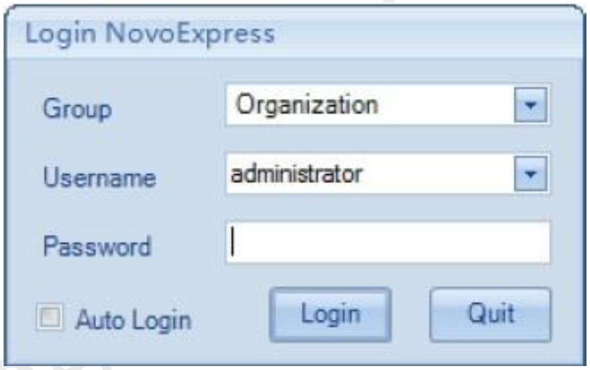
HKU Med LKS Faculty of Medicine
Centre for PanorOmic Sciences
香港大學泛組學科研中心

Imaging and Flow Cytometry Core

NovoCyte Advanteon BVYG Standard Operation Protocol
Basic Operation

1. NovoExpress Software Log In

Log into NovoExpress software with your own login name and password. Make sure *Auto Login* is unchecked.
**Please contact Faculty Core Facility Staff to establish a new user account*.

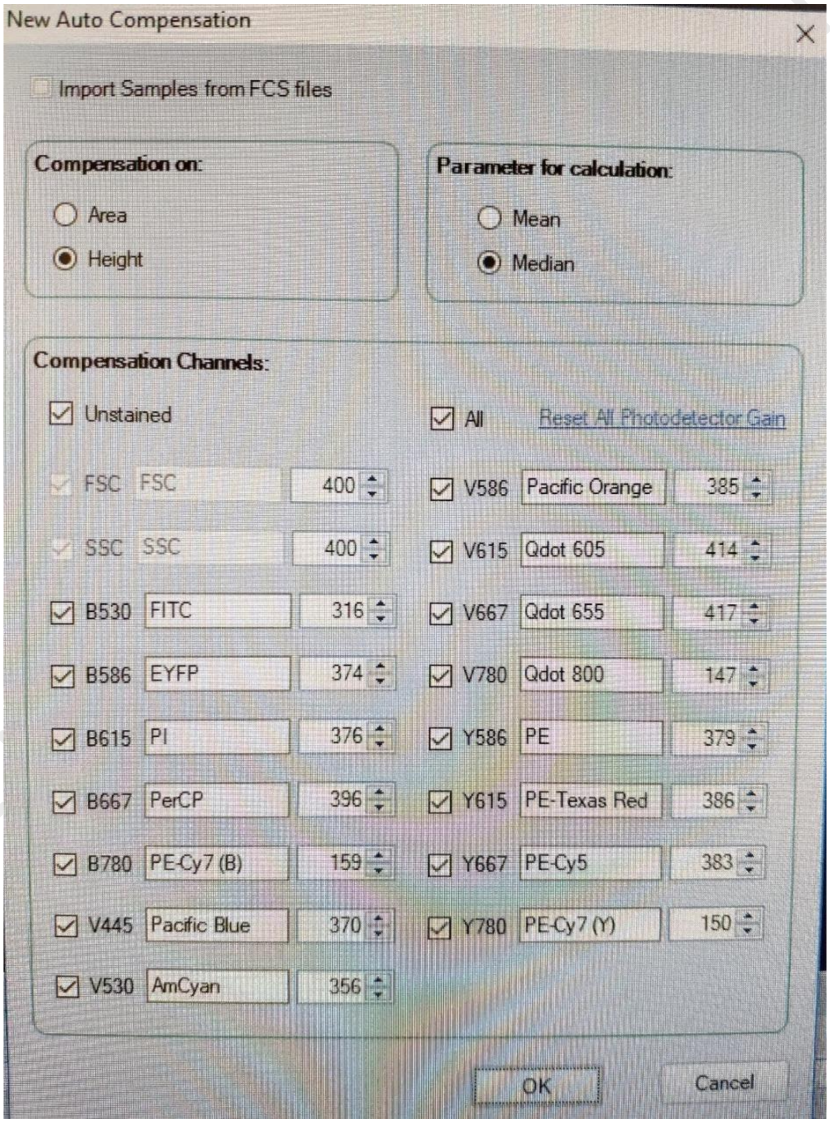


Group: Organization
Username: administrator
Password:

2. Compensation (Perform when needed)

Step 1. Set-up Compensation Controls
a. In the ***Home*** tab of the **Menu Bar**, click the ***Auto Compensation*** button.

b. Select Compensation on: *Height*, Parameter for calculation: *Median* and **check the boxes of channels involved**. Then click ***OK***


New Auto Compensation

☐ Import Samples from FCS files

Compensation on:
○ Area
● Height

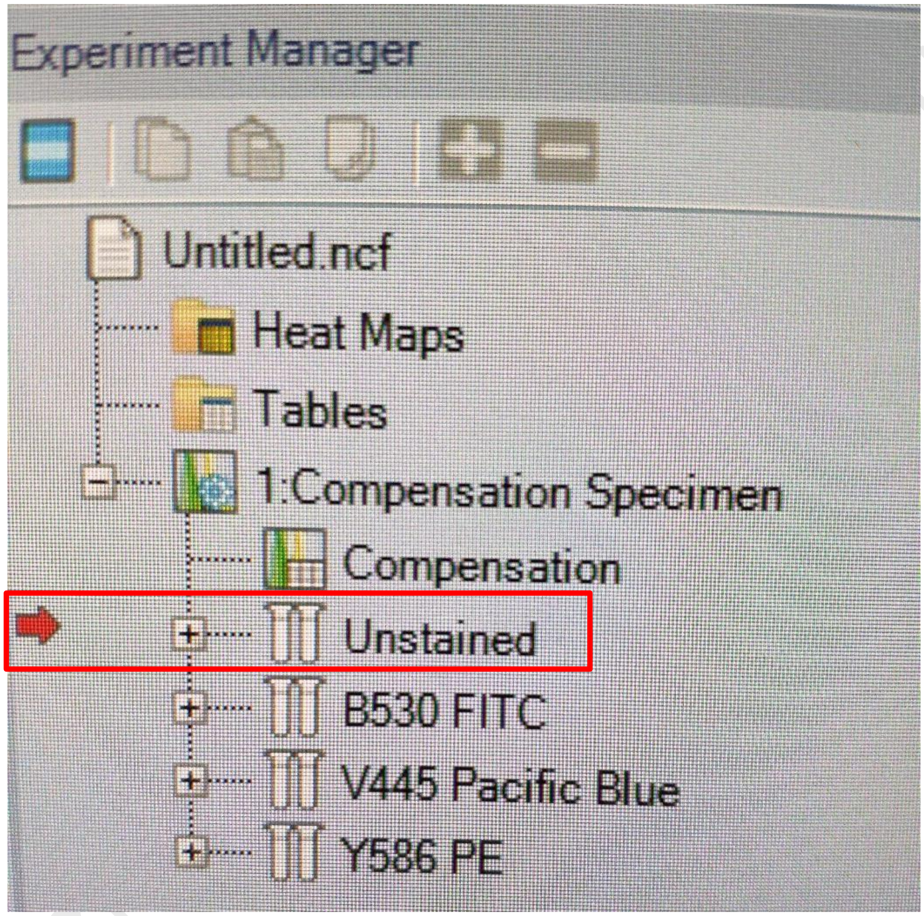
Parameter for calculation:
○ Mean
● Median

Compensation Channels:

☑ Unstained
☑ All
Reset All Photodetector Gain

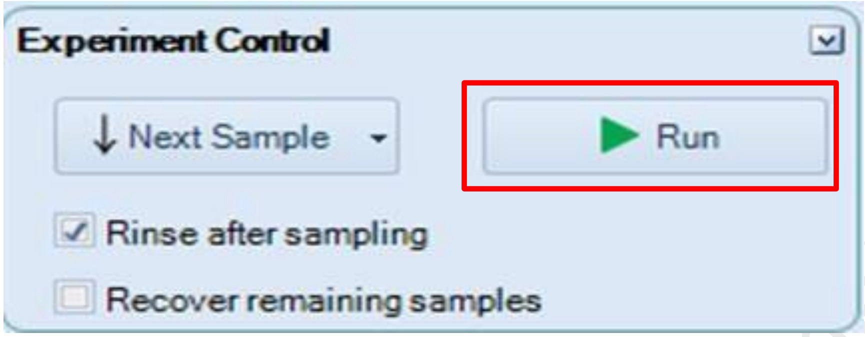
☑ FSC FSC 400
☑ SSC SSC 400
☑ B530 FITC 316
☑ B586 EYFP 374
☑ B615 PI 376
☑ B667 PerCP 396
☑ B780 PE-Cy7 (B) 159
☑ V445 Pacific Blue 370
☑ V530 AmCyan 356
☑ V586 Pacific Orange 385
☑ V615 Qdot 605 414
☑ V667 Qdot 655 417
☑ V780 Qdot 800 147
☑ Y586 PE 379
☑ Y615 PE-Texas Red 386
☑ Y667 PE-Cy5 383
☑ Y780 PE-Cy7 (Y) 150

c. Compensation Control Specimen is created in the Experiment Manager panel with corresponding empty control samples (e.g. Unstained, B530 FITC, V445 Pacific Blue, and Y586 PE).



Step 2. Compensation Control Acquisition

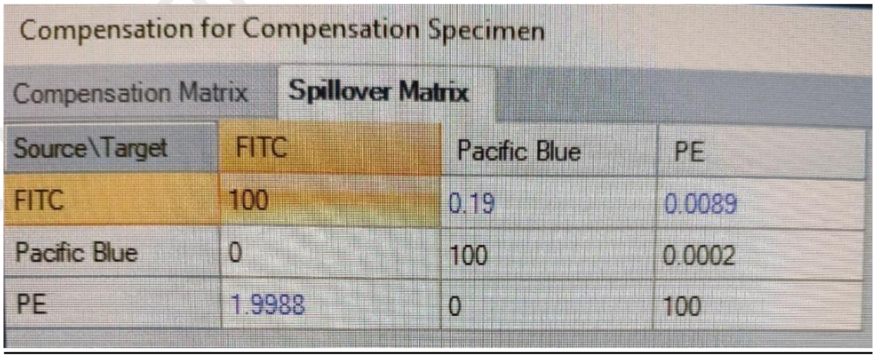
a. Double Click your sample and let the red arrow point to it
b. Then click ***Run*** on the Cytometer Control Panel.



c. Repeat the steps a and b to the following single stained tubes.

d. After all controls have been acquired, the compensation matrix is calculated automatically.

Step 3. To view or adjust the compensation matrix, click the ***Compensation Matrix*** button In the ***Home*** tab of the Menu Bar and the Compensation Matrix window will show.


Compensation for Compensation Specimen

Compensation Matrix | Spillover Matrix

Source\Target	FITC	Pacific Blue	PE
FITC	100	0.19	0.0089
Pacific Blue	0	100	0.0002
PE	1.9988	0	100

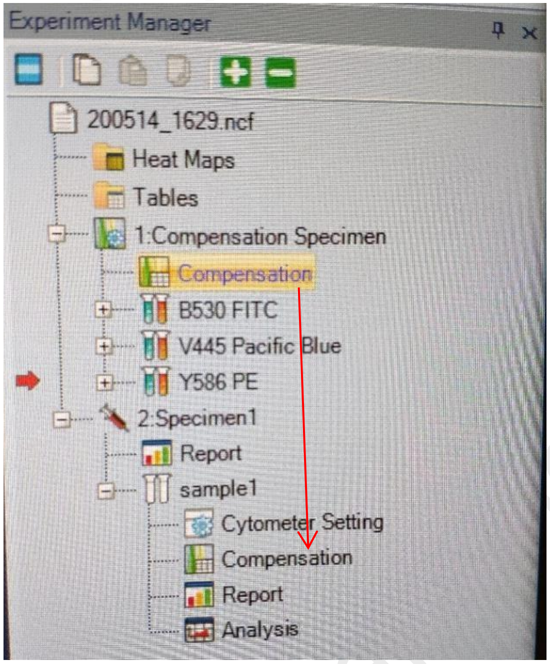
Step 4. Apply Compensation Matrix to Experiment Sample

a. Click the ***New Sample(s)*** button to create a new sample (sample1) of *Specimen*

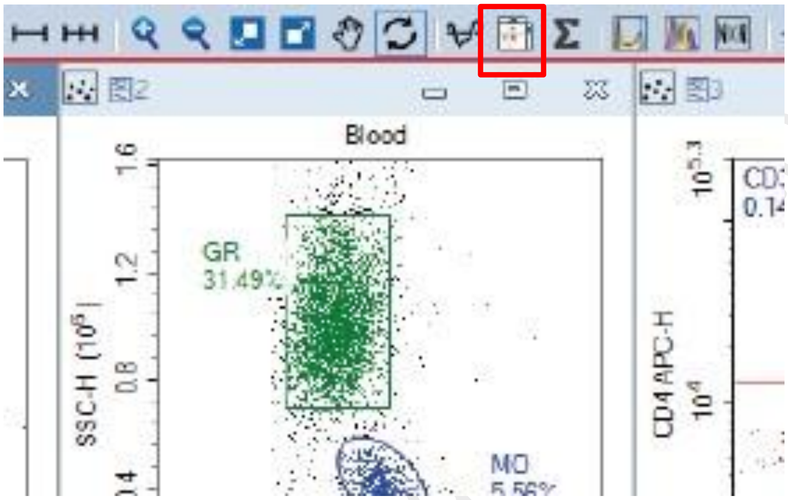
1.

b. **Drag** the *Compensation* node under the *Compensation Specimen* and **Drop over** the desired sample.

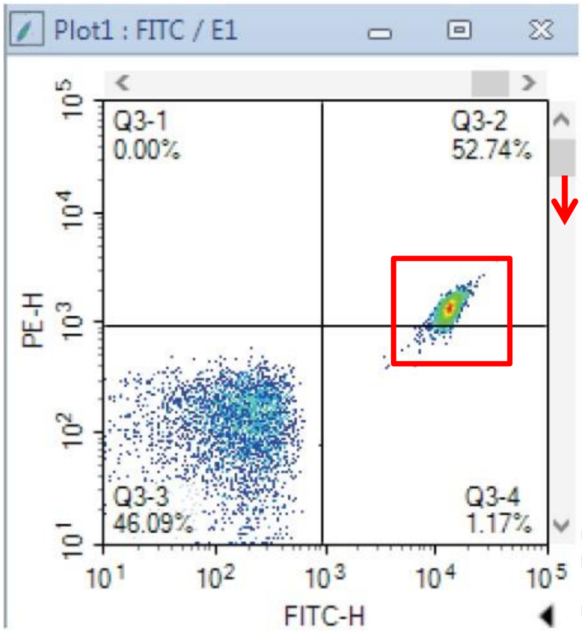
c. Repeat step *a* and *b* to create new sample of *Specimen 2* if needed.

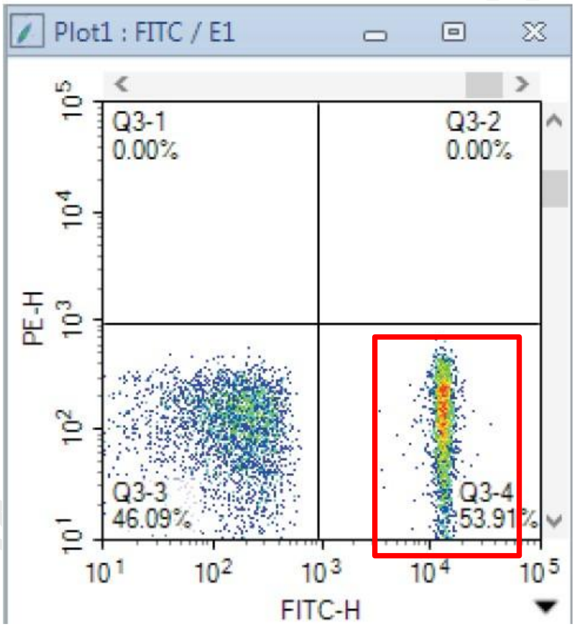


d. To fine tune the Compensation, click on the plot you want to adjust and click the ***Quick Compensation*** button In the ***Home*** tab of the <u>Menu Bar</u> **OR** the ***quick compensation icon*** in the tool bar.

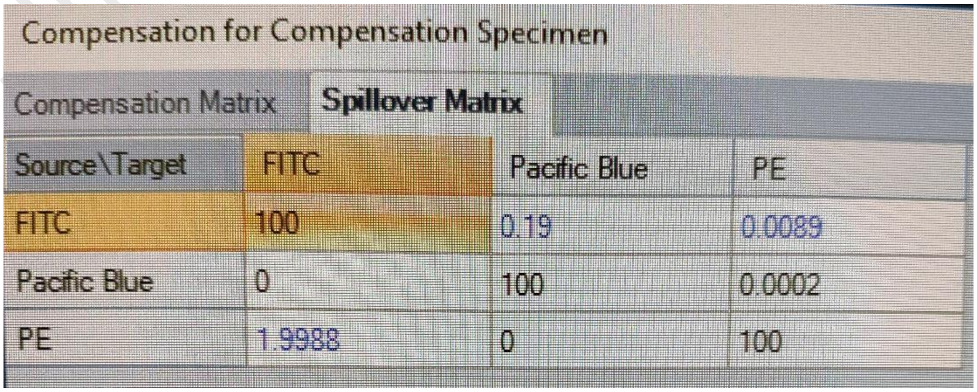


Scrollbars appear on any two parameters plots with fluorescent parameters opened on the workspace. Quickly adjust compensation by **dragging the scrollbar.**





e. To view or adjust the compensation matrix, click the ***Compensation Matrix*** button In the ***Home*** tab of the Menu Bar and the Compensation Matrix window will show.


Compensation for Compensation Specimen

Compensation Matrix | **Spillover Matrix**

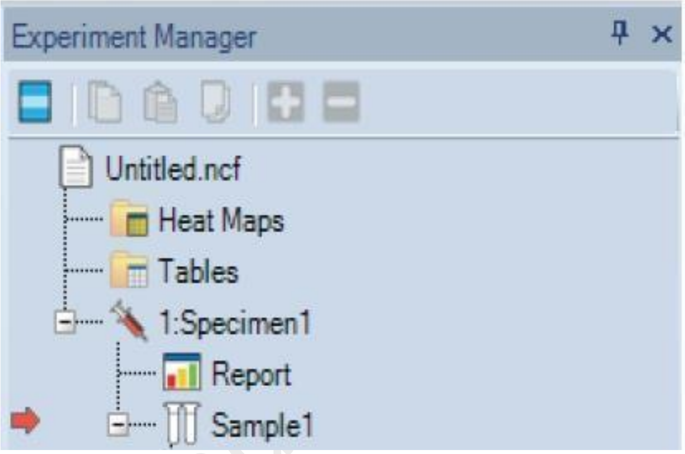
Source\Target	FITC	Pacific Blue	PE
FITC	100	0.19	0.0089
Pacific Blue	0	100	0.0002
PE	1.9988	0	100

3. Sample setting without auto-compensation:

Step 1. Set up new sample

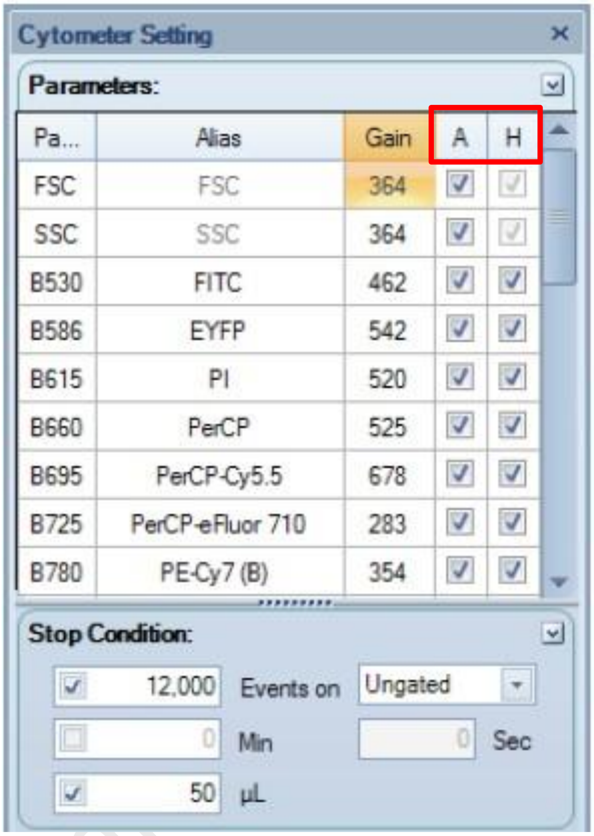
a. Click the ***New Sample(s)*** button to create a new sample (sample1) of *Specimen 1*.

b. Double click *Sample 1* on the Experiment Manager until the red arrow is pointing to Sample 1.



Step 2. Select Channels

a. Click on the "**A**" and "**H**" of the parameters panel in Cytometer setting to Select OR Unselect ALL.



Cytometer Setting

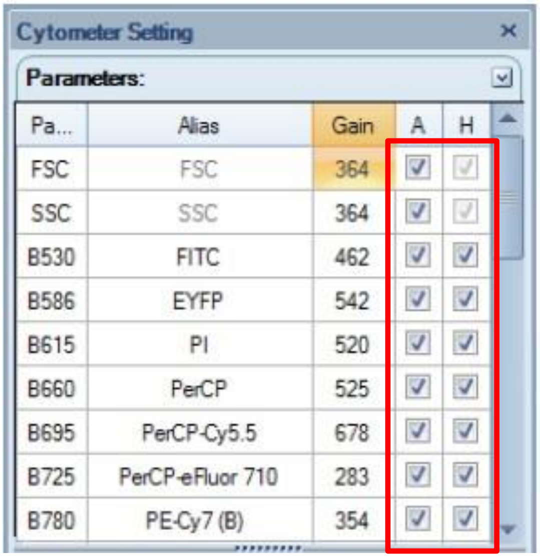
Parameters:

Pa...	Alias	Gain	A	H
FSC	FSC	364	☑	☑
SSC	SSC	364	☑	☑
B530	FITC	462	☑	☑
B586	EYFP	542	☑	☑
B615	PI	520	☑	☑
B660	PerCP	525	☑	☑
B695	PerCP-Cy5.5	678	☑	☑
B725	PerCP-eFluor 710	283	☑	☑
B780	PE-Cy7 (B)	354	☑	☑

Stop Condition:

☑ 12,000 Events on Ungated
☐ 0 Min 0 Sec
☑ 50 µL

b. ***Check the box of A or H*** of the interested channels to select. Please always check A for FSC (H is checked by default).



Cytometer Setting

Parameters:

Pa...	Alias	Gain	A	H
FSC	FSC	364	☑	☑
SSC	SSC	364	☑	☑
B530	FITC	462	☑	☑
B586	EYFP	542	☑	☑
B615	PI	520	☑	☑
B660	PerCP	525	☑	☑
B695	PerCP-Cy5.5	678	☑	☑
B725	PerCP-eFluor 710	283	☑	☑
B780	PE-Cy7 (B)	354	☑	☑

Step 3. Conditions Setup

a. Set up the data recording stop conditions by **checking the box next to the condition** *Events* and/or *Time* and/or *Volume*. Acquisition will stop when ANY one of the selected condition(s) is fulfilled. *Volume is compulsorily selected.

Stop Condition:

☑ 10,000 Events on Ungated

☐ 0 Min 0 Sec

☑ 10 µL

Events

Time

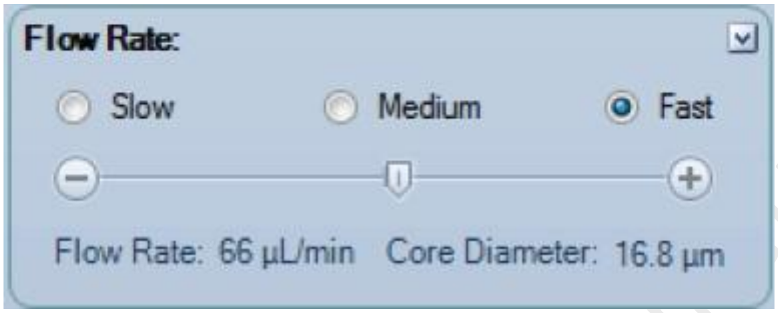
Volume

Range of each conditions:

Events	1 – 10,000,000
Time	0-60 min; 0-59 Sec
Volume	5 – 5000 µL

b. Select flow rate by click the radio button of *Slow* (14 µL/min), *Medium* (35 µL/min), and *Fast* (66 µL/min) OR use the slider to adjust the flow rate from 5~120 µL/min.

* Current sample's flow rate and the corresponding core diameter are shown in the bottom of the panel.

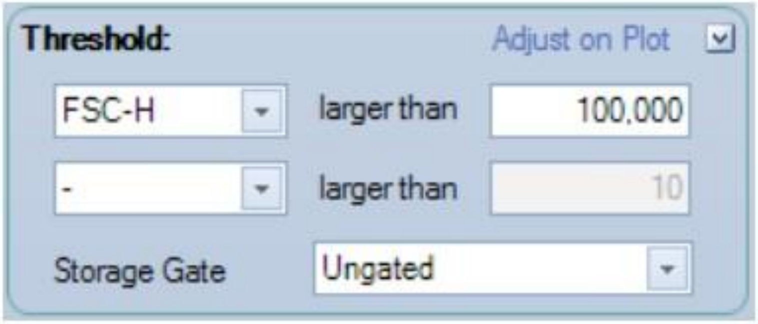


Flow Rate:

○ Slow ○ Medium ● Fast

Flow Rate: 66 µL/min Core Diameter: 16.8 µm

c. Set the appropriate threshold by select the appropriate parameters and type in the appropriate number on the *Threshold* panel.



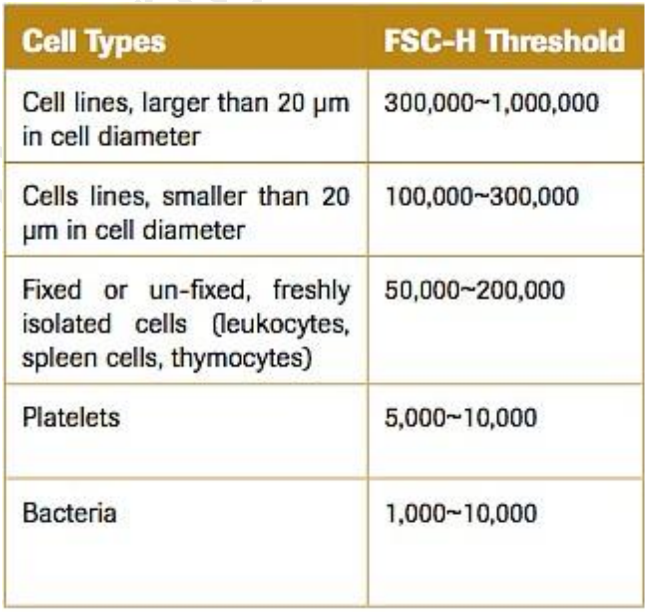
Threshold:

FSC-H larger than 100,000

\- larger than 10

Storage Gate Ungated

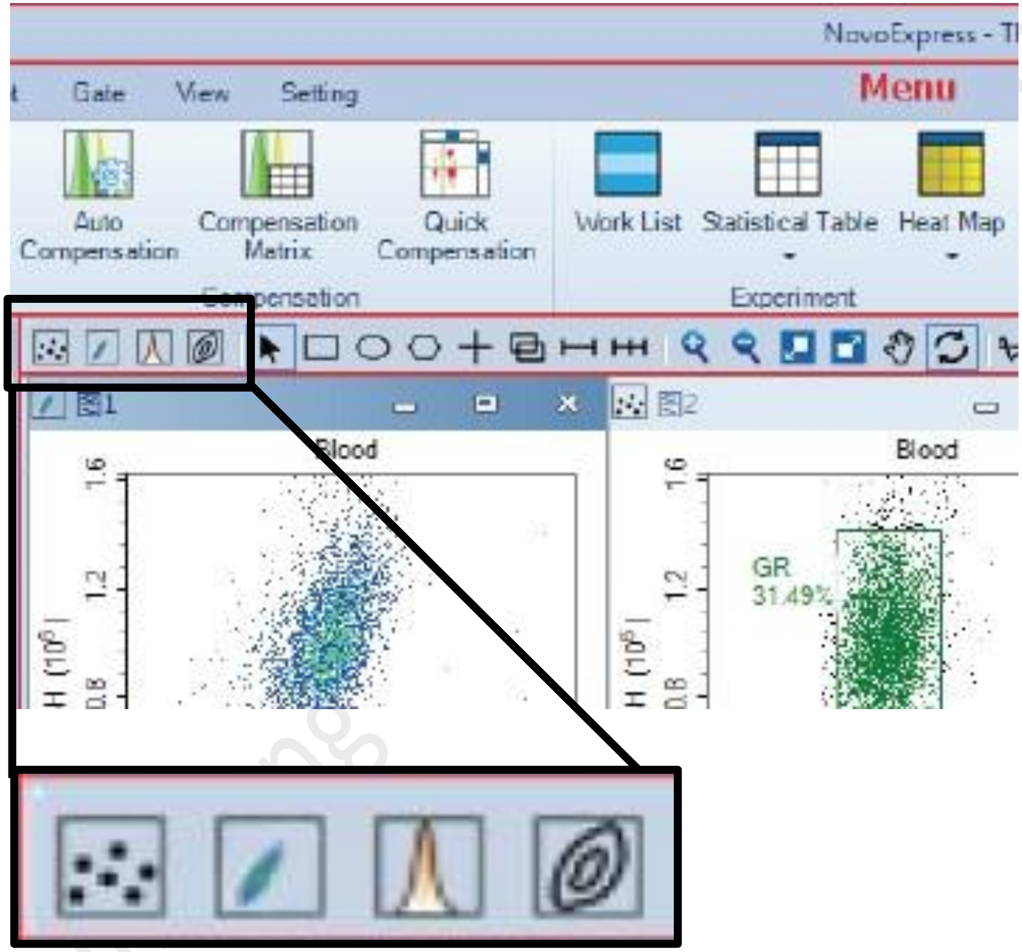
Suggested Threshold on Different cell type:



Cell Types	FSC-H Threshold
Cell lines, larger than 20 µm in cell diameter	300,000~1,000,000
Cells lines, smaller than 20 µm in cell diameter	100,000~300,000
Fixed or un-fixed, freshly isolated cells (leukocytes, spleen cells, thymocytes)	50,000~200,000
Platelets	5,000~10,000
Bacteria	1,000~10,000

Step 4. Draw Plots

a. Click the icon of the interested **plot type** above the workspace



Dot Density Histogram Contour

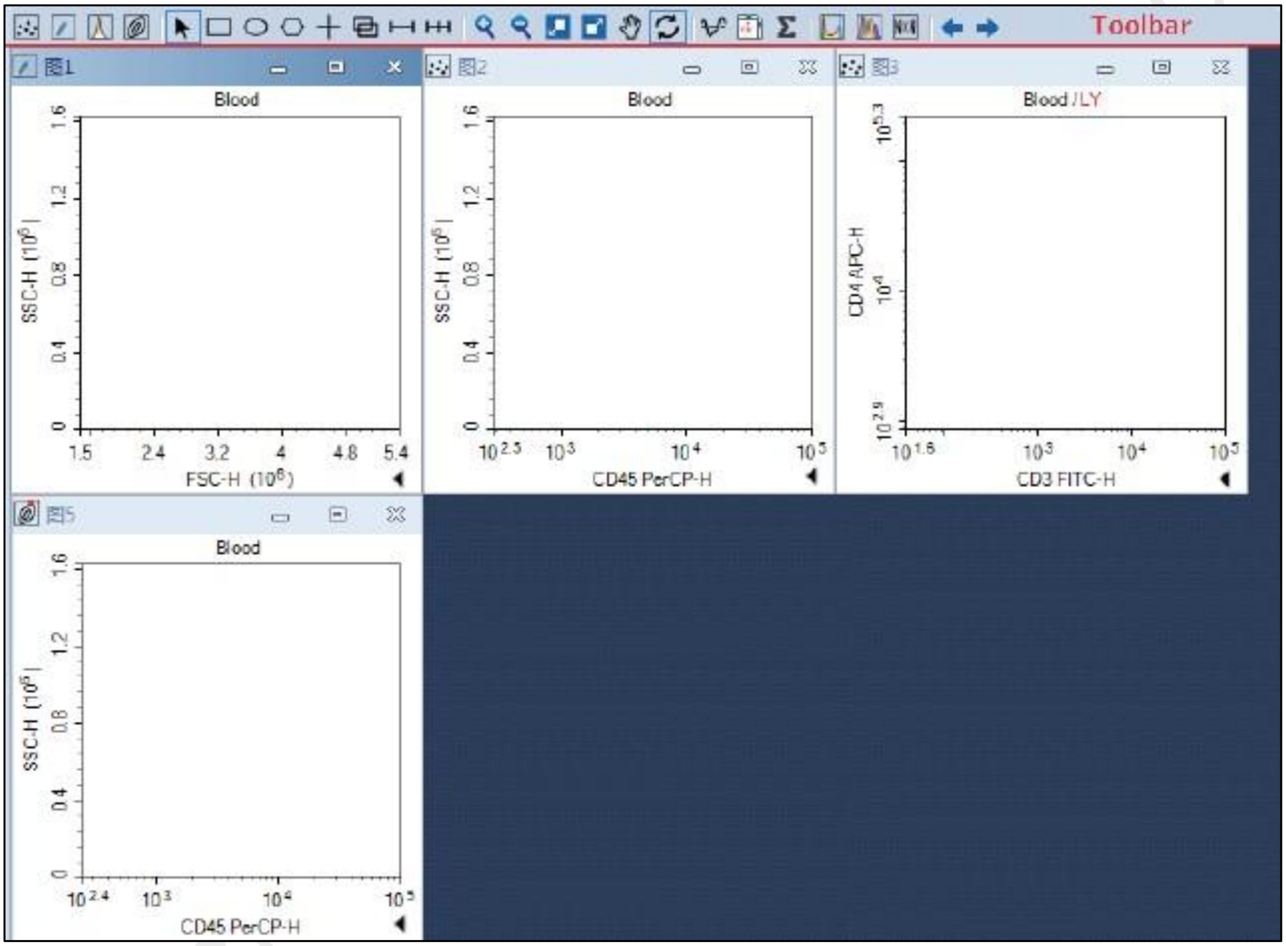
Plot type	**Number of parameters**	**Description**
Dot plot	2	The intensities of two parameters are represented by the coordinates of an event (one dot) on the plot.
Density plot	2	The intensities of two parameters are represented by the coordinates of an event (one dot) on the plot with colour-coded density display.

Contour plot	2	The intensities of two parameters are represented by the coordinates on the plot with contour line to show density.
Histogram plot	1 (x axis only)	The intensity of a parameter is represented along the x-axis, and the number of events at each intensity value is represented along the y-axis.

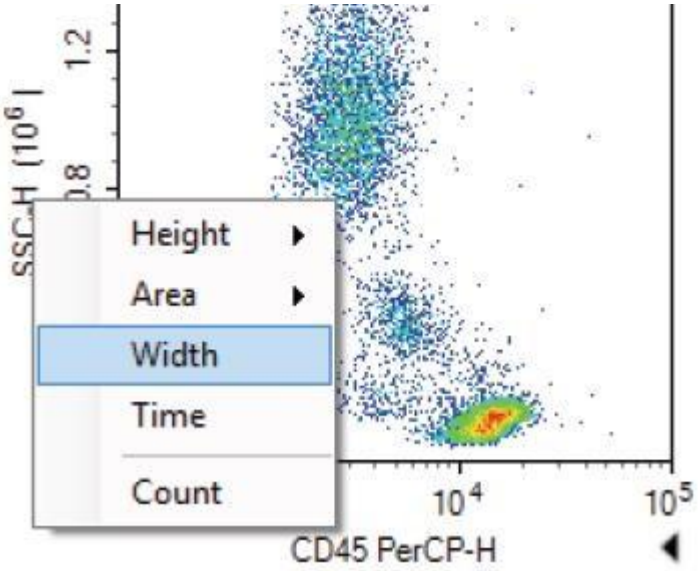
b. Create the following plots with the following sequence.

FSC-H VS SSC-H (Mother population of interest) >
FSC-H VS FSC-A (Single Cell Gate) >
Live-Dead VS SSC-A (if applicable) >

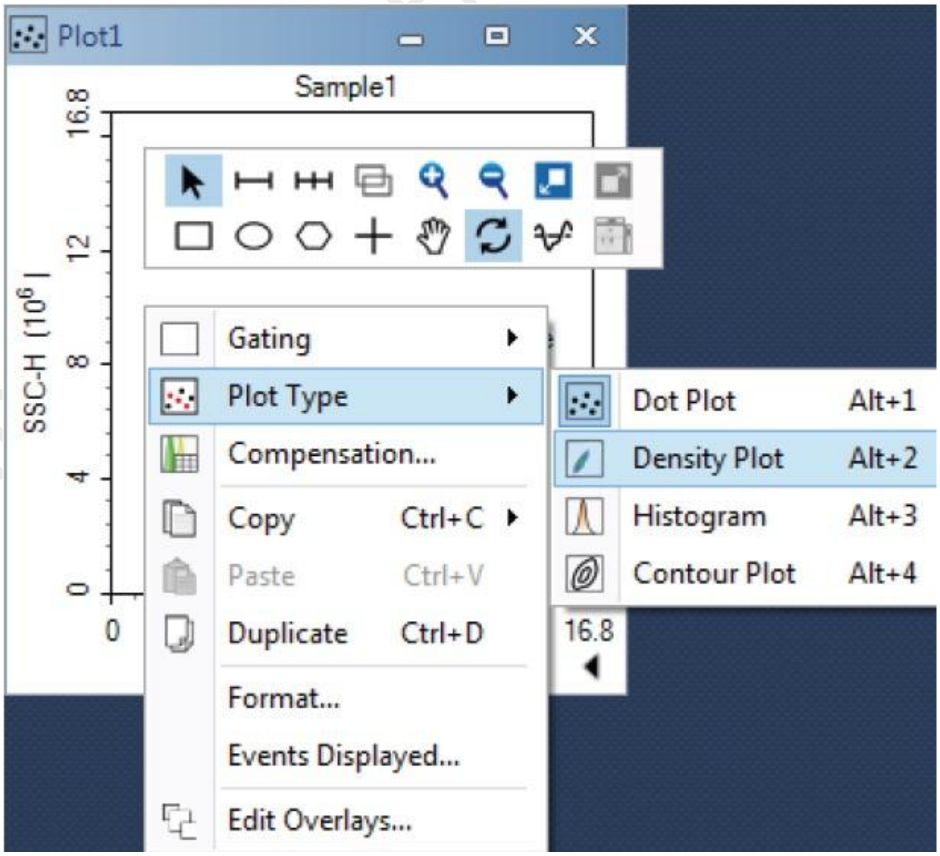
Fluorescence Plots (if applicable)



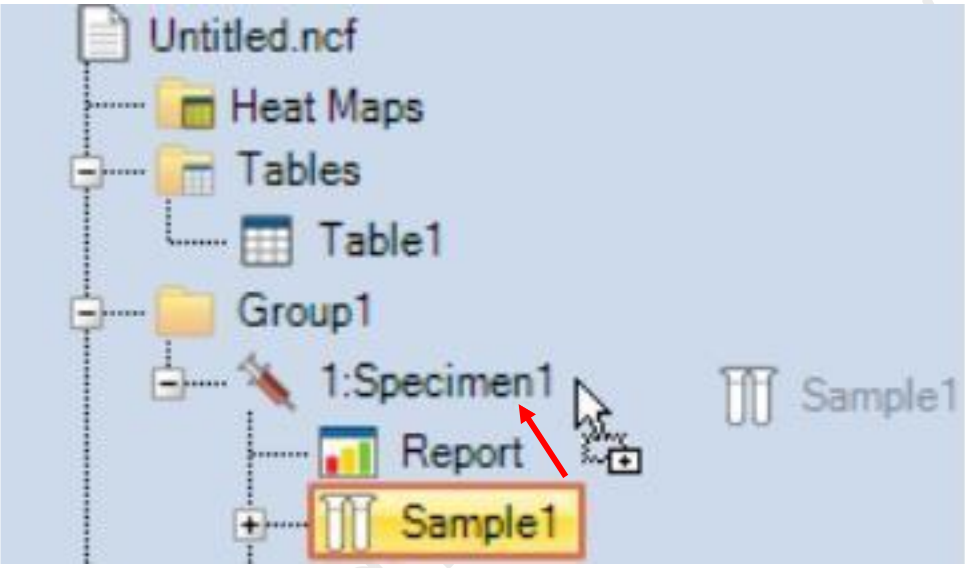
c. To change the parameters of a plot, **mouse over the axis label** and **right-click** to open the drop-down menu of parameters list. Select the parameter of interest.



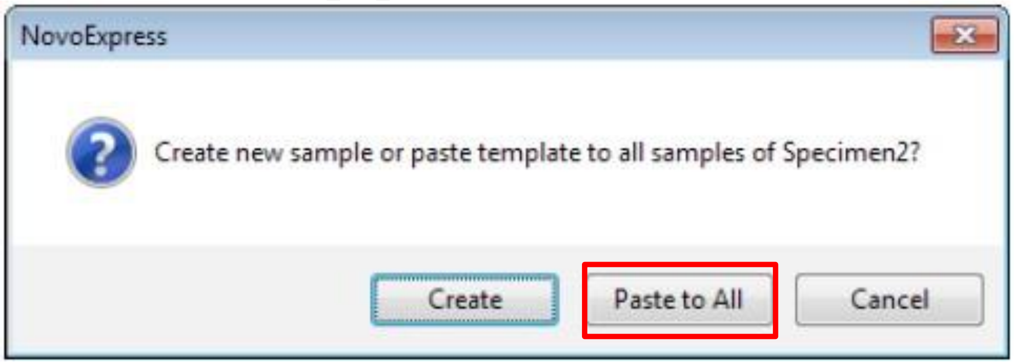
d. **Right-click** within a plot to change the plot type if needed.



e. To copy all the settings and plots to other samples in Specimen 1, **drag *Sample 1* and drop over *Specimen 1*** on the Experiment Manager.



f. Click *Paste to All*.

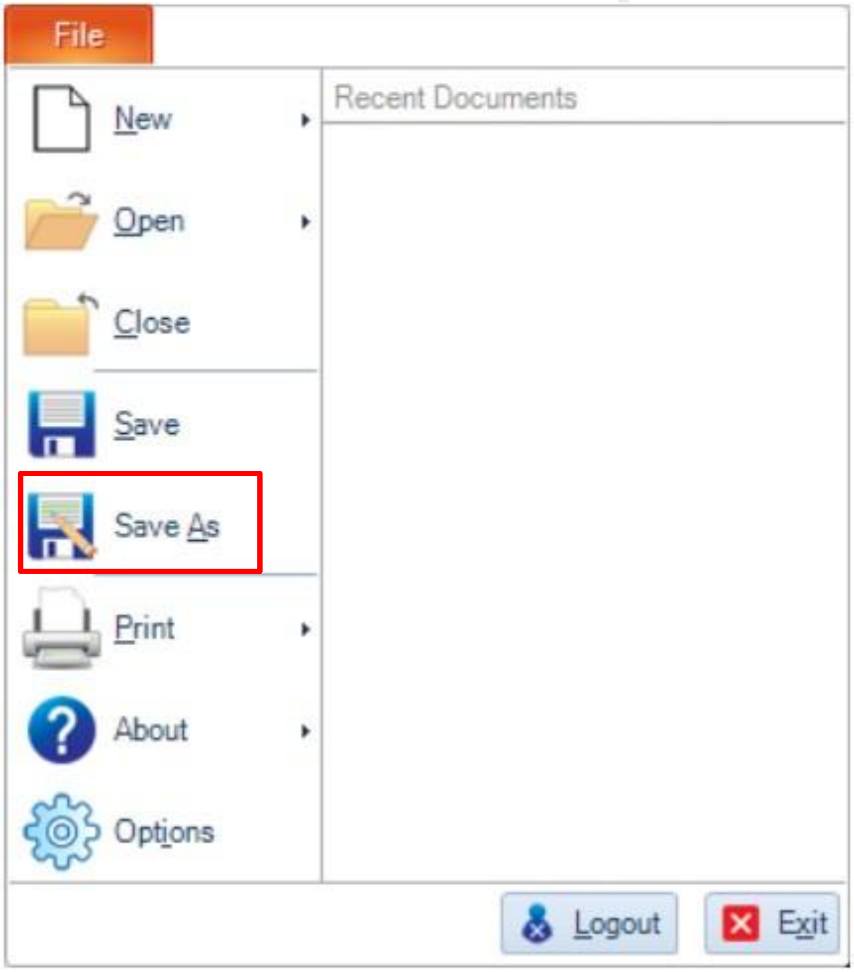


Create new sample or paste template to all samples of Specimen2?

Step 5. Save Experiment

a. Click ***File*** on the Menu bar.

b. Click *Save As*.



c. Save the experiment (.ncf) in the folder below.

Computer> Experiment Data (D:)> Users> Department> YOUR FOLDER

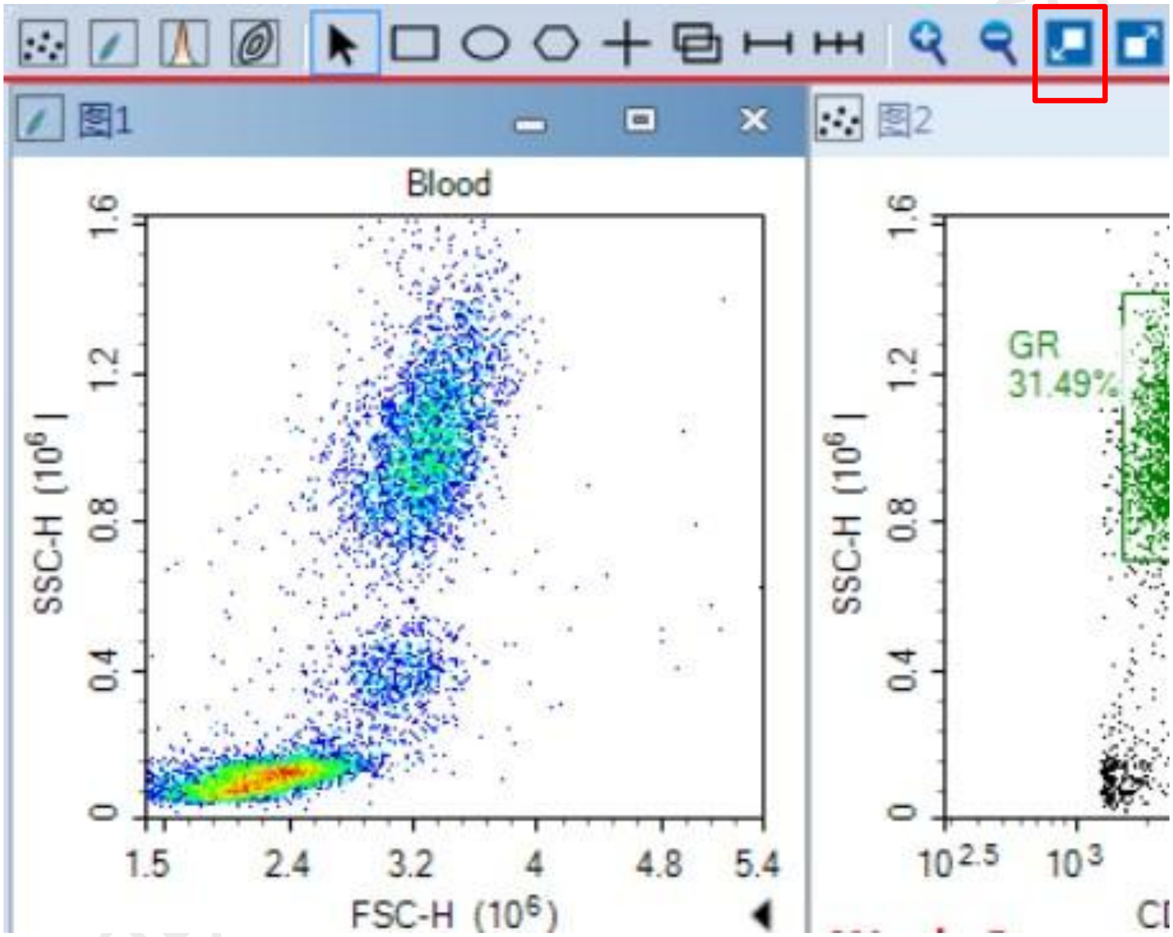
*Default location: Computer> D: > Novoexpress Data. Please do NOT save as default

Click *Save*.

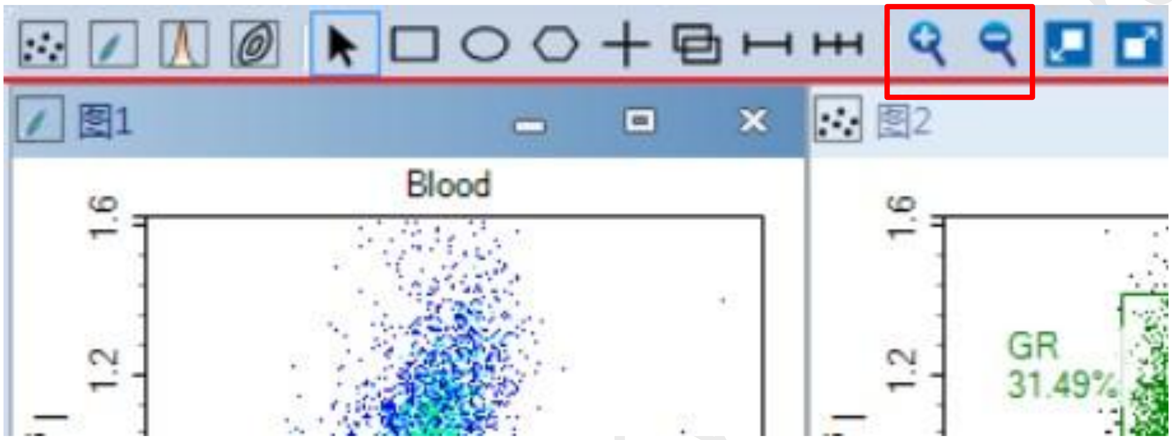
4. Data Analysis during acquisition

Step 1. Set the appropriate display range of the plot.

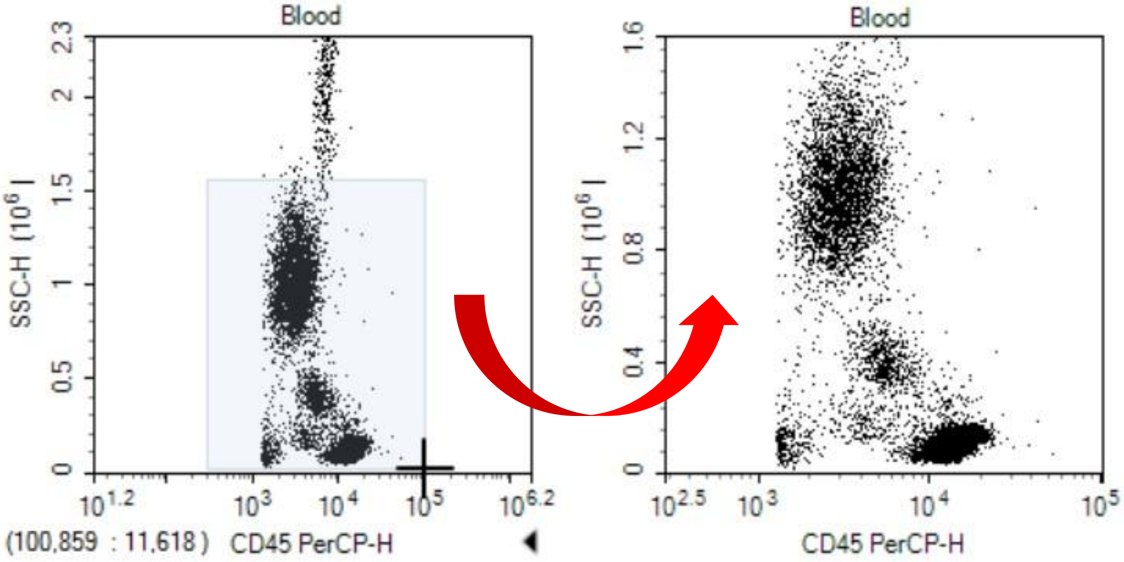
a. Select the FSC-H Vs SSC-H plot (The colour of the header of the plot will be darker). Click ***Auto range* button** to optimize the data display range.



b. To fine tune the data display range, **click *zoom in / zoom out* buttons**.

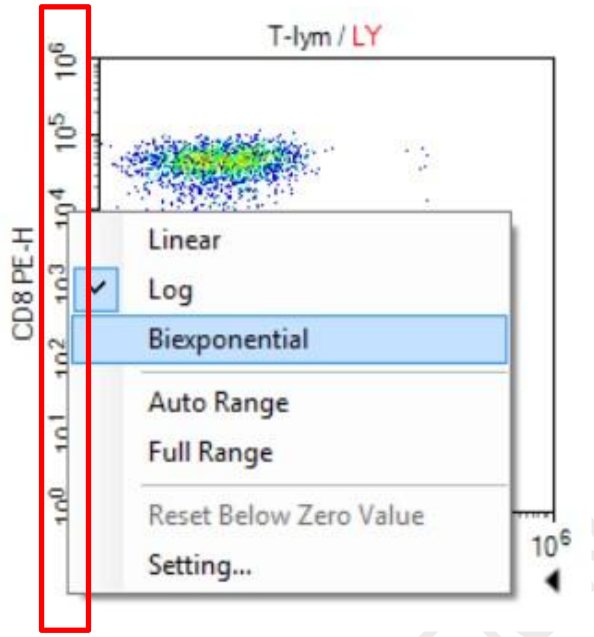


c. **Drag** on the interested region on the plot if you click ***zoom in*.**



d. **Click within a plot if you click *zoom out*.** The range increases by 20% of the current range. Click repeatedly until the desired range is reached.

e. To change the scale of parameters, ***right click* on the coordinate label** to open and select the axis scaling (i.e. Linear, Log or Biexponential). Click ***Setting*** for more options.



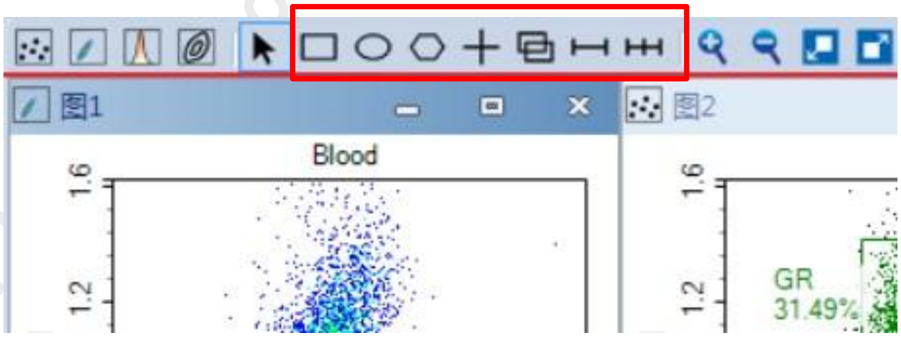
f. If you cannot achieve a desirable range by using the plot range tools, **adjust the *Gain*** of the corresponding channels in Cytometer Control - Parameters.

To adjust photodetector gain of one parameter, ***double click the current Gain number*** of the specified parameter, the photodetector gain adjustment slider will show. ***Drag the slider bar or directly enter the value*** to change the photodetector gain.
***Gain can only be adjusted during acquisition.**

Pa...	Alias	Gain	A	H
FSC	FSC	364		☑
SSC	SSC	364		☑
B530	FITC	462		☑
B586	EYFP	643		☑
B615	PI	520		☑
B660	PerCP	525		☑

Step 2. Gating

a. Draw Gates to gate out the target population on the FSC-H VS SSC-H plot with gating tools.

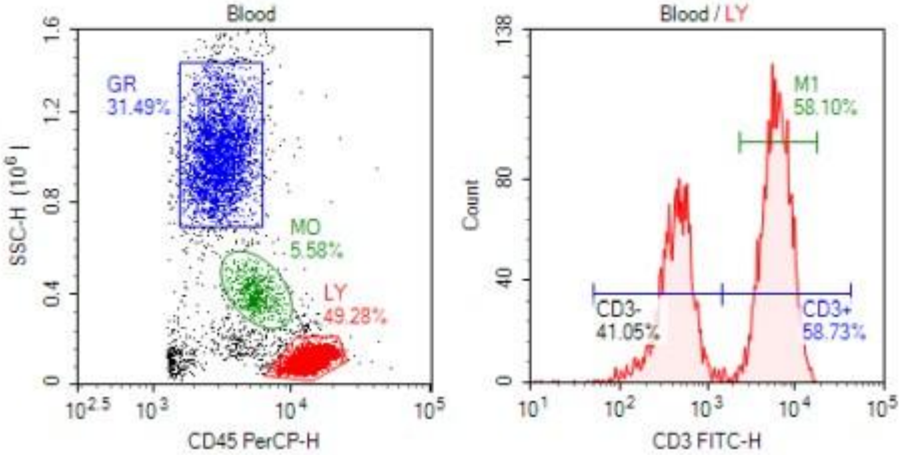


rectangular gates □, elliptical gates ○, polygonal gates ⬡, quadrant gates +, logic gates ⧉, range gates ├─┤, and bi-range gates ├┼┤

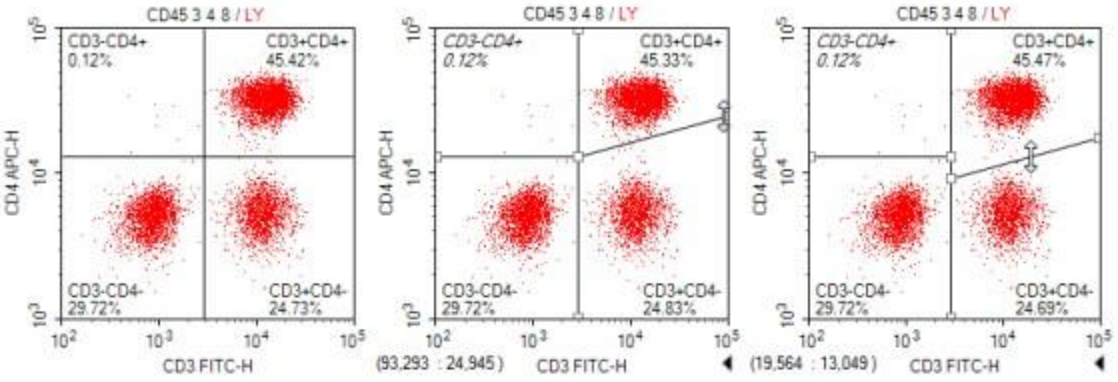
Dot Plot, Density Plot, Contour Plot – All gates suitable
Histogram Plot – Range / Bi-range gate suitable

b. To create *rectangular/ elliptical/ range/ bi-range gate*, click the corresponding icon and drag in the plot to enclose the target population within the shape. Release the mouse button to create the gate.

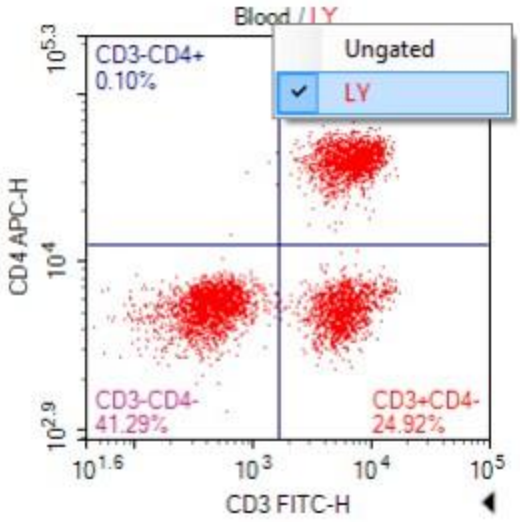
To create *polygonal gate*, click the corresponding icon and left click in the plot to create the first vertex of the polygon. Click in a new location to create the second vertex of the polygon. Continue moving around the target population and creating vertices until the target population is enclosed. On the last vertex, double-click to complete the polygon and create the gate.



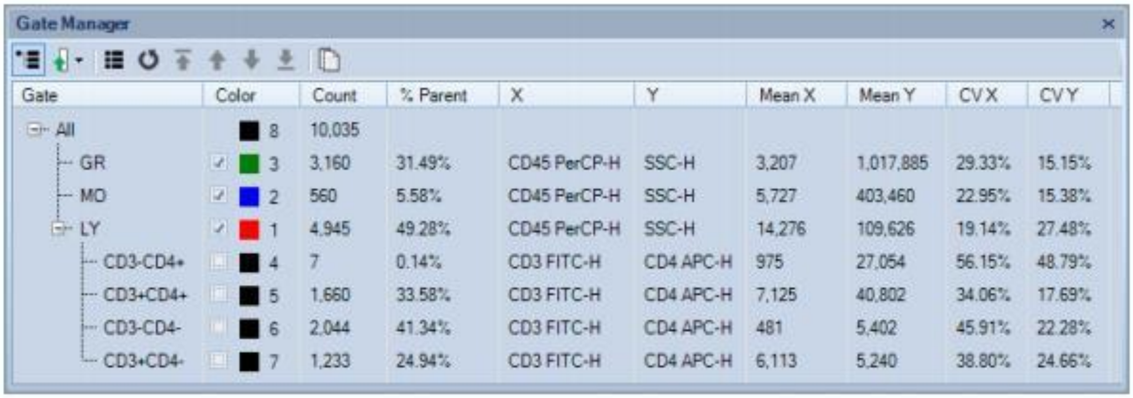
To create *quadrant gate*, click the corresponding icon and Click in the plot to create the center of the quadrants and create the gate. As shown below, the center, endpoints, and lines of the quadrant gate can be moved to enclose the correct populations.



c. To create gate subpopulation, ***right-click at the plot header*** of a plot to display a drop-down menu. Select the mother gate and create a new gate for your target.



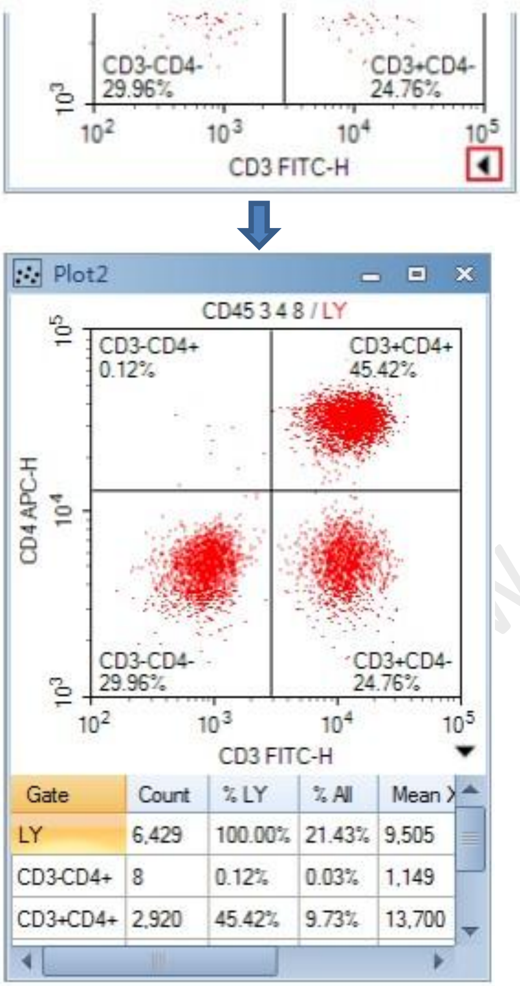
d. The <u>Gate Manager</u> panel displays all gates of the active sample in list mode or tree mode. It provides user interface to modify gate name, color and color precedence and also shows gate hierarchy and gate statistics.



Gate	Color	Count	% Parent	X	Y	Mean X	Mean Y	CV X	CV Y
All	⬛ 8	10,035							
GR	☑ 🟩 3	3,160	31.49%	CD45 PerCP-H	SSC-H	3,207	1,017,885	29.33%	15.15%
MO	☑ 🟦 2	560	5.58%	CD45 PerCP-H	SSC-H	5,727	403,460	22.95%	15.38%
LY	☑ 🟥 1	4,945	49.28%	CD45 PerCP-H	SSC-H	14,276	109,626	19.14%	27.48%
CD3-CD4+	☐ ⬛ 4	7	0.14%	CD3 FITC-H	CD4 APC-H	975	27,054	56.15%	48.79%
CD3+CD4+	☐ ⬛ 5	1,660	33.58%	CD3 FITC-H	CD4 APC-H	7,125	40,802	34.06%	17.69%
CD3-CD4-	☐ ⬛ 6	2,044	41.34%	CD3 FITC-H	CD4 APC-H	481	5,402	45.91%	22.28%
CD3+CD4-	☐ ⬛ 7	1,233	24.94%	CD3 FITC-H	CD4 APC-H	6,113	5,240	38.80%	24.66%

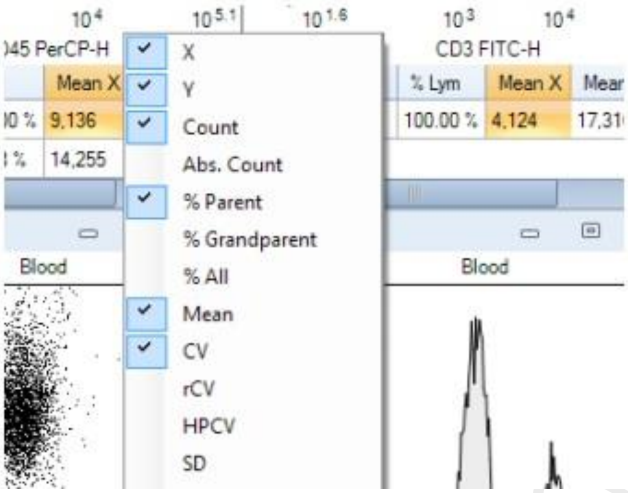
Step 3. Statistics

a. To edit statistics, click the button on the lower right corner of a plot to expand the plot and display the statistics chart first.



Gate	Count	% LY	% All	Mean
LY	6,429	100.00%	21.43%	9,505
CD3-CD4+	8	0.12%	0.03%	1,149
CD3+CD4+	2,920	45.42%	9.73%	13,700

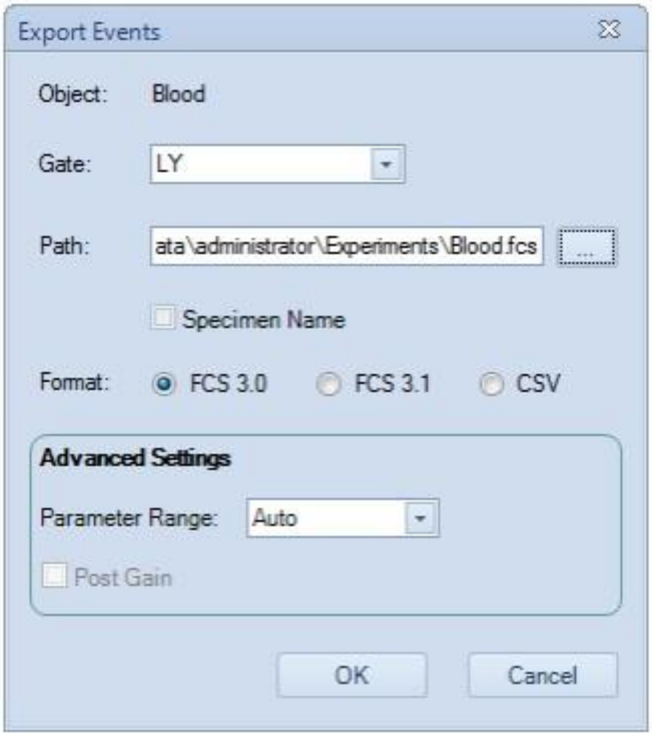
b. ***Right-click*** **within the chart** and select the parameters to hide or display.



5. Data Export

Step 1. Export FCS file

a. Select the sample, specimen, group, or experiment file node with data to be exported in the Experiment Manager Panel. ***Right-click*** the node and select ***Export →Export to FCS Files***⋯The Export Events window will open.



Export Events

Object: Blood

Gate: LY

Path: ata\administrator\Experiments\Blood.fcs

☐ Specimen Name

Format: ● FCS 3.0 ○ FCS 3.1 ○ CSV

Advanced Settings

Parameter Range: Auto

☐ Post Gain

b. Choose "All" for the *Gate* option. Click "…" button next to entry box of *Path.* Select your saving destination in Experiment Data Drive (D:/)

Experiment-Data (D:)> user > Department> Your NAME

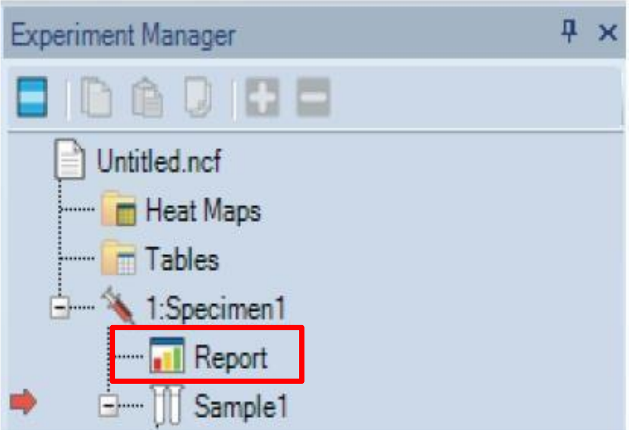
Select "*FSC3.0*" for Format and Click *OK.*

- Data files (including the experiment files) can be stored in D drive for 3 months ONLY. While experiment template files may be stored for a longer duration.

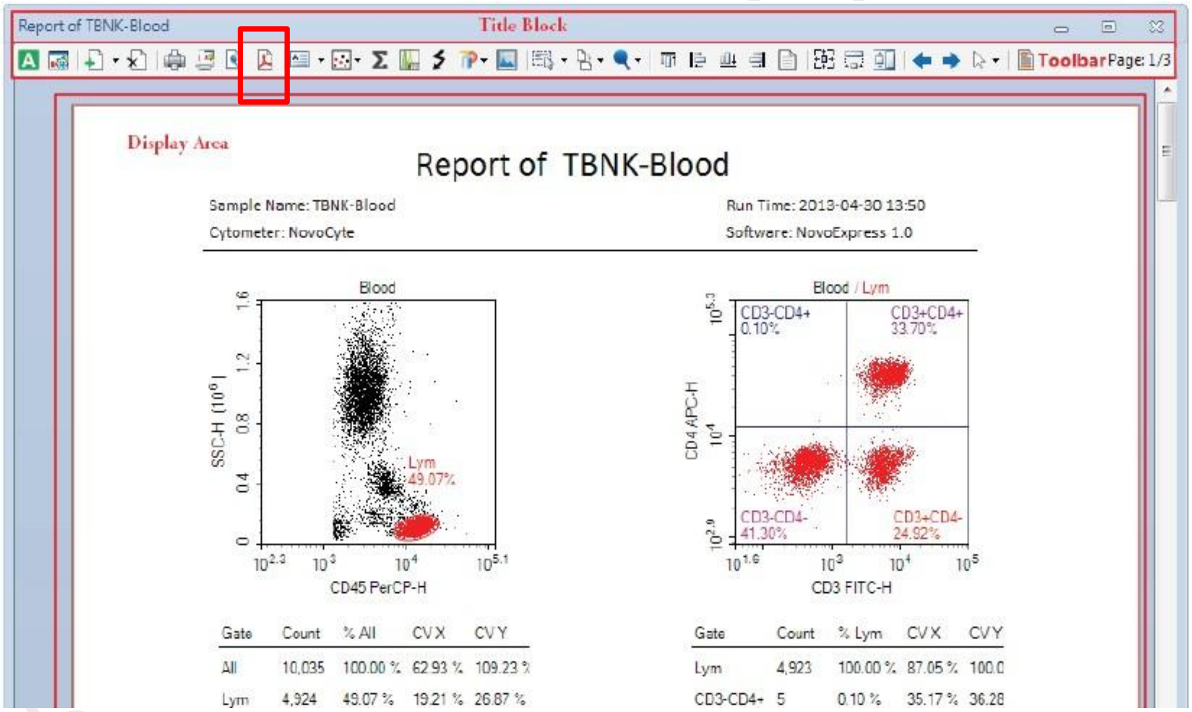
- Data on the computer (D drive) will be removed regularly without prior notice.

Step 2. Export PDF file (optional)

a. To Export Plots and Statistics to a PDF, **double-click the *Report* node** in the Experiment Manager panel and Report Window will popup.



b. Click the *PDF* button of the tool bar.



Report of TBNK-Blood

Sample Name: TBNK-Blood
Cytometer: NovoCyte
Run Time: 2013-04-30 13:50
Software: NovoExpress 1.0

Gate	Count	% All	CV X	CV Y
All	10,035	100.00 %	62.93 %	109.23 %
Lym	4,924	49.07 %	19.21 %	26.87 %

Gate	Count	% Lym	CV X	CV Y
Lym	4,923	100.00 %	87.05 %	100.0
CD3-CD4+	5	0.10 %	35.17 %	36.28

Select your saving destination in Experiment Data Drive (D:/) D:/user/Your department/Your NAME

c. Click *Save* buttons on the top left-hand corner of the window when you finish your experiment.

Step 3. Export experiment template file (optional)

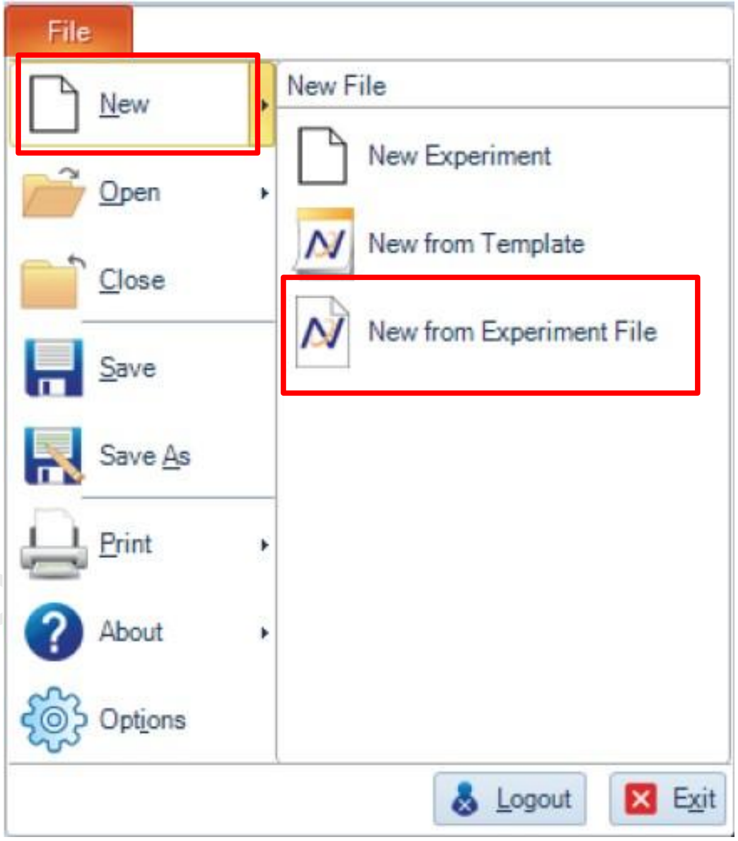
a. To export experiment template, select the experiment file node to be exported in the Experiment Manager Panel. ***Right-click*** the node and select ***Export →Export as Experiment Template.***

6. System Cleaning

a. Place tubes of at least 1mL of cleaning solution 1, 2 and 3 (FACS Clean, FACS Rinse, Milli-Q H_2O).

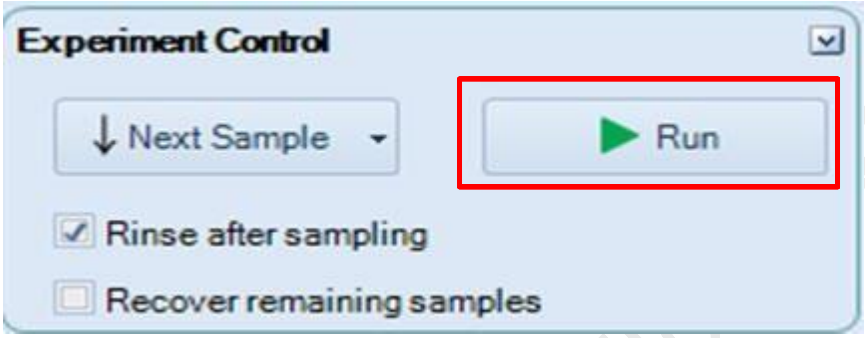
b. Click *File* on the Menu bar.

c. Click *New > New from Experiment File*.



d. If you haven’t stained with PI, then Select *Desktop* > ***clean.ncf*** . Then click *OK*
e. If you have stained with PI, then Select *Desktop* > ***clean-PI .ncf*** . Then click *OK*

f. Double click the sample 1, then Click “Run”



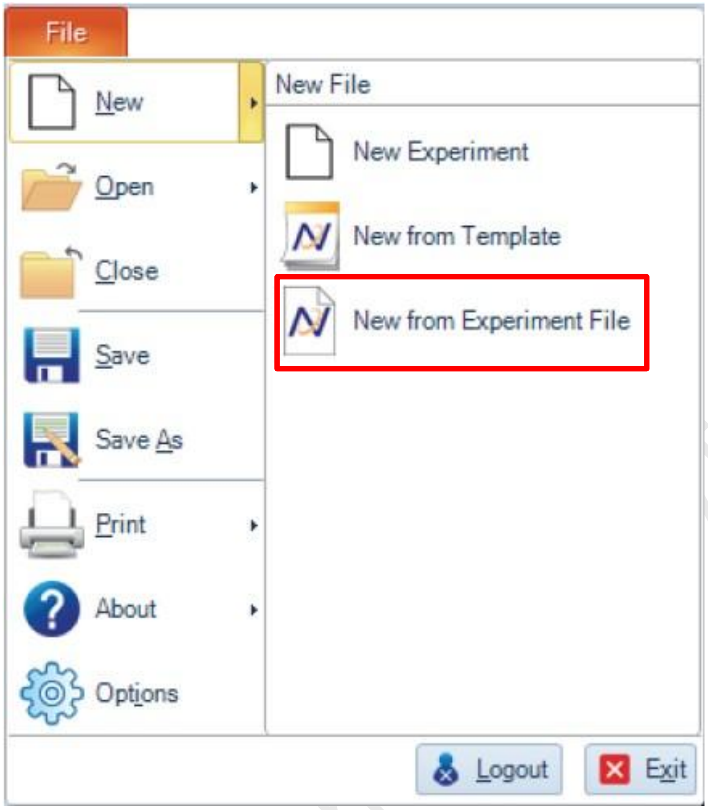
☑ Rinse after sampling
☐ Recover remaining samples

g. After running sample 1, double click the sample 2, then click “Run”
h. After running sample 2, double click the sample 3, then click “Run”

7. Re-use Experiment as template

a. Click *File* on the Menu bar.

b. Click *New* > *New from Experiment File*.



c. Select your target experiement file (.ncf) . Then click *OK*

d. Click *File*> *Save As* to save the new experiment.

<u>8. NovoExpress Software Log out</u>

a. Click *File* on the <u>Menu</u> bar.

b. Click *Logout*