On-chip Sort

Simplified User Guide for Standard Cell Sorting

2D Chip-Z1001
80μm Channel

Manual Ver. 1.0.0
Soft Ver. 1.8.7

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Chip 2D-Z1001

On-chip Sort Sorting Steps

Turn on On-chip Sort

Create a Sample file

Load buffer

Prime

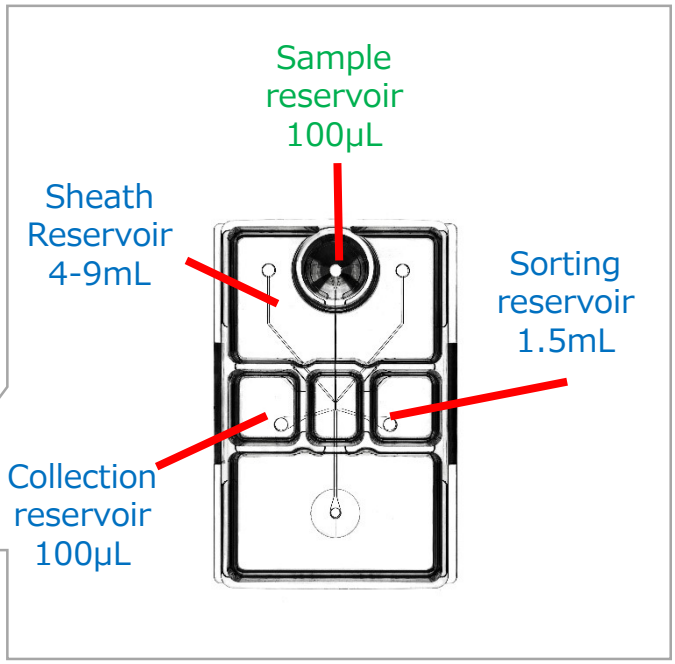
Load sample

Gain adjustment

Gate setting

Start Sort

Stop



Run a small amount of sample for control voltage adjustment

Run a small amount of sample to set gates, and click Select population for the gate of interest

※Please connect both On-chip Sort and extension cable to 3-pin plugs. It may cause signal failure.

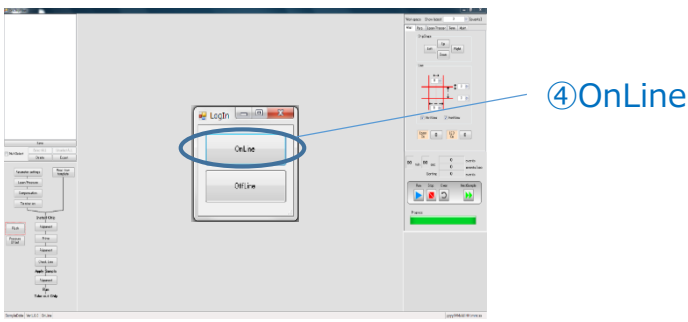

3-pin plug

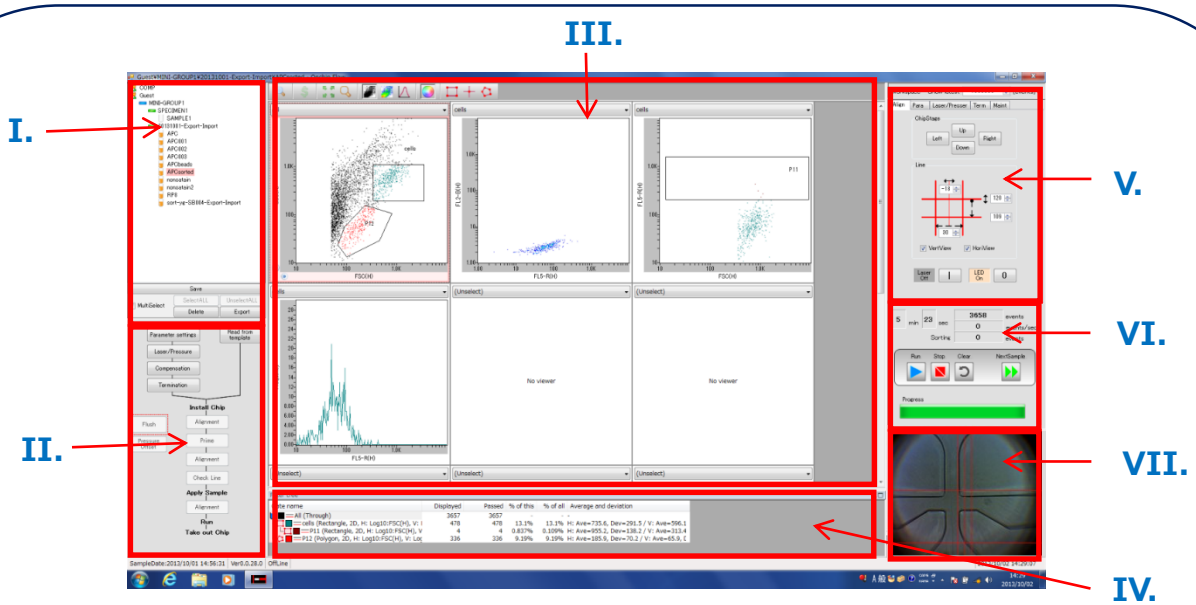
① Turn on On-chip Sort (Flick the power switch on the power box)

② Turn on the PC

③ Double click on the "OnChipFlow" software icon

④ Click "OnLine"





I. Sample management
Selects and manages Sample files

II. Processing zone
Confirms working steps

III. Work space
Displays dot plot, gating, histogram, scatters. Set axis for scatter and fluorescent light parameters.

IV. Population management
Provides information of each gated population

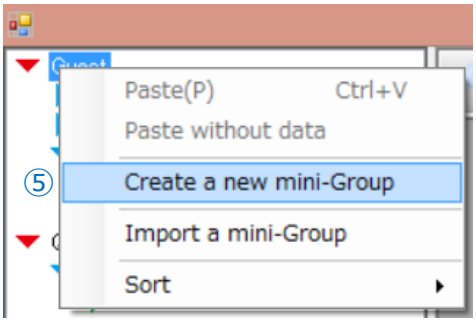
V. Instrument settings
Adjusts chip location, lasers, and detectors' control voltages etc

VI. Operation controls
Regulates analysis and sorting process

VII. Camera view
Image from CCD camera

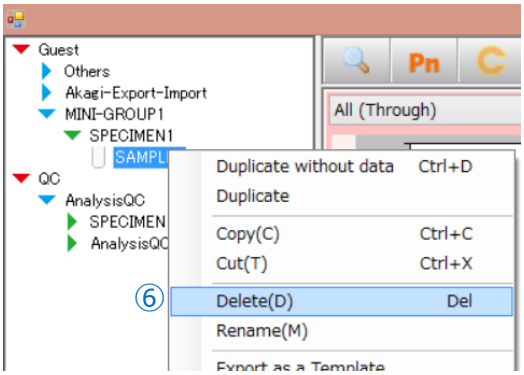
Sample management tree is formed with 4 tiers.

- Guest layer/QC layer
  - MINI-GROUP layer
    - SPECIMEN layer
      - SAMPLE layer
        - Empty Sample file
        - Sample file with data

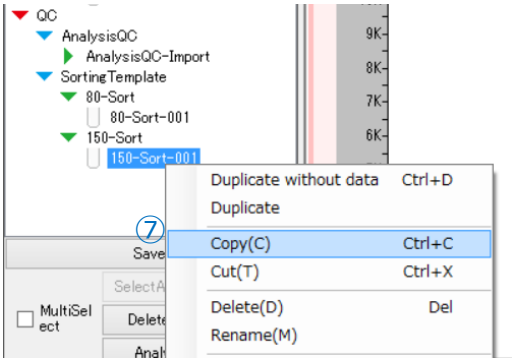


⑤ Right click on “Guest”, and left click on “Create a Mini-Group”. The newly created Mini-Group should contain all the lower tiers (Specimen folder and Sample file).

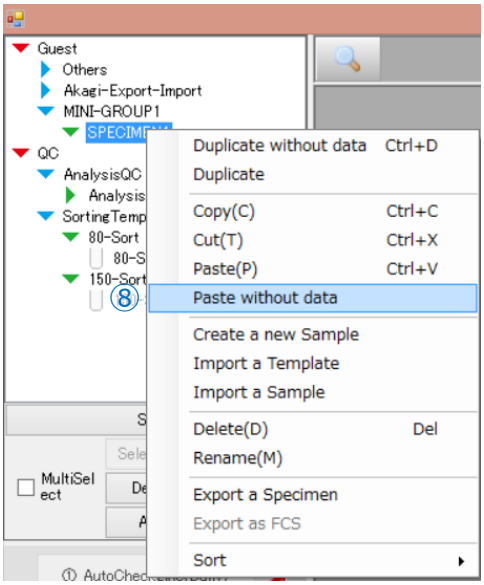
⑥ Right click on the “Sample File” created together with the Mini-Group, and click “Delete”. This folder does not contain any of the crucial parameters for sorting.



⑦ Under QC folder, find the appropriate Sorting template file. Right click on the file, then select “Copy”.



⑧ On the Specimen folder created earlier, right click and select “Paste without data”. Click on the new Sample file.



NOTE

These steps on this page is only required when you create a Mini-Group or a Specimen folder. Once this is complete, then the same setting can be carried over to a new file by clicking “Duplicate without data”.

⑨ Parameter settings
Click on “Parameter settings” under II. Processing zone. A setting window will be displayed.

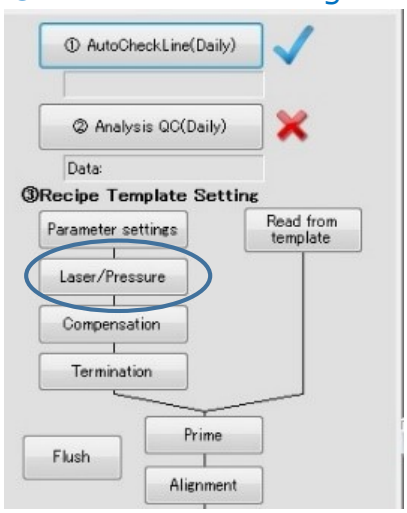
⑩ Threshold setting
Setting a threshold is important to distinguish noise/debris to cell populations. Avoid cutting out the target cell population. (FSC threshold of 50-100 is suitable for cells 10-20μm in size) (Finalize the threshold value after flowing a small amount sample)

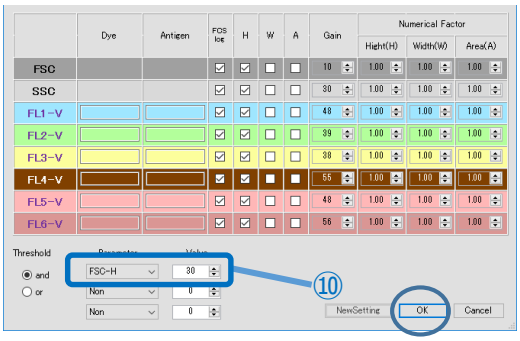
⑪ Click “OK” to confirm.

⑫ Laser/Pressure setting
Click “Laser/Pressure” under II. Processing zone to display the window for detailed laser and pressure setting. Tick boxes for the lasers to be used.

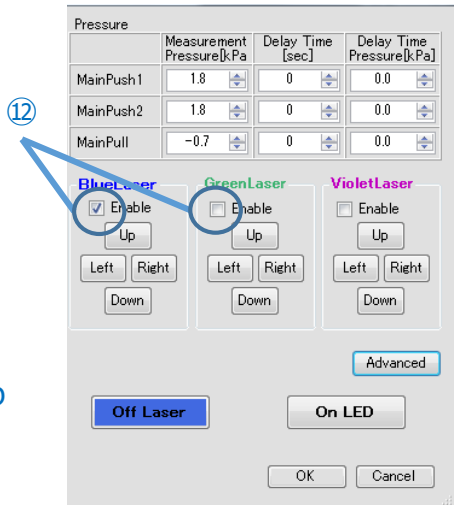
⑬ Putting chip inside Chip holder
Remove chip from the bag and set it inside the chip holder. Install the chip with the sample reservoir at the rear of the chip holder (waste reservoir in front).

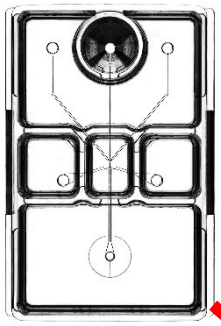
⑨ ”Parameter settings”



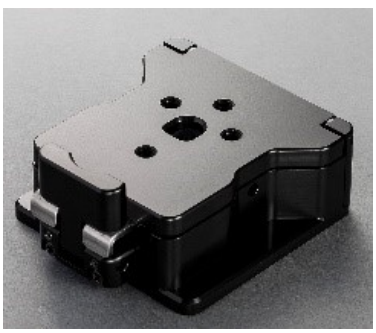


⑪ Click “Ok” to confirm





Chip holder



⑬ Install a new chip

⑬ Sample reservoir at rear

Buffers

□On-chip Sheath

Used as sheath liquid without adjustments.

NOTE Open inside a clean bench

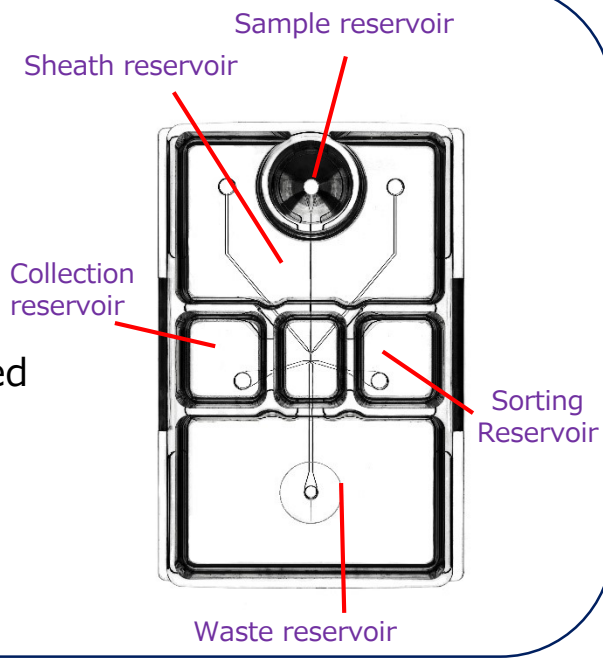
☆On-chip Sample buffer

Buffer used to suspend the sample. Comes in the form of 1x and 2x solution for convenience.

1x solution used for resuspending pellet
2x solution used for 1:1 sample dilution

Sorting chip: Names and functions

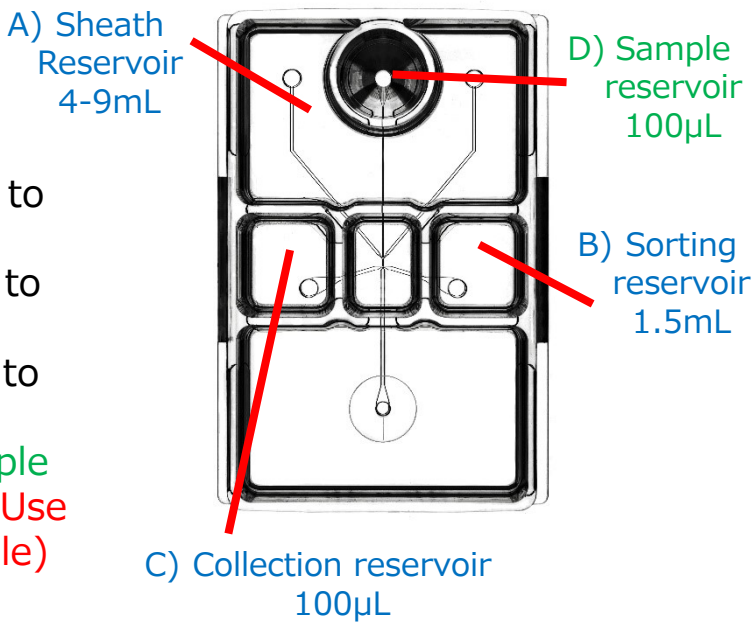
- Sheath reservoir
  Load sheath liquid or media for sample flow focusing
- Sample reservoir
  Load sample
- Waste reservoir
  Unsorted sample and waste is collected
- Collection reservoir
  Target cells are collected after sorting
- Sorting reservoir
  Reservoir for generating sorting pulse flow



⑭ Loading buffer on chip
Do not add your sample.

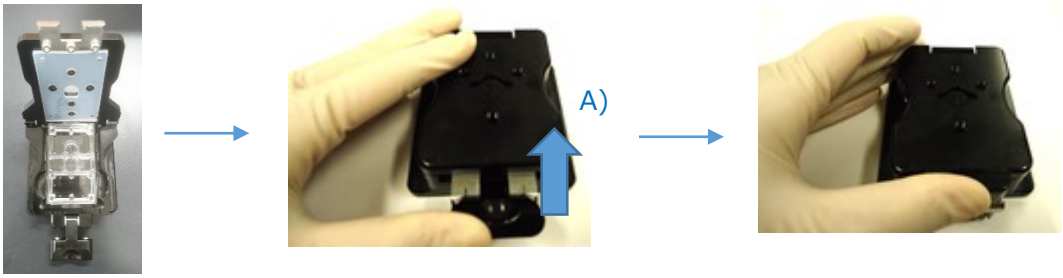
For 2D-Z1001

A) Load 4-9mL of sheath liquid to Sheath reservoir.
B) Load 1.5mL of sheath liquid to Sorting reservoir.
C) Load 100µL of sheath liquid to Collection reservoir.
D) Load 100µL of On-chip Sample buffer to Sample reservoir. (Use Sample buffer without sample)

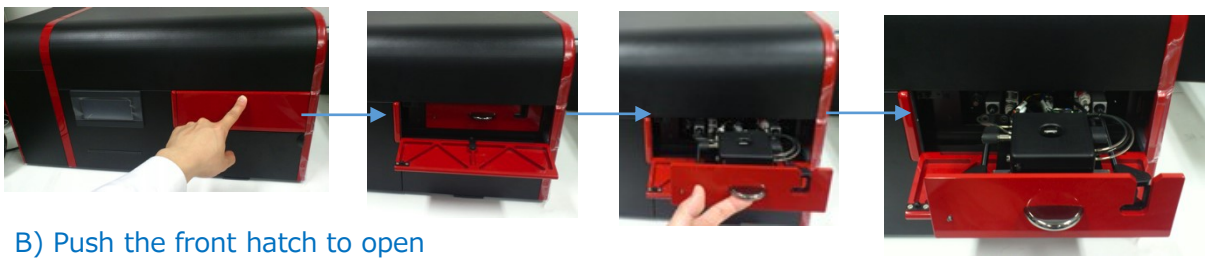


⑮ Inserting chip holder

A) Close the top lid of the chip holder and lock using the tab.



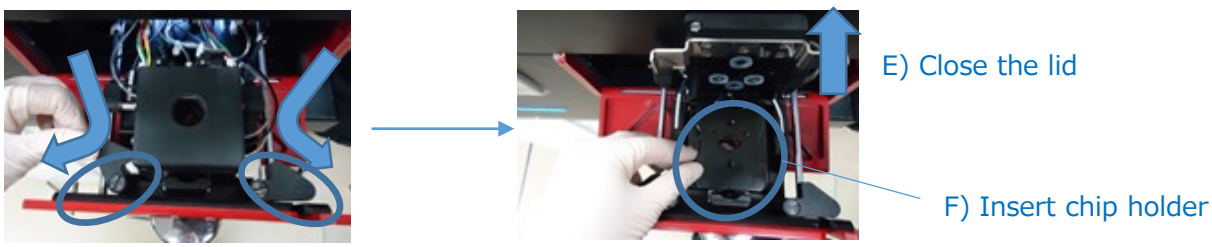
B) Open the front hatch, then C) pull out the loading port until it locks with a click sound.



B) Push the front hatch to open

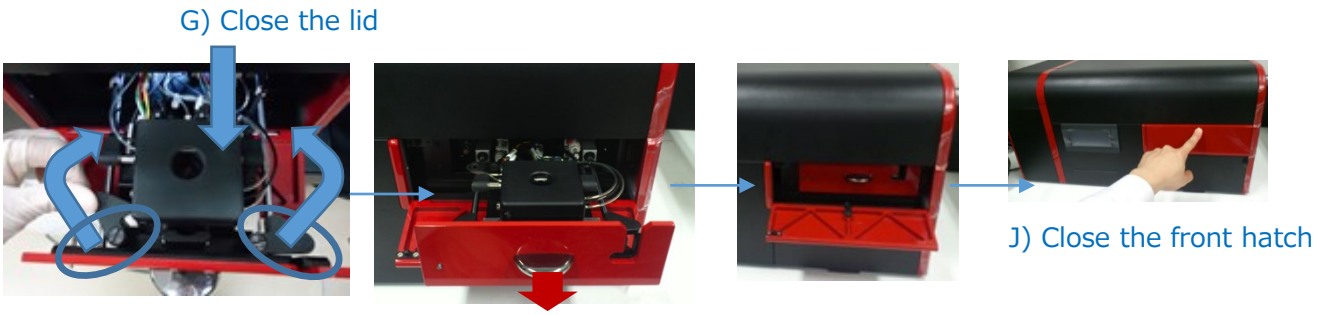
C) Pulling out till the end will lock the loading port

D) Undo the locks on the left and right sides, E) lift up the lid, F) then insert the chip holder. Make sure chip holder is firmly in position.



D) Undo the locks on left and right

G) Close the unit, H) Lock, I) Pull the loading port, then J) close the front hatch



H) Lock the lid

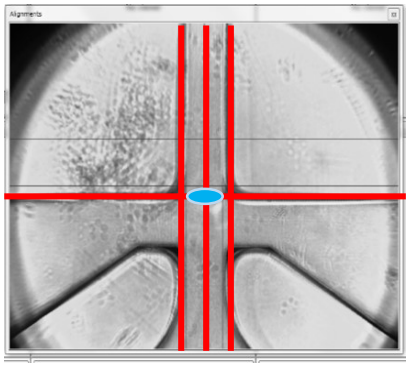
I) Pulling the loading port will automatically set it in position

⑯ Alignment

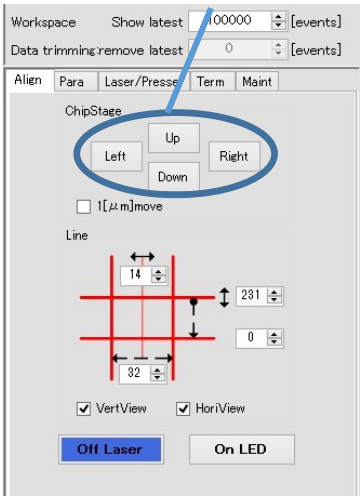
Chip stage positioning (with automatic adjustment)

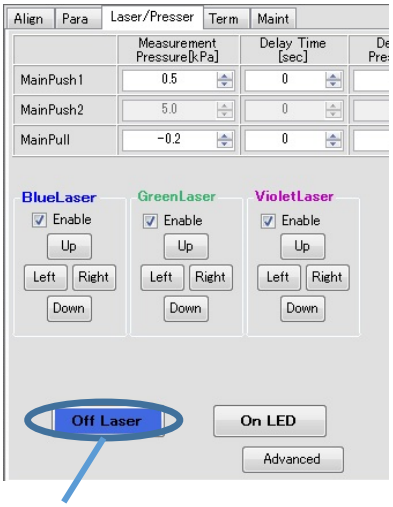
Confirm that two red vertical lines on either side of the central line and red horizontal line in A) are aligned with the channel walls of the microfluidic chip. If they are not aligned, correct the position using B) ChipStage buttons under “Align” tab in V. Instrument settings.

A) Chip is correctly aligned



B) ChipStage Control





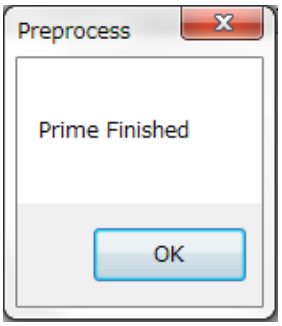
C) Laser ON/OFF

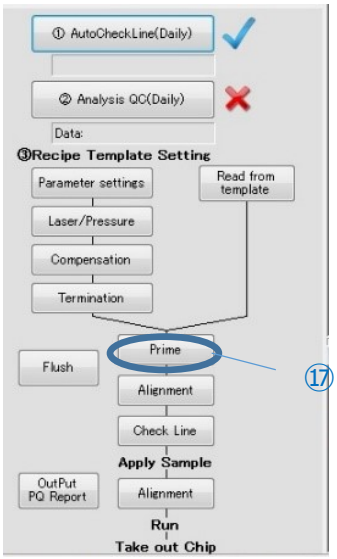
⑰ Prime

Click on “Prime” button located in II. Processing zone.

A message will appear when it is done.

Click “OK”.

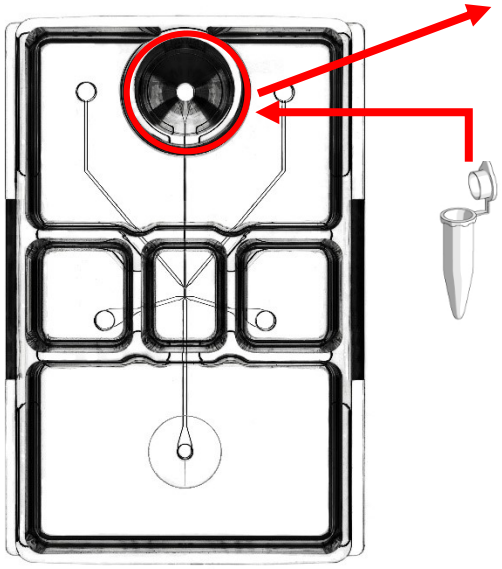




⑰ ”Prime” button

⑱ Sample loading

Follow step ⑯ B)-E) to remove the chip holder from the instrument.
A) Remove buffer from Sample reservoir
B) Load sample. Ensure the sample is suspended in sample buffer!



Confirm that each reservoir contains buffer as shown in ⑮.
Remove waste.
Volume in Sorting collection reservoir can be reduced.

⑲ Follow step ⑮ A), F)-J) to insert the chip holder in the instrument.
Check chip stage position as shown in ⑯

<Proceed to analysis and sorting>

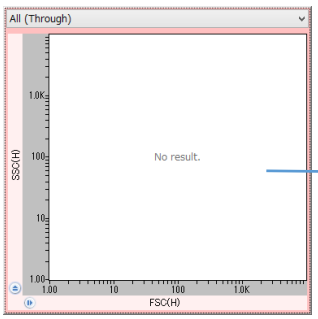
⑳ Push "Run" button in VI. Operation controls to flow small amount of sample first.

㉑ Push "Stop" button to stop the run, and open the plot of interest.

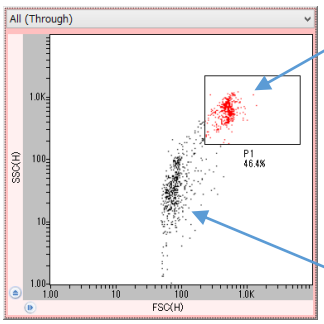
Preparation prior to sorting

A) Open dot plot or histogram you would like to analyze in III. Work space

SSC vs FSC



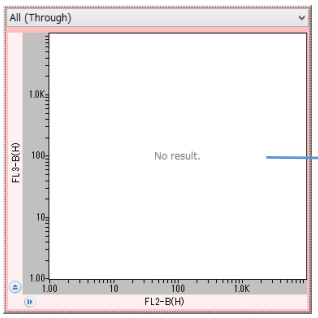
(Example)



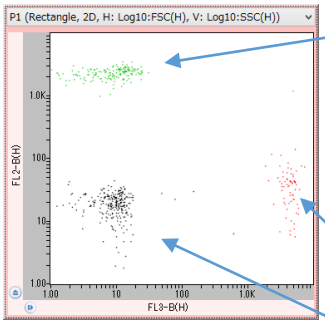
Cell population

Noise/Dust

FL-2 vs FL-3



(Example)

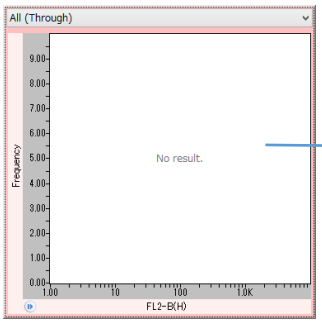


FITC +ve cells

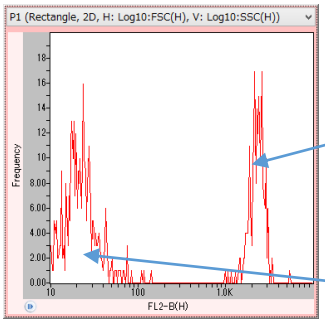
PE +ve cells

FITC/PE –ve cells

FL-2 Histogram

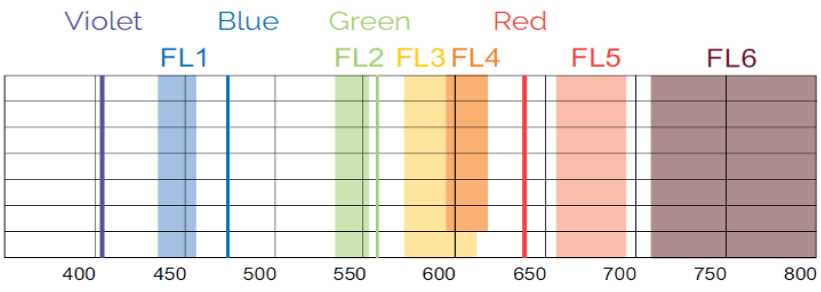


(Example)



FITC +ve cells

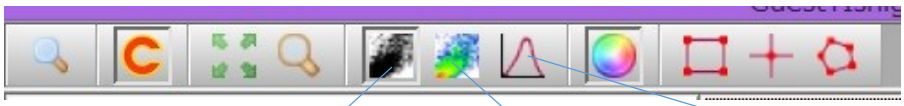
FITC –ve cells



	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

B) Plot settings

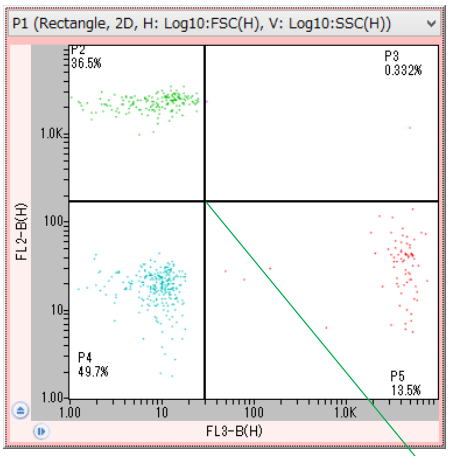
Buttons located at the top of III. Work space

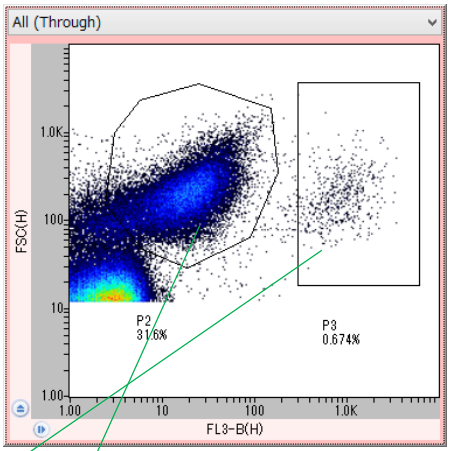


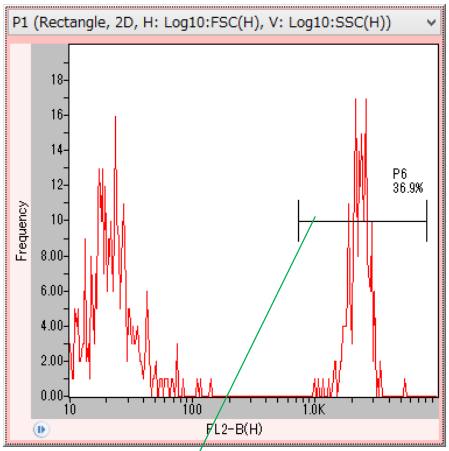
Dot plot

Pseudo color plot

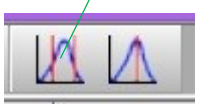
Histogram



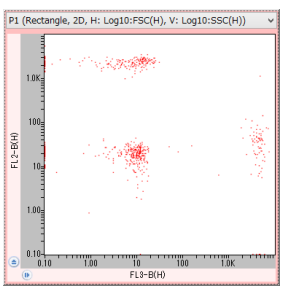


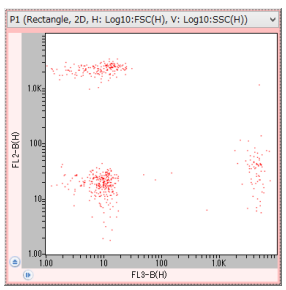


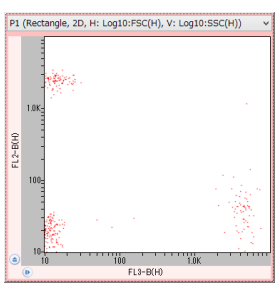
Gate



Click here to change axis information



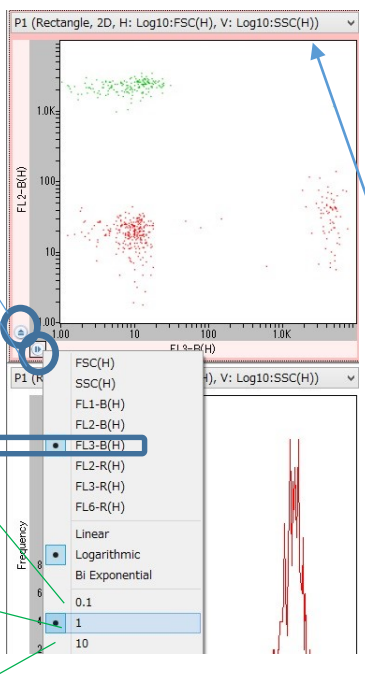




0.1~

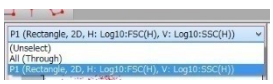
1~

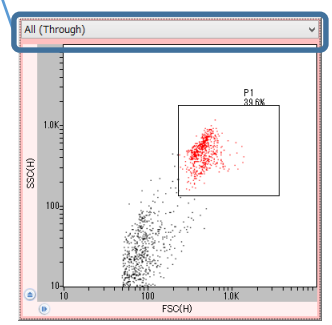
10~



Changing axis

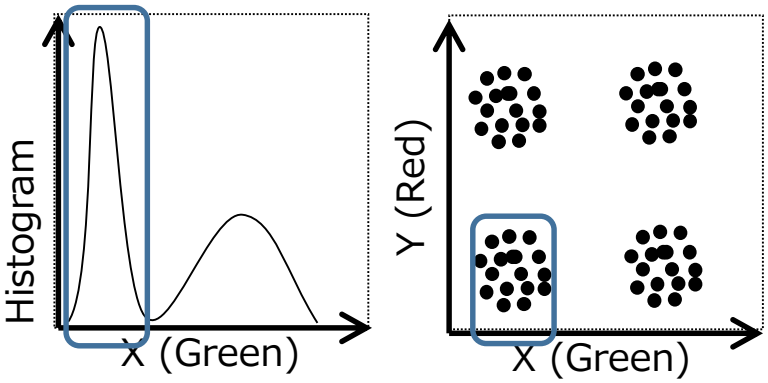
Select a gated population to display only that population





C) Gain adjustment

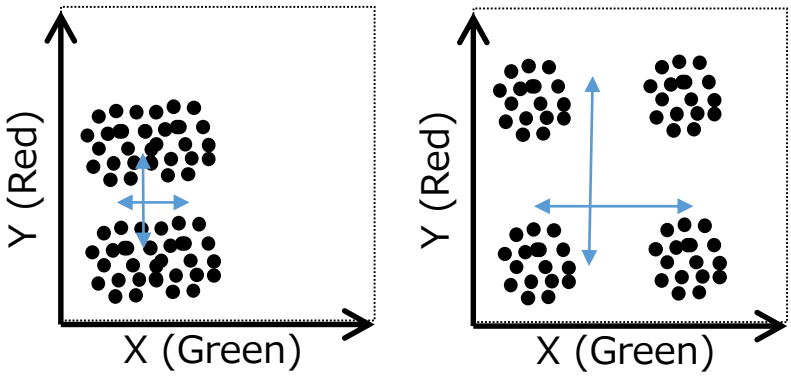
Adjust Gain for PMTs by inputting values in “Gain” under “Para” tab in



Ideal example

Gain value for each FL should be adjusted so that negative cell population is located close to the edge, and positive and negative populations are well separated.

(Samples with weak fluorescence may be hard to separate. Users may have to adjust increase the fluorescent intensity by, for example, changing antibody concentration.)



D) Fluorescence compensation

Users may need to carry out compensation when two or more fluorescence are used. There is no need for compensation if only one color is used.

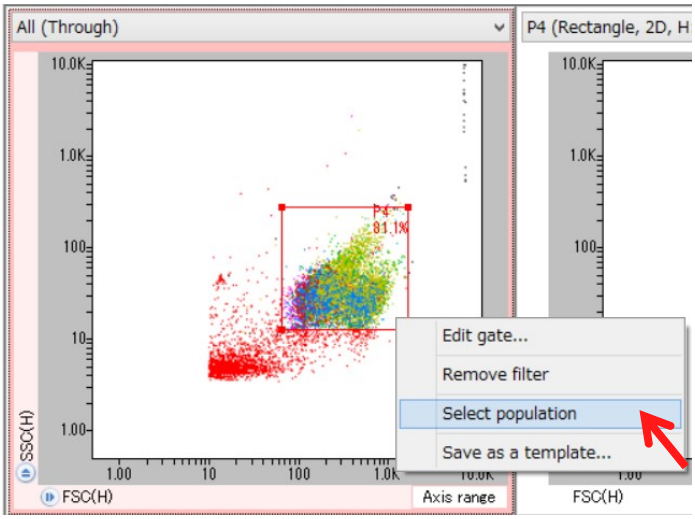
Please refer to Section 5-14-7 of User Manual for more details.

Ensure fluorescent compensation button is ON and sorting gate is selected for sorting with compensation. It may not function properly otherwise. Please also make sure the Sample file is empty.

Fluorescent compensation button → At the top of III. Work space

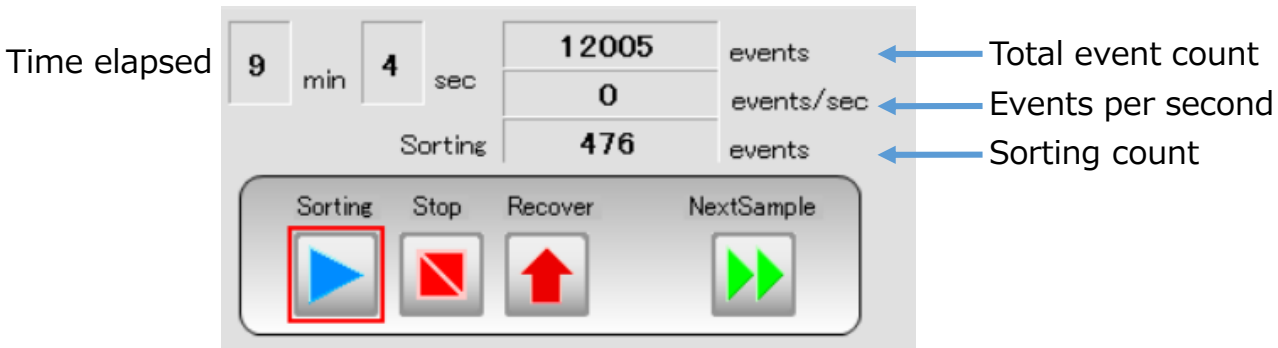
Sorting gate → Click ” Select population” for the target population

㉒ Run a small amount of sample, check on the plot, and gate the target population.
Right click on the selected gate, and choose ”Select population”.



㉓ Sorting will start once ”Sorting” button in VI. Operation controls is pressed.

VI. Operation controls



㉔ Press Stop” once required sorting number is reached, take out the sample holder, and retrieve the collected sample from sorting collection reservoir.