

Flow Cytometry/FACS – Bio-Rad S3e Cell Sorter



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Brief Description

The S3e cell sorter is equipped with two lasers and four fluorescent detectors, plus both forward and side scatters. Samples are analyzed using the traditional jet-in-air technology, and events can be sorted at high speeds while maintaining sensitivity and high-purity.

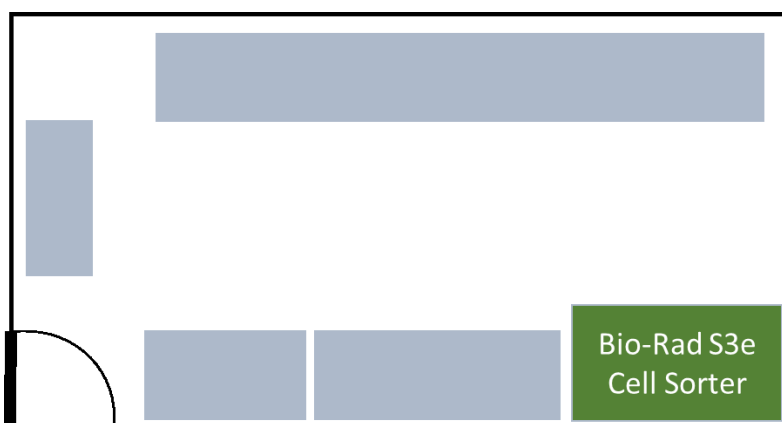
The S3e cell sorter is largely efficient and user-friendly by automating the complex presort setup with a unique technology (ProDrop technology). In detail, this cell sorter brings forward several automated features, such as Startup/shutdown, Stream-to-laser alignment (Hands-free alignment), Drop delay calculation, Droplet break-off monitoring and feedback, Phase and deflection adjustments to sort stream, Daily QC reporting and trending and Collection volume monitoring to prevent overfilling

Reference

Bio-Rad S3e™ Cell Sorter 145-1006

Location

Room 5.22 (Common Equipment Room), 5º floor, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA)



Suggestion for “Materials and Methods”

Flow cytometry data were acquired on a Bio-Rad S3e Cell Sorter and analyzed using the ProSort software, version 1.6.

Flow cytometry data were acquired on a Bio-Rad S3e Cell Sorter operated by the ProSort software, version 1.6, and analyzed using the Cytospec software from Purdue University Cytometry Laboratories.

Bacillus subtilis cells were sorted as alive GFP and dead GFP cells using a Bio-Rad S3e Cell Sorter operated by the ProSort software, version 1.6.

Suggestion for “Acknowledgements”

This work was partially supported by PPBI - Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122) co-funded by national funds from OE - "Orçamento de Estado" and by european funds from FEDER - "Fundo Europeu de Desenvolvimento Regional".

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Features and Specifications

- Double-Laser System: 488 nm and 561 nm lasers of 100 mW for cell and particle interrogation
- 4 Fluorescence Detectors, photomultiplier tube (PMT) -based
- Forward scatter (FSC) detector, photomultiplier tube (PMT) -based
- Side scatter (SSC) detector, photomultiplier tube (PMT) -based
- Droplet Frequency: 37–43 kHz
- Sorting type: True jet-in-air for high-performance sorting
- Sorting rate: No hardware limitations for sort rate, limited only by droplet frequency and application
- Sorting purity: >99% pure
- Nozzle Size: 100 µm
- Sorting direction: 2-way sorting
- Sorting collection: Up to 5 x 5 ml tubes each direction; Up to 5 x 1.5 ml tubes each direction; Microscope slides; 8-well strip each direction
- Sample and collection temperature control system: 4–37°C
- Onboard fluidics and dilution of 8x sheath fluid with deionized water

Lasers/Filter-Sets

Laser	Filter	Emission Filter	Fluorophore or Dye	Fluorophore Excitation Maximum	Fluorophore Emission Maximum
488	FL1	BP 525/30	EGFP/GFP	488	507
			FITC	495	519
			Alexa Fluor 488	499	519
			EYFP/YFP	514	527
	FL2	BP 586/25	PE	496, 565	578
			RFP	554	584
	FL3	BP 615/25	PI	536	617
			PE-Texas Red	496, 565	619
561	FL2	BP 586/25	PE	496, 565	578
			tdTomato	554	581
			RFP	554	584
			DsRed	554	586
	FL3	BP 615/25	mCherry	587	610
			PI	536	617
			PE-Texas Red	496, 565	619
	FL4	655/LP	PE-Alexa Fluor 647	496, 565	669
			PE-Cy5	496, 565	670
			PE-Cy5.5	496, 565	693
			PE-Cy7	496, 565	785

BP stands for bandpass filter

LP stands for longpass filter

Additional Equipment

Computer

- Type: Desktop
- Brand: Dell Inc.
- Model: OptiPlex 3020
- Features: Intel I5 4590 up to 3.7 GHz (quad-core); 4GB RAM; 500 GB HDD
- OS: Windows 7 Professional
- Available Software: **ProSort**

Monitor

- Brand: Asus
- Model: VE248

System Overview



WARNINGS and Advice

- **Before** you get started for the first time on this cell sorter consult the responsible people for proper training
- **For the moment, bacterial pathogens, cell lines or any other agent of Biosafety level 2 or higher are not allowed, as well as radioactively labelled or flammable samples**
 - Do not forget to check the fluidics **before you start operating the cell sorter**
 - Use **only MiliQ water** in the water container
 - While transferring a cap assembly to a new container, avoid touching the assembly to the outside surfaces of the containers. If it is necessary to lay the cap assemblies down, set only on pre-sterilized surfaces
 - It is recommended that resuspended adherent samples are mixed at high speed to prevent clumping
 - Samples must be filtered prior to running. This will minimize clogs in the sample tube and nozzle tip
 - **Do not open** the nozzle door
 - Keep the green containment door closed as often as possible during the sorting procedure
 - **Never try to access** the laser beam paths
 - Any adjustment to the toolbars should be avoid. Only knowledgeable users must perform alterations to the current settings. Please, refer to the responsible people for any constraint
 - Optimal settings are automatically determined during the quality control procedure. Adjusting any of these values will affect the sorting performance
 - **Do not** adjust any setting of the global preferences menu
 - **Please confirm the bead lot before starting the quality control procedure.** If the bead lot is not in the dropdown list, please refer to the responsible people
 - **Use always beads from the same lot to perform one quality control procedure**
 - When the remaining run time is down to 5 minutes, the system will automatically shut down. The fluidics will need to be refilled and the start-up procedure must be performed. To prevent this, perform the fluidics “hot swap”.

- Operate quickly when performing the “hot swap” procedure, as time is limited to three minutes for the operation. Bring containers of water or sheath fluid in advance.
- The maximum total volumes for a 5 and 1.5 ml collection tubes are 4 and 1 ml, respectively. Going above these will cause spilling and risk contamination
- You must empty the waste and refill the fluidic containers at the end of the use
- Perform a high-pressure cleaning protocols if you are the last user of the day. Otherwise, perform a low-pressure cleaning procedure
- Use only 70% ethanol and MiliQ water as cleaning solutions
- Please let us know if some solution or fluidic is running out
- Make sure to save all the data to your own storage device or cloud as we routinely wipe out the computer’s data
- Do not forget to register your utilization in the logbook. **Remember that future maintenance costs and repairs will be supported by the groups according to the logbook data**
- **This equipment is only available for booking in the ITQB intranet**

Available Techniques

- Fluorescence-Activated Cell Sorting (FACS)
- Flow Cytometry Analysis

Set Up

Sample Preparation

Cells Suspension

To avoid clogs when working with cells, resuspend cells into a single cell suspension and always use a 40 µm or smaller filter prior to cell sorting

Initial Set Up: Start-up and Fluidics Procedure (≈ 20 minutes)

In case you are not the first user of the current day, please confirm with the previous user whether a low-pressure cleaning final set up has been performed. If so, skip both start-up and quality control procedures. NEVERTHELESS, DO NOT FORGET TO CHECK THE FLUIDICS. IF NEEDED, PERFORM THE “HOT SWAP” PROCEDURE (See Swapping Fluidics During Sorting: “Hot Swap”).


1. Open the bulk fluidics door




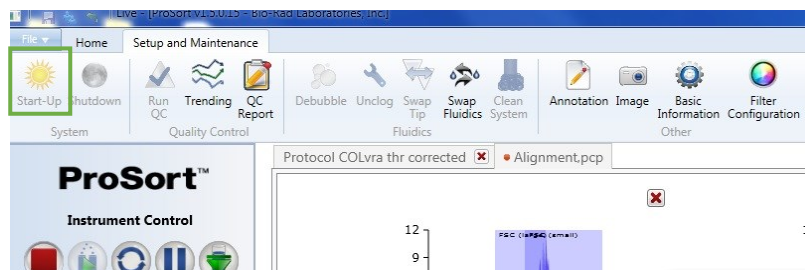
2. Detach the quick disconnect from the cap assembly by pushing in the metal button and lifting the quick disconnect until it engages the magnetic holder above it



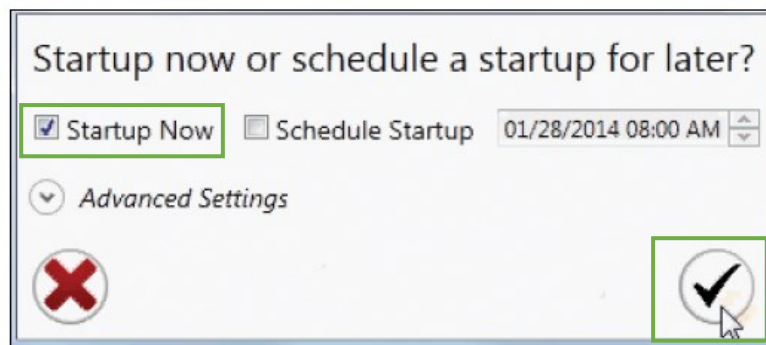
3. Slightly lift and pull out the container
4. Uncap the cap assembly from the container

5. Refill, change or empty the container
6. Carefully place the cap assembly into the container and tighten the cap
7. Place the container into position
8. Attach the quick disconnect to the cap assembly (you should hear a click)
9. Switch on the computer
10. Double-click in the **ProSort**  icon in the desktop
11. Log in into the group account (Contact the responsible people for more log in options)

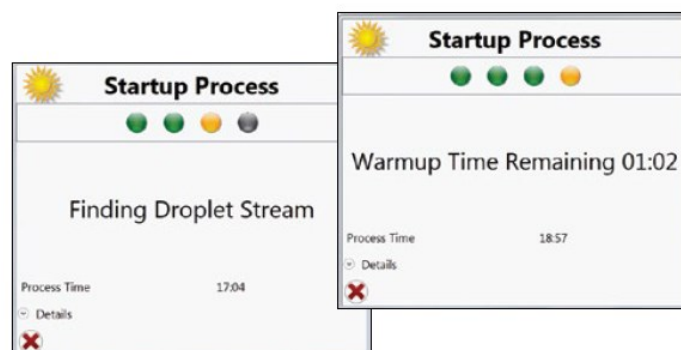
12. In the **Setup and Maintenance** tab, click in the **Start-up**  button



13. Box the **Start-up Now** option, and then click in the checkmark button of the **Start-up Settings** dialog



14. The start-up will be initiated and should take approximately 20 minutes

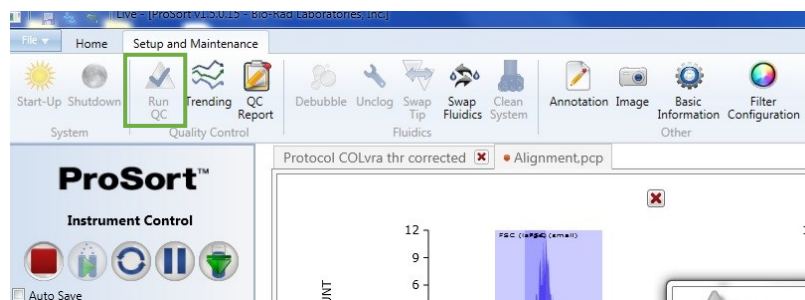


Initial Set Up: Quality Control (≈ 10 minutes)

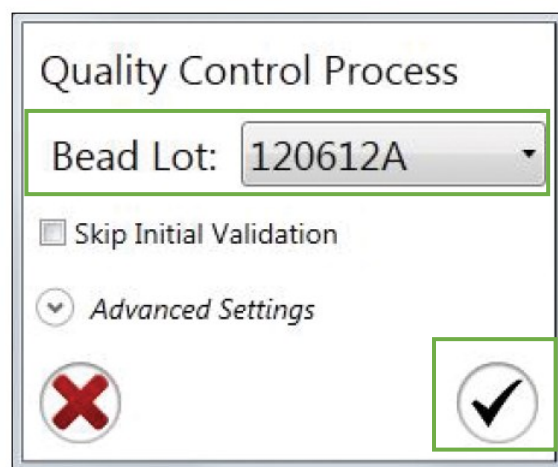
In case you are not the first user of the current day, please confirm with the previous user whether a low-pressure cleaning final set up has been performed. If so, skip both start-up and quality control procedures. NEVERTHELESS, DO NOT FORGET TO CHECK THE FLUIDICS. IF NEEDED, PERFORM THE “HOT SWAP” PROCEDURE (See Swapping Fluidics During Sorting: “Hot Swap”).

1. Vortex a bottle of ProLine Calibration Beads and place 8-10 drops into a 5 ml sorter tube. Do not dilute these particles.
2. Place the tube with beads into the sample input station and move the loading stage into the run position

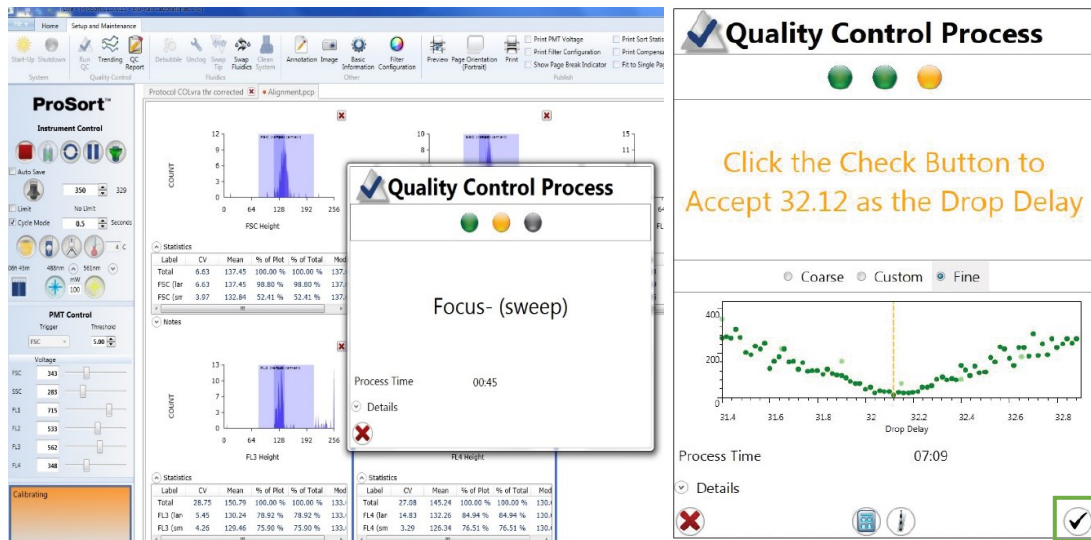
3. Click in the **Run QC** button in the **Setup and Maintenance** tab



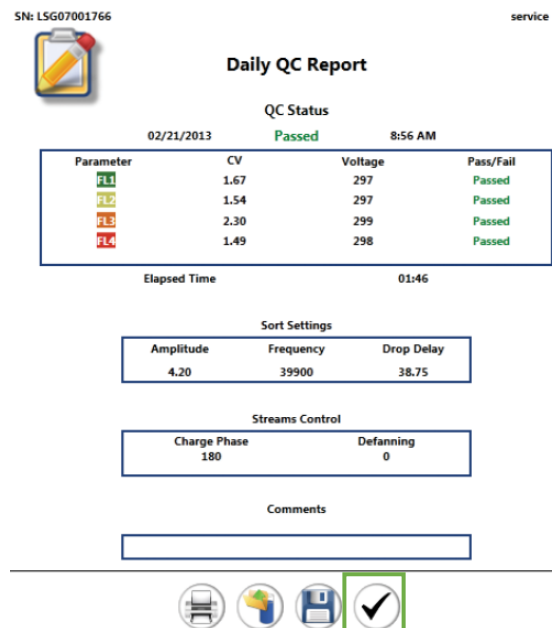
4. Choose the correct bead lot in the **Bead Lot** dropdown list of the **Quality Control Process** dialog and then click in the **checkmark** button to begin the **Quality Control** procedure



5. Once the drop delay calculation has been completed, click in the **checkmark** button to accept the drop delay. The QC procedure estimates the optimal drop delay and performs the side and forward-scatter detectors calibration



6. A QC report window will appear showing the QC test results. If all parameters have passed the QC test, click in the **checkmark** button to close the window

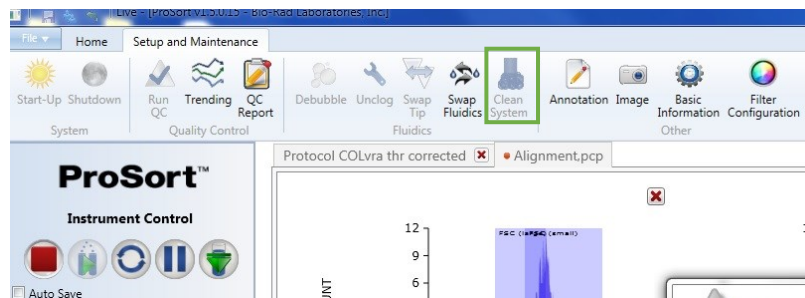


7. The system is ready to acquire and sort samples

Final Set Up: Low-Pressure Cleaning

The low-pressure cleaning procedure should only be performed between uses. If you are the last user, please perform the shutdown procedure. See Final Set Up: Shut Down to do so.

1. Fill a 5 ml tube with a minimum of 3 ml of cleaning solution (70% ethanol)
2. Load the tube into the sample station and move it into the run position
3. In the **Set Up and Maintenance** tab, click in the **Clean System** button



4. A **Clean Warning** dialog will open with a prompt warning for the fact that the tube solution will be rapidly consumed. Click in the **Yes** option
5. In the **Clean System Modes** window, select the **Low Pressure Clean** option and click in the **checkmark** button
6. A **Clean System** process window will be displayed
7. When the cleaning protocol is complete, the prompt **“Please put Loading Stage into the Wash Position”** will appear in the **Clean System** process window. Move the loading stage into the wash position to close such window



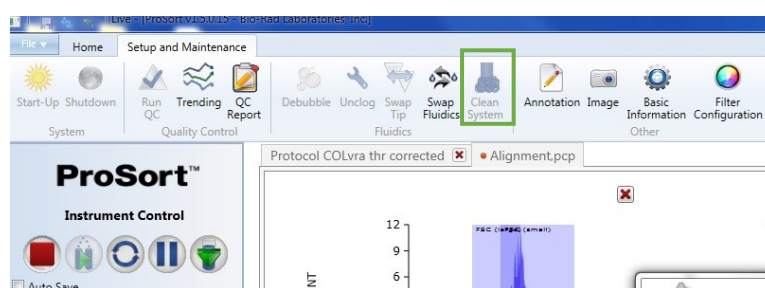
8. Repeat this procedure using MiliQ water as cleaning solution

9. Empty the waste and refill the fluidics containers using the “Hot Swap” procedure (See Swapping Fluidics During Sorting: “Hot Swap”)

Final Set Up: Shut Down

The shutdown procedure is only recommended for the end of day’s use. If you are not the last user, please perform a low-pressure cleaning procedure. See Final Set Up: Low-Pressure Cleaning to do so.

1. Fill a 5 ml tube with a minimum of 3 ml of cleaning solution (70% ethanol)
2. Load the tube into the sample station and move it to the run position
3. In the **Set Up and Maintenance** tab, click in the **Clean System** button

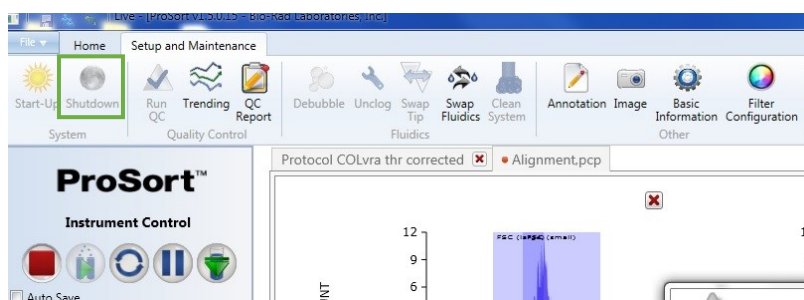


4. A **Clean Warning** dialog will open with a prompt warning for the fact that the tube solution will be rapidly consumed. Click in the **Yes** option
5. In the **Clean System Modes** window, select the **High Pressure Clean** option and click in the **checkmark** button
6. A **Clean Warning** dialog will open with a prompt warning for the fact that the high-pressure cleaning will leave the sorter uncalibrated. Click in the **Yes** option
7. A **Clean System** process window will be displayed

8. When the cleaning protocol is complete, the prompt “**Please put Loading Stage into the Wash Position**” will appear in the **Clean System** process window. Move the loading stage into the wash position to close such window



9. Click in the **Shutdown**  button in the **Setup and Maintenance** tab

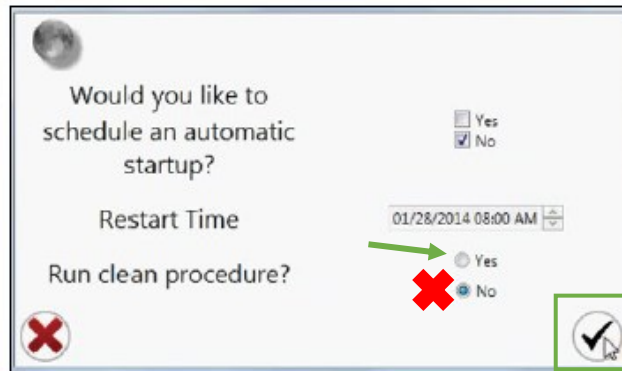


10. Select the **Yes** option to confirm that the system should be shut down at this time

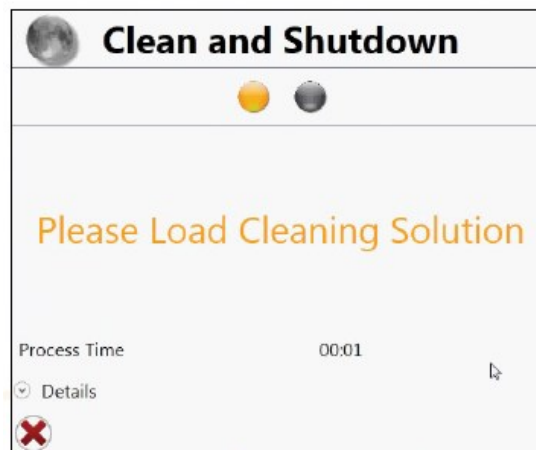
11. Select the **Yes** option to continue without saving protocols. Otherwise, select **Abort**, save the protocols and repeat the previous steps.

12. If you intend to use the **Automatic Start-up** option, box the **Yes** option and set both start date and time in the **Automatic Start-up** window

13. Yet in this window, select the **Yes** option for the **Run Clean Procedure** question and click in the **checkmark** button



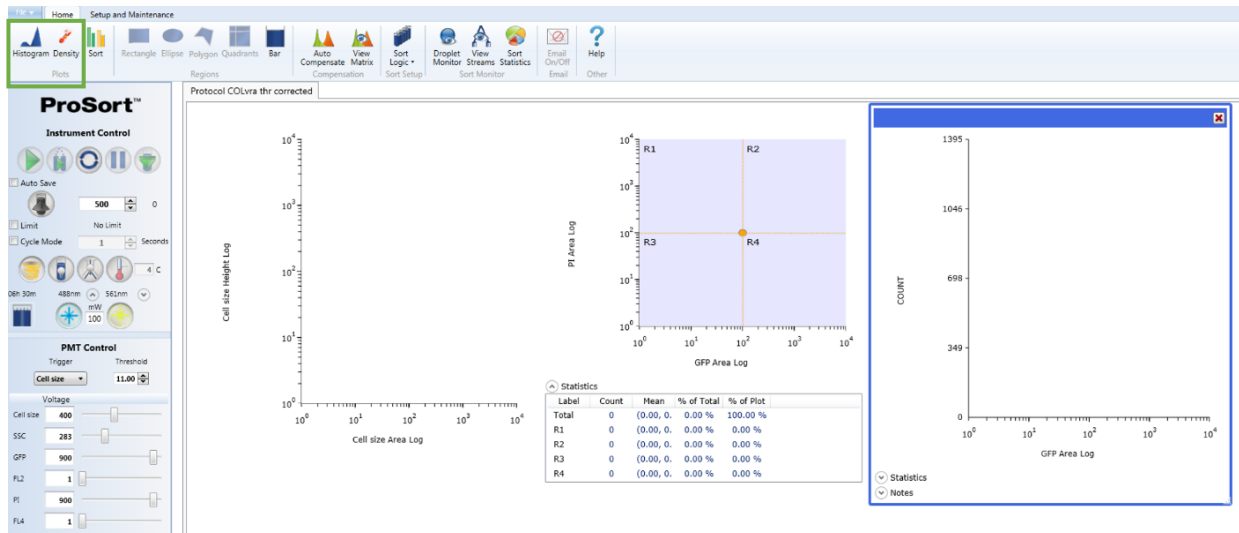
14. When the prompt **"Please Load Cleaning Solution"** appears in the **Clean and Shutdown** window, load a tube of water MiliQ and move the loading stage into the run position



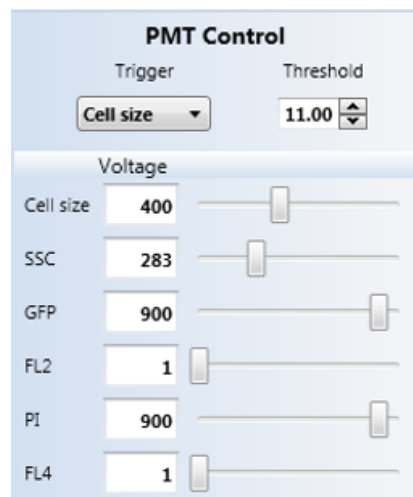
15. After the cleaning procedure move the loading stage into the wash position
16. At this time the system is fully shut down. The software will return to the opening log in screen
17. Close the **ProSort** software and switch off the computer
18. Empty the waste and refill the fluidics containers

Acquisition

1. Go to **File -> New Protocol** or **Open Protocol** (see Protocols and Workspace)
2. Create at least one histogram and dot plot by clicking in the **Histogram** and **Density** buttons of the **Home** tab

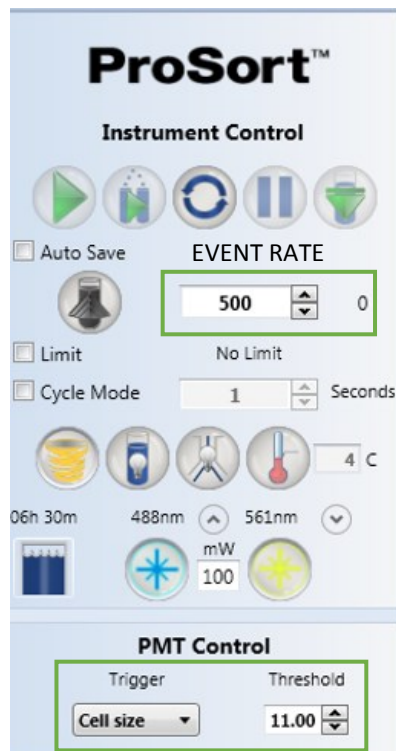



3. Load the sample into the sample input station and move the loading stage into the run position
4. Detectors can be renamed for each parameter based on the fluorophores or markers used



5. Box the **Cycle Mode** option to allow refresh of the data displayed

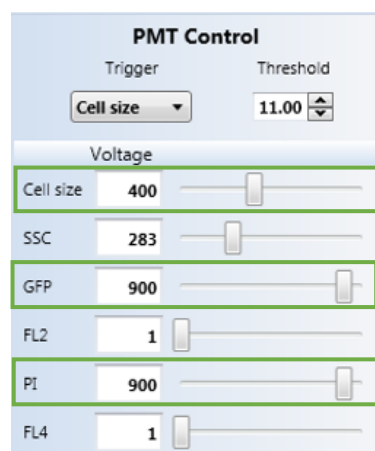
- Define the target event rate, trigger parameter and threshold value



- In the **Instrument Control Panel** window, click in the **Start**  button to begin sample acquisition



- Adjust photomultiplier tube (PTM) voltages to place populations

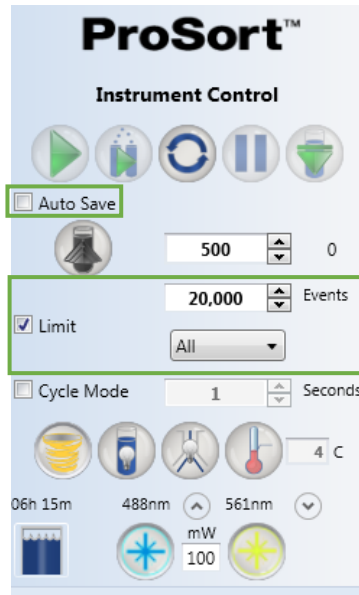


- Adjust the target event rate, trigger parameter and threshold value



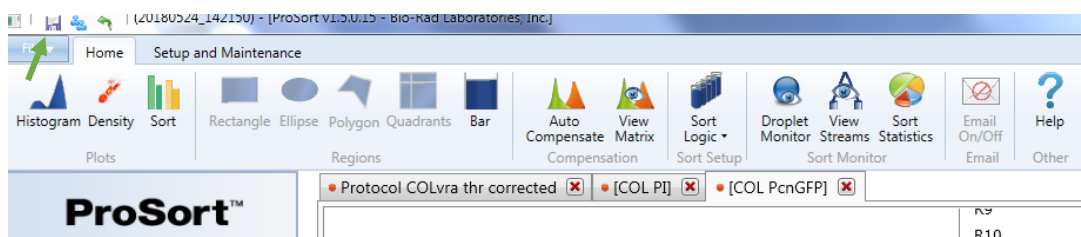
10. Stop sample acquisition by clicking the **Stop** button

11. Box and define the event limit. **Gate Limit** can be chosen, and **Cycle Mode** can be unboxed at this time. You can enable the **Auto Save** option to automatically save the file after the event limit is reached



Cycle Mode can be enabled, or the **refresh** button can be used to refresh the data as adjustments are being made

12. Re-collect the sample and save the FCS format file if **Auto Save** has not been boxed



13. Unlock the loading stage and remove the sample tube

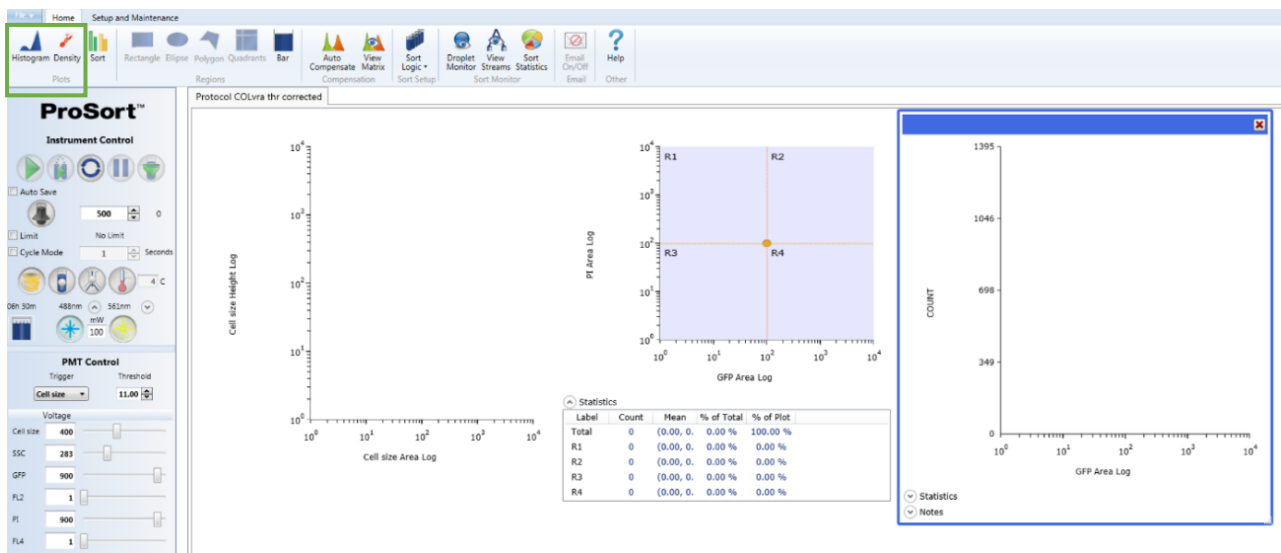
14. Move the loading stage into the wash position before running the next sample

When running multicolour experiments, compensation is necessary. To use the **Auto Compensation** wizard, single stained compensation control samples must be saved in addition to the multi-coloured samples

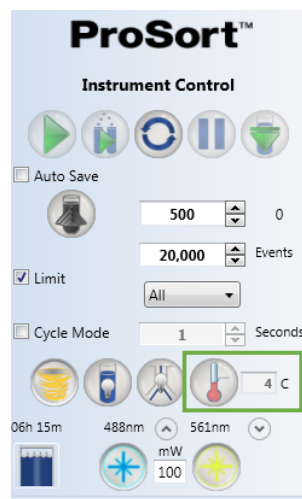
15. Run a single stained compensation control for each parameter
16. Set the event limit high enough to visualize a positive population, then collect and save the file
17. Use the **Auto Compensation** wizard to set compensation for multi-coloured samples (see Auto Compensation Wizard)

Sorting

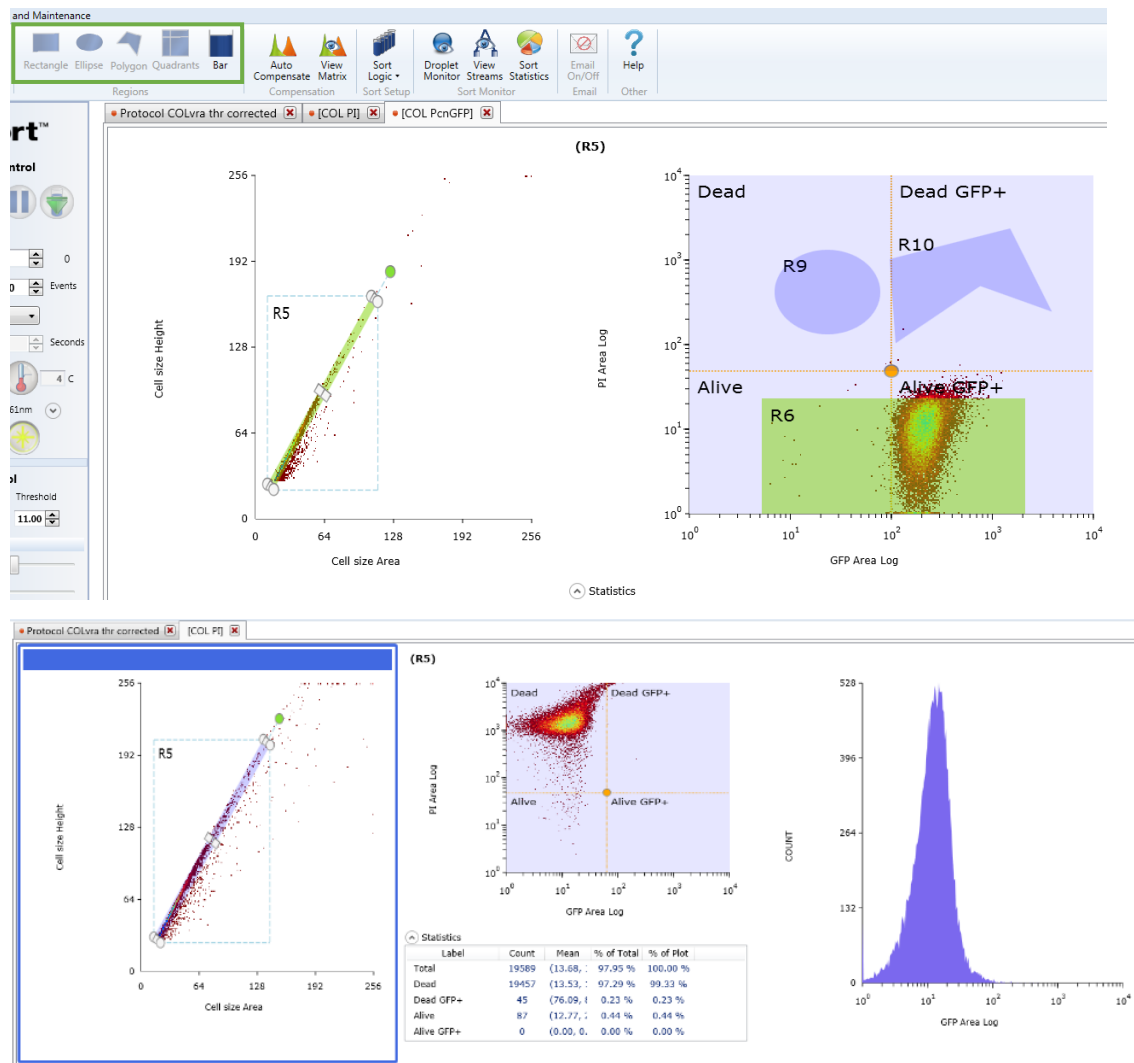
1. Go to **File -> New Protocol** or **Open Protocol** (see Protocols and Workspace)
2. Create at least one histogram and dot plot by clicking in the **Histogram** and **Density** buttons of the **Home** tab



3. If desired, adjust temperature control for sample input and collection area in the **Temperature Control** area of the **Instrument Control Panel**

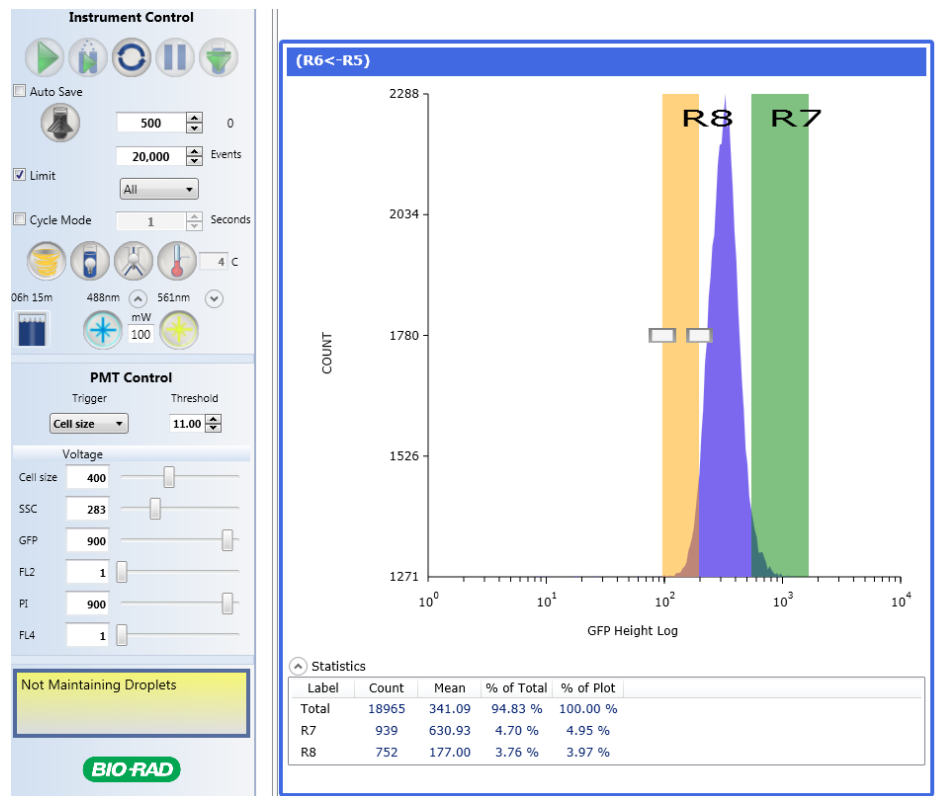


4. Acquire a small amount of the sample to be sorted
5. Set sorting regions and gates (in the **Set Up and Maintenance** tab) on plots with the populations of interest



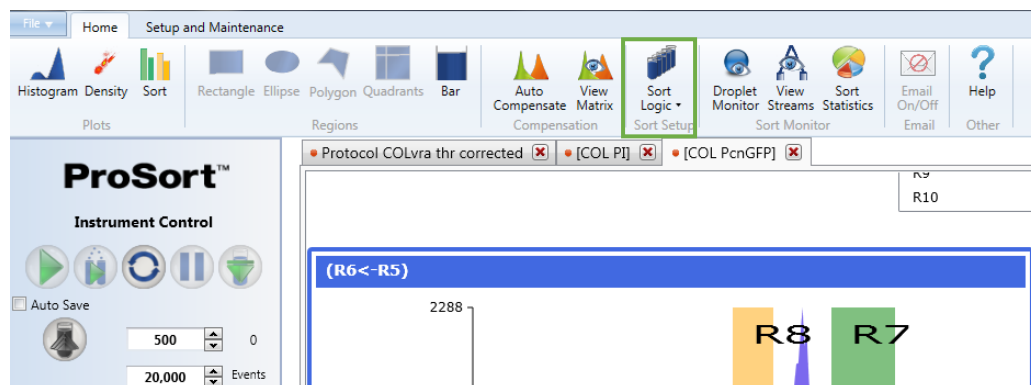
Hint: Create a signal intensity versus time dot plot. The signal intensity and time should be displayed on the y and x axes, respectively. Then, select as many dots as possible linearly disposed as if in a direct proportion. The dots contained in this region stand only for single events. Use, therefore, this region of single events to fine sorting the sample. Notice that, single events can also be fine-tuned using a FSC height versus FSC area dot plot.

6. Once all regions are set, right click in the desired region to be sorted and select the sort direction



7. Repeat with a second region if two-way sorting is desired

8. Click in the **Sort Logic** button and select a collection vessel in the **Sort Logic** dropdown menu



9. Set the parameters in the **Sort Logic** window by selecting the sort mode (**Purity**, **Enrich** or **Single**) and event limit for each collection position

Sort Logic

Tube (1.5mL)

Sort Priority: Left Sort (←) Right Sort (→)

☐ Move to Next Row on Sort Limit

Prevent Overflow Initial Volume (mL) 0.01

R5 & R6 & R7

Position	Sort Mode	Limit	Limit Value
1	None	<input type="checkbox"/>	
2	Enrich	<input checked="" type="checkbox"/>	3,000
3	None	<input type="checkbox"/>	
4	None	<input type="checkbox"/>	
5	None	<input type="checkbox"/>	
6	None	<input type="checkbox"/>	
7	None	<input type="checkbox"/>	
8	None	<input type="checkbox"/>	
9	None	<input type="checkbox"/>	
10	None	<input type="checkbox"/>	

Buttons: Refresh (↺), Confirm (✓)

The **Enrich** sort mode stands for sorting positive-match events, although there may be contamination with negative-match events (advised for low concentrations)

The **Purity** sort mode stands for sorting only positive-match events, although there may be double or more events sorted at once

The **Single** sort mode stands for sorting only single positive-match events

10. Define the sort collection tube volume if you intend to track the collected volume for preventing collection overflow. Set the volume of buffer or cushion liquid already in the collection tubes

Sort Logic

Tube (1.5mL)

Sort Priority: Left Sort (←) Right Sort (→)

☐ Move to Next Row on Sort Limit

Prevent Overflow Initial Volume (mL) 0.01

R5 & R6 & R7

Position	Sort Mode	Limit	Limit Value
1	None	<input type="checkbox"/>	
2	Enrich	<input checked="" type="checkbox"/>	3,000
3	None	<input type="checkbox"/>	
4	None	<input type="checkbox"/>	
5	None	<input type="checkbox"/>	
6	None	<input type="checkbox"/>	
7	None	<input type="checkbox"/>	
8	None	<input type="checkbox"/>	
9	None	<input type="checkbox"/>	
10	None	<input type="checkbox"/>	

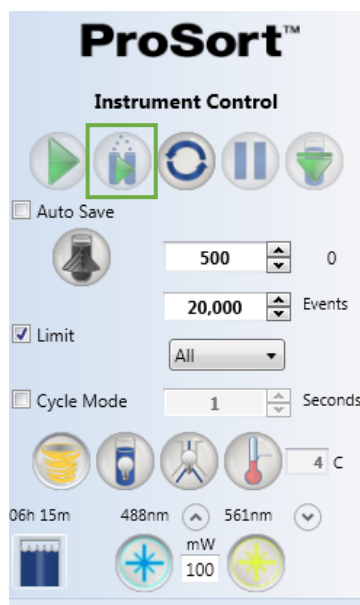
Buttons: Refresh (↺), Confirm (✓)

11. Place the sort collection tubes in the sort collection device at the previously selected positions




12. Make sure the 5 ml tubes (if it is the case) have at least 0.5 ml of buffer to cushion the sorted cells

13. Close the sort chamber door and click in the **Start Sorting** button of the **Instrument Control Panel**



14. The **Sort Statistics** window will appear, and sorting will be initiated. This window can be reopened by clicking the **Sort Statistics** button in the toolbar

			
Sort Statistics			
<input checked="" type="checkbox"/> Show Aggregate Statistics			
Left Sort		Right Sort	
Sort Logic	R5 & R6 & High Pop	R5 & R6 & Low Pop	
Mode	Single	Single	
Current Target	1	2	
Sort Count	12,000	12,000	
Sort Volume (mL)	0.0356	0.0356	
Abort Count	12734	11979	
Sorts Per Second	15	8	
Aborts Per Second	16	8	
Sort %	1.50 %	1.50 %	
Abort %	1.59 %	1.49 %	
Efficiency	48.39 %	50.00 %	
Event Rate		Total Events	
0		802,030	
Sort Time	00:22:06		

Other Guidelines

Swapping Fluidics During Sorting: “Hot Swap”

New containers or bottles should be prepared before clicking the Swap Fluidics

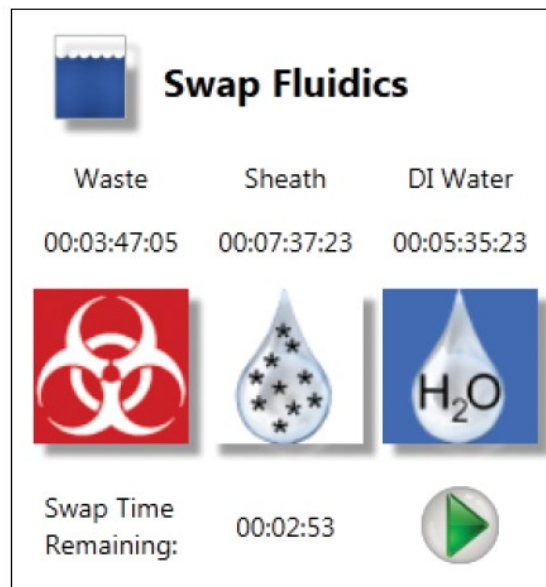


button, as the swap time is limited to only 3 minutes before the system starting to shut down due to low fluid levels.

Read carefully all procedure before starting the “**Hot Swap**”

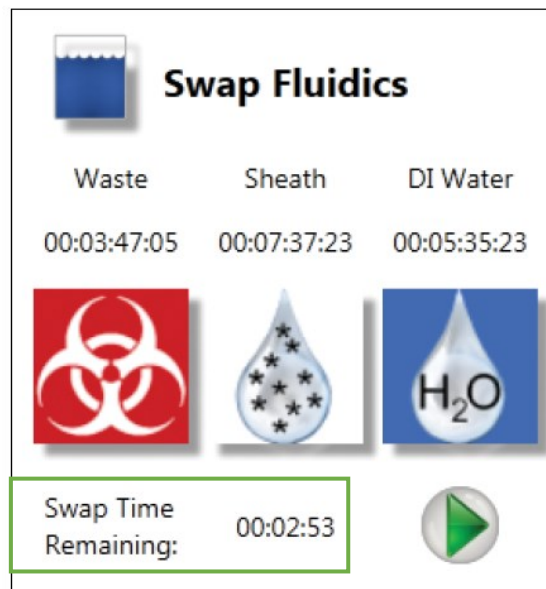


1. Click in the **Swap Fluidics** button of the **Setup and Maintenance** tab. The **Swap Fluidics** window will open




2. Click in the **Play**  button of the **Swap Fluidics** window

After clicking the **Play** button, a countdown timer will start in the bottom of the **Swap Fluidics** window



Operate quickly and carefully from now on

3. Open the fluidics door located on the left side of the sorter
4. Detach the quick disconnect from the cap assembly by pushing the metal button
5. Lift the quick disconnect until it engages the magnetic holder. This will hold the quick disconnect away from the exchange area
6. Slightly lift and pull out the container
7. Uncap the cap assembly from the container
8. Refill, change or empty the container
9. Carefully place the cap assembly into the container and tighten the cap
10. Place the container into position
11. Attach the quick disconnect to the cap assembly (you should hear a click)
12. Click in the **Play**  button of the **Swap Fluidics** window again to finish the swapping procedure

Auto Compensation Wizard

The compensation wizard assists with establishing the proper compensation matrix coefficient for each parameter. Single controls for each parameter and the associated FCS files must be saved prior to compensation.

1. Run single controls for each parameter and your multi-stained sample.
2. Save the acquisitions as FCS files.
3. Go to **Home -> Compensation Wizard**
4. Select the parameters for compensation
5. Click the file icon for each selected parameter
6. Locate and select the single control FCS file for each parameter
7. Select whether to use height or area for all parameters
8. Click the forward blue arrow to open the **Automatic Compensation Calculation Wizard** window

The screenshot shows the 'Auto Compensate' wizard window. The title bar says 'Auto Compensate'. Below the title bar, it says 'This wizard will assist in establishing the proper Compensation Matrix coefficients.' The window contains several sections for selecting FCS files for different parameters. Each section has a label, a text box for the file path, and a file icon button. The sections are: 'Global Negative Population' with file 'C:\Users\user\Four color 1.1 Data\unstained neg.fcs', 'CD3 FITC event source' with file 'C:\Users\user\Four color 1.1 Data\FITC comp.fcs', 'CD8 PE event source' with file 'C:\Users\user\Four color 1.1 Data\PE comp.fcs', 'CD45 PETR event source' with file 'C:\Users\user\Four color 1.1 Data\PETR comp.fcs', and 'CD4 PECy7 event source' with file 'C:\Users\user\Four color 1.1 Data\PECy7 comp.fcs'. At the bottom, there is a 'Compensate on' dropdown menu set to 'Area'. The window has a red 'X' button on the left and a blue right arrow button on the right, both highlighted with green boxes.

Auto Compensate
This wizard will assist in establishing the proper Compensation Matrix coefficients.

Global Negative Population
C:\Users\user\Four color 1.1 Data
unstained neg.fcs

CD3 FITC event source
C:\Users\user\Four color 1.1 Data
FITC comp.fcs

CD8 PE event source
C:\Users\user\Four color 1.1 Data
PE comp.fcs

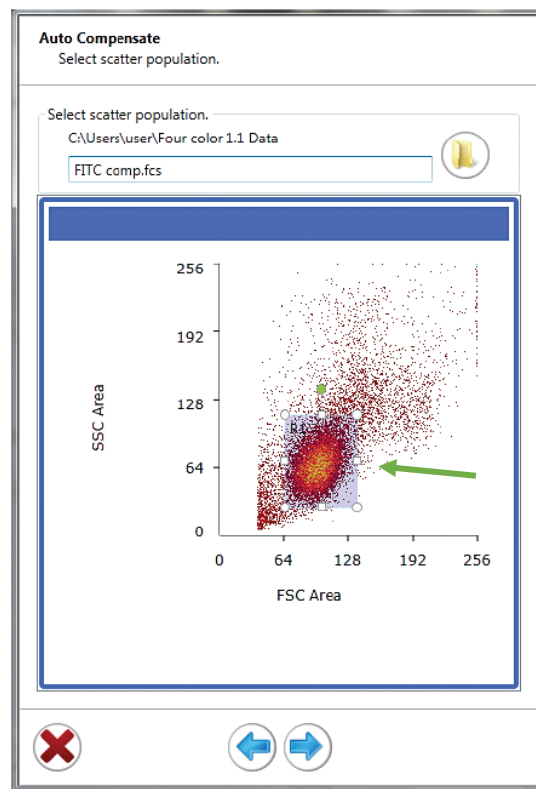
CD45 PETR event source
C:\Users\user\Four color 1.1 Data
PETR comp.fcs

CD4 PECy7 event source
C:\Users\user\Four color 1.1 Data
PECy7 comp.fcs

Compensate on Area

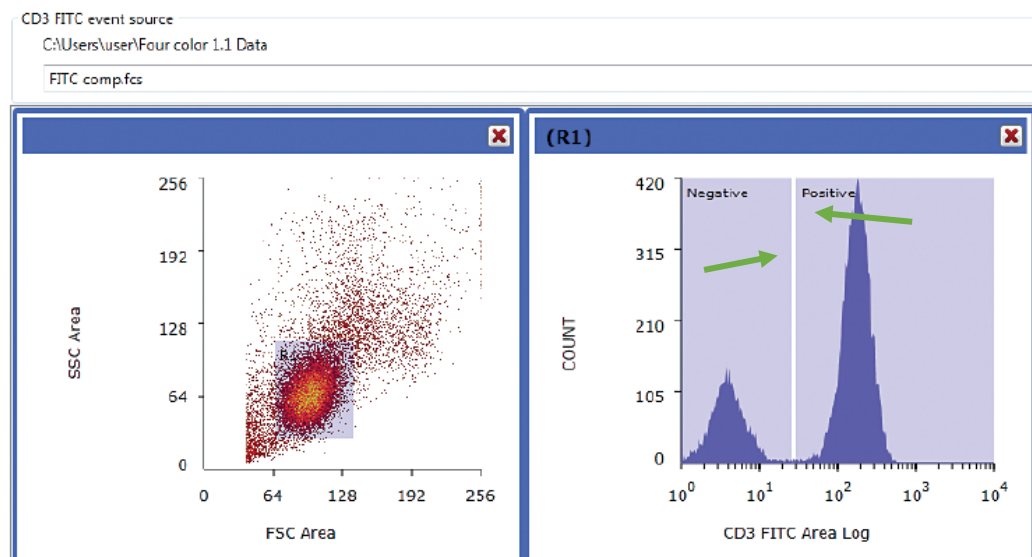
Buttons: [Red X] [Blue Arrow]

9. Adjust the scatter, negative, and positive regions as necessary. **Note** that the positive and negative population gates **must not overlap**



Auto Compensate

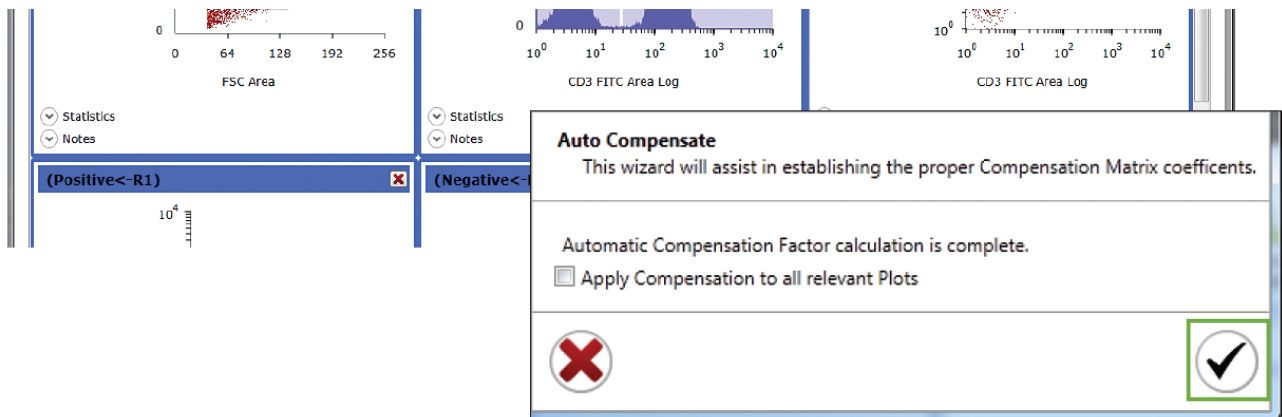
Setup the Positive and Negative population gates.



10. Click the blue forward arrow to go to the next control

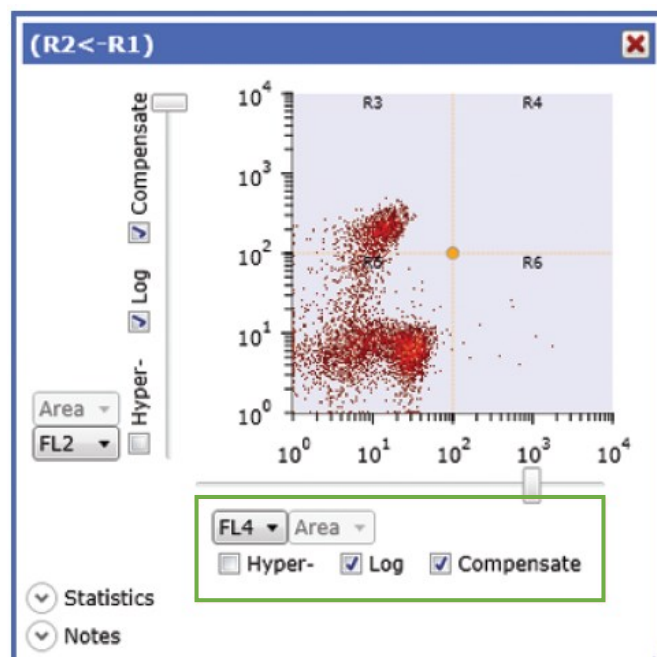
11. Repeat steps 9 and 10 for remaining controls

12. After all controls are adjusted, click the checkmark button. This will complete the automatic compensation calculation



13. Check the compensation box on the axis of the desired histogram or density plot to display either the compensated, acquired or sort data

14. The compensation matrix may be viewed at this point if desired



The hyper log feature allows data that have values lesser than zero to be correctly displayed. This data display format is used only for compensated data. It converts a small portion of the scale around zero to linear and adds a small amount of negative log scaling to each access, preventing negative events from piling up on the axis. This allows better visualization of compensated data.

Note that the area parameter is greyed out and cannot be changed because compensate is checked. Compensation can only be applied to area or height depending on which selection was chosen during auto compensation.

Protocols and Workspace

Create a New Protocol

1. Go to **File -> New Protocol**
2. A new protocol will open as a new tab in the workspace
3. From the **Home** tab, you can add a histogram or density plot to your workspace

Opening Protocols

You can open a protocol from either a protocol format file or FCS format file

1. Go to **File -> Open Protocol**
2. A Windows dialog will open to locate and open the desired protocol
3. Search for the protocols directory
4. To the right of the file name field, there is a dropdown box with the protocol or FCS file options
5. If you select the FCS file open, a protocol can also be opened from a saved FCS file (see below how to perform this in another way)

Saving Protocols

Each protocol is saved with the following information: plots; regions; gates; trigger; threshold; PMT voltages; sort logic; sort limits; sort modes; compensation settings; filter configuration

1. Go to **File -> Save Protocol**
2. This will save a protocol format file in the currently assigned directory

Loading Instrument Settings

You can load pre-existing instrument settings from a protocol format file into a currently open protocol

1. Open the protocol to be used or create a new protocol and save
2. Go to **File -> Load Instrument Settings**
3. Select the protocol that contains the settings to be loaded
4. This will load pre-existing instrument settings into the currently open protocol

Opening FCS Files

You can open a FCS file into two different ways: embedding the data into the currently open protocol; opening the data into the protocol in which it was saved

a) To embed the data into the currently open protocol, go to **File -> Open FCS Data Only**

b) To open the data into its corresponding protocol, go to **File -> Open FCS with Protocol**

Remember that you can save FCS files after running acquisition or sorting procedures. In addition, the FCS files will not only save the procedures statistics as will also store the protocol in use

Further Protocols, Tutorials and Guidelines

Contact the responsible people to acquire the following tutorials and instruction manuals:

- Instruction Manual
- Quick Guide
- Daily Procedures
- Filter Sets
- ProFlow Sort Grade 8x Sheath Fluid Instructions Handout
- ProFlow Sort Grade Water Instructions Handout
- ProLine Universal Calibration Beads Instructions Handout