet drive amplitude changes too much. The error message recommends that the user run the Sort Calibration again. It is ok to continue sorting despite the error message, but the Sort Calibration should be re-run before initiating the next sort.

5. Shutdown with Daily Cleaning

a. Click on the Cytometer tab (top of the screen) to display the Cytometer tool ribbon.

b. Click on Hardware and Software Shutdown.



c. Go to the Shutdown Wizard and click on the Start button to begin the cleaning.

d. Follow the software prompts for Normal Cleaning.
   - Provide 10 mL of 10% bleach (regular bleach diluted 1:10) in a 15 mL tube
   - Provide 12 mL of sterile DI water in a 15 mL tube

e. When prompted, click on the Shutdown button and OK to confirm.
   - The MA900 Cell Sorter will power off.
   - The software will close.

f. Wipe down the inner surfaces of the deflection plates and the outer surfaces of the waste catcher using a few Kim-wipes wetted with 70% ethanol.

g. Turn off the air compressor and blue switch on the air-line

h. Turn off the BSC main blower (optional).

i. Close the BSC front sash (optional).

j. Shut down the computer (optional).

Training Exercise A: Simple 5-color Sort into Tubes

1. Experiment Setup

a. Click on the Experiment tab at the top left of the screen

b. Click on the New button. This will open the Experiment Template Screen.

c. Note the template options:

- Public Templates
- My Templates
- Recent Experiments

d. Under Public Templates, click on the Blank Template.

e. Under Experiment Information, overwrite the default experiment name.

2. Measurement Settings

a. Uncheck all the FL Area boxes EXCEPT FL1, FL5, FL6, FL9, and FL10

b. Change the FL1 and FL10 fluorochrome names:

- Change FL1 from FITC to Alexa Fluor 488
- Change FL10 from APC to Alexa Fluor 647

c. Enter the marker names for FL1, FL5, FL6, FL9, and FL10

- For FL1 enter “CD8”
- For FL5 enter “NK”
- For FL6 enter “CD19”
- For FL9 enter “CD3”
- For FL10 enter “CD4”

d. Check the boxes that activate the 405, 488, and 638 lasers.

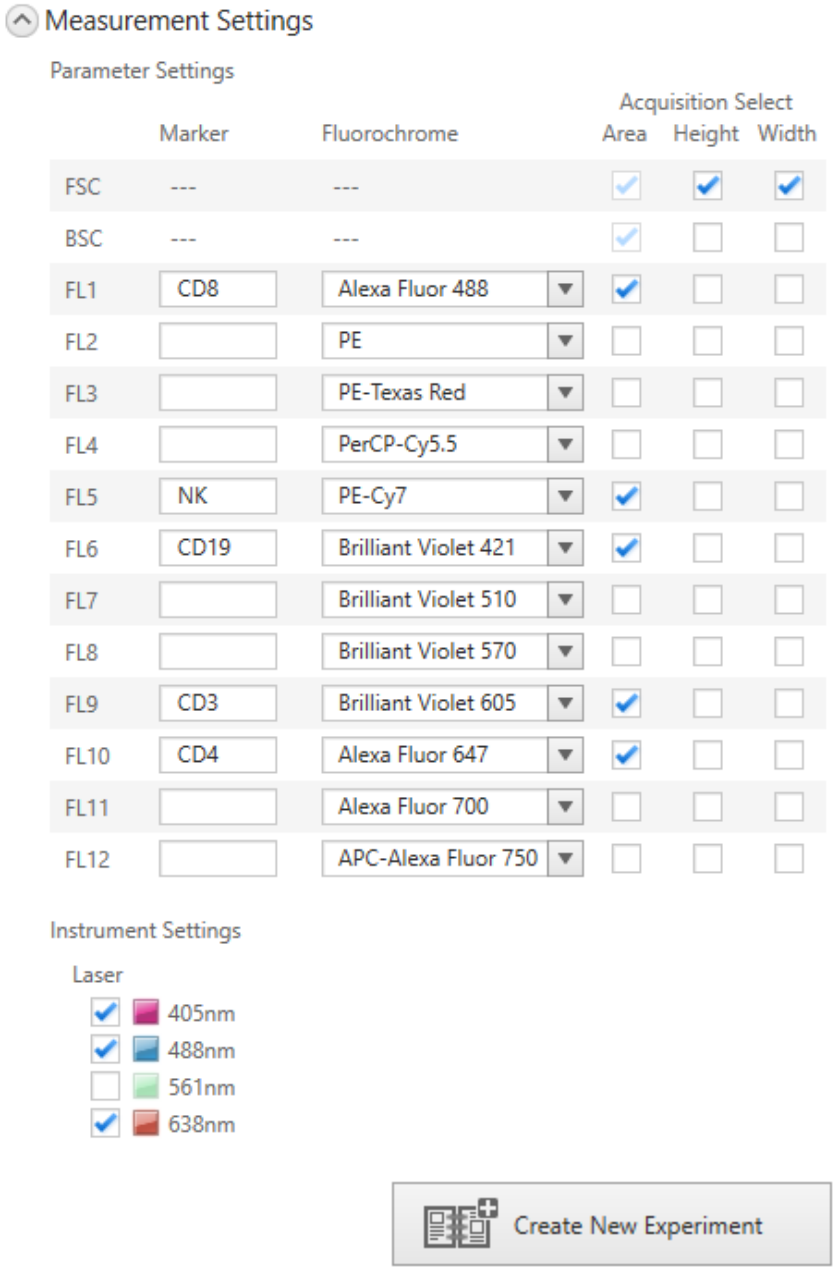
e. Uncheck the box that activates the 561 laser.

- The 561 laser is used primarily for fluorescent proteins (e.g. RFP, mCherry, etc.)
- The 561 laser is not recommend for antibody stained samples, especially when PE and BV570, PE and BV605, or PE-TR and BV605 are combined.

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. Run the Unstained Sample

a. Mount the sample tube.

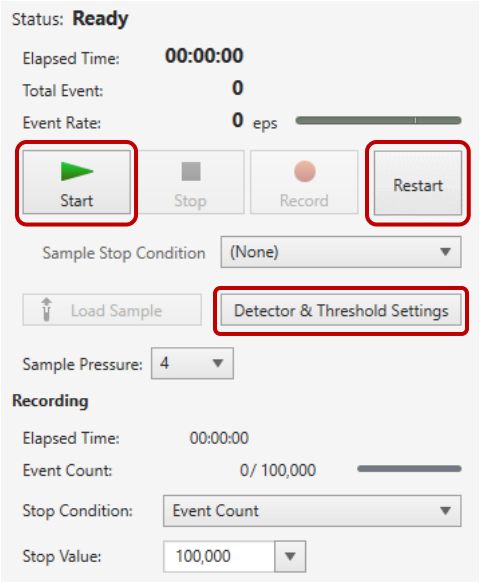
b. Click on the Start button [▶]

c. The sample tube will be lifted and sealed inside 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 (default is 4).

f. Sample flow is stable after 30 seconds. Do not start data recording or cell sorting until stable sample flow is achieved.



5. Adjust the Detectors and Threshold

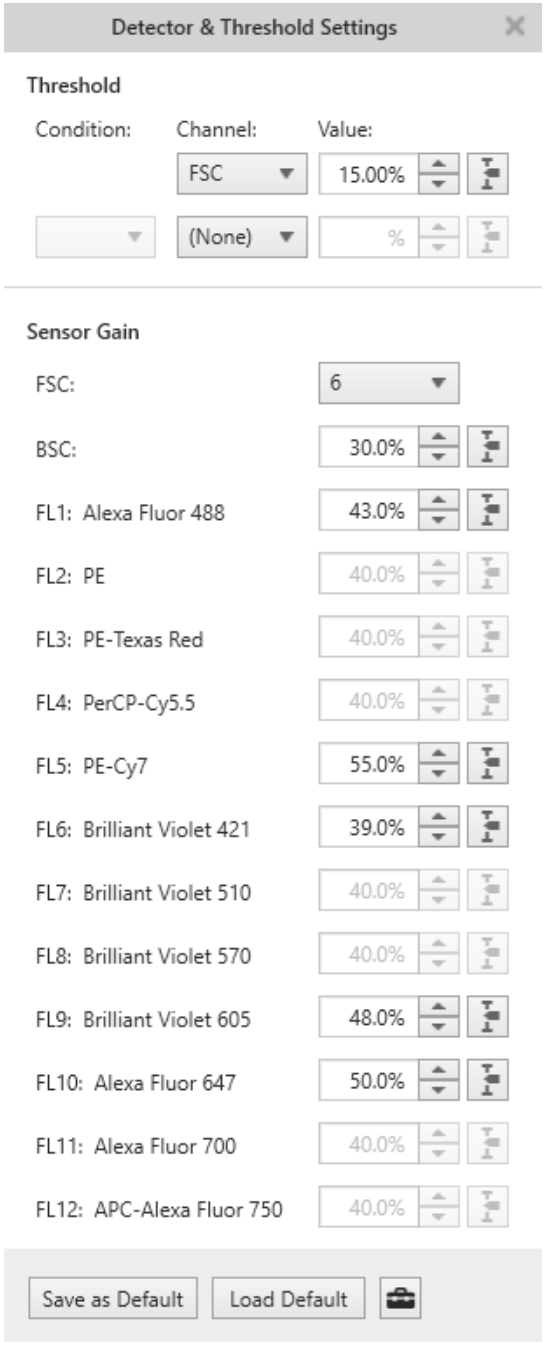
a. Click on the Detector & Threshold Settings button.

b. Go to the Detector & Threshold Settings Window and adjust the FSC Sensor Gain to position the cells of interest to the center of Plot 1.

c. Adjust the BSC Sensor Gain as needed.

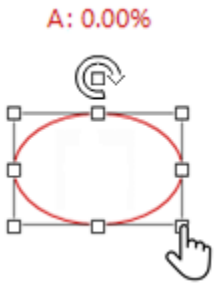
d. Adjust the Threshold Value to eliminate excess debris (small particles of no interest or concern).

e. Click on the Restart button as needed to clear old data from the display buffer.



6. Plot 1: Light Scatter (FSC and BSC)

a. Position Gate A around the cells of interest.



- Click anywhere inside Gate A to select.
- Click and hold to grab handles.
- Grab anywhere inside Gate A and drag to move.
- Grab and drag any corner handle to re-size.
- Grab and drag the top handle to rotate.
- Grab and drag the label to move it.

b. Convert Gate A to a Polygon.

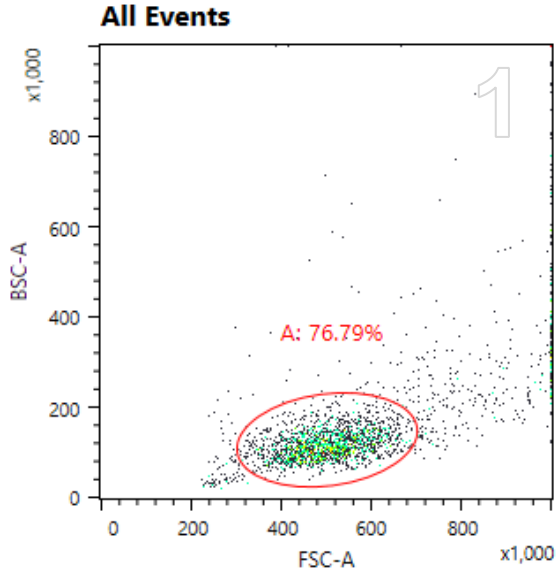
- Right-click on Gate A and hover over Convert to:
- Click on Polygon

c. Modify the Polygon

- Click, grab, drag, and release any vertex (—■—) to reposition it.
- Right-click on any line segment (——) and click on Add Vertex to add a vertex.
- Right-click on any vertex (—■—) and select Delete Vertex to remove it.

d. Click on Undo (CTRL+Z) as needed to restore Gate A to an ellipse.

e. Double click inside Gate A to create child plot gated on A, this will be Plot 2.

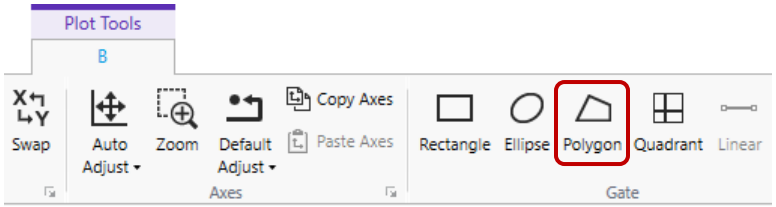


7. Plot 2: Doublet Elimination

a. Leave the X-axis as FSC-A. Change the Y-axis to FSC-H.

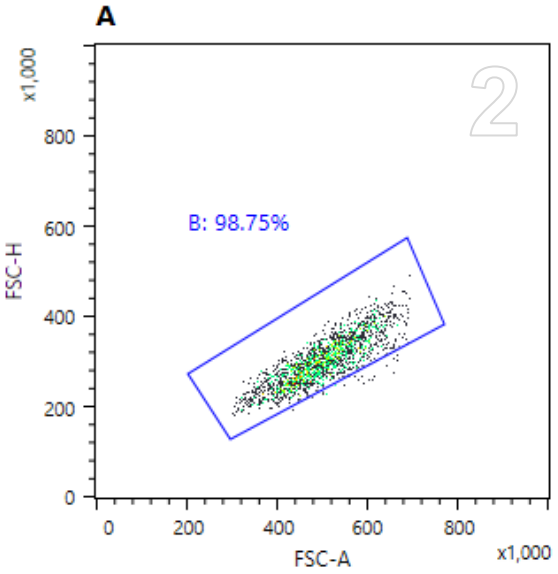
- FSC-A is the total forward light scatter signal measured as the cell passed through the laser.
- FSC-H is the maximum intensity of the forward light scatter measured as the cell passed through the laser.
- For single cells, the ratio of FSC-A to FSC-H is 1.
- For cell aggregates (e.g. doublets) the ratio of FSC-A to FSC-H is less than 1.

b. Go to the Plot tools ribbon at the top of the screen and click on Polygon.



c. Draw a polygon gate around the single cells, this will be Gate B.

d. Double click inside Gate B to create a child plot gated on B, this will be Plot 3.



8. Plots 3-6: Fluorescence

a. Go to Plot 3 and change the X-axis to CD3 Brilliant Violet 605.

b. Also on Plot 3, change the Y-axis to CD19 Brilliant Violet 421.

c. Right-click on Plot 3 and select Duplicate to create Plot 4.

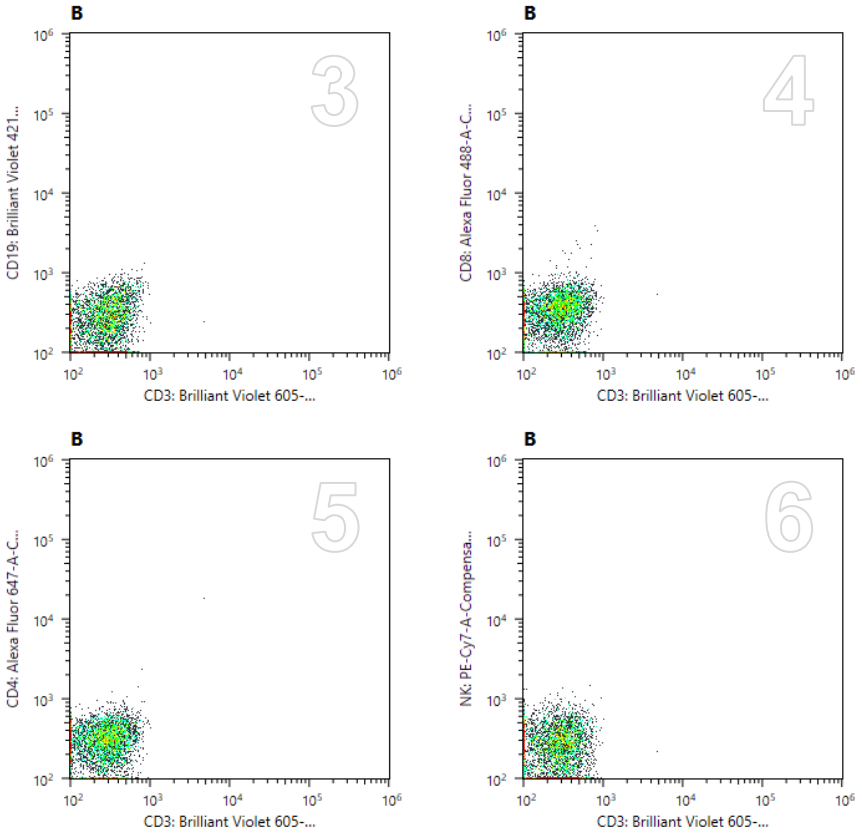
d. On Plot 4, change the Y-axis to CD8 Alexa Fluor 488.

e. Right-click on Plot 4 and select Duplicate to create Plot 5.

f. On Plot 5, change the Y-axis to display CD4 Alexa Fluor 647.

g. Right-click on Plot 5 and select Duplicate to create Plot 6.

h. On Plot 6, change the Y-axis to NK PE-Cy7.

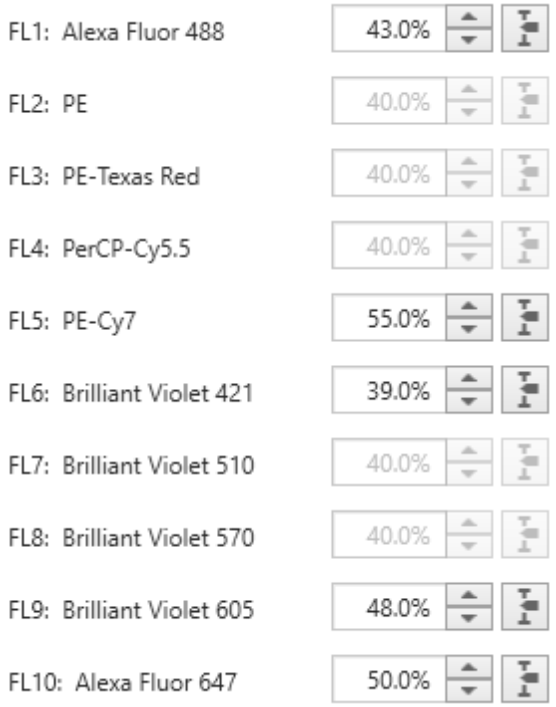


9. Adjust the Fluorescence Detectors (PMT Voltages)

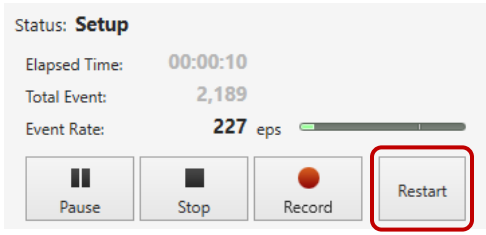
a. Click on the Detectors & Threshold Settings button.

b. Adjust the PMT Voltage % for FL1, FL5, FL6, FL9, and FL10.

c. The data dots should fill the lower left corner of each fluorescence plot.



d. Click on the Restart button as needed to clear old data from the display buffer.



e. After all adjustments are made, close the Detectors & Threshold Settings window.

10. Record Data

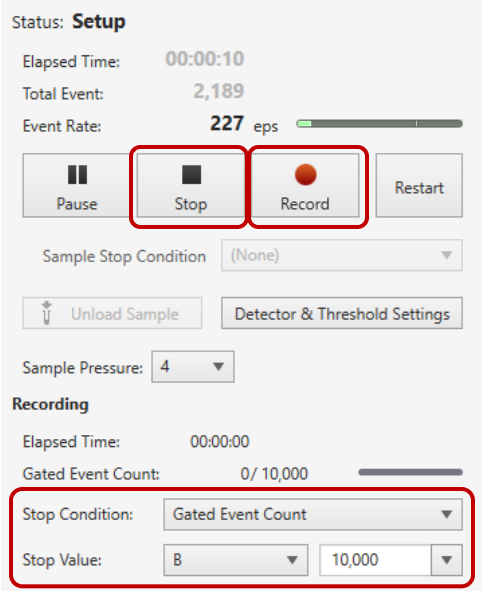
a. Under Recording, change the Stop Condition to Gated Event Count

b. Select “B” from the gate select dropdown list.

c. Set the Stop Value to 10,000.

d. Click on the Record button [●]

e. When the recording is complete, click on the Stop button [■] to stop the sample.

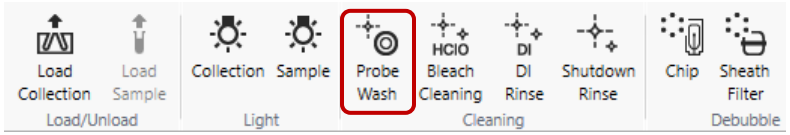


11. Automatic Probe Wash

a. After every sample Stop [■] the sample probe is automatically cleaned.

- Sheath fluid backflushes from the sorting chip through the sample line and sample probe to wash away cells.
- The outside of the sample probe is washed with a cascade of sheath fluid.

b. A Probe Wash can also be initiated on demand from the Cytometry tool ribbon:

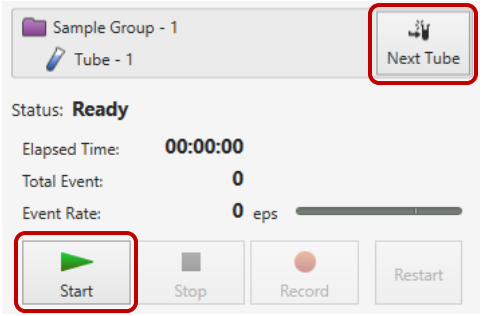


12. Run the Stained Sort Sample

a. Click on the Next Tube button to create a worksheet for the next tube.

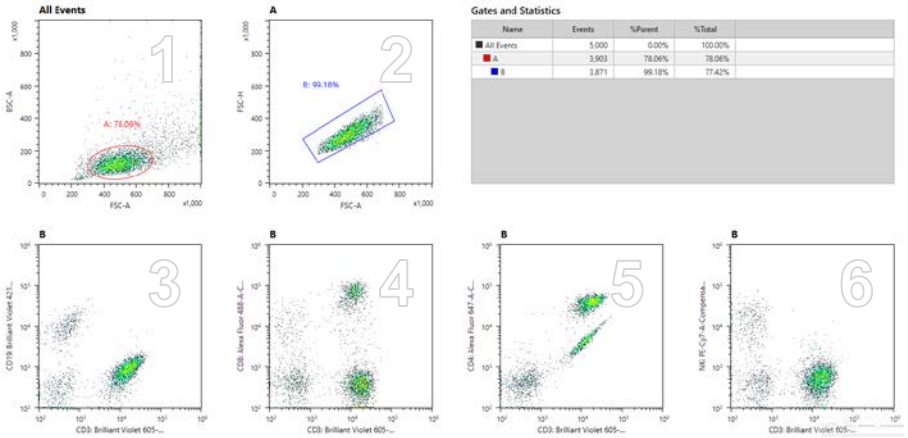
b. Mount the sample tube.

c. Click on the Start button [▶]



d. Let the sample run for 30 seconds (the boost), then click on the Restart button to clear any data that accumulated during the boost.

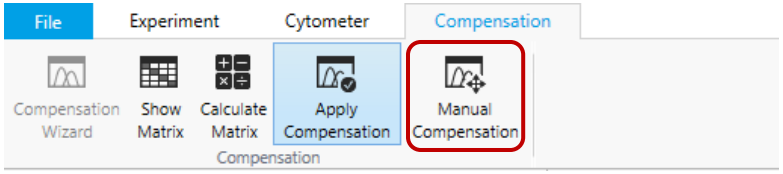
e. Let data accumulate in the plots until all the populations can be seen, then click on the Pause button [❚❚] to conserve the sample while you adjust color compensation, create sort gates, and configure the sort settings.



13. Adjust Color Compensation (Manual Method)

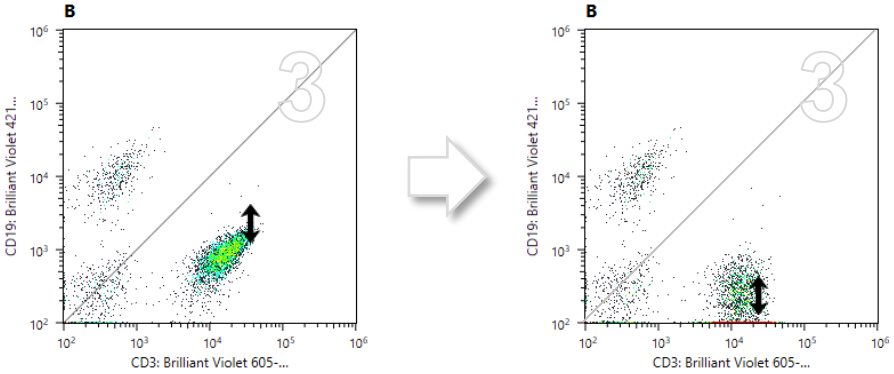
a. Click on the Compensation tab at the top of the screen to display the Compensation tool ribbon.

b. Click on Manual Compensation



c. Go to Plot 3 (CD19 Brilliant Violet 421 vs. CD3 Brilliant Violet 605)

d. Click and grab the CD3+CD19- population. Drag down until the CD3+CD19- population is even with the CD3-CD19- population with respect to the Y-axis.



e. Click and grab the CD3-CD19+ population. Drag left until the CD3-CD19+ population is even with the CD3-CD19- population with respect to the X-axis.

f. Perform the same procedure on any other plots that require color compensation adjustment (e.g. Plot 5: CD4 Alexa Fluor 647 vs. CD3 Brilliant Violet 605).

g. Go to the Compensation tool ribbon and click on Manual Compensation to deactivate the mode.

h. The adjusted color compensation will be applied to all the data in the sample group, as Apply Compensation is active by default.

14. Create Four Sort Gates

a. Click on Plot 3, then go to the Plot tools ribbon and click on Rectangle.

b. Drag and drop a rectangular gate around the CD3-CD19+ cell population.

c. Click on Plot 4, then go to the Plot tools ribbon and click on Rectangle.

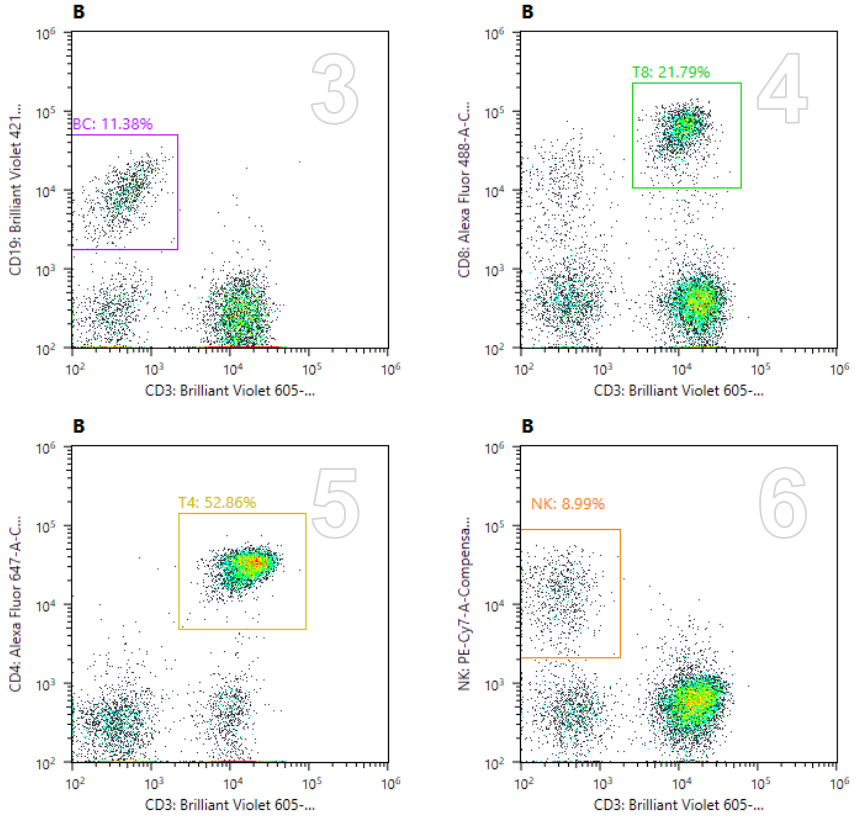
d. Drag and drop a rectangular gate around the CD3+CD8+ cell population.

e. Click on Plot 5, then go to the Plot tools ribbon and click on Rectangle.

f. Drag and drop a rectangular gate around the CD3+CD4+ cell population.

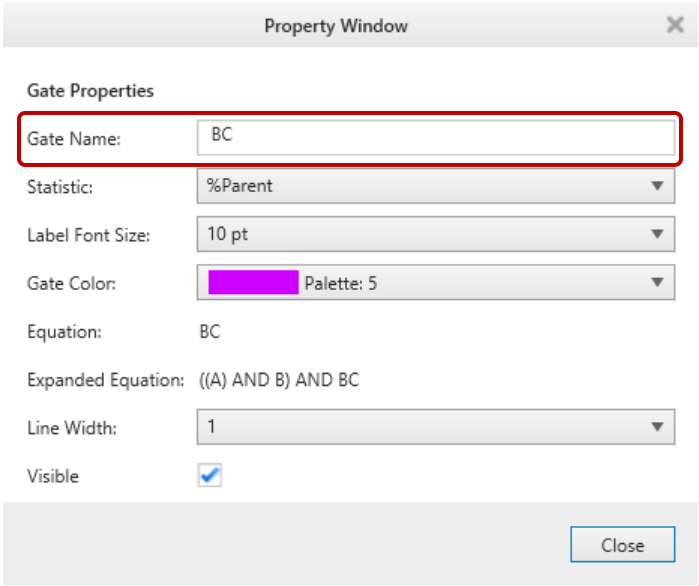
g. Click on Plot 6, then go to the Plot tools ribbon and click on Rectangle.

h. Drag and drop a rectangular gate around the CD3-NK+ cell population.



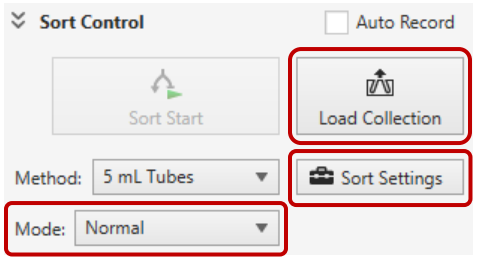
15. Rename the Sort Gates

a. Go to Plot 3 and Right-Click on the gate around the CD3-CD19+ cell population.
b. Select Properties from the quick pick list. This will open the Property Window.
c. Go to the Property Window and type in a new Gate Name (e.g. BC).
d. Do not close the Property Window, simply press [Enter] to apply the gate name.
e. Go to Plot 4 and click on the gate around the CD3+CD8+ cell population.
f. Go back to the Property Window and type in a new Gate Name and press [Enter].
g. Go to Plot 5 and click on the gate around the CD3+CD4+ cell population.
h. Go back to the Property Window and type in a new Gate Name and press [Enter].
i. Go to Plot 6 and click on the gate around the CD3-NK+ cell population.
j. Go back to the Property Window and type in a new Gate Name and press [Enter].
k. Click on the Close button to close the Property Window

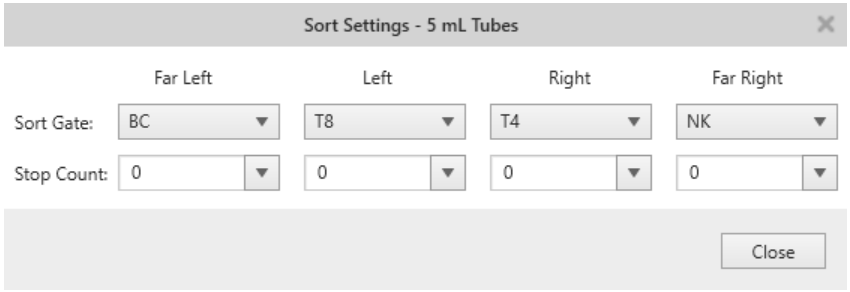


16. Configure the Sort Settings

a. Make sure you have the Four Tube Holder mounted in the Sort Collection Area.
b. Mount four 5 mL tubes. Each tube should contain about 200 µL of PBS.
c. Go to the Sort Control at the bottom of the screen and click on Load Collection.
d. Click on the Mode: drop down list and select Semi-Purity.



e. Click on the Sort Settings button. This will open the Sort Settings Window.



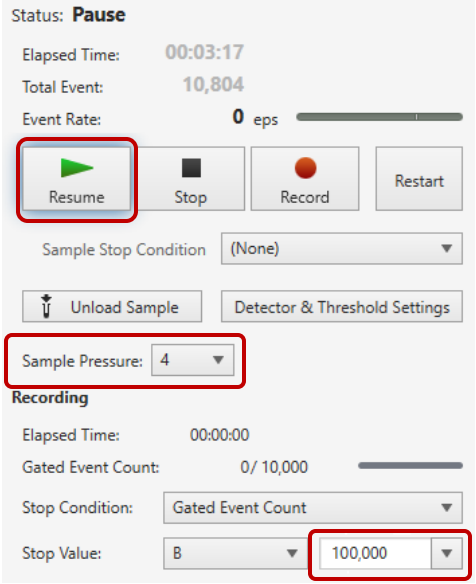
f. Go to the Sort Settings Window and select a population to sort to each tube.
g. If the Stop Count is left at zero, sorting will continue until it is stopped manually.
h. You may enter a Stop Count number for each tube or select a number from each drop down list.
i. Each Stop Count is independent. Sorting into each tube will stop when its sort count is met, but sorting will continue into the other tubes.

17. Resume the Sample

a. Click on the Resume button [▶] to restore the sample flow.

b. Adjust the Sample Pressure to get the Event Rate you want.

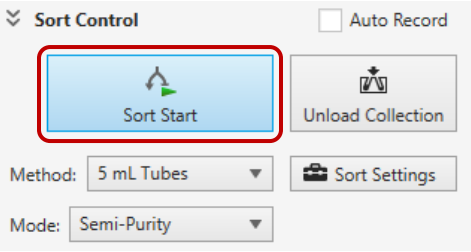
c. Change the Recording Stop Value to an appropriate number.



18. Start the Sort

a. Check the position of all the gates and make adjustments as needed.

b. Go to the Sort Control and click on the Sort Start button.



19. Record Data

a. Make sure the Recording Stop Condition and Stop Value are set appropriately.

b. Click on the Record button [●].

c. Recording will stop automatically when the stop condition is met.

d. Sorting will continue. Sorting is independent of recording.

20. Sort Statistics

Sort Statistics	Far Left	Left	Right	Far Right
Sort Gate:	BC	T8	T4	NK
Elapsed Time:	00:03:47	00:03:47	00:03:47	00:03:47
Remaining Time:				
Sort Count:	18,467	35,473	85,111	13,571
Sort Rate:	64 eps	133 eps	333 eps	46 eps
Sort Efficiency:	95 %	95 %	96 %	95 %
Abort Count:	836 (4 eps)	1,678 (10 eps)	2,989 (13 eps)	648 (1 eps)

a. Look at the Sort Statistics displayed in table at the bottom of the screen.

b. If you had entered Stop Counts (see 16-h above), the estimated Remaining Time would be displayed for each tube.

c. Sort Count is the number of target cells sorted into each tube.

d. Abort Count is the number of target cells aborted (not sorted) because of coincidence with another cell in the droplet.

e. The Sort Efficiency is the percent of total target cells that have been sorted.
   • Sort Efficiency = (# sorted / (# sorted + # aborted)) * 100

f. If the Sort Efficiency is to low, do one or both of the following things:
   • Reduce the Event Rate by lowering the sample pressure.
   • Consider using a different Mode. Normal mode will give the highest efficiency. (*To change the Mode, you will need to stop the current sort, click on next tube button, load the collection, start the sample, select the new mode, and start a new sort.)*

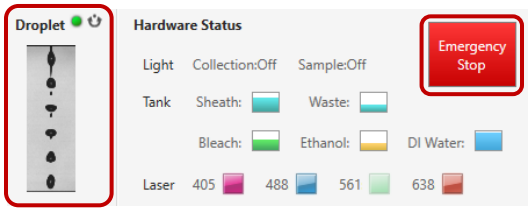
21. Unclogging the Chip

a. If a large clump of cells is introduced into the system it may clog the sample path.

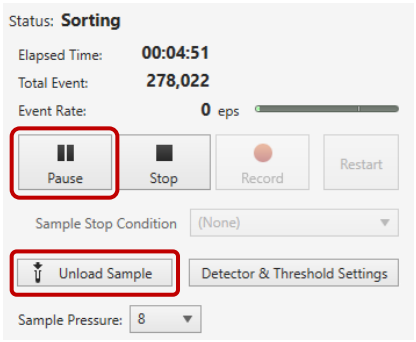
b. If the Event Rate suddenly drops to zero you likely have a clog in the sample path.

c. Look at the Droplet Viewer to make sure the clog is NOT in the nozzle.

- A normal droplet image indicates that the clog is NOT in the nozzle.
- If the stream image is severely disrupted, then the nozzle is likely clogged. Click on the Emergency Stop to exchange the chip. This occurrence is rare.
- If stream looks OK, continue with the Sample Path Unclogging step d) below.

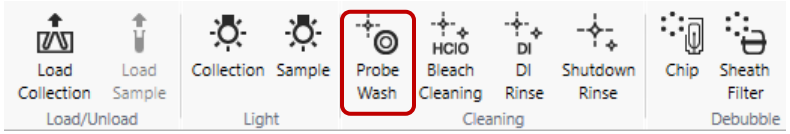


d. Click on the Pause button [❚❚] , then click on the Unload Sample button.



e. Click on the Cytometer tab (top of the screen) and click on Probe Wash.

f. When the probe wash is done, click on the Load Sample button.



g. Click on the Resume button [▶] to restore the sample flow.

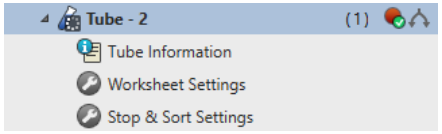
h. Sorting will continue in a minute or so, as cells enter the sorting chip again.

22. Stop the Sort

a. Make sure you have at least 30,000 sorted cells in each tube.

b. Make sure you have recorded data. Look at the Active Experiment Layout in the lower left corner of the screen.

- A red ball with a green check next to the Tube Name indicates that data has been recorded.
- The double arrow icon indicates that a sort was performed on that tube.



c. Click on the Stop button [■] to stop the sample and the sort.

d. Remove the Collection Tube Holder from the Sort Collection Area.

23. Post Sort Analysis

a. Check to make sure the system is clean before running any Post Sort Analysis.

- Load a sample tube with PBS only.
- Click on Next Tube.
- Click on the Start button [▶]
- Change the Sample Pressure to 10.
- Click on Restart.
- Continue running the sample until the Event Rate is less than 1 eps.
- Change the sample Pressure to 6.
- Click on the Stop button [■]

b. Load one of the collection tubes.

c. Click on the Start button [▶]

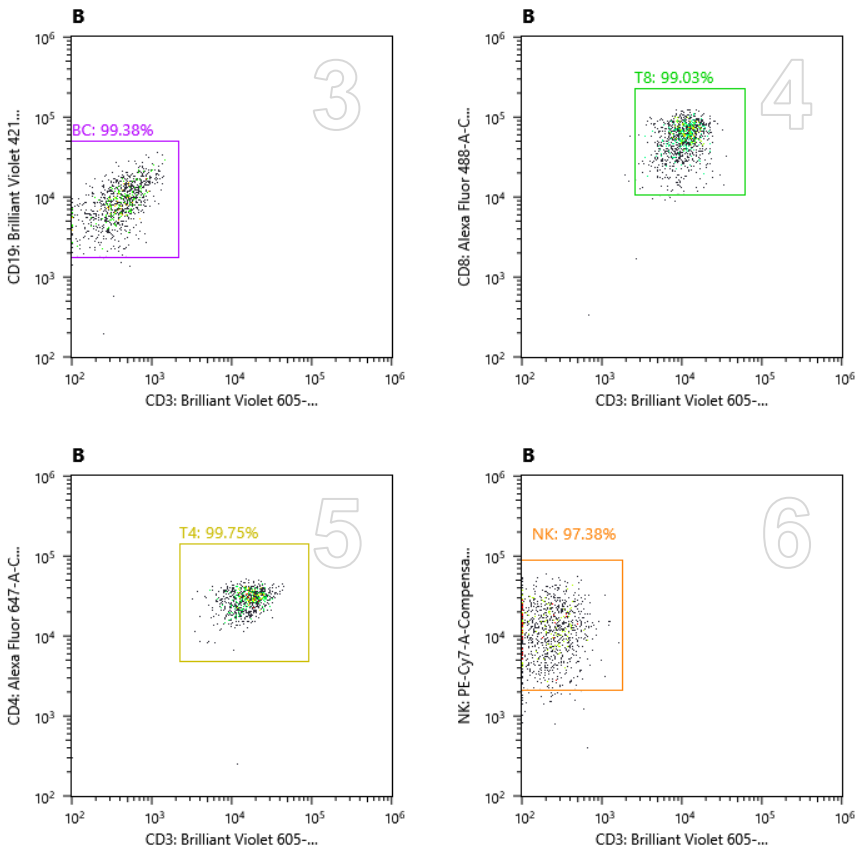
d. Change the Recording Stop Value to 1,000.

e. When the Elapsed Time reached 30 seconds, click on the Record button [●].

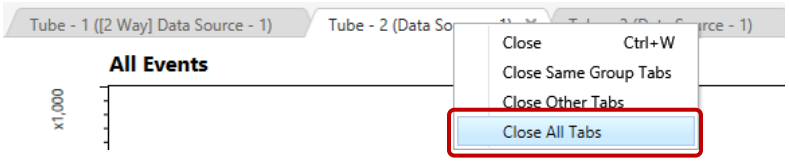
f. Repeat 23-a through 23-e for each collection tube.

g. Note the purity of each sorted population (see examples on next the page).

23. Post Sort Analysis (continued)



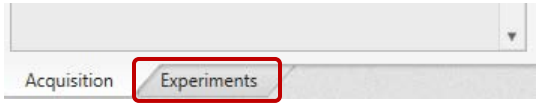
24. Close the Experiment



a. Right-click on any Worksheet Tab and click on Close All Tabs.
b. The last worksheet will remain open.
c. Right-click on the remaining Worksheet Tab and click on Close.

25. Re-open the Sort Sample Tube

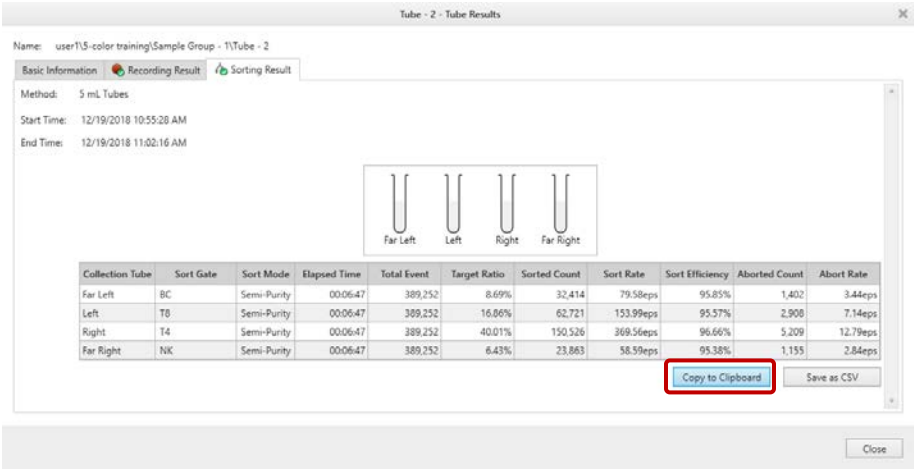
a. Go to the Experiment pane (lower left corner of the screen)
b. Click on the Experiments tab at the bottom to access the experiment archive.



c. Double-click on the name of the experiment you just closed (e.g. 5-color Training).
d. Double-click on the name of the sort sample tube (e.g. Tube-2).
e. The worksheet for the sort sample tube will open in the workspace.

26. Print or Copy and Paste Plots and Results

a. Right-click anywhere on the worksheet and click on Print. Print the report to PDF.
b. Right-click on any plot and click on Copy Picture.
c. Go to Paint or PowerPoint and paste the plot picture.
d. Right-click on the Worksheet and click on Copy Worksheet Picture.
e. Go to Paint or PowerPoint and paste the worksheet picture.
f. Go back to the Experiment pane.
g. Right-click on the sort sample tube name and click on Show Results
h. Click on the Sorting Result tab.
i. Click on Copy to Clipboard button.
j. Go to Paint or PowerPoint and paste the Sorting Result Table.



Training Exercise B: Multi-color Setup (8-color)

1. Experiment Setup

a. Click on the Experiment tab at the top left of the screen

b. Click on the New button. This will open the Experiment Template Screen.

c. Under Public Templates, click on the Blank Template.

d. Under Experiment Information, overwrite the default experiment name.

2. Measurement Settings

a. Uncheck the Area boxes FL3, FL4, FL8, and FL11

b. Change the FL1 and FL10 fluorochrome names:

- Change FL1 from FITC to Alexa Fluor 488
- Change FL10 from APC to Alexa Fluor 647
- Change FL12 from APC-Alexa Fluor 750 to APC-Cy7

c. Enter the marker names for FL1, FL2, FL5, FL6, FL7, FL9, FL10, and FL12

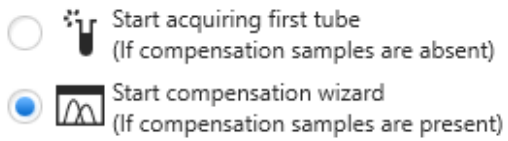
- For FL1 enter “CD8”
- For FL2 enter “CD45RO”
- For FL5 enter “NK”
- For FL6 enter “CD19”
- For FL7 enter “CD45”
- For FL9 enter “CD3”
- For FL10 enter “CD4”
- For FL12 enter “CD45RA”

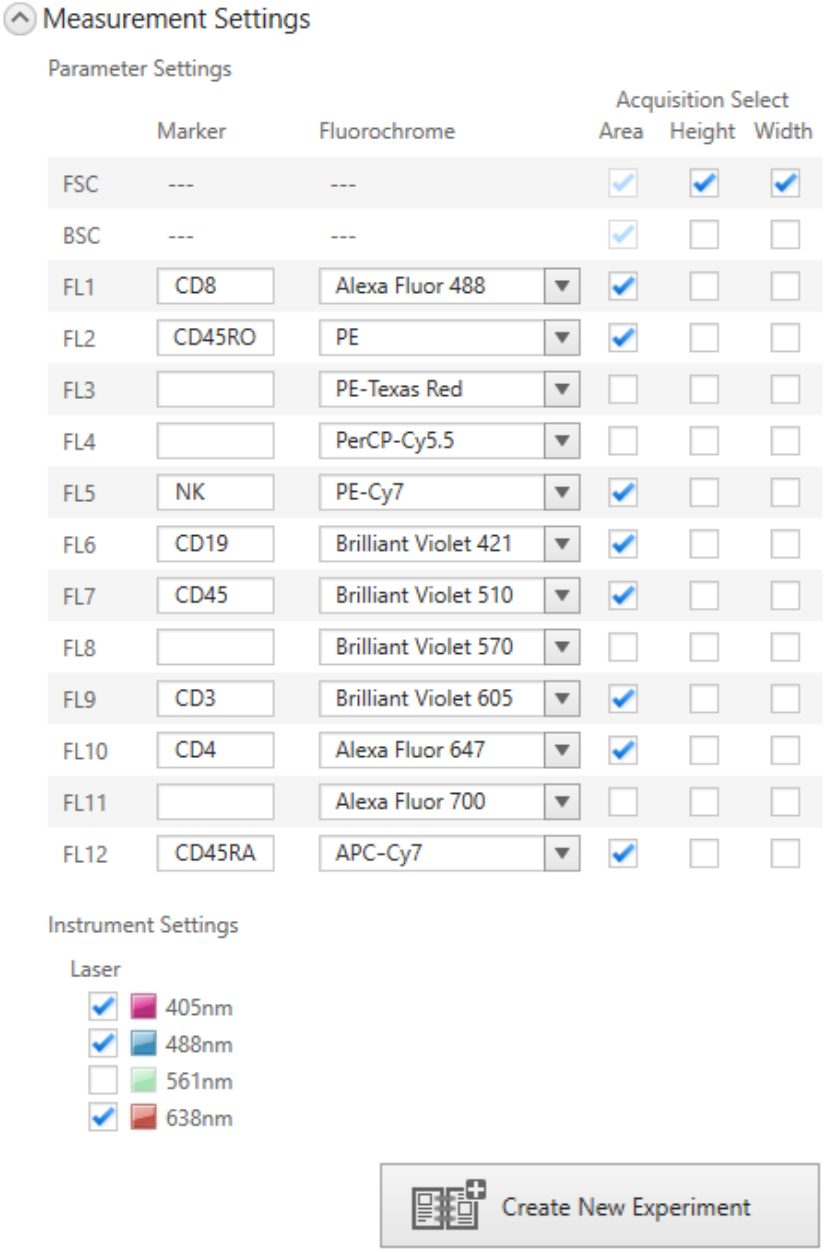
d. Check to activate the 405, 488, and 638 lasers. Uncheck the 561 laser.

3. Create the Experiment

a. Click on the Create New Experiment button.

b. The New Experiment Startup Procedure dialog box will appear, select the bottom option to launch the Compensation Wizard. Click OK to continue.





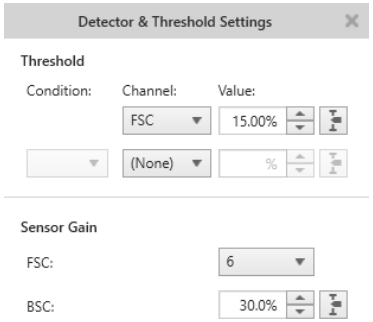
4. Compensation Wizard

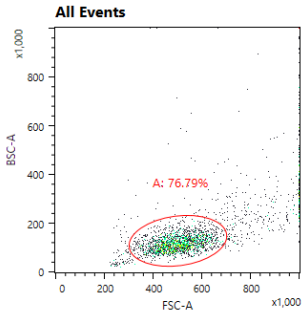
a. Follow the Compensation Wizard prompts to run an unstained control sample, run single-color controls, and calculate a color compensation matrix.

b. Run the unstained control sample as prompted by the Compensation Wizard.

c. Adjust FSC, BSC, and Threshold values as you did in Exercise A.

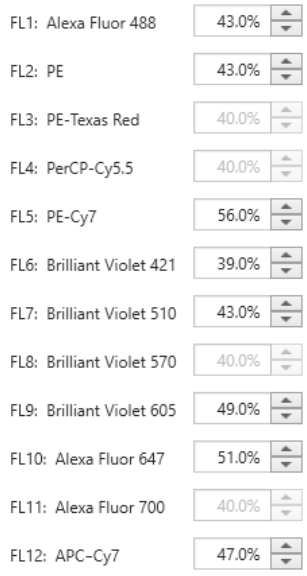
d. Position Gate A around the cells of interest.

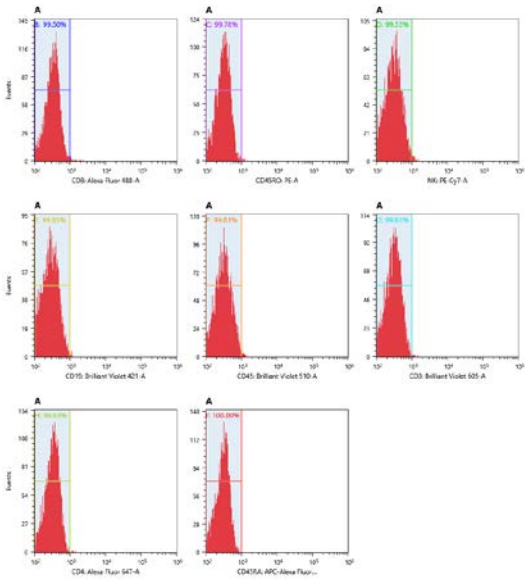




e. Adjust the PMT Voltage % for FL1, FL2, FL5, FL6, FL7, FL9, FL10, and FL12.

f. The data distribution (negative peak) in each histogram should be centered in the first decade of the log scale. Do not move the gates that demark the first decade.



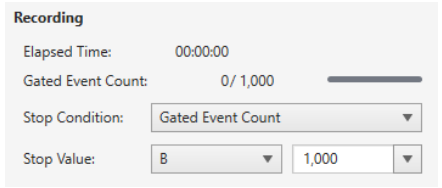


g. Run the single-color control samples as prompted by the Compensation Wizard.

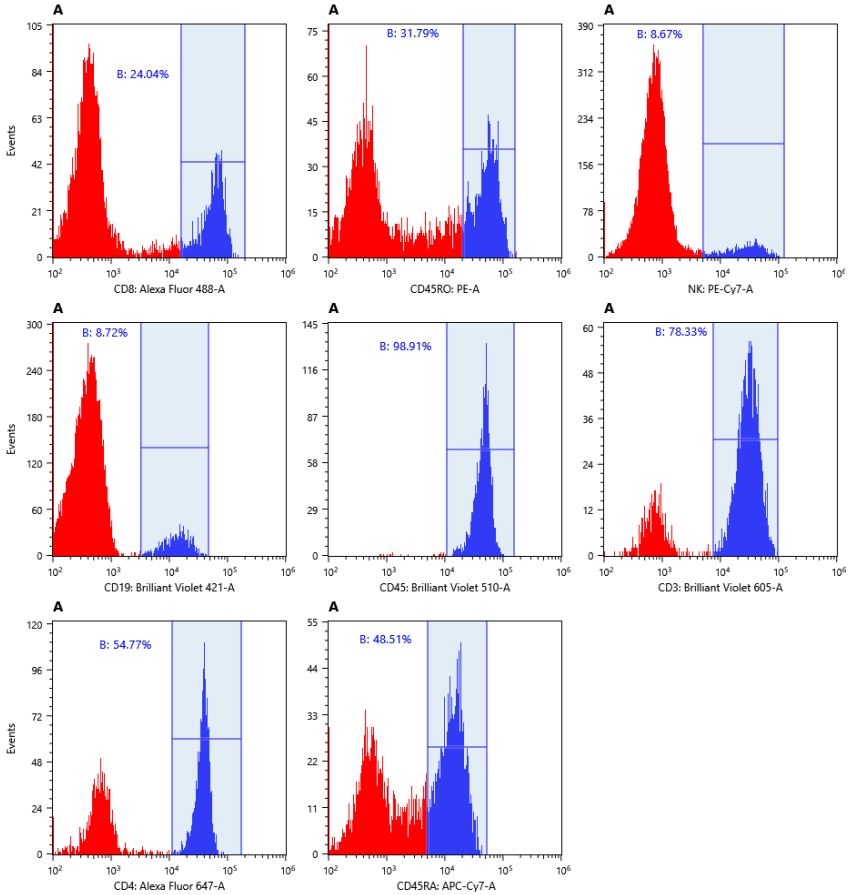
h. To increase the event rate, change the Sample Pressure setting to 8.

i. Be sure to let the sample flow stabilize for 30 seconds before recording data.

j. To record the same number of positive cells from each control sample, change the Recording Stop Values to Gate B and 1,000. Make this change prior to each recording.



k. Adjust Gate B tightly around the positive cells before each recording.



4. Compensation Wizard (continued)

l. Calculate the compensation matrix as prompted by the Compensation Wizard.

m. Apply Compensation is active by default. The color compensation matrix will be applied to all the data in the experiment.

Fluorochrome	CD8: Alexa Fluor 488	CD45RO: PE	NK: PE-Cy7	CD19: Brilliant Violet 421	CD45: Brilliant Violet 510	CD3: Brilliant Violet 605	CD4: Alexa Fluor 647	CD45RA: APC-Cy7
CD8: Alexa Fluor 488	100.00	12.04	0.11	-0.02	0.22	0.00	-0.09	-0.01
CD45RO: PE	1.30	100.00	1.15	0.04	0.04	2.06	0.21	0.00
NK: PE-Cy7	0.21	0.89	100.00	-0.07	-0.10	-0.08	-0.15	5.76
CD19: Brilliant Violet...	-0.14	-0.11	-0.10	100.00	9.70	0.15	-0.38	-0.10
CD45: Brilliant Violet...	0.36	0.13	0.01	12.94	100.00	40.13	9.24	0.60
CD3: Brilliant Violet 6...	-0.03	3.05	0.60	1.79	0.24	100.00	31.48	1.68
CD4: Alexa Fluor 647	-0.08	-0.07	0.05	-0.01	-0.04	0.32	100.00	5.48
CD45RA: APC-Alexa...	-0.20	-0.20	3.81	-0.16	-0.29	0.04	11.13	100.00

5. Run and Record the 8-color Sample

a. Upon closing the Compensation Wizard, the software will go to Tube-1.

b. Load the 8-color stained sample and click on the Start button [▶]

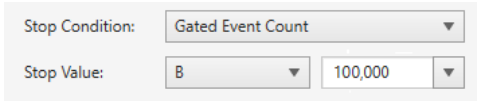
c. Position Gate A around the cells of interest.

d. Double click inside Gate A to create Plot 2.

e. Go to Plot 2. Leave the X-axis as FSC-A. Change the Y-axis to FSC-H.

f. Draw a polygon gate around the single cells, this will be Gate B.

g. Under Recording, change the Stop Condition to Gated Event Count



h. Set the Stop Values to Gate B and 100,000.

i. Click on the Record button [●] to record data from the 8-color sample.

j. When the recording is complete, click on the Stop button [■] to stop the sample.

6. Create Fluorescence Plots

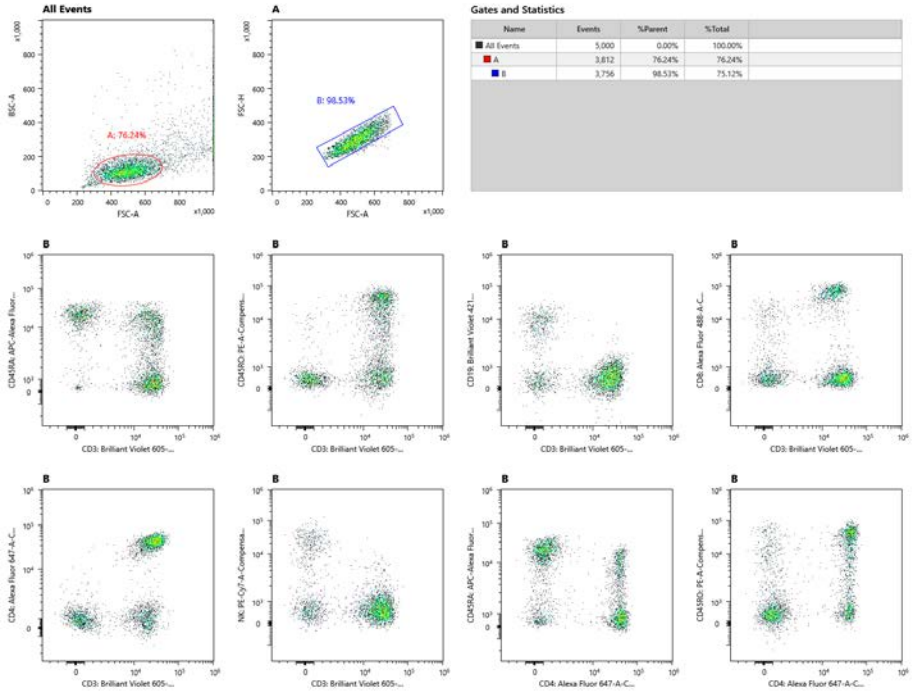
a. Go to Plot 2 and double click inside Gate B to create Plot 3.

b. Go to Plot 3.

- Change the X-axis to CD3: Brilliant Violet 605
- Change the Y-axis to CD45RA: APC-Cy7
- Right-click on Plot 3 and click on Duplicate to create Plot 4.

c. Go to Plot 4 and change the axes as you wish and duplicate to create Plot 5.

d. Make more plots to look at various antibody combinations.



7. Change to Biexponential Scaling (optional)

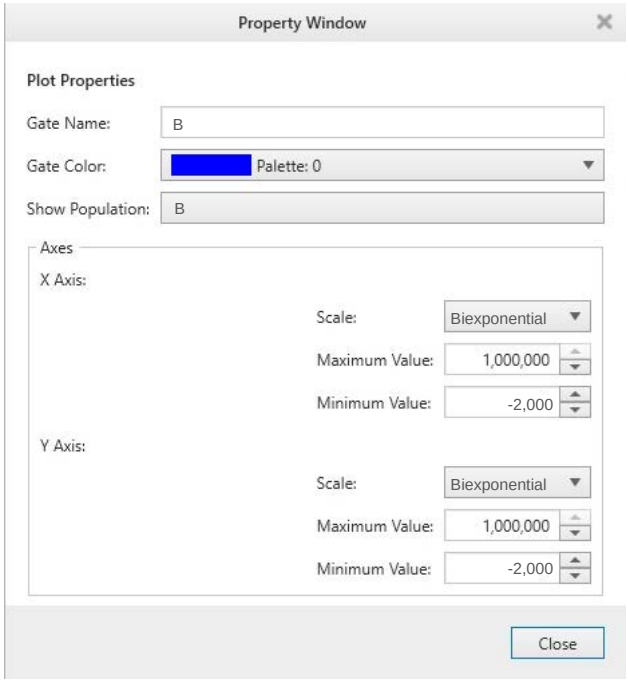
a. Right-click on Plot 3 and click on Properties.

b. Go to the Property Window.

c. For both the X Axis and Y Axis, change the Scale type to Biexponential.

d. For both the X Axis and Y Axis, change the Minimum Value to -2,000.

e. Look at Plot 3. Re-adjust the Minimum Value as need.



f. DO NOT close the Property Window.

g. Click on the next plot and adjust the scaling.

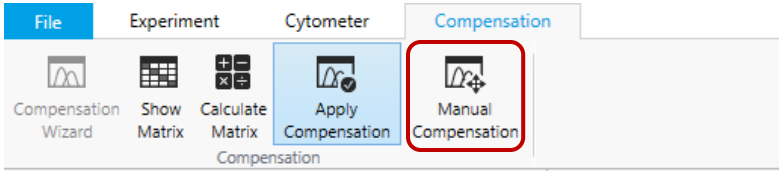
h. Click on the other plots and adjust scaling until all adjustments are made.

i. Click on the Close button to close the Property Window.

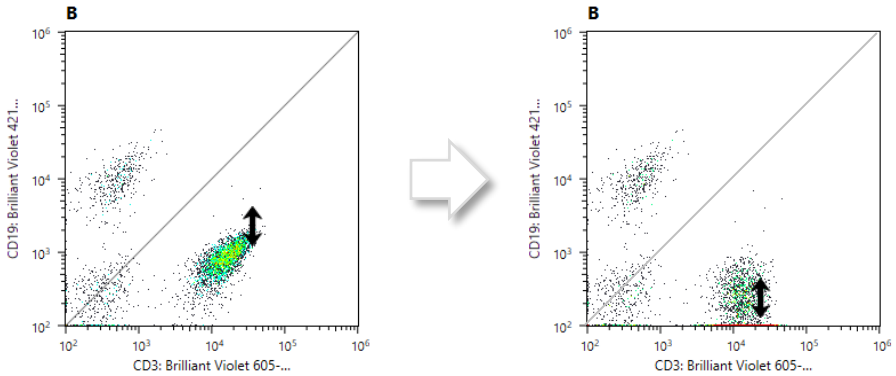
8. Adjust Color Compensation Manually

a. Click on the Compensation tab at the top of the screen to display the Compensation tool ribbon.

b. Click on Manual Compensation



c. Go to any plot. Click, grab, and drag populations to adjust color compensation.



d. Perform the same procedure on any other plots that require adjustment.

e. Go to the Compensation tool ribbon and click on Manual Compensation to deactivate the mode.

f. The adjusted color compensation will be applied to all data.

g. To go back to the original color compensation settings, go to the Compensation tool ribbon and click on Calculate Matrix. The matrix will be re-calculated using the control data in the Compensation Panel.

9. Replace a Compensation Control

a. Go to the Experiment Manager.

b. Expand the Compensation Panel section.

c. Right-click on the control you want to replace.

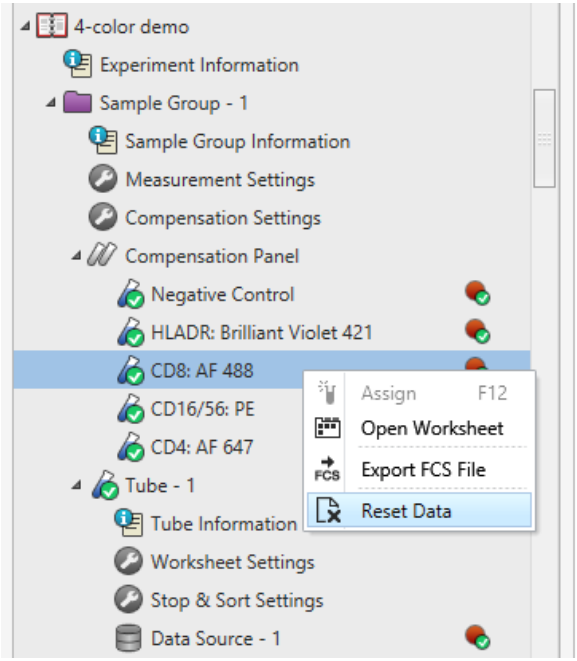
d. Select Reset Data from the menu.

e. Right-click again and select Assign to re-activate the tube for data acquisition.

f. Mount the sample tube for the control and click on Start.

g. Record data.

h. Go to the Compensation Tab and click on Calculate Matrix.



10. Edit Statistics (optional)

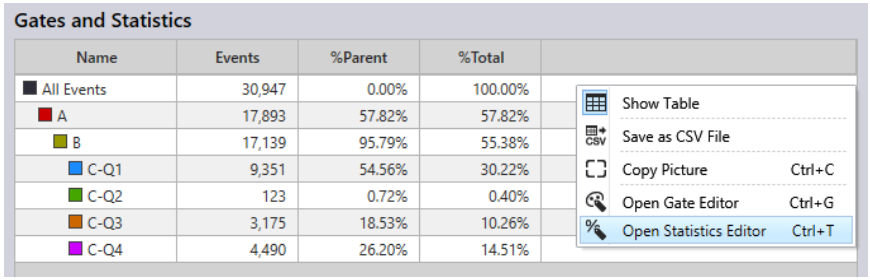
a. Create Quadrant gates on plots as desired.

b. To edit the statistics, right click on the table and select Open Statistics Editor.

c. Select the desired gate and check on the desired statistics.

d. Click on Apply

e. Repeat steps c and d to add statistics for other gates.

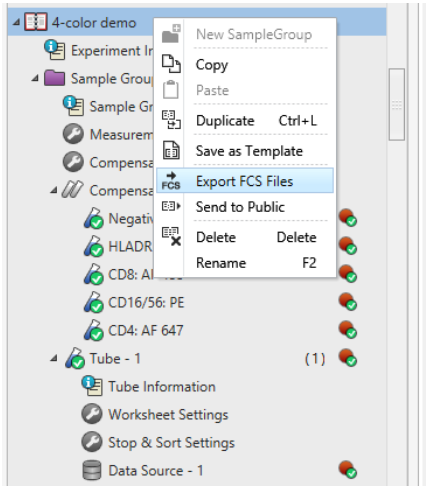


11. Export Data to .FCS Files

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

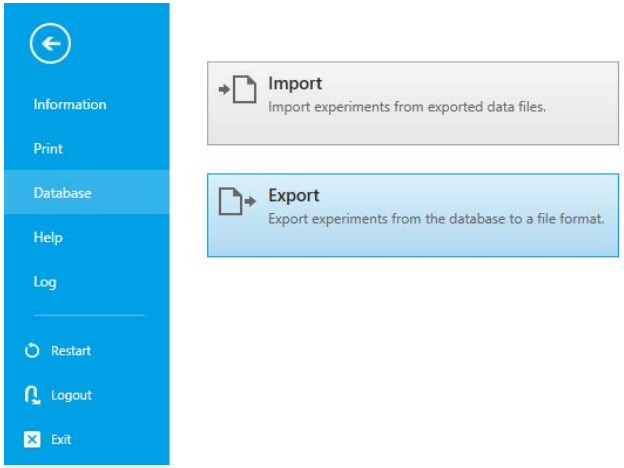
g. Click on Export

12. Export Data to .expdat Database Files

a. Go to the File menu

b. Click on Database

c. Click on the Export button



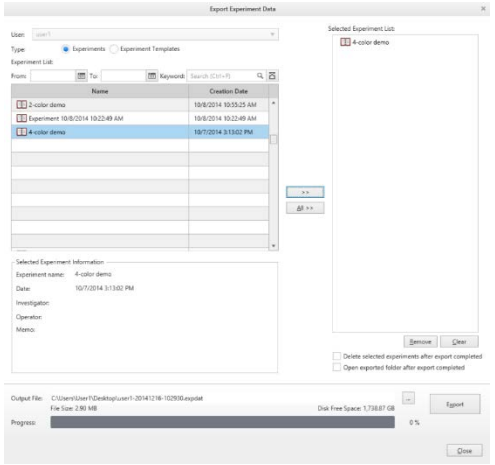
d. The Export Experiment Data dialog box will open

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: Multi-well Plate Sorting

1. Mount a 96-well Plate

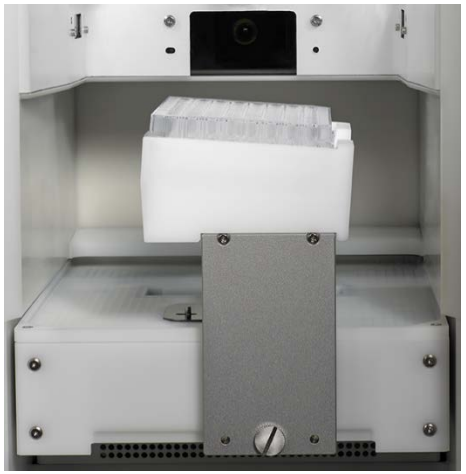
a. Attach the Plate Support Arm to the collection stage.
   - Remove the knurled screw from the collection stage
   - Put the support arm in place (the two pins go into holes flanking the screw hole).
   - Use the screw to secured the support arm, tighten the screw firmly.

b. Mount a 96-well plate into the white plastic Plate Holder:
   - There are metal springs are in the back end and right side of the plate holder.
   - Ensure that plate position A1 is in the front left corner of the plate holder (away from the springs).
   - Leave the lid on the plate for plate position calibration.

c. Mount the plate holder onto the support arm.

d. Ensure that all pins are aligned correctly.

e. Keep the lid on the plate for plate position calibration.



2. Setup the Sort Sample

a. Click on the Next Tube button to create a worksheet for the next tube.

b. Make sure the 8-color sample is still mounted.

c. Click on the Start button [▶]

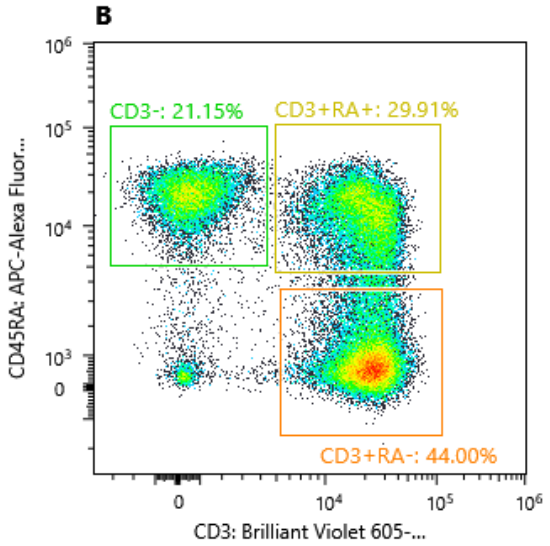
d. Let the sample run for 30 seconds (the boost), then click on the Restart button to clear any data that accumulated during the boost.

e. Let data accumulate in the plots until all the populations can be seen, then click on the Pause button [❚❚] to conserve the sample while you create sort gates, adjust the plate position, and configure the sort settings.

3. Create and Rename Sort Gates

a. Go to Plot 3 and create three Rectangular gates around the three major populations.

b. Rename the gates (e.g. CD3-RA+, CD3+RA+, CD3+RA-).

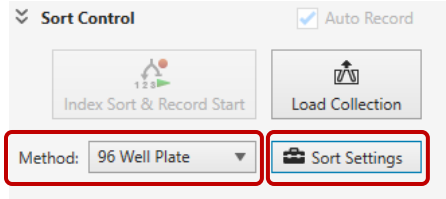


4. Adjust the Plate Position

a. Go to Sort Control at the bottom of the screen.

b. Change the Method to 96-Well Plate.

c. Click on the Sort Settings button to open the Sort Settings window.



d. Go to the Sort Settings window and click on the Plate Adjustment tab.

e. Under Sort Test, select the Sort Test Type: Four Corners and Center Well.

***Option**: For very large cells that may affect droplet trajectory, consider doing the plate alignment with your Sample and the Mode you plan to use in the real sort. For this, you would first need to preview and gate on a representative cell population in order to select it in the Plate Adjustment drop-down menu. You may also skip to Step 5e below to alter the Sort Mode parameters.*

f. Make sure the lid is on the plate and the collection area door is closed.

g. Under Sort Test, click on the Start button.

h. When the test is complete, remove the plate holder from the plate support arm.

i. Examine the position of each sort test puddle.

j. Make position adjustments as needed

- Click the position you want to adjust (A1, A12, H1, or A12)
- Click the appropriate Droplet Position arrow button to change the position of droplet deposition.
- Each click moves the droplet position one step increment.
- The default step increment (1.0 mm) is good for 96-well.

k. Click on the Unload button to return the support arm to the loading position.

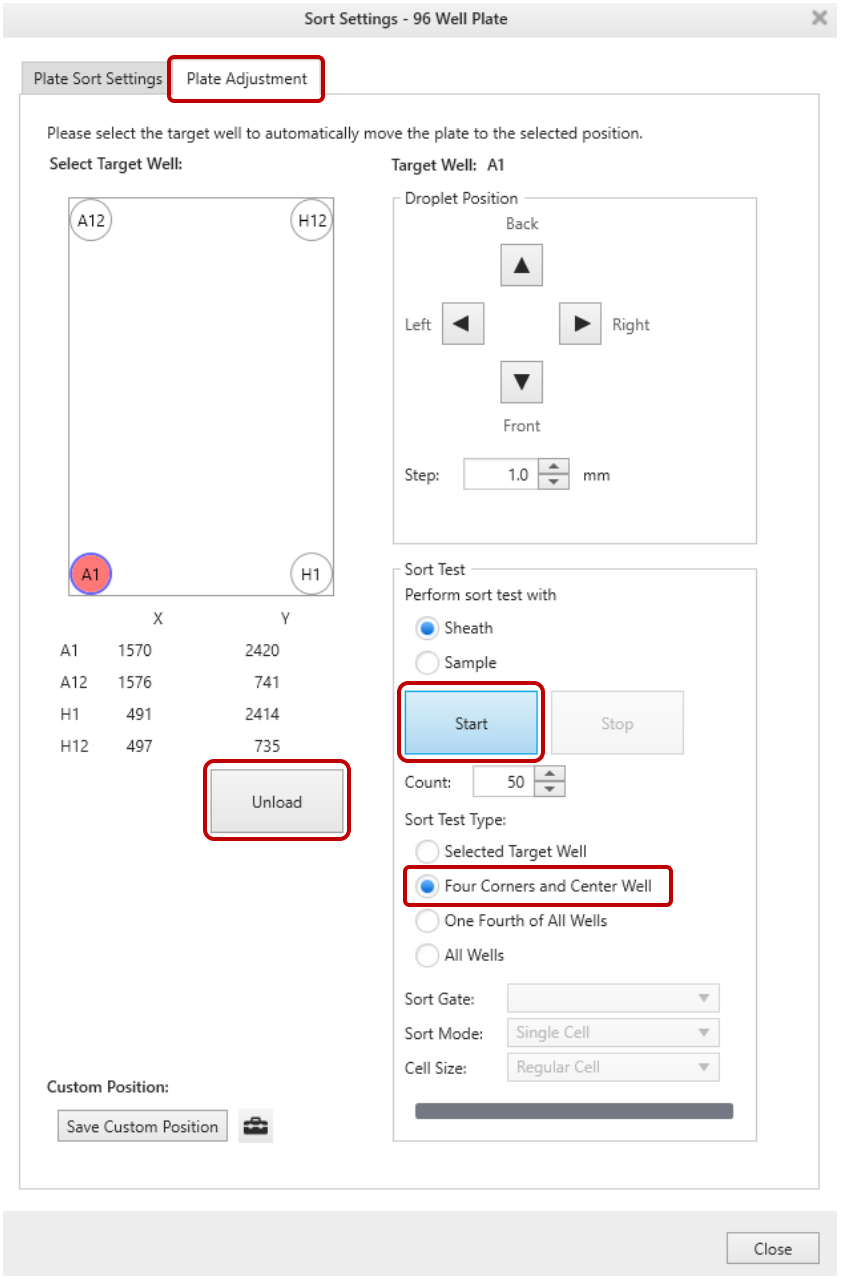
l. Re-mount the plate holder onto the plate support arm.

m. Perform the Sort Test again.

n. Make more adjustments as needed and repeat the test as needed.

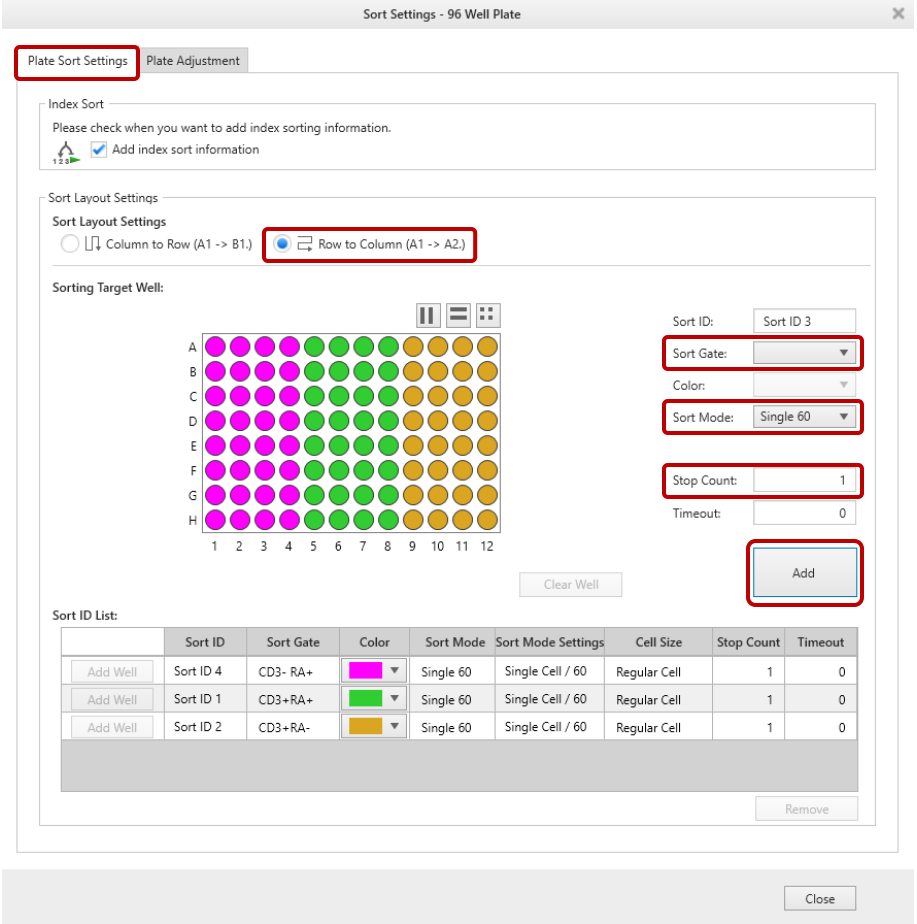
o. When finished you DO NOT need to save anything.

p. Click on the Plate Sort Settings tab and go to the next step.



5. Configure the Plate Sort Settings

a. Click on the Plate Sort Settings tab.
b. Check the box to activate Add Index Sort Information
c. Go to the Sorting Target Well section and drag the cursor over the first four columns of the plate to select those well locations.
d. Change the Sort Gate to CD3-RA+
e. Change the Sort Mode to Custom. This will open the Custom Sort Settings window.
   - Modify Custom1
   - For Name, enter Single 60.
   - For Mode, select Single Cell. *Single-cell mode ensures that you do not sort two targets accidentally (as may happen in Purity Modes). You should use some kind of single-cell mode when sorting for a single cell per well. See MA900 Sort Modes Guide PDF or the MA900 User Manual for more information.*
   - Change the Centering Mask value to 60. *Sorting will occur only when the cell position is in the middle 60% of the droplet period, which will ensure the droplet is not empty. The existing single-cell mode is at 20% centering, making this mode more wasteful when sorting rare targets.*
   - Click on OK to finish.
   - This custom mode will apply only to this experiment. To create a custom mode that can be applied to all future experiments, go to File > Information > User Preference > Sort > Custom Sort Settings > Edit. Then follow the steps above.
f. Change the Stop Count value to 1. One cell will be deposited into each well.
g. Leave the Timeout value set at 0 seconds (disabled).
h. Click on the Add button to add this sorting job to the Sort ID List.
i. Drag the cursor over the next four columns to select those wells.
j. Change the Sort Gate to CD3+RA+
k. Keep the Sort Mode (Single 60), Stop Count (1), and Timeout value (0) the same.
l. Click on the Add button to add this sorting job to the Sort ID List.
m. Drag the cursor over the remaining four columns to select those wells.
n. Change the Sort Gate to CD3+RA-
o. Keep the Sort Mode (Single 60), Stop Count (1), and Timeout value (0) the same.
p. Click on the Add button to add this sorting job to the Sort ID List.



q. Change the Sort Layout Settings to Rows to Column (A1 -> A2). This way the plate will be processed by rows, ensuring that some of each sort job will be performed even if the sample runs out midway through the plate.
r. When finished click on the Close button.

6. Initiate the Index Sort

a. Click on the Resume button [▶] to get the sample running again.

b. Adjust the sample pressure to get an event rate around 300-500 eps.

c. Click on the Restart button to clear out the old data.

d. Inspect and adjust gate positions as needed.

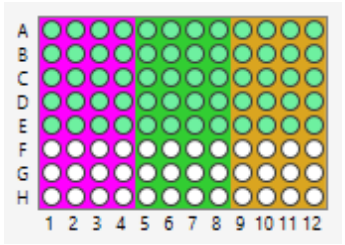
e. Look at the Droplet Viewer to confirm that the sort calibration is valid (solid green).

f. Make sure the plate lid has been removed.

g. Go to Sort Control at the bottom of the screen.

h. Click on the Index Start & Record Start button.

7. Plate Sort Monitoring and Completion

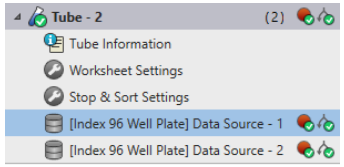


a. The plate progress can be monitored by watching the diagram at the bottom center of the screen.

b. When the plate is completed a notification window will appear. Click on the Continue button to sort another plate using this sample. Then:
   - Exchange the plate
   - Click on the Resume button [▶] to get the sample running again
   - Click on the Index Start & Record Start button.

c. When complete, click on the Finish button, you are done sorting from this sample.

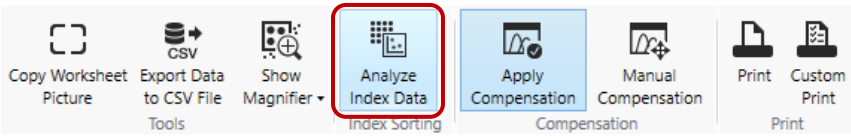
8. Analyze Index Sort Data



a. Load an Index Sort Data Source:
   - Go to the Experiment Manager (lower left).
   - Double-click on [Index 96 Well] Data Source-1.

b. Click anywhere on the open Worksheet area to activate the Worksheet Tools Ribbon.

c. Go to the Worksheet Tools ribbon and click on Analyze Index Data.



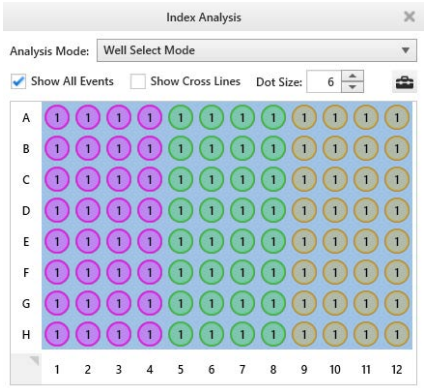
c. The Index Analysis dialog box will open.

9. Well Select Mode:

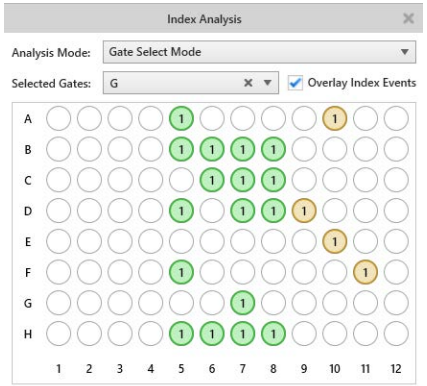
a. Click on any well location in the diagram.

b. A colored dot will appear on all the data plots indicating the position of the cell that was sorted to that well location.

c. Drag the cursor over the entire plate to select all the well locations. All sorted cells will be represented by colored dots.



10. Gate Select Mode:



a. Change the Analysis Mode to Gate Select Mode.

b. Draw a new gate on any plot.

c. Change the Select Gates to the gate you just created.

d. Well locations will be highlighted for every sorted cell that meets the criteria of the newly created gate.

e. Check the Overlay Index Events box to display plot dots for each sorted cell.

f. Move the gate to display different sets of well locations.

g. Multiple gates can be created and selected.

11. Export Index Sort Data to a CSV File

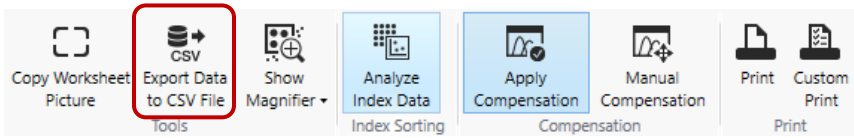
a. Close the Index Analysis window.

b. Load an Index Data Source:
   - Go to the Experiment Manager (lower left)
   - Double-click on [Index 96 Well] Data Source-1

c. Click anywhere on the Worksheet to activate the Worksheet Tools Ribbon.

d. Click Analyze Index Data (to export only plated cell data). Do not click Analyze Index Data if you require the entire dataset for all cells processed during the sort.

e. Go to the Worksheet Tools ribbon again and click on Export Data to CSV File.



f. Choose a path where the file can be saved (e.g. the Desktop) & click on Export.

g. Go to the file location and open the file using Excel or Notepad.

h. The spreadsheet contains one line for each sorted cell.

i. The right-most column contains the well locations.

j. The other columns contain the intensity values for each light scatter and fluorescence parameter.

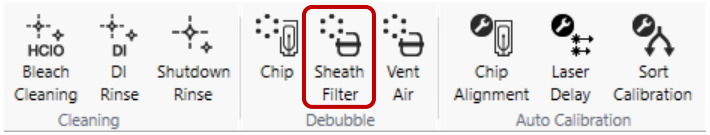
Troubleshooting

1. Sheath De-bubble

Most droplet stability 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. Click on the Cytometer tab (top of screen) to get the Cytometer tools ribbon.

b. Click on Sheath Filter De-bubble, then click Confirm to continue.



2. 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 the Stop button [■]

b. Click on the Cytometer tab (top of screen) to get the Cytometer tools ribbon.

c. Click on Chip De-bubble.

d. After the Chip 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 Chip De-bubble until the droplet formation appears normal.

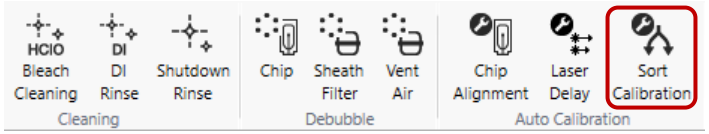
e. The system will automatically return to the Calibrated state (solid green ball). However, you may want to run the Sort Calibration to ensure accurate sorting.

f. If Chip De-bubble does not work, try a Sheath Filter De-bubble instead.

3. Automatic Setup Failure

a. If the Automatic Setup fails, check the following items and then perform Sort Calibration again.

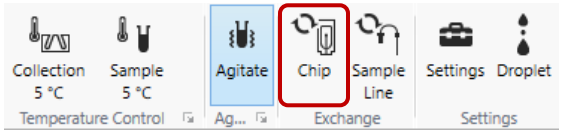
- Confirm that the collection optics are in the default configuration
- Check the sheath tank lid seal and release valve for air leaks.
- Purge air bubbles from the fluidics system by performing the Sheath De-bubble routine (see Item 1 above).
- Check the deflection plates and clean them as needed.
- Check the stream illumination laser windows and clean them as needed.
- Click on the Cytometer tab (top of screen) to get the Cytometer tools ribbon.



- Click on the Sort Calibration button.
- Follow the software prompts for Sort Calibration.
- If Sort Calibration fails again, then reset the current chip.

b. Reset the Current Chip

- Click on the Cytometer tab (top of screen) to get the Cytometer tools ribbon.
- Go to the Cytometer tools ribbon and click on Chip Exchange.



- Follow the software prompts for Chip Exchange.
- 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 again, contact technical support:

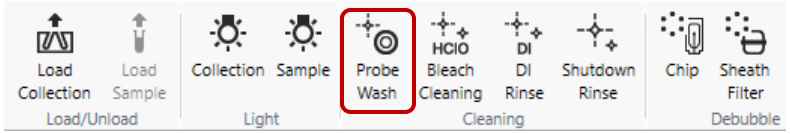
Sony Biotechnology Technical Support

800-275-5963

4. 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 by the following steps:

a. DO NOT click on Stop.
b. Click on the Pause button [❚❚]
c. Click on the Unload Sample button.
d. Click on the Cytometer tab (top of the screen) to get the Cytometer tools ribbon.
e. Go to the Cytometer tools ribbon and click on Probe Wash

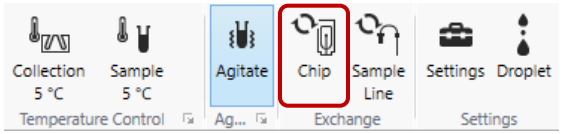


f. Watch the sheath fluid drip from the sample probe. If need, repeat the Probe Wash until the sheath fluid drips at a normal rate, indicating that the sample path is clear.
g. Inspect the sample. If needed, filter the sample again to remove clumps.
h. Re-mount the sample tube
i. Click on the Load Sample button.
j. Click on the Resume button [▶]
k. Data should appear again within 90 seconds.
l. Sorting will resume automatically.
m. If data does not re-appear, the next steps are to run Bleach/DI Rinse.

5. 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 the Stop button [■]
b. Exchange the Chip
   • Click on the Cytometer tab (top of screen) to get the Cytometer tools ribbon.
   • Go to the Cytometer tools ribbon and click on Chip Exchange.



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. For most cells 5-10 million cells per mL is good.
b. Clean lymphocyte preps can be at up to 20 million cells per mL.
c. Some cells form clumps at higher concentrations.

3. Filter Sort Samples to Remove Cell Clumps RIGHT BEFORE sorting

a. 35 µm nylon mesh is recommended.
b. The Falcon™ Tube with Cell Strainer Cap (Corning 352235) works well.
c. The sample may need to be re-filtered if sample path clogging occurs frequently.

4. How to Run a Very Small Volume Sort Sample

a. Mount a sample tube containing only PBS or other buffer. Do not use water.
b. Click on the Start button [▶]
c. Let the buffer run for 30 seconds (the boost).
d. Click on the Pause button [❚❚]
e. Click on the Unload Sample button.
f. Replace the buffer tube with the sort sample tube.
g. Click on the Load Sample button.
h. Click on the Resume button [▶]
i. The cells 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.
k. Start the sort.

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

a. Change the Sample Pressure setting to 6.
b. Mount a sample tube containing only PBS or other buffer. Do not use water.
c. Click on the Start button [▶]
d. Let the buffer run for 30 seconds (the boost)
e. Click on the Pause button [❚❚]
f. Click on the Unload Sample button.
g. Replace the buffer tube with the sample tube
h. Click on the Load Sample button.
i. Click on the Resume button [▶] and the Restart button to reset the timer.
j. Let the sample run for 20 seconds to fill the sample line with cells.
k. Click on the Pause button [❚❚]
l. Click on the Unload Sample button.
m. Replace the sample tube with the buffer tube.
n. Click on the Load Sample button.
o. Click on the Record button [●]
p. Record data until the recording stop is met or until the data rate diminishes.
q. Click the Stop button [■] to stop the sample.

6. Stop the Sort Before the Sample Runs Dry

a. Refer to the tables below for the approximate sample consumption rate (µL/min) at each sample pressure setting.
b. Use these values to estimate when the sample will run dry.

• 100 µm Sorting Chip

[Sample Pressure]	1	2	3	4	5	6	7	8	9	10
Flow rate (µl/min)	6	11	16	21	27	37	47	58	68	89

• 70 µm Sorting Chip

[Sample Pressure]	1	2	3	4	5	6
Flow rate (µl/min)	8	18	28	42	62	80