

SONY®

Spectral Cell Analyzer LE-SP6800A

Quick Start Guide



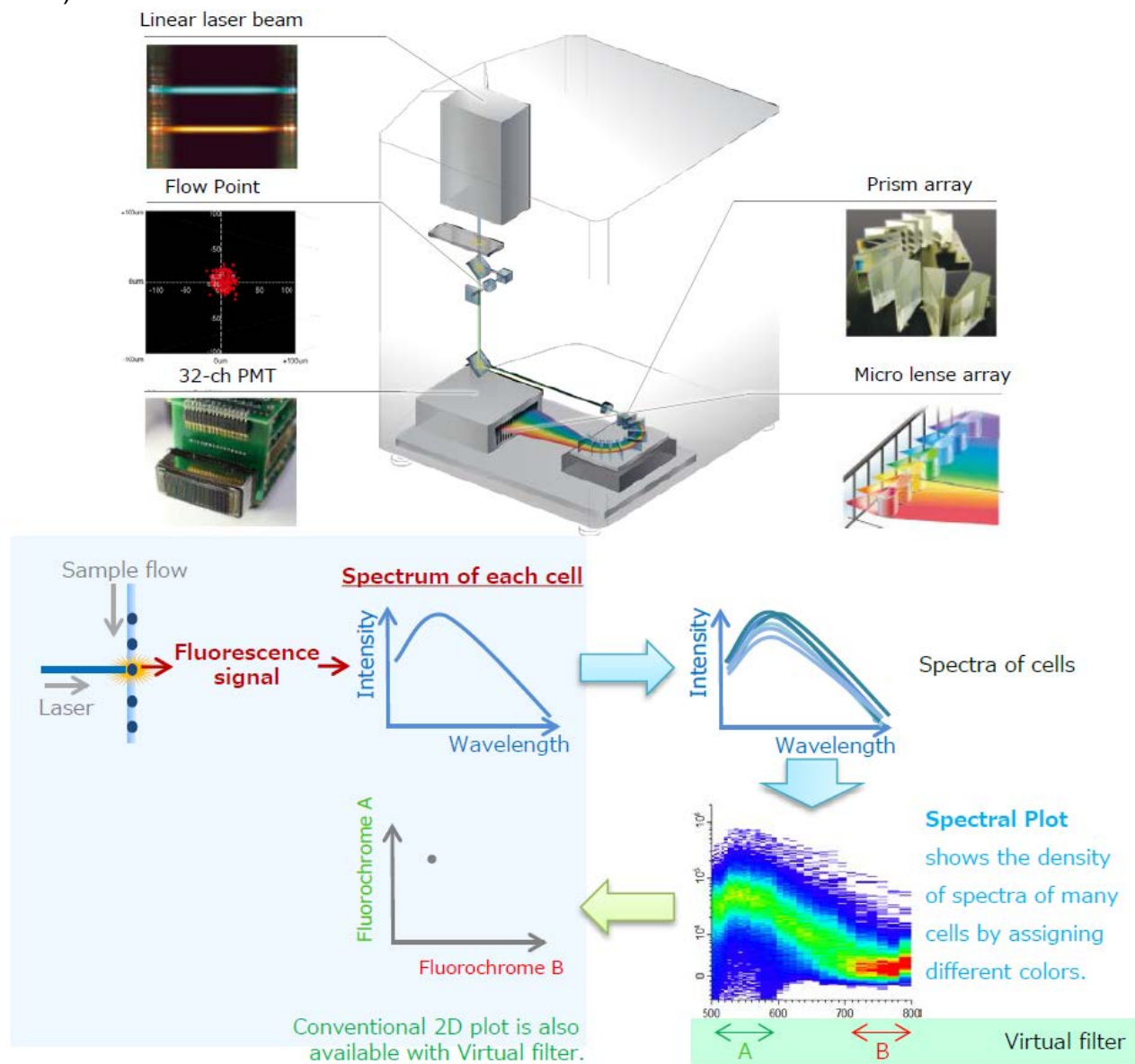
About this Quick Start Guide

This Quick Start Guide describes the basic operation of hardware and software for the LE-SP6800 series Spectral Cell Analyzer. Note: For detailed operation, refer to the supplied Operator's Guide. Hardware and software depicted herein may have been upgraded.

SP6800 Features

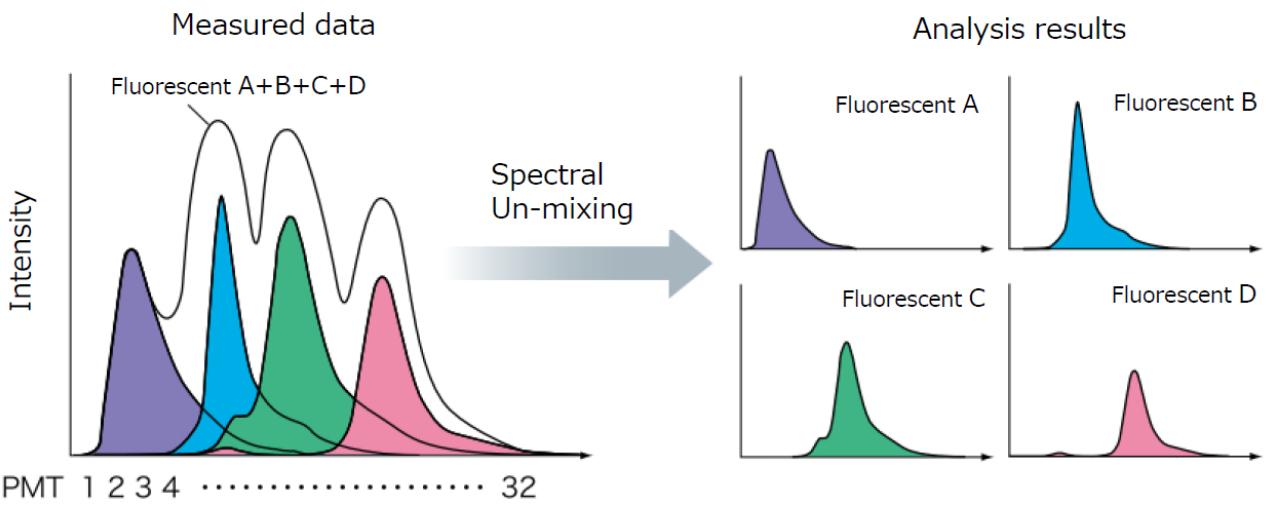
1. Spectral Analysis

SP6800 Spectral Analyzer enables further accurate analysis by un-mixing and analyzing spectra of cells with unique optical systems (10 consecutive prisms and 32-channel PMT).



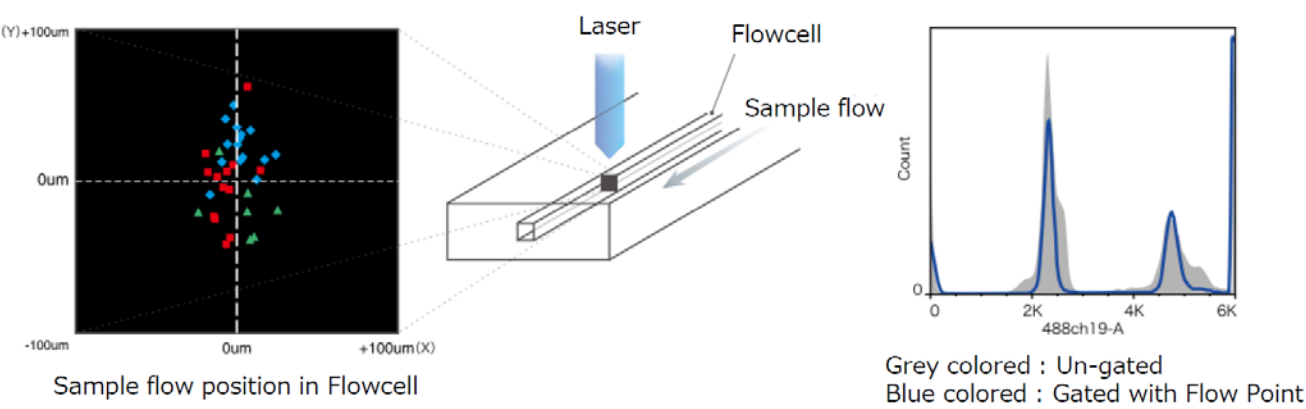
2. Spectral Unmixing

The SP6800's spectral un-mixing algorithms utilizes all the emitted fluorescence to separate different spectrums, enabling the separation of very similar or highly overlapping fluorescent spectra.



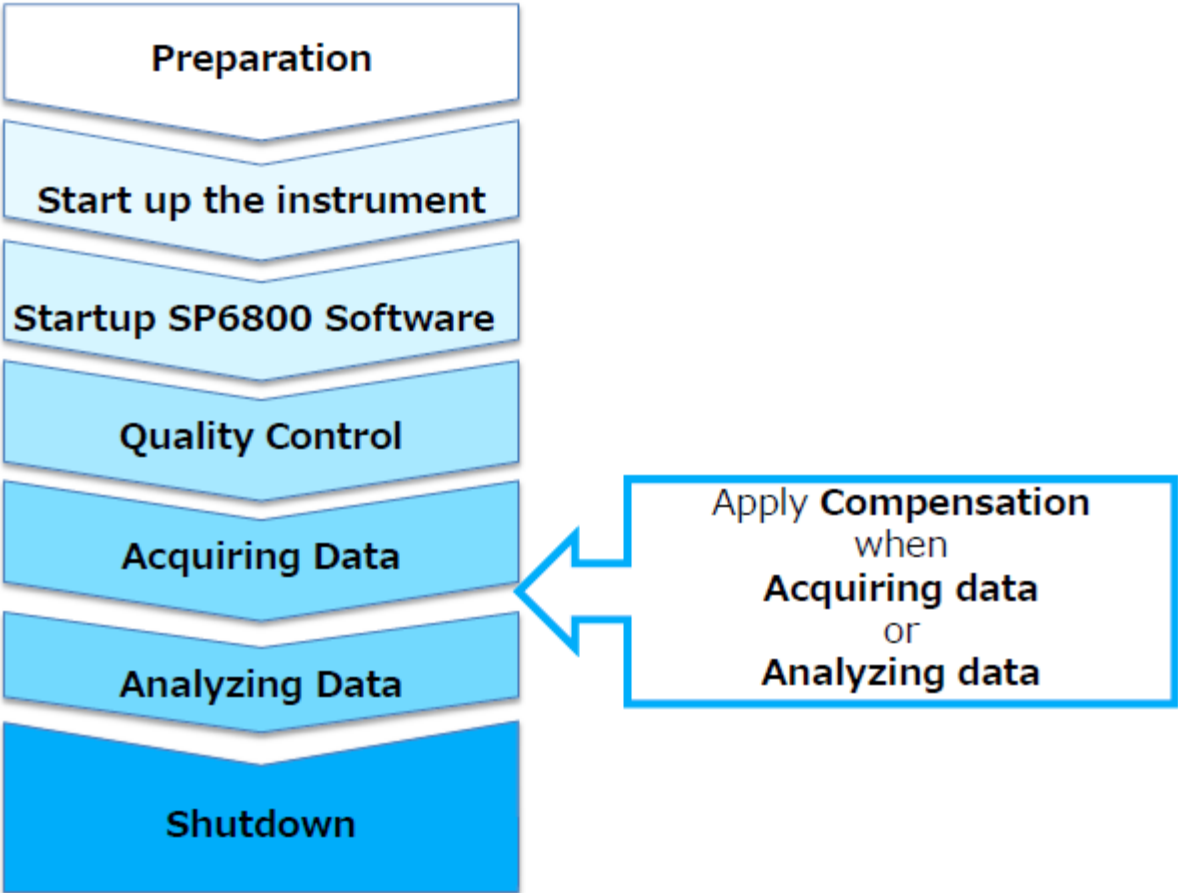
3. Flow Point

Using an advanced laser positioning and tracking system adapted from Blu-ray Disc™ player technology, this system allows the visualization of the core stream and each event's location in the flowcell.



4. Quality Control functions

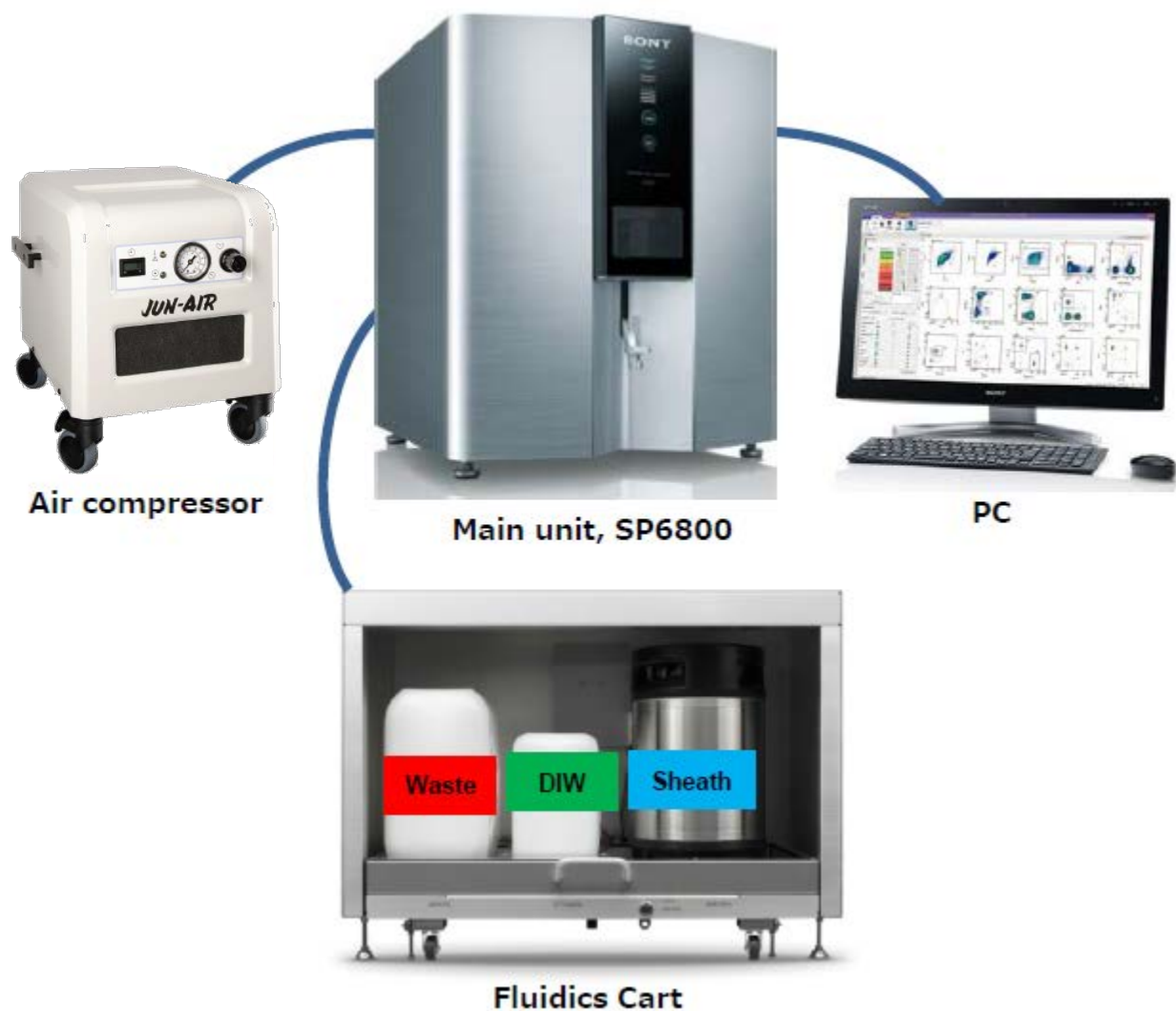
The performance of the SP6800 is being monitored on a daily and monthly basis to ensure stability and sensitivity of the system.



System configuration

The SP6800 system comprises the main unit, fluidics cart, PC and air compressor.

- Instrument control and data analysis is performed on the PC.
- The fluidics cart contains tanks for collecting waste, and for supplying sheath and DI water.



Preparation

Tanks

Fill the sheath tank (10 liters) and DI water tank (5 liters), and empty the waste tank (10 liters). Approximately 1 liter of sheath fluid is needed for analyzing a day. Recommended sheath fluid is ClearFlow™ Sheath Fluid (AE700592).

Opening/closing the lid of the pressurized sheath tank

- Check that the instrument power is turned off.
- Disconnect the sheath air line (clear) and sheath fluid line (blue) from the top of the sheath tank.
- Pull the ring-pull air release valve up to release the residual pressurized air
- Lift the lever, lower the lid into the tank, rotate it by 90° and remove the lid.
- Refill the tank with appropriate sheath fluid, e.g. ClearFlow™ Sheath Fluid (AE700592).
- Attach the lid in the reverse order of removal. Check that the lid and the air release valve are closed tightly.



Beads for Quality Control (QC)

- Alignment check beads (used for **Daily QC**): LE-AE700510
- 8-peak beads (used for **Monthly QC**): LE-AE700522

Preparation of bead solutions for QC

- Invert the reagent bottle at least twice to disperse the beads uniformly.
- Open the bottle and place 2 drops in a sample tube (5ml).
- Add 450 µl DI water into the sample tube.
- Agitate the sample tube to mix the solution.

Startup

1. Turn on the air compressor and wait for the air pressure comes up to **0.4kPa**.

Power SW



Set to "I" position.

Pressure gage



0.4kPa.



Air compressor

2. Turn on the main unit.

Breaker (inside the unit)



Set to upward.

Power button (on front panel)



Push "POWER" button.



Main unit, SP6800A

3. Turn on PC, keyboard and mouse.

PC power switch



Push "POWER" button.

Keyboard power switch

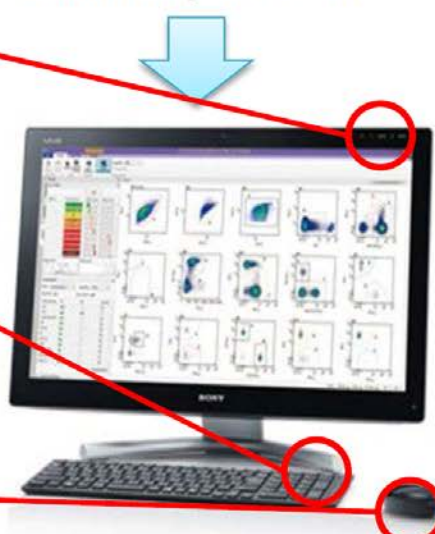


Set to "ON".

Mouse power switch



Set to "ON".



PC ,keyboard and mouse

4. Starting Windows 8

- Click the mouse > **LOG IN WINDOW** appears.
- Log into Windows8 using your login credentials.

5. Startup SP6800 software

- Click “SP6800 Software” icon to start the SP6800 Software.



- Log into SP6800 Software with assigned ID and Password.

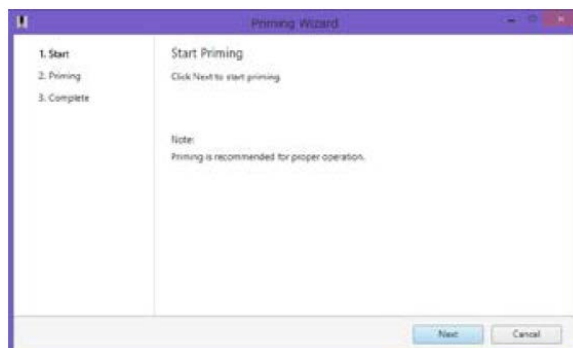


Standard account: user1 → [no password]

Administrator access: administrator → administrator

6. Priming

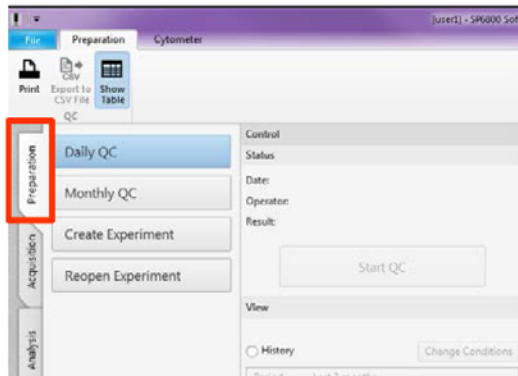
- The Priming wizard will open automatically when you start the SP6800 software the first time on a given day.
- Click Next. Priming takes about 5 min and is required to startup and stabilize system pressure and fluidics.



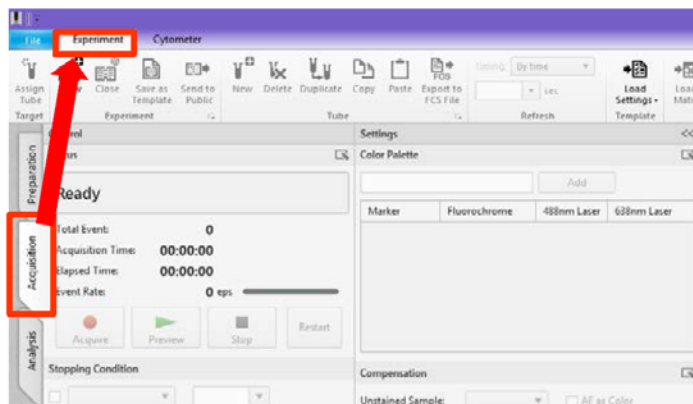
Selecting the process mode

1.Preparation : On Preparation tab following can be selected.

- **Daily QC** : For daily check with Align check beads.
- **Monthly QC** : For checking performance with 8-peak beads.
- **Create Experiment** : For creating NEW Experiment .
- **Reopen Experiment** : For using existed Experiment.

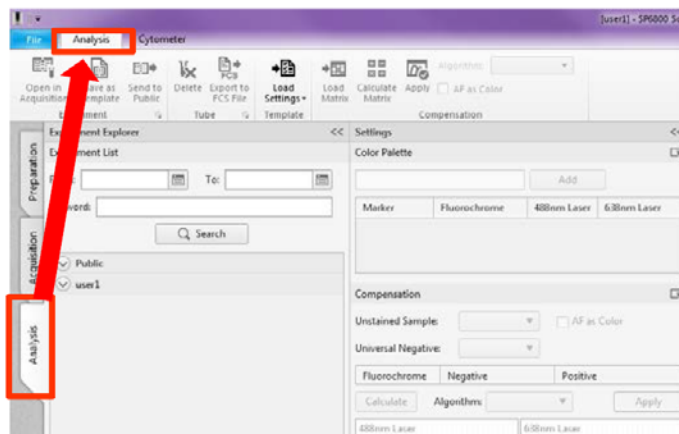


2.Data acquisition : Select **Experiment** on **Acquisition** tab .



3.Data analysis : Select **Analysis** on **Analysis** tab.

Note : NO need to start up main unit nor air compressor for only **Analysis** w/o **Acquisition**. In this case, **Disconnect** appears on status.



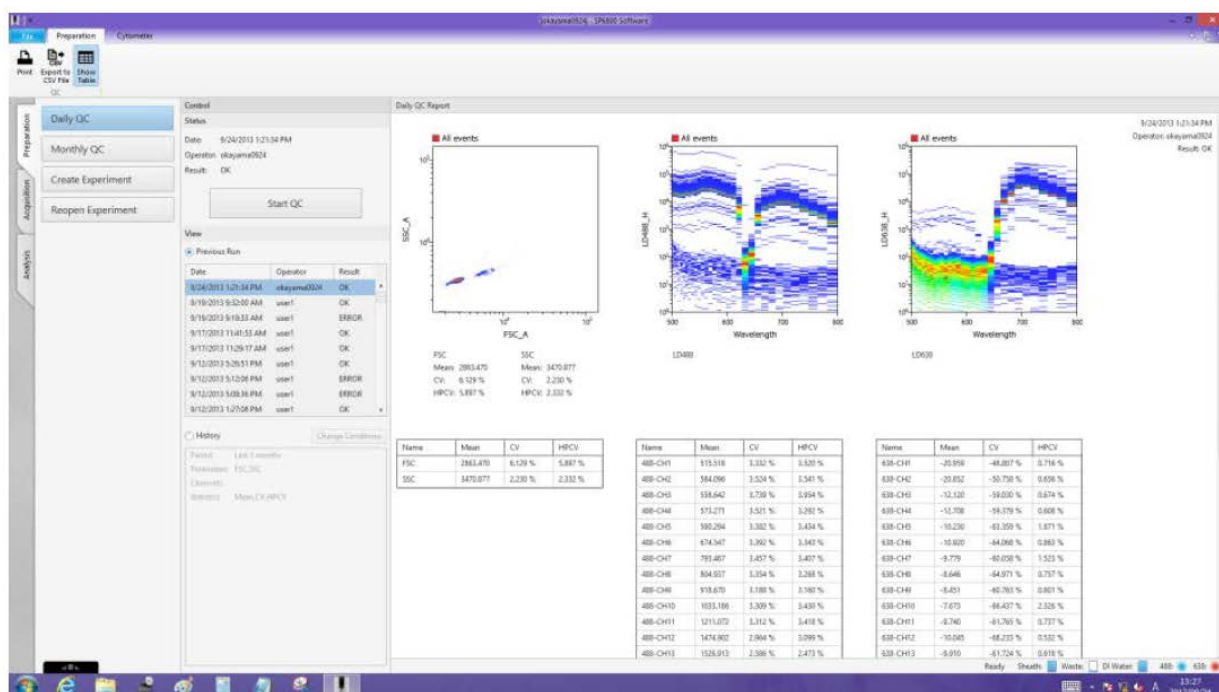
Running Quality Controls (QC)

Daily QC

Performs device checks and automatic calibration of flow rate and optical alignment, including checks using alignment check beads.

Note : This procedure is recommended to be run everyday before starting acquisition.

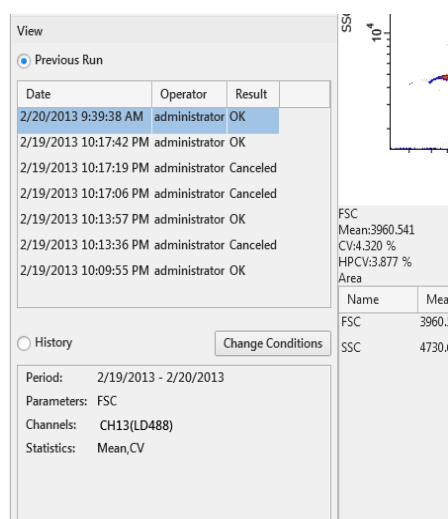
- Prepare 10% solution of Align check beads (LE-AE700510): Mix 2 drops of beads with 450 μ l DI water.
- Click **Daily QC** on the **Preparation** tab
- Click **Start QC** on the **Control** panel
- Follow the instructions in the displayed wizard
- The results are saved and added to the **Previous run** list in the **View** panel.
- **Check the result of Daily QC:** Three plots (of FSC/SSC, 488nm spectrum and 638nm spectrum) and data (of FSC/SSC and 32-channel red/blue) are displayed in the **Daily QC Report** panel.



Running Quality Controls (QC)

1. View previous Daily QC results

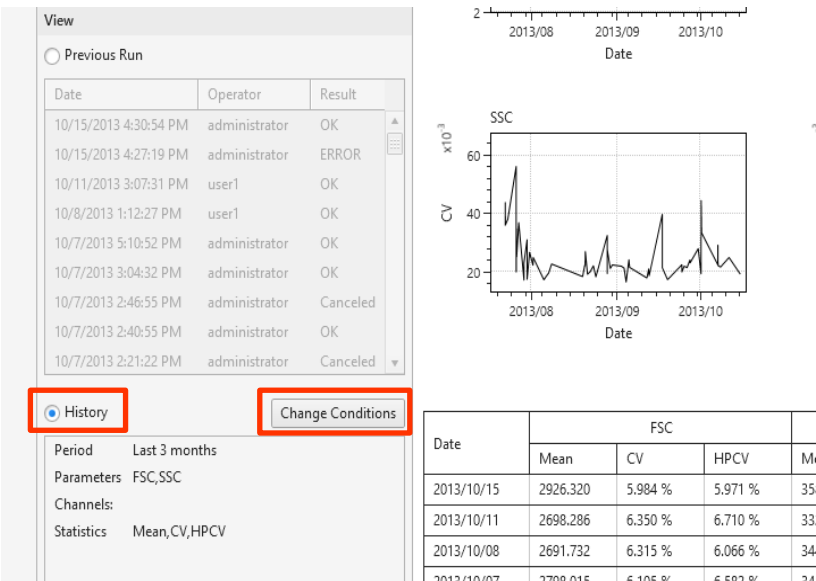
Select the **Previous Run** radio button, then click the desired file in the list to display the results for the previous Daily QC.



2. Check multiple Daily QC results on an L-J chart.

Select the **History** radio button to display the Daily QC results for the specified period on an L-J chart.

To change the display period and parameters, click **Change Conditions**.

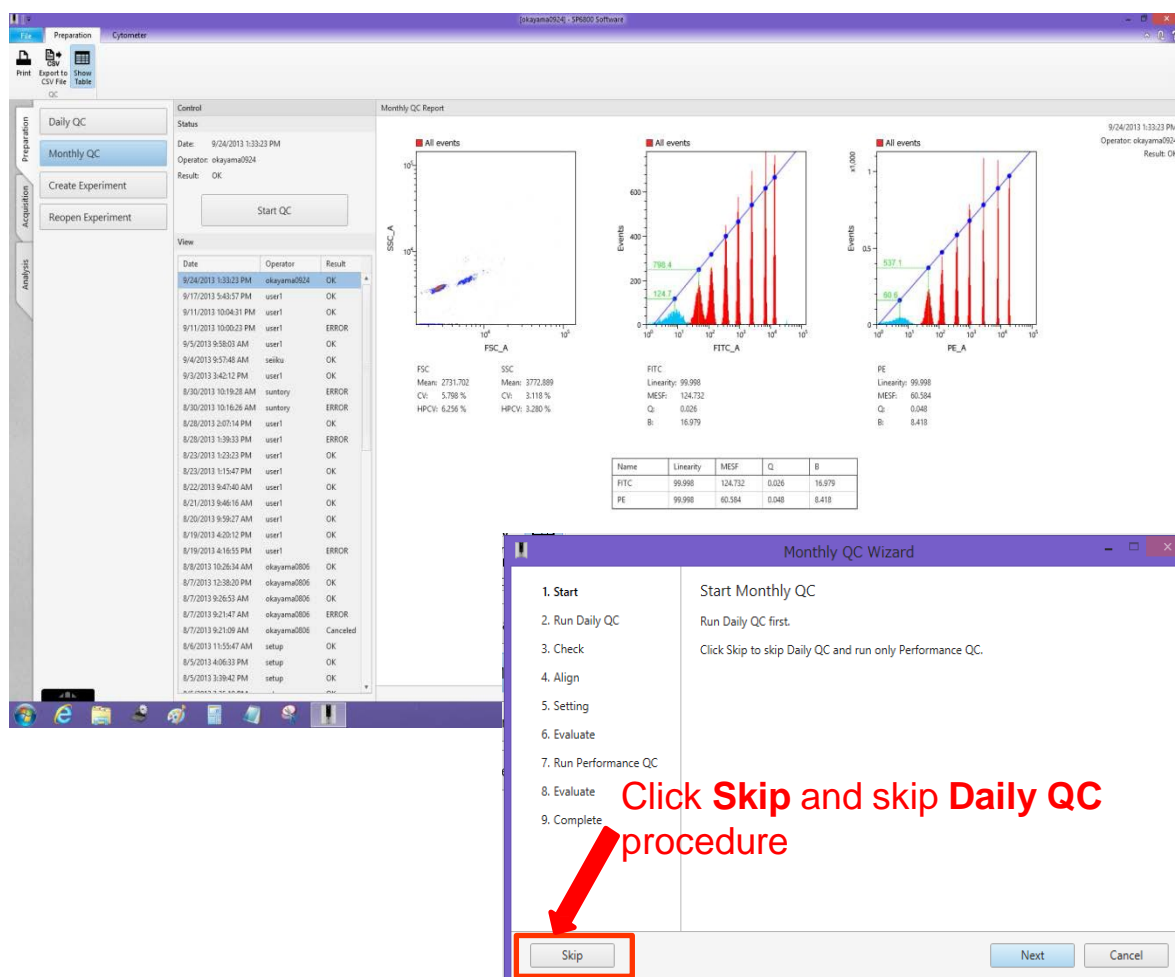


Running Quality Controls (QC)

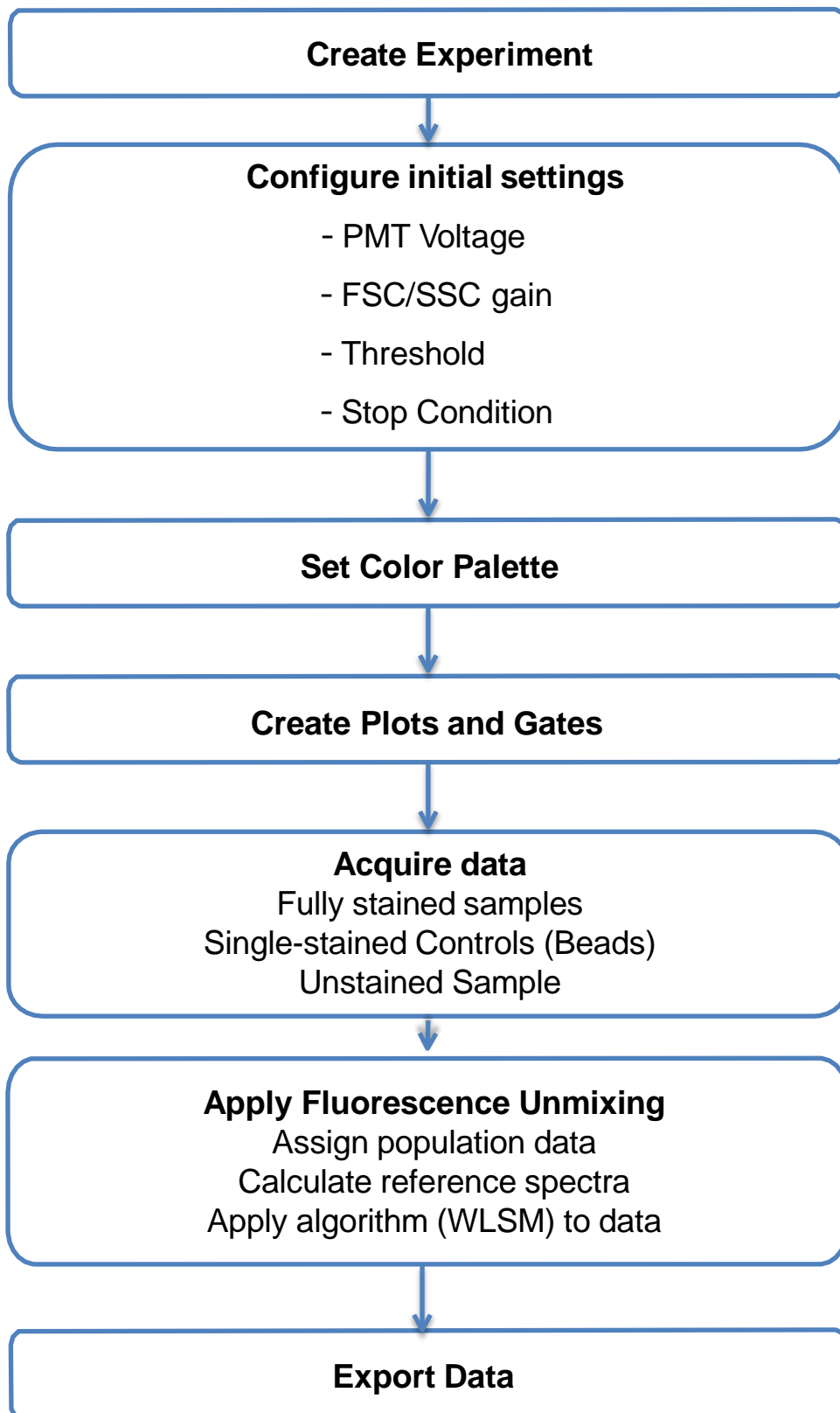
Monthly QC

Performs Daily QC procedure at first. Additionally performs checks of fluorescence sensitivity, linearity, Q value, and B value using 8-peak beads.

- Prepare 10% solution of Align check beads (LE-AE700510): Mix 2 drops of beads with 450 µl DI water.
- Click Monthly QC on the Preparation tab.
- Click Start QC on the Control panel.
- Follow the instructions in the displayed wizard. Note : Click Skip in case no need Daily QC.
- The results are saved and added to the Previous run list in the View panel.
- panel.
- The results are displayed in the Monthly QC Report panel.



Data acquisition



Acquiring Procedure

Create an Experiment

- Click **Create Experiment** and create your new Experiment to acquire data.

Configure initial settings

- Select 1- or 2-laser excitation mode.
- Check plots with changing the following items.
 - The 32-channel **PMT voltage** (%) with the brightest sample. The **FSC/SSC gain**.
 - The **Threshold**.
 - Stop Condition** or **Stop Gate** (option).
- Finish adjustments.

Set Color Palette

- Input **Fluorochrome** and click **Add**.
- Each **Fluorochrome** can be named by input **Marker** name.

Set Plot and Gate

- Add plots on Work sheet area.
- Add a gate to the tube plot.

Acquiring Data

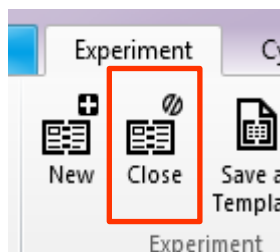
- Record data.
- Stop measurement

Note : Recording will automatically stop at the specified condition with a check mark in the **Stopping Condition** checkbox.
When recording ends, sample line cleaning starts automatically and the new tube is added in **Sample list**.

Exit Experiment

- Click **Close** on **Experiment** tab.

Note : The experiment is saved and go to **Analysis** tab.



Apply Fluorescence Unmixing

In spectral analysis FCM SP6800, the reference spectrum for each fluorochrome is identified based on single-stained controls. This information is used to perform fluorescence unmixing. Obtaining clean reference spectra is the key to high-accuracy analysis.

- The spectrum for each fluorochrome is derived from the positive control (Positive) and negative control (Negative). Single-stained samples using cells or beads are consisting of “Positive = fluorochrome spectrum + Negative”, hence the spectrum is determined by both the Positive and Negative. The clean reference spectrum for each fluorochrome is calculated by subtracting the negative signal from the positive signal. This information is also stored in the **Reference Spectra Library** and can be imported for use in other experiments.
- A Sample that shows the same autofluorescence as the multi-stained test samples should be used as Unstained Sample. It is recommended that the gate range be the same as the range intended for use in analysis of multi-stained test samples.
- The goal of a **Universal Negative** is to reduce the workload of registering negative controls when preparing many single-stained samples containing the same type of cells or beads.

The algorithm commonly used for fluorescence unmixing is called „Weighted Least Square Method“ (WLSM). It calculates the individual contribution of every fluorochrome defined in the color palette to the resulting spectral vector for every event.

Apply Fluorescence Unmixing

Unmixing procedure

Specify the populations

To calculate the fluorescence unmixing algorithm, specify the following populations. Previous data of each fluorochrome can be applied.

Name		Population
Positive	Mandatory	Positive populations from single-stained controls
Negative	Mandatory	Negative populations from single-stained controls
Universal Negative	Optional:	Negative population for single-stained controls Only used in case one negative population can be applied to all (or several) single-stained controls
Unstained Sample	Optional	Unstained control of cell sample. Used to calculate autofluorescence.

Calculate the Unmixing matrix

- Acquire single-stained controls. Compensation Beads are recommended, since these lead to a clear separation between the positive and negative population.
- Create gates to define positive and negative populations for every single-stained control.
- Define positive and negative reference populations for every fluorochrome specified in the color palette and unmixing matrix.
- Click **Calculate** to calculate the reference spectra for every fluorochrome
- Click **Apply** to display the fluorescence unmixing result for fully-stained samples

Data acquisition

Acquiring Procedure using three fluorochromes

- ① Create an experiment / Configure initial settings
- ② Set Color Palette
- ③ Acquire data for compensation
- ④ Specify the populations
- ⑤ Calculate the compensation matrix
- ⑥ Apply Compensation
- ⑦ Acquire data of test sample

The screenshot displays the Sony SP6800 Software interface with various panels and controls. Red boxes and arrows indicate the sequence of steps for data acquisition:

- Step 1:** Located in the 'Laser' section, where the 488nm and 638nm lasers are checked.
- Step 2:** Located in the 'Color Palette' section, where the 'unstained' sample is selected.
- Step 3:** Located in the 'Acquire' button in the 'Preparation' panel.
- Step 4:** Located in the 'Tube List' section, where the 'unstained' sample is selected.
- Step 5:** Located in the 'Compensation' section, where the 'Algorithm' is set to 'FSC'.
- Step 6:** Located in the 'Compensation' section, where the '638nm Laser' is selected.
- Step 7:** Located in the 'Acquire' button in the 'Preparation' panel.

The 'Monitor' panel at the bottom left shows the 'Event Rate' graph and the 'Flow Point' graph. The 'Detector & Threshold' panel at the bottom right shows the 'Window Extension' set to 'Normal' and the 'Threshold Value (%)' set to 7.1.

Name	Events	Parent%	Total%
All events	7,930	100.00 %	100.00 %

Preparation

Prepare fluorochrome stained samples

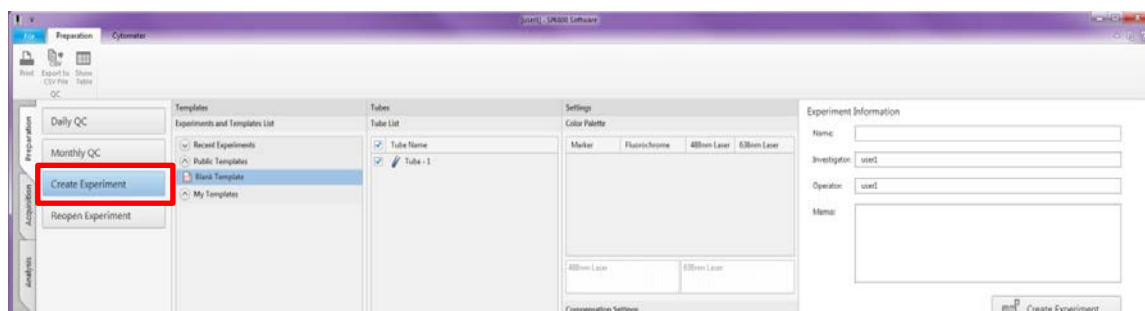
In this case, prepare 5 types of sample shown in the table below.

Tube names are configured by the user (called NEGA/FITC/PE/APC/MIX here).

	Fluorochromes	Tube name
1	Unstained	Negative
2	FITC	FITC
3	PE	PE
4	APC	APC
5	FITC/PE/APC	Mix

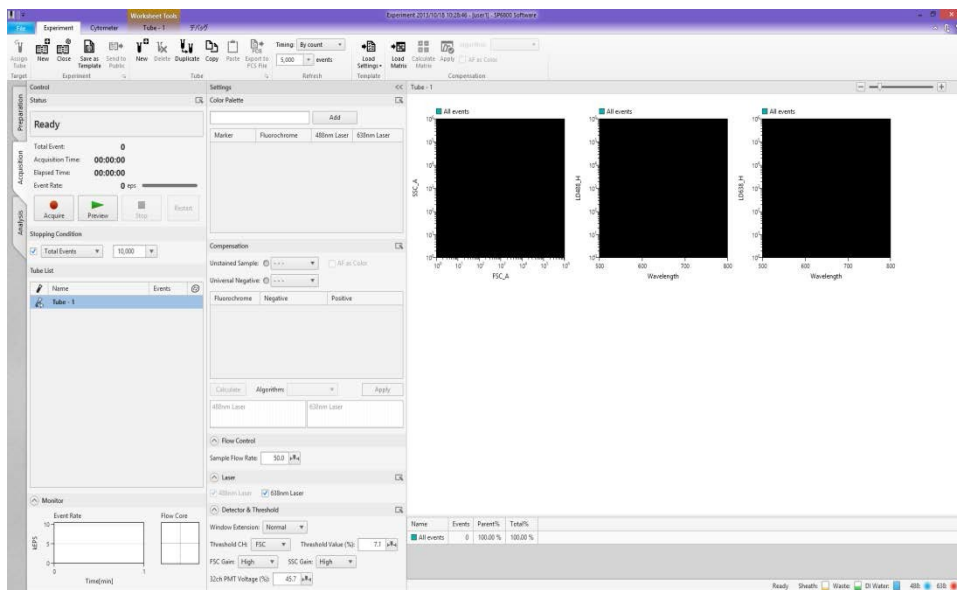
Create an experiment

- Click **New Experiment** on the **Preparation** tab.



- Select **Template** from the **Experiments and Templates List**.
 - To create a new template, select **Blank Template**.
 - To select a previous template, select from the list in **Recent Experiments** or from the **My Templates** menu.
- Further experiment information will be provided in the **Tubes** and **Settings** columns of the **New Experiment** menu. These can only be changed later during acquisition.
- Enter details in the **Experiment Information** list on the right.
 - Name** : Enter the name of Experiment.
Note : If a **Name** is not entered, a [Experiment + *timestamp*] format name is assigned.
 - Investigator** : Enter the name of the investigator.
 - Operator** : Enter the name of the operator.
 - Memo** : Use to save further text details for your experiment.

- Click **Create Experiment** and the **Acquisition** tab will open automatically.



Configure the initial settings for the instrument

1. Flow Control

- Set the sample flow rate.

2. Set the laser mode

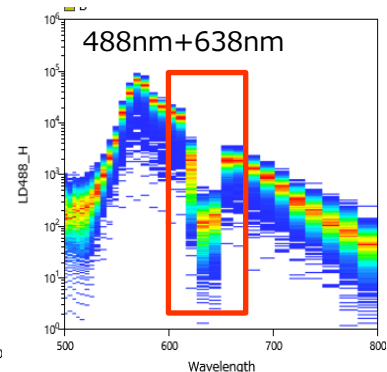
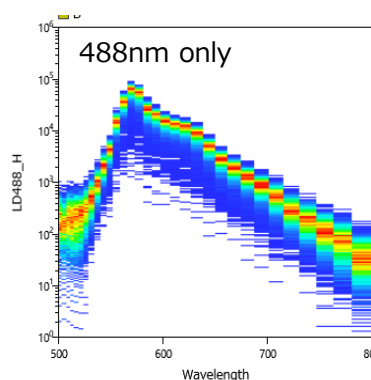
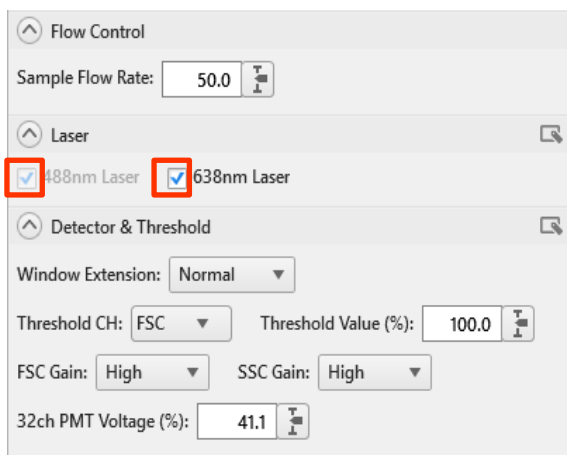
You can select 1-, 2- or 3-laser excitation mode.

Available laser excitation:

488 nm (blue): always required as trigger signal

405 nm (violet)

638 nm (red): causes a dip in the fluorescence spectrum due to the presence of a notch filter blocking scatter light from the red laser.



3. Window Extension

- The window can be modified according to the cell size for measurement.
- Standard setting is **Normal** (default).

4. Set the threshold

- Select the threshold channel and threshold value in the range 0 to 100.0%.
- The default threshold channel is **FSC**

5. Set the FSC/SSC gain

- The gain can be set to 1-17.
- Set the gain as high as possible, without saturating the detectors. Check that there are no events on the axis .
- 17 is the default setting. Use the zoom function to enlarge your population of interest if necessary.

6. Set the 32-channel PMT voltage (%)

- Enter the voltage level to be applied to the 32-channel PMT detector.
- Select a value in the range 0 to 100%. The default value varies due to the result of the Daily QC.
- Set the voltage as high as possible, without saturating the detectors when the sample is flowing, while viewing the 32-channel spectrum.

The screenshot shows a software interface with three main sections: 'Flow Control', 'Laser', and 'Detector & Threshold'. The 'Flow Control' section has a 'Sample Flow Rate' input field set to 50.0. The 'Laser' section has checkboxes for '488nm Laser' and '638nm Laser', both of which are checked. The 'Detector & Threshold' section contains several controls: 'Window Extension' is a dropdown menu set to 'Normal'; 'Threshold CH' is a dropdown menu set to 'FSC'; 'Threshold Value (%)' is an input field set to 100.0; 'FSC Gain' is a dropdown menu set to 'High'; 'SSC Gain' is a dropdown menu set to 'High'; and '32ch PMT Voltage (%)' is an input field set to 41.1. Each input field has a small icon to its right, likely for zooming or focusing.

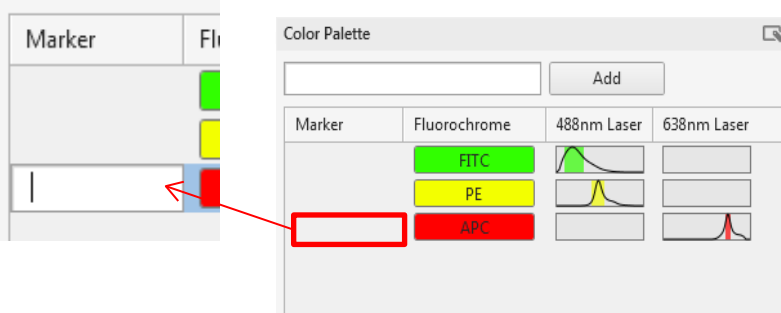
Define the measurement conditions

1. Add a fluorochrome to the color palette

- Register fluorochromes beforehand for axis labels on plots and for fluorescence unmixing.
- Register **FITC**.
 - Enter “**F**” or “**f**” in the Color Palette text box.
 - Fluorochromes that start with the letter F are displayed. Select FITC from the pull-down menu.
 - Click **Add** to add FITC to the Dye Name list.
- Register **PE**.
 - Enter “**P**” or “**p**” in the text box and select PE in the same way.
- Register **APC**.
 - Enter “**A**” or “**a**” in the text box and select APC in the same way.

2. Add Marker name (optional)

- Click Marker field of each palette> **Text Box** appears> Enter desired Marker name

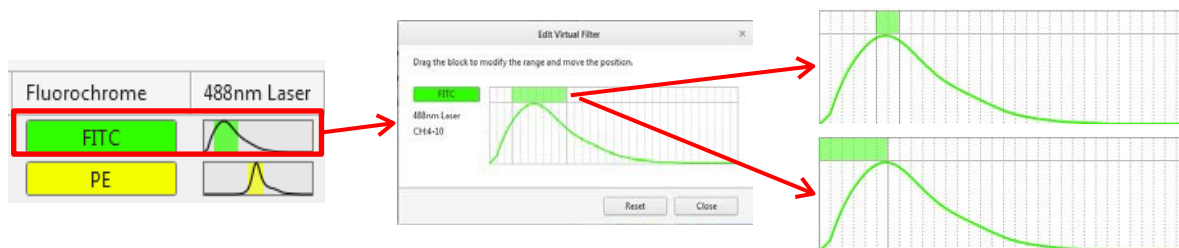


3. To remove registered fluorochrome

- Right-click a fluorochrome you want to remove in the **Dye Name** list and select **Remove**.

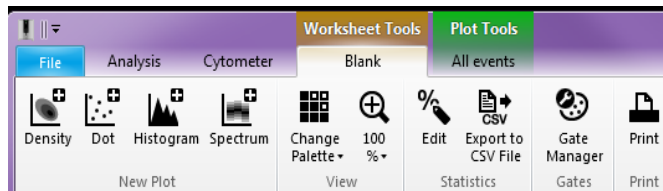
4. Modify Virtual Filter

- Double-click a filter range in the **488nm Laser** or **638nm Laser** list.
- The **Edit Virtual Filter** window appears. Modify the filter range by changing the width of the color bar.
- The modified filter range is applied to the current experiment only.



4. Set up plots on the worksheet area

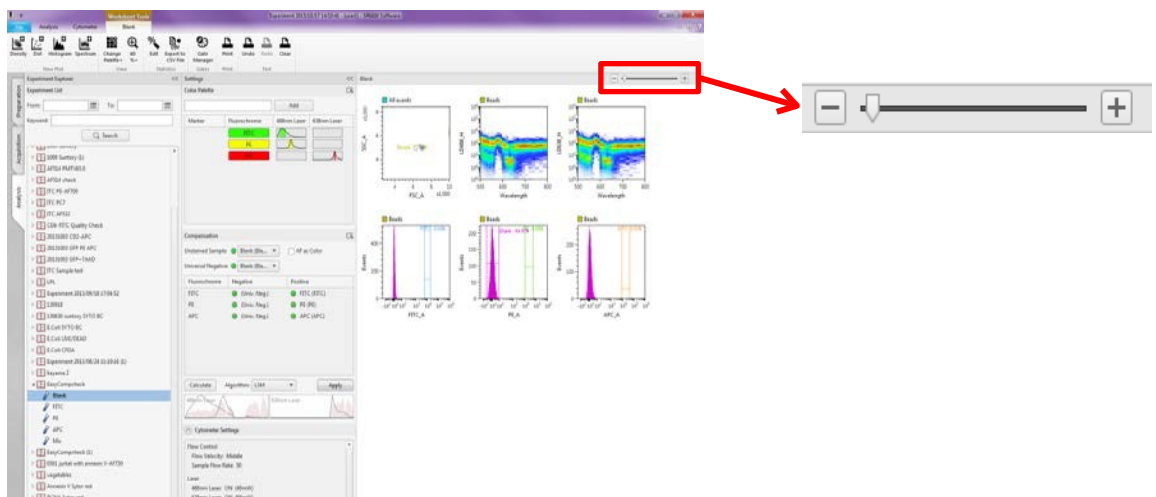
- Three plots are added by default for a new experiment.
 - FSC/SSC plot
 - Spectrum plot of 488-laser
 - Spectrum plot of 638-laser
- Add a new plot
Click the **Worksheet** tab, then click the desired plot type in the **New Plot** group to add a new plot.



- Plot types
 - **Dot**
 - **Density**
 - Color of events encodes the frequency of populations in a heat map.
 - **Histogram :**
 - Y axis parameter: Number of **Events**
 - **Spectrum :**
 - X Axis parameter: Wavelength or Channel number of fluorescence detector.
 - Y Axis parameter: Fluorescence intensity of fluorochrome caused by 488 nm or 405/638 nm laser excitation.
 - Choose either 488 nm or 405/638 nm laser spectrum plot.

5. Change the size of the plots

- Drag the slider on the bottom right of the worksheet to change the size of plots.
Note : The plots are rearranged automatically.






6. Add new tubes

- In the initial setting one new tube is created when Experiment was created.
- A new tube is added to the end of **Tube List** when data recording of the previous tube is finished.
- Multiple tubes can be added by clicking New or Duplicate in the **Tube** menu on **Experiment** tab.
 - Click **New** to add new tube with worksheet.
 - Select the desired tube and click **Duplicate** and the new tube with the same work sheet as desired tube is added.

7. Modify the name of the tube

- Double-click a tube name in **Tube List** and enter a name for the tube.

Tube List			
	Name	Events	
	PE		

Start the sample flow

1. Set sample

- Start with a fully-stained sample, where you expect the brightest fluorescence intensity
- Load the sample in a 5 ml Falcon round tube and place the tube in the single loader.

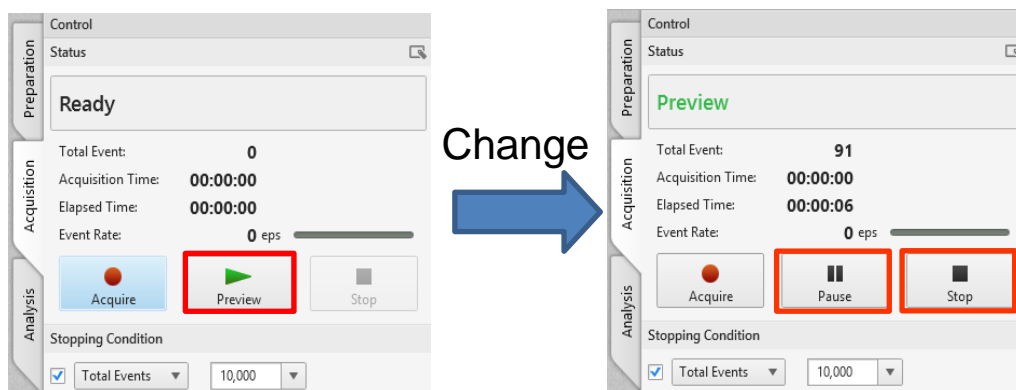
Important : Make sure the sample volume does **NOT exceed 2 ml**. An excess might causes splashes, which will impair the tube detectors



2. Start sample flow

- Click **Preview**.
- The sample tube is loaded and the sample starts flowing.

Note : The **Preview** button changes to a **Pause** button.



Note : If you click **Acquire** sample flow and data recording will start at the same time. In this case, we use the **Preview** button to record the data after adjusting measuring parameters .

3. Pause sample flow

- Click **Pause** .
- The pinch valve closes and stops the flow of the sample only.

Note : To restart, click **Preview**.

4. Stop sample flow

- Click **Stop** .

Note : If **Stop Condition** or **Stop Gate** is set before click **Acquire**, sample flow will stop automatically when the setting condition is achieved.

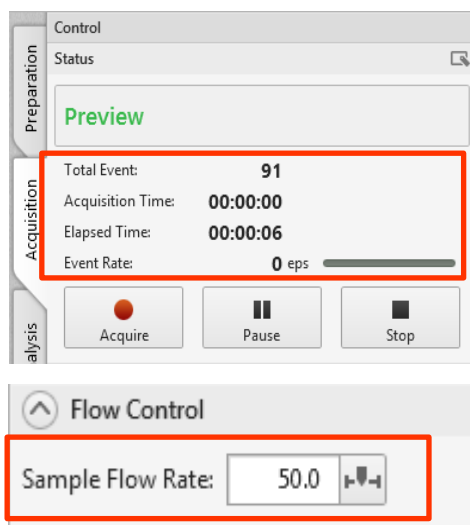
Adjust the event rate

The following parameters are displayed when you click **Preview** to start the sample flow.

- **Total Event** : Total number of events
- **Acquired Time** : Recording time
- **Elapsed Time** : Total elapsed time
- **Event Rate** : Event rate

Modify the value of **Sample Flow Rate** in **Flow Control** to adjust the event rate.

Note : It is not recommended to run the sample at a Flow Rate higher than 50.0%



Check the plots

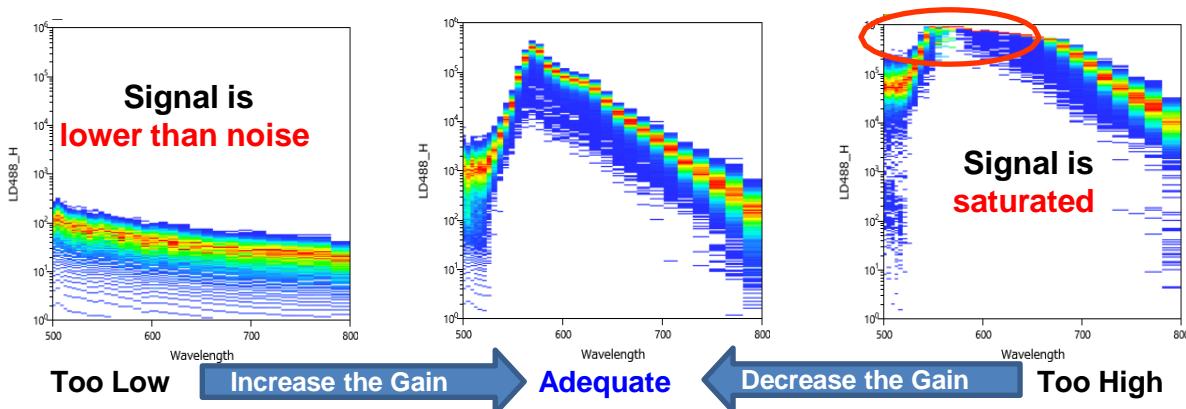
1. Modify the FSC / SSC gain

- Check the **Density** plot of FSC/SSC and modify the **FSC / SSC gain** to lower level if the signal is saturated.

2. Modify the 32-ch PMT voltage

- Set the gain by checking the **Spectrum** plot of the flowing brightest sample.
- The gain should be as high as possible, without saturating the detectors.

Note : To check the PMT Voltage, use the value of **Height** of each Wave Length as a parameter of Y Axis of **Spectrum** plot.



3. Re-modify the 32ch PMT Voltage

Re-modify the **32ch PMT Voltage** if the signal level in **Spectrum** plot is too High or too Low.

Note : Click **Restart** to reset the plot

Record (= Acquire) Data

1. Define the stop condition

Place a check mark in the **Stopping Condition** checkbox to stop recording automatically at the specified condition.

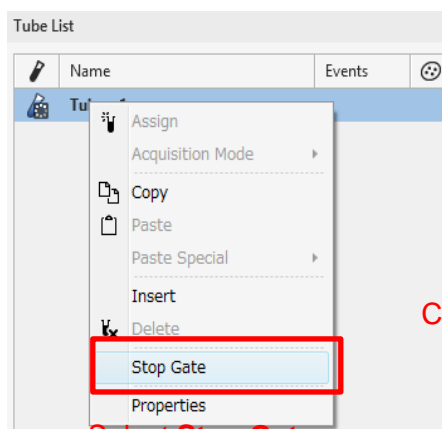
The following three stop conditions are available:

- **Total Events**
- **Elapsed Time**
- **Acquisition Time**

2. Define a stop gate

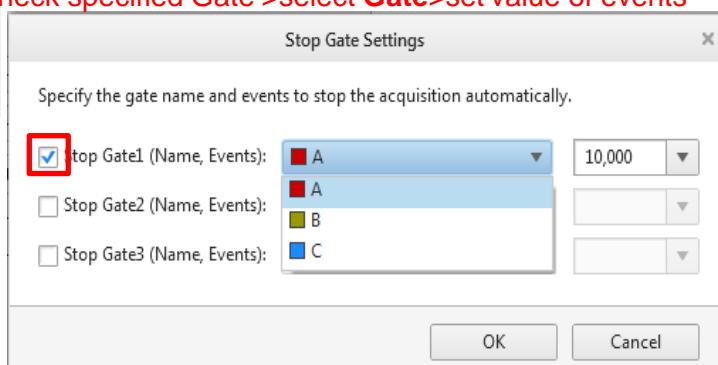
If you want the acquisition to stop after a certain number of events has been collected in a pre-defined gate of the sample, use the following procedure:

- Right-click on the desired tube in **Tube List** and select **Stop Gate**.
- **Stop Gate Settings** dialog box appears> Check Stop Gate> Select the desired Gate in pull-down menu>Set a value of events



Select Stop Gate

Check specified Gate > select Gate > set value of events



3. Start acquiring data

- Click **Acquire** to start acquiring data.

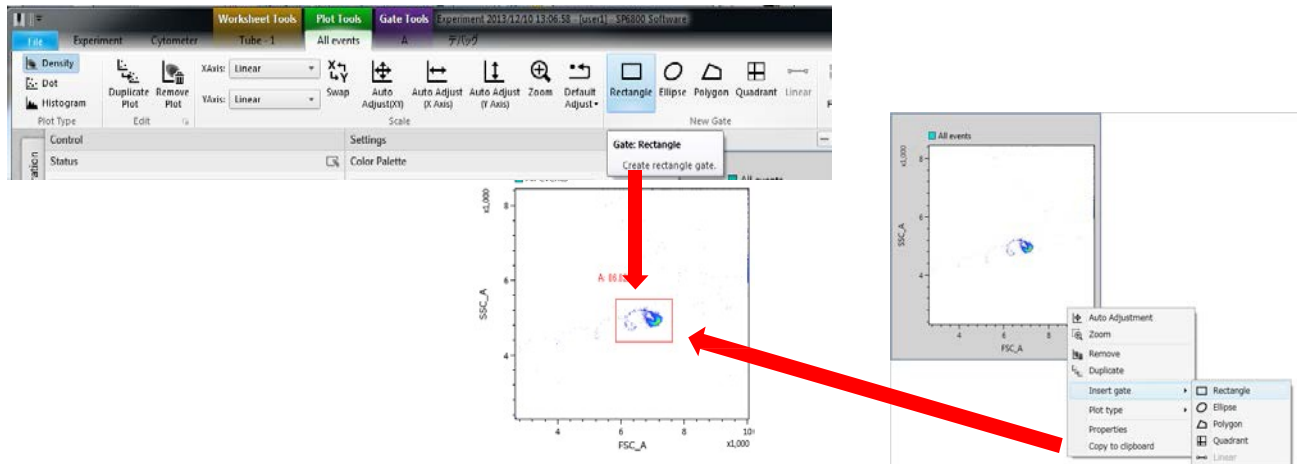
Note : Without setting the **Stopping Condition**, click **Stop** to stop acquiring data.

Applying Fluorescence Unmixing

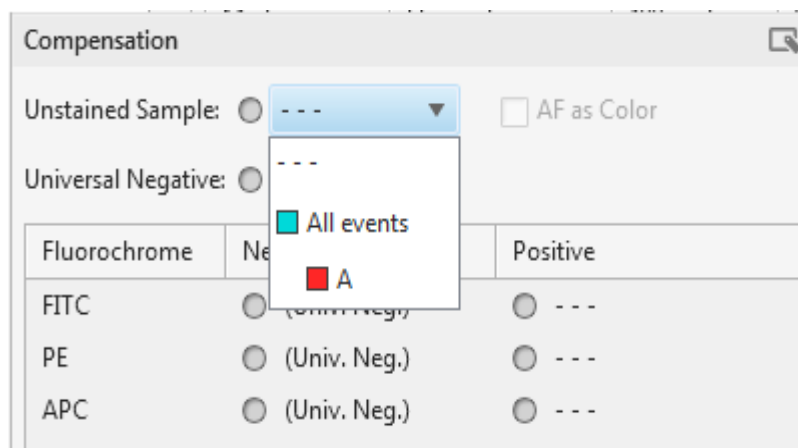
Calculate the Unmixing Matrix and apply Fluorescence Unmixing to your samples.

Specify **Unstained Sample** and **Universal Negative**

1. Put the Unstained Sample and start the sample flow.
2. Right-click the FSC/SSC bivariate plot on the worksheet > select **New Gate** menu on the **Plot Tools** tab > select **Rectangle**.
3. Click on the plot and drag the mouse to specify a region to add a gate surrounding the population. Gate "A" is created.



4. Click near **Unstained Sample** on the **Compensation** pane and select "A" from the list of displayed gates in pull-down menu.



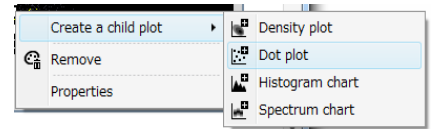
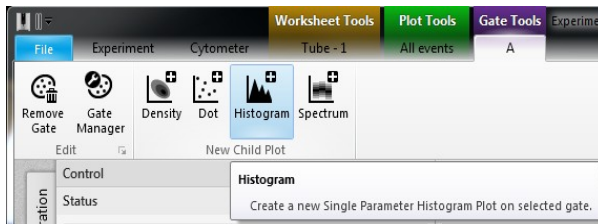
5. Similarly, click near **Universal Negative** and select "A" again from the list of displayed gates in pull-down menu.

Note : **Universal Negative** can be ONLY used in case

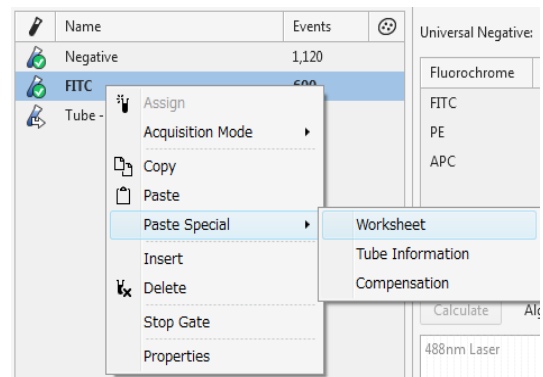
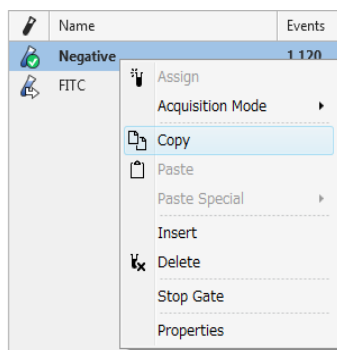
- Each Negative is common
- With NO Nonspecific Adsorption Effect

Specify Positive and Negative for each fluorochrome (e.g. FITC)

1. Select the **FITC** tube from the **Sample List**.
2. Click on the FSC/SSC bivariate plot and drag the mouse to specify a region to add a gate surrounding the population. Gate "A" is created.
3. Select gate A > select **New Child Plot** on **Gate Tools** tab > click **Histogram** > A child plot for the selected population is added.



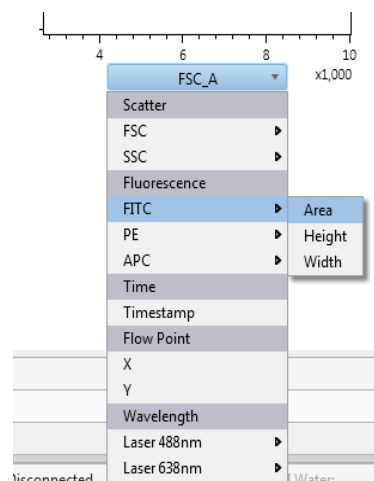
Note : Right-click on **Negative** in **Tube List** > select **Copy** > right-click on **FITC** tube > select **Paste Special** → **Worksheet** > You can apply the setting of work sheet created with **Negative** tube.



4. Change the axis of the added child plot.

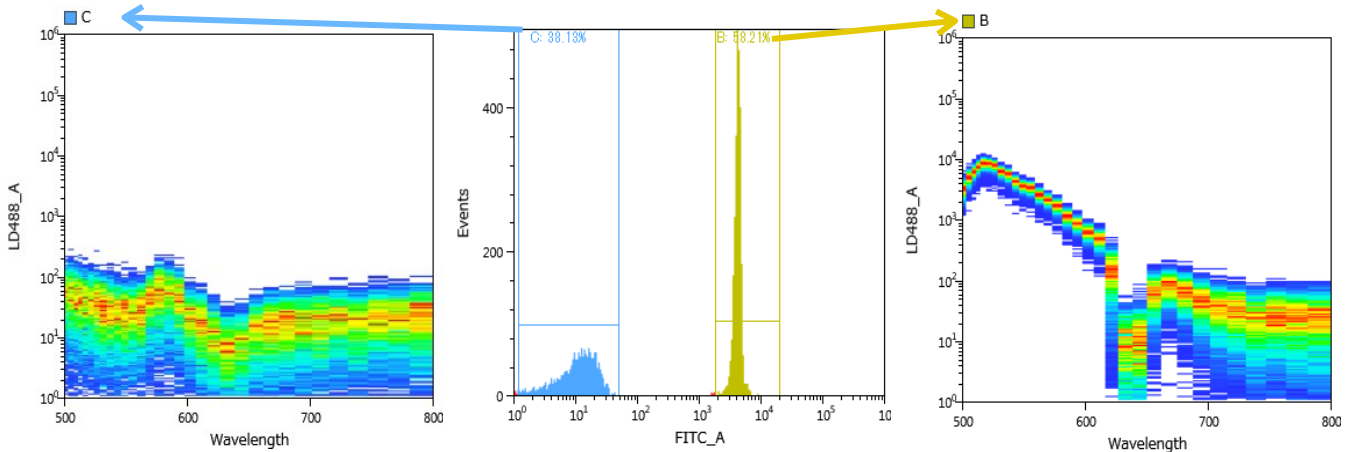
Right-click the X-axis label and select **FITC** > select **Area**.

Note : You can also get **Histogram** plot by select **Events** for Y-axis on **Dot** plot or **Density** Plot.



Specify Positive and Negative populations

1. Select **New Gate** on **Plot Tools** tab and then select **Linear** for the gate type.
2. Click and drag to add a gate that encompasses the positive population. Gate **B** is created.
3. Next, add a gate that encompasses the negative population. Gate “C” is created.



4. Click the **FITC Positive** pull-down menu in the **Unmixing** table and select “**B**” from the list of displayed gates.
5. Click the **FITC Negative** pull-down menu on the **Unmixing** table and select “**B**” from the list of displayed gates.

Note : The population assigned as **Universal Negative** is applied to the Negative population of all fluorescence channels automatically. You can change the negative population individually.

Compensation

Unstained Sample: ---

AF as Color ☐

Universal Negative: ---

Fluorochrome	Negative	Positive
FITC	<input type="radio"/> (Univ. Neg.)	<input type="radio"/> ---
PE	<input type="radio"/> (Univ. Neg.)	<input type="radio"/> ---
APC	<input type="radio"/> (Univ. Neg.)	<input type="radio"/> ■ All events

■ A

■ B

■ C

Calculate

Algorithm: LSM

Apply

9-4-3. Procedure for other single-stained fluorescence controls.

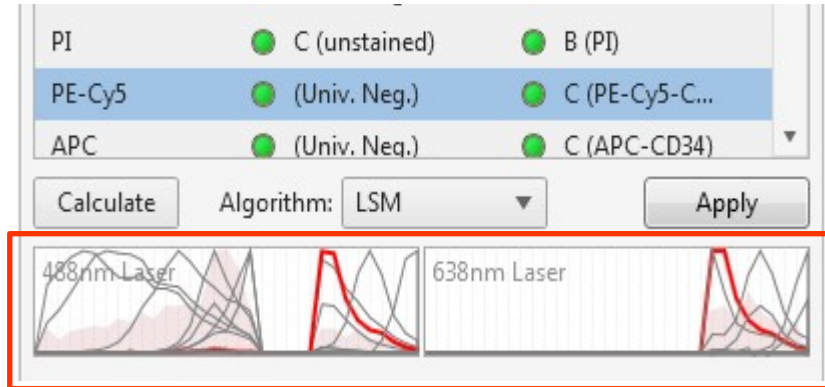
Repeat the same steps for all other single-stained fluorescence controls.

Calculate fluorescence unmixing matrix

Click **Calculate**.

After the calculation has finished, you can check the shape of each reference spectrum each fluorochrome at the bottom of the Unmixing table.

If the spectrum shape is distorted, check the size and shape of gates for Positive and Negative populations until the correct spectrum shape is obtained.

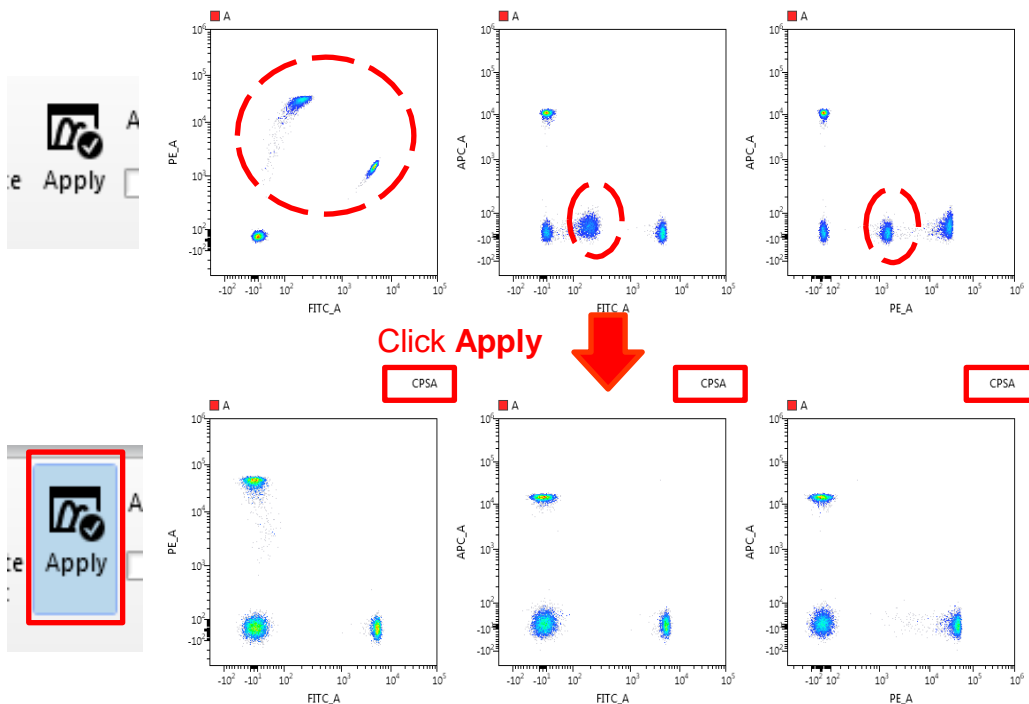


Apply

Note : If spectral unmixing is applied, the Apply button turns blue and the name of the active algorithm is displayed at the right upper side the each plots.

Check spectral unmixing results

1. Open the **Reference Spectrum Adjuster** menu from the Analysis tab.
2. Adjust the biexponential scaling for every fluorochrome.
3. Check and adjust the reference spectrum if necessary.

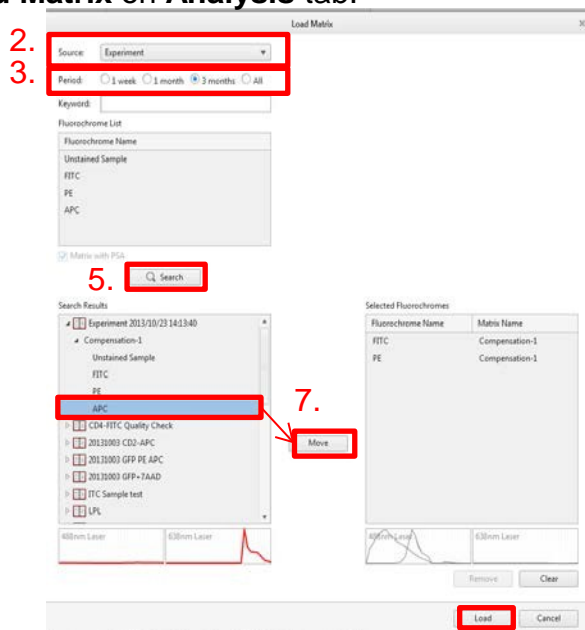


Compensation is applied to the bivariate plots

Import reference spectra (Load Matrix)

If you have used the same fluorochrome for analysis in the past, it is possible to import the respective reference spectra.

1. Click **Load Matrix** on **Analysis** tab.



2. Define the data source, where you would like to search for the reference spectrum, e.g. **Experiment**.
3. Define the search period of time.
4. Select the desired Fluorochrome in **Fluorochrome List**. If you start searching without selecting a specific Fluorochrome, software will search for all fluorochromes defined in the color palette at once.
5. The list of the Unmixing matrices, that contain the specified fluorochrome appears in **Search Results**.

Note : If you want to select a reference spectrum that does not fit the current laser setting of your experiment, the message “Cytometer setting mismatch” appears.

6. Click desired Fluorochrome in **Compensation Matrix** > click **Move** to display the desired Fluorochrome in **Selected Fluorochromes**.
7. Repeat the procedure 6. and click **Load** when you finish selecting all Fluorochromes.

Note : Negative populations cannot be imported, because the library only contains already calculated reference spectra.

Fluorochrome	Negative	Positive
FITC	<input type="radio"/> ---	<input checked="" type="radio"/> Imported
PE	<input type="radio"/> ---	<input checked="" type="radio"/> Imported
APC	<input type="radio"/> ---	<input checked="" type="radio"/> Imported

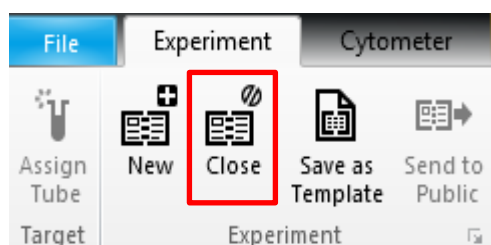
Acquire test samples

Measure the test sample

1. Put a MIX-stained test sample in a 5 ml round tube, and place the tube in the loader.
2. Change the name of the tube.
3. Click **Preview** to start sample flow.
4. Make sure that the **Total Event** in **Stopping Condition** is checked and the value is set to the desired number of events to be recorded.
5. Click **Acquire** to record data.
6. Recording automatically stops. Eject the tube.
7. Repeat these steps for all sample tubes.

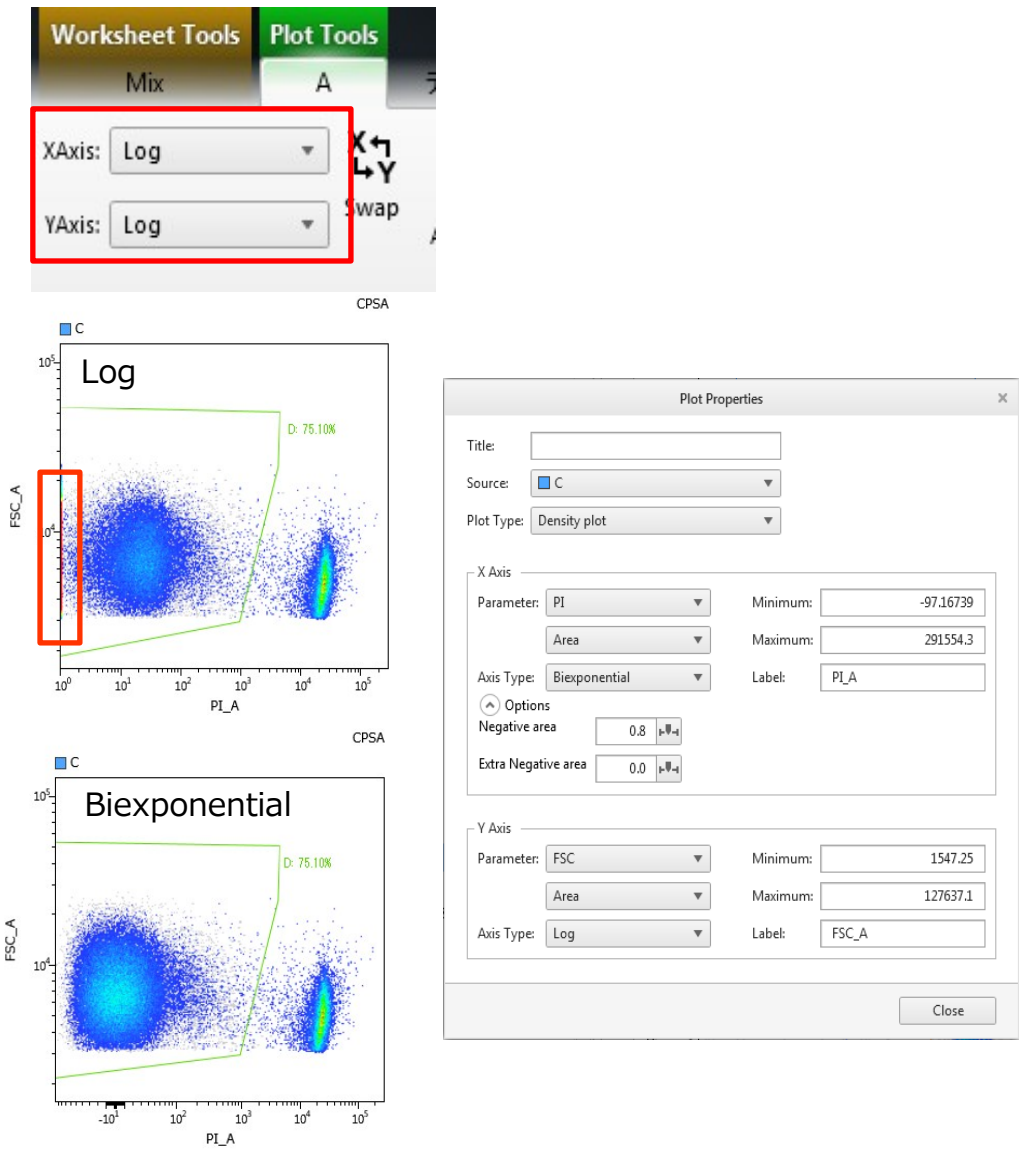
Close Experiment

- After finishing all measurement, click **Close** in **Experiment** menu on **Experiment** tab to close Experiment.
- The experiment will be automatically opened in the **Analysis** tab.



Change and adjust the scaling of the plot axis

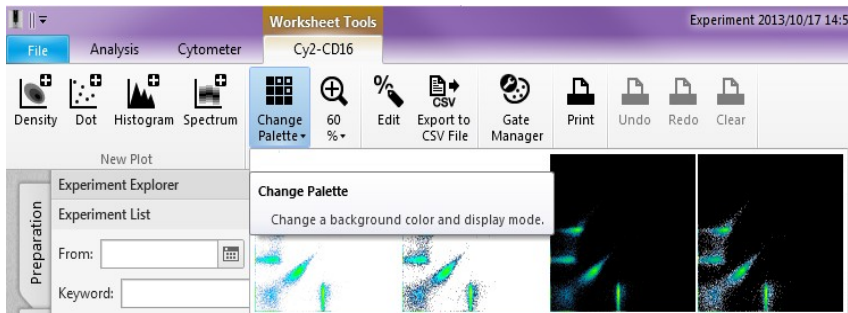
Change the axis display, if many events appear piled up on the lower end of the axis. The default axis type for fluorescence intensity is Log scale. You can change the scaling of the axis in the **Plot** tools tab by choosing the appropriate scaling type using the pull-down menu for X and Y axis. Alternatively, you can open the **Plot Properties** menu by right-clicking on the plot and selecting **Properties**.



Change background color of plots

If the sample you want to display occurs rarely, you can change the sample display mode to view the events using the **Change Palette** function on the ribbon.

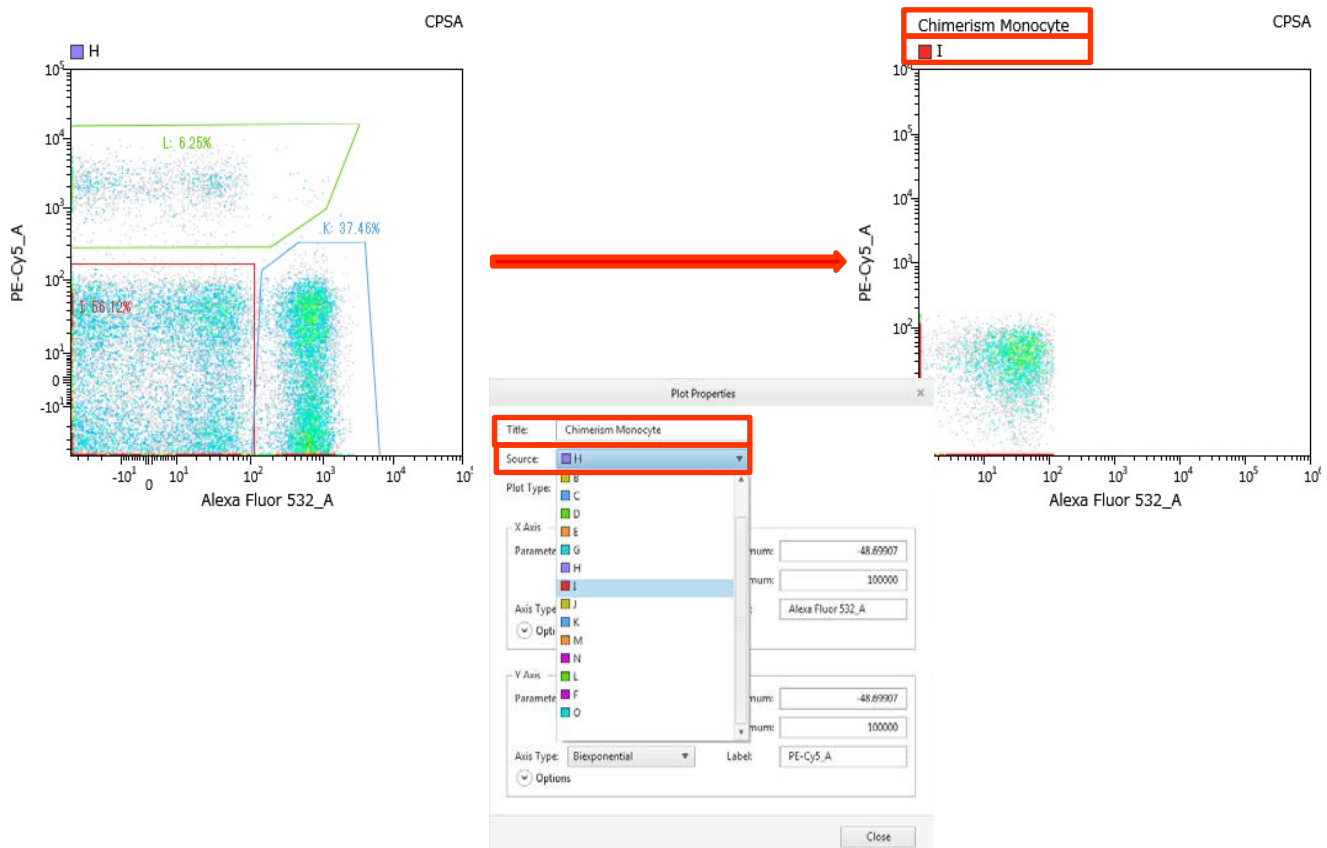
Note : Four options are available: white or black background with rare events highlighting ON or OFF.



Change properties of gates

You can change the expression of gates

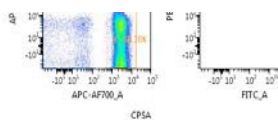
- A colored ■ marker and gate name are displayed on the top left of each plot, indicating the parent gate of the plot.
- Right-click and select **Properties** to open the **Plot Properties** window, then select from the **Source** pull-down menu to change the parent plot.
- You can also assign a title to a plot.



Display the statistics table

The statistics table is displayed below the worksheet. You can change the space allocated for the statistics table.

Note : Click **Gate Manager** button on **Worksheet Tools** tab or right- click the statistics table to open the **Gate Manager and Statistics Editor**. Use the appropriate tool if you wish to change or add items.



The statistics table

Name	Events	Parent's	Total's
All events	100,000	100.00%	100.00%
A	94,994	94.99%	94.99%
B	93,632	93.63%	93.63%
C	93,648	100.00%	93.65%
D	70,327	75.19%	70.33%
E	62,421	88.76%	62.42%
G	34,961	36.01%	34.96%
H	26,999	43.19%	26.96%
I	15,129	56.12%	15.13%
J	6,278	42.48%	6.28%
K	10,090	37.46%	10.10%
M	5,079	50.30%	5.08%
N	4,236	43.95%	4.24%
L	1,685	6.25%	1.69%
O	20,727	33.21%	20.73%
P	52	0.07%	0.05%

- Create a plot
- Open Gate Manager
- Open Statistics Editor
- Remove
- Properties

[illegible]

Name	Equation	Type	Mean	Min.	Max.	SD	CV	Median	Mode	HF/CV
A	A	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
B	(A) AND B	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
C	(A) AND B AND C	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
D	(B) AND B AND C AND D	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E	(E) AND E AND C AND D AND E	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
F	(E) AND B AND C AND D AND E AND C	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
H	(E) AND B AND C AND D AND E AND H	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I	(E) AND B AND C AND D AND E AND H AND I	<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
TFC		<input type="radio"/> Any	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Preparation

- DI Water: 2ml
- 1% solution of Hypochloric acid (Bleach, FACSClean): 2 ml

Shutting Down the Instrument



- Click **Hardware shutdown** on **Cytometer** tab.> Select **daily cleaning**.
- Follow the instructions of **Shutdown Wizard**.
Note : 2ml of DI Water and 1% solution of Hypochlorous acid are used.
Note : SP6800 will automatically power off after **daily cleaning** is finished.


Exiting SP6800 Software

- Click **X** (Close) in the top right corner of the window and follow the instructions to exit.

Turn off the Instruments

1. Make sure SP6800 is power off.
Note : If SP6800 is power on press the POWER button in its center panel for 10 seconds or more to force shutting down.
Note : Turn off the breaker inside the left door.
2. Set the power switch of the air-compressor to OFF.

Maintenance item	Frequency	Description
Refill the sheath tank		Refill the sheath tank with sheath fluid. See "4-1. Tanks" or Page95 of the Operator's Guide.
Refill the DI Water tank	When the message of "DIW Tank Empty" appears	Refill the DI Water tank with DI Water. See Page95 of the Operator's Guide.
Cleaning the sample line and sample probe	At the end of each day's operation	Run Daily Cleaning . See 10-2. Shutting Down the Instrument or page 89 of the Operator's Guide.
De-bubbling the sheath filter	Check sheath filter daily to weekly, and de-bubble as necessary.	Loosen the air release port and vent air bubbles. See page 91 of the Operator's Guide. 
Disposing compressor waste fluid	Once per week to month	Empty the auto drain tank of the air compressor.
Replacing the DI water tank and air filter of waste tank	Once per month	Shut down the main unit, remove the air filter, and replace with a new filter. See Page95 of the Operator's Guide. 
Replacing the sheath tank and the sheath filter		As for replacing the sheath tank - Shut down the main unit, pull the ring-pull air release valve up to vent the residual air-pressure air in the tank, and replace with a new sheath tank. As for replacing the sheath filter - When you set the new sheath filter, connect the connector at bottom side at first, then connect the one at top side and DO de-bubbling (see above).

Issue	Cause	Solution
<ul style="list-style-type: none"> Sample is not measured Number of measured samples is shortage 	Clogged sample	<ul style="list-style-type: none"> Clean the sample line and sample probe. Filter the sample before measurement using a nylon mesh to prevent sample fluid clogging.
	Pressure leaking from the tube	Use only tubes that have a flat surface at the top. <ul style="list-style-type: none"> Recommended tubes : BD Falcon polystyrene 5 ml tubes Cat. 352003,352008,352054, 352058,352235
	Air bubbles inside the sheath filter	Loosen the air release port and vent air bubbles. See <i>page of 95 of the Operator's Guide</i> . 
	Pressure leaking from the Sheath tank	Confirm the sound of leaking air is heard and the lid of the sheath tank is attached firmly to the tank. Re-attach the lid of the Sheath tank. See <i>4-1. Tanks</i> .
Failure to recognize sample tube	Tube sensor error	<ul style="list-style-type: none"> Do not mark or label the top 15 mm of the tube using a marking pen, adhesive label, etc. Ensure that the sample in the tube NEVER excess 2 ml.
Pressure error	Something is wrong around the air compressor	Check follows if the pressure is under 0.4MPa. <ul style="list-style-type: none"> - Confirm the Out put Valve is open. - Confirm the Power switch is set to ON. - Confirm the AC cable is connected to the outlet
Daily/Monthly QC Failed	Clogged sample	Run Priming and clean the sample line.
	Trouble around the beads	Try QC again with new samples.
		Agitate the sample tube WELL to mix the solution and try QC again.