

On-chip Sort / Flow

User's Manual

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Soft Ver. 1.7.50
Spec2.1



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About this manual

■ Cautions regarding the protection, safety and modifications of this product

- For the protection and safety of this product and system that regulate this product, please follow the safety criteria stated in this manual. If it was not handled according to the manual, we cannot guarantee the safety of this product.
- The protection function of this product may be lost if it is not handled according to the manual.
- Please use parts and consumables provided by us for repairs and replacement.
- We do not allow any modifications done to this product.
- This manual contains the symbol below to indicate safety cautions.



: indicates safety cautions.

Instructions are provided to prevent accidents in cases where mishandling may be a threat to users' life and body due to accidents involving, for example, lasers and electrical shocks.

■ Cautions regarding the manual

- The content of this manual may change without notice. Also, the images displayed in this manual may differ slightly to what is actually displayed in the software.
- Although we ensure the manual is at its best quality, there may be some mistakes and omissions. Please contact us if you noticed any mistakes.
- We do not allow for reprint or copy of this manual in parts or in full without permissions from us.

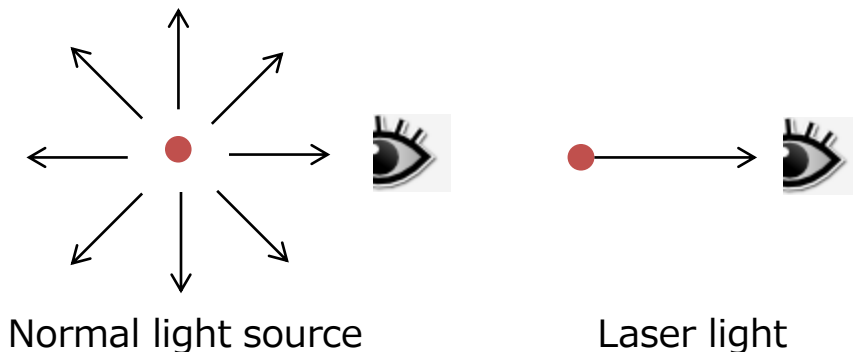
※ **NOTE** indicates important precautions or instructions

Safety and Restrictions

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0-1 Laser Safety Measures

0-1-1 Laser hazards



Laser light emitted from a laser has high power density even if the amount emitted is small. It has the potential to cause extremely serious and irreparable damage to human skin and eyes. Figure on the left is used to describe the situation. A normal light source disperses light in three dimensions, and thus the amount of light entering the eye is small. In contrary, laser light source projects light in a single direction, and thus the amount of light entering the eye is the full amount from the light source, regardless of the distance from the light source. Therefore, laser light shining directly on the eyeball can cause burns on the cornea and retina, potentially causing blindness. Such symptoms can be triggered by laser exposure that continues for only one second, so laser light must be handled with great care.



0-1-2 Precautions for safe operation

This instrument is equipped with up to three types of laser (blue (30mW), violet (30mW), red (50mW) and/or green (30mW)). Any one of them has enough output power to injure the retina if its beam enters the eye directly. Therefore, the lasers are completely enclosed in the instrument, with a structure that prevents laser light from leaking out of the instrument. However, if the instrument covers are removed or modified, exposure to harmful laser emission is a real possibility. To avoid injury to skin or eyes with no prospect of recovery, never remove or modify the instrument covers, or make mechanical repairs in areas that could come into contact with the laser warning level.

The user can use the camera installed inside the instrument to check whether the lasers are lit.



0-2 Electrical Safety

- The maximum power consumption of the instrument is 240VA. The safety of the instrument power supply is important for electrical safety. This power supply is a power supply box outside the instrument body. It is compatible with inputs of AC100–240V and supplies a rated output of 600-650W at a voltage of 12V and 24V. The switch is on the power supply box, and a blue lamp on the front of the unit body lights when the power supply is switched on. The blue lamp flashes while the instrument is in operation.
- Follow the guidelines below to avoid electric shock.
- Only connect the instrument to the power supply intended for it. Do not use extension cables. If you discover damage to any cable or plug, immediately contact us or a maintenance company.
- Except where stated otherwise in the user's manual, do not perform any maintenance or service actions on the instrument.



0-3 Biohazard Countermeasures

All biological specimens and materials that come in contact could potentially carry life-threatening disease. Follow the guidelines below to prevent exposure to biohazardous (and potentially biohazardous) pathogens.

- Handle all biological specimens and materials as having the potential to infect with disease. Devise appropriate preventive measures for used chips, and then dispose of them in accordance with local regulations. Never put them in your mouth. Wear appropriate protective clothing, safety goggles and gloves.
- When disposing of used chips, fill each reservoir of each chip before disposal with an aqueous solution of sodium hypochlorite (which may be substituted with chlorine-type bleach for home use) at a final concentration of at least 2% for at least one hour. Alternatively, seal chips in an autoclave bag or similar container and autoclave them before disposal. Dispose of waste fluid in accordance with local regulations. Devise suitable preventive measures and wear appropriate protective clothing, safety goggles and gloves.

- A chip filled with sample should be charged with sample with the chip holder attached, and carried with the chip holder still in place. If sample material etc. leaks into the chip holder, seal the chip in an autoclave bag without removing it from the chip holder, and autoclave the entire chip holder.

Chip Holder Sterilization Process

As explained in Chapter 1, the fluidic chip is used while it is mounted inside a chip holder, so it is possible that the chip holder could also be contaminated by the sample. The chip holder can be treated in an autoclave. The silicon rubber inside the holder can also be autoclaved and used repeatedly.

Refer to the following guidelines for information about laboratory safety. NCCLS documents can be ordered online.

- NIH Guidelines
- NOTICE PERTINENT TO THE MARCH 2013 REVISIONS OF THE NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES
- Schmid I, Nicholson JKA, Giorgi JV etc., Biosafety Guidelines for Sorting of Unfixed Cells. Cytometry.1997;28: 99-177
- Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline. Wayne,PA: National Committee for Clinical Laboratory Standards, 1997 NCCLS Document M29-A
- Procedures for the Handling and Processing of Blood Specimens; Approved Guideline. Wayne, PA: National Committee for Clinical Laboratory Standards, 1990 NCCLS Document H18-A



0-4 General Safety

Your hand or fingers could be trapped or injured during preparation of the instrument for operation. Avoid this by following these preventive measures.

- Take care to avoid trapping your hands or fingers while loading and unloading chips.
- Take care to avoid trapping your hands or fingers while changing consumable filters and silicon gaskets.
- The instrument weighs at least 25kg, so take care when installing it. Use the instrument without moving it after installation. Call us if the instrument is moved, because it may require adjustment.
- Do not place anything on top of the instrument.
- Take particular care to avoid liquids coming into contact with the instrument.



0-5 Warning Labels

	Label	Affixed location	Hazard
User Warnings		Power supply box	Power supply box
	 	Side of main unit	Risk of exposure to laser beam
		Side of main unit	Risk for human body and nature ecosystem
Warnings for instrument installation and service maintenance	 	Inside main unit	Risk of exposure to laser beam (The user must not remove covers from the instrument)
		Back of main unit	Move the instrument with care because it weighs at least 25kg. (This label is for maintenance use. The user should not move the instrument).



0-6 Usage Restrictions

This instrument is for research purposes only. Do not use it for any purposes other than research.



0-7 Restrictions for setting

Leave at least 5cm between the sides and back of the instrument when installing it. Place the power supply box at least 10cm away from the main unit.



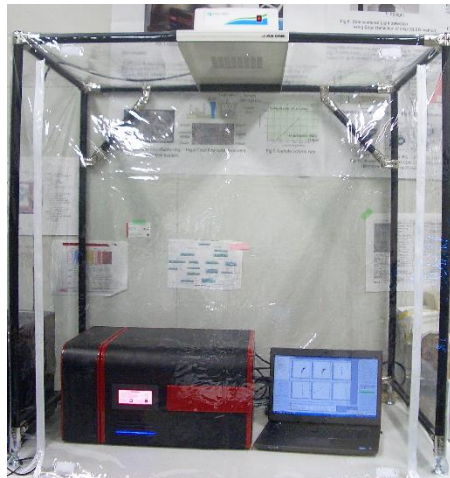
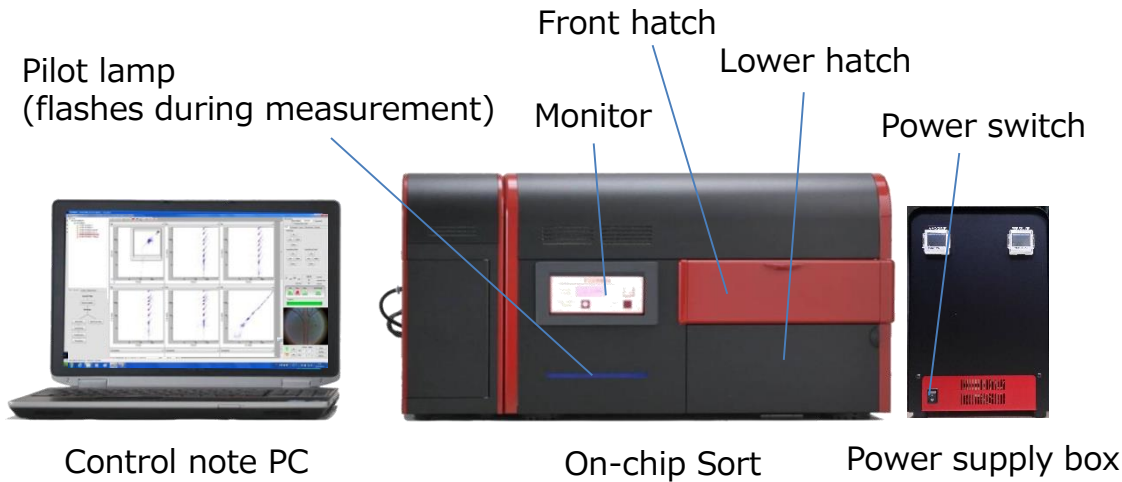
0-8 Environmental Restrictions

The optimal temperature and humidity for the instrument is 23 ± 5 °C and 40-80%, respectively. Please avoid any condensation.

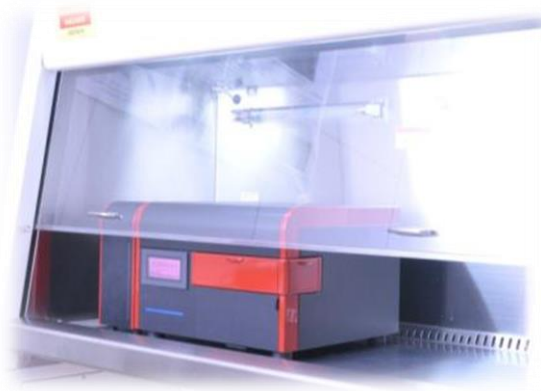
Chapter 1: Instrument Configuration

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1-1 Overview of the Instrument



« in a clean booth»



« in a bio-safety cabinet»

- On-chip Sort irradiate lasers onto cells that are flowing within a disposable and interchangeable microfluidic chip, and detects the scattered light and fluorescence signals that are generated when cells pass through the laser-illuminated area. This enables it to identify and separate individual target cells of the specified type from the group of non-target cells.

- The system consists of a regulating notebook computer, the instrument, and the power supply box. The control notebook computer and the instrument are connected by a LAN cable and a USB cable, while the power supply box and instrument body are connected by a power supply cable.

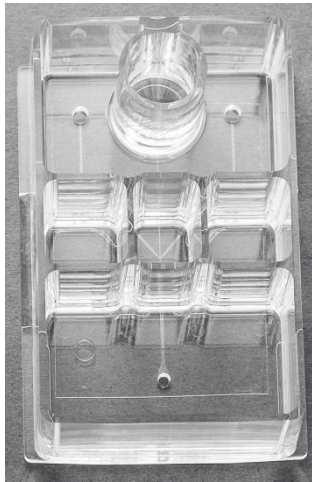
- The power supply box is compatible with AC100–240V. The power switch is on the power supply box.

- This instrument was kept to a size that can be installed in a general-purpose safety cabinet, so that its use of disposable chips gives it an advantage in biohazard and sterilization processes.

1-2 Fluidic System

1-2-1 Microfluidic Chip

On-chip Sort can use the microfluidic chip shown in the photograph on the left.



Sorting Chip (2D Chip-Z1001)

For analysis :

Main Push: ~ 8.0 kPa

Main Pull: ~ -2.5 kPa

For sorting :

Main Push: $1.0 \sim 3.0$ kPa

Main Pull: $-0.5 \sim -1.0$ kPa

Sorting chip (2D Chip-Z1001)

Material: COP

Sample volume : $20 \sim 300 \mu\text{L}$

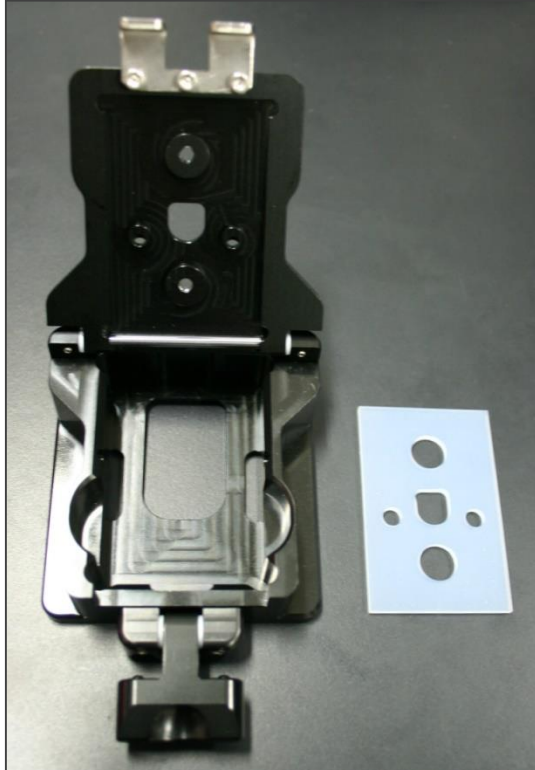
Sheath volume : 9 mL

Flow channel size (width x depth): $80 \mu\text{m} \times 80 \mu\text{m}$

Reflection edges for side scatters and fluorescence

1-2-2 Chip holder for large volume chip (Product code number : 40003)

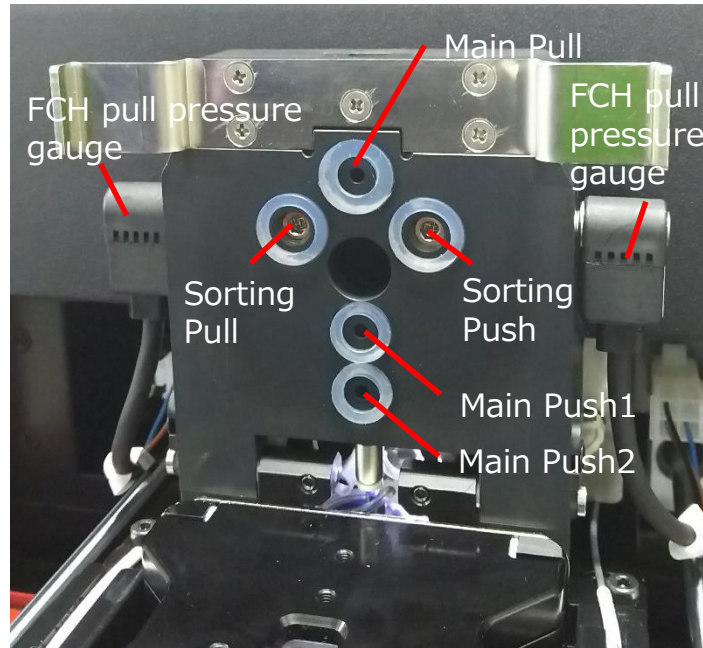
Silicon Packing for Chip holder (Product code number : 40015)



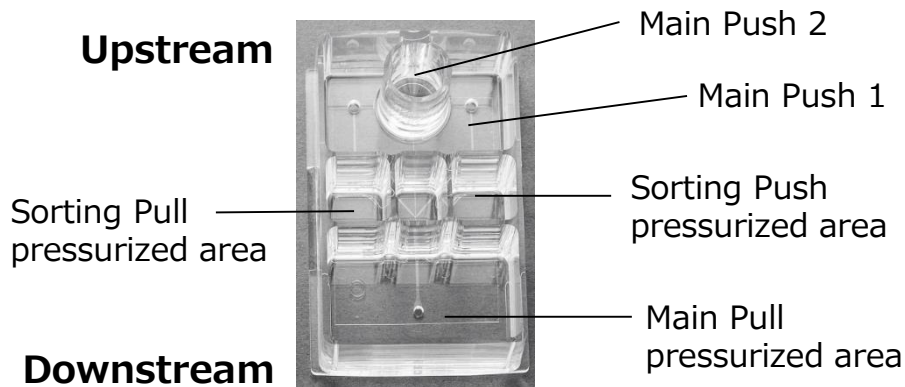
- The chip holder for large volume chip is a holder for keeping the microfluidic chip in place inside the instrument. The chip has an airtight connection with the pressure control unit through the chip holder, and air pressure is applied to it.
- It consists of the body and Silicon packing (gasket). The chip holder and Silicon packing can be sterilized by autoclave (121°C, 20min).
- Spare chip holders and silicon packings can be purchased from On-chip. Please contact our distributor for more information.

1-3 Pressure System

1-3-1 Pressure Control Unit



Pressure Control Unit



- The pressure control unit is the part that conveys the air pressure controlled by the cylinder pumps to the disposable microfluidic chip. There are multiple electromagnetic valves and pressure gauges inside this unit, and the parts that contact the chip reservoirs are made of silicon rubber.

- This unit has five pressure ports (Main Push1, Main Push2, Main Pull, Sorting Push, Sorting Pull), with a donut-shaped pad of silicon rubber around each port. As the photograph to the lower left shows, they form airtight connections with the four types of reservoir on the microfluidic chip.

- The sorting push and sorting pull ports have built-in filters to prevent the entry of foreign objects.

- The values of the pressure gauges connected on both sides can be checked from the monitor on the front of the instrument.

1-3-2 Loading Port

- The loading port is the area in which the microfluidic chip, mounted in the chip holder, is set inside the instrument.
- The procedure for opening it is as follows: Applying excessive force to close the port could break it.



① Press the top of the front hatch.



② The front hatch opens, revealing the loading port



③ Hook a fingertip into the silver knob on the loading port to pull it out.



④ The loading port makes a click when it is pulled all of the way out, so stop at that position.



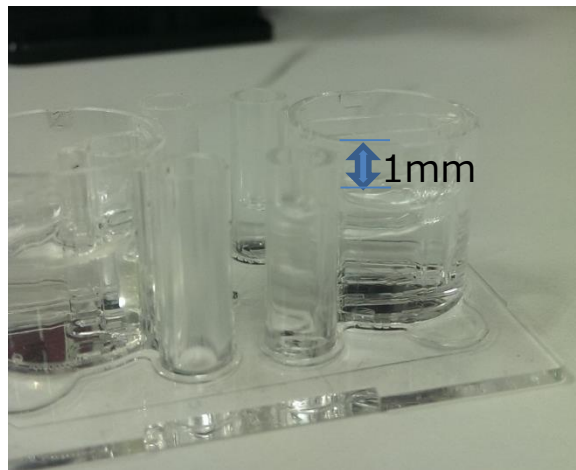
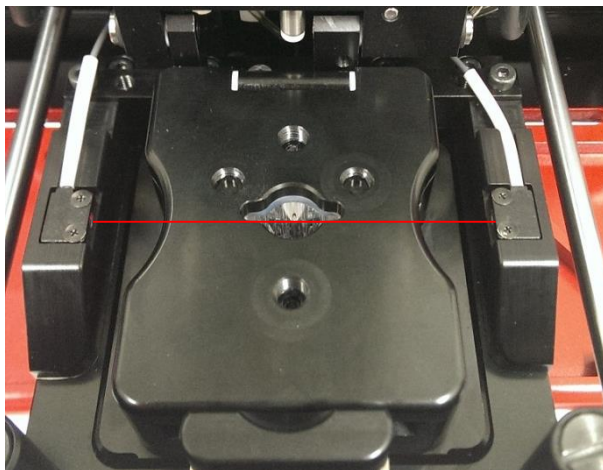
⑤ To return the loading port to its original position, first pull it forward and slowly let it return.



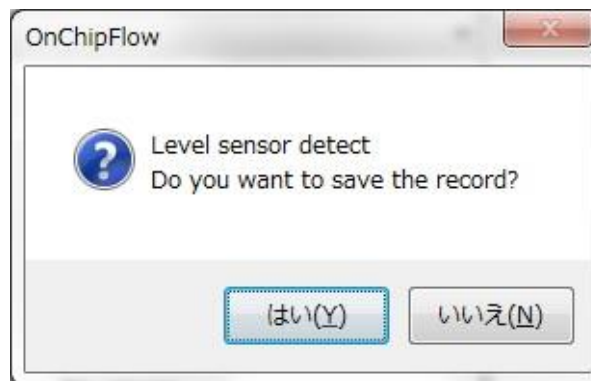
⑥ The loading port should be placed back to its original position

1-3-3 Liquid Level Sensor

This sensor is installed in the loading port, right next to where sorting reservoirs are positioned. This sensor is useful for staving off the trouble that a liquid flows from chip into the instrument. It will automatically terminate the run when the liquid level reaches 1mm below the top of the reservoir.



This message is displayed when "Check Line" process is stopped by the level sensor (see 3-3-8 Check Line).



This message is displayed when measurement is stopped by the level sensor. If you want to save the data, click the button **はい(Y)** or Yes.



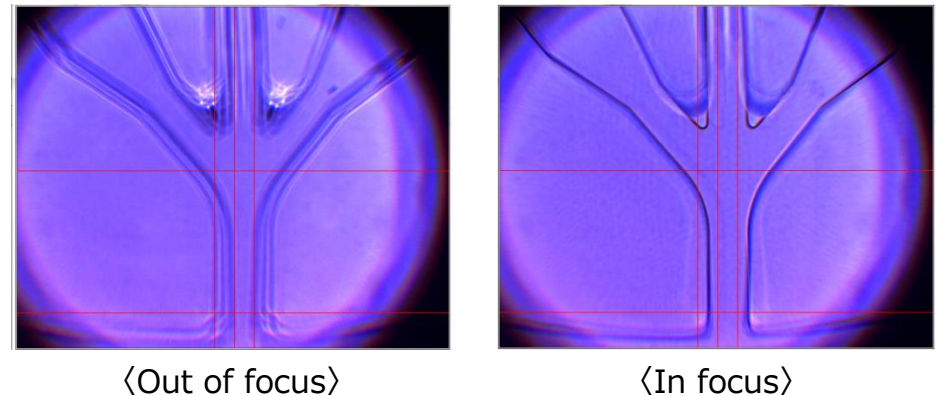
- Do not rely on the Liquid Level Sensor for termination of analysis and sorting.
- The Liquid Level Sensor is a supporting tool for prevention of liquid overflow.
- It is not a tool to prevent all types of liquid overflow, and thus users must be aware of the amount of liquid and the time of sorting for the prevention of any troubles.
- Remove liquid from the waste reservoir frequently to avoid any overflow.

1-3-4 Objective lens

You can check the image from the objective lens using the built-in CCD camera when the instrument and the PC is connected.

It is necessary to adjust the focus of the objective lens by moving it up and down when the microfluidic chip is out of focus (the channel wall is not clearly focused) as shown in the image below.

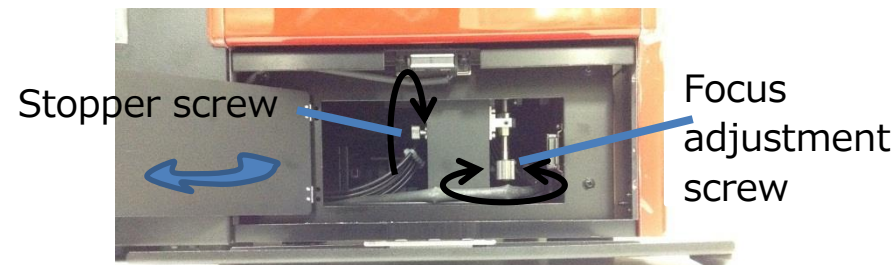
- ① Open the lower hatch by pulling the knob. Interlock operates when lower hatch is open. The lasers are turned off automatically.



- ② There is the inner hatch behind the lower hatch. Pull the knob of the inner hatch toward you and open the inner hatch.

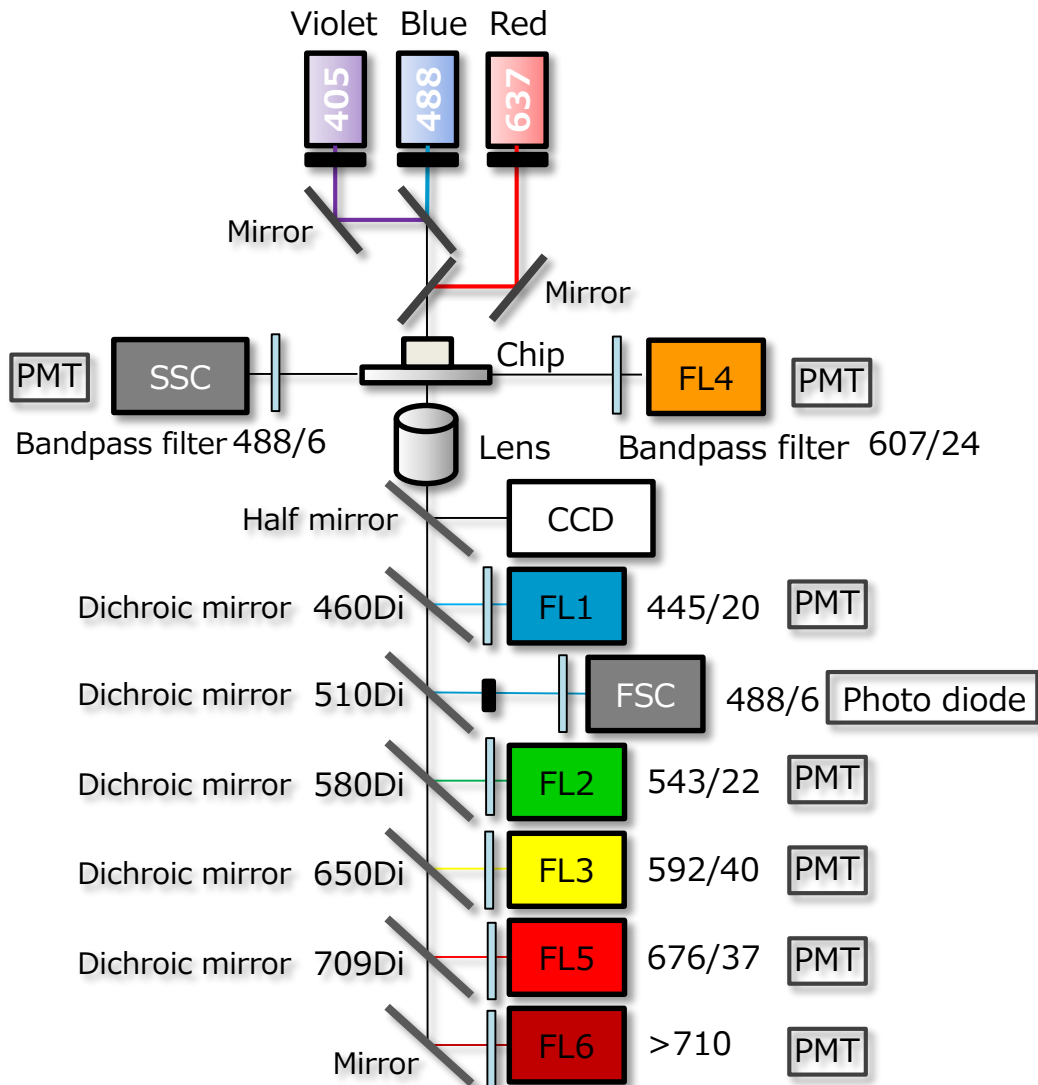


- ③ Loosen the stopper screw on the left. Adjust the height of the objective lens using the focus adjustment screw on the right. Tighten the stopper screw again to fix the focus adjustment screw in position.



1-4 Optical System

1-4-1 Overview

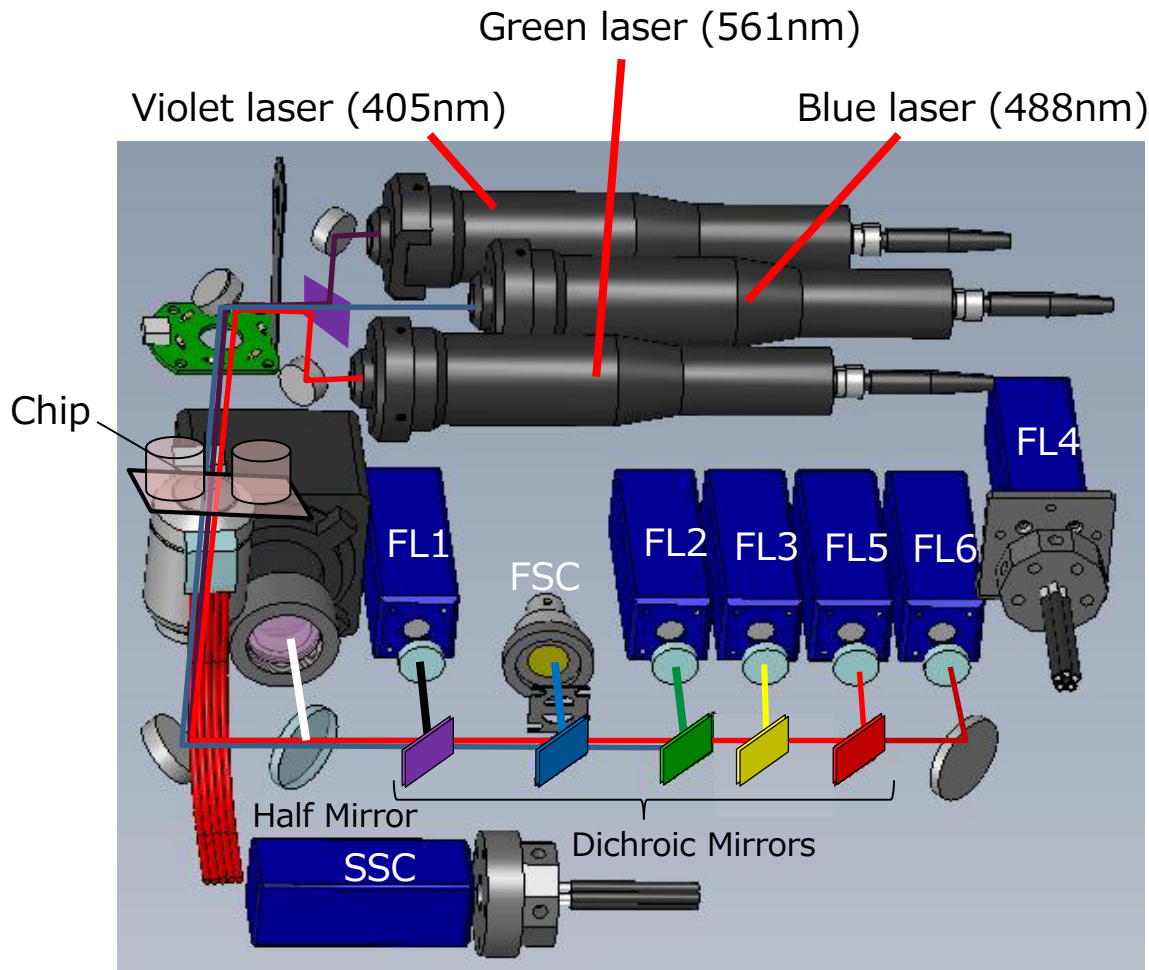


On-chip Sort is equipped with up to three lasers (violet, blue, and red or green), and can measure up to eight parameters, including forward scatter (FSC), side scatter (SSC), and six colors of fluorescence (FL1, 2, 3, 4, 5 and 6).

A laser is irradiated into the channel of the microfluidic chip, and FSC and FL1, 2, 3, 5, and 6 are detected in the transmitted light direction. SSC and FL4 are detected from the side of the chip.

The CCD is a system for observing the microchannel in the chip and the position illuminated by the laser.

1-4-2 Layout of the lasers and detectors



Lasers are irradiated from above on the microchannel inside the microfluidic chip. Past the objective lens, detectors are arranged in the sequence of CCD camera, FL1, FSC, FL2, FL3, FL5 and FL6. Dichroic mirrors are used to distribute the light to each detector, and the wavelength range detected by each detector is limited by the bandpass filters.

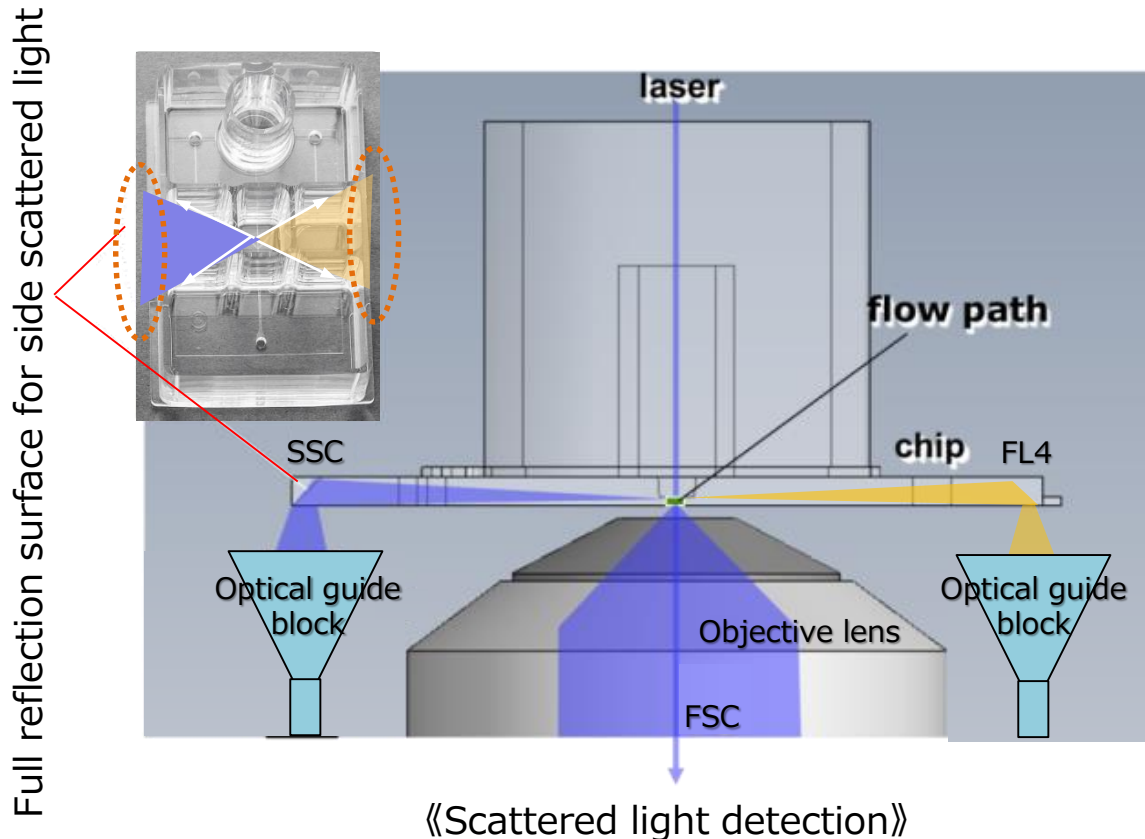
FL1-5: Bandpass filters
FL6: Longpass filters

Photodetectors

FSC: Silicon photo diode

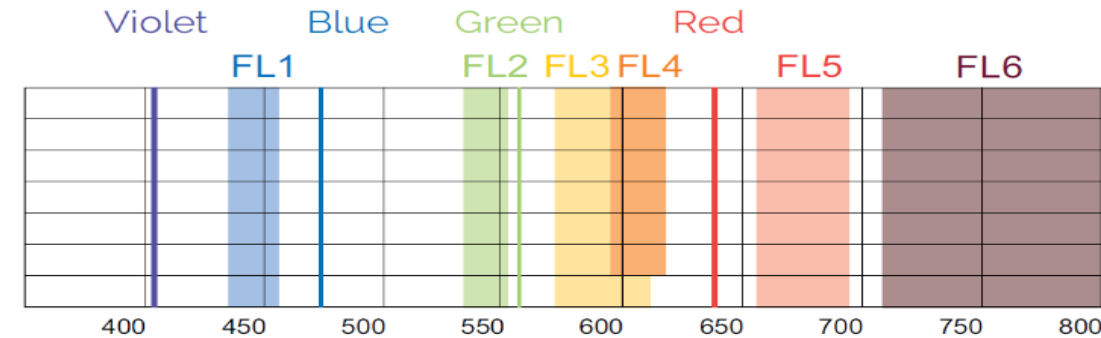
SSC, FL1, 2, 3, 4, 5, 6: Light sensor module

1-4-3 Optical system within the chip



- With a disposable chip, the optical system is displaced every time the chip is changed, necessitating a robust optical system less vulnerable to the influence of displacement. In the side scatter detection optical system, full reflection within the chip base and the light guide block are used to achieve a robust optical system for disposable chips.
- The microfluidic channels are used to narrow the sample flow to the width of $10\mu\text{m}$ or less and align the cells to a single line by sandwiching the sample with sheath flow from both sides. Lasers are irradiated from above the chip. Forward scatters (FSC) are detected vertically, and side scatters (SSC) are detected through the chip and reflected downwards by total reflection edge of the microfluidic chip.

1-4-4 Detectable fluorescence colors



On-chip Sort can be installed with up to 3 lasers (Violet, Blue, and Red or Green) and up to 6 fluorescence detectors (FL1-6). Up to 10 colors from 13 colors listed on the left can be measured. 10 color limitation is due to the signal processing circuit.

	FL1	FL2	FL3	FL4	FL5	FL6
Violet	DAPI, AF405, PacBlue, V450, BV421	PacOrange, V500, BV510	PacOrange, BV605		BV650	BV711, BV786
Blue		FITC, AF488, GFP, CFP, YFP	PE	PI, 7AAD	PerCP, PerCP-Cy5.5, PE-Cy5	PE-Cy7
Green			DsRed, mCherry, tdTomato, TagRFP			
Red					APC, AF647	APC-Cy7, APC-H7, AF700

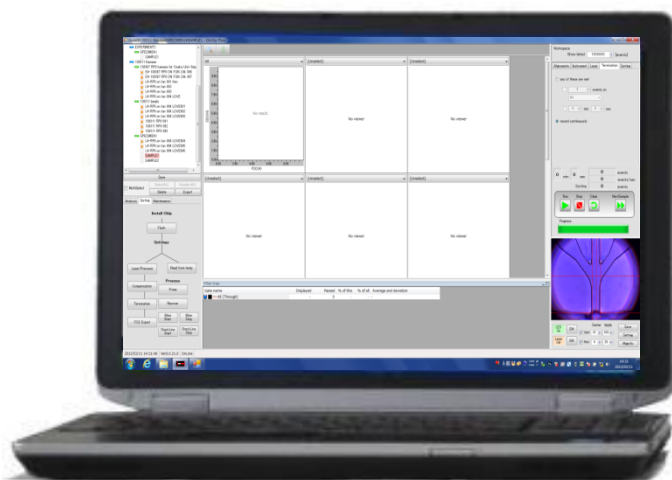
Optics

Laser	Up to 3 Lasers from 405 nm, 488 nm, 561 nm, 637 nm, 785 nm
Measurement parameter	Forward scatter (FSC), Side scatter (SSC), 6 PMTs (up to 10 parameters)
Pulse analysis	Height, Area, Width
Detection wavelength	FL1 (445/20 nm), FL2 (543/22 nm), FL3 (591.5/40 nm) FL4 (607/24 nm), FL5 (676/37 nm), FL6 (>710 nm)

1-5 Control PC

The specification for the notebook computer that controls On-chip Flow/Sort is as follows:

It is connected with the body of the instrument by a LAN cable and a USB cable.



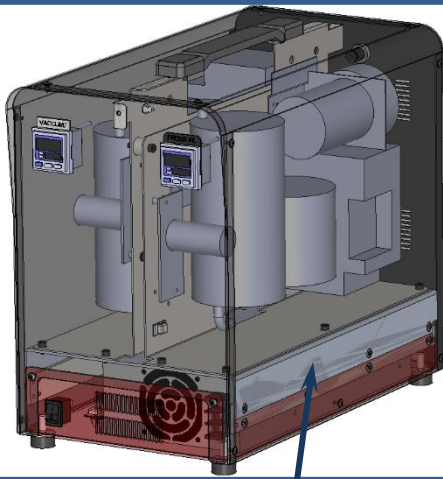
Control notebook computer

- LCD:15.6 inch(1920 x 1080)
- OS:Windows10 64bit
- CPU: 2.70GHz 2.71GHz (Core i7)
- Memory: 8GB

- NOTE**
- ① If the notebook computer has WiFi turned on, or if it has antivirus software installed, faults and possible interruption can occur in communications with the instrument, so care is required.
 - ② Please use a high spec LAN cable (cat.7) for LAN cable between the instrument and PC.

1-6 Power Supply Box

The detailed specification of the power supply is as follows:



Internal unit power supply (ACE650F by COSEL)
Rated power output 600W (50-150V)
650W (170-250V)

- Safety standards
UL60950-1, C-UL (CSA60950-1), EN60950-1,
EN50178 compliant, Electrical Appliance and Material
Safety
Law compliant.
- CE Mark compliant
Low-voltage directive
- Environmental compliance
RoHS directive
- EMI standard
FCC-B, CISPR22-B, EN55022-B VCCI-B compliant
- EMS (immunity) :
EN61204-3
EN61000-6-2
EN61000-4-2 compliant (static electrical discharge)
EN61000-4-3 compliant (radiated, radio-frequency,
electromagnetic field)
EN61000-4-4 compliant (fast transient burst)
EN61000-4-5 compliant (lightning surge)
EN61000-4-6 compliant (conducted disturbances
induced by radio-frequency,
electromagnetic field)
EN61000-4-8 compliant (power frequency magnetic field
immunity)
EN61000-4-11 compliant (voltage dips and short
interruptions)



This Power Supply Box is for use of On-chip Sort.

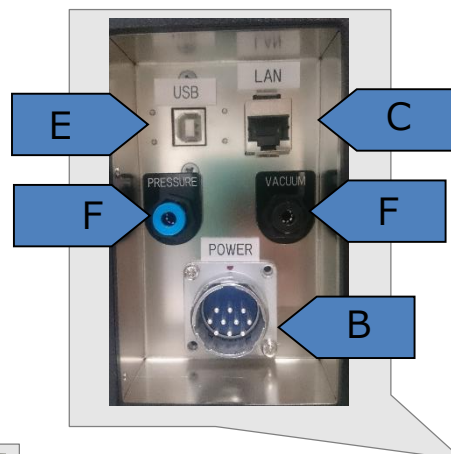
1-7 Connection Method

The connection methods and cable types between the instrument body and the PC are as follows:



NOTE

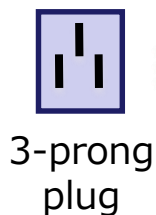
Power supply cable A must always be plugged into a 3-prong outlet.



- A Power supply cable
- B Power connection cable
- C LAN cable
- D PC power supply cable
- E USB cable
- F Pressure supply tube



Main Unit
Side



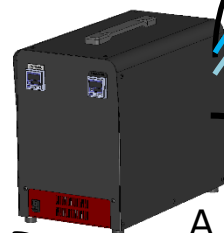
3-prong
plug



2-prong
plug



B



A



C



E



B

F

F

D

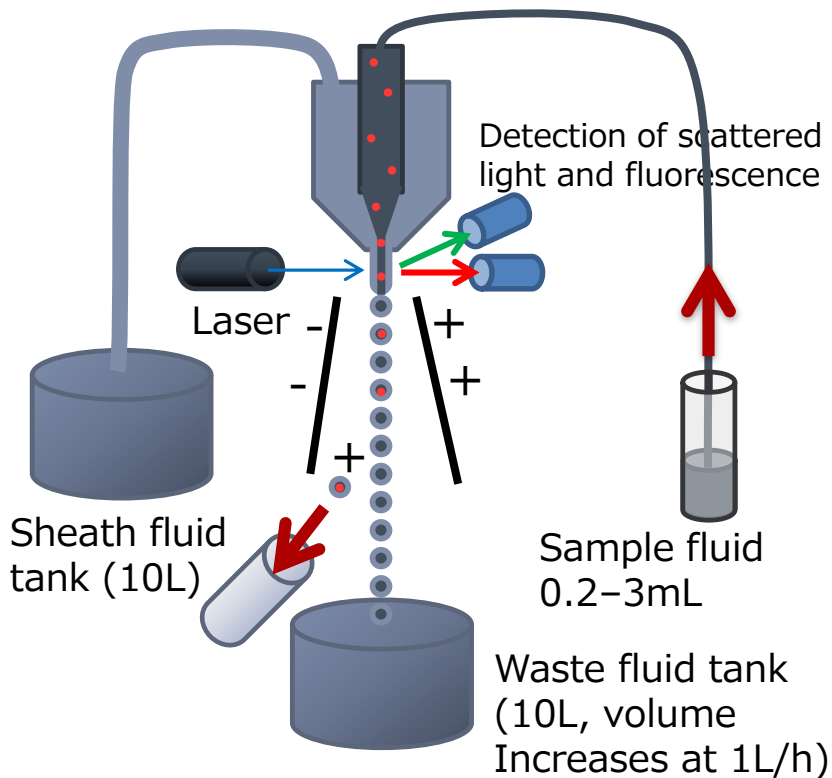


Chapter 2: Principles of Flow Cytometry

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2-1 Overview

2-1-1 Principles of the general flow cytometry method



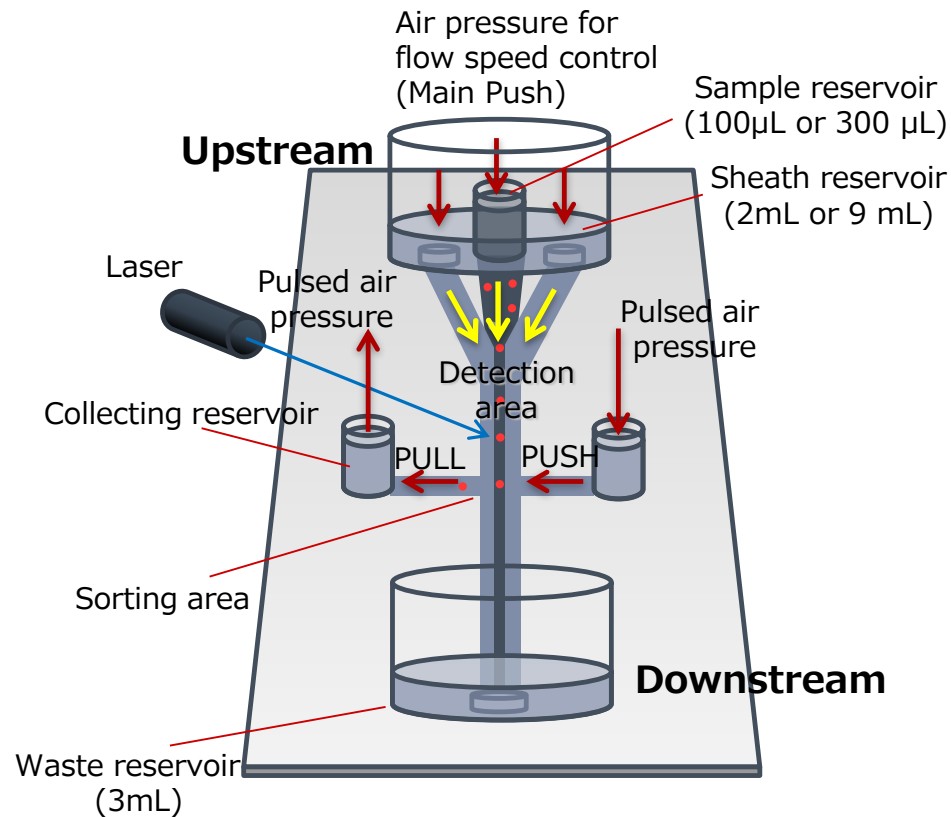
Configuration of a conventional
Jet-in-Air type cell sorter

In conventional flow cytometry methods, the flow channel system is fixed and the sample fluid and sheath fluid are pushed into the flow channel system by application of 3–5 atmospheric pressure. The sample fluid, surrounded by sheath fluid, flows through the aperture, which produces a high flow speed of approximately 10m/s. It is irradiated by lasers, and the scattered light and fluorescence are detected. In a cell sorter, ultrasound is applied in a nozzle to form droplets, and droplets that include target cells are electrostatically charged, so that individual droplet units can be separated by electrostatic force in a high electric field.

Shortcomings of the conventional method includes:

- 1) Instrument adjustment takes a long time.
- 2) Consumption of sheath fluid is high, with large volumes of waste fluid. (approximately 1L/h)
- 3) Cells are extensively damaged
 - Cells are pressurized at 3 atmospheric pressure or more
 - Cells collide with collection tubes at a high speed of 10m/s
 - Cells are exposed to ultrasound
 - Cells are exposed to an electric field
- 4) There is cross-contamination between samples

2-1-2 Principles of sorting by On-chip Sort



Sorting within the disposable microfluidic chip

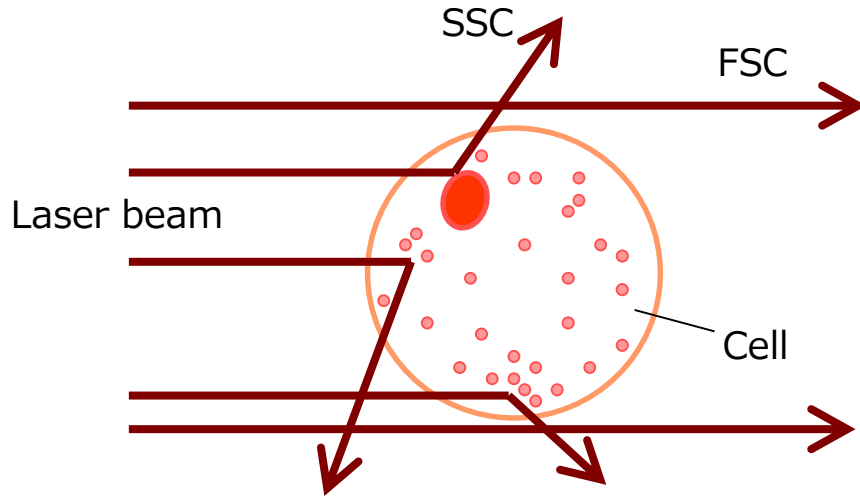
A laser is irradiated onto cells that are flowing within a disposable microfluidic chip, and the fluorescence signals that are generated when cells pass through the laser beam are detected in order to identify and separate individual target cells of the specified type from the group of non-target cells.

Sample solution within the sample reservoir is narrowed down to a flow width of 10 μ m or less by sheath fluid sandwiching from both sides, so that cells flow in a single file of individual cells. The scattered light and fluorescence which are generated when cells pass through the detection area are used to judge in real time whether or not a cell is of the target type. If a cell is of the target type, it is taken into the collecting reservoir by push-pull pulse flows from both sides in the moment that the target passes through the sorting area. Non-target cells flow into the waste reservoir. This process sorts target cells from non-target cells.

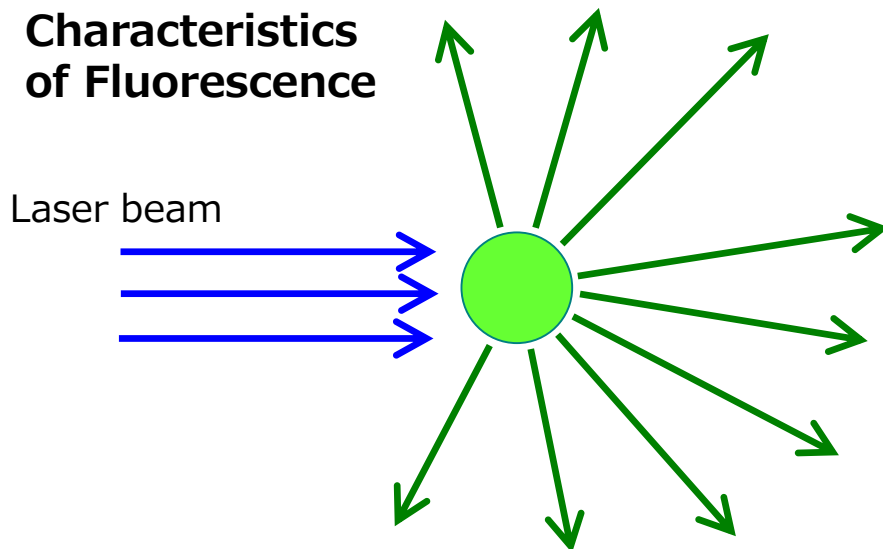
The flow speed is approximately 30cm/s, which is approximately 1/30 the speed of the Jet in Air method. The pressure used to make the sample flow is 1/100 of the conventional sorter. The sorting force is only pulse flow, so the damage to cells are minimal.

2-2 Signal Formation

Characteristics of Scattered Light



Characteristics of Fluorescence



FSC

Light which scatters forward at a small angle to the direction of the laser light axis is called forward scatter (FSC). The range of scatter angle distribution tends to vary according to the size of the scattering body. The FSC signal is mainly a signal generated by gathering light scattered in small angle, so it mainly reflects information on the sizes of the scattering body (cell).

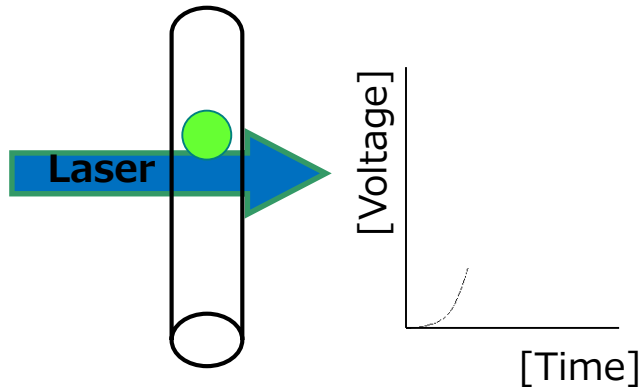
SSC

Light which scatters roughly perpendicular to the laser light axis is called side scatter (SSC). The SSC signal is mainly a signal generated by gathering light scattered in large angle, so it mainly reflects information on the fine structures smaller than the scattering body (cell). Therefore, the two-dimensional distribution of FSC and SSC can be analyzed for the size and complexity of the cell, and it is used for classifying unstained white blood cells.

Characteristics of Fluorescence

Fluorophores are excited by laser light, absorbing part of its energy and emitting the remainder as light. This light is called fluorescence. Fluorescence is distributed towards longer wavelengths than the excitation wavelength of the laser. The excitation wavelength and fluorescence wavelength differ according to the category of fluorophore. The main methods for cell labelling are the nuclear stain method and the fluorescent antibody stain method.

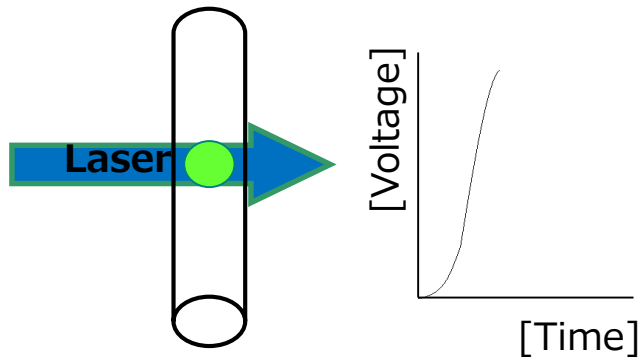
《 Signal Formation 》



When cells and particles pass through the laser beam, they emit fluorescence.

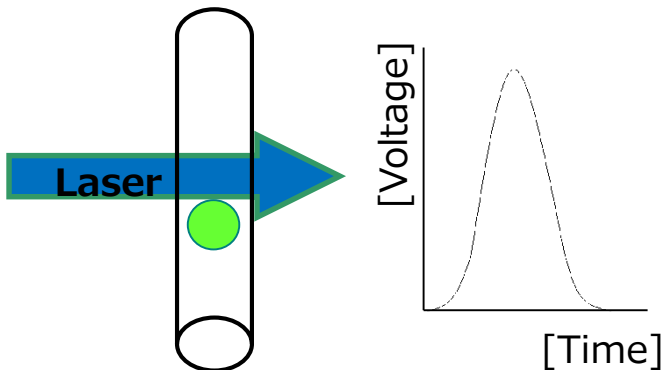
The photomultiplier (PMT) detects this fluorescence and converts it to an electronic signal called a pulse.

The pulse starts when the particle has advanced into the laser beam.



When the particle has reached the middle of the beam, the pulse intensity and the strengths of the beam and signal reach their peaks.

The peak intensity and pulse height are measured at this point.



The pulse ends when the particle leaves the beam.

2-3 Signal Detection

Threshold is the value which, when exceeded by the signal value, initiates assessment. The threshold is relative to the Base. The Base level is derived from the background light that enters the detector even when no cell is passing. This background light is caused by the autofluorescence of the flow cell, fluorescent molecules in the buffer, and light leaking into the instrument. It changes over time, so the threshold is set to avoid detecting such variation, so that only cells and other particles are detected.

This instrument can measure following three signal parameters:

- Height (H)

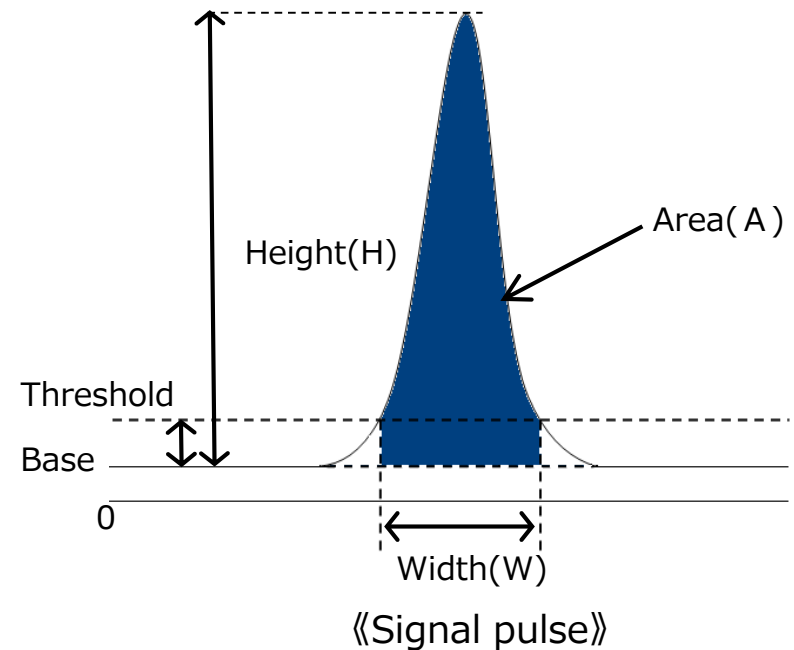
This is the pulse height relative to the signal base level. It is proportional to the size of the signal. This value is almost entirely independent of flow speed. On this instrument, height is used as the standard analytical quantity.

- Width (W)

This is the width, in the time scale, of the signal that exceeds the threshold. This corresponds to the time taken for the cell to pass through the laser beam area. It depends on cell size, flow speed, and laser beam width.

- Area (A)

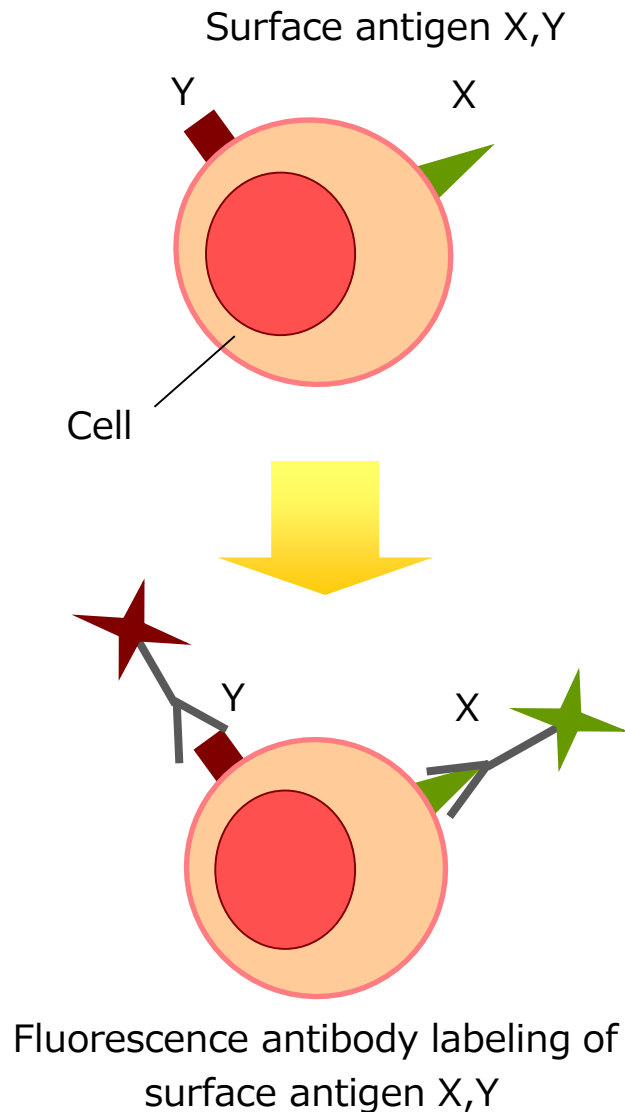
The area of the pulse within the width. The area of FSC depends on cell size, flow speed, and laser beam width. The area of FL1 etc. depends on cell size, fluorescence label quantity, flow speed, and laser beam width.



« Example of analysis using area »

In analysis of cell cycles etc., distinguish between individual cells and cell masses, and derive the histogram for the DNA stain quantity in single cell. The method for distinguishing cell masses is to use the correlation diagram between area and height

2-4 Fluorescent Labeling



Fluorescent Labeling by antibody

Prepare an antibody that specifically binds to the antigen in the target cells, and attach a fluorophore to that antibody. This fluorescence antibody probe is used as a specific fluorescence label for the antigen within the cell.

Fluorescent Labeling of Cell Surfaces

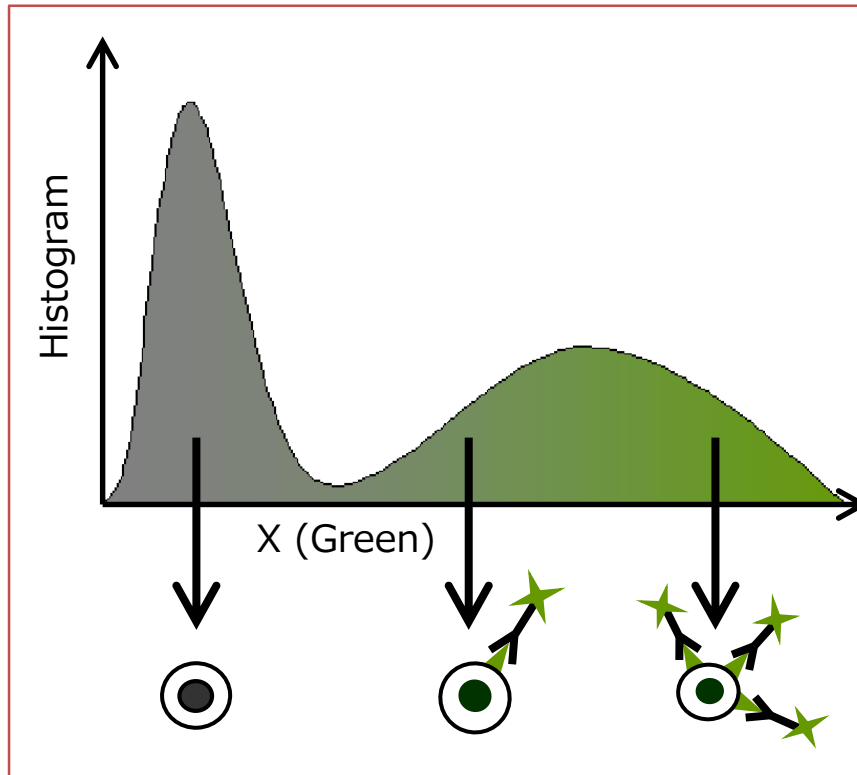
There are various types within the cell surface antigens used for antibody fluorescence labeling, and CD (cluster designation) categories are known as international classifications of monoclonal antibodies which join to molecules (**surface antigens**) present on various cell surfaces, of which human white blood cells are the main type. White blood cells and other cells express various molecules, produced from glycoproteins etc., on cell surfaces, and it is possible to identify subtle differences between cells by distinguishing the differences in those molecules. These molecules can be identified as antigens to which monoclonal antibodies bond, and are called **surface antigens** or **surface markers**.

Surface markers can label cells from the outside, so they are capable of fluorescent labeling of living cells.

Fluorescent Labeling within Cells

Fluorescence antibody labeling within a cell requires opening a hole in the cell membrane large enough to pass an antibody through, so it cannot be done with a living cell. Fluorescent labeling of substances such as cytokeratin is one example.

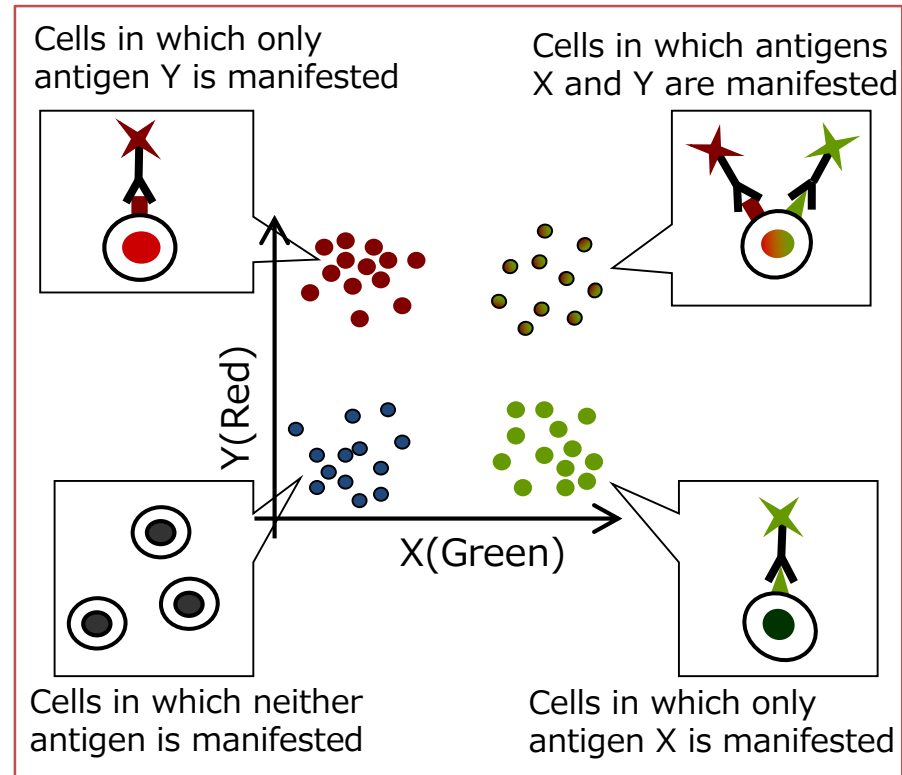
2-5 Analysis of Fluorescence Signals



The position at which a cell appears on the graph above varies according to the presence of antigen expression and the amount of expression.

Cells which do not have antigens (in this case, non-target cells) appear at a position on the graph close to the x-axis origin.

On the other hand, a cell carrying a large amount of antigen (a target cell) will appear at a position with a large x-axis value.



This kind of compound analysis is possible if a cell is stained with two types of antibody and pigment.

Chapter 3: Software Configuration

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3-1 Basic Configuration of the Software

I. Sample management

V. Instrument Settings

III. Work space

I. Sample management

Manage and use sample files in a tree

II. Processing zone

Check and run task processes

III. Work space

Dot plots, gating, histograms, axis setting for scattered light and fluorescence parameters

IV. Population management

Population information for each gate

V. Instrument settings

Setting chip positioning, laser, Gain detectors, etc.

VI. Operation controls

Control of analysis and sorting actions

VII. Camera view

CCD image

Each boundary lines can be moved up, down, left, and right.



II. Processing zone

IV. Population management

VII. Camera view

VI. Operation controls

Measurement date : 2015/10/09 16:54:17 | Ver1.7.29.0 | Offline | Laser1:0h; Laser2:0h; Laser3:0h; SortingPulse:0; PowerOnTime:0h; MeasurementTime:0h; MPU:20141007; FPGA:20140106; SBC:sn-36253001-00037-283; PC:Ver1.7.29.0; 2015/10/12 02:20:59

Displays measurement date, software version, laser operation time etc

3-2 I. Sample management


Sample files can be managed and used in a tree.


The tree area has a four-tiered structure, and normal sample files are saved in the lower tiers under “Guest”. Under that, there are the Experiment tier, the Specimen tier, and the Sample tier that is actually used for saving data. The Experiment and Specimen tiers can be opened and closed. For example, if Specimen is opened, the status of the sample files on lower tiers can be seen as shown on the right.


Sample file selected is in white letters and highlighted in blue. **20131001-Export-Import**

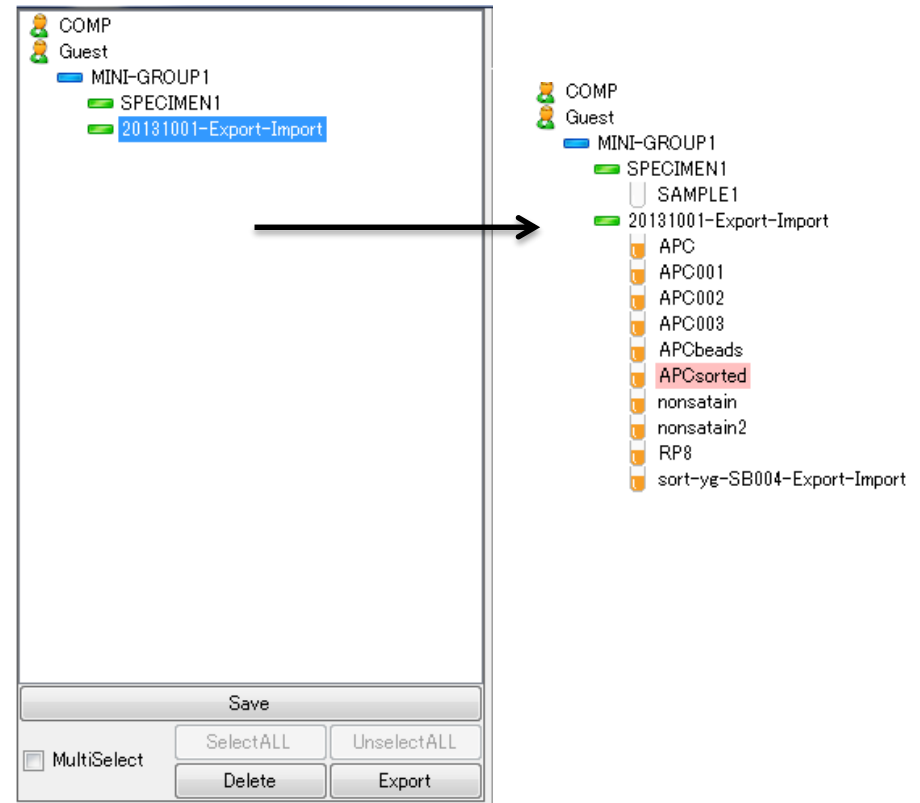
The blue background changes to pink after another operation is done, such as clicking on a button. **APCsorted**

There are two kinds of sample files:

An empty sample file is represented with an icon of a empty test tube. 

A sample file with measurement data is represented with an icon of a test tube with orange solution. 

A sample file with fluorescence compensation has its file name in blue letters.  **sort001**



3-2-1 Guest layer

When each layer is selected then right clicked, a pop-up menu appears.

NOTE Please make sure you left click a sample file first. Right clicking on an unselected file will open a pop-up menu for already selected file or folder.

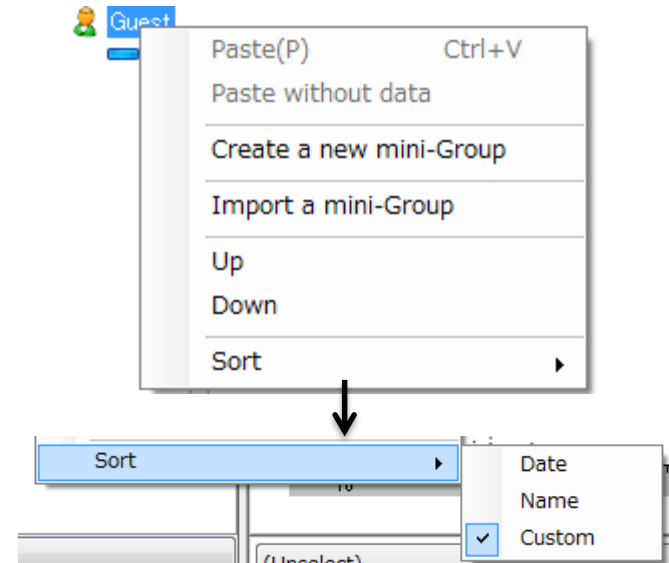
A file or a folder in mini-group, specimen or sample layer can be copied and pasted to one layer above (e.g. a mini-group can be pasted to Guest layer. "Paste without data" keep the layer information and settings, but create a sample file without any measurement data.

A new file can be created in guest, mini-group and specimen layer to one layer below (e.g. right click, then "Create a new sample" on a specimen file will create a sample file in sample layer). An exported file or folder can be imported in the same manner.

"Sort" at any layer will change the order of files within a folder that contains more than one file.

NOTE Selecting "Date" or "Name" will change the order of files/folders in layers other than Guest.
"Custom" is recommended.

When Custom is selected, then files can be moved up or down.

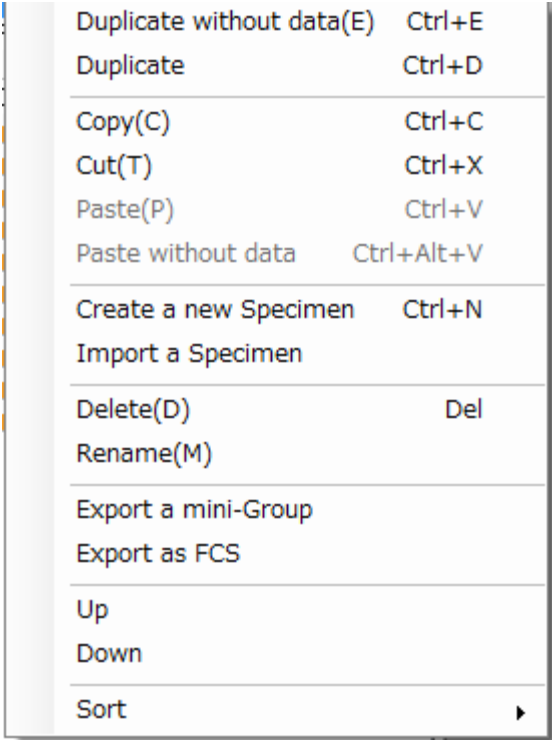


3-2-2 MINI-GROUP layer

In MINI-GROUP layer and below, one can create an exact copy of a sample file by selecting “Duplicate” or an empty file with all the gating and settings inherited by selecting “Duplicate without data”.

Similarly, one can use a copy and cut functions by selecting “Copy” and “Cut”, respectively. For “Cut”, the name of a folder or a sample file turns gray, and the item can be pasted to a desired location. Doing other actions such as coping another file or folder cancels the cut action, and the file name turns back to black lettering.

These folders and files can be exported and saved in On-chip Flow’s original format (Export a mini-Group, etc) or in FCS file format (Export as FCS).

A screenshot of a context menu for a MINI-GROUP layer. The menu is displayed over a light gray background. It contains several options, each with a keyboard shortcut to its right. The options are: 'Duplicate without data(E)' (Ctrl+E), 'Duplicate' (Ctrl+D), 'Copy(C)' (Ctrl+C), 'Cut(T)' (Ctrl+X), 'Paste(P)' (Ctrl+V), 'Paste without data' (Ctrl+Alt+V), 'Create a new Specimen' (Ctrl+N), 'Import a Specimen', 'Delete(D)' (Del), 'Rename(M)', 'Export a mini-Group', 'Export as FCS', 'Up', 'Down', and 'Sort'. The 'Sort' option has a small right-pointing arrow next to it.

Duplicate without data(E)	Ctrl+E
Duplicate	Ctrl+D
Copy(C)	Ctrl+C
Cut(T)	Ctrl+X
Paste(P)	Ctrl+V
Paste without data	Ctrl+Alt+V
Create a new Specimen	Ctrl+N
Import a Specimen	
Delete(D)	Del
Rename(M)	
Export a mini-Group	
Export as FCS	
Up	
Down	
Sort	

3-2-3 SPECIMEN layer

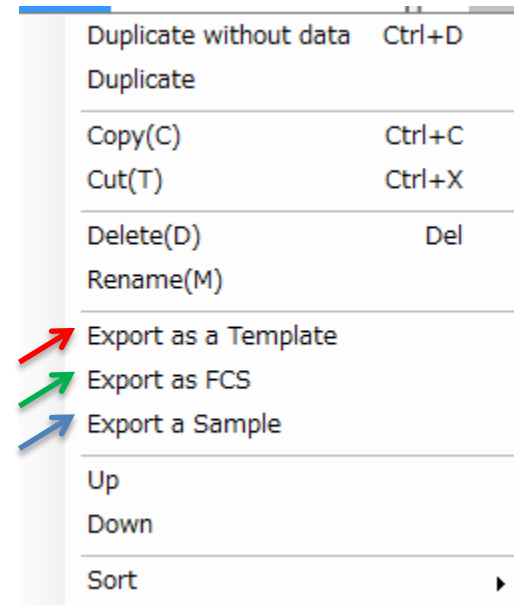
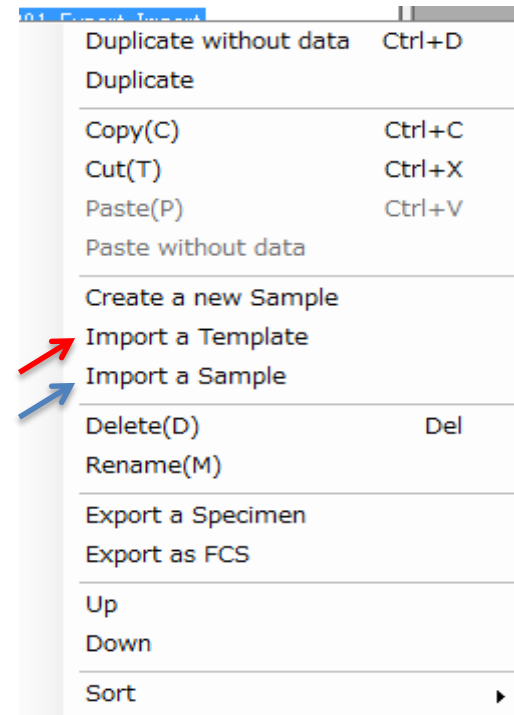
In SPECIMEN layer, you can import a sample file (Import a Sample) that was previously exported, but a sample file previously exported as a Template file can also be imported (Import a Template). In this case, an empty sample file without any data is created.

3-2-4 SAMPLE layer

When you select 'Export as a Template' column, your sample file is exported as your template file. In this case, an empty sample file without any saved data is exported.

NOTE: Sample layer appears at the lowest layer in On-chip Flow software, but it appears as a folder once it is exported as it is actually an aggregate of multiple files.

Sample files exported in FCS format is exported as a singular file with .fcs extension.



3-2-5 File management button

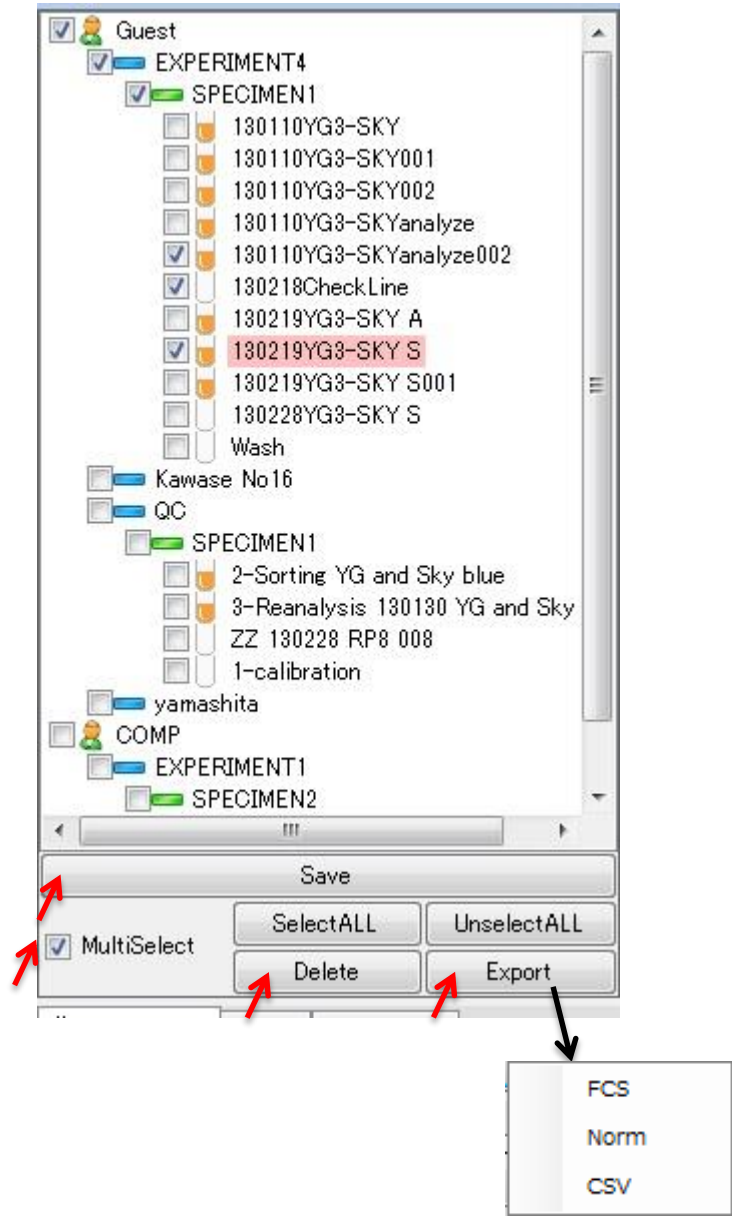
•The buttons under the tree can be used to perform operations on sample files and the tiers above them.

•**Save button**: In a sample file with saved measurement data, changes to gates and values etc. are not saved, so to save changes, it is necessary to press the Save button before opening another file.

•**MultiSelect check box**: If this checkbox is checked, checkboxes appear on all tiers of the tree, making it possible to select multiple items. Also, **SelectALL button** and **UnselectALL button** becomes enabled.

•**Delete button**: Delete selected sample file etc.

•**Export button**: The selected tier can be exported with its tier structure and saved data. Exports can be in FCS for FCS format, Norm for On-chip's format, and CSV for spreadsheet format.



3-3 II. Processing zone

Check and run task processes

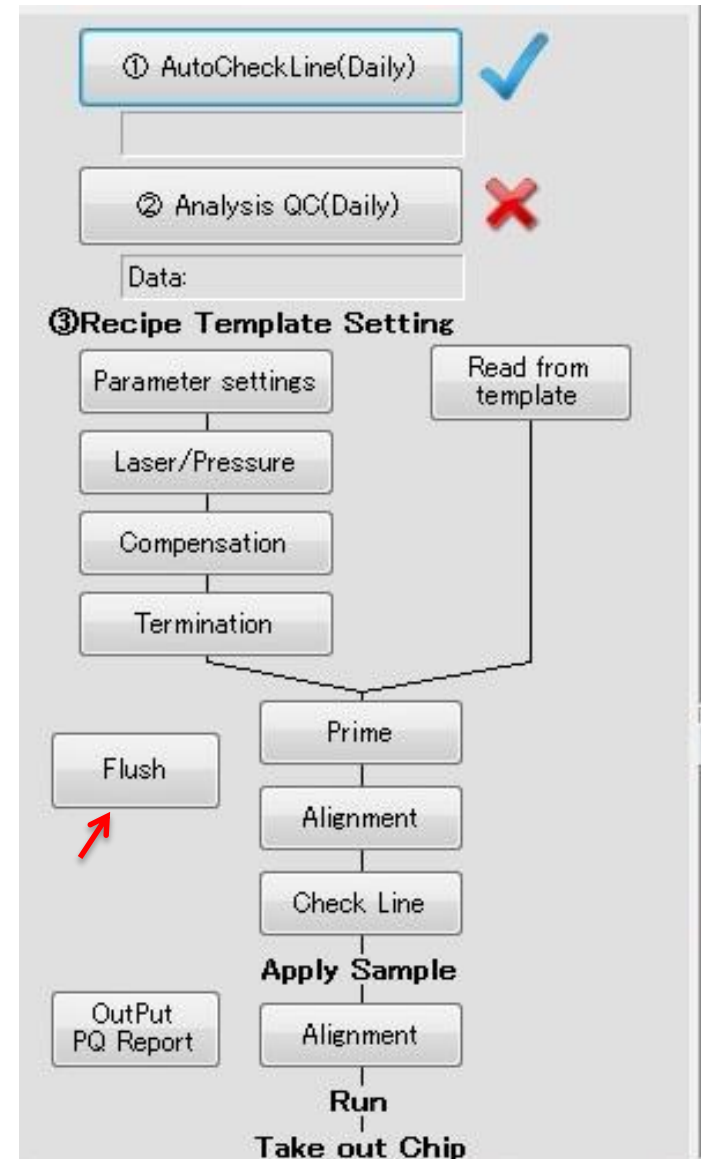
3-3-1 Overview

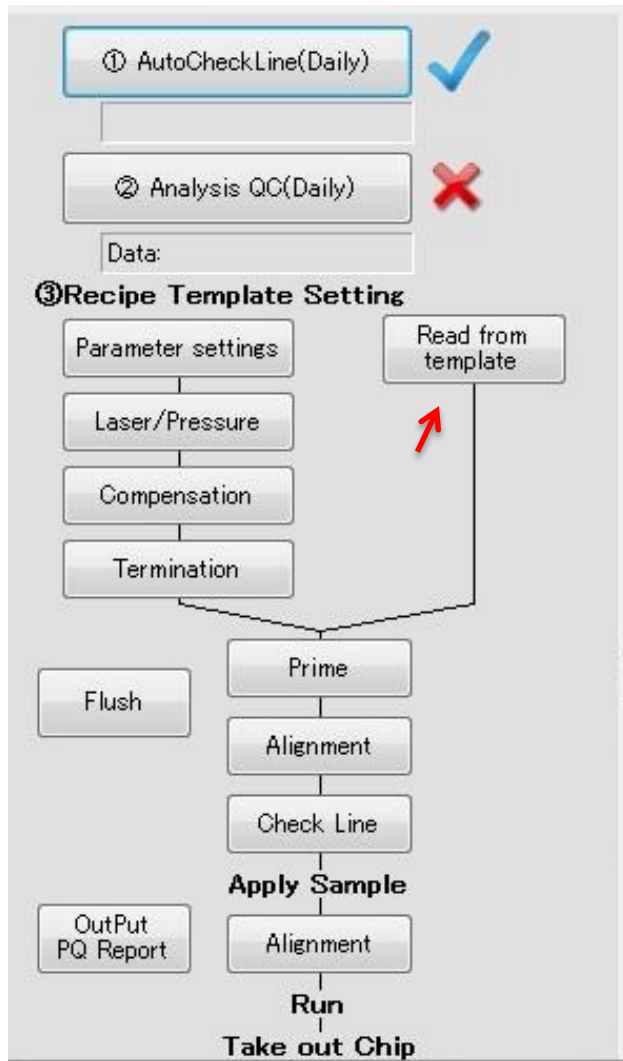
Buttons for steps required for performing analysis and sorting are located in Processing zone. Operations such as detector sensitivity adjustment and chip alignment can be controlled here. Tasks performed by the user are displayed as workflows.

Pressing the **Flush button** zooms the Camera View image and enables monitoring of the chip. (Zoomed window will return to its original position by clicking on the x button at top right corner.)

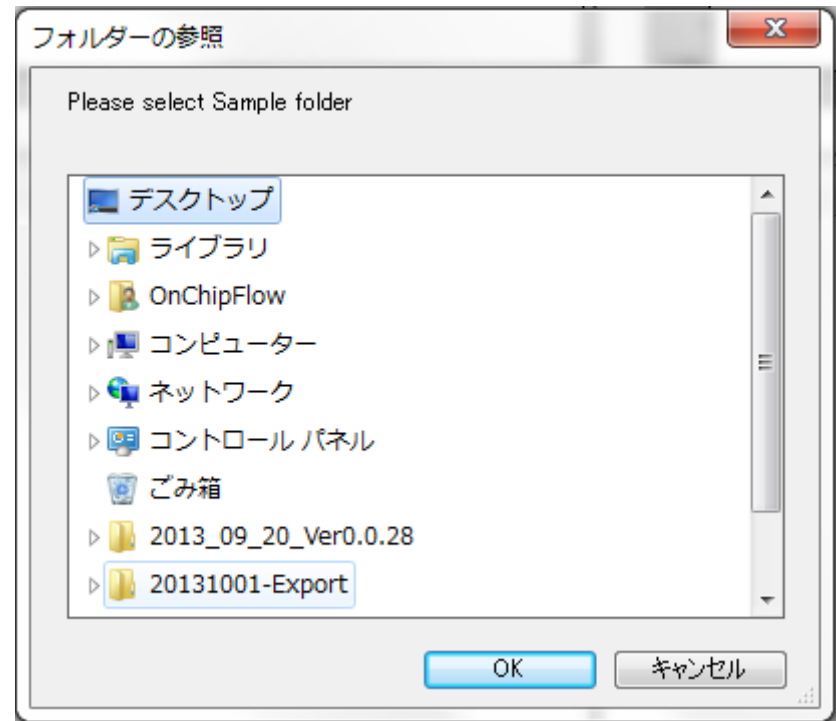


Flush button change color red and name changes to 'Stop Flushing'. It generates a flow from upstream on the chip (the sheath and sample reservoirs) to downstream (the waste reservoir), flushing bubbles out of the flow channel by flow much greater than the Prime button. Small dust particles and cell blockages are also cleared. Press 'Stop Flushing' to end the flushing process.





Press the **Read from temp** button to display a window from which to open templates (a folder that contains empty exported and saved sample files) to refer to. In this case, there is no need to make any changes to the setting, but fine tuning using buttons located on the left are also possible. Read from temp. basically has the same effect as “Duplicate without data” or “Paste without data” on a sample file.



A tick will appear next to each button when actions other than **Flush** or **Pressure Offset** are completed.

3-3-2 Parameter Settings

Press the **Parameter Settings** button to display a setup window for scattered light and fluorescence parameters.

When the names of used fluorophores and subject substances are entered in the Dye and Antigen fields, they are displayed on the **III Workspace** axis and elsewhere.

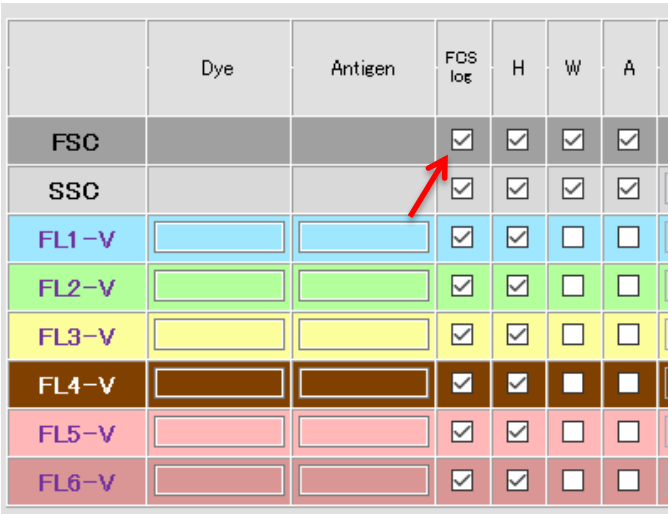
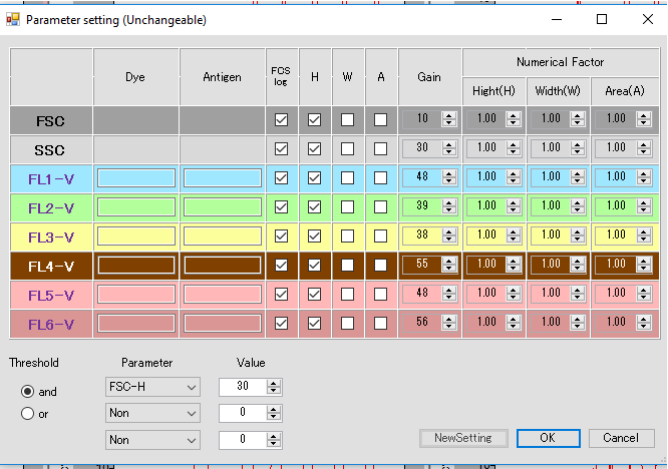
FSC: Forward scattered light, SSC: Side scattered light

Fluorescence parameters can be selected from FL1-V, FL2-V, FL3-V, FL4-V, FL5-V and FL6-V. Pulse Height (H), Width (W) and Area (A) for each fluorescence can be selected.

If Log is checked, it is reflected in **FCS file exports**. Even if it is not checked, it is still possible to switch between log and linear in **III Workspace**.

If even one of H (Height), W (Width), or A (Area) is checked, that parameter is reflected in the axis settings for **III Workspace** and in FCS file exports.

NOTE Number of checked parameters must be **within 10**. Check **the threshold parameter is checked**.



The sensitivity for each parameter is adjusted by Gain. When Gain is increased, the signals are detected stronger, and thus the plot will shift to higher value. Gain must be set as to multiple fluorescence signals would be within the measurement range. Please refer to Chapter 5, 5-14-4 Gain adjustment for more details.

Numerical Factor changes the signal value similarly to Gain, but Numerical Factor is a factor for multiplication of signal intensity. Therefore, it only change data position in plot chart. (It may take up to 3 seconds for it to take effect. During which time buttons for actions are disabled)

Threshold can be set for all signals including FSC and SSC from blue laser derived excitation parameters. Threshold can be set for max 3 signals with logic “and” or “or”. The value of threshold is the same as the unit displayed in the panel of **III Workspace**. When Numerical Factor of a parameter selected for threshold is changed, the apparent threshold on the plot (data cut-off position) shifts according to the Numerical Factor value, but the actual value of threshold is not changed. In such cases, input a value calculated by apparent threshold divided by Numerical Factor. For example, if you want to set 50 as the minimum value of FSC-H in a plot, you must set 20 as Threshold of FSC when the Numerical Factor of FSC-H is 2.5. Press **OK** after changes to settings are made.

NOTE

Changes to parameter setting will not take effect unless “OK” button is clicked. After changing parameter setting, gates setting and plots setting are reset.

Align	Para	Laser/Presser	Term	Stirring	Maint
	Gain	Numerical Factor			
		Hight(H)	Width(W)	Area(A)	
FSC	10	1.00	1.00	1.00	
SSC	30	1.00	1.00	1.00	
FL1	40	1.00	1.00	1.00	
FL2	28	1.00	1.00	1.00	
FL3	60	1.00	1.00	1.00	
FL4	0	1.00	1.00	1.00	
FL5	47	1.00	1.00	1.00	
FL6	50	1.00	1.00	1.00	

Setting of Gain and Numerical Factor

Threshold	Parameter	Value
<input checked="" type="radio"/> and	FSC-H	50
<input type="radio"/> or	Non	0
	Non	0

Setting of Threshold

3-3-3 Laser/Pressure

Setting of Main pressure

A setting window appears when you push the **Laser/Pressure** button. Pressure setting window allows users to check and make changes to the values of MainPush (a pressure for pushing of upper stream) and MainPull (a pressure for pulling of down stream).

NOTE

In the sorting mode, MainPush and MainPull are set with pre-determined values, and are not changed.

Flow speed becomes high with increasing value on the positive side in MainPush1 and on the negative side in MainPull. Note that signal depends on the flow speed of the particle. (MainPush2 is blacked out, as this is not used).

Optical adjustment of Laser

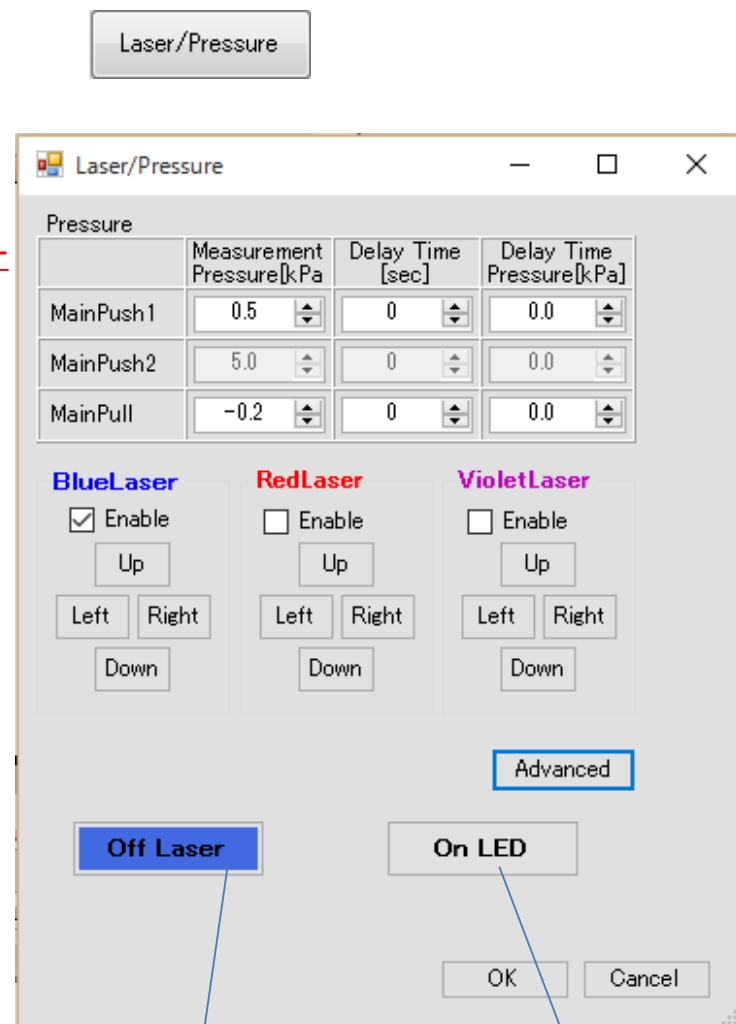
The blue laser is optically fixed. Therefore, the blue laser becomes the standard of the optics adjustment.

The positions of other lasers can be adjusted by clicking Up, Down, Right and Left button.

Turn ON of Lasers and LED light

When you adjust the position of laser beam, you must turn ON of laser by click switch button. The laser switch button displays the current state of the laser (ON/OFF). The Laser beam can be observed by VII. Camera View. Similarly, LED can also be turned on and off using the LED button. When laser turns ON, LED turns OFF automatically.

After changes are made, click OK button.



Laser switch button

LED switch button

Laser/Pressure

Pressure	Measurement Pressure[kPa]	Delay Time [sec]	Delay Time Pressure[kPa]
MainPush1	0.5	0	0.0
MainPush2	5.0	0	0.0
MainPull	-0.2	0	0.0

BlueLaser

☒ Enable

Up

Left Right

Down

RedLaser

☐ Enable

Up

Left Right

Down

VioletLaser

☐ Enable

Up

Left Right

Down

Advanced

Off Laser On LED

OK Cancel

By click “**Advanced**” button, a window for more detailed setting opens.

Do not make any changes to the settings in this window except for when determining the signal base level (checking for optical misalignment). To reset to the initial value, do not copy the values shown in the left figure. Please refer to the QC data created at the time of installment.

Laser/Pressure

FlowSpeed LaserSetting

	Measurement Pressure[kPa]	Delay Time [sec]	Delay Time Pressure[kPa]
MainPush1	1.8	3	0.0
MainPush2	1.8	3	0.0
MainPull	-0.7	0	0.0

Gain

FSC	10
SSC	30
FL1	32
FL2	35
FL3	33
FL4	0
FL5	40
FL6	55

Ch1(TRG) Ch2

☒ FSC ☐ SSC ☐ FL1 ☐ FL2 ☐ FL3 ☐ FL4 ☐ FL5 ☐ FL6

PROFILE mode

Trigger

PROFILE Width 8000

Ch1 Threshold 500

Start Stop

PROFILE Only AC/DC AC

Alignments

BlueLaser RedLaser VioletLaser

☒ Enable ☐ Enable ☐ Enable

Up Up Up

Left Right Left Right Left Right

Down Down Down

ChipStage ☐ 1[μm]move

Up Left Right Left Right Left Right

Off Laser

OK Cancel

FSC

FL2

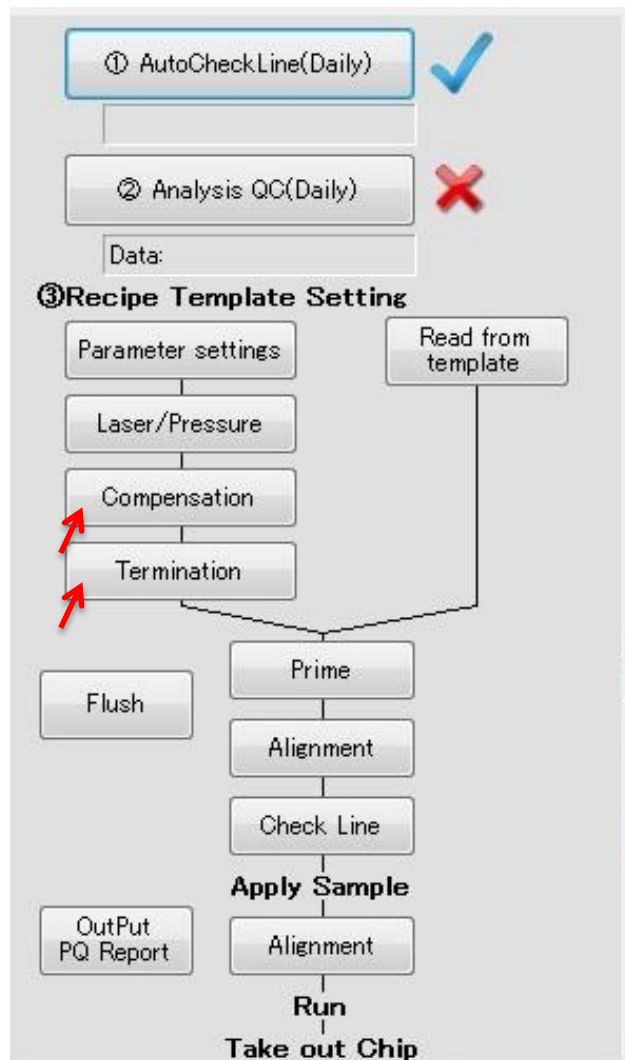
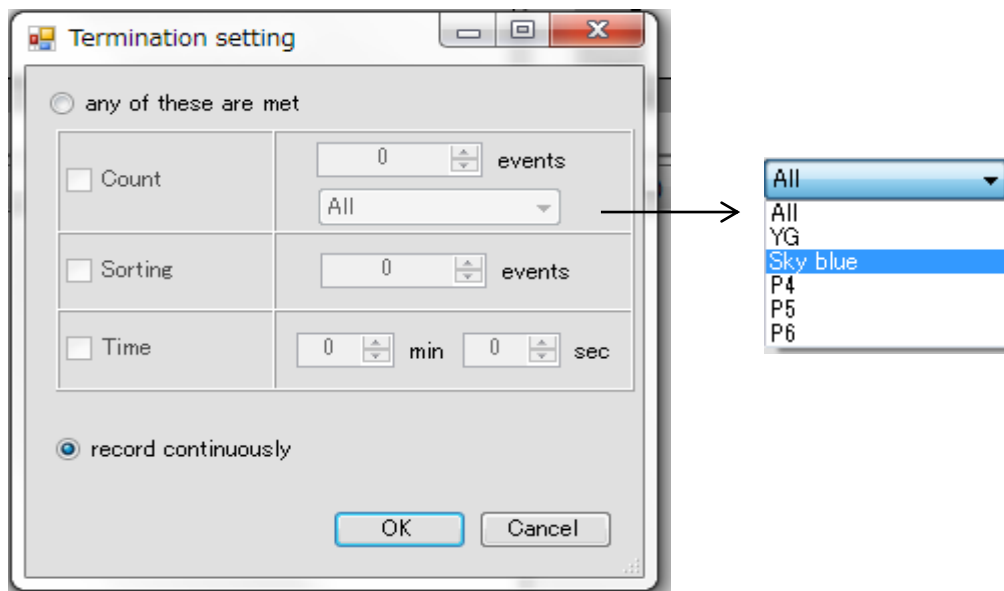
3-3-4 Compensation

Press the **Compensation button** to display a setup screen for fluorescence compensation. Please refer to **Chapter 5, 5-14-7 Compensation** for more information.

3-3-5 Termination

Press the **Termination button**, the termination setting window appears. Termination condition of analysis or sorting can be set in this window.

As termination conditions, total counts, counts of selected population, sorting events, and measurement time are available. When record continuously is selected, the measurement will not stop until either **Stop** or **Recover** (for sort only) button is not pressed as shown in **3-7 VI Operation controls**.



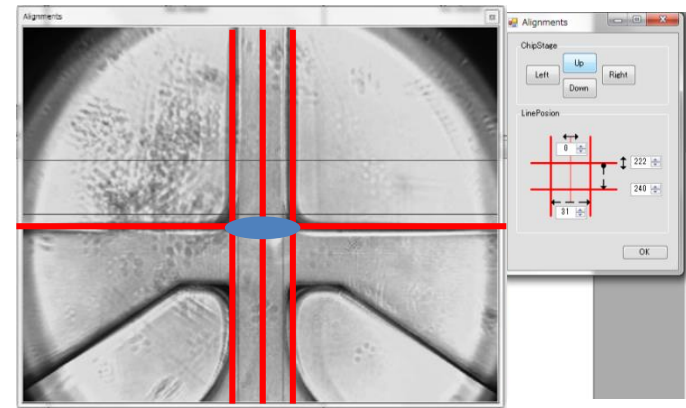
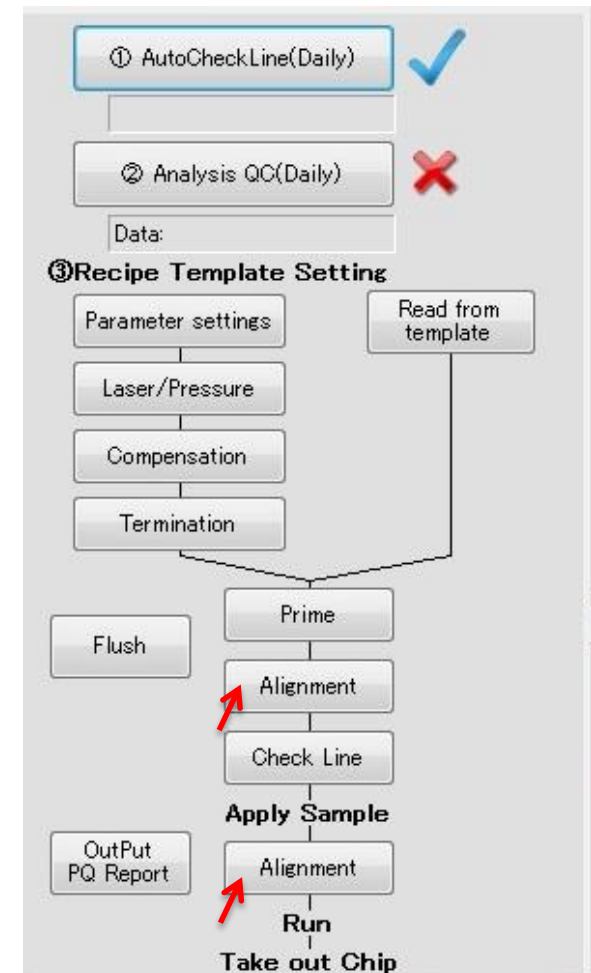
3-3-6 Alignment

Auto alignment function is available for this instrument, so this step is skipped. If the edges of the chip are not aligned with the x and y lines, click the Auto Alignment button again.

Clicking “**Alignment**” button will open an expanded **VII. Camera view** window for manual position adjustment.

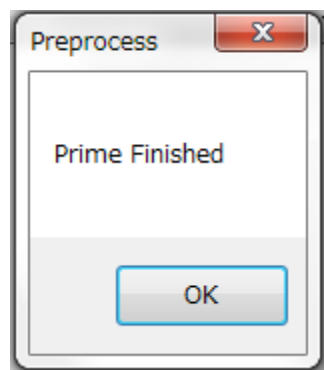
NOTE

If the lines and the blue laser are not aligned, please re-adjust it according to the procedure in 3-8-1 Alignment parameter setting.



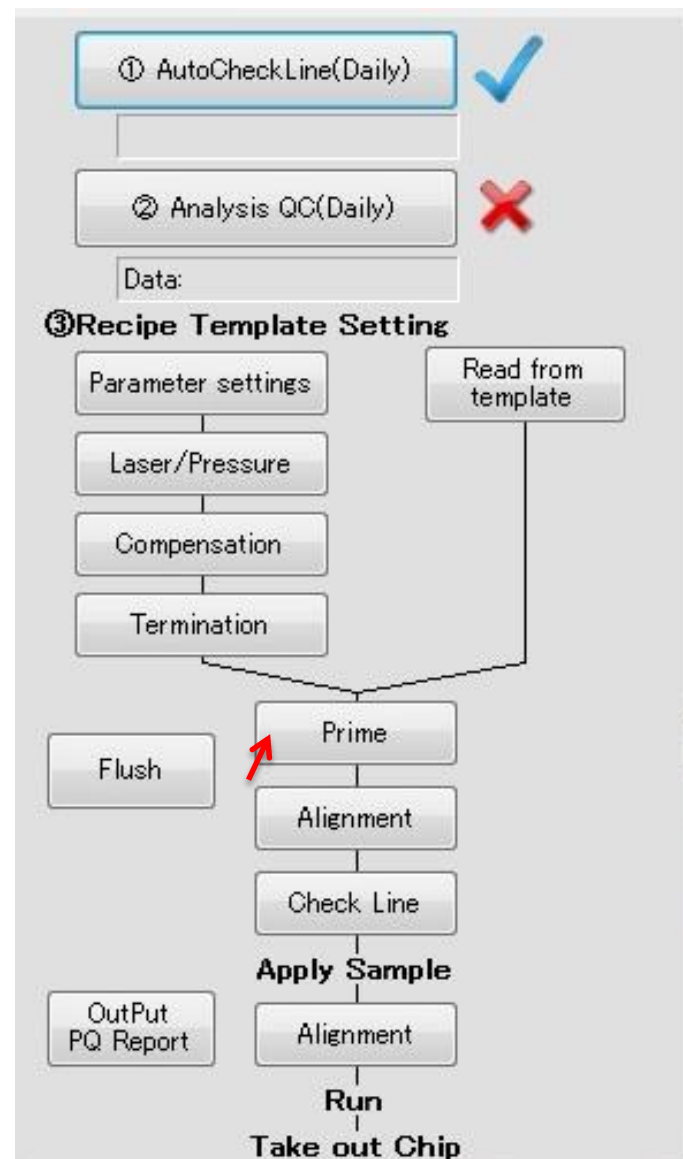
3-3-7 Prime

Prime button initiates a priming process that applies pressure for sheath fluid to flow through the microchannel. During priming, the button blink in red. When prime ends, a pop-up window appears to inform that prime finished.



NOTE

The pop-up windows hides behind the main panel when you click on the main panel. You can move to the next step without pressing OK. Pop-up window recovery is described in 3-9 Troubleshooting.



3-3-8 Auto Check Line

Sorting Only

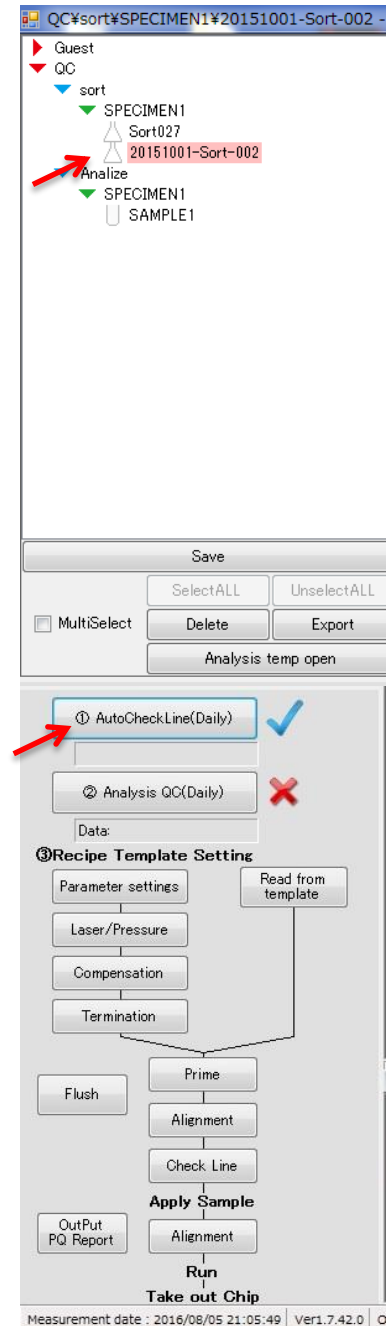
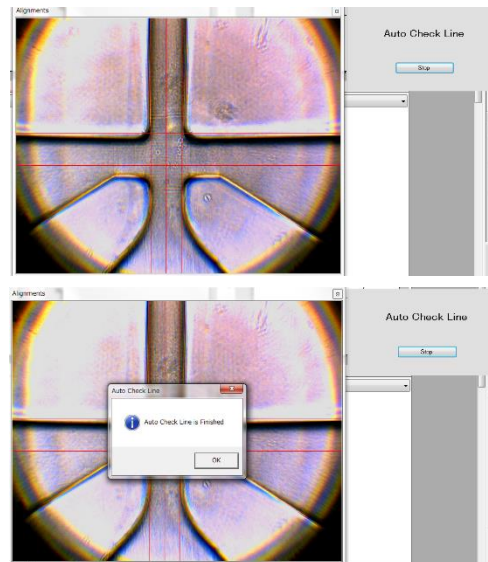
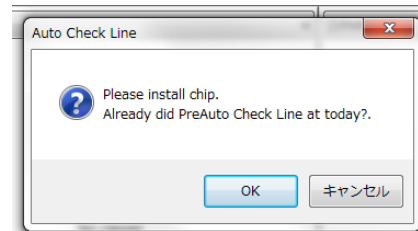
AutoCheckLine should be carried out once a day prior to sorting.
AutoCheckLine is a quality control inspection for the air pressuring system vital for sorting of cells.

Insert the Sealed Chip (provided at the time of instrument installation) in the Chip holder, then put the holder inside the instrument. Select the sample file found at the bottom of the QC layer, by expanding the layers under **I. Sample management**. Click “AutoCheckLine (Daily)” button under **II. Processing zone**.

A message (below) window such as one on the right will appear (“Please install chip. Already did Pre Auto Check Line at today?”). Click “OK” to proceed. After pressing OK, an magnified CCD image appears, and AutoCheckLine will begin.

When the process is completed successfully, a message window with a dialog “Auto Check Line is Finished” will appear. Press “OK” to end process.

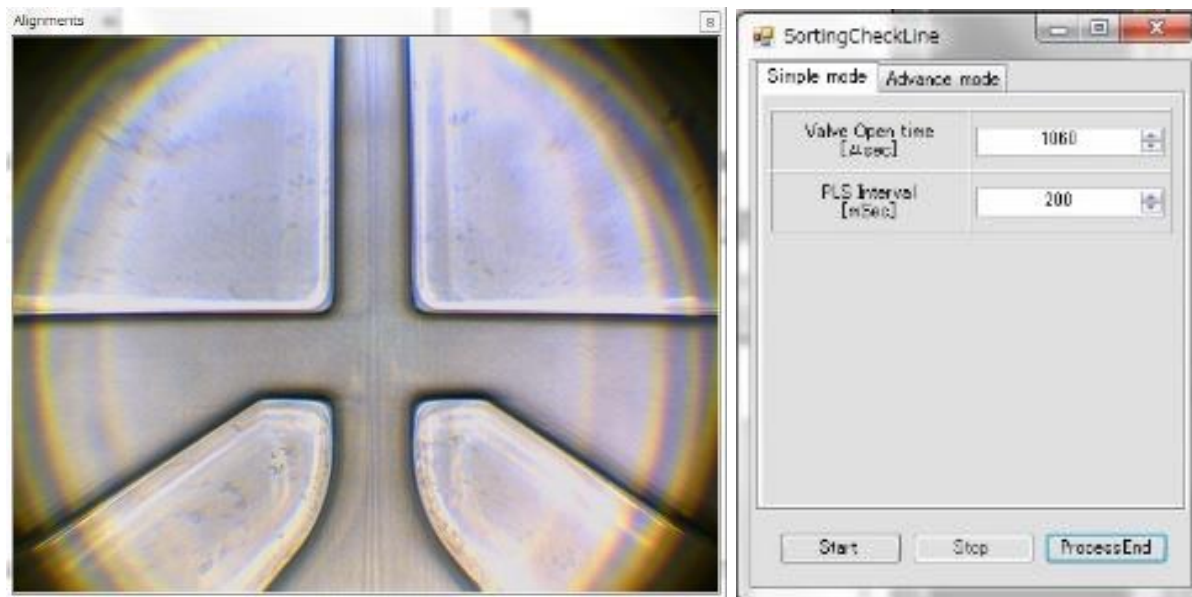
※Please carry out the Auto Check Line process again if an error message appears. It is possible that there is a problem with the instrument if error message still appears after several tries.



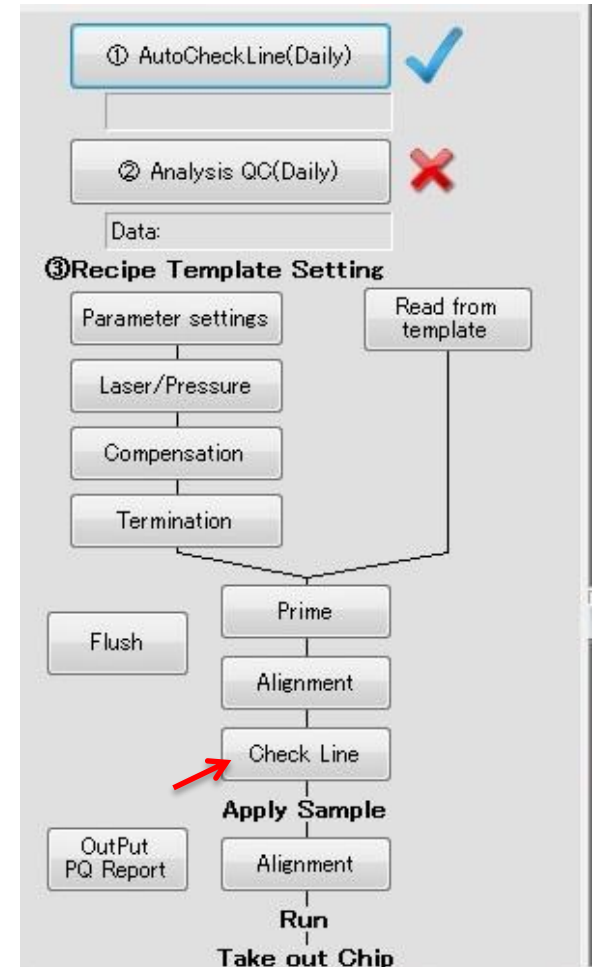
3-3-9 Manual Check Line

Sorting Only

For any reason Auto Check Line is not available, the Check Line process can be carried out manually. Clicking “**Check Line**” button expands the CCD image under **VII. Camera View**, and a pop up window for valve open time adjustment will appear. The red reference lines disappear at this point. For more information on Check Line, please refer to **Chapter 5-11 Check Line**.



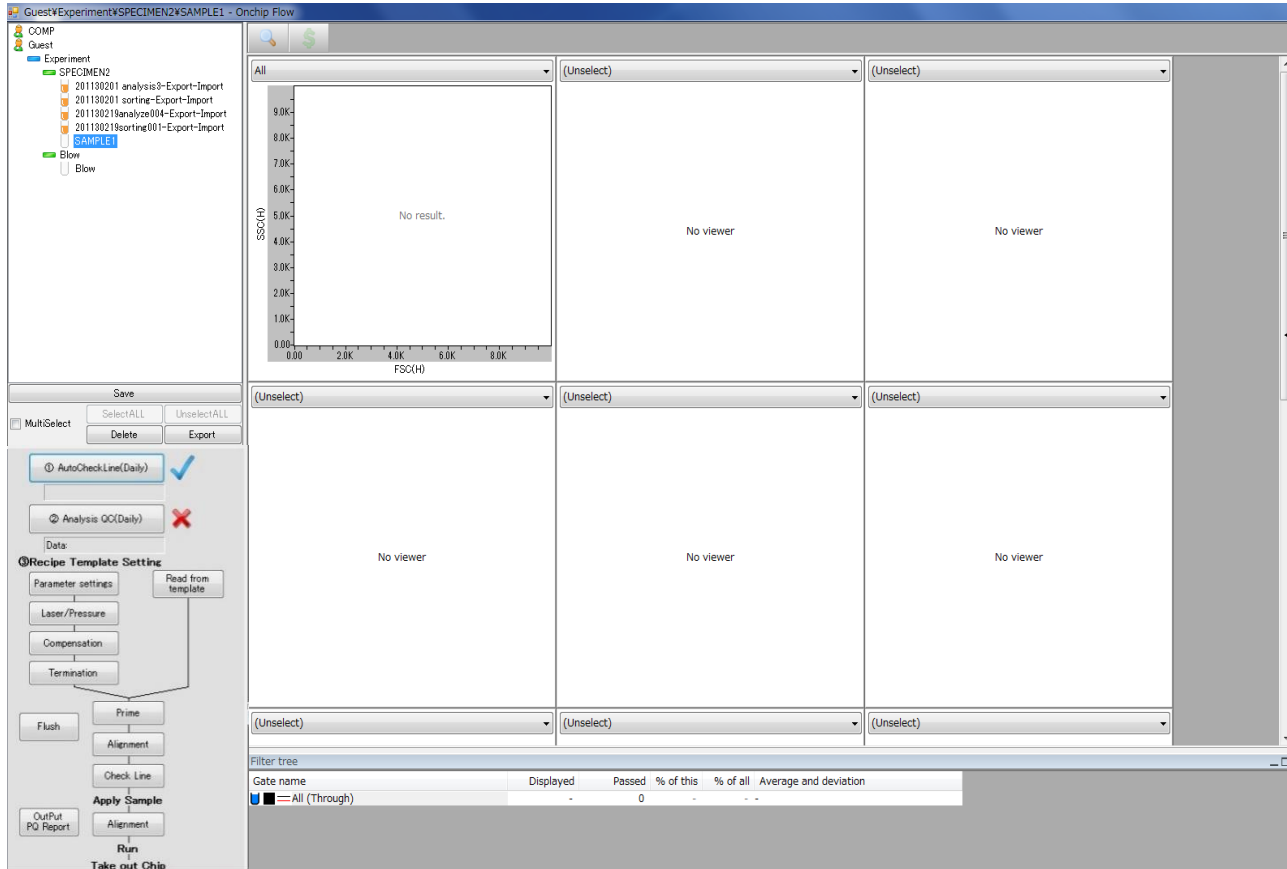
Pressing “**Start**” initiates sorting pulses. Ensure the fluid line is not entering the sorting channels on either side. If there was any flow into one of the sorting channels, there may be a blockage within the channel. Carry out the Check Line process after priming the chip. If the flow line is not entering the channels on the right or left, press “**Stop**” then click “**ProcessEnd**” to end.



3-4 III. Work space

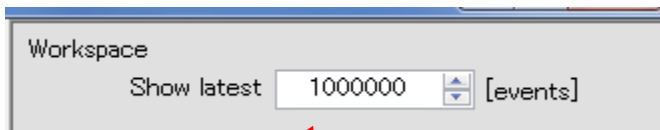
Dot plots, gating, histograms

Axis setting for scattered light and fluorescence parameters



When a new sample file is created, the top left panel of the 12 displays the FSC (linear) and SSC (linear) axes, by default.

← Scroll down to display other panels.



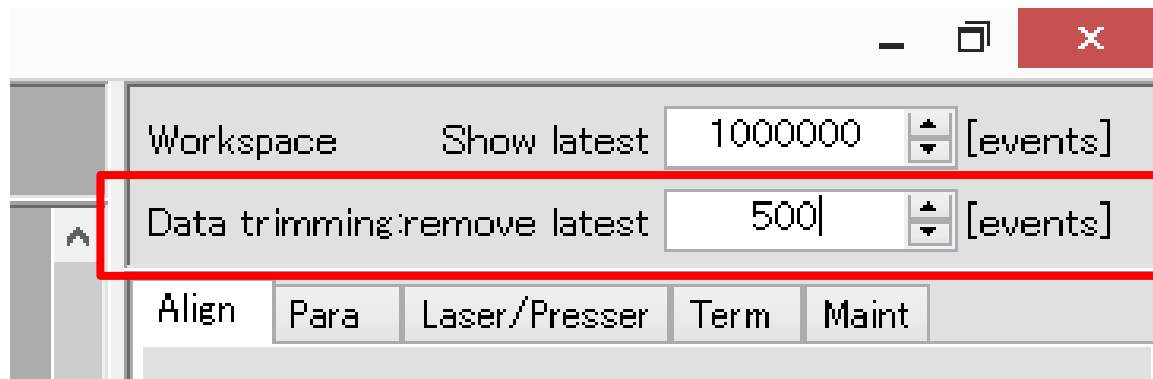
The setting space at the top of **V Instrument Settings** can be used to set the number of recent events to display on panels during measurement and sorting.

Data trimming function

Data trimming can tune events counts.

Enter an appropriate event number to be removed from the latest counts.
The data trimming function can remove the plot that arise by air bubbles when you completely run through the sample.

NOTE: Removed data can still be recovered until Save button is pressed. Removed data cannot be recovered after the data is saved.



Save button located on the left of the screen

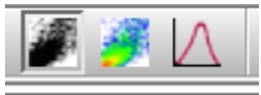


3-4-1 Panel display

The background of the selected panel turns red.

The axes can be selected from the scattered light and fluorescence color parameters selected in **3-3-2 Parameter Settings**.

Axis scales can be selected from linear, log, and bi-exponential. With log and bi-exponential, the minimum value can be selected from 10, 1, and 0.1.

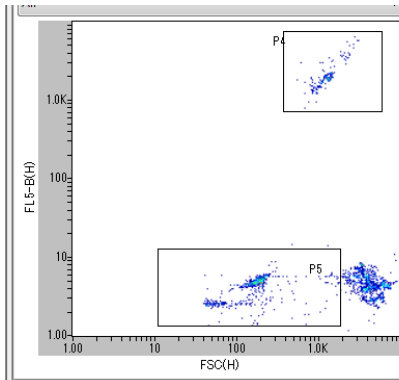


Dot plot (single color, or multi color), **density plot**, and **histogram** can be changed.

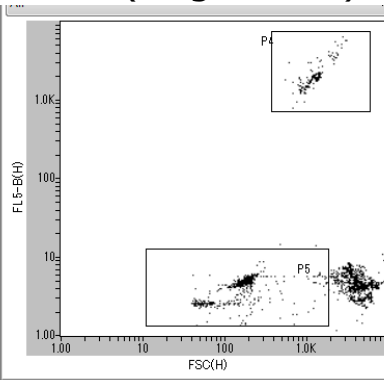


If dot plot and histogram are selected, the **Color Mode Selection button** is displayed.

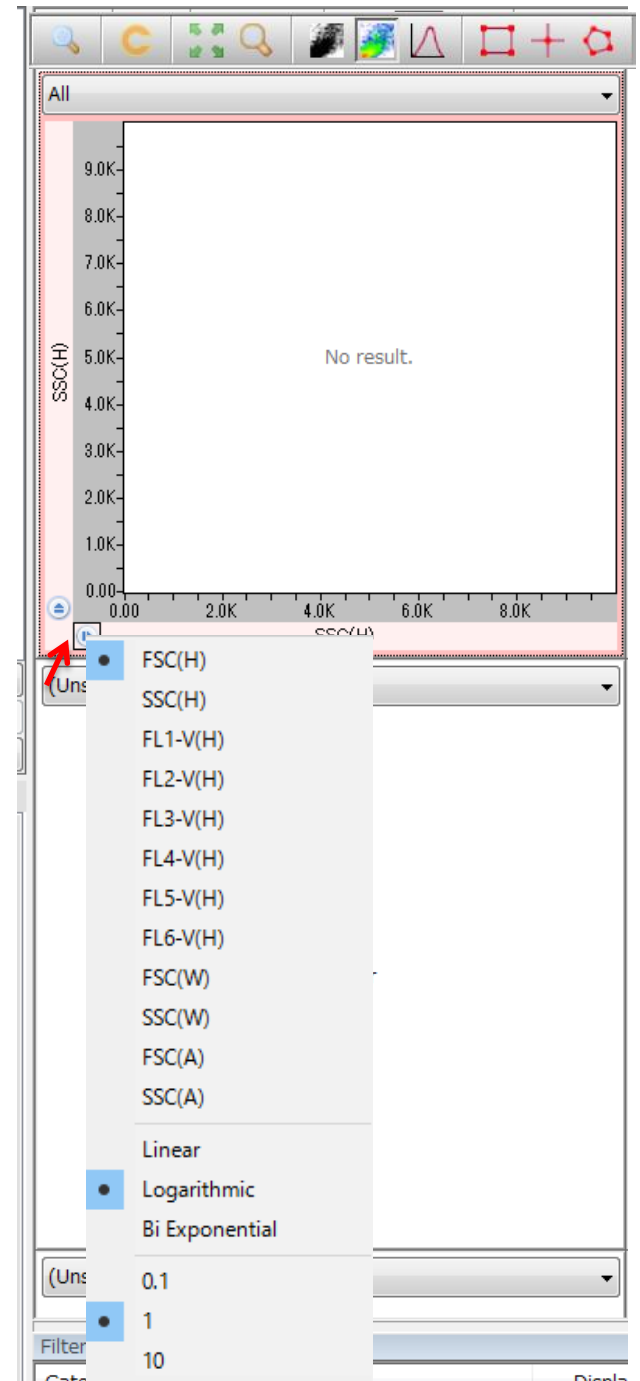
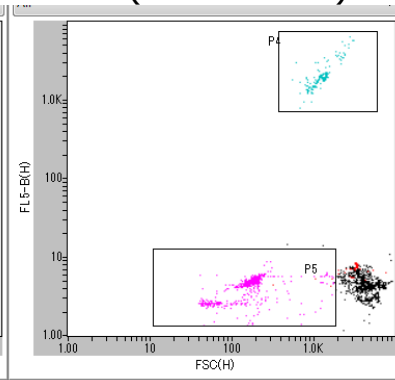
Density plot



Dot plot
(single color)



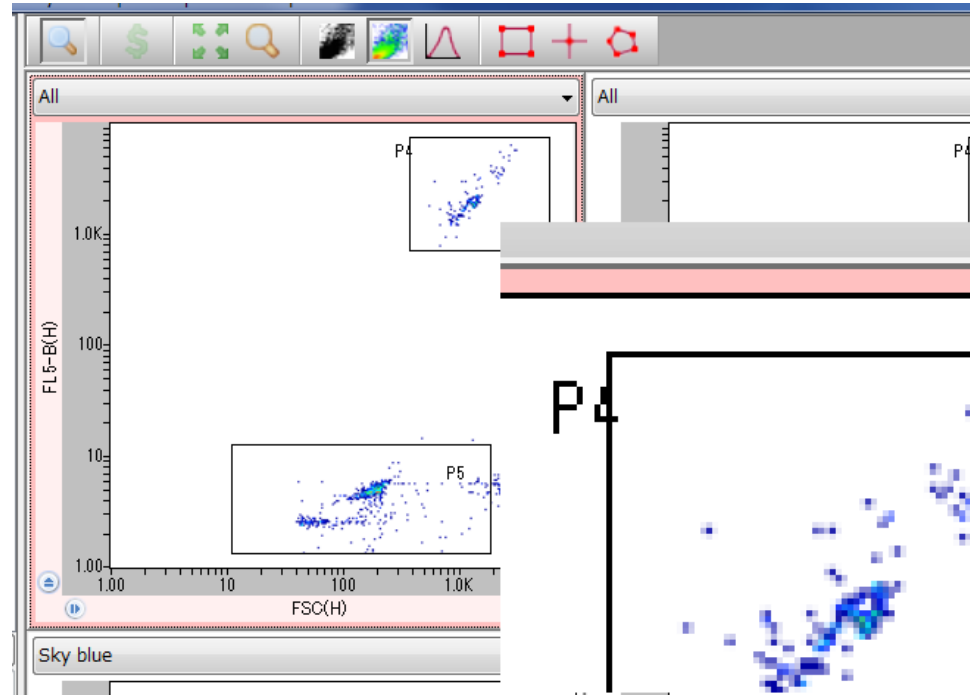
Dot plot
(multi color)



3-4-2 Toolbar

Magnifying glass

Magnifies area where the pointer is.



Enable compensation

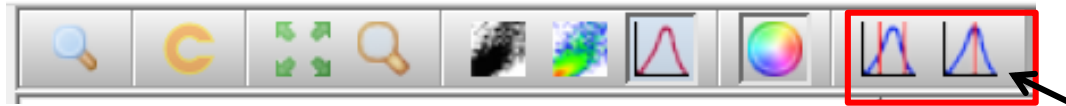
Applies or removes the compensation setting to the data.



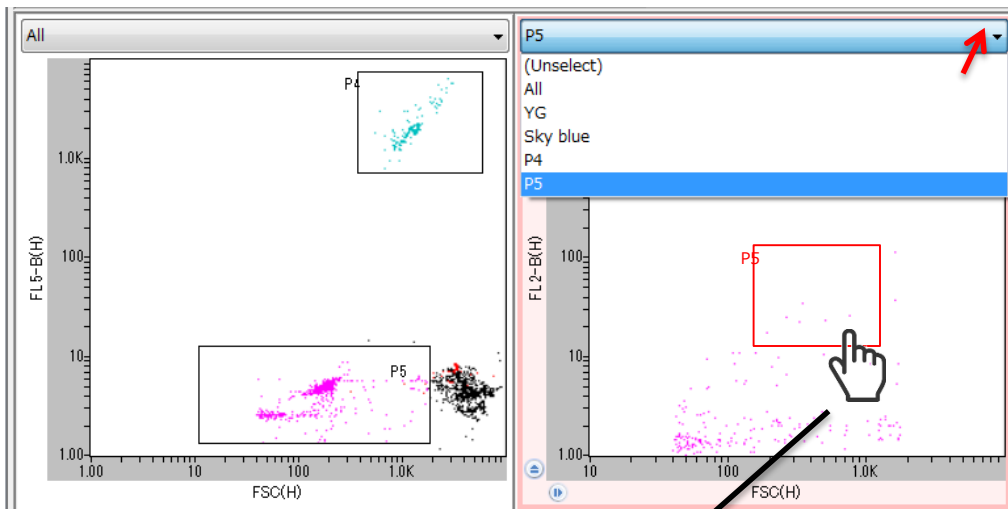


Gates

Box, **cross**, and **polygon** gate can be created for dot plot or density plot of the selected panel.



Sandwich or **splitter** gate can be created for histogram of the selected panel.



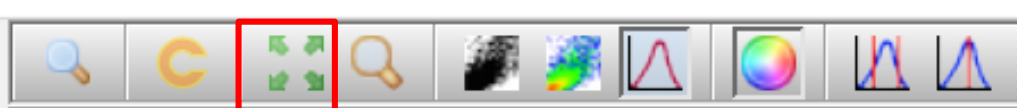
The selected gate turns red. The name and the size can be changed on the panel.

When a new gate is created, name of the populatoin is automatically added to the dropdown box at the top of the panel, where users can select which population to be displayed on the panel. At the same time, the population tree is displayed in **IV Population management** under **III Workspace**.

Drag and drop to anther panel

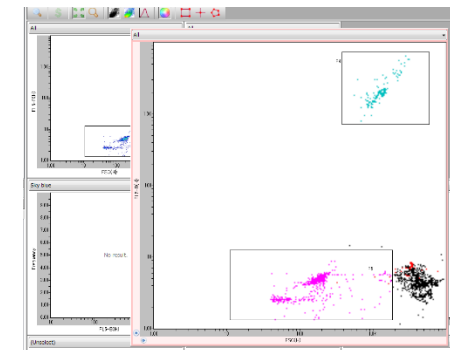
Gate can be displayed in a different panel by selecting the gate and drag-and-drop to a panel.

Gate name	Displayed
All (Through)	1630
YG (Rectangle, 2D, H: Log10:FL2-B(H), V: Sky blue (Rectangle, 2D, H: Log10:FL2-B(H), V: P4 (Rectangle, 2D, H: Log1:FSC(H), V: Log	49
P4 (Rectangle, 2D, H: Log1:FSC(H), V: Log	176
P5 (Rectangle, 2D, H: Log1:FSC(H), V: Log	578



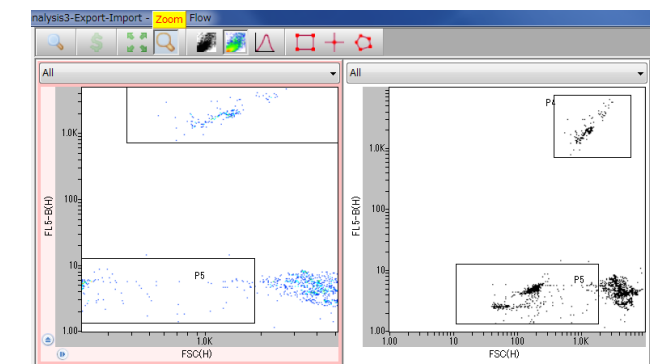
Maximize button

Magnifies the selected panel. Push the maximize button to return to the original size.



Zoom button

Push **Zoom** button on a selected panel, and click to set the start point of a rectangle. Move the pointer from the start point along a diagonal line, to create a rectangle that specifies the area to zoom.



Example: The figure on the left shows the red panel enlarged, and the right shows it at the original scale.



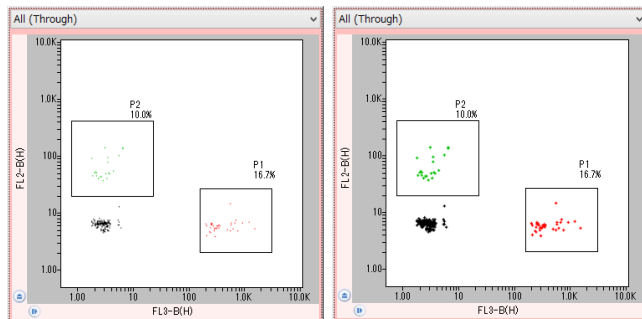
Rename gates button

Rename gates from P1.

(Caution: all names will be reset)

x^2 button

The size of a dot expands 2 times. (shown on the left)



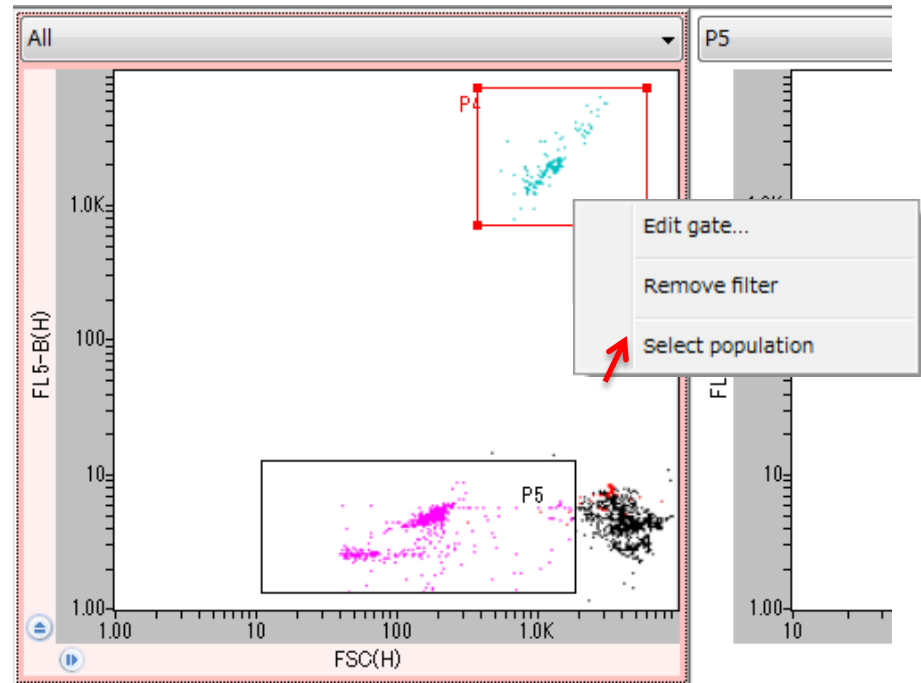
(x1)

(x2)

3-4-3 Population management

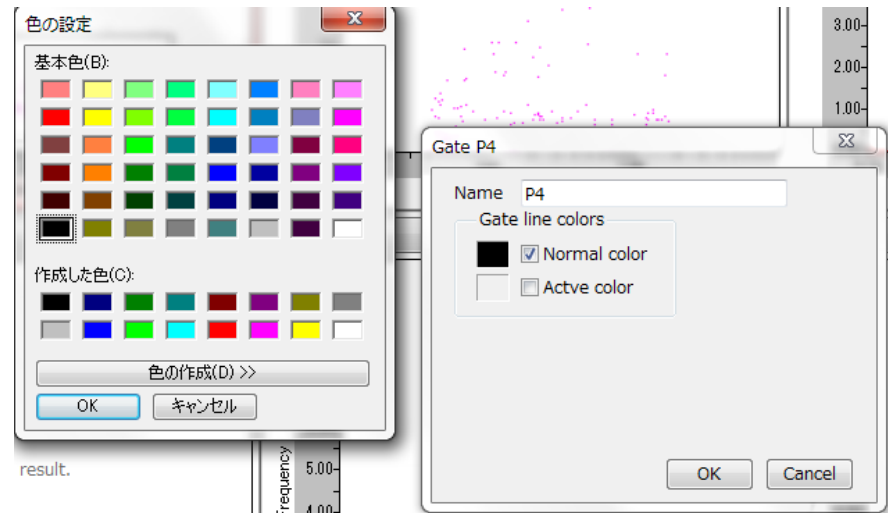
When a gate is selected, gate frame changes to the active color. Right click on the active gate to delete the gate (Remove filter) and change settings (Edit gate).

Choose “Select population” for sorting. The frame of the gate becomes bold, and the selected population will be sorted.



Edit Gate opens a window for changing name and color.

Check both “Normal color” box and “Active color” box, and click on the rectangle on the left. A panel with various colors appear, and users can select the color of the gate in unselected state and selected state. The defaults for normal and active are black and red, respectively.

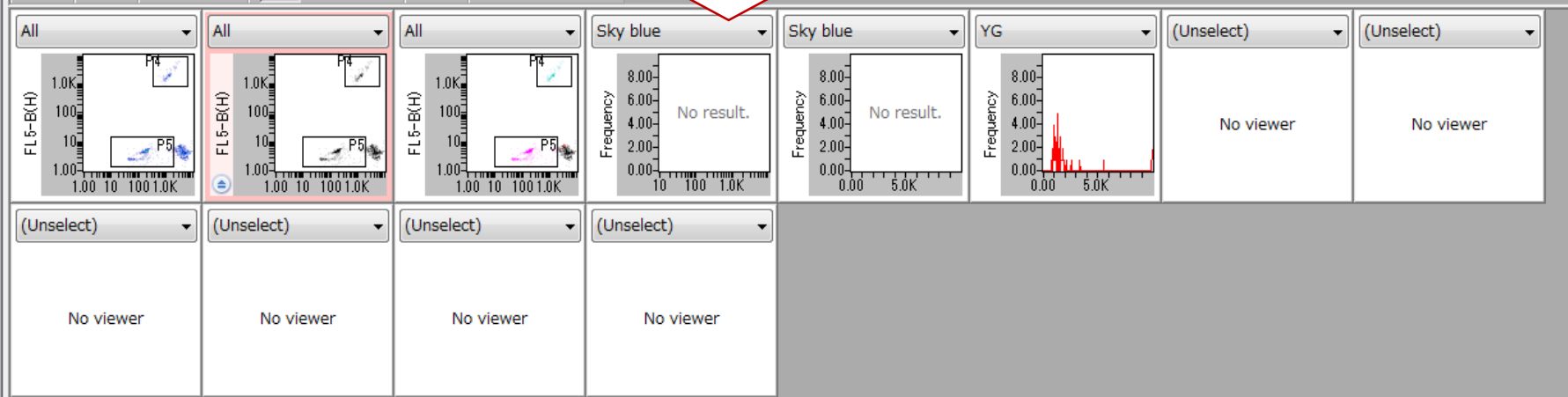
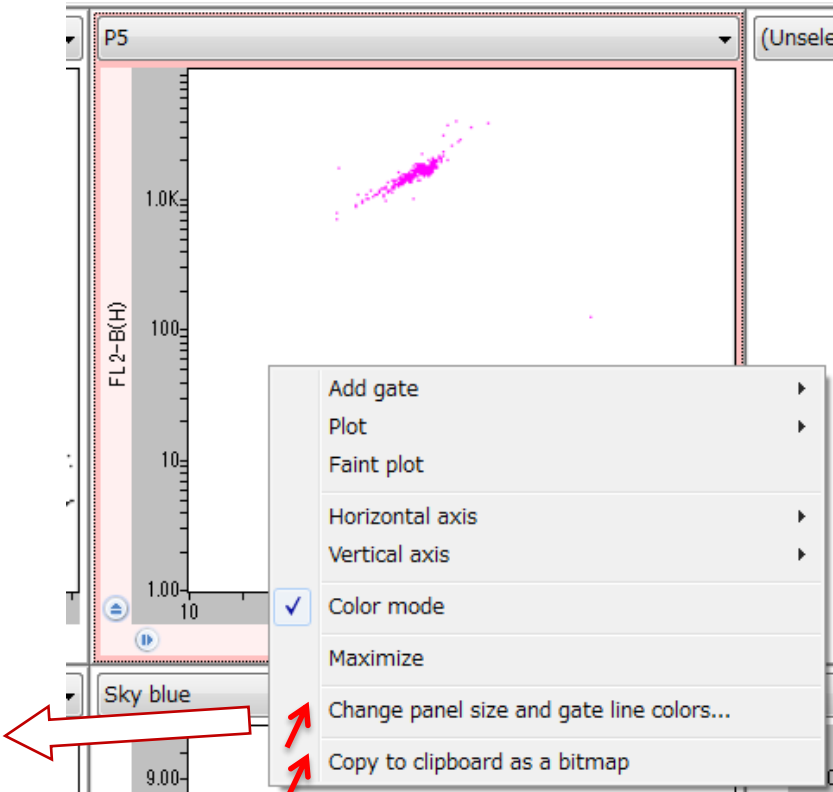
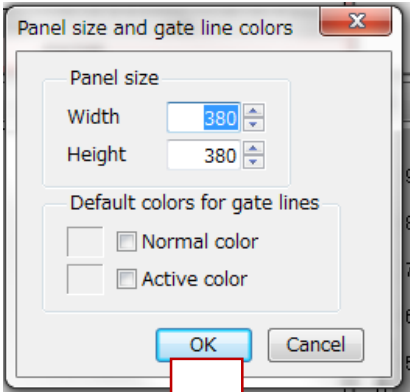


3-4-4 Panel setting

Actions and commands described in **3-4-2 Toolbar** can be applied from a window that appears by right clicking a panel. Moreover, plot color changes and axis changes described in **3-4-1 Panel display** can also be applied.

Panel size changes are applied to all the panels.

Selected panel can also be exported as a bitmap file.



3-5 IV. Population management

Population information of each gate

Population tier is shown in tree structure

% respective to parent population

Gate name and details (e.g. graph axis)

% respective to all population

Gate name	Displayed	Passed	% of this	% of all	Average and deviation
All (Through)	1630	1630	-	-	-
YG (Rectangle, 2D, H: Log10:FL2-B(H), V: Sky blue (Rectangle, 2D, H: Log10:FL2-B(H), V: P4 (Rectangle, 2D, H: Log1:FSC(H), V: Log	49	49	3.01%	3.01%	H: Ave=13.1, Dev=8.4 / V: Ave=7.0, Dev=
	-	0	0.000%	0.000%	-
	176	176	10.8%	10.8%	H: Ave=1336.8, Dev=464.1 / V: Ave=217:
P5 (Rectangle, 2D, H: Log1:FSC(H), V: Log	578	578	35.5%	35.5%	H: Ave=228.1, Dev=284.5 / V: Ave=4.2, I

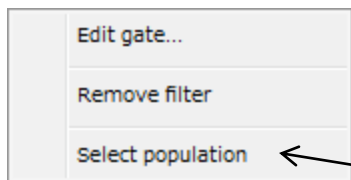
Gate frame color:
Normal and selected

Population color

Gate type: displayed as polygon, box etc

Displayed: The number of events displayed within the gate. The number of events included in the latest gate modified during or after analysis or sorting.

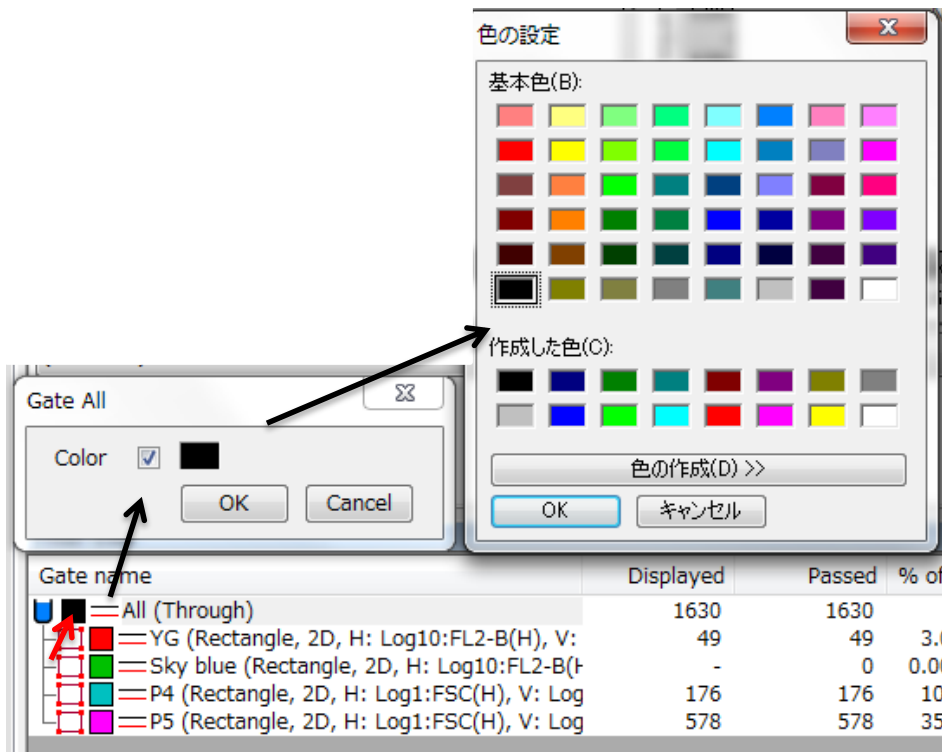
Passed: The number of events that passed the gate. The number may differ to the number displayed when gate is modified during or after analysis or sorting.



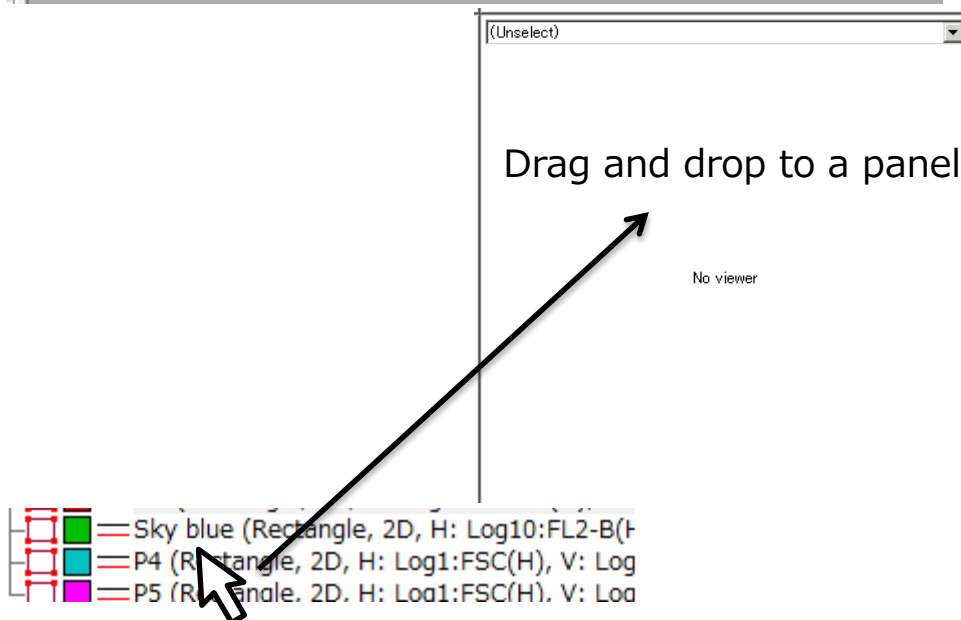
A pop-up menu appears when population name is right clicked. The content is the same as explained in III. Workspace.

"ALL" cannot be deleted (Remove filter).

At the time of sorting, select "Select population". The selected gate is bolded, and the selected population is sorted.



A dialogue opens when the color box of population is right clicked. Color pallet for color setting appears when the color box on the new dialogue is clicked. The dots on the plot are displayed in these selected colors in color mode.



Similarly to the gate, selected population appears automatically when the population is drag and dropped to a panel.

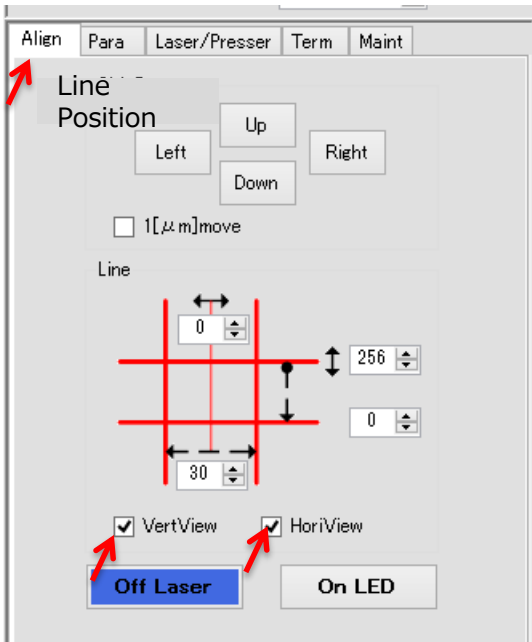
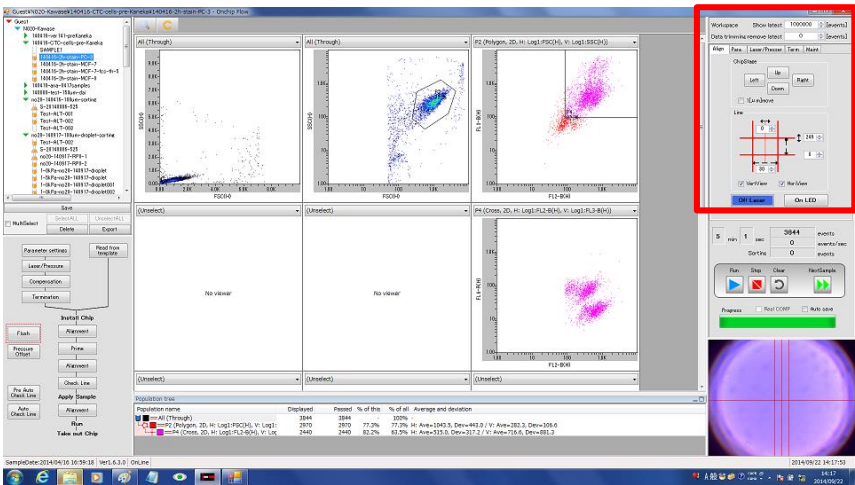
3-6 V. Instrument Settings

Settings for chip position, laser, Gain of detectors can be changed here. It is generally the same as **II. Processing zone**.

3-6-1 Alignments tab (position alignment)

The basic operation is generally the same as **3-3-6 Alignment**. The difference is that the red reference lines shown in VII. Camera view is can be hidden by unchecking the VerView and HoriView boxes. Lasers and LED can also be turned ON/OFF as shown in **3-3-3 Laser Pressure**

V. Instrument Settings Zone



3-6-2 Parameter settings

Para tab contains one section of **II. Processing zone, 3-3-2 Parameter Settings** that opens by clicking **Parameter Settings button**, and enables setting of Gain, and Numerical Factor of Height, Width and Area.

Align	Para	Laser/Presser	Term	Stirring	Maint
	Gain	Numerical Factor			
		Height(H)	Width(W)	Area(A)	
FSC	10	1.00	1.00	1.00	
SSC	30	1.00	1.00	1.00	
FL1	40	1.00	1.00	1.00	
FL2	28	1.00	1.00	1.00	
FL3	60	1.00	1.00	1.00	
FL4	0	1.00	1.00	1.00	
FL5	47	1.00	1.00	1.00	
FL6	50	1.00	1.00	1.00	

3-6-3 Laser/Pressure

Laser/Pressure tab enables operations same as the window that appears when clicking **Laser/Pressure** button in **II Processing zone, 3-3-3 Laser/Pressure**.

- ① Chip position can be observed when Laser is OFF and LED is ON.
- ② Laser beam spot can be observed when Laser is ON.
- ③ Nothing can be observed when both Laser and LED are OFF.

Align Para **Laser/Presser** Term Maint

	Measurement Pressure[kPa]	Delay Time [sec]	Delay Time Pressure[kPa]
MainPush1	0.5	0	0.0
MainPush2	5.0	0	0.0
MainPull	-0.2	0	0.0

BlueLaser
☒ Enable
Up
Left Right
Down

GreenLaser
☒ Enable
Up
Left Right
Down

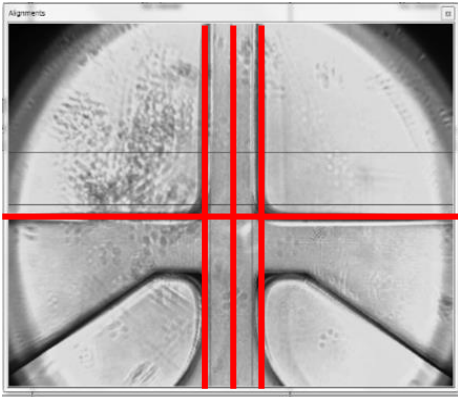
VioletLaser
☒ Enable
Up
Left Right
Down

Off Laser

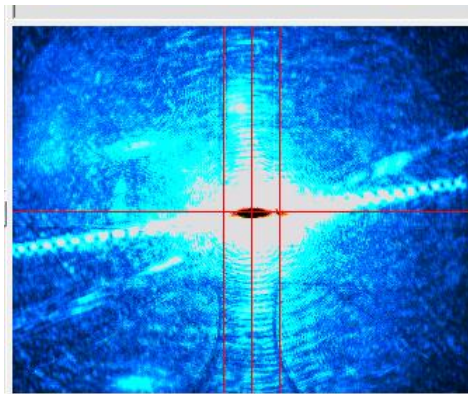
On LED

Advanced

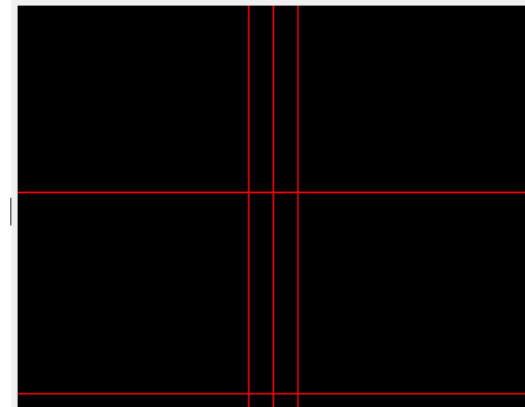
①



②



③



3-6-4 Termination

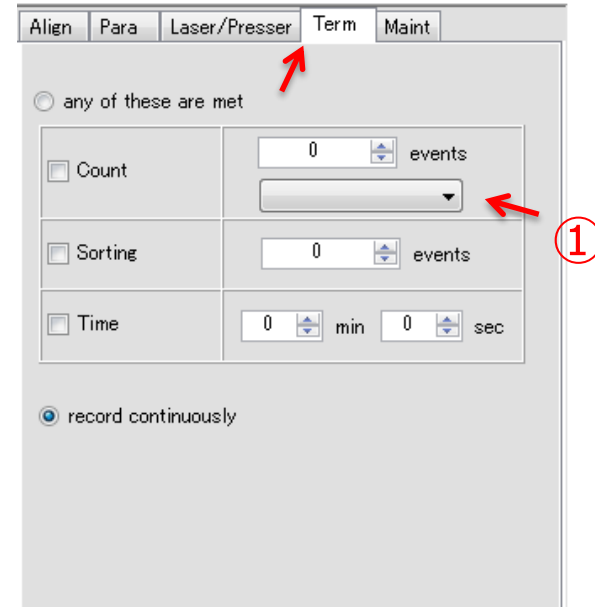
Operation is the same as described in 3-3-5 Termination. Select Term tab, then select "any of these are met".

Count terminates measurement after number of plots within specified gate reaches the input value. Select the gate from dropdown box ①.

Sorting terminates sorting process after specified number of cells are sorted.

Time terminates measurement after specific time. Tick one or more of these items to activate termination. The first condition that reaches the target number will be applied.

Select "record continuously" to turn off termination.



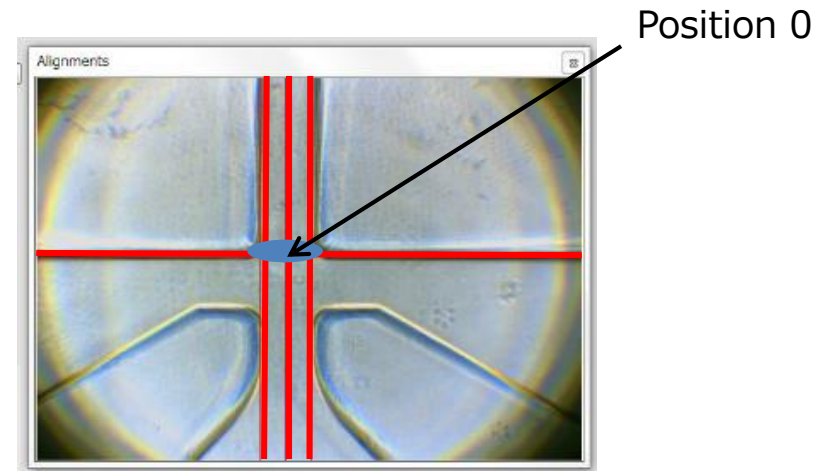
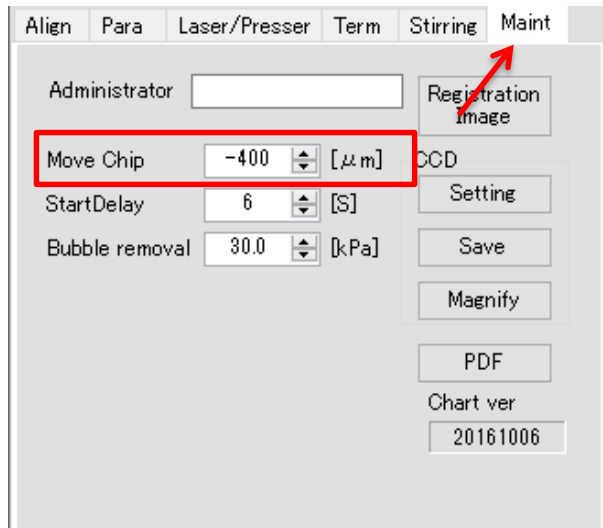
The screenshot shows a software window with tabs: Align, Para, Laser/Presser, Term, and Maint. The 'Term' tab is selected. Inside the 'Term' tab, there is a radio button labeled 'any of these are met'. Below this, there are three rows of controls: 'Count' with a checkbox, a numeric input set to '0', and a dropdown menu; 'Sorting' with a checkbox, a numeric input set to '0', and the text 'events'; and 'Time' with a checkbox, two numeric inputs set to '0', and the labels 'min' and 'sec'. At the bottom, there is a radio button labeled 'record continuously'. A red arrow points to the 'Term' tab, and another red arrow points to the dropdown menu in the 'Count' row, which is also labeled with a circled '1'.

3-6-5 Maintenance

When you click the “Start” button to initiate analysis/sorting, the installed chip automatically moves to the detection position entered in Move Chip box. The detection position is important for timing from the detection to sorting. This parameter “Move Chip” can be changed under the “Maint” tab.

This parameter changes depending on type of chip and reagent used. For example, in the case of -575 as the value of MoveChip, the detection position is 575 μ m upstream from the 0 position.

This parameter should only be changed if the purity and yield of the sorted sample starts to decrease. Changing the Move Chip changes the timing for sort. More details in 4-1-2.



Controls for analysis and sorting

Execution of measurement and monitor progress.

Run button starts analysis or sorting. The button blinks during the measurement.

Stop button stops analysis or sorting process.

Clear button refreshes data plots in chart during measurement. This button is useful for getting new plots data after changing Gain of detector.

NextSample button shifts over to next sample in the data tree view under **I. Sample Management**. If there is no empty sample data in the same specimen, an empty sample data file is created retaining the same parameters of current sample file.

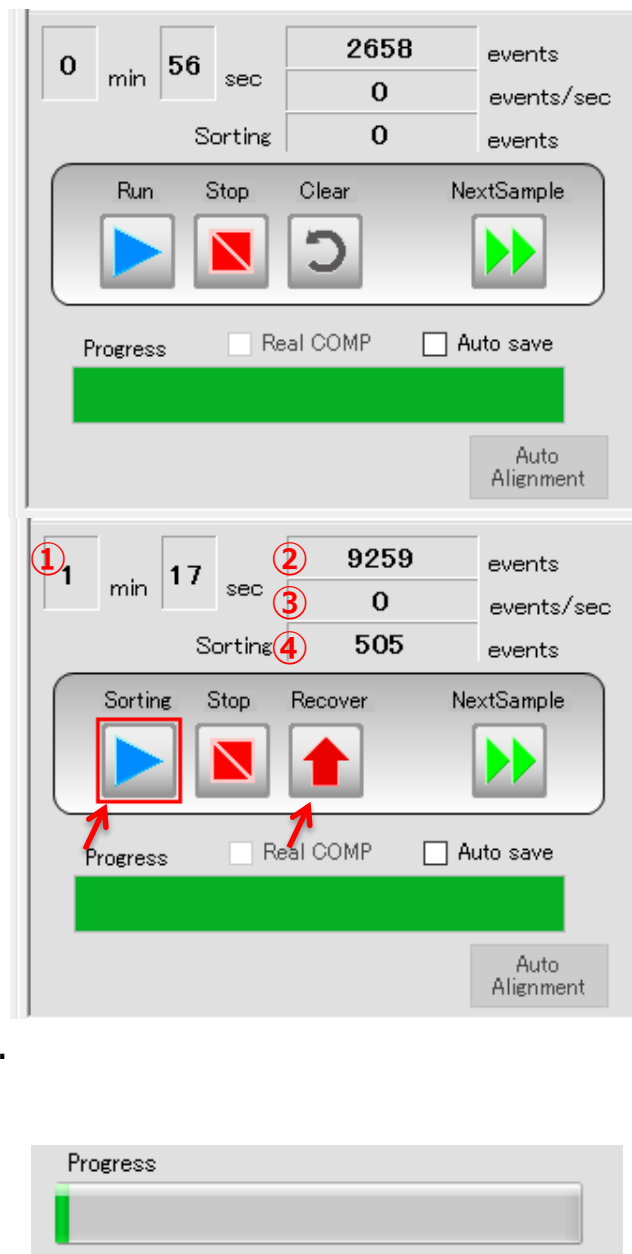
Sorting button appears where the Run button is when sorting gate is set by "Select Population" in III Workspace or IV Population Management. Sorting button is rounded by red frame. Sorting button blinks when it is clicked.

Recover button appears when Sorting button is clicked. Unlike the Stop button that stops the run, Recover actively flow sheath fluid into collection reservoir in order to drive cells that remained in the microchannel into collection reservoir. The yield increases by this process, but the purity of collected sample may decrease.

AutoAlignment button is used to performs alignment of the chip.

Green bar indicates the analysis/sorting progress. Other values are:

- ① : Operation time ② : Total event count
- ③ : Event rate ④ : Number of sorting events



3-8 VII. Camera view

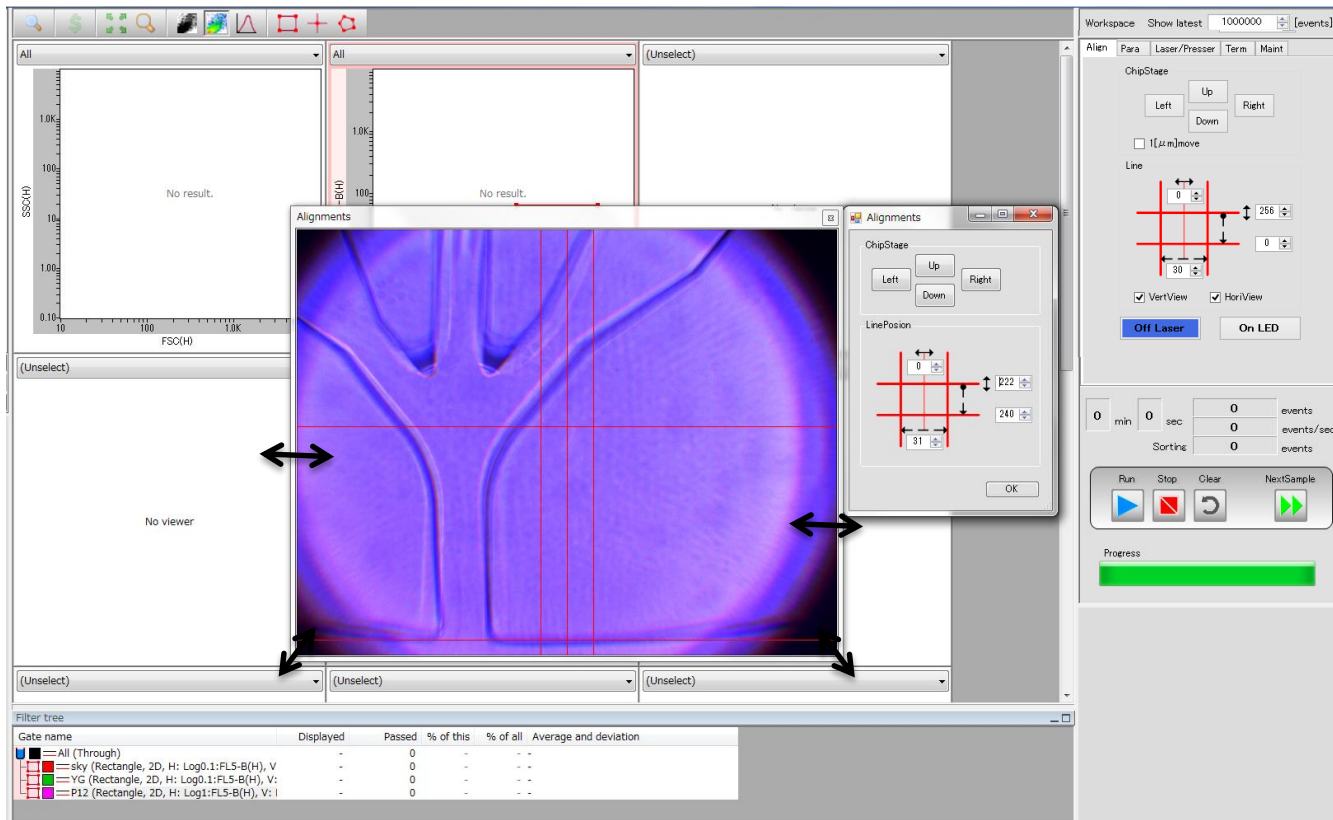
CCD image

The image of the screen lower right is a live image of flow channel by CCD camera.

LED turns OFF during analysis and sorting. Laser is then turned on.

The CCD image is magnified at the time of Alignment and CheckLine for clear view.

Crossing off the window at top right corner returns the zoomed CCD image back to its original location. The zoomed screen size can be changed freely.



3-8-1 Alignment parameter setting

This instrument is recognizing the chip coordinates using the edges created by sorting channels.

Auto alignment may not take effect if the sorting channels are not seen in the CCD image when the chip is inserted.

If the sorting channel is out of the screen, move the chip stage manually so that the sorting channels can be seen, and try auto alignment again.

Pull out the chip tray, and insert the tray back to initiate auto alignment.

If auto alignment is not successful despite following the steps above, an CCD image of correct location must be registered.

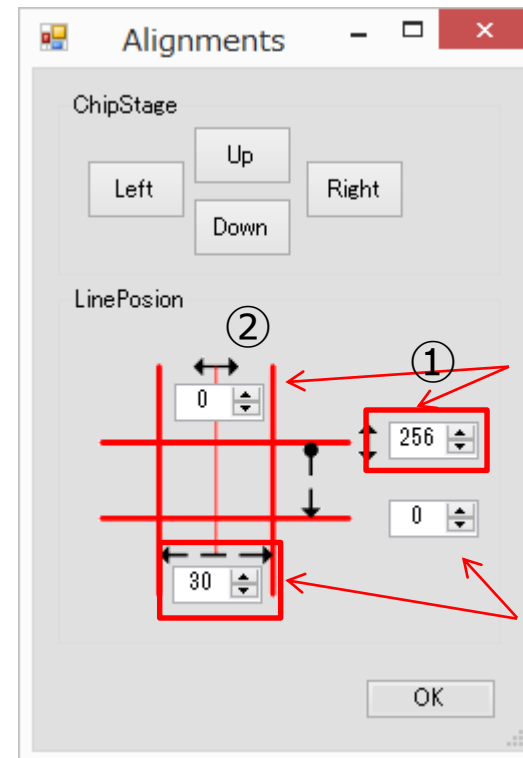
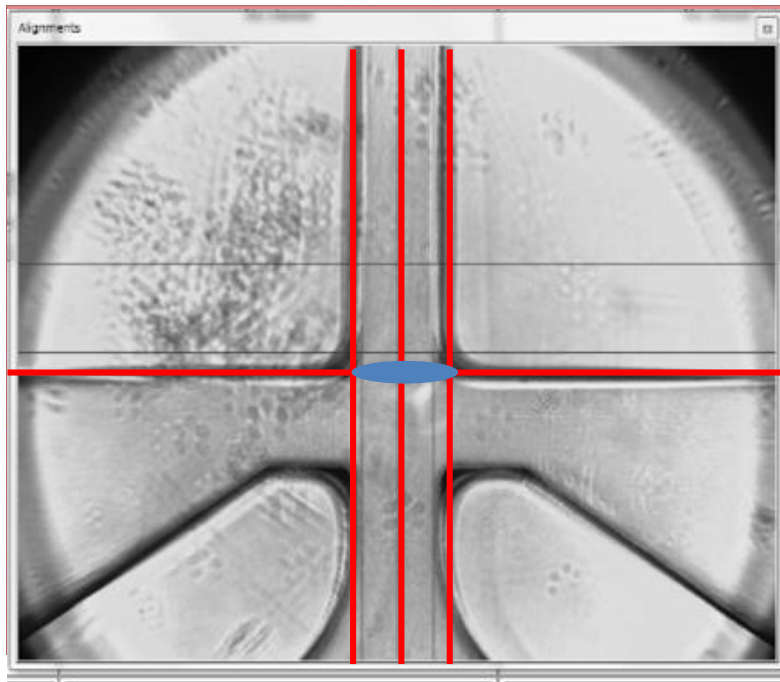
Check the laser and reference lines.

Insert a chip, and turn on the blue laser as shown in figure below.

① Move the red reference line on x-axis to the position of the laser.

② Align the center line of the y-axis reference line to the center of the laser beam spot.

Turn off the laser.



Must be adjusted depending on the laser position.

Fixed

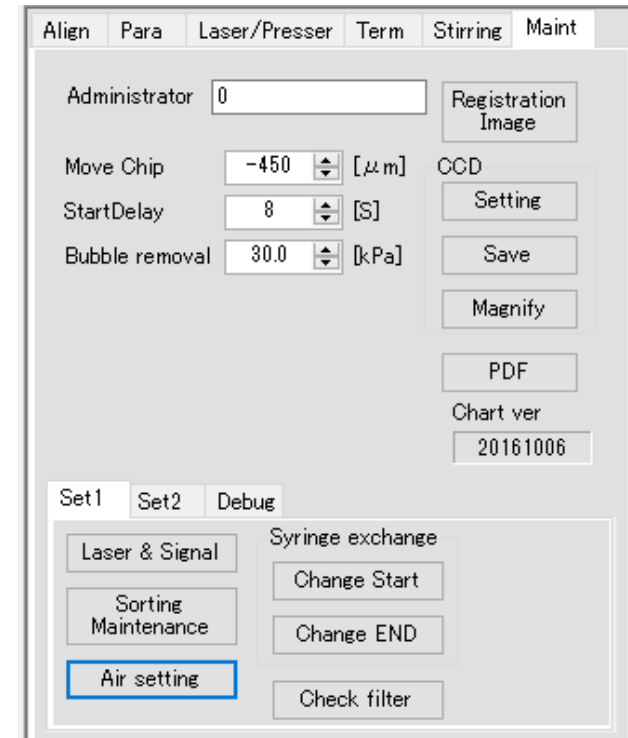
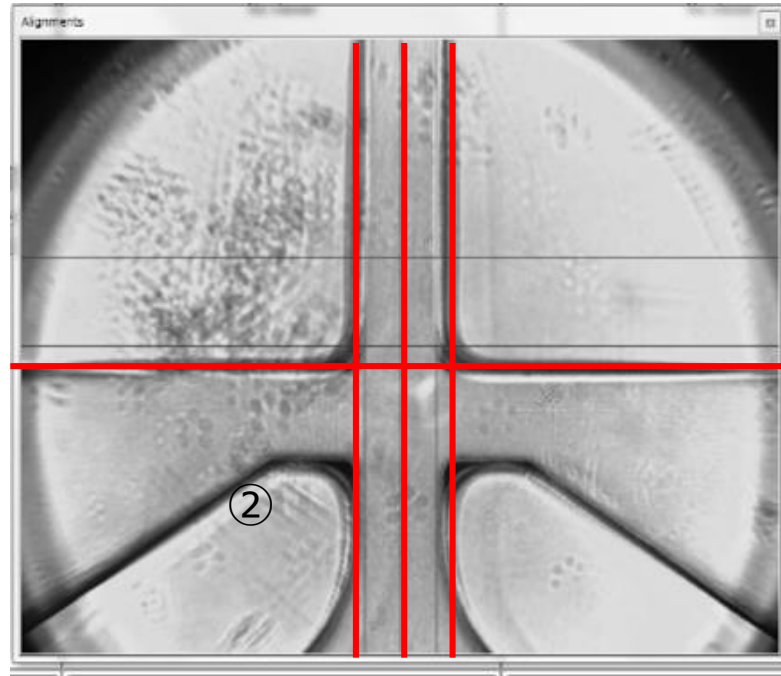
3-8-2 Image Registration

Move the chip to where the reference lines are.

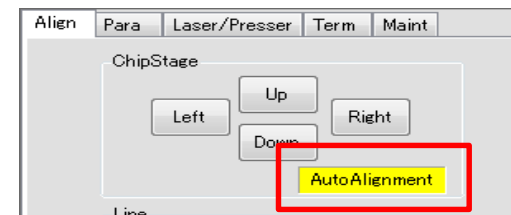
The vertical channel edges must align with the vertical reference lines.

③ Click on "Registration Image" button under Maint tab to complete image registration.

Caution: The software must be restarted for this to take effect.



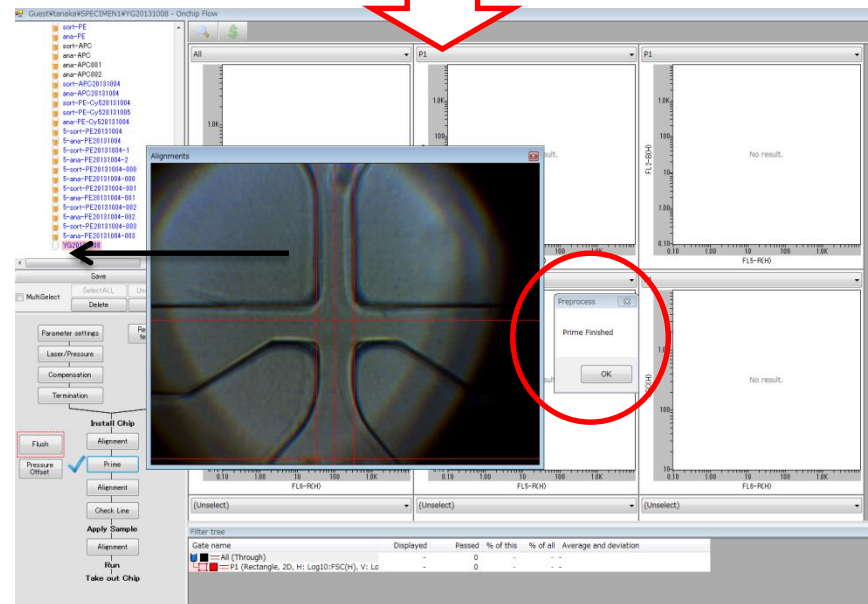
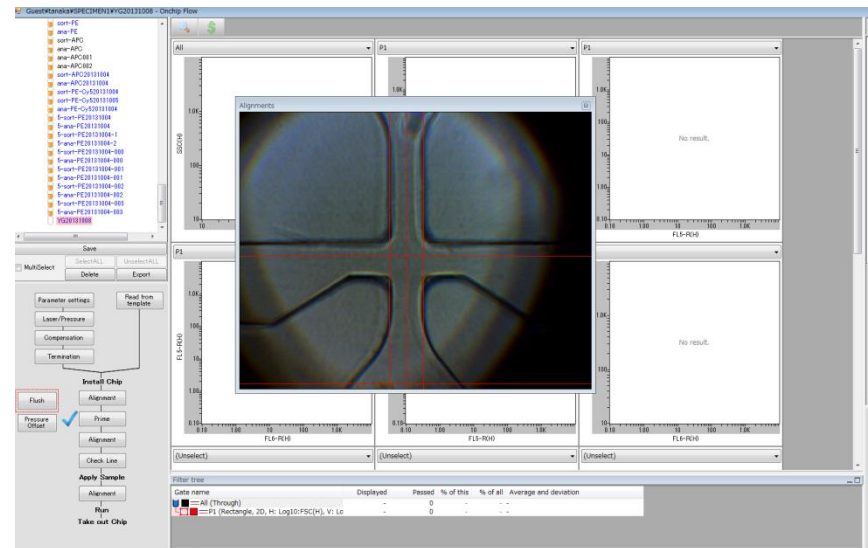
"AutoAlignment" sign appears during alignment process

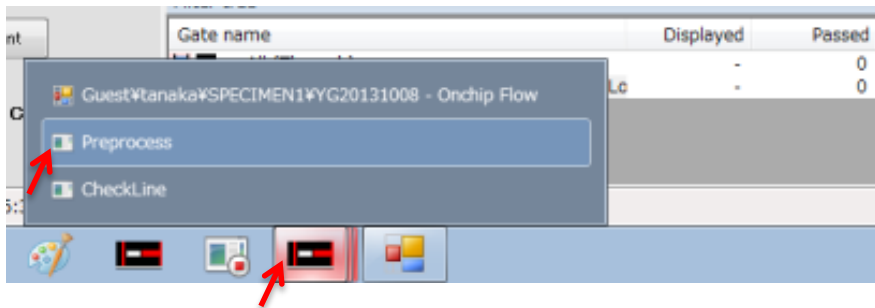


3-9 Troubleshooting

For when OK button for operation confirmation does not appear

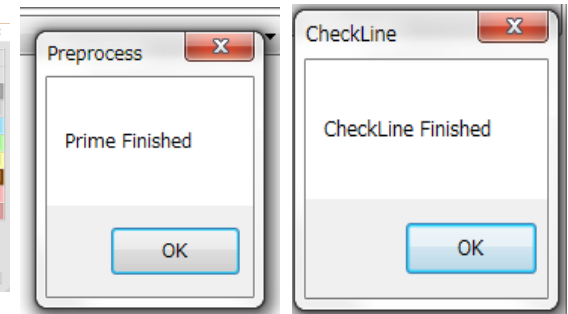
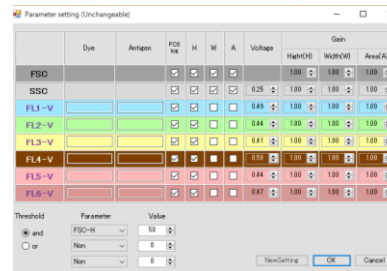
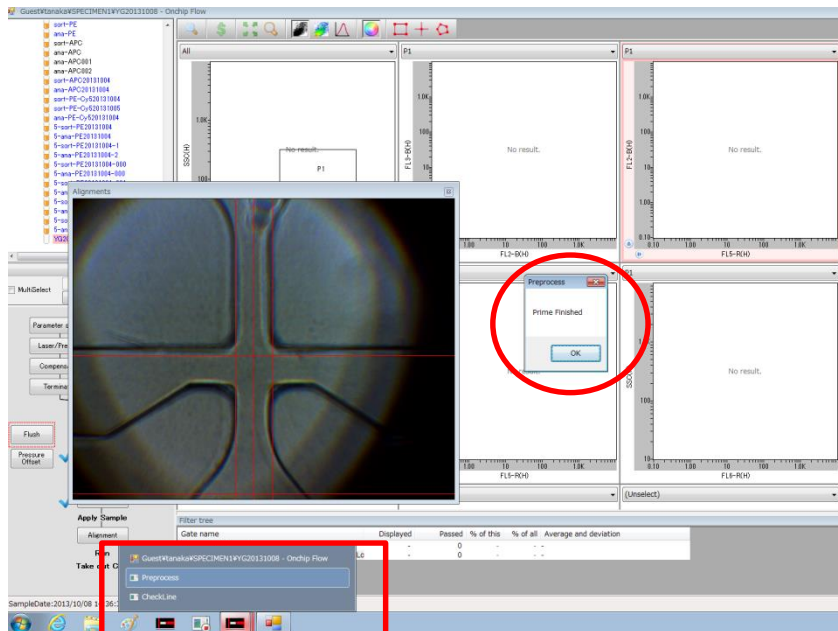
A dialogue with OK button appears after operation of certain actions, such as Prime. However, this dialogue may not be found as live image window always appear at the front, as shown in the figure on the right. In such cases, the dialogue may be hidden behind the live image window, and thus the dialogue may be found by moving the live image window to the side.





There may be some occasions where the dialogue still does not appear after taking actions mentioned previously. The small window moves to the back after clicking the main window. In such cases, click on the software icon at taskbar, and check if multiple windows are open. Select the window for it to appear at the front.

Examples of windows that may be hidden behind are as follows:



In operations other than Alignment, some small windows may be hidden behind the main window.

Chapter 4: QC (Quality Control)

4-1	About QC	4-1
4-1-1	Analysis QC (Daily)	4-1
4-1-2	Sorting QC	4-5

4-1 About QC

QC (Quality Control) is work to maintain the condition of the instrument at a constant level. We recommend regularly performing QC on the fluorescence detectors and sorting as QC for On-chip Sort.

QC of On-chip Sort

- ☐ Detection sensitivity (Sort/Flow common)
- ☐ Sorting (Sort only)

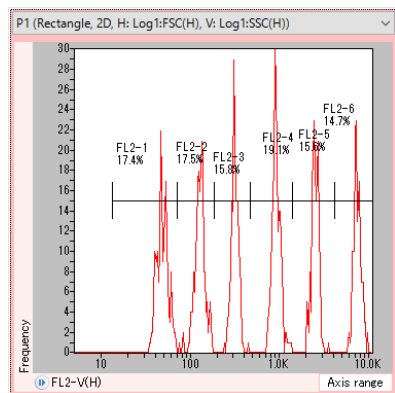
4-1-1 Analysis QC (Daily)

Analysis QC checks for the detection sensitivity of each fluorescence detectors using Rainbow calibration particle 6 peaks beads. Average and deviations are calculated for each peaks generated from the calibration peaks, and is checked for any offsets from the original values set at the time of installation.

If any values seems to be out of the tolerance range, the value is displayed in red.

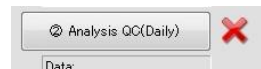
H: Ave=56.9(1.44), Dev=27.7(2.49), Cv=48.7%

The instrument is said it is functioning fine when all values are within the permissible range.



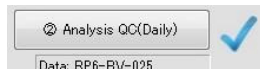
Population name	Displayed	Passed	% of this	% of all	Average and deviation
FL2-1 (Sandwich, Histogram, Log1:FL2-V(H))	175	175	17.4%	7.53%	H: Ave=49.2(1.09), Dev=7.1(1.05), Cv=14.5%
FL2-2 (Sandwich, Histogram, Log1:FL2-V(H))	176	176	17.5%	7.57%	H: Ave=129.8(1.12), Dev=17.1(1.12), Cv=13.2%
FL2-3 (Sandwich, Histogram, Log1:FL2-V(H))	159	159	15.8%	6.84%	H: Ave=320.7(1.12), Dev=30.4(0.77), Cv=9.5%
FL2-4 (Sandwich, Histogram, Log1:FL2-V(H))	192	192	19.1%	8.26%	H: Ave=940.9(1.14), Dev=104.4(0.97), Cv=11.1%
FL2-5 (Sandwich, Histogram, Log1:FL2-V(H))	157	157	15.6%	6.76%	H: Ave=2567.8(1.13), Dev=234.9(0.73), Cv=9.1%
FL2-6 (Sandwich, Histogram, Log1:FL2-V(H))	148	148	14.7%	6.37%	H: Ave=7716.9(1.12), Dev=734.5(0.86), Cv=9.5%

<Unexecuted>



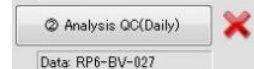
"X" flashing

<Complete>



"✓" flashing

<Error>



"X" flashing and an error message

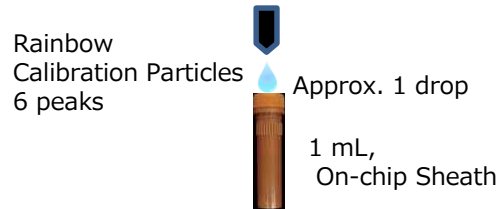
Analysis QC(Daily) protocols

Step 1

Add 50 μ L of calibration beads ※¹ to 1mL of On-chip Sheath※² to adjust the event rate to about 20 event/sec. (Homogenize the sheath fluid and beads before adding beads to the liquid. **Avoid leaving the lid open and at room temperature, as culture medium in On-chip Sheath would precipitate.**)

※¹ Spherotech Rainbow Calibration Particles 6 peaks 3.2(+/- 0.1) μ m; Cat No. RCP-32-5

※² On-chip Sheath; Cat No. 2001012

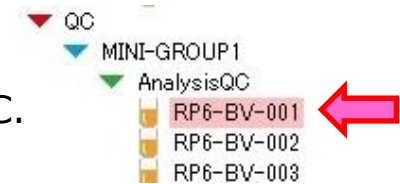


Step 2

Load appropriate amount of sheath into each well of 2D Chip-Z1001 chip. (More details in Chapter 5)

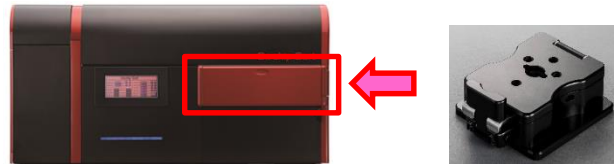
Step 3

Select the sample file at the top of AnalysisQC specimen folder under QC.



Step4

Insert the chip inside the chip holder, then in the instrument. Click **Prime**. After the priming, remove buffer from sample reservoir (refer to Chapter 5). Set 50 μ L of the sample prepared in Step 1. Ensure the chip is auto-aligned properly.

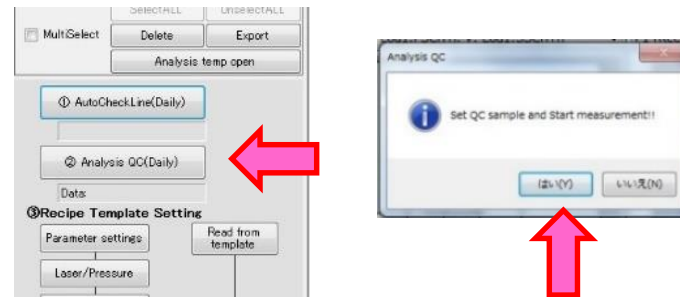


NOTE

In QC of detectors, detection conditions such as Gain, flow speed and chip type must be kept the same.

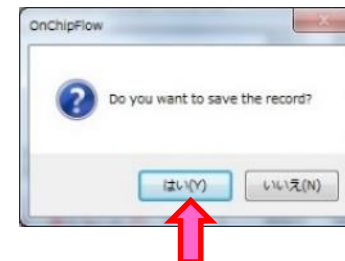
Step 5

Click "AnalysisQC(Daily)" button, then click Yes (or はい).



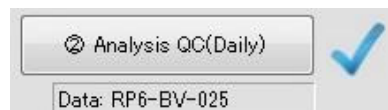
Step 6

When the analysis is complete, save the data by clicking Yes (or はい).

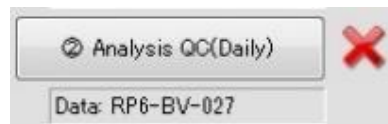


Step 7

A tick "✓" next to the Analysis QC(Daily) button indicates that the detection system is functioning correctly.



When the values obtained are out of the permissible range, the software will display an error message with blinking cross "✗" next to the Analysis QC(Daily) button.



If the QC gives an error, run the same process for two more times. If any of these runs give a tick "✓", the instrument is functioning fine.

If this error seems to persist, please contact us.

On-chip Biotechnologies, Co. Ltd.

E-mail: info@on-chip.co.jp TEL : 042-385-0461

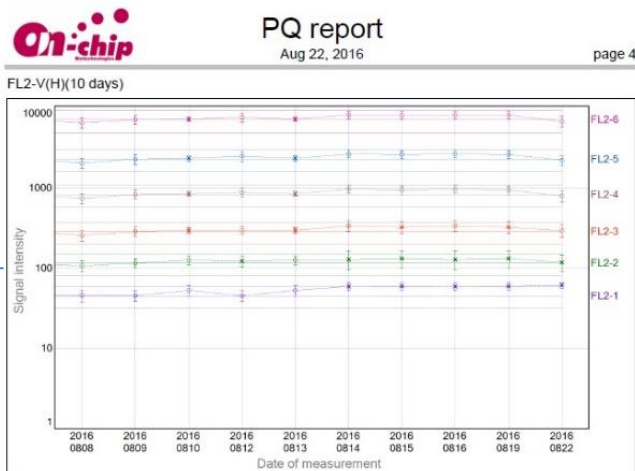
Step 8

The results of QC can be exported as PQ report in PDF format.

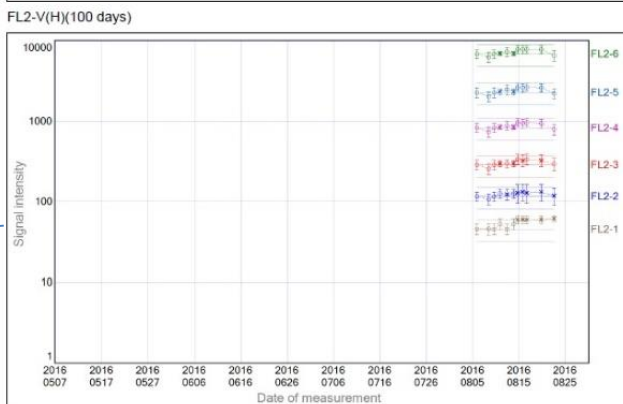
Click on "OutPut PQ Report" button and specify where to save the file.



Report for
recent 10 days



Report for
recent 100 days



$\overline{\Phi}$ ← Tolerance limit
 Φ ← Average and error bars
 $\overline{\Phi}$ ← Tolerance limit

Φ ← Dev
 Φ ← Ave
 Φ ← Dev

4-1-2 Sorting QC

Step 0 Two types of samples for sorting QC

2 types of samples are prepared: target particles (Sky Blue) and non-target particles (YG). Non-target particle (YG 3 μ m) should be adjusted to 50 events/sec, while target particles (Sky Blue 3 μ m) should come to about 3% of the total population. Preparation of the two kinds of samples is as following.

☐ Preparation of target particle suspension (Sky Blue)

Target particle suspension is a mixture of 2 μ L of Sky Blue (size:3 μ m)^{※1} and 1mL of On-chip sample buffer^{※3}.

☐ Preparation of non-target particle suspension (YG)

Non-target particle suspension is a mixture of 15 μ L of YG (size:3 μ m)^{※2} and 1mL of On-chip sample buffer^{※3}.

NOTE

Non-target (YG) and target (Sky Blue) suspensions should be prepared each time. The beads may coagulate and would affect the QC results when stored for a long time.

Step 0

☐ Target particle suspension (Sky Blue)

2 μ L of SkyBlue^{※1}

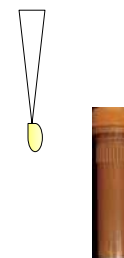


1mL of On-chip sample buffer^{※3}

Sample buffer must be x1 equivalent

☐ Non-target particle suspension (YG)

15 μ L of YG^{※2}



1mL of On-chip sample buffer^{※3}

Sample buffer must be x1 equivalent

- ※1 Sky Blue 3 μ m : SPHERO™ Fluorescent Particles Sky Blue, 0.2% w/v, 3 μ m (Spherotech, No:FP-4070-2, Vol: 2ml)
- ※2 YG 3.0 μ m: Fluoresbrite™ Calibration Grade YG Microspheres, 3.16 μ m (Polysciences, No:18861, Vol: 1ml)
- ※3 On-chip Sample buffer On-chip Biotechnologies, Cat. No. 2001013 OR sample buffer prepared using culture medium or PBS (Sample buffer prepared using On-chip Sample buffer 2x or On-chip Sample buffer preparation kit, with more details in Chapter 5, 5-2-2).
On-chip brand Sample buffer must be used.

Procedure of Sorting QC

Step1

Select 2-Sorting under detector specimen of data tree view.

Step2

Set a chip in the chip holder and load 50 μ L of 1x Sample buffer into sample reservoir. 3mL, 1.5mL and 100 μ L of sheath fluid into sheath reservoir, sorting reservoir and collection reservoir, respectively. Perform "Prime" and "Check line". (See Chapter 5 for more details)

NOTE 1x Sample buffer must be loaded to the sample reservoir

Step3

Remove sample buffer in sample reservoir, and load 1:1 mixture of Sky Blue and YG into sample reservoir. (e.g. set a mixture of 20 μ L of Sky Blue and 20 μ L of YG into sample reservoir.) Return the loading port to activate chip auto alignment.

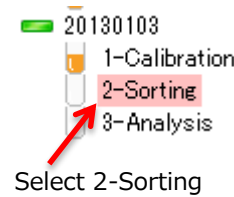
Step4

Ensure the button says **Sorting**, then click the button to start.

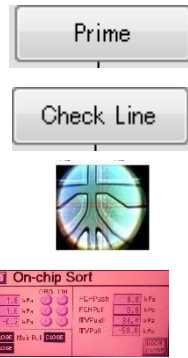
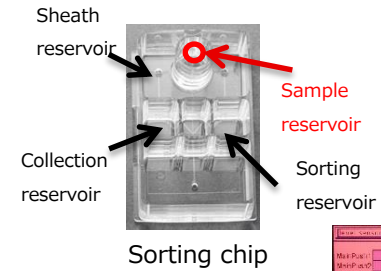
Step5

After total sorting events reached 100-200 events, click **Stop**. To check the purity and yield of sorted sample, reanalyze the sorted particles (Step 6).

Step 1

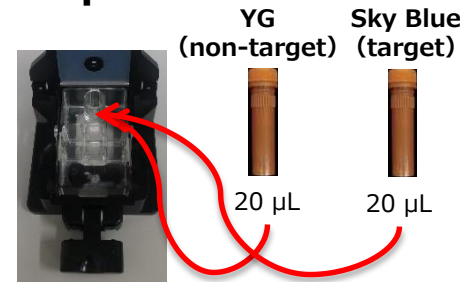


Step 2



Carry out Prime and Check Line

Step 3



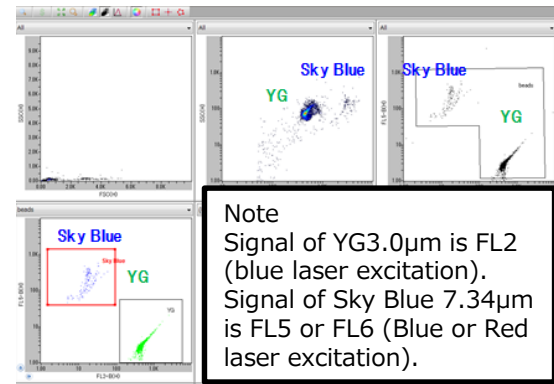
Set chip into the chip holder, and load 1:1 mixture of Sky Blue and YG into sample reservoir (e.g. 20 μ L+20 μ L). Activate auto alignment.

Step 4

Magnified



Step 5



Sorting 100~200 events



After 100-200 sorting events, click "Stop"

Step6

Select 3-Reanalysis in the specimen tree view. Set a new chip in the chip holder, and carry out "Prime" process using sheath fluid. After Prime, remove liquid from sample reservoir.

Step 6



Select 3-Reanalysis



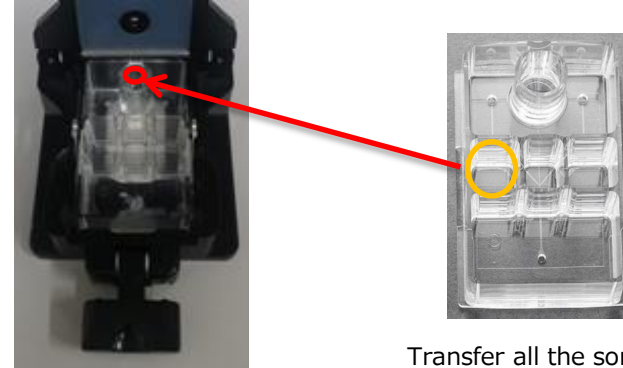
Set a new chip in the chip holder, and carry out "Prime" sheath fluid.

Step7

Transfer all the sorted sample from the collection reservoir of the chip in Step 5 to the new chip.

NOTE In the case of reanalysis, the speed does not have to be the same as speed of sorting. Although the change in flow speed changes signal intensity, the QC result would not be affected.

Step 7



Transfer all the sorted sample of the collection reservoir of the chip in Step 5 to the new chip in Step 6.

Step8

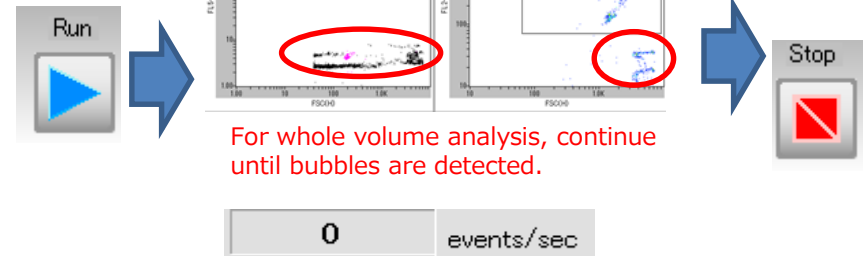
Click **Run** and carry out whole volume analysis. After whole volume analysis, click **Stop** and save the data.

NOTE

The whole volume of the sample must be run through for reanalysis. The indications for the whole volume analysis completion are:

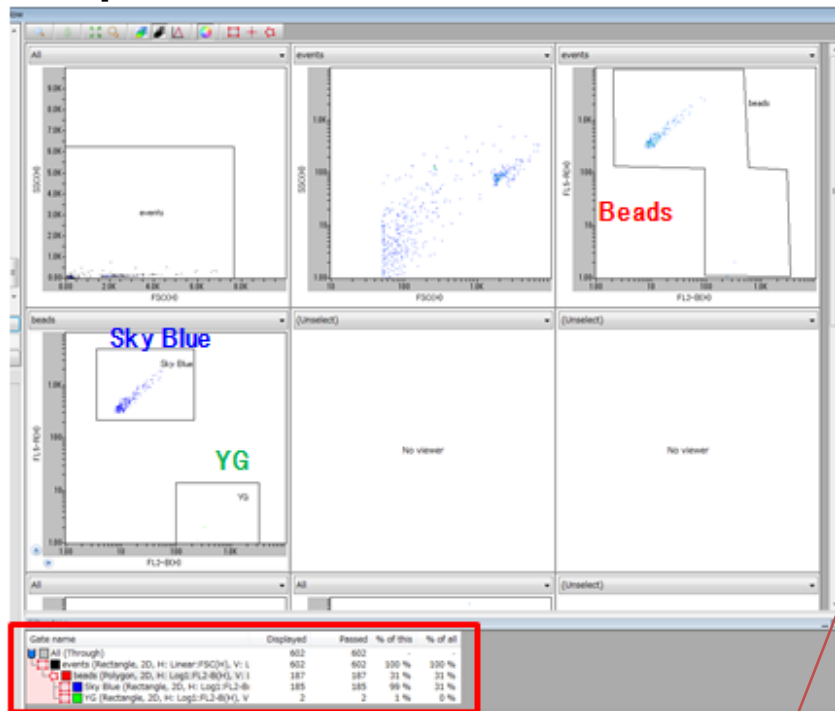
- ◆Plots unique to air bubbles appear
- ◆Event rate becomes almost 0
- ◆The laser spot seen in Camera View blinks

Step 8



Or until event rate reaches 0

Step 9



Step9

Calculation of Yield (sorting efficiency) and Purity

Yield

Yield (sorting efficiency%) =

$$\frac{\text{Number of sorted target particles (SKY)}}{\text{Number of sorting pulses (Sorting events)}} \times 100$$

Sorting 100~200 events

Purity

Purity (%) =

$$\frac{\text{Number of sorted target (SKY)}}{\text{Total number of sorted particles (total=Sky+YG)}} \times 100$$

Gate name	Displayed	Passed	% of this	% of all
All (Through)	602	602	-	-
events (Rectangle, 2D, H: Linear:FSC(H), V: L	602	602	100 %	100 %
beads (Polygon, 2D, H: Log1:FL2-B(H), V: L	187	187	31 %	31 %
Sky Blue (Rectangle, 2D, H: Log1:FL2-Bi	185	185	99 %	31 %
YG (Rectangle, 2D, H: Log1:FL2-B(H), V	2	2	1 %	0 %

NOTE

The tolerance for >60% and >80% for Yield and Purity, respectively. If the yield is below this tolerance level, the pulse timing may be incorrect. Follow the instruction on next page to adjust the timing. If the purity is below the tolerance level, the open time of CheckLine (See Chapter 5 for more details) may be too long (i.e. the value is too large), thus it may require small adjustment.

Sorting timing adjustment protocol

(In the case of low Yield only)

If the yield is low, then the sorting pulse timing may be off. Follow the steps below to adjust the timing.

Step1

Add sample to sample reservoir and remove liquid from waste reservoir.

Step 2

Select 2-Sorting in data tree view and carry out “Duplicate without data” to make empty data file for sorting. Similarly, make empty data file for re-analysis by “Duplicate without data” from 3-Reanalysis.

Step 3

Go to Maint tab and increase the Move Chip value by 50 (e.g. from -450 to -500), sort the beads again and carry out reanalysis. If the yield is higher than the previous value, increase the value by another 50. If the yield is lower than the previous value, decrease Move Chip value by 50. Repeat this process until the optimal sorting yield is achieved. **Change value each time by about ± 50 .**

NOTE

Please be cautious about the following points:

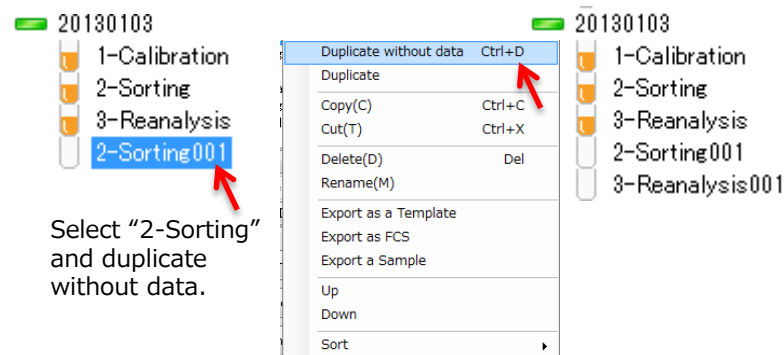
- ◆ Confirm that the yield is determined with all particles from collection reservoir. If particles are left to the collection reservoir, the yield is estimated as a lower value.
 - ◆ Immediately re-analyze the sorted sample because sorted particles in collection reservoir may flow back into the main channel when the chip is left untouched for a long period of time.
- When yield does not improve, please contact our customer center.

Step 1



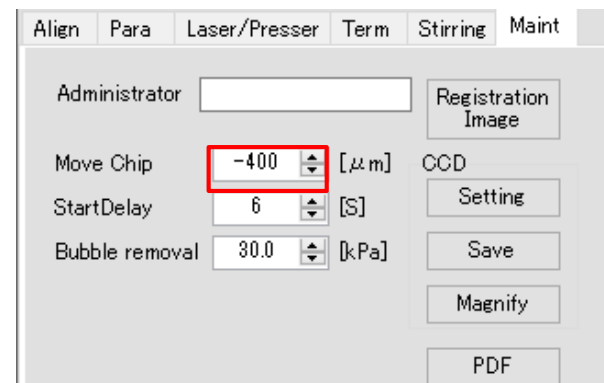
Remove waste

Step 2



Select “2-Sorting” and duplicate without data.

Step 3



Chapter 5: Analysis and Sorting

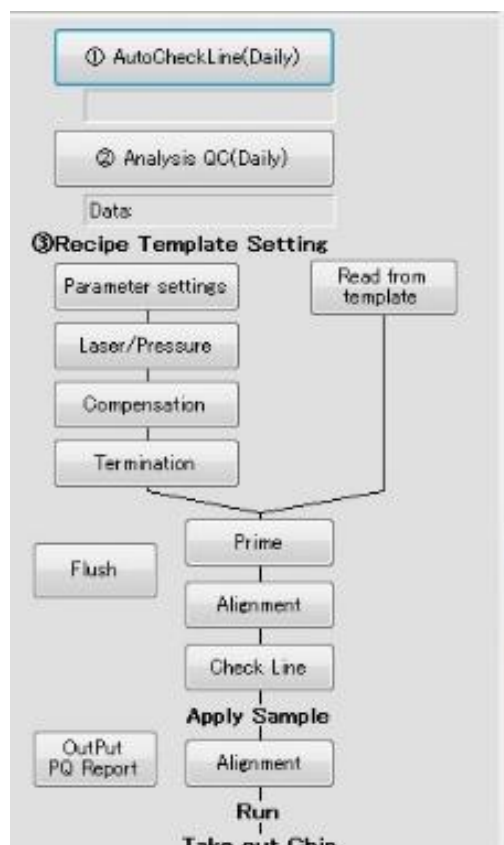
5-1	Basic Operation Procedure.....	5-1
5-2	Buffer Preparation (Sheath Fluid and Sample Buffer).....	5-2
5-2-1	Using On-chip Standard Buffer Solutions.....	5-2
5-2-2	Using Prepared Solutions as Buffer Solutions.....	5-3
	①For PBS-base solutions.....	5-3
	②Using medium as base.....	5-4
5-3	Instrument and Software Start up.....	5-5
5-3-1	Power on.....	5-5
5-3-2	Software Start up and Log in.....	5-5
5-4	Sample File Creation and Selection.....	5-6
5-4-1	Creating a New File with Settings Carried Over.....	5-6
5-4-2	Creating a New File.....	5-7
5-5	Parameter Settings.....	5-8
5-6	Laser/Pressure Settings.....	5-11
5-7	Auto Termination Condition Settings.....	5-13
5-8	Loading Buffer Solution.....	5-14
5-8-1	Using Sorting Chip 2D-Z1001.....	5-15
5-9	Inserting Chip.....	5-16
5-10	Priming.....	5-17
5-11	Check Line.....	5-18
5-12	Sample Loading.....	5-19
5-13	Laser Alignment.....	5-21

5-14	Run (Sorting or Analysis)·····	5-22
5-14-1	Gate Settings·····	5-23
5-14-2	Gate Type Selection·····	5-24
5-14-3	Gate Extension·····	5-25
5-14-4	Gain Adjustment·····	5-26
5-14-5	Threshold Settings·····	5-27
5-14-6	Gain Adjustment·····	5-28
5-14-7	Fluorescence Compensation·····	5-29
5-14-8	Displaying Gating Steps·····	5-39
5-14-9	Sorting·····	5-40
5-14-10	Sorting Initiation and Termination·····	5-41
5-14-11	Sorted Sample Collection·····	5-42
5-15	Collected Sample Purity Assessment·····	5-43
5-16	Maintenance·····	5-45
5-16-1	Washing Chip Holder and Silicon Packing (Gasket)···	5-45
5-16-2	Software Updates·····	5-46

5-1 Basic Operation Procedure (common)

On the On-chip software, the operation steps are displayed under Processing Zone.

Once the instrument and the software are running and buffer solutions (sheath fluid and sample buffer) preparation is complete, follow the steps displayed in Processing Zone. The process will get a tick (✓) when the procedure is complete.

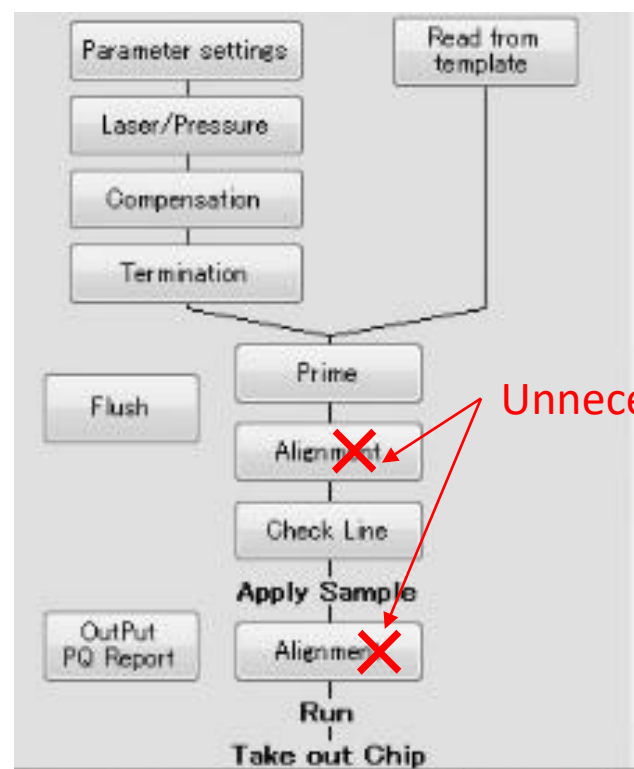


Processing Zone

Follow the steps shown
in Processing Zone

When a New File is created

Operating at the same
conditions as saved run



Processing Zone

5-2 Buffer Adjustment

The distinctive feature of On-chip Sort is that the user can select the sheath fluid and sample buffer solution to their preference.

Two examples are explained herein. Please select according to the goal of the experiment.

1. Using On-chip Standard Buffer Solutions (5-2-1)
2. Using Prepared Solutions (5-2-2)

5-2-1 Using On-chip Standard Buffer Solutions

There are standard buffer solutions (sheath fluid and sample buffer) available that allows users to sort samples in one single step. All On-Chip buffer solutions are sterilized.

- ☐ Sheath Fluid: On-chip Sheath (Cat. No. 2001012), 200mL
- ☐ Sample Buffer: On-chip Sample Buffer (Cat. No. 2001013), 50mL

☐ On-chip Sheath

(Cat. No. 2001012) 200mL

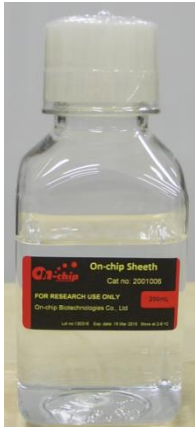
Ready-to-use sheath fluid without any further adjustments.

- Contents-
- Proprietary serum-free cell preservation fluid
- Through Path Plus

-Feature-

Sorted cells can be stored in a refrigerator (4°C) for one day

NOTE This is a mixture close to a culture medium, and thus opening in a clean bench is recommended.



☐ On-chip Sample Buffer (for sorting)

(Cat. No. 2001013) 50mL

Used as a suspension medium for the cells. Also used for adjusting the Check Line.

- Contents-
- 1x viscosity adjustment agent and serum-free cell preservation fluid
- Through Path Plus

-Feature-

High viscosity
Maintains a stable cell sedimentation speed

NOTE This contains culture media-like reagent, and thus opening in a clean bench is recommended.

NOTE As the viscosity of the sample must be accurate, cells should be washed two to three times using On-chip Sample Buffer prior to resuspension. Due to the reduced sedimentation rate, the sample must be centrifuged at a rotational speed 1.5 to 3 times higher than the normal speed.



5-2-2 Using Prepared Solutions as Buffer Solutions

Users can choose from various solutions, such as culture media, to use as buffer solutions (sheath fluid and sample buffer) by adding the following products to the intended buffer solutions.

① <For PBS-base solutions>

- ☐ Sheath Fluid: Through Path plus 40x (Cat. No. 2001004-6), 10mL x 6 bottles
- ☐ Sample Buffer: On-chip Sample Buffer 2x (Cat. No. 2001015), 50mL

☐ **Through Path plus 40x (for sheath fluid production)** (Cat. No. 2001004-6)



Allows the use of intended buffers as sheath fluid by adding 1/40 of the buffer volume
(Prior filtration of buffer is recommended)

- Contents-
- Hydrophilic coating for microfluidic channels
- Features-
- Prevents blockage of the microfluidic channels by cells
- Biocompatible and harmless to cells

<Sheath Fluid Preparation>

E.g. 1 Add 1mL of Through Path Plus 40x to 39mL of RPMI1640 (10% FBS, 1% penicillin/streptomycin), and filtrate the mixture.

E.g. 2 Add 100μL of Through Path Plus 40x to 3.9mL of PBS, and filtrate the mixture.

<Using Adjusted Sheath Fluid>

Use the culture medium mixture with Through Path plus 40x as the sheath fluid for sorting and analysis. Each sorting run requires around 3mL of sheath mixture solution.

☐ **On-chip Sample Buffer 2x (for sample buffer adjustment) (for sorting)** (Cat. No. 2001015)



Quick and easy preparation of sample buffer using media of preference.

- Contents-
- 2x viscosity adjustment with PBS base
- Features-
- Mixed in 1:1 ratio
- High viscosity
- Maintains a stable cell sedimentation speed

<Example of Sample Buffer Use>

1. Mix On-chip Sample Buffer 2x to the same amount of sample solution to produce sample buffer solution.
As On-chip Sample Buffer 2x does not contain Through Path plus, it should be added when necessary.

NOTE Please make sure you mix the solution in 1:1 ratio as the viscous agent is at 2 time concentrate. The sorting efficiency will decrease if the viscosity changes.

2. Use the sample buffer mixture prepared above when adjusting the Check Line.

②<Using medium as base>

- Sheath Fluid: Use the medium of your preference as it is
- Sample Buffer: On-chip Sample Buffer Production Kit (Cat. No. 2001011), 15mL x 3

□ **On-chip Sample Buffer Production Kit** (For sorting users)
(On-chip Sample Buffer Production Kit (Cat. No. 2001011)), 15mL×3

Add 10mL of your preferred medium to this product to produce sample buffer.

-Contents-

- Viscosity adjustment agent (powder)
- ※The viscosity agent has been proven non-toxic to most standard cells.

-Features-

- Mix medium 15mL and this product
- Maintains a stable cell sedimentation speed
- ※This product assumes the use of cell culture medium with standard viscosity, such as RPMI.
- The volume must be carefully considered for the use of viscous solutions.
- Please contact the support team.

<Example use of On-chip Sample Buffer Production Kit>

① Add 15mL of medium to this product according to the protocol, and use the mixture as sample buffer. As the kit does not contain Through Path plus, it should be added when necessary.

NOTE Make sure all the powder is dissolved for correct viscosity. The sorting efficiency decreases when the viscosity changes.

② The sample buffer prepared above can be used for CheckLine.



※ Make sure the power is drawn using a 3-prong plug, including the extension cord.
The signal may become unstable if disregarded.



3-prong plug

5-3 Starting the Instrument and Software

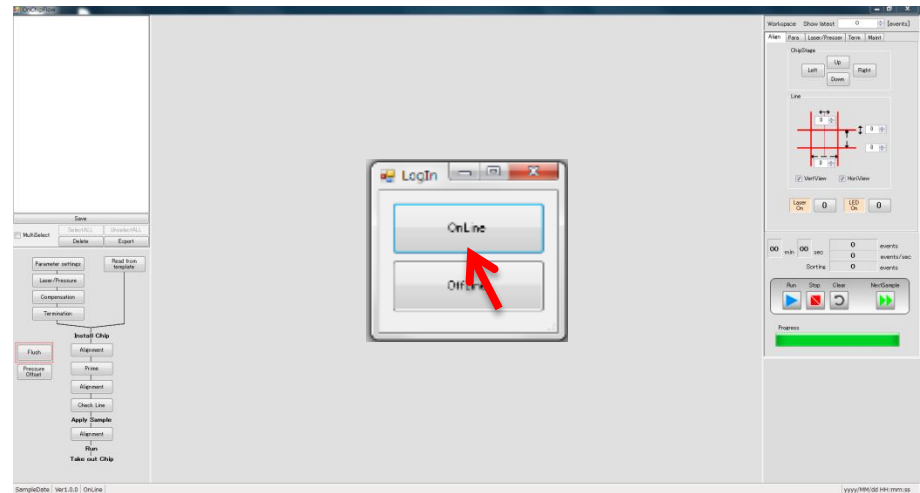
5-3-1 Power on

- 1) Turn on On-chip Sort.
(Turn the switch on the power supply box)
- 2) Turn the laptop computer on.



5-3-2 Software Start up and Log in

- 1) Double click the OnChipFlow icon.
- 2) Click "Online".



5-4 Creating and Selecting Sample File

The users are required to create a new sample file when starting a new sorting or analysis. Two examples are explained herein.

- ☐ Creating a New File with Settings Carried Over (5-4-1)
- ☐ Creating a New File (5-4-2)

5-4-1 Creating a New File with Settings Carried Over

Step 1

Select an existing sample file under Sample management, right click, and select "**Duplicate without data**" to copy the sample file.

The new file will be given the same name from the original file and a new number assigned by the software. The measurement conditions and parameters (such as gates and Gain) are carried over to the new sample file.

e.g. Original file name: ABCDE
 ↓ Duplicate without data
 New sample file name: ABCDE001

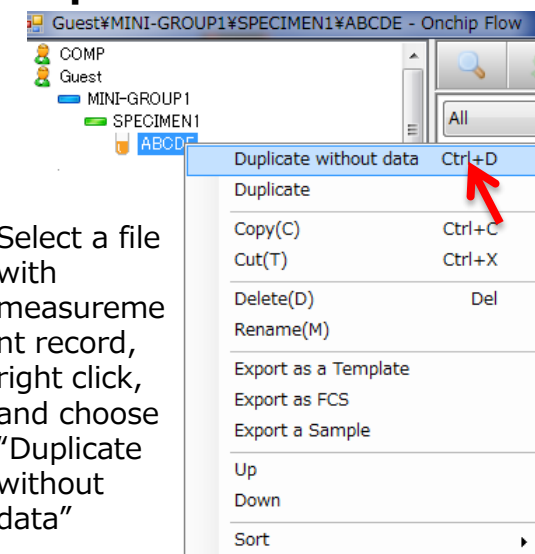
Step2

Any files can be renamed by right clicking the file and selecting “**Rename**”.

NOTE

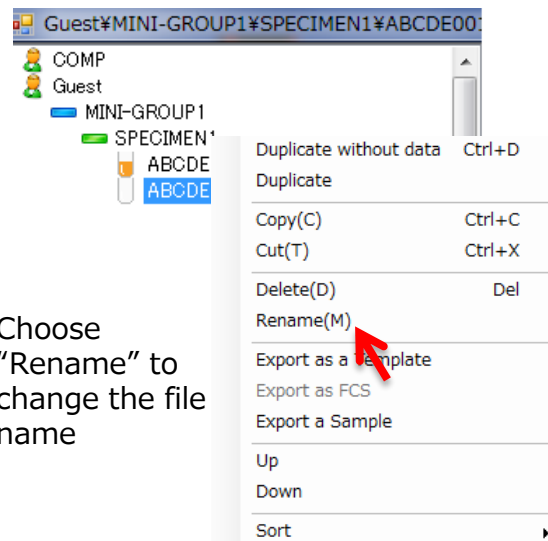
You can create a new Mini-Group and a new sample file with the same measurement conditions within the new Mini-Group. You can also import a template by clicking “**Read from template**” in Processing Zone. Please refer to Chapter 3 for more information regarding importing a template.

Step 1



Select a file with measurement record, right click, and choose "Duplicate without data"

Step2



Choose
"Rename" to
change the file
name

5-4-2 Creating a New File

Step1

Right click on a Specimen file under Sample Management, and select **“Create a new sample”** to create a new sample file with a name “SAMPLE1”.

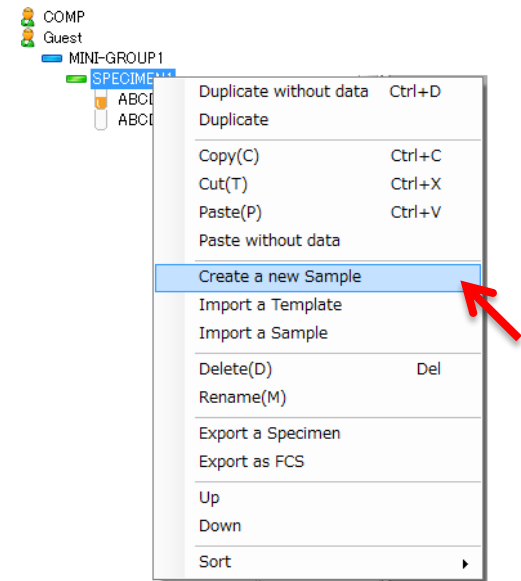
NOTE

When a new Sample File is created according to the procedure above, the measurement settings and parameters are not carried over, and thus measurement settings (such as Gain and MainPush pressure settings) must be adjusted appropriately for the new sample. Please refer to 5-5 under “Parameter Settings”.

NOTE

Please refer to “Chapter 3: Software Configuration” for instructions to create other types of files (such as creating a new Mini-Group and a new SPECIMEN). Please contact our support team for any further questions.

Step1



A new file “SAMPLE1” is created when you select “Create a new sample”.



5-5 Parameter Settings

This section describes the steps required in the case where a new Sample File was created by clicking "Create a new sample".

Step1

Select the "**Parameter settings**" button to open the advanced setting dialog box.

Step2

Select the H (height) boxes for the required FL. If the Dye and Antigen fields are filled, they are reflected in the displays.

E.g. For ○○-Alexa Fluor® 488, the maximum excitation is 495 nm and maximum emission is 519, and thus the blue laser is used to excite and emission is detected using FL-3, so H (height) box of FL3-B must be ticked. Please refer to a table of commercially sold dye-color relationship (such as one shown in 1-1-4).

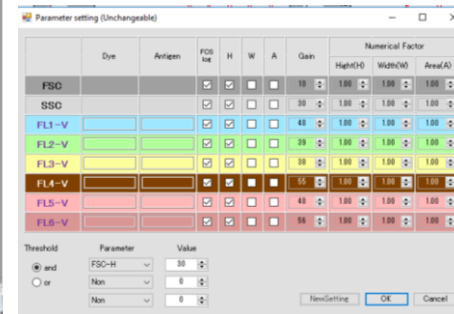
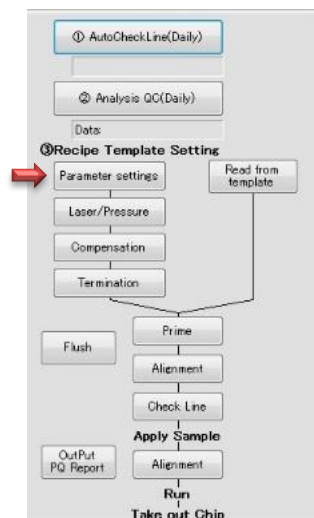
NOTE Log display in the work space is possible, regardless of whether or not log is selected here. However, if the FCS format is used for export, only one of log or linear can be selected. If log is not selected, linear is used in the exported document. W (width) and A (area) can also be selected. Up to 10 parameters can be selected.

Step3

Gain can be set for SS in the range of 0 to 100. However, in some cases the signal may saturate and prevent detection if the setting is 40 or higher, and thus it is better to set the Gain to around 30 and check the signal before adjusting. FL-1 to FL-6 can be set in the range of 0 to 100. The actual range in use is 30 to 80.

NOTE Gain can be adjusted during a measurement.

Step 1



Select the "**Parameter settings**" button to open the advanced setting dialog box.

Step 2

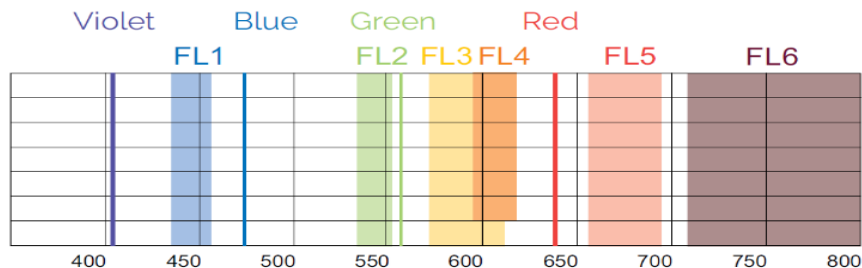
FCS log	H	W	A
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Select the appropriate detectors (FL-) and laser color (-B, -R or -V) according to the dyes used.

Step 3

Gain
10
30
48
39
38
55
48
56

Settings the Gain



	FL1	FL2	FL3	FL4	FL5	FL6
Violet	DAPI, AF405, PacBlue, V450, BV421	PacOrange, V500, BV510	PacOrange, BV605		BV650	BV711, BV786
Blue		FITC, AF488, GFP, CFP, YFP	PE	PI, 7AAD	PerCP, PerCP-Cy5.5, PE-Cy5	PE-Cy7
Green			DsRed, mCherry, tdTomato, TagRFP			
Red					APC, AF647	APC-Cy7, APC-H7, AF700

* References for **Parameter settings** *

Maximum of 10 colors from 13 dyes from the table on the left can be measured using 3 lasers (Violet, Blue, Green and Red) and 6 detectors (FL1-6). Maximum number of 10 colors is due to the specifications of the signal treatment circuit.

Step 4

Numerical Factor		
Hight(H)	Width(W)	Area(A)
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00

Step4

The initial setting of the Numerical Factor is 1.00.
It is recommended to run the measurement using the initial setting.

The value of Numerical Factor can be altered at later stage if necessary.

NOTE

Please do not set the Numerical Factor value to 0. The plot will not be displayed.

Use the initial setting of 1.00

Step5

Setting the threshold condition (Refer to 3-3-2 Parameter Settings for further details)

The detection trigger must be the signal derived from blue laser excitation. If multiple selections from FS, SS, FL1-B to FL6-B are selected as detection trigger signals (using checkboxes), select AND or OR as the condition.

◆ If AND is selected, detection occurs if all the selected signals satisfy their corresponding threshold conditions.

◆ If OR is selected, detection occurs if any one of the selected signals satisfies its threshold conditions.
If there is only one type of trigger signal, there is no difference between AND and OR.

In the case of bacterial measurement, it is necessary to detect small targets, so high sensitivity detection of scattered light is required. In this regard, the use of 'FSC and SSC with AND condition' or 'FSC and FL2 with AND condition in the case of fluorescent dye-stained sample' eliminates noises caused by small particles suspended in the solution, and thus allow for high sensitivity detection. For cells with size 0.3μm or less cannot be detected using FSC, but nuclear staining with dyes such as SYTO9 or SYBR Green I and appropriate FL2 signal threshold condition set up enable detection due to fluorescent signals.

NOTE Set the FSC Threshold to a level that noises are no longer detected. Specifically, set the threshold to a level that the noise disappears during the measurement with the laser positioned away from the flow channel.

Step 6

When set up is complete, select "OK".

NOTE Changes to the threshold settings will not be applied if the window is closed before clicking OK. Once the changes are applied, gates set in III Workspace and all information on the open panels will be reset.

Step 5

	Dye	Antigen	FSC log	H	W	A	Gain	Numerical Factor		
								Height(H)	Width(W)	Area(A)
FSC			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	10	1.00	1.00	1.00
SSC			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	30	1.00	1.00	1.00
FL1-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	48	1.00	1.00	1.00
FL2-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	39	1.00	1.00	1.00
FL3-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	38	1.00	1.00	1.00
FL4-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	55	1.00	1.00	1.00
FL5-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	48	1.00	1.00	1.00
FL6-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	56	1.00	1.00	1.00

Threshold	Parameter	Value
<input checked="" type="radio"/> and	FSC-H	30
<input type="radio"/> or	Non	0
	Non	0

NewSetting OK Cancel

Up to three Threshold values can be set from all blue laser-triggered parameters including FSC and SSC. AND condition or OR condition can be selected.

Insert the Value calculated by the apparent Threshold value divided by the Numerical Factor value. E.g. In the case FSC-H Numerical Factor is set to 2.5 and you would like to set the Threshold value on 50, then $50 \div 2.5 = 20$. Therefore, insert 20 in the Value section.

When Threshold condition is set, press "OK".

Step 6

OK Cancel

5-6 Laser/Pressure Settings

Step 1

Select the “**Laser/Pressure**” button under Processing zone to display the detailed setting dialog.

Step2

Set the Main push (kPa) value according to the chip

Step3

Click on the tick box for the lasers required. Check that the lasers are at appropriate position (where the red lines cross). If not, please follow the instructions on STEP4 and STEP5.

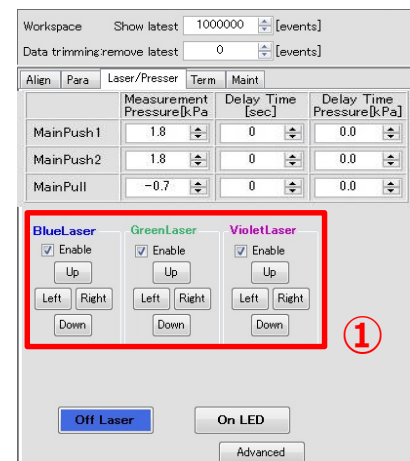
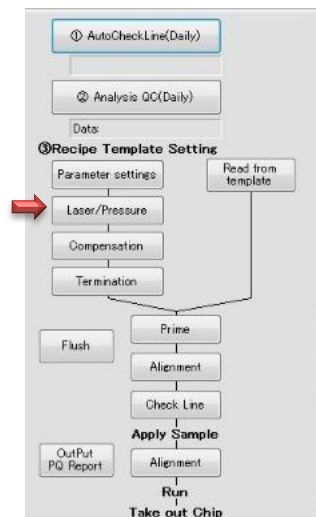
NOTE

Whether blue laser is functioning or not can be checked by pressing “ON Laser” and “OFF Laser”. Use red and violet lasers by referring to the excitation/emission information of the dyes used.

NOTE

Lasers do not turn on for instruments without red and/or violet lasers installed even when the boxes are ticked.

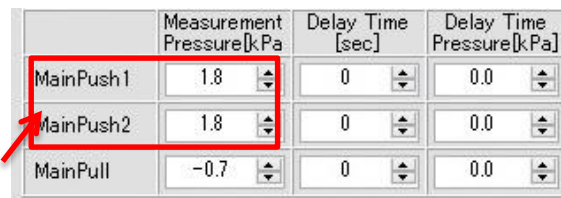
Step 1



Detailed setting dialog is displayed

Press “**Laser/Pressure**” button

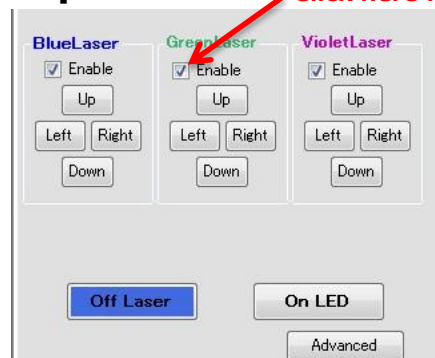
Step 2



MainPush1と2(kPa)の設定(1と2は同じにする)

MainPush1&2 pressure (kPa) set up (1&2 value should be the same)

Step 3



Click here for Green Laser

Tick on the boxes for the lasers used

Step 4

Turn only the blue laser on by unticking the red and violet laser boxes, then pressing the ON/OFF laser button. Confirm that the blue laser is positioned at the intersection of the central vertical and horizontal lines.

NOTE

If the laser spot is not positioned at the intersection of the horizontal and central vertical reference lines, click "Align" tab, move the reference lines, then move the red lines to the center of the laser spot. For the Line adjustment, please refer to Chapter 3: Software Configuration (Alignment under 3-6 Instrument Settings).

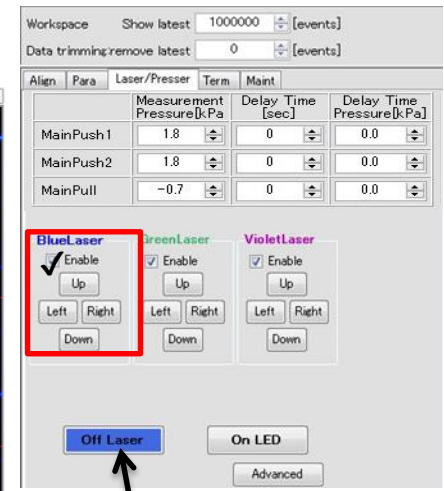
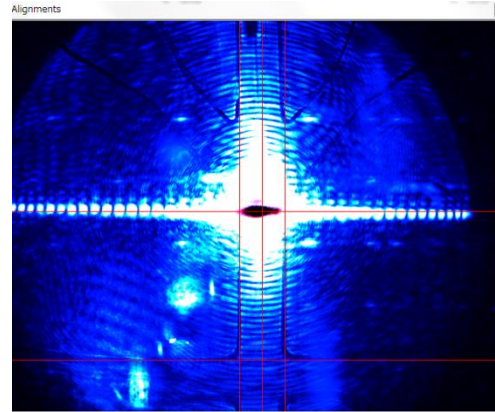
Step 5

Turn on the green laser (or violet laser) by ticking the green (or violet) laser box. Move the laser spot to the center of the blue laser spot (the red or violet laser spot must be positioned at the center of the intersection formed with central vertical and horizontal line).

NOTE

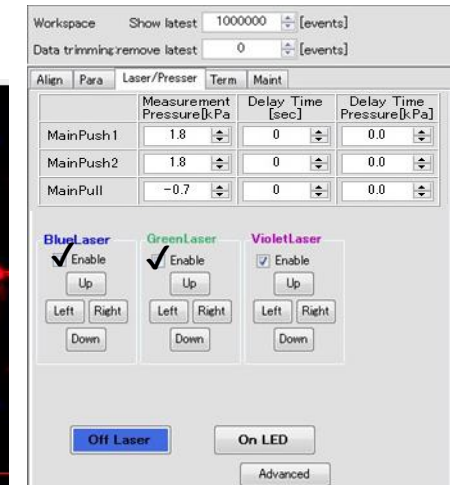
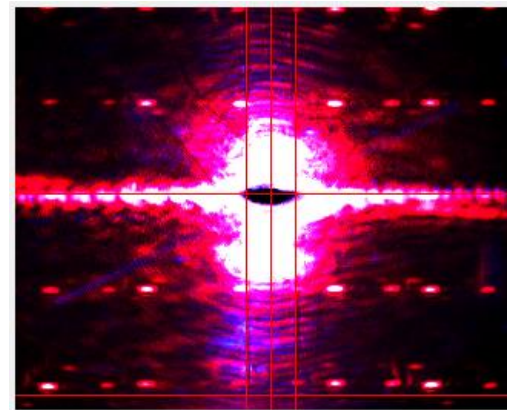
It is easier to adjust the position of green and violet laser one at a time.

Step 4



Turn on the blue laser, and align the beam spot to the intersection of the vertical and horizontal lines.

Step 5



Turn on the Green laser by ticking the green laser box. Move the laser spot to the center of the blue laser spot (the green laser spot must be positioned at the center of the intersection formed with central vertical and horizontal line). The image above is for when the red laser is installed.

5-7 Auto Termination Condition Settings

Step 1

Select the **"Termination"** button under Processing zone to display the detailed setting dialog.

Step2

1. If you do not wish to use the auto termination function, select "record continuously".
2. If you wish to use the auto termination function, select "any of these are met", choose a termination condition (Count, Sorting, Time), then insert a number. If "Count" termination condition is chosen, select the target gate you wish to use the count for.

☐ "Count" terminates the run once the number of particle counted within the target gate is reached.

E.g. If you wish to terminate the run after 100 beads are counted within the "Sky blue" gate

☒ Count 100 events
 Sky blue

☐ "Sorting" terminates the run once the number of sorted particle within the sorting gate is reached

E.g. If you wish to terminate the run after 200 sorting

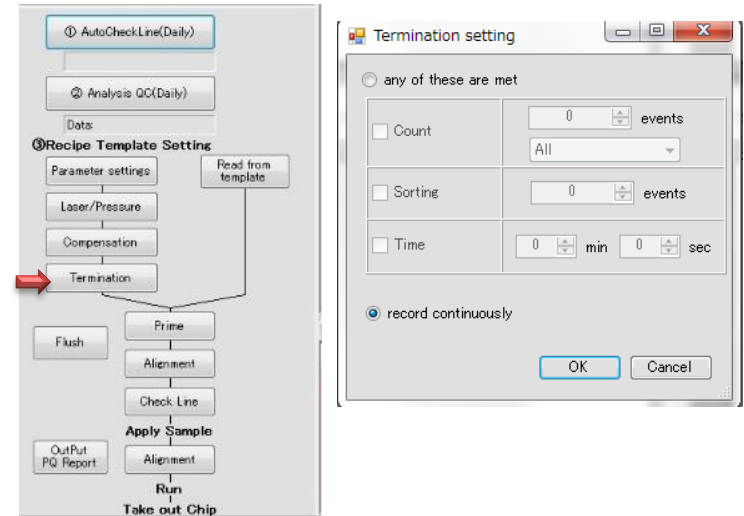
☒ Sorting 200 events

☐ "Time" terminates the run after a specified time.

E.g. If you wish to terminate the run after 10 minutes

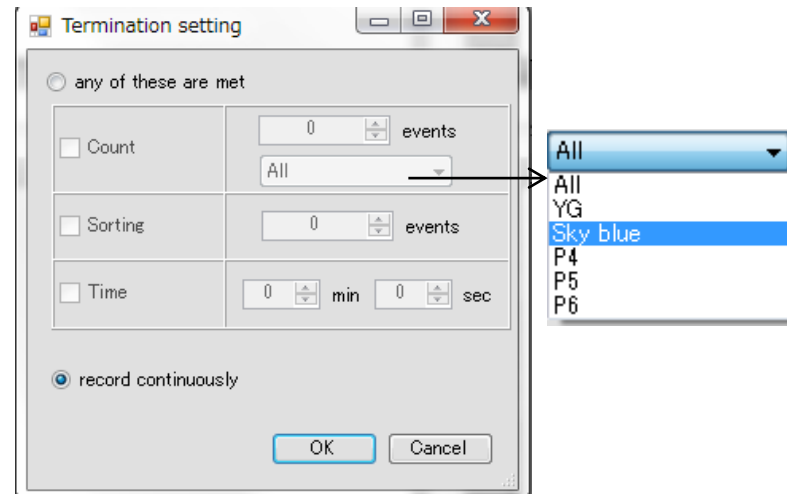
☒ Time 10 min 00 sec

Step 1



Press **"Termination"** button

Step 2

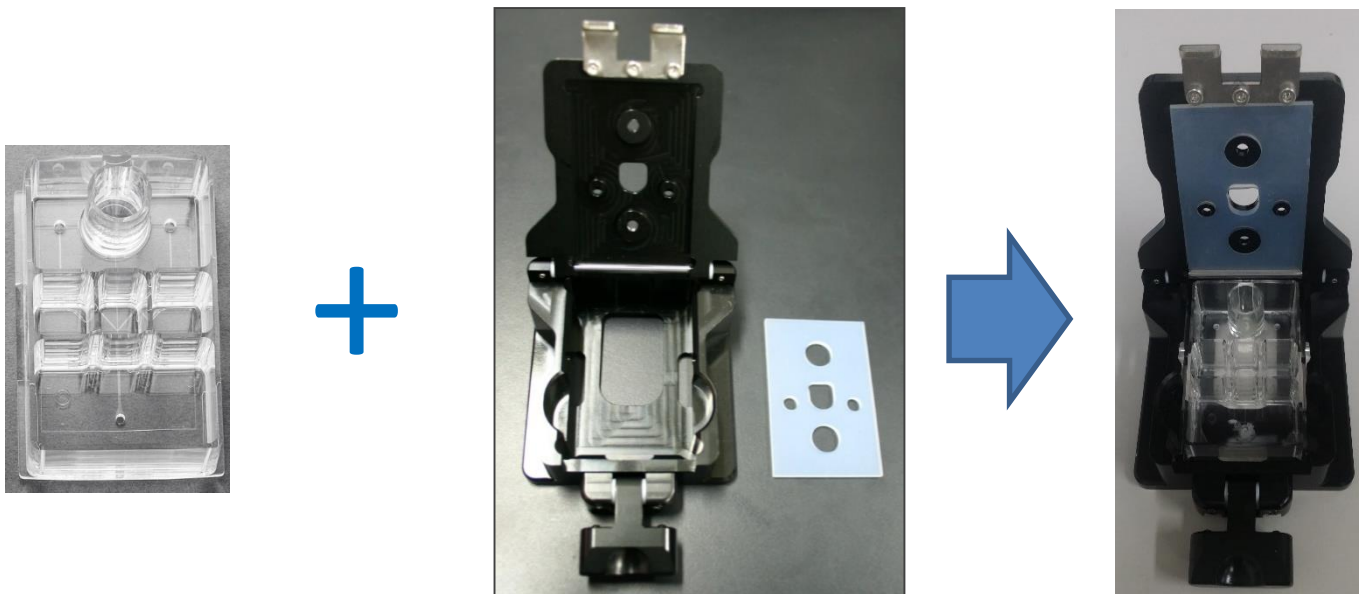


5-8 Loading Buffer Solution

Load the sample buffer made in **Section 5-2** before sample measurement.

Install a new microfluidic chip inside the chip holder for easy loading of buffers.

NOTE Loading of buffers inside a safety cabinet, clean bench or a clean hood is recommended.



5-8-1 Loading solutions on Chip 2D-Z1001

Step1

Load 4-9mL of sheath fluid in Sheath Reservoir.

Step2

Load 1.5mL and 100 μ L of sheath buffer in Sorting Reservoir (right) and Sorting Collection Reservoir (left), respectively.

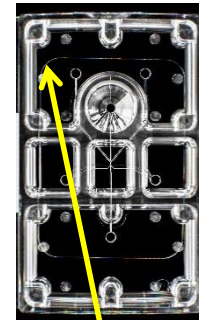
Step3

Load 20-300 μ L of On-chip Sample buffer in Sample Reservoir

NOTE

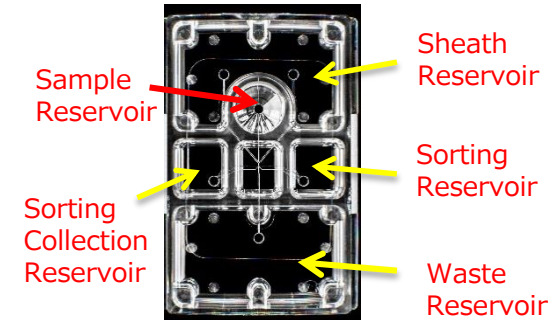
If you would like to use other solutions such as culture medium as a sample buffer, please follow the instruction in Section 5-2-2. We recommend using On-chip sample buffer.

Step 1

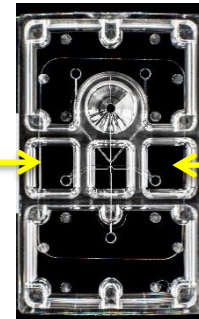


Sheath fluid
4-9mL

2D-Z1001



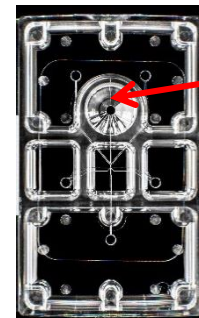
Step 2



Sheath fluid
0.1mL

Sheath fluid
1.5mL

Step 3



On-chip Sample buffer 1x
20~300 μ L

5-9 Inserting Chip

Step1.

Insert a loaded chip inside a Chip Holder, close the lid, and lock the holder by pulling up the tab.

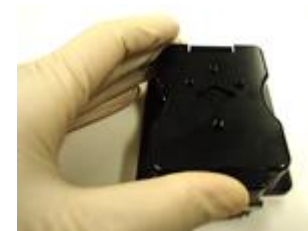
Step2

Position the Chip holder inside the Loading Port (make sure the holder is securely positioned).

Step3

Pull down the pressure control unit, fix the chip holder and the control unit using the locks on the right and left.

Step 1



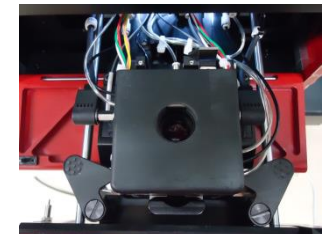
Lock for close

Step 2



Set a chip holder in Loading Port

Step 3



5-10 Priming (common)

Step1.

Press “**Prime**” button.

◆Prime treatment is automatic.

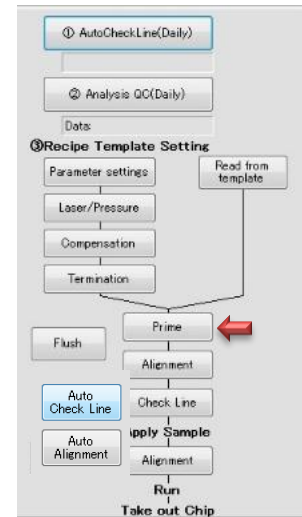
NOTE

Priming must be carried out every time a new chip is used, as this process removes air bubbles within the flow channels and apply a hydrophilic coating

Step2

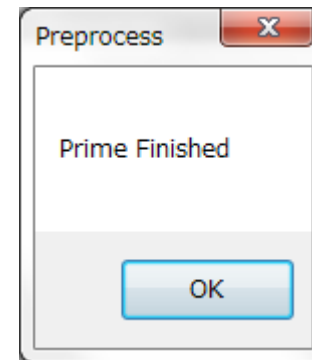
Prime button flashes red during the prime treatment. A dialog appears after 30 seconds to notify the prime treatment finished. Press “**OK**” to proceed.

Step 1



Press “**Prime**” button

Step 2



Press “**OK**”

Prime button flashes red during the prime treatment.

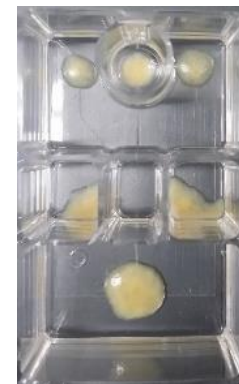
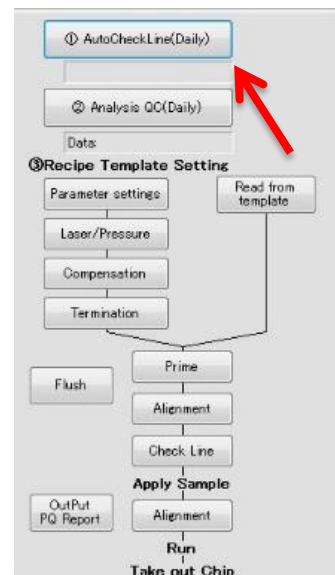
5-11 Auto Check Line (Daily QC)

Insert the **Sealed chip** by following step 5 to 9. Ensure to carry out this process using the parameters based on the templates stored in QC.

Press "**Auto CheckLine**" button under the Processing zone. This process may take several minutes. Press "**OK**" to end the process.

Please contact your local distributor or contact the Japanese headquarters if error messages are displayed after repeating the process 2-3 times.

Contacts: On-chip Biotechnologies, Co., Ltd.
support@on-chip.co.jp



Sealed chip

5-12 Sample Loading

After Check Line (or Priming) is complete, remove the chip from Chip Holder, load the samples on chip by following the instructions below.

NOTE

Sample loading should ideally be carried out inside a safety cabinet, on a clean bench or inside a bench top clean hood.

NOTE

We recommend filtrating the samples prior to sample loading.

Step1

Remove Chip Holder from the instrument, and open the lid of Chip Holder.

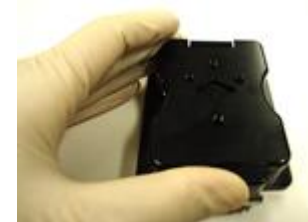
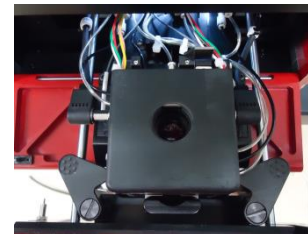
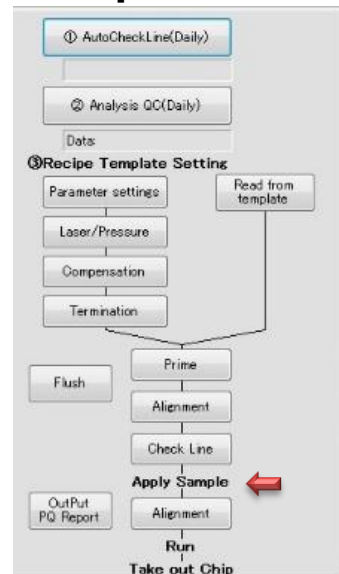
Step2

Remove sample buffer from Sample Reservoir, then load the sample to be measured.
20 to 300 μL of sample can be introduced.

NOTE

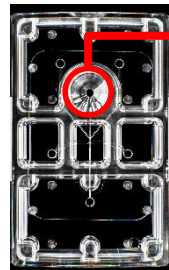
Samples must have the viscosity adjusted to the same viscosity as On-chip Sample buffer 1x. Please refer to 5-3 for buffer preparation.

Step 1

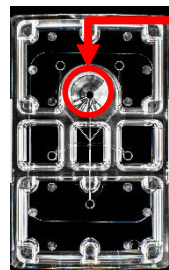


Step 2

2D-Z1001



Remove sample buffer from Sample Reservoir



20 to 300 μL of sample can be introduced (viscosity adjusted)



Step3

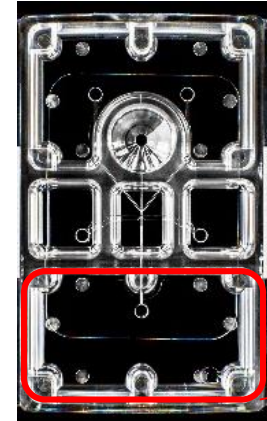
Sheath fluid refill and removal.

- ☐ Remove all waste from Waste Reservoir.
- ☐ Refill sheath fluid.
 - ◇ Refill the sheath fluid to Sheath Reservoir to make up to 4-9mL.
 - ◇ Refill the sheath fluid to Sorting Reservoir to make up to 1.5mL.
 - ◇ Remove fluid from Sorting Collection Reservoir, leaving 20-500 μ L. Discard the rest.

NOTE

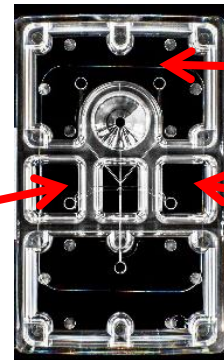
Sheath fluid may run out during sorting if sheath fluid is not refilled to reservoirs.

Step3



2D-Z1001

Remove all fluids from Waste Reservoir



Add up to 1.5mL of sheath fluid

Add up to 4-9mL of sheath fluid

Leave 100 μ L of sheath fluid

5-13 Laser Alignment

The method to check that the laser spot is on the correct position (intersection of the red lines) is explained.

Step 1

Select "Laser/Pressure" tab under Instrument Settings.

Step 2

Turn on blue laser only.

Untick the boxes for red/green and violet lasers, and check that the beam spot of blue laser is on the intersection of the red lines.

NOTE If the beam spot is not on the intersection, move the beam position by clicking on the "Up", "Down", "Left" and "Right" buttons below the tick box for the blue laser.

Step 3 (For instrument with Red, Green or Violet lasers only)

Tick the box for red (green) laser (or violet laser) and turn on the laser. Confirm that the laser spots for red and violet lasers are on the blue laser spot (at the intersection).

NOTE

We recommend checking the lasers one by one.

Step 1

Select "Laser/Pressure" tab

Step 2

Turn only the blue laser. Untick for red and violet laser, and confirm that blue laser is on the intersection of the red lines.

Step 3

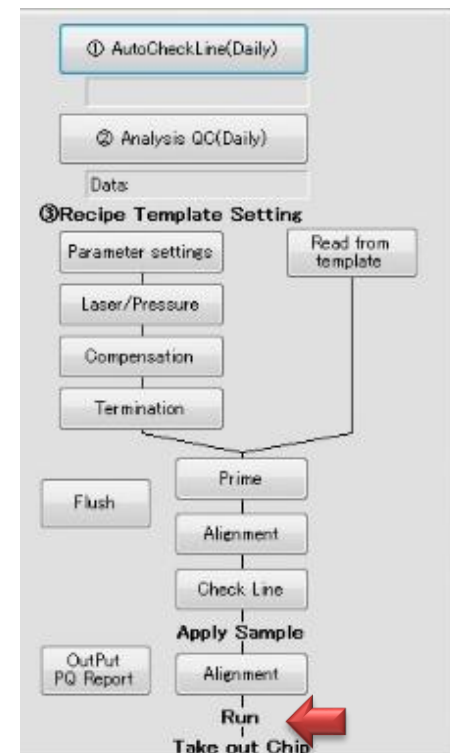
Turn on the red laser (violet laser). Confirm that the laser spots are aligned with the blue laser.



5-14 Run (Sorting or Analysis)

This section describes and explains the procedures for creating a new Sample File and set up.

5-14-1	Gate Settings	5-23
5-14-2	Gate Type Selection	5-24
5-14-3	Gate Development	5-25
5-14-4	Gain Adjustment	5-26
5-14-5	Threshold Settings	5-27
5-14-6	Numerical Factor Adjustment	5-28
5-14-7	Fluorescence Compensation	5-29
5-14-8	Gate Tier Display	5-39
5-14-9	Sorting	5-40
5-14-10	Sorting Initiation and Termination	5-41
5-14-11	Sorted Sample Collection	5-42



5-14-1. Gate Settings

When a new Sample File is selected, an empty Work Space is displayed.

Step1

Press “**Run**” button under Operation control to flow the sample.

NOTE

For a new Sample File, only FSC(H) vs SSC(H) plot in linear scale is displayed on the left top window. To show the fluorescence signal results, click on the button that appears next to the scale title, and select from the list of detectors available.

NOTE

Gain can be modified during a measurement from the “Para” tab.

Step2

Press “**Stop**”.

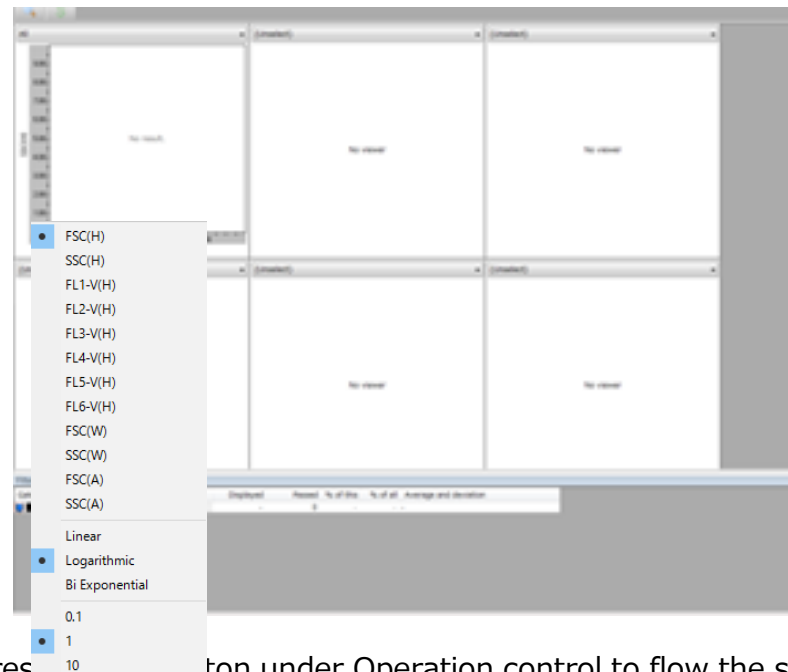
A dialog with a message “Do you want save the record?” will open, and:

- Click “Yes” to save the data
- Click “No” to not save the data and choose a gate.

NOTE

If the data is not saved, all the data will be lost at the time of next measurement (the data will not be overridden).

Step1



Press “**Stop**” button under Operation control to flow the sample

Step 2

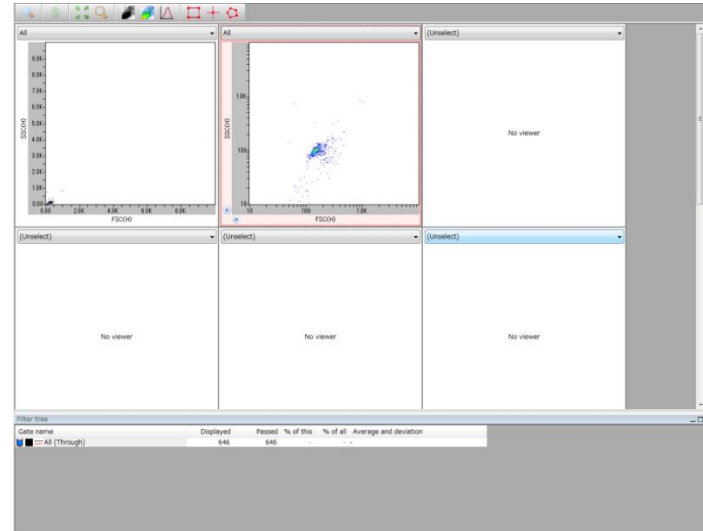


Press “**Stop**”.

A dialog with a message “Do you want save the record?” will open, and:

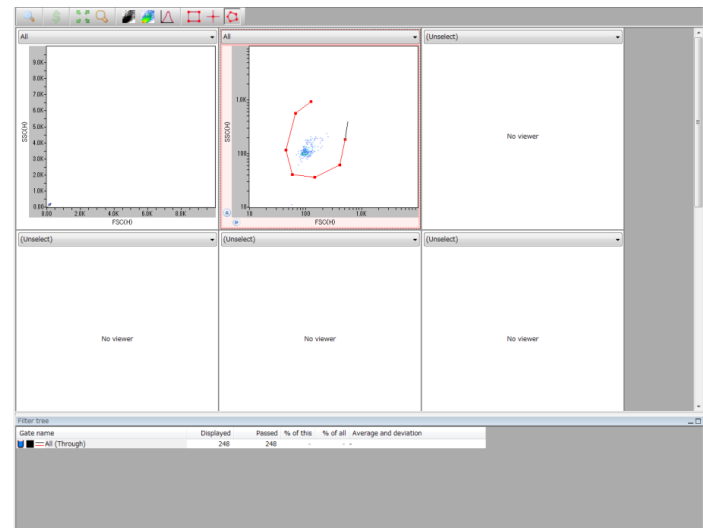
- Click “Yes” to save the data
- Click “No” to not save the data and choose a gate.

Step 1



Select the population to be gated, and select the type of gate to surround the target population

Step 2



Surround the target population (gating)

5-14-2 Gate Type Selection

Selecting a gate within a FSC(H) vs SSC(H) plot for a population to be analyzed or sorted.

Step 1

Select an appropriate gate type from the Work Space Window.



Step 2

Surround the target population (gating).
Name the population if necessary.

5-14-3 Gate Development

Develop the identified group (population) to the intended plot.

Step1

Select the population to develop from the panel.

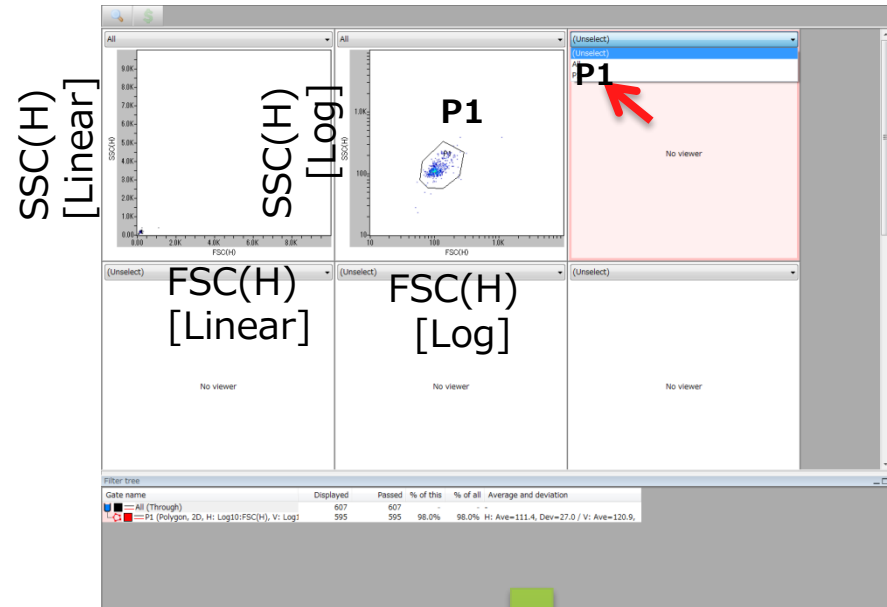
Step2

The selected (gated) population can be displayed in a separate window with scattered light (FSC, SSC) and fluorescence (FL) on the vertical and horizontal axes.

NOTE

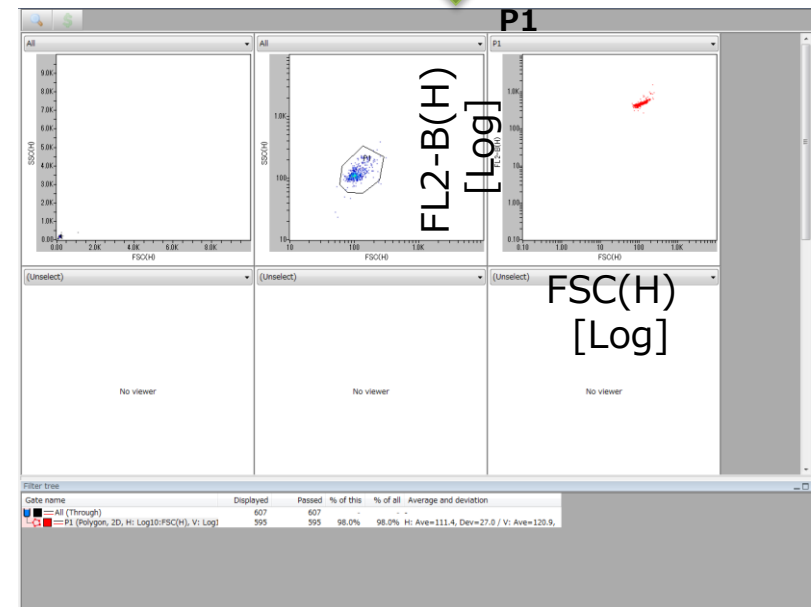
If necessary, change sensitivity settings for SSC and FL. Sensitivity can be adjusted while the sample is flowing (RUN is in effect) by adjusting Gain in the Instrument tab. (Refer to the section on principles of flow cytometry for details of sensitivity adjustment).

Step1



Step2

Open P1 by FL2-B(H) vs FCH(H)



5-14-4 Gain Adjustment

For detectors other than FSC, the visible intensity of scattered light and fluorescence can be changed by changing the Gain. This adjustment resembles changing the exposure time on a fluorescent microscope.

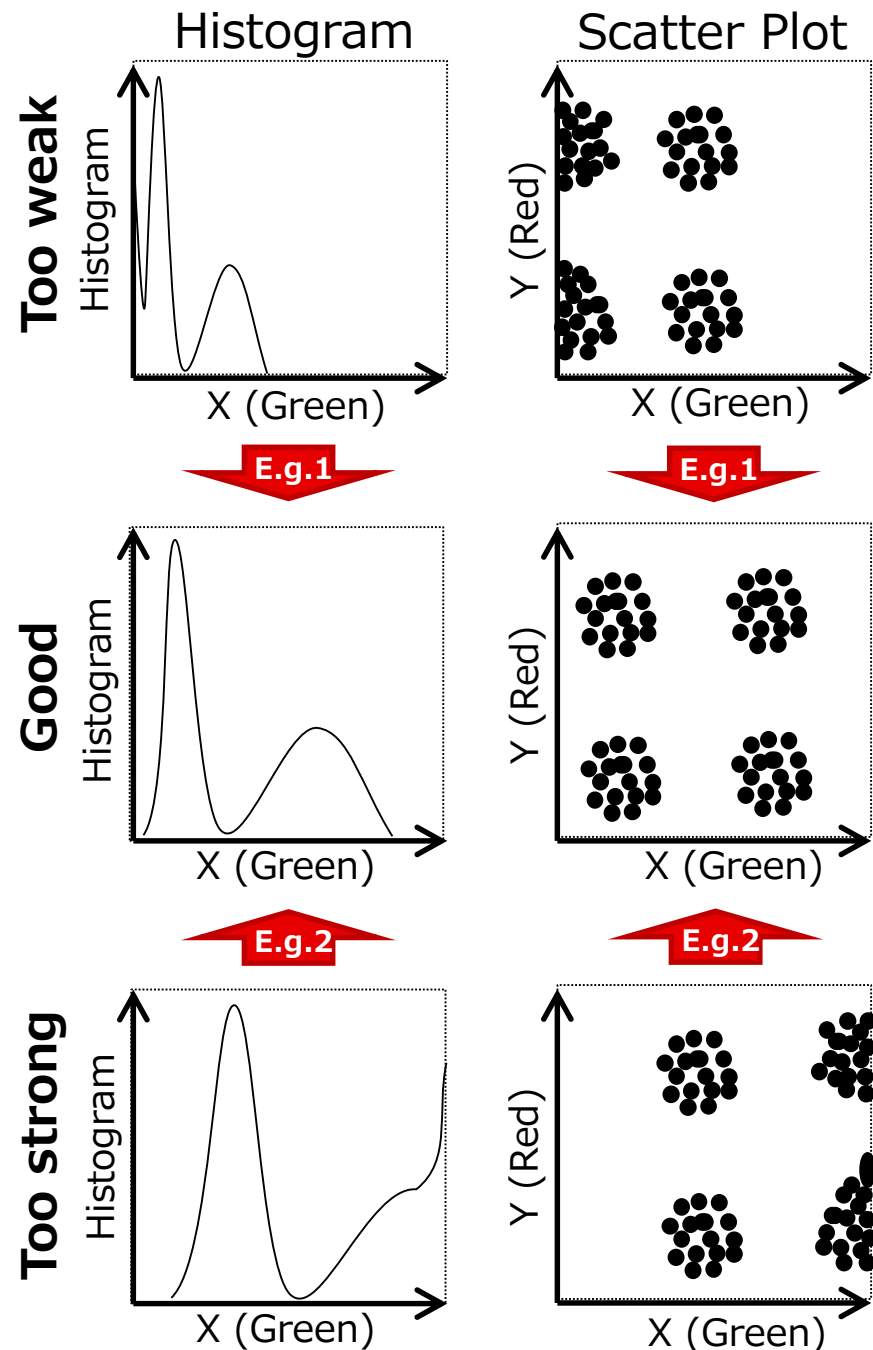
Changes on the histogram and on the scatter plot, with the example of changing the Gain for X (Green) are explained.

If the Gain is increased, the overall signal is shifted in the direction of greater strength. If the signal is weaker for cells that should be strongly stained, as in E.g.1, or if there is a distribution close to (or below) the minimum display value that appears “stuck”, raise the Gain.

If the distribution of the stained population is “stuck” beyond the maximum display value of the plot, as in E.g. 2, the whole population can be brought into the display by lowering the Gain.

When determining the Gain, it is important to prepare stained positive controls in advance, as well as unstained cells and single color controls (cells stained with only one color).

If the Gain is changed during a measurement, the signals before and after the change will be plotted together, so it is better to stop and restart the measurement again.



Refer to 3-3-2 Parameter Settings for changing Gain Settings. Figure on the right shows the Gain can be modified by changing the Voltage value of each detectors other than FSC.

5-14-5 Threshold Settings

Threshold settings determines the minimum signal value that is detected. If the threshold value is too low, small particles suspended in solution are detected, and thus the target event count is considerably smaller than the total event count value. Furthermore, the file size becomes large due to the number of signals detected. If the threshold value is too high, the target cells may not be detected. **Set the threshold value to H value of signals to be triggered by the blue laser.** Decide on the value according to a measurement result. Threshold is usually set according to FSC value, but removal of non-fluorescent cells is also possible by setting threshold in FL.

The screenshot shows the 'Parameter setting (Unchangeable)' window. It contains a table of detector parameters and a threshold settings section at the bottom.

	Dye	Antigen	FSC log	H	W	A	Gain	Numerical Factor		
								Hight(H)	Width(W)	Area(A)
FSC			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	10	1.00	1.00	1.00
SSC			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	30	1.00	1.00	1.00
FL1-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	48	1.00	1.00	1.00
FL2-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	39	1.00	1.00	1.00
FL3-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	38	1.00	1.00	1.00
FL4-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	55	1.00	1.00	1.00
FL5-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	48	1.00	1.00	1.00
FL6-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	56	1.00	1.00	1.00

Threshold	Parameter	Value
<input checked="" type="radio"/> and	FSC-H	30
<input type="radio"/> or	Non	0
	Non	0

Buttons: NewSetting, OK, Cancel

5-14-6 Numerical Factor Adjustment

Numerical Factor controls the appearance of the plot by multiplying the signals measured by detectors. Unlike the voltage adjustment, Numerical Factor will not affect the original signals.

Similarly to the Gain, signals will consequently shift to the more intense direction when the Numerical Factor value is increased.

	Dye	Antigen	FSC log	H	W	A	Gain	Numerical Factor		
								Height(H)	Width(W)	Area(A)
FSC			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	10	1.00	1.00	1.00
SSC			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	30	1.00	1.00	1.00
FL1-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	48	1.00	1.00	1.00
FL2-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	39	1.00	1.00	1.00
FL3-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	38	1.00	1.00	1.00
FL4-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	55	1.00	1.00	1.00
FL5-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	48	1.00	1.00	1.00
FL6-V			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	56	1.00	1.00	1.00

Threshold: ☒ and ☐ or

Parameter: FSC-H, Non, Non

Value: 30, 0, 0

NewSetting OK Cancel

The value of FSC must be adjusted by Numerical Factor as voltage of FSC cannot be changed. However, original threshold will also be multiplied, and thus the cut-off point set by threshold will also shift. Therefore, threshold value should be adjusted before changing the Numerical Factor. After changing the Numerical Factor, the original threshold can be calculated by dividing the apparent threshold value by the Numerical Factor value.

H values other than FSC should be adjusted at Change Voltage instead of Numerical Factor for convenience.

Numerical Factor for A and W can also be changed during a measurement. Unlike voltage, all the signals will be affected when Numerical Factor is changed during a measurement.

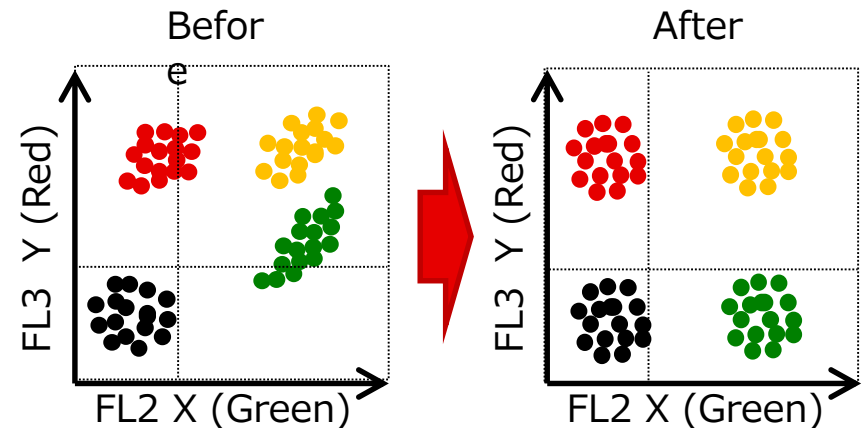
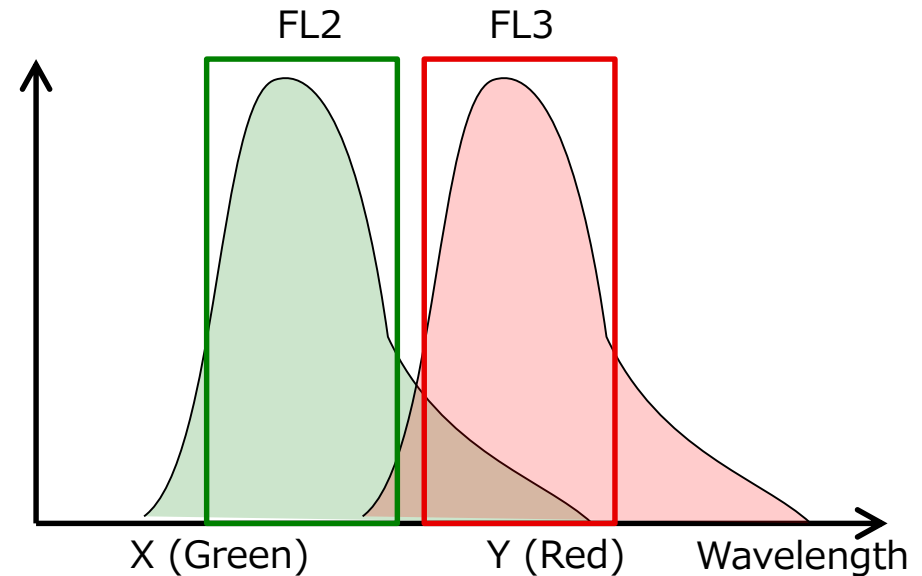
5-14-7 Fluorescence Compensation

Compensation is a correction process where signals from two or more fluorescence leaked into a detector is calculated and removed from the measured signal.

Figure on the top right shows an example of a green and a red fluorescence spectrum on a histogram. Most of the emission spectrum of the green fluorescence is covered by FL2, but considerable amount is leaked into the detection band of FL3. Furthermore, the red spectrum also leaks into FL2 detection band. Therefore, detected signals are formed from multiple fluorescent signals, and are stronger than single fluorescence signals.

In this case, cells stained in green (green population shown in the plots on the bottom left) can be detected by FL3, though it does not fluoresce in red. The population will shift towards the FL3 direction, and the population seems as if they are also stained red. The same situation occurs for red stained cells as they shift slightly towards the FL2 direction.

The objective of fluorescence compensation is to calculate the fluorescence intensity that the cells actually have by measuring the amount of leaks and subtracting the amount from each signal.



Auto Compensation

Preparation of sample for correction value calculation

For automatic calculation of correction values, prepare samples below, analyze 500 events or above (use the same condition as the real sample in case of sorting) and save the data.

1. Target sample to be corrected
2. Non-stained or isotype control sample (no-color)
3. Single-color stain samples

An example using FITC, PE, APC anti-body tri-color

1. is the real FITC, PE, APC tri-stained sample. It is “Mix” in the figure on the right. Make sure all parameters to be corrected for are turned ON, and the signal intensity is adjusted by Voltage.

2. is the non-stained or isotype control sample, named “no color” on the right. Click Copy without data on “Mix” sample to carry over the settings. Changing fluorescence intensity is not ideal at this stage, and should be adjusted in 1.

3. should be samples highlighted with single dyes: FITC single stain, PE single stain, and APC single stain samples. Copy without data “no-color” data to carry over the parameters.

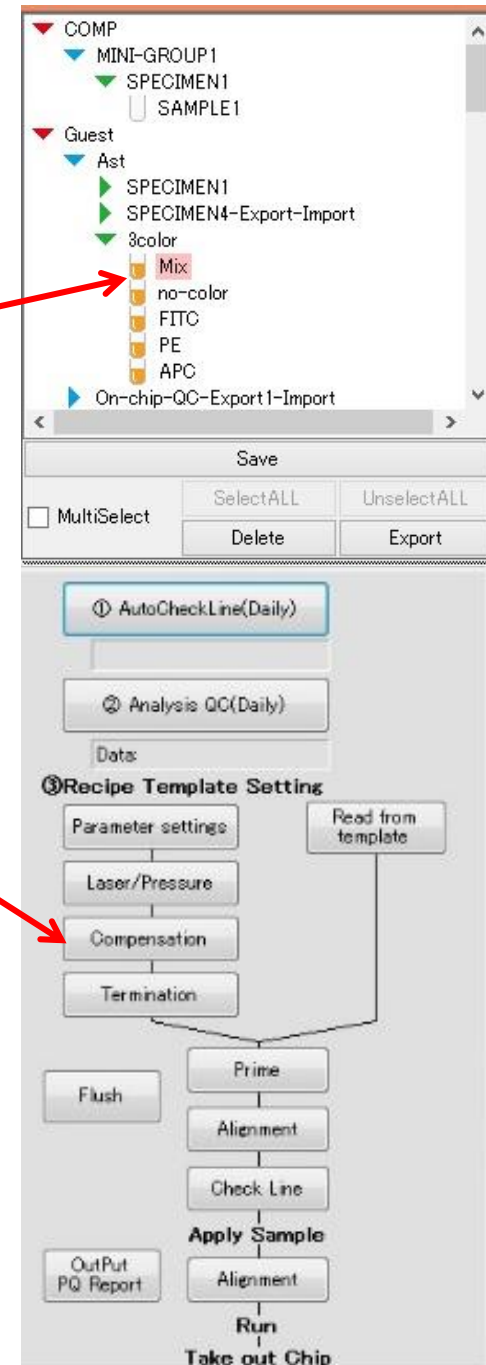
Auto compensation is to be carried out using the samples above.

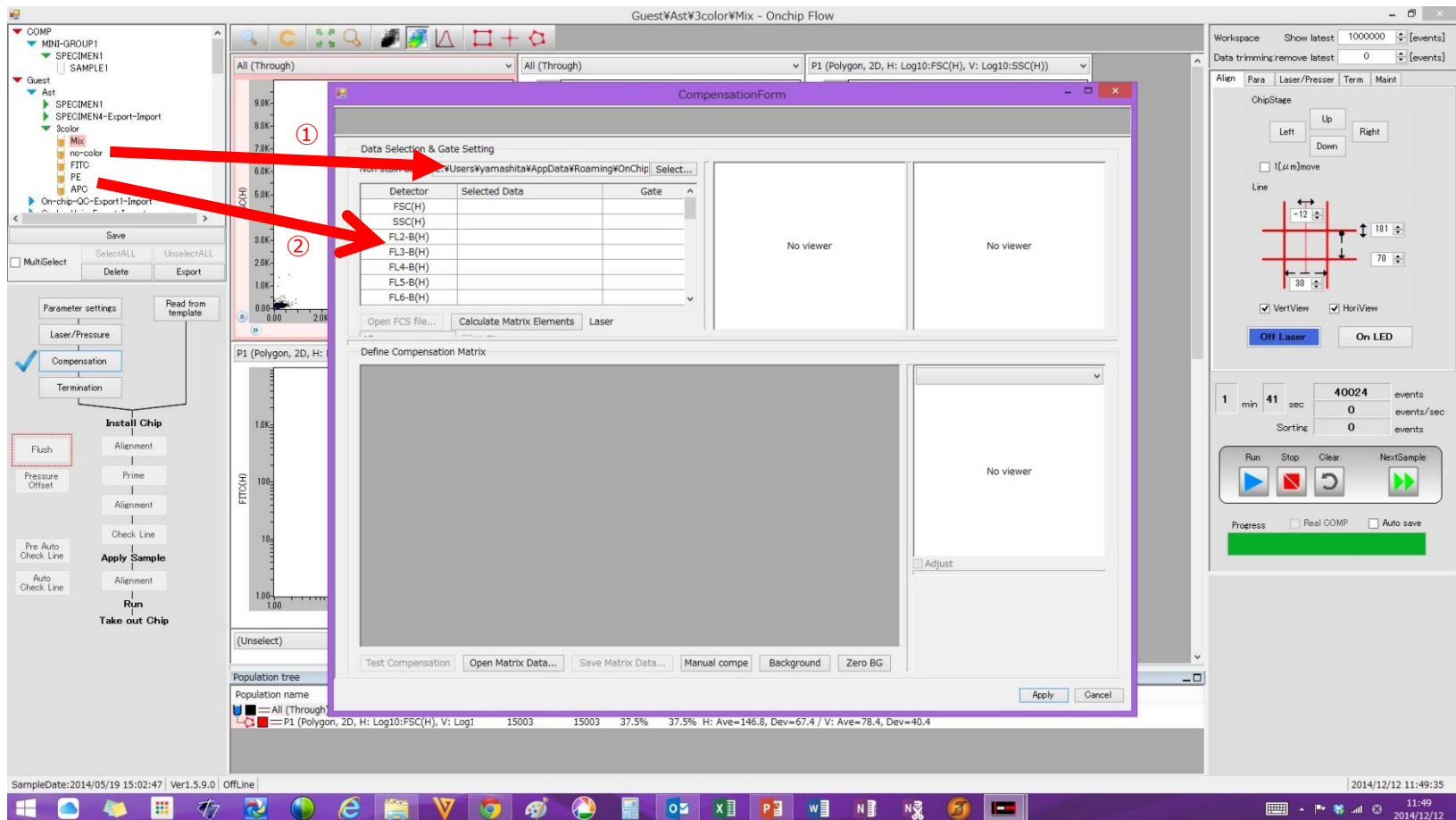


Open Compensation Window

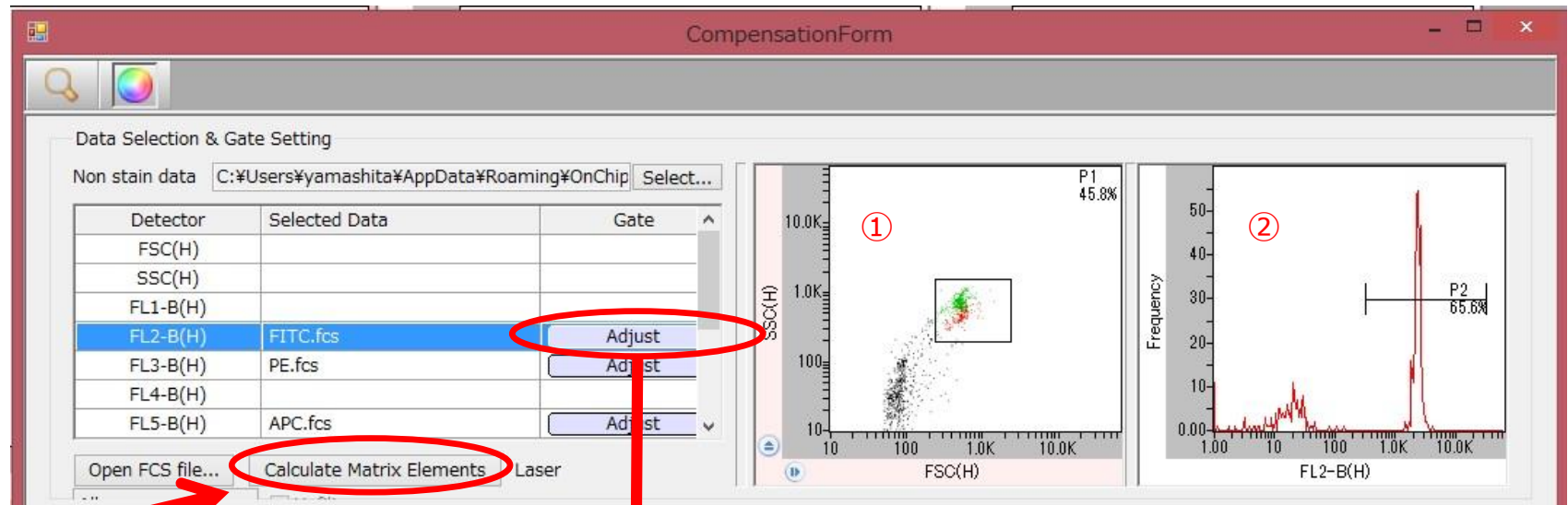
① Select the “Mix” sample file for fluorescence correction. (The aim is to carry out sorting with compensation based on this sample file).

② Press **Compensation** button. (Compensation dialog opens.)





- ① Drag and drop "no-color" sample file to "Non stain data" section of the compensation dialog.
- ② Drag and drop single stained data to appropriate channels.
 - FITC single stain sample file goes to FL-2
 - PE single stain sample file goes to FL-3
 - APC single stain sample file goes to FL-5

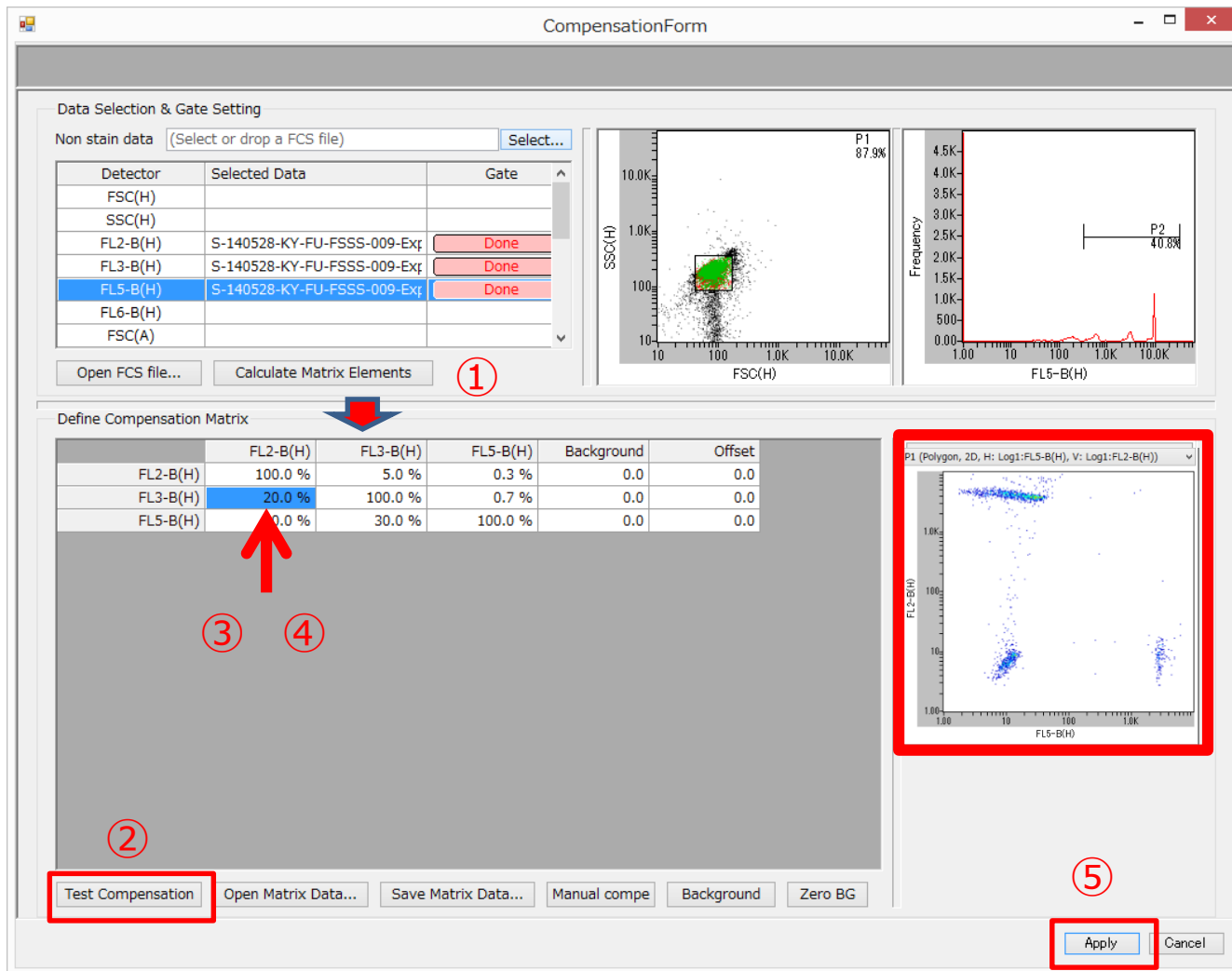


⑤

Detector	Selected Data	Gate
FSC(H)		
SSC(H)		
FL1-B(H)		
FL2-B(H)	FITC.fcs	Done
FL3-B(H)	PE.fcs	Adjust
FL4-B(H)		
FL5-B(H)	APC.fcs	Adjust

③

- ① Set a gate for cell population in SSC vs FSC (log vs log) plot.
- ② Set a gate for positive targets in a fluorescence channel.
- ③ Click "Adjust" after setting gates. "Adjust" becomes "Done".
- ④ Repeat step ①-③ for other channels
- ⑤ Click "Calculate Matrix Elements" when all the channels are "Done".



① When "Calculate Matrix Elements" is pressed, calculated fluorescence leakage percentage is displayed under "Define Compensation Matrix".

② Click "Test Compensation" to load the sample data selected initially. (Nothing is displayed in this case)

③ Select a cell of be checked, and the selected chart is automatically displayed on the right.

④ Adjust each leakage percentage.

⑤ Click "Apply" after adjustment. The window closes automatically.

Caution: The entered leakage percentage will not be registered until Enter key is pressed on the keyboard.

Manual Compensation

If single stain samples cannot be prepared, fluorescence corrections can be conducted manually. Follow the steps below.

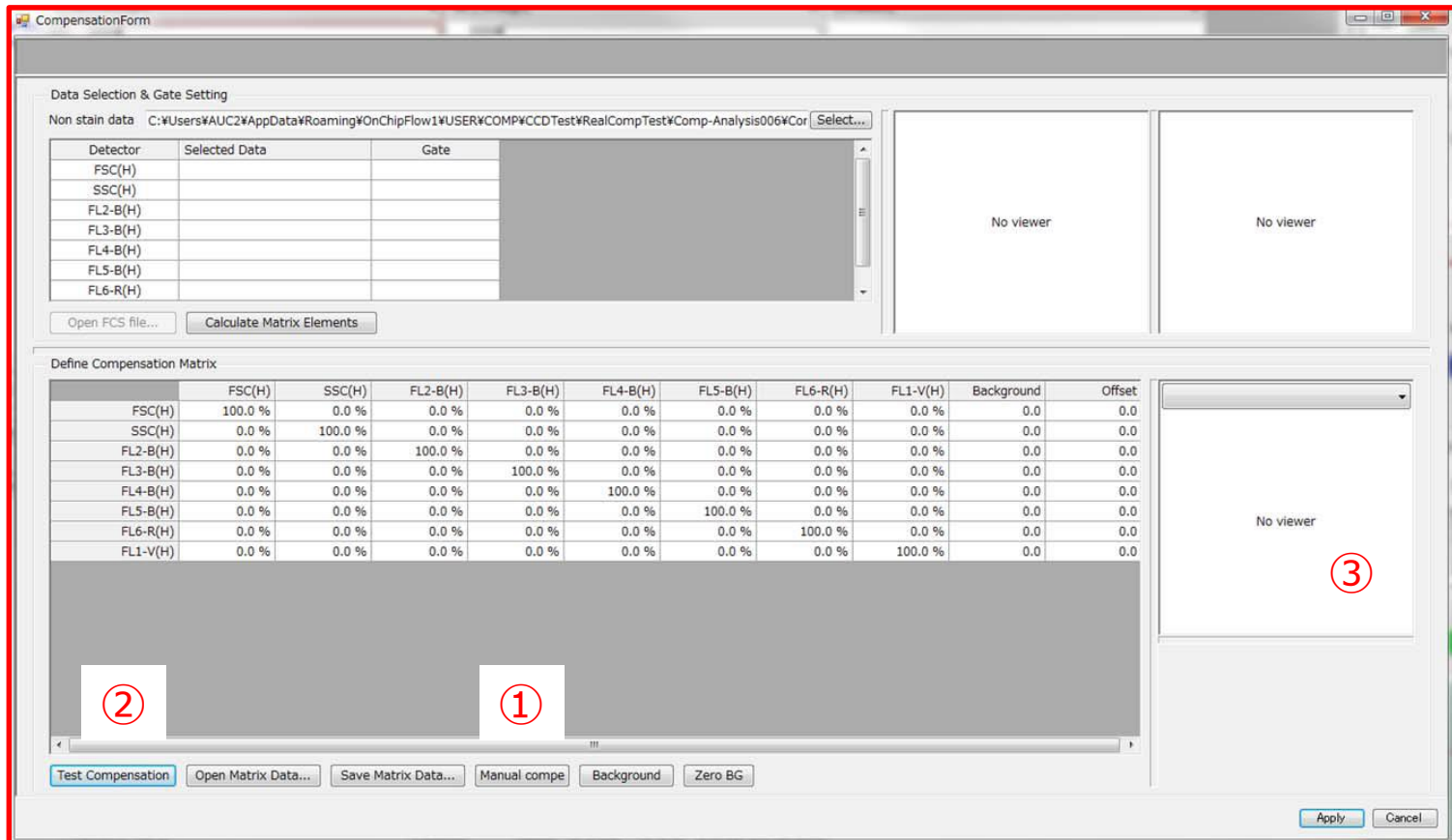
The screenshot shows the OnChip Flow software interface. The left panel displays a tree view of sample data, including 'RealCompTest' and 'SPECIMEN2'. The main window shows a 'CompensationForm' dialog with the following sections:

- Data Selection & Gate Setting**: A table for selecting data and gates.
- Define Compensation Matrix**: A table for defining the compensation matrix.
- Buttons**: 'Test Compensation', 'Open Matrix Data...', 'Save Matrix Data...', 'Manual compe', 'Background', 'Zero BG', 'Apply', and 'Cancel'.

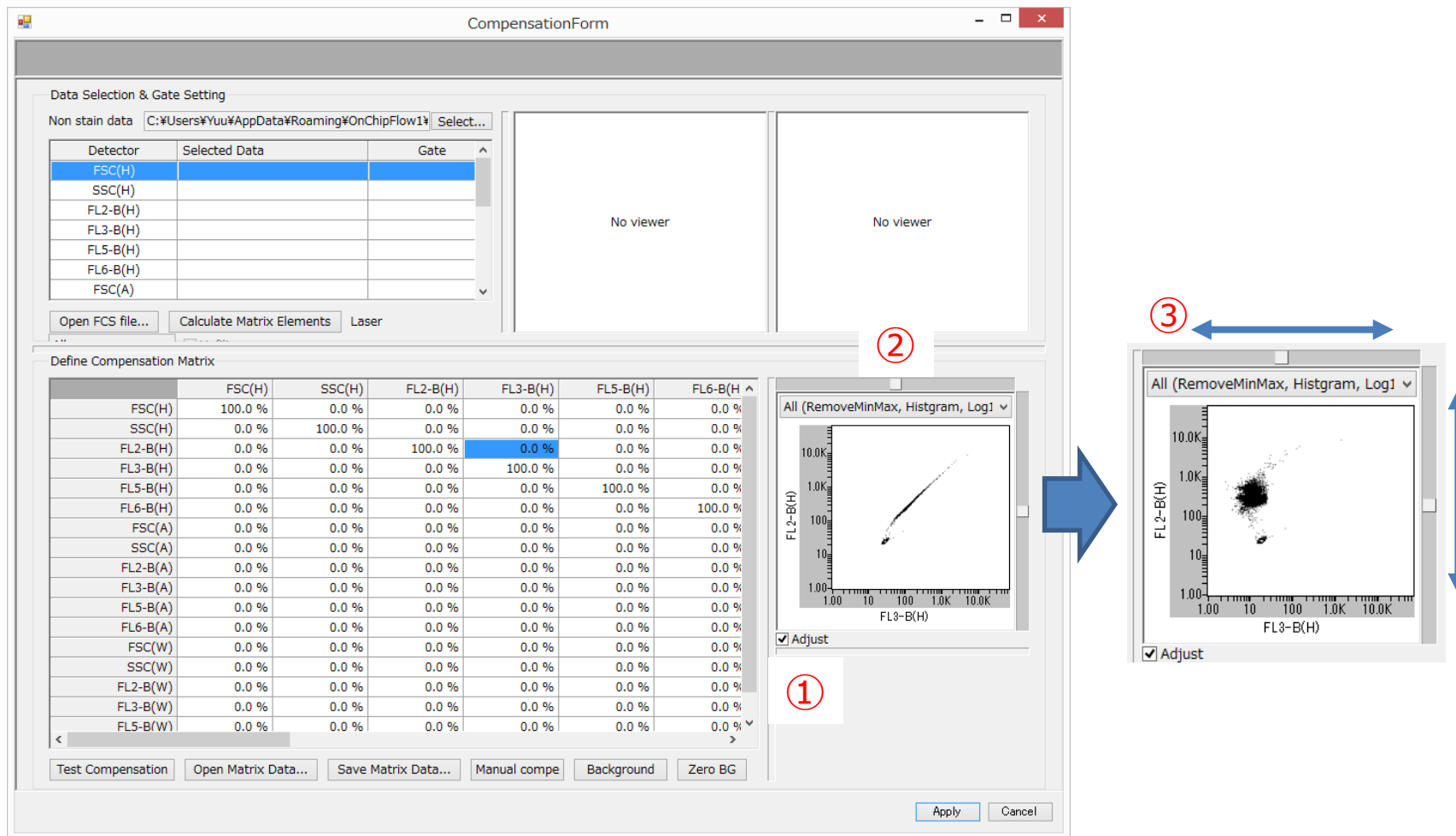
Red arrows and numbers indicate the steps:

1. Select the sample data to be corrected, and press "Compensation" button.
2. Compensationform (compensation dialog) opens.
3. Drag and drop the sample file to be corrected in Non Stain data section.

1. Select the sample data to be corrected, and press "Compensation" button.
2. Compensationform (compensation dialog) opens.
3. Drag and drop the sample file to be corrected in Non Stain data section.



- ① Click "Manual compe".
- ② Click "Test Compensation".
- ③ Sample data is displayed on the bottom right chart.



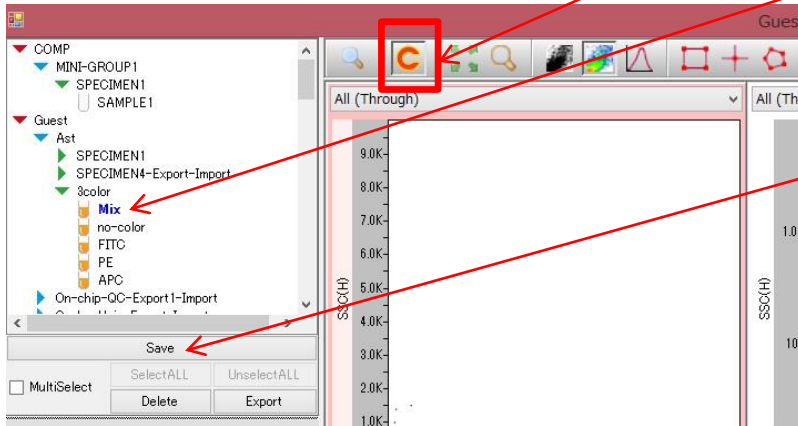
- ① Click "Adjust".
- ② Slide bar appears on the plot.
- ③ Move the slide bar to adjust the correction value. (Moving the bar to the edge adjust the value by $\pm 10\%$)
- ④ Click "Apply" after adjustment to close the window.

Execution of Compensation

Click “**Enable compensation**” button to apply the fluorescence correction result.

Names of sample files turns blue to indicate compensation is applied.

To apply compensation on data that has been measured already, click “Save”.



Sorting with Compensation Applied

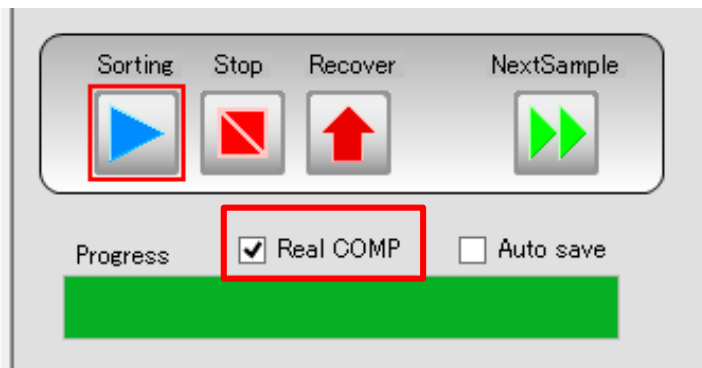
① Create a new empty sample file by “Duplicate without data” a sample that correction has been applied (“Mix” sample in this case).

② Ensure **Compensation** button is applied.



③ “Real COMP” box shown in the left figure is ticked automatically.

Caution: Choose the sorting gate, press **SAVE** and **Duplicate without data**.



5-14-8 Gating Tier Display

This section explains the structure of gating tiers. This means upper and lower tiers are linked by AND condition.

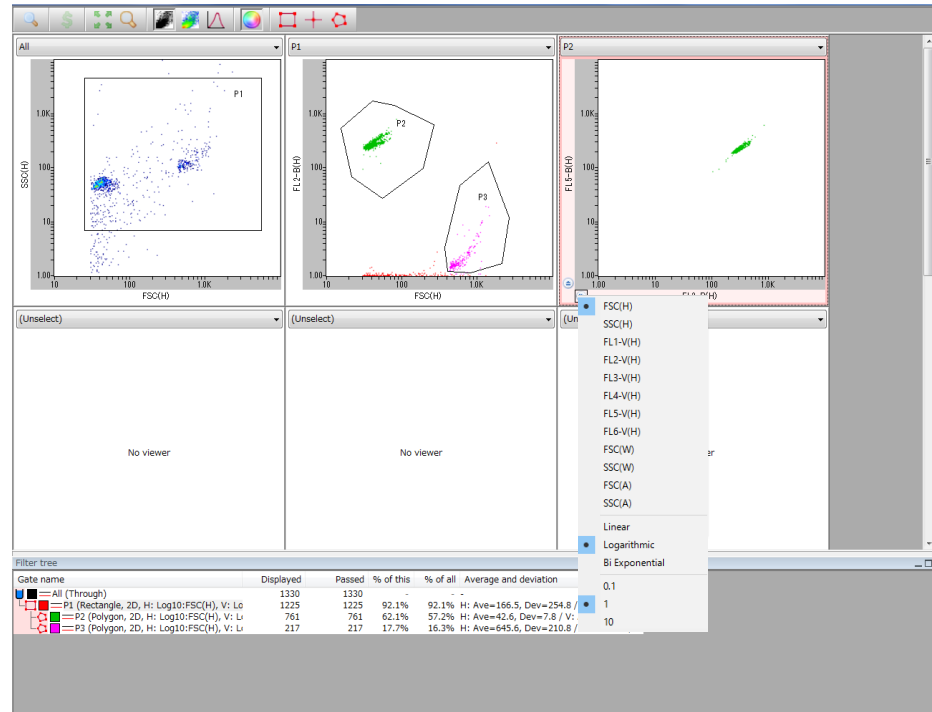
- (1) Population tier status is displayed under Population Management.

Gate name	Displayed
All (Through)	1330
P1 (Rectangle, 2D, H: Log10:FSC(H), V: Lo	1225
P2 (Polygon, 2D, H: Log10:FSC(H), V: L	761
P3 (Polygon, 2D, H: Log10:FSC(H), V: L	217
P15 (Rectangle, 2D, H: Log10:FSC(H), V	108

- (2) The population is highlighted when gated. displayed in color when gating is performed. The color of the population can be modified if necessary (refer to Chapter 3 Software Configuration for details).

NOTE

There can be up to ten steps of gates during sorting. On the eleventh and lower steps, a popup window is displayed to notify sorting cannot take place.



Gate name	Displayed	Passed	% of this	% of all	Average and deviation
All (Through)	2011	2011	-	-	-
P1 (Rectangle, 2D, H: Log10:FSC(H), V: Lo	1823	1823	90.7%	90.7%	H: Ave=529.2, Dev=658.8 / V: Ave=18.5, Dev=18.5
P2 (Polygon, 2D, H: Log10:FSC(H), V: L	1614	1614	88.5%	80.3%	H: Ave=370.5, Dev=96.6 / V: Ave=204.4, Dev=204.4
P31 (Rectangle, 2D, H: Log0.1:FL5-B	1551	1551	96.1%	77.1%	H: Ave=1.3, Dev=0.5 / V: Ave=206.4, Dev=206.4
P33 (Rectangle, 2D, H: Log0.1:FL2	1450	1450	93.5%	72.1%	H: Ave=201.3, Dev=16.9 / V: Ave=1.2, Dev=1.2
P38 (Rectangle, 2D, H: Log0.1:F	1391	1400	96.6%	69.6%	H: Ave=1.2, Dev=0.3 / V: Ave=201.6, Dev=201.6
P3 (Polygon, 2D, H: Log10:FSC(H), V: L	102	102	5.60%	5.07%	H: Ave=2723.9, Dev=699.3 / V: Ave=9.8, Dev=9.8

Sorting is possible only up to the tenth step.

Gate name	Displayed	Passed	% of this	% of all	Average and deviation
All (Through)	2011	2011	-	-	-
P1 (Rectangle, 2D, H: Log10:FSC(H), V: Lo	1823	1823	90.7%	90.7%	H: Ave=529.2, Dev=658.8 / V: Ave=18.5, Dev=18.5
P2 (Polygon, 2D, H: Log10:FSC(H), V: L	1614	1614	88.5%	80.3%	H: Ave=370.5, Dev=96.6 / V: Ave=204.4, Dev=204.4
P31 (Rectangle, 2D, H: Log0.1:FL5-B	1551	1551	96.1%	77.1%	H: Ave=1.3, Dev=0.5 / V: Ave=206.4, Dev=206.4
P33 (Rectangle, 2D, H: Log0.1:FL2	1450	1450	93.5%	72.1%	H: Ave=201.3, Dev=16.9 / V: Ave=1.2, Dev=1.2
P38 (Rectangle, 2D, H: Log0.1:F	1391	1400	96.6%	69.6%	H: Ave=1.2, Dev=0.3 / V: Ave=201.6, Dev=201.6
P39 (Cross, 2D, H: Log1:FL6	-	0	0.000%	0.000%	-
P40 (Cross, 2D, H: Log1:FL6	-	0	0.000%	0.000%	-
P41 (Cross, 2D, H: Log1:FL6	86	86	6.14%	4.28%	H: Ave=47.0, Dev=10.5 / V: Ave=16.7, Dev=16.7
P42 (Cross, 2D, H: Log1:FL6	1305	1305	93.2%	64.9%	H: Ave=86.2, Dev=11.6 / V: Ave=16.4, Dev=16.4

There are no step limits for analysis.

5-14-9 Sorting

Once the population gate is set, start sorting for the target population.

Step1

A. Selecting from Work Space

A popup window appears when the cursor is placed on the target gate. Click "Select Population" in the popup window.

B. Selecting from Population Space

Place the cursor on the target population and right click to display a popup window. Select "Select Population" in the popup window.

When "Select Population" is selected, the "Run" button in Operation Controls is exchanged with "Sorting" button.



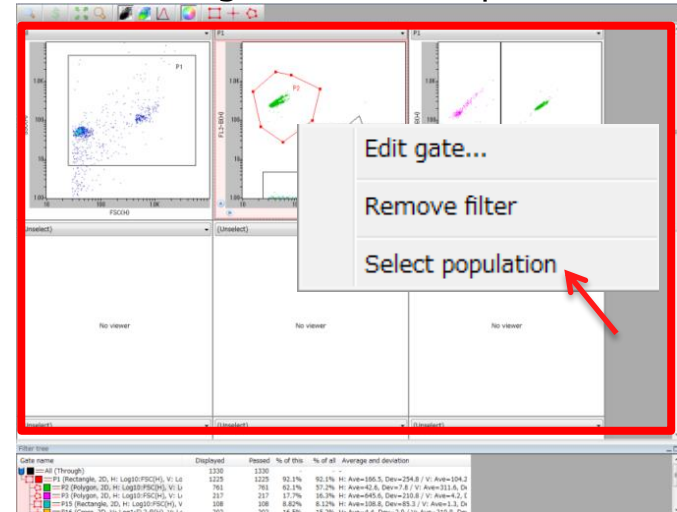
Step 2

When the population is determined, press "**Sorting**" to start sorting.

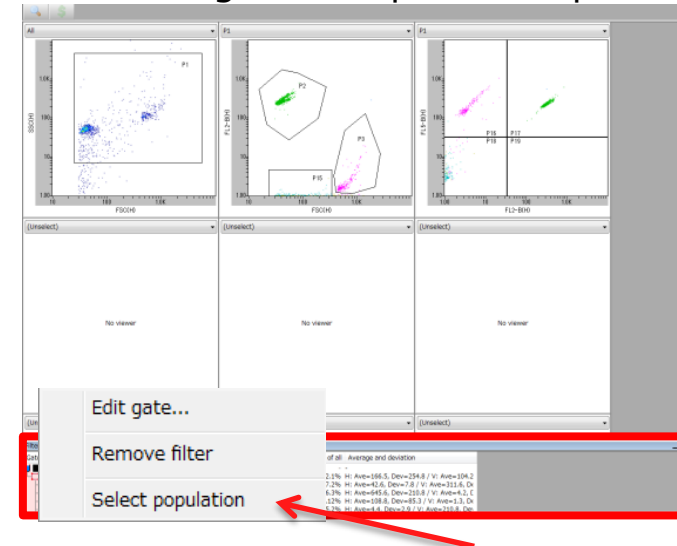


Step1

A. Selecting from Work Space



B. Selecting from Population Space



Place the cursor on the target gate, and select "Select Population" in the popup window.

5-14-10 Sorting Initiation and Termination

Step1

Time (min, sec), Event (total event count), Event/sec (event count per second) and Sorting events (total sorting count) are displayed in real time under Operation Controls after sorting starts (**Sorting**).

NOTE

When Liquid Level Sensor is activated, a warning window appears, and sorting automatically terminates.
Click "Yes" to save data, and collect the samples from Collection Reservoir.

Step2

Click either "**Stop**" or "**Recover**" to end sorting.

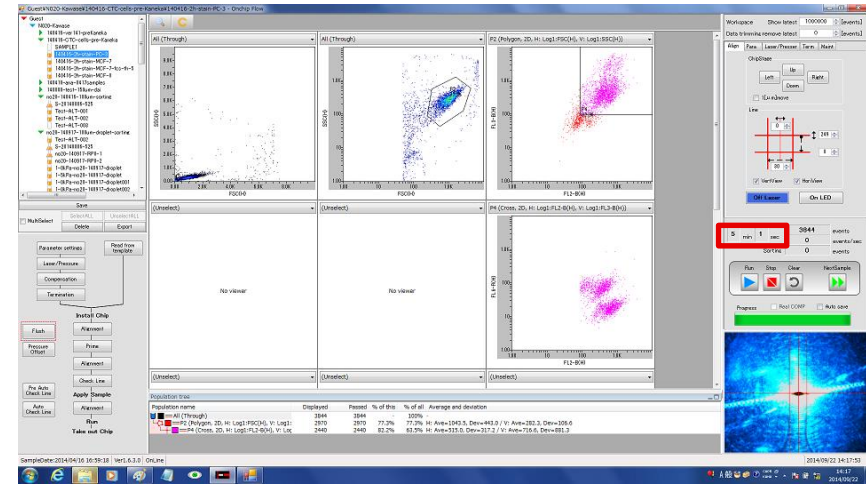
A. "**Recover**"

If "**Recover**" button in the Processing zone is pressed to end sorting, a mechanical operation is performed to expel all sample fluid remaining in the lateral flow channels to the Collection Reservoir. This may increase sample yield, but there is a risk that purity is reduced.

B. Press "**Stop**"

If "**Stop**" in Operation control is pressed to terminate sorting, all flow in the channels stops.

Step1



When Liquid Level Sensor is activated, a message on the left appears, and sorting is automatically terminated.

Step2



Stop: For higher purity
Recover: For higher yield

5-14-11 Sorted Sample Collection

Step1

Remove Chip Holder from the instrument and Open the lid of Chip Holder.

Step2

Collect all fluid from the Sorting Collection Reservoir.

NOTE

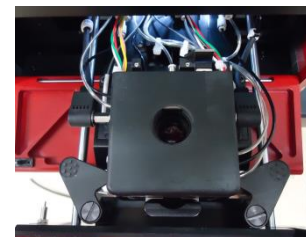
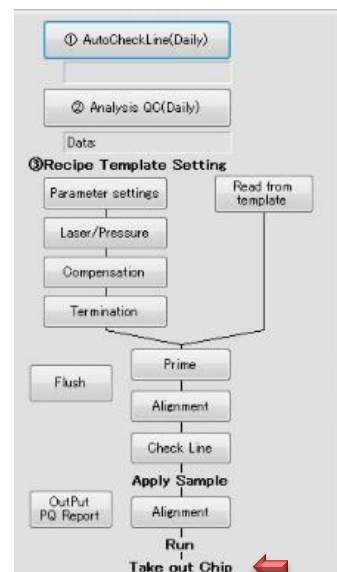
To check whether the target samples are collected or not, please refer to next page.

NOTE

If you would like to continue using the same chip for sorting,

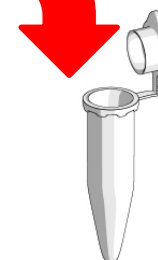
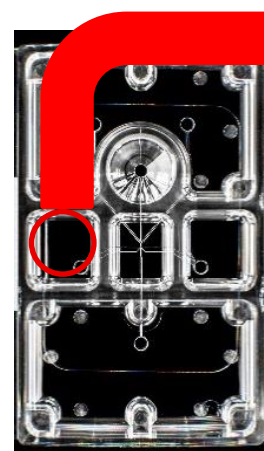
- ◆ Remove all waste from Waste Reservoir
- ◆ Refill sheath fluid (fill up to 4-7mL, 1.5mL and 50μL for Sheath, Sorting, Collection Reservoir, respectively)

Step1



Remove and open Chip Holder

Step2



Collect all fluid from Collection Reservoir

5-15 Purity Assessment of Collected Sample

To check whether or not the target cells have been collected, sorted samples are re-analyzed.

Step1

Prepare a new chip, prime with sample buffer and sheath fluid, and remove sample buffer from the Sample Reservoir.

Step2

Load 10 to 50 μ L of collected sample to Sample Reservoir

Step3

Select the file used for sorting, right click, select "Duplicate without data", and rename accordingly.

Right click the sorted gate and left click "Select Population". Maximum value for MainPush and MainPull is 8.0 and -2.5kPa, respectively.

Step 1



Prepare a new chip, prime with sheath fluid, and remove fluid from Sample Reservoir.

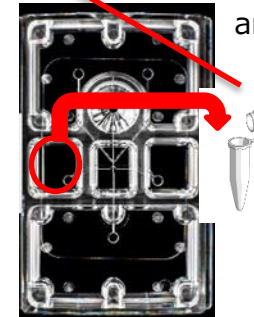
Step 2



Chip for re-analysis

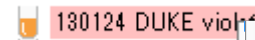


or

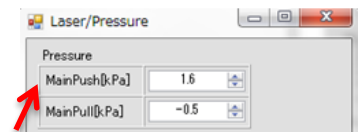
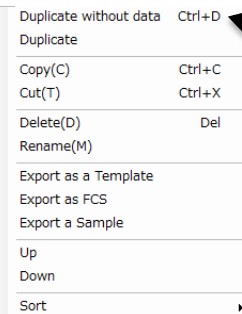


Remove certain amount of sorted samples from Collection Reservoir, or move all samples once to another container and re-analyze.

Step 3



Right Click

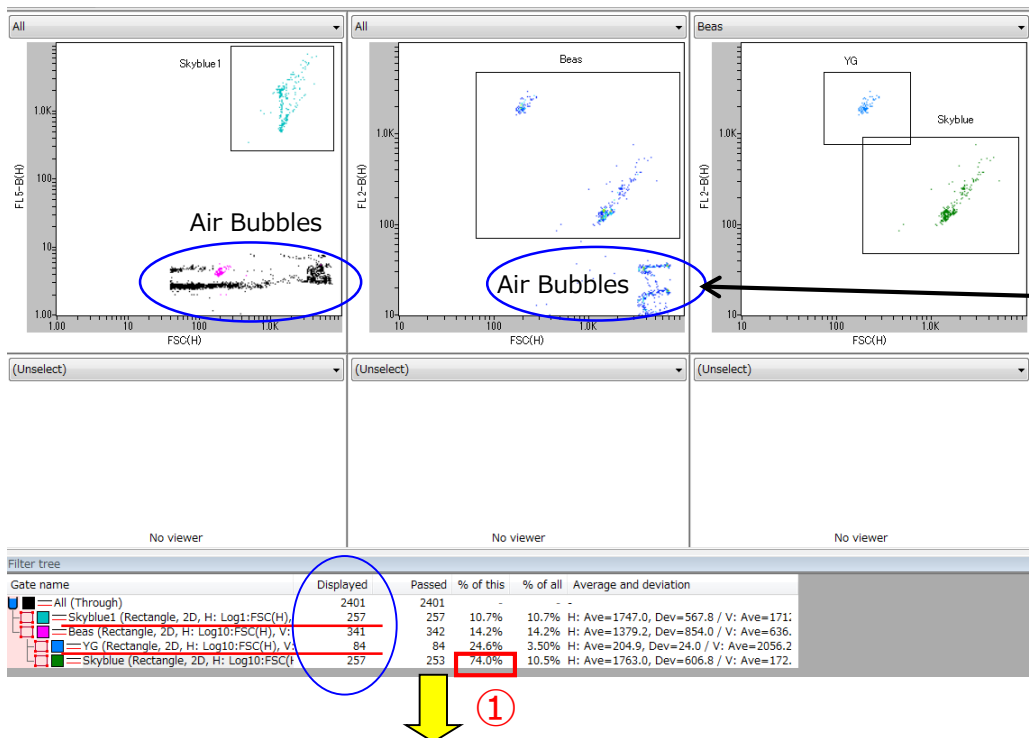


Set up MainPush

Step 4



Press **“Run”** and start re-analysis.
Press **“Stop”** after all fluid from
Sample Reservoir runs out.



Step4

Push **“Run”** to start re-analysis.



Once the full volume of sample has run, click **“Stop”**, and calculate the yield and purity.

NOTE

When sample completely runs out, bubbles enter the channel, causing disturbance to the CCD image and plots resembling debris will start to appear.

NOTE

The gate for the analysis and sorting may slightly differ due to the difference between chips.

Yield and Purity Calculation

Values under “Displayed” represent the total number of particles surrounded by the gate. (Value for All is the number of all particles in the plot, and thus includes the number of debris.)

Therefore, from the figure above (Skyblue as target particle and YG as non-target), there are 257 target particles and 84 non-target particles detected during the re-analysis.

In this case, **Yield is calculated by the number of target particles analyzed ÷ total sorting event count × 100**. Purity can be derived by gating all particles first, then gating only the target population. The percentage (①) shown on figure above equals to the purity, and it is 74%. **Purity can also be calculated by target particle count ÷ total particle count × 100**.

5-16 Maintenance

5-16-1 Washing Chip Holder and Silicon Packing (Gasket)

After finishing tests, wash the used Chip Holder with deionized water and alcohol, and dry in a clean environment.

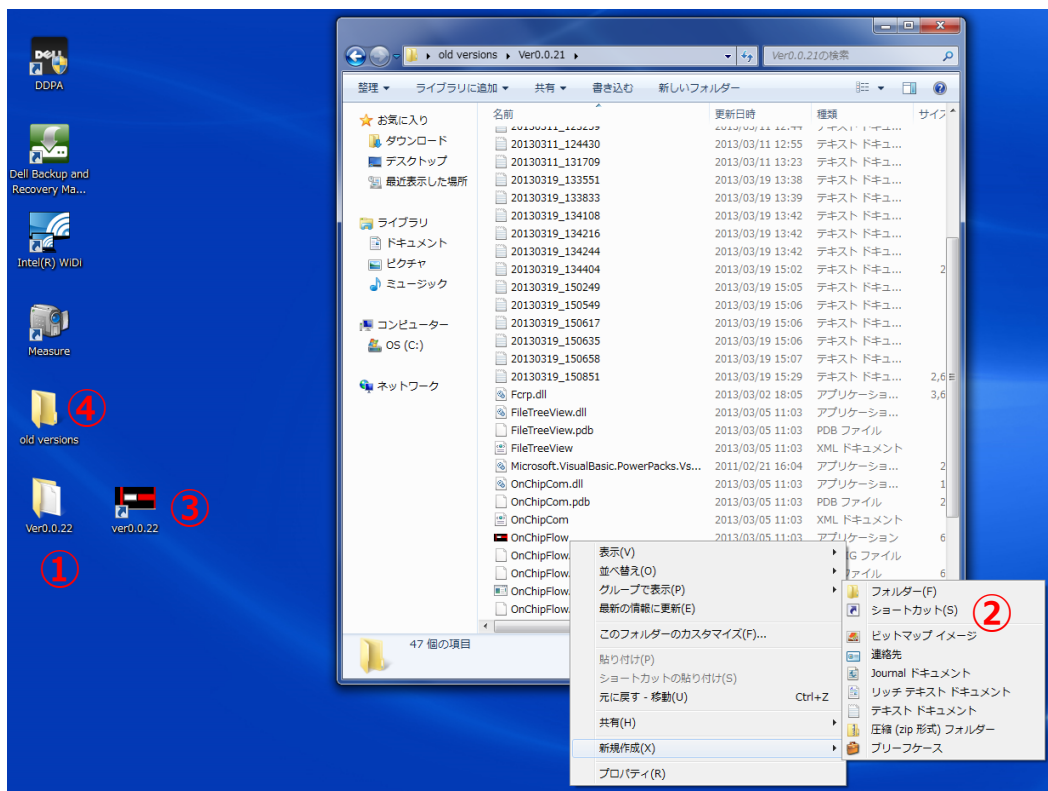
Attach the Silicon Packing (gasket) before using Chip Holder. If there is dust on the gasket, wipe off the dust with fiber-free cloth and some alcohol.



Align the bumps and indentations on the surfaces of the Silicon Packing (gasket) and Chip Holder. When the Silicon Packing (gasket) is attached correctly, the Silicon Packing (gasket) at the arrowed position may appear to protrude beyond the Chip Holder, but this is because the Chip Holder is tilted, and it is not a problem.

Chip Holder fitted with Silicon Packing (gasket)

5-16-2 Updating Software Update



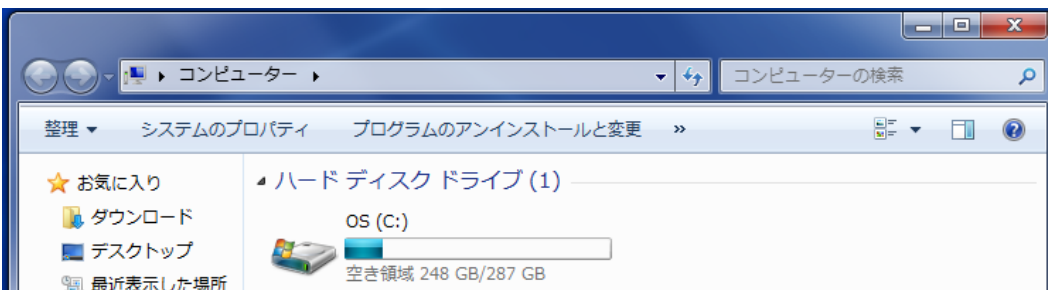
Procedures for updating to a new version of the software

- ① Save the software folder on the desktop.
- ② Create a shortcut to the OnChipFlow application file in the folder, and paste it onto the desktop.
- ③ Insert the version number in the shortcut name, to distinguish from the shortcut to the old version.
- ④ Move the folder containing the old version of the software to another location.
- ⑤ Delete the shortcut to the old version.

If the storage space in HDD of associated PC is running low, back up data and delete these from the PC.

Open the software and export old Experiment and Sample Files from Sample Management (Refer to Section 3-2 for more details), and save them to an external hard drive or similar location. Delete backed-up data from Sample Management. Important data should be kept backed up at all times.

5-16-3 Data Management



Chapter 6: Troubleshooting

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6-1 Troubes and Countermeasure for CheckLine

There may be an air leak, so apply the following countermeasures.

Countermeasure 1) If the central sample line cannot be seen at all in the check line process, there may be a blockage with bubbles, so prime again. (Refer to Chapter 5-10 Priming.) It may be difficult to remove bubbles because the chip is defectively molded, so if priming does not improve the situation, replace the chip with a new one.

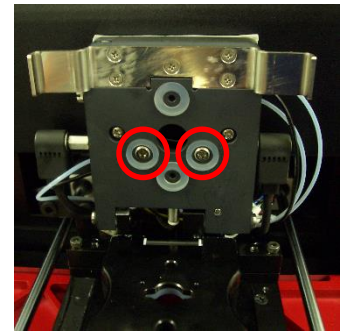
Countermeasure 2) If the Open Time value when performing check line is 100 or more higher than the value at the time of installation, contact our support group because there may be a fault with the X1 valve.

Countermeasure 3) There may be a leak from a gap between the chip holder and the pressure control unit, so remove foreign objects from between the rubber gasket of the pressure control unit and the chip holder. (Wipe with a lint-free cloth moistened with alcohol)

Countermeasure 4) There may be a leak from a gap between the chip and the chip holder, so remove the Silicon packing from the chip holder, wash the body and the Silicon packing with deionized water etc., then wipe away moisture and wipe with a lint-free cloth moistened with alcohol. (Refer to Chapter 5-16-1 Washing chip holders and silicon packings)

Countermeasure 5) If the chip is damaged, there may be a leak from a gap between the chip and the chip holder, so replace the chip with a new one. Please contact us if there are any defectively molded chips.

Countermeasure 6) There may be a blockage in a pressure control unit filter, so replace the filters. (Refer to Chapter 4-2-2 Changing the pressure control unit filter)



Locations to remove debris from in countermeasure 3 (parts circled in red on the pressure control unit)



Chip holder and Silicon packing to wash for countermeasure 4.



Pressure control unit filter used in countermeasure 6

6-2 Points to Check if Data is Abnormal

1) If plot is not displayed at all

- 1-1 Check the base levels for signals with thresholds set (explained in Section 6-5).
- 1-2 Check that the value of threshold in Parameter Settings is not too large. [Refer to Chapter 5-14-5 Setting the threshold condition](#)
- 1-3 Communications between the PC and the instrument may not be working well, so reboot both.
- 1-4 The sample may be blocked in the chip, or air may have entered at the bottom, so pipette the inside of the sample reservoir.
- 1-5 Check that the first gate is surrounding the detected data. [Refer to Chapter 5-14-1 Setting FSC and SSC Gates](#)
- 1-6 The fluorescence signal strength may not be appropriate in some cases. While running the process, adjust the Gain. [Refer to Chapter 5-14-4 Gain Adjustment](#)
- 1-7 Check whether the intended laser has been checked. [Refer to Chapter 5-13 Laser Alignment](#)
- 1-8 Check the chip alignment to confirm that the laser is hitting the flow channel correctly. [Chapter 5-10 Flow Channel Alignment](#)
- 1-9 The chip may be damaged, so in some cases, it may better to replace it with a new one.

2) If sorting purity is poor

- 2-1 Check that the appropriate solutions are used and that they were prepared by the right method. [Refer to Chapter 5-2 Buffer Adjustment](#)
- 2-2 Check that the line during check line is not too far over to the collecting reservoir side. [Refer to Chapter 5-11 Check Line](#)
- 2-3 Check that the concentration of non-target cells is $10^7/\text{mL}$ or less. Purity is reduced if the concentration of non-target cells is too high.

- 2-4 The sorting timing may be off. Refer to QC for the adjustment method for sorting timing. [Refer to Chapter 4-1-3 Sorting QC](#)
 - 2-5 In some cases purity may be improved by putting 30–50μL of sheath fluid in the collecting reservoir in advance when sorting.
 - 2-6 Check that the chip is in focus, and contact us if it is not.
- 3) If sorting efficiency is poor
- 3-1 Check whether the line during check line is too far from the collecting reservoir side. [Refer to Chapter 5-8 Check Line](#)
 - 3-2 Check whether the sorting timing is off.
 - 3-3 Confirm that the flow channel is not blocked.
 - 3-4 Avoid reusing the chip as efficiency declines.
 - 3-5 Check that the concentration of Through Path Plus is appropriate. [Refer to Chapter 5-2 Buffer Adjustment](#)
 - 3-6 Check that the concentration of viscosity agent is appropriate. [Refer to Chapter 5-2 Buffer Adjustment](#)
- 4) If sorting pulses are not generated
- 4-1 Confirm that select population is On. [Refer to Chapter 5-14-9 Sorting](#)

6-3 If the Laser does not turn on

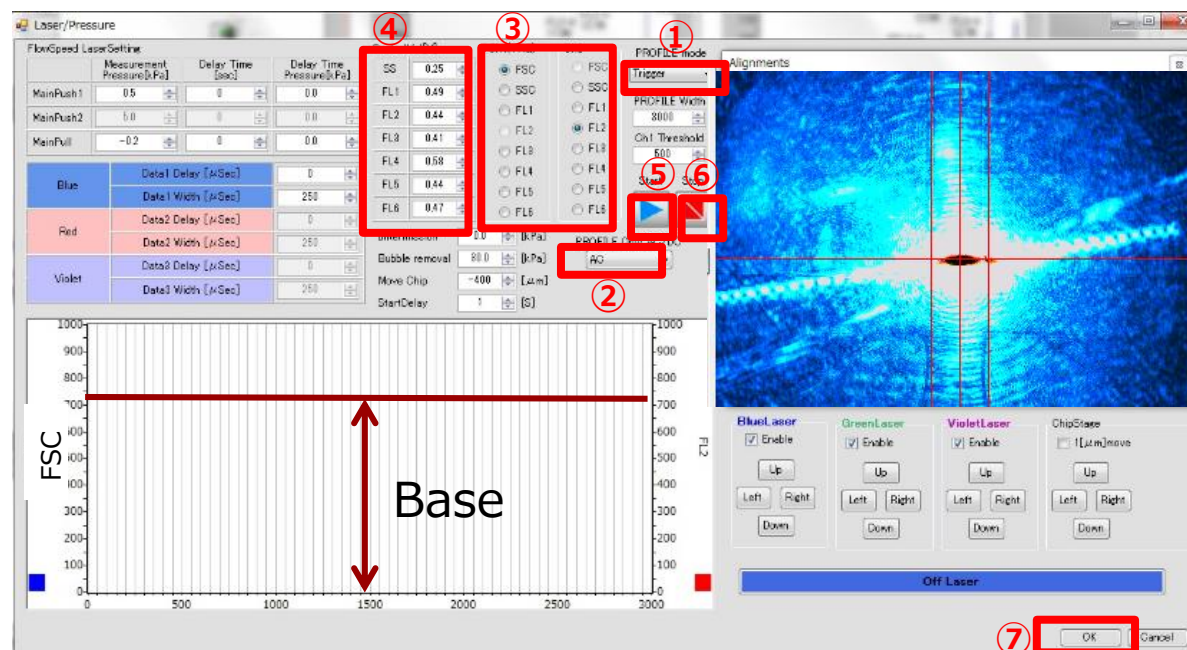
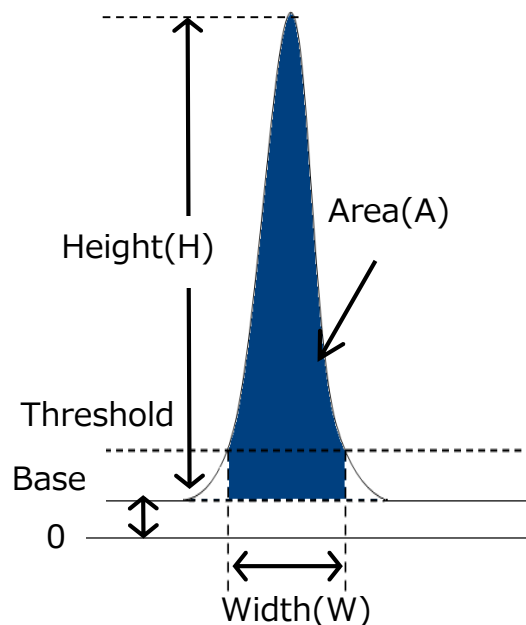
If the laser does not turn on during analysis or sorting despite having a check mark added as a used laser, stop the Run and turn the laser on and off repeatedly until it turns on. Refer to **Chapter 5-13 Laser Alignment**

If the lower hatch (for objective lens adjustment) is open or not fully closed, the safety device automatically turns off the laser. Therefore, the lower hatch must be closed for the laser to turn on. Please ensure it is closed properly.

6-4 Action to take when liquid is drawn inside the instrument

If sheath fluid or sample fluid leaks from a reservoir and is drawn into the pressure control unit side, replacement of several parts is required. Contact us immediately.

6-5 How to Check Signal Base Levels (check displacement in the optical system)



If no signal is detected, the first thing to check is the base level of the signal (also called the DC (direct current) level) of FSC. Signal detection requires that the following conditions be satisfied.
Dynamic range of detection signal > Base+Height

If the Base is so high that it is almost at the same level as the dynamic range, the measurement of Height becomes impossible, and signals more than the threshold will not be detected.

How to Check Signal Base Levels

Press the **Laser/Pressure** button in the Analysis tab to display the window on the left. ① Switch Trigger to Continuous, ② Switch to DC, ③ Select up to two types of signal to investigate, ④ Set the Gain for the detector, ⑤ Click **Start** to display the base level of the selected signal on the plot, and ⑥ Stop. If the displayed level is 1,000 or less, it is normal.

Please contact us if it is higher than 2000, the optical system requires adjustment.

Once this check is complete, turn ① back to Trigger, return ② to AC (alternating current), and ⑦ Click **OK** to close the window.

Note Checking the signal base level of FSC is important if the instrument has been moved.

6-6 When PC freezes during measurement

Please turn OFF of main switch of the instrument, remove the chip, and restart PC. After restarting PC, turn ON the main switch. Please contact us if the PC keeps on freezing during measurements.

6-7 If a fluorescence signal is at uppermost limit

If the Gain of a detector such as FL1 is too high, the signal data of detectors (SS, FL1-6) will look as if they hit the uppermost limit. In such cases, reduce the Gain of the detectors while checking the signal strength appearing on the plot.