

**DanStem and NNF CPR
Flow Cytometry Core Facility**

Sony SH800 Cell Sorter



Quick Reference Guide

Introduction to Sony SH800

The SH800 is a simple, ready-to-use, cell sorter developed by Sony Corporation.

Main Features

1. Fully-automated setup

All setup steps, including optical alignment, droplet formation, side stream calibration, and delay time adjustment, are automated using Sony-developed CoreFinder™ technology. This completely eliminates all the complicated setup work required with conventional cell sorters.

2. High-accuracy sorting

Equipped with built-in analog-to-digital converter module for high-speed signal processing, using Sony electronics technology cultivated in the development of consumer devices. High-speed signal processing enables the SH800 to achieve high-purity sorting performance.

3. Simple maintenance

The SH800 uses a unique Sony-developed plastic sorting chip, created using micro-fabrication technology gained through experience with optical discs. This allows disposable components to be employed, instead of using fixed, expensive quartz flow cells. The sample line and sample probe are also designed for easy maintenance.

4. Desktop size footprint

Employs integrated laser technology and compact mechanical design, making it approximately 1/3rd the size of conventional cell sorters.

[Width 55 cm (21 ³/₄ in.) / Depth 55 cm (21 ³/₄ in.) / Height 72cm (28 ³/₈ in.)]

5. Easy-to-use software

Powered by SH800 software, created by Sony, that performs all functions, including automatic setup, sorting, and acquired data analysis.

System Configuration and Name of the Parts



Host computer



Fluidics cart



SH800 main unit



Compressor

Note!
Droplet formation may become unstable if the fluidics lines between the main unit and fluidics cart are exposed to air flow due to air conditioners, fans, open windows, or other sources.

- The host computer controls data acquisition and other hardware modules, and performs analysis on acquired data.
- The SH800 main unit contains the lasers, detectors, electrical systems, and fluidics flow systems.
- The fluidics cart contains the sheath tank, waste tank, and ethanol tank.

Startup Preparation

a. Sheath tank



Fluidics cart

b. Waste tank

c. Ethanol tank

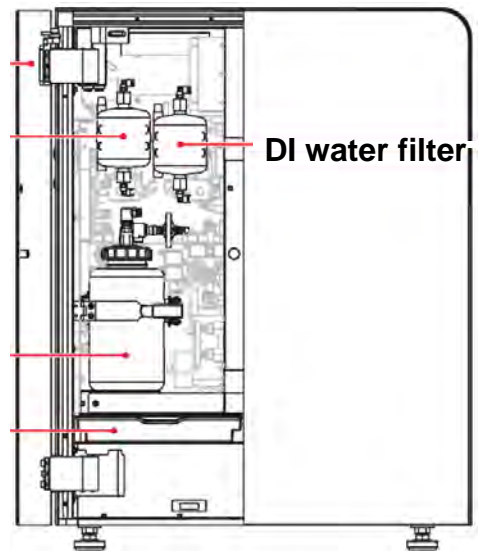


d. Sheath filter

DI water filter

e. DI water tank

Drip tray



SH800 main unit (left side)

f. Automatic setup beads and sorting chip

1. Check that the sheath tank in the fluidics cart (a) is full with sheath fluid (PBS bag).
2. Check that the waste tank in the fluidics cart is empty (b).
3. During normal operation, ethanol is **not required** in the ethanol tank in the fluidics cart (c).
* Fill the ethanol tank with ethanol only when cleaning the sheath line using ethanol (page 31).
4. Open the fluidics maintenance door (left side) and check that there are no bubbles in the sheath filter (d). Check only. If there are bubbles, de-bubble the filter after turning on the power (page 8).
5. Open the fluidics maintenance door (left side) and make sure the DI water tank is full (e) with milliQ water.
6. Prepare the automatic setup beads (LE-B3001) and a sorting chip (LE-C3110) (f).

Refilling the Sheath Tank



Sheath tank lid

Before startup, always check the sheath fluid level and refill the sheath tank if necessary (5 to 10 liters, 1.3 to 2.6 gallons). The sheath fluid consumption rate is about 2.5 liters/8 hours. If the sheath fluid runs out, acquisition will be stopped, so it is recommended to always start with a full tank.

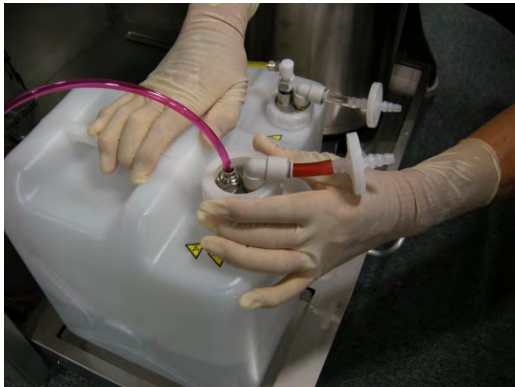
1. Check that the power is turned off.
2. Check that the compressed air supply is turned off.
3. Pull out the fluidics cart tray.
4. Disconnect the sheath air line (clear) and sheath fluid line (blue) from the top of the sheath tank.
5. Release the residual air pressure from the tank using the ring-pull air relief valve, then remove the tank from the tray.
6. Lift the lever securing the lid of the tank to open the lid.
7. Lower the lid slightly into the tank, turn it 90 degrees, and then remove the lid from the tank.
8. Refill the tank with sheath fluid.
9. Insert and secure the lid, and reconnect the sheath lines in the reverse order of removal.
10. Place the tank back in the fluidics cart.

Note!

Droplet formation may be adversely affected if the temperature of the added sheath fluid is significantly different from that of the sheath fluid in the tank.

Store replacement sheath fluid, if possible, in a room with the same temperature conditions as the room housing the main unit. Alternatively, place the replacement sheath fluid in the same room as the main unit and allow it to acclimatize before refilling the sheath tank.

Emptying the Waste Tank



Waste tank cap

- Before startup, always dispose of waste fluid and replace with an empty waste tank.
1. Check that the power is turned off.
 2. Pull out the fluidics cart tray.
 3. Remove the cap of the waste tank and remove the nozzle.
 4. Remove the tank from the fluidics cart.
 5. Place an empty tank in the fluidics cart.
 6. Reinsert the nozzle into the waste tank, tighten the cap, and stow the tray.

Dispose of waste fluid in accordance with laboratory rules.

Refilling the DI Water Tank



DI water tank
(inside fluidics maintenance
door on left side of main unit)

- Before startup, always make sure the DI water is full.
1. Check that the power is turned off.
 2. Disconnect the line from the top of the DI water tank.
 3. Undo the metal retaining clamp and remove the tank.
 4. Remove the cap from the tank, refill with DI water up to the lip of the tank, and reattach the cap.
 5. Place the tank back in the unit and reattach the clamp.

DI water is principally used to clean the sample probe and sample line after each measurement. Refilling the tank at the beginning of each day will ensure there is sufficient fluid for a day of testing.

Turning System Power On

① Compressor



Turn compressor on
1 minute before
turning on the main
unit

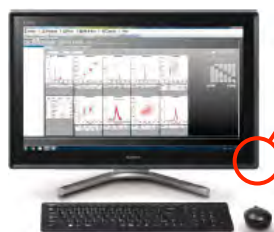
② SH800 main unit



Note!
Do not open the flip-up
door before turning on
the air supply!
Doing so may damage
the unit!

Power switch

④ Keyboard



③ Computer

⑤ Mouse



Turn on the system power in the following sequence.

1. Press the power button on the right of the front panel of the SH800 main unit.
A green indicator lights up and system initialization starts ("Standby" appears after about 30 seconds).
2. Turn on the host computer using the power switch on the top right of the screen.
3. Turn on the keyboard using the power switch on the top right.
4. Turn on the mouse using the power switch on the underside.

Checking and De-bubbling the Sheath Filter

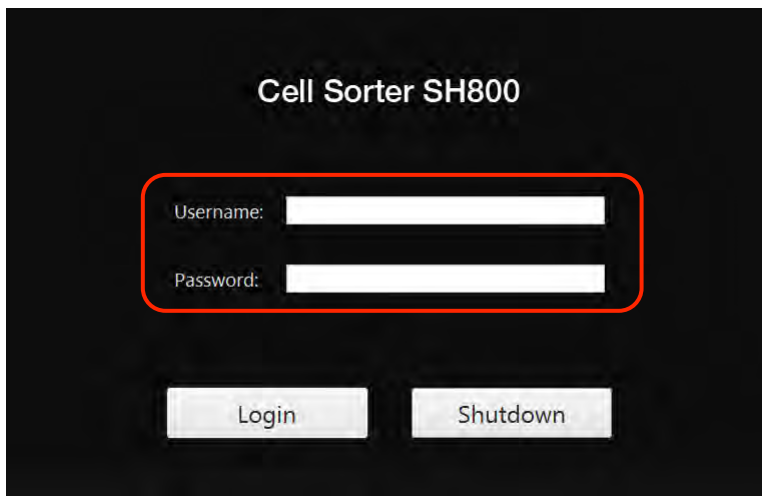


**Sheath filter
(inside fluidics maintenance
door on left side of main unit)**

After startup, always check that there are no bubbles inside the sheath filter.
Remove any air trapped inside the filter.

1. Check that “Standby” appears on the LCD display on the front of the main unit after initialization finishes.
2. Open the fluidics maintenance door, remove the sheath filter from the retaining clamp (without disconnecting the connectors), tap the side of the filter 2 or 3 times with your hand, and check for bubbles in the filter.
If there is no air trapped in the filter, place the sheath filter back and reattach the clamp (no further action is required). Reinsert the filter with the arrow on the side of the filter facing up.
3. If there is air trapped in the filter, open the air release port on the top of the filter to expel the trapped air. Hold a kimtowel wipe around the port to collect any sheath fluid overflow from the air release port.
4. Flip the sheath filter upside down and repeat the procedure to release trapped air from the air release port on the bottom of the filter.
5. Reinsert the filter with the arrow on the side of the filter facing up and reattach the clamp.

System Startup



- Double-click the SH800 Software icon

SH800 login window

1. When the Windows login screen appears, enter your User ID and Password, and then log in.
User ID: **fc**m Password: **password**
2. Wait until the SH800 has finished the initialization and the monitor displays "Standby"
3. After Windows starts, double-click the SH800 icon on the desktop to launch SH800 software.
When the login window above appears, enter your Username and Password, and then press [Login].
Username: **user1** Password: (leave blank)

After logging in, the Initial Instruments Setup window appears.

System Startup

Cell Sorter SH800

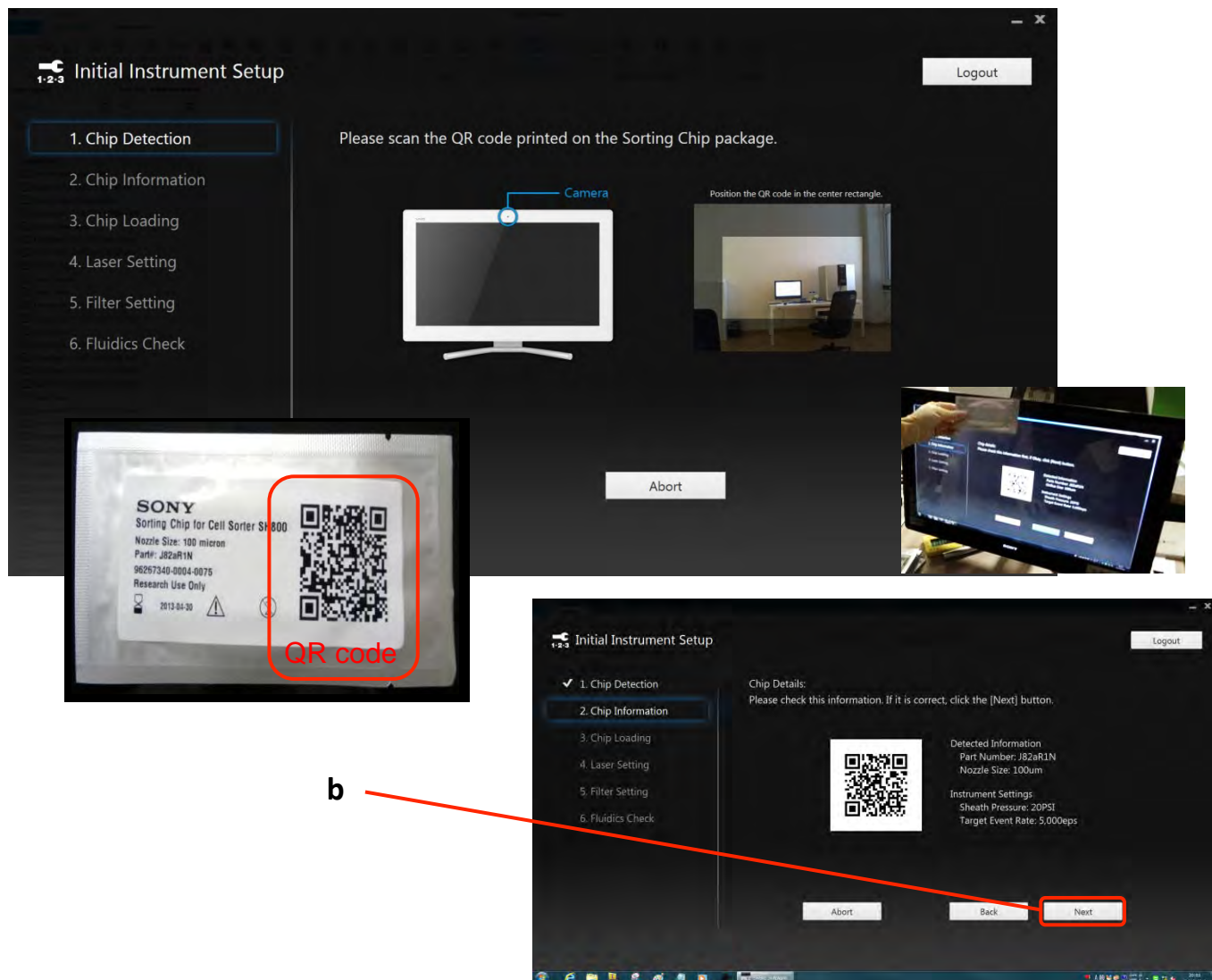
Username: user1

Password:

Login Shutdown Maintenance

1. When the login window above appears, enter your Username and Password (a), and then press [Login] (b).
Username: **user1** Password: (leave blank)
2. If you would like to shutdown the instrument press [Shutdown] (c)
3. To access different cleaning routines press [Maintenance]

Chip Detection

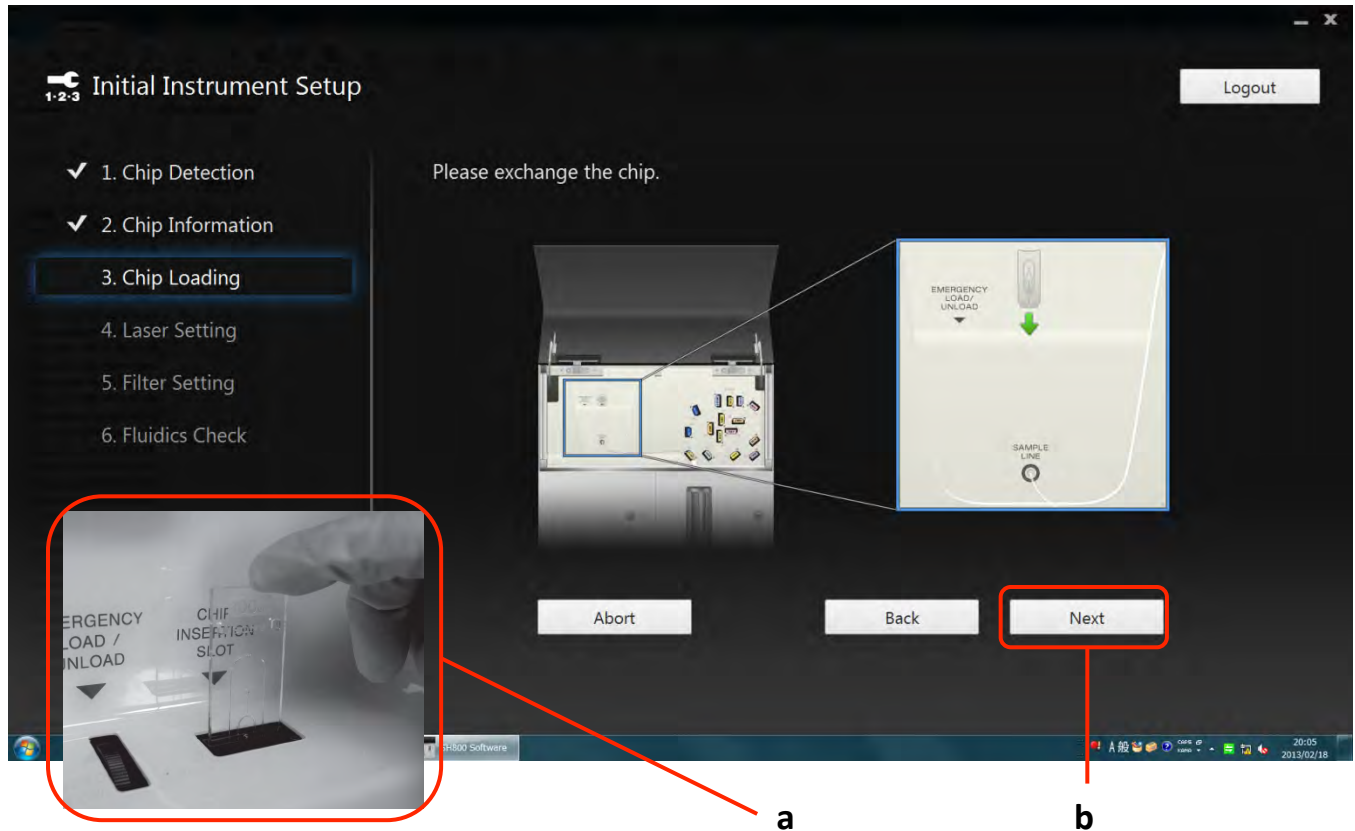


Register a sorting chip with the SH800 main unit.

1. Hold the QR code on the sorting chip package in front of the built-in camera on the host computer.
2. Check the information displayed on the screen for the sorting chip, and then click [Next] (b).
The Chip Loading window appears.

- The sorting chip can be used for 24 hours after it is registered.

Loading a Chip



Load the sorting chip in the SH800 main unit.

1. Open the flip-up door on the top of the front panel of the main unit.
2. Remove the sorting chip ejected automatically from the chip insertion slot.
3. Insert the new registered sorting chip (a).

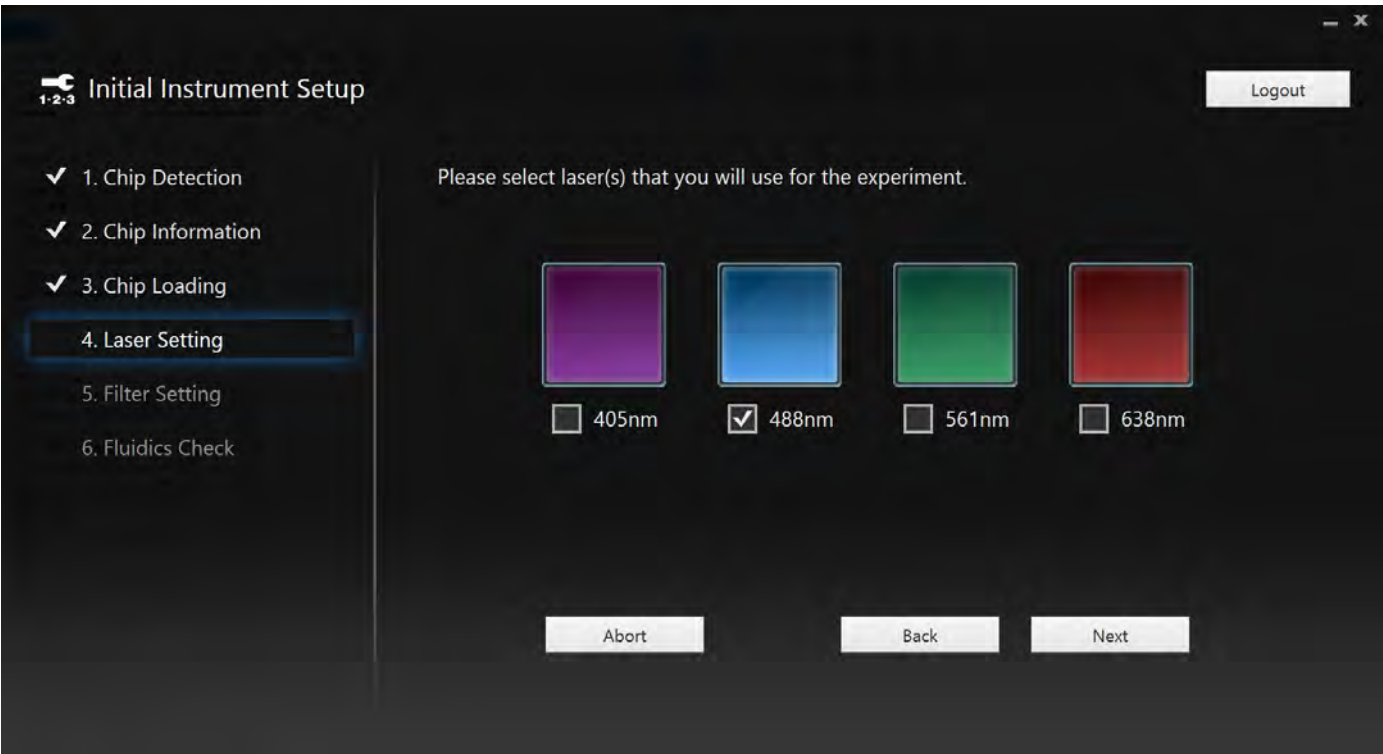
Insert the chip part-way into the chip insertion slot with the “100 μ m” label facing toward you. The chip loader automatically loads the chip.

* Do not exert excessive force when inserting the chip.

4. Click [Next] (b).

The Laser Setting window appears.

Laser Selection

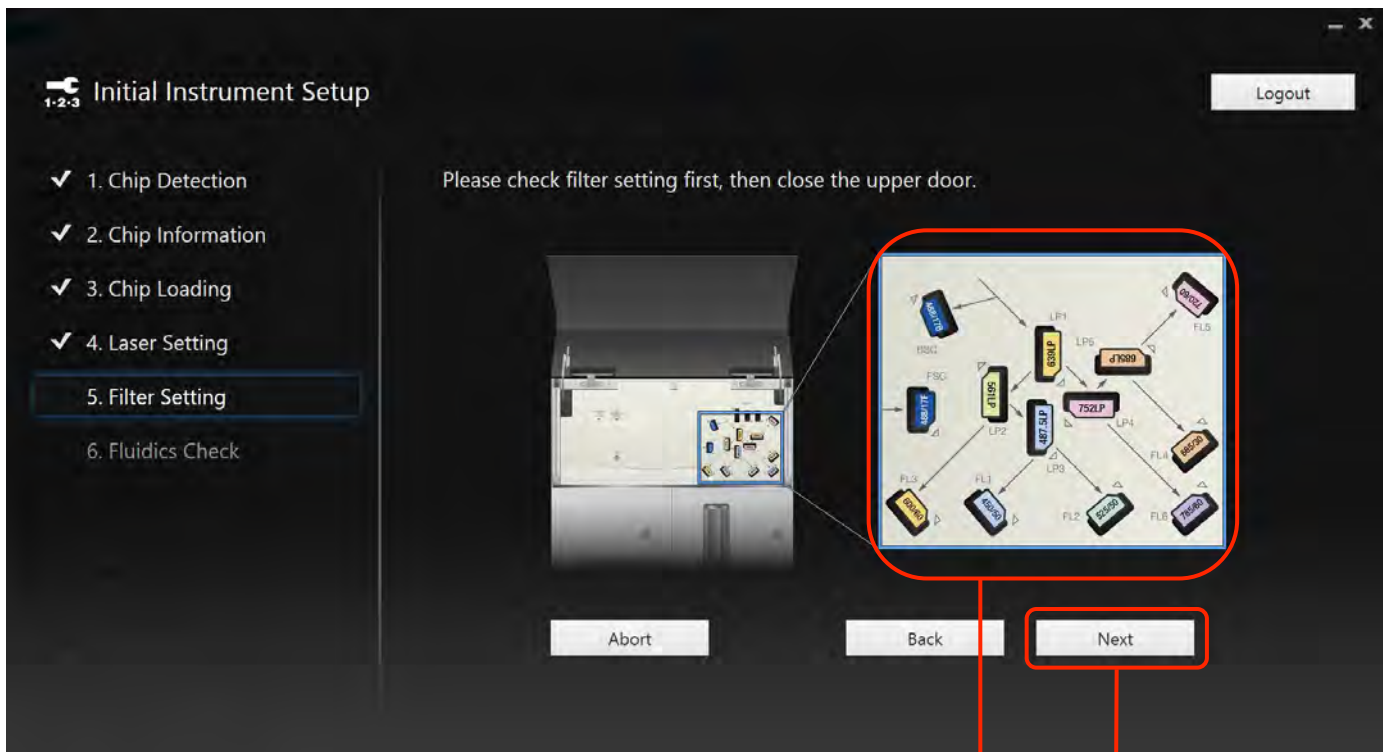


Example. Display for 4-laser model

Choose your desired laser configuration for your experiment

- 1. Placing a check mark in a checkbox selects the corresponding laser.
- 2. The blue (488 nm) laser is mandatory for the system setup.
- 3. You can change these settings later in the experiment
- 4. Click [Next]

Check the Optical Filter Pattern



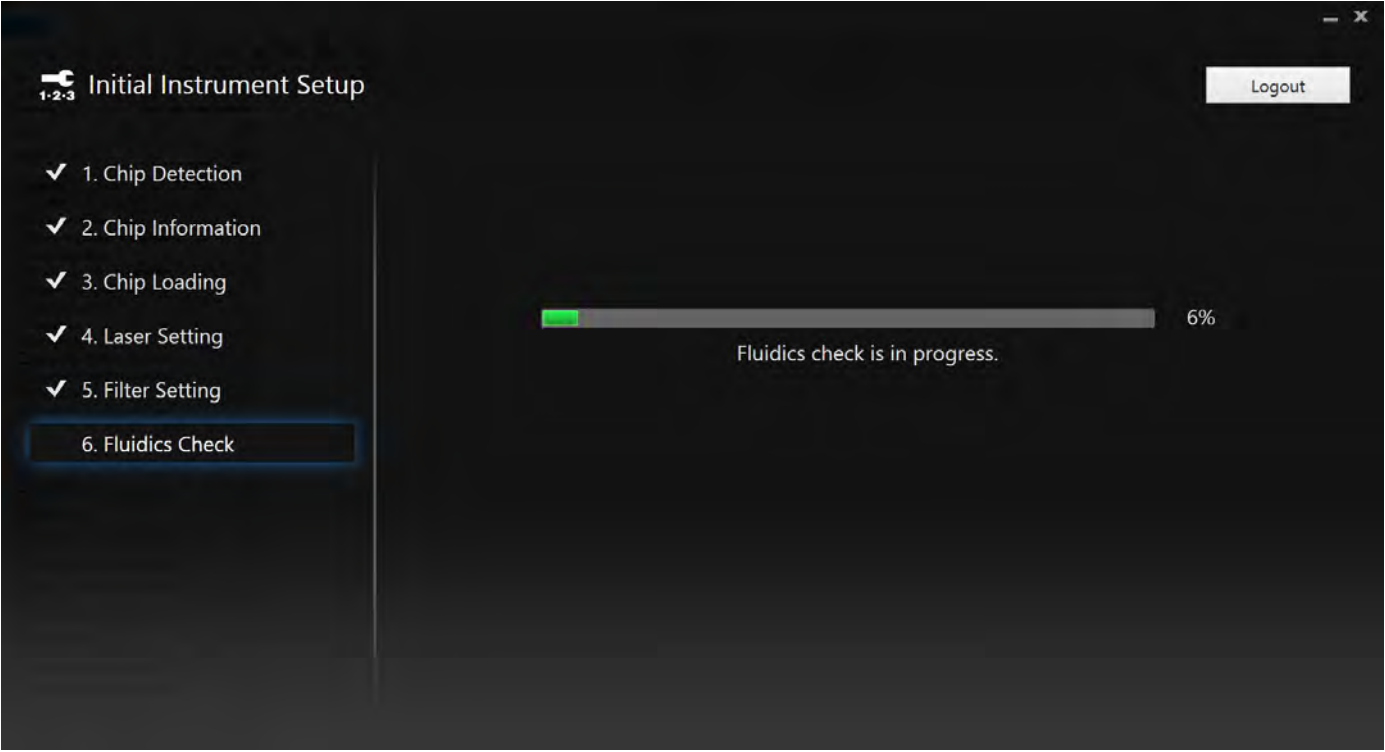
a

b

Check that the optical filter pattern is correct for the selected combination of lasers.

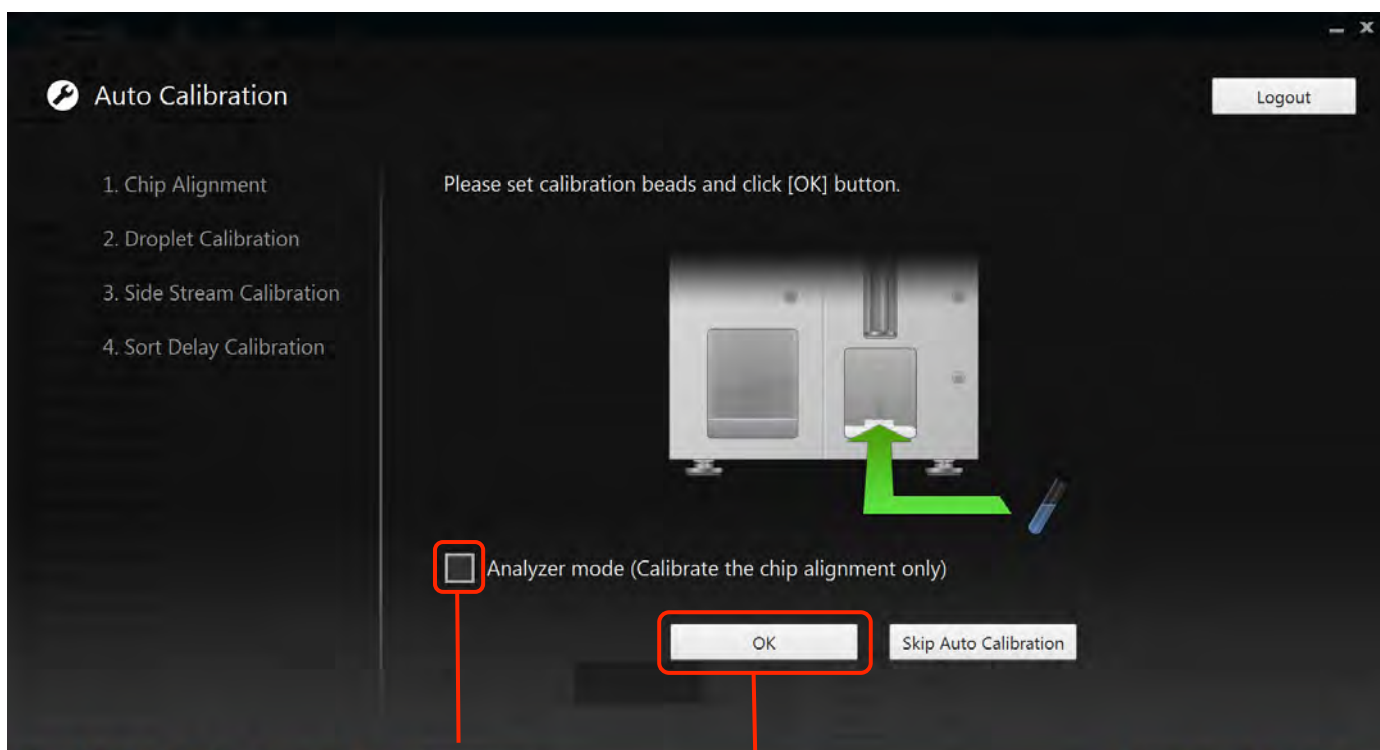
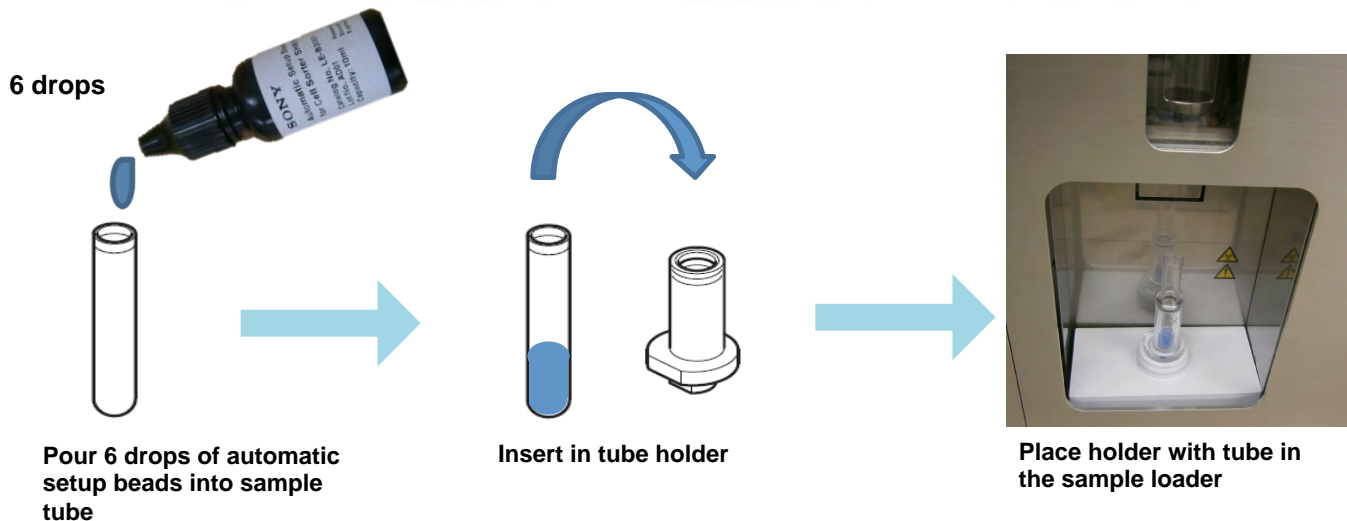
1. Check that the physical optical filter pattern is the same as the displayed filter pattern (a).
2. Click [Next] (b).

Fluidics Check



- 1. Automatic fluidics check starts
- 2. Takes 5 min to complete
- 3. Startup of the fluidics, followed by repeated de-bubbling of the sorting chip
- 4. Stabilization of pressure and flow

Set Automatic Setup Beads



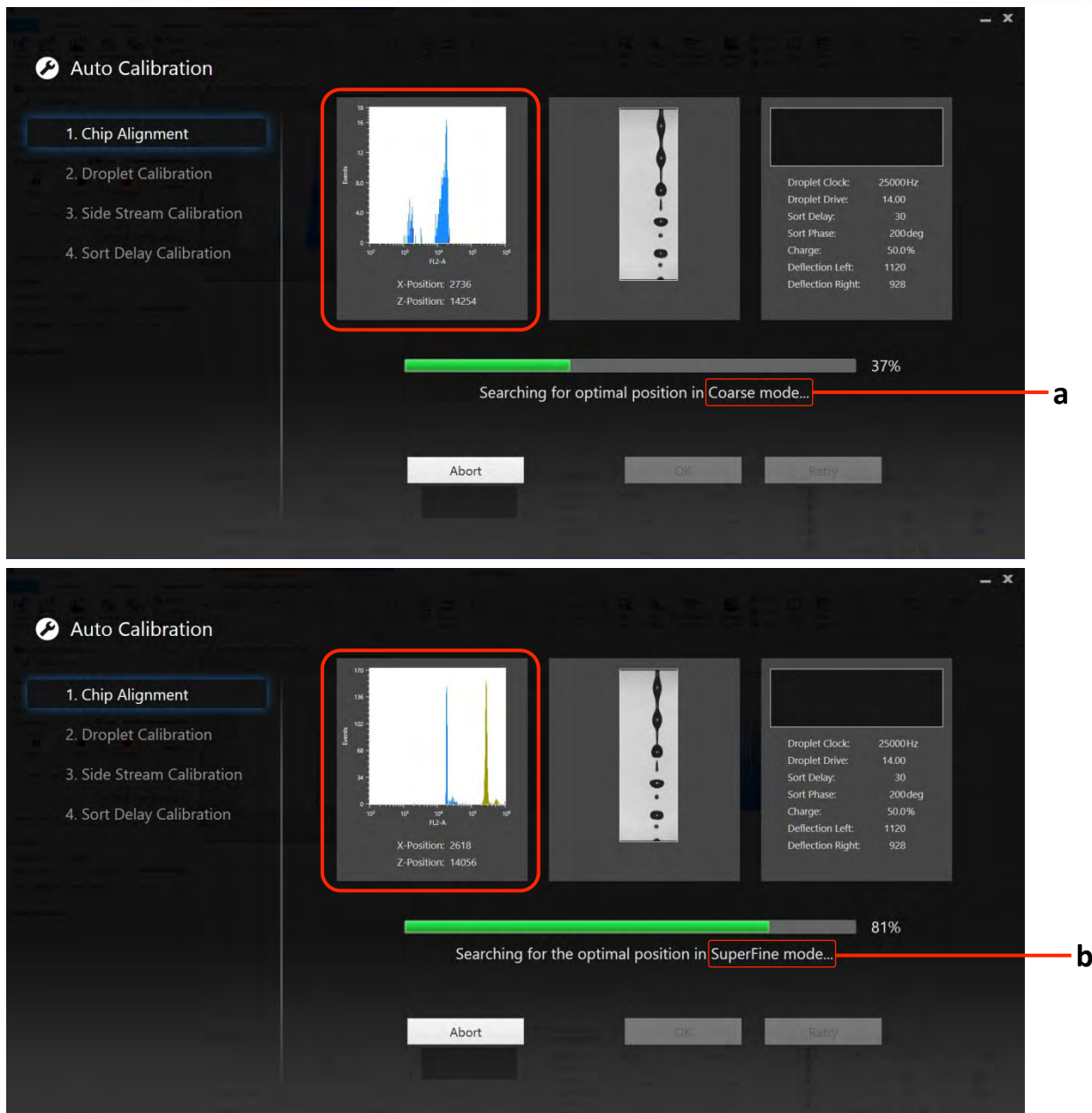
b

a

Start automatic calibration.

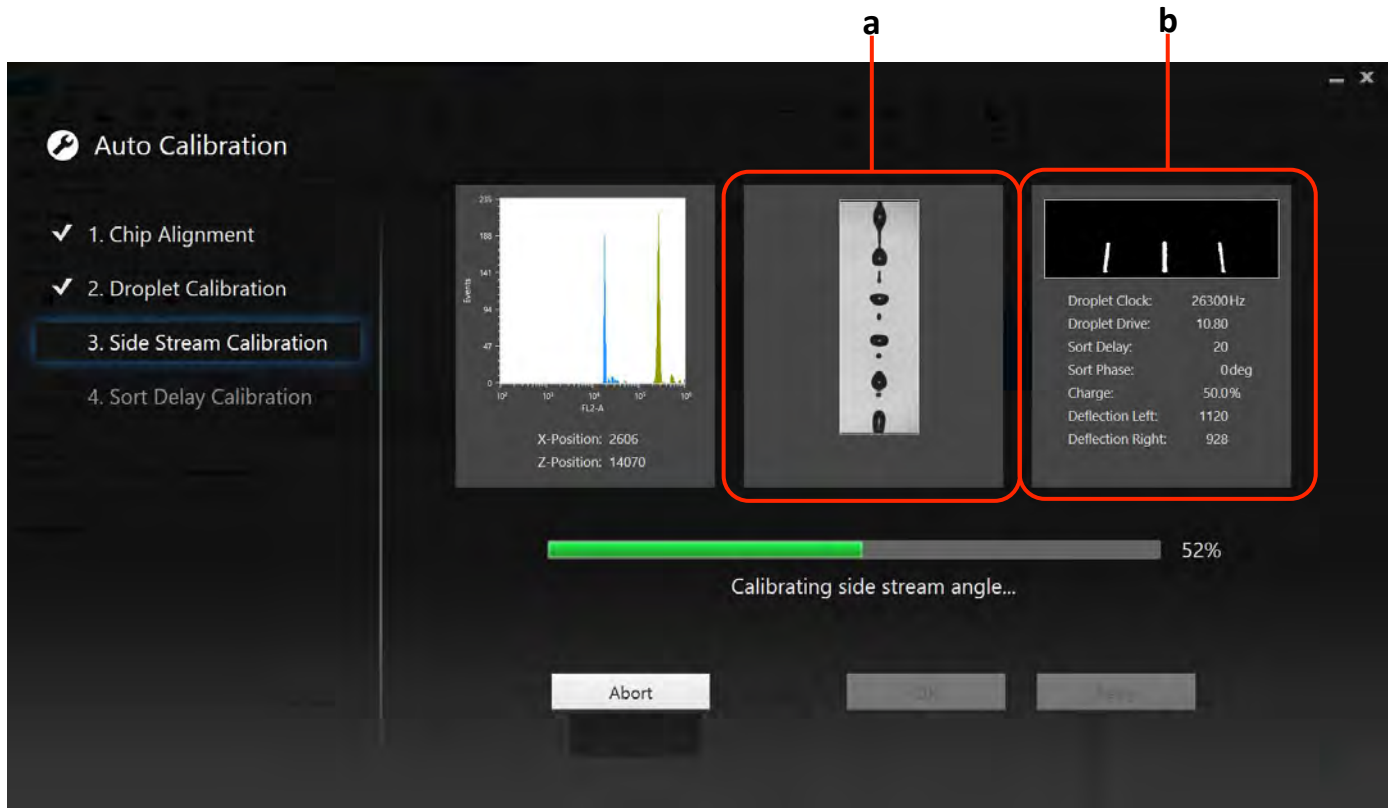
1. Load a sample tube with automatic setup beads, then click [OK] (a).
* If running samples without sorting, click [Analyzer mode] to reduce the time taken for calibration (b).
2. Calibration starts and automatically performs the 4 calibration steps listed on the left side of the screen.

Chip Alignment



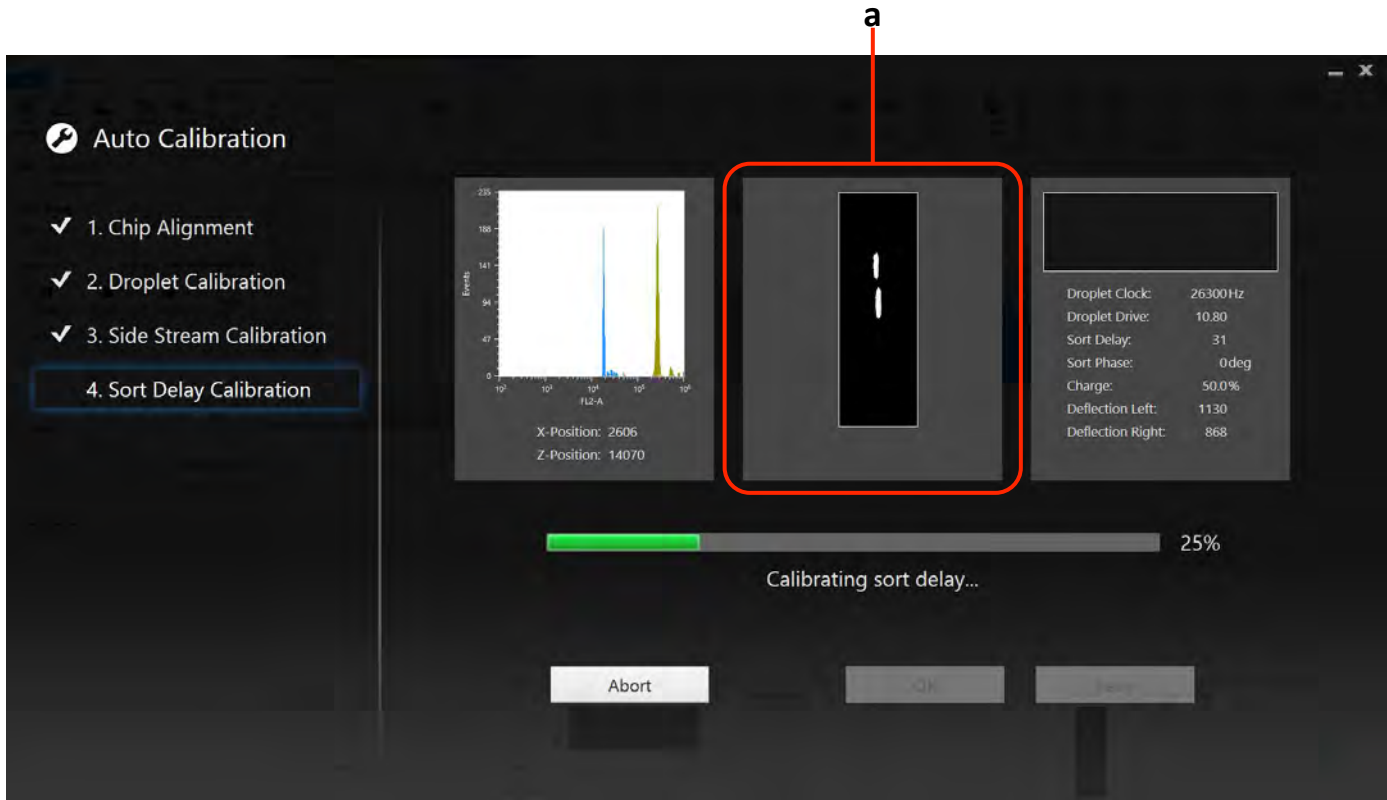
1. During Chip Alignment the sorter sets the optimal position of the sorting chip in front of the optical lense, where the peaks of the bead populations exhibit highest and most focussed fluorescence intensity.
2. Alignment according to two bead populations: Beads with high and low fluorescence intensity.
3. First Step: Coarse mode (a), then Fine mode and finally SuperFine mode (b)

Droplet and Side Stream Calibration



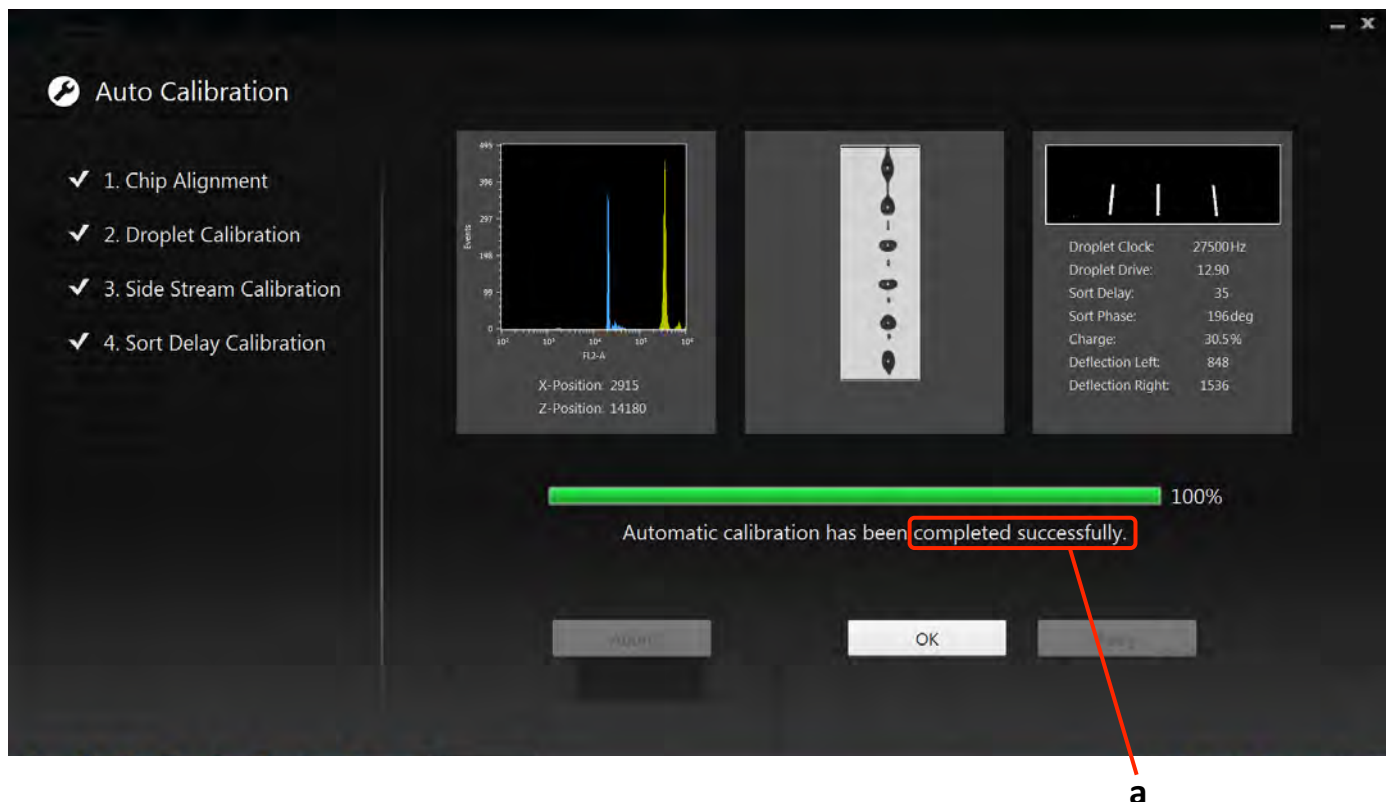
1. During Droplet Calibration the sorter identifies the optimal Droplet Clock frequency to form stable droplets (a)
2. During Side Stream Calibration the sorter sets the optimal Droplet Drive amplitude to produce stable side streams.
3. The sorter sets the optimal voltage on the deflection plates to hit the target values for the angle of the side streams (b)

Sort Delay Calibration



1. The sorter calibrates the time for the optimal sort delay (a)
2. First whole drops are being screened and then fine-tuning by changing the sort phase is done.

End of Automatic Calibration



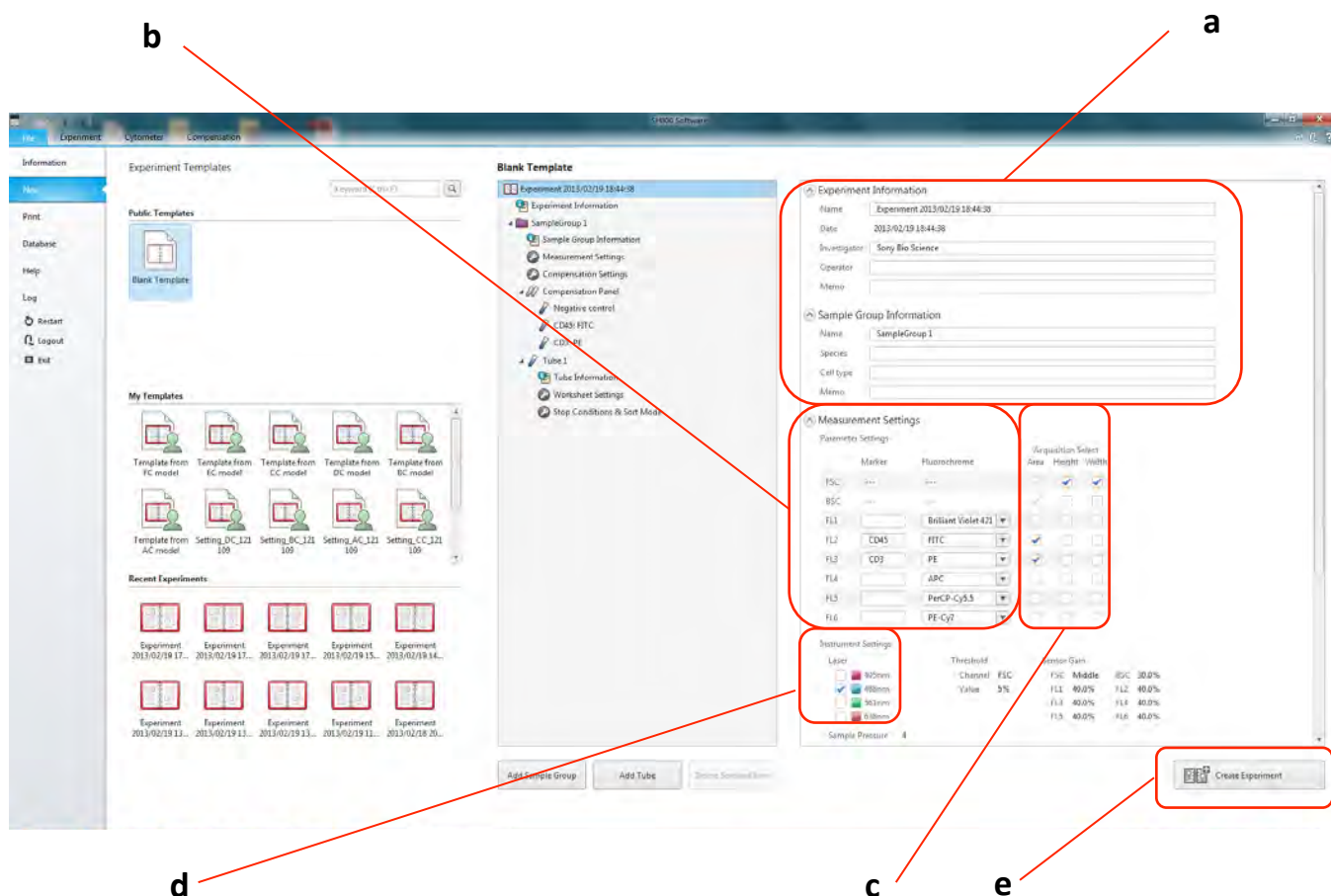
Auto Calibration final display

(Histogram and droplet appearance may vary slightly depending on the system)

Check the following after automatic calibration finishes.

1. The status is updated after each calibration step is finished.
 2. After all steps are finished, check that automatic calibration has “completed successfully” (a).
 3. If successful, click [OK] to display the Create Experiment window.
- Even when started in Analyzer mode, you can still perform automatic calibration for sorting from SH800 software to enable sorting operations (page 17).

Creating an Experiment

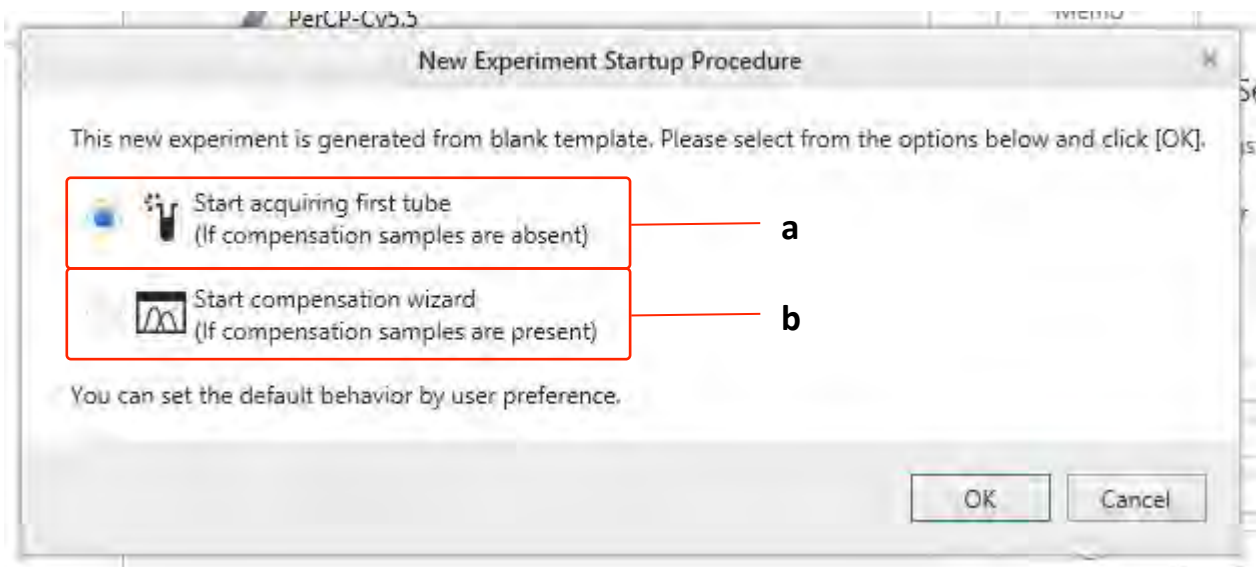


Create Experiment window

Create an experiment for measurement.

1. Clicking [OK] at the end of automatic calibration automatically displays the Create Experiment window. Alternatively, you can click [New] in the [File] tab in SH800 software to display the window at any time.
2. Enter the name of the experiment, operator, cell type, and other information in [Experiment Information] (a).
3. Enter the name of markers and select the corresponding fluorochemicals (b).
4. Select the acquisition parameters (Area, Height, Width) to be measured for the scattered light and fluorescent channels (c). "Area" is enabled by default for the FSC and BSC channels.
5. Select the laser wavelengths to be used (d).
6. When finished, click [Create Experiment] to display the Experiment Explorer in the main window (e).

New Experiment Startup Procedure

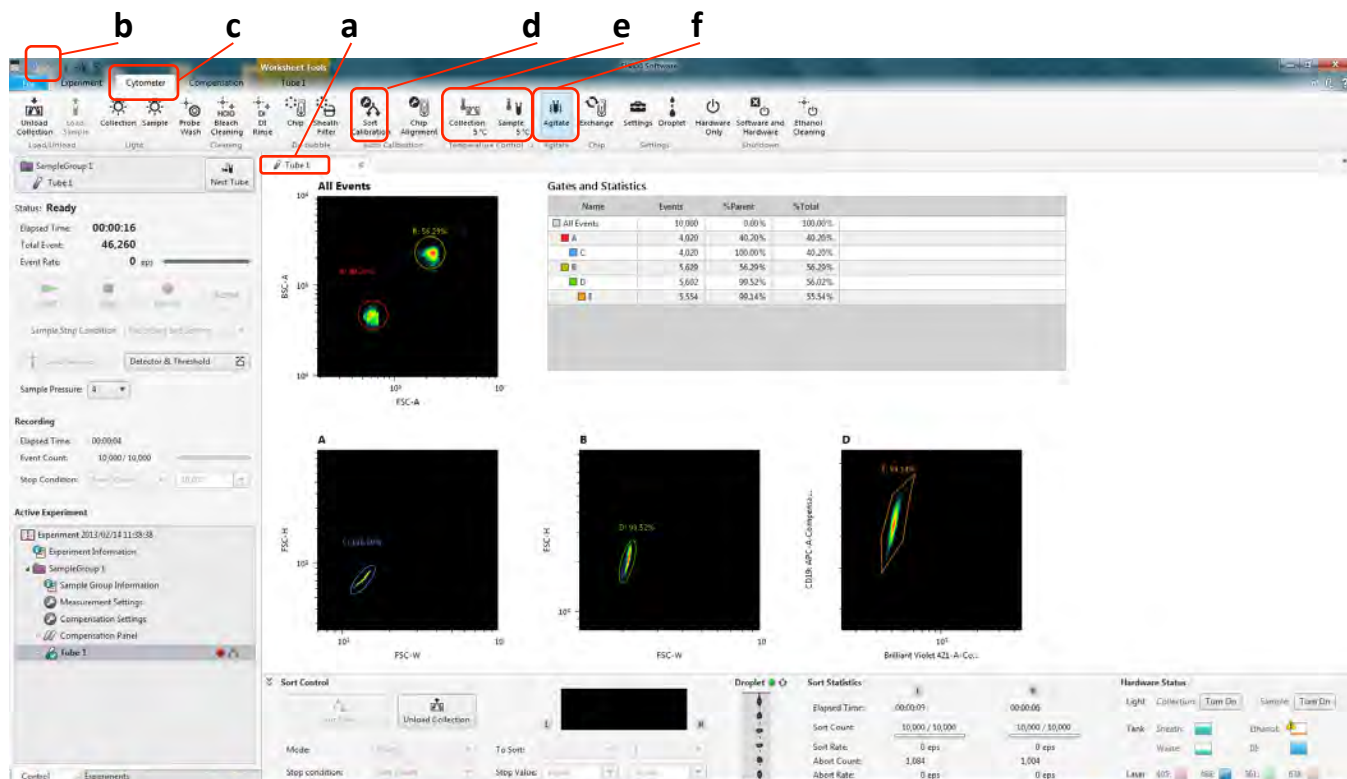


New Experiment Startup Procedure

Create an experiment for measurement.

1. Clicking [Create Experiment] at the end of Experiment Setup, will automatically open the [New Experiment Startup Procedure] window.
2. You can choose to directly start by acquiring the first tube. This option is recommended, if you don't want to run automatic compensation procedure (a).
3. You can also start the compensation wizard, if you wish to run automatic compensation procedure (b).
4. It is also possible to start the compensation wizard later in the software. Go to [Compensation] tab and click [Compensation Wizard].
5. It is also possible to manually adjust compensation in the software. Go to [Compensation] tab and click [Manual Compensation].

SH800 Software Overview

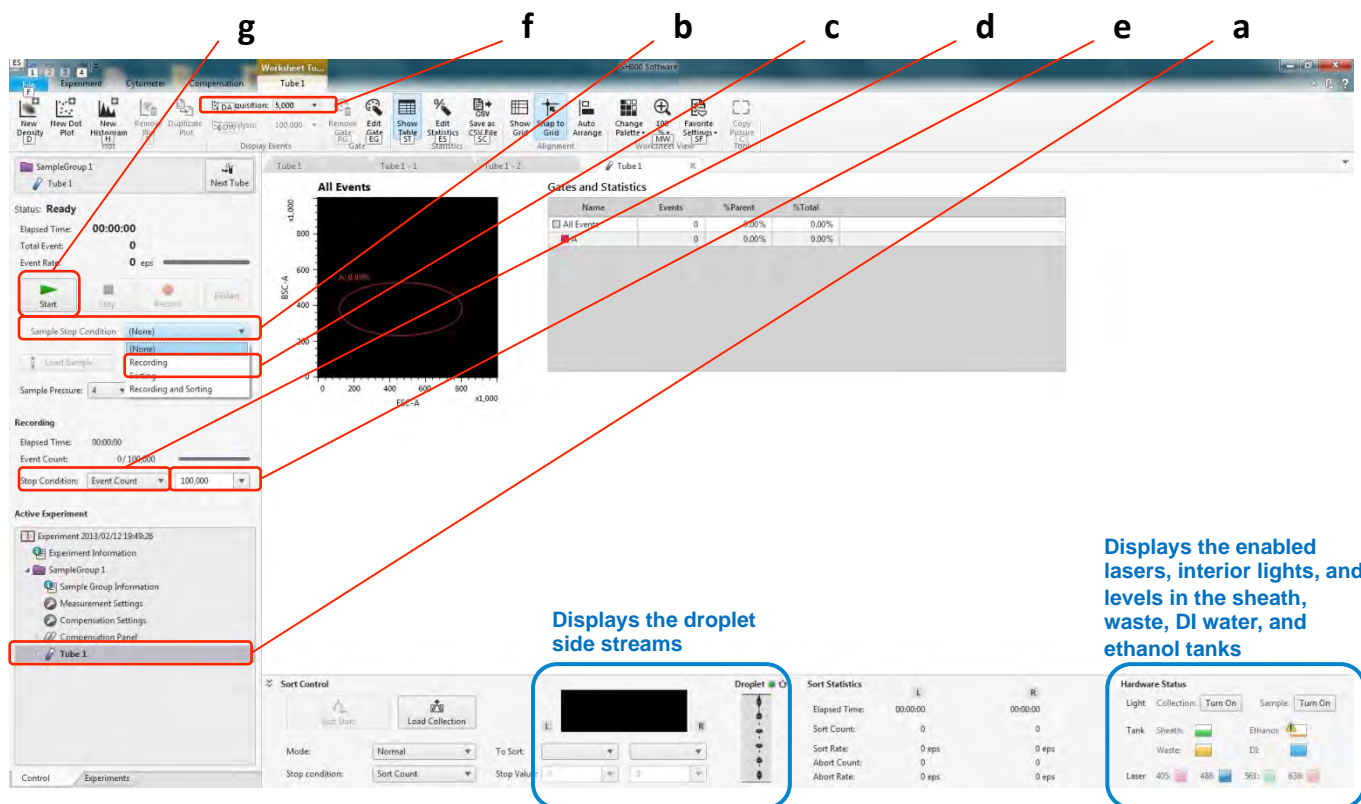


Main window

SH800 Software Features

- Intuitive interface based on tabs and ribbons
 - Select a tab or plot to display the applicable operations for that object in the ribbon
 - Tab with tube icon indicates the target tube for data acquisition (a)
- Undo/Redo function
Undo/Redo using icons in top left corner of the window (b) or using Ctrl-z/Ctrl-y key combinations
- Independent zoom and automatic layout of plots and tables on the worksheet
- Configuration of main SH800 cytometer functions
Click the [Cytometer] tab (c).
 - Sorting automatic calibration (sorting also supported after starting in Analyzer mode) (d)
 - Temperature control (cooling) of samples after sorting (e)
 - Sample agitation On/Off (f)

Configuring Data Acquisition Settings



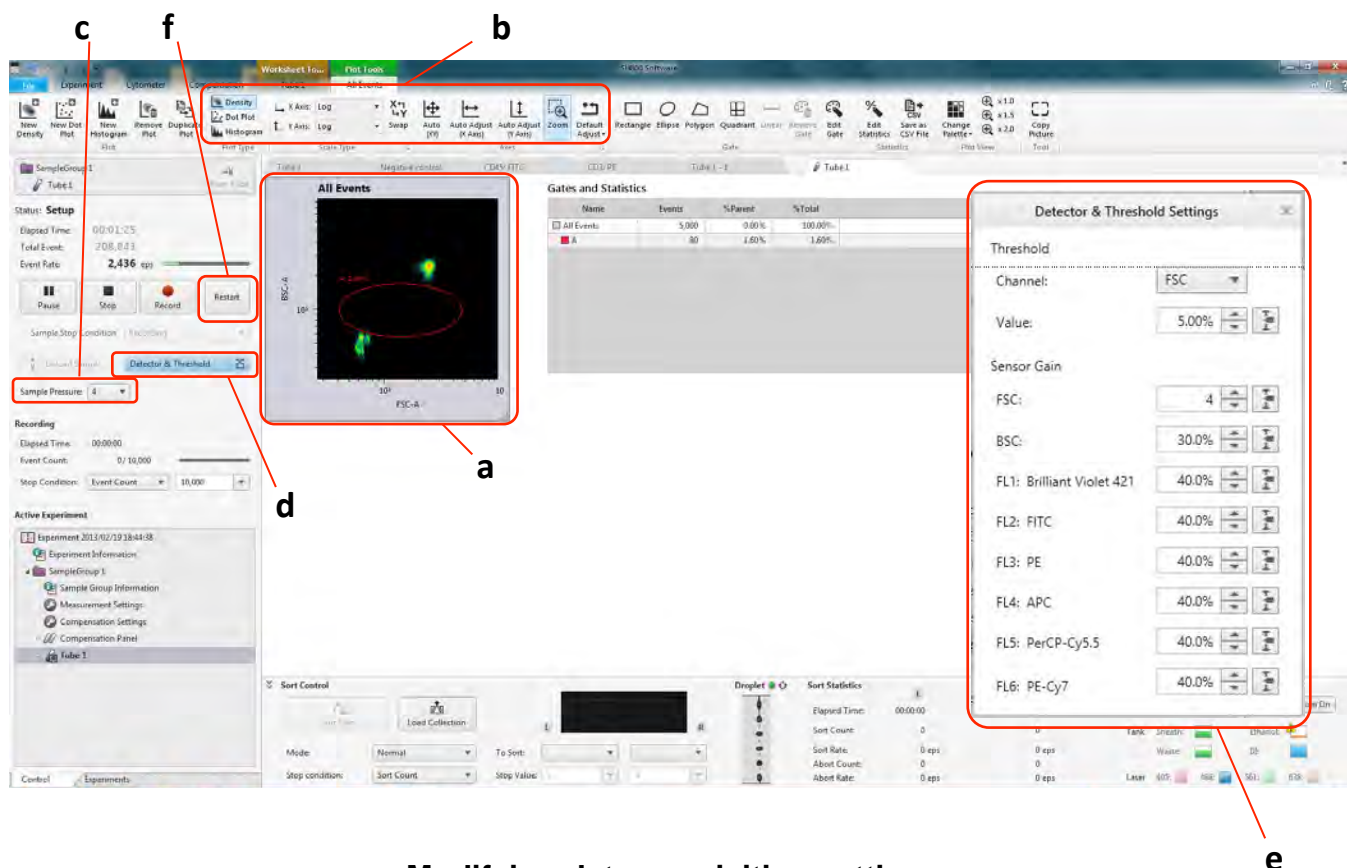
Configuring data acquisition settings

Tube 1 is created in the Experiment Explorer (a). All subsequent measurements are tied to this tube. The first step is to configure settings for data acquisition by selecting parameters from the pull-down menus.

* You can change the name of the tube by right-clicking it and selecting [Properties] or by selecting [Tube Information] in the Experiment Explorer.

1. Set the sample stop condition (b).
2. Select the trigger channel (normally set to FSC) (c).
3. Select the recording stop condition (d).
4. Select the recording stop condition limit value (up to 1,000,000,000 events) (e).
5. Select the number of events to display (screen update condition) (f).
The default value is 5,000 events. If the targeted cells have few events, the display may be updated before there are sufficient events to display.
6. Place a sample tube in the sample loader, and click the [Start] button to begin data acquisition (g).

Modifying Data Acquisition Settings



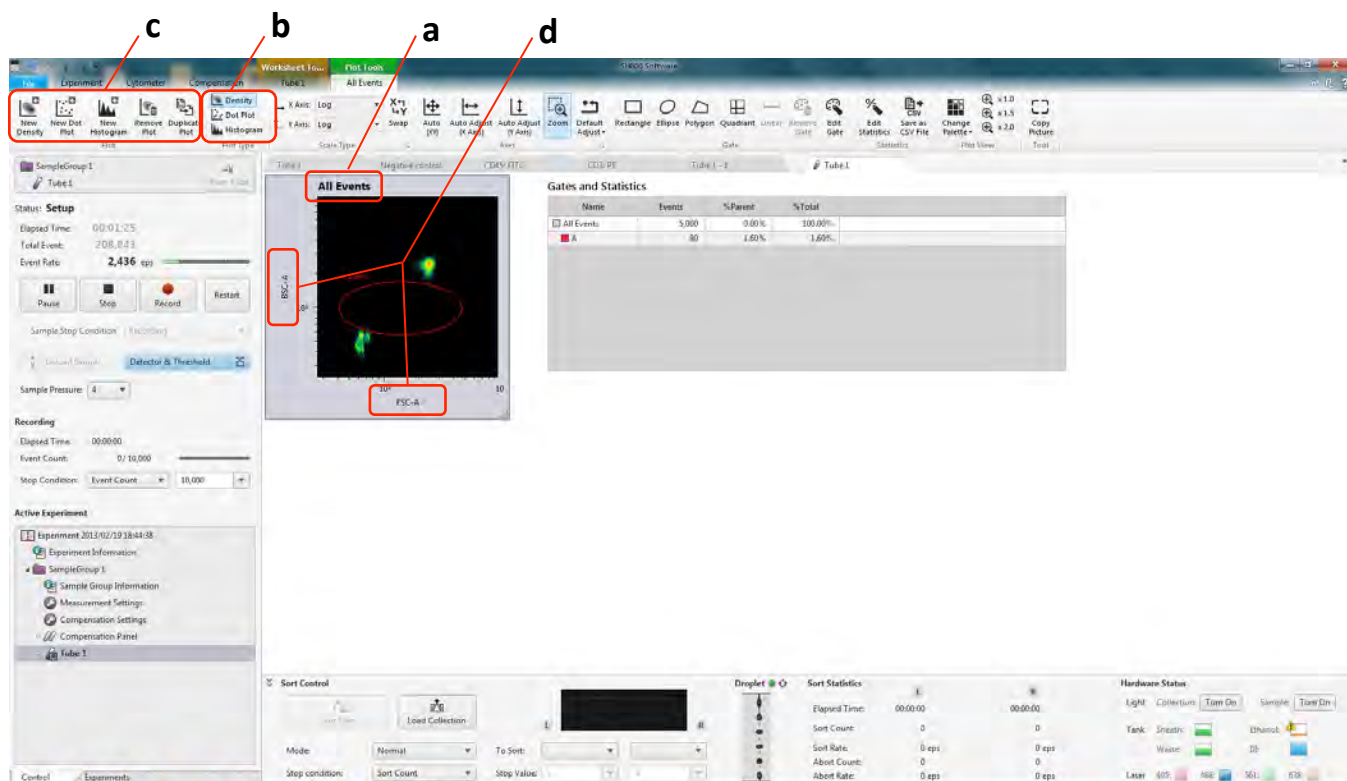
Modifying data acquisition settings

Modify the data acquisition settings in the data acquisition control pane.

This procedure describes an FSC-A vs. BSC-A plot as an example.

1. Click a plot to select it (a).
The Plot Tools ribbon is displayed.
2. Set the plot type, X/Y axis types (Linear, Log, or Biexponential), zoom, and other settings (b).
3. Adjust [Sample Pressure] to set the desired event rate (c).
4. Click [Detector & Threshold] (d) and adjust the detection sensitivity of the detectors, selecting values so that events appear on the plots (e).
5. Click [Restart] to confirm that the desired results are being achieved (f).

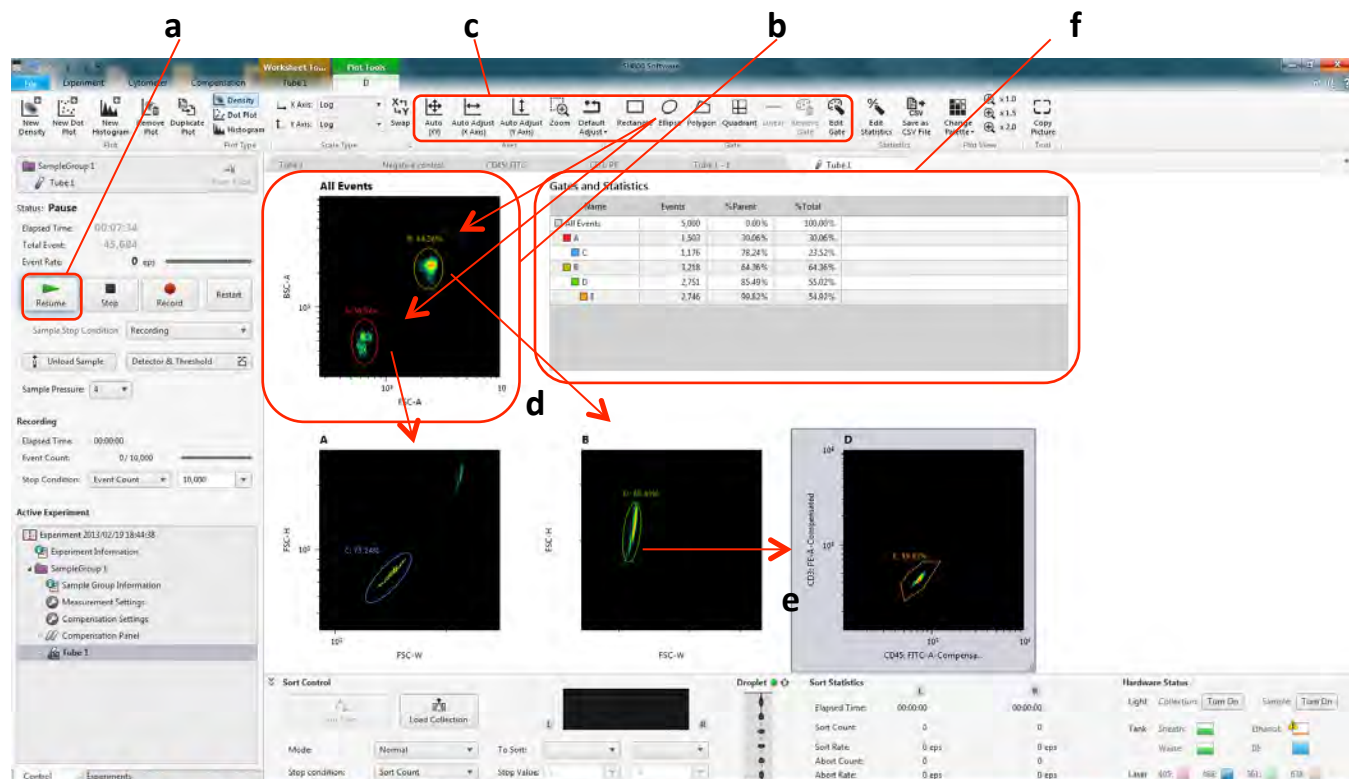
Plot Display Settings



Plot Display Settings

1. Click [All Events] (or other Gate name appearing in the top left corner), a drop down menu will open, where you can choose the population for display in the respective plot (a).
2. Choose the plot type – Density, Dot Plot and Histogram (b).
3. Add a New Plot to your analysis (Density, Dot Plot or Histogram), Remove or Duplicate Plots (c).
4. Clicking on the Legend of X- and Y-axis of plots will open drop down menus, where you can choose the parameter you want to display on the respective axis (d).

Gating Acquired Data



Gating acquired data

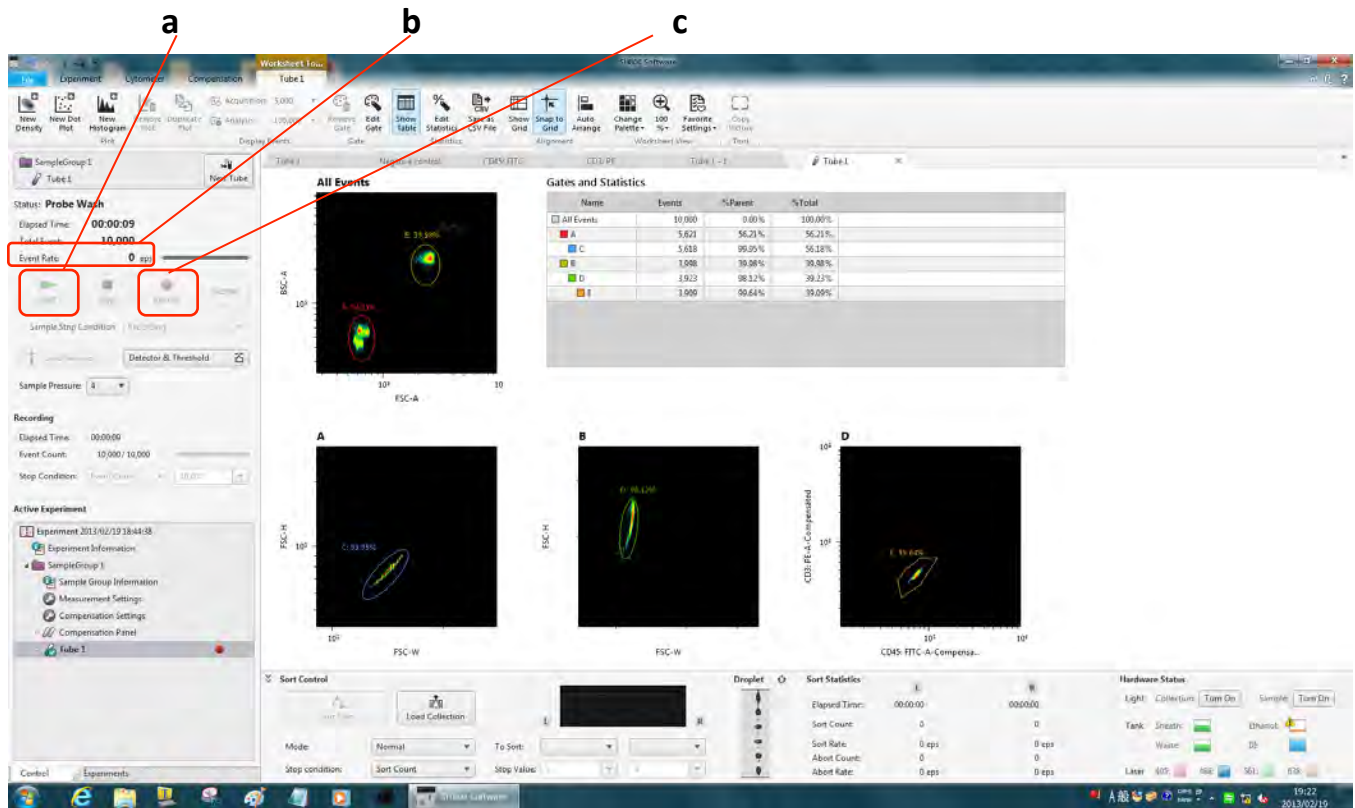
Add gates to isolate event populations on the worksheet.

1. Click [Pause] to reduce consumption of the sample (a).
2. Click the “All Events” plot to select it (b).
The Plot Tools ribbon is displayed.
3. Select the gate shape that best fits the acquired data from [Gate] in the Plot Tools ribbon, and draw a gate around the events in the plot (c).
4. Double-click the gate to add a child plot that contains only the data within the gate (d).
The index letter for the source gate is displayed at the top left of the child plot.
Change the X/Y axis settings and other parameters as necessary, and proceed with analysis.
5. Add and then double-click a gate in a child plot to add a grandchild plot to the worksheet (e).

You add gates to child plots and grandchild plots in the same way as the “All Events” plot.

The gates, including the parent-child relationship, are added to the Gates and Statistics table (f).

Acquiring Data



Acquiring data

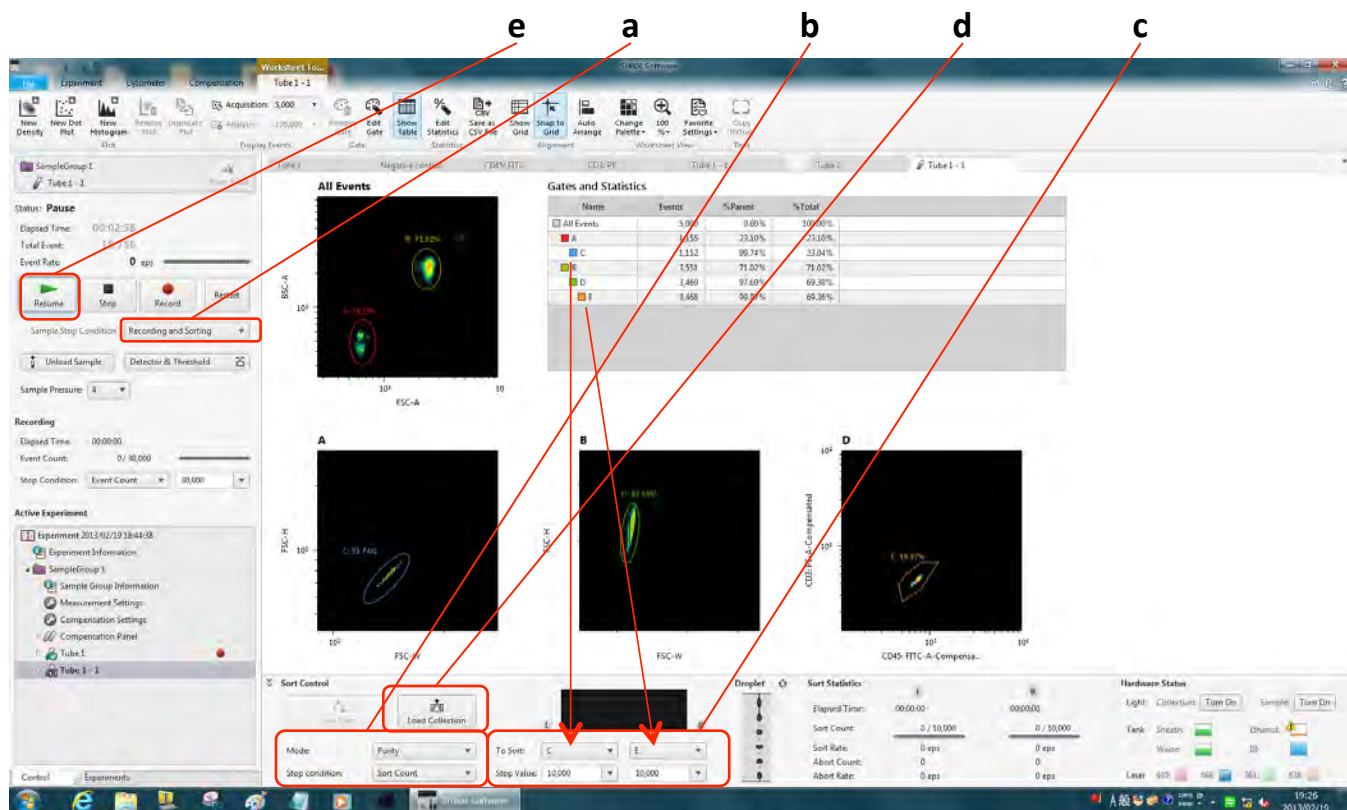
Acquire and record data after adjusting detector settings and adding gates.

1. Click [Resume] to restart the sample flow (a).
2. Monitor [Event Rate] and check that the value is stable (b).
3. Click [Record] to start recording the acquired data (c).

When the stop condition is satisfied, acquisition and recording automatically stops, and the sample tube is unloaded.

To stop recording before the stop condition is satisfied, click [Stop].

Configuring Sorting

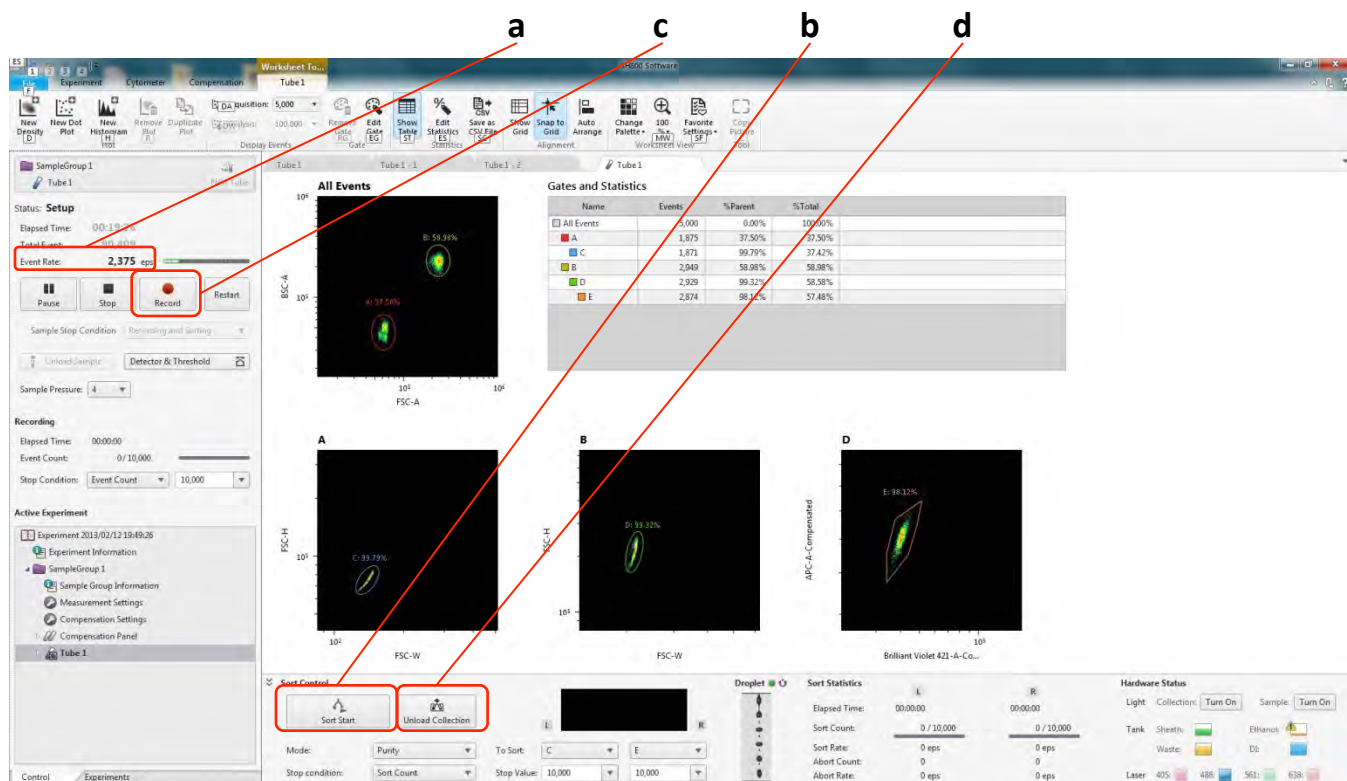


Sorting settings

Configure the sorting parameters in the sort control pane in the main window. Click [Next Tube] and prepare “Tube 1-1” for sorting.

1. Set [Sample Stop Condition] to “Recording and Sorting” (a).
2. Set the sort [Mode] and [Stop Condition] (b).
3. Select the samples to sort from the [To Sort] pull-down menus, and set the stop condition values (c).
4. Open the sorting area door, and place collection tubes and tube holder on the collection stage. Make sure the collection tube holder is **not sitting at an angle**. It is recommended that the collection tubes be prepared with suitable cell capture media to prevent damage to cells. Fill 1/2 to 2/3 with.
5. Close the door and click [Load Collection] to move the collection tubes into position (d).
6. Click [Resume] to restart the sample flow (e).
Note that sorting does not start at this point.

Starting Sorting

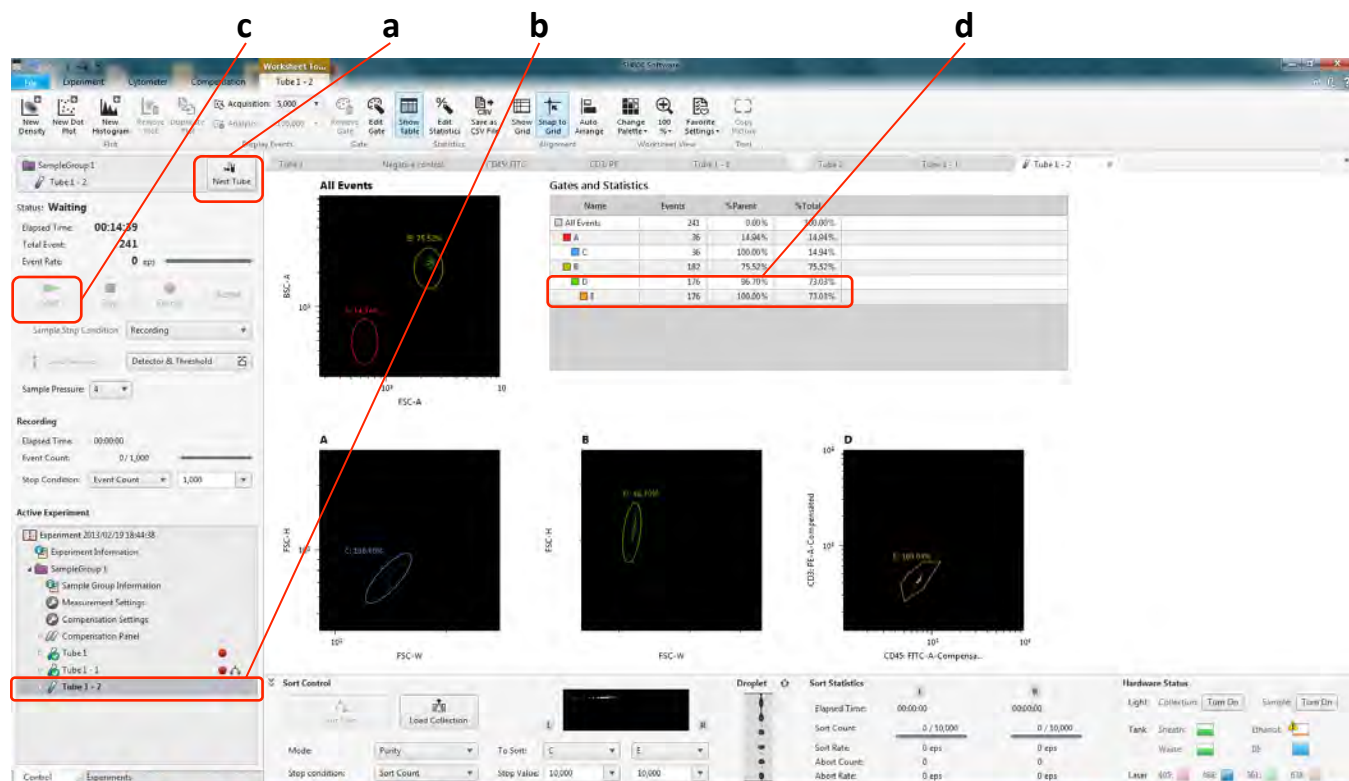


Sorting

Start sorting.

1. Monitor [Event Rate] and check that the value is stable (a).
2. When the value stabilizes, click [Sort Start] to commence sorting (b).
Sorting automatically stops when the [Stop Value] condition is satisfied.
3. Click [Record] to record the acquired data, if required (c).
Recording automatically stops when the recording [Stop Condition] is satisfied.
4. When finished, click [Unload Collection] to unload the collection tube holder and remove the tubes (d).

Reference: Verifying Purity



Verifying purity

Verify the purity of the sorted samples.

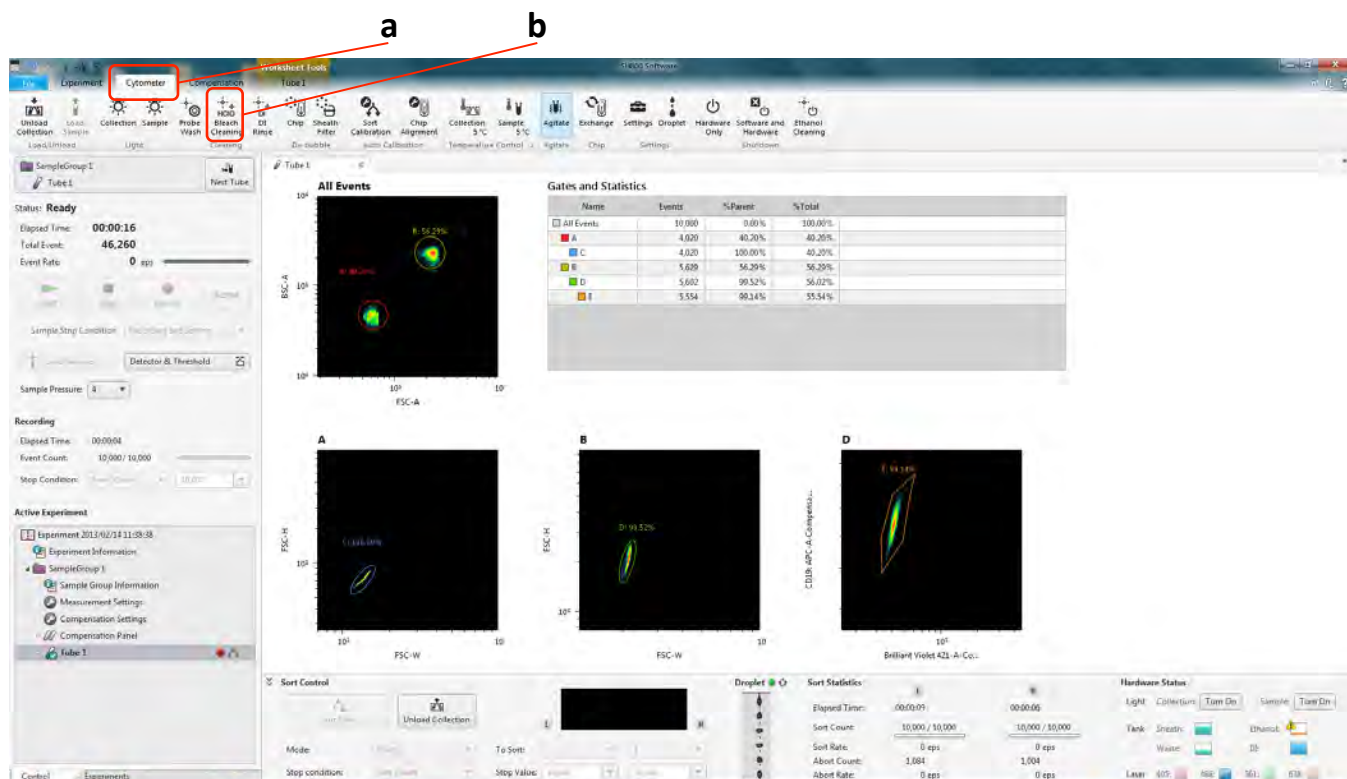
Prepare an appropriate dilution of the sorted sample in the collection tube, then wash the sample probe and sample line with DI water to remove any residual sample.

1. Click [Next Tube] (a).
This creates "Tube 1-2" with the same settings and gating inherited from "Tube 1" (b).
2. Load a sample tube containing about 15 ml of DI water, set [Sample Pressure] to the maximum value of 10, and click [Start] to start cleaning (c).
3. Click [Stop] when the [Event Rate] is as close to zero as possible.
4. Click [Next Tube] again to create a new tube, load the sorted sample tube, reduce the [Sample Pressure] to a value of 5 and click [Start] (c).
Data acquisition/analysis starts, and automatically stops when the [Stop Count] condition is satisfied.
5. Verify the purity in the Gates and Statistics table (d).



Follow the instructions in the wizard to perform adjust compensation.

Cleaning the Sample Line (Bleach)



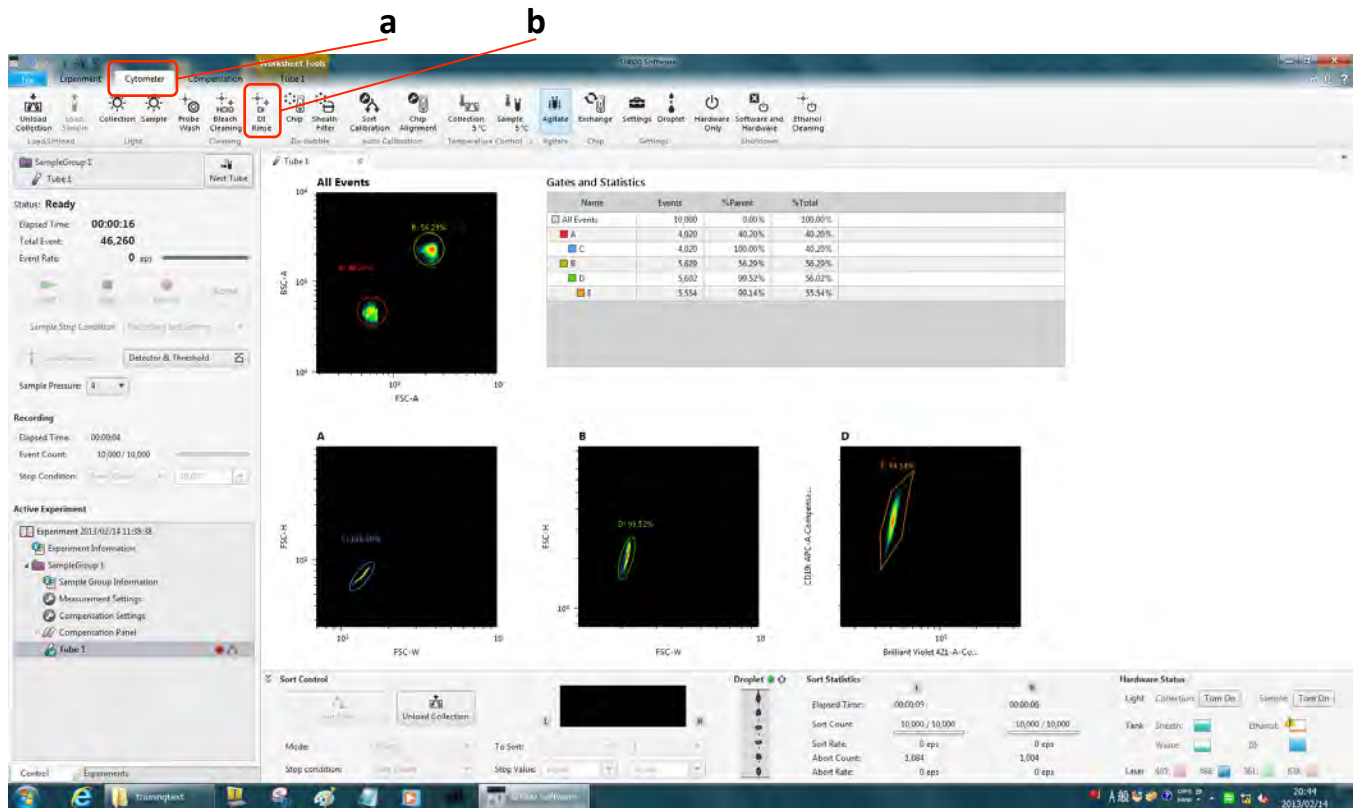
Sample line cleaning (bleach)

Clean the sample fluidics system, comprising the sample probe and sample line, with bleach.

Prepare a 1% bleach solution.

1. Click the Cytometer tab of the ribbon (a).
2. Click [Bleach Cleaning] (b), and follow the instructions to load a 15 ml tube with 10 ml of bleach solution in the sample loader.
Do not overfill with bleach. The level of DI water in the next cleaning phase must be higher than the level of bleach in order to remove all bleach from the sample probe.
3. When cleaning is finished, unload the sample tube.

Cleaning the Sample Line (DI water)



Sample line cleaning (DI water)

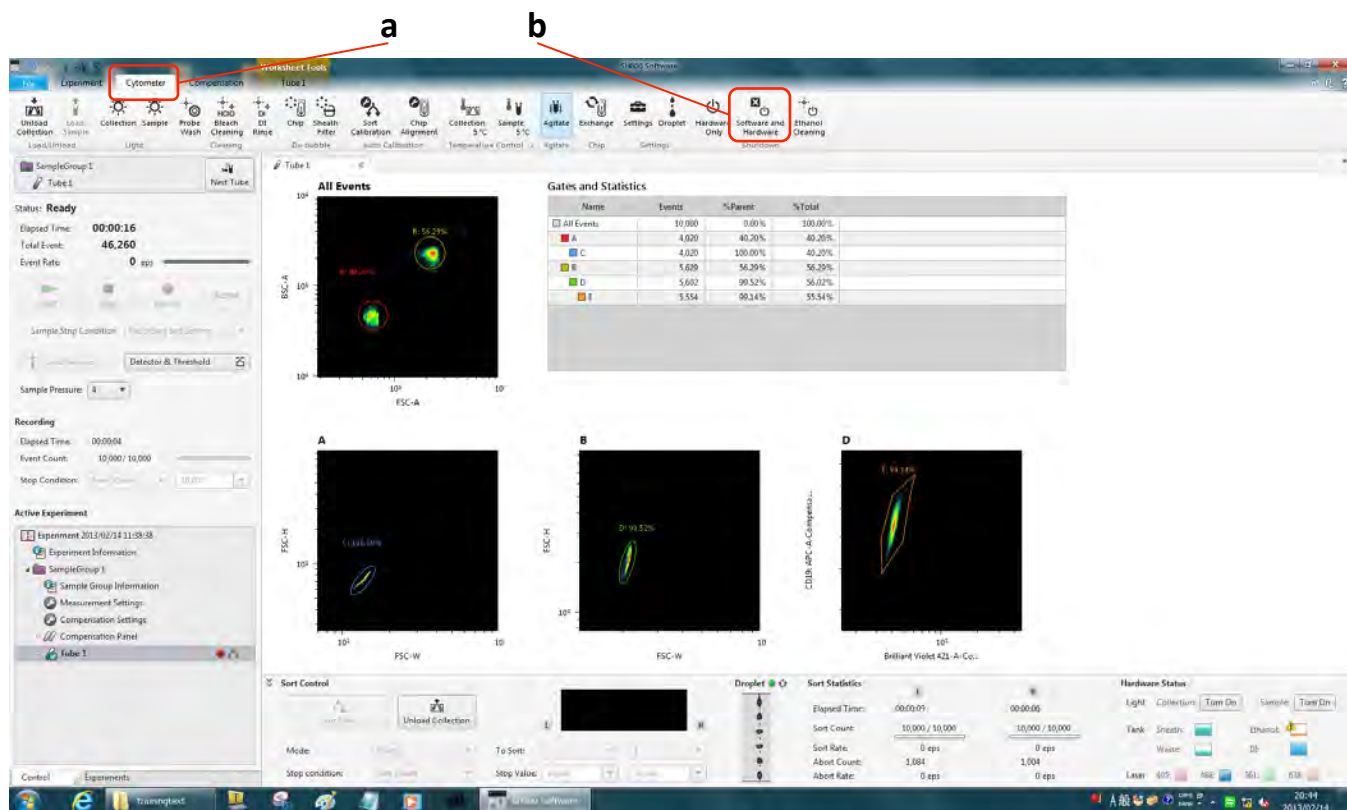
Clean the sample fluidics system, comprising the sample probe and sample line, with DI water.

Always perform DI water cleaning before shutting down.

Prepare a 15 ml tube with 12 ml of DI water (always add more DI water to cleaning tubes than bleach to remove all traces of bleach from the sample probe)

1. Click the Cytometer tab of the ribbon (a).
2. Click [DI Rinse] (b), and follow the instructions to load a 15 ml tube with 12 ml of DI water in the sample loader.
3. When cleaning is finished, unload the sample tube.

Shutting down



Shutdown

Shut down the system.

1. Click the Cytometer tab of the ribbon (a).
2. Click [Software and Hardware] in the Shutdown group of the ribbon (b).
The SH800 main unit and SH800 Software automatically shut down.
3. Shut down the host computer.
4. Turn off the air compressor.

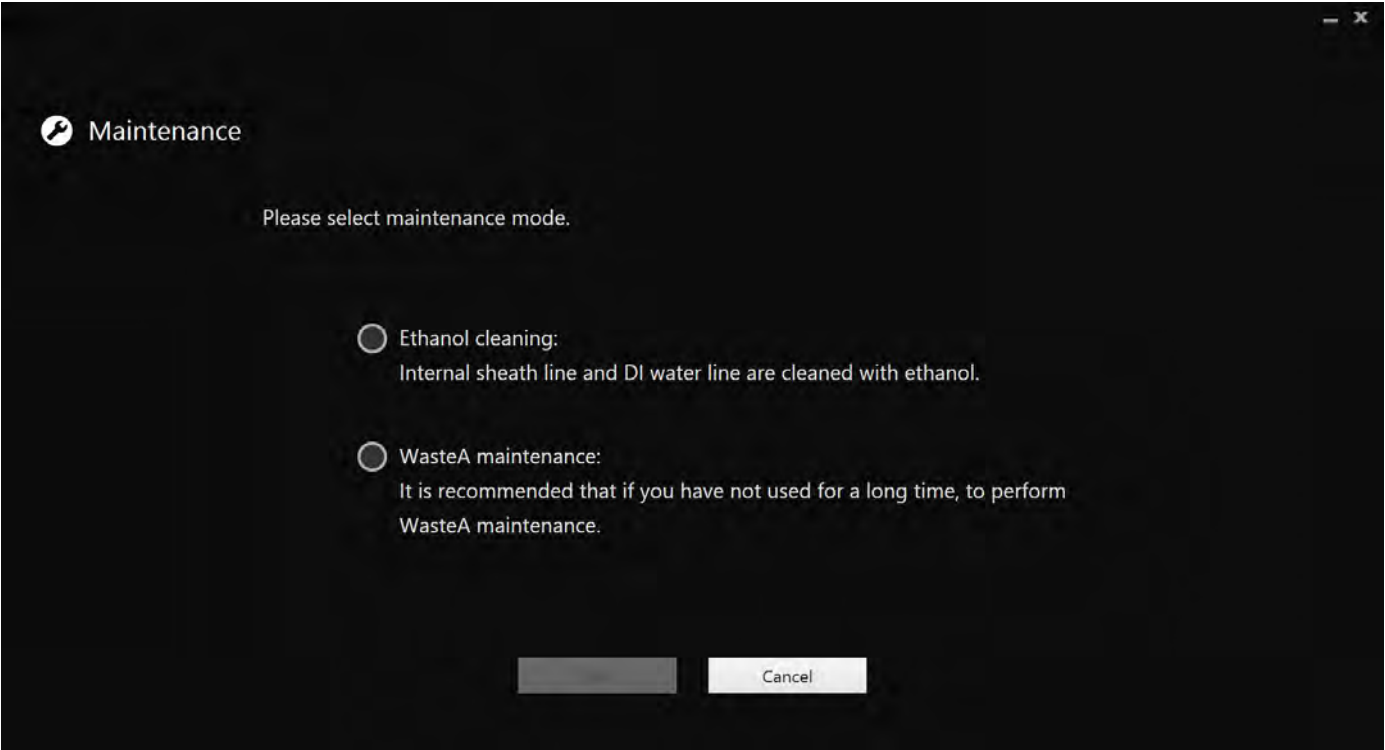
***** If the instrument is connected to house air, disconnect the system from house air and vent the sheath tank at the end of the day.**

Note:

If you shut down SH800 Software only, you can still safely shut down the SH800 main unit. **Press and hold the power button on the front panel for a few seconds.**

If you leave a sample in the sample loader after shutting down, **check that the air compressor is on** and then press the power button on the front panel. The sample door will open after initialization finishes. Remove the sample tube and then press the power button for a few seconds to shut down the main unit.

Maintenance



Choose your desired maintenance protocol and press [Start]

Ethanol Cleaning:

Internal sheath line and DI water line are cleaned with ethanol.

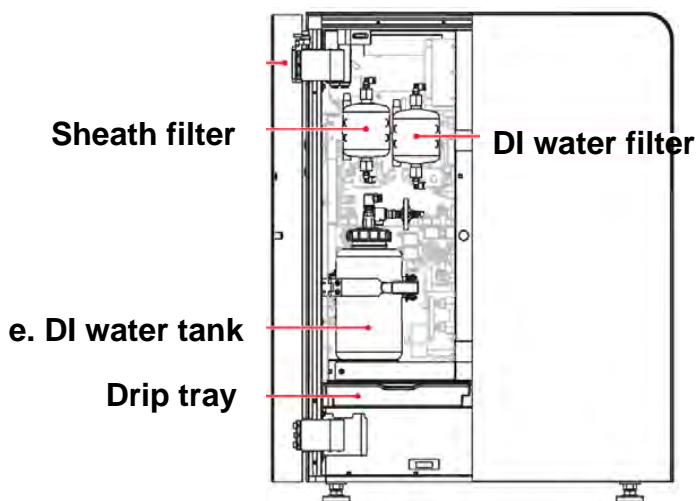
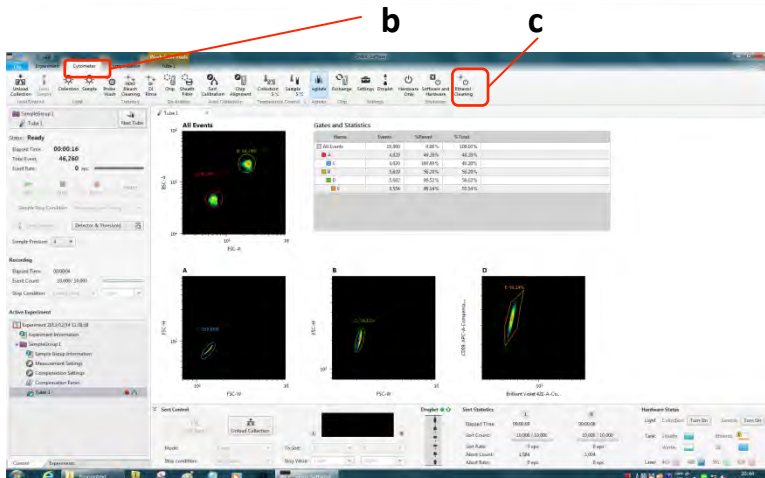
WasteA maintenance:

If the instrument hasn't been used for a longer time, please run WasteA maintenance. Fluidics system is cleaned using Bleach solution. DI water lines and internal sheath lines are cleaned to prevent buildup of salts.

Ethanol Cleaning



a Cleaning chip and bypass line (supplied)



SH800 main unit (left side)

Clean and disinfect the sheath line using ethanol.

Note: Take all necessary safety precautions when handling ethanol.

Prepare 1 l of 70% ethanol.

1. Prepare a cleaning chip (yellow label package) and the supplied sheath filter and DI water filter bypass lines (a). Usually, they can be found in the drip tray behind the left door of the instrument (b).

Note: Ethanol will damage the filters if the bypass lines are not connected.

2. Click the Cytometer tab of the ribbon (b).
3. Click [Ethanol Cleaning] (c), and follow the instructions to refill the ethanol tank (f), refill the DI water tank (e), attach the sheath filter bypass line (for details see next page), and start cleaning.

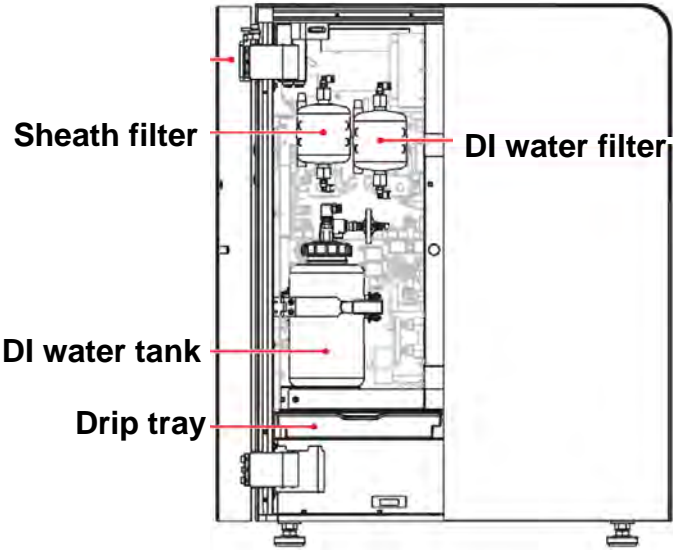
Completely fill the DI water tank when prompted as ethanol cleaning consumes about 700 to 800 ml of DI water.

4. When finished, remove the bypass line and reattach the sheath filter.

Ethanol Cleaning



a Cleaning chip and bypass line (supplied)



SH800 main unit (left side)



Default situation

Filters are correctly installed, bypass lines are stored in the drip tray under the filters.



Filters are bypassed

sheath filter (left) with yellow bypass line, DI water filter (right) with green bypass line.

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