

Multi-Application Cell Sorter

Operator's Guide

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The supplied installation disc includes the Operator's Guide for this unit in PDF format.
For more details, see "*Using the PDF Manual*" (page 8).

- Reproduction or duplication, in whole or part, of the software or operator's guide supplied with the unit, as well as renting or leasing of the software without the authorization of the right holder is prohibited under copyright law.
- Sony assumes no responsibility for damages, loss of income, or any claims from a third party arising out of use of the unit or supplied software.
- For complete terms and conditions of the warranty for the unit, refer to the warranty card included in the package.
- The software supplied with the unit cannot be used with any other units.
- Note that the specifications of the unit and supplied software are subject to change for improvement without prior notice.

Using the PDF Manual

The Operator's Guide can be read on a computer with Adobe Reader installed.

You can download Adobe Reader free from the Adobe website.

- 1** Open the Operator's Guide folder in the installation disc.
- 2** Select and click on the Operator's Guide that you want to read.

If you have lost or damaged the installation disc, you can purchase a new one from your Sony distributor.

Model Name and Function Table

This document covers the following models. The following functions are supported for each model.

Name	Model	2-way/4-way sorting ¹⁾	Multi-well plate sorting ²⁾	Sample line	Sorting chip ³⁾
LE-MA900	LE-MA900B, C, E, F, G, H	Yes	-	PEEK sample line	70 µm 100 µm
LE-MA900P	LE-MA900BP, CP, EP, FP, GP, HP	Yes	96-well plates (6 to 96) 384-well plates	PEEK sample line	70 µm 100 µm

1) SH800 series and FX500 series collection tube holders (5 ml/15 ml) cannot be used.

2) In this document, 6, 12, 24, 48, 96, and 384-well plates are collectively referred to as “multi-well plates” or “96-well plates.”

3) 130 µm sorting chip is not supported.

Tip

Electron beam irradiated consumables are not supported.

Components and Documentation

System Components

The LE-MA900 system comprises the following items.

- Main unit (1)
- Fluidics cart (1)
- Installation disc (Operator's Guide, Software) (1)
- Power cord (1)
- PC connection cable (1)
- PEEK sample line (1)
- Sheath filter (1)
- Sheath tank (1)
- Ethanol tank (1)
- Ethanol tank probe (1)
- Air filter for ethanol tank (1)
- Bleach tank (1)
- Bleach tank probe (1)
- Air filter for bleach tank (1)
- DI water tank (1)
- DI water tank probe (1)
- Air filter for DI water tank (1)
- Waste tank (1)
- Waste tank nozzle (1)
- Air filter for waste tank (1)
- Air line (clear) for fluidics cart (1)
- Sheath line (blue) for fluidics cart (1)
- DEBUBBLE line (orange) for fluidics cart (1)
- Waste line (red) for fluidics cart (1)
- Cart connection cable (1)
- Ethanol line (yellow) for tank (1)
- Sheath line (blue) for tank (1)
- Bleach line (yellow) for tank (1)
- Waste line (red) for tank (1)
- DI water line (green) for tank (1)
- Air line (clear) for tank (1)
- Sample tube holder (0.5 ml) (1)
- Sample tube holder (1.5 ml) (1)
- Sample tube holder (5 ml) (1)
- Sample tube holder (15 ml) (1)
- Cleaning tube holder (30 ml) (1)
- Collection tube holder (5 ml) (1)
- Collection tube holder (15 ml) (1)
- 96/384-well plate holder¹⁾ (1)
- 384-well PCR adapter¹⁾ (1)
- 96-well PCR plate holder¹⁾ (1)
- Plate holder adapter¹⁾ (1)
- Retaining screw¹⁾ (1)
- Splash guard¹⁾ (1)

- Tube for bleach line cleaning (1)

1) Supplied with the LE-MA900P model only.

Documentation Structure

The system documentation comprises the following manuals.

- Safety Guide
Contains safety information, usage precautions, and specifications.
Read carefully before operating the unit.
- Operator's Guide
Describes the required preparation and basic operating procedures in order to use the instrument, adjustments and procedures for more advanced use of the instrument, and the configuration items in the Cell Sorter Software windows and dialogs.
"Chapter 3 Basic Operation" provides an overview of the basic procedures, from setting up and calibrating the instrument through to acquiring data, analyzing data, and sorting.
Refer to the subsequent chapters for detailed information about operating procedures and instrument settings.

Main Features

The LE-MA900 series is a benchtop, high-speed, multilaser flow cytometer and cell sorter designed for research laboratory use. It uses a novel, replaceable, microfluidics, cell “sorting chip” that features high reliability and greatly simplifies cell sorting setup. It can be easily and quickly exchanged as needed for quick turnaround between measurements to minimize system downtime and to improve user work flow.

- Flow channels embedded within the chip for precision sheath fluid and sample fluid flow control
- Uniform droplet formation
- Automated chip loading and unloading system
- Easy optimization of sort setup after changing sorting chip
- Bleach cleaning and ethanol cleaning of the fluidics system. A cleaning wizard supports bleach cleaning and ethanol cleaning of all flow channels in the fluidics system, including the sample line and the sheath line from the sheath tank to the main unit.

The LE-MA900 interrogates cells in samples using up to four lasers on two axes (488 nm/561 nm, 405 nm/638 nm). It measures forward scatter, back scatter, and a maximum of 12 channels of fluorescent light. The excitation wavelengths support the use of a wide variety of common fluorochromes for use in cell analysis.

The LE-MA900 can measure intrinsic properties, such as size and internal complexity using forward scatter and back scatter analysis. It can simultaneously measure a wide range of extrinsic properties using fluorescent light emission analysis.

The operator can specify multiple gates that isolate cells with defined characteristics for further processing. Multiple parameters enable real-time identification of selected cell subpopulations at speeds of up to 70,000 events per second (eps) during analysis. The LE-MA900 supports high-speed, four-way sorting to isolate subpopulations for further study or testing.

An array of automated mechanisms is incorporated to enable ease of use. These systems include:

- Sorting chip alignment

- Sample tube loading
- Collection tube¹⁾ loading and alignment
- Fluorescence compensation
- Droplet formation, frequency, and calibration
- Device status monitoring function (QC function)
- Sample line, sheath line, and sorting chip cleaning
- Temperature control of sample tube and collection tubes
- Temperature control of sheath fluid for stable fluidics system

1) In this document, sample tubes, and multi-well plates used for collecting sorted samples are collectively referred to as “collection tubes.”

Manual controls for fluorescence compensation and droplet formation are also supported in Cell Sorter Software.

The hardware is designed to be simple, with major functions controlled by the simple. An LCD monitor on the front of the unit displays status information and notification of error messages.

The LE-MA900 connects to standard 100 V to 240 V AC 50/60 Hz power supplies, and has a 19.5 °C to 27.5 °C (67.1 °F to 81.5 °F) recommended operating temperature range. It communicates with the host computer via the PC connection cable. The LE-MA900 requires the connection to a laboratory compressed-air supply for operation. It can be supplied to an external air compressor unit. A fluidics cart supplies the main unit with sheath fluid, DI water, bleach, and ethanol.

A host computer running Cell Sorter Software controls all aspects of instrument operation, including cell analysis and sorting. The operator uses the software to create experiments for measuring data and to create plots and define statistics for analyzing data. The export functions are fully compliant with Flow Cytometry Standard version 3.0 or 3.1. The software provides quick access to all functions from a single window.

The LE-MA900 has a compact footprint size. The benchtop unit has external dimensions (W/D/H) of 550 × 550 × 720 mm (21 3/4 × 21 3/4 × 28 3/8 inches) and weighs 105 kg (231 lb. 7.8 oz.).

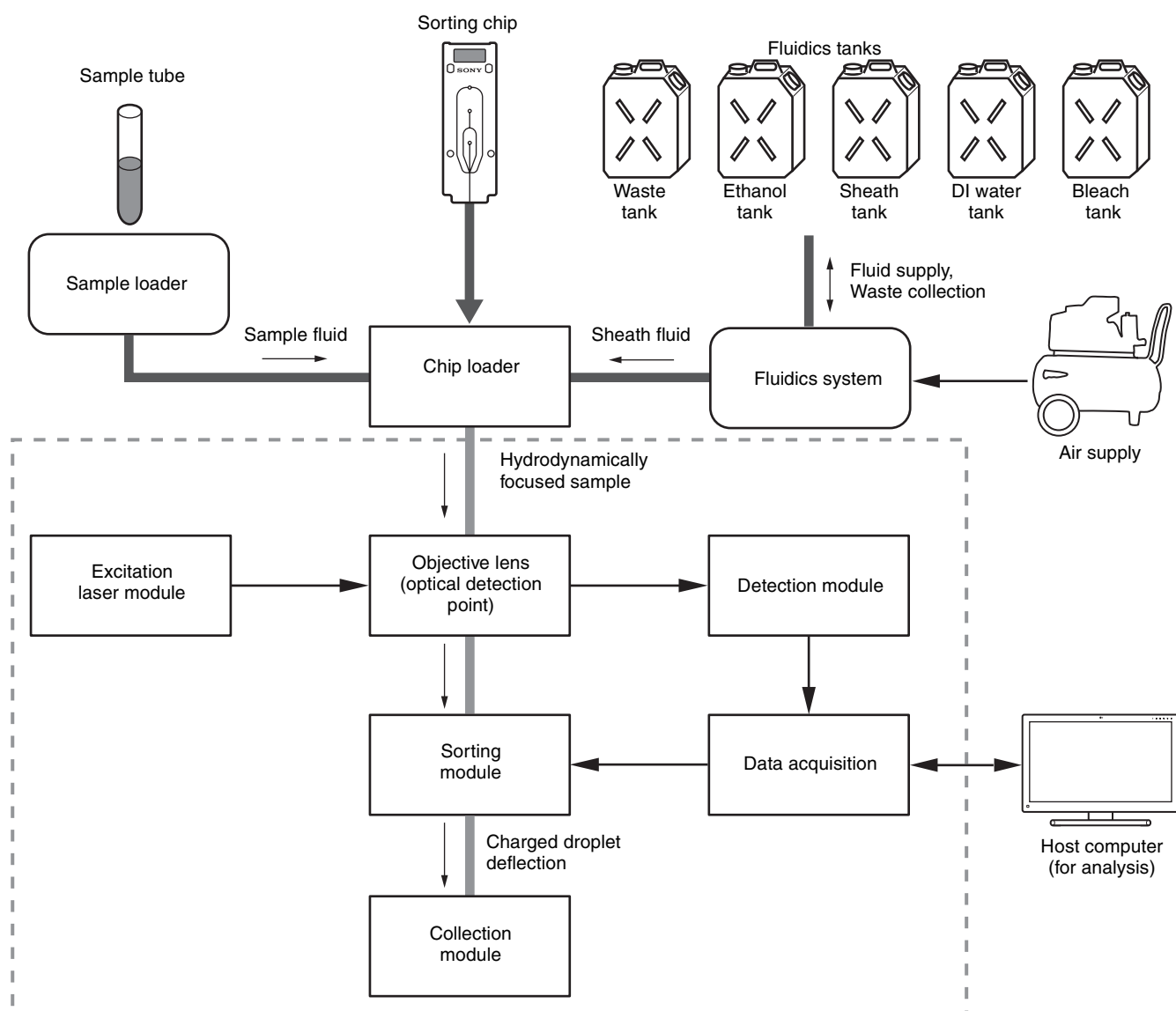
Note

Care must be taken to place the instrument in an area of a room where there is no direct air draft in order to ensure stable stream operation.

Tip

This manual describes the basic principles flow cytometry as it pertains to understanding how the LE-MA900 operates. It is not intended as a reference on the subject of flow cytometry. There are many reference sources available, both in print and online, that you should consult for flow cytometry specifics, such as sample preparation, dye selection, analysis techniques, results publication methods, and other topics.

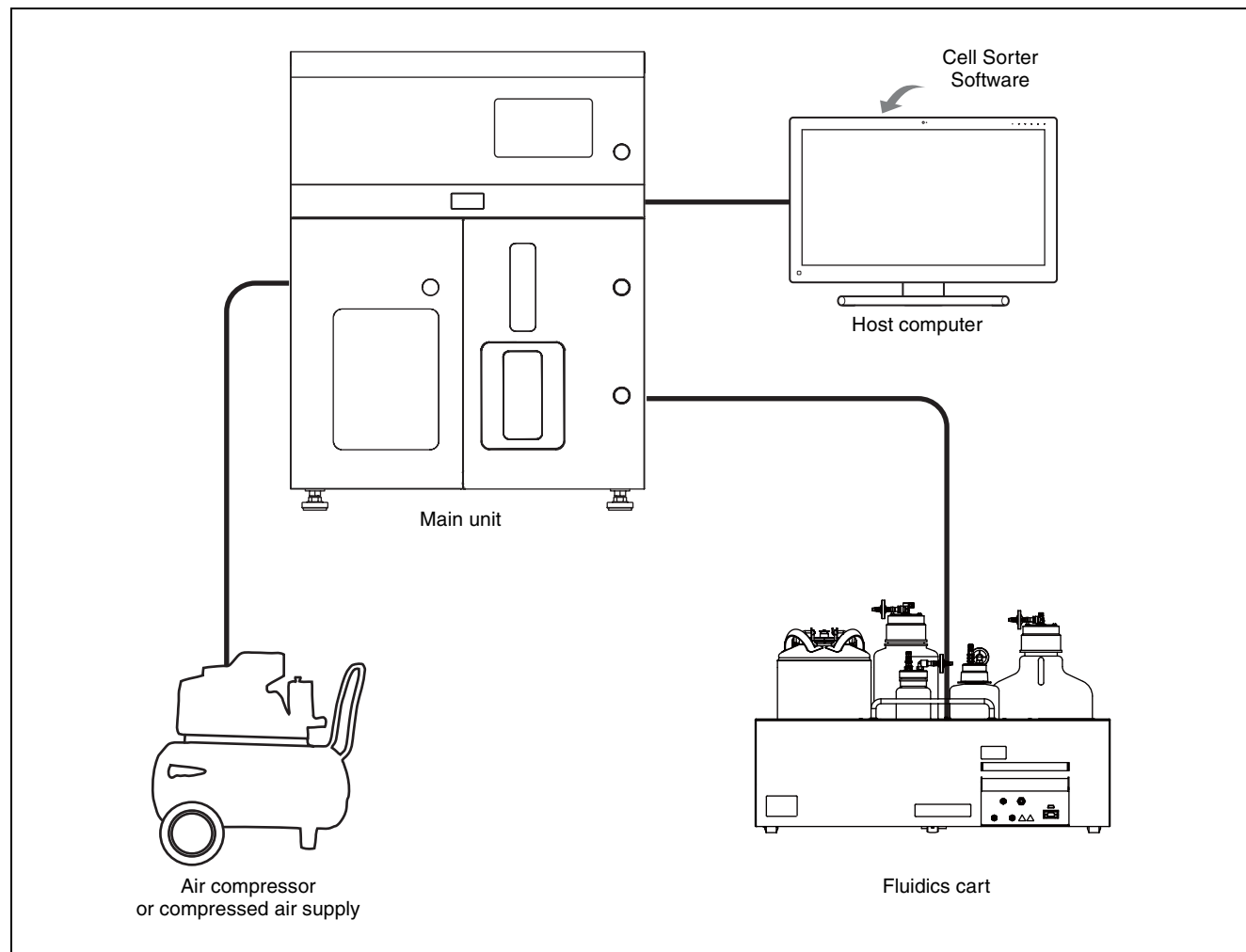
Cell Sorter Block Diagram



- The flow of waste fluid, bleach, ethanol, and DI water is not depicted in this diagram. All waste fluid is collected in the waste tank in the fluidics cart.

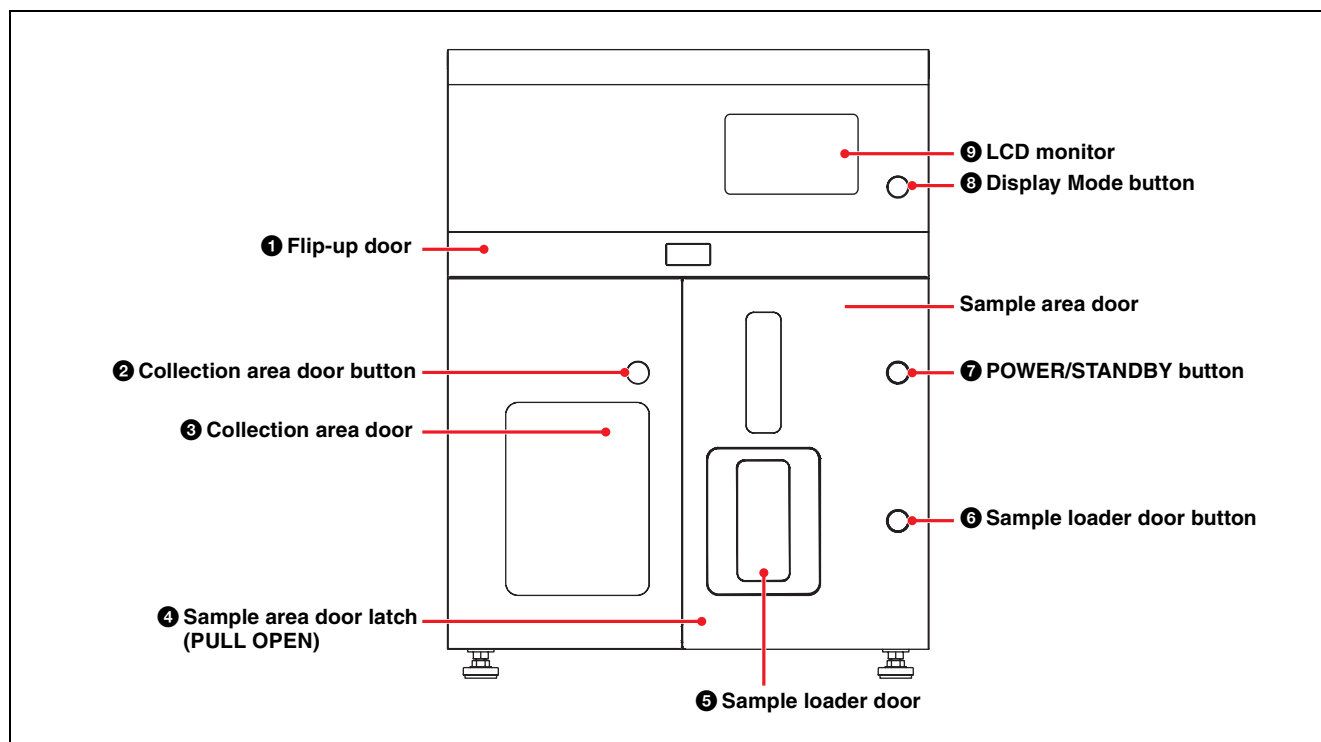
System Configuration

The LE-MA900 system comprises the LE-MA900 main unit, fluidics cart, host computer, and compressed air supply.



Name and Function of Parts

Front Panel



1 Flip-up door

Provides access for attaching the sorting chip, and sample line.

The door also provides access to the optical filters, the sample line and probe for routine maintenance and cleaning.

Notes

- Make sure to close the flip-up door to run samples.
- The flip-up door opens and closes with assistance from the compressed air supply. Make sure the compressed air supply is turned on and supplying the rated air pressure when operating the door. For safety, a lock function operates to prevent the door from opening and closing when the air supply is turned off. Do not attempt to operate the door when the compressed air supply is turned off.

2 Collection area door button

Opens the collection area door.

3 Collection area door

Provides access to the collection area for loading and unloading collection tubes for sorting. It also provides access for cleaning and routine maintenance.

A safety interlock switch disables sorting operation while the door is open.

Note

A rubber packing seal is used to ensure the integrity of the collection area door. If the door does not open when the door button is pressed, the door may be sticking to the packing. In this case, open the door by simultaneously pressing the button with one hand while pulling gently on the bottom edge of the door with your other hand.

4 Sample area door latch (PULL OPEN)

Provides access to the sample area for cleaning and routine maintenance.

You can open the door when the main unit is turned off or when running the [Sample Area Maintenance] wizard. The door is locked and cannot be opened during cell analysis and sorting operation.

5 Sample loader door

Provides access to the sample loader for loading and unloading samples for testing.

Note

Observe the following precaution when opening and closing the sample loader door.

- Turn on the power before operating the sample loader door button.

6 Sample loader door button (OPEN/CLOSE)

Opens and closes the sample loader door.

Once a sample has been lifted into the injection chamber within the sample loader and acquisition started, the

button is disabled to prevent opening and closing of the sample loader door. The button is also disabled while the sample area door is open.

7 Power button (POWER/STANDBY)

Switches the LE-MA900 main unit power supply between ON and OFF. When power is ON, press and hold for about two seconds to switch power OFF.

8 Display Mode button

Switches the LCD monitor display on/off.

9 LCD monitor

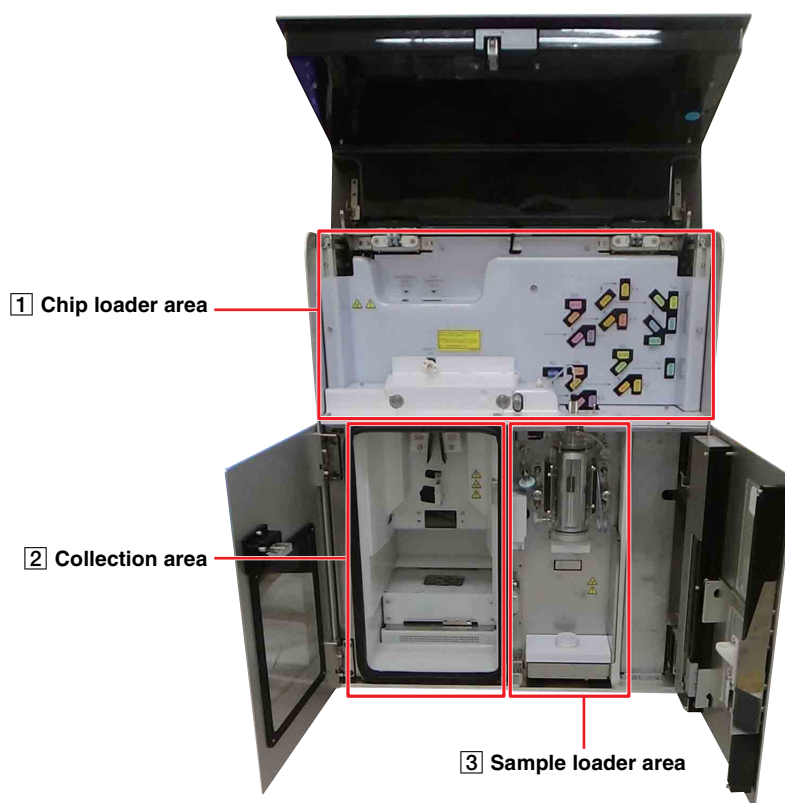
Displays instrument status information.

For details, see "LCD Monitor" (page 31).

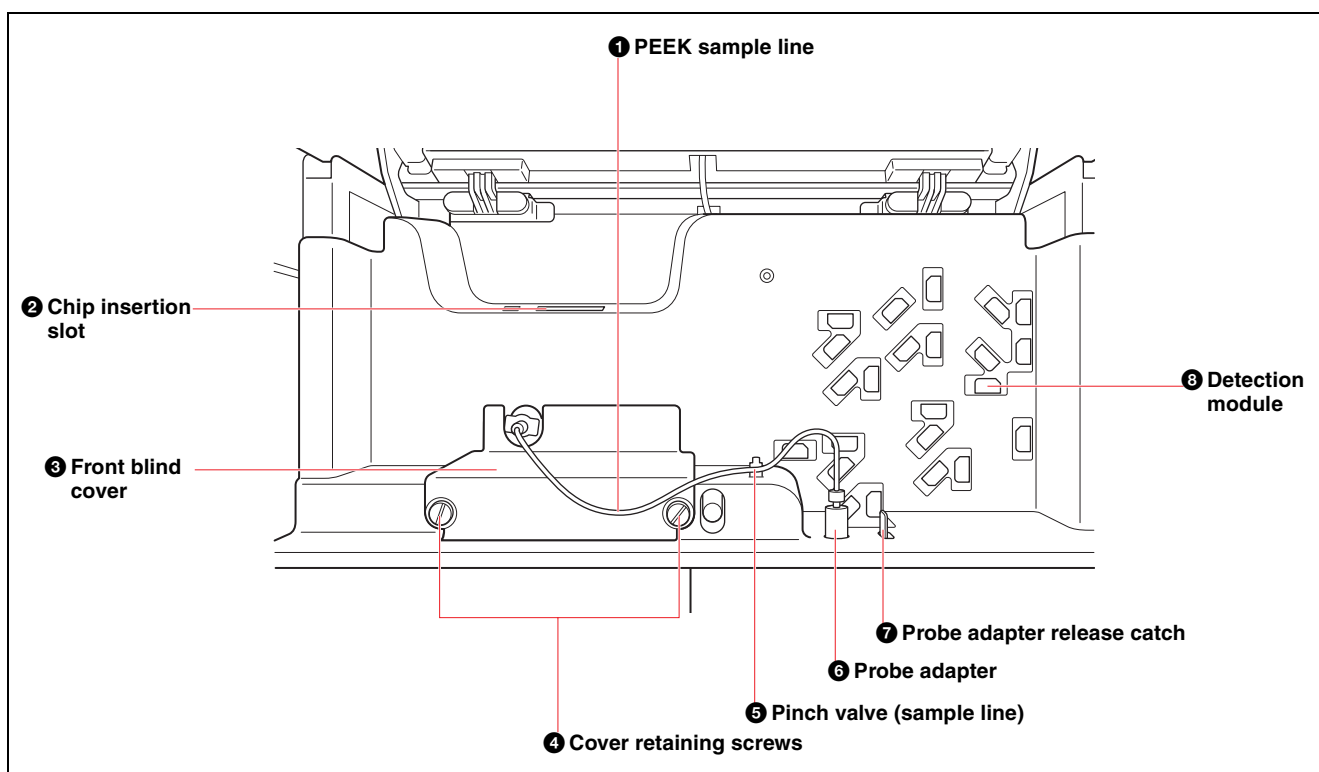
CAUTION

- Do not place any objects in front of the doors.
- Be aware of the door edges when the flip-up door, collection area door, fluidics maintenance door, or sample area door is open.
- When opening/closing the flip-up door, check to make sure your fingers and other objects are clear of the edges. Failure to do so may result in damage or injuries.

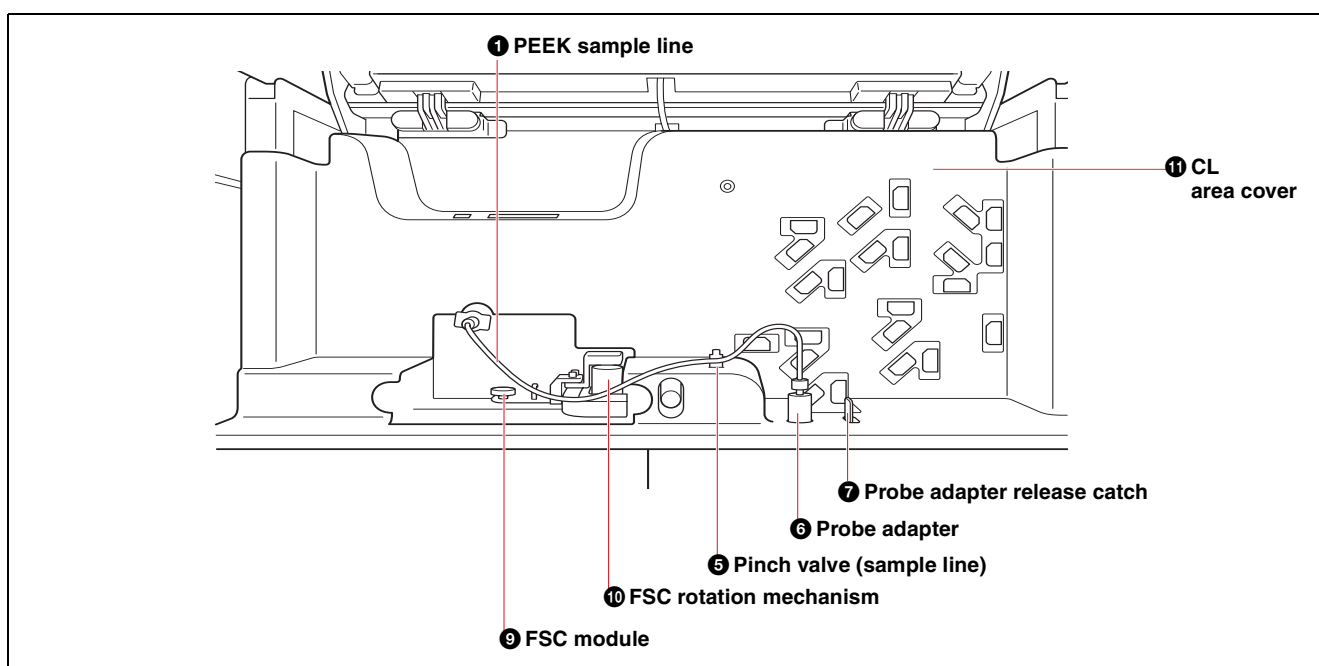
Internal View (Front)



1 Chip loader area



With front blind cover removed



1 PEEK sample line

Connects directly to the sorting chip, transporting the sample fluid.
The PEEK sample line combines the sample line and sample probe in a single component.
The PEEK sample line is an exchangeable component.

Notes

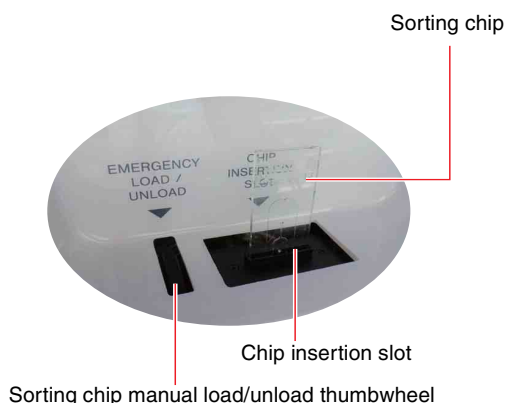
- Make sure the sample line is fully inserted into the pinch valve. Failure to do so may result in fluid leakage.

- Touching the connector of sample line connector will directly affect the chip alignment. If you touch this connector after the chip alignment has been adjusted, the chip must be realigned using the chip alignment function.
- Always leave the sample line connected when the unit is not in use for protection against dust.

For details, see “Changing the PEEK Sample Line” (page 175).

② Chip insertion slot

Provides access to the chip loader for inserting and removing the sorting chip.



The chip unloading thumbwheel is used to eject the sorting chip if the chip will not unload automatically. For details, see “Ejecting the Sorting Chip Manually” (page 201).

③ Front blind cover

Provides access to the interior of the instrument for cleaning.

④ Cover retaining screws

Secures the front blind cover.

Remove these screws to remove the front blind cover.

⑤ Pinch valve (sample line)

Controls the flow of sample fluid between the sample probe and the sorting chip.

⑥ Probe adapter

Holds the inserted sample probe when installing a PEEK sample line.

⑦ Probe adapter release catch

Used when removing the sample probe.

⑧ Detection module

Comprises an array of dichroic beamsplitter longpass filters (LPF) to separate the collected fluorescent light into separate channels, and bandpass optical filters (BPF) to filter the light entering each detector.

There are 14 detectors: forward scatter (FSC), back scatter (BSC), and up to 12 fluorescent light (FL1 to FL12) channels, depending on the model, for multicolor analysis.

Notes

- The detection module uses components that are susceptible to static electricity, which may cause an error in operation. Do not touch the detection module during operation to prevent the transfer of static electricity.
- If you sense a discharge of static electricity near the detection module during data acquisition, it is recommended that acquisition be paused and then resumed. This procedure restores normal gain settings, even in the event that static electricity affects the operation of the detection module.

⑨ FSC module

Detects the forward scatter (FSC) light.

Rotate forward to gain access when performing maintenance.

For details, see “Cleaning the Lens Guard” (page 186).

⑩ FSC rotation mechanism

Used to rotate the FSC module toward you when performing maintenance.

For details, see “Cleaning the Lens Guard” (page 186).

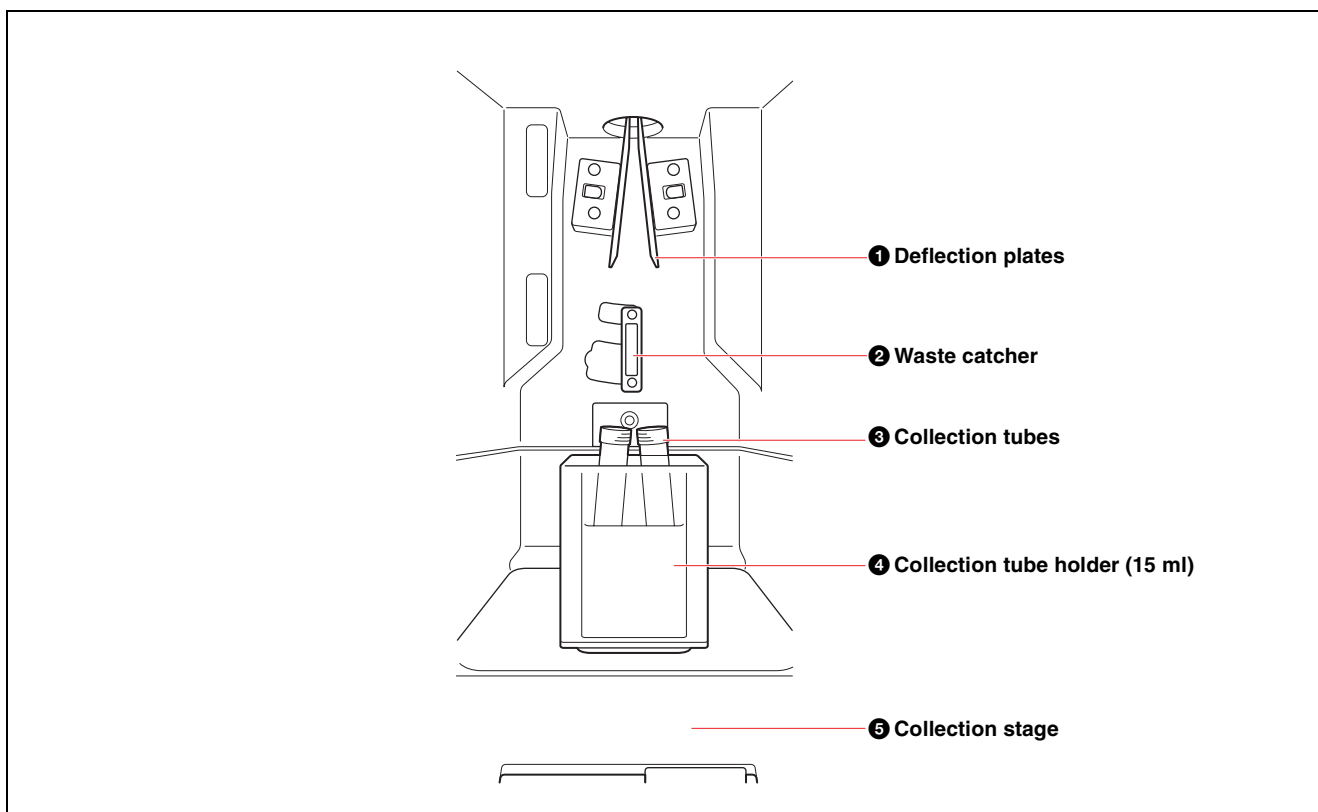
⑪ CL area cover

Remove to gain access to clean the lens guard.

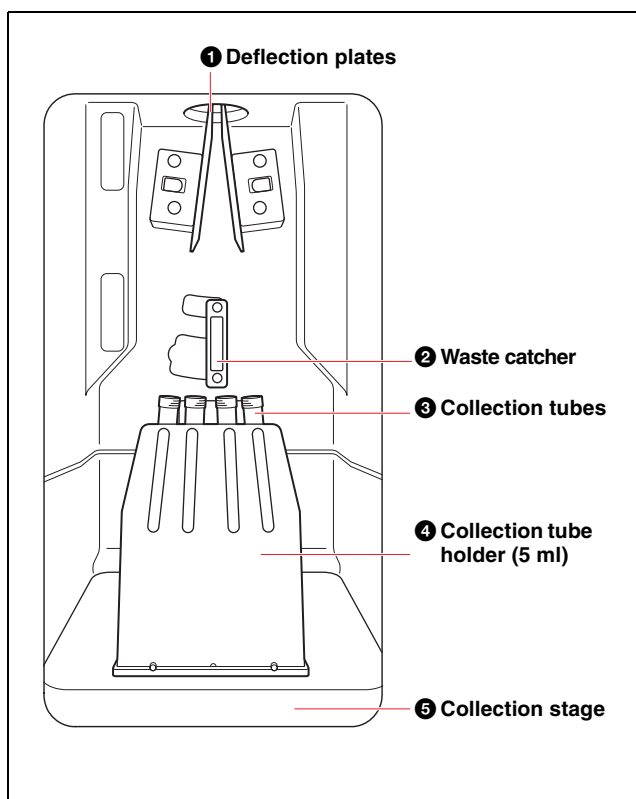
For details, see “Cleaning the Lens Guard” (page 186).

2 Collection area

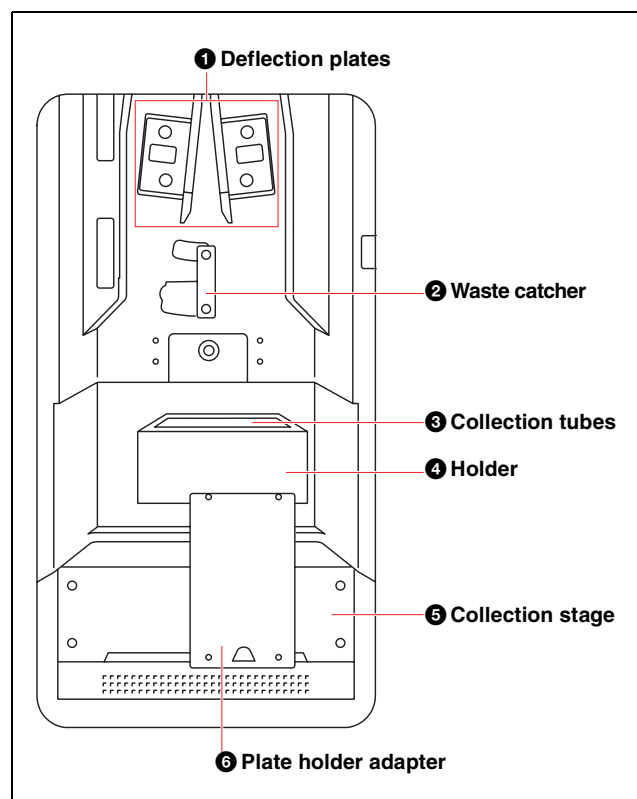
LE-MA900 model



LE-MA900/LE-MA900P models (common)



LE-MA900P model (when using multi-well plate)



DANGER

Fluids may contain biological, chemical, or other agents. When handling collection tubes, always wear laboratory-

use gloves, mask, protective goggles, and other protective clothing.

1 Deflection plates

Deflects electrostatically-charged droplets ejected from the nozzle of the sorting chip, sorting the droplets into the designated collection tubes.

Droplets that contain target cells for sorting are charged as they are ejected from the sorting chip nozzle, while other droplets containing non-target cells are not charged and collected in the waste catcher. The target cells for sorting are specified using gates in Cell Sorter Software.

The trajectories of droplets that are sorted in the collection tubes are called side streams. For details, see “Side stream monitor: Displays the image from the side stream monitor camera.” (page 156).

DANGER

The deflection plates are charged at an extremely high voltage. Contact with charged deflection plates can result in serious injury or electrical shock. Do not touch the deflection plates while power is connected to the main unit.

Note

Side stream calibration and data acquisition can be adversely affected by bright light shining directly on the side stream monitor. Do not place the front of the instrument near sources of bright light, such as lamps or unshaded windows.

Tip

Sorting may not occur correctly if there is any condensation on the deflection plates or if they have salt deposits. Clean the deflection plates.

2 Waste catcher

Collects the uncharged, non-target droplets.

The collected droplets are passed to the waste tank in the fluidics cart.

3 Collection tubes

Collects the electrostatically-charged, target droplets.

The instrument supports the following collection tube devices. Each type of collection tube is used with a corresponding holder that mounts on the collection stage.

Collection tubes	Holder
15 ml conical tubes	Collection tube holder (15 ml) ¹⁾
5 ml round tubes	Collection tube holder (5 ml) ¹⁾
6-well plates	96/384-well plate holder
12-well plates	
24-well plates	
48-well plates	
96-well plates	
8-well PCR strips	96-well PCR plate holder ²⁾
96-well PCR plates	
384-well plates	96/384-well plate holder
384-well PCR plates	96/384-well plate holder + 384-well PCR adapter

- 1) SH800 series and FX500 series collection tube holders (5 ml/15 ml) cannot be used.
- 2) Use an Eppendorf® PCR cooler (cold storage system for 96-well plates and PCR tubes).

For details about collection tubes and loading tubes, see “Preparing Collection Tubes and Plates” (page 36).

4 Holder

Holds the collection tube device.

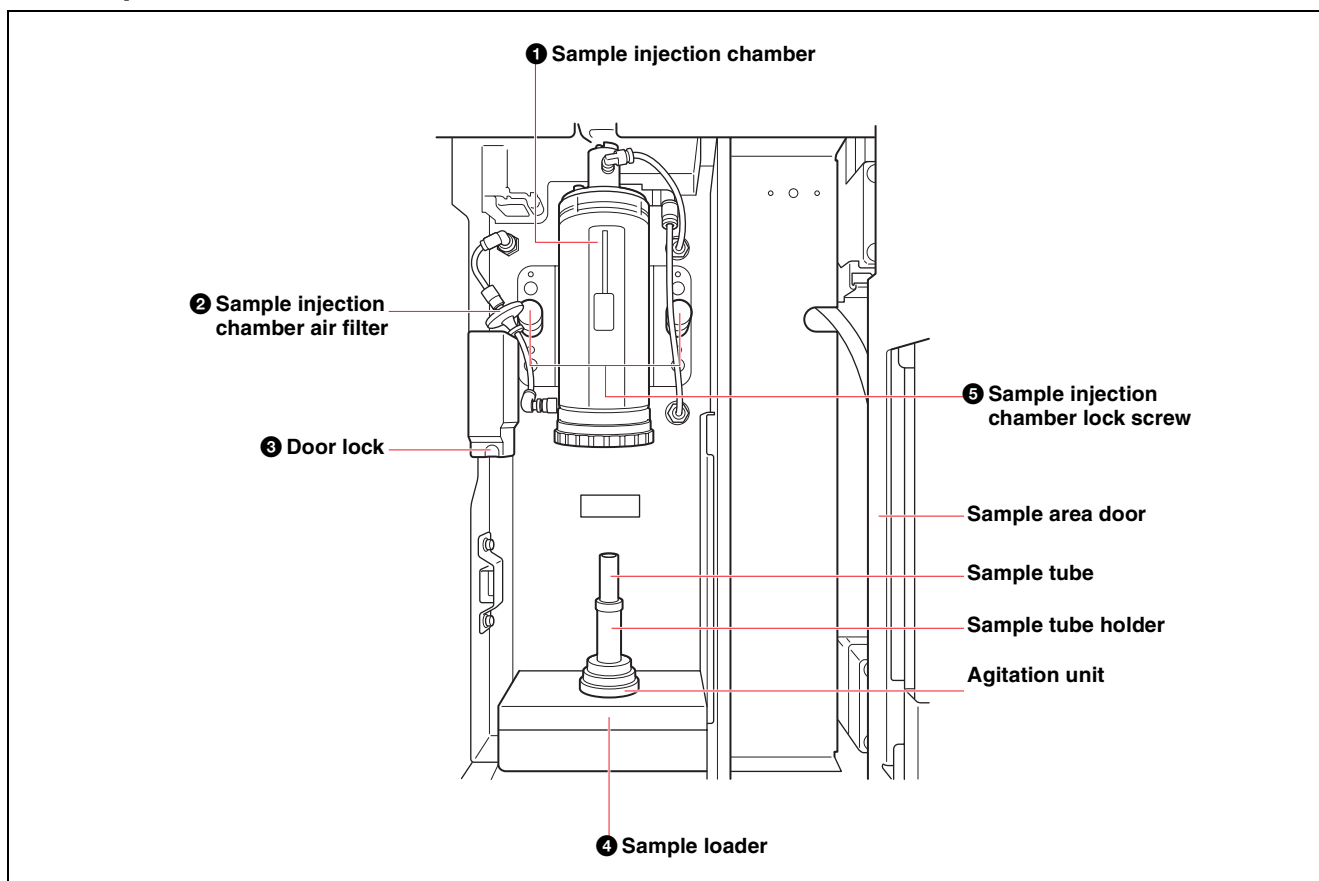
5 Collection stage

Loads the collection tube device holder.

6 Plate holder adapter (96/384-well plate compatible, LE-MA900P model only)

Used for loading multi-well plate collection devices. When sorting into 15 ml conical tubes or 5 ml round tubes, remove the adapter and load the corresponding collection tube holder.

3 Sample loader area



Notes

- Always wear laboratory-use gloves, mask, protective goggles, and other protective clothing when handling sample fluid to provide protection against biohazards.
- Clean the fluidics system regularly using a sodium hypochlorite liquid solution (bleach) to prevent clogging of the sample line and sample probe.
- Load the sample tube with its cap removed.

1 Sample injection chamber

Extracts sample fluid from the sample tube, through the sample probe, and forces the fluid into the sorting chip.

2 Sample injection chamber air filter

Filters the air supply to the sample injection chamber. The sample injection chamber air filter is an exchangeable component.

3 Door lock

Locks the sample area door automatically when it closes. The lock is unlocked when the power is turned off when in maintenance mode.

4 Sample loader

Loads a sample tube holder containing a single sample tube containing sample fluid into the sample loader agitation unit.

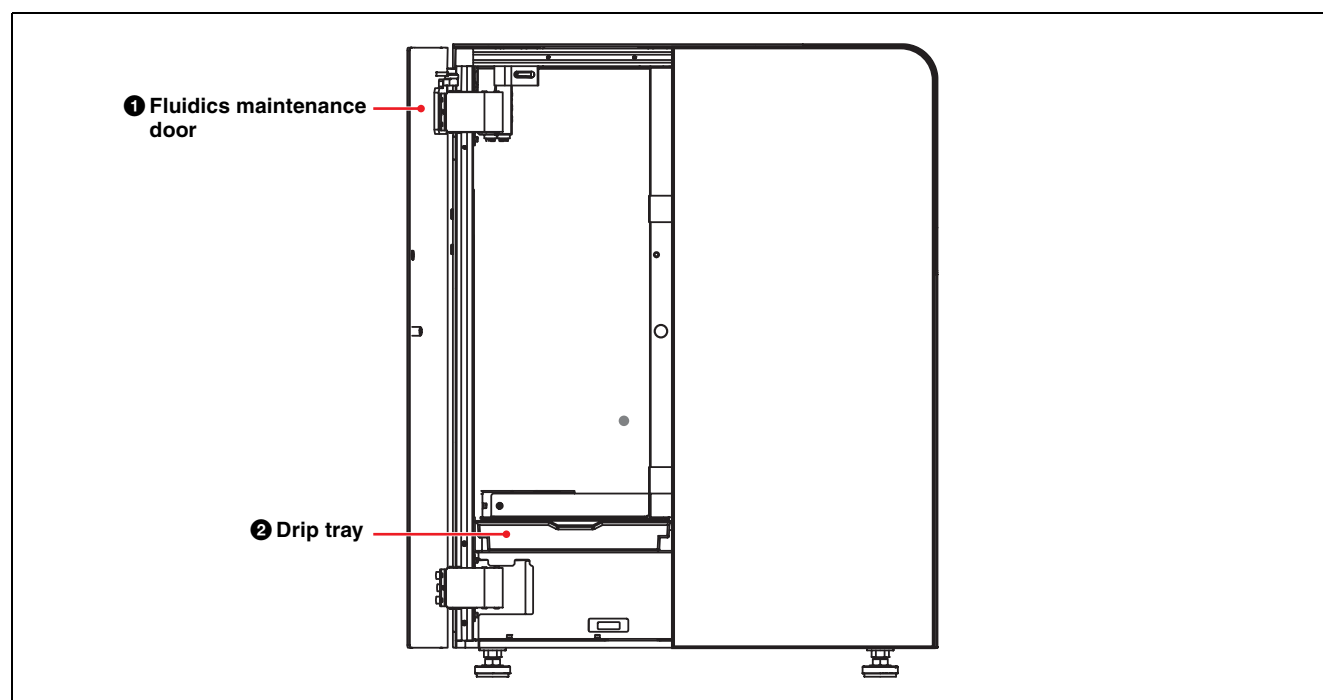
Samples can be loaded in 0.5 ml or 1.5 ml micro tubes, 5 ml round tubes, or 15 ml conical tubes. A sample tube holder is provided for each size of sample tube.

For details, see “Loading a Sample Tube in a Sample Tube Holder” (page 34).

5 Sample injection chamber lock screw

Secures the sample injection chamber in place. Remove the screws to remove the sample injection chamber.

Internal View (Side)



① Fluidics maintenance door

Provides access to the fluidics system for performing maintenance.

Fluidics system maintenance should be performed by qualified service personnel only. Do not touch interior components.

Note

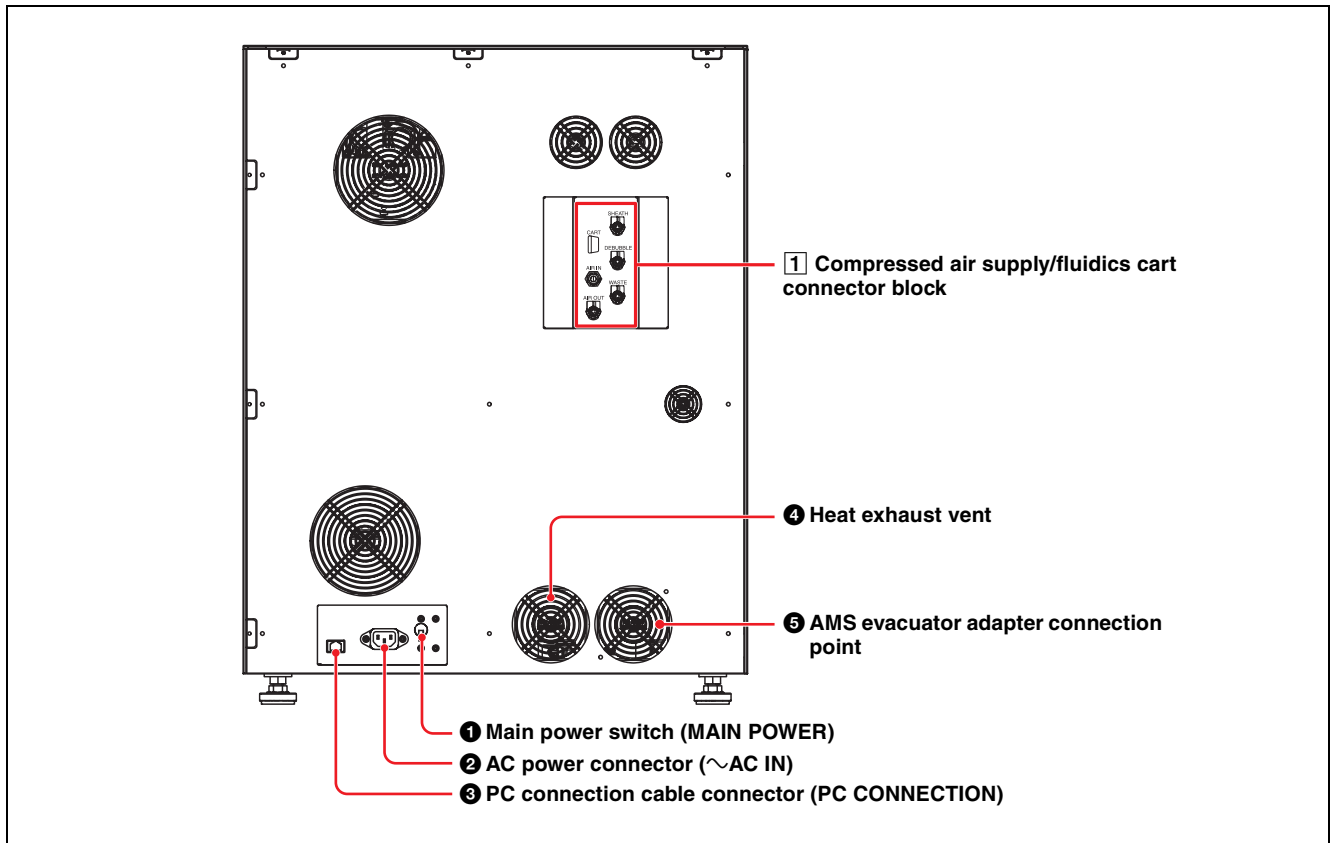
Be aware of the sharp door edges when the door is open. Do not touch the surface directly with bare hands during operation. Internal components may generate significant heat during operation.

② Drip tray

Collects liquid leakages due to damaged tubing or improperly fitted connectors. The drip tray should be checked regularly for signs of leakage.

An alert is shown on the host computer if waste fluid is detected in the drip tray. If this occurs, contact your Sony representative for service.

Rear Panel



1 Main power switch (MAIN POWER)

Turns the power supply for the LE-MA900 ON (up) and OFF (down). The breaker automatically disconnects the power supply if an electrical fault is detected.

2 AC power connector (~AC IN)

Connects to a standard AC power supply (100/240 V AC, 50/60 Hz). Use only the supplied power cord to connect the LE-MA900 main unit to a power supply.

Notes

- Use only within the specified supply voltage range.
- The power cord must be connected to a power outlet which is grounded.

3 PC connection cable connector (PC CONNECTION)

Connects directly to the host computer using the supplied PC connection cable.

Notes

- Do not disconnect the PC connection cable during operation. Data acquisition cannot be performed if the cable is disconnected.
- Do not connect any device other than the host computer using the PC connection cable. Device operation is not guaranteed if an external device is connected.

CAUTION

For safety, do not connect the connector for peripheral device wiring that might have excessive voltage to this port.

Follow the instructions for this port.

4 Heat exhaust vent

Discharges heat generated from inside the unit.

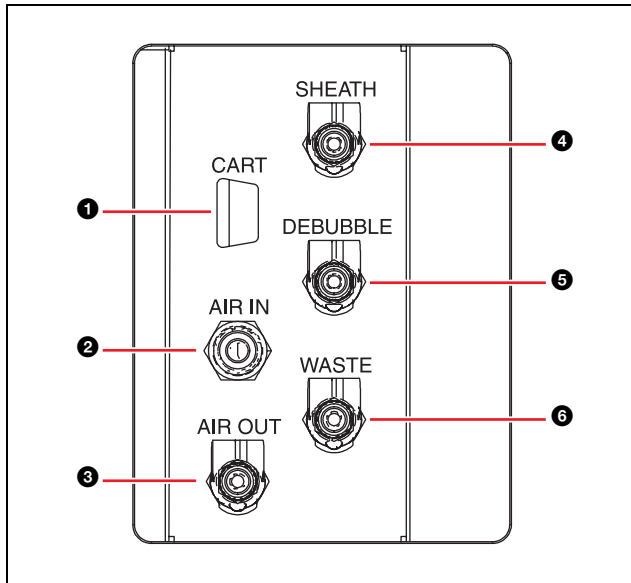
5 AMS evacuator adapter connection point

Connect to an AMS device using a dedicated adapter.

Note

Maintain at least 10 cm (4 in) space behind the main unit to allow for the dissipation of heat and to provide access to the power connector, main power switch, electrical connectors, fluidics cart connectors, and compressed air supply connectors.

1 Compressed air supply/fluidics cart connector block



1 Cart connection cable connector (CART)

Connects to the cart connection cable connector on the fluidics cart. It connects the sensors for the three fluid tanks in the fluidics cart.

Tighten the lock screw of the connector firmly so that the connection cannot become loose.

Note

Use only the supplied cable to connect the main unit to the fluidics cart. Using third-party cables may cause incorrect operation or device failure. Using the cable without tightening the lock screw may cause incorrect operation or device failure.

2 Compressed air supply connector (AIR IN)

Connects to the laboratory compressed air supply or a standalone air compressor.

The LE-MA900 is designed to connect to 500 kPa to 650 kPa (73 psi to 94 psi) compressed air supply for stable output. Use a compressed air supply with rated pressure in the range 500 kPa to 650 kPa (73 psi to 94 psi).

Use polyurethane tubing with an outer diameter of 6 mm and an inner diameter of 4 mm.

Note

Insert a cutoff valve in the air tube so that compressed air can be turned off as necessary.

3 Fluidics cart air line connector (AIR OUT)

Connects to the sheath air line connector (AIR IN) on the sheath tank in the fluidics cart using the supplied air connection line (clear tubing). It supplies compressed air to the sheath tank.

4 Fluidics cart sheath line connector (SHEATH)

Connects to the SHEATH connector on the fluidics cart using the supplied sheath line (blue tubing).

5 Fluidics cart de-bubble line connector (DEBUBBLE)

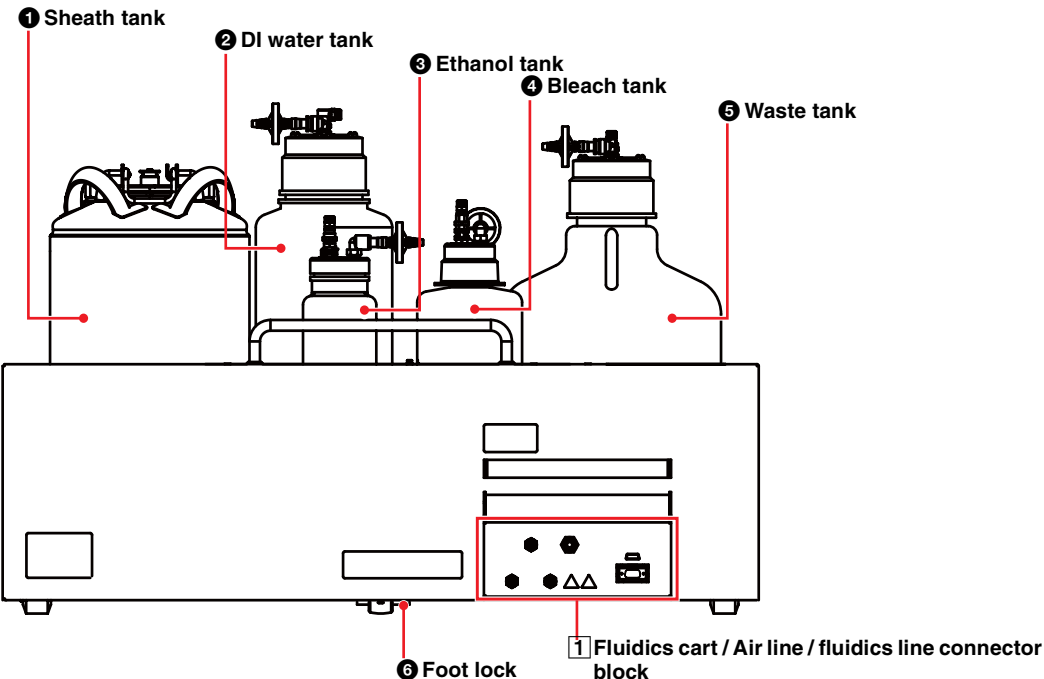
Connects to the DEBUBBLE connector on the fluidics cart using the supplied de-bubble line (orange tubing).

6 Fluidics cart waste line connector (WASTE)

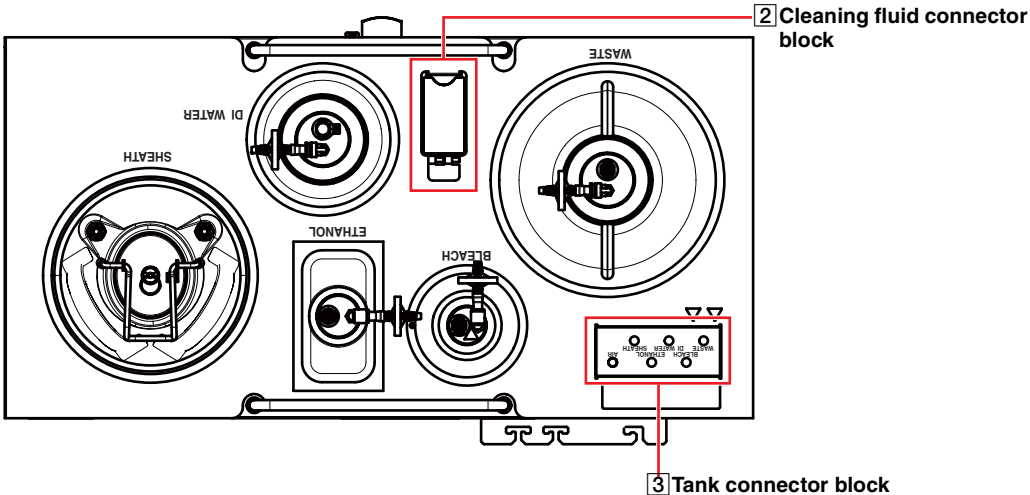
Connects to the waste connector on the fluidics cart using the supplied waste line (red tubing).

Fluidics Cart

Cart (rear side)



Cart (top side)



1 Sheath tank

Supplies the sheath fluid used as the flow medium for transporting the sample fluid in a laminar flow through the sorting chip and into the collection tubes. The tank is pressurized using regulated compressed air supplied from the main unit.

A weight sensor in the cart sensor unit detects the sheath fluid level for display on the LCD monitor and host computer. An alarm is generated when the sheath fluid falls below a set level. The tank has a capacity of 10 liters (2.6 gallons US).

Notes

- Never use the supplied sheath tank for anything other than the intended purpose.
- When opening the sheath tank cap, check that “Standby” is displayed on the LCD monitor or that the power to the main unit is turned off. If “Standby” is not displayed, place the unit in Standby mode using the following procedure.

- ① Click [Settings] on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog appears.

- ② Click [Advanced Settings].

The [Advanced Settings] dialog appears.

- ③ Click [Standby] on the [Pressure Options] tab.

- ④ Check that “Standby” is displayed on the LCD monitor.

When “Standby” is displayed, disconnect the tank air line (clear tubing) from the top of the sheath tank. For details, see “Refilling the Sheath Tank” (page 165).

- Do not force the lid down, or place objects on the lid, or move the tank while the tank is pressurized.

Note

The sheath tank and sheath fluid line must not be disturbed to ensure stable, measurement conditions. Position the sheath fluid line where it will not be affected by drafts from air conditioning or vibration of the air compressor.

CAUTION

Overfilling the sheath tank may lead to significant damage to the internal components of the instrument. Never overfill the tank above the capacity of 10 liters (2.6 gallons US).

2 DI water tank

Supplies deionized (DI) water used for flushing the fluidics system.

A sensor detects the level of DI water within the tank, and a message is displayed when the level drops below the minimum threshold.

The tank has a capacity of 5 liters (1.3 gallons US).

3 Ethanol tank

Supplies the cleaning fluid (ethanol) used to clean the fluidics system during maintenance. A sensor detects the ethanol level for display on the LCD monitor and host computer. An alarm is generated when the ethanol falls below a set level. The tank has a capacity of 2 liters (0.5 gallons US).

4 Bleach tank

Supplies the cleaning fluid (sodium hypochlorite solution) used to clean the fluidics system during maintenance. A sensor detects the sodium hypochlorite solution level for display on the LCD monitor and host computer. The tank has a capacity of 2 liters (0.5 gallons US).

5 Waste tank

Collects waste fluid, comprising sample fluid, sheath fluid, and cleaning fluid, from various locations in the main unit. A cart sensor detects the waste fluid level for display on the LCD monitor and host computer. The tank has a capacity of 10 liters (2.6 gallons US).

DANGER

Waste fluid may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, as required, when emptying or replacing the waste tank. The handling of waste fluid should be performed in accordance with biological hazard handling safety procedures.

6 Foot lock

Secure in position to ensure the fluidics cart does not move during data acquisition.

7 Sheath filter

Filters the sheath fluid flowing from the sheath tank and the DI water from the DI water tank. The sheath filter is user exchangeable. The filter can be autoclaved for asepsis.

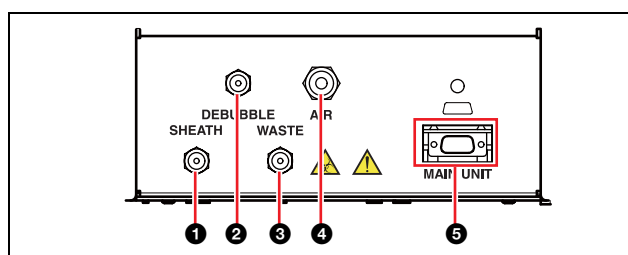
8 Sheath filter door

Provides access to the sheath filter for performing maintenance.

CAUTION

Be aware of the door edges when the door is open.

1 Fluidics cart / Air line / fluidics line connector block



1 Fluidics cart sheath line (blue) connector

Connects to the SHEATH connector on the main unit using the supplied sheath line (blue tubing).

2 Fluidics cart de-bubble line (orange) connector

Connects to the DEBUBBLE connector on the main unit using the supplied de-bubble line (orange tubing).

3 Fluidics cart waste fluid line (red) connector

Connects to the waste connector on the main unit using the supplied waste line (red tubing).

④ Fluidics cart air line connector (AIR)

Connects to the fluidics cart air line connector (AIR OUT) on the MA900 (clear tubing). The air line supplies regulated compressed air for the sheath tank.

Notes

- The standard fluidics lines are about 3 m (9.8 ft) in length.
- Use only the supplied cable to connect the main unit to the fluidics cart. Using third-party cables may cause incorrect operation or device failure.

⑤ Cart connection cable connector (MAIN UNIT)

Connects to the cart connection cable connector on the main unit.

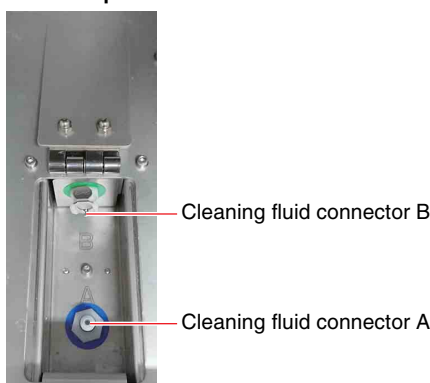
Using the cable without tightening the lock screw may cause incorrect operation or device failure.

Notes

- Use only the supplied cable to connect the main unit to the fluidics cart. Using third-party cables may cause incorrect operation or device failure. Using the cable without tightening the lock screws may cause incorrect operation or device failure.
- The standard cable is about 3 m (9.8 ft) in length.

② Cleaning fluid connector block

With lid open

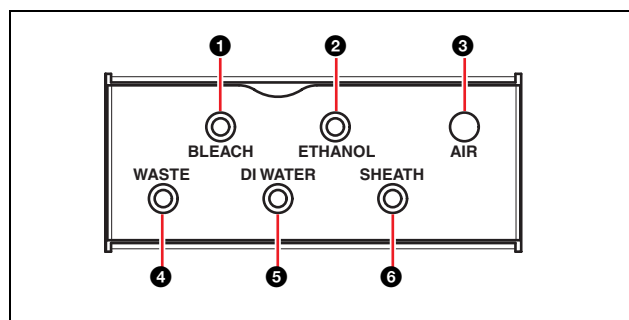


Used when running ethanol cleaning and bleach cleaning in maintenance mode.

Connect the corresponding tank connection lines to cleaning fluid connector A or B to switch the cleaning mode.

For details, see “Running Maintenance Mode” (page 192).

③ Tank connector block



① Bleach tank connector (BLEACH)

Connects to the bleach tank using the supplied bleach line (yellow tubing).

② Ethanol tank connector (ETHANOL)

Connects to the ethanol tank using the supplied ethanol tank line (yellow tubing).

③ Fluidics cart air line connector (AIR)

Connects to the sheath tank using the supplied air line (clear tubing), and supplies compressed air to the sheath tank.

④ Waste tank connector (WASTE)

Connects to the waste tank using the supplied waste line (red tubing).

⑤ DI water tank connector (DI)

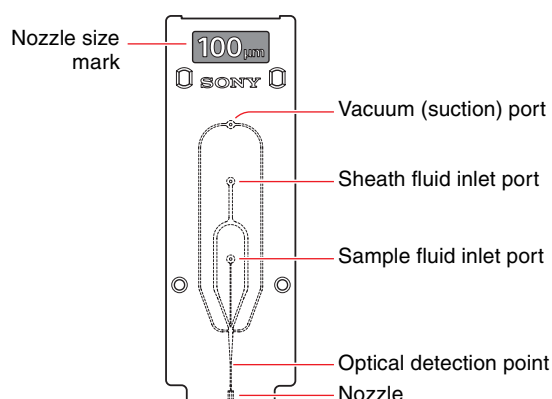
Connects to the DI water tank using the supplied DI water tank line (green tubing).

⑥ Sheath tank connector (SHEATH)

Connects to the sheath tank using the supplied sheath tank line (blue tubing).

Sorting chip

The LE-MA900 uses a disposable sorting chip.



Sample fluid and sheath fluid enter the chip through inlet ports on the front surface of the chip. The sample fluid and sheath fluid are forced into the chip by air pressure from the sample loader and the sheath tank, respectively. The sample fluid and accompanying sheath fluid flows through the chip where the sample fluid is focused into a

stream of cells. The cells in the sample fluid then pass the optical detection point where they are interrogated by the lasers, before being ejected from the nozzle. A transducer (not shown) vibrates the chip at an ultrasonic frequency to break the fluid stream into droplets for sorting.

Applying negative pressure (suction) to the vacuum port removes bubbles and clogged samples from the fluidics channels in the sorting chip.

The nozzle size mark indicates the size of the nozzle located at the bottom of the chip.

The sorting methods available depend on the diameter of the sorting chip nozzle and the size of cells in the sample. For details, see “Sorting” (page 59).

Notes

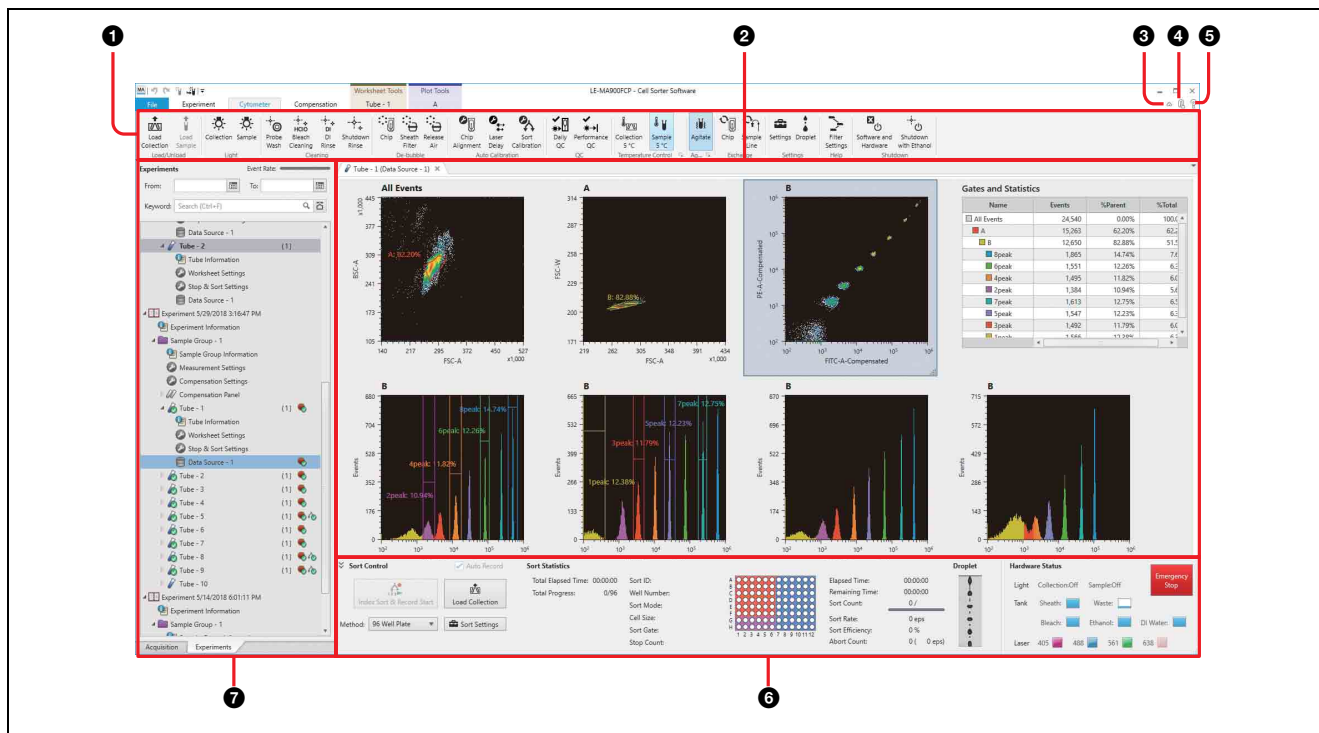
- Do not touch the sorting chip near the optical detection point. Fingerprints, scratches and smudges near the optical detection point can adversely affect data acquisition results.
- Do not reuse the same sorting chip beyond the recommended use time.

Main Window

Tips

- The menus and buttons displayed vary depending on the user account privileges.
- The screenshots displayed in this document may vary from the actual software.

Operating window



For details about the items displayed, see “Chapter 7 Window Description” (page 119).

1 Ribbon

Displays the tool buttons that can be used in the displayed window.
The content of the ribbon varies depending on the selected tab.

2 Worksheet

Work area for displaying and editing the results from data acquisition and analysis.

3 Ribbon show/hide control (Minimize/Restore)

Clicking / alternately shows the ribbon or hides the ribbon.

4 Logout

Logs out the currently logged in user, and displays the login window (page 45).

5 Help

Displays the Help window for accessing the Operator’s Guide.

6 Sorting control pane

Configures and controls droplet sorting.
It also displays the current status of sorting, fluid tank levels, lights, and laser sources.
The displayed items vary depending on the model and the sorting method.
The sorting control pane is a collapsible window element. The panel can be shown or hidden as required.

Tip

When the unit is calibrated in analyzer mode, the sorting control pane is automatically hidden.

7 Experiment/Acquisition control area

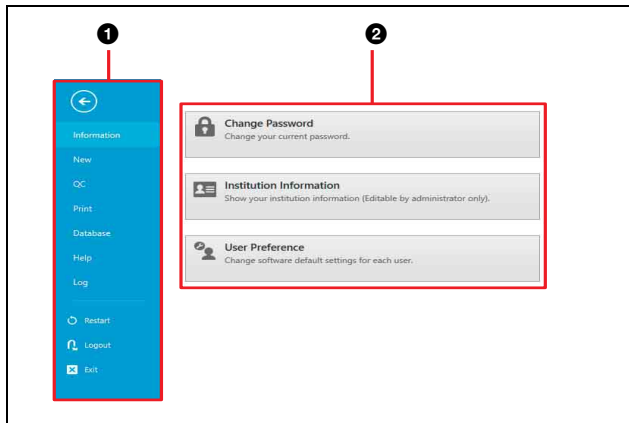
Used to configure and control data acquisition and work with experiments.

[Acquisition] tab: Displays the controls used for acquiring and recording data.

[Experiments] tab: Displays experiments and the components in an experiment, used to acquire and analyze data, in a hierarchical list view.

[File] window

Example: [Information] tab



① Menu items

Displays the following menu items.

[Information]: Displays commands used to configure user passwords and institution information, and to add/manage user accounts.

[New]: Creates a new experiment based on saved templates and/or recent experiments.

[QC]: Displays the QC results.

[Print]: Prints analysis results and other reports.

[Database]: Displays commands used to perform experiment database operations and to check hard disk free space.

[Help]: Displays commands used to display the Operator's Guide and to check software version information.

[Log]: Displays commands used to display the chip alignment log, sorting calibration log, and user log.

[Restart]: Restarts Cell Sorter Software.

When the software restarts, it loads the same conditions that existed at the time the [Restart] button was pressed, but without assigning any experiment as the active experiment.

Tip

This function should only be used if Cell Sorter Software operation has become unstable. It should not be used during normal operation.

[Logout]: Logs out the currently logged in user, and displays the login window.

[Exit]: Exits Cell Sorter Software.

② Operation area

Displays function buttons and information related to the selected menu item.

Cell Sorter Software Operating Environment

The host computer must satisfy the following requirements in order to run Cell Sorter Software.

Operating system (OS): Microsoft Windows 8.1/10

Operating System, Professional, 64 bit

CPU: Intel Core i7-6700 3.4 GHz or later

Memory: 16 GB or greater

Network interface: 1000BASE-T (1)

Main storage: 1 TB HDD or greater

Monitor resolution: 1920 × 1080 (16:9 Full HD)

Graphics resolution: 1920 × 1080 (Full HD) or greater

Camera: 1.3 megapixels or higher effective pixels

OS language setting: Symbol for the decimal point is “.” (period).

Note

If the decimal point is set to a character other than “.” (period), the following error message is displayed when starting Cell Sorter Software.

Invalid Language setting was detected.

Please change “Decimal symbol” setting to “.” (period)” according to the Operator's Guide.

Press [OK] to close this application.

If this occurs, use the following procedure to change the decimal point to “.” (period), then restart Cell Sorter Software.

1 Open [Control Panel] > [Clock, Language, and Region] > [Region and Language].

2 Open [Additional settings...] on the [Formats] tab.

3 Set [Decimal symbol] to “.” (period) on the [Numbers] tab.

Tips

- For details about recommended antivirus software, contact your Sony representative.
- It is the responsibility of the user to manage the Windows ID/password.

LCD Monitor

The LCD monitor on the front of the LE-MA900 displays status information during operation.

The LCD monitor is turned on/off using the Display Mode button on the front panel.



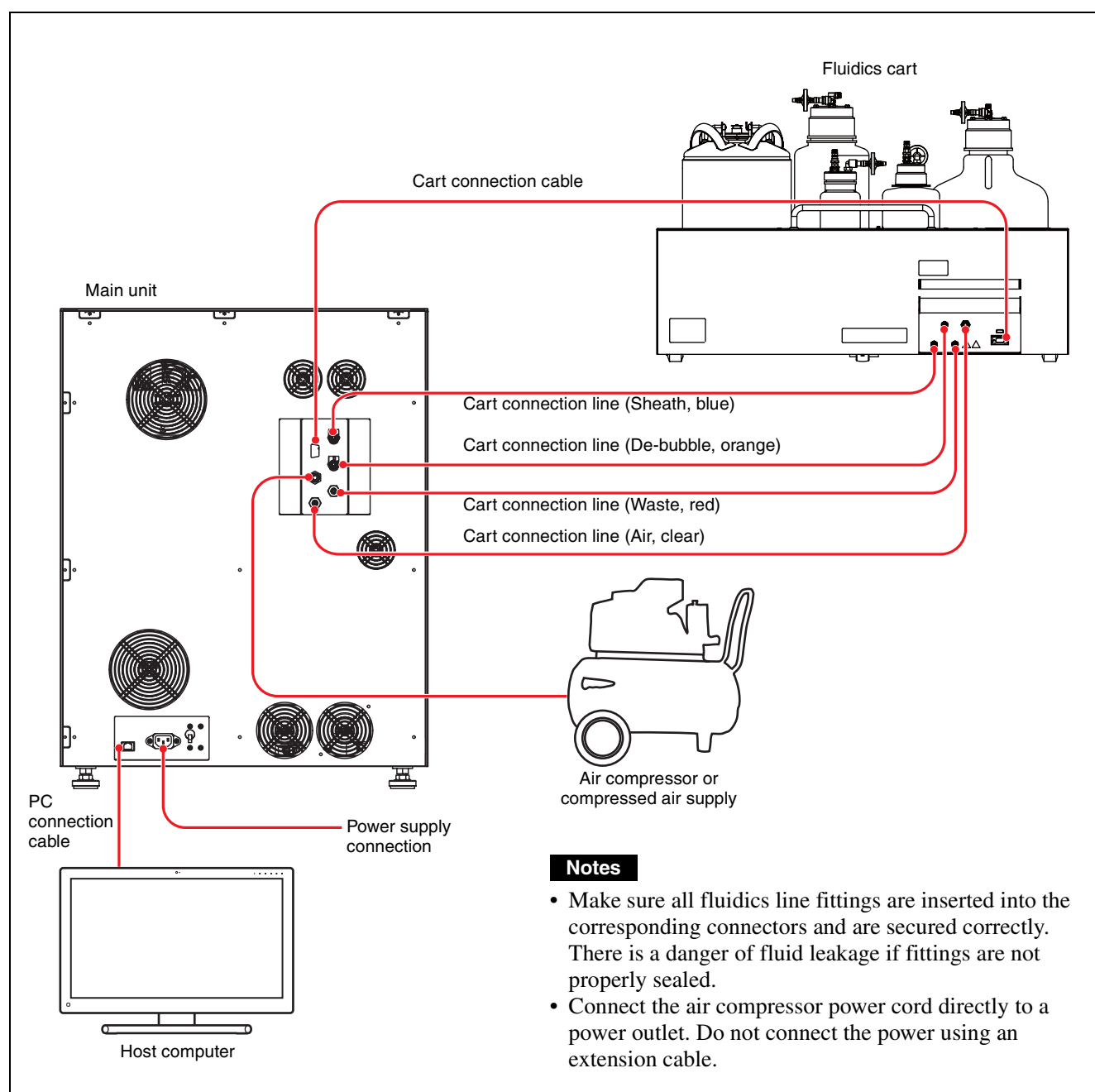
Tip

The LCD monitor shows the status of the instrument. This display is sometimes different from the Cell Sorter software display which shows software status.



Checking Device Connections

Check that the main unit, fluidics cart, host computer, and compressed air supply are connected correctly. For details about connectors, connection cable, and connection lines, see “Connections” (page 33).



Connections

The connectors, connection cable, and connection lines to connect are as follows.

Connector on main unit (label)	Connection cable/line	Connector on fluidics card (label)
SHEATH	Fluidics cart sheath line (blue tubing)	SHEATH
DEBUBBLE	Fluidics cart de-bubble line (orange tubing)	DEBUBBLE
WASTE	Fluidics cart waste line (red tubing)	WASTE
AIR OUT	Fluidics cart air line (clear tubing)	AIR
CART	Cart connection cable	MAIN UNIT

Preparing a Sample

Loading a Sample Tube in a Sample Tube Holder

Insert the sample fluid for analysis or sorting in a sample tube, and place the tube in the corresponding sample tube holder.

The instrument supports the following sample tubes. Each sample tube is used with a corresponding holder that mounts in the sample loader.

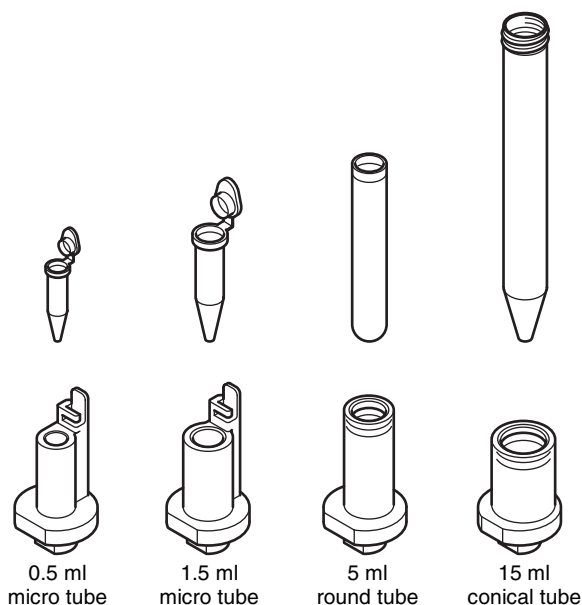
Sample tube	Holder
0.5 ml micro tube	Sample tube holder (0.5 ml)
1.5 ml micro tubes	Sample tube holder (1.5 ml)
5 ml round tubes	Sample tube holder (5 ml)
15 ml conical tubes	Sample tube holder (15 ml)

A sample tube holder is provided for each size of sample tube.

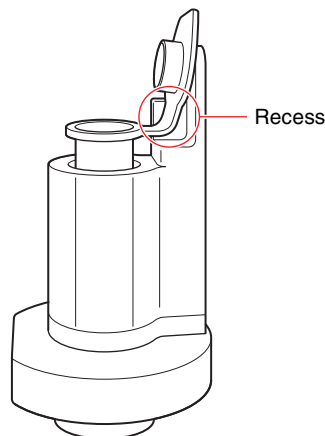
Load the sample tube into the corresponding size sample tube holder.

Note

Always remove the cap from the sample tube before measurement.



The tube holders for micro tubes have a recess at the top to hold the cap clear of the tube opening.



Notes

- Always wear laboratory-use gloves, mask, protective goggles, and other protective clothing when handling sample fluid to provide protection against biohazards. Also, take care when loading the sample tube in the tube holder to avoid sample fluid spills and breakage of the sample tube.
- Before using a tube, check that there is no dust or other foreign matter in the tube.
- Before placing a sample tube in the sample tube holder, agitate the sample to prevent aggregation in the sample and maintain sorting performance.
- Before placing the sample tube in the sample tube holder, check the volume of sample fluid to avoid introducing air bubbles in the fluidics line. Air bubbles cause droplet formation to become unstable. Unstable droplets may cause fluid to adhere to the surface of optical components, and stable performance cannot be maintained.

Preparing Automatic Setup Beads

During auto calibration, aliquot 1 ml or more of undiluted, automatic setup beads in a sample tube, and place the tube in the corresponding sample tube holder. Always use automatic setup beads in undiluted form.

Sample Temperature Control

The LE-MA900 is fitted with thermal control units in the sample injection chamber and collection area to maintain sample fluid temperature control while running an experiment. The temperature control device controls temperature and can be set to 5 °C (41 °F) or 37 °C (98.6 °F). The collection stage can be set to 5 °C (41 °F) only. Settings can be configured in Cell Sorter Software.

Notes

- The temperature control function in the software cannot be used to cool 96/384-well plate holders, 96-well PCR plate holders, or 384-well PCR adapters. However, the holders contain an embedded cooling agent and can be cooled beforehand in a laboratory


refrigerator (-20°C (-4°F)), and then placed on the collection stage.

Cooling time: 8 hours or longer

Temperature retention time: About 1 hour (varies with ambient temperature)

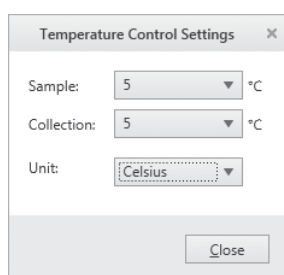
- Note that holders/adapters may freeze if immediately placed on the collection stage when removed from the refrigerator.

For details about starting the LE-MA900 instrument and Cell Sorter Software, see “System Startup” (page 44) and “Logging In” (page 45).

- 1 Click the  dialog launcher in the [Temperature Control] group on the [Cytometer] tab of the ribbon.

The [Temperature Control Settings] dialog appears.

- 2 Select the temperature setting for the sample fluid, then click [Close].



Sample: 5°C (41°F) or 37°C (98.6°F)

Collection: 5°C (41°F) only

Note

When sorting with the temperature set to 5°C (41°F), place the collection tube holder in a refrigerator to cool the holder before loading it on the collection stage.

- 3 To turn a temperature control device on/off, click [Sample] or [Collection] in the [Temperature Control] group on the [Cytometer] tab of the ribbon.

The buttons toggle between on and off.

Note

You can turn the sample cooling ON or OFF as needed.

Precautions when temperature control is set to 5°C (41°F)

- You can cool the collection tubes to approximately 5°C (41°F) by attaching the collection holder, but cooling the collection holder beforehand significantly reduces the time required to cool the collection tubes.
- While the temperature is set to 5°C (41°F), do not unload the collection tube holder from the collection stage. Insert and remove the collection tubes directly from the collection tube holder.
- Loading a collection tube holder that is warm or that has become warm may result in a sudden change in

temperature, and may prevent correct operation of the unit. When unloading and loading the collection tube holder, temporarily turn off the temperature control function, wait about 30 seconds, and then set the temperature to 5°C (41°F) again.

- The metallic parts of the multi-well plate holder become cold when the holder is cooled. Always load and unload by holding the plastic sides.

Preparing Collection Tubes and Plates

Prepare the collection tube device for collecting droplets containing target cells for sorting.

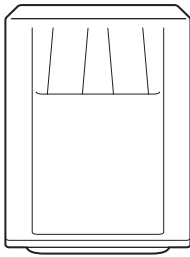
The instrument supports the following collection tube devices. Each type of collection tube is used with a corresponding holder that mounts on the collection stage.

Collection tubes/ multi-well plates	Holder
15 ml conical tubes	Collection tube holder (15 ml)
5 ml round tubes	Collection tube holder (5 ml)
6-well plates	96/384-well plate holder
12-well plates	
24-well plates	
48-well plates	
96-well plates	
8-well PCR strips	96-well PCR plate holder ¹⁾
96-well PCR plates	
384-well plates	96/384-well plate holder
384-well PCR plates	96/384-well plate holder + 384-well PCR adapter

1) Use an Eppendorf® PCR cooler (cold storage system for 96-well plates and PCR tubes).

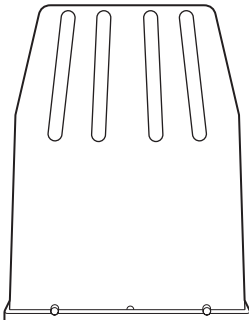
Common to all models

- Collection tube holder (15 ml)



If sorting using collection tubes on the LE-MA900P model, see “Removing and Attaching the Plate Holder Adapter (LE-MA900P)” (page 38).

- Collection tube holder (5 ml)



LE-MA900P

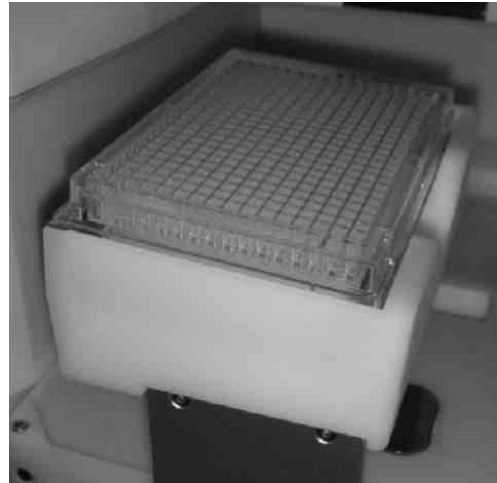
- 96/384-well plate holder (with 96-well plate)



- 96-well PCR plate holder (with 96-well PCR plate)



- 96/384-well plate holder (with 384-well plate)



For details about loading, see “Loading a Multi-well Plate (LE-MA900P)” (page 38).

- 96/384-well plate holder (with 384-well PCR adapter and 384-well PCR plate)



For details about loading, see “Loading a 384-well PCR Plate (LE-MA900P)” (page 38).

When sorting into a 96/384-well plate or 384-well PCR plate, always adjust the sort position before starting as described in “Index Sorting” (page 114).

DANGER

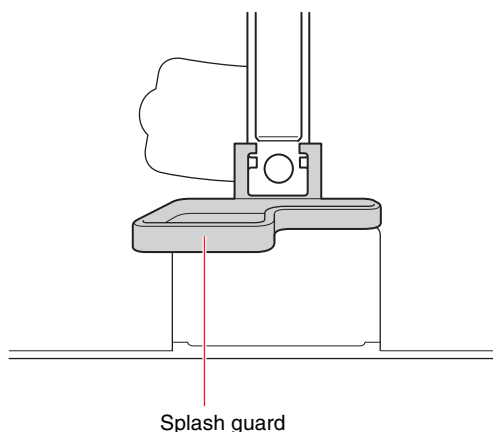
Fluids may contain biological, chemical, or other agents. When handling collection tubes, always wear laboratory-use gloves, mask, protective goggles, and other protective clothing.

Note

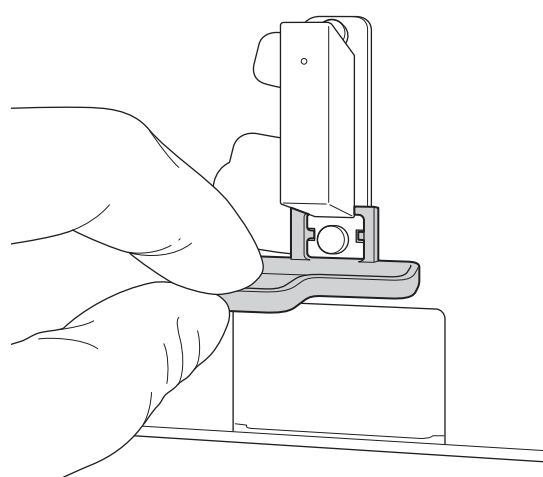
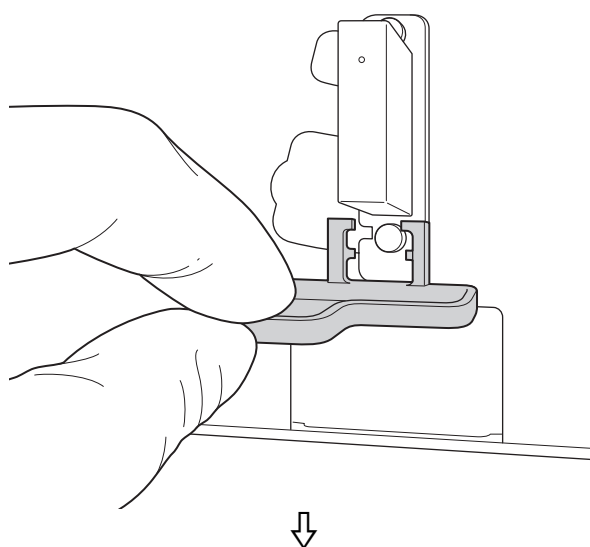
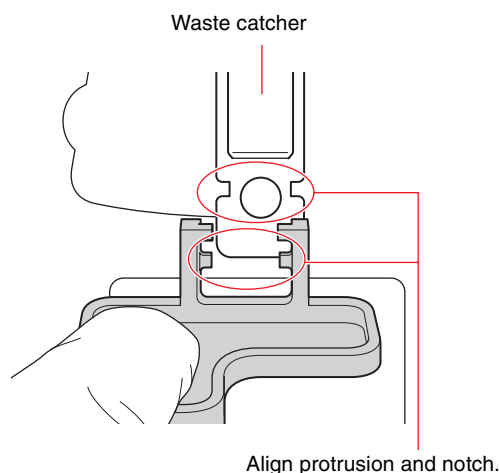
Before using a tube, check that there is no dust or other foreign matter in the tube. Never use cracked or damaged tubes.

Attaching the Splash Guard (for use with multi-well plates) (LE-MA900P)

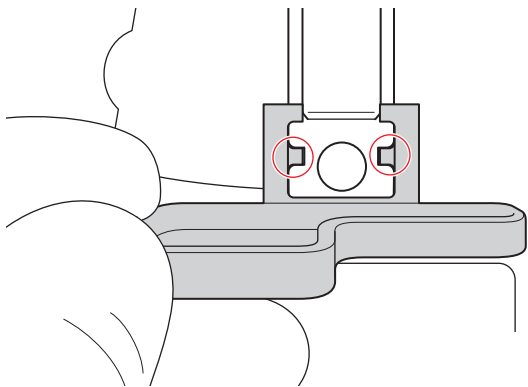
The splash guard can be attached (optional) if splashing of the stream is observed while sorting cells into multi-well plates.



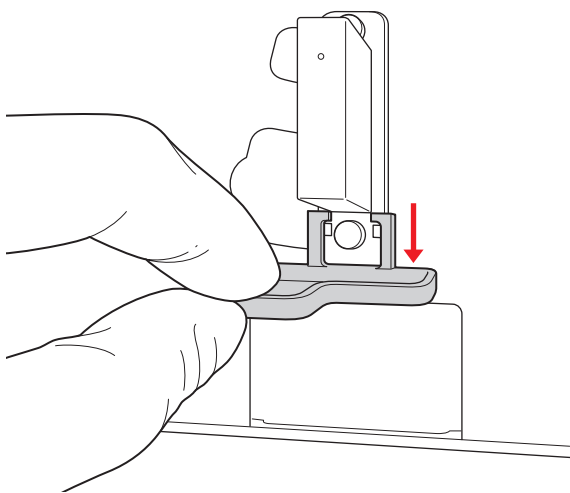
- 1 Hold the splash guard in the orientation shown in the diagram, and insert the protrusion on the splash guard into the notch on the waste catcher.



Insert the protrusions on both sides.



- 2** Push the splash guard in until it sits clicks into position.



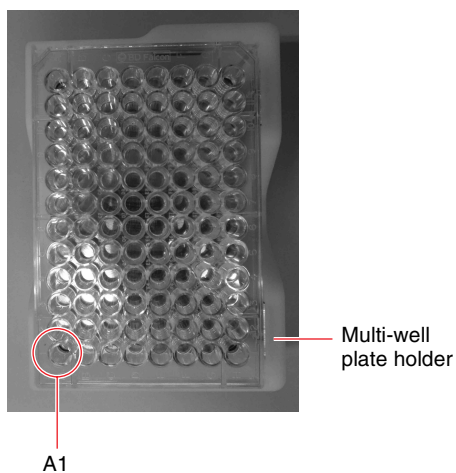
Tip

Remove the splash guard when unloading multi-well plates after sorting is finished.

Loading a Multi-well Plate (LE-MA900P)

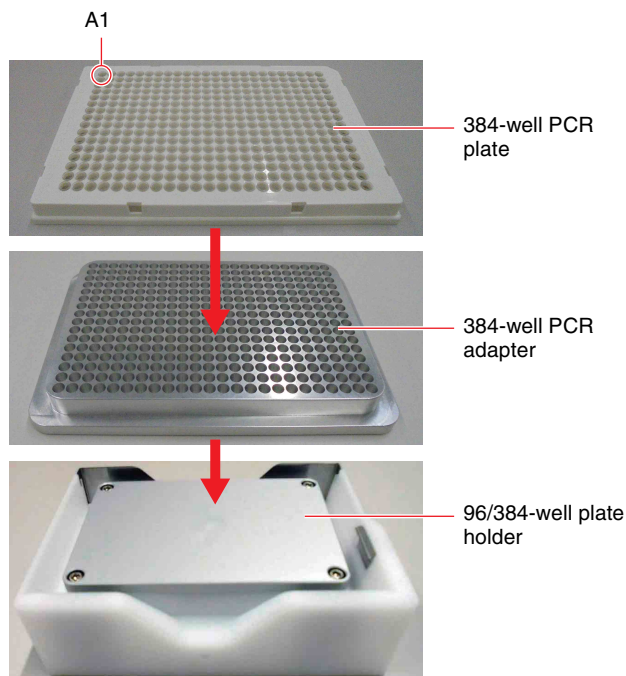
Place the multi-well plate into the plate holder with the A1 well at the front left of the instrument.

Rear of instrument



Loading a 384-well PCR Plate (LE-MA900P)

Place the 96/384-well PCR adapter in the 384-well plate holder, and place a 384-well PCR plate on the adapter. Place the 384-well PCR plate into the adapter with the A1 well at the front left of the instrument.



Note

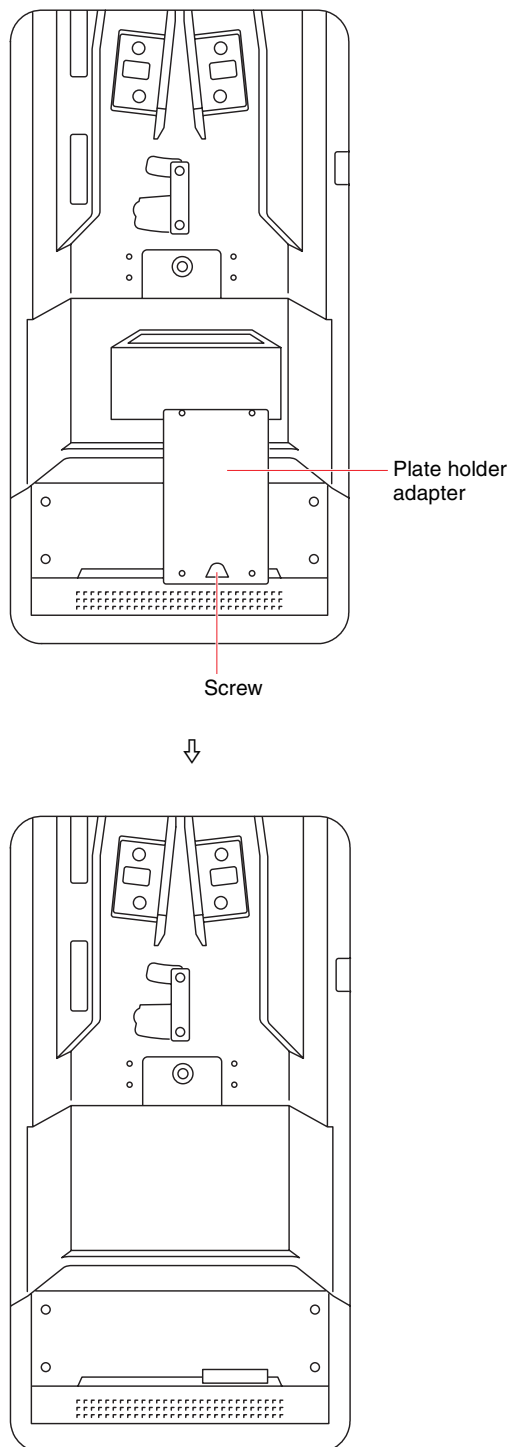
Always use a 384-well PCR plate holder and 96/384-well PCR adapter when using 384-well PCR plates. Do not use a 96-well PCR plate holder.

Removing and Attaching the Plate Holder Adapter (LE-MA900P)

When sorting using collection tubes, the plate holder adapter must first be removed.

Remove the screw securing the adapter using a coin or screwdriver, and remove the adapter.

For multi-well plate sorting, re-attach the adapter.



Configuring Users

This section describes how to manage user accounts in Cell Sorter Software.

For details about starting the LE-MA900 instrument and Cell Sorter Software, see “System Startup” (page 44) and “Logging In” (page 45).

About the administrator account

The administrator account user name is set to “administrator” by default. The administrator account cannot be used to operate the LE-MA900.

If the administrator account password has not been configured, the change password screen appears for the administrator account when launching Cell Sorter for the first time.

Specify each item and click [OK] to set the password.

Set Password dialog box showing fields for Username (administrator), New Password, and Confirm Password. OK and Cancel buttons are at the bottom.

[New Password]: Enter a new password, comprising 8 to 20 alphanumeric characters (required item).

[Confirm Password]: Enter the same password again for confirmation (required item).

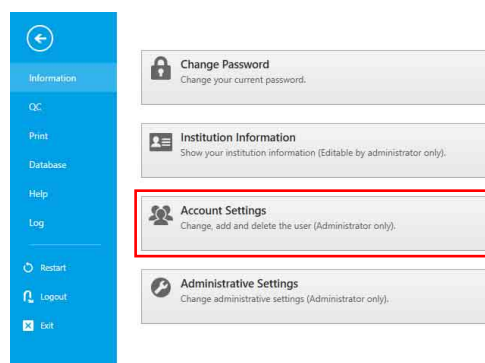
Adding Users

Only administrators can add new users.

Tip

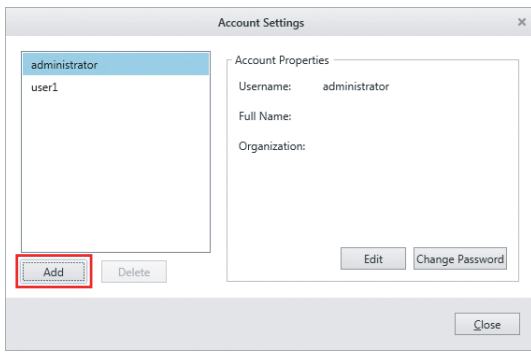
It is the responsibility of the administrator to manage the administrator account password.

- 1 Click the [File] tab, then click [Information] in the menu on the left.
- 2 Click [Account Settings].



The [Account Settings] dialog appears.

3 Click [Add].



The [Add Account] dialog appears.

4 Set each field, then click [OK].



[Username]: Enter the user name, comprising up to 32 alphanumeric characters, used when logging in to Cell Sorter Software (required item).

[Password]: Enter the password, comprising a combination of 8 to 20 alphanumeric characters, used when logging in to Cell Sorter Software (required item).

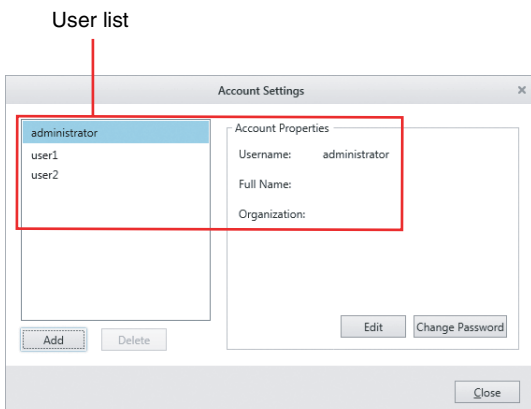
[Confirm Password]: Enter the same password again for confirmation (required item).

[Full Name]: Enter the full name of the user.

[Organization]: Enter the name of the organization.

[Accessibility]: Place a check mark in the checkbox if you want to temporarily disable a general user account.

The user is registered in the user list in the [Account Settings] dialog.



Editing User Settings

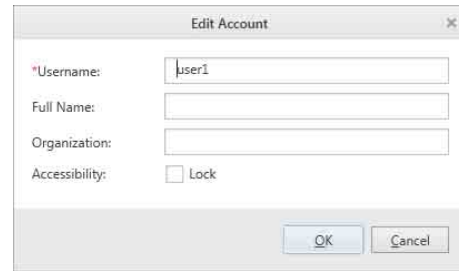
Only administrators can edit user settings.

1 Select the user you want to edit in the [Account Settings] dialog, then click [Edit].

Selecting a user in the list displays the settings for the user in the area on the right.

The [Edit Account] dialog appears.

2 Edit the settings, then click [OK].



For details, see step 4 (page 40) in “Adding Users”.

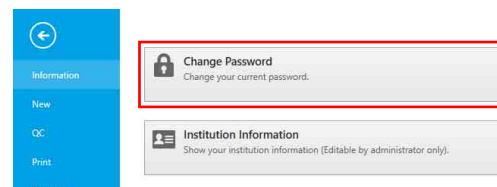
The account settings are updated.

Changing Passwords

Individual users can change their password used to log in to Cell Sorter Software.

1 Click the [File] tab, then click [Information] in the menu on the left.

2 Click [Change Password].



The [Change Password] dialog appears.

3 Set each field, then click [OK].



[Old Password]: Enter the current password (required item).

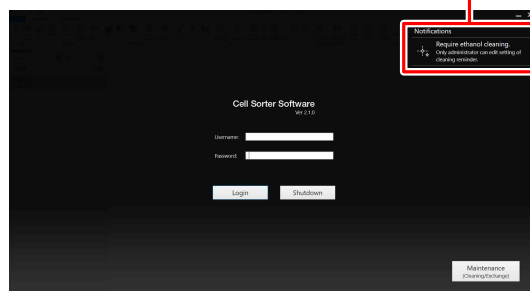
[New Password]: Enter a new password, comprising a combination of 8 to 20 alphanumeric characters (required item).
[Confirm Password]: Enter the same password again for confirmation (required item).

The password is updated.

Setting a Cleaning Reminder

The instrument must be cleaned periodically using ethanol to control contamination and side stream instability resulting from poor system maintenance. You can set to display a message (cleaning reminder) on the login screen after a certain preset time has elapsed without performing ethanol cleaning.

Cleaning reminder



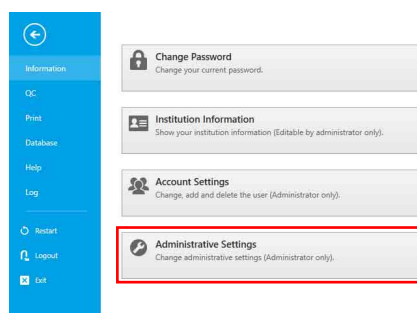
Tip

You can click the cleaning reminder to display the [Cleaning Reminder] screen and then start ethanol cleaning.

For details, see “When a Cleaning Reminder is Displayed” (page 195).

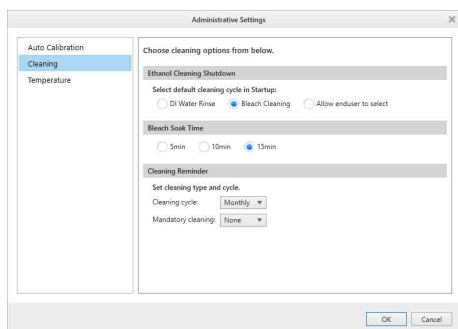
Use the following procedure to set the cleaning interval for sending cleaning reminder notifications. Only administrators can set the cleaning interval.

- 1** Click the [File] tab, then click [Information] in the menu on the left.
- 2** Click [Administrative Settings].



The [Administrative Settings] dialog appears.

- 3 Select [Cleaning], set each item in [Cleaning Reminder], and then click [OK].



[Cleaning cycle]: Select the ethanol cleaning interval.

- None (no notifications)
- Daily
- Weekly
- Monthly (default value)

[Mandatory cleaning]: Select the mandatory ethanol cleaning interval.

If the interval specified here is exceeded, login is disabled until ethanol cleaning has been performed.

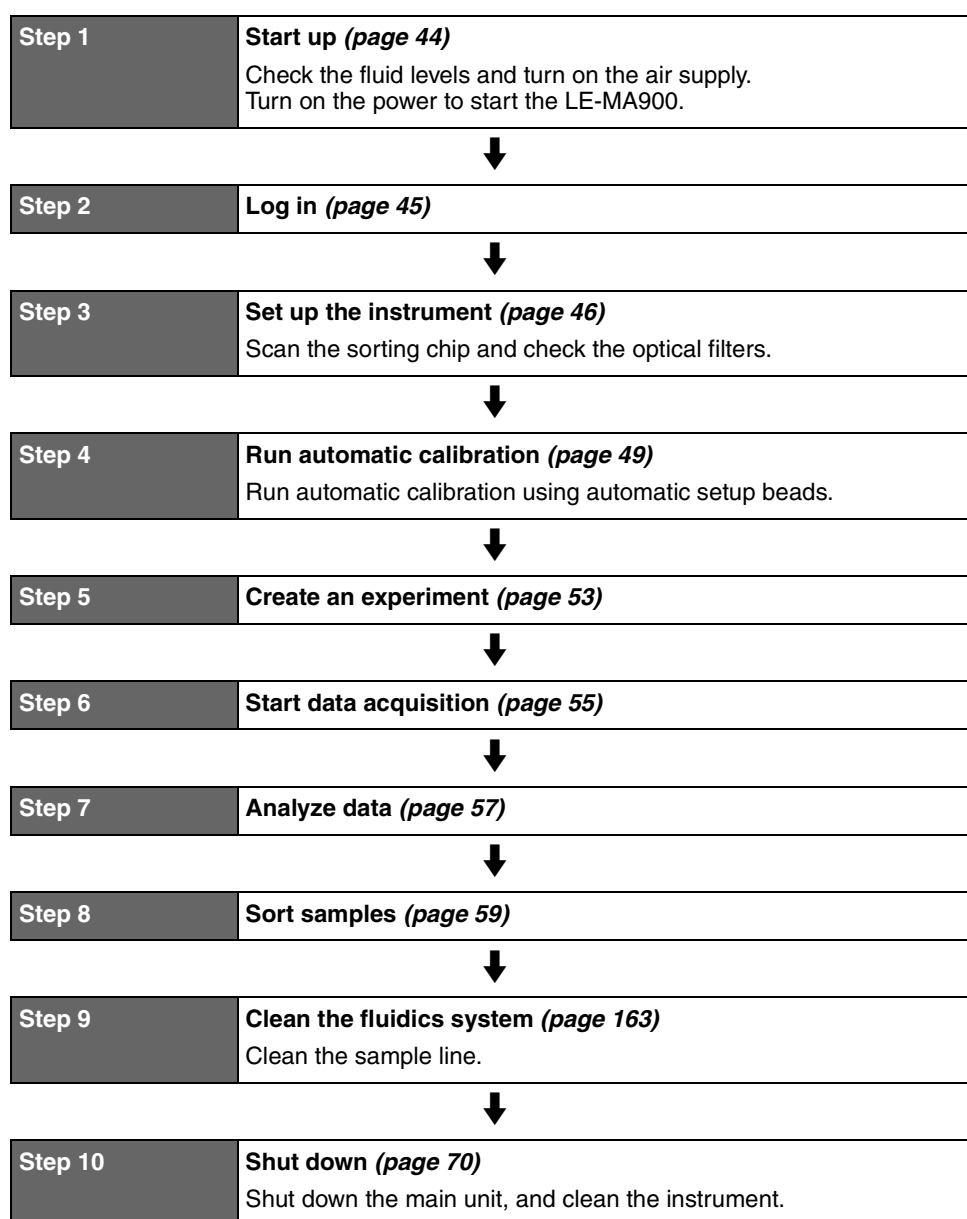
- None (no notifications) (default value)
- 1 week
- 2 weeks
- 1 month (30 days)

The intervals chosen for ethanol cleaning determine when the notification for cleaning is displayed.

You can check the cleaning history on the [Ethanol Cleaning Log] screen. For details, see “Ethanol cleaning log” (page 129).

This chapter describes the basic operating procedure for running a simple experiment, acquiring and analyzing data, and sorting selected populations in the sample.

Workflow



System Startup

Turn on the power supply and start the LE-MA900 using the following procedure.

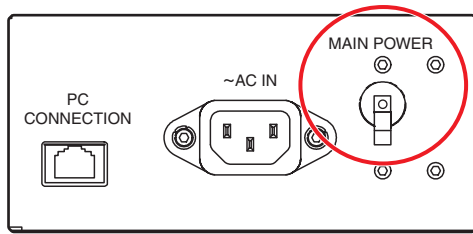
Note

Do not open the flip-up door at any time before turning on the compressed air supply. Doing so may damage the unit.

- 1 Check that the compressed air supply is connected and is supplying the rated pressure.
- 2 Check the level in the tanks on the fluidics cart, and refill or empty the tanks as required.

Also, check that the fluidics lines, sheath air line, and cart connection cable are connected correctly.

- 3 Turn on the MAIN POWER switch on the rear panel of the main unit.

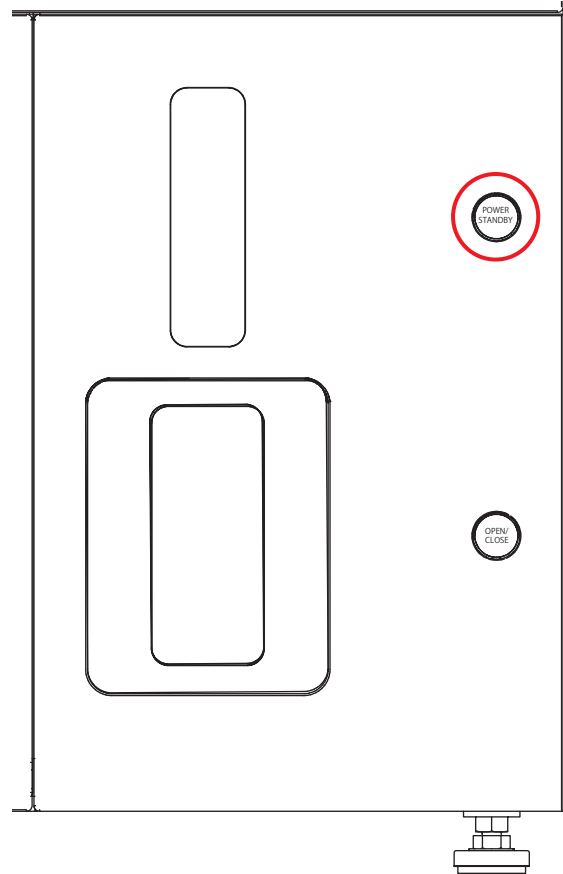


Note

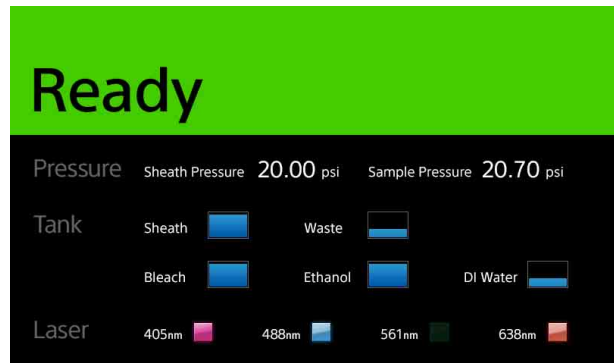
During normal operation, the MAIN POWER switch can be left turned on all the time. Use the POWER/STANDBY button to turn power on/off on a daily basis.

- 4 Turn on the power using the POWER/STANDBY button on the front panel of the main unit.

The system goes through an initialization process during which self-diagnostics are run to test the proper working condition of all subsystems.



The LE-MA900 status appears on the LCD monitor.



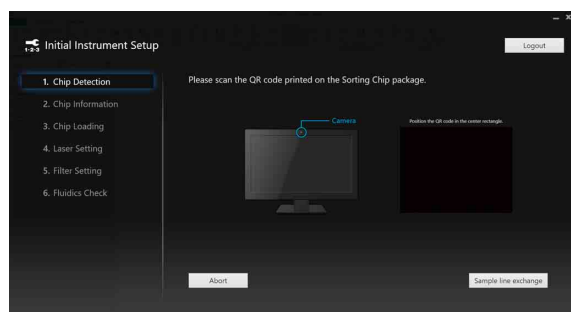
Logging In

Turn on the PC, start Cell Sorter Software, and log in to the system.

- 1 Check that “Standby” status is displayed on the LCD monitor.
- 2 Select Cell Sorter Software on the Windows Start screen to start the software.
The login window appears.
- 3 Enter your user name and password, then click [Login].



If login is successful, the [Initial Instrument Setup] wizard appears.



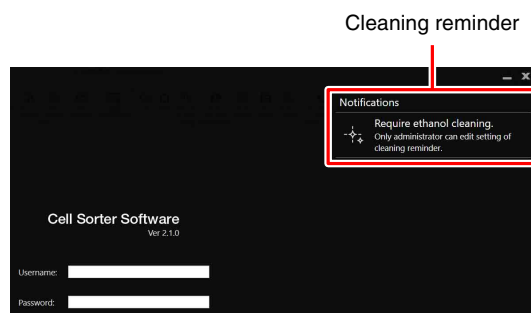
Tip

To change the sample line before initial instrument setup, click [Sample line exchange] and follow the instructions in the on-screen wizard.

For details, see “Changing the PEEK Sample Line” (page 175).

If periodic ethanol cleaning has not been performed

A cleaning reminder notification appears at the top right of the screen. Click the cleaning reminder to display the [Cleaning Reminder] screen and then start ethanol cleaning.



For details, see “When a Cleaning Reminder is Displayed” (page 195).

Configuring the Instrument

Load a sorting chip, select the lasers to be used, and check the optical filters by following the on-screen instructions on the [Initial Instrument Setup] screen.

Using the Sorting Chip

- 1 Hold the QR code on the sorting chip packaging in front of the camera on the host computer.

QR code



The camera automatically scans the QR code.

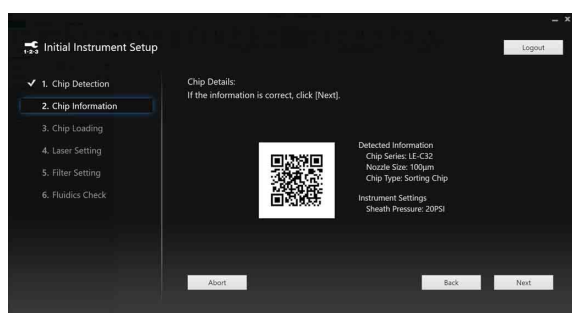
Tip

You can click [Abort] to bypass instrument configuration and proceed directly to the main window. In this mode, Cell Sorter Software can be used only for analysis of existing recorded data in experiments.

When loading a sorting chip, the QR code on the chip packaging must be scanned using the built-in camera on the host computer to uniquely identify the chip and register the serial number and nozzle size of the chip with the LE-MA900. This function maintains a history for the chip in order to ensure optimum performance.

When the QR code is successfully scanned, the product number and nozzle size are displayed, together with basic instrument settings. The serial number for the chip is registered internally.

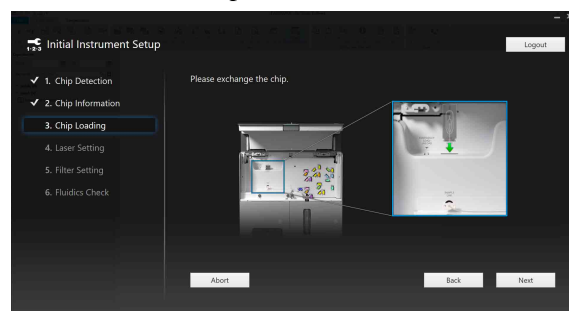
- 2 Check that the displayed information is correct, then click [Next].



When [Next] is clicked, the currently loaded sorting chip is ejected from the chip loader.

Tip

If you wish to load a different chip, click [Back] to return to the previous screen, then scan the QR code for the different chip.



- 3 Open the flip-up door.
- 4 Remove the current sorting chip, if one is present, and insert the new chip into the chip slot at the top of the chip loader.



Insert the chip into the slot with the Sony logo and nozzle size mark at the top facing toward you. Take care not to insert the sorting chip facing backwards.

Place the previous chip in its original packaging. The chip and its corresponding QR code should be kept together at all times so that they are not mixed up with other chips.

Notes

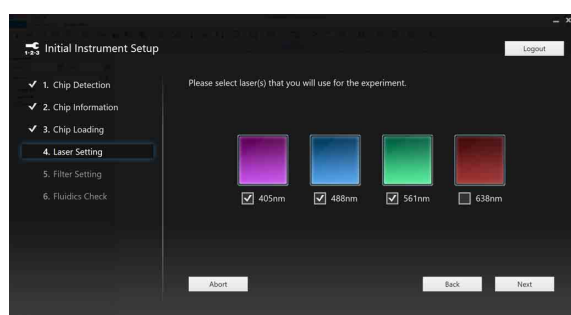
- An error message appears if the sorting chip is inserted in the incorrect orientation.
- Do not insert any objects other than Sony sorting and cleaning chips designed for the LE-MA900 into the chip insertion slot.
- The packaging for the loaded sorting chip should be stored in a safe location.
- Replace the sorting chip if it is discolored, dirty, or stained from previous use.
- Do not drop the sorting chip on a hard surface or bend the chip intentionally. If there is a problem with the sorting chip, contact your Sony distributor.

- Always wear gloves and other protective clothing, mask, and goggles, when changing the sorting chip.
- If the sorting chip will not load correctly, run the instrument in maintenance mode as described in “Running Maintenance Mode” (page 192). If the problem persists, contact your Sony distributor for service.
- Replace the sorting chip if it is used for extended periods or after the usage period expires, and when it becomes clogged, discolored, or stained.

5 Click [Next].

The sorting chip is loaded and the [Laser Setting] screen appears.

6 Select the excitation lasers to be used for the experiment, then click [Next].



The excitation spectra of the fluorochrome labels you wish to detect largely determine the lasers needed for an experiment.

For a list of fluorochromes versus excitation laser wavelength for each model, see “Fluorochrome Detection Matrix” (page 218).

Tips

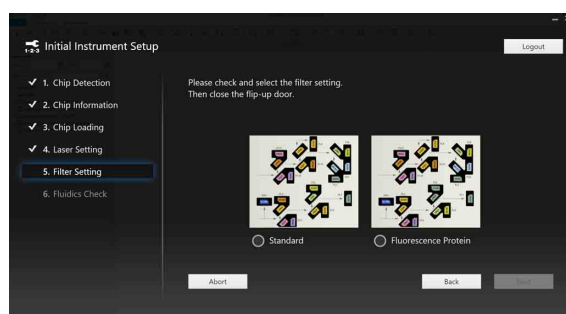
- The 488 nm excitation laser is used to measure forward scatter and back scatter light.
- It is recommended that lasers not required for an experiment be deselected.

The [Filter Setting] screen appears.

7 Select the optical filter pattern setting, insert all the optical filters in the slots specified in “Fluorochrome Detection Matrix” (page 218) and close the flip-up door, then click [Next].

The number of optical filters varies depending on the lasers installed in the instrument.

Example: When four lasers are installed



The image displayed shows the optical filter pattern to be installed.

Notes

- Make sure that the correct filter is inserted in each slot for the recommended filter pattern. Correct data cannot be obtained if a filter is inserted in the wrong slot.
- Exercise extreme care when handling the optical filters. The surfaces of each optical filter must be free of dust, smudges, fingerprints, and scratches for accurate data acquisition and cell sorting operation.

For details about handling optical filters, see “Cleaning and Handling the Optical Filters” (page 202).

RESERVE slots

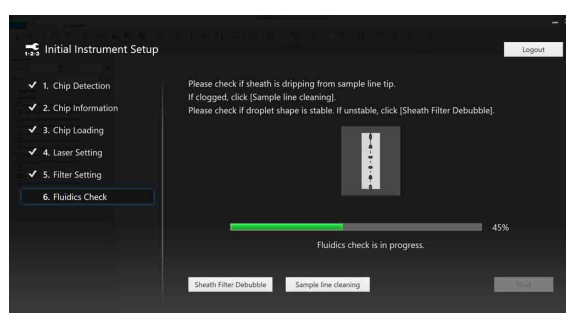
On the following models, dummy filters are installed in the RESERVE slots.

LE-MA900B, LE-MA900H, LE-MA900BP, LE-MA900HP

For details about filter patterns, see “Optical Filter Patterns” (page 216).

The [Fluidics Check] screen appears.

8 Click [Next].



When fluidics check starts, sheath fluid droplets appear from the tip of the sample probe. If droplets do not appear, the sample line or the sample probe may be clogged. Click [Sample line cleaning] on the bottom right of the screen to clean the sample line.

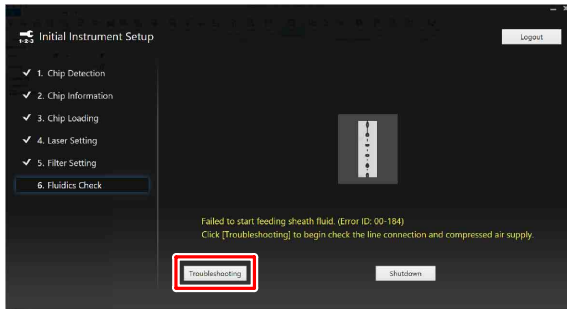
During the fluidics check, the sorting chip de-bubble function is run periodically, making the droplets

appear unsettled. If droplet formation is unstable, even when the sorting chip is de-bubbled, air may have accumulated in the sheath filter. Click [Sheath Filter Debubble] at the bottom of the screen to de-bubble the sheath filter.

When the fluidics check is completed, the [Auto Calibration] screen appears.

If fluid flow does not start

[Troubleshooting] is displayed at the bottom left of the screen. Click [Troubleshooting] to launch the troubleshooting wizard. Follow the on-screen instructions to resolve the issue.



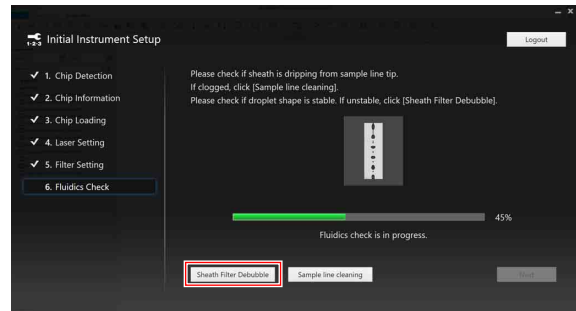
Tip

If you click the [Shutdown] button, the LE-MA900 will shut down.

De-bubbling the Sheath Filter

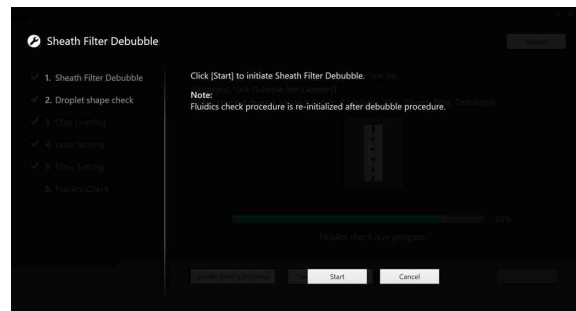
You can de-bubble the sheath filter before running automatic calibration.

- 1 Click [Sheath Filter Debubble] on the [Fluidics Check] screen of the [Initial Instrument Setup] wizard.



The [Sheath Filter Debubble] wizard appears.

- 2 Click [Start] to the sheath filter de-bubbling wizard.



- 3 Click [Start] to begin de-bubbling the sheath filter. When de-bubble is completed, click [Next].
- 4 Check that the droplet formation displayed on the screen is stable, then click [Next].
- If unstable, click [Debubble again] and repeat step 3.
- 5 Click [OK] to exit the [Sheath Filter Debubble] wizard.

Tip

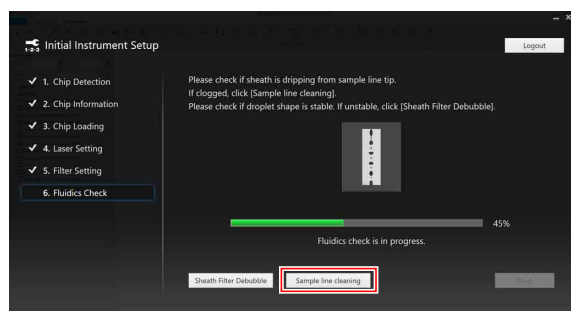
Running the sheath filter de-bubble function initializes the fluidics check in the Initial Instrument Setup.

When the [Sheath Filter Debubble] wizard finishes, the [Fluidics Check] screen appears. When the fluidics check is completed, the [Auto Calibration] screen appears.

Cleaning the Sample Line

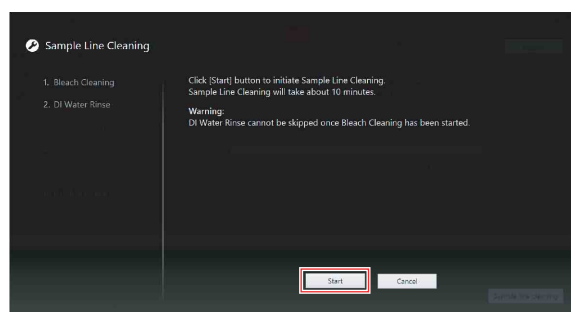
You can clean the sample line before running automatic calibration.

- 1 Click [Sample line cleaning] on the [Fluidics Check] screen of the [Initial Instrument Setup] wizard.



The [Sample Line Cleaning] wizard appears.

- 2 Click [Start] to begin cleaning the sample line.



Follow the on-screen instructions.

In sample line cleaning, the fluidics system is cleaned using a sodium hypochlorite solution (bleach cleaning) and DI water, in that order.

Tips

- You can skip each cleaning step by clicking [Skip], but cleaning with DI water after cleaning with bleach cannot be skipped.
- You can repeat the cleaning steps by clicking [Cleaning again].

For details about cleaning using Sodium Hypochlorite solution, see “Cleaning Sample Line using Bleach” (page 174).

When sample line cleaning finishes, the [Fluidics Check] screen appears. When the fluidics check is completed, the [Auto Calibration] screen appears.

Running Automatic Calibration

Follow the on-screen instructions on the [Auto Calibration] screen to perform the following adjustments.

- Sorting chip alignment
- Laser delay adjustment
- Droplet stream calibration
- Side stream calibration
- Sort delay calibration

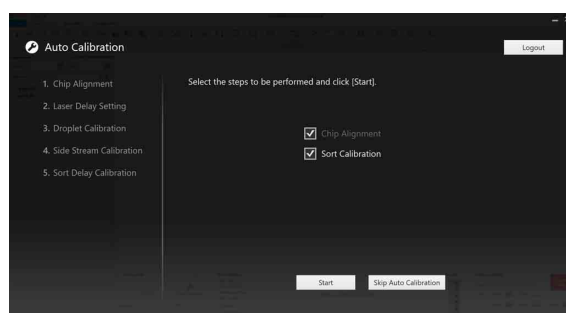
Note

If the LE-MA900 is installed in a Biosafety Cabinet, wait 15 minutes after fluidics check before running auto calibration to allow the temperature within the cabinet to stabilize.

Tips

- You can skip corresponding adjustments if not running sorting.
- You can click [Skip Auto Calibration] to bypass automatic calibration and proceed directly to the Cell Sorter Software main window. Bypassing automatic calibration is useful for quickly accessing the main window to perform analysis of existing data. Do not bypass automatic calibration if you plan to run new samples.
- You can align the sorting chip, calibrate the sorting system, and run QC later using [Chip Alignment], [Laser Delay], [Sort Calibration], and [Daily QC] on the [Cytometer] tab of the ribbon from the main window.

- 1 Select the steps to be performed during Auto Calibration.



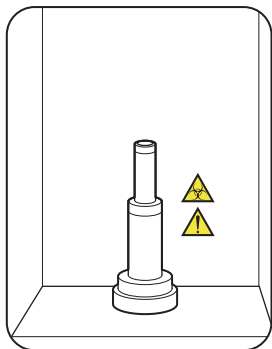
Note

If performing analysis only (without sorting samples), you can clear the [Sort Calibration] checkbox and click [OK] to skip droplet stream calibration, side stream calibration, and sort delay calibration. In this case, main window is immediately displayed when sorting chip alignment finishes.

- 2 Insert 1 ml or more of undiluted, automatic setup beads in a sample tube, and place the tube in the corresponding sample tube holder.

For details about sample tubes, sample tube holders, and automatic setup bead preparation, see “Preparing a Sample” (page 34).

- 3 Place the sample tube holder containing the sample tubes in the sample loader.



Align the D-shaped notch on the bottom of the sample tube holder with the slot in the top of the agitation unit when loading the sample tube holder.

Notes

- Always wear laboratory-use gloves, mask, protective goggles, and other protective clothing when handling sample fluid to provide protection against biohazards.
- Also, take care when loading the sample tube in the tube holder to avoid sample fluid spills and damage to the sample tube.
- Before using a tube, check that there is no dust or other foreign matter in the tube.
- Do not subject the instrument to vibration during operation. Shock or vibration may adversely affect alignment and calibration.

- 4 Press the sample loader door button to close the sample loader door.

Tip

Even if you leave the door open, the sample loader door will close automatically when you click the [OK] button in the next step.

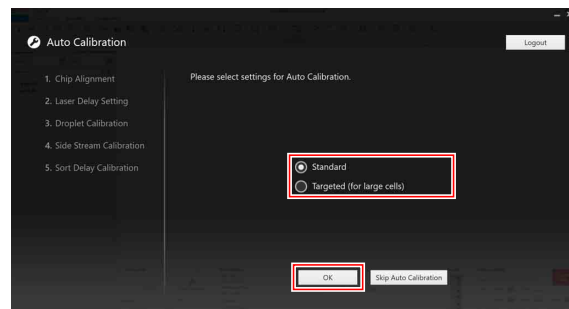
- 5 Click [OK] on the [Auto Calibration] screen.

Using 100 μ m sorting chips

A screen for selecting auto calibration settings appears.

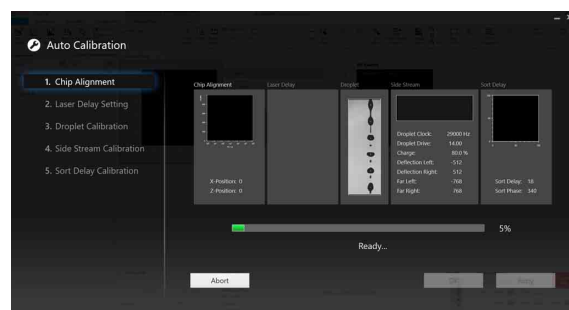
Select [Standard] or [Targeted], and click [OK].

“Targeted” is a setting suitable for sorting of large cells.



The automatic calibration sequence starts.

A progress bar displays the status at each stage of the calibration process.



As automatic calibration proceeds, a histogram for the automatic setup beads, the droplet camera image and the side stream monitor image are displayed.

Tip

During automatic calibration, you can click [Abort] to return to the previous screen.

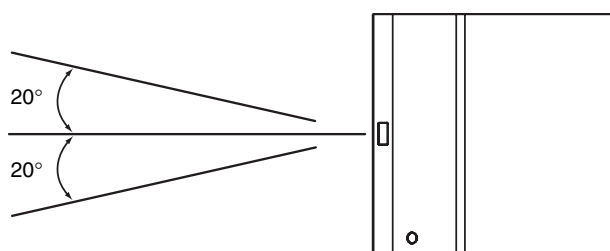
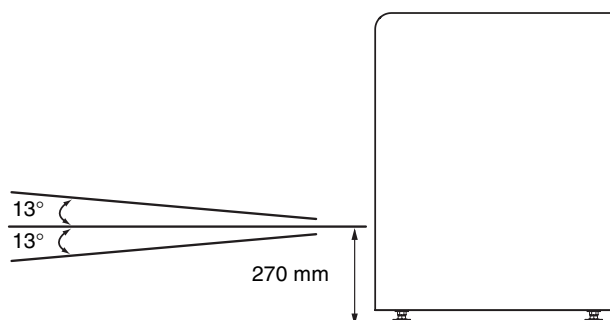
When automatic calibration finishes, [Abort] is disabled, and [Retry] and [OK] are enabled. Clicking [Retry] restarts the automatic calibration procedure.

- 6 When calibration finishes, click [OK].

The [Create Experiment] window appears for creating a new experiment.

Note

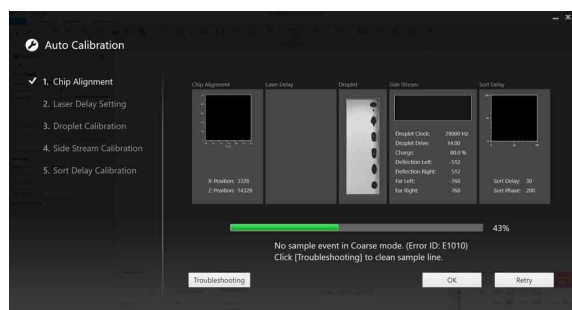
During automatic calibration, precise measurements are done using the side stream monitor. Accordingly, do not place any bright light sources (halogen lamps, for example) within the following angular ranges. Doing so may cause automatic calibration to malfunction.

Top view**Side view****If an error occurs during auto calibration**

If an error occurs during sorting chip alignment or side stream calibration, [Troubleshooting] appears at the bottom left of the screen.

Click [Troubleshooting] to launch the troubleshooting wizard. Follow the on-screen instructions to resolve the issue.

If [Troubleshooting] is not displayed when an error occurs, see “*Error Messages*” (page 229).



Running QC

You can run Daily QC and Performance QC to check the instrument status.

- **Daily QC**

Checks the chip alignment and the optical characteristics automatically using automatic setup beads.

- **Performance QC**

Checks the fluorescence detection characteristics and detector sensitivity using 8-peak beads. Run checks once a month.

Note

Any fluorescent dye that separates from the automatic setup beads may affect the results of Performance QC. Before running Performance QC, run [Bleach Cleaning] and [DI Rinse] to clean the sample line.

Tip

The QC results can be exported to a CSV file to monitor the instrument performance over time.

See “[QC] Window” (page 125).

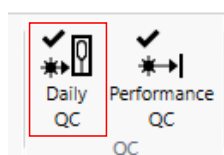
Running Daily QC

Daily QC measures the rCV, Area, and Height parameters of the QC target channel using automatic setup beads.

Tip

Daily QC cannot be run when using the fluorescent protein filter pattern.

- 1 Click [Daily QC] on the [Cytometer] tab.



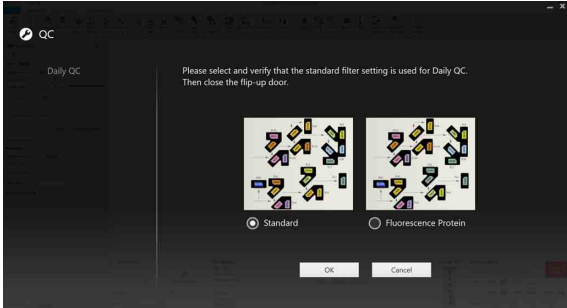
The [Daily QC Wizard] window appears.

- 2 Add 0.5 ml or more of undiluted, automatic setup beads in a sample tube, and place the tube in the corresponding sample tube holder.
- 3 Place the sample tube holder containing the sample tubes in the sample loader.
- 4 Press the sample loader door button to close the sample loader door.

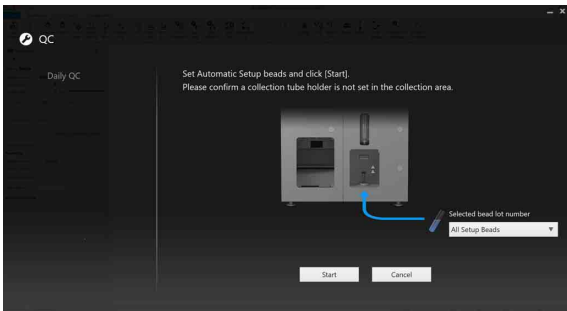
Tip

Even if you leave the door open, the sample loader door will close automatically when you click the [OK] button in the next step.

- 5 Check that the optical filter pattern is a standard pattern, then select the optical filter pattern setting, and click [OK].



- 6 Select [All Setup Beads] in [Selected bead lot number], and click [Start].



The results are displayed when Daily QC is completed.

**Tip**

After Daily QC is completed, return to the fluorescent protein filter pattern if you are acquiring data using the fluorescent protein filter pattern.

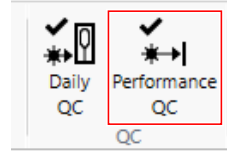
Running Performance QC

Performance QC measures the Linearity and Separation Index(S) parameters of the QC target channel using 8-peak beads.

Tips

- Store 8-peaks beads in a cool location, and use immediately after preparation.
- Shake the 8-peak beads bottle vigorously before use to obtain a uniform bead concentration.
- Run Performance QC once a month.
- Performance QC cannot be run when using the fluorescent protein filter pattern.

- 1 Click [Performance QC] on the [Cytometer] tab.



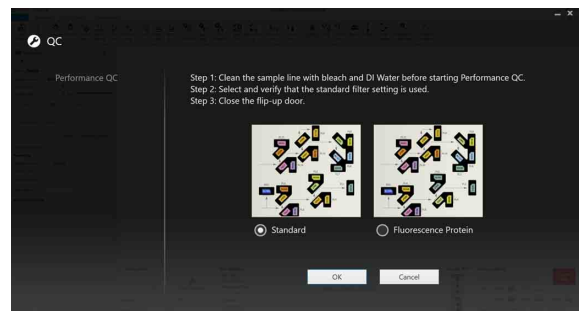
The [Performance QC Wizard] window appears.

- 2 Add 4 drops of 8-peak beads in a sample tube and mix with 1 ml of DI water or PBS (saline) solution, then place the tube in the sample holder.
- 3 Place the sample tube holder containing the sample tubes in the sample loader.
- 4 Press the sample loader door button to close the sample loader door.

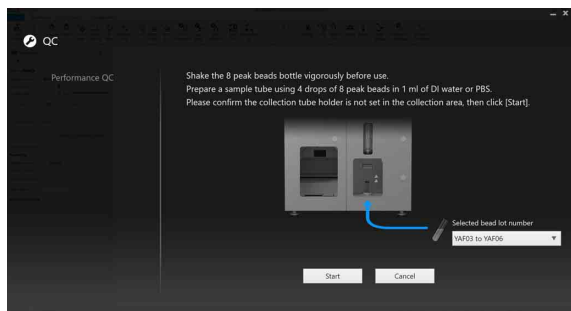
Tip

Even if you leave the door open, the sample loader door will close automatically when you click the [OK] button in the next step.

- 5 Check that the optical filter pattern is the standard pattern, then select the optical filter pattern setting, and click [OK].



- 6 Select the 8-peak beads lot number in [Selected bead lot number], and click [Start].



The results are displayed when Performance QC is completed.



Tip

After Performance QC is completed, return to the fluorescent protein filter pattern if you are acquiring data using the fluorescent protein filter pattern.

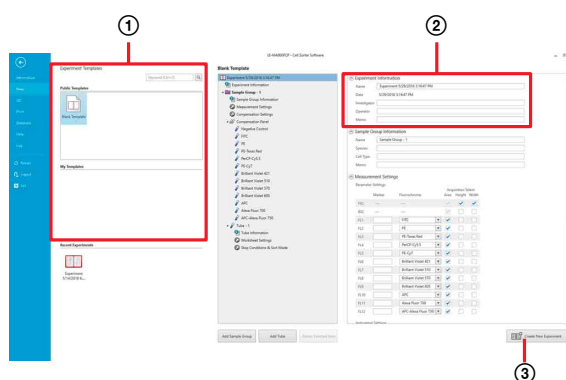
Creating an Experiment

When calibration finishes, you create an experiment that is used to acquire data from samples in the [Create Experiment] window.

Creating a New Experiment

This section describes how to create a new experiment using existing templates and/or recent experiments.

- 1 If the [Create Experiment] window is not displayed, click [New] in the [File] tab of the ribbon.
- 2 Create an experiment.



- ① Select a template.
Clicking a template or an experiment on the left displays the structure of the selected experiment on the right side of the window.

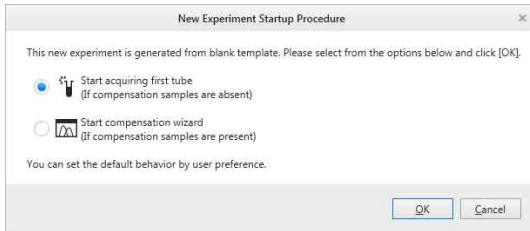
For details about changing the structure of an experiment, see “Creating a Customized Experiment” (page 78).

- ② Enter a name for the experiment in [Name].
You can configure sample groups, sample tubes, pulse parameters, and other settings for data acquisition.

For details about each item, see “[Create Experiment] Window” (page 130).

- ③ Click [Create New Experiment].
If Blank Template was selected, the [New Experiment Startup Procedure] dialog appears. Proceed to step 3.
If an existing template or experiment was selected, the main window appears. See “Acquiring Data” (page 55).

- 3 Select the action to perform, then click [OK].



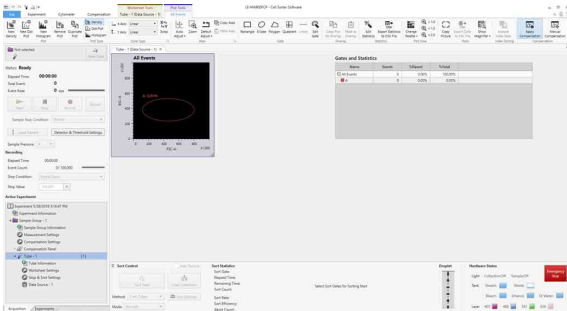
[Start acquiring first tube]: Displays the main window and assigns the first tube in preparation for data acquisition.

[Start compensation wizard]: Displays the [Compensation Wizard] to perform fluorescence compensation in preparation for measurement.

For details about the [Compensation Wizard], see “Compensating Fluorescence Spillover” (page 66).

The experiment is created, and the main window appears.

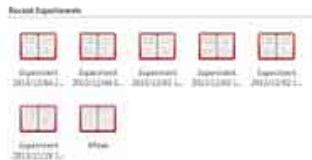
The created experiment is automatically made the active experiment, and the first tube is automatically assigned as the active tube.



To create an experiment using an existing experiment

- 1 In step 2-① of the procedure above, select an experiment you want to use from [Recent Experiments] instead of a template.

[Recent Experiments] displays the most recently used experiments.



- 2 Check the experiment details, and enter a name for the experiment in [Name] under [Experiment Information].
- 3 Click [Create New Experiment].

The experiment is created, and the main window appears.

To save an experiment as a template

You can save an existing experiment as a shared template (Public Templates) or a private template (My Templates).

For details, see “Saving an Experiment as a Template” (page 87).

Acquiring Data

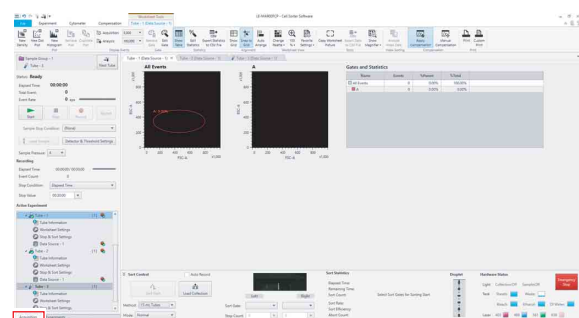
After an experiment is created, you can acquire and record data for samples in the experiment from the main window. Recorded data is displayed in real-time, and can also be exported for analysis at a later time.

Before starting acquisition

Check the following items.

- Check the fluid levels in each of the tanks
- Check that the main unit and fluidics cart are connected correctly.

You control data acquisition and recording in the [Acquisition] tab of the Experiment/Acquisition control pane.



[Acquisition] tab

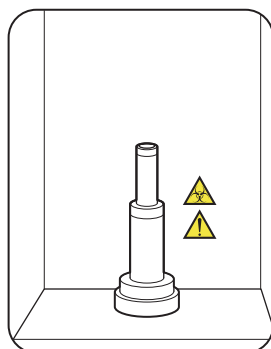
- 1 Press the sample loader door button to open the sample loader door.

- 2 Insert sample fluid in a sample tube, and place the tube in the corresponding sample tube holder.

For details about sample tubes and sample tube holders, see “Preparing a Sample” (page 34).


- 3 Place the sample tube holder in the sample loader.

If the sample loader door is closed, press the sample loader door button to open the sample loader door.

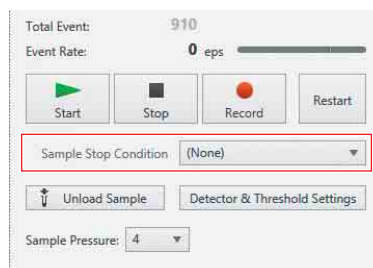


- 4 Press the sample loader door button to close the sample loader door.

Tip

The sample loader door will close automatically, even if you leave the door open, when you click  (Start) in step 8 to start acquisition.

- 5 Specify the measurement stop condition in [Sample Stop Condition].



The following options are available.

- None (data acquisition will stop automatically when the number of detected events exceeds 345,600,000 or the elapsed time exceeds 12:00:00, whichever occurs first)
- Recording (stops data acquisition automatically when the recording stop condition is satisfied)
- Sorting (stops data acquisition automatically when the sorting stop condition is satisfied)
- Recording and Sorting (stops data acquisition automatically when both the recording and sorting stop conditions are satisfied)

Tip

[Recording] cannot be selected for index sorting. For details about index sorting, see “Index Sorting” (page 114).

For details, see “Configuring Detector Settings” (page 87).

- 6 Configure the detector settings.

Adjust the gain, threshold, and other settings.

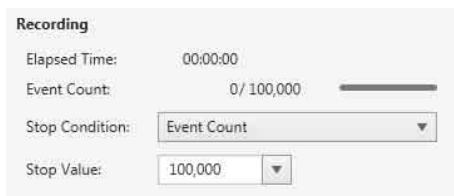
For details, see “Configuring Detector Settings” (page 87).

- 7 Specify the recording stop condition under [Recording], if a recording stop condition was selected above.

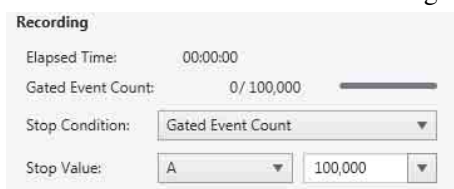
The following options are available.

- None
- Elapsed Time (total elapsed time)
- Event Count (total number of events)
- Gated Event Count (number of events for the specified gate)

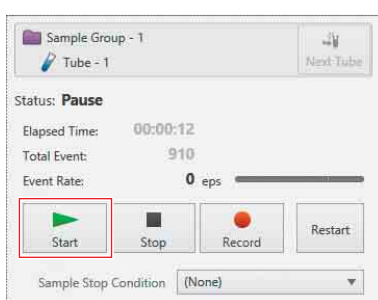
You can also enter a value directly from the keyboard.



When [Gated Event Count] is selected, select the gate for the automatic stop condition and specify the number of events within the selected gate.



8 Click (Start).




Sample fluid starts flowing from the sample tube into the sorting chip, and data acquisition starts.

9 Click (Record).

Data recording starts.

If an automatic stop condition was specified, data acquisition and recording stops automatically when the stop condition has been satisfied.

Tip

 (Record) cannot be selected for index sorting.

For details about index sorting, see “Index Sorting” (page 114).

10 If an automatic stop condition was not specified (None), click (Stop) to stop data acquisition and recording.

The sample tube unloads automatically.

[Acquisition] Tab Main Functions

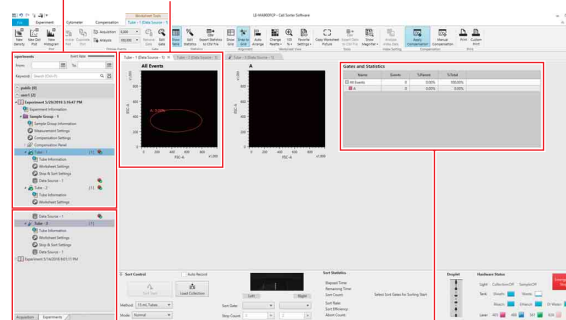
During data acquisition, the acquired data information is displayed on plots and in the statistics table on the worksheet.

Control the data acquisition, recording and pausing as appropriate, while monitoring the data acquisition status. You can change the type of plots, change gates, and edit statistics in the [Gates and Statistics] table, as required.

Controls data acquisition and recording.

Plots

Displays acquired data in graphical form.



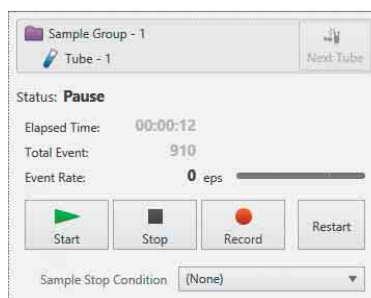
Displays the experiment structure hierarchically.


[Gates and Statistics] table
Displays statistics calculated automatically from acquired data.


Controlling Data Acquisition and Recording

After data acquisition starts, the elapsed time since the start of data acquisition, the total number of events acquired, and the event rate measured in events/second (eps) are displayed on the [Acquisition] tab. You control acquisition, as required, while monitoring the acquisition status.

For details about the displayed items, see “[Acquisition] Tab (control pane)” (page 151).





 **(Start):** Starts/resumes data acquisition. This button does not start data recording.

 **(Pause):** Pauses data acquisition. The elapsed time continues counting while acquisition is paused.


 **(Stop):** Stops data acquisition manually.

 **(Acquire):** Records the measured data.

 **(REC stop):** Stops data recording manually.

 **(Restart):** Resets the total events and elapsed time counters to 0 and restarts data acquisition. Clicking this button while recording will delete all data recorded up to that point.

Tips

- If an automatic stop condition has been specified, data acquisition stops automatically when the stop condition has been satisfied.
- Clicking the  (Start) button to start data acquisition automatically switches the worksheet display to the assigned sample tube data, even if another sample tube data is displayed on the worksheet.

Customizing the Worksheet

You can change the type of plots, modify gates on plots, and customize the content in the [Gates and Statistics] table displayed on the worksheet during data acquisition. You customize the items using the tabs on the ribbon.

Example: [Worksheet Tools] tab



1 [Display Events] group

Sets the number of events displayed on plots.

2 [Gate] group

Tools for editing gates.

3 [Statistics] group

Shows/hides the [Gates and Statistics] table, customizes the content displayed in the table, and exports the table as a CSV file.

4 [Worksheet View] group

Changes the view and scale of plots on the worksheet.

For details about each item, see “[Worksheet Tools] Tab (ribbon)” (page 144).

Analyzing Data

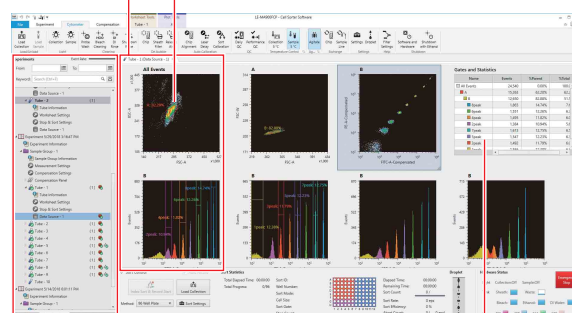
After data acquisition finishes, you analyze the acquired data events. The most recent data is displayed on the worksheet immediately after acquisition finishes. To analyze data for a past experiment, search for and select the target experiment on the [Experiments] tab of the Experiment/Acquisition control pane.

Plots

Displays acquired data in graphical form. Plots can be added/edited.

Gates

Groups events with common characteristics, isolating them from all other events.



[Experiments] tab

Displays experiments that can be selected for analysis.

[Gates and Statistics] table

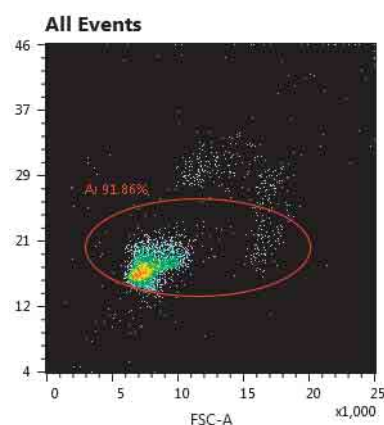
Displays statistics calculated automatically from acquired data. You can customize the statistics displayed in the table.

This section describes how to add plots to the worksheet, how to add gates to a plot, and how to display statistics for the gates.

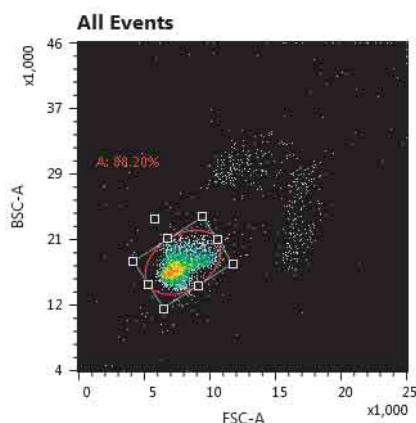
For details, see “Chapter 5 Analysis” (page 93).

1 Select the [All Events] plot on the worksheet.

The [All Events] plot is the top level in the hierarchy. All plots for gates are derived from [All Events]. If necessary, adjust the axes scales using the command buttons in the [Axes] group of the [Plot Tools] tab of the ribbon.



- 2 Adjust the gate size and position to surround the target population.



- 3 Double-click the gate in the plot to add a child plot for that gate.

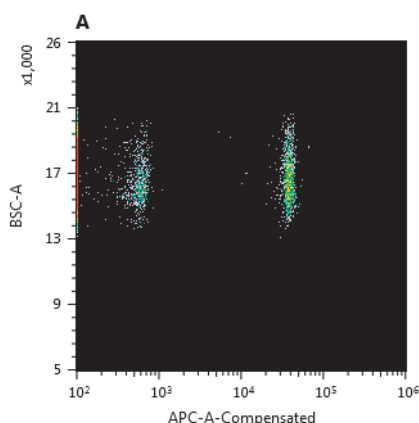
You can also add a child plot from the context menu by right-clicking the gate in the [Gates and Statistics] table.

Tips

- You can add a new plot using the command buttons in the “[Plot] group” (page 144) on the [Plot Tools] tab or the [Worksheet Tools] tab of the ribbon.
- You can change the type of the plot using the command buttons in the “[Plot Type] group” (page 148) of the [Plot Tools] tab of the ribbon.

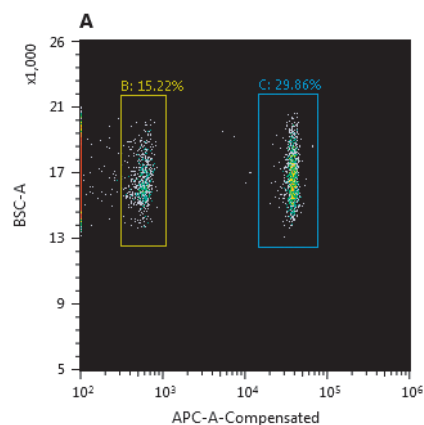
- 4 Click the axis labels and select the parameters to be displayed from the popup menu.

Adjust the axes scales to display the events on the plot, as required.



- 5 Select a gate type in the [Gate] group of the [Plot Tools] tab, and draw gates around both of the populations in the child plot.

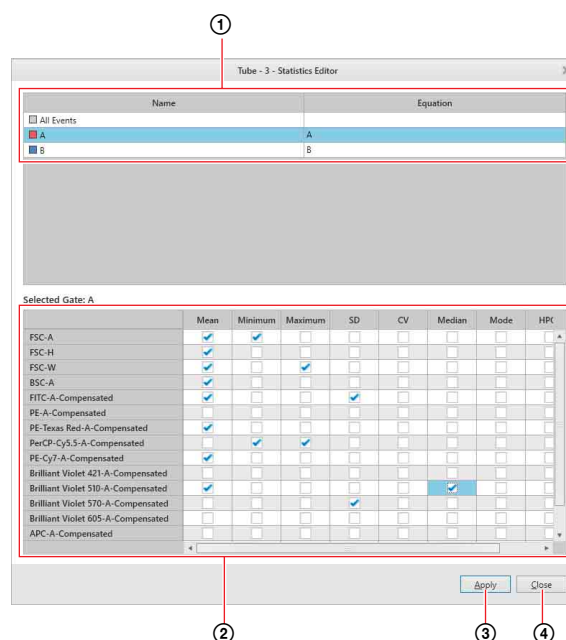
The gates are automatically named in alphabetical order. You can change the name and color of gates in the [Property Window] dialog by right-clicking a gate and selecting [Properties] from the context menu.



- 6 Click [Edit Statistics] in the [Statistics] group on the [Worksheet Tools] tab or [Plot Tools] tab.

The [Statistics Editor] dialog appears.

- 7 Select the statistics to display in the [Gates and Statistics] table.



- Select a gate.
- Place check marks in the checkboxes for the statistics to display.
- Click [Apply] to update the [Gate and Statistics] table.
- Click [Close] to close the dialog.

The selected statistics are displayed in the [Gates and Statistics] table.



Name	Events	%Parent	%Total	Mean	Minimum	Ma...
All Events	20,142	0.00%	100.00%			
A	95	0.47%	0.47%			
FSC-A				563,225	372,618	
FSC-H				68,458		
FSC-W				19		
BSC-A				934,206		
FITC-A-Com...				-26		
PE-Texas Re...				63,198		
PerCP-Cy5.5...					-363	
PE-Cy7-A-C...				330,360		

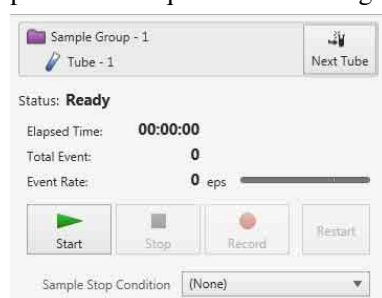
Sorting

Once events have been isolated on plots using gates, the same events can be sorted into different collection tubes for further analysis. Sorting enables the targeted cells to be recovered from a sample to increase the concentration of the cells or to isolate cells for increased purity.

Sorting into 15 ml Tubes (2-way)

- 1 Check that data acquisition and recording are stopped or paused.

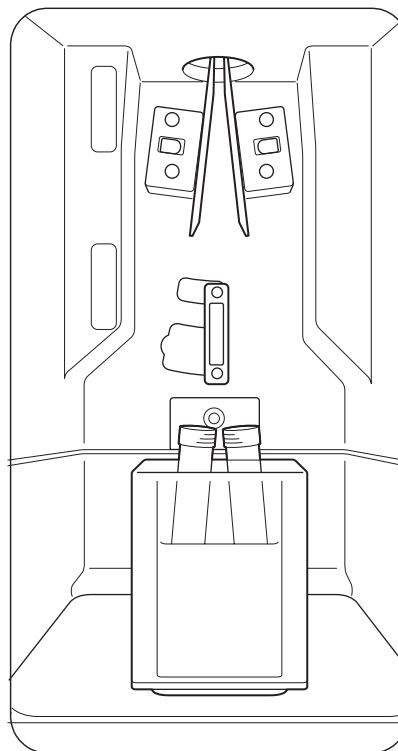
If data acquisition is in progress, click  (Stop) to stop data acquisition/recording or  (Pause) to pause data acquisition/recording.



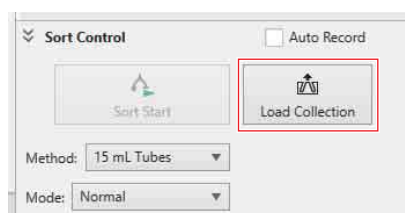
- 2 Place the collection tubes into the corresponding holder.

For details, see “Preparing Collection Tubes and Plates” (page 36).

- 3 Open the collection area door, place the holder on the collection stage, and close the door.



- 4 Click [Load Collection] in the [Sort Control] pane.



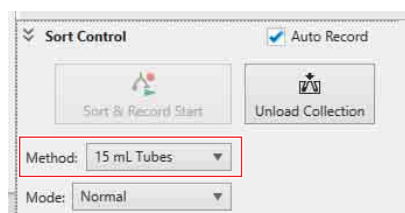
The collection tube device is loaded.

Note

Do not open the collection area door during sorting.

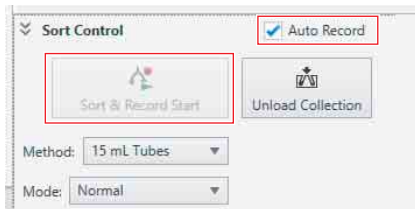
- 5 Select [5 mL Tubes] in [Method].

Always select the sorting method matching the collection tube device loaded in steps 2 to 3.



The sorting parameters vary depending on the selected sorting method.

To start recording at the same time as sorting, place a check mark in the [Auto Record] checkbox. [Sort Start] changes to [Sort & Record Start].



6 Set each item.

For details about each item, see “[Sort Control] Pane” (page 155).



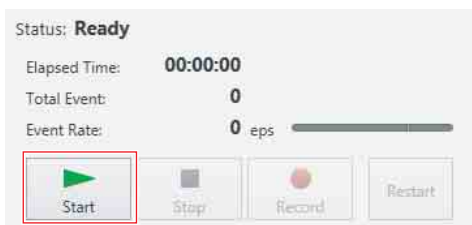
[Mode]: Selects the sorting mode. The purity and yield will vary depending on the sorting mode. Select the sorting mode to match the intended purpose. When sorting larger cells, selecting [Single Cell (3 drops)] may improve the sorting performance. To adjust the position for sorting of target cells within a droplet, you can adjust the settings using [Custom] - [Edit setting].

For details, see “[Sort Mode Setting] dialog” (page 158).

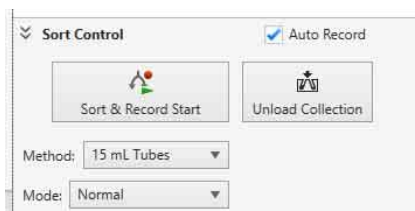
[Sort Gate]: Selects the gates for the target events to sort into the left and right collection tubes.

[Stop Count]: Specifies the event count conditions to stop recording automatically. You can select a value or enter a value from the keyboard. Entering a value of “0” disables the stop condition.

7 Click [Start] or [Resume] in the data acquisition control pane to begin data acquisition.



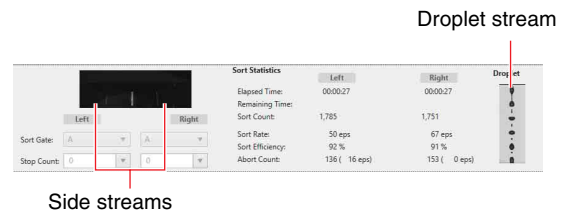
8 Click [Sort Start] or [Sort & Record Start] in the [Sort Control] pane to begin sorting.



Basic sorting status is displayed, together with progress bars if an automatic stop condition is specified.

Sort Statistics		
	L	R
Elapsed Time:	00:00:00	00:00:00
Remaining Time:		
Sort Count:	0	0
Sort Rate:	0 eps	0 eps
Sort Efficiency:	0 %	0 %
Abort Count:	0 (0 eps)	0 (0 eps)

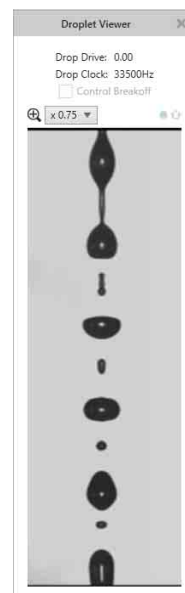
You can monitor the side streams and droplet flow in the main window (when using collection tube holder (15 ml)).



Notes

- The side stream laser is turned off by default. To turn the laser on, see [Side Stream Laser] in “[Cytometer Settings] dialog” (page 139).
- When using the collection tube holder (5 ml), turn the side stream laser off.

Double-clicking the image displays the side streams in a separate window for monitoring droplets.



If an automatic stop condition is configured, a stop message appears when the stop condition is satisfied and sorting stops.

9 Click [OK] to close the confirmation message dialog.

Tip



If an event is detected that falls within more than one gate selected as the target for sorting, sorting into the left-most side stream tube takes precedence.

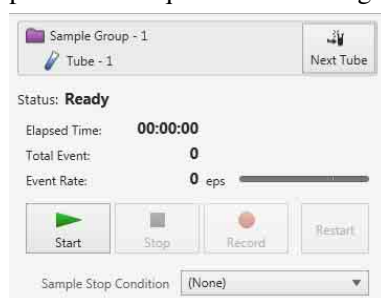
Note

Do not sort sample fluid above the permitted volume into the tube and do not overfill the tube with buffer solution to prevent sample fluid overflow from the tube.

Sorting into 5 ml Tubes (4-way)

- 1 Check that data acquisition and recording are stopped or paused.

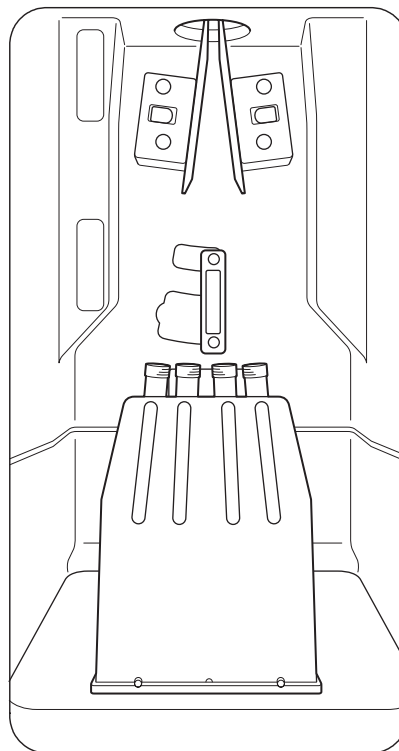
If data acquisition is in progress, click  (Stop) to stop data acquisition/recording or  (Pause) to pause data acquisition/recording.



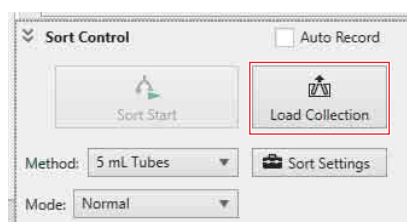
- 2 Place the collection tubes into the corresponding holder.

For details, see “Preparing Collection Tubes and Plates” (page 36).

- 3 Open the collection area door, place the holder on the collection stage, and close the door.



- 4 Click [Load Collection] in the [Sort Control] pane.



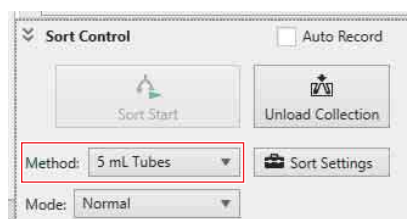
The collection tube device is loaded.

Note

Do not open the collection area door during sorting.

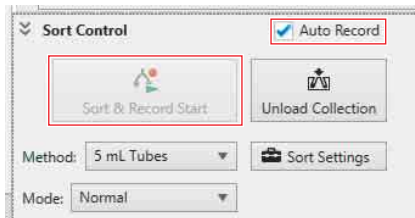
- 5 Select [5 mL Tubes] in [Method] (sorting method).

Always select the sorting method matching the collection tube device loaded in steps 2 to 3.

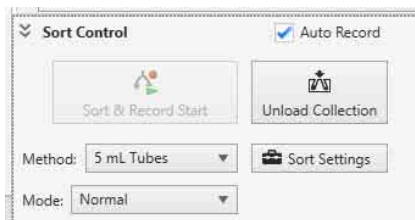


The sorting parameters vary depending on the selected sorting method.

To start recording at the same time as sorting, place a check mark in the [Auto Record] checkbox. [Sort Start] changes to [Sort & Record Start].



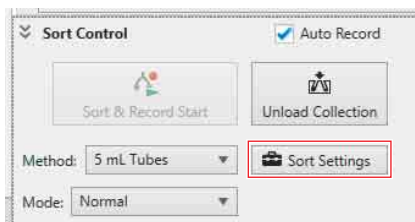
- 6 Select a sorting mode in [Mode].



When sorting larger cells, selecting [Single Cell (3 drops)] may improve the sorting performance. To adjust the position for sorting of target cells within a droplet, you can adjust the settings using [Custom] - [Edit setting].

For details, see “[Sort Mode Setting] dialog” (page 158).

- 7 Click [Sort Settings].



The [Sort Settings - 5mL Tubes] dialog appears.

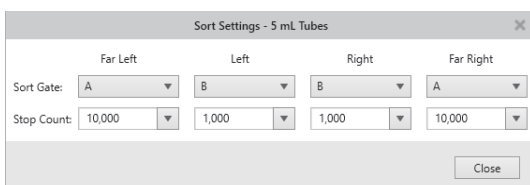
- 8 Set each item, then click [Close].

[Sort Gate]: Selects the gates for the target events to sort into the collection tubes.

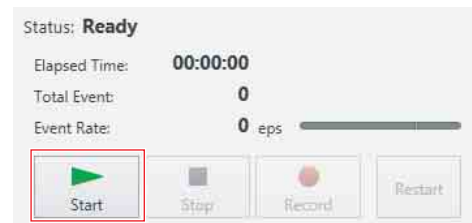
[Stop Count]: Specifies the event count conditions to stop recording automatically.

You can select a value or enter a value from the keyboard.

Entering a value of “0” disables the stop condition.



- 9 Click [Start] or [Resume] in the data acquisition control pane to begin data acquisition.



- 10 Click [Sort Start] or [Sort & Record Start] in the [Sort Control] pane to begin sorting.



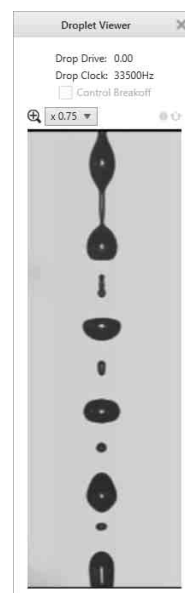
Basic sorting status is displayed, together with progress bars if an automatic stop condition is specified.

Sort Statistics	Far Left	Left	Right	Far Right	Droplet
Sort Gate:	A	B	B	A	
Elapsed Time:	00:00:10	00:00:10	00:00:10	00:00:10	
Remaining Time:	00:00:10	00:00:04	00:00:03	00:00:21	
Sort Count:	731 / 10,000	714 / 1,000	748 / 1,000	680 / 10,000	
Sort Rate:	67 eps	50 eps	50 eps	67 eps	
Sort Efficiency:	97 %	93 %	97 %	93 %	
Abort Count:	17 (0 eps)	51 (16 eps)	17 (16 eps)	51 (0 eps)	

Note

When using the collection tube holder (5 ml), turn the side stream laser off.

Double-clicking the droplet image displays a separate window for monitoring the droplet status.



If an automatic stop condition is configured, a stop message appears when the stop condition is satisfied and sorting stops.

- 11 Click [OK] to close the confirmation message dialog.

Tip

If an event is detected that falls within more than one gate selected as the target for sorting, the left-most stream takes precedence.



Note

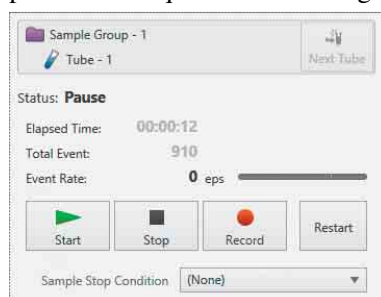
Do not sort sample fluid above the permitted volume into the tube and do not overfill the tube with buffer solution to prevent sample fluid overflow from the tube.

Sorting into Multi-well Plates (LE-MA900P)

This section describes the procedure for sorting into a 96-well plate. The procedure is identical for sorting into 6, 12, 24, 48, and 384-well plates.

- 1 Check that data acquisition and recording are stopped or paused.

If data acquisition is in progress, click  (Stop) to stop data acquisition/recording or  (Pause) to pause data acquisition/recording.



- 2 Attach the splash guard.

Always attach the splash guard when sorting into multi-well plates.

For details, see “Attaching the Splash Guard (for use with multi-well plates) (LE-MA900P)” (page 37).

- 3 Place the 96-well plate into the corresponding holder.

For details, see “Preparing Collection Tubes and Plates” (page 36).

- 4 Open the collection area door, place the holder on the collection stage, and close the door.

**Notes**

- Securely mount the holder adaptor to ensure successful sorting. Check orientation of the plate (well A1) to ensure correct sorting.
- Do not sort sample fluid above the permitted volume into the wells and do not overfill the wells with buffer solution to prevent sample fluid overflow from the wells.
- Take care not to spill samples when removing the well plate from the multi-well plate holder.
- Securely mount the holder adaptor to ensure successful sorting.
- Load a multi-well plate according to the method selected in [Method] on the [Sort Control pane].
- Do not open the collection area door during sorting.
- Do not attempt to remove a multi-well plate while the collection stage is moving.

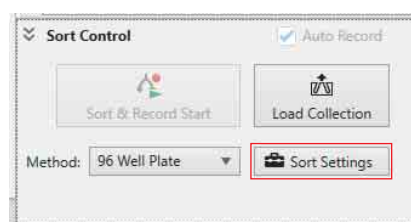
- 5 Select the appropriate plate sorting method in [Method].

Always select the sorting method matching the collection tube device loaded in steps 2 to 3.



The sorting parameters vary depending on the selected sorting method.

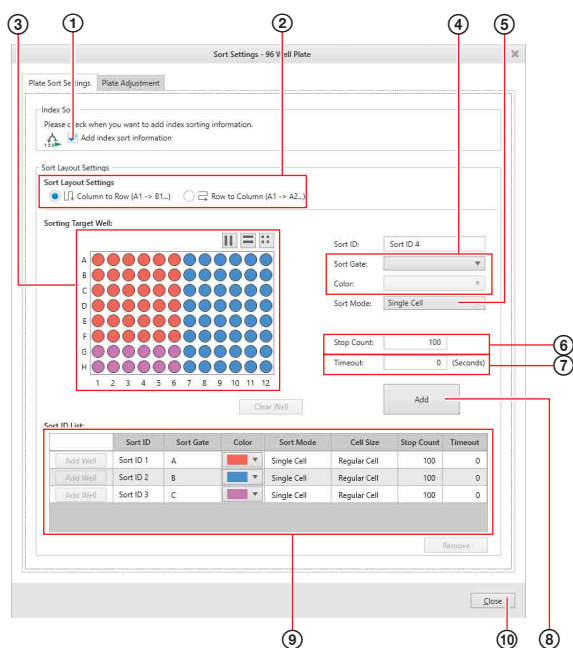
- 6 Click [Sort Settings].



The [Sort Settings] dialog appears.

- 7 Configure the settings for sorting into wells on the [Plate Sort Settings] tab.

The description in this section shows a 96-well plate as an example. The operation is the same for all other multi-well plates.



- ① If using index sorting, place a check mark in the [Add index sort information] checkbox. In index sorting, you can associate the cells sorted in each well with the events for those cells on plots.

For details, see “Index Sorting” (page 114).

- ② Select the sorting sequence in [Sort Layout Settings].
- [Column to Row]: Sorting occurs in the sequence A1 → B1 → C1...H1 → A2 → B2...G12 → H12.
 - [Row to Column]: Sorting occurs in the sequence A1 → A2 → A3...A12 → B1 → B2...H11 → H12.

- ③ Select the target well to set. You can select the well using the following buttons or by clicking the well on the palette. You can select multiple wells using the Shift and Ctrl keys.

: Select wells in alternate columns.

The selection toggles between odd columns and even columns each time the button is clicked.

: Select wells in alternate rows.

The selection toggles between odd rows and even rows each time the button is clicked.

: Select wells in groups of four.

The selection toggles as follows each time the button is clicked.

Top left → Top right → Bottom left → Bottom right → Top left...

The ID is displayed in [Sort ID].

You can edit the entry in [Sort ID] manually.

- ④ Select the gate whose population you want to sort into the well in [Sort Gate]. You can change the color of the gate in [Color].

- ⑤ Select a sorting mode in [Sort Mode]. The default is “Single Cell.” “Single Cell” mode is recommended for reliable sorting.

When sorting larger cells, selecting [Single Cell (3 drops)] may improve the sorting performance. To adjust the detection range for sorting of target cells within a droplet, you can adjust the settings using [Edit setting].

For details, see “Configuring Custom Sorting Mode” (page 117).

- ⑥ Enter the number of events to sort per well in [Stop Count].

Note

If a value exceeding the values below is entered in [Stop Count], a message appears warning you of the risk that sample fluid may overflow the well (operation does not stop).

96 Well Plate: 50,000 events

- ⑦ Enter the timeout time in [Timeout] in units of seconds.

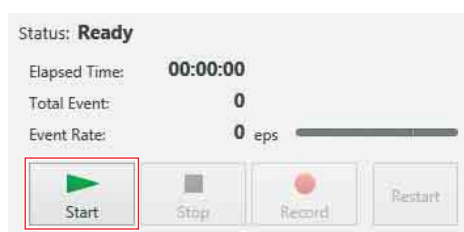
- ⑧ Click [Add]. The settings are added to [Sort ID List].

- ⑨ Repeat steps ③ to ⑧ to set parameters for other wells as necessary. You can modify the parameters for a well by clicking the corresponding cells in [Sort ID List].

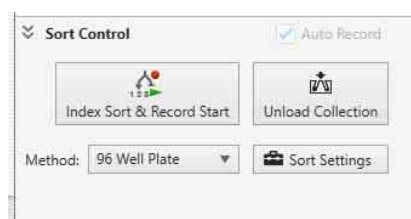
- ⑩ When finished, click [Close] to close the dialog.

For details about the [Plate Adjustment] tab, see “Adjusting the Sort Position” (page 112).

- 8 Click [Start] or [Resume] in the data acquisition control pane to begin data acquisition.



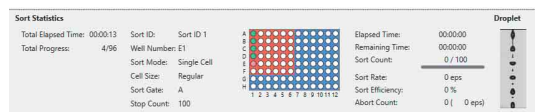
- 9 Click [Sort Start] in the [Sort Control] pane to begin sorting.



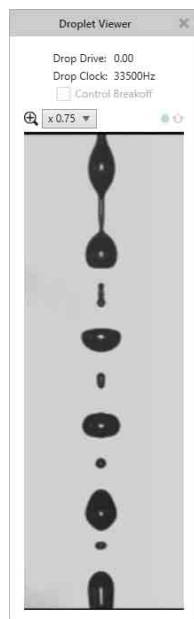
Tip

Sorting and recording start at the same time.

Basic sorting statistics are displayed, together with progress bars if an automatic stop condition is specified.



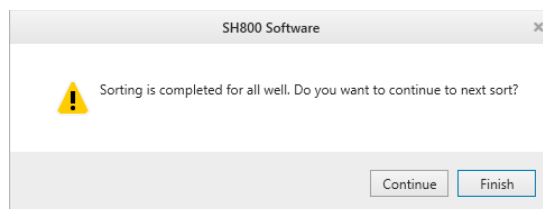
Double-clicking the image displays the side streams in a separate window for monitoring droplets.



Double-clicking the plate image or right-clicking and selecting [Show Plate Sorting Monitor] from the context menu displays the [Plate Sorting Monitor] window for monitoring progress. You can also display the window by right-clicking a tube in the tube list and selecting [Show Sorting Report] from the context menu.

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
B	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
C	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
D	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
E	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
F	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
G	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C
H	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 C	100 C	100 C	100 C	100 C

After one set has been sorted, the following message appears.



10 Click [Finish] to finish sorting.

Clicking [Continue] unloads the collection tube holder so you can load the next multi-well plate.

Compensating Fluorescence Spillover

Fluorescence compensation is used to remove fluorescence spillover components from each channel to obtain the signal for the target fluorochrome only for each channel.

In fluorescence compensation, a compensation panel comprising an unstained negative control and a singly-stained, positive control samples for each fluorochrome are recorded.

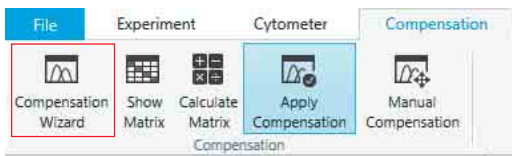
This section describes the fluorescence compensation procedure using the [Compensation Wizard].

For details about specifying a target gate for fluorescence compensation or adjusting fluorescence compensation manually, see “Adjusting Fluorescence Compensation” (page 90).

Note

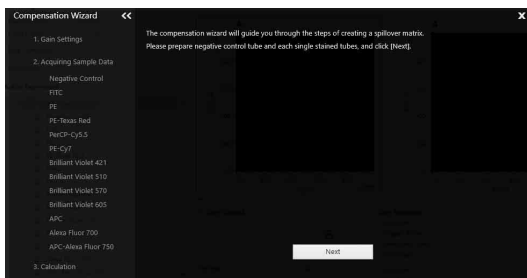
Do not change the gain of the channel detectors while recording the control tubes or samples for a compensation panel. The fluorescence compensation process is valid only for the instrument settings in place when all control tubes in a compensation panel are recorded. If the sensor gain of any detector is changed after recording the control tubes, the fluorescence compensation will no longer be valid and the control tubes will need to be loaded and recorded again.

- 1 Click [Compensation Wizard] on the [Compensation] tab of the ribbon.



The [Compensation Wizard] launches.

- 2 Click [Next].



The following screen appears.

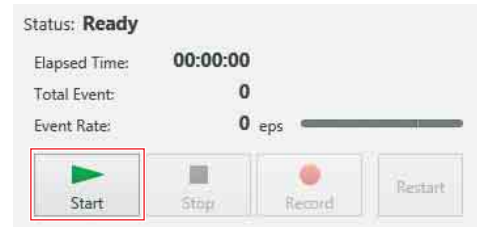
Follow the on-screen instructions to record control tubes.

The operating procedure is displayed.



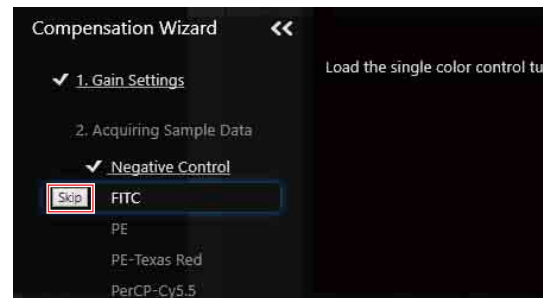
A control tube is automatically assigned as the active tube and the worksheet for the tube opens.

- 3 Load the control tube displayed in the wizard, then click [Start] on the data acquisition control pane.



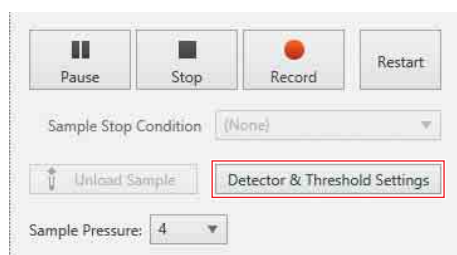
Tip

If, for any reason, you choose not to use a fluorochrome channel, you can click [>>>Skip] to bypass the control tube.



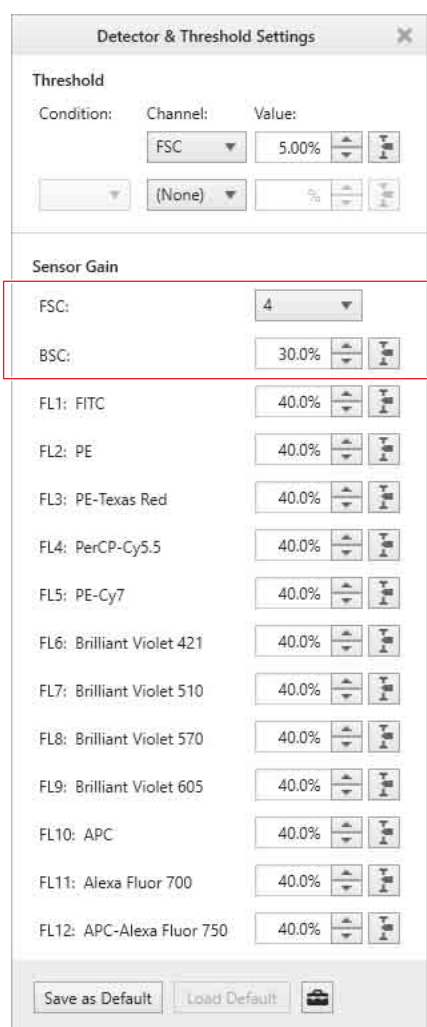
Data acquisition starts for the tube and events are added to the plots on the worksheet in real-time.

- 4 Click [Detector & Threshold Settings].



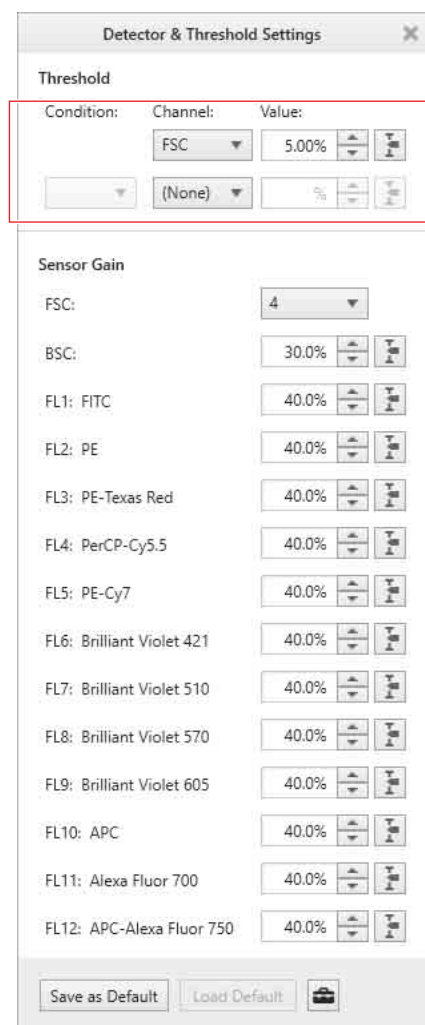
The [Detector & Threshold Settings] dialog appears.

- 5 Adjust the sensor gain of the FSC and BSC detectors to place the population on scale.



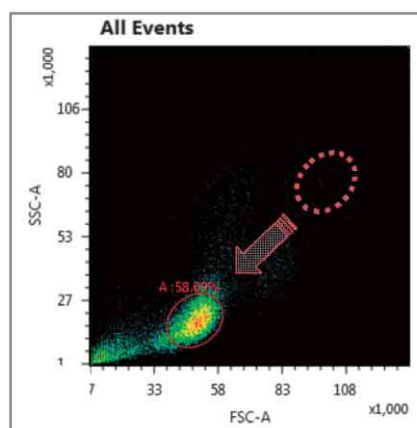
You adjust the gain while viewing the FSC vs. BSC density plot. You can also adjust the axis display scales to display a magnified portion of the plot.

- 6 Adjust the trigger threshold level to remove background noise without affecting the targeted population.



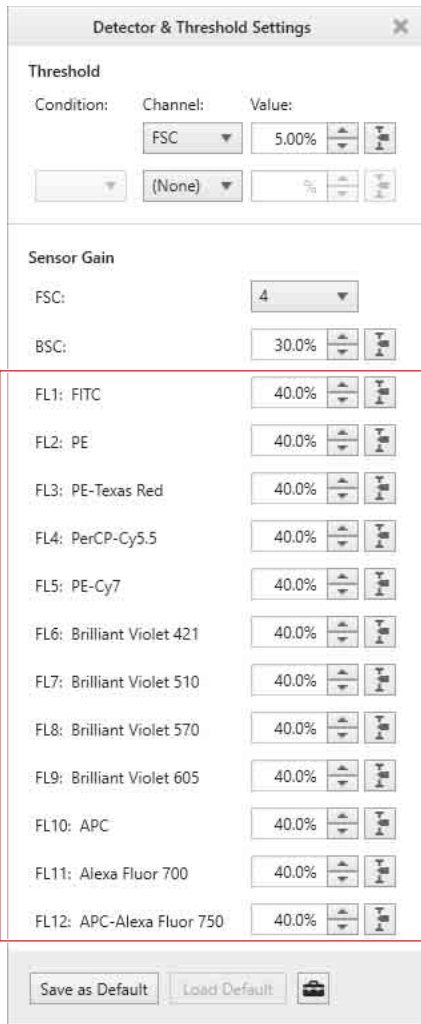
To use two triggers, see “Configuring Detector Settings” (page 87).

- 7 Adjust the size and position of the gate automatically added to the plot to encompass the desired population, then click [Next] in the [Compensation Wizard].

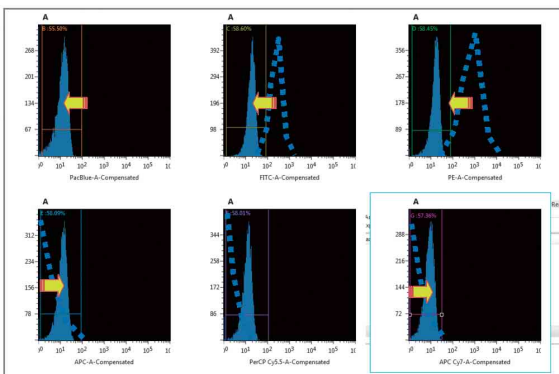


- 8** Adjust the gain levels of the FL1 to FL12 fluorescence channels to place the negative populations on scale in all channels.

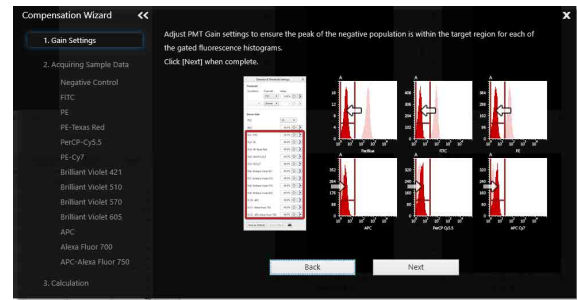
You adjust the gain while viewing the histogram plots for each fluorescence channel.



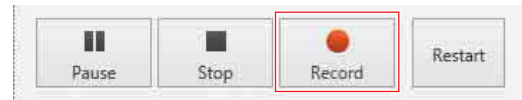
The negative peaks represent the signals detected due to cellular autofluorescence. The negative peaks should be on scale, and not wedged up against the axis in all channels.



- 9** After adjusting the detector gain settings, click [Next].



- 10** Click [Record] in the data acquisition control pane.

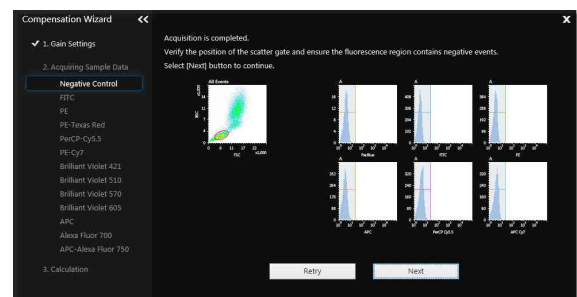


The LE-MA900 starts recording the data acquired for the negative control tube. Recording stops automatically after satisfying the stop condition.

Tip

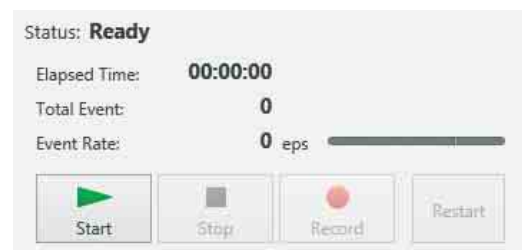
You can stop data acquisition and recording manually using [Stop] if a sufficient number of events have been recorded.

- 11** Click [Next].



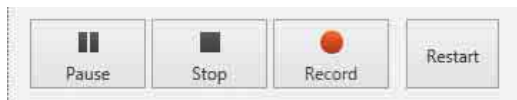
Data acquisition and recording for the negative control tube is completed. You are then prompted to load and record the positive control tubes.

- 12** Load the next control tube displayed in the wizard, then click [Start] on the data acquisition control pane.



The positive controls must be loaded in the sequence displayed in the Experiment Explorer, as prompted by the [Compensation Wizard].

- 13** Check that data acquisition has started, then click [Record] in the data acquisition control pane.



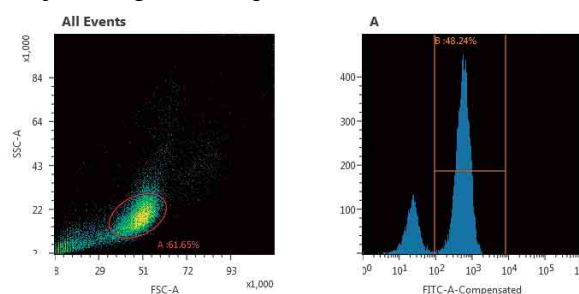
Recording stops automatically after satisfying the record stop condition. The positive population is displayed on the histogram plot.

Tip

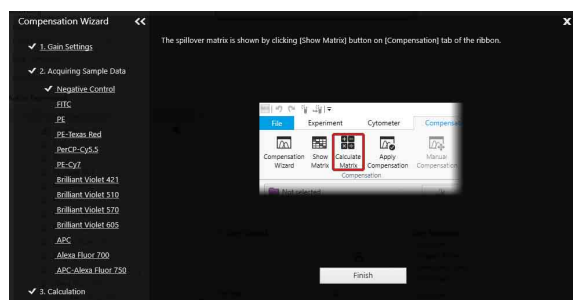
You can stop data acquisition and recording manually using [Stop] if a sufficient number of events have been recorded and the plot view has stabilized.

- 14 Check the position of the gate on the density plot and the gate for the positive population on the histogram, then click [Next].

Adjust the gate, as required.

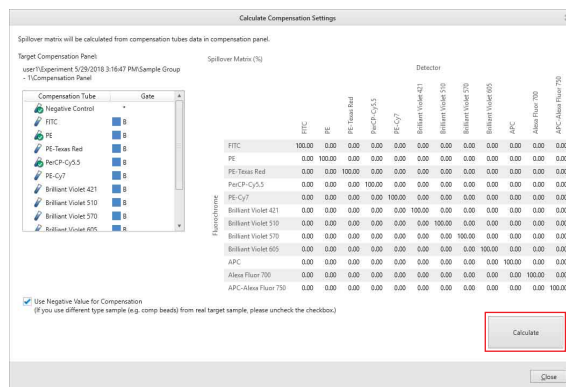


- 15 Repeat steps 11 to 14 to record each of the control tubes in sequence.
- 16 After recording all control tubes, click [Calculate Matrix] on the [Compensation] tab of the ribbon.



The [Calculate Compensation Settings] dialog appears.

- 17 Check the target compensation panel, then click [Calculate].



The spillover matrix and the negative fluorescence compensation values are calculated.

- 18 Check the fluorescence compensation values, then click [Close].

- 19 Click [Finish] to exit the [Compensation Wizard].

Tip

To check whether the fluorescence compensation value calculated using the Compensation Wizard is correct using the measurement data of a control tube, it is necessary to duplicate the control tube to a normal sample tube.

- ① In the compensation panel, right-click a recorded positive control tube and select [Duplicate to Normal Tube] from the context menu.
- ② Open the worksheet for the duplicated sample tube and check whether the compensation value is correct.

Probe Wash

Probe wash is automatically performed after each sample run to reduce sample carryover. Probe wash can also be run manually for added cleaning.

- 1 Verify that data acquisition and recording are not ongoing.

The fluidics system cannot be washed during data acquisition.

- 2 Click [Probe Wash] in the [Cleaning] group on the [Cytometer] tab of the ribbon.



The sorting chip, sample line, and sample probe are flushed with sheath fluid.

For details about cleaning manually using bleach, see “Cleaning Sample Line using Bleach” (page 174).

Shutting Down

Shut down the LE-MA900 using the procedure described below. The fluidics system should be cleaned according to the instructions in the shutdown wizard before shutting down.

The Shutdown Wizard describes the bleach cleaning procedure. For details, see “Cleaning Sample Line using Bleach” (page 174).

Shutting Down the Main Unit and Software

Note

Always leave the sorting chip in the chip loader after shutting down. The chip seals the inlet ports when the LE-MA900 is not in use.

- 1 Click [Unload Collection] to unload and remove the collection tubes.



Tip

Remove the splash guard when unloading multi-well plates.

- 2 Clean the sample loader, collection area, and the collection stage using ethanol to prevent salt precipitation within the unit and to prevent biological contamination.

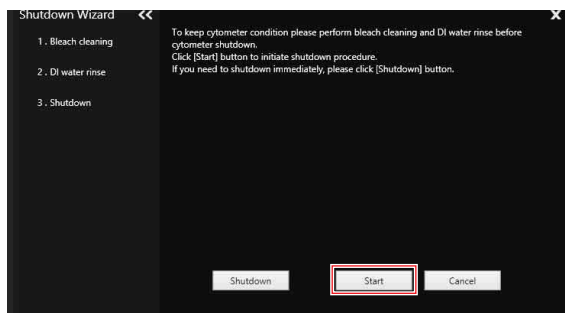
For details about cleaning, see “Regular Cleaning” (page 180).

- 3 Click [Software and Hardware] in the [Shutdown] group on the [Cytometer] tab of the ribbon.



The Shutdown Wizard appears.

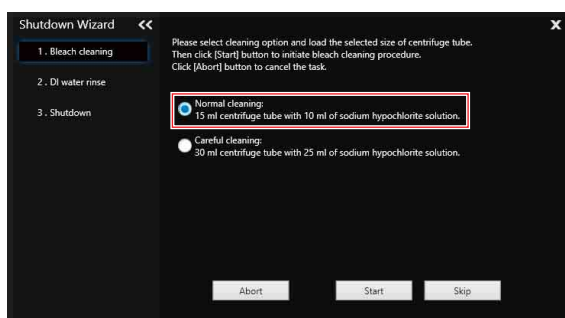
- 4 Click [Start].



Tip

If you click [Shutdown], the LE-MA900 shuts down without doing any cleaning.

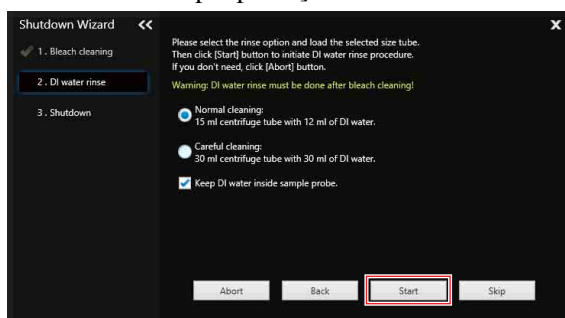
- 5 Select the sample tube size for bleach cleaning and click the [Start] button.



Bleach cleaning of the sample line starts.

- 6 Select the sample tube size for DI water cleaning and click the [Start] button.

To shutdown the instrument with the sample probe filled with DI water, place a check mark in [Keep DI water inside sample probe].



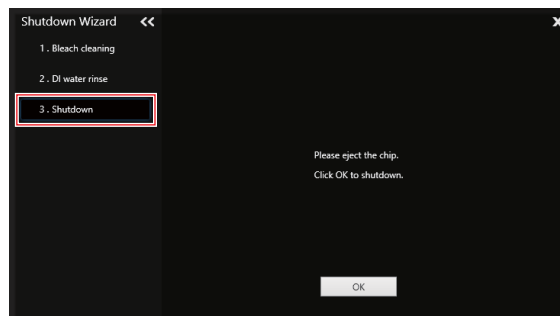
DI water cleaning starts.

When cleaning with DI water finishes, the following screen appears.

Note

After cleaning, the fluidics lines are filled with DI water to prevent the formation of salt deposits.

- 7 Click [OK], and shut down the LE-MA900.

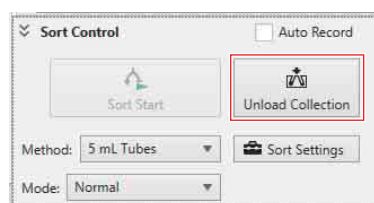


The fluidics system is depressurized and all fluidics lines are sealed.

Cleaning the Sheath Line and Shutting Down

Use the following procedure to clean the sheath lines and shut down the LE-MA900.

- 1 Click [Unload Collection] to unload and remove the collection tubes.



Tip

Remove the splash guard when unloading multi-well plates.

- 2 Clean the sample loader, collection area, and the collection stage using ethanol to prevent salt precipitation within the unit and to prevent biological contamination.

For details about cleaning, see “Regular Cleaning” (page 180).

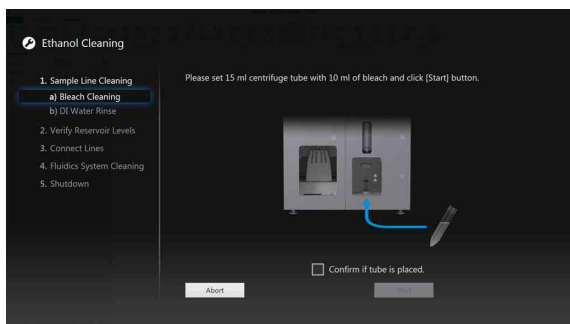
- 3 Click [Shutdown with Ethanol] in the [Shutdown] group on the [Cytometer] tab of the ribbon, and follow the procedure in the wizard to shut down.



The Shutdown Wizard appears.

- 4 Start ethanol cleaning.

Follow the on-screen instructions.



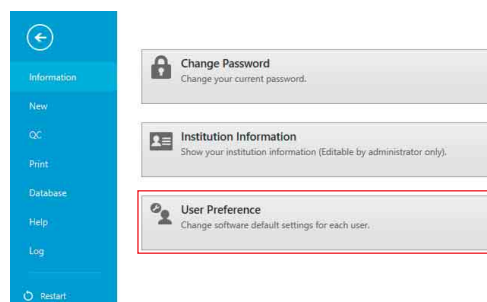
The instrument shuts down when ethanol cleaning finishes.

When the system is restarted, resume with the instructions on the screen to complete the cleaning cycle.

Specifying User Preferences

Various configuration settings and values can be saved by each user in user preferences. User preferences are loaded automatically when a user logs in, saving having to configure the instrument you want to perform acquisition, analysis, or sorting.

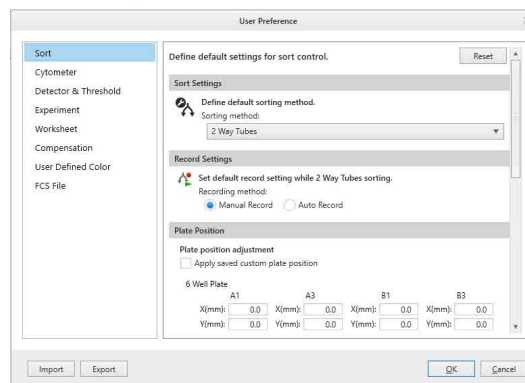
- 1 Click [Information] on the [File] tab of the ribbon, then click [User Preference].



The [User Preference] dialog appears.

- 2 Set each item.

The values set here become the default values for each setting.



For details about each item, see “[User Preference] dialog” (page 121).

Exchanging the Sorting Chip

The sorting chip must be changed regularly to ensure an unobstructed flow path.

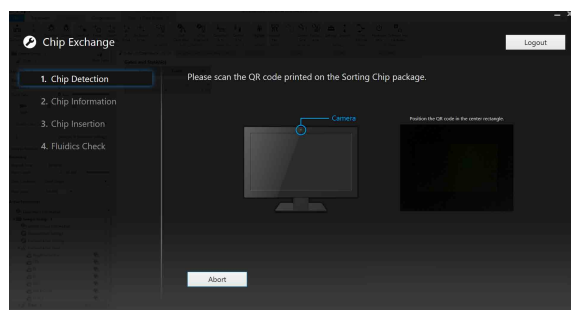
- 1 Click [Chip] in the [Exchange] group on the [Cytometer] tab of the ribbon.

A confirmation dialog appears.

- 2 Click [Yes].

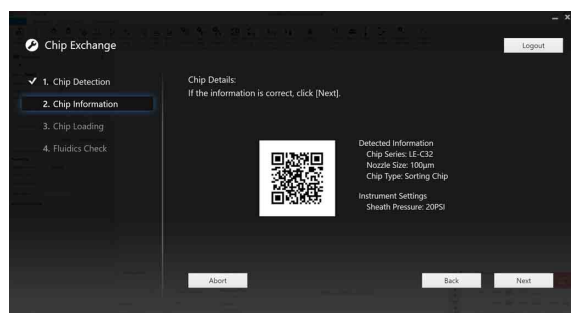
The [Chip Exchange] wizard appears.

- 3 Scan the QR code on the packaging of the replacement chip.



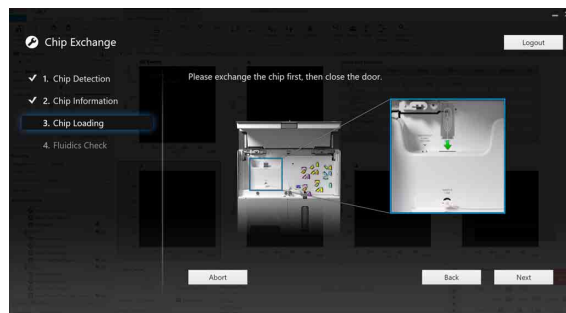
If the scan is successful, the chip information is displayed.

- 4 Click [Next].

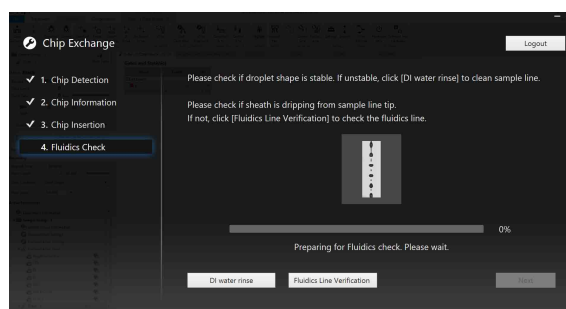


You are prompted to exchange the chip.

- 5 Open the flip-up door, and remove the current sorting chip.
- 6 Insert the replacement chip in the chip insertion slot, then close the flip-up door.



- 7 Click [Next].



The fluidics check starts.

- 8 Click [Next].

The automatic calibration sequence starts. Follow the on-screen instructions to align the sorting chip and calibrate the instrument.

Notes

- Do not insert any objects other than Sony sorting and cleaning chips designed for the LE-MA900 into the chip insertion slot.
- The packaging for the loaded sorting chip should be stored in a safe location.
- Replace the sorting chip if it is discolored, dirty, or stained from previous use.
- Do not drop the sorting chip on a hard surface or bend the chip intentionally. If there is a problem with the sorting chip, contact your Sony distributor.
- Always wear gloves and other protective clothing, mask, and goggles, when changing the sorting chip.
- If the sorting chip will not load correctly, run the instrument in maintenance mode as described in “Running Maintenance Mode” (page 192). If the problem persists, contact your Sony distributor for service.
- The sorting chip must be changed regularly if subjected to prolonged exposure to irradiation by the lasers to prevent burn-in, especially by the 405 nm laser. You can monitor the length of time the chip has been exposed to the light from each laser in the [Chip Information] tab of the [Cytometer Settings] dialog, accessed by clicking [Settings] on the [Cytometer] tab of the ribbon.



Inheriting Auto Calibration Results Between Users

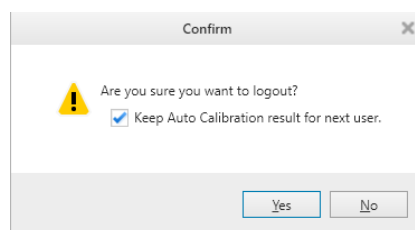
When using the same sorting chip, the auto calibration results can be inherited between users. This reduces the time taken for auto calibration when another user logs in. Allowing auto calibration results to be passed between users is determined when logging out.

Tips

- Depending on the prior calibration performed, the inherited autocalibration results will bring in settings for chip alignment or sort calibration.
- An administrator can select whether the inherited auto calibration results are made default for the next user.

For details, see [Administrative Settings] in “[Information] window” (page 119).

- 1 On the logout confirmation screen, select [Keep Auto Calibration result for next user.] checkbox and click [Yes].



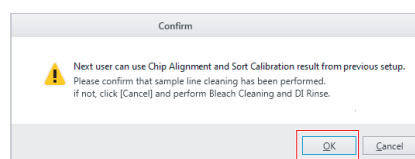
A confirmation message appears.

Notes

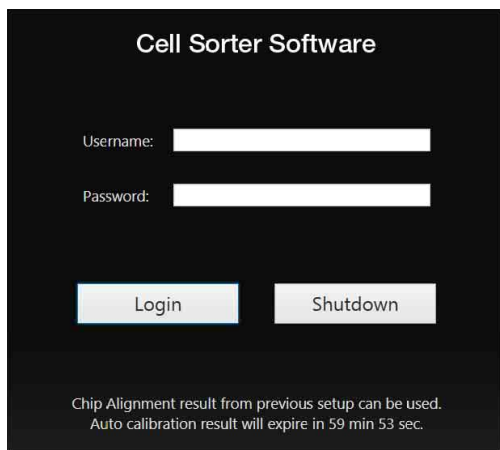
- If the level in the sheath tank is too low, droplet formation is unstable, the fluidics system stops due to an error, or other instrument status problem, the auto calibration result may not be inherited by another user, even if auto calibration was completed successfully.
- To enable the auto calibration result to be inherited by another user, run [Bleach Cleaning] and [DI Rinse] to clean the sample line before logging out.

- 2 Check the message details, then click [OK].

If the sample line has not been cleaned, click [Cancel] and then run [Bleach Cleaning] and [DI Rinse].



The login screen appears, displaying a timer showing when the auto calibration result will be cleared.



completed, the [Create Experiment] window appears.

- If [Skip] is clicked, the [Create Experiment] window appears immediately.

For details about sorting calibration, see “Running Automatic Calibration” (page 49).

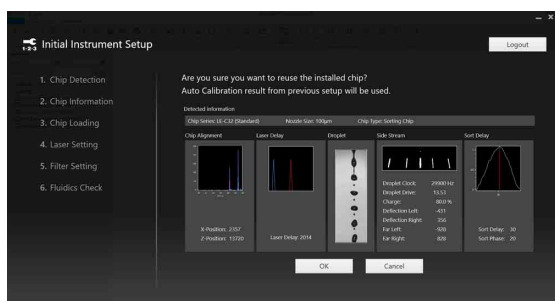
Tips

- When the auto calibration results are inherited, the sheath fluid flow continues.
- The auto calibration results are maintained for approximately 60 minutes.
- The auto calibration results may also be cleared, depending on the instrument status, even when the auto calibration results are inherited.
- If maintenance mode is run while the auto calibration results are inherited, the auto calibration results are cleared.

3 Log in.

The inherited auto calibration results are displayed.

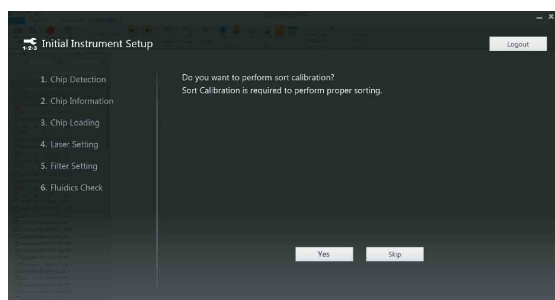
4 Check the settings, then click [OK].



The [Create Experiment] window appears for building a new experiment.

Tips

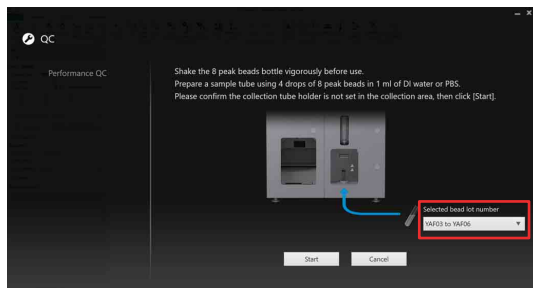
- If only the chip position alignment result is inherited, the follow screen appears before the [Create Experiment] window appears.



- To perform sorting, click [Yes] to run sorting calibration. After the sorting calibration is

Updating the Bead Lot File

Update the bead lot information and select the correct lot number before running Performance QC.

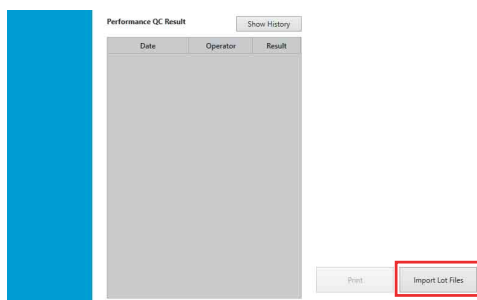


Note

Use the same bead lot as selected in the software to ensure that QC will run correctly.

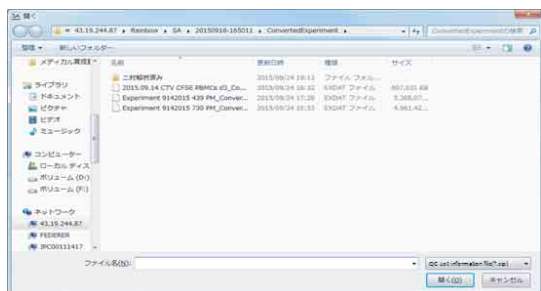
Contact your Sony representative for obtaining the bead lot file.

- 1 Click the [QC] tab, and click [Import Lot Files].



The file selection dialog appears.

- 2 Select a QC beads lot file (.bl), and click [Open].



The beads lot file is imported.

Backing Up/Restoring the Database

You can back up all data in the Cell Sorter Software database. You can also restore all experiment data in a backed up database, as required.

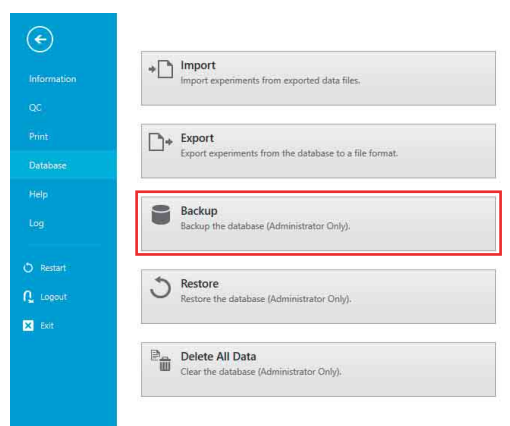
This operation is available only when logged in using an administrator account.

Tip

Backup/restore operations are supported on NTFS-format file systems. Other format file systems are not supported.

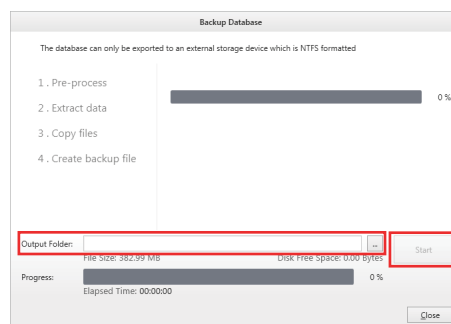
Backing up the Database

- 1 Click [Backup] in the [Database] window on the [File] tab.



The [Backup Database] dialog appears.

- 2 Click (Browse) to specify the backup data destination, then click [Start].



The database backup commences and a progress bar is displayed.

When the database is backed up, a confirmation message appears.

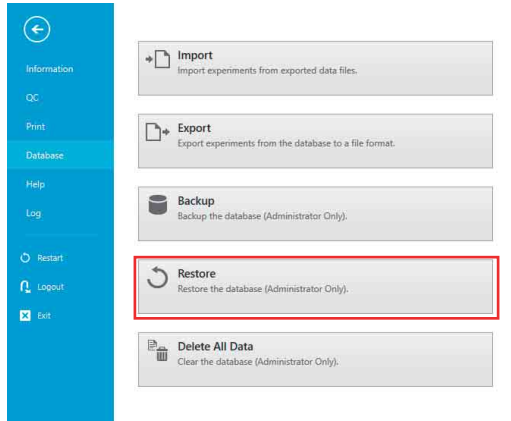
- 3 Click [OK].
- 4 Click [Close] to close the [Backup Database] dialog.

Restoring a Database


Tip

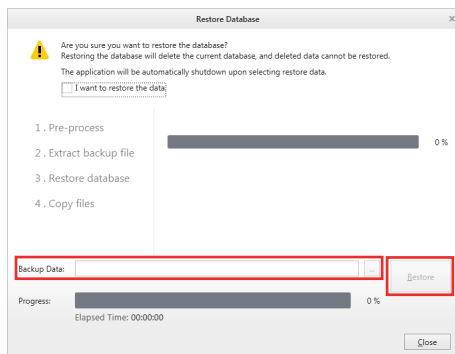
Restoring a previous database backup loads the user accounts, experiment data, templates, and other information in the backup, erasing and replacing all the information in the current database.

- 1 Click [Restore] in the [Database] window on the [File] tab.



The [Restore Database] dialog appears.

- 2 Click  (Browse) to specify the backup data to restore, then click [Restore].



A confirmation message appears.

- 3 Click [Yes].

Cell Sorter Software launches and the database restore operation starts.

When the database is restored, a confirmation message appears.

- 4 Click [OK].
- 5 Click [Close] to close the [Restore Database] dialog.

This chapter describes how to customize experiments, and how to configure experiment components, detector settings, and fluorescence compensation.

For details creating an experiment quickly, see “Creating an Experiment” (page 53).

Creating a Customized Experiment

You can customize the structure of an experiment created using templates or recent experiments by adding sample groups and sample tubes to the experiment, and configuring the settings of each component.

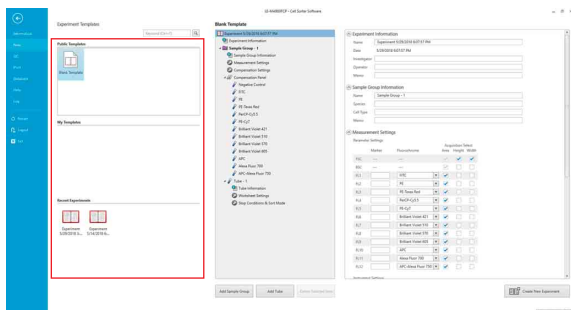
You can also customize an experiment after it has been created. For details, see “Editing an Experiment” (page 83).

- 1 Click [New] on the [File] tab of the ribbon or click [New] in the [Experiment] group on the [Experiment] tab of the ribbon.

The [Create Experiment] window appears.

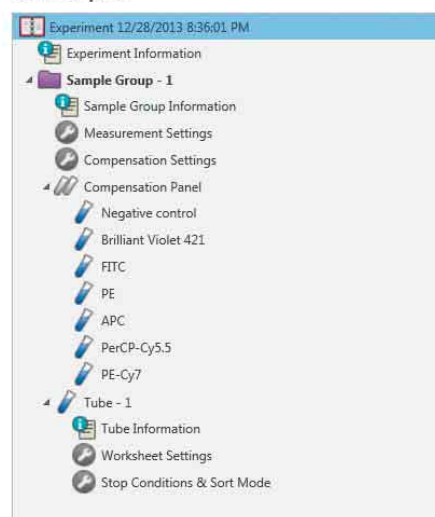
- 2 Select a template or a recent experiment.

Clicking a template or an experiment on the left displays the structure of the selected experiment on the right side of the window.



- 3 Modify the structure of the experiment, as desired.

Blank Template



For details about adding/deleting sample groups and sample tubes, see “To add a sample group” (page 79), “To add a sample tube” (page 80), and “To delete a sample group or tube” (page 81).

Tip

Changes made to the settings on the right side of the window are immediately reflected in the experiment structure.

- 4 Modify the experiment and sample group information, as desired.

Tip

The name of the experiment is supplied automatically, although it is recommended that you change it to a more appropriate name.

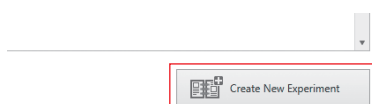
- 5 Select the detector channel pulse parameters for data acquisition in the [Measurement Settings] pane.

For details, see the [Measurement Settings] dialog description in “Configuring an Experiment” (page 81).

Tips

- The fluorochrome and marker names are optional, but recommended.
- Check that the fluorochrome and marker names assigned to detection channels correspond with the laser configuration and optical filter pattern. For details, see “Fluorochrome Detection Matrix” (page 218).

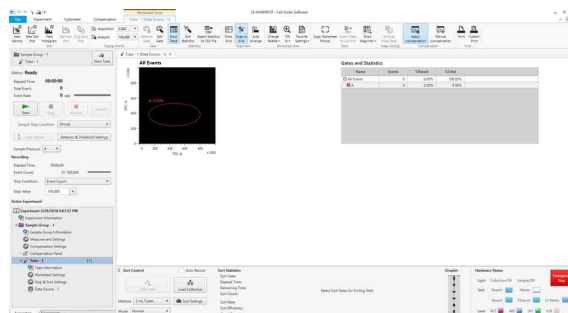
- 6 When finished building the experiment, click [Create New Experiment] in the bottom right-hand corner of the window.



If “Blank Template” was selected, the [New Experiment Startup Procedure] dialog (page 53) appears.

If an existing template or experiment was selected, the main window appears.

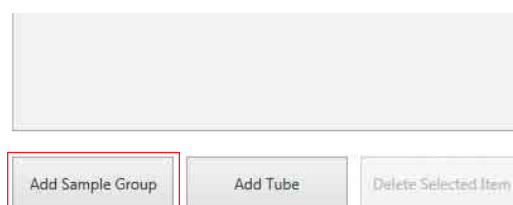
The created experiment is automatically made the active experiment, and the first tube is automatically assigned as the active tube.



To add a sample group

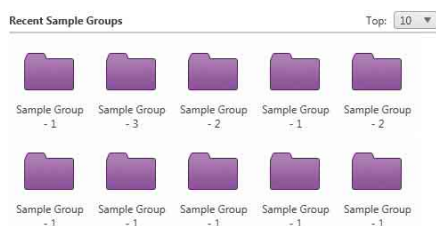
Use the following procedure to add a sample group to an experiment.

- 1 Click [Add Sample Group] in the [Create Experiment] window.



The [Add Sample Group] dialog appears.

- 2 Select a sample group template or recent sample group in [Sample Group Templates].



- 3 Enter a name for the sample group, and modify other settings as required.

The name of the sample group is a required item.

Sample Group Information

Name	Sample Group
Species	
Cell type	
Memo	

- 4 Select the detector channel pulse parameters for data acquisition in the [Measurement Settings] pane.

Measurement Settings

Marker	Fluorochrome	Acquisition Select	Area	Height	Width
FSC		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
BSC		<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL1	FITC	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL2	PE	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL3	PE-Texas Red	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL4	PerCP-Cy5.5	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL5	PE-Cy7	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL6	Brilliant Violet 421	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL7	Brilliant Violet 510	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL8	Brilliant Violet 570	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL9	Brilliant Violet 605	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL10	APC	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL11	Alexa Fluor 700	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FL12	APC-Alexa Fluor 750	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Instrument Settings

Laser	Threshold	Sensor Gain
<input checked="" type="checkbox"/> 405nm	Channel: FSC	FSC 4
<input checked="" type="checkbox"/> 488nm	Value: 5.00%	FL1 40.0%
<input checked="" type="checkbox"/> 561nm		FL2 40.0%
<input type="checkbox"/> 638nm		FL3 40.0%
		FL4 40.0%
		FL5 40.0%
		FL6 40.0%
		FL7 40.0%
		FL8 40.0%
		FL9 40.0%
		FL10 40.0%
		FL11 40.0%
		FL12 40.0%

Sample Pressure: 4

AD Advanced Settings

Only control tubes that have a selected detection channel pulse parameter are included in the

compensation panel for the sample group in the experiment structure.

Tips

- The fluorochrome and marker names are optional, but recommended.
- Check that the fluorochrome and marker names assigned to detection channels correspond with the laser configuration and optical filter pattern. For details, see “*Fluorochrome Detection Matrix*” (page 218).

- 5 Modify the structure of the sample group, as required.

For details, see “*To add a sample tube*” (page 80) and “*To delete a sample group or tube*” (page 81).

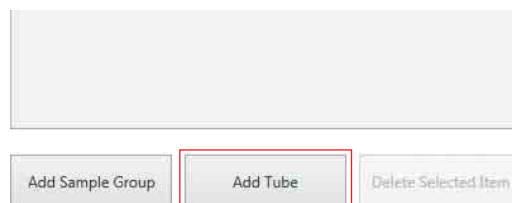
- 6 When finished, click [Add Sample Group] in the bottom right-hand corner of the window.

The sample group is added to the experiment.

To add a sample tube

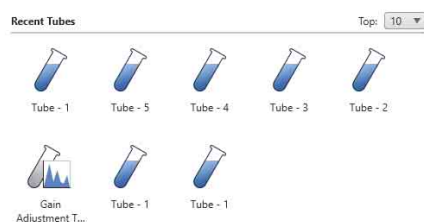
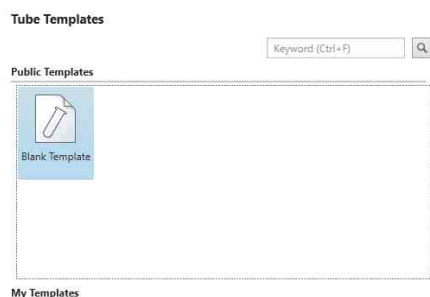
Use the following procedure to add a sample tube to the selected sample group.

- 1 Click [Add Tube] in the [Create Experiment] window.



The [Add Tube] dialog appears.

- 2 Select a sample tube template or recent sample tube in [Tube Templates].



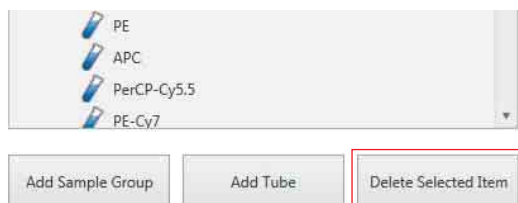
- 3 Enter a name for the sample tube, and modify other settings as required.

The name of the sample tube is a required item.

- 4 When finished, click [Add Tube] in the bottom right-hand corner of the window.

To delete a sample group or tube

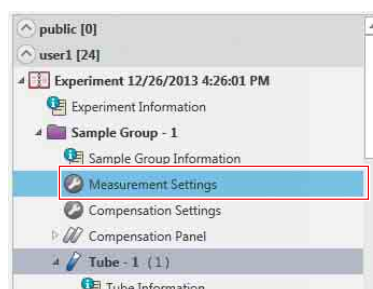
Select the sample group or tube you want to delete in the structure tree in the [Create Experiment] window and click [Delete Selected Item].



Configuring an Experiment

This describes how to configure each component in an experiment.

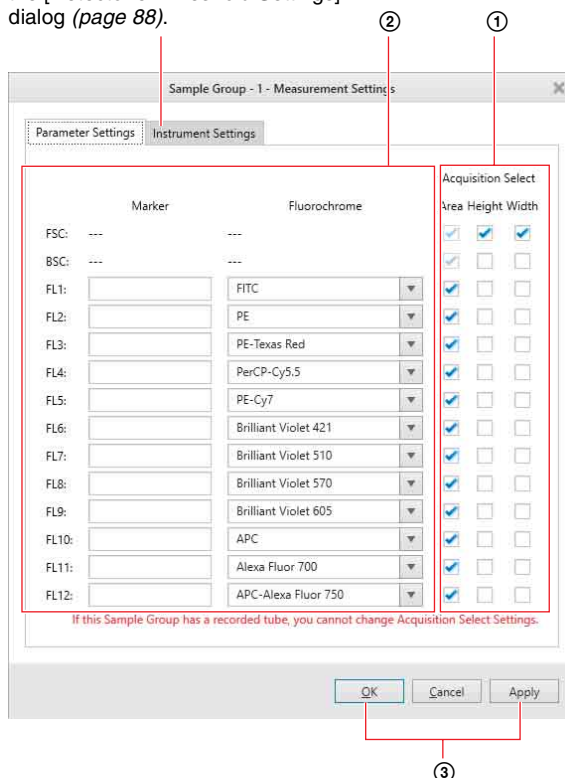
- 1 Expand the target experiment in the Experiment Explorer, and double-click [Measurement Settings] in the sample group you want to configure.



The [Measurement Settings] dialog appears.

- 2 Set each item.

These parameters are configured in the [Detector & Threshold Settings] dialog ([page 88](#)).



- ① Select the pulse parameters (Area, Height, Width) for each detection channel.
The LE-MA900 supports the acquisition of three pulse parameters (area, height, width) in the detector for each of the input channels (FSC, BSC, FL1 to FL12) for each tube in a sample group. You select the detection channels for

which to acquire data by selecting acquisition pulse parameters for those channels. Any combination of pulse parameters can be selected for each detector channel.

Tips

- The Area, Height, and Width pulse parameters are selected by default for the FSC detector channel. Only the Area pulse parameter is selected by default for all other detector channels.
- The Area pulse parameters for the FSC and BSC detector channels are always enabled. Only detector channels with a pulse parameter selection will appear in the compensation panel.
- FSC, BSC, and FL1 to 5 have a common value for the Width (W) pulse parameter, and FL6 to 12 also have a common value.

- ② Specify a marker name and/or fluorochrome name for each fluorescence detector channel. You can also enter a fluorochrome name directly from the keyboard. Marker names and fluorochrome names, if specified, appear in the axis labels on plots, and fluorochrome names appear for control tubes in the compensation panel.

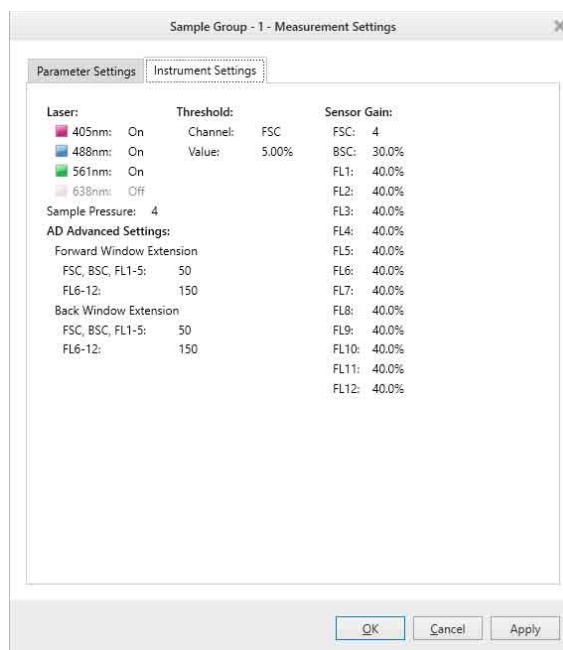
Tips

- Forward scatter (FSC) and back scatter (BSC) are measured using the incident laser light, not fluorescent light. Fluorochrome names and marker names cannot be entered for FSC and BSC.
- Fluorochrome names and marker names can be modified before, during, or after data acquisition.
- Marker names and fluorochrome names are optional, but recommended. If names are not specified, the fluorescence channel numbers (FL1 to FL12) will be displayed as the axis labels on plots by default, providing limited feedback as to the type of data displayed on plots.

- ③ Click [Apply] or [OK] to apply the measurement settings. Clicking [OK] applies the settings and closes the dialog.

Tip

The parameters on the [Instrument Settings] tab are configured in the [Detector & Threshold Settings] dialog (page 87).



Editing an Experiment

You can modify an existing experiment, as required. You can also edit an experiment using the [Experiment] tab of the ribbon.

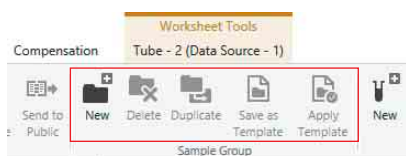
For details about tools available on the [Experiment] tab, see “[Experiment] Tab (ribbon)” (page 132).

Editing a Sample Group

You can add, delete, and perform other edit functions on a sample group in an experiment.

Edit operations are performed using buttons in the [Sample Group] group on the [Experiment] tab of the ribbon.

You can also perform the same operations by right-clicking a sample group in the Experiment Explorer.



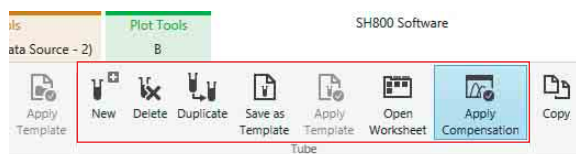
For details about each button, see “[Sample Group] group” (page 132) on the [Experiment] tab of the ribbon.

Editing a Sample Tube

You can add, delete, and perform other edit functions on a sample tube in an experiment.

Edit operations are performed using buttons in [Tube] on the [Experiment] tab of the ribbon.

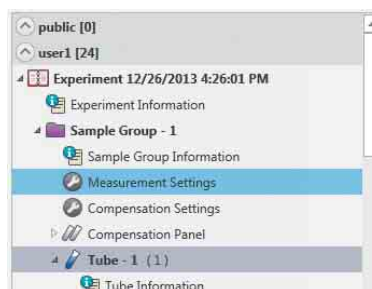
You can also perform the same operations by right-clicking a sample tube in the Experiment Explorer.



For details about each button, see “[Tube] group” (page 133) on the [Experiment] tab of the ribbon.

Changing Component Settings

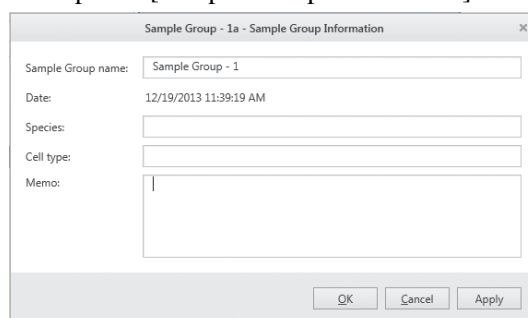
- 1 Expand the target experiment in the Experiment Explorer, and double-click the component you want to configure.



The corresponding dialog for the selected component appears.

- 2 Specify each parameter, then click [Apply] or [OK].

Example: If [Sample Group Information] is selected



For details about items displayed in the dialogs, see “Experiment Explorer” (page 152).

For details about [Measurement Settings], see “Configuring an Experiment” (page 81).

Exporting/Importing an Experiment

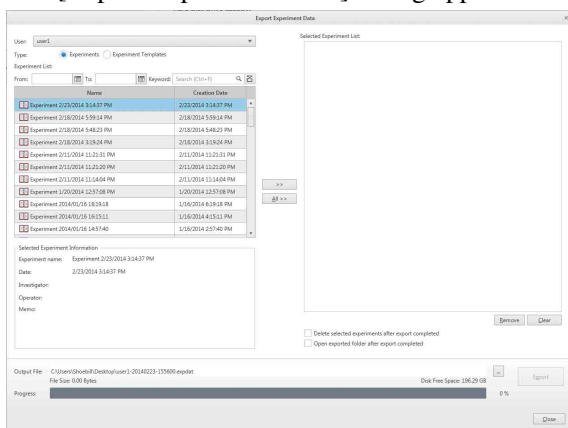
You can import and export experiment data to back up the data, to reopen a previous experiment, or to review the experiment on another LE-MA900 system.

Exporting an Experiment

When data acquisition for an experiment is finished, the data for the experiment can be exported to an external file.

- 1 Click [Database] on the [File] tab of the ribbon, then click [Export].

The [Export Experiment Data] dialog appears.




- 2 Select the owner of the data in [User], and select to export experiments or templates using the [Type] radio buttons.
- 3 Select an experiment you want to export in [Experiment List], and click [>>] to move it to [Selected Experiment List].

Clicking [All >>] moves all the experiments to [Selected Experiment List].

Tips

- To remove the exported experiments from the Experiment Explorer after the export is finished, place a check mark in [Delete selected experiments after export completed].
- To open the export destination folder after the export is finished, place a check mark in [Open exported folder after export completed].

- 4 Click  (Browse) to specify the destination folder in which the experiment data is to be exported.

- 5 Click [Export].

The export status is displayed in the progress bar. A confirmation dialog appears when exporting is completed.

- 6 Click [OK].

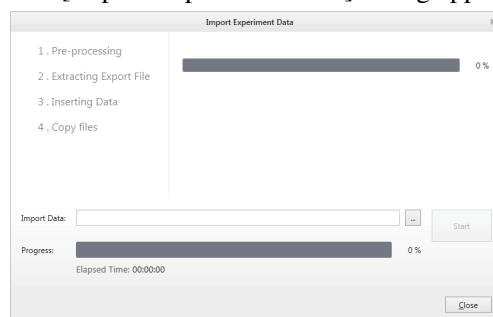
- 7 Click [Close] to close the dialog.


Importing an Experiment

You can import previously exported experiment data into the database.

- 1 Click [Database] on the [File] tab of the ribbon, then click [Import].

The [Import Experiment Data] dialog appears.



- 2 Click  (Browse) to specify the folder where the experiment data you want to import (.expdat file) is stored.

- 3 Click [Start].

The import status is displayed in the progress bar. A confirmation dialog appears when importing is completed.

- 4 Click [OK].

- 5 Click [Close] to close the dialog.

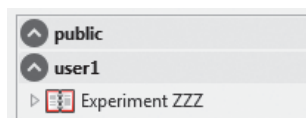
Sharing Experiments

You can share experiments with other users. Shared experiments allow multiple users to work with the experiment data on worksheets.

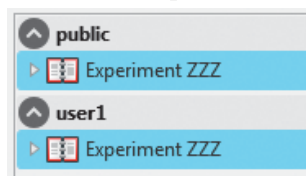
To share an experiment

- 1 Select the experiment you want to share in the Experiment Explorer, and click [Send to Public] on the [Experiment] tab of the ribbon.

Alternatively, you can right-click an experiment and select [Send to Public] from the context menu.



The shared experiment is duplicated in the [public] node in the Experiment Explorer.




Tip

Other users can access the shared experiment under the [public] node. After an experiment is shared, the shared experiment and the original private experiment exist as independent experiments, with no interaction between the two.

Searching Experiments

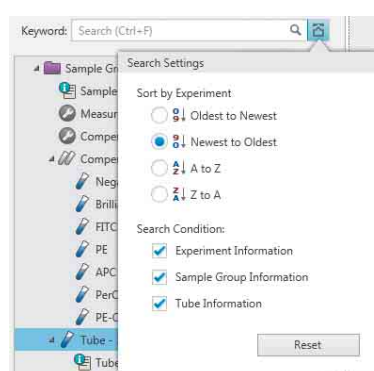
You can search experiments by date and keyword.

- 1 Enter the search criteria in the Experiment Explorer of the [Experiments] tab on the left side of the main window.

Clicking  displays a calendar for specifying a date.



- 2 Click  to set optional addition search options.



[Oldest to Newest]: Displays the search results in chronological order from oldest to newest.

[Newest to Oldest]: Displays the search results in chronological order from newest to oldest.

[A to Z]: Displays the search results in ascending alphabetical order.

[Z to A]: Displays the search results in descending alphabetical order.

[Search condition]: Selects the experiment components to search for the entered text keyword.

[Reset]: Resets the search options to their defaults.

Experiments matching the search criteria are displayed in the Experiment Explorer.

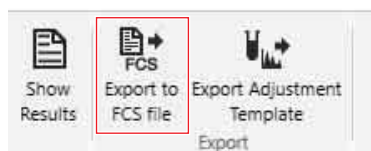
Saving Tube Data as FCS Files

You can export data recorded for tubes in flow cytometry industry standard FCS 3.0 or FCS 3.1 format for use in third-party applications.

Note

Cell Sorter Software does not support the importing of FCS format files.

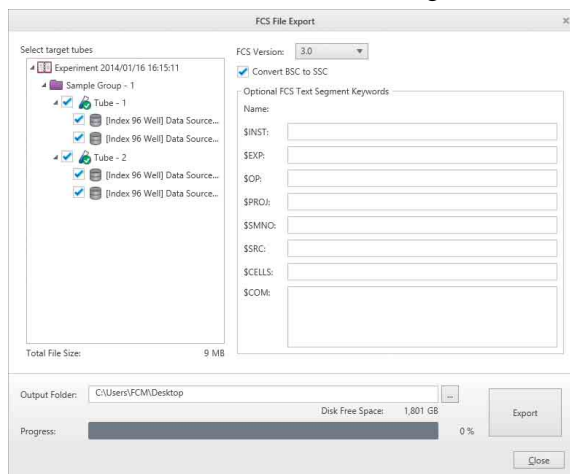
- 1 Select the target tube in the Experiment Explorer on the [Experiments] tab on the left of the main screen.
- 2 Click [Export to FCS file] in the [Export] group on the [Experiment] tab of the ribbon.



The [FCS File Export] dialog appears.

- 3 Place check marks in [Select target tubes] for tubes whose data is to be exported, specify the FCS format and export destination folder, then click [Export].

You can also select the required data source if there is more than one data source for a single tube.



The tube data is exported.

The name of the exported file is the same as the name of the tube, with an “.fcs” filename extension.

Tips

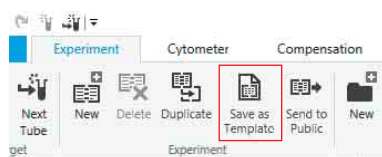
- [Convert BSC to SSC]: Select to convert the data name of back scatter data from BSC (back scatter) to SSC (side scatter) when creating FCS files.
- The required FCS text segment keywords are exported automatically. The following optional FCS text segment keywords can also be specified.

Keyword	Description
\$INST	Institution at which data was acquired
\$EXP	Name of investigator initiating the experiment
\$OP	Name of flow cytometer operator
\$PROJ	Name of the experiment project
\$SMNO	Specimen (e.g., tube) label
\$SRC	Source of the specimen (patient name, cell type)
\$CELLS	Description of objects measured
\$COM	Comment

Saving an Experiment as a Template

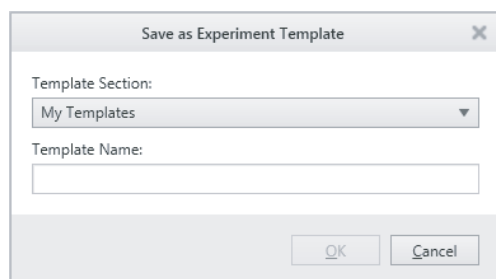
You can save an experiment, sample group, or sample tube as a shared template (Public Templates) or a private template (My Templates.)

- 1 Select the target experiment in the Experiment Explorer on the [Experiments] tab on the left of the main screen.
- 2 Click [Save as Template] in the [Experiment] group on the [Experiment] tab of the ribbon.



The [Save as Experiment Template] dialog appears.

- 3 Select the template type, enter a name, and then click [OK].



The experiment is saved as a template.

Tip

Overlay displays are not saved as templates.

Configuring Detector Settings

The following instrument settings must be optimized in an experiment to acquire accurate data.

- Trigger threshold channel
- Trigger threshold value
- Sensor gain of the forward scatter, back scatter, and fluorescence detectors

The instrument settings are saved with the sample group in an experiment. Duplicating an experiment or creating a new experiment using templates will load the saved instrument settings into the new experiment. Running an experiment using the same instrument settings as a previous experiment enables direct comparisons to be made between the results obtained, even if the experiments were conducted at different times.

Note

The gain parameter values directly affect the fluorescence compensation calculation of the spillover matrix. Do not change the gain of the channel detectors after or while recording the control tubes in a compensation panel. The fluorescence compensation process is valid only for the instrument settings in place when all control tubes in a compensation panel are recorded. If the gain of a detector for any channel is changed for any reason after recording the control tubes, the fluorescence compensation will no longer be valid and the control tubes will need to be recorded again.

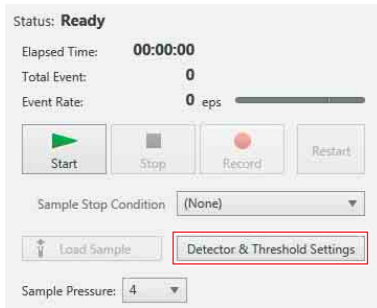
For details about recording control tubes in a compensation panel, see “Compensating Fluorescence Spillover” (page 66).

Basic Configuration Method

You can also configure many of the same parameters using the [Compensation Wizard] and on the [Control] tab of the [Cytometer Settings] dialog.

For details, see “Compensating Fluorescence Spillover” (page 66) and “[Cytometer Settings] dialog” (page 139).

- 1 Click [Detector & Threshold Settings] in the [Acquisition] tab of the Experiment/Acquisition control pane.



The [Detector & Threshold Settings] dialog appears.

2 Set each item.



[Threshold]

Specifies the threshold for event detection. You can specify up to two channels for the trigger condition.

[Condition]: Specifies the handling of the two trigger channels.

- **[And]:** Detects events that simultaneously satisfy both triggers.
- **[Or]:** Detects events that satisfy either one or the other of the two triggers.

[Channel]: Selects the channel (FSC, BSC, FL1 to FL12) used to trigger the detection of events. When a second trigger is specified, select a channel for the 2nd trigger that is different to the channel of the 1st trigger. The default values of the 1st and 2nd triggers are [FSC] and [(None)], respectively, but a new default value can be set as required using [Save as Default].

[Value]: Sets the threshold value for the trigger channel as a percentage of the output signal of the detector for the trigger channel. The default value

is 5%, but a new default value can be set as required using [Save as Default].

[Sensor Gain]

Specifies the sensor gain of the detectors.

[FSC]: Specifies the gain of the forward-scatter (FSC) detector. The gain setting has a 16-step range.

[BSC]: Specifies the gain of the back scatter (BSC) detector as a percentage of the output signal of the detector. The default value is 30.0%.

[FL1] to [FL12]: Specifies the gain of the corresponding fluorescence channel detector as a percentage of the output signal of the detector. The default value is 40.0%.

[Save as Default]: Saves the current settings in the [Detector & Threshold Settings] dialog as the default values.

The default values are used when creating an experiment or sample group using the Blank Template. The default values can also be configured in the [User Preference] dialog (*page 121*).

[Load Default]: Overwrites the current settings in the [Detector & Threshold Settings] dialog using the default values saved using the [User Preference] dialog. After loading, you can change the default values using [Save as Default]. [Load Default] is disabled if the default values have not been changed.

[Set]: Clicking this button displays the [User Preference] dialog (*page 121*) for setting user default values for the [Detector & Threshold Settings] dialog.

Tips

- Only the gain of fluorescence channels that have pulse parameters selected for acquisition can be adjusted. The gain settings of other channels are disabled.
- If the gain is too high, the detector output circuit may become saturated, providing incorrect signal output. In this case, reduce the gain and then readjust the instrument settings to find the optimum operating point.

3 Click [X] to close the dialog.

The settings are saved.

Loading Reference Data Method

You can load data acquired on another instrument as reference data in order to obtain equivalent measurement results.

Note

If the instrument is configured using this method, use the same kind of sample as the sample used to obtain the

reference data. Note that because the detector characteristics will vary from instrument to instrument, data identical to the reference instrument data cannot be obtained.

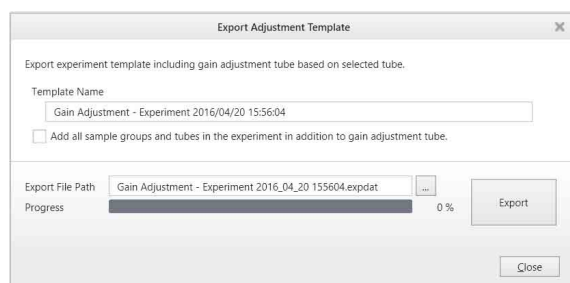
Creating a gain adjustment template

You can create a gain adjustment template (template for instrument settings) for an experiment that includes data used as reference data. The experiment template contains a gain adjustment tube which includes the reference data.

- 1 In the Experiment Explorer on the [Experiments] tab, right-click a tube with acquired data to use as the reference, and select [Export Adjustment Template] from the context menu.

The [Export Adjustment Template] dialog appears.

- 2 Enter a name for the template in [Template Name], enter the folder path and file name in [Export File Path], and click [Export].



An experiment template file containing the reference data is exported.

Creating an experiment

You can create an experiment and apply instrument settings using an experiment template with gain adjustment tube.


Use the following procedure to configure the instrument.

- 1 Import an experiment adjustment template file containing reference data.

The imported template is added to [My Templates].

For details about importing a template, see “Importing an Experiment” (page 84).

- 2 Create a new experiment using the imported experiment adjustment template.

The gain adjustment tube is indicated by the  icon.

For details about creating an experiment, see “Creating an Experiment” (page 53).

- 3 Assign the gain adjustment tube as the active tube and perform data acquisition.

For details, see “Acquiring Data” (page 55).

- 4 Configure the detectors based on the reference data.

Configure the detector settings, including gain adjustment and threshold value adjustment.

Two types of data, the reference data and acquired data are displayed on a plot for the gain adjustment tube.

Adjust the detectors until the acquired data matches the reference data.

For details about detector settings, see “Basic Configuration Method” (page 87).

- 5 Update the detector default values as required.

Click [Save] in the [Detector & Threshold Settings] dialog to save the current settings as the default values.

Tip

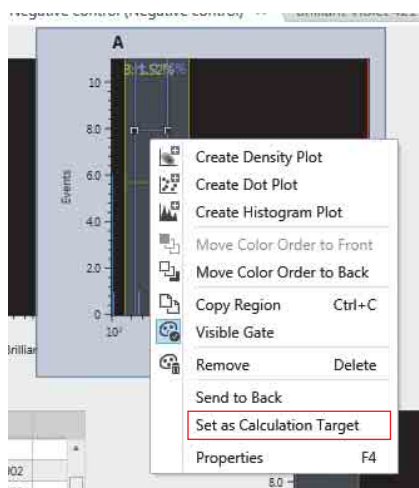
There are various other methods that can be used to optimize the detector gain settings for an experiment. For example, a more rigorous approach to detector gain optimization might be to measure the coefficient of variation (CV) for positive controls at various detector gain levels to determine the minimum gain levels for which the CV plateaus off in each detection channel sensor. A detector gain set at this minimum gain level will provide the optimal signal-to-noise ratio required for maximum sensitivity and maximum separation between negative and positive peaks.

Adjusting Fluorescence Compensation

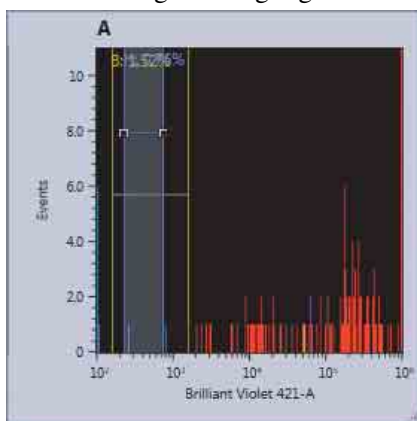
Using a Target Gate in the Gate Hierarchy

You can specify a target gate within the gate hierarchy to be used as the negative control region or as a singly-stained positive control region for calculating the compensation matrix.

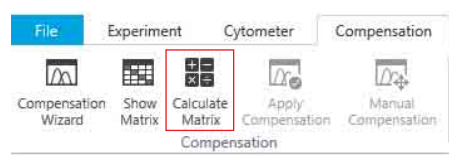
- 1 Right-click a gate on a plot for a control tube in the compensation panel to set the gate as the calculation target for the compensation matrix, and select [Set As Calculation Target] from the context menu.



The selected gate is highlighted.

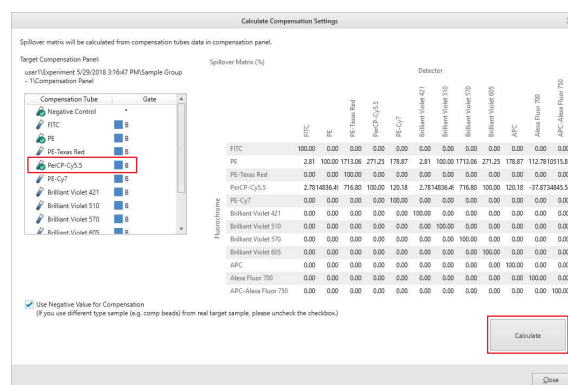


- 2 Click [Calculate Matrix] on the [Compensation] tab of the ribbon.



The [Calculate Compensation Settings] dialog appears.

- 3 Check that the specified gate is the target of the calculation, and click [Calculate] to calculate the spillover matrix.



- 4 Click [Close] to close the dialog.

Adjusting the Spillover Matrix Manually

You can modify the compensation settings after using the [Compensation Wizard] or you can enter fluorescence compensation values manually to edit the spillover matrix directly.

Tips

- You can save the current fluorescence compensation settings using the [Compensation Settings] dialog.

For details about displaying the [Compensation Settings] dialog, see “Manually adjusting the spillover matrix” (page 91).

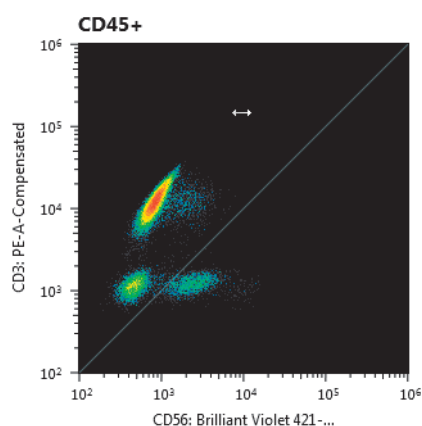
- Adjusting the position of events shown on a plot also adjusts the position of all events on other plots. Accordingly, you should add plots for all fluorescence parameters to the worksheet before adjusting compensation manually in order to monitor the affect of adjusting the compensation on each plot.

To adjust compensation graphically on plots

You can adjust fluorescence compensation graphically on the worksheet using a mouse.

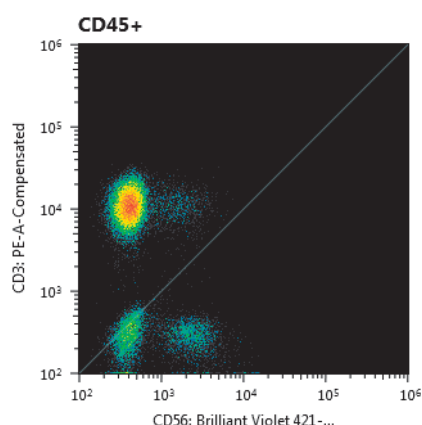
- 1 Display the worksheet for the tube whose compensation you want to modify.
- 2 Enable [Apply Compensation] on the [Compensation] tab of the ribbon.
- 3 Click [Manual Compensation] on the [Compensation] tab of the ribbon to activate manual compensation mode.

A diagonal line appears on plots that have fluorescence parameters on the X and Y axes.



- 4 Place the mouse cursor over the plot, and drag the plot to change compensation for the target parameter.

The mouse cursor changes to a horizontal double-ended arrow cursor in the left half of the plot, and a vertical cursor in the right half of the plot. The displayed events move on the plot to reflect the changed compensation.



- 5 When finished, click [Manual Compensation] again to deactivate manual compensation mode.

You can also deactivate manual compensation mode by selecting another worksheet to display.

Tips

- Adjusting the position of events shown on a plot also adjusts the position of all events on other plots. Accordingly, you should add plots for all fluorescence parameters to the worksheet before adjusting compensation manually in order to monitor the affect of adjusting the compensation on each plot.
- The actual compensation values in the spillover matrix can be monitored simultaneously in the [Compensation Settings] dialog. It is recommended that you save the compensation settings from the [Compensation Settings] dialog before adjusting compensation manually. You can also click [Reset] in the [Compensation Settings] dialog to reset the compensation settings.

Manually adjusting the spillover matrix

You can adjust the fluorescence compensation manually in the [Compensation Settings] dialog. You can adjust the values using visual feedback alone, or enter spillover matrix values directly to reproduce the compensation settings of another experiment.

Note

Editing the spillover matrix directly to adjust compensation is inherently difficult. Exercise care when editing the spillover matrix directly.


- 1 Display the worksheet for the tube whose compensation you want to modify.
- 2 Click [Apply Compensation] on the [Experiment] tab of the ribbon.
- 3 Display the [Compensation Settings] dialog by doing one of the following.

- Select [Compensation Settings] in the Experiment Explorer and click [Show Settings] on the [Experiment] tab of the ribbon.
- Double-click [Compensation Settings] in the Experiment Explorer.
- Select any component within the sample group and click [Show Matrix] on the [Compensation] tab of the ribbon.

The [Compensation Settings] dialog appears.

- 4 Click a cell in the matrix to select its value.

You can adjust a value using one of the following methods.

- Turn the mouse scroll wheel button.
- Enter a value directly in the spillover matrix.
- Click the  spin boxes.
- Drag the slider.

Sample Group: 1 - Compensation Settings

Spillover Matrix (%)

	APC	PE	PE-Cyan Red	PerCP-Cy5.5	PerCP	Brilliant Violet 421	Brilliant Violet 570	Brilliant Violet 570	Brilliant Violet 605	APC	APC-Alexa Fluor 700
APC	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PE	2.81	100.00	200.00	200.00	178.87	2.81	100.00	200.00	178.87	112.78	200.00
PE-Cyan Red	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PerCP-Cy5.5	2.78	200.00	200.00	100.00	120.15	2.78	200.00	200.00	180.00	120.15	27.87
PerCP	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 421	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 570	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
Brilliant Violet 605	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
APC-Alexa Fluor 700	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00

Save Load Reset

Close

The plots on the worksheet are updated in real-time.

Tips

- The number of fluorochromes in the experiment determines the dimension of the spillover matrix. Spillover elements can be modified, but they cannot be added nor deleted.
- To save the current compensation settings, click [Save]. To load compensation settings for the sample group, click [Load].
- To reset the compensation settings, click [Reset].

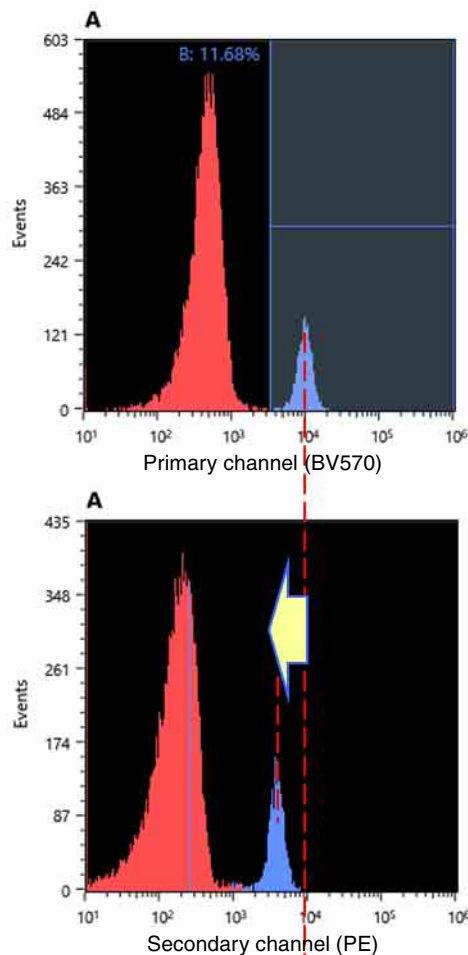
5 Click [Close] to close the dialog.

Cross beam excitation

When using tandem fluorochromes, fluorescence leakage may occur between lasers with different optical axes.

Setting up compensation for cross beam excitable fluorochromes

When performing multicolor experiments using tandem fluorochromes, cross beam excitation may be observed with some fluorochrome combinations. For example, the Brilliant Violet 570 (BV570) and phycoerythrin (PE) share a similar emission spectrum. BV570 can be excited by 405nm laser and partially excited by the 561 nm laser. Therefore when using these two fluorochromes together in a multicolor panel, the spillover should be correctly compensated. To reduce the spillover of BV570 into the PE channel, ensure that the PMT gains for the primary (BV570) and secondary (PE) channel is set so that the positive peak of the BV570 control has a higher mean fluorescence intensity in the primary channel than the secondary. Ideally, this difference should be at least half a log decade. Setting the voltages in this manner reduces the spillover and results in less spreading error.



Note

When the PMT gain is adjusted, all control tubes must be recorded again.

Data is analyzed using various tools available on the worksheet.

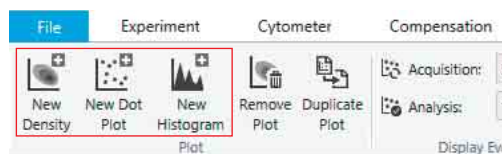
This chapter describes gate settings used for analysis of the acquired data and for sorting, generating reports of data analysis, and related tasks.

For details about content displayed on the main window, see “*Main Window*” (page 132).

Adding a Plot

You can add plots to the worksheet.

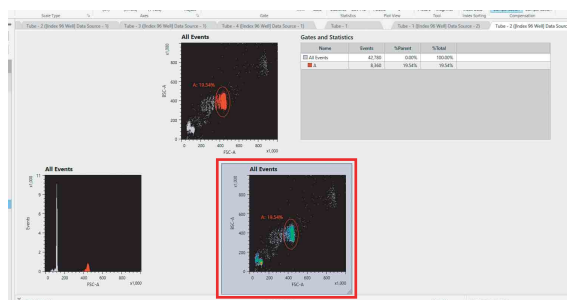
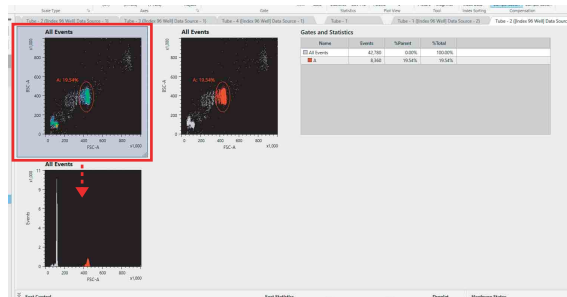
- 1 Click a button for a new plot of the desired plot type to be added in the [Plot] group on the [Worksheet Tools] tab or the [Plot Tools] tab of the ribbon.



The plot is added to the worksheet. The new plot created represents the [All Events] population.

- 2 Change the layout of plots, as required.

You can change the position of plots by selecting the target plot and dragging it to a new location.



Edit the plots, add gates, and perform other actions on plots, as required.

See “*Editing Plots*” (page 94) and “*Adding Gates*” (page 99).

Editing Plots

Cell Sorter Software displays data on the following types of plots. You can change the plot type, change the axis scale, and modify other aspects of plots.

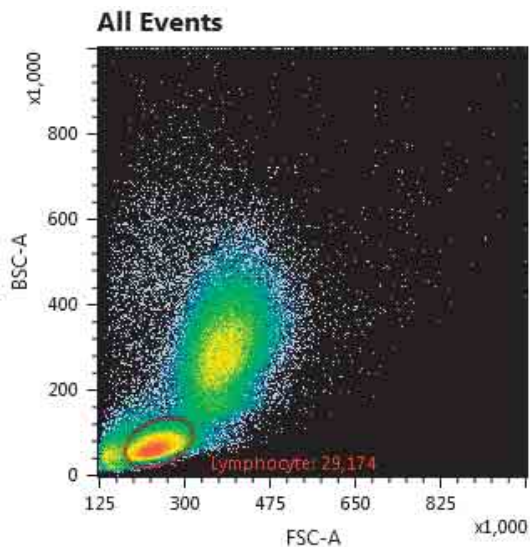
For details about the buttons on the [Plot Tools] tab of the ribbon, see “[Plot Tools] Tab (ribbon)” (page 148).

For details about adding gates to a plot, see “Adding Gates” (page 99).

Density plots

Density plots display a frequency distribution of fluorescence intensity for two acquisition parameters (bivariate distribution).

Density plots use color to show the distribution of the number of detected events having the same value. High-density regions are shown in red, low-density regions are shown in violet, and regions of intermediate density are shown using the colors of the visible spectrum in between. Density plots are most useful for displaying populations where the number of detected events is relatively large.



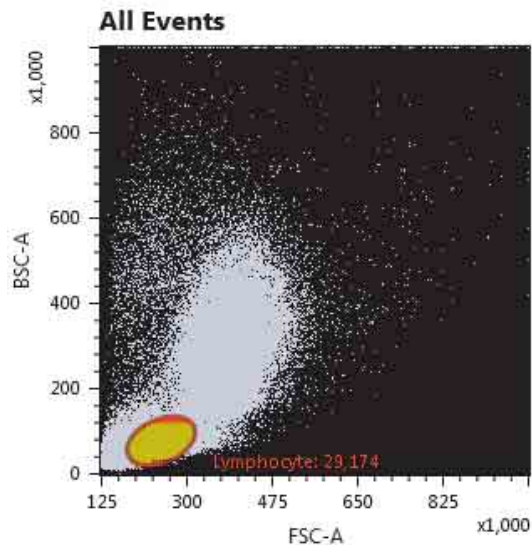
Dot plots

Dot plots display a frequency distribution of fluorescence intensity for two acquisition parameters (bivariate distribution).

Dot plots are most useful for displaying populations where the number of detected events is relatively small. Events on a dot plot are colored based on their classification (gate and population).

Tip

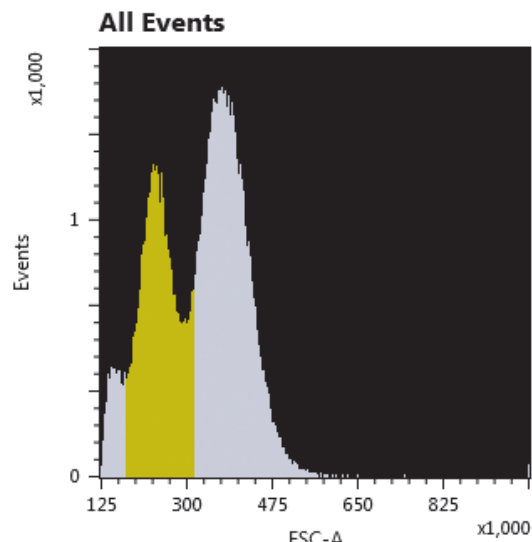
As the number of events on a dot plot increases, the plot may become saturated making it difficult to discern patterns. In this case, reduce the number of events displayed or use a density plot to view the data.



Histogram plots

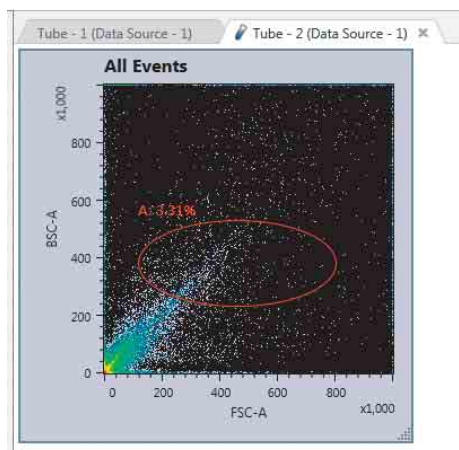
Histogram plots display a frequency distribution of fluorescence intensity for a single acquisition parameter (univariate distribution).

Histogram plots are effective for identifying the peaks and troughs in measurement data.



Changing Plot Type

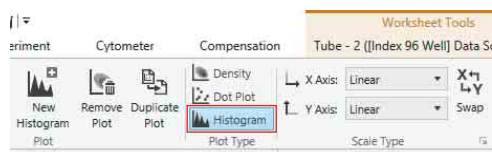
- 1 Select a plot whose type you want to change on the worksheet.



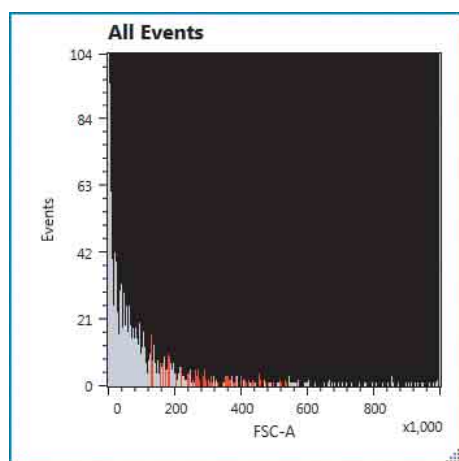
- 2 Click the target plot type in the [Plot Type] group on the [Plot Tools] tab of the ribbon.

You can also right-click a plot and select a plot type from the [Plot Type] submenu of the context menu.

For example, the diagram below shows a density plot being changed to a histogram plot.



The type of the selected plot is changed.



Tip

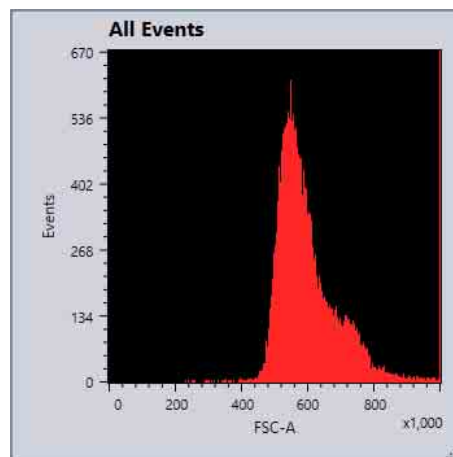
The plot type of an overlay display plot cannot be changed. To change the plot type, first remove the overlay.

For details about operation, see “Deleting Overlay Layers” (page 106).

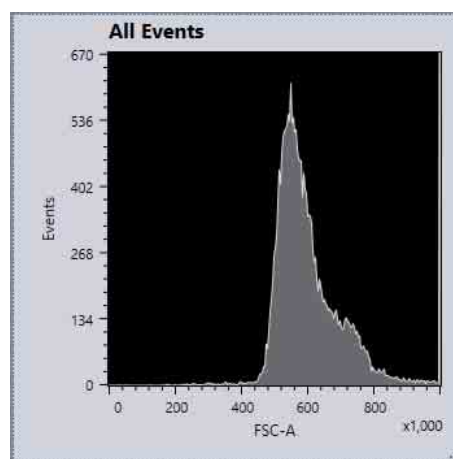
Changing the Histogram Display View

You can select the following display views for histogram plots.

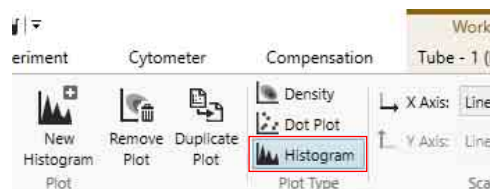
Filled area view



Outline view



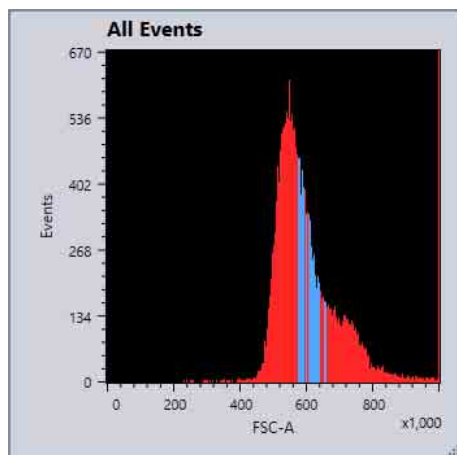
Select a histogram plot on the worksheet, and click [Histogram] repeatedly in the [Plot Type] group on the ribbon to toggle between filled area view and outline view.



About color display of events

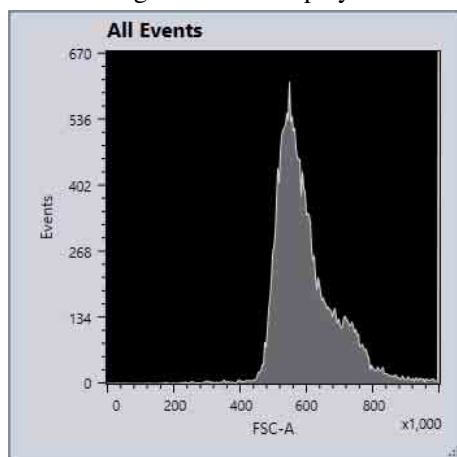
In filled area view

Events included within are gate are displayed in the specified colors.



Outline view

The colors of each gate are not displayed.



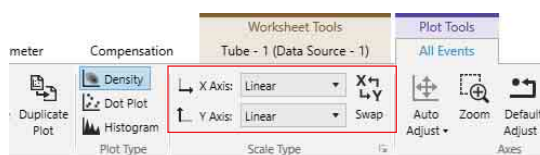
For details about each button, see “[Plot Tools] Tab (ribbon)” (page 148).

Removing a Plot

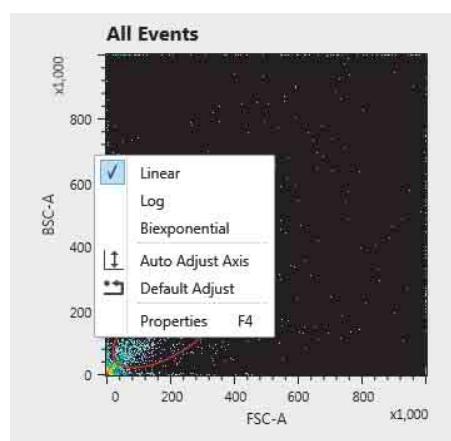
Select a plot to be deleted on the worksheet, then click [Remove Plot] in the [Plot] group on the [Plot Tools] tab of the ribbon.

Changing Axis Scale Type

Select a plot on the worksheet, then click the desired scale type for each axis in the [Scale Type] group on the [Plot Tools] tab of the ribbon.



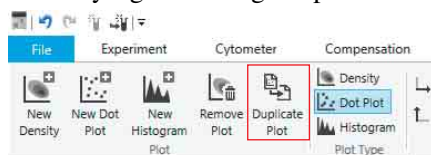
You can also change the scale by right-clicking the plot and selecting from the context menu.



Duplicating a Plot

- 1 Select a plot to be duplicated on the worksheet, then click [Duplicate Plot] in the [Edit] group on the [Plot Tools] tab of the ribbon.

You can perform the same operation from the context menu by right-clicking the plot.



The selected plot is duplicated.

- 2 Edit the duplicated plot, as required, using the buttons on the [Plot Tools] tab of the ribbon.

You can perform the same operation from the context menu by right-clicking the plot.

For details about each item, see “[Scale Type] group” (page 148).

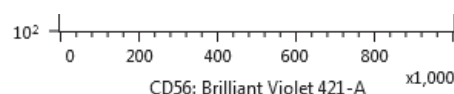
You can select the following scale types.

Tip

The axis scale type is applied only to the display of data on plots. Internally, all data is stored as linear data.

Linear

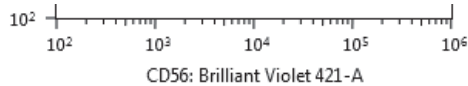
Linear scales are always used for the display of forward scatter and back scatter.



Log (logarithmic)

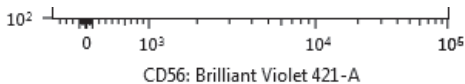
Logarithmic (log) scales are useful for displaying cells that exhibit fluorescence with intensities across a wide dynamic range. A logarithmic scale helps to make

individual peaks easier to visualize. However, log scales cannot adequately represent data with a low mean and high variance.



Biexponential

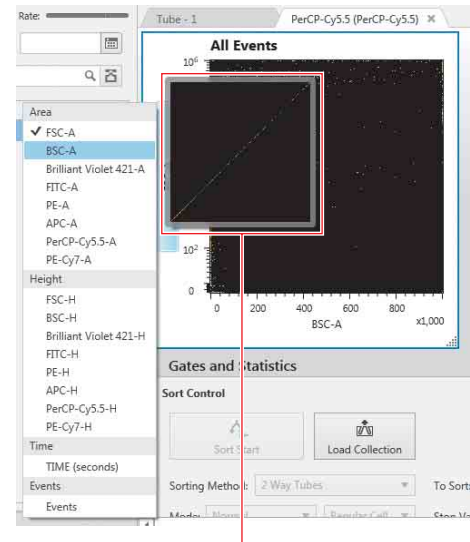
Biexponential scales combine a linear scale at the lower end of the axis (around zero) and a logarithmic scale at the higher end of the axis, with an algorithmic transition between the two scales. A biexponential scale makes it easier to analyze events close to zero or below zero that are difficult to observe on a logarithmic scale.



A common problem occurs when displaying fluorescence data on a logarithmic scale after applying fluorescence compensation. Fluorescence compensation introduces variations in measurement data due to variations in detector gain, background noise, and other factors. The result is that some data values can become close to zero or even negative. When values close to zero are plotted on a log scale, the values are wedged up against the axis making them difficult to discern. In addition, values below zero cannot be displayed at all. This can lead to these events, which are real events, being excluded from gates or other analysis. It can also lead to inaccurate fluorescence compensation when attempting to adjust compensation manually using visual cues. In these cases, a biexponential scale can be used to avoid some of the pitfalls of logarithmic scales, while maintaining the overall utility of logarithmic scales.

Changing the Axis Parameters

Click the axis title and select the desired parameter from the popup menu.

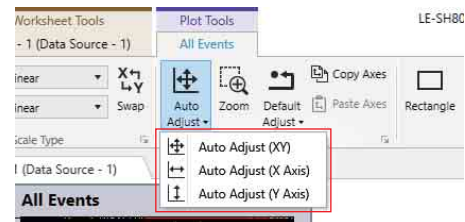


A preview of the plot appears as the mouse hovers over each parameter.

Adjusting Scale Ranges

To adjust a scale range automatically

Select a plot, then click [Auto Adjust] in the [Axes] group on the [Plot Tools] tab of the [Plot Tools] ribbon, and select the auto adjustment method.




[Auto Adjust (XY)]: Automatically adjusts the scale of the X axis and Y axis.

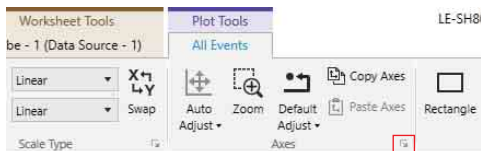
[Auto Adjust (X Axis)]: Automatically adjusts the scale of the X axis only.

[Auto Adjust (Y Axis)]: Automatically adjusts the scale of the Y axis only.

To specify scale ranges manually

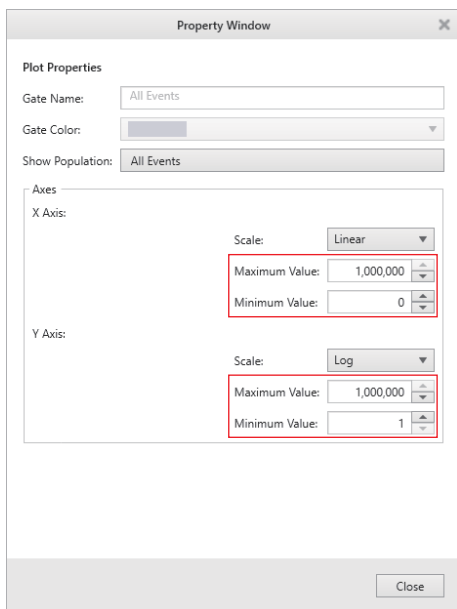
- 1 Select a plot, then click the  dialog launcher in the [Axes] group on the [Plot Tools] tab of the ribbon.

You can also right-click a plot, and select [Properties] in the context menu to display the [Property Window] dialog.



The [Property Window] dialog appears.

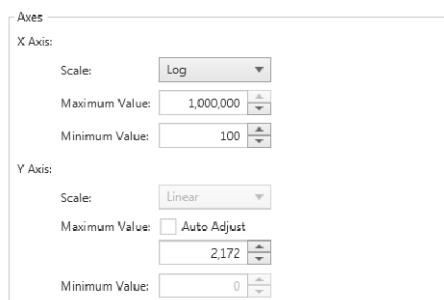
- 2 Enter values for the minimum and maximum values for the X axis and Y axis in [Axes].



- 3 Click [Close] to close the dialog.

Tip

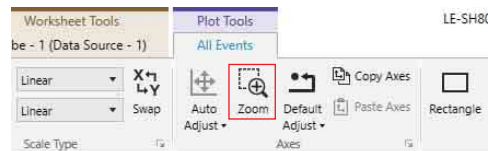
On histogram plots, by default the Y-axis range is adjusted automatically according to the data values, but the range can also be set manually. To set the range manually, clear the [Auto Adjust] checkbox, and enter the maximum value of the Y-axis manually. The minimum value is fixed at 0. Placing a check mark in the [Auto Adjust] checkbox selects automatic adjustment again.




To zoom in using a mouse

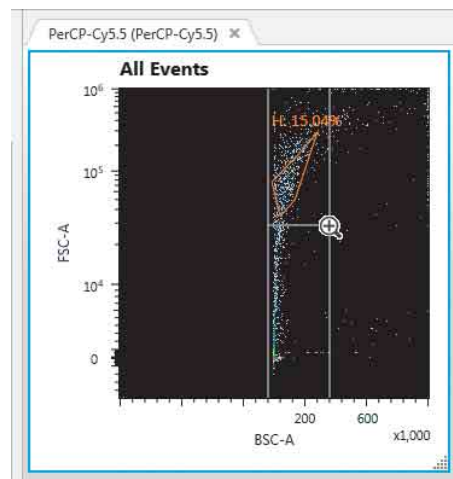
You can enlarge a portion of the plot you want to display using the mouse.

- 1 Select a plot, then click [Zoom] in the [Axes] group on the [Plot Tools] tab of the ribbon.

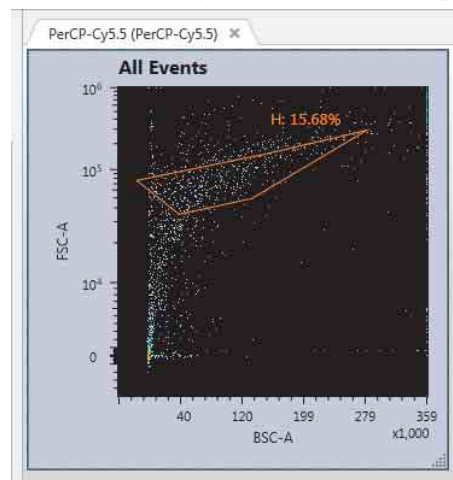


The mouse cursor changes to  when the mouse is placed over the selected plot.

- 2 Drag a selection rectangle over the portion of the plot to display then release the mouse.



The selected range is scaled to fill the plot area.



Tip

Gates change shape on plots to track the scale type and zoom factor, but may not be always reproduced accurately. In particular, errors may occur with polygon gates and ellipse gates on the linear scale or the biexponential scale.

Adding Gates

You can isolate populations of events by drawing gates around the events on plots. Gates of different types (Rectangle, Ellipse, Polygon, Quadrant, Linear) are available which can be drawn to match the distribution of events. Adding a gate allows you to display a new plot representing the population of events that fall within the gate.

Gates are automatically added to the gate hierarchy in the [Gates and Statistics] table when they are added to plots.

You can also create Boolean gates which are defined by a logical combination of existing gates using Boolean logic. Boolean gates are defined not by drawing gates on plots, but by specifying an equation for the gate. The populations defined by Boolean gates can be displayed on plots, just like populations from physical gates. All statistics functions available for physical gates are also available for Boolean gates.

Physical gates and Boolean gates can be selected as the sorting criteria when sorting.

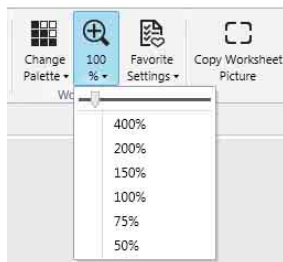
For details, see “Sorting” (page 108).

Adding a Gate

You add a physical gate directly to plots.

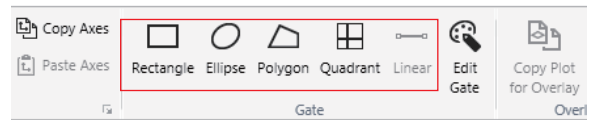
- 1 Select a plot on which to add a gate.

Change the plot zoom, as required, in the [Worksheet View] group on the [Worksheet Tools] tab of the ribbon to make it easier to draw the gate.



- 2 Click the desired gate type button in the [Gate] group on the [Plot Tools] tab of the ribbon, and draw a gate on the plot.

Alternatively, right-click the plot, then select [Create gate] and the desired shape of gate from the context menu.



[Rectangle]: Adds a rectangle gate.

[Ellipse]: Adds an ellipse gate.

[Polygon]: Adds a polygon gate. A polygon gate is added to a plot by clicking each vertex of the polygon using the mouse. Double-click to close the polygon.

[Quadrant]: Adds a quadrant gate. A quadrant gate is created on a plot by clicking the mouse at the intersection point of the four quadrants.

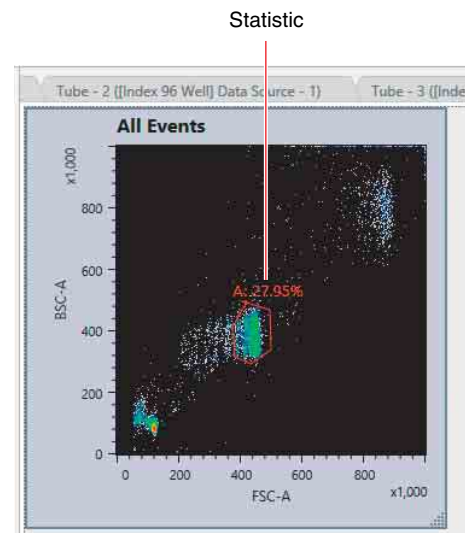
The four quadrants (“<gate_name>-Q1” to “<gate_name>-Q4”) are added to the plot.

Drag the intersection point to change the size of the quadrants.

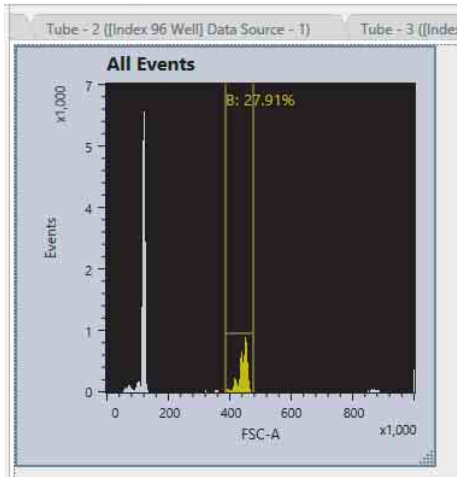
[Linear]: Adds a linear gate. Linear gates can be added to histogram plots only.

The gate is added to the plot, together with a single statistic for the gate.

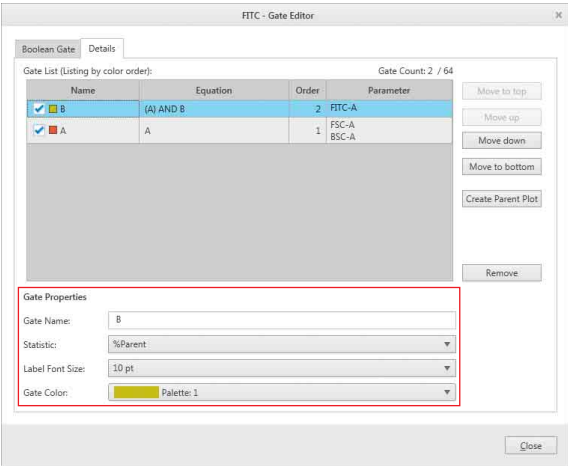
Example: Polygon gate



Example: Linear gate



- 3 Edit the name of the gate, the displayed statistic, color, and other preferences, as required.
- Select the gate, click [Edit Gate] in the [Gate] group on the [Plot Tools] tab of the ribbon to display the [Gate Editor] dialog, and then select the [Details] tab.

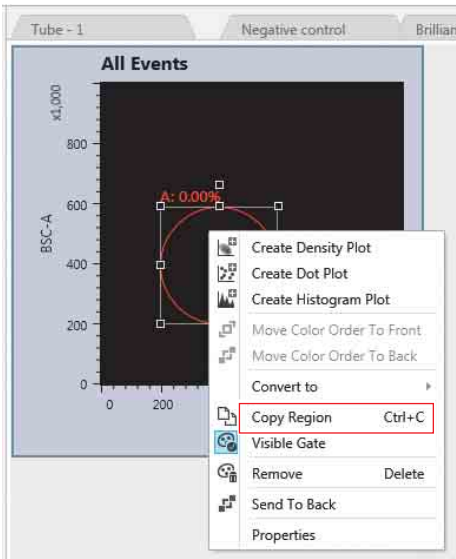


For details about each item, see “[Gate Editor] dialog” (page 146).

Copying a Gate

You can copy a gate drawn on a plot to another plot.

- 1 Right-click a gate to copy, then select [Copy Region] from the context menu.



- 2 Paste the gate on the target plot.

Tips

- You can also right-click a gate in the [Gates and Statistics] table to copy a gate within the gate hierarchy using commands in the context menu.
- Adjustment to the scale of the copy destination is not performed. It is copied as a shape.
- Only linear gates can be pasted in a histogram. And linear gates cannot be pasted in a plot other than a histogram.

Using Boolean Gates

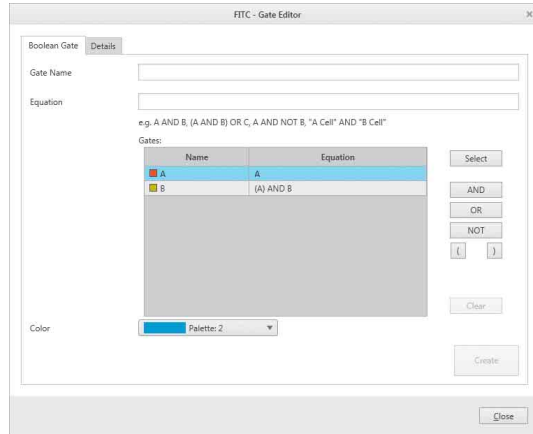
- 1 Select a plot to display the [Plot Tools] tab.
- 2 Click [Edit Gate] in the [Gate] group on the [Plot Tools] tab of the ribbon.



The [Gate Editor] dialog appears.

- 3** Enter the equation for the Boolean gate on the [Boolean Gate] tab.

Enter an equation as follows.



- 5** Set the axis labels, scale types, and other properties for the new plot.

For details, see “Editing Plots” (page 94).

- ① Click the [Equation] text box to place the cursor in the box.
- ② Select a gate in the [Gates] list and click [Select], or double-click the name of the gate. The gate is added to the equation.
- ③ Click [AND], [OR], [NOT], [(], or [)], as required. The logic operator is added to the equation. You can also enter the equation directly from the keyboard.
- ④ Repeat steps ② and ③ to build the equation for the gate.

Tip

The equations for all gates can be viewed on the [Details] tab.

- ⑤ Change the name of the Boolean gate, as required. Click in the [Gate Name] text box, and enter a new name. The names of gates must be unique.
- ⑥ Select a color for the Boolean gate from the [Color] drop-down menu.
- ⑦ Click [Create].
If the equation specified for the gate is verified as a valid definition for a gate, the gate is added to the bottom of the gate hierarchy in the [Gates and Statistics] table.
- ⑧ Click [Close] to close the dialog.

- 4** Double-click the Boolean gate in the gate hierarchy.

A default FSC v. BSC density plot with linear X axis and Y axis is added to the worksheet.

Editing Gates

You can change the shape of and settings for gates on plots.

To change the shape of a gate

Select the gate and drag the drawing handles to change the shape.

For quadrant gates, drag the intersection point of the quadrants to another position to change the gate.

To add/delete a vertex of a polygon gate, right-click the vertex point and select [Add Vertex] or [Remove Vertex] from the context menu.

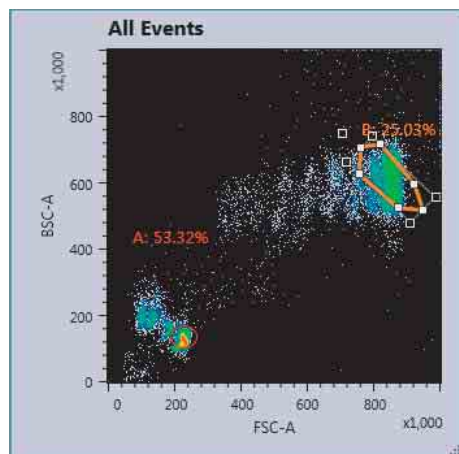
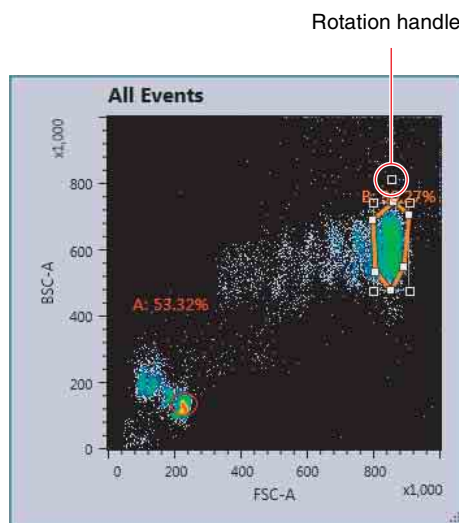
To move a gate

Select the gate and drag the gate to the desired position.

To rotate a gate

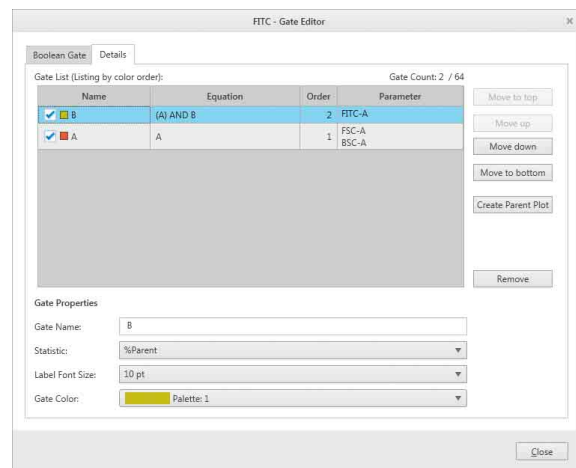
You can rotate polygon and ellipse gates.

Select a polygon or ellipse gate and drag the rotation handle to rotate the gate.



To change gate settings

Select the gate, click [Edit Gate] in the [Gate] group on the [Plot Tools] tab of the ribbon to display the [Gate Editor] dialog, and then select the [Details] tab.



For details about each item, see "[Gate Editor] dialog" (page 146).

To hide a gate

Select a gate you want to hide, then click [Visible] in the [Style] group on the [Gate Tools] tab of the ribbon.

Alternatively, right-click the gate you want to hide and select [Visible Gate] from the context menu.

To delete a gate

Select the gate to be deleted, then click [Remove Gate] in the [Gate] group on the [Gate Tools] tab of the ribbon. Alternatively, right-click the gate and select [Remove] from the context menu.

You can also delete a gate using the Delete key on the keyboard.

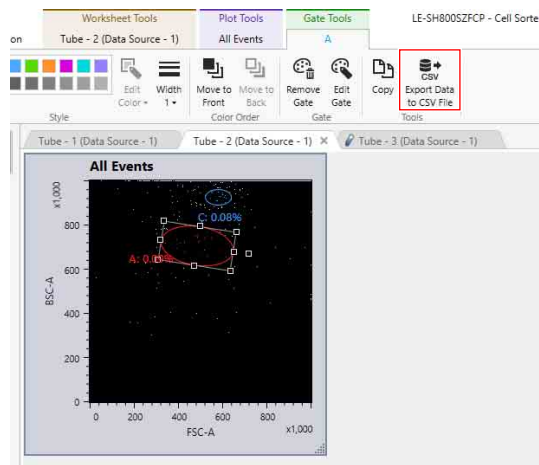
Note

Deleting a gate will delete all data and statistics for the gate, including all child gates and plots derived from the gate.


To export the event data within a gate as a CSV file

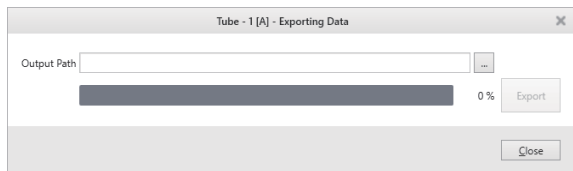
You can export the event data within a gate as a CSV file on a gate-by-gate basis.

- 1 Select a gate whose event data you want to export, then click [Export Data to CSV File] in the [Tools] group on the [Gate Tools] tab of the ribbon.



The [Exporting Data] dialog appears.

- 2 Click  (Browse) to specify the folder to save the CSV file, then click [Export].



The CSV file is exported.

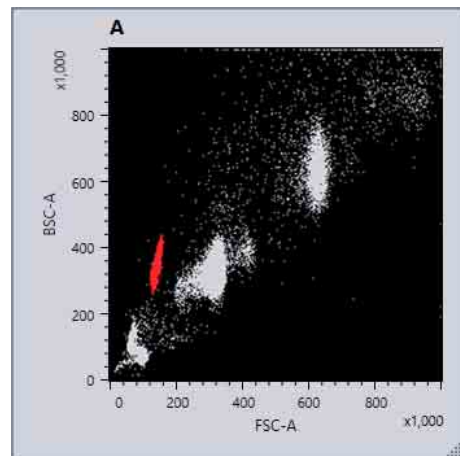
- 3 Click [Close] to close the dialog.

Displaying Multiple Plots Overlaid

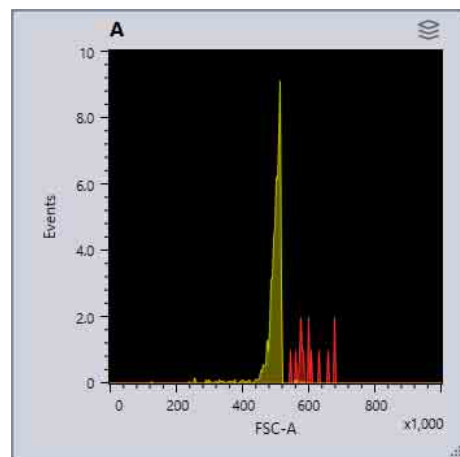
You can display the data for up to eight plots overlaid so that you can compare data acquired in an experiment. Overlay display is supported for the following combinations of plots.

		Overlay source		
		Density plots	Dot plots	Histogram plots
Overlay destination	Density plots	Yes (displayed as dot plots)	Yes (displayed as dot plots)	No
	Dot plots	Yes (displayed as dot plots)	Yes	No
	Histogram plots	No	No	Yes

Example: Dot plot + dot plot



Example: Histogram plot + histogram plot



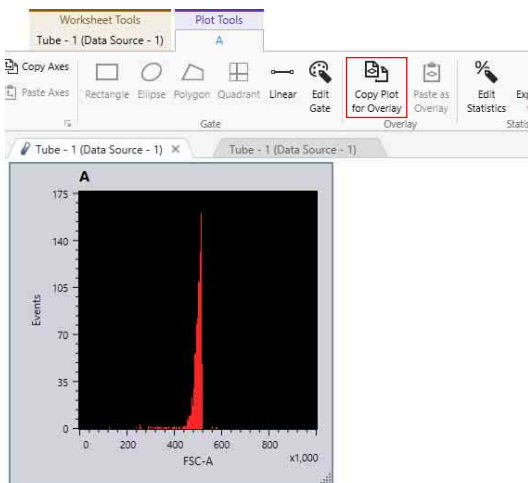
Tip

A plot of another tube can be displayed overlaid on the plot of the gain adjustment tube, but the plot of a gain adjustment tube cannot be overlaid on the plot of another tube.

Displaying Plots Overlaid (Overlay Display)

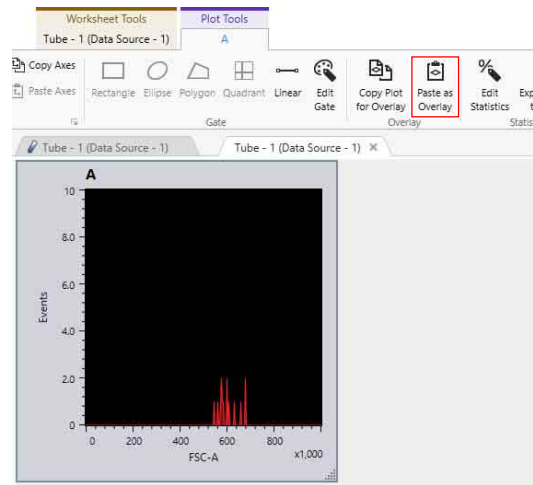
- 1 Open the worksheet of tube containing a plot that you want to display overlaid.

Open the worksheets for both the overlay source tube and overlay destination tube.
- 2 Select a plot from the overlay source, then click [Copy Plot for Overlay] in the [Overlay] group on the [Plot Tools] tab of the ribbon.

**Tips**

- [Copy Plot for Overlay] cannot be selected for a plot that has not been recorded. First acquire and record data. For details about operation, see *“Controlling Data Acquisition and Recording”* (page 56).
- [Copy Plot for Overlay] cannot be selected for the All Events plot. First add a gate, and display a plot for the gate. For details about operation, see *“Adding Gates”* (page 99).
- [Copy Plot for Overlay] cannot be selected for a plot that is displayed overlaid. First remove the overlay display. For details about operation, see *“Deleting Overlay Layers”* (page 106).

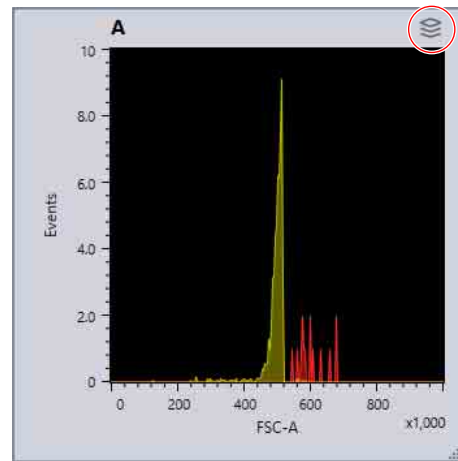
- 3 Select a plot from the overlay destination, then click [Paste as Overlay] in the [Overlay] group on the [Plot Tools] tab of the ribbon.



The plots are displayed overlaid.

During overlay display, an overlay display icon is displayed at the top right of the plots.


The scale and axes names correspond to the overlay destination.

**Tips**

- Leave the overlay source experiment open; do not close it.
- Child gates in the overlay source are not displayed.
- The layer information of the overlay source layer information is not exported when exporting the data of overlaid plots.
- The overlay source worksheet is not closed automatically when the overlay destination worksheet is closed. Close it manually.

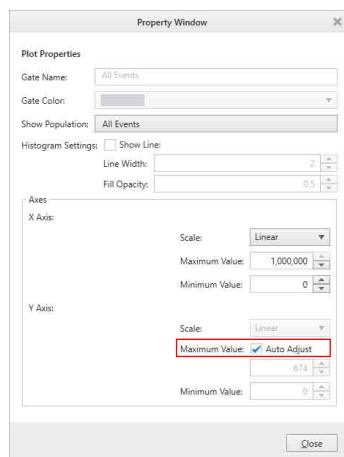
To align the scales of histogram plots

On histogram plots, you can adjust the scales automatically or switch the scales to match the overlay destination scale.

- 1 Select a histogram plot, then click the  dialog launcher in the [Axes] group on the [Plot Tools] tab of the ribbon.

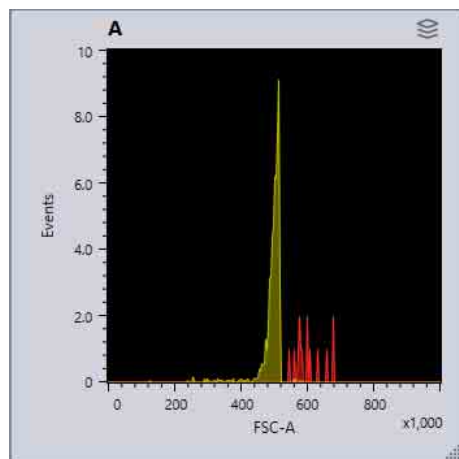
The [Property Window] dialog appears.

- 2 Select whether to adjust scales automatically using the [Auto Adjust] under [Maximum Value].



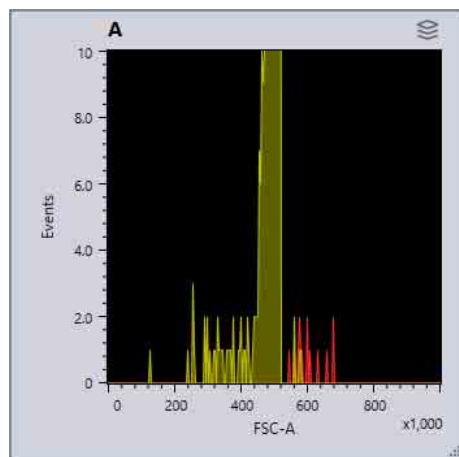
When on

The scales are adjusted automatically.



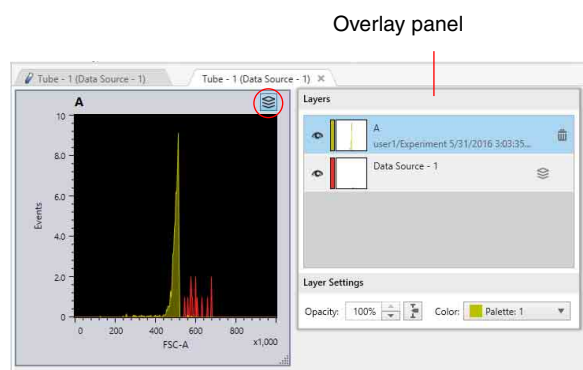
When off

The scales are aligned to the overlay destination.

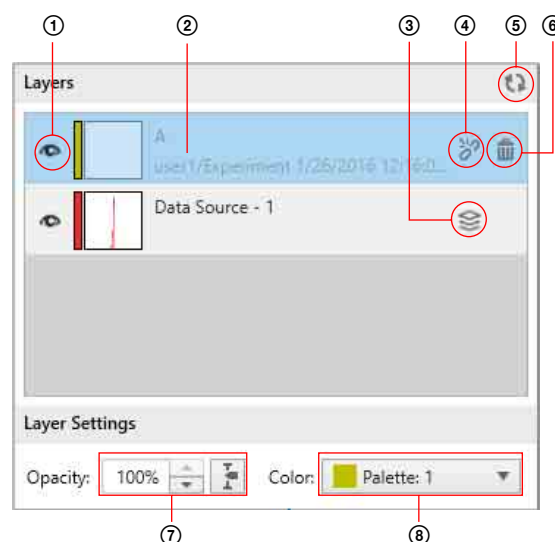


Customizing the Overlay Display

Click to display the overlay panel on the right side.



You can show/hide layers and change colors in the overlay panel.



- ① **Layer show/hide selector**
Click repeatedly to toggle between show (👁) and hide (👁).
- ② **Layer name**
Hover the mouse over the layer name to display user/experiment/sample group/sample tube/gate information.
- ③ **Base plot icon**
Indicates the base plot.
- ④ **Unload icon**
Indicates that overlay display is unavailable because the overlay source worksheet is not open.
- ⑤ **Refresh icon**
Updates the overlay display to the latest status.
- ⑥ **Delete icon**
Deletes the selected layer.
- ⑦ **[Opacity]**
Adjusts the opacity of the selected layer.

⑧ [Color]

Click to display a color palette in order to change the color of the selected layer.


Tip

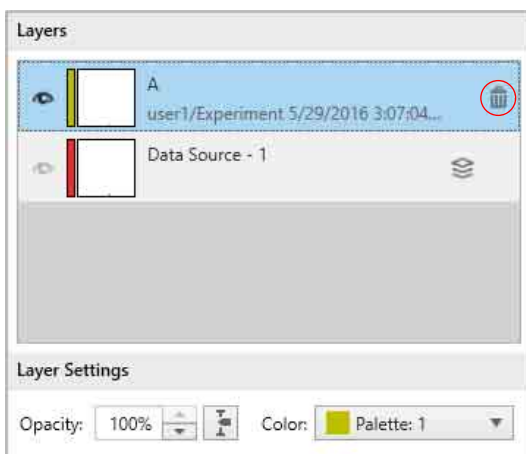
If the overlay source worksheet is closed and the display is refreshed, the worksheet is reopened and the layer is shown. However, if the overlay gate of the overlay source is deleted, the display is not refreshed. Recreate the overlay display again.

Deleting Overlay Layers

You can delete a single overlaid layer or all layers.

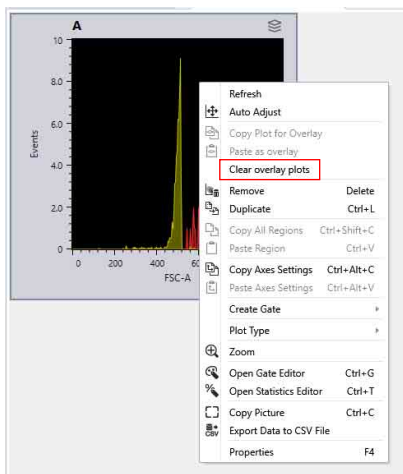
To delete a single layer

Open the overlay panel, select the layer you want to delete, and click .



To delete all layers

Right-click the plot, and select [Clear overlay plots] from the context menu.



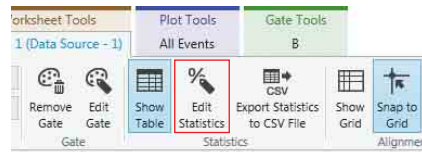
Displaying Statistics

You can change the statistics items displayed for each gate in the [Gates and Statistics] table.

For details about statistics, see “[Gates and Statistics] Table” (page 150).

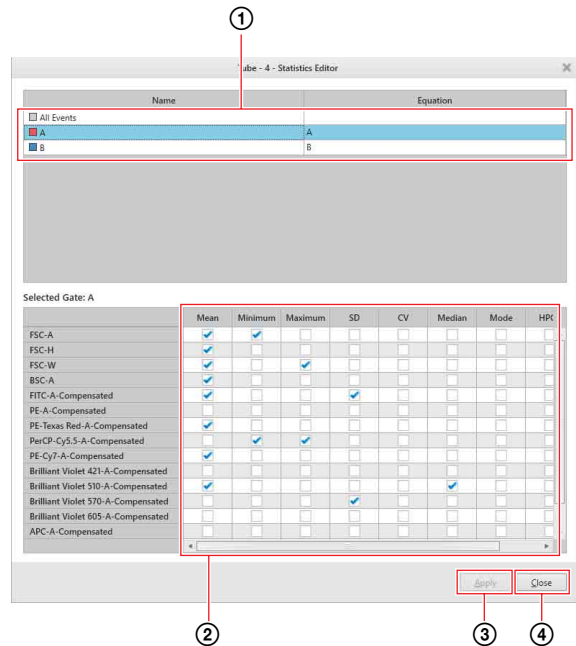
- 1 Click [Edit Statistics] in the [Statistics] group on the [Worksheet Tools] tab of the ribbon.

Alternatively, right-click the [Gates and Statistics] table and select [Open Statistics Editor] from the context menu.



The [Statistics Editor] dialog appears.

- 2 Specify the items to be displayed in the [Gates and Statistics] table.



- ① Select a gate.
The statistics values for the population in the selected gate are displayed in the lower area.
- ② Place a check mark in the checkboxes for the statistics you want to display for each parameter.
- ③ Click [Apply] to update the statistics displayed in the [Gate and Statistics] table.
- ④ Click [Close] to close the dialog.

To change the width of columns

Drag the right edge of a table column to change the width of the column.

To change the display order of columns

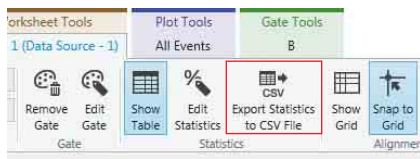
Select the title of the column to move, and drag it to the desired location.

Tip


The [Name] column cannot be moved.

To export statistics as a CSV file

- 1 Click [Export Statistics to CSV File] in the [Statistics] group on the [Worksheet Tools] tab of the ribbon.



The [Exporting statistics table] dialog appears.

- 2 Click  (Browse) to specify the folder to save the CSV file, then click [Export].



The CSV file is exported.

- 3 Click [Close] to close the dialog.

This chapter describes the various sorting modes and detailed sorting criteria.

The LE-MA900 can sort cells contained in a selected subpopulation in the sample fluid at high speed. The instrument sorts droplets containing the selected target cells using gates drawn on plots on a worksheet. Sorted droplets can be collected in 5 ml round tubes, 15 ml conical tubes, and on multi-well plates. In addition, gates can be combined using Boolean logic to uniquely identify a population for sorting using multiple attributes, providing powerful sorting capabilities.

Sorting Mode

Overview

In an ideal sort, cells travel past the optical detection point in single file and are sorted one cell at a time in separate droplets. In practice, however, cells appear randomly and can occur in close proximity within the same droplet or in adjacent droplets (called coincidences).

When a cell is detected during sorting, it is generally not possible to know with great accuracy if the signal represents the passage of a large single cell, small multiple cells adhering to one another, or other non-cellular material. Accordingly, it is more common to refer to each signal detection not as a cell, but as an event.

The sorting mode sets the rules for sorting droplets containing detected cells. These rules determine the sorting decision for each event based on the type of event and the distance between adjacent events. For example, a decision might be made to sort an event regardless of an adjacent unwanted event if the goal is to isolate as many cells as possible from a small population. Conversely, a decision might be made to not sort an event if there is an adjacent unwanted event that could reduce the purity of the sorted population.

Tip

The trigger threshold setting in the [Detector & Threshold Settings] dialog (*page 88*) can be critical for effective sorting. If the threshold level is set too high, small cells and cell debris (noise) that fall below the threshold value may be unintentionally sorted together with events above the trigger threshold, depending on the sorting mode setting. To increase sorting purity, it is recommended that the trigger threshold value be set to a relatively low value.

Note

Check that the collection tubes are securely inserted in the collection tube holder, and that the holder is loaded correctly. Stop sorting before the collection tubes become full.

About custom sorting mode

In addition to the preset sorting modes, each user can configure up to five custom sorting modes that allow you to adjust the position of target cells within a droplet for sorting.

For details about configuration, see “Configuring Custom Sorting Mode” (page 117).

The LE-MA900 supports the following eight preset sorting modes for obtaining the desired sorting results. The modes provide different levels of purity¹⁾ and yield²⁾.

	Single Cell ← Increasing purity					Increasing yield →			
	Single Cell (page 110)	Ultra Purity (page 111)	Purity (page 111)	Semi-Purity (page 110)	Normal (page 110)	Semi-Yield (page 111)	Yield (page 111)	Ultra-Yield (page 111)	
	3drops								
100 μm/ 70 μm sorting chip									
Number of droplets sorted	3	1	1	1	1	1	1 or 2	2	3

△: Target cell, □: Non-targeted (conflicting) cell

: Sorted droplet

: Waste droplet

: Multi-drop inclusion

: Multi-drop exclusion

-----: Droplet virtual division lines

1) Purity measures the proportion of target cells collected as a percentage of all cells collected.

2) Yield measures the proportion of target cells collected as a percentage of target cells in the sample. It indicates the percentage of the target population that is collected.

A high-degree of purity of targeted cells can be obtained if the targeted cell events are separated from other unwanted events. In this case, the droplets containing the targeted events are sorted, while all unwanted events and empty droplets are passed to waste disposal. In practice, however, there is a trade-off to be made between yield, recovery, and purity.

Sorting involves a combination of several factors that can affect the outcome. The position of a cell within a droplet can only be determined within a certain degree of accuracy, fluctuations can occur in the fluid flow due to the passage of large cells or variations in the sheath fluid pressure, and the droplet charging pulse timing may be slightly out of phase with droplet formation. The following considerations must be taken into account when choosing the sorting mode to use in order to meet the sorting goal.

- If a targeted event appears close to the top or bottom edge of a droplet, the probability of correct recovery is reduced. The targeted event may actually be output in an adjacent droplet. One option is to sort successive droplets in order to catch the event to increase yield and recovery.
- If a targeted event appears close to the edge of a droplet and an unwanted event occurs in the adjacent droplet, or vice versa, the decision whether to sort the droplets or not depends on the goal: isolating a pure population or recovering as many targeted cells as possible. Sorting the targeted event will increase the yield but with a subsequent reduction in purity. Conversely, rejecting the targeted event will maintain high purity but with reduced yield. Likewise, a similar decision occurs if a targeted event and an unwanted event appear in the same droplet.
- If two, different targeted events for collection in separate collection tubes appear in the same droplet or close to one another in adjacent droplets, the sorting decision must determine which of the two targeted events is sorted correctly or whether to abort sorting of the two events.
- If two or more cells, including target and/or unwanted cells, appear at the optical detection point in very close proximity, a single event will be triggered if the detected signal does not fall below the threshold level between the cells. In this case, it is impossible to distinguish the event as separate cells in a single-pass sort. If the event contains targeted and unwanted cells, it will exhibit some of the characteristics of both types of cell. If the sort criteria only define a limited subset of targeted cell characteristics, the event may satisfy that criteria and be sorted accordingly, with a corresponding reduction in purity due to the presence of the unwanted event. Sorting using multiple gates to

isolate target cells for multiple characteristics can help isolate targeted events.

In addition, hardware-related factors can affect sorting. If two or more cells, including target and/or unwanted cells, appear at the optical detection point in reasonably close proximity, separate events will be triggered if the detected signal falls below the threshold level between cells. However, if the acquisition electronics are not yet ready to receive the second event, the physical presence of the second event is detected but the event is not analyzed. This time is called the dead time. These events are aborted automatically and cannot be sorted, reducing the potential yield.

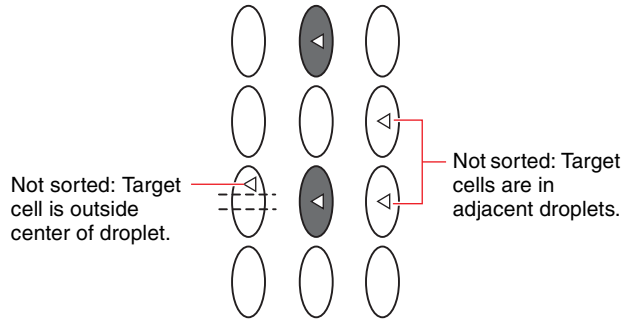
From this discussion, it can be seen that sorting only a single targeted population where the targeted events occur in the center of droplets that contain no other unwanted events will always produce the best sort results in terms of purity. Sorting all events for a single targeted population will always produce the best results in terms of yield.

Single Cell Mode

In Single Cell mode, only droplets containing a single targeted cell within the center region of the cell are sorted if the adjacent droplets are empty. If an adjacent droplet contains the same type of target cell, the droplet is not sorted.

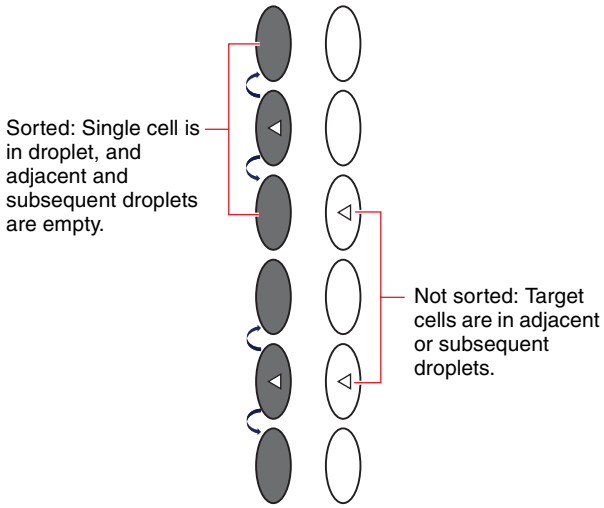
1-droplet sorting

A droplet is sorted if it contains a single targeted event located within the center region of the droplet and both of the adjacent droplets are empty. One droplet is sorted for each event matching the sorting criteria.



3-droplet sorting (3 drops)

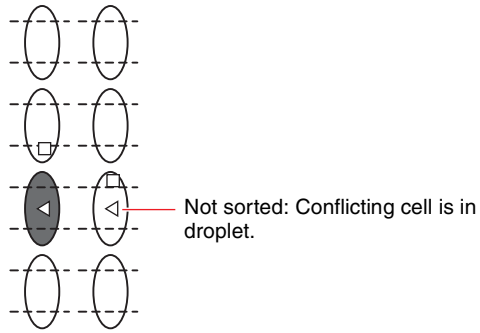
A droplet is sorted if it contains a single targeted event and the adjacent and subsequent droplets on both sides are all empty. Three droplets are sorted for each event matching the sorting criteria. In comparison with 1-droplet sorting, an improvement in sorting performance can be expected when sorting cells of 15 μm or larger, but this does not guarantee cells can be sorted.



Normal Mode

Normal mode is the default sorting mode.

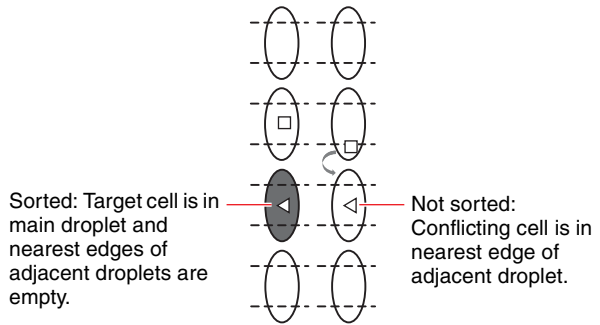
A droplet is sorted if it contains one or more targeted events of a single type. One droplet is sorted for each event matching the sorting criteria.



Semi-Purity Mode

In Semi-Purity mode, the purity achieved is higher than in Normal mode.

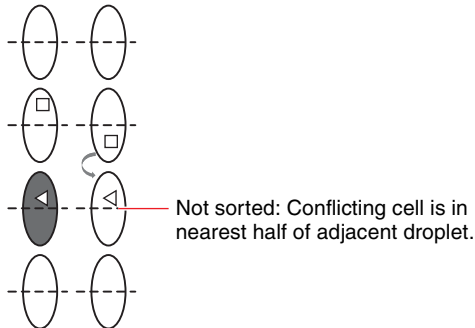
A droplet is sorted if it contains one or more targeted events of a single type and the nearest edge region of both of the adjacent droplets is empty or non-conflicting. One droplet is sorted for each event matching the sorting criteria.



Purity Mode

In Purity mode, cells are sorted with high purity.

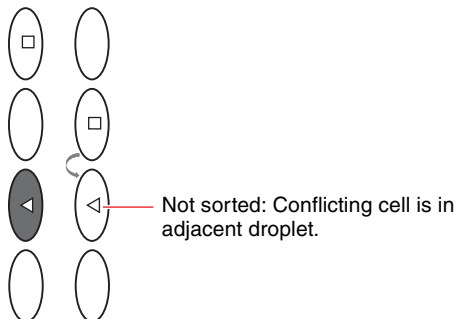
A droplet is sorted if it contains one or more targeted events of a single type and the nearest edge region of both of the adjacent droplets is empty or non-conflicting. One droplet is sorted for each event matching the sorting criteria.



Ultra Purity Mode

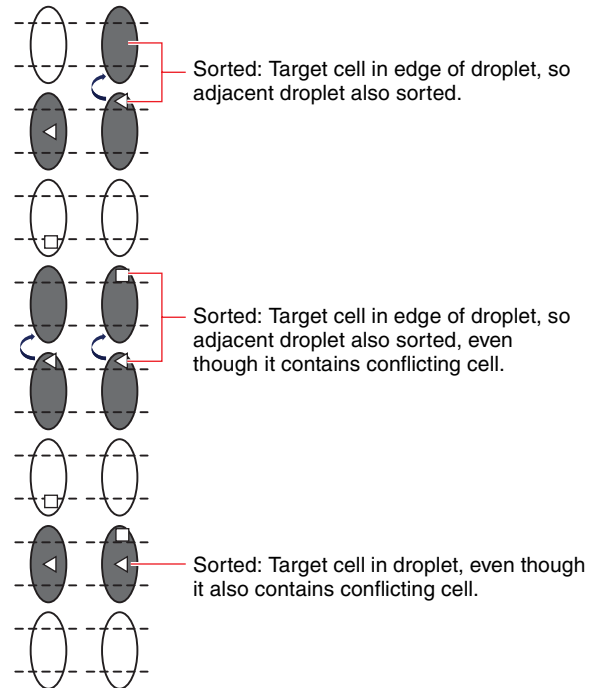
In Ultra Purity mode, cells are sorted with the highest purity.

A droplet is sorted if it contains one or more targeted events of a single type and the both of the adjacent droplets are empty or non-conflicting. One droplet is sorted for each event matching the sorting criteria.



Semi-Yield Mode

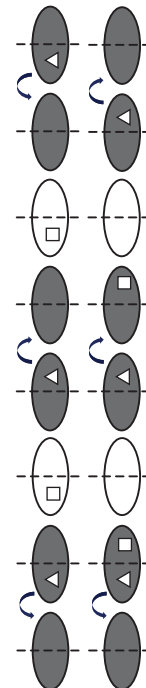
In Semi-Yield mode, a droplet is sorted if it contains one or more targeted events, even if the droplet contains conflicting events. In addition, if a targeted cell is near the edge of the droplet, the adjacent droplet is also sorted, even if it too contains conflicting events. One or two droplets are sorted for each event matching the sorting criteria.



Yield Mode

In Yield mode, a droplet is sorted, together with the adjacent droplet nearest to the target event, if it contains one or more targeted events, even if the droplets contain conflicting events.

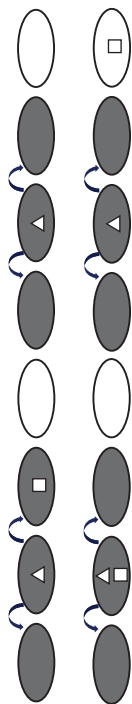
Two droplets are sorted for each event matching the sorting criteria.



Ultra Yield Mode

In Ultra Yield mode, a droplet is sorted, together both adjacent droplets, if it contains one or more targeted events, even if the droplets contain conflicting events.

Three droplets are sorted for each matching sorting criteria.



Adjusting the Sort Position

You can adjust the position where droplets fall within wells when sorting into a multi-well plate.

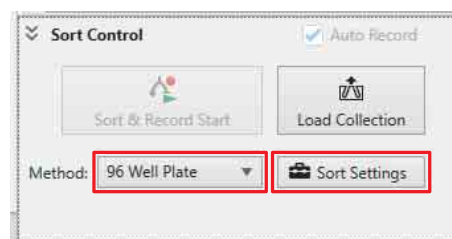
The description in this section shows a 96-well plate as an example. The operation is the same for all other multi-well plates.

When sorting into a 384-well plate or 384-well PCR plate, always adjust the sort position before starting.

Tip

The sort position cannot be adjusted when running sorting using collection tubes.

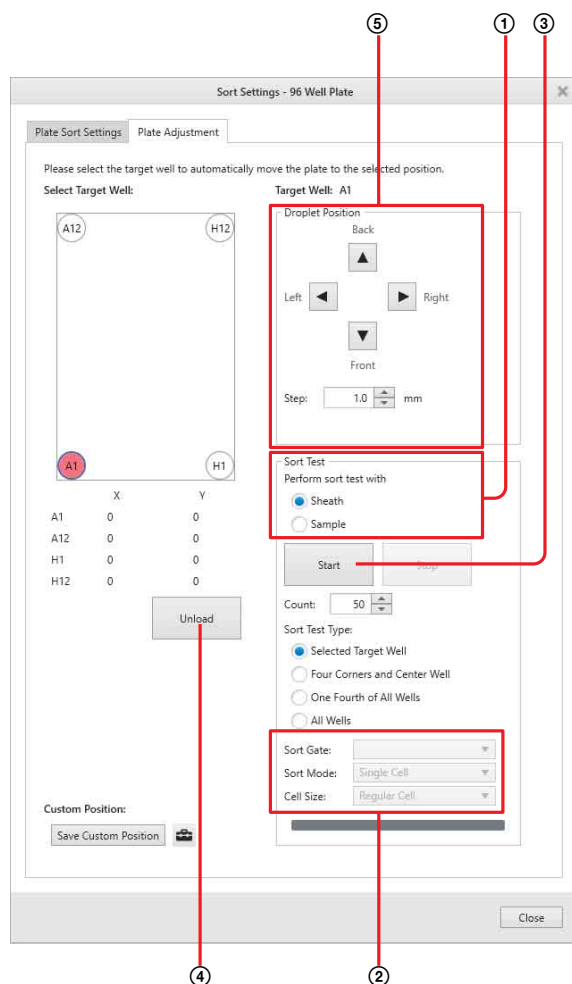
- 1** Place a cover over the multi-well plate so that you can see the position where droplets fall.
- 2** Select a multi-well plate sorting method in [Method] in the [Sort Control] pane, and click [Settings].



The [Sort Settings] dialog appears.

- 3** Adjust the sort position on the [Plate Adjustment] tab.


Adjust the position where droplets fall by visually monitoring the top of the multi-well plate.





- (5) Visually check the position of the droplets on the multi-well plate cover.
- (6) Repeat the procedure for wells A12 and H12, as required.

⑦ Wipe off droplets after confirming their position.

Tip

Clicking  displays the [User Preference] dialog (page 121) for setting user default preferences.

4 When finished, click [Close] to close the dialog.

- ① Select whether to use a sample for test sorting in [Sort Test] - [Perform sort test with].
 - [Sheath]: Run test sorting with sheath fluid only (no sample flow).
 - [Sample]: Run test sorting with sample fluid while running actual sorting.
- ② When [Sample] is selected in [Perform sort test with], select the sorting target gate in [Sort Gate], the sorting mode in [Sort Mode], and the cell size in [Cell Size].
- ③ Click [Start].
- ④ Click [Unload].
- ⑤ Visually check the position of the droplets on the multi-well plate cover.
- ⑥ Adjust the sort position of droplets using the arrow buttons in [Droplet Position].
For example, if the position for well A1 was 1mm too far left and 1.5mm too far up:
 - (1) Click well A1.
 - (2) Move the position 1 mm to the right using  and 1.5 mm down using  in [Droplet Position].
 - (3) Click [Start] in [Sort Test].
 - (4) Click [Unload].

Index Sorting

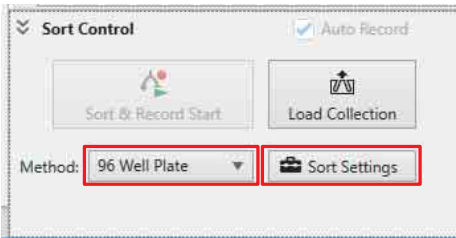
You can associate the cells sorted in each well with the events for those cells on plots. Using the index sorting function allows you to select a specific well and view the data for cells collected in that well. This section describes sorting using a 96-well plate, but the procedure is identical for sorting onto 384-well plates.

Note

Index sorting is not available for sorting using collection tubes.

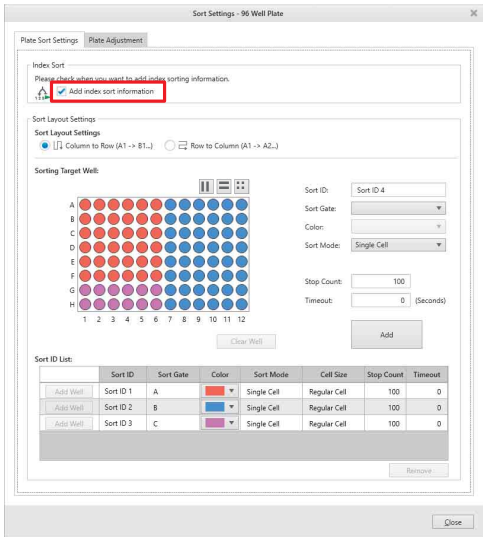
Configuring Index Sorting

- 1 Select a sorting method in [Method] in the [Sort Control] pane, and click [Sort Settings].



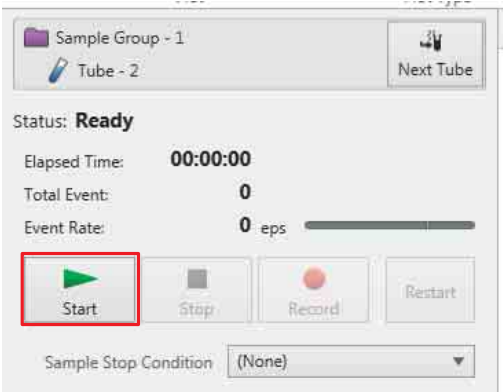
The [Sort Settings] dialog appears.

- 2 Place a check mark in [Add index sort information] on the [Plate Sort Settings] tab.



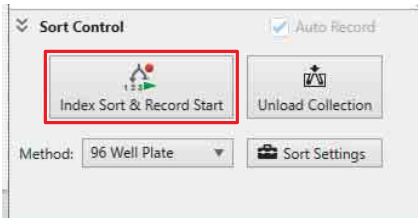
- 3 When finished configuring other items, click [Close] to close the dialog.

- 4 Click [Start] on the data acquisition control pane to start acquiring data.



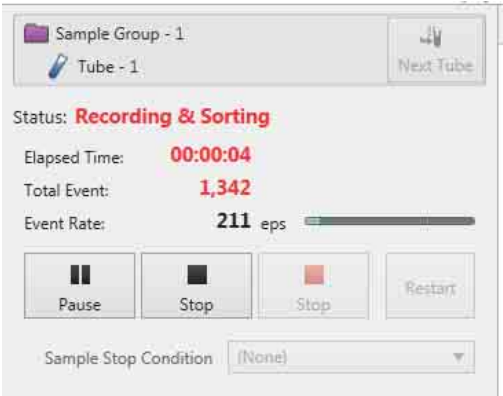
- 5 Click [Index Sort Start] on the [Sort Control] pane to start sorting.

When configured for indexed sorting, “Index” is displayed on the button.



Tip

Starting sorting also automatically starts recording.



Analyzing Index Sort Data

This section describes how to analyze data acquired during index sorting.

This function allows you to examine the position on plots of cells sorted in a specific well.

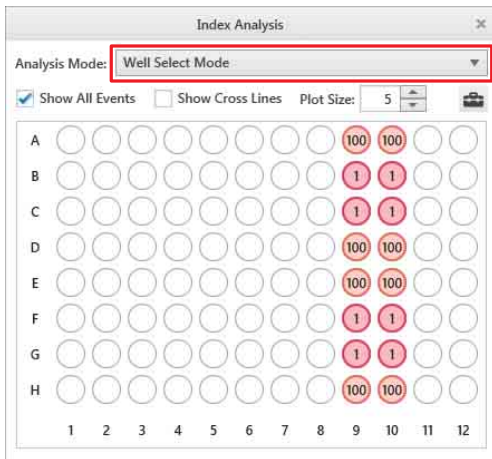
- 1 Click [Analyze Index Data] in the [Index Sorting] group on the [Worksheet Tools] tab of the ribbon.



The [Index Analysis] dialog appears.

- 2 Select a mode in [Analysis Mode].

The wells displayed vary depending on the selected mode.



[Well Select Mode]: Select this mode when you want to display the position on plots of cells sorted in each well. You can select multiple wells at the same time.

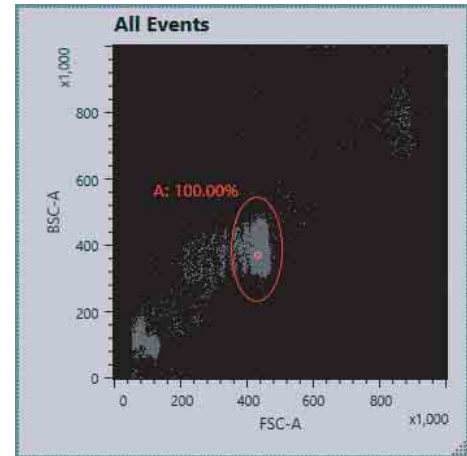
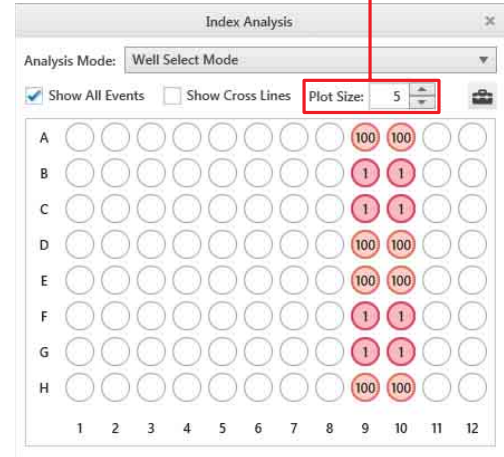
[Gate Select Mode]: Select this mode to check which wells the cells within the selected gate are sorted.

If [Well Select Mode] is selected

Displays all wells in the multi-well plate. Clicking a well in the dialog displays the position on plots of the cells collected in that well.

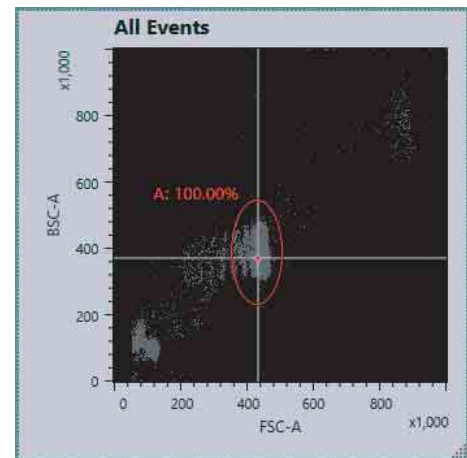
Placing a check mark in [Show All Events] displays all data acquired during sorting (includes events that were sorted and events that were not sorted) on plots.

Specifies the size of the dot displayed on plots indicating the data position.




To view the dot position accurately

Placing a check mark in [Show Cross Lines] displays horizontal and vertical lines intersecting the dot position for more accurate positioning.



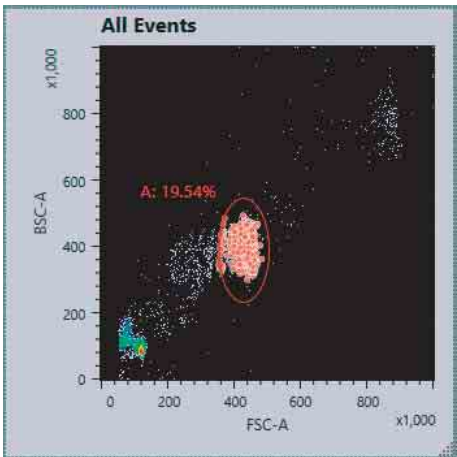
Tip

Clicking  displays the [User Preference] dialog (page 121) for setting user default preferences.


If [Gate Select Mode] is selected

Displays only those wells that contain events encompassed by the gate selected in [Selected Gate]. Placing a check mark in [Overlay Index Events] displays the wells into which the cells within the gate are sorted on plots.

Displays the wells into which the cells within the selected gate are sorted.



Tip

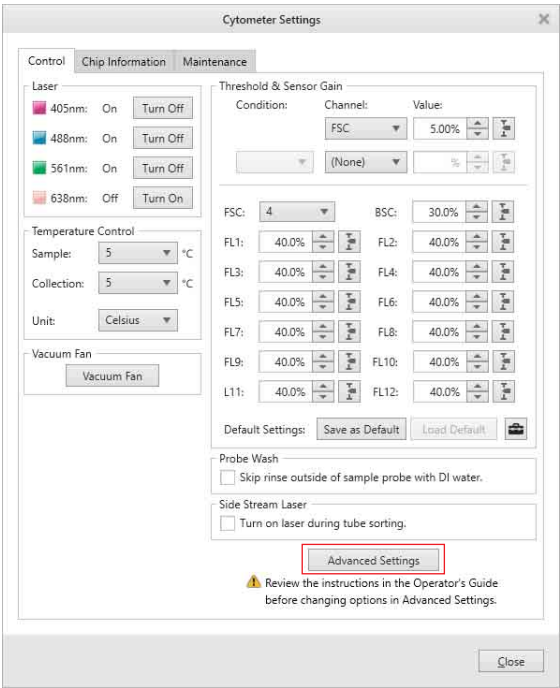
Clicking  displays the [User Preference] dialog (page 121) for setting user default preferences.

Adjusting Sort Parameters Manually

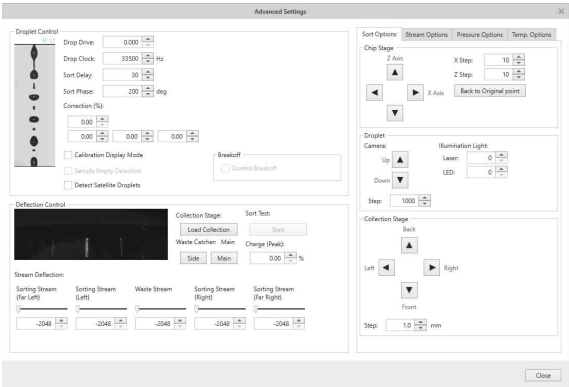
During startup, the LE-MA900 automatically calibrates sort setup parameters using automatic setup beads. However, there may be cases where you wish to override the automatic control system and adjust the sorting parameters manually.

These parameters are for experienced operators who are already familiar with manual sorting controls. The manual controls are located in the [Advanced Settings] window.

- 1 Click [Settings] on the [Cytometer] tab of the ribbon. The [Cytometer Settings] dialog appears.
- 2 Click [Advanced Settings].



The [Advanced Settings] dialog appears.



For details about each item, see “[Advanced Settings] dialog” (page 141).

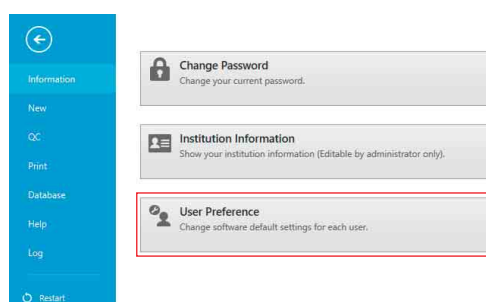
Configuring Custom Sorting Mode

Each user can configure up to five custom sorting modes that allow you to adjust the position of target cells within a droplet for sorting.

Tip

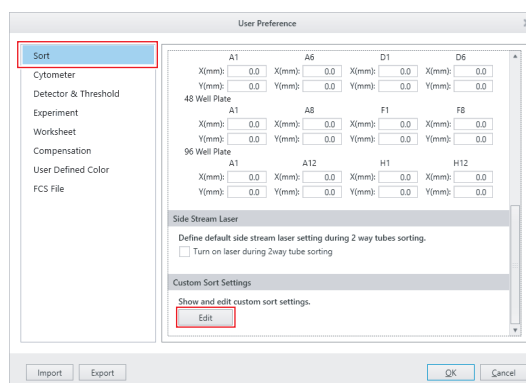
Cells near the edge of droplet may cause unstable side streams. [Centering mask] in custom sorting mode allows you to adjust the balance between cell purity and yield of side streams.

- 1 Click [Information] on the [File] tab of the ribbon, then click [User Preference].



The [User Preference] dialog appears.

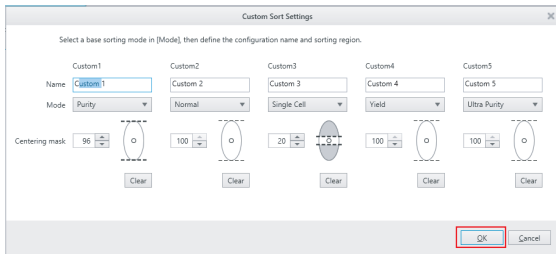
- 2 Select [Sort] from the menu on the left side, then click [Edit] in [Custom Sort Settings].



The [Custom Sort Settings] screen appears.

- 3 Set each item, then click [OK].

Select a base sorting mode in [Mode], then set a configuration name and the position range of cells within a droplet for sorting.



[Name]: Enter a name for the custom mode.

[Mode]: Select the base sorting mode.

When a sorting mode is selected, the default value for that mode is entered in [Centering mask].

[Centering mask]: Specify the position of target cells within a droplet for sorting.

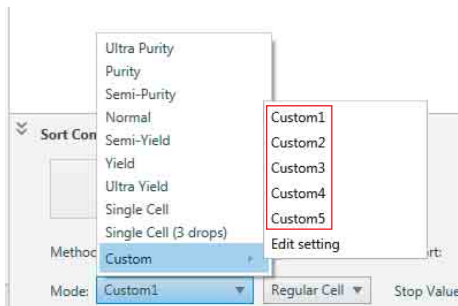
Tips

- The [Name] field cannot be left blank. Always specify a name.
- Duplicated names cannot be specified.
- Specifying the same name as a preset sorting mode is not possible.

The screen returns to the [User Preference] dialog.

4 Click [OK].

The custom mode can now be selected for [Mode] on the [Sort Control] pane.



Tips

- The menus and buttons displayed vary depending on the user account privileges.
- The screenshots displayed in this document may vary from the actual software.

[File] Window

The [File] window (Backstage View) is displayed by clicking the [File] tab of the ribbon.

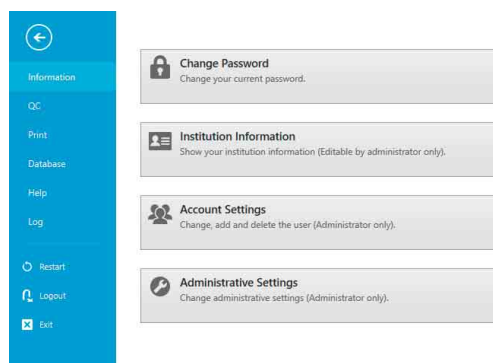
For details about [Restart], [Logout], and [Exit], see “[File] window” (page 30).

[Information] window

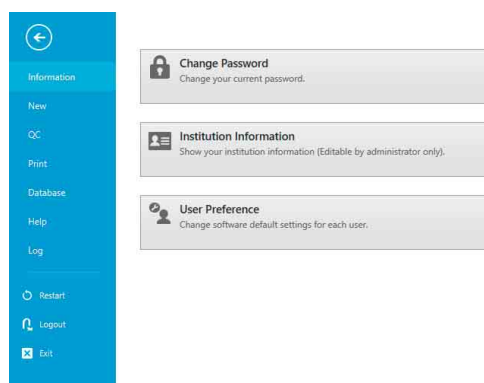
The [Information] window is displayed by clicking [Information] in the menu on the left side.

The [Information] window is used to change login passwords, set institution information, and add/modify user accounts.

Example: Administrator account login



Example: User account login



[Change Password]: Allows you to change the password used to log in to Cell Sorter Software. For details, see “*Changing Passwords*” (page 40).

[Institution Information]: Allows you to view information on the institution using the unit. You can also configure settings if you are logged in using an Administrator account. Settings cannot be configured if you are logged in using a general user account.

Click this button to display the [Institution Information] dialog (page 120).

[Account Settings]: Displays the [Account Settings] dialog (page 40) for managing user accounts for Cell Sorter Software. This button is displayed for Administrator accounts only.

[User Preference]: Displays the [User Preference] dialog (page 121) for specifying software preferences, which can be specified independently by each user. This button is displayed for Administrator accounts only.

[Administrative Settings]: Displays the [Administrative Settings] dialog (page 120) for configuring administrative settings on the instrument. This button is displayed only when logged in using an administrator account.

[Institution Information] dialog

The [Institute Information] dialog is displayed by clicking [Information] > [Institute Information] on the [File] tab of the ribbon.

The [Institution Information] dialog is used to specify information about the institution using the unit. Only administrators can modify this information.



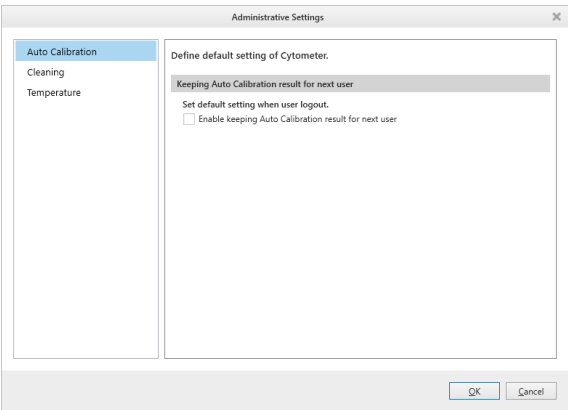
- [Institution]:** Enter the name of the institution.
- [Address]:** Enter the street address of the institution.
- [City]:** Enter the name of the city or town.
- [State]:** Enter the name of the state or province.
- [Zip Code]:** Enter the postal code number.
- [Country]:** Enter the name of the country or region.
- [OK]:** Applies the settings and closes the dialog.
- [Cancel]:** Cancels the settings and closes the dialog.

[Administrative Settings] dialog

The [Administrative Settings] dialog is displayed by clicking [Administrative Settings] in the [Information] window.

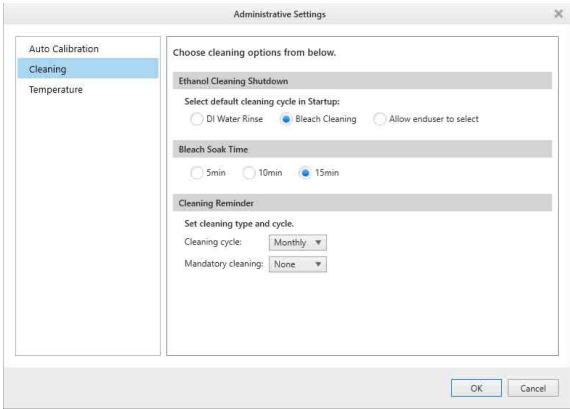
The [Administrative Settings] dialog is used to configure administrative settings on the instrument.

- [Auto Calibration]**
Configures the inheritance of auto calibration results for other users.



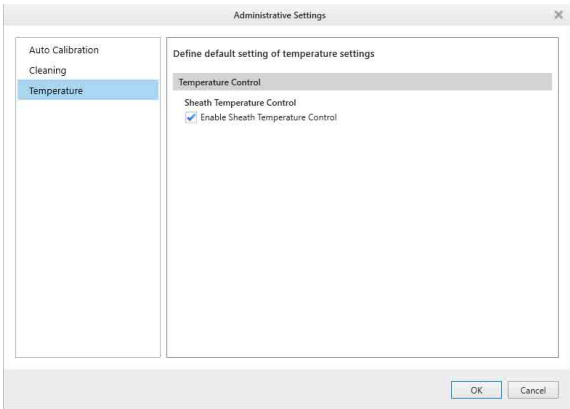
- [Keeping Auto Calibration result for next user]**
- [Enable keeping Auto Calibration result for next user.]:** Place a check mark in the checkbox to enable inheritance of auto calibration results by the next user after logging out.

- [Cleaning]**
Sets various items for cleaning.



- [Ethanol Cleaning Shutdown]:** Selects the cleaning process to perform when restarting after running ethanol cleaning prior to shutting down.
 - DI Water Rinse
 - Bleach Cleaning
 - Allow end user to select
- [Bleach Soak Time]:** Selects the bleach soak time when running bleach cleaning.
- [Cleaning Reminder]:** Sets the ethanol cleaning intervals for sending cleaning reminder notifications.
 - [Cleaning cycle]:** Selects the ethanol cleaning interval.
 - None (no notifications)
 - Daily
 - Weekly
 - Monthly (default value)
 - [Mandatory cleaning]:** Selects the mandatory ethanol cleaning interval. If the interval specified here is exceeded, login is disabled until ethanol cleaning has been performed.
 - None (no notifications) (default value)
 - 1 week
 - 2 weeks
 - 1 month (30 days)

- [Temperature]**
Sets the temperature adjustments.



[Temperature Control]: Sets the temperature adjustment.

[Enable Sheath Temperature Control]: Sets the default value of [Sheath Temp. Control] on the [Temp. Options] tab (page 143) of the [Cytometer Settings] - [Advanced Settings] dialog. Place a check mark in the checkbox to enable sheath fluid temperature control by default.

[User Preference] dialog

The [User Preference] dialog is displayed by clicking [Information] > [User Preference] on the [File] tab of the ribbon.

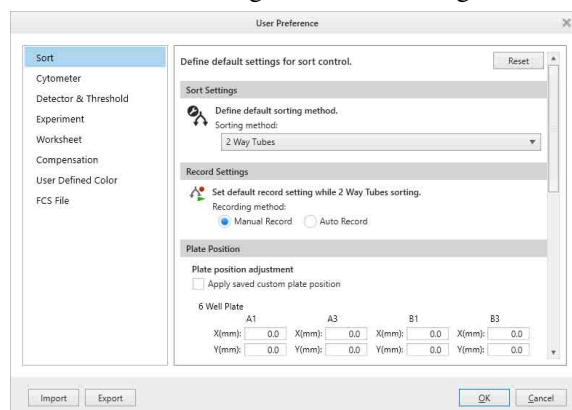
The [User Preference] dialog is used to specify software preferences, unique to each user. User preferences are loaded automatically when a user logs in, saving having to configure the instrument you want to perform acquisition, analysis, or sorting.

Tips

- You can click [Reset] to restore the default values for each setting.
- You can click [Export] to export the settings to a user preference settings file. The file has a “.userdat” file name extension.
- You can click [Import] to import a user preference settings file to load and update settings.

• [Sort] tab

Defines default settings related to sorting.



[Sort Settings]: Specifies the default sorting configuration.

[Sort method]: Selects the sorting method. The available options vary depending on the model.

[Record Settings]

[Recording method]: Selects the recording method for 2-way/4-way sorting.

- Manual Record
- Auto Record (recording starts when sorting starts)

[Plate Position]: Adjusts the position of wells for each type of plate.

[Apply saved custom plate position]:

Applies saved plate position adjustment values.

[Side Stream Laser]

[Turn on laser during tube sorting]:

Selects whether to turn on the side-stream laser when sorting using collection tubes.

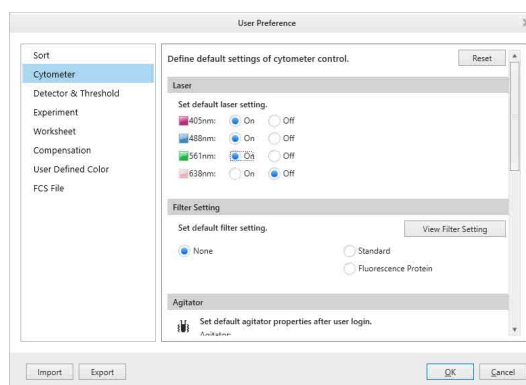
[Custom Sort Settings]

[Edit]: Sets custom sorting modes that allow you to adjust the position of target cells within a droplet for sorting.

For details about configuration, see “Configuring Custom Sorting Mode” (page 117).

• [Cytometer] tab

Defines default settings related to the instrument.



[Laser]

[Laser]: Turns the excitation lasers used for measurement on/off.

Tip

The laser settings are not inherited by the next user, even if inheritance of auto calibration results between users has been enabled. The settings follow the [User Preference] settings for those users. However, if all lasers are turned off, the laser settings are inherited.

[Filter Setting]: Selects the optical filter pattern setting.

Click [View Filter Setting] to display the selected optical filter pattern.

- None (no default setting)
- Standard Filter Settings
- Filter settings for Fluorescence protein

[Agitator]

[Agitator]: [Agitator]: Turns the agitation function on/off.

Tip

Agitation is performed as required during automatic calibration, even if the agitation function is turned off.

[Speed]: Selects the agitation speed.

- High
- Low

[Temperature Control]

[Sample Temperature Control]: Selects the temperature setting of the sample injection chamber.

- Off
- On (5 °C/41 °F)
- On (37 °C/98.6 °F)

[Collection Temperature Control]: Selects the temperature setting of the collection stage.

- Off
- On (5 °C/41 °F)

[Probe Wash]

[Skip rinse outside of sample probe.]:

Sets whether to skip the rinse of the outside of the sample probe between measurements. If a check mark is placed in this checkbox, clean the outside of the probe as required.

Tip

When the probe is cleaned after auto calibration finishes, the inside of the probe is also cleaned, regardless of this setting.

[Area Light Control]

[Sample tube area light automatic control]:

Specifies whether the sample loader light turns on automatically loading/unloading a sample.

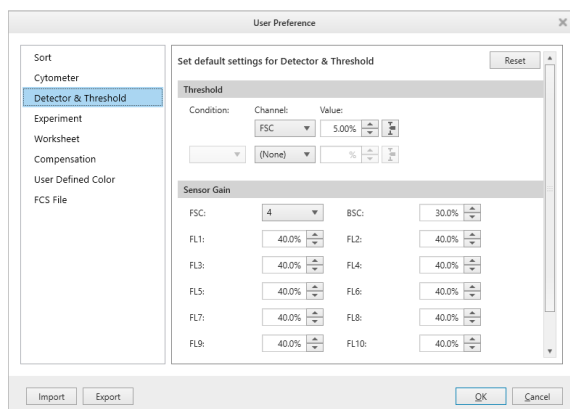
- Off
- On

[Collection area light automatic control]:

Specifies whether the collection area light turns on automatically when loading/unloading collection tubes.

• [Detector & Threshold] tab

Defines default settings for the threshold value of the trigger channel and for the detectors.



[Threshold]: Specifies the threshold for event detection.

You can specify up to two channels for the trigger condition.

[Condition]: Specifies the handling of the two channels.

- [And]: Detects events that simultaneously satisfy both triggers.
- [Or]: Detects events that satisfy either one or the other of the two triggers.

[Channel]: Selects the channel (FSC, BSC, FL1 to FL12) used to trigger the detection of events.

When a second trigger is specified, select a channel for the 2nd trigger that is different to the channel of the 1st trigger. The default values of the 1st and 2nd triggers are [FSC] and [(None)], respectively, but a new default value can be set as required using [Save as Default].

[Value]: Specifies the threshold value for the trigger channel as a percentage of the output signal of the detector for the trigger channel.

[Sensor Gain]: Specifies the gain of each detector.

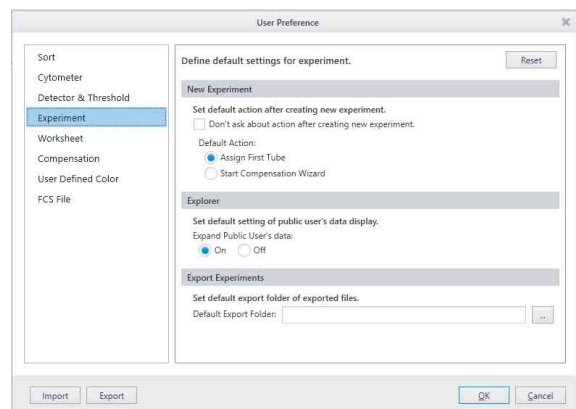
[FSC]: Specifies the gain of the forward-scatter (FSC) detector. The gain setting has a 17-step range.

[BSC]: Specifies the gain of the back scatter (BSC) detector as a percentage of the output signal of the detector.

[FL1] to [FL12]: Specifies the gain of the corresponding fluorescence channel detector as a percentage of the output signal of the detector.

• [Experiment] tab

Defines default settings related to experiments.



[New Experiment]

[Don't ask about action after creating new experiment.]:

Sets whether to display a dialog prompting you to select the next action after creating an experiment using the Blank template.

[Default Action]: Selects the default action to take after creating an experiment.

- **Assign First Tube**
Assigns the first tube in preparation for data acquisition.
- **Start Compensation Wizard**
Select this to perform fluorescence compensation in preparation for measurements.

[Explorer]

[Expand Public User's data]: Selects whether to display the list of shared experiments.

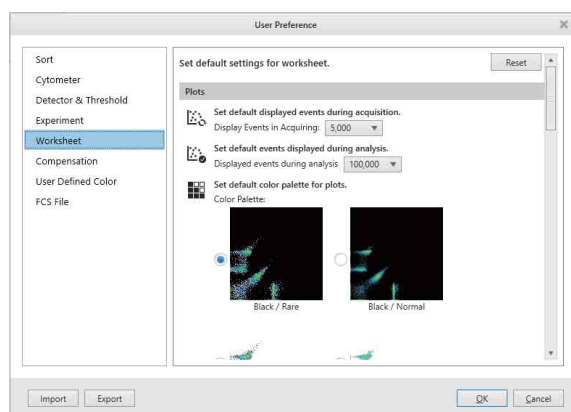
- On
- Off

[Export Experiments]

[Default Export Folder]: Specifies the destination folder used when exporting experiment data.

• [Worksheet] tab

Defines default settings related to the worksheet.



[Plots]

[Display Events in Acquiring]: Selects the number of events to display during data acquisition.

[Display events during analysis]: Selects the number of events to display when analyzing data.

[Color Palette]: Selects the color palette (background color and display of outlying (rare) events).

- Black/Rare
- Black/Normal
- White/Rare
- White/Normal

[Histogram Settings]: Makes settings for the display of histogram plots.

- For filled area view, select [No Line]. For outline view, select [Show Line]. When [Show Line] is selected, set the type of line in [Line Settings].
- [Line Settings]: Select the line thickness in [Width]. Set the opacity of the filled area in [Opacity]. You can check the type of line in [Preview].

[Gate]

[Label Font Size]: Selects the font size of text labels shown on gates. You can check the size in [Preview].

[Gate Line Width]: Selects the line width used to draw gates. You can check the line width in [Preview].

[Automatic zoom tracking function]:

Changes the shape and size of gates on plots to track the zoom size of plots and change of axis type.

[Default Scatter Plot]

[Plot Type]: Selects the default type of scatter plot added to a new worksheet.

- Density
- Dot Plot

[Axis Type]: Selects the scale type of an axis on scatter plots added to a new worksheet.

- Linear
- Log (logarithmic)
- Biexponential

[Gate type]: Selects the default gate type.

- Ellipse
- Polygon
- Rectangle

[Fluorescence Plots]

[Axis Type]: Selects the scale type of an axis on new plots displaying fluorescence data.

- Linear
- Log
- Biexponential

Tip

This setting is not applied to fluorescence plots for compensation panels.

[Index Analysis]

[Plot Size]: Specifies the size that events are displayed on plots (for emphasis) when using the index sorting function. You can check the size in [Preview].

[Alignment]

[Auto Arrange]: Selects whether to automatically align the plots and [Gates and Statistics] table to the grid of the worksheet.

- On
- Off

[Snap to Grid]: Selects whether to align the plots and [Gates and Statistics] table to the grid when moving or adding them on the worksheet.

- On
- Off

[Show Grid]: Selects whether to display the grid on the worksheet. The size of the grid is set in [Grid Size].

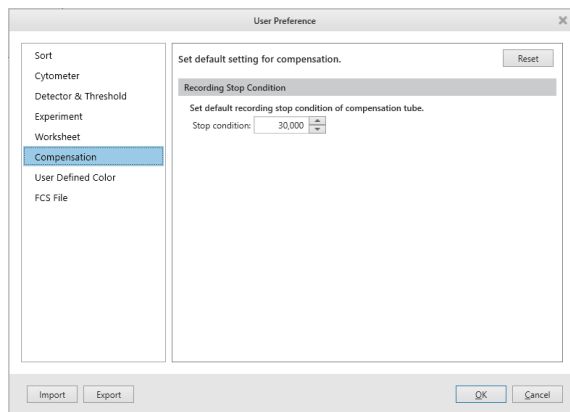
- On
- Off

[View]

[Expand]: Sets the zoom factor of the worksheet display.

[Compensation] tab

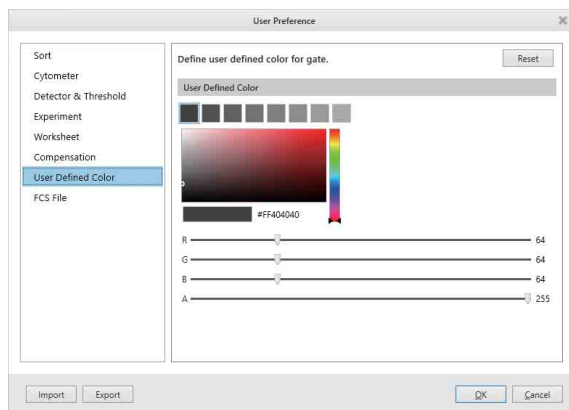
Defines default settings related to fluorescence compensation.

**[Recording Stop Condition]**

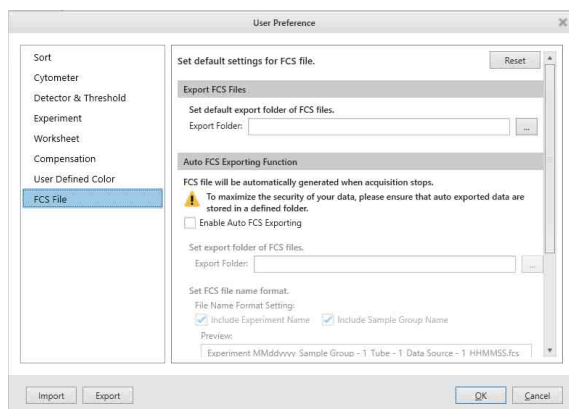
[Stop condition]: Selects the number of events before recording stops automatically.

[User Defined Color] tab

Specifies custom colors for gates.

**[FCS File] tab**

Specifies default settings related to FCS files.

**[Export FCS Files]**

[Export Folder]: Specifies the destination folder used when exporting FCS files.

[Auto FCS Exporting Function]

[Enable Auto FCS Exporting]: Enables the automatic exporting of experiment data to FCS files.

Note that data security may be reduced if automatic export is enabled.

[Export Folder]: Specifies the destination folder used when exporting FCS files.

[File Name Format Setting]: Specifies the file naming format.

You can check the file name in [Preview].

- Include Experiment Name
- Include Sample Group Name

[FCS Version]: Selects the FCS file version.

- 3.0
- 3.1

[Convert BSC to SSC]: Selects whether to convert the data name of back scatter data from BSC (back scatter) to SSC (side scatter) when creating FCS files.

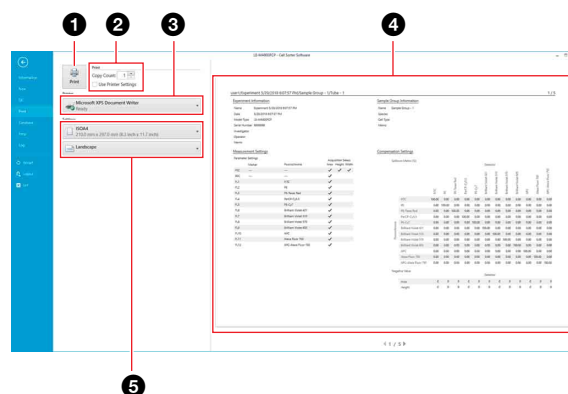
[Maximum Save Events]: Specifies the maximum number of events to save.

[Print] Window**Note**

Printing cannot be executed during data acquisition and recording.

The [Print] window is displayed by opening the worksheet of the tube you want to print and clicking [Print] in the menu on the left side.

The [Print] window can print analysis results, statistics, and plots displayed on the worksheet.

**1 [Print] button**

Sends the print job to the printer.

2 [Print]

Sets the number of copies to print.

Place a check mark in the [Use Printer Settings] checkbox to set advanced printer settings.

③ [Printer]

Selects the printer for printing.

④ Print preview area

Displays a preview image of the print job.

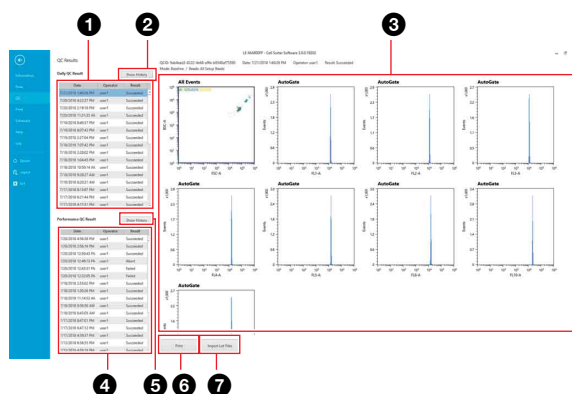
⑤ [Settings]

Sets the paper size and page orientation.

[QC] Window

The [QC] window is displayed by clicking [QC] in the menu on the left side.

The [QC] window is used to check the results of Daily QC and of Performance QC.



① [Daily QC Result]

Displays a list of previously run Daily QC checks. Selecting a Daily QC item displays the QC results in [QC Report] on the right side.

② [Show History]

Clicking this button displays the “[Daily QC History] dialog” (page 125) for showing changes in Daily QC results over time.

③ [QC Report]

Displays the QC results for the QC check selected in the [Daily QC Result] or [Performance QC Result] list.

④ [Performance QC Result]

Displays a list of previously run Performance QC checks. Selecting a Performance QC item displays the QC results in [QC Report] on the right side.

⑤ [Show History]

Clicking this button displays a dialog for showing changes in Performance QC results over time. Clicking [Select Display] displays the “[Select Display] dialog” (page 126). Clicking [Print] displays the “[Print] Window” (page 124).

⑥ [Print]

Displays the print preview area for previewing the printing of QC results for the QC check selected in the [Daily QC Result] or [Performance QC Result] list. For details about the print preview area, see “[Print] Window” (page 124)).

⑦ [Import Lot File]

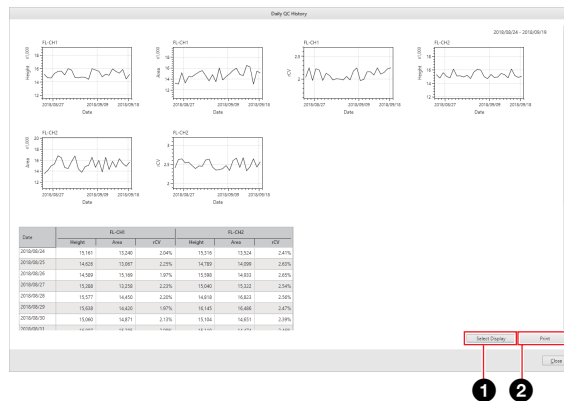
Imports the beads lot file for automatic setup beads.

For details, see “Updating the Bead Lot File” (page 76).

[Daily QC History] dialog

The [Daily QC History] dialog is displayed by clicking [Show History] in the [QC] window.

You can check the changes in Daily QC results over time in the [Daily QC History] dialog.



① [Select Display]

Clicking this button displays the “[Select Display] dialog” (page 126) for selecting the interval and parameters to display.

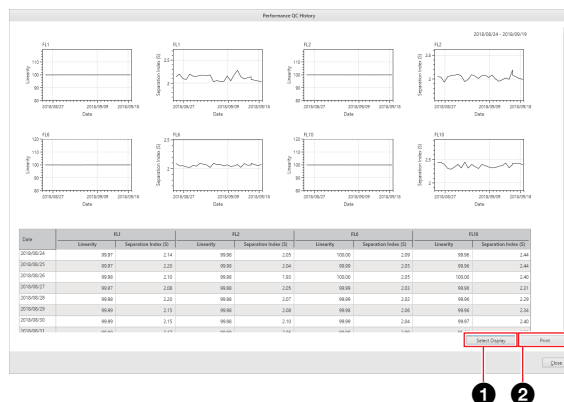
② [Print]

Clicking this button displays the “[Print] Window” (page 124).

[Performance QC History] dialog

The [Performance QC History] dialog is displayed by clicking [Show History] in the [QC] window.

Performance QC parameters can be displayed as a function of time using Levy Jennings plots.



① [Select Display]

Clicking this button displays the “[Select Display] dialog” (page 126) for selecting the interval and parameters to display.

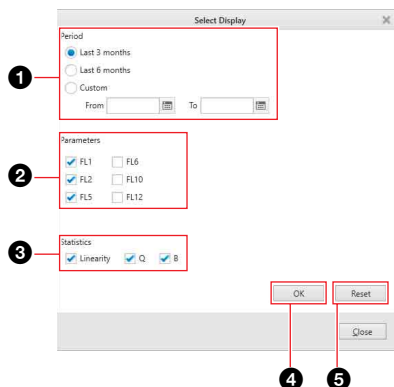
② [Print]

Clicking this button displays the “[Print] Window” (page 124).

[Select Display] dialog

The [Select Display] dialog is displayed by clicking [Select Display] in the [Daily QC History] dialog or the [Performance QC History] dialog.

You can select the QC results interval and the target parameters in the [Select Display] dialog.



① [Period]

Selects the interval for the QC results you want to display.

When [Custom] is selected, specify the start and end dates to display.

② [Parameters]

Place a check mark in the checkboxes for the parameters to display. You can select multiple parameters.

③ [Statistics]

Place a check mark in the checkboxes for the statistics to display. You can select multiple statistics.

④ [OK]

Applies the settings.

⑤ [Reset]

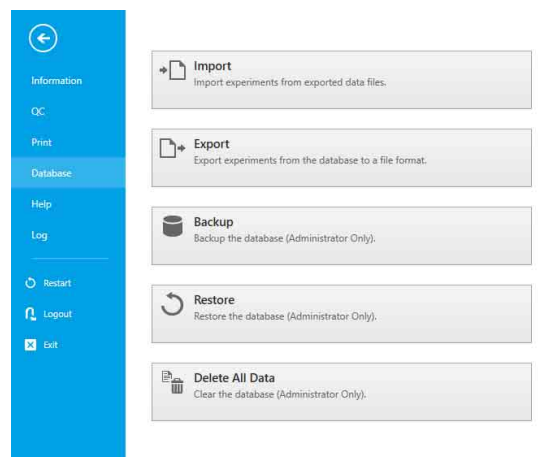
Restores the display items to the default values.

[Database] Window

The [Database] window is displayed by clicking [Database] in the menu on the left side.

The [Database] window is used to perform experiment database operations and to check hard disk free capacity.

Example: Administrator account login



[Import]: Displays the [Import Experiment Data] dialog (page 127) for importing experiment data.

You can import experiment data created using another LE-MA900 for use on this unit.

[Export]: Displays the [Export Experiment Data] dialog (page 127) for exporting experiment data.

You can export experiment data created on this unit for use by another LE-MA900.

[Backup]: Displays the [Backup Database] dialog for backing up the experiment database. This button is displayed for administrator accounts only.

Backing up the database allows the data to be restored at a later time, if necessary.

For details, see “Backing Up/Restoring the Database” (page 76).

[Restore]: Displays the [Restore Database] dialog for restoring a previously backed up experiment database. This button is displayed for administrator accounts only.

For details, see “Backing Up/Restoring the Database” (page 76).

[Delete All Data]: Displays the [Delete All Data] dialog (page 127) for resetting the experiment database. This button is displayed for administrator accounts only.

Note

Resetting the database removes all data, including user accounts, experiments, and analysis results.

[Hard Disk Drive Capacity]: Displays the available free space on the hard disk.

A warning will appear when the free space on the hard disk falls below 50 GB. The assigning of sample tubes is disabled when the free space falls below 35 GB.

[Database Capacity]: Displays the available space on the database.

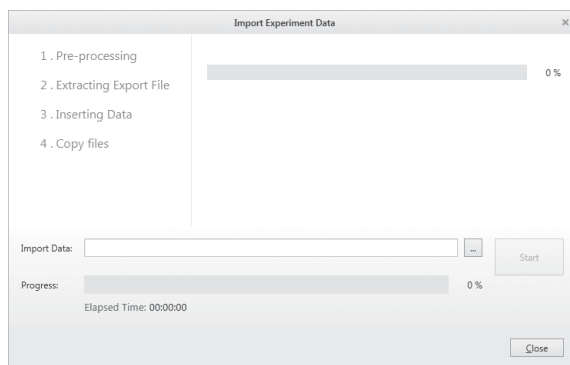
Up to 10 GB of data can be saved to the database in Cell Sorter Software. Various types of measurement conditions and configuration settings are saved in the

database. Measurement data is not stored in the database, and has no effect on the available database capacity.

[Import Experiment Data] dialog

The [Import Experiment Data] dialog is displayed by clicking [Import] in the [Database] window.

The [Import Experiment Data] dialog is used to import data created on another LE-MA900 into this instrument.



[Import Data]: Specifies the experiment data to import.

Click (Browse) to specify a file to import.

[Progress] bar: Displays the import progress.

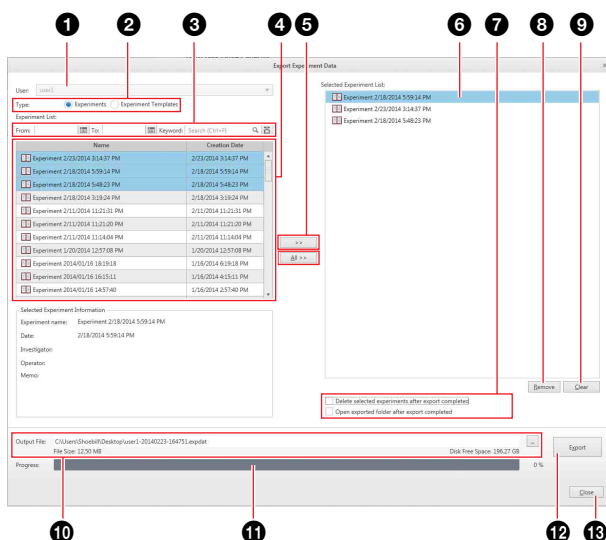
[Start]: Starts importing the specified data.

[Close]: Closes the dialog.

[Export Experiment Data] dialog

The [Export Experiment Data] dialog is displayed by clicking [Export] in the [Database] window.

The [Export Experiment Data] dialog is used to export data created on this unit.



① User

Selects the owner of the experiments to export.

② [Type]

Selects whether to export experiments or experiment templates.

③ Search fields

Searches for experiments by date and keyword.

④ Experiment list

Displays a list of experiments that satisfy the specified search criteria.

Select the experiment(s) you want to export.

Selecting an experiment in the upper list displays information about the experiment in the lower list.

⑤ [>>] / [All >>]

Clicking [>>] adds the experiment data selected in the experiment data list to [Selected Experiment List].

Clicking [All >>] adds all the experiments in [Experiment List] to [Selected Experiment List].

⑥ [Selected Experiment List]

Lists the experiments to be exported.

⑦ [Delete selected experiments after export completed]

Deletes the exported experiments from the database after exporting has finished.

[Open exported folder after export completed]

Opens the export destination folder after exporting has finished.

⑧ [Remove]

Removes the experiment selected in [Selected Experiment List] from the list.

⑨ [Clear]

Clears the [Selected Experiment List].

⑩ [Output File]

Specifies the export destination file. Click (Browse) to specify the file.

⑪ Progress

Displays the export progress.

⑫ [Export]

Starts exporting the experiment data.

⑬ [Close]

Closes the dialog.

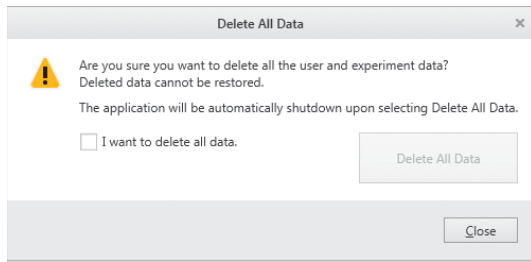
[Delete All Data] dialog

The [Delete All Data] dialog is displayed by clicking [Delete All Data] in the [Database] window.

The [Delete All Data] dialog is used to remove all data, including user accounts and experiments.

Notes

- Resetting the database resets all data, including user accounts, experiments, and analysis results.
- After resetting the database, Cell Sorter Software automatically shuts down.

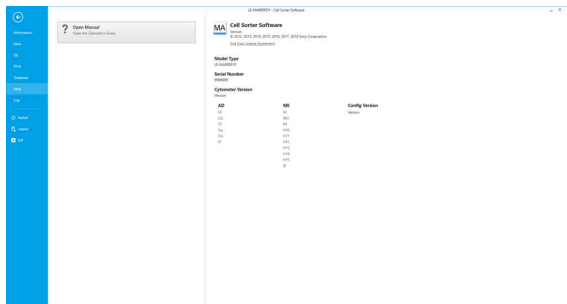


[I want to delete all data]: Place a check mark in the checkbox to confirm you want to reset the database.
[Delete All Data]: Resets the database, deleting all data.
[Close]: Closes the dialog.

[Help] Window

The [Help] window is displayed by clicking [Help] in the menu on the left side.

The [Help] window is used to display the Operator's Guide (this document) and to check software version information.

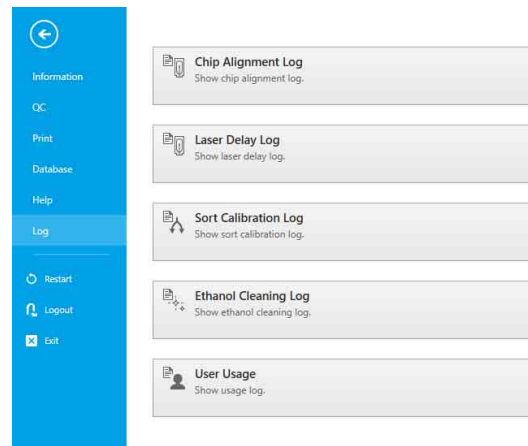


[Open Manual]: Displays the Operator's Guide.
[Cytometer Version]: Displays version information for instrument components.
[Model Type]: Displays the LE-MA900 model being used.
[Serial Number]: Displays the serial number of the LE-MA900 being used.
[Cell Sorter Software]: Displays the Cell Sorter Software version information.
 Clicking [End User License Agreement] displays the license agreement.

[Log] Window

The [Log] window is displayed by clicking [Log] in the menu on the left side.

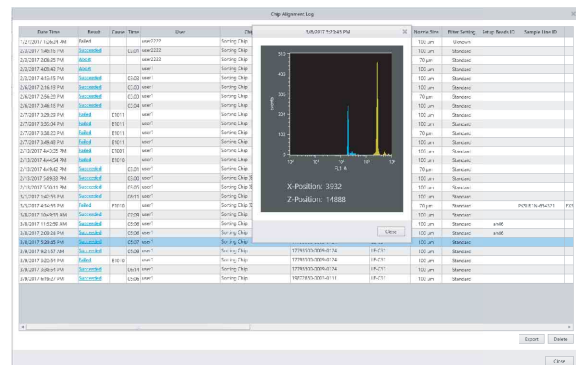
The [Log] window is used to display the chip alignment log, sort calibration log, and user usage log.



[Chip Alignment Log]: Displays the position alignment log for the sorting chip.
[Laser Delay Log]: Displays the laser delay adjustment history log.
[Sort Calibration Log]: Displays the sort calibration log.
[Ethanol Cleaning Log]: Displays the ethanol cleaning history log.
[User Usage]: Displays the user usage log.

Chip alignment log

Displays the time and date when the sorting chip was aligned, together with other sorting chip information. Clicking a result in the [Result] column displays an image of the chip alignment result. Clicking [Export] exports the contents of the log to a CSV-format file.



Laser delay log

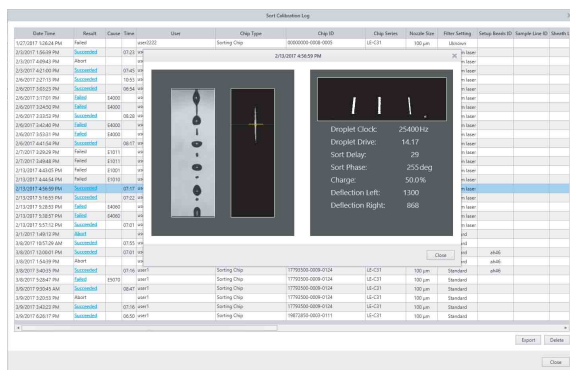
Displays the time and date when the laser delay was adjusted, together with adjustment results information. Clicking a result in the [Result] column displays an image of the laser delay adjustment result. Clicking [Export] exports the contents of the log to a CSV-format file.

Sorting calibration Log

Displays the time and date when sorting was calibrated, together with other calibration information.

Clicking a result in the [Result] column displays an image of the sort calibration result.

Clicking [Export] exports the contents of the log to a CSV-format file.



Ethanol cleaning log

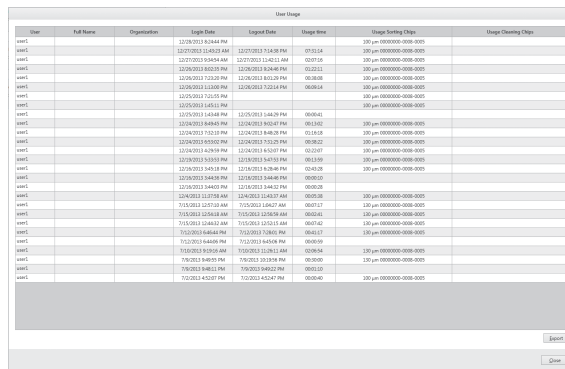
Displays the time and date when ethanol cleaning was performed, together with other information.

Clicking [Export] exports the contents of the log to a CSV-format file.

User usage log

Displays the date and time users logged in to LE-MA900 and other user information.

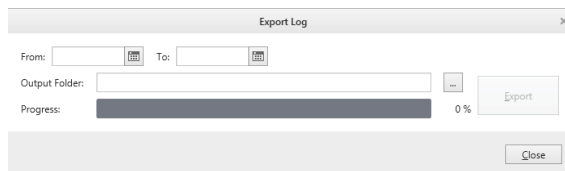
Clicking [Export] exports the contents of the log to a CSV-format file.





[Export Log] dialog

The [Export Log] dialog is displayed by clicking [Export] in the chip alignment log, sort calibration log, or user usage log dialogs.

The [Export Log] dialog is used to export the contents of the displayed log as a CSV-format file.



[From] / [To]: Specifies the interval of the log to be exported. Click  to enter a date.

[Output Folder]: Specifies the output destination folder. Click  to browse for a destination folder.

Progress: Displays the export progress.

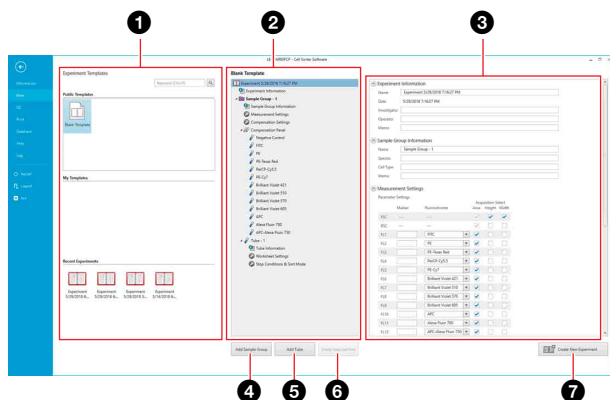
[Export]: Starts exporting the log.

[Close]: Closes the dialog.

[Create Experiment] Window

The [Create Experiment] window is displayed by clicking [New] on the [File] tab of the ribbon or [New] in the [Experiment] group on the [Experiment] tab of the ribbon.

The [Create Experiment] window is used to create a new experiment using templates.



1 [Experiment Templates] list

Displays the available experiment templates and experiments that can be used as templates.

You can search the template list by keywords.

[Public Templates]: Displays experiment templates that were created as shared templates.

[Blank Template]: An empty template.

[My Templates]: Displays private experiment templates that you have created.

[Recent Experiments]: Displays recent experiments that can be used as templates.

2 Experiment structure

Displays the internal structure of the experiment template selected in the list.

You can add and delete sample groups and sample tubes, as required.

3 Experiment settings

Displays configurable information and instrument settings for the experiment.

You can also edit settings after an experiment has been created.

[Experiment Information]: Specifies the name and other basic information of the experiment. The date cannot be modified.

For details about each item, see “[Experiment Information] dialog” (page 153).

[Sample Group Information]: Specifies information relating to the sample groups.

For details about each item, see “[Sample Group Information] dialog” (page 154).

[Measurement Settings]: Specifies parameter settings and instrument settings.

For details about each item, see the [Measurement Settings] dialog (page 81).

[Tube Information]: Specifies information relating to the sample tubes.

For details about each item, see “[Tube Information] dialog” (page 154).

[Compensation Settings]: Displays compensation settings.

For details about each item, see “[Compensation Settings] dialog” (page 143).

[Sort & Stop Settings]: Displays the sorting and automatic stop conditions.

4 [Add Sample Group]

Displays the [Add Sample Group] dialog (page 130) for adding a sample group to the experiment structure.

5 [Add Tube]

Displays the [Add Tube] dialog (page 131) for adding a sample tube to the selected sample group in experiment structure.

6 [Delete Selected Item]

Deletes the selected item from the experiment structure.

7 [Create New Experiment]

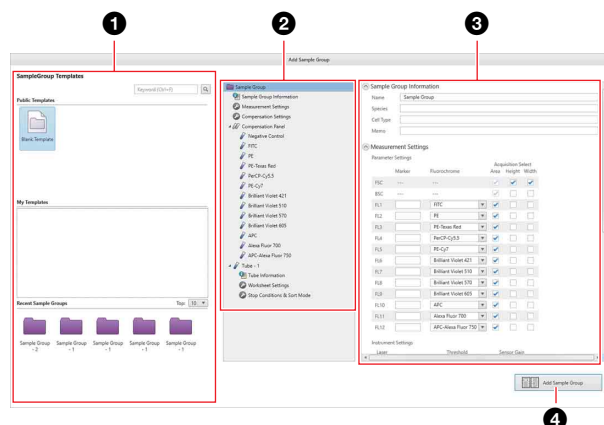
Creates a new experiment with the specified settings.

The experiment is added to the Experiment Explorer in the main window. The created experiment is automatically made the active experiment.

[Add Sample Group] dialog

The [Add Sample Group] dialog is displayed by clicking [Add Sample Group] in the [Create Experiment] window.

The [Add Sample Group] dialog is used to add a sample group to the experiment structure.



❶ [Sample Group Templates] list

Displays the available sample group templates and recent sample groups that can be used as templates.

You can search the template list by keywords.

[Public Templates]: Displays sample group templates that were created as shared templates.

[Blank Template]: An empty template.

[My Templates]: Displays private sample group templates that you have created.

[Recent Sample Groups]: Displays recent sample groups that can be used as templates.

❷ Sample group structure

Displays the internal structure of the sample group template selected in the list.

❸ Sample group settings

Displays configurable information and instrument settings for the sample group.

You can also edit settings after an experiment has been created.

For details about configurations items, see “*Experiment settings*” (page 130) in the [Create Experiment] Window.

❹ [Add Sample Group]

Adds a new sample group with the specified settings to the experiment structure.

❷ Sample tube structure

Displays the internal structure of the sample tube template selected in the list.

❸ Sample tube settings

Displays configurable information and instrument settings for the sample tube.

You can also edit settings after an experiment has been created.

For details about configurations items, see “*Experiment settings*” (page 130) in the [Create Experiment] Window.

❹ Worksheet

Displays the plots and gate statistics configured for the selected template or sample tube.

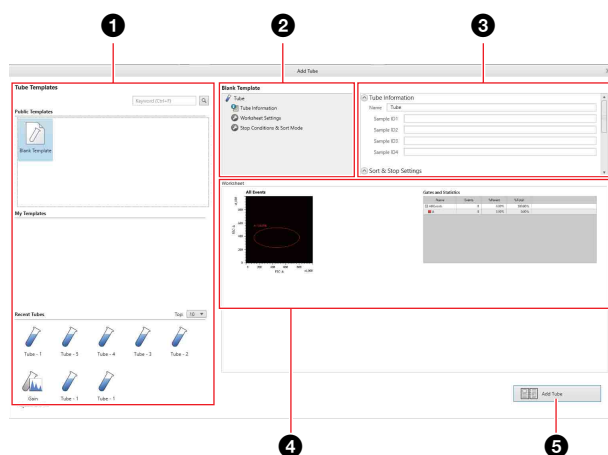
❺ [Add Tube]

Adds a new sample tube with the specified settings to the selected sample group in the experiment structure.

[Add Tube] dialog

The [Add Tube] dialog is displayed by clicking [Add Tube] in the [Create Experiment] window.

The [Add Tube] dialog is used to add a sample tube to the selected sample group in the experiment structure.



❶ [Tube Templates] list

Displays the available tube templates and sample tubes that can be used as templates.

You can search the template list by keywords.

[Public Templates]: Displays sample tube templates that were created as shared templates.

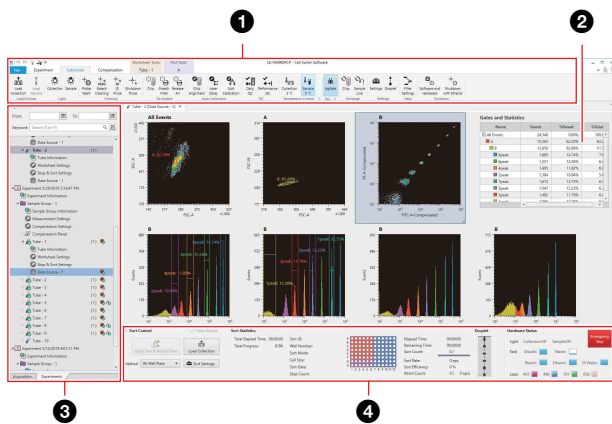
[Blank Template]: An empty template.

[My Templates]: Displays private tube templates that you have created.

[Recent Tubes]: Displays recent sample tubes that can be used as templates.

Main Window

The main window is where you acquire and record data, perform analysis, run cell sorting, and other functions. The main window has the following configuration.



1 Ribbon

- [File] tab ([page 119](#))
- [Experiment] tab ([page 132](#))
- [Cytometer] tab ([page 136](#))
- [Compensation] tab ([page 143](#))
- [Worksheet Tools] tab ([page 144](#))
- [Plot Tools] tab ([page 148](#))
- [Gate Tools] tab ([page 149](#))

2 Worksheet, [Gates and Statistics] table ([page 150](#)), context menu ([page 159](#))

3 [Acquisition] tab ([page 151](#)) / [Experiments] tab ([page 152](#)), context menu ([page 159](#))

4 [Sort Control] pane ([page 155](#))

[Experiment] Tab (ribbon)

The [Experiment] tab of the ribbon has the following buttons.

[Target] group

Contains tool buttons for specifying the active sample tube used for data acquisition, recording, and sorting.



[Assign Tube]: Assigns the tube selected in the Experiment Explorer as the active tube for data acquisition, recording, and cell sorting.

[Next Tube]: Assigns the next tube. If there are no further tubes, it duplicates the current tube and assigns the new tube.

[Experiment] group

Contains tool buttons for creating, deleting, and performing other actions on experiments.



[New]: Displays the [Create Experiment] window ([page 130](#)) for creating a new experiment.

[Delete]: Deletes the experiment selected in the Experiment Explorer.

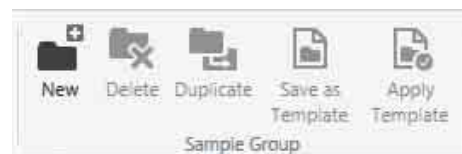
[Duplicate]: Duplicates the experiment selected in the Experiment Explorer.

[Save as Template]: Displays the [Save as Experiment Template] dialog ([page 133](#)) for saving the experiment selected in the Experiment Explorer as a template used when creating a new experiment.

[Send to Public]: Creates a duplicate of the selected experiment and places the duplicate under the [Public] node for sharing with other users.

[Sample Group] group

Contains tool buttons for creating, deleting, and performing other actions on sample groups.



[New]: Creates a new, blank sample group in the experiment selected in the Experiment Explorer.

[Delete]: Deletes the sample group selected in the Experiment Explorer.

[Duplicate]: Duplicates the sample group selected in the Experiment Explorer.

[Save as Template]: Displays the [Save as Sample Group Template] dialog ([page 133](#)) for saving the sample group selected in the Experiment Explorer as a template used when creating a new experiment.

[Apply Template]: Displays the [Apply Template] dialog ([page 134](#)) for selecting a template for a new sample group selected in the Experiment Explorer.

[Tube] group

Contains tool buttons for creating, deleting, and performing other actions on sample tubes.



[New]: Creates a new, blank sample tube in the sample group selected in the Experiment Explorer.

[Delete]: Deletes the sample tube selected in the Experiment Explorer.

[Duplicate]: Duplicates the sample tube selected in the Experiment Explorer.

[Save as Template]: Displays the [Save as Tube Template] dialog (*page 133*) for saving the sample tube selected in the Experiment Explorer as a template used when adding a sample tube.

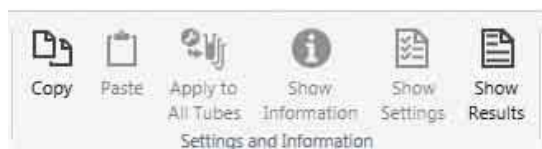
[Apply Template]: Displays the [Apply Template] dialog (*page 134*) for selecting a template for a new sample tube selected in the Experiment Explorer.

[Open Worksheet]: Opens the worksheet tab for the tube selected in the Experiment Explorer.

[Apply Compensation]: Applies fluorescence compensation to the plots on the worksheet for the tube selected in the Experiment Explorer.

[Settings and Information] group

Contains tool buttons for copying, pasting, and displaying settings and information.



[Copy] / [Paste]: Copies/Pastes the item selected in the Experiment Explorer.

[Apply to All Tubes]: Applies the worksheet settings for the tube selected in the Experiment Explorer to all sample tubes within the sample group.

Click [Worksheet Settings] for a tube in the Experiment Explorer and then click [Apply To All Tubes] to apply the same worksheet settings to the other tubes in the sample group for easy comparison during analysis.

[Show Information]: Displays the information dialog for the item ([Experiment Information], [Sample Group Information], etc.) selected in the Experiment Explorer.

You can also display the settings dialog by double-clicking [Experiment Information] and other items in the Experiment Explorer.

[Show Settings]: Displays the configuration dialog for the settings item ([Measurement Settings], [Compensation Settings], etc.) selected in the Experiment Explorer.

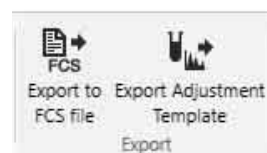
You can also display the settings dialog by double-clicking [Experiment Information] and other items in the Experiment Explorer.

[Show Results]: Displays the [Tube Results] dialog (*page 134*) for displaying the recording results and sorting results for the tube selected in the Experiment Explorer.

If [96 Well Plate], or [384 Well Plate] sorting was performed, the data source results that were recorded last are also displayed. If you click this button while a data source is selected, the [Data Source Results] dialog (*page 135*) appears, allowing you to view the results of each data source.

[Export] group

Contains a tool button for exporting recorded data for a sample tube.



[Export to FCS Files]: Displays the [FCS File Export] dialog (*page 135*) for exporting the data recorded for the tubes in the experiment selected in the Experiment Explorer in FCS 3.0 or FCS 3.1 format.

Tip

The importing of FCS files is not supported in Cell Sorter Software.

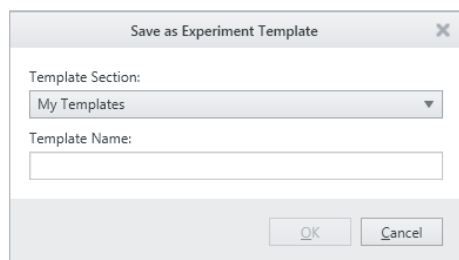
[Export Adjustment Template]: Creates a gain adjustment template (template for instrument settings) for an experiment that includes data used as reference data.

Select an experiment in the Experiment Explorer and click this button to display the [Export Experiment Data] dialog (*page 127*).

[Save as Experiment/Sample Group/Tube Template] dialog

The [Save as Experiment/Sample Group/Tube Template] dialog is displayed by selecting an experiment, sample group, or sample tube in the Experiment Explorer and clicking [Save as Template] on the [Experiment] tab of the ribbon or by right-clicking and selecting [Save as Experiment Template]/[Save as Sample Group Template]/[Save as Tube Template] from the context menu.

The [Save as Experiment/Sample Group/Tube Template] dialog is used to save the selected experiment, sample group, or sample tube as a template.

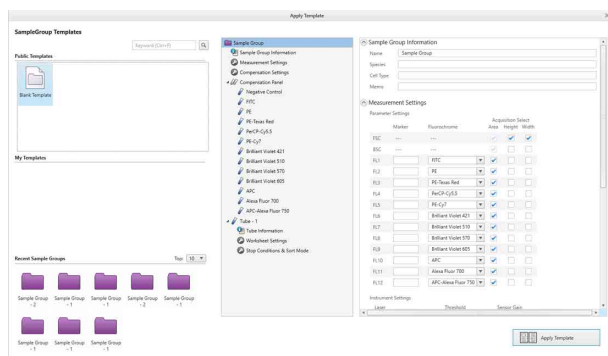


For details, see “Saving an Experiment as a Template” (page 87).

[Apply Template] dialog

The [Apply Template] dialog is displayed by selecting a new sample group or sample tube in the Experiment Explorer and clicking [Apply Template] on the [Experiment] tab of the ribbon.

The [Apply Template] dialog is used to select a template to apply to the sample group or sample tube.



The configurable items are the same as the [Add Sample Group] dialog (page 130) or [Add Tube] dialog (page 131) for sample groups.

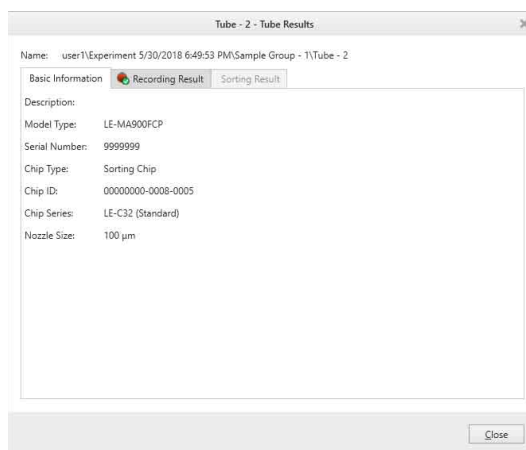
[Tube Results] dialog

The [Tube Results] dialog is displayed by selecting a sample tube in the Experiment Explorer and clicking [Show Results] in the [Settings and Information] group on the [Experiment] tab of the ribbon.

The [Tube Results] dialog is used to check the recording results and sorting results of the selected sample tube.

• [Basic Information] tab

Displays basic information identifying the sample tube.



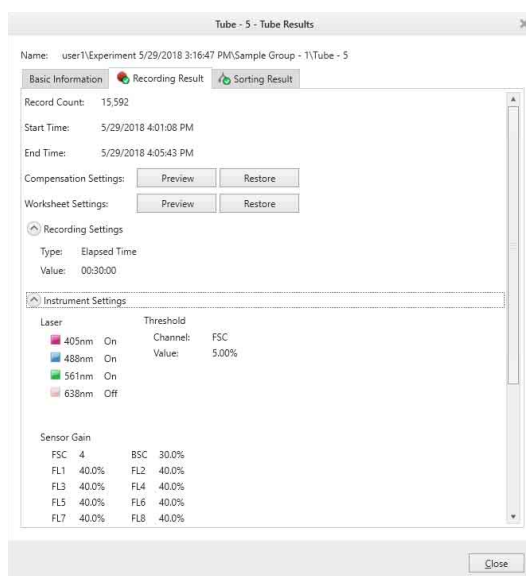
[Chip ID]: Displays the ID number of the sorting chip.

[Nozzle Size]: Displays the nozzle size of the sorting chip.

[Chip Series]: Displays the series name of the sorting chip.

• [Recording Result] tab

Displays recording results.



[Record Count]: Displays the number of events recorded.

[Start Time]: Displays the time when recording started.

[End Time]: Displays the time when recording ended.

[Compensation Settings]: Previews/restores fluorescence compensation settings, saved when data recording ends.

- [Preview]: Displays the fluorescence compensation settings.

- [Restore]: Restores the fluorescence compensation settings. Click [Yes] in the confirmation dialog to proceed.

[Worksheet Settings]: Previews/restores worksheet settings, saved when data recording ends.

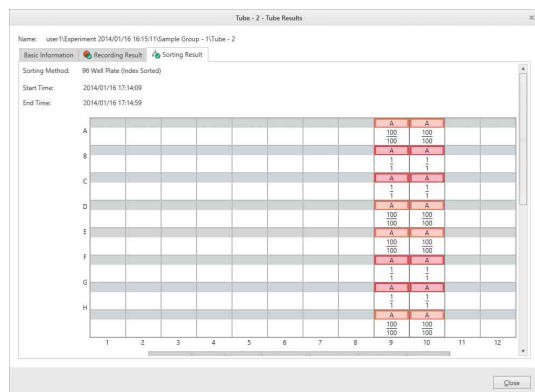
- **[Preview]:** Displays the worksheet settings.
- **[Restore]:** Restores the worksheet settings. Click **[Yes]** in the confirmation dialog to proceed.

[Recording Settings]: Displays the recording settings.

[Instrument Settings]: Displays the instrument settings.

• **[Sorting Result] tab**

Displays the sorting results.



[Method]: Displays the sorting method.

[Start Time]: Displays the time when recording started.

[End Time]: Displays the time when recording ended.

[Sort Gate]: Displays the gate specified for sorting.

[Sort Mode]: Displays the sorting mode specified for sorting.

[Elapsed Time]: Displays the elapsed time since starting sorting.

[Total Events]: Displays the number of events detected during sorting.

[Target Ratio]: Displays the percentage of events detected during sorting that fall within the sort gate.

[Sorted Count]: Displays the number of events sorted.

[Sort Rate]: Displays the rate at which events are sorted in units of events per second (eps).

[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.

[Aborted Count]: Displays the number of target events in the sort gate that do not match the sort mode criteria and which are aborted during sorting.

[Abort Rate]: Displays the rate at which events are aborted during sorting in units of events per second (eps).

[Sort Result]: Indicates whether sorting is finished or not.

[Copy to Clipboard]: Copies the sorting results data to the clipboard as an image for pasting in other software, for example to produce reports.

[Save as CSV]: Saves the sorting results as CSV-format file.

[Compensation Settings]: Previews/restores fluorescence compensation settings, saved when sorting ends.

• **[Preview]:** Displays the fluorescence compensation settings.

• **[Restore]:** Restores the fluorescence compensation settings. Click **[Yes]** in the confirmation dialog to proceed.

[Worksheet Settings]: Previews/restores worksheet settings, saved when sorting ends.

• **[Preview]:** Displays the worksheet settings.

• **[Restore]:** Restores the worksheet settings. Click **[Yes]** in the confirmation dialog to proceed.

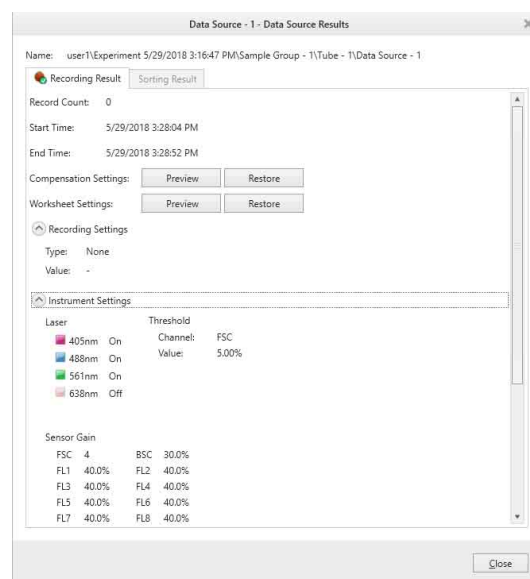
[Sort Settings]: Displays the sort settings.

[Instrument Settings]: Displays the instrument settings.

[Auto Parameters]: Displays automatically configured sorting parameters.

[Data Source Results] dialog

The **[Data Source Results]** dialog is displayed by selecting a data source in the Experiment Explorer and clicking **[Show Results]** in the **[Settings and Information]** group in the **[Experiment]** tab of the ribbon. The **[Data Source Results]** dialog allows you to view the results of each data source.

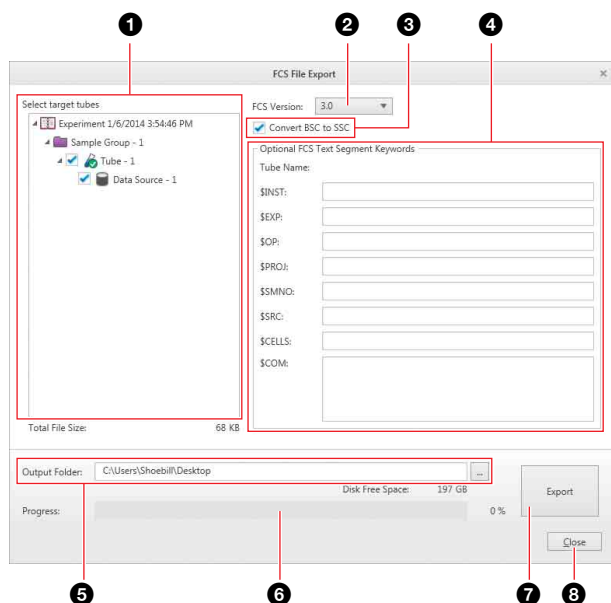


The **[Data Source Results]** dialog displays all the information from the **[Tube Results]** dialog, except the **[Basic Information]** tab.

For details, see “**[Tube Results] dialog**” (page 134).

[FCS File Export] dialog

The **[FCS File Export]** dialog is displayed by selecting an experiment in the Experiment Explorer and clicking **[Export to FCS file]** in the **[Export]** group on the **[Experiment]** tab of the ribbon or by right-clicking and selecting **[Export FCS Files]** from the context menu.



1 [Select target tubes]

Selects the sample tubes to export. Place a check mark in the checkbox for each target tube.

2 [FCS Version]

Selects the FCS file version (3.0 or 3.1).

3 [Convert BSC to SSC]

Selects whether to convert the data name of back scatter data from BSC (back scatter) to SSC (side scatter) when creating FCS files.


4 [Optional FCS Text Segment Keywords]

You can specify optional FCS text segment keywords, as required. Required FCS text segment keywords are exported automatically.

The following optional FCS text segment keywords can be specified.

- \$INST: Institution at which data was acquired
- \$EXP: Name of investigator initiating the experiment
- \$OP: Name of instrument operator
- \$PROJ: Name of the experiment project
- \$SMNO: Specimen (e.g., tube) label
- \$SRC: Source of the specimen (patient name, cell type)
- \$CELLS: Description of objects measured
- \$COM: Comment

5 [Output Folder]

Specifies the export destination folder. Click  (Browse) to specify the destination folder. The files are named in "<tube_name>.fcs" format.

6 Progress

Displays the export progress.

7 [Export]

Starts the export.

8 [Close]

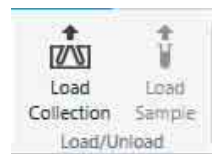
Closes the dialog.

[Cytometer] Tab (ribbon)

The [Cytometer] tab of the ribbon has the following buttons.

[Load/Unload] group

Contains tool buttons for loading and unloading sample tubes and the collection tubes.

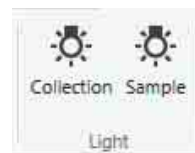


[Load Collection/Unload Collection]: Loads/unloads the collection tube in the collection area.

[Load Sample/Unload Sample]: Loads/unloads the sample tube in the sample loader.

[Light] group

Contains tool buttons for turning the collection stage and sample loader lights on/off.



[Collection]: Turns the collection stage light on/off.

[Sample]: Turns the sample loader light on/off.

[Cleaning] group

Contains tool buttons for cleaning the fluidics system.



[Probe Wash]: Flushes the sorting chip, the inside and the outside of the sample probe using sheath fluid.

Tip

The outer surface of the sample probe is not washed when [Skip rinse outside of sample probe] is enabled.

[Bleach Cleaning]: Displays the [Bleach Cleaning] dialog (page 139) for cleaning the sorting chip, sample line, and sample probe with a Sodium Hypochlorite solution.

For details about cleaning using Sodium Hypochlorite solution, see "Cleaning Sample Line using Bleach" (page 174).

[DI Rinse]: Displays the [DI Rinse] dialog (*page 139*) for flushing the sorting chip, sample line, and sample probe with DI water.

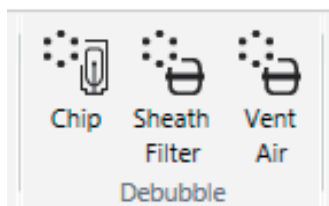
[Shutdown Rinse]: Displays the [Shutdown DI Rinse] dialog (*page 139*) for flushing the sorting chip, sample line, and sample probe with DI water before shutting down the instrument.

Note

After cleaning, the fluidics lines are filled with DI water to prevent the formation of salt deposits. Running data acquisition in this state may damage cells.

[Debubble] group

Contains command buttons for removing air bubbles from the sorting chip and sheath filter.



[Chip]: Removes air bubbles from the sorting chip.

[Sheath Filter]: Removes air bubbles from the sheath filter and the sheath line.

[Vent Air]: Removes the remaining air from the sheath filter.

[Auto Calibration] group

Contains tool buttons for aligning the chip and calibrating sorting.



[Chip Alignment]: Displays the [Chip Alignment] screen of the [Auto Calibration] wizard for aligning the sorting chip.

Follow the on-screen instructions to align the sorting chip. During automatic alignment, a progress bar and status are displayed, allowing you to check the progress and current state.

Tip

After auto alignment calibration, clean the outside of the sample probe using a loaded tube containing DI water or similar fluid.

[Laser Delay]: Runs the laser delay adjustment.

Clicking this button displays the [Laser Delay] wizard.

Follow the on-screen instructions to adjust the laser delay. During laser delay adjustment, a progress bar and status are displayed.

Tip

Clicking [Laser Delay] after inserting a sorting chip and while chip alignment is not completed successfully will display a warning message and laser delay adjustment will not be executed.

[Sort Calibration]: Displays the [Auto Calibration] wizard for calibrating sorting for performing droplet calibration, side stream calibration, and sort delay calibration automatically.

Run sorting calibration after inserting a sorting chip and the sorting chip alignment is successful.

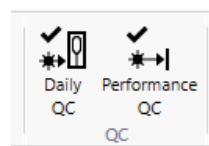
For details, see “Running Automatic Calibration” (page 49).

Tip

Clicking the [Sort Calibration] button after inserting a sorting chip and while chip alignment is not completed successfully will display a warning message and sorting calibration will not be executed.

[QC] group

Contains tool buttons for running Daily QC and Performance QC.



[Daily QC]: Displays the [Daily QC] wizard for running Daily QC.

Follow the on-screen instructions to run Daily QC. During Daily QC, a progress bar and status are displayed, allowing you to check the progress and current state.

Run Daily QC after successfully completing chip alignment and laser delay adjustment.

Tip

Clicking [Daily QC] after inserting a sorting chip and while chip alignment is not completed successfully will display a warning message and Daily QC will not be executed.

[Performance QC]: Displays the [Performance QC] wizard for running Performance QC.

Follow the on-screen instructions to run Performance QC. During Performance QC, a progress bar and status are displayed, allowing you to check the progress and current state.

Run Performance QC after successfully completing chip alignment and laser delay adjustment.

Tip

Clicking [Performance QC] after inserting a sorting chip and while chip alignment is not completed

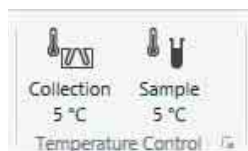
successfully will display a warning message and Performance QC will not be executed.

[Temperature Control] group

Contains tool buttons for setting the temperature of the collection stage and sample injection chamber.


The following temperature control options are available.

- Collection stage: 5 °C (41 °F) only
- Sample injection chamber: 5 °C (41 °F) or 37 °C (98.6 °F)



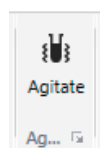
[Collection]: Turns the collection temperature control on/off and displays the current temperature setting.

[Sample]: Turns the sample injection chamber temperature control on/off and displays the current temperature setting.


Clicking the  dialog launcher displays the [Temperature Control Settings] dialog (page 139) for specifying the temperature settings.

[Agitate] group

Contains a tool button for controlling agitation of the sample fluid.



[Agitate]: Turns the sample loader agitation unit on/off.

 : Displays the [Agitate Settings] dialog (page 139) for setting the agitation speed.

[Exchange] group

Contains command buttons for changing the fluidics system components.



[Chip]: Ejects the sorting chip and displays the [Chip Exchange] wizard for inserting a different sorting chip.

For details, see “Exchanging the Sorting Chip” (page 73).

[Sample Line]: Displays the [Sample Line Exchange] wizard for changing the sample line.

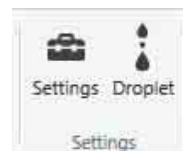
For details, see “Changing the PEEK Sample Line” (page 175).

Tip

The sorting chip must be replaced or reinserted after changing the sample line.

[Settings] group

Contains tools button for configuring advanced settings related to the instrument.

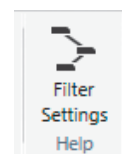


[Settings]: Displays the [Cytometer Settings] dialog (page 139) for configuring the instrument, adjusting sort parameters, and other instrument functions.

[Droplet]: Displays a detailed image of the droplets.

[Help] group

Contains a tool button for displaying the optical filter pattern.



[Filter Settings]: Displays the configured optical filter pattern. This cannot be modified.

[Shutdown] group

Contains tool buttons used when shutting down the LE-MA900.



[Software and Hardware]: Displays the [Shutdown Wizard] screen for cleaning the LE-MA900 shutting down the instrument and Cell Sorter Software.

For details, see “Shutting Down the Main Unit and Software” (page 70).

[Shutdown with Ethanol]: Displays the [Ethanol Cleaning Wizard] for shutting down the LE-MA900 main unit Cell Sorter Software after cleaning the unit with ethanol.

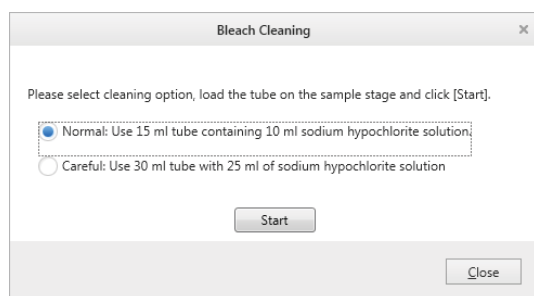
For details, see “Cleaning the Sheath Line and Shutting Down” (page 71).

[Bleach Cleaning] dialog

The [Bleach Cleaning] dialog is displayed by clicking [Bleach Cleaning] in the [Cleaning] group on the [Cytometer] tab of the ribbon.

The [Bleach Cleaning] dialog is used to start cleaning of the sorting chip, sample line, and sample probe using a Sodium Hypochlorite (bleach) solution.

For details, see “Cleaning Sample Line using Bleach” (page 174).

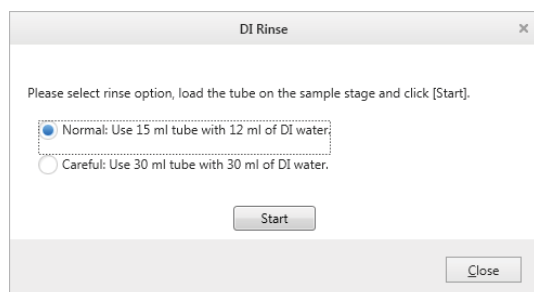


[DI Rinse] dialog

The [DI Rinse] dialog is displayed by clicking [DI Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon.

The [DI Rinse] dialog is used to start flushing of the sorting chip, sample line, and sample probe using DI water.

Select the size of conical tube containing DI water to use, then click [Start] to load the tube and begin cleaning.

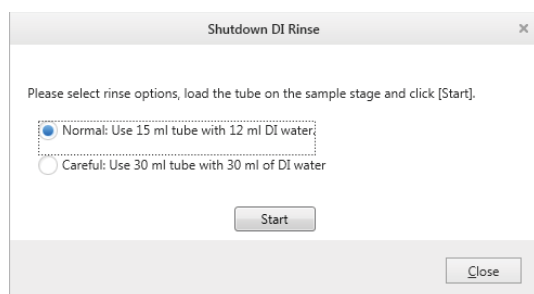


[Shutdown DI Rinse] dialog


The [Shutdown DI Rinse] dialog is displayed by clicking [Shutdown Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon.

The [Shutdown DI Rinse] dialog is used to clean the sorting chip, sample line, and sample probe using DI water, and then automatically shutting down.

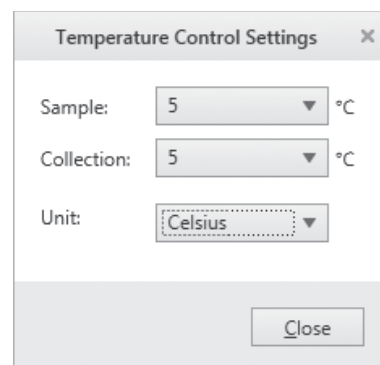
Select the size of conical tube containing DI water to use, then click [Start] to load the tube and begin cleaning.



[Temperature Control Settings] dialog

The [Temperature Control Settings] dialog is displayed by clicking the  dialog launcher in the [Temperature Control] group on the [Cytometer] tab of the ribbon.

The [Temperature Control Settings] dialog is used to set the temperature of the sample injection chamber and collection stage area.




[Sample]: Sets the control temperature of the sample injection chamber to 5 °C (41 °F) or 37 °C (98.6 °F). The selected temperature values are displayed on the corresponding buttons in the [Temperature Control] group on the ribbon.

[Collection]: Selects the control temperature of the collection stage area. Only 5 °C (41 °F) is available.

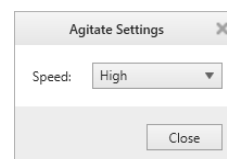
[Unit]: Selects the units of temperature (Celsius or Fahrenheit).

[Close]: Closes the dialog.

[Agitate Settings] dialog

The [Agitate Settings] dialog is displayed by clicking the  dialog launcher in the [Agitate] group on the [Cytometer] tab of the ribbon.

The [Agitate Settings] dialog is used to set the agitation speed.



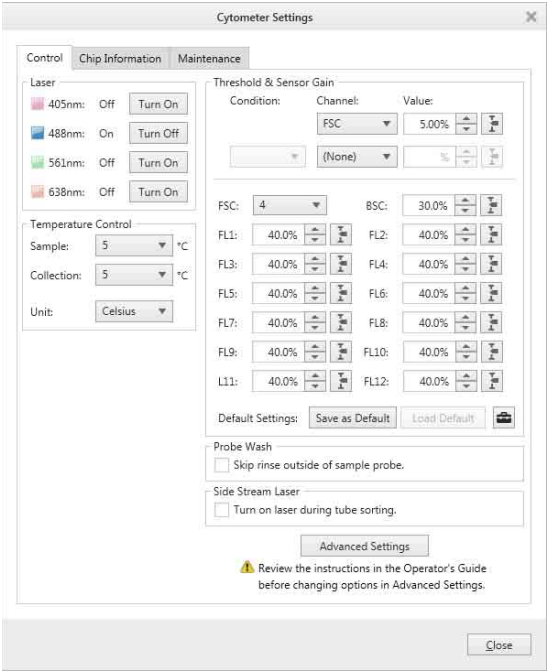
[Speed]: Set the speed to [High] or [Low].

[Cytometer Settings] dialog

The [Cytometer Status] dialog is displayed by clicking [Settings] in the [Settings] group on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog is used to configure instrument settings, adjust sort parameters, and other functions.

- **[Control] tab**
This tab is used to configure instrument settings.



- [Laser]:** Turns each laser on/off.
- [Temperature Control]:** Sets the control temperature of the sample injection chamber and collection stage.
For details about each item, see “[Temperature Control Settings] dialog” (page 139).

- [Threshold & Sensor Gain]:** Sets the threshold trigger for detecting events and the gain of each detector.
 - **[Condition]:** Specifies the handling of the two trigger channels.
 - **[Channel]:** Selects the channel (FSC, BSC, FL1 to FL12) used to trigger the detection of events.
 - **[Value]:** Sets the input threshold signal level of the trigger channel as a percentage of the maximum signal from the detection module.
 - **[FSC]:** Selects the gain of the forward-scatter detector. The gain setting has a 17-step range.
 - **BSC, FL1 to FL12:** Sets the gain of the corresponding back scatter and fluorescence detectors as a percentage of the maximum level.
 - **[Default Settings]:** Used to save current settings as default settings and to load previously saved settings.

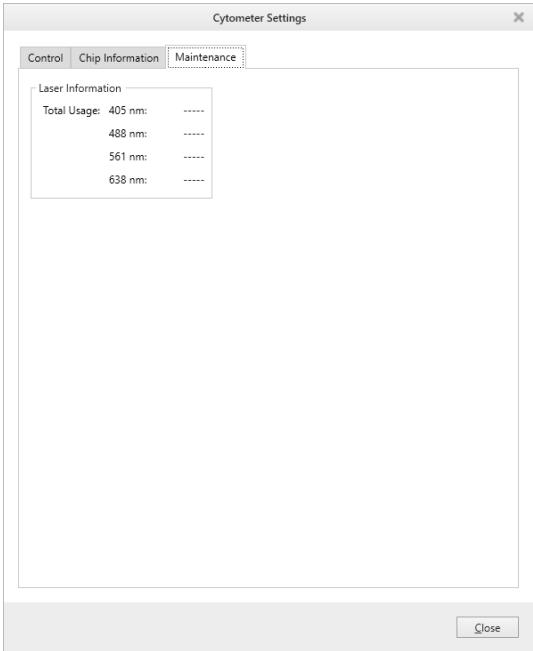
- [Probe Wash]:** To skip the rinse of the outside of the sample probe between measurements, enable [Skip rinse outside of sample probe.]. If a check mark is placed in this checkbox, clean the outside of the probe as required.
- [Side Stream Laser]:** Sets whether to turn the laser when sorting using collection tubes.

- [Advanced Settings]:** Displays the [Advanced Settings] dialog (page 141) for manually adjusting sort parameters.

- **[Chip Information] tab**
Displays information about the loaded sorting chip. This is useful for checking the length of time the sorting chip has been in use.



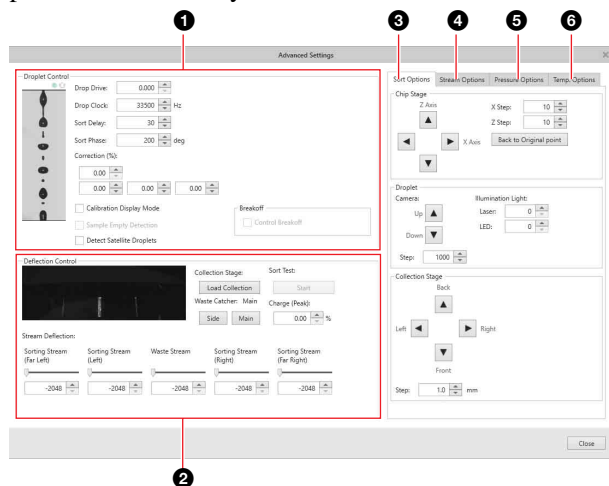
- **[Maintenance] tab**
Displays the status of the fluidics lines and the laser usage time.



- [Laser Information]:** Displays the usage time of each laser.

• [Advanced Settings] dialog

The [Advanced Settings] dialog is used to adjust sort parameters manually.



1 [Droplet Control]

Contains adjustments related to droplet formation.

[Drop Drive]

The drop drive setting adjusts the amplitude of the piezoelectric element drive voltage. The frequency sets the cycle time of vibration of the sorting chip. The vibration of the sorting chip causes the stream to break into droplets after the stream is emitted from the chip nozzle. The drop drive is optimized during auto calibration.

[Drop Clock]

The drop clock setting adjusts the frequency of the vibration of the piezoelectric element drive voltage. The frequency sets the cycle time of vibration of the sorting chip. The drop drive is optimized during auto calibration.

[Sort Delay]

The sort delay setting adjusts the number of vibrations applied to the sorting chip as cells pass between the optical detection point and the breakoff point. It is used to determine the timing of the electrostatic charging pulse applied to the sheath fluid.

The sort delay is optimized during auto calibration.

[Sort Phase]

The sort phase setting adjusts the phase difference of the charging pulse waveform as cells pass between the optical detection point and the breakoff point. It is used to determine the timing of the electrostatic charging pulse applied to the sheath fluid.

The sort phase is optimized during auto calibration.

[Correction (%)]

The correction setting adjusts the shape of the charging waveform applied to successive droplets.

- Top spinbox: Controls the overall charging waveform correction adjustment.
- Lower spinboxes: Fine-tunes the correction applied to the first droplet, second droplet, and third droplet, respectively.

When droplets are electrostatically charged, the next droplet receives an induced charge of the opposite polarity, which can affect the angle of deflection as the droplets pass through the deflection plates (called fanning). A defanning algorithm adjusts the charging waveform applied to successive droplets to reduce fanning.

[Calibration Display Mode]

The calibration display mode changes the image displayed by the droplet camera so that only droplets containing automatic setup beads are displayed.

This mode is used for fine manual control of the sort delay, sort phase, and related sort parameters.

[Control Breakoff]

The control breakoff setting uses a feedback system to control the breakoff position automatically.

When this function is enabled, the drop drive is adjusted so as to maintain the optimized breakoff point and side streams obtained during auto calibration.

When auto calibration finishes, the Control Breakoff function is automatically enabled.

Tips

- The drop drive and drop clock cannot be adjusted manually while the Control Breakoff function is enabled.
- When breakoff feedback control is enabled, if the breakoff point moves and cannot be recovered within a predetermined time, sorting stops and an error message is displayed on the screen.

[Sample Empty Detection]

The empty detection function automatically stops data acquisition when the sample tube becomes empty. The function detects disturbances in the droplet stream as the sample fluid in the sample tube is used up, and then stops data acquisition.

Tips

- The function detects disturbances in the droplets caused when the sample tube becomes empty and air enters the droplets or when aggregated cells enter the flow. You can also disable this function.

- If a sample empty state is detected, click [Chip] in the [Debubble] group on the [Cytometer] tab of the ribbon in Cell Sorter Software, and repeatedly de-bubble the sorting chip until the breakoff point returns to its original position.

[Detect Satellite Droplets]

The satellite droplet detection function detects irregular shaped droplets during sorting. When enabled, this function pauses the sample acquisition and sorting if satellite drops are generated in the stream.

Tips

- Satellite droplet detection is supported only for 70 μm sorting chips.
- In rare cases, the satellite droplet detection function may pause the sample acquisition by mistake. If this occurs, disable the satellite droplet detection function to continue data acquisition.

2 [Deflection Control]

Contains controls for adjusting the side stream deflection angle, loading/unloading the collection stage, and running a sorting test.

[Stream Deflection]

Adjusts the angle of deflection of each side stream.

[Collection Stage]

Loads/unloads the collection stage.

[Sort Test]

Runs a sorting test.

[Waste Catcher]

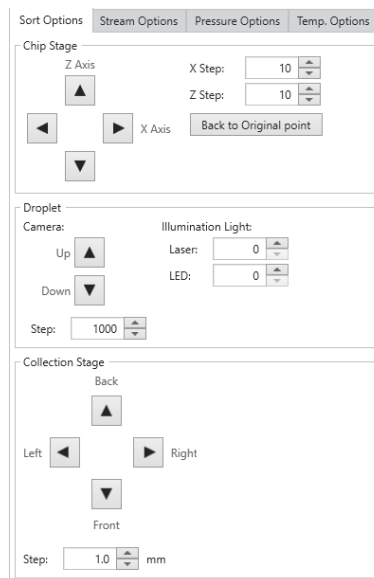
Moves the collection area waste catcher to the side.

[Charge (Peak)]

Sets the peak value of the charging voltage waveform.

3 [Sort Options] tab

The sort option settings are used to adjust the position of the sorting chip, droplet image, and collection stage manually.



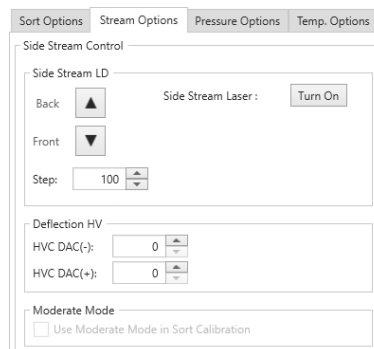
[Chip Stage]: Adjusts the positioning of the sorting chip within the chip loader.

[Droplet]: Adjusts the vertical positioning of the droplet camera, laser for droplet image fluorescence detection, and the brightness of the infrared LED for droplet imaging.

[Collection Stage]: Adjusts the horizontal position of the collection stage.

4 [Stream Options] tab

Adjusts side stream settings.



[Side Stream LD]: Adjusts the irradiation position of the side stream laser.

[Deflection HV]: Adjusts the voltage applied to each of the deflection plates.

[Moderate Mode]: Sets whether to use moderate mode for sorting calibration.

5 [Pressure Options] tab

Sets the sample fluid flow controls and the window extension settings.

[Flow Control]: Adjusts the sample pressure and sheath pressure.

Clicking [Standby] stops the sheath and sample fluid flow. Clicking [Ready] starts sheath flow again in preparation for a sample.

[Pulse Calculation]: Sets the forward window extension (FWE) and back window extension (BWE) settings used to modify the pulse parameters for data acquisition.

Tip

The setting of the forward window extension and back window extension affect the data acquisition results for Area pulse parameters.

[Laser Delay]: Adjusts the laser delay.

[Sample Loader]: Loads/unloads the sample loader.

[Sample area maintenance]: Displays the wizard for cleaning the sample area.

6 [Temp. Options] tab

Sets temperature adjustments.

[Device Temperature]: Sets the display units for temperature.

- Celsius
- Fahrenheit

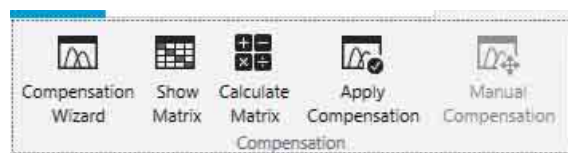
[Sheath Temp. Control]: Switches the temperature adjustment of sheath fluid on/off.

[Compensation] Tab (ribbon)

The [Compensation] tab of the ribbon has the following buttons.

[Compensation] group

Contains tool buttons related to fluorescence compensation.



[Compensation Wizard]: Displays the [Compensation Wizard] for recording control tubes in a compensation panel.

The compensation settings should normally be configured using the [Compensation Wizard] to record a negative (unstained) control tube and up to six positive (stained) control tubes in a compensation panel. The [Compensation Wizard] acquires data for compensation samples and then automatically calculates the fluorescence compensation spillover matrix for the sample group.

For details, see “Compensating Fluorescence Spillover” (page 66).

[Show Matrix]: Displays the [Compensation Settings] dialog (page 143) for viewing the spillover matrix, and loading/saving the spillover matrix.

[Calculate Matrix]: Displays the [Calculate Compensation Settings] dialog (page 144) for calculating the coefficients of the fluorescence compensation spillover matrix based on the compensation panel.

[Apply compensation]: Selects whether fluorescence compensation is applied to plots on the worksheet. Clicking this button toggles compensation on/off.

[Manual Compensation]: Toggles manual fluorescence compensation mode on/off for the plots on the worksheet.

[Compensation Settings] dialog

The [Compensation Settings] dialog is displayed by clicking [Show Matrix] on the [Compensation] tab of the ribbon.

The [Compensation Settings] dialog is used to view the spillover matrix, and load/save the spillover matrix.

Sample Group: 1 - Compensation Settings

Spillover Matrix (%)

	FTTC	PE	PE-Texas Red	PerCP-Cy5.5	PE-Cy7	Brilliant Violet 421	Brilliant Violet 510	Brilliant Violet 605	Brilliant Violet 610	APC	APC-Alexa Fluor 700	APC-Alexa Fluor 750
FTTC	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PE	2.81	100.00	200.00	200.00	178.87	2.81	100.00	200.00	200.00	178.87	112.78	200.00
PE-Texas Red	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PerCP-Cy5.5	2.78	200.00	200.00	100.00	120.18	2.78	200.00	200.00	100.00	120.18	-37.87	200.00
PE-Cy7	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 421	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 510	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 605	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
APC-Alexa Fluor 700	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
APC-Alexa Fluor 750	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00

☒ Negative Value

Save Load Reset

Close

Tip

Fluorochromes cannot be added or deleted using the [Compensation Settings] dialog.

To make changes, see “[Measurement Settings] dialog” (page 81).

[Spillover Matrix (%)]: Displays the fluorescence compensation spillover matrix.

Spillover matrix values display the fluorescence intensity due to each fluorochrome as a percentage of the total intensity in each channel.

Spillover matrix coefficients can be modified manually.

Note

Changing compensation values directly is only recommended for experienced users.

[Negative Value]: Displays the spillover matrix for the negative control tube for both area and height pulse parameters.

[Save]: Saves the compensation settings as an XML file. You can load saved compensation settings into another sample group or another experiment.

[Load]: Loads previously saved compensation settings from an XML file.

[Reset]: Resets all compensation settings.

[Close]: Closes the dialog.

[Calculate Compensation Settings] dialog

The [Calculate Compensation Settings] dialog is displayed by clicking [Calculate Matrix] on the [Compensation] tab of the ribbon.

The [Calculate Compensation Settings] dialog is used to calculate the coefficients of the fluorescence compensation spillover matrix based on the compensation panel.

Calculate Compensation Settings

Spillover matrix will be calculated from compensation tubes data in compensation panel.

Target Compensation Panel:
user1\Experiment 5/29/2018 3:16:47 PM\Sample Group 1 - TC Compensation Panel

Spillover Matrix (%)

	FTTC	PE	PE-Texas Red	PerCP-Cy5.5	PE-Cy7	Brilliant Violet 421	Brilliant Violet 510	Brilliant Violet 605	Brilliant Violet 610	APC	APC-Alexa Fluor 700	APC-Alexa Fluor 750
FTTC	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PE	2.81	100.00	1713.06	271.25	178.87	2.81	100.00	1713.06	271.25	178.87	112.78	10515.87
PE-Texas Red	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PerCP-Cy5.5	2.78	14026.48	716.90	100.00	100.18	2.78	14026.48	716.90	100.00	100.18	-37.87	14041.54
PE-Cy7	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 421	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 510	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
Brilliant Violet 605	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
APC-Alexa Fluor 700	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
APC-Alexa Fluor 750	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00

☒ Use Negative Value for Compensation
(If you use different type sample (e.g. comp beads) from real target sample, please uncheck the checkbox)

Calculate

Close

[Target Compensation Panel]: Displays the compensation panel for which to calculate fluorescence compensation.

[Use Negative Value for Compensation]: Enables calculation of the spillover matrix using a negative control. If an appropriate negative control is not available, deselect the checkbox to calculate the spillover matrix without using a negative control. The checkbox default setting is on (enabled).

[Spillover Matrix (%)]: Displays the spillover matrix. Spillover matrix values display the fluorescence intensity due to each fluorochrome as a percentage of the total intensity in each channel.

[Calculate]: Calculates the spillover matrix coefficients.

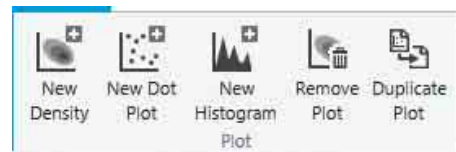
[Close]: Closes the dialog.

[Worksheet Tools] Tab (ribbon)

The [Worksheet Tools] tab of the ribbon has the following buttons.

[Plot] group

Contains tool buttons for adding, deleting, and duplicating plots.



For details, see “Adding a Plot” (page 93).

[New Density]: Adds a new density plot to the worksheet.

[New Dot Plot]: Adds a new dot plot to the worksheet.

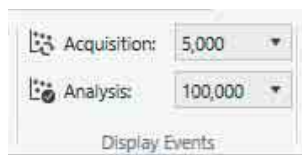
[New Histogram]: Adds a new histogram plot to the worksheet.

[Remove Plot]: Removes the selected plot from the worksheet.

[Duplicates Plot]: Duplicates the selected plot on the worksheet.

[Display Events] group

Contains tool buttons for setting the number of events displayed on plots.



[Acquisition]: Sets the number of events to display on plot during data acquisition.

[Analysis]: Sets the number of events to display on plot when analyzing data.

[Gate] group

Contains tool buttons for editing gates.



For details, see “Editing Gates” (page 102).

[Remove Gate]: Deletes the selected gate.

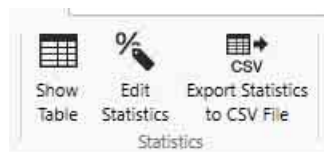
Note

Deleting a gate will also delete all gates and plots that are derived from the selected gate.

[Edit Gate]: Displays the [Gate Editor] dialog (page 146) for editing gates.

[Statistics] group

Contains tool buttons for working with statistics.



For details, see “Displaying Statistics” (page 106).

[Show Table]: Shows/hides the [Gates and Statistics] table.

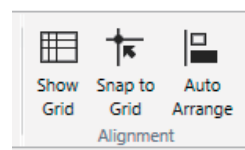
[Edit Statistics]: Displays the [Statistics Editor] dialog for configuring the attributes displayed in the [Gates and Statistics] table.

For details about the [Statistics Editor] dialog, see “Displaying Statistics” (page 106).

[Export Statistics to CSV File]: Displays the [Exporting statistics table] dialog (page 147) for exporting the data in the [Gates and Statistics] table as a CSV-format file.

[Alignment] group

Contains tool buttons for displaying a grid on the worksheet and for aligning displayed items with the grid.



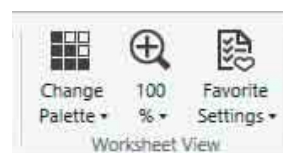
[Show Grid]: Displays the grid on the worksheet.

[Snap to Grid]: Aligns the plots and [Gates and Statistics] table on the worksheet with the grid when they are moved or added.

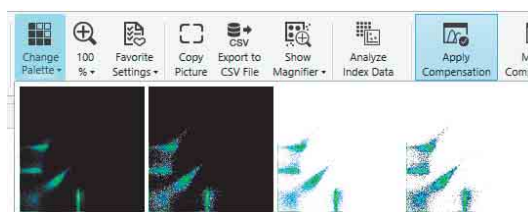
[Auto Arrange]: Automatically aligns the plots and [Gates and Statistics] table on the worksheet to the grid.

[Worksheet View] group

Contains tool buttons related to the display of items on the worksheet, and for saving the current settings.



[Change Palette]: Displays a drop-down palette for changing the display style of the selected plot.



- Black background
- Black background with highlighted outlying events
- White background
- White background with highlighted outlying events

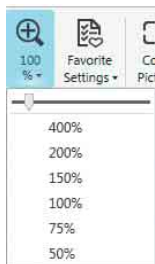
Highlighting outlying events is useful for identifying and analyzing rare events.

Zoom%: Displays a drop-down menu for changing the display zoom factor of the worksheet.

The current zoom factor is displayed on the button icon.

This setting is applied to all plots.

Click this button and select the zoom factor from the menu, or use the slider to set the zoom factor.

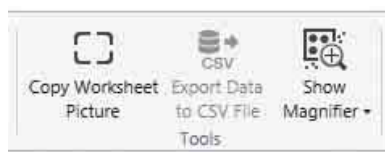


[Favorite Settings]: Allows you to store favorite worksheet settings.

- [Save as Favorite]: Saves the current worksheet state as the default settings.
- [Restore from Favorite]: Applies saved settings to the current worksheet.

[Tools] group

Contains tool buttons for copying, exporting, and displaying data on the worksheet.



[Copy Worksheet Picture]: Copies the selected worksheet to the clipboard for pasting in other software, for example to produce reports.

[Export Data to CSV File]: Exports the recorded data for the sample tube as a CSV file. Clicking this button displays the [Exporting Data] dialog (*page 147*).

If conducting index sorting, you can export only the index sorted data by selecting [Well Select Mode] in the [Index Analysis] dialog (*page 115*).

[Show Magnifier]: Shows/hides a worksheet magnifier. You can also set the zoom factor.



[Index Sorting] group

Contains a command button for analyzing data using the index sorting function.

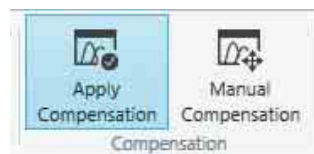


[Analyze Index Data]: Displays the [Index Analysis] dialog for emphasizing the position on plots of cells sorted in each well in multi-well plates.

For details about the [Index Analysis] dialog, see “Analyzing Index Sort Data” (*page 115*).

[Compensation] group

Contains tool buttons related to fluorescence compensation.

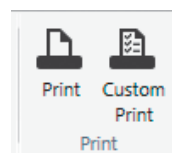


[Apply compensation]: Selects whether fluorescence compensation is applied to plots on the worksheet. Clicking this button toggles compensation on/off.

[Manual Compensation]: Toggles manual fluorescence compensation mode on/off for the plots on the worksheet.

[Print] group

Contains tool buttons for printing statistics and plots displayed on the worksheet.



[Print]: Displays the [Print] window (*page 124*) for printing the worksheet.

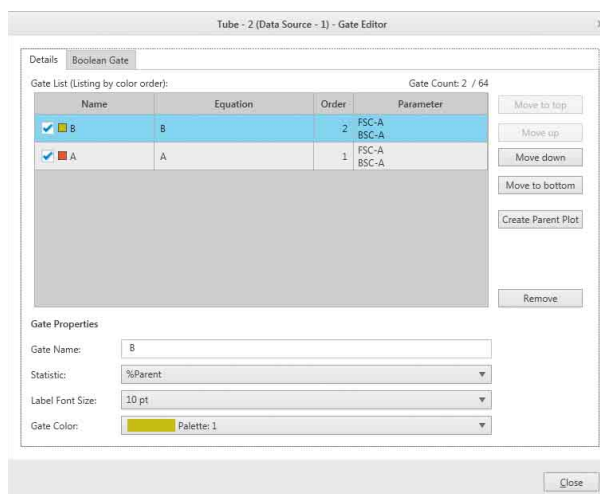
[Custom Print]: Displays the [Custom Print] dialog (*page 147*) for selecting the items to print.

[Gate Editor] dialog

The [Gate Editor] dialog is displayed by clicking [Edit Gate] on the [Worksheet Tools] tab, [Plot Tools] tab, or [Gate Tools] tab of the ribbon.

The [Gate Editor] dialog is used to edit gates.

- **[Details] tab**
Edits attributes of gates.



[Gate List]: Displays a list of the configured gates.

You can show or hide a gate on plots using the checkbox beside the gate name.

[Move to top]: Moves the gate with the selected color to the front on dot plots and histogram plots.

[Move up]: Moves the gate with the selected color one step toward the front on dot plots and histogram plots.

[Move down]: Moves the gate with the selected color one step toward the back on dot plots and histogram plots.

[Move to bottom]: Moves the gate with the selected color to the back on dot plots and histogram plots.

[Create Parent Plot]: Adds a parent plot to the worksheet.

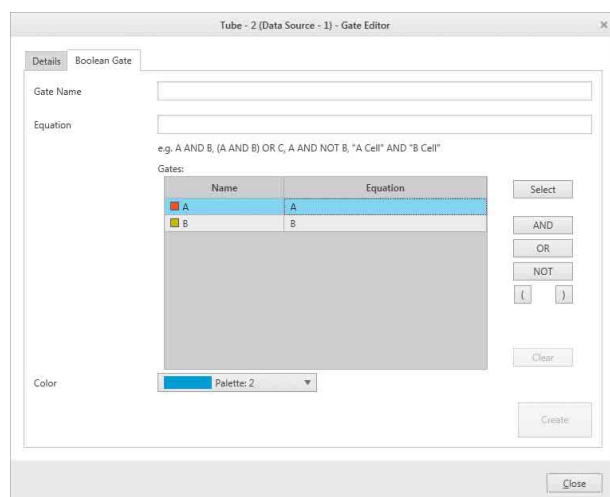
[Remove]: Deletes the selected gate.

[Close]: Closes the dialog.

• [Boolean Gate] tab

Adds a Boolean gate to the gate hierarchy.

For details about the [Boolean Gate] tab, see “Using Boolean Gates” (page 100).



[Exporting statistics table] dialog

The [Exporting statistics table] dialog is displayed by clicking [Export Statistics to CSV File] on the [Worksheet] tab or [Plot Tools] tab of the ribbon.

The [Exporting statistics table] dialog is used to export the contents of the [Gates and Statistics] table as a CSV-format file.



[Output Path]: Specifies the export destination folder.

Click (Browse) to specify for a destination folder.

Progress bar: Displays the export progress.

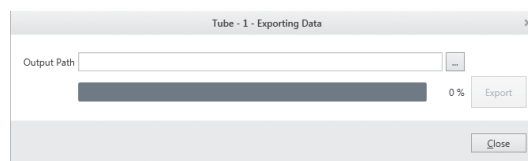
[Export]: Starts the export.

[Close]: Closes the dialog.

[Exporting Data] dialog

The [Exporting Data] dialog is displayed by clicking [Export Data to CSV File] on the [Worksheet] tab of the ribbon.

The [Exporting Data] dialog is used to export recorded data for sample tubes as a CSV-format file.



[Output Path]: Specifies the export destination folder.

Click (Browse) to specify for a destination folder.

Progress bar: Displays the export progress.

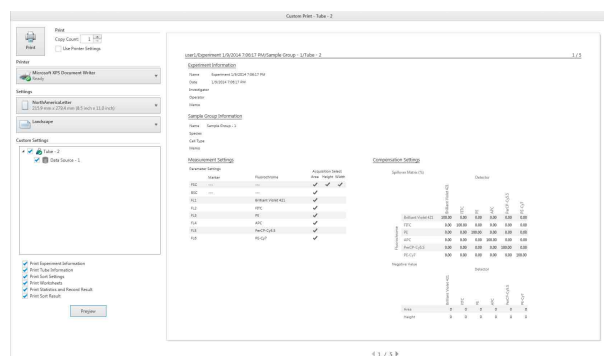
[Export]: Starts the export.

[Close]: Closes the dialog.

[Custom Print] dialog

The [Custom Print] dialog is displayed by clicking [Custom Print] in the [Print] group on the [Worksheet Tools] tab of the ribbon.

The [Custom Print] dialog is used to select items for printing.



[Custom Settings]: Selects the sample tubes for printing. Select the checkbox for a tube to print the worksheet for the tube.

[Print Experiment Information]: Selects whether to print the experiment information settings.

[Print Tube Information]: Selects whether to print the sample tube information settings.

[Print Sort Settings]: Selects print of the sort settings.

[Print Worksheets]: Selects printing of the worksheet settings.

[Print Statistics and Record Result]: Selects printing of the [Gates and Statistics] table and recorded results.

[Print Sort Result]: Selects print of the sorting results.

[Preview]: Displays a print preview image on the right side of the screen.

For details about other items, see “[Print] Window” (page 124).

[Plot Tools] Tab (ribbon)

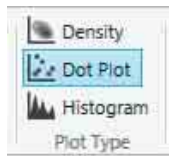
The [Plot Tools] tab of the ribbon has the following buttons.

Refer to the [Worksheet Tools] tab for a description of the [Plot] group buttons (page 144), [Statistics] group buttons (page 145), [Tools] group buttons (page 146), and [Compensation] group buttons (page 146).

[Plot Type] group

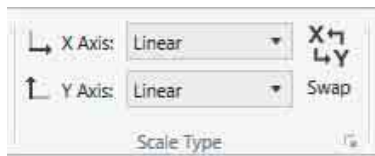
Contains tool buttons for changing the type of the selected plot.

For histogram plots, select a histogram plot on the worksheet, and click [Histogram] repeatedly to toggle between filled area view and outline view.



[Scale Type] group

Changes the type of scale displayed on axes on plots.



[X Axis] / [Y Axis]: Changes the type of scale displayed on the X axis and Y axis, respectively, of the selected plot.

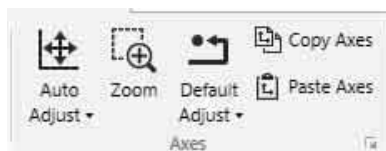
For details about axis scale types, see “Changing Axis Scale Type” (page 96).

[Swap]: Swaps the position of the X axis and Y axis scales.

: Displays the [Property Window] dialog (page 149) for specifying axis types and scale ranges.

[Axes] group

Contains tool buttons for adjusting axis scales.



[Auto Adjust]: Automatically adjusts plot axes.

- [Auto Adjust (XY)]: Automatically adjusts the ranges of the X axis and Y axis of the selected plot to their minimum and maximum measured values.
- [Auto Adjust (X Axis)]: Automatically adjusts the ranges of the X axis of the selected plot to their minimum and maximum measured values.

- [Auto Adjust (Y Axis)]: Automatically adjusts the ranges of the Y axis of the selected plot to their minimum and maximum measured values.

[Zoom]: Zooms in on the area selected by dragging the mouse on a plot.

[Default Adjust]: Restores the default X axis and Y axis ranges. Click the down arrow to set the number of decades displayed on log scales.

[Copy Axes] / [Paste Axes]: Copies and pastes the plot axes into another plot.

: Displays the [Property Window] dialog (page 149) for specifying axis types and scale ranges.

[Gate] group

Contains tool buttons for drawing gates on plots and editing gate properties.



For details, see “Adding Gates” (page 99) and “Editing Gates” (page 102).

[Rectangle]: Creates a rectangular gate on a dot plot or density plot.

[Ellipse]: Creates an elliptical gate on a dot plot or density plot.

[Polygon]: Creates a polygonal gate on a dot plot or density plot.

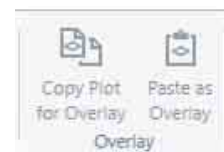
[Quadrant]: Creates a quadrant gate on a dot plot or density plot.

[Linear]: Creates a linear gate on a histogram plot.

[Edit Gate]: Displays the [Gate Editor] dialog (page 146) for editing gates.

[Overlay] group

Contains tool buttons for overlay display of plots.

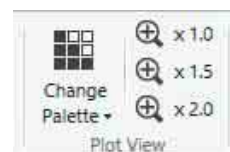


[Copy Plot for Overlay] / [Paste as Overlay]:

Copies and pastes an overlay source plate onto an overlay destination plot.

[Plot View] group

Contains tool buttons for changing the display of plots.




[Change Palette]: Displays a drop-down palette for changing the display style of the selected plot.

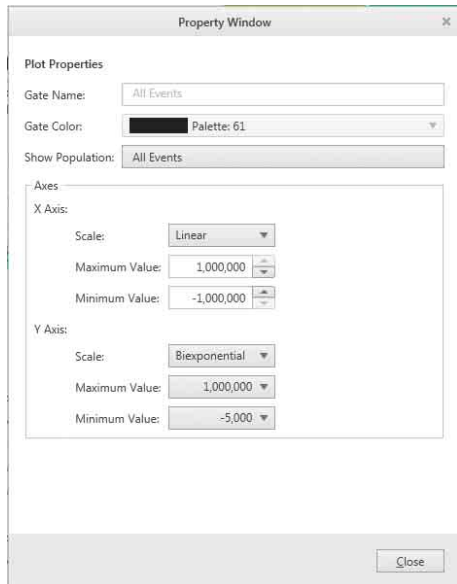
For details, see “[Change Palette]” (page 145).

x1.0, x1.5, x2.0: Displays the plot at 100% (default), 150%, and 200% size, respectively.

[Property Window] dialog

The [Property Window] dialog is displayed by clicking the  dialog launcher in the [Scale Type] group or [Axes] group on the [Plot Tools] tab of the ribbon. You can also display the dialog by right-clicking the worksheet, plot, gate, or a [Gates and Statistics] table cell, and selecting [Properties] from the context menu. The [Property Window] dialog is used to change the attributes of plots, and to specify the scale type and range of axes.

Example: Density plot / dot plot properties



[Gate Name]: Displays the name of the gate (required). You can edit the name, as required. Note that the names of gates must be unique.

[Gate Color]: Selects the color of the gate on plots.

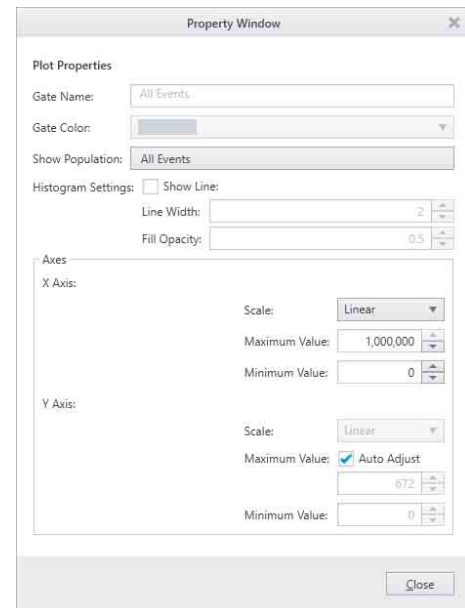
[Show Population]: Selects the population to display on plots.

[Axes]: Specifies the scale type and range of the X and Y axes.

- [Scale]: Selects the scale type.
- [Maximum Value]: Specifies the maximum value of the scale.
- [Minimum Value]: Specifies the minimum value of the scale.

[Close]: Closes the dialog.

Example: Histogram plots



[Gate Name]: Displays the name of the gate.

[Gate Color]: Displays the color of the gate.

[Show Population]: Selects the population to display on plots.

[Histogram Settings]: Sets the display view of histogram plots.

- [Show Line]: Toggles between outline view and filled area view.
- [Line Width]: Selects the line thickness.
- [Fill Opacity]: Selects the opacity of the filled area.

[Axes]: Specifies the scale type and range of the X and Y axes.

- [Scale]: Selects the scale type.
- [Maximum Value]: Specifies the maximum value of the scale.
- [Minimum Value]: Specifies the minimum value of the scale.
- Placing a check mark in [Auto Adjust] selects automatic adjustment of the Y axis.

[Close]: Closes the dialog.

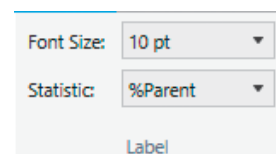
[Gate Tools] Tab (ribbon)

The [Gate Tools] tab of the ribbon has the following buttons.

Refer to the [Worksheet Tools] tab for a description of the [Gate] group buttons (page 145).

[Label] group

Contains tool buttons for specifying gate labels.

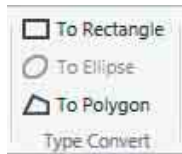


[Font Size]: Selects the font size of text labels.

[Statistic]: Selects the statistic that is displayed beside gates on plots.

[Type Convert] group

Contains tool buttons for converting the selected gate to a different gate type.



[Style] group

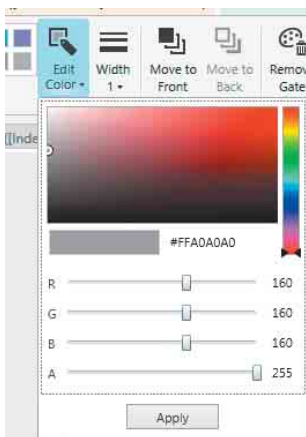
Contains tool buttons for changing the display style of gates.



[Visible]: Shows/hides the selected gate.

Color palette: Changes the color of the selected gate.

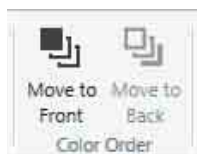
[Edit Color]: Allows you to edit the color in the lower color palette (user defined colors) to any color. Click a color in the color palette, click [Edit Color], then select a new color and click [Apply].



[Width]: Selects the line width of the selected gate.

[Color Order] group

Contains tool buttons for moving the gate with the selected color toward the front or back.

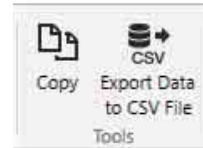


[Move to Front]: Moves the gate with the selected color one step toward the front.

[Move to Back]: Moves the gate with the selected color one step toward the back.

[Tools] group

Contains a tool button for copying gates.



[Copy]: Copies the selected gate.

[Export to CSV File]: Exports the event data in the selected gate as a CSV-format file.

[Gates and Statistics] Table

The [Gates and Statistics] table displays the following information.

1	2	3	4	5	
Name	Events	%Parent	%Total	Mean	
All Events	0	0.00%	0.00%		
A	0	0.00%	0.00%		
B	0	0.00%	0.00%		
Brilliant V...				0	
FITC-A				0	
PE-A				0	
APC-A				0	
PerCP-Cy...				0	
PE-Cy7-A				0	

1 [Name]

Displays the names of gates and parameters.

2 [Events]

Displays the number of events.

3 [%Parent]

Displays the proportion of events of each gate relative to its parent.

4 [%Total]

Displays the proportion of events of each gate relative to the total of all events.

5 Other statistics values

Displays the values of statistics configured in the [Statistics Editor] dialog.

- [Mean]: Average value of events in population
- [Minimum]: Minimum value in a population
- [Maximum]: Maximum value in a population
- [SD]: Standard deviation of events from the mean value in a population
- [CV]: Coefficient of variation of events in a population
- [Median]: Median value (equal number of events above and below)
- [Mode]: Value with highest event count (corresponds to the highest peak on a histogram plot)
- [HPCV]: Half-peak coefficient of variation (coefficient of variation from width of distribution at half peak height)
- [rCV]: Robust coefficient of variation of events in a population

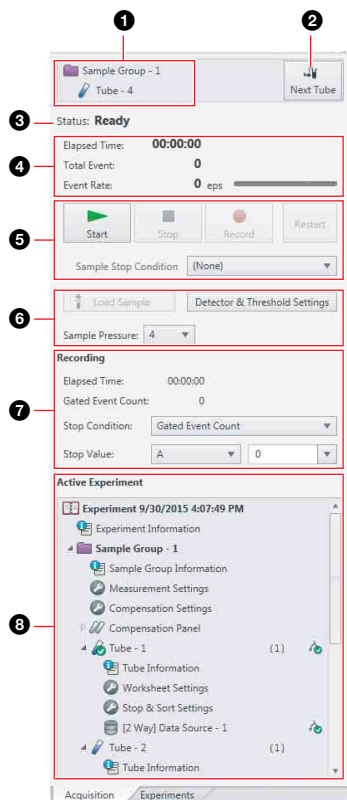
For details, see “Displaying Statistics” (page 106).

6 Gate hierarchy

Displays the hierarchical relationship between gates. The color to the left of the gate name shows the color of the gates on plots.

[Acquisition] Tab (control pane)

The [Acquisition] tab controls data acquisition and recording.



1 Currently assigned sample tube and sample group

2 [Next Tube]

Assigns the next tube.

If there are no further tubes, it duplicates the current tube and assigns the new tube.

3 Instrument status

Displays the status of the instrument.

The following states are displayed.

- Initializing
- Standby
- Ready
- Pause
- Measurement
- Backflash
- No Chip
- Chip Debubble
- Sheath Filter Debubble
- Vent Air from Sheath Filter
- Error
- Waiting
- Bleach Cleaning

- DI Water Cleaning
- Shutdown

4 Data acquisition status

Displays the status of data acquisition.

[Elapsed Time]: Displays the cumulative elapsed time since the start of data acquisition.

[Total Events]: Displays the total number of events detected during data acquisition.

[Event Rate]: Displays the rate events are detected during data acquisition in events per second (eps).



Tip

It is recommended that the event rate be reduced if the indicator exceeds 10k when sorting.

5 Data acquisition controls

(Start): Starts/resumes data acquisition.

It does not start data recording.

(Pause): Pauses data acquisition.

The elapsed time continues counting while acquisition is paused.

(Stop): Stops data acquisition manually.

(Record): Records the acquired data.

(REC stop): Stops data recording manually.

(Restart): Resets the total events and elapsed time counters to 0 and restarts data acquisition.

Note

Avoid pausing sample acquisition for a prolonged time, to prevent sample from aggregating and clogging the sample line.

Tip

If an automatic stop condition has been specified, data acquisition and/or recording stops automatically when the corresponding stop condition has been satisfied.

[Sample Stop Condition]: Selects the stop condition for stopping data acquisition automatically.

- None (data acquisition will stop automatically when the number of detected events exceeds 345,600,000 or the elapsed time exceeds 12:00:00, whichever occurs first)
- Recording (stops data acquisition automatically when the recording stop condition is satisfied)
- Sorting (stops data acquisition automatically when the sorting stop condition is satisfied)
- Recording and Sorting (stops data acquisition automatically when both the recording and sorting stop conditions are satisfied)

Tip

[Recording] cannot be selected for index sorting.

For details about index sorting, see “Index Sorting” (page 114).

6 Instrument controls

[Load Sample] / [Unload Sample]: Loads/unloads the sample tube.

These operations are enabled only when data acquisition is paused.

[Detector & Threshold Settings]: Displays the [Detector & Threshold Settings] dialog.

For details about the [Detector & Threshold Settings] dialog, see “Configuring Detector Settings” (page 87).

[Sample Pressure]: Sets the sample fluid pressure (range of 1 to 10).

7 Recording controls

[Elapsed Time]: Displays the cumulative elapsed time since the start of data recording.

[Event Count]: Displays the number of recorded events.

[Stop Condition]: Selects the stop condition for stopping data recording automatically.

- None
- Elapsed Time
Specifies elapsed time condition to stop recording automatically.
- Event Count
Specifies the event count condition to stop recording automatically.
- Gated Event Count
Specifies the event count condition of the selected gate to stop recording automatically. Specify the target gate of the stop condition, and set the number of events.

Tip

Recording control items cannot be configured for index sorting.

For details about index sorting, see “Index Sorting” (page 114).

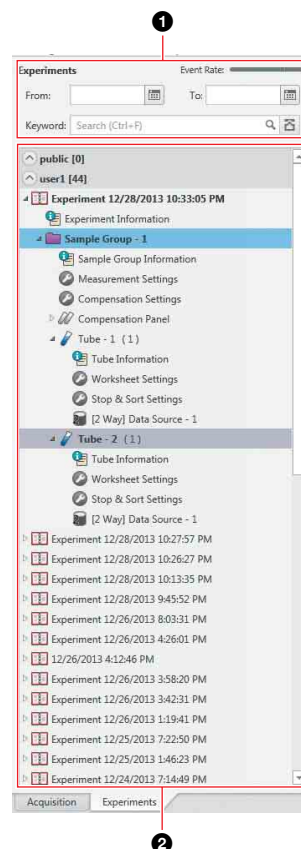
8 Experiment Explorer (Active Experiment)

Displays the structure of the active experiment enabled for data acquisition, recording, and cell sorting.

For details about the Experiment Explorer, see “Experiment Explorer” (page 152) on the [Experiments] tab (control pane).

[Experiments] Tab (control pane)

The [Experiments] tab displays all current public (shared) and private experiments.



1 Search criteria

You can search experiments by date and keyword. The search results are displayed in the Experiment Explorer.

For details about searching, see “Searching Experiments” (page 85).

2 Experiment Explorer

Displays a list of all current experiments. The internal structure of each experiment is displayed in a hierarchical list view.

[public]: Lists shared experiments. Shared experiments can be viewed by all users.

User_name: Lists private experiments created by the logged-in user. Private experiments cannot be viewed by other users.

An experiment contains the following structural components.


Experiment


[Experiment Information]: Contains information about the experiment. Double-clicking this component displays the [Experiment Information] dialog (page 153).


Sample group: Displays the name of a sample group. A sample group contains the following components.


[Sample Group Information]: Contains information about the sample group. Double-

clicking this component displays the [Sample Group Information] dialog (page 154).


 **[Measurement Settings]:** Contains instrument settings. Double-clicking this component displays the [Measurement Settings] dialog (page 81).


 **[Compensation Settings]:** Contains the fluorescence compensation settings for the fluorochromes used. Double-clicking this component displays the [Compensation Settings] dialog (page 143).


 **[Compensation Panel]:** Contains one negative (unstained) control tube and up to 12 positive (fluorochrome stained) control tubes. A compensation control tube is required for each fluorochrome used to mark samples in the sample group.

The  icon indicates sample tubes for which data has been recorded or which have been used for sorting. These tubes cannot be reassigned as the active tube.

Double-clicking a tube displays the worksheet for the tube.


 **[Negative control]:** Tube for recording data for the negative control sample used to compensate for cellular autofluorescence.


 **Positive control tubes:** Tubes for recording single-stained positive control samples used to calculate the fluorescence compensation spillover matrix, one tube for each fluorochrome used to mark samples.

 **Sample tube:** Tubes containing sample for data acquisition, recording, and cell sorting. Each tube also has worksheet settings and sort & stop settings associated with it.


Double-clicking a tube displays the worksheet for the tube.


To make a tube the active tube for data acquisition, select the tube and click [Assign Tube] in the [Target] group on the [Experiment] tab of the ribbon.


During acquisition the tube icon changes to .


The  icon indicates sample tubes for which data has been recorded or which have been used for sorting. These tubes cannot be reassigned as the active tube.


The following icons are displayed for each tube on the right side in the Experiment Explorer.


 : Indicates data is being recorded.


 : Indicates data has been recorded.


 : Indicates tube is being sorted.

 : Indicates that the tube has been used for sorting into the collection tubes.

 **[Tube Information]:** Contains information about the tube. Double-clicking this component displays the [Tube Information] dialog (page 154).

 **[Worksheet Settings]:** Contains information about the plots, gates, and statistics on the worksheet. Double-clicking this component displays the [Worksheet Settings] dialog (page 154).

 **[Stop & Sort Settings]:** Contains the sort settings, and the stop conditions for data acquisition, recording, and sorting for the tube. Double-clicking this component displays the [Stop & Sort Settings] dialog (page 154).

 **Data source:** Indicates the presence of saved data and type of sort results.

Data source naming convention

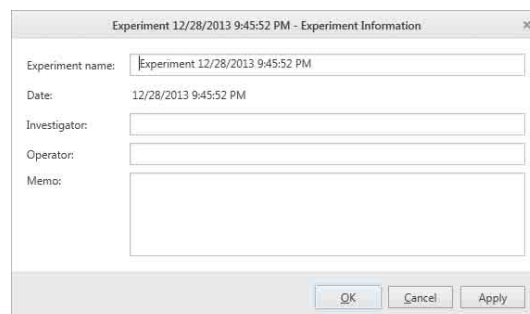
The sorting method is prefixed to the name of the data source.

Sorting method	Data source name
5 mL Tubes	[5 mL Tubes] Data Source - #
15 mL Tubes	[15 mL Tubes] Data Source - #
6 Well Plate	[6 Well] Data Source - #
12 Well Plate	[12 Well] Data Source - #
24 Well Plate	[24 Well] Data Source - #
48 Well Plate	[48 Well] Data Source - #
96 Well Plate	[96 Well] Data Source - #
384 Well Plate	[384 Well] Data Source - #
Index sorting Example: 96 Well Plate	[Index 96 Well] Data Source - # * The data source name has an "Index" prefix.

[Experiment Information] dialog

The [Experiment Information] dialog is displayed by double-clicking [Experiment Information] for an experiment in the Experiment Explorer.

The [Experiment Information] dialog is used to view and edit information about the experiment.



[Experiment Name]: Name of the experiment (required item).

[Date]: Displays the date the experiment was created. This parameter cannot be modified.

[Investigator]: Name of the lead researcher.

[Operator]: Name of the operator.

[Memo]: Descriptive comments.

[OK]: Applies the settings and closes the dialog.

[Cancel]: Cancels settings and closes the dialog.

[Apply]: Applies the settings without closing the dialog.

[Sample Group Information] dialog

The [Sample Group Information] dialog is displayed by double-clicking [Sample Group Information] in an experiment in the Experiment Explorer.

The [Sample Group Information] dialog is used to view and edit information about the sample group.

[Sample Group Name]: Unique name of the sample group (required item).

[Date]: Displays the date the sample group was created. This parameter cannot be modified.

[Species]: Name of the target biological species.

[Cell type]: Name of the cell type within the biological species.

[Memo]: Descriptive comments.

[OK]: Applies the settings and closes the dialog.

[Cancel]: Cancels settings and closes the dialog.

[Apply]: Applies the settings without closing the dialog.

[Tube Information] dialog

The [Tube Information] dialog is displayed by double-clicking [Tube Information] for a sample tube in the Experiment Explorer.

The [Tube Information] dialog is used to view and edit information about the sample tube.

[Tube Name]: Unique name of the sample tube (required item).

[Date]: Displays the date the sample tube was created. This parameter cannot be modified.

[Sample ID1] to [Sample ID4]: ID labels for identifying the sample tube.

[OK]: Applies the settings and closes the dialog.

[Cancel]: Cancels settings and closes the dialog.

[Apply]: Applies the settings without closing the dialog.

[Worksheet Settings] dialog

The [Worksheet Settings] dialog is displayed by double-clicking [Worksheet Settings] for a sample tube in the Experiment Explorer.

The [Worksheet Settings] dialog is used to save the current worksheet settings (number of plots, type of plots, statistics settings, etc.) as the favorite settings. Saving current worksheet settings as the favorite settings allows you to restore the settings at a later time or to apply the settings to a different sample tube.

Tip

Data that is acquired and recorded for a tube is not included in the worksheet settings.

[Current Worksheet Settings]

[Modified]: Displays the date and time that the current worksheet settings were modified.

[Restore Favorite]: Restores the saved favorite worksheet settings as the current worksheet settings.

[Save as Favorite]: Saves the current worksheet settings as the favorite (default) worksheet settings.

[Favorite Worksheet Settings]

[Modified]: Displays the date and time that the favorite worksheet settings were saved.

[Preview]: Displays a preview of the worksheet with the saved favorite worksheet settings.

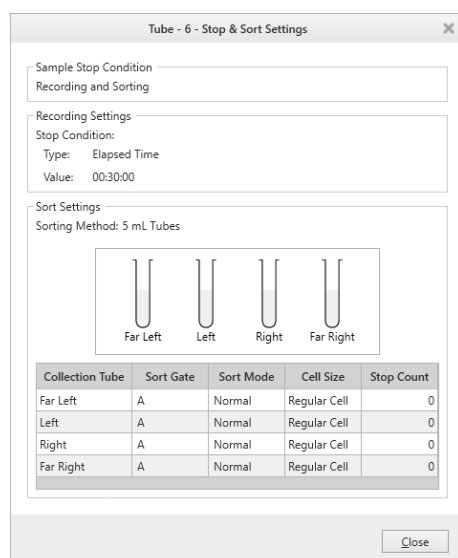
[Close]: Closes the dialog.

[Stop & Sort Settings] dialog

The [Stop & Sort Settings] dialog is displayed by double-clicking [Stop & Sort Settings] for a sample tube in the Experiment Explorer.

The stop and sort settings can be viewed in the [Stop & Sort Settings] dialog.

Example: 5 mL tube sorting



[Sample Stop Condition]: Displays the data acquisition stop condition.

[Recording Settings]

[Stop Condition]: Displays the recording stop condition.

[Sort Settings]

[Sorting Method]: Displays the sorting method for the sample tube.

You can change the order items are displayed by dragging and dropping the title cells.

[Close]: Closes the dialog.


[Sort Control] Pane

The [Sort Control] pane is used to configure and control cell sorting.

For details, see “Sorting” (page 59).

The items displayed in the [Sort Control] pane vary depending on the selected sorting method. This section describes the displayed items using 5 ml tube sorting, 15 ml tube sorting, and 96-well plate sorting as examples.

Tip

You can show/hide the [Sort Control] pane by clicking  in the top left corner of the pane. In analyzer mode, the [Sort Control] pane is automatically hidden.

For 5 ml tube sorting

The following items are displayed when [5 mL Tubes] is selected in [Method].



① [Sort Control] pane

Configures and controls droplet sorting.

[Auto Record]: Place a check mark in the checkbox to start recording at the same time as sorting.

[Sort Start] / [Sort Stop]: Starts/stops sorting.

[Load Collection] / [Unload Collection]: Loads/unloads the collection stage.

[Method]: Selects the sorting method. Set the sorting method to match the collection devices loaded on the collection stage.

The sorting parameters vary depending on the selected sorting method.

[Sort Settings]: Specifies the sorting conditions.

Clicking this button displays the [Sort Settings] dialog.

For details about the [Sort Settings] dialog, see “Sorting into 5 ml Tubes (4-way)” (page 61).

[Mode]: Selects the sorting mode.

Sorting mode

- Ultra Purity
- Purity
- Semi Purity
- Normal
- Semi Yield
- Yield
- Ultra Yield
- Single Cell
- Single Cell (3 drops)
- Custom: You can specify up to five user-defined modes.

Tip

To adjust the position for sorting of target cells within a droplet, you can adjust the settings using [Edit setting].

For details, see “[Sort Mode Setting] dialog” (page 158)

For details about each mode, see “Sorting Mode” (page 108).

Tips

- When sorting larger cells, selecting [Single Cell (3 drops)] may improve the sorting performance.
- The side streams may become unstable when sorting cells of size 20 μm or larger.
- If droplet marks are visible on the collection tube holder after sorting, wipe clean with a lint-free, soft, clean cloth sprayed with ethanol and then another moistened with water.

② [Sort Statistics] pane

Displays the statistics about events during sorting.

[Sort Gate]: Displays the gates for the target events to sort into the collection tubes.

[Elapsed Time]: Displays the cumulative elapsed time since the start of sorting.

[Remaining Time]: Displays the estimated remaining sorting time.

[Sort Count]: Displays the number of events sorted.

[Sort Rate]: Displays the rate at which events are sorted in units of events per second (eps).

[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.

[Abort Count]: Displays the number of target events and event rate in the sort gate that do not match the sort mode criteria and which are aborted during sorting.

3 [Droplet] pane

Displays the droplet stream immediately beneath the sorting chip nozzle. The droplet stream is used to monitor droplet formation and to adjust the breakoff point.

Double-clicking the image displays the droplet stream in the [Droplet Viewer] window “[Droplet Viewer] window” (page 158) for monitoring droplets.

4 [Hardware Status] pane

Displays the status of the interior lights in the sample loader and collection stage area, and the level in each of the tanks in the fluidics cart. It also displays the lasers enabled for the experiment.

[Light]

[Collection]: Turns the collection stage light on/off.

[Sample]: Turns the sample injection chamber light on/off.

[Tank]: Indicates the fluid level in the sheath tank and waste tank.

A warning icon appears when the waste tank is close to full, or when the sheath tank is close to empty.

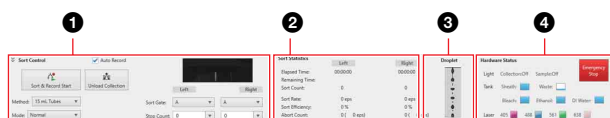
[Laser]: Indicates the lasers used for data acquisition.

[Emergency Stop]: Aborts acquisition and stops sample flow.

When stopped using this button, the instrument and Cell Sorter software need to be restarted in order to continue.

For 15 ml tube sorting

The following items are displayed when [15 mL Tubes] is selected in [Method].



1 [Sort Control] pane

Configures and controls droplet sorting.

[Auto Record]: Place a check mark in the checkbox to start recording at the same time as sorting.

[Sort Start] / [Sort Stop]: Starts/stops sorting.

[Load Collection] / [Unload Collection]: Loads/unloads the collection stage.

[Method]: Selects the sorting method. Set the sorting method to match the collection devices loaded on the collection stage.

The sorting parameters vary depending on the selected sorting method.

[Mode]: Selects the sorting mode.

Sorting mode

- Ultra Purity
- Purity
- Semi Purity
- Normal
- Semi Yield
- Yield
- Ultra Yield
- Single Cell
- Single Cell (3 drops)
- Custom: Up to five user-defined modes

Tip

To adjust the position for sorting of target cells within a droplet, you can adjust the settings using [Edit setting].

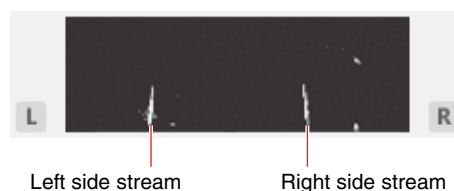
For details, see “[Sort Mode Setting] dialog” (page 158).

For details about each sorting mode, see “Sorting Mode” (page 108).

Tips

- When sorting larger cells, selecting [Single Cell (3 drops)] may improve the sorting performance. The cell size is fixed to [Large Cell] when [Single Cell (3 drops)] is selected.
- The side streams may become unstable when sorting cells of size 20 μm or larger.
- If droplet marks are visible on the collection tube holder after sorting, wipe clean with a lint-free, soft, clean cloth sprayed with ethanol and then another moistened with water.

Side stream monitor: Displays the image from the side stream monitor camera.



For details about adjusting the deflection angle of side streams, see “[Advanced Settings] dialog” (page 141).

[Sort Gate]: Selects the gates for the target events to sort into the left and right collection tubes.

[Stop Count]: Specifies the event count conditions to stop recording automatically for each of the left and right collection tubes.

You can select a value or enter a value from the keyboard.

Entering a value of “0” disables the stop condition.

② [Sort Statistics] pane

Displays the statistics about events during sorting.

[Elapsed Time]: Displays the cumulative elapsed time since the start of sorting.

[Remaining Time]: Displays the estimated remaining sorting time.

[Sort Count]: Displays the number of events sorted.

[Sort Rate]: Displays the rate at which events are sorted in units of events per second (eps).

[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.

[Abort Count]: Displays the number of target events and event rate in the sort gate that do not match the sort mode criteria and which are aborted during sorting.

③ [Droplet] pane

Displays the droplet stream immediately beneath the sorting chip nozzle.

The droplet stream is used to monitor droplet formation and to adjust the breakoff point.

Double-clicking the image displays the side streams in the [Droplet Viewer] window (page 158) for monitoring droplets.

④ [Hardware Status] pane

Displays the status of the interior lights in the sample loader and collection stage area, and the level in each of the tanks in the fluidics cart. It also displays the lasers enabled for the experiment.

[Light]

[Collection]: Turns the collection area light on/off.

[Sample]: Turns the sample injection chamber light on/off.

Tank: Indicates the fluid level in the sheath tank and waste tank.

A warning icon appears when the waste tank is close to full, or when the sheath tank is close to empty.

[Laser]: Indicates the lasers used for data acquisition.

[Emergency Stop]: Aborts acquisition and stops sample flow.

When stopped using this button, the instrument and Cell Sorter software need to be restarted in order to continue.

For multi-well plate sorting

The following items are displayed when a multi-well plate is selected in [Method].

Example: When [96 Well Plate] is selected



The [Droplet] and [Hardware Status] panes are the same as for 15 ml tube sorting.

① [Sort Control] pane

Configures and controls droplet sorting.

[Sort Start] / [Sort Stop]: Starts/stops sorting.

[Load Collection] / [Unload Collection]: Loads/unloads the collection stage.

[Method]: Selects the sorting method. Set the sorting method to match the collection devices loaded on the collection stage.

The sorting parameters vary depending on the selected sorting method.

To start recording at the same time as sorting, place a check mark in the [Auto Record] checkbox.

[Sort Settings]: Specifies the default sorting configuration.

Clicking this button displays the [Sort Settings] dialog.

For details about the [Sort Settings] dialog, see “Sorting” (page 59) and “Adjusting the Sort Position” (page 112).

② [Sort Statistics] pane

Displays the statistics about events during sorting.

[Total Elapsed Time]: Displays the cumulative elapsed time since the start of sorting of the whole plate.

[Total Progress]: Displays the ratio of sorted wells to the total number of wells.

[Sort ID]: Displays the ID of the well currently being sorted.

[Well Number]: Displays the number of the well currently being sorted.

[Sort Mode]: Displays the sorting mode of the well currently being sorted.

[Cell Size]: Displays the current cell size setting.

[Sort Gate]: Displays the sort gate of the well currently being sorted.

[Stop Count]: Displays the number of events for the automatic stop condition of the well currently being sorted.

Plate (image): Displays the layout of the wells.

Double-clicking the plate image or right-clicking and selecting [Show Sorting Report] from the context menu displays the plate image in a separate window for monitoring progress.

[Elapsed Time]: Displays the cumulative elapsed time since the start of sorting.

[Remaining Time]: Displays the estimated remaining sorting time.

[Sort Count]: Displays the number of events sorted.

[Sort Rate]: Displays the rate at which events are sorted in units of events per second (eps).

[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.

[Abort Count]: Displays the number of target events and event rate in the sort gate that do not match the sort mode criteria and which are aborted during sorting.

[Sort Mode Setting] dialog

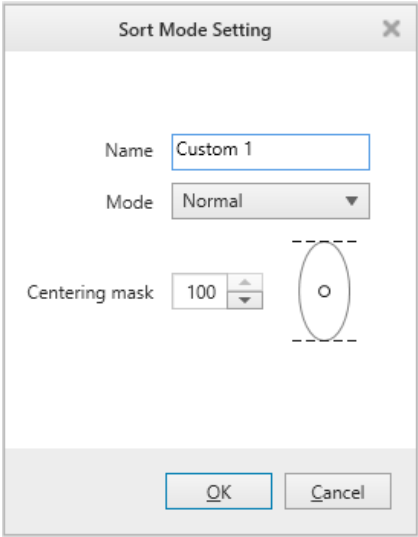
The [Sort Mode Setting] dialog is displayed by selecting [Edit setting] from [Custom] under [Sort Mode].

The [Sort Mode Setting] dialog is used to adjust the position of target cells within a droplet for sorting.

The adjustment values are valid for the currently selected tube only.

To apply the value to other tubes, register the value in [User Preference].

To display [User Preference], see the tip (page 113) in step 3 in “Adjusting the Sort Position” (page 112).



[Name]: Enter a name for the custom mode (required item).

[Mode]: Select the base sorting mode.

When a sorting mode is selected, the default value for that mode is entered in [Centering mask].

[Centering mask]: Specify the position of target cells within a droplet for sorting.

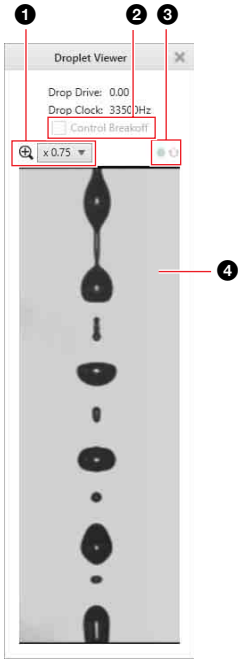
Tips

- The [Name] field cannot be left blank. Always specify a name.
- Specifying the same name as a preset sorting mode is not possible.

[Droplet Viewer] window

The [Droplet Viewer] window is displayed by double-clicking the image in the [Droplet] pane at the bottom of the main window.

The [Droplet Viewer] window is used to monitor droplet formation and as a guide when adjusting the breakoff point.



1 Zoom factor
Selects the zoom factor (×1.00, ×0.75, or ×0.50)

2 [Control Breakoff]
The control breakoff setting uses a feedback system to control the breakoff position automatically.

When enabled, the amplitude and frequency settings are adjusted automatically to maintain a stable breakoff point position with coherent side streams. When adjusting the breakoff point, first set the amplitude and frequency settings to obtain a stable breakoff point. After establishing a stable breakoff point, click the feedback control checkbox to enable the breakoff feedback control.

3 Status icon
●: Indicates the stability of droplet formation.

Indication	Status
Flashing slowly	Waiting for stream to stabilize
Flashing rapidly	Adjustment in progress
On	Stable

⚙: Indicates that the droplet control breakoff function is active.

No icon: Indicates unstable or absence of droplet formation.

4 Droplet monitor
Displays the droplet stream.

Tips

- The manual amplitude and frequency controls are disabled in this mode.
- When breakoff feedback control is enabled, if the breakoff point moves and cannot be recovered within a predetermined time, sorting stops and an error message is displayed on the screen.

Context Menu

The context menu displays common operations that can be accessed by right-clicking components in the Experiment Explorer or items on the worksheet.

Experiment Explorer Context Menu

This section describes the context menu items displayed for components in the Experiment Explorer.

Experiments

The following menu commands are displayed when right-clicking an experiment icon in the Experiment Explorer.

- [New Sample Group]:** Creates a blank sample group.
- [Copy] / [Paste]:** Copies the experiment and pastes a new experiment into the Experiment Explorer.
- [Duplicate]:** Duplicates the selected experiment.
- [Save as Experiment Template]:** Saves the selected experiment as a private template.
- [Export FCS File]:** Displays the [FCS File Export] dialog (*page 135*) for exporting the tubes in the selected experiment in FCS 3.0 or FCS 3.1 format.
- [Send to Public]:** Creates a duplicate of the selected experiment and places the duplicate under the [Public] node for sharing with other users.
- [Delete]:** Deletes the selected experiment.
- [Rename]:** Renames the selected experiment.

Compensation Panel

The following menu commands are displayed when right-clicking a compensation panel icon in the Experiment Explorer.

- [Compensation Wizard]:** Displays the [Compensation Wizard] (*page 66*) for recording control tubes in a compensation panel.
- [Show Matrix]:** Displays the [Compensation Settings] dialog (*page 143*) for viewing the spillover matrix, and loading/saving the spillover matrix.
- [Calculate Matrix]:** Displays the [Calculate Compensation Settings] dialog (*page 144*) for calculating the coefficients of the fluorescence compensation spillover matrix based on the compensation panel.
- [Export FCS Files]:** Displays the [FCS File Export] dialog (*page 135*) for exporting the tubes in the selected compensation panel in FCS 3.0 or FCS 3.1 format.

Control tubes in a compensation panel

The following menu commands are displayed when right-clicking a control tube in a compensation panel icon in the Experiment Explorer.

- [Assign]:** Assigns the selected control tube as the active tube for data acquisition and recording.
- [Open Worksheet]:** Opens the worksheet tab for the selected control tube.
- [Duplicate to Normal Tube]:** Duplicates the recorded data of the selected fluorochrome to a normal tube.
- [Export FCS File]:** Displays the [FCS File Export] dialog (*page 135*) for exporting the selected control tube in FCS 3.0 or FCS 3.1 format.
- [Reset Data]:** Deletes the data recorded for the control tube.
- [Show Results]:** Displays the recorded results of the selected fluorochrome.
Selecting this menu item displays the [Tube Results] dialog (*page 134*).

Sample groups

The following menu commands are displayed when right-clicking a sample group icon in the Experiment Explorer.

- [New Tube]:** Creates a new sample tube.
Selecting this item creates a new sample tube in the sample group.
- [Copy] / [Paste]:** Allows you to copy and paste all settings (including sample tubes) in the selected sample group. However, the recorded data of the sample tubes will not be pasted.
- [Paste to All Tubes in Sample Group]:** Pastes copied worksheet settings to all sample tubes in the selected sample group.
This item is available when you copy the worksheet settings of a sample tube, and then right-click another sample group.
- [Duplicate]:** Duplicates the selected sample group.
- [Save as Sample Group Template]:** Displays the [Save as Sample Group Template] dialog (*page 133*) for saving the selected sample group as a private template.
- [Apply Template]:** Displays the [Apply Template] dialog (*page 134*) for selecting a template to apply to the selected sample group.
- [Export FCS file]:** Displays the [FCS File Export] (*page 135*) dialog for exporting the recorded data for the sample tubes included in the sample group as an FCS 3.0 or FCS 3.1 format file.
- [Delete]:** Deletes the selected sample group.
- [Rename]:** Renames the selected sample group.

Measurement settings

The following menu commands are displayed when right-clicking a [Measurement Settings] icon for a sample group in the Experiment Explorer.

[Copy] / [Paste]: Copies the measurement settings for the selected sample group and pastes them into the measurement settings for another sample group.

[Show Settings]: Displays the [Measurement Settings] dialog (*page 81*) for viewing measurement settings.

Compensation settings

The following menu commands are displayed when right-clicking a [Compensation Settings] icon for a sample group in the Experiment Explorer.

[Copy] / [Paste]: Copies the compensation settings for the selected sample group and pastes them into the compensation settings for another sample group.

[Show Settings]: Displays the [Compensation Settings] dialog (*page 143*) for viewing compensation settings.

Sample tubes

The following menu commands are displayed when right-clicking a sample tube icon in the Experiment Explorer.

[Assign]: Assigns the selected sample tube as the active tube for data acquisition, recording, and cell sorting.

[Open Worksheet]: Opens the worksheet tab for the selected sample tube.

[Apply Compensation]: Applies fluorescence compensation to the plots on the worksheet for the selected sample tube.

[Copy] / [Paste]: Copies the sample tube and pastes it into another sample tube.

[Duplicate]: Duplicates the selected sample tube.

[Save as Tube Template]: Saves the selected sample tube as a private template.

[Apply Template]: Displays the [Apply Template] dialog (*page 134*) for selecting a template to apply to the selected sample tube.

[Export FCS Files]: Displays the [FCS File Export] dialog (*page 135*) for exporting the selected sample tube in FCS 3.0 or FCS 3.1 format.

[Delete]: Deletes the selected sample tube.

[Rename]: Renames the selected sample tube.

[Show Plate Sorting Monitor]: Displays the [Plate Sorting Monitor] dialog (*page 65*) for monitoring the status when sorting onto multi-well plates.

[Show Results]: Displays the [Tube Results] dialog (*page 134*) for displaying the recording results and sorting results for the selected sample tube.

Tube worksheet settings

The following menu commands are displayed when right-clicking a [Worksheet Settings] icon for a sample tube in the Experiment Explorer.

[Copy] / [Paste]: Copies the worksheet settings for the selected sample tube and pastes them into the worksheet settings for another sample tube.

[Save as Favorite]: Saves the current worksheet settings as the favorite (default) worksheet settings.

[Restore Favorite]: Restores the saved favorite worksheet settings as the current worksheet settings.

[Apply to All Tubes In Same Sample Group]: Applies the worksheet settings for the selected sample tube to all sample tubes within the sample group.

[Show Settings]: Displays the [Worksheet Settings] dialog (*page 154*) for displaying, saving, and loading worksheet settings.

Data sources

The following menu commands are displayed when right-clicking a data source icon in the Experiment Explorer.

[Open Data Source]: Displays the data source on the worksheet.

[Delete]: Deletes the selected data source.

[Rename]: Renames the selected sample tube.

[Export FCS File]: Displays the [FCS File Export] dialog (*page 135*) for exporting the selected data source in FCS 3.0 or FCS 3.1 format.

[Show Results]: Displays the [Data Source Results] dialog (*page 135*) for displaying the recording results and sorting results for the selected data source.

Worksheet Context Menu

This section describes the context menu items displayed for items displayed on the worksheet and the worksheet itself.

Worksheet

The following menu commands are displayed when right-clicking on the worksheet.

[Refresh]: Updates the information displayed on the worksheet.

[New Density]: Adds a new density plot to the worksheet.

[New Dot Plot]: Adds a new dot plot to the worksheet.

[New Histogram]: Adds a new histogram plot to the worksheet.

[Show Table]: Shows/hides the [Gates and Statistics] table.

[Show Grid]: Displays the grid on the worksheet.

[Snap to Grid]: Aligns the plots and [Gates and Statistics] table on the worksheet with the grid when they are moved or added.

[Auto Arrange]: Automatically aligns the plots and [Gates and Statistics] table on the worksheet to the grid.

[Fit to Screen]: Expands the worksheet to fill the entire screen.

[Edit Gate]: Displays the [Gate Editor] dialog (*page 146*) to edit gates.

[Edit Statistics]: Displays the [Statistics Editor] dialog (*page 106*) to configure the statistics displayed in the [Gates and Statistics] table.

[Overlay Gate List]: Displays the [Overlay Gate List] dialog (*page 162*).

[Favorite Settings] >

[Save as Favorite]: Saves the current worksheet settings as the favorite (default) worksheet settings.

[Restore Favorite]: Restores the saved favorite worksheet settings as the current worksheet settings.

[Copy Worksheet Picture]: Copies the worksheet to the clipboard for pasting in other software, for example to produce reports.

[Print]: Displays the [Print] window (*page 124*) for printing the analysis results displayed on the worksheet.

[Custom Print]: Displays the [Custom Print] dialog (*page 147*) for selecting the items to print.

[Properties]: Displays the [Property Window] dialog (*page 149*).

Plots

The following menu commands are displayed when right-clicking a plot.

[Refresh]: Updates the information displayed on the worksheet.

[Auto Adjust]: Automatically adjusts the ranges of the X axis and Y axis of all plots to their minimum and maximum measured values.

[Copy Plot for Overlay] / [Paste as Overlay]: Copies and pastes an overlay source plate onto an overlay destination plot.

[Clear overlay plots]: Clears the overlay display of plots.

[Remove]: Removes the selected plot.

[Duplicate]: Duplicates the selected plot.

[Copy All Regions] / [Paste Region]: Copies all gates on a plot and pastes them onto another plot.

[Create Gate] >

[Rectangle]: Creates a rectangular gate on a dot plot or density plot.

[Ellipse]: Creates an elliptical gate on a dot plot or density plot.

[Polygon]: Creates a polygonal gate on a dot plot or density plot.

[Quadrant]: Creates a quadrant gate on a dot plot or density plot.

[Linear]: Creates a linear gate on a histogram plot.

[Plot Type] >

[Density]: Changes the type of the selected plot to a density plot.

[Dot Plot]: Changes the type of the selected plot to a dot plot.

[Histogram]: Changes the type of the selected plot to a histogram plot.
Click [Histogram] repeatedly to toggle between filled area view and outline view.

[Zoom]: Zooms in on the area selected by dragging the mouse on a plot.

[Open Gates Editor]: Displays the [Gate Editor] dialog (*page 146*) to edit gates.

[Open Statistics Editor]: Displays the [Statistics Editor] dialog (*page 106*) to configure the statistics displayed in the [Gates and Statistics] table.

[Copy Picture]: Copies the selected plot to the clipboard for pasting in other software, for example to produce reports.

[Properties]: Displays the [Property Window] dialog (*page 149*).

Gates

The following menu commands are displayed when right-clicking a gate.

[Create Density Plot]: Adds a density plot for the events within the selected gate.

[Create Dot Plot]: Adds a dot plot for the events within the selected gate.

[Create Histogram Plot]: Adds a histogram plot for the events within the selected gate.

[Move Color Order to Front]: Moves the gate with the selected color one step toward the front.

[Move Color Order to Back]: Moves the gate with the selected color one step toward the back.

[Convert to] >

[Rectangle]: Converts the selected gate to a rectangle gate.

[Ellipse]: Converts the selected gate to an ellipse gate.

[Polygon]: Converts the selected gate to a polygon gate.

[Copy Region] / [Paste Region]: Copies the selected gate and pastes it into another plot.

[Visible Gate]: Shows/hides the selected gate.

[Remove]: Deletes the selected plot.

[Send to Back]: Moves the selected gate to the back.

[Properties]: Displays the [Property Window] dialog (*page 149*).

[Gates and Statistics] table

The following menu commands are displayed when right-clicking within the [Gates and Statistics] table.

[Show Table]: Shows/hides the [Gates and Statistics] table.

[Save as CSV File]: Displays the [Exporting statistics table] dialog (*page 147*) for exporting the data in the [Gates and Statistics] table as a CSV-format file.

[Copy Picture]: Copies the [Gates and Statistics] table to the clipboard for pasting in other software, for example to produce reports.

[Open Gates Editor]: Displays the [Gate Editor] dialog (*page 146*) to edit gates.

[Open Statistics Editor]: Displays the [Statistics Editor] dialog (*page 106*) to configure the statistics displayed in the [Gates and Statistics] table.

[Gates and Statistics] table cells

The following menu commands are displayed when right-clicking within a [Gates and Statistics] table cell.

- [Create Density Plot]:** Adds a density plot for the events within the selected gate.
- [Create Dot Plot]:** Adds a dot plot for the events within the selected gate.
- [Create Histogram Plot]:** Adds a histogram plot for the events within the selected gate.
- [Create Parent Plot]:** Adds a parent plot to the worksheet.
- [Copy Selected Gate]:** Copies the selected gate.
- [Cut Selected Gate]:** Cuts the selected gate.
- [Paste Selected Gate]:** Pastes a previously copied or cut gate.
- [Visible Gate]:** Shows/hides the selected gate.
- [Remove Selected Gate]:** Deletes the selected gate.
- [Show Table]:** Shows/hides the [Gates and Statistics] table.
- [Save as CSV File]:** Displays the [Exporting statistics table] dialog (*page 147*) for exporting the data in the [Gates and Statistics] table as a CSV-format file.
- [Open Gate Editor]:** Displays the [Gate Editor] dialog (*page 146*) to edit gates.
- [Open Statistics Editor]:** Displays the [Statistics Editor] dialog (*page 106*) to configure the statistics displayed in the [Gates and Statistics] table.
- [Refresh List]:** Updates the content displayed in the [Gates and Statistics] table.
- [Properties]:** Displays the [Property Window] dialog (*page 149*).

[Overlay Gate List] dialog

The [Overlay Gate List] dialog is displayed by right-clicking the worksheet and selecting [Overlay Gate List] from the context menu.

The [Overlay Gate List] dialog displays a list of gates that are overlaid on other worksheets.



[Overlay Gate List]: Displays a list of the overlaid gates.

You can select the gate selection state using the checkbox beside the gate name.

[Select All]: Selects all overlaid gates.

[Select Unused]: Deletes overlay plots and selects the unused gates.

[Remove]: Removes the selected gates.

[Close]: Closes the dialog.

This chapter describes the routine maintenance procedures required to maintain the performance and longevity of the LE-MA900.

DANGER

Fluids may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, as required, when performing maintenance.

Maintenance Schedule

The maintenance procedures outlined in this manual should be performed according to the schedule below.

Preparations for the Day Before Measurement

Maintenance item	Schedule	See
Refilling the sheath tank	<ul style="list-style-type: none"> It is recommended to fill the tank the day before operation to allow air bubbles to escape. 	"Refilling the Sheath Tank" (page 165)

Maintenance Checks at Startup

Maintenance item	Schedule	See
Disposing of waste fluid Replacing the waste tank	<ul style="list-style-type: none"> Check that the tank is not full when starting up and shutting down Replace when nearly full or when prompted in Cell Sorter Software 	"Emptying/Changing the Waste Tank" (page 168)
Checking that there is sufficient volume in the sheath tank	<ul style="list-style-type: none"> When starting up 	"Refilling the Sheath Tank" (page 165)
Refilling the sheath tank	<ul style="list-style-type: none"> When prompted in Cell Sorter Software 	
Refilling the DI water tank	<ul style="list-style-type: none"> Refill when prompted in Cell Sorter Software 	"Refilling the DI Water Tank" (page 169)
Refilling the ethanol tank	<ul style="list-style-type: none"> Refill when prompted in Cell Sorter Software 	"Refilling the Ethanol Tank" (page 170)
Refilling the bleach tank	<ul style="list-style-type: none"> Refill when prompted in Cell Sorter Software 	"Refilling the Bleach Tank" (page 171)

Maintenance Checks at Shutdown

Sample fluidics system cleaning

Maintenance item	Schedule	See
Sample fluidics system cleaning	<ul style="list-style-type: none"> When shutting down at day's end As required 	<i>"Cleaning Sample Line using Bleach"</i> (page 174)

General cleaning

Maintenance item	Schedule	See
Cleaning the sample loader	<ul style="list-style-type: none"> When shutting down 	<i>"Regular Cleaning"</i> (page 180)
Cleaning the collection area	<ul style="list-style-type: none"> When shutting down 	
Cleaning the droplet camera windows	<ul style="list-style-type: none"> When shutting down 	
Cleaning the laser windows	<ul style="list-style-type: none"> When shutting down 	
Cleaning collection tube holders, multi-well plate holder	<ul style="list-style-type: none"> When shutting down 	
Cleaning the splash guard (MA900P model)	<ul style="list-style-type: none"> When shutting down As required 	<i>"Cleaning the Deflection Plates"</i> (page 189)
Cleaning the lens guard	<ul style="list-style-type: none"> Weekly As required 	
Cleaning the deflection plates	<ul style="list-style-type: none"> When shutting down As required (when dirty) 	
Cleaning the waste catcher	<ul style="list-style-type: none"> When shutting down As required (when dirty) 	<i>"Cleaning the Waste Catcher"</i> (page 190)

Monthly Maintenance

Maintenance item	Schedule	See
Replacing the waste tank air filter	<ul style="list-style-type: none"> Every month 	<i>"Changing Tank Air Filters"</i> (page 200)
Autoclaving the DI water tank, sheath tank and waste tank	<ul style="list-style-type: none"> Monthly As required 	<i>"Autoclaving the Tanks"</i> (page 172)

Periodic Maintenance

Maintenance item	Schedule	See
Changing the PEEK sample line	<ul style="list-style-type: none"> Every 3 months As required 	<i>"Changing the PEEK Sample Line"</i> (page 175)
Autoclaving the probe adapter	<ul style="list-style-type: none"> Every 3 months As required 	
Changing the sheath filter	<ul style="list-style-type: none"> Every 3 to 12 months As required 	<i>"Changing the Sheath Filter"</i> (page 178)
Changing the DI water tank and ethanol tank air filter	<ul style="list-style-type: none"> Every 12 months 	<i>"Changing Tank Air Filters"</i> (page 200)
Changing the waste catcher O-ring	<ul style="list-style-type: none"> Every 12 months 	<i>"Cleaning the Waste Catcher"</i> (page 190)
Changing the waste tank	<ul style="list-style-type: none"> Every 12 months 	–

Miscellaneous

Maintenance item	Schedule	See
Running Waste Line A maintenance	<ul style="list-style-type: none"> When not used for 1 week or longer As required 	<i>"Running Waste Line maintenance (Waste A Maintenance)"</i> (page 193)
Changing the sheath fluidics lines	<ul style="list-style-type: none"> When a cleaning reminder is displayed on the login screen 	<i>"When a Cleaning Reminder is Displayed"</i> (page 195)
Cleaning the sample loader O-ring	<ul style="list-style-type: none"> As required 	<i>"Changing the Sample Injection Chamber O-Ring"</i> (page 196)
Releasing air trapped in sheath filter and the sorting chip	<ul style="list-style-type: none"> As required 	<i>"Releasing Air in the Sheath Filter and Sorting Chip"</i> (page 199)
Autoclaving the sheath filter	<ul style="list-style-type: none"> As required 	<i>"Autoclaving the Sheath Filter"</i> (page 200)
Ejecting the sorting chip manually	<ul style="list-style-type: none"> As required 	<i>"Ejecting the Sorting Chip Manually"</i> (page 201)
Cleaning the optical fibers	<ul style="list-style-type: none"> As required 	<i>"Cleaning and Handling the Optical Filters"</i> (page 202)
Disconnecting/reconnecting the fluidics cart	<ul style="list-style-type: none"> As required 	<i>"Disconnecting and Reconnecting the Fluidics Cart"</i> (page 203)

Fluidics Cart

Status of fluidics cart tanks

The status of the five fluidics tanks in the tray is displayed on the LCD monitor and in [Hardware Status] in the bottom right of the main window in Cell Sorter Software.



Refilling the Sheath Tank

The sheath tank should always be checked before starting operation. Refill the tank when the level is insufficient. Also, the tank must be refilled when prompted to do so in Cell Sorter Software.

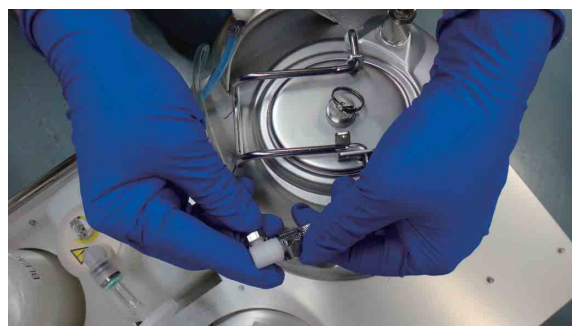
Notes

- Sheath fluid is a sterile solution. Avoid contact with the contents of the tank to maintain sheath fluid sterility.
- Never use the supplied sheath tank for any other purpose.
- When opening the sheath tank cap, check that “Standby” is displayed on the LCD monitor or that the power to the main unit is turned off. If “Standby” is not displayed, place the unit in Standby mode using the following procedure.
 - ① Click [Settings] on the [Cytometer] tab of the ribbon.
The [Cytometer Settings] dialog appears.
 - ② Click [Advanced Settings].
The [Advanced Settings] dialog appears.
 - ③ Click [Standby] on the [Pressure Options] tab.
 - ④ Check that “Standby” is displayed on the LCD monitor.
- Do not force the lid down or place objects on the lid when the tank is pressurized.
- Store replacement sheath fluid in a room with the same temperature environment as the instrument, or allow the sheath fluid to acclimatize in the same room as the instrument before refilling the sheath tank. Droplet formation may become unstable if the sheath fluid added to the tank is a different temperature to the sheath fluid remaining in the tank.

Note

It is recommended that the sheath tank be filled one day before measurement to ensure all air bubbles are removed from the sheath fluid.

- 1 Disconnect the air line (clear) from the top of the sheath tank.



Note

It is important to disconnect the air line before disconnecting the sheath fluid line.

- 2** Release the residual air pressure from the tank using the ring-pull air relief valve, then remove the tank from the fluidics cart.



- 3** Disconnect the sheath fluid line (blue) from the top of the tank.



- 4** Lift the lever securing the lid of the tank to open the lid.



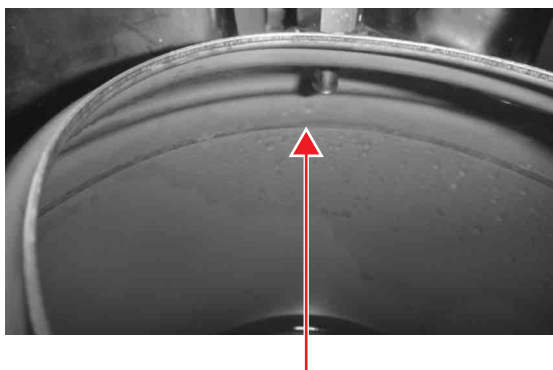
- 5** Lower the lid slightly into the tank, turn it 90 degrees, and then remove the lid from the tank.



6 Refill the tank with sheath fluid.

Notes

- Do not touch the surfaces of the sheath tank lid that come into contact with sheath fluid. Take care not to spill any fluid.
- Do not use a pump or other object that has not been sterilized for filling the tank. It is recommended that the tank be placed immediately beneath the tap of the source sheath fluid container so that the tank is filled using gravity alone.
- Overfilling the sheath tank may damage the unit. Do not fill the tank beyond the full line inside the tank.



Fill with sheath fluid to below this line.

7 Attach the lid.

Note

When inserting the sheath tank lid, make sure that the black rubber seal is attached to the lid.

8 Wipe off any residue liquid or dirt attached to the connector, and connect the sheath air line and sheath fluid line, in the reverse order of removal, until they lock into place.

9 Clean any fluid from the connectors and outside surfaces of the tank, and place the tank back in the designated position in the fluidics cart.

Note

Place the sheath tank near the center of the tray so that it is not in contact with the wall. The sheath fluid level detector may not operate correctly if the tank is in contact with the wall.

10 If the sheath tank was refilled while the instrument is in use, click [Settings] on the [Cytometer] tab of the ribbon to display the [Advanced Settings] dialog, and then click [Ready] on the [Pressure Options] tab.

Notes

- If necessary, move the fluidics cart to a place where it is easier to access. Be careful not to pull on the cart connection lines or cart connection cable with undue force.
- To continue sorting, click [Sort Calibration] on the [Cytometer] tab of the ribbon to run auto calibration.

For details, see “Running Automatic Calibration” (page 49).

Emptying/Changing the Waste Tank

Check the fluid level in the waste tank daily before starting the unit. If it is full, dispose of the waste fluid or replace the tank with a new tank. Also, the waste fluid must be disposed or the waste tank must be replaced when prompted to do so in Cell Sorter Software.

Note

When handling the waste tank, always follow the laboratory rules for the handling of waste fluids.

DANGER

Waste fluid may contain virulent or hazardous substances that may be highly contagious or toxic, depending on the sample under test. Always wear gloves and other protective clothing, mask, and goggles, as required, when handling waste fluid.

- 1 Check that the LE-MA900 main unit is turned off, or check that “Standby” is displayed on the LCD monitor.
- 2 Move the fluidics cart to a place where it is easier to access.

Note

Be careful not to pull on the tubes connected to the main unit with undue force.

- 3 Disconnect the tank waste line (red tubing) from the waste tank.
- 4 Remove the waste tank from the fluidics cart.
- 5 Unscrew the cap of the waste tank, and empty the tank, disposing the waste fluid, or prepare a new waste tank.

Note

Dispose of the waste tank and fluid in accordance with the laboratory rules and local ordinances and regulations.

- 6 Place an empty waste tank or a new tank in the fluidics cart.

Notes

- After emptying the waste or replacing the waste tank, check the following items.
 - Tube couplings are securely connected
 - Waste line is connected securely to the connector on the cap of the waste tank
 - Cap is tightened and there is no fluid leakage

- The tank connection lines and tank fittings are color-coded for visual confirmation of proper connections.
- On the waste tank, check that the color of the tubing connected to the waste tank and the color marking on the cap are both red.
- Place the waste tank near the center of the tray so that it is not in contact with the wall. The waste fluid level detector may not operate correctly if the tank is in contact with the wall.
- If the air filter attached to the waste tank becomes blocked, air cannot escape the waste tank as it fills with waste fluid.
- If the waste tank air filter gets wet, the filter may become clogged, causing the pressure inside the tank to rise because the air cannot escape, and may cause malfunction of the unit. If the waste tank is full, do not tilt or otherwise jostle the waste tank to prevent the filter from getting wet.

Refilling the DI Water Tank

Insert the line connector and push until it locks into place. Carefully Wipe off any residue fluid from the connector and outside surfaces of the tank.

The level of fluid in the DI water tank can be monitored in the main window in Cell Sorter Software and on the LCD monitor.

Precautions when handling DI water

- Refill the DI water tank with fresh DI water before startup, disposing of any unused fluid.
- Regularly autoclave the DI water tank to prevent the outbreak of bacteria and other matter.
- Use DI water (or ultrapure water) uncontaminated by bacteria or biological matter to control the growth of bacteria.

- 1** Disconnect the DI water line from the cap on the DI water tank, and place it in a clean beaker or other container.



Press the metal release catch to withdraw the connector.

- 2** Remove the DI water tank from the fluidics cart for easy access to the cap of the tank.
- 3** Remove the cap and tank probe from the DI water tank and place in a clean, safe location.



- 4** Refill the DI water tank with the specified volume of DI water, and reattach the cap.
- 5** Place the tank in the designated position in the fluidics cart, and connect the DI water line connector.

Refilling the Ethanol Tank

The level of fluid in the ethanol tank can be monitored in the main window in Cell Sorter Software and on the LCD monitor.

Precautions when handling ethanol

- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Never pour ethanol into a tank other than the ethanol tank, and conversely, never pour other liquids into the ethanol tank.
- Make sure that the cap on the ethanol tank is correctly and securely fastened at all times.
- When the ethanol tank is not in use, remove all ethanol from the tank.

Note

The Cell Sorter is compatible with standard disinfectant ethanol (70%).

- 1 Disconnect the ethanol line from the cap on the ethanol tank, and place it in a clean beaker or other container.



Press the metal release catch to withdraw the connector.

Note

Do not touch the surfaces of the ethanol connector that come into contact with ethanol. Take care not to spill any fluid.

- 2 Remove the ethanol tank from the fluidics cart for easy access to the cap of the tank.
- 3 Remove the cap and tank probe from the ethanol tank and place in a clean, safe location.



- 4 Refill the ethanol tank with the specified volume of ethanol, and reattach the cap.

- 5 Place the tank in the designated position in the fluidics cart, and connect the ethanol line connector.

Insert the line connector and push until it locks into place. Carefully Wipe off any residue fluid from the connector and outside surfaces of the tank.

Refilling the Bleach Tank

The level of fluid in the bleach tank can be monitored in the main window in Cell Sorter Software and on the LCD monitor.

Precautions when handling bleach

- Ensure the laboratory is well ventilated during cleaning with bleach. Inhaling bleach vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never pour bleach into a tank other than the bleach tank, and conversely, never pour other liquids into the bleach tank.
- Make sure that the cap on the bleach tank is correctly and securely fastened at all times.
- When the bleach tank is not in use, remove all bleach from the tank.

Note

Use 1% concentration sodium hypochlorite bleach solution when bleach cleaning the sheath line.

- 1 Disconnect the bleach line from the cap on the bleach tank, and place it in a clean beaker or other container.



Press the metal release catch to withdraw the connector.

Note

Do not touch the surfaces of the bleach connector that come into contact with bleach. Take care not to spill any fluid.

- 2 Remove the bleach tank from the fluidics cart for easy access to the cap of the tank.
- 3 Remove the cap and tank probe from the bleach tank and place in a clean, safe location.



- 4 Refill the bleach tank with the specified volume of bleach, and reattach the cap.

- 5 Place the tank in the designated position in the fluidics cart, and connect the bleach line connector.

Insert the line connector and push until it locks into place. Carefully Wipe off any residue fluid from the connector and outside surfaces of the tank.

Autoclaving the Tanks

Autoclaving the DI Water Tank

The DI water tank (including the cap and nozzle) can be autoclaved.

Notes

- Do not autoclave the air filter.



- The DI water tank may not fit inside small laboratory autoclave equipment.
- Do not autoclave the DI water tank if there are any scratches or abnormalities in the DI water tank, cap, or nozzle. Doing so may cause damage.
- If a label attached to the tank peels off or the display deteriorates, handle the tank according to the conditions described in the operating instructions.
- The tank is made of durable material that can be autoclaved up to 120 times.

- 1 Remove the DI water tank rack from the fluidics cart, and dispose of the DI water.

Note

Dispose of fluid in accordance with the laboratory rules and local ordinances and regulations.

- 2 Remove the DI water tank air filter.

For details about removing, see “Changing Tank Air Filters” (page 200).

Note

The air filter is not autoclavable. Always remove it.

- 3 Unscrew the cap of the DI water tank and remove the DI water tank cap and nozzle.

The DI water tank cap and nozzle are autoclavable.

- 4 Autoclave the DI water tank, cap, and nozzle under the following conditions.

Temperature: 121 °C (250 °F)
Pressure: 103.4 kPa (15 psig)
Duration: 20 minutes

- 5 Attach the cap, nozzle, and air filter to the DI water tank.

For details about attaching an air filter, see “Changing Tank Air Filters” (page 200).

- 6 Return the DI water tank to the fluidics cart.

Autoclaving the Sheath Tank

The sheath tank (including the cap and nozzle) can be autoclaved.

Notes

- The sheath tank may not fit inside small laboratory autoclave equipment.
- Do not autoclave the sheath tank if there are any scratches or abnormalities in the sheath tank, cap, or nozzle. Doing so may cause damage.

- 1 Remove the sheath tank rack from the fluidics cart, and dispose of the sheath fluid.

Note

Dispose of fluid in accordance with the laboratory rules and local ordinances and regulations.

- 2 Perform steps 1 to 5 in “Refilling the Sheath Tank” (page 165) to remove the cap of the sheath tank.

The cap is autoclavable.

- 3 Autoclave the sheath tank, cap, and nozzle under the following conditions.

Temperature: 121 °C (250 °F)
Pressure: 103.4 kPa (15 psig)
Duration: 20 minutes

- 4 Refill the tank with sheath fluid.

For details, see step 6 and later in “Refilling the Sheath Tank” (page 165).

- 5 Return the sheath tank to the fluidics cart.

Autoclaving the Waste Tank

The waste tank (including the cap and nozzle) can be autoclaved.

Notes

- Do not autoclave the air filter.
- The waste tank may not fit inside small laboratory autoclave equipment.
- Do not autoclave the waste tank if there are any scratches or abnormalities in the waste tank, cap, or nozzle. Doing so may cause damage.

- If a label attached to the tank peels off or the display deteriorates, handle the tank according to the conditions described in the operating instructions.
- The tank is made of durable material that can be autoclaved up to 120 times.

1 Remove the waste tank from the fluidics cart, and carry the waste tank to the autoclave equipment.

2 Remove the waste tank air filter.

For details about removing, see “Changing Tank Air Filters” (page 200).

Note

The air filter is not autoclavable. Always remove it.

3 Unscrew the cap of the waste tank and remove the waste tank cap and nozzle.

The waste tank cap and nozzle are autoclavable.

4 Autoclave the waste tank, cap, and nozzle under the following conditions.

Temperature: 121 °C (250 °F)

Pressure: 103.4 kPa (15 psig)

Duration: 20 minutes

5 Dispose of the waste fluid.

Note

Dispose of waste fluid in accordance with the laboratory rules and local ordinances and regulations.

6 Attach the cap, nozzle, and air filter to the waste tank.

For details about attaching an air filter, see “Changing Tank Air Filters” (page 200).

7 Return the tank to the fluidics cart.

Temperature: 121 °C (250 °F)

Pressure: 103.4 kPa (15 psi)

Duration: 20 minutes

3 Install the probe adapter.

For details about installing the probe adapter, see “Changing the Probe Adapter” (page 177).

Autoclaving the Probe Adapter

The probe adapter can be autoclaved.

Notes

- Do not autoclave the sample probe.
- Do not autoclave the probe adapter if there are any scratches or abnormalities. Doing so may cause damage.

1 Remove the probe adapter.

For details about removing the probe adapter, see “Changing the Probe Adapter” (page 177).

2 Autoclave the probe adapter under the following conditions.

Cleaning Sample Line using Bleach

Clean the fluid fluidics system regularly using a protein-dissolving bleach (sodium hypochlorite solution) to disinfect the lines that carry cells and other material for analysis. The fluidics system should be cleaned on a regular basis, or as required, when running samples that contain, for example, infectious substances.

Use a 1% to 3% concentration sodium hypochlorite (bleach) solution to clean the sorting chip and PEEK sample line.

- 1 In Cell Sorter Software, click [Bleach Cleaning] on the [Cytometer] tab of the ribbon.

Note

The bleach cleaning sequence cannot be stopped once it is started. Once started, the sequence must be followed to completion.

- 2 Load a 15 ml conical sample tube containing 10 ml of 1% to 3% sodium hypochlorite (bleach) solution in the sample loader.

Tip

When performing bleach cleaning during measurement, perform the cleaning sequence once using a 15 ml conical sample tube. When performing bleach cleaning before shutdown at the end of a day, perform the cleaning sequence three times using a 15 ml conical sample tube or once using a 30 ml cleaning tube.

- 3 Click [Start] to begin cleaning.

The bleach solution passes through the PEEK sample line and sorting chip.

During cleaning, a progress bar is displayed.

Abort cleaning, if required, before the level of bleach in the sample tube falls below the tip of the sample probe.

- 4 When finished, remove the sample tube.

- 5 Click [DI Rinse] on the [Cytometer] tab of the ribbon.

- 6 Load a 15 ml conical sample tube containing 12 ml of DI water in the sample loader.

To prevent the accumulation of excess bleach, the level of DI water must exceed the level of bleach added in step 2.

- 7 Click [Start] to begin cleaning.

Abort cleaning, if required, before the level of DI water in the sample tube falls below the tip of the sample probe.

- 8 When finished, remove the sample tube.

- 9 Click [Probe Wash] on the [Cytometer] tab of the ribbon to flush the PEEK sample line and probe.

Notes

- Always fill the tube with the specified volume of bleach when cleaning with bleach. If the volume of fluid is insufficient, the cleaning fluid may be ejected from the sorting chip in a spray, contaminating the optical lenses and other components.
- If the main unit shuts down during waste line cleaning, always restart the main unit and run [DI rinse]. If bleach solution remains in the instrument, it may damage the main unit.
- 1% to 3% bleach solution is used for sample line cleaning. 1% bleach solution should be used for “*Cleaning sheath lines using bleach (Bleach Cleaning)*” (page 192).

Changing the PEEK Sample Line

The PEEK sample line must be replaced regularly to prevent the buildup of salts restricting the sample flow.

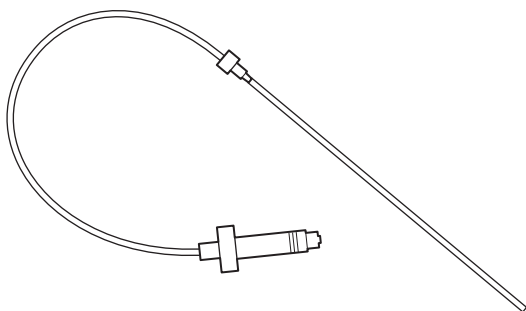
Notes

- The PEEK sample line should be checked for any signs of damage or wear. A damaged PEEK sample line may split and cause sample fluid leakage.
- The PEEK sample line does not support autoclaving (however, the probe adapter does support autoclaving).
- Always leave the PEEK sample line connected when the unit is not in use for protection against dust.
- Use the following procedure to attach the PEEK sample line. There is a risk of fluid leakage if the sample line is not attached correctly.

For details, see “Changing the Probe Adapter” (page 177).

Tip

Sample fluid may contain biological, chemical, or other agents. Always wear biology laboratory gloves and other protective clothing, mask, and goggles when changing the PEEK sample line.



- 1 In Cell Sorter Software, click [Sample Line] in the [Exchange] group on the [Cytometer] tab of the ribbon.

The [Sample Line Exchange] wizard appears.

- 2 Select [PEEK sample line] and click [Start] to begin changing the PEEK sample line.

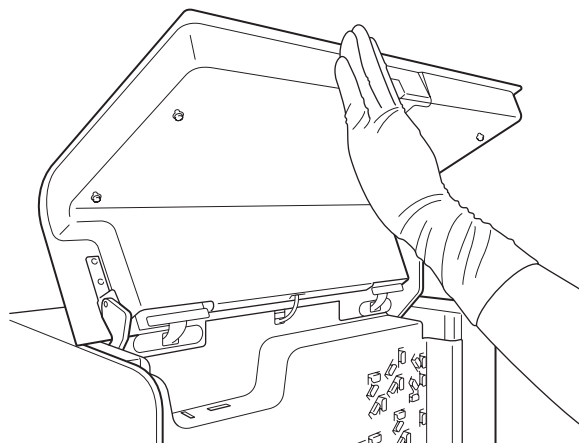
Notes

- Always follow the instructions displayed in the wizard when changing the PEEK sample line.
- The sorting chip must be replaced or reinserted after changing the PEEK sample line.

The PEEK sample line can also be changed using [Sample line exchange] on the Initial Instrument Setup screen or using [Sample line exchange] in maintenance mode. For details, see “Logging In”

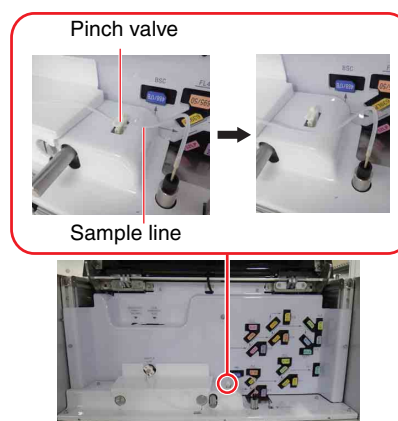
(page 45) and “Running Maintenance Mode” (page 192).

- 3 Open the flip-up door.

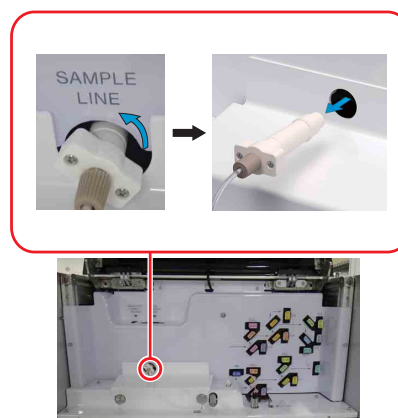


- 4 Remove the sample line from the pinch valve, then click [Next].

To remove, grasp the sample line on both sides of the pinch valve and pull the sample line out towards you.



- 5 Grasp the T-shaped portion of the sample line connector, and gently unscrew it counterclockwise to remove it from the inlet port on the chip loader, then click [Next].



Note

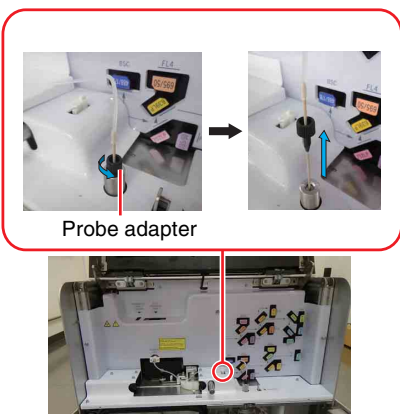
Take care when removing the sample line. Sample fluid can damage electronic circuits if any droplets

fall down between the gaps in the sample loader cover and come into contact with internal components.

- 6 Grasp the black fitting on the PEEK sample line and gently unscrew it counterclockwise to remove it, then click [Next].

Notes

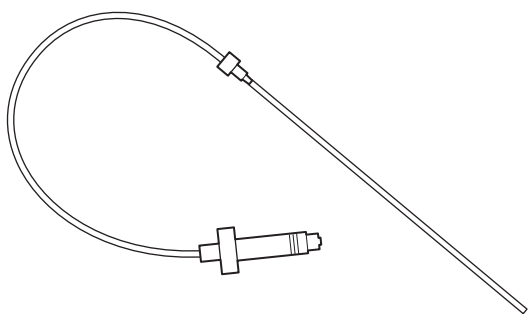
- Unscrew the black fitting while holding the probe adapter down.
- If the sample probe cannot be pulled out, the black fitting may be turning in the reverse direction due to twists in the sample line. Unscrew slowly without releasing the black fitting.



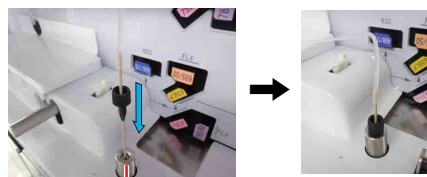
Notes

- Take care when handling the sample probe. It is a high-precision component, which can be damaged easily if handled carelessly.
- Discard the sample line in accordance with the laboratory rules and local ordinances and regulations.

- 7 Prepare a new PEEK sample line, then click [Next].



- 8 Insert the PEEK sample line into the probe adapter, grasp the black fitting, and gently screw it clockwise to secure it, then click [Next].



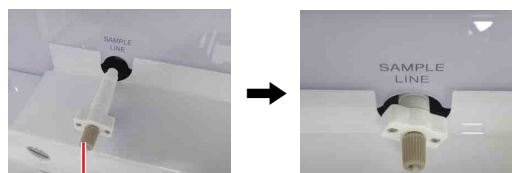
Probe adapter

Note

Fully insert and tighten the threaded portion of the fitting. If the fitting is not fully secured, the correct sample pressure may not be applied

- 9 Attach the new sample line to the inlet port on the front of the chip loader, then click [Next].

When attaching the sample line, insert the sample line straight in without twisting the sample line, hold part A and tighten by turning it clockwise until you hear a clicking sound.



Part A

Note

When turning the connector, always hold part A, not the T-shaped portion. Holding the connector by the T-shaped portion may cause tightening that exceeds the appropriate tightening torque, and adversely affect instrument performance.

Tip

Check the sample line connector regularly for any signs of looseness.

- 10 Check that the sample line connector is attached correctly, then click [Next].

When installed correctly, the stepped portion of the sample line connector fits under the cover. View the connector from the side and check the stepped portion is not visible.

Check that stepped portion is not visible.

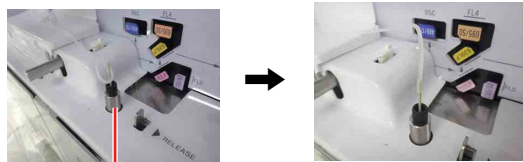


Correct



Incorrect

- 11 Turn the probe adapter, removing any twists in the sample line, then click [Next].



Probe adapter

- 12 Insert the sample line into the pinch valve, then click [Next].

Make sure the sample line is inserted all the way to the back of the pinch valve.



Notes

- Insert the sample line securely into the pinch valve. If the sample line is not correctly inserted in the pinch valve, sample fluid may leak.
- Make sure the sample line connector is securely fastened and that the sample line is correctly inserted in the pinch valve. If the sample line is not inserted correctly, sheath fluid may flow in the reverse direction, potentially causing serious damage to internal components and increasing the risk of biohazard contamination.

- 13 Click [OK] to exit the [Sample Line Exchange] wizard.

Note

When changing the sample line in maintenance mode, the chip loading screen appears before exiting the [Sample Line Exchange] wizard. Reinsert the unloaded sorting chip, then click [Next].

When the sample line has been changed, the chip exchange screen appears.

Changing the Probe Adapter

The probe adapter can be removed, disinfected using autoclaving, and then reinserted back into the unit.

WARNING

Sample fluid may contain biological, chemical, or other agents. Always wear biology laboratory gloves and other protective clothing, mask, and goggles when changing the probe adapter.

Note

Change the probe adapter using the following procedure after removing the PEEK sample line. In particular, when disinfecting the probe adapter for reuse, always follow the procedure correctly to change the adapter.

- 1 Remove the PEEK sample line.

For details about removal, see steps 1 to 6 in “Changing the PEEK Sample Line” (page 175).

- 2 Remove the probe adapter.

Push and hold the sample probe release catch to the right, then pull the probe straight up and out.



- 3 Disinfect the probe adapter, as required.

Autoclave the probe adapter under general autoclave conditions (121 °C (250 °F), 2 atmospheres, approx. 20 minutes).

Note

Make sure the probe adapter is autoclaved if reusing the probe adapter.

To reinstall

- 1 Install the probe adapter.

Push and hold the sample probe release catch to the right, insert the probe straight down, and then let go of the release catch. Check that the release catch returns all the way to the left.

- 2 Install the PEEK sample line.

For details about installation, see steps 7 to 13 in “Changing the PEEK Sample Line” (page 175).

Changing the Sheath Filter

The sheath filter in the fluidics cart should be replaced regularly to prevent small particles of dust that can cause fluctuations in the fluid flow and bacteria that can cause contamination of the sample.

Sheath filter



Perform the following procedure by clicking [Skip Auto Calibration] in “Configuring the Instrument” (page 46) or “Running Automatic Calibration” (page 49) with the main window displayed.

- 1 In Cell Sorter Software, click [Settings] on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog appears.

- 2 Click [Advanced Settings].

The [Advanced Settings] dialog appears.

- 3 Click [Standby] in the [Pressure Options] tab of the [Advanced Settings] dialog.

The sheath fluid flow stops.

- 4 Open the sheath filter door on the fluidics cart, remove the sheath filter from the retaining clamp without disconnecting the sheath lines (two blue tube and one orange tube).



Retaining clamp

- 5 Disconnect the sheath lines from the three connectors.

Press the metal release catch and withdraw each connector.

Tip

The connectors that connect to the sheath filter have stop valves that prevent leakage of fluid when disconnected.



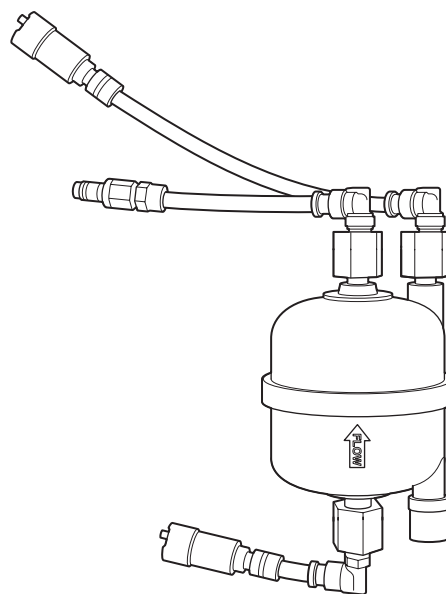
Connectors

- 6 Discard the used sheath filter.

Note

Discard the sheath filter as biohazard waste in accordance with the laboratory rules and local ordinances and regulations.

- 7 Hold a new sheath filter with the arrow on the side of the filter facing up.



- 8 Connect the three sheath filter connectors to the connectors on the fluidics cart.

Note

If the arrow on the side of the sheath filter is pointing up, the filter cannot be connected correctly as described below.

- The connectors will not fit.
- The sheath filter will not fit correctly in the retaining clamp on the fluidics cart.

Tips

- Connect the connectors to the tubes with the same color. If the tubes are not corrected correctly, the sheath filter cannot be inserted correctly into the retaining clamp on the fluidics cart.
- Insert each connector and push it until it locks into place. A click sound occurs when the connectors lock into place.

- 9 Insert the sheath filter into the retaining clamp.

- 10 In Cell Sorter Software, click [Sheath Filter] in the [Debubble] group on the [Cytometer] tab of the ribbon.

The [Sheath Filter Debubble] dialog appears.

- 11 Click [Start]. When the de-bubble function is completed, click [Close].

Repeat two more times to remove residual air from the sheath filter.

Note

The sheath filter de-bubble process takes a short time to finish. Run the sheath filter de-bubble function at least three times to make sure that all air and small particles have been removed from the filter and

sheath line. Failing to do so may cause any solid matter not removed from accumulating and clogging the sorting chip.

12 Purge the air as described in “*Releasing Air in the Sheath Filter and Sorting Chip*” (page 199).

13 Click [Ready] in the [Pressure Options] tab of the [Advanced Settings] dialog.

The sheath fluid flow restarts.

Regular Cleaning

The collection area and sample loader should be cleaned regularly to prevent the precipitation of salts from the sheath fluid and to prevent the spread of biological matter. Cleaning the instrument regularly also prevents trouble occurring during cultivation processes after sorting.

Checking for Leaks

WARNING

When cleaning leakages, always wear biology laboratory gloves and other protective clothing, mask, and goggles.

1 Check that there are no fluid leaks in the drip tray inside the fluidics maintenance area.


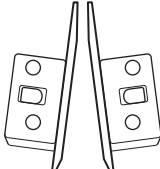

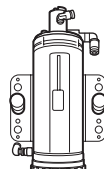
If the problem persists, contact your Sony distributor for service.






2 Check that there are no leaks in the fluidics cart.

Check for loose connectors or clogged filters as described in “*Troubleshooting*” (page 226).

If the problem persists, contact your Sony distributor for service.

Removable parts and cleaning methods

Part		Cleaning method		
		Ethanol	Autoclave	Water rinse
Lens guard		Yes	Yes	Yes
Deflection plates		Yes	No	No
Waste catcher		Yes	No	Yes
Sample injection chamber		Yes	No	Yes

Part		Cleaning method		
		Ethanol	Autoclave	Water rinse
Sheath tank		Yes	Yes	Yes
Waste tank		Yes	Yes	Yes
DI water tank		Yes	Yes	Yes
Ethanol tank		Yes	No	Yes
Bleach tank		Yes	No	Yes

Notes

- Be aware of the sharp door edges when the door is open.
- Always wear biology laboratory gloves and other protective clothing, mask, and goggles.
- Do not operate Cell Sorter Software or other hardware while cleaning the collection area or sample loader.

Cleaning the Collection Area

Clean the droplet camera windows, laser windows, collection stage, and collection tube holder in the collection area using water and ethanol.

Notes

- When cleaning the collection area, pull the collection stage out to its maximum travel, remove the collection tube holder, and clean it separately.
- When cleaning the collection area, also remove the deflection plates. For details, see “*Cleaning the Deflection Plates*” (page 189).

- 1 If the main unit is turned on, select [Instrument Cleaning] in maintenance mode, and follow the on-screen instructions to clean the instrument.

If the main unit has been shut down, proceed to step 2.

For details about maintenance mode, see “*Running Maintenance Mode*” (page 192).

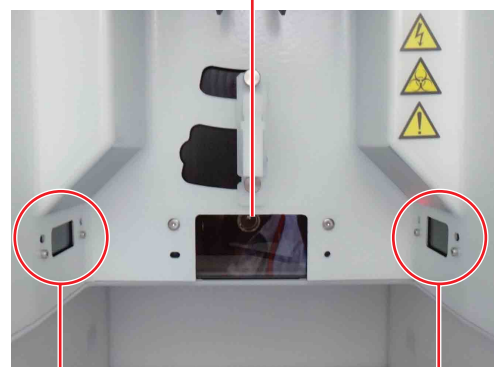
- 2 Press the collection area door button to open the collection area door.

- 3 Moisten a clean, soft, lint-free cloth with water, and wipe clean.

Droplet camera window and laser windows

Carefully clean the droplet camera window and laser windows to prevent reflections affecting the side stream monitor. If the windows become dirty, an error may occur during automatic calibration.

Droplet camera window



Laser window

Laser window

Collection stage and collection tube holder

Clean the collection stage and collection tube holders carefully, especially the upper portion of the collection stage, where the unit is most exposed to salts and biological matter.



Drainage channel

Clean the drainage channel located behind the collection area.

- 1 Press the sides of the waste catcher in the drainage channel and lift the waste catcher up and out.

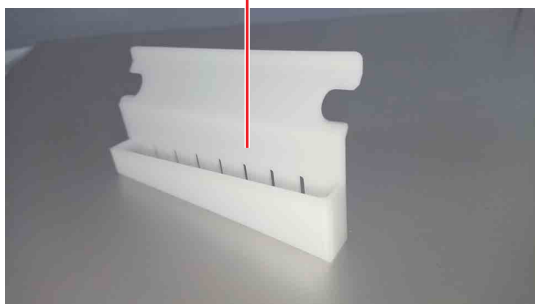


Drainage channel

- 2 Clean the inside of the waste catcher tray and the drainage channel using water and ethanol.

Use a clean, soft, lint-free cloth to remove liquids and stains.

Waste catcher tray



Cleaning the Sample Area

Clean carefully to remove all salt deposits using water and ethanol.

Note

When cleaning the sample loader, remove the sample tube holder and clean it separately.

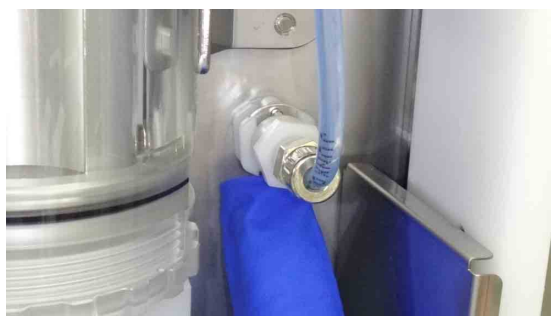
- 1 Check that the main unit is shut down.
If the main unit is turned on, run maintenance mode.
For details about maintenance mode, see “Running Maintenance Mode” (page 192).
- 2 Open the sample area door.



- 3 Remove the PEEK sample line, and probe adapter.

For details about removing, see “Changing the PEEK Sample Line” (page 175).

- 4 Press the metal release catch and disconnect the two outer-most cleaning tubes.



- 5 Remove the sample injection chamber air filter.



- 6** Unscrew the two sample injection chamber lock screws (①), and remove the sample injection chamber (②).

① Unscrew both sample injection chamber lock screws.



② Remove chamber.

Note

Grasp both sides securely so that the sample injection chamber is not dropped. Dropping it may cause damage.

- 7** Unscrew the cap on the bottom of the sample injection chamber, and withdraw the O-ring holder.

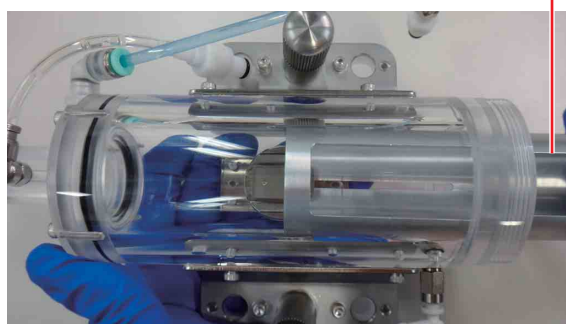
Screw cap



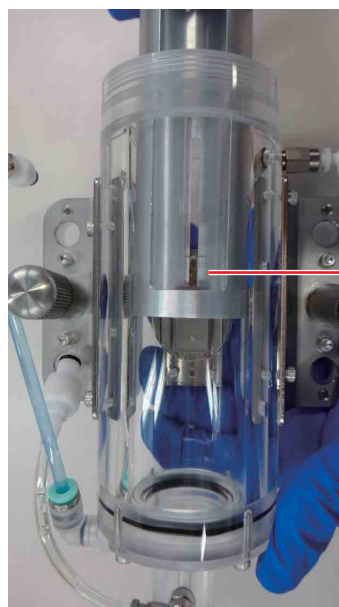
O-ring holder

- 8** Remove the pipe cooler inside the sample injection chamber from the top.

Pipe cooler



- 9** Moisten a clean, soft, lint-free laboratory-use cloth with clean water, and wipe the sample injection chamber, cap, pipe cooler, and sample loader area.



Insert with slot facing inward.

Note

Insert the pipe cooler such that the slot in the pipe cooler engages the metal fitting of the sample injection chamber.



Sample injection chamber metal fitting

- 10** Moisten a clean, soft, lint-free laboratory-use cloth with ethanol, and wipe clean.

- 11** Reinsert the pipe cooler.

Hold the pipe cooler with the slot facing the instrument, and insert it into the sample injection chamber from the bottom.

- 12** Attach the O-ring holder to the sample injection chamber.

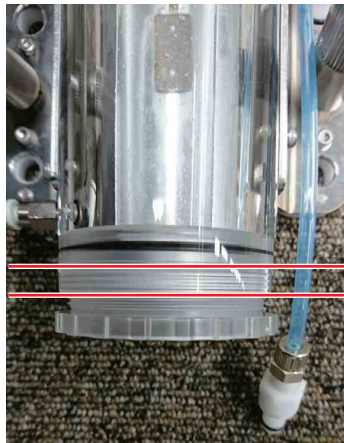


Note

When attaching the O-ring holder, check that the two protrusions on the O-ring holder are aligned with the two notches in the sample injection chamber unit.

13 Attach the screw cap.

Attach so that the cap is parallel.



Parallel attachment

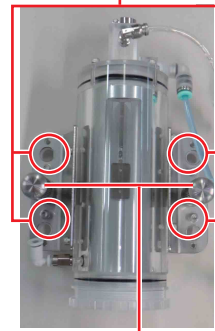
Note

When attaching the cap, check that the thread is aligned correctly (not inclined at an angle) and then tighten. If the cap is not square when attached, the thread may be damaged and air may leak from the sample injection chamber, adversely affecting the sample fluid flow.

14 Remount the sample injection chamber.

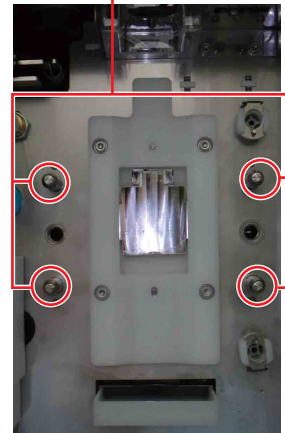
Align the four holes on the sides of the sample injection chamber with the four pins at the rear of the sample loader area, push the chamber on, and tighten the two sample injection chamber lock screws.

Sample injection chamber holes (4)



Sample injection chamber lock screws (2)

Sample loader area pins (4)



Notes

- Grasp both sides securely so that the sample injection chamber is not dropped when mounting. Dropping it may cause damage.
- If the cap of the sample injection chamber is not tightened securely, air may leak from the sample injection chamber, adversely affecting the sample fluid flow.

15 Reattach the two outer cleaning tubes and the sample injection chamber air filter.

Notes

- Push the sample injection chamber air filter into the correct position shown below. If the filter is near the front (incorrect), the slide door may be difficult to close due to interference.

Correct



Push air filter toward the rear to clear the door.

Incorrect



Air filter protrudes at the front, blocking the door.

- Push the coupling so that the outer cleaning tube (blue tubing) is in the correct position shown below. If the tube points toward the front (incorrect), the slide door may be difficult to close due to interference with the tube.

Correct



Push coupling toward the rear so tubes clear the door.

Incorrect



Coupling points toward the front, blocking the door.

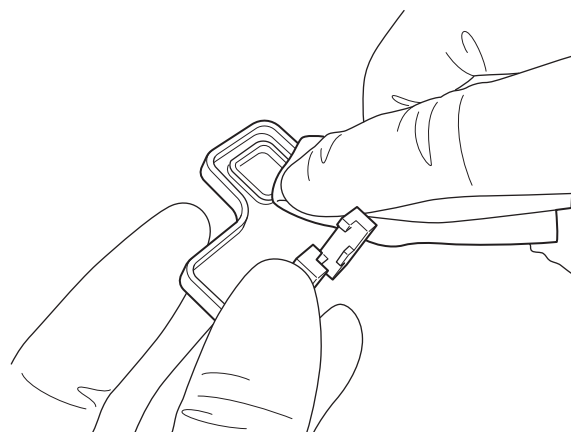
16 Attach the PEEK sample line, and probe adapter.

For details about attaching, see “*Changing the PEEK Sample Line*” (page 175).

17 Close the sample area door.

Cleaning the Splash Guard (LE-MA900P Model)

- 1 Moisten a clean, soft, lint-free cloth with water, and wipe clean.



- 2 Moisten a clean, soft, lint-free laboratory-use cloth with ethanol, and wipe clean.

Cleaning the Lens Guard

The FSC module must be moved to clean the lens guard.

- 1 Check that the main unit is shut down.
- 2 Loosen the two cover retaining screws, and remove the front blind cover.



- 3 Unscrew the screw securing the FSC module.



- 4** Pull out the FSC module toward you.



- 5** Rotate the FSC module counterclockwise to gain access.



- 6** Unscrew the lens guard retaining screw, and remove the lens guard.



- 7** Clean the lens guard.

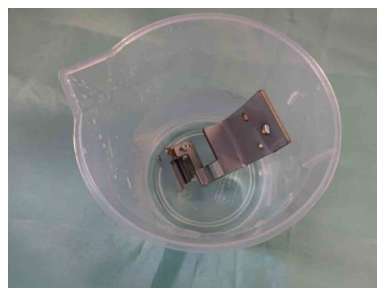
- ① Remove the A WL screw from the lens guard.



- ② Half fill a beaker with DI water.



- ③ Place the lens guard in the beaker and allow it to sit for five minutes.

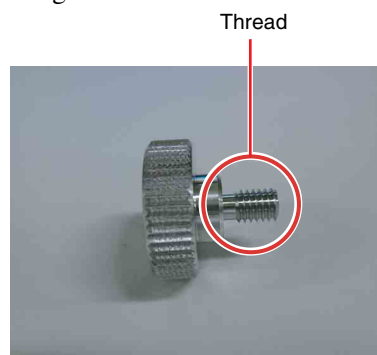


- ④ Remove the lens guard, and wipe it dry using a dry, lint-free laboratory-use cloth.



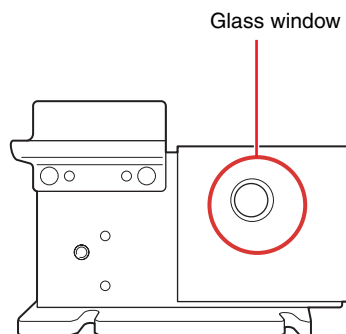
- 8** Gently wipe screw A WL with ethanol.

Check that the thread is not damaged. Replace if damaged.

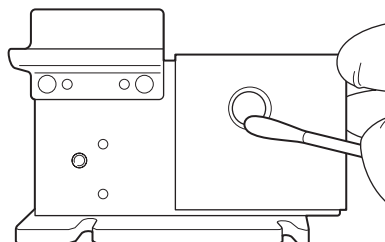


9 Clean the FSC module.

Remove any salt deposits adhering to the glass window.



- ① Wipe off any salt deposits adhering to the glass window using a cloth moistened with DI water.



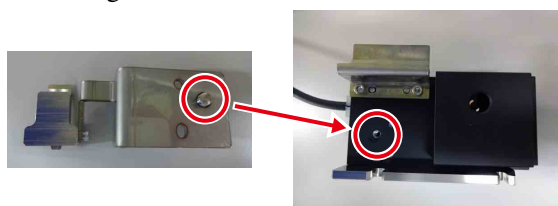
- ② After the DI water dries off, wipe the glass window clean using a cloth moistened with ethanol (99.5%). Check that there is no residue or dust on the surface.

Reassembling the lens guard

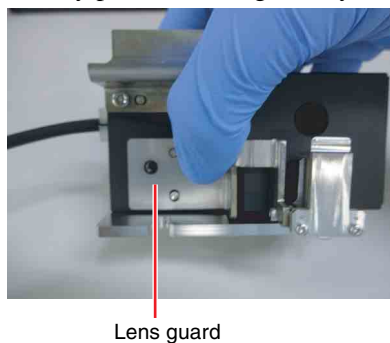
Attach the lens guard removed for cleaning, and attach the FSC module.

1 Attach the lens guard.

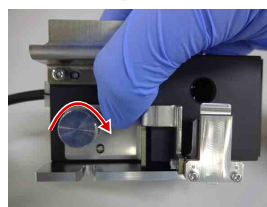
- ① Align the pin on the rear of the lens guard with the hole on the right of the FSC module, and attach the lens guard.



- ② Gently press the lens guard by hand.

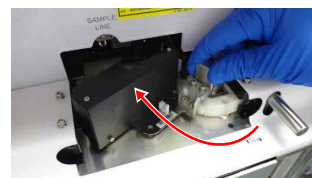


- ③ Secure in position using the retaining screw.

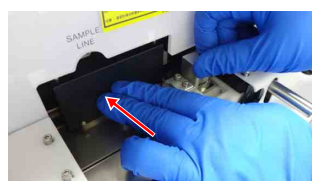


2 Move the FSC module back into position.

- ① Rotate the FSC module clockwise until it stops.



- ② Push the FSC module in all the way.

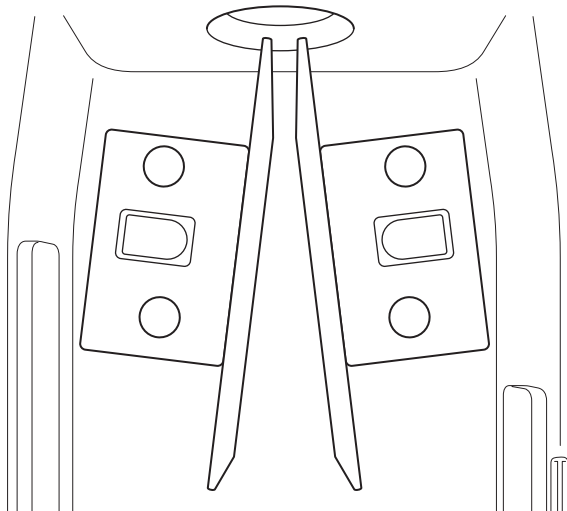


- ③ Secure in position using the retaining screws.

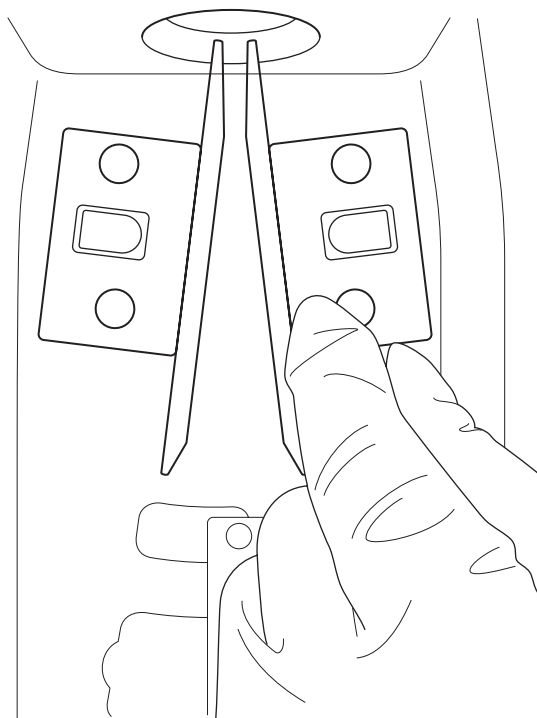


Cleaning the Deflection Plates

The deflection plates in the collection area should be removed and cleaned when cleaning the collection area to maintain sorting performance.



- 1 Check that the main unit is shut down.
- 2 Open the collection area door on the front panel.
- 3 Remove the two bolts securing each deflection plate by hand, and remove the deflection plates.

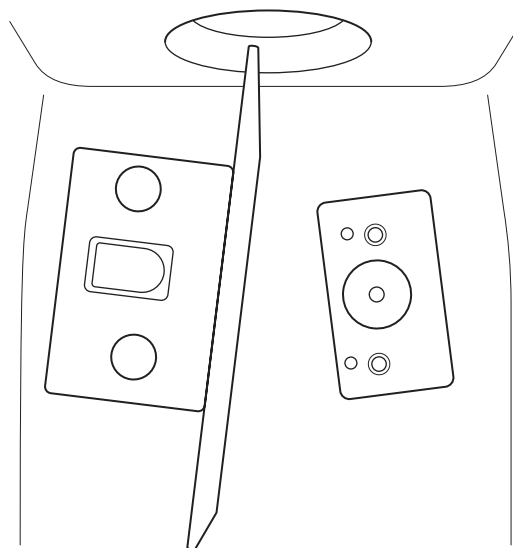


Note

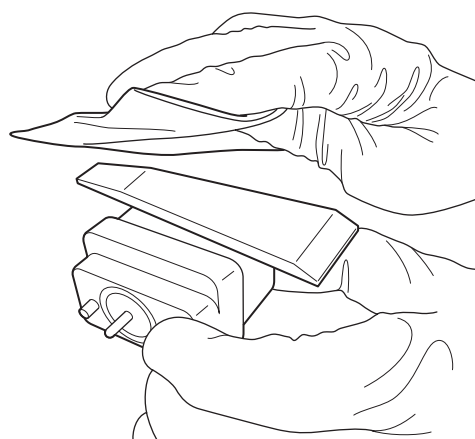
When the deflection plates are removed, the rubber packing may remain within the main unit. If this occurs, remove the rubber packing from the main

unit and insert into the grooves on the deflection plates.

- 4 Clean the deflection plate mounting points and bolts using a lint-free cleaning cloth moistened with water.

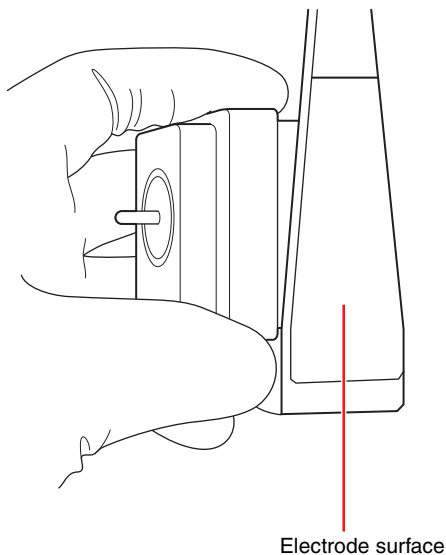


- 5 Clean the deflection plates.



Note

The electrode surfaces of the deflection plates have been treated with a water-repellent coating. Wiping salt crystal deposits with a cloth may damage the coating. Wash the electrode surfaces with water to dissolve salt crystals, then wipe clean.



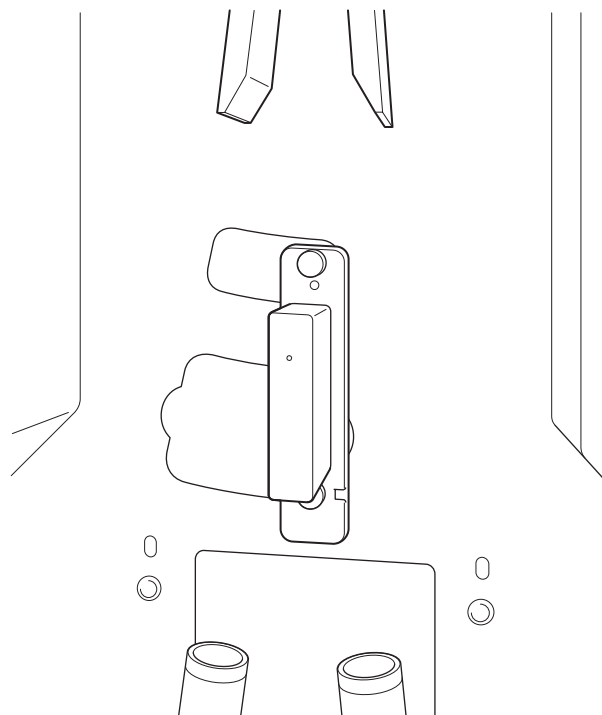
- 6** Clean the items in steps **5** to **6** using a lint-free cleaning cloth moistened with ethanol.
- 7** Attach the deflection plates in the correct orientation.
- 8** Insert the two bolts and tighten by hand.

Note

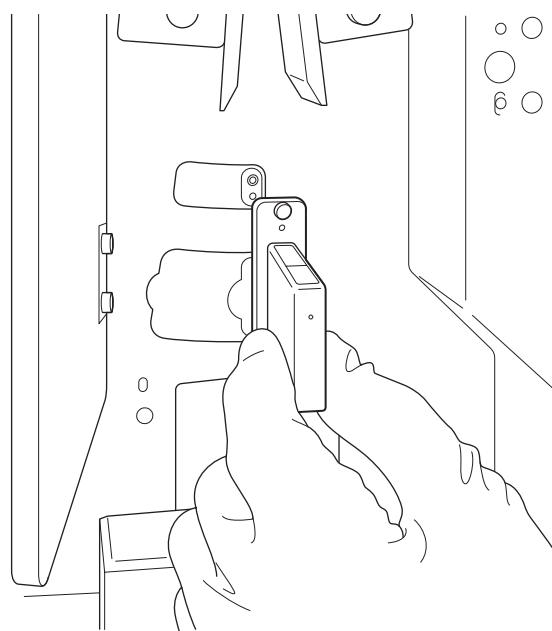
Do not overtighten using tools.

Cleaning the Waste Catcher

The waste catcher in the collection area should be removed and cleaned when cleaning the collection area to ensure the area is not contaminated between sorts.



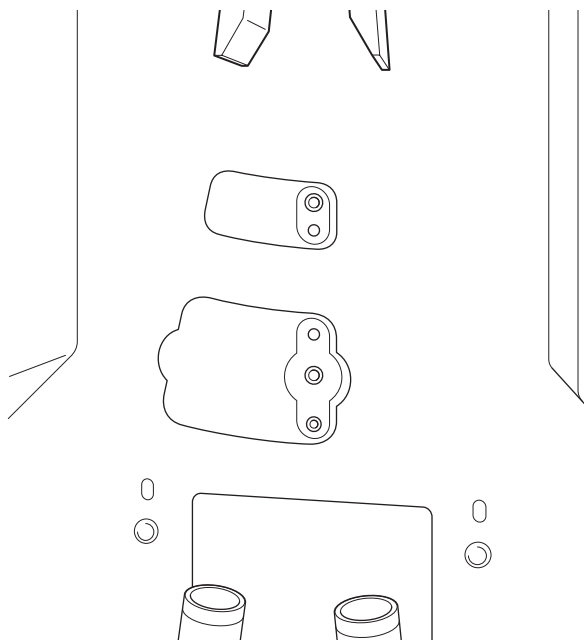
- 1** Check that the main unit is shut down.
- 2** Open the collection area door on the front panel.
- 3** Remove the two screws securing the waste catcher, and remove the catcher.



Note

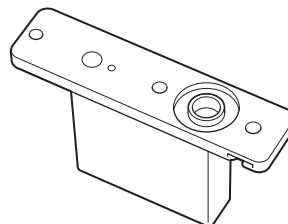
When the waste catcher is removed, the rubber packing may remain within the main unit. If this occurs, remove the rubber packing from the main unit and insert into the groove on the waste catcher.

- 4** Clean the waste catcher mounting bracket, holes, and screws using a lint-free cleaning cloth moistened with water.

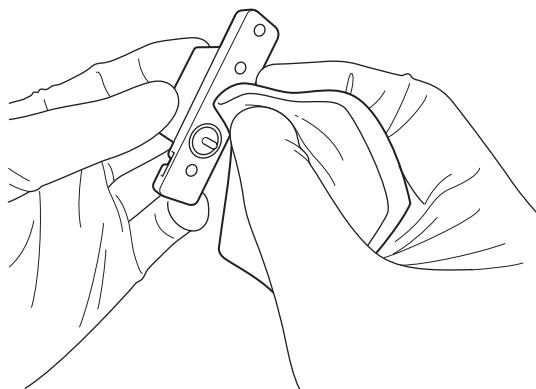


fluid leakage if not fitted correctly (especially, the O-ring) and risk of overflow if the port is obstructed.

- The O-ring on the rear of the waste catcher should be replaced every 12 months.
- Electrostatic charge may accumulate in the collection area after cleaning the plastic covers with a cloth that is too dry. This charge can adversely affect the side stream position, causing an error in the automatic side stream calibration process. If this occurs, wipe the areas around the deflection plates with a cloth soaked in DI water to dissipate any accumulated electrostatic charge.
- Check that the waste catcher screw is not wet when tightening the screw. A malfunction may occur if the screw is wet when installed.



- 5** Clean the waste catcher.



- 6** Clean the items in steps **4** to **5** using a lint-free cleaning cloth moistened with ethanol.

Wipe any moisture off the screws using a dry, lint-free cleaning cloth.

- 7** Reattach the waste catcher.

Notes

- Make sure that the waste fluid outlet of the waste catcher is correctly aligned with the port on the mounting plate, and that the waste catcher is securely fastened to the mounting plate. There is a risk of waste

Running Maintenance Mode

The following maintenance can be performed in maintenance mode.

- Ethanol cleaning
- Bleach cleaning
- Ethanol priming
- Waste A maintenance
- Sample line exchange
- Sample area maintenance

You can run maintenance mode from the Cell Sorter Software login screen.

For each cleaning fluid, connect the corresponding tank connection lines to cleaning fluid connector A or B to switch the cleaning mode.

The tank connection line to connect to connector A or B changes depending on the cleaning mode. Follow the instructions in the maintenance wizard to switch modes.

For details about the cleaning fluid connectors, see “[\[2\] Cleaning fluid connector block](#)” (page 27).

Precautions when cleaning with ethanol

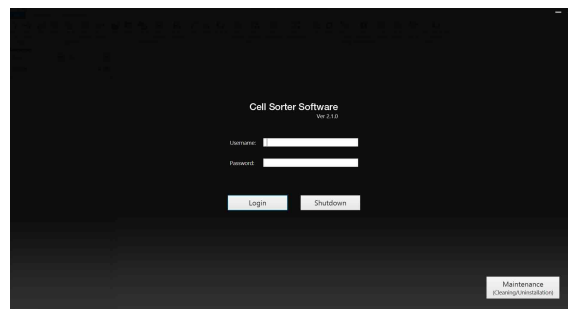
- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Do not pour any fluid other than the specified fluid into the fluidics system tanks.
- Make sure that the cap on the ethanol tank is correctly and securely fastened at all times.

Tip

The LE-MA900 is compatible with standard disinfecting agents (ethanol 70%).

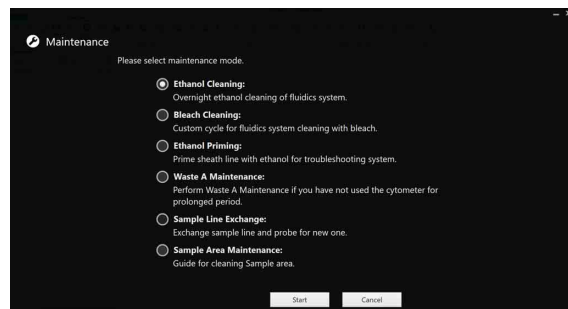
Running ethanol cleaning

- 1 Click [Maintenance] on the bottom right of the Cell Sorter Software login screen.



The [Maintenance] screen appears.

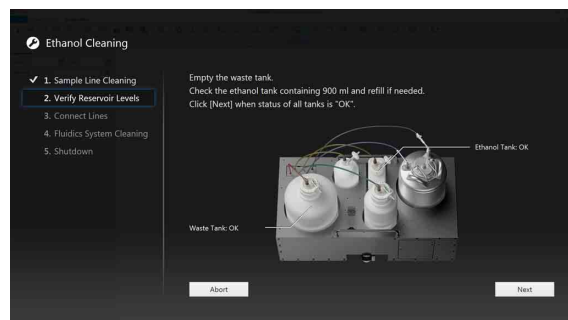
- 2 Select [Ethanol Cleaning], then click [Start].



The [Ethanol Cleaning] wizard appears.

- 3 Start ethanol cleaning.

Follow the on-screen instructions.



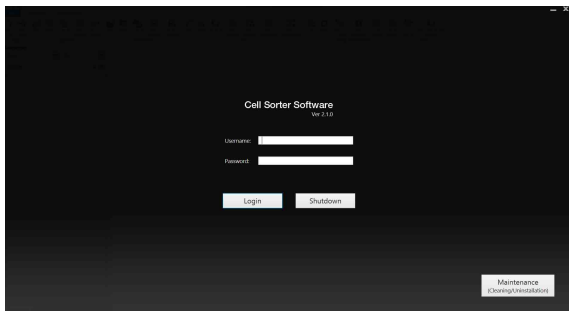
The instrument shuts down when ethanol cleaning finishes.

Cleaning sheath lines using bleach (Bleach Cleaning)

The entire fluidics system, including the sheath tank, sheath line, DI water line, and sample line, should be cleaned with bleach solution regularly to prevent the growth of bacteria and other agents. The fluidics system should be cleaned on a regular basis, or as required, when running samples that contain, for example, infectious substances.

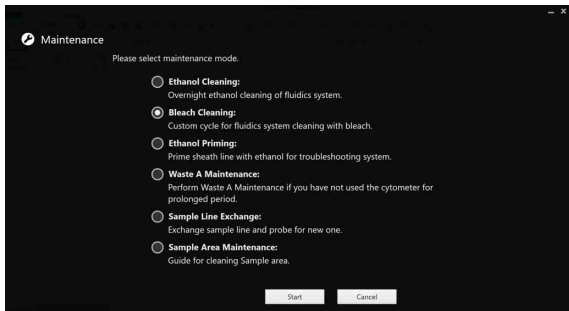
Prepare a cleaning chip before starting the cleaning process. Use a bleach solution (1% concentration sodium hypochlorite) and DI water.

- 1 Click [Maintenance] on the bottom right of the Cell Sorter Software login screen.



The [Maintenance] screen appears.

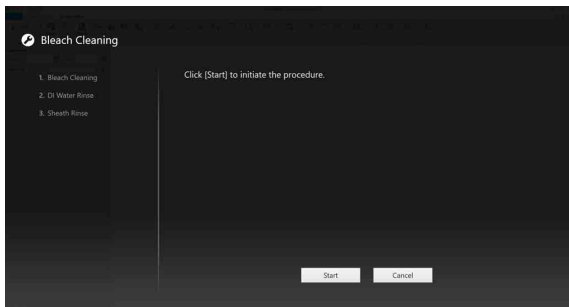
- 2 Select [Bleach Cleaning], then click [Start].



The [Bleach Cleaning] wizard appears.

- 3 Start sheath line cleaning.

Follow the on-screen instructions.



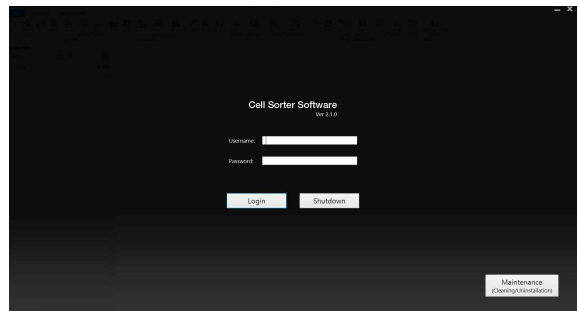
When the sheath line cleaning finishes, the login screen reappears.

Priming with ethanol (Ethanol Priming)

Prime the sheath fluidics line to clear air trapped in the sheath line or other factor causing unstable droplet formation.

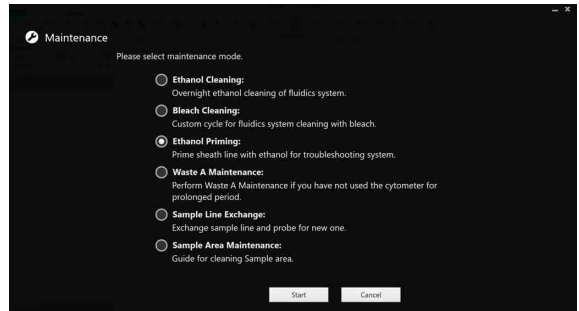
Prepare a cleaning chip before starting the cleaning process. Use 70% ethanol and DI water.

- 1 Click [Maintenance] on the bottom right of the Cell Sorter Software login screen.



The [Maintenance] screen appears.

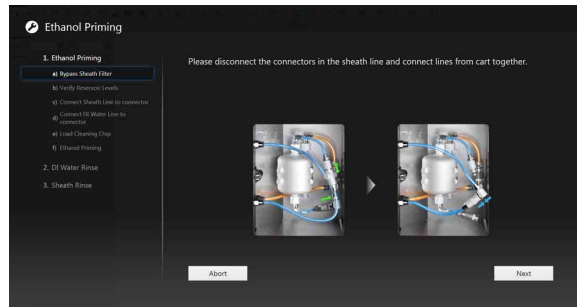
- 2 Select [Ethanol Priming], then click [Start].



The [Ethanol Priming] wizard appears.

- 3 Start ethanol priming.

Follow the on-screen instructions.

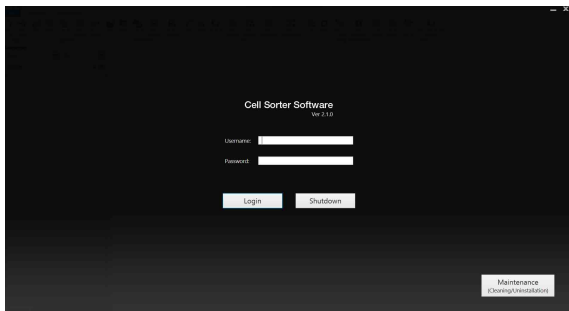


When ethanol priming finishes, the login screen reappears.

Running Waste Line maintenance (Waste A Maintenance)

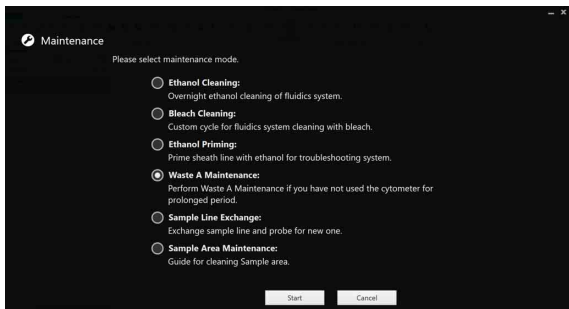
The instrument should be started in maintenance mode to prevent the buildup of salts and clean waste lines (Waste Line).

- 1 Click [Maintenance] on the bottom right of the Cell Sorter Software login screen.



The [Maintenance] screen appears.

- 2 Select [Waste A Maintenance], then click [Start].

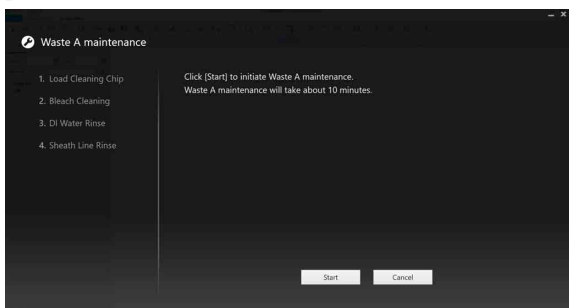


The [Waste A Maintenance] wizard appears.

- 3 Start Waste A Line maintenance.

Follow the on-screen instructions.

In Waste A Line maintenance, bleach cleaning, DI water cleaning, and sheath line cleaning are performed, in that order.



For details about cleaning using sodium hypochlorite (bleach) solution, see “Cleaning Sample Line using Bleach” (page 174).

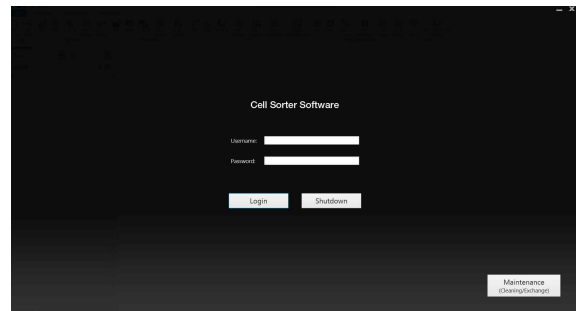
When Waste A Line maintenance finishes, the login screen reappears.

Note

A cleaning chip is required for Waste A Line maintenance.

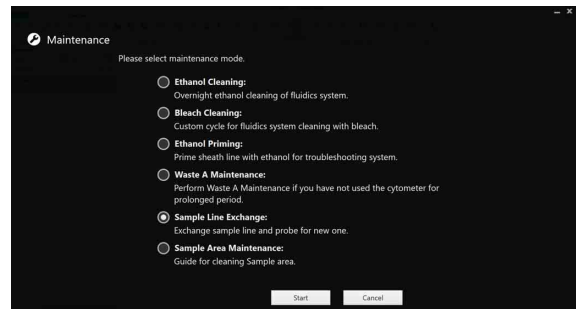
Changing the Sample Line (Sample Line Exchange)

- 1 Click [Maintenance] on the bottom right of the Cell Sorter Software login screen.



The [Maintenance] wizard appears.

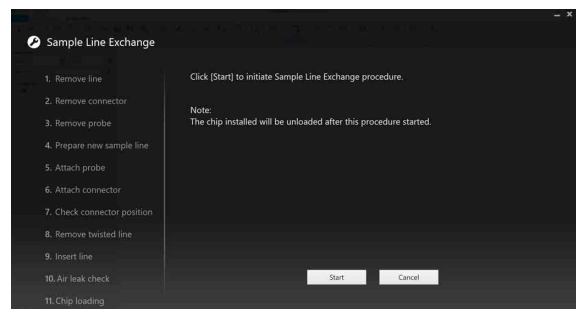
- 2 Select [Sample Line Exchange], then click [Start].



The [Sample Line Exchange] wizard appears.

- 3 Start changing the sample line.

Follow the on-screen instructions.



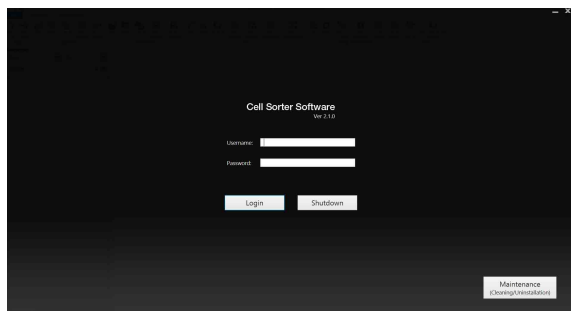
For details about changing the sample line, see “Changing the PEEK Sample Line” (page 175).

When the sample line has been changed, the login screen reappears.

Running sample area maintenance

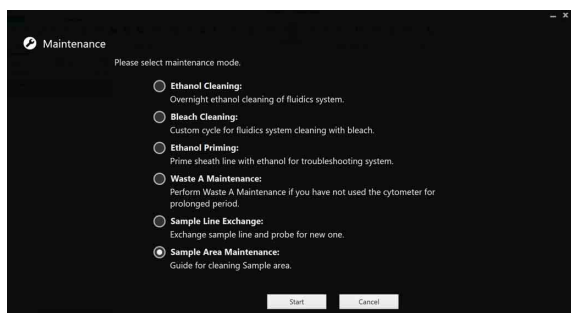
This process cleans the sample area to remove any salt deposits.

- 1 Click [Maintenance] on the bottom right of the Cell Sorter Software login screen.



The [Maintenance] screen appears.

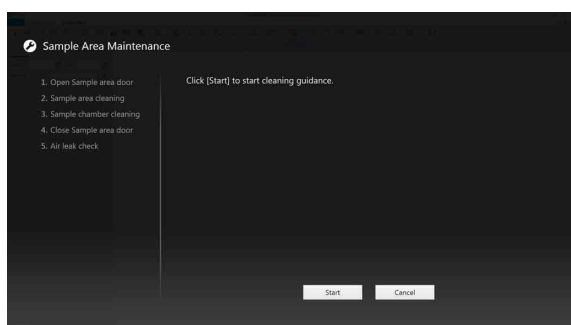
- 2 Select [Sample area maintenance], then click [Start].



The [Sample Area Maintenance] wizard appears.

- 3 Start sample area maintenance.

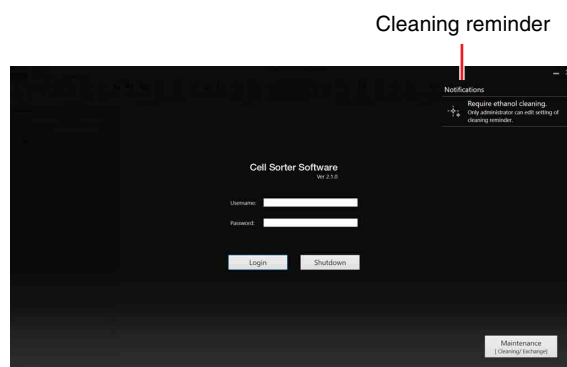
Follow the on-screen instructions.



When sample area maintenance finishes, the login screen reappears.

When a Cleaning Reminder is Displayed

The sheath lines must be cleaned periodically to reduce contamination and side stream instability resulting from poor system maintenance. A message (cleaning reminder) appears on the login screen after a certain preset time has elapsed without performing ethanol cleaning or bleach cleaning.



Tip

Only an administrator can set the ethanol cleaning intervals for sending cleaning reminder notifications.

For details about configuration, see “Setting a Cleaning Reminder” (page 41).

Precautions when cleaning with ethanol or bleach

- Ensure the laboratory is well ventilated during cleaning with ethanol or bleach. Inhaling ethanol or bleach vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Do not pour any fluid other than the specified fluid into the fluidics system tanks.
- Make sure that the cap on the ethanol tank or bleach tank is correctly and securely fastened at all times.
- Make sure that the cap on the waste tank is correctly and securely fastened at all times.
- Make sure that the waste fluid line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the waste tank itself.

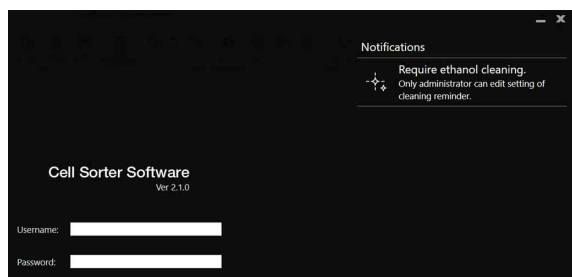
Note

Prepare a fresh batch of bleach solution (1% concentration sodium hypochlorite) before each cleaning.

Tip

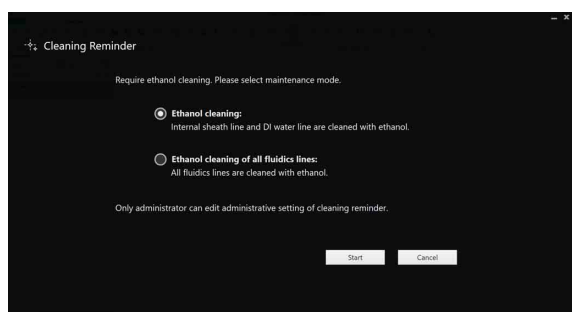
The LE-MA900 is compatible with standard disinfecting agents (ethanol 70%).

- 1 Click the cleaning reminder.



The [Cleaning Reminder] screen appears.

- 2 Select [Ethanol cleaning] or [Bleach Cleaning], then click [Start].



The [Ethanol cleaning for all fluidics lines] or [Bleach Cleaning] wizard appears.

Follow the on-screen instructions to clean the sheath line/DI water line or all fluidics lines with ethanol or bleach.

When ethanol cleaning or bleach cleaning finishes, the login screen reappears.

Tip

Do not autoclave the sheath filter used during ethanol cleaning.

Changing the Sample Injection Chamber O-Ring

An O-ring is fitted into the base of the sample injection chamber that seals the chamber when the sample tube is lifted into the chamber. The O-ring should be cleaned regularly, and replaced every 3 to 6 months to ensure the integrity of the seal.

- 1 Check that the main unit is shut down.
If the main unit is turned on, run maintenance mode.
For details about maintenance mode, see “Running Maintenance Mode” (page 192).

- 2 Open the sample area door.



- 3 Remove the PEEK sample line, probe adapter, sample injection chamber air filter, and the two outer cleaning tubes if connected.
- 4 Unscrew the two sample injection chamber lock screws (①), and remove the sample injection chamber (②).

① Unscrew both sample injection chamber lock screws.



② Remove chamber.

Note

Grasp both sides securely so that the sample injection chamber is not dropped. Dropping it may cause damage.

- 5 Unscrew the cap on the bottom of the sample injection chamber, and withdraw the O-ring holder.

Note

Take care not to damage the O-ring or the O-ring groove. If damaged, air may leak from the sample injection chamber, adversely affecting the sample fluid flow.



- 6 Remove the two O-rings from the O-ring holder.

To clean the O-ring, first wipe with a clean, soft, lint-free cloth that has been slightly moistened with water, then wipe with a similar cloth moistened with ethanol.

Also, clean the groove in the O-ring holder and the top surface and circumference of the sample tube agitation unit that forms the seal with the O-ring.



- 7** Insert new O-rings into the O-ring grooves, and run your finger around the outer thin O-ring and inner thick O-ring to ensure they are seated properly.



Inner thick O-ring

Outer thin O-ring

Note

If the O-rings are not seated properly, air may leak from the sample injection chamber, adversely affecting the sample fluid flow.

- 8** Attach the O-ring holder to the sample injection chamber.

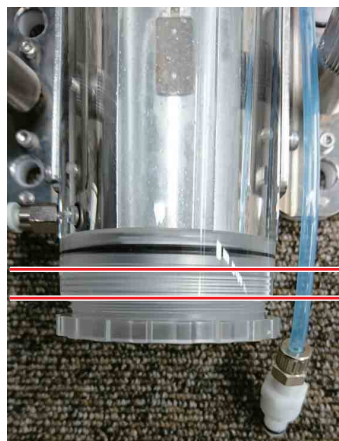


Note

When attaching the O-ring holder, check that the two protrusions on the O-ring holder are aligned with the two notches in the sample injection chamber unit.

- 9** Attach the screw cap.

Attach so that the cap is parallel.



Parallel attachment

Note

When attaching the cap, check that the thread is aligned correctly (not inclined at an angle) and then tighten. If the cap is not square when attached, the thread may be damaged and air may leak from the sample injection chamber, adversely affecting the sample fluid flow.

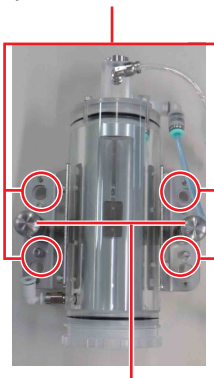
- 10** Remount the sample injection chamber.

Align the four holes on the sides of the sample injection chamber with the four pins at the rear of the sample loader area, push the chamber on, and tighten the two sample injection chamber lock screws.

Note

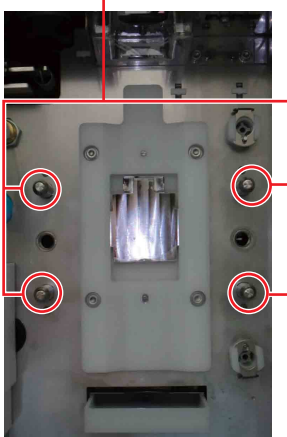
If the cap of the sample injection chamber is not tightened securely, air may leak from the sample injection chamber, adversely affecting the sample fluid flow.

Sample injection chamber holes (4)



Sample injection chamber lock screws (2)

Sample loader area pins (4)



Note

Grasp both sides securely so that the sample injection chamber is not dropped when mounting. Dropping it may cause damage.

- 11 Attach the PEEK sample line, probe adapter, sample injection chamber air filter, and the two outer cleaning tubes as required.

For details about attaching, see “*Changing the PEEK Sample Line*” (page 175).

- 12 Close the sample area door.

Releasing Air in the Sheath Filter and Sorting Chip

During operation, air may become trapped in the sheath filter, interrupting the smooth flow of sheath fluid to the sorting chip. Air may also become trapped in the sorting chip itself, preventing the formation of regular-shaped droplets.

To release air trapped in the sheath lines and sheath filter using the de-bubble function

- 1 Click [Sheath Filter] in the [Debubble] group on the [Cytometer] tab of the ribbon.

The [Sheath Filter Debubble] dialog appears.

- 2 Click [Start], and then click [Close] when finished.

The sheath filter de-bubble process takes a short time to finish.

If changing the sheath filter, run the sheath filter de-bubble process at least three times to make sure that all air and small particles have been removed from the filter and the sheath line.

To release air trapped in the sheath filter

Click [Vent Air] in the [Debubble] group on the [Cytometer] tab of the ribbon.

To release air trapped in the sorting chip using the de-bubble function

If the formation of droplets is irregular even though there is no air trapped in the sheath filter, de-bubble the sorting chip. Click [Chip] in the [Debubble] group on the [Cytometer] tab of the ribbon.

Changing Tank Air Filters

The DI water tank, ethanol tank, and waste tank are fitted with an air filter. The filter allows air to flow into or out of the tank as the fluid level in the tank changes.

The air filters on the three tanks are connected using the same type of connector. This section describes the replacement procedure using the DI water tank as an example.

- 1 Grasp the short length of tubing with one hand, and push the quick release collet into the connector using your other hand.



- 2 Pull the tubing out of the connector.



- 3 Insert the replacement air filter tubing into the connector and push firmly into place.

Note

The filter may become clogged if fluid gets inside the filter, adversely affecting unit operation. If any fluid enters the filter while emptying, replacing, or refilling tanks, replace the filter as soon as possible.

Autoclaving the Sheath Filter

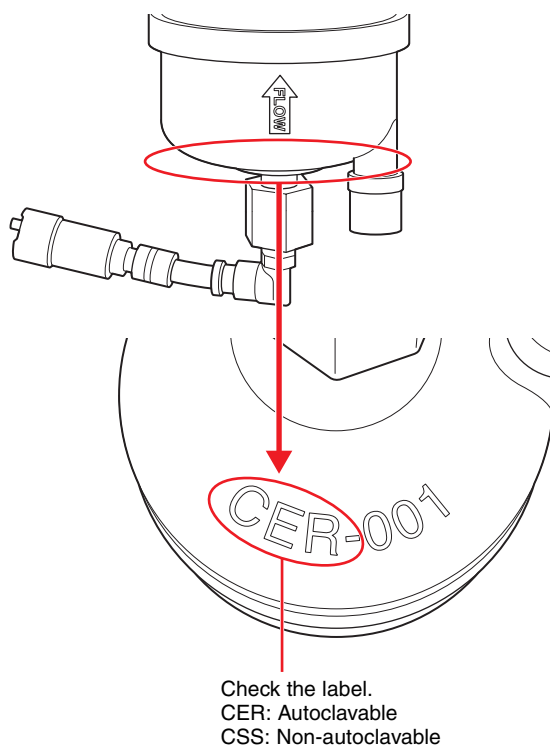
You can autoclave the sheath filter if using autoclavable parts to sterilize the filters.

Notes

- Couplings and connectors can be autoclaved while attached.
- Each filter can be autoclaved up to ten times.
- Do not autoclave a filter if there is any ethanol within the filter.

Autoclaving the Sheath Filter

If necessary, you can autoclave the sheath filter and DI water filter.



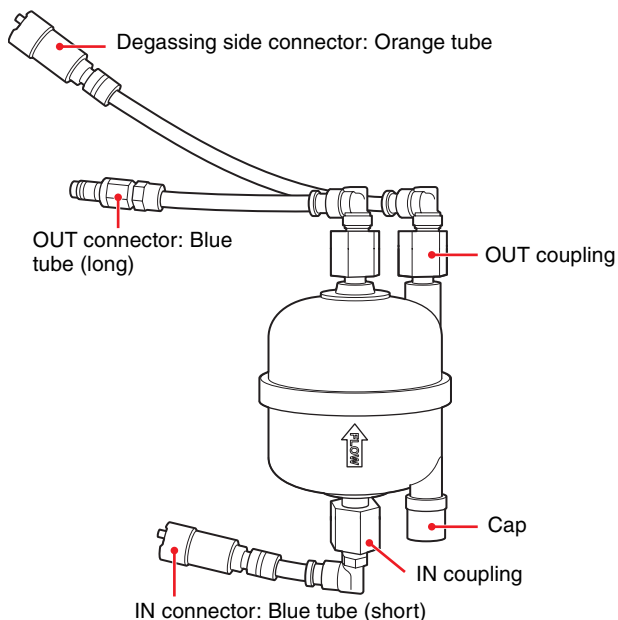
- 1 Remove the filter from the fluidics cart.

For details about removing filters, see “Changing the Sheath Filter” (page 178).

- 2 Remove the cap from the bottom end of the filter.

Note

You can identify the orientation of the filter by the FLOW arrow on the filter's body.



3 Autoclave the filter under the following conditions.

Temperature: 121 °C (250 °F)

Pressure: 2 atmospheres

Duration: 30 minutes

4 After autoclaving, attach the cap to the bottom end of the filter, and place the filter back in the fluidics cart.

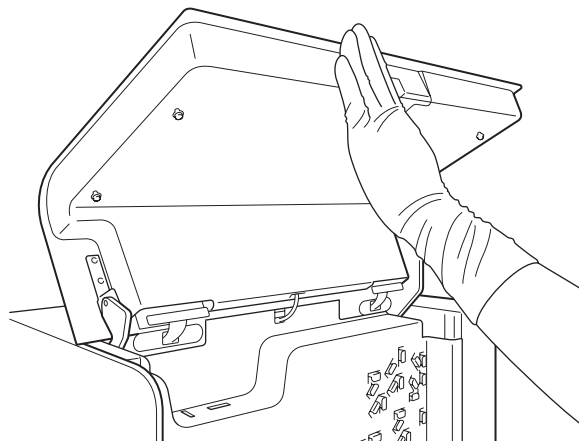
Notes

- The IN and OUT couplings differ at their tube ends. If the connectors are removed at all, check the connector shape and tube length and then attach to the couplings. Check that the tubes are fully inserted in the couplings.
- After attaching the filter, release the air from inside the filter by performing the procedures in the “Releasing Air in the Sheath Filter and Sorting Chip” (page 199).

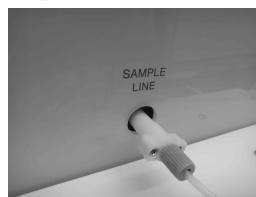
Ejecting the Sorting Chip Manually

If for any reason the chip will not eject normally, the chip can be removed manually.

1 Check that the compressed air supply is turned on, and open the flip-up door to access the sorting chip.



If the sample line connector is out at the front, go to step 6.



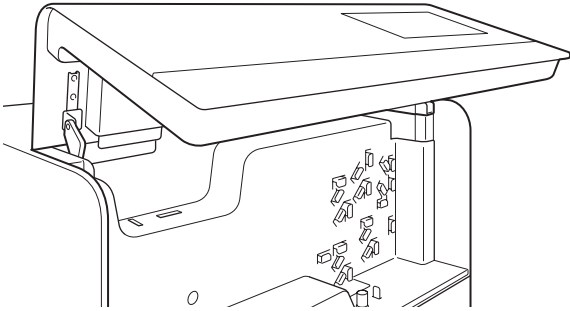
If the sample line connector is not out at the front, go to step 2.



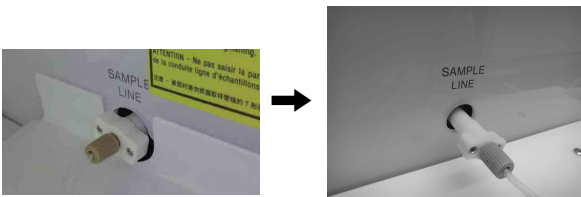
2 Turn off the compressed air supply.

Have an assistant help if you cannot reach the air supply control valve of the compressor supplying compressed air while holding the flip-up door open.

- 3 Lower the flip-up door slowly until it can support its own weight.



- 4 Shut down the main unit.
- 5 Grasp the sample line connector firmly and pull it out towards you to the position, if not in position already.



Pulling the sample line connector withdraws the sorting chip connector interface, allowing the chip to be removed.

- 6 Eject the chip by pushing the top of the thumbwheel from front to back.



Note

Touching the thumbwheel or the sample line will directly affect the chip alignment. If you touch either and then opt to leave the chip inserted, the chip must be realigned. To align the chip, click [Chip Alignment] on the [Cytometer] tab of the ribbon.

- 7 When finished, turn on the compressed air supply.
- 8 Close the flip-up door.
- 9 Turn on the main power supply.

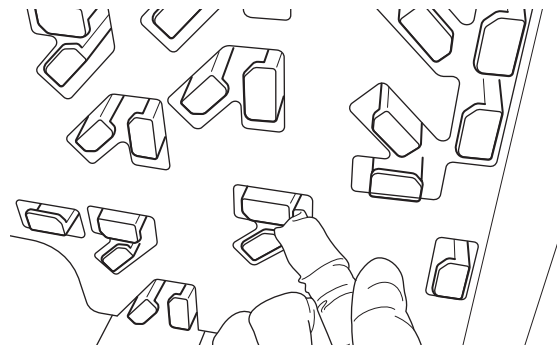
Cleaning and Handling the Optical Filters

The bandpass optical filters and longpass optical filters in the detection module require cleaning to remove any dust on the surface of the filters. The filters should be checked and cleaned every 6 to 12 months, or more often if the optical filter pattern is frequently changed.

Note

The optical filters are high-precision components that require the utmost care. If an optical filter is scratched or damaged in any way, it must be replaced.

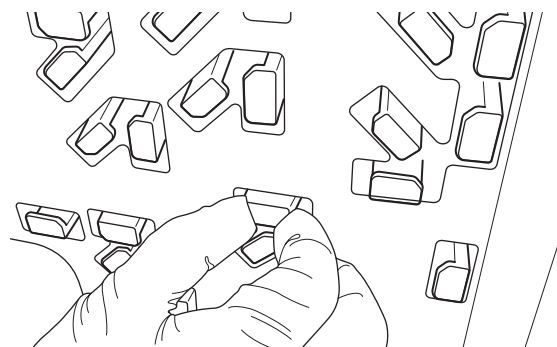
- 1 Remove an optical filter by placing your finger under the lip of the retaining holder and pulling directly out.



- 2 Grip the sides of the optical filter and withdraw it from the filter slot.

Note

Do not touch the surfaces of the optical filters.

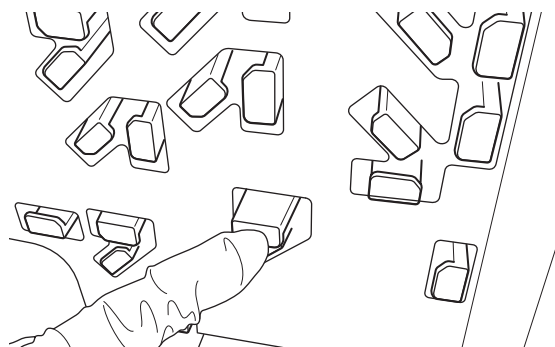


It is recommended that only one optical filter be withdrawn at any one time to ensure that it is returned to the original slot.

- 3 Remove loose dust or particles using an air blower, similar to those used in photography.

Direct the airflow across the surface at an oblique angle. If necessary, gently wipe the surface using a clean, soft, lint-free cloth moistened in anhydrous alcohol (ethanol solution with very low water content). Use a fresh surface of the cloth for each wipe. Maintain a continuous wiping motion at a constant speed until the cloth passes the edge of the optical filter.

- 4 After cleaning, insert the optical filter into the slot and push the lip of the optical filter until it clicks into place.



Notes

- Avoid touching or wiping the coated mirror (metal mirror) surfaces at all times, except when cleaning.
- Avoid handling exposed coatings with bare fingers.
- Always wear biology laboratory gloves and other protective clothing, mask, and goggles to prevent oils from your hands contaminating the optical glass, and to prevent solvents and chemicals coming into contact with your skin or eyes.

Disconnecting and Reconnecting the Fluidics Cart

If you wish to move the fluidics cart after installation, it may be necessary to disconnect the connection lines and tanks, and then reconnect them after moving the fluidics cart.

WARNING

Waste fluid may contain virulent or hazardous substances that may be highly contagious or toxic, depending on the sample under test. When handling waste fluid, always wear biology laboratory gloves and other protective clothing, mask, and goggles.

Notes

- If you wish to move the LE-MA900 main unit, contact your Sony distributor. Do not attempt to move it yourself. The main unit contains high-precision optical and laser instruments that require alignment after installation that only your Sony distributor can provide.
- When handling waste fluid, always wear biology laboratory gloves and other protective clothing, mask, and goggles.

- 1 With the software shut down, turn off the power supply using the MAIN POWER switch on the rear panel of the main unit.

- 2 Close the cap of the sheath tank, and remove the air tube connected to the pressurized sheath tank.

Press and hold the button on the coupling and remove the air tube.

Note

Keep your fingers clear when closing the cap of the sheath tank.

- 3 Move the sheath tank to a location where it will not get in the way.

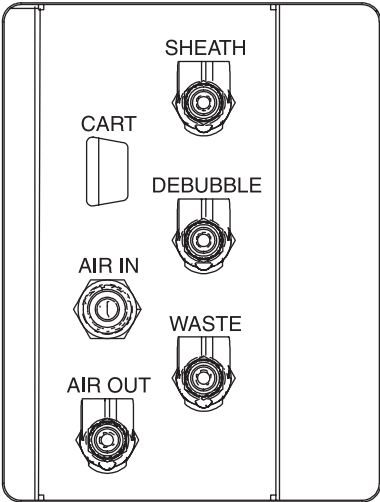
Grasp the handle of the sheath tank when moving it.

Note

When moving the sheath tank, take care not to drop it or place it on your feet.

- 4 Remove the five waste lines connected to the WASTE connector.

Press and hold the button on the coupling and remove the tube.



Note

Do not touch the surfaces of the waste line that come into contact with waste fluid. Take care not to spill any waste fluid.

- 5 Disconnect the cart connection cable.

Unscrew the retaining screws and pull the connector out.



Cart connection cable

- 6 Move the fluidics cart or re-route fluidics lines, as required.

- 7 Reconnect the fluidics lines and cart connection cable,

Insert each connector and push until it locks into place.

Refer to the connection diagram in “*Checking Device Connections*” (page 32) to ensure correct connection.

Note

Check to make sure there is no foreign matter on the connector before connecting it. If there is any foreign matter adhering to the connector, clean it before inserting the connector.

- 8 Turn on the power supply to the main unit.

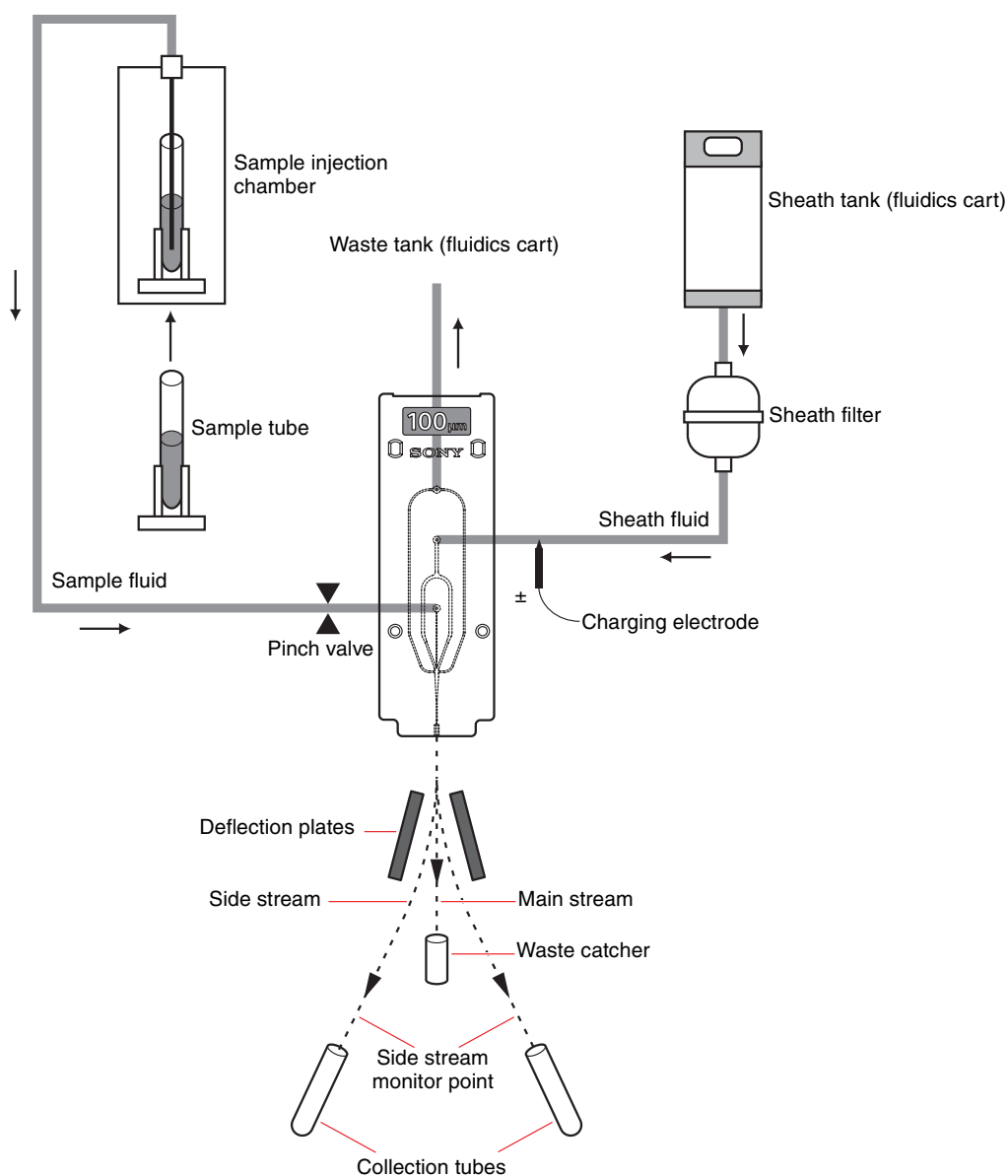
Check for any fluid spills, and clean as necessary.

- 9 Check the operation of the fluid level sensors in the main window in Cell Sorter Software or on the LCD monitor.

Check the cable electrical connection if the status information does not appear.

This chapter describes some of the basic concepts of flow cytometry and cell sorter operating principles of the LE-MA900.

Fluid Flow



Sample Fluid

Sample preparation is an important part of the testing procedure in flow cytometry. Sample fluid must contain cells in a monodispersed suspension that has been filtered using an appropriate filter mesh to remove aggregates. This is required to maintain a consistent sample concentration. If the sample is not filtered, the sample may clog or adhere to the surfaces of fluidics components, and stable performance cannot be maintained. For more information about sample preparation, refer to reference sources.

Before running samples, check that the sorting chip nozzle size is appropriate for the size of cells in the sample fluid. As a generally accepted rule of thumb, the nozzle size should be approximately five times (min) the diameter of the largest cell in the sample fluid to maintain stable sample fluid flow without clogging the chip.

The standard sorting chip has a 100 μm nozzle, which is suitable for most cell sorting applications. Cells ranging from approximately 1 μm to 20 μm in diameter can be analyzed and sorted.

Note

The size of the sorting chip nozzle affects the droplet size, the gap between droplets, and the consumption of sample fluid and sheath fluid.

Sample fluid can be loaded in 0.5 ml micro tubes, 1.5 ml micro tubes, 5 ml round tubes or 15 ml conical tubes for use with the LE-MA900. Each type of tube fits into a corresponding tube holder for the sample tube. The tube holder all fit into a D-cut slot in the base of the sample loader.

After the sample is loaded and [Start] is clicked in Cell Sorter Software, the sample door closes and the sample tube containing the sample is raised into the injection chamber. The chamber is hermetically sealed and then pressurized using regulated compressed air. The pressure inside the injection chamber forces the sample fluid up through the sample probe and into the sample line connected to the probe at the top of the chamber. Sample fluid flows through the sample line and into the sample fluid inlet port on the front of the sorting chip, via a pinch valve which opens/closes the sample line to control the sample flow.

The sample fluid flow rate is controlled by the [Sample Pressure] setting on the main window in Cell Sorter Software. The sample pressure determines the speed of the sample flow, which indirectly controls the distance between cells in the sample fluid flow as they are injected into the sheath fluid in the sorting chip.

An agitation unit agitates the sample fluid during testing to ensure a uniform sample concentration. Agitation on/off and speed are controlled from Cell Sorter Software.

Sheath Fluid

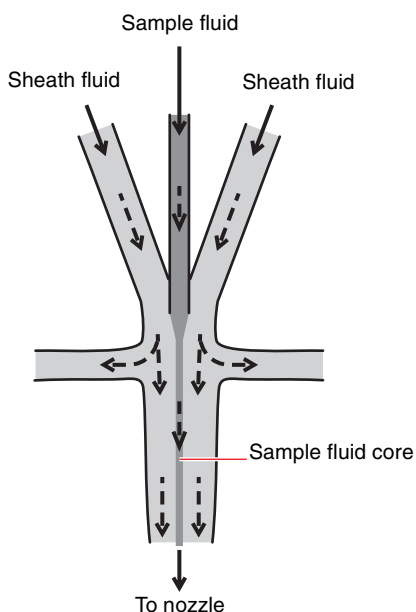
Sheath fluid is supplied from the sheath tank in the fluidics cart. A level sensor in the fluidics cart monitors the level in the tank. The sensor output is displayed on the LCD monitor and on the bottom right of the main window in Cell Sorter Software. A warning is displayed if the level gets close to empty. The tank is pressurized using regulated compressed air, which is used to force the sheath fluid through the fluidics system components at constant pressure, avoiding the pressure fluctuation problems common to pumps.

When [Start] is clicked in Cell Sorter Software to begin sorting, sheath fluid is forced under pressure through the sheath filter and into the sheath fluid inlet port on the front of the sorting chip. A charging electrode mounted near the inlet port is used to electrostatically charge the sheath fluid for sorting.

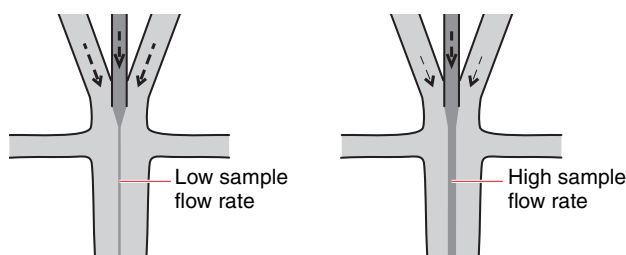
The sheath fluid flow rate is controlled by the [Sheath Pressure] setting in Cell Sorter Software. The sheath pressure determines the speed of the sheath fluid flow through the sorting chip.

Hydrodynamic Focusing

Sample fluid and sheath fluid are forced into the sorting chip using air pressure. The sample fluid is injected into the center of the sheath fluid stream, hydrodynamically focusing the sample fluid within the sheath fluid, according to the principles of fluid dynamics. The internal micro-channel structure of the sorting chip encases the sample fluid within the sheath fluid to ensure high-sensitivity, stable optical detection characteristics.



The relative difference in pressure between the sheath fluid flow and the sample fluid flow forces the diameter of the sample fluid core to vary. When the sample pressure is lowered, the diameter of the sample fluid core decreases, “focusing” the sample fluid into a narrow core stream. When the sample pressure is raised, the diameter increases with a corresponding increase in the sample event detection rate.

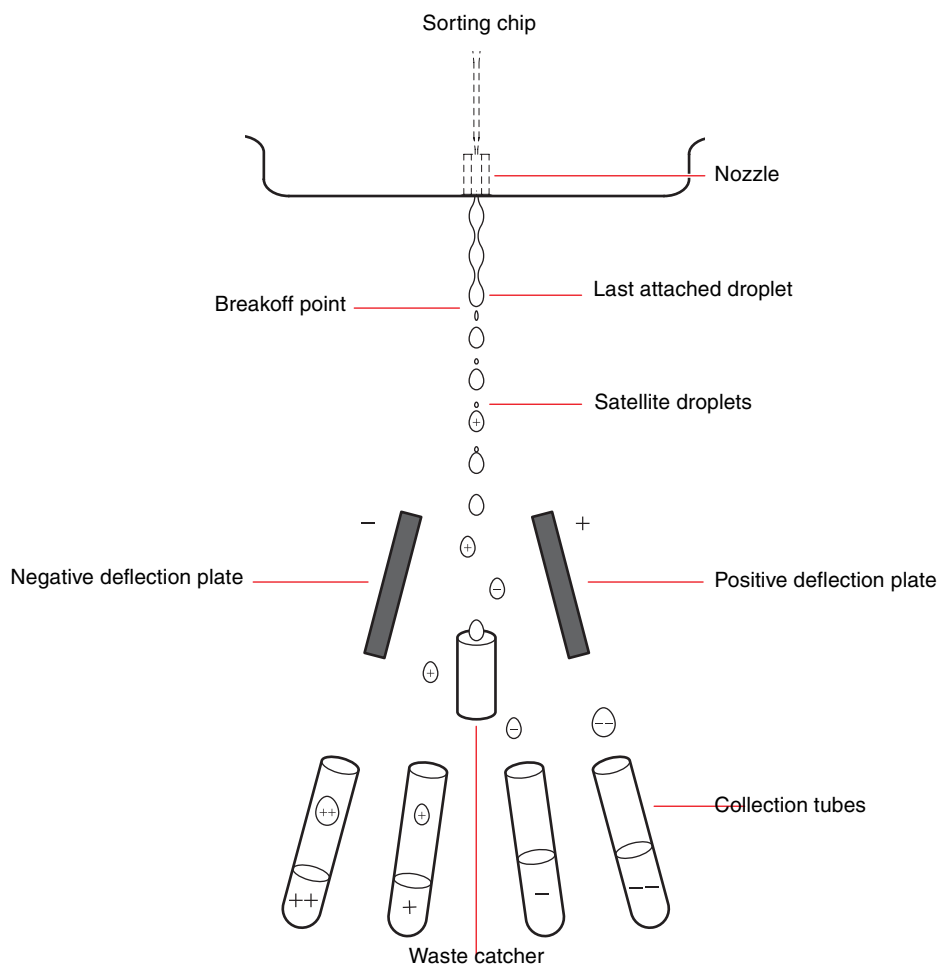


The flow of sample cells encased in sheath fluid passes the optical detection point where the fluid core is interrogated by up to four lasers of different wavelengths.

The highest measurement resolution is obtained when the sample fluid flow is focused into a single-file stream of cells moving past the optical detection point at low sample flow pressure, maximizing the exposure to excitation laser energy that each cell receives.

The diameter of the channel in the sorting chip narrows as it approaches the nozzle at the bottom of the chip, increasing the flow speed while maintaining the relative proportion between the sheath and sample fluid. Steady fluid flow ejected from the nozzle is critical for accurate sorting, hence vibrations of the main unit or the sheath line on the rear panel must be avoided during sorting.

Droplet Sorting



When the sorting chip is inserted in the chip loader, a transducer causes the chip to vibrate at an ultrasonic frequency as fluid flows through the chip. The vibrations cause the fluid flow to be ejected in a jet of uniform droplets from the nozzle at the bottom of the chip.

At a point a short distance beneath the sorting chip, called the breakoff point, the fluid flow breaks into a very regular stream of uniform droplets. The size of the droplets is determined by the nozzle size, frequency of vibration, and the speed of the fluid flow. The amplitude of vibration (drop drive voltage) controls the position of the breakoff point, and the frequency of vibration determines the distance between droplets.

If the last attached droplet satisfies the sorting criteria, the droplet containing the cell receives an electrostatic charge just before the fluid flow reaches the breakoff point from a charging electrode located near the sheath fluid inlet on the sorting chip. The last attached droplet retains the electrical charge after it breaks off from the fluid flow. The electrostatic charge is distributed on the periphery of the droplet and does not affect any sample cells contained within the droplet.

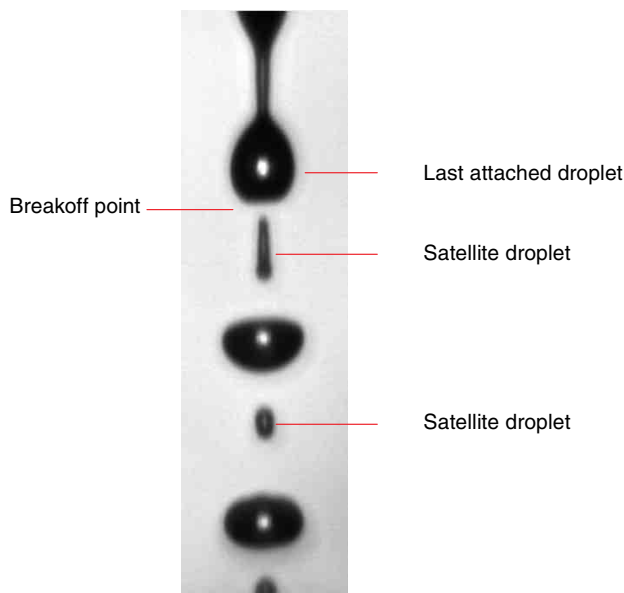
The polarity (positive or negative) of the charge applied to a droplet depends on the cell sorting criteria defined by the characteristics of the cell populations that you want to isolate for sorting using gates specified in Cell Sorter Software. This information is then used to drive the charging pulse for sorting. Unwanted droplets are left uncharged and are collected by the waste catcher.

In an ideal sort, all cells travel pass the optical detection point in single file, and are sorted one cell at a time in separate droplets, with an interval between droplets. In practice, however, cells can appear very close together at the optical detection point, which means they may be found within the same droplet or in adjacent droplets. The sorting mode selection in Cell Sorter Software determines whether to sort or abort droplets whenever these “coincidences” occur.

See “Sorting Mode” (page 108).

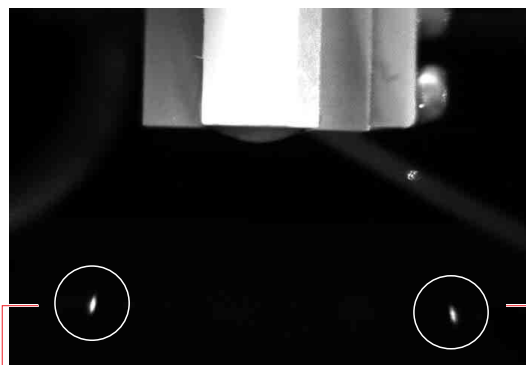
The time taken for droplets to traverse the distance between laser interrogation at the optical detection point and the breakoff point is called the sort delay. The sort delay controls the timing of the electrostatic charging of the target droplet for stable sorting.

The droplet camera monitors the droplet stream before and after the breakoff point. The stroboscopic output image of the droplet camera is displayed in the bottom of the main window in Cell Sorter Software. The output image is also used by control circuits to automatically adjust the amplitude and frequency of vibration of the sorting chip to control the position of the breakoff point and the timing of the electrostatic charging pulse. Manual droplet control is also supported.



High-voltage deflection plates on either side of the droplet stream deflect the charged droplets, using electrostatic attraction and repulsion, to the left and right as the droplets fall between the deflection plates, sorting the droplets into four side streams (four-way sorting). The polarity of the charge on each droplet determines the direction of the stream (outer left, inner left, inner right, outer right). Uncharged droplets (main stream) are not deflected and fall into the waste catcher. The side streams are directed toward the collection tubes on the collection stage.

The side stream monitor detects the position of the side streams. The side stream laser irradiates the side streams, and an image captured by the side stream camera is displayed at the bottom of the main window in Cell Sorter Software. The output image is also used by control circuits to automatically adjust the angle of deflection of the side streams so that they are directed into the collection tubes. The collection stage supports 15 ml conical tubes and 5 ml round tubes inserted in collection tube holders.



Left side stream

Right side stream

WARNING

The deflection plates are charged at extremely high voltages. Contact with charged deflection plates can result in serious injury or electrical shock. Do not touch the deflection plates while power is connected to the main unit.

The collection area is sealed during operation to prevent contamination of the air by aerosols generated by falling droplets and to prevent air circulation in the room affecting the accuracy of the droplet sorting. Negative pressure (vacuum suction) helps reduce the risks associated with aerosol generation.

WARNING

Waste fluid may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, as required, when handling samples. The handling of waste fluid should be performed in accordance with biological hazard handling safety procedures.

The formation of droplets at the breakoff point and the angular deflection of side streams are calibrated at startup using automatic setup beads.

For details about calibration, see “Running Automatic Calibration” (page 49).

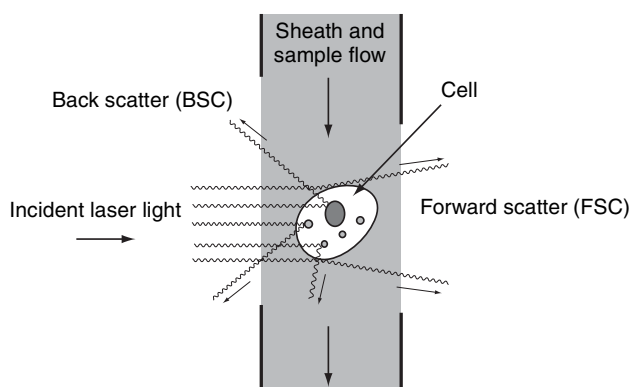
Optical System

This section describes the signals that can be generated using the LE-MA900.

Light and Signal Generation

Light Scatter

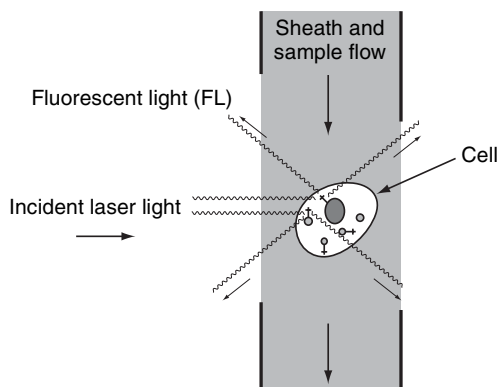
When laser light hits a cell passing the optical detection point in the sorting chip, light is scattered in all directions. Light scattered in the forward direction is called forward scatter (FSC). Light scattered to the sides and back toward the light source is called back scatter (BSC).



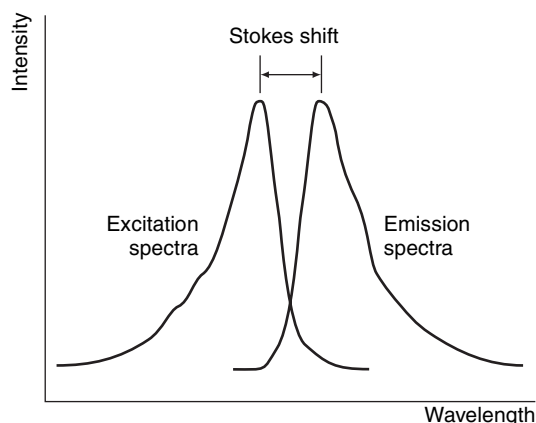
In general terms, forward scatter can provide information about the size of the cell or can indicate the state of living cells. Back scatter can provide information about the internal detail of the cell, indicating complexity, granularity, and irregularities in a cell.

Fluorescence

When laser light hits a cell passing the optical detection point in the sorting chip, the light excitation can cause fluorochromes in molecules or antibodies that have been labeled with fluorescent markers to fluoresce in the sample, producing fluorescent light (FL). Fluorochromes for markers emit high-level fluorescent light when excited by lasers at certain wavelengths and low-level fluorescent light at other wavelengths. The wavelength of the excitation lasers should be selected to match the fluorochromes for the markers.

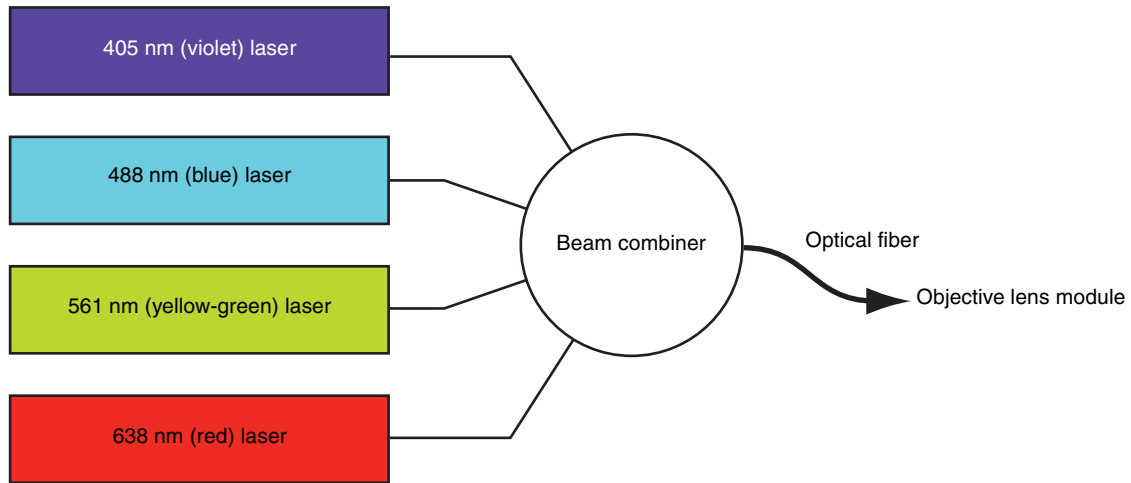


Fluorescence occurs when an electron in a molecule is raised to an excited state as the electron absorbs a photon of the incident excitation light. The electron loses some of this energy before the molecule emits a different photon and the electron returns to its ground state. The emitted photon has less energy than the absorbed photon, so the emitted light has a longer wavelength than the incident light and hence a different color. The difference between the peak excitation wavelength and peak emitted wavelength is called the Stokes shift. Some fluorochromes are available that exhibit a narrow Stokes shift while others exhibit a wide Stokes shift. Multicolor fluorescent light analysis can be used to provide a wealth of structural and other functional information about cells.



Laser Interrogation

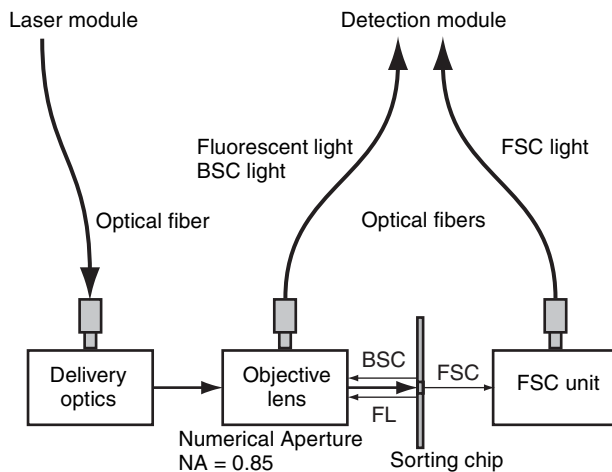
Light source



* 4-laser models

The excitation laser module supplies the laser light used to interrogate individual cells as they flow past the optical detection point. The excitation laser beams are combined into a single collinear beam and coupled to a single-mode fiber to the objective lens module. The excitation laser module uses Automatic Power Control (APC) to provide optical measurement accuracy and reproducibility.

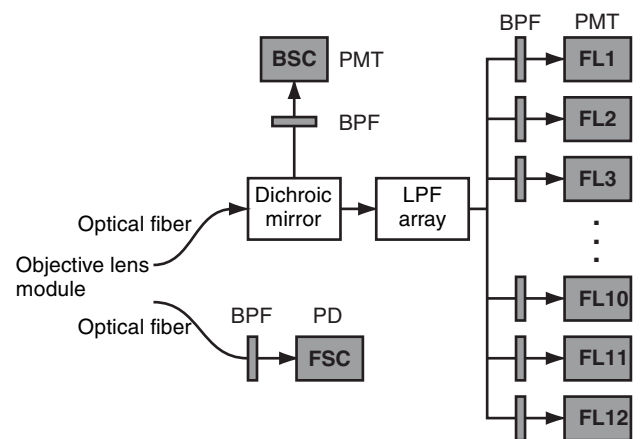
Light collection



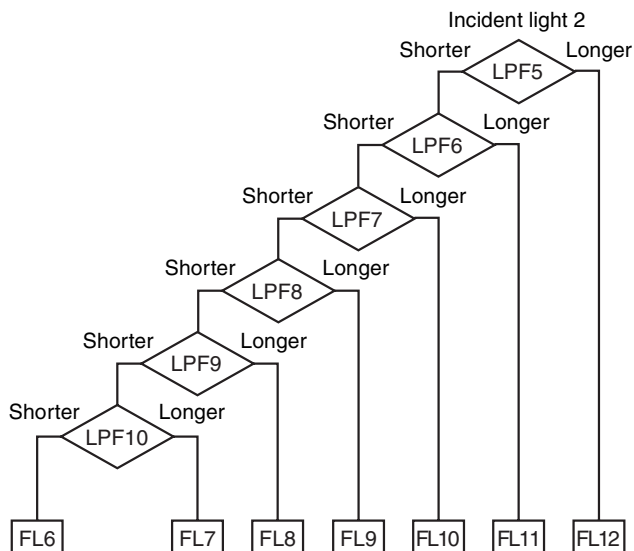
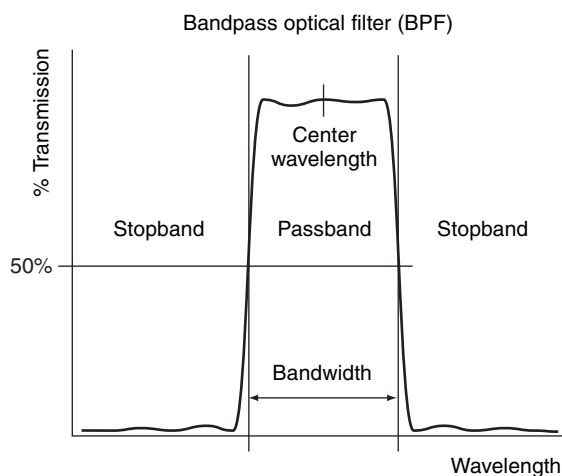
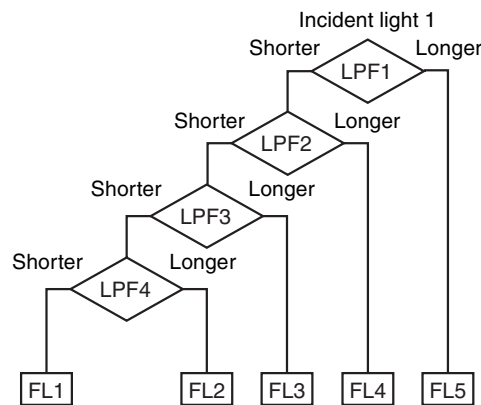
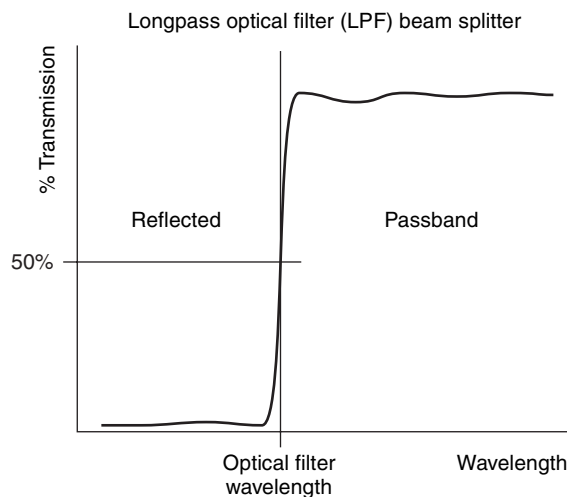
The objective lens module focuses the beam from the four different wavelength lasers separated along two optical paths onto the optical detection point on the sorting chip. There are two spots at the optical detection point. The 488 nm and 561 nm lasers are focused on spot 1, and the 405 nm and 638 nm lasers are focused on spot 2.

Detection

The detection module takes the light collected from the objective lens module and separates it into 14 channels of light, comprising forward scatter light (FSC), back scatter light (BSC), and 12 channels of fluorescent color light (FL1 to FL12). The output from each channel is received by a detector (photodiode (PD) or photomultiplier tube (PMT)) that converts the light energy into an electrical signal for processing by the acquisition module.



The detection module is comprised by an array of dichroic longpass optical filters (LPF) and bandpass optical filters (BPF). Dichroic longpass optical filters reflect light below a certain wavelength and transmit light above a certain wavelength. Bandpass optical filters transmit light in a narrow band of wavelengths and block light of all other wavelengths. For bandpass optical filters, the first number in the name indicates the center wavelength and the second indicates the bandpass width (e.g. 525/50 is a 525 nm bandpass optical filter with 50 nm bandwidth).



Forward scatter light passes through a collimator and a bandpass optical filter (BPF), and is detected by a photodiode (PD). The bandpass optical filter determines the wavelength of the laser source for which forward scatter is measured.

Note

The LE-MA900 is configured by default to measure forward scatter light at a wavelength of 488 nm.

Back scatter light passes through a bandpass optical filter and is detected and amplified by a photomultiplier tube (PMT) detector. The bandpass optical filter determines the wavelength of the laser source for which back scatter is measured.

Note

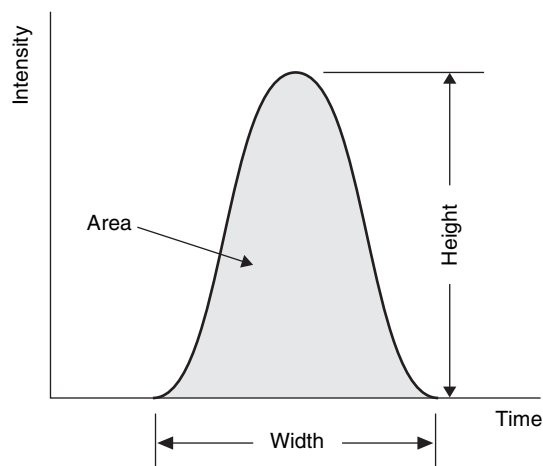
The LE-MA900 is configured by default to measure back scatter light at a wavelength of 488 nm.

Fluorescent lights passes through an array of longpass optical filters (LPFs), separating the fluorescent light into individual fluorescent channels. Each color then passes through a bandpass optical filter and is detected and amplified by a PMT detector.

The LE-MA900 can detect 12 channels of fluorescent light simultaneously. The laser configuration and the optical filter pattern determine the fluorescent markers that can be detected.

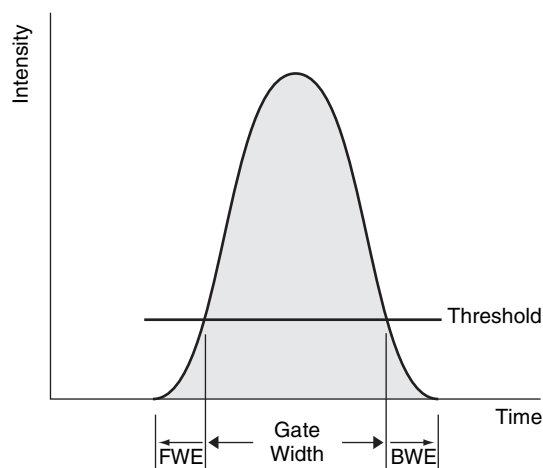
For a list of fluorochromes that can be detected for each model, see “Fluorochrome Detection Matrix” (page 218).

The output from the photodiode and PMT detectors is passed to the acquisition module. The pulse shape that is detected by each detector varies depending on the size and type of cell, and the type of light being collected. The LE-MA900 measures three parameters per pulse (area, height, and width) to provide a wealth of information about cells. The width of the pulse provides an indication of the size of a cell, the height of the pulse provides an indication of the peak fluorescence intensity (brightness) of the cell, and the area provides an indication of the total fluorescent material contained in the cell. The pulse parameters can be selected independently for each channel.



When sorting or analyzing cells, you can set a threshold level for the pulse height for one of the channels (BSC, FSC, FL1 to FL12) to discriminate between real events and background noise. The threshold level for a channel sets the minimum light detector output that is required to record an event. When the trigger is set, data is acquired only for events for which the detector output exceeds the threshold level. Values below the threshold level are ignored. The FSC channel threshold level is the most commonly used trigger, as this channel is less susceptible to the effects of background noise.

The pulse at the threshold level can also be effectively widened to increase the region of the pulse included in the detected signal. This is achieved by adding pulse data that was digitized just before the pulse rises above the threshold value and just after the pulse falls below the threshold value. These two additional components are called forward window extension (FWE) and back window extension (BWE), respectively. Window extensions are used to provide more accurate measurements of the area pulse parameter. When the window extensions are set to zero, only the area above the threshold level is included in the area calculation. By setting window extensions before and after the threshold crossing points, a more accurate determination of the total area under the pulse can be achieved.



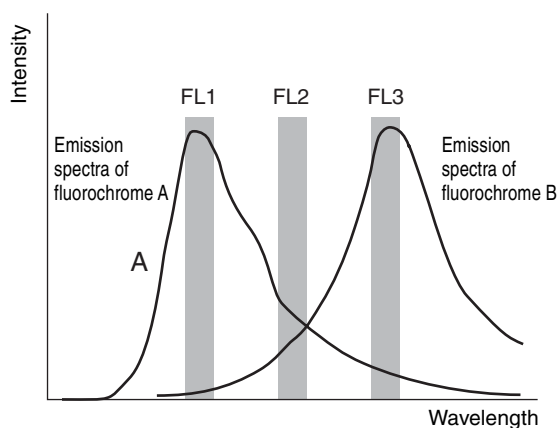
The optimum length of the window extensions depends on the rise height and width of the pulse. Hence, the

optimum window extension will vary from pulse to pulse within the same sample, and from experiment to experiment. If the window extensions are too narrow, the measurement for the area pulse parameter will be smaller than its real value. If the window extensions are too wide, then the window extension may begin to overlap with other pulses, degrading the calculation.

When a cell is detected during sorting, it is generally not possible to know with great accuracy if the signal represents the passage of a large single cell, small multiple cells adhering to one another, or other non-cellular material. Accordingly, it is more common to refer to each signal detection not as a cell, but as an event.

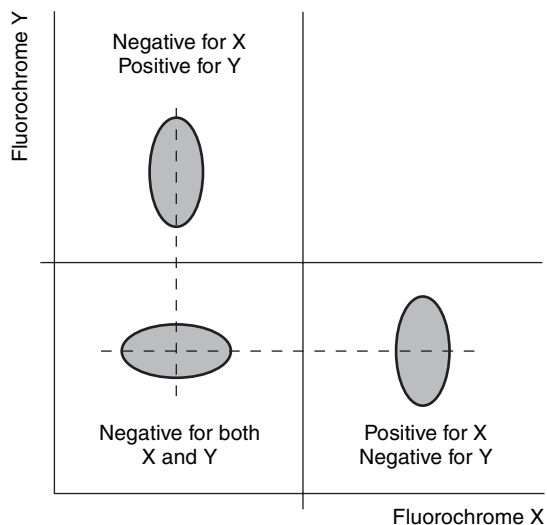
Fluorescence Compensation

When detecting the emitted fluorescent light for a fluorochrome, components of the emitted light can appear in one or more of the fluorescent light detectors (FL1 to FL12) due to the wide emission spectrum of each fluorochrome. When detecting the emitted light for more than one fluorochrome, components of the spectrum of all fluorochromes can appear in multiple detectors, a phenomenon called spillover. The problem then becomes one of working out the proportion of the light detected in each detector due to the individual fluorochromes. For example, in the following diagram, the output of detector FL1 is almost totally due to the spectra of fluorochrome A. The output of detector FL3 is predominantly due to the spectra of fluorochrome B, but it does contain a small component (approximately 10%) due to fluorochrome A. However, the output of FL2 contains significant components from the spectra of both fluorochromes A and B. This problem increases considerably as the number of fluorochromes being simultaneously detected increases.



Fluorescence compensation is the process of calculating the components of the detected signals due to each fluorochrome in the sample and subtracting the “unwanted” components from each channel. This converts the channel detector output from signals of fluorescence intensity at a given wavelength to signals of fluorescence intensity due to each fluorochrome.

Compensation adjusts the mean value of fluorescence intensity of populations that are not stained with a particular fluorochrome to the mean value of a negative control sample, which by definition is not stained with any fluorochromes. When compensated, the populations effectively appear aligned into quadrants on 2D plots.



Notes

- The visual representation of the compensated data on a 2D plot will vary depending on the axis type (log, linear, or biexponential).
- The negative and positive populations may or may not appear as diverse populations on 2D plots, depending on the type of cells in the sample and the selected fluorochromes. For example, if samples contain cells that are actively acquiring or losing the antigen with the fluorochrome marker, the two populations may tend to merge into one another.
- Cell Sorter Software also supports manual specification of the fluorescent compensation parameters. See “*Adjusting the Spillover Matrix Manually*” (page 90).

Series Lineup

The LE-MA900 cell sorter is available in six different models equipped with a combination of one, two, three, or four excitation lasers of different wavelengths. The models are indicated by a two-letter suffix (AC to FC).

LE-MA900

Model	No. of lasers	Laser wavelengths (nm)
LE-MA900B	2	488, 638
LE-MA900C	2	405, 488
LE-MA900E	3	405, 488, 638
LE-MA900F	4	405, 488, 638, 561
LE-MA900G	3	405, 488, 561
LE-MA900H	3	488, 638, 561

LE-MA900P

Model	No. of lasers	Laser wavelengths (nm)
LE-MA900BP	2	488, 638
LE-MA900CP	2	405, 488
LE-MA900EP	3	405, 488, 638
LE-MA900FP	4	405, 488, 638, 561
LE-MA900GP	3	405, 488, 561
LE-MA900HP	3	488, 638, 561

- 488 nm blue laser (standard)
- 405 nm violet laser
- 561 nm yellow-green laser
- 638 nm red laser

The fluorochromes that can be excited and the resulting emission spectra that can be detected by each laser configuration depend on the wavelength of the lasers and the optical filter pattern. The fluorochrome detection matrix for each laser configuration is given in the “*Fluorochrome Detection Matrix*” (page 218).

Tip

On multi-laser models, you specify the lasers used for experiments when Cell Sorter Software launches.

Notes

- Do not use the 405 nm laser for extended periods of time. Extended use can damage the sorting chip.

- The use of optical adjustment procedures not specified in this document or installation/removal of optical components may result in hazardous radiation exposure. Do not remove or otherwise modify the excitation laser or detection module.

Optical Filter Patterns

The detection module takes the scattered and fluorescent light collected from cells and separates it into 14 channels of light, comprising the forward scatter channel, back scatter channel, and 12 channels of fluorescent light of various wavelengths.

The LE-MA900 uses a combination of longpass optical filters (in slots labeled LP1 to LP10) and bandpass optical filters (in slots labeled BSC, and FL1 to FL12).

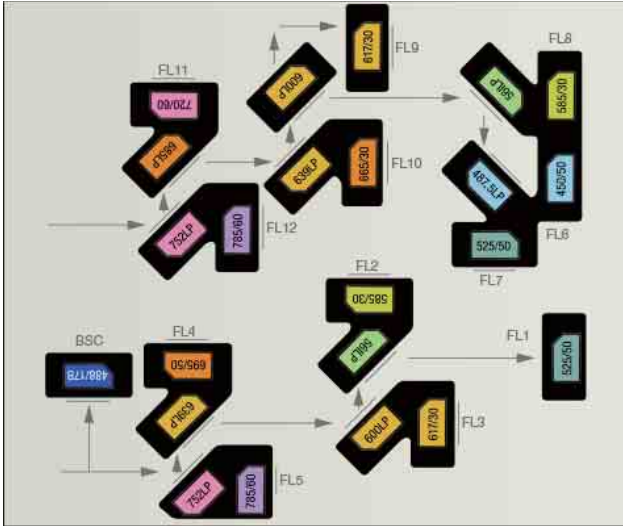
The LE-MA900 uses the following standard optical filter patterns.

Optical filters	Filter characteristics
LP1	752LP
LP2	639LP
LP3	600LP
LP4	561LP
LP5	752LP
LP6	685LP
LP7	639LP
LP8	600LP
LP9	561LP
LP10	487.5LP
BSC	488/17B
FL1	525/50
FL2	585/30
FL3	617/30
FL4	695/50
FL5	785/60
FL6	450/50
FL7	525/50
FL8	585/30
FL9	617/30
FL10	665/30
FL11	720/60
FL12	785/60

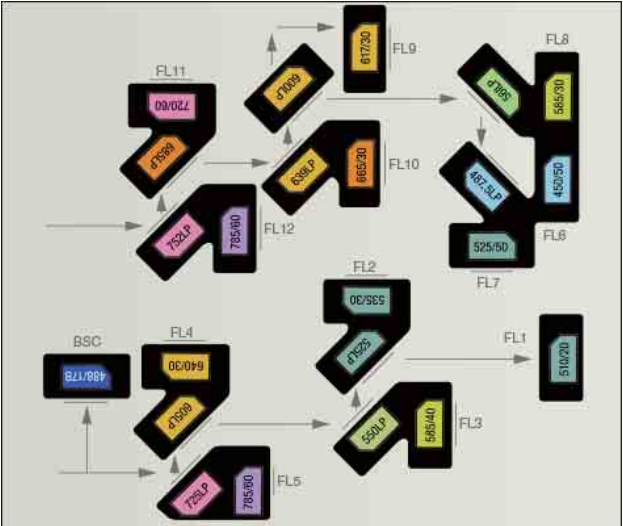
Tips

- On the following models, LP8 to 10 and FL6 to 9 filters are not included, and dummy filters are installed in the RESERVE slots.
- MA900B, MA900H, MA900BP, MA900HP
- The LP1 to LP10 longpass optical filters are labeled with the wavelength rating of the optical filter and an “LP” suffix (e.g. “639LP” for 639 nm longpass optical filter). The FL1 to FL12 bandpass optical filters are labeled with the center wavelength of the optical filter and the bandwidth of the optical filter (e.g. “525/50” for 525 nm bandpass optical filter 50 nm bandwidth). The BSC bandpass optical filter label has a “B” suffix.

Optical Filter Pattern



Fluorescent Protein Optical Filter Pattern (Option)



Optical filters	Filter characteristics
LP1	725LP
LP2	605LP
LP3	550LP
LP4	525LP
LP5	752LP
LP6	685LP
LP7	639LP
LP8	600LP
LP9	561LP
LP10	487.5LP
BSC	488/17B
FL1	510/20
FL2	535/30
FL3	585/40

Optical filters	Filter characteristics
FL4	640/30
FL5	785/60
FL6	450/50
FL7	525/50
FL8	585/30
FL9	617/30
FL10	665/30
FL11	720/60
FL12	785/60

Note

The filter characteristic of the LP1 filter is 725LP. Do not confuse it with the 752LP characteristic of the standard filter pattern.

Fluorochrome Detection Matrix

1 laser (488 nm)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)

2 lasers (488 and 638 nm lasers)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
638 nm laser	APC (Allophycocyanin)	660	FL10 (665/30)
	Cy5	670	FL10 (665/30)
	Alexa Fluor 647	668	FL10 (665/30)
	APC-Cy5.5	695	FL11 (720/60)
	Alexa Fluor 700	719	FL11 (720/60)
	APC-Cy7	779	FL12 (785/60)
	APC-Alexa Fluor 750	775	FL12 (785/60)
	APC-Fire 750	750	FL12 (785/60)

2 lasers (405 and 488 nm lasers)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL6 (450/50)
	Alexa Fluor 405	421	FL6 (450/50)
	DAPI	455	FL6 (450/50)
	Pacific Blue	455	FL6 (450/50)
	mCFP	475	FL6 (450/50)
	Hoechst 33342	483	FL6 (450/50)
	AmCyan1	491	FL7 (525/50)
	T-Sapphire	511	FL7 (525/50)
	Qdot 525	525	FL7 (525/50)
	Qdot 545	545	FL7 (525/50)
	Pacific Orange	551	FL7 (525/50)
	Brilliant Violet 570	570	FL8 (585/30)
	Qdot 585	588	FL8 (585/30)
	Qdot 605	603	FL9 (617/30)
	Qdot 655	654	FL10 (665/30)
	Qdot 705	705	FL11 (720/60)
	Qdot 800	800	FL12 (785/60)
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)

3 lasers (405, 488, and 638 nm lasers)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL6 (450/50)
	Alexa Fluor 405	421	FL6 (450/50)
	DAPI	455	FL6 (450/50)
	Pacific Blue	455	FL6 (450/50)
	mCFP	475	FL6 (450/50)
	Hoechst 33342	483	FL6 (450/50)
	AmCyan1	491	FL7 (525/50)
	Brilliant Violet 510	510	FL7 (525/50)
	T-Sapphire	511	FL7 (525/50)
	Qdot 525	525	FL7 (525/50)
	Qdot 545	545	FL7 (525/50)
	Pacific Orange	551	FL7 (525/50)
	Brilliant Violet 570	570	FL8 (585/30)
	Qdot 585	588	FL8 (585/30)
	Qdot 605	603	FL9 (617/30)
	Brilliant Violet 605	605	FL9 (617/30)
	Brilliant Violet 650	650	FL10 (665/30)
	Qdot 655	654	FL10 (665/30)
	Qdot 705	705	FL11 (720/60)
	Brilliant Violet 711	711	FL11 (720/60)
	Brilliant Violet 785	785	FL12 (785/60)
	Qdot 800	800	FL12 (785/60)
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	SYBR Green I	522	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	Venus	528	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
638 nm laser	APC (Allophycocyanin)	660	FL10 (665/30)
	Cy5	670	FL10 (665/30)
	Alexa Fluor 647	668	FL10 (665/30)
	APC-Cy5.5	695	FL11 (720/60)
	Alexa Fluor 700	719	FL11 (720/60)
	APC-Cy7	779	FL12 (785/60)
	APC-Alexa Fluor 750	775	FL12 (785/60)
	APC-Fire 750	780	FL12 (785/60)

3 lasers (405, 488, and 561 nm lasers)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL6 (450/50)
	Alexa Fluor 405	421	FL6 (450/50)
	DAPI	455	FL6 (450/50)
	Pacific Blue	455	FL6 (450/50)
	mCFP	475	FL6 (450/50)
	Hoechst 33342	483	FL6 (450/50)
	AmCyan1	491	FL7 (525/50)
	Brilliant Violet 510	510	FL7 (525/50)
	T-Sapphire	511	FL7 (525/50)
	Qdot 525	525	FL7 (525/50)
	Qdot 545	545	FL7 (525/50)
	Pacific Orange	551	FL8 (585/30)
	Brilliant Violet 570	570	FL8 (585/30)
	Qdot 585	588	FL9 (617/30)
	Qdot 605	603	FL9 (617/30)
	Brilliant Violet 605	605	FL10 (665/30)
	Brilliant Violet 650	650	FL10 (665/30)
	Qdot 655	654	FL11 (720/60)
	Qdot 705	705	FL11 (720/60)
	Brilliant Violet 711	711	FL12 (785/60)
	Brilliant Violet 785	785	FL12 (785/60)
	Qdot 800	800	FL12 (785/60)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	SYBR Green I	522	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	Venus	528	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)
561 nm laser	mOrange	562	FL2 (585/30)
	DsRed-Monomer	586	FL2 (585/30)
	tdTomato	581	FL2 (585/30)
	mCherry	610	FL3 (617/30)
	Alexa Fluor 594	618	FL3 (617/30)
	mPlum	649	FL4 (695/50)

3 lasers (488, 561, and 638 nm lasers)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	SYBR Green I	522	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	Venus	528	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
561 nm laser	mOrange	562	FL2 (585/30)
	DsRed-Monomer	586	FL2 (585/30)
	tdTomato	581	FL2 (585/30)
	mCherry	610	FL3 (617/30)
	Alexa Fluor 594	618	FL3 (617/30)
	mPlum	649	FL4 (695/50)
638 nm laser	APC (Allophycocyanin)	660	FL10 (665/30)
	Cy5	670	FL10 (665/30)
	Alexa Fluor 647	668	FL10 (665/30)
	APC-Cy5.5	695	FL11 (720/60)
	Alexa Fluor 700	719	FL11 (720/60)
	APC-Cy7	779	FL12 (785/60)
	APC-Alexa Fluor 750	775	FL12 (785/60)
	APC-Fire 750	780	FL12 (785/60)

4 lasers (405, 488, 561, and 638 nm lasers)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL6 (450/50)
	Alexa Fluor 405	421	FL6 (450/50)
	DAPI	455	FL6 (450/50)
	Pacific Blue	455	FL6 (450/50)
	mCFP	475	FL6 (450/50)
	Hoechst 33342	483	FL6 (450/50)
	AmCyan1	491	FL7 (525/50)
	Brilliant Violet 510	510	FL7 (525/50)
	T-Sapphire	511	FL7 (525/50)
	Qdot 525	525	FL7 (525/50)
	Qdot 545	545	FL7 (525/50)
	Pacific Orange	551	FL7 (525/50)
	Brilliant Violet 570	570	FL8 (585/30)
	Qdot 585	588	FL8 (585/30)
	Qdot 605	603	FL9 (617/30)
	Brilliant Violet 605	605	FL9 (617/30)
	Brilliant Violet 650	650	FL10 (665/30)
	Qdot 655	654	FL10 (665/30)
	Qdot 705	705	FL11 (720/60)
	Brilliant Violet 711	711	FL11 (720/60)
	Brilliant Violet 785	785	FL12 (785/60)
	Qdot 800	800	FL12 (785/60)

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	SYBR Green I	522	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	Venus	528	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (695/50)
	PE-Cy5	670	FL4 (695/50)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (695/50)
	PE-Cy5.5	695	FL4 (695/50)
	PerCP-Cy5.5	695	FL4 (695/50)
	PerCP-eFluor 710	710	FL4 (695/50)
	PE-Cy7	779	FL5 (785/60)
561 nm laser	mOrange	562	FL2 (585/30)
	DsRed-Monomer	586	FL2 (585/30)
	tdTomato	581	FL2 (585/30)
	mCherry	610	FL3 (617/30)
	Alexa Fluor 594	618	FL3 (617/30)
	mPlum	649	FL4 (695/50)
638 nm laser	APC (Allophycocyanin)	660	FL10 (665/30)
	Cy5	670	FL10 (665/30)
	Alexa Fluor 647	668	FL10 (665/30)
	APC-Cy5.5	695	FL11 (720/60)
	Alexa Fluor 700	719	FL11 (720/60)
	APC-Cy7	779	FL12 (785/60)
	APC-Alexa Fluor 750	775	FL12 (785/60)
	APC-Fire 750	780	FL12 (785/60)

Appendix B Miscellaneous

[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
Event rate (eps)	Sample concentration (1 × 10 ⁶ /ml)	60	160	260	350	450	650	840	1,000	1,200	1,600
	Sample concentration (1 × 10 ⁷ /ml)	600	1,300	2,100	2,800	3,600	5,100	6,600	8,100	9,600	12,600

[Sample Pressure]		1	2	3	4	5	6
Flow rate (μl/min)		8	18	28	42	62	80
Event rate (eps)	Sample concentration (1 × 10 ⁷ /ml)	1,000	2,200	3,500	5,300	7,700	10,000
	Sample concentration (2 × 10 ⁷ /ml)	2,000	4,500	7,000	11,000	15,000	20,000

The values above are reference values. Values may vary depending on the environment.

Troubleshooting

This section lists common problems that may occur and the actions to take to resolve each problem. Check these items before calling for service. If the problem still persists after carrying out the recommended actions, contact your Sony distributor.

Problem	Possible Cause/Recommended Solution
The event rate does not rise during data acquisition, sorting, or adjustment.	<p>The sample line may be clogged. Click [Probe Wash] in the [Cleaning] group on the [Cytometer] tab of the ribbon to clean the sample line. If the problem persists, click [DI Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon to flush the clog.</p> <p>If the problem persists, change the sample line (see <i>“Changing the PEEK Sample Line”</i> (page 175)).</p> <p>After DI Rinse or changing the sample line, run automatic calibration again.</p> <p>Air may be leaking from the sample injection chamber.</p> <p>Tighten the screw caps securely by hand, and clean the sample injection chamber O-rings.</p>
Measurement values cannot be acquired, or sorting performance cannot be maintained.	<p>The sorting chip may be clogged or contain trapped air. Click [Chip] in the [Debubble] group on the [Cytometer] tab of the ribbon several times as required to clear the chip. If the problem persists, click [DI Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon to flush the clog or trapped air. If the problem persists, replace the sorting chip.</p> <p>If the problem persists, the FSC module may require cleaning (see <i>“Clean the FSC module.”</i> (page 188)).</p> <p>After DI Rinse or replacing sorting chip, run automatic calibration again.</p> <p>If the problem persists, the objective module lens may require cleaning. Contact your Sony distributor for service.</p> <p>Air may be leaking from the sample injection chamber.</p> <p>Tighten the screw caps securely by hand, and clean the sample injection chamber O-rings.</p>
Droplet image in droplet viewer shakes up and down, or flows in a curved path.	<p>The sorting chip may be clogged or contain trapped air.</p> <p>If the problem occurs during fluidics check</p> <p>Click [Sample line cleaning], skip [Bleach Cleaning] and run [Water Rinse].</p> <p>If the problem persists, after completing the fluidics check, click [Skip Auto Calibration] and eject and then re-insert the sorting chip using [Chip Exchange] on the [Cytometer] tab of the ribbon.</p> <p>If the problem occurs after automatic calibration</p> <p>Click [Chip Debubble] on the [Cytometer] tab of the ribbon to de-bubble the sorting chip, repeating several times.</p> <p>If the problem persists, run [DI Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon.</p> <p>If the problem still persists, eject and then re-insert the sorting chip using [Chip Exchange] on the [Cytometer] tab of the ribbon.</p> <p>In other cases</p> <p>Check that there is no air leaking from the cap of the sheath tank.</p> <p>On the login screen, click [Maintenance] and run [Ethanol Priming].</p> <p>If the problem persists after repeated attempts, there may be a foreign object in the sorting chip.</p> <p>Replace the sorting chip.</p> <p>If the problem persists, contact your Sony distributor for service.</p>
Shape of droplets is irregular.	<p>The sorting chip may contain trapped air.</p> <p>Click [Chip] in the [Debubble] group on the [Cytometer] tab of the ribbon several times as required to clear the chip.</p> <p>If the problem persists, there may be salt deposits in the flow channels. Log off and restart in maintenance mode (<i>“Running Maintenance Mode”</i> (page 192)).</p> <p>The sorting chip is ejected automatically. Check that the surface of the chip is not dirty or smudged, then perform chip realignment. If the sorting chip is dirty or smudged, replace the chip.</p> <p>The sorting parameters may be set incorrectly. The sorting parameters affect the droplet shape, breakoff point, and electric charge. Increase the amplitude or decrease the frequency to make droplets larger. Decrease the amplitude or increase the frequency to make droplets smaller.</p>

Problem	Possible Cause/Recommended Solution
Sheath fluid is not flowing.	Check the following items. <ul style="list-style-type: none"> • Check that all fluidics connectors on the fluidics cart, main unit, and the cap of the sheath tank are sealed securely. • Check the pressure of the compressed air supply to the main unit. • Check that the sheath line is attached correctly.
Electrical discharge between deflection plates causes sorting errors.	Discharge may occur due to fluid droplets adhering to the surface of the plates. Turn off the power, open the collection area door, and wipe the deflection plates dry. If residue remains on the plates, remove and clean the plates.
The plot position does not change after increasing the gain.	Increasing the gain too much can cause output saturation. In this case, reduce the gain and readjust settings to find the optimum operating point.
Sample tube hits the sample probe.	Use only standard tubes and fully insert the tube in the sample tube holder when loading a sample. An ill-fitting or non-standard tube can damage the sample probe.
Fluid is leaking from the sample probe.	Check that the sample line is fully inserted in the pinch valve.
Fluid is leaking from the top in the collection area.	The sample line connector and chip connector with waste line may not be securely fastened. Fasten securely.
Fluidics component (pump, fluid connectors, etc.) is leaking fluid.	The seals may have deteriorated due to age, causing ethanol or waste fluid to leak. Clean the area, and contact your Sony distributor for service. <p>Note</p> Always wear gloves and other protective clothing, mask, and goggles, as required, when cleaning spills and leakages.
Droplet camera image is erratic.	The droplet camera may require cleaning. Contact your Sony distributor for service.
Droplets are not being formed or the droplet camera image is not displayed.	The droplet camera may be defective. Contact your Sony distributor for service.
Data is not acquired in a given fluorescence detection channel or condition less than favorable.	The optical filters may not be inserted in the correct filter pattern or may require cleaning. Check that the filters are clean and inserted in the correct slots.
Gain settings appear to have deviated during measurement.	The optical filters may have been subjected to static electricity discharge. Pause or stop data acquisition, and then resume data acquisition to restore normal gain settings.
No output from the lasers (laser temperature error).	<ul style="list-style-type: none"> • Check that the front blind cover has been attached correctly. If not attached correctly, the interlock function will prevent the lasers from turning on. • If the ambient operating temperature exceeds the maximum operating temperature of 35 °C (95 °F), the lasers shutdown automatically and an error message is displayed. To clear the error, shut down the main unit and wait for the ambient temperature to cool sufficiently before restarting the main unit. If the error persists even after allowing the ambient temperature to cool below 35 °C (95 °F), contact your Sony distributor for service. • If the ambient temperature drops below the minimum operating temperature of 10 °C (50 °F), the lasers shut down automatically and an error message is displayed. To clear the error, shut down the main unit and wait for the ambient temperature to rise above 10 °C (50 °F) before restarting the main unit. • If the error persists even after the temperature has risen above 10 °C (50 °F), contact your Sony distributor for service.
Waste fluid is not being collected in the waste tank.	Waste fluid may be leaking in the fluidics cart due to an unsecured waste line connector on the waste tank. Make sure the waste line connector is securely connected on the waste tank. Replace the waste tank air filter if more than one month has elapsed since the air filter was last replaced. If the problem persists, contact your Sony distributor for service.
Communication error between the LE-MA900 and the host computer.	Shutdown and restart both the LE-MA900 main unit and the host computer.

Problem	Possible Cause/Recommended Solution
POWER/STANDBY button indicator is flashing.	<p>If the doors can be opened and closed, remove the sample tube and collection tubes before performing the following procedure.</p> <ol style="list-style-type: none"> 1 Press and hold the POWER/STANDBY button until the indicator stops flashing. 2 Press the POWER/STANDBY button again to start the main unit. If the POWER/STANDBY button indicator does not flash, continue normal operation. 3 If the POWER/STANDBY button indicator starts flashing again, press and hold the POWER/STANDBY button until the indicator stops flashing, then turn off the main power switch on the rear panel. 4 Contact your Sony distributor for service.
An error occurs in automatic calibration or automatic calibration stops and does not proceed.	<ul style="list-style-type: none"> • There may be air bubbles trapped in the sorting chip. Remove the air bubbles from the sorting chip. See <i>"Releasing Air in the Sheath Filter and Sorting Chip"</i> (page 199). • If an error occurs while adjusting the side streams, the sensor windows may be dirty, preventing normal side stream detection. Clean the windows using a soft, lint-free cloth lightly moistened with water. Clean the black sensor windows on the left, right, and back. • The temperature within the unit may have changed. With the main unit up and running, leave standing until the room temperature stabilizes, then run automatic calibration again. • If the problem persists, there may be salt deposits in the flow channels. Log off and run the unit in maintenance mode (<i>"Running Maintenance Mode"</i> (page 192)). • If the problem persists, change the sorting chip. • If the problem persists, contact your Sony distributor for service.
Side streams are not visible during automatic calibration.	The transparent protective covers in the collection area may be charged with static electricity. Clean the covers using a soft, lint-free cloth moistened with water. For details, see <i>"Regular Cleaning"</i> (page 180).
The output pressure of the air compressor drops.	Connect the power cord for the air compressor directly to a power outlet. Do not connect using an extension cord.
The waste tank is bulging.	The waste tank air filter may be blocked. Replace the waste tank air filter. See <i>"Changing Tank Air Filters"</i> (page 200).
The instrument will not operate after adding a printer in [Control Panel] on the host computer.	Adding a printer in [Control Panel] while the LE-MA900 main unit and host computer are connected may cause the LE-MA900 main unit to stop functioning. Press and hold the POWER/STANDBY button on the front panel to turn off the power, then turn the power on again and restart Cell Sorter Software. To add a printer, first turn off the LE-MA900 power supply or disconnect the network cable before adding the printer.
The main window in Cell Sorter Software does not update (e.g. the event rate does not update).	If the main window display does not update, click the LE-MA900 icon in the task bar to minimize the main window. Then, click the LE-MA900 icon again to restore the window. If pull-down menus still do not open in the main window, open the [Detector & Threshold Settings] dialog (page 88) and operate a [Threshold] pull-down menu, for example.
Button labels displayed for dialogs on the main window are transparent.	If the dialog display is corrupted, press the ESC key to close the dialog (clicking the [X] icon in the top right corner of the dialog has no effect). Then, reopen the dialog from the main window. If the problem persists, click the LE-MA900 icon in the task bar to minimize the main window and then click it again to restore the window.
The sheath tank pressure does not rise. There is the sound of air escaping from the sheath tank.	<ul style="list-style-type: none"> • The clamp tightening may be insufficient. Tighten the clamp securely. • There may be foreign matter adhering to the sheath tank O-ring. Wipe clean with a soft, lint-free cloth. If the problem persists, replace the O-ring.
The chip connector cannot be removed after unscrewing the retaining screws.	<p>Use the following procedure for removal.</p> <ol style="list-style-type: none"> 1 Retighten the two screws on the chip connector. 2 Remove the two retaining screws only, without removing the chip connector using excessive force. 3 Insert one of the retaining screws into the screw hole between the two screws used to secure the chip connector, and tighten slowly. The chip connector should become loose.

Error Messages

This section lists the error messages that may be displayed in Cell Sorter Software. When an error occurs, check these items for the actions to take to resolve each error. If the problem still persists after carrying out the recommended actions, make a note of the error message and then contact your Sony distributor.

Error message	Solution
Unload Chip Error. Discard and load a new chip. If the error reoccurs, please contact your service representative. (Error ID : 00 - 100)	Shut down the main unit and turn off the compressed air supply. Pull the sample line out from the chip connector, and remove the sorting chip manually.
Load Chip Error. Error on loading mechanism. Please reinsert the chip. If the error reoccurs, please contact your service representative. (Error ID : 00 - 101)	Insert a new sorting chip and try to load the chip again. If the problem persists, contact your Sony distributor for service.
Host Communication Error. 1 Shutdown the cytometer. 2 Make sure the cable is connected correctly and firmly and restart the cytometer and software. If the error reoccurs, please contact your service representative. (Error ID : 00 - 102)	Check the connection between the main unit and the host computer and restart Cell Sorter Software. If the problem persists, contact your Sony distributor for service.

Error message	Solution
<p>Unload Sample tube Error. If the error reoccurs, please contact your service representative. (Error ID : 00 - 104)</p>	<p>Use the following procedure.</p> <ol style="list-style-type: none"> 1 Check that the compressed air supply is supplying rated pressure. 2 Shut down the main unit, and turn off the compressed air supply. 3 Clean or replace the sample loader O-ring. <p>If the problem persists, contact your Sony distributor for service.</p>
<p>Unload Sample tube Error. Failed to reduce the sample pressure. If the error reoccurs, please contact your service representative. (Error ID : 00 - 509)</p>	
<p>Unload Sample tube Error. Failed to down the sample loader. If the error reoccurs, please contact your service representative. (Error ID : 00 - 506)</p>	
<p>Unload Sample tube Error. Failed to move the support guide. If the error reoccurs, please contact your service representative. (Error ID : 00 - 507)</p>	
<p>Unload Sample tube Error. Failed to move the sample stage. If the error reoccurs, please contact your service representative. (Error ID : 00 - 508)</p>	
<p>Load Sample tube Error. Please reload the tube. If the error reoccurs, please contact your service representative. (Error ID : 00 - 105)</p>	
<p>Load Sample tube Error. Failed to move the waste tray. Please reload the tube. If the error reoccurs, please contact your service representative. (Error ID : 00 - 501)</p>	
<p>Load Sample tube Error. Failed to lift up the sample loader. Please reload the tube. If the error reoccurs, please contact your service representative. (Error ID : 00 - 502)</p>	<p>Use the following procedure.</p> <ol style="list-style-type: none"> 1 Check that the compressed air supply is supplying rated pressure. 2 Shut down the main unit, and turn off the compressed air supply. 3 Clean or replace the sample loader O-ring. <p>If the problem persists, contact your Sony distributor for service.</p>
<p>Load Sample tube Error. Failed to move the support guide. Please reload the tube. If the error reoccurs, please contact your service representative. (Error ID : 00 - 503)</p>	
<p>Load Sample tube Error. Failed to move the sample stage. Please reload the tube. If the error reoccurs, please contact your service representative. (Error ID : 00 - 504)</p>	
<p>Sheath Fluid Tank is empty. (Error ID : 00 - 106)</p>	<p>The sheath tank is empty.</p>
<p>Waste Fluid Tank is full. Empty the waste tank following the instructions in the operator's guide. (Error ID : 00 - 107)</p>	<p>Empty the waste tank. <i>See "Emptying/Changing the Waste Tank" (page 168).</i></p>
<p>Compressed air is not supplied. Confirm if the compressed air hose is connected or the sheath tank is set up properly. (Error ID : 00 - 110)</p>	<p>Check that the compressed air supply is supplying rated pressure.</p>

Error message	Solution
Fluidics cart is not connected. Connect the fluidics cart correctly following the instructions in the operator's guide. (Error ID : 00 - 111)	Shut down the main unit, connect or reconnect the cart connection cable between the main unit and the fluidics cart, and then restart. When reconnecting the cable, tighten the connector screws to tighten the connector. If the error occurred during data acquisition, click [Restart] to restart Cell Sorter Software, re-open the experiment, and start data acquisition again.
The ambient temperature exceeds the recommended operating range. Shut down the cytometer. (Error ID : 00 - 112)	The main unit may be overheating. Shut down the main unit, and allow the temperature to fall within the rated range before restarting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.
The temperature of the sample area is too high. (Error ID : 00 - 113)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The temperature of the collection area is too high. (Error ID : 00 - 114)	
Leakage is suspected in the drip tray. (Error ID : 00 - 115)	Open the fluidics maintenance door and check for leakages. When doing so, do not touch anything other than the drip tray. If the problem persists, contact your Sony distributor for service.
The sheath tank on the fluidics cart is not detected. Check that the sheath tank is attached. (Error ID : 00 - 116)	Check that the sheath tank is positioned correctly in the fluidics cart. Check that the connection cable between the main unit and the fluidics cart is connected securely. If the problem persists, contact your Sony distributor for service.
The waste tank on the fluidics cart is not detected. Check that the waste tank is attached. (Error ID : 00 - 117)	Check that the waste tank is empty and is positioned correctly in the fluidics cart. Check that the connection cable between the main unit and the fluidics cart is connected securely. If the problem persists, contact your Sony distributor for service.
The temperature control of the sample area failed. Shut down the cytometer. (Error ID : 00 - 118)	Check that there is no object shielding the base of the main unit. If the problem persists, contact your Sony distributor for service.
The temperature control of the collection area failed. Shut down the cytometer. (Error ID : 00 - 119)	
The laser initialization timed out. (Error ID : 00 - 120)	The main unit may be overheating. Shut down the main unit, and allow the temperature to fall within the rated range before restarting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.

Error message	Solution
System power supply error on the PCT board. (Error ID : 00 - 121)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
AC/DC power supply error on the PCT board. (Error ID : 00 - 122)	
250 V power supply error on the HVC board. (Error ID : 00 - 123)	
+5 V power supply error on the HVC board. (Error ID : 00 - 124)	
-5 V power supply error on the HVC board. (Error ID : 00 - 125)	
-12 V power supply error on the HVC board. (Error ID : 00 - 126)	
Piezo short error on the HVC board. (Error ID : 00 - 127)	
12 V power supply error on the MD1 board. (Error ID : 00 - 128)	
24 V power supply error on the MD1 board. (Error ID : 00 - 129)	
5 V power supply error on the MD1 board. (Error ID : 00 - 130)	
Temperature error from the X axis in the chip stage motor on the MD2 board. (Error ID : 00 - 131)	
Temperature error from the Z axis in the chip stage motor on the MD2 board. (Error ID : 00 - 132)	
Temperature error from the chip load motor on the MD2 board. (Error ID : 00 - 133)	
Temperature error from the collection load motor on the MD2 board. (Error ID : 00 - 134)	
Temperature error from the LDS motor on the MD2 board. (Error ID : 00 - 135)	
12 V power supply error on the MD2 board. (Error ID : 00 - 136)	
24 V power supply error on the MD2 board. (Error ID : 00 - 137)	
5 V power supply error on the MD2 board. (Error ID : 00 - 138)	
12 V power supply error on the MD3 board. (Error ID : 00 - 139)	
24 V power supply error on the MD3 board. (Error ID : 00 - 140)	
5 V power supply error on the MD3 board. (Error ID : 00 - 141)	
Temperature error from the agitation motor on the MD3 board. (Error ID : 00 - 142)	
Pump error on the MD1 board. (Error ID : 00 - 143)	
Temperature error from the droplet camera motor on the MD2 board. (Error ID : 00 - 144)	The main unit may be overheating. Shut down the main unit, and allow the temperature to fall within the rated range before restarting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.

Error message	Solution
Power supply error on the MEC board. (Error ID : 00 - 146)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Error from FAN1 on the MD1 board. (Error ID : 00 - 148)	
Error from FAN2 on the MD1 board. (Error ID : 00 - 149)	
Error from FAN3 on the MD1 board. (Error ID : 00 - 150)	
Error from FAN on the MD3 board. (Error ID : 00 - 151)	
Circuit error on the LD1 board. (Error ID : 00 - 152)	
Circuit error on the LD2 board. (Error ID : 00 - 153)	
Circuit error on the LD3 board. (Error ID : 00 - 154)	
Temperature control of the LD1 failed. (Error ID : 00 - 155)	
Temperature control of the LD2 failed. (Error ID : 00 - 156)	
Temperature control of the LD3 failed. (Error ID : 00 - 157)	
Temperature control of the prism failed. (Error ID : 00 - 158)	
Power supply error for LD. (Error ID : 00 - 159)	
Power supply error for peltier. (Error ID : 00 - 160)	
Thermistor error for LD1. (Error ID : 00 - 161)	Shut down the main unit, then try again. If the problem persists, contact your Sony distributor for service.
Thermistor error for LD2. (Error ID : 00 - 162)	
Thermistor error for LD3. (Error ID : 00 - 163)	
Thermistor error for prism. (Error ID : 00 - 164)	
Temperature error of the 561 nm laser. (Error ID : 00 - 165)	The main unit may be overheating. Shut down the main unit, and allow the temperature to fall within the rated range before restarting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.
Error on the eeprom. (Error ID : 00 - 166)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The ambient temperature is too low. (Error ID : 00 - 167)	The main unit may be too cold. Shut down the main unit, and allow the temperature to rise within the rated range before restarting. If the problem persists at temperatures exceeding 10 °C (50 °F), contact your Sony distributor for service.

Error message	Solution
Communication error on the PCT board. (Error ID : 00 - 168)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Communication error on the MD1 board. (Error ID : 00 - 169)	
Communication error on the MD2 board. (Error ID : 00 - 170)	
Communication error on the MD3 board. (Error ID : 00 - 171)	
Communication error on the CLM board. (Error ID : 00 - 172)	
Communication error on the HVC board. (Error ID : 00 - 173)	
No Reply error on the PCT board. (Error ID : 00 - 174)	
No Reply error on the MD1 board. (Error ID : 00 - 175)	
No Reply error on the MD2 board. (Error ID : 00 - 176)	
No Reply error on the MD3 board. (Error ID : 00 - 177)	
No Reply error on the CLM board. (Error ID : 00 - 178)	
No Reply error on the HVC board. (Error ID : 00 - 179)	
Load Chip Error. Wrong side out. (Error ID : 00 - 180)	Insert a sorting chip in the correct orientation, and try to load the chip again. If the problem persists, contact your Sony distributor for service.
Load Chip Error. Up side down. (Error ID : 00 - 181)	
Load Chip Error. Failed to hold the chip. Please reinsert the chip. (Error ID : 00 - 500)	
Load Chip Error. Failed to detect the chip. Please reinsert the chip. (Error ID : 00 - 182)	
Leakage is suspected in the collection area. (Error ID : 00 - 183)	Search the collection area and check to make sure there is no fluid leakage. If there is fluid leakage, wipe clean and wait a while before restarting. If the problem persists, contact your Sony distributor for service.
Leakage is suspected in the drip tray. Check if there is leakage in the drip tray and clean it off. If the detection will be continued, the cytometer will be disabled in two minutes. (Warning ID : 00 - 245)	Inspect the drip tray and check for any fluid leakages. When doing so, do not touch anything other than the drip tray. If the problem persists, contact your Sony distributor for service.
Leakage is suspected in the collection area. Check if there is leakage in the collection area and clean it off. If the detection will be continued, the cytometer will be disabled in two minutes. (Warning ID : 00 - 246)	Search the collection area and check to make sure there is no fluid leakage. If there is fluid leakage, wipe clean and wait a while before restarting. If the problem persists, contact your Sony distributor for service.
Failed to start feeding sheath fluid. (Error ID : 00-184) Click [Troubleshooting] to begin check the line connection and compressed air supply.	Click [Troubleshooting] to launch the troubleshooting wizard, and follow the on-screen instructions. <i>See "If fluid flow does not start" (page 48).</i>
Failed to fit the chip. (Error ID : 00 - 185)	Insert a sorting chip in the correct orientation, and try to load the chip again. If the problem persists, contact your Sony distributor for service.

Error message	Solution
Failed to release the chip. (Error ID : 00 - 186)	Shut down the main unit and turn off the compressed air supply. Pull the sample line out from the chip connector, and remove the sorting chip manually.
Unload Chip Error. Failed to hold the chip. (Error ID : 00 - 187)	
Unload Chip Error. Failed to release the chip. (Error ID : 00 - 188)	
The model number is invalid. (Error ID : 00 - 189)	
The laser configuration is invalid. (Error ID : 00 - 190)	
The ADC configuration is invalid. (Error ID : 00 - 191)	
Power supply error on the ADC board. (Error ID : 00 - 192)	
Power supply error on the ADC2 board. (Error ID : 00 - 193)	
Timed out to close the sample door. (Error ID : 00 - 194)	
Timed out to control sheath pressure. (Error ID : 00 - 195)	
Flash write error. (Error ID : 00196)	
Collection load motor temperature error on the MD2 board. (Error ID : 00-197)	
Timed out to control sheath pressure. (Error ID : 00 - 198)	
Timed out to control sample pressure. (Error ID : 00 - 199)	
Sheath Fluid Tank is almost empty. (Warning ID : 00 - 200)	The sheath tank is nearly empty. Finish measurement within the specified time.
Waste Fluid Tank is almost full. Empty the waste tank following the instructions in the operator's guide. (Warning ID : 00 - 201)	Empty the waste tank. <i>See "Emptying/Changing the Waste Tank" (page 168).</i>
Collection area door is open. (Warning ID : 00 - 202)	Close the collection area door.
Chip loader flip-up door is open. Close the chip loader flip-up door. (Warning ID : 00 - 203)	Close the flip-up door.
Warning for the 561 nm laser. Chip loader flip-up door is open. Close the chip loader flip-up door. (Warning ID : 00 - 210)	Laser has reached the end of its service life. The laser output does not drop immediately. The laser is still operational, but should be replaced as soon as possible.
Warning for the unsuccessful communication on MD2 board. (Warning ID : 00 - 214)	This warning does not affect automatic calibration operation, although it may appear during automatic calibration. In this case, continue the automatic calibration process.
Warning for the unsuccessful communication on PCT board. (Warning ID : 00 - 212)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Warning for the unsuccessful communication on MD1 board. (Warning ID : 00 - 213)	
Warning for the unsuccessful communication on MD3 board. (Warning ID : 00 - 215)	
Warning for the unsuccessful communication on CLM board. (Warning ID : 00 - 216)	
Warning for the unsuccessful communication on HVC board. (Warning ID : 00 - 217)	

Error message	Solution
LD1 laser power warning. (Warning ID : 00 - 229)	A reduction in laser output power has been detected. Contact your Sony distributor for service.
LD2 laser power warning (Warning ID : 00 - 230)	
LD3 laser power warning (Warning ID : 00 - 231)	
LD4 laser power warning (Warning ID : 00 - 232)	
LD1 laser current warning. (Warning ID : 00 - 233)	A laser error has been detected. Contact your Sony distributor for service.
LD2 laser current warning. (Warning ID : 00 - 234)	
LD3 laser current warning. (Warning ID : 00 - 235)	
The LD1 laser is approaching its end of life expiration. (Warning ID : 00 - 236)	Laser is approaching the end of its service life. The laser output does not drop immediately. The laser is still operational, but should be replaced as soon as possible.
The LD2 laser is approaching its end of life expiration. (Warning ID : 00 - 237)	
The LD3 laser is approaching its end of life expiration. (Warning ID : 00 - 238)	
The LD4 laser is approaching its end of life expiration. (Warning ID : 00 - 239)	
Failed to record LD1 laser total hours of use. (Warning ID : 00 - 240)	Total irradiation time was not recorded correctly. The laser is still operational, but you should contact your Sony distributor for service.
Failed to record LD2 laser total hours of use. (Warning ID : 00 - 241)	
Failed to record LD3 laser total hours of use. (Warning ID : 00 - 242)	
Failed to record LD4 laser total hours of use. (Warning ID : 00 - 243)	
Waste catcher position warning. (Warning ID : 00-247)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The temperature control of the collection area has failed and has turned off. Wait a while before turning on it again. (Warning ID : 00-260)	
Motor position has reached to the limit. (Motor limit over) (Warning ID : 00-261)	
Calculated well position has reached to the limit. These position cannot be updated. (Warning ID : 00-262)	
Power supply error on the LCD board. (Warning ID : 00-263)	An error occurred on the LCD board. The laser is still operational, but you should contact your Sony distributor for service.
The cytometer has been disconnected. Acquiring and sorting has stopped.	Check the connections to the main unit.
Communication to the cytometer has been lost. Exit and relaunch the software.	Check the connections to the main unit and relaunch Cell Sorter Software.
The initial setup did not complete successfully. Exit and relaunch the software.	Exit Cell Sorter Software and try starting again.
Acquiring and sorting has stopped because available free size of Hard Disk Drive is not enough.	Insufficient free space on the hard disk. Delete unnecessary files to increase the free capacity.
The application is already running. This instance will now exit.	Shut down the Cell Sorter Software instance that is already running.
Failed to save settings.	Try saving the settings again.
Parameter count in a selected file is different from current settings.	Select a file with the same number of parameters as the current settings.
A selected file has the wrong format.	Select a file in the correct format.

Error message	Solution
Unable to maintain stable droplet breakoff.	An error occurred when the control breakoff function was turned on. If a droplet formation problem is detected during measurement, acquisition stops. Try running [Sort Calibration]. If the problem persists after running [Sort Calibration] again, contact your Sony distributor for service.
The selected drive is not external storage device. Please select an external storage device.	Specify an external storage device.
The drive is not formatted as NTFS. Please use an NTFS formatted drive.	Use only NTFS-formatted drives.
You do not have write permission for the selected folder. Please add write permission to the folder or select another folder with write permission.	You do not have write access privileges for the selected folder. Change the folder access privileges or specify a folder with write access privileges.
The available disk free space is low.	Insufficient free space on the hard disk. Delete unnecessary files to increase the free capacity.
This chip has been used over the duration limit. Please exchange for a new chip.	Replace the sorting chip.
This chip used more than 24 hours ago. Please use new chip.	
System resources are scarce and may cause the application performance to be worse. Would you like to close unnecessary worksheets?	Close any unnecessary worksheets.
A number of plots are opened and may cause the application performance to be worse. Please close unnecessary plots or worksheets.	
System resources are scarce and may cause the application to shutdown. Would you like to start the recovery process?	Stop data acquisition and measurement, and shut down Cell Sorter Software.
System resources are scarce because of multiple applications/processes running in the background. This may impact the performance of the Cell Sorter Software. Please close unnecessary applications/processes.	Close any unnecessary running applications and processes.
Invalid Biexponential parameters were selected.	Specify valid biexponential parameters.
Cannot assign this tube because available free space on the HDD is insufficient. Please increase the available free space on the HDD.	Cannot assign due to insufficient free space on HDD. Delete unnecessary files to increase the free capacity.
Detected UNKNOWN state. Please check the Cell Sorter.	An unknown problem has been detected. Check the LE-MA900.
Unable to maintain stable droplet breakoff. The measurement will be stopped and Control Breakoff will be disabled.	Data acquisition stops. Check the instrument settings, as required.
Cannot receive droplet camera image continuously. If you have some trouble, please stop acquisition.	The droplet camera image cannot be acquired. If a problem occurs, stop data acquisition.
Cannot receive side stream camera image continuously. If you have some trouble, please stop acquisition.	The side stream monitor image cannot be acquired. If a problem occurs, stop data acquisition.
Cannot make appropriate droplet for sorting.	It is impossible to maintain the droplet shape determined during auto alignment. De-bubble the sorting chip to remove any trapped air. If the problem persists, run [Sort Calibration] again.
Unable to perform ethanol cleaning with full waste tank. Please empty the waste tank and retry.	Cannot start ethanol cleaning due to insufficient free capacity in the waste tank. Empty the waste tank.
Droplet condition has changed. Please re-perform Sort Calibration for stable sorting.	The droplet shape has changed. Run [Sort Calibration] again to restore stable sorting. Use the unit within the specified ambient temperature range. Use only in environments where the ambient temperature does not change suddenly. If the error message is displayed repeatedly, remove the chip using [Chip Exchange], then re-insert the same chip and run [Sort Calibration]. A message indicating that the chip is being used a second time appears, but the chip can be used.

Error message	Solution
Sample flow is paused due to change in droplet condition. Click [Stop] and re-perform Sort Calibration for stable sorting.	The sample flow was paused due to changes in the droplet shape. Click [Stop] and run [Sort Calibration] again to restore stable sorting. If the error message is displayed repeatedly, remove the chip using [Chip Exchange], then re-insert the same chip and run [Sort Calibration]. A message indicating that the chip is being used a second time appears, but the chip can be used.
Sample flow is paused due to change in droplet condition. Click [Stop] and re-perform Sort Calibration for stable sorting. To continue sorting without re-calibration, deselect [Detect Satellite Droplets] under [Settings] - [Advanced Settings].	The sample flow was paused due to changes in the droplet shape. Click [Stop] and run [Sort Calibration] again to restore stable sorting. To continue sorting without running the calibration again, click [Settings] on the [Cytometer] tab of the ribbon to display the [Advanced Settings] dialog, and disable [Detect Satellite Droplets]. If the error message is displayed repeatedly, remove the chip using [Chip Exchange], then re-insert the same chip and run [Sort Calibration]. A message indicating that the chip is being used a second time appears, but the chip can be used. See "[Detect Satellite Droplets]" (page 142).
Failed to initialize the sheath pressure regulator. Sheath pressure is too high. Please restart the cytometer and software. If the error reoccurs, please contact your service representative. (Error ID : 00 - 510)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to initialize the sample pressure regulator. Sample pressure is too high. Please restart the cytometer and software. If the error reoccurs, please contact your service representative. (Error ID : 00 - 511)	
High sheath pressure is detected while initializing the regulator. Please contact your service representative. (Warning ID : 00 - 264)	A pressure value outside of rated values has been detected. The laser is still operational, but you should contact your Sony distributor for service.
High sample pressure is detected while initializing the regulator. Please contact your service representative. (Warning ID : 00 - 265)	
Low sheath pressure is detected while initializing the regulator. Please contact your service representative. (Warning ID : 00 - 268)	
Low sample pressure is detected while initializing the regulator. Please contact your service representative. (Warning ID : 00 - 269)	
Failed to move the waste tray. (Warning ID : 00 - 266)	The waste fluid tray is not operating correctly. Contact your Sony distributor for service.
Failed to agitate the sample tube. (Warning ID : 00 - 267)	The agitation unit of the sample loader is not operating correctly. Contact your Sony distributor for service.
Low chip chuck pressure is detected. Please contact your Sony distributor for service. (Warning ID : 00 - 274)	A pressure value outside of rated values has been detected. The laser is still operational, but you should contact your Sony distributor for service.
High chip chuck pressure is detected. Please contact your Sony distributor for service. (Warning ID : 00 - 275)	
Failed to lock the sample area door. (Warning ID : 276)	The sample area door could not be locked. Check the condition of the sample area door.
Invalid deflection voltage is detected. Check if deflection plate is wet or dirty and clean it off. (Warning ID : 00 - 277)	A voltage error was detected with the deflection plates. Check the deflection plates for any dust or moisture, and wipe clean as necessary. If the problem persists, contact your Sony distributor for service.

Error message	Solution
Sheath tank will be empty if you use automatic bleach/DI cleaning at shutdown. If you want to continue a current sorting, manual bleach/DI cleaning should be execute in the Instrument Cleaning wizard at shutdown. (Warning ID : 278)	The remaining volume of sheath fluid is insufficient if automatic cleaning at shutdown is performed. To continue measurement, perform cleaning manually using the cleaning wizard at shutdown.
Sorting error has been detected. Acquisition was stopped.	A sorting error has occurred. Check that there are no abnormal objects in the collection area. Wait for a short while before restarting sorting. If the problem persists, contact your Sony distributor for service.
Failed to collection load!!	Loading of the collection stage failed. Check that there are no abnormal objects in the collection area. Wait for a short while before loading again. If the problem persists, contact your Sony distributor for service.
Laser output error of the 561nm laser. (Error ID : 00 - 517)	A laser output error has been detected. Contact your Sony distributor for service.
The MD1 board configuration is invalid. (Error ID : 00 - 518)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Emergency stop was activated. Shut down the cytometer. (Error ID : 00 - 519)	The [Emergency Stop] button was pressed. Shut down the main unit.
Chip chuck pressure error. (Error ID : 00 - 520)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Sample door error. (Error ID : 00 - 521)	
Sample door error. (Error ID : 00 - 522)	Shut down the main unit, and check that the FSC module is attached. If not attached, reattach the FSC module and then restart the main unit. If the module is attached, wait a while and then restart. If the problem persists, contact your Sony distributor for service.
The sheath temperature is too high. Please call a service representative. (Error ID : 00 - 523)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The sheath temperature is too high. Please call a service representative. (Error ID : 00 - 524)	
Thermistor error. Please call a service representative. (Error ID : 00 - 525)	
Thermistor error. Please call a service representative. (Error ID : 00 - 526)	
Thermistor error. Please call a service representative. (Error ID : 00 - 527)	
Failed to initialize instrument. (Error ID : E1001)	
No sample events detected in Coarse mode. (Error ID : E1010) Click [Troubleshooting] to clean sample line.	Stable measurement could not be obtained of the automatic setup beads signal. Check the state of the fluidics lines and automatic setup beads, then run auto calibration again. If the problem persists, contact your Sony distributor for service.
No sample events detected in Fine mode. (Error ID : E1011) Click [Troubleshooting] to clean sample line.	
No sample events detected in SuperFine mode. (Error ID : E1012) Click [Troubleshooting] to clean sample line.	
The statistics on the found point is not proper value. (Error ID : E1013)	
The statistics on the found point is not proper value. (Error ID : E1014)	
Failed to calibrate the line offset. Event rate is unstable. (Error ID : E1020)	



Error message	Solution
Failed to initialize instrument. (Error ID : E2000)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to initialize instrument. (Error ID : E2001)	An error occurred when moving the collection stage. Check that there are no abnormal objects in the collection area, then run auto calibration again. If the problem persists, contact your Sony distributor for service.
Failed to initialize instrument. (Error ID : E2003)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to form appropriate droplet pattern. (Error ID : E3000)	A droplet shape suitable for sorting could not be obtained. Check the state of the fluidics lines, then de-bubble the sorting chip. See <i>"Releasing Air in the Sheath Filter and Sorting Chip"</i> (page 199).
Sheath Temperature is unstable. (Error ID : E3200)	Click [Retry], and run again. If the problem persists, shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to calibrate the side stream position. (Error ID : E4000)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to calibrate the side stream position. (Error ID : E4010)	Click [Troubleshooting] to launch the wizard, and follow the on-screen instructions. See <i>"If an error occurs during auto calibration"</i> (page 51).
Failed to calibrate the side stream position. (Error ID : E4020)	
Failed to calibrate the side stream angle. (Error ID : E4050)	
Failed to calibrate the side stream position. Droplet is unstable. (Error ID : E4060)	A droplet shape suitable for sorting could not be obtained. Check the state of the fluidics lines, then de-bubble the sorting chip. See <i>"Releasing Air in the Sheath Filter and Sorting Chip"</i> (page 199).
Failed to calibrate the sort delay. (Error ID : E5000)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to calibrate the sort delay. Event rate is unstable. (Error ID : E5010)	An error occurred during sort delay calibration. Check the state of the fluidics lines and automatic setup beads, then run auto calibration again. If the problem persists, contact your Sony distributor for service.
Failed to calibrate the sort delay. Captured beads image is too bright. (Error ID : E5020)	An error occurred during sort delay calibration. Check the state of the fluidics lines and automatic setup beads, then run auto calibration again. If the same error message appears repeatedly, salt precipitation may have occurred on the lens guard. Shut down the main unit, and clean the lens guard. For details, see <i>"Cleaning the Lens Guard"</i> (page 186).

Error message	Solution
Failed to calibrate the sort delay. Captured beads image is too dim. (Error ID : E5030)	An error occurred during sort delay calibration. Check the state of the fluidics lines and automatic setup beads, then run auto calibration again. If the problem persists, contact your Sony distributor for service.
Failed to calibrate the sort delay. Captured beads image is too bright. (Error ID : E5040)	
Failed to calibrate the sort delay. Captured beads image is too dim. (Error ID : E5050)	
Failed to calibrate the sort delay. Captured beads image is unstable. (Error ID : E5060)	
Failed to calibrate the sort delay. No sample event is detected. (Error ID : E5070)	
Laser Delay Time is too long. (Error ID : E8010)	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Laser Delay Time is too short. (Error ID : E8011)	
Event rate is unstable. (Error ID : E9001)	Stable measurement could not be obtained of the automatic setup beads or the 8-peak beads signal. Check the state of the fluidics lines and beads, then run QC again. If the problem persists, contact your Sony distributor for service.

Optional Accessories

For details about purchasing optional accessories, contact your Sony distributor.

Item	Product Number
Sorting chip (100 µm)	LE-C3210
Sorting chip (70 µm)	LE-C3207
Cleaning chip	LE-C3100
Automatic setup beads	LE-B3001
8-peak beads	LE-AE700522
PEEK sample line	LE-U3F021
Waste tank air filter	LE-U3F011A
Sample loader O-ring	LE-U3M001
Waste catcher O-ring	LE-U3M009
Rubber packing for collection area door	LE-U3M012
Fluorescence protein filter set	LE-U7F005
Splash guard	LE-U3M013
Lens guard	LE-U6F002
Retaining screw	LE-U6F006
Waste catcher	LE-U6F009
96-well PCR plate holder	LE-A3104
384-well plate holder ¹⁾	LE-A3107
384-well PCR adapter	LE-A3108
Probe adapter	LE-U3F023
Sheath filter	LE-U7F004
Sheath tank	LE-U7F001
Waste tank	LE-U7F002
DI water tank kit	LE-U7F003

1) You can use a 384-well plate holder as a 96/384-well plate holder.

Software License

LGPL

This product uses the following LGPL software. The customer is entitled to obtain, modify, and redistribute the source code for the software.

DirectShow.NET

Source codes can be obtained from the “\Others\SourceCode” folder on the supplied installation disc.

However, Sony cannot accept inquiries regarding the content of these source codes.

For license information, refer to the “\Others\License” folder on the supplied installation disc.

Apache License

This product uses the Apache license software, ZXing and Unity.

For license information, refer to the “\Others\License” folder on the supplied installation disc.

Microsoft Public License (Ms-PL)

This product uses the Microsoft Public License (Ms-PL) software, WPF Toolkit, DotNetZip, and Fluent Ribbon Control Suite.

For license information, refer to the “\Others\License” folder on the supplied installation disc.

The MIT License (MIT)

This product uses the MIT License software, “OxyPlot.”

For license information, refer to the \Others\License folder on the supplied installation disc.

Third-party Software

This product uses third-party software Entity Framework, .NET Framework, Visual C++ Redistributable Packages, and Microsoft SQL Server. For license information, refer to the “\Others\License” folder on the supplied installation disc.

Environmental Notices

Disposal of old electrical & electronic equipment (applicable in the European Union and other countries with separate collection systems)



This symbol on the product or on its packaging indicates that this product shall not be treated as household waste. Instead it shall be handed over to the appropriate collection point for the recycling of electrical and electronic equipment. By ensuring that this product is disposed of correctly, you will help to prevent potential negative consequences for the environment and human health, which could be caused by inappropriate waste handling of this product. The recycling of materials will help to conserve natural resources. For more detailed information about recycling of this product, please contact your local Civic Office, your household waste disposal service or the shop where you purchased the product.

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