NovoExpress (Opteon)

Software Guide

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

Notices

Manual Part Number

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Software Revision

This guide is valid for the 2.1.0 revision of the Agilent NovoExpress (Opteon) program.

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Safety Notices

⚠WARNING

A WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury.

⚠CAUTION

A CAUTION is used with the safety alert symbol, indicating a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE

A NOTICE indicates a situation which, if not avoided, will result in damage to or destruction of the instrument or data.

In This Guide

This guide covers the following module: NovoExpress (Opteon) Software

1 Prologue

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

2 Installation

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

3 Use NovoExpress (Opteon) Software

This chapter provides information needed to use NovoExpress (Opteon) software, including overview of NovoExpress (Opteon) software interface, **Title Block, Menu, Workspace Toolbar**, **Cytometer Setting** panel, **Fluorochrome Setting** panel, **Cytometer Control** panel, **Plate Manager** panel, **Experiment Manager** panel, **Cytometer Status** panel, **Gate Manager** panel, **Status Bar**, **Instrument Configuration** window, **Manage Plate Types** window, **New Reference Control Specimen** window, **Reference Spectra** window, **Option Setting** window, **Fluorochrome Library** window.

4 Sample Acquisition

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

5 Data Analysis

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

6 Plate Manager

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

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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**.

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 Instrument and associated accessories, including prompted message ID, software messages, possible causes, recommended solutions, and procedures to create technical support files.

11 Version History

Contents

1 Prologue

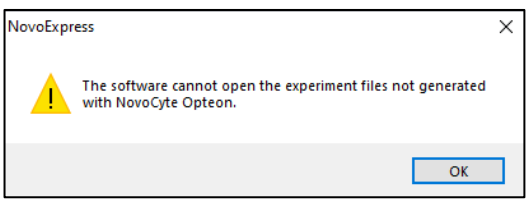
This chapter provides basic information for NovoExpress (Opteon) and the software guide, including overview of NovoExpress (Opteon), scope of this guide, keyboard shortcuts and glossary.

About NovoExpress (Opteon)

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

NOTE

NovoExpress (Opteon) software is compatible with NovoCyte Opteon Spectral Flow Cytometer and associated accessories only. When opening any experiment files (*.ncf) generated by NovoExpress software (e.g., NovoExpress v1.6.2 and below) in NovoExpress (Opteon) software, following error message will be prompted. Please contact Agilent technical support for any further questions.



Using this Guide

This guide contains an overview and information on using NovoExpress (Opteon) software. For information on the operation and maintenance of NovoCyte Opteon Spectral 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
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 + 7	Create a Spectrum Density plot
Ctrl + 8	Create a Spectrum Dot plot
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 + 7	Change to Spectrum Density plot
Alt + 8	Change to Spectrum Dot plot
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 gate or Vertical Range gate
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. Instrument 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 (Opteon) 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.
Analysis	The process of plotting, gating, and comparing statistical information on collected parameters of samples.
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
Fluorochrome Bi-Variate Plot	A matrix of plots with selected unmixed fluorescent 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 used to compensate the fluorescence spillover between different fluorochromes. It is inversely related to the Spillover Matrix.
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.
Distance Filter	When the timing distance of two events of a sample is less than or equal to the set value, these two events will be filtered out, or aborted. The software will not display the aborted events.
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
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 (Opteon) Software saved experimental data files. A file can store experimental data from multiple samples.
Experiment Manager	The NovoExpress (Opteon) 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 (Opteon) 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 fluorochromes 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 (Opteon) 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.
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
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 fluorescent or scattered light intensity. In the NovoExpress (Opteon) Software, plots include dot plots, density plots, contour plots, histograms, cell cycle plot, cell proliferation plot, spectrum density plot and fluorochrome bi-variate plot
Polygon Gate	Draws a polygon gate.
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 Instrument 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, Instrument 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.
Quick compensation	Use scrollbars in two-parameter plots to quickly adjust the Spillover Matrix after unmixing or fluorescence compensation of a sample.
Quadrant Gate	Draws a 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
Reference Control	A series of single-stained and/or unstained samples which provide the reference fluorescence spectra (spectral signatures) necessary to unmix the data by spectral flow cytometer.
Reference Spectra	Spectral signatures of a fluorochrome or an unstained sample from all fluorescence detection channels of all lasers. It is essential for conducting spectral unmixing and compensation (when Virtual Filter is used).
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.
Raw Workspace	The workspace which allows users to view and create plots of raw fluorescence channel data, FSC, BSSC, VSSC, Width and Time, and is mainly used to analyze raw data.
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 (Opteon) 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.
Spectrum Density Plot	A density plot with the fluorescence channel(s) of selected lasers plotted in horizontal axis, and the Mean Fluorescence Intensity (MFI) value plotted in vertical axis.
Spectrum Dot Plot	A dot plot with the fluorescence channel(s) of selected lasers plotted in horizontal axis, and the Mean Fluorescence Intensity (MFI) value plotted in vertical axis.
Spillover Matrix	A matrix where each row contains value of spillover of a fluorochrome into 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.

Technical Support Request	A function which automatically collects instrument configurations, NovoExpress (Opteon) 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.
Unmix	The mathematical method used in spectral flow cytometer to deconvolute the abundance of individual fluorochrome in a multi-color sample using the spectral signature of each individual fluorochrome as the reference.
Unmixed Workspace	The workspace which allows users to view and create plots of unmixed fluorescence data, FSC, BSSC, VSSC, Width and Time. It is mainly used to analyze unmixed data.
Virtual Filter	An individual optical bandpass filter or a combination of multiple consecutive ones to allow using compensation to analyze spectral flow cytometer data in the same way as conventional flow cytometers.
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 (Opteon) Software, the middle area where plots are displayed.

2 Installation

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

Installation Requirements

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

Hardware

- Processor: quad-core 2.5 GHz
- Computer Memory: 16 GB
- Hard Drive: 50 GB free space
- Screen Resolution: 1366 X 768 pixels

Software

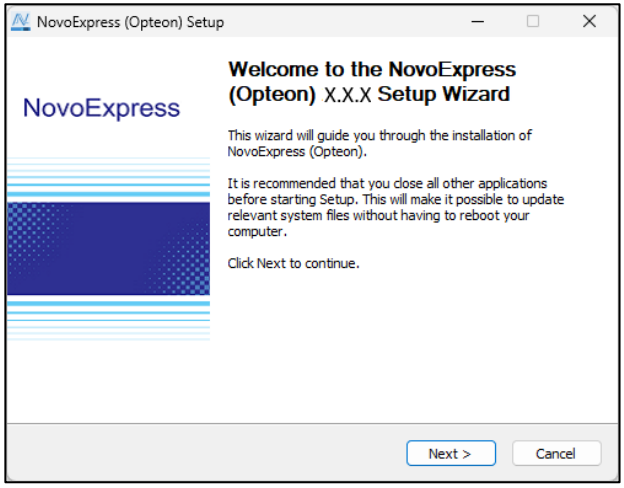
- Operating System: Windows 10 (x64)/Windows 11 (x64)
- PDF Reader Software

Install NovoExpress (Opteon) Software

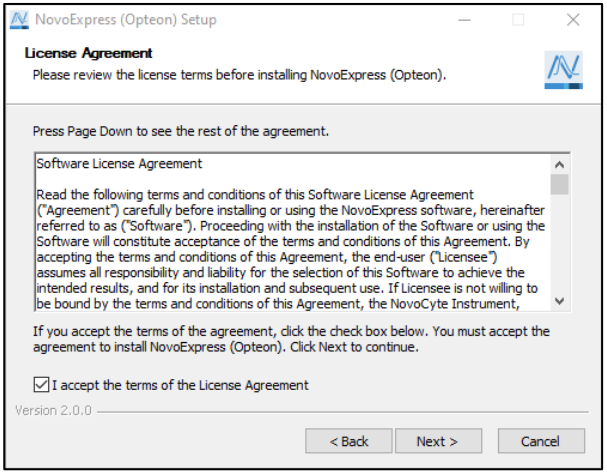
Following the instructions below to Install NovoExpress (Opteon) Software:

1 Download NovoExpress (Opteon) Software installation package from https://www.agilent.com/chem/NovoExpress and unzip it. Double-click **Setup.exe** file in the NovoExpress (Opteon) installation directory to start the installation process.

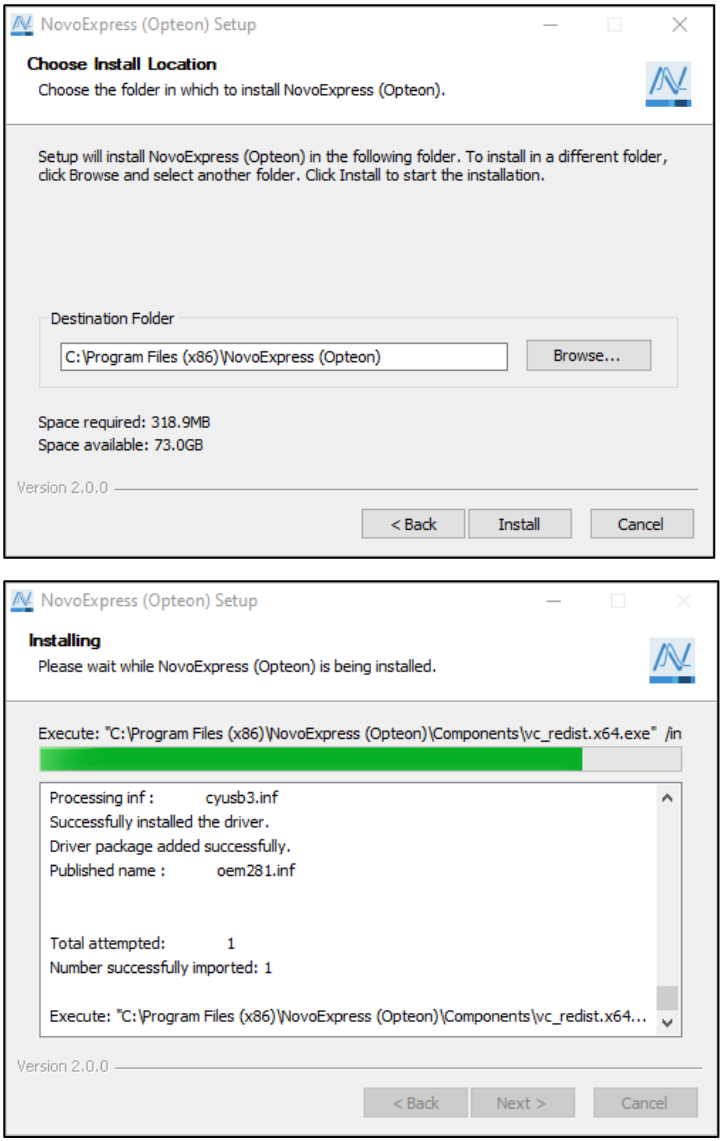
2 The NovoExpress (Opteon) Software installation wizard will be prompted as shown below. Click **Next** to continue.



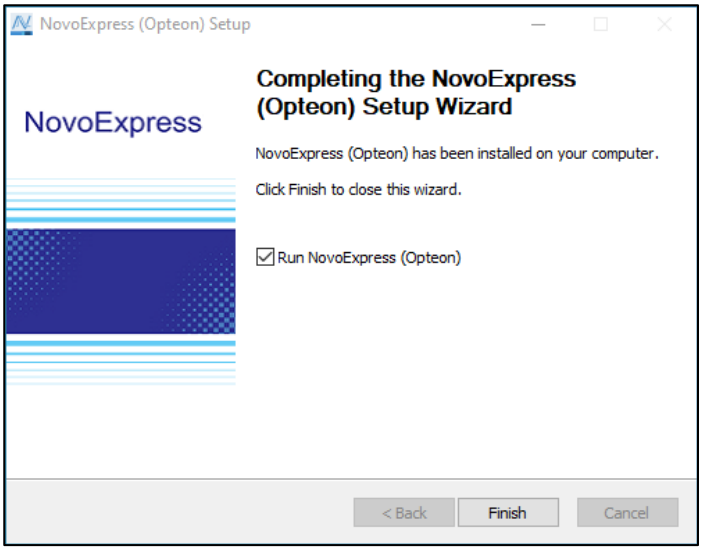
3 Read the license agreement and accept all terms by selecting the checkbox and clicking **Next**.



4 Choose the installation location. By default, the NovoExpress (Opteon) Software will be installed in **C:\Program Files (x86)\NovoExpress (Opteon)**. To install the software into 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.



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 (Opteon)** box, and click **Finish**.

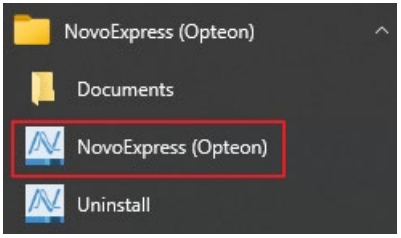


Start NovoExpress (Opteon) Software

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

- Double Click NovoExpress (Opteon) Shortcut from Desktop

- Click NovoExpress (Opteon) from Start Menu



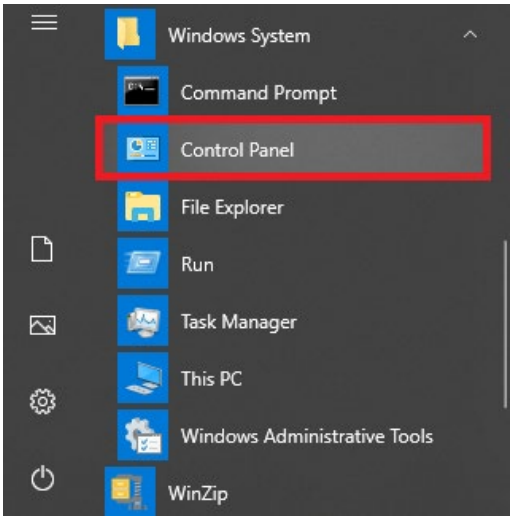
Uninstall NovoExpress (Opteon) Software

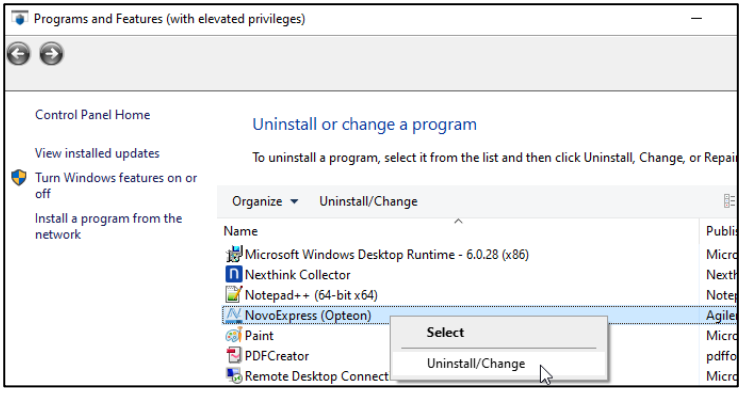
The NovoExpress (Opteon) Software can be uninstalled by the following methods:

- From **Control Panel**

  Click **Start** > **Windows System** > **Control Panel** > **Programs and Features**

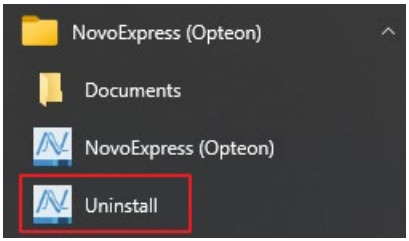
  In the prompted window, select **NovoExpress (Opteon)** and select **Uninstall/Change**.





- From **Start** Menu

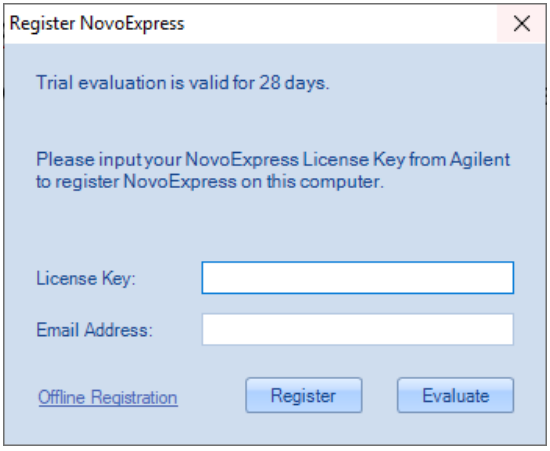
  Click **Uninstall** under **NovoExpress (Opteon)** folder



NovoExpress (Opteon) License

Free Trial

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

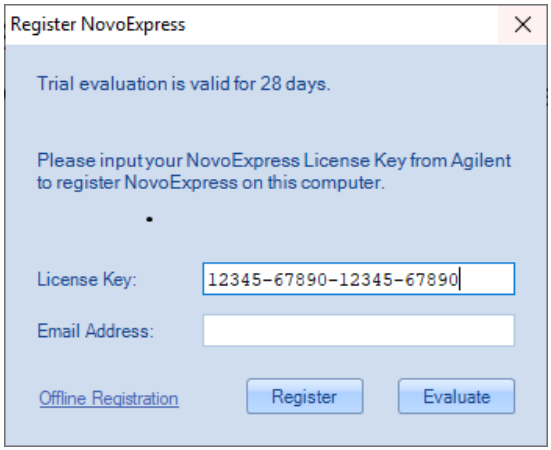


Registration will be needed to use the software after the trial expires. User can enter the NovoExpress (Opteon) Software License Key which comes with instrument. User can also purchase it separately from Agilent.

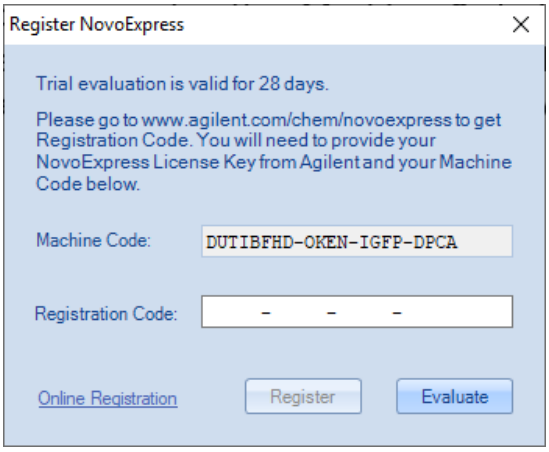
NovoExpress (Opteon) Registration

The **Register NovoExpress** window will automatically pop up when starting the NovoExpress (Opteon) software if the software is not registered yet. You can also click **File** > **About** > **Register NovoExpress** to open the **Register NovoExpress** window. There are two ways to register NovoExpress (Opteon).

- 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 (Opteon).



- 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 (Opteon).

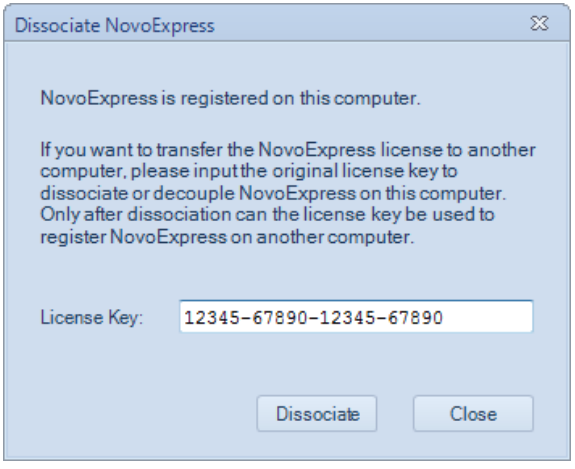


NOTE

A license key is required for NovoExpress (Opteon) 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 (Opteon) Dissociation

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



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

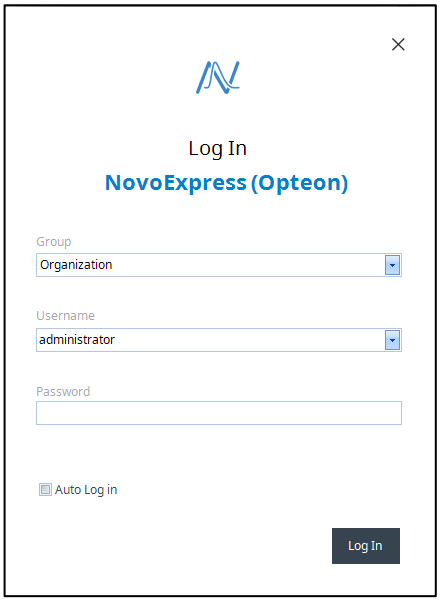
NOTE Only the users with the **Administrator** privilege can conduct this license dissociation process.

User Management

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

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

The NovoExpress (Opteon) 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.



User Groups

NovoExpress (Opteon) 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.

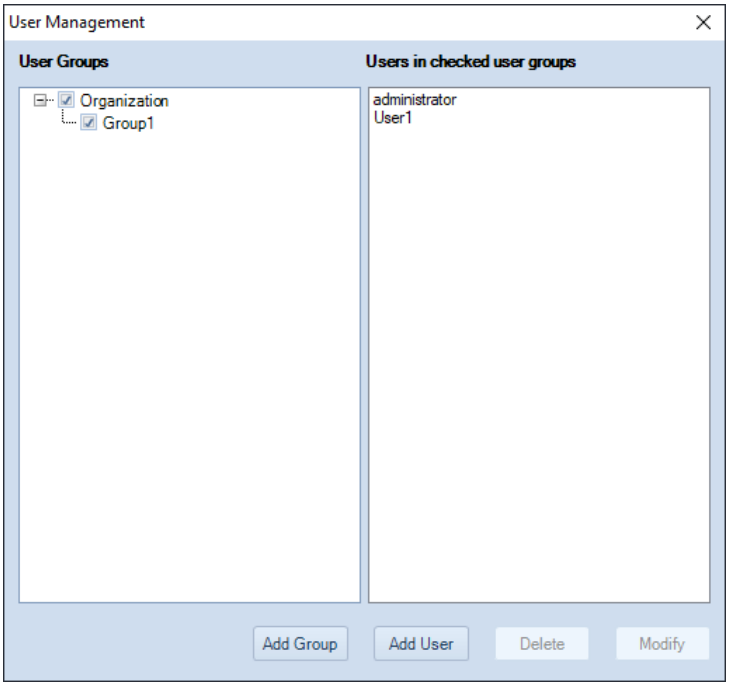
Add a User Group

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

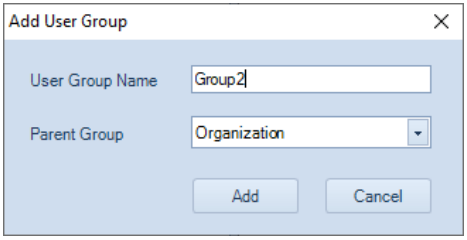
1 Log into the software using an account with **Administrator** privilege.

2 Select the **Setting** tab.

3 Click **User** > **Management**. The **User Management** window will appear.



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.

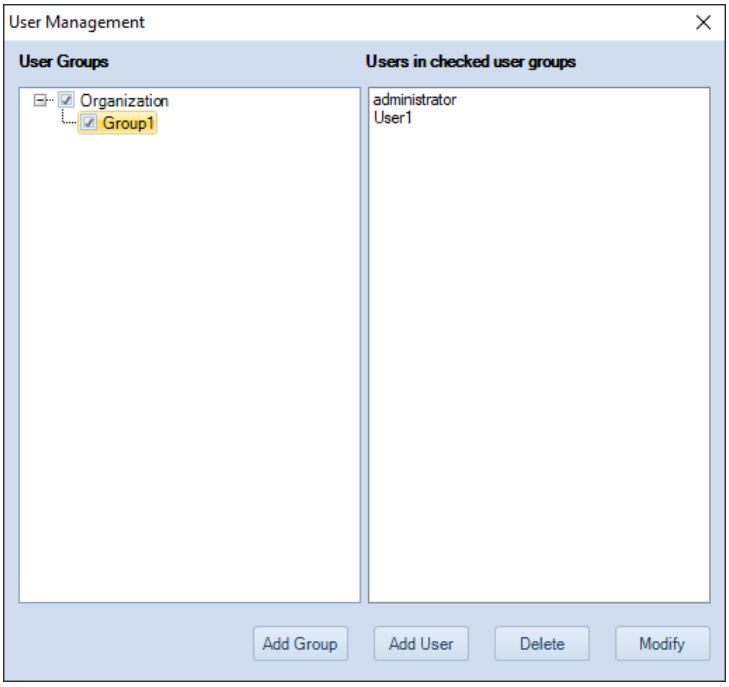
Modify a User Group

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

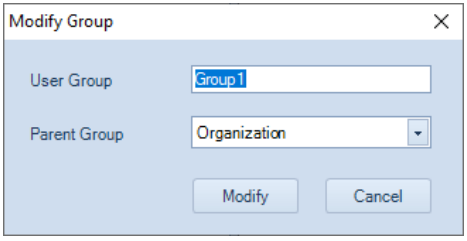
1 Log into the software using an account with **Administrator** privilege.

2 Select the **Setting** tab.

3 Click **User** > **Management**. The **User Management** window will appear.



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.

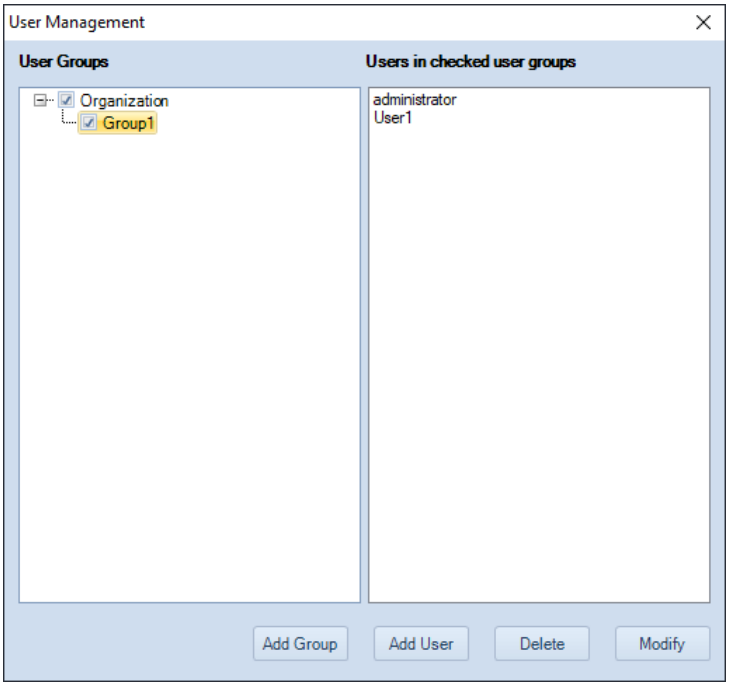
Delete a User Group

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

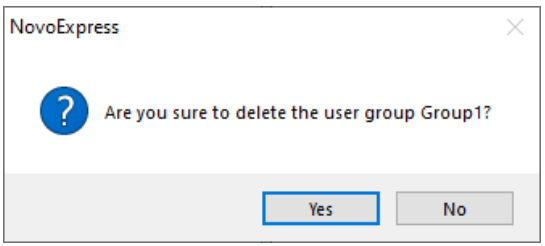
1 Log into the software using an account with **Administrator** privilege.

2 Select the **Setting** tab.

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.

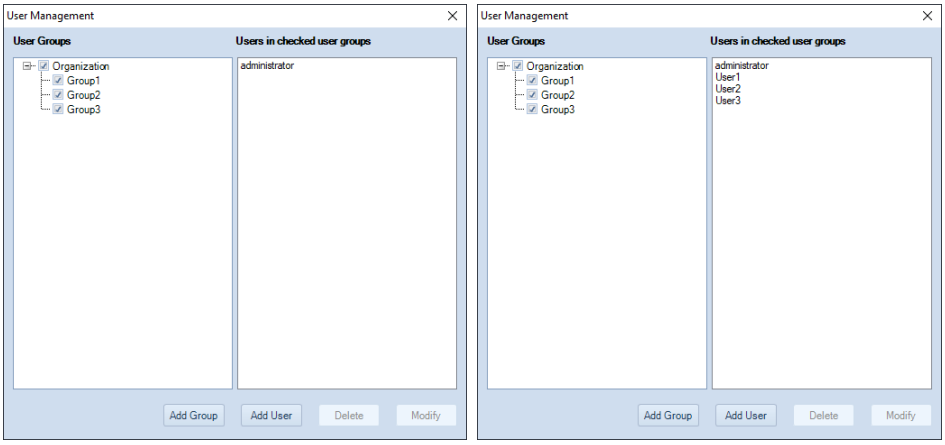


NOTE When the selected user group is deleted, the user accounts in the group will automatically be moved to the deleted group's parent group.

NOTE The root parent group **Organization** cannot be deleted.

Display the Users in a User Group

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



Users

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

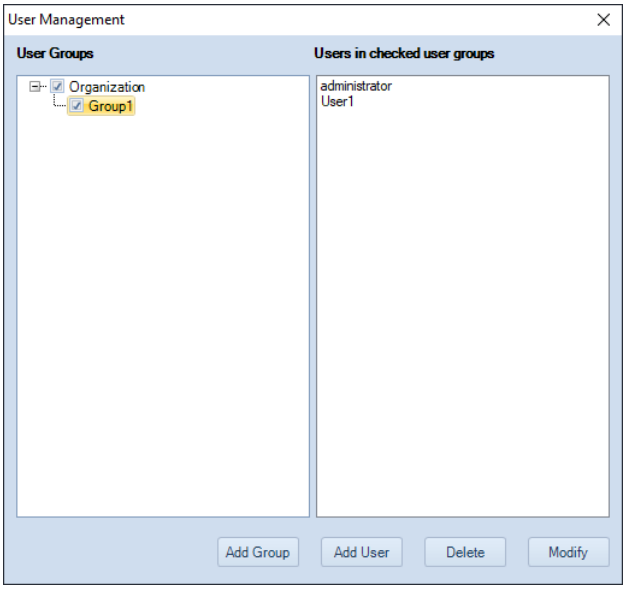
Add a New User

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

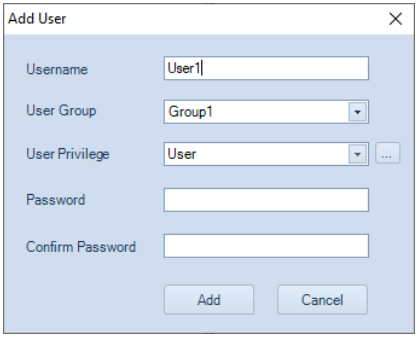
1 Log into the software using an account with **Administrator** privilege.

2 Select the **Setting** tab.

3 Click **User** > **Management**. The **User Management** window will appear.



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.

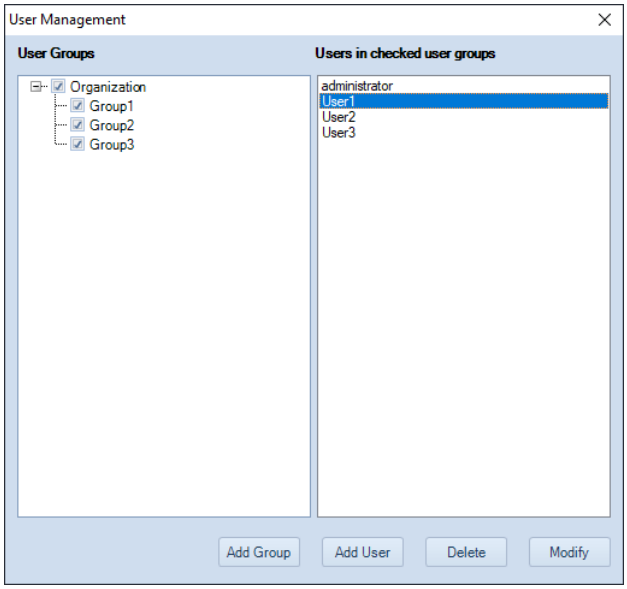
6 Click **Add** and the new user account is created.

Modify User Information

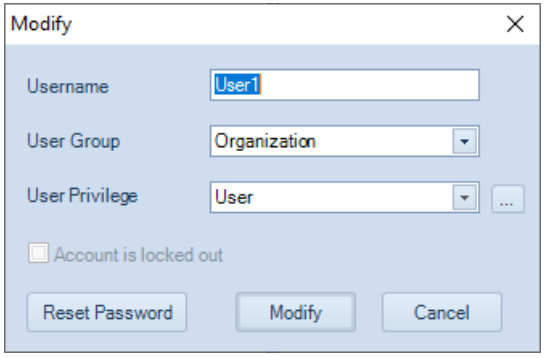
Accounts with **Administrator** privilege can modify username, user privilege, access privilege, and reset password for each user, while other accounts can only modify its own username, user group name, and password.

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

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



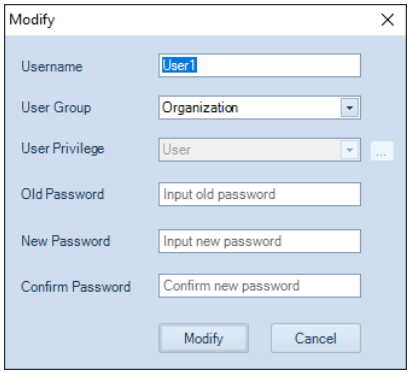
c Click **Modify**. The **Modify** window below will appear allowing the user to make changes to the account.



- From individual user accounts:

a After logging in, select the **Setting** tab.

b Click **User** > **Modify**. The **Modify** window shown below 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.

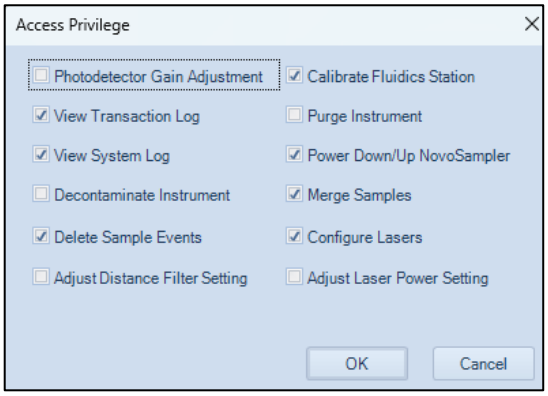


Access Privilege

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

- **Photodetector Gain Adjustment**
- **View Transaction Log**
- **View System Log**
- **Decontaminate Instrument**
- **Delete Sample Events**
- **Adjust Distance Filter Setting**
- **Calibrate Fluidics Station/Cart**
- **Purge Instrument**
- **Power Down/Up NovoSampler**
- **Merge Samples**
- **Configure Lasers**
- **Adjust Laser Power Setting**

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.



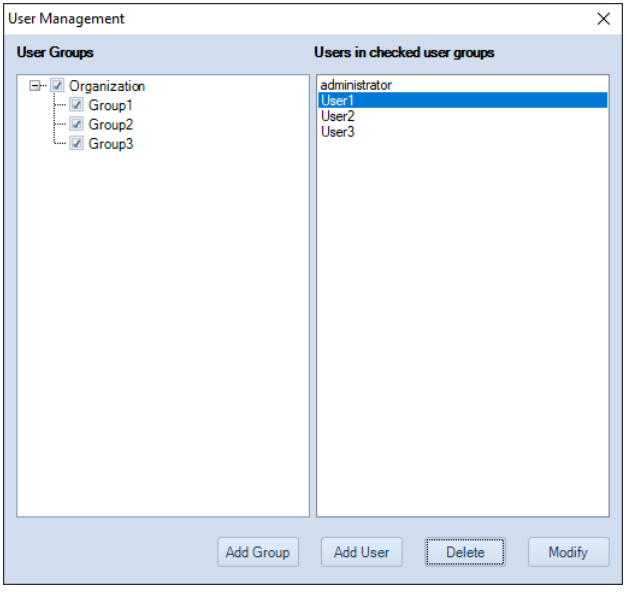
Delete a User

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**.

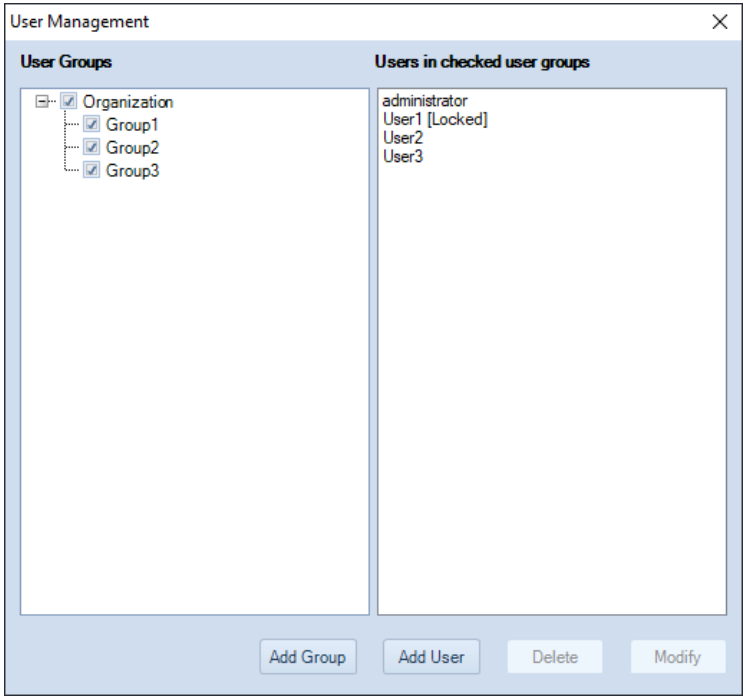
Unlock a User

If a user account is locked (refer to Settings in this guide for more details), it can only be unlocked through an account with **Administrator** privilege. To unlock a user account:

1 Login to the software as the administrator or an account with **Administrator** privilege.

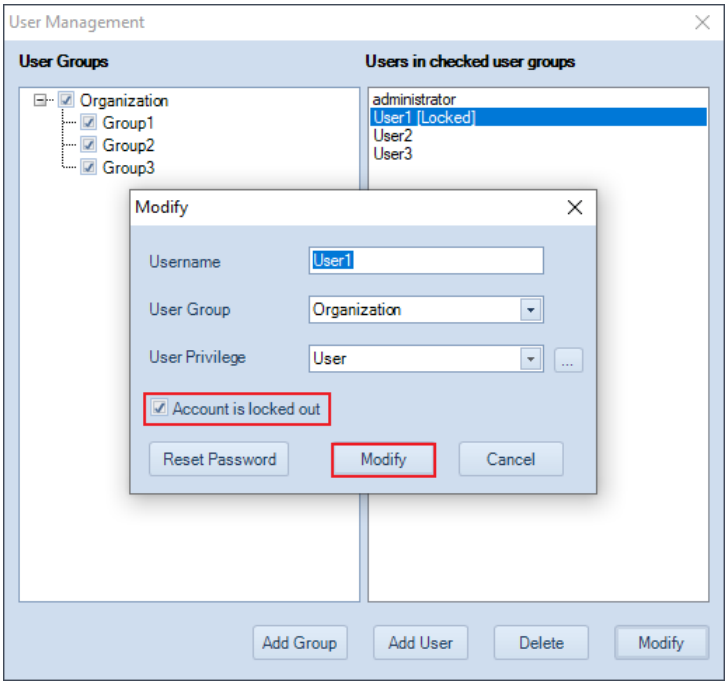
2 Select the **Setting** tab.

3 Click **User** > **Management**. The **User Management** window will appear. When a user account is locked, a suffix of **[Locked]** is displayed after the name of the user account as shown below.



4 Select the user account that you would like to unlock.

5 Click **Modify** from **User Management** window, uncheck **Account is locked out** and click **Modify** in the prompted window.



6 In the confirmation window, click **Yes**.

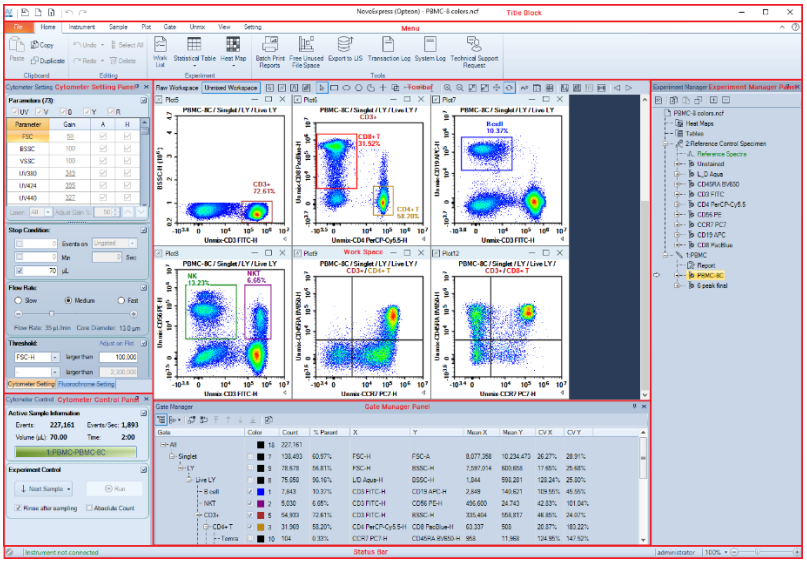
3 Use NovoExpress (Opteon) Software

This chapter provides information needed to use NovoExpress (Opteon) software, including overview of NovoExpress (Opteon) software interface, **Title Block, Menu, Workspace Toolbar**, **Cytometer Setting** panel, **Fluorochrome Setting** panel, **Cytometer Control** panel, **Plate Manager** panel, **Experiment Manager** panel, **Cytometer Status** panel, **Gate Manager** panel, **Status Bar**, **Instrument Configuration** window, **Manage Plate Types** window, **New Reference Control Specimen** window, **Reference Spectra** window, **Option Setting** window, **Fluorochrome Library** window.

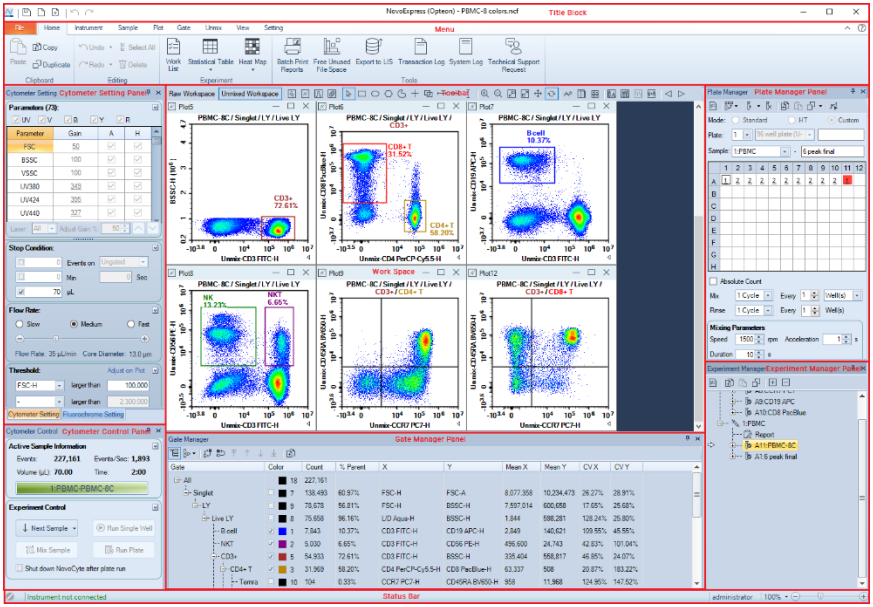
NovoExpress (Opteon) Software Interface

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

- If NovoSampler S 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**.

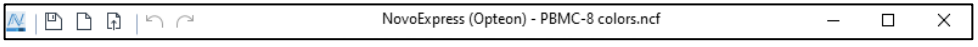


- If NovoSampler S is connected, user will see following interface. Compared to the software interface with instrument alone, differences are observed in the **Menu Bar**, **Cytometer Control** panel, **Experiment Manager** panel, and **Work List**. The **Plate Manager** panel is only visible when NovoSampler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



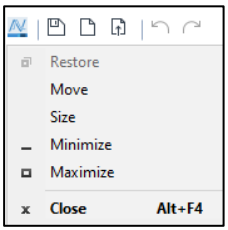
Title Block

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



Please refer to table below for description of each icon located in **Title Block**.

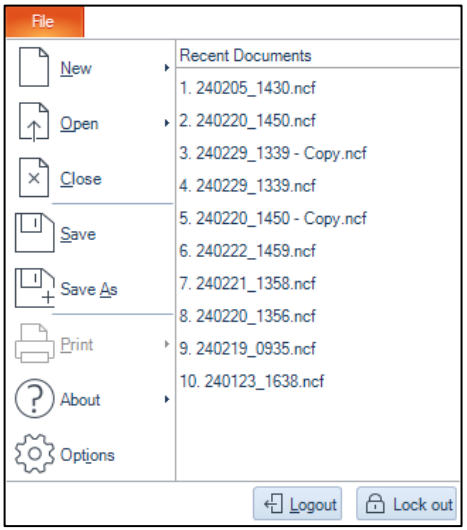
Icon	Description
	Clicking this icon prompts a drop-down menu with options to resize (e.g., Restore, Minimize) the display window and close the software.

	
	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.
	Redo drawing gates, delete gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot related functions.
NovoExpress (Opteon) - Untitled.ncf	Displays the data file name (*.ncf).
	Minimizes window.
	Maximizes window.
	Restores window.
	Closes window.

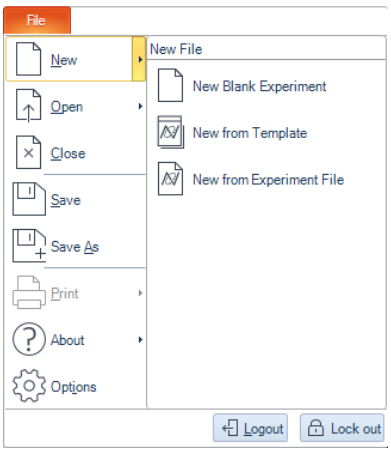
Menu

The **Menu** contains functions for instrument control and data analysis which includes **File, Home, Instrument, Sample, Plot, Gate, Unmix, View** and **Setting** tabs.

File

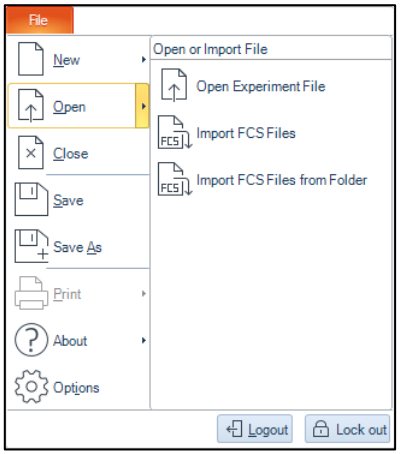


Please refer to table below for description of each icon located in **File** tab.

Icon	Description
	**New:** Click to create experiment file.  • **New Blank 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. The **Absolute Counting** and **Sample Recovery** setting (i.e., enabled or disabled) from the existing experiment file will be automatically applied to the newly created

sample. Both the **User-Defined Keywords**, and the keywords listed in **Edit Keywords** window in the existing experiment file will also be automatically included in the newly created sample. Please refer to Sample Keywords in this guide for more information.

Open: Click to open experiment (*.ncf) file.



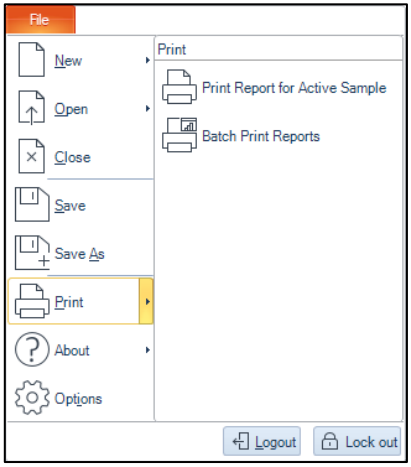
- **Open Experiment File**: Opens an experiment file.
- **Import FCS files**: Imports selected FCS file(s) to the current specimen.
- **Import FCS Files from Folder**: Imports all FCS files in a selected folder to the current FCS specimen.

Close: Click to close the current experiment file and creates a new experiment file.

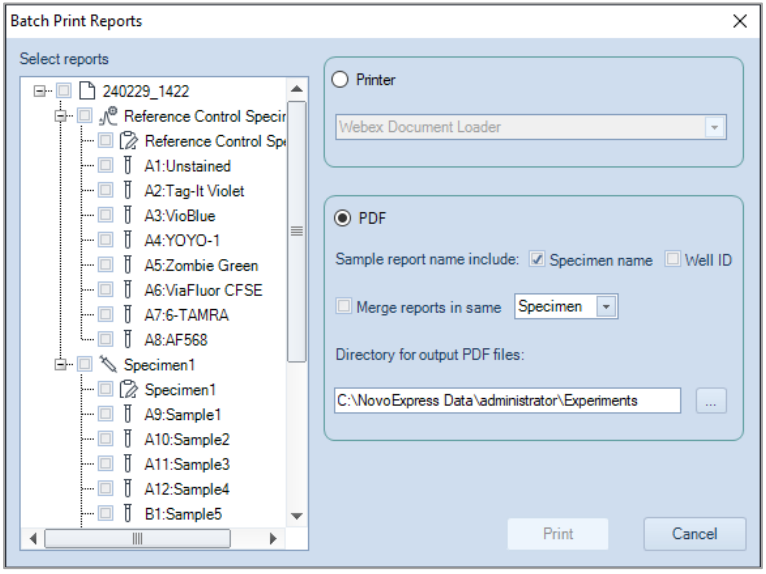
Save: Click to save the experiment data, experiment setup, and data analysis of the current experiment file.

Save As: Click to save the current experiment file to a specified location.

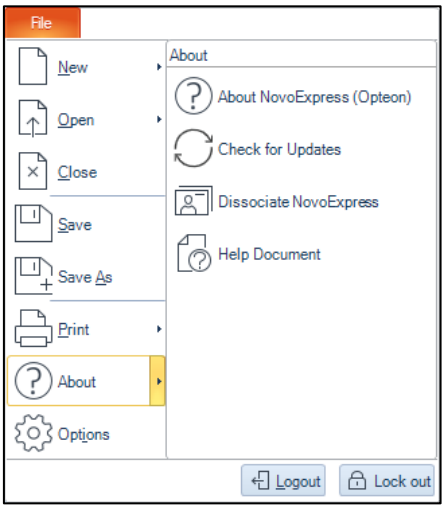
Print: Click to print sample report/batch print reports



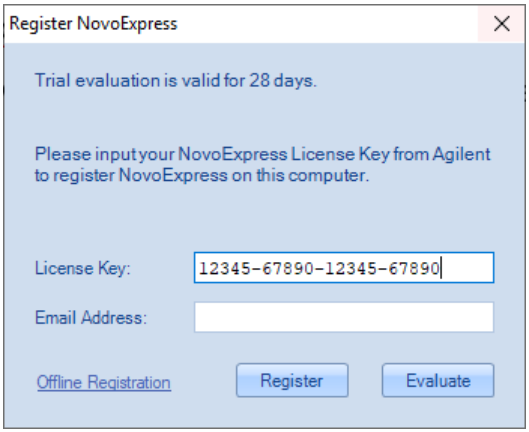
- **Print Report for Active Sample**: Prints the report for the current sample.
- **Batch Print Reports**: Opens the **Batch Print Reports** window. Refer to Batch Print Reports in this guide for more details.



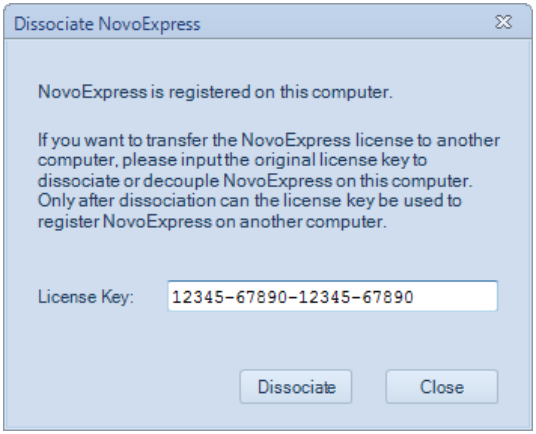
About: Click to view the software information (e.g., version, copyright), check for any available software updates, register or dissociate NovoExpress license, and view the help document (i.e., software guide) information.



- **About NovoExpress (Opteon)**: Displays the software version and copyright information.
- **Check for Updates**: When NovoCyte workstation is properly connected to internet, clicking this function will automatically check for software updates.
- **Register NovoExpress**: When clicked, following window will appear to allow user to register the NovoExpress (Opteon) software. This function will be available only when NovoExpress (Opteon) License is not registered. Refer to NovoExpress (Opteon) Registration in this guide for more details.

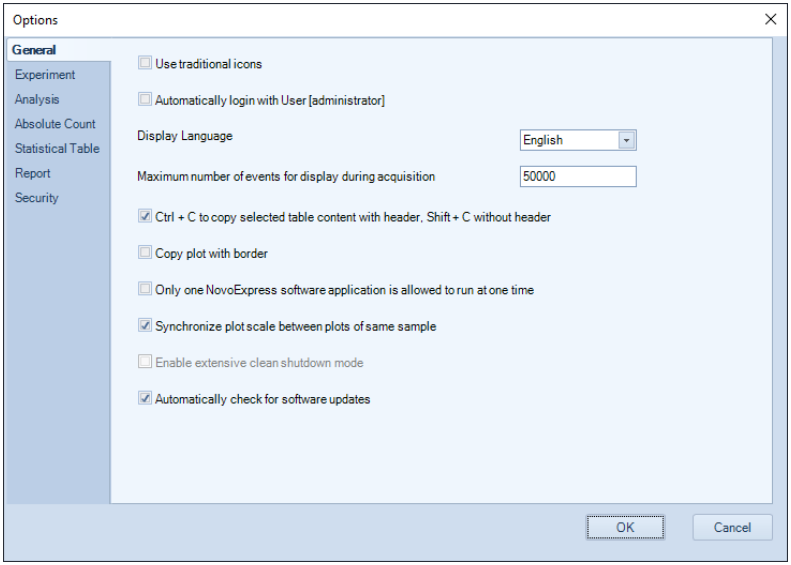


- **Dissociate NovoExpress**: This function will be available only when the software is currently registered. When clicked, following window will appear to allow user to dissociate or decouple NovoExpress (Opteon) from this computer. Refer to NovoExpress (Opteon) Dissociation in this guide for more details.



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

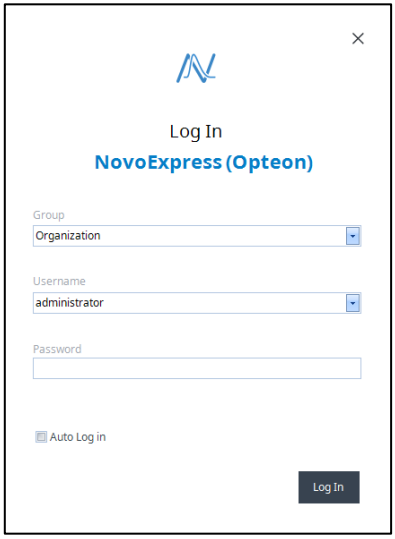
Options: Click to open the **Options** window. Refer to Option Settings in this guide for details.



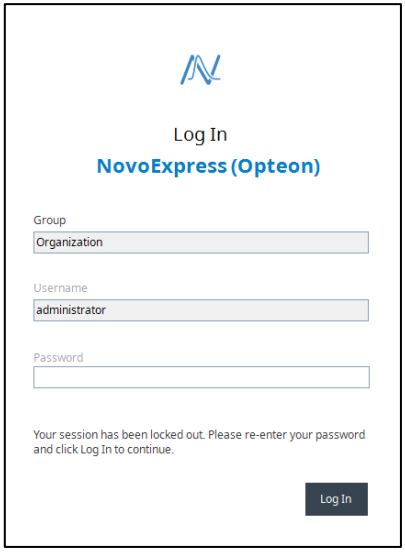
Recent Documents

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

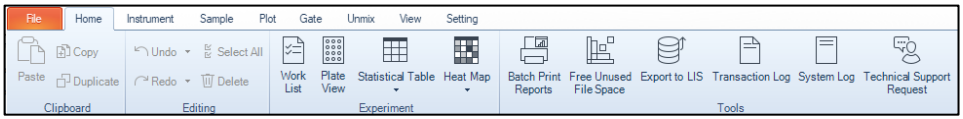
Logout: Exits and logs out of the current account. The login window will appear. User needs to enter the correct username and password again and click **Log In** to log back into the software.



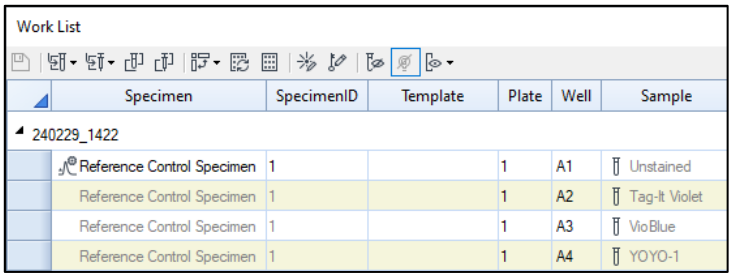
Lock out: Lock out from the current session. When clicked, following window will be prompted. User needs to enter the correct password again and click **Log In** to continue operation of the software.



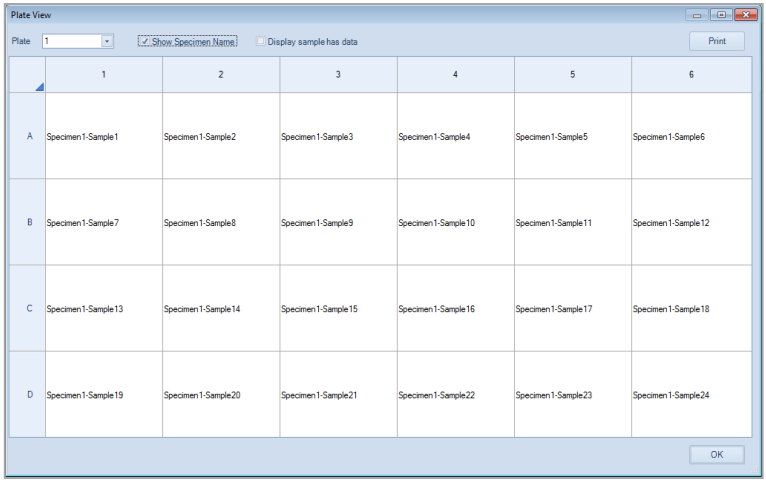
Home



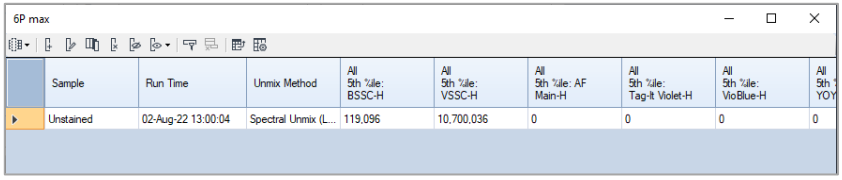
Please refer to table below for description of each icon located in **Home** tab.

Icon	Description
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:** Undo drawing gates, deleting gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot-related functions.
	**Redo:** 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.
Experiment	
	**Work List**: Users may view and edit the **Work List**. Refer to Work List in this guide for more details. 
	**Plate View**: Click to open **Plate View** window. This function is only available when NovoSampler S is used. This 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

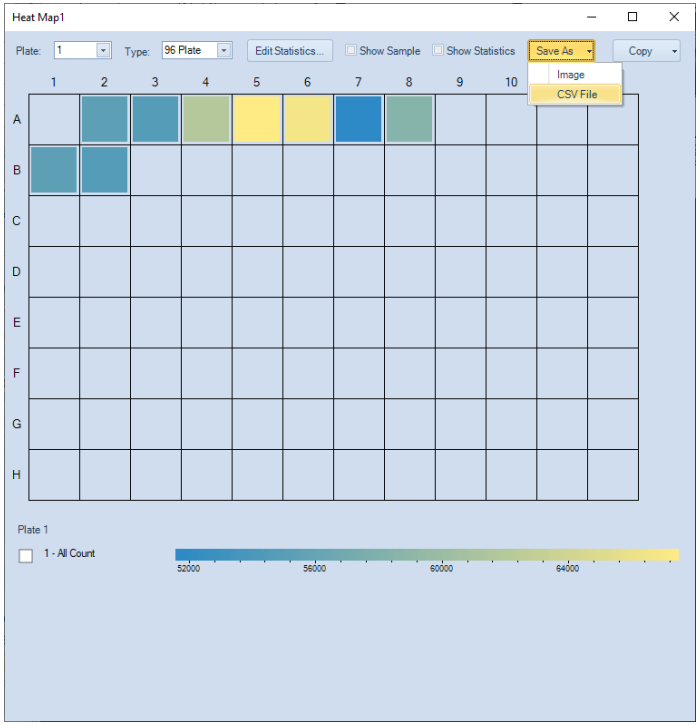
all the created plates. Select a **Plate ID** from the drop-down menu and view the sample information for this plate. Check the **Show Specimen Name** checkbox to enable the specimen's name associated with each well to be shown. Check the **Display sample has data** checkbox to also show samples which have collected data.



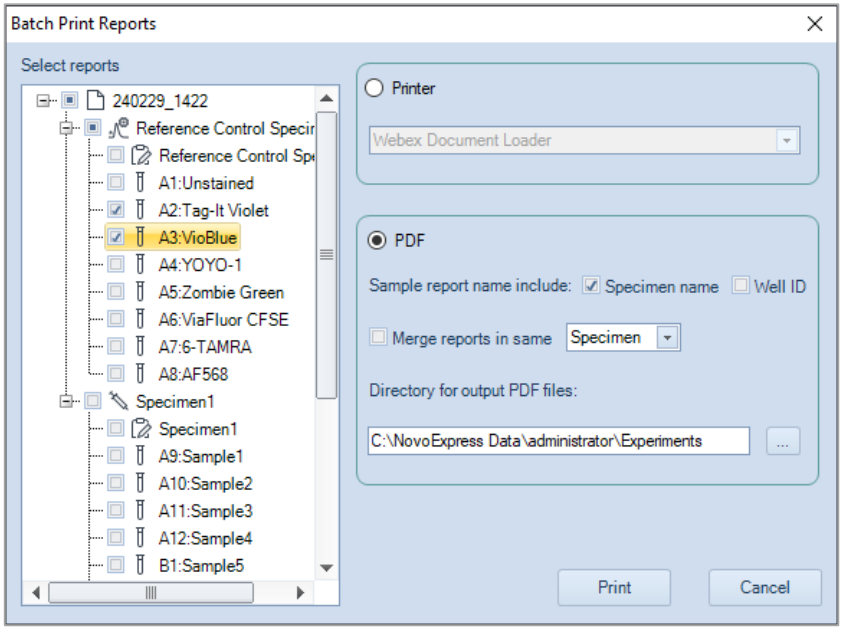
Statistical Table: Creates a statistical analysis table. Please refer to Statistical Tables in this guide for more details.



Heat Map: Creates a heat map for the defined parameter in a plate layout format. Please refer to Heat Map in this guide for more details.

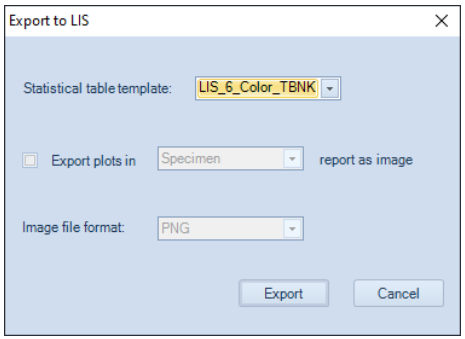


Tools	
	**Batch Print Reports**: Click to print or create PDF of multiple test reports. Please refer to Batch Print Reports in this guide for more details.



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.

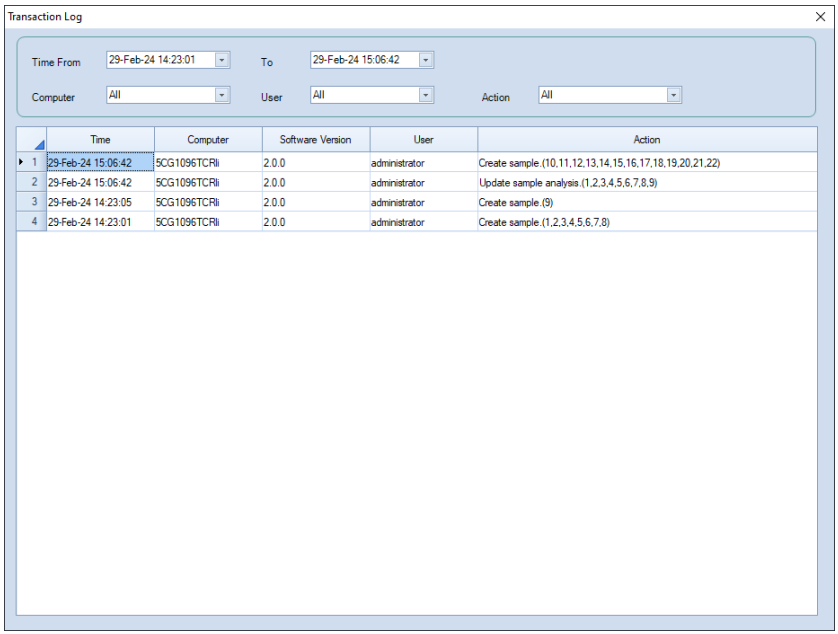
Export to LIS: Allows user to export data analysis results in the designated format to CSV file and such CSV file can be parsed by a Laboratory Information System (LIS) or read by other programs. When clicked, following window will be prompted.



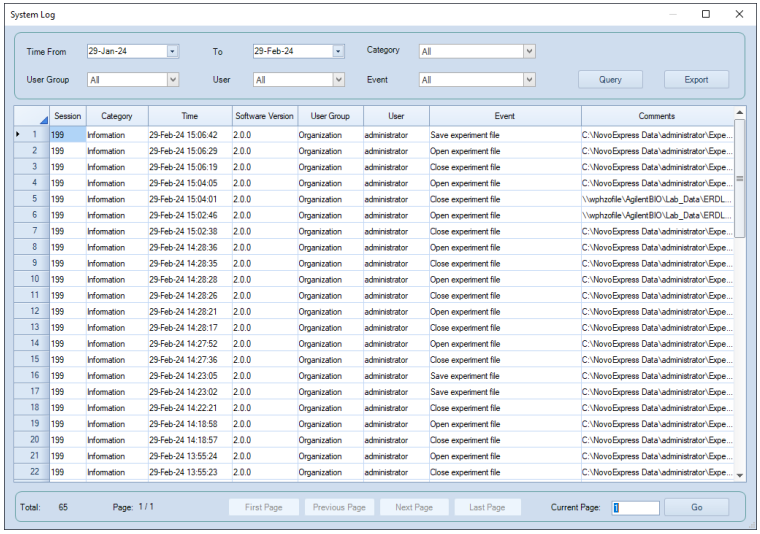
- **Statistical table template**: Sets the proper statistical table template to export the data. To add a statistical table template, export the statistical table as a template to the default folder "**User data root folder\Statistical Table Templates**". Refer to Statistical Table Management for details.

- **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 (Opteon) Data\administrator**" and can be changed. Refer to Setting for details of changing the default user data root folder.

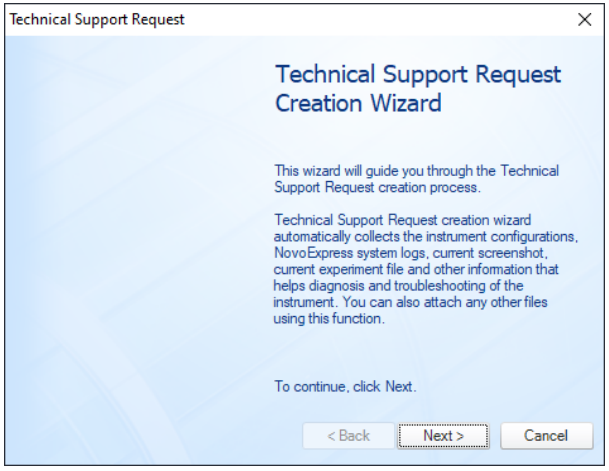
Transaction Log: Displays the **Transaction Log** window. Records can be filtered by **Time**, **Computer**, **User**, and **Action**. Only accounts with the **View Transaction Log** privilege can access the **Transaction Log**.



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.



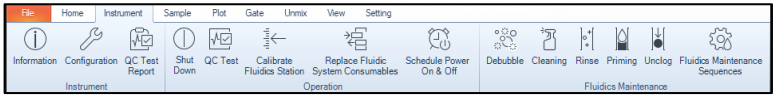
Technical Support Request: **Technical Support Request Creation Wizard** automatically collects instrument configurations, NovoExpress (Opteon) system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of instrument. You can also attach any other files using this function. Refer to Technical Support Request in this guide for details.



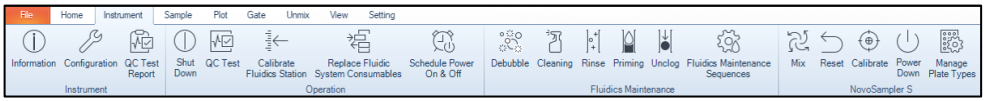
Instrument

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 S is connected, additional functions are available.

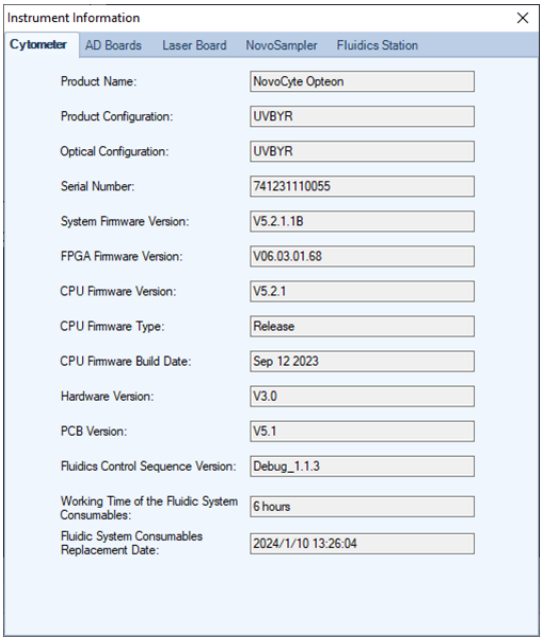


When NovoCyte Opteon spectral flow cytometer is connected



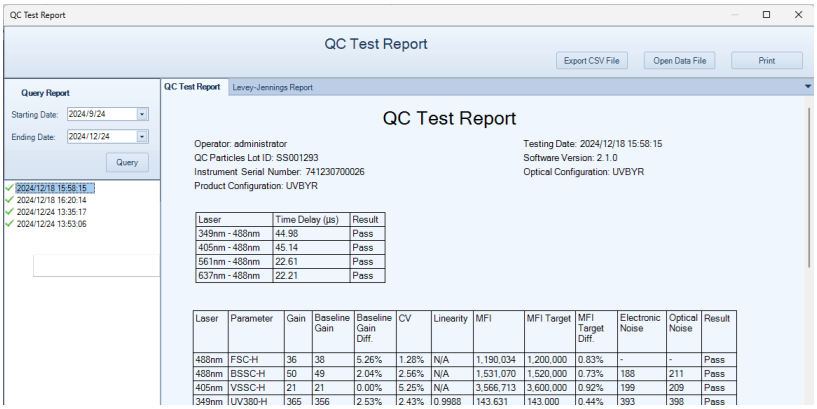
When NovoCyte Opteon spectral flow cytometer and NovoSampler S are connected

Please refer to table below for description of each icon located in **Instrument** tab.

Icon	Description
Instrument	
	**Information**: Click to display the instrument information including Product Model, AD boards, Laser board, NovoSampler S (if connected) and fluidics station S (or cart) information. This function is only available when the instrument is connected and powered on. 

Configuration: Displays instrument model, optical configuration and detection channel and options for the NovoSampler S. Refer to Instrument Configuration in this guide 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. Please refer to QC Test in this guide for details.



Operation

NOTE All the functions or actions listed in this tab are only available when the instrument or associated accessories is properly connected and powered on.

Shut Down: Starts the shutdown process. After completion, the instrument will automatically turn off. To clean sample injection probe while shutting down instrument, refer to NovoCyte Opteon Spectral Flow Cytometer Operator's Guide for more information.

QC Test: Runs the instrument QC test.

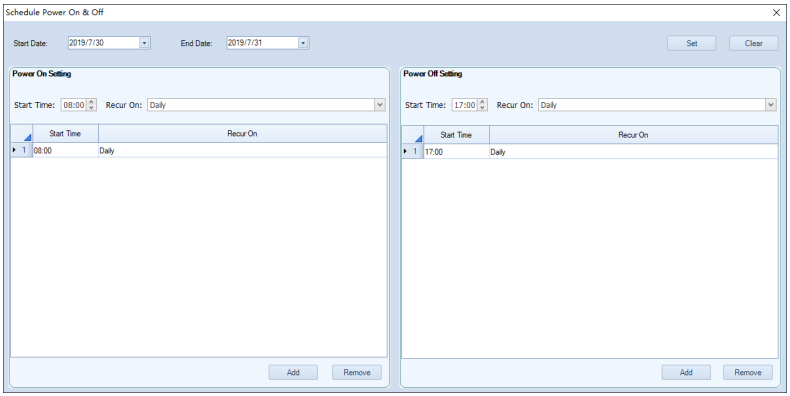
Calibrate Fluidics Station (or **Fluidics Cart**): Long distance transportation, movement and other reasons may cause the fluidics station S (or Fluidics Cart) system malfunction. Use this function to re-calibrate the fluidics station S (or Fluidics Cart). After clicking the button, it will ask to remove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

Replace Fluidic System Consumables: Click to open the **Replace Fluidic System Consumables** window and follow the instructions to replace fluidic system consumables. Refer to NovoCyte Opteon Spectral Flow Cytometer Operator's Guide for more information.

NOTE The software monitors the accumulated running time of the fluidic system consumables to ensure the consumables are changed in a timely manner for optimal flow cytometry results. When the accumulated running time is

reached, NovoExpress (Opteon) software will prompt a message to remind the users to replace the consumables.

Schedule Power On & Off: Click 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.

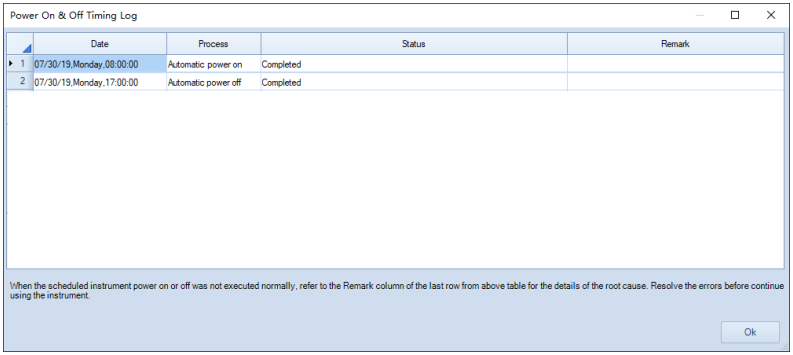


- **Start Date**: Select the start date of the instrument power on and off. The start date cannot be set to 7 days or more over the current date.
- **End Date**: Select the end date of the instrument power on and off.
- **Start Time**: Select the start time of instrument power on or off.
- **Recur On**: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

- **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.



Fluidics Maintenance

NOTE All the functions or actions listed in this tab are only available when the instrument is properly connected and powered on.

Debubble: Removes the air bubbles from the fluidic system.

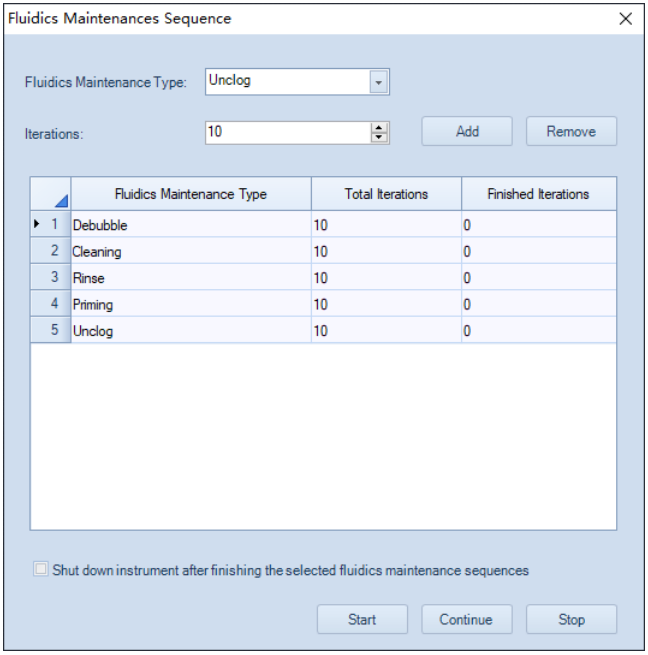
Cleaning: Uses a cleaning solution to decontaminate the biohazards that may exist in the fluidic system.

Rinse: Rinses the fluidic system using a rinsing solution.

Priming: After the instrument has not been in use for certain 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.

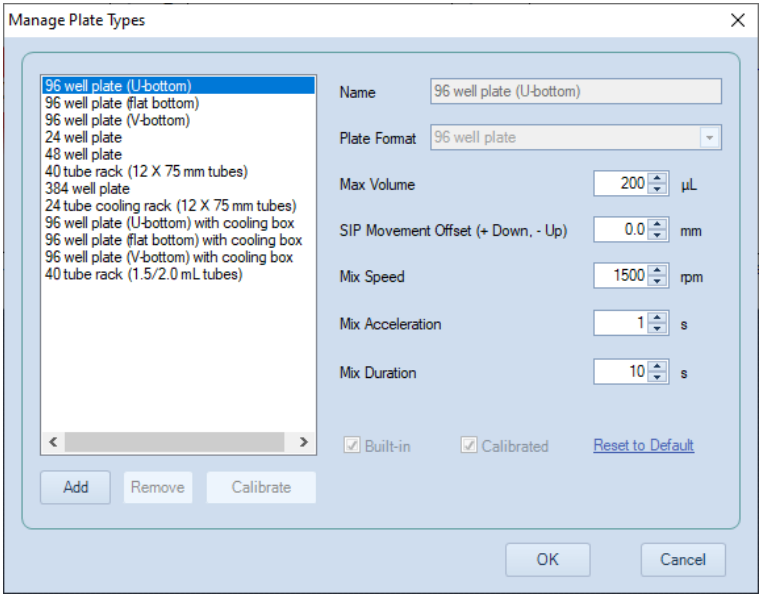
Fluidics Maintenance Sequences: program and run multiple fluidics maintenance sequences in pre-defined order.



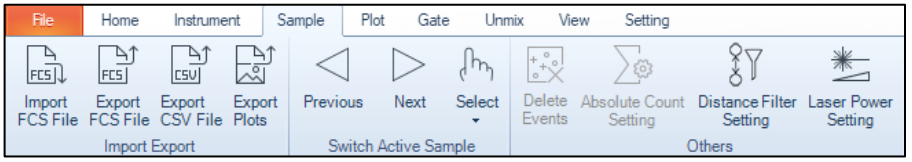
- **Fluidics Maintenance Type**: Select fluidics maintenance type (i.e., **Debubble, Cleaning**, **Rinse**, **Priming**, **Unclog**)
- **Iterations**: Set the number of iterations for the selected fluidics maintenance sequence.
- **Add**: Click to add the selected fluidics maintenance sequence to the 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 Instrument 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 NovoCyte Opteon Spectral Flow Cytometer Operator's Guide for detailed procedure.

	**Decontamination**: If Instrument 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 NovoCyte Opteon Spectral Flow Cytometer Operator's Guide for detailed procedure.
NovoSampler S NOTE	The functions or actions listed in this tab (except **Manage Plate Types**) are only available when the NovoSampler S is properly connected.
	**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 S in case of misalignment or error
	**Calibrate**: Click to calibrate the NovoSampler S with the instrument.
	**Power Down/Up**: When clicked, NovoSampler S will be powered on (if currently powered off) and allow the operation of the NovoSampler S. After execution, the status indication LED on NovoSampler S will light up in green color. NovoExpress (Opteon) will prompt a message to restart the software. After the software restarts, a dialog box requesting to calibrate the NovoSampler S will be shown. Click **Calibrate** to start the automatic calibration procedure. The software features for control and use of the NovoSampler S will be available after restart of the NovoExpress (Opteon) Software. If the NovoSampler S is already powered on, clicking this button will power off the NovoSampler S, and the status indication LED on NovoSampler S will turn off. NovoExpress (Opteon) 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 (Opteon) 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.



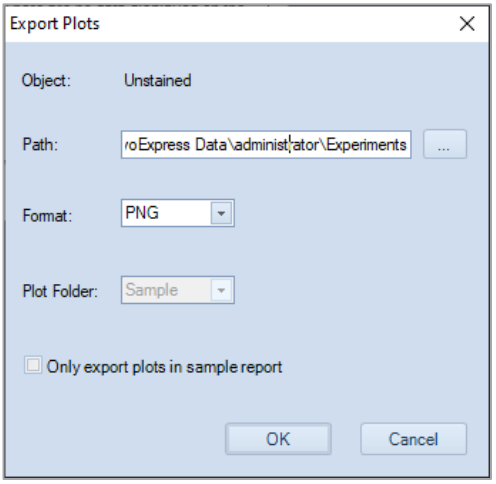
Sample



Please refer to table below for description of each icon located in **Sample** tab.

Icon	Description
Import and Export	
	**Import FCS File**: Import data from FCS format files.
	**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, Bitmap, GIF, TIFF into designated path. Check **Only export plots in sample report** to export plots in the sample report only.

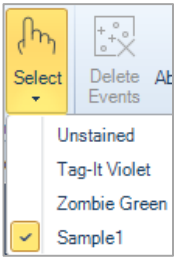


Switch Active Sample

Previous: Switches the Active Sample to the previous sample.

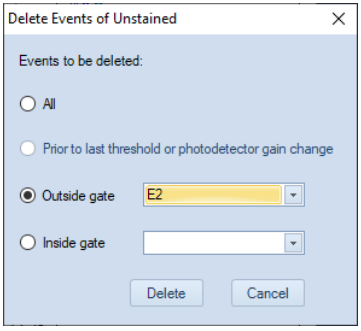
Next: Switches the Active Sample to the next sample.

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



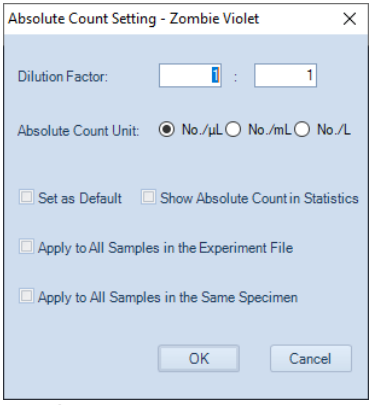
Others

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.



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.



- **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 (Opteon) 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./µL, No./mL, or No./L) to present 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.

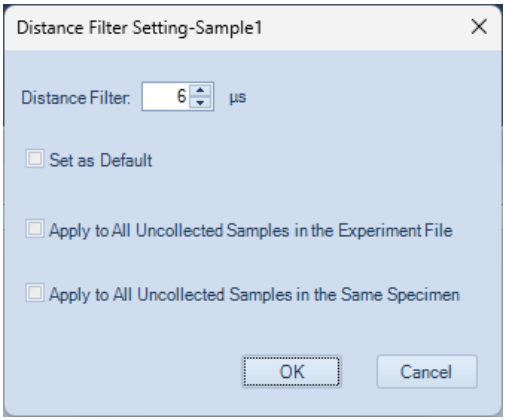
- **Show Absolute Count in Statistics**:
  Show **Absolute Count** column in the statistical table of the plots in the workspace.

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 is described in Calculation of Statistics in this guide.

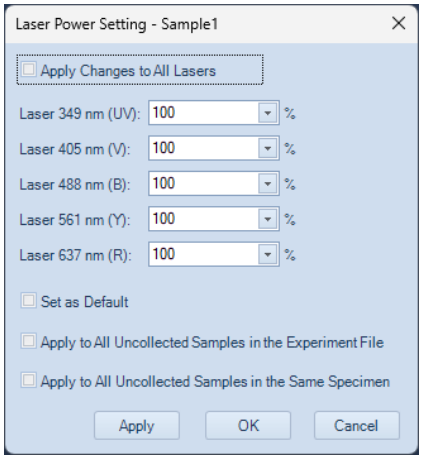
Distance Filter Setting: Sets up distance filter values for active sample. This window can be edited only when **Adjust Distance Filter Setting** is checked in **Access Privilege** window.



- **Distance Filter**:
  Allows user to set the distance filter value in the range of 0-12 µs. This option is automatically disabled once the data collection for selected active sample is started.
- **Set as Default**:
  When checked, the distance filter value user set in **Distance Filter** area above will be set as default for any newly created samples. The software will at the same time apply this value to **Default Distance Filter Setting for New Samples** in **Options > Other** tab.
- **Apply to All Uncollected Samples in the Experiment File**:
  Check to apply the distance filter value user set in **Distance Filter** area above to all uncollected samples in the current experiment file.
- **Apply to All Uncollected Samples in the Same Specimen**:

Check to apply the distance filter value user set in **Distance Filter** area above to all uncollected samples in the same specimen.

Laser Power Setting: Sets up laser power values for active sample. When clicked, following window will be prompted. User can set the laser power for selected sample by either directly entering the power percentage (i.e., 20%-100%) or selecting one from drop down menu of associated laser. This window can be edited only when **Adjust Laser Power Setting** is checked in **Access Privilege** window.



NOTE Under following situations, user cannot edit this window.

- Adjust Laser Power Setting privilege is not enabled in the Access Privilege window.
- Data collection for the selected sample is already completed
- For reference control specimen, as long as one of the control samples is collected, the laser power setting of all other control samples cannot be edited.

Apply Changes to All Lasers: When checked, any changes to the power setting of a laser will be automatically applied to the other remaining lasers.

Set as Default: Check to set the current setting as the default setting for all newly created samples.

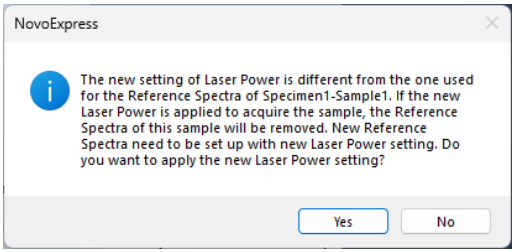
Apply to All Uncollected Samples in the Experiment File: When checked, the software will apply the laser power setting defined in this window to all uncollected samples within the same experiment file.

Apply to All Uncollected Samples in the Same Specimen: When checked, the software will apply the laser power setting defined in this window to all uncollected samples within the same specimen.

NOTE When all reference control samples are not collected, and user selects any of the reference control samples, the Apply to All Uncollected Samples in the Same Specimen option will be automatically checked by default (i.e., any changes to the laser power setting in this window will be automatically applied to other reference control samples). User cannot edit this option.

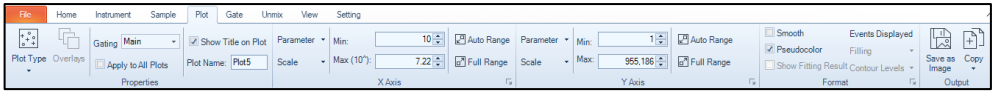
NOTE

When the reference spectra exist for a selected experiment sample, and user changes the laser power setting in this window, the following window will be prompted after user clicks **OK** or **Apply**.



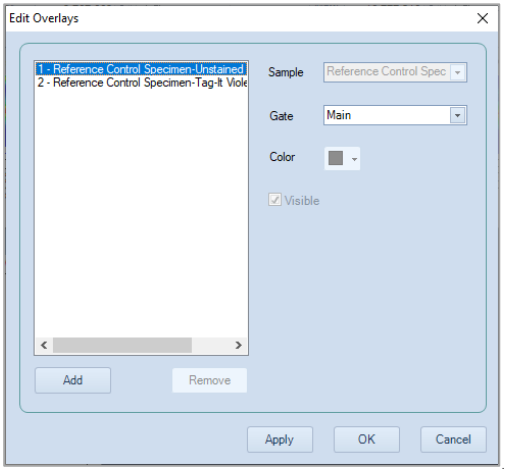
If user clicks **Yes**, the new laser power setting will be applied to selected sample, and the associated existing reference spectra will be deleted.

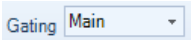
Plot



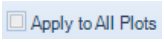
Please refer to table below for description of each icon located in **Plot** tab.

Icon	Description
Properties	
	**Plot Type**: Changes the plot type for the selected plot.
	**Overlays**: Click to open the **Edit Overlays** window. User can edit overlays in this prompted window. Overlay is only available for dot plots and histograms. Please refer to Overlays in this guide for more details.



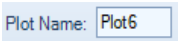


Gating: The gate and its events are selected as the current data source for plotting.



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

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.

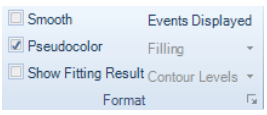


Plot Name: Sets the name of a plot.

X -Axis, Y –Axis

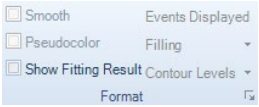
Allows user to set plot properties for the X and Y-axes including the plotted parameter, scale, and the display range.
User can set the minimum and maximum displayed values in **Min** and **Max** box for the parameter selected from **Parameter** drop-down list or click **Auto Range** to automatically sets the range of the X and Y-Axes according to the range of the dataset or click **Full Range** to set the full range of the X and Y-Axes. The default range is 2^{24}=16,777,216. User can also set designated plot scale (i.e., **Linear, Log** or **Biexponential**) from the drop-down list of **Scale.**

Format

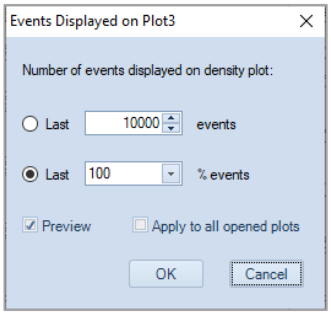


Format: Controls format settings for the plots. Available settings depend on the type of the selected plots.

- **Smooth**: Smooths the data in histogram and density plots.



- **Pseudocolor**: Density plots are displayed in pseudo-color to visualize event density.
- **Events Displayed**: Click to set the number of events to be displayed in the dot plot, density plot or contour plot. When clicked, following window will be prompted. Please refer to Plot Formatting in this guide for more details.



- **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.

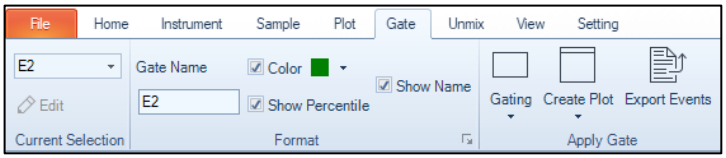
Output

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.

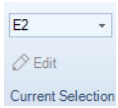
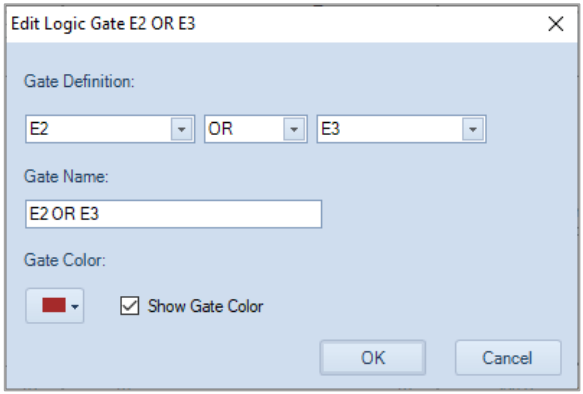
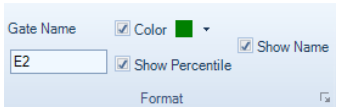
Copy: Selects from the drop-down menu to copy to the clipboard. The plots can then be pasted into Microsoft Word, PowerPoint, Excel, and other documents.
Pull-down options include:

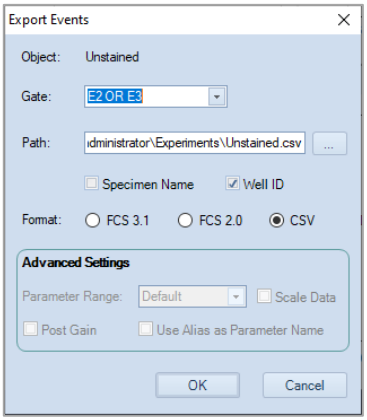
- **Copy Plot (Bitmap)**: Copies the selected plot as a Bitmap.
- **Copy Plot with Statistics (Bitmap)**: Copies the selected plot and associated statistical information as a Bitmap.
- **Copy Vector Graphics (EMF)**: Copies the selected plot as an EMF.
- **Copy Statistics (Text)**: Copies the statistics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

Gate

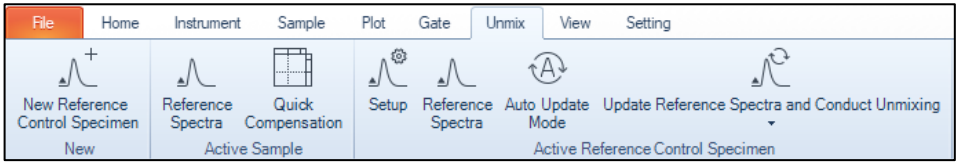


Please refer to table below for description of each icon located in **Gate** tab.

Icon	Description
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. Please refer to Gates in this guide for more details. 
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	

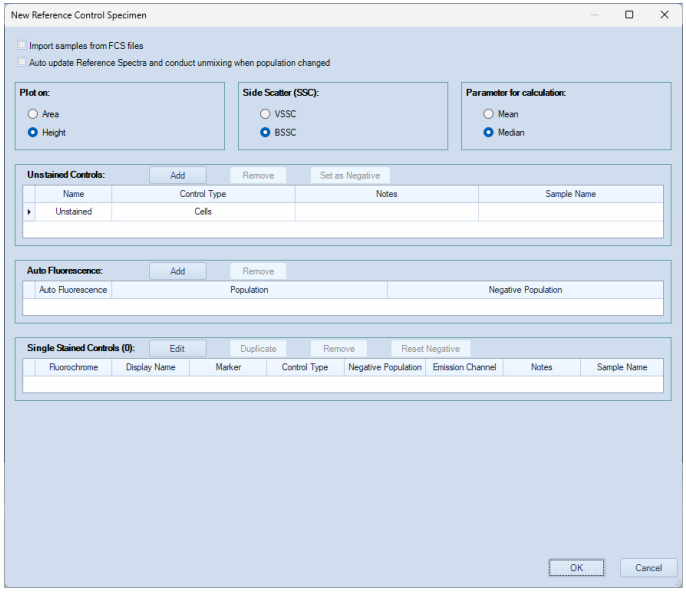
	**Gating**: Applies the current gate to the selected plot.
	**Create Plot**: Creates a new plot and applies the current gate to the new plot.
	**Export Events**: Click to export data for the events inside the current gate in either FCS or CVS format. When clicked, following window will be prompted. Please refer to Export Gate Events in this guide for more details. 

Unmix



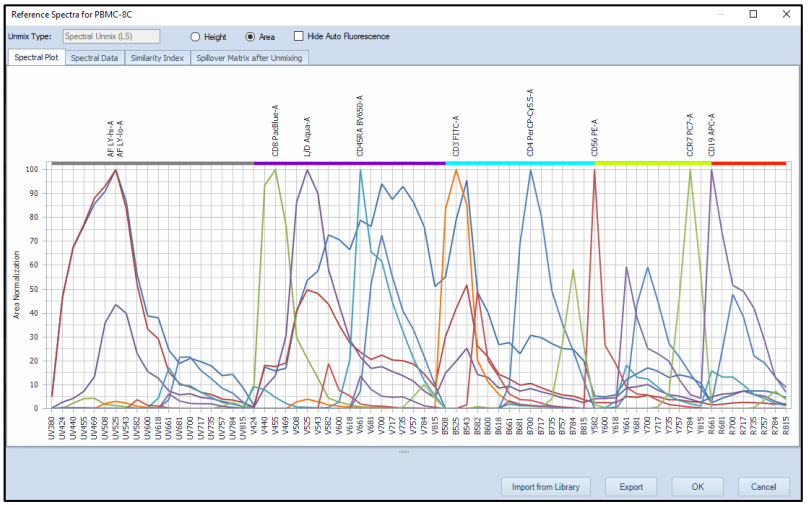
Please refer to table below for description of each icon located in **Unmix** tab.

Icon	Description
New	
	**New Reference Control Specimen**: Click to open the **New Reference Control Specimen** window. User can create and define the Reference Control specimen and samples in this window. Please refer to New Reference Control Specimen in this guide for more details.

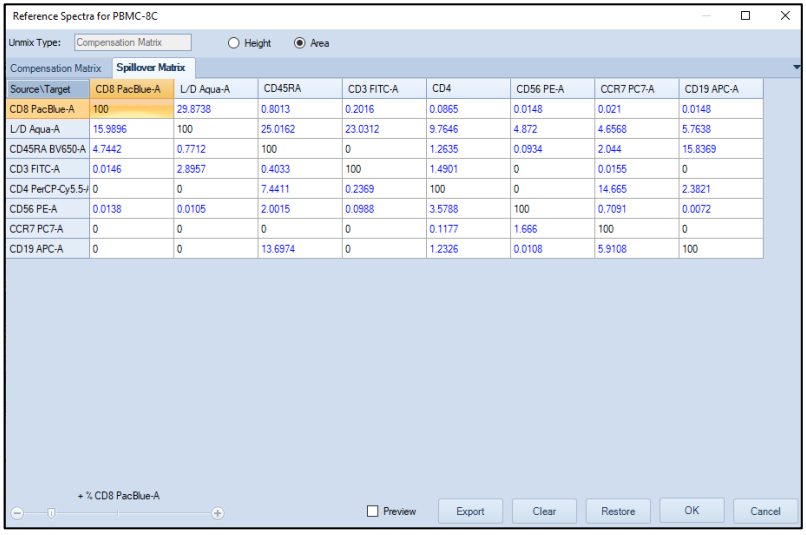


Active Sample

Reference Spectra: Click to view the Reference Spectra information for active sample in the prompted window. Depending on whether Spectral Unmix or Compensation is selected to analyze the data, this window may display different information. This function is not available if no active sample is selected. Please refer to Reference Spectra in this guide for more details.



When Spectral Unmix is selected

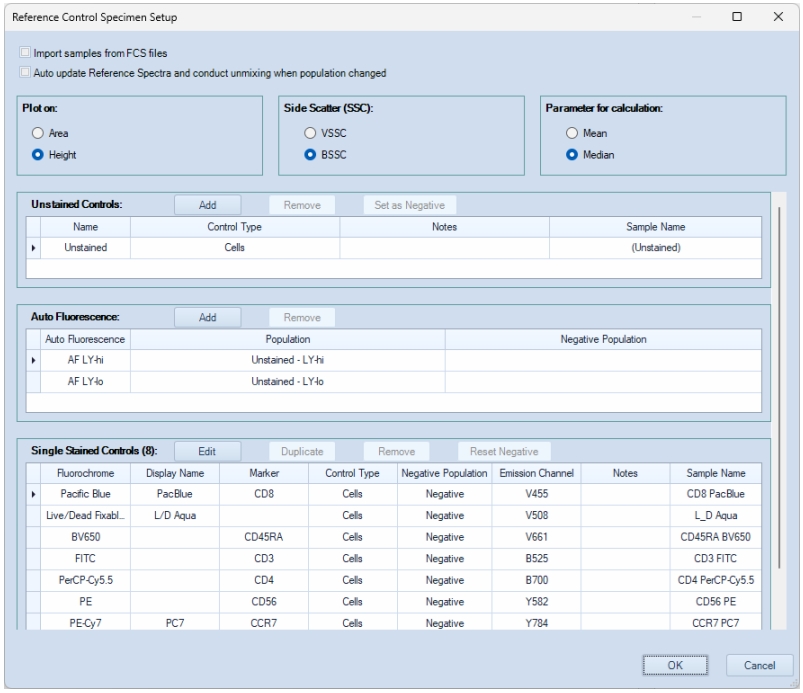


When Compensation is selected

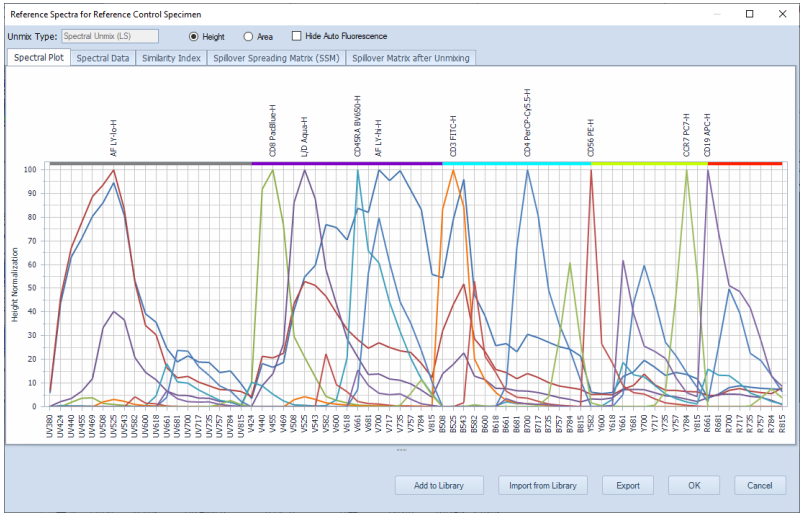
Quick Compensation: Click to display the Compensation scrollbars on the active plot and enable quick compensation. Refer to Quick Compensation in this guide for more details.

Active Reference Control Specimen: Following functions are available only when active sample is selected to be Reference Control sample.

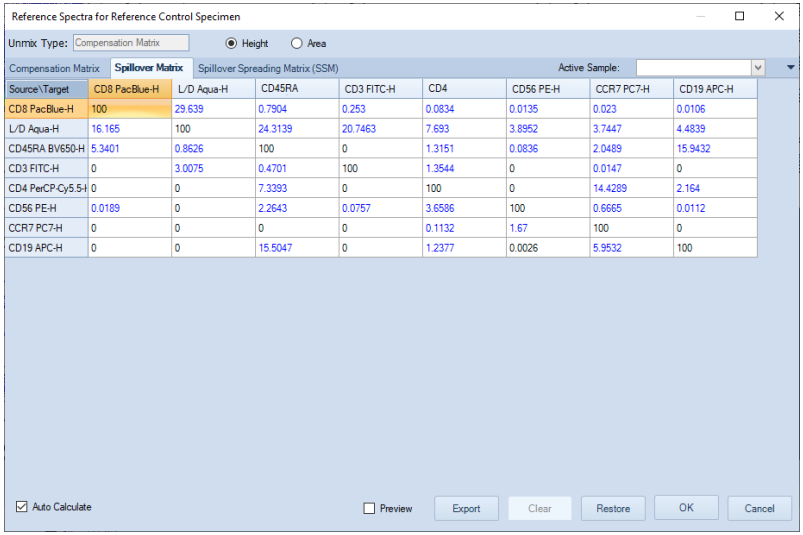
Setup: Click to open the **Reference Control Specimen Setup** window. User can also access this window by double clicking the **Reference Control Specimen** node from **Experiment Manager** panel. User can modify the Reference Control specimen settings defined previously. Refer to New Reference Control Specimen in this guide for details of each function.



Reference Spectra: Click to open and view the Reference Spectra for Reference Control specimen in prompted window. User can also access this window by double clicking the **Reference Spectra** under **Reference Control Specimen** node from **Experiment Manager** panel. Depending on whether Spectral Unmix or Compensation is selected to analyze the data, this window may display different information. Refer to Reference Spectra in this guide for details of each function.



When Spectral Unmix is selected

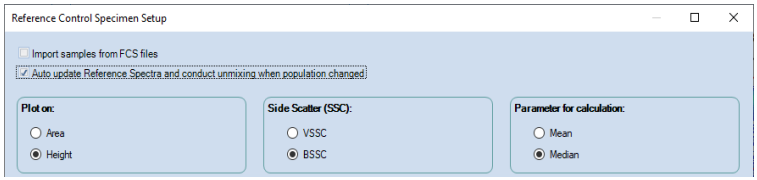


Source\Target	CD8 PacBlue-H	L/D Aqua-H	CD45RA	CD3 FITC-H	CD4	CD56 PE-H	CCR7 PC7-H	CD19 APC-H
CD8 PacBlue-H	100	29.639	0.7904	0.253	0.0834	0.0135	0.023	0.0106
L/D Aqua-H	16.165	100	24.3139	20.7463	7.693	3.8952	3.7447	4.4839
CD45RA BV650-H	5.3401	0.8626	100	0	1.3151	0.0836	2.0489	15.9432
CD3 FITC-H	0	3.0075	0.4701	100	1.3544	0	0.0147	0
CD4 PerCP-Cy5.5-H	0	0	7.3393	0	100	0	14.4289	2.164
CD56 PE-H	0.0189	0	2.2643	0.0757	3.6586	100	0.6665	0.0112
CCR7 PC7-H	0	0	0	0	0.1132	1.67	100	0
CD19 APC-H	0	0	15.5047	0	1.2377	0.0026	5.9532	100

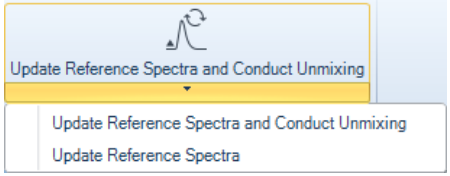
When Compensation is selected

Auto Update Mode: Allows user to enable or disable the Reference Spectra auto update mode. By default, this function is disabled. User can click this icon once to enable the auto update mode (icon will be highlighted) and click it again to disable the auto update mode (icon will not be highlighted). When auto update mode is enabled, and there are any changes to the spectra (e.g., changes to the negative or positive

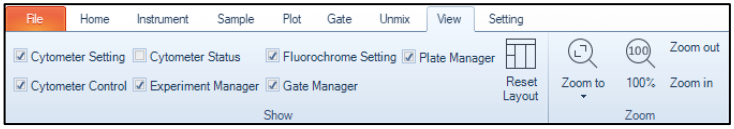
population gating of single-stained control sample), the software will automatically update the Reference Spectra and re-do the Spectral Unmixing (or Compensation). User can also enable this mode by checking **Auto update Reference Spectra and conduct unmixing when population changed** option from **New Reference Control Specimen** window (Refer to New Reference Control Specimen in this guide for details).



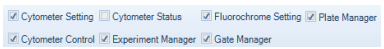
Update Reference Spectra and Conduct Unmixing: Click to select **Update Reference Spectra and Conduct Unmixing** to manually update the Reference Spectra for active Reference Control specimen, and start the unmixing immediately, or select **Update Reference Spectra** to only update the Reference Spectra for active Reference Control specimen but no unmixing afterward. User can also access these two functions by right clicking the **Reference Spectra** under **Reference Control Specimen** node from **Experiment Manager** panel. This function is available only when **Auto Update** mode is disabled, and there are changes made to the Reference Spectra Specimen.



View

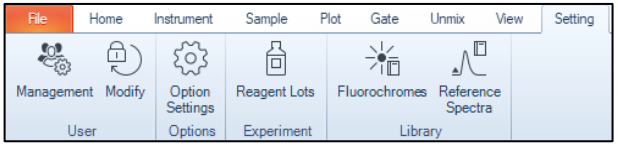


Please refer to table below for description of each icon located in **View** tab.

Icon	Description
Show	
	Shows or hides the corresponding panel. **Plate Manager** is available only when NovoSampler S is connected.
	**Reset Layout**: Resets the layout of the panels to the default layout.

Zoom	
	**Zoom to**: Selects the scaling of the size of the plots inside the workspace in the drop-down menu (50%-200%). 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.

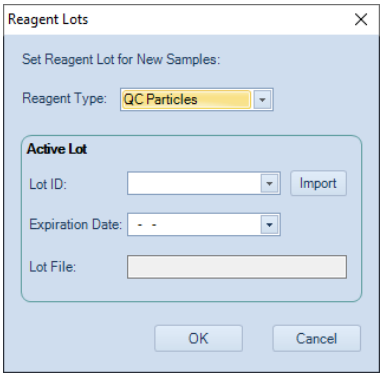
Setting



Please refer to table below for description of each icon located in **Setting** tab.

Icon	Description
User	
	**Management**: Use this function to manage all user groups and user accounts which includes adding, deleting, and modifying user groups and user accounts. This function is only available for accounts with the **Administrator** privilege.
	**Modify**: Use this function to change the username and password of the currently logged in account.
Options	
	**Option Settings**: Click to define **General, Experiment, Analysis, Absolute Count, Statistical Table, Report**, and **Security** related settings in prompted window and sub-windows. Refer to Option Settings in this guide for details.
Experiment	

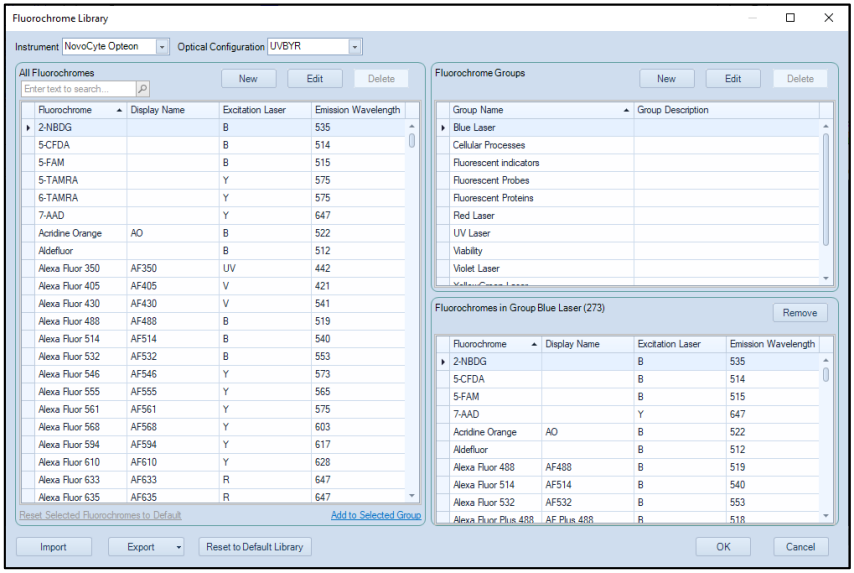
Reagent Lots: Allows user to define reagent lot related settings.



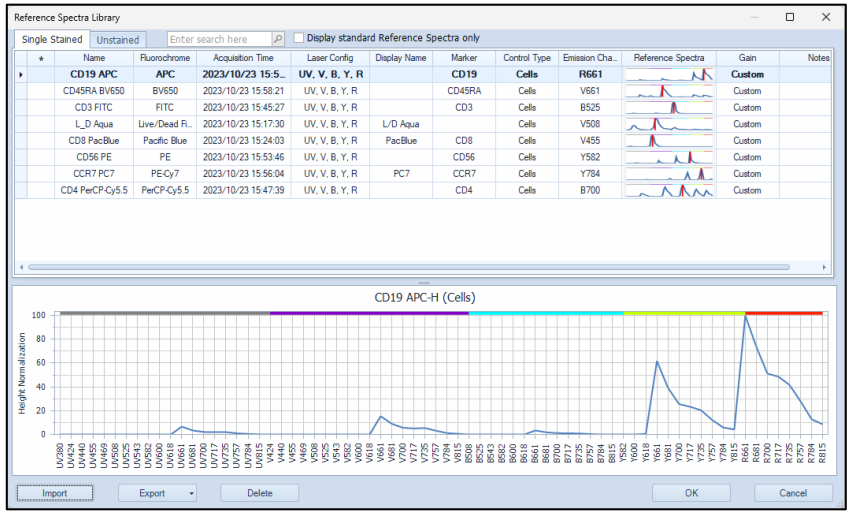
- **Reagent Type**: Sets the type of the reagent.
- **Lot ID**: Sets the ID of the active lot.
- **Import**: Import the lot file download from https://www.agilent.com/en/support/qc-particles-lot-file.The Lot ID will be listed after importing the lot file.
- **Expiration Date**: Sets the expired date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

Library

Fluorochromes: Allows user to view and edit fluorochromes stored in the library. Refer to Fluorochrome Library in this guide for details.



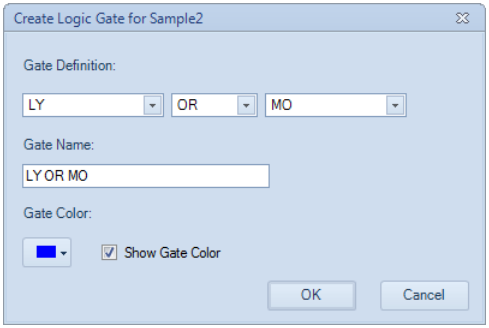
Reference Spectra: Allows user to view and edit Reference Spectra stored in the library. Refer to Reference Spectra Library in this guide for details.



Workspace Toolbar

Please refer to table below for description of each icon located in **Workspace Toolbar**.

Icon	Description
Raw Workspace	**Raw Workspace**: Click to switch to **Raw Workspace**. In this workspace, user can view and create plots of raw fluorescence channel data, FSC, BSSC, VSSC, Width and Time. It is mainly used to analyze raw data (i.e., not the unmixed or compensated data). Any plot (except **Spectrum Density** plot) created in **Raw Workspace** will be automatically created (i.e., synchronized) in **Unmixed Workspace**. User cannot create **Cell Cycle** plot, **Cell Proliferation** plot and **Fluorochrome Bi-variate** plot in Raw Workspace.
Unmixed Workspace	**Unmixed Workspace**: Click to switch to **Unmixed Workspace**. In this workspace, user can view and create plots of unmixed (or compensated) fluorescence channel data, FSC, BSSC, VSSC, Width and Time. It is mainly used to analyze unmixed or compensated data (i.e., not the raw data). Any plot (except **Cell Cycle** plot, **Cell Proliferation** plot) created in **Unmixed Workspace** will be automatically created (i.e., synchronized) in **Raw Workspace**. User cannot create **Spectrum Density** plot when working in this workspace

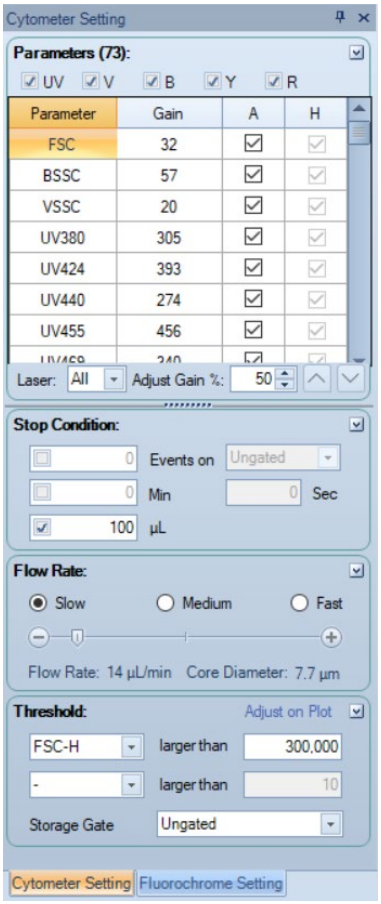
	**Dot Plot**: Creates a dot plot.
	**Density Plot**: Creates a density plot.
	**Histogram**: Creates a histogram.
	**Contour Plot**: Creates a contour plot.
	**Gate Pointer**: Use to select charts, gates, and statistical data.
	**Rectangular Gate**: Draws a rectangular gate. To use, select the tool, 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.
	**Vertical Range Gate**: Draws a vertical range gate. This function is only available for **Spectrum Density** plot.
	**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 in this guide for detailed procedures.
	**Quick Compensation**: Allows user to quickly adjust the Spillover Matrix through scrollbars. To use, select the tool. Scrollbars appear on plots to allow for adjustment of Spillover Matrix. This function is only available in **Unmixed Workspace**.
	**Show Statistics**: Shows or hides the statistical tables below plots.
	**Cell Cycle Plot**: Creates a cell cycle analysis plot. This function is available only when user works in **Unmixed Workspace**. Refer to Cell Cycle Analysis in this guide for more details.
	**Cell Proliferation Plot**: Creates a cell proliferation plot. This function is only available in **Unmixed Workspace**. Refer to Cell Proliferation Analysis in this guide for more details.

	**Spectrum Density Plot**: Creates a spectrum density plot. This function is only available in **Raw Workspace**. Refer to the Spectrum Density (Dot) Plot in this guide for more details
	**Spectrum Dot Plot**: Creates a spectrum dot plot. This function is only available in the **Raw Workspace**. Refer to the Spectrum Density (Dot) Plot in this guide for more details
	**Fluorochrome Bi-Variate Plots**: Opens **Fluorochrome Bi-Variate Plots** window. This function is only available in the **Unmixed Workspace**. Refer to Fluorochrome Bi-Variate Plots in this guide for more details.
	**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.

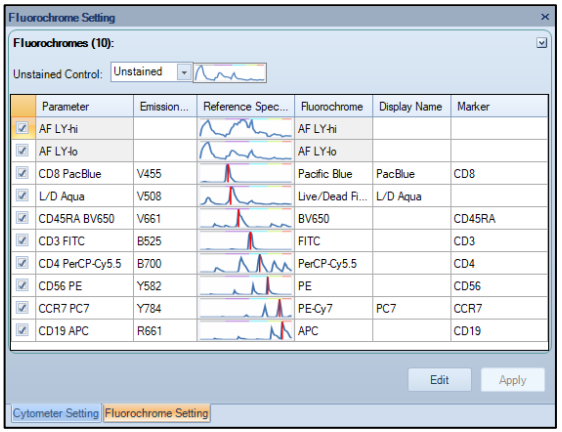
Cytometer Setting Panel

The **Cytometer Setting** panel sets the data collection parameters, stop conditions, flow rate, and threshold. This panel is displayed by default. User can click **Fluorochrome Setting** button on the bottom of this panel to switch to **Fluorochrome Setting** panel (refer to Fluorochrome Setting for details). For more details of **Cytometer Setting** panel, please refer to Cytometer Setting in this guide.

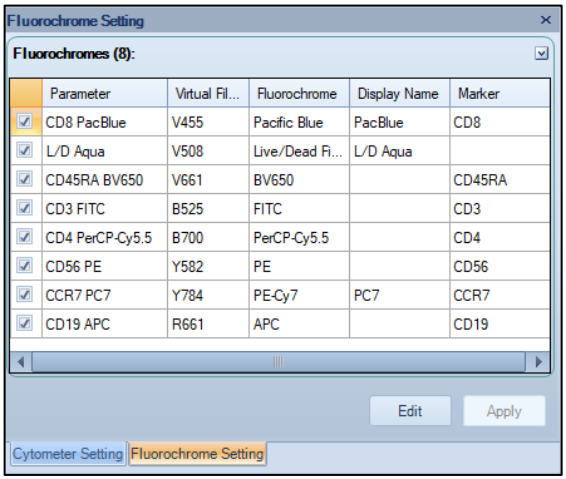


Fluorochrome Setting Panel

The **Fluorochrome Setting** panel allows user to view and edit the fluorochromes for current selected sample. User can click the **Fluorochrome Setting** button on bottom of the **Cytometer Setting** panel or click **Fluorochrome Setting** node in **Experiment Manager** to access this window. Depending on whether **Spectral Unmix** or **Compensation** is selected to analyze the data, this window may display different information. Please refer to Fluorochrome Setting in this guide for more details.



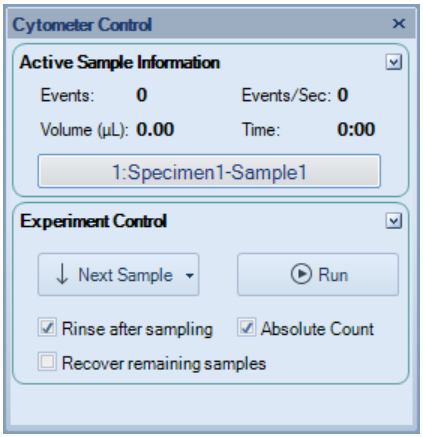
When Spectral Unmix is selected



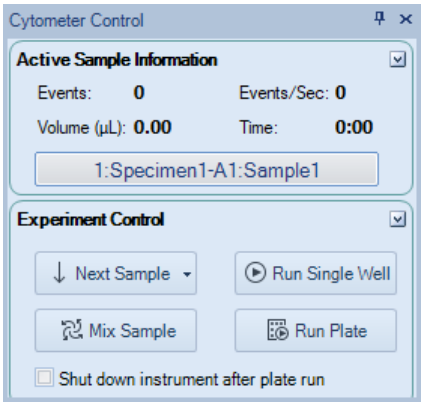
When Compensation is selected

Cytometer Control Panel

The **Cytometer Control** panel contains the **Active Sample Information** and the **Experiment Control** panel. The **Experiment Control** panel contains different functions when NovoSampler S is connected. Please refer to Cytometer Control for in this guide for more details.



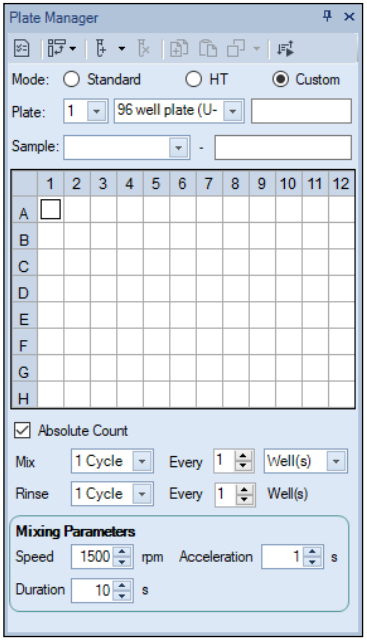
When NovoSampler S is not connected



When NovoSampler S is connected

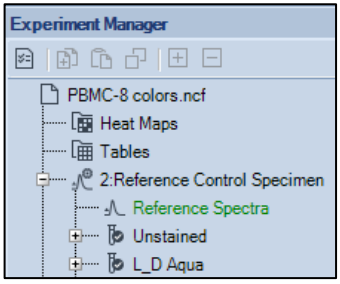
Plate Manager

The **Plate Manager** allows user to set up the working mode, plate type, mixing and rinsing conditions when NovoSampler S is used. Please refer to Plate Manager in this guide for more details.

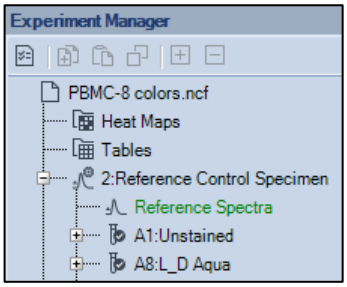


Experiment Manager

The **Experiment Manager** contains the sample hierarchy structure and functions for copying, pasting templates, and importing and exporting sample data. additional information (e.g., Well ID) is displayed when NovoSampler S is connected. Please refer to Experiment Manager in this guide for more details.



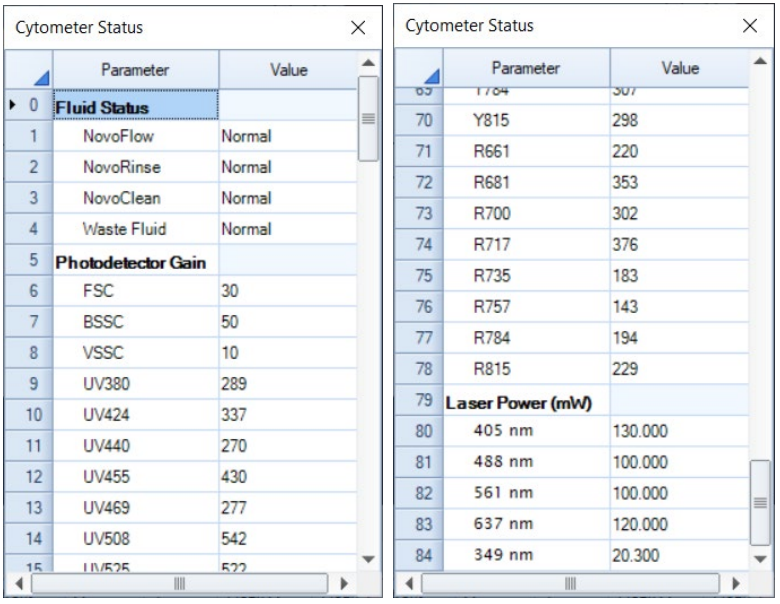
When NovoSampler S is not connected



When NovoSampler S is connected

Cytometer Status Panel

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



Cytometer Status

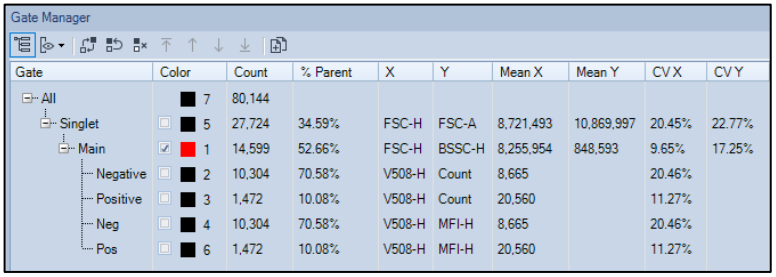
	Parameter	Value
0	**Fluid Status**	
1	NovoFlow	Normal
2	NovoRinse	Normal
3	NovoClean	Normal
4	Waste Fluid	Normal
5	**Photodetector Gain**	
6	FSC	30
7	BSSC	50
8	VSSC	10
9	UV380	289
10	UV424	337
11	UV440	270
12	UV455	430
13	UV469	277
14	UV508	542
15	UV525	522

Cytometer Status

	Parameter	Value
69	1784	307
70	Y815	298
71	R661	220
72	R681	353
73	R700	302
74	R717	376
75	R735	183
76	R757	143
77	R784	194
78	R815	229
79	**Laser Power (mW)**	
80	405 nm	130.000
81	488 nm	100.000
82	561 nm	100.000
83	637 nm	120.000
84	349 nm	20.300

Gate Manager Panel

The **Gate Manager** displays all gates of the active sample in list mode or tree mode. This panel is displayed by default after initial installation of the software. User can click **Reset Layout** or check **Gate Manager** option from **View** tab of software main window to display it again after closes it. This panel allows user to modify gate name, color and color precedence and also shows gate hierarchy and gate statistics. Please refer to Gate Manager in this guide for more details.



Gate	Color	Count	% Parent	X	Y	Mean X	Mean Y	CV X	CV Y
All	7	80,144							
Singlet	5	27,724	34.59%	FSC-H	FSC-A	8,721,493	10,869,997	20.45%	22.77%
Main	1	14,599	52.66%	FSC-H	BSSC-H	8,255,954	848,593	9.65%	17.25%
Negative	2	10,304	70.58%	V508-H	Count	8,665		20.46%	
Positive	3	1,472	10.08%	V508-H	Count	20,560		11.27%	
Neg	4	10,304	70.58%	V508-H	MFI-H	8,665		20.46%	
Pos	6	1,472	10.08%	V508-H	MFI-H	20,560		11.27%	

Status Bar

The status bar located at the bottom of the software main window 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 greyed out.

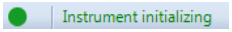
Green Indicator

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

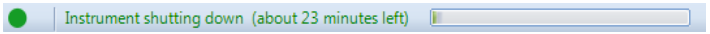
User may observe following green indicator during normal operation of the instrument.

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

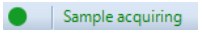
- The status bar displays **Instrument initializing** as shown below after powering on the instrument. The initializing sequence flushes the fluidic lines in the instrument to prepare for sample acquisition.



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

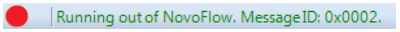


- The status bar displays **Sample acquiring** during sample acquisition.

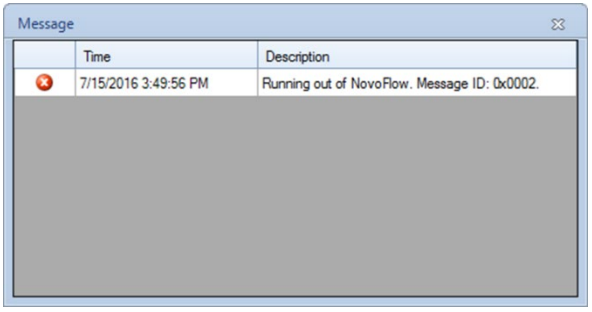


Flashing Red Indicator

If an error occurs, the indicator flashes red. An error message is displayed with the cause and possible solutions. An example red indicator is shown below. Please refer to Troubleshoot in this guide for details.

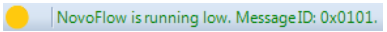


User can click on the indicator to view detailed description of the error in the prompted message box.

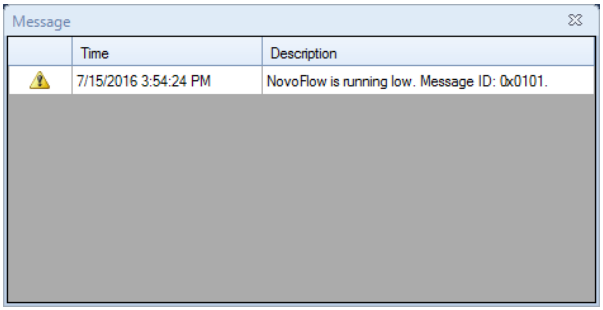


Flashing Yellow Indicator

If a warning occurs, the indicator flashes yellow. A warning message displays the cause of the problem and possible solutions. An example yellow indicator is shown below. Please refer to Troubleshoot in this guide for details.



User can click on the indicator to view the detailed description in the prompted message box.

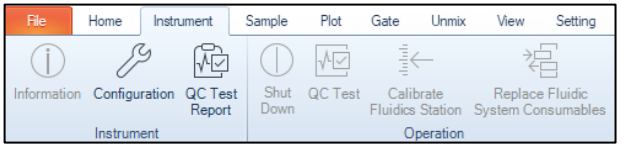


Grey Indicator

If the instrument is not connected to the computer, the indicator is grey. The instrument is either powered off or there is problem with the USB connection between the computer and instrument. Also, if multiple instances of the NovoExpress (Opteon) 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 grey.

Instrument Configuration

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



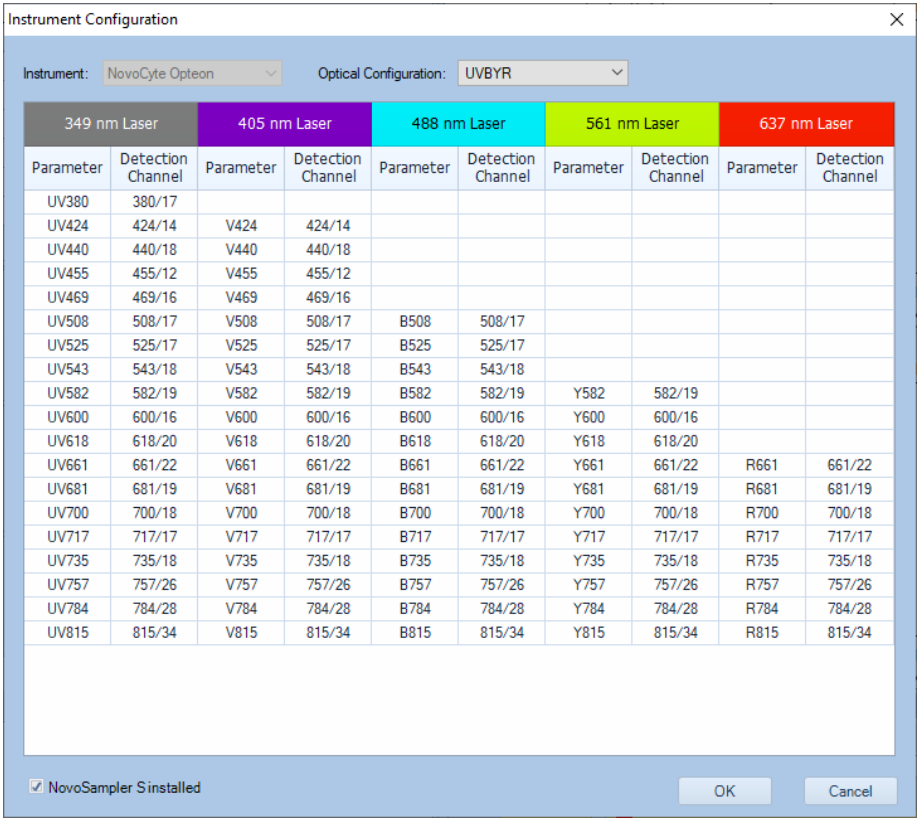
NovoExpress (Opteon) Software automatically detects the channel parameters, excitation lasers, and detection channels of a connected instrument. The software also detects if the NovoSampler S is connected with NovoCyte Opteon Spectral flow cytometer.

If the instrument is powered off or not connected to the workstation, user can use the **Instrument Configuration** window to select the designated optical configuration to view the associated information.

Instrument Configuration with Instrument Connected

The **Instrument Configuration** window displays the instrument type, current optical configuration, detection channel parameters for currently connected instrument, and status of NovoSampler S when the instrument is connected to the workstation and powered on. This window will display channel parameter, excitation laser, detection channel wavelength in tabular format.

The status of the NovoSampler S 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 S and this box will be automatically checked.



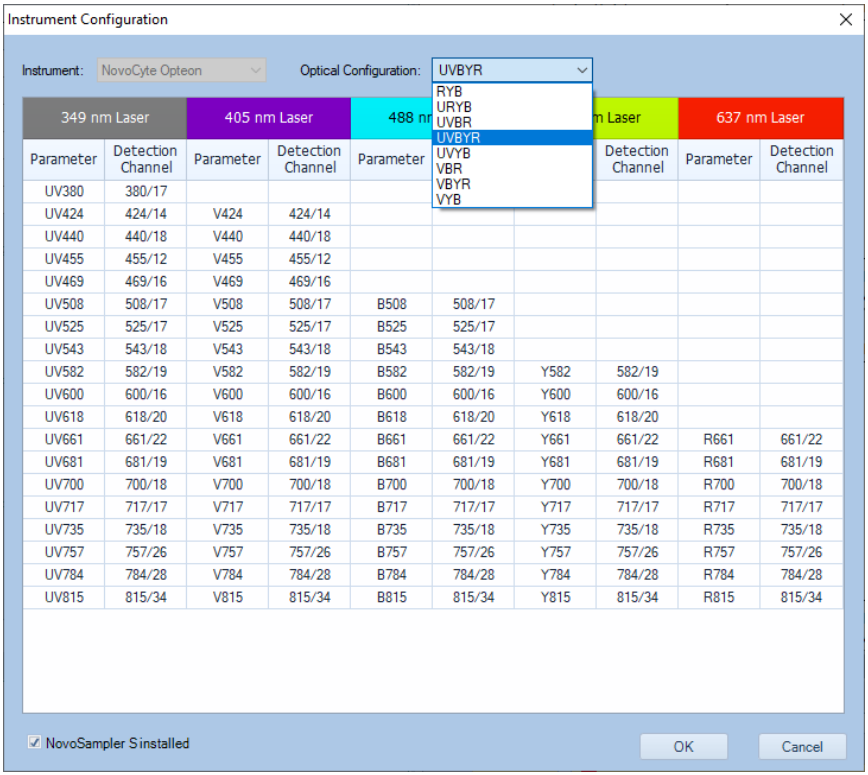
349 nm Laser		405 nm Laser		488 nm Laser		561 nm Laser		637 nm Laser	
Parameter	Detection Channel	Parameter	Detection Channel	Parameter	Detection Channel	Parameter	Detection Channel	Parameter	Detection Channel
UV380	380/17								
UV424	424/14	V424	424/14						
UV440	440/18	V440	440/18						
UV455	455/12	V455	455/12						
UV469	469/16	V469	469/16						
UV508	508/17	V508	508/17	B508	508/17				
UV525	525/17	V525	525/17	B525	525/17				
UV543	543/18	V543	543/18	B543	543/18				
UV582	582/19	V582	582/19	B582	582/19	Y582	582/19		
UV600	600/16	V600	600/16	B600	600/16	Y600	600/16		
UV618	618/20	V618	618/20	B618	618/20	Y618	618/20		
UV661	661/22	V661	661/22	B661	661/22	Y661	661/22	R661	661/22
UV681	681/19	V681	681/19	B681	681/19	Y681	681/19	R681	681/19
UV700	700/18	V700	700/18	B700	700/18	Y700	700/18	R700	700/18
UV717	717/17	V717	717/17	B717	717/17	Y717	717/17	R717	717/17
UV735	735/18	V735	735/18	B735	735/18	Y735	735/18	R735	735/18
UV757	757/26	V757	757/26	B757	757/26	Y757	757/26	R757	757/26
UV784	784/28	V784	784/28	B784	784/28	Y784	784/28	R784	784/28
UV815	815/34	V815	815/34	B815	815/34	Y815	815/34	R815	815/34

Instrument Configuration with Instrument Disconnected

When there is no instrument connected to the workstation or the instrument is powered off, user can view and select any one of available optical configurations

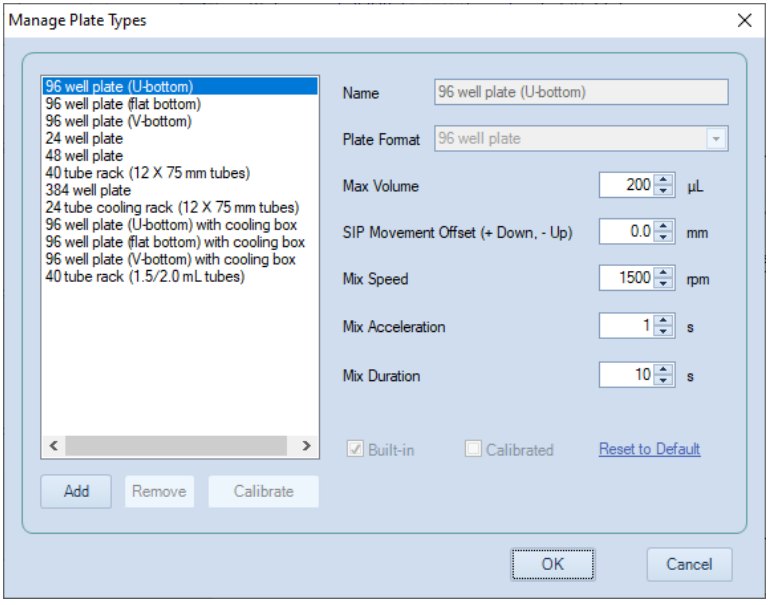
of the instrument displayed in the **Optical Configuration** field. The information displayed in this window will be automatically updated based on the selection. After selecting the optical configuration, click **OK**, and the software will restart. After restarting, the **Cytometer Setting** panel, 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 display the corresponding information of the connected instrument.

User can also view the NovoSampler S related software interface and functions by checking the **NovoSampler S installed** checkbox located at the lower left corner of **Instrument Configuration** window and clicking **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 S 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 S**.



NOTE When NovoSampler S 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 plate 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 panel in the **Manage Plate Types** window. Please refer to NovoSampler S Operator's Guide for more details on compatible plates.

All functions in this window are described below.

- **Name**: The name of the selected plate type. User cannot edit this area if the built-in plate type is selected. However, when new plate (i.e., user defined plate) is added, user can edit its name in this area.
- **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 define 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

Define 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 default value of zero.

NOTE

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

NOTICE

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

- **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.

Auto-Sampler	Plate Type	Range of Mixing Speed (rpm)	Default Mixing Speed (rpm)
NovoSampler S	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 (12 X 75 mm tubes)	200 - 1200	1000

	384 well plate	200 - 3000	2500
	24 tube cooling rack (12 X 75 mm tubes)	200 - 1200	1000
	96 well plate (U, V and Flat bottom) with cooling box	200 - 2000	1500
	40 tube rack (1.5/2.0 mL tubes)	200 - 1500	1500

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

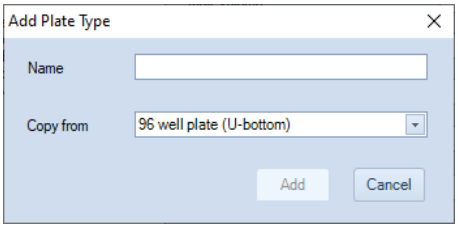
NOTE When NovoSampler S is selected to work in **Standard** and **High-Throughput** mode, the software will automatically apply the default settings for mixing cycle, rinse cycle, mixing speed, acceleration, mixing duration, sampling volume and flow rate.

- **Mix Acceleration**: The acceleration time for the orbital shaker to reach the specified **Mix Speed**. **Mix Acceleration** can be set 1 second (default) to 10 seconds for NovoSampler S.
- **Mix Duration**: The mixing time for the orbital shaker to mix the sample when NovoSampler S works in **Custom** mode. **Mix Duration** can be set from 1 second to 120 seconds. However, the default mix duration may vary when NovoSampler S works in other mode (i.e., **Standard** and **High Throughput** mode). Please refer to Plate Manager in this guide for more details.
- **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.

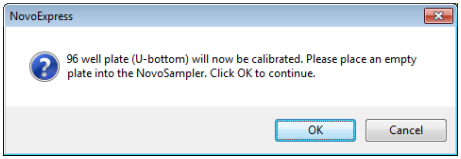
NOTE The built-in plate type has been tested with biological samples, including human peripheral blood sample and cultured cell lines, to determine the default mixing settings. At the default mixing settings, the biological samples can be effectively mixed and uniformly suspended inside each well.

- **Calibrated**: Indicate if the selected plate type is calibrated. Only calibrated plate type can be used in experiments.
- **Reset to Default**: Click this button to reset the **Max Volume** and **SIP Movement Offset** of the selected plate type to default, and reset the **Max Volume**, **SIP Movement Offset, Mix Speed, Mix Acceleration, and Mix Duration** of the selected plate type to default. Only built-in plate types can be reset to default.

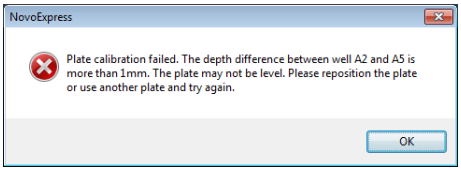
- **Remove**: Click this button to delete the selected plate type. Only user defined plate types can be removed.
- **Add**: Click this button to add a user defined plate type. Enter plate type name for new plate type in the prompted window. The **Copy from** function helps to quickly set up a customer 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 S 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.



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.



NOTICE 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 40 tube rack (12 X 75 mm tubes) or 24 tube cooling rack (12 X 75 mm tubes), ensure a 12 X 75 mm tube is fully inserted in each position of the tube rack. Before calibrating the 40 tube rack (1.5/2.0 mL tubes), ensure to cut off the cap from a 1.5 or 2.0 mL tube and fully insert the tube in each position of the tube rack.

NOTE

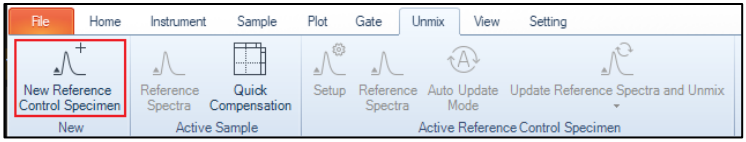
Once plate type calibration is initiated, the **Calibrate** button in the **Manage Plate Types** window will automatically become **Stop Calibration** button. Click **Stop Calibration** will stop the plate type calibration.

New Reference Control Specimen

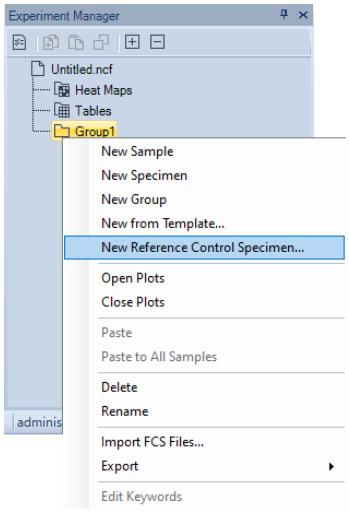
The **New Reference Control Specimen** window allows user to create and define new Reference Control specimen and sample.

This window can be accessed using one of the methods below.

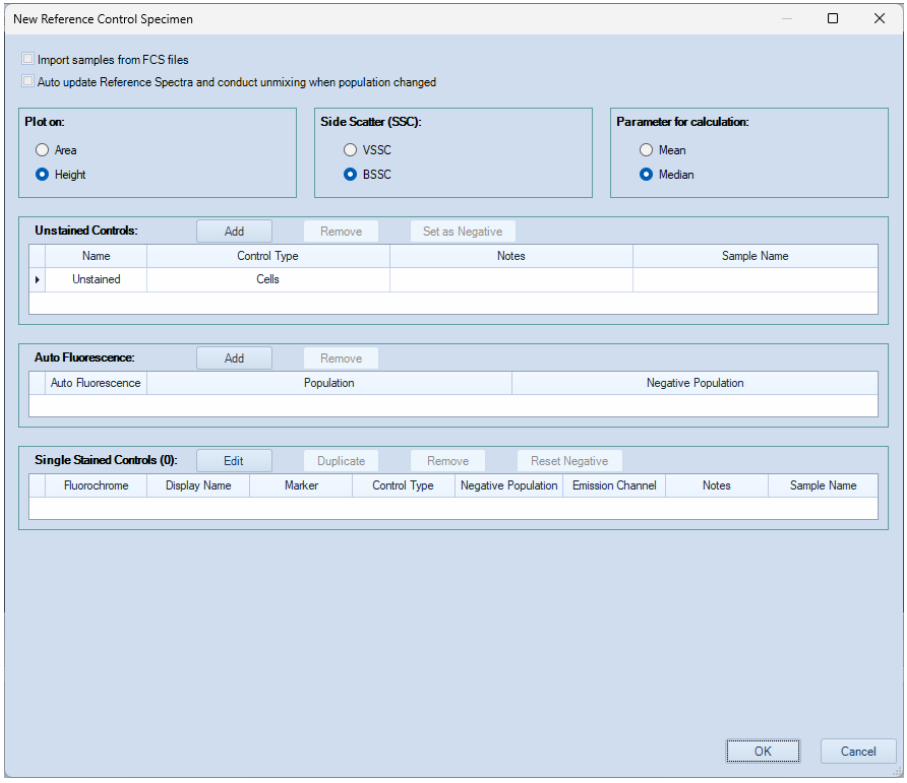
- Click the **New Reference Control Specimen** icon from the **Unmix** tab.



- Right click file node or group node in **Experiment Manager** panel and select **New Reference Control Specimen**.

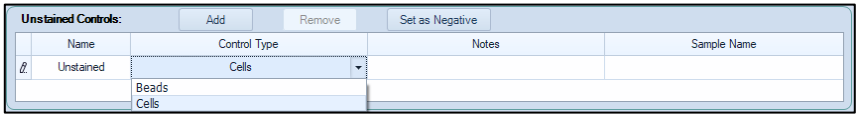


In the prompted window, user can view and define the Unstained Control sample, auto fluorescence settings, and single stained control sample, etc.

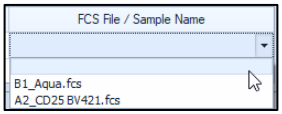


The functions included in this window are described below.

- **Import samples from FCS file:** Check to create Reference Control specimen from imported FCS file. Refer to Conduct Spectral Unmixing from Imported FCS Files for more details. This option is unchecked by default.
- **Auto update Reference Spectra and conduct unmixing when population changed:** When checked, if there are any changes to the gated population (e.g., changes to the gating of autofluorescence population, negative or positive population gating of single stained control sample), the associated Reference Spectra will be automatically updated followed by automatic data unmixing (or compensation) accordingly. This option is unchecked by default.
- **Plot on:** Allows user to select **Height** or **Area** when creating the plot.
- **Side Scatter (SSC):** Allows user to select **VSSC** (405nm SSC) or **BSSC** (488nm SSC) when creating the dot plot for unstained and single stained sample. The **BSSC** is checked by default. For instrument not equipped with VSSC module, this option will be automatically disabled.
- **Parameter for calculation:** Allows user to select **Mean** or **Median** when calculating the Reference Spectra. The **Median** is checked by default.
- **Unstained Controls:** Allows user to define the Unstained Control sample.
  - **Name:** Displays the name of the Unstained Control sample. User can double click associated cell and enter the desired name. **Unstained** is displayed by default.
  - **Control Type**: Displays the type of the Unstained Control sample. User can select **Beads** or **Cells** from the drop-down menu of associated cell. **Cells** is selected by default.



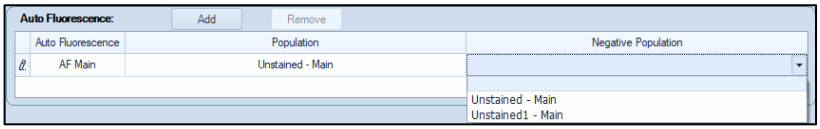
  - **Notes:** Displays the relevant notes associated with the unstained sample. User can double click the associated cell and enter the notes.
  - **Sample Name**: Displays the sample name of the Unstained Control sample. When user imports the control sample from an FCS file, the header of this column will be automatically changed to **FCS File/Sample Name** and user can select the designated FCS file from the drop-down menu of selected cell.



- **Add**: Click to add a new Unstained Control sample.
- **Remove**: Click to remove selected Unstained Control sample.
- **Set as Negative**: Click to set the main population of the selected Unstained Control sample as the negative population for all Single Stained Control samples.

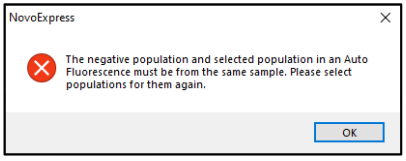
- **Auto Fluorescence:** Allows user to define the auto fluorescence control tag.
  - **Auto Fluorescence**: Displays the name of the auto fluorescence control tag. A default name (i.e., **AF Main**) is automatically generated based on associated population. User cannot edit it.
  - **Population**: Displays the population of the auto fluorescence control tag. User can select designated population from the drop-down menu of associated cell.

  - **Negative Population**: Displays the negative population of the auto fluorescence control tag. User can select designated population from the drop-down menu of associated cell as the negative population. When defined, the software will subtract it from the main population during calculation of the auto fluorescence Reference Spectra.



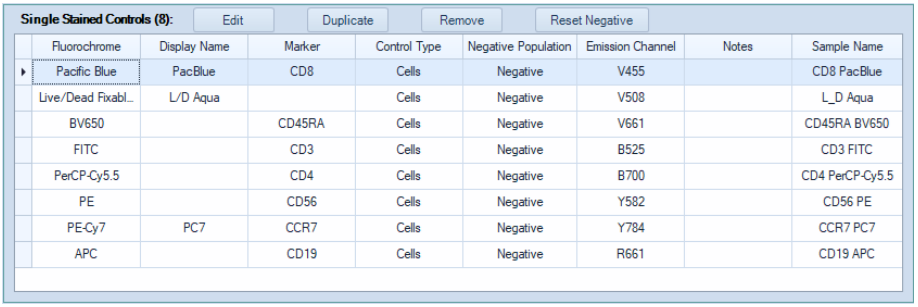
NOTE

Ensure to select the same unstained sample for the Negative Population and Population. Following error may be prompted if different samples are selected and user clicks **OK** in **New Reference Control Specimen** window.



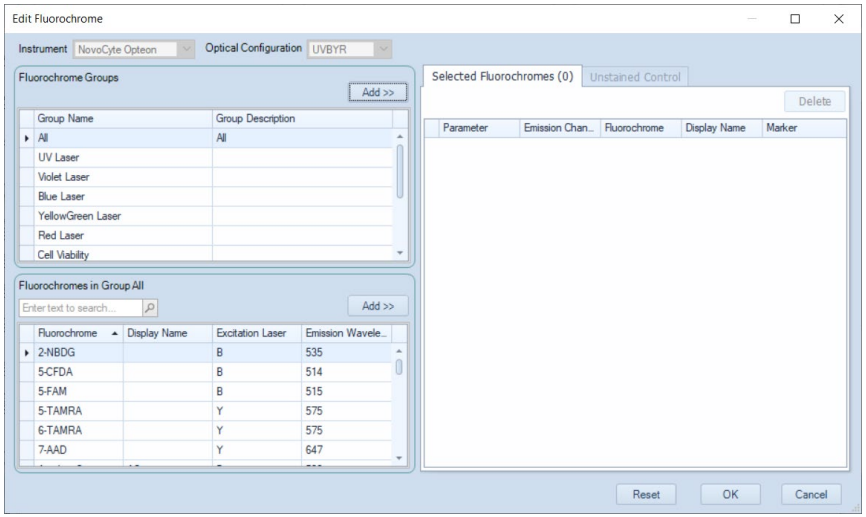
- **Add**: Click to add a new auto fluorescent control tag.
- **Remove**: Click to remove selected auto fluorescent control tag.

- **Single Stained Controls:** Allows user to define the Single Stained Control sample.



Fluorochrome	Display Name	Marker	Control Type	Negative Population	Emission Channel	Notes	Sample Name
Pacific Blue	PacBlue	CD8	Cells	Negative	V455		CD8 PacBlue
Live/Dead Fixabl...	L/D Aqua		Cells	Negative	V508		L_D Aqua
BV650		CD45RA	Cells	Negative	V661		CD45RA BV650
FITC		CD3	Cells	Negative	B525		CD3 FITC
PerCP-Cy5.5		CD4	Cells	Negative	B700		CD4 PerCP-Cy5.5
PE		CD56	Cells	Negative	Y582		CD56 PE
PE-Cy7	PC7	CCR7	Cells	Negative	Y784		CCR7 PC7
APC		CD19	Cells	Negative	R661		CD19 APC

  - **Edit**: Click to add new Single Stained Control sample in prompted **Edit Fluorochrome** window below. Refer to Fluorochrome Setting in this guide for more details of this window.



  - **Duplicate**: Click to duplicate the selected single stained control sample and add the duplicated single stained control sample as new control sample with suffix of duplicating number added to the end of the sample name. For example, if user selects fluorochrome 5-CFDA, clicks **Duplicate**, a duplicated single stained control sample with name of **5-CFDA Cells 1** will be automatically added below the selected sample. Note the default control type is **Cells**. When user changes the control type to **Beads**, the sample name will be changed accordingly.

NOTE When user clicks **Edit** from the **Single Stained Controls** panel to open the **Edit Fluorochrome** window, double click the selected fluorochrome, and click **OK**. The software will automatically add the selected fluorochrome into the **Single Stained Controls** panel as a new control sample with the associated sample name same as the fluorochrome name. If user double clicks the selected fluorochrome more than once, the software will add a duplicated sample with the suffix of **Control Type** and a duplicating number added to the end of the sample name.

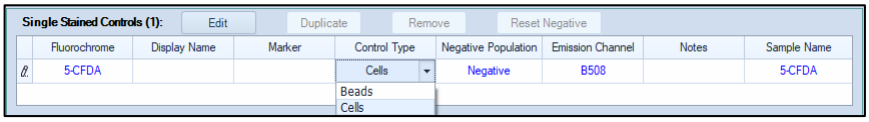
NOTE The suffix of a duplicated control sample can also be changed in the **Experiment Manager, Cytometer Control (Active Sample Information), Worklist, and Plate Manager** window.

NOTE When user edits the **Single Stained Controls** panel in **Reference Control Specimen Setup** window or **New Reference Control Specimen** window, any changes to the **Emission Channel** or **Virtual Filter** information will be automatically applied to any duplicated single stained control samples with the same fluorochrome.

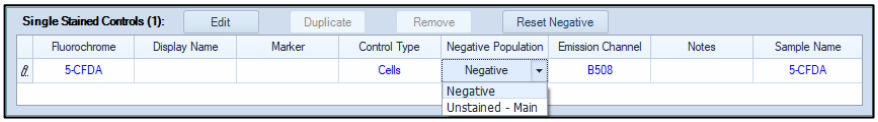
- **Remove**: Click to remove selected Single Stained Control sample.
- **Reset Negative**: Click to reset the negative population for the Single Stained Control samples to its default negative population.
- **Fluorochrome:** Displays the name of the fluorochrome selected for Single Stained Control sample. User cannot edit it.
- **Display Name:** Displays the name to be displayed for the associated fluorochrome. User can double click associated cell and enter the desired name. When defined, the newly created plot and statistical table will use **Display Name** as parameter name. When not defined, the newly created plot and statistical table will use **Fluorochrome** as parameter name.
- **Marker:** Displays the antibody or biomarker for the associated fluorochrome. User can double click associated cell and edit the name.

NOTE The default name of the Single Stained Control sample will be combination of defined **Marker** and **Display Name (or Fluorochrome)**. For example, if user defines **CD3** for **Marker**, **BV510** for **Display Name**, then the name of the single stained sample will be **CD3 BV510**.

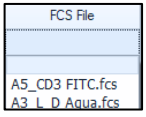
- **Control Type**: Displays the type of the Single Stained Control sample. User can select **Beads** or **Cells** from the drop-down menu of associated cell. **Cells** is selected by default.



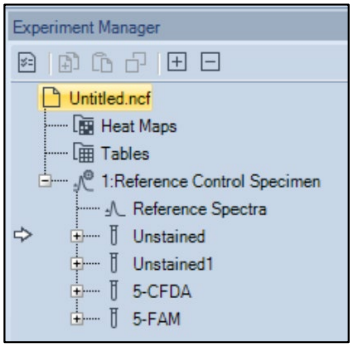
- **Negative Population:** Displays the negative population of the Single Stained Control. User can select designated population from the drop-down menu of associated cell to define it as the negative population.



- **Emission Channel:** Displays the emission channel information for associated fluorochrome. Software will use this information to create histogram of Single Stained Control sample by default. Any changes to this information will be automatically applied to **any** duplicated single stained control samples with the same fluorochrome.
- **Notes:** Displays the relevant notes associated with the single stained sample. User can double click the associated cell and enter the notes.
- **Sample Name**: Displays the sample name of the Single Stained Control sample.
- **FCS File**: This column is displayed only after user imports FCS files as single stained control samples. When displayed, it shows the FCS file name of the Single Stained Control sample. User can select the designated FCS file from the drop-down menu of selected samples.



- **OK:** Click to make all changes effective. The Reference Control specimen with node icon of  and defined Reference Control samples (e.g., Unstained and Single Stained control samples) will be automatically created in **Experiment Manager** (see example below).



Selected plots associated with defined control sample(s) will be also automatically created in each workspace (refer to table below for the type of plots software automatically creates in each workspace).

Reference Control Sample	Raw Workspace	Unmixed Workspace
Unstained Control Sample	• Density Plot of FSC Height (or Area) versus BSSC (or VSSC) Height (or Area) • Spectrum Density Plot	• Density Plot of FSC Height (or Area) versus BSSC (or VSSC) Height (or Area) • Density plot of defined auto fluorescence and defined fluorochrome Height (or Area) versus BSSC (or VSSC) Height (or Area)
Single Stained Control Sample	• Density Plot of FSC Height (or Area) versus BSSC (or VSSC) Height (or Area) • Spectrum Density Plot • Histogram plot of emission channel versus event count	• Density Plot of FSC Height (or Area) versus BSSC (or VSSC) Height (or Area) • Density plot of defined fluorochrome Height (or Area) versus defined auto fluorescence and other defined fluorochrome Height (or Area)

- **Cancel:** Click to cancel the operation.

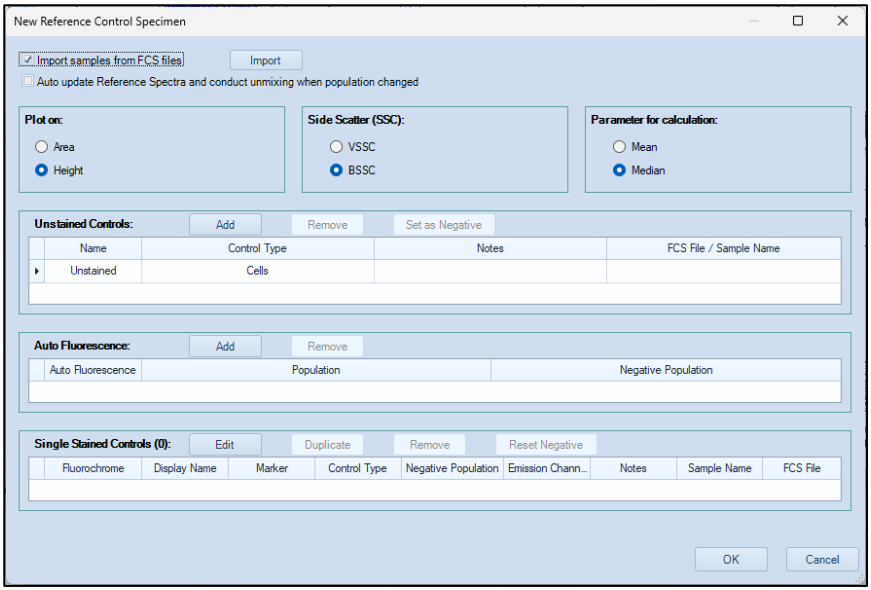
Conduct Spectral Unmixing from Imported FCS Files

To perform Spectral Unmixing directly from the imported FCS file:

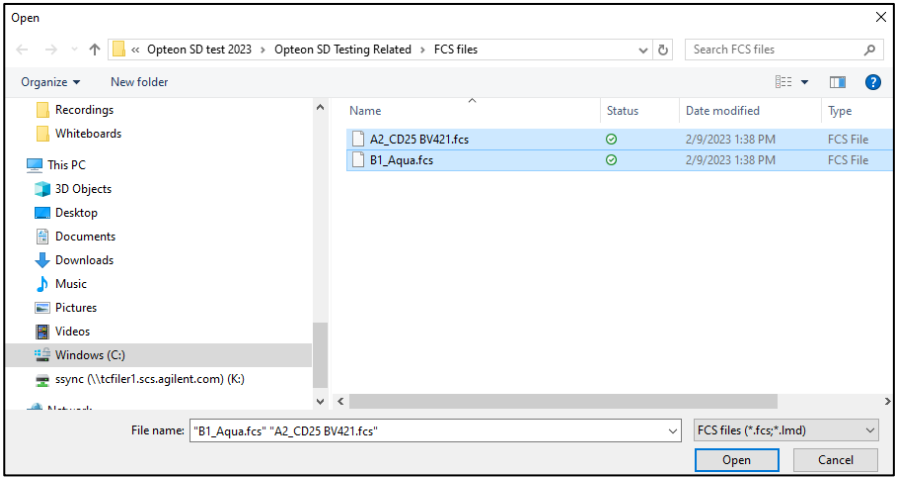
1 Click **Unmix** > **New Reference Control Specimen** to open the **New Reference Control Specimen** window. Select the **Import Samples from FCS files** and click **Import** button.

NOTE

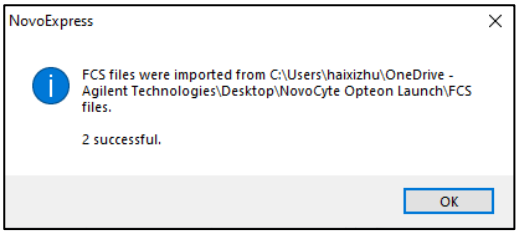
This window can also be accessed by right clicking the experiment file and selecting **New Reference Control Specimen** in the **Experiment Manager** window.



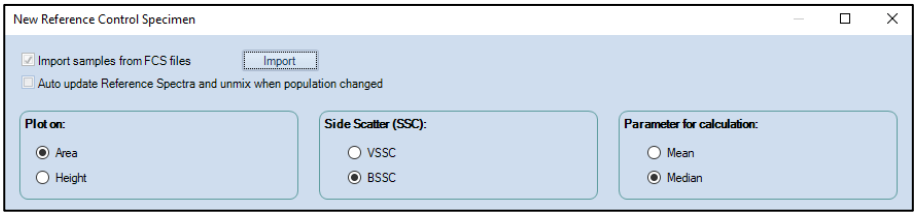
2 In the prompted window, select the single file or multiple files while pressing the Shift key in the keyboard. Click **Open** to import the selected file(s) to the software.



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

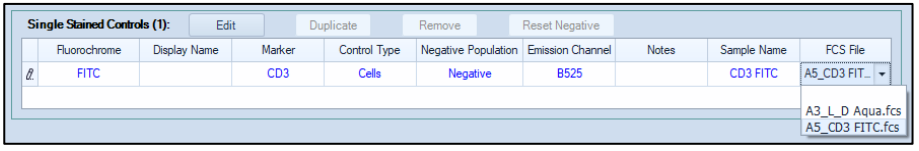


4 Set the unmixing parameters in the **New Reference Control Specimen** window (i.e., **Area** or **Height**, **VSSC** or **BSSC**, **Median** or **Mean**).



5 Ensure the selected FCS file for each unstained and single stained sample is correct. To select different single stained FCS file, click the FCS file name, select the desired file from the pull-down menu.

NOTICE The software will automatically identify the fluorochrome and marker associated with unstained and single stained sample based on the keyword of the file name (e.g., CD3 FITC). Users need to verify and select the correct FCS file for each unstained and Single Stained Control sample.



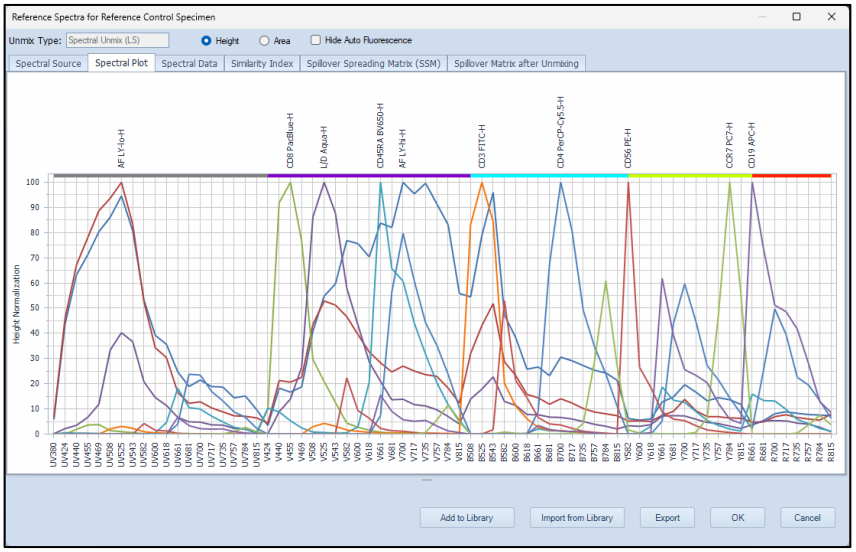
6 Click **OK** and NovoExpress (Opteon) will automatically create the corresponding unstained and Single Stained Control samples, calculate the Reference Spectra based on the samples imported. Once generated, the Reference Spectra can be applied to other experiment samples.

Reference Spectra

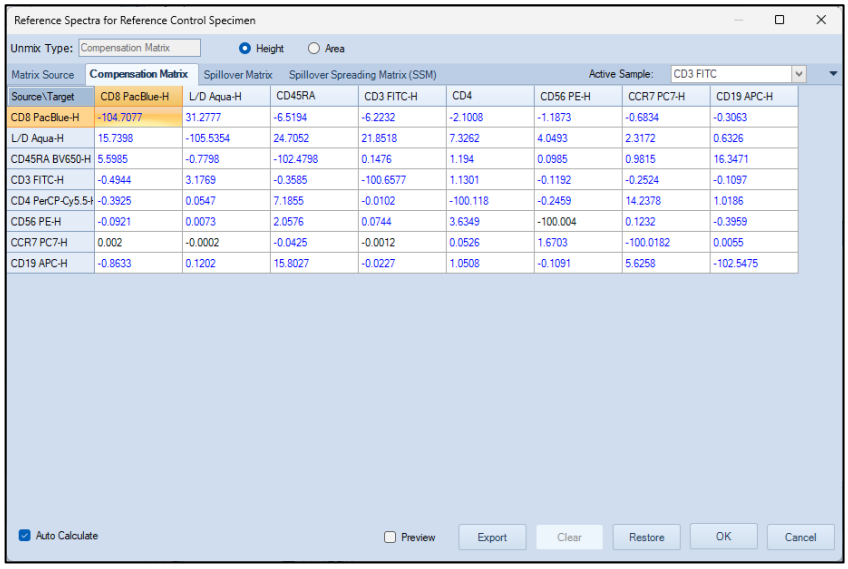
User can view and edit the Reference Spectra information for associated specimen or sample through the **Reference Spectra** window. This window will

display different information depending on whether user selects Spectral Unmix or Compensation to analyze the data (Refer to Right-Click Menu of Experiment Manager in this guide for instructions to switch between Spectral Unmix and Compensation).

- When Spectral Unmixing is selected, user can view and edit **Spectral Source, Spectral Plot, Spectral Data, Similarity Index, Spillover Spreading Matrix (SSM),** and **Spillover Matrix after Unmixing** associated information, etc. in this window**.**



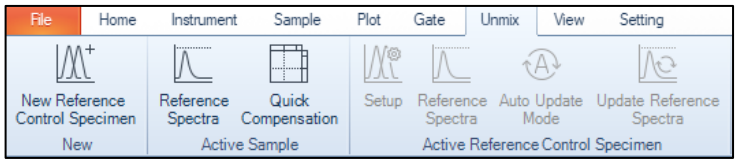
- When Compensation is selected, user can view and edit **Compensation Matrix, Spillover Matrix, Spillover Spreading Matrix (SSM)** associated information, etc. in this window.



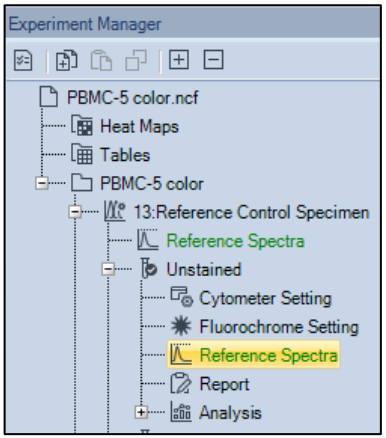
The **Reference Spectra** window consists of functions and information for Reference Control specimen, Unstained Control sample, Single Stained Control sample and experiment sample. For demonstration purpose, the **Reference Spectra** window of Reference Control Specimen is used as example to show and describe each function. For other control sample or experiment sample, certain functions are not available and will be noted in associated description below.

To access **Reference Spectra** window of selected specimen or sample, use one of the methods below.

- Click to select the target specimen or sample in **Experiment Manager**. If Reference Control Specimen, Unstained Control sample, or Single Stained Control sample is selected, click **Active Reference Control Specimen > Reference Spectra** from the **Unmix** tab of software main window. If experiment sample is selected, click **Active Sample > Reference Spectra** from the **Unmix** tab of software main window.



- Double click **Reference Spectra** node of associated specimen or sample from the **Experiment Manager**.



Following options are available in **Reference Spectra** window for any specimen or sample no matter whether user selects Spectral Unmix or Compensation.

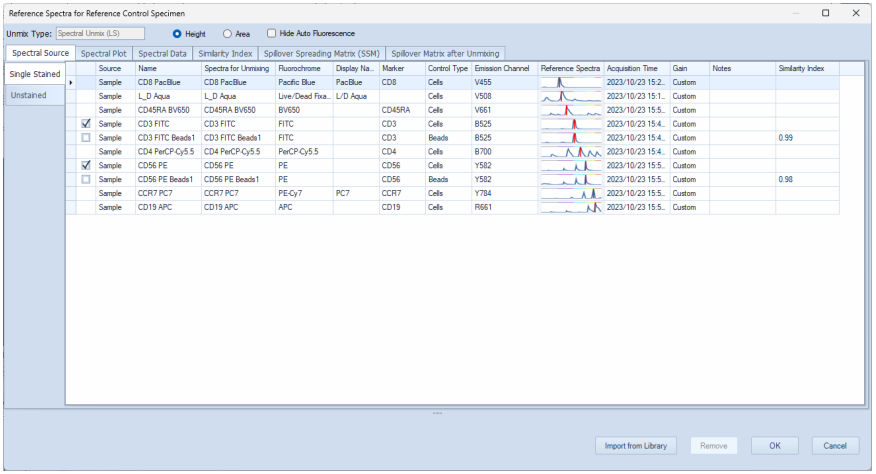
- **Unmix Type:** Displays the unmix type.
- **Height:** Check to use Height parameter for analysis.
- **Area**: Check to use Area parameter for analysis.

Reference Spectra for Reference Control for Spectral Unmix

Following options are available only when Spectral Unmix is selected**.**

- **Only display the fluorochrome used**: When checked, only the fluorochrome checked in **Fluorochrome Setting** panel will be displayed in **Spectral Plot, Spectral Data, Similarity Index, Spillover Spreading Matrix (SSM), and Spillover Matrix after Unmixing** panel. This option will not be available if all fluorochromes are checked in **Fluorochrome Setting** window.
- **Hide Auto Fluorescence**: When checked, the auto fluorescence information will not be displayed in **Spectral Plot, Spectral Data, Similarity Index, Spillover Matrix after Unmixing** panel of this window.
- **Spectral Source**: This panel will be available only if the duplicated single stained control sample or spectra exists. User can click **Single Stained** sub-tab to view the spectra information for all defined single stained control samples, including **Source, Name, Spectra for Unmixing, Fluorochrome, Display Name, Marker, Control Type, Emission Channel, Reference Spectra, Acquisition Time, Gain, Notes, Similarity Index**. Note the **Name** column displays the sample name, and **Spectra for Unmixing** column displays the spectra info software used to unmix the associated control sample. By default, the software will use the spectra acquired from data collection for associated

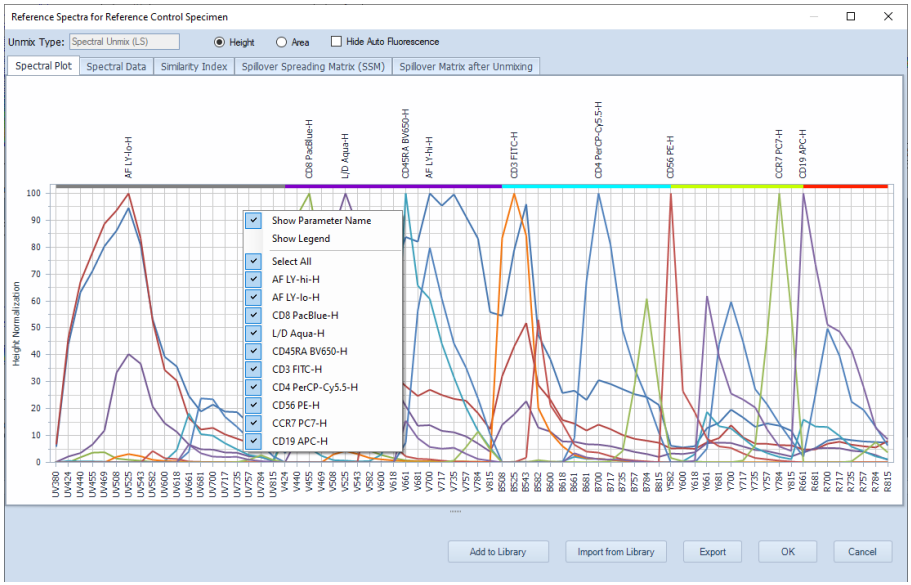
control samples. User can select different spectra from its drop-down menu. The **Similarity Index** column displays the calculated similarity index value between the checked sample and present sample. This value automatically updates when user checks different samples.



NOTE When **Spectral Source** tab is displayed, if there are more than one spectra associated with one fluorochrome, only one spectra can be checked and will be used for spectrum unmixing. The information in other tabs of the **Reference Spectra** window (e.g., **Spectral Plot**) will be automatically updated accordingly.

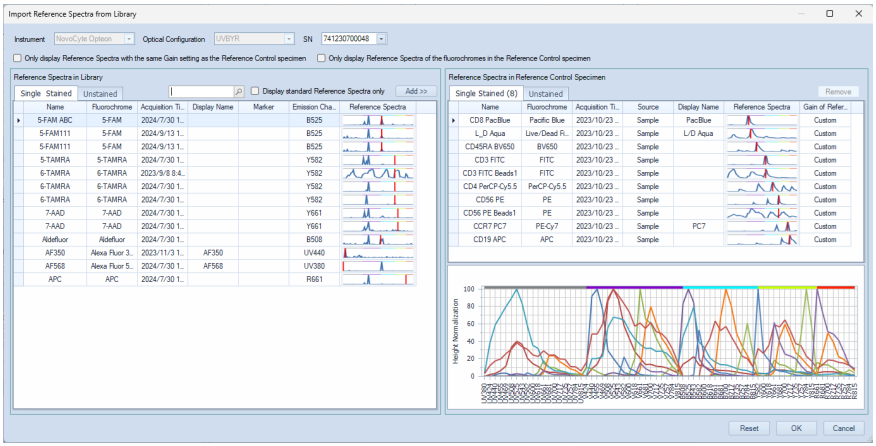
NOTE When **Spectral Source** tab is displayed, user can only edit **Spectra for Unmixing** column and check/uncheck the check box in front of each spectra of corresponding sample.

- **Spectral Plot**: This panel is available only when the duplicated single stained control sample or spectra exists. When clicked, user can view spectral plot for selected fluorochrome of associated sample or specimen. When right clicking the plot, user can select to display the fluorochrome name on top of the plot by checking **Show Parameter Name**, or display the plot legend by checking **Show Legend**, or check/uncheck the designated fluorochrome to have the associated spectra to be displayed/not displayed.



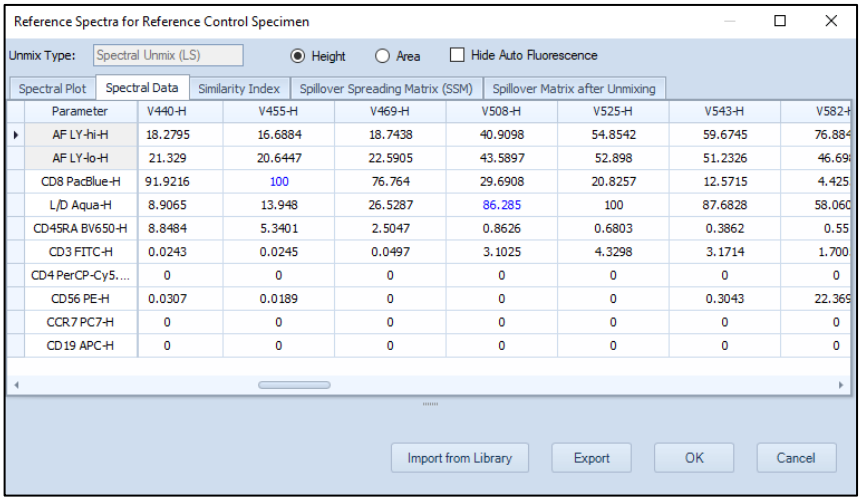
Following additional functions are available in **Spectral Plot** panel.

- **Add to Library:** This option is available only for Reference Control sample or specimen. When clicked, the displayed Reference Spectra will be added into the **Reference Spectra Library**. Please refer to Add Reference Spectra to Library in this guide for more details.
- **Import from Library:** When clicked, following window will be prompted. User can import the selected Reference Spectra from the Reference Spectra library to the target Reference Control sample or specimen. Please refer to Import Reference Spectra from Library in this guide for more details.



- **Export:** Click to export the displayed spectral plot in format of **PNG, JPEG, Bitmap, GIF, TIFF,** or **Enhanced Metafile**.

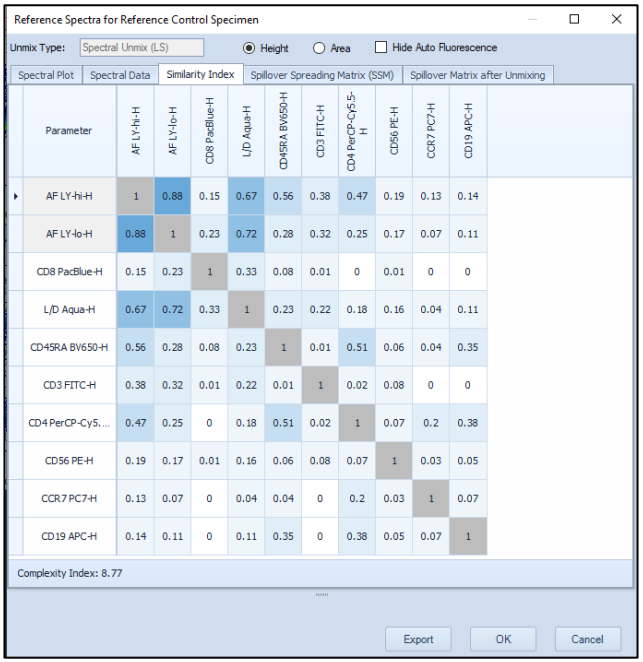
- **Spectral Data:** When clicked, user can view the normalized MFI value of each channel for Reference Spectra of selected sample (or specimen). The value for associated emission channel is displayed in blue color. User can also click **Export** in the bottom of this panel to export the displayed spectral data in format of **CSV.** For Reference Control sample or specimen, the **Import from Library** option will be available for user to import the selected Reference Spectra from the Reference Spectra library. Please refer to Import Reference Spectra from Library in this guide for more details.



Parameter	V440-H	V455-H	V469-H	V508-H	V525-H	V543-H	V582-H
AF LY-hi-H	18.2795	16.6884	18.7438	40.9098	54.8542	59.6745	76.88
AF LY-lo-H	21.329	20.6447	22.5905	43.5897	52.898	51.2326	46.69
CD8 PacBlue-H	91.9216	100	76.764	29.6908	20.8257	12.5715	4.425
L/D Aqua-H	8.9065	13.948	26.5287	86.285	100	87.6828	58.060
CD45RA BV650-H	8.8484	5.3401	2.5047	0.8626	0.6803	0.3862	0.55
CD3 FITC-H	0.0243	0.0245	0.0497	3.1025	4.3298	3.1714	1.700
CD4 PerCP-Cy5....	0	0	0	0	0	0	0
CD56 PE-H	0.0307	0.0189	0	0	0	0.3043	22.369
CCR7 PC7-H	0	0	0	0	0	0	0
CD19 APC-H	0	0	0	0	0	0	0

- **Similarity Index**: When clicked, user can view the **Similarity Index** value between each fluorochromes. The **Complexity Index** value of all

fluorochromes is displayed in the lower left corner of this window. User can also click **Export** in the bottom of this window to export the displayed Similarity Index data in format of **CSV.**



Parameter	AF LY-hi-H	AF LY-lo-H	CD8 PacBlue-H	L/D Aqua-H	CD45RA BV650-H	CD3 FITC-H	CD4 PerCP-Cy5.5-H	CD56 PE-H	CCR7 PC7-H	CD19 APC-H
AF LY-hi-H	1	0.88	0.15	0.67	0.56	0.38	0.47	0.19	0.13	0.14
AF LY-lo-H	0.88	1	0.23	0.72	0.28	0.32	0.25	0.17	0.07	0.11
CD8 PacBlue-H	0.15	0.23	1	0.33	0.08	0.01	0	0.01	0	0
L/D Aqua-H	0.67	0.72	0.33	1	0.23	0.22	0.18	0.16	0.04	0.11
CD45RA BV650-H	0.56	0.28	0.08	0.23	1	0.01	0.51	0.06	0.04	0.35
CD3 FITC-H	0.38	0.32	0.01	0.22	0.01	1	0.02	0.08	0	0
CD4 PerCP-Cy5....	0.47	0.25	0	0.18	0.51	0.02	1	0.07	0.2	0.38
CD56 PE-H	0.19	0.17	0.01	0.16	0.06	0.08	0.07	1	0.03	0.05
CCR7 PC7-H	0.13	0.07	0	0.04	0.04	0	0.2	0.03	1	0.07
CD19 APC-H	0.14	0.11	0	0.11	0.35	0	0.38	0.05	0.07	1

NOTE

Similarity Index measures the fluorochrome pair uniqueness on a scale from 0 to 1. Values close to 0 indicate that the full spectrum signatures of the two fluorochromes are very different from each other, and values close to 1 indicate that the signatures are very similar to each other.

NOTE

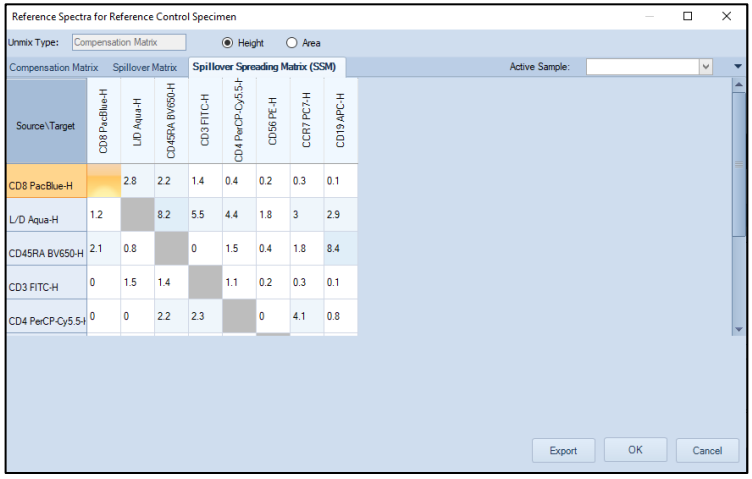
Complexity Index measures the uniqueness of all fluorochromes in a full spectrum cytometry panel. In general, the lower the value, the easier it will be to work with the fluorochromes in the panel as the overall spread in the panel will be low. The higher the value, the more challenging it will be to work with the fluorochromes in the panel as the overall spillover spread is higher. This value in general may increase with panel size.

- **Spillover Spreading Matrix (SSM):** SSM is a measure of the performance of an instrument for a given panel. Click to display the **Spillover Spreading Matrix (SSM)** values of associated fluorochromes. SSM values are calculated based on formula below.

$$SS(\mathrm{X} \rightarrow \mathrm{Y}) = \sqrt{\frac{\Delta(\sigma_Y^2)}{\Delta Median_X}} = \sqrt{\frac{(\sigma_Y^P)^2 - (\sigma_Y^N)^2}{Median_X^P - Median_X^N}}$$

where $SS(\mathrm{X} \rightarrow \mathrm{Y})$ is the spillover spreading value from fluorochrome X to Y. σ_Y is the difference between 84th percentile and 50th percentile of population. P represents the positive population of single stained X, N represents the negative population of single stained X or unstained.

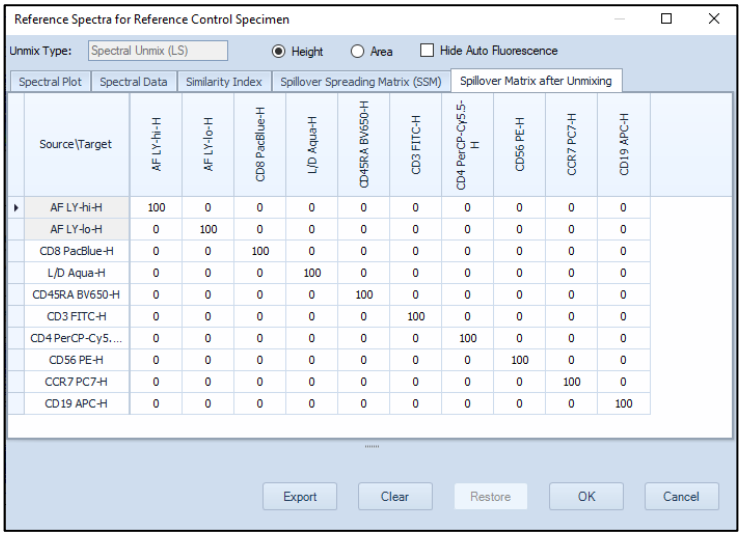
User can click **Export** at the bottom of this panel to export the matrix in format of **CSV.**



NOTE

SSM panel is only available for Reference Control Specimen and Single Stained Control Sample when user selects either Spectral Unmixing or Compensation to analyze the data.

- **Spillover Matrix after Unmixing**: Click to view and edit the Spillover Matrix value after Spectral Unmixing. User can click **Export** in the bottom of this panel to export the displayed information in format of **CSV.** To edit the Spillover Matrix, double click the cell and enter the new value. To clear all the edited Spillover Matrix, click **Clear**. To restore the Spillover Matrix to the one that is last saved, click **Restore**.



NOTE

Spillover Matrix is a matrix where each row contains value of spillover of a fluorochrome into the others. In an example of calculating spillover, the spillover of FITC into the PE using median or mean height measurements is shown below. The values below are measured from the single stained FITC sample.

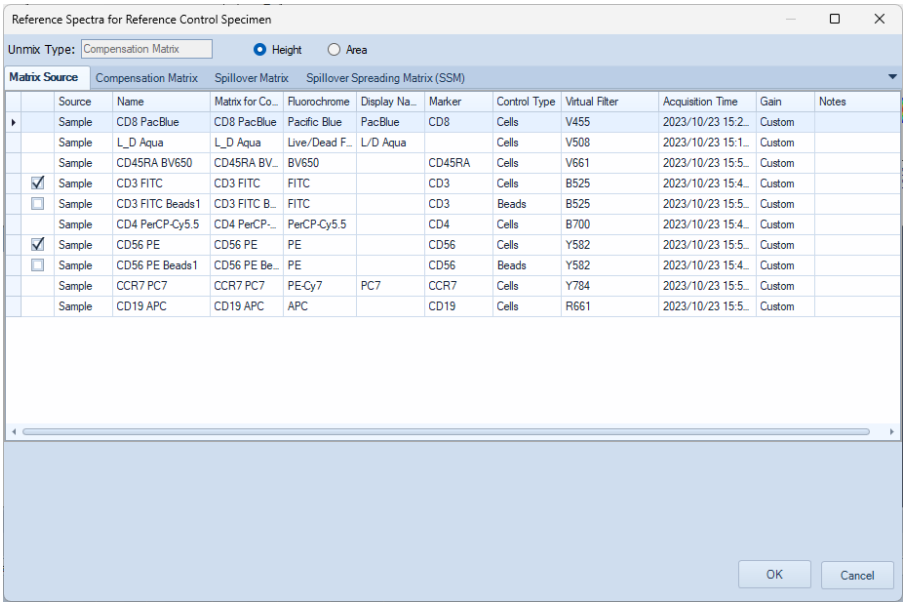
$$\text{Spillover of FITC into PE} = \frac{(X_{PE,positive} - X_{PE,negative})}{(X_{FITC,positive} - X_{FITC,negative})}$$

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

Reference Spectra for Reference Control for Compensation

Following options are available only when Compensation is selected.

- **Matrix Source**: This tab is only available when a duplicated single stained control sample exists. User can view the information for all defined single stained control samples, including **Source, Name, Matrix for Compensation, Fluorochrome, Display Name, Marker, Control Type, Virtual Filter, Acquisition Time, Gain, Notes**. Note the **Name** column displays the sample name, and **Matrix for Compensation** column displays the matrix info the software used to compensate the associated control sample. By default, the software will use the matrix acquired from data collection for the associated control sample. User can select a different matrix from its drop-down menu.



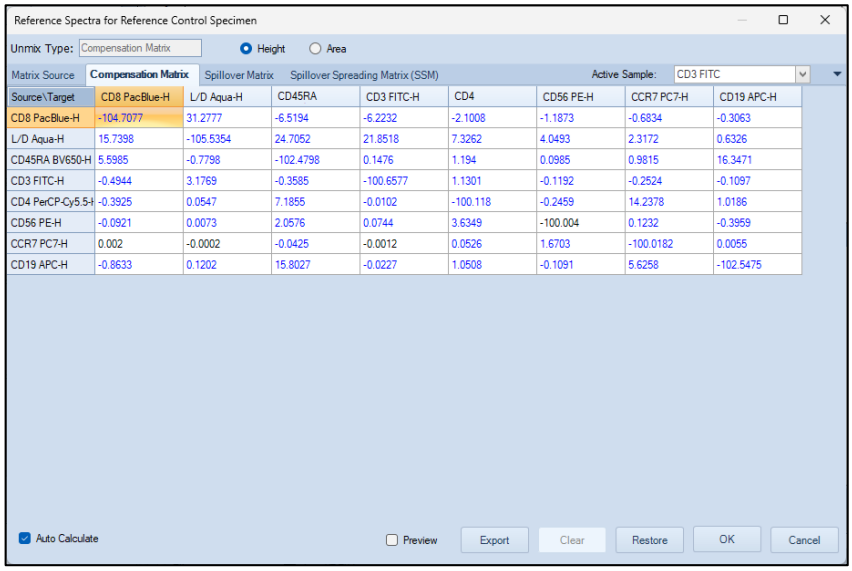
NOTE

When **Matrix Source** tab is displayed, if there are more than one compensation matrix associated with one fluorochrome, only one matrix can be checked and will be used for data compensation. The information in other tabs of the **Reference Spectra** window (e.g., **Compensation Matrix**) will be automatically updated accordingly.

NOTE

When **Matrix Source** tab is displayed, user can only edit **Matrix for Compensation** column and check/uncheck the check box in front of each matrix of corresponding sample.

- **Compensation Matrix**: Click to display the compensation matrix coefficients of associated fluorochromes.



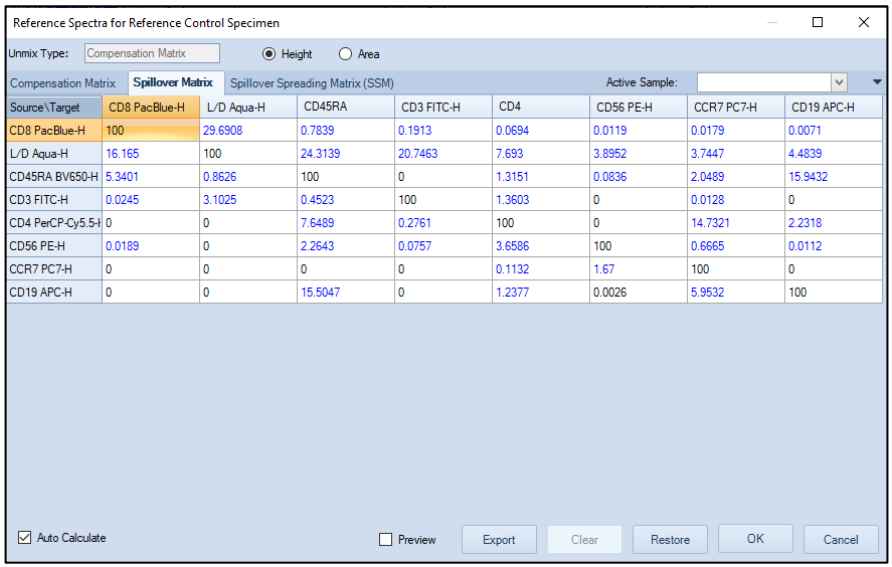
NOTE

Compensation Matrix is used to directly calculate the compensated fluorescent intensity of each fluorochrome through sum of associated negative of column percentage multiplied by measured fluorescent intensity value from associated Virtual Filter(s). It is inversely related to the Spillover Matrix.

Following additional functions are available in **Compensation Matrix** panel.

- **Active Sample**: Allows user to view and select the active sample from the drop-down menu. The plots in **Unmixed Workspace** will be automatically updated based on user selection of the active sample. This option is available only for Reference Control Specimen.
- **Auto Calculate**: When checked, the software will automatically re-do the auto compensation if there is any change to the Spillover Matrix coefficients.
- **Preview**: When checked, the plots in **Unmixed Workspace** will be continually updated in real time without exiting out of the current window as adjustments are made to the compensation matrix.
- **Export:** Click to export the compensation matrix to CSV file.
- **Clear**: Click to reset the compensation matrix. The compensation matrix coefficients will be reset to zero.
- **Restore**: Click to restore the compensation matrix to the last saved one.

- **Spillover Matrix:** Click to display the Spillover Matrix coefficients of associated fluorochromes.



Source\Target	CD8 PacBlue-H	L/D Aqua-H	CD45RA	CD3 FITC-H	CD4	CD56 PE-H	CCR7 PC7-H	CD19 APC-H
CD8 PacBlue-H	100	29.6908	0.7839	0.1913	0.0694	0.0119	0.0179	0.0071
L/D Aqua-H	16.165	100	24.3139	20.7463	7.693	3.8952	3.7447	4.4839
CD45RA BV650-H	5.3401	0.8626	100	0	1.3151	0.0836	2.0489	15.9432
CD3 FITC-H	0.0245	3.1025	0.4523	100	1.3603	0	0.0128	0
CD4 PerCP-Cy5.5-H	0	0	7.6489	0.2761	100	0	14.7321	2.2318
CD56 PE-H	0.0189	0	2.2643	0.0757	3.6586	100	0.6665	0.0112
CCR7 PC7-H	0	0	0	0	0.1132	1.67	100	0
CD19 APC-H	0	0	15.5047	0	1.2377	0.0026	5.9532	100

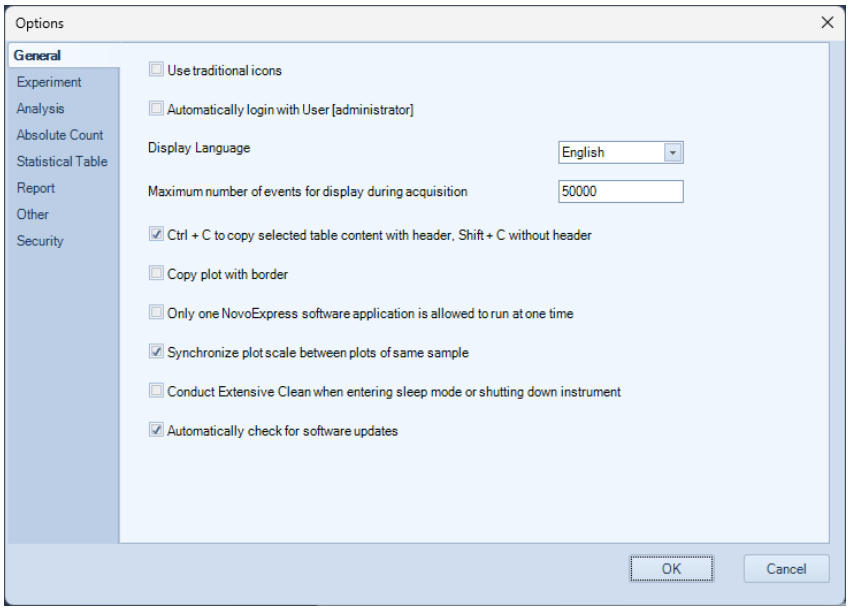
Following additional functions are available in **Spillover Matrix** panel.

- **Active Sample**: Allows user to view and select the active sample from the drop-down menu. The plots in **Unmixed Workspace** will be automatically updated based on user selection of the active sample. This option is available only for Reference Control Specimen.
- **Auto Calculate**: When checked, the software will automatically re-do the auto compensation if there is any change to the Spillover Matrix coefficients. By default, this option is checked. User can uncheck it to enter the manual calculation mode. In manual calculation mode, a slide bar will appear allowing user to quickly modify the coefficient for the selected cell. Alternatively, user can also double click individual cell in the matrix to edit the value directly. Please note user cannot edit the matrix value when **Auto Calculate** is checked.
- **Preview**: When checked, the plots in **Unmixed Workspace** will be continually updated in real time without exiting current window as adjustments are made to the spillover matrix.
- **Export:** Click to export the Spillover Matrix to CSV file.
- **Clear**: Click to reset the Spillover Matrix. The Spillover Matrix coefficients will be reset to zero.
- **Restore**: Click to restore the Spillover Matrix to the last saved one.

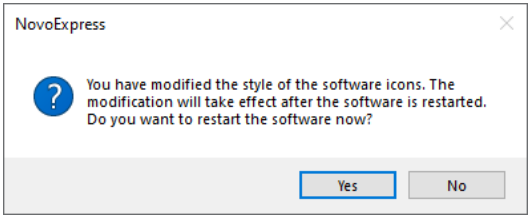
Option Settings

When user clicks **Setting > Options > Option Settings** from the software **Menu Bar**, the **Options** window will be prompted. User can define each setting in **General, Experiment, Analysis, Absolute Count, Statistical Table, Report**, and **Security** sub-window.

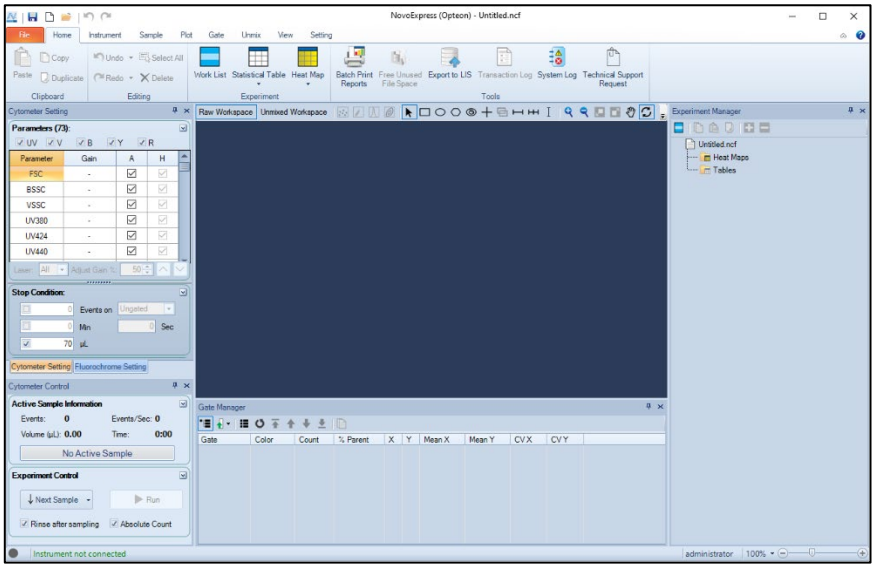
- **General:** Allows user to define general settings. This sub-window will be displayed by default when user clicks **Option Settings** from the software menu bar.



- **Use traditional icons**: Allows user to select to display all software icons in traditional style. By default, this option is unchecked. When checked, following window will be promoted.



After user clicks **Yes**, the software will automatically restart, and the traditional icons will be applied after user logs back into the software as shown below.



- **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**: Indicates the language software use to display.

- **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 (Opteon) is allowed to run at a time. An error message will be displayed when user tries to run a second instance of NovoExpress (Opteon).

- **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.

  User can also click **Synchronize Plot Scale** button on workspace toolbar to quick switch this setting. Refer to Workspace Toolbar for more information.

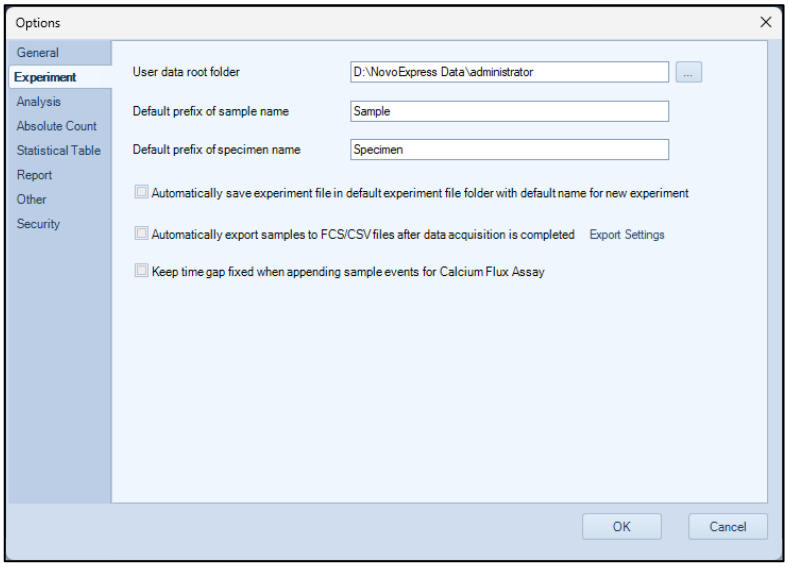
- **Conduct Extensive Clean when entering sleep mode or shutting down instrument**

  When selected, the instrument will automatically perform the **Extensive Clean** procedure during instrument shut down or when instrument enters sleep mode. The total time required for instrument to shut down or enter the sleep mode is around 30 min. This option is available only when instrument is properly connected and powered on. Please contact Agilent technical support for further questions on this procedure.

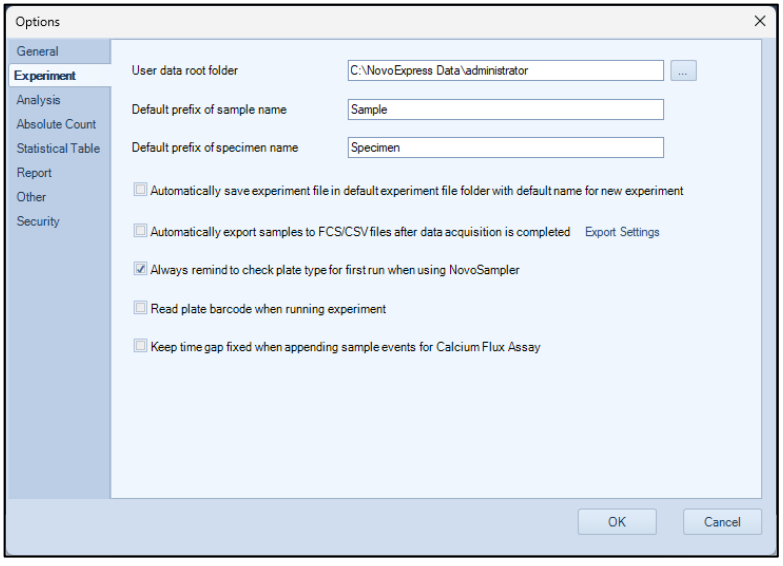
- **Automatically check for software updates**:

  When selected, the software will automatically check for any software updates when the workstation is connected to internet.

- **Experiment**: Allows user to define experiment related settings. This window displays additional settings when NovoSampler S is connected.



When NovoSampler S is not connected



When NovoSampler S is connected

Following functions are included in **Experiment** panel.

- **User data root folder**:

  Sets the default folder for saving the experiment file.

- **Default prefix of sample name**:

  Sets the prefix for the name of the experiment sample.

- **Default prefix of specimen name**:

  Sets the prefix for the name of the experiment specimen.

- **Automatically save experiment file in default experiment file folder with default name for new experiment**:

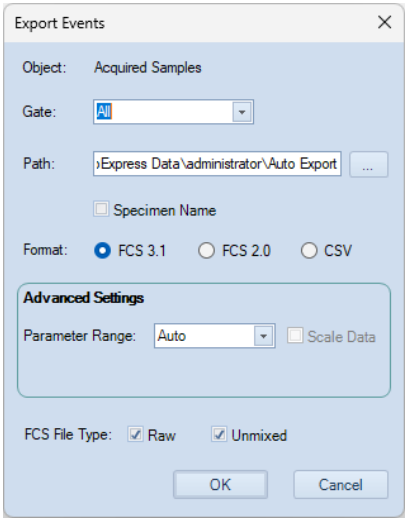
  This setting determines how to save a current experiment file when a new experiment is started.

  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.

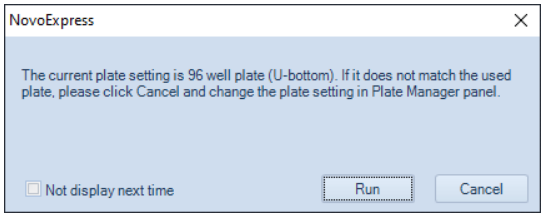
- **Automatically export samples to FCS/CSV files after data acquisition is completed**:

  If selected, the software will automatically generate and export an 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 S is

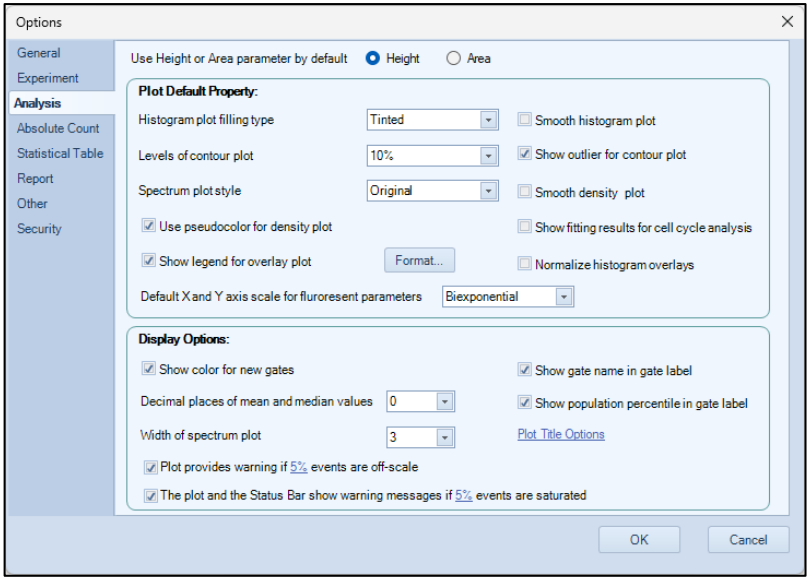
connected. With this setting checked, a window will be prompted to remind user to check if the correct plate type is selected before starting the experiment. It is highly recommended to enable this setting to remind user to check and make sure the correct plate type is selected for the experiment to avoid collision of the sample injection probe.



- **Read plate barcode when running experiment:** This option is available only when NovoSampler S 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 S 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.



Following functions are included in **Analysis** panel.

- **Use Height or Area parameter by default**:

  Allows user to select to use **Height** or **Area** for data analysis by default. This setting when checked will automatically apply to **New Reference Control Specimen** window, **Reference Spectra** window, **Fluorochrome Bi-Variate Plot** window, and all plots newly created in **Raw** or **Unmixed Workspace**.

- **Plot Default Property**: Allows user to 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%**).
  - **Show outlier for contour plot**:

    If selected, outlier events are shown as dots on contour plots.
  - Spectrum **plot style**:

    Select to display the spectrum density plot and spectrum dot plot in either original or smoothed style.
  - Smooth **density plot**:

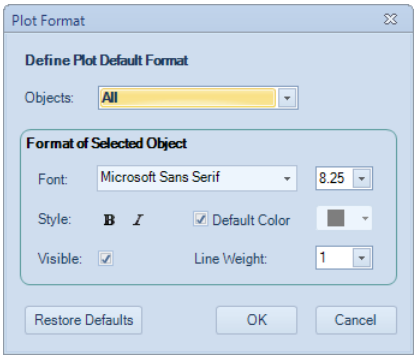
    If selected, density plots are smoothed.
  - **Use pseudocolor for density plot**:

    If selected, density plots are displayed in pseudocolor.
  - Showing **fitting results for cell cycle analysis**:

    If selected, the fitting results for the cell cycle analysis are displayed on the plot.
  - Show **legend for overlay plots**:

    If selected, plots with overlays include a legend.
  - 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 in this guide for more details about plot format.



- Normalize **histogram overlays**:

  If selected, the histogram overlays are normalized to a 100% scale.

- **Default X and Y axis scale for fluorescent parameters:**

  Allows user to select **Biexponentia**l, **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.

- **Display Options**: Allows user to set plot display related 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.

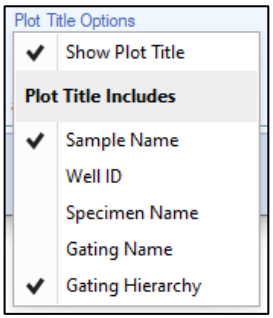
  - **Width of spectrum plot**:

    Set the plot width for spectrum density plot and spectrum dot plot.

  - **Plot Title Options**:

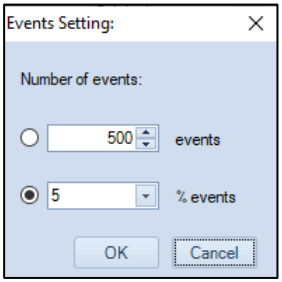
    If clicked, a drop-down menu will show as below. User can select to display plot title and what to be included in the plot title (e.g., **Sampler**

Name, Well ID (if NovoSampler S is connected), **Specimen Name, Gating Name, Gating Hierarchy**) on workspace plots.



- **Plot provides warning if XXX events are off-scale**:

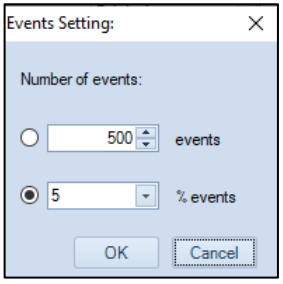
  If selected, the number or percentage of off-scale events will be displayed in created dot, density, histogram and contour plot after the data acquisition. By default, this function is enabled. User can click **XXX** area to open **Events Setting** window to define the off-scale event settings. By default, 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.



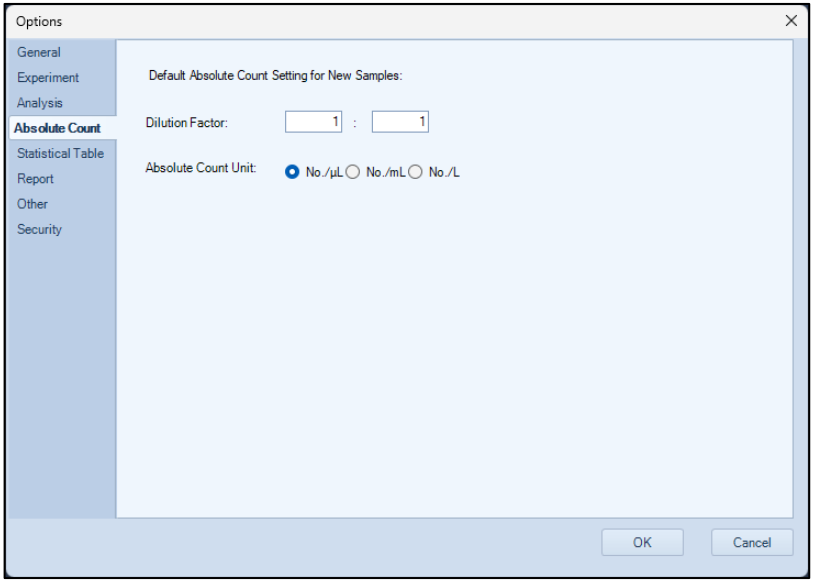
- **The Plot and the Status Bar show warning messages if XXX events are saturated**:

  If selected, user will see the number or percentage of saturated events displayed in created dot, density, histogram and contour plot after the data acquisition, and a warning message displayed in the software status bar during data acquisition. By default, this function is enabled. User can click **XXX** area to open **Events Setting** window to define the saturated event settings. By default, 5% saturated 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 saturated event settings. Once defined, after the data collection, when there are any saturated 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 saturated event information.



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



Following functions are included in **Absolute Count** panel.

- **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

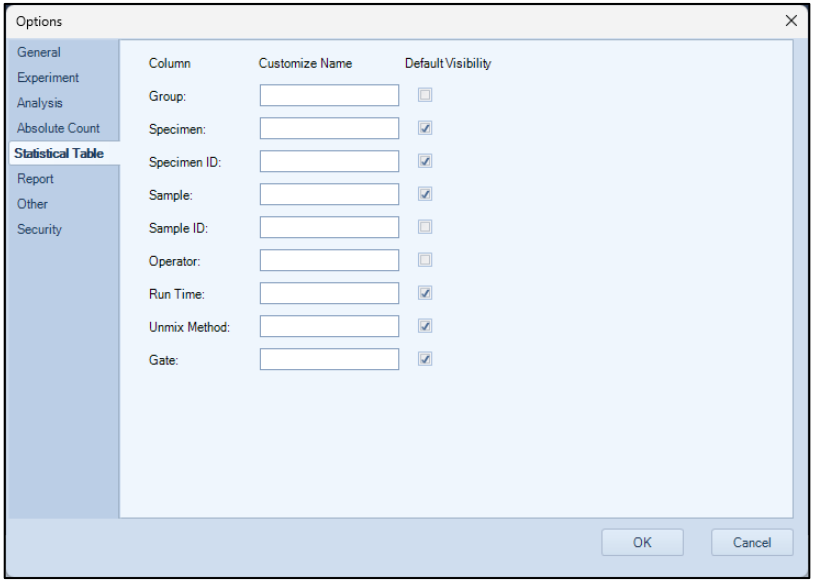
(Opteon) software will show the absolute counting results for the original sample by multiplying the concentration of the sample run on instrument by 10.

- **Absolute Count Unit**:

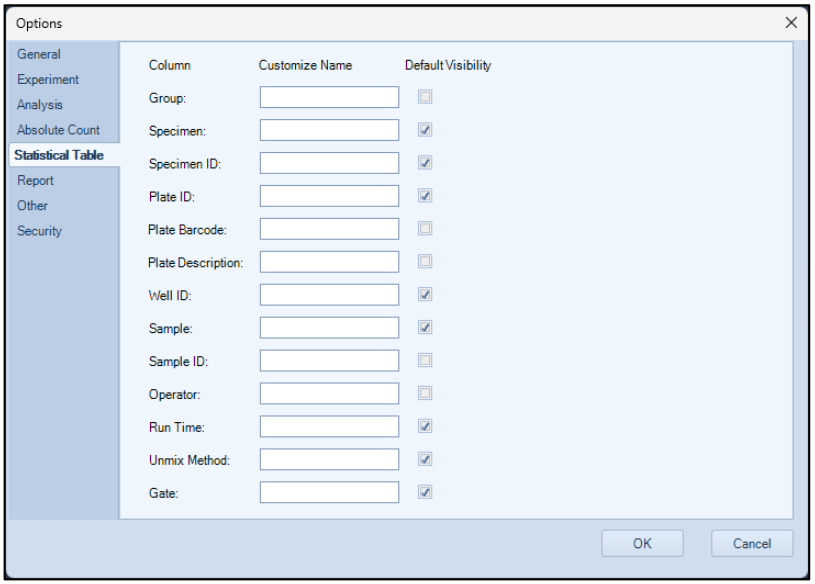
  Sets the default absolute count unit for new samples. The Absolute Count Unit parameter is used to set the unit for the absolute counting. User can select one of the units (i.e., No./µL, No./mL, or No./L) to present absolute count result for number of interested particles per microliter, per milliliter,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

  Further information on absolute count calculations is described in Calculation of Statistics in this guide.

- **Statistical Table**: Allows user to re-name the associated column header **(**i.e., Group name, Specimen name, Specimen ID, Plate ID, Plate Barcode, Plate Description, Well ID, Sample name, Sample ID, Operator Name, Run time, Unmix method, Gate name) in the statistical table. If **Default Visibility** is checked, the associated column will appear in the statistical table by default. Some options **(**i.e., **Plate ID, Plate Barcode, Plate Description, Well ID)** are only available when NovoSampler S is connected.

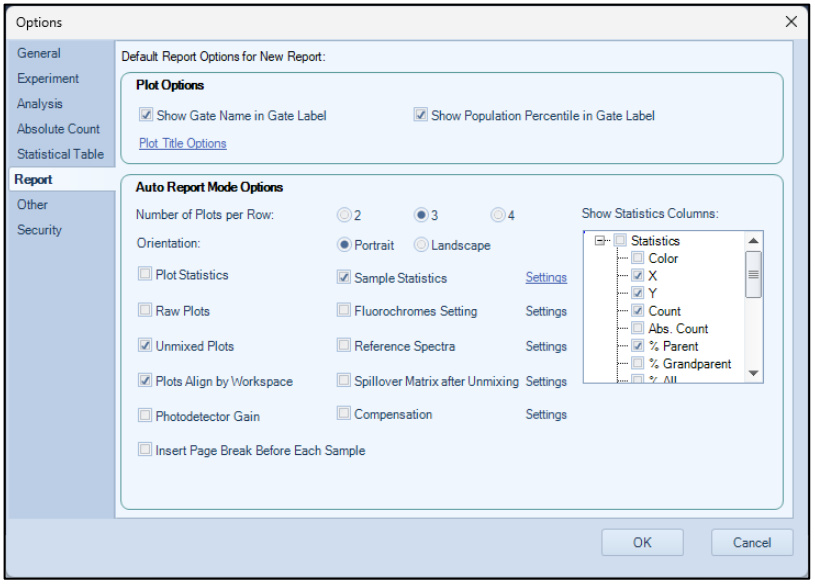


When NovoSampler S is not connected

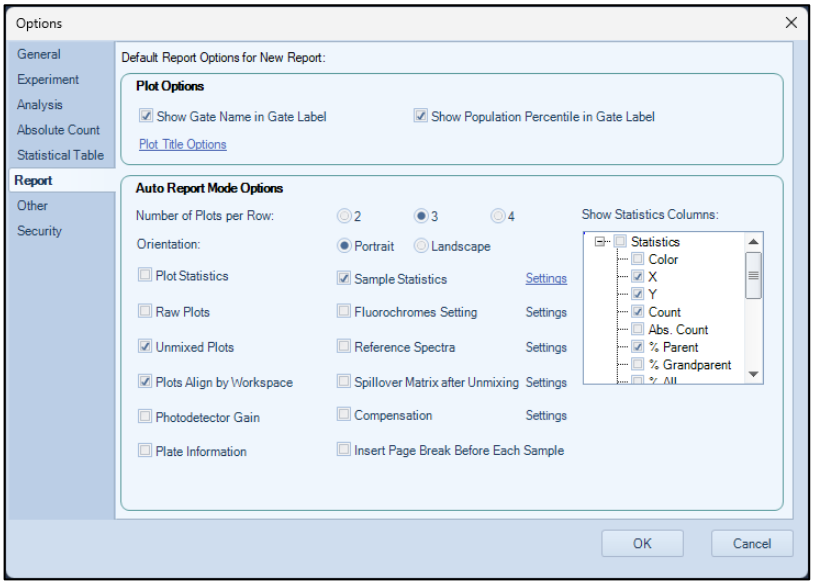


When NovoSampler S is connected

- **Report**: Allows user to define report related settings. This window displays additional setting **(**i.e., **Plate Information)** when NovoSampler S is connected.



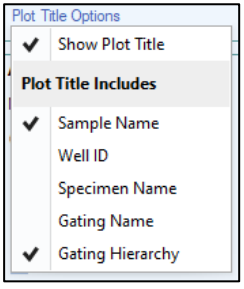
When NovoSampler S is not connected



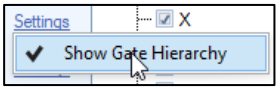
When NovoSampler S is connected

Following functions are included in **Report** panel.

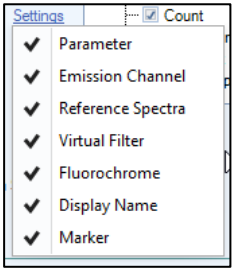
- **Plot Options:** The settings in this panel are used for customizing plots inside new report. They are applied to both auto and manual report mode.
  - **Show Gate Name in Gate Label**: If selected, gate name is displayed in gate label on the plot.
  - **Show Population Percentile in Gate Label**: 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. User can select to display plot title and what to be included in the plot title (e.g., **Sample Name, Well ID** (if NovoSampler S is connected), **Specimen Name, Gating Name, Gating Hierarchy**) on report plots.



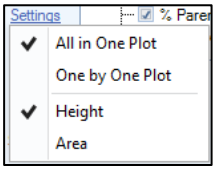
- **Auto Report Mode Options:** The settings in this panel are used for customizing automatically created report only.
  - **Number of Plots per Row**: Sets how many plots are shown in one row.
  - **Orientation:** Select to display the report in **Portrait** or **Landscape** orientation.
  - **Plot Statistics**: If selected, shows gate statistics of plot.
  - **Sample Statistics**: If selected, shows gate statistics of sample in the report and the associated **Settings** option will be available for user. User can click it and select to include gate hierarchy in the report.



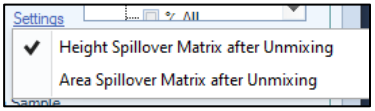
  - **Raw Plots**: If selected, shows all the plots from **Raw Workspace** in the report.
  - **Fluorochrome Setting**: If selected, shows fluorochrome setting information of sample in the report and the associated **Settings** option will be available for user. User can click it and select to include fluorochrome parameter, emission channel, Reference Spectra, fluorochrome name, display name, and marker information in the report.



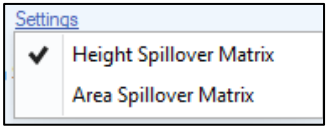
  - **Unmixed Plots**: If selected, shows all plots from **Unmixed Workspace** in the report.
  - **Reference Spectra**: If selected, shows Reference Spectra information of sample in the report and the associated **Settings** option will be available for user. User can click it and select to display in the report all Reference Spectra in one plot by checking **All in One Plot** or display one Reference Spectra in one plot by checking **One by One Plot**, or display Reference Spectra Height (or Area) plot by checking **Height** or **Area**.



- **Plots Align by Workspace**: If selected, display the plots in **Raw Workspace** first, then the plots in **Unmixed Workspace**. If not selected, display the plots in the order of sample defined in **Experiment Manager**.
- **Spillover Matrix After Unmixing**: If selected, shows Spillover Matrix information of sample in the report and the associated **Settings** option will be available for user. User can click it and select to display height Spillover Matrix information by checking **Height Spillover Matrix after Unmixing**, or display area Spillover Matrix information by checking **Area Spillover Matrix after Unmixing** in the report. This information will only be included in the report when user selects Spectral Unmixing to deconvolute the data.

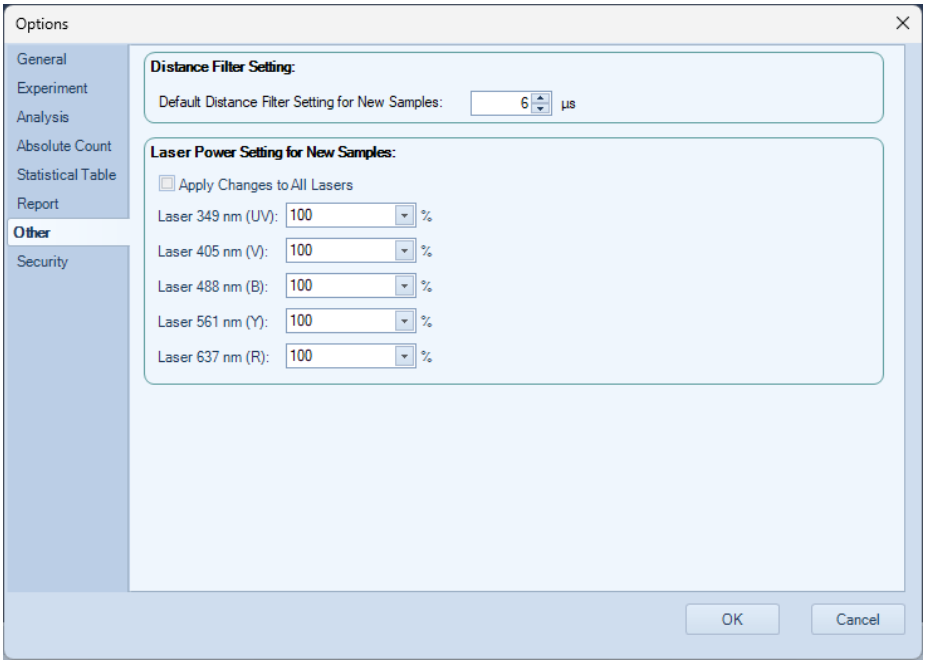


- **Photodetector Gain**: If selected, shows photodetector gain information in the report.
- **Compensation**: If selected, shows compensation information in report and the associated **Settings** option will be available for user. User can click it and select to display Height parameter-based Spillover Matrix information by checking **Height Spillover Matrix**, or display Area parameter-based Spillover Matrix information by checking **Area Spillover Matrix** in the report. This information will only be included in the report when user selects Compensation to analyze the data.



- **Plate Information**: If selected, shows plate information. This option is available only when NovoSampler S 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.

- **Other**: Allows user to define the distance filter and laser power settings for new samples. Only user logged in with associated privilege can edit this panel.



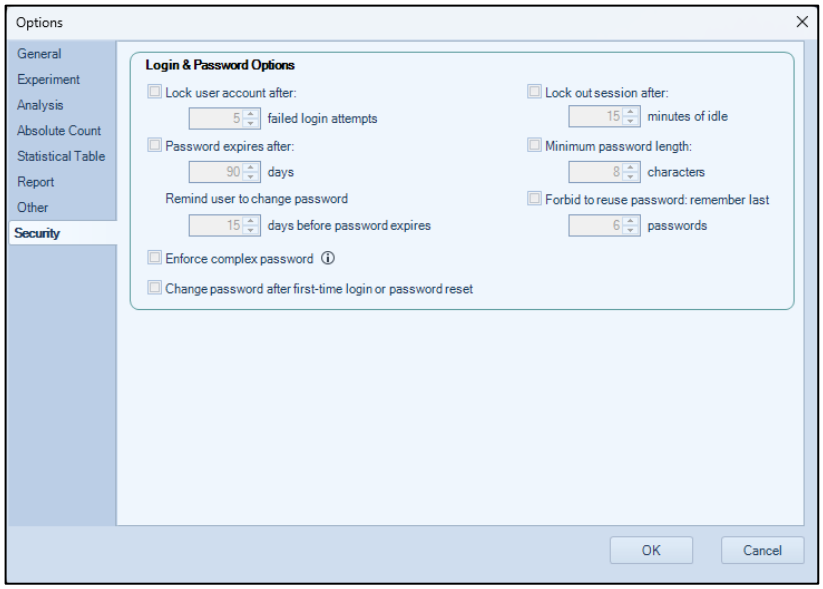
  - **Distance Filter Setting**: Allows user to define the distance filter related setting for new samples. Only user logged in with privilege of **Adjust Distance Filter Setting** can edit this setting. By default, 6 µs is selected as the default value. User can set it in the range of 0-12 µs.
  - **Laser Power Setting for New Samples**: Allows user to define the laser power setting for new samples. Only user logged in with privilege of **Adjust Laser Power Setting** can edit this setting.

    To set the laser power, user can either directly enter the power percentage (i.e., 20%-100%) or select one from drop down menu of the associated laser. By default, 100 (i.e., 100% of laser power) is selected.

    When **Apply Changes to All Lasers** option is checked, any changes to the power setting of a laser will be automatically applied to other remaining lasers.

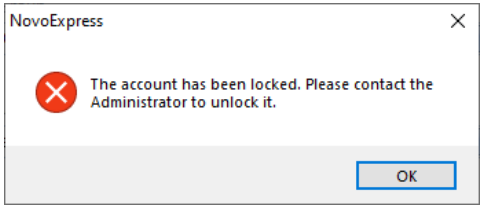
NOTE When user creates a new sample from template, the laser power will be automatically set to be same as the one from the template. Otherwise, the laser setting defined in this panel will be automatically applied to any newly created sample (s).

- **Security**: Allows user to define security related settings(i.e., customizing login and password options for all user account). Only user logged in as **Administrator** can edit this panel.

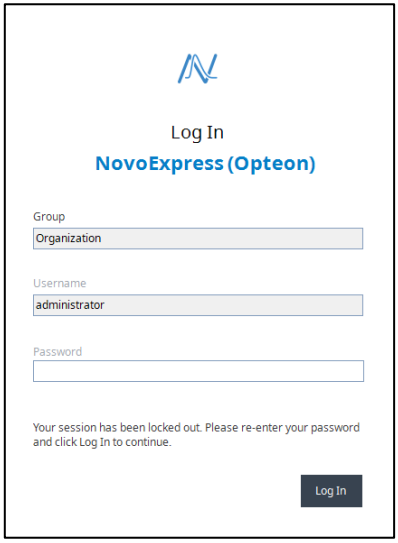


Following functions are included in **Security** panel.

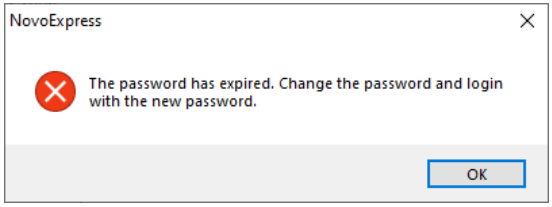
- **Lock User Account After**: when checked, user can enter the number of allowed failed login attempts in the box below this area. Once enabled, if user entered incorrect password during account login or unlock after more than the selected number of attempts are tried, the account will be locked out and following window will be prompted as shown in figure below. User needs to click **OK** and log in as **Administrator** to unlock this account.

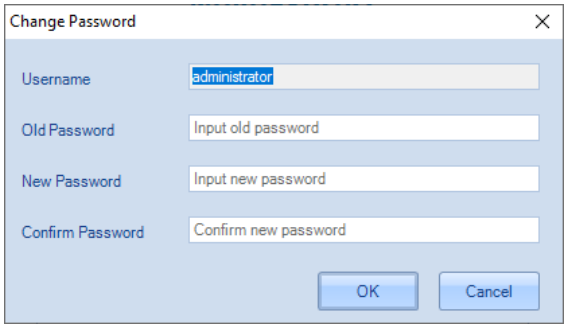


- **Lock Out Session After**: when checked, user can enter the number of minutes of idle in the box below this area. Once enabled, if there is no operation of software from user after selected number of minuets, a window as shown below will be prompted indicating the software is locked out. User needs to enter the correct password again and click **Log In** to continue operation of the software.

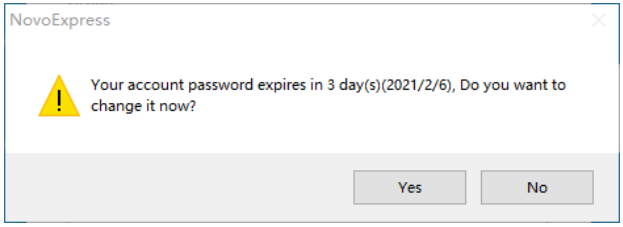


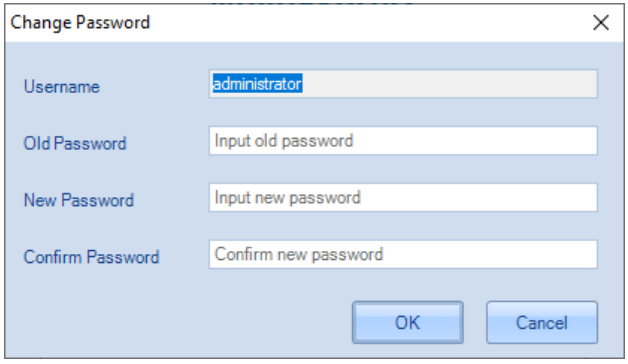
- **Password Expires After**: when checked, user can enter the total and remaining number of days current password remains valid in the box below this area. Once enabled, if user does not change the password after the selected total number of days, following window will be prompted. User needs to click **OK,** change the password and log in with the new password before continue operating the software.



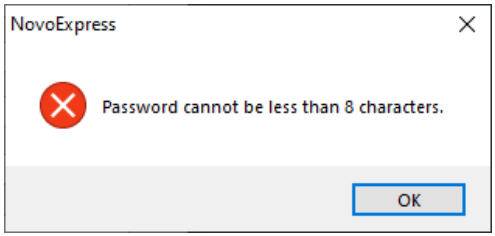


If user does not change the password after the selected remaining number of days, following window will be prompted to remind user to change the password. User can click **Yes** and change the password in the prompted window or click **No** to continue using the software.

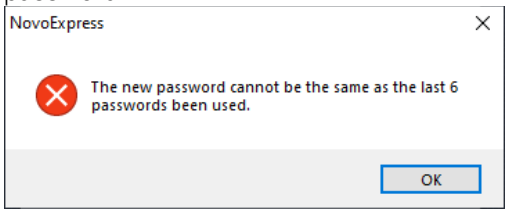




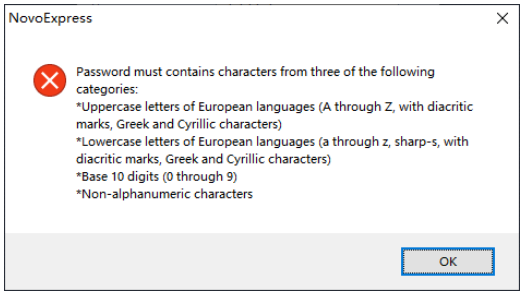
- **Minimum Password Length**: when checked, user can enter the minimum number of characters (4 – 32 characters) required for a valid password. Once enabled, if user enter any password that contains less characters than the one defined in this area, following window will be prompted. User needs to click **OK** to go back to the previous window and revise the password.



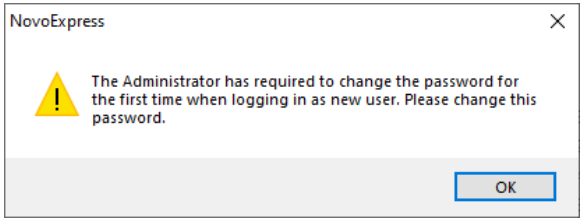
- **Forbid to Reuse Password: Remember Last**: when checked, user can enter the number of password software can remember (i.e., passwords user cannot reuse). Once enabled, if user enters a password that is same as any password software remembered, and prompt window below. For example, if user enter 6 in this area, then the new password user enters cannot be same as any one of these 6 remembered passwords. User needs to click **OK** to go back to the previous window and revise the password.

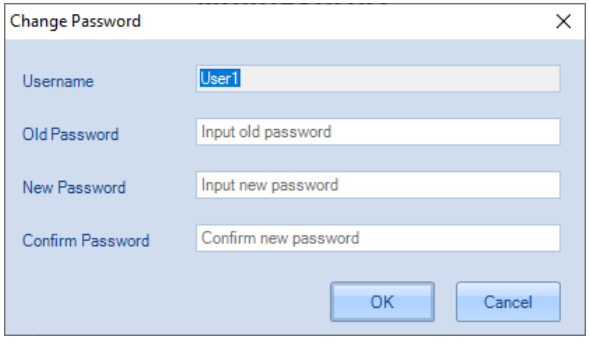


- **Enforce Complex Password**: when checked, a valid password user entered must contain characters from three of the following categories. Once enabled, any password does not meet the requirement will be invalid and following window will be prompted. User needs to click **OK** to go back to the previous window and revise the password.
  - Uppercase letters of European Languages (A to Z with diacritic marks, Greek and Cyrillic characters)
  - Lowercase letters of European Languages (a to z, sharp-s, with diacritic marks, Greek and Cyrillic characters)
  - Base 10 digits (0-9)
  - Non-alphanumeric characters



- **Change Password After First-Time Login or Password Reset**: when checked and enabled, following window will be prompted after user logs into the software for the first time or resets the password. User needs to click **OK** and change the password in the next prompted window as shown below.

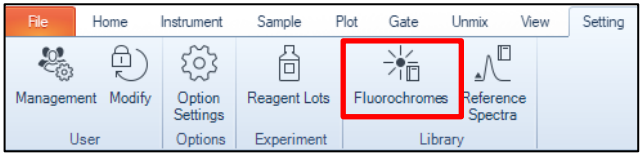


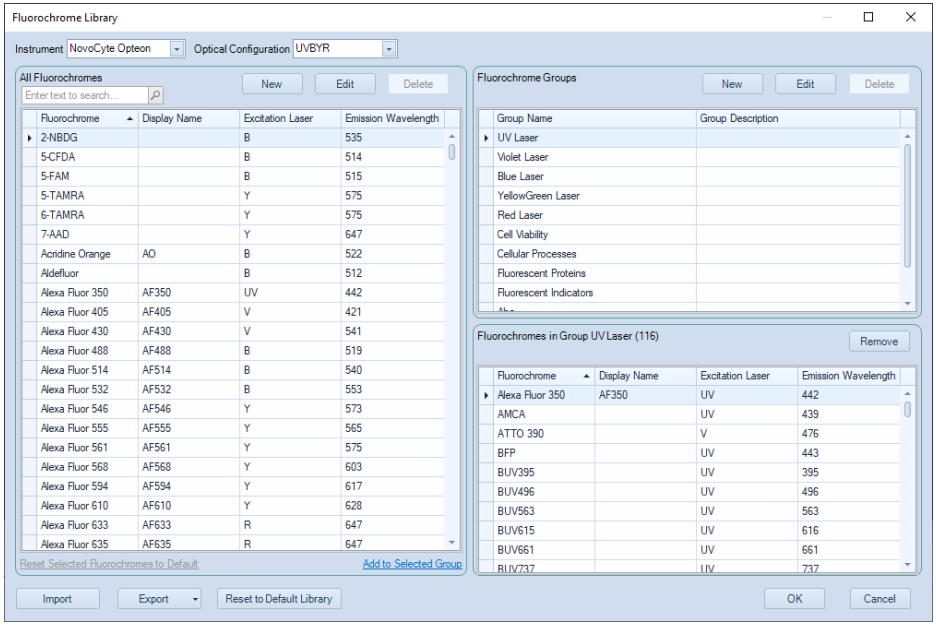


Fluorochrome Library

When user clicks **Setting > Library > Fluorochrome** from the software **Menu Bar**, the **Fluorochrome Library** window will be prompted. NovoExpress (Opteon) software includes a broad selection of fluorochromes available in the

Fluorochrome Library already (software default library). User can view, add and edit fluorochromes in the library in this window.





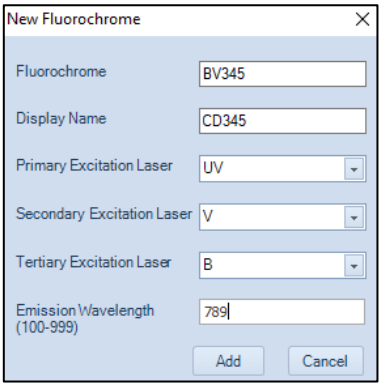
Following functions are included in **Fluorochrome Library** window.

- **Instrument**: Displays the current instrument model (i.e., NovoCyte Opteon).
- **Optical Configuration**: Allows user to view and select the optical configuration (e.g., UVBYR). The list of associated fluorochromes will be automatically updated accordingly.
- **All Fluorochromes**: This panel lists all available fluorochromes stored in the library. Each fluorochrome is listed with information of fluorochrome name, display name, excitation laser, emission wavelength. To quickly locate the desired fluorochrome, user can enter the keyword in the search box, and the list below will be automatically updated.

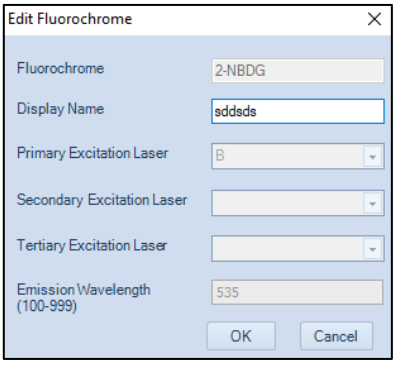
  Following functions are also available in this panel.

  - **New:** When clicked, following window will be prompted. User can add new fluorochrome into the library by entering the fluorochrome name, display

name, primary excitation laser, secondary excitation laser, tertiary excitation laser, emission wavelength information, and clicking **Add** in the prompted window.

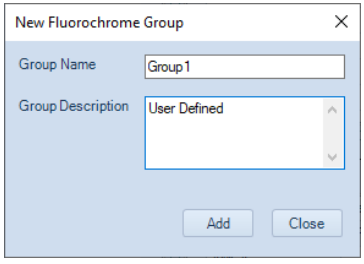


- **Edit:** Used to edit a selected fluorochrome in the library. First, click a fluorochrome to be edited in the list. The Edit button will then be enabled. Click the Edit button and following window will be prompted. User can edit the selected fluorochrome information in the prompted window, click **OK** to complete the edit. Note only the **Display Name** can be edited if the selected fluorochrome is in the software default library.

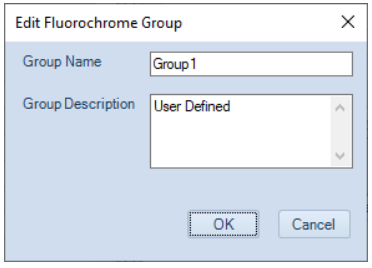


- **Delete:** Click to delete the selected fluorochrome from the library. Only user defined fluorochromes can be deleted. Fluorochrome in the software default library cannot be deleted.
- **Reset Selected Fluorochromes to Default:** Click to reset the selected fluorochrome back to software default library settings.
- **Add to Selected Group:** Click to add the selected fluorochrome to highlighted group within **Fluorochrome Groups** panel on the right.

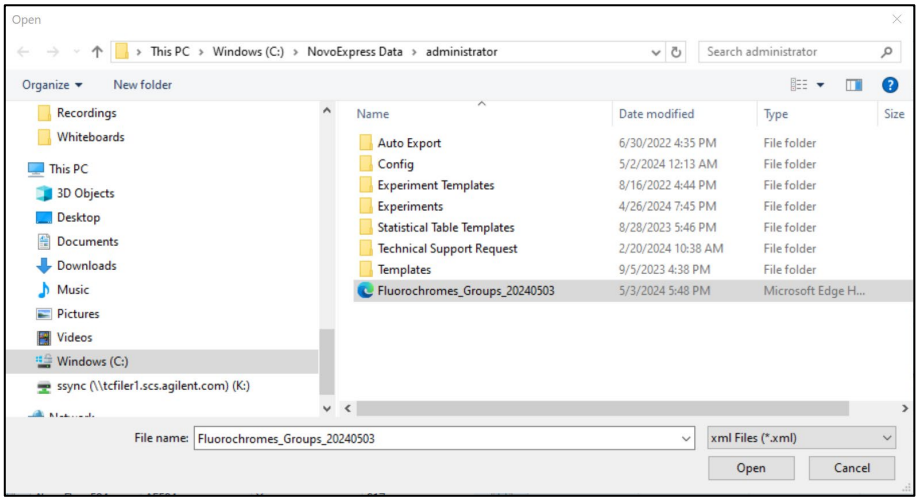
- **Fluorochrome Groups**: This panel lists all available fluorochrome groups stored in the library. By default, the fluorochrome in the software default library are categorized into default fluorochrome groups based on the primary excitation lasers and the characteristics of the fluorochrome. User can add new group to the list or edit the groups already defined. Each group is listed with information of group name and group description. Following functions are available in this panel.
  - **New:** When clicked, following window will be prompted. User can add new fluorochrome group into the library by entering the group name and group description and clicking **Add** in the prompted window.



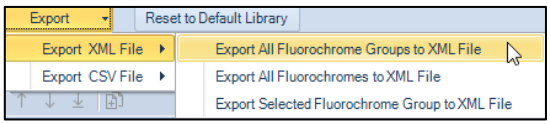
  - **Edit:** When clicked, following window will be prompted. User can edit the selected fluorochrome group information in the prompted window, click **OK** to complete. Note only the **Group Description** can be edited if the selected fluorochrome group is in the software default fluorochrome library.



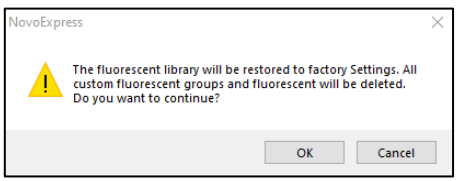
  - **Delete:** Click to delete the selected fluorochrome group. Note the software default fluorochrome group cannot be deleted.
- **Fluorochromes in Group XXX**: This panel lists all available fluorochromes in the selected group in the Fluorochrome Groups panel. Each fluorochrome is listed with information of fluorochrome name, display name, excitation laser, emission wavelength. User can click **Remove** to remove the selected fluorochrome from the selected group.
- **Import**: Click and select fluorochrome file in the prompted window as shown below to import the desired fluorochromes into the library.



- **Export**: When clicked, user can select to export all fluorochrome groups, or all fluorochromes, or selected fluorochrome group to either XML file or CSV file through the associated drop-down menu.



- **Reset to Default Library**: Restore the library back to software default settings. Any user defined fluorochrome and group will be automatically deleted. When clicked, following window will be prompted. User can click **OK** to confirm restoring all the settings or click **Cancel** to cancel the operation.



Reference Spectra Library

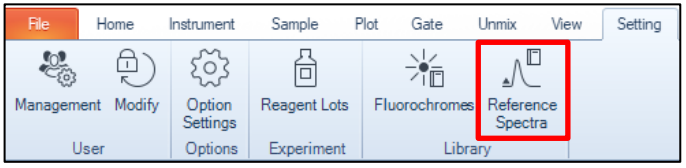
The **Reference Spectra Library** window allows user to view the Reference Spectra stored by software, add the Reference Spectra of Reference Control specimen, Unstained Control sample or Single Stained Control sample to the Reference

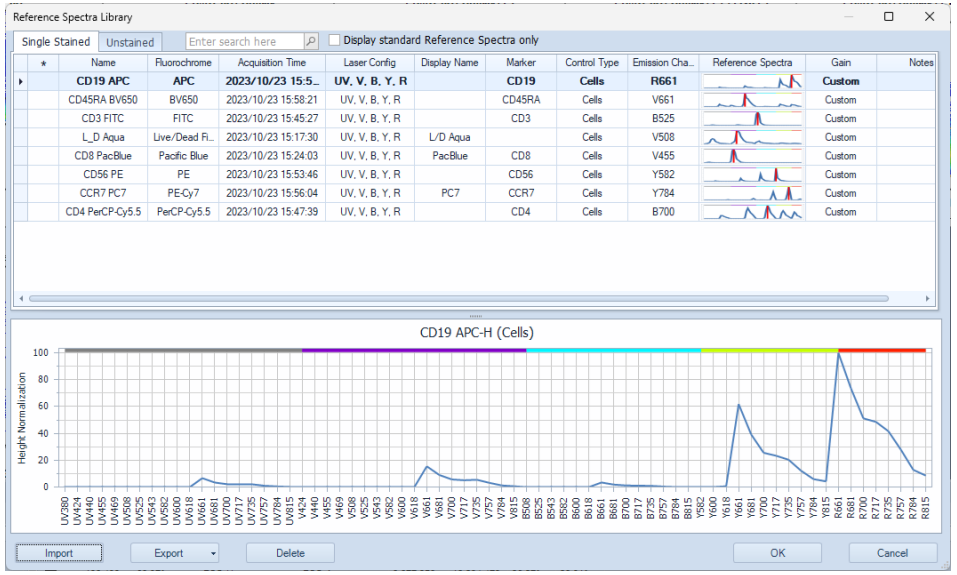
Spectra Library, and import the Reference Spectra from the Reference Spectra Library to Reference Control specimen, Unstained Control sample, Single Stained Control sample or experiment sample.

To access this window, user can click **Setting > Library > Reference Spectra** from the software **Menu Bar**, the **Reference Spectra Library** window will be prompted. User can view and edit Reference Spectra information stored in the library in this window. The spectral plot (**Height** or **Area** versus channel wavelength) associated with selected fluorochrome is displayed in the bottom of this window. User can switch between **Height** and **Area** through right-clicking menu of the plot.

NOTE

The **Reference Spectra Library** is empty under factory initial setting. User needs to add the Reference Spectra to the library manually first. Refer to Add Reference Spectra to Library in this guide for instructions.



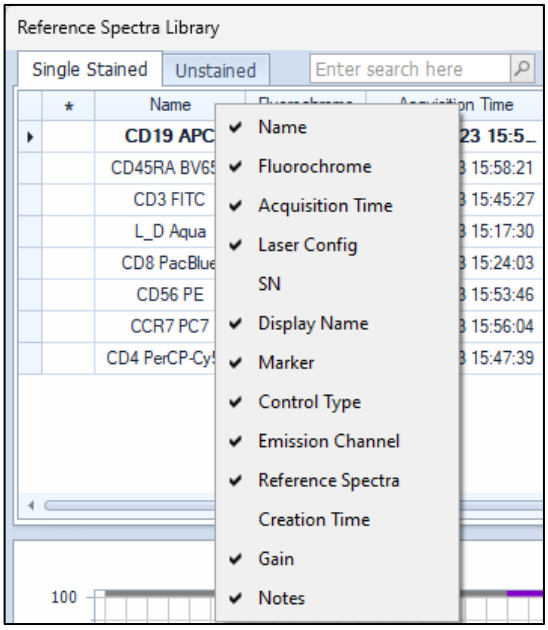


Following additional functions are included in **Reference Spectra Library** window.

- **Single Stained**: Click to display the Reference Spectra information of Single Stained Control sample stored in the library. The information of **Name, Fluorochrome, Acquisition Time, Laser Config, SN, Display Name, Marker,**

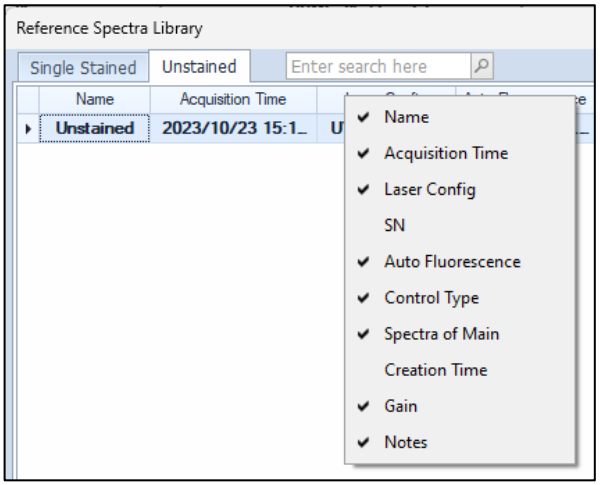
Control Type, Emission Channel, Reference Spectra, Creation Time, Gain, and **Notes** are listed in tabular format, and the spectra plot of the selected fluorochrome will be displayed under the list.

User can right click the table header to select the information to be displayed.



- **Unstained**: Click to display the Reference Spectra information of Unstained Control sample stored in the library. The information of **Name, Acquisition Time, Laser Config, SN, Auto Fluorescence, Control Type, Spectra of Main, Creation Time, Gain,** and **Notes** are listed in tabular format, and the spectra plot of the selected fluorochrome will be displayed under the list.

  User can right click the table header to select the information to be displayed.

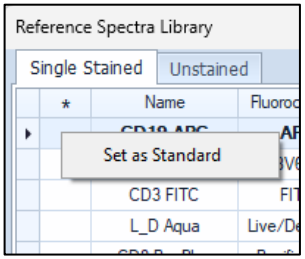


- **Display Standard Reference Spectra only**: Check to display only the standard Reference Spectra in table below. This option is not available in **Unstained** panel. Note any standard Reference Spectra is marked with * in the front of associated fluorochrome.

NOTE

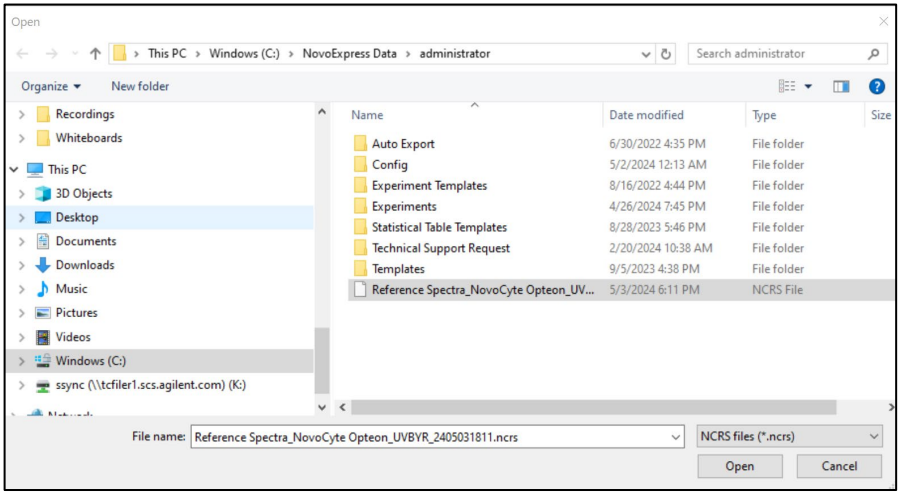
When multiple Reference Spectra from the same fluorochrome with the same marker and control type exist in the Reference Control Library, user can select one and define it as Standard Reference Spectra. This provides user with convenience of identifying the desired Reference Spectra.

To set standard Reference Spectra, user can right click the row header or the * column associated with the selected fluorochrome, click **Set as Standard**. Clicking it again will cancel setting it as standard Reference Spectra. Note only one Reference Spectra from the same fluorochrome, marker and control type can be defined as standard Reference Spectra.

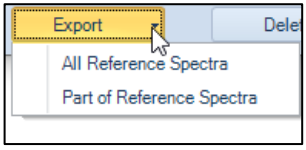


- **Import**: Click to import the selected Reference Spectra (ncrs file) into the library.

NOTE User can only import the reference spectra exported from the library before. All exported Reference Spectra are stored as ncrs file.

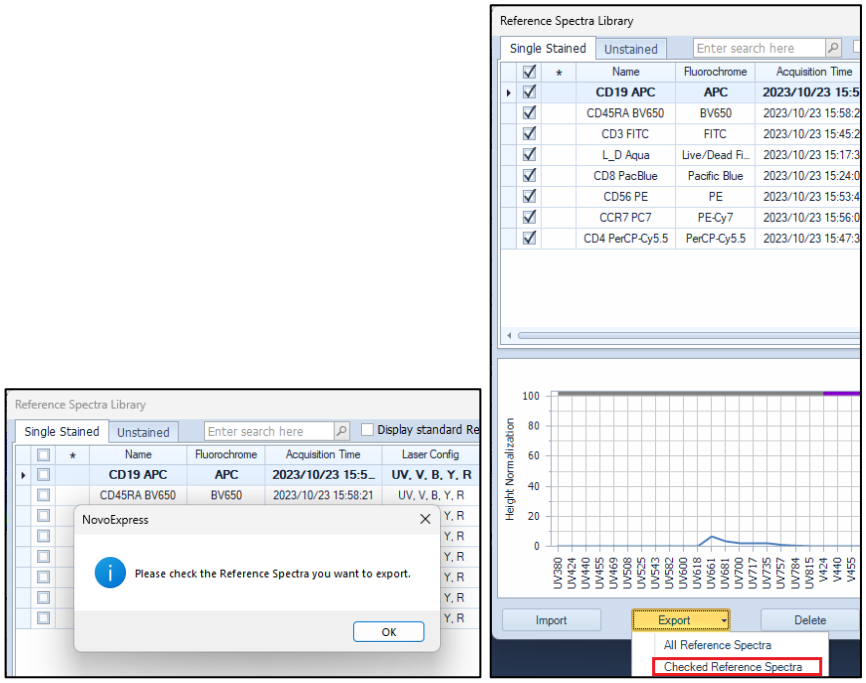


- **Export**: When clicked, user can select to export all Reference Spectra, or part of Reference Spectra to a NCRS file in the associated drop-down menu.



To export all Reference Spectra, click **All Reference Spectra**, select the target folder, and click **Save**. By default, the Reference Spectra will be saved as **Reference Spectra_YYMMDDHHmm.ncrs**. For example, a **Reference Spectra_2404301035.ncrs** file indicates that this file was generated at 10:35 on April 30, 2024.

To export part of Reference Spectra, click **Part of Reference Spectra**, additional checkbox column will display in **Reference Spectra Library** window as shown below. User can click **OK** to confirm and close the prompted window, check the checkbox in front of selected Reference Spectra. Click **Export > Checked Reference Spectra**, select the target folder, and click **Save**. By default, the Reference Spectra will be saved as **Reference Spectra_NovoCyte Opteon_UVBYR_YYMMDDHHmm.ncrs.** For example, a **Reference Spectra_NovoCyte Opteon_UVBYR_2404301035.ncrs** file indicates that this file was generated on NovoCyte Opteon with optical configuration of UVBYR at 10:35 on April 30, 2024.



- **Delete**: Click to delete the selected Reference Spectra from the library.

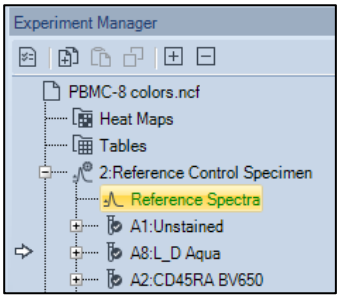
Add Reference Spectra to Library

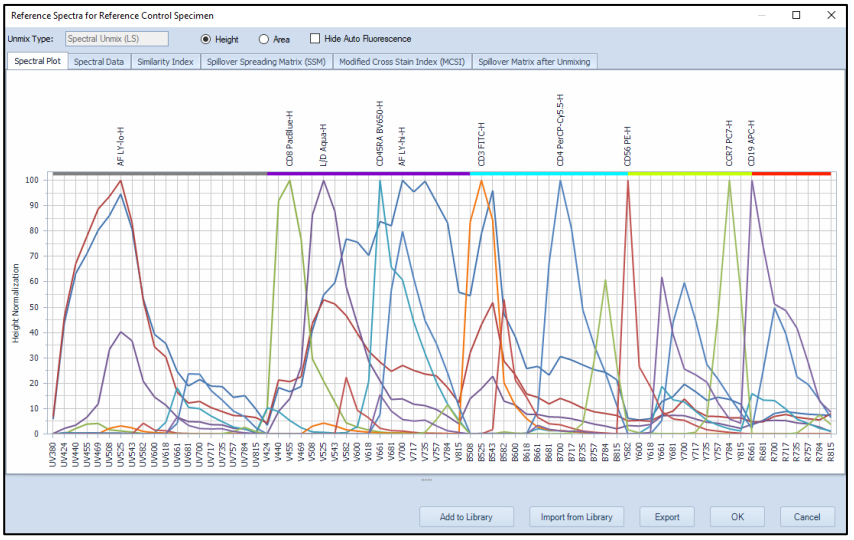
User can add the Reference Spectra of Reference Control specimen, Unstained Control sample or Single Stained Control sample to the Reference Spectra Library by following the procedures below.

NOTE User cannot add the Reference Spectra of experiment sample to the library.

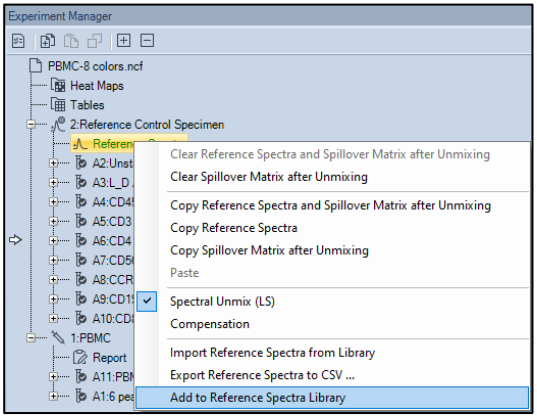
1 Double click **Reference Spectra** node of an associated Reference Control specimen or sample from the **Experiment Manager**. In the prompted window, click **Add to Library.**

NOTE Reference Control Specimen is displayed with icon of in **Experiment Manager** panel. Any sample(s) displayed under the Reference Control Specimen node is Reference Control sample (s).

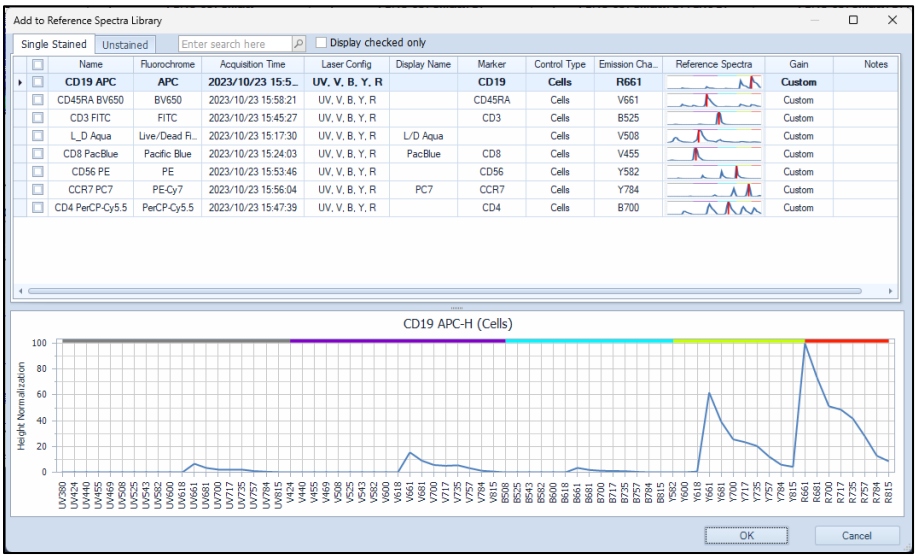




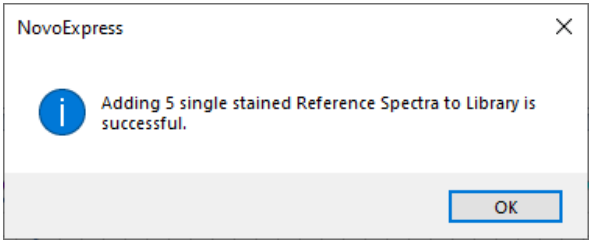
Alternatively, user can right click **Reference Spectra** node of associated specimen or sample in **Experiment Manager** panel and select **Add to Reference Spectra Library.**



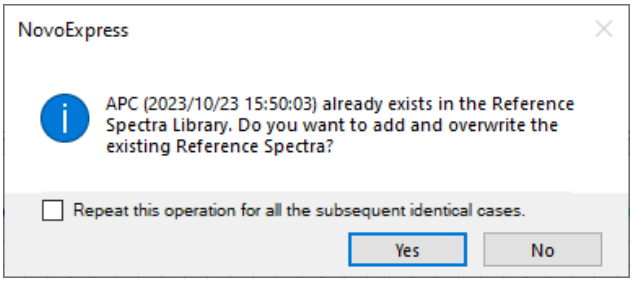
2 When adding Reference Spectra of **Reference Control Specimen**, following **Add to Reference Spectra Library** window will be prompted.



This window consists of two sub-panels, i.e., **Single Stained** and **Unstained** sub-panels. User can click either sub-panel and check the checkbox in front of the desired Reference Spectra and click **OK** to add the spectra into the library. Following message will be prompted indicating the spectra is successfully added to the library.

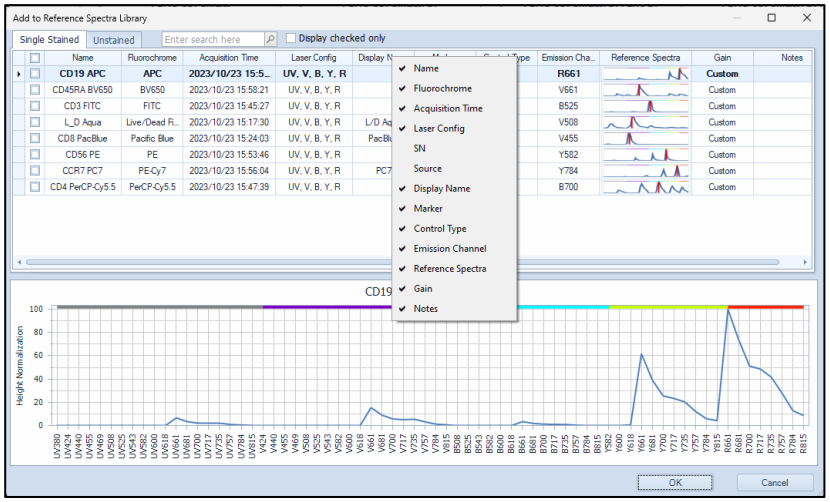


Under the situation when the selected Reference Spectra already exist in the library, following message will be prompted.



User can click **Yes** to overwrite the existing Reference Spectra or click **No** to cancel the operation. If **Repeat the operation for all subsequent identical cases** is checked, software will apply user's selection (Yes or No) in this window to all other Reference Spectra.

User can also view the Reference Spectra information of Single Stained Control sample or Unstained Control sample in associated sub-panel in the tabular format and corresponding spectra density plot below. The information listed in **Single Stained** sub-panel includes **Fluorochrome, Acquisition Time, Laser Config, SN, Source, Display Name, Marker, Control Type, Emission Channel, Reference Spectra, Gain,** and **Notes.** The information listed in **Unstained** sub-panel includes **Name, Acquisition Time, Laser Config, SN, Source, Auto Fluorescence, Control Type, Spectra of Main, Gain,** and **Notes.** User can right click the table header to select the information to be displayed.

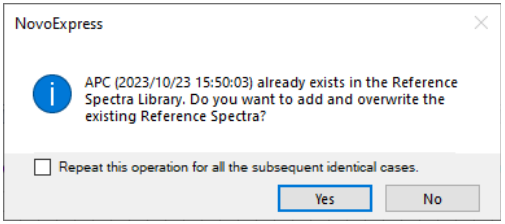


User can also enter the keyword in the search box on top of each sub-panel to quickly locate the desired Reference Spectra. The table below will automatically be updated based on the filtering result.

When **Display Checked Only** is checked, only the checked Reference Spectra will be listed in the table below.

3 When adding Reference Spectra of individual Single Stained Control or Unstained Control Sample to Library, the **Add to Reference Spectra Library** window will not be prompted. Instead, following message will be prompted indicating the spectra is successfully added to the library.

Under the situation when the selected Reference Spectra already exist in the library, following message will be prompted.

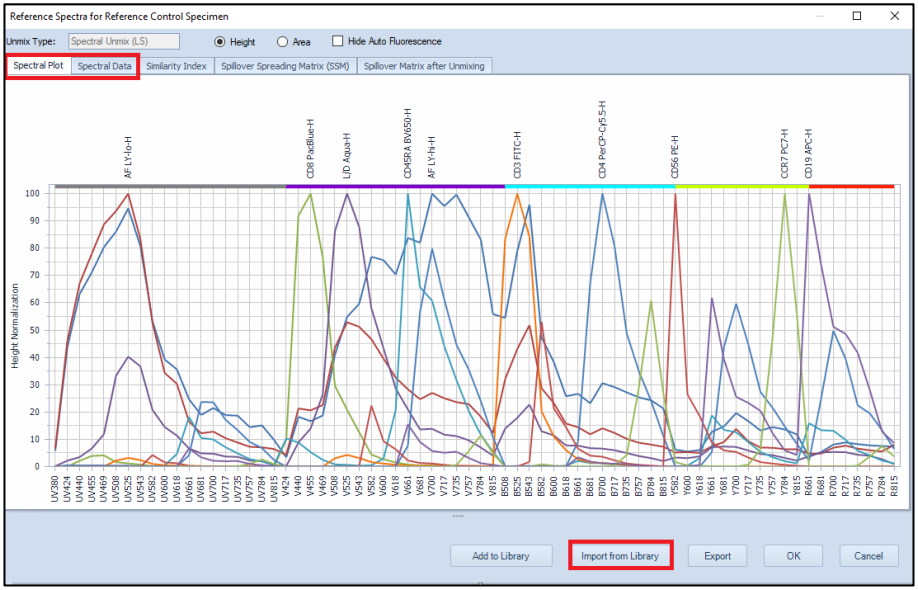


User can click **Yes** to overwrite the existing Reference Spectra or click **No** to cancel the operation. If **Repeat the operation for all subsequent identical cases** is checked, software will apply user's selection (Yes or No) in this window for future operation, and will not prompt this message window.

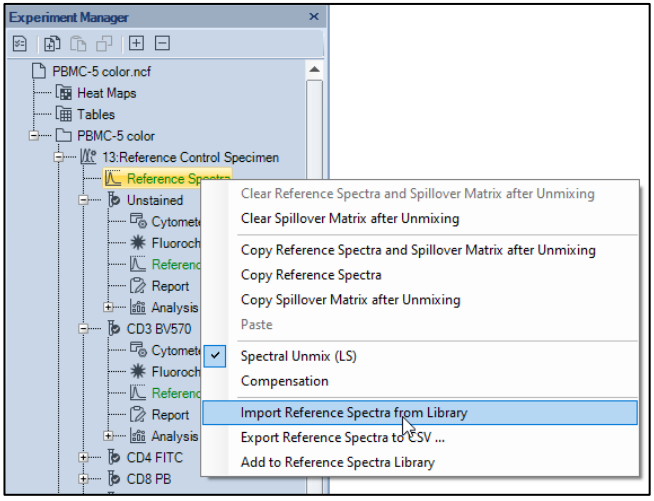
Import Reference Spectra from Library

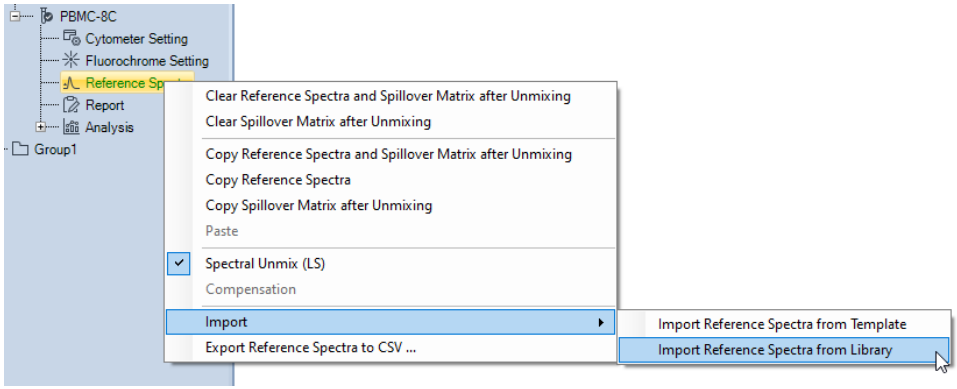
In the case of importing the Reference Spectra from the Reference Spectra Library to Reference Control specimen, Unstained Control sample, Single Stained Control sample or experiment sample is needed, following methods can be used.

- Double click **Reference Spectra** node of associated specimen or sample from the **Experiment Manager** panel. In prompted window, select **Spectral Plot** or **Spectral Data** tab, click **Import from Library**.

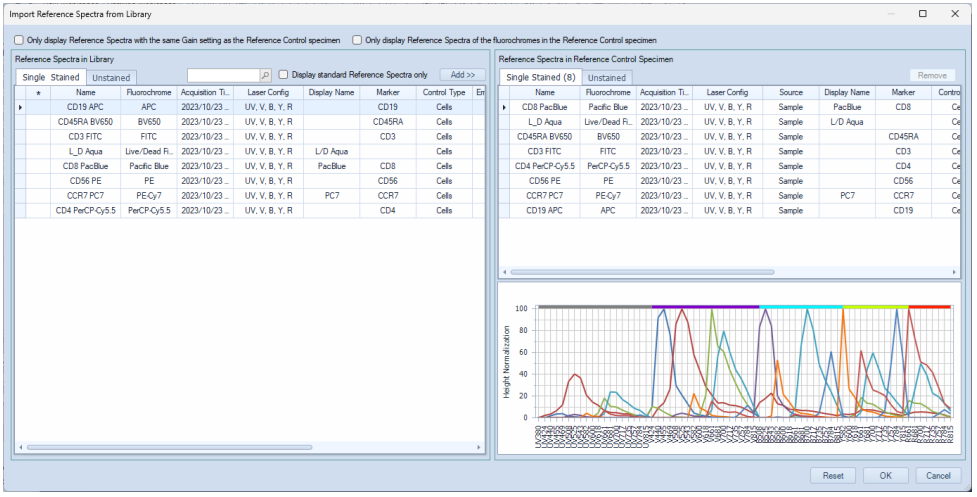


- Right click **Reference Spectra** node in **Experiment Manager** panel. For Reference Control specimen or Reference Control sample, select **Import Reference Spectra from Library**. For experiment sample, select **Import > Import Reference Spectra from Library.**





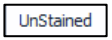
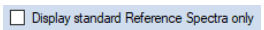
When user clicks **Import Reference Spectra from Library,** following window will be prompted.



Following functions or information are included in **Import Reference Spectra from Library** window.

- **Only display Reference Spectra with the same Gain setting as XXX**: When checked, only the Reference Spectra in library with the same gain as the selected specimen (or sample) will be displayed and available for user selection.
- **Only display Reference Spectra of the fluorochrome in XXX**: When checked, only the Reference Spectra in library from the fluorochrome contained in the Reference Control specimen (or experiment sample) will be displayed and available for user selection. This option is not available when importing the Reference Spectra to Unstained Control sample.

- **Reference Spectra in Library**: This panel will display the Reference Spectra in library based on user selection of **Only display Reference Spectra with the same Gain setting, Only display Reference Spectra of the fluorochrome.** This panel consists of two sub-panels (i.e., **Single Stained** and **Unstained**) when importing the Reference Spectra to Reference Control specimen or experiment sample. The associated spectra information is displayed in tabular format which may vary depending on which sub-panel user selects. Please refer to table below for detailed description of each function.

Functions/Terminology	Description
	Click to view the Reference Spectra information of Single Stained Control sample stored in the library in the table below, including **Fluorochrome, Acquisition Time, Laser Config, SN, Display Name, Marker, Control Type, Emission Channel, Reference Spectra, Creation Time, Gain,** and **Notes.** User can right click the table header to select the information to be displayed. This sub-panel is not available when importing the Reference Spectra from the library to Unstained Control sample.
	Click to view the Reference Spectra information of Unstained Control sample stored in the library in the table below, including **Name, Acquisition Time, Laser Config, SN, Auto Fluorescence, Control Type, Spectra of Main, Creation Time, Gain,** and **Notes.** User can right click the table header to select the information to be displayed. This sub-panel is not available when importing the Reference Spectra from the library to Single Stained Control sample.
	When user enters the keyword of the spectra, the table below will be automatically updated to display the search result.
	Check to display only the standard Reference Spectra in table below. This option is not available when **Unstained** tab is selected.
	Click to add selected spectra to Reference Control specimen or sample panel on the right side. Newly added spectra will be displayed in blue font.

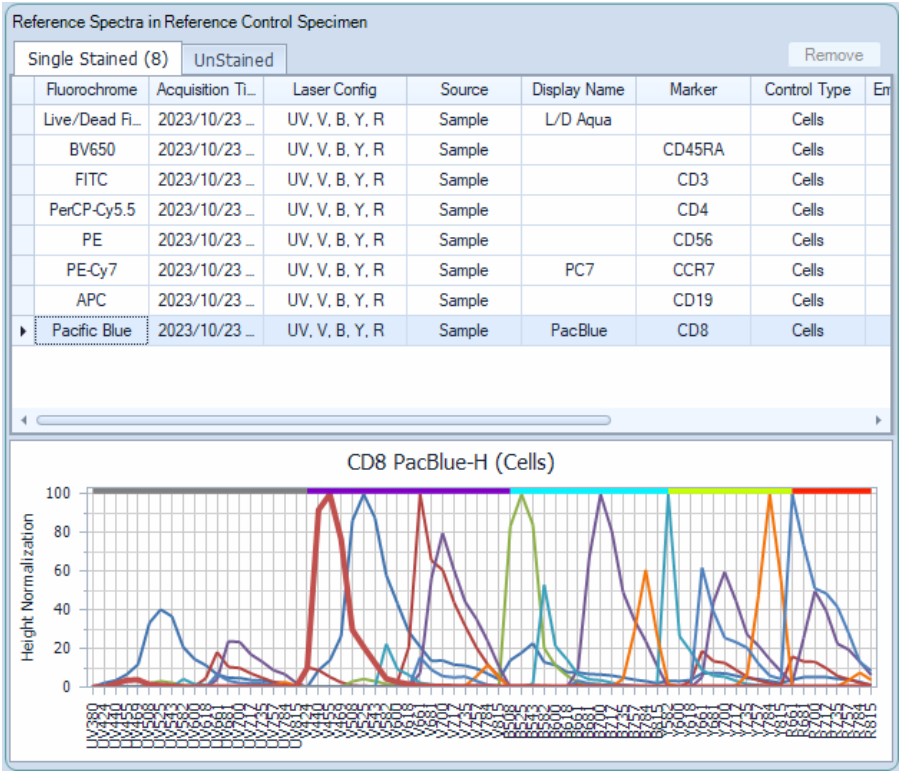
- **Reference Spectra in XXX**: The right side of **Import Reference Spectra from Library** window lists current Reference Spectra associated with selected specimen or sample. This panel is displayed in the same structure layout as **Reference Spectra in Library** panel. Please refer to table below for description of each function and parameter included in this panel.

Functions/Terminology	Description
	Click to view the Reference Spectra information of Single Stained Control sample in the table below, including

	Fluorochrome, Acquisition Time, Laser Config, Source, Display Name, Marker, Control Type, Emission Channel, Reference Spectra, Gain, and **Notes.** User can right click the table header to select the information to be displayed. This sub-panel is available only when importing the Reference Spectra from the library to Reference Control specimen or Single Stained Control sample.
UnStained	Click to view the Reference Spectra information of Unstained Control sample in the table below, including **Name, Acquisition Time, Laser Config, Source, Auto Fluorescence, Control Type, Spectra of Main, Gain,** and **Notes.** User can right click the table header to select the information to be displayed. This sub-panel is available only when importing the Reference Spectra from the library to Reference Control specimen or Unstained Control sample.
Remove	Click to remove selected spectra from the library.

The bottom side of this panel displays the spectral plot of all fluorochrome listed in table above when user clicks **Single Stained** tab, or spectral plot of autofluorescence of the unstained user selected when user clicks **Unstained** tab.

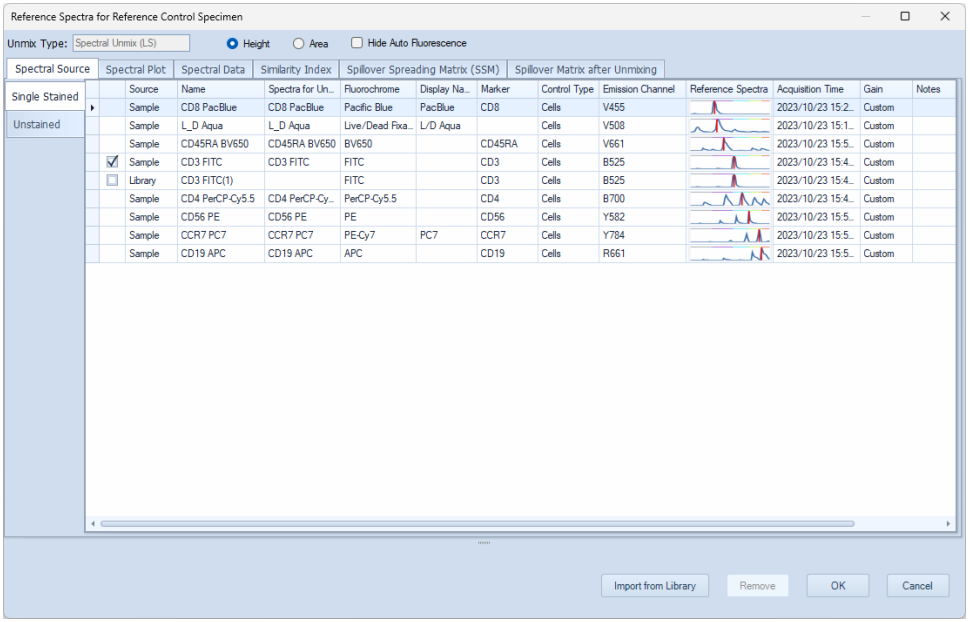
When user clicks to select any fluorochrome from the list in **Single Stained** tab, the associated spectral plot will be bolded automatically. User can also click spectral plot of any fluorochrome and associated fluorochrome in the table above will be automatically highlighted. As an example shown below, the **Pacific Blue** is highlighted, and the associated spectral plot is bolded (in dark brown color).



After the Reference Spectra is imported from the library to the Reference Control specimen or sample, the **Spectra Source** tab will be automatically added to the **Reference Spectra** window. The table will display additional **Source** column indicating the source of associated Reference Spectra (e.g., **Library, Sample**). There are two sub-tabs associated with **Spectra Source** tab, namely **Single Stained** and **Unstained**. User can view and edit the Reference Spectra in each sub-tab.

NOTE

Under the situation when one fluorochrome has both spectra from Reference Spectra Library and from sample, user can select either of them for conducting Spectral Unmixing, as shown below.


Reference Spectra for Reference Control Specimen
Unmix Type: Spectral Unmix (LS)
Height
Area
Hide Auto Fluorescence
Spectral Source
Spectral Plot
Spectral Data
Similarity Index
Spillover Spreading Matrix (SSM)
Spillover Matrix after Unmixing
Single Stained
Unstained
Source Name Spectra for Un... Fluorochrome Display Na... Marker Control Type Emission Channel Reference Spectra Acquisition Time Gain Notes
Sample CD8 PacBlue CD8 PacBlue Pacific Blue PacBlue CD8 Cells V455 2023/10/23 15:2... Custom
Sample L_D Aqua L_D Aqua Live/Dead Fixa... L/D Aqua Cells V508 2023/10/23 15:1... Custom
Sample CD45RA BV650 CD45RA BV650 BV650 CD45RA Cells V661 2023/10/23 15:5... Custom
Sample CD3 FITC CD3 FITC FITC CD3 Cells B525 2023/10/23 15:4... Custom
Library CD3 FITC(1) FITC CD3 Cells B525 2023/10/23 15:4... Custom
Sample CD4 PerCP-Cy5.5 CD4 PerCP-Cy... PerCP-Cy5.5 CD4 Cells B700 2023/10/23 15:4... Custom
Sample CD56 PE CD56 PE PE CD56 Cells Y582 2023/10/23 15:5... Custom
Sample CCR7 PC7 CCR7 PC7 PE-Cy7 PC7 CCR7 Cells Y784 2023/10/23 15:5... Custom
Sample CD19 APC CD19 APC APC CD19 Cells R661 2023/10/23 15:5... Custom
Import from Library
Remove
OK
Cancel

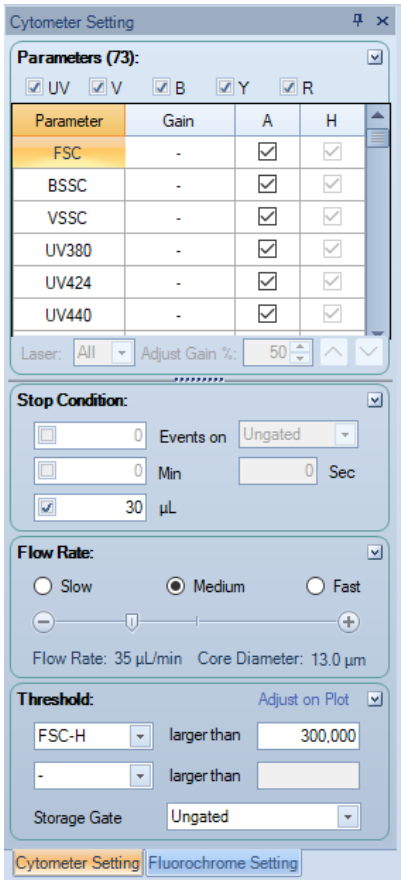
4 Sample Acquisition

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

Cytometer Setting/Fluorochrome Setting

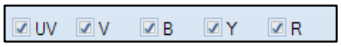
The **Cytometer Setting** and Fluorochrome Setting panel is displayed in the same area in the software. **Cytometer Setting** panel is displayed by default. User can click **Fluorochrome Setting** to switch to **Fluorochrome Setting** panel (Refer to Fluorochrome Setting for more details).

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



Laser Control

There are five checkboxes on the top of the **Cytometer Setting** panel which allows user to select the laser needed for running the experiment.



By default, all the lasers are checked, and all corresponding fluorescence channels will be enabled and displayed in the table below. When user unchecks any laser, the corresponding fluorescence channels will be automatically disabled (i.e., associated **Gain, A** and **H** column will be greyed out). The instrument will not collect any fluorescence data from unchecked laser.

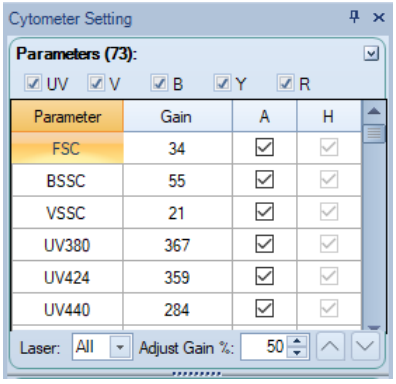
NOTE

User cannot edit all or part of laser settings if any of the following is true.

- When user logs into the software without the privilege of **Configure Lasers**.
- When the instrument is in the process of collecting data.
- When the opened experiment file contains data already acquired.
- When the instrument is collecting data for one or more Reference Control samples, the laser control setting for all the Reference Control samples are not available.
- When any of the threshold setting is set on a fluorescence signal, the associated laser cannot be unchecked.
- When any fluorochrome is added to the **Fluorochrome Setting** window, the associated laser cannot be unchecked.

Parameters Settings

The parameters settings specify which parameters are collected during sample acquisition. User can view all the parameters that the instrument is capable of collecting, select the Height (H) and/or Area (A) parameter to be collected, and define the photodetector gain value associated with each channel.



Parameter

This column displays all the available channels instrument will collect data from. User cannot edit this column.

Gain

This column displays the photodetector gain values associated with each channel. The default gain values are automatically displayed when instrument is properly connected and powered up.

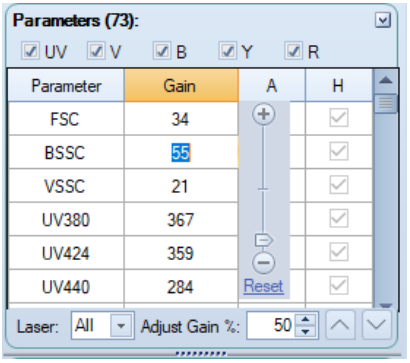
There are three ways to adjust photodetector gain of selected channel(s).

NOTE The photodetector gain adjustment range is 1-1,000 for FSC, and 1-3,000 for BSSC, VSSC and all fluorescence channels.

NOTE Any change to the photodetector gain of any fluorescence channel of a Reference Control sample will be automatically applied to the other Reference Control samples in the same Reference Control Specimen.

NOTE When data acquisition of at least one of the Reference Control samples completes, the photodetector gain of the other fluorescence channels in the same Reference Control Specimen cannot be changed.

- To adjust photodetector gain of one channel, double click the gain value of associated channel, the photodetector gain adjustment tool will show as below:

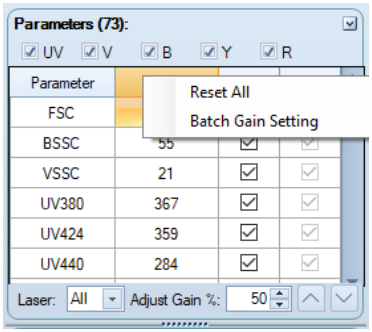


Drag the slider bar or directly enter the target gain value to change the photodetector gain. Click the **Reset** link button to reset back to factory default value. If currently logged in user account which does not have **Photodetector Gain Adjustment** privilege, only **Reset** button will be available. To grant **Photodetector Gain Adjustment** privilege, refer to Access Privilege in this guide for more details.

Every channel has its default photodetector gain setting. An underlined photodetector gain value as shown above indicates the photodetector gain has been modified from the factory default value. A non-underlined photodetector gain indicates that it is the factory default setting.

NOTE

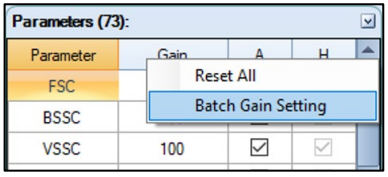
User can right click the **Gain** column header and select the **Reset All** to reset all the photodetector gains to the factory default value.



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 events acquired before photodetector gain adjustment are not needed, click the **Restart** button to restart the acquisition. Refer to Experiment Control in this guide for details. You can also delete the events acquired before photodetector gain adjustment after the acquisition is completed by using the **Delete Events** function (refer to Sample in this guide).

- To batch adjust the gain of selected channel(s), user can right click the **Gain** column header and click **Batch Set Gain**.

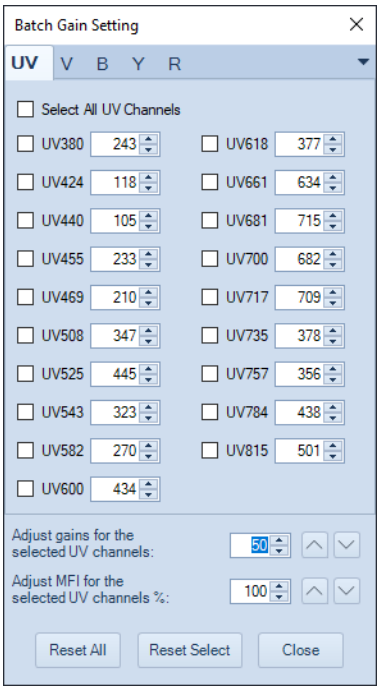


In prompted window, select the specific laser (e.g., UV), check **Select All XX Channels** to select all corresponding channels, or check the checkbox in front of designated channel, enter the new gain value or click the arrow next to the value to increase or decrease the gain value.

Alternatively, user can check the channels for selected laser, enter the proper gain value in **Adjust Gains for the Selected XX Channels** area, or enter the MFI percentage in **Adjust MFI for the Selected XX Channels %** area, click to increase the gain by entered absolute value or percentile value or click to decrease the gain by entered absolute value or percentile value.

The displayed gain values in the **Parameter** panel will be automatically updated after above adjustment.

User can also click **Reset All** to reset all gain values to the default ones or click **Reset Select** to reset the gain value of selected channel in this window to the default ones.



NOTE This function is available only when instrument is properly connected and powered on, and **Photodetector Gain Adjustment** privilege is enabled for current user account.

- To quickly adjust the gain for all the channels of designated laser(s), select **All** or designated laser from drop-down menu of **Laser** which is located in the bottom of the parameter list and enter the gain value in **Adjust Gain** area, click ^ to increase the gain by entered value or click v to decrease the gain by entered value for selected laser(s).

NOTE This function is available only when **Photodetector Gain Adjustment** privilege is enabled for current user account and when instrument is powered on and properly connected.

NOTE When NovoExpress (Opteon) is restarted or new blank experiment file is created, the photodetector gains will be reset to default value too.

H and A

These two columns display all the **H** (height) or **A** (area) options for associated channels. Instrument will collect data from only the checked **H** (height) or **A** (area).

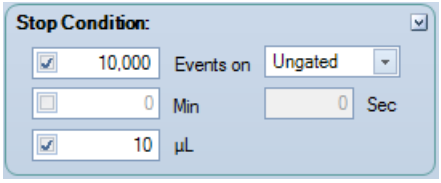
The **H** (height) of all channels is automatically checked by default and user cannot uncheck it.

The **A** (area) associated with FSC, BSSC and SSC can be checked or unchecked individually by user. When user checks any **A** (area) associated with a fluorescent channel, **A** (area) checkboxes of all listed fluorescent channels will be automatically checked.

NOTE The selection of parameters (**Gain**, **Area** and **Height**) can no longer be modified once sample acquisition has started.

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



- **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 1~30,000,000.
- **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 5 and 5000 µL.

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.

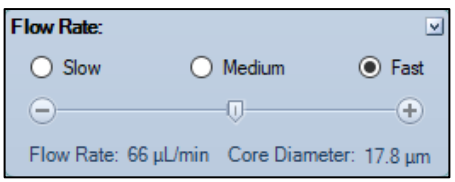
NOTE After sample acquisition has started, stop conditions based on number of events can be modified but stop conditions based on time and volume cannot be changed.

NOTE The number of events displayed in a plot during sample acquisition can be set in **Settings**. Refer to Setting in this guide for more details. 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 (Refer to Parameters Settings in this guide) before acquisition in order to reduce the file size. If events have already been acquired or collected, you can delete events (Refer to Sample in this guide) to discard parts of unnecessary events in the sample.

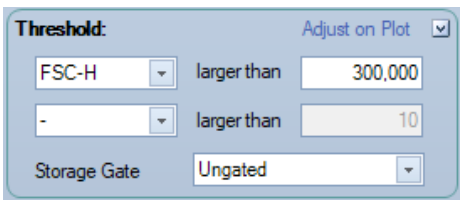
Flow Rate Settings

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



Threshold Settings

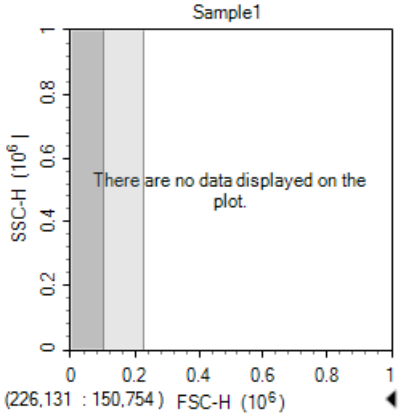
The threshold settings determine which events are recorded during sample acquisition. Only events that exceed the set threshold values are recorded.



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 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 from **Raw Workspace**, 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.



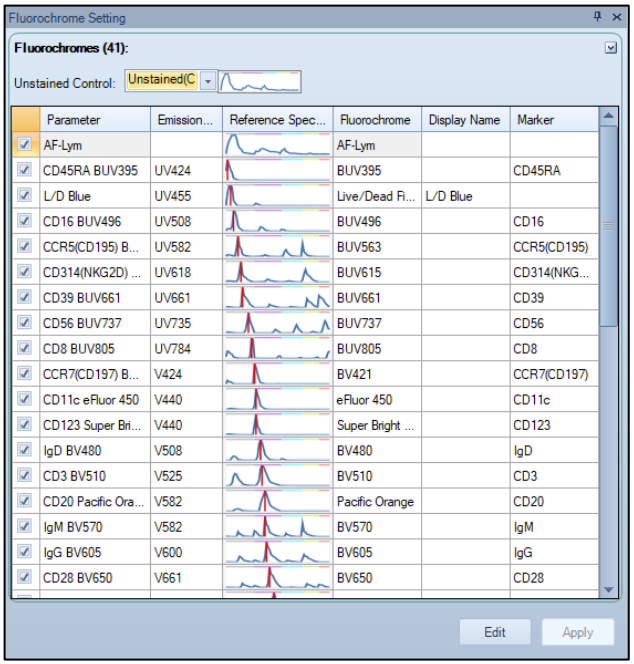
NOTE

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 in this guide for more details. You can also delete the previous events after the acquisition is completed by using the **Delete Events** function (refer to Sample in this guide).

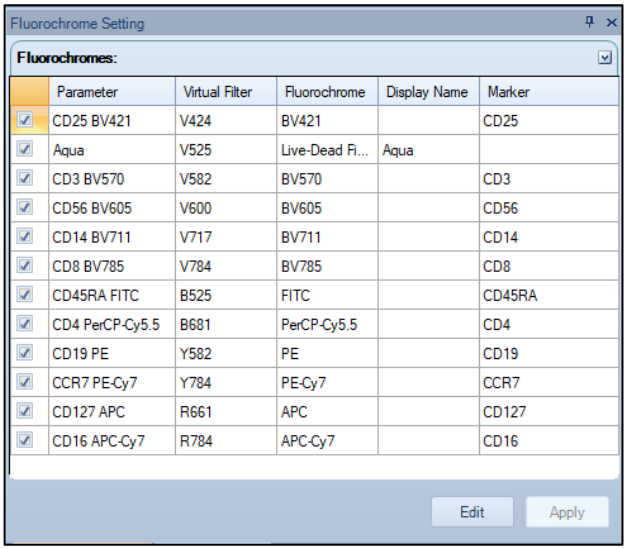
Fluorochrome Setting

The **Fluorochrome Setting** panel allows user to view and edit the information of the selected fluorochromes for current sample. User can click **Fluorochrome Setting** from **Cytometer Setting** panel, or double click **Fluorochrome Setting** node from **Experiment Manager** to access this panel.

This panel will display different information when user selects spectral unmixing or compensation to analyze data.



Fluorochrome Setting Panel when Unmixing is selected.

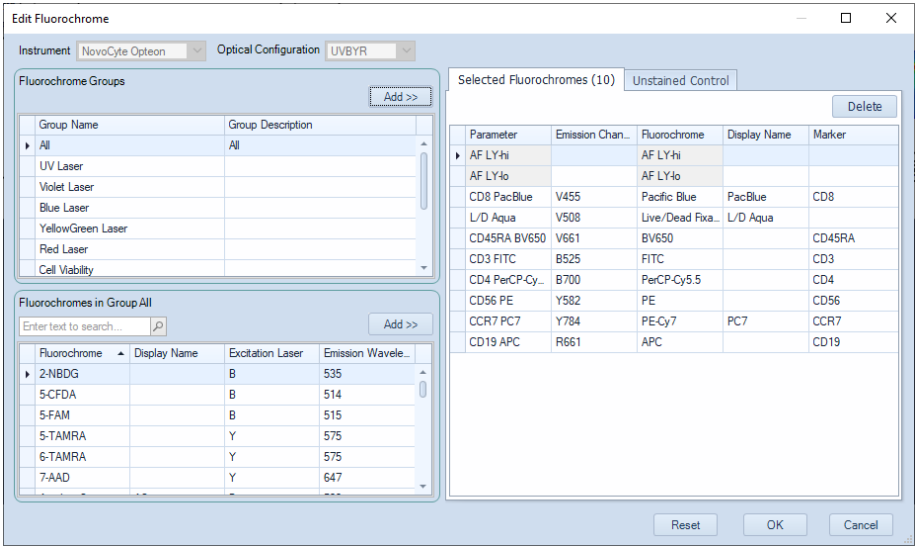


Fluorochrome Setting Panel when Compensation is selected.

Following functions or information are included in **Fluorochrome Setting** window.

- **Unstained Control:** Allows user to select the Unstained Control in the associated drop-down menu. The auto fluorescence signal associated with selected Unstained Control will be listed below and Reference Spectra will be displayed next to the drop-down menu. Any checked auto fluorescence signal will be used for unmixing. This option is available only when user selects unmixing to analyze data.
- **Parameter:** Shows the parameter name of the associated fluorochrome. If the display name exists, the software will automatically use the marker name combined with display name as parameter name. If the display name does not exist, the software will name the parameter as marker name plus fluorochrome name. For example, if the marker name is **CD25**, and the Fluorochrome name is **BV421**, then the parameter is named as **CD25 BV421**. User cannot edit this area.
- **Emission Channel:** Shows the excitation laser type and emission wavelength of the selected fluorochrome. For example, V424 means the excitation laser is 405 nm (violet) and emission wavelength is 424 nm. User cannot edit this area if associated reference spectrum has been defined by software. This information is displayed only when user selects unmixing to analyze data.
- **Reference Spectra:** Shows the available Reference Spectra plot of the selected fluorochrome. User cannot edit this area. This information is displayed only when user selects unmixing to analyze data.

- **Fluorochrome:** Shows the name of the selected fluorochrome. User cannot edit this area.
- **Display Name:** Shows the display name of the selected fluorochrome. User can double click the cell to edit the name.
- **Marker:** Shows the marker name of the selected fluorochrome. User can double click the cell to edit it.
- **Virtual Filter:** Shows the information of Virtual Filter associated with selected fluorochrome. User can click the cell to select the Virtual Filter. This information is displayed only when user selects compensation to analyze data.
- **Edit:** Allows user to edit the fluorochrome settings for current sample. When clicked, following window will be prompted. This function is not available when Reference Control sample (i.e., Unstained or Single Stained control sample) is selected as active sample.

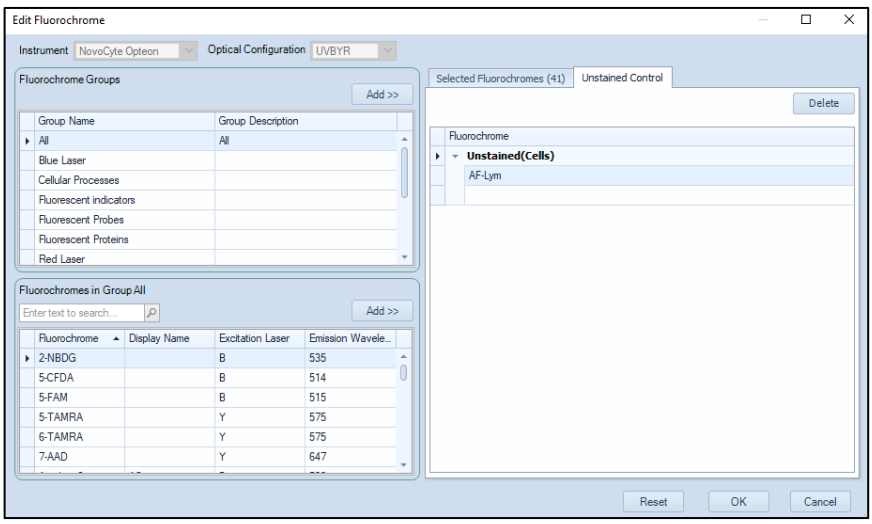


Following functions or information are included in **Edit Fluorochrome** window.

- **Instrument:** Displays the instrument information of currently connected instrument if no sample data have been collected or displays the information of the instrument which was used to acquire the data. User cannot edit this part.
- **Optical Configuration:** Displays the optical configuration of currently connected instrument if no sample data have been collected or displays

the optical configuration of the instrument which was used to acquire the data. User cannot edit this part.

- **Fluorochrome Groups:** This panel displays all available fluorochrome groups currently defined in **Fluorochromes Library**. When user click **Add**, all fluorochromes associated with selected group will be added into **Selected Fluorochromes** panel located on the right side of **Edit Fluorochrome** window.
- **Fluorochrome in Group XXX:** Displays all fluorochromes included in the group user selected in **Fluorochrome Groups** panel. When user click **Add**, the selected fluorochrome(s) will be added into **Selected Fluorochromes** panel located on the right side of **Edit Fluorochrome** window.
- **Selected Fluorochromes:** Click this tab to display current fluorochromes associated with selected sample. User can click **Delete** to delete the selected fluorochrome. User cannot edit the **Parameter** and **Fluorochrome** column. When there is no Reference Spectra associated with selected fluorochrome, the **Emission Channel** information of associated fluorochrome can be edited by double clicking the cell and entering the new value. Otherwise, the **Emission Channel** information cannot be edited. The **Display Name** and **Marker** information of all displayed fluorochromes can be edited by double clicking the cell and entering the new value.
- **Unstained Control:** This tab is only available when unmixing is selected for data analysis. When clicked, it will display autofluorescence information associated with Unstained Control sample. User can click **Delete** to delete the selected fluorochrome.



- **Reset:** Click to reset all the settings to default.

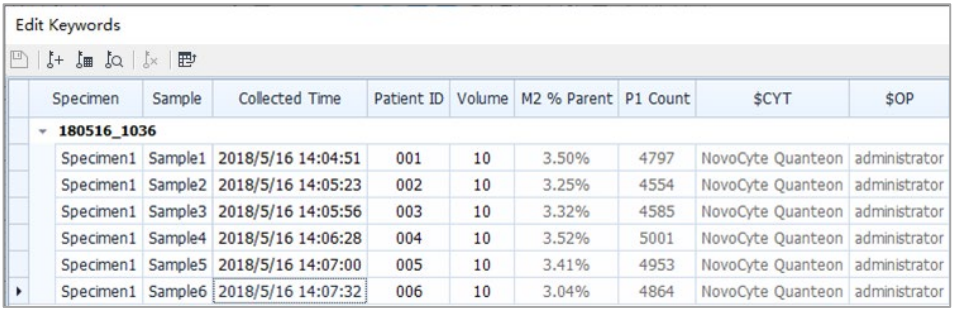
- **Apply:** Click to apply any changes to the **Fluorochrome Setting** panel and the unmixing result will be automatically updated. This function is only available when there are any changes to the information in **Fluorochrome Setting** window.

Sample Keywords

Before starting the experiment or after the sample acquisition, user can create, edit and export keywords of selected sample(s).

Edit Keywords Window

Most keywords associated functions can be accessed through **Edit Keywords** window as shown below.

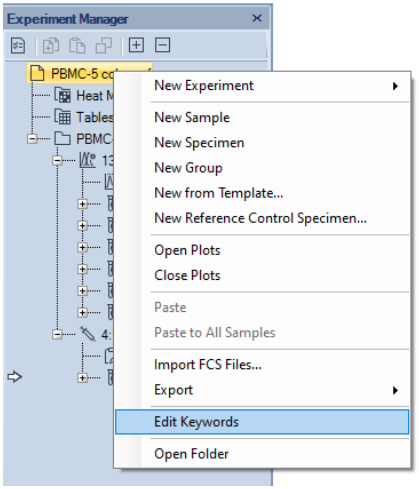


Specimen	Sample	Collected Time	Patient ID	Volume	M2 % Parent	P1 Count	$CYT	$OP
180516_1036								
Specimen1	Sample1	2018/5/16 14:04:51	001	10	3.50%	4797	NovoCyte Quanteon	administrator
Specimen1	Sample2	2018/5/16 14:05:23	002	10	3.25%	4554	NovoCyte Quanteon	administrator
Specimen1	Sample3	2018/5/16 14:05:56	003	10	3.32%	4585	NovoCyte Quanteon	administrator
Specimen1	Sample4	2018/5/16 14:06:28	004	10	3.52%	5001	NovoCyte Quanteon	administrator
Specimen1	Sample5	2018/5/16 14:07:00	005	10	3.41%	4953	NovoCyte Quanteon	administrator
Specimen1	Sample6	2018/5/16 14:07:32	006	10	3.04%	4864	NovoCyte Quanteon	administrator

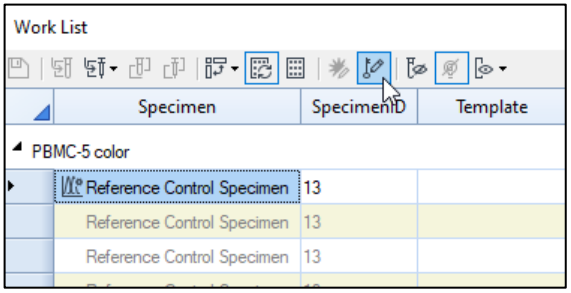
To open the **Edit Keywords** window, use one of the following methods:

- From **Experiment Manager** panel: Select experiment file, group(s), specimen(s), or sample(s). Right click the selected item(s) and click **Edit Keywords** in the right click menu.

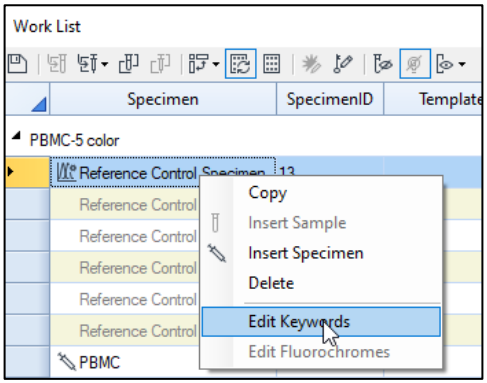
NOTE To select more than one group, specimen or sample, press **Ctrl** or **Shift** key while clicking each item.



- From **Work List** window: Click **Home** > **Experiment** > **Work List** to open the **Work List** window. Open the **Edit Keywords** window with one of the methods below:
  - Click  from the toolbar. Keywords associated with all samples will be listed in prompted **Edit Keywords** window.



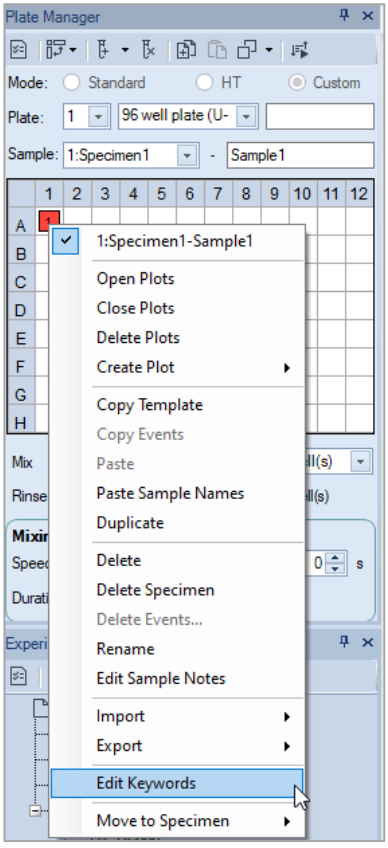
  - Highlight the row(s) associated with selected sample(s). Right click and select **Edit Keywords** from the right-click menu. Keywords associated with the selected sample (s) will be listed in prompted **Edit Keywords** window.



- From **Plate Manager** panel: Right click the selected well(s) and click **Edit Keywords**. This function is available only when NovoSampler S is connected.

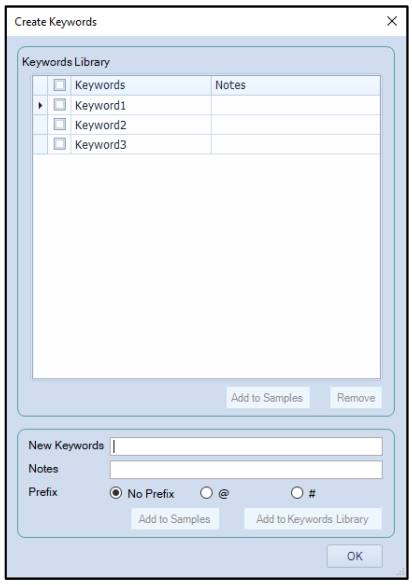
NOTE

To select more than one well, press **Ctrl** or **Shift** key while clicking each well in the Well Map.

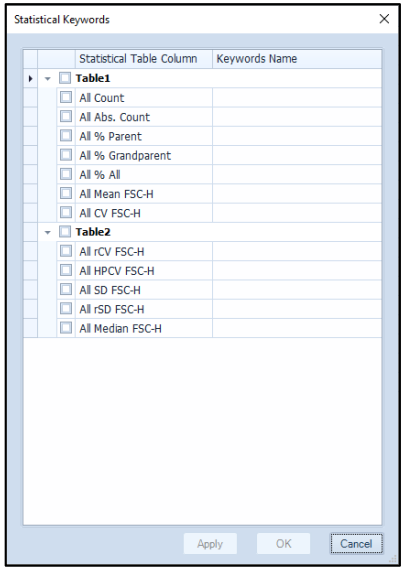


Edit Keywords window includes the following toolbars.

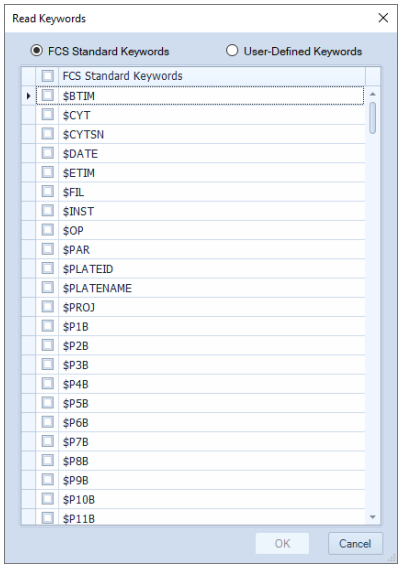
Icon	Description
	Save: Save the changes to the current keywords list. This function is disabled (i.e., greyed out) when there is no change to the current list.
	Create Keywords: When clicked, following window will be prompted. User can create new keywords or add existing keywords from **Keyword Library** to the samples. Please refer to Create Keywords in this guide for details.



Statistical Keywords: When clicked, following window will be prompted. User can add selected statistics from statistical table as a new keyword in **Edit Keywords** window. Please refer to Add Statistics as Keywords in this guide for details.

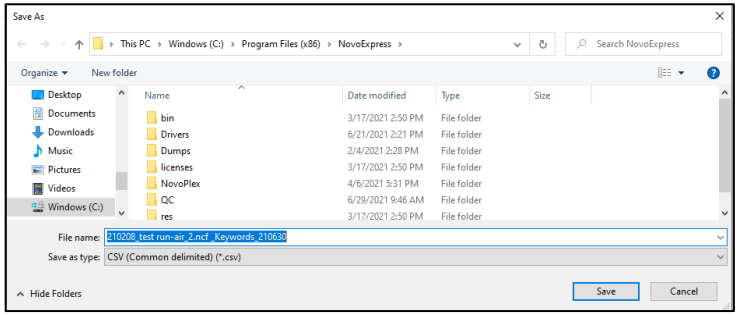


Read Keywords: When clicked, following window will be prompted. User can read and add selected **FCS Standard Keywords** or **User-Defined Keywords** into **Edit Keywords** window. Please refer to Read Keywords from Sample in this guide for more details.



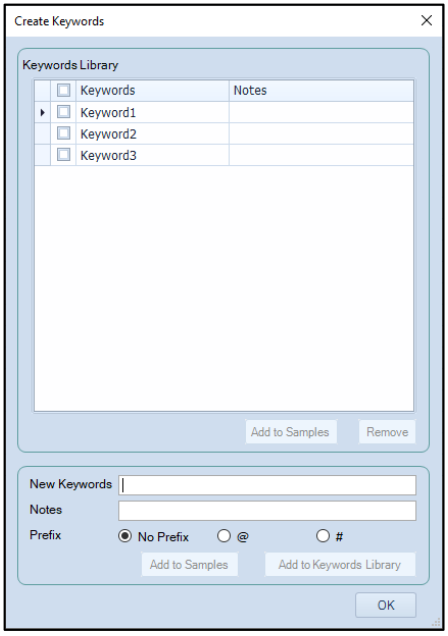
Delete Keywords: When clicked, the selected keyword column in **Edit Keywords** window will be deleted.

Export CSV File: When clicked, following window will be prompted. User can export the keywords to a CSV file. In the prompted window as shown below, select the target folder and edit the file name, and click **Save** to export the keywords listed in **Edit Keywords** window as a CSV file in designated folder. By default, the exported CSV file will be saved in the folder where the experiment file is stored (e.g., **D:\NovoExpress (Opteon) Data)** in format of **Experiment File Name_Keywords_YYMMDD.CSV**.



Create Keywords

User can create new keywords or add existing keywords from **Keyword Library** to the samples in **Edit Keywords** window. To do this, click from the toolbar in **Edit Keywords** window to open the **Create Keywords** window as shown below.



This window consists of two panels.

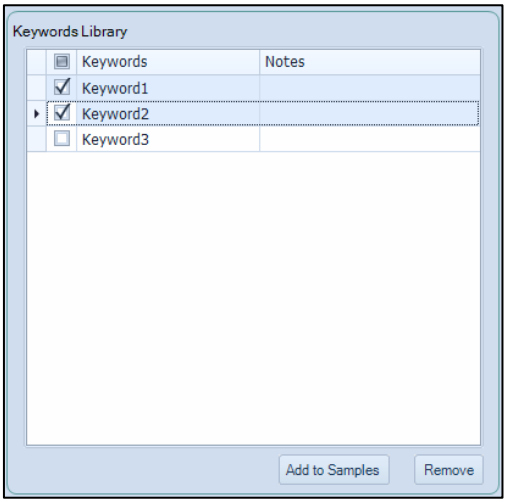
- The top panel is the **Keywords Library** which lists all keywords available in the **Keywords Library**. Keywords Library serves as a convenient way to save the commonly used keywords to be able to add to samples quickly.

  To add keyword(s) from the **Keywords Library** to the selected samples, select the checkbox in front of one or more keywords from the list, click **Add to Samples.** The selected keyword(s) will show up as column(s) in the **Edit Keywords** window.

  User can also add specific keyword(s) to the **Keywords Library** or remove specific keyword(s) from the **Keywords Library**. To add keyword(s) to **Keywords Library**, please refer to the method described below.

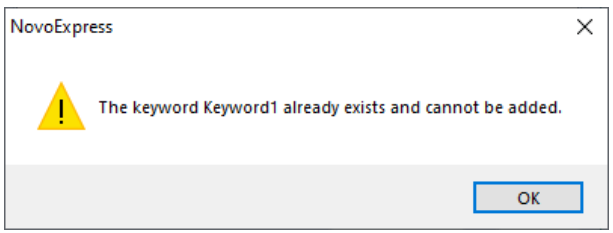
  To remove keywords, select the checkbox in front of one or more keywords from the list in **Keywords Library**, and click **Remove**.

  Click **OK** in the bottom will close the **Create Keywords** window.

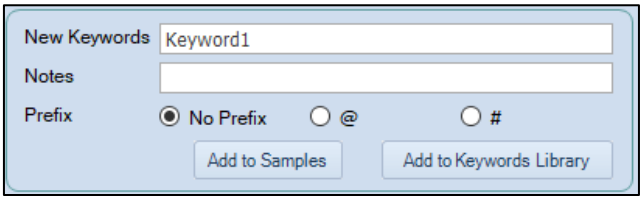


NOTE

If the selected keyword already exists in **Edit Keywords** window, when user clicks **Add to Samples**, following window will be prompted indicating the selected keywords cannot be added.

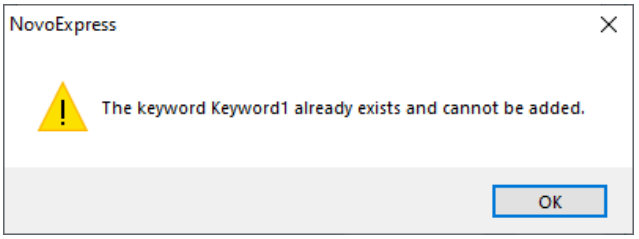


- The bottom panel allows user to create new keywords in the selected format and add to **Edit Keywords** window or **Keyword Library**. To do this, enter the newly defined keyword in **New Keywords** field, enter associated notes in **Notes** field if needed, select the prefix of the new keyword, click **Add to Samples** to add the new keyword to **Edit Keywords** window, or click **Add to Keywords Library** to add the new keyword to the **Keyword Library**. By default, the newly created keyword does not come with a prefix (i.e., **No Prefix** option in **Prefix** field is automatically selected). User can add prefix of **@** or **#** to the new keyword by selecting the associated option in **Prefix** field. After creating the new keyword, user can click **OK** in the bottom to close the **Create Keywords** window.



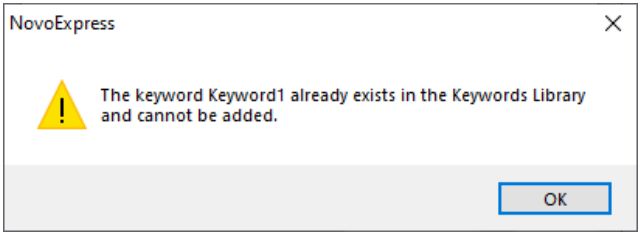
NOTE

If the new keyword already exists in **Edit Keywords** window, when user clicks **Add to Samples**, following window will be prompted indicating the key word cannot be added.



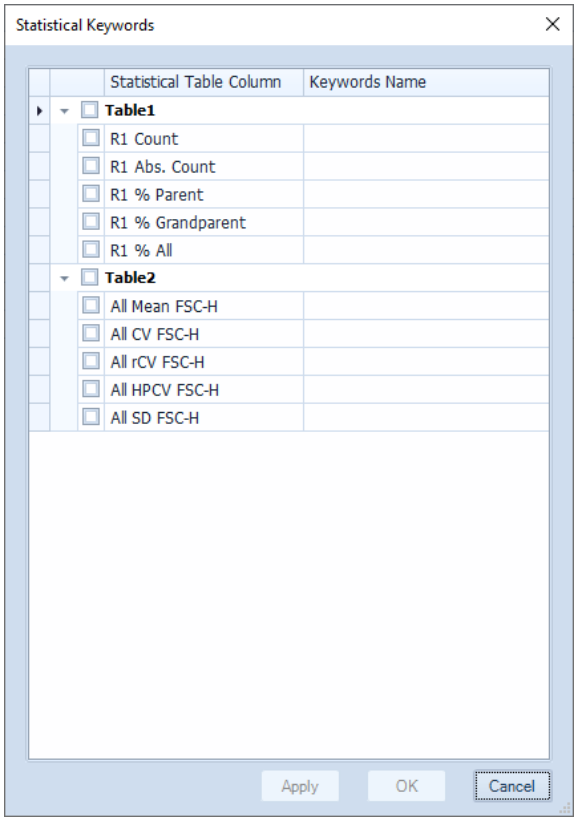
NOTE

If the new keyword already exists in **Keywords Library**, when user clicks **Add to Keywords Library**, following window will be prompted indicating the keyword cannot be added.



Add Statistics as Keywords

User can add statistics from **Statistical Table** as new keywords in **Edit Keywords** window. To do this, click from the toolbar in **Edit Keywords** window to open the **Statistical Keywords** window as shown below.



Select the checkbox in front of one or more statistics from the list, click **Apply** to add the selected statistics as new keyword(s) to **Edit Keywords** window.

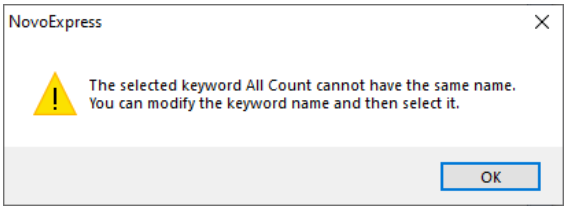
NOTE If there is no default type statistical table created for the selected experiment file, no statistics will be displayed in this window.

NOTE If there is more than one default type statistical table created for selected experiment file, the associated statistics will be displayed in groups respectively in this window.

NOTE If the name of statistics is different from associated keyword name, the keyword name will be displayed in **Edit Keywords** window when user clicks **Apply**. If there is no keyword name associated with corresponding statistics, the name of statistics will be displayed in **Edit Keywords** window when user clicks **Apply**.

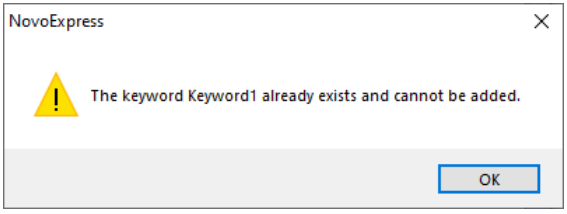
NOTE

If more than one statistical parameters with the same name are selected, when user clicks **Apply**, following window will be prompted. User needs to click **OK** to cancel the operation, rename the statistics before clicking **Apply** again.



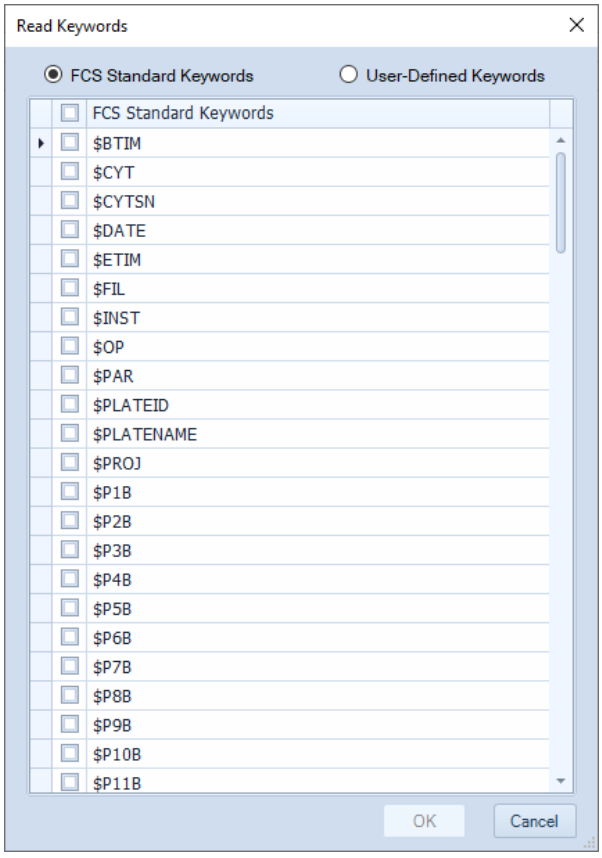
NOTE

If the name of statistics already exists in **Edit Keywords** window, when user clicks **Apply**, following window will be prompted indicating the keyword cannot be added.



Read Keywords from Sample

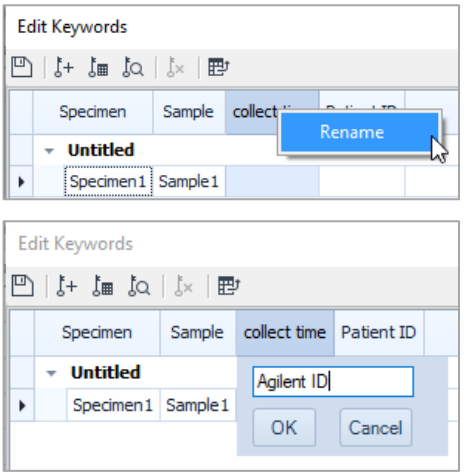
User can read **FCS Standard Keywords** or **User-Defined Keywords** from selected samples in **Edit Keywords** window. To do this, click from the toolbar in **Edit Keywords** window to open the **Read Keywords** window as shown below.



Select **FCS Standard Keywords** or **User-Defined Keywords** to display the corresponding keywords from the selected sample. Check the checkbox in front of selected keyword(s), click **OK**. The selected keyword(s) will be added to the **Edit Keywords** window.

Rename and Edit Keywords

User can rename **Statistical Keywords** and **User-Defined Keywords** in **Edit Keywords** window. To do this, right click the keyword name, click **Rename**, enter the new name, click **OK**.



User can also edit the value of **User-Defined Keywords** in **Edit Keywords** window by double clicking the associated cell and entering the new value.

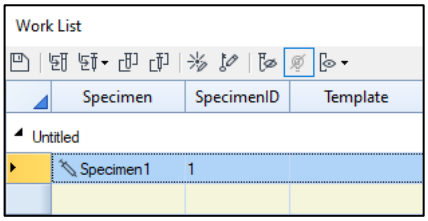
NOTE

The **User-Defined Keywords** can also be edited in **Work List**. Please refer to Edit Keywords in Work List in this guide for more details.

Work List

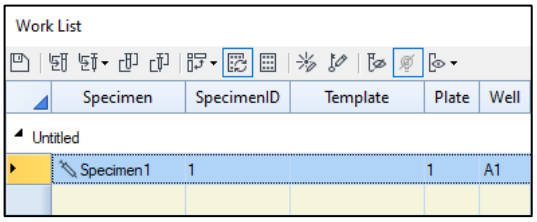
Before starting the experiment, the **Work List** can be used to set up the sample list. The **Work List** allows users to create new specimens and samples, import specimens or samples from a template, duplicate specimens or samples, and copy and paste sample information. Alternatively, user can perform above operation through **Experiment Manager** (Refer to Experiment Manager in this guide for).

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



When NovoSampler S is not connected

When NovoSampler S 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.

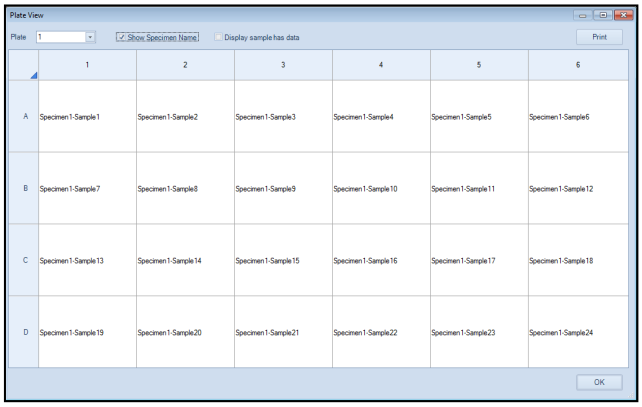


When NovoSampler S is connected

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.**
	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 S 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 S is used. When selected, a blue outline will show on the icon. When Well ID of a sample is modified

manually, the NovoExpress (Opteon) 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 S 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.



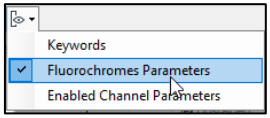
Edit Fluorochromes: Click to open **Fluorochrome Setting** window**.** This option is available only when experiment sample is selected. Please refer to Fluorochrome Setting in this guide for more details.

Edit Keywords: Click to open **Edit Keywords** window**.** Please refer to Edit Keywords Window in this guide for more details.

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

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

Display Column: Click to select to display or hide the **Keywords, Fluorochromes Parameters, Enabled Channel Parameters** column.



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:

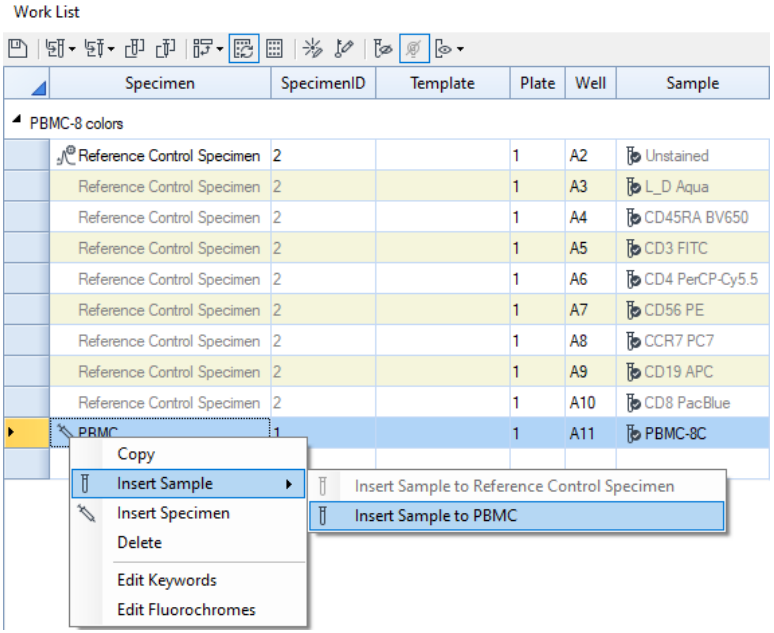
- From the **Experiment Manager** or **Plate Manager** panel, click the **Work List** icon at the top of the window.

- 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, highlight to select a sample row and right click. 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.



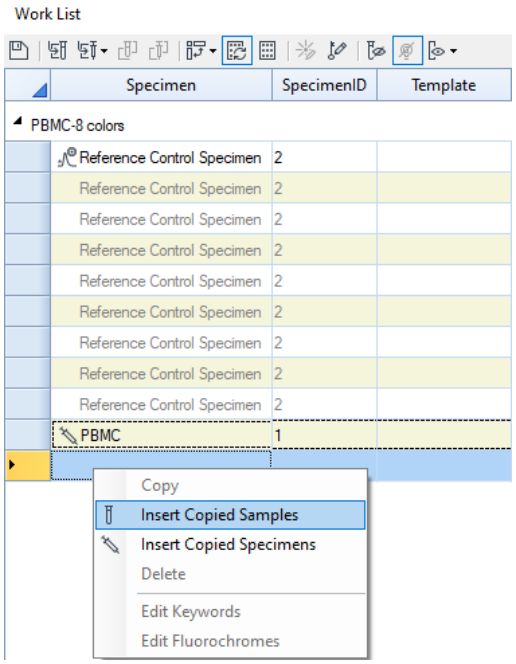
Copy and Insert the Copied Specimen or Sample

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.



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.

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**.

NOTE When importing or exporting sample template or specimen template, only the **User-Defined Keywords** will be automatically included.

Create a Duplicate Specimen or Sample

To duplicate a specimen or sample:

1 To select the specimens or samples for duplicating, click and drag in the first column of the **Work List**. The selected rows become highlighted.

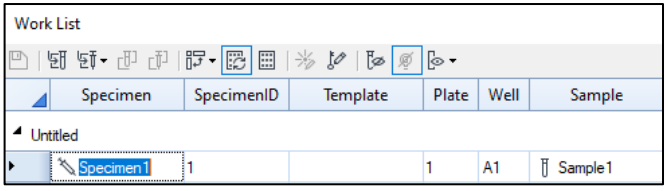
2 After selecting the samples, click the **Duplicate as Sample** icon , to duplicate the samples and add them under the last specimen of the **Work List**.

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.



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

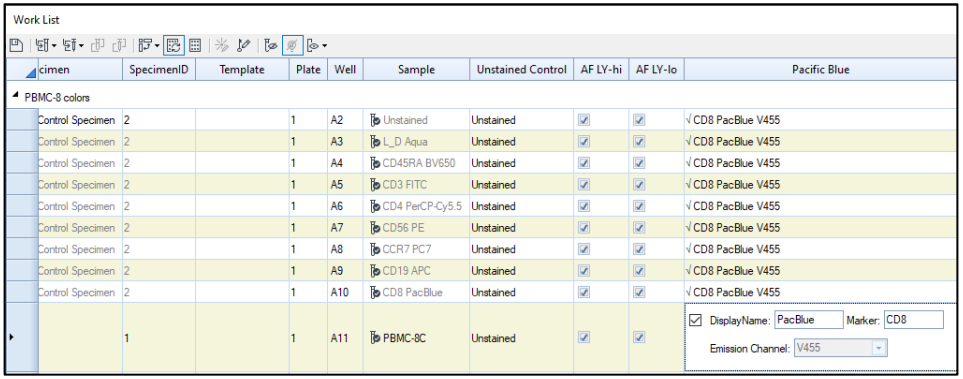
When a specimen, specimen ID, or template is entered into in an empty row, a new specimen is created after the edit is done.

Edit Fluorochrome Parameters

To modify a fluorochrome parameter for experiment sample, double click on the cell of selected fluorochrome in the **Work List**. In the prompted menu, enter the new value for **Display Name, Marker** and press **Enter** key.

The **Emission Channel** value cannot be edited when it has Reference Spectra. The fluorochrome parameter for Reference Control sample cannot be edited.

User can also check or uncheck the checkbox to select whether to include associated fluorochrome into the spectral unmixing calculation.

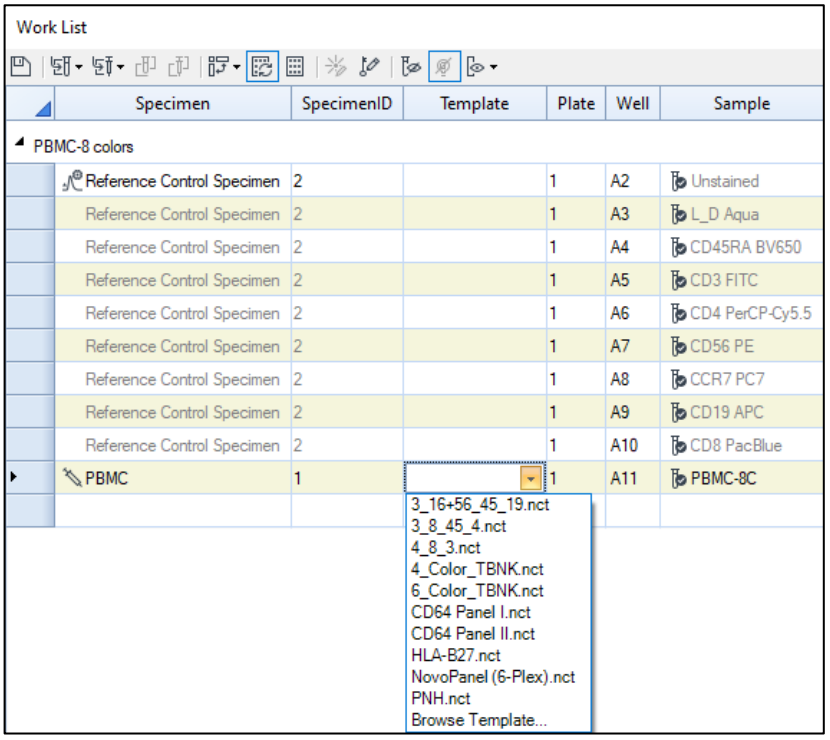


Unmixing with Auto Fluorescence

When user selects to display **Fluorochromes Parameters** in the **Work List**, the cells associated with auto fluorescence will be displayed with checkbox. These checkboxes are checked by default and associated auto fluorescence will be automatically included into the spectral unmixing calculation. User can uncheck the checkbox(es) to exclude the associated auto fluorescence from the spectral unmixing calculation.

Edit Template

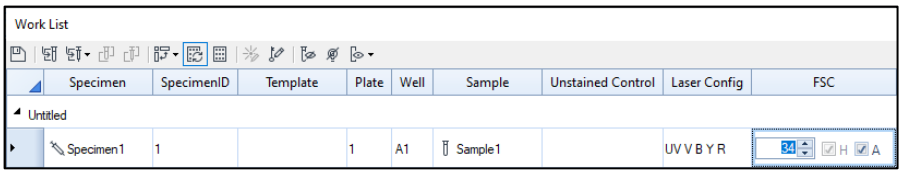
To modify a template for regular sample, double-click on the **Template** cell in the **Work List**. After a new template is selected, the template is applied into the current specimen. Refer to Templates in this guide for additional details. The template for Reference Control specimen cannot be edited.



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

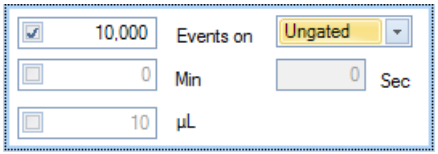
Edit Acquisition Parameters

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

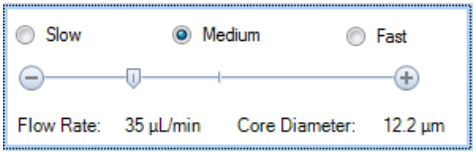


Edit Stop Conditions, Sample Flow Rates, and Threshold Settings

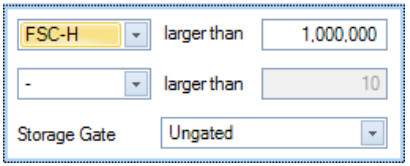
To set the sample stop conditions for newly created sample, double-click the stop condition cell to enter edit mode.



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



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



Edit Keywords in Work List

User can only view and edit the **User-Defined Keywords** in **Work List**.

To edit a **User-Defined Keyword**, double click the associated cell and enter the new value. **Statistical Keywords** and **FCS Standard Keywords** will not be visible from **Work List**.

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

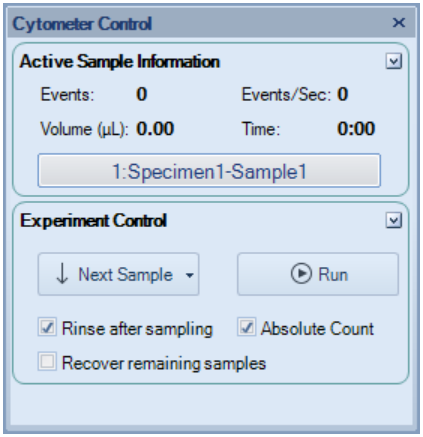
NOTE

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**.

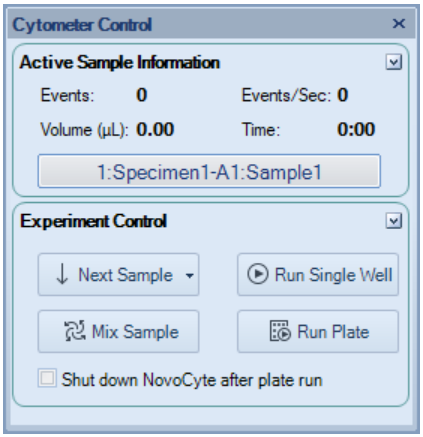
Cytometer Control

The **Cytometer Control** panel contains the **Active Sample Information** and the **Experiment Control** panel. The **Experiment Control** panel displays additional buttons when NovoSampler S is connected.

- When NovoSampler S is not connected



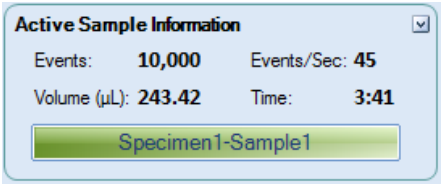
- When NovoSampler S is connected



Active Sample Information

In the **Experiment Manager** panel, the active sample is indicated by the white 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 S 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

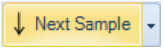
The **Experiment Control** panel displays different functions depending on whether the instrument is connected to NovoSampler S.

When NovoSampler S is not connected

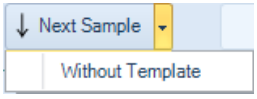
The **Experiment Control** panel includes the **Next Sample** and the **Run** buttons.

- **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**, **Fluorochrome Setting**, **Reference Spectra, Report** and **Analysis**.

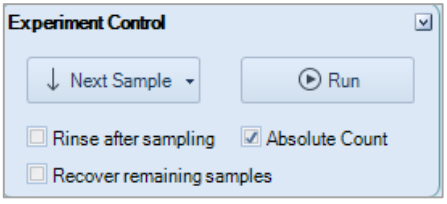
  To create a new sample without the template settings, click on the arrow on the right side of the **Next Sample** button and select **Without Template**. The new sample contains the same **Cytometer Settings** as the previous samples, but **Fluorochrome Setting**, **Reference Spectra, Report** and **Analysis** settings are not transferred.

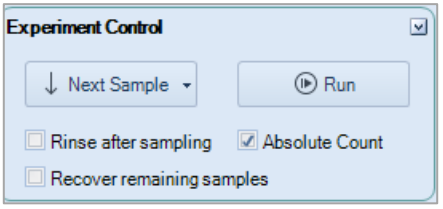
- **Run**

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

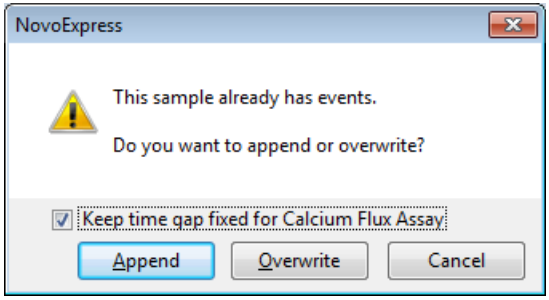
  If the active sample does not contain event data, the **Run** button appears with a solid triangle. Click the **Run** button to begin sample acquisition.

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

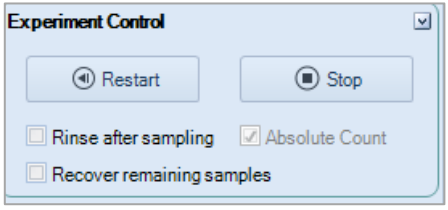
Clicking the **Run** button causes a dialog window to appear. Click **Append** to add additional events to the existing events. Click **Overwrite** to delete the existing events and collect new events. If the "Keep fixed time gap when appending sample events for Calcium Flux Assay" function is enabled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



- **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.

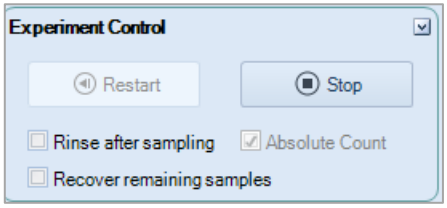
  When **Restart** is clicked, the previously acquired events will be deleted and the acquisition status including sampling volume and sampling time will be reset to zero. Then the sample acquisition will restart until one of the defined stop conditions is met. The **Restart** button is only visible after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

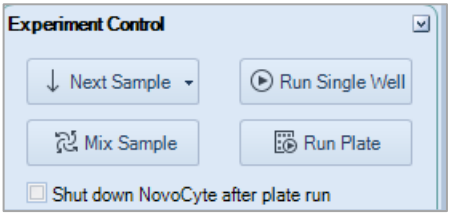


- **Rinse after sampling**: check to enable SIP rinse function after each sample acquisition.
- **Absolute Count:** check to enable sample collection in **Absolute Count** mode.
- **Recover Remaining Samples**: check to enable recover remaining sample already aspirated into the sampling tubing but not 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 S is used).

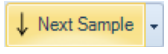
NOTICE Do not remove the sample tube, tube rack or microwell plate to prevent spill of the sample. Ensure the moving path of SIP is not blocked by any obstacles.

When NovoSampler S is connected

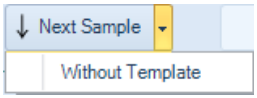
The **Experiment Control** panel includes the **Next Sample**, **Run Single Well, Mix Sample** and **Run Plate** buttons.



- **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**, **Fluorochrome Setting**, **Reference Spectra, 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 Setting** as the previous samples, but **Fluorochrome Setting**, **Reference Spectra, Report** and **Analysis** 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

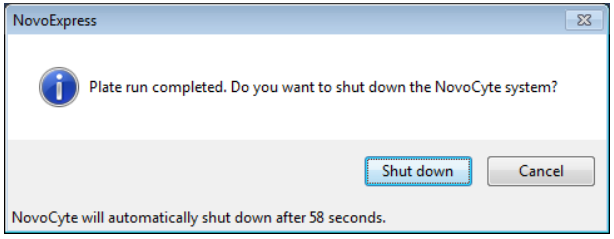
When **Run Single Well** is selected, the sample injection probe will automatically be rinsed after sample acquisition. This SIP rinse function will minimize the carryover between samples.

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

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 (Opteon) can automatically shut down the instrument after plate run is completed. When the plate run is completed, NovoExpress (Opteon) will prompt a 60 second 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 (Opteon) will shut down the instrument automatically.



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

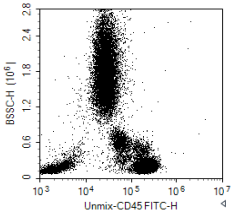
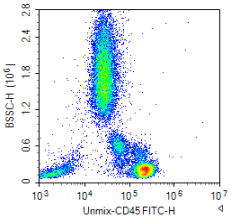
NOTE User can also schedule automatic instrument power on or off at selected time and frequency by clicking **Schedule Power On & Off** from the **Operation** panel. Please refer to Instrument in this guide for more details about this function.

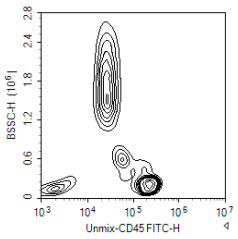
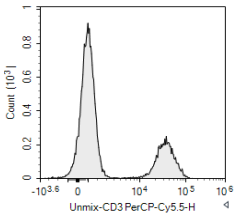
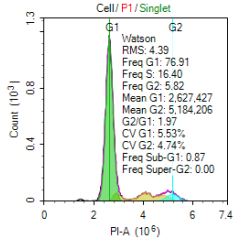
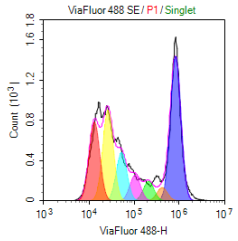
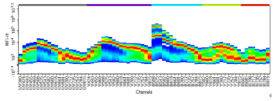
5 Data Analysis

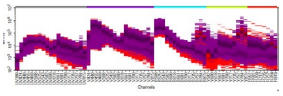
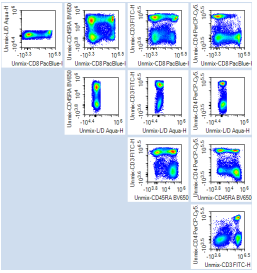
This chapter provides information for data analysis tools, including **Plots, Gates, Statistics, Spectral Unmixing and Compensation, Cell Cycle Analysis, Cell Proliferation Analysis, Statistical Tables, Heat Maps**.

Plots

The NovoExpress (Opteon) Software includes dot plots, density plots, contour plots, histograms, cell cycle plot, cell proliferation plot, spectrum density plot and fluorochrome 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.	

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. Refer to Cell Cycle Analysis in this guide 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. Refer to Cell Proliferation Analysis in this guide for more information.	
Spectrum Density Plot	Single-parameter	The fluorescence channel(s) of selected laser is represented along the horizontal axis, and the Mean Fluorescence Intensity (MFI) value is represented along the vertical axis. This function is not available when **Unmixed Workspace** is selected. Refer to Spectrum Density (Dot) Plot	

			in this guide for more information.	
Spectrum Dot Plot		Single-parameter	The fluorescence channel(s) of a selected laser is represented along the horizontal axis, and the Mean Fluorescence Intensity (MFI) value is represented along the vertical axis. This function is not available when the **Unmixed Workspace** is selected. Refer to Spectrum Density (Dot) Plot in this guide for more information.	
Fluorochrome Bi-variate Plot		Two-parameter	Create a matrix of plots with selected fluorescence parameters plotted against each other. Refer to Fluorochrome Bi-Variate Plots in this guide for more information.	

Create a Plot

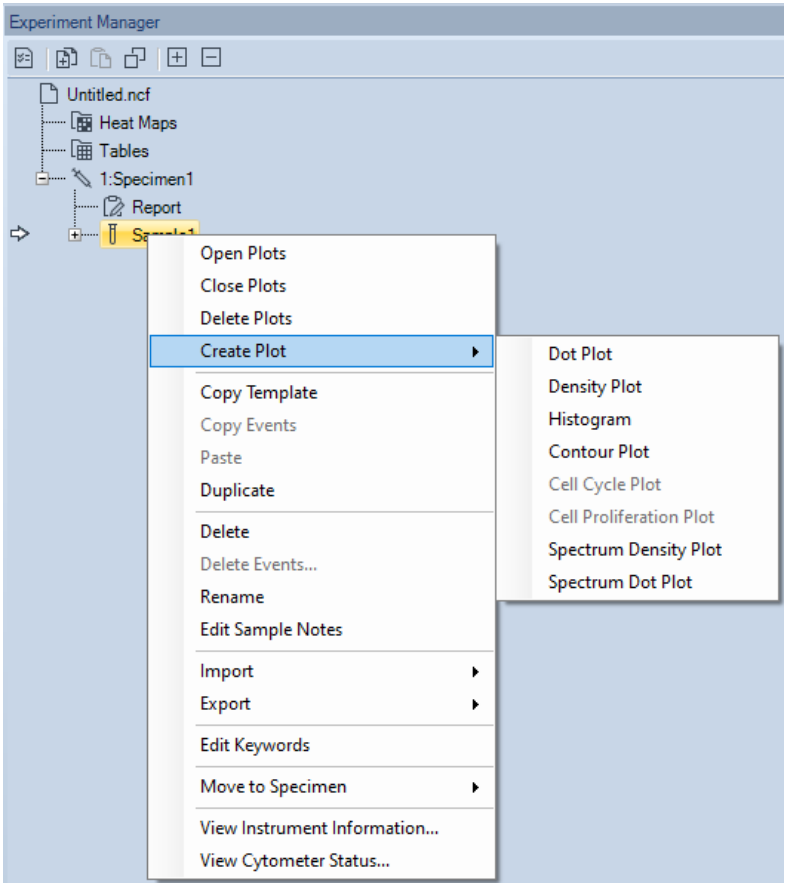
In the NovoExpress (Opteon) Software, user can create **Dot Plot, Density Plot, Histogram Plot, Contour Plot, Spectrum Density Plot, Spectrum Dot Plot** in **Raw Workspace**, and create **Dot Plot, Density Plot, Histogram Plot, Contour Plot, Cell Cycle Plot, Cell Proliferation Plot, Fluorochrome Bi-Variate Plot** in **Unmixed Workspace.** Most plots can be created through the workspace toolbar, or the **Experiment Manager**, or the gate of existing plot. The **Fluorochrome Bi-Variate Plot** can only be created through the toolbar. In addition, plots can be duplicated in the Workspace, copied in the **Experiment Manager**, and imported from templates.

Create a Plot through 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 through 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.



Create a Plot through a Gate of Existing Plot

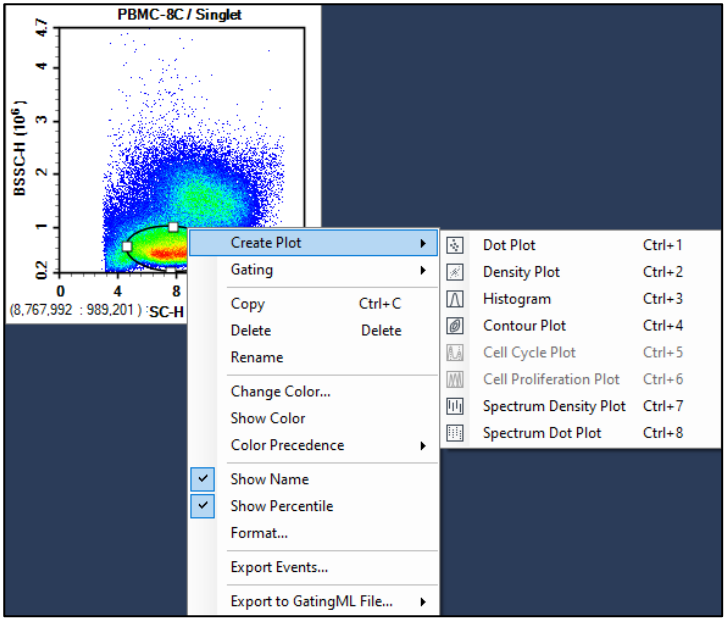
In the Workspace, new plots can be created using gates from previously created plots. New plots created through gates will only display events within the gate. There are multiple methods for creating a new plot through a gate:

- 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**

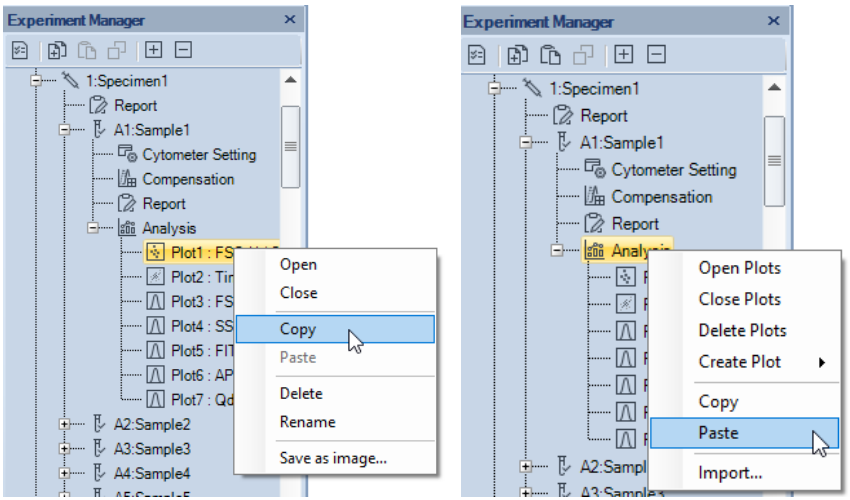
  In the **Experiment Manager** or **Gate Manager** panel, right click on a gate heading. Select **Create Plot** and select a plot type.

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 **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.

NOTE When importing or exporting sample template or specimen template, only the **User-Defined Keywords** will be automatically included.

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.

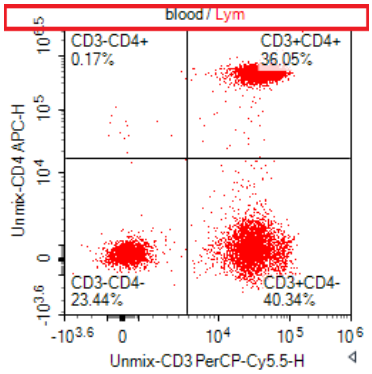
Edit Plots

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

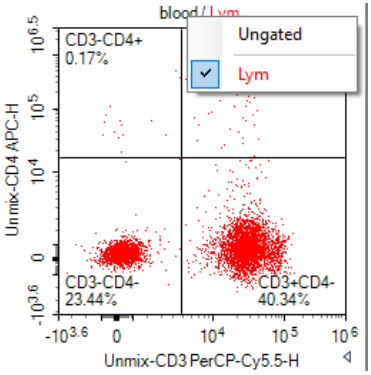
newer plot. The new plot can then be used to analyze the subpopulation or to further gate for more specific populations.

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 Lym.



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.

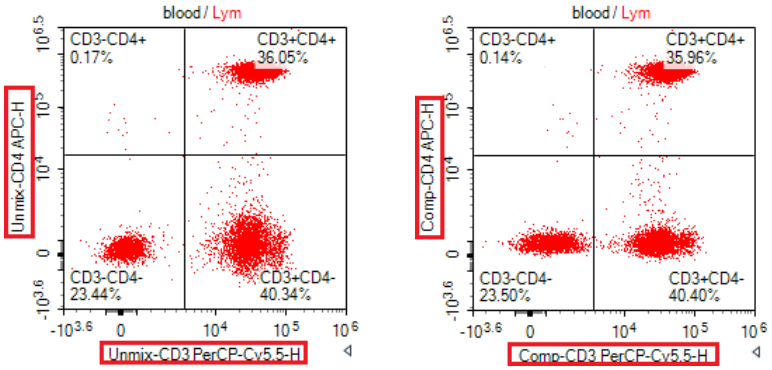


- Right-click in the plot, select **Gating** and select the gate. If **Ungated** is selected, the plot is not gated and all events are displayed in the plot.
- Click on the plot to be gated to select the plot. In the **Plot** tab of the **Menu Bar**, select the gate in the drop-down menu from the properties 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 are 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. When user selects Spectral Unmix, Unmix**-** is automatically added to each plotted fluorescent parameter. When user selects Compensation, **Comp-**is automatically added to each plotted fluorescent parameter.

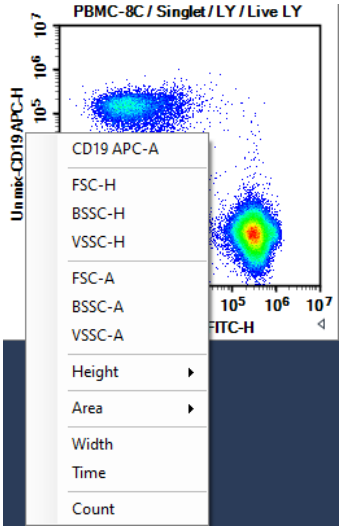


NOTE

User cannot select fluorochrome parameter (e.g., CD3 PerCP-Cy5.5) in **Raw Workspace**. User cannot select fluorescent channel parameter (e.g., UV380) as plot parameter in **Unmixed Workspace**.

To change the plot parameters:

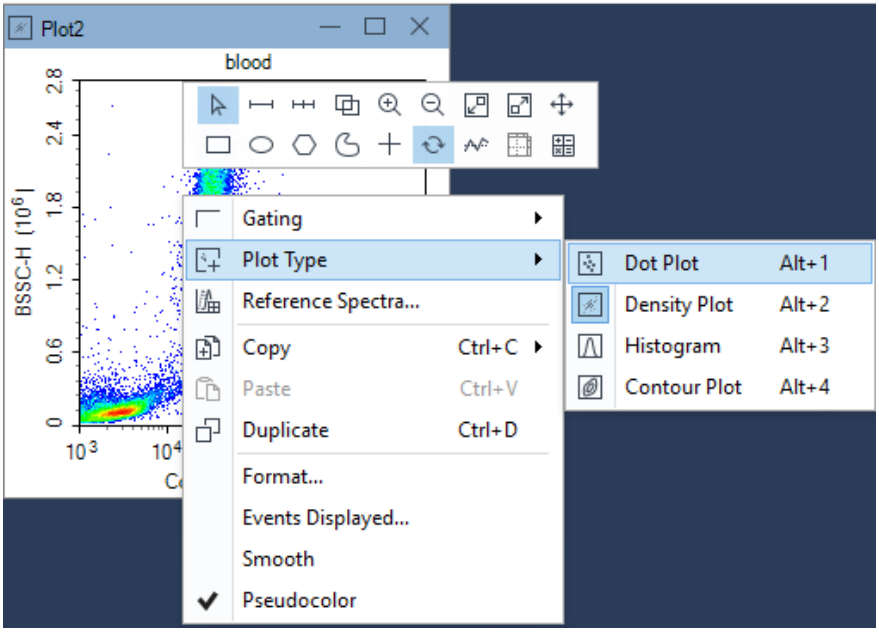
Double click or right-click on the plot label and select the desired parameter. In the prompted drop-down menu, the scatter and fluorescent parameters are listed in height and area measurements. Additional parameters include Width, the width of the individual event signal, Time, the time of the individual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



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 (e.g., dot, density, or contour plot) to a single-parameter plot (e.g., 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.

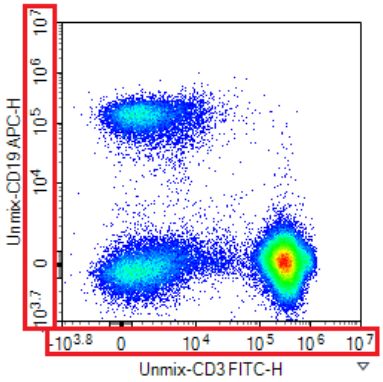
Delete Plots

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.



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.

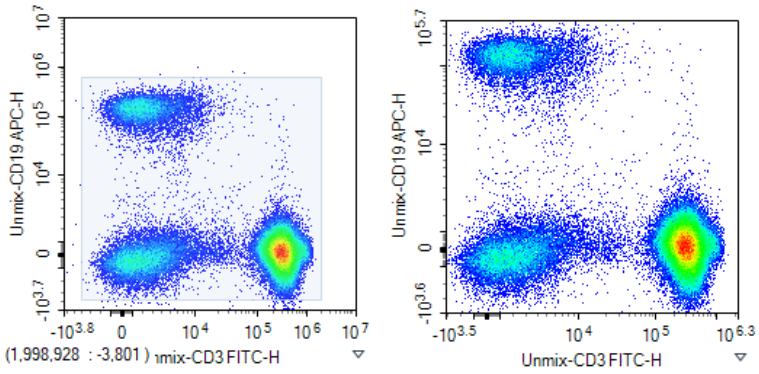
There are multiple methods for changing the coordinate range including zooming, the auto range tool, the move tool, and manually entering the coordinate range. Select these tools either from workspace toolbar or plot right-click popup mini toolbar.

- 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.



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.

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**.

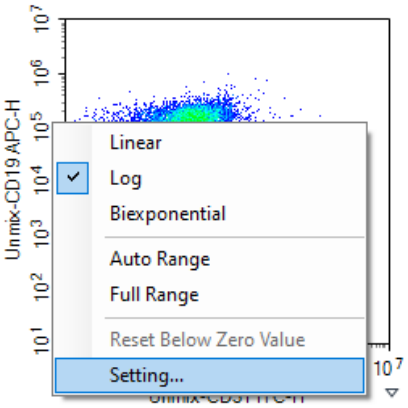
To use this tool, click and drag in a plot to move the display region. The coordinate range then automatically adjusts.

NOTE

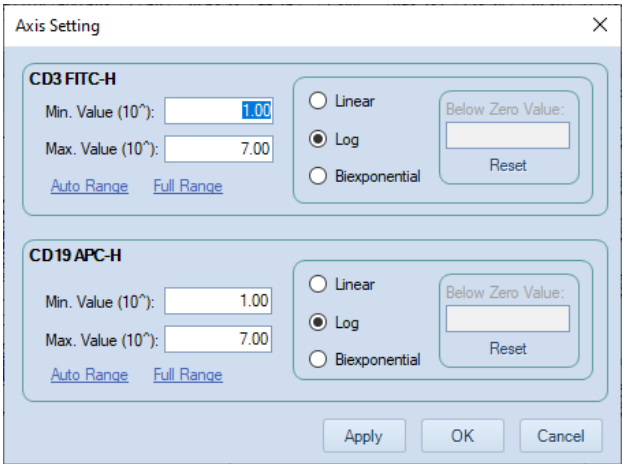
To pan the plot only along one parameter, click and drag along the parameter's coordinate label. This method causes the plot to pan along the selected parameter, while the second parameter's coordinate range will remain the same.

- 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.

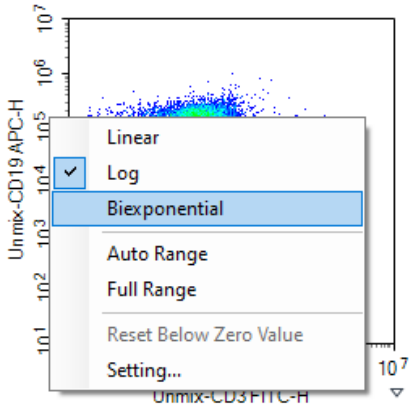


- Manually Setting the Coordinate Range in the **Plot** tab of **Menu Bar**.

Set the Coordinate Scale

The available coordinate scaling types available in the NovoExpress (Opteon) 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 Spillover Compensation has resulted in negative values.

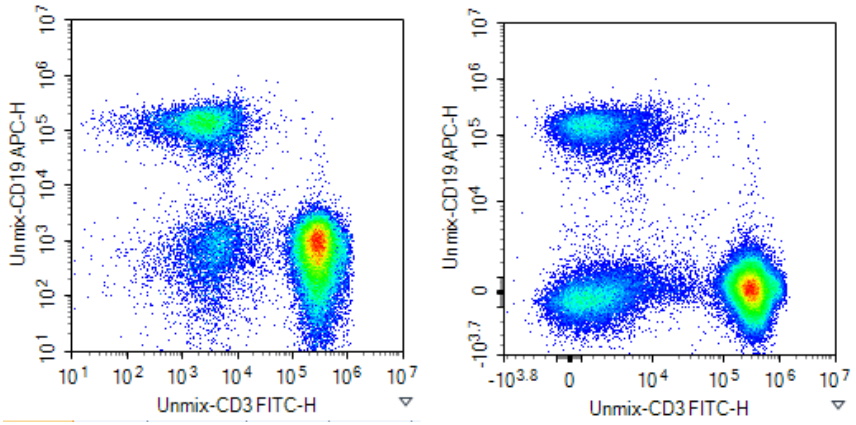
To set the coordinate scaling, right-click on the coordinate label and select the axis scaling.



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

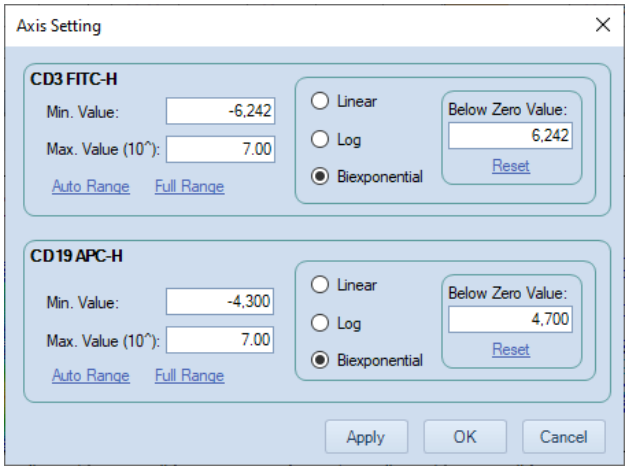
Biexponential display uses biexponential scale to transform data, especially for those where cells become piled up in the first decade at the axis. This is displayed as fluorescence values <0 even for uncompensated data. Biexponential transformation incorporates linear scaling for low values together 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.



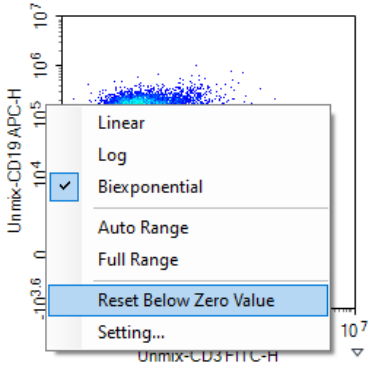
- Below Zero Value of biexponential scale

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

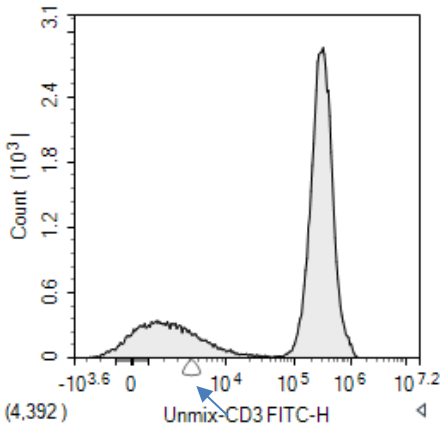
  Manually enter the **Below Zero Value** in **Axis Setting** dialog or click **Reset** button to let NovoExpress (Opteon) software calculate the value automatically. When resetting, software calculates the **Below Zero Value** according to the events data in current gating of plot. The minimum value of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



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.



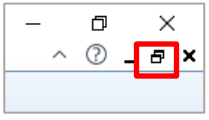
To adjust the Below Zero Value directly on plot, move the cursor to a coordinate axis with biexponential scale, a triangular symbol will appear on the position of below zero value. Click and drag the triangular symbol to adjust the Below Zero Value, and the plot will reflect the change dynamically while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



Adjust the Size of Plots

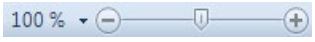
- Maximizing and restoring a plot window

  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.



- 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

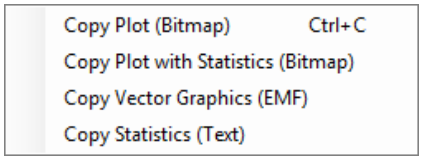
Adjusting the size of the plot window does not affect the coordinate range of the plots. To adjust the coordinate ranges, Refer to Set the Coordinate Range in this guide.

Copy or Save Plots

Plots from the NovoExpress (Opteon) 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 to copy the selected plot in bitmap format.

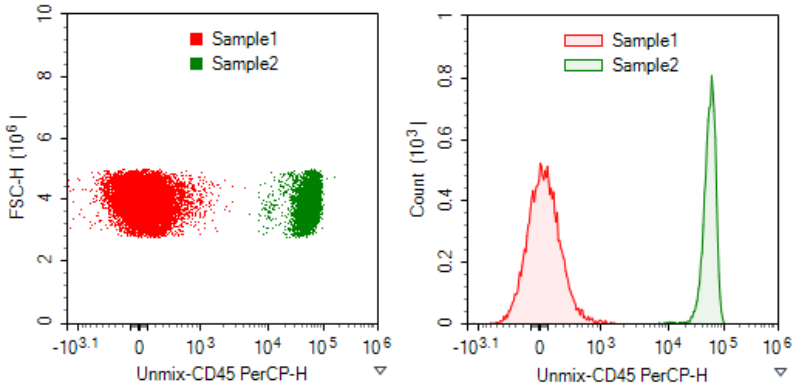


- To save a plot

  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.

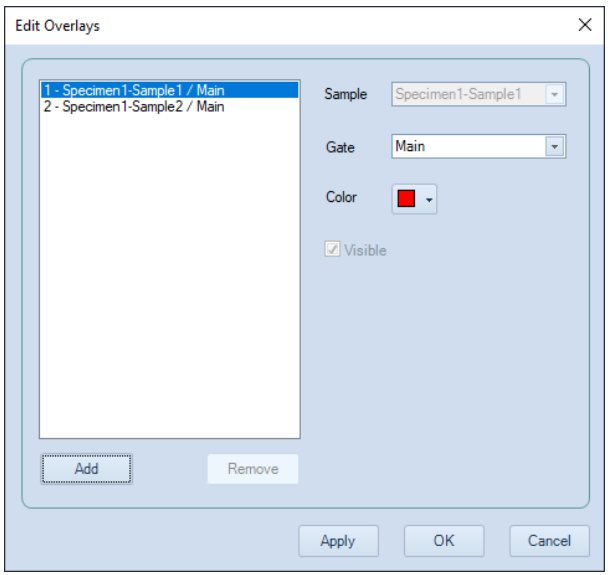


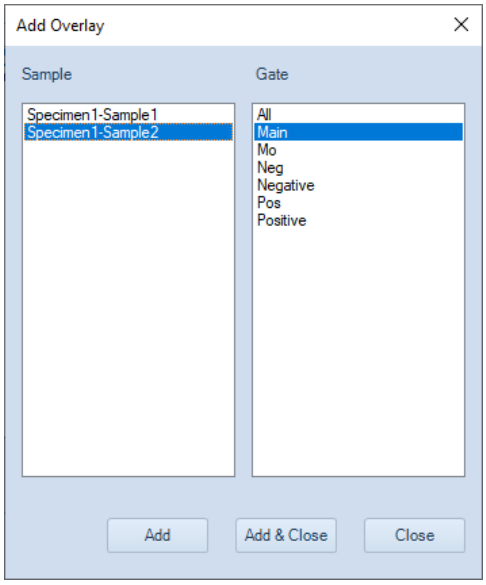
- Add overlays by using the drag and drop method:

  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:

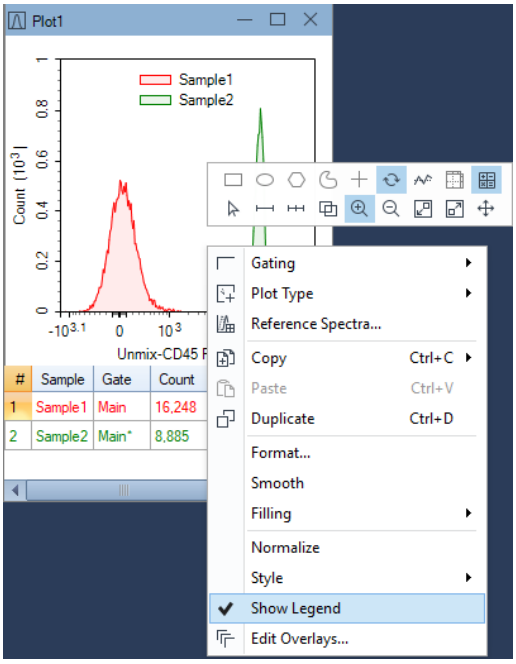
Right-click a plot to access the shortcut menu, select **Edit Overlays** to generate the **Edit Overlays** dialog window as shown below. In the dialog, all overlays of the plot are listed. One can select an overlay, set the overlay's sample, gate or color, and make a choice to show or hide the selected 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.





- 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.

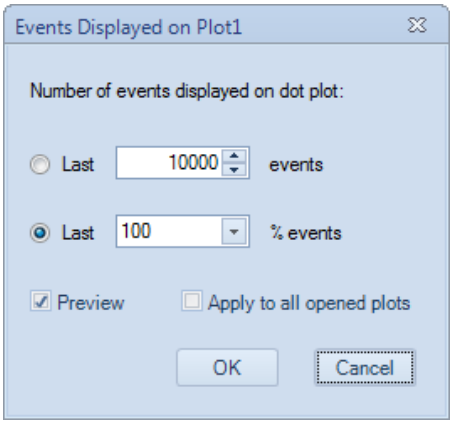


Plot Formatting

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….**

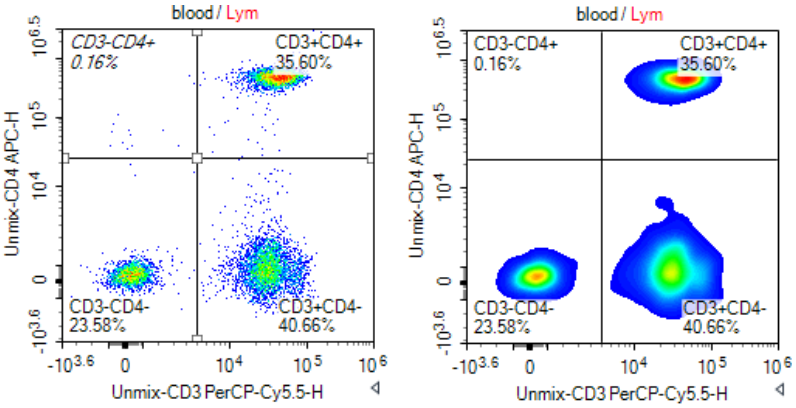


In the window, selecting **Preview** modifies the dot plot display as the user is changing the settings. Selecting **Apply** to all open plots applies the setting to all open dot plots.

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.

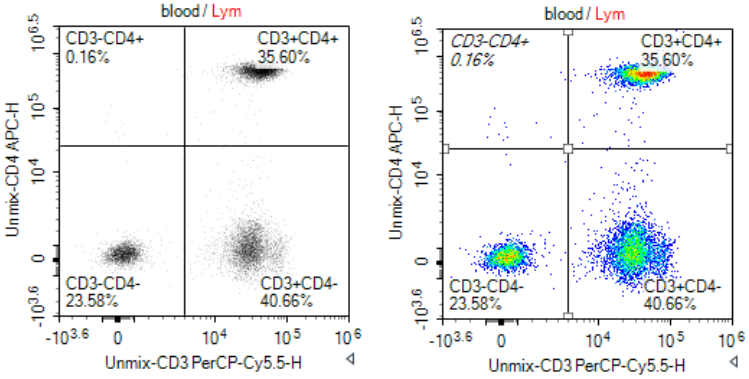
- **Smooth** density plot: In this view, the density plot data are smoothed. To use this view, right-click on the density plot and select **Smooth** or select **Smooth** from the **Plot** tab of the **Menu Bar**. A comparison of a standard pseudocolor density plot (left) and a smooth pseudocolor density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

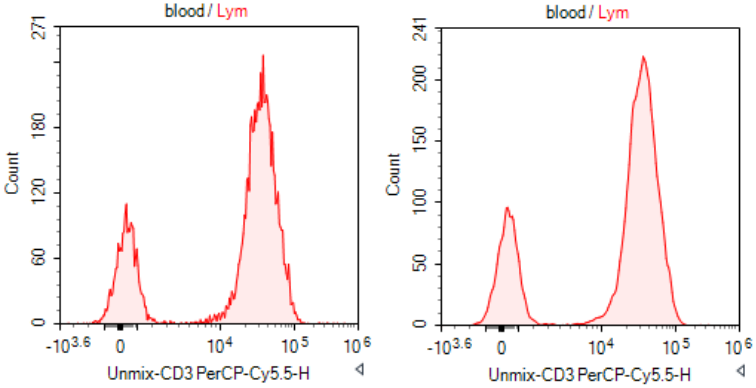
shown in warmer colors (colors toward the right of the color bar below) and areas of the plot with a lower density of events are shown in cooler colors (colors toward the left of the color bar below).

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.

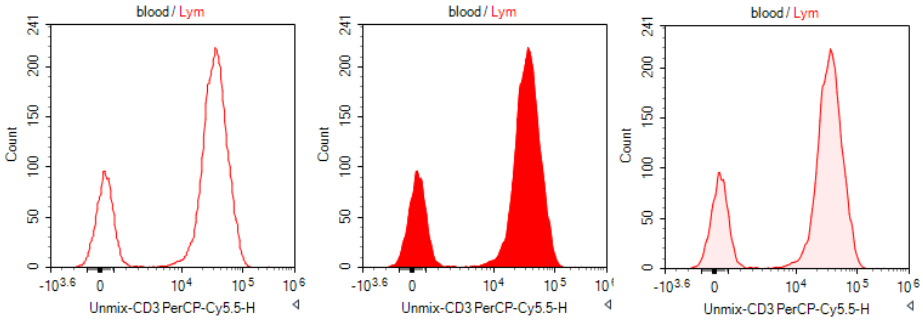


Histogram Plot Formatting

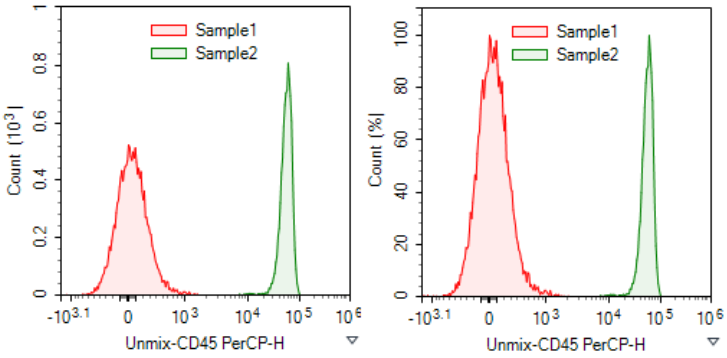
- **Smooth** histogram: To smooth the edges of a histogram, right-click on a histogram plot and select Smooth or select **Smooth** from the **Plot** tab of the **Menu Bar**. A comparison of a standard histogram plot (left) and a smooth histogram plot (right) is shown below.



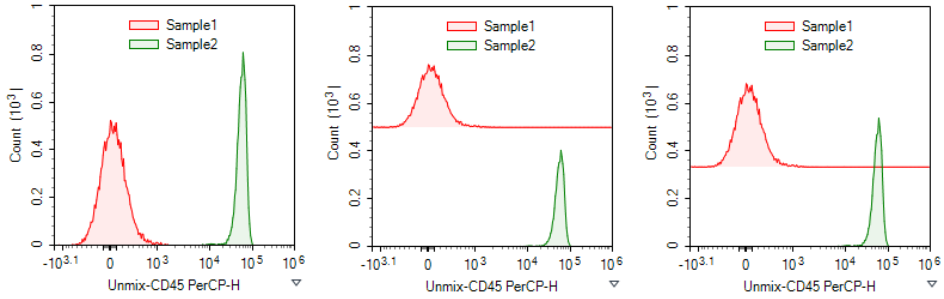
- Histogram fill type: Histogram plots can be viewed with different fill types. To select a fill type, right-click a histogram plot and select **Filling** and select the fill type or select the fill type from the **Filling** drop-down menu in the **Plot** tab of the **Menu Bar**. A comparison of the **filling** types is shown below. The options include **None** (left), **Filled** (middle), and **Tinted** (right).



- Histogram Normalization: When overlaying histogram plots, user can right-click the histogram plot and select **Normalize** to normalize the histogram. The Y axis value of each point on histogram plot is converted to the percentage of its max value. The Y axis of normalized histogram plot changes to percentage accordingly. An example of overlayed histogram before the normalization (left), and after the normalization (right) is shown below.



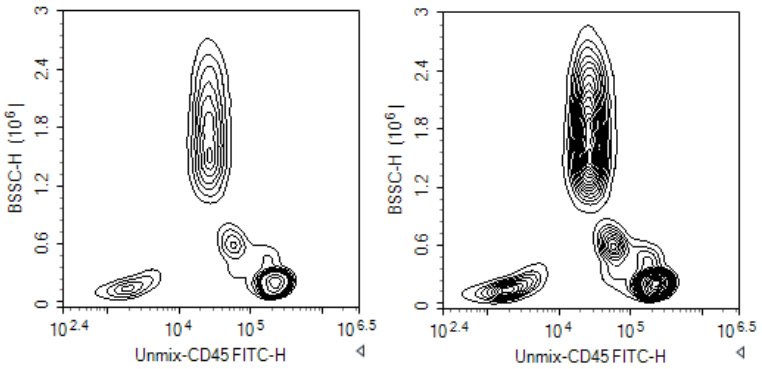
- Histogram layering: When overlaying histogram plots, different overlay styles can be selected. To select an overlay style, right-click in a histogram plot with layers and select Style to select the overlay style. The overlay style options, as shown below, include **Overlaid** (left), **Offset** (middle),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



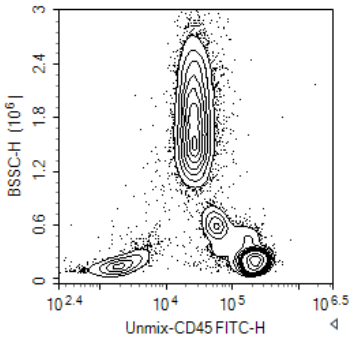
Contour Plot Formatting

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).

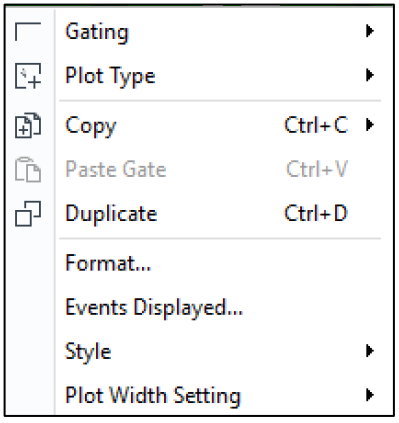


- **Show Outlier**: If selected, outlier events are shown as dots on contour plots. A contour plot is shown below with **Show Outlier** selected.

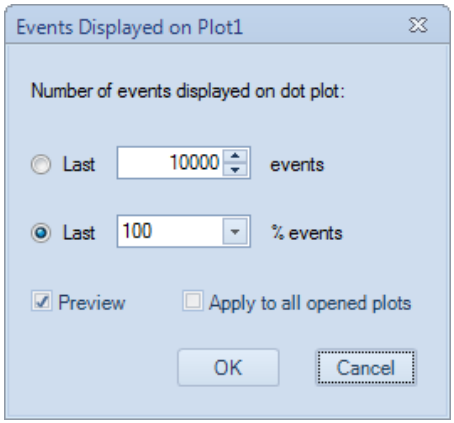


Spectrum Density (Dot) Plot Formatting

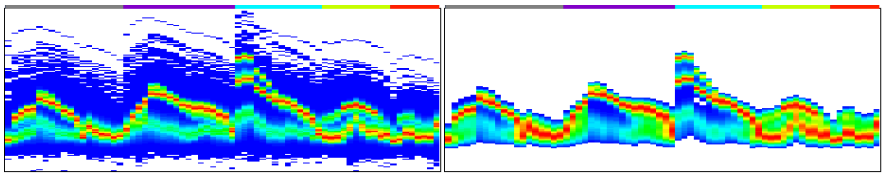
With spectrum density (dot) plots, the following formatting options are available from the right click menu.



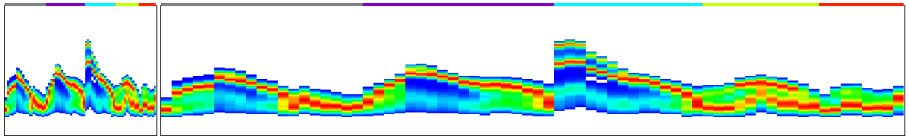
- **Events Displayed**: Allows user to set the number of events to be displayed in the plot in a prompted window. Refer to Plot Formatting for more details on this function.



- **Style**: Allows user to select different plot style (i.e., Original or Smooth). A spectrum density plot is shown below with Original plot style (left) and a Smooth plot style (right).

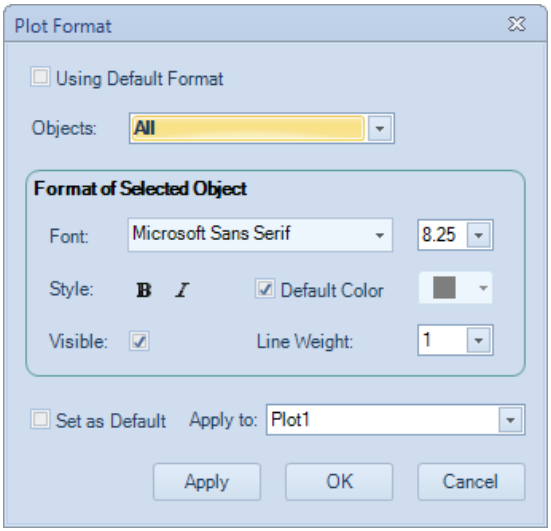


- **Plot Width Setting**: Different plot width levels are available for spectrum density plots. Higher width levels indicate a wider plot. The available width levels can be selected from 1 to 4. A spectrum density plot is shown below with a width level of 1 (left) and a width level of 4 (right).



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.



- **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**:

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**:

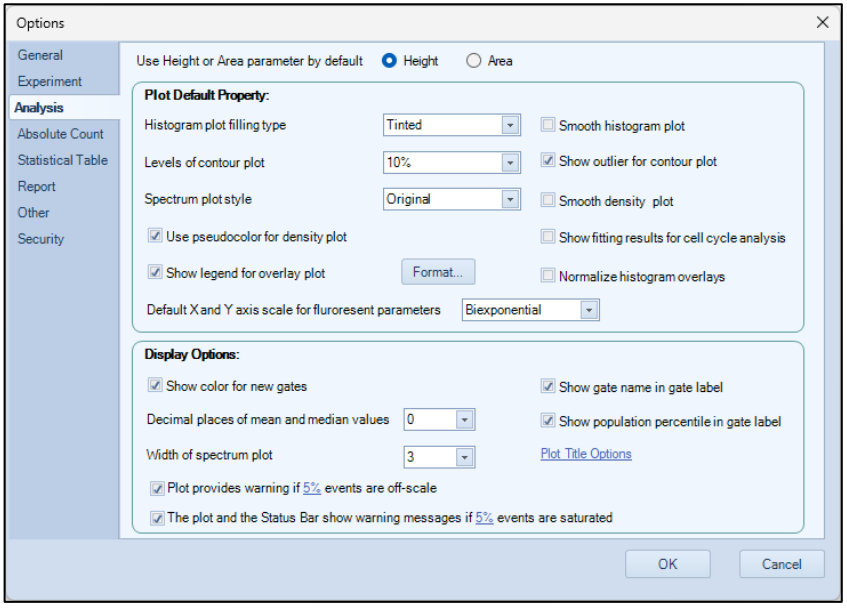
  Click to apply changes and close this window.

- **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. Refer to Setting in this guide for details.



Quick Compensation

The NovoExpress (Opteon) Software's quick compensation method gives users the option to use a slider bar for quick and intuitive adjustment of fluorescence Compensation Matrix or Spillover Matrix after unmixing.

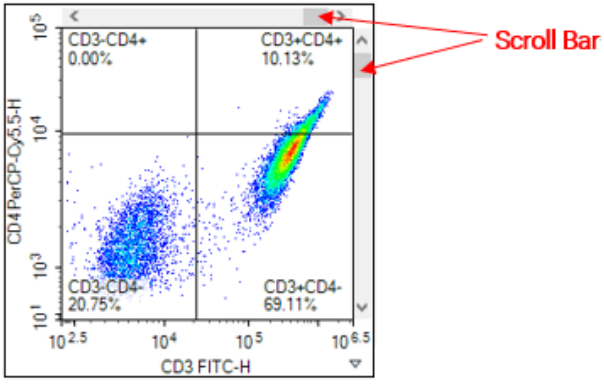
To open the Quick Compensation Adjustment, ensure to work in the **Unmixed Workspace** from the **Unmix** tab of the **Menu Bar**, click the **Quick Compensation** button.

NOTE

User can also access the quick compensation function from **Fluorochrome Bi-Variate Plots** window. Refer to Fluorochrome Bi-Variate Plots in this guide for details.

Scrollbars will appear on plot which meets following requirements.

- Plot created in **Unmixed Workspace**.
- Two-parameter plot
- Plot of fluorescent parameters on both axes
- Both X-axis and Y-axis are **Height** or **Area** parameters



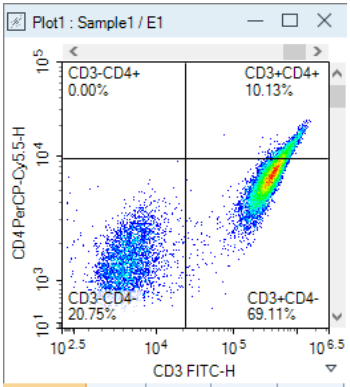
To hide the quick compensation scrollbars, click again on the **Quick Compensation** button.

Please follow the instructions below to quickly adjust compensation using the scroll bar. For illustration purpose, density plot of selected single stained sample is used.

1 Set the X-axis parameter as the sample's fluorochrome parameter. Set the Y-axis as the spillover parameter to be corrected. For example, to correct for the

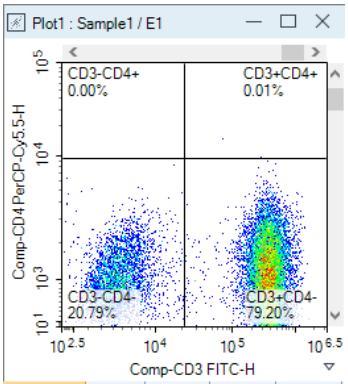
spillover of CD3 FITC-H into CD4 PerCP-Cy5.5-H, analyze a sample stained only with CD3 FITC and use a plot with the X-axis set to CD3 FITC-H and the Y-axis set to CD4 PerCP-Cy5.5-H.

2 Create a quadrant gate to gate the positive and negative populations as shown below.



Gate	Count	% E1	Mean X	Mean Y
E1	16,107	100.00%	322,750	5,771
CD3-CD4+	0	0.00%	0	0
CD3+CD4+	1,632	10.13%	717,830	11,745
CD3-CD4-	3,343	20.75%	5,937	1,573
CD3+CD4-	11,132	69.11%	359,970	6,155

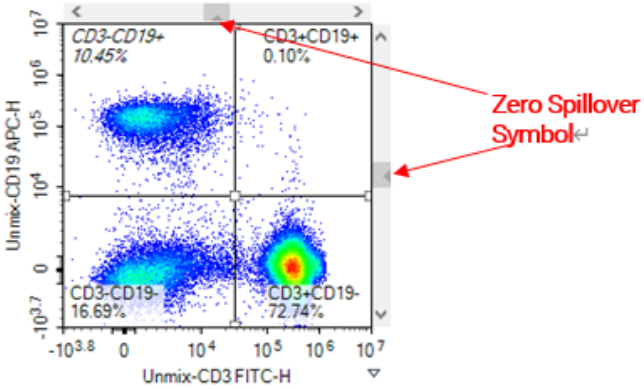
3 Drag the vertical scrollbar to adjust compensation. The associated tooltip will automatically display the compensation percentage.



Gate	Count	% E1	Mean X	Mean Y
E1	16,107	100.00%	321,871	1,042
CD3-CD4+	0	0.00%	0	0
CD3+CD4+	2	0.01%	176,163	12,244
CD3-CD4-	3,348	20.79%	5,079	1,136
CD3+CD4-	12,757	79.20%	405,034	1,016

NOTE

When **Spectral Unmix** is used, there is a triangle symbol automatically displayed in both X and Y axis scroll bar as shown below. This triangle symbol indicates where the zero spillover (i.e., the value without post unmixing adjustment) is at. User can double click this symbol to return to zero spillover state.



NOTE

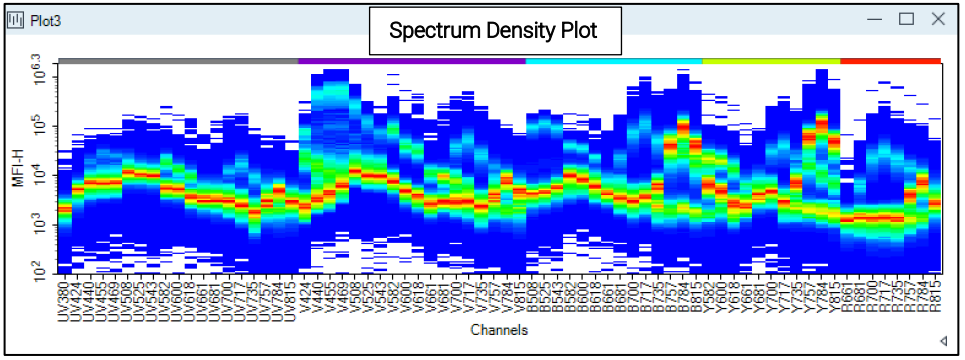
The spillover value range user can adjust is between is -99% and 99% when **Spectral Unmix** is used, or between 0 and 800 when **Compensation** is used.

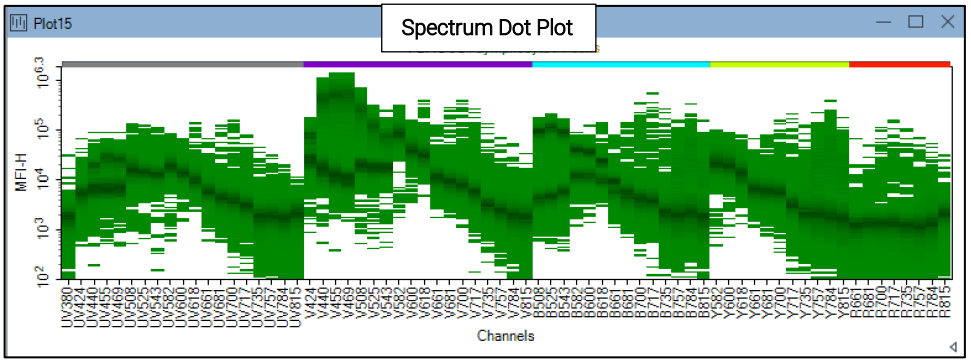
NOTE

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

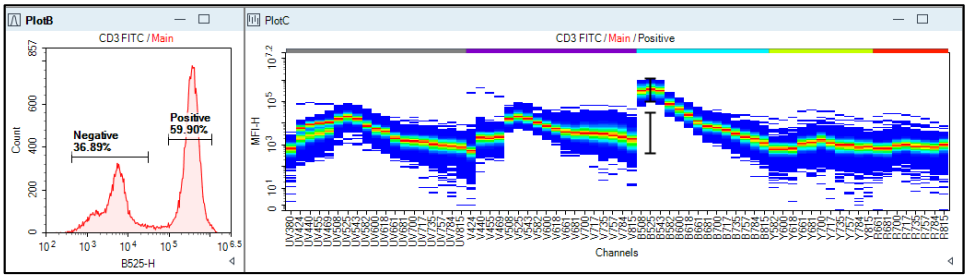
Spectrum Density (Dot) Plot

Spectrum Density Plot (or Spectrum Dot Plot) can be created in the **Raw Workspace** with Mean Fluorescence Intensity (MFI) from each available fluorescence channel displayed in a density plot or dot plot format.





When user creates the single stained or unstained Reference Control sample, the Spectrum Density Plot for this sample will be automatically created in **Raw Workspace**. For Single Stained Control sample, after the data acquisition, the MFI associated with the pre-defined emission channel for the associated fluorochromes will be automatically gated for positive and negative populations These two gates are synchronized with the ones on associated histogram plot as shown below.



- **Channels**: Displays all the channels in the order of laser wavelength (i.e., UV, V, B, Y, R) and emission wavelength (from low to high) by default. User can select the channels for designated laser from the right click menu (refer to Right Click Menu of Spectrum Density (Dot) Plot in this guide for more details).
- **MFI-H or MFI-A**: Displays the value of MFI- A or MFI-H (Default). User can switch between MFI-H and MFI-A from the right click menu (refer to Right Click Menu of Spectrum Density (Dot) Plot in this guide for more details).

NOTE

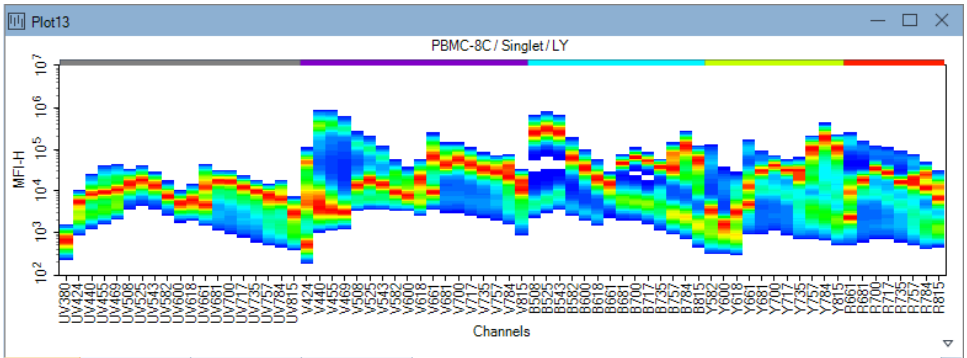
The upper border of the spectrum density (dot) plot is color-banded to indicate corresponding excitation laser.

Manually Create Spectrum Density (Dot) Plot

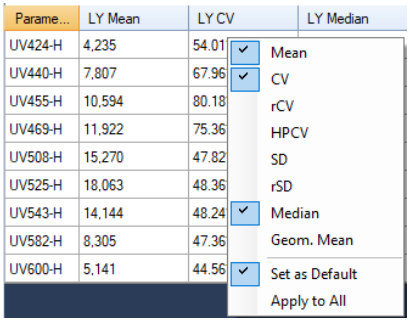
Under the situation when manually creating a spectrum density (dot) plot is required (e.g., for experiment sample), user can select **Raw Workspace**, click the associated icon from the workspace toolbar. Alternatively, user can create a new spectrum density (dot) plot through the **Experiment Manager**, or the gate of existing plot. Refer to Create a Plot in this guide for more details.

View Statistical Information

User can view the statistical information (e.g., CV, Mean, Median) of associated channels in tabular format from Spectrum Density (dot) plot. To access this table, click ◃ located in the lower right corner of the plot. By default, the CV, mean and median value of associated channels will be displayed. User can also right click anywhere inside the table, check the statistical info to be displayed, and the table will be automatically updated based on the selection. User can also check **Set as Default** to make the selection become default for Spectrum Density (dot) Plot created afterwards. When **Apply to All** is checked, the selections will be applied to all spectrum density (dot) plots of the same sample.



Parame...	LY Mean	LY CV	LY Median
UV380-H	709	45.31%	661
UV424-H	4,235	54.01%	3,897
UV440-H	7,807	67.96%	7,161
UV455-H	10,594	80.18%	8,617
UV469-H	11,922	75.36%	9,779
UV508-H	15,270	47.82%	14,488
UV525-H	18,063	48.36%	17,349
UV543-H	14,144	48.24%	13,525
UV582-H	8,305	47.36%	7,831

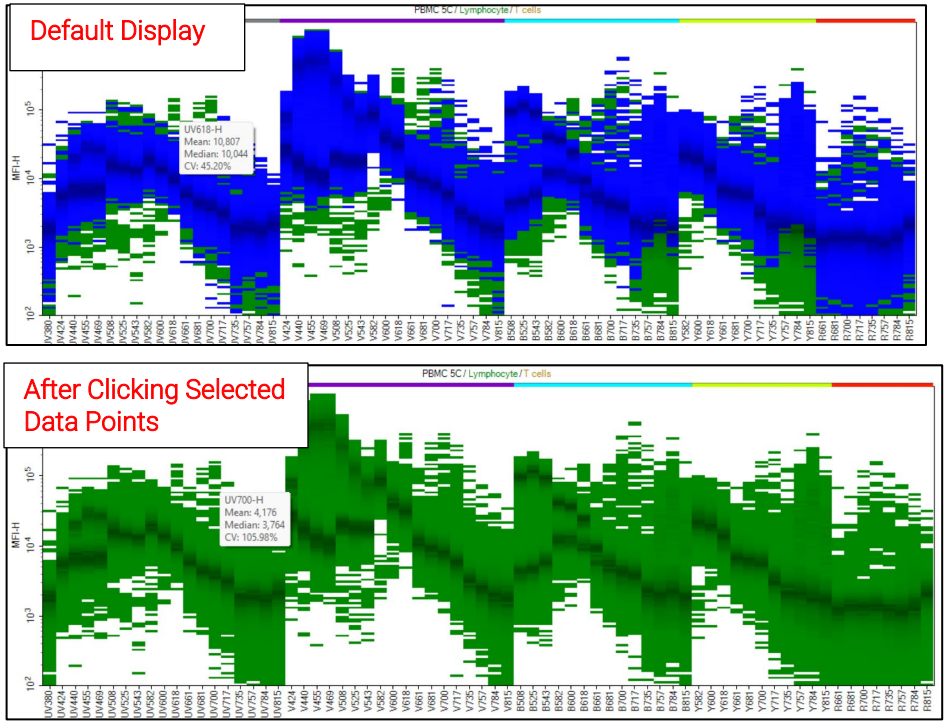


Set Color Displaying Priority for Spectrum Dot Plot

User can left click the selected data points from spectrum dot plot to set the associated color as highest priority displaying color. To go back to the default color, user can left click the blank area of the plot.

NOTE

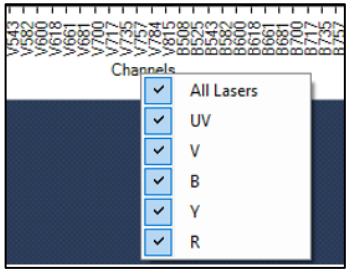
This function is only available for the Spectrum Dot Plot.



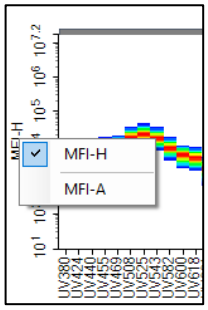
Right-Click Menu of Spectrum Density (Dot) Plot

The following additional functions are available from the right click menu of Spectrum Density (dot) plot.

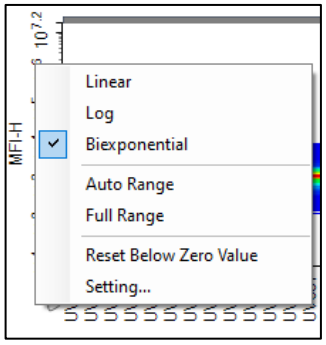
- When right clicking **Channels** or any emission channel in the plot, user can check the laser in the prompted menu to display only the channels associated with selected laser(s) in the plot.



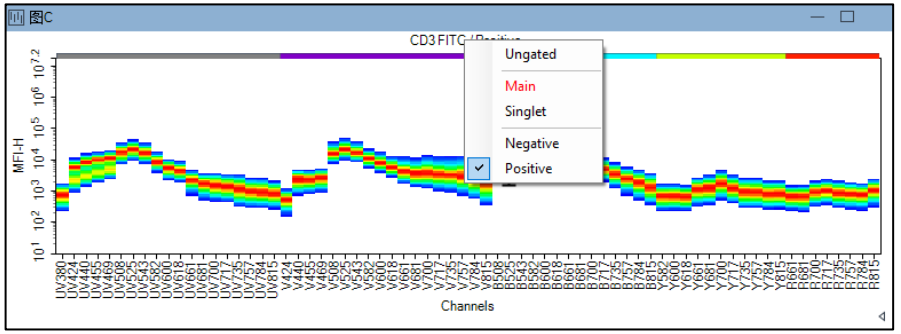
- When right clicking **MFI** in the plot, user can check either **MFI-H** or **MFI-A** to switch the displayed parameter between **MFI-H** and **MFI-A**.



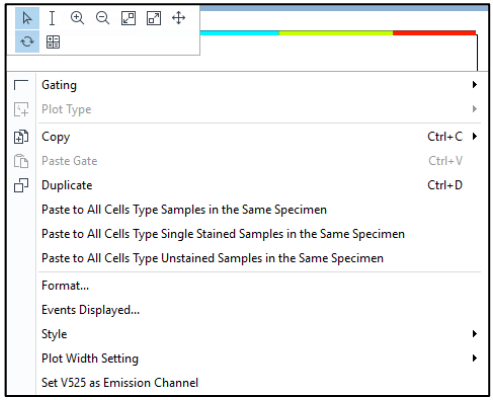
- When right clicking MFI axis, user can select to display the MFI values in desired scale (e.g., **Linear, Log, Biexponential, Auto range**, or **Full range**). User can also select to reset below zero value. When user clicks **Setting**, user can edit the additional axis setting of the plot. Please refer to Set the Coordinates of the Axis in this guide for more details of each function in this right click menu.

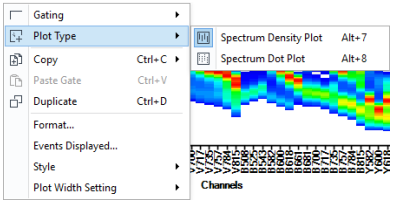
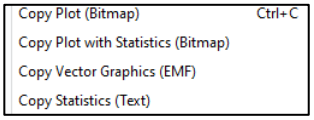


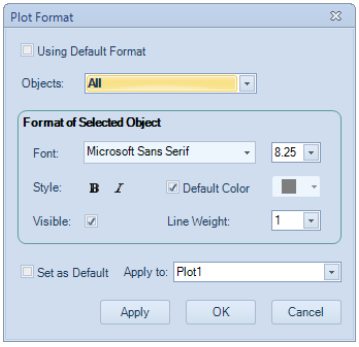
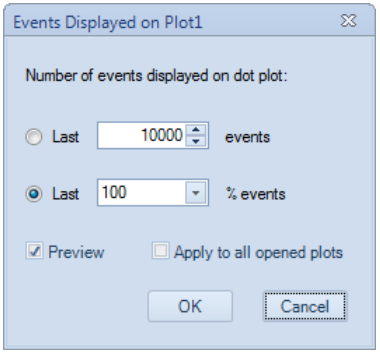
- When right clicking plot header in the plot, user can select to display the spectrum density (dot)of the events within a selected gate.

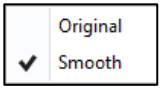
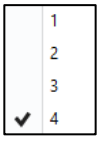


- When right clicking the plot area in the spectrum density (dot) plot, user can access additional functions which are listed below.



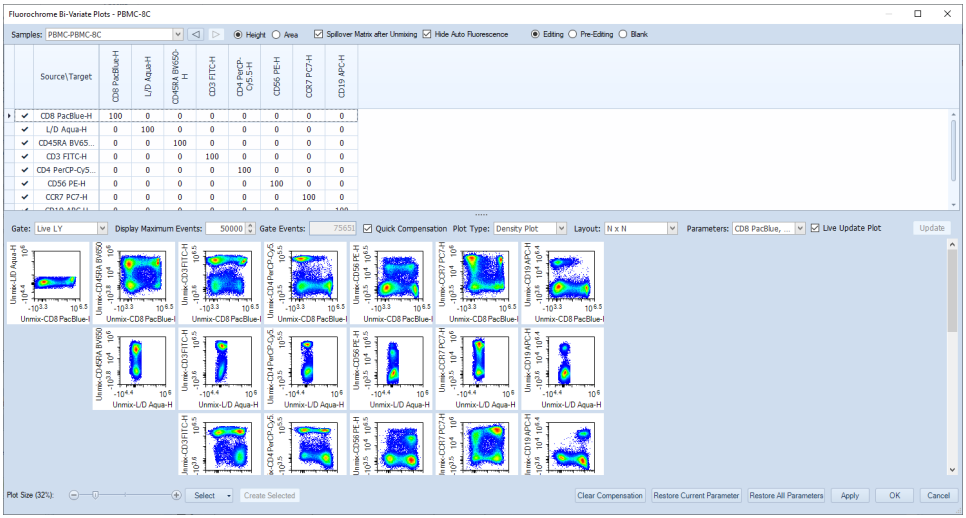
Function	Description
Gating	Click to select to select gate from the drop-down menu to display the spectrum density plot within the checked gates.
Plot Type	Click and switch the plot between Spectrum Density Plot and Spectrum Dot Plot from the expanded sub-menu. This option is not available when user right clicks the software to automatically create a spectrum density plot of the reference control sample. 
Copy	Click to copy the spectrum density plot and associated information into clipboard. User can select to copy plot only in Bitmap image format, or copy plot with statistics in Bitmap image format, or copy plot in EMF image format, or copy statistics only in text from the drop-down menu. 
Paste Gate	Click to paste the copied Vertical Range Gate to the target spectrum density plot. First, select a Vertical Range Gate, right click and select **Copy** from drop-down list. Then move to the target spectrum density plot, right click and select **Paste Gate** from drop-down list.
Duplicate	Click to duplicate a spectrum density plot for the same sample.

Paste to All Cells (Beads) Type Samples in the same Specimen	Click to past the spectrum density plot to all control samples defined in Reference Control specimen with the same cell (bead) type. This option is not available for experiment sample.
Paste to All Cells (Beads) Type Single Stained Samples in the same Specimen	Click to past the spectrum density plot to all single stained samples in Reference Control specimen with the same cell (bead) type. This option is not available for experiment sample.
Paste to All Cells (Beads)Type Unstained Samples in the same Specimen	Click to past the spectrum density plot to all unstained samples in Reference Control Specimen with the same cell (bead) type. This option is not available for experiment sample.
Format	Clicks to defines plot appearance in prompted window below. Refer to Plot Format in this guide for more details. 
Events Displayed	Click to set the number of events to be displayed in the plot in the prompted window. Refer to Spectrum Density (Dot) Plot Formatting in this guide for more details. 
Style	Click to set the plot style (i.e., Original or Smooth) for the spectrum density plot in the drop-down menu. Refer to Spectrum Density Plot Formatting in this guide for more details.

	
Plot Width Setting	Click to set the plot width for the spectrum density plot in the drop-down menu. Refer to Spectrum Density Plot Formatting in this guide for more details. 
Set XXX as Emission Channel	Click to select a specific channel on a spectrum density plot, right click and select this option to manually apply the Vertical Range Gate to the selected emission channel. XXX is automatically updated when selecting the emission channel. The gates will be automatically updated on both the Spectrum Density Plot and histogram plot after this operation. Alternatively, user can click and hold the gate and drag to the designated channel to set it as emission channel. This option is available only for Single Stained Control sample.

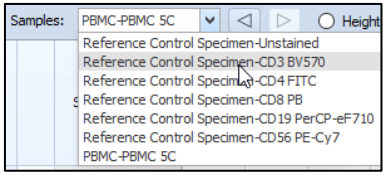
Fluorochrome Bi-Variate Plots

The Fluorochrome Bi-Variate plots can be created through **Fluorochrome Bi-Variate Plots** window. It is very useful to check the results of spectral unmixing and apply necessary adjustment of compensation to be unmixed data. To open this window, ensure to select **Unmixed Workspace**, and click  icon in the workspace toolbar.



Following options are available in **Fluorochrome Bi-Variate Plots** window.

- **Samples**: Select which sample in the experiment file to be used for the plots. By default, the current Active Sample will be selected when the window is opened. Used ◁ ▷ icon to switch samples forward and backward.



- **Height**: Select to set the parameter of the plots to Height.
- **Area**: Select to set the parameter of the plots to Area.
- **Spillover Matrix after Unmixing**: Check to display the Spillover Matrix after unmixing in tabular format. This option is available only when Spectral Unmix is selected.
- **Compensation Spillover Matrix**: Check to display the Spillover Matrix after compensation in tabular format. This option is available only when compensation is selected.
- **Hide Auto Fluorescence**: Check to hide the autofluorescence parameters. This option is available only when Spectral Unmix is selected.
- **Editing**: Check to enter the editing mode. The **Quick Compensation** option in this window will be available for user to check and perform quick compensation on the plot. The Spillover Matrix and all the plots will be automatically updated after the Quick Compensation is applied.

- **Pre-Editing**: Check to display the Spillover Matrix currently applied to the sample and associated plots. The **Quick Compensation** option in this window will be disabled.
- **Blank**: Check to display blank Spillover Matrix and associated plots. The **Quick Compensation** option in this window will be disabled.

NOTE

Editing, Pre-Editing, and **Blank** options allow user to quickly check the effect of three conditions by checking the changes in the **Fluorochrome Bi-Variate Plots**.

- **Gate**: Select the gates of the selected sample in the drop-down box. When selected, only the data in the selected gate will be displayed on the plots.
- **Display Maximum Events**: Select the maximum number of events to be displayed in the plot.

NOTE

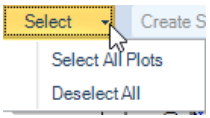
Define proper **Display Maximum Events** will help with the software response speed. Less events displayed in the plot, faster the response.

- **Gate Events**: Display the number of events in the selected gate. It is read-only and user cannot edit it.
- **Quick Compensation**: When checked, user can perform quick compensation on the selected plot. This option is available only when user is working in **Editing** mode (i.e., **Editing** is checked in this window). Refer to Quick Compensation in this guide for detailed instructions of performing quick compensation on a plot.
- **Plot Type**: Select plot type (i.e., dot plot, density plot, and contour plot) for the created fluorochrome bi-variate plots.
- **Layout**: Select to create the plot in 1x N or N x N layout. When 1 x N is selected, the X axis of each plot will be the parameter defined in **Parameters** box.
- **Parameters**: Select the parameter(s) to be plotted in the Fluorochrome Bi-Variate Plots.
- **Live Update Plot**: Check to automatically update the plot when **Gate, Display Maximum Events** settings in this window are changed.
- **Update**: Click to manually update the plot when **Gate, Display Maximum Events** settings in this window are changed. This button will be automatically disabled when **Live Update Plot** is checked.

NOTE

It is recommended to manually update the plot when the software response speed is compromised.

- **Plot Size**: Move the slider to change the size of the plots.
- **Select**: Click on the pop-up selection menu to select or unselect all plots. The plot can also be selected by clicking individual plot. Multiple plots can be manually selected by holding the Shift or Ctrl key. Once selected, a red border will be displayed on the plot. Clicking on the selected plot one more time will cancel the selection. Selected plots can be added into the **Unmixed Workspace** by clicking "**Create Selected**" button.



- **Create Selected**: Click to create the selected plots in **Unmixed Workspace**.
- **Clear Compensation**: Click to clear any changes made after unmixing. This option is available only when user is working in **Editing** mode (i.e., **Editing** is checked in this window).
- **Restore Current Parameter**: Click to restore the Spillover Matrix values of selected fluorochrome back to the ones displayed in **Pre-Editing** mode (i.e., Spillover Matrix currently applied to the sample and associated plots). This option is available only when user is working in **Editing** mode (i.e., **Editing** is checked in this window).
- **Restore All Parameters**: Click to restore the Spillover Matrix values of all fluorochrome back to ones displayed in **Pre-Editing** mode (i.e., Spillover Matrix currently applied to the sample and associated plots).. This option is available only when user is working in **Editing** mode (i.e., **Editing** is checked in this window).
- **Apply**: Click to apply the changes to selected sample.

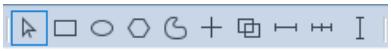
NOTE

The settings user defined in **Fluorochrome Bi-variate Plots** window, including **Spillover Matrix after Unmixing, Hide Auto Fluorochrome, Display Maximum Events, Quick Compensation, Plot Type, Layout, Live Update Plot, Plot Size** 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.

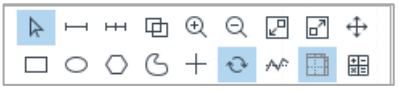
The Workspace Toolbar includes icons for creating rectangular gates, elliptical gates, polygonal gates, freehand Gates, quadrant gates, logic gates, range gates, bi-range gates, and vertical range gates.



NOTE

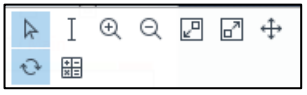
The Vertical Range gate is only available for **Spectrum Density Plot**.

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



NOTE

When user right click **Spectrum Density Plot**, only the Vertical Range gate is available for gating as shown below.



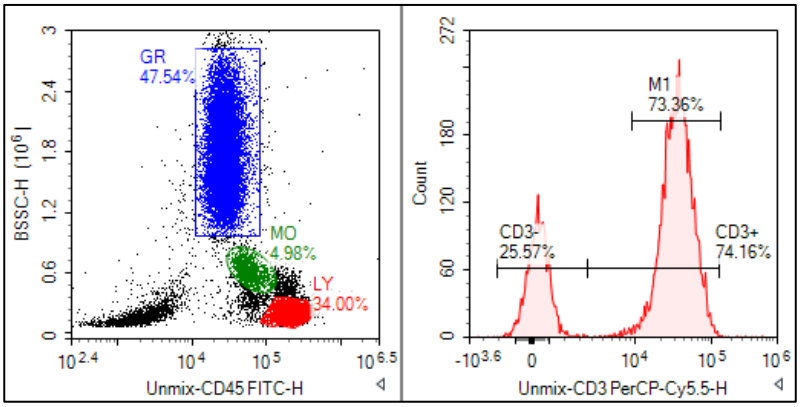
All gate types (except vertical gate) can be created on dot plots and density plots. Range gates and bi-range gates (except vertical gate) can be created on histograms. Vertical Range gate can be created only on Spectrum Density Plot. Gates can also be combined to create a logic gate.

Create Gates

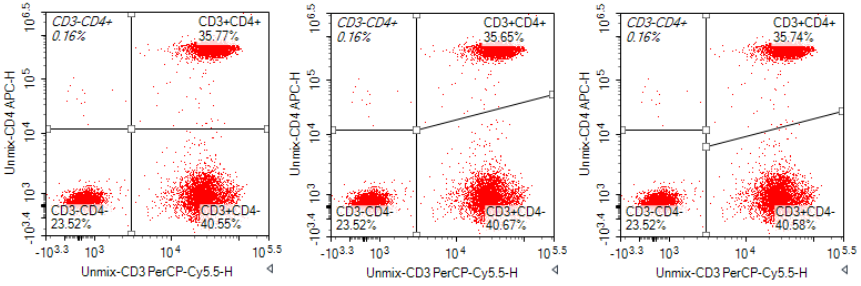
- To create a rectangular gate: Click the rectangular gate icon, in the Workspace Toolbar. Click and drag in the plot to enclose the target population within the rectangle. Release the mouse button to create the gate.
- To create an elliptical, range, or bi-range gate: Follow similar procedures as for 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.

  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.

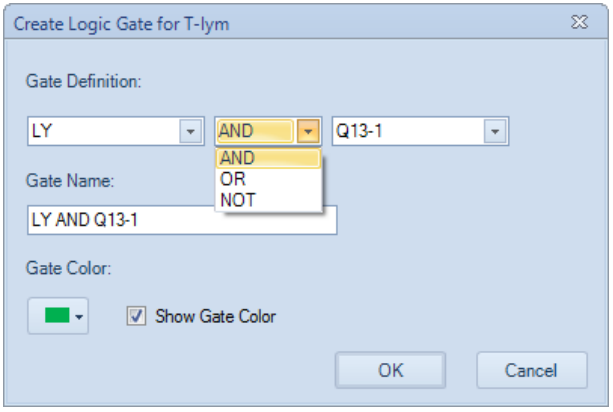


- To create a logic gate: Click the logic gate icon, in the Workspace Toolbar to open the **Create Logic Gate** window. In this window, the user can create a logic gate for the sample in the selected plot.

  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.



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

- To create a vertical gate: Ensure to select **Raw Workspace**, click the Vertical Range gate icon in the Workspace Toolbar. Left click in the Spectrum Density Plot and drag vertically in the plot to enclose the target population within the gate. Release the mouse button to create the gate.

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.

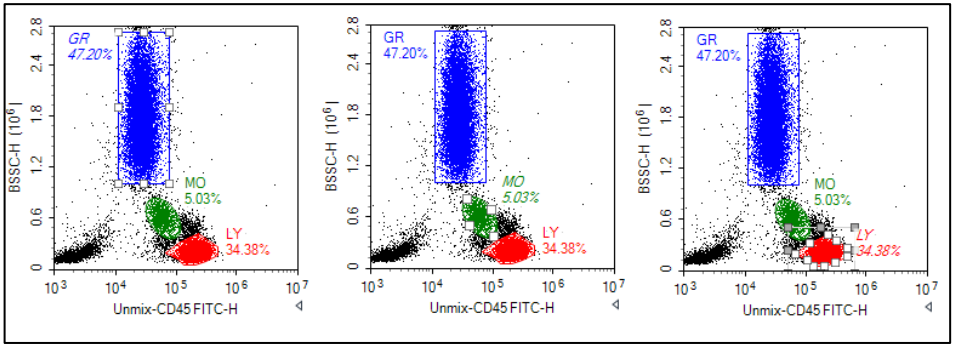
There are multiple methods to select a gate for editing. Options include:

- 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.

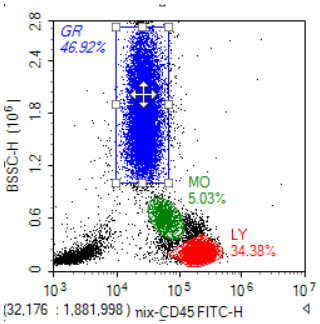
After the gate is selected, the gate's control points are displayed (as white boxes). To change the size of a gate, click and drag the control points to modify the gate.

NOTE

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



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

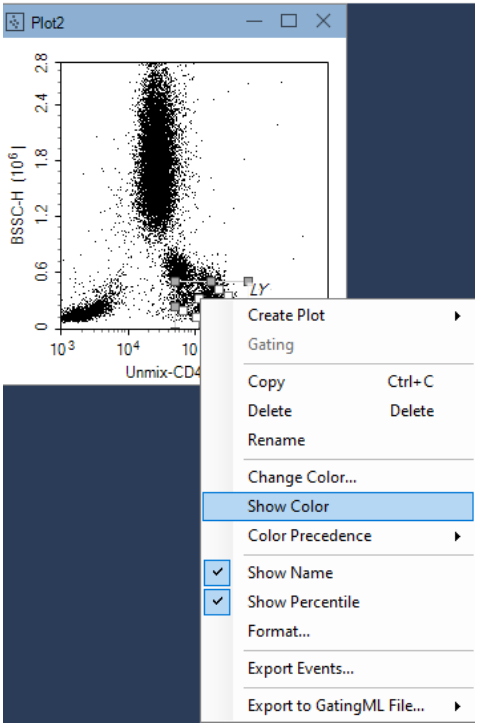
The NovoExpress (Opteon) Software allows users to format the color and labels of gates. Gate color determines the color of events displayed on the dot plot, as well as the color of the histogram when the gate is applied to a histogram.

Set the Color of the Gate

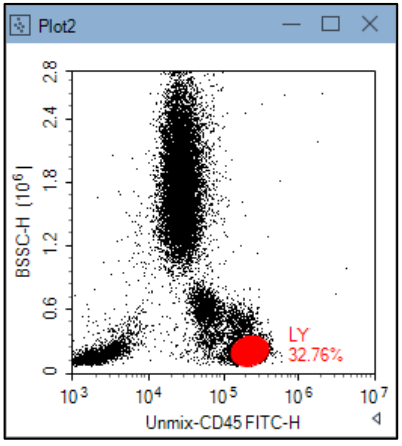
The color of each gate can be set. In dot plots, the events included in the gates are displayed in the chosen color. If additional dot plots are created, these events are displayed in the same color for easy identification.

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 plot shown below have a gate with the color unselected.

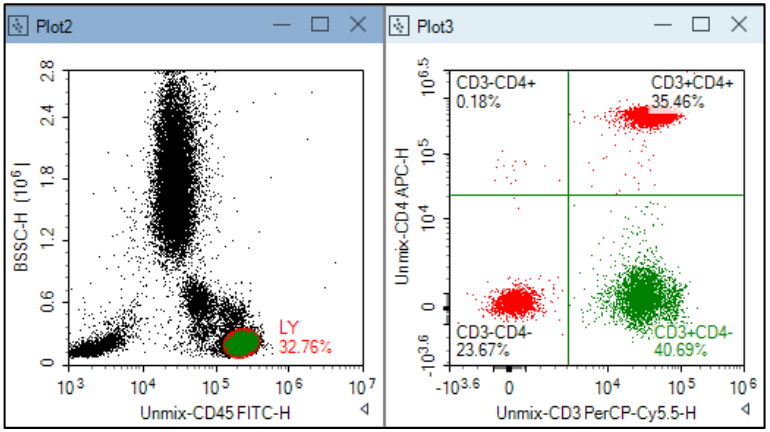


The plot below has a gate with the color selected.



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 in this guide.



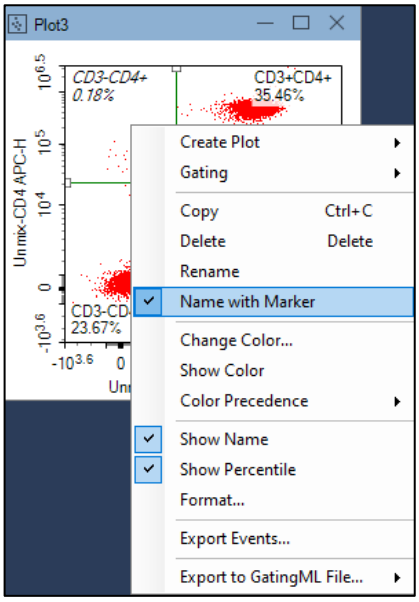
Gate Labels

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.

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 a parameter is associated with marker, quadrant and bi-range gates can be used to easily label positive and negative populations. To use this setting, the parameter must be associated with marker in **New Reference Control Specimen** window. Right-click on the quadrant or bi-range gate and select **Name with Marker** to rename the gates according to the 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.
- 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.

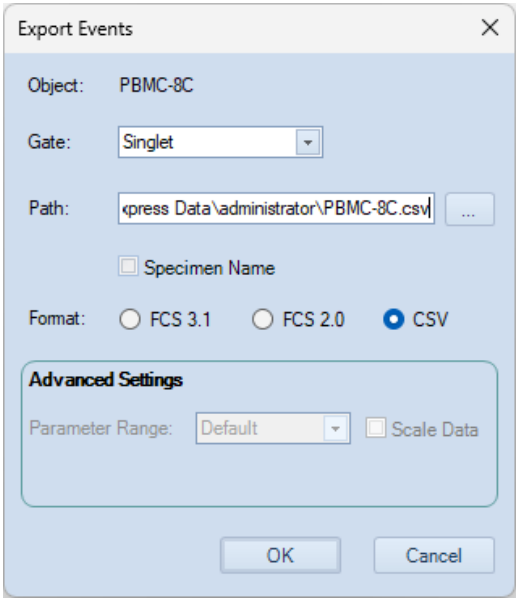
- Select a gate. Drag and drop the gate into a different plot. The dragged gates are pasted into the plot where it was dropped.
- To duplicate a gate within the same plot, select a gate and use the keyboard shortcut Ctrl D or select the gate and use the **Duplicate** button from the **Home** tab of the **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.

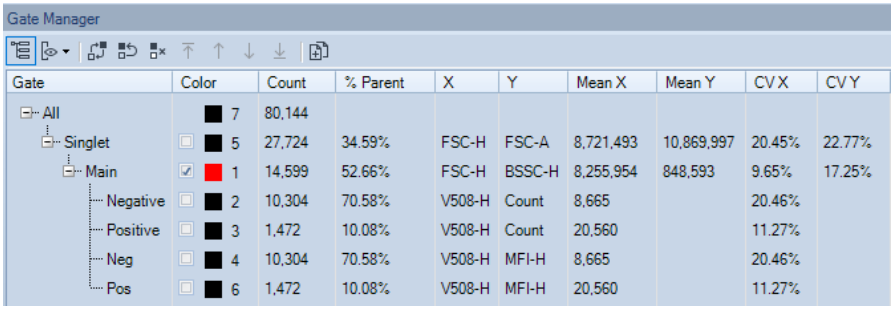


3 In the window, set the export path, file format, parameter range and post gain. Click **OK** to export the data.

NOTE For more information regarding this window, Refer to Export Data in this guide.

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	CV X	CV Y
All	7	80,144							
Singlet	5	27,724	34.59%	FSC-H	FSC-A	8,721,493	10,869,997	20.45%	22.77%
Main	1	14,599	52.66%	FSC-H	BSSC-H	8,255,954	848,593	9.65%	17.25%
Negative	2	10,304	70.58%	V508-H	Count	8,665		20.46%	
Positive	3	1,472	10.08%	V508-H	Count	20,560		11.27%	
Neg	4	10,304	70.58%	V508-H	MFI-H	8,665		20.46%	
Pos	6	1,472	10.08%	V508-H	MFI-H	20,560		11.27%	

Toolbar of Gate Manager

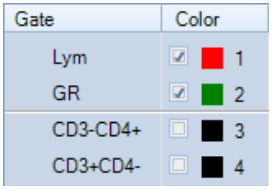
Icon	Description
	Show Gate Hierarchy: When checked, the table displays output in tree mode. Child gates are indented.
	Show Columns: Choose which statistical columns to display in the table. Refer to Calculation of Statistics in this guide for further information on calculation of gate statistics.
	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.
	Clear Color: Click to uncheck all checkboxes in front of each color and remove all the previously defined color.
	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 in this guide.

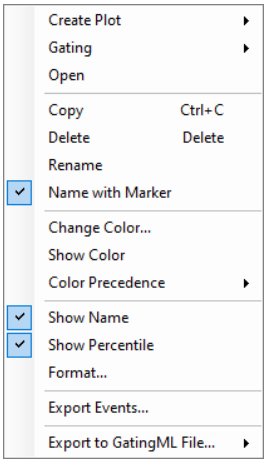
To modify gate color precedence, check the **Modify Color Precedence** tool in the toolbar. The color column in **Gate Manager** is shown below:



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 checkbox 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.

Name with Marker: If a fluorescence parameter is associated with marker, this labels the gate using the marker 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.

Export to GatingML File: Exports the gating information of selected gates or all gates to a Gating-ML file. Please refer to Export to Gating-ML File in this guide for more details.

NOTE

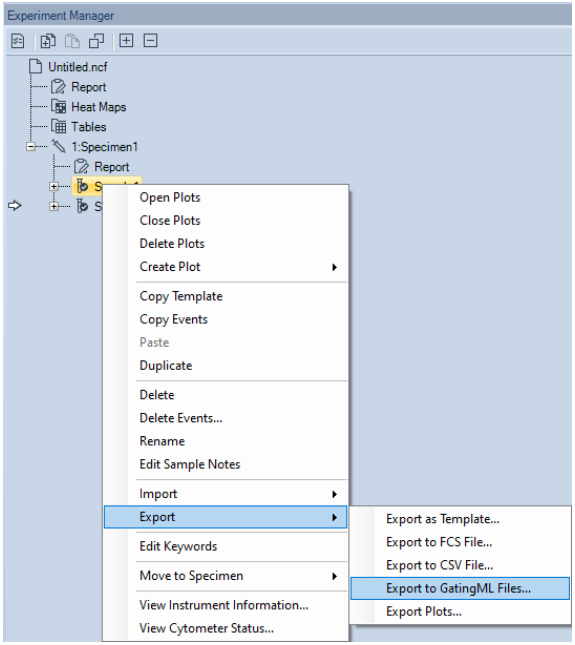
When multiple gates are selected in **Gate Manager**, only **Delete, Show Color, Color Precedence, Show Name** and **Show Percentile** are available.

Export to Gating-ML File

The gating information of the selected gates can be exported to a Gating-ML file. The exported Gating-ML file can be further analyzed by a third party software which also supports the Gating-ML standard, using the **Export to GatingML File** function.

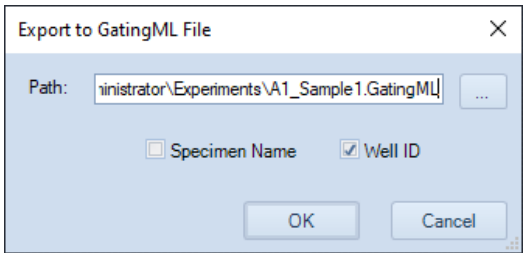
This function can be accessed with one of the following methods:

- From **Experiment Manager** panel: Right click the selected sample, click **Export→ Export to GatingML Files…** to export gating information associated with the selected sample.

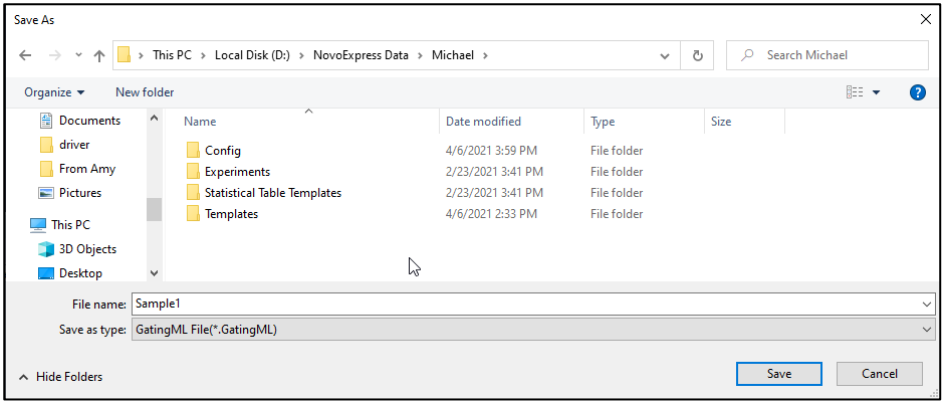


In the prompted window, the **Path** field will be automatically filled with the default target folder (i.e., the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously) and the default file name (i.e., **Sample**

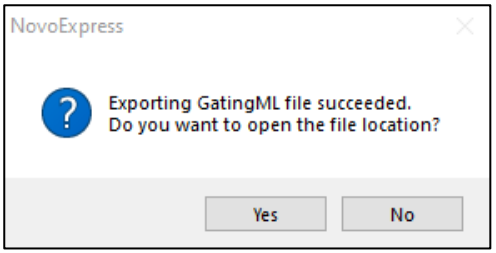
Name.GatingML). User can check the box in front of **Specimen Name** to include the specimen name as part of the file name, and/or check the box in front of **Well ID** (when NovoSampler S is connected) to include Well ID as part of the name.



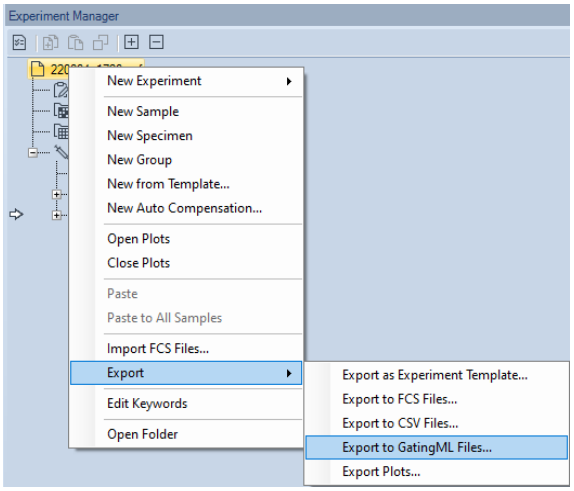
To use different folder and file name, user can directly edit the **Path** field, or click ... to the right of the **Path** text box to open the window below, select the appropriate path and edit the file name, and click **Save**. The updated folder and file name will be automatically filled into **Path** field in **Export to Gating-ML File** window.



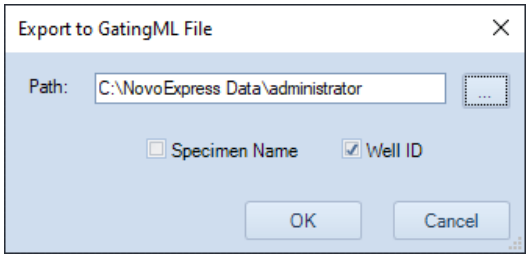
Click **OK** in **Export to GatingML File** window to export Gating-ML file, the file contains all the gating information of the sample. Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.



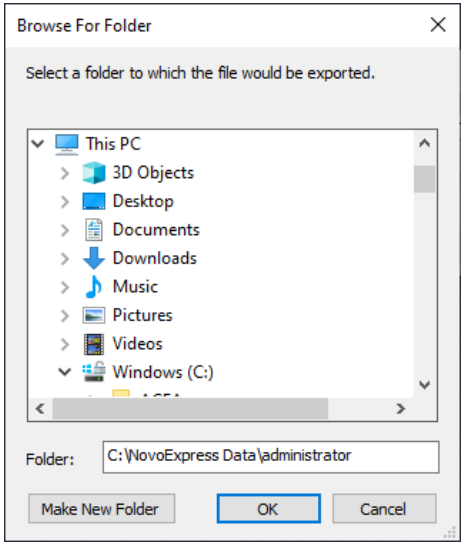
- From **Experiment Manager** panel: Right click an experiment file, a group, or a specimen, and click **Export→ Export to GatingML Files…** to export gating information of all samples associated with the selected experiment, group or specimen correspondingly. Each sample corresponds to a Gating-ML file respectively.



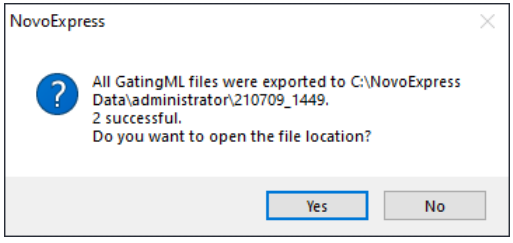
In the prompted window, the **Path** field will be automatically filled with the default folder (i.e., the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously). The default name of exported file will be in the format of **Sample Name.GatingML**, and user cannot edit the file name in this window. User can also check the box in front of **Specimen Name** to include the specimen name as part of the file name, and/or check the box in front of **Well ID** (when NovoSampler S is connected) to include Well ID as part of the name.



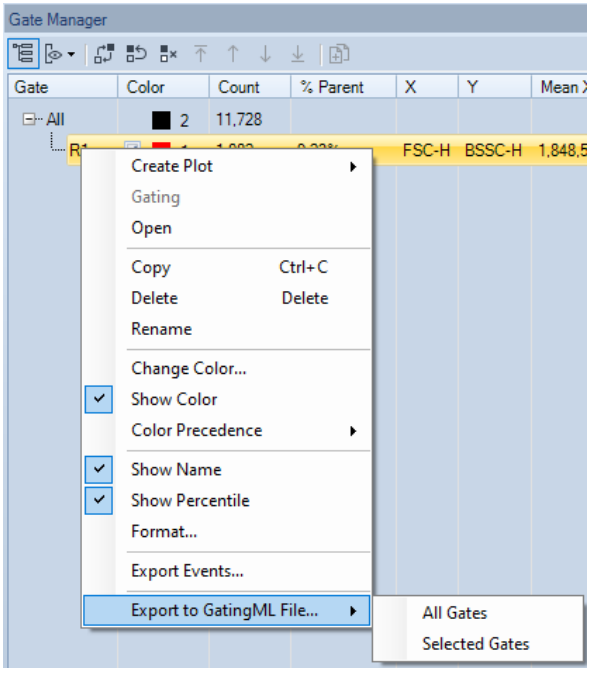
To use a different folder, user can directly edit the **Path** field, or click ... to the right of the **Path** text box, select the appropriate path, and click **OK**. The updated folder will be automatically filled into **Path** field in **Export to GatingML File** window.



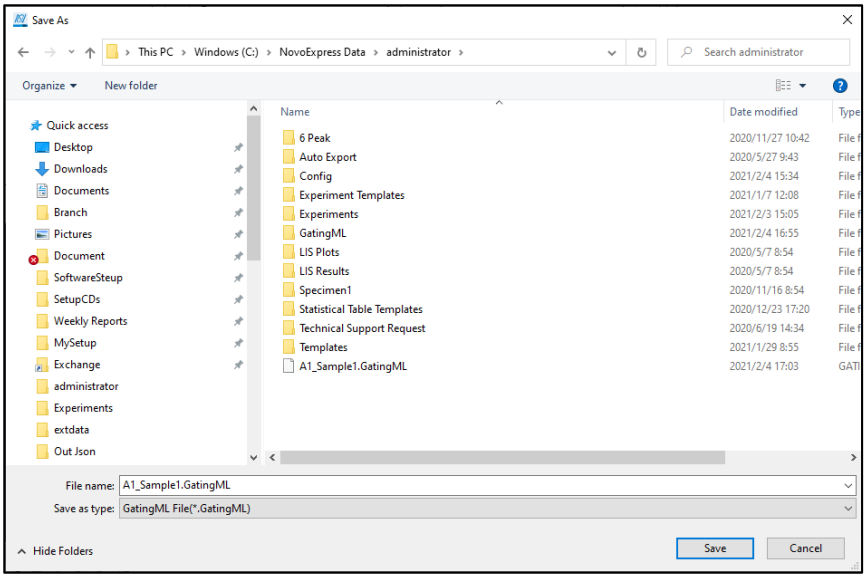
Click **OK** in **Export to GatingML File** window to export Gating-ML file. The file contains all gates of the sample. Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.



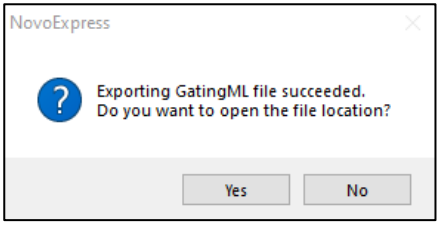
- From **Gate Manager** panel: Right click the selected gates, click **Export to GatingML File…** and select **All Gates** to export all gates of the selected sample to the Gating-ML file, or select **Selected Gates** to export the selected gates and their associated parent gates to the Gating-ML file.



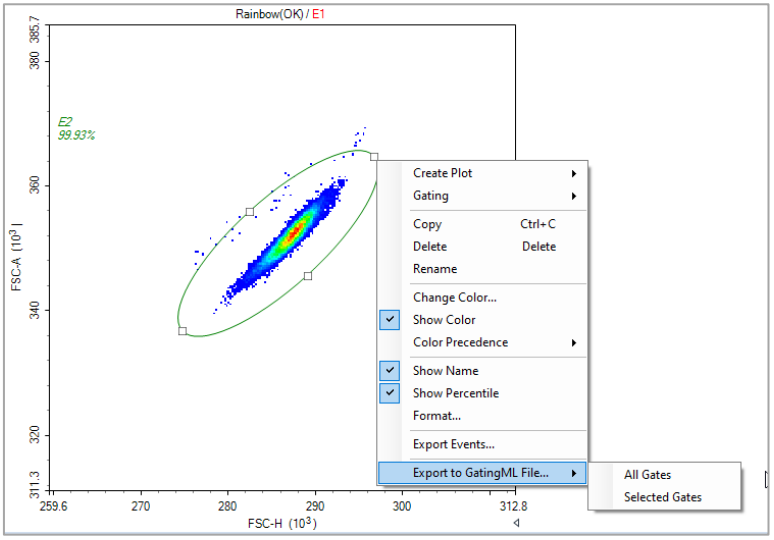
In prompted window as shown below, user can select the target folder and edit the file name. The default folder is the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously. The default file name is **Sample Name.GatingML**. Click **Save** to continue.



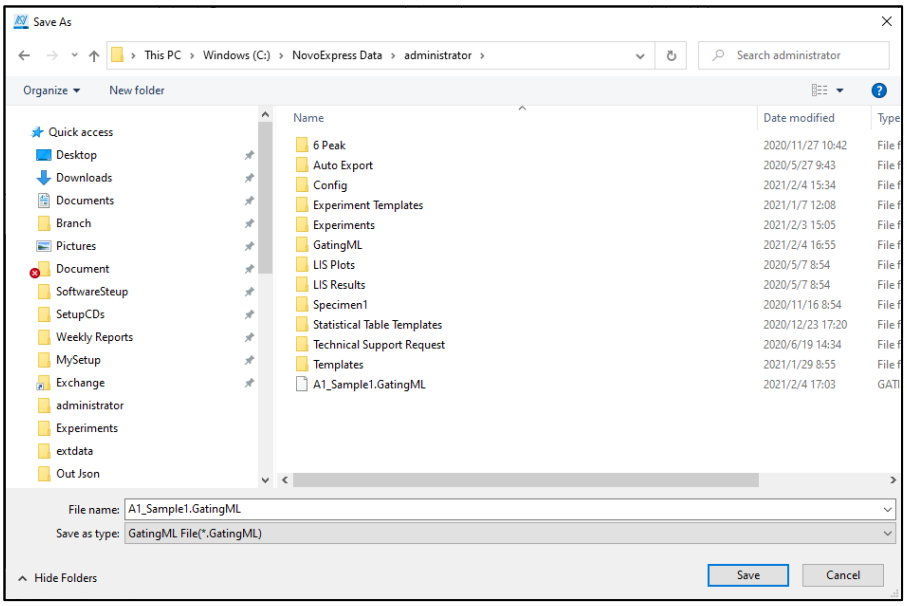
Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.



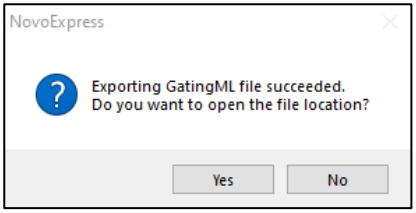
- From **Plot** area: Right click the selected gate, click **Export to GatingML File…** and select **All Gates** to export all gates of the active sample to the **Gating-ML** file, or select **Selected Gates** to export the selected gates and their associated parent gates to the **Gating-ML** file.



In prompted window as shown below, user can select the target folder and edit the file name. The default folder is the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously. The default file name is **Sample Name.GatingML.** Click **Save** to continue.



Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.

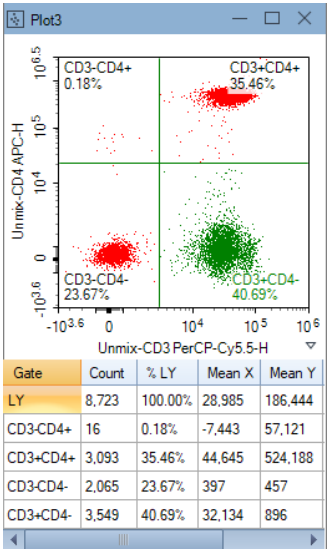


Statistics

In the NovoExpress (Opteon) 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.

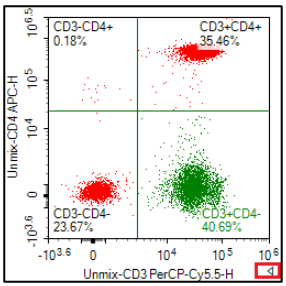


Gate	Count	% LY	Mean X	Mean Y
LY	8,723	100.00%	28,985	186,444
CD3-CD4+	16	0.18%	-7,443	57,121
CD3+CD4+	3,093	35.46%	44,645	524,188
CD3-CD4-	2,065	23.67%	397	457
CD3+CD4-	3,549	40.69%	32,134	896

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.



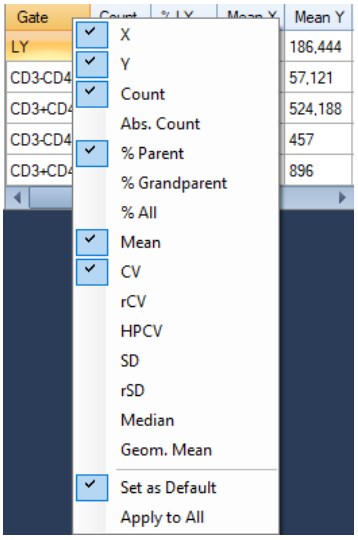
- From the Workspace Toolbar, click the **Show Statistics** button to show/hide the statistics chart of a plot.

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	% LY	Mean X	Mean Y
LY	8,723	100.00%	28,985	186,444
CD3-CD4+	16	0.18%	-7,443	57,121
CD3+CD4+	3,093	35.46%	44,645	524,188
CD3-CD4-	2,065	23.67%	397	457
CD3+CD4-	3,549	40.69%	32,134	896

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.

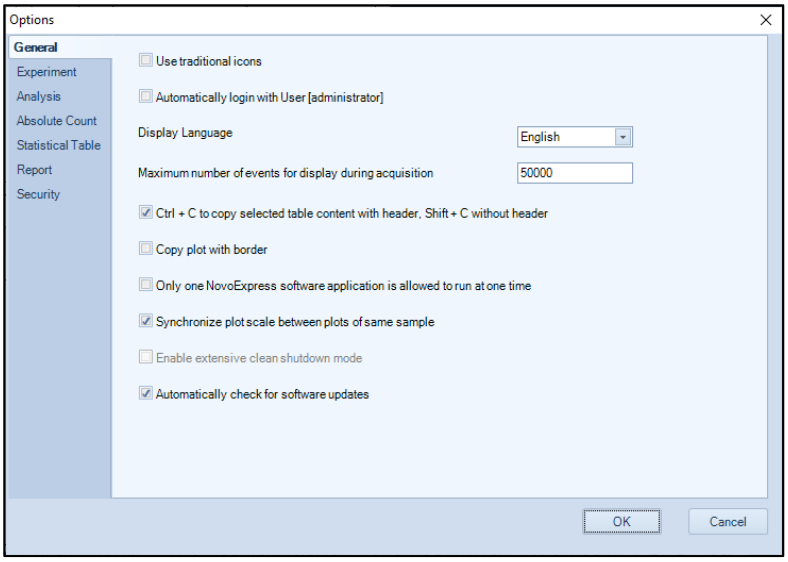


Copy Statistical Information to Clipboard

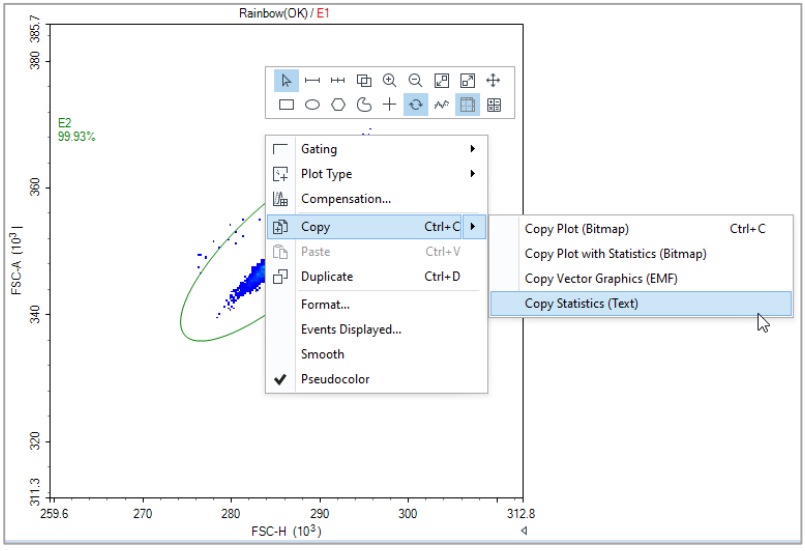
Data from the statistics chart can be copied to the clipboard. The copied data can by pasted to a spreadsheet program, such as Microsoft Excel, for further analysis.

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**.



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



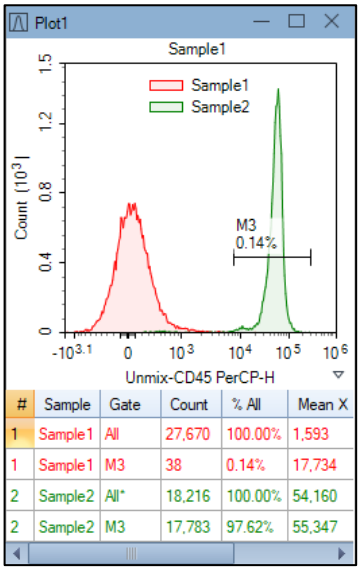
Statistics Layers

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.



#	Sample	Gate	Count	% All	Mean X
1	Sample1	All	27,670	100.00%	1,593
1	Sample1	M3	38	0.14%	17,734
2	Sample2	All*	18,216	100.00%	54,160
2	Sample2	M3	17,783	97.62%	55,347

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 Spillover Compensation applied to the data.

In addition, the calculations update automatically if the data set, gates, Spillover 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**

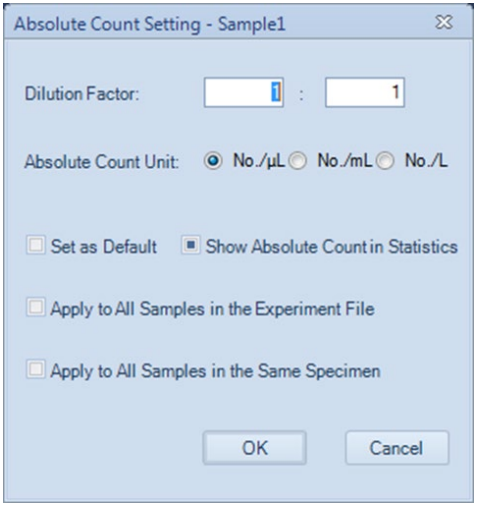
  The number of events collected in the specified gate.

- **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**.



- **% Parent**

  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

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

  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

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

  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

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

  Where SD is the standard deviation and $\overline{X}$ is the mean.

- **rCV (Robust CV)**

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

$\mathrm{rCV} = \mathrm{rSD} \,/\, \mathrm{Median}$

- **HPCV**

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

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

  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

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

  Where, n is the number of events and Xi 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

  $$\mathrm{Stain\_Index} = (MFI_1 - MFI_2)/(2 \times SD_2)$$

  Where MFI_1 is the Mean Fluorescence Intensity of the positive population, MFI_2 is the Mean Fluorescence Intensity of the negative population and SD_2 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.

- **% Gate**

  Percentage of events included within the gate shown in **Gate Manager** relative to the number of events within the gate that user selected from **%Gate** drop-down menu.

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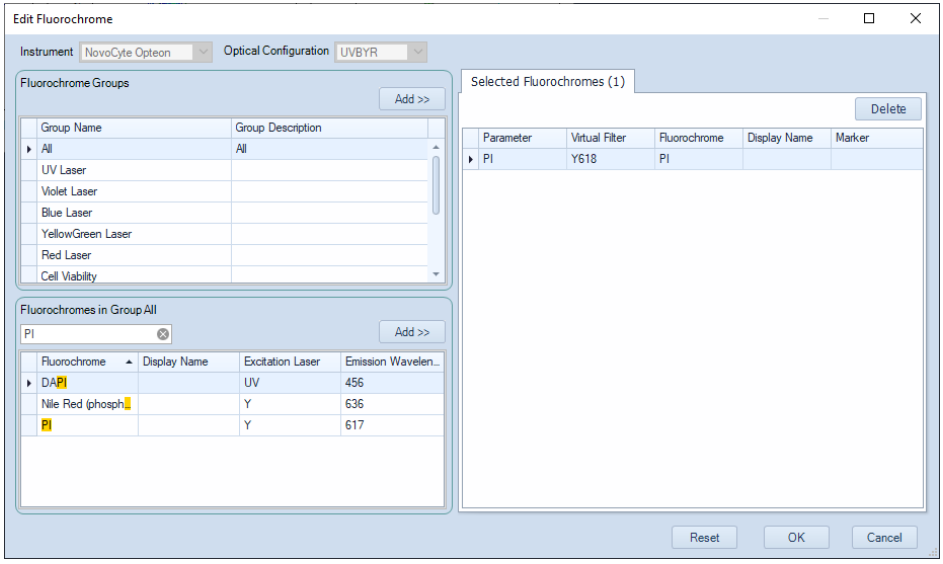
Cell Cycle Analysis

The NovoExpress (Opteon) 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. This feature is only available when user works in Unmixed workspace.

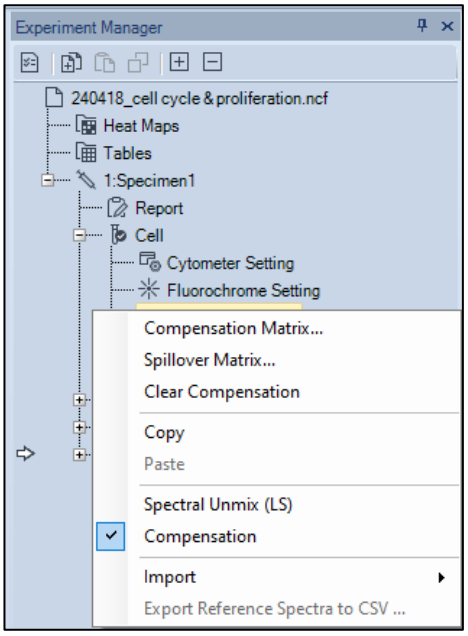
Automated Cell Cycle Analysis

- Add Cell Proliferation Fluorochrome
  In the **Fluorochrome Setting** panel, click **Edit** and add the cell proliferation fluorochrome (e.g., **PI**) in prompted **Edit Fluorochrome** window. Refer to Fluorochrome Setting in this guide for more details.



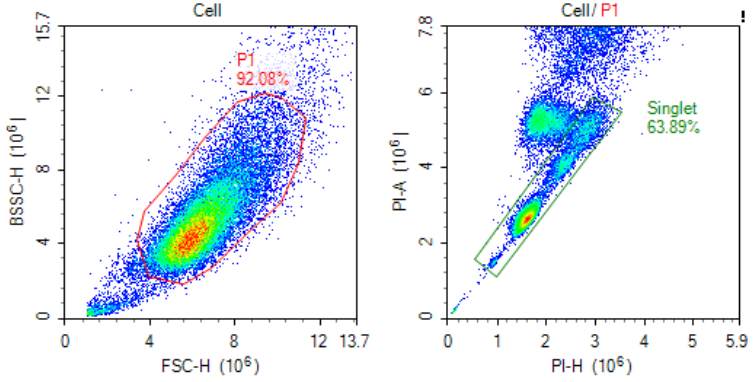
- Select **Compensation** to analyze the data.

  Right click the **Reference Spectra** node of experiment sample in **Experiment Manager**, select **Compensation**.



- Gating Single Cell Population

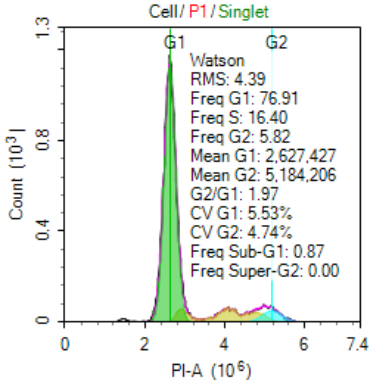
  After collecting the DNA stained cells, use an FSC-H /SSC-H density plot to get the target population and exclude cell debris. From the target population, create a Height versus Area density plot on the fluorochrome corresponding to the DNA stain, and gate for the single cell population and exclude cell 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 are 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:

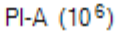
Item	Description
Watson	The model used for cell cycle fitting
RMS	The root means square error of the fit of the G1, S, and G2 phases. A smaller value indicates a better fit.
Freq G1	Percentage in G1 phase
Freq S	Percentage in S phase
Freq G2	Percentage in G2 phase
Mean G1	The mean fluorescence intensity 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.

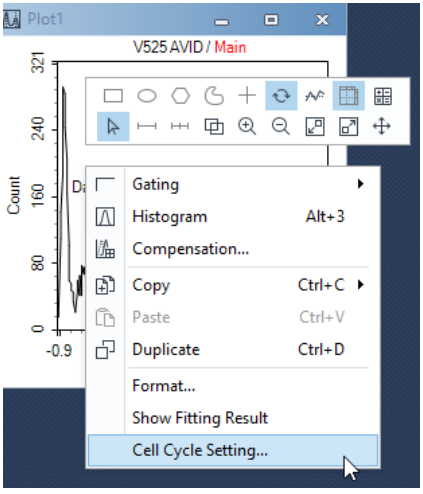
- Constrain G1 and G2 peaks

  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.

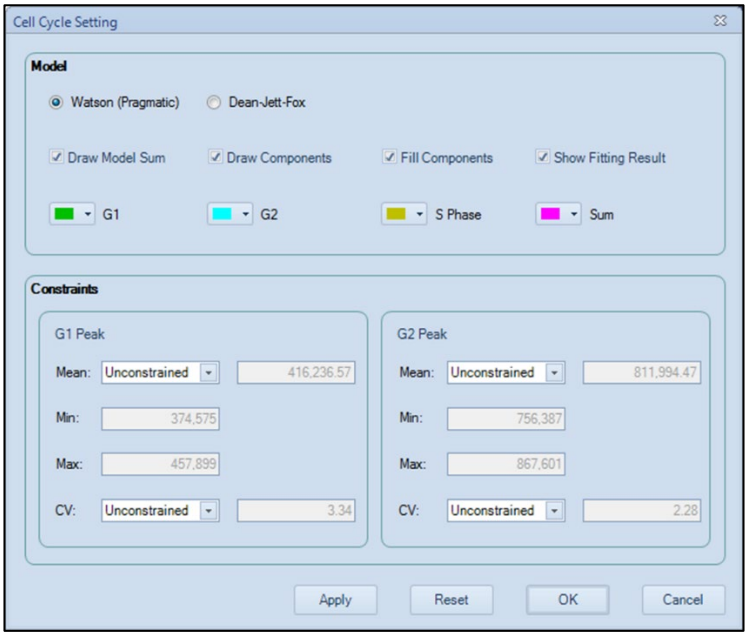


- **Cell Cycle Setting** window

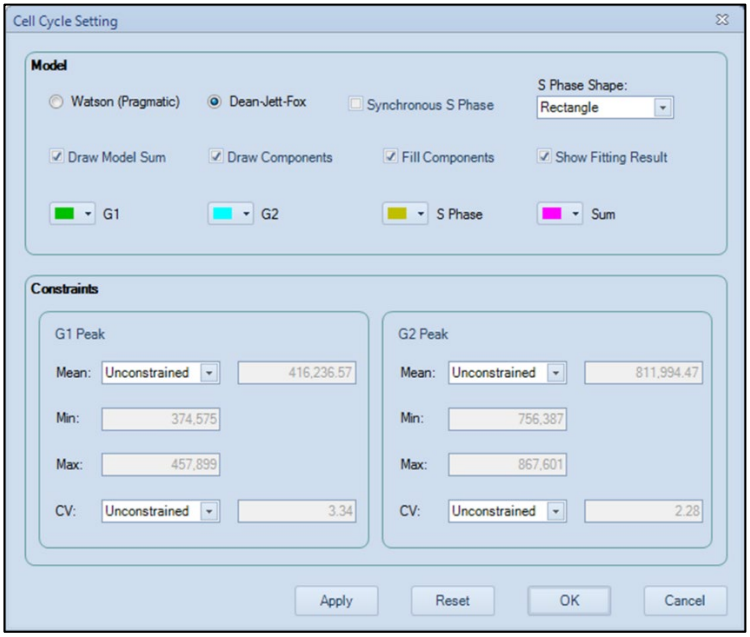
  Right-click on the cell cycle plot and select **Cell Cycle Setting** to open the **Cell Cycle Setting** window.



  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.



When Watson model is selected



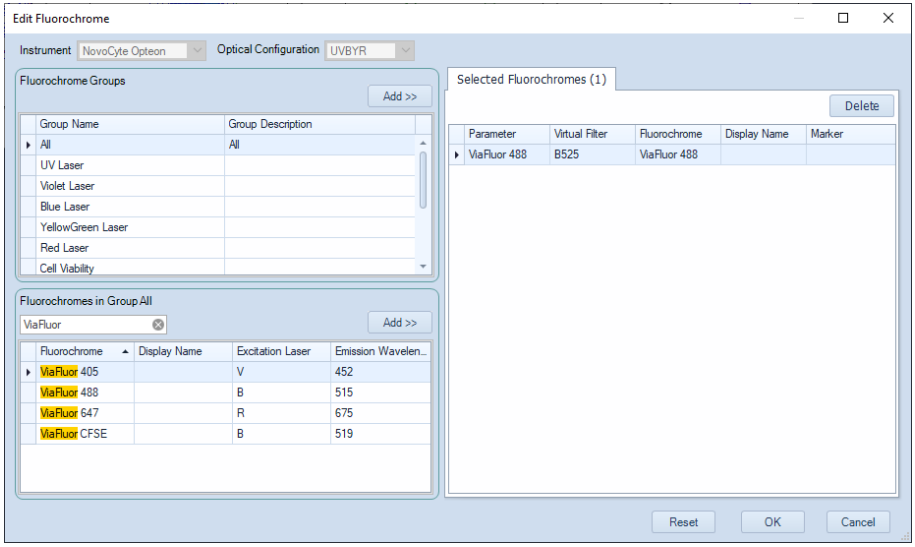
When Dean-Jett-Fox model is selected

Cell Proliferation Analysis

The NovoExpress (Opteon) Software includes a cell proliferation analysis feature that allows for the quantification of cell numbers as a function of time. This feature is only available when user works in Unmixed workspace.

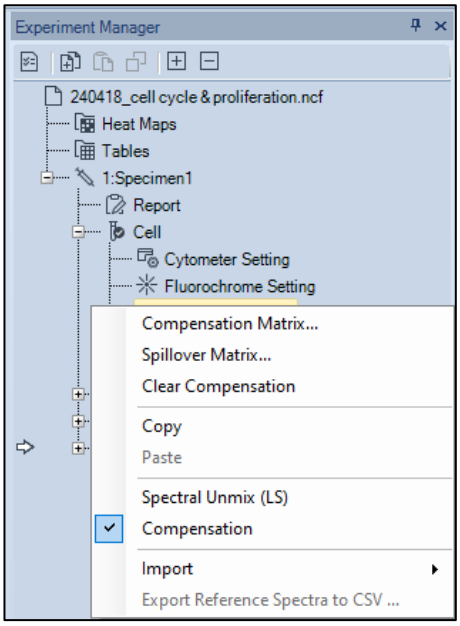
Automated Cell Proliferation Analysis

- Add Cell Proliferation Fluorochrome
  In the **Fluorochrome Setting** panel, click **Edit** and add the cell proliferation fluorochrome (e.g., ViaFluor 488) in prompted **Edit Fluorochrome** window. Refer to Fluorochrome Setting in this guide for more details.



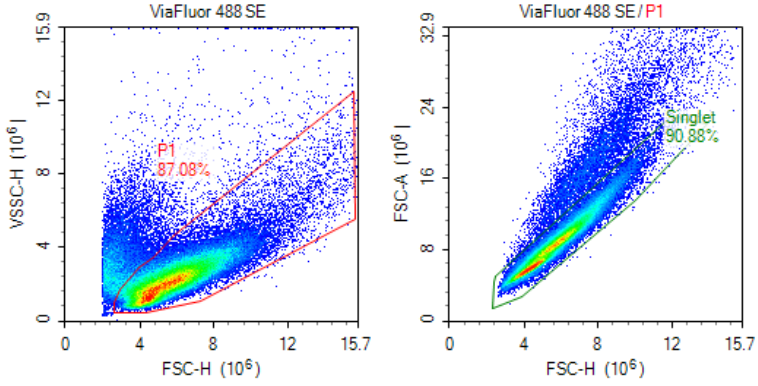
- Select **Compensation** to analyze the data.

  Right click the **Reference Spectra** node of experiment sample in **Experiment Manager**, select **Compensation**.



- Gating Target Cell Population

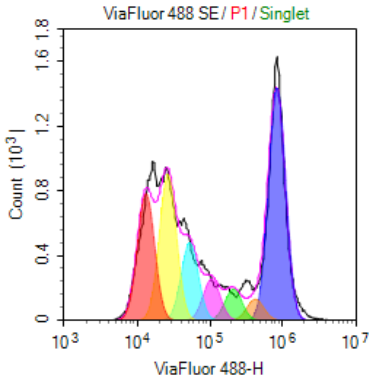
  On the density plot of FSC-H/SSC-H, create a gate which includes the target cell population you are interested in. From the target population, create a Height versus Area density plot on FSC, and gate for the single cell population and exclude cell aggregates. This is shown in the figure below.



- 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 the fluorochrome that relates to the proliferation staining dye, such as ViaFluor 488-H. Apply the previously

created cell gate to the plot and make sure all the events inside this gate are within the plot scale. The software automatically attempts to fit the data using the modeling algorithm and calculates the results.



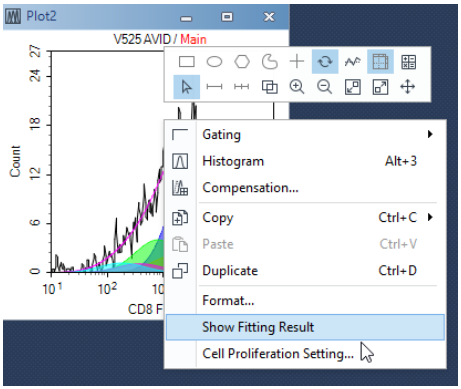
To display the statistics, right click the resulting cell proliferation histogram and select **Show Fitting Result**.

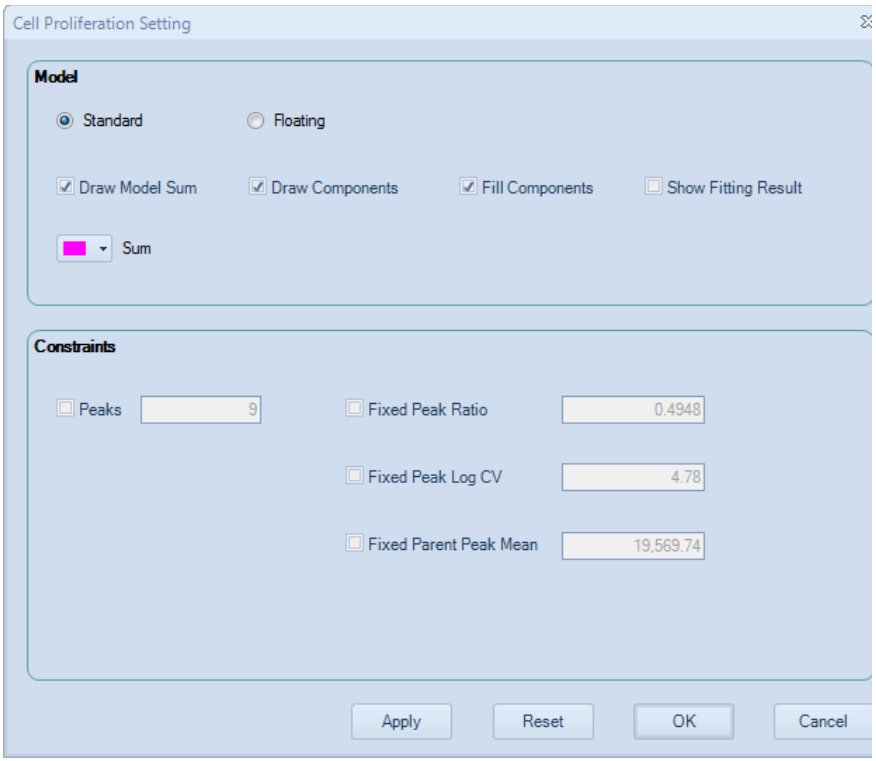
Item	Description
Model	The name of model used for analysis and generates results, including **Standard** and **Floating** models.
RMS	Root Mean Square error. It is an estimate of the "goodness of fit" of the model.
Peaks	The count of Peaks.
Peak Log CV	Log CV of peak
Peak Ratio	The average ratio of all the peak positions. $\text{Peak Ratio} = \frac{1}{n-1}\sum_{i=1}^{n-1}\frac{meanG_i}{meanG_{i-1}}$ where $meanG_i$ is the mean of the peak of i^{th} generation, n is the count of peaks include the parent peak.
Freq Divided	The percentage of the original cells that are divided. $\text{Freq Divided} = \frac{\sum_{i=1}^{n-1}\frac{N_i}{2^i}}{\sum_{i=0}^{n-1}\frac{N_i}{2^i}} \times 100\%$ where i=0 means the original generation.
Prol. Index	Proliferation Index. It is the sum of the number of divisions in each generation divided by the number of original cells that are divided.

	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	Expansion Index. It is the number of cells divided by the number of original cells. Exp. Index = $\frac{\sum_{i=0}^{n-1} N_i}{\sum_{i=0}^{n-1}\frac{N_i}{2^i}}$
Div. Index	Division Index. It is the sum of the number of divisions in each generation divided by the number of original cells. 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	Replication Index. It is the number of non-original cells divided by the number of original cells that divided. 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.

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.





- 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 S, 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

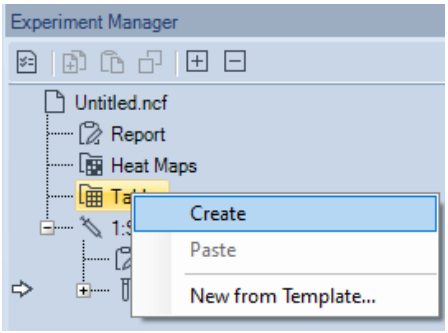
	Selects samples to show
	Deletes rows
	Exports table as a CSV file
	Opens the **Options** > **Statistical Table** window

Create Different Types of Statistical Tables

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

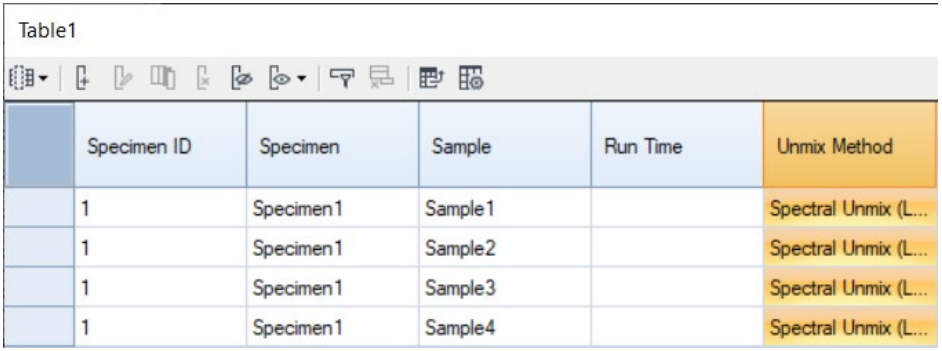
- Creating a New Statistical Table

  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.



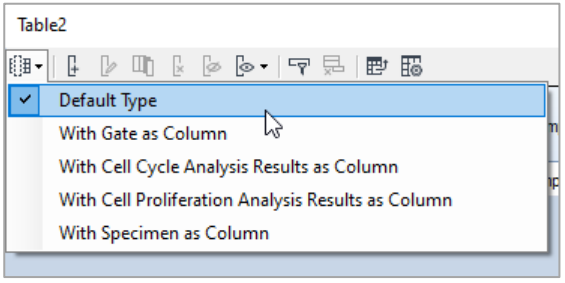
- Creating a New Statistical Table from Template

  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.

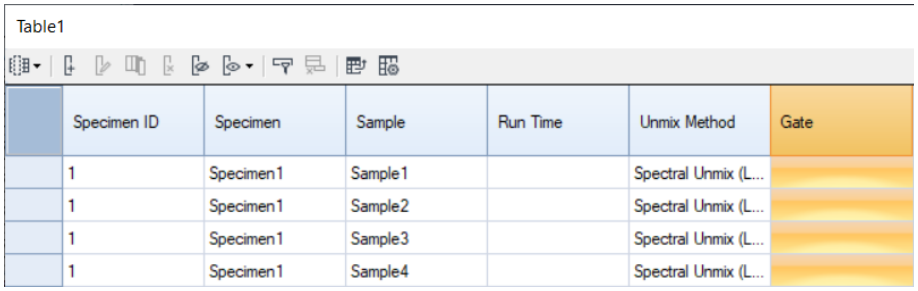
- Selecting the Type of Statistical Table

  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**.

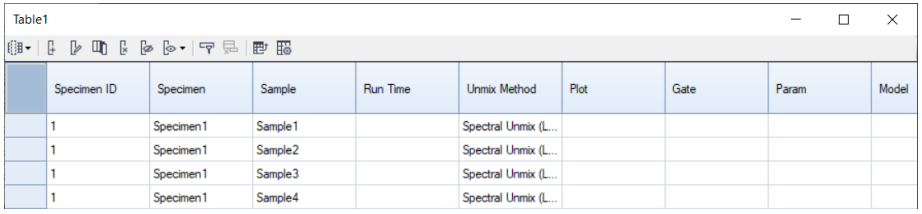
  New tables are created as The Default Type. To change the table type, click the **Table Type** button from the toolbar and select the new table type from the drop-down menu.



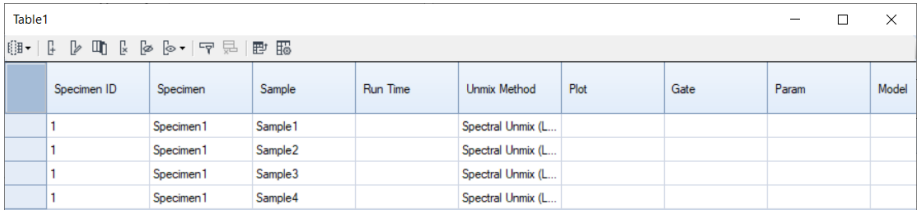
  Shown below is an example for a table **With Gate as Column**.



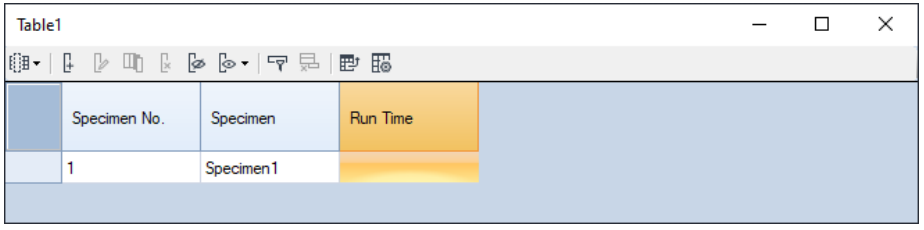
Shown below is an example for a table **With Cell Cycle Analysis Results as Column**.



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



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



- 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.

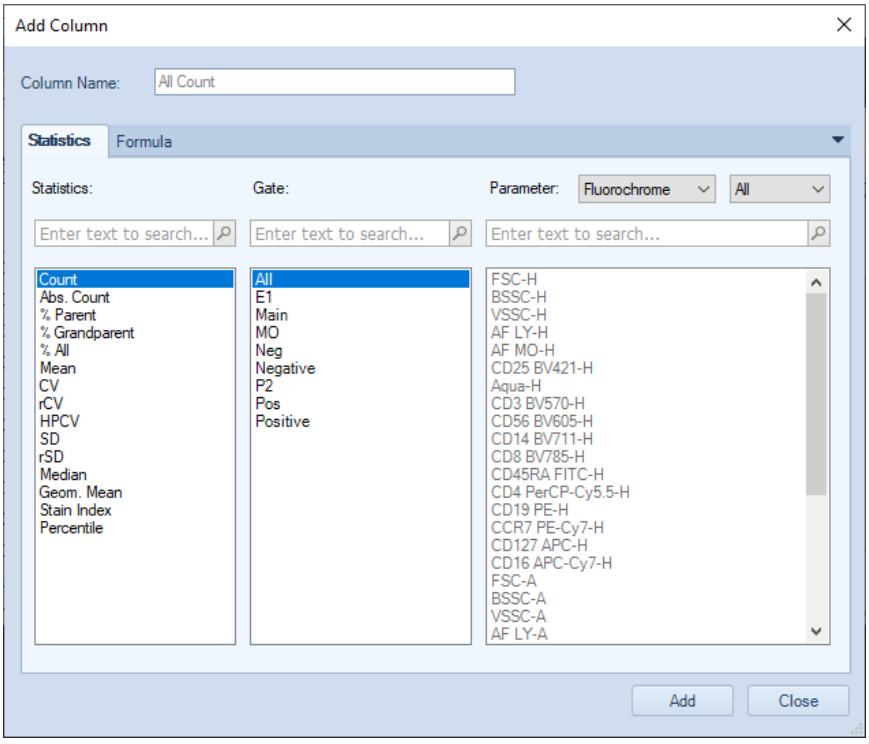
Add and Edit Columns

- 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.



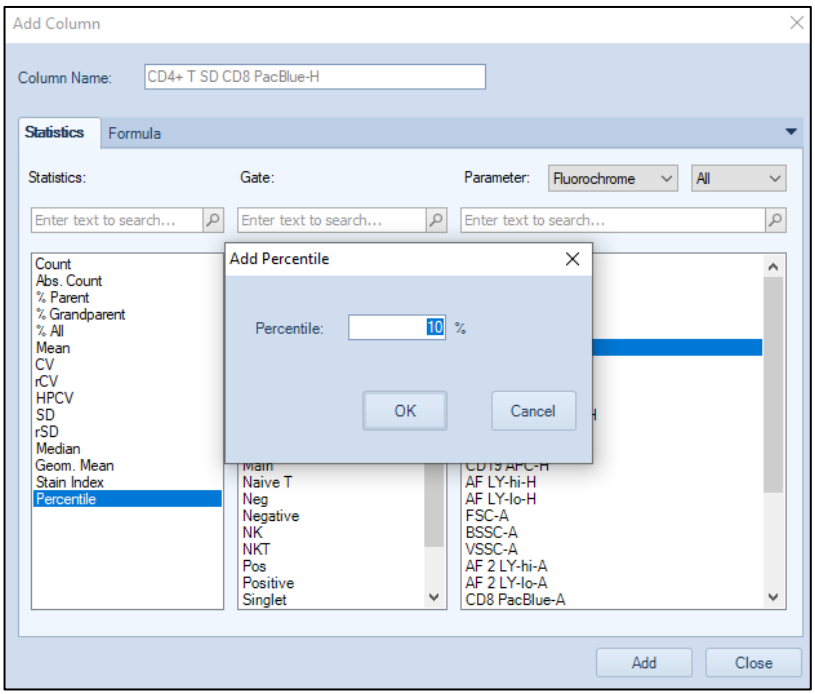
NOTE To add multiple items from each column, highlight the items to be added while holding the Ctrl or Shift key. Click **Add** to add the selected items to the table.

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 in this guide for detailed description of **Stain Index**.

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** and10th 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.

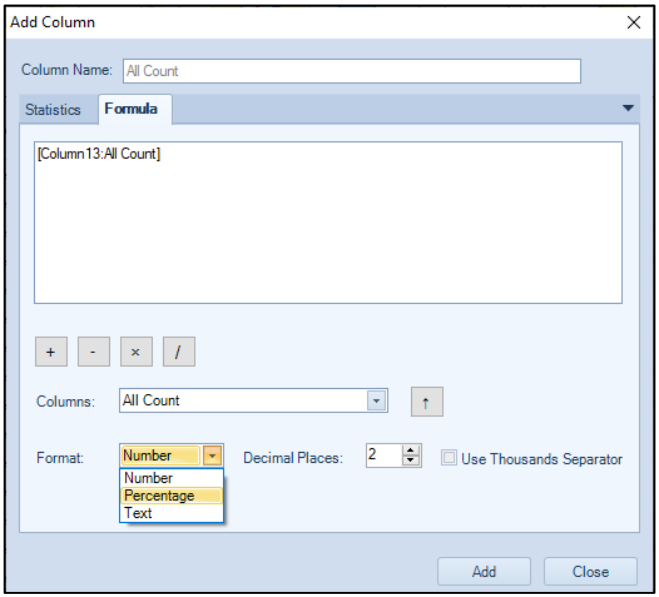
  By default, the column name is automatically generated. The name can be modified by the **Column Name** box at the top of the **Edit Column** window.

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.



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.

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.

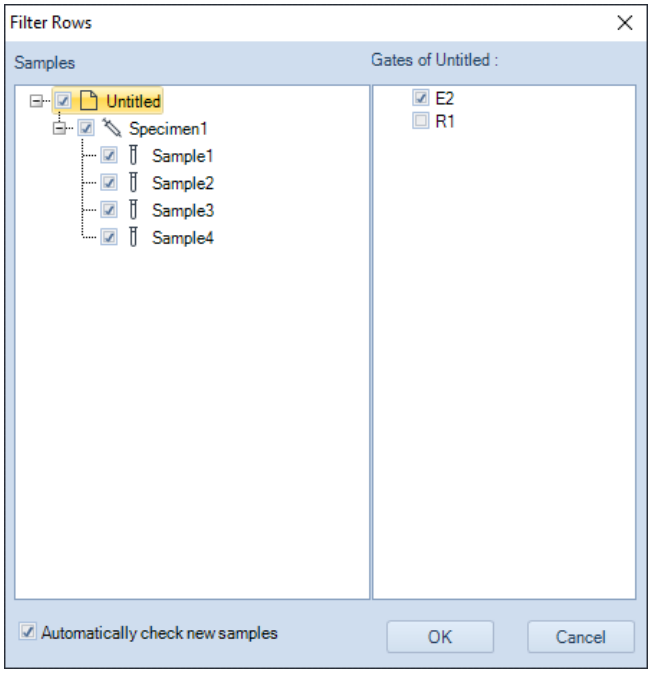
Statistical Table Rows

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.



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.

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 to 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 **Group, Specimen**, **Specimen ID**, **Sample**, **Sample ID**, **Operator**, **Run Time, Unmix Method** and **Gate** columns. When NovoSampler S is connected, user can set **Customize Name** and **Default Visibility** of additional **Plate ID, Plate Barcode**, and **Well ID** columns. Please refer to Option Settings in this guide for more details.

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.

- Export Statistical Table as Template

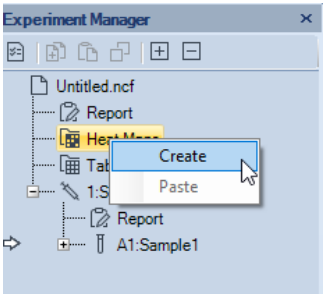
  In the **Experiment Manager** panel, select the table. Right-click and select **Export Template..**. Enter the name for the template in the prompted window and click **Save** to create a template. The template will be saved as a *.nst file.

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

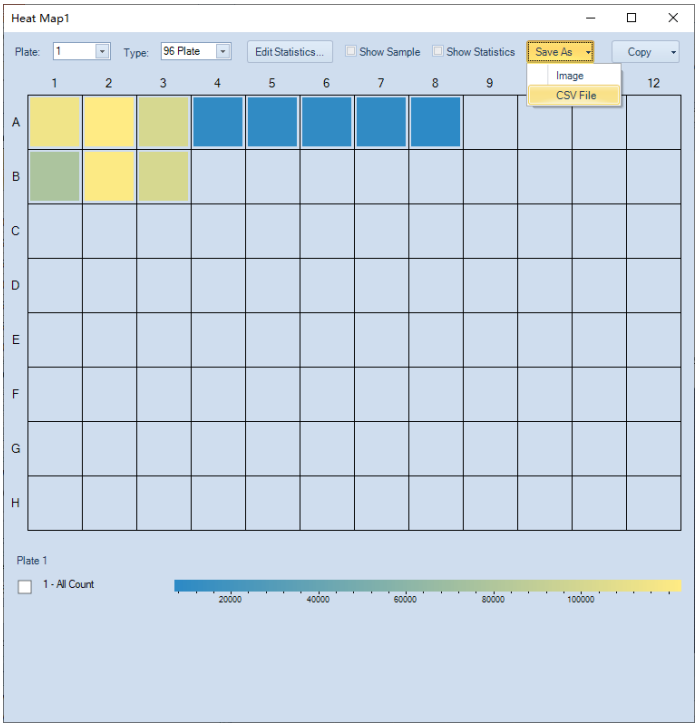
In the **Experiment Manager** panel, under the experiment file name, right-click on the **Heat Maps** node and select **Create**.



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.



NOTE If there are multiple samples in the same well, only the first sample will be used to generate the heat map.

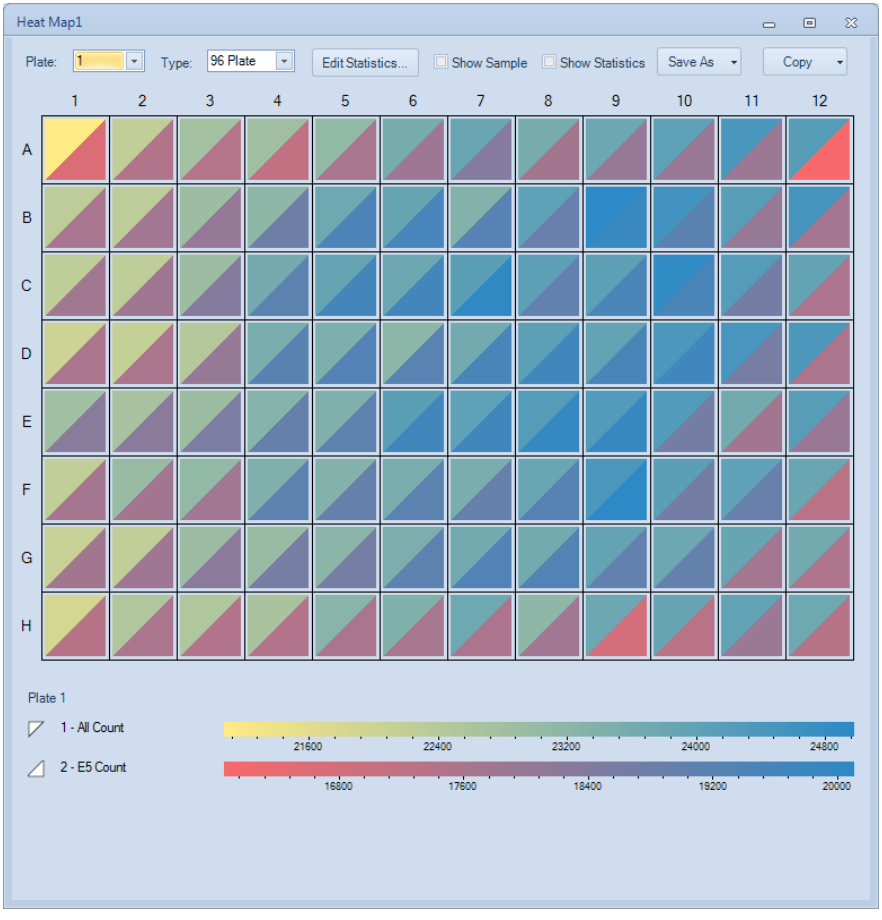
NOTE If there are samples outside the current plate type, "*" will be displayed in the upper left corner of the heat map.

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.

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.

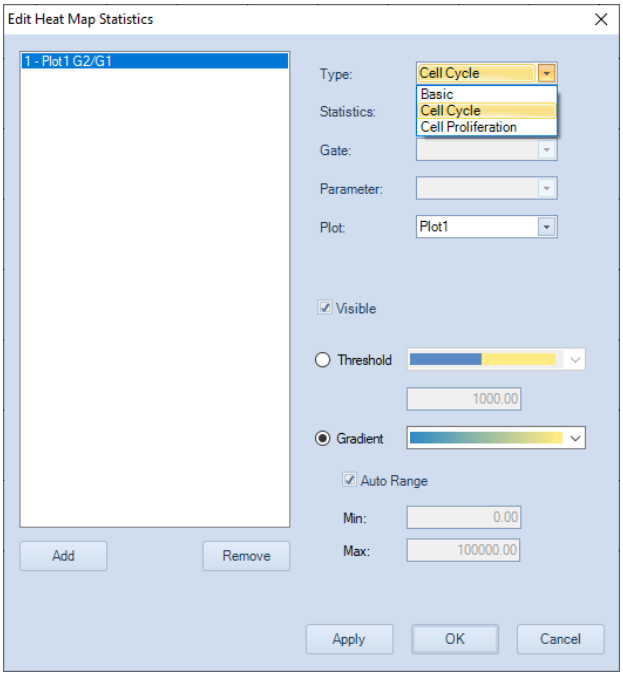
Add Statistics

- 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 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.



- **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.

  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.

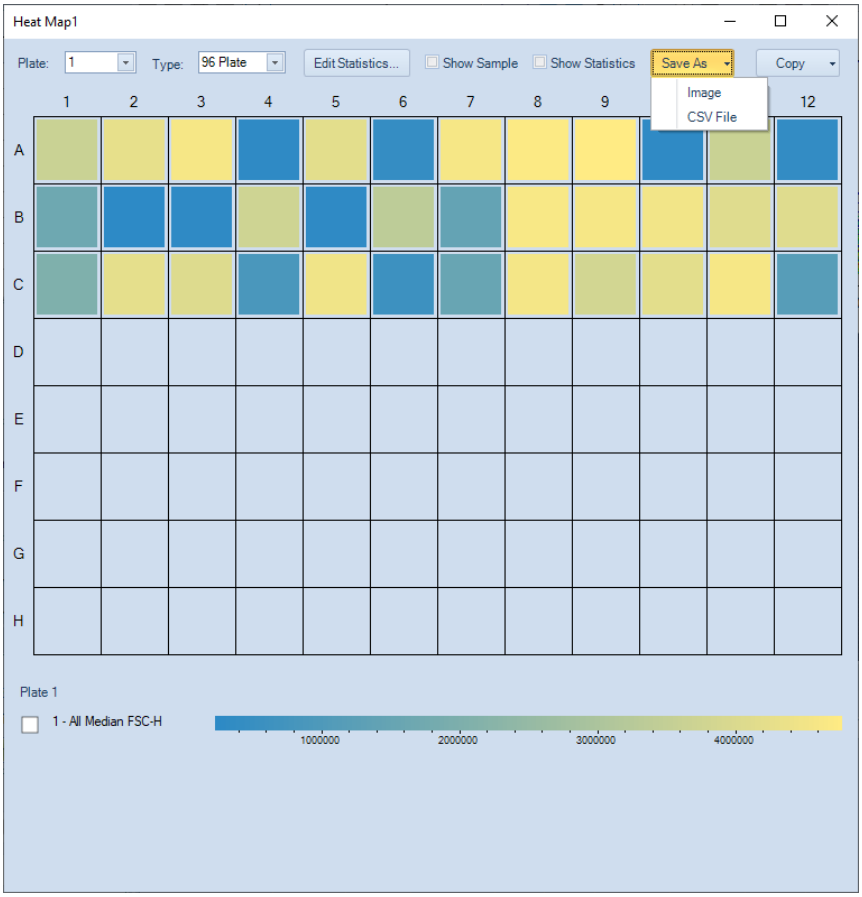
Update Heat Map

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.

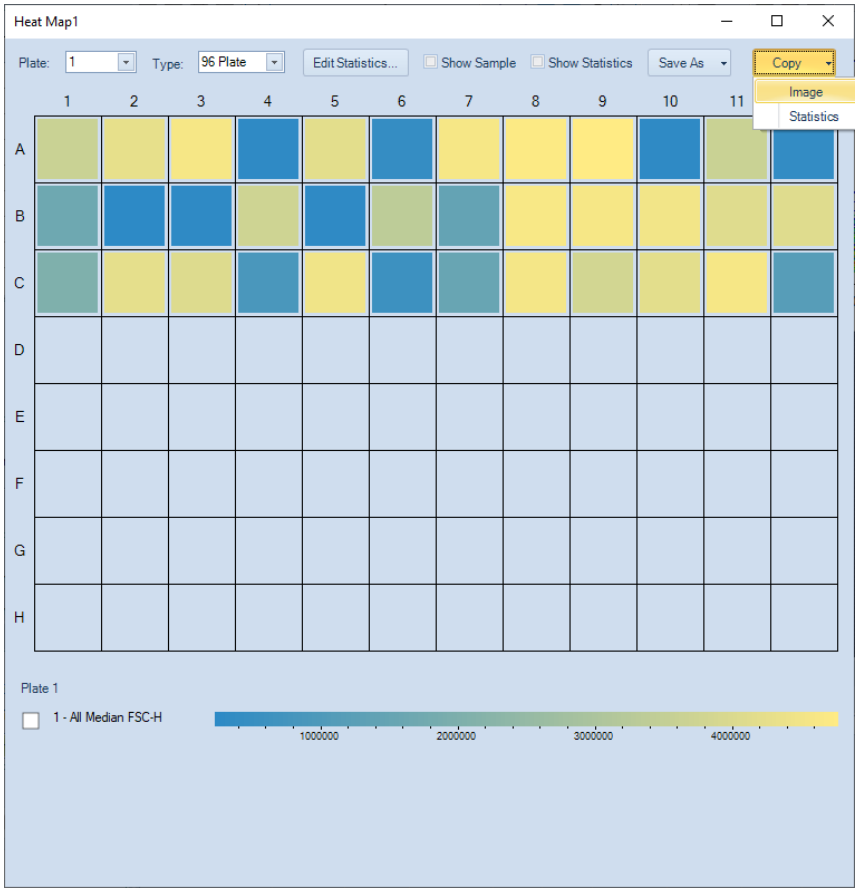
- Select **Image** or **CSV File** from the drop-down menu of **Save As.**



- 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**.

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. 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

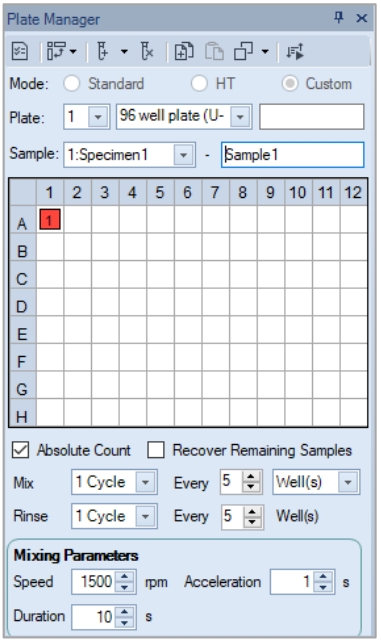
  In the **Experiment Manager** panel, select a heat map. Right-click and select **Rename**. Enter the new name to rename the heat map.

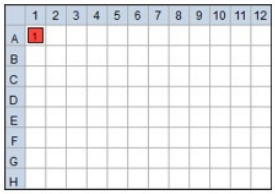
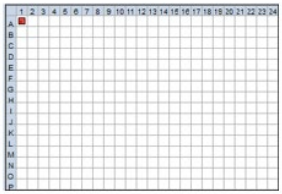
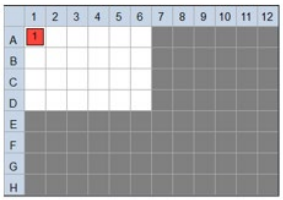
6 Plate Manager

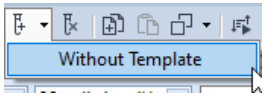
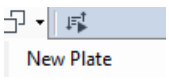
This chapter provides the information for the **Plate Manager** panel, including overview of **Plate Manager** panel and context menu.

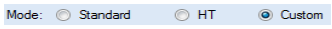
Overview of Plate Manager Panel

The **Plate Manager** panel will only be available when the NovoSampler S is installed, or the **NovoSampler S installed** checkbox is checked in the **Instrument Configuration** window in the NovoExpress (Opteon) 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.



Icon	Description
  	The **Plate Manager** panel displays a 16 x 24 grid indicating a 384-well plate map (for NovoSampler S 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. 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. **The state of each well can be:** • This icon indicates that this well is selected. Multiple wells can be selected at the same time. • 1 The number is the Specimen ID and indicates to which specimen the selected sample belongs to. • 1 An underlined number indicates that the selected sample has acquired data. • 1 The arrow in the upper right corner indicates that the well has multiple samples. Context menu of the well will list all samples. • 1 The italicized number indicates the acquisition sequence of the selected sample. • 1 A red background indicates the well contains the Active Sample. • 1 A green background indicates that the well contains sample in queue to be collected. • 1 / 1 An alternating green and dark green background indicate that the well contains sample currently in acquisition. • 1/ 1 An alternating green and red background indicate that the well contains sample currently in acquisition and also is the Active Sample. **The following operations can be operated on the plate grid:** • 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.
	Work List: View and edit **Work List**.
	Samples Created Order: Selects whether to automatically proceed horizontally across the plate or vertically across the plate when creating new samples.
	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. **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.



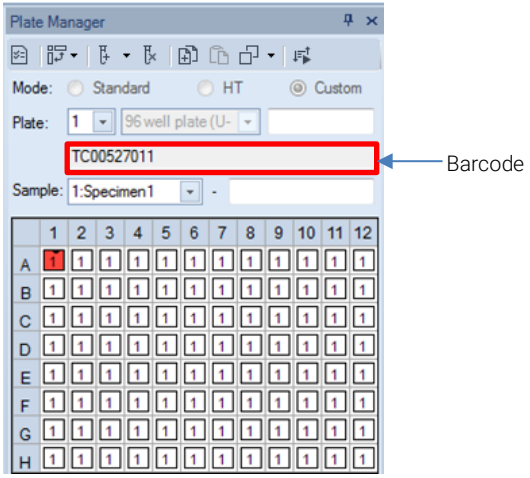
Mode: Allows user to select the working mode (Standard, High-Throughput and Custom) of the NovoSampler S in this area. Default mixing condition, rinse condition, and flow rate will be automatically applied when **Standard** or **HT** mode is selected. Please refer to associated operator's guide for more details. User can only customize these settings when **Custom** mode is selected.

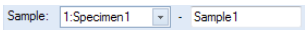
NOTE Selecting the working mode is only available before the sample is created.

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.

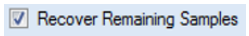
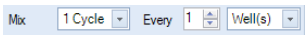
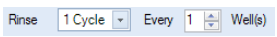
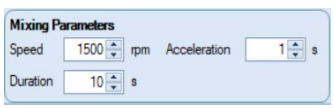
NOTE The plate type cannot be modified once the sample acquisition has begun.

NOTE When NovoSampler S 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 S Operator's Guide for more details.





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** ....
	This function is available only when NovoSampler S 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 S works in Standard or High-Throughput (HT) mode. When Custom mode is selected, user can select to disable or enable this function.
	**This function is available only when NovoSampler S 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 checkbox 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.
	**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**: 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:** User can view or set the mixing parameters for the orbital shaker in this panel. When NovoSampler S 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 S 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 S works in different mode.

Plate Type	Default Mix Speed (rpm)		
	Standard Mode	HT Mode	Custom Mode
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 (12 X 75 mm tubes)	1000	1000	1000
384 well plate	2500	2500	2500
24 tube cooling rack (12 X 75 mm tubes)	1000	1000	1000
96 well plate (U, V and Flat bottom) with cooling box	1500	1500	1500
40 tube rack (1.5/2.0 mL tubes)	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 1 second.

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 S works in different mode.

Plate Type	Default Mix Duration (s)		
	Standard Mode	HT Mode	Custom Mode
96 well plate (U, V and Flat bottom)	8	1	10
24 well plate	10	4	10
48 well plate	10	5	10
40 tube rack (12 X 75 mm tubes)	10	5	10
384 well plate	8	1	10
24 tube cooling rack (12 X 75 mm tubes)	10	5	10

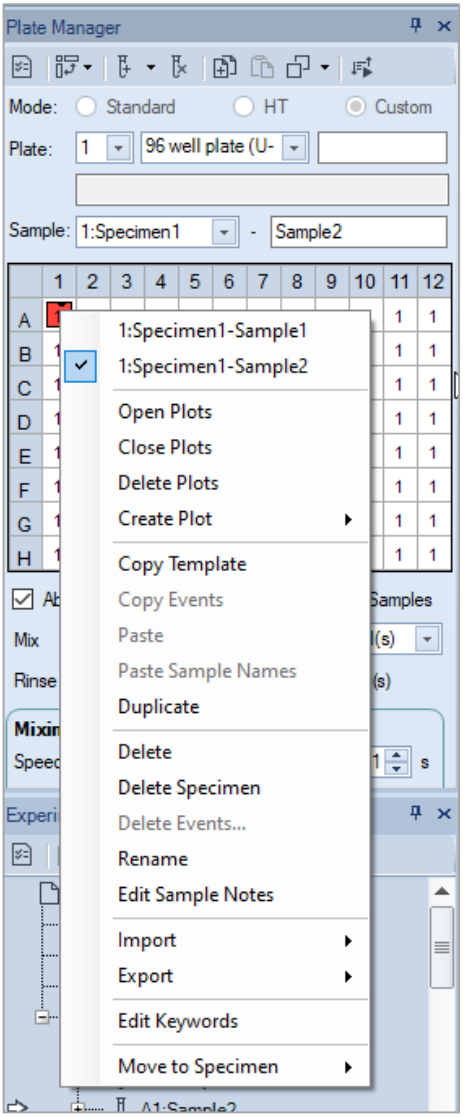
96 well plate (U, V and Flat bottom) with cooling box	8	3	10
40 tube rack (1.5/2.0 mL tubes)	10	5	10

Context Menu

In addition to the **Tools** menu, the **Plate Management** panel also provides a rich set of context menu actions.

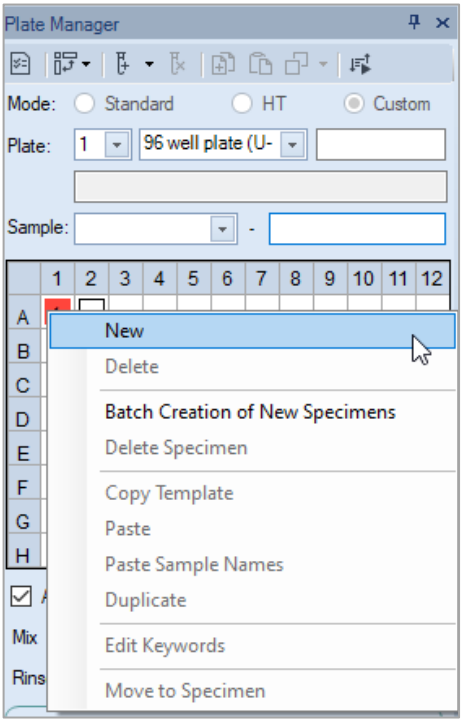
Context menu when a single well is selected

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 specimen. For additional information regarding these functions, refer to Right-Click Menu in this guide.

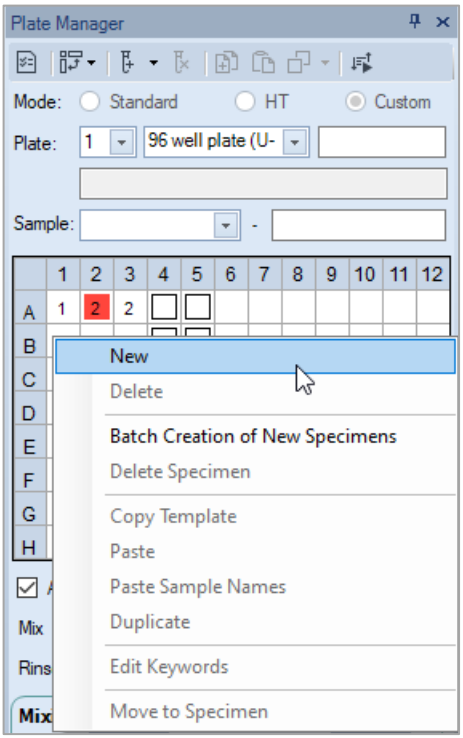


If the selected well has no sample defined, the right-click menu generally has two items available, **New** and **Batch Creation of New Specimens**. **New** will create new sample without a template. Batch Creation of New Specimens allows user to create new specimen(s) with customized specimen and sample organization. Refer to Context menu when multiple wells are selected for more details on this function.

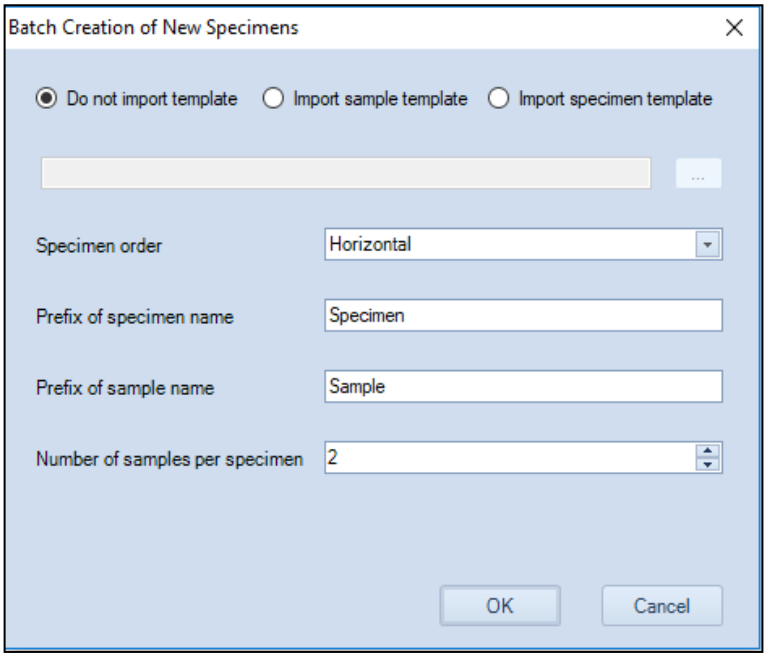
NOTE **Paste Sample Names** will be available if user copied the sample name in previous operation.



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 needs to define item(s) in this window, click **OK** to complete or cancel to go back to the previous window.



- 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).

    - 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 (Opteon) 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.

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

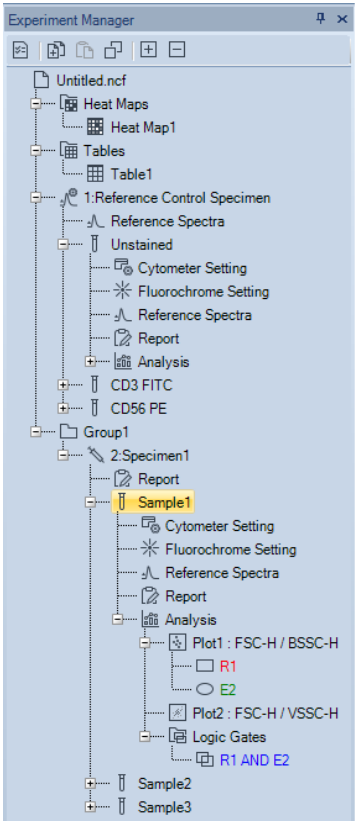
Icon	Description
	Work List: View and edit the work list. Contains information on the sample names and collection parameters. Refer to Work List in this guide for more information.
	Copy the selected node.
	Paste the copied content.
	Create a copy of the currently selected sample or specimen.
	Expand all child nodes of the current node.
	Collapse all child nodes of the current node.

Hierarchy

Description

In the NovoExpress (Opteon) Software, the hierarchy structure for experiment sample from high to low is groups, specimen, and samples. If Reference Control Specimen is created, additional node of Reference Control Specimen and

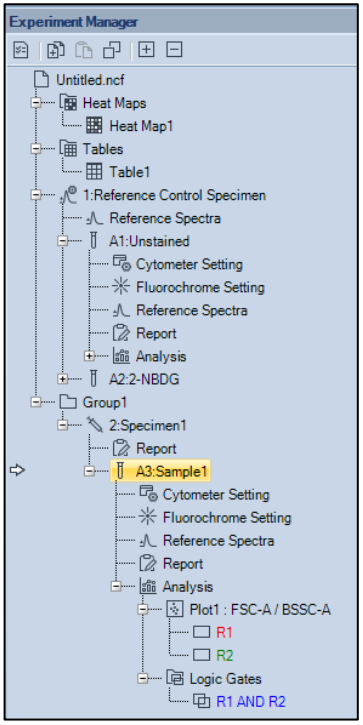
associated Reference Control sample(s) nodes will be displayed in the **Experiment Manager** panel.



In the figure above, the white arrow indicates the active sample. In the NovoExpress (Opteon) 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 S 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, refer to Plate Manager Panel in this guide.



Items in **Experiment Manager** can be identified by the icon before it which is summarized in the table below. Each item has associated right-click menu to allow users to conduct further operations conveniently.

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 under this node.
Heat Map	Individual created Heat map under Heat Maps node.
Tables	This node contains statistical tables for the experiment file. Right-clicking this node allows for the creation of new statistical table under this node.

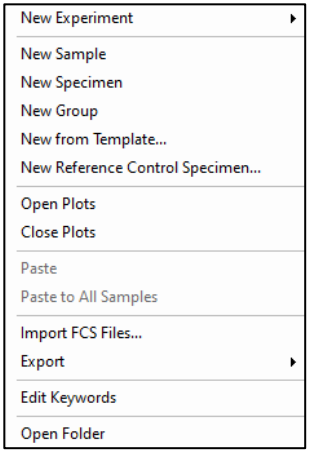
 Table	Individual statistical analysis table
 Reference Control Specimen	This node represents a Reference Control Specimen. A Reference Control Specimen can contain multiple Reference Control samples (i.e., Unstained and Single Stained Control samples) and the Reference Spectra of the Reference Control specimen and samples. This node appears only after the Reference Control Specimen is created by user. Refer to New Reference Control Specimen in this guide for more details. Note: If the population gating or autofluorescence setting for Reference Control samples changes, the **Reference Control Specimen** node, associated **Reference Spectra** node and Reference Control sample node will be displayed in italicized format. When the Reference Spectra of Reference Control sample is updated but Spectral Unmix (or Compensation) is not conducted, only the **Reference Control Specimen** node will be displayed in italicized format.
 Reference Spectra	Reference Spectra can appear under both Reference Control Specimen and individual Reference Control sample node. Double click it can open **Reference Spectra** window for associated specimen which contains all the Reference Spectra information included in this specimen, or for associated sample which only contains Reference Spectra information for this sample. Refer to Reference Spectra for more details.
 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 an experiment specimen in the organizational hierarchy. A experiment specimen can contain multiple experiment 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.
 Sample	This node represents a Sample (i.e., Reference Control sample or experiment sample) in the organizational hierarchy. The sample is the most basic organizational unit and contains sample data collection parameters, instrument settings, fluorochrome settings, Reference Spectra, 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	Double click to open **Cytometer Settings** panel which contains the sample parameters, the acquisition stop conditions, and the sample flow rate and threshold settings. Refer to Cytometer Setting in this guide for additional information.
 Fluorochrome Setting	Double click to open **Fluorochrome Setting** panel which contains the information of the selected fluorochromes for current sample including fluorochrome parameters, the emission channel, Reference Spectra, etc. Refer to Fluorochrome Setting in this guide for additional information.

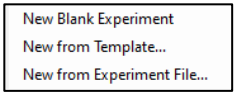
 Report	Contains a report of the data analysis. There is a Report node under specimen and sample. • Reports under Specimen can include plots and statistical analysis for all samples under the specimen. • Reports under the Sample can include plots and statistical analysis only for the sample. Refer to Reports in this guide 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
 White arrow	The white arrow indicates the sample is the active sample.
 Flashing green arrow	Flashing green and dark green arrows indicate the sample is being collected.
 Alternatively flashing white and green arrows	Flashing red and green arrows indicate the active sample is being collected.

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.

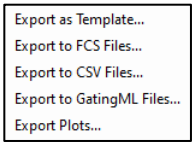
Icon	Description
 File	**New Experiment**: When clicked, following options are available in the expanded menu.





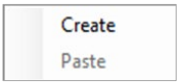
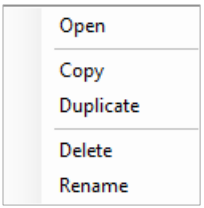
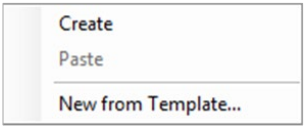
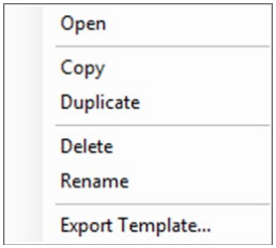
- **New Blank Experiment**: Create a blank experiment file.
- **New from Template**: Create an experiment file from a template.
- **New from Experiment File**: Create an experiment file from an existing experiment file. The **Absolute Counting** and **Sample Recovery** setting (i.e., enabled or disabled) from the existing experiment file will be automatically applied to the newly created sample.

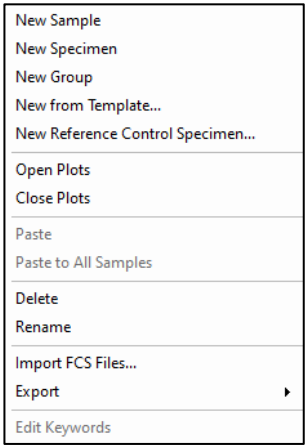
New Sample: Creates a new specimen with a new sample included.
New Specimen: Creates a new specimen.
New Group: Creates a new group.
New from Template: Imports selected group, specimen, and samples from a template.
New Reference Control Specimen: Click to open **New Reference Control Specimen** window which allows user to create a Reference Control specimen containing Unstained Control and Single Stained Control samples. Refer to New Reference Control Specimen in this guide for more details.
Open Plots: Opens all plots from all the samples.
Close Plots: Closes all plots from all 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: When clicked, following options are available.



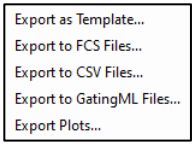
- **Export as Template**: Exports the file as a template file.
- **Export to FCS Files**: Exports all samples as FCS files.
- **Export to CSV Files**: Exports all samples as CSV files.
- **Export to GatingML Files:** Exports all gates in each sample as Gating-ML files.
- **Export Plots**: Exports all plots from all samples as image files.

Edit Keywords: Opens **Edit Keywords** window**.** The **Edit Keywords** window will list the keywords information for all the samples in the

	selected file**.** Please refer to Edit Keywords Window in this guide for more details. **Open Folder**: Opens the folder containing the experiment file.
 Heat Maps 	**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. **Export Template**: Exports the selected statistical table as template (.nst).
 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 Reference Control Specimen: Click to open **New Reference Control Specimen** window which allows user to create a Reference Control specimen containing Unstained Control and Single Stained Control samples. Refer to New Reference Control Specimen in this guide for more details.
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: When clicked, following options are available.

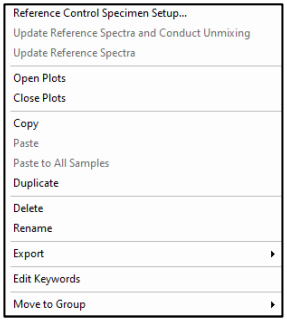


- **Export as Template**: Exports the group as a template file.
- **Export to FCS Files**: Exports all samples as FCS files.
- **Export to CSV Files**: Exports all samples as CSV files.
- **Export to GatingML Files:** Exports all gates in each sample as Gating-ML files.
- **Export Plots**: Exports all plots in current group as image files.

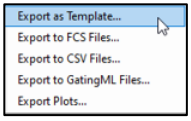
Edit Keywords: Opens **Edit Keywords** window**.** The **Edit Keywords** window will list **the** keyword**s** information for all samples in **the** selected group. Please refer to Edit Keywords Window in this guide for more details**.**

Reference Control Specimen

Reference Control Specimen Setup: Click to open the **Reference Control Specimen Setup** window. User can edit the reference control samples related setting in this window. This window contains the same functions as **New Reference Control Specimen** window does. Please refer to New Reference Control Specimen in this guide for details.
Auto Calculate Compensation: Click to allow software to automatically conduct the compensation. This option is available only when user selects Compensation to analyze the data. By

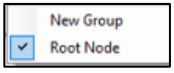


default, it is checked and disabled. When there are any changes to the Spillover Matrix, this option will become available.
Update Reference Spectra and Conduct Unmixing: Click to update the Reference Spectra and conduct automatic Spectral Unmix or Compensation. This function is available only when **Auto Update** mode is disabled, and the gated population is changed.
Update Reference Spectra: Click to update the Reference Spectra only. This function is available only when **Auto Update** mode is disabled, and the gated population is changed.
Open Plots: Opens all plots from all of the samples within the Reference Control specimen.
Close Plots: Closes all plots from all of the samples within the Reference Control specimen.
Copy: Copies the template of the Reference Control specimen.
Paste: Pastes the template of a copied Reference Control 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 Reference Control specimen.
Duplicate: Creates a duplicate of the Reference Control specimen.
Delete: Deletes the Reference Control specimen.
Rename: Renames the Reference Control specimen
Export: When clicked, following options are available in the expanded menu.

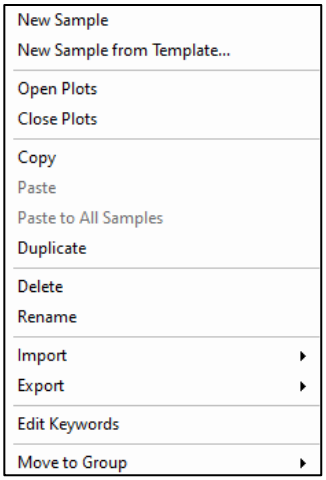


- **Export as Template**: Exports the Reference Control specimen as a template file.
- **Export to FCS Files**: Exports all Reference Control samples as FCS files.
- **Export to CSV Files**: Exports all Reference Control samples as CSV files.
- **Export to GatingML Files:** Exports all gates in each Reference Control sample as Gating-ML files.
- **Export Plots**: Exports all plots in current Reference Control specimen as image files.

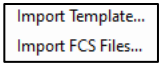
Edit Keywords: Opens **Edit Keywords** window. The **Edit Keywords** window will list the keyword**s** information for all samples in the selected specimen. Please refer to Edit Keywords Window in this guide for more details**.**
Move to Group: When clicked, user can select to move the Reference Control specimen to the root node or new group in the expanded menu.



Specimen

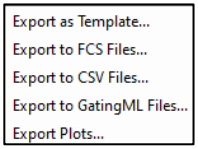


New Sample: Creates a new sample in the specimen.
New Sample from Template: Imports the samples of the first specimen from a selected template.
Open Plots: Opens all plots from all of the samples within the specimen.
Close Plots: Closes all plots from all of the samples within the specimen.
Copy: Copies 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: When clicked, following options are available.



- **Import Template**: Imports template and apply to the selected specimen.
- **Import FCS Files**: Selects one or more FCS files imported as samples.

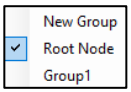
Export: When clicked, following options are available.



- **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 to GatingML Files:** Exports all gates in each sample as Gating-ML files.
- **Export Plots**: Exports all plots in current specimen as image files.

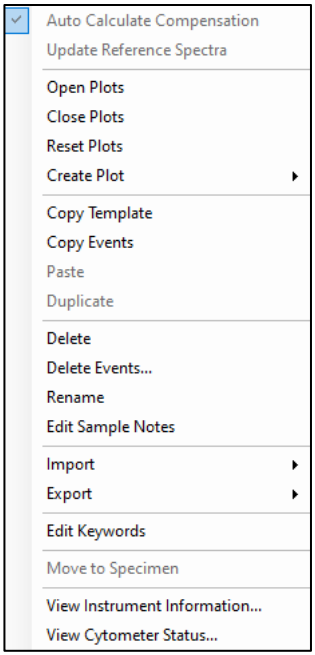
Edit Keywords: Opens **Edit Keywords** window. The **Edit Keywords** window will list the keyword**s** information for all samples in the selected specimen. Please refer to Edit Keywords Window in this guide for more details**.**

Move to Group: When clicked, user can select to move the specimen to the existing group, new group or root node in expanded menu.



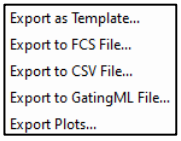
Auto Calculate Compensation: Click to allow software to automatically conduct the compensation. This option is available

Sample

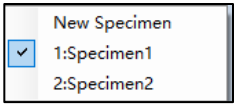


only when user selects Compensation to analyze the data. By default, it is checked and disabled. When there are any changes to the Spillover Matrix, this option will become available.
Update Reference Spectra: Click to update the Reference Spectra only. This function is available only for Reference Control sample, and when **Auto Update** mode is disabled, and user changes the gated population.
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 in this guide).
Rename: Renames the sample.
Edit Sample Notes: Edits the sample notes.
Import: Click to import a sample template or FCS file in the expanded menu.

Export: Click to export the sample as a template, FCS file, CSV file, exports all gates as a Gating-ML file or exports all plots of the sample as image files in the expanded menu.



Edit Keywords: Opens **Edit Keywords** window. The **Edit Keywords** window will list the keywords information for the sample. Please refer to Edit Keywords Window in this guide for more details.
Move to Specimen: Move the sample to the selected specimen or new specimen.

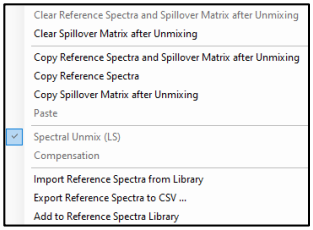


View Cytometer Status: Displays the cytometer status when the sample is collected.
View Instrument Information: Displays the instrument information when the file is created or the first sample is collected.
View Merge Information: This option is only available when selected sample is merged from other samples. User can view the

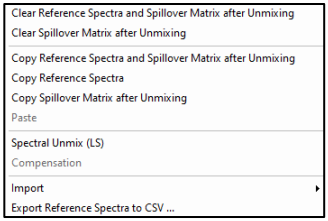
	merge information (e.g., samples which current sample was merge from). Please refer to Merge Samples in this guide for more details.
Cytometer settings 	**Copy**: Copies the sample's instrument settings. **Paste**: Pastes the copied instrument settings to the sample. **Import**: Imports the instrument settings from a selected template.
Fluorochrome Setting 	**Copy**: Copies the sample's fluorochrome settings. **Paste**: Pastes the copied fluorochrome settings to the sample. **Import**: Imports the fluorochrome settings from a selected template.

Reference Spectra

Right click menu of **Reference Spectra** node associated to Reference Control specimen or sample when user selects Spectral Unmix to deconvolute the data.



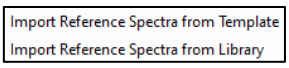
Right click menu of **Reference Spectra** node associated to experiment sample when user selects Spectral Unmix to deconvolute the data.



Right click menu of **Reference Spectra** node associated to Reference Control specimen or sample when user selects Compensation to analyze the data.

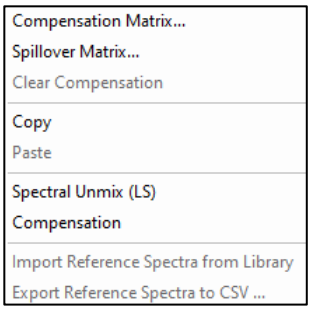
Following options are available when Spectral Unmix (LS) is used.

- **Clear Reference Spectra and Spillover Matrix after Unmixing**: Clear the sample's Reference Spectra and Spillover Matrix after unmixing. This option is only available for multi-color experiment sample, but not available for Reference Control specimen, Unstained Control sample and Single Stained Control sample.
- **Clear Spillover Matrix after Unmixing**: Clear the sample's Spillover Matrix after unmixing.
- **Copy Reference Spectra and Spillover Matrix after Unmixing**: Copy the sample's Reference Spectra and Spillover Matrix after unmixing.
- **Copy Reference Spectra**: Copy the Reference Spectra of selected sample.
- **Copy Spillover Matrix after Unmixing**: Copy the Spillover Matrix after unmixing of selected sample.
- **Paste**: Pastes the copied Reference Spectra or Spillover Matrix to the selected sample.
- **Spectral Unmix (LS)/Compensation**: Select Spectral Unmix (LS) or Compensation to analyze the data. This option is not available for Unstained Control sample and Single Stained Control sample. For experiment samples, only when the sample has not been analyzed by either method, i.e. its associated Reference Spectra or Compensation is empty, user can select one method to analyze the data.
- **Import Reference Spectra from Library**: Imports Reference Spectra from library. Refer to Import Reference Spectra from Library in this guide for more details.
- **Export Reference Spectra to CSV**: Exports Reference Spectra to a CSV file.
- **Add to Reference Spectra Library**: Add the Reference Spectra to library. This option is not available when user selects Compensation to analyze the data. When Spectral Unmix (LS) analysis method is selected, this option is only available for Reference Control samples, but not available for experiment sample.
- **Import:** This option is available for experiment sample only. User can click it to import the Reference Spectra from template or import the Reference Spectra from the library to the experiment sample in the prompted window. Please refer to Import Reference Spectra from Library in this guide for more details.

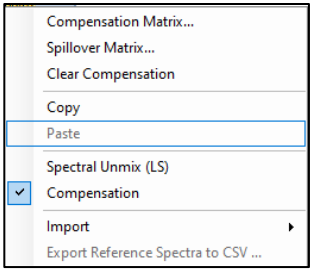


Following options are available if Compensation is used.

- **Compensation Matrix**: View and edit the Compensation Matrix in prompted **Reference Spectra** window. Refer to Reference Spectra in this guide for more details.



Right click menu of **Reference Spectra** node for experiment sample node when user selects Compensation to analyze the data.

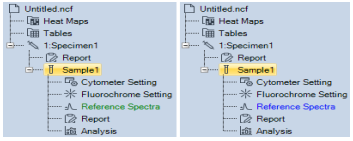


- **Spillover Matrix**: View and edit the Spillover Matrix in prompted **Reference Spectra** window. Refer to Reference Spectra in this guide for more details.
- **Clear Compensation**: Clear the Compensation Matrix and Spillover Matrix of selected sample. This option is available only for experiment sample.
- **Copy**: Copy the Compensation Matrix of selected sample.
- **Paste**: Paste the copied Compensation Matrix to selected sample.
- **Spectral Unmix (LS)/Compensation**: Check to switch to using Spectral Unmix (LS) or Compensation to analyze the data. This option is not available for Unstained Control sample and Single Stained Control sample. For experiment samples, only when the sample has not been analyzed by either method, i.e. its associated Reference Spectra or Compensation is empty, user can select one method to analyze the data.
- **Import**: This option is available for experiment sample only. User can click it to import the Reference Spectra from template to the experiment sample in the prompted window. Import Reference Spectra from Library is not available since Compensation is selected for analysis.

- **Export Reference Spectra to CSV**: Exports Compensation Matrix and Spillover Matrix to a CSV file.

NOTE

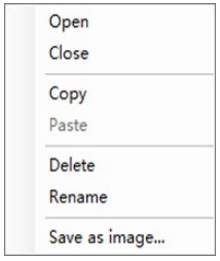
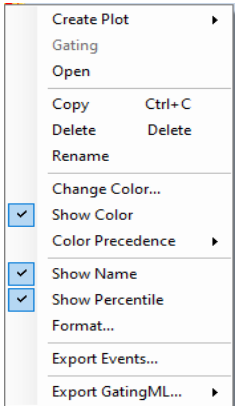
When **Spectral Unmix (LS)** is used, the Reference Spectra node will be displayed in green color if the associated specimen or sample has Reference Spectra. When **Compensation** is used, the Reference Spectra node will be displayed in blue color if the Compensation Matrix of associated specimen or sample is not empty.

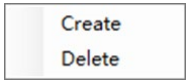
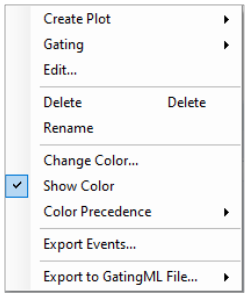


Report

Specimen Report:

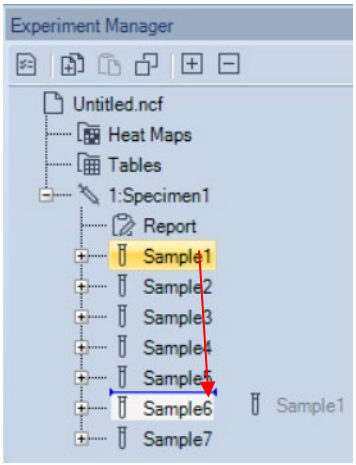
Open: Opens the report.
Print: Prints the report.
Copy: Copies the report template.
Paste: Pastes the copied report template to the sample. Specimen reports and sample reports are not able to copy and paste each other.
Import: Imports a report template to the sample from a selected template file.

Sample Report: 	
 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 Marker**: If a fluorescence parameter is associated with marker, this labels the gate using the marker specified. **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. **Export GatingML:** Exports gates as Gating-ML file.
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. **Color Precedence**: Modifies color precedence of the gate. **Export Events**: Exports data for the events inside the current gate in either FCS or CVS format. **Export to GatingML File**: Exports all or selected gates as Gating-ML file.

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 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, refer to Drag and Drop the Templates in this guide.



NOTE During the drag, press the ESC key to cancel the drag operation.

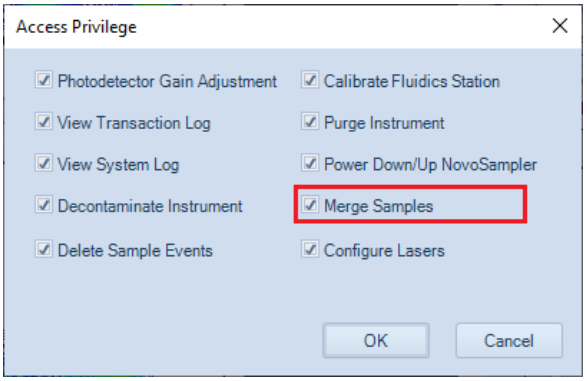
NOTE **Experiment Manager** supports making multiple selections within tree node to allow batch operation of multiple objects. Use Shift key to select continuous nodes on the tree and use Ctrl key to select discontinuous nodes on the tree, just like selecting multiple files on Windows Explorer. Only 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,

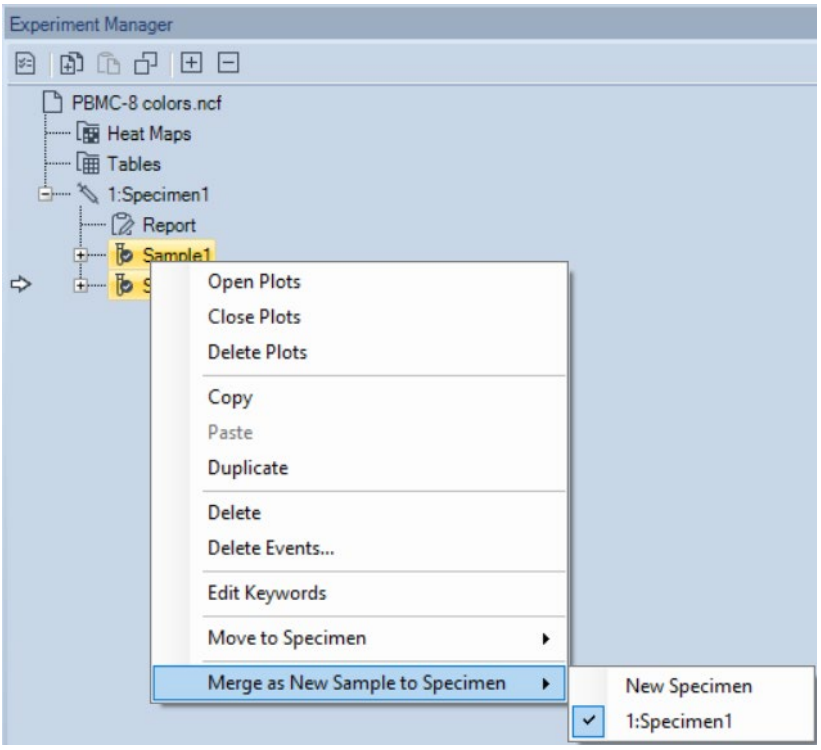
1 Ensure the **Merge Samples** privilege is enabled in the **Access Privilege** window.



NOTE When user logs into the software with Administrator account, the Merge Samples privilege is automatically enabled by default.

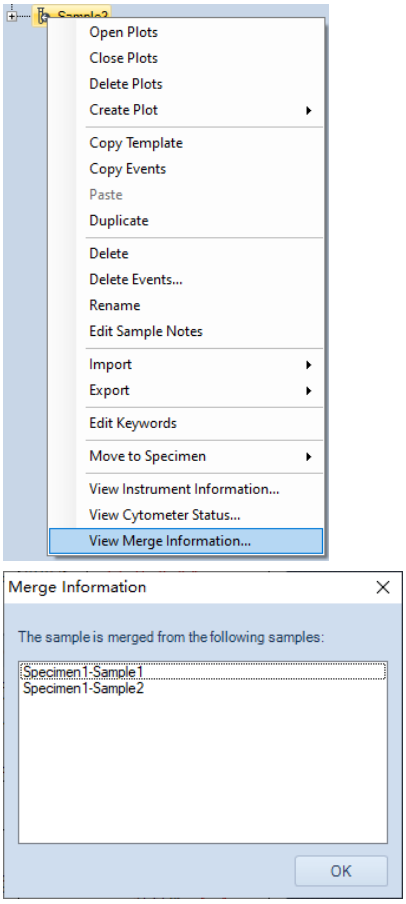
2 Select the samples from the **Experiment Manager** panel or **Plate Manager** panel (if NovoSampler S is connected) while holding the **Ctrl** or **Shift** key on the keyboard.

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.



4 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 .

5 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).

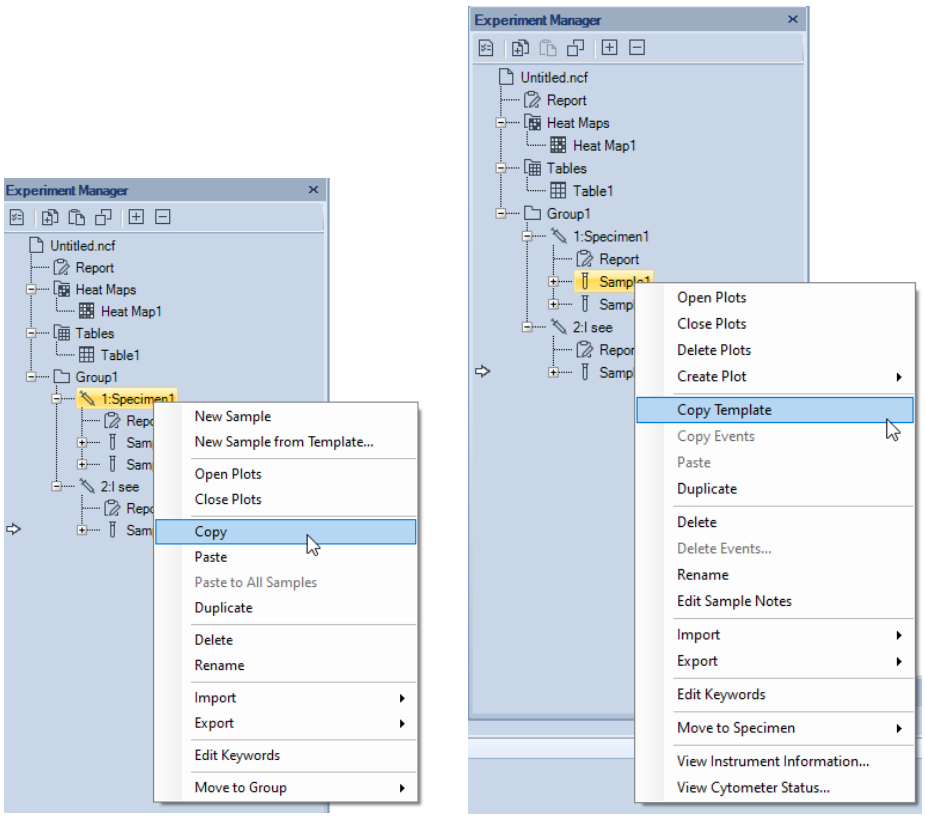


Templates

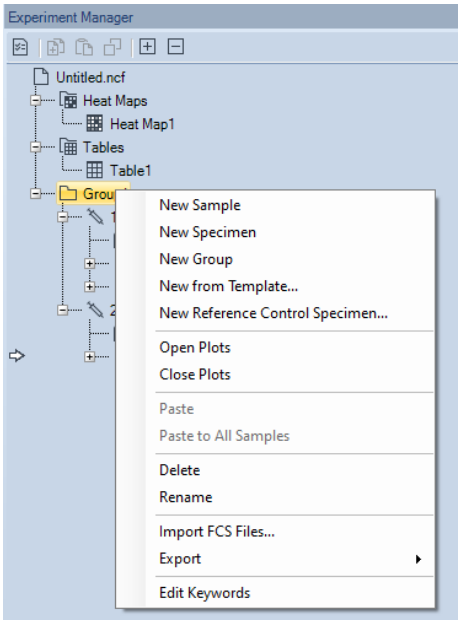
The NovoExpress (Opteon) Software allows for the use of templates to quickly set up experiment settings. These files can contain the settings for groups, specimens, or samples. There are multiple methods for a template to be applied including: copying and pasting, dragging and dropping, adding through 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:



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.

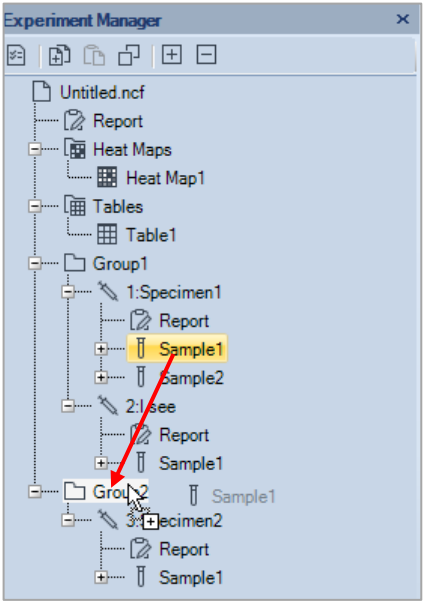
The source node	Target node
Specimen	**Specimen**: The target specimen report is replaced by the template of source specimen report and samples in the target specimen are replaced by copying samples in the source specimen and pasting to them. Group, Experiment File: Creates the same specimen as the source 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.

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.
Fluorochrome Setting	**Fluorochrome Setting, Sample**: The fluorochrome setting of target sample is replaced. **Specimen, Group, Experiment File**: The fluorochrome setting of all samples within the target node are replaced.
Reference Spectra	**Reference Spectra, Sample**: The Reference Spectra and Fluorochrome setting of target sample is replaced. **Specimen, Group, Experiment File**: The Reference Spectra and Fluorochrome setting of all samples within the target node are replaced. Note: If the photodetector gain value is different between the source Reference Spectra and the target sample, the Reference Spectra applied to target sample will be calculated automatically based on gain value of the target sample. The unmixing results may not be accurate if this gain difference is significant.
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

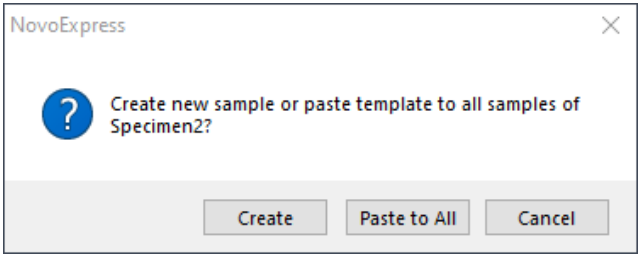
The template from source nodes can also be applied to target nodes by dragging and dropping, as shown below:



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.

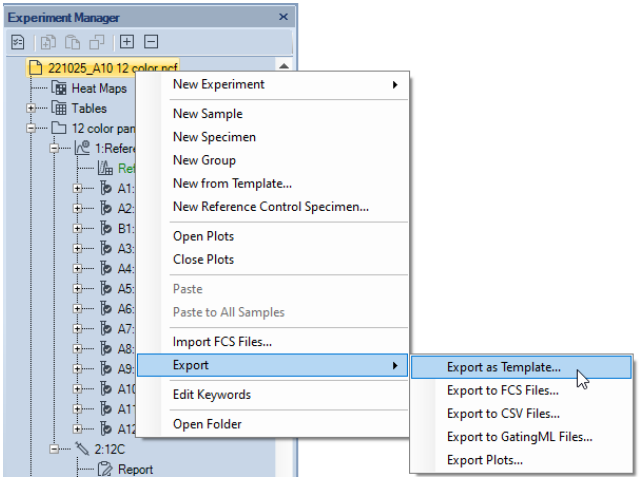
Use the Toolbar

In the **Experiment Manager** panel, select the source node to be copied. The node will be highlighted in yellow (e.g., 1: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.

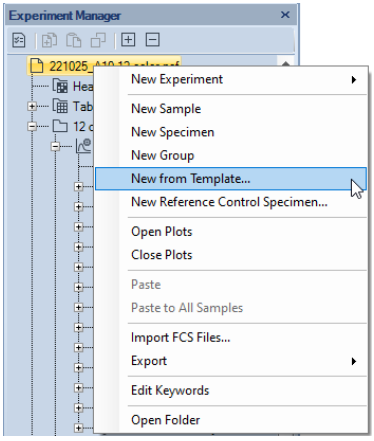


NOTE When exporting sample template or specimen template, only the **User-Defined Keywords** will be automatically included.

Import Template

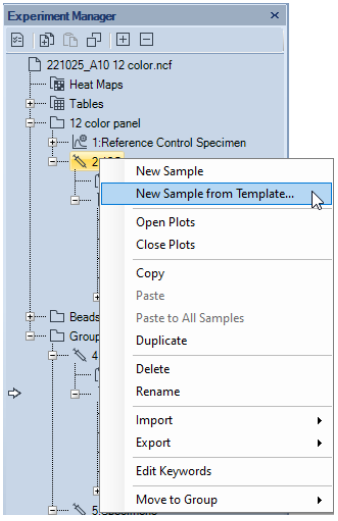
- Import to Experiment File

  If importing to an experiment file, right-click the experiment file node and select **New from Template…**.



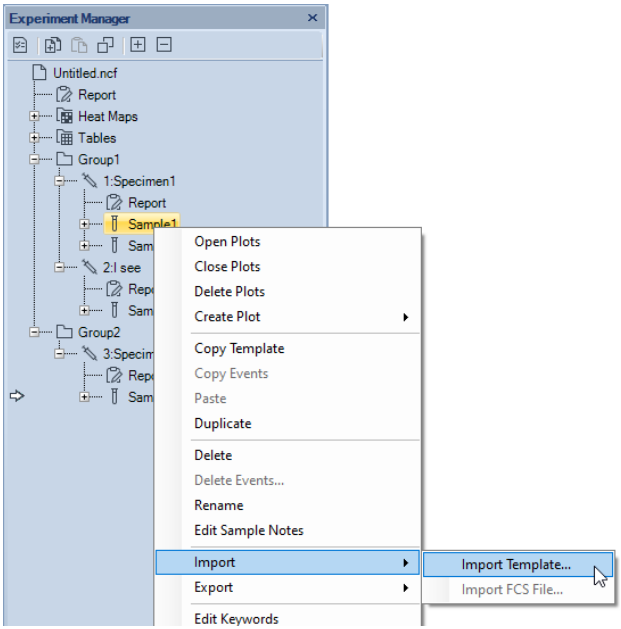
- Import to Specimen

  If importing to a specimen, right-click the specimen node and select **New Sample from Template…**.



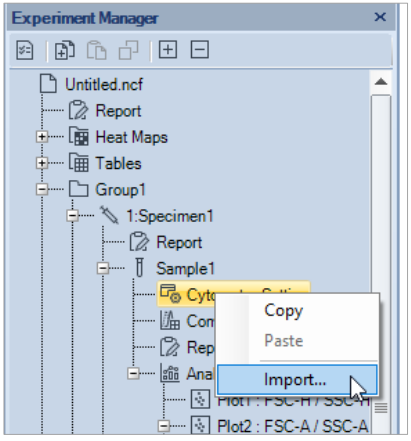
- Import to Sample

  If importing to a sample, right-click the sample node and select **Import**, then **Import Template…**.



- Import to **Cytometer Setting**, **Fluorochrome Setting, Reference Spectra, Report**, or **Analysis**

  If importing to **Cytometer Setting**, **Fluorochrome Setting, Reference Spectra**, **Report**, or **Analysis**, right-click the target node and select **Import**…



NOTE When importing sample template or specimen template, only the **User-Defined Keywords** will be automatically included.

Import and Export Data

The NovoExpress (Opteon) 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**.

NOTE

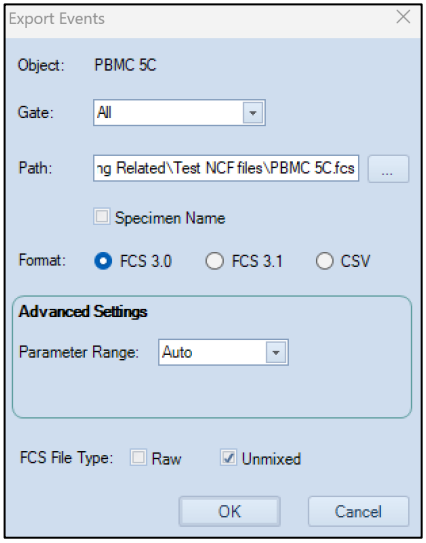
When importing a FCS file, all the **FCS Standard Keywords** will be automatically included. Other keywords (if any) will be imported as **User-Defined Keywords**. If the imported keyword has the same name as the existing keyword, software will automatically add **(1)** behind the imported keyword. For example, if the imported keyword is **ADT** which already exists, the software will import it as **ADT(1)**.

Export Data

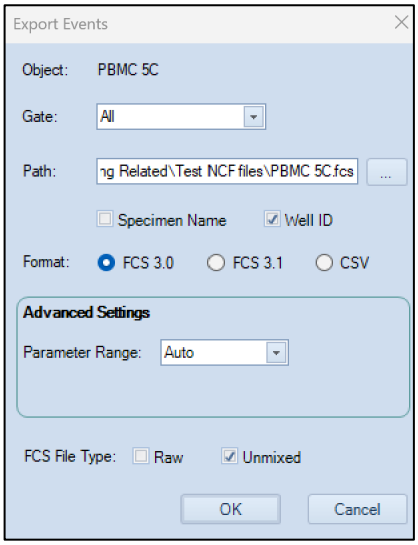
Select the sample, specimen, group, or experiment file node with data to be exported. Right-click the node and select **Export** > **Export to FCS Files⋯** or **Export to CSV Files⋯**. The **Export Events** window will open.

NOTE

When exporting data to FCS file, raw and unmixed data will be exported in separate FCS files. A suffix of **_unmixed** will be automatically added to the FCS file with unmixed data. When exporting data to CSV file, raw and unmixed data will be exported in one CSV file.



When NovoSampler S is not connected



When NovoSampler S 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.
- **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 S 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.
- **FCS File Type**: Allows user to check **Raw** to export the raw data, or check **Unmixed** to export the unmixed data, or check both to export both raw and unmixed data. A suffix of **Unmixed** will be automatically added to the end of the exported FCS file when user exports the unmixed data. For example, when user checks **Unmixed**, and clicks **OK**, the exported FCS file will be named as **Sample Name_unmixed.fcs**.

After setting the above options, click **OK** to begin exporting the data.

NOTE When exporting to FCS file, two FCS files will be exported. One FCS file consists of the data of the channel and fluorochrome parameters. Another FCS file (with _unmixed automatically included in the end of the file name) consists of the data of only the fluorochrome parameters.

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.

NOTE When exporting a FCS file, both **Statistical Keywords** and **User-Defined Keywords** will be exported automatically with no prefix added. However, the **FCS Standard Keywords** will be exported with prefix of **$** automatically added.

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 Reference Spectra and Report.

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**.

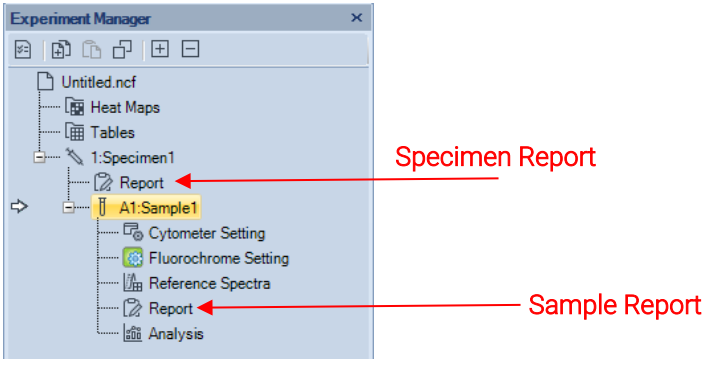
Reports can be created using an auto mode and a manual mode. In the auto mode, the software generates the report using a fixed format to include user created plots, statistics (**Gate, Count, % of Parent, Mean X and Mean Y**), and basic information (**Sample Name, Run Time, Cytometer, and Software**). 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.

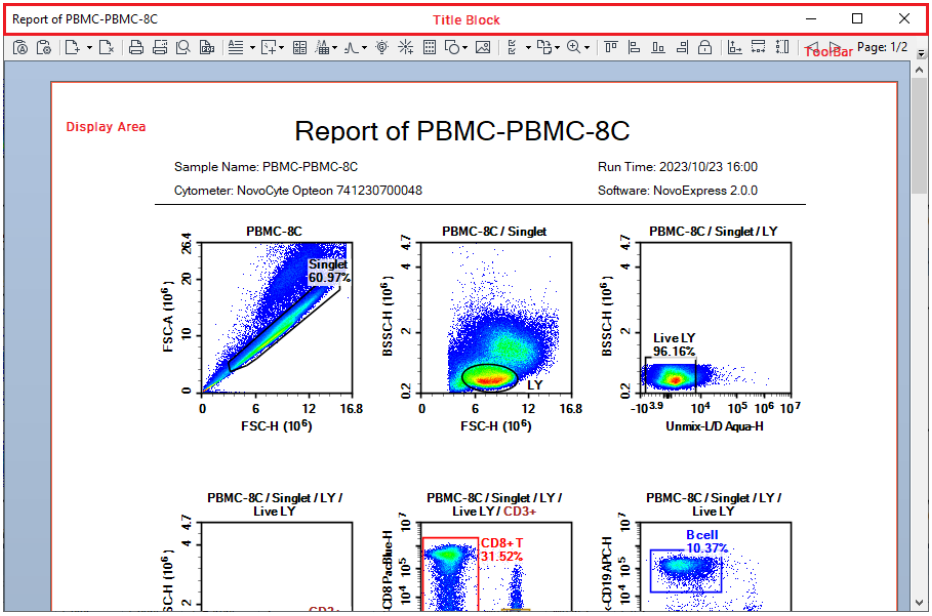
From the **Experiment Manager** panel, the report can either be a sample report or a specimen report. A sample report can contain plots, statistical information, compensation matrices, and collection information for the sample. A specimen report contains such information for all of its samples and basic 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.

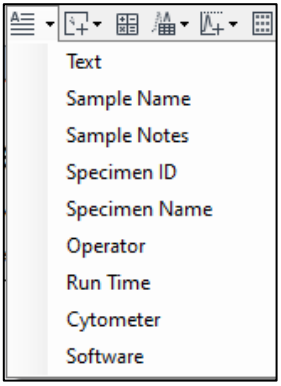
The title bar contains the name of the current report in the window.

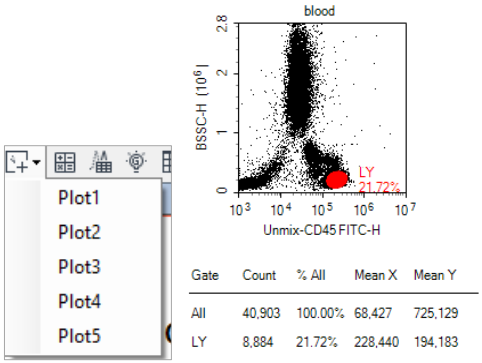
The toolbar contains the functions available to generate and edit the report.

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, Spillover Compensation, and plots.

The toolbar functions are described below:

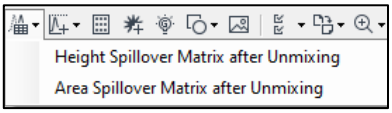
Icon	Description
	Auto Report Mode: Clicking on this button switches between the automatic report generation mode and the manual mode. When selected and in automatic

	mode, the icon appears with a blue border. When unselected and in manual mode, the icon appears without the blue border. 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 Options Settings in this guide 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 in this guide.
	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, software. 
	Insert Plot: Click the icon to list the plots for the sample, including dot plots, density plots, histogram plots, contour plots, spectrum density plot and cell cycle plots. Select the plot to insert. The plot is inserted with the statistics as shown below.

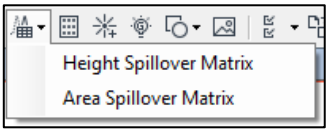


Insert Sample Statistics: Inserts a table of gate statistics for a sample. In a sample report, click the button to insert a gate statistics table for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a gate statistics table for the selected sample.

Insert Spillover Matrix: When user selects Spectral Unmix, clicking this button will prompt window below allowing user to Insert the Height or Area parameter-based Spillover Matrix after unmixing.

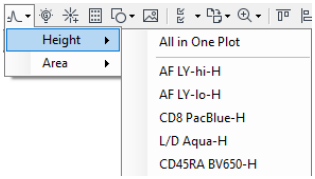
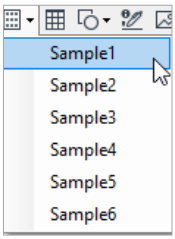


When user selects Compensation, clicking this button will prompt window below allowing user to Insert the Height or Area parameter-based Spillover Matrix.



In a sample report, click the button to insert the selected Spillover Matrix for the selected sample. In a specimen report, click the button and select a sample from the drop-down menu to insert the selected Spillover Matrix for the selected sample. The Spillover Matrix cannot be edited within the report. If the Spillover Matrix is modified in the main interface, it will be updated automatically in the report window.

Insert Reference Spectra: Inserts the Height or Area parameter-based Reference Spectra into the report. In a sample report, click the button to insert the selected Reference Spectra for the selected sample. In a specimen report, click the button and select a sample from the drop-down menu to insert the selected Reference Spectra for the selected sample. User can also select **All in One Plot** to insert all Reference Spectra of selected sample in one plot. This option is not available when user selects Compensation to analyze the data.

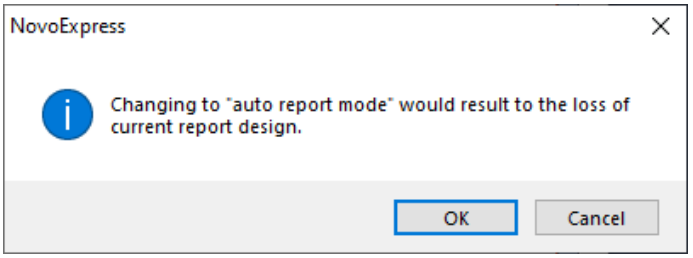
	
	Insert Photodetector Gain: Inserts a table of photodetector gain setting for a sample. In a sample report, click the button to insert a table of photodetector gain setting for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a table of photodetector gain setting for the selected sample.
	Insert Fluorochromes Information: Inserts a table of fluorochrome information for a sample. In a sample report, click the button to insert a table of fluorochrome information for the sample. In a specimen report, click the button and select a sample from the drop-down menu to insert a table of fluorochrome information 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 S is connected. 
	Insert Shape: Click to insert a horizontal line, vertical line, or rectangle.
	Insert Picture: Click to select a picture to insert in the prompted window. The picture will be resized to an appropriate size with the original aspect ratio and inserted in the report.
	Select: The default is **Select All**. From the arrow on the right of the button, **Select Similar Objects** is also available.
	Page Orientation: 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. 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.



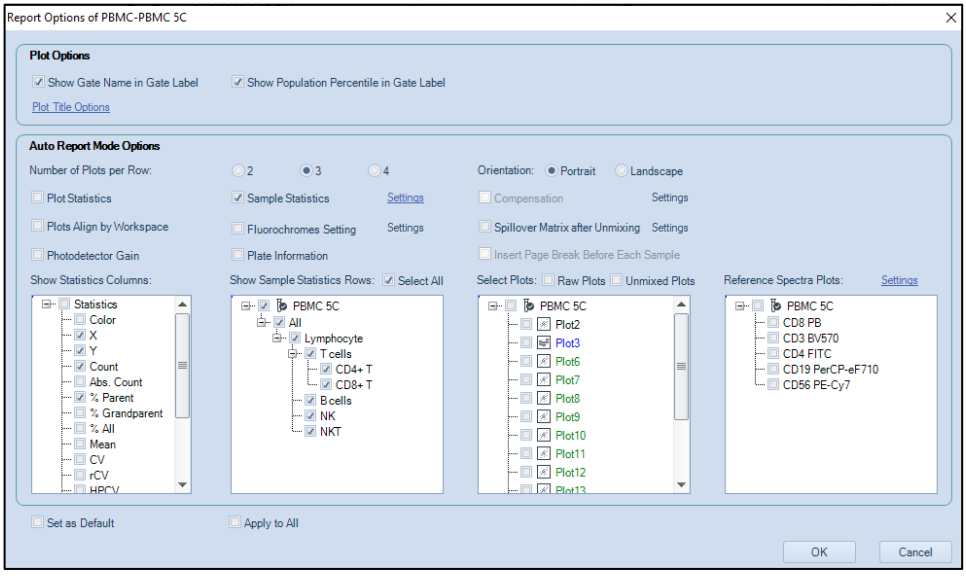
To switch the mode from automated to manual, click on **Auto Report Mode** button in the toolbar . The user can now make modifications to the report.

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:



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**:

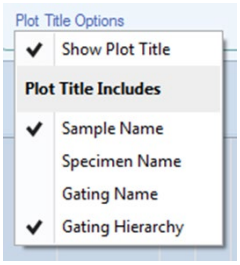
  If selected, gate name is displayed in gate label on the plot.

- **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 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.

- **Orientation**:

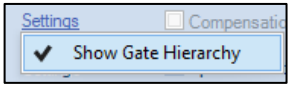
  Sets the page layout orientation to portrait or landscape.

- **Plot Statistics**:

  If selected, shows gate statistics of plot.

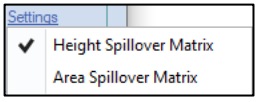
- **Sample Statistics**:

  If selected, shows gate statistics of sample and the **Settings** option on the right will become available. User can click it to select to show the gate hierarchy.



- **Compensation**:

  If selected, shows compensation matrix and the **Settings** option on the right will become available. User can click it to select to show the **Height Spillover Matrix** or **Area Spillover Matrix**. This setting will not be available if Spectral Unmix was used to deconvolute the data.



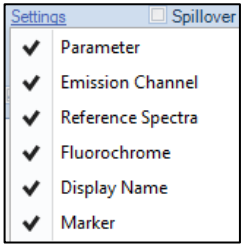
- **Plots Align by Workspace**:

  If selected, shows the plots in the order of fluorescence channel and fluorochromes. If not selected, shows the plots in the order defined in **Experiment Manager**.

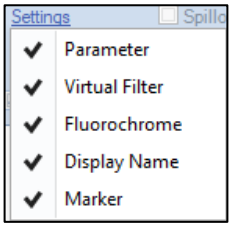
- **Fluorochromes Setting**:

  If selected, shows the fluorochrome settings, and the **Settings** option on the right will become available.

  When Spectral Unmix was used to deconvolute the data, clicking **Settings** allows user to select to show the **Parameter, Emission Channel, Reference Spectra, Fluorochrome, Display Name, Marker** information.

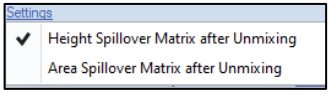


  When Compensation was used for data analysis, clicking **Settings** allows user to select to show **Parameter, Virtual Filter, Fluorochrome, Display Name, Marker** information.



- **Spillover Matrix after Unmixing**:

  If selected, shows Spillover Matrix after unmixing and the **Settings** option on the right will become available. User can click it to show the **Height Spillover Matrix after Unmixing** or **Area Spillover Matrix after Unmixing**. This setting will not be available if Compensation was used for data analysis.



- **Photodetector Gain**:

  If selected, shows the photodetector gain setting.

- **Plate Information**:

  If selected, shows the plate information. This option is available only when NovoSampler S 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 All**:

  Selects all associated items to display on report.

- **Select Plots**:

  Selects plots to display on report.

- **Raw Plots**:

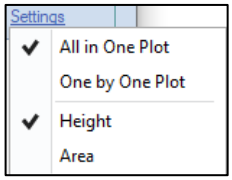
  Check to display the plots from **Raw Workspace**.

- **Unmixed Plots**:

Check to display the plots from **Unmixed Workspace**.

- **Reference Spectra Plots**:

  Shows Reference Spectra plot on the report. User can click the **Settings** option on the right to select to show all the Reference Spectra in one plot by checking **All in One Plot,** or show each Reference Spectra individually by checking **One by One plot, and** select either **Height** or **Area** parameter is used when plotting the Reference Spectra. This setting will not be available if Compensation was used for data analysis.



- **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

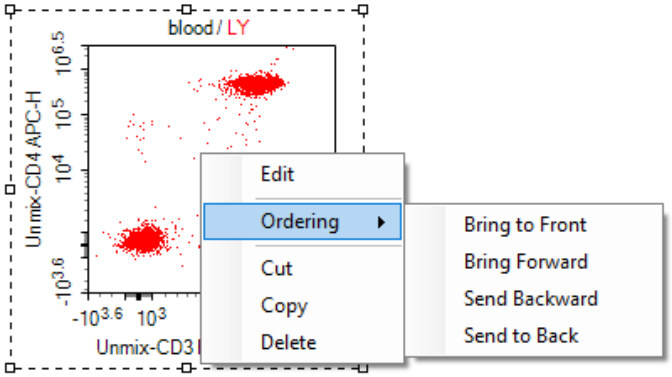
Objects can be added to the report through the toolbar, Workspace, and **Experiment Manager** panel. To add objects using the toolbar, use the insert functions described in Report Interface. From both the Workspace and **Experiment Manager** panel, objects corresponding to the sample can be dragged and dropped into the report.

Select Report Objects

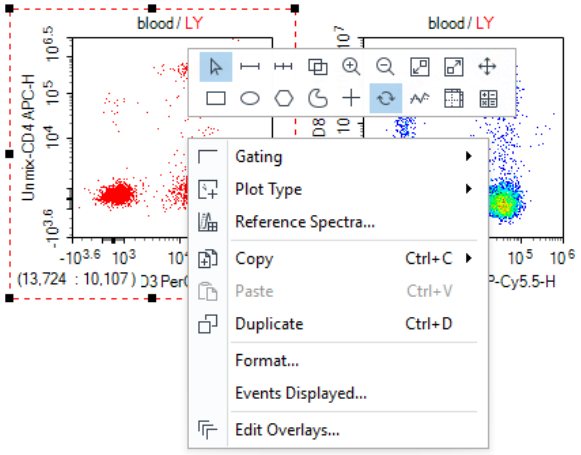
Click on an object to select it in the report. An object can have one of two selected states. After clicking on an object, the object will be highlighted. In this state, the object is bordered by a black dashed line with white control points. If the object is

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:



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.

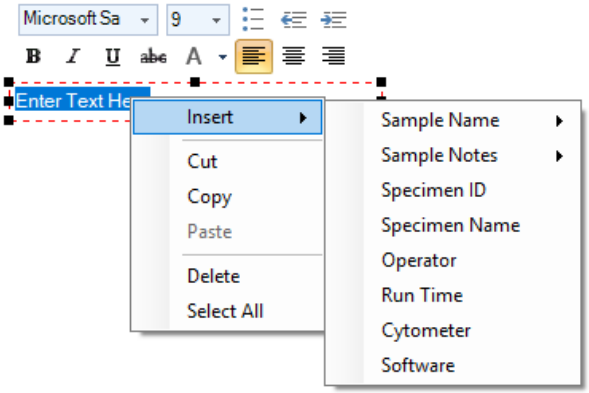
When multiple items are selected, the first item is bordered by a black dashed line with white control points and additional items are bordered by black dashed lines with black control points.

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

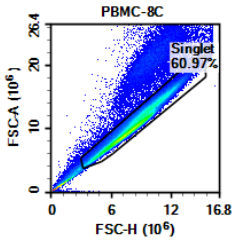
Double-click on the textbox or right-click on the textbox and select **Edit** to enter editing mode. In this mode, text formatting tools will appear. Right-click on the textbox and select **Insert** to insert sample information including sample name, sample notes, specimen ID, specimen name, operator, 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.

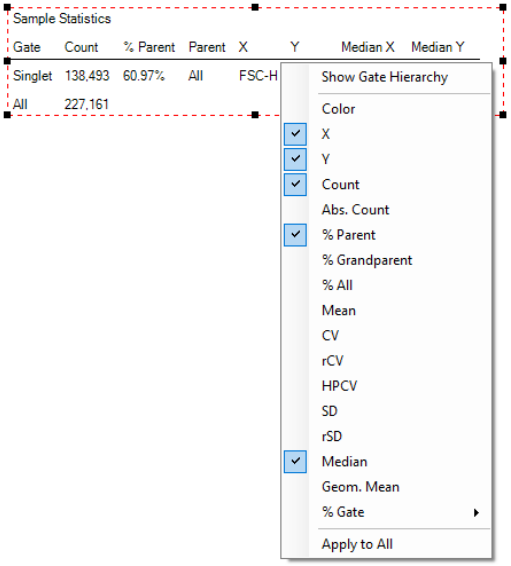


Edit Plots and Statistics

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.

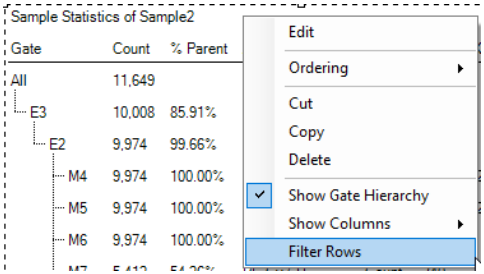
Double-click on the statistics box or right-click on the statistics box and select **Edit** to enter editing mode. Right-click in the selected statistics box to choose the columns to display, as shown below.



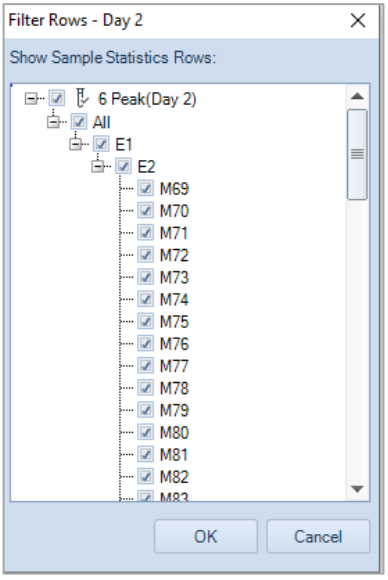


Filter Statistics

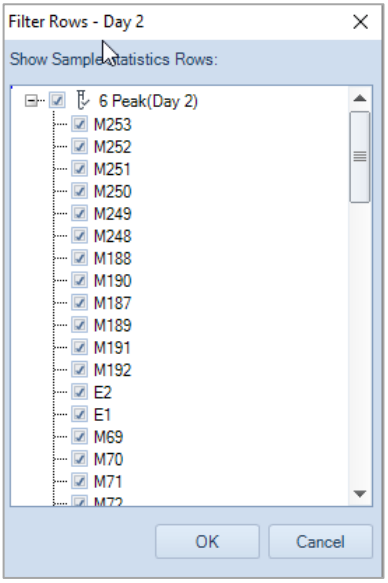
User can right click the statistics box of the report, click **Filter Rows** to open the **Filter Rows** window. Check to select the gate(s) to display associated sample statistical information in the report and click **OK** to confirm.



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.

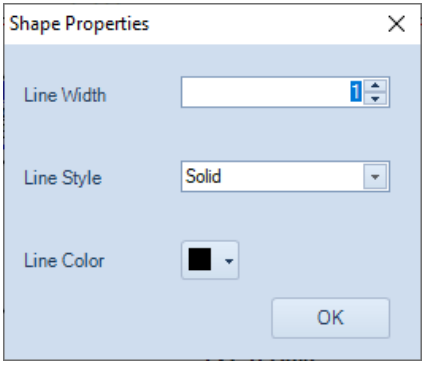


When the statistics box does not show the gate hierarchy, the **Filter Rows** window does not display the gate hierarchy either.



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 ender 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.

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.

- 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.

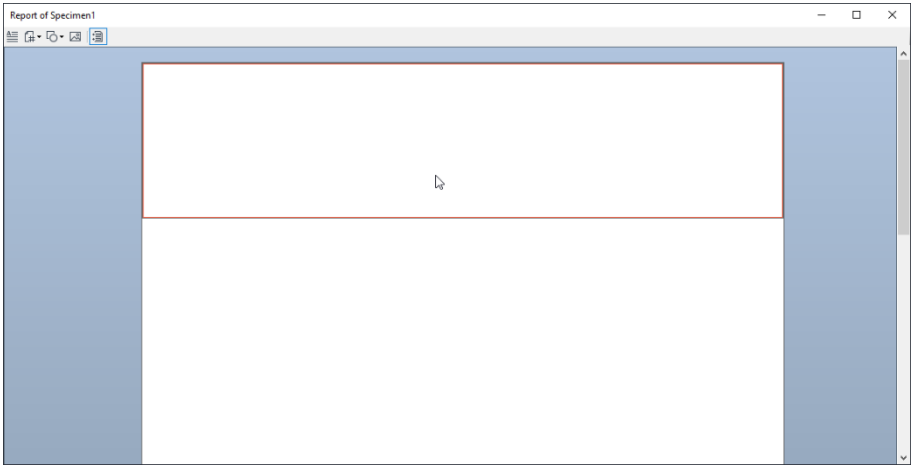
Insert or Delete Pages

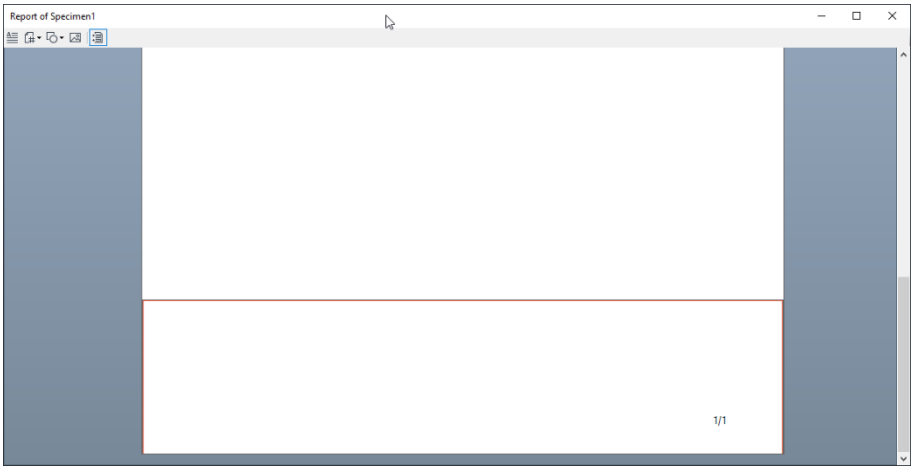
To insert or delete pages, use the **Insert Page** and **Delete Page** button from the toolbar. For more information, Refer to Report Interface in this guide.

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.





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.

- 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.

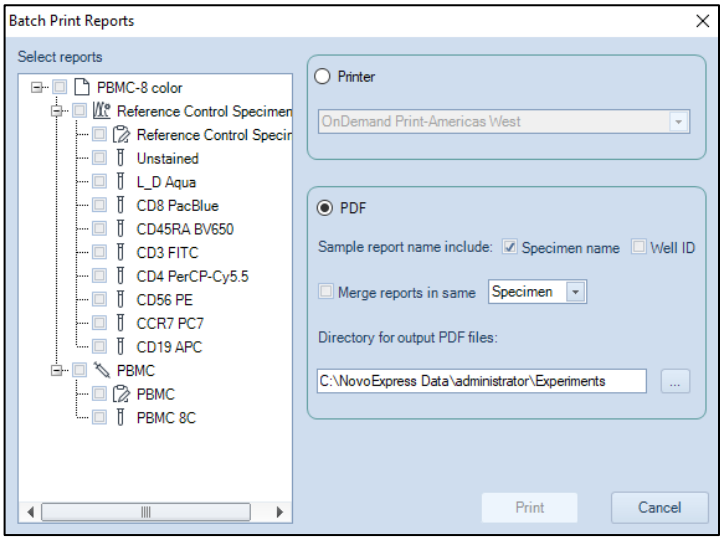
- To Convert to PDF:

  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.



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.

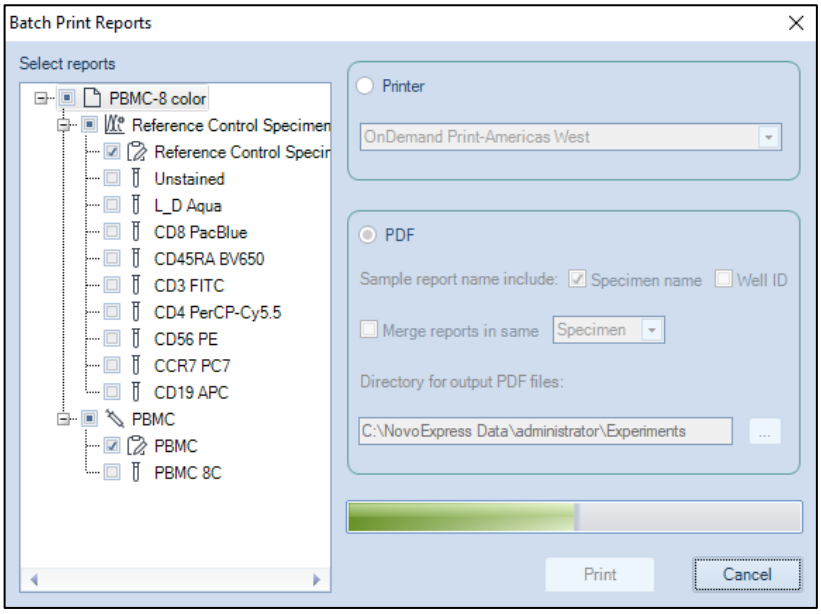
If printing, select a printer and click **Print**.

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.



During the printing process, click **Cancel** to stop the printing.

9 QC Test

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

The NovoExpress (Opteon) software **QC Test** function automatically conducts the QC procedure using Instrument QC particles. Click **Instrument** > **QC Test** from the NovoExpress (Opteon) 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 (Opteon) software.

Instrument 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.

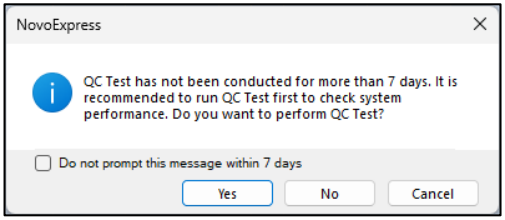
The QC Test procedure generally includes following steps:

- Prepare QC particles sample.
- Fill in Test Information
- Electronic Noise Test
- Optical Noise Test

- Calibrate Time Delay
- Adjust Photodetector Gain
- Run QC test.
- System Test (Optional)
- View QC reports and Levey-Jennings plot

NOTE

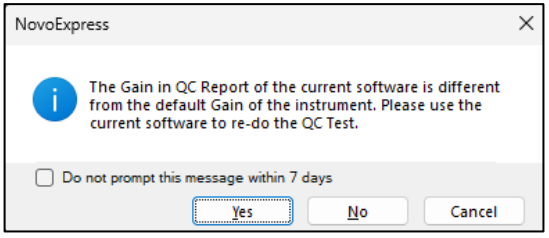
It is highly recommended to run the QC test on daily basis. Under the situation when user has not performed the QC test in the past seven days, the following window will be prompted when user starts sample collection for the first time each day.



User can click **Yes** to proceed to perform the QC test or click **No** to skip the QC test. When **Do not prompt this message within 7 days** is checked, the software will not prompt this window in the next 7 days.

NOTE

Under the situation when the default gain recorded in the instrument is different from the one recorded in latest QC report, following window will be prompted when user starts sample collection for the first time each day.



User can click **Yes** to proceed to re-do the QC test or click **No** to skip the QC test. When **Do not prompt this message within 7 days** is checked, the software will not prompt this window in the next 7 days.

NOTE The software prompted windows displayed in this chapter is for illustration purpose only. User may observe different windows depending on the Instrument 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.

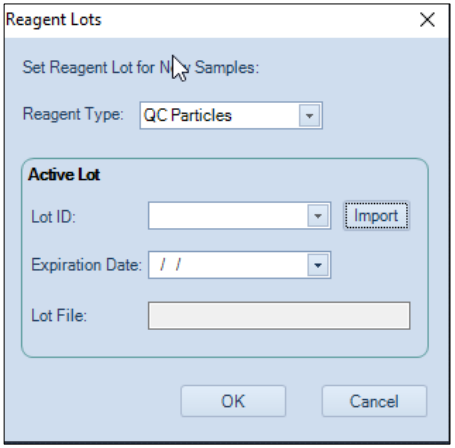
5 Place the tube onto the instrument and start the QC test. If NovoSampler S is used, place the tube to the A1 well of a 40 tube rack (12X75 mm tubes) and run the QC Test immediately. If the prepared QC particle sample is not intended to be run immediately, store the sample in a 2-6 ℃ refrigerator away 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 during the QC Test. If user selects to perform system test, ensure to prepare at least 1 mL of QC particle sample.

Run QC Test

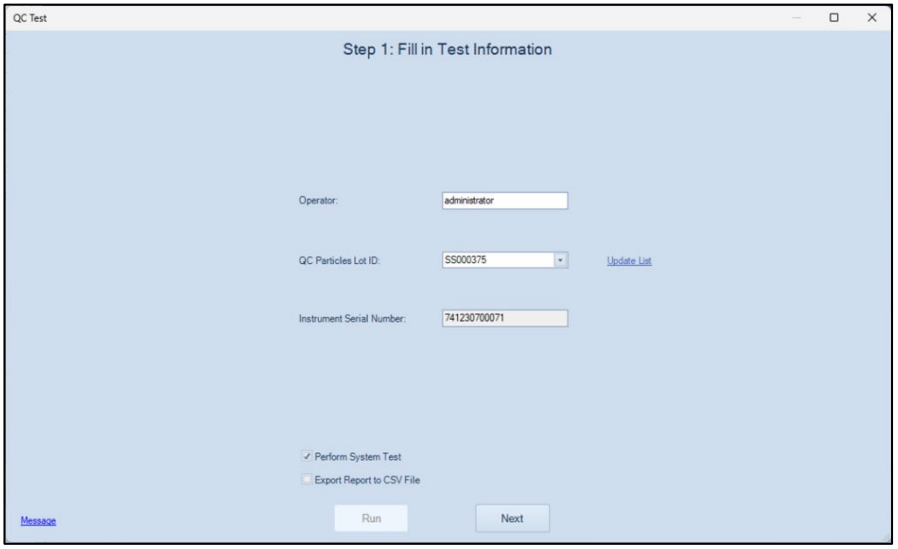
1 Import the Instrument QC Particles Lot file.

Download the Lot File for the specific batch of QC Particles from https://www.agilent.com/en/support/qc-particles-lot-file. Launch and log into NovoExpress (Opteon) software, click **Setting > Experiment > Reagent Lots** to open **Reagent Lots** window, click **Import** to import the lot file. The **Lot ID** will be automatically listed after importing the lot file. Click **OK** to continue.

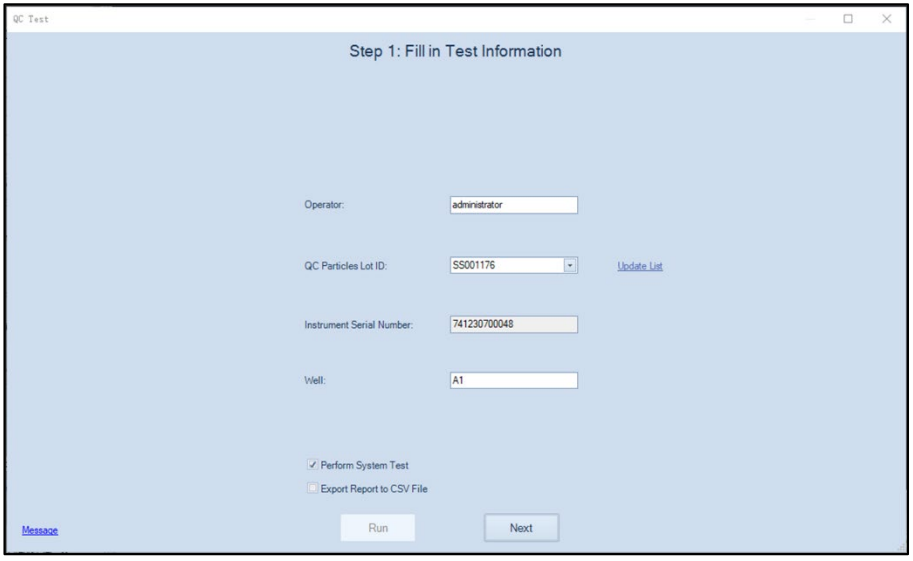


2 Ensure the instrument is in **Ready** status. If NovoSampler S is used, open the cover of NovoSampler S and place the sample plate on the tray. Ensure the plate is fully seated with the correct orientation onto the shaker.

3 In the NovoExpress (Opteon) 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.



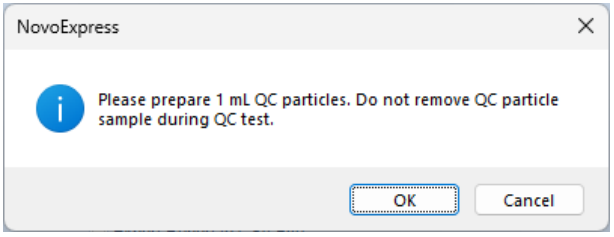
NovoExpress (Opteon) QC Test Window when NovoSampler S is not connected



NovoExpress (Opteon) QC Test Window when NovoSampler S is connected

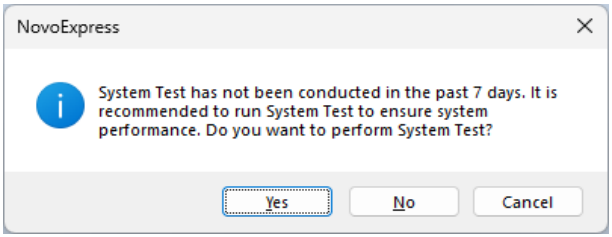
In the window, enter the name of the operator, select the **QC particles lot ID**, select the **Well ID** (if NovoSampler S is used) and click **Next**. If the correct QC particles lot ID is not listed, please refer to the first step to download and save the QC particles Lot File.

User can check **Perform System Test** to perform system test after QC test is completed. The software will prompt the message below after user clicks **Next**. User needs to ensure 1 mL of QC particle sample is prepared and then click **OK** to continue.



NOTE

System test is optional but recommended to ensure optimized data analysis results. If user unchecks **Perform System Test**, and the last time the user performed this test was more than 7 days ago, the software will prompt the message below after user clicks **Next**. User can click **Yes** to perform system test or click **No** to skip the system test or click **Cancel** to keep current selection and close this message window.

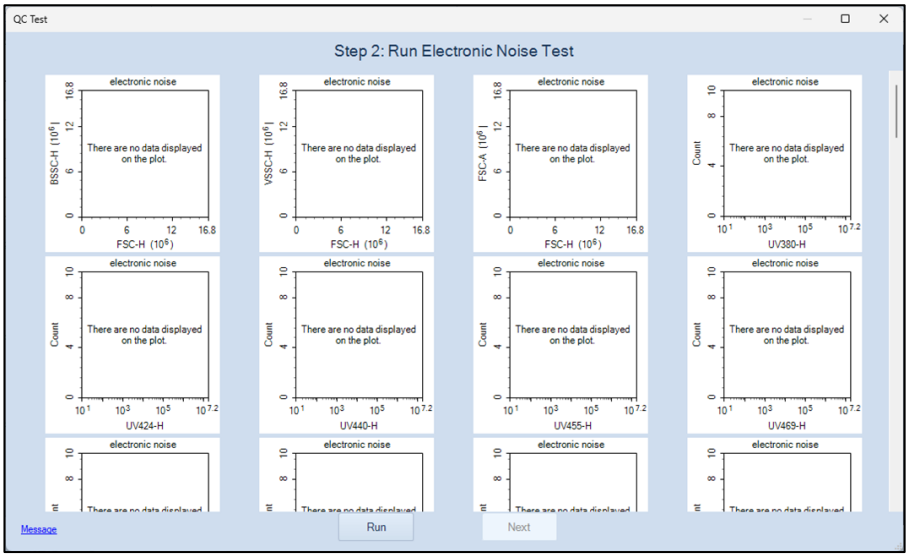


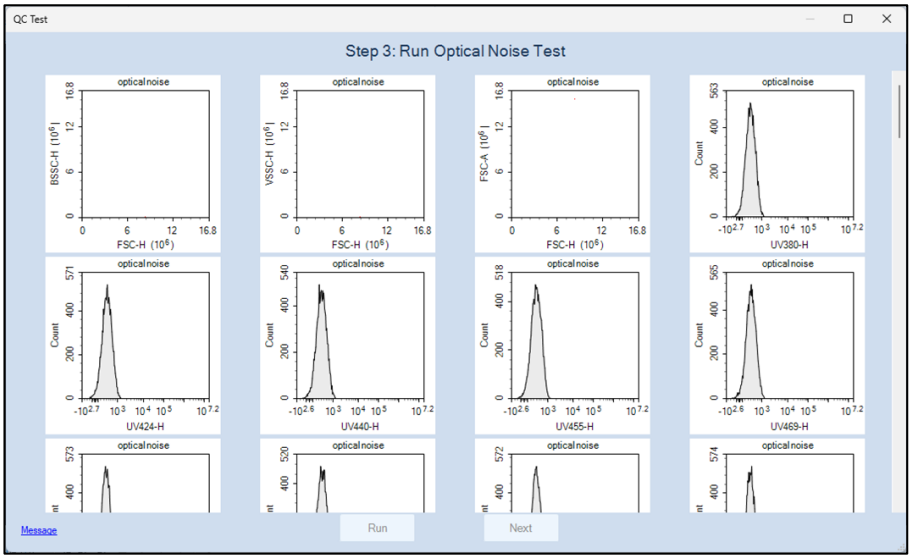
User can check **Export Report to CSV File** to export report as a csv file after QC test is completed. The exported CSV file (in the format of YYMMDD_hhmmss.csv) is automatically saved in **C:\Program Files (x86)\NovoExpress (Opteon)\Config\QC \QC Report Files** by default.

NOTE

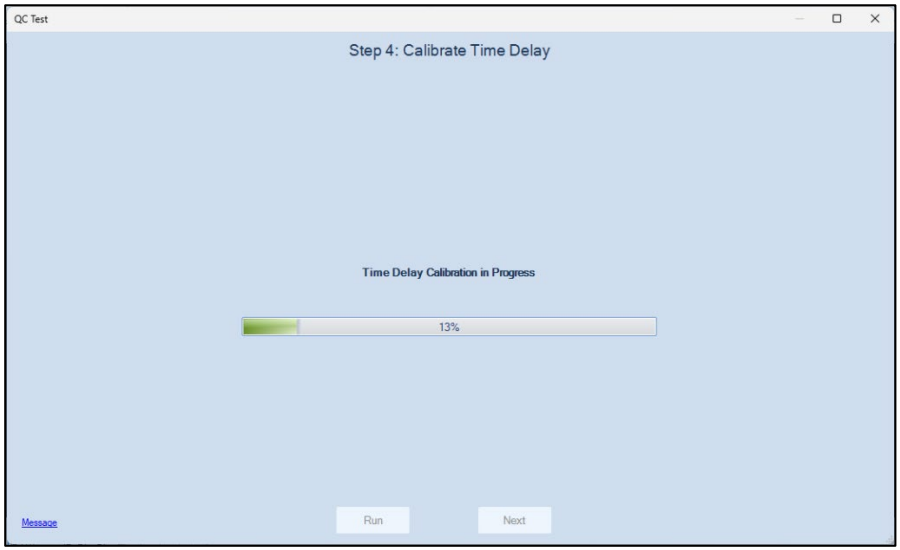
The default name of the exported CSV file is in the format of QC test date and time. For example, if the QC test is performed at 10:30:40 am on January 18, 2019, the default name of the exported CSV file will be 190118_103040.csv.

4 Click **Run** to begin the test. The software will collect electronic and optical noise (see figure below) before collecting the events. The results will be displayed in real-time in following windows.

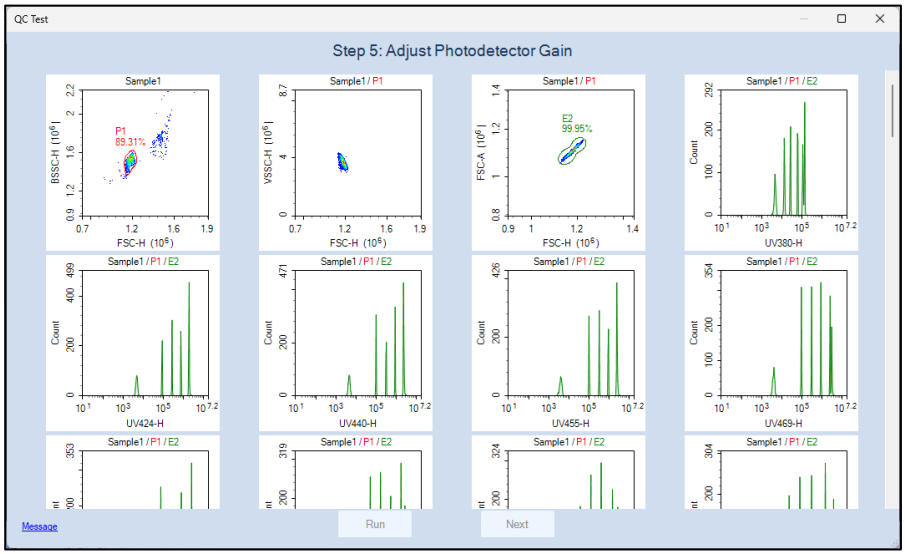




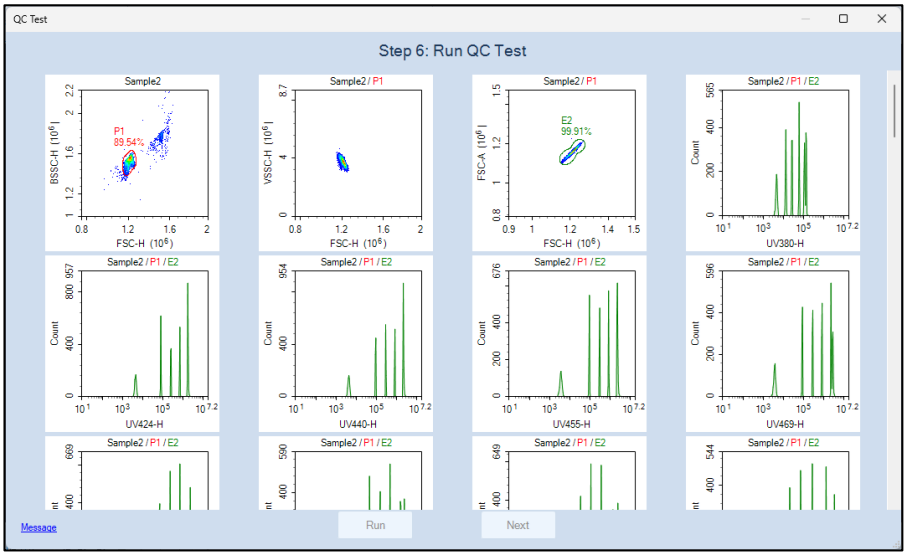
5 After electronic and optical noise are collected, the software will automatically proceed to calibrate time delay. After the calibration completes, the result (i.e., **Pass** or **Fail**) will be displayed in the QC test report.



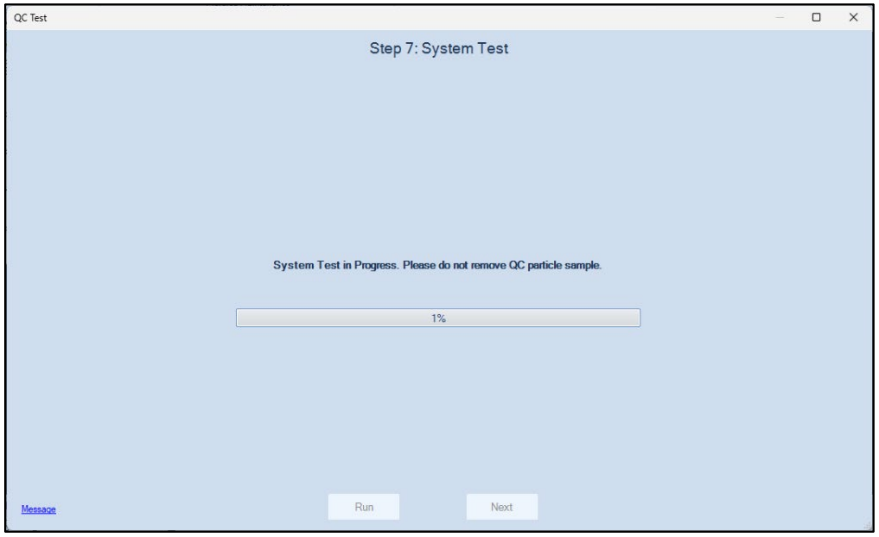
6 After time delay is calibrated, the software will automatically proceed to adjust photodetector gain if the time delay between 488 nm laser and at least one of other laser are within the target range.



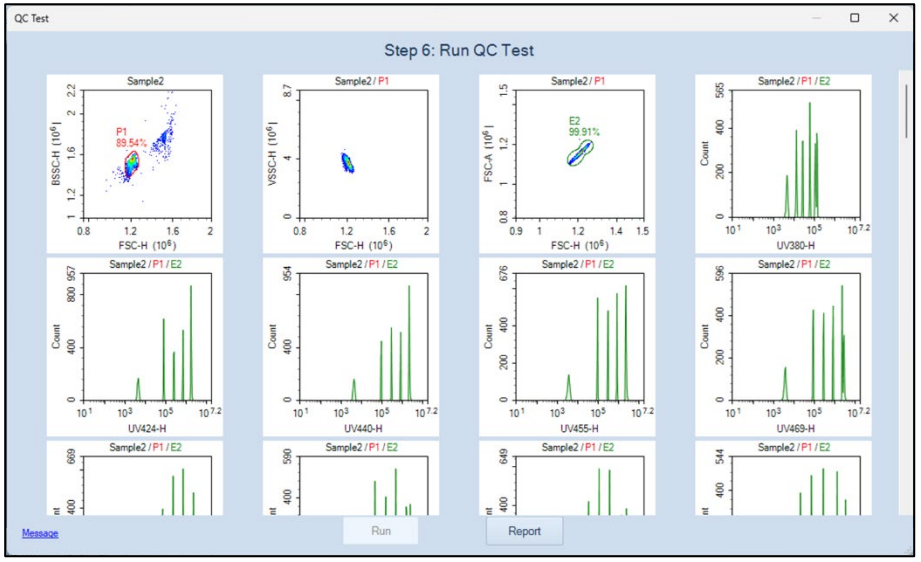
7 After photodetector gain is adjusted, the software will automatically proceed to collect data from the prepared QC particle sample. The results will be displayed in real-time in following window.



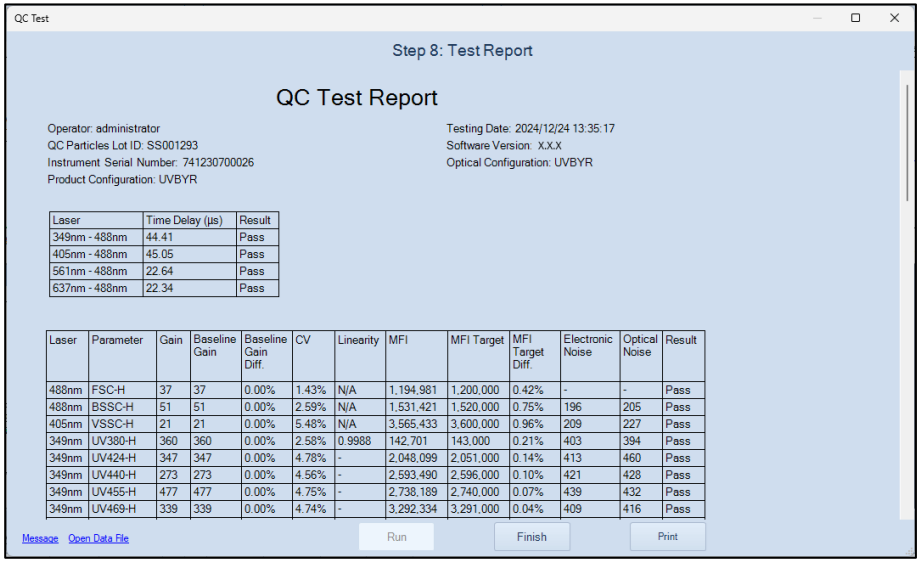
8 After data collection is complete, if user checked **Perform System Test**, the software will automatically proceed to perform the system test as shown below and display the QC result in **QC Test Report** window afterwards.



If user did not check **Perform System Test**, the software will automatically skip the system test and prompt window below. User needs to click the **Report** button to open the **QC Test Report** window to view the test results.



9 From the **QC Test Report** window, user can view the QC test results for various parameters (e.g., Time Delay, CV, MFI, Result). User can click **Message** to view the QC test related messages or click **Open Data File** to open the associated experiment file software automatically created or click **Finish** to close this window or click **Print** to print the test report.



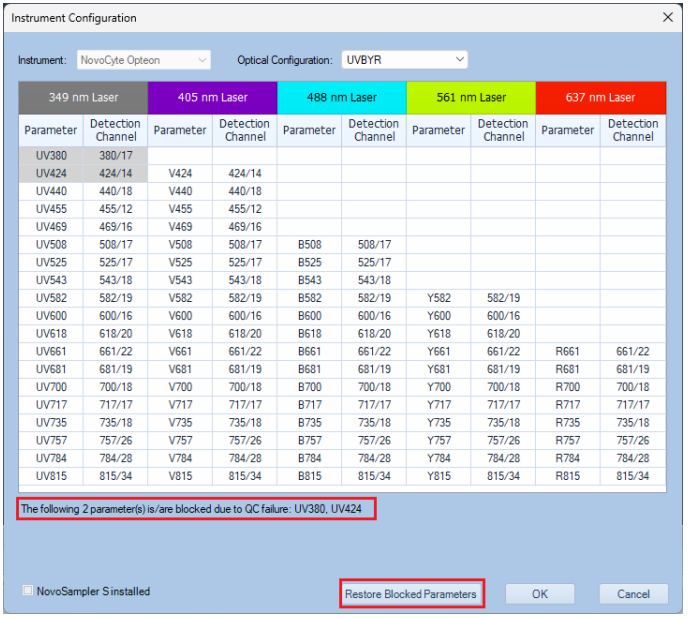
NOTE

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.

NOTE

Under the situation when the QC test reports show **Failed** for any of the channels, if user closes the QC test report window, the following dialogue window will be prompted.

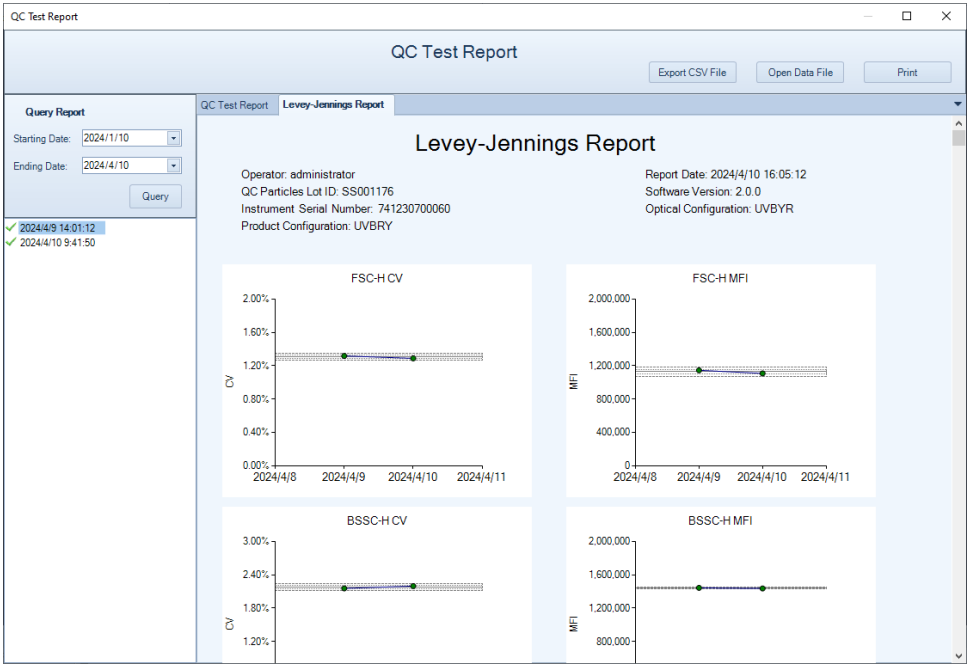
After user clicks **Yes,** these channels will not be displayed in the **Parameter** panel, and the **Instrument Configuration** window will display these channels in grey color with message at the lower side as shown below. The blocked channels will be excluded from data collection and unmixing.



To restore these channels (i.e., include them in the next sample data collection), user can open the **Instrument Configuration** window, and click **Restore Blocked Parameters**, then click **OK** in the prompted window. The software will be automatically restarted to make the changes effective.

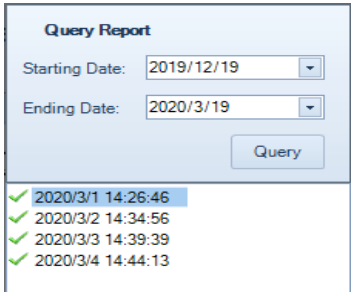
View QC Test Report

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:



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: administrator
QC Particles Lot ID: SS000375
Instrument Serial Number: 741230700071
Product Configuration: UVBYR

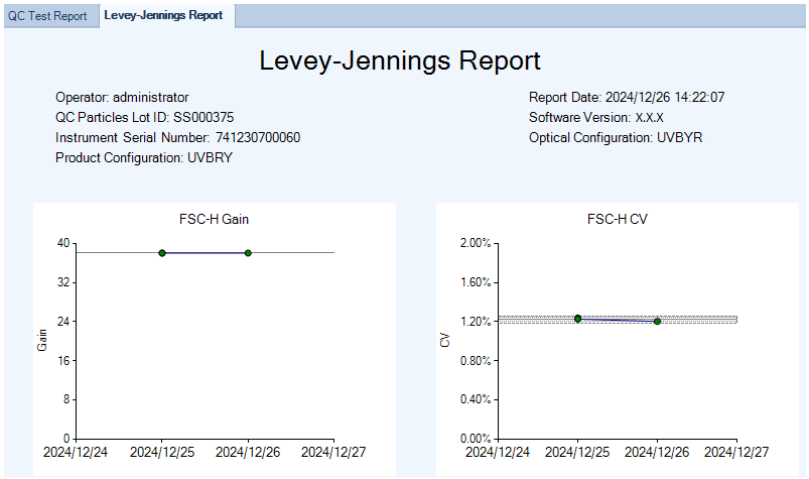
Testing Date: 2024/9/26 15:35:43
Software Version: X.X.X
Optical Configuration: UVBYR

Laser	Time Delay (µs)	Result
349nm - 488nm	44.73	Pass
405nm - 488nm	45.08	Pass
561nm - 488nm	22.45	Pass
637nm - 488nm	22.60	Pass

Laser	Parameter	Gain	Baseline Gain	Baseline Gain Diff.	CV	Linearity	MFI	MFI Target	MFI Target Diff.	Electronic Noise	Optical Noise	Result
488nm	FSC-H	34	33	3.03%	1.58%	N/A	1,213,829	1,200,000	1.15%	-	-	Pass
488nm	BSSC-H	52	52	0.00%	2.34%	N/A	1,531,084	1,520,000	0.73%	197	185	Pass
405nm	VSSC-H	18	18	0.00%	6.51%	N/A	3,727,163	3,600,000	3.53%	238	253	Pass
349nm	UV380-H	391	397	1.51%	2.14%	0.9997	137,443	137,000	0.32%	414	415	Pass
349nm	UV424-H	346	380	8.95%	4.52%	-	1,794,409	1,792,000	0.13%	461	449	Pass
349nm	UV440-H	268	291	7.90%	4.29%	-	2,293,923	2,289,000	0.22%	387	414	Pass
349nm	UV455-H	509	552	7.79%	4.32%	-	2,427,400	2,425,000	0.10%	400	411	Pass
349nm	UV469-H	290	317	8.52%	4.41%	-	2,896,606	2,892,000	0.16%	408	430	Pass
349nm	UV508-H	514	568	9.51%	4.58%	-	3,600,772	3,596,000	0.13%	441	447	Pass
349nm	UV525-H	482	535	9.91%	4.56%	-	2,533,219	2,530,000	0.13%	439	461	Pass
349nm	UV543-H	428	478	10.46%	4.48%	-	2,513,706	2,507,000	0.27%	425	447	Pass
349nm	UV582-H	565	631	10.46%	4.32%	-	3,567,657	3,562,000	0.16%	395	406	Pass
349nm	UV600-H	631	693	8.95%	4.14%	-	3,645,678	3,637,000	0.24%	440	476	Pass
349nm	UV618-H	519	565	8.14%	4.12%	-	3,870,301	3,873,000	0.07%	438	437	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.

10 Troubleshoot

This chapter provides the information for troubleshooting 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 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 S.	Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps.
		NovoSampler S is not calibrated.	Re-calibrate NovoSampler S.
		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 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.	• Calibrate Fluidics Station S (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.	• Calibrate Fluidics Station S (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.	• Calibrate Fluidics Station S (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.	• Calibrate Fluidics Station S (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.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x0007	**System electric current is out of range**	System Error.	• Restart the instrument. • Contact Agilent technical support if the error persists.

0x0008	**Firmware configuration error**	System Error.	• 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.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x000F	**NovoSampler communication lost**	The cable between the NovoSampler S and instrument is not securely connected.	Reconnect the cable between the NovoSampler S and instrument.
		NovoSampler S is not communicating with instrument.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x0010	**NovoSampler has not been calibrated**	NovoSampler S is newly installed or re-connected.	Follow the prompted instructions to calibrate the NovoSampler S.
0x0011	**NovoSampler calibration failed……**	NovoSampler S is not installed properly.	Re-install and calibrate the NovoSampler S following the procedures described in Installation in associated operator's guide. Ensure the NovoSampler S is seated flat on the stage and tightly close to the instrument.
		NovoSampler S cover is opened during calibration.	Close the NovoSampler S cover and redo the calibration.
		The optocoupler or motor of NovoSampler S is not working properly.	• Restart the instrument and redo the calibration. • Contact Agilent technical support if the error persists.
0x0012	**The movement of plate is out of range**	The movement of the orbital shaker is blocked.	• Check the path of the orbital shaker to make sure there are no objects blocking the movement. Clear the block if there is anything. • Re-calibrate the NovoSampler S.
		NovoSampler S is not installed properly.	Re-install and calibrate the NovoSampler S following the

			procedures described in Installation in associated operator's guide.
0x0013	**Cover of NovoSampler is opened during moving plate**	The cover of NovoSampler S is open during moving plate. Or the cover is not closed well.	Close the cover. NovoSampler S 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 (Opteon) to clear the error. If instrument 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 (Opteon) to clear the error. If instrument is connected, replace the sheath in-line filter following the procedures described in Replacing Fluidic System Consumables in instrument operator's guide.
		Sample injection probe or flow cell is clogged.	Follow the prompted instructions from NovoExpress (Opteon) to clear the error.
0x0015	**NovoSampler firmware error**	NovoSampler S firmware is not working properly.	Re-install or upgrade the NovoSampler S firmware.
0x0016 0x0017 0x0018 0x400B 0X400F	**…… laser does not emit**	Specified laser is not connected properly, or laser is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x0019 0x001A 0x001B 0x400C 0x4010	**…… laser communication error**	Specified laser is not communicating with the instrument properly or the laser is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x001C	**Sample injection probe reset failed**	Bad connection or optocoupler is not working properly.	• 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.	• 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.	• 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.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x0023	**Resetting NovoSampler to home position failed**	NovoSampler S is not working properly.	• Click **Instrument** → **NovoSampler S** → **Reset** in NovoExpress (Opteon) software to reset NovoSampler S. • Restart the NovoSampler S.
0x0100	**Instrument cover opened**	Instrument cover is opened or not tightly closed.	Close the instrument cover.
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.	• Calibrate Fluidics Station S (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.	• Calibrate Fluidics Station S (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.	• Calibrate Fluidics Station S (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.	• Calibrate Fluidics Station S (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 S is opened. Or the cover is not closed well.	Close the cover.
0x0106	**NovoSampler is disconnected when powered up**	The NovoSampler S is disconnected when powered up	• Shut down instrument. • Reconnect the cable of the NovoSampler S to instrument. • Turn on instrument and follow the prompts to calibrate the NovoSampler S. • Contact Agilent technical support if the error persists.
0x0109	**Fluidics Station is not connected**	Cable between the fluidics station S and the instrument is not properly connected.	• Power down the instrument. • Reconnect the cable between the fluidics station S 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 S (or Cart) is not working properly.	• Reconnect the fluidics station S (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**	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.

	run the Priming procedure		
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 S is not working properly.	No action is needed. The NovoSampler S 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.
0x0113	**The sample tube compartment door is open**	The sample compartment door is not closed properly or the door sensor is not working properly.	• Close the door. • Contact Agilent technical support if the error persists
0x1001	**Fluidics procedure run error**	Fluidics procedure file is damaged.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x300C	**Data Communication error between main board and AD board**	AD Board or Main Board is not working properly	• Restart the instrument. • Contact Agilent technical support if the error persists
0x300D	**Photodetector module communication error**	Photodetector module is not working properly or not properly connected to the AD board.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x3102	**Failed to read photodetector information**	Photodetector sensor is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x3106	**Photodetector module not detected**	Photodetector module is not working properly or not properly connected to the AD board.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x6100	**Communication error between NovoSampler and the orbital shaker**	The orbital shaker is not communicating to NovoSampler S is not working properly.	• Restart NovoSampler S through NovoExpress (Opteon). • Restart the instrument. • Contact Agilent technical support if the error persists.
0x7000	**Fluidics cart firmware error**	NovoCyte Fluidics Cart firmware is not working properly.	• Restart the instrument. • Replace the NovoCyte Fluidics Cart. • Contact Agilent technical support if the error persists.

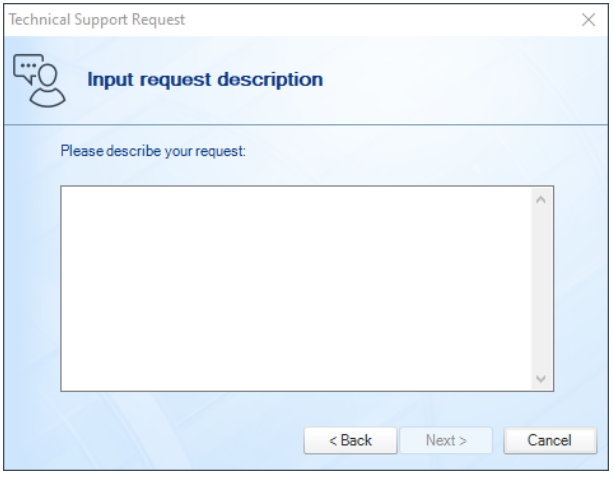
0x7001	**NovoFlow liquid level sensor is not connected**	NovoFlow liquid level sensor is not connected or does not work properly.	• Reconnect the NovoFlow liquid level sensor. • Contact Agilent technical support if the error remains after reconnection. The sensor may be damaged and need to be replaced.
0x7002	**Waste liquid level sensor is not connected**	Waste liquid level sensor is not connected or does not work properly.	• 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.	• 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.
0xA020	**Laser board firmware error**	Laser board firmware is not working properly.	• Re-install or upgrade the laser board firmware.
0xA021	**Laser board communication lost**	Laser board is not properly connected to the instrument.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0xA100	**The temperature of the Photodetector module is abnormal and cannot work normally**	Photodetector module is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
	Communication error (code: xx, xx). Please restart Instrument and NovoExpress).	USB cable between the instrument and workstation is not connected properly.	• Reconnect the USB cable between the instrument and the workstation. • Restart the instrument, workstation and NovoExpress (Opteon) software. • Contact Agilent technical support if the error persists.

Technical Support Request

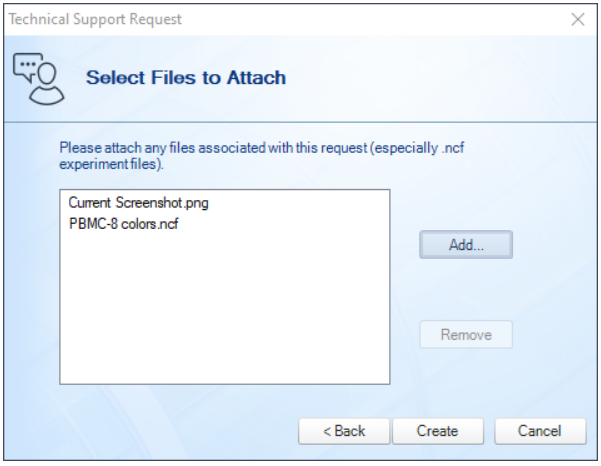
In case you need to contact Agilent technical supports, use the **Technical Support Request** from **Home** menu to create a request. **Technical Support Request Creation Wizard** automatically collects instrument configurations, NovoExpress (Opteon) system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of NovoCyte Opteon Spectral 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.

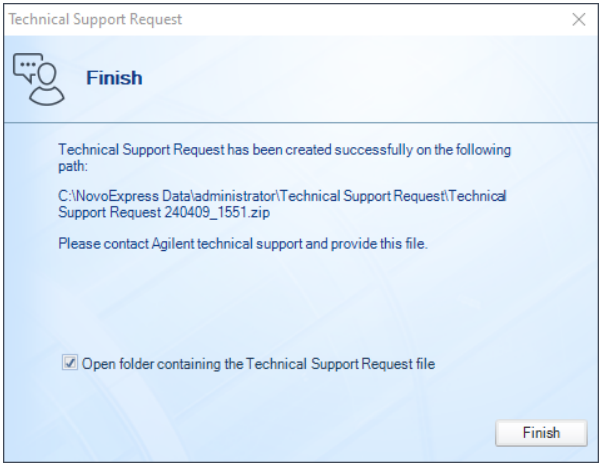


3 Click **Next** button and select the files to be attached. Any related files including experiment files (*. ncf) can be attached.



4 Click **Create** button to start creating technical support request.

5 Click **Finish**.



After the request creating process is completed, send the created request files to Agilent technical support.

11 Version History

Date	Changed by	Version/Description
05/01/2024		Edition 1.00: Initial release.
07/22/2024		Edition 1.01: Added Distance Filter function in Option Setting and Sample tab in software main window, revised QC procedure with system test function added.
03/03/2025		Edition 1.02: Updated Chapter 3, 5, 9 to include newly added functions from NovoExpress (Opteon) Software v2.1.0.

In This Guide

The manual describes the following:

- Prologue
- Installation
- Use NovoExpress (Opteon) Software
- Sample Acquisition
- Data Analysis
- Plate Manager
- Experiment Manager
- Reports
- QC Test
- Troubleshoot
- Version History

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Edition 1.02 March 2025

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