



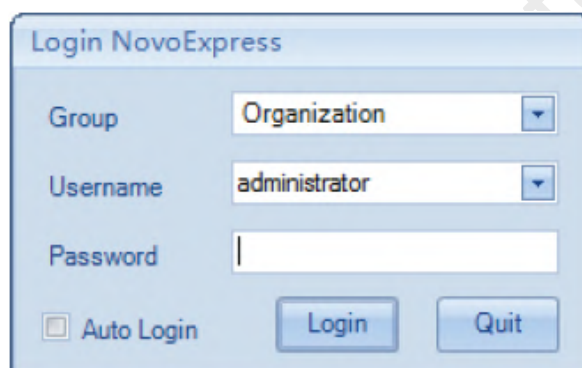
## Imaging and Flow Cytometry Core

### NovoCyte Quanteon 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.*



\*Username *Ocleaning* do not have password. Leave password entry box empty and click *Login*.

Ocleaning account is for cleaning purpose only, **DO NOT USE THIS ACCOUNT TO PERFORM EXPERIMENT.**

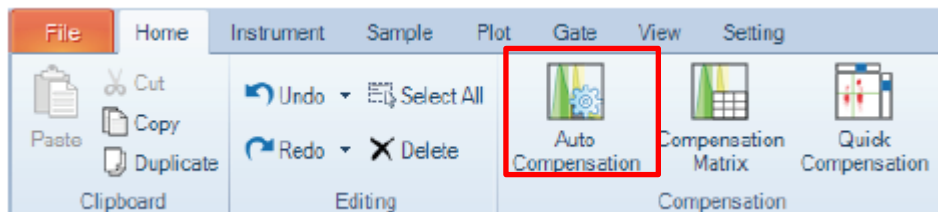
## Imaging and Flow Cytometry Core

### 2. Compensation (Perform when needed)

Step 1. Select appropriate **plate type** in the *Plate Manager*.

Step 2. 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**



## Imaging and Flow Cytometry Core

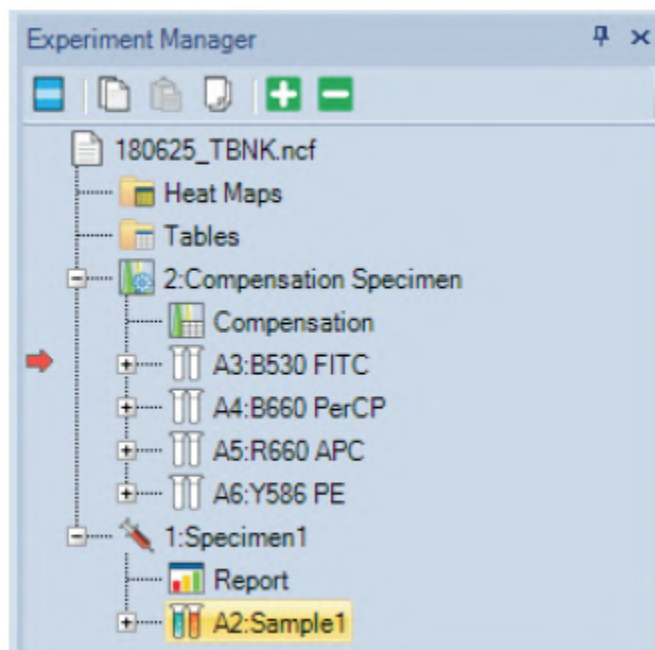
The image shows a software window titled "New Auto Compensation". It contains several sections for configuring flow cytometry compensation. At the top, there is a checkbox "Import Samples from FCS files". Below this, there are two sections: "Compensation on:" with radio buttons for "Area" and "Height" (selected), and "Parameter for calculation:" with radio buttons for "Mean" and "Median" (selected). The main section is "Compensation Channels:", which lists various channels with checkboxes and dropdown menus for selecting the compensation parameter. The channels are organized in three columns. The first column includes "Unstained", "FSC", "SSC", "B530 FITC", "B586 EYFP", "B615 PI", "B660 PerCP", "B695 PerCP-Cy5.5", "B725 PerCP-eFluor 7", and "B780 PE-Cy7 (B)". The second column includes "All", "R660 APC", "R695 Alexa Fluor 680", "R725 Alexa Fluor 700", "R780 APC-Cy7", "V445 Pacific Blue", "V530 AmCyan", "V586 Pacific Orange", "V615 Qdot 605", and "V660 Qdot 655". The third column includes "V695 Qdot 705", "V725 BV711", "V780 Qdot 800", "Y586 PE", "Y615 PE-Texas Red", "Y660 PE-Cy5", "Y695 PE-Cy5.5", "Y725 PE-Alexa Fluor", and "Y780 PE-Cy7 (Y)". Each channel has a checkbox and a dropdown menu. At the bottom right, there are "OK" and "Cancel" buttons.

Channel	Parameter
<input checked="" type="checkbox"/> Unstained	All
<input checked="" type="checkbox"/> FSC	FSC
<input checked="" type="checkbox"/> SSC	SSC
<input checked="" type="checkbox"/> B530	FITC
<input checked="" type="checkbox"/> B586	EYFP
<input checked="" type="checkbox"/> B615	PI
<input checked="" type="checkbox"/> B660	PerCP
<input checked="" type="checkbox"/> B695	PerCP-Cy5.5
<input checked="" type="checkbox"/> B725	PerCP-eFluor 7
<input checked="" type="checkbox"/> B780	PE-Cy7 (B)
<input checked="" type="checkbox"/> R660	APC
<input checked="" type="checkbox"/> R695	Alexa Fluor 680
<input checked="" type="checkbox"/> R725	Alexa Fluor 700
<input checked="" type="checkbox"/> R780	APC-Cy7
<input checked="" type="checkbox"/> V445	Pacific Blue
<input checked="" type="checkbox"/> V530	AmCyan
<input checked="" type="checkbox"/> V586	Pacific Orange
<input checked="" type="checkbox"/> V615	Qdot 605
<input checked="" type="checkbox"/> V660	Qdot 655
<input checked="" type="checkbox"/> V695	Qdot 705
<input checked="" type="checkbox"/> V725	BV711
<input checked="" type="checkbox"/> V780	Qdot 800
<input checked="" type="checkbox"/> Y586	PE
<input checked="" type="checkbox"/> Y615	PE-Texas Red
<input checked="" type="checkbox"/> Y660	PE-Cy5
<input checked="" type="checkbox"/> Y695	PE-Cy5.5
<input checked="" type="checkbox"/> Y725	PE-Alexa Fluor
<input checked="" type="checkbox"/> Y780	PE-Cy7 (Y)

- c. Compensation Control Specimen is created in the Experiment Manager panel with corresponding empty control samples of specific position of the tube rack or plates (e.g. A3: B530 FITC, A3 is the position of the rack or plate and refers to FITC single stain controls).

The compensation controls tubes should be placed in the rack or plate according to the positions given (i.e. Put FITC single stain tubes in A3 position).

## Imaging and Flow Cytometry Core

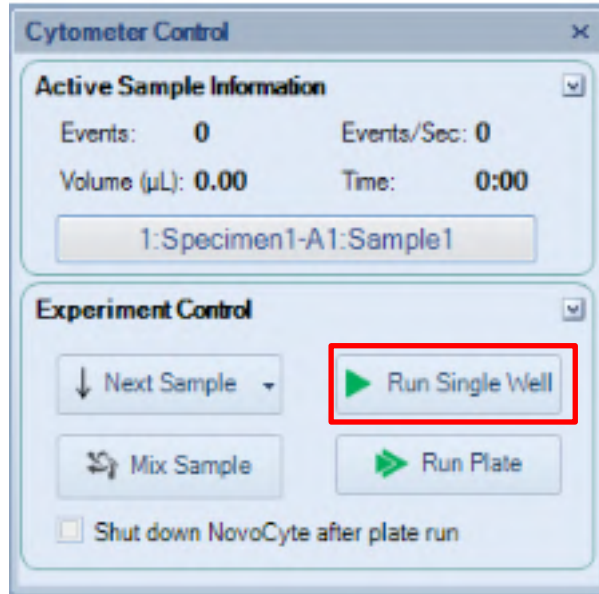


You may change the position by right-click of the sample name and *Rename* the control (e.g. A3: B530 FITC can be renamed to B3: B530 FITC, FITC single stain tube position now changes from A3 to B3).

## Imaging and Flow Cytometry Core

### Step 3. Compensation Control Acquisition

- Click **Run Plate** on the Cytometer Control Panel.



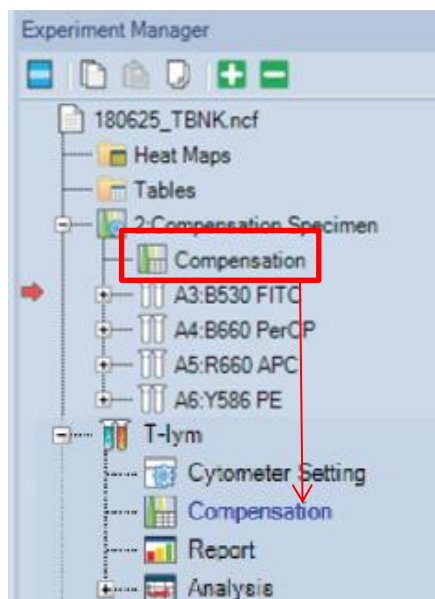
- Select wells of compensation controls. Click **Run** and then **OK** to proceed.
- After all controls have been acquired, the compensation matrix is calculated automatically.

### Step 4. Apply Compensation Matrix to Experiment Sample

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

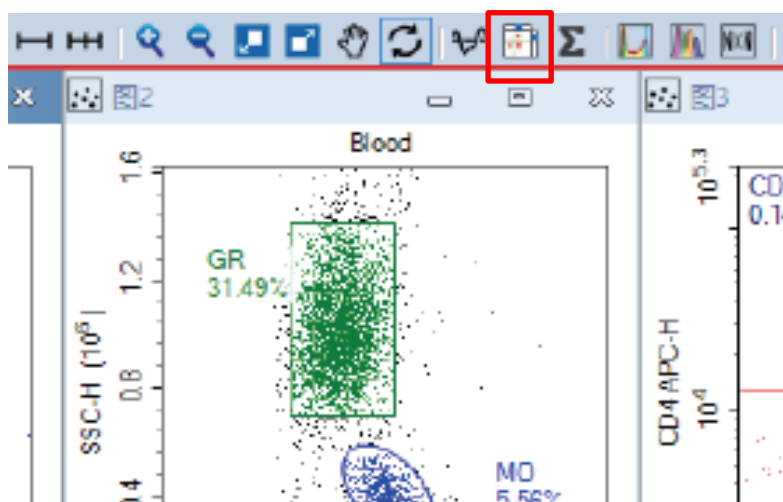
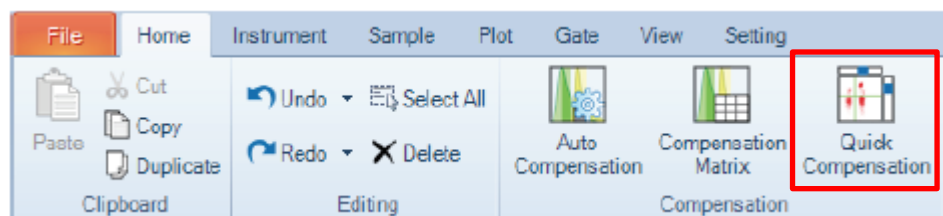


## Imaging and Flow Cytometry Core



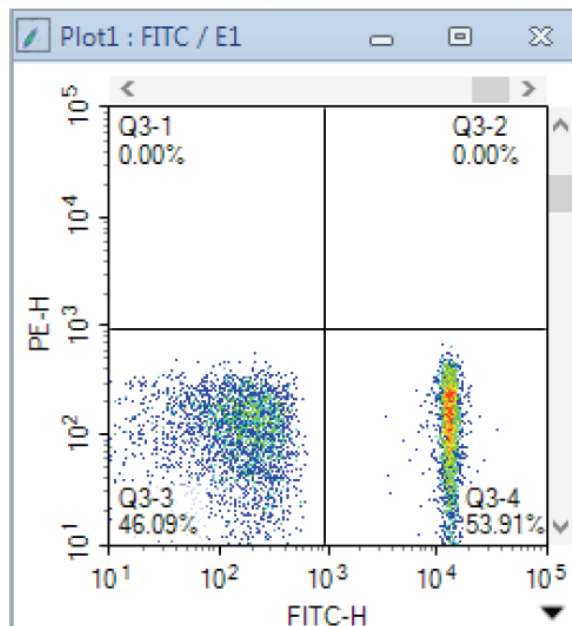
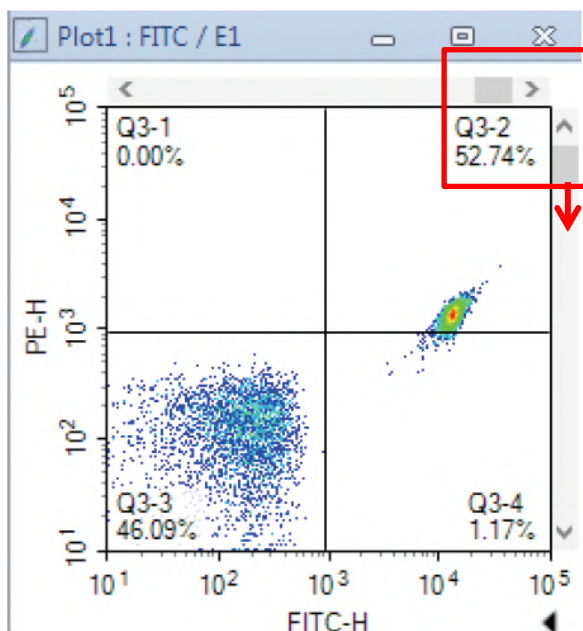
## Imaging and Flow Cytometry Core

- b. 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 Menu Bar OR the **quick compensation icon** in the tool bar.

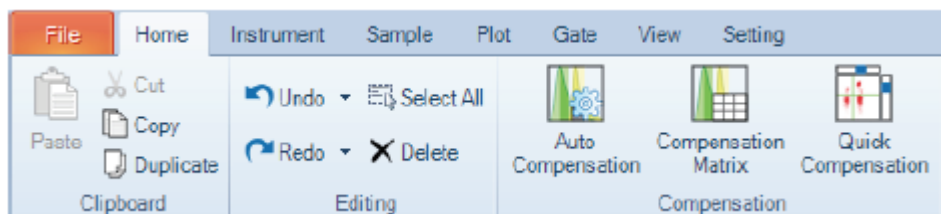


Scrollbars appear on any two parameters plots with fluorescent parameters opened on the workspace. Quickly adjust compensation by **dragging the scrollbar**

## Imaging and Flow Cytometry Core



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







## Imaging and Flow Cytometry Core

Compensation for T-lym

Compensation Matrix Spillover Matrix

Source\Target	CD3 FITC	CD8 PE	CD45 PerCP	PE-Cy7	CD4 APC	APC-Cy7
CD3 FITC	-100.3801	8.9548	-2.1786	0	0.4603	0
CD8 PE	4.2611	-100.3911	27.5831	0	-5.84	0
CD45 PerCP	0.0185	0.0386	-100.6337	0	21.6362	0
PE-Cy7	0	0	0	-100	0	0
CD4 APC	-0.0005	-0.0011	2.8983	0	-100.6231	0
APC-Cy7	0	0	0	0	0	-100

- % CD3 FITC

☐ Preview Clear Restore **OK** Cancel

To adjust, **check *Preview*** box and adjust the corresponding value. The corresponding plots will refresh with updated value real time. Adjust until satisfied. Then click ***OK*** to apply.

Click *Restore* to restore the Auto-compensation matrix value.

## Imaging and Flow Cytometry Core

### 3. Sample acquisition with NovoSampler Q

Step 1. Create experiment samples from the Plate Manager

- a. Select appropriate Plate type. Choose **40-tube rack** for 5-mL flow tubes

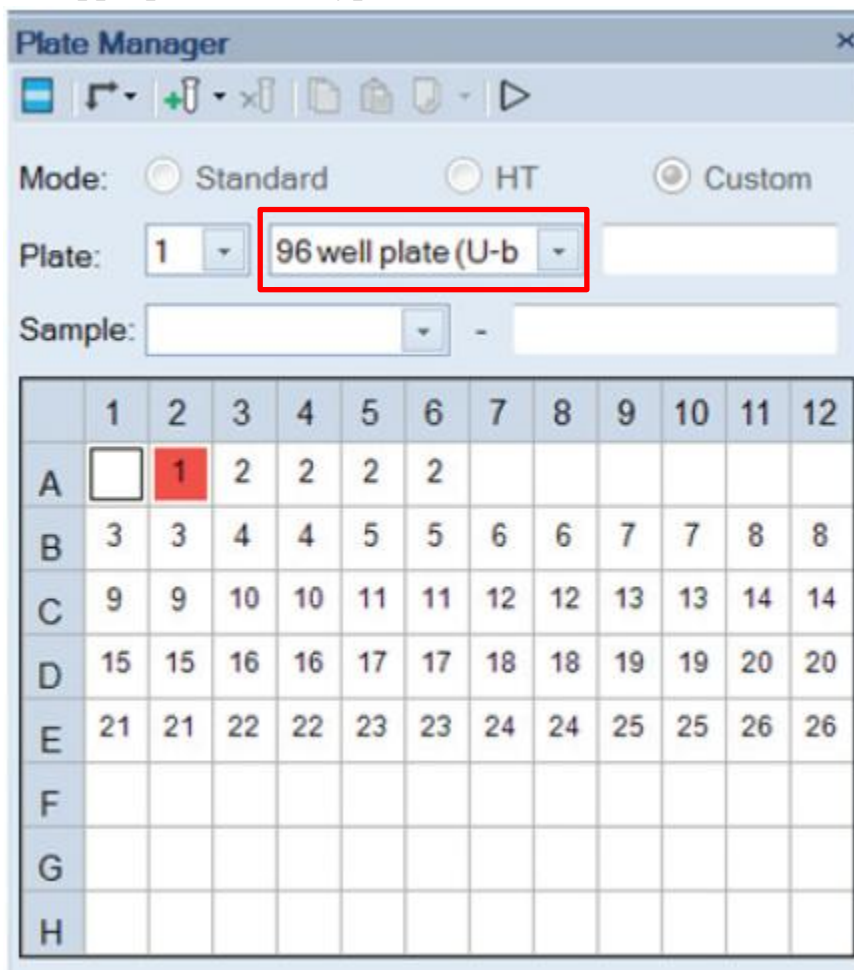


Plate Manager

Mode: ☐ Standard ☐ HT ☒ Custom

Plate: 1 **96 well plate (U-b)**

Sample: -

	1	2	3	4	5	6	7	8	9	10	11	12
A		1	2	2	2	2						
B	3	3	4	4	5	5	6	6	7	7	8	8
C	9	9	10	10	11	11	12	12	13	13	14	14
D	15	15	16	16	17	17	18	18	19	19	20	20
E	21	21	22	22	23	23	24	24	25	25	26	26
F												
G												
H												

- b. Highlight the position with samples on the plate by holding left Click and Drag AND/OR hold Ctrl and left-click to multi-select specific wells. Black square indicates selected well.

## Imaging and Flow Cytometry Core

	1	2	3	4	5	6	7	8	9	10	11	12
A		1	2	2	2	2						
B	3	3	4	4	5	5	6	6	7	7	8	8
C	9	9	10	10	11	11	12	12	13	13	14	14
D	15	15	16	16	17	17	18	18	19	19	20	20
E	21	21	22	22	23	23	24	24	25	25	26	26
F												

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

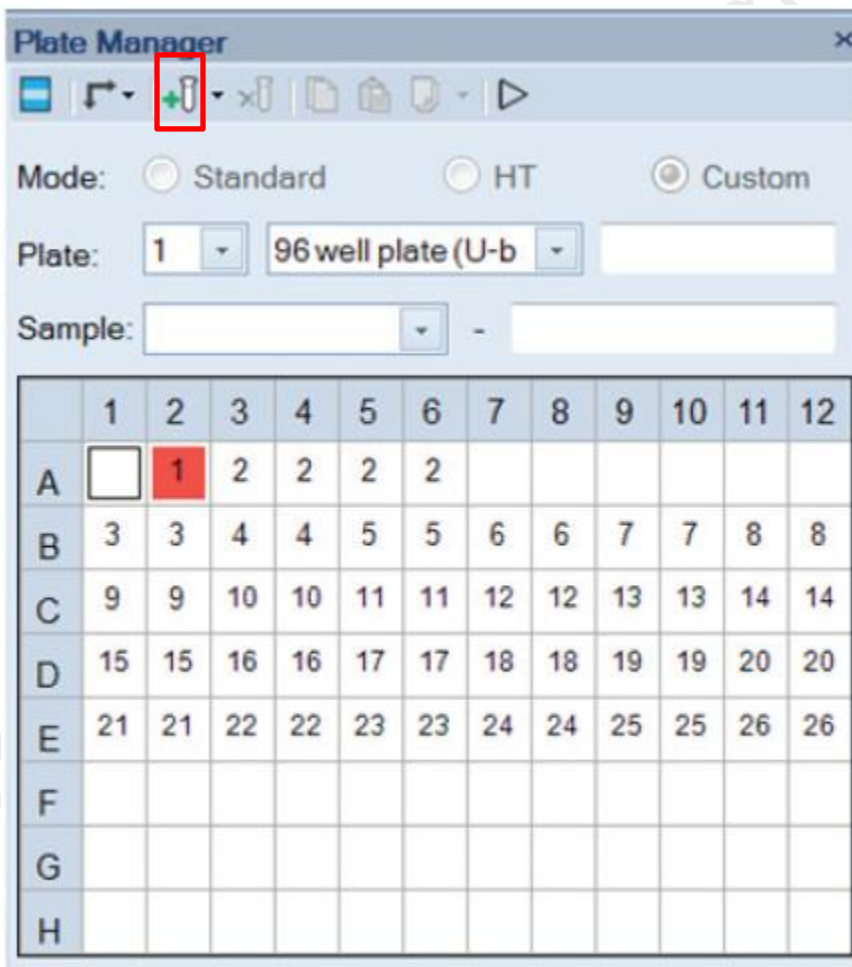


Plate Manager

Mode: ☐ Standard ☐ HT ☒ Custom

Plate: 1 96 well plate (U-b)

Sample: -

	1	2	3	4	5	6	7	8	9	10	11	12
A		1	2	2	2	2						
B	3	3	4	4	5	5	6	6	7	7	8	8
C	9	9	10	10	11	11	12	12	13	13	14	14
D	15	15	16	16	17	17	18	18	19	19	20	20
E	21	21	22	22	23	23	24	24	25	25	26	26
F												
G												
H												

d. Repeat step 1b and 1c to create new sample of *Specimen 2* if needed.

e. Check *Absolute count* if absolute counting is required.



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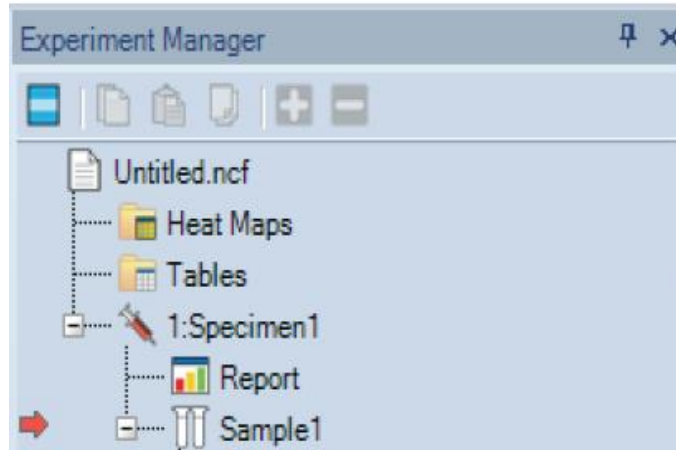
## Imaging and Flow Cytometry Core

\*Dead volume will increased from 10uL to 30uL with Absolute count checked.

CPoS - Imaging and Flow Cytometry Core

## Imaging and Flow Cytometry Core

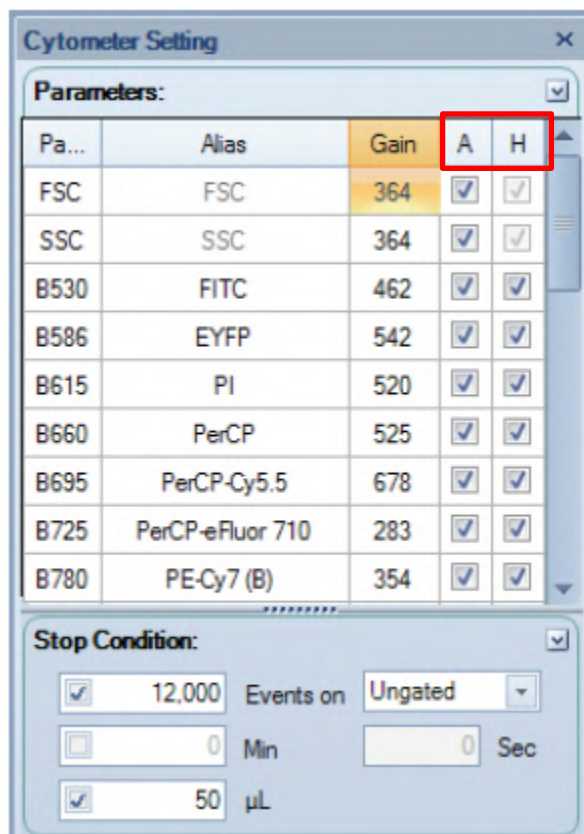
- f. Double click *Sample 1* on the Experiment Manager until the red arrow is pointing to Sample 1.



## Imaging and Flow Cytometry Core

### Step 2. Select Channels

- a. Click on the “A” and “H” of the parameters panel in Cytometer setting to Select OR Unselect ALL.



The image shows a 'Cytometer Setting' dialog box. It contains a 'Parameters' table with columns for 'Pa...', 'Alias', 'Gain', 'A', and 'H'. The 'A' and 'H' columns are highlighted with a red box. Below the table is a 'Stop Condition' section with checkboxes and input fields for 'Events on', 'Min', 'Sec', and 'μL'.

Pa...	Alias	Gain	A	H
FSC	FSC	364	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
SSC	SSC	364	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B530	FITC	462	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B586	EYFP	542	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B615	PI	520	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B660	PerCP	525	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B695	PerCP-Cy5.5	678	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B725	PerCP-eFluor 710	283	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
B780	PE-Cy7 (B)	354	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

**Stop Condition:**

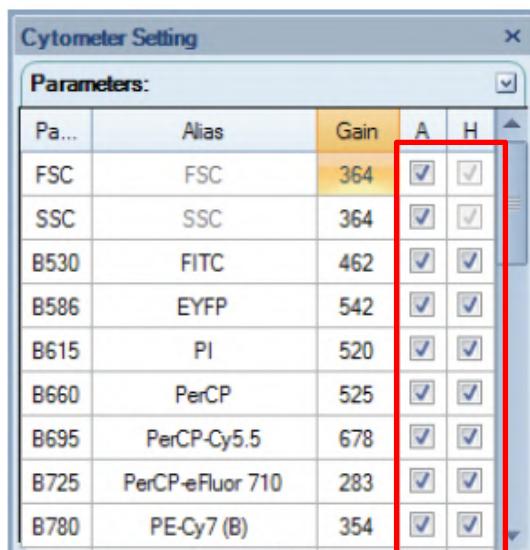
☒ 12,000 Events on

☐ 0 Min  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).

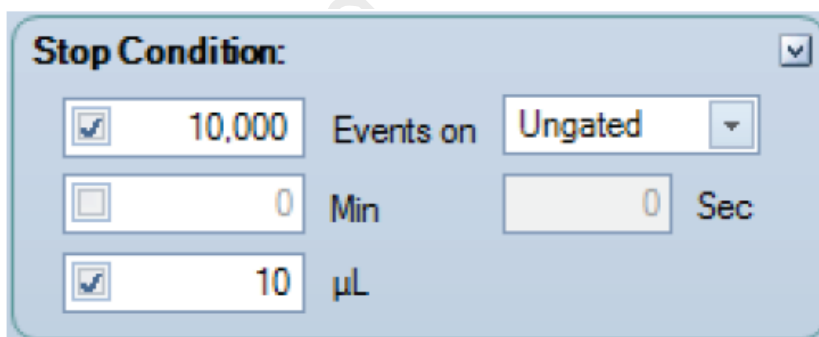
## Imaging and Flow Cytometry Core



### Step 3. Conditions Setup

- 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:

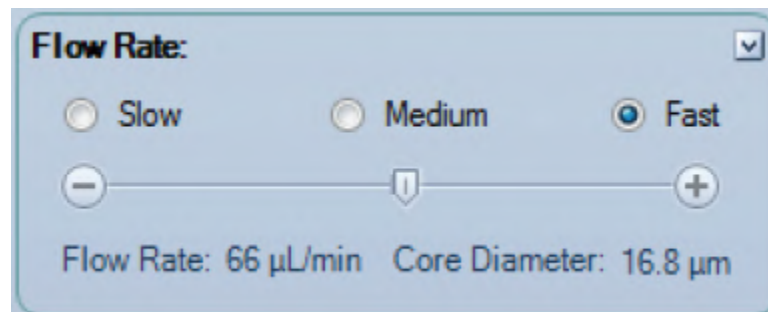
Events	1 – 10,000,000
Time	0-60 min; 0-59 Sec
Volume	5 – 5000 uL

\* Volume may be limited to the Plate format. Please refer to the Appendix.

## Imaging and Flow Cytometry Core

- b. Select flow rate by click the radio button of *Slow* (14  $\mu\text{L}/\text{min}$ ), *Medium* (35  $\mu\text{L}/\text{min}$ ), and *Fast* (66  $\mu\text{L}/\text{min}$ ) OR use the slider to adjust the flow rate from 5~120 $\mu\text{L}/\text{min}$ .

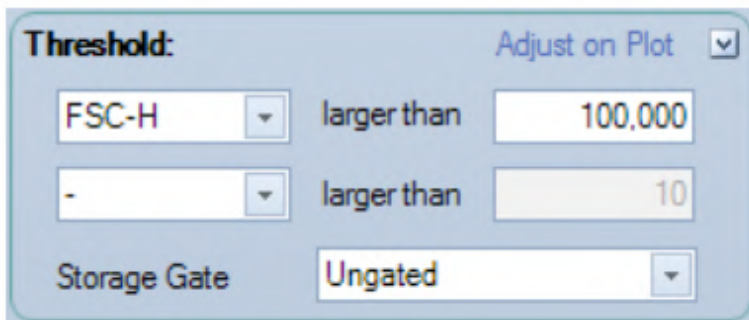
\* Current sample's flow rate and the corresponding core diameter are shown in the bottom of the panel.





## Imaging and Flow Cytometry Core

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



The screenshot shows the 'Threshold' panel in a flow cytometry software. It has a title 'Threshold:' and a button 'Adjust on Plot' with a dropdown arrow. Below the title, there are two rows of parameter selection. The first row shows 'FSC-H' in a dropdown menu, followed by 'larger than' and a text box containing '100,000'. The second row shows '-' in a dropdown menu, followed by 'larger than' and a text box containing '10'. At the bottom, there is a 'Storage Gate' label and a dropdown menu showing 'Ungated'.

Suggested Threshold on Different cell type:

Cell Types	FSC-H Threshold
Cell lines, larger than 20 $\mu\text{m}$ in cell diameter	300,000~1,000,000
Cells lines, smaller than 20 $\mu\text{m}$ 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

- f. Setup mixing and rinsing conditions under Plate Manager.  
\*For 96-well plate, the default is 1000 rpm.



## Imaging and Flow Cytometry Core

Mix  Every  Well(s)

Rinse  Every  Well(s)

**Mixing Parameters**

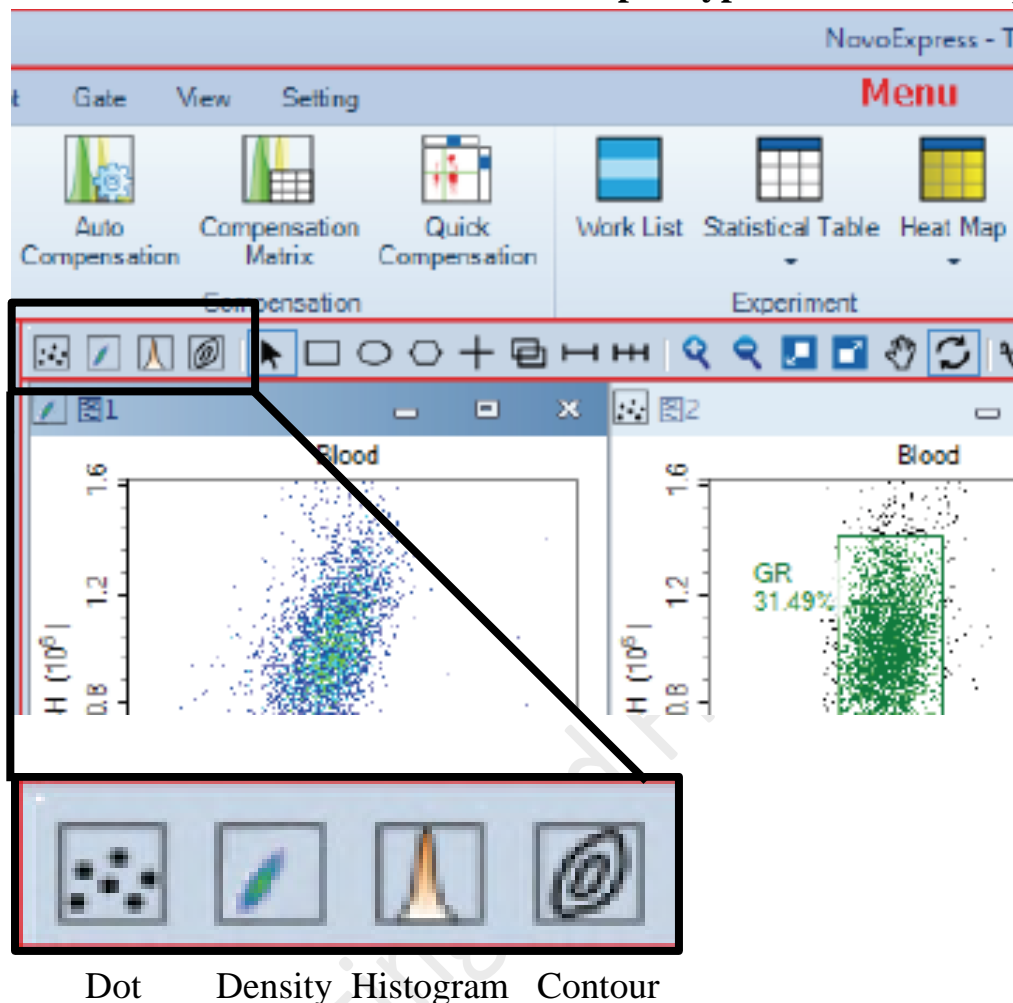
Speed  rpm Acceleration  s

Duration  s

## Imaging and Flow Cytometry Core

### Step 4. Draw Plots

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



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	The intensity of a parameter is represented along the

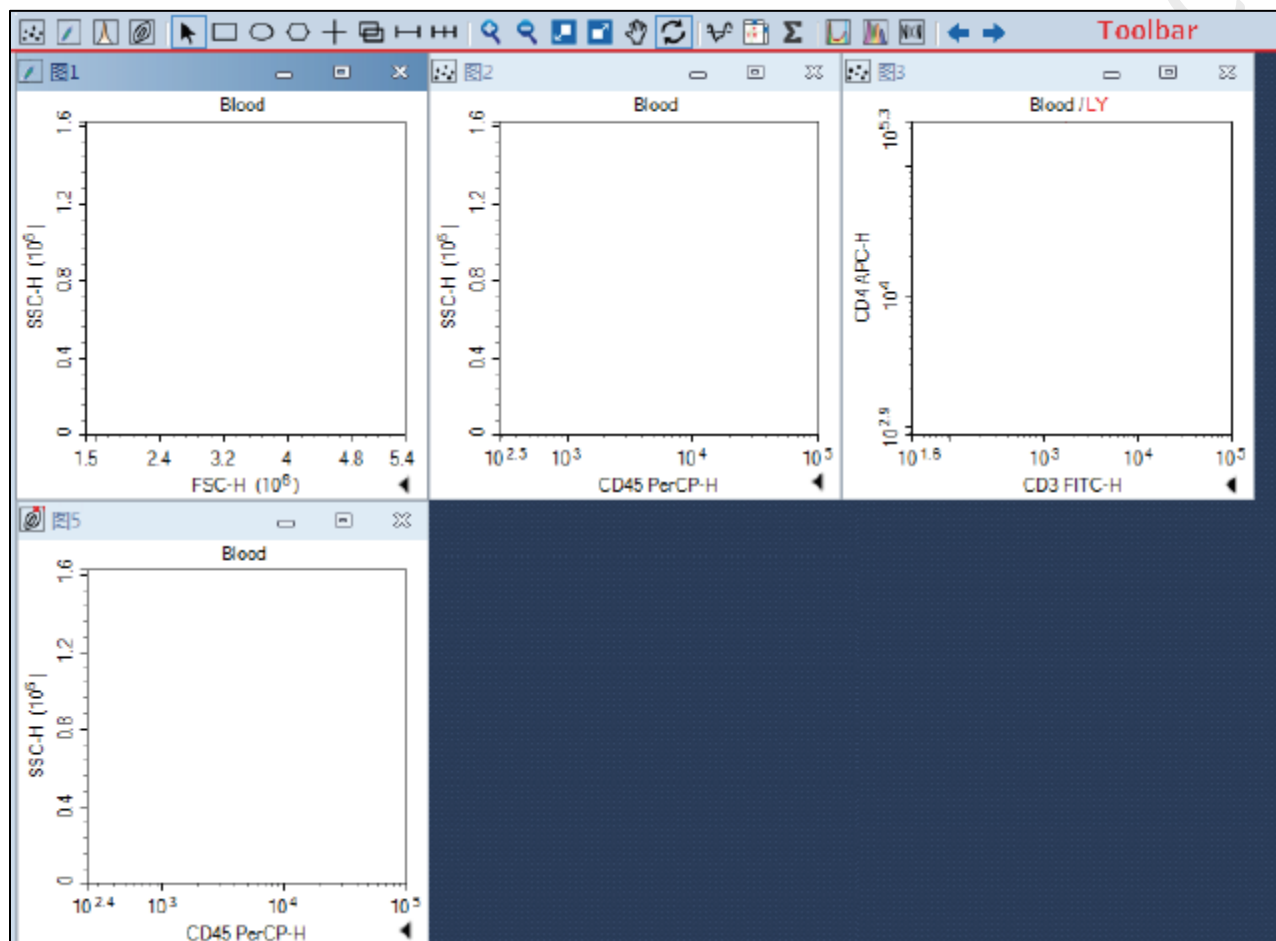


## Imaging and Flow Cytometry Core

	(x axis only)	x-axis, and the number of events at each intensity value is represented along the y-axis.
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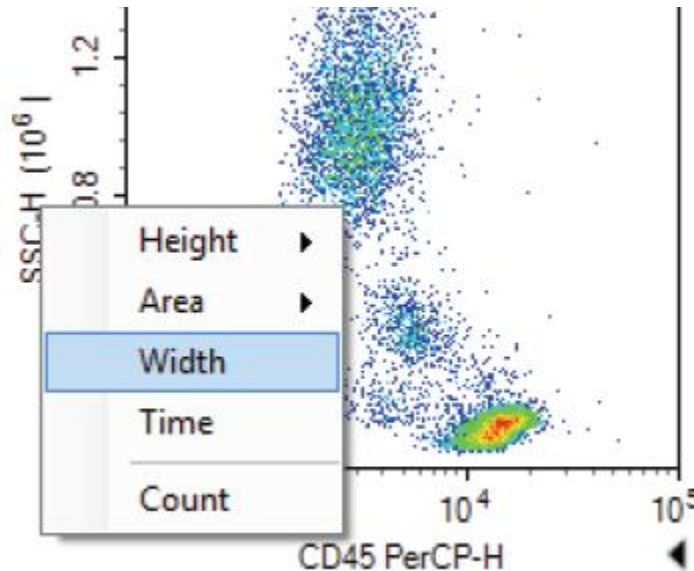
## Imaging and Flow Cytometry Core

- 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)



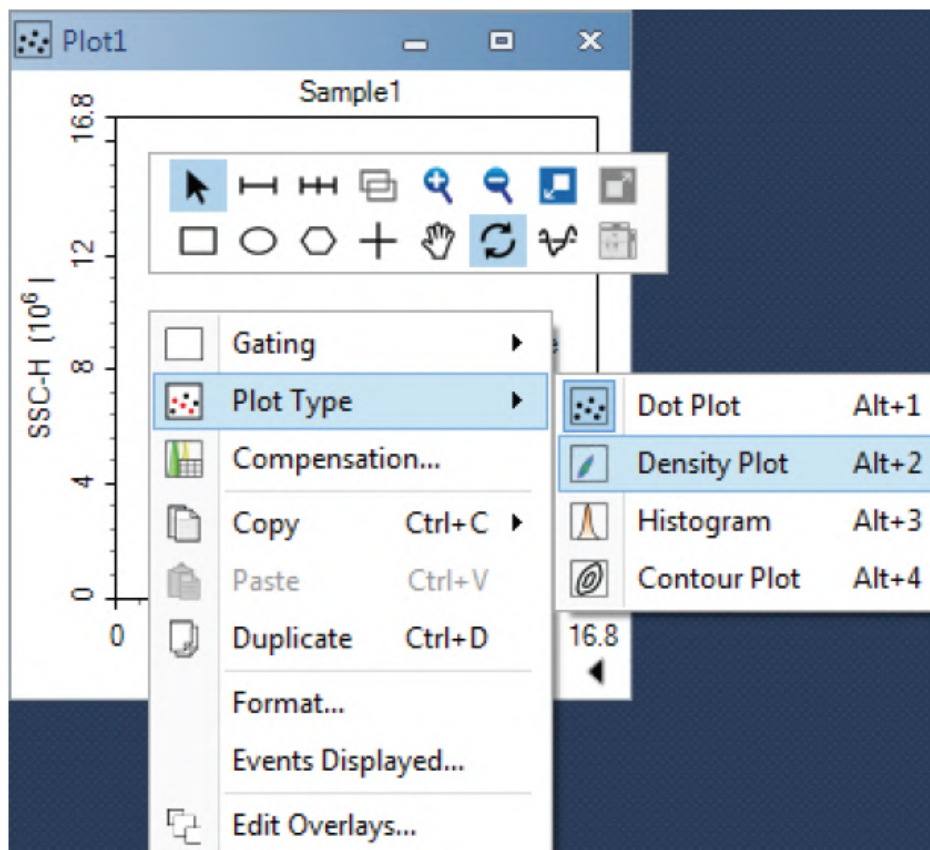
## Imaging and Flow Cytometry Core

- 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.



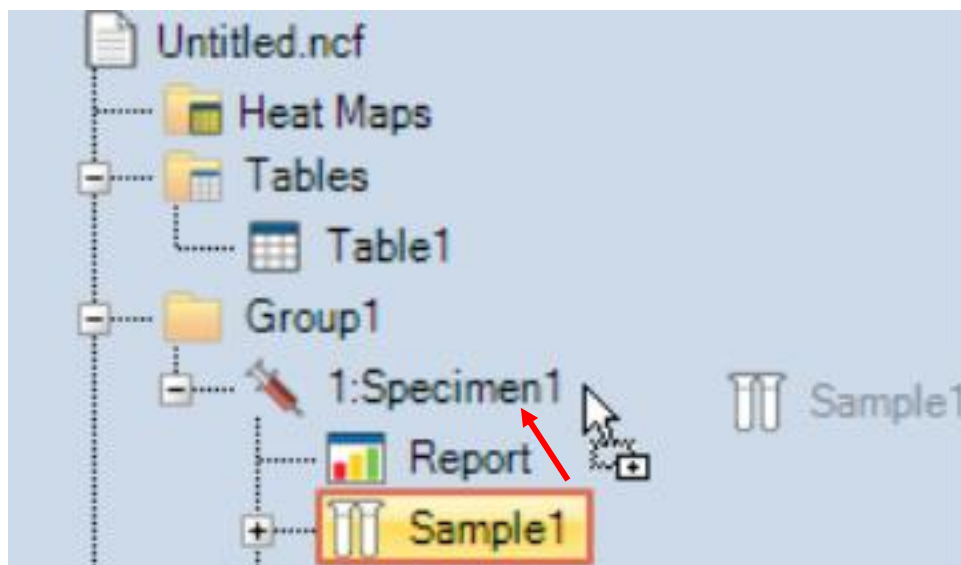
- d. **Right-click** within a plot to change the plot type if needed.

## Imaging and Flow Cytometry Core

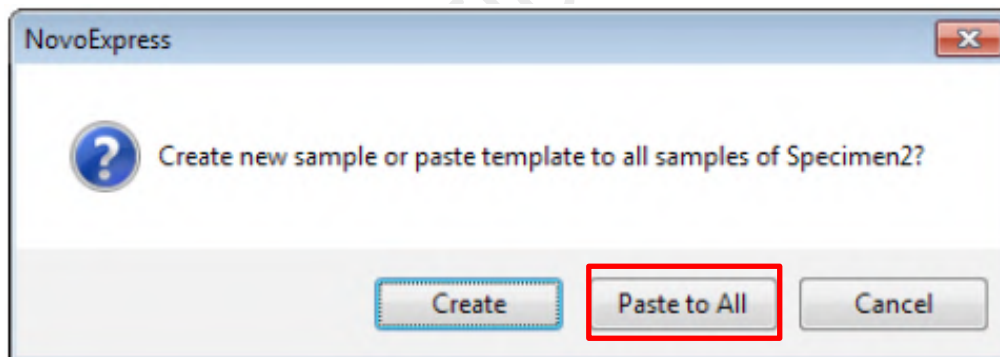


## Imaging and Flow Cytometry Core

- d. 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.



- e. Click *Paste to All*.





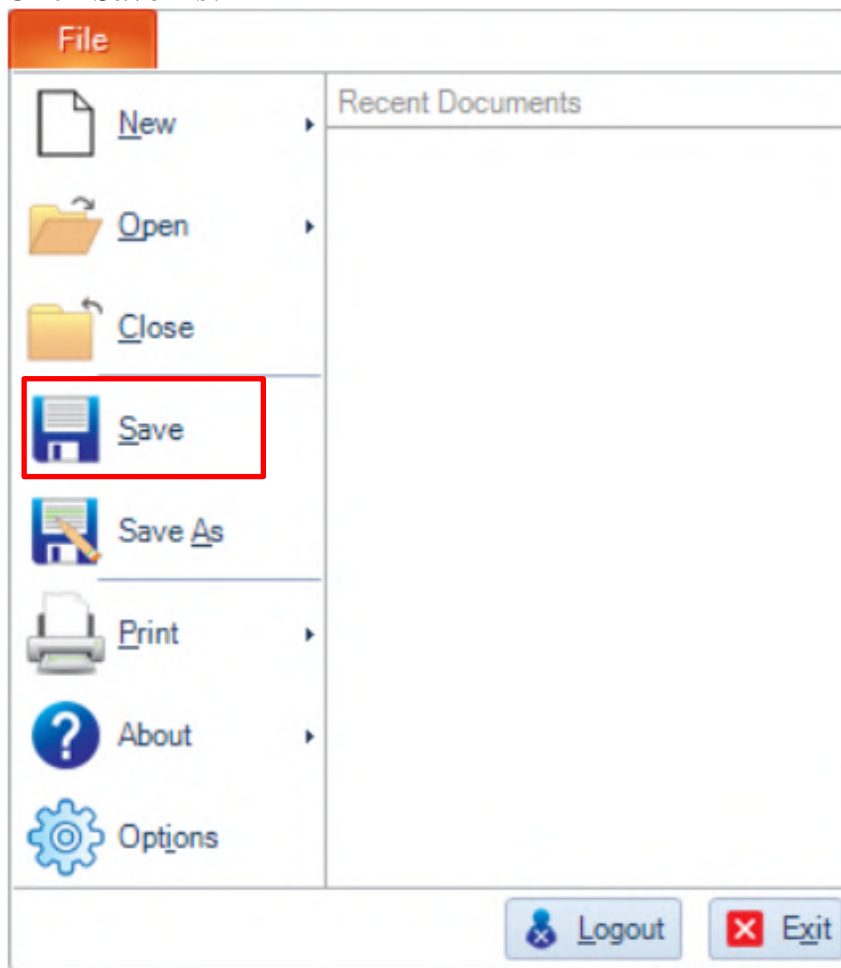
## Imaging and Flow Cytometry Core

### 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 (F: )> User> Department> YOUR FOLDER**

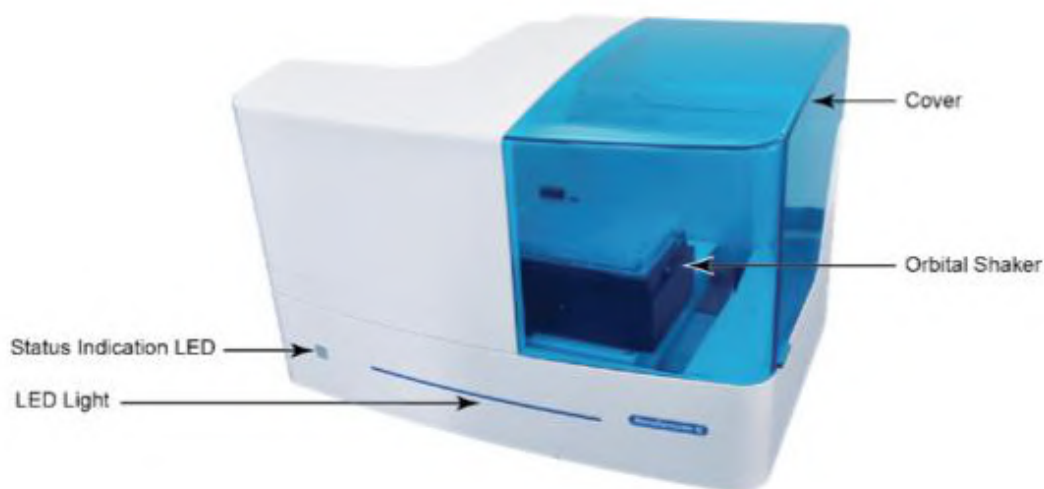
\*Default location: Computer> D: > Novoexpress Data. Please do **NOT** save as default

Click *Save*.

## Imaging and Flow Cytometry Core

Step 6. Load tube rack/ plate

- a. Lift the cover of the NovoSampler Q.



- b. Place the tube rack with your sample tubes or plate on the orbital Shaker with A1 position on the top left-hand corner. Make sure the rack or plate is placed within the 4 metal poles.

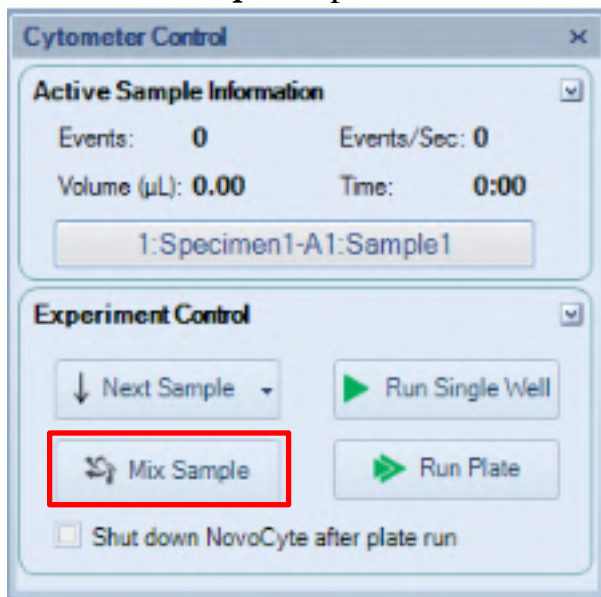


- c. Put down the cover.

## Imaging and Flow Cytometry Core

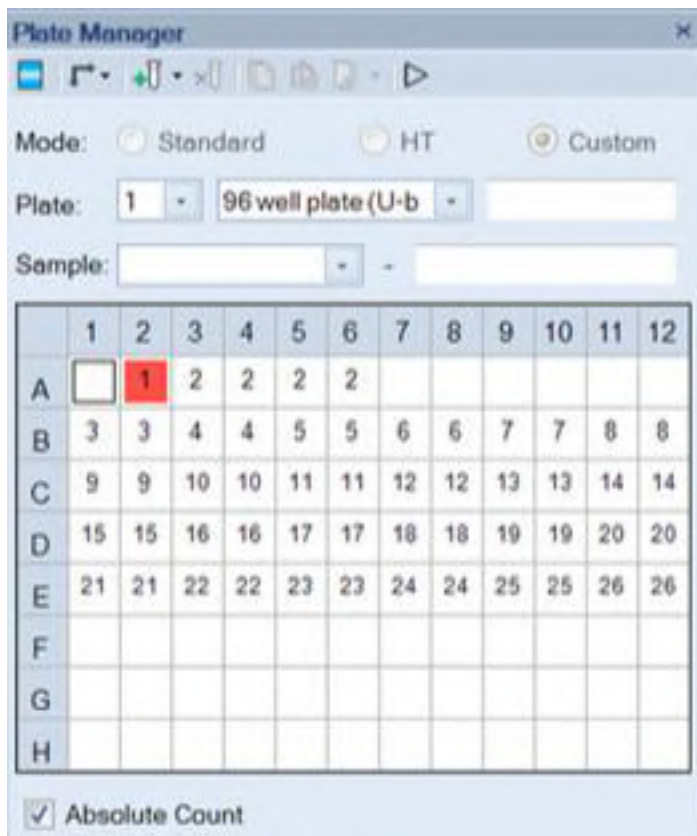
### Step 7. Sample Acquisition

- Click **Mix Sample** to perform orbital shaking.

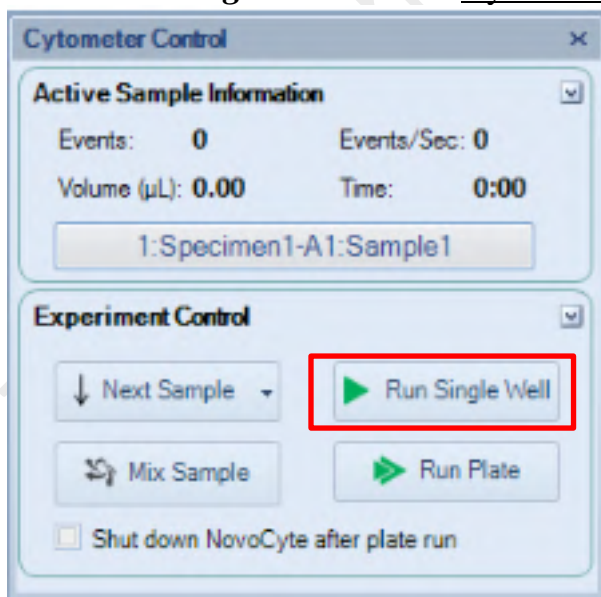


- To run **SINGLE** well/ tube, **double-click** on the interested well on Plate Manager and the selected position highlight in red.  
\*A2 is selected in the picture.

## Imaging and Flow Cytometry Core

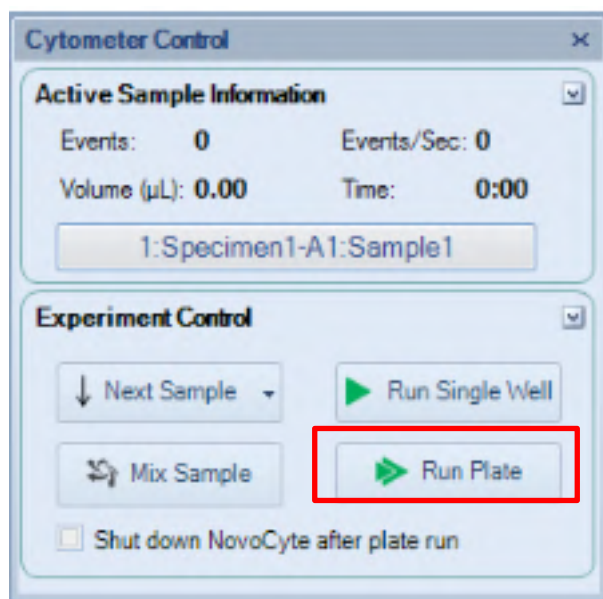


- c. Click **Run Single Well** on the Cytometer Control Panel.



## Imaging and Flow Cytometry Core

- d. To run multiple tubes / wells automatically, click **Run Plate** on the Cytometer Control Panel.



- e. Double check if the plate type is correct as it state. Click **Run** to continue, or else click *Cancel* and correct the plate type in Plate Manager panel.



## Imaging and Flow Cytometry Core

- f. Select the tubes or wells you would like to be acquired on the Plate View.  
Selected wells highlight in Blue. Then click **Run**.

Select well(s) to run

Plate 1 ☐ Show Specimen Name ☐ Display sample has data

	1	2	3	4	5	6	7	8	9	10	11	12
A			Compensat...	Compensat...	Compensat...	Compensat...						
B	Wells1-Sa...	Wells1-Sa...	Wells2-Sa...	Wells2-Sa...	Wells3-Sa...	Wells3-Sa...	Wells4-Sa...	Wells4-Sa...	Wells5-Sa...	Wells5-Sa...	Wells6-Sa...	Wells6-Sa...
C	Wells7-Sa...	Wells7-Sa...	Wells8-Sa...	Wells8-Sa...	Wells9-Sa...	Wells9-Sa...	Wells10-Sa...	Wells10-Sa...	Wells11-Sa...	Wells11-Sa...	Wells12-Sa...	Wells12-Sa...
D	Wells13-Sa...	Wells13-Sa...	Wells14-Sa...	Wells14-Sa...	Wells15-Sa...	Wells15-Sa...	Wells16-Sa...	Wells16-Sa...	Wells17-Sa...	Wells17-Sa...	Wells18-Sa...	Wells18-Sa...
E	Wells19-Sa...	Wells19-Sa...	Wells20-Sa...	Wells20-Sa...	Wells21-Sa...	Wells21-Sa...	Wells22-Sa...	Wells22-Sa...	Wells23-Sa...	Wells23-Sa...	Wells24-Sa...	Wells24-Sa...
F												
G												
H												

- g. Click **OK** to continue.

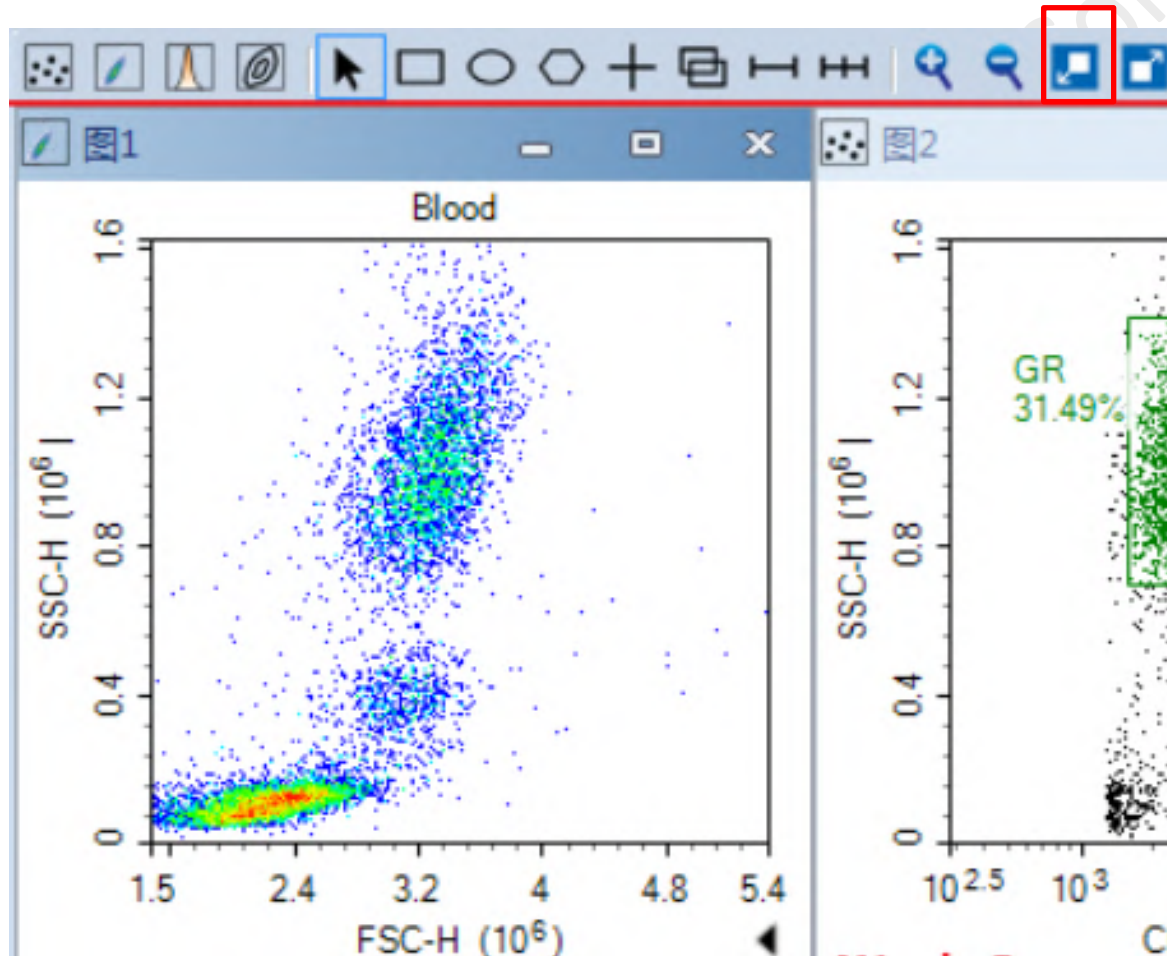


## Imaging and Flow Cytometry Core

### 4. Data Analysis during acquisition

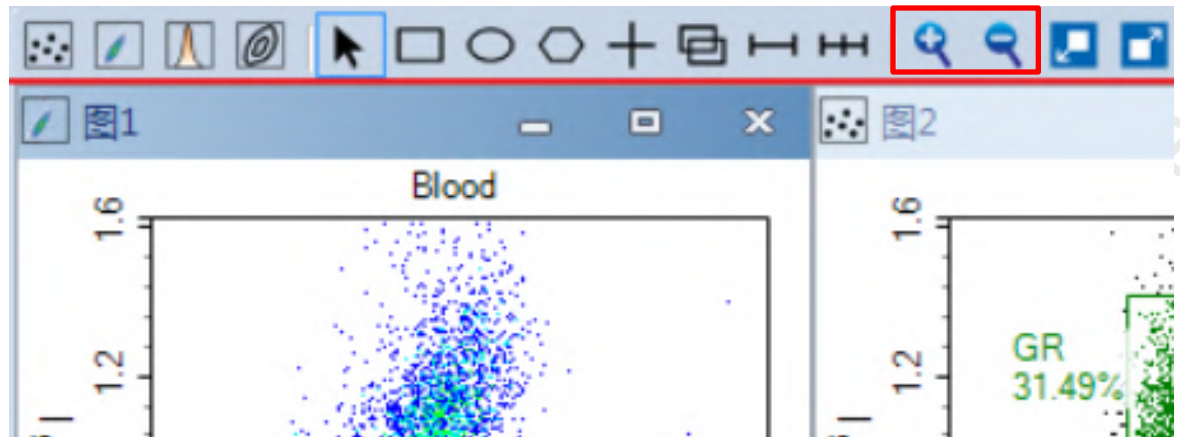
Step 1. Set the appropriate display range of the plot.

- 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.

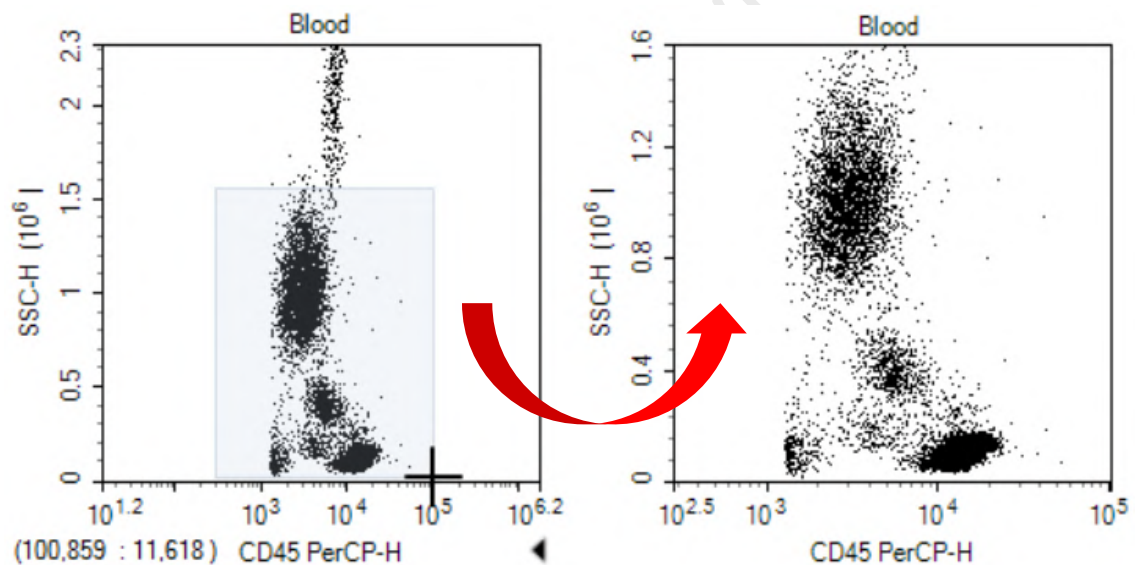


- To fine tune the data display range, click **zoom in / zoom out** buttons.

## Imaging and Flow Cytometry Core



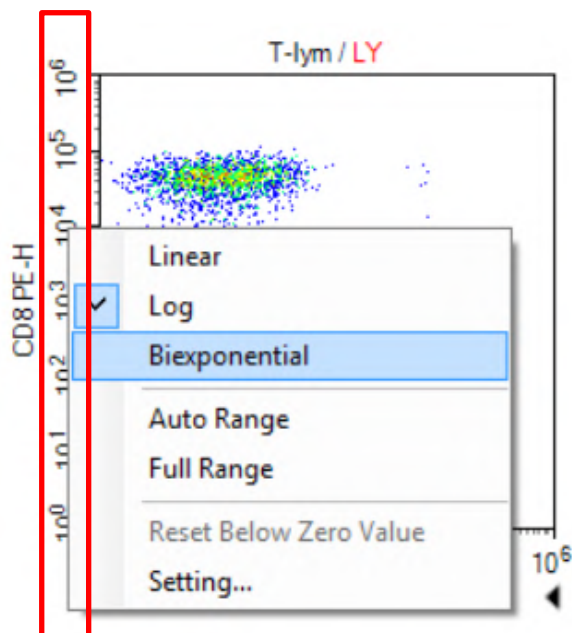
- c. **Drag** on the interested region on the plot if you click *zoom in*.



- 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.



## Imaging and Flow Cytometry Core



- 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.

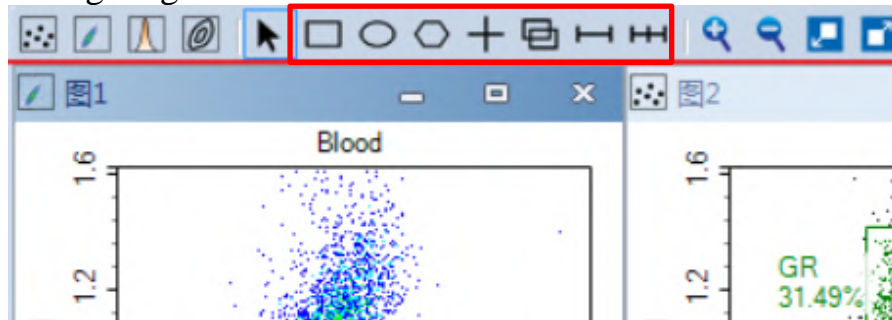
**\*Gain can only be adjusted during acquisition.**








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

## Imaging and Flow Cytometry Core

### Step 2. Gating

- 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 

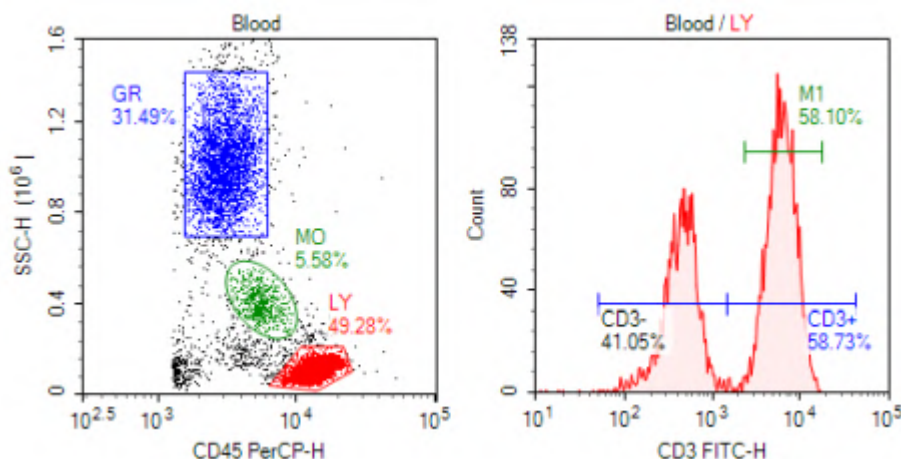
Dot Plot, Density Plot, Contour Plot – All gates suitable

Histogram Plot – Range / Bi-range gate suitable

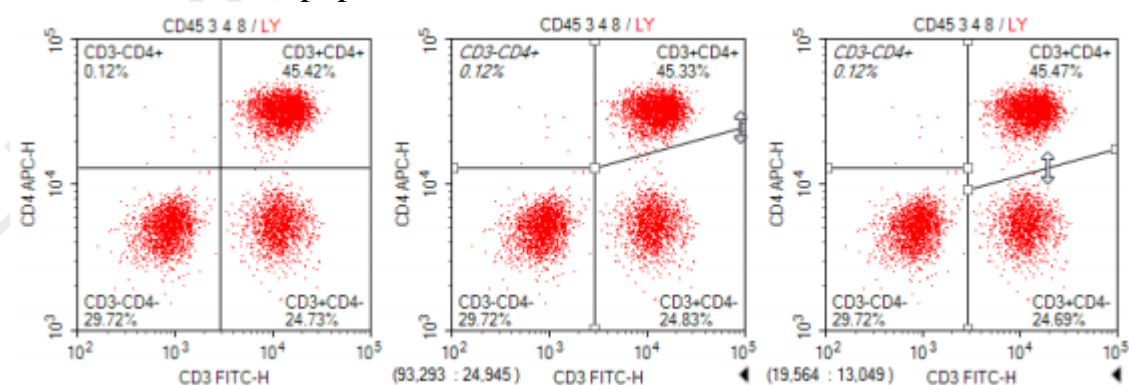
## Imaging and Flow Cytometry Core

- 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.

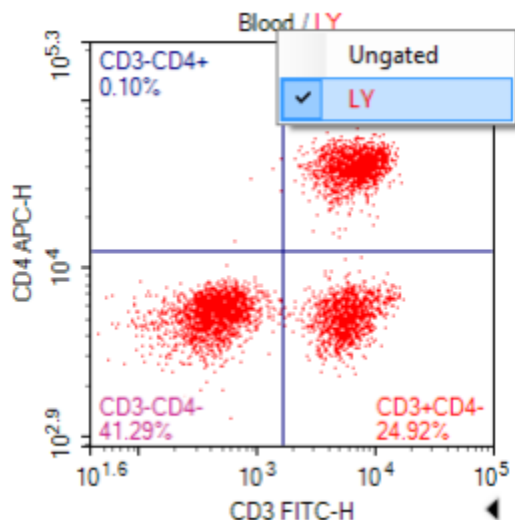


To create *quadrant gate*, click the corresponding icon and Click in the plot to create the center of the quadrants and create the gate. As shown below, the center, endpoints, and lines of the quadrant gate can be moved to enclose the correct populations.



## Imaging and Flow Cytometry Core

- 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.



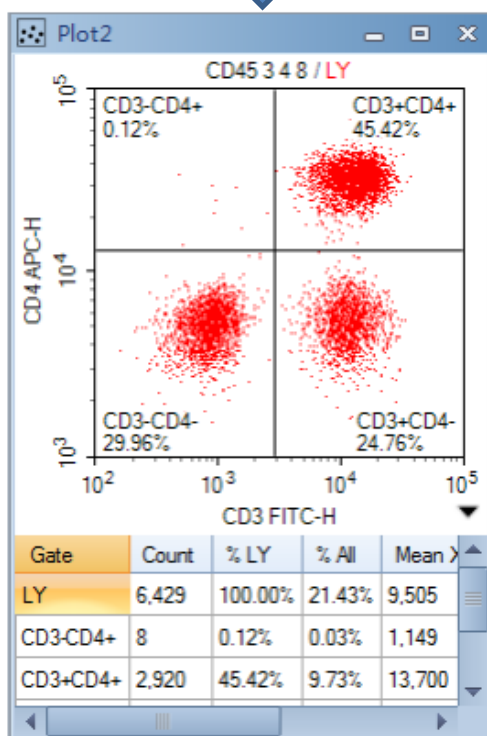
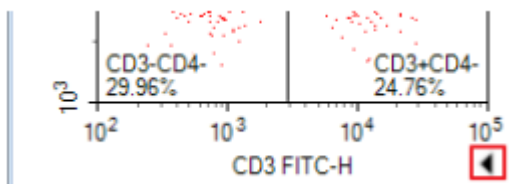
- d. The Gate Manager panel displays all gates of the active sample in list mode or tree mode. It provides user interface to modify gate name, color and color precedence and also shows gate hierarchy and gate statistics.

Gate Manager									
Gate	Color	Count	% Parent	X	Y	Mean X	Mean Y	CVX	CVY
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%

## Imaging and Flow Cytometry Core

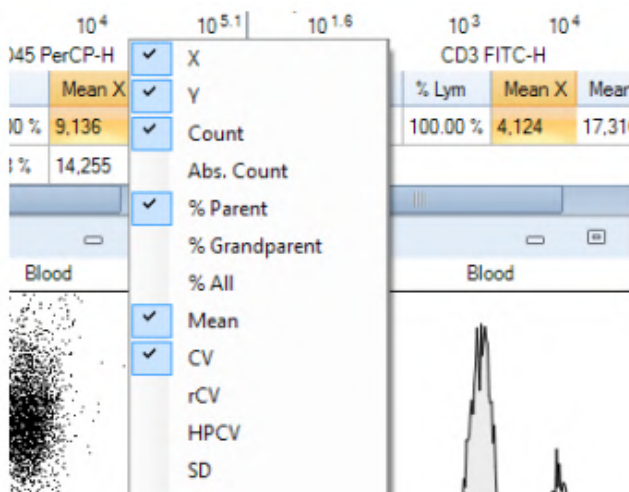
### Step 3. Statistics

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



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

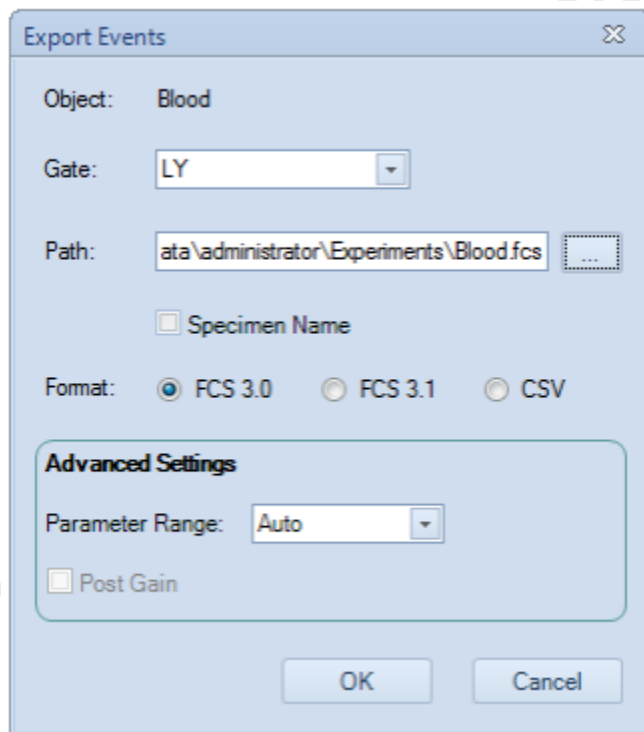
## Imaging and Flow Cytometry Core



### 5. Data Export

Step 1. Export FCS file

- 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.



The 'Export Events' dialog box is shown. It has the following fields and options:

- Object:** Blood
- Gate:** LY (dropdown menu)
- Path:** ata\administrator\Experiments\Blood.fcs (text box with a browse button)
- ☐ Specimen Name
- Format:** ☒ FCS 3.0 ☐ FCS 3.1 ☐ CSV
- Advanced Settings:**
  - Parameter Range:** Auto (dropdown menu)
  - ☐ Post Gain
- Buttons:** OK, Cancel

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



## Imaging and Flow Cytometry Core

**Experiment Data (F:)> user > Department> Your NAME**

Select “FSC3.0” for Format and Click *OK*.

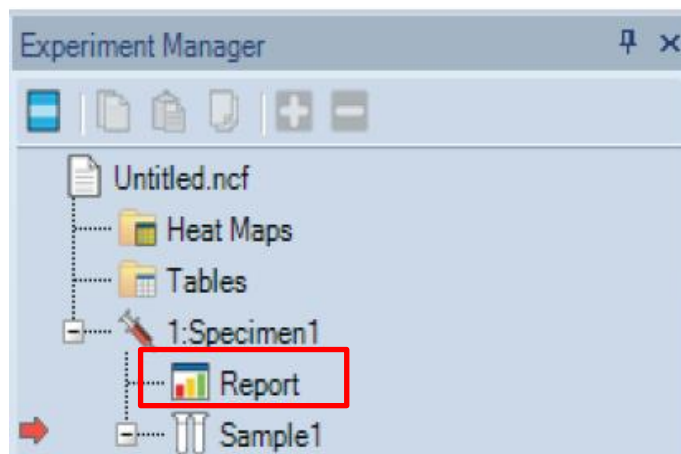
- Data files (including the experiment files) can be stored in F drive **for 3 months ONLY**. While experiment template files may be stored for a longer duration.
- Data on the computer (F drive) will be removed regularly without prior notice.



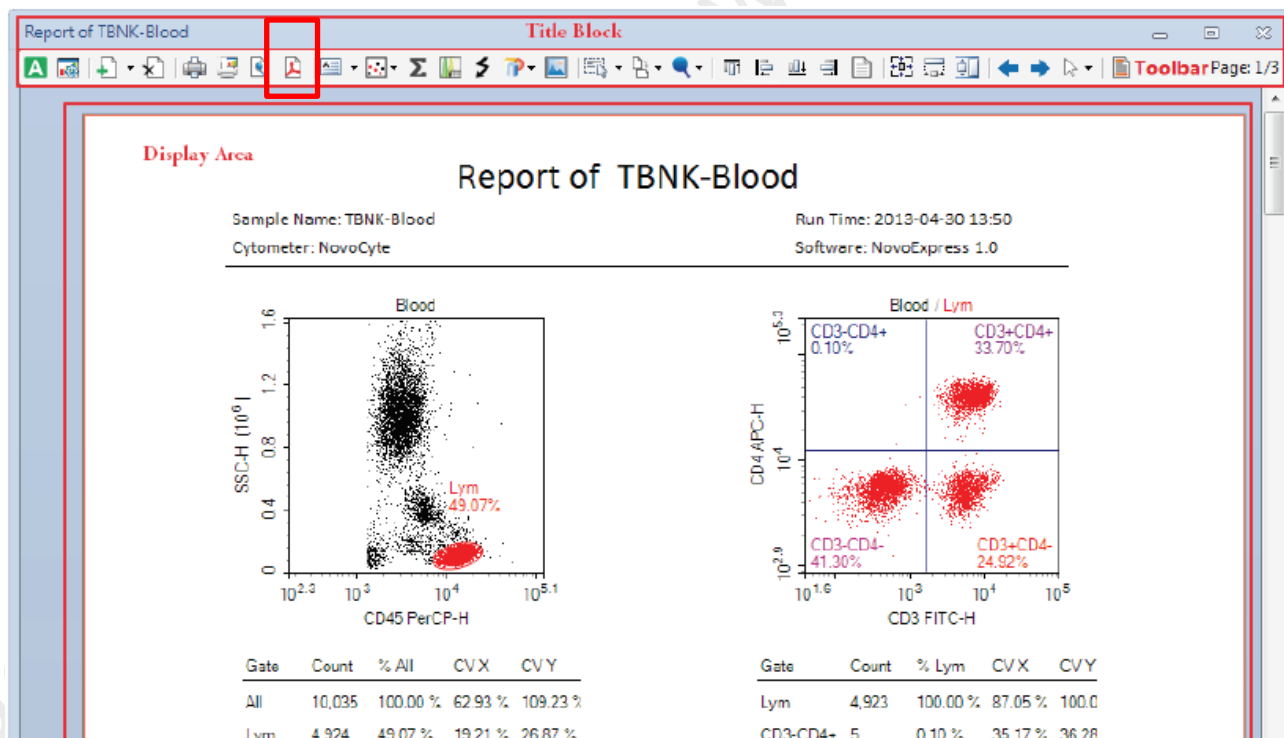
## Imaging and Flow Cytometry Core

Step 2. Export PDF file (optional)

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



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



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



## Imaging and Flow Cytometry Core



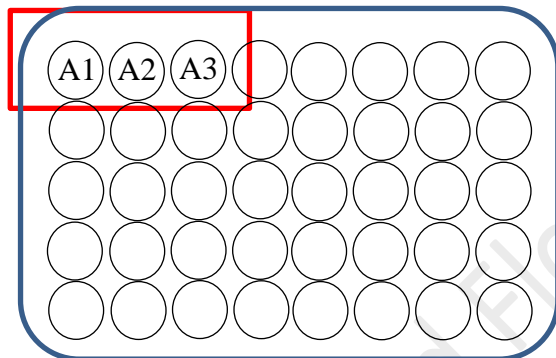
- c. Click *Save* buttons on the top left-hand corner of the window when you finish your experiment

### 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 (You may use the “Ocleaning” account to perform)

- a. Place tubes of at least 1ml of cleaning solution 1, 2 and 3 and put them in A1-A3 of the 40-tube rack respectively.

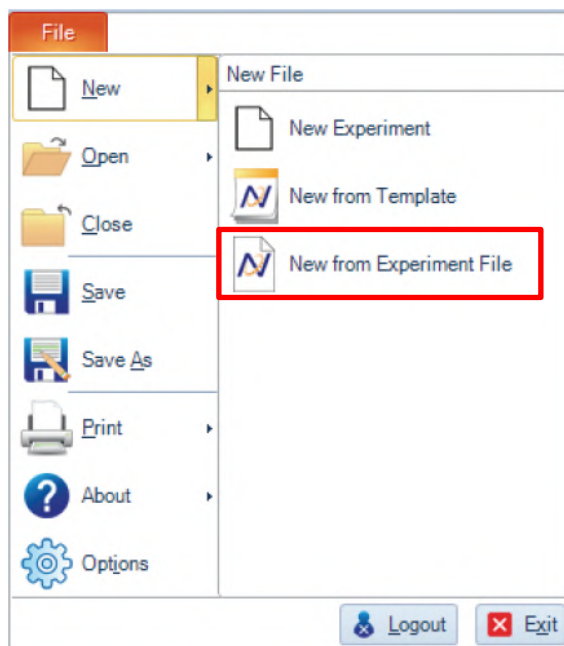


- a. Click *File* on the Menu bar.



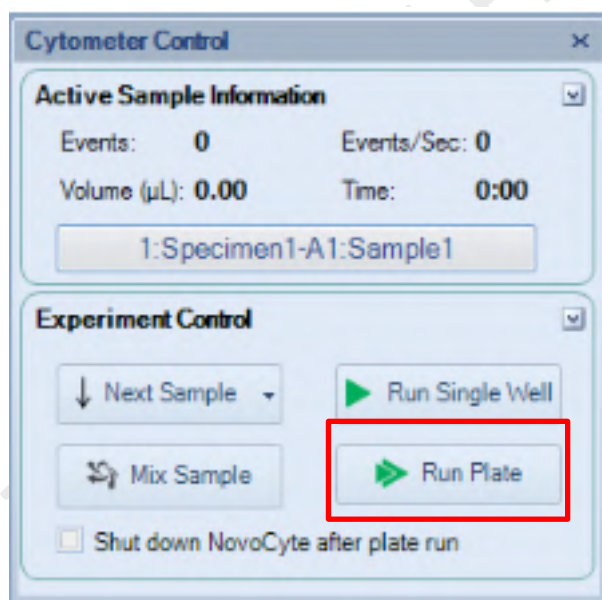
- b. Click *New* > *New from Experiment File*.

## Imaging and Flow Cytometry Core



c. Select *Desktop > cleaning.ncf* . Then click **OK**

d. Click *Run Plate* on the Cytometer Control Panel.



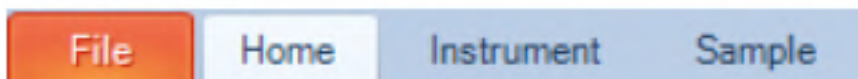
e. Select all wells. Then click *Run*. (Click **OK** to continue).

f. Select *Desktop > cleaning.ncf*. Click *Save* and *Yes* to overwrite.

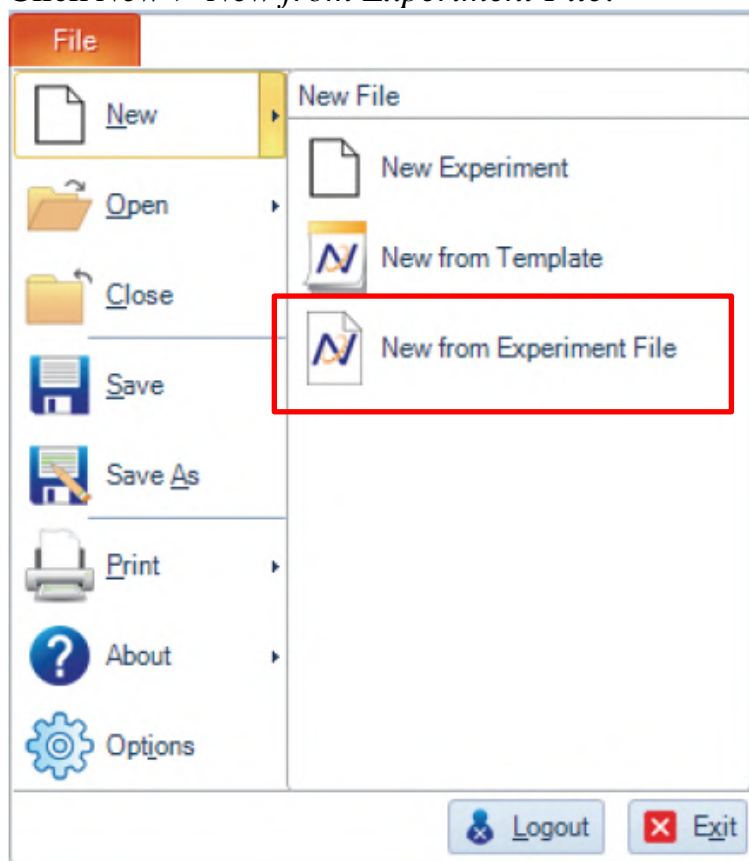
## Imaging and Flow Cytometry Core

### 7. Re-use Experiment as template

a. Click *File* on the Menu bar.



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



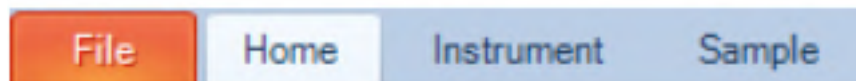
c. Select your target experiment file (.ncf) . Then click *OK*

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

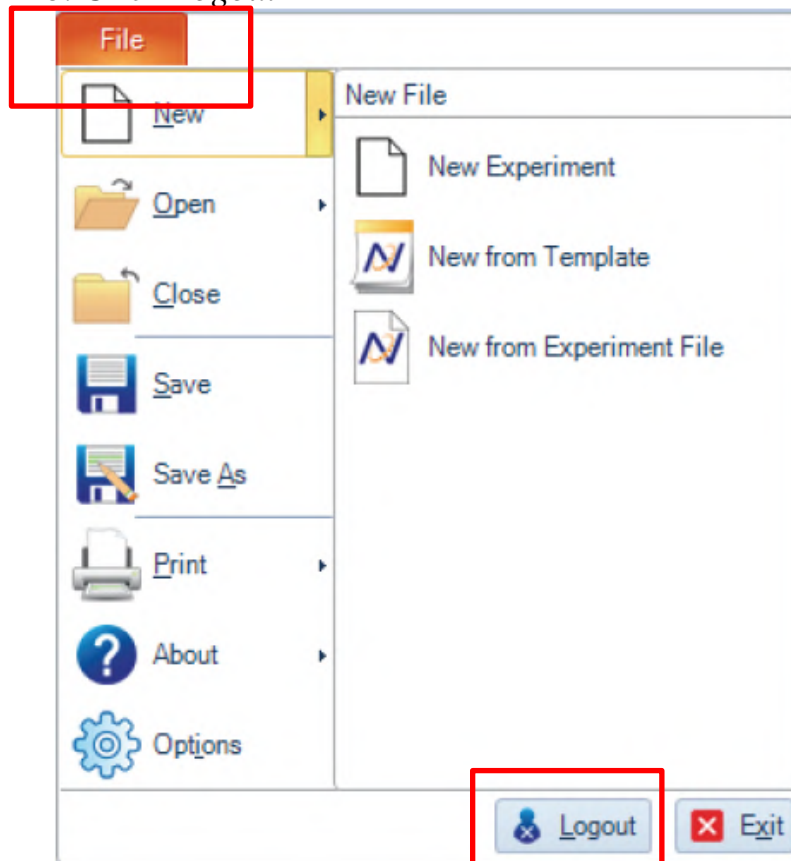
## Imaging and Flow Cytometry Core

### 8. NovoExpress Software Log out

a. Click *File* on the Menu bar.



b. Click *Logout*



## Imaging and Flow Cytometry Core

### APPENDIX

Minimum Sample Volume Requirements for the NovoSampler Q is shown in table 3-1 below

Parameter	Plate Type	Standard Mode/HT Mode	Custom Mode	
			With Absolute Count Checked	With Absolute Count Unchecked
Minimum Sample Volume (μL)	12 × 75 mm tube (with ACEA 40 tube rack)	20.5	40.5	20.5
	24-well plates	285	305	285
	48-well plates	110	130	110
	96-well plates (flat-bottom)	41.5	61.5	41.5
	96-well plates (V-bottom)	15.6	35.6	15.6
	96-well plates (U-bottom)	15	35	15
	384-well plates	18	38	18

**Table 3-1 Minimum Sample Volume Requirement for Each Plate and Tube Type**



The recommended sample concentration range is  $1 \times 10^6$  to  $5 \times 10^6$  cells/mL.