



NovoExpress

Software Guide

For Research Use Only. Not for use in diagnostic procedures.

Notices

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Software Revision

This guide is valid for the 1.5.0 revision of the Agilent NovoExpress program and compatible programs, until superseded.

Instrument Manufacturing

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

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A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

In This Guide

This guide covers the following module: NovoExpress Software

1 Prologue

This chapter provides basic information for NovoExpress and the software guide, including overview of NovoExpress, scope of this guide, keyboard shortcuts and glossary.

2 Installation

This chapter describes the installation requirements, procedures to install, start and uninstall the NovoExpress software, NovoExpress license registration and dissociation, and user management feature.

3 Use NovoExpress Software

This chapter provides information needed to use NovoExpress software, including overview of NovoExpress software interface, Title Block, Menu, Workspace Toolbar, Cytometer Setting panel, Cytometer Control panel, Plate Manager panel, Experiment Manager panel, Cytometer Status panel, Gate Manager panel, Status Bar, Instrument Configuration window and Manage Plate Types window.

4 Sample Acquisition

This chapter provides the information for sample acquisition function, including Cytometer Setting panel, Work List, and Cytometer Control panel.

5 Data Analysis

This chapter provides information for data analysis tools, including Plots, Gates, Statistics, Fluorescence Compensation, Cell Cycle Analysis, Cell Proliferation Analysis, Statistical Tables, Heat Maps and Post Gain.

6 Plate Manager

This chapter provides the information for the Plate Manager panel, including overview of Plate Manager panel and context menu.

7 Experiment Manager

In This Guide

This chapter provides the information for the Experiment Manager function, including Experiment Manager Toolbar, Hierarchy, Templates, Import and Export Data.

8 Reports

This chapter provides information for the Report function, including Report Interface, Automatically Generate Reports, Report Options, Report Editor, Report Output and Batch Print Reports.

9 QC Test

This chapter describes the procedures to run QC test and view the report for Agilent flow cytometers.

10 Troubleshoot

This chapter provides the information for troubleshooting Agilent flow cytometer instrument and associated accessories, including prompted message ID, software messages, possible causes, recommended solutions, and procedures to create technical support files.

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1

Prologue

This chapter provides basic information for NovoExpress and the software guide, including overview of NovoExpress, scope of this guide, keyboard shortcuts and glossary.

About NovoExpress

NovoExpress Software provides users with the ability to control data collection and analysis on Agilent flow cytometer and associated accessories. The software contains features to control Quality Control (QC) test, sample acquisition, data analysis, and report generation.

Using this Guide

This guide contains an overview and information on using NovoExpress software. For information on the operation and maintenance of Agilent flow cytometer and associated accessories, please refer to associated operator's guide.

Keyboard Shortcuts

Shortcuts	Command
Overall Situation	
Ctrl + [Switch the active sample to the previous sample
Ctrl +]	Switch the active sample to the next sample
Ctrl + 1	Create a dot plot
Ctrl + 2	Create a density plot

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Ctrl + 3	Create a histogram
Ctrl + 4	Create a contour plot
Ctrl + 5	Create a cell cycle diagram
Ctrl + 6	Create a cell proliferation diagram
Ctrl + N	New file
Ctrl + O	Open the file
Ctrl + S	Save the file
Ctrl + W	Close the file
F3	Next sample without template
F4	Next sample
F5	Run / Stop / Run single well / Stop Single Well
F6	Run Plate / Stop
F7	Pause / Continue
F8	Restart
Plot	
Alt + 1	Change to dot plot
Alt + 2	Change the density plot
Alt + 3	Change to histogram
Alt + 4	Change to contour plot
Alt + 5	Change to cell cycle diagram
Alt + 6	Change to cell proliferation diagram
Alt + Q	Quick compensation
Ctrl + +	Zoom In
Ctrl + - (Minus Key)	Zoom Out
Ctrl + A	Auto Range
Ctrl + B	Bi-Range gates
Ctrl + C	Copy the plot or gate
Ctrl + D	Duplicate a plot or gate
Ctrl + E	Elliptical gate
Ctrl + F	Full Range
Ctrl + H	Range gates

Prologue

Ctrl + J	Freehand gate
Ctrl + L	Logic gates
Ctrl + M	Move
Ctrl + P	Polygonal gate
Ctrl + Q	Quadrant gate
Ctrl + R	Rectangular gate
Ctrl + T	Adjust threshold
Ctrl + V	Paste gate
Ctrl + Y or Ctrl + Shift + Z	Redo
Ctrl + Z	Undo
Experiment Manager	
Ctrl + C	Copy
Ctrl + D	Duplicate
Ctrl + V	Paste
F2	Rename

Glossary

Term	Definition
Absolute count	Number of cells or particles per unit volume. Agilent flow cytometer is a volumetric instrument, thus, exact volumes of acquired sample can be determined without the need for counting beads. After the dilution factor and unit of measure (default is # of events per μL) are defined by user, NovoExpress can display number of events within specified gate per unit volume in the statistical information chart.
Active sample	The sample currently displayed and being analyzed. The Cytometer Control and Cytometer Setting panels display basic information regarding the active sample. In the Experiment Manager panel, the active sample is indicated by a red arrow. To switch the active sample, double-click on a new sample in the Experiment Manager panel, use the Switch Active Sample buttons from the Sample tab of the Menu Bar , or use the combination of Ctrl + [or Ctrl +] on the keyboard to go backward or forward.
Adjust on Plot	A function to adjust threshold value on plot.

Prologue

Alias	Alternative and customizable name for fluorescence parameter.
Analysis	The process of plotting, gating, and comparing statistical information on collected parameters of samples.
Auto compensation	Use a specimen containing an unstained sample and single stained samples to automatically calculate a compensation matrix.
Auto Range	A function which allows user to display the range for the X-axis and Y-axis to fit the experimental data.
Backflush	Clear blockages in the sample pipeline.
Batch Print Reports	A function to print or create PDF of multiple specimen and sample reports.
Bi-Range Gate	Draws a gate with two ranges
Bi-Variate Plot	A matrix of plots with selected parameters plotted against each other.
Blank samples	Samples without collected event data. A blank sample must first be created before sample collection begins. Created blank samples contain the default settings from the previously created sample. See the icon for a blank sample in Hierarchy .
Cell cycle plot	Uses a histogram of DNA content to derive cell cycle phase populations based on curve fitting to the histogram.
Cell proliferation plot	Generates c modeling results to analyze different cell generations during the cell proliferation procedure.
Cleaning	Clear biological hazards that may exist in the pipeline.
Compensation Matrix	A matrix which contains each photo detector's value that is subtracted in order to determine the final calculated true fluorescence.
Contour plot	Two-parameter plot. Each axis can plot a parameter. The plot uses contour lines to indicate the density of populations on the plot.
Core Diameter	The diameter of hydrodynamic focusing which corresponds to the flow rate.
Debubble	Clear bubbles present in the sample line.
Decontamination	Decontaminate the instrument when it is known to have contamination or to prevent the occurrence of contamination
Density plot	Two-parameter plot. Each axis can plot a parameter. The color of a point on the plot will be an indicator of the number of events at that point.
Dot plot	Two-parameter plot. Each axis can plot a parameter. Multiple overlapping points will be displayed the same as a single point in a dot plot.
Elliptical Gate	Draws an elliptical gate

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Event	Refers to a particle that passes the acquisition threshold and has a set of data on intensity collected. Events are due to particles including microspheres and cells.
Experiment File	The NovoExpress Software saved experimental data files. A file can store experimental data from multiple samples.
Experiment Manager	The NovoExpress Software uses samples, specimens, and groups in a hierarchy structure to organize the experimental data, instrument settings, analysis and other information. The organization is displayed in the Experiment Manager panel.
Export to LIS	A function allows a user to export data analysis results in the designated format to CSV file and such CSV file can be parsed by a Laboratory Information System (LIS) or read by other programs.
Extensive Rinse	Extensively rinse tubing
FCS	Data file standard for flow cytometry. NovoExpress is compatible with FCS 3.0 and 3.1.
Fluidics maintenance sequences	A function to program and run multiple fluidics maintenance sequences in pre-defined order
Fluorescence compensation	Different fluorescent dyes emit different emission spectrums. When emission spectrums overlap, this is known as spectrum overlap. When the overlap occurs within a detection channel, fluorescence compensation can be used to mathematically compensate by removing the signal that does not belong in the channel.
Freehand Gate	Draws a freehand gate.
Free Unused File Space	When events of a sample or all samples are deleted, the file space is not automatically released. Click on this icon to free the file space. The file space can also be released by saving the file to the hard drive. Large files may take longer to release.
Full Range	Sets the full range of the X and Y-Axes.
Gain	A parameter corresponds to the output current of a photodetector for fluorescent signal detection.
Gate	Used to select a specific population of events. Gate types include rectangular, elliptical, polygonal, freehand, range, and bi-range gates. Gates can also be combined to create logic gates. Gates are used to further analyze specific populations.
Group	A part of the NovoExpress Software's hierarchy structure. The group can contain multiple specimens.
Heat Map	Heat map can be used to visualize the data in a well plate format. It uses different color to display the result of a specified statistical parameter.
Histogram Plot	Single-parameter plot. The X-axis will plot a parameter, and the Y-axis will plot the number of events.

Prologue

HT	Abbreviation for High Throughput.
Instrument settings	The settings include the sample, the stop condition, the sample flow rate, and the threshold settings.
Layer	The option to superimpose multiple plots to make a comparison. This tool is available with histograms and dot plots. The overlay plots then contain these superimposed layers.
LIS (Laboratory Information System)	Result of a statistical table and plots can be exported and parsed by LIS System)
Logic gate	The combination of individual gates using the logic operators AND, OR, or NOT to create logic gates.
Merge Samples	Merge two or more samples when they were collected from same instrument (i.e. same instrument serial number, and same optical configuration), with same data acquisition parameters selected in the Cytometer Setting panel, with same data acquisition mode (i.e. Absolute Count mode applied or not), and the total events from merged samples are less than 10 million.
Overlay	Display the data from multiple samples and gates in one plot with different colors.
Parameters	Refers to fluorescent or scattering intensity measurements. Parameters can be differentiated by the specific light channel or measurement type (height or area).
Plot	Tool for displaying sample information, including fluorescence or scattered light intensity. In the NovoExpress Software, plots include dot plots, density plots, histograms, contour plots and cell cycle diagrams.
Polygon Gate	Draws a polygon gate.
Post Gain	In certain situation, user may want to align a particular peak on different samples in the same plot. Post Gain function allows such adjustment performed after data acquisition.
Priming	Use after the instrument has been inactive for a long period of time to fill the tubing with fresh sheath fluid and clear any bubbles
Purge	If Agilent flow cytometer needs to be shipped, click this button and follow the procedure shown on the popup window to purge the fluidic system before packaging and shipment.
QC test	In QC test, Agilent Flow Cytometer QC Particles are used to check the instrument performance. Measured data are used to determine if the instrument parameters fall within a standard range to ensure stable and reliable operation of the instrument.
QC test reports	QC test reports contain parameters for individual QC test results, and it can also plot results over a period of time in the Levey-Jennings reports.

Prologue

Quick compensation	Use scrollbars in two-parameter plots to quickly adjust the fluorescence compensation of a sample.
Quadrant Gate	Draws an quadrant gate.
Range Gate	Draws a gate with single range.
Reagent Lots	Sets the type of the reagent in prompted window.
Rectangular Gate	Draws a rectangular gate
Report	Reports can be either a specimen report or a sample report. A sample report can contain plots, statistical information, compensation matrices, and collection information for the sample. A specimen report will contain information for all the samples included in the specimen.
Reset Layout	Resets the layout of the panels to the default layout.
Rinse	A function to rinse fluidic system.
Sample	The basic unit of experimental data organization. Samples contain the information from sample data collection, instrument settings, fluorescence compensation, reporting, analysis and data.
Sample currently in acquisition	Usually, sample currently in acquisition is same as active sample; however, they do not have to be same sample. After sample acquisition is started, user can switch active sample to other sample for analysis purpose. In Experiment Manager panel, sample currently in acquisition has a flash arrow (alternating green and dark green). If sample currently in acquisition is same as active sample, the arrow alternating red and green.
Sample flow rate	The flow rate of the sample can be used to control the number of events collected per second.
Specimen	A part of the NovoExpress Software's hierarchy structure. Specimen can be composed of many samples; multiple samples of the same test items can be placed in a specimen; a clinical specimen can correspond to a patient.
Spillover Matrix	A matrix contains value of spillover of each fluorochrome into each of the others. It is inversely related to the compensation matrix.
Statistical table	A customizable table of statistical information for batch data analysis. It can contain multiple samples, multiple gates, and statistical information for multiple parameters.
Stop condition	A defined number of events, length of time, or volume of collection, where sample collection is stopped immediately after reaching the condition.
Storage Gate	Filter out events outside the selected gate.
System Log	A window records information including user login and log out, and instrument operating activities including data acquisition, fluidics maintenance, etc.

Prologue

Technical Support Request	A function which automatically collects instrument configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of instrument.
Templates	Set contains the group, instrument settings, specimens and samples, fluorescence compensation, reporting, analysis, etc., can be saved as *.nct file format.
Threshold	The minimum value of defined parameters where if the signal is lower than the defined value, the data will be discarded. By setting an appropriate threshold value, the target events can be effectively captured. A threshold value too high will discard target events, while a threshold too low will include a large noise from small events being collected.
Transaction Log	A window which displays recorded information related to changes to the experiment file, such as Time, Computer, Software Version, User, and Action.
Unclog	Clear blockages in the flow cell.
Work List	Displays the samples as rows in a table. Sample settings are also listed and can be set in the table, including specimen name, sample name, parameters, stop condition, sample flow rate, threshold, compensation, and analysis is reporting information. Allows the user to quickly create and manage multiple samples.
Workspace	In the main interface of the NovoExpress Software, the middle area where plots are displayed.

2 Installation

This chapter describes the installation requirements, procedures to install, start and uninstall the NovoExpress software, NovoExpress license registration and dissociation, and user management feature.

Installation Requirements

Before installing the NovoExpress Software, ensure that your computer meets the following minimum requirements:

Hardware:

- Processor: 1 GHz
- Computer Memory: 2 GB
- Hard Drive: 10 GB free space
- Screen Resolution: 1024 X 768 pixels or higher

Software:

- Operating System: Windows 7 SP1/Windows 10
- PDF Reader Software

NOTE

To analyze high event-count samples (event count greater than 1 million), 8 GB memory and quad-core 2.5 GHz CPU are required.

Install NovoExpress Software

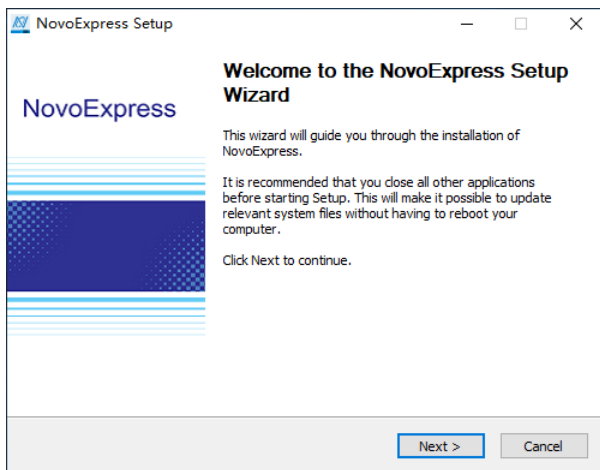
Install NovoExpress Software using the following instructions:

- 1 Download the NovoExpress Software installation package from website <https://www.agilent.com/chem/novoexpress> and unzip it. Double-click the

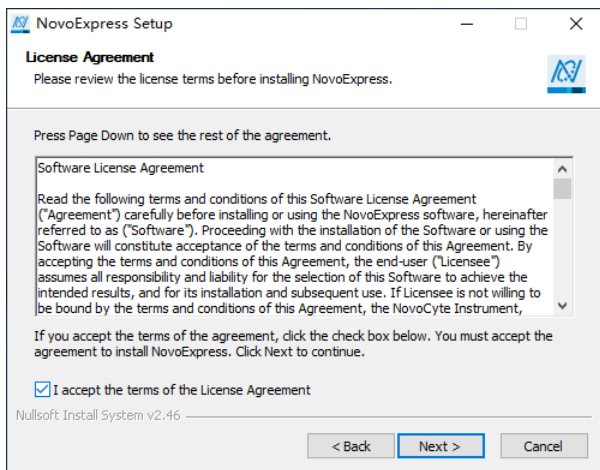
Installation

Setup.exe file in the NovoExpress installation directory to start the installation process.

- 2 The NovoExpress Software installation wizard will display. Click **Next** to continue.

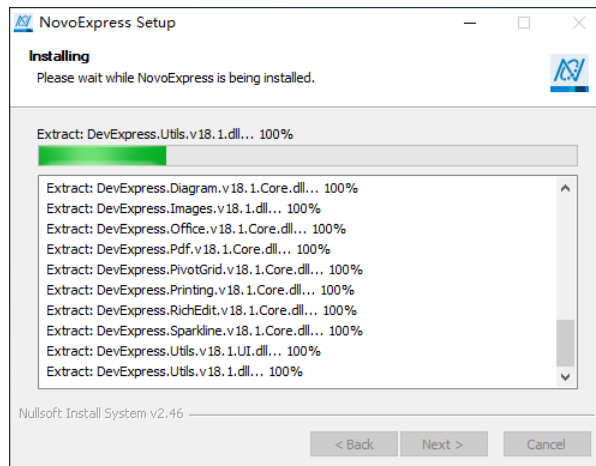
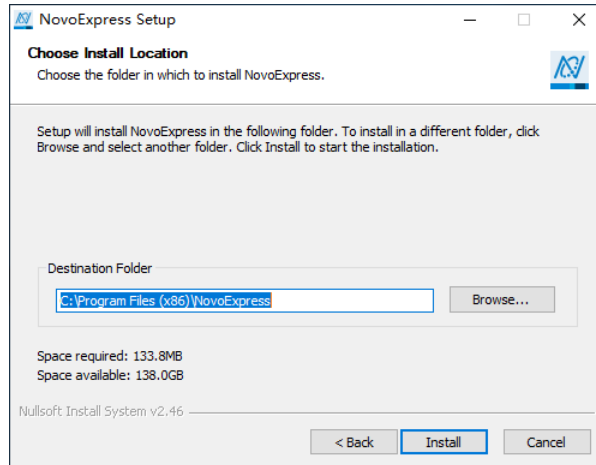


- 3 Please read the license agreement and accept by selecting the check box and clicking **Next**.



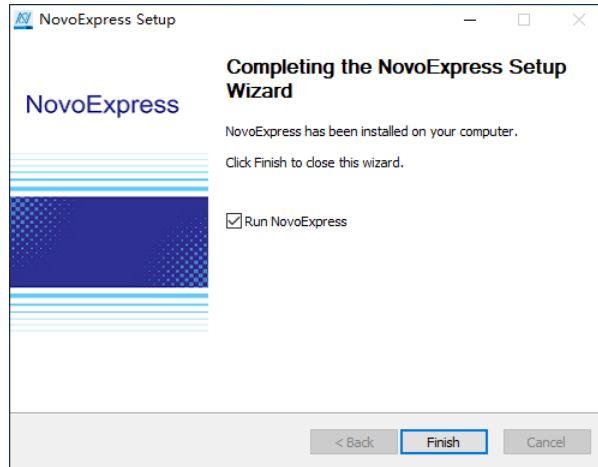
- 4 Choose the installation location. By default, the NovoExpress Software will be installed in **C:\Program Files (x86)\NovoExpress**. To install the software to another location, enter the target location or click **Browse** to select a destination folder. If the selected path does not exist, the installation wizard will automatically create the directory. After selecting the destination folder, click **Install** to continue.

Installation



- 5 After the installation is complete, click **Finish** to finish the installation and start the software. If you would not like to immediately start the software, uncheck the **Run NovoExpress** box, and click **Finish**.

Installation



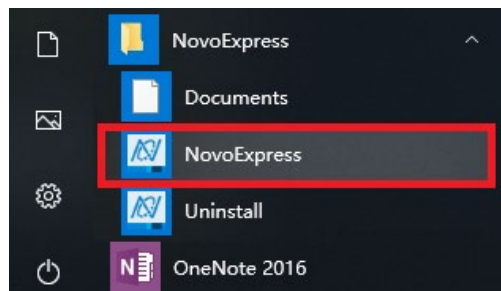
Start NovoExpress Software

After the successful installation of the NovoExpress Software, the program can be started by the following methods:

- **Desktop Shortcut**



- **Start Menu**



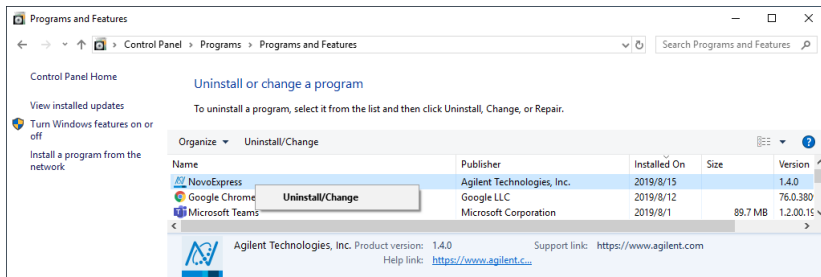
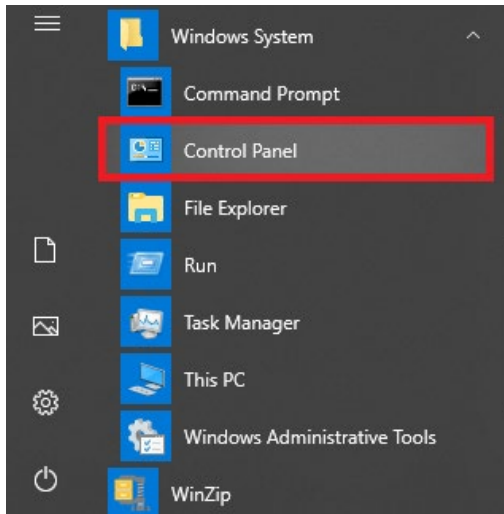
Uninstall NovoExpress Software

The NovoExpress Software can be uninstalled by the following methods:

- **Control Panel**

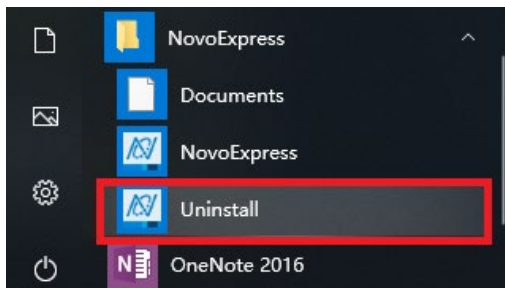
Start > Windows System > Control Panel > Programs and Features

In the new window, select **NovoExpress** and select **Uninstall/Change**.



- **Start Menu**

Installation



NovoExpress License

Free Trial

NovoExpress is available for a 30-day free trial. The **Register NovoExpress** window will automatically pop up every time starting the NovoExpress software during the trial expiration. Click the **Evaluate** button to start the free trial of **NovoExpress**.

A screenshot of the 'Register NovoExpress' dialog box. The window title is 'Register NovoExpress'. The text inside reads: 'Trial evaluation is valid for 28 days.' Below this, it says: 'Please input your NovoExpress License Key from Agilent to register NovoExpress on this computer.' There are two input fields: 'License Key:' and 'Email Address:'. At the bottom, there is a link for 'Offline Registration', a 'Register' button, and an 'Evaluate' button.

Registration will be needed to use the software after the trial expires. User can enter the NovoExpress Software License Key which comes with instrument. User can also purchase it separately from Agilent.

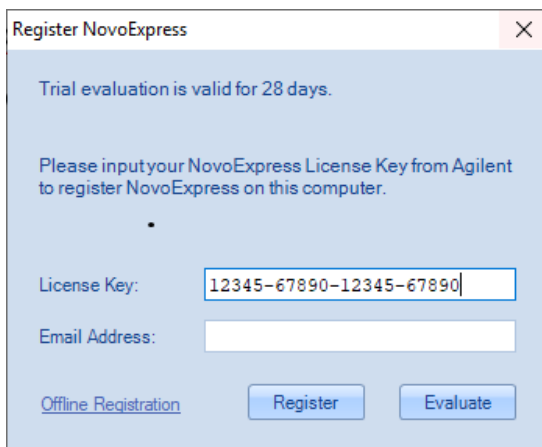
NovoExpress Registration

The **Register NovoExpress** window will automatically pop up when starting the NovoExpress software if the software is not registered yet. You can also click **File**

Installation

> **About** > **Register NovoExpress** to open the **Register NovoExpress** window. There are two ways to register NovoExpress.

- If the computer is connected to the internet, enter a valid Agilent issued license key and a valid email address and click the **Register** button to register NovoExpress.



Register NovoExpress

Trial evaluation is valid for 28 days.

Please input your NovoExpress License Key from Agilent to register NovoExpress on this computer.

•

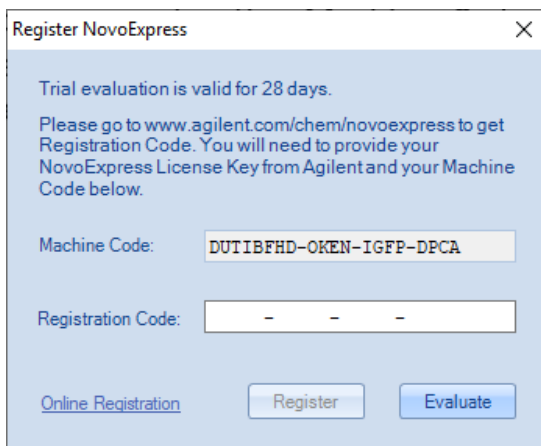
License Key:

Email Address:

[Offline Registration](#)

- If the computer is not connected to the internet, click the **Offline Registration** button to switch to offline registration mode. Write down the **Machine Code** displayed in the window and go to another computer that is connected to the internet and open the **Get Registration Code** web page (<https://www.agilent.com/chem/novoexpress>). On the web page, enter the **Machine Code** into the specified textbox, type a valid Agilent issued license key, and then click **Get Registration Code**. Write down the **Registration Code**, enter it to the **Register NovoExpress** window, and click **Register** button to register NovoExpress.

Installation

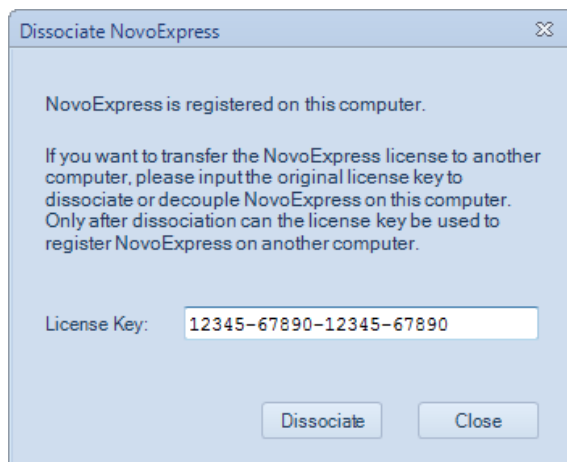


NOTE

A license key is required for NovoExpress software registration or license transfer. Software will automatically record the license key. However, it is still recommended to keep a safe record of license key assigned to each computer or user.

NovoExpress Dissociation

NovoExpress supports license transfer to another computer. Each license may be transferred up to 5 times. If you want to transfer the NovoExpress license to another computer, log in the software with **Administrator** account, click **File > About > Dissociate NovoExpress** to open the **Dissociate NovoExpress** window.



Installation

The license key recorded during software registration will automatically filled in the text box. User can click the **Dissociate** button to dissociate or decouple NovoExpress from this computer. After dissociation, the license key can be used to register NovoExpress on another computer.

NOTE

Please connect NovoCyte workstation to the internet when dissociating the license. Contact Agilent technical support for how to dissociate the license if no connection to the internet is available.

NOTE

Only the users with the **Administrator** privilege can conduct this license dissociation process.

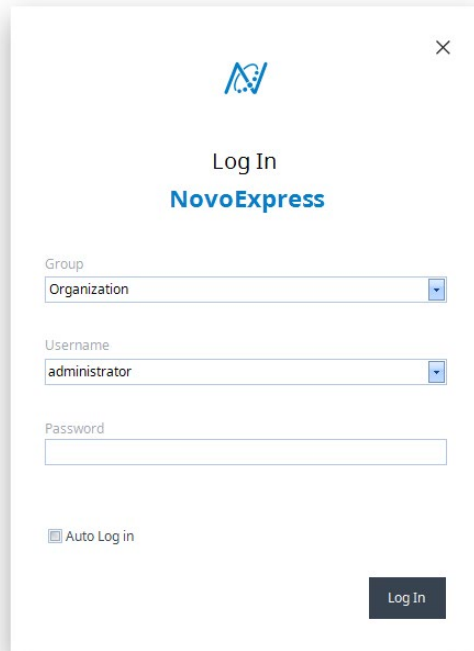
User Management

A user management feature is included with the NovoExpress Software allowing for separate user settings to be saved in different accounts.

When starting the NovoExpress Software, a login window will appear. By checking the **Auto Login** box, the software will automatically login with the associated user account and the login window will not appear in the future.

The NovoExpress Software initially includes a system administrator account with username as "**administrator**". The default password for this account is "**administrator**". This system administrator account has the highest privilege and the username "administrator" cannot be changed. This system administrator and users with the **Administrator** privilege can add, delete, and modify information for all the other users and user groups. There is no limit to the number of user groups or user accounts. Each user belongs to a specific user group. The methods for adding, modifying and deleting a user group are described in [User Groups](#). The methods for adding, modifying, and deleting a user account are described in [Users](#). A user can directly enter the username and password to log in the software, or select the specific user group first, select the username, and enter the correct password to log in the software.

Installation



The image shows a 'Log In' dialog box for NovoExpress. At the top center is the NovoExpress logo, a stylized 'N' with a blue and green gradient. Below the logo, the text 'Log In' is centered, followed by the 'NovoExpress' logo in a larger, bold font. The dialog contains three input fields: 'Group' with a dropdown menu showing 'Organization', 'Username' with a dropdown menu showing 'administrator', and 'Password' with an empty text box. Below these fields is a checkbox labeled 'Auto Log in'. At the bottom right is a dark grey button with the text 'Log In'. A close button (an 'X' icon) is located in the top right corner of the dialog.

User Groups

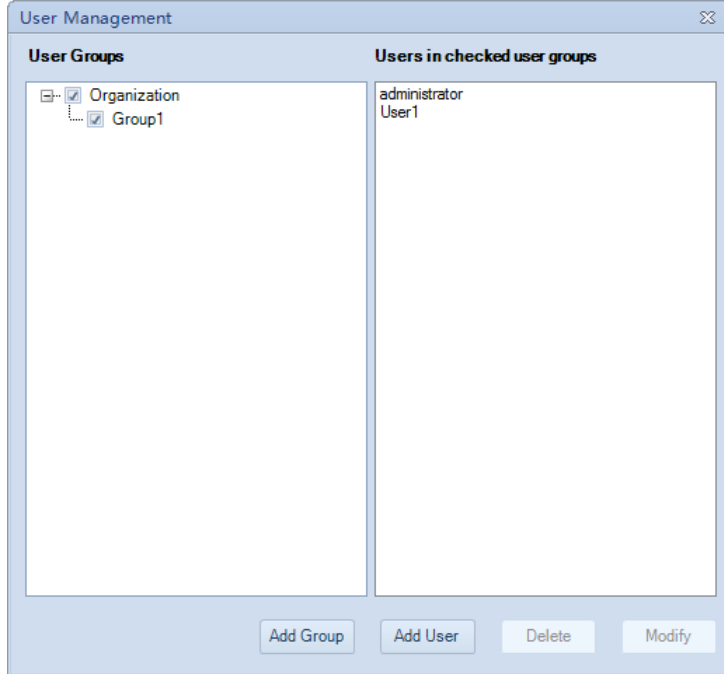
NovoExpress contains a user group management feature to allow groups of multiple users. Only users with **Administrator** privilege can add, modify, and delete user groups. The root parent group “**Organization**” is included by default. Users can only add group under this root group.

Add a User Group

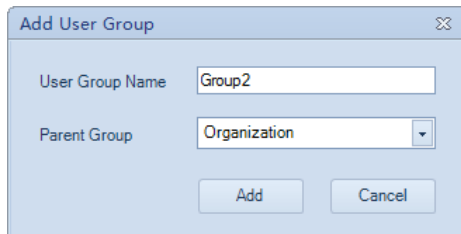
New user groups can only be added through an account with **Administrator** privilege. To add a new user group:

- 1 Log into the software using an account with **Administrator** privilege.
- 2 Select the **Setting** tab.
- 3 Click **User > Management**. The **User Management** window will appear.

Installation



- 4 Click **Add Group**. The **Add User Group** window will appear.



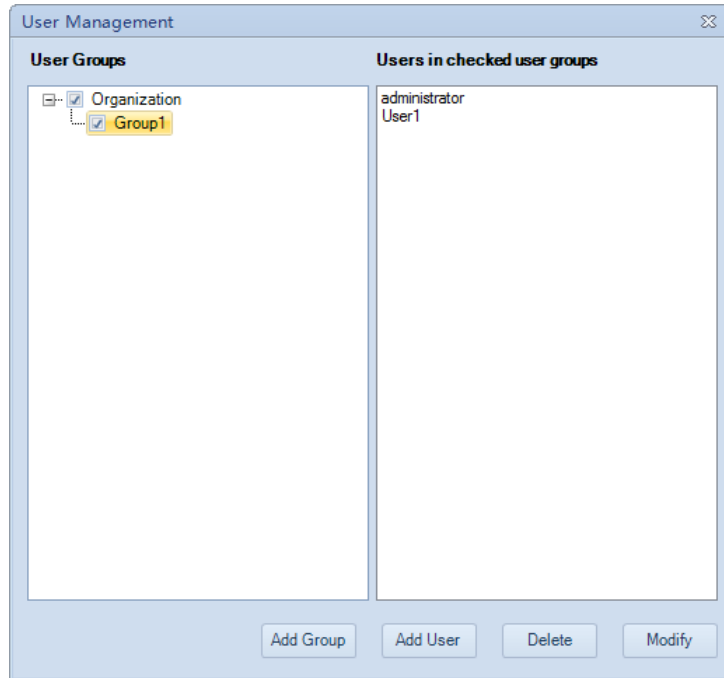
- 5 Enter the group name and select the desired parent group for the created user group in the prompted window.
- 6 Click **Add** and the new user group is created.

Modify a User Group

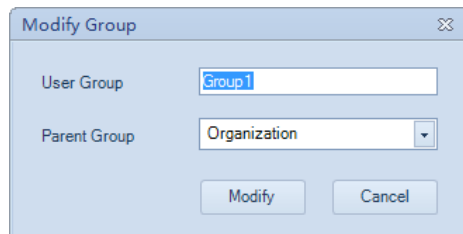
User groups can only be modified through an account with **Administrator** privilege. To modify a user group:

- 1 Log into the software using an account with **Administrator** privilege.
- 2 Select the **Setting** tab.
- 3 Click **User > Management**. The **User Management** window will appear.

Installation



- 4 Select the group to be modified. Click **Modify**. The **Modify Group** window will appear.



- 5 Modify the group name and select the desired parent group for the modified user group in the prompted window.
- 6 Click **Modify** and the user group is modified.

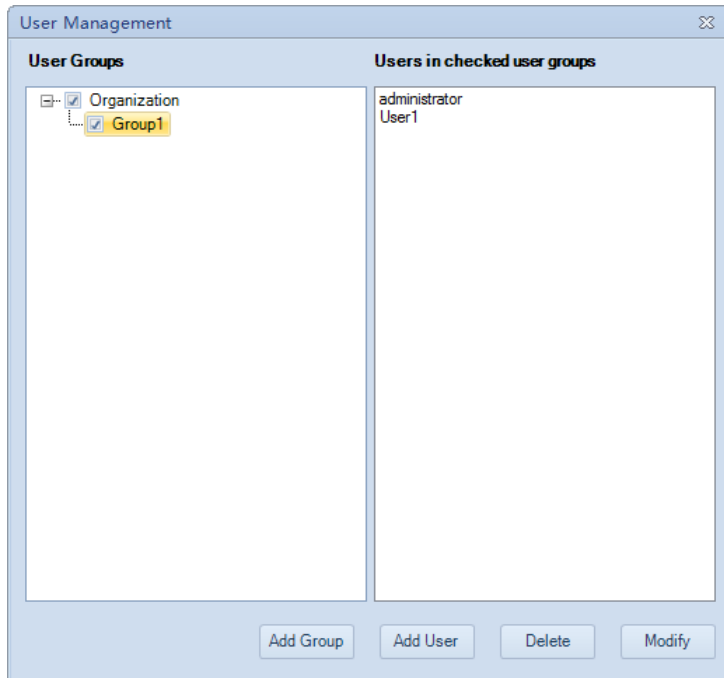
Delete a User Group

User groups can only be deleted through an account with **Administrator** privilege. To delete a user group:

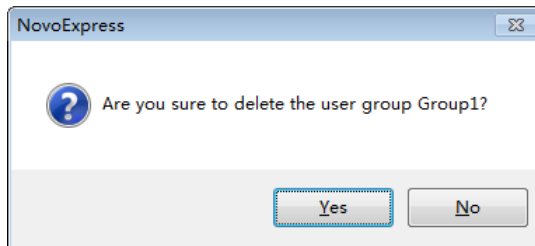
- 1 Log into the software using an account with **Administrator** privilege.
- 2 Select the **Setting** tab.

Installation

- 3 Click **User > Management**. The **User Management** window will appear. Select the group to be deleted. Click **Delete**.



- 4 Click **Yes** in the prompted window and the selected user group is deleted.



NOTE

When the selected user group is deleted, the user accounts in the group will automatically be moved to the deleted group's parent group.

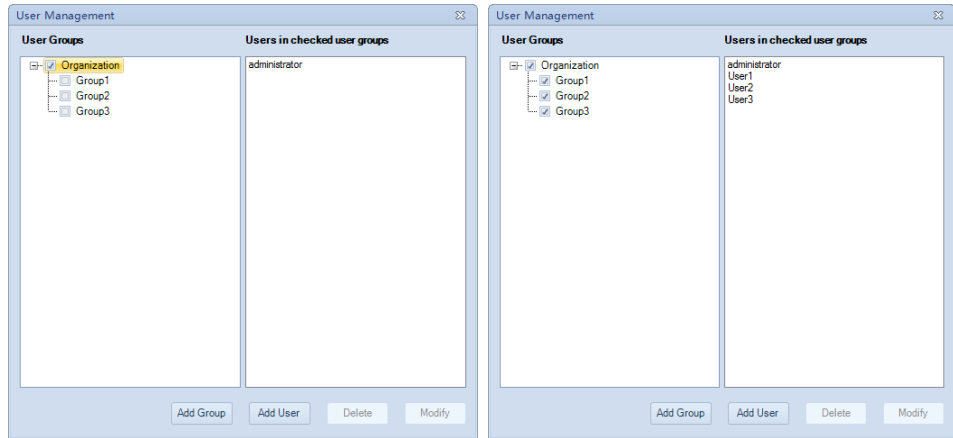
NOTE

The root parent group Organization cannot be deleted.

Installation

Display the Users in a User Group

The right half of the **User Management** window displays the users contained in the user groups with check box checked in the left half of the window. All displayed usernames are automatically listed in alphabetical order.



Users

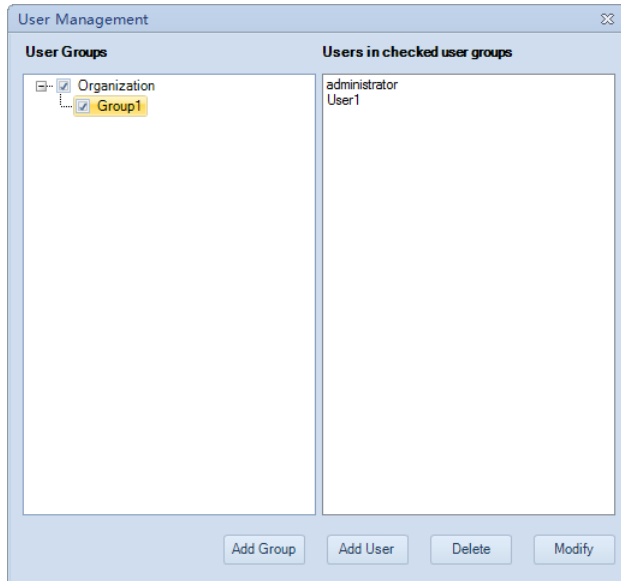
NovoExpress contains a user management feature to allow different settings and privileges for each user account. Only users with the **Administrator** privilege can add, modify, and delete user accounts.

Add a New User

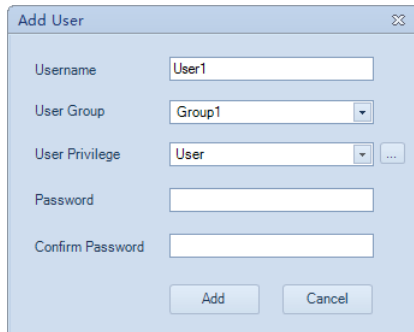
New users can only be added through an account with **Administrator** privilege. To add a new user:

- 1 Log into the software using an account with **Administrator** privilege.
- 2 Select the **Setting** tab.
- 3 Click **User > Management**. The **User Management** window will appear.

Installation



- 4 Select a user group. Click **Add User**. The **Add User** window will appear.



- 5 Enter username and password and assign user privilege (**User** or **Administrator**) for the created account in the prompted window.
- 6 Click **Add** and the new user account is created.

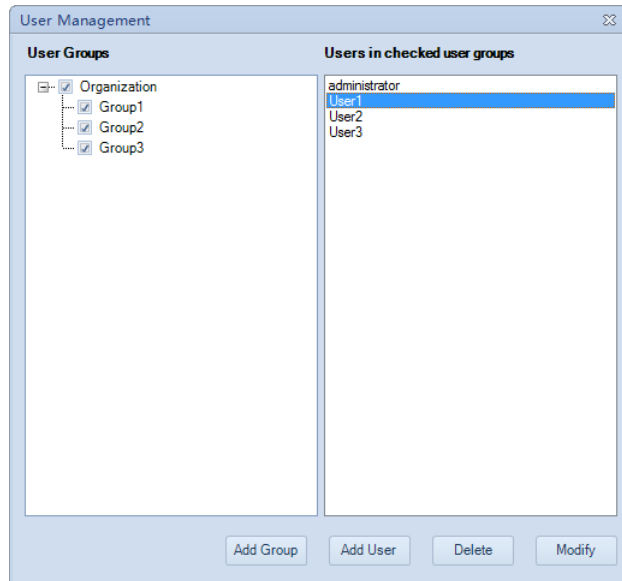
Modify User Information

Accounts with **Administrator** privilege can modify username, user privilege, access privilege, and password for each user, while other accounts can only modify its own username, user group name, and password.

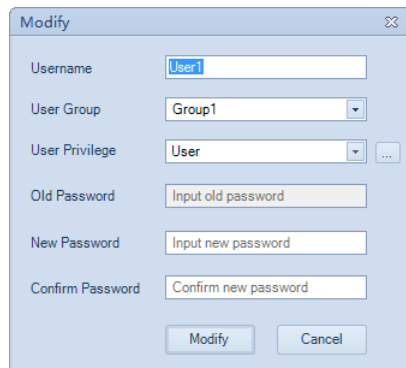
- From the administrator account or an account with **Administrator** privilege:
 - a After logging in, select the **Setting** tab.

Installation

- b** Click **User > Management**. The **User Management** window will appear. Select the user group, and then the user account you would like to modify.

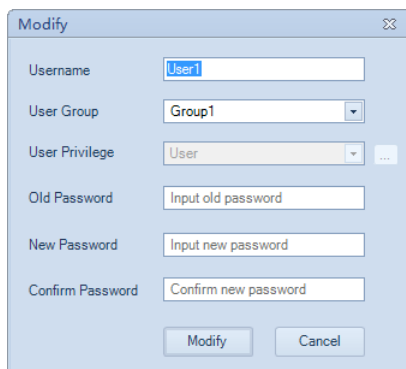


- c** Click **Modify**. The **Modify** window will appear allowing the user to make changes to the account.



- From individual user accounts:
 - a** After logging in, select the **Setting** tab.
 - b** Click **User > Modify**. The **Modify** window will appear allowing the user to make changes to the logged in account. Please note User Privilege and Access Privilege can only be modified by accounts with **Administrator** privilege.

Installation




The image shows a 'Modify' dialog box with the following fields and controls:

- Username:** Text input field containing 'User1'.
- User Group:** Dropdown menu showing 'Group1'.
- User Privilege:** Dropdown menu showing 'User' and a '...' button to its right.
- Old Password:** Text input field containing 'Input old password'.
- New Password:** Text input field containing 'Input new password'.
- Confirm Password:** Text input field containing 'Confirm new password'.
- Buttons:** 'Modify' and 'Cancel' buttons at the bottom.

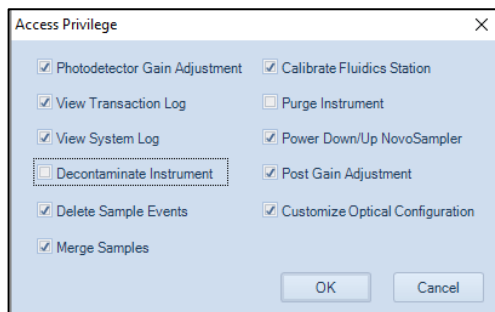
Access Privilege

Several functions in NovoExpress are only accessible to accounts with specified Access Privileges, including:

- **Photodetector Gain Adjustment**
- **View Transaction Log**
- **View System Log**
- **Decontaminate Instrument**
- **Delete Sample Events**
- **Merge Samples**
- **Calibrate Fluidics Station/Cart**
- **Purge Instrument**
- **Power Down/Up NovoSampler**
- **Post Gain Adjustment**
- **Customize Optical Configuration**

To modify the **Access Privilege** for users, click the  button in the **Modify** window and then select or unselect checkboxes in the popup **Access Privilege** window. Click **OK** to confirm changes.

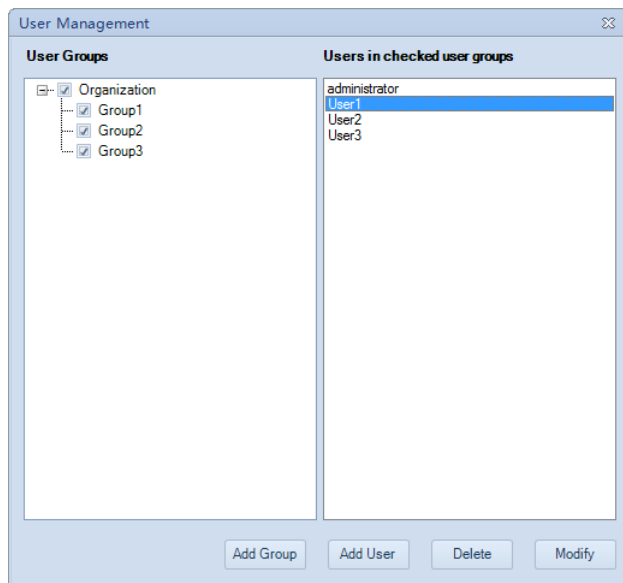
Installation



Delete a User

User accounts can only be deleted through an account with **Administrator** privilege. To delete a user account:

- 1 Login to the software as the administrator or an account with **Administrator** privilege.
- 2 Select the **Setting** tab.
- 3 Click **User > Management**. The **User Management** window will appear.



- 4 Select the user account that you would like to delete.
- 5 Click **Delete**.
- 6 In the confirmation window, click **Yes**.

3 Use NovoExpress Software

This chapter provides information needed to use NovoExpress software, including overview of NovoExpress software interface, Title Block, Menu, Workspace Toolbar, Cytometer Setting panel, Cytometer Control panel, Plate Manager panel, Experiment Manager panel, Cytometer Status panel, Gate Manager panel, Status Bar, Instrument Configuration window and Manage Plate Types window.

NovoExpress Software Interface

After starting the NovoExpress Software, the initial interface (i.e. main window) may look different depending on whether NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected.

- If NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected, user will see following interface which includes a Title Block, Menu, **Cytometer Setting** Panel, Toolbar, **Experiment Manager** Panel, **Cytometer Control** Panel, Workspace, and Status Bar.

Use NovoExpress Software

The screenshot displays the NovoExpress software interface with the following panels and data:

Cytometer Setting Panel:

Pa...	Alias	Gain	A	H
FSC	FSC	-	<input type="checkbox"/>	<input type="checkbox"/>
SSC	SSC	-	<input type="checkbox"/>	<input type="checkbox"/>
B530	CD3 FITC	826	<input type="checkbox"/>	<input type="checkbox"/>
B572	CD8 PE	546	<input type="checkbox"/>	<input type="checkbox"/>
B615	PI	398	<input type="checkbox"/>	<input type="checkbox"/>
B675	CD45 PerCP	300	<input type="checkbox"/>	<input type="checkbox"/>
B780	PECy7	505	<input type="checkbox"/>	<input type="checkbox"/>
R675	CD4 APC	300	<input type="checkbox"/>	<input type="checkbox"/>
R780	APC-Cy7	505	<input type="checkbox"/>	<input type="checkbox"/>

Work Space: Displays flow cytometry plots for Blood. The top row shows SSC-H (10⁶) vs FSC-H (10⁶) and SSC-H (10⁶) vs CD45 PerCP-H. The bottom row shows a histogram of CD3 FITC-H and a contour plot of CD45 PerCP-H vs CD3 FITC-H. Gated populations include GR (31.45%), MO (5.56%), LY (49.27%), CD3-CD4+ (0.14%), CD3-CD4 (41.34%), and CD3-CD4- (24.94%).

Gate Manager Panel:

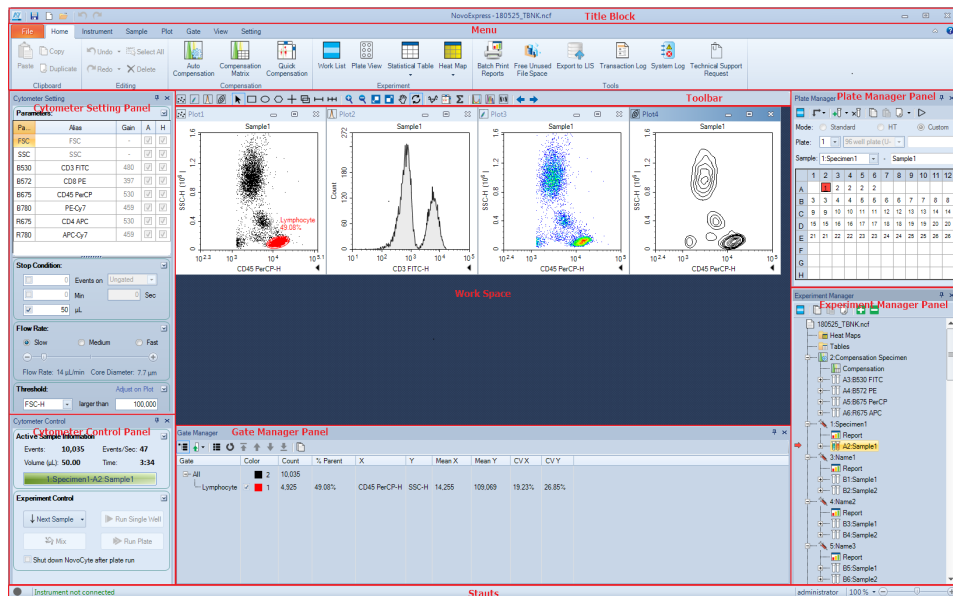
Gate	Color	Count	% Parent	X	Y	Mean X	Mean Y	CVX	CVY
All		8	10.035						
GR	3	3,160	31.49%	CD45 PerCP-H	SSC-H	3,207	1,017,885	29.33%	15.15%
MO	2	558	5.56%	CD45 PerCP-H	SSC-H	5,715	403,275	22.71%	15.27%
LY	1	4,944	49.27%	CD45 PerCP-H	SSC-H	14,272	109,519	19.19%	27.36%
CD3-CD4+	7	7	0.14%	CD3 FITC-H	CD4 APC-H	975	27,054	56.15%	48.79%
CD3-CD4-	6	1,680	33.58%	CD3 FITC-H	CD4 APC-H	7,125	40,802	34.06%	17.69%
CD3-CD4-	5	2,044	41.34%	CD3 FITC-H	CD4 APC-H	481	5,402	45.91%	22.28%
CD3-CD4-	4	1,233	24.94%	CD3 FITC-H	CD4 APC-H	6,113	5,240	38.80%	24.66%

Experiment Manager Panel: Shows a tree view of the experiment structure, including TBNK.ncf, Heat Maps, Tables, Compensation Specimen, Unstained, FITC, PE, PerCP, APC, and Report. The Report section includes CD3_CDR8_CD45_CD4, Human CD3 8 45 4, and Human CD3 16-56 45 19.

Status Bar: Instrument not connected. Administrator | 100% | 0

- If NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, user will see following interface. Compared to the software interface with instrument alone, differences are observed in the **Menu Bar**, **Cytometer Control** panel, **Experiment Manager** panel, and **Work List**. The **Plate Manager** panel is only visible when NovoSampler (or NovoSampler Pro or NovoSampler Q) is installed.

Use NovoExpress Software



Title Block

The **Title Block** displays the data file name in the center. It also provides options for opening, saving, and closing an experiment file.

NovoExpress - Untitled.ncf	
Icon	Description
	Clicking this button displays a drop-down menu with options to resize the display window and close the software.
	Saves the experiment file.
	Creates a new experiment file.
	Opens an experiment file.
	Undo drawing gates, deleting gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot related functions.

Use NovoExpress Software



Redo drawing gates, delete gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot related functions.

NovoExpress - Untitled.ncf

Displays the data file name (*.ncf).



Minimizes window.



Maximizes window.



Restores window.

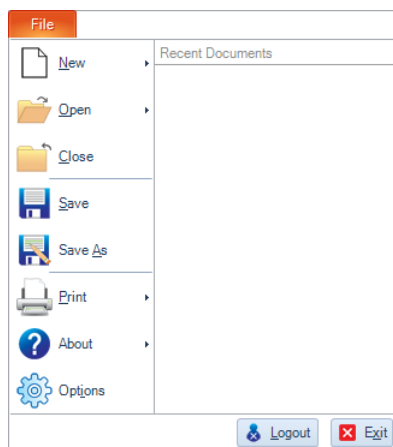


Closes window.

Menu

The **Menu** contains functions for instrument control and data analysis.

File

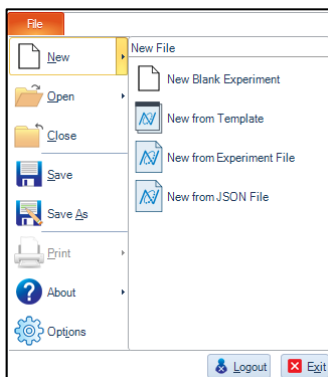


Icon	Description
------	-------------



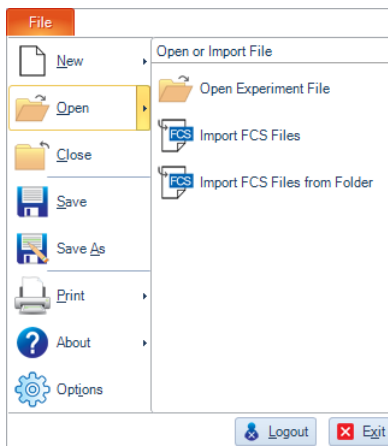
New experiment file

Use NovoExpress Software



- **New Experiment:** Creates a new experiment file.
- **New from Template:** Imports a template (*.nct) for the new experiment.
- **New from Experiment File:** Creates a new experiment file with the experiment setting and data analysis method inherited from an existing experiment file.
- **New from JSON File:** Creates a new experiment file with the experiment setting and data analysis method inherited from an existing JSON file. This option is only available when NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon is connected.

Open experiment (*.ncf) file



- **Open Experiment File:** Opens an experiment file.
- **Import FCS files:** Imports selected file to the current specimen.
- **Import FCS Files from Folder:** Imports all FCS files in a selected folder to the current FCS specimen.

Closes the current experiment file and creates a new experiment file.



Use NovoExpress Software



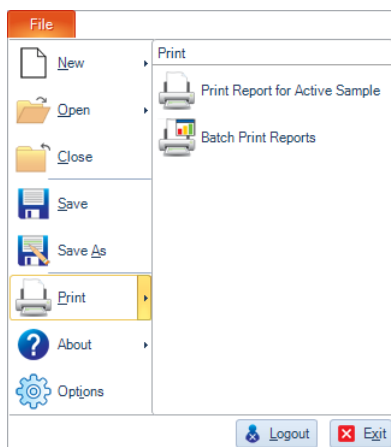
Saves the experiment data, experiment setup, and data analysis of the current experiment file.



Saves the current experiment file to a specified location.



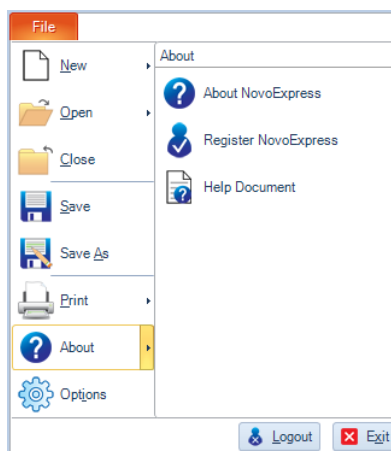
Prints sample report/batch print reports



- **Print Report for Active Sample:** Prints the report for the current sample.
- **Batch Print Reports:** Opens the **Batch Print Reports** window.

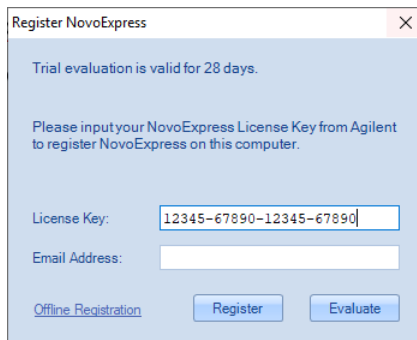


About: Contains the software version, copyright information, help files, and registration information.



- **About NovoExpress:** Displays the software version and copyright information.
- **Register NovoExpress:** Registers the NovoExpress software.

Use NovoExpress Software



- **Help Document:** Opens the help file (i.e. NovoExpress Software Guide).



Options: Opens the **Options** window. See [Setting](#) for details.

Recent Documents

Recent Documents: Shows recently opened data files. Up to 10 files can be displayed. Click the file name to directly open the corresponding file.

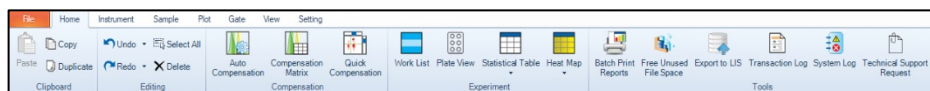


Logout: Exits and logs out of the current account. The login window will appear.



Exit: Exits the software.

Home



Icon	Description
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Clipboard



Copy: Copies the selected gate or selected data.



Paste: Pastes the copied gate to other plots or pastes the copied item in the **Experiment Manager**.



Duplicate: Creates a duplicate of the plot (the gates will not be included.) or creates a duplicate sample or specimen.

Editing



Undo drawing gates, deleting gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot-related functions.

Use NovoExpress Software



Redo drawing gates, delete gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot-related functions.



Select All: Selects all the gates in the current plot.



Delete: Deletes the selected gate.

Compensation



Auto Compensation: Opens the **Automatic Compensation Settings** window.



Compensation Matrix: Displays the current compensation matrix for the selected sample.



Quick Compensation: Displays the Compensation scrollbars on the active plot for quick compensation.

Experiment



Work List: Users may view and edit the **Work List**.



Plate View: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. User can view the sample information for each well in the prompted window.



Statistical Table: Creates a statistical analysis table.



Heat Map: Creates a heat map for the defined parameter in a plate layout format.

Tools



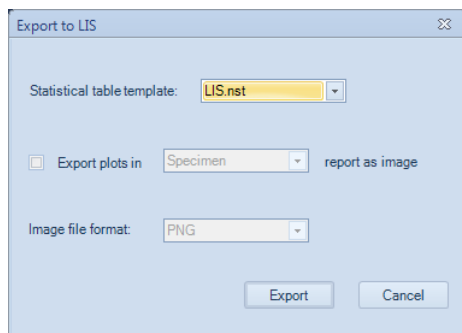
Batch Print Reports: Prints or creates PDF of multiple test reports.



Free Unused File Space: When events of a sample or all samples are deleted, the file space is not automatically released. Click on this icon to free the file space. The file space can also be released by saving the file to the hard drive. Large files may take longer to release.



Export to LIS:



This function allows a user to export data analysis results in the designated format to CSV file and such CSV file can be parsed by a Laboratory Information System (LIS) or read by other programs.

- **Statistical table template:** Sets the proper statistical table template to export the data. To add a statistical table template, export the statistical table as a template to the default folder "**User data root folder\Statistical Table Templates**". Refer to [Statistical Table Management](#) for details.
- **Export plots in Specimen/Sample report as image:** Once selected, plots in the specimen report or sample report will be exported as images in the selected image file format to the designated folder.
- **Image file format:** Sets the image file format of the export plots, including PNG, JPEG, Bitmap, GIF and TIFF format.
- **Export:** Exports the data and the plots into designated folders. The data will be exported into *.csv file in UTF-8 code. * is the same as the experiment name. The created csv file will be automatically saved in "**User data root folder\LIS Results**" folder, and plots will be saved in "**User data root folder\LIS Plots**" folder. The default user data root folder is "**D:\NovoExpress Data\administrator**" and can be changed. Refer to [Setting](#) for details of changing the default user data root folder.



Transaction Log: Displays the **Transaction Log** window. Records can be filtered by **Time**, **Computer**, **User**, and **Action**. Only accounts with the **View Transaction Log** privilege can access the **Transaction Log**.

Use NovoExpress Software

Transaction Log

Time From: 11/27/2013 5:09:38 PM To: 2/26/2014 9:23:09 AM

Computer: All User: All Action: All

	Time	Computer	Software Version	User	Action
1	11/27/2013 5:09:38 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (1)
2	11/27/2013 5:10:37 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample settings (1)
3	11/27/2013 5:10:58 PM	WIN-TM100TLF99T	1.0.0	User1	Add sample events (1)
4	11/27/2013 5:13:48 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (2)
5	11/27/2013 5:13:48 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample analysis (1)
6	11/27/2013 5:13:48 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample settings (1)
7	11/27/2013 5:14:11 PM	WIN-TM100TLF99T	1.0.0	User1	Add sample events (2)
8	11/27/2013 5:38:14 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (3)
9	11/27/2013 5:38:14 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample analysis (1,2)
10	11/27/2013 5:38:14 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample settings (2)
11	11/27/2013 5:38:47 PM	WIN-TM100TLF99T	1.0.0	User1	Add sample events (3)
12	11/27/2013 5:40:33 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (4)
13	11/27/2013 5:40:33 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample analysis (3)
14	11/27/2013 5:40:54 PM	WIN-TM100TLF99T	1.0.0	User1	Add sample events (4)
15	11/27/2013 5:45:28 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (5)
16	11/27/2013 5:45:28 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample analysis (2,4)
17	11/27/2013 5:45:28 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample settings (4)
18	11/27/2013 5:45:50 PM	WIN-TM100TLF99T	1.0.0	User1	Add sample events (5)
19	11/27/2013 5:49:36 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (6)
20	11/27/2013 5:49:36 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample analysis (4,5)
21	11/27/2013 5:49:36 PM	WIN-TM100TLF99T	1.0.0	User1	Update sample settings (2,4,5)
22	11/27/2013 5:49:59 PM	WIN-TM100TLF99T	1.0.0	User1	Add sample events (6)
23	11/27/2013 5:51:31 PM	WIN-TM100TLF99T	1.0.0	User1	Create sample (7)



System Log: Displays the **System Log** window. Only accounts which have the **View System Log** privilege can access the **System Log**. The **System Log** window records information including user login and log out, and instrument operating activities including data acquisition, fluidics maintenance, etc.

System Log

Time From: 2018/5/4 To: 2018/6/4 Category: All

User Group: All User: All Event: All

Query Export

Session	Category	Time	Software Version	User Group	User	Event	Comments	
1	49	Information	2018/6/4 16:21:48	1.3.0	Organization	administrator	Open experiment file	D:\NovoExpress Data\Administrator\Exp...
2	49	Information	2018/6/4 16:21:45	1.3.0	Organization	administrator	User login	
3	49	Information	2018/6/4 16:21:41	1.3.0	Organization	administrator	Software start	Instrument disconnected
4	48	Information	2018/6/4 16:21:36	1.3.0	Organization	administrator	Software ext	
5	48	Information	2018/6/4 16:21:36	1.3.0	Organization	administrator	User logout	00:10:43
6	48	Information	2018/6/4 16:21:36	1.3.0	Organization	administrator	Close experiment file	D:\NovoExpress Data\Administrator\Exp...
7	48	Information	2018/6/4 16:16:42	1.3.0	Organization	administrator	Save experiment file	D:\NovoExpress Data\Administrator\Exp...
8	48	Information	2018/6/4 16:10:53	1.3.0	Organization	administrator	User login	
9	48	Information	2018/6/4 16:10:49	1.3.0	Organization	administrator	Software start	Instrument disconnected
10	47	Information	2018/6/4 16:10:48	1.3.0	Organization	administrator	Software ext	User logout
11	47	Information	2018/6/4 16:10:47	1.3.0	Organization	administrator	User logout	00:07:33
12	47	Information	2018/6/4 16:03:14	1.3.0	Organization	administrator	User login	
13	47	Information	2018/6/4 16:03:08	1.3.0	Organization	administrator	Software start	Instrument disconnected
14	46	Information	2018/6/1 17:02:46	1.3.0	Organization	administrator	Software ext	
15	46	Information	2018/6/1 17:02:46	1.3.0	Organization	administrator	User logout	00:20:02
16	46	Information	2018/6/1 16:42:44	1.3.0	Organization	administrator	User login	
17	46	Information	2018/6/1 16:42:40	1.3.0	Organization	administrator	Software start	Instrument disconnected
18	45	Information	2018/6/1 16:42:19	1.3.0	Organization	administrator	Software ext	
19	45	Information	2018/6/1 16:42:19	1.3.0	Organization	administrator	User logout	00:12:44
20	45	Information	2018/6/1 16:29:35	1.3.0	Organization	administrator	User login	
21	45	Information	2018/6/1 16:29:30	1.3.0	Organization	administrator	Software start	Instrument disconnected
22	44	Information	2018/6/1 16:21:10	1.3.0	Organization	administrator	Software ext	

Total: 410 Page: 1 / 3 First Page Previous Page Next Page Last Page Current Page: 1 Go



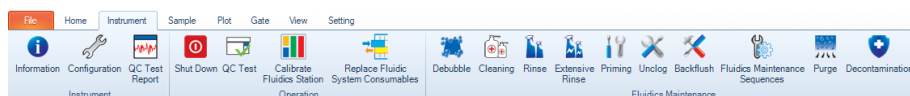
Technical Support Request: **Technical Support Request Creation Wizard** automatically collects instrument configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting

Use NovoExpress Software

of instrument. You can also attach any other files using this function. Refer to Troubleshoot in this guide for details.

Instrument

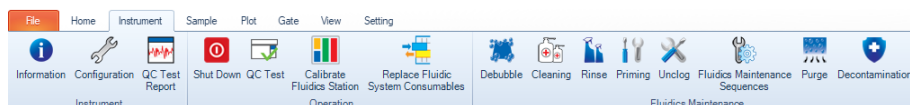
This panel allows user to view the instrument configuration, conduct instrument operation and maintenance. The availability of these functions depends on the model of instrument connected to the workstation. When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, additional functions are available.



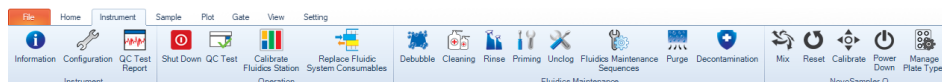
When NovoCyte flow cytometer is connected




When NovoCyte flow cytometer and NovoSampler (or NovoSampler Pro) are connected



When NovoCyte Quanteon (or Advanteon or Penteon) flow cytometer is connected



When NovoCyte Quanteon (or Advanteon or Penteon) flow cytometer and NovoSampler Q are connected

Icon	Description
Instrument	
	<p>Information: Click to display the instrument information.</p> <ul style="list-style-type: none"> When NovoCyte flow cytometer is connected, following window is prompted. User can view the hardware information (e.g. Product Model) for the instrument and fluidics station (or cart).

The screenshot shows a dialog box titled "Instrument Information" with a close button (X) in the top right corner. The "Cytometer" tab is selected, and the "Fluidics Station" sub-tab is active. The dialog displays the following information:

Product Name:	NovoCyte
Product Model:	3000
Optical Configuration:	3000
Serial Number:	451141011615
System Firmware Version:	V4.7.9E
FPGA Firmware Version:	Ver05.13.40.1B
CPU Firmware Version:	V3.14.7.5
CPU Firmware Type:	Release
CPU Firmware Build Date:	Aug 12 2019
Hardware Version:	V3.4
PCB Version:	V3.3
Fluidics Control Sequence Version:	V4.1.0
Working Time of the Fluidic System Consumables:	0 hours
Fluidic System Consumables Replacement Date:	2020/3/19 14:01:43

- When NovoCytometer Quanteon and Advanteon flow cytometer is connected, following window is prompted. User can view the hardware information (e.g. Product Model) for the instrument, AD boards, COM board and fluidics station (or cart).

The screenshot shows a dialog box titled "Instrument Information" with a close button (X) in the top right corner. The "Cytometer" tab is selected, and the "Advanteon" sub-tab is active. The dialog displays the following information:

Product Name:	NovoCytometer Advanteon
Product Configuration:	V8B7R4
Optical Configuration:	V8B7R4
Serial Number:	711191110136
System Firmware Version:	V3.4.3A
FPGA Firmware Version:	V09.01.02.22
CPU Firmware Version:	V3.4.3
CPU Firmware Type:	Release
CPU Firmware Build Date:	Aug 9 2019
Hardware Version:	V2.0
PCB Version:	V5.0
Fluidics Control Sequence Version:	Release_3.0.0
Working Time of the Fluidic System Consumables:	52 hours
Fluidic System Consumables Replacement Date:	2020/6/8 13:14:32

Use NovoExpress Software

- When NovoCyte Penteon flow cytometer is connected, following window is prompted. User can view the hardware information (e.g. Product Model) for the instrument, AD boards, COM board, Laser board and fluidics station (or cart).

Field	Value
Product Name:	NovoCyte Penteon
Product Configuration:	U7V7B6Y6R4
Optical Configuration:	U7V7B6Y6R4
Serial Number:	731200300039
System Firmware Version:	V4.0.1B
FPGA Firmware Version:	V09.02.02.2C
CPU Firmware Version:	V4.0.1
CPU Firmware Type:	Release
CPU Firmware Build Date:	Jun 29 2020
Hardware Version:	V2.0
PCB Version:	V5.0
Fluidics Control Sequence Version:	Debug_3.3.2
Working Time of the Fluidic System Consumables:	52 hours
Fluidic System Consumables Replacement Date:	2020/6/8 13:14:32



Configuration: Displays and modifies the excitation laser and detection channel and options for the NovoSampler, NovoSampler Pro or NovoSampler Q. See [Instrument Configuration](#) for details.



QC Test Report: Displays results from QC tests. The results can be viewed individually or plotted over a time interval in a Levey-Jennings chart.

Operation



Shut Down: Starts the shutdown process. After completion, the instrument will automatically turn off. To clean sample injection probe while shutting down instrument, see associated instrument operator's guide for more information.



QC Test: Runs the instrument QC test.



Calibrate Fluidics Station (or Fluidics Cart): Long distance transportation, movement and other reasons may cause the fluidics station (or Fluidics Cart) system malfunction. Use this function to re-calibrate the fluidics station (or Fluidics Cart). After clicking the button, it will ask to remove the instrument reagent containers from the fluidics station (or Fluidics Cart) before calibration. Only accounts with the **Calibrate Fluidics Station (or Fluidics Cart)** privilege can calibrate the fluidics station (or Fluidics Cart).



Replace Fluidic System Consumables: Click the button to open the **Replace Fluidic System Consumables** window and follow the instructions to replace fluidic system consumables. See associated instrument operator's guide for more information.

NOTE

Agilent flow cytometer monitors the accumulated running time of the fluidic system consumables to ensure the consumables are changed in timely manner for optimal flow cytometry results. When the accumulate

running time is reached, NovoExpress software will prompt a message to remind the users to replace the consumables.



Schedule Power On & Off: Click this button to open the **Schedule Power On & Off** window. User can schedule the instrument to be powered on or off at user defined time with user defined frequency. This function is available only when NovoCyte Quanteon, Advanteon or Penteon flow cytometer is connected.

- **Start Date:** Select the start date of the instrument power on and off. The start date cannot be set to 7 days or more over the current date.
- **End Date:** Select the end date of the instrument power on and off.
- **Start Time:** Select the start time of instrument power on or off.
- **Recur On:** Select the recurring frequency of instrument power on or off.
- **Add:** Click to add the selected start time and recurring frequency to the schedule table.
- **Remove:** Click to remove the selected schedule from the schedule table.
- **Set:** Click to confirm and make the schedule effective.
- **Clear:** Click to clear the schedule.

After the scheduled power on and off was executed, and the software is launched with the instrument properly connected, following **Power On & Off Timing Log** window will pop up. User can view the date, status and remark of the executed power on and off function in this window.

- **Start Date:** Select the start date of the instrument power on and off. The start date cannot be set to 7 days or more over the current date

Use NovoExpress Software

Fluidics Maintenance



Debubble: Removes the air bubbles from the fluidic system.



Cleaning: Uses a cleaning solution to decontaminate the biohazards that may exist in the fluidic system.



Rinse: Rinses the fluidic system using a rinsing solution.



Extensive Rinse: Performs an extensive rinse of the fluidic system. This function is available only when NovoCyte flow cytometer is connected.



Priming: After the instrument has not been in use for a period of time, this function clears the air bubbles and primes the fluidic system with fresh sheath fluid.



Unclog: Clears blockage from the flow cell.



Backflush: Clears blockage from the sample injection probe. This function is available only when NovoCyte flow cytometer is connected.



Fluidics Maintenance Sequences: program and run multiple fluidics maintenance sequences in pre-defined order.

Fluidics Maintenance Sequence

Fluidics Maintenance Type:

Iterations:

	Fluidics Maintenance Type	Total Iterations	Finished Iterations
▶ 1	Debubble	10	0
2	Cleaning	10	0
3	Rinse	10	0
4	Priming	10	0
5	Unclog	10	0

Shut down instrument after finishing the selected fluidics maintenance sequences

- **Fluidics Maintenance Type:** Select fluidics maintenance type (e.g. **Cleaning, Rinse, Extensive Rinse, Priming, Unclog, Backflush** for NovoCyte, and **Cleaning, Rinse, Priming, Unclog** for NovoCyte Quanteon, Advanteon and Penteon)
- **Iterations:** Set the number of iterations for the selected fluidics maintenance sequence.
- **Add:** Click to add the selected fluidics maintenance sequence to the table.
- **Remove:** Click to remove the selected fluidics maintenance sequence from the table.
- **Total Iterations:** The total number of iterations.
- **Finished Iterations:** The number of completed iterations.
- **Shut down instrument after finishing the selected fluidics maintenance sequences:** Check to shut down the instrument after finishing the selected fluidics maintenance sequences.
- **Start:** Click to start the fluidics maintenance sequences in the pre-defined order (top to bottom).
- **Stop:** Click to stop the fluidics maintenance sequences. The fluidics maintenance sequence currently running will continue until completed, but the remaining scheduled fluidics maintenance sequences will be aborted.
- **Continue:** Click to continue the remaining fluidics maintenance sequences which was not completed.



Purge: If Agilent flow cytometer needs to be shipped, click this button and follow the procedure shown on the popup window to purge the fluidic system before packaging and shipment. Only accounts with the **Purge Instrument** privilege enabled can perform the purge operation. Refer to [Purge Fluidic System before Shipment](#) in the associated instrument operator's guide for detailed procedure.



Decontamination: If Agilent flow cytometer is known to have contamination or to prevent the occurrence of contamination, click this button and follow the instruction shown in the popup window to decontaminate the instrument. Only accounts with the **Decontaminate Instrument** privilege can perform this decontamination operation. Refer to [Decontamination](#) in the associated instrument operator's guide for detailed procedure.

NovoSampler (or NovoSampler Pro or NovoSampler Q)



Mix: Click to mix the sample. The **Mix** button from the **Cytometer Control** panel has the same function.



Reset: Click to move the sample tray to the home position. This will allow a reset of the NovoSampler (or NovoSampler Pro or NovoSampler Q) in case of misalignment or error



Calibrate: Click to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q) with the instrument.



Power Down/Up: When clicked, NovoSampler (or NovoSampler Pro or NovoSampler Q) will be powered on (if currently powered off) and allow the operation of the NovoSampler (or NovoSampler Pro or NovoSampler Q). After execution, the status indication LED on NovoSampler (or NovoSampler Pro or NovoSampler Q) will light up in green color. The NovoExpress will prompt a message to restart the software. After the software restart, a dialog box requesting to calibrate the NovoSampler (or

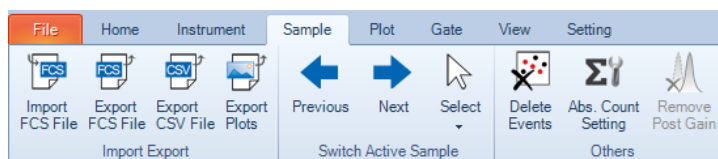
Use NovoExpress Software

NovoSampler Pro or NovoSampler Q) will be shown. Click **Calibrate** to start the automatic calibration procedure. The software features for control and use of the NovoSampler (or NovoSampler Pro or NovoSampler Q) will be available after restart of the NovoExpress Software. If the NovoSampler (or NovoSampler Pro or NovoSampler Q) is already powered on, clicking this button will power off the NovoSampler (or NovoSampler Pro or NovoSampler Q), the status indication LED on NovoSampler (or NovoSampler Pro or NovoSampler Q) will be off. The NovoExpress will prompt a message to restart the software. After the software restart, only the instrument stand-alone features will be available. This feature is only visible when logged into NovoExpress Software with the account with **Power Down/Up NovoSampler** privilege.



Manage Plate Types: Click to add, remove, and modify plate settings in prompted window. Refer to [Manage Plate Types](#) in this guide for more details.

Sample



Icon	Description
------	-------------

Import and Export



Import FCS File: Users may select FCS format files to import.



Export FCS File: Exports the current data into a FCS file.



Export CSV File: Exports the current data into a CSV file.



Export Plots: Exports the plots of the active sample into files in PNG, JPEG,

Switch Active Sample



Previous: Switches the Active Sample to the previous sample.



Next: Switches the Active Sample to the next sample.



Select: Switches to an Active Sample from the prompted drop-down menu.

Others



Delete Events: Deletes events from a sample. Users may select events inside a gate, outside a gate, or all events to delete. If the threshold or the photodetector gain has been adjusted during data acquisition, the **Prior to last threshold or photodetector gain change** radio button will be available to allow event deletion before the last adjustment. Only accounts with the **Delete Sample Events** privilege can perform this operation.

Delete Events of Sample1

Events to be deleted:

All

Prior to last threshold or Photodetector gain change

Outside gate R1

Inside gate

Delete Cancel

NOTE

After deleting the events from a sample, the file size does not automatically decrease. When saving the file, the software will prompt a window, asking if the unused file space is to be released or not. To manually release the unused space, refer to [Home](#) in this guide for details.



Absolute Count Setting: Sets up absolute counting conditions for active sample.

Absolute Count Setting - Sample1

Dilution Factor: 1 : 1

Absolute Count Unit: No./ μ L No./mL No./L

Set as Default Show Absolute Count in Statistics

Apply to All Samples in the Experiment File

Apply to All Samples in the Same Specimen

OK Cancel

- **Dilution Factor:**
The **Dilution Factor** is conversion coefficient used to calculate the absolute counting results for the original sample. For instance, if the original sample is diluted 10 times and is run on the instrument, enter 1:10 in the Dilution Factor. NovoExpress software will show the absolute counting results for the original sample by multiplying the concentration of the sample run on instrument by 10.
- **Absolute Count Unit:**
The Absolute Count Unit is parameter to set the unit for the absolute counting. User can select one of the units (i.e. No./ μ L, No./mL, or No./L) to present

Use NovoExpress Software

absolute count result for number of interested particles per microliter, per milliliter, or per liter.

- **Set as Default:**

Set the Absolute Count setting (i.e. Dilution Factor and Absolute Count Unit) as default for new samples.

NOTE

Changing the settings in **File > Options > Absolute Count** tab also sets the settings as default.

- **Show Absolute Count in Statistics:**

Show **Absolute Count** column in the statistical table of the plots in the workspace.

NOTE

There are three states of the checkbox:

- : Show **Absolute Count** column for all plots of the sample.
- : No change.
- : Hide **Absolute Count** column for all plots of the sample.

- **Apply to All Samples in the Experiment File:**

Set Absolute Count Setting for all samples in the experiment file.

- **Apply to All Samples in the Same Specimen:**

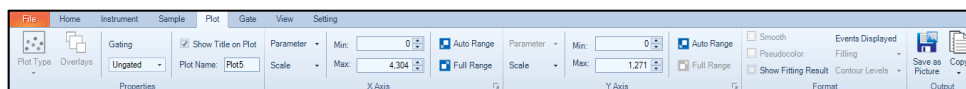
Set Absolute Count Setting for all samples belonging to the same specimen as the active sample.

Further information on absolute count calculations are described in [Calculation of Statistics](#).



Remove Post Gain: Click this button to remove Post Gain of all parameters of the Active Sample. Only accounts with the **Post Gain Adjustment** privilege can perform this operation. To adjust Post Gain, refer to [Post Gain](#).

Plot



Icon

Description

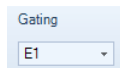
Properties



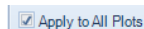
Plot Type: Changes the plot type for the selected plot.



Overlays: Opens the **Edit Overlays** window. Overlay is only available for dot plots and histograms.



Gating: The gate and its events are selected as the current data source for plotting.



Apply to All Plots: Apply the selected gate to all plots.

Use NovoExpress Software

Show Title on Plot

Show Title on Plot: Toggle on and off the display of a title on plots. This is set for all data analysis plots in the Workspace.

Plot Name: Plot 1

Plot Name: Sets the name of a plot.

X -Axis, Y -Axis

Parameter ▾ Min: 0 Auto Range
Scale ▾ Max: 16,777,216 Full Range
X Axis

Parameter ▾ Min: 194,422 Auto Range
Scale ▾ Max: 2,300,256 Full Range
Y Axis

- Sets plot properties for the X and Y-axes including the plotted parameter, scale (linear, logarithmic, or biexponential), and the display range.
- Custom Range: Uses the input boxes to set the minimum and maximum displayed values for the selected parameter.
- Automatic Range : Automatically sets the range of the X and Y-Axes according to the range of the dataset.
- Full Range : Sets the full range of the X and Y-Axes. The default range is $2^{24}=16,777,216$.

Format

Smooth Events Displayed
 Pseudocolor Filling ▾
 Show Outlier Contour Levels ▾
Format

Smooth Events Displayed
 Pseudocolor Filling ▾
 Show Fitting Result Contour Levels ▾
Format

Format: Controls format settings for the plots. Available settings depend on the type of the selected plots.

- **Smooth:** Smooths the data in histogram and density plots.
- **Pseudocolor:** Density plots are displayed in pseudocolor to visualize event density.
- **Events Displayed:** Opens a window to set the number of events to be displayed in the plot.
- **Show Fitting Result:** Displays the results for the cell cycle analysis.
- **Filling:** Sets the histogram fill mode: None, Filled, or Tinted.
- **Contour Level:** Sets the contour levels in contour plots. The three levels are: 10%, 5%, and 2%.
- **Show Outlier:** Show outlier events as dots on contour plot.

Output



Save As Image: The plot can be exported in JPEG, BMP, PNG, GIF, TIFF, and EMF formats. EMF format is a vector format which, when exporting, uses 600dpi resolution TIFF format.

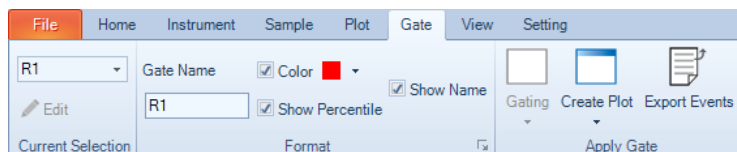


Copy: Selects from the drop-down menu to copy to the clipboard. The plots can then be pasted into Microsoft Word, PowerPoint, Excel, and other documents. Pull-down options include:

- **Copy Plot (Bitmap):** Copies the selected plot as a Bitmap.

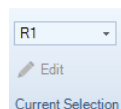
- **Copy Plot with Statistics (Bitmap):** Copies the selected plot and associated statistical information as a Bitmap.
- **Copy Vector Graphics (EMF):** Copies the selected plot as an EMF.
- **Copy Statistics (Text):** Copies the statistics for the selected plot in text format.

Gate



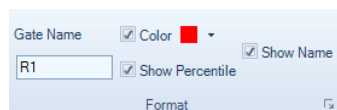
Icon	Description
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Current Selection



Provides a drop-down menu of all available gates. A gate can be selected from this list. The **Edit** button is available only when a logic gate is selected. Click the **Edit** button to open the **Edit Logic Gate** window for modifying the setting of a logic gate.

Format



Formats the selected gate.

Gate Name: Sets the name for the selected gate.

Color: Sets the color for the selected gate.

Show Percentile: Shows the percentage of the gated events relative to the total number of events on the plot.

If the Show population percentile in gate label in **Setting > Analysis** is not checked, the Show Percentile option here will be disabled.

Show Name: Shows the gate name in gate label on plot.

If the **Show gate name in gate label** option in **Setting > Analysis** is not checked, the **Show Name** option here will be disabled.

Apply Gate



Gating: Applies the current gate to the selected plot.



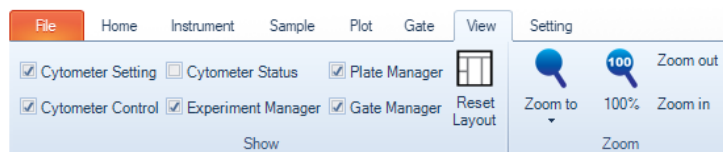
Create Plot: Creates a new plot and applies the current gate to the new plot.

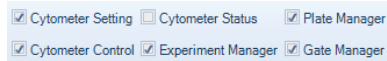







Export Events: Exports data for the events inside the current gate in either FCS or CVS format.

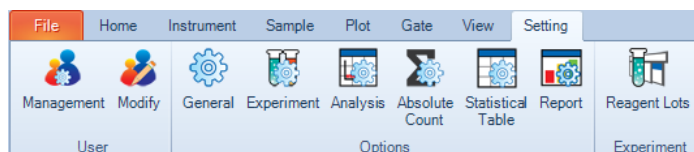
Use NovoExpress Software

View




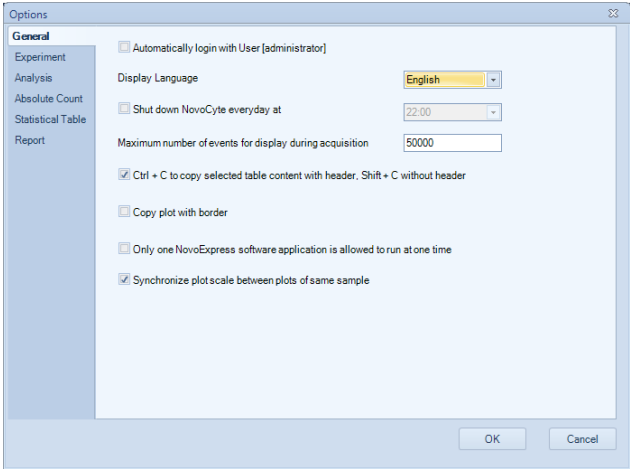
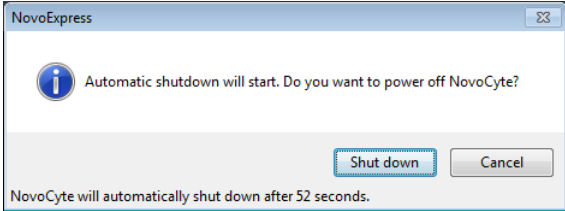


Icon	Description
<p>Show</p> 	Shows or hides the corresponding panel. Plate Manager is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
	Reset Layout: Resets the layout of the panels to the default layout.
<p>Zoom</p> 	Zoom to: Selects the scaling of the size of the plots inside the workspace in the drop-down menu (50%-150%). This function is equivalent to adjusting the sliding bar at the bottom-right corner of the software.
	100%: Restores the size of the plots inside the workspace to the default setting. This function is equivalent to adjusting the sliding bar at the bottom-right corner of the software to 100% value.
	Zoom out: Decreases the size of the plots inside the workspace. This function is equivalent to adjusting the sliding bar at the bottom-right corner of the software. One click corresponds to 1% increment.
	Zoom in: Increases the size of the plots inside the workspace. One click corresponds to 1% increment.

Setting



Use NovoExpress Software



Icon	Description
User	
	Management: Use this function to manage all user groups and user accounts. It allows for adding, deleting, and modifying user groups and user accounts. This function is only available on the accounts with the Administrator privilege.
	Modify: Use this function to change the username and password of the currently logged in account.
Options	
	General: Allows user to define general settings.
	
	<ul style="list-style-type: none">• Automatically login with User: If this box is checked, the current user will automatically be logged in when the software starts and the login window will not appear.• Display Language: Sets the display language.• Shut down NovoCyte every day at: If this box is checked, the instrument will automatically shut down at the selected time. For example, if the time is set to 22:00, the software will prompt a message window as shown below at 22:00 o'clock every day. The system will automatically shut down in 1 min if there is no operation from the user.
	


Only user with **Administrator** privilege can enable this function. If the instrument is not in the "Ready" status at the set time (i.e. the instrument is performing fluidic maintenance or data acquisition etc.), the software will wait until the instrument enters the "Ready" status before shutting down the instrument. If the instrument cannot enter the "Ready" status 30 mins after the set time, automatic shutdown function will be cancelled automatically.

NOTE

The NovoExpress software should be opened at the selected time to perform this function.


NOTE

User can also double click the icon  in the status bar to access this function. User can view the current status of this function (i.e. "Instrument will be shut down at the set time" or "Automatically shutting down instrument is disabled") by placing the mouse on the icon .

User can also enable or disable this function by left clicking the  icon in the status bar.

NOTE

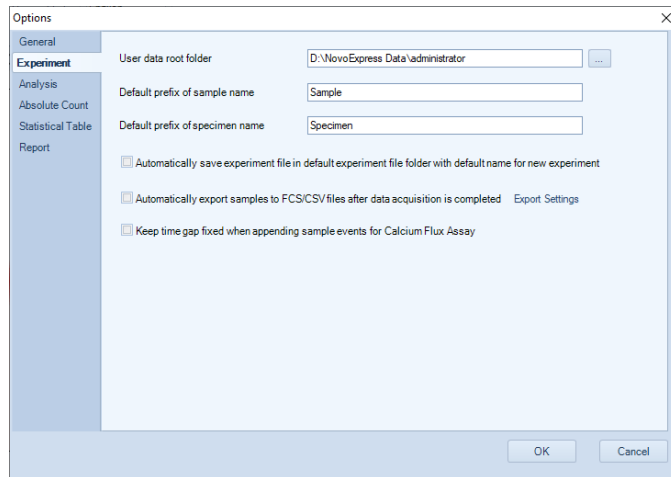
This function is available when NovoCyte instrument is connected. When NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon flow cytometer is connected, user can schedule the instrument to be automatically powered on and/or off by clicking the **Schedule Power On and Off** function from the **Operation** panel. Refer to [Instrument](#) in this guide for details.

- **Maximum number of events for display during acquisition:**
Sets a limit to the number of events displayed on plots during sample acquisition. For example, if this is set to 20,000 events, only the last 20,000 events collected will be displayed. The maximum setting is 50,000 events.
- **Ctrl + C to copy selected table content with header, Shift + C without header:**
When selected, statistical data copied from either the Statistical Table or the table below the plots will include a header when copied using Ctrl + C and will not include a header when selected using Shift + C.
- **Copy plot with border:**
When selected, plots copied will include a dotted line border.
- **Only one NovoExpress software application is allowed to run at one time:**
When selected, only one instance of NovoExpress is allowed to run at a time. An error message will be displayed when user tries to run a second instance of NovoExpress.
- **Synchronize plot scale between plots of same sample:**
When selected, the axis range and scale of same parameter on different plots of same sample will be automatically synchronized when user change axis range or scale.
Click **Synchronize Plot Scale** button  on workspace toolbar to quick switch this setting. Refer to [Workspace Toolbar](#) for more information.

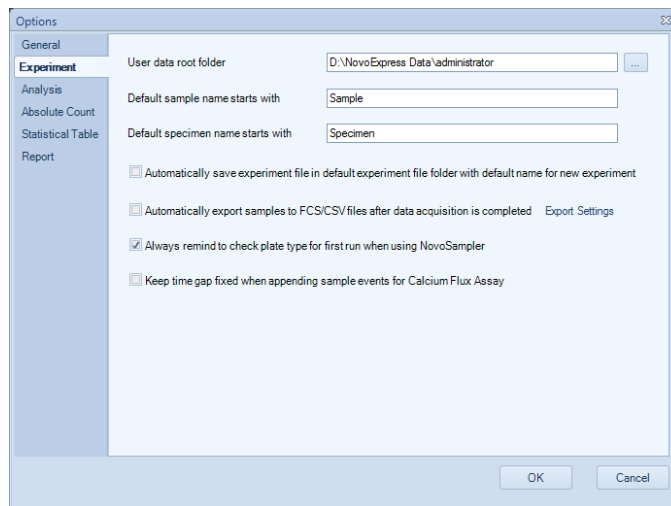


Experiment: Allows user to define experiment related settings. This window displays additional settings when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected.

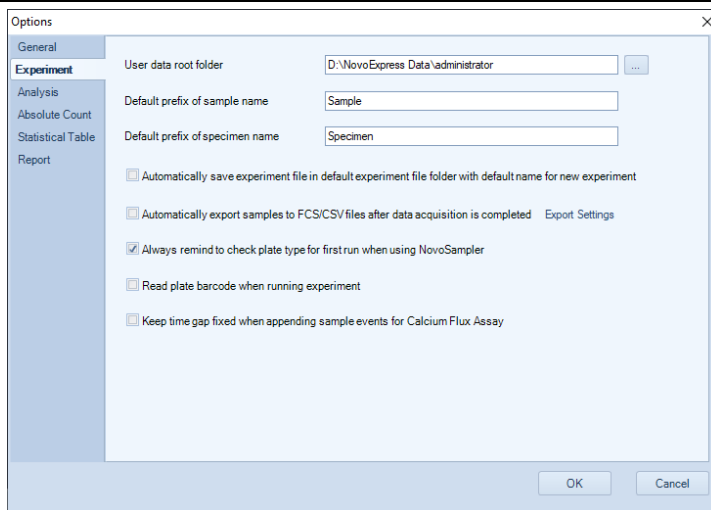
Use NovoExpress Software



When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

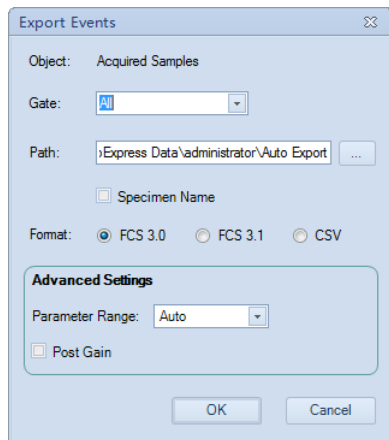


When NovoSampler or NovoSampler Pro is connected

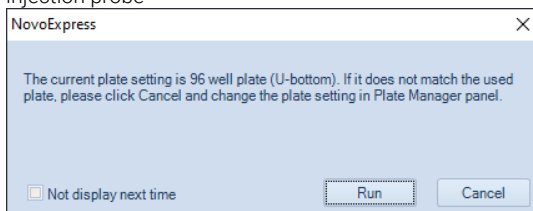


When NovoSampler Q is connected

- **User data root folder:**
Sets the default folder directive for saving the experiment file.
- **Default prefix of sample name:**
Sets the prefix for the name of the sample.
- **Default prefix of specimen name:**
Sets the prefix for the name of the specimen.
- **Automatically save experiment file in default experiment file folder with default name for new experiment:**
This setting determines how to save a current experiment file when a new experiment is started.
If selected, the current file is automatically saved when a new experiment is started. The file is saved at the default experiment file folder with the name based on the current time in the format of **YYMMDD_hhmm.ncf**.
If not selected, a **Save As** window appears when a new experiment is started. The user then has the option to set a name and location to save the current experiment file.
- **Automatically export samples to FCS/CSV files after data acquisition is completed:**
If selected, the software will automatically generate and export a FCS or CSV file after data acquisition is completed. Click **Export Settings** to open the **Export Events** window. Export Settings, including file format (i.e. FCS 3.0, FCS 3.1, or CSV), can be defined in this window. Please refer to [Export Data](#) in this guide for more details.



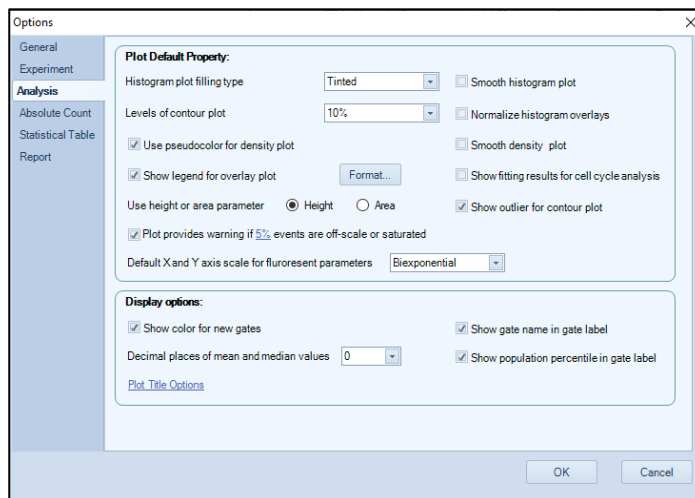
- **Always remind to check plate type for first run when using NovoSampler:** This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected. With this setting checked, a window will be prompted to remind user to check if the correct plate type is selected before starting the experiment. It is highly recommended to enable this setting to remind user to check and make sure the correct plate type is selected for the experiment to avoid collision of the sample injection probe



- **Read plate barcode when running experiment:** This option is available only when NovoSampler Q is connected. If this option is selected, the software will automatically read the barcode attached to the plate. Please refer to [Read Plate Barcode](#) in NovoSampler Q Operator's Guide for more details
- **Keep time gap fixed when appending sample events for Calcium Flux Assay:** If this option is selected, the software will keep the time gap fixed when appending sample events for some specific assay (e.g. calcium flux assay).

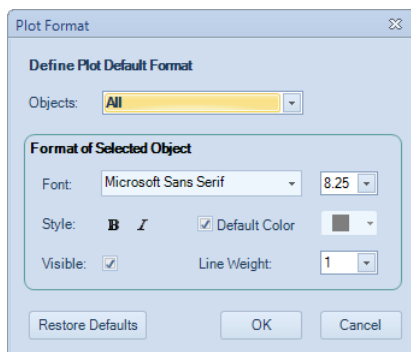


Analysis: Allows user to define analysis related settings.

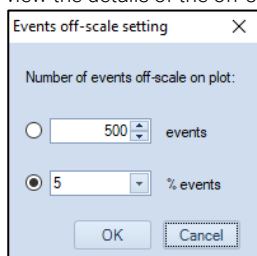


Set the default plot properties when creating a new plot:

- **Histogram plot filling type:**
Sets the filling type for the histogram plots. Options include **None**, **Filled**, and **Tinted**.
- **Smooth histogram plot:**
If selected, this option smooths data on histogram plots.
- **Levels of contour plot:**
Sets the contour plot level (**2%**, **5%**, and **10%**).
- **Normalize histogram overlays:**
If selected, the histogram overlays are normalized to a 100% scale.
- **Use pseudocolor for density plot:**
If selected, density plots are displayed in pseudocolor.
- **Smooth density plot:**
If selected, density plots are smoothed.
- **Show legend for overlay plots:**
If selected, plots with overlays include a legend.
- **Showing fitting results for cell cycle analysis:**
If selected, the fitting results for the cell cycle analysis are displayed on the plot.
- **Use height or area parameter:**
Sets the default parameter for plots to either Height or Area.
- **Show outlier for contour plot:**
If selected, outlier events are shown as dots on contour plots.
- **Format:**
Click **Format...** button to open the **Plot Format** window to define plot default format. Click the **Restore Defaults** button in the window to restore factory default plot format. Refer to [Plot Format](#) for more details about plot format.



- **Plot provides warning if XXX events are off-scale or saturated:**
If selected, the number or percentage of off-scale events will be displayed in created dot, density, histogram and contour plot after the data acquisition. By default, this function is enabled. User can click XXX area to open **Events off-scale setting** window to define the off-scale event settings. By default, 5% off-scale events are selected in this window. User can check the radio button in front of **events** or **%events** and enter the proper number to define the off-scale settings. Once defined, after the data collection, when there is any off-scale events in either X or Y axis, the upper right corner of the associated plot will display "!", user can hover the mouse onto it, and view the details of the off-scale event information.



- **Default X and Y axis scale for fluorescent parameters:**
Allows user to select **Biexponential**, **Log** or **Linear** to be the default scale from the associated drop down menu. By default, software will select **Biexponential** here. When a new **Dot Plot**, **Density Plot**, **Histogram Plot** or **Contour Plot** is created, the selected scale will be automatically applied. This setting will not apply to any previously created plots.

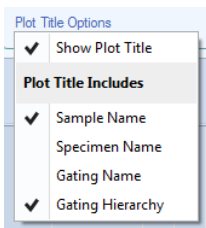
Set plot Display Options:

- **Show color for new gates:**
If selected, new gates are displayed with default color. If not selected, new gates are in black.
 - **Show gate name in gate label:**
If selected, gate name is displayed in gate label on the plot.
 - **Decimal places of mean and median values:**
Sets the number of digits displayed after the decimal point when computing mean and median values.
 - **Show population percentile in gate label:**
-

If selected, gate label is displayed with the percentage of the population within the gate.

- **Plot Title Options:**

If clicked, a drop-down menu will show as below:



- **Show Plot Title:**

If selected, plot titles are displayed on workspace plots.

- **Sample Name:**

If selected, the sample name is displayed in the workspace plot title.

- **Specimen Name:**

If selected, the specimen name is displayed in the workspace plot title.

- **Gating Name:**

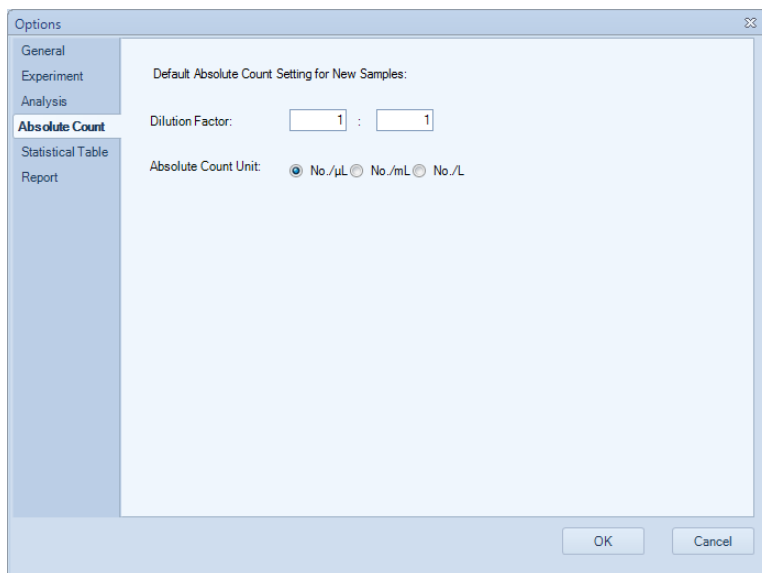
If selected, the gating name is displayed in the workspace plot title.

- **Gating Hierarchy:**

If selected, the gating hierarchy is displayed in the workspace plot title.



Absolute Count: Allows user to define absolute count related settings.



Set default Absolute Count setting for new samples.

- **Dilution Factor:**

Sets the default dilution factor for new samples. The **Dilution Factor** is a conversion coefficient used to calculate the absolute counting results for the

original sample. For instance, if the original sample is diluted 10 times and is run on instrument, enter 1:10 in the Dilution Factor. NovoExpress software will show the absolute counting results for the original sample by multiplying the concentration of the sample run on instrument by 10.

- **Absolute Count Unit:**
Sets the default absolute count unit for new samples. The Absolute Count Unit parameter is used to set the unit for the absolute counting. User can select one of the units (i.e. **No./ μ L**, **No./mL**, or **No./L**) to present absolute count result for number of interested particles per microliter, per milliliter, or per liter.

Further information on absolute count calculations is described in [Calculation of Statistics](#).



Statistical Table: Allows user to define statistical table related settings. This window displays additional settings (e.g. **Plate ID**, **Well ID**) when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected.

Column	Customize Name	Default Visibility
Group:	<input type="text"/>	<input type="checkbox"/>
Specimen:	<input type="text"/>	<input checked="" type="checkbox"/>
Specimen ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample ID:	<input type="text"/>	<input type="checkbox"/>
Operator:	<input type="text"/>	<input type="checkbox"/>
Run Time:	<input type="text"/>	<input checked="" type="checkbox"/>
Gate:	<input type="text"/>	<input checked="" type="checkbox"/>

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

Use NovoExpress Software

Options

Column	Customize Name	Default Visibility
Group:	<input type="text"/>	<input type="checkbox"/>
Specimen:	<input type="text"/>	<input checked="" type="checkbox"/>
Specimen ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Plate ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Plate Description:	<input type="text"/>	<input type="checkbox"/>
Well ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample ID:	<input type="text"/>	<input type="checkbox"/>
Operator:	<input type="text"/>	<input type="checkbox"/>
Run Time:	<input type="text"/>	<input checked="" type="checkbox"/>
Gate:	<input type="text"/>	<input checked="" type="checkbox"/>

OK Cancel

When NovoSampler or NovoSampler Pro is connected

Options

Column	Customize Name	Default Visibility
Group:	<input type="text"/>	<input type="checkbox"/>
Specimen:	<input type="text"/>	<input checked="" type="checkbox"/>
Specimen ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Plate ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Plate Barcode:	<input type="text"/>	<input type="checkbox"/>
Plate Description:	<input type="text"/>	<input type="checkbox"/>
Well ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample ID:	<input type="text"/>	<input type="checkbox"/>
Operator:	<input type="text"/>	<input type="checkbox"/>
Run Time:	<input type="text"/>	<input checked="" type="checkbox"/>
Gate:	<input type="text"/>	<input checked="" type="checkbox"/>

OK Cancel

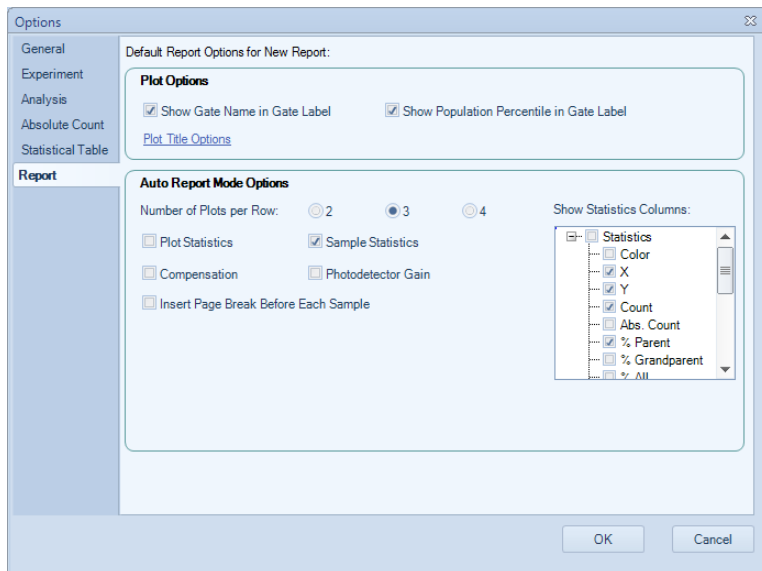
When NovoSampler Q is connected

- **Group**
Customize Name allows the user to re-name the **Group** column header in the statistical table. If **Default Visibility** is selected, the **Group** column will appear in the statistical table by default.
 - **Specimen:**
Customize Name allows the user to re-name the **Specimen** column header in the statistical table. If **Default Visibility** is selected, the **Specimen** column will appear in the statistical table by default.
 - **Specimen ID:**
Customize Name allows the user to re-name the **Specimen ID** column header in the statistical table. If **Default Visibility** is selected, the **Specimen ID** column will appear in the statistical table by default.
 - **Plate ID:**
Customize Name allows the user to re-name the **Plate ID** column header in the statistical table. If **Default Visibility** is selected, the **Plate ID** column will appear in the statistical table by default. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
 - **Plate Barcode:**
Customize Name allows the user to re-name the **Plate Barcode** column header in the statistical table. If **Default Visibility** is selected, the **Plate Barcode** column will appear in the statistical table by default. This option is available only when NovoSampler Q is connected.
 - **Plate Description:**
Customize Name allows the user to re-name the **Plate Description** column header in the statistical table. If **Default Visibility** is selected, the **Plate Description** column will appear in the statistical table by default. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
 - **Well ID:**
Customize Name allows the user to re-name the **Well ID** column header in the statistical table. If **Default Visibility** is selected, the **Well ID** column will appear in the statistical table by default. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
 - **Sample:**
Customize Name allows the user to re-name the **Sample** column header in the statistical table. If **Default Visibility** is selected, the **Sample** column will appear in the statistical table by default.
 - **Sample ID:**
Customize Name allows the user to re-name the **Sample ID** column header in the statistical table. If **Default Visibility** is selected, the **Sample ID** column will appear in the statistical table by default.
 - **Operator:**
Customize Name allows the user to re-name the **Operator** column header in the statistical table. If **Default Visibility** is selected, the **Operator** column will appear in the statistical table by default.
 - **Run Time:**
Customize Name allows the user to re-name the **Run Time** column header in the statistical table. If **Default Visibility** is selected, the **Run Time** column will appear in the statistical table by default.
 - **Gate:**
-

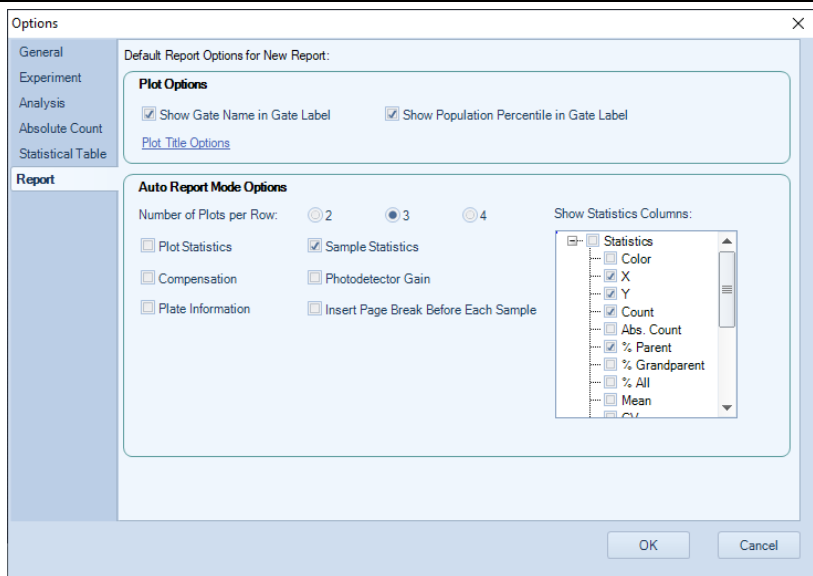
Customize Name allows the user to re-name the **Gate** column header in the statistical table. If **Default Visibility** is selected, the **Gate** column will appear in the statistical table by default.



Report Options: Allows user to define report related settings. This window displays additional setting (i.e. **Plate Information**) when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected.



When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

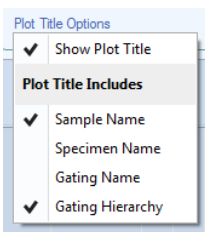


When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

Set Default Report Options for New Report.

The settings in **Plot Options** panel are used for customizing plots inside report. They are effective for both auto and manual report mode.

- **Show Gate Name in Gate Label:** If selected, gate name is displayed in gate label on the plot.
- **Show Population Percentile in Gate Label:** If selected, gate label is displayed with the percentage of the population within the gate.
- **Plot Title Options:** If clicked, a drop down menu will show as below:



- **Show Plot Title:**
If selected, plot titles are displayed on report plots.
- **Sample Name:**
If selected, the sample name is displayed in the report plot title.
- **Specimen Name:**
If selected, the specimen name is displayed in the report plot title.
- **Gating Name:**
If selected, the gating name is displayed in the report plot title.
- **Gating Hierarchy:**
If selected, the gating hierarchy is displayed in the report plot title.

The settings in **Auto Report Mode Options** panel are used for customizing auto report. They are only effective for auto report mode.

- **Number of Plots per Row:** Sets how many plots are shown in one row.
 - **Plot Statistics:** If selected, shows gate statistics of plot.
 - **Sample Statistics:** If selected, shows gate statistics of sample.
 - **Compensation:** If selected, shows compensation matrix.
 - **Photodetector Gain:** If selected, shows photodetector gain of parameters.
 - **Plate Information:** If selected, shows plate information. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
 - **Insert Page Break Before Each Sample:** Only available for specimen report. If selected, a page break will be inserted before each sample.
 - **Show Statistics Columns:** Selects statistical items to display.
-



Reagent Lots:

Reagent Lots

Set Reagent Lot for New Samples:

Reagent Type: **QC Particles**

Active Lot

Lot ID: Import

Expiration Date: / /

Lot File:

OK Cancel

- **Reagent Type:** Sets the type of the reagent.
 - **Lot ID:** Sets the ID of the active lot.
 - **Import:** Import the lot file download from <https://www.agilent.com/chem/novocyte-qc-lot-files>. The lot ID will be listed after importing the lot file.
 - **Expiration Date:** Sets the expired date of the active lot.
 - **Lot File:** Display the lot file associated to the selected **Lot ID**.
-

Workspace Toolbar



Icon	Description
	Dot Plot: Creates a dot plot.

Use NovoExpress Software



Density Plot: Creates a density plot.



Histogram: Creates a histogram.



Contour Plot: Creates a contour plot.



Gate Pointer: Use to select charts, gates, and statistical data.



Rectangular Gate: Draws a rectangular gate. To use, select the tool, click and drag inside a plot to draw a rectangle gate.



Elliptical Gate: Draws an elliptical gate. To use, select the tool, click and drag inside a plot to draw an elliptical gate.



Polygon Gate: Draws a polygon gate. To use, select the tool, click inside a plot to begin creating the polygon. Click at additional locations inside the plot to add vertices to the polygon. To close the gate, either click on the first point of the polygon or double click on the last point.



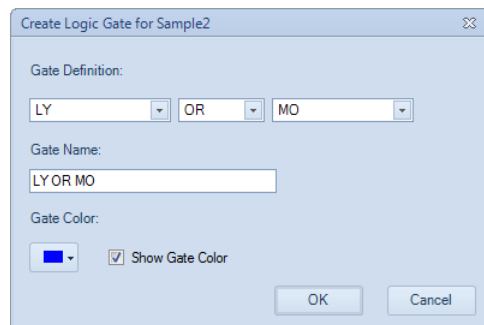
Freehand Gate: Draws a freehand gate. To use, select the tool, click inside start point in a plot to begin creating the freehand gate. Move mouse cursor inside the plot to draw the gate. Double click the end point to close the gate.



Quadrant Gate: Draws a quadrant gate. To use, select the tool, and click inside the plot to divide it into quadrants.



Logic Gate: Creates logical gates. Click the icon to open the **Create Logic Gate** window. In the window, select two current gates and define the logic gate with AND or OR. Select one gate if you wish to define logic gate with NOT. The logic gate name and color can also be defined in this window.



Range Gate: Draws a range gate.



Bi-Range Gate: Draws a bi-range gate.

Use NovoExpress Software



Zoom In: Zooms in on a specified area of a plot. To use, select the tool, click and drag inside a plot to create a rectangle. The plot will be zoomed in on the area inside the rectangle. To zoom in only along one axis, click and drag along either the X-axis or the Y-axis on the plot.



Zoom Out: Zooms out on a specified area of a plot. To use, select the tool, and click on a plot to zoom out. Continue to click to zoom out further. To zoom out only along one axis, click on either the X-axis or Y-axis on the plot.



Auto Range: The display range for the X-axis and Y-axis are automatically adjusted to fit the experimental data.



Full Range: The display range for a plot is set to the maximum.



Move: Use to adjust the display parameters of a plot by dragging the plot area. To use, select the tool, click and drag inside the plot to achieve the desired display range. To only adjust along one axis, click and drag along either the X-axis label or Y-axis label. The display range will only be adjusted along the selected axis.



Synchronize Plot Scale: When selected, the axis range and scale of same parameter on different plots of same sample will be automatically synchronized when user change axis range or scale.



Adjust Threshold: Use this tool to adjust threshold value on plot. Refer to [Threshold Settings](#) for detailed procedures.



Adjust Post Gain: Use this tool to adjust Post Gain. Only accounts with the **Post Gain Adjustment** privilege can perform this operation. Refer to [Post Gain](#) for detailed procedures.



Quick Compensation: Allows for quick fluorescence compensation through scrollbars. To use, select the tool. Scrollbars appear on plots to allow for adjustment of fluorescence compensation.



Show Statistics: Shows or hides the statistical tables below plots.



Cell Cycle Plot: Creates a cell cycle analysis plot.



Cell Proliferation Plot: Creates a cell proliferation plot.



Bi-Variate Plots: Opens **Bi-Variate Plots** window.



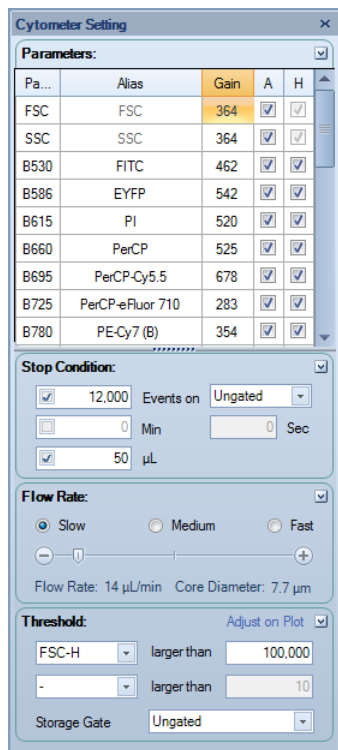
Previous Plot: Switches the active plot to the previous plot. Use this tool when the plot window is maximized.



Next Plot: Switches the active plot to the next plot. Use this tool when the plot window is maximized.

Cytometer Setting Panel

The **Cytometer Setting** panel sets the data collection parameters, stop conditions, flow rate, and threshold. Please refer to [Cytometer Setting](#) for cytometer settings.



Cytometer Control Panel

The **Cytometer Control** panel contains the **Active Sample Information** and the **Experiment Control** panel. The **Experiment Control** panel displays additional buttons when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected. Please refer to [Cytometer Control](#) for cytometer controls.

Use NovoExpress Software

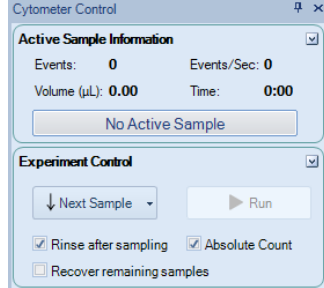
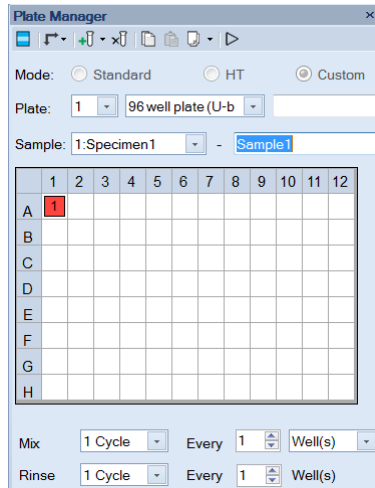


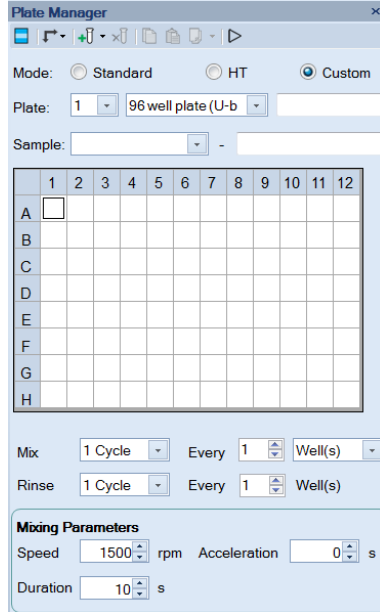
Plate Manager

The **Plate Manager** allows user to set up the working mode, plate type, mixing and rinsing conditions when NovoSampler or NovoSampler Pro or NovoSampler Q is used. Please refer to [Plate Manager](#) for more details.

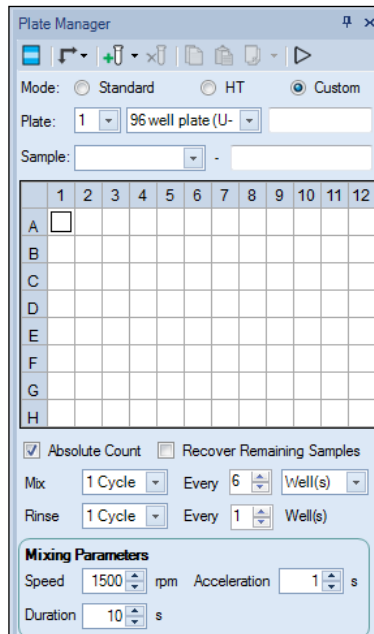


When NovoSampler is connected

Use NovoExpress Software



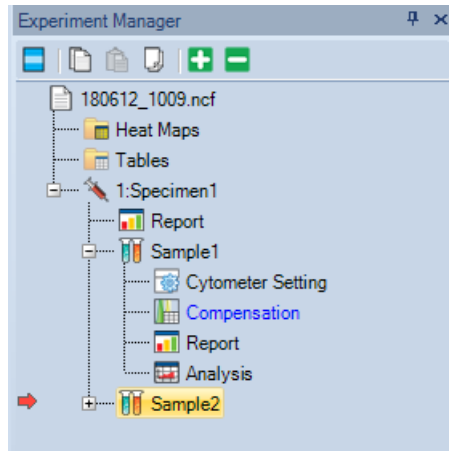
When NovoSampler Pro is connected



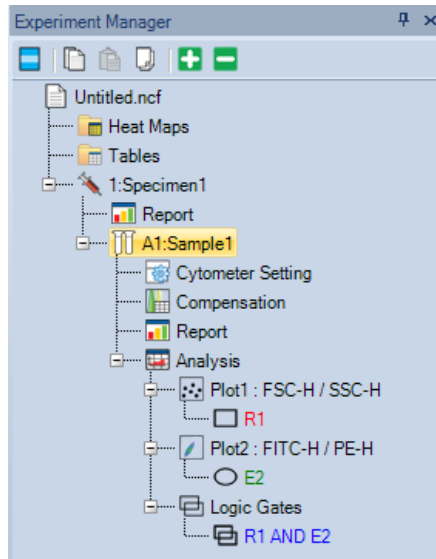
When NovoSampler Q is connected

Experiment Manager

The **Experiment Manager** contains the sample hierarchy structure and functions for copying, pasting templates, and importing and exporting sample data. Please refer to [Experiment Manager](#) for experiment management.



When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

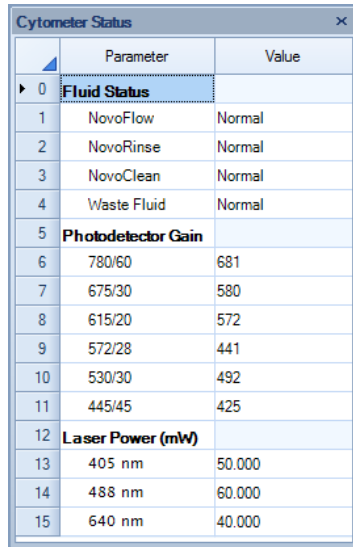


When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

Cytometer Status Panel

The **Cytometer Status** panel displays the fluidic component status, photodetector gains, and laser powers. This panel is hidden by default. To show this panel, click on the **Cytometer Status** box in the **Show** group of the **View** tab.

- Cytometer Status Panel for NovoCyte Instrument (e.g. 3000 System)



	Parameter	Value
0	Fluid Status	
1	NovoFlow	Normal
2	NovoRinse	Normal
3	NovoClean	Normal
4	Waste Fluid	Normal
5	Photodetector Gain	
6	780/60	681
7	675/30	580
8	615/20	572
9	572/28	441
10	530/30	492
11	445/45	425
12	Laser Power (mW)	
13	405 nm	50.000
14	488 nm	60.000
15	640 nm	40.000

Use NovoExpress Software

- Cytometer Status Panel for NovoCyte Quanteon Instrument (e.g. 4025 System)

	Parameter	Value
0	Fluid Status	
1	NovoFlow	Normal
2	NovoRinse	Normal
3	NovoClean	Normal
4	Waste Fluid	Normal
5	Photodetector Gain	
6	FSC	364
7	SSC	364
8	B530	462
9	B586	542
10	B615	520
11	B660	525
12	B695	678
13	B725	283
14	B780	354
15	R660	629
16	R695	718
17	R725	327
18	R780	349
19	V445	503
20	V530	540
21	V586	610
22	V615	535
23	V660	623
24	V695	684
25	V725	505
26	V780	183
27	Y586	618
28	Y615	573
29	Y660	529
30	Y695	622
31	Y725	415
32	Y780	255
33	Laser Power (mW)	
34	405 nm	100.000
35	488 nm	100.000
36	561 nm	100.000
37	637 nm	100.000

Use NovoExpress Software

- Cytometer Status Panel for NovoCytte Advanteon Instrument (e.g. V8B7R4 system)

	Parameter	Value
0	Fluid Status	
1	NovoFlow	Normal
2	NovoRinse	Normal
3	NovoClean	Normal
4	Waste Fluid	Normal
5	Photodetector Gain	
6	FSC	400
7	SSC	400
8	B530	377
9	B586	406
10	B615	338
11	B667	525
12	B695	426
13	B725	292
14	B780	100
15	V445	378
16	V530	401
17	V586	366
18	V615	294
19	V667	374
20	V695	466
21	V725	220
22	V780	100
23	Y586	340
24	Y615	372
25	Y667	370
26	Y695	436
27	Y725	260
28	Y780	100
29	Laser Power (mW)	
30	405 nm	50.000
31	488 nm	60.000
32	561 nm	50.000

Use NovoExpress Software

- Cytometer Status Panel for NovoCyte Penteon Instrument (e.g. U7V7B6Y6R4 system)

Cytometer Status		
	Parameter	Value
▶ 0	Fluid Status	
1	NovoFlow	Normal
2	NovoRinse	Normal
3	NovoClean	Normal
4	Waste Fluid	Normal
5	Photodiode Gain	
6	FSC	400
7	SSC	400
8	B525	480
9	B586	561
10	B615	533
11	B667	526
12	B695	771
13	B725	495
14	R667	574
15	R695	737
16	R725	461
17	R780	260
18	UV445	539
19	UV525	595
20	UV586	605
21	UV615	745
22	UV667	665
23	UV725	615
24	UV780	475
25	V445	620
26	V525	679
27	V586	713
28	V615	618
29	V667	644
30	V725	383
31	V780	409
32	Y586	702
33	Y615	601
34	Y667	557
35	Y695	704
36	Y725	567
37	Y780	367
38	Laser Power (mW)	
39	405 nm	100.000
40	488 nm	100.000
41	561 nm	100.000
42	637 nm	100.000
43	349 nm	20.000

Gate Manager Panel

The **Gate Manager** 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%

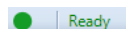
Status Bar

The status bar located at the bottom of the monitor displays the instrument's status through color indicators. If the instrument is connected, the indicator can be green, red, or yellow, indicating a normal condition, an error, or a warning, respectively. If the instrument is not connected to the computer, the indicator is black.

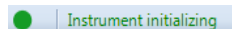
Green Indicator

The indicator light is green if the instrument is connected and without warnings or errors.

The status bar will display Ready if the instrument has completed the initializing sequence and is ready for additional commands.

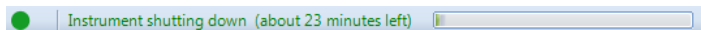


The status bar displays Instrument initializing after powering on the instrument. The initializing sequence flushes the fluidic lines in the instrument to prepare for sample acquisition.

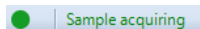


Use NovoExpress Software

The status bar displays Instrument shutting down after shutting down the instrument. The shutting down sequence flushes the fluidic lines and automatically powers off the instrument when complete.

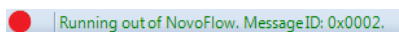


The status bar displays Sample acquiring during sample acquisition.

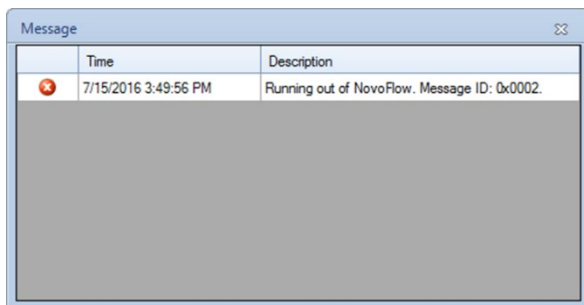


Flashing Red Indicator

If an error occurs, the indicator flashes red. An error message is displayed with the cause and possible solutions. Please see [Troubleshoot](#) for details.

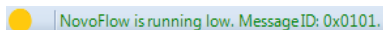


Clicking on the indicator displays the message box.



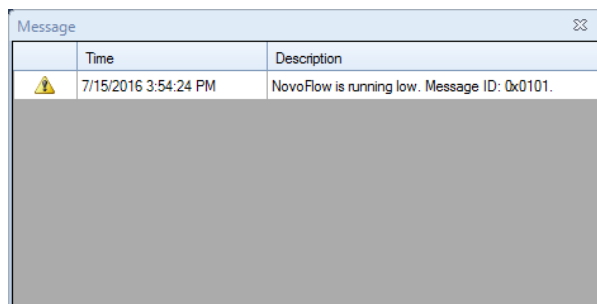
Flashing Yellow Indicator

If a warning occurs, the indicator flashes yellow. A warning message displays the cause of the problem and possible solutions. Please see [Troubleshoot](#) for details.




Clicking on the indicator displays the message box.

Use NovoExpress Software

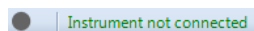


The screenshot shows a 'Message' dialog box with a warning icon. The message text is 'NovoFlow is running low. Message ID: 0x0101.' The dialog box has a title bar with 'Message' and a close button. Below the message text is a large grey rectangular area.

	Time	Description
	7/15/2016 3:54:24 PM	NovoFlow is running low. Message ID: 0x0101.

Black Indicator

If the instrument is not connected to the computer, the indicator is black. This may be due to the instrument being off or a problem with the USB connection between the computer and instrument. Also, if multiple instances of the NovoExpress Software are running, only the first instance of the software will connect to the instrument. The remaining instances will not connect to the instrument and the indicator light will be black.



Instrument Configuration

To open the **Instrument Configuration** window, click the **Configuration** icon from the **Instrument** tab of the **Menu Bar**. User can view or modify the instrument configuration from this window.

NOTE

Please refer to associated instrument operator's guide for procedures to modify the instrument configuration.

NovoExpress Software automatically detects the fluorescence parameters, excitation lasers, and detection channels of a connected Agilent flow cytometer. The software also detects if the NovoSampler or NovoSampler Pro is connected while connecting NovoCyte instrument and detects if the NovoSampler Q is connected while connecting NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon instrument.

Use NovoExpress Software

If using the software while the instrument is powered off or not connected to the workstation, use the **Instrument Configuration** window to select the correct instrument and configuration to display the correct fluorescence parameters.

Instrument Configuration with Instrument Connected

The **Instrument Configuration** window displays the instrument type, the name and schematic of the current optical configuration, parameter window, and status of NovoSampler, NovoSampler Pro or NovoSampler Q. When the instrument is connected to the workstation and powered on, the software will detect the excitation laser and photodetectors connected to the system and display the schematics of the compatible optical configurations. The schematic of each configuration shows the position and type of each bandpass filter, dichroic mirror, photodetector, and the excitation laser. The parameter window shows the Parameter, Excitation Laser, Detection Channel and the Default Alias for each fluorescence channel. The Default Alias can be modified by double-clicking.

The status of the NovoSampler, NovoSampler Pro or NovoSampler Q is displayed in the lower left corner of this window. When the workstation is connected to the instrument and the instrument is powered on, it can automatically detect the installed NovoSampler, NovoSampler Pro or NovoSampler Q and this box will be automatically checked.

- Instrument Configuration with NovoCyte Connected (e.g. 3000 System)

Instrument Configuration (Automatically Detected)

Instrument: NovoCyte Optical Configuration: 3000

SSC

488/10 ↓

445/45 ↓

530/30 ↓

615/20 ↓

780/60 ↓

425SP ↓

572/28 ↓

535LP ↓

600LP ↓

675/30 ↓

650LP ↓

735LP ↓

V

V B

V B R

V B R

V B R

V B R

V B R

Light Collection Optics

405 nm

488 nm

640 nm

NovoSampler installed

Par...	Excit...	Detectio...	Default Alias
B530	488	530/30	FITC
B572	488	572/28	PE
B615	488	615/20	PE-Texas Red
B675	488	675/30	PerCP
B780	488	780/60	PE-Cy7
R675	640	675/30	APC
R780	640	780/60	APC-Cy7
V445	405	445/45	Pacific Blue
V530	405	530/30	AmCyan
V572	405	572/28	Pacific Orange
V615	405	615/20	Qdot 605
V675	405	675/30	Qdot 655
V780	405	780/60	Qdot 800

OK Cancel

Use NovoExpress Software

- Instrument Configuration with NovoCyte Quanteon Connected (e.g. 4025 System)

Instrument Configuration (Automatically Detected)

Instrument: NovoCyte Quanteon Optical Configuration: 4025 Default

Par...	Excit...	Detectio...	Default Alias
B525	488	525/45	FITC
B586	488	586/20	EYFP
B615	488	615/20	PI
B667	488	667/30	PerCP
B695	488	695/40	PerCP-Cy5.5
B725	488	725/40	PerCP-eFluor 7...
B780	488	780/60	PE-Cy7 (B)
R667	637	667/30	APC
R695	637	695/40	Alexa Fluor 680
R725	637	725/40	Alexa Fluor 700
R780	637	780/60	APC-Cy7
V445	405	445/45	Pacific Blue
V525	405	525/45	AmCyan
V586	405	586/20	Pacific Orange
V615	405	615/20	Qdot 605
V667	405	667/30	Qdot 655
V695	405	695/40	Qdot 705
V725	405	725/40	BV711
V780	405	780/60	Qdot 800
Y586	561	586/20	PE
Y615	561	615/20	PE-Texas Red
Y667	561	667/30	PE-Cy5
Y695	561	695/40	PE-Cy5.5
Y725	561	725/40	PE-Alexa Fluor...

NovoSampler Q installed

OK Cancel

- Instrument Configuration with NovoCyte Advanteon Connected (e.g. V8B7R4 System)

Use NovoExpress Software

Instrument Configuration (Automatically Detected)

Instrument: **NovoCytte Advanteon** Optical Configuration: **V8B7R4**

Par...	Excit...	Detectio...	Default Alias
B525	488	525/45	FITC
B572	488	572/28	PE
B615	488	615/20	PE-Texas Red
B667	488	667/30	PerCP
B695	488	695/40	PE-Cy5.5
B725	488	725/40	PerCP-eFluor 710
B780	488	780/60	PE-Cy7
R667	640	667/30	APC
R695	640	695/40	Alexa Fluor 680
R725	640	725/40	Alexa Fluor 700
R780	640	780/60	APC-Cy7
V445	405	445/45	Pacific Blue
V525	405	525/45	AmCyan
V572	405	572/28	Pacific Orange
V615	405	615/20	Qdot 605
V667	405	667/30	Qdot 655
V695	405	695/40	Qdot 705
V725	405	725/40	BV711
V780	405	780/60	Qdot 800

NovoSampler Q installed

OK Cancel

- Instrument Configuration with NovoCytte Penteon Connected (e.g. U7V7B6Y6R4 System)

Instrument Configuration (Automatically Detected)

Instrument: **NovoCytte Penteon** Optical Configuration: **U7V7B6Y6R4** [Rename](#) [Create Copy](#) [Delete](#)

Par...	Excit...	Detectio...	Default Alias
B525	488	525/45	FITC
B586	488	586/20	EYFP
B615	488	615/20	PI
B667	488	667/30	PerCP
B695	488	695/40	PerCP-Cy5.5
B725	488	725/40	PerCP-eFluor 7...
R667	640	667/30	APC
R695	640	695/40	Alexa Fluor 680
R725	640	725/40	Alexa Fluor 700
R780	640	780/60	APC-Cy7
UV445	349	445/45	BUV395
UV525	349	525/45	BUV496
UV586	349	586/20	BUV563
UV615	349	615/20	BUV615
UV667	349	667/30	BUV661
UV725	349	725/40	BUV737
UV780	349	780/60	BUV805
V445	405	445/45	Pacific Blue
V525	405	525/45	AmCyan
V586	405	586/20	Pacific Orange
V615	405	615/20	Qdot 605
V667	405	667/30	Qdot 655
V725	405	725/40	BV711
V780	405	780/60	Qdot 800

NovoSampler Q installed

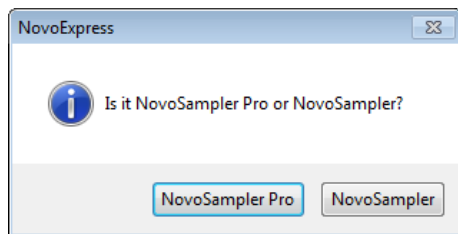
OK Cancel

Instrument Configuration with Instrument Disconnected

When there is no instrument connected to the workstation or the instrument is powered off, user can select **NovoCyte**, **NovoCyte Quanteon**, **NovoCyte Advanteon** or **NovoCyte Penteon** instrument, and all the available optical configurations of the selected instrument will be displayed in the Optical Configuration field. User can select any one of the configurations and view the associated optical schematic. After selecting the optical configuration, click **OK**, and the software will restart. After restarting, the **Cytometer Setting** panel, the **Cytometer Control** panel, the **Experiment Manager** panel, and the **Work List** are all updated according to the new configuration settings. When the instrument is connected to the workstation and powered on, the software will automatically detect the current hardware setting, and restore the correct optical configuration

User can also view the NovoSampler, NovoSampler Pro or NovoSampler Q related software interface and functions.

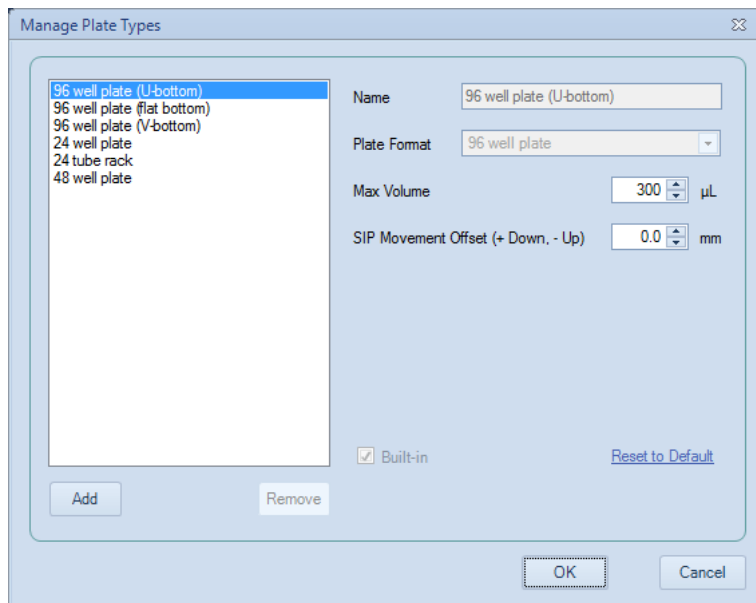
- If **NovoCyte** is selected from the drop down menu of Instrument, check the **NovoSampler installed** checkbox located at the lower left corner of **Instrument Configuration** window, choose **NovoSampler Pro** or **NovoSampler** in the prompted dialog box, and click **OK**. The software will prompt a dialog box, asking to restart software. After restarting, the **Cytometer Control** panel, the **Experiment Manager** panel and the **Work List** are all updated, and the **Plate Manager** panel will be visible



If **NovoCyte Quanteon**, **Advanteon** or **Penteon** is selected from the drop down menu of **Instrument**, check the **NovoSampler Q installed** checkbox located at the lower left corner of **Instrument Configuration** window, and click **OK**. The software will prompt a dialog box, asking to restart software. After restarting, the **Cytometer Control** panel, the **Experiment Manager** panel and the **Work List** are all updated, and the **Plate Manager** panel will be visible.

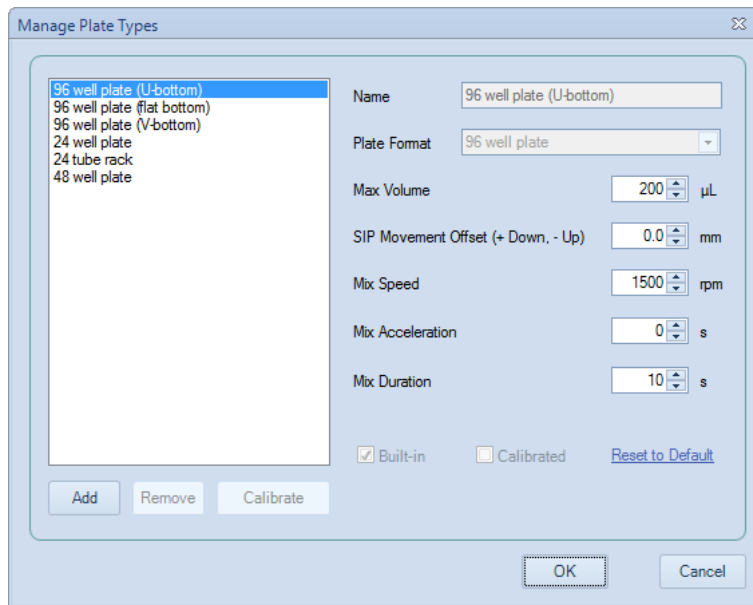
Manage Plate Types

If NovoSampler, or NovoSampler Pro, or NovoSampler Q is used, user can add, remove, and modify plate settings in the **Manage Plate Types** window. To open this window, click the **Manage Plate Types** button from **Instrument > NovoSampler or NovoSampler Pro or NovoSampler Q**.

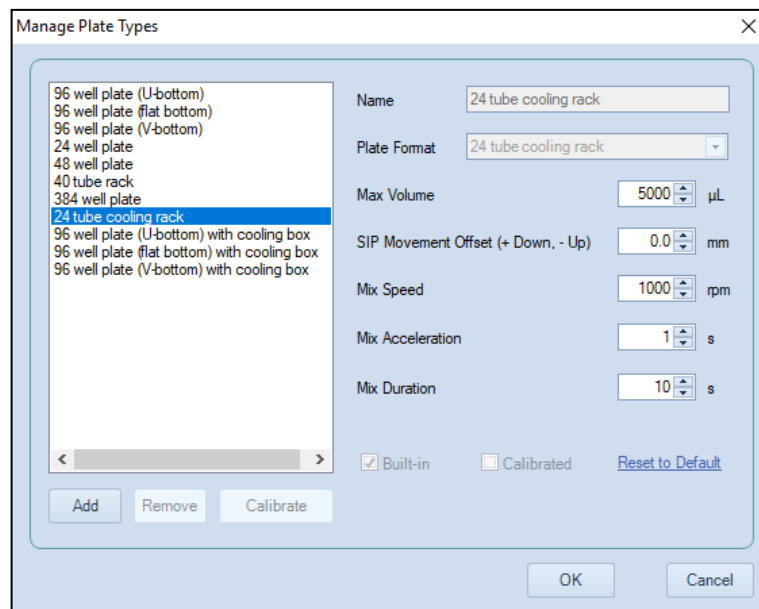


Manage Plate Types Window When NovoSampler is Connected

Use NovoExpress Software



Manage Plate Types Window When NovoSampler Pro is Connected



Manage Plate Types Window When NovoSampler Q is Connected

Use NovoExpress Software

NOTE

When NovoSampler Pro or NovoSampler Q is used, a new plate type must be calibrated before it could be used. Once calibrated, the same type of plate can be used for an experiment without any calibration needed.

NOTE

Plate Type is defined by the format of the and the geometry of the well. For example, two U-bottom 96-well plates are considered as different types if they have different height of the well.

NOTE

Specify the plate name in detail to help easy recognition of different plate type, such as Nunc 96-Well Polypropylene Plates, Corning Carbo-BIND 96-well plates, etc.

All the available plate types are listed in the left box in the **Manage Plate Types** window. Please refer to associated operator's guide for more details.

- **Name:** The name of the selected plate type. Edit it to rename the customer plate other than the built-in plate types. The name of the built-in plate type cannot be changed.
- **Plate Format:** The plate format of the selected plate type. This property cannot be changed.
- **Max Volume:** Set the limit for the maximum sampling volume user can input in the **Stop Condition of Cytometer Setting** panel for an experiment. **Max Volume** can be set in a range between 10 μL to 5000 μL .

NOTE

Input an appropriate **Max Volume** to avoid running out of sample for a well during an experiment.

- **SIP Movement Offset (+ Down, - Up):** The sample injection probe height offset relative to the default height when aspirating the sample from each well or tube. Positive value means the height is decreased. Negative value means the height is increased. The **SIP Movement Offset** can be set in a range between -10 mm to 5 mm with a zero default value.

NOTE

Set **SIP Movement Offset** to be Positive can help to reduce the dead volume from each well.

NOTE

Be cautious to set the **SIP Movement Offset**. Incorrect setting would result in collision of **SIP** to the bottom of the well. When NovoSampler Pro or NovoSampler Q is used, the SIP height is automatically calibrated during plate type calibration. Normally, there is no need to adjust the **SIP Movement Offset**.

Use NovoExpress Software

- **Mix Speed:** The mix speed of the orbital shaker. Refer to table below for the range of mix speed and default mix speed for each compatible plate. This function is available only when NovoSampler Pro or NovoSampler Q is connected.

Auto-Sampler	Plate Type	Range of Mixing Speed (rpm)	Default Mixing Speed (rpm)
NovoSampler Pro	96 well plate (U, V and Flat bottom)	60 - 3000	1500
	24 well plate	60 - 3000	2500
	24 tube rack	60 - 1800	1500
	48 well plate	60 - 3000	1500
NovoSampler Q	96 well plate (U, V and Flat bottom)	200 - 3000	1500
	24 well plate	200 - 3000	2500
	48 well plate	200 - 3000	1500
	40 tube rack	200 - 1200	1000
	384 well plate	200 - 3000	2500
	24 tube cooling rack	200 - 1200	1000
	96 well plate (U, V and Flat bottom) with cooling box	200 - 2000	1500

NOTE

Determine the proper mixing speed and volume before running the experiment can help to avoid any potential spills during the experiment running.

NOTE

When NovoSampler Pro or NovoSampler Q is selected to work in **Standard** and **High-Throughput** mode, the software will automatically apply the default settings for mixing cycle, rinse cycle, mixing speed, acceleration, mixing duration, sampling volume and flow rate.

- **Mix Acceleration:** The acceleration time for the orbital shaker to reach the specified **Mix Speed**. **Mix Acceleration** can be set from 0 second (default) to 10 seconds for NovoSampler Pro, or 1 second (default) to 10 seconds for NovoSampler Q. This function is available only when NovoSampler Pro or NovoSampler Q is connected.
- **Mix Duration:** The mixing time for the orbital shaker to mix the sample when NovoSampler Pro or NovoSampler Q works in custom mode. **Mix Duration** can be set from 1 second to 120 seconds. However, the default mix duration

Use NovoExpress Software

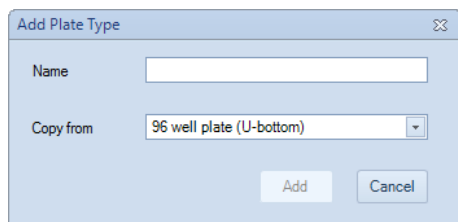
may vary when NovoSampler Pro or NovoSampler Q works in other mode (i.e. Standard and High Throughput mode). Please refer to [Plate Manager](#) in this guide for more details. This function is available only when NovoSampler Pro or NovoSampler Q is connected.

- **Built-in:** Checked if the selected plate is the built-in plate type. Built-in plate types cannot be renamed or deleted. The built-in information is read only.

NOTE

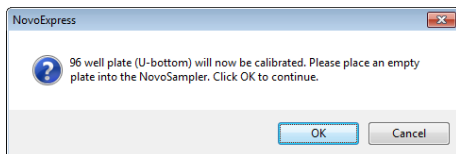
The built-in plate type has been tested with biological samples, including human peripheral blood sample and cultured cell lines, to determine the default mixing settings. At the default mixing settings, the biological samples can be effectively mixed and uniformly suspended inside each well.

- **Calibrated:** Indicate if the selected plate type is calibrated. Only calibrated plate type can be used in experiments. This function is available only when NovoSampler Pro or NovoSampler Q is connected.
- **Reset to Default:** Click this button to reset the **Max Volume** and **SIP Movement Offset** of the selected plate type to default for NovoSampler, and reset the **Max Volume, SIP Movement Offset, Mix Speed, Mix Acceleration, and Mix Duration** of the selected plate type to default for NovoSampler Pro and NovoSampler Q. Only built-in plate types can be reset to default.
- **Remove:** Click this button to delete the selected plate type. Only non-built-in plate types can be removed.
- **Add:** Click this button to add a customer plate type. Input plate type name for new plate type in the prompted window. The **Copy from** function helps to quickly set up a customer plate type based on the format of an existing plate type. Choose a proper existing plate type from the **Copy from** drop-down list. The **Plate Format, Max Volume** and **SIP Movement Offset** settings are copied from the **Copy From** plate type.

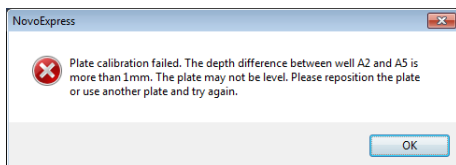


- **Calibrate:** This function is available only when NovoSampler Pro or NovoSampler Q is connected. Click this button to calibrate the selected plate type. Only calibrated plate type can be used in experiments. The **Calibrated** checkbox indicates if the selected plate type is already calibrated. Figure below shows the dialog box prompted when the **Calibrate** button is clicked.

Use NovoExpress Software



Click **OK** after properly placing a plate of the selected plate type on the orbital shaker. It will take several minutes to complete the plate type calibration process. If the plate is not placed properly, the calibration could fail and a dialog box shown below will be prompted. Reposition the plate properly and conduct the calibration again.



NOTE

Before loading multi-well plates or tube racks, identify the correct orientation to position the plate or the tube rack onto the orbital shaker. Make sure the sample plate is positioned properly on the orbital shaker and completely contact the surface of the orbital shaker without any tilting. Incorrect placement may cause misalignment between the sample wells and sample injection probe, resulting in damage to the sample injection probe or the orbital shaker.

NOTE

Before calibrating the 24 tube rack, 40 tube rack or 24 tube cooling rack, ensure a 12 X 75 mm tube is fully inserted in each position of the tube rack.

NOTE

Once plate type calibration is initiated, the **Calibrate** button in the **Manage Plate Types** window will automatically become **Stop Calibration** button. Click **Stop Calibration** will stop the plate type calibration.

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4 Sample Acquisition

This chapter provides the information for sample acquisition function, including Cytometer Setting panel, Work List, and Cytometer Control panel.

Cytometer Setting

The **Cytometer Setting** panel contains the **Parameters**, **Stop Condition**, **Flow Rate**, and **Threshold** controls. The panel displays the settings of the sample being acquired.

The screenshot shows the 'Cytometer Setting' dialog box with the following sections:

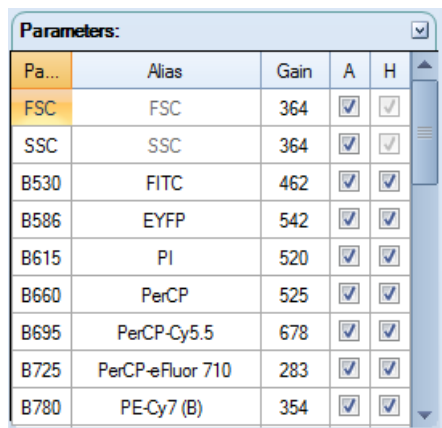
- Parameters:** A table with columns Pa..., Alias, Gain, A, and H. The Gain column is highlighted in yellow.
- Stop Condition:** Includes checkboxes for '12,000 Events on Ungated', '0 Min', and '50 µL'.
- Flow Rate:** Includes radio buttons for 'Slow', 'Medium', and 'Fast', and a slider showing 'Flow Rate: 14 µL/min' and 'Core Diameter: 7.7 µm'.
- Threshold:** Includes dropdowns for 'FSC-H' and 'Adjust on Plot', and input fields for 'larger than 100,000' and 'larger than 10'. It also has a 'Storage Gate' dropdown set to 'Ungated'.

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"/>

Sample Acquisition

Parameters Settings

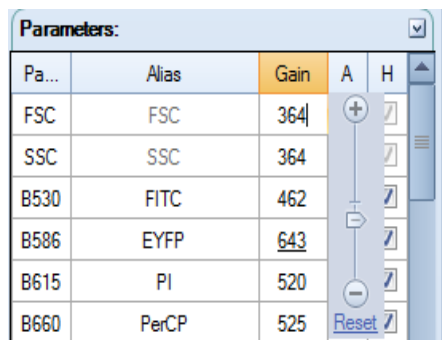
The parameters settings specify which parameters are collected during sample acquisition.



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"/>

The list includes all of the parameters that the instrument is capable of collecting. Area and height measurements can be collected for each parameter. To enable data collection for specific parameters, check the corresponding check boxes under the **A** (area) and **H** (height) columns. The selection of parameters can no longer be modified once sample acquisition has started. The FSC height and SSC height parameters are required for data collection and cannot be unselected. Fluorescent parameters can be renamed by double clicking the name under the alias column.

To adjust photodetector gain of one parameter, double click the cell on the specified parameter row and **Gain** column, the photodetector gain adjustment tool will show as below:



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	643	<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"/>

Drag the slider bar or directly enter the target value to change the photodetector gain. Click the **Reset** link button to set as default value. If currently logged in user

Sample Acquisition

does not have **Photodetector Gain Adjustment** privilege, only **Reset** button will be available. To grant **Photodetector Gain Adjustment** privilege, refer to [Access Privilege](#).

Every channel has its default photodetector gain setting. An underlined photodetector gain value as shown above for the B586 channel means the photodetector gain has been modified and is not the default value. A non-underlined photodetector gain indicates that it is the default setting.

NOTE

Click the **Gain** column header and select the **Reset All** context menu item to reset the photodetector gains of all parameters to default value. When NovoExpress is restarted or new blank experiment file is created, the photodetector gains will be reset to default value too.

Click the **A** or **H** column header to check or uncheck area or height check boxes for all parameters.

NOTE

For NovoCyte instrument, different parameters may share the same photodetector, for example B675 and R675. If the photodetector gain of one parameter is changed, the other one will be changed too.

For NovoCyte Quanteon, NovoCyte Advanteon and NovoCyte Penteon instrument, the photodetector gain for each parameter can be changed independently.

NOTE

The photodetector gain can be changed during sample acquisition. When changing photodetector gain during acquisition, the plot will only display events after the gain adjustment. Please note the events are not deleted, and will be shown on the plots when the acquisition is completed. If previous events before photodetector gain adjustment are not wanted, click the **Restart** button to restart the acquisition. Refer to [Experiment Control](#) for the Restart function. You can also delete the previous events after the acquisition is completed by using the Delete Events function (refer to [Sample](#)).

NOTE

For NovoCyte instrument, the photodetector gain of FSC and SSC cannot be changed.

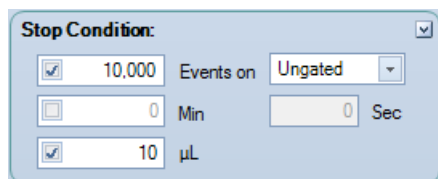
For NovoCyte Quanteon, NovoCyte Advanteon and NovoCyte Penteon instrument, the photodetector gain of FSC and SSC can be changed.

Stop Condition Settings

The **Stop Condition** Settings are used to stop sample acquisition after a specific set of conditions has been met. The conditions may include number of events

Sample Acquisition

collected, collection time, and / or collection volume. To enable a condition, check the box next to the condition.



The screenshot shows a 'Stop Condition' dialog box with a title bar and a close button. It contains three rows of settings:

- Row 1: A checked checkbox, a text box containing '10,000', the text 'Events on', a dropdown menu showing 'Ungated', and a small downward arrow.
- Row 2: An unchecked checkbox, a text box containing '0', the text 'Min', a text box containing '0', and the text 'Sec'.
- Row 3: A checked checkbox, a text box containing '10', and the text 'µL'.

Stop Conditions

- **Events:** Used to specify the number of events to acquire. Acquisition stops when the set number of events has been collected. When the drop-down menu is set to Ungated, the acquisition stops after the total number of events reaches the set value. If the drop-down menu is set to a gate, the acquisition stops after the number of events in the gate reaches the set value. The number of events collected can range between 1~10,000,000.
- **Time:** Used to stop sample acquisition after a set sample collection time. The collection time can be set between 0 and 60 minutes and 0 to 59 seconds.
- **Volume:** Used to stop sample acquisition after a set sample volume has been analyzed. The sample volume can be set between 10 and 5000 µL for NovoCyte instrument, and between 5 and 5000 µL for NovoCyte Quanteon, NovoCyte Advanteon and NovoCyte Penteon instrument.

Multiple stop conditions can be concurrently set. When multiple stop conditions are set, the sample acquisition stops after the first stop condition is met. If no stop conditions are set, the sample acquisition stops after one of the system's maximum limits for events, time, and volume as described is reached.

NOTE

After sample acquisition has started, stop conditions based on number of events can be modified but stop conditions based on time and volume cannot be changed.

NOTE

The number of events displayed in a plot during sample acquisition can be set in Settings. See [Setting](#). The maximum number of events displayed is 50,000 events.

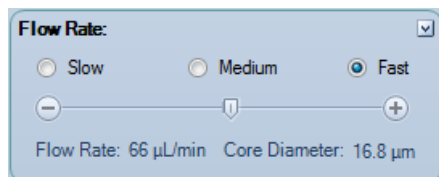
NOTE

File size can be excessively large if you acquire a large number of events, i.e., 1,000,000 events. Therefore, it is always important to consider disabling unnecessary parameters ([Parameters Settings](#)) before acquisition in order to reduce the file size. If events have already been acquired or collected, you can delete events (See [Sample](#)) to discard parts of unnecessary events in the sample.

Sample Acquisition

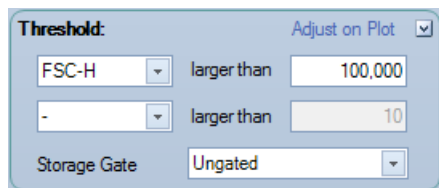
Flow Rate Settings

The three standard settings for flow rate include **Slow** (14 $\mu\text{L}/\text{min}$), **Medium** (35 $\mu\text{L}/\text{min}$), and **Fast** (66 $\mu\text{L}/\text{min}$). In addition, custom flow rates can be set using the slider bar. Sample flow rates can range between 5~120 $\mu\text{L}/\text{min}$. The bottom of the panel includes information on the current sample's flow rate and the corresponding core diameter.




Threshold Settings

The threshold settings determine which events are recorded during sample acquisition. Only events that exceed the set threshold values are recorded.

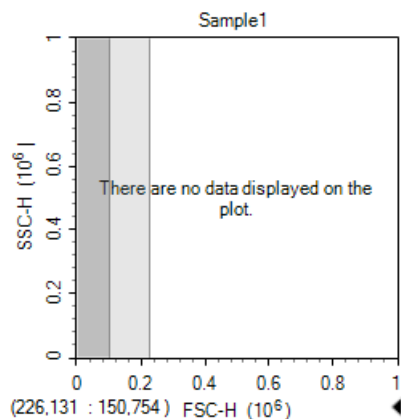


To set the threshold:

For sample acquisition, the primary threshold can be set on either FSC or SSC height, or a fluorescence signal height if firmware supports. If desired, a secondary threshold can also be set on the height of a second parameter. The Storage Gate is used to filter out events outside the gate. All events exceeding the primary and secondary threshold will be recorded when Storage Gate is set to Ungated. Threshold values can range from 10 to 500,000,000.

To adjust threshold value on plot, first click the **Adjust on Plot** link button in the **Threshold** window or the **Adjust Threshold** tool  in the workspace toolbar. Then move the cursor to the target position on a plot with either primary, secondary or both thresholds set as displayed axis parameter. As shown below, the right edge of the dark gray area is the current threshold value and the right edge of the light gray area is the target threshold value to be set. Left-clicking the cursor sets the threshold value to the new value which is shown on the lower left corner.

Sample Acquisition



NOTE

Threshold channels cannot be changed after data acquisition begins. Threshold values and Storage Gate can be changed during acquisition, but the events already acquired will not be processed. When changing threshold values during acquisition, the plot will only show events after threshold adjustment. Please note the events are not deleted, and will be shown when the acquisition is completed. If previous events are not wanted, click the **Restart** button to restart the acquisition. Refer to [Experiment Control](#) for Restart function. You can also delete the previous events after the acquisition is completed by using the Delete Events function (refer to [Sample](#)).

Work List




Before starting the experiment, the **Work List** can be used to set up the sample list. The **Work List** allows users to create new specimens and samples, import specimens or samples from a template, duplicate specimens or samples, and copy and paste sample information. Please see [Experiment Manager](#) for additional details.

The **Work List** displays all created specimens and associated samples. User can view the specimen name, specimen ID, template, sample type, plate number, well ID, sample name, acquisition parameters, stop conditions, threshold settings, compensation settings, and analysis and report information, etc.

Sample Acquisition




Work List												
Specimen	SpecimenID	Template	Sample	FSC	SSC	B530	B572	B660	R660	Stop Condition	Flow Rate	Threshold
Untitled												
Specimen 1	1		Sample 1	FSC H A	SSC H A	FITC H A	PE H A	PE-Cy5 H A	APC H A	10 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Specimen 1	1		Sample 2	FSC H A	SSC H A	FITC H A	PE H A	PE-Cy5 H A	APC H A	10 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, there are three additional icons    shown on the menu bar. The created Work List will also include the **Plate ID** and **Well ID** columns. The other functions remain the same.

Work List						
Specimen	SpecimenID	Template	Plate	Well	Sample	
180625_TBNK						
Compensation Specimen	2		1	A3	B530 FITC	
Compensation Specimen	2		1	A4	B572 PE	
Compensation Specimen	2		1	A5	B675 PerCP	
Compensation Specimen	2		1	A6	R675 APC	
Specimen 1	1		1	A2	Sample 1	
Name 1	3		1	B1	Sample 1	
Name 1	3		1	B2	Sample 2	
Name 2	4		1	B3	Sample 1	
Name 2	4		1	B4	Sample 2	

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

Icon	Description
	Apply Modification: Click this button to save the changes made to the Work List . After applying the modification, the Experiment Manager panel updates to reflect the changes.
	Import Sample from Template: Click this button to import a new sample to the work list with selected template. User needs to select the template file to open. The template of the first sample in the file gets imported to the Work List .
	Import Specimen from Template: Click this button to import a new specimen to the work list with selected template. User needs to select the template file to open. The template of the first specimen in the file gets imported to the Work List .

Sample Acquisition




Duplicate as Sample: Click this button to duplicate selected sample as new sample and add to the **Work List**. Refer to [Create a Duplicate Specimen or Sample](#) in this guide for more details.



Duplicate as Specimen: Click this button to duplicate selected specimen as new specimen and add to the **Work List**. Refer to [Create a Duplicate Specimen or Sample](#) in this guide for more details.



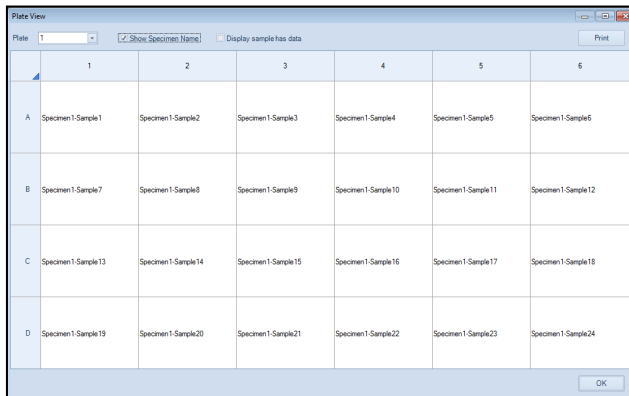
Well ID Setting Direction: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. When new samples are created in the **Work List**, the **Well ID** will be assigned sequentially. Use the **Well ID Setting Direction** button  in the Work List window to set whether new samples are created in a row-by-row sequence or a column-by-column sequence for a plate.



Automatic Setting Well ID: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. When selected, a blue outline will show on the icon. When Well ID of a sample is modified manually, the NovoExpress Software will automatically assign new Well ID for blank samples below that sample in an order set in the Well ID Setting Direction based on the plate type information.



Plate View: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. Click this icon opens the **Plate View** window. **Plate View** window is read only and is used to view the sample information for each well on a plate view. The Plate drop-down menu lists the Plate ID for all the created plates. Select a **Plate ID** from the drop-down menu and view the sample information for this plate. Check the **Show Specimen Name** checkbox to enable the specimen name associated with each well to be shown. Check the **Display sample has data** checkbox to also show samples which have collected data.



The screenshot shows the 'Plate View' window with a dropdown menu set to 'Plate 1'. It contains two checkboxes: 'Show Specimen Name' (checked) and 'Display sample has data' (unchecked). The main area is a table with 4 rows (A-D) and 6 columns (1-6). Each cell contains a specimen name like 'Specimen1-Sample1' through 'Specimen1-Sample24'. A 'Print' button is at the top right and an 'OK' button is at the bottom right.

	1	2	3	4	5	6
A	Specimen1-Sample1	Specimen1-Sample2	Specimen1-Sample3	Specimen1-Sample4	Specimen1-Sample5	Specimen1-Sample6
B	Specimen1-Sample7	Specimen1-Sample8	Specimen1-Sample9	Specimen1-Sample10	Specimen1-Sample11	Specimen1-Sample12
C	Specimen1-Sample13	Specimen1-Sample14	Specimen1-Sample15	Specimen1-Sample16	Specimen1-Sample17	Specimen1-Sample18
D	Specimen1-Sample19	Specimen1-Sample20	Specimen1-Sample21	Specimen1-Sample22	Specimen1-Sample23	Specimen1-Sample24



Hide Disabled Parameters: Click to hide the acquisition parameters that are currently disabled.



Hide Samples Containing Events: Click to hide the samples that contain events.

Sample Acquisition



Hide Photodetector Gain: Click to hide the photodetector gain value in parameters columns.

NOTE

The **Plate ID** and **Well ID** in the **Work List** can be modified to assign **Plate ID** and **Well ID** for the samples.

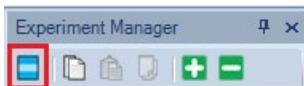
NOTE

When collecting repeats from a sample well is desired, multiple samples can be assigned to the same **Well ID**.

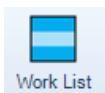
Open the Work List

The **Work List** can be opened using two methods:

- From the **Experiment Manager** or **Plate Manager** panel, click the **Work List** icon at the top of the window.



- From the **Home** tab in the **Menu Bar**, click on the **Work List** icon in the **Experiment** group.



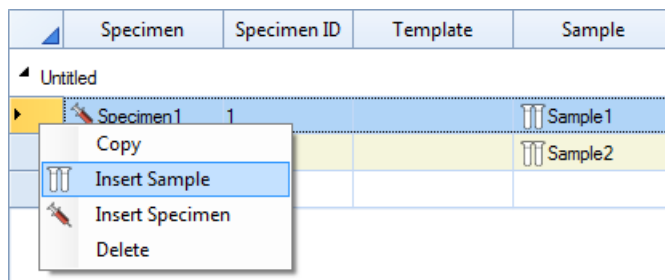
Work List Management

Insert a New Specimen or Sample

To create a new specimen or sample, right click on the first column of a sample row or an empty row. Click **Insert Specimen** to create a new specimen, or click **Insert Sample** to create a new sample. The new sample will be placed in the selected row and under the corresponding specimen for that row.

Sample Acquisition

Specimen	Specimen ID	Template	Sample
Specimen1	1		Sample1
			Sample2

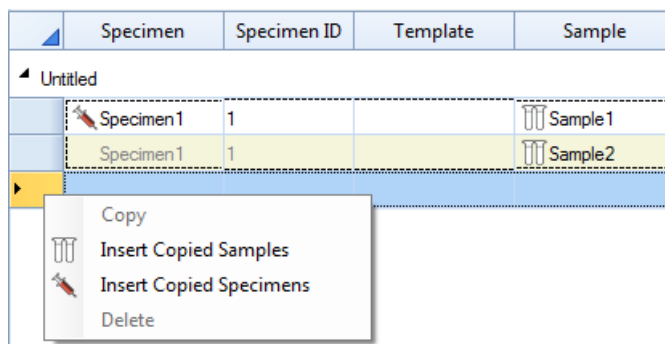


Copy and Insert the Copied Specimen or Sample

Pertaining to copying and inserting of specimens or samples:

- 1 To select the specimens or samples for copying, click and drag in the first column of the **Work List**. The selected rows become highlighted.
- 2 To copy the selected specimens or samples, right click and select **Copy** or use the keyboard shortcut Ctrl C. A dash line borders the copied rows.
- 3 To insert the copied samples, right click and select **Insert Copied Samples**. The samples are inserted at the selected location. Select **Insert Copied Specimens** or use the keyboard shortcut Ctrl V to insert the specimens at the selected location.

Specimen	Specimen ID	Template	Sample
Specimen1	1		Sample1
Specimen1	1		Sample2



Delete Specimen or Sample


Pertaining to deleting of specimens or samples:

- 1 To select the specimens or samples for deleting, click and drag in the first column of the **Work List**. The selected rows become highlighted.
- 2 To delete the selected specimens or samples, right click and select **Delete** or press the Delete key on the keyboard.


Sample Acquisition

Import a Specimen or Sample from a Template

- To import a specimen from a template:


In the **Work List** window, click on the **Import Specimen from Template** icon , from the toolbar at the top of the window and select the template file to open. The template of the first specimen in the file gets imported to the **Work List**.


- To import a sample from a template:

In the **Work List** window, click on the **Import Sample from Template** icon , from the toolbar at the top of the window and select the template file to open. The template of the first sample in the file gets imported to the **Work List**.

Create a Duplicate Specimen or Sample

To duplicate a specimen or sample:



- To select the specimens or samples for duplicating, click and drag in the first column of the **Work List**. The selected rows become highlighted.
- After selecting the samples, click the **Duplicate as Sample** icon , to duplicate the samples and add them under the last specimen of the **Work List**.

Or, after selecting the specimens, click the **Duplicate as Specimen** icon , to duplicate the specimens and create them as new specimens.

Edit a Work List Cell

Edit Specimen, Specimen ID and Sample Names

To modify a specimen, specimen ID or sample cell, double-click on the cell in the **Work List**. Enter the new value and press Enter key.

	Specimen	Specimen ID	Template	Sample
▲ Untitled				
▶	Specimen 1	1		 Sample 1
	Specimen 1	1		 Sample 2

When a sample name is entered into an empty specimen, a new sample is created after Enter key is pressed.

When a specimen, specimen ID, or template is entered into in an empty row, a new specimen is created after the edit is done.

Sample Acquisition

Edit Template

To modify a template, double-click on the **Template** cell in the **Work List**. After a new template is selected, the template is applied into the current specimen. See [Templates](#) for additional details.

	Specimen	Specimen ID	Template	Sample
▲	Untitled			
▶	Specimen 1	1	Template1.nc	Sample 1
	Specimen 1	1	Template1.nct	Sample 2
	Specimen 2	2	Template2.nct	Sample 1
	Specimen 2	2	Template2.nct	Sample 2

To edit template in an empty row, the template's first specimen would be imported into **Work List** after the edit is done.

Edit Acquisition Parameters

To modify the acquisition parameters, double-click on the channel cell to enter edit mode. In this mode, the fluorescence parameter alias and photodetector gain can be modified, and the height and area measurements can be enabled or disabled.

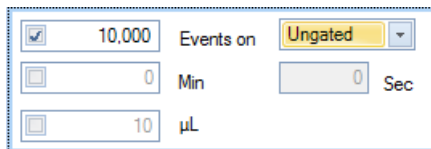


When in non-edit mode, the character * after photodetector gain text indicates the gain is not default value. For more information about photodetector gain, refer to [Parameters Settings](#).

FITC 474* H A

Edit Stop Conditions, Sample Flow Rates, and Threshold Settings

To set the sample stop conditions, double-click the stop condition cell to enter edit mode.



To set the sample flow rate, double-click the flow rate cell to enter edit mode.

Sample Acquisition

To set the sample threshold, double-click the threshold cell to enter edit mode.

Copy and Paste Cells

Pertaining to copying and pasting cells between samples:

- 1 To select the cells for copying, click and drag in the **Work List**. The selected cells become highlighted.
- 2 To copy the selected cells, right-click and select **Copy** or use the keyboard shortcut Ctrl C. A dashed line borders the copied cells.
- 3 To paste the selected cells, select the target cells and right click and select **Paste** or use the keyboard shortcut Ctrl V. The target cells location must have matching columns. After pasting, a green background in the cells indicates that the pasting was successful.

SSC	B530	B586	Stop Condition	Flow Rate
-----	------	------	----------------	-----------

SSC H A	FITC H A	EYFP H A	10000 Ungated	Medium, 35 µL/min
SSC H A	FITC H A	EYFP H A	10000 Ungated	Slow, 14 µL/min
SSC H A	FITC H A	EYFP H A	10000 Ungated	Slow, 14 µL/min

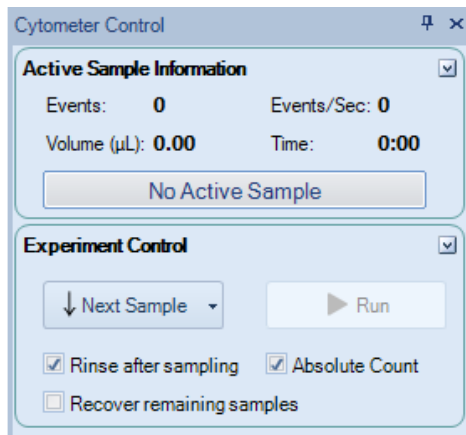
NOTE

You can copy sample names in a column from a spreadsheet program like Microsoft Excel, then select sample cells of multiple rows in **Work List** and press Ctrl V to paste them into **Work List**.

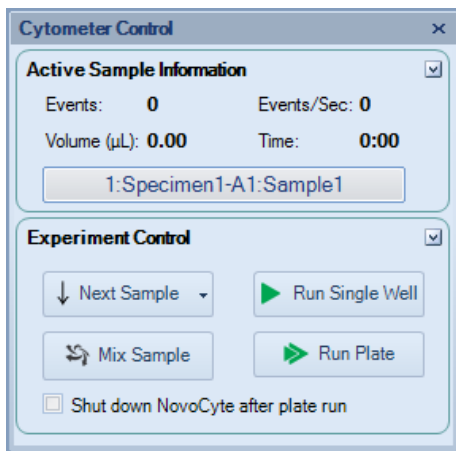
Cytometer Control

The **Cytometer Control** panel contains the **Active Sample Information** and the **Experiment Control** panel. The **Experiment Control** panel displays additional buttons when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected.

- When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected



- When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

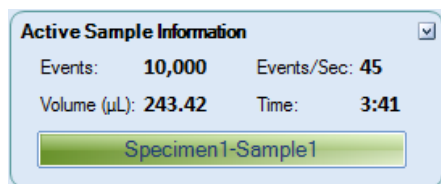


Sample Acquisition

Active Sample Information

In the **Experiment Manager** panel, the active sample is indicated by the red arrow. The active sample can be switched by double-clicking on a new sample or by using the keyboard shortcuts Ctrl + and Ctrl – to switch to the next and previous sample, respectively.

In the **Active Sample Information** panel, the number of events collected, the average events collected per second, the collected volume, and the collection time are displayed. During sample acquisition, this information is updated in real-time.



The current sample information box at the bottom of the panel displays the sample name of the current sample. When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, the sample Well ID is also displayed. To rename the sample or change Well ID from this box, double-click the name to enter edit mode. The specimen name cannot be edited from this box.



Experiment Control

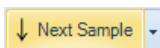
The **Experiment Control** panel displays different functions depending on whether the instrument is connected to NovoSampler (or NovoSampler Pro or NovoSampler Q)

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

The **Experiment Control** panel includes the **Next Sample** and the **Run** buttons.

- Next Sample

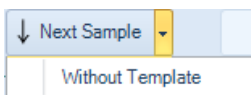
The **Next Sample** button can be used to switch the active sample to the next sample in the **Experiment Manager** panel. If the active sample is the last sample in the **Experiment Manager**, clicking the **Next Sample** button creates a new sample. The new sample has the same template as the previous sample with the same **Cytometer Setting, Compensation, Report and Analysis**.



To create a new sample without the template settings, click on the arrow on the right side of the **Next Sample** button and select **Without Template**. The

Sample Acquisition

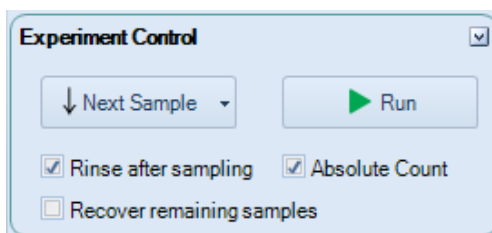
new sample contains the same cytometer settings as the previous samples, but analysis, report and compensation settings are not transferred.



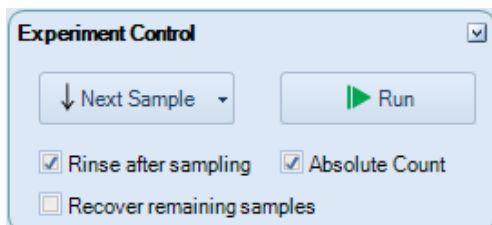
- Run

The **Run** button is used to begin sample acquisition.

If the active sample does not contain event data, the **Run** button appears with a solid green triangle. Click the **Run** button to begin sample acquisition.

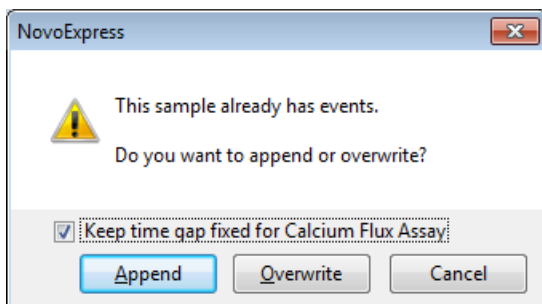


If the active sample already contains event data, the **Run** button appears with a striped green triangle.



Clicking the **Run** button causes a dialog window to appear. Click **Append** to add additional events to the existing events. Click **Overwrite** to delete the existing events and collect new events. If the “Keep fixed time gap when appending sample events for Calcium Flux Assay” function is enabled in the **Experiment Setting** window, the check box in front of the “**Keep time gap fixed for Calcium Flux Assay**” will be automatically selected.

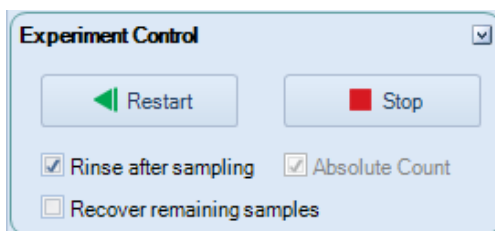
Sample Acquisition



- Restart

The **Restart** button is used to restart sample acquisition while sample acquisition is in process and the previously acquired events are desired to be deleted. **Restart** button is particularly useful when user wants to adjust the photodetector gain or threshold first to a proper value and then collect the data.

When **Restart** is clicked, the previously acquired events will be deleted and the acquisition status including sampling volume and sampling time will be reset to zero. Then the sample acquisition will restart until one of the defined stop conditions is met. The **Restart** button is only visible after acquisition has started.

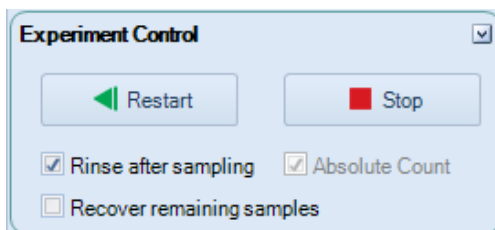


NOTE

The **Run** button is only available when the instrument status is Ready. The **Run** button is not available when the instrument is not connected, when the instrument is powered off, when there is an instrument error, or during the initialization, shutting down, and reagent maintenance sequences.

During sample acquisition, the **Run** button switches into a **Stop** button. Click the **Stop** button to manually stop the acquisition.

Sample Acquisition



- **Rinse after sampling:** check to enable SIP rinse function after each sample acquisition.
- **Absolute Count:** check to enable sample collection in Absolute Count mode.

NOTE

This function is available for NovoCyte Quanteon, NovoCyte Advanteon and NovoCyte Penteon instrument.

- **Recover Remaining Samples:** check to enable recover remaining sample already aspirated into the sampling tubing but not used for acquisition back into the sample tube. The Sample Injection Probe (SIP) will move down after sample acquisition to return any remaining samples back to the sample tube (if tube holder is used) or plate well (if NovoSampler is used).

NOTE

Do not remove the sample tube, tube rack or microwell plate to prevent spill of the sample. Ensure the moving path of SIP is not blocked by any obstacles.

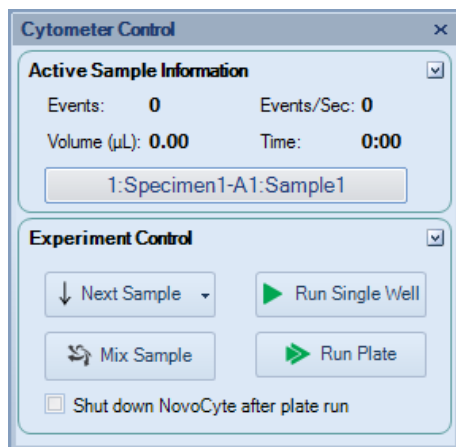
NOTE

This function is available for NovoCyte Quanteon, NovoCyte Advanteon and NovoCyte Penteon instrument.

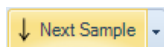
When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

The **Experiment Control** panel includes the **Next Sample**, **Run Single Well**, **Mix Sample** and **Run Plate** buttons.

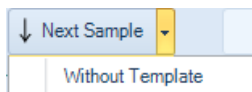
Sample Acquisition



- **Next Sample:** Click this button to switch the active sample to the next sample in the **Experiment Manager** panel. If the active sample is the last sample in the **Experiment Manager**, clicking the **Next Sample** button creates a new sample. The new sample has the same template as the previous sample with the same **Cytometer Setting, Compensation, Report and Analysis**.



To create a new sample without the template settings, click on the arrow on the right side of the **Next Sample** button and select **Without Template**. The new sample contains the same cytometer settings as the previous samples, but analysis, report and compensation settings are not transferred.



- **Run Single Well:** Click this button to run a single well in the plate.

NOTE

When **Run Single Well** is selected, the sample will not be mixed before sample acquisition. To mix the sample, click the **Mix Sample** button in the **Cytometer Control** panel.

NOTE

When **Run Single Well** is selected, the sample injection probe will automatically be rinsed after sample acquisition. This SIP rinse function will minimize the carryover between samples.

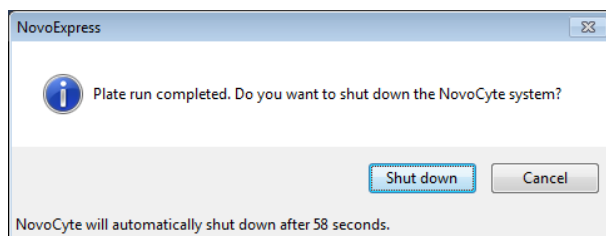
- **Mix Sample:** Click this button to mix the sample in the plate.
- **Run Plate:** Click this button to run multiple wells in the plate.

Sample Acquisition

NOTE

Before acquiring a sample, ensure the sample is the Active Sample and the Well ID is correct. In the **Experiment Manager** panel, double-click a sample node to set the sample to be the Active Sample. The active sample will be indicated by a red arrow on the left of the sample in the **Experiment Manager** panel.

- **Shutdown NovoCyte after plate run:** When checked, NovoExpress can automatically shut down the instrument after plate run is completed. When the plate run is completed, NovoExpress will prompt a 60 seconds count down message box to confirm whether or not to shut down the instrument. The dialog box is shown below. If no action taken after 60 seconds, NovoExpress will shut down the instrument automatically.



NOTE

Automatic shutdown function is only effective for **Run Plate**. **Run Single Well** will not trigger the shut down process.

NOTE


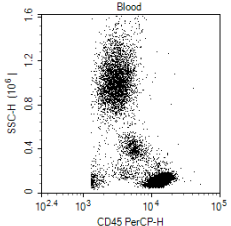

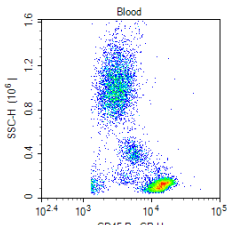
User can also schedule automatic instrument power on or off at selected time and frequency by clicking **Schedule Power On & Off** from the **Operation** panel. This function is available only when NovoCyte Quanteon, Advanteon or Penteon flow cytometer is connected. Please refer to [Instrument](#) in this guide for more details about this function.

5 Data Analysis

This chapter provides information for data analysis tools, including Plots, Gates, Statistics, Fluorescence Compensation, Cell Cycle Analysis, Cell Proliferation Analysis, Statistical Tables, Heat Maps and Post Gain.

Plots

The NovoExpress Software includes dot plots, density plots, contour plots, histograms, cell cycle plot, cell proliferation plot and bi-variate plot.

Plot Type	Icon	Display Parameters	Description	Example
Dot Plot		Two-parameter	The intensities of two parameters are represented by the coordinates of the plot. Each point on the plot represents at least one event with the corresponding intensity values.	
Density		Two-parameter	The intensities of two parameters are represented by the coordinates of the plot. The color of each point represents the density, or number of events, at the corresponding intensity values.	

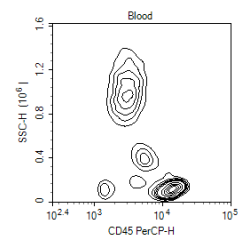
Data Analysis

Contour



Two-parameter

The intensities of two parameters are represented by the coordinates of the plot. Contour lines are drawn to represent the density distribution of the population.

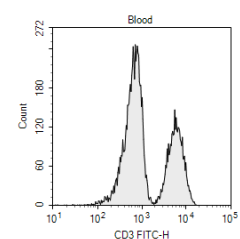


Histogram



Single-parameter

The intensity of a parameter is represented along the horizontal axis, and the number of events at each intensity value is represented along the vertical axis.

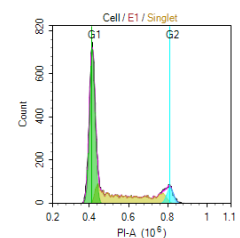


Cell Cycle Analysis



Single-parameter

DNA content is represented along the horizontal axis, and the number of events at each value is represented along the vertical axis. The cell cycle fitting algorithm is used to separate the population into G1, S, and G2 phases of the cell cycle. See [Cell Cycle Analysis](#) for more information.

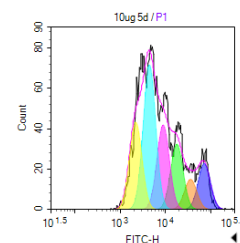


Cell Proliferation Analysis



Single-parameter

Cell Proliferation Analysis can be used to analyze the samples containing cell proliferation information and show the fitting results. See [Cell Proliferation Analysis](#) for more information.



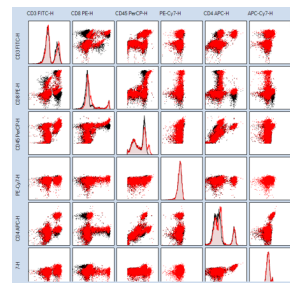
Data Analysis

Bi-variate
Plot



Two-
parameter

Create a matrix of plots with selected parameters plotted against each other. See [Bi-Variate Plots](#) for more information.



Create a Plot

In the NovoExpress Software, plots (except bi-variate plot) can be created through the toolbar, the **Experiment Manager**, and the **Gate Manager**. In addition, plots can be duplicated in the Workspace, copied in the **Experiment Manager**, and imported from templates.

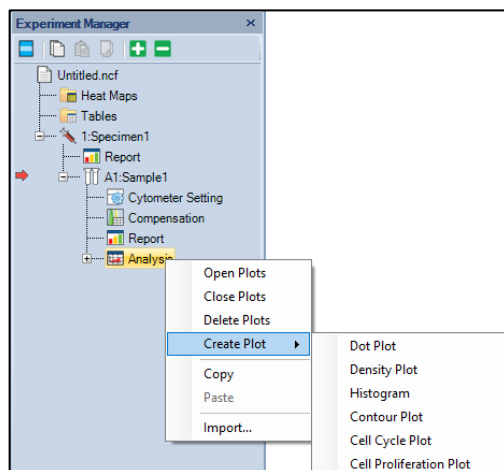
Create a Plot with the Toolbar

Use the plot buttons in the Workspace toolbars to create new plots. The button will create a new plot for the active sample.



Create a Plot with the Experiment Manager

In the **Experiment Manager** panel, right-click on either the sample or the **Analysis** node under the sample. Select **Create Plot** and select the plot type.



Data Analysis

Create a Plot from a Gate

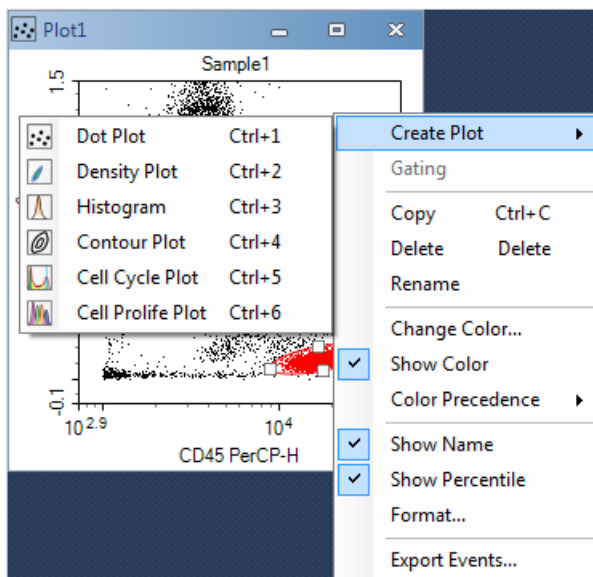
In the Workspace, new plots can be created using gates from previously created plots. New plots created through gates will only display events within the gate. There are multiple methods for creating a new plot through a gate:

- Double-Clicking a Gate

Double-clicking on a selected gate creates a new plot. The new plot has the same parameters and plot type as the plot containing the gate. The plot type and parameters can then be modified.

- Selecting a Gate within the Workspace

Click on a gate within a plot to select the gate. The gate label is italicized to indicate that it is selected. Right-click on the gate and select **Create Plot** and select the plot type. The new plot will have the same parameters as the plot containing the gate. The plot parameters can then be modified.



- Selecting a Gate using the **Menu Bar**

In the **Gate** tab of the **Menu Bar**, a gate can be selected from the drop-down menu in the **Current Selection** group. Click the **Create Plot** icon in the **Apply Gate** group to select a plot type.

- Selecting a Gate using the **Experiment Manager** or **Gate Manager**

In the **Experiment Manager** or **Gate Manager** panel, right click on a gate heading. Select **Create Plot** and select a plot type.

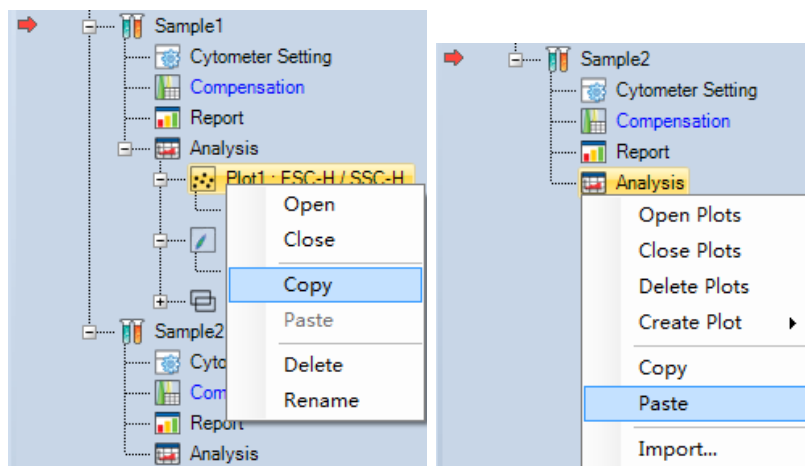
Data Analysis

Create a Duplicate Plot

Click a plot in the workspace to select it. To duplicate the selected plot, click the **Duplicate** icon in the **Home** tab of the **Menu Bar** or use the keyboard shortcut Ctrl D. The plot type and parameters of the new plot will match the previous plot, but the gates will not be replicated.

Copy and Past a Plot with the Experiment Manager

When using this method, the parameters of a plot from one sample can be applied to plot the data of a different sample. In the **Experiment Manager** panel, locate the initial plot by expanding the **Analysis** node under the corresponding sample. Right-click the plot to be copied and click **Copy**. Select the **Analysis** node under the sample where the plot will be pasted. Right-click on this **Analysis** node and click **Paste**. The new plot uses parameters from the copied plot to plot data from the new sample. This can also be accomplished by doing a click and drag on the plot to be copied and dropping it into the desired sample.



NOTE

If the **Analysis** node is copied, all of the plots for the sample are included. Pasting this to the **Analysis** node of a new sample replicates all of the plots. Any plots currently in the sample are replaced.

NOTE

Pasting to a specimen node pastes to all of the samples under the specimen.

Import from a Template

In the **Experiment Manager** panel, select the **Analysis** node under the sample where the plots are to be imported. Right-click on the **Analysis** node and click

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Import.... Select the template file to open. Upon selecting, the plots from the first sample in the template file are imported into the selected sample.

Open and Close a Plot Window

There are multiple methods for opening and closing a plot window.

To open a plot window:

- In the **Experiment Manager** panel, double-click on a plot node or right-click and select **Open** to open the plot.
- In the **Experiment Manager** panel, double-click on a gate node or right-click and select **Open** to open the plot containing the gate.
- In the **Experiment Manager** panel, right-click and select **Open Plots** on a sample to open all of the plots associated with the sample.
- In the **Experiment Manager** panel, right-click and select **Open Plots** on a specimen to open all of the plots associated with the specimen.
- In the **Experiment Manager** panel, right-click and select **Open Plots** on a group to open all of the plots associated with the group.

To close a plot window:

- Click the **Minimize** button in the top right corner of a plot window to close the plot.
- In the **Experiment Manager** panel, right-click and select **Close Plots** on a plot to close the plot.
- In the **Experiment Manager** panel, right-click and select **Close Plots** on a sample to close all of the plots associated with the sample.
- In the **Experiment Manager** panel, right-click and select **Close Plots** on a specimen to close all of the plots associated with the specimen.
- In the **Experiment Manager** panel, right-click and select **Close Plots** on a group to close all of the plots associated with the group.

NOTE

Click the **Close** button  in the top right corner of a plot window to delete the plot.

Edit Plots

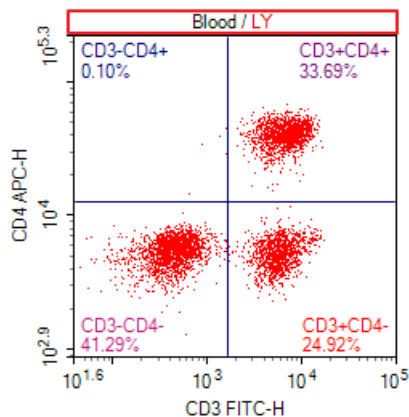
Plot Gating

To analyze subpopulations, plots can be set to only display events from within a specific gate. For this method, a gate from a previous plot will be applied to a

Data Analysis

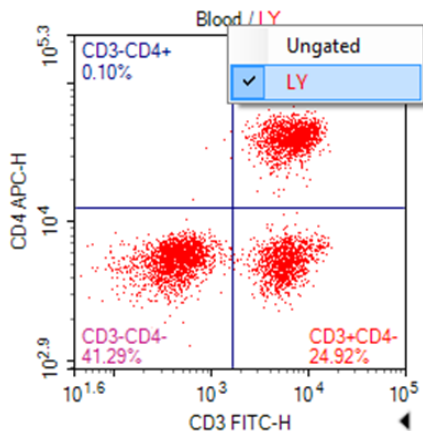
newer plot. The new plot can then be used to analyze the subpopulation or to further gate for more specific populations.

If plots are gated, the gate will be displayed on the header of the plot as shown below. The header will display the sample name and the gate. In the example below, the sample name is Blood and the gate is LY.



There are multiple methods for gating a plot. These methods include:

- In the plot header, right-click to display a drop-down menu. In the drop-down menu, select the gate. If **Ungated** is selected, the plot is not gated and all events are displayed in the plot.



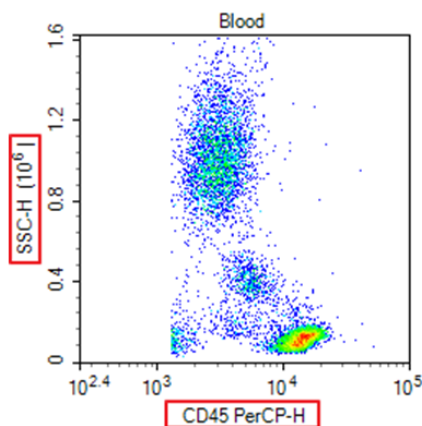
- Right-click in the plot, select **Gating** and select the gate. If **Ungated** is selected, the plot is not gated and all events are displayed in the plot.

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- Click on the plot to be gated to select the plot. In the **Plot** tab of the **Menu Bar**, select the gate in the drop-down menu from the properties group. If Ungated is selected, the plot is not gated and all events are displayed in the plot.
- Select a gate in the **Gate** tab of the **Menu Bar**, and click **Gating**, select the plot to be gated. If All following plots is selected, all the plots listed will be gated.
- Select a gate in the workspace, and drag it into the title of the plot to be gated.

Parameter Plot Settings

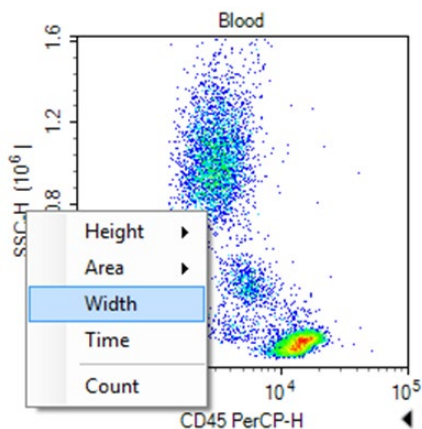
As shown in the figure below, the plot parameters are labeled next to the axes.



To change the plot parameters:

Right-click on the plot label and select the desired parameter. In the drop-down menu, the scatter and fluorescent parameters are separated in height and area measurements. Additional parameters include Width, the width of the individual event signal, Time, the time of the individual event signal, and Count, the number of events at a specific parameter.

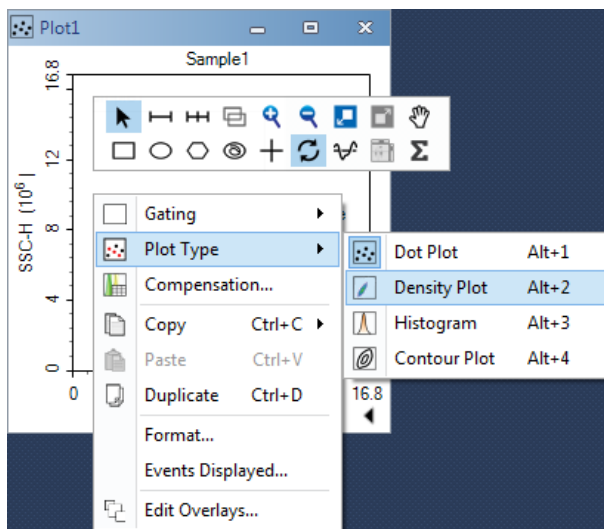
Data Analysis



Set Plot Types

There are two methods for setting or changing the plot type of a plot.

- Within a plot, right-click and select **Plot Type** and select the desired type of plot.



- Click on the plot to be modified to select the plot. In the **Plot** tab of the **Menu Bar**, click on the **Plot Type** icon and select the desired type of plot from the drop-down menu.

NOTE

When the plot is switched from a two-parameter type (dot, density, or contour plot) to a single-parameter plot (histogram), all two dimensional gates (rectangular, ellipse, polygon, and quadrant gates) are deleted.

Rename Plots

There are three methods for renaming a plot.

- Click on the plot to be renamed to select the plot. In the **Plot** tab of the **Menu Bar**, edit the plot name in the **Plot Name** box.
- In the **Experiment Manager** panel, right-click on the plot node and select **Rename** to enter a new name.
- In the **Experiment Manager** panel, click the plot node, and type a new name directly.

Delete Plots

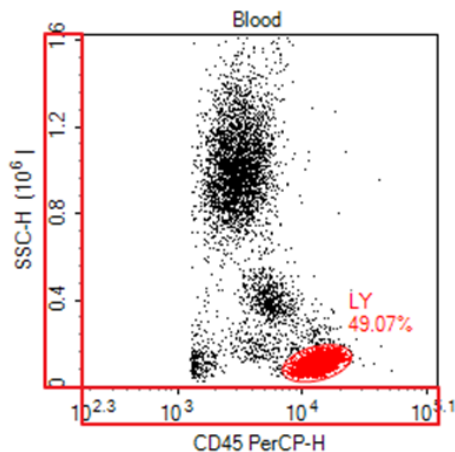
There are multiple methods for deleting a plot.

- Click the **Close** button in the top right corner of the plot window to delete the plot.
- In the **Experiment Manager** panel, right-click on the plot node and select **Delete** to delete the plot.
- In the **Experiment Manager** panel, right-click on the sample node or **Analysis** node and select **Delete Plots** to delete all of the plots associated with the sample.

Set the Coordinates of the Axis


As shown in the figure below, the coordinates of an axis are labeled next to the axis. The axis multiplier is labeled within parentheses in the axis label.

Data Analysis






Set the Coordinate Range

By default, the coordinates for each parameter will be shown over a full range. During analysis, it may be necessary to reduce the display range to focus on a specific population.

There are multiple methods for changing the coordinate range including zooming, the auto range tool, the move tool, and manually entering the coordinate range. Select these tools either from workspace toolbar  or plot right-click popup mini toolbar.



- Pointer : When the pointer is selected, maximum or minimum axis value can be directly adjusted on the plot.

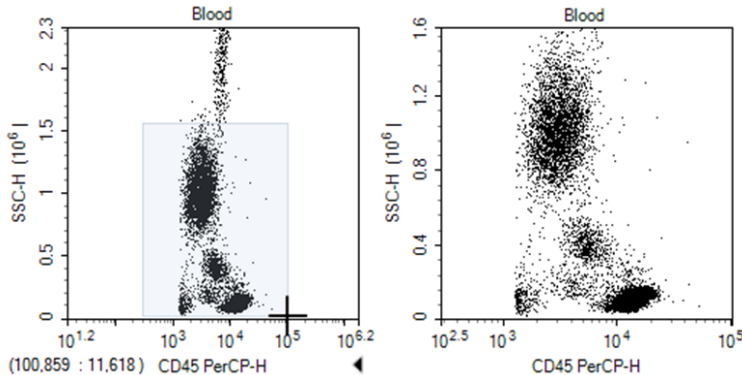
Move the cursor to the maximum or minimum position of the X or Y coordinate. The cursor will change to  for X coordinate or  for Y coordinate. Click and move the cursor to change the maximum or minimum value of the corresponding value. Double clicking the arrow will set the axis to Auto Range on the corresponding coordinate.

- Zoom In : This tool enlarges the display by narrowing the coordinate range.

There are multiple methods to access this tool. This tool can be activated by clicking on the icon in the Workspace toolbar, using the keyboard shortcut Ctrl +, or right-clicking on a plot and selecting **Zoom In**.


To use the tool, click and drag in a plot over the area to be enlarged. A rectangle is drawn, and the range of the rectangle becomes the range of the zoomed in plot.

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NOTE

To zoom in only along one parameter, click and drag along the parameter's coordinate label. This method zooms in on the selected parameter, while the second parameter's coordinate range remains unchanged.

- Zoom Out : This tool compresses the display by widening the coordinate range.


There are multiple methods to access this tool. This tool can be activated by clicking on the icon in the Workspace toolbar, using the keyboard shortcut Ctrl -, or right-clicking on a plot and selecting **Zoom Out**.


To use the tool, click within a plot. The range increases by 20% of the current range. Click repeatedly until the desired range is reached.

NOTE


To zoom out only along one parameter, click on the parameter's coordinate label. This method zooms out on this parameter, while the second parameter's coordinate range remains unchanged.

- Auto Range/Full Range

Auto Range : This tool automatically sets the coordinate range based on the maximum and minimum values of the data set.

Full Range : This tool automatically sets the coordinate range to the maximum and minimum values possible for the parameter.

There are multiple methods to access these tools. These tools can be activated by clicking on the icons in the Workspace toolbar, using the keyboard shortcuts Ctrl A for Auto Range and Ctrl F for Full Range, or right-clicking on a plot and selecting **Auto Range** or **Full Range**.

- Move : This tool allows the user to pan the graph with the coordinate range automatically adjusting.

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There are multiple methods to access this tool. This tool can be activated by clicking on the icon in the Workspace toolbar, using the keyboard shortcut Ctrl M, or right-clicking on a plot and selecting **Move**.

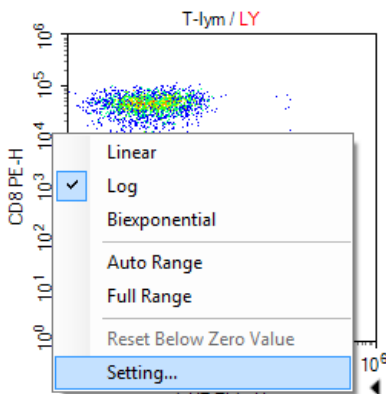
To use this tool, click and drag in a plot to move the display region. The coordinate range then automatically adjusts.

NOTE

To pan the plot only along one parameter, click and drag along the parameter's coordinate label. This method causes the plot to pan along the selected parameter, while the second parameter's coordinate range will remain the same.

- Manually Setting the Coordinate Range through the **Axis Setting** Window

To access the **Axis Setting** window, right-click on the coordinate label of a plot and select **Setting**.

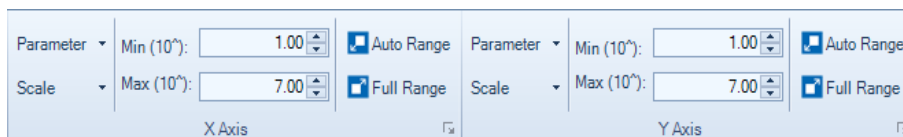


The **Axis Setting** window includes boxes to set the maximum and minimum value for both parameters.

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- Manually Setting the Coordinate Range in the **Plot** tab of **Menu Bar**.

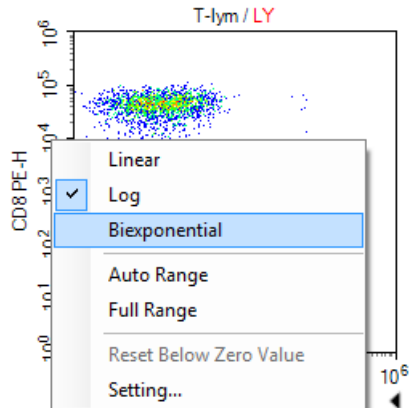


Set the Coordinate Scale

The available coordinate scaling types available in the NovoExpress Software include linear, logarithmic, and biexponential. In general, linear scaling is used for scatter channels, logarithmic scaling is used for the fluorescent channels, and biexponential scaling is used for fluorescent channels where fluorescence compensation has resulted in negative values.

To set the coordinate scaling, right-click on the coordinate label and select the axis scaling.

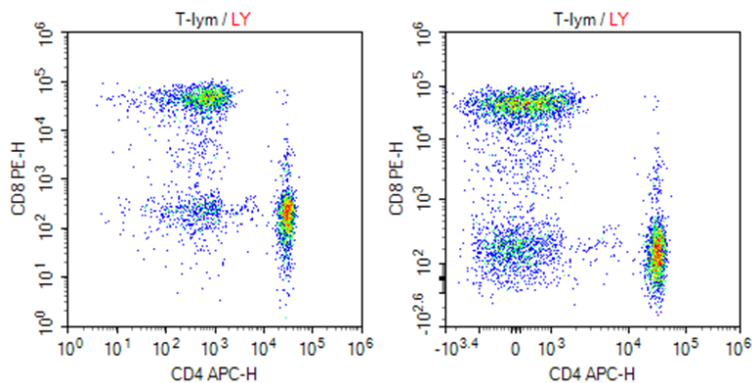
Data Analysis



The axis scaling can also be set through the **Plot** tab of the **Menu Bar** using the **Scale** drop-down menu for each axis.

Display a Biexponential Plot

Biexponential display uses biexponential scale to transform data, especially for those where cells become piled up in the first decade at the axis. This is displayed as fluorescence values <0 even for uncompensated data. Biexponential transformation incorporates linear scaling for low values together with log scaling for high values. Biexponential scaling gets rid of cells being piled up at axes origins, allowing visualization of cells with negative or dim fluorescence. The plots below are the result of different scales, left side is with logarithmic scale and right is with biexponential scale.



- Below Zero Value of biexponential scale

Biexponential transformation can be seen as combination of near linear and near logarithmic scales. It goes smoothly from near linear within the reflection

Data Analysis

point to the near logarithmic within range further away from the reflection point. The width of near linear transformation interval can be changed, which is calculated by the Below Zero Value of biexponential scale in NovoExpress software.

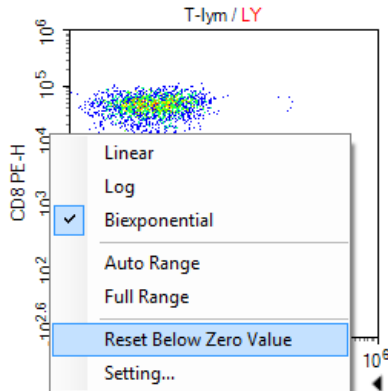
Manually enter the **Below Zero Value** in **Axis Setting** dialog or click **Reset** button to let NovoExpress software calculate the value automatically. When resetting, software calculates the **Below Zero Value** according to the events data in current gating of plot. The minimum value of the axis will be automatically set by the linear minimum of biexponential scale, which is determined by the current **Below Zero Value**.

The screenshot shows the 'Axis Setting' dialog box with the following details:

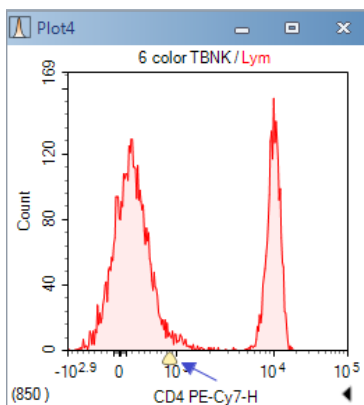
- CD3 FITC-H**
 - Min. Value: -46
 - Max. Value (10[^]): 6.00
 - Scale: Biexponential (selected)
 - Below Zero Value: 21
- CD4 APC-H**
 - Min. Value: -2,506
 - Max. Value (10[^]): 6.00
 - Scale: Biexponential (selected)
 - Below Zero Value: 1,212

Another way to reset the **Below Zero Value** is to right-click on the coordinate label and select the **Reset Below Zero Value** menu item.

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To adjust the Below Zero Value directly on plot, move the cursor to a coordinate axis with biexponential scale, a triangular symbol will appear on the position of below zero value. Click and drag the triangular symbol to adjust the Below Zero Value, and the plot will reflect the change dynamically while dragging the triangular symbol.

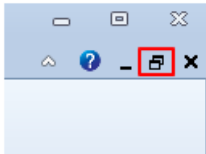


Adjust the Size of Plots

- Maximizing and restoring a plot window

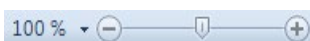
Plot windows can be maximized by clicking on the maximize button , in the top right corner of the window or by double-clicking in the plot. To restore the plot after maximizing it, either click on the restore button (as shown below) or double-click in the plot.

Data Analysis



- Resizing all plot windows

To resize all of the plot windows, use the zoom slider on the right side of the Status Bar (as shown below) or the Zoom tool in the **View** tab of the **Menu Bar**.



NOTE

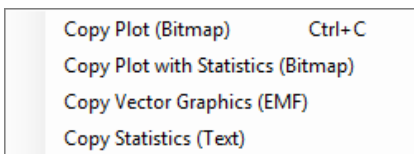
Adjusting the size of the plot window does not affect the coordinate range of the plots. To adjust the coordinate ranges, see [Set the Coordinate Range](#).

Copy or Save Plots


Plots from the NovoExpress Software can be copied and saved.

- To copy a plot to the clipboard

Right-click in a plot. Select **Copy** and select the format to copy the plot. Plots can also be copied using the **Copy** button in the **Plot** tab of the **Menu Bar**. Using the keyboard shortcut Ctrl C copies the selected plot in bitmap format.



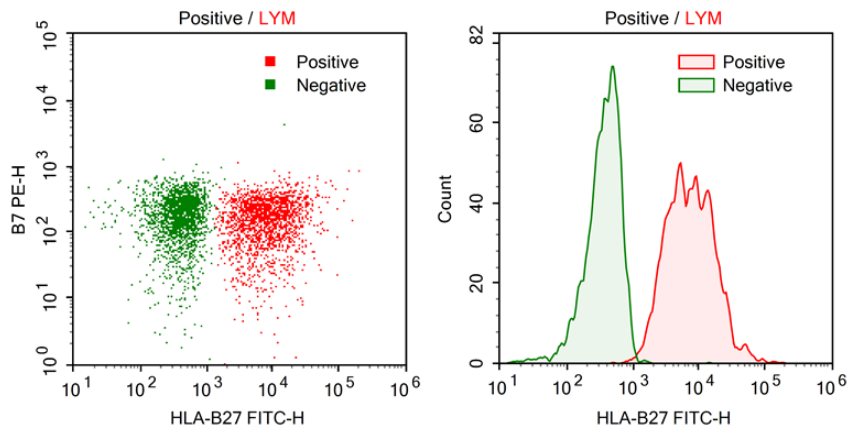
- To save a plot

Select a plot by clicking on it, and click the **Save as Image** button  from the **Plot** tab of the **Menu Bar**. The image format can be selected in the **Save Image** window.

Overlays

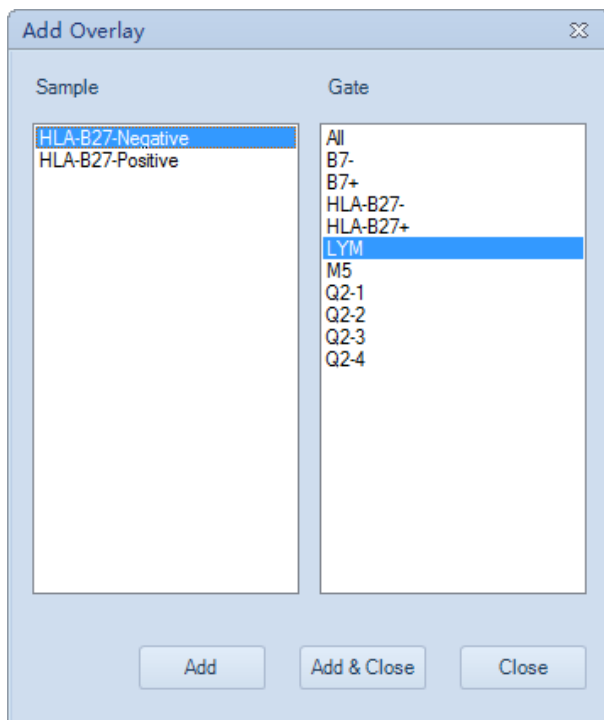
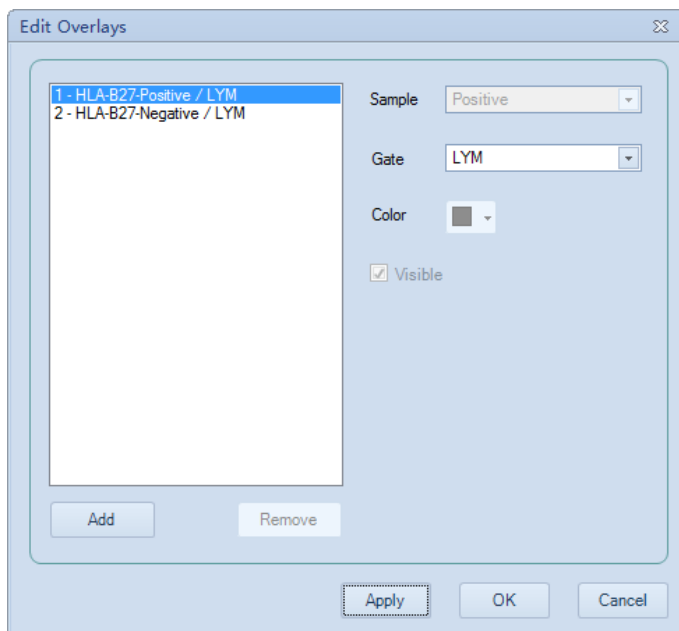
Multiple overlays can be included in dot plots or histogram plots. When a plot is created, it only contains the data from one sample. Overlays can display the data from multiple samples and gates in one plot with different colors. Below show the example of the dot and histogram plots with overlays from different samples.

Data Analysis



- Add overlays by using the drag and drop method:
Hold down the Ctrl key on the keyboard, use the mouse to drag a sample or multiple samples to a plot from the **Experiment Manager**, and the new overlays are added to the plot. The gate of new overlay is from the sample of the overlay, it always takes the same name as the gate of plot. It will be the All gate, if no gate with the name is found in the sample of the overlay.
- Edit overlays:
Right-click a plot to access the shortcut menu, select **Edit Overlays** to generate the **Edit Overlays** dialog window as shown below. In the dialog, all overlays of the plot are listed. One can select an overlay, set the overlay's sample, gate or color, and make a choice to show or hide the selected overlay on the plot. Adding new overlays or deleting overlays can also be done here. Click **Add** button to open the **Add Overlay** window, press Ctrl or Shift key while clicking the selected sample (s), click **Add** or **Add & Close** in this window to add selected sample (s) to the **Edit Overlays** window. Click **Apply** and **OK** to complete adding the new overlays to the current plot.

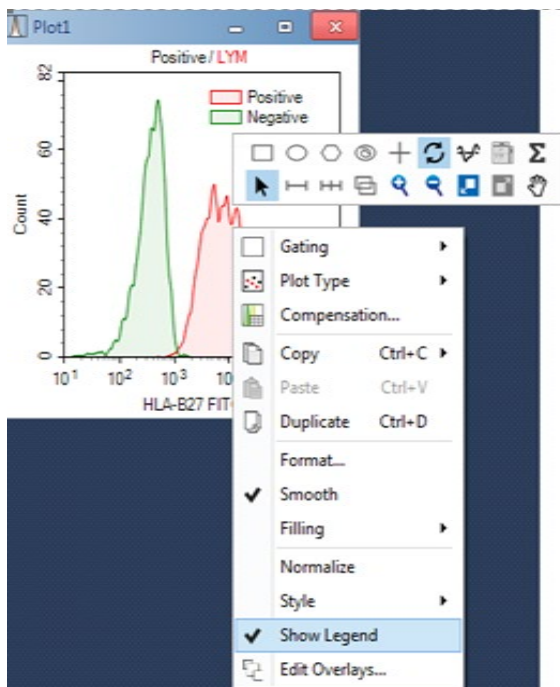
Data Analysis



Data Analysis

- Display legend:

When the overlays are added, the legend will automatically appear on the overlay. User can move the legend to any location inside the plot by left-clicking and dragging the legend. To remove the legend from the plot, right-click the legend and uncheck the Show legend in the menu.



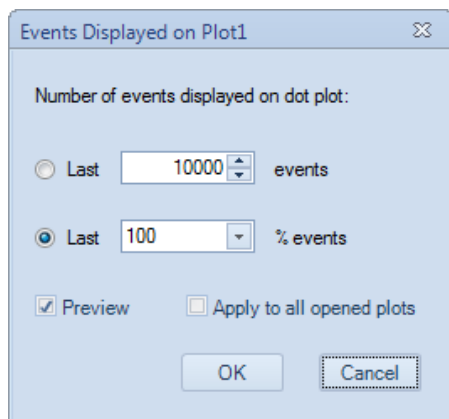
Plot Formatting

Each plot type has different formatting and settings options. This section describes the formatting options associated with each plot type. To format a plot, right click on a plot window and select corresponding format menu item.

Dot Plot Formatting

With dot plots, there is an option to only display the most recently collected events. This option allows the user to set a number or percentage of the most recently collected events to display. To open the **Events Displayed** window, right click on the dot plot and select **Events Displayed....**

Data Analysis

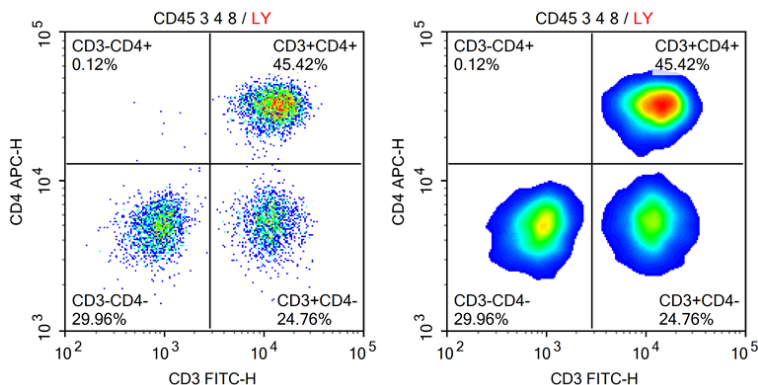


In the window, selecting **Preview** modifies the dot plot display as the user is changing the settings. Selecting **Apply** to all open plots applies the setting to all open dot plots.

Density Plot Formatting

With density plots, there is also an option to only display the most recently collected events. Please refer to [Dot Plot Formatting](#) for more details. Besides, following formatting options are available from the right click menu of density plot.

- **Smooth** density plot: In this view, the density plot data are smoothed. To use this view, right-click on the density plot and select **Smooth** or select **Smooth** from the **Plot** tab of the **Menu Bar**. A comparison of a standard pseudocolor density plot (left) and a smooth pseudocolor density plot (right) is shown below.



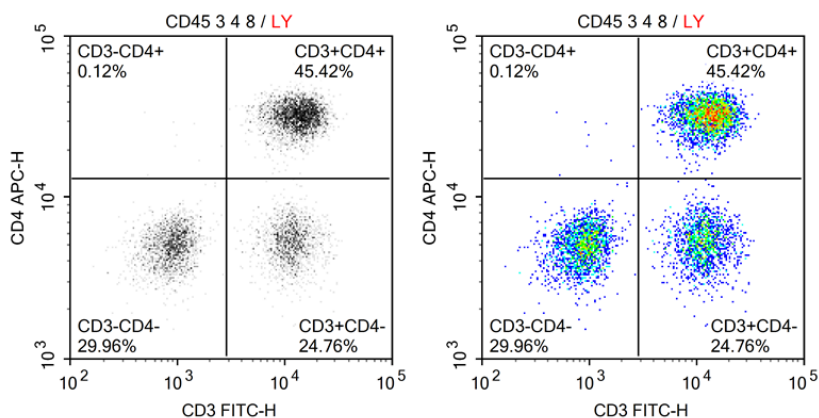
- **Pseudocolor** density plot: By default, density plots are displayed in pseudocolor. In this view, areas of the plot with a higher density of events are

Data Analysis

shown in warmer colors (colors toward the right of the color bar below) and areas of the plot with a lower density of events are shown in cooler colors (colors toward the left of the color bar below).

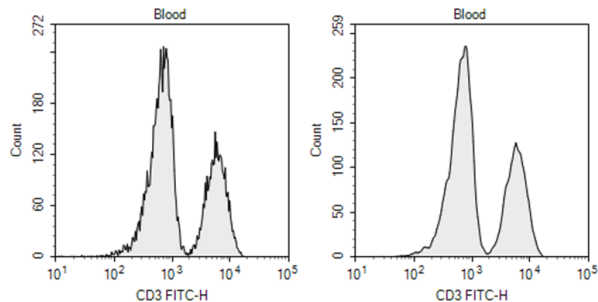


To switch from a pseudocolor to a gray-scale density plot, right-click on a density plot and unselect **Pseudocolor** or unselect **Pseudocolor** from the **Plot** tab of the **Menu Bar**. A comparison of a grayscale density plot (left) and a pseudocolor density plot (right) is shown below.



Histogram Plot Formatting

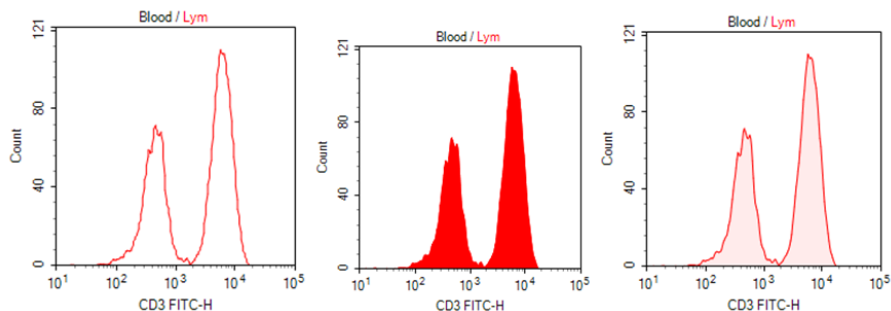
- **Smooth** histogram: To smooth the edges of a histogram, right-click on a histogram plot and select Smooth or select **Smooth** from the **Plot** tab of the **Menu Bar**. A comparison of a standard histogram plot (left) and a smooth histogram plot (right) is shown below.



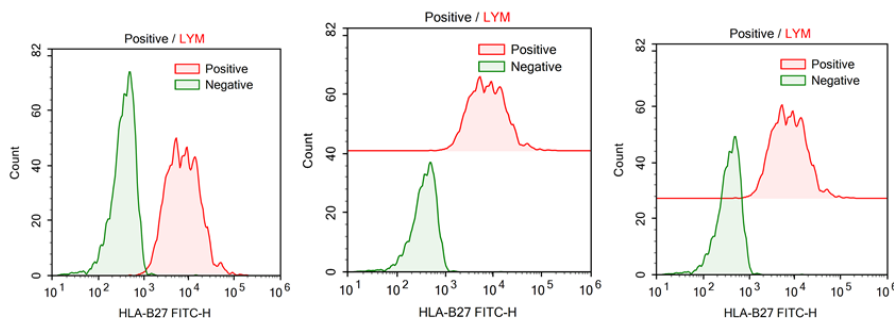
- Histogram fill type: Histogram plots can be viewed with different fill types. To select a fill type, right-click a histogram plot and select **Filling** and select the fill

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type, or select the fill type from the **Filling** drop-down menu in the **Plot** tab of the **Menu Bar**. A comparison of the **filling** types is shown below. The options include **None** (left), **Filled** (middle), and **Tinted** (right).



- Histogram layering: When overlaying histogram plots, different overlay styles can be selected. To select an overlay style, right-click in a histogram plot with layers and select Style to select the overlay style. The overlay style options, as shown below, include **Overlaid** (left), **Offset** (middle), and **Half Offset** (right).

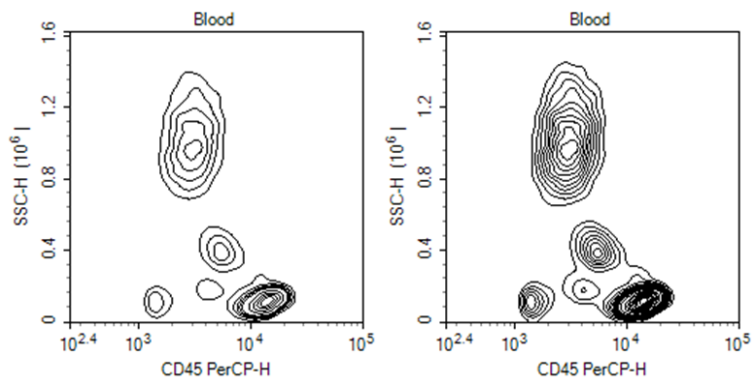


Contour Plot Formatting

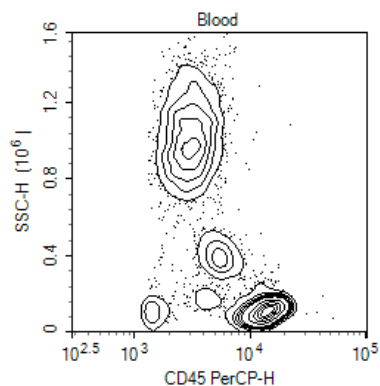
With contour plots, there is also an option to only display the most recently collected events. Please refer to [Dot Plot Formatting](#) for more details. Besides, following formatting options are available from the right click menu of contour plot.

- **Contour levels:** Different contour levels are available for contour plots. Higher contour levels indicate a larger density interval in between contour lines on the plot. The available contour levels include **10%**, **5%**, and **2%**. A contour plot is shown below with a 10% contour level (left) and a 5% contour level (right).

Data Analysis



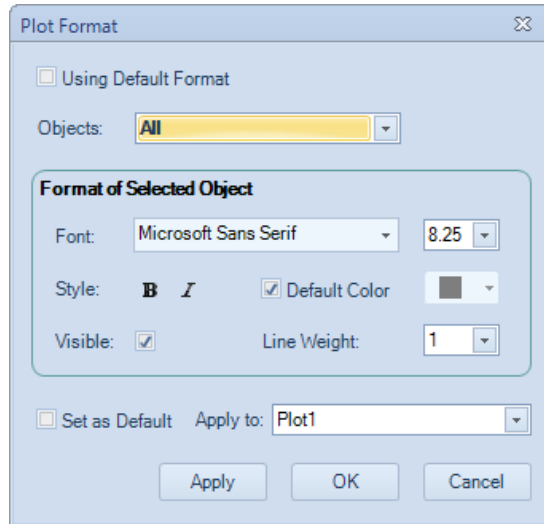
- **Show Outlier:** If selected, outlier events are shown as dots on contour plots. A contour plot is shown below with **Show Outlier** selected.



Plot Format

The **Plot Format** defines plot appearance. The font, size, style, color, line weight, and visibility can be customized. To open the **Plot Format** window, right click inside plot area and select the **Format...** menu item in the popup menu.

Data Analysis



- **Using Default Format:**
Check this box to set plot using system default format.
- **Objects:**
Select objects that the format is applied to. The objects are listed in tree mode. Select parent node will apply to all its child nodes.
- **Font:**
Set font name and font size for the selected objects.
- **Style:**
Set font style for the selected objects. Check button **B** for bold, button **I** for italic.
- **Default Color:**
Set color for the selected objects. Check **Default Color** box if the software default color is to be used. For gate label on plot or plot title, the default color is the color of the gate. For the other objects, the default color is black.
- **Visible:**
Check this box to set the object visible.
- **Line Weight:**
Select line weight of selected objects.
- **Set as Default:**

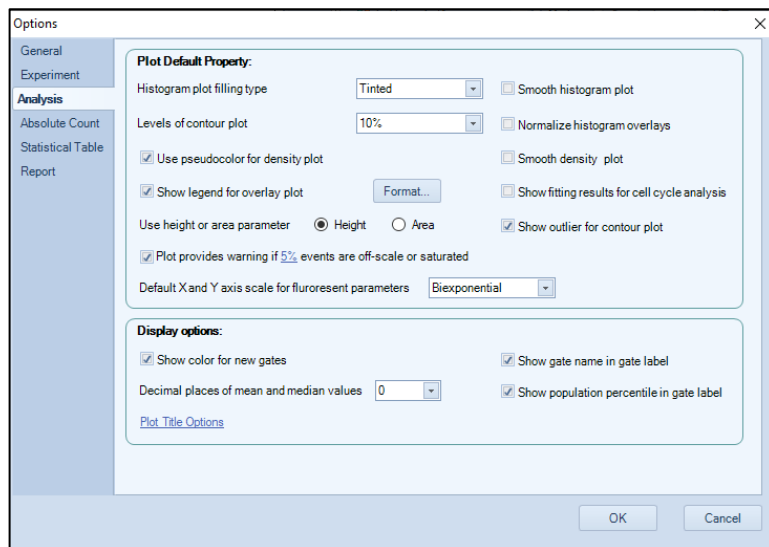
Data Analysis

Check this box to set the format settings as the default when **Apply** or **OK** button is clicked.


- **Apply to:**
Select which plot(s) to apply the format settings.
- **Apply:**
Click to apply changes and keep this window open.
- **OK:**
Click to apply changes and close this window.
- **Cancel:**
Click to close this window without applying any changes.

Default Plot Settings

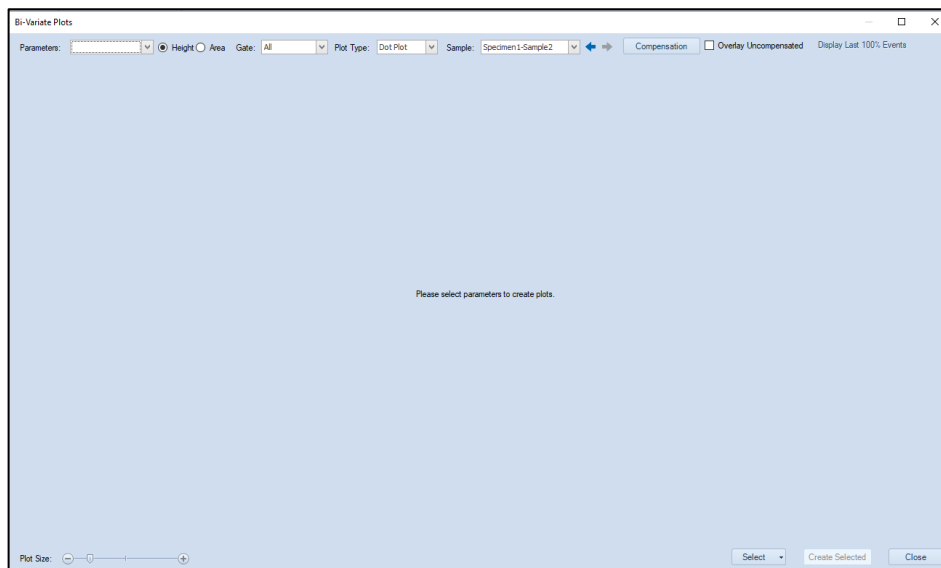
To change the default settings for plots, go to **File > Options** and select the **Analysis** tab. See [Setting](#) for details.



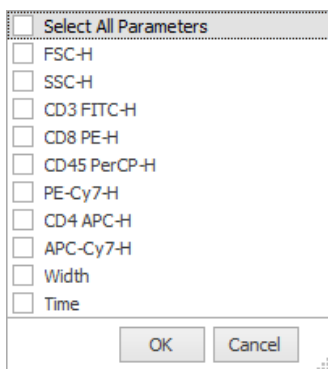
Bi-Variate Plots

Bi-Variate Plots window can be used to create a matrix of plots with selected parameters plotted against each other. To create bi-variate plots, click  icon in the workspace toolbar to open **Bi-Variate Plots** window.

Data Analysis




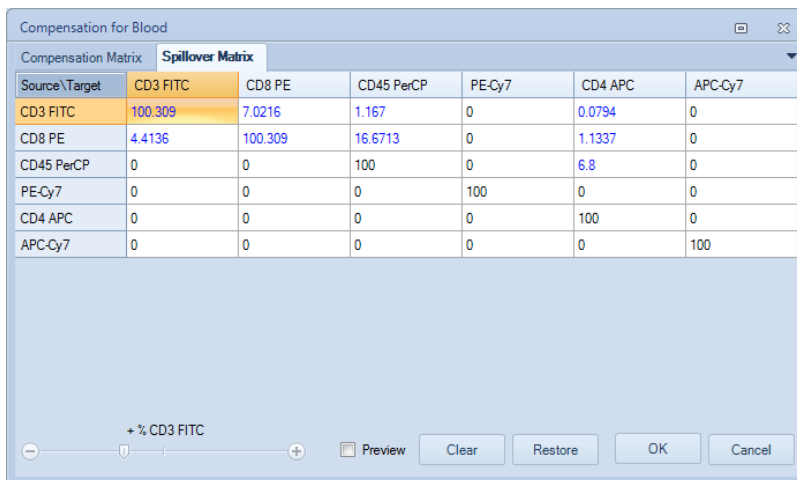
- **Parameters:** List all the parameters of the selected sample in the **Sample** drop-down box. Clicking the **OK** button after selecting the parameters in the drop-down list will create the N×N plots of the selected parameters. Check **Select all parameters** to check all the parameters.



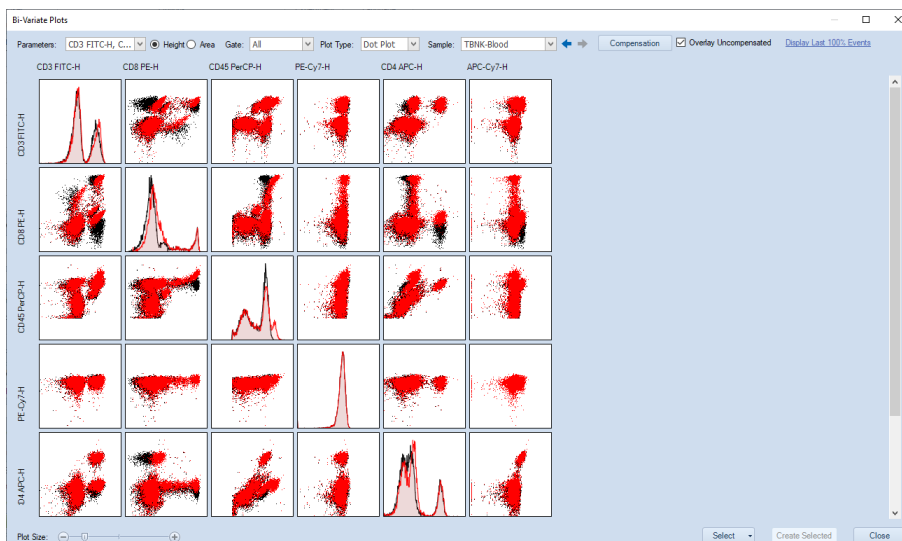
- **Height:** Select to set the parameter of the plots to Height.
- **Area:** Select to set the parameter of the plots to Area.
- **Gate:** List all the gates of the selected sample in the **Sample** drop-down box. The first item is **Ungated**. When select a gate, only the data in the gate will be displayed on the plots.
- **Plot Type:** Select plot type for the created bi-variate plots, including Dot Plot, Density Plot, and Contour Plot.

Data Analysis

- **Sample:** Select which sample in the experiment file to be plotted. By default, the current active sample will be selected when the window is opened. Used  icon to switch samples forward and backward.
- **Compensation:** Click to open **Compensation** window to change the compensation settings. The created plots will be refreshed according to the modified compensation settings. For details of the fluorescence compensation setting, refer to [Active Compensation](#) for detailed description.

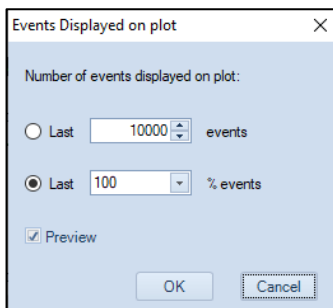


- **Overlay Uncompensated:** Select to overlay uncompensated data on the created bi-variate plots.

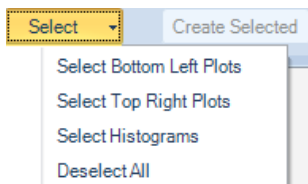


Data Analysis

- **Display Last XXX Events:** Displays the most recently collected events in the plot. This option allows the user to set a number or percentage of the most recently collected events to display. When user clicks the XXX area, following window will pop up. User can select and edit either the number or percentage of events to be displayed for all Bi-variate plot. When **Preview** is checked, user can preview the resulted changes to all plots.



- **Plot Size:** Move the slider can change the size of the plots.
- **Select:** Click on the pop-up selection menu to select different section of the bi-variate plots (Bottom Left Plots, Top Right Plots, and Histograms) or unselect all plots. The plot can also be selected by clicking individual plot. To select a group of plots, pressing the left mouse button and drag an area across all the plots to be selected. Once selected, a red border will show on the plot. Clicking on the selected plot one more time will cancel the selection. Selected plots can be added into the workspace by clicking "**Create Selected**" button.











- **Create Selected:** Click to create the selected plots in the workspace.
- **Close:** Click to close **Bi-Variate Plots** window.

NOTE

The settings user defined in Bi-variate Plots window, including Parameter, Height, Area, Gate, Plot Type, Sample, Compensation, Overlay, and Events to be displayed will be automatically recorded by the software. When user re-open this window or restart the software, these settings will be automatically applied.

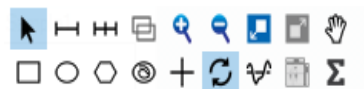
Gates

Gates allow for the analysis of subpopulations from the total population collected. As described in [Plot Gating](#) gates can be applied to subsequent plots to focus in on a specific population. These plots can then be further gated and new plot created to focus on a more specific population.

The Workspace Toolbar includes icons for creating rectangular gates , elliptical gates , polygonal gates , Freehand Gates , quadrant gates , logic gates , range gates , and bi-range gates .









The gating tools can be also selected from the right-click popup mini toolbar on plots.



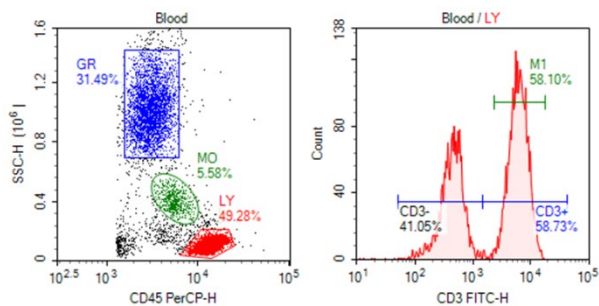
All gate types can be created on dot plots and density plots. Range gates and bi-range gates can be created on histograms. Gates can also be combined to create a logic gate.


Create Gates

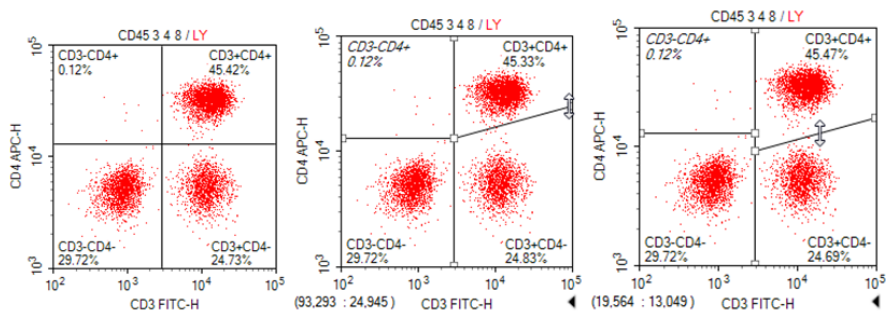
- To create a rectangular gate: Click the rectangular gate icon , in the Workspace Toolbar. Click and drag in the plot to enclose the target population within the rectangle. Release the mouse button to create the gate.
- To create an elliptical , range , or bi-range gate : Follow similar procedures as for creating the rectangular gate.
- To create a polygonal gate: Click the polygonal gate icon , in the Workspace Toolbar. 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 a Freehand Gate: Click the **Freehand Gate** icon , in the Workspace Toolbar. Left click start point in the plot to begin creating the Freehand Gate. Move the mouse cursor inside the plot to draw curve. Double-click the end point to complete and create the gate.


Data Analysis

The following figures include a rectangular gate GR, an elliptical gate MO, a polygonal gate LY, a range gate M1, and a bi-range gate separating CD3- and CD3+.



- To create a quadrant gate: Click the quadrant gate icon , in the Workspace Toolbar. 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.



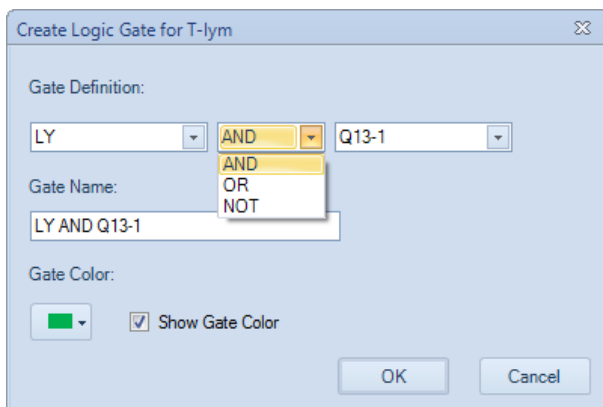
- To create a logic gate: Click the logic gate icon , in the Workspace Toolbar to open the **Create Logic Gate** window. In this window, the user can create a logic gate for the sample in the selected plot.

There are three types of logic gates: **AND** gates, **OR** gates, and **NOT** gates. In the window, a drop-down menu includes the three logic gate types.

When **AND** or is selected from the drop-down menu, there are two additional drop-down menus to select gates. With an **AND** gate, the new gate includes events that are included in both of the selected gates. With an **OR** gate, the new gate includes events that are included in either one of the selected gates.

When **NOT** is selected from the drop-down menu, there is one additional drop-down menu to select a gate. With a **NOT** gate, the new gate includes all of the events excluded from the selected gate.

Data Analysis



In the **Experiment Manager** panel, logic gates can be found under the sample's **Analysis** node.


NOTE

If you want to create multiple gates of the same type, double click the gate icon (a blue outer line will show on the gate icon). The gating tool will then remain active and you can create multiple gates of the selected type. Once completed, press the Esc key on the keyboard to exit.

Edit Gates

All gates can be moved and resized after being created. If a gate is edited, all gate statistics and subsequent plots dependent on the gates are updated to reflect the changes.

There are multiple methods to select a gate for editing. Options include:

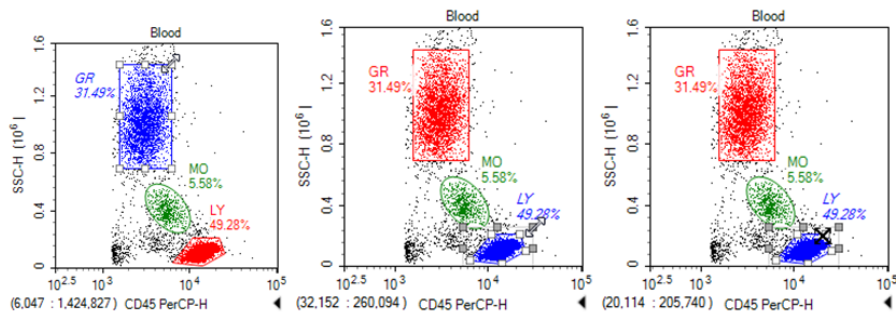
- Click the pointer icon  , in the Workspace Toolbar to activate the cursor. Select the gate by clicking on a vertex or edge of the gate.
- Click on the gate label.
- From the **Gate** tab of the **Menu Bar**, select the gate from the drop-down menu in the **Current Selection** group.
- Double-click on an area within the gate. This does not work for quadrant gates.

After the gate is selected, the gate's control points are displayed (as white boxes). To change the size of a gate, click and drag the control points to modify the gate.

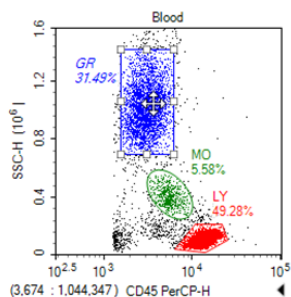
NOTE

Extra grey control points are displayed when a polygon gate is selected, which can be used to scale the gate as a whole.

Data Analysis



To move the gate, select the gate as described above and drag within the gate or press keyboard arrows. While moving, the cursor should change into the crossed arrow symbol.



After editing is complete, click outside of the gate to unselect the gate.

To delete a gate, select the gate as described above and press the Delete key on the keyboard. When a gate is deleted, the subsequent gates and plots that depend on it are reset.

Gate Display Format

The NovoExpress Software allows users to format the color and labels of gates. Gate color determines the color of events displayed on the dot plot, as well as the color of the histogram when the gate is applied to a histogram.

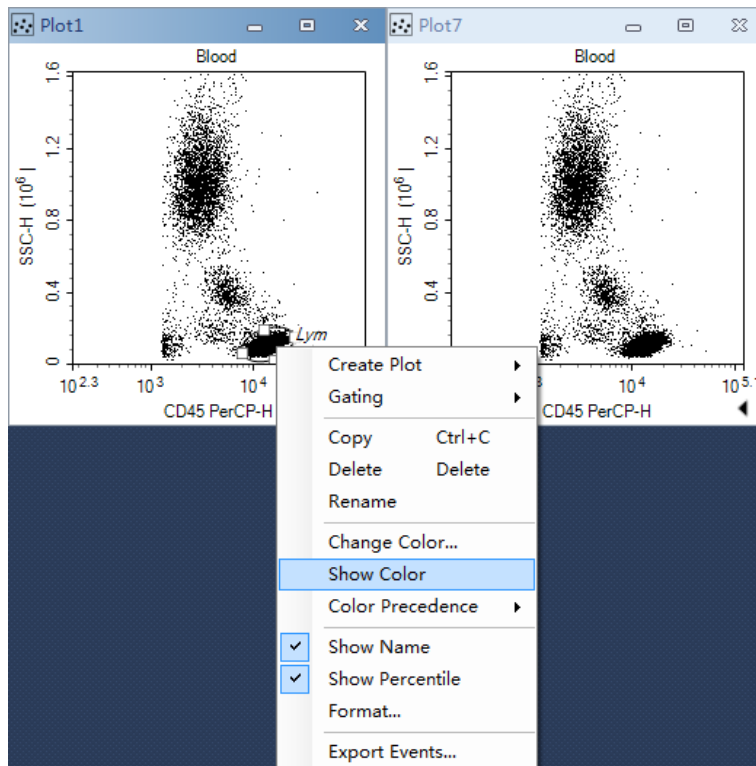
Set the Color of the Gate

The color of each gate can be set. In dot plots, the events included in the gates are displayed in the chosen color. If additional dot plots are created, these events are displayed in the same color for easy identification.

Data Analysis

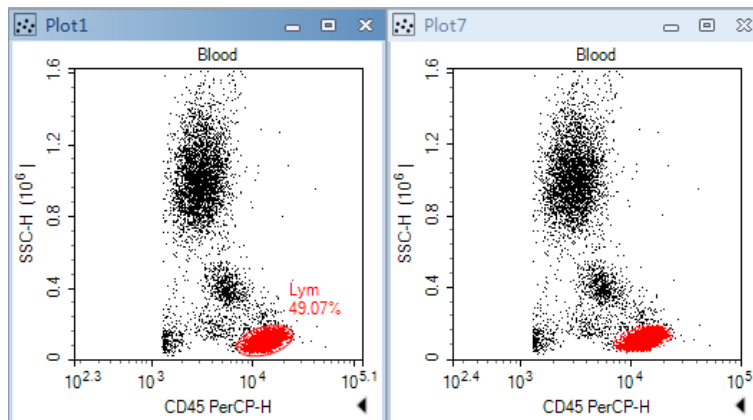
To remove the color from gates, select the gate and right-click on the gate and unselect **Show Color** or select the gate and unselect Color from the **Gate** tab of the **Menu Bar**.

The plots shown below have a gate with the color unselected.



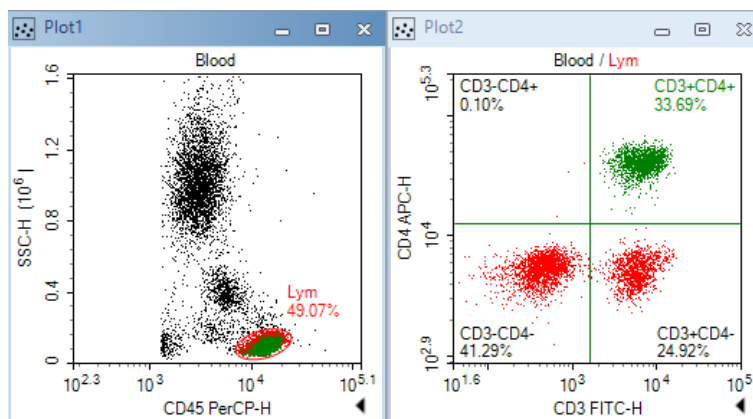
The plots below have a gate with the color selected.

Data Analysis



To change the color of a gate, select the gate, right-click and select **Change Color...** or select the gate and change the color from the drop-down menu next to Color in the **Gate** tab of the **Menu Bar**. Gate color can also be changed via color column of **Gate Manager**.

When an event is inside more than one gate, its color on dot plot is determined by the color precedence of the gates. The plots below show that gate CD3+CD4+ has higher color precedence than does gate Lym. To view or modify color precedence of gate, refer to [Gate Manager](#).



Gate Labels

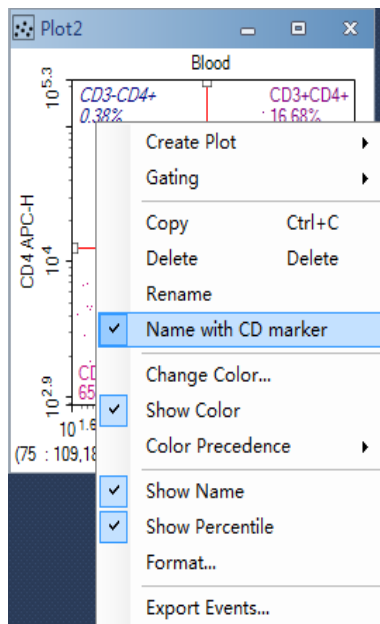
Gate labels are displayed near the gates and include the gate name and the percentage of the events included in the gate relative to the total number of events displayed on the plot.

Data Analysis

To change the name of a gate, select the gate and click on the gate name to edit or select the gate and edit it from the **Gate Name** box of the **Gate** tab in the **Menu Bar**.

To hide the population percentage, unselect **Show Value** from the **Gate** tab of the **Menu Bar**, or unselect **Show population percentile** in the gate label in the **Analysis** Tab of **Options**.

If the alias of a parameter is labeled in the **Parameters** panel as a **CD** (Cluster of Differentiation) marker, quadrant and bi-range gates can be used to easily label positive and negative populations. To use this setting, the parameter's alias must be labeled as CD and a number. Right-click on the quadrant or bi-range gate and select **Name with CD** to rename the gates according to the **CD** markers.



Apply a Gate to a Plot

There are multiple methods for applying a gate to a plot. When gates are applied to plots, the plots only display events included within the specified gate. Gates can be applied to a plot if the creation of the gate is not dependent on the plot.

To apply a gate to a plot:

- Within the workspace, hold down the keyboard Ctrl key while dragging the gate to the plot. The dragged gate is applied to the plot where it was dropped.
- Select a gate in the workspace, and drag it to the title of the plot to be gated.

Data Analysis

- Select the gate and right-click on the gate. Select **Gating** and select the plots to have the gate applied.
- Select the gate from the **Gate** tab of the **Menu Bar**, select the **Gating** button and select the plots to have the gate applied.

Copy and Paste Gates

There are multiple methods to copy and paste a gate:

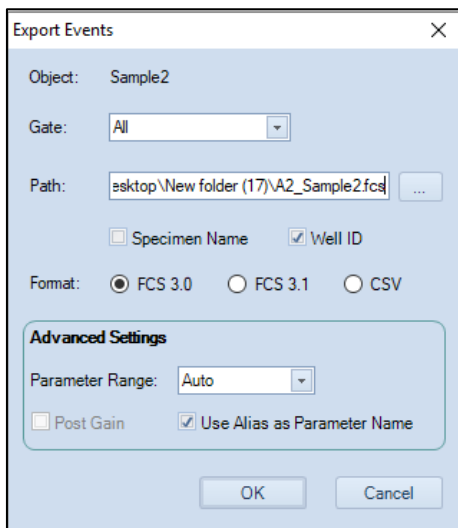
- Select the gate and use the keyboard shortcut Ctrl C to copy the gate. Select a plot and use the keyboard shortcut Ctrl V to paste the gate into the selected plot.
- Select a gate. Drag and drop the gate into a different plot. The dragged gates are pasted into the plot where it was dropped.
- To duplicate a gate within the same plot, select a gate and use the keyboard shortcut Ctrl D or select the gate and use the **Duplicate** button from the **Home** tab of the **Menu Bar**. The duplicate plot appears at the same location as the original gate.

Export Gate Events

The data from a gate can be exported in either CSV or FCS file format. To export:

- 1 Select the gate.
- 2 In the **Gate** tab of the **Menu Bar**, click the **Export Events** button to open the window shown below.

Data Analysis



- 3 In the window, set the export path, file format, parameter range and post gain. Click **OK** to export the data.

NOTE

For more information regarding this window, see [Export Data](#).

Gate Manager

The **Gate Manager** 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	CVX	CVY
All	8	10,035							
GR	2	3,160	31.49%	CD45 PerCP-H	SSC-H	3,207	1,017,885	29.33%	15.15%
MO	1	560	5.58%	CD45 PerCP-H	SSC-H	5,727	403,460	22.95%	15.38%
LY	3	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%

Toolbar of Gate Manager



Icon	Description
	Show Gate Hierarchy: When checked, the table displays output in tree mode. Child gates are indented.
	Show Columns: Choose which statistical columns to display in the table. Refer to Calculation of Statistics for further information on calculation of gate statistics.
	Modify Color Precedence: When checked, the table displays output as list mode. The table is sorted by color precedence – the gate with highest color precedence is displayed on the top.
	Reset to Default Color Precedence: Sets color precedence of all gates to default values. By default, newer gates have higher precedence than do older gates. Child gates have higher precedence than do parent gates. Logic gates have higher precedence than do gates which compose the logic gates.
	To Top: Sets color precedence of selected gate to the highest precedence. Only available when Modify Color Precedence is checked.
	Up: Sets color precedence of selected gate higher. Only available when Modify Color Precedence is checked.
	Down: Sets color precedence of selected gate lower. Only available when Modify Color Precedence is checked.
	To Bottom: Sets color precedence of selected gate to the lowest precedence. Only available when Modify Color Precedence is checked.
	Copy Text: Copies all gate name(s) and statistics as text to clipboard.

Modify Gate Color and Color Precedence

A gate can be set with a color, and the color will be used to draw the gate label. On dot plots, events inside a gate are shown as colored dots defined by the gate color. When an event is inside more than one gate, its color on a dot plot is determined by the color precedence of the gates. To understand more about gate color and color precedence, refer to [Set the Color of the Gate](#).

To modify gate color precedence, check the **Modify Color Precedence** tool in the toolbar. The color column in **Gate Manager** is shown below:

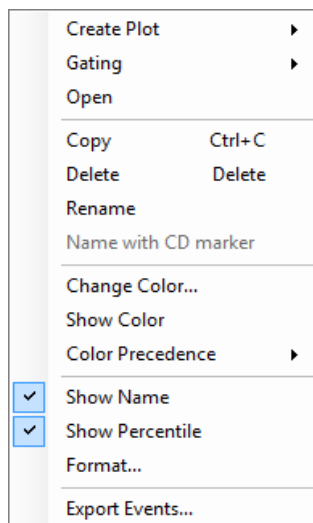
Data Analysis

Gate	Color
Lym	<input checked="" type="checkbox"/> ■ 1
GR	<input checked="" type="checkbox"/> ■ 2
CD3-CD4+	<input type="checkbox"/> ■ 3
CD3+CD4-	<input type="checkbox"/> ■ 4

The number in the **Color** column is the gate color precedence. Number 1 indicates highest color precedence. The gate rows are sorted by color precedence. To change the color precedence of a gate, drag the gate row and drop it to desired position. Click the check box to set whether to show gate color (black color indicates no color is shown). Click the color square box to change gate color in a pop up tool window.

Context menu

The context menu is shown below for right clicking on only one gate.



Create Plot: Creates a new plot including the events from the selected gate.

Gating: Selects plots to apply the gate.

Open: Opens the plot containing the gate.

Copy: Copies the gate.

Delete: Deletes the gate.

Rename: Renames the gate.

Data Analysis

Name with CD Marker: If a fluorescence parameter is labeled as a CD (Cluster of Differentiation) marker in the **Parameter** panel by setting the **Alias** as CD and a number, this labels the gate using the CD marker(s) specified.

Change Color: Modifies the color of the gate.

Show Color: Sets whether to display the gate in color.

Color Precedence: Modifies color precedence of the gate.

Show Name: Shows the gate name in gate label on plot. If the **Show gate name in gate label** option in **Setting > Analysis** is not checked, the **Show Name** menu item here will be disabled.

Show Percentile: Shows the percentage of the gated events relative to the total number of events on the plot. If the **Show population percentile in gate label** option in **Setting > Analysis** is not checked, the Show Percentile menu item here will be disabled.

Format: Opens **Plot Format** dialog to define gate format.

Export Events: Exports data for the events inside the current gate in either FCS or CVS format.

NOTE

When multiple gates are selected in **Gate Manager**, only Delete, Show Color, Color Precedence, Show Name and Show Percentile are available.

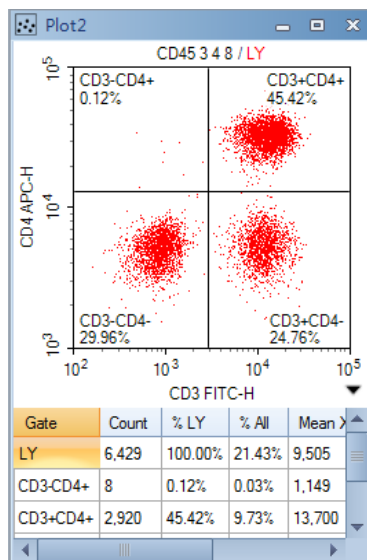
Statistics

In the NovoExpress Software, a table of statistical information can be found under plots.

Display Statistical Information

In the following figure, the statistical information chart is displayed below the plot.

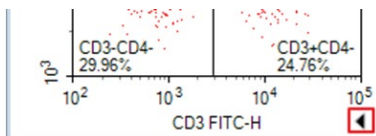
Data Analysis



Open the Statistics Chart

There are two methods to open the statistical information chart.

- From the plot, click the button on the lower right corner to expand the plot and display the statistics chart.



- From the Workspace Toolbar, click the **Show Statistics** button to show/hide the statistics chart of a plot.

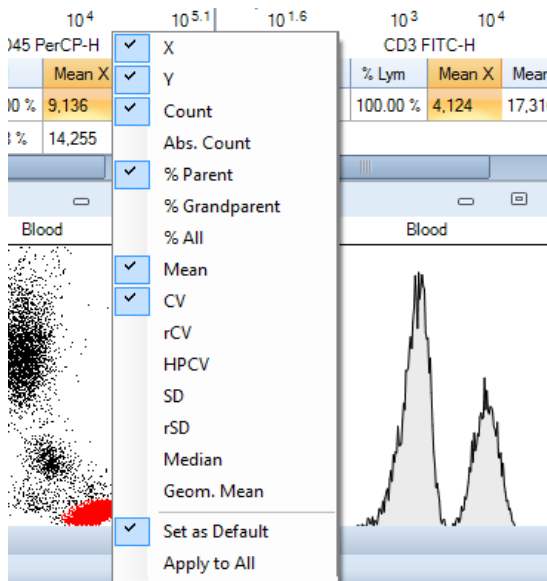
Statistics Layout

In the statistics chart, the first column is the **Gate** column, and the remaining columns list the statistical parameters. As labeled by the **Gate** column, the first row of the chart contains statistical information for all events, and the remaining rows contain statistical information for individual gates.

Gate	Count	% All	Mean X	Mean Y
All	30,000	100.00%	14,412	497,847
MO	1,681	5.60%	12,411	260,983
GR	18,163	60.54%	6,696	703,977

Data Analysis

To hide or show individual statistical parameters in the chart, right-click within the chart and select the parameters to hide or display. Check **Set as Default** to set the current setting as the default setting of new plot. And click **Apply** to All to hide or show individual statistical parameters to all plots of current sample.



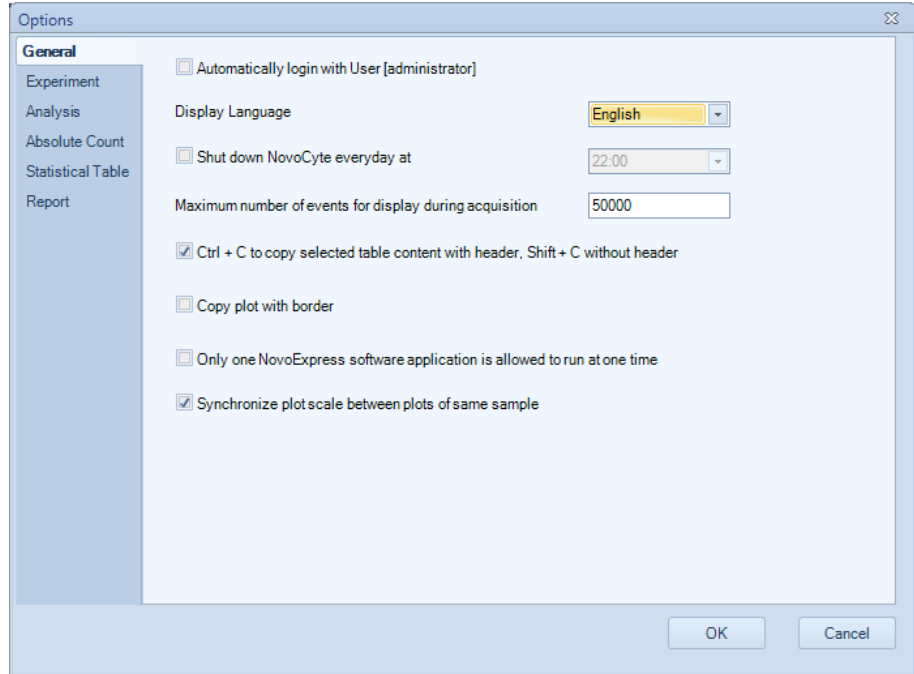
Copy Statistical Information to Clipboard

Data from the statistics chart can be copied to the clipboard. The copied data can be pasted to a spreadsheet program, such as Microsoft Excel, for further analysis.

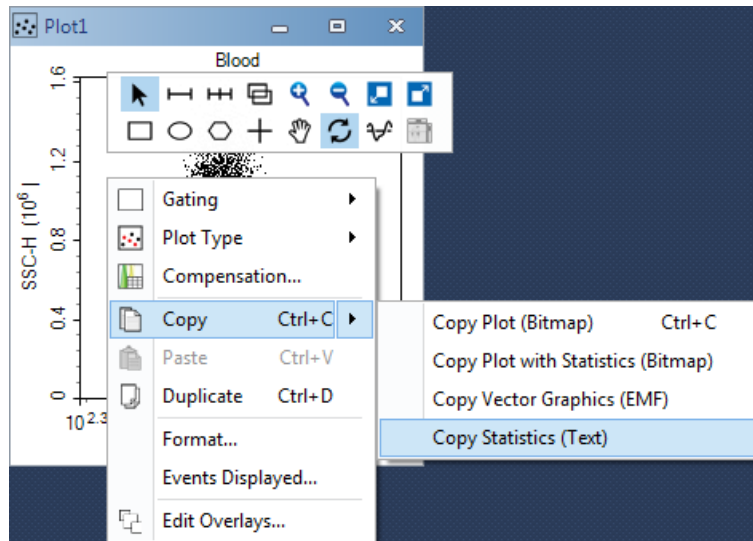
There are two methods to copy statistical information to the clipboard:

- Select the statistical information from the chart by clicking and dragging or by using the keyboard shortcut Ctrl A to select all. Use the keyboard shortcut Ctrl C or Shift C to copy the selected information. With Ctrl C and Shift C, one may copy the information with the column header and one may copy the information without the header. This can be set under **File > Options > General**.

Data Analysis



- Right-click in the plot, select **Copy**, and select **Copy Statistics (Text)**.



Data Analysis

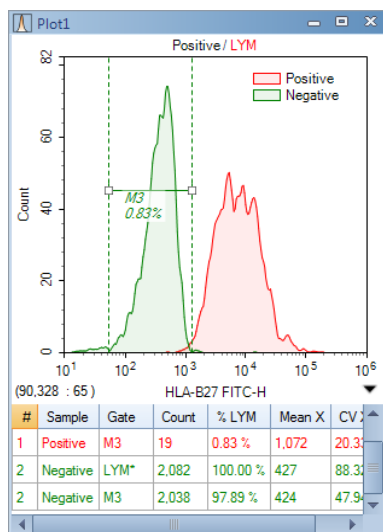
Statistics Layers

For plots with multiple layers, the statistical table includes additional columns. The first column, #, indicates the layer on the plot. The second column, Sample, indicates the sample plotted in the layer. The third column, Gate, indicates the gate. The remaining columns describe the statistical parameters.

If a gate belongs to a sample which is different from the first layer's sample, an asterisk appears next to the gate name to indicate such situation. In the figure below, the statistics of the second row is for gate LYM which applies to the sample named Negative as indicated by the asterisk next to the gate name to distinguish it from the LYM gate which applies to the sample named Positive.

#	Sample	Gate	Count	% LYM	Mean X
1	Positive	LYM	2,281	100.00%	10,808
2	Negative	LYM*	2,082	100.00%	427

For gates in a layered plot, the statistical information is displayed for all layers. In the figure below, statistical information for Gate M3 is displayed for both the layer corresponding to the Positive sample and the layer corresponding to the Negative sample. The statistics in the last row is for gate M3 which belongs to the sample named Positive; since this is the same as the first layer, no asterisk shows next to the gate name.



Data Analysis

Calculation of Statistics

In calculating the statistics, linear scale data are used regardless of the coordinate scale displayed by the plot. The calculation also takes into account any fluorescence compensation applied to the data.

In addition, the calculations update automatically if the data set, gates, fluorescence compensation, or plot parameters are modified.

The statistics include the total number of events, absolute count, percentage gated, mean, coefficient of variation, half-peak coefficient of variation, median, and geometric mean.

- Count

The number of events collected in the specified gate.

- Abs. Count

The abbreviation of Absolute Count. The concentration of events defined as:

$$\text{Absolute Count} = \text{Count} / \text{Ve} / \text{DF} / \text{Absolute Count Unit}$$

Where Count is the number of events in the gate, Ve is the sample acquisition volume, DF is the dilution factor, and Absolute Count Unit is the absolute number of units. To set the dilution factor and the absolute number of units, click on **Absolute Count Setting** from the **Sample** tab of the **Menu Bar**.

Absolute Count Setting - Sample1

Dilution Factor: :

Absolute Count Unit: No./µL No./mL No./L

Set as Default Show Absolute Count in Statistics

Apply to All Samples in the Experiment File

Apply to All Samples in the Same Specimen

OK Cancel

- %Parent

Data Analysis

Percentage of events included within the gate relative to the number of events within parent gate.

- % Grandparent

Percentage of events included within the gate relative to the number of events within grandparent gate.

- % All

Percentage of events included within the gate relative to the total number of events collected.

- Mean

The mean is defined as

$$\bar{X} = \frac{1}{n} \sum_{i=1}^n X_i$$

Where n is the number of events and X_i is the parameter value of the number i event.

- SD

The standard deviation indicates the variation in the data set and is defined as

$$\sqrt{\frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X})^2}$$

Where n is the number of events, X_i is the parameter value of the number i event, and \bar{X} is the mean of the set.

- rSD (Robust SD)

The robust SD is relatively insensitive to outliers comparing to the classical standard deviation. It is equal to 0.75 multiplied by the interquartile range (IQR). The interquartile range is the 75th percentile channel minus the 25th percentile channel.

The RSD is defined as

$$rSD = 0.75 \times IQR = 0.75 \times (Q3 - Q1)$$

Where Q1 is the 25th percentile channel and Q3 is the 75th percentile channel.

- CV

The coefficient of variation indicates the variation of the data set expressed as a percentage and is defined as

Data Analysis

$$CV = (SD / \bar{X}) \times 100\%$$

Where *SD* is the standard deviation and \bar{X} is the mean.

- rCV (Robust CV)

Robust CV is calculated by Robust SD divided by population median.

$$rCV = rSD / \text{Median}$$

- HPCV

The half-peak coefficient of variation is expressed as a percentage and is defined as

$$HPCV = FWHM / (2.36\bar{X}) \times 100\%$$

Where **FWHM** is the full width at half maximum of the peak and \bar{X} is the mean of the set.

- Median

The median value separates the data set so that number of events larger and the number of events smaller than the median are equal.

- Geom. Mean

The geometric mean is defined as

$$\bar{X}_{geo} = 10^{\frac{1}{n} \sum_{i=1}^n \log X_i}$$

Where, *n* is the number of events and *X_i* is the parameter value of the number *i* event. Note that the geometric mean cannot be calculated for events with negative values. If you include the geometric mean for populations with negative values, the resulting statistics will be invalid.

- Stain Index

The Stain Index is a normalized functional measure of the reagent brightness, defined as

$$\text{Stain_Index} = (MFI_1 - MFI_2) / (2 \times SD_2)$$

Where *MFI₁* is the Mean Fluorescence Intensity of the positive population, *MFI₂* is the Mean Fluorescence Intensity of the negative population and *SD₂* is the standard deviation for the negative population. The Stain Index function is only available in statistical table but not statistics chart below the plot.

Fluorescence Compensation

In multicolor flow cytometry, where a sample is stained with a combination of different fluorophores, each of the different fluorophores has a unique emission spectrum, and in many cases, the different emission spectra overlap. If the overlap occurs within a specific fluorophore's channel, fluorescence compensation can be used to correct for the overlap by removing the signal from the fluorophores that do not correspond to the channel.

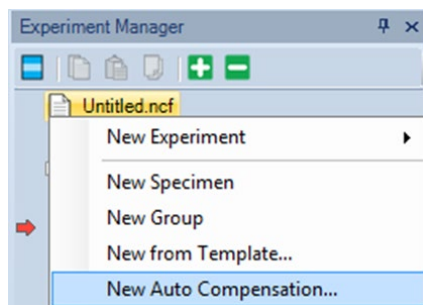
The NovoExpress Software provides three methods for the user to adjust fluorescence compensation. These methods include an automatic method, a compensation matrix adjustment method, and a quick compensation method.

Automatic Compensation

The automatic compensation method automatically calculates the compensation matrix and also allows for the compensation matrix to be applied to additional samples. Automatic Compensation matrix can be generated from samples acquired on instrument and from samples imported with FCS files. [Conduct Automatic Compensation from Imported FCS Files](#) describes the details on how to generate automatic compensation matrix using imported FCS files.

Set Automatic Compensation

In the **Home** tab of the **Menu Bar**, click the **Auto Compensation** button, or in the **Experiment Manager** panel, right click on the file name or a group and select **New Auto Compensation...** The **New Auto Compensation** window appears.



In the **New Auto Compensation** window, the user can select the channels for compensation, whether to compensate using area or height measurements, and whether to calculate compensation based on the measured mean or median.

To set up the automatic compensation:

Data Analysis

- 1 Select the channels to compensate: Use the checkboxes to select the channels to compensate. If an unstained sample is to be used to assist in compensation, select the **Unstained** box.
- 2 Choose to compensate either using the Area or Height measurements.
- 3 Choose to compensate based on either mean or median values.

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](#)

<input checked="" type="checkbox"/> B530 FITC	<input checked="" type="checkbox"/> V445 Pacific Blue
<input checked="" type="checkbox"/> B572 PE	<input checked="" type="checkbox"/> V530 AmCyan
<input checked="" type="checkbox"/> B615 PE-Texas Red	<input checked="" type="checkbox"/> V572 Pacific Orange
<input checked="" type="checkbox"/> B675 PerCP	<input checked="" type="checkbox"/> V615 Qdot 605
<input checked="" type="checkbox"/> B780 PE-Cy7	<input checked="" type="checkbox"/> V675 Qdot 655
<input checked="" type="checkbox"/> R675 APC	<input checked="" type="checkbox"/> V780 Qdot 800
<input checked="" type="checkbox"/> R780 APC-Cy7	

OK Cancel

New Auto Compensation window for NovoCyte instrument (e.g. 3000 System)

Data Analysis

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](#)

<input checked="" type="checkbox"/> FSC FSC	<input checked="" type="checkbox"/> R667 APC	<input checked="" type="checkbox"/> V695 Qdot 705
<input checked="" type="checkbox"/> SSC SSC	<input checked="" type="checkbox"/> R695 Alexa Fluor 680	<input checked="" type="checkbox"/> V725 BV711
<input checked="" type="checkbox"/> B530 FITC	<input checked="" type="checkbox"/> R725 Alexa Fluor 700	<input checked="" type="checkbox"/> V780 Qdot 800
<input checked="" type="checkbox"/> B586 EYFP	<input checked="" type="checkbox"/> R780 APC-Cy7	<input checked="" type="checkbox"/> Y586 PE
<input checked="" type="checkbox"/> B615 PI	<input checked="" type="checkbox"/> V445 Pacific Blue	<input checked="" type="checkbox"/> Y615 PE-Texas Red
<input checked="" type="checkbox"/> B667 PerCP	<input checked="" type="checkbox"/> V530 AmCyan	<input checked="" type="checkbox"/> Y667 PE-Cy5
<input checked="" type="checkbox"/> B695 PerCP-Cy5.5	<input checked="" type="checkbox"/> V586 Pacific Orange	<input checked="" type="checkbox"/> Y695 PE-Cy5.5
<input checked="" type="checkbox"/> B725 PerCP-eFluor 7	<input checked="" type="checkbox"/> V615 Qdot 605	<input checked="" type="checkbox"/> Y725 PE-Alexa Fluor
<input checked="" type="checkbox"/> B780 PE-Cy7 (B)	<input checked="" type="checkbox"/> V667 Qdot 655	<input checked="" type="checkbox"/> Y780 PE-Cy7 (Y)

OK Cancel

New Auto Compensation window for NovoCyte Quanteon instrument (e.g. 4025 System)

New Auto Compensation

Import Samples from FCS files

Compensation on:

Area
 Height

Parameter for calculation:

Mean
 Median

Compensation Channels:

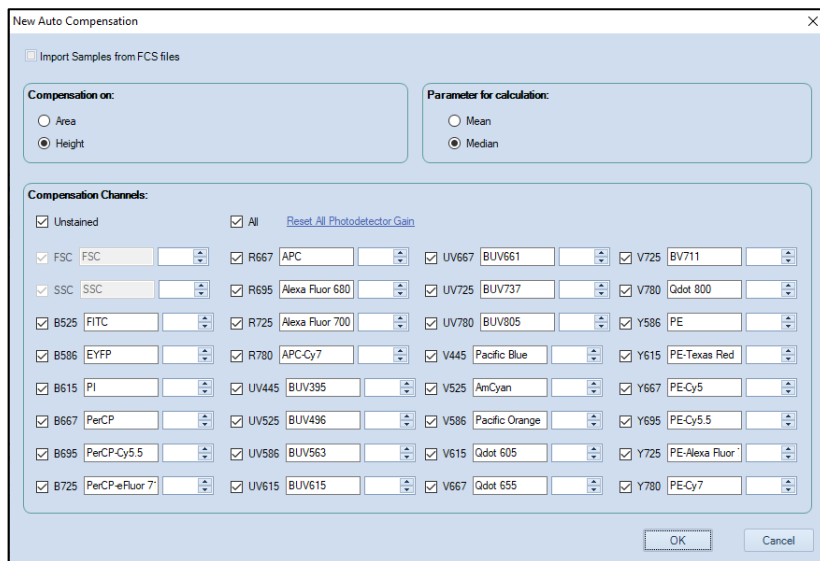
Unstained All [Reset All Photodetector Gain](#)

<input checked="" type="checkbox"/> FSC FSC	<input checked="" type="checkbox"/> B780 PE-Cy7 (B)	<input checked="" type="checkbox"/> V780 Qdot 800
<input checked="" type="checkbox"/> SSC SSC	<input checked="" type="checkbox"/> V445 Pacific Blue	<input checked="" type="checkbox"/> Y586 PE
<input checked="" type="checkbox"/> B530 FITC	<input checked="" type="checkbox"/> V530 AmCyan	<input checked="" type="checkbox"/> Y615 PE-Texas Red
<input checked="" type="checkbox"/> B586 EYFP	<input checked="" type="checkbox"/> V586 Pacific Orange	<input checked="" type="checkbox"/> Y667 PE-Cy5
<input checked="" type="checkbox"/> B615 PI	<input checked="" type="checkbox"/> V615 Qdot 605	<input checked="" type="checkbox"/> Y695 PE-Cy5.5
<input checked="" type="checkbox"/> B667 PerCP	<input checked="" type="checkbox"/> V667 Qdot 655	<input checked="" type="checkbox"/> Y725 PE-Alexa Fluor
<input checked="" type="checkbox"/> B695 PerCP-Cy5.5	<input checked="" type="checkbox"/> V695 Qdot 705	<input checked="" type="checkbox"/> Y780 PE-Cy7 (Y)
<input checked="" type="checkbox"/> B725 PerCP-eFluor 7	<input checked="" type="checkbox"/> V725 BV711	

OK Cancel

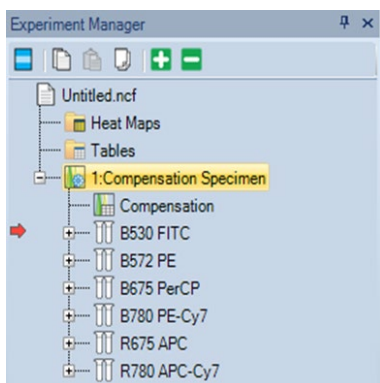
New Auto Compensation window for NovoCyte Advanteon instrument (e.g. V8Y6B7 System)

Data Analysis



New Auto Compensation window for NovoCyte Penteon instrument (e.g. U7V7B6Y6R4 System)

- 4 Edit photodetector gain of each channel. Click **Reset All photodetector Gain** to reset photodetector gains of all channels to default values. Every instrument has the default photodetector gain setting. An underlined photodetector gain text as shown above for the B530 channel indicates it has been modified and is not the default value. A non-underlined photodetector gain indicates that it is the default setting.
- 5 Click **OK**. In the **Experiment Manager** panel, a **Compensation Specimen** node gets created with blank control samples created for the compensation calculation. The samples include the channels selected in the **New Auto Compensation** window.



Data Analysis

To modify the automatic compensation settings, right-click on the **Compensation Specimen** node in the **Experiment Manager** panel. Select **Auto Compensation Setup...** The **Auto Compensation Setup** window appears. Modifications to the automatic compensation settings can be made in this window. Click **OK** to save the modifications.

Prepare Samples for Automatic Compensation

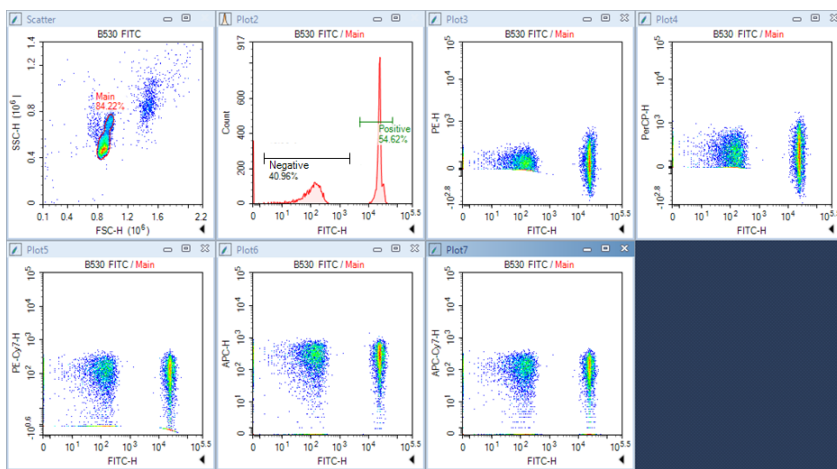
To calculate the compensation, experimental data will need to be collected for each of the control samples in the **Compensation Specimen** node. For the unstained sample, the sample should be prepared without any fluorophores added. For the other control samples in the **Compensation Specimen** node, the samples should be stained with only the corresponding stain. For example, a FITC control sample should be stained with only FITC.

In addition, control samples can also be copied and pasted or imported from a FCS file into the sample, but for the compensation to be correct, the user must ensure that the samples meet the correct staining conditions and photodetector gain settings.

Automatic Calculation of the Compensation Matrix

- Automatic Gating

After the acquisition of a compensation control sample, the sample data are automatically compensated. For each of the compensation specimens, the software automatically gates the main population in a density plot. In addition, the positive and negative groups are identified and gated on a histogram plot.



Data Analysis

NOTE

If an unstained sample is used, the Main gate in the unstained sample is used as the negative group for the control samples in calculating compensation.

In most cases, the automatic gating finds the appropriate populations, but if necessary, the user can also adjust the gates using the following method:

- Open the scatter plot for the sample and modify the position or size of the polygonal Main gate to enclose the correct main population.
- Another option is to create a new gate in the scatter plot to enclose the correct main population, and then apply the new gate to the histogram containing the Positive and Negative gates. See [Apply a Gate to a Plot](#) for more information on applying a gate to a plot.
- Adjust the Positive and Negative gates in the histogram to enclose the correct population.

NOTE

It is possible to remove the Main gate, but the Positive and Negative gates cannot be deleted. If the Main gate is removed, it has to be recreated manually on the unstained sample.

NOTE

After modifying the gates, if the user would like to restore the gate to the default position, in the **Experiment Manager** panel, select the sample, right-click, and select **Reset Plots**.

- Automatic Calculation of the Spillover Values

After the acquisition of the compensation specimen is completed, the fluorescence spillover of each dye is automatically calculated and displayed in the spillover matrix. In the matrix, the fluorophore is listed in the row and the spillover channel is listed in the column.

In an example of calculating spillover, the spillover of FITC into the PE channel using median or mean height measurements is shown below. The values below are measured from the single stained FITC sample.

$$\text{Spillover of FITC into PE} = \frac{(X_{PE,positive} - X_{PE,negative})}{(X_{FITC,positive} - X_{FITC,negative})}$$

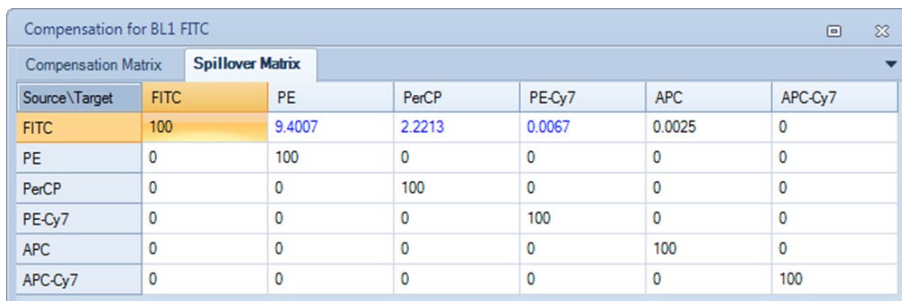
Where $X_{PE,positive}$ is the median or mean in the PE-H channel of the positive FITC population, and so on.

If median is used for calculation, the above spillover value will be adjusted slightly so that after compensation, the median in PE-H channel of the negative FITC population and the median in the PE-H channel of the positive FITC population are closely aligned.

Data Analysis

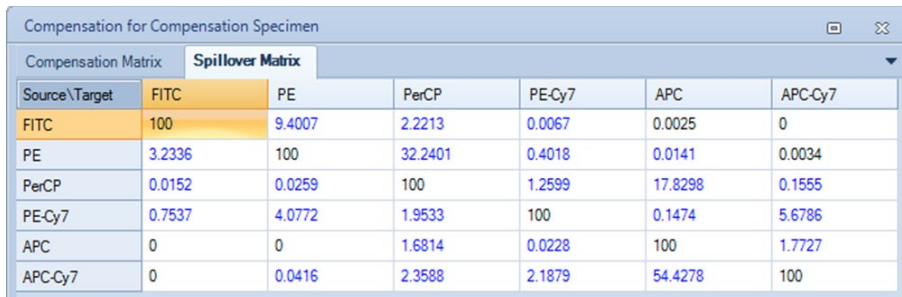
- Automatic Calculation of the Spillover Matrix

In the following figure, the spillover of the single stained FITC sample is automatically calculated. In the matrix, the fluorophore is listed in the row and the spillover channel is listed in the column. In this figure, the FITC fluorophore spillover is 9.4007% into PE, 2.2213% into PerCP, 0.0067% into PE-Cy7 and 0.0025% into APC.



Source\Target	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7
FITC	100	9.4007	2.2213	0.0067	0.0025	0
PE	0	100	0	0	0	0
PerCP	0	0	100	0	0	0
PE-Cy7	0	0	0	100	0	0
APC	0	0	0	0	100	0
APC-Cy7	0	0	0	0	0	100

Following figure shows the entire spillover matrix automatically calculated. To open this, double click the **Compensation** node of **Compensation Specimen** in the **Experiment Manager** Panel. The spillover values of this matrix are from all single stained samples' spillover matrices.



Source\Target	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7
FITC	100	9.4007	2.2213	0.0067	0.0025	0
PE	3.2336	100	32.2401	0.4018	0.0141	0.0034
PerCP	0.0152	0.0259	100	1.2599	17.8298	0.1555
PE-Cy7	0.7537	4.0772	1.9533	100	0.1474	5.6786
APC	0	0	1.6814	0.0228	100	1.7727
APC-Cy7	0	0.0416	2.3588	2.1879	54.4278	100

Application of Automatic Compensation Results

To apply the compensation matrix to experimental samples, in the **Experiment Manager** panel copy the compensation matrix under the Compensation Specimen and paste it to the **Compensation** node under the desired sample.

WARNING

The compensation matrix is calculated at specified photodetector gains. It should only be applied to samples that were acquired with the same photodetector gains.

Data Analysis

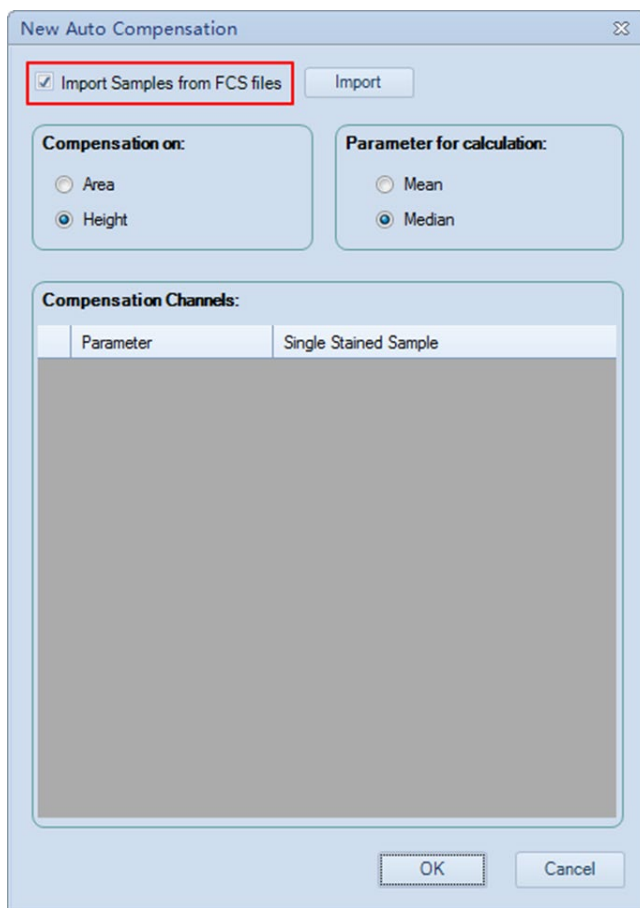
Conduct Automatic Compensation from Imported FCS Files

To perform auto-compensation directly from the imported FCS file:

- 1 Click **Home > Auto Compensation** to open the **New Auto Compensation** window. Select the **Import Samples from FCS files**.

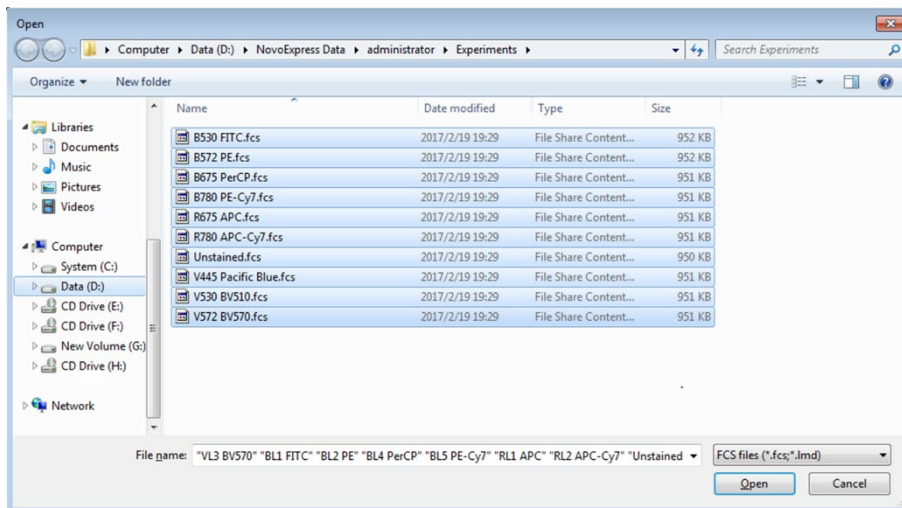
NOTE

This window can also be accessed by right clicking the experiment file and selecting **New Auto Compensation** in the **Experiment Manager** window.



- 2 Select the single file or multiple files while pressing the Shift key in the keyboard. Click **Open** to import the selected file(s) to the software.

Data Analysis



- 3 Ensure all the selected FCS files are successfully imported to the software. Click **OK** to continue.

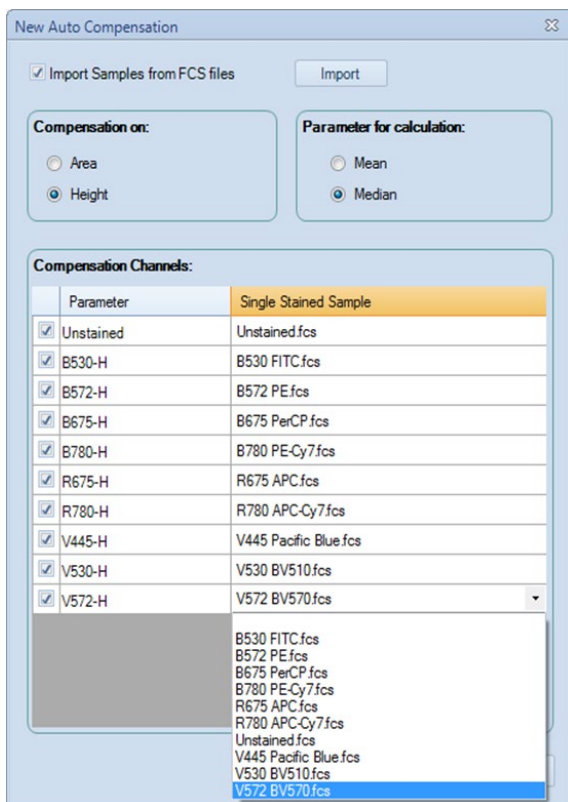


- 4 Set the compensation parameters in the **New Auto Compensation** window (i.e. Area or Height, Median or Mean). Ensure the selected channel parameter for each single stained sample is correct. To select different single stained sample file, click the file name, select the desired file from the pull-down menu. If a channel parameter does not need to be included for the compensation, click the blank area in the pull-down menu. The check box in front of the corresponding channel parameter will be automatically unselected.

WARNING

The software will automatically associate the imported FCS file with the selected channel parameter based on the keyword of the file name (e.g. B530). Users need to verify and select the correct FCS file for each fluorescence detection channel.

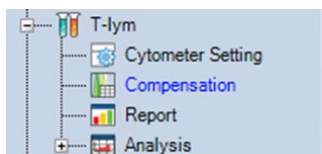
Data Analysis



- 5 Click **OK** and NovoExpress will automatically calculate the compensation matrix based on the samples imported. Once generated, the compensation matrix can be applied to other experiment samples.

Active Compensation

The compensation matrix for each sample can be set to correct for fluorescence spillover in each channel. To open the compensation matrix for a specific sample, in the **Experiment Manager** panel, double-click on **Compensation** under the sample.

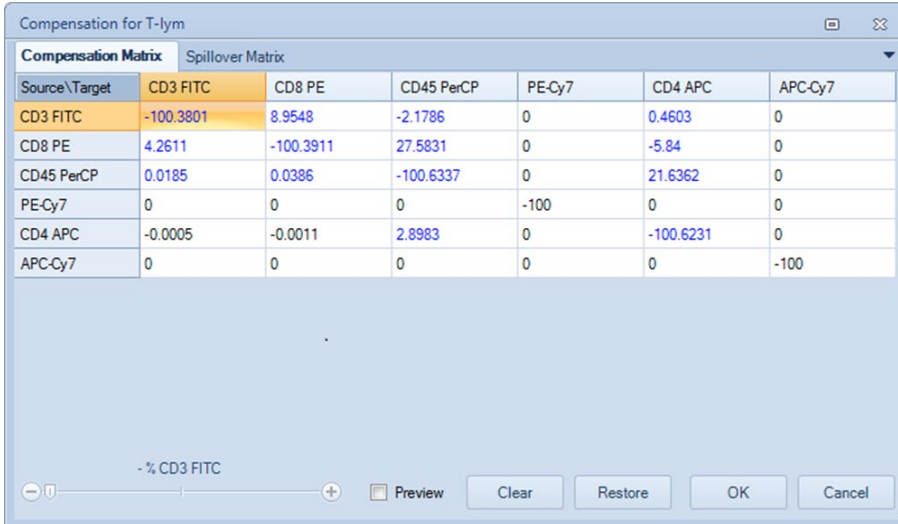


Data Analysis

NOTE

The text of the **Compensation** node in the **Experiment Manager** panel will be blue when the matrix is filled and black with the compensation matrix is empty.

In the compensation and spillover matrix, the fluorescent probe is listed as rows and the spillover channel is listed as columns.



The screenshot shows a dialog box titled "Compensation for T-lym" with two tabs: "Compensation Matrix" and "Spillover Matrix". The "Compensation Matrix" tab is active, displaying a table with the following data:

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

Below the table, there is a slider bar labeled "- % CD3 FITC" with a "Preview" checkbox and buttons for "Clear", "Restore", "OK", and "Cancel".

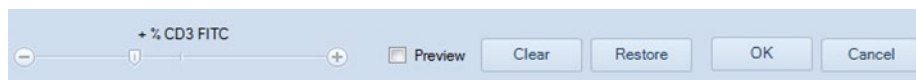
Relationship between the Spillover and Compensation Matrix

The spillover matrix and the compensation matrix are inversely related. When the spillover matrix is modified, the software automatically updates the compensation matrix.

Edit the Spillover Matrix

The NovoExpress Software has two methods for editing the spillover matrix.

- The spillover matrix elements can be manually entered. To manually enter values, select the cell in the matrix to edit and directly enter the value. Values can range between 0 and 800 when entered manually.
- The spillover matrix elements can be modified using the slider bar. To use the slider bar, select the cell in the matrix and use the slider bar to modify the value. Values can range between 0 and 800 when using the slider bar.



Additional options in the Matrix Window:

Data Analysis

- **Preview:**

If the **Preview** box is checked, the plots will be continually updated in real time without exiting out of the matrix window as adjustments are made to the compensation and spillover matrices.

- **Clear:**

Click the **Clear** button to reset the compensation and spillover matrices. The matrix elements will be reset to zero.


- **Restore:**

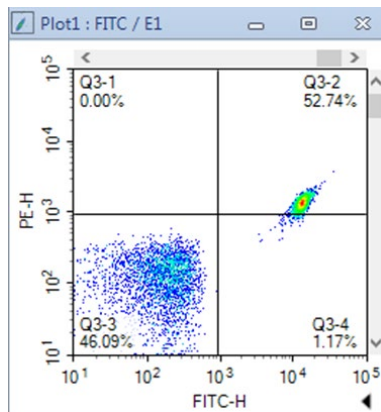
Click the **Restore** button to restore the matrix to the last saved matrix.

Quick Compensation Adjustment

The NovoExpress Software's quick compensation method gives users the option to use a slider bar for quick and intuitive adjustment of fluorescence compensation.

Open the Quick Compensation Adjustment

From the **Home** tab of the **Menu Bar**, click the **Quick Compensation** button . Scrollbars appear on any two parameters plots with fluorescent parameters opened on the workspace. Quickly adjust compensation by adjusting the scrollbar.



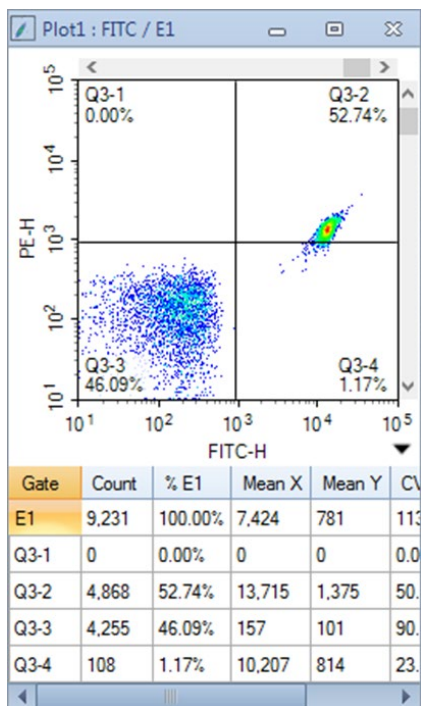
To hide the quick compensation scrollbars, click again on the **Quick Compensation** button.

Use the Quick Compensation Adjustment

To use the quick compensation:

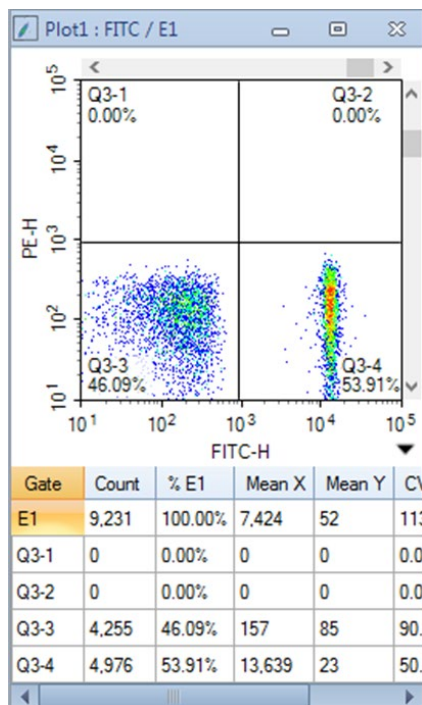
Data Analysis

- 1 For a single-stained sample, create a density plot. Set the X-axis parameter as the sample's single-stained fluorophore channel. Set the Y-axis as the spillover channel to correct. For example, to correct for the spillover of FITC-H into PE-H, analyze a sample stained only with FITC and use a plot with the X-axis set to FITC-H and the Y-axis set to PE-H.
- 2 Create a quadrant gate to gate the positive and negative populations as shown below.



- 3 Drag the vertical scrollbar to adjust compensation. When the Y-axis parameter mean or median in the positive and negative populations are approximately equal, the sample is properly compensated.

Data Analysis



NOTE

Clicking on the blank area of the scrollbar adjusts the compensation by 0.1% increments, and clicking on the arrows of the scrollbar adjusts the compensation by 0.01% increments.

NOTE

When using quick compensation on bi-exponential plots, the display may be slow to update.

Cell Cycle Analysis

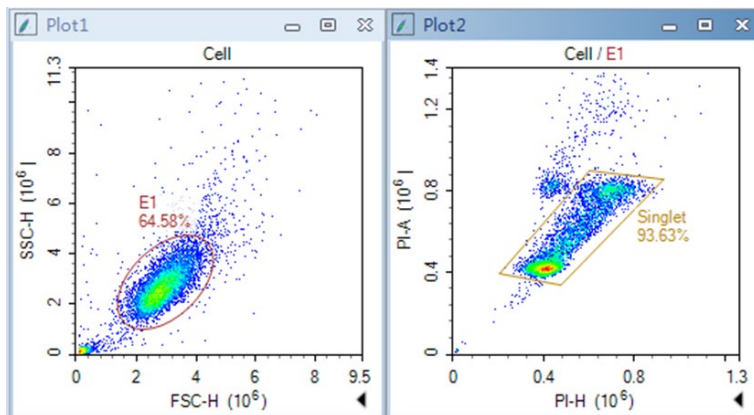
The NovoExpress Software includes a cell cycle analysis feature that allows for the quantification of cells in each phase of the cell cycle based on DNA content.

Automated Cell Cycle Analysis

- Gating Single Cell Population

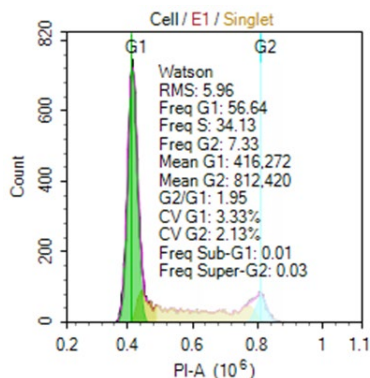
Data Analysis

After collecting the DNA stained cells, use a FSC-H /SSC-H density plot to get the target population and exclude cell debris. From the target population, create a Height versus Area density plot on the fluorescent channel corresponding to the DNA stain, and gate for the single cell population and exclude cell aggregates. This is shown in the figure below.



- Cell Cycle Analysis

Click on the **Cell Cycle Plot** button in the Workspace Toolbar to create a cell cycle plot. Set the X-axis of the plot to a channel for the DNA content stain, such as PI-A. Apply the previously created single cell gate to the plot and make sure all events inside the single cell gate is on plot scale. The software automatically attempts to fit the data and if successful, the results are calculated. To display the statistics, right click on the resulting cell cycle histogram and select **Show Fitting Result**.



- Fitting results:

Item	Description
------	-------------

Data Analysis

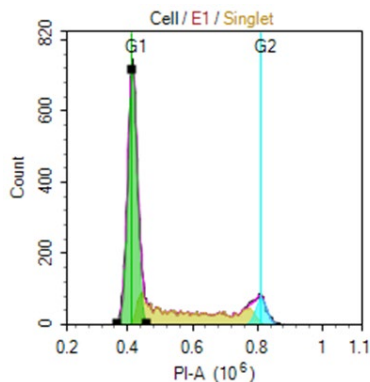
Watson	The model used for cell cycle fitting
RMS	The root mean square error of the fit of the G1, S, and G2 phases. A smaller value indicates a better fit.
Freq G1	Percentage in G1 phase
Freq S	Percentage in S phase
Freq G2	Percentage in G2 phase
Mean G1	The mean fluorescence intensity of the G1 phase
Mean G2	The mean fluorescence intensity of the G2 phase
G2/G1	The ratio comparing the mean fluorescence intensity of the G1 to G2 phase
CV G1	The coefficient of variation of the G1 phase
CV G2	The coefficient of variation of the G2 phase
Freq Sub-G1	Percentage in Sub-G1
Freq Super-G2	Percentage in Super-G2

Manual Cell Cycle Analysis

In some cases, the automatic fitting is not successful or additional constraints need to be applied to increase the accuracy of the fitting.

- Constrain G1 and G2 peaks

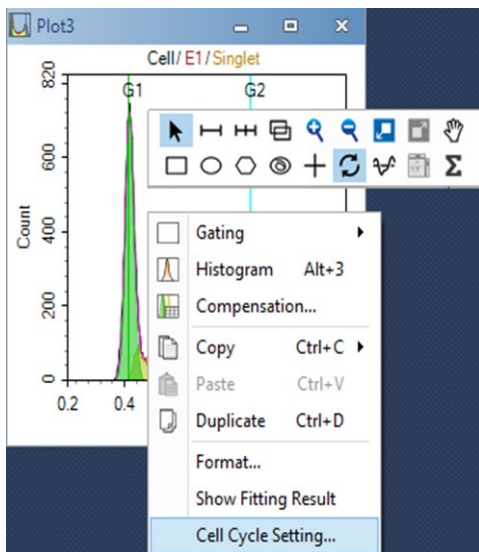
To modify the G1 or G2 peaks, click on the peak. Black boxes appear on the left, center, and right of the peak. Dragging the boxes adjusts the mean and CV used in the fitting. After the modification, the cell-cycle results update automatically.



Data Analysis

- **Cell Cycle Setting** Window

Right-click on the cell cycle plot and select **Cell Cycle Setting** to open the **Cell Cycle Setting** window.



In the **Cell Cycle Setting** window, there are two mathematical models can be selected, the **Watson** model and the **Dean-Jett-Fox** model. For Dean-Jett-Fox model, the S Phase Shape can be fitted with three options: **Rectangle**, **Trapezoid**, and **Polynomial**. Normally, select **Rectangle** if the S phase looks relatively flat, select **Trapezoid** if the S phase is inclined, and select **Polynomial** if the S phase presents the middle low and the sides are high. When analyzing the experimental data for cell cycle S phase synchronization, **Synchronous S Phase** should be enabled. Constraints on the fitting can be applied including the mean of G1 and G2 peaks, the CV of G1 and G2 peaks, and the ratio between the mean of G1 and G2 peaks. In addition, the color of the fitting curves can be set for better visualization of the fitting results.

Data Analysis

Cell Cycle Setting ✕

Model

Watson (Pragmatic) Dean-Jett-Fox

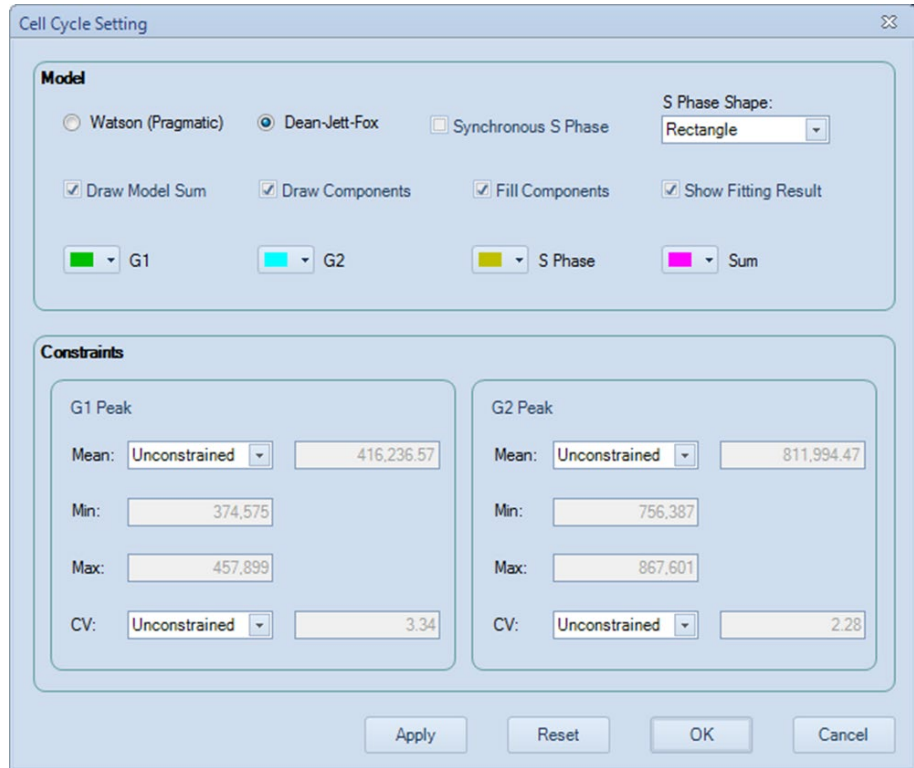
Draw Model Sum Draw Components Fill Components Show Fitting Result

G1 G2 S Phase Sum

Constraints

Parameter	Constraint	Value
G1 Peak	Mean:	Unconstrained 416,236.57
	Min:	374,575
	Max:	457,899
	CV:	Unconstrained 3.34
G2 Peak	Mean:	Unconstrained 811,994.47
	Min:	756,387
	Max:	867,601
	CV:	Unconstrained 2.28

Data Analysis



The image shows a 'Cell Cycle Setting' dialog box with two main sections: 'Model' and 'Constraints'.

Model Section:

- Radio buttons: Watson (Pragmatic), Dean-Jett-Fox, Synchronous S Phase
- Checkboxes: Draw Model Sum, Draw Components, Fill Components, Show Fitting Result
- Dropdown: S Phase Shape: Rectangle
- Color selection: G1 (green), G2 (cyan), S Phase (yellow), Sum (magenta)

Constraints Section:

Parameter	Value
G1 Peak Mean	Unconstrained (416,236.57)
G1 Peak Min	374,575
G1 Peak Max	457,899
G1 Peak CV	Unconstrained (3.34)
G2 Peak Mean	Unconstrained (811,994.47)
G2 Peak Min	756,387
G2 Peak Max	867,601
G2 Peak CV	Unconstrained (2.28)

Buttons: Apply, Reset, OK, Cancel

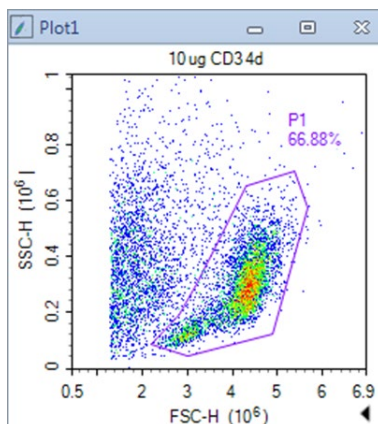
Cell Proliferation Analysis

Automated Cell Proliferation Analysis

- Gating Target Cell Population

On the density plot of FSC-H/SSC-H, create a gate which includes the target cell population you are interested in, for example P1 as show below.

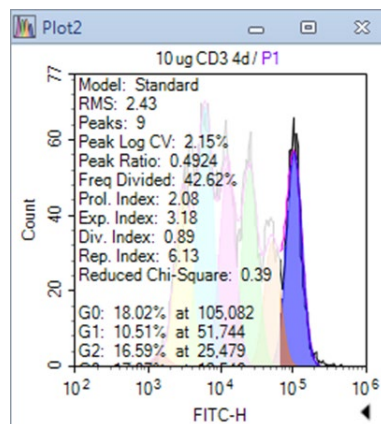
Data Analysis



- Cell Proliferation Analysis

Click on the **Cell Proliferation Plot** icon in the Workspace Toolbar to create a cell Proliferation plot. Set the X-axis of the plot to a detection channel that relates to the proliferation staining dye, such as CFSE-H. Apply the previously created cell gate to the plot and make sure all the events inside this gate is within the plot scale. The software automatically attempts to fit the data using the modeling algorithm and calculates the results. To display the statistics, right click the resulting cell proliferation histogram and select **Show Fitting Result**.

Item	Description
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Model	The name of model used for analysis and generates results, including Standard and Floating models.
RMS	Root Mean Square error. It is an estimate of the “goodness of fit” of the model.

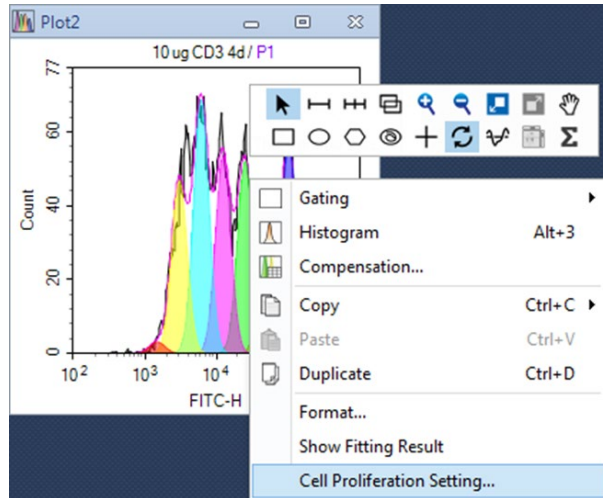
Data Analysis

Peaks	The count of Peaks.
Peak Log CV	Log CV of peak
Peak Ratio	<p>The average ratio of all the peak positions.</p> $\text{Peak Ratio} = \frac{1}{n-1} \sum_{i=1}^{n-1} \frac{\text{mean}G_i}{\text{mean}G_{i-1}}$ <p>where $\text{mean}G_i$ is the mean of the peak of i^{th} generation, n is the count of peaks include the parent peak.</p>
Freq Divided	<p>The percentage of the original cells that are divided.</p> $\text{Freq Divided} = \frac{\sum_{i=1}^{n-1} \frac{N_i}{2^i}}{\sum_{i=0}^{n-1} \frac{N_i}{2^i}} \times 100\%$ <p>where $i=0$ means the original generation.</p>
Prol. Index	<p>Proliferation Index. It is the sum of the number of divisions in each generation divided by the number of original cells that are divided.</p> $\text{Prol. Index} = \frac{\sum_{i=1}^{n-1} i \frac{N_i}{2^i}}{\sum_{i=1}^{n-1} \frac{N_i}{2^i}}$
Exp. Index	<p>Expansion Index. It is the number of cells divided by the number of original cells.</p> $\text{Exp. Index} = \frac{\sum_{i=0}^{n-1} N_i}{\sum_{i=0}^{n-1} \frac{N_i}{2^i}}$
Div. Index	<p>Division Index. It is the sum of the number of divisions in each generation divided by the number of original cells.</p> $\text{Div. Index} = \frac{\sum_{i=0}^{n-1} i \frac{N_i}{2^i}}{\sum_{i=0}^{n-1} \frac{N_i}{2^i}}$
Rep. Index	<p>Replication Index. It is the number of non-original cells divided by the number of original cells that divided.</p> $\text{Rep. Index} = \frac{\sum_{i=1}^{n-1} N_i}{\sum_{i=1}^{n-1} \frac{N_i}{2^i}}$
Reduced Chi-Square	Reduced Chi-Square equals the Chi-Square value divided by the free degree. It is an estimate of the "goodness of fit" of the model.
Freq Gi	The percentage of the i^{th} generation. It equals the number of cells of i^{th} generation divided by the number of cells.
Mean Gi	The mean of the peak of the i^{th} generation.

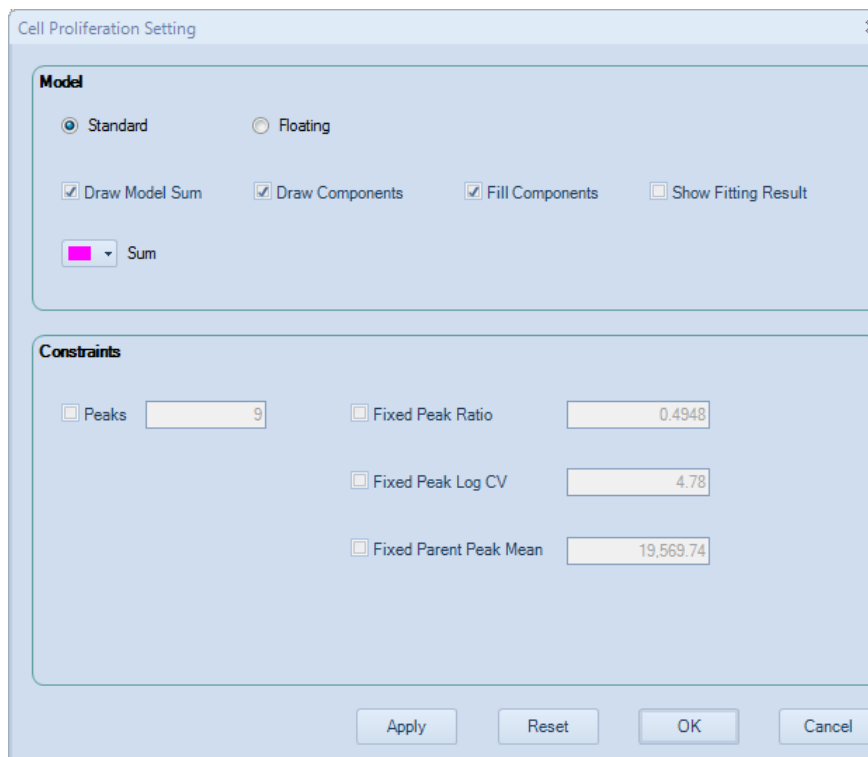
Data Analysis

Cell Proliferation Setting

You can select model to fit the data, format to display the results, and set constraints for analyzing cell proliferation data. On a generated Cell Proliferation analysis plot, right click and select **Cell Proliferation Setting** to open the **Cell Proliferation Setting** window as shown below.



Data Analysis














- Select Cell Proliferation Model
The default cell proliferation model is Standard model, which is suitable for the case that there are overlaps between peaks of generations. The peak ratios between generations are the same. Floating model is only suitable for the case that peaks of generations are distinct, and almost no overlap between them. The peak ratios between generations are distinct.
- Format the Modeling Display Results
Select **Draw Model Sum**, **Draw Components**, and **Fill Components** to format the cell proliferation modeling results displayed on the plot. Select **Show Fitting Results** to enable the statistical results shown on the plot.
- Set Cell Proliferation Constraints
In the **Cell Proliferation Setting** window, Constraints on the fitting can be applied including Peak Count, Peak Ratio, Peak Log CV, and mean value of the parent peak.

Statistical Tables

The statistical tables provide a summary from multiple samples, gates, and parameters enabling batch analysis and data comparison. For data generated using NovoSampler (or NovoSampler Pro), additional columns including **Plate ID, Well ID and Plate Description** are available. For data generated using NovoSampler Q, additional columns including **Plate ID, Well ID, Plate Barcode** and **Plate Description** are available.

Many of the features of the statistical table can be accessed through the toolbar in the **Statistical Table** window.



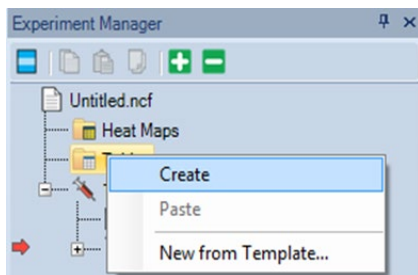
Icon	Description
	Sets the type of statistical table
	Adds a column to the table
	Edits a column
	Duplicates columns
	Deletes columns
	Hides columns
	Selects columns to show
	Selects samples to show
	Deletes rows
	Exports table as a CSV file
	Opens the Options > Statistical Table window


Create Different Types of Statistical Tables

Use the following method to create and format a new statistical table.

- Creating a New Statistical Table

In the **Experiment Manager** panel, under the experiment file name, right-click on the **Tables** node and select **Create**. A new table is created.



Alternatively, click on the **Statistical Table** button  in the **Home** tab of the **Menu Bar**. This also creates a new statistical table.

The new statistical table will contain **Specimen ID**, **Specimen**, **Sample**, and **Run Time** columns, and lists all of the samples in the experiment file.

The screenshot shows the 'Default Type' window containing a table with the following data:


	Specimen ID	Specimen	Sample	Run Time	Gate1 Count	Gate2 Mean: FSC-H
▶	1	Specimen1	Sample1	2014/2/26 11:47:28	19,904	1,824,762
	1	Specimen1	Sample2	2014/2/26 11:49:19	19,583	1,822,929
	2	Specimen2	Sample1	2014/2/26 11:51:15	20,165	1,816,893
	2	Specimen2	Sample2	2014/2/26 11:54:32	19,207	1,811,706

- Creating a New Statistical Table from Template

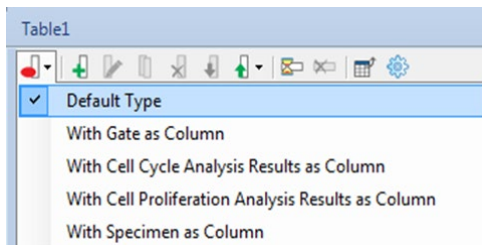
In the **Experiment Manager** panel, under the experiment filename, right-click on the **Tables** node and select **New from Template**, a new table is created.

- Selecting the Type of Statistical Table

There are five types of statistical tables to choose from: **Default Type**, a table **With Gate as Column**, a table **With Cell Cycle Analysis Results as Column**, a table **With Cell Proliferation Analysis Results as Column**, and a table **With Specimen as Column**.

New tables are created as The Default Type. To change the table type, click the **Table Type** button  from the toolbar and select the new table type from the drop-down menu.

Data Analysis



Shown below is an example for a table **With Gate as Column**.

Specimen ID	Specimen	Sample	Run Time	Gate	Count	Mean: FSC-H
1	Specimen1	Sample1	2014/2/26 11:47:28	Gate1	19,904	2,031,011
1	Specimen1	Sample1	2014/2/26 11:47:28	Gate2	15,222	1,824,762
1	Specimen1	Sample2	2014/2/26 11:49:19	Gate1	19,583	2,016,302
1	Specimen1	Sample2	2014/2/26 11:49:19	Gate2	15,099	1,822,929

Shown below is an example for a table **With Cell Cycle Analysis Results as Column**.

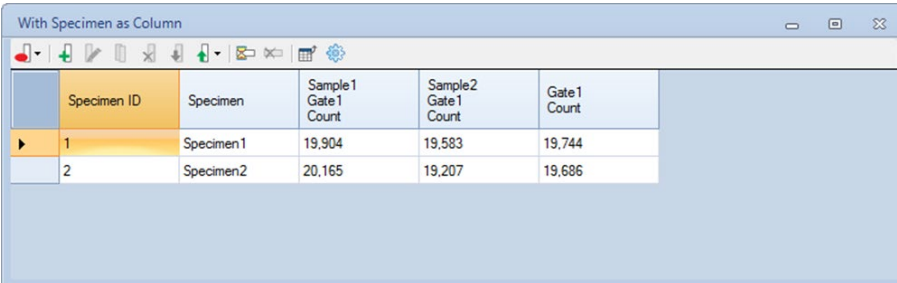
Specimen	Sample	Plot	Param	Model	RMS	G2/G1
Specimen1	Sample1	Plot4	PE-A	Watson Pragmatic	6.72	1.95
Specimen1	Sample2	Plot4	PE-A	Watson Pragmatic	7.43	1.94
Specimen2	Sample1	Plot4	PE-A	Watson Pragmatic	8.66	1.95
Specimen2	Sample2	Plot4	PE-A	Watson Pragmatic	8.88	1.95

Shown below is an example for a table **With Cell Proliferation Analysis Results as Column**.

Specimen ID	Specimen	Sample	Run Time	Plot	Gate	Param	Model	Freq G0
1	Specimen1	CFSE 4d	2013/5/7 17:26:52	Plot6	P1	FITC-H	Standard	98.79%
1	Specimen1	5ug CD3 4d	2013/5/7 17:32:03	Plot6	P1	FITC-H	Standard	17.59%
1	Specimen1	10 ug CD3 4d	2013/5/7 17:39:32	Plot6	P1	FITC-H	Standard	35.16%
1	Specimen1	CFSE 5d	2013/5/8 15:15:31	Plot6	P1	FITC-H	Standard	96.49%
1	Specimen1	5 ug 5d	2013/5/8 15:24:15	Plot6	P1	FITC-H	Standard	10.10%
1	Specimen1	10ug 5d	2013/5/8 15:30:16	Plot6	P1	FITC-H	Standard	10.03%
1	Specimen1	CFSE 6d	2013/5/9 16:38:05	Plot6	P1	FITC-H	Standard	98.51%
1	Specimen1	5ug 6d	2013/5/9 16:46:25	Plot6	P1	FITC-H	Standard	8.58%

Data Analysis

Shown below is an example for a table **With Specimen as Column**. Each specimen takes up one row in the table.



Specimen ID	Specimen	Sample1 Gate1 Count	Sample2 Gate1 Count	Gate1 Count
1	Specimen1	19,904	19,583	19,744
2	Specimen2	20,165	19,207	19,686

- Add columns and rows to the table and close the window.
- Rename the statistical table by selecting it from the **Experiment Manager** panel. Right-click and select **Rename** to rename the table.

NOTE

If columns or rows are not added to the table before closing the window, the table will not be saved.

Statistical Table Columns

Two types of columns can be added to the statistical table. These include statistical columns and formula columns. Formula columns are new parameters based on statistical parameters and user-defined formulas.

After columns are created, they can be edited, deleted, duplicated, hidden, moved, and sorted.

Add and Edit Columns

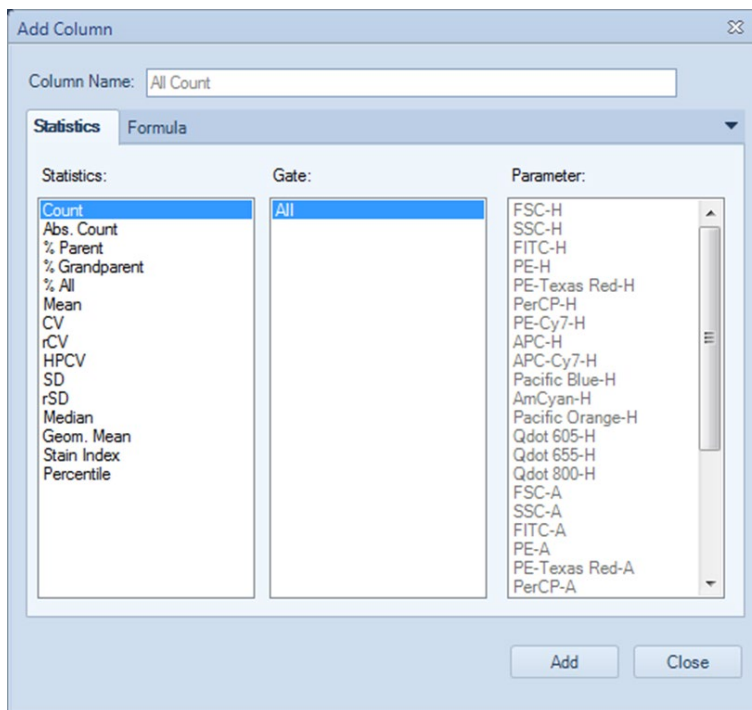
- Drag and Drop to Add Columns

For the Default statistical table type, dragging and dropping a gate into the statistical table creates a percent population column for the gate. For this method, select a gate from a plot in the Workspace and drag and drop it into the statistical table window. A percent population column is created. Gates can also be selected from the **Experiment Manager** panel and dragged and dropped into the statistical table window to create the percent population column.

- **Add Column** Window

Click on the **Add Column** button in the toolbar. The **Add Column** window will appear. Select the statistical value, the gate, and the parameter. Click the **Add** button to add the column to the table.

Data Analysis



NOTE

To add multiple items from each column, highlight the items to be added while holding the Ctrl or Shift key. Click **Add** to add the selected items to the table.

NOTE

For a table **With Specimen as Column**, the **Sample Name** should be specified when adding columns. If selecting **"All"** in the sample list box, the statistical results will be the average of all samples in each specimen. For Abs. Count calculation, if Abs. Count Unit defined for samples in the specimen is different, the Abs. Count result will be empty.

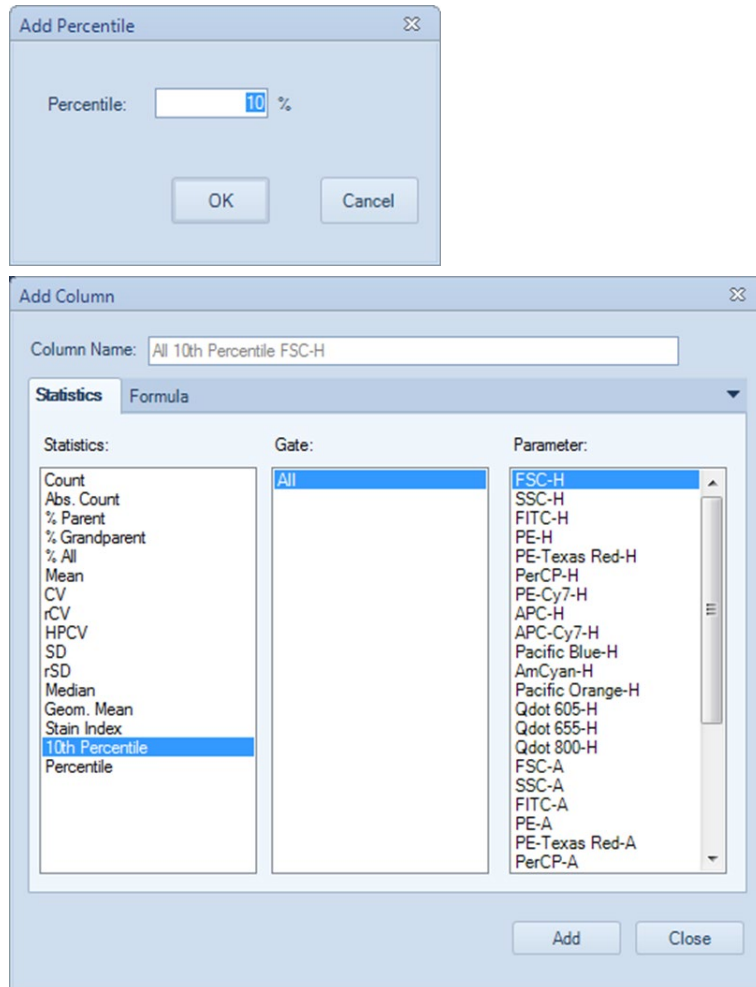
NOTE

To calculate the Stain Index, you need to select two gates. The gate with smaller MFI will be used as negative population gate while the gate with larger MFI is used as positive population gate. Refer to [Calculation of Statistics](#) for detailed description of Stain Index.


Data Analysis

NOTE

To add Percentile statistics, click on **Percentile**, enter the Percentile value in the **Add Percentile** window, such as 10 for calculating the 10th percentile. Then click **OK** and 10th Percentile item will be added in the statistics column.



- Edit a Column

Select the column in the statistical table window and click the **Edit Column** button , in the toolbar. The **Edit Column** window opens. Select the modifications and click **OK** to edit the column.

By default, the column name is automatically generated. The name can be modified by the **Column Name** box at the top of the **Edit Column** window.

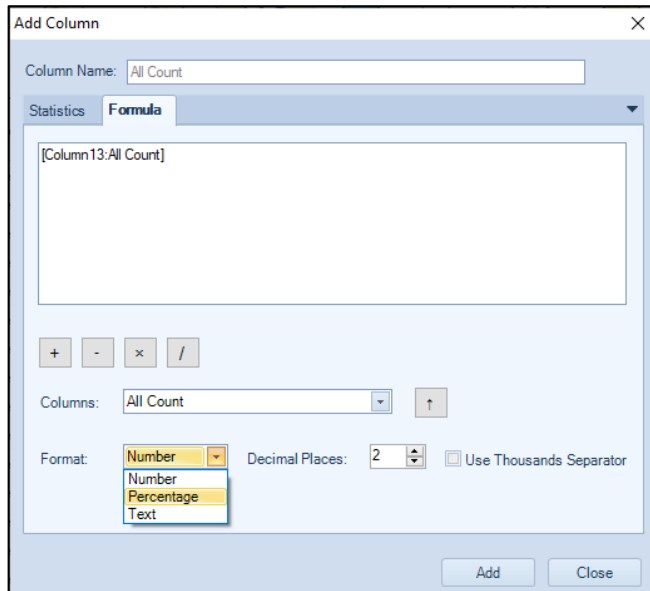
Data Analysis

Formula Columns

In the **Add Column** and the **Edit Column** windows, click the **Formula** tab to enter a user-defined formula. The formula can be defined using existing column values and basic arithmetic operations. Click **Add** or **OK** to define the formula and create a new column. User can select **Number**, **Percentage** or **Text** in the drop down menu of **Format**, select the **Decimal Places** and check **Use Thousands Separator** to add thousands separator to the formula. When **Percentage** is selected, user cannot check **Use Thousands Separator** box (i.e. greyed out). When **Text** is selected, user cannot select the **Decimal Places** and check **Use Thousands Separator** box.

NOTE

If the formula is displayed in red, there is an error in the equation.



The screenshot shows the 'Add Column' dialog box with the 'Formula' tab selected. The 'Column Name' field is 'All Count'. The 'Format' dropdown menu is open, showing 'Number', 'Percentage', and 'Text' options. The 'Decimal Places' field is set to '2'. The 'Use Thousands Separator' checkbox is checked. The 'Add' and 'Close' buttons are visible at the bottom.

Select Multiple Columns


In the header row of the table, click and drag in the top half of the cell to select multiple columns.

NOTE


Click and drag in the lower half of the cell to move the column.

Data Analysis

Duplicate Columns


Select the column, and click the **Duplicate Column** button  , in the toolbar to duplicate the selected column.

Delete Columns

Select the column, and click the **Delete Column** button  , in the toolbar to delete the selected column.

Show and hide Columns

Select the column, and click the **Hide Column** button  , in the toolbar to hide the selected column.

To show the column again, click the **Show Columns** button  , and select the column to show from the drop-down menu.

Move Columns

In the header row of the table, click and drag in the lower half of the cell to move columns.

NOTE

Click and drag in the upper half of the cell to select multiple columns.

Sort by Columns


In the header row of the table, double-click on a column header. The rows of the table are sorted in ascending order based on the selected column. Double-click on the header again to sort in descending order.

Statistical Table Rows

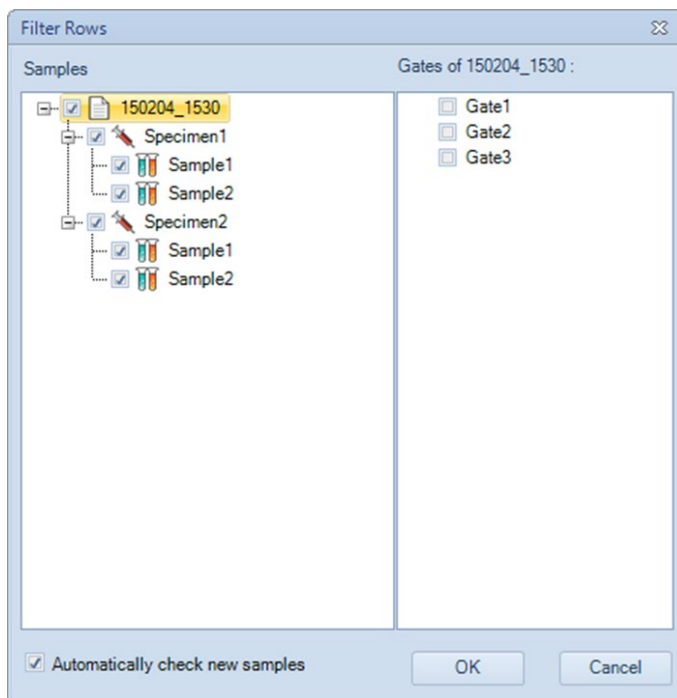
In the statistical table, the rows list separate populations for analysis.

Add Rows

- **Filter Rows** Window

In the toolbar, click the **Filter Rows** button  . The **Filter Rows** window will appear. Check the boxes to be used as rows to the table. **Unchecked** boxes will be excluded from the table. Click **OK** to recreate or delete the rows in the table.

Data Analysis



If **Automatically check new samples** is selected, the samples created later will be added into the statistical table automatically. For **with Specimen as Column** statistical table type, if **Automatically check new specimens** is selected, the specimens created later will be added into the statistical table automatically.

- Drag and Drop to Add Rows

Drag and drop a sample, a specimen, or a group from the **Experiment Manager** panel into the statistical table window can create new rows for each added sample.

For the **With Gate as Column** statistical table type, drag and drop a gate from the **Experiment Manager** panel or plot in the workspace into the statistical table window to create rows for the selected gate. If multiple samples contain a gate with the same name as the dropped gate, a row is created in the table for each of those samples' gates.


For the **With Cell Cycle Analysis Results as Columns** statistical table type, drag and drop a cell cycle analysis plot from the **Experiment Manager** panel or workspace into the statistical table window to create a row for the sample. If multiple samples have a cell cycle analysis plot with the same name, a row is added to the table for each of those samples.

Data Analysis

Select Multiple Rows

Click and drag in the column to the left of the Sample row to select multiple rows. Alternatively, hold down Ctrl and click in the column to the left of the Sample row to select multiple noncontiguous rows.

Delete Row

Select the row, and click the **Delete Rows** button , in the toolbar to delete the selected row. Alternatively, select the row and press the keyboard Delete key.

Statistical Tables Export or Copy Text

Statistical table results can be exported to CSV file or copied to clipboard as text.


Export Statistical Tables as CSV File

In the **Sample** tab toolbar, click the **Export CSV File** button . Enter the file path and click **Save** to export the file.

Copy Statistical Table as Text to the Clipboard

Select the cells to be copied by clicking and dragging with the mouse, or select all using the keyboard shortcut Ctrl A. Use the keyboard shortcut Ctrl C to copy the selected cells to the clipboard. The copied table can be pasted to a program, such as Microsoft Excel, for further analysis.

Statistical Table Options

In the toolbar, click the **Statistical Table Options** button  to open the **Statistical Table** tab of **Options** window, set **Customize Name** and **Default Visibility** of **Specimen**, **Specimen ID**, **Case Number**, **Sample**, **Sample ID**, **Operator**, **Run Time** and **Gate** columns.

Data Analysis

The screenshot shows a software dialog box titled "Options" with a close button (X) in the top right corner. On the left side, there is a vertical navigation menu with the following items: "General", "Experiment", "Analysis", "Absolute Count", "Statistical Table" (which is highlighted), and "Report". The main area of the dialog is titled "Statistical Table" and contains a table with three columns: "Column", "Customize Name", and "Default Visibility".

Column	Customize Name	Default Visibility
Group:	<input type="text"/>	<input type="checkbox"/>
Specimen:	<input type="text"/>	<input checked="" type="checkbox"/>
Specimen ID:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample:	<input type="text"/>	<input checked="" type="checkbox"/>
Sample ID:	<input type="text"/>	<input type="checkbox"/>
Operator:	<input type="text"/>	<input type="checkbox"/>
Run Time:	<input type="text"/>	<input checked="" type="checkbox"/>
Gate:	<input type="text"/>	<input checked="" type="checkbox"/>

At the bottom right of the dialog box, there are two buttons: "OK" and "Cancel".

Statistical Table Management

Statistical tables can be managed in the **Experiment Manager** panel under the **Tables** node.

- Copy and Paste Statistical Tables

In the **Experiment Manager** panel, dragging a statistical table and dropping it into the **Tables** node will create a new table with identical information.

Alternatively, a statistical table can be copied and then pasted into the **Tables** node to also create a new table with identical information.

- Delete Statistical Tables

In the **Experiment Manager** panel, select the table. Right-click and select **Delete** to delete the table. Alternatively, select the table and press the Delete key on the keyboard.

- Rename Statistical Tables

In the **Experiment Manager** panel, select the table. Right-click and select **Rename**. Enter the new name to rename the table.

Data Analysis

- Export Statistical Table as Template

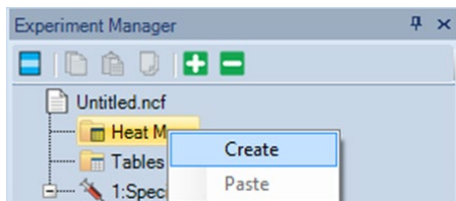
In the **Experiment Manager** panel, select the table. Right-click and select **Export Template...** Enter the name for the template in the prompted window and click **Save** to create a template. The template will be saved as a *.nst file.

Heat Maps

The heat map can be used to visualize the data in a well plate format. It uses different color to display the result of a specified statistical parameter. The color is determined by the color scale set for the statistical parameter to be analyzed. Multiple heat maps can be opened at the same time, and one heat map supports up to four statistical items.

Create a New Heat Map

In the **Experiment Manager** panel, under the experiment file name, right-click on the **Heat Maps** node and select **Create**.

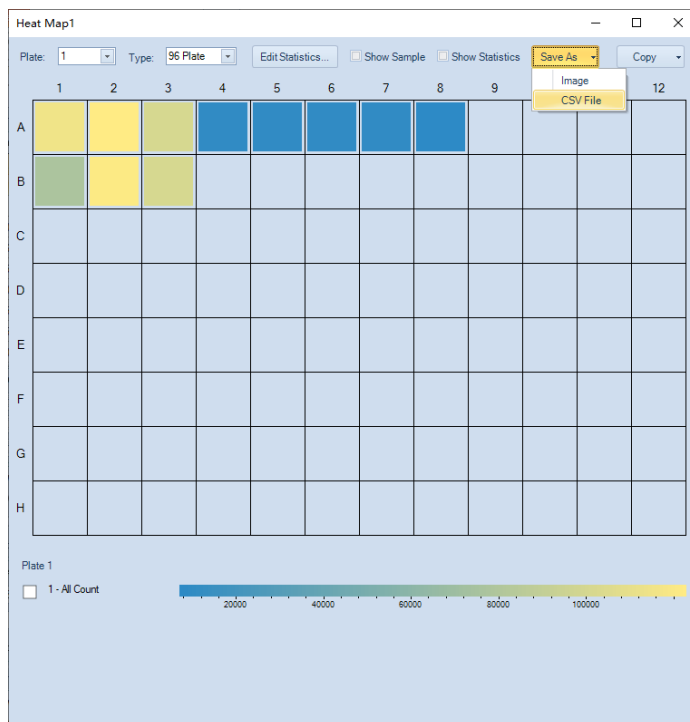


Alternatively, click on the **Heat Map** icon  in the **Home** tab of the **Menu Bar**. The **Heat Map** window will show up.

Heat Map Window

The heat map window contains heat map and legends. The well plate ID, plate type, and whether to show the sample name and statistics can be changed, and the heat map statistics can be edited. In addition, the heat map and legend can be copied and saved as image (i.e. PNG, JPEG, BMP, GIF, TIFF, or EMF) or a CSV file.

Data Analysis



NOTE

If there are multiple samples in the same well, only the first sample will be used to generate the heat map.

NOTE

If there are samples outside the current plate type, “*” will be displayed in the upper left corner of the heat map.

Heat Map Grid

A heat map can be generated for up to four statistic parameters. Each parameter will be displayed in a heat map with a corresponding section as illustrated below, based on the number of parameters to be displayed together.



Zero Statistic



One Statistic



Two Statistics



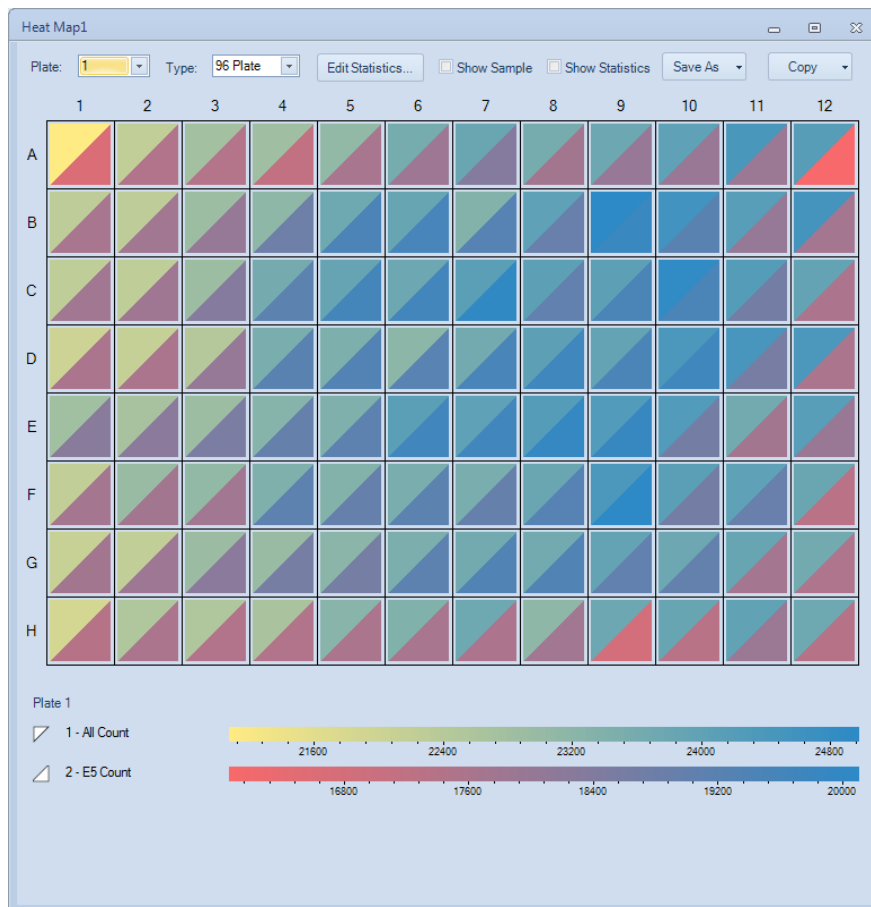
Three Statistics



Four Statistics

Heat Map for Multiple Statistics

The following figure shows a heat map with two statistics.



NOTE

The gate, color scale, and color scale range of each statistic parameter can be set separately.

Add Statistics

- Drag and Drop to Add Statistic Parameter

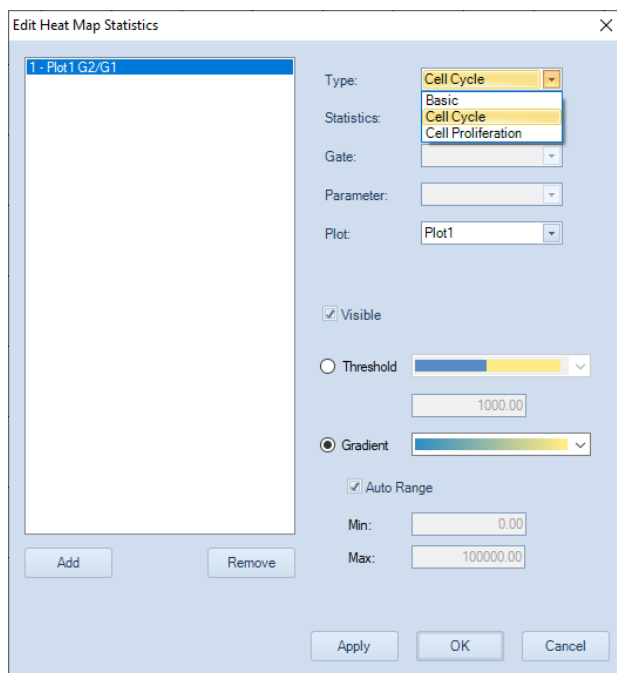
Directly dragging and dropping a gate into the heat map will add the Count parameter from the selected into the heat map. To do so, select a gate from a plot in the Workspace, drag and drop it into the created heat map. If there is no statistic parameter defined in the heat map, this action will add the Count

Data Analysis

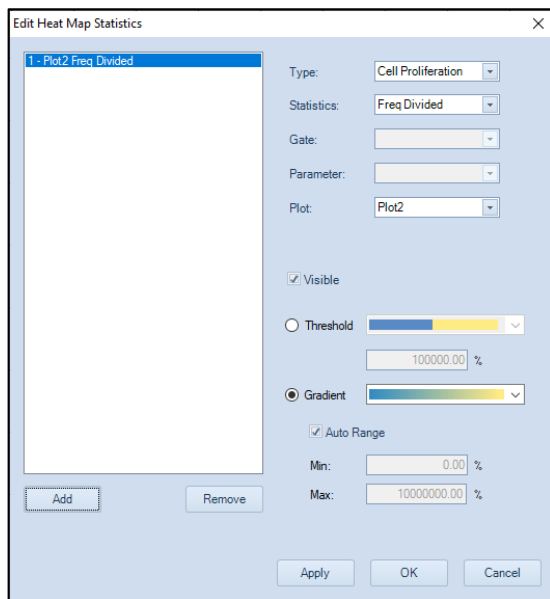
statistics of the selected into the heat map. Otherwise, the current statistic parameter will be replaced by the Count statistics of the selected gate. To add the Count of a gate as a new statistic parameter, press the Ctrl key while drag and drop a gate. You can also select the gate from the **Experiment Manager** panel and drag and drop it into the heat map to add Count statistics of the gate.

Edit Heat Map Statistics Window

You can add, edit, and remove statistics in **Edit Heat Map Statistics** window. To do so, click the **Edit Statistics...** button in the heat map. The **Edit Heat Map Statistics** window will appear.



Data Analysis



- **Add:** Click to add a new statistic to the list. Up to 4 statistics parameters can be added into the list.
- **Remove:** Click to delete the selected statistics parameter.
- **Type:** Select Basic, Cell Cycle, or Cell Proliferation to be displayed in the heat map from the drop down list.

NOTE

User can select Cell Cycle or Cell Proliferation only when associated analysis results are included for selected sample.

- **Statistics:** Select the statistics type to be displayed in the heat map from the drop down list.
- **Gate:** Select the gate whose associated statistics is displayed in the heat map from the drop down list.
- **Parameter:** Select the parameter to be displayed in the heat map from the drop down list.
- **Plot:** Select the plot to be displayed in the heat map from the drop down list. This option is available only when Cell Cycle or Cell Proliferation is selected in the drop down list of **Type**.
- Select color scale and range: There are two color scale displaying patterns you can choose for each statistic in the heat map, **Threshold** mode and **Gradient** mode.

Data Analysis

Threshold pattern defines the statistics parameter with two colors, depending on if the result is larger or smaller than the defined threshold. Select different color scheme from the drop-down list in the **Threshold** option and enter the **Threshold** into the text box below.

Gradient pattern shows the statistics parameter in a color gradient. Select different color scheme from the drop-down list in the **Gradient** option. The scale of the statistics parameter can be defined as **Auto Range** (software identifies the minimum and maximum value and calculate the range automatically) or you can manually define the range by entering the minimum value in the **Min.** text box and the maximum value in the **Max.** text box.

Update Heat Map

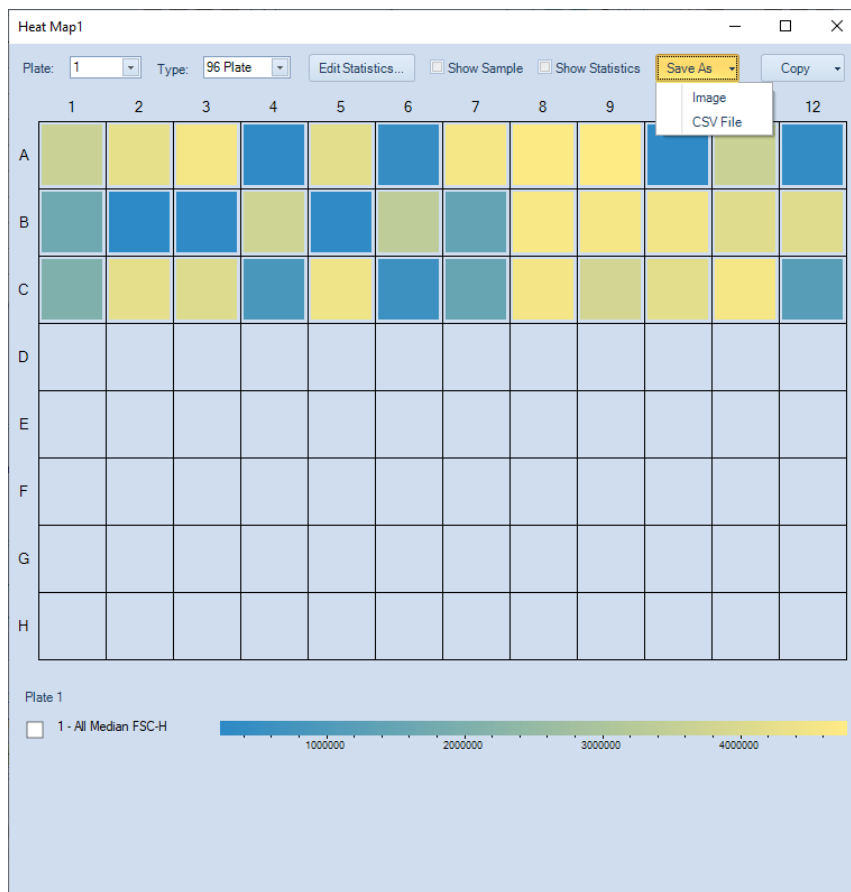
When the statistics of a sample are changed in value or in scale, the heat map will be automatically updated in real time.

Save Heat Map

The heat map and legend can be saved as image or a CSV file.

- Select **Image** or **CSV File** from the drop down menu of **Save As**.

Data Analysis

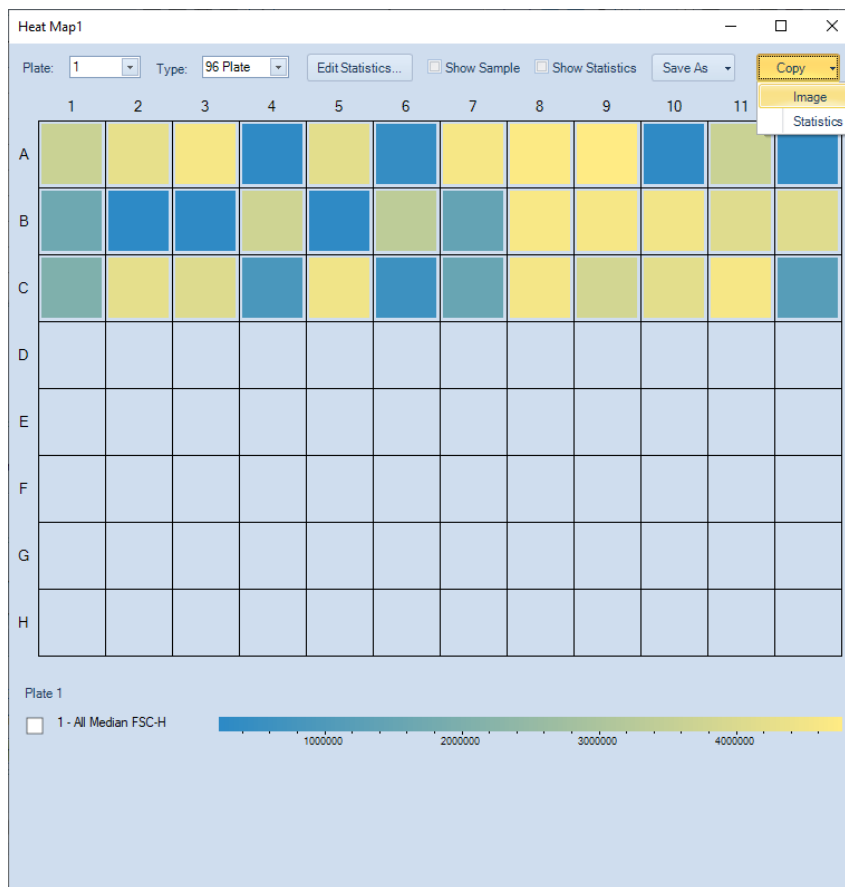


- Select the target folder, and enter the file name, click **Save**. The currently displayed heat map will be saved in designated folder as an image file (i.e. PNG, JPEG, BMP, GIF, TIFF, or EMF) or a CSV file. The default prefix of the saved file is in the format of **Experiment File Name_Heat Map Name**. For example, if the experiment file name is **190417_1507.ncf**, and name of the current heat map is **HM20201012**, the prefix of the saved file will be **190417_1507_HM20201012**.

Data Analysis

Copy Heat Map

The heat map and statistics result can be copied to clipboard. To do this, select **Image** or **Statistics** from the drop down menu of **Copy**. The currently displayed heat map or statistics will be copied to clipboard. User can paste it to a third party editing software (e.g. Microsoft Excel) for further analysis.



Heat Maps Management

Heat maps can be managed in the **Experiment Manager** panel under the **Heat Maps** node.

- Copy and Paste Heat Map

In the **Experiment Manager** panel, drag a heat map and drop it into the **Heat Maps** node will create a new heat map identical to the original one.

Data Analysis

Alternatively, a heat map can be copied and then pasted into the **Heat Maps** node to create a new heat map identical to the original one.

- Duplicate Heat Map

In the **Experiment Manager** panel, select a heat map. Right-click and select **Duplicate** to create a new heat map identical to the original one.

- Delete Heat Map

In the **Experiment Manager** panel, select a heat map. Right-click and select **Delete** to delete the selected heat map. Alternatively, select the heat map and press the Delete key on the keyboard.

- Rename Heat Map

In the **Experiment Manager** panel, select a heat map. Right-click and select **Rename**. Enter the new name to rename the heat map.

Post Gain

In certain situations, user may want to align a particular peak on different samples on the same plot. Post Gain function in NovoExpress software allows such adjustment to be done after data acquisition.

NOTE


Post Gain does not affect data acquisition. The threshold value entered in the **Cytometer Setting** panel is based on event data value before Post Gain.

NOTE

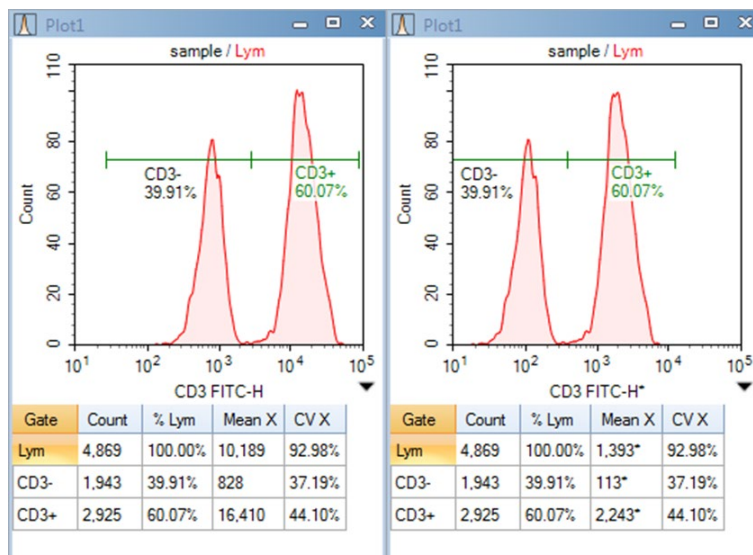
Only accounts with the **Post Gain Adjustment** privilege can adjust Post Gain.

Adjust Post Gain

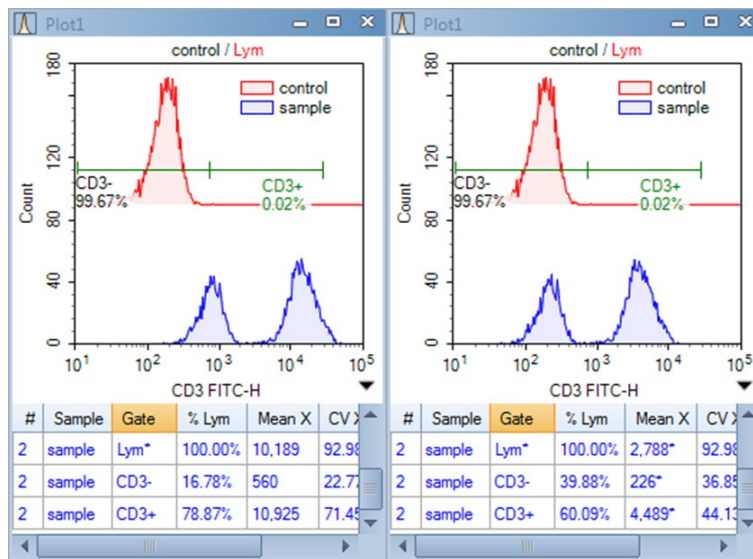
You can adjust the post gain value for each parameter of a sample.

First create a histogram of the desired parameter and click **Adjust Post Gain** button  on the workspace toolbar. Move mouse to the histogram plot area, hold down the left button of the mouse and drag the histogram curve, then drag and drop the peak of interest to the target location. In the example below the left plot is prior to the post gain adjustment and the right plot is after the post gain adjustment.

Data Analysis



If you want to align peaks of different samples, first create a histogram plot of the parameter with multiple overlays of these samples. Set the style of the histogram plot as offset, and then use the mouse to drag each histogram curve to align them. In the examples below the left plot is prior to alignment and the right plot after alignment using the post gain function.



Data Analysis

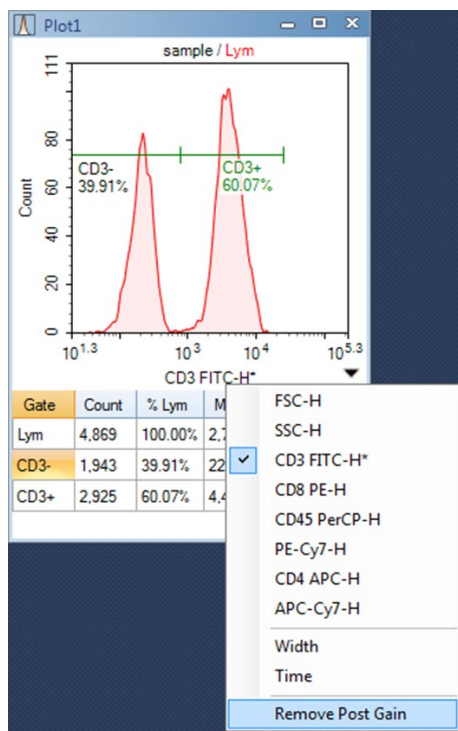
After a parameter is set using post gain, a * mark will be shown with parameter name in the statistics information of a plot or in the analysis table, and the value of any statistics with post gain will be shown with a * mark.

NOTE

Post gain will have no effect on the calculation of compensation, such that, compensated data will be calculated from the original data first, and the post gain will then be applied to the data. Control samples in an auto compensation specimen cannot be set using post gain.

Clear Post Gain

If you want to clear the post gain of a parameter, on area of axis parameter of any plot which contains the parameter, click the **Clear Post Gain** in the shortcut menu. If the menu item is not in the menu, it means that no post gain is set to the parameter.



If you want to clear post gain of all parameters of a sample, click **Clear Post Gain** menu under the main menu Sample, the post gain of active sample will be clear. If

Data Analysis

the menu item is gray, it means that no post gain is set to the parameters of the active sample.

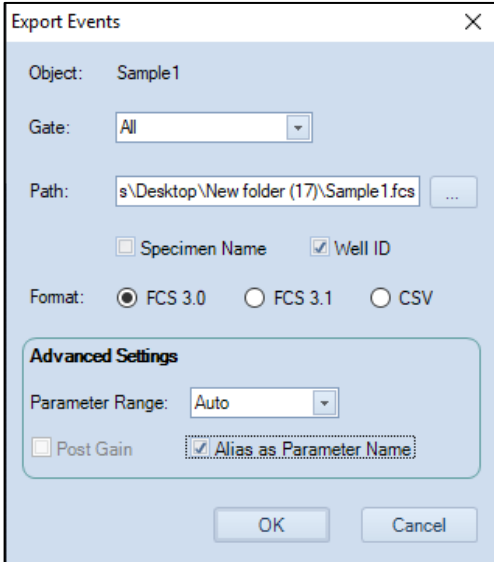
Apply Post Gain

When pasting an **Analysis** node of a sample to other sample on the experiment management tree, post gain will be pasted to the target sample too.

When exporting a sample to a .nct template file in the experiment management tree, post gain is contained in that template file too. When importing a template file to an **Analysis** node of a sample, post gain will also be imported and applied to the target sample.

Export Post Gained Data

In **Export Events** dialog, check the **Post Gain** option to enable exporting post gained data to FCS or CSV file. If the **Post Gain** option is unchecked, data without post gain will be exported.



The screenshot shows the 'Export Events' dialog box with the following settings:

- Object: Sample 1
- Gate: All
- Path: s:\Desktop\New folder (17)\Sample 1.fcs
- Specimen Name:
- Well ID:
- Format: FCS 3.0, FCS 3.1, CSV
- Advanced Settings:
 - Parameter Range: Auto
 - Post Gain:
 - Alias as Parameter Name:

If **Post Gain** option is checked, the sample data exported to FCS file are post gained data, and if there is compensation matrix in the sample, the data are first compensated, and then post gained. If the FCS file is again imported in NovoExpress, post gain can be readjusted or cleared, as well as the compensation matrix. If the FCS file is imported into a third party software, the third party

Data Analysis

software will treat the data like an original data, and does not know about the original compensation matrix and Post Gain information.

NOTE

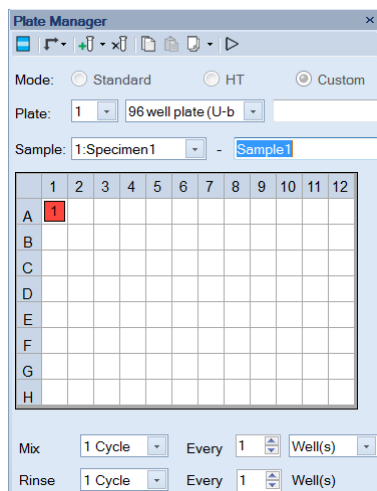
When export post gained data to CSV file, the name of parameters with post gain will be followed with a * mark.

6 Plate Manager

This chapter provides the information for the Plate Manager panel, including overview of Plate Manager panel and context menu.

Overview of Plate Manager Panel

The **Plate Manager** panel will only be available when the NovoSampler (or NovoSampler Pro or NovoSampler Q) is installed or the **NovoSampler (or NovoSampler Pro or NovoSampler Q) installed** checkbox is checked in the **Instrument Configuration** window in the NovoExpress software offline mode. This management tool provides users with an intuitive and convenient way to manage samples on a multi-well plate or tube rack. The **Plate Manager** also allows user to set up the working mode, plate type, mixing and rinsing conditions.



When NovoSampler is connected

Plate Manager

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	<input type="checkbox"/>											
B												
C												
D												
E												
F												
G												
H												

Mix: 1 Cycle Every 1 Well(s)

Rinse: 1 Cycle Every 1 Well(s)

Mixing Parameters

Speed: 1500 rpm Acceleration: 0 s

Duration: 10 s

When NovoSampler Pro is connected

Plate Manager

Mode: Standard HT Custom

Plate: 1 96 well plate (U-)

Sample: -

	1	2	3	4	5	6	7	8	9	10	11	12
A	<input type="checkbox"/>											
B												
C												
D												
E												
F												
G												
H												

Absolute Count Recover Remaining Samples

Mix: 1 Cycle Every 6 Well(s)

Rinse: 1 Cycle Every 1 Well(s)

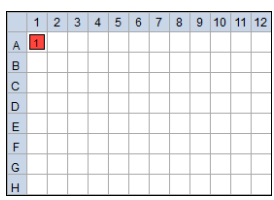
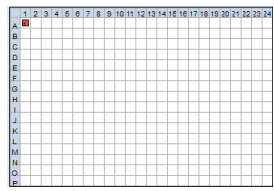





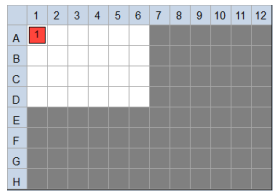






Mixing Parameters

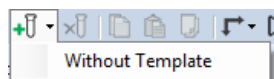
Speed: 1500 rpm Acceleration: 1 s

Duration: 10 s

When NovoSampler Q is connected

Plate Manager

Icon	Description
	<p>The Plate Manager panel displays a 16 x 24 grid indicating a 384-well plate map (for NovoSampler Q only) and an 8 x 12 grid indicating a 96-well plate map. In settings for a 24 or 40 tube-rack or a plate type other than 384-well and 96-well plate, an 8 x12 grid will be displayed while invalid wells will be grayed out.</p> <p>In one plate, multiple samples can be set with the same Well ID. In the grid, one well will show one sample at one time. The currently visible sample in a well is defined as selected sample of a well.</p>
	<p>The state of each well can be:</p> <ul style="list-style-type: none">  This icon indicates that this well is selected. Multiple wells can be selected at the same time.  The number is the Specimen ID and indicates to which specimen the selected sample belongs to.  An underlined number indicates that the selected sample has acquired data.  The arrow in the upper right corner indicates that the well has multiple samples. Context menu of the well will list all samples.  The italicized number indicates the acquisition sequence of the selected sample.
	<ul style="list-style-type: none">  A red background indicates the well contains the Active Sample.  A green background indicates that the well contains sample in queue to be collected.  An alternating green and dark green background indicate that the well contains sample currently in acquisition.  An alternating green and red background indicate that the well contains sample currently in acquisition and also is the Active Sample.
<p>The following operations can be operated on the plate grid:</p>	<ul style="list-style-type: none"> Click: Click the header of a row or a column to select the entire row or column. Click the top left corner cell selects all wells in the plate. Right-click: Displays context menu. Double-click: Set selected sample to be the Active Sample. Drag: Drag to select multiple wells in a rectangular range. Ctrl + Click: Hold Ctrl to select multiple individual wells. Shift + Click: Hold Shift to select consecutive wells between two wells.
	<p>Work List: View and edit Work List.</p>
	<p>Samples Created Order: Selects whether to automatically proceed horizontally across the plate or vertically across the plate when creating new samples.</p>



New Sample(s) on Selected Well(s): Creates new samples on the selected wells. The created samples will have the same template as the Active Sample with the same compensation matrix, report, data analysis, and **Cytometer Setting**. The samples will be created in the specimen with name specified in **Specimen Name** box. If specified specimen name is empty or does not exist, a new specimen will be created. The created sample name will use the name specified in the **Sample Name** box as a prefix. If the sample name box is empty, the default sample name prefix (sample 1, sample 2 ...) will be used.

Without Template: Creates new samples on the selected wells without template applied. The compensation matrix, report, and data analysis template will be empty. But the cytometer setting will be the same as the current cytometer setting in the **Cytometer Setting** panel.



Delete Sample(s) on Selected Well(s): Deletes the selected samples from the selected wells. If one well has multiple samples defined, only the selected sample will be deleted.

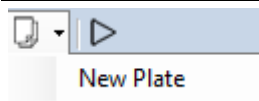


Copy Sample(s) on Selected Well(s): Copies templates of selected samples from the selected wells. If no sample has defined in the selected wells, this function is disabled. If part of selected wells has samples defined, only the wells which have sample defined will be copied.



Paste Sample(s) on Selected Well(s): Pastes sample template or sample events to the selected wells according to copied content.

- If all the selected wells have samples defined, then it applies templates of the copied samples to the selected samples of selected wells one by one repeatedly.
- If all the selected wells have no samples defined, then it creates samples with the same templates as copied samples on the selected wells. The samples will be created in the specimen with the name specified in **Specimen Name** box.
- If part of the selected wells has samples defined and the rest part have no samples defined, or the selected samples of the selected wells are in different specimen, this tool will be disabled.
- If **Copy Events** is selected from context menu of a well, this tool will be available when only one well is selected and the selected sample of the selected well contains no events.



Duplicate Sample(s) on Selected Well(s): Creates new samples on empty wells, duplicating the template of the selected samples of the selected wells.

- If part of the selected wells has samples defined and the rest part have no samples defined, or the selected samples of the selected wells are in different specimen, this tool will be disabled.
- If the specimen contains more than one sample, and all the samples are selected, clicking this icon will create duplicated samples under a newly created specimen.

Plate Manager

New Plate: Creates new plate and specimen, and creates new samples in the new specimen, duplicating the template of the selected samples of the selected wells. The new samples have the same well ID with the selected wells.



Show Run Order: When selected, a blue outline shows on the icon . The sample acquisition sequence will be shown in the plate view.

Mode: Standard HT Custom

Mode: Allows user to select the working mode (Standard, High-Throughput and Custom) of the NovoSampler (or NovoSampler Pro or NovoSampler Q) in this area. Default mixing condition, rinse condition, stop condition and flow rate will be automatically applied when **Standard** or **HT** mode is selected. Please refer to associated operator's guide for more details. User can only customize these settings when **Custom** mode is selected.

NOTE

Selecting the working mode is only available before the sample is created.

Plate: 1 96 well plate (U)

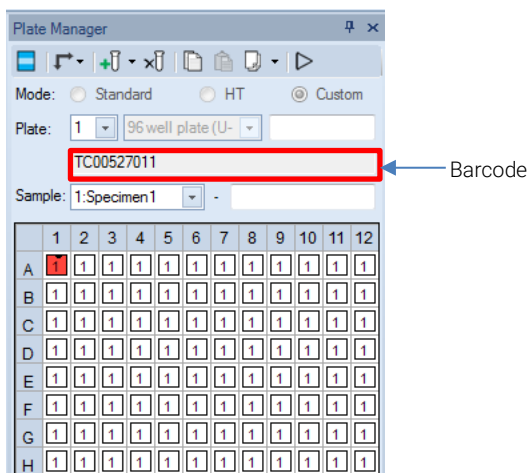
This area is designed to input plate information. The left drop-down box is the **Plate ID**. The **Plate ID** is a positive integer and can be modified by highlighting the area and entering a new number. The middle drop-down box is the **Plate Type**. The drop-down box lists all the available plate type defined in the **Manage Plate Types** window. Pull down the list and select the corresponding plate type for the experiment. The right box is the Plate Name. Input plate name in this box to help identify the plate.

NOTE

The plate type cannot be modified once the sample acquisition has begun.

NOTE

When NovoSampler Q is connected and the barcode reading function is enabled, user can view the scanned barcode information from the row below this area (see example below). Please refer to [NovoSampler Q Operator's Guide](#) for more details.



Sample: 1:Specimen1 - Sample 1

This area is designed to input the specimen and sample information. The left drop-down box is the **Specimen ID** and **Specimen Name**, separated by a colon. The right box is the **Sample Name**.

When creating new samples, the samples will be created in a specimen with specimen name specified in the **Specimen Name** box. If the **Specimen Name** box is empty or does not exist, a new specimen will be created. The default new specimen name is **Specimen1**, **Specimen2**.... The default sample name is **Sample1**, **Sample2**

Absolute Count

This function is available only when NovoSampler Q is connected. With **Absolute Count** is selected, user can analyze the exact number of cells or particles per unit volume. This function is disabled by default when NovoSampler Q works in Standard or High-Throughput (HT) mode. When Custom mode is selected, user can select to disable or enable this function.

Recover Remaining Samples

This function is available only when NovoSampler Q is connected. With Recover Remaining Samples selected, user can recover the remaining sample aspirated into the sampling tube back to the sample tube or plate well after sample acquisition. This function is disabled by default when Standard or High-Throughput (HT) are selected. When Custom mode is selected, user can select to disable or enable this function.

NOTE The Recover Remaining Samples function is disabled by default. To enable this function, user need to check the check box in the front.

NOTE Any changes to this setting (i.e. disable or enable) during the sample collection will only be effective from the next well forward.

Plate Manager

Mix 1 Cycle Every 1 Well(s)

Mix: Sets the mixing conditions. **Mix** can be set for **None** (no mixing), **1 Cycle**, **2 Cycles**, **3 Cycles** for every defined number of wells or every defined number of minutes.

NOTE Default mixing conditions (i.e. Mix 1 Cycle Every 1 Well) will be automatically applied and cannot be modified by user when Standard or HT mode is selected.

Rinse 1 Cycle Every 1 Well(s)

Rinse: Sets the rinse cycle to clean the sample injection probe. **Rinse** can be set for **None** (no rinse), **1 Cycle**, **2 Cycles**, **3 Cycles** for every defined number of wells.

NOTE There is always one rinse cycle at the end of each plate run even the **Rinse** setting is set to **None**.

NOTE Default rinse conditions (i.e. Rinse 1 Cycle Every 1 Well when Standard mode is selected, or None when HT mode is selected) will be automatically applied and cannot be modified by user when Standard or HT mode is selected.

Mixing Parameters
Speed 1500 rpm Acceleration 0 s
Duration 10 s

Mixing Parameters: This panel is available only when NovoSampler Pro or NovoSampler Q is connected. User can view or set the mixing parameters for the orbital shaker in this panel. When NovoSampler Pro or Q works in **Custom** mode, these mixing parameters for each plate type are defined in the **Manage Plate Type** window and can be modified in this area for the current experiment. When NovoSampler Pro or NovoSampler Q works in **Standard** or **HT** mode, default mixing parameters will be automatically applied and cannot be modified by user.

Speed: The mix speed of the orbital shaker. Please refer to table below for the default mix speed when NovoSampler Pro or NovoSampler Q works in different mode.

	Plate Type	Default Mix Speed (rpm)		
		Standard Mode	HT Mode	Custom Mode
NovoSampler Pro	96 well plate (U, V and Flat bottom)	1500	1500	1500
	24 well plate	2500	2500	2500
	24 tube rack	1500	1500	1500
	48 well plate	1500	1500	1500
NovoSampler Q	96 well plate (U, V and Flat bottom)	1500	1500	1500
	24 well plate	2500	2500	2500
	48 well plate	1500	1500	1500
	40 tube rack	1000	1000	1000
	384 well plate	2500	2500	2500
	24 tube cooling rack	1000	1000	1000
	96 well plate (U, V and Flat bottom) with cooling box	1500	1500	1500

Acceleration: The acceleration time for the orbital shaker to reach the specified Mix Speed. By default, the mix acceleration is set to 0 second for NovoSampler Pro, and 1 second for NovoSampler Q.

Duration: The mixing time for the orbital shaker to mix the sample in one cycle. Please refer to table below for the default mix duration when NovoSampler Pro and NovoSampler Q works in different mode.

	Plate Type	Default Mix Duration (s)		
		Standard Mode	HT Mode	Custom Mode
NovoSampler Pro	96 well plate (U, V and Flat bottom)	10	10	10
	24 well plate	10	10	10
	24 tube rack	10	10	10
	48 well plate	10	10	10
NovoSampler Q	96 well plate (U, V and Flat bottom)	8	3	10
	24 well plate	10	4	10
	48 well plate	10	5	10
	40 tube rack	10	5	10
	384 well plate	8	2	10
	24 tube cooling rack	10	5	10
	96 well plate (U, V and Flat bottom) with cooling box	8	3	10

Context Menu

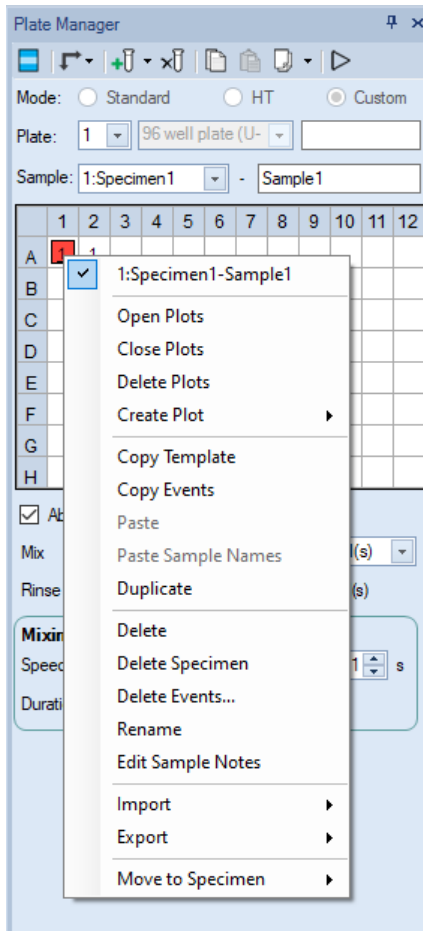
In addition to the **Tools** menu, the **Plate Management** panel also provides a rich set of context menu actions.

Context menu when a single well is selected

If the selected well has sample already defined, the right-click menu displays a list of the samples defined in the selected well and the software functions available to be used. This context menu is the same as the context menu by right-clicking a sample node in the **Experiment Manager** panel. From this context menu, you can quickly select the defined sample, open, close, delete, and create plots, copy and paste templates, copy and delete events, delete, rename and duplicate the sample, edit sample notes, import template or the data from a FCS file, or export as a template or the data in FCS and CSV formats, move the sample to the selected

Plate Manager

specimen. For additional information regarding these functions, refer to [Right-Click Menu](#) in this guide.



If the selected well has no sample defined, the right-click menu generally has two items available, **New** and **Batch Creation of New Specimens**. **New** will create new sample without a template. Batch Creation of New Specimens allows user to create new specimen(s) with customized specimen and sample organization. Refer to [Context menu when multiple wells are selected](#) for more details on this function.

NOTE

If **Paste Sample Names** will be available if user copied the sample name in previous operation.

Plate Manager

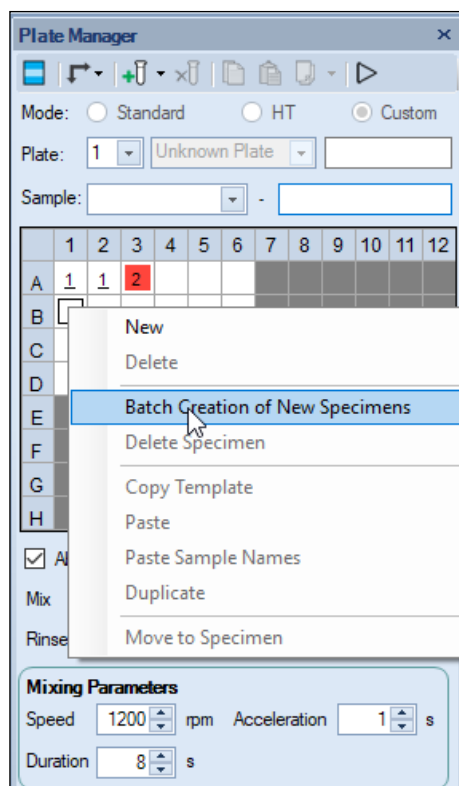
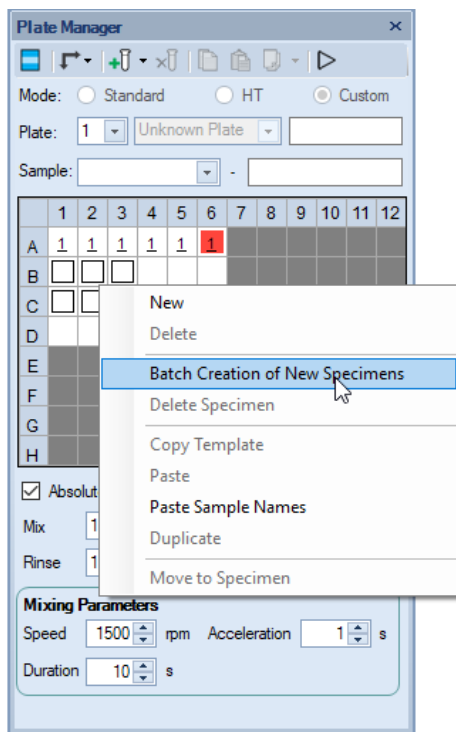


Plate Manager

Context menu when multiple wells are selected



- **New:** Same as the **New Sample(s) on Selected Well(s)** tool in the toolbar.
- **Delete:** Same as the **Delete Sample(s) on Selected Well(s)** tool in the toolbar.
- **Batch Creation of New Specimens:** Create new specimen(s) with customized specimen and sample organization. When clicked, software will prompt following window. User need to define item(s) in this window, click **OK** to complete or cancel to go back to the previous window.

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Batch Creation of New Specimens

Do not import template Import sample template Import specimen template

_____ ...

Specimen order Horizontal

Prefix of specimen name Specimen

Prefix of sample name Sample

Number of samples per specimen 2

OK Cancel

- Do not import template: Select this option if no templates need to be imported
- Import sample template: Select this option if sample template needs to be imported for newly created samples. Click ... to load the template file (.nct file).
- Import specimen template: Select this option if specimen template needs to be imported for newly created specimens. Click ... to load the template file (.nct file).
- Specimen order: direction of creating the specimens for selected wells. For example, when A1, A2, B1, B2 are selected, and Horizontal is selected as the specimen order, and 2 samples per specimen is selected, software will automatically create two new specimens and new samples in these four wells. Sample A1 and A2 will be placed under the first newly created specimen. Sample B1 and B2 will be placed under the second newly created specimen.
- Prefix of specimen name: Set the prefix for the name of the newly created specimen(s).
- Prefix of sample name: Set the prefix for the name of the newly created sample(s).

Plate Manager

- Number of samples per specimen: Set the number of newly created samples per each newly created specimen.
- **Delete Specimen:** Delete the selected specimen and associated samples on selected wells.
- **Copy Template:** Same as the **Copy Sample(s) on Selected Well(s)** tool in the toolbar.
- **Paste:** Same as the **Paste Sample(s) on Selected Well(s)** tool in the toolbar.
- **Paste Sample Names:** Rename samples of the selected wells with the names copied from a spreadsheet program like Microsoft Excel or create new samples with copied name on selected wells if there is no sample defined. If part of selected wells has samples defined and the rest part have no samples defined, this menu will be disabled.

NOTE

If you have an Excel spreadsheet defining the sample names in a plate format, use **Paste Sample Names** function from the right-click context menu to copy the sample name from the spreadsheet and quickly create new samples for a plate in NovoExpress Software.

-
- **Duplicate:** Same as the **Duplicate Sample(s) on Selected Well(s)** tool in the toolbar.
 - **Move to Specimen:** Move the selected sample(s) to the selected specimen or new specimen.

Plate Manager

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7

Experiment Manager

This chapter provides the information for the Experiment Manager function, including Experiment Manager Toolbar, Hierarchy, Templates, Import and Export Data.

Experiment Manager Toolbar



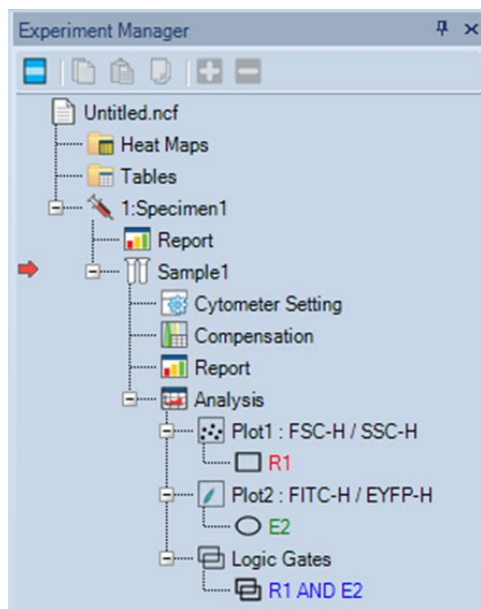
Icon	Description
	Work List: View and edit the work list. Contains information on the sample names and collection parameters. Refer to Work List for more information.
	Copy the selected node.
	Paste the copied content.
	Create a copy of the currently selected sample or specimen.
	Expand all child nodes of the current node.
	Collapse all child nodes of the current node.

Hierarchy

Description

In the NovoExpress Software, the hierarchy structure from high to low is groups, specimen, and samples.

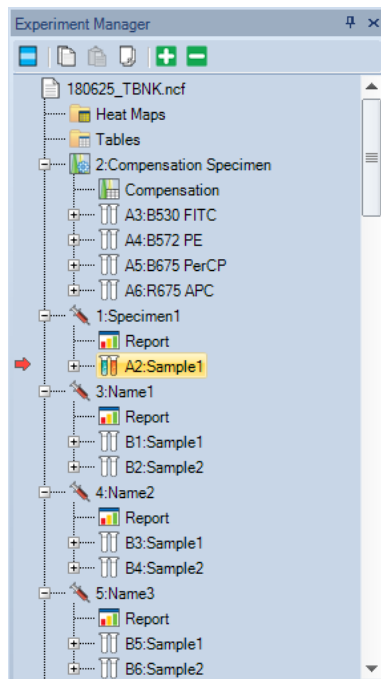
Experiment Manager









In the figure above, the red arrow indicates the active sample. In the NovoExpress Software, the active sample collection parameters are displayed in the **Cytometer Setting** and **Cytometer Control** panels. In the **Experiment Manager** panel, double-clicking on a sample node will make it the active sample. When switching to a new active sample, the **Cytometer Setting** and **Cytometer Control** panels will update with the new active samples' information, and the plots in the Workspace will be replaced with the new active sample's plots.

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, The samples listed in the **Experiment Manager** panel include the Well ID and the sample name, separated by a colon (see figure below). Click the sample node to select the sample, right-click and select Rename to rename the sample. When renaming the sample, both the Well ID and the sample name can be modified. Specimen is also associated with a Specimen ID. The Specimen ID is the number before the specimen name, separated by a colon. The Specimen ID is used to identify specimen and can be modified. For additional information regarding the Specimen ID, see [Plate Manager Panel](#) in this guide.

Experiment Manager



Icon	Description
 File	The experiment file (*.ncf file format)
 Heat Maps	This node contains Heat Maps for the experiment file. Right-clicking this node allows for creating new heat map.
 Statistical tables	This node contains statistical tables for the experiment file. Right-clicking this node allows for the creation of new statistical tables.
 Statistical table	Statistical analysis table
 Group	This node represents a Group in the organizational hierarchy. A group can contain multiple specimens and subgroups, and specimens will always be placed in front of subgroups.
 Specimen	This node represents a Specimen in the organizational hierarchy. A specimen can contain multiple samples. Each specimen contains a specimen report. In the node text " 1:Specimen 1 ", the former number is Specimen ID, the latter text is Specimen Name.


Experiment Manager




Sample

This node represents a Sample in the organizational hierarchy. The sample is the most basic organizational unit and contains sample data collection parameters, instrument settings, fluorescence compensation settings, reports, analysis, and data. The sample icon will display differently depending on the status of the sample.

A blank sample without any data collected will appear as 

A sample listed for sample acquisition and during acquisition will appear as 

A sample with data collected will appear as 



Cytometer settings

Cytometer settings contain the sample parameters, the acquisition stop conditions, and the sample flow rate and threshold settings. See [Cytometer Setting](#) for additional information.



Fluorescence compensation

Fluorescence compensation matrix for the sample. If the matrix is empty, the node is displayed in black. If the matrix is filled, the matrix is displayed in blue. See [Fluorescence Compensation](#) for additional information.



Report

Contains a report of the data analysis and is found under both specimen and sample nodes.

Reports under specimen nodes can include plots and statistical analysis for all samples under the specimen.

Reports under the sample nodes can include plots and statistical analysis only for the sample.

See [Reports](#) for additional information.



Analysis

Analysis contains the plots and gates for a sample. Under the **Analysis** node, there are plot nodes and a logic gate node. Plot nodes are listed for individual plots of the sample, and each plot node contains the gates created for the plot. A separate logic gate node contains all of the logic gates created for the sample.

Plot

A plot created for the analysis of a sample

Gate

A gate created within a plot



Logic gate group

Contains all logic gates for a sample



Logic gate

Logic gates



Red arrow

The red arrow indicates the sample is the active sample.



Flashing green and dark green arrows

Flashing green and dark green arrows indicate the sample is being collected.

Experiment Manager


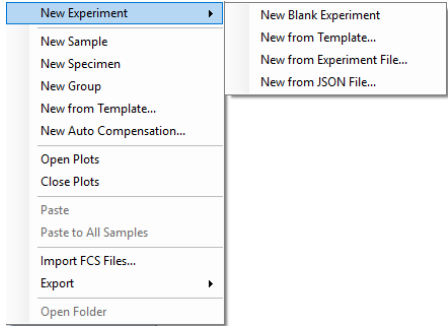
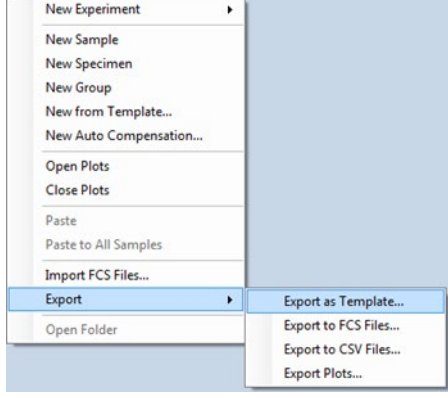


Flashing red and green arrows indicate the active sample is being collected.

Alternatively
flashing red and
green arrows

Right-Click Menu

From the **Experiment Manager** panel, right-clicking each node will bring up a menu of functions. The table below lists the specific functions available by right-clicking each node type.

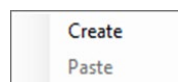
Icon	Description
 File	
	New Experiment: <ul style="list-style-type: none">• New Blank Experiment: Create a blank experiment file.• New from Template: Create an experiment file from a template.• New from Experiment File: Create an experiment file from an existing experiment file.• New from JSON File: Create an experiment file from an existing JSON file. This option is available when NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon is connected.
	New Sample: Creates a new specimen with a new sample included. New Specimen: Creates a new specimen. New Group: Creates a new group. New from Template: Imports selected group, specimen, and samples from a template. New Auto Compensation: Creates a compensation specimen containing samples to compute a compensation matrix. Open Plots: Opens all plots from all of the samples. Close Plots: Closes all plots from all of the samples. Paste: Creates a new specimen with the copied specimen template. Paste to All Specimens: Pastes the copied specimen template to all specimens. Paste to All Samples: Pastes the copied sample template to all samples. Import FCS Files: Selects a folder to import all FCS files within the folder or subfolders as samples. Files up to 10 subfolders deep from the selected folder will be added and organized according to the folder structure. Export:

Experiment Manager

- **Export as Template:** Exports the file as a template file.
 - **Export to FCS Files:** Exports all samples as FCS files.
 - **Export to CSV Files:** Exports all samples as CSV files.
 - **Export Plots:** Exports all plots from all samples as image files.
- Open Folder:** Opens the folder containing the experiment file.
-



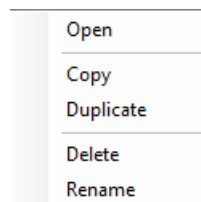
Heat Map



Create: Creates a new heat map.
Paste: Pastes a copied heat map.



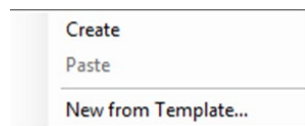
Heat Map



Open: Opens the selected heat map.
Copy: Copies the selected heat map.
Duplicate: Duplicates the selected heat map.
Delete: Deletes the selected heat map.
Rename: Renames the selected heat map.



Statistical Tables



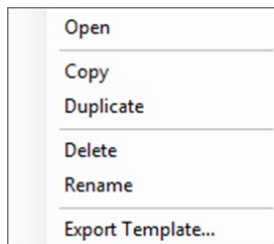
Create: Creates a new statistical table.
Paste: Pastes a copied statistical table.
New from Template: Creates a new statistical table from an exist template.



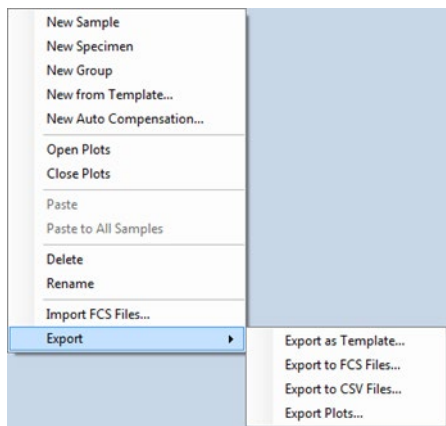
Statistical Table

Open: Opens the selected statistical table.
Copy: Copies the selected statistical table.
Duplicate: Duplicates the selected statistical table.
Delete: Deletes the selected statistical table.
Rename: Renames the selected statistical table.

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Group



New Sample: Creates a new specimen with a new sample included.

New Specimen: Creates a new specimen.

New Group: Creates a new group.

New from Template: Imports selected group, specimen, and samples from a template.

New Auto Compensation: Creates a compensation specimen containing samples to compute a compensation matrix.

Open Plots: Opens all plots from all of the samples within the group.

Close Plots: Closes all plots from all of the samples within the group.

Paste: Creates a new specimen with the copied specimen template.

Paste to All Specimens: Pastes the copied specimen template to all specimens in the group.

Paste to All Samples: Pastes the copied sample template to all samples in the group.

Delete: Deletes the group.

Rename: Renames the group.

Import FCS Files: Selects a folder to import all FCS files within the folder or subfolders as samples. Files up to 10 subfolders deep from the selected folder will be added and organized according to the folder structure.

Export:

- **Export as Template:** Exports the group as a template file.
- **Export to FCS Files:** Exports all samples as FCS files.
- **Export to CSV Files:** Exports all samples as CSV files.
- **Export Plots:** Exports all plots in current group as image files.

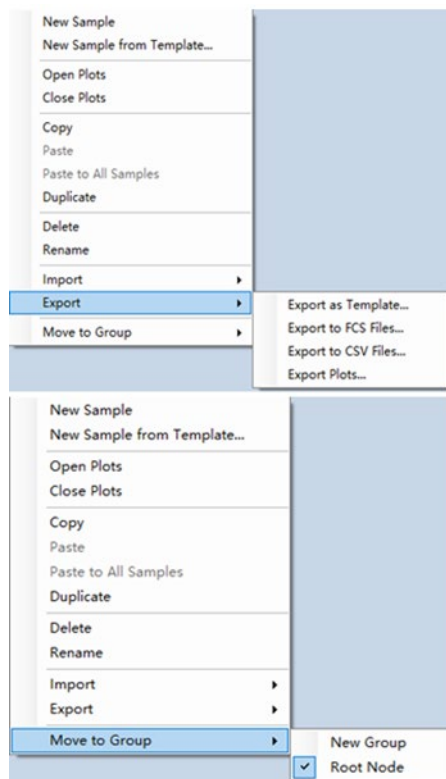


Specimen

New Sample: Creates a new sample in the specimen.

New Sample from Template: Imports the samples of the first specimen from a selected template.

Experiment Manager



Open Plots: Opens all plots from all of the samples within the specimen.

Close Plots: Closes all plots from all of the samples within the specimen.

Copy: Copies the template of the specimen.

Paste: Pastes the template of a copied specimen, or creates a new sample with the copied sample template.

Paste to All Samples: Pastes the copied sample template to all samples in the specimen.

Duplicate: Creates a duplicate of the specimen.

Delete: Deletes the specimen.

Rename: Renames the specimen.

Import:

- **Import Template:** Imports template and apply to the selected specimen.

- **Import FCS Files:** Selects one or more FCS files imported as samples.

Export:

- **Export as Template:** Exports the specimen as a template file.

- **Export to FCS Files:** Exports all samples as FCS files.

- **Export to CSV Files:** Exports all samples as CSV files.

- **Export Plots:** Exports all plots in current specimen as image files.

Move to Group: Move the specimen to the existing group or new group.



Sample

Open Plots: Opens all plots from the sample.

Close Plots: Closes all plots from the sample.

Delete Plots: Deletes all plots from the sample.

Create Plot: Creates a new plot for the sample.

Copy Template: Copies the template of the sample.

Copy Events: Copies the events of the sample.

Paste: Pastes the copied template or events of a sample.

Duplicate: Creates a duplicate of the sample.

Delete: Deletes the sample.

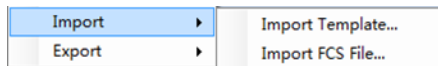
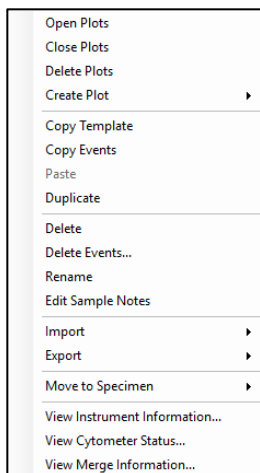
Delete Events: Deletes all or part of the sample's events. Only accounts with the **Delete Sample Events** privilege can perform this operation (Refer to [Sample](#)).

Rename: Renames the sample.

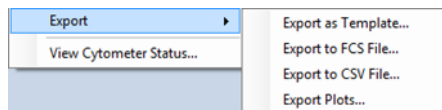
Edit Sample Notes: Edits the sample notes.

Import: Imports a sample template or FCS file, as shown below:

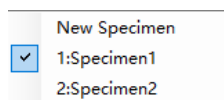
Experiment Manager



Export: Exports the sample as a template, FCS file, CSV file, or exports all plots of the sample as image files.



Move to Specimen: Move the sample to the selected specimen or new specimen.



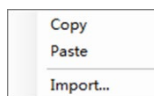
View Cytometer Status: Displays the cytometer status when the sample is collected.

View Instrument Information: Displays the instrument information when the file is created or the first sample is collected.

View Merge Information: This option is only available when selected sample is merged from other samples. User can view the merge information (e.g. samples which current sample was merge from). Please refer to [Merge Samples](#) in this guide for more details.



Cytometer settings



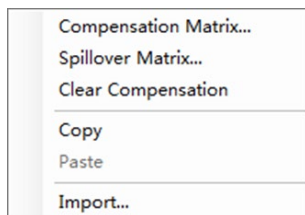
Copy: Copies the sample's instrument settings.

Paste: Pastes the copied instrument settings to the sample.

Import: Imports the instrument settings from a selected template.



Fluorescence compensation



Compensation Matrix: Opens the sample's compensation matrix.

Spillover Matrix: Opens the sample's spillover matrix.

Clear Compensation: Clears fluorescence compensation information of the sample.

Copy: Copies the sample's fluorescence compensation.

Paste: Pastes the copied fluorescence compensation to the sample.

Import: Imports fluorescence compensation from a selected sample template.



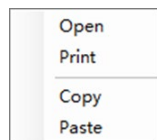
Open: Opens the report.

Print: Prints the report.

Experiment Manager

Report

Specimen Report:



Sample Report:



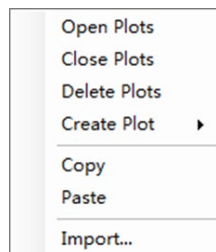
Copy: Copies the report template.

Paste: Pastes the copied report template to the sample. Specimen reports and sample reports are not able to copy and paste each other.

Import: Imports a report template to the sample from a selected template file.



Analysis



Open Plots: Opens all plots from the sample.

Close Plots: Closes all plots from the sample.

Delete Plots: Deletes all plots from the sample.

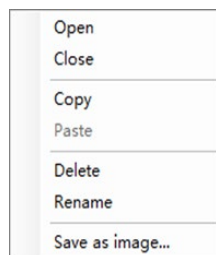
Create Plot: Creates a new plot for the sample.

Copy: Copies the sample analysis template.

Paste: Pastes the copied analysis template to the sample.

Import: Imports an analysis template to the sample from a selected template file.

Plot



Open: Opens the plot.

Close: Closes the plot.

Copy: Copies the plot.

Paste: Pastes a copied gate to the plot.

Delete: Deletes the plot.

Rename: Renames the plot.

Save as image...: Save plot as an image file.

Gate

Create Plot: Creates a new plot including the events from the selected gate.

Gating: Selects plots to apply the gate.

Open: Opens the plot containing the gate.

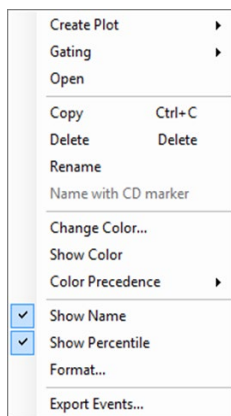
Copy: Copies the gate.

Delete: Deletes the gate.

Rename: Renames the gate.

Name with CD Marker: If a fluorescence parameter is labeled as a CD (Cluster of Differentiation) marker

Experiment Manager



in the **Parameter** panel by setting the **Alias** as CD and a number, this labels the gate using the CD markers.

Change Color: Modifies the color of the gate.

Show Color: Sets whether to display the gates in color.

Color Precedence: Modifies color precedence of the gate.

Show Name: Shows the gate name in gate label on plot. If the **Show gate name in gate label** option in **Setting > Analysis** is not checked, the Show Name menu item here will be disabled.

Show Percentile: Shows the percentage of the gated events relative to the total number of events on the plot. If the **Show population percentile in gate label** option in **Setting > Analysis** is not checked, the Show Percentile menu item here will be disabled.

Format: Opens **Plot Format** dialog to define gate format.

Export Events: Exports data for the events inside the current gate in either FCS or CVS format.



Logic gate group

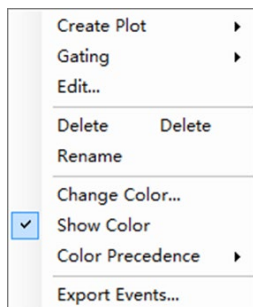


Create: Creates a logic gate.

Delete: Deletes all logic gates.



Logic gate



Create Plot: Creates a new plot including the events from the selected gate.

Gating: Selects plots to apply the gate.

Edit: Opens the logic gate editing window.

Delete: Deletes the logic gate.

Rename: Renames the logic gate.

Change Color: Modifies the color of the logic gate.

Show Color: Sets to display the logic gates in color.



Gating: Selects plots to apply the gate.

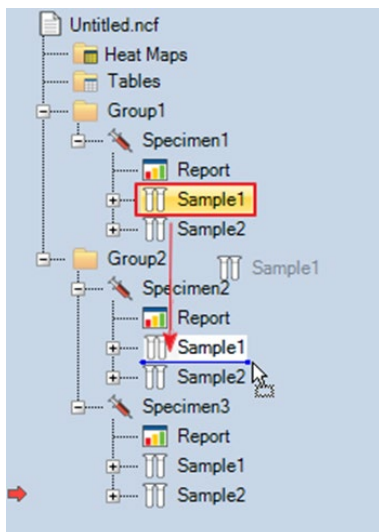
Create Plot: Creates a new plot with the gate applied to the plot.

Move Items

Items in the **Experiment Manager** can be easily re-organized by drag and drop action. Select the item using the left key of the mouse. Move the mouse while

Experiment Manager

holding the left key to drag the item. When the mouse cursor turns like , one may move the item to the position indicated by the blue line. When the mouse cursor turns like , one may apply the analysis as the template to another item. For more information, see [Drag and Drop the Templates](#).



NOTE

During the drag, press the ESC key to cancel the drag operation.

NOTE

Experiment Manager supports making multiple selections within tree node to allow batch operation of multiple objects. Use Shift key to select continuous nodes on the tree and use Ctrl key to select discontinuous nodes on the tree, just like selecting multiple files on Windows Explorer. Only nodes of same type can be selected simultaneously. The selection menu may have less items available when in multiple selection mode.

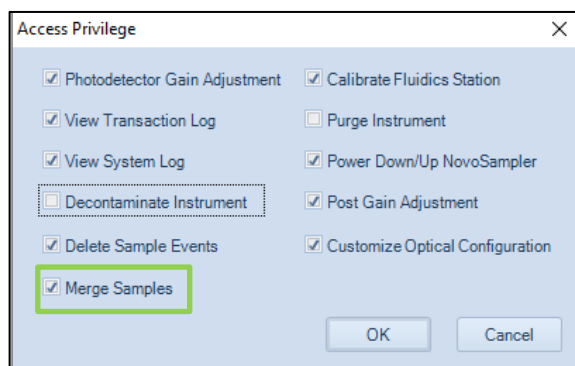
Merge Samples

When two or more samples were collected from the same instrument (i.e. same instrument serial number, and same optical configuration), with same data acquisition parameters selected in the **Cytometer Setting** panel, with same data acquisition mode (i.e. Absolute Count mode applied or not), and the total events from merged samples are less than 10 million, these samples can be merged into one sample.

To merge multiple samples,

Experiment Manager

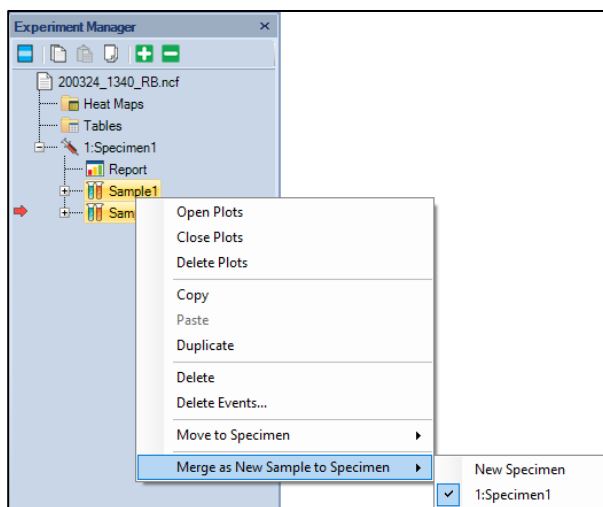
- 1 Ensure the **Merge Samples** privilege is enabled in the **Access Privilege** window.




NOTE

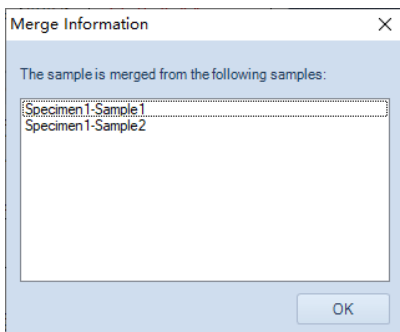
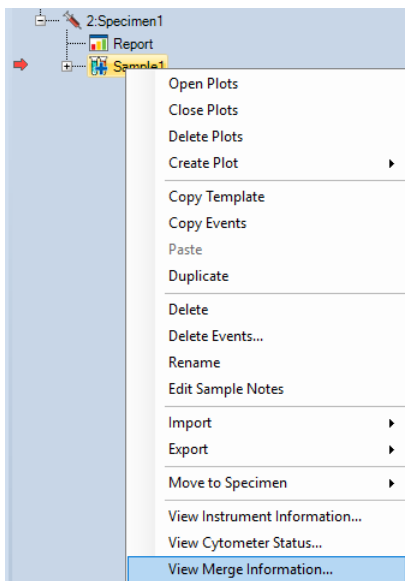
When user logs into the software with Administrator account, the Merge Samples privilege is automatically enabled by default.

- 2 Select the samples from the **Experiment Manager** panel or **Plate Manager** panel (if NovoSampler, NovoSampler Pro or NovoSampler Q is connected) while holding the **Ctrl** or **Shift** key on the keyboard
- 3 Right click any sample, click **Merge as New Sample to Specimen**. User can select either **New Specimen** to have the merged samples placed under a newly created specimen, or select **Specimen X** to have the merged sample placed under the selected existing specimen.



Experiment Manager

- The original samples will be merged in the order of acquisition time with a fixed time interval of 30 seconds added between each original sample to account for the time needed for fluidic rinsing and sample preparation. The icon of the merged sample is displayed as .
- User can right click the merged sample, and select **View Merge Information** to view the information of merged sample (e.g. Name of original specimen and sample).

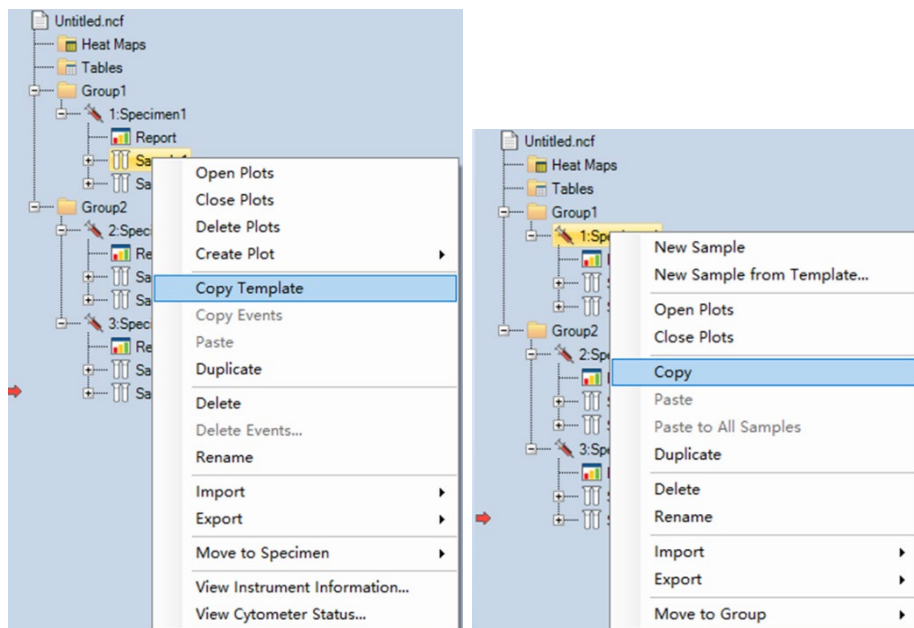


Templates

The NovoExpress Software allows for the use of templates to quickly set up experiment settings. These files can contain the settings for groups, specimens, or samples. There are multiple methods for a template to be applied including: copying and pasting, dragging and dropping, adding through the toolbar, and importing and exporting templates.

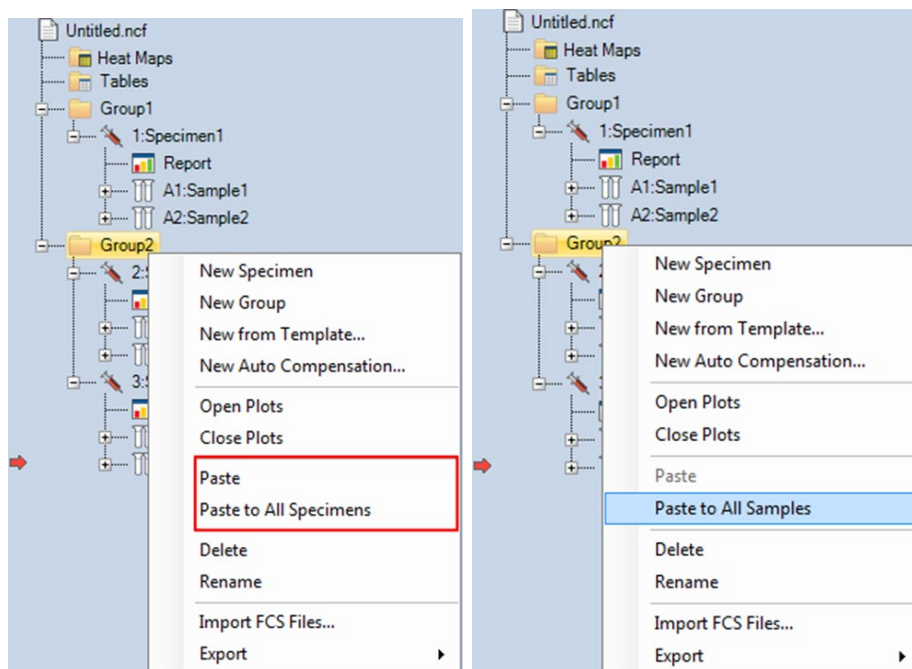
Copy and Paste the Template

In the **Experiment Manager** panel, select a specimen, right-click, and select **Copy** to copy the template of the specimen. To copy the template of a sample, select the sample, right-click, and select **Copy Template** to copy the template of the sample.



To paste, right-click on the target node and select **Paste** or **Paste to All Samples** or **Paste to All Specimens** to apply the template, as shown below:

Experiment Manager



The following table lists template information transferred when copying from a source node type and pasting to a target node type. In addition to copying and pasting, the table below also applies to dragging and dropping ([Drag and Drop the Templates](#)) and using the toolbar ([Use the Toolbar](#)) except for one exception described in [Drag and Drop the Templates](#).

The source node	Target node
Specimen	Specimen: The target specimen report is replaced by the template of source specimen report and samples in the target specimen are replaced by copying samples in the source specimen and pasting to them. Group, Experiment File: Creates the same specimen as the source specimen in the target group of experiment file.
Specimen Report	Specimen Report, Specimen: The target specimen report or specimen report of target specimen is replaced by template of source specimen report. Group, Experiment File: The specimen reports of all specimens within the target node are replaced by the template of source specimen report.
Sample	Sample: The cytometer settings, compensation, sample report and analysis templates are replaced. If the target sample contains events, cytometer settings are not replaced. Specimen: Create the same sample in the target specimen Groups, Experiment File: All samples in the target node are replaced by copying the source sample and pasting them to the target node.

Experiment Manager

Cytometer Setting	Cytometer Setting, Sample: The cytometer settings template is replaced. If the target sample node contains previously collected events, only the parameter aliases are replaced. Specimen, Groups, Experiment File: The cytometer settings templates for all samples within the target node are replaced.
Compensation	Compensation, Sample: The compensation of target sample is replaced. Specimen, Group, Experiment File: The compensations of all samples within the target node are replaced.
Sample Report	Sample Report, Sample: The report template is replaced by the report template of source sample. Specimen, Group, Experiment File: The sample report templates of all samples within the target node are replaced.
Analysis	Analysis, Sample: The analysis template of target sample including plots and gates is replaced by the analysis template of source sample. Specimen, Group, Experiment File: The analysis templates of all samples within the target node are replaced.
Plot	Analysis, Plot: If the target node contains a plot with the same name as the source node, the plot will be replaced. Otherwise, the plot will be created. Specimen, Group, Experiment File: Either replace or create the plot in all of the samples of the target node.
Gate (does not include logic gates)	Plot (does not include cell cycle plots): If the target node contains a gate with the same name as the source node, the gate will be replaced. Otherwise, the gate is created. Only range and bi-range gates can be drawn into a one-dimensional histogram. i.e., a rectangular gate cannot be applied to a histogram. Also, changing a two-dimensional gate (such as FITC vs. PE, that has a rectangular gate drawn in it) to a histogram will delete rectangular gates. Analysis, Sample: Source gate will be pasted to the plot with the same plot name as source gate's plot in the target sample if it exists. Specimen, Group, Experiment File: Source gate will be pasted to all samples in the target node.

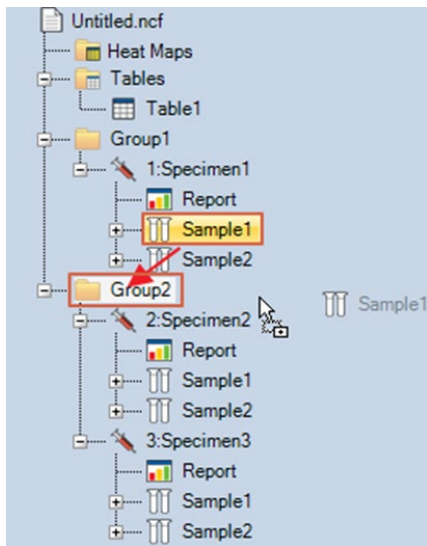
NOTE



Specimen reports and sample reports cannot copy and paste each other.

Drag and Drop the Templates

The template from source nodes can also be applied to target nodes by dragging and dropping, as shown below:

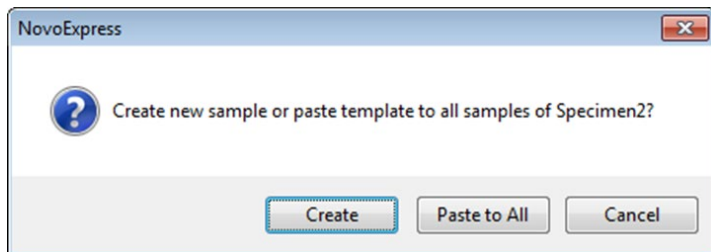
Experiment Manager



When dragging a node over another, if the mouse turns to , dropping will apply template. If the mouse turns to , dropping will move and reposition the selected item. For more details for moving objects, see [Move Items](#).

NOTE

Drag a sample node to a specimen node, a pop-up dialog box will ask you to create new sample or paste a template to all samples of that specimen; drag a specimen node to a group node, a pop-up dialog box will ask you whether create new specimen or paste a template to all specimen of that group.



NOTE




During the drag, press the ESC key to cancel the drag operation.

NOTE

Drag a sample node to the workspace (empty area or inside plot window) will apply the data analysis template of the current Active Sample to the dragged sample and display the data from the dragged sample. In another word, it will keep current analysis template and switch the Active Sample to the dragged sample.

Experiment Manager

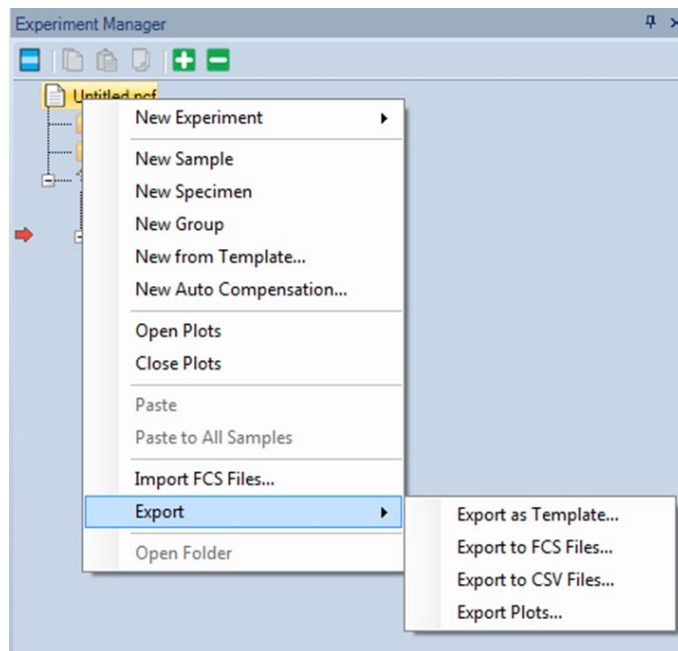
Use the Toolbar

In the **Experiment Manager** panel, select the source node to be copied. The node will be highlighted in yellow (e.g.  Specimen1). To copy the selected node, click the **Copy** button , from the **Experiment Manager** panel toolbar. To paste the copied node, select the target node in the **Experiment Manager** panel, and click the **Paste** button , from the toolbar.

Import and Export Templates

Export Template

In the **Experiment Manager** panel, select the sample, specimen, group, or experiment file to export. Right-click the selected node and select **Export > Export as Template...**. The template will be exported as a *.nct template file.

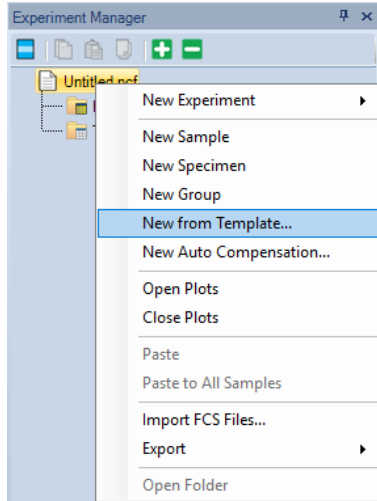


Import Template

- Import to Experiment File

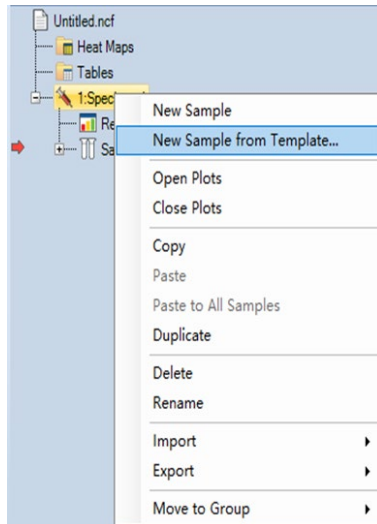
If importing to an experiment file, right-click the experiment file node and select **New from Template...**

Experiment Manager



- Import to Specimen

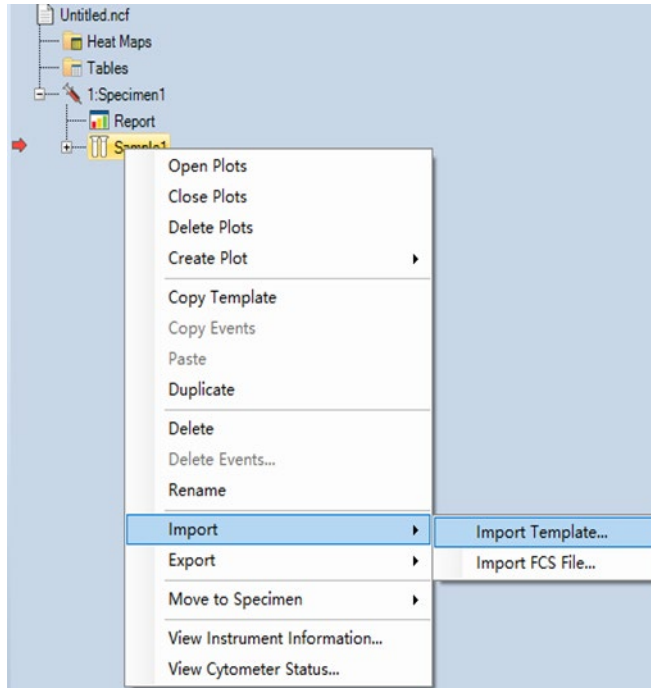
If importing to a specimen, right-click the specimen node and select **New Sample from Template...**



- Import to Sample

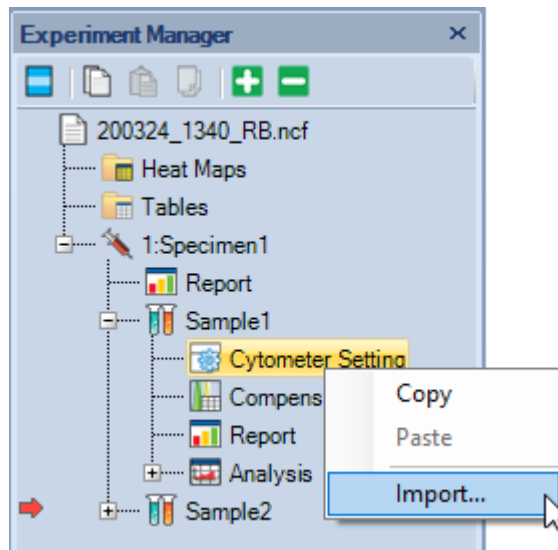
If importing to a sample, right-click the sample node and select **Import**, then **Import Template...**

Experiment Manager



- Import to **Cytometer Setting**, **Compensation**, **Report**, or **Analysis**

If importing to **Cytometer Setting**, **Compensation**, **Report**, or **Analysis**, right-click the target node and select **Import...**



Import and Export Data

The NovoExpress Software is capable of importing FCS 2.0, 3.0 and 3.1 formatted files for data analysis, and it is able to export FCS 3.0, FCS 3.1, and CSV formatted files.

Import Data

There are multiple methods for importing FCS files through the **Experiment Manager** panel:

- Select the experiment file node or a group node. Right-click and select **Import FCS Files....** Select a folder containing the FCS files to import. All FCS files within the folder will be imported. Files up to 10 subfolders deep will be imported and organized according to the folder structure.
- Select a specimen node. Right-click and select **Import FCS Files....** Select FCS files to import as samples under the specimen node.

NOTE

To import multiple samples, hold down the Ctrl key while selecting samples to select more than one sample.

- Select a blank sample node. Right-click and select **Import**, then **Import FCS File...**

Select the FCS file, and data from the file will be imported to the blank sample. It is not possible to import data to a sample already containing collected data. To import data to a sample already containing data, first clear the sample of any events by right-clicking the sample and selecting **Delete Events**.

Export Data

Select the sample, specimen, group, or experiment file node with data to be exported. Right-click the node and select **Export > Export to FCS Files...** or **Export to CSV Files...**. The **Export Events** window will open.

Experiment Manager

The 'Export Events' dialog box is shown with the following settings:

- Object: Sample2
- Gate: All
- Path: Desktop\New folder (17)\A2_Sample2.fcs
- Specimen Name
- Format: FCS 3.0 FCS 3.1 CSV
- Advanced Settings:
 - Parameter Range: Auto
 - Post Gain
 - Use Alias as Parameter Name

Buttons: OK, Cancel

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

The 'Export Events' dialog box is shown with the following settings:

- Object: Sample2
- Gate: All
- Path: Desktop\New folder (17)\A2_Sample2.fcs
- Specimen Name Well ID
- Format: FCS 3.0 FCS 3.1 CSV
- Advanced Settings:
 - Parameter Range: Auto
 - Post Gain
 - Use Alias as Parameter Name


Buttons: OK, Cancel

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

The **Export Events** window has the following settings:

- **Object:** This is the node to be exported. If the object is a sample, only the sample will be exported. If the object is a specimen, group, or experiment file, all samples within the object will be exported.

Experiment Manager

- **Gate:** The default gate setting is All. In this setting, all events are exported for each of the exported samples. A specific gate can be selected using the drop-down menu. When a gate is selected, only events within the gate are exported. If an exported sample does not contain the selected gate, all events within the sample are exported. Also, if an exported sample contains the gate but the gate does not include any events, all of the sample's events are exported.
- **Path:** The path specifies the location to save the exported files. The user can type in the textbox or use the button  , to change the path. When the object is a single sample, the exported data file is saved directly at the path. When the object is a specimen, group, or experiment file, the exported data files is saved in subfolders representative of the sample hierarchy organization in the **Experiment Manager**.
- **Specimen Name:** Check this box to include specimen name in the name of the exported file.
- **Well ID:** Check this box to include **Well ID** in the name of the exported file. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
- **Format:** This specifies the exported data file format. The default setting depends on whether the window was opened using **Export > Export to FCS Files...** or **Export > Export to CSV Files...**. However, the format can be changed post selection using this setting. If you want to import the FCS files to FlowJo with version below v10, please select **FCS 3.0**.
- **Parameter Range:** When exporting as a FCS formatted files, there is the option to set the recommends visualization parameter range. The three options are **Default**, **Auto**, and **Plots**. When **Default** is selected, the parameter range is the full range of the instrument (10 to 224). When **Auto** is selected, the software automatically calculates the best range based on the distribution of the sample data. When **Plots** is selected, the parameter range is determined from parameter ranges used in plots.
- **Post Gain:** This option is enabled only when the samples specified to export have Post Gain defined. When checked, exported event value is the value after Post Gain adjusted.
- **Use Alias as Parameter Name:** Check this option to use the parameter alias as keyword when exporting FCS file.

After setting the above options, click **OK** to begin exporting the data.

Experiment Manager

NOTE

Parameter range of options does not affect the number of events exported. No matter what choice was made it will export all the events within specified gate. Export FCS file with **Auto** or **Plots** parameter range could help third-party software to select the appropriate range when showing plots.

Copy and Paste Events

In the **Experiment Manager** panel, data of collected events can be copied and pasted to blank samples.

- Select the sample containing the events to be copied. Right-click and select **Copy Events**.
- Select the empty sample. Right-click and select **Paste**, or select the **Paste** button, from the toolbar. Data from all of the collected events in the original sample is pasted into the blank sample. Note that only events and cytometer settings are pasted. To copy and paste Analysis, select the sample containing the Analysis to be copied. Right click and select **Copy Template**. Select the sample to paste the Analysis into. Right click and select **Paste**. Alternatively, dragging and dropping Analysis from one sample to another will also copy and paste the Analysis. Similarly, this can be performed with Compensation and Report.

NOTE

This is useful when using the **Auto Compensation** feature during analysis to apply single stained compensation controls to the generated Auto Compensation samples.

Experiment Manager

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8

Reports

This chapter provides information for the Report function, including Report Interface, Automatically Generate Reports, Report Options, Report Editor, Report Output and Batch Print Reports.

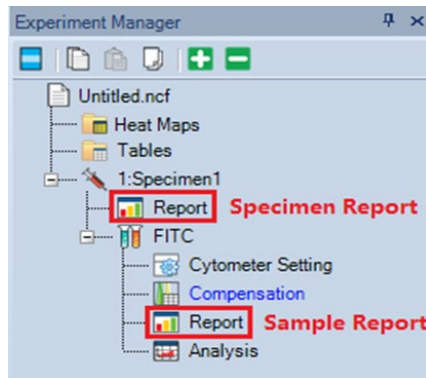
Reports can be created using an Auto mode and a manual mode. In the Auto mode, the software generates the report using a fixed format to include user created plots, statistics (Gate, Count, % of Parent, Mean X and Mean Y), and basic information (Sample Name, Run Time, Cytometer, and Software). In the manual mode, the user is able to add or remove elements and adjust formatting.

NOTE

Real-time changes made to a plot within the Report are also applied to sample's Analysis back in the main interface. Statistics are automatically adjusted.

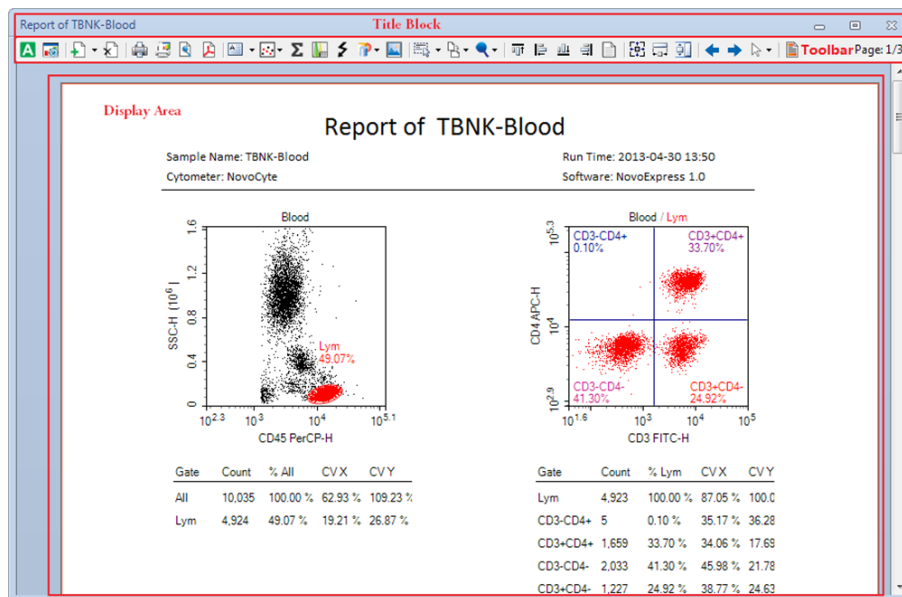
From the **Experiment Manager** panel, the report can either be a sample report or a specimen report. A sample report can contain plots, statistical information, compensation matrices, and collection information for the sample. A specimen report contains such information for all of its samples and basic information of its own. Double clicking on **Report** in the **Experiment Manager** allows the user to view the report interface window.

After the reports are created, a batch print function ([Batch Print Reports](#)) can be used to generate PDF files.



Report Interface

The report interface window is shown below. It can be opened by double-clicking on the report node in the **Experiment Manager** panel.



The interface window is divided into three main sections: Title Block, Toolbar, and Display Area.

The title bar contains the name of the current report in the window.

The toolbar contains the functions available to generate and edit the report.

The display area is the main area of the window. Using this area, the user is able to edit the objects displayed and add and delete pages. The objects here forth are referred to as report items and include text, graphics, statistical information, fluorescence compensation, and plots.

The toolbar functions are described below:

Icon	Description
	Auto Report Mode: Clicking on this button switches between the automatic report generation mode and the manual mode. When selected and in automatic mode, the icon appears with a blue border . When unselected and in manual mode, the icon appears without the blue border .

Reports

NOTE

The original automatic report can be restored after making manually changes in manual mode by re-selecting auto report mode.



Report Options: Click to open **Report Options** dialog. Refer to [Report Options](#) for detail information.



Insert Page: This function inserts a page into the report. By default, clicking the button inserts a blank page after the current page being viewed. In the display window, the current page being viewed has a red border. From the arrow to the right of the button, a drop-down menu allows the user to specify **Insert Before Current Page** or **Insert After Current Page**.



Delete Page: Deletes the current page. If the report is a single page, the page cannot be deleted.



Print: Prints the report.



Batch Print Reports: Open the **Batch Print Reports** dialog. Refer to [Batch Print Reports](#).



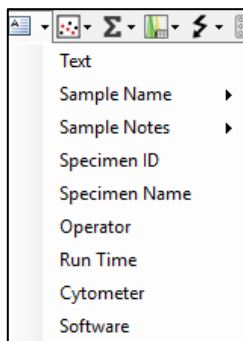
Print Preview: Displays a print preview of the report.



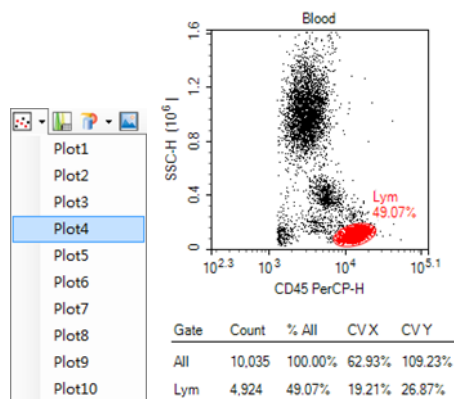
PDF: Generates a PDF file of the report.



Insert Text: Inserts a textbox. The user can edit and format the text. Text types include text, sample name, sample notes, specimen ID, specimen name, operator, run time, cytometer, and software. In addition, specimen reports contain specimen information text type.



Insert Plot: Click the icon to list the plots for the sample, including dot plots, density plots, histogram plots, contour plots, and cell cycle plots. Select the plot to insert. The plot is inserted with the statistics as shown below.



Insert Sample Statistics: Inserts a table of gate statistics for a sample. In a sample report, click the button to insert a gate statistics table for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a gate statistics table for the selected sample.



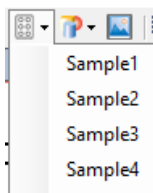
Insert Compensation: Inserts a spillover matrix. In a sample report, click the button to insert the spillover matrix for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert the spillover matrix for the selected sample. The spillover matrix cannot be edited from within the report. If the spillover matrix is modified in the main interface, the matrix in the report window will update.



Insert Photodetector Gain: Inserts a table of photodetector gain setting for a sample. In a sample report, click the button to insert a table of photodetector gain setting for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a table of photodetector gain setting for the selected sample.



Plate Information: Inserts plate information for selected sample, including plate ID, barcode of the plate (if scanned), and description. It is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.













Insert Shape: Click to insert a horizontal line, vertical line, or rectangle.



Insert Picture: Click open the **Open** window, select a picture to insert. The picture will be resized to an appropriate size with the original aspect ratio and inserted in the report.

Reports


	Select: The default is Select All . From the arrow on the right of the button, Select Similar Objects is also available.
	Page Rotation: Switches the page layout orientation between portrait and landscape.
	Zoom: Sets the zoom percentage of the displayed page. When report displays is zoomed in or zoomed out (zoom percentage is not 100%), user cannot double-click report item to enter into edit mode.
	Align: Select two or more objects in a page to activate these features. When selecting multiple objects, the first object has white squares on the border, while additional objects have black squares. From the four alignment options (align top, left, bottom, or right), select the edge to be aligned. The objects will be moved so that the selected edge of all selected objects aligns with the object first selected.
	Lock Position: Check this item to forbid moving any object of the report. Align Top, Align Left, Align Bottom, Align Right buttons will be disabled when Lock Position is checked.
	Make Same Size / Width / Height: Select two or more objects in a page to activate these features. When selecting multiple objects, the first object has white squares on the border, while additional objects will have black squares. From the three resize options (make same size, width, or height), select the desired resize dimension. The selected objects will be resized to match the selected dimension of the object first selected.
	Previous: In a sample report, click to switch to the previous sample's report. In a specimen report, click to switch to the previous specimen's report.
	Next: In a sample report, click to switch to the next sample's report. In a specimen report, click to switch to the next specimen's report.
	Select: Select to view or edit another report.
	Set Page Header and Footer: Click to show header and footer editing interface.
Page: 1/3	Page: Displays the current page and the total number of pages.

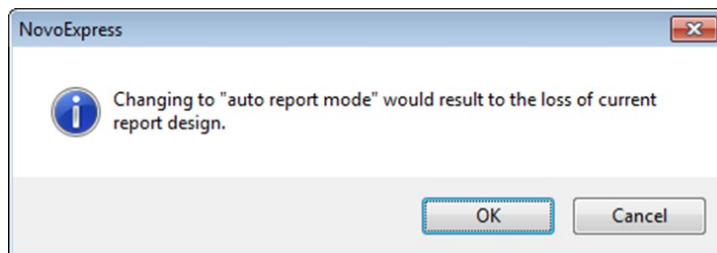
Automatically Generate Reports


The report can be generated through an automated or a manual mode. In the automated mode, the user performs the analysis and creates the plots in the main software interface. The report will be automatically created with the plots and statistical information added without the need for additional input from the user.

Reports

The user is not able to add, delete, or modify the contents of the report in this mode.

To switch the mode from manual to automated, click on the **Auto Report Mode** button in the toolbar . The prompt below will appear to confirm the switch to the automated mode. Click **OK**, to automatically generate the report.



To switch the mode from automated to manual, click on **Auto Report Mode** button in the toolbar . The user can now make modifications to the report.

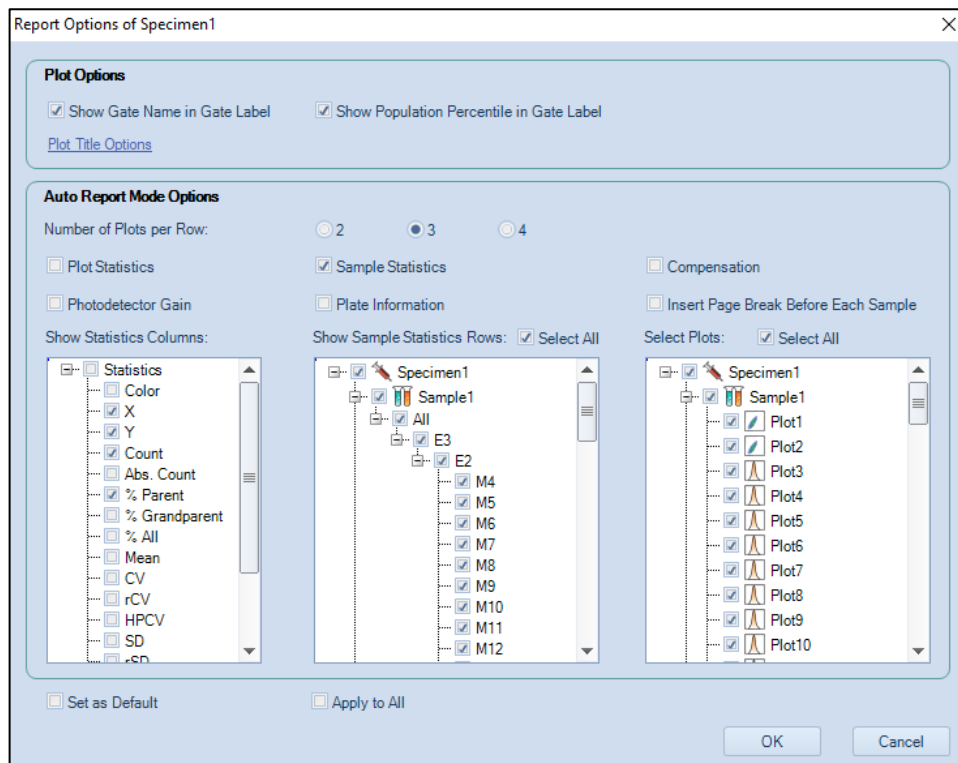
NOTE

When creating a report, it may be best to use the automated mode to generate an initial report and then switch to manual mode to modify the report.

Report Options

Report Options dialog provides user interface for user to customize report of auto and manual mode. To open **Report Options** dialog, click the **Report Options** button  in the report window toolbar. The **Report Options** dialog is shown below:

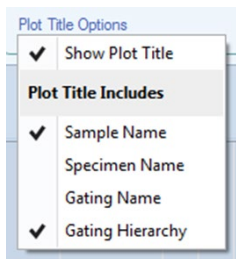
Reports



The settings in **Plot Options** panel are used for customizing plots inside report. They are effective for both auto and manual report mode.

- **Show Gate Name in Gate Label:**
If selected, gate name is displayed in gate label on the plot.
- **Show Population Percentile in Gate Label:**
If selected, gate label is displayed with the percentage of the population within the gate.
- **Plot Title Options:**
If clicked, a drop down menu will show as below.

Reports



- **Show Plot Title:**
If selected, plot title is displayed on the report plot.
- **Sample Name:**
If selected, the sample name is displayed in the report plot title.
- **Specimen Name:**
If selected, the specimen name is displayed in the report plot title.
- **Gating Name:**
If selected, the gating name is displayed in the report plot title.
- **Gating Hierarchy:**
If selected, the gating hierarchy is displayed in the report plot title.

The settings in **Auto Report Mode Options** panel are used for customizing auto report. They are only effective for auto report mode.

- **Number of Plots per Row:**
Sets how many plots are shown in one row.
- **Plot Statistics:**
If selected, shows gate statistics of plot.
- **Sample Statistics:**
If selected, shows gate statistics of sample.
- **Compensation:**
If selected, shows compensation matrix.
- **Photodetector Gain:**
If selected, shows the photodetector gain setting.
- **Plate Information:**

Reports

If selected, shows the plate information. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.

- **Insert Page Break Before Each Sample:**
Only available for specimen report. If selected, a page break will be inserted before each sample.
- **Show Statistics Columns:**
Selects statistical items to display.
- **Show Sample Statistics Rows:**
Selects sample statistical items to display.
- **Select Plots:**
Selects plots to display on report.
- **Select All:**
Selects all associated items to display on report.
- **Set as Default:**
Sets above settings as default setting for new reports.
- **Apply to All:**
Applies above settings to all report in the experiment.

Report Editor

In the manual mode, the user is able to freely edit the report. Options include adding, removing, and editing objects in the report

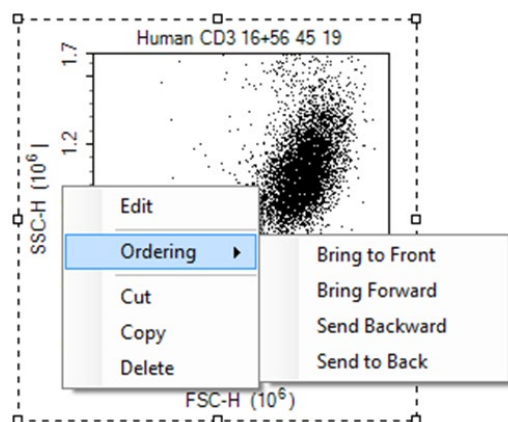
Add Report Objects

Objects can be added to the report through the toolbar, Workspace, and **Experiment Manager** panel. To add objects using the toolbar, use the insert functions described in [Report Interface](#). From both the Workspace and **Experiment Manager** panel, objects corresponding to the sample can be dragged and dropped into the report.

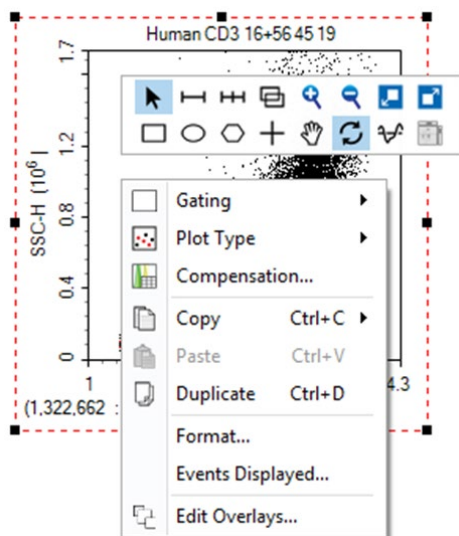
Select Report Objects

Click on an object to select it in the report. An object can have one of two selected states. After clicking on an object, the object will be highlighted. In this state, the object is bordered by a black dashed line with white control points. If the object is then double-clicked, it is in the edit state, and the object is bordered by a red dashed line with black control points. Different operations can be performed on the object depending on the selected state. The two states are shown below.

This is the object after being selected:




This is the object selecting and in the editing state:



Reports

To have multiple objects selected simultaneously:


- In the toolbar, click the **Select All** button , to select all of the objects in the report.
- Left-click and drag in the report to enclose objects inside of the dashed rectangle. Objects within the dashed rectangle will be selected when the mouse button is released.
- Select an initial object. In the toolbar, click **Select All**, then **Select Similar Objects**. All objects in the report of the same type as the initial object will be selected.
- Select an object. Press and hold the Ctrl key to select additional items.

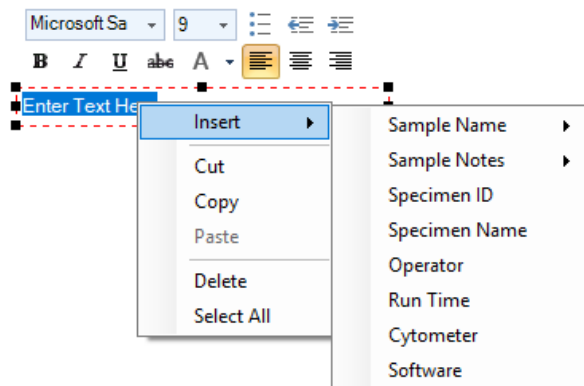
When multiple items are selected, the first item is bordered by a black dashed line with white control points and additional items are bordered by black dashed lines with black control points.

Edit Report Objects

Double-click on a selected object or right-click on the object and select **Edit** to enter the editing mode for that object. This section will describe the editing options available for the objects.

Edit Text

Double-click on the textbox or right-click on the textbox and select **Edit** to enter editing mode. In this mode, text formatting tools will appear. Right-click on the textbox and select **Insert** to insert sample information including sample name, sample notes, specimen ID, specimen name, operator, run time, cytometer and software information, as shown below. This can also be accessed by clicking the **Insert Text**  icon's drop-down menu in the tool bar.

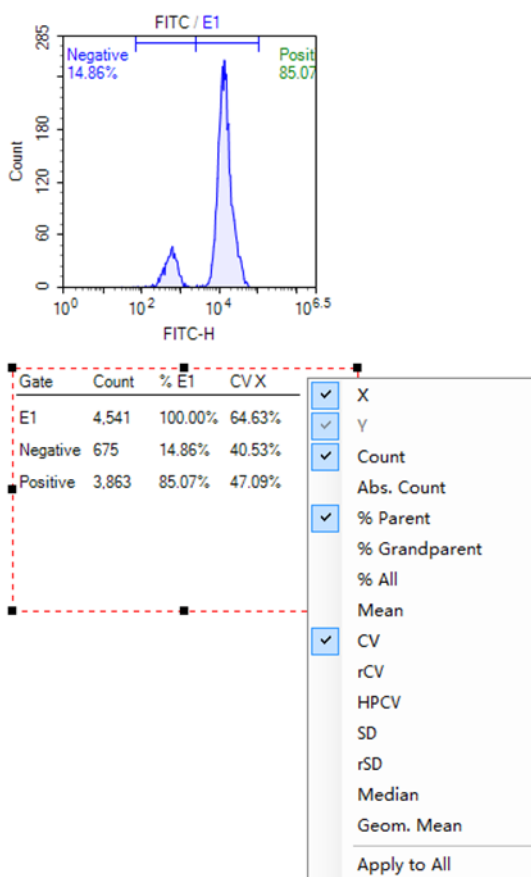


Reports

Edit Plots and Statistics

Double-click on the plot or right-click on the plot and select **Edit** to enter editing mode. Plots can be edited in the report by right-clicking in the plot to access the plot tools. Modifications made to the plot will also be updated to the plot in the main interface. In addition, if the plots are modified in the main interface, the plots in the report will also update automatically.

Double-click on the statistics box or right-click on the statistics box and select **Edit** to enter editing mode. Right-click in the selected statistics box to choose the columns to display, as shown below.

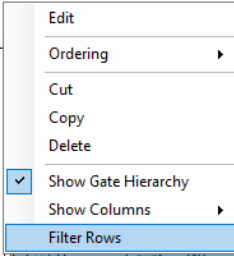


Filter Statistics

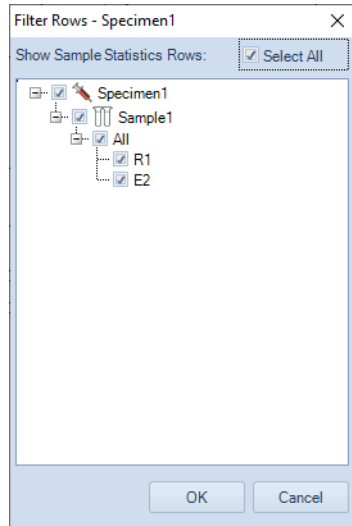
User can right click the statistics box of the report, click **Filter Rows** to open the **Filter Rows** window. Check to select the gate(s) to display associated sample statistical information in the report, and click **OK** to confirm.

Reports

Gate	Count	% Parent
All	11,649	
E3	10,008	85.91%
E2	9,974	99.66%
M4	9,974	100.00%
M5	9,974	100.00%
M6	9,974	100.00%

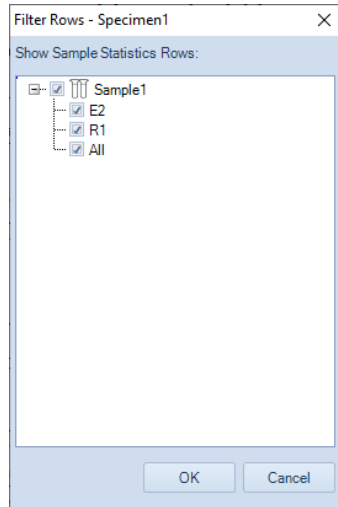


When the statistics box shows the gate hierarchy, the **Filter Rows** window displays the gate hierarchy too. When the child gate is checked, the parent gate will be automatically checked.



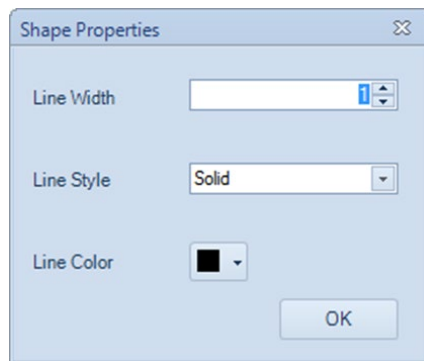
When the statistics box does not show the gate hierarchy, the **Filter Rows** window does not display the gate hierarchy either.

Reports



Edit Shapes

Double-click on an inserted shape (horizontal lines, vertical lines, or rectangles) or right-click on the shape object and select **Edit** to enter editing mode. A **Shape Properties** window will appear. In the window, line width, style, and color can be set.







Edit Pictures

Double-click on the picture or right-click on the picture and select **Edit** to enter editing mode. The **Open** window will appear, and users can select an image to replace the current picture.

Reports

Align Report Project Items

There are multiple methods to align objects in a report.

- Use the mouse to drag an object within the report. As the object is dragged, smart guides appear when the object is aligned with other objects in the report. Drag the object until it is aligned with the appropriate other objects and release the mouse button to set the object at the new location.
- Select the object and use the ↑, ↓, ←, → keys on the keyboard to move the object. Move the object until the object appears to be aligned with the appropriate other objects.
- Select multiple objects. Select the appropriate align tool from the toolbar depending on the edge of the object to be aligned (**Align Top**  ; **Align Left**  ; **Align Bottom**  ; **Align Right** ). The objects will be aligned along the selected edge relative to the position of the first selected object. (The first selected object will be displayed with white control points, while other selected objects will be displayed with black control points.)

Resize Objects

There are two methods to resize objects in a report:

- Select an object. Click and drag on the control points of the object to resize the object .
- Select multiple objects. Select the tool to Make Same Size, Make Same Width, or Make Same Height. The objects will be resized to match the appropriate dimensions of the first selected object. (The first selected object is displayed with white control points, while other selected objects are displayed with black control points.)

Order Object Levels

When objects are overlapped in the report, the object that is displayed is determined by the ordering of the object. To change the ordering of an object, select the object. Right-click on the object and select **Ordering**. Options then include **Bring to Front**, **Bring Forward**, **Send Backward**, and **Send to Back**. Select the appropriate operation for the object. Objects toward the front are displayed over objects further back.

Cut, Copy, Paste, and Delete

- **Cut**: Select an object. Use the keyboard shortcut Ctrl X or right-click and select **Cut** to cut an object.

Reports


- **Copy:** Select an object. Use the keyboard shortcut Ctrl C or right-click and select **Copy** to copy an object. The copied object can be pasted to the office software such as Word, PowerPoint, and Excel.
- **Paste:** After cutting or copying an object, the object can be paste from the clipboard using the keyboard shortcut Ctrl V or right-click and select **Paste**. The object will be pasted at the specified location.
- **Delete:** Select an object. Use the keyboard Delete key or right-click and select **Delete** to delete an object.

Insert or Delete Pages

To insert or delete pages, use the **Insert Page** and **Delete Page** button from the toolbar. For more information, see [Report Interface](#).

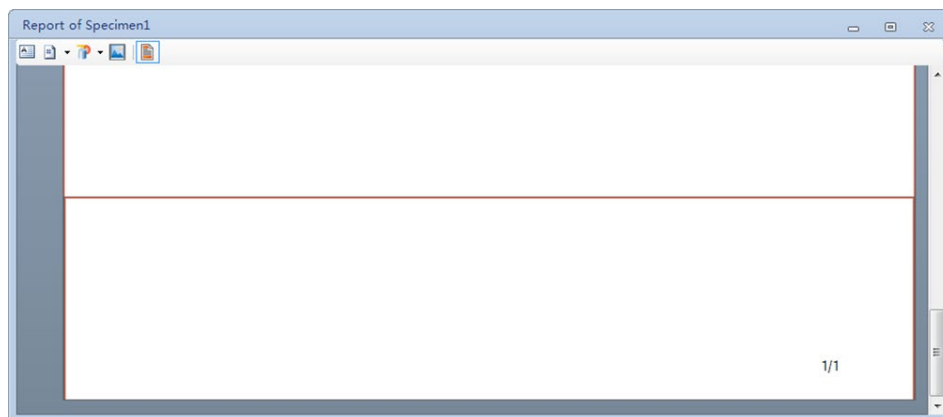
Header and Footers

Headers and Footers Working Interface






The header and footer display information at the top and bottom of the pages, respectively, in the report. To edit the header and footer, click the **Set Page Header and Footer** button , from the toolbar. As shown below, the rectangular region at the top and bottom of the page for the header and footer is outlined. A toolbar containing functions to edit the header and footer is also available. At this time, the header and footer can be edited.



Reports



Toolbar for Header and Footer Functions:

Icon	Description
	Insert Text: Inserts a textbox.
	Insert Page Number: Inserts page numbers. Select between two styles: 1,2,3 ... or 1/3, 2/3
	Insert Shape: Inserts a horizontal line, vertical line, or rectangle.
	Insert Picture: Inserts a picture.
	Set Page Header and Footer: Click to exit header and footer editing mode.

Edit Headers and Footers

To edit the header, click in the header region at the top of the page. Once selected, the region is bordered by a red rectangle. The toolbar can now be used to add objects to the header.


To edit the footer, click in the footer region at the bottom of the page. After selected, the region is bordered by a red rectangle. The toolbar can now be used to add objects to the footer. The default footer includes an object for page number.

The methods for editing the header and footer are consistent with the rest of the report interface with the following exceptions.

- The level of the objects in the header and footer cannot be ordered. Newer created objects are automatically created more towards the top. Objects in the header and footer are behind objects created in the main report interface.

Reports

- Smart guides are not available to help align objects in the header and footer.
- Copying and pasting objects is unavailable in the header and footer.
- Variables such as sample name, specimen ID. Specimen name, operator, run time, and cytometer and software information cannot be inserted into textboxes in the header or footer.



After editing the header and footer is complete, click the **Set Page Header and Footer** button , to return to the main report interface. The header and footer will display on all pages of the report.

Copy the Header and Footer Settings to Other Reports

To transfer the header and footer from one report to another, copy and paste the report as a template as described in [Templates](#).

Report Output

The reports can be printed or converted to a PDF file.

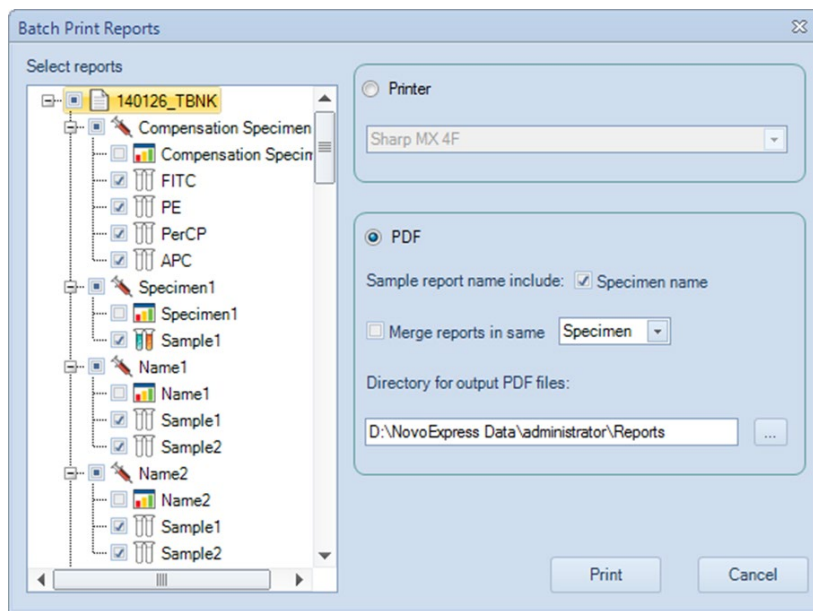
- To Print:
Click the toolbar **Print** button . Alternatively, right-click the report node in the **Experiment Manager** panel and select **Print**. The print window will appear. Select the correct printer and print the report.
- To Convert to PDF:
Click the toolbar **PDF** button . The **Save As** window will appear to save the report as a PDF file.

Batch Print Reports

To batch print reports:

In the main interface window, click the **Batch Print Reports** button in the **Home** tab of the **Menu Bar**. Alternatively, select **File > Print > Batch Print Reports**. The **Batch Print Reports** window will appear.

Reports



The reports are listed on the left side of the **Batch Print Reports** window. Use the checkboxes to select the reports to be printed. On the right side of the window, select either to print the report or generate PDF files for the report.

If printing, select a printer and click **Print**.

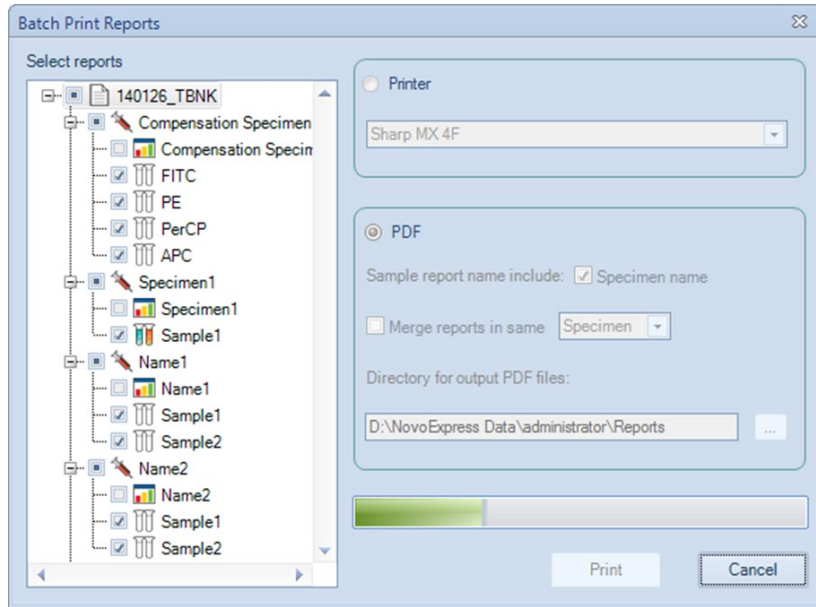
If generating a PDF, select a file path to save the PDF files. If the **Specimen name** box is checked, the PDF files will be saved in the format **specimen name_sample name_YYYYMMDD_hhmmss**. If the box is unchecked, the PDF files will be saved in the format **sample name_YYYYMMDD_hhmmss**. Click **Print** to begin generating the PDF files.

NOTE

One PDF file will be created for each report selected by default. If Merge reports in the same specimen/group/experiment is checked, all reports of one specimen/group/experiment will be printed into one PDF file.

After clicking **Print** a progress bar will appear as shown below.

Reports



During the printing process, click **Cancel** to stop the printing.

This chapter describes the procedures to run QC test and view the report for Agilent flow cytometers.

The NovoExpress software **QC Test** function automatically conducts the QC procedure using Agilent flow cytometer QC particles. Click **Instrument > QC Test** from the NovoExpress software to initialize the QC test. The **QC Test Report** tracks the QC test results over time and can be reviewed through **Instrument > QC Test Report** from the NovoExpress software.

Agilent Flow Cytometer QC particles are polystyrene microspheres with uniform size (average diameter of 3 μm). The QC particles contain 5 kinds of microspheres with differing fluorescence intensities and one blank microsphere. These fluorescent microspheres are internally stained with fluorescent dye, which can be excited by all the lasers with known MESF (Molecules of Equivalent Soluble Fluorochrome) values for each fluorescent peak, from which the linearity can be calculated.

There are three test results:

- **Pass**
Indicating that all the performance parameters meet the requirements.
- **Acceptable**
Indicating that the performance parameters are not optimal compared to the manufacturing QC criteria. However, continued use of the instrument is still allowed and the experiment results should not be impacted.
- **Fail**
Indicating that the performance parameters do not meet the requirements. A label in red will show on report to indicate the reason of failure. Please contact your local Agilent Technologies representative when the QC test fails.

The QC Test procedure generally includes three steps:

- Prepare QC particles sample
- Run QC test
- View QC reports and Levey-Jennings plot

QC Test

NOTE

The software prompted windows displayed in this chapter is for illustration purpose only. User may observe different windows depending on the Agilent flow cytometer models.

Prepare QC Particle Sample

- 1 Label a 12 × 75 mm tube for the QC test. Add 1 mL dilution buffer (0.8 mL PBS and 0.2 mL NovoRinse solution) into the tube.
- 2 Take one bottle of QC particles from storage. Invert the bottle to mix the particles thoroughly.
- 3 Add two drops of QC Particles into the test tube.
- 4 Vortex the test tube to mix the QC particles thoroughly.
- 5 Place the tube onto the instrument and start the QC test. If NovoSampler or NovoSampler Pro or NovoSampler Q is used, transfer 150 µL of the QC particles sample from the test tube to the A1 well of a 96-well plate and run the QC Test immediately. If the prepared QC particle sample is not intended to be run immediately, store the sample in a 2-6 °C refrigerator away from light. The prepared QC particle sample should be used within four hours after preparation.

NOTE

Make sure the QC particle sample is prepared properly following the above procedure. Properly prepared QC particle sample would give enough QC particle counts (~10,000 QC particles) during the QC Test.

Run QC Test

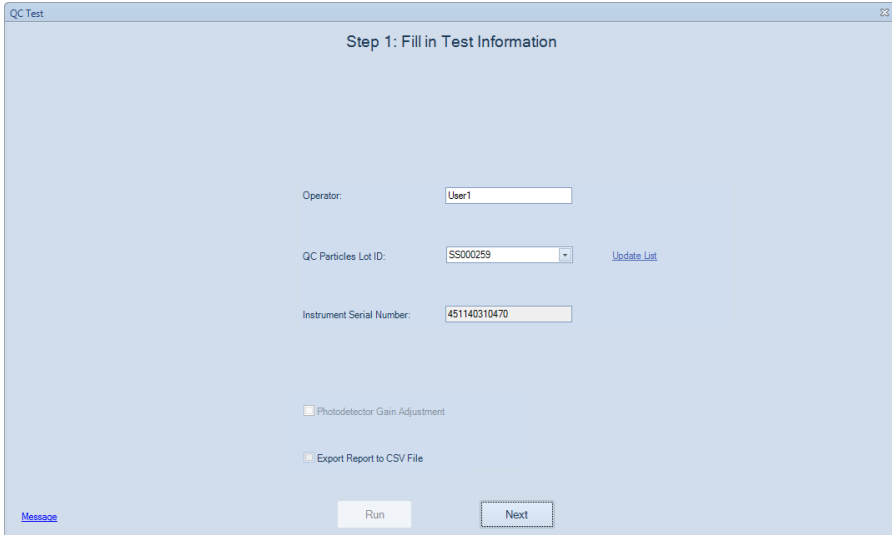
- 1 Save the Agilent Flow Cytometer QC Particles Lot file.

Download the Lot File for the specific batch of QC Particles from <https://www.agilent.com/chem/novocyte-qc-lot-files>. Save the Lot File in a specific directory in the NovoExpress Software's installation directory. (Save the file in **C:\Program Files (x86)\NovoExpress\QC \QC Beads**, if the installation directory is **C:\Program Files (x86)\NovoExpress**). Saving the Lot file is only done once for each new batch of QC Particles. After saving the Lot

QC Test

File to the directory, the lot ID for the specific batch will be listed when running the QC test.

- 2 Ensure the instrument is in **Ready** status. If NovoSampler (or NovoSampler Pro or NovoSampler Q) is used, open the cover of NovoSampler (or NovoSampler Pro or NovoSampler Q) and place the sample plate on the tray. Ensure the plate is fully seated with the correct orientation onto the shaker.
- 3 In the NovoExpress Software, click the **QC Test** button in the **Instrument** tab of the **Menu Bar** to open the **QC Test** window. The window is shown below.



The screenshot shows the 'QC Test' window with the title 'Step 1: Fill in Test Information'. The window contains the following fields and options:

- Operator: User1
- QC Particles Lot ID: SS000259 (with an 'Update List' link)
- Instrument Serial Number: 451140310470
- Photodetector Gain Adjustment
- Export Report to CSV File

At the bottom, there is a 'Message' link, a 'Run' button, and a 'Next' button.

NovoExpress QC Test Window when NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected

QC Test

The screenshot shows a software window titled "QC Test" with a sub-header "Step 1: Fill in Test Information". The window contains the following fields and controls:

- Operator: administrator
- QC Particles Lot ID: 55000392 (with an "Update List" link)
- Instrument Serial Number: 71119010022
- Well: A1
- Photodetector Gain Adjustment
- Export Report to CSV File
- Buttons: Run, Next
- A "Message" link is located in the bottom left corner.

NovoExpress QC Test Window when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

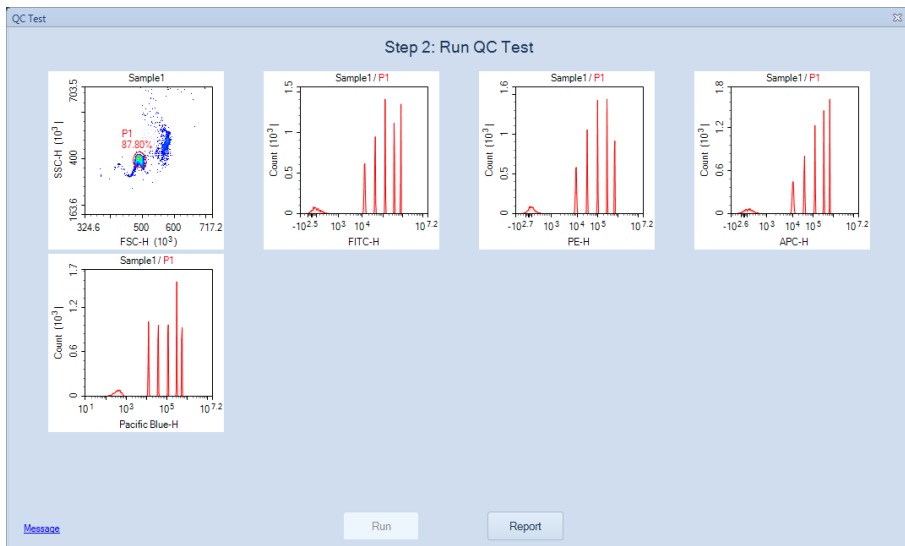
In the window, enter the name of the operator, select the **QC particles lot ID**, select the **Well ID** (if NovoSampler or NovoSampler Pro or NovoSampler Q is used) and click **Next**. If the correct QC particles lot ID is not listed, please refer to the first step to download and save the QC particles Lot File. Check **Export Report to CSV File** to export report as a csv file after QC test is completed. The exported CSV file (in the format of YYMMDD_hhmmss.csv) is automatically saved in **C:\Program Files (x86)\NovoExpress\QC \QC Report Files** by default.

NOTE

The default name of the exported CSV file is in the format of QC test date and time. For example, if the QC test is performed at 10:30:40 am on January 18, 2019, the default name of the exported CSV file will be 190118_103040.csv.

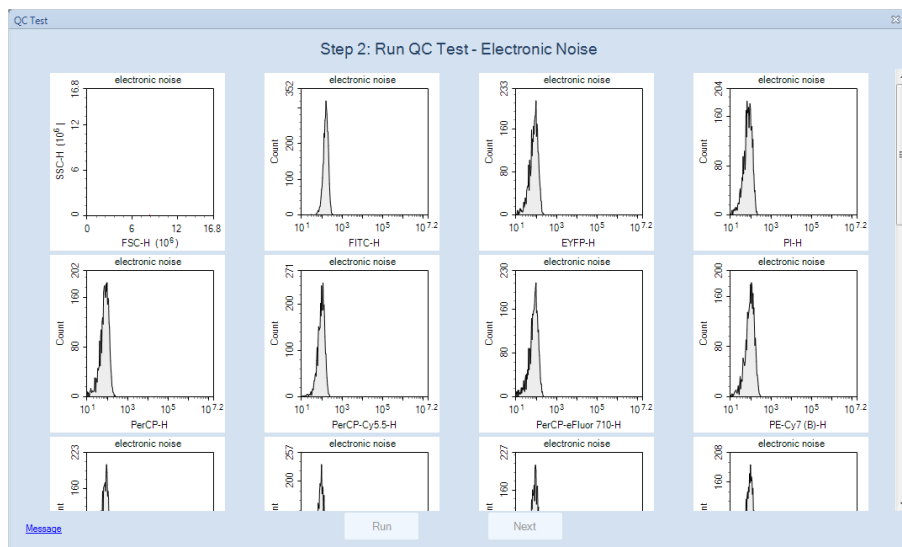
- 4 Click **Run** to begin the test. The instrument will begin collecting events and the results will be displayed in real-time on the **QC Test** window.

QC Test

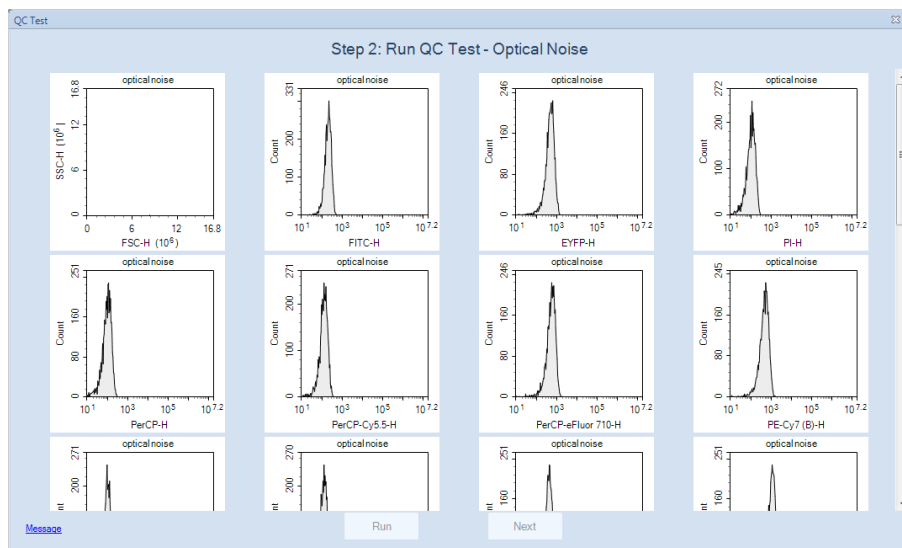


NOTE

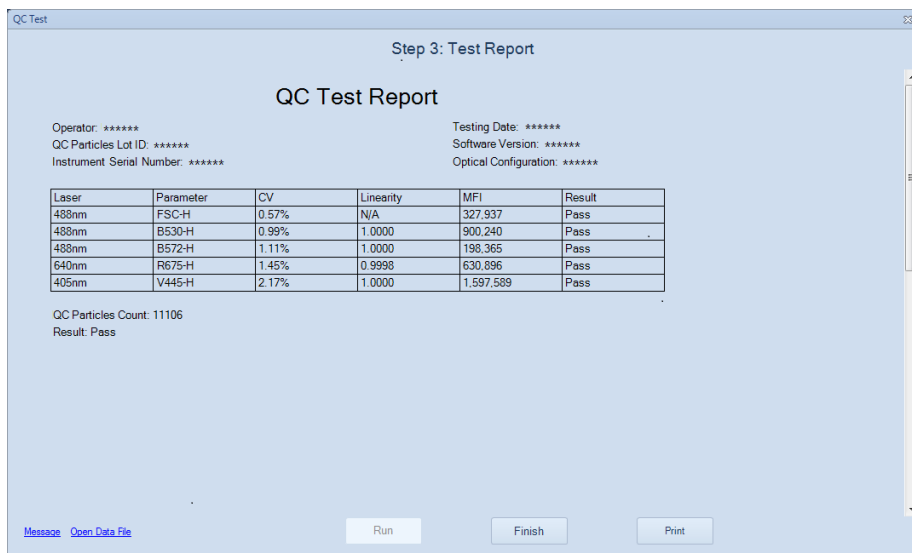
When NovoCyte Quanteon, Advanteon or Penteon is used, the software will collect electronic and optical noise (see figure below) before collecting the events.



QC Test



- 5 After data collection is complete, click the **Report** button to view the test results.



From the figure, the QC test will provide results for various parameters. Click **Print** to print the test report.

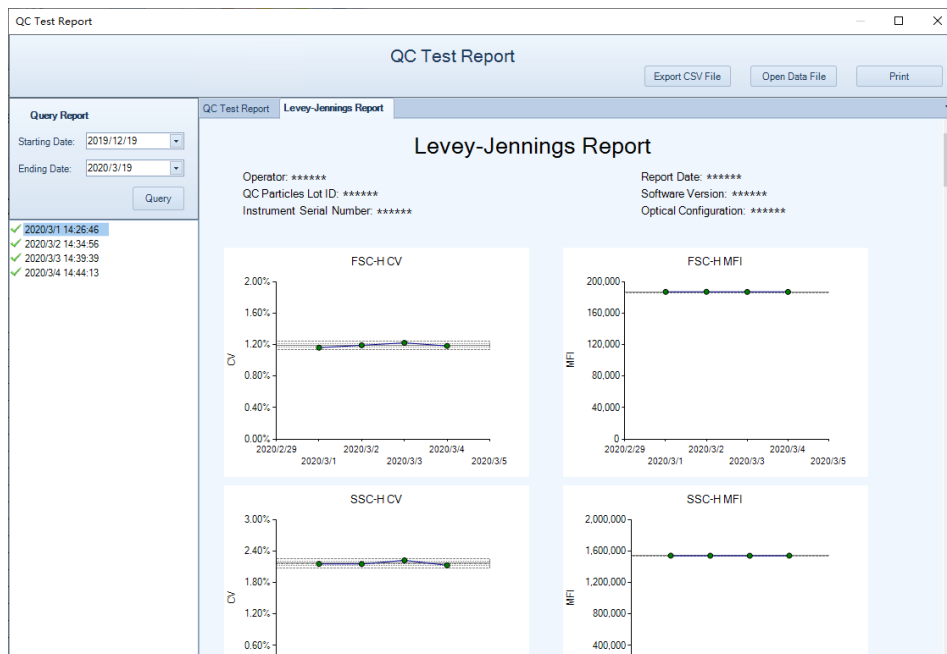
QC Test

NOTE

To view the history of the resulting QC data, click **QC Test Report** from the **Instrument** tab of the **Menu Bar** (described in [View QC Test Report](#)). Highlight the date to be viewed and select the **QC Test Report** tab.

View QC Test Report

The QC test report function stores previous QC test results and provides a data analysis feature to track instrument performance changes over a period time. To open the **QC Test Report** window, click **QC Test Report** from the **Instrument** tab of the **Menu Bar**. The window is shown below.



The **QC Test Report** window contains the following sections.

Interface	Description
Title Area	Displays the report name and the Export CSV File , Open Data File and Print button.
Query Area	Query a time interval for QC test reports. As shown:

Query Report

Starting Date: 2019/12/19

Ending Date: 2020/3/19

Query

- ✓ 2020/3/1 14:26:46
- ✓ 2020/3/2 14:34:56
- ✓ 2020/3/3 14:39:39
- ✓ 2020/3/4 14:44:13

QC Test Report

Displays the QC test report for a selected report from the query results. As shown:

QC Test Report Levey-Jennings Report

QC Test Report

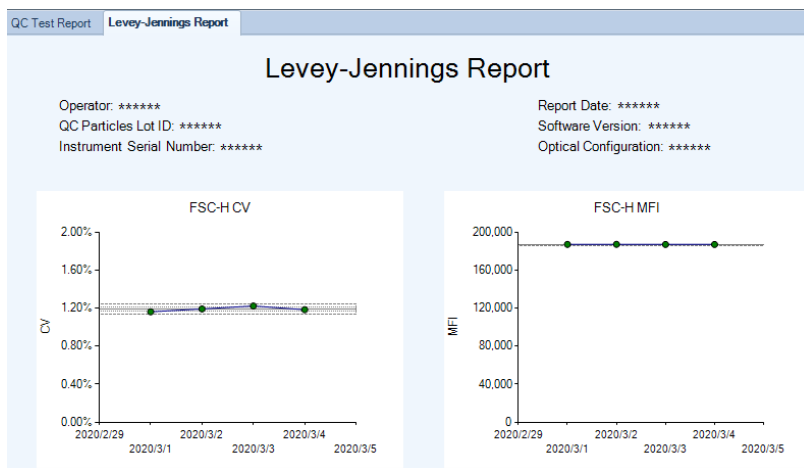
Operator: ***** Testing Date: *****
 QC Particles Lot ID: ***** Software Version: *****
 Instrument Serial Number: ***** Optical Configuration: *****

Laser	Parameter	Gain	CV	Linearity	MFI	MFI Target	MFI Target Difference	Electronic Noise	Optical Noise	Result
561nm	FSC-H	400	2.42%	N/A	191,691	-	-	-	-	Pass
561nm	SSC-H	400	1.99%	N/A	520,428	-	-	180	183	Pass
488nm	B530-H	464	3.80%	0.9999	1,272,305	1,310,000	2.88%	81	118	Pass
488nm	B586-H	520	2.38%	-	3,354,282	3,400,000	1.34%	70	521	Pass
488nm	B615-H	555	2.37%	-	1,902,220	1,910,000	0.41%	71	96	Pass
488nm	B660-H	489	2.25%	-	869,716	875,000	0.60%	68	90	Pass
488nm	B695-H	676	2.37%	-	392,604	395,000	0.61%	69	100	Pass
488nm	B725-H	469	2.46%	-	585,968	590,000	0.68%	69	212	Pass
488nm	B780-H	176	3.08%	-	83,330	82,500	1.01%	74	83	Pass
637nm	R660-H	535	2.87%	0.9991	759,111	765,000	0.77%	71	108	Pass
637nm	R695-H	699	2.64%	-	577,618	580,000	0.41%	69	94	Pass
637nm	R725-H	461	2.61%	-	992,301	1,000,000	0.77%	78	142	Pass
637nm	R780-H	198	3.39%	-	188,854	185,000	2.08%	68	149	Pass
405nm	V445-H	509	4.89%	1.0000	5,667,486	5,550,000	2.12%	72	439	Pass
405nm	V530-H	563	3.94%	-	4,218,506	4,150,000	1.65%	71	112	Pass
405nm	V586-H	567	3.88%	-	1,815,414	1,750,000	3.74%	64	87	Pass
405nm	V615-H	573	4.00%	-	941,057	900,000	4.56%	72	80	Pass
405nm	V660-H	574	3.96%	-	583,807	560,000	4.25%	75	88	Pass
405nm	V695-H	727	3.97%	-	312,138	300,000	4.05%	77	107	Pass
405nm	V725-H	364	3.96%	-	203,240	195,000	4.23%	76	133	Pass
405nm	V780-H	267	4.53%	-	53,692	50,000	7.38%	75	83	Pass
561nm	Y586-H	619	1.67%	0.9998	2,019,922	2,025,000	0.25%	72	107	Pass
561nm	Y615-H	601	1.16%	1.0000	3,495,527	3,475,000	0.59%	71	93	Pass
561nm	Y660-H	478	1.73%	-	1,387,690	1,375,000	0.92%	74	99	Pass
561nm	Y695-H	621	1.25%	-	746,132	745,000	0.15%	73	249	Pass
561nm	Y725-H	510	1.36%	-	733,519	735,000	0.20%	71	126	Pass
561nm	Y780-H	387	2.14%	-	136,789	135,000	1.32%	71	79	Pass

QC Particles Count: 10775
 Result: Pass

Levey-Jennings Report

Displays the QC test data over time to observe trends in instrument performance. As shown:



Export CSV File Export the QC result as a csv file.

Open Data File Open the QC data file of the selected report.

Print Click the **Print** button in the top right corner of the window to print QC test reports or Levey-Jennings reports.

QC Test

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10 Troubleshoot

This chapter provides the information for troubleshooting Agilent flow cytometer instrument and associated accessories, including prompted message ID, software messages, possible causes, recommended solutions, and procedures to create technical support files.

Troubleshoot Instrument

The following table lists possible causes for warning and error prompts from the status bar when connecting with Agilent flow cytometer instrument.

Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0001	Collision of SIP	The movement of the SIP is blocked by some obstacles.	Locate and clear the obstacles. The instrument will automatically start error handling and move the SIP to the home position.
		Incorrect plate selected in plate manager.	Select the correct plate in the plate manager window.
		Incorrect positioning of plate in NovoSampler, NovoSampler Pro or NovoSampler Q.	Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps.
		NovoSampler (or NovoSampler Pro or NovoSampler Q) is not calibrated.	Re-calibrate NovoSampler (or NovoSampler Pro or NovoSampler Q).
		Dirty SIP or SIP Cleaning apparatus.	Clean the SIP or SIP cleaning apparatus following the procedures described in Preventative Maintenance in associated instrument operator's guide.
0x0002	Running out of NovoFlow	NovoFlow solution is not sufficient to continue to run any samples.	Refill the NovoFlow container following the procedures described in Add Instrument Reagents in

Troubleshoot

			associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0003	Running out of NovoRinse	NovoRinse solution is not sufficient to continue to run any samples.	Refill the NovoRinse container following the procedures described in Add Instrument Reagents in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0004	Running out of NovoClean	NovoClean solution is not sufficient to continue to run any samples.	Refill the NovoClean container following the procedures described in Add Instrument Reagents in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0005	Waste container is full	Waste container is too full to continue to run any samples.	Empty the waste container following the procedures described in Empty Waste in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0006	System voltage is out of range	System Error.	<ul style="list-style-type: none"> • Restart the instrument. • Contact Agilent technical support if the error persists.
0x0007	System electric current is out of range	System Error.	<ul style="list-style-type: none"> • Restart the instrument. • Contact Agilent technical support if the error persists.

Troubleshoot

0x0008	Firmware configuration error	System Error.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x0009 0x000A 0x000B 0x4009 0x400D laser self-test error	Specified laser is not functioning properly.	The instrument will automatically reset the laser and run a laser self-test. It takes approximately 5 to 10 minutes.
0x000C 0x000D 0x000E 0x400A 0x400E laser is not connected	Specified laser is not detected.	<ul style="list-style-type: none"> Restart the instrument Contact Agilent technical support if the error persists.
0x000F	NovoSampler communication lost	The cable between the NovoSampler(or NovoSampler Pro or NovoSampler Q) and instrument is not securely connected.	Reconnect the cable between the NovoSampler (or NovoSampler Pro or NovoSampler Q) and instrument.
		NovoSampler (or NovoSampler Pro or NovoSampler Q) is not communicating with instrument.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x0010	NovoSampler has not been calibrated	NovoSampler (or NovoSampler Pro or NovoSampler Q) is newly installed or re-connected.	Follow the prompted instructions to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q).
0x0011	NovoSampler calibration failed.....	NovoSampler (or NovoSampler Pro or NovoSampler Q) is not installed properly.	Re-install and calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q) following the procedures described in Installation in associated operator's guide . Ensure the NovoSampler is seated flat on the stage and tightly close to the instrument.
		The NovoSampler (or NovoSampler Pro or NovoSampler Q) cover is opened during calibration.	Close the NovoSampler (or NovoSampler Pro or NovoSampler Q) cover and redo the calibration.
		The optocoupler or motor of NovoSampler Q is not working properly.	<ul style="list-style-type: none"> Restart the instrument and redo the calibration. Contact Agilent technical support if the error persists.
0x0012		The movement of the orbital shaker is blocked.	<ul style="list-style-type: none"> Check the path of the orbital shaker to make sure there are no objects blocking the

Troubleshoot

	The movement of plate is out of range		<p>movement. Clear the block if there is anything.</p> <ul style="list-style-type: none"> Re-calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q).
		NovoSampler (or NovoSampler Pro or NovoSampler Q) is not installed properly.	Re-install and calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q) following the procedures described in Installation in associated operator's guide.
0x0013	Cover of NovoSampler is opened during moving plate	The cover of NovoSampler (or NovoSampler Pro or NovoSampler Q) is open during moving plate. Or the cover is not closed well.	Close the cover. NovoSampler (or NovoSampler Pro or NovoSampler Q) will automatically reset and be ready for operation.
0x0014	Pressure is out of limit	Waste container is not correctly connected to the instrument. Or waste tubings are bending and clogged.	Follow the prompted instructions from NovoExpress to clear the error. If NovoCyte Flow Cytometer is connected, check the quick coupling connectors to ensure the waste container is correctly connected to the instrument. Ensure the waste tubings are not bending and clogged.
		Sheath in-line filter is clogged.	Follow the prompted instructions from NovoExpress to clear the error. If NovoCyte Flow Cytometer is connected, replace the sheath in-line filter following the procedures described in Replacing Fluidic System Consumables in associated instrument operator's guide.
		Sample injection probe or flow cell is clogged.	Follow the prompted instructions from NovoExpress to clear the error.
0x0015	NovoSampler firmware error	NovoSampler (or NovoSampler Pro or NovoSampler Q) firmware is not working properly.	Re-install or upgrade the NovoSampler (or NovoSampler Pro or NovoSampler Q) firmware.
0x0016 0x0017 0x0018 0x400B 0X400F laser does not emit	Specified laser is not connected properly or laser is not working properly.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x0019 0x001A 0x001B laser communication error	Specified laser is not communicating with the	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.

Troubleshoot

0x400C 0x4010		instrument properly or the laser is not working properly.	
0x001C	Sample injection probe reset failed	Bad connection or optocoupler is not working properly.	<ul style="list-style-type: none"> Click the OK button in the prompted dialog box or wait 10 seconds for automatically error handling. Restart the instrument. Contact Agilent technical support if the error persists.
0x001D	Sampling Pump reset failed	Bad connection or optocoupler is not working properly.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x001E	The SIP module needs to be upgraded to support NovoSampler Pro	SIP module is not compatible with connected NovoSampler Pro.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x0020	System initialization is paused	Liquid level in the reagent containers is not within normal range when instrument is powered up.	<ul style="list-style-type: none"> Make sure that the instrument reagent containers are placed correctly and the liquid level in the containers is within the normal range. Click OK on the prompted dialog to continue system initialization.
0x0021	Sheath fluid pump reset failed	Bad connection or optocoupler is not working properly.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x0022	Resetting NovoSampler Pro to zero position failed	NovoSampler Pro is not working properly.	<ul style="list-style-type: none"> Click Instrument → NovoSampler Pro → Reset in NovoExpress software to reset NovoSampler Pro. Restart the NovoSampler Pro.
0x0023	Resetting NovoSampler to home position failed	NovoSampler (or NovoSampler Pro or NovoSampler Q) is not working properly.	<ul style="list-style-type: none"> Click Instrument → NovoSampler (or NovoSampler Pro or NovoSampler Q) → Reset in NovoExpress software to reset NovoSampler (or NovoSampler Pro or NovoSampler Q). Restart the NovoSampler (or NovoSampler Pro or NovoSampler Q).
0x0100	Instrument cover opened	Instrument cover is opened or not tightly closed.	Close the instrument cover.

Troubleshoot

0x0101	NovoFlow running low	NovoFlow solution is below the pre-set volume limit.	Refill the NovoFlow container following the procedures described in Add Instrument Reagents in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0102	NovoRinse running low	NovoRinse solution is below the pre-set volume limit.	Refill the NovoRinse container following the procedures described in Add Instrument Reagents in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0103	NovoClean running low	NovoClean solution is below the pre-set volume limit.	Refill the NovoClean container following the procedures described in Add Instrument Reagents in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0104	Waste container is close to full	Waste is above the pre-set volume limit.	Empty the waste container following the procedures described in Empty Waste in associated instrument operator's guide.
		If the liquid level is within the normal range, maybe the sensor is not working properly. Or the sensor needs to recalibrate.	<ul style="list-style-type: none"> • Calibrate Fluidics Station (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0105	Cover of NovoSampler is opened	Cover of NovoSampler (or NovoSampler Pro or	Close the cover.

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		NovoSampler Q) is opened. Or the cover is not closed well.	
0x0106	NovoSampler is disconnected when powered up	The NovoSampler (or NovoSampler Pro or NovoSampler Q) is disconnected when powered up	<ul style="list-style-type: none"> • Shut down instrument. • Reconnect the cable of the NovoSampler (or NovoSampler Pro or NovoSampler Q) to instrument. • Turn on instrument and follow the prompts to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q). • Contact Agilent technical support if the error persists.
0x0109	Fluidics Station is not connected	Cable between the fluidics station and the instrument is not properly connected.	<ul style="list-style-type: none"> • Power down the instrument. • Reconnect the cable between the fluidics station and the instrument. • Power up the instrument. • Contact Agilent technical support if the error persists.
0x010A 0x010B 0x010C 0x010D	... liquid level sensor failure	Specified liquid level sensor in the fluidics station (or Cart) is not working properly.	<ul style="list-style-type: none"> • Reconnect the fluidics station (or Cart) cable. • Restart the instrument. • Contact Agilent technical support if the error persists.
0x010F	Sheath filter is clogged. Please replace the sheath filter and run the Priming procedure	Sheath in-line filter is clogged.	Replace the sheath in-line filter following the procedures described in Replacing Fluidics System Consumables in associated instrument operator's guide.
0x0110	Recovering collision error	Plate stops at an incorrect position.	No action is needed. Instrument will automatically recover the error.
0x0111	Orbital shaker homing position reset failure	Orbital shaker of NovoSampler (or NovoSampler Pro or NovoSampler Q) is not working properly.	No action is needed. The NovoSampler (or NovoSampler Pro or NovoSampler Q) can be used normally.
0x0112	0.05 µm sheath fluid ultrafiltration (UF) filter is clogged	0.05 µm sheath fluid ultrafiltration (UF) filter is clogged.	Replace the 0.05 µm sheath fluid ultrafiltration (UF) filter following the procedures described in Replacing Fluidics System Consumables in associated instrument operator's guide.
0x1001	Fluidics procedure run error	Fluidics procedure file is damaged.	<ul style="list-style-type: none"> • Restart the instrument. • Contact Agilent technical support if the error persists.

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0x300A	Calibrating DA coefficient failed	AD board is not working properly.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x3100	Failed to read optical filter information	Optical filter sensor is not working properly.	<ul style="list-style-type: none"> Restart NovoExpress software. Reinstall the optical filter. Replace an optical filter. Restart the instrument. Contact Agilent technical support if the error persists.
0x3101	Failed to read dichroic mirror information	Dichroic mirror sensor is not working properly.	<ul style="list-style-type: none"> Restart NovoExpress software. Reinstall the dichroic mirror. Replace a dichroic mirror. Restart the instrument. Contact Agilent technical support if the error persists.
0x3102	Failed to read photodetector information	Photodetector sensor is not working properly.	<ul style="list-style-type: none"> Restart the instrument. Contact Agilent technical support if the error persists.
0x3103	The optical filter information has been changed	The optical filter has been replaced.	Follow the software prompted instructions to perform the appropriate operation.
0x3104	The dichroic mirror information has been changed	The dichroic mirror has been replaced.	Follow the software prompted instructions to perform the appropriate operation.
0x3105	The photodetector information has been changed	The photodetector has been replaced.	Follow the software prompted instructions to perform the appropriate operation.
0x6100	Communication error between NovoSampler and the orbital shaker	The orbital shaker is not communicating to NovoSampler Pro (or NovoSampler Q) is not working properly.	<ul style="list-style-type: none"> Restart NovoSampler Pro (or NovoSampler Q) through NovoExpress. Restart the instrument. Contact Agilent technical support if the error persists.
0x7000	Fluidics cart firmware error	NovoCyte Fluidics Cart firmware is not working properly.	<ul style="list-style-type: none"> Restart the instrument. Replace the NovoCyte Fluidics Cart. Contact Agilent technical support if the error persists.
0x7001	NovoFlow liquid level sensor is not connected	NovoFlow liquid level sensor is not connected or does not work properly.	<ul style="list-style-type: none"> Reconnect the NovoFlow liquid level sensor. Contact Agilent technical support if the error remains after reconnection. The sensor

Troubleshoot

			may be damaged and need to be replaced.
0x7002	Waste liquid level sensor is not connected	Waste liquid level sensor is not connected or does not work properly.	<ul style="list-style-type: none"> • Reconnect the waste liquid level sensor. • Contact Agilent technical support if the error remains after reconnection. The sensor may be damaged and need to be replaced.
0x7100	NovoCyte Fluidics Cart communication lost	NovoCyte Fluidics Cart is not properly connected to the instrument.	<ul style="list-style-type: none"> • Ensure the DVI cable between the fluidics cart and the instrument is properly connected. • Restart the instrument. • Contact Agilent technical support if the error persists.
0xA003	The temperature of the photodetectors is abnormal	Ambient temperature outside the operating range.	<ul style="list-style-type: none"> • Ensure to have the instrument working at normal ambient temperatures. • Restart the instrument. • Contact Agilent technical support if the error persists.
		More than four temperature sensors of the photodetector module are not working properly.	<ul style="list-style-type: none"> • Restart the instrument. • Contact Agilent technical support if the error persists.
0xA020	Laser board firmware error	Laser board firmware is not working properly.	<ul style="list-style-type: none"> • Re-install or upgrade the laser board firmware.
0xA021	Laser board communication lost	Laser board is not properly connected to the instrument.	<ul style="list-style-type: none"> • Restart the instrument. • Contact Agilent technical support if the error persists.
0xA100	The temperature of the photodetectors is abnormal	One to three temperature sensors of the photodetector module may not work properly.	No action is needed. The instrument can use the temperature from other sensors and ensure the data is processed normally.
	Communication error (code: xx, xx). Please restart Instrument and NovoExpress.	USB cable between the instrument and workstation is not connected properly.	<ul style="list-style-type: none"> • Reconnect the USB cable between the instrument and the workstation. • Restart the instrument, workstation and NovoExpress software. • Contact Agilent technical support if the error persists.

Technical Support Request

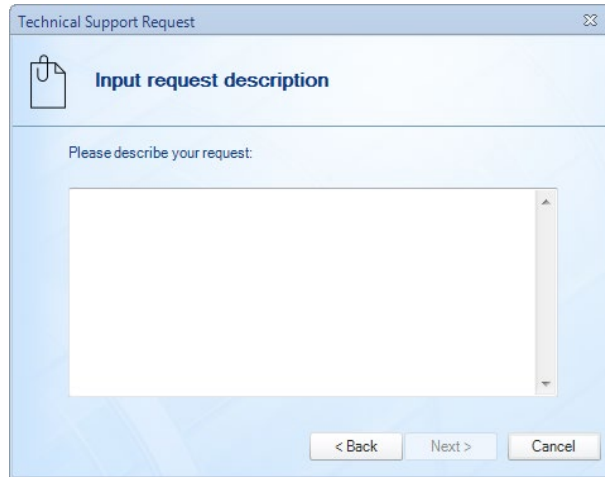
In case you need to contact Agilent technical supports, use the **Technical Support Request** from **Home** menu to create a request. **Technical Support Request Creation Wizard** automatically collects instrument configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of Agilent flow cytometer. You can also attach any other files using this function.

- 1 Click **Home** > **Technical Support Request** to open the **Technical Support Request Creation Wizard**.



- 2 Click **Next** button and input request description.

Troubleshoot



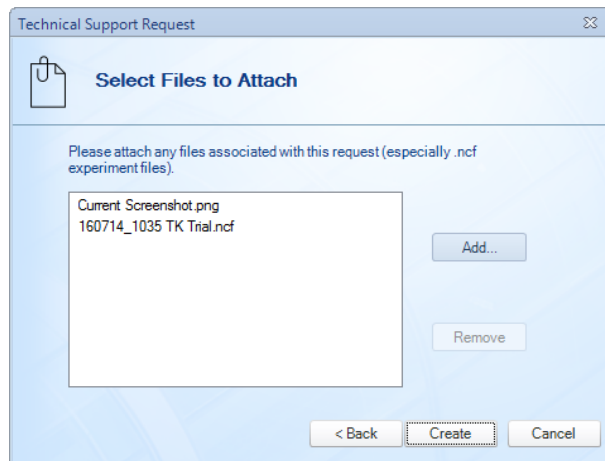
Technical Support Request

Input request description

Please describe your request:

< Back Next > Cancel

- 3 Click **Next** button and select the files to be attached. Any related files including experiment files (*.ncf) can be attached.



Technical Support Request

Select Files to Attach

Please attach any files associated with this request (especially .ncf experiment files).

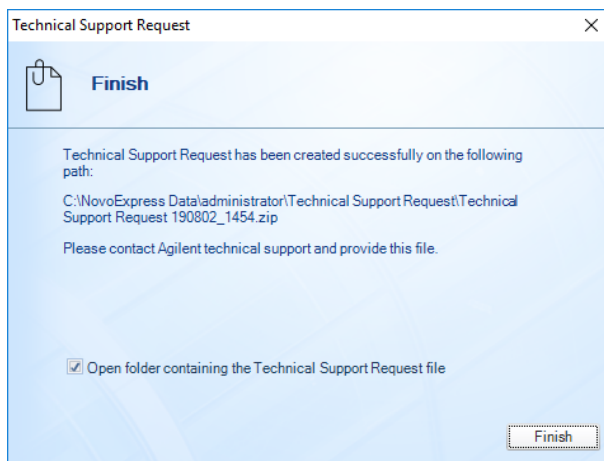
Current Screenshot.png
160714_1035 TK Trial.ncf

Add...
Remove

< Back **Create** Cancel

- 4 Click **Create** button to start creating technical support request.
- 5 Click **Finish**.

Troubleshoot



After the request creating process is completed, send the created request files to Agilent technical support.

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Version History

Date/Version	Changed by	Description
10/30/2020		Initial release.

Version History

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In This Guide

The manual describes the following:

- Prologue
- Installation
- Use NovoExpress Software
- Sample Acquisition
- Data Analysis
- Plate Manager
- Experiment Manager
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- QC Test
- Troubleshoot
- Version History

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