NovoExpress

Software Guide

Notices

Manual Part Number

XA150013

Edition 3.02, July 2023

Copyright

© Agilent Technologies, Inc. 2023

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Agilent Technologies, Inc.

6779 Mesa Ridge Road, Suite 100,

San Diego, CA 92121, USA

Software Revision

This guide is valid for the v1.6.2 revision of the Agilent NovoExpress program and compatible programs, until superseded.

Instrument Manufacturing

Agilent Biosciences (Hangzhou) Co., Ltd.

Building 4, No.208 Zhenzhong Road, Xihu District, Hangzhou, Zhejiang Province, 310030

P. R. China

Printed in P. R. China

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

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

WARNING

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

In This Guide

This guide covers the following module: NovoExpress Software

1 Prologue

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

2 Installation

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

3 Use NovoExpress Software

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

4 Sample Acquisition

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

5 Data Analysis

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

6 Plate Manager

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

7 Experiment Manager

In This Guide

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

8 Reports

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

9 QC Test

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

10 Troubleshoot

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

11 Version History

Appendix A: Permission List

Contents

In This Guide 3

Contents 5

1 Prologue 12

About NovoExpress 12

Using this Guide 12

Keyboard Shortcuts 13

Glossary 15

2 Installation 21

Installation Requirements 21

Install NovoExpress Software 21

Start NovoExpress Software 24

Uninstall NovoExpress Software 25

Log into NovoExpress 26

Register and Dissociate NovoExpress 27

Free Trial 27

Register NovoExpress Software 28

Dissociate NovoExpress Software 31

Manage User Access and Data Security 32

General 33

Access Control 36

Data Security 52

e-Signature 54

Audit Trail 56

3 Use NovoExpress Software 60

NovoExpress Software Interface 60

Title Block 62

Menu 63

File 63

Home 75

Instrument 84

Sample 92

Plot 95

Gate 97

View 99

Setting 100

Workspace Toolbar 116

File Explorer Panel 119

Cytometer Setting Panel 124

Cytometer Control Panel 125

Plate Manager 126

Experiment Manager 128

Cytometer Status Panel 129

Gate Manager Panel 133

Status Bar 133

Green Indicator 133

Flashing Red Indicator 134

Flashing Yellow Indicator 134

Black Indicator 135

Instrument Configuration 135

Instrument Configuration with Instrument Connected 136
Instrument Configuration with Instrument Disconnected 139
Manage Plate Types 140

4 Sample Acquisition 146

Cytometer Setting 146
Parameters Settings 147
Stop Condition Settings 149
Flow Rate Settings 150
Threshold Settings 150
Sample Keywords 151
Edit Keywords Window 152
Create Keywords 157
Add Statistics as Keywords 159
Read Keywords from Sample 161
Rename and Edit Keywords 162
Work List 163
Open the Work List 165
Work List Management 166
Edit a Work List Cell 169
Cytometer Control 171
Active Sample Information 172
Experiment Control 173

5 Data Analysis 178

Plots 178
Create a Plot 180
Open and Close a Plot Window 183

Edit Plots 184
Set the Coordinates of the Axis 188
Adjust the Size of Plots 194
Copy or Save Plots 195
Overlays 195
Plot Formatting 198
Bi-Variate Plots 205
Gates 209
Create Gates 210
Edit Gates 212
Gate Display Format 213
Apply a Gate to a Plot 216
Copy and Paste Gates 216
Export Gate Events 217
Gate Manager 218
Export to Gating-ML File 220
Statistics 228
Display Statistical Information 228
Calculation of Statistics 233
Fluorescence Compensation 236
Automatic Compensation 236
Active Compensation 255
Quick Compensation Adjustment 259
Cell Cycle Analysis 262
Automated Cell Cycle Analysis 262
Manual Cell Cycle Analysis 264
Cell Proliferation Analysis 267

Automated Cell Proliferation Analysis 267
Cell Proliferation Setting 269
Statistical Tables 271
Create Different Types of Statistical Tables 272
Statistical Table Columns 274
Statistical Table Rows 278
Statistical Tables Export or Copy Text 280
Statistical Table Options 280
Statistical Table Management 282
Heat Maps 283
Create a New Heat Map 283
Heat Map Window 283
Edit Heat Map Statistics Window 286
Update Heat Map 287
Save Heat Map 287
Copy Heat Map 289
Heat Maps Management 289
Post Gain 290
Adjust Post Gain 290
Clear Post Gain 292
Apply Post Gain 293
Export Post Gained Data 294

6 Plate Manager 295

Overview of Plate Manager Panel 295
Context Menu 303
Context menu when a single well is selected 303
Context menu when multiple wells are selected 306

7 Experiment Manager 309

Experiment Manager Toolbar 309

Hierarchy 309

Description 309

Right-Click Menu 313

Move Items 321

Merge Samples 322

Templates 324

Copy and Paste the Template 325

Drag and Drop the Templates 327

Use the Toolbar 329

Import and Export Templates 329

Import and Export Data 332

Import Data 332

Export Data 333

Copy and Paste Events 337

8 Reports 338

Report Interface 339

Automatically Generate Reports 343

Report Options 344

Report Editor 347

Add Report Objects 347

Select Report Objects 347

Edit Report Objects 349

Align Report Project Items 353

Resize Objects 354

Order Object Levels 354
Cut, Copy, Paste, and Delete 354
Insert or Delete Pages 354
Header and Footers 355
Report Output 357
Batch Print Reports 357

9 QC Test 360

Prepare QC Particle Sample 361
Run QC Test 361
View QC Test Report 367

10 Troubleshoot 370

Troubleshoot Instrument 370
Technical Support Request 379

11 Version History 381

Appendix A: Permission List 382

1 Prologue

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

About NovoExpress

NovoExpress Software provides users with the ability to control data collection and analysis on Agilent flow cytometer and associated accessories. The software contains features to control Quality Control (QC) test, sample acquisition, data analysis, and report generation. With NovoExpress Compliance License registered, NovoExpress software contains additional features (e.g., permission control and data security) to support GxP and FDA 21 CFR Part 11 compliance.

WARNING **It is the responsibility of the user and their organization to ensure proper use of NovoExpress to achieve compliant data acquisition and processing. Besides the functions NovoExpress provides, the user and their organization must establish proper Standard Operating Procedures (SOPs) to address relevant non-technical requirements. For example, internal audit programs must also be established to ensure that system operators follow the SOPs.**

Using this Guide

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

Keyboard Shortcuts

Shortcuts	Command
Overall Situation	
Ctrl + [	Switch the active sample to the previous sample
Ctrl +]	Switch the active sample to the next sample
Ctrl + 1	Create a dot plot
Ctrl + 2	Create a density plot
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 + G	Electronically sign the experiment file or experiment template file
Ctrl + N	New file
Ctrl + O	Open the file
Ctrl + S	Save the file
Ctrl + W	Close the file
F3	Next sample without template
F4	Next sample
F5	Run / Stop / Run single well / Stop Single Well
F6	Run Plate / Stop
F7	Pause / Continue
F8	Restart
Plot	
Alt + 1	Change to dot plot
Alt + 2	Change the density plot
Alt + 3	Change to histogram
Alt + 4	Change to contour plot
Alt + 5	Change to cell cycle diagram
Alt + 6	Change to cell proliferation diagram

Alt + Q	Quick compensation
Ctrl + +	Zoom In
Ctrl + - (Minus Key)	Zoom Out
Ctrl + A	Auto Range
Ctrl + B	Bi-Range gates
Ctrl + C	Copy the plot or gate
Ctrl + D	Duplicate a plot or gate
Ctrl + E	Elliptical gate
Ctrl + F	Full Range
Ctrl + H	Range gates
Ctrl + J	Freehand gate
Ctrl + L	Logic gates
Ctrl + M	Move
Ctrl + P	Polygonal gate
Ctrl + Q	Quadrant gate
Ctrl + R	Rectangular gate
Ctrl + T	Adjust threshold
Ctrl + V	Paste gate
Ctrl +Y or Ctrl + Shift + Z	Redo
Ctrl + Z	Undo
Experiment Manager	
Ctrl + C	Copy
Ctrl + D	Duplicate
Ctrl + V	Paste
F2	Rename

Glossary

Term	Definition
Absolute count	Number of cells or particles per unit volume. Agilent flow cytometer is a volumetric instrument, thus, exact volumes of acquired sample can be determined without the need for counting beads. After the dilution factor and unit of measure (default is # of events per µL) are defined by user, NovoExpress can display number of events within specified gate per unit volume in the statistical information chart.
Active sample	The sample currently displayed and being analyzed. The **Cytometer Control** and **Cytometer Setting** panels display basic information regarding the active sample. In the **Experiment Manager** panel, the active sample is indicated by a red arrow. To switch the active sample, double-click on a new sample in the **Experiment Manager** panel, use the **Switch Active Sample** buttons from the **Sample** tab of the **Menu Bar**, or use the combination of Ctrl + [or Ctrl +] on the keyboard to go backward or forward.
Adjust on Plot	A function to adjust threshold value on plot.
Alias	Alternative and customizable name for fluorescence parameter.
Analysis	The process of plotting, gating, and comparing statistical information on collected parameters of samples.
Auto compensation	Use a specimen containing an unstained sample and single stained samples to automatically calculate a compensation matrix.
Auto Range	A function which allows user to display the range for the X-axis and Y-axis to fit the experimental data.
Backflush	Clear blockages in the sample pipeline.
Batch Print Reports	A function to print or create PDF of multiple specimen and sample reports.
Bi-Range Gate	Draws a gate with two ranges
Bi-Variate Plot	A matrix of plots with selected parameters plotted against each other.
Blank samples	Samples without collected event data. A blank sample must first be created before sample collection begins. Created blank samples contain the default settings from the previously created sample. See the icon for a blank sample in Hierarchy.
Cell cycle plot	Uses a histogram of DNA content to derive cell cycle phase populations based on curve fitting to the histogram.
Cell proliferation plot	Generates c modeling results to analyze different cell generations during the cell proliferation procedure.
Cleaning	Clear biological hazards that may exist in the pipeline.

Compensation Matrix	A matrix which contains each photo detector's value that is subtracted in order to determine the final calculated true fluorescence.
Contour plot	Two-parameter plot. Each axis can plot a parameter. The plot uses contour lines to indicate the density of populations on the plot.
Core Diameter	The diameter of hydrodynamic focusing which corresponds to the flow rate.
Debubble	Clear bubbles present in the sample line.
Decontamination	Decontaminate the instrument when it is known to have contamination or to prevent the occurrence of contamination
Density plot	Two-parameter plot. Each axis can plot a parameter. The color of a point on the plot will be an indicator of the number of events at that point.
Dot plot	Two-parameter plot. Each axis can plot a parameter. Multiple overlapping points will be displayed the same as a single point in a dot plot.
Elliptical Gate	Draws an elliptical gate
Event	Refers to a particle that passes the acquisition threshold and has a set of data on intensity collected. Events are due to particles including microspheres and cells.
Experiment File	The NovoExpress Software saved experimental data files. A file in the format of *.ncf which stores experimental data from multiple samples.
Experiment Template File	A file in the format of *.ncet which includes settings for running a plate (e.g., mixing, rinsing and absolute count), settings for specimens, samples, file report, heat maps and statical tables, etc.
Experiment Manager	The NovoExpress Software uses samples, specimens, and groups in a hierarchy structure to organize the experimental data, instrument settings, analysis and other information. The organization is displayed in the **Experiment Manager** panel.
Export to LIS	A function allows a user to export data analysis results in the designated format to CSV file and such CSV file can be parsed by a Laboratory Information System (LIS) or read by other programs.
Extensive Rinse	Extensively rinse tubing
FCS	Data file standard for flow cytometry. NovoExpress is compatible with FCS 3.0 and 3.1.
Fluidics maintenance sequences	A function to program and run multiple fluidics maintenance sequences in pre-defined order
Fluorescence compensation	Different fluorescent dyes emit different emission spectrums. When emission spectrums overlap, this is known as spectrum overlap. When the overlap occurs within a detection channel, fluorescence compensation can be used to mathematically compensate by removing the signal that does not belong in the channel.

File Explorer	A software user interface panel allowing user to manage (e.g. move, rename, open) experiment file (*.ncf), experiment template file (*.ncet), template file (*.nct), or statistical table template file (*.nst) within the software main window.
Freehand Gate	Draws a freehand gate.
Free Unused File Space	When events of a sample or all samples are deleted, the file space is not automatically released. Click on this icon to free the file space. The file space can also be released by saving the file to the hard drive. Large files may take longer to release.
Full Range	Sets the full range of the X and Y-Axes.
Gain	A parameter corresponds to the output current of a photodetector for fluorescent signal detection.
Gate	Used to select a specific population of events. Gate types include rectangular, elliptical, polygonal, freehand, range, and bi-range gates. Gates can also be combined to create logic gates. Gates are used to further analyze specific populations.
Group	A part of the NovoExpress Software's hierarchy structure. The group can contain multiple specimens.
Heat Map	Heat map can be used to visualize the data in a well plate format. It uses different color to display the result of a specified statistical parameter.
Histogram Plot	Single-parameter plot. The X-axis will plot a parameter, and the Y-axis will plot the number of events.
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 fluorescence or scattered light intensity. In the NovoExpress Software, plots include dot plots, density plots, histograms, contour plots and cell cycle diagrams.
Polygon Gate	Draws a polygon gate.
Post Gain	In certain situation, user may want to align a particular peak on different samples in the same plot. Post Gain function allows such adjustment performed after data acquisition.
Priming	Use after the instrument has been inactive for a long period of time to fill the tubing with fresh sheath fluid and clear any bubbles
Purge	If Agilent flow cytometer needs to be shipped, click this button and follow the procedure shown on the popup window to purge the fluidic system before packaging and shipment.
QC test	In QC test, Agilent Flow Cytometer QC Particles are used to check the instrument performance. Measured data are used to determine if the instrument parameters fall within a standard range to ensure stable and reliable operation of the instrument.
QC test reports	QC test reports contain parameters for individual QC test results, and it can also plot results over a period of time in the Levey-Jennings reports.
Quick compensation	Use scrollbars in two-parameter plots to quickly adjust the 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
Report	Reports can be either a specimen report or a sample report. A sample report can contain plots, statistical information, compensation matrices, and collection information for the sample. A specimen report will contain information for all the samples included in the specimen.
Reset Layout	Resets the layout of the panels to the default layout.
Rinse	A function to rinse fluidic system.
Sample	The basic unit of experimental data organization. Samples contain the information from sample data collection, instrument settings, fluorescence compensation, reporting, analysis and data.
Sample currently in acquisition	Usually, sample currently in acquisition is same as active sample; however, they do not have to be same sample. After sample acquisition is started, user can switch active sample to other sample for analysis

	purpose. In **Experiment Manager** panel, sample currently in acquisition has a flash arrow (alternating green and dark green). If sample currently in acquisition is same as active sample, the arrow alternating red and green.
Sample flow rate	The flow rate of the sample can be used to control the number of events collected per second.
Specimen	A part of the NovoExpress Software's hierarchy structure. Specimen can be composed of many samples; multiple samples of the same test items can be placed in a specimen; a clinical specimen can correspond to a patient.
Spillover Matrix	A matrix contains value of spillover of each fluorochrome into each of the others. It is inversely related to the compensation matrix.
Statistical table	A customizable table of statistical information for batch data analysis. It can contain multiple samples, multiple gates, and statistical information for multiple parameters.
Stop condition	A defined number of events, length of time, or volume of collection, where sample collection is stopped immediately after reaching the condition.
Storage Gate	Filter out events outside the selected gate.
System Log	A window records information including user login and log out, and instrument operating activities including data acquisition, fluidics maintenance, etc.
Technical Support Request	A function which automatically collects instrument configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of instrument.
Templates	Set contains the group, instrument settings, specimens and samples, fluorescence compensation, reporting, analysis, etc., can be saved as *.nct file format.
Threshold	The minimum value of defined parameters where if the signal is lower than the defined value, the data will be discarded. By setting an appropriate threshold value, the target events can be effectively captured. A threshold value too high will discard target events, while a threshold too low will include a large noise from small events being collected.
Transaction Log	A window which displays recorded information related to changes to the experiment file, such as **Time**, **Computer**, **Software Version**, **User**, and **Action**.
Unclog	Clear blockages in the flow cell.
Work List	Displays the samples as rows in a table. Sample settings are also listed and can be set in the table, including specimen name, sample name, parameters, stop condition, sample flow rate, threshold, compensation,

	and analysis is reporting information. Allows the user to quickly create and manage multiple samples.
Workspace	In the main interface of the NovoExpress Software, the middle area where plots are displayed.

2 Installation

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

Installation Requirements

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

Hardware

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

Software

- Operating System: Windows 7 SP1 (x64)/Windows 10 (x64)/Windows 11

NOTE

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

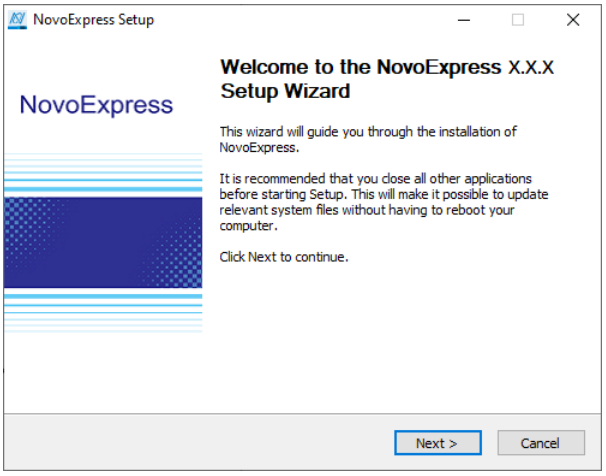
Install NovoExpress Software

Install NovoExpress Software using the following instructions:

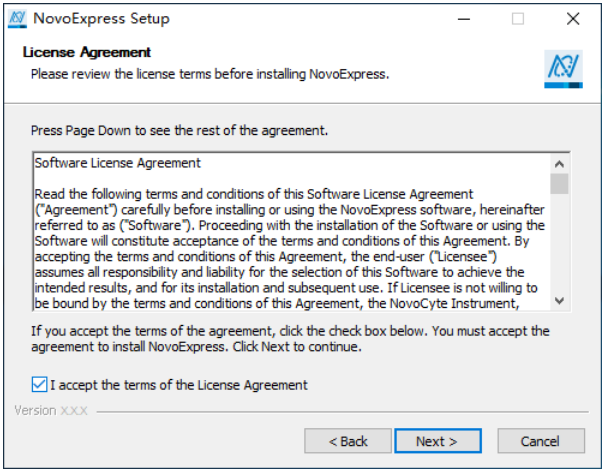
1 Download the NovoExpress Software installation package from website https://www.agilent.com/chem/novoexpress and unzip it. Double-click the

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

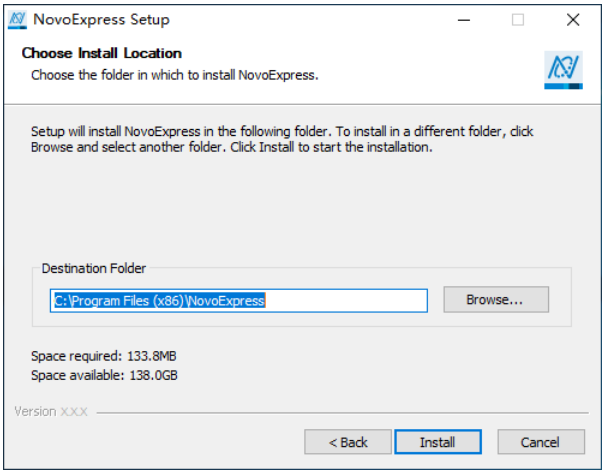
2 The NovoExpress Software installation wizard will display. Click **Next** to continue.

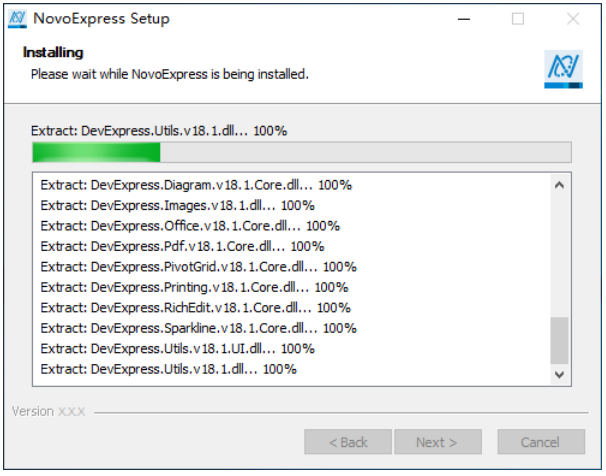


3 Please read the license agreement and accept by selecting the check box and clicking **Next**.

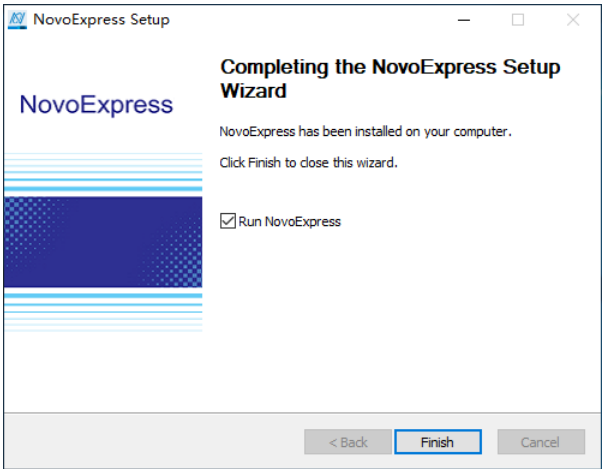


4 Choose the installation location. By default, the NovoExpress Software will be installed in **C:\Program Files (x86)\NovoExpress**. To install the software to another location, enter the target location or click **Browse** to select a destination folder. If the selected path does not exist, the installation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





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

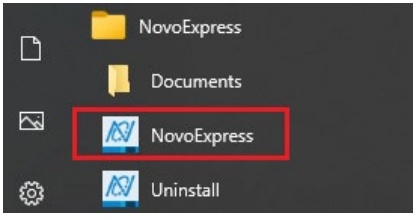


Start NovoExpress Software

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

- **Desktop Shortcut**

- **Start Menu**



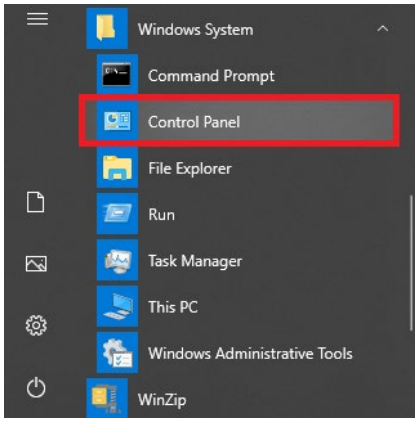
Uninstall NovoExpress Software

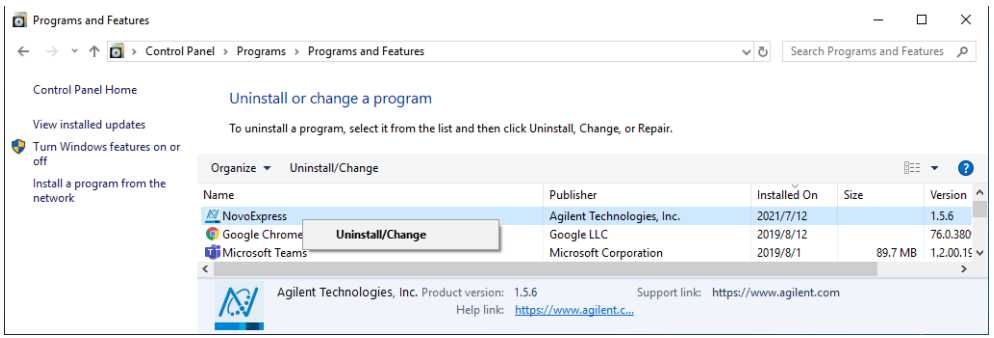
The NovoExpress Software can be uninstalled by the following methods:

- **Control Panel**

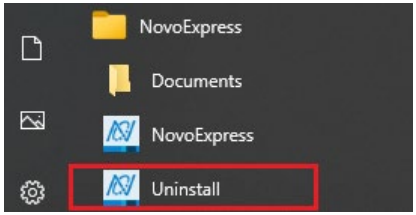
  Start > **Windows System** > **Control Panel** > **Programs and Features**

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





- **Start Menu**

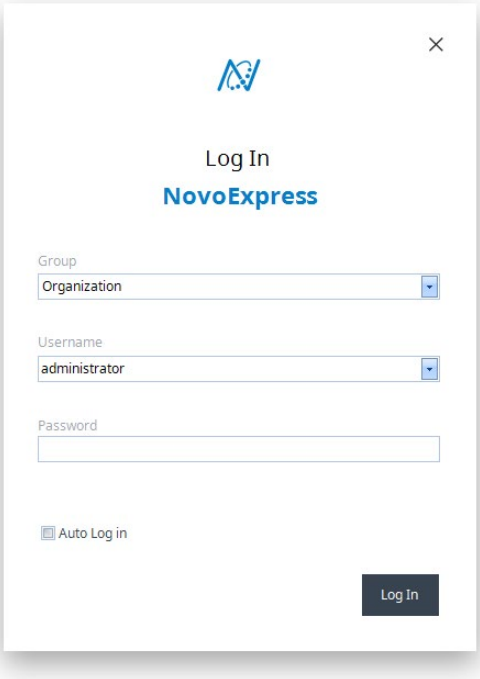


Log into NovoExpress

When starting the NovoExpress Software, a login window will appear as shown below. User can select the proper user group from the drop-down list of **Group**, enter or select the correct username from drop-down list of **Username**, enter the correct password and click **Log In** to log into NovoExpress software. If **Allow auto login** option is enabled in **Security Settings >Access Control > Login** window (Refer to Login in this guide), user can also check the **Auto Login** box, and the software will remember the password and automatically login with the associated user account and the login window will not appear in the future.

The user account and user group management feature can be used to assign separate user settings to different accounts.

The NovoExpress Software initially includes a system administrator account with username as "**administrator**". The default password for this account is "**administrator**". The username "administrator" cannot be changed. User account with designated permission can add, delete, and modify information for 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 or a user account are described in Access Control in this guide.

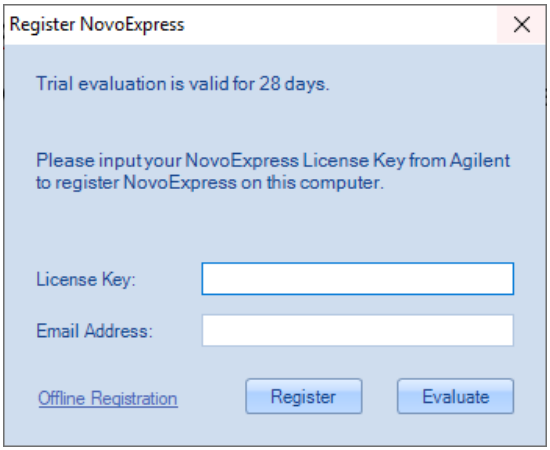


Register and Dissociate NovoExpress

NovoExpress software requires proper registration of NovoExpress License and NovoExpress Compliance License before user can access all the functionality. The NovoExpress License is required to access basic software functions after the free 30-day trial. The NovoExpress Compliance License is required in addition to NovoExpress License to access additional software functions supporting GxP and 21 CFR Part 11 compliance. When transferring license to another computer is needed, user needs to dissociate both licenses first and re-register NovoExpress.

Free Trial

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



Registration will be needed to use the software after the trial expires. Please refer to Register NovoExpress in this guide for more details.

Register NovoExpress Software

NovoExpress License and NovoExpress Compliance License are needed to register NovoExpress software after the free 30-day trial. The NovoExpress License needs to be registered before registering NovoExpress Compliance License.

NOTE

NovoExpress License comes with the instrument. NovoExpress Compliance License is available to order through Agilent. Please contact your local Agilent sales representative for ordering information.

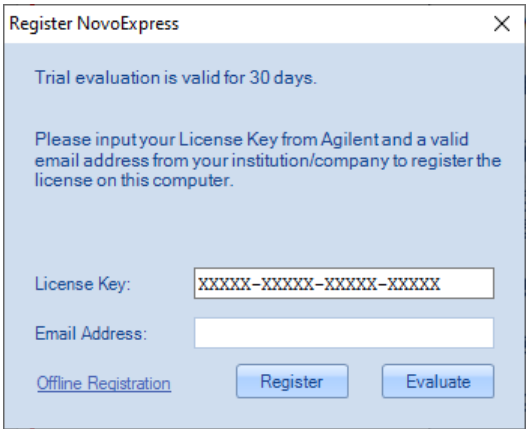
NOTE

Only the users with permission of **Register/Dissociate NovoExpress** can conduct this license registration process. Refer to Roles in this guide for more details.

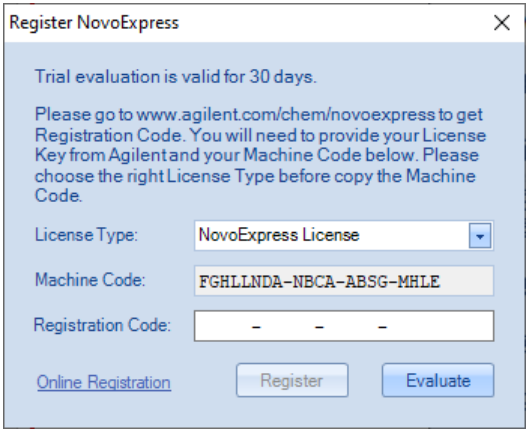
Register NovoExpress License

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

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



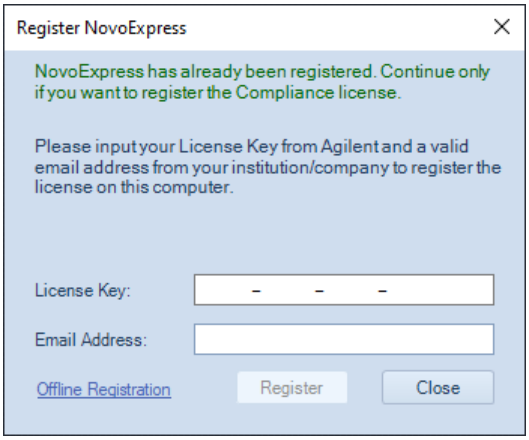
- If the computer is not connected to the internet, click the **Offline Registration** button to switch to offline registration mode. Write down the **Machine Code** displayed in the window and go to another computer that is connected to the internet and open the **Get Registration Code** web page (https://www.agilent.com/chem/novoexpress). 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 License.



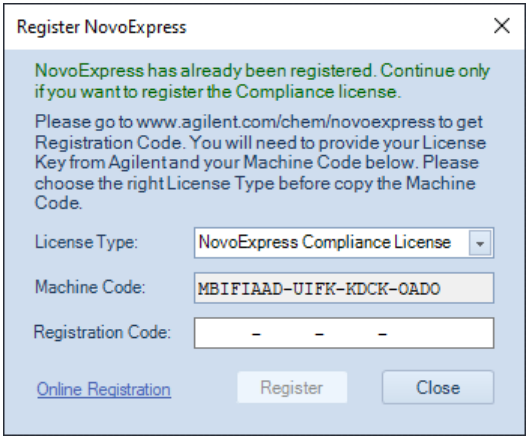
Register NovoExpress Compliance License

To register NovoExpress Compliance License, ensure NovoExpress License is properly registered first (refer to Register NovoExpress License in this guide), click **File** > **About** > **Register NovoExpress** to open the **Register NovoExpress** window.

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

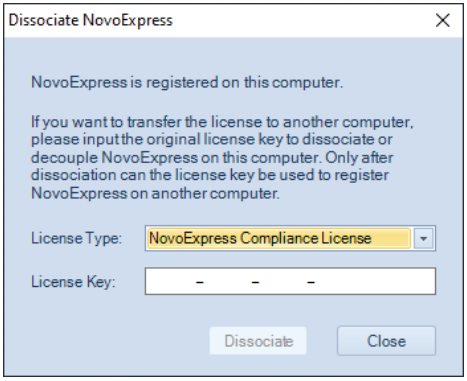


- If the computer is not connected to the internet, click the **Offline Registration** button to switch to offline registration mode. Select **NovoExpress Compliance License** from the drop-down menu of **License Type**. Write down the **Machine Code** displayed in the window and go to another computer 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 Compliance 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 Compliance License.



Dissociate NovoExpress Software

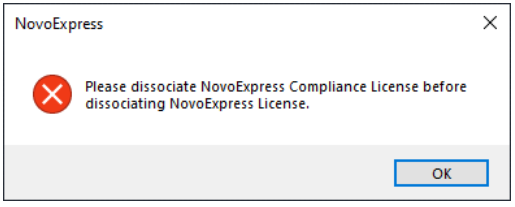
NovoExpress supports license transfer to another computer. Each license may be transferred up to 5 times. If you want to transfer the NovoExpress License or NovoExpress Compliance License to another computer, log in the software with permission of **Register/Dissociate NovoExpress**, click **File** > **About** > **Dissociate NovoExpress** to open the **Dissociate NovoExpress** window.



User can select the license to be dissociated from the drop-down list of **License Type.** The corresponding license key recorded during software registration will automatically filled in the **License Key** box. User can then click the **Dissociate** button to dissociate or decouple NovoExpress from this computer. After dissociation, the license key can be used to register NovoExpress on another computer.

NOTE

When dissociating NovoExpress License, ensure to dissociate NovoExpress Compliance License (if registered) first. Following window will be prompted if user dissociates NovoExpress License before dissociating NovoExpress Compliance License.



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 permission of **Register/Dissociate NovoExpress** can conduct this license dissociation process. Refer to Roles in this guide for more details.

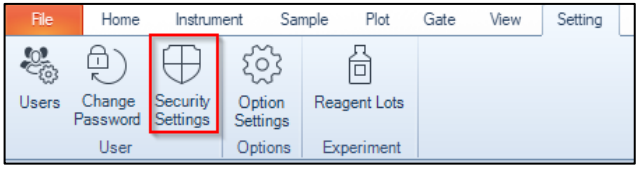
NOTE

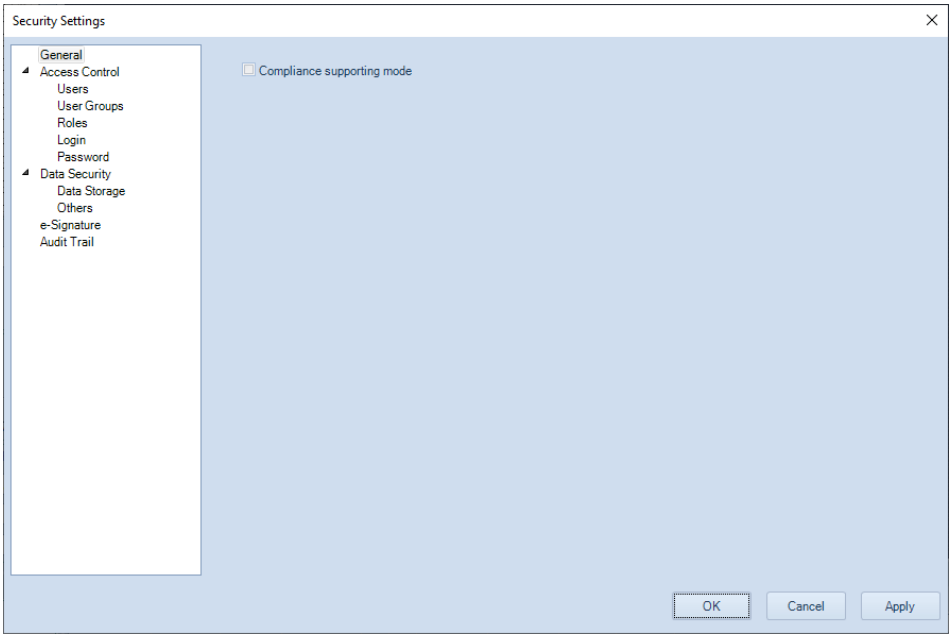
The license key is required for NovoExpress software registration or license transfer. Software will automatically record the license key. However, as a good practice, always keep a safe record of license key assigned to each computer or user.

Manage User Access and Data Security

User can manage selected user account or group, enable Compliance Supporting Mode, customize accessibility for selected user account, define data storage settings, e-Signature settings, audit trail settings through the **Security Settings** window.

To access this window, click **Security Settings** from the **User** panel under **Setting** tab in software main window after successfully logged into the software.

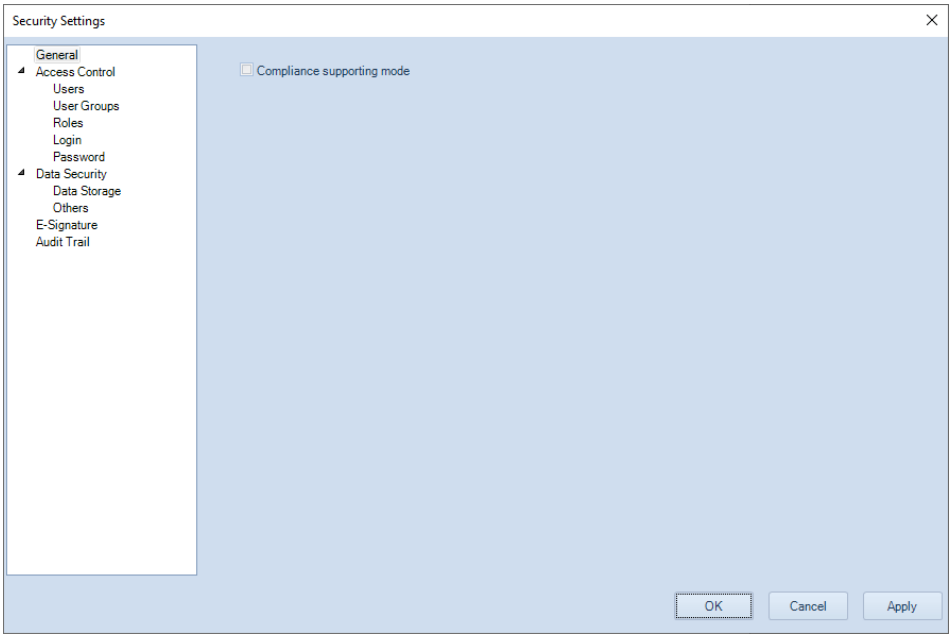




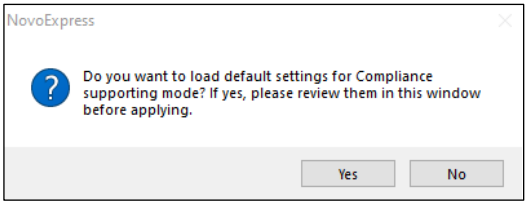
The **Security Settings** window includes five pages, namely **General, Access Control, Data Security, e-Signature** and **Audit Trail**. The **Access Control** page includes five subpages, namely **Users, User Groups, Roles, Login** and **Password**. The **Data Security** page includes two subpages, namely **Data Storage** and **Others**.

General

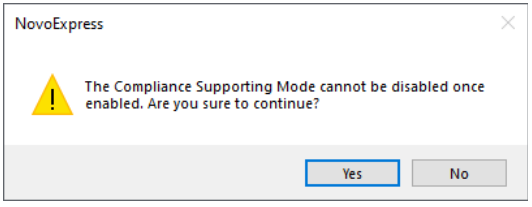
This page allows user to switch the software to work in the Compliance Supporting Mode.



To enable **Compliance Supporting Mode**, check the checkbox in front of **Compliance Supporting Mode**, click **Yes** in following prompted window.

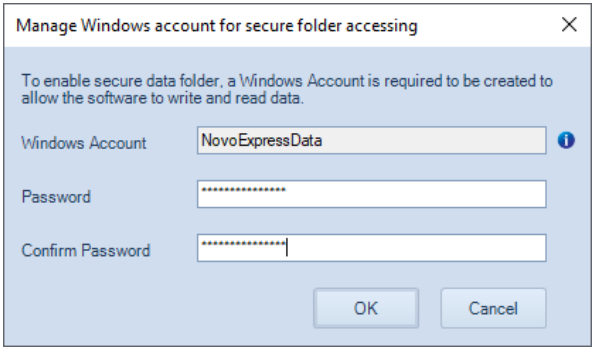


The default settings for **Compliance Supporting Mode** will be automatically loaded. User can view and edit all these settings in each page of **Security Settings** window and click **OK** or **Apply**. Following window will be prompted, user can click **Yes** to confirm and make all settings effective.



When **Compliance Supporting Mode** option is checked for the first time, following window will be prompted which requires user to create a Windows Account to

allow the software to write and read data in the secure data folder in the hard drive of the Workstation. User needs to enter and confirm a user defined password that complies to the Windows system password policy (if applicable) for displayed Windows Account (i.e., **NovoExpressData**) and then click **OK**. User needs to restart the software to make the changes effective. The software will be not able to open files in root data folder if the software is not restarted.



NOTE

The **Compliance Supporting Mode** option is automatically disabled if the NovoExpress Compliance License is not registered, or if any experiment file (*.ncf) or experiment template file (*.ncet) is currently open.

NOTE

The **Compliance Supporting Mode** option cannot be disabled after enabled.

NOTE

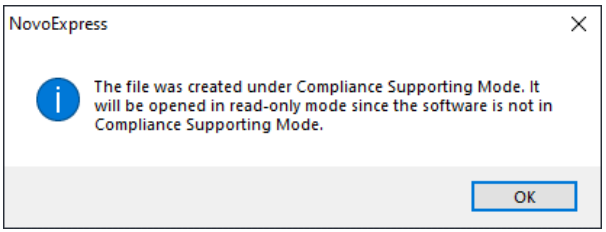
When **Compliance Supporting Mode** is enabled, the experiment file (*.ncf) and experiment template file (*.ncet) will be automatically encrypted to increase data security.

NOTE

User cannot import FCS file to any experiment file (*.ncf) created or modified with **Compliance Supporting Mode** enabled.

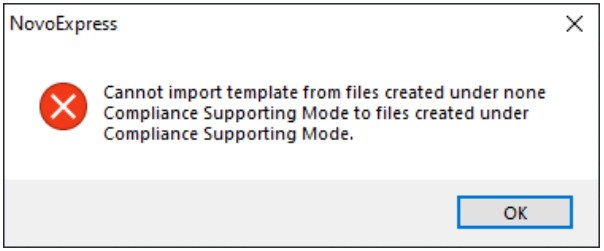
NOTE

If the software is not working in **Compliance Supporting Mode,** and user opens an experiment file (*.ncf) or experiment template file (*.ncet) created when software was working in **Compliance Supporting Mode**, following window will be prompted. User can open these files as read-only (i.e., user cannot edit it)



NOTE

When software works in **Compliance Supporting Mode,** user cannot import any template file (*.nct) or Statistical table template (*.nst) which was created when software was not working in **Compliance Supporting Mode.** Following window will be prompted.

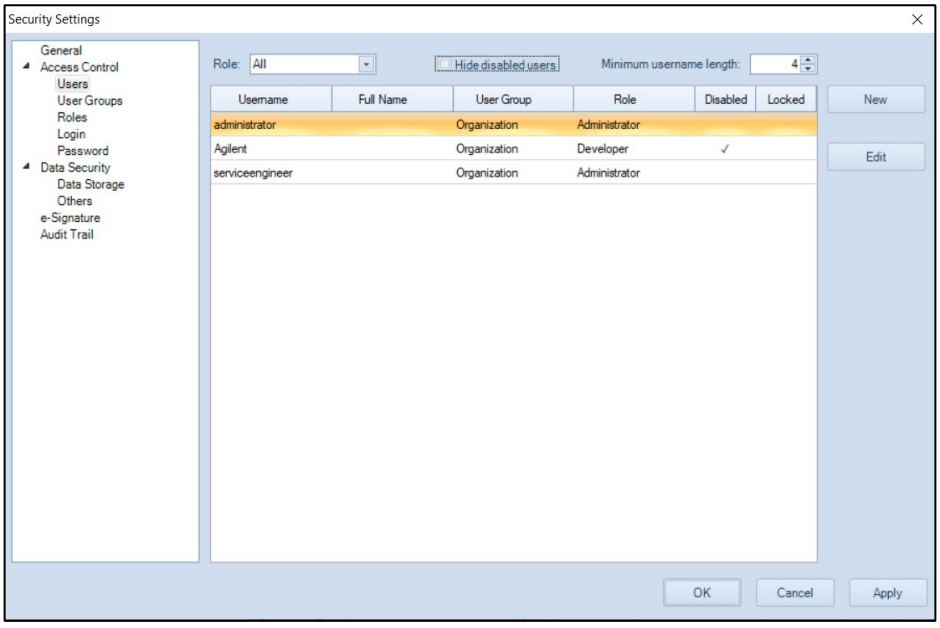


Access Control

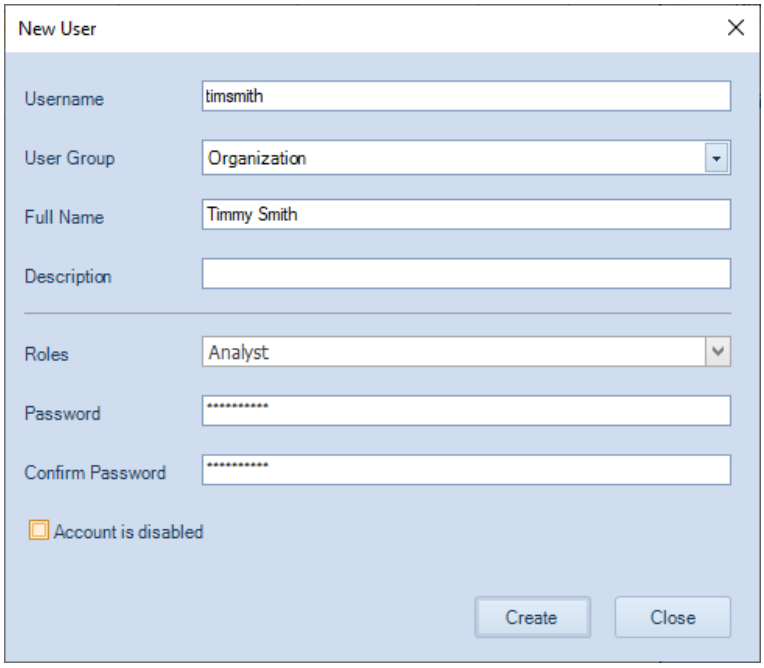
This page includes five subpages, namely **Users, User Groups, Roles, Login** and **Password** to allow user to manage software access related settings. Only user with the **Modify Access Control Settings** permission can access the functions in this page. Please refer to Roles in this guide for more details on permissions.

Users

This subpage allows user to view and edit access settings for each available user account.



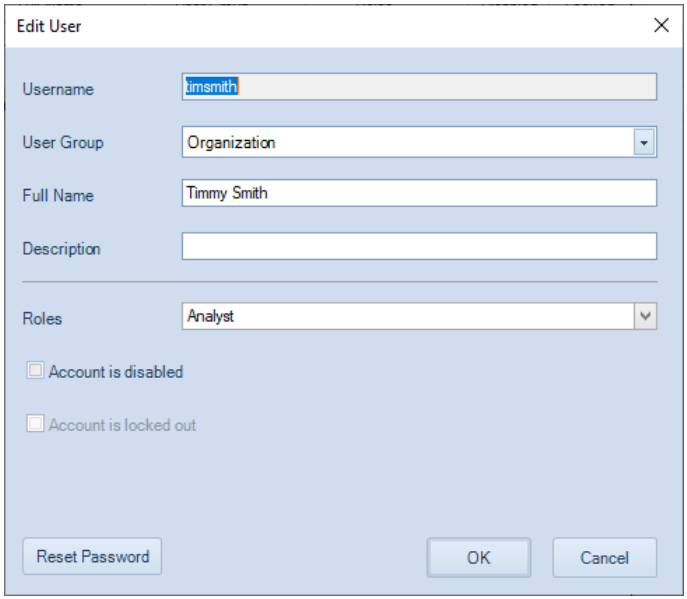
- **Role**: Allows user to select a role to filter users list below. The list of users will be automatically updated based on the filtering criteria. By default, **All** is selected and user accounts with all available roles are listed. Please refer to Roles in this guide for more details.
- **Hide disabled users**: when checked, all the disabled user accounts will be automatically hidden.
- **Minimum username length**: Allows user to set the minimum username length when creating a new user account.
- **New**: Allows user to create new user account. When clicked, following window will be prompted.



User needs to enter the new **Username**, select the proper **User Group**, enter the **Full Name**, select the proper **Roles**, enter and confirm the **Password**, click **Create** to create a new user account.

User can also check **Account is disabled** to disable the associated user account. The access and e-signature associated with the user account will be disabled. User account can be re-enabled by other user with permission of **Modify Access Control Settings**.

- **Edit**: Allows user to edit selected user account. When clicked, following window will be prompted.

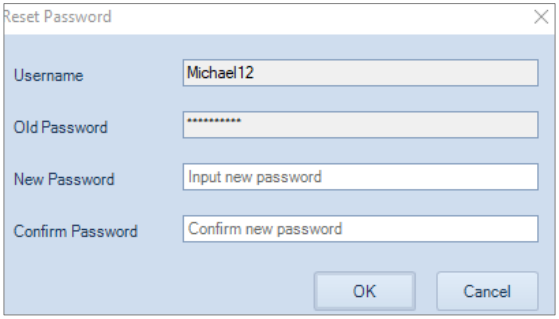


User can edit associated **User Group**, **Full Name**, **Description**, and **Roles** of the selected user account in this window. Click **OK** to complete editing users.

User can also check **Account is disabled** to disable the associated user account. The access and e-signature associated with the user account will be disabled.

When the user account is locked out, **Account is locked out** option will become available and automatically checked. User can uncheck this option to unlock user account.

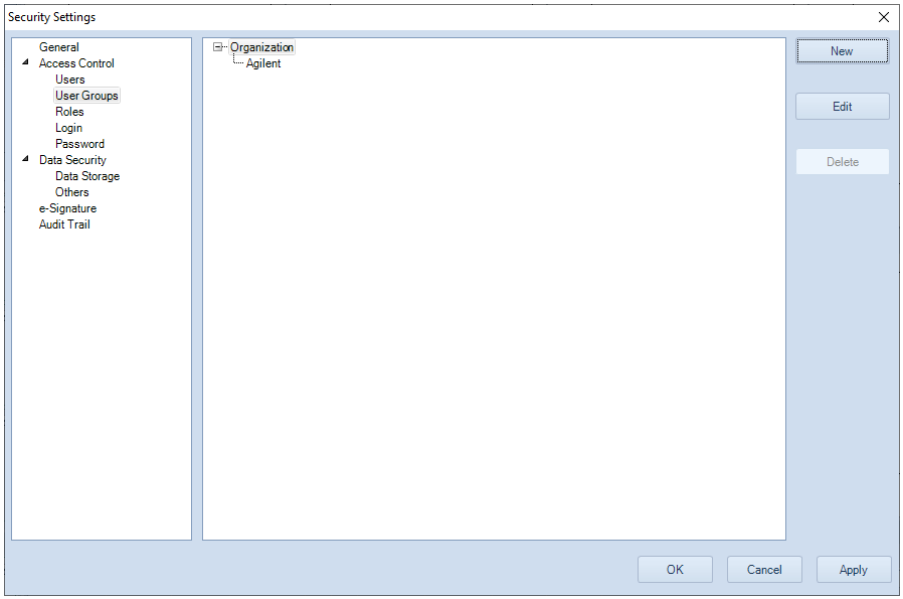
User can also click **Reset Password** to reset the password for selected user account. To do this, enter and confirm the new password in prompted window, and click **OK**.



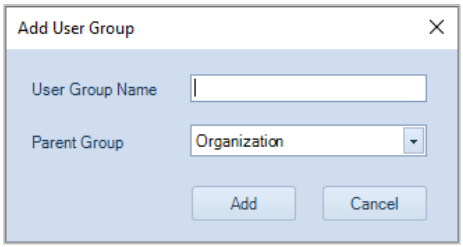
NOTE The built-in user account **serviceengineer** is used by Agilent service engineer only.

User Groups

This subpage allows user to view and edit selected user group.



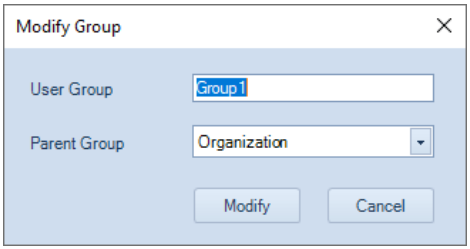
- **New**: Allows user to create a new user group. When clicked, following window will be prompted.



User can enter the new **User Group Name**, select the proper **Parent Group**, and click **Add** to create a new user group.

NOTE The root parent group "**Organization**" is included by default. User can only add a new user group under it.

- **Edit**: Allows user to edit the selected user group. When clicked, following window will be prompted.

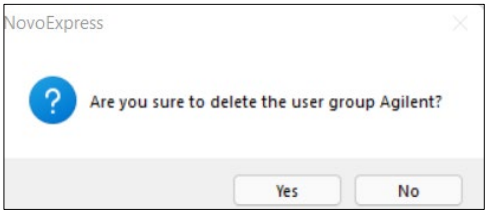


User can enter the new **User Group Name**, select the proper **Parent Group**, and click **Modify** to complete editing user group.

NOTE

When editing the root parent group **Organization**, the **Parent Group** option is automatically disabled.

- **Delete**: Allows user to delete the selected user group. When clicked, following window will be prompted. User can click **OK** to complete.



NOTE

When user select the root parent group (e.g., **Organization**), this function will be automatically disabled (i.e., user cannot delete root parent group).

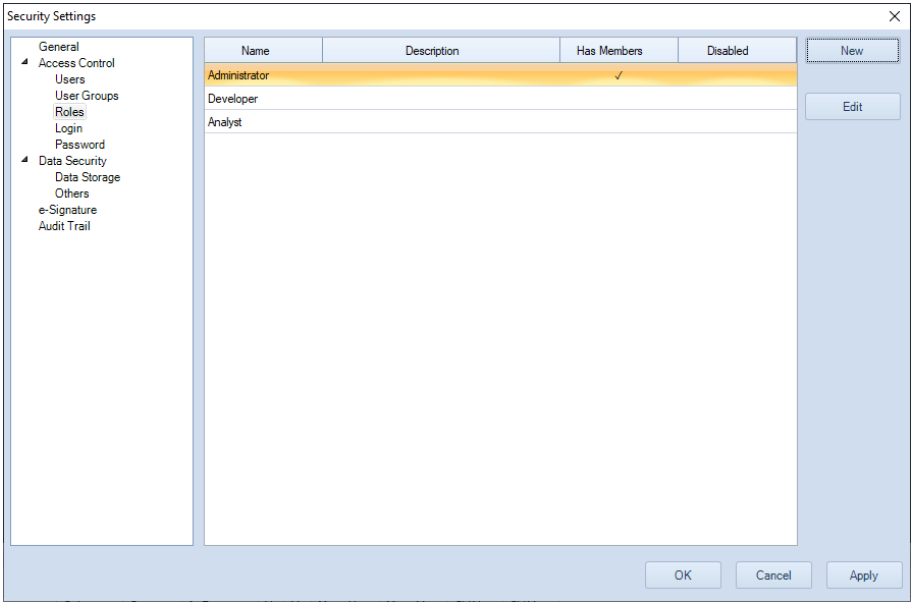
Roles

This subpage allows user to create new role(s), edit existing role(s), associate a role with selected user account, etc. There are three default roles predefined in the software, namely **Administrator, Developer, and Analyst**. Each role holds different permissions and can be assigned to each user account. One user account can have multiple roles with the combined permissions from all the roles. Whether a user has a specific permission is decided by the roles user has.

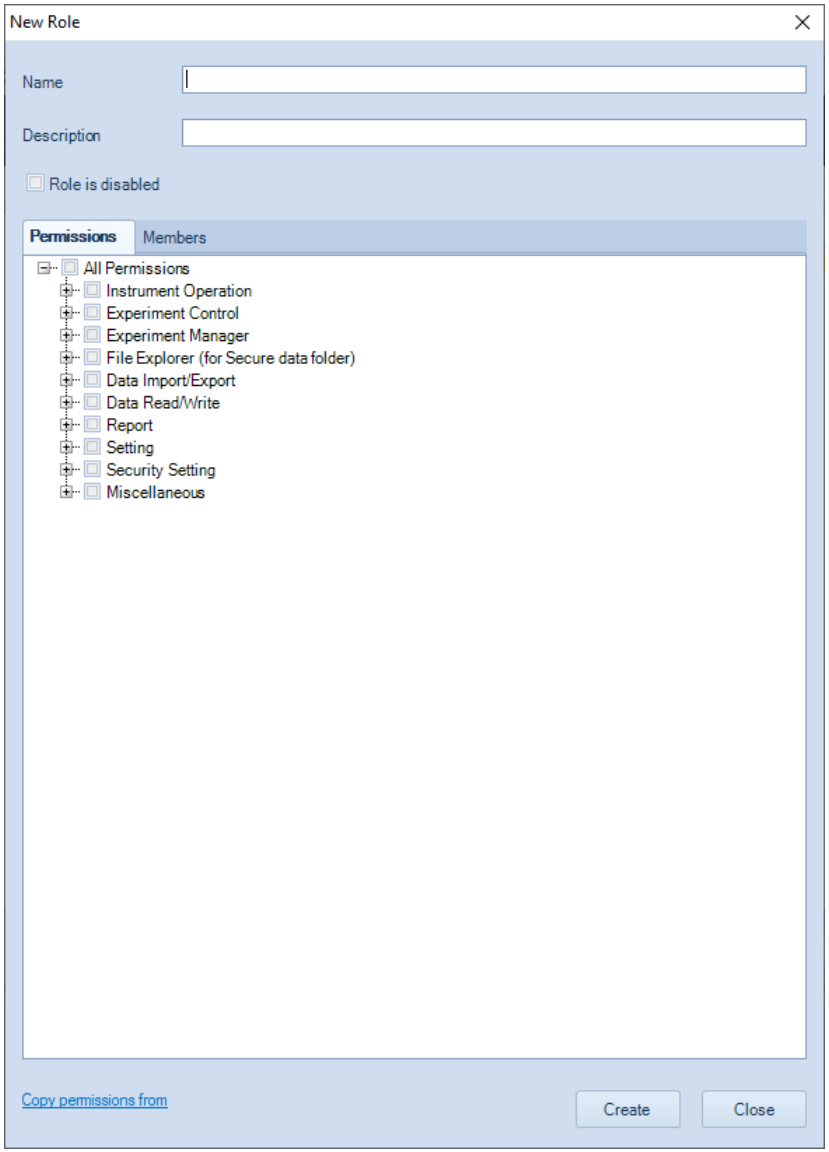
In this subpage, user can view all available roles in the tabular format. If any role is currently associated with one or more user account. If a role is currently disabled, there will be a checkmark in the corresponding **Disabled** column.

NOTE

Ensure to obtain proper permissions (or roles) for your user account before using NovoExpress software. Certain software functions may not be available to user without proper permissions. Please refer to Appendix A: Permission List for the complete list of available permissions and the ones predefined for default roles.



- **New**: Allows user to create a new role with selected permissions and associate user account with this role. When clicked, following window will be prompted.



There are two sub-windows associated with this window, namely **Permissions** and **Members**. The **Permissions** sub-window allows user to define permissions for the selected role. The **Members** sub-window allows user to assign user accounts as member of the selected role. This window also includes a **Copy Permissions From** function which allows user to quickly clone permissions from a selected existing role to the new role as a starting point to define permissions for the new role.

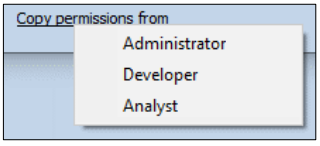
To create a **New Role**,

a Enter the **Name** of the new role and **Description** (optional).

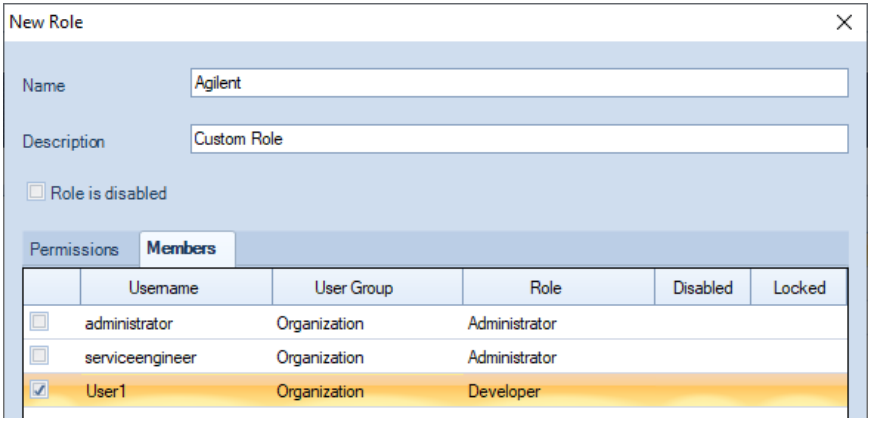
b As needed, check **Role** is disabled to disable the new role. A role can only be disabled when there are no members associated with it.

c Assign the permissions for the new role using one of the methods below.

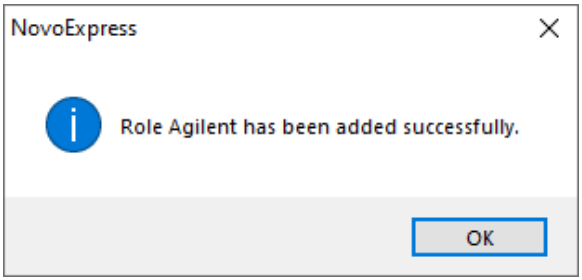
- Click **Permission** tab to display all available permissions, and check or uncheck the box in front of each individual permission item.
- In either **Permissions** tab or **Members** tab, click **Copy Permissions From,** and select the desired role from the prompted list. All the existing roles should be available for selection. This serves a way to quickly create a role with similar permissions as an existing role.



d If any user account needs to be assigned as member of the newly created role, click the **Members** tab and check to select the user account.

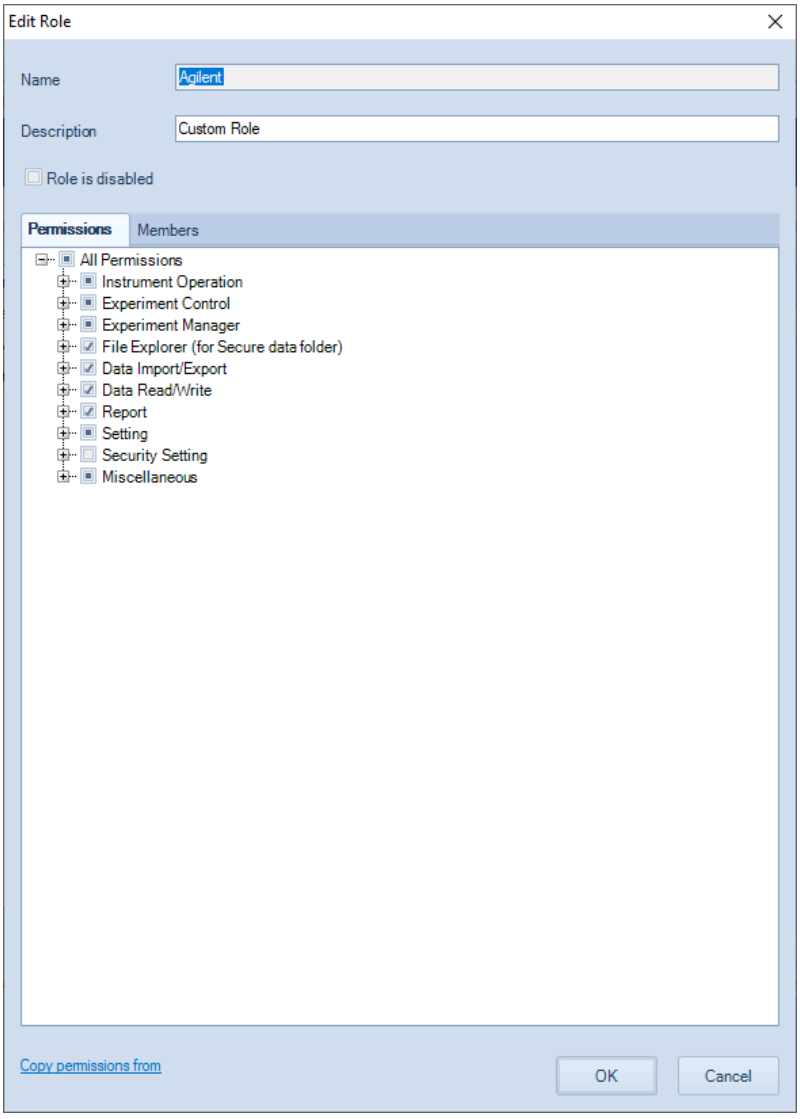


e Click **Create** and the window below will be prompted.



f Click **OK**. The new role is created and displayed in **Roles** subpage.

- **Edit**: Allows user to edit selected role. First, select the role by clicking it on the table in the Roles subpage. The selected role will be highlighted. Click **Edit** and following window will be prompted.



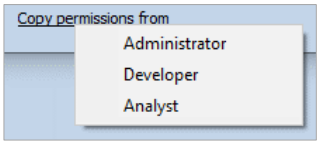
To edit the **Description** of the selected role, enter the new description in the associated text box.

To disable the selected role, check **Role is disabled**. A role can only be disabled when there are no members associated with it.

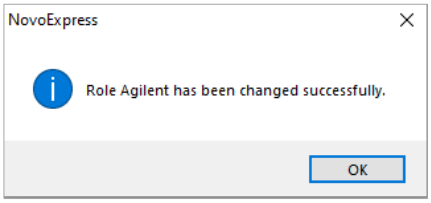
To modify permission(s) for the selected role, click **Permissions** tab, check or uncheck the box in front of each individual permission item.

To re-assign the designated user account as member of the selected role, click **Members** tab, uncheck the user account to dissociate the selected role from it, or check the user account to associate the selected role with it.

Use **Copy Permissions From** function to quickly set up a role with similar permissions as an existing role

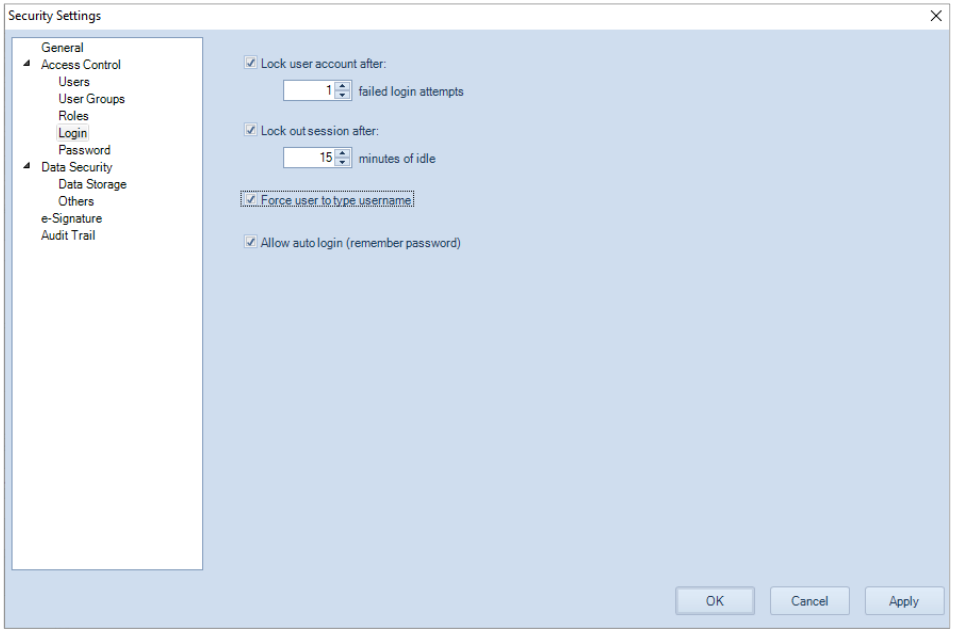


After all the edits are completed, click **OK** in **Edit Role** window, and click **OK** in following prompted window to complete.

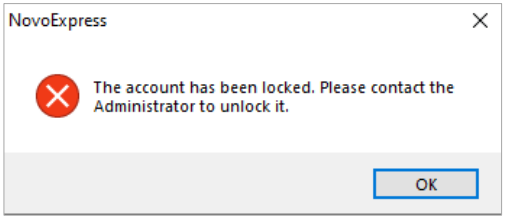


Login

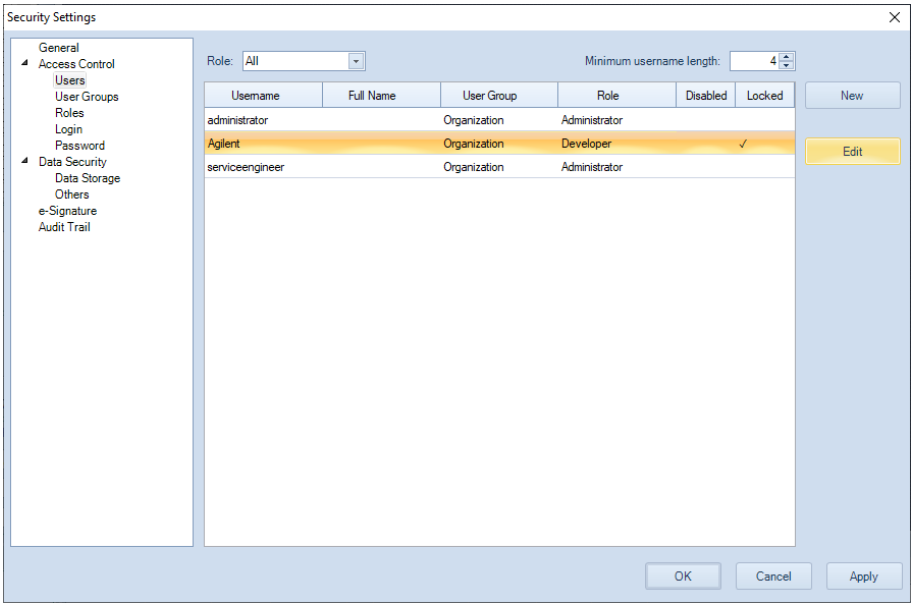
This subpage allows user to define the login related settings for all user accounts.



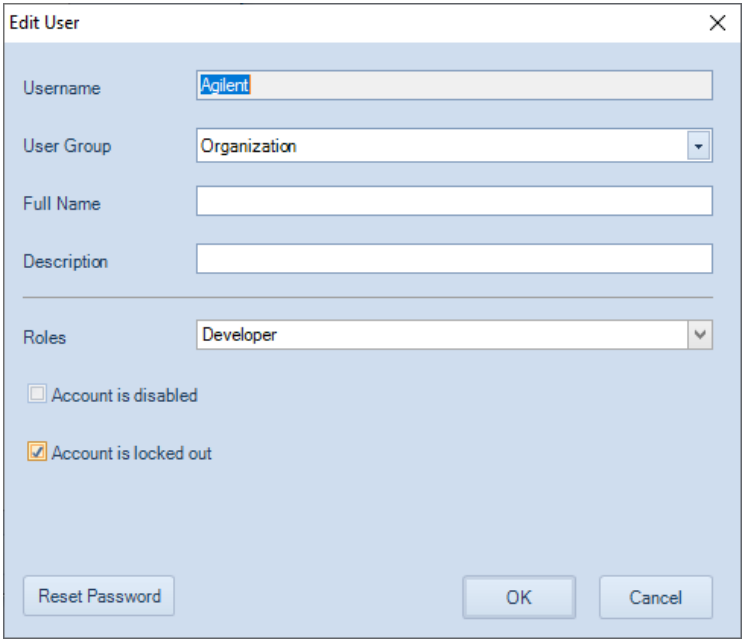
- **Lock user account after**: when checked, user account will be locked after specified number of failed login attempts. The software will inform user how many failed attempts left before locking the user account. Once the account is locked, following window will be prompted.



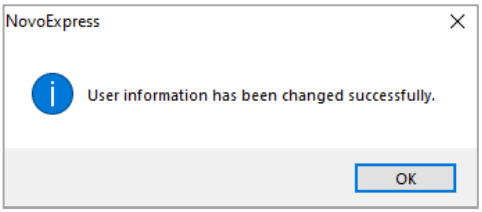
To unlock the account, click **OK** in above window, first log into the software with another user account with permission of **Modify Access Control Settings**. Then open the **Security Setting** window, click **Users**, highlight to select the account that is locked, and click **Edit**.



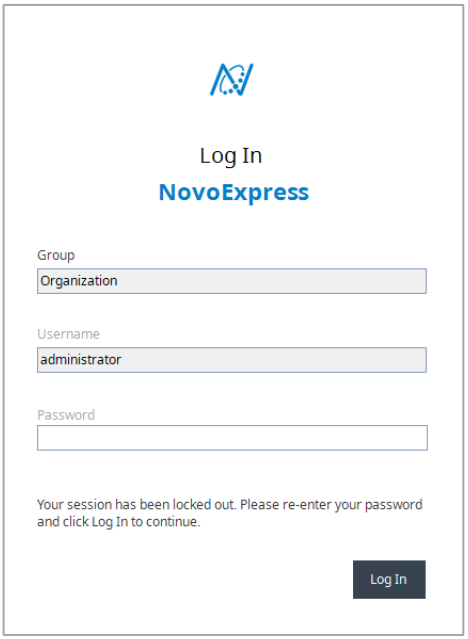
Uncheck **Account is locked out** option in the prompted window, and click **OK.**



Click **OK** in the prompted window.



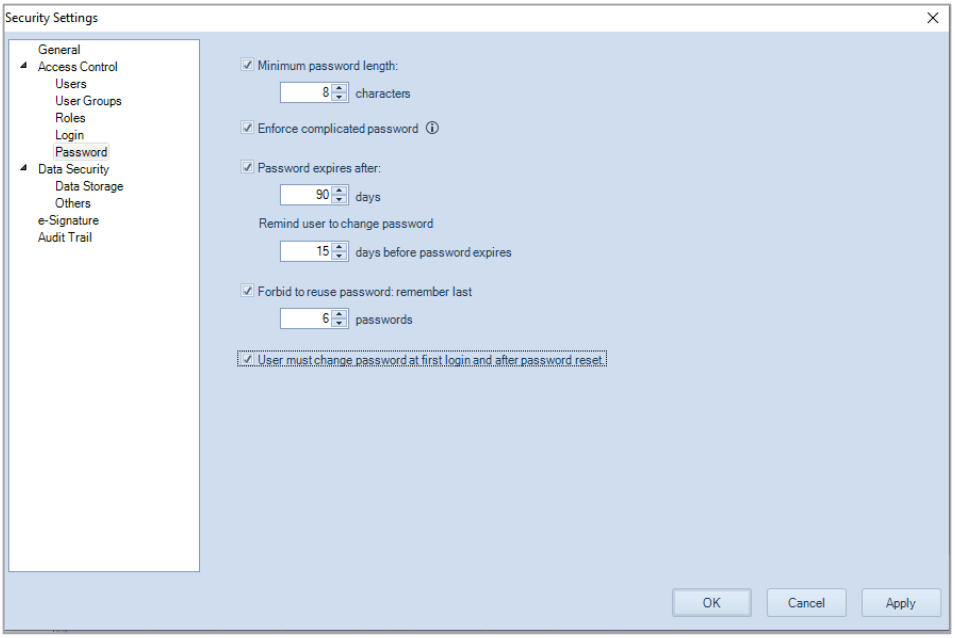
- **Lock out session after**: when checked, after specified minutes of idle (i.e. without any operations on the software), following window will be prompted. User needs to enter the correct password again and click **Log In** to continue operation of the software.



- **Force user to type username**: when not checked, user is able to select username from the drop-down list in the login window. When checked, user needs to type in username manually using the keyboard.
- **Allow auto login (remember password)**: when checked, the **Automatically login with user** option will be available in **System** sub-window of **Option Settings** window. If both options are checked, the software will remember the login password and user will be automatically logged in when software launches. This option is not available if **Compliance Supporting Mode** is enabled.

Password

This subpage allows user to define the password related settings for all user accounts.



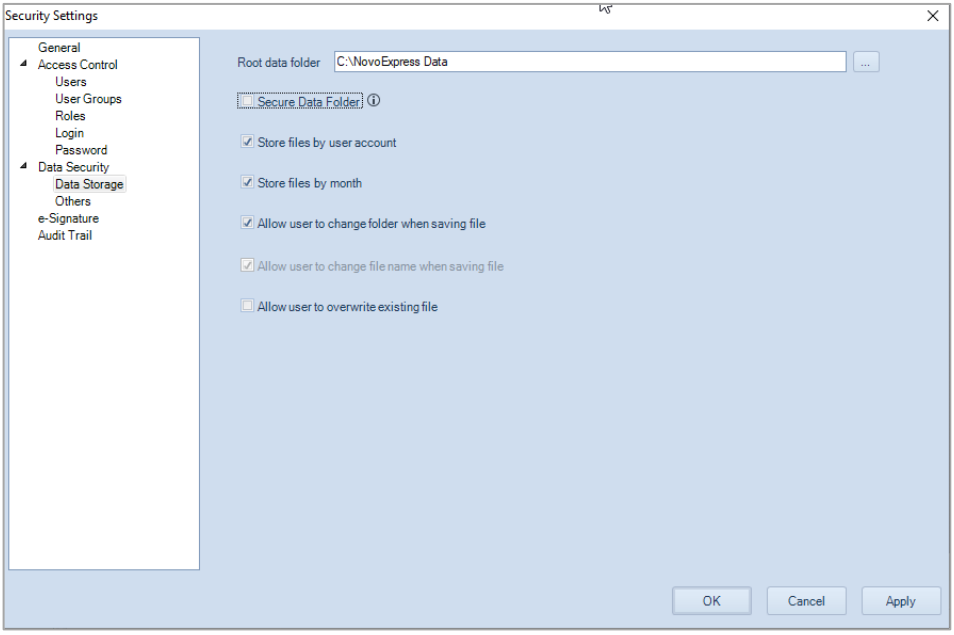
- **Minimum password length**: Allows user to set the minimum password length.
- **Enforce complicated password**: when checked, the password must contain characters from three of the following categories: uppercase letters, lowercase letters, digits, and special characters.
- **Password expires after**: when checked, user is required to change the password when login after specified days. User can also specify the days when software remind user how many days are left before changing password is required.
- **Forbid to reuse password**: when checked, software will remember the specified instance of passwords user used in the past and forbid user to reuse them.
- **User must change password at first login and after password reset**: When checked, user is required to change the password during the first login and after password is reset.

Data Security

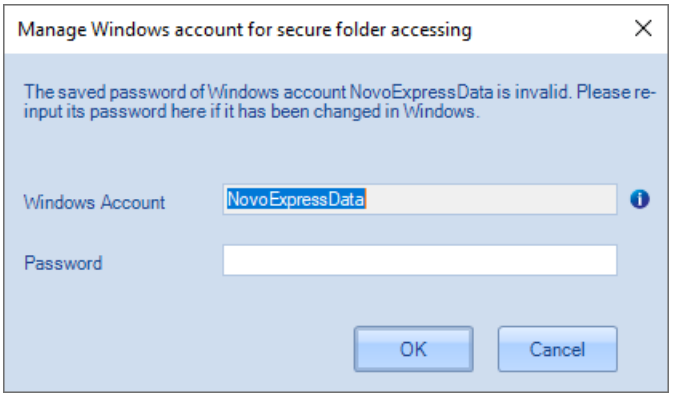
This page includes two subpages, namely **Data Storage** and **Others,** allowing user to manage data storage and exporting related settings. Only user with the permission of **Modify Data Security Settings** can access the functions in this page. Please refer to Roles in this guide for more details on permissions.

Data Storage

This subpage allows user to define the data related operations for all user accounts.



- **Root Data Folder**: Allows user to set the root data folder where experiment files are saved to.
- **Secure data folder**: If checked and enabled, Windows users other than the System Administrator is not able to delete, move or rename any files stored in the root data folder and its subfolders through Windows Explorer. When this option is checked and enabled for the first time, the software will prompt window below asking user to create a Windows account for the software to write and read data to and from that folder. If system administrator changes the password of this Windows account, NovoExpress will prompt window below requesting user to enter the new password.



- **Store files by user account**: If checked, the software will automatically create a folder under root data folder with associated username as the folder name, and automatically save the experiment files created by the user to this folder.
- **Store files by month**: If checked, the software will automatically create folders under root data folder with the folder name as the month when the experiment files were created, and automatically save experiment files to this folder.

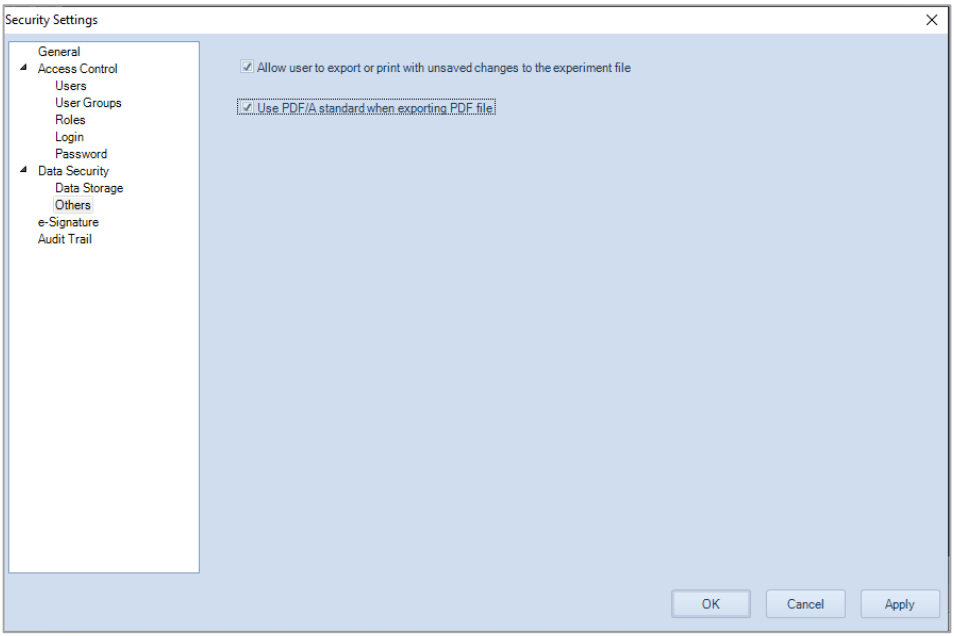
NOTE

If both **Store files by user account** and **Store files by month** are checked, the User Account will be the parent folder with sub-folders organized in month according to who created the experiment files and when the experiment files are created.

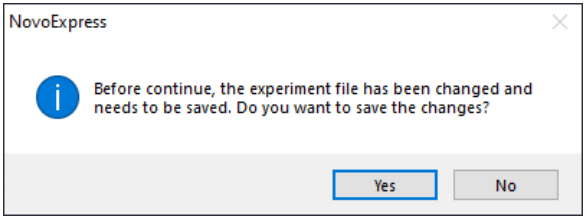
- **Allow user to change folder when saving file**: If not checked, new experiment files are saved to the default folder defined either by **Store files by user account** or **Store files by month**. If checked, user is allowed to select other folder where new experiment files are saved to. If **Secure Data Folder** is checked and enabled, user can only save the file to root data folder or its subfolders.
- **Allow user to change file name when saving file**: if not checked, software will automatically name the newly created experiment file as the default experiment file name in the format of **Experiment Date_Experiment Time.ncf**. For example, if the experiment file is created at 19:30 on 03/15/2022, the default file name will be **220315_1930.ncf**. This option is not available if **Allow user to change folder when saving file** is checked.
- **Allow user to overwrite existing file**: If checked, the existing files can be overwritten. This option is not available if **Compliance Supporting Mode** is enabled, or **Secure Data Folder** is checked.

Others

This subpage allows user to define the data export related settings for all users.



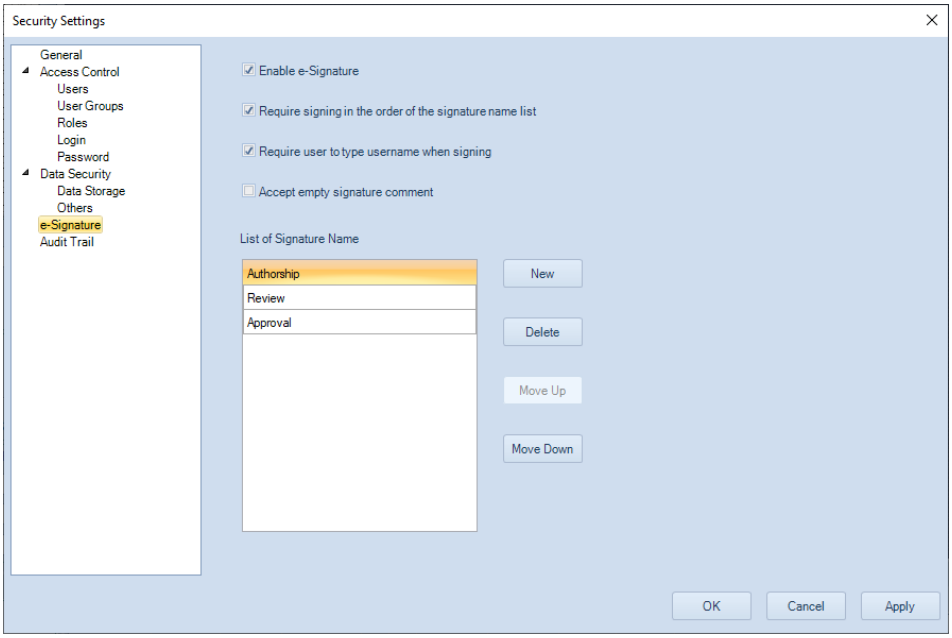
- **Allow user to export or print with unsaved changes to the experiment file**: if not checked, when user try exporting or printing an experiment file with unsaved changes, following error message will prompt. User needs to save the changes before exporting or printing.



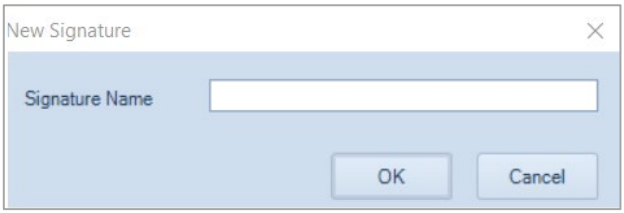
- **Use PDF/A standard when exporting PDF file:** if checked, PDF/A standard will be used when exporting PDF files.

e-Signature

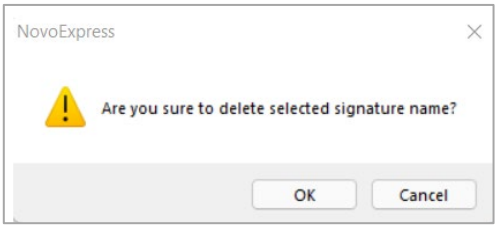
This page allows user to manage e-signature related settings. Only when NovoExpress Compliance License is registered, and user with permission of **Modify e-Signature Settings** can access the functions in this page. Please refer to Roles in this guide for more details on permissions.



- **Enable e-Signature**: Check to enable user to electronically sign the experiment file and experiment template file. If unchecked, all the settings in this page, the **Sign** function in **File** menu will not be available (i.e., greyed out). Please refer to File in this guide for more details on these two functions.
- **Require signing in the order of signature name list**: if checked, the file needs to be signed in the order defined by **List of Signature Name** displayed in this page.
- **Require user to type username when signing**: If checked, user needs to enter username and password before signing a file.
- **Accept empty signature comment**: if checked, user does not need to enter any comment before signing a file.
- **List of Signature Name**: Allows user to view and edit the current defined list of signature names. The default signature names are **Authorship**, **Review**, and **Approval**. User can also create a new signature name, delete a selected signature name or change the order of signature name.
    - **New**: When clicked, following window will be prompted. User can enter the new signature name and click **OK.** The new signature name will be created and listed in the end of signature name list.



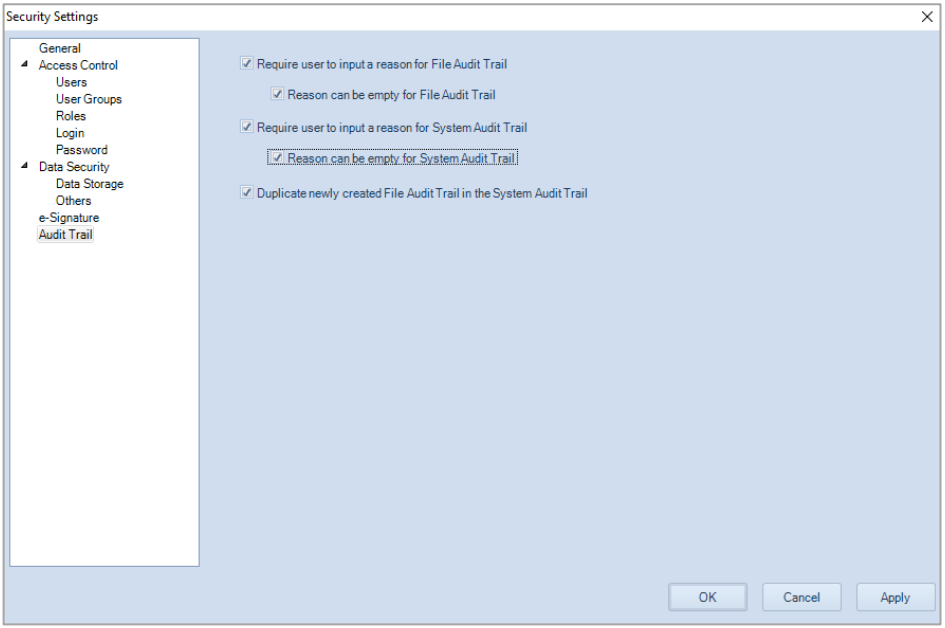
- **Delete**: When clicked, following window will be prompted. User can click **OK** to delete the selected signature name from the list. Any experiment file previously signed using this deleted signature name will not be affected by this operation.



- **Move up**: Click to move the selected signature name up in the list.
- **Move down**: Click to move the selected signature name down in the list.

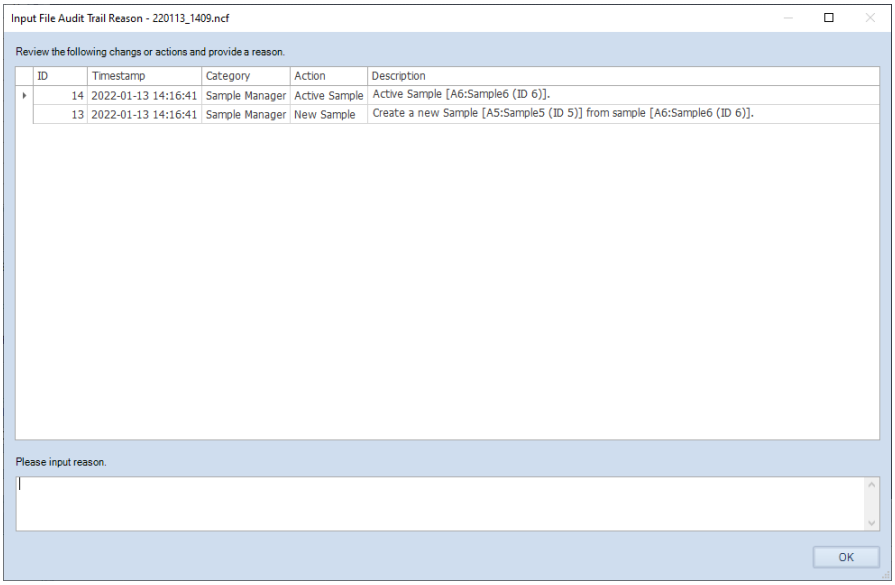
Audit Trail

This page allows user to manage audit trail related settings. Only when NovoExpress Compliance License is registered, and user logged in with permission of **Modify Audit Trail Settings** can access the functions in this page. Please refer to Roles in this guide for more details on permissions.



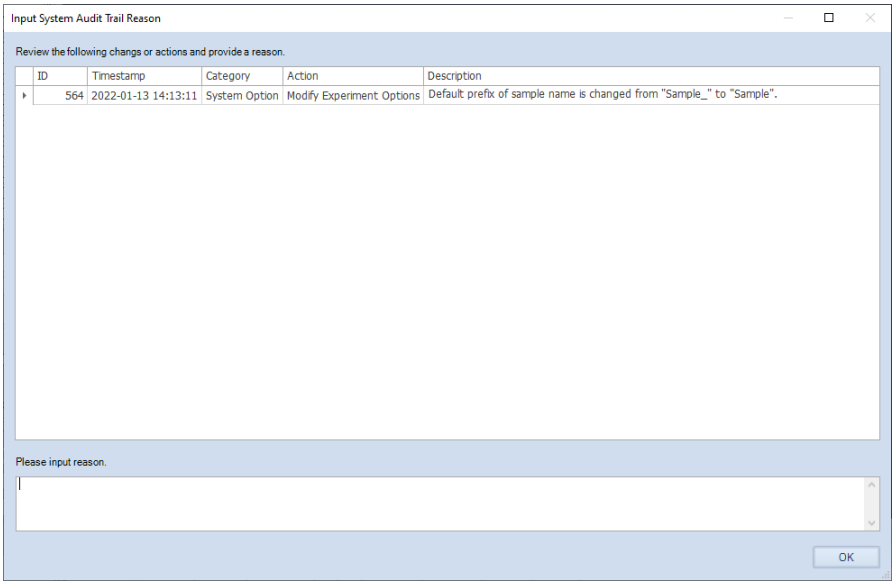
- **Require user to input a reason for File Audit Trail**: when checked, any time when a **File Audit Trail** is recorded, the following window will be prompted. User can review the changes or actions listed in this window, enter the reason for the changes (if **Reason can be empty for File Audit Trail** is not checked) and click **OK** to close this window.

  When **Reason can be empty for File Audit Trail** is checked, it is optional to enter the reason or comments in the **Input File Audit Trail Reason** window. User can click **OK** directly to close the window.



- **Require user to input a reason for System Audit Trail**: when checked, any time when a **System Audit Trail** is recorded, the following window will be prompted. User can review the changes or actions listed in this window, enter the reason for the changes (if **Reason can be empty for System Audit Trail** is not checked) and click **OK** to close this window.

  When **Reason can be empty for System Audit Trail** is checked, it is optional to enter the reason or comments in the **Input System Audit Trail Reason** window and can click **OK** directly to close the window.



- **Duplicate newly created File Audit Trail in the System Audit Trail**: if checked, all the **File Audit Trail** will be also shown in the **System Audit Trial**. Changing this setting will only affect the new created audit trails.

NOTE

Both File and System Audit Trail cannot be turned off. The software always records File and System Audit Trails. File Audit Trails are embedded into the data file (*.ncf) or experiment template file (*.ncet) and System Audit Trails are saved in the **SystemLog** file within software installation folder.

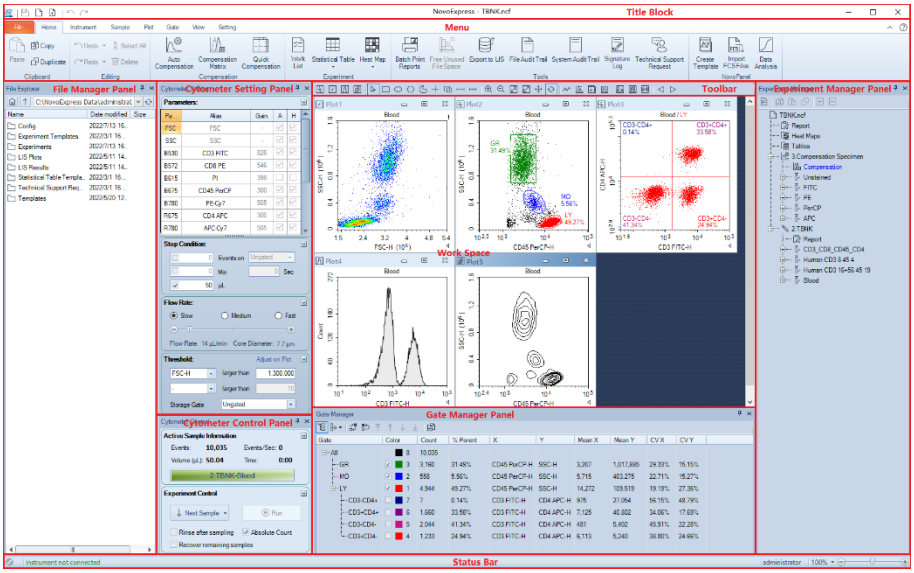
3 Use NovoExpress Software

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

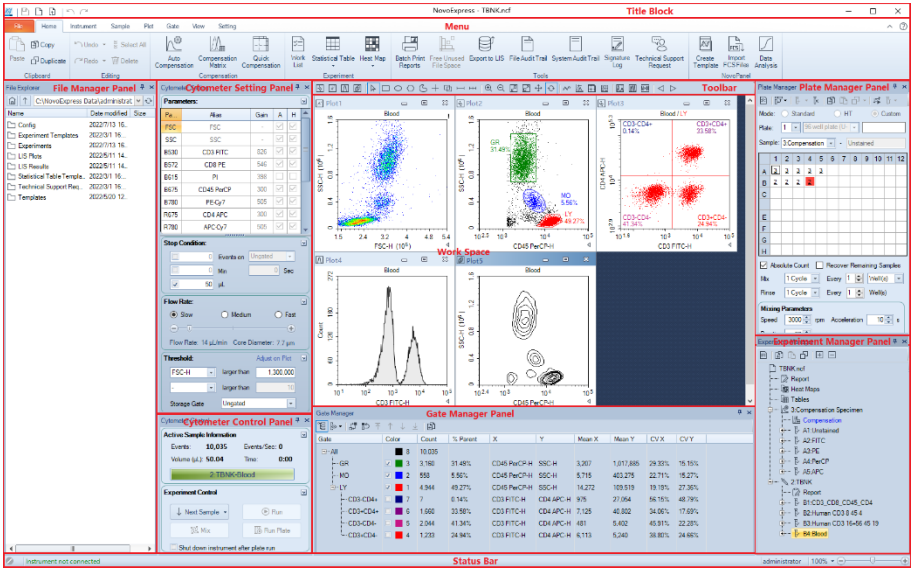
NovoExpress Software Interface

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

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

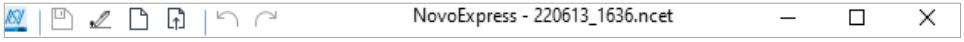


- If NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, user will see following interface. Compared to the software interface with instrument alone, differences are observed in the **Menu Bar**, **Cytometer Control** panel, **Experiment Manager** panel, and **Work List**. The **Plate Manager**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



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.

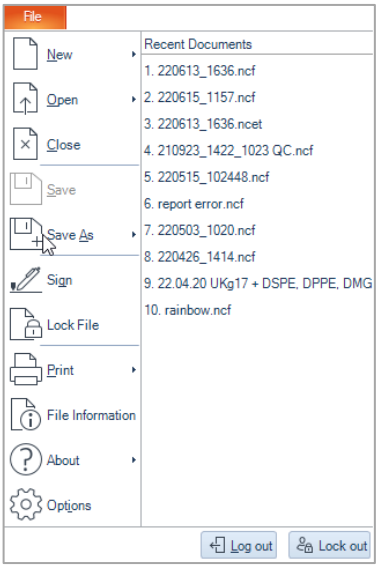


Icon	Description
	Clicking this button displays a drop-down menu with options to resize the display window and close the software.
	Saves the experiment file.
	Electronically sign the experiment file or experiment template file. Refer to e-Signature and File in this guide for more details.
	Creates a 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 - Untitled.ncf	Displays the name of the software (i.e., NovoExpress) and name of the currently opened experiment file.
	Minimizes window.
	Maximizes window.
	Restores window.
	Closes window.

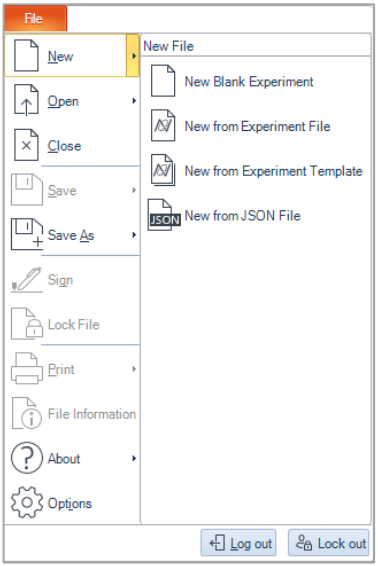
Menu

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

File

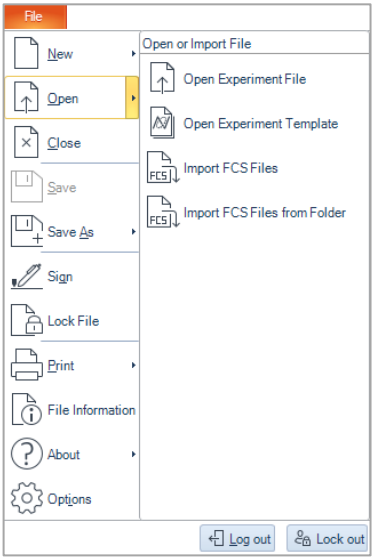


Icon	Description
	New: Click to create new experiment file.



- **New Blank Experiment**: Creates a new blank experiment file.
- **New from Experiment File**: Creates a new experiment file with the experiment setting and data analysis method inherited from an existing experiment file. When NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon is connected, 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.
- **New from Experiment Template**: Creates a new experiment file with the experiment setting and data analysis method inherited from an existing experiment template.
- **New from JSON File**: Creates a new experiment file with the experiment setting and data analysis method inherited from an existing JSON file. This option is only available when NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon is connected or selected in **Instrument Configuration**.

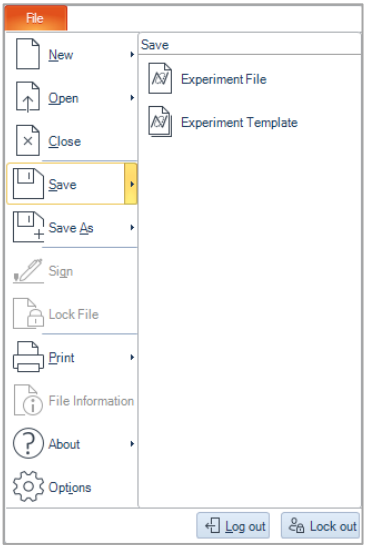
Open: Click to open or import file.



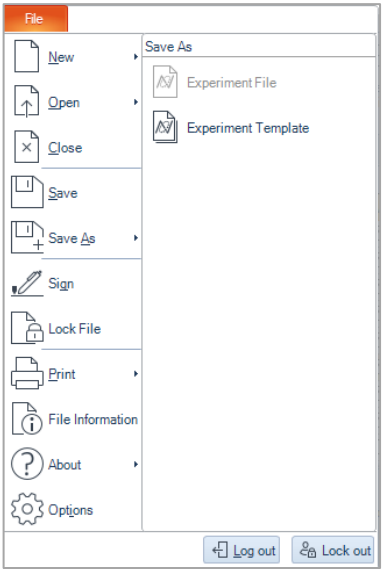
- **Open Experiment File**: Opens an experiment file.
- **Open Experiment Template**: Opens an experiment template.
- **Import FCS files**: Imports selected file to the current specimen.
- **Import FCS Files from Folder**: Imports all FCS files in a selected folder to the current FCS specimen.

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

Save: Click to save the experiment data, experiment setup, and data analysis of the current experiment file or experiment template. This function will be automatically disabled if the current experiment file or template is already saved.



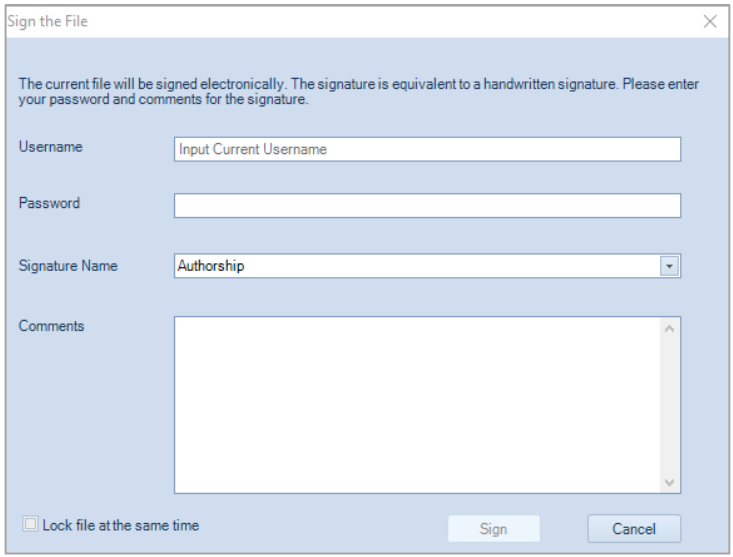
Save As: Click to saves the current file as either experiment file or experiment template to a specified location. The **Experiment File** option will be automatically disabled if the **Compliance Supporting Mode** is enabled in **Security Setting** window.



Sign: Click to sign the current experiment file or template electronically. This function is available to user with permission of **Sign Experiment File** or **Sign Experiment Template**, and when **Enable e-Signature** option is enabled in **e-Signature** page in the

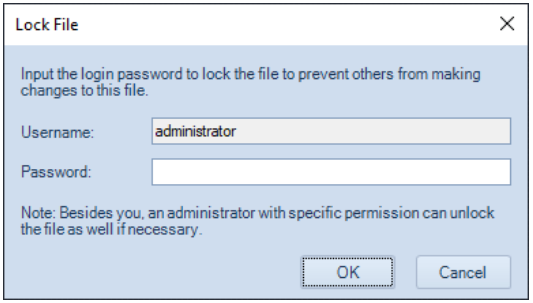
Security Setting window, and the file is open by software. Refer to e-Signature in this guide for more details.
When clicked, following window will be prompted,

NOTE When a new experiment file is created, ensure to save it first before sign it.

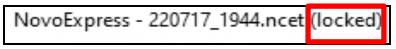


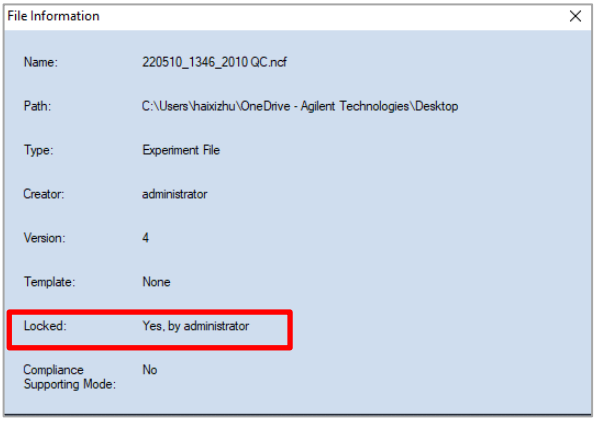
- **Username**: Enter the username of current user account. User cannot edit this area if **Require user to type username when signing** option in **Security Setting** window is not checked.
- **Password**: Enter the password of current user account.
- **Signature Name**: Allows user to select the proper signature name from the drop-down list. If **Require signing in the order of signature name list** in **Security Setting** window is checked, only the current available signature name will be shown in the **Signature Name** drop-down list on this window. Refer to e-Signature in this guide for more details.
- **Comments**: Allows user to enter signature related comments. This is optional if **Accept empty signature comment** is checked in **Security Setting** window.
- **Lock file at the same time**: When checked, the file will be automatically locked after signed. Refer to Lock File in this guide for more details.

Lock File: Allows user to lock/unlock an experiment file or experiment template file to prevent/allow further editing of the file. Once a file is locked, any changes (including e-signature) to the file cannot be saved unless user unlocks it first. This function is available only when **NovoExpress Compliance License** is registered, and **Lock/Unlock Experiment File** or **Lock/Unlock Template File** permission is enabled for current user account.
When clicked, following window will be prompted. User needs to enter password of current user account and click **OK** to lock the file.



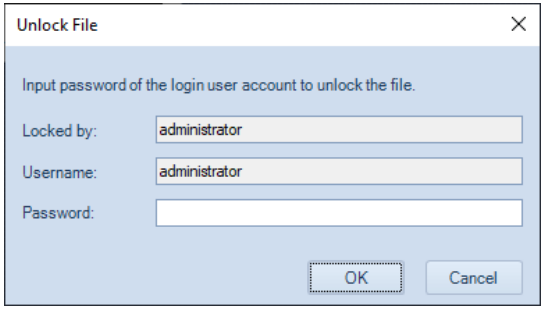
After the file is locked, the file name in the **Title Block** area and **File Information** window will automatically updated to show the locking status. An example is shown below.



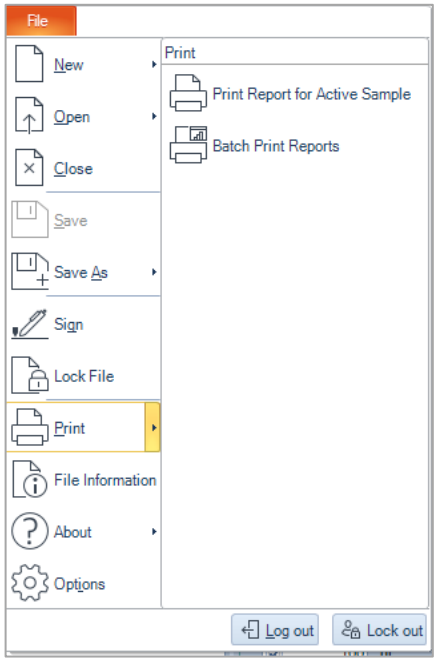


NOTE When **Lock file at the same time** is checked in **Sign the File** window, the will be automatically locked after signed. Refer to Sign in this guide for n details.

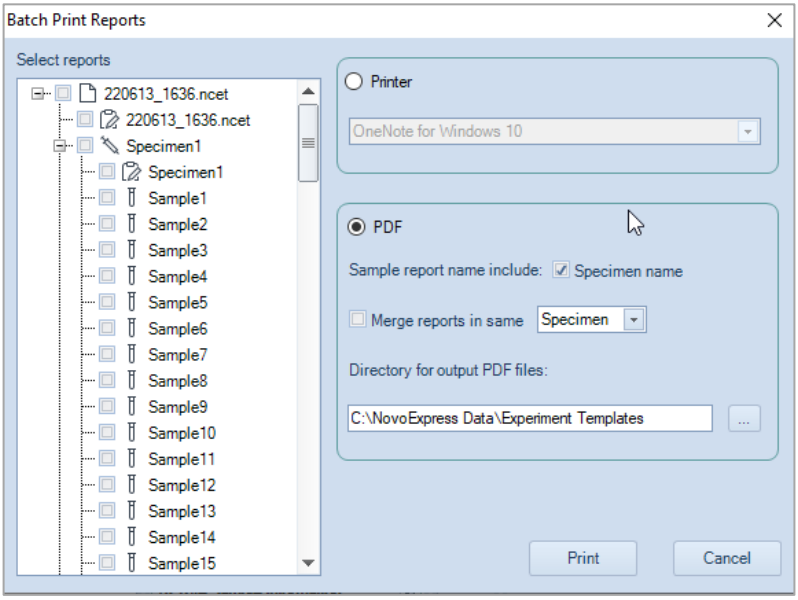
Once a file is locked, user can unlock it by clicking this icon again. In prompted window, enter the password associated with displayed user account, click **OK**. Only user who locked the file or user who has the permission of **Unlock File Locked by Others** can unlock the file.



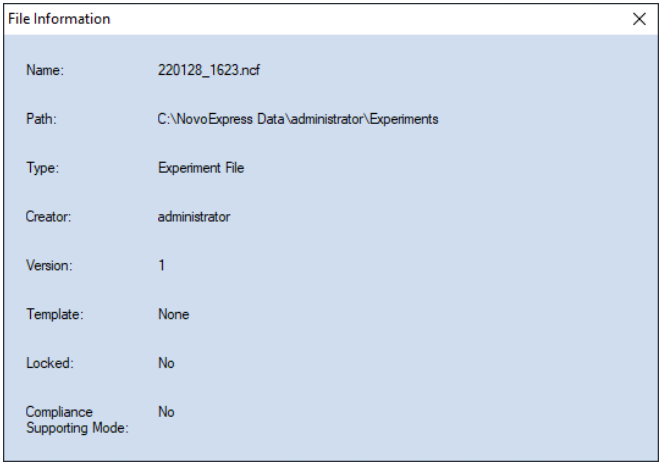
Print: Allows user to print sample report/batch print reports



- **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.



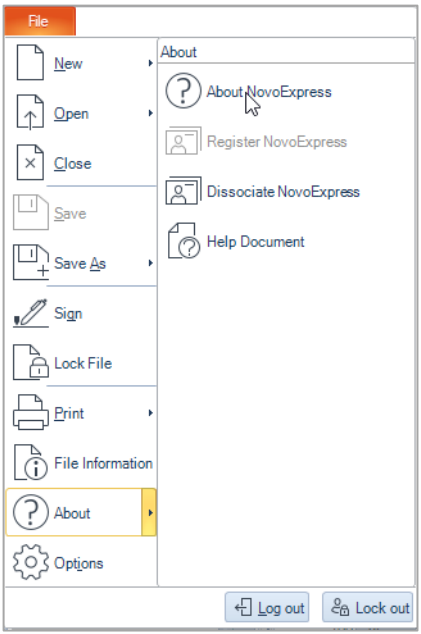
File Information: Contains experiment file or experiment template file related information (e.g., Name, Type, Path). When clicked, following window will appear.



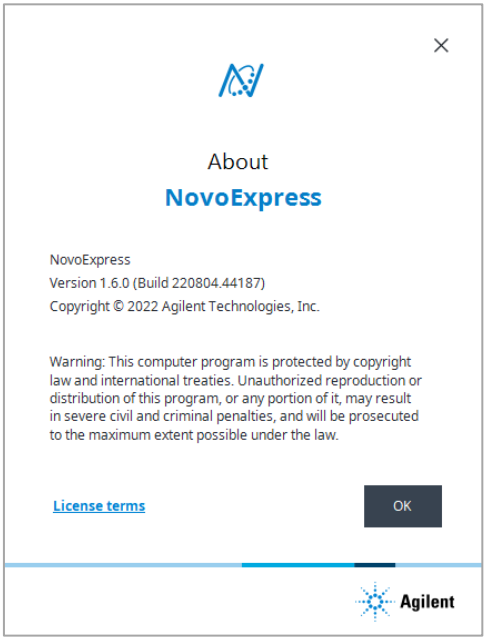
- **Name**: Displays the name of current experiment or experiment template file.
- **Path**: Displays the path where the file is currently stored.
- **Type**: Displays the file type (i.e., Experiment File or Experiment Template).
- **Creator**: Displays the username of user who created the file.
- **Version**: Displays the version of the file. A version number of 1 is automatically assigned to any new file. This version number increase by 1 each time when the file is changed and saved.

- **Template**: Displays the path and name of the template file which the current experiment file was created from.
- **Locked**: Indicates whether the file is locked or not. If the file is locked, the username of the user who locks the file will be displayed as well.
- **Compliance Supporting Mode**: Indicates whether the **Compliance Supporting Mode** is enabled when the file was created and modified.

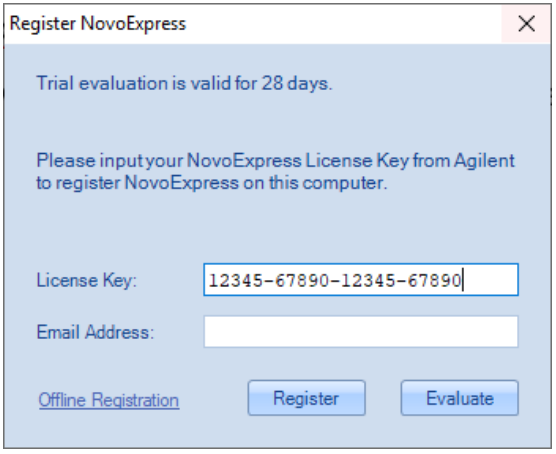
About: Contains the software version, copyright information, help files, software registration and dissociation information.



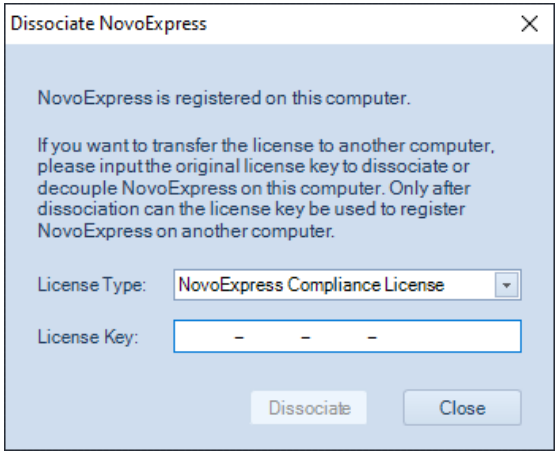
- **About NovoExpress**: When clicked, following window will appear to allow user to view the software version and copyright information.



- **Register NovoExpress**: When clicked, following window will appear to allow user to register the NovoExpress software. This function will be automatically disabled if both NovoExpress License and NovoExpress Compliance License are successfully registered. Refer to Register NovoExpress Software in this guide for more details.

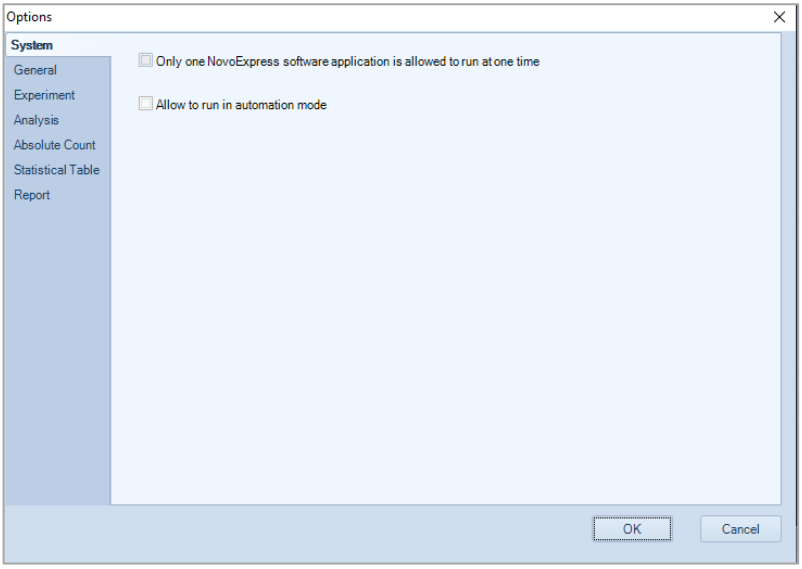


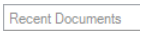
- **Dissociate NovoExpress**: When clicked, following window will appear to allow user to dissociate or decouple NovoExpress from this computer. Refer to Dissociate NovoExpress Software in this guide for more details.



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

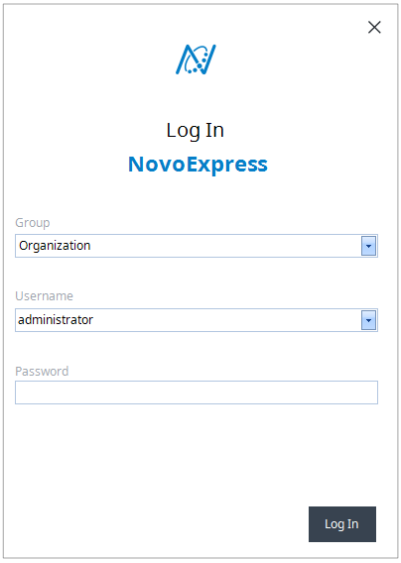
Options: Click to open the **Option Settings** window. Refer to Setting in this guide for details.



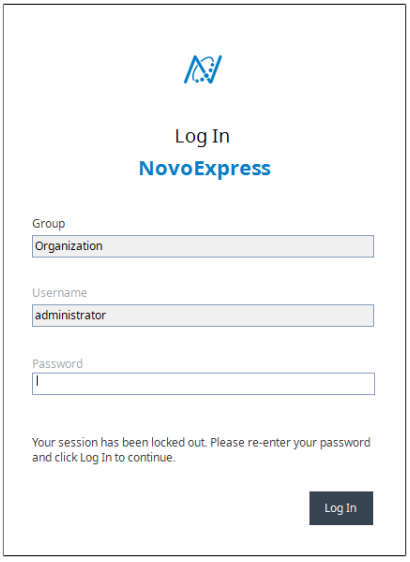


Recent Documents: Shows recently opened data files. Up to 10 files can be displayed. Click the file name to directly open the corresponding file.

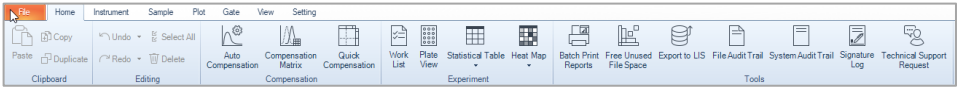
Logout: Click to exit and log out the current account. The login window below will appear. User needs to enter the correct username and password again and click **Log In** to log back into the software.



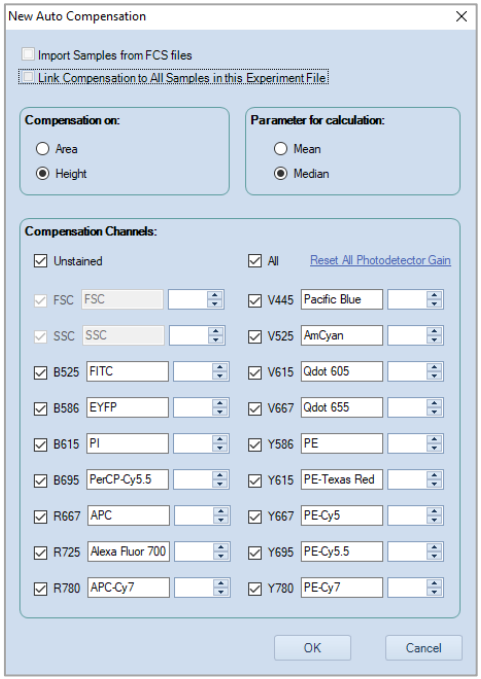
Lock out: Lock out the current software session. When clicked, following window will be prompted. User needs to enter the correct password again and click **Log In** to continue operation of the software.



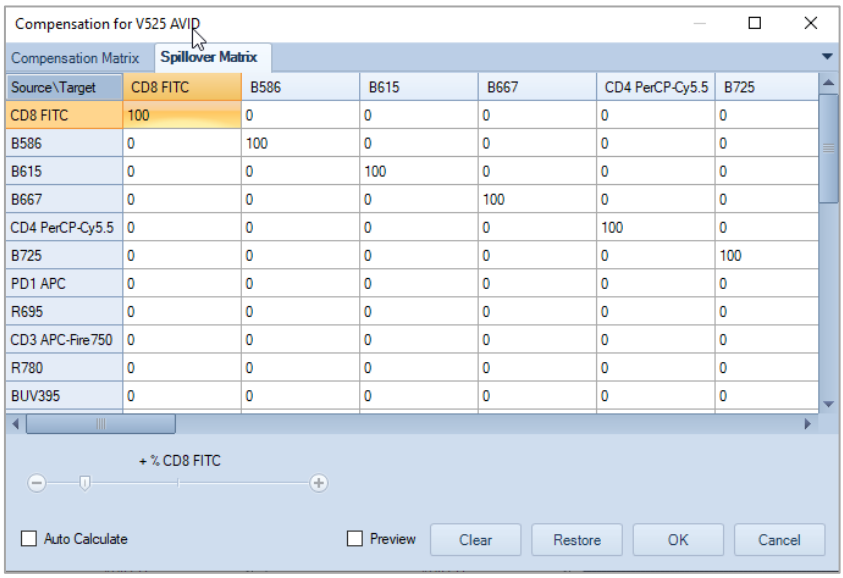
Home



Icon	Description
Clipboard	
	Copy: Click to copy the selected gate or selected data.
	Paste: Click to paste the copied gate to other plots or pastes the copied item in the **Experiment Manager**.
	Duplicate: Click to create a duplicate of the plot (the gates will not be included.) or creates a duplicate sample or specimen.
Editing	
	Click to undo drawing gates, deleting gates, zooming in or zooming out on a plot, etc. This operation is only effective for plot-related functions.
	Click to 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: Click to select all the gates in the current plot.
	Delete: Click to delete the selected gate.
Compensation	
	Auto Compensation: Click to open the **New Automatic Compensation** window. Refer to Fluorescence Compensation in this guide for more details.



Compensation Matrix: Click to displays the current compensation matrix for the selected sample. Refer to Fluorescence Compensation in this guide for more details.



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

Experiment

Work List: Click to open the **Work List**. Refer to Work List in this guide for more details.

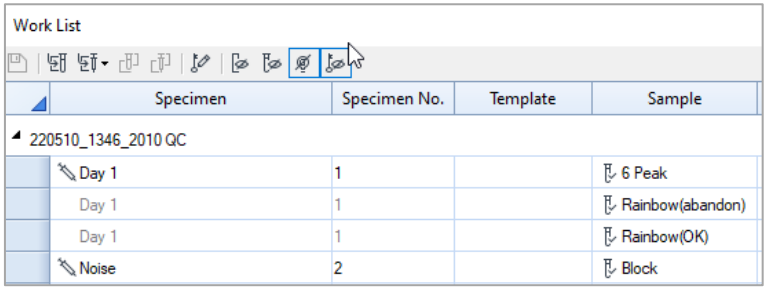
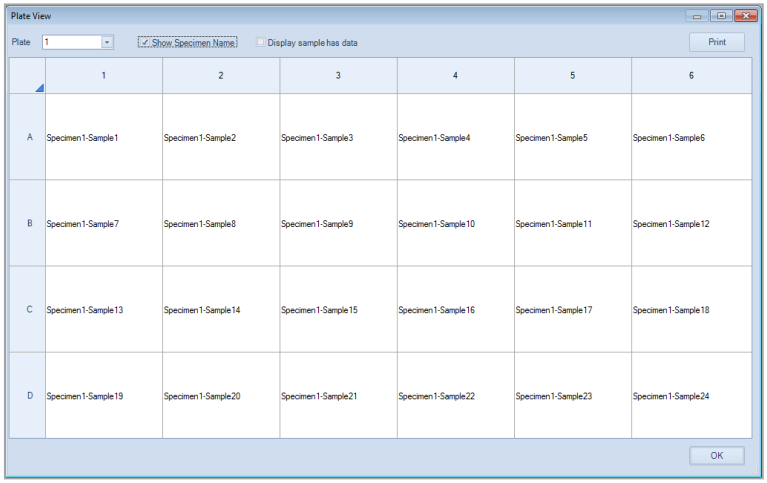
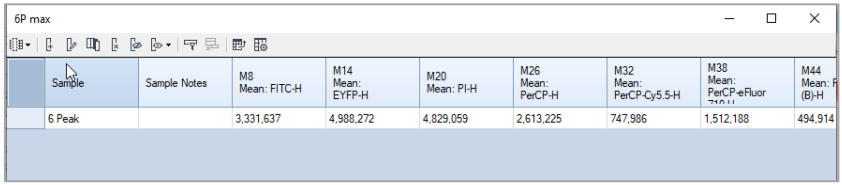


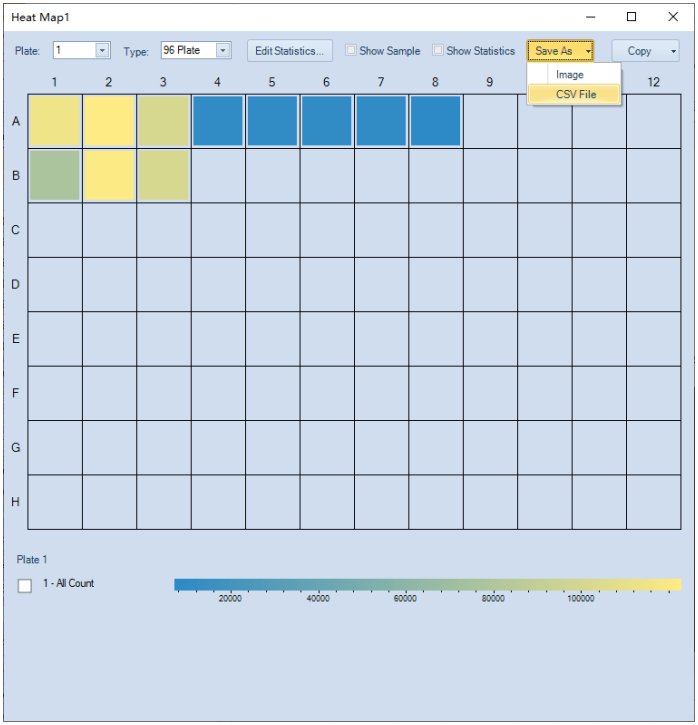
Plate View: Click to open **Plate View** window. This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used.
This window is read only and is used to view the sample information for each well on a plate view. The **Plate** drop-down menu lists the **Plate ID** for all 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 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: Click to create a statistical analysis table. Please refer to Statistical Tables in this guide for more details.

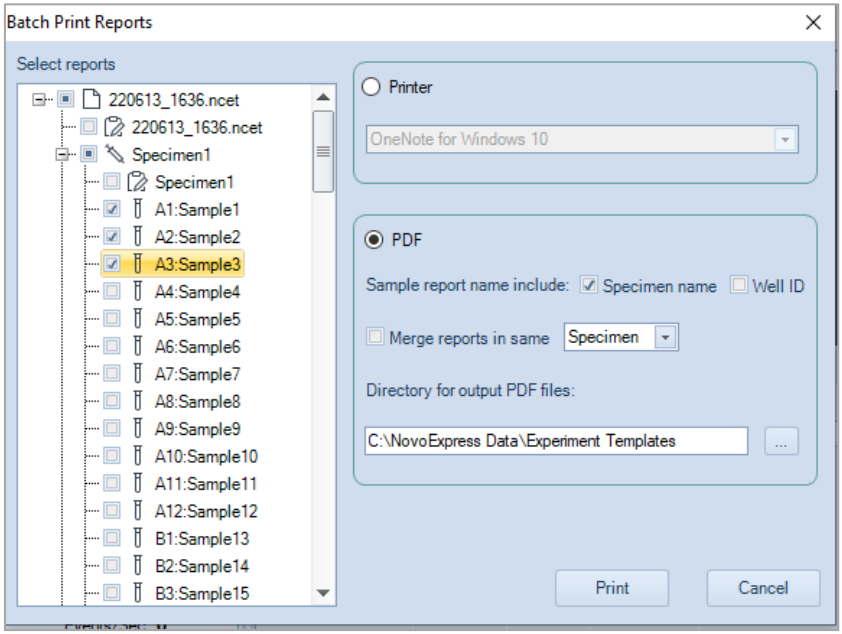


Heat Map: Click to create a heat map for the defined parameter in a plate layout format. Please refer to Heat Map in this guide for more details.



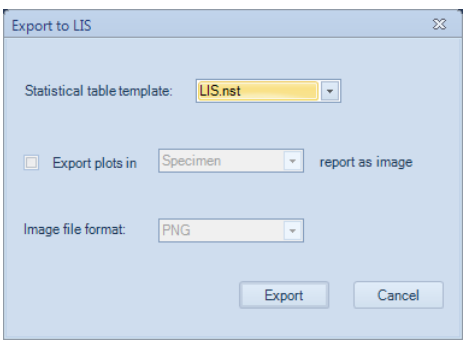
Tools

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.

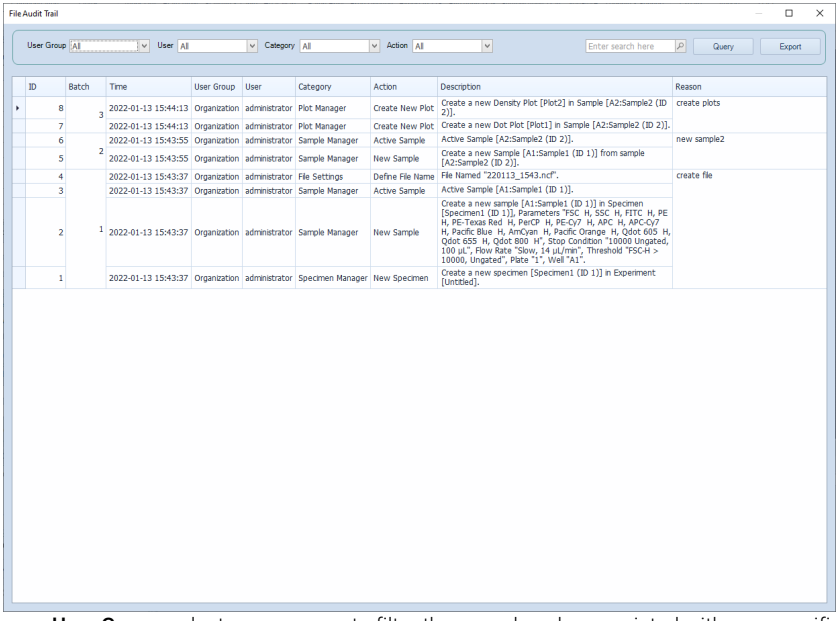
Export to LIS: Allows user to export data analysis results in the designated format to CSV file and such CSV file can be parsed by a Laboratory Information System (LIS) or read by other programs. When clicked, following window will be prompted.



- **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.

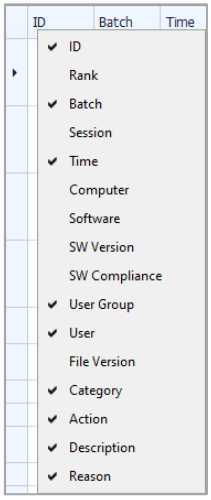
- **Export plots in Specimen/Sample report as image**: Once selected, plots in the specimen report or sample report will be exported as images in the selected image file format to the designated folder.
- **Image file format**: Sets the image file format of the export plots, including PNG, JPEG, Bitmap, 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 Data\administrator**" and can be changed. Refer to Setting for details of changing the default user data root folder.

File Audit Trail: Allows user to view and export the operation activities associated with the experiment file. This function is available only when a ncf file or ncet file is open, and **View File Audit Trail** permission is enabled in **Security Settings** window for current user. Please refer to Audit Trail in this guide for more details.
When clicked, following window will be prompted.



- **User Group**: select a user group to filter the records only associated with one specific user group.
- **User**: select a user to filter the records only associated with one specific user.
- **Category**: select the category (e.g., Cytometer Setting, Sample) to filter the records.
- **Action**: select the type of action (e.g., Modify Parameter) to filter the records.
- : enter a keyword in the text box and click to search the associated records.
- **Query**: click to query the records.
- **Export**: click to export the records shown in the table to a CSV file.

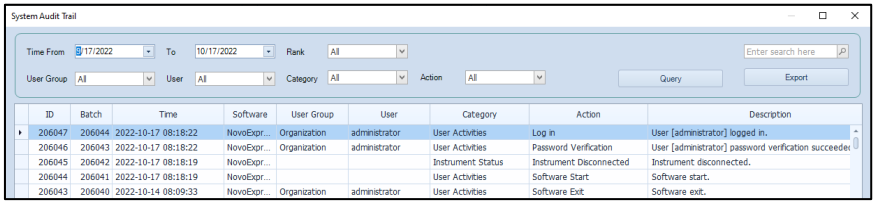
User can also right click table header row and customize the item to be displayed in this table.



System Audit Trail: Allows user to view and export system operation related activities. This function is available only when **View System Audit Trail** permission is enabled in **Security Settings** window for current user. Please refer to Audit Trail in this guide for more details.

NOTE If "**Duplicate newly created File Audit Trails in the System Audit Trail**" is enabled in **Security Settings** window, all File Audit Trails will also be displayed in **System Audit Trail** window.

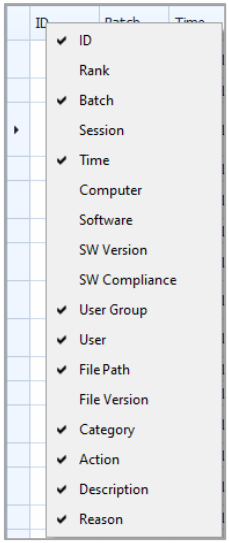
When clicked, following window will be prompted.



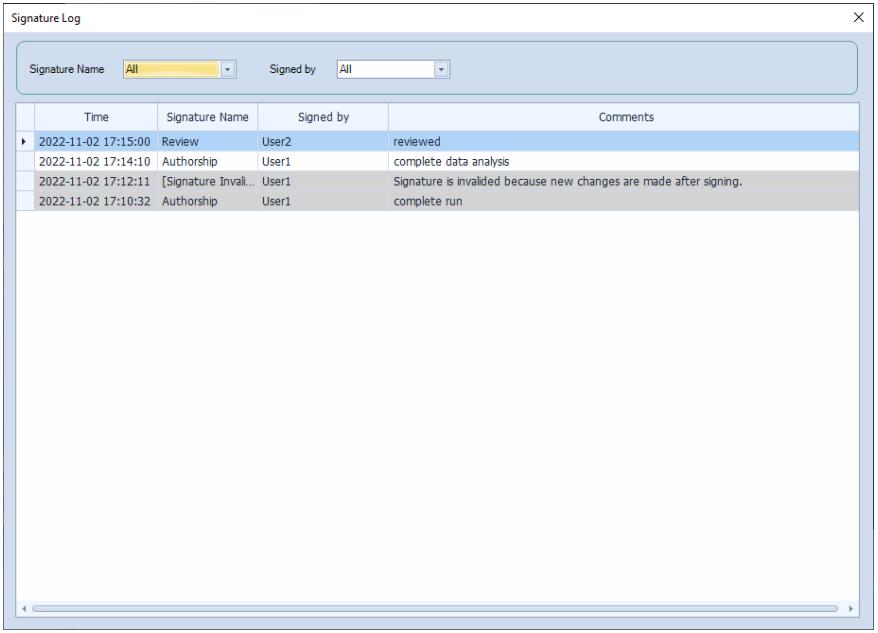
- **Time From**: Allows user to specify the start date for the filtered records. By default, it is one month before the current date.
- **To**: Allow user to specify the end date for the filtered records. By default, it is set to be the current date.
- **Rank**: Allows user to select type of information to be displayed. User can select to display all information, or select **Information, Warning** or **Error** from the drop-down list.
- **User Group**: Allows user to specify user group whose associated recorded information will be displayed.
- **User**: Allows user to specify the user whose associated recorded information will be displayed.
- **Category**: Allows user to select the category of recorded information. For example, Instrument Operation, Cytometer Settings, etc.
- **Action**: Allows user to select the type of action (e.g., New Sample) for recorded information.

- : Allows user to enter a keyword and search the recorded information containing the keywords.
- **Query**: click to filter the recorded information. The table below will be automatically updated.
- **Export**: click to export the displayed records to a CSV file.

User can also right click table header row and check the item to be displayed in this table.



Signature Log: Allows user to view the signature history of current file. When clicked, following window will be prompted.



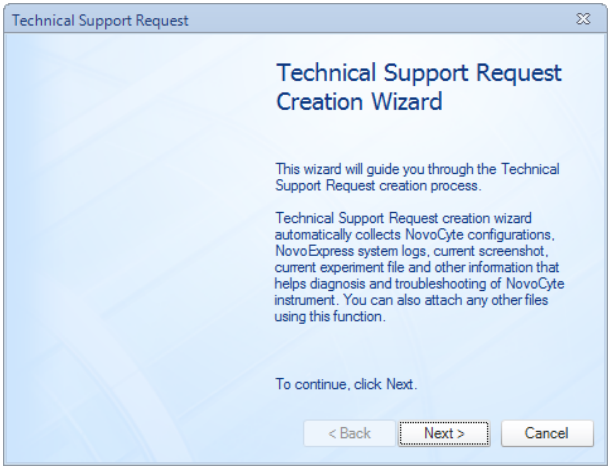
- **Signature Name**: Allows user to filter the records to only show the signature records from selected signature name.
- **Signed by**: Allows user to filter the records to only show the signature records from selected user account.

The bottom panel of this window allows user to view the filtered signature records.

- **Time**: Indicates when the file was signed.
- **Signature Name**: Indicates the signature name user selected when signing the file. This area will display **[Signature Invalided]** when changes are made after the file has been signed.
- **Signed by**: Indicates username of the user account who signed the file.
- **Comments**: displays the comments user entered when signing the file or other signature related information (e.g., invalid signature).

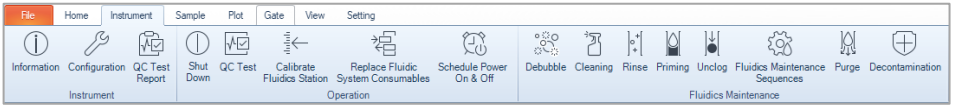
NOTE A valid signature is shown in white background while invalid signature is shown as gray background.

Technical Support Request: Click to open **Technical Support Request Creation Wizard** which automatically collects instrument configurations, NovoExpress 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.



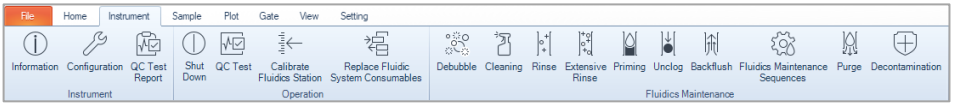
Instrument

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 (or NovoSampler Pro or NovoSampler Q) is connected, additional functions are available.

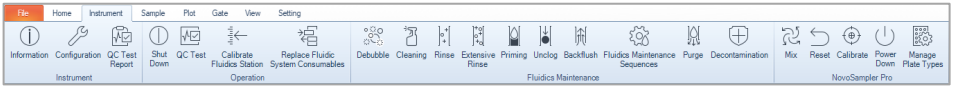


When NovoCyte flow cytometer is connected

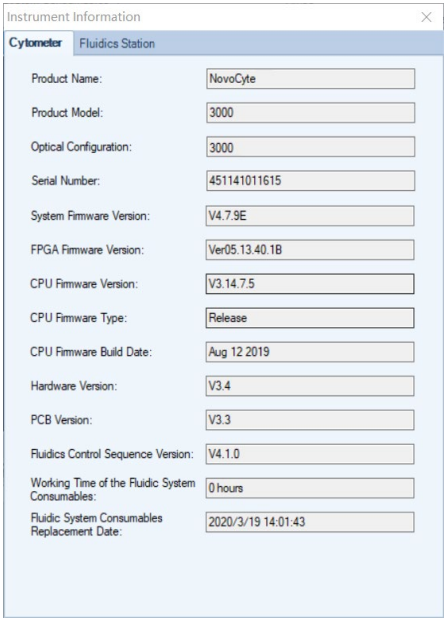
When NovoCyte flow cytometer and NovoSampler (or NovoSampler Pro) are connected

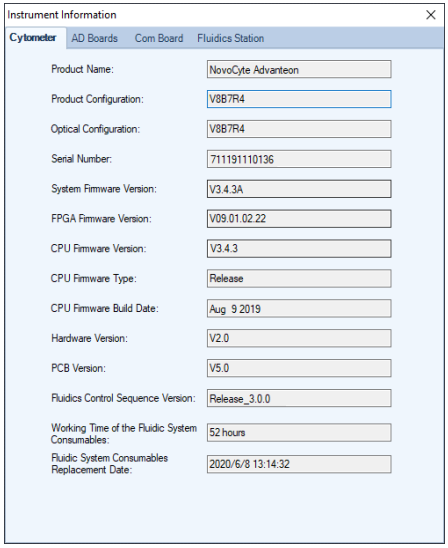


When NovoCyte Quanteon (or Advanteon or Penteon) flow cytometer is connected

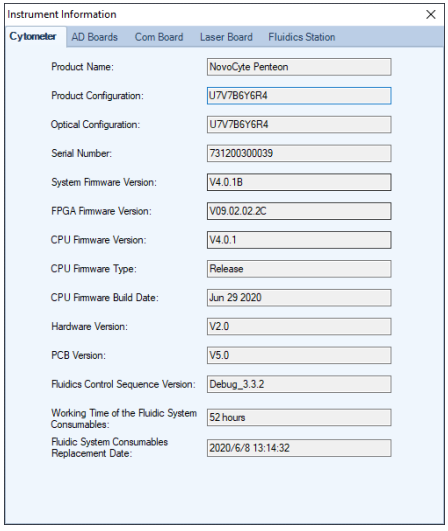


When NovoCyte Quanteon (or Advanteon or Penteon) flow cytometer and NovoSampler Q are connected

Icon	Description
Instrument	
ⓘ	**Information**: Click to display the instrument information. This option is automatically disabled when instrument is not connected. • When NovoCyte flow cytometer is connected, following window is prompted. User can view the hardware information (e.g., Product Model) for the instrument and fluidics station (or cart).  • When NovoCyte Quanteon and Advanteon flow cytometer is connected, following window is prompted. User can view the hardware information (e.g., Product Model) for the instrument, AD boards, COM board and fluidics station (or cart).

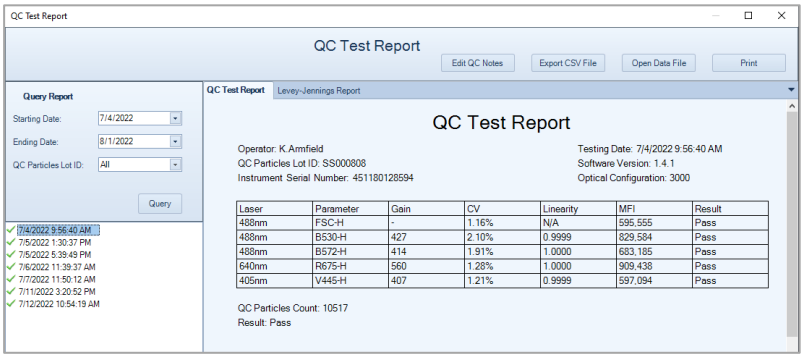


- When NovoCyte Penteon flow cytometer is connected, following window is prompted. User can view the hardware information (e.g., Product Model) for the instrument, AD boards, COM board, Laser board and fluidics station (or cart).



Configuration: Click to display and modify the excitation laser and detection channel and options for the NovoSampler, NovoSampler Pro or NovoSampler Q. Please refer to Instrument Configuration in this guide for details.

QC Test Report: Click to display 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 connected.

Shut Down: Click to start the instrument shutdown process. After completion, the instrument will automatically be turned off. To clean sample injection probe while shutting down instrument, see corresponding instrument operator's guide for more information.

QC Test: Click to run the instrument QC test. Please refer to QC Test in this guide for details.

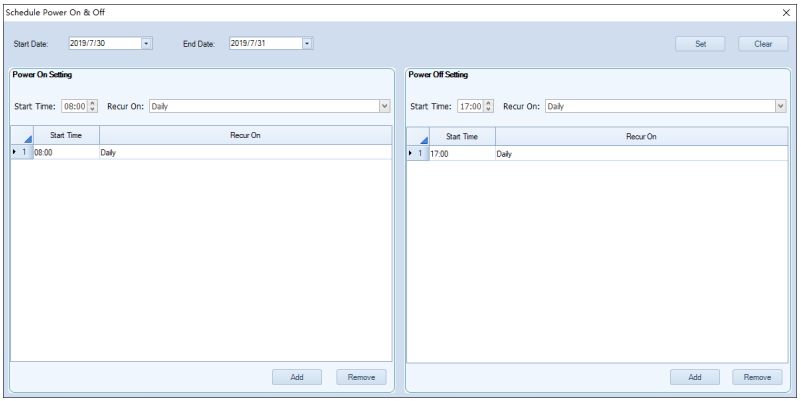
Calibrate Fluidics Station (or **Fluidics Cart**): Long distance transportation, movement and other reasons may cause the fluidics station (or Fluidics Cart) system malfunction. Use this function to re-calibrate the fluidics station (or Fluidics Cart). When clicked, user needs to follow the instructions in prompted window to remove the instrument reagent containers from the fluidics station (or Fluidics Cart) and perform the calibration. Only accounts with the permission of **Calibrate Fluidics Station** (or **Fluidics Cart**) can calibrate the fluidics station (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. See corresponding instrument operator's guide for more information.

NOTE Agilent flow cytometer monitors the accumulated running time of the fluic system consumables to ensure the consumables are changed in a timely manner for optimal flow cytometry results. When the accumulated runnin time is reached, NovoExpress 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. This function is available to user with permission of **Schedule Power On & Off.**

NOTE This function is applicable only to NovoCyte Quanteon, Advanteon or Penteon flow cytometer.

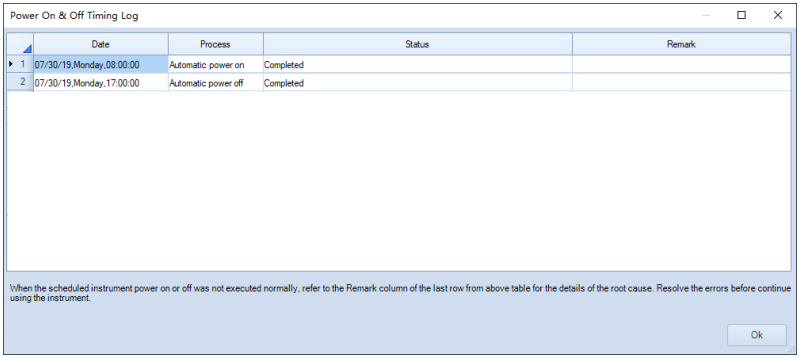


- **Start Date**: Select the start date of the instrument power on and off. The start date cannot be set to 7 days or more over the current date.
- **End Date**: Select the end date of the instrument power on and off.
- **Start Time**: Select the start time of instrument power on or off.
- **Recur On**: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

Debubble: Click to remove the air bubbles from the fluidic system.

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

Rinse: Click to rinse the fluidic system using a rinsing solution.

Extensive Rinse: Click to perform an extensive rinse of the fluidic system.

NOTE This function is applicable only to NovoCyte flow cytometer.

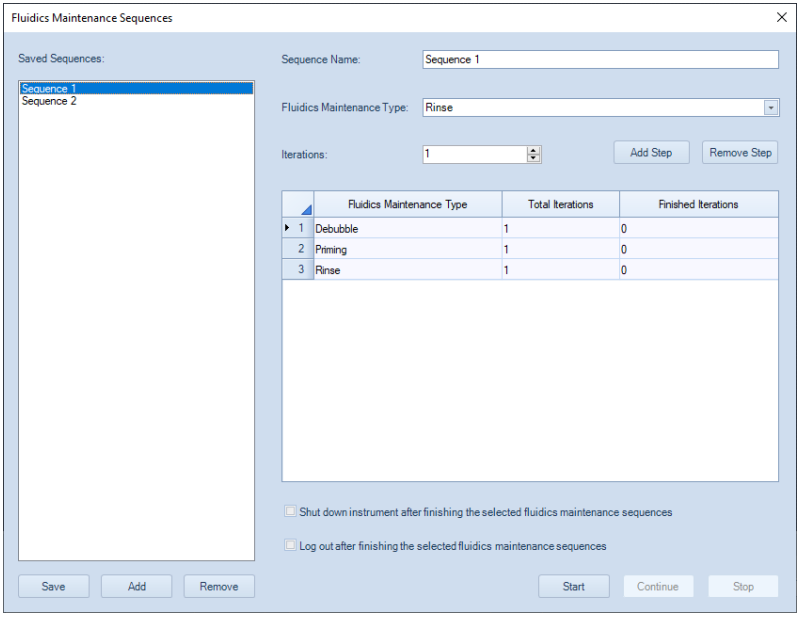
Priming: After the instrument has not been in use for a period of time, user can click this button to clear the air bubbles and primes the fluidic system with fresh sheath fluid.

Unclog: Click to clear blockage from the flow cell.

Backflush: Click to clear blockage from the sample injection probe.

NOTE This function is applicable only to NovoCyte flow cytometer.

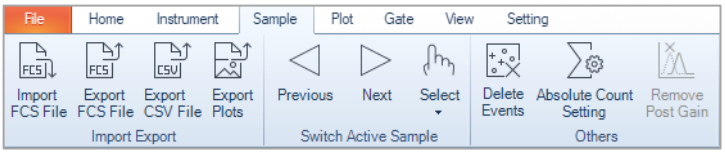
Fluidics Maintenance Sequences: Click to program and run multiple fluidics maintenance sequences in pre-defined order.

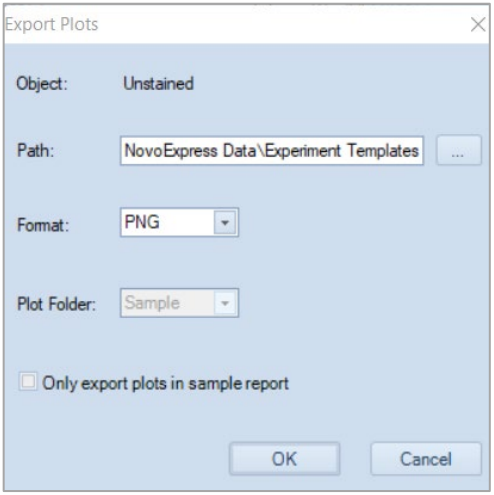


- **Saved Sequences**: Displays all the sequences user have saved.
- **Save**: Click to save any changes to the selected sequence.
- **Add**: Click to add the newly created maintenance sequences to **Saved Sequences** area.
- **Remove**: Click to delete the selected sequence from **Saved Sequences** area.
- **Sequence Name**: Displays the name of the selected sequence from **Saved Sequences** area.
- **Fluidics Maintenance Type**: Select fluidics maintenance step (e.g., **Cleaning**, **Rinse**, **Extensive Rinse**, **Priming**, **Unclog**, **Backflush** for NovoCyte, and **Cleaning**, **Rinse**, **Priming**, **Unclog** for NovoCyte Quanteon, Advanteon and Penteon)
- **Iterations**: Set the number of iterations for the selected fluidics maintenance step.
- **Add Step**: Click to add the selected fluidics maintenance step to the table.
- **Remove Step**: Click to remove the selected fluidics maintenance step 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**: When checked before clicking **Start**, after all programmed fluidics maintenance steps completes, only the instrument will automatically be shut down while NovoExpress will not be closed.
- **Log out after finishing the selected fluidics maintenance sequences**: When checked before clicking **Start**, NovoExpress software will be automatically logged out after all programmed fluidics maintenance steps completes.
- **Start**: Click to start the fluidics maintenance steps in the pre-defined order (top to bottom).
- **Stop**: Click to stop the fluidics maintenance steps. The fluidics maintenance step currently running will continue until completed, but the remaining scheduled fluidics maintenance steps will be aborted.

	• **Continue**: Click to continue the remaining fluidics maintenance steps which was not completed.
	Purge: If Agilent flow cytometer needs to be shipped, click this button and follow the procedure shown on the popup window to purge the fluidic system before packaging and shipment. Only accounts with the permission of **Purge Instrument** enabled can perform the purge operation. Refer to associated instrument operator's guide for detailed procedure.
	Decontamination: If Agilent flow cytometer is known to have contamination or to prevent the occurrence of contamination, click this button and follow the instruction shown in the popup window to decontaminate the instrument. Only accounts with the permission of **Decontaminate Instrument** can perform this decontamination operation. Refer to associated instrument operator's guide for detailed procedure.
NovoSampler (or NovoSampler Pro or NovoSampler Q)	
NOTE	The functions or actions listed in this tab (except **Manage Plate Types**) are only available when the NovoSampler or NovoSampler Pro or NovoSampler Q is 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 (or NovoSampler Pro or NovoSampler Q) in case of misalignment or error.
	Calibrate: Click to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q).
	Power Down/Up: When clicked, NovoSampler (or NovoSampler Pro or NovoSampler Q) will be powered on (if currently powered off) and allow the operation of the NovoSampler (or NovoSampler Pro or NovoSampler Q). After execution, the status indication LED on NovoSampler (or NovoSampler Pro or NovoSampler Q) will light up in green color. The NovoExpress will prompt a message to restart the software. After the software restart, a dialog box requesting to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q) will be shown. Click **Calibrate** to start the automatic calibration procedure. The software features for control and use of the NovoSampler (or NovoSampler Pro or NovoSampler Q) will be available after restart of the NovoExpress Software. If the NovoSampler (or NovoSampler Pro or NovoSampler Q) is already powered on, clicking this button will power off the NovoSampler (or NovoSampler Pro or NovoSampler Q), the status indication LED on NovoSampler (or NovoSampler Pro or NovoSampler Q) will be off. The NovoExpress will prompt a message to restart the software. After the software restart, only the instrument stand-alone features will be available. This feature is only visible when logged into NovoExpress Software with the account with permission of **Power Down/Up NovoSampler**.
	Manage Plate Types: Click to add, remove, and modify plate settings in prompted window. Refer to Manage Plate Types in this guide for more details.

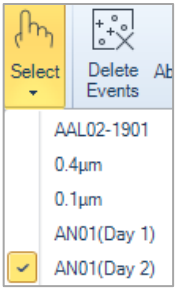
Sample



Icon	Description
Import and Export	
	Import FCS File: Click to import selected FCS format files to the active sample. Refer to Import Data in this guide for more details.
	Export FCS File: Click to export the current data into a FCS file. Refer to Export Data in this guide for more details.
	Export CSV File: Click to export the current data into a CSV file. Refer to Export Data in this guide for more details.
	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: Click to switch the Active Sample to the previous sample.

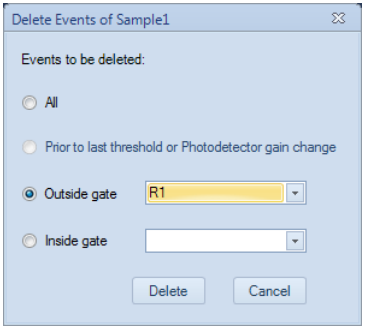
Next: Click to switch the Active Sample to the next sample.

Select: Click to switch the Active Sample to the one selected from the prompted drop-down menu.



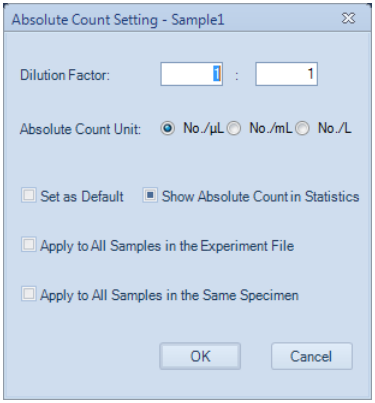
Others

Delete Events: Click to delete events from a sample. When clicked, following window will be prompted. User can 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 permission of **Delete Sample Events** 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: Click to set up absolute counting conditions for active sample. When clicked, following window will be prompted.



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

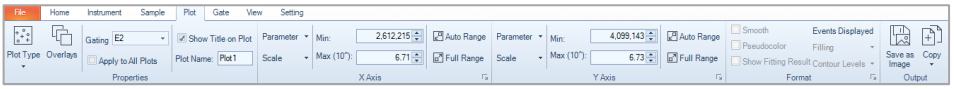
- : Show **Absolute Count** column for all plots of the sample.
- : No change.
- : Hide **Absolute Count** column for all plots of the sample.

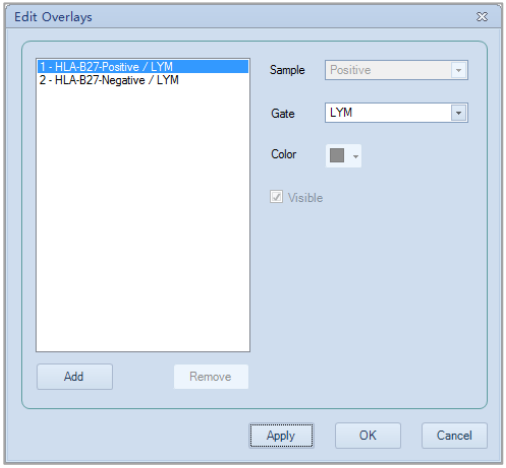
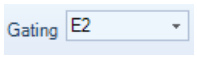
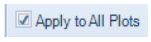
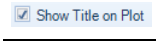
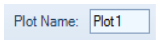
- **Apply to All Samples in the Experiment File**:
  Set Absolute Count Setting for all samples in the experiment file.
- **Apply to All Samples in the Same Specimen**:
  Set Absolute Count Setting for all samples belonging to the same specimen as the active sample.

Please refer to Calculation of Statistics in this guide for further information on absolute count calculations.

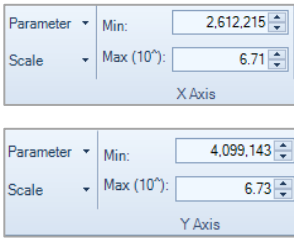
	Remove Post Gain: Click to remove Post Gain of all parameters of the Active Sample. Only accounts with the permission of **Post Gain Adjustment** can perform this operation. To adjust Post Gain, refer to Post Gain in this guide.

Plot



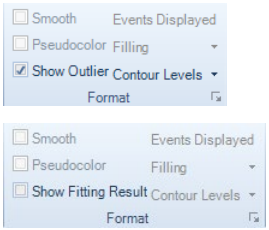
Icon	Description
Properties	
	Plot Type: Click to change the plot type for the selected plot.
	Overlays: Click to open the **Edit Overlays** window. User can edit overlays in this prompted window. Overlay is only available for dot plots and histograms. Please refer to Overlays in this guide for more details. 
	**Gating**: Select the gate and its events to be the current data source for plotting.
	**Apply to All Plots**: Check to apply the selected gate to all plots.
	**Show Title on Plot**: Check to 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



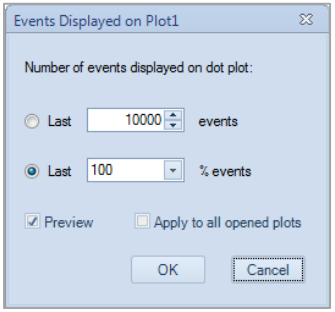
Allows user to set plot properties for the X and Y-axes including the plotted parameter, scale, and the display range.
User can set the minimum and maximum displayed values in **Min** and **Max** box for the parameter selected from **Parameter** drop down list, or click **Auto Range** to automatically 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: Allows user to control format settings for the plots. Available settings depend on the type of the selected plots.

- **Smooth**: Check to smooth the data in histogram and density plots.
- **Pseudocolor**: Check to display Density plots 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.



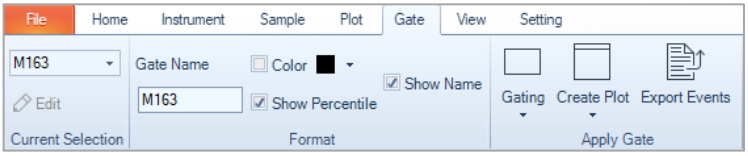
- **Show Fitting Result**: Check to display the results for the cell cycle analysis.
- **Filling**: Select the histogram fill mode (i.e., None, Filled, or Tinted) from the associated drop-down list.
- **Contour Level**: Select the contour level (i.e., 10%, 5%, or 2%) in contour plots from associated drop down list.
- **Show Outlier:** Check to show outlier events as dots on contour plot.

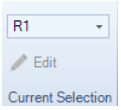
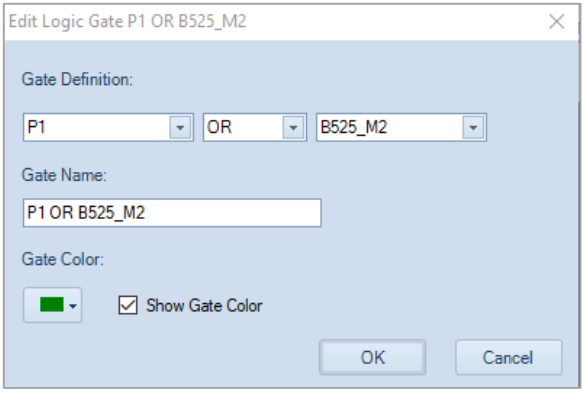
Output

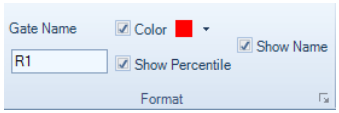
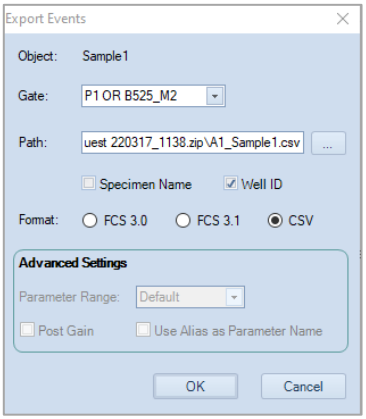
Save As Image: Click to save the plot in JPEG, BMP, PNG, GIF, TIFF, and EMF formats. EMF format is a vector format which, when exporting, uses 600dpi resolution TIFF format.

	Copy: Selects from the drop-down menu to copy plot, EMF or statistics to the clipboard. The plots can then be pasted into third party software (e.g., Microsoft Word, PowerPoint, Excel). The drop-down options include: • **Copy Plot (Bitmap)**: Copies the selected plot as a Bitmap. • **Copy Plot with Statistics (Bitmap)**: Copies the selected plot and associated statistical information as a Bitmap. • **Copy Vector Graphics (EMF)**: Copies the selected plot as an EMF. • **Copy Statistics (Text)**: Copies the statistics for the selected plot in text format.

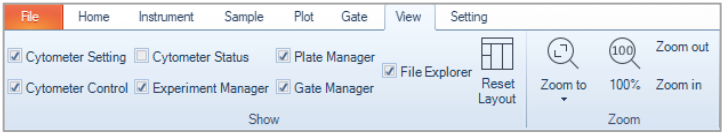
Gate

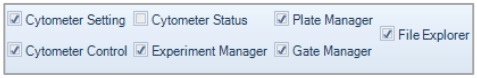


Icon	Description
Current Selection	
	Provides a drop-down menu of all available gates. A gate can be selected from this list. The **Edit** button is available only when a logic gate is selected. Click the **Edit** button to open the **Edit Logic Gate** window below for modifying the setting of a logic gate. Please refer to Gates in this guide for more details. 
Format	

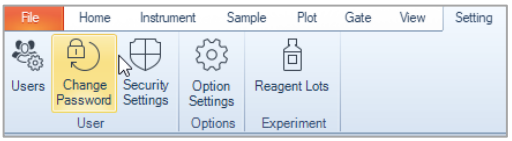
	Allows user to format the selected gate. • **Gate Name**: Sets the name for the selected gate. • **Color**: Sets the color for the selected gate. • **Show Percentile**: Check to show the percentage of the gated events relative to the total number of events on the plot. If the **Show population percentile in gate label** in **Setting** > **Option Settings** > **Analysis** is not checked, the **Show Percentile** option here will be disabled. • **Show Name**: Check to show the gate name in gate label on plot. If the **Show gate name in gate label** option in **Setting** > **Option Settings** > **Analysis** is not checked, the **Show Name** option here will be disabled.
Apply Gate	
	Gating: Applies the current gate to the selected plot from the drop-down list.
	Create Plot: Creates a new plot with selected type from the drop-down list 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. 

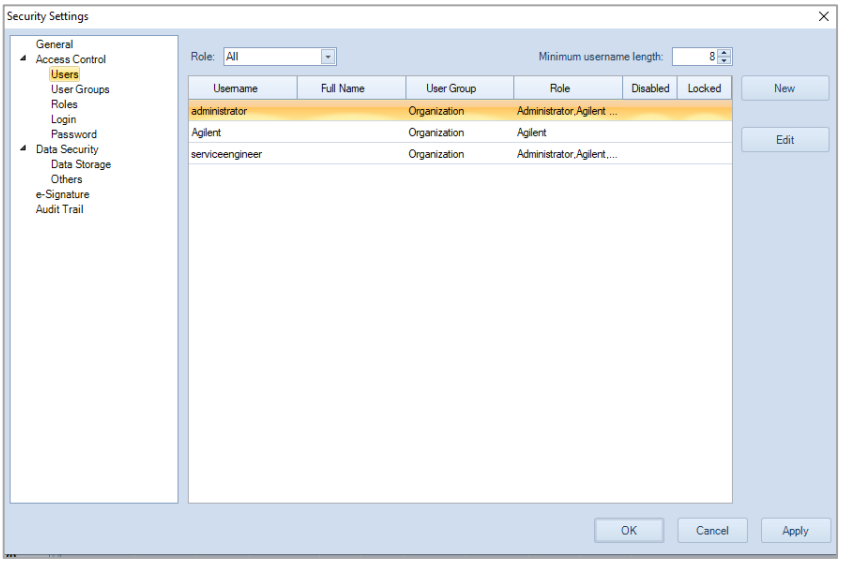
View

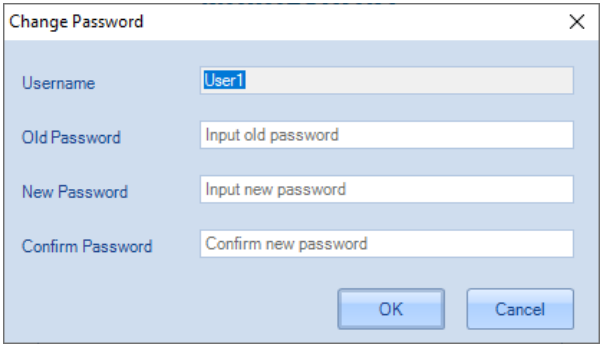


Icon	Description
Show	
	Allows user to check to show or uncheck to hide the corresponding panel. **Plate Manager** is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
	Reset Layout: Click to reset the layout of the panels to the default layout.
Zoom	
	Zoom to: Selects the scaling of the size of the plots inside the workspace in the drop-down menu (50%-150%). This function is equivalent to adjusting the sliding bar at the bottom-right corner of the software.
	100%: Click to restore 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: Click to decrease 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: Click to increase the size of the plots inside the workspace. One click corresponds to 1% increment.

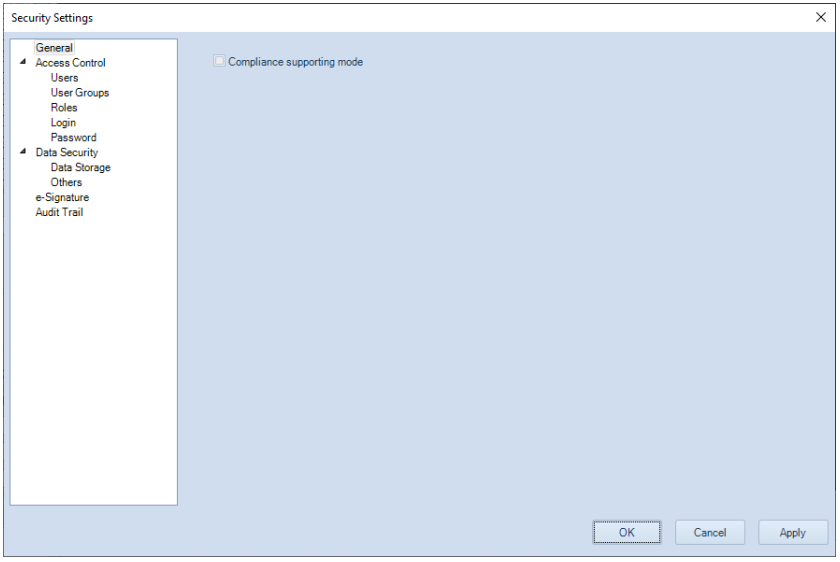
Setting



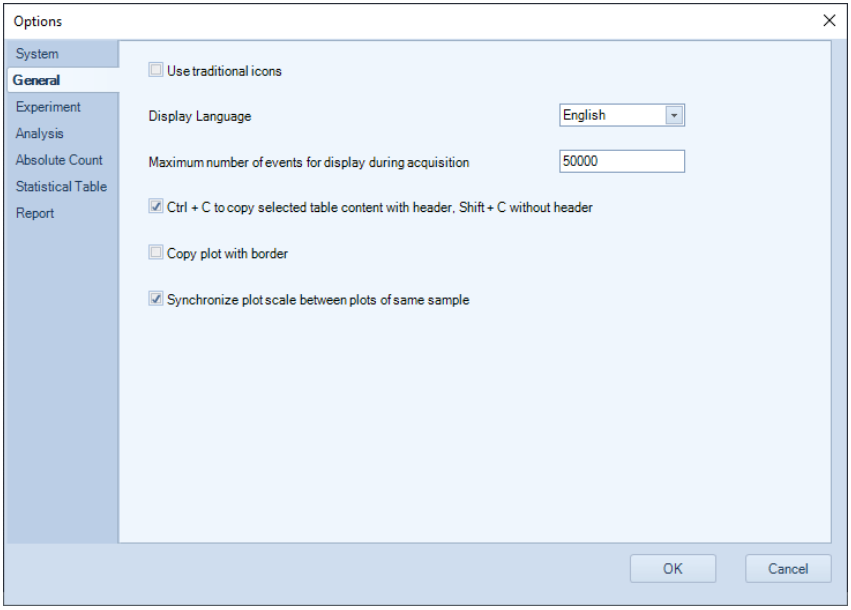
Icon	Description
User	
	Users: Click to open the **Users** sub-window of **Security Settings** window. User can create new user account or edit existing user account in this sub-window. This function is only available to user account with permission of **Modify Access Control Settings**. Please refer to Users in this guide for details. 
	Change Password: When clicked, user can change the password of the currently login user account in the prompted window.



Security Settings: When clicked, following window will be prompted. User can select to enable **Compliance Supporting Mode**, customize accessibility for selected user account, define data storage settings, e-Signature settings, audit trail settings, etc. in this window. Please refer to Manage User Access and Data Security in this guide for details.

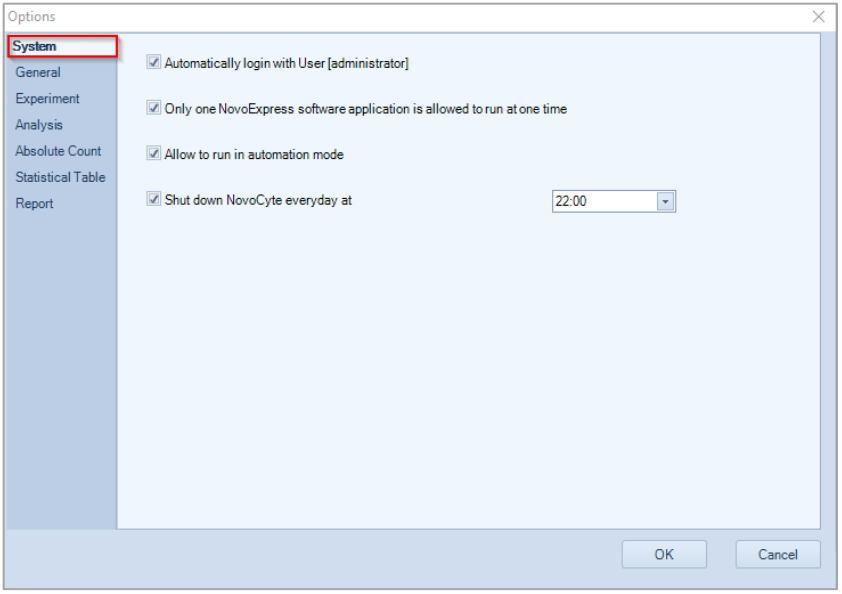


Option Settings: Allows user to define system and experiment related settings. When clicked, following window will be prompted.

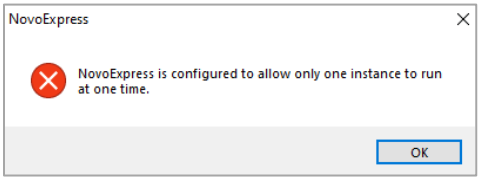


The **Options** window includes seven sub-windows, namely **System, General, Experiment, Analysis, Absolute Count, Statistical Table** and **Report**. User can click each associated tab on the left side of this window to open designated sub-window.

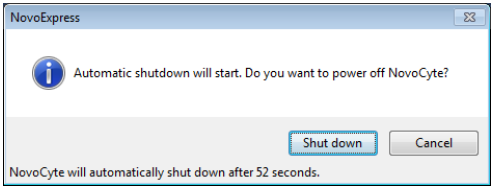
System: Allows user to define general system settings.



- **Automatically login with User:** This option will not be available if **Allow auto login (remember password)** is not checked in **Security Settings → Access Control → Login** window. When user check this option, the software will remember the login password and user will be automatically logged in when software launches.
- **Only one NovoExpress software application is allowed to run at one time:** When checked, only one instance of NovoExpress software is allowed to run at a time. Following error message will be prompted when more than one instance of software is launched.



- **Allow to run in automation mode:** Check to allow user to run NovoExpress in automation mode. This option is disabled if **Compliance Supporting Mode** is enabled or when **Secure Data Folder** is checked in **Security Settings → Data Security → Data Storage** window. Please contact Agilent technical support for more details of NovoExpress automation mode and functions.
- **Shut down NovoCyte every day at**: This option is available on for user with permission of **Schedule Power On & Off**. When checked, the instrument will automatically shut down at the selected time. For example, if the time is set to 22:00, the software will prompt a message window as shown below at 22:00 o'clock every day. The system will automatically shut down in 1 min if there is no operation from the user.



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

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

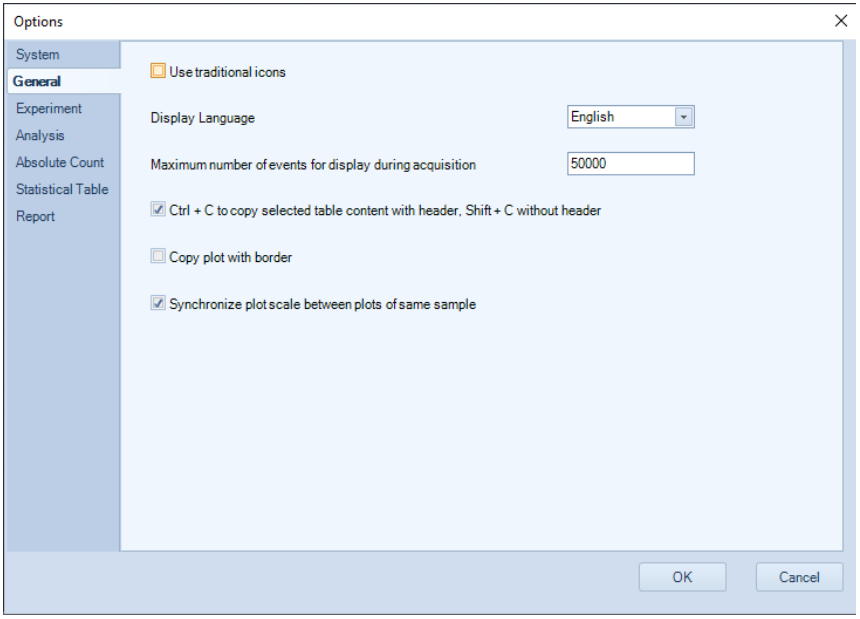
NOTE User can also double click the icon in the status bar to access this function. User can view the current status of this function (i.e., "Instrument will be shut down at the set time" or "Automatically shutting down instrument is disabled") by placing the mouse on the icon .

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

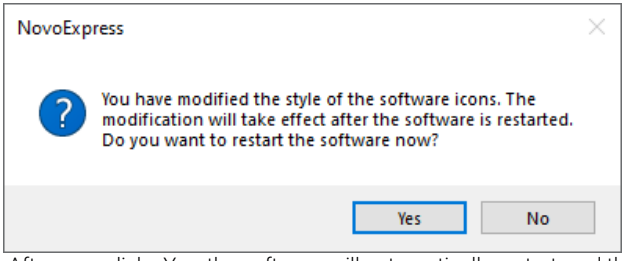
NOTE

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

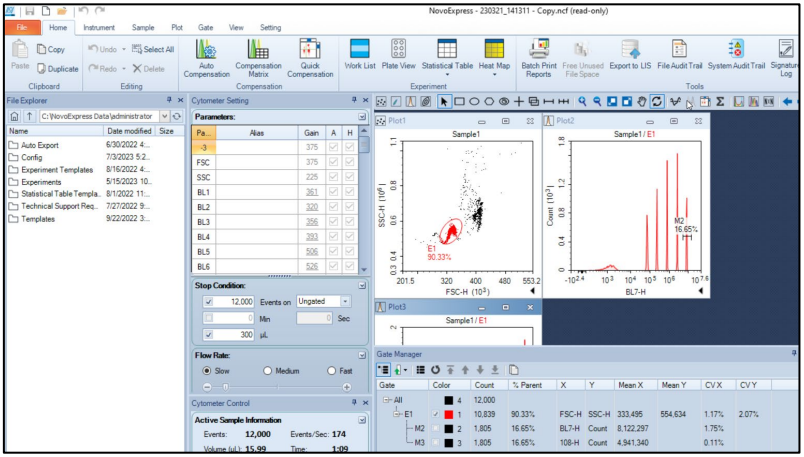
General: Allows user to define general settings. When clicked, following sub-window will be prompted.



- **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.

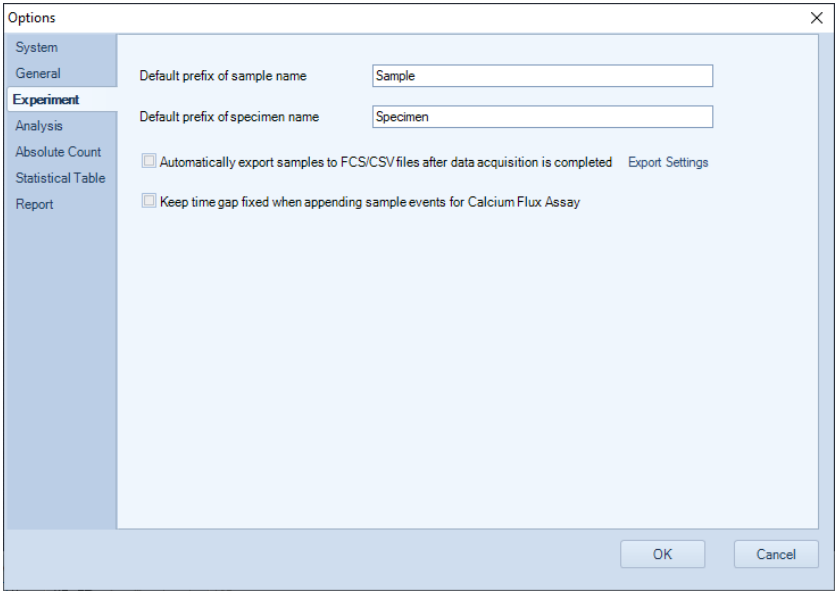


After user clicks Yes, the software will automatically restart, and the traditional icons will be applied after user logs back into the software as shown below.

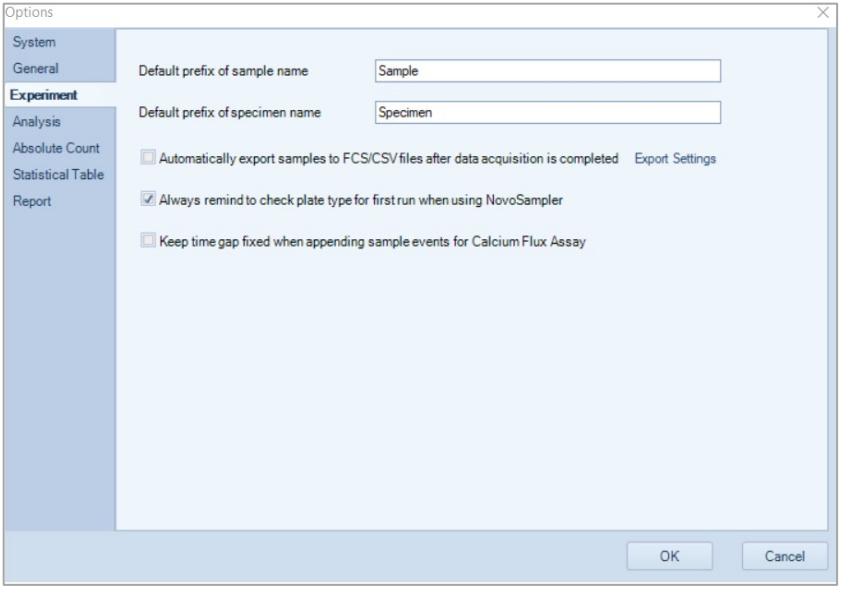


- **Display Language:** Sets the display language.
- **Maximum number of events for display during acquisition**: Sets a limit to the number of events displayed on plots during sample acquisition. For example, if this is set to 20,000 events, only the last 20,000 events collected will be displayed. The 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.
- **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 quickly switch this setting. Refer to Workspace Toolbar for more information.

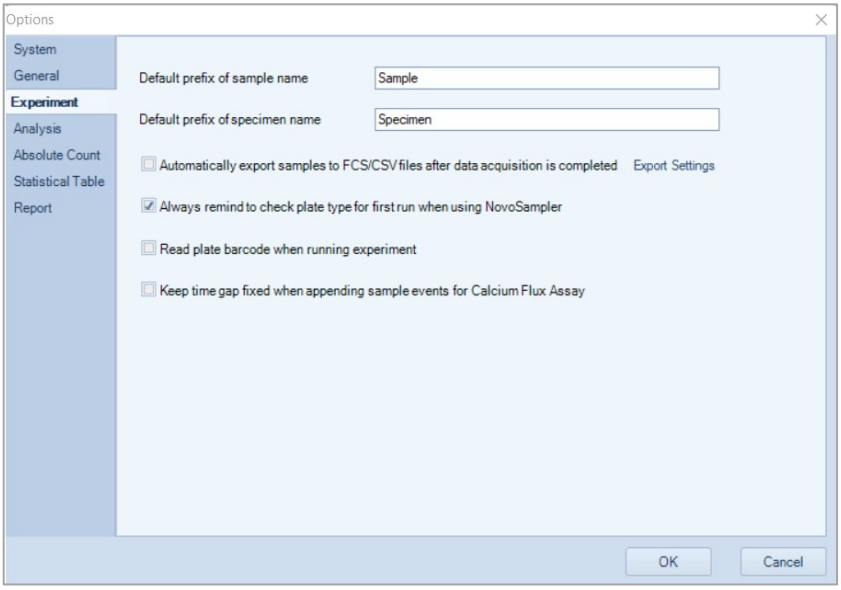
Experiment: Allows user to define experiment related settings. This window displays additional settings when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected.



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

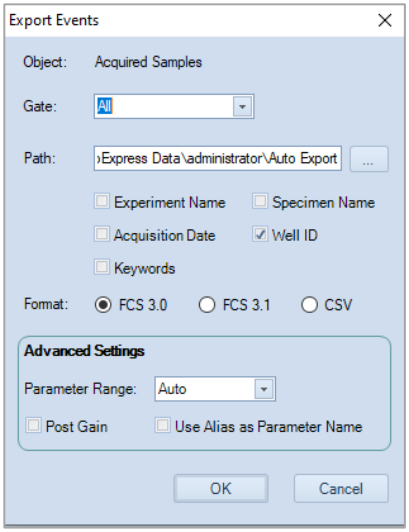


When NovoSampler or NovoSampler Pro is connected

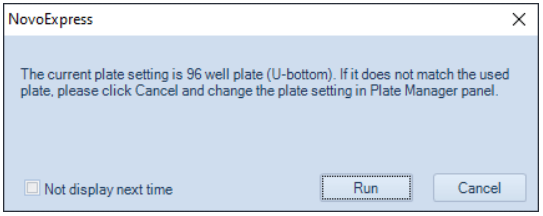


When NovoSampler Q is connected

- **Default prefix of sample name**: Sets the prefix for the name of the sample.
- **Default prefix of specimen name**: Sets the prefix for the name of the specimen.
- **Automatically export samples to FCS/CSV files after data acquisition is completed**: If selected, the software will automatically generate and export a FCS or CSV file after data acquisition is completed. Click **Export Settings** to open the **Export Events** window. Export Settings, including file format (i.e., FCS 3.0, FCS 3.1, or CSV), can be defined in this window. Please refer to Export Data in this guide for more details.

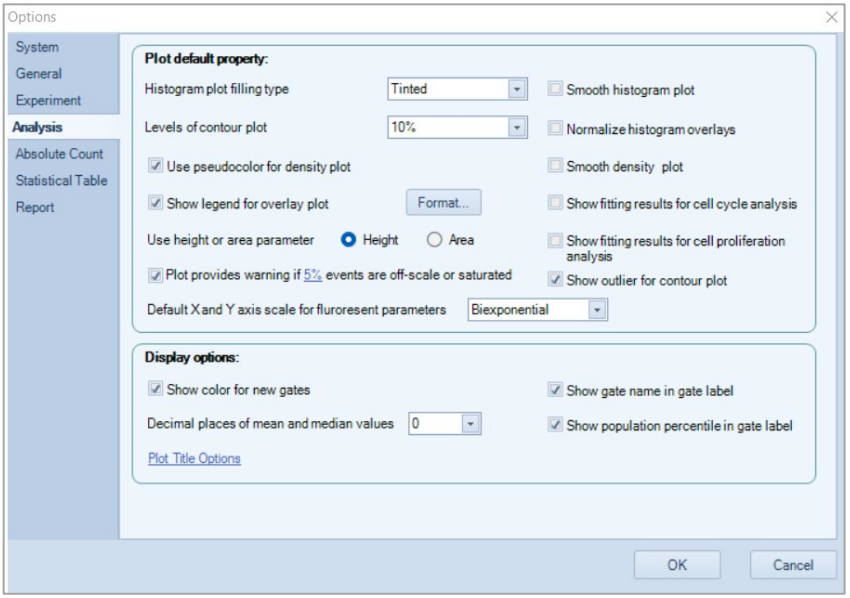


- **Always remind to check plate type for first run when using NovoSampler:** This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected. When checked, a window will be prompted to remind user to check if the correct plate type is selected before starting the experiment.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



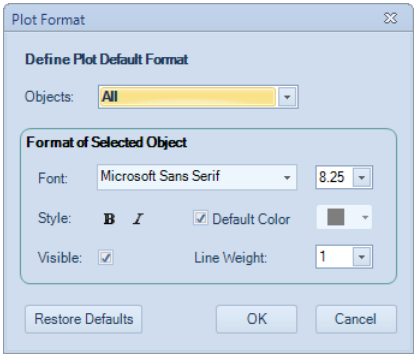
- **Read plate barcode when running experiment:** This option is available only when NovoSampler Q is connected. If this option is selected, the software will automatically read the barcode attached to the plate. Please refer to NovoSampler Q Operator's Guide for more details
- **Keep time gap fixed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

Analysis: Allows user to define analysis related settings.

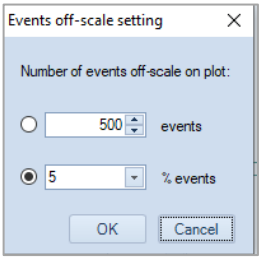


Plot Default Property: Set the default plot properties when creating a new plot.

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



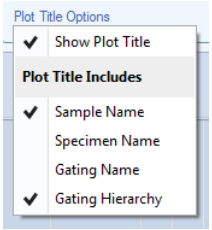
- **Plot provides warning if XXX events are off-scale or saturated**: If selected, the number or percentage of off-scale events will be displayed in created dot, density, histogram and contour plot after the data acquisition. By default, this function is enabled. User can click XXX area to open **Events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



- **Default X and Y axis scale for fluorescent parameters:** Allows user to select **Biexponential**, **Log** or **Linear** to be the default scale from the associated drop-down menu. The default setting is **Biexponential**. 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.

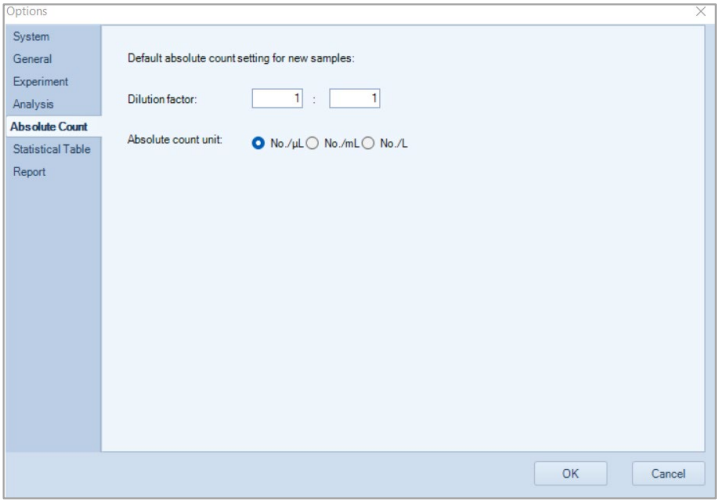
Display Options: Set plot Display Options.

- **Show color for new gates**: If selected, new gates are displayed with default color. If not selected, new gates are in black.
- **Show gate name in gate label**: If selected, gate name is displayed in gate label on the plot.
- **Decimal places of mean and median values**: Sets the number of digits displayed after the decimal point when computing mean and median values.
- **Show population percentile in gate label**: If selected, gate label is displayed with the percentage of the population within the gate.
- **Plot Title Options**: If clicked, a drop-down menu will show as below:



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

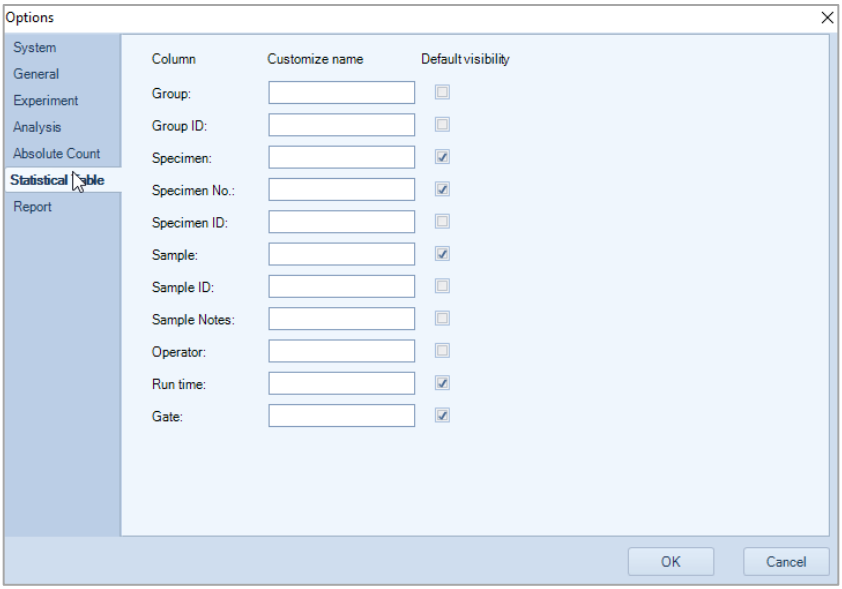
Absolute Count: Allows user to define Absolute Count related settings.



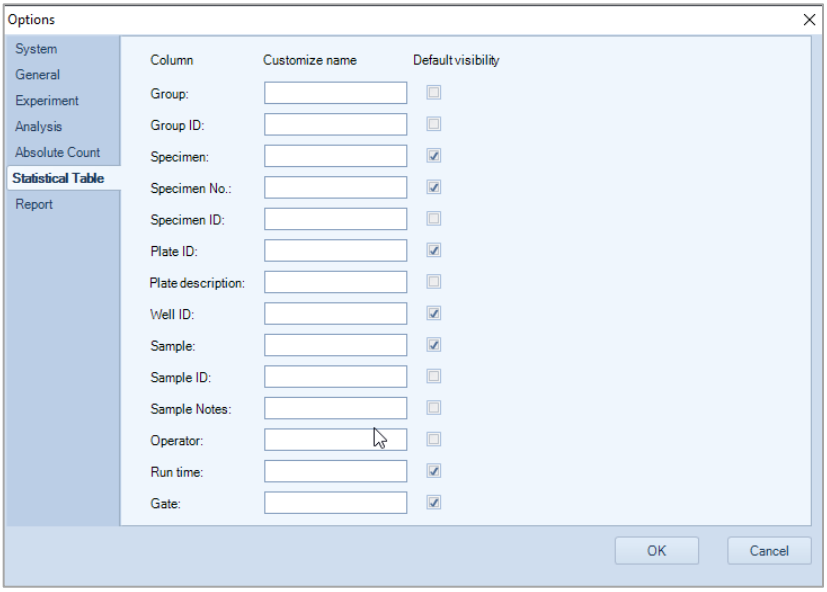
- **Dilution Factor**: Sets the default dilution factor for new samples. The **Dilution Factor** is a conversion coefficient used to calculate the absolute counting results for the original sample. For instance, if the original sample is diluted 10 times and is run on instrument, enter 1:10 in the Dilution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

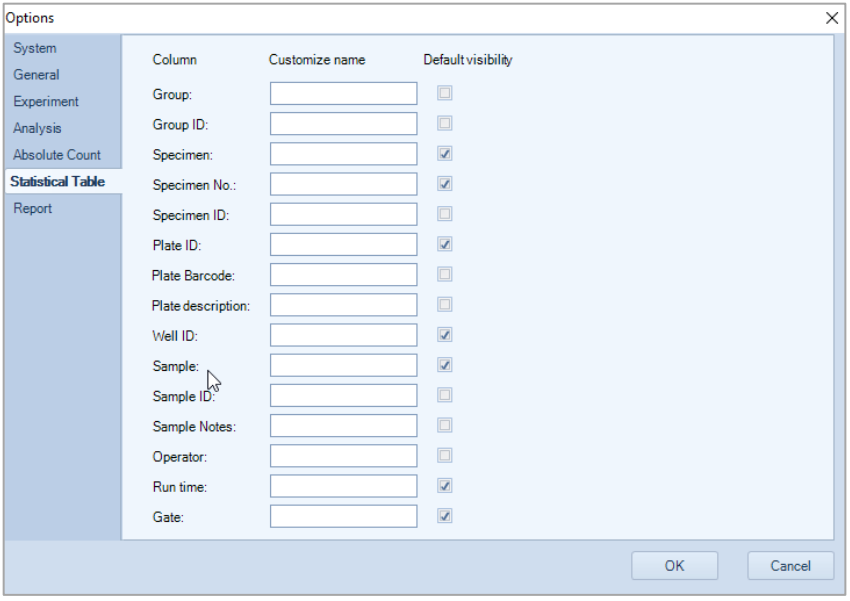
Statistical Table: Allows user to define statistical table related settings (e.g., **Group, Group ID, Specimen, Specimen No, Specimen ID, Sample, Sample ID, Sample Notes, Operator, Run time, Gate**). This window displays additional settings (e.g., **Plate ID, Plate description, Well ID)** when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected. The **Plate Barcode** will only be available when NovoSampler Q is connected. User can rename the associated column header in statistical table by entering the new name in **Customize Name** textbox. When user checks the checkbox in **Default visibility** column, the associated column will appear in any newly created statistical table. By default, **Specimen, Specimen No, Sample, Run time, Gate, Plate ID** (if applicable), **Well ID** (If Applicable) are automatically checked.



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

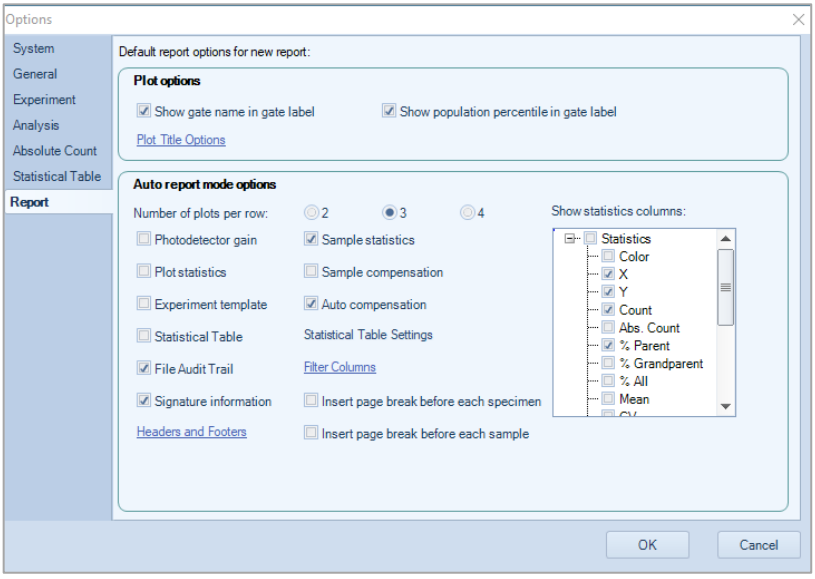


When NovoSampler or NovoSampler Pro is connected

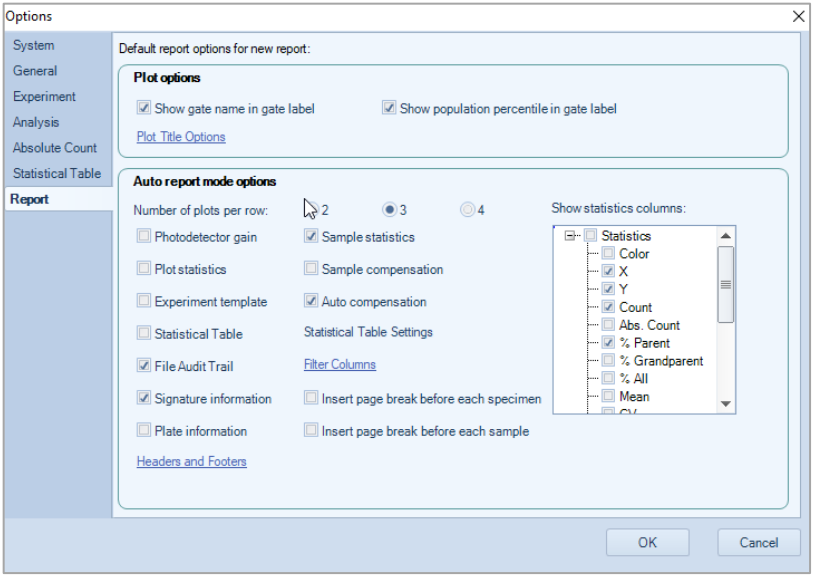


When NovoSampler Q is connected

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



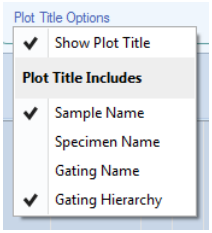
When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected



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

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

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



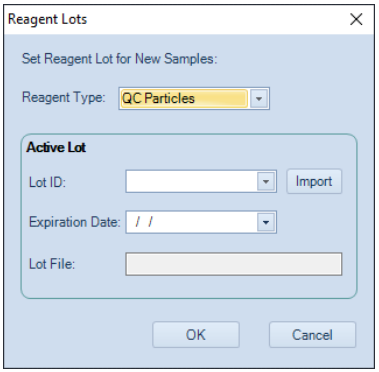
  - **Show Plot Title**: If selected, plot titles are displayed on report plots.
  - **Sample Name**: If selected, the sample name is displayed in the report plot title.
  - **Specimen Name**: If selected, the specimen name is displayed in the report plot title.
  - **Gating Name**: If selected, the gating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

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

- **Number of Plots per Row**: Sets how many plots are shown in one row.
- **Photodetector Gain**: If selected, shows photodetector gain value.
- **Sample Statistics**: If selected, shows gate statistics of sample.
- **Plot Statistics**: If selected, shows gate statistics of plot.
- **Sample Compensation**: If selected, shows sample compensation matrix.
- **Experiment template**: If selected, shows experiment template.
- **Auto Compensation**: Only available for file report. If selected, shows auto compensation information in file report for new created experiment.
- **Statistical Table**: Only available for file report. If selected, shows statistical table.
- **Statistical Table Settings**: Click to edit the statistical table settings in the prompted window.
- **File Audit Trail**: If selected, shows File Audit Trail information.
- **Filter Columns**: Click to select the column to be displayed for File Audit Trail table.
- **Signature Information**: If selected, shows signature information.
- **Plate Information**: If selected, shows plate information. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
- **Insert Page Break Before Each Specimen**: Only available for file report. If selected, a page break will be inserted before each specimen.
- **Insert Page Break Before Each Sample**: Only available for specimen report. If selected, a page break will be inserted before each sample.
- **Headers and Footers**: Click to customize the report header and footer in the prompted window. Refer to Headers and Footers in this guide for details.

- **Show Statistics Columns**: Selects statistical items to display.

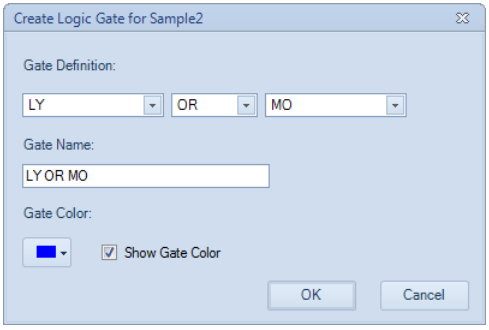
Reagent Lots: Set reagent lots for new samples. When clicked, following window will be prompted.



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

Workspace Toolbar

Icon	Description
	Dot Plot: Creates a dot plot.
	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.
	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.

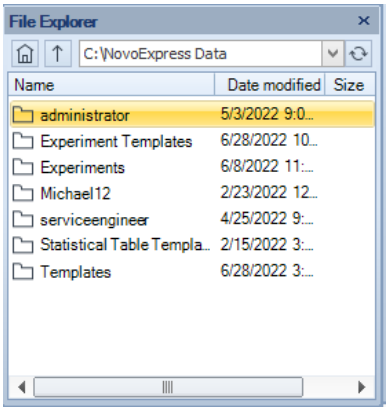
	Zoom Out: Zooms out on a specified area of a plot. To use, select the tool, and click on a plot to zoom out. Continue to click to zoom out further. To zoom out only along one axis, click on either the X-axis or Y-axis on the plot.
	Auto Range: The display range for the X-axis and Y-axis are automatically 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.
	Adjust Post Gain: Use this tool to adjust Post Gain. Only accounts with the permission of **Post Gain Adjustment** can perform this operation. Refer to Post Gain for detailed procedures.
	Quick Compensation: Allows for quick fluorescence compensation through scrollbars. To use, select the tool. Scrollbars appear on plots to allow for adjustment of fluorescence compensation.
	Show Statistics: Shows or hides the statistical tables below plots.
	Cell Cycle Plot: Creates a cell cycle analysis plot.
	Cell Proliferation Plot: Creates a cell proliferation plot.
	Bi-Variate Plots: Opens **Bi-Variate Plots** window.
	Previous Plot: Switches the active plot to the previous plot. Use this tool when the plot window is maximized.
	Next Plot: Switches the active plot to the next plot. Use this tool when the plot window is maximized.

File Explorer Panel

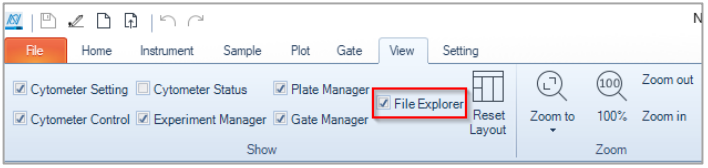
The **File Explorer** panel allows user to manage (e.g., move, rename, open) experiment file (*.ncf), experiment template file (*. ncet), template file (*.nct), or statistical table template file (*.nst). If any file is renamed or moved through this panel, the action will be recorded and displayed in the **System Audit Trail** window.

NOTE

When **Secure Data Folder** is checked in **Security Settings** window, user other than the Windows system administrator will not be able to edit (i.e., delete, remove, or rename) the secure data folder through Windows Explorer. In this case, user can edit the files through **File Explorer** panel in NovoExpress Software.



By default, the **File Explorer** panel will be displayed on the left side of the software main window. User can also manually check or uncheck **File Explorer** from **Show** panel under **View** tab to show or hide the **File Explorer** panel.

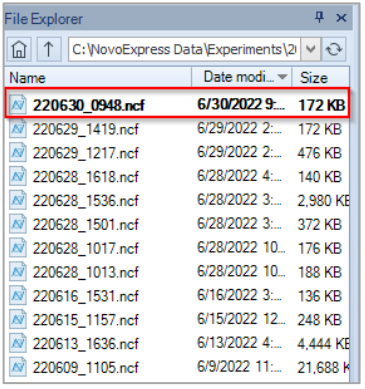


The **File Explorer** panel consists of two parts. The top part allows user to manage folder displayed in the window below. The bottom part displays all available folders user can manage.

Item	Description
	Home Folder: Click to display the root data or root user folder and associated sub-folder. If **Store files by month** option is selected but **Store files by user account** is not selected in **Security Setting**

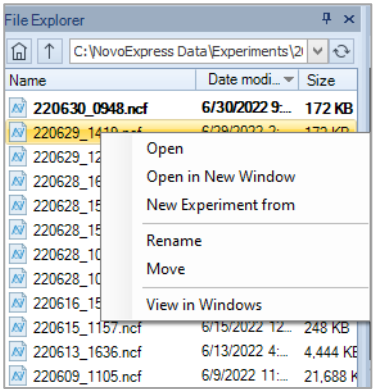
	window, the root data folder (e.g., C:\NovoExpress Data) and associated sub-folders will be displayed. If **Store files by user account** option is selected in **Security Setting** window, the root user folder (e.g., C:\NovoExpress Data\Username) and associated sub-folder will be displayed.
↑	**Up Folder**: Click to move to the upper level of the current folder.
C:\NovoExpress Data\administrator\Statis	Displays the path of current root folder. User can select different folder from the drop-down list to display desired folder and associated sub-folder.
	Refresh: Click to refresh the displayed folders.

To open an experiment file (*.ncf) or experiment template file (*.ncet) file through **File Explorer**, user can double click the selected file, or select **Open** from the right-click menu. The file already opened will be displayed with bolded file name.



Context menu

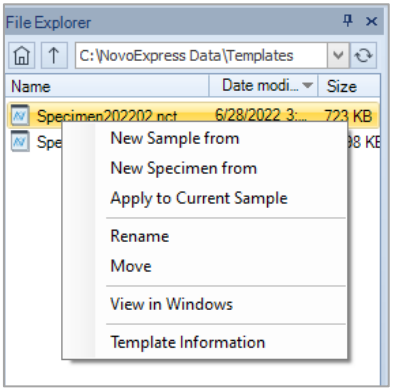
- When user right clicks an experiment file or experiment template file, following context menu will be prompted.



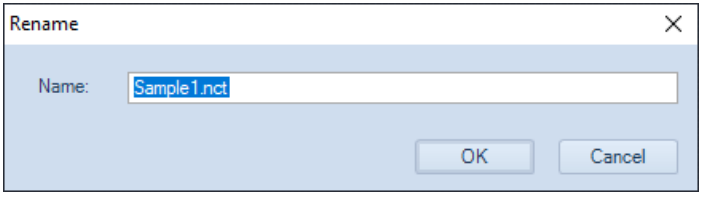
- **Open**: Click to open the selected file in currently launched software instance.
- **Open in New Window**: Click to open the selected file in a newly launched software instance. This option is not available if **Only one NovoExpress software application is allowed to run at one time** is enabled in **System** tab in **Options** window.
- **New Experiment from**: Click to create a new experiment using the selected file as a template in currently launched software instance.
- **Rename**: Click to prompt the window below. User can enter the new name and click **OK** to rename the selected file.

- **Delete:** Click to delete the selected file. This function is not available if **Compliance Supporting Mode** is enabled in **Security Settings** window.
- **Move**: Click to move the selected file to designated folder.
- **View in Windows**: Click to open the folder where the selected file is stored using Windows Explorer. This function is not available if **Secure Data Folder** option is enabled in **Security Settings** window.

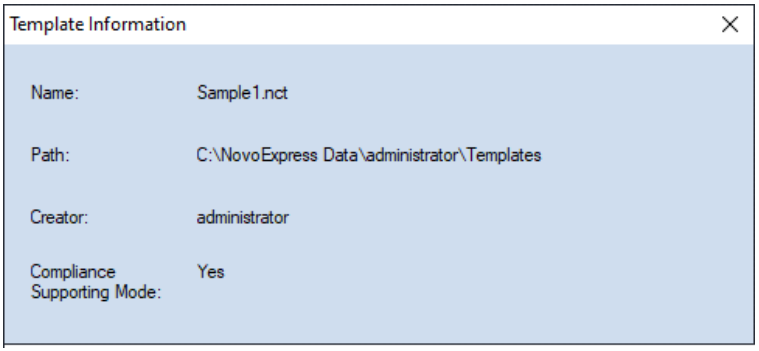
- When user right clicks a template file, following context menu will be prompted.



- **New Sample from**: Click to create a new sample using the selected template file.
- **New Specimen from**: Click to create a new specimen using the selected template file.
- **Apply to Current Sample**: Click to apply the selected template file to the active sample.
- **Rename**: Click to prompt the window below. User can enter the new name and click **OK** to rename the selected file.



- **Delete:** Click to delete the selected file. This function is not available if **Compliance Supporting Mode** is enabled in **Security Settings** window.
- **Move**: Click to move the selected file to designated folder.
- **View in Windows**: Click to open the folder where the selected file is stored using Windows Explorer. This function is not available if **Secure Data Folder** option is enabled in **Security Settings** window.
- **Template Information**: When clicked, following window will be prompted to allow user to view template related information.



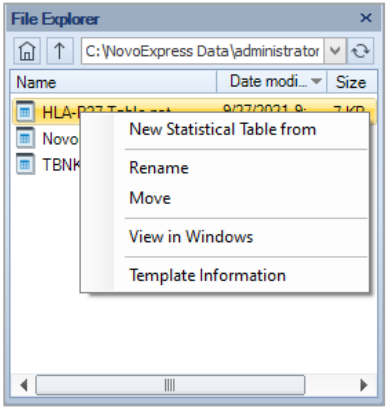
Name: Displays the name of current template file.

Path: Displays the path where the template file is stored.

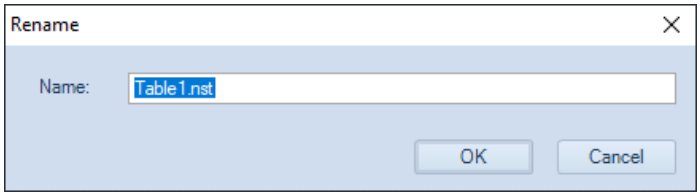
Creator: Displays the username of the user who created the template file.

Compliance Supporting Mode: Indicates whether the Compliance Supporting Mode was enabled when the template file was created.

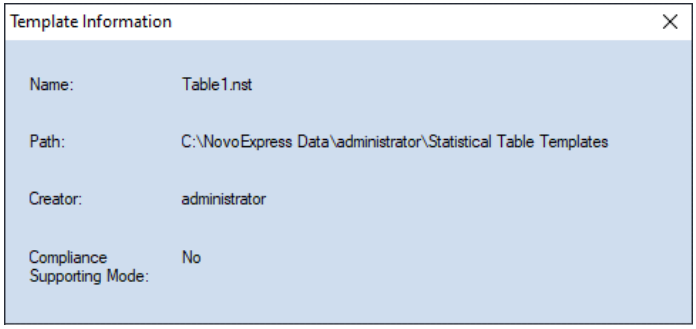
- When user right clicks a Statistical Table Template File, following menu will be prompted.



  - **New Statistical Table from**: Click to create a new statistical table using the selected template file.
  - **Rename**: Click to prompt the window below. User can enter the new name and click **OK** to rename the selected file.



- **Delete:** Click to delete the selected file. This function is not available if **Compliance Supporting Mode** is enabled in **Security Settings** window.
- **Move**: Click to move the selected file to designated folder.
- **View in Windows**: Click to open the folder where the selected file is stored using Windows Explorer. This function is not available if **Secure Data Folder** option is enabled in **Security Settings** window.
- **Template Information**: When clicked, following window will be prompted to allow user to view template related information.



Name: Displays the name of current template file.

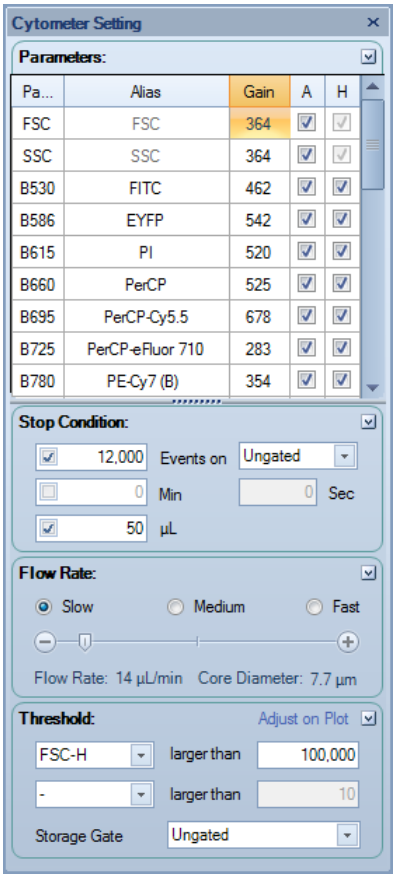
Path: Displays the path where the template file is stored.

Creator: Displays the username of the user who created the template file.

Compliance Supporting Mode: Indicates whether the Compliance Supporting Mode was enabled when the template file was created.

Cytometer Setting Panel

The **Cytometer Setting** panel sets the data collection parameters, stop conditions, flow rate, and threshold. Please refer to Cytometer Setting in this guide for more details.



Cytometer Control Panel

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

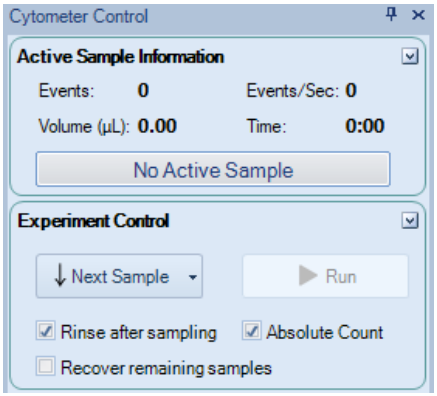
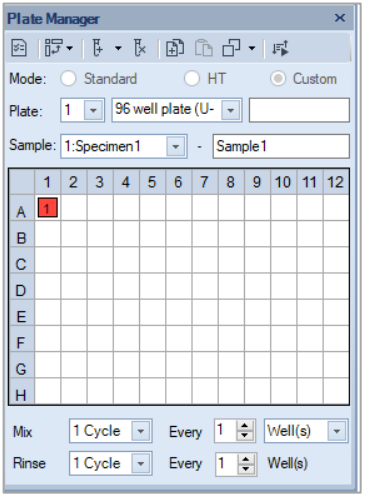
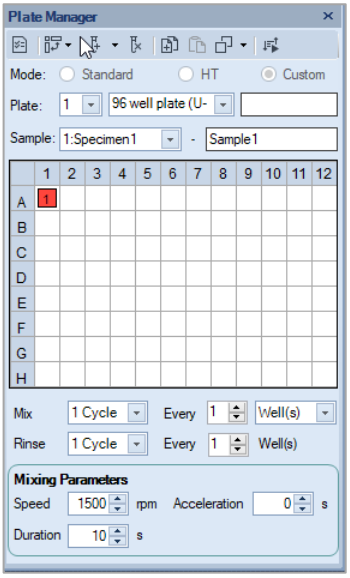


Plate Manager

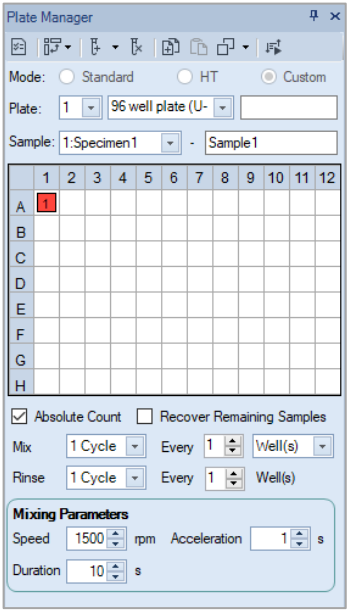
The **Plate Manager** allows user to set up the working mode, plate type, mixing and rinsing conditions when NovoSampler or NovoSampler Pro or NovoSampler Q is used. Please refer to Plate Manager for more details.



When NovoSampler is connected



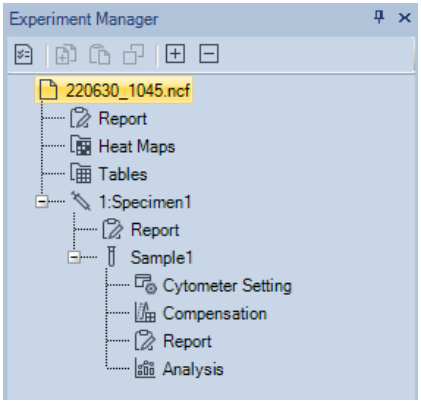
When NovoSampler Pro is connected



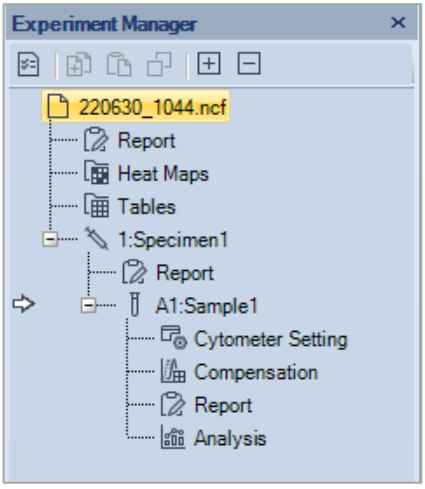
When NovoSampler Q is connected

Experiment Manager

The **Experiment Manager** contains the sample hierarchy structure and functions for copying, pasting templates, and importing and exporting sample data. Please refer to Experiment Manager in this guide for more details.



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

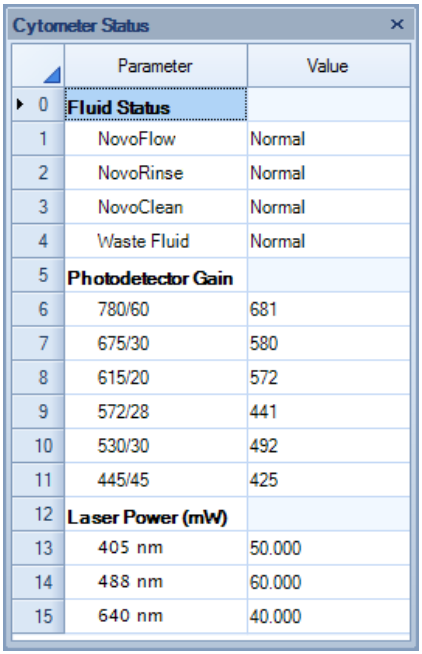


When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

Cytometer Status Panel

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

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



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

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

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

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

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	400
7	SSC	400
8	B530	377
9	B586	406
10	B615	338
11	B667	525
12	B695	426
13	B725	292
14	B780	100
15	V445	378
16	V530	401
17	V586	366
18	V615	294
19	V667	374
20	V695	466
21	V725	220
22	V780	100
23	Y586	340
24	Y615	372
25	Y667	370
26	Y695	436
27	Y725	260
28	Y780	100
29	**Laser Power (mW)**	
30	405 nm	50.000
31	488 nm	60.000
32	561 nm	50.000

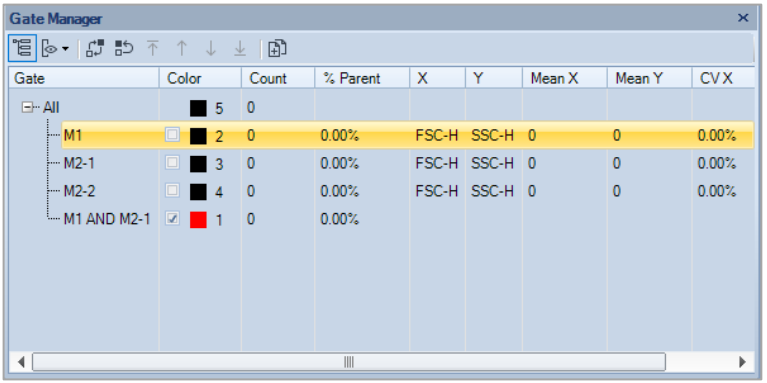
- Cytometer Status Panel for NovoCyte Penteon Instrument (e.g., U7V7B6Y6R4 system)

Cytometer Status

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

Gate Manager Panel

The **Gate Manager** displays all gates of the active sample in list mode or tree mode. It provides user interface to modify gate name, color and color precedence and also shows gate hierarchy and gate statistics. Please refer to Gate Manager in this guide for more details.



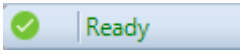
Status Bar

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

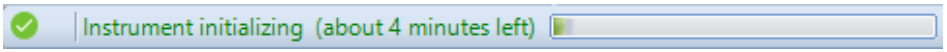
Green Indicator

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

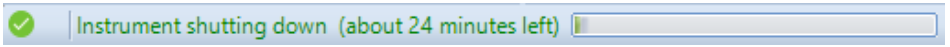
The status bar will display Ready if the instrument has completed the initializing sequence and is ready for additional commands.



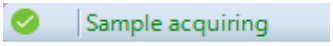
The status bar displays Instrument initializing after powering on the instrument. The initializing sequence flushes the fluidic lines in the instrument to prepare for sample acquisition.



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

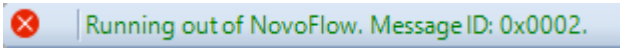


The status bar displays Sample acquiring during sample acquisition.

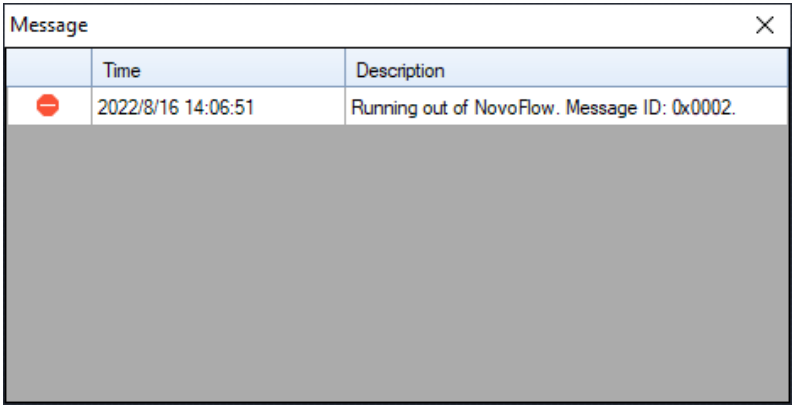


Flashing Red Indicator

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

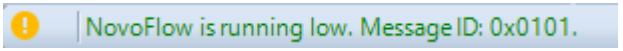


Clicking on the indicator displays the message box.

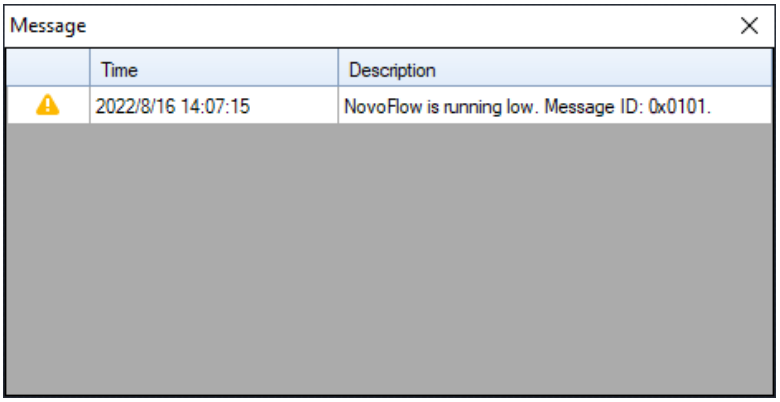


Flashing Yellow Indicator

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

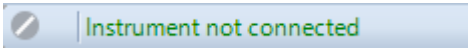


Clicking on the indicator displays the message box.



Black Indicator

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



Instrument Configuration

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

NOTE

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

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

connected while connecting NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon instrument.

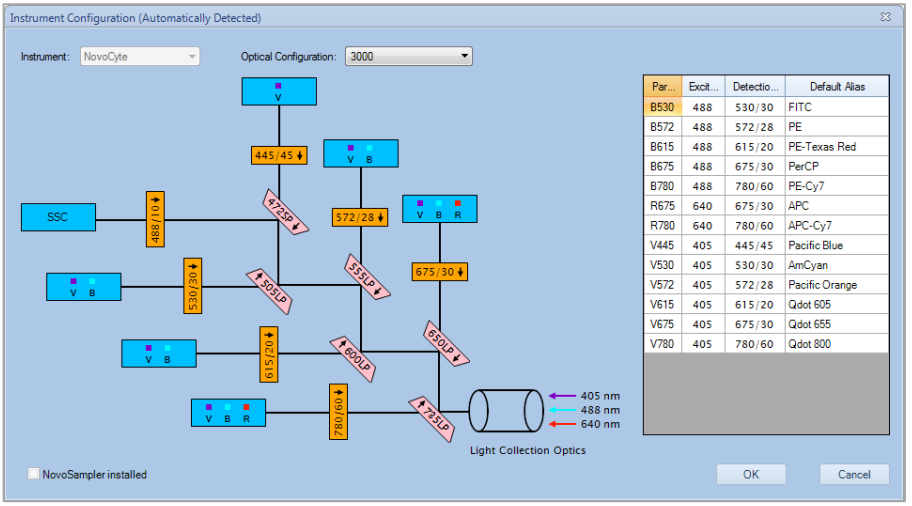
If using the software while the instrument is powered off or not connected to the workstation, use the **Instrument Configuration** window to select the correct instrument and configuration to display the correct fluorescence parameters.

Instrument Configuration with Instrument Connected

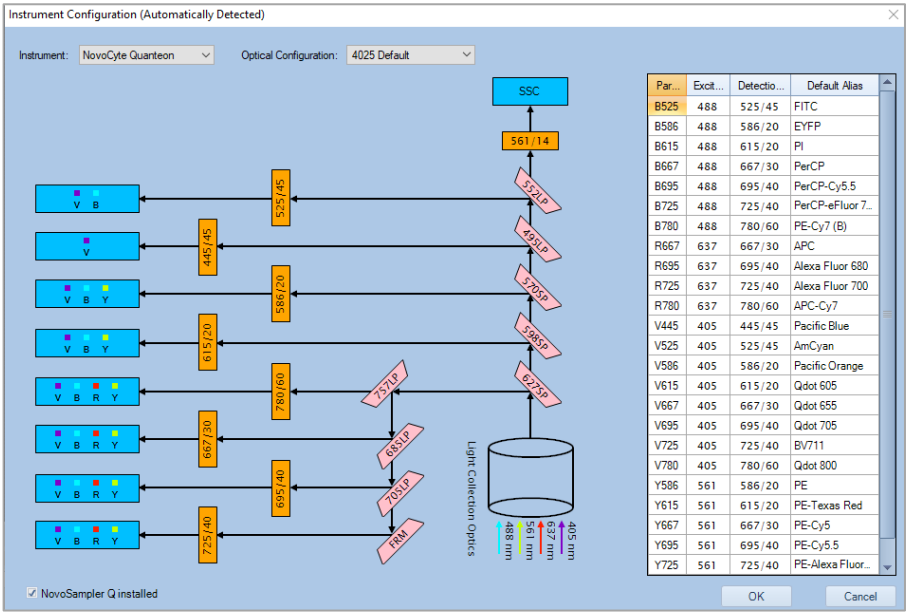
The **Instrument Configuration** window displays the instrument type, the name and schematic of the current optical configuration, parameter window, and status of NovoSampler, NovoSampler Pro or NovoSampler Q. When the instrument is connected to the workstation and powered on, the software will detect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

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

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

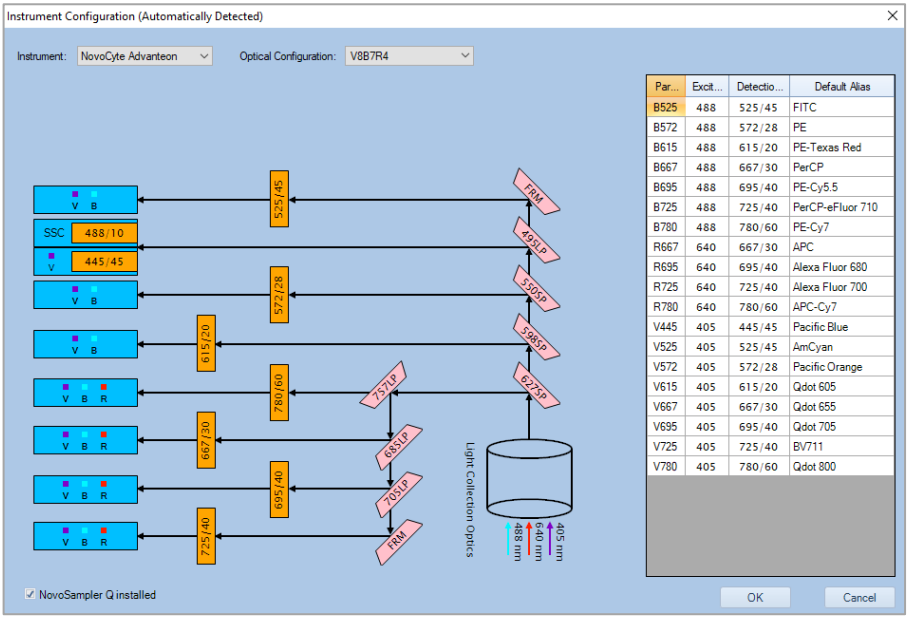


- Instrument Configuration with NovoCyte Quanteon Connected (e.g., 4025 System)

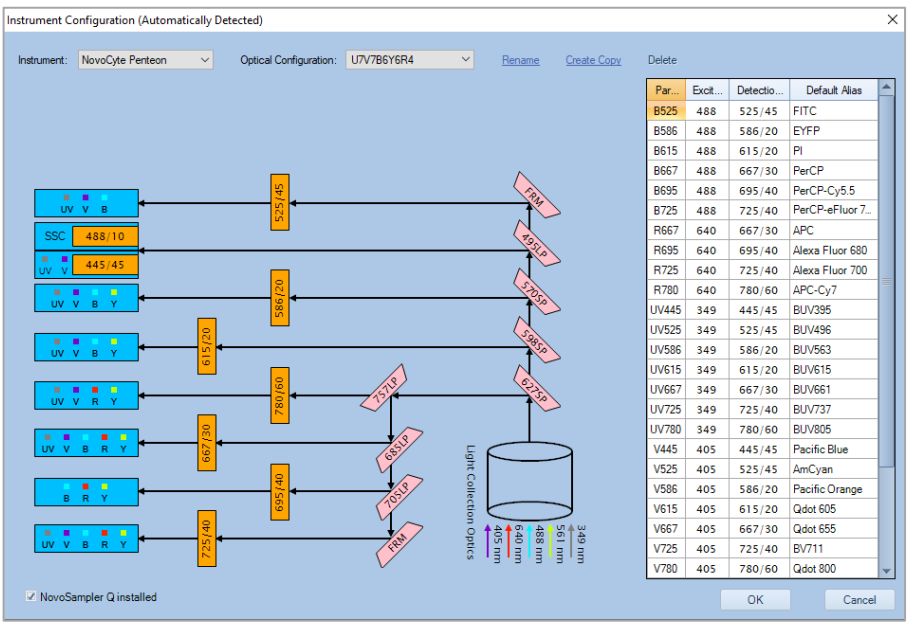


Par...	Excit...	Detectio...	Default Alias
B525	488	525/45	FITC
B586	488	586/20	EYFP
B615	488	615/20	PI
B667	488	667/30	PerCP
B695	488	695/40	PerCP-Cy5.5
B725	488	725/40	PerCP-eFluor 7...
B780	488	780/60	PE-Cy7 (B)
R667	637	667/30	APC
R695	637	695/40	Alexa Fluor 680
R725	637	725/40	Alexa Fluor 700
R780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

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



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

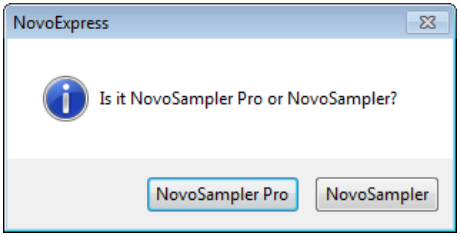


Instrument Configuration with Instrument Disconnected

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

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

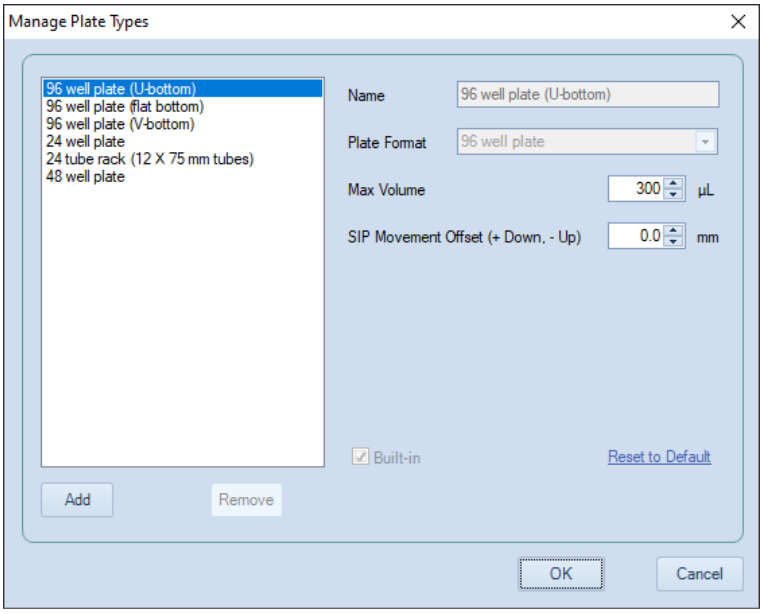
- If **NovoCyte** is selected from the drop-down menu of Instrument, check the **NovoSampler installed** checkbox located at the lower left corner of **Instrument Configuration** window, choose **NovoSampler Pro or NovoSampler** in the prompted dialog box, and click **OK**. The software will prompt a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



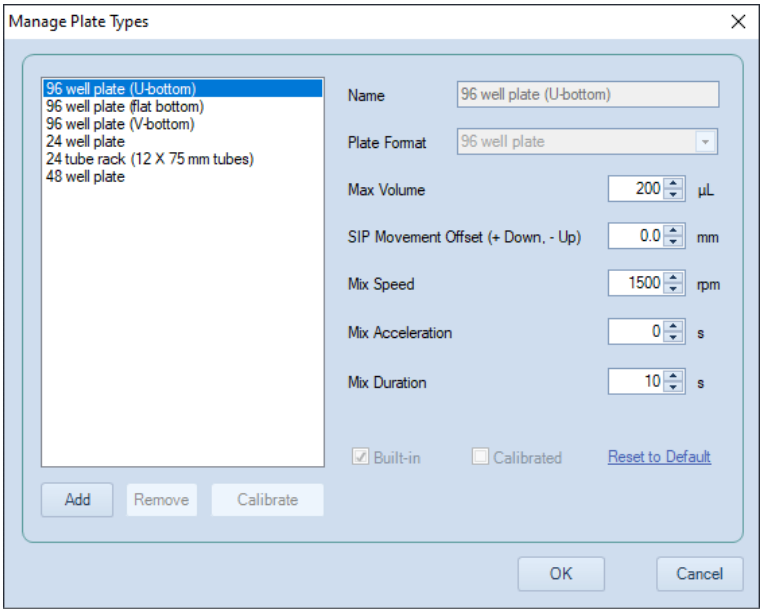
- If **NovoCyte Quanteon, Advanteon or Penteon** is selected from the drop-down menu of **Instrument**, check the **NovoSampler Q installed** checkbox located at the lower left corner of **Instrument Configuration** window, and click **OK**. The software will prompt a dialog box, asking to restart software.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

Manage Plate Types

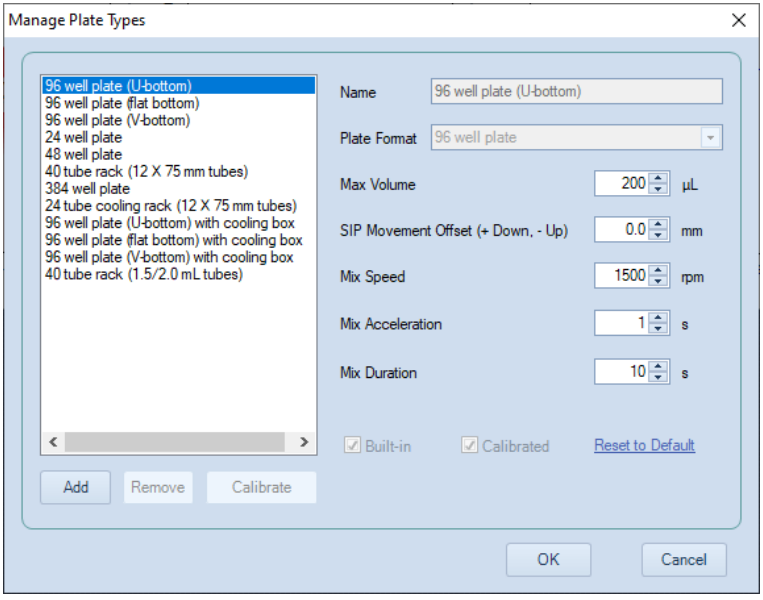
If NovoSampler, or NovoSampler Pro, or NovoSampler Q is used, user can add, remove, and modify plate settings in the **Manage Plate Types** window. To open this window, click the **Manage Plate Types** button from **Instrument** > **NovoSampler** or **NovoSampler Pro** or **NovoSampler Q**.



Manage Plate Types Window When NovoSampler is Connected



Manage Plate Types Window When NovoSampler Pro is Connected



Manage Plate Types Window When NovoSampler Q is Connected

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

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

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

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

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

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

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

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

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

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

Auto-Sampler	Plate Type	Range of Mixing Speed (rpm)	Default Mixing Speed (rpm)
NovoSampler Pro	96 well plate (U, V and Flat bottom)	60 - 3000	1500
	24 well plate	60 - 3000	2500
	24 tube rack (12 X 75 mm tubes)	60 - 1800	1500
	48 well plate	60 - 3000	1500
NovoSampler Q	96 well plate (U, V and Flat bottom)	200 - 3000	1500
	24 well plate	200 - 3000	2500
	48 well plate	200 - 3000	1500
	40 tube rack (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 Pro or NovoSampler Q is selected to work in **Standard** and **High-Throughput** mode, the software will automatically apply the default settings for mixing cycle, rinse cycle, mixing speed, acceleration, mixing duration, sampling volume and flow rate.

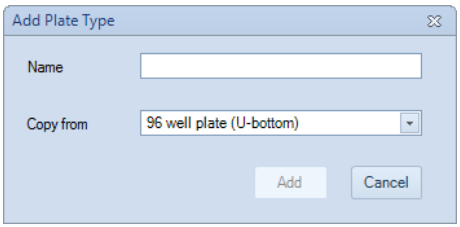
- **Mix Acceleration**: The acceleration time for the orbital shaker to reach the specified **Mix Speed**. **Mix Acceleration** can be set from 0 (default) to 10 seconds for NovoSampler Pro, or 1 second (default) to 10 seconds for NovoSampler Q. This function is available only when NovoSampler Pro or NovoSampler Q is connected.

- **Mix Duration**: The mixing time for the orbital shaker to mix the sample when NovoSampler Pro or NovoSampler Q works in custom mode. **Mix Duration** can be set from 1 second to 120 seconds. However, the default mix duration may vary when NovoSampler Pro or NovoSampler Q works in other mode (i.e., Standard and High Throughput mode). Please refer to Plate Manager in this guide for more details. This function is available only when NovoSampler Pro or NovoSampler Q is connected.
- **Built-in**: Checked if the selected plate is the built-in plate type. Built-in plate types cannot be renamed or deleted. The built-in information is read only.

NOTE

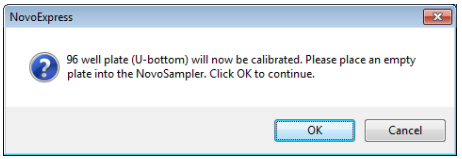
The built-in plate type has been tested with biological samples, including human peripheral blood sample and cultured cell lines, to determine the default mixing settings. At the default mixing settings, the biological samples can be effectively mixed and uniformly suspended inside each well.

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

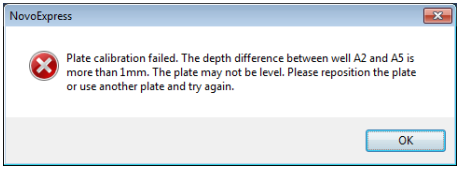


- **Calibrate**: This function is available only when NovoSampler Pro or NovoSampler Q is connected. Click this button to calibrate the selected plate

type. Only calibrated plate type can be used in experiments. The **Calibrated** checkbox indicates if the selected plate type is already calibrated. Figure below shows the dialog box prompted when the **Calibrate** button is clicked.



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



NOTE

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

NOTE

Before calibrating the 24 tube rack (12 X 75 mm tubes), 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 aa 1.5 or 2.0 mL tube and fully insert the tube end in each position of the tube rack.

NOTE

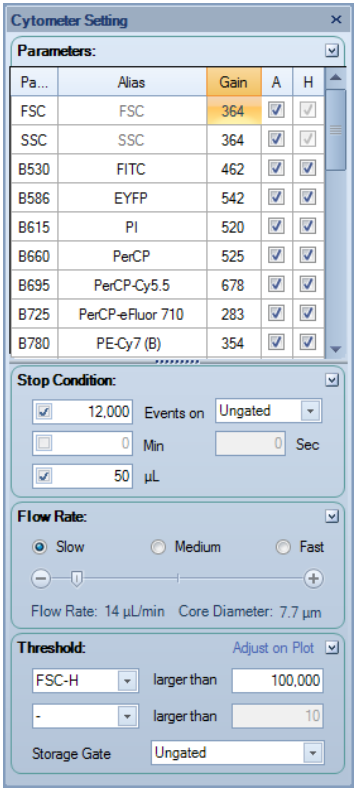
Once plate type calibration is initiated, the **Calibrate** button in the **Manage Plate Types** window will automatically become **Stop Calibration** button. Click **Stop Calibration** will stop the plate type calibration.

4 Sample Acquisition

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

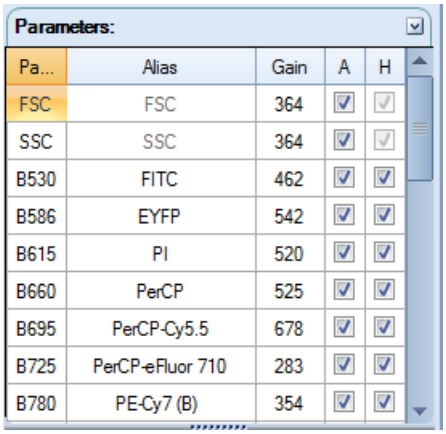
Cytometer Setting

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



Parameters Settings

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

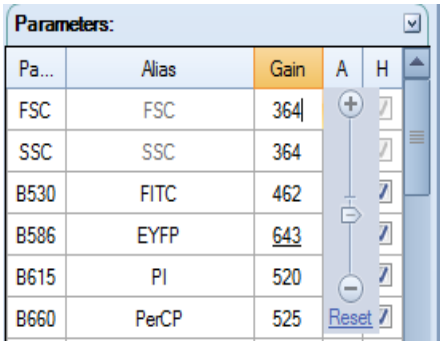


The list includes all the parameters that the instrument is capable of collecting. Area and height measurements can be collected for each parameter. To enable data collection for specific parameters, check the corresponding check boxes under the **A** (area) and **H** (height) columns. The selection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

To adjust photodetector gain of one parameter, ensure to log into the software with permission of **Adjust Photodetector Gain,** double click the cell on the specified parameter row and **Gain** column, the photodetector gain adjustment tool will show as below:

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.



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

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

NOTE

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

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

NOTE

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

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

NOTE

The photodetector gain can be changed during sample acquisition. When changing photodetector gain during acquisition, the plot will only display events after the gain adjustment. Please note the events are not deleted and will be shown on the plots when the acquisition is completed. If previous events before photodetector gain adjustment are not wanted, click the **Restart** button to restart the acquisition. Refer to Experiment Control in this guide for 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).

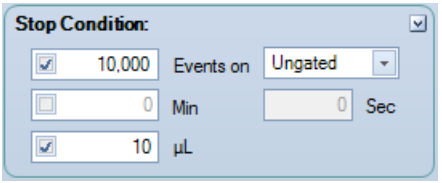
NOTE

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

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

Stop Condition Settings

The **Stop Condition** Settings are used to stop sample acquisition after a specific set of conditions has been met. The conditions may include number of events collected, collection time, and / or collection volume. To enable a condition, check the box next to the condition.



Stop Conditions

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

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

NOTE

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

NOTE

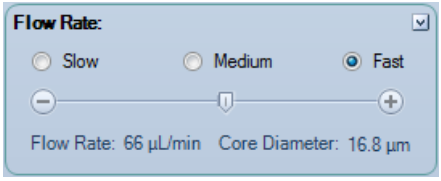
The number of events displayed in a plot during sample acquisition can be set in Settings. Refer to Setting in this guide for more details. The maximum number of events displayed is 50,000 events.

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.

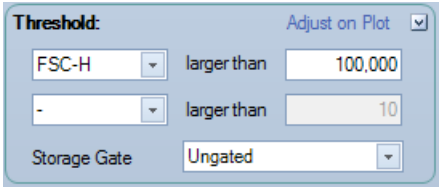
Flow Rate Settings

The three standard settings for flow rate include **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.

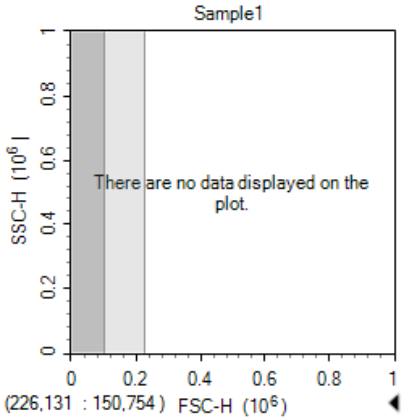


To set the threshold:

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

secondary threshold will be recorded when Storage Gate is set to Ungated. Threshold values can range from 10 to 500,000,000.

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



NOTE

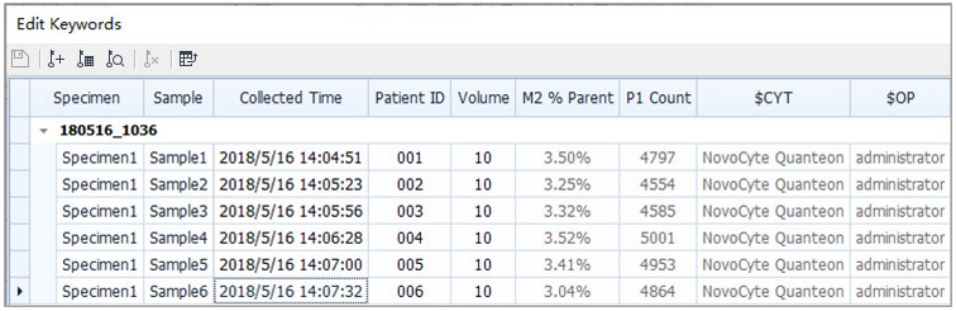
Threshold channels cannot be changed after data acquisition begins. Threshold values and Storage Gate can be changed during acquisition, but the events already acquired will not be processed. When changing threshold values during acquisition, the plot will only show events after threshold adjustment. 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).

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.



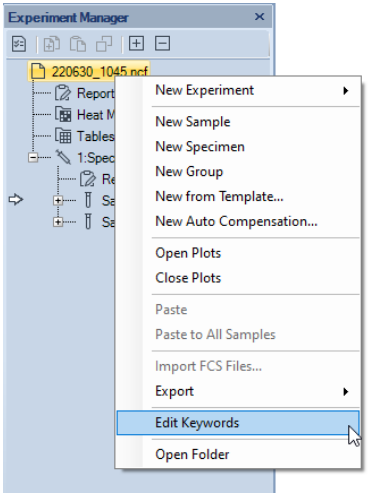
Specimen	Sample	Collected Time	Patient ID	Volume	M2 % Parent	P1 Count	$CYT	$OP
180516_1036								
Specimen1	Sample1	2018/5/16 14:04:51	001	10	3.50%	4797	NovoCyte Quanteon	administrator
Specimen1	Sample2	2018/5/16 14:05:23	002	10	3.25%	4554	NovoCyte Quanteon	administrator
Specimen1	Sample3	2018/5/16 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:

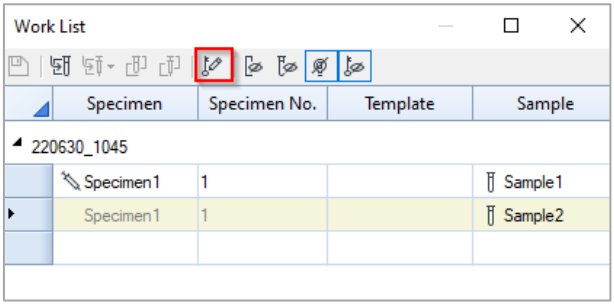
- From **Experiment Manager** panel: Select experiment file, group(s), specimen(s), or sample(s). Right click the selected item(s) and click **Edit Keywords** in the right click menu.

NOTE

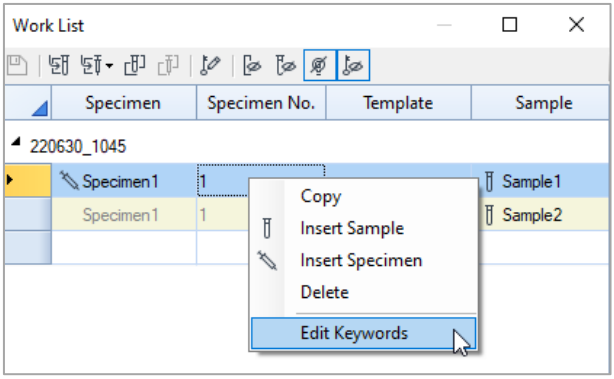
To select more than one group, specimen or sample, press **Ctrl** or **Shift** key while clicking each item.



- 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.



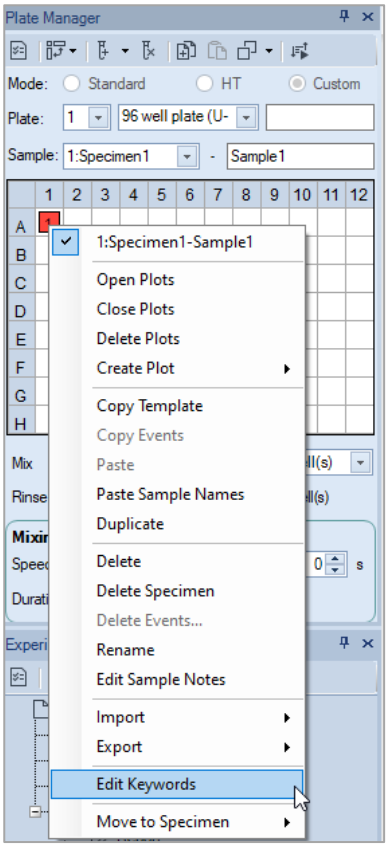
- Highlight the row(s) associated with selected sample(s). Right click and select **Edit Keywords** from the right click menu. Keywords associated with the selected sample (s) will be listed in prompted **Edit Keywords** window.



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

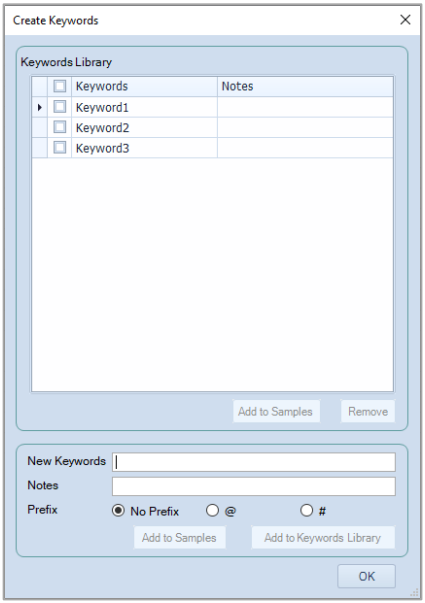
NOTE

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

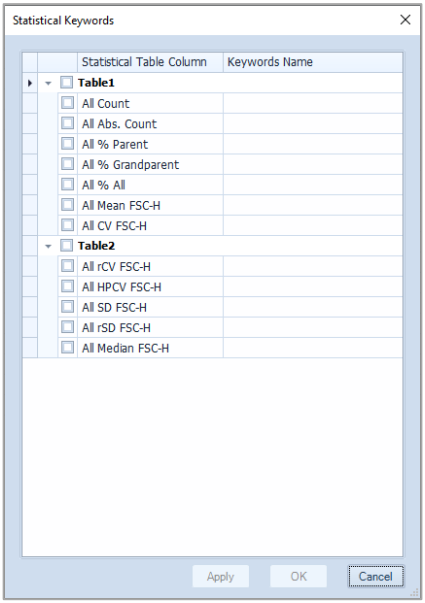


Edit Keywords window includes the following toolbars.

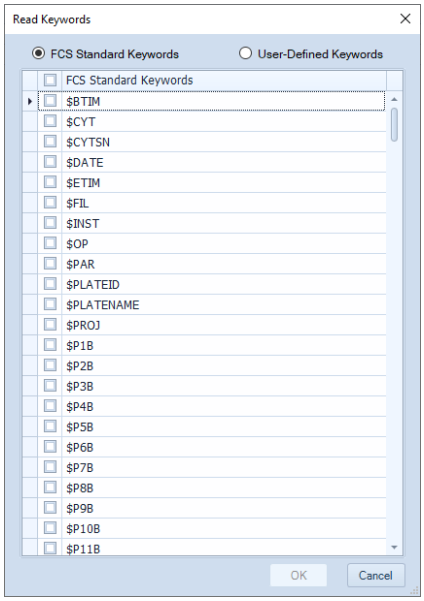
Icon	Description
	Save: Save the changes to the current keywords list. This function is disabled (i.e., greyed out) when there is no change to the current list.
	Create Keywords: When clicked, following window will be prompted. User can create new keywords or add existing keywords from **Keyword Library**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

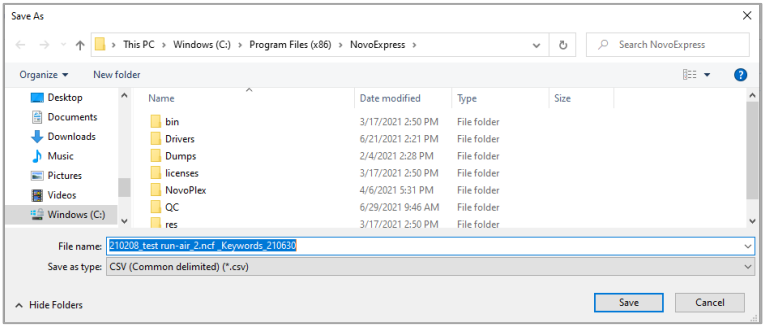


Read Keywords: When clicked, following window will be prompted. User can read and add selected **FCS Standard Keywords** or **User-Defined Keywords** into **Edit Keywords** window. Please refer to Read Keywords from Sample in this guide for more details.



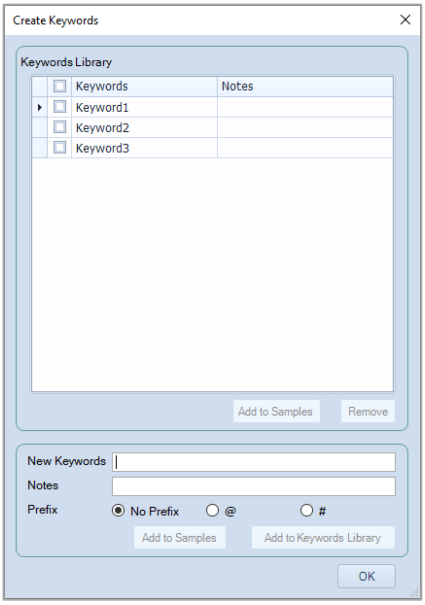
Delete Keywords: When clicked, the selected keyword column in **Edit Keywords** window will be deleted.

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 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.

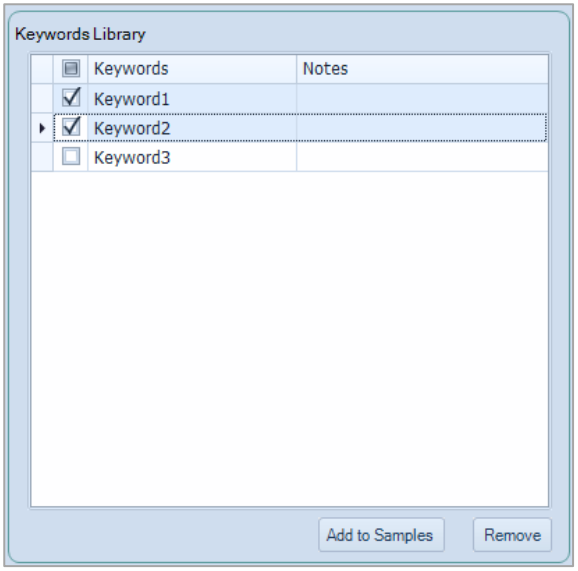
- The top panel is the **Keywords Library** which lists all keywords available in the **Keywords Library**. Keywords Library serves as a convenient way to save the commonly used keywords to be able to add to samples quickly.

  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.

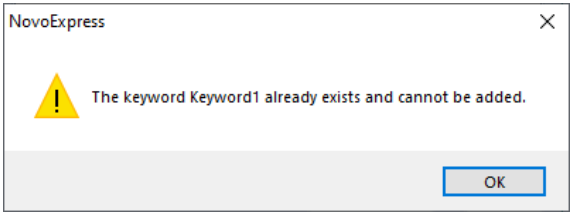
  To remove keywords, select the checkbox in front of one or more keywords from the list in **Keywords Library**, and click **Remove**.

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

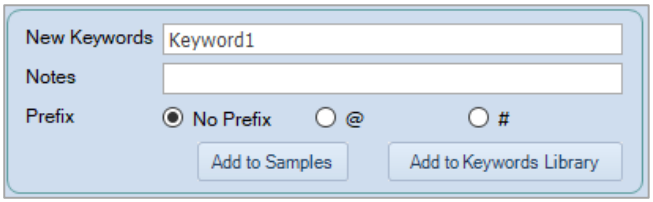


NOTE

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.

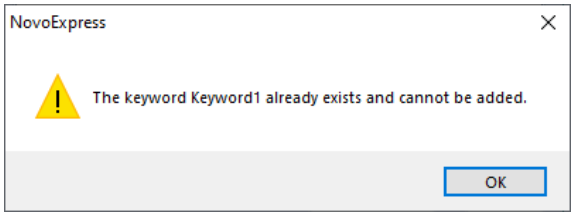


- The bottom panel allows user to create new keywords in the selected format and add to **Edit Keywords** window or **Keyword Library**. To do this, enter the newly defined keyword in **New Keywords** field, enter associated notes in **Notes** field if needed, select the prefix of the new keyword, click **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.



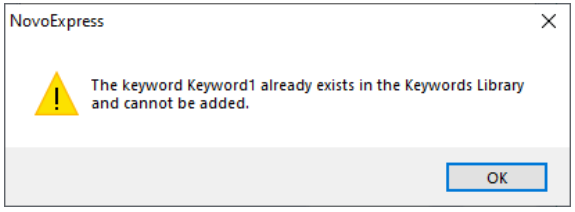
NOTE

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.



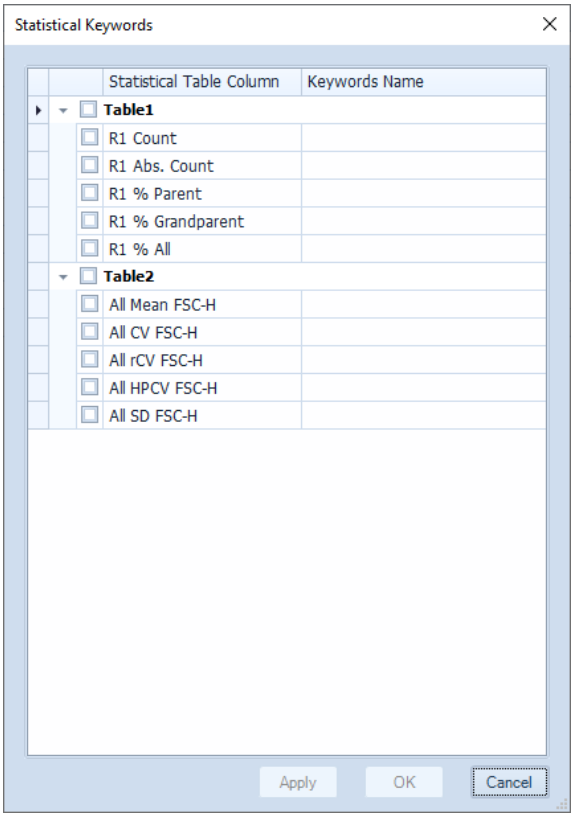
NOTE

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

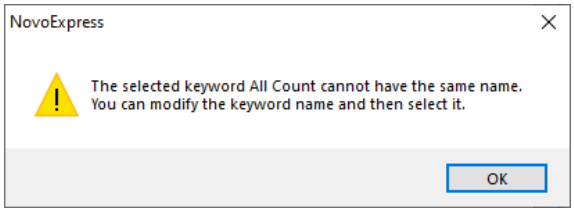
If there is more than one default type statistical table created for selected experiment file, the associated statistics will be displayed in groups respectively in this window.

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**.

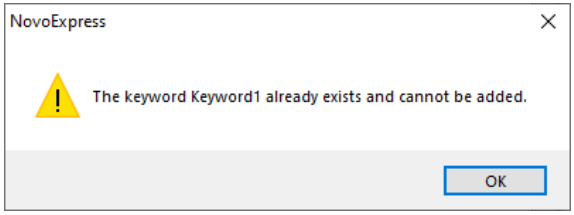
NOTE

If more than one statistical parameter with the same name is 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.



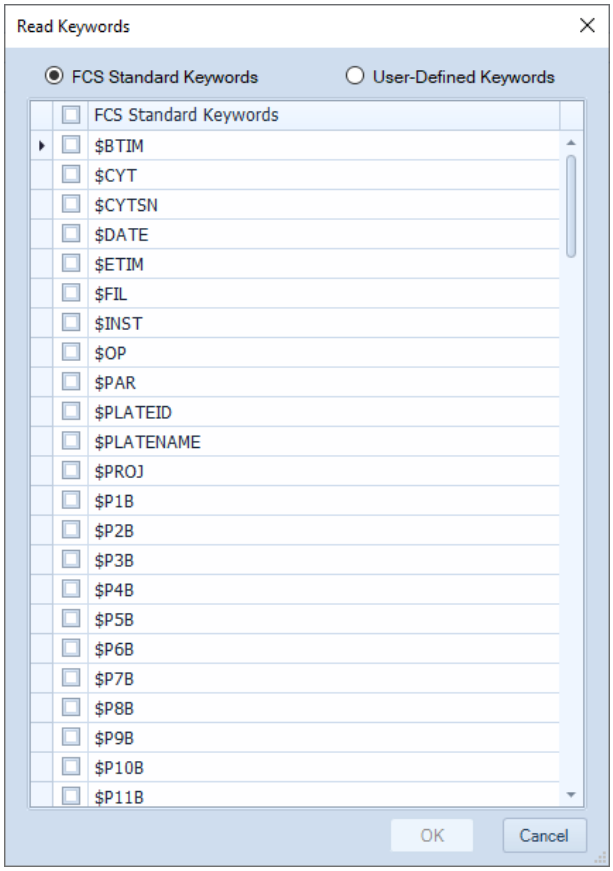
NOTE

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

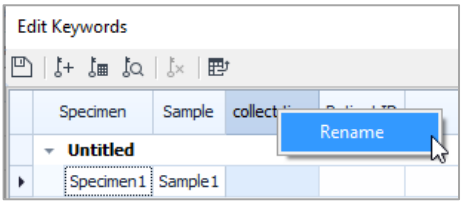
User can read **FCS Standard Keywords** or **User-Defined Keywords** from selected samples in **Edit Keywords** window. To do this, click from the toolbar in **Edit Keywords** window to open the **Read Keywords** window as shown below.

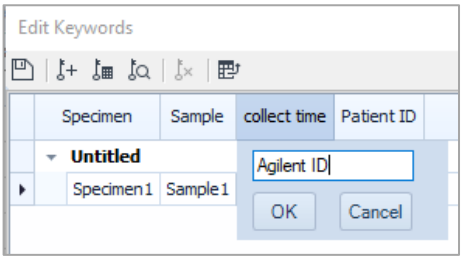


Select **FCS Standard Keywords** or **User-Defined Keywords** to display the corresponding keywords from the selected sample. Check the checkbox in front of selected keyword(s), click **OK**. The selected keyword(s) will be added to the **Edit Keywords** window.

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.

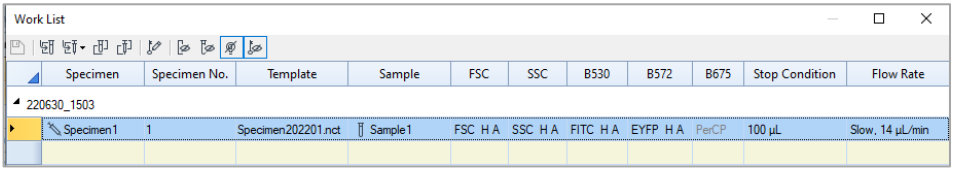
NOTE

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

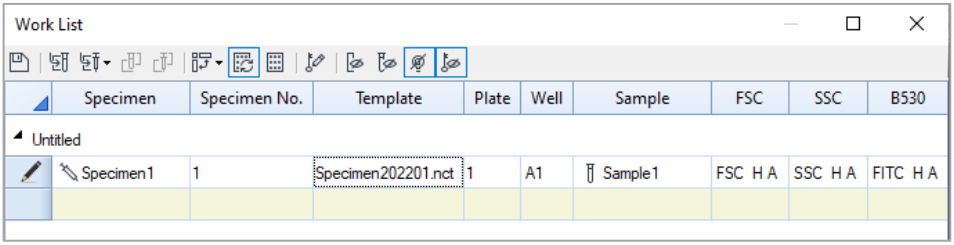
Before starting the experiment, the **Work List** can be used to set up the sample list. The **Work List** allows users to create new specimens and samples, import specimens or samples from a template, duplicate specimens or samples, and copy and paste sample information. Please refer to Experiment Manager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

The **Work List** displays all created specimens and associated samples. User can view the specimen's name, specimen No, template, sample type, plate number, well ID, sample name, acquisition parameters, stop conditions, threshold settings, compensation settings, and analysis and report information, etc.



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

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



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

Icon	Description
	Apply Modification: Click this button to save the changes made to the **Work List**. After applying the modification, the **Experiment Manager** panel updates to reflect the changes.
	Import Sample from Template: Click this button to import a new sample to the work list with selected template. User needs to select the template file to open. The template of the first sample in the file gets imported to the **Work List.**
	Import Specimen from Template: Click this button to import a new specimen to the work list with selected template. User needs to select the template file to open. The template of the first specimen in the file gets imported to the **Work List.**
	Duplicate as Sample: Click this button to duplicate selected sample as new sample and add to the **Work List**. Refer to Create a Duplicate Specimen or Sample in this guide for more details.
	Duplicate as Specimen: Click this button to duplicate selected specimen as new specimen and add to the **Work List**. Refer to Create a Duplicate Specimen or Sample in this guide for more details.
	Well ID Setting Direction: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. When new samples are created in the **Work List**, the **Well ID** will be assigned sequentially. Use the **Well ID Setting Direction** button in the Work List window to set whether new samples are created in a row-by-row sequence or a column-by-column sequence for a plate.
	Automatic Setting Well ID: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. When selected, a blue outline will show on the icon. When Well ID of a sample is modified manually, the NovoExpress Software will automatically assign new Well ID for blank samples below that sample in an order set in the Well ID Setting Direction based on the plate type information.

	Plate View: This function is only available when NovoSampler (or NovoSampler Pro or NovoSampler Q) is used. Click this icon opens the **Plate View** window. Please refer to Home 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 Disabled Parameters: Click to hide the acquisition parameters that are currently disabled.
	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**.**
	Hide Keywords: Click to display or hide the **User-Defined Keywords.** Please refer to Sample Keywords in this guide for more information.

NOTE

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

NOTE

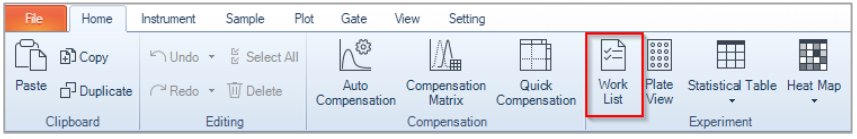
When collecting repeats from a sample well is desired, multiple samples can be assigned to the same **Well ID**.

Open the Work List

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

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

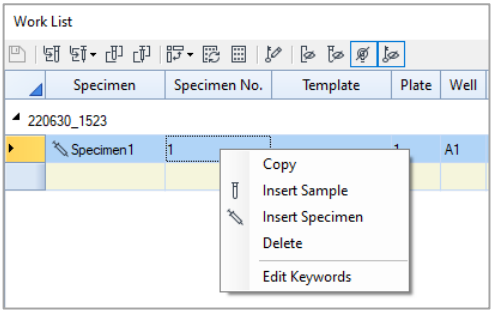
- From the **Home** tab in the **Menu Bar**, click on the **Work List** icon in the **Experiment** group.



Work List Management

Insert a New Specimen or Sample

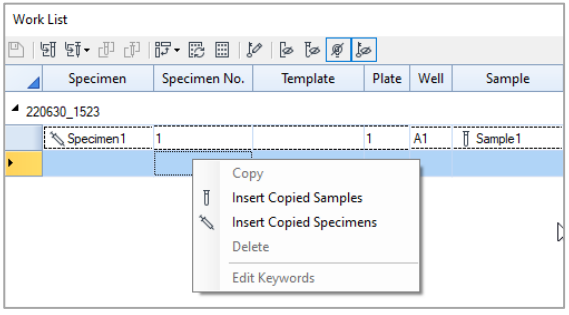
To create a new specimen or sample, right click on the first column of a sample row or an empty row. Click **Insert Specimen** to create a new specimen or click **Insert Sample** to create a new sample. The new sample will be placed in the selected row and under the corresponding specimen for that row.



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:

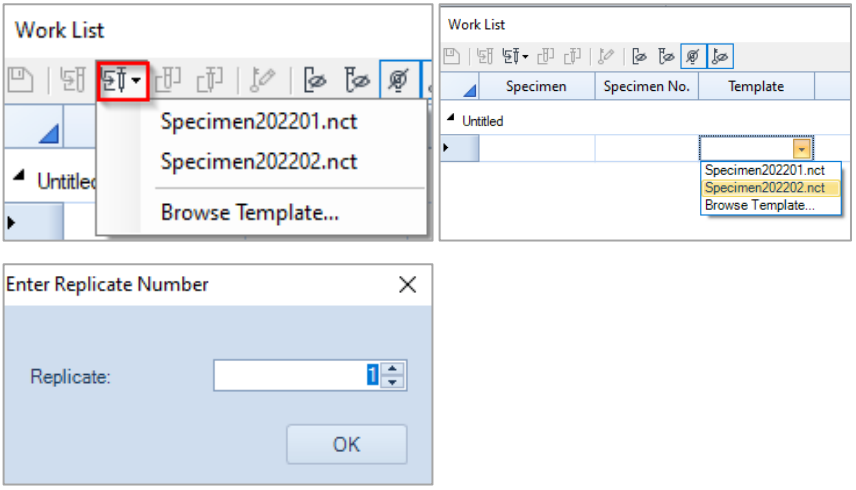
1 To select the specimens or samples for duplicating, click and drag in the first column of the **Work List**. The selected rows become highlighted.

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.

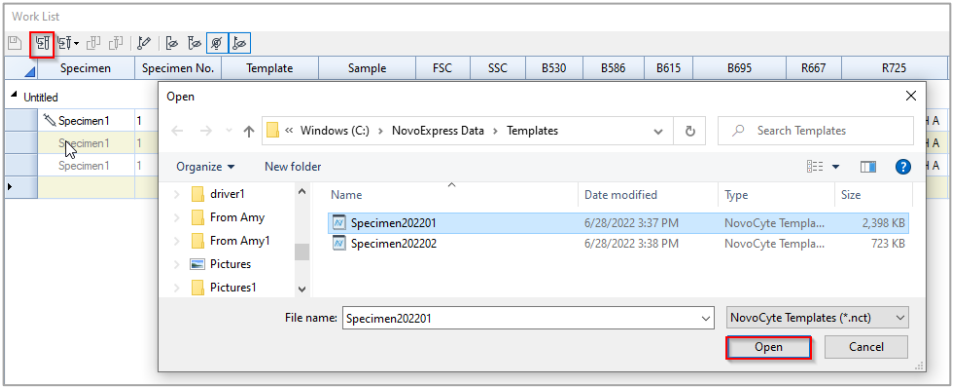
Create Replicate Specimen

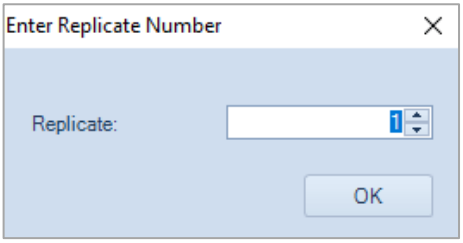
User can create replicate specimens in **Work List**. To do this, click **Import Specimen from Template** icon or double click an empty cell (not associated with any existing specimen) in **Template** column, select a template from the drop-down menu, enter the number of replicates in prompted window and click **OK**. The software will automatically create and add corresponding number of replicate specimens to the **Work List** using the selected template.



Create Replicate Sample

User can create replicate samples in **Work List**. To do this, click to select the sample, click **Import Sample from Template** icon and select the designated template from the prompted window, click **Open**. The **Enter Replicate Number** window will be prompted. Enter the number of replicates and click **OK**. The software will automatically create and add corresponding number of replicate samples to the **Work List** using the selected template.

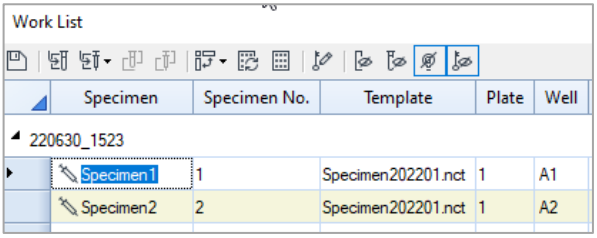




Edit a Work List Cell

Edit Specimen, Specimen No and Sample Names

To modify a specimen, specimen No or sample cell, double-click on the cell in the **Work List**. Enter the new value and press Enter key.

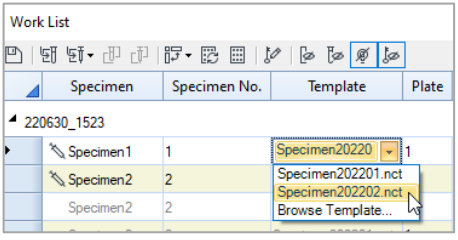


When a sample name is entered into an empty specimen, a new sample is created after Enter key is pressed.

When a specimen, specimen No, or template is entered into in an empty row, a new specimen is created after the edit is done.

Edit Template

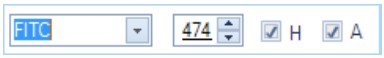
To modify a template, double-click on the **Template** cell in the **Work List**. After a new template is selected, the template is applied into the current specimen. Refer to Templates in this guide for additional details.



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

Edit Acquisition Parameters

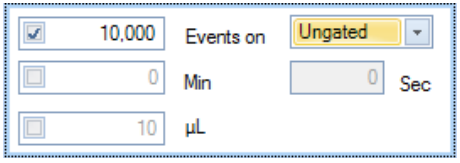
To modify the acquisition parameters, double-click on the channel cell to enter edit mode. In this mode, the fluorescence parameter alias and photodetector gain can be modified, and the height and area measurements can be enabled or disabled.



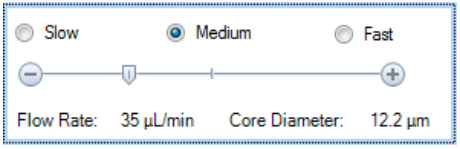
When in non-edit mode, the character * after photodetector gain text indicates the gain is not default value. For more information about photodetector gain, refer to Parameters Settings in this guide.

Edit Stop Conditions, Sample Flow Rates, and Threshold Settings

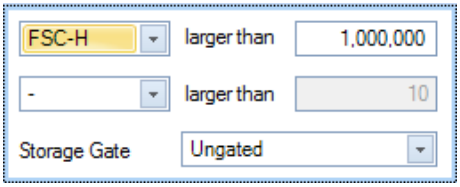
To set the sample stop conditions, double-click the stop condition cell to enter edit mode.



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



To set the sample threshold, 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

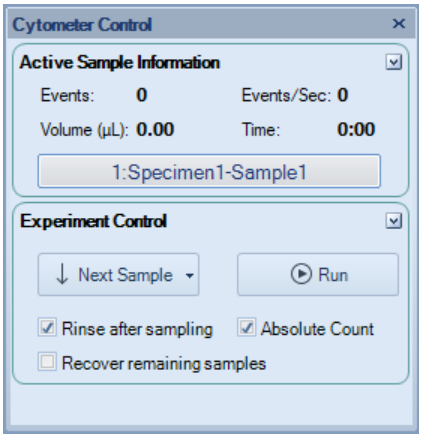
NOTE

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**.

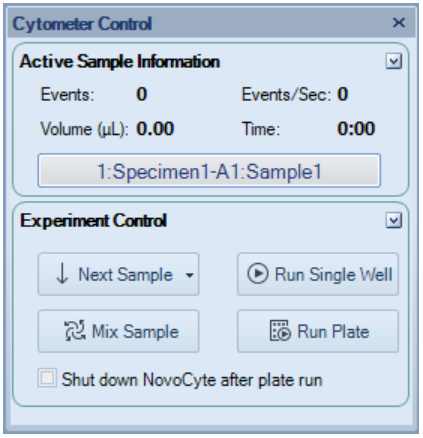
Cytometer Control

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

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



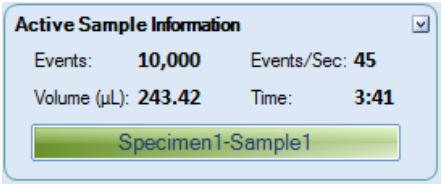
- When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected



Active Sample Information

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

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



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

Experiment Control

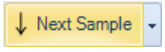
The **Experiment Control** panel displays different functions depending on whether the instrument is connected to NovoSampler (or NovoSampler Pro or NovoSampler Q)

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

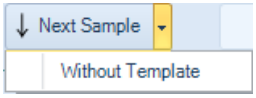
The **Experiment Control** panel includes the **Next Sample** and the **Run** buttons.

- **Next Sample**

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

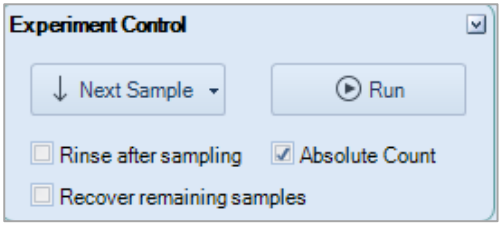
  To create a new sample without the template settings, click on the arrow on the right side of the **Next Sample** button and select **Without Template**. The new sample contains the same cytometer settings as the previous samples, but analysis, report and compensation settings are not transferred.

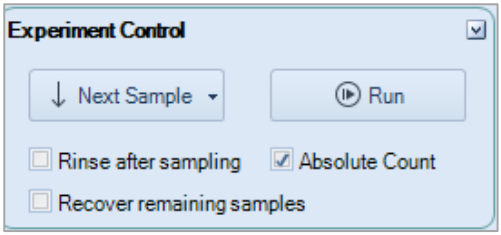
- **Run**

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

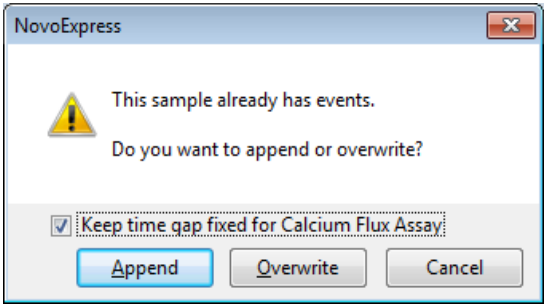
If the active sample does not contain event data, the **Run** button appears with a solid triangle. Click the **Run** button to begin sample acquisition.



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 check box in front of the "**Keep time gap fixed for Calcium Flux Assay**" will be automatically selected.

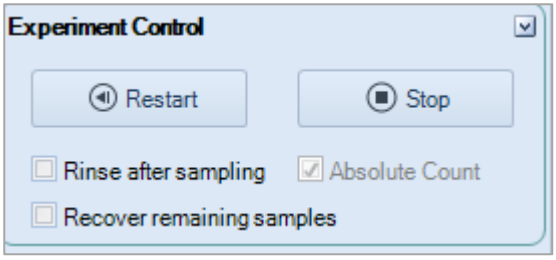


- **Restart**

  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.

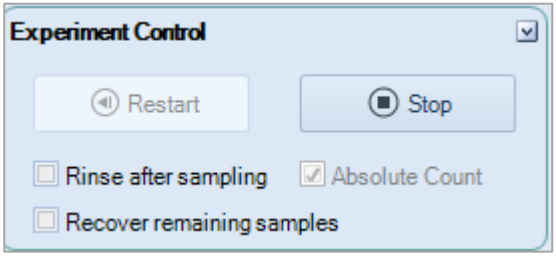
When **Restart** is clicked, the previously acquired events will be deleted and the acquisition status including sampling volume and sampling time will be reset to zero. Then the sample acquisition will restart until one of the defined stop conditions is met. The **Restart** button is only visible after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

NOTE

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

- **Recover Remaining Samples**: check to enable recover remaining sample already aspirated into the sampling tubing but not used for acquisition back into the sample tube. The Sample Injection Probe (SIP) will move down after

sample acquisition to return any remaining samples back to the sample tube (if tube holder is used) or plate well (if NovoSampler is used).

NOTE

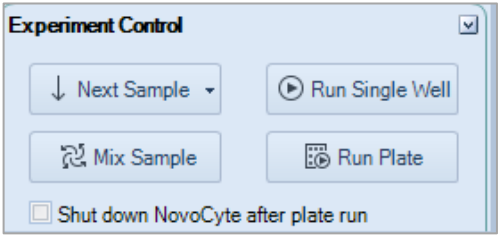
Do not remove the sample tube, tube rack or microwell plate to prevent spill of the sample. Ensure the moving path of SIP is not blocked by any obstacles.

NOTE

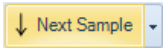
This function is available for NovoCyte Quanteon, NovoCyte Advanteon and NovoCyte Penteon instrument.

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

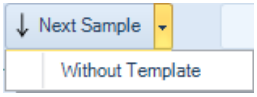
The **Experiment Control** panel includes the **Next Sample**, **Run Single Well, Mix Sample** and **Run Plate** buttons.



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

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

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

NOTE

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

NOTE

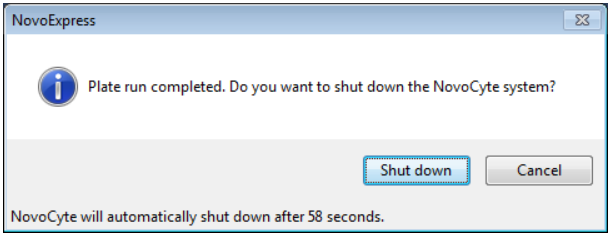
When **Run Single Well** is selected, the sample injection probe will automatically be rinsed after sample acquisition. This SIP rinse function will minimize the carryover between samples.

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

NOTE

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

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



NOTE

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

NOTE

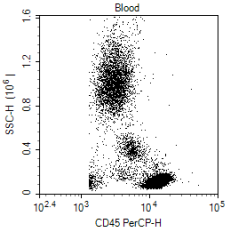
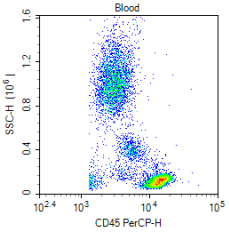
User can also schedule automatic instrument power on or off at selected time and frequency by clicking **Schedule Power On & Off** from the **Operation** panel. This function is available only when NovoCyte Quanteon, Advanteon or Penteon flow cytometer is connected. Please refer to Instrument in this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

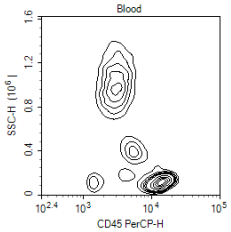
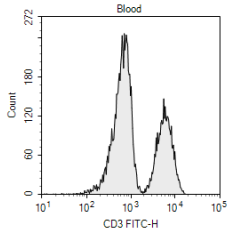
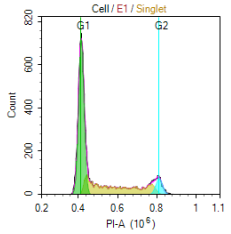
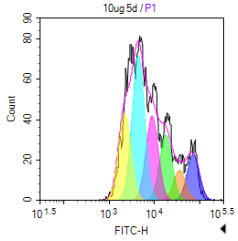
5 Data Analysis

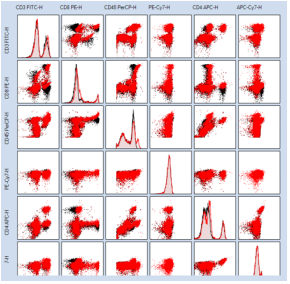
This chapter provides information for data analysis tools, including **Plots, Gates, Statistics, Fluorescence Compensation, Cell Cycle Analysis, Cell Proliferation Analysis, Statistical Tables, Heat Maps** and **Post Gain**.

Plots

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

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

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.	

Bi-variate Plot		Two-parameter	Create a matrix of plots with selected parameters plotted against each other. See Bi-Variate Plots for more information.	

Create a Plot

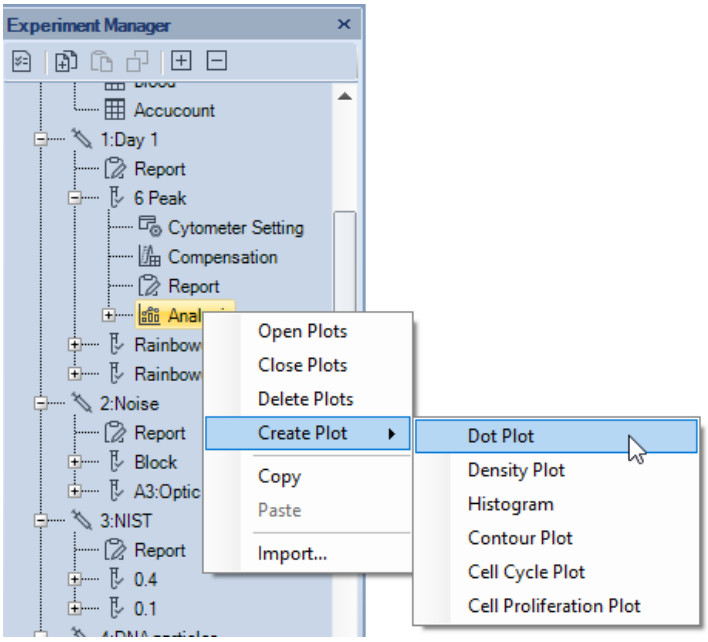
In the NovoExpress Software, plots (except bi-variate plot) can be created through the toolbar, the **Experiment Manager**, and the **Gate Manager**. In addition, plots can be duplicated in the Workspace, copied in the **Experiment Manager**, and imported from templates.

Create a Plot with the Toolbar

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

Create a Plot with the Experiment Manager

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



Create a Plot from a Gate

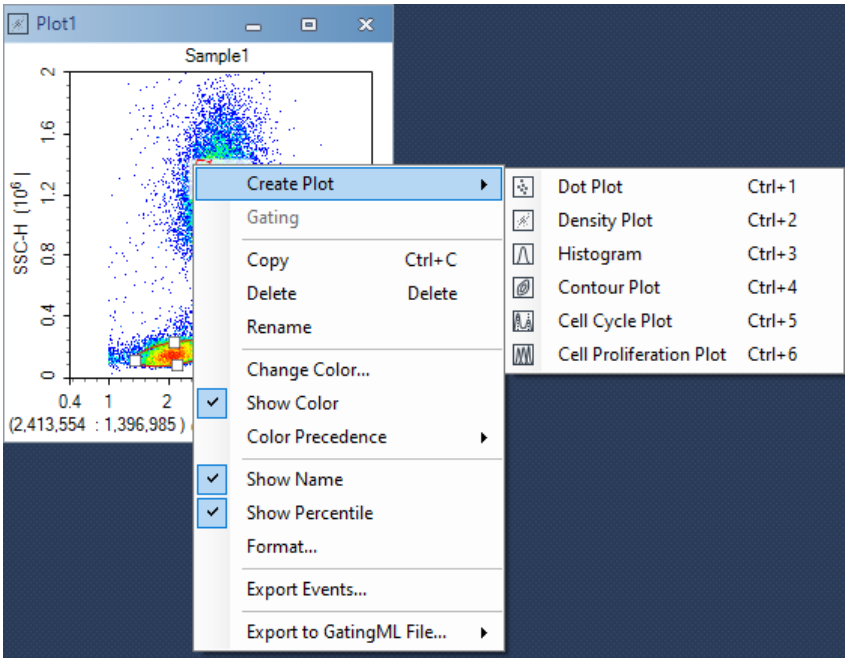
In the Workspace, new plots can be created using gates from previously created plots. New plots created through gates will only display events within the gate. There are multiple methods for creating a new plot through a gate:

- Double-Clicking a Gate

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

- Selecting a Gate within the Workspace

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



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

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

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

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

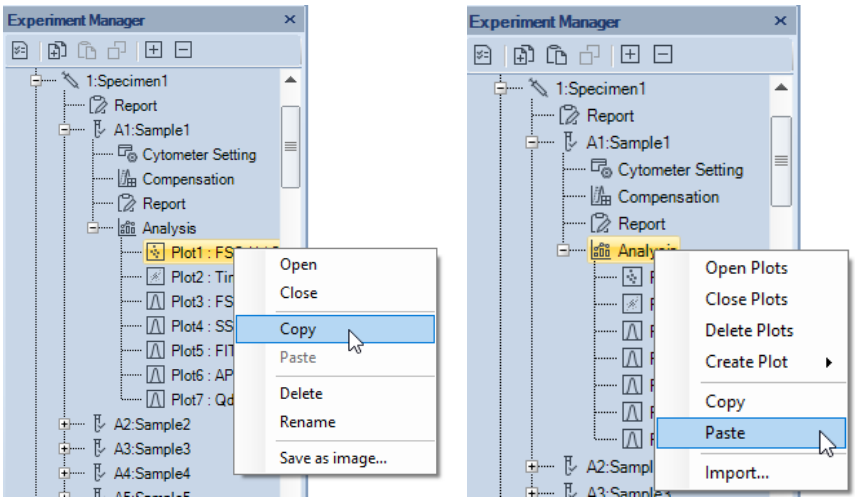
Create a Duplicate Plot

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.

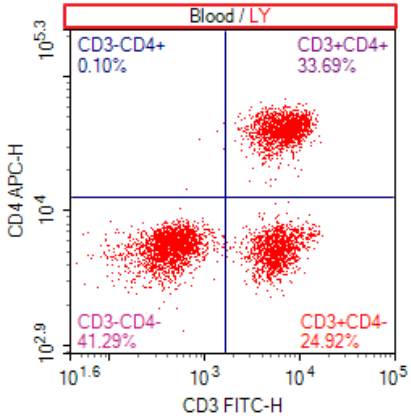
NOTE Click the **Close** button in the top right corner of a plot window to delete the plot.

Edit Plots

Plot Gating

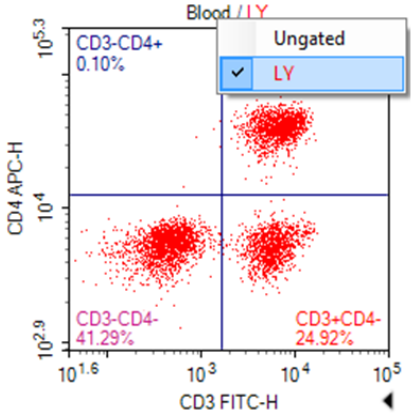
To analyze subpopulations, plots can be set to only display events from within a specific gate. For this method, a gate from a previous plot will be applied to a newer plot. The new plot can then be used to analyze the subpopulation or to further gate for more specific populations.

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 LY.



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

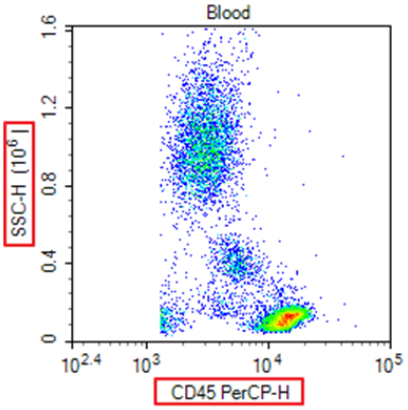
- In the plot header, right-click to display a drop-down menu. In the drop-down menu, select the gate. If **Ungated** is selected, the plot is not gated, and all events are displayed in the plot.



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

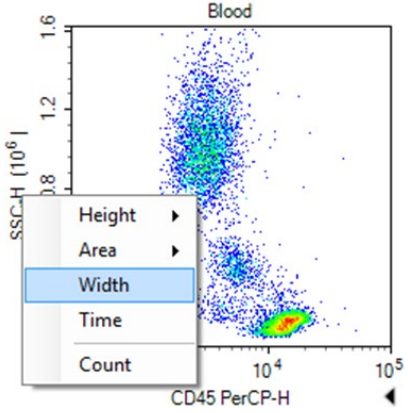
Parameter Plot Settings

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



To change the plot parameters:

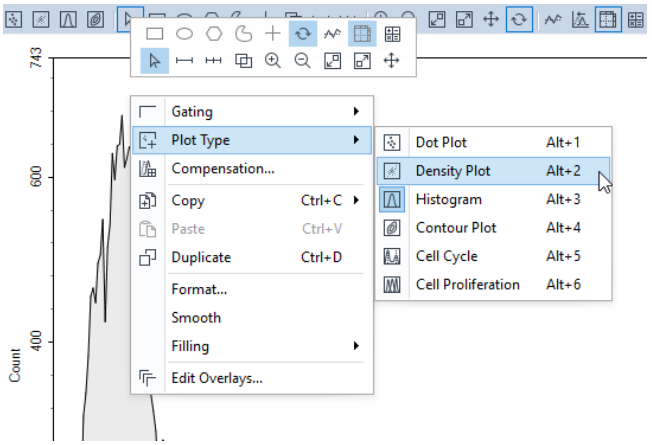
Right-click on the plot label and select the desired parameter. In the drop-down menu, the scatter and fluorescent parameters are separated in height and area measurements. Additional parameters include Width, the width of the individual event signal, Time, the time of the individual event signal, and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



Set Plot Types

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

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



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

NOTE

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

Rename Plots

There are three methods for renaming a plot.

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

Delete Plots

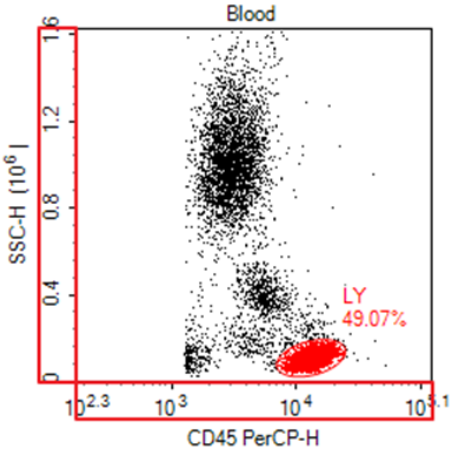
There are multiple methods for deleting a plot.

- Click the **Close** button in the top right corner of the plot window to delete the plot.
- In the **Experiment Manager** panel, right-click on the plot node and select **Delete** to delete the plot.

- In the **Experiment Manager** panel, right-click on the sample node or **Analysis** node and select **Delete Plots** to delete all of the plots associated with the sample.

Set the Coordinates of the Axis

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



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.

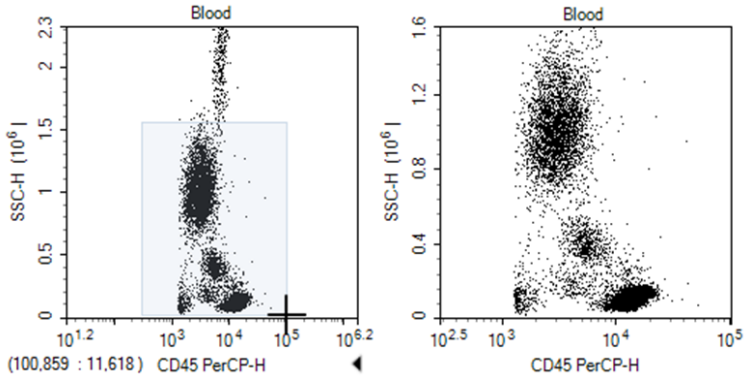
  Move the cursor to the maximum or minimum position of the X or Y coordinate. The cursor will change to for X coordinate or for Y coordinate. Click and move the cursor to change the maximum or minimum

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**.

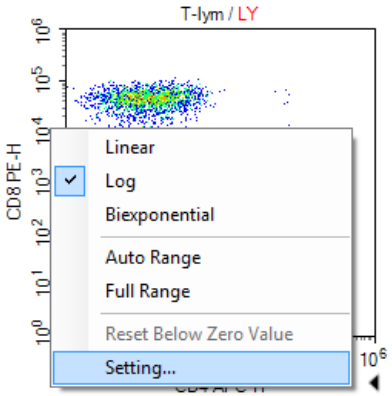
  To use this tool, click and drag in a plot to move the display region. The coordinate range then automatically adjusts.

NOTE

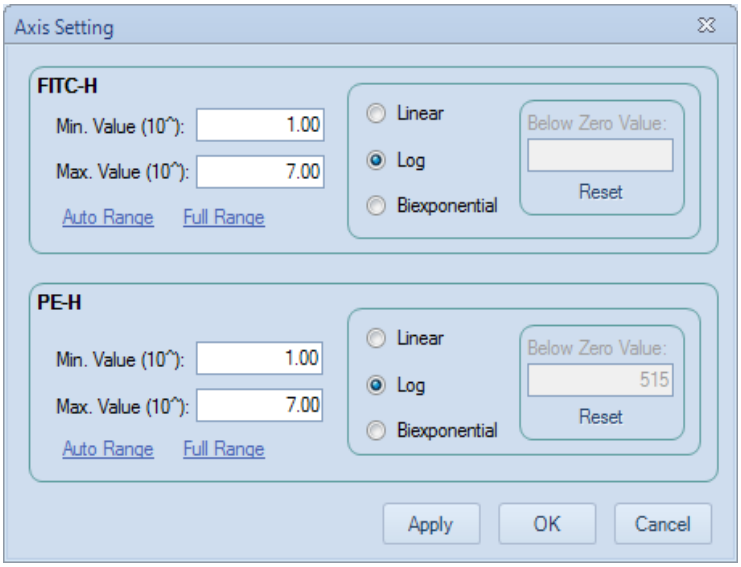
To pan the plot only along one parameter, click and drag along the parameter's coordinate label. This method causes the plot to pan along the selected parameter, while the second parameter's coordinate range will remain the same.

- 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.

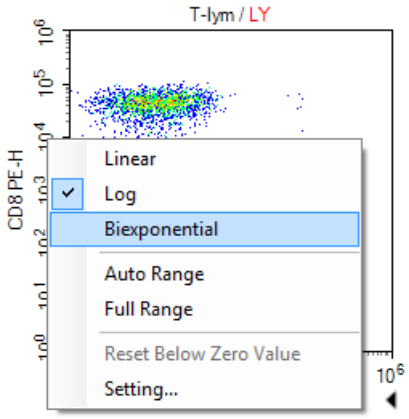


- Manually Setting the Coordinate Range in the **Plot** tab of **Menu Bar**.

Set the Coordinate Scale

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

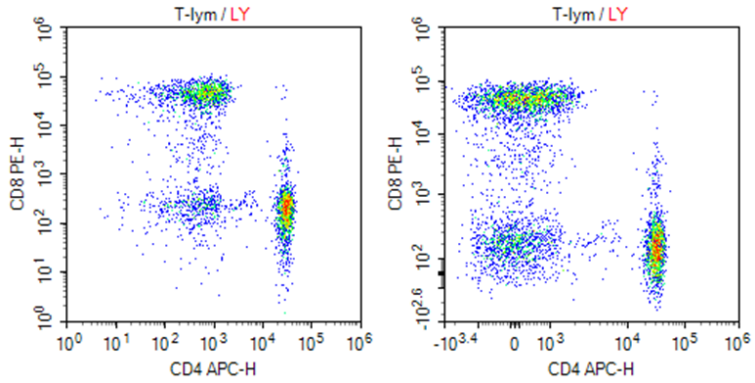
To set the coordinate scaling, right-click on the coordinate label and select the axis scaling.



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.

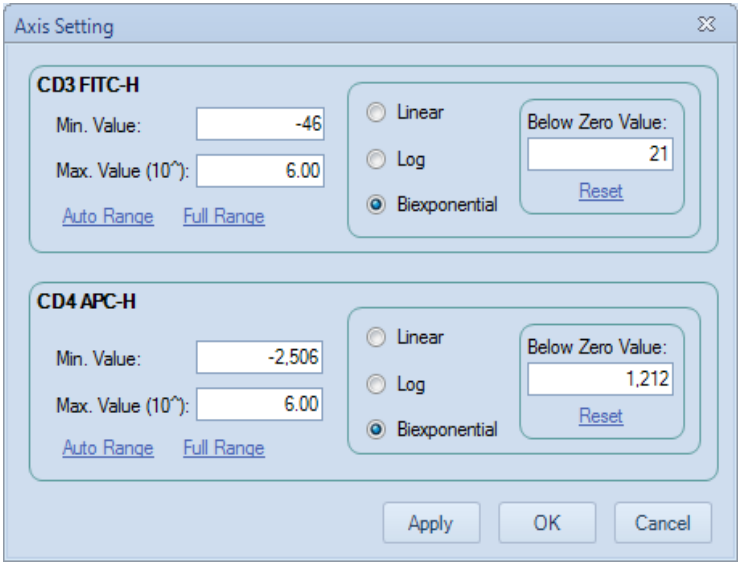


- Below Zero Value of biexponential scale

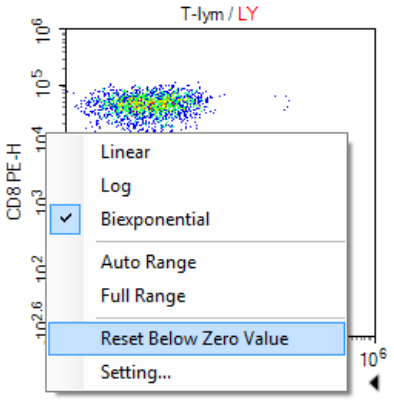
  Biexponential transformation can be seen as combination of near linear and near logarithmic scales. It goes smoothly from near linear within the reflection

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 software.

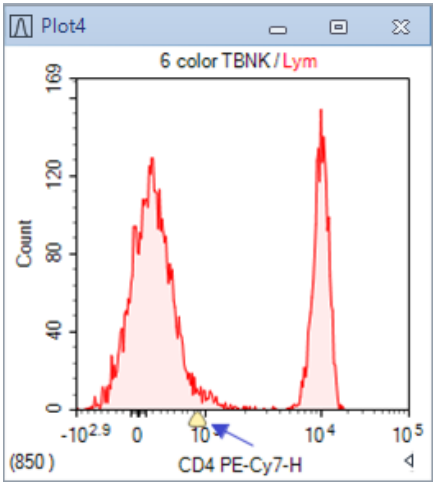
Manually enter the **Below Zero Value** in **Axis Setting** dialog or click **Reset** button to let NovoExpress software calculate the value automatically. When resetting, software calculates the **Below Zero Value** according to the events data in current gating of plot. The minimum value of the axis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



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.



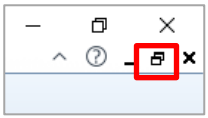
To adjust the Below Zero Value directly on plot, move the cursor to a coordinate axis with biexponential scale, a triangular symbol will appear on the position of below zero value. Click and drag the triangular symbol to adjust the Below Zero Value, and the plot will reflect the change dynamically while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

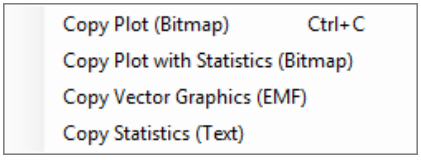
Adjusting the size of the plot window does not affect the coordinate range of the plots. To adjust the coordinate ranges, refer to Set the Coordinate Range in this guide.

Copy or Save Plots

Plots from the NovoExpress 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 will copy the selected plot in bitmap format.

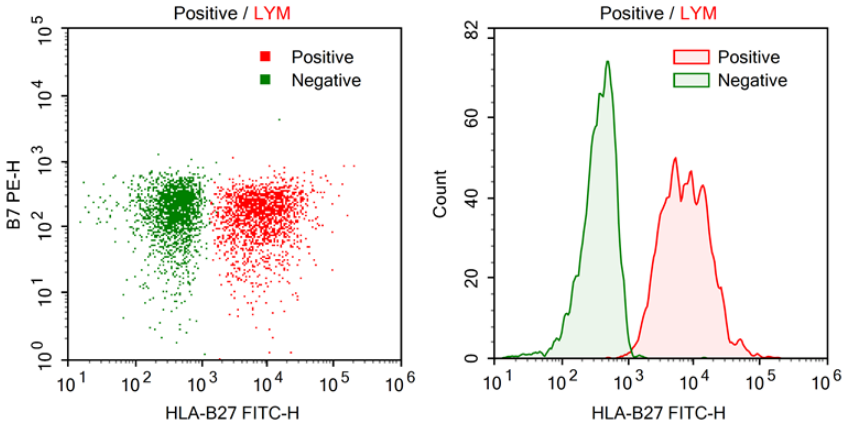


- To save a plot

  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.

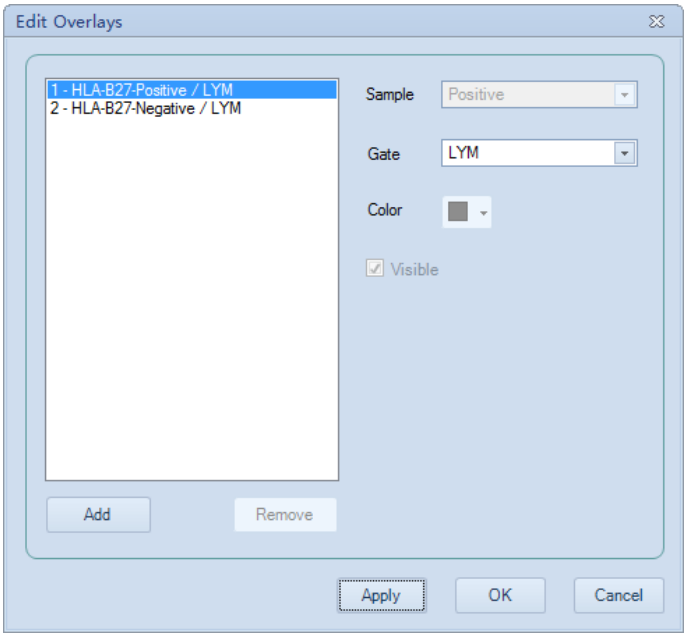


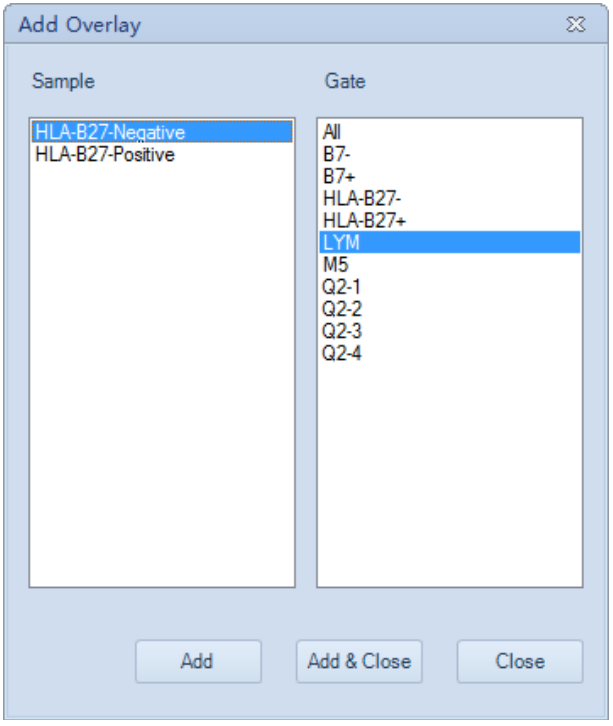
- Add overlays by using the drag and drop method:

  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:

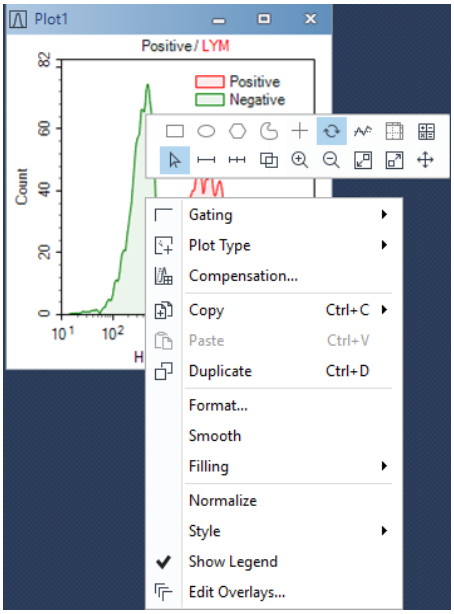
  Select the plot, click **Plot > Overlays** from the software main menu or right-click the plot to access the shortcut menu, select **Edit Overlays** to prompt the **Edit Overlays** dialog window as shown below. In the dialog, all overlays of the plot are listed. One can select an overlay, set the overlay's 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.


Edit Overlays
1 - HLA-B27-Positive / LYM
2 - HLA-B27-Negative / LYM
Sample
Positive
Gate
LYM
Color
Visible
Add
Remove
Apply
OK
Cancel


Add Overlay
Sample
Gate
HLA-B27-Negative
HLA-B27-Positive
All
B7-
B7+
HLA-B27-
HLA-B27+
LYM
M5
Q2-1
Q2-2
Q2-3
Q2-4
Add
Add & Close
Close

- 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.

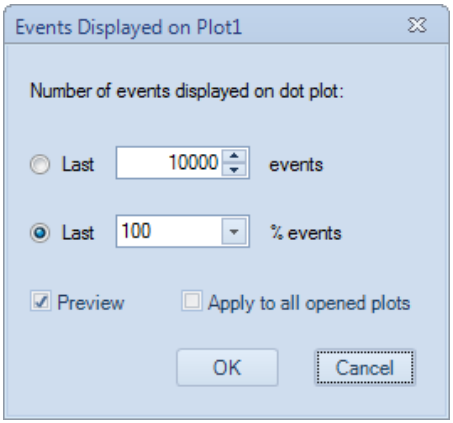


Plot Formatting

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….**

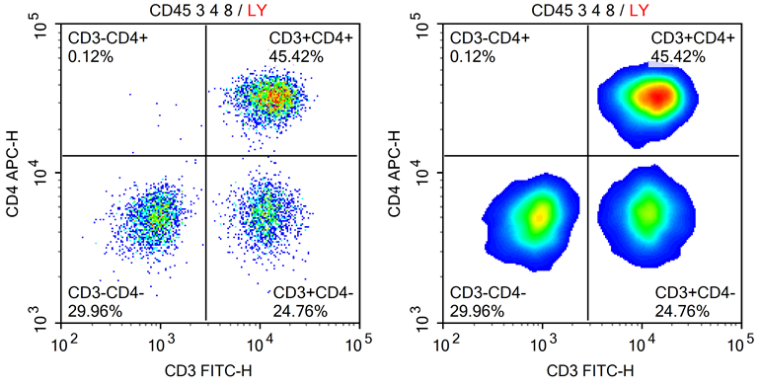


In the window, selecting **Preview** modifies the dot plot display as the user is changing the settings. Selecting **Apply** to all open plots applies the setting to all open dot plots.

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.

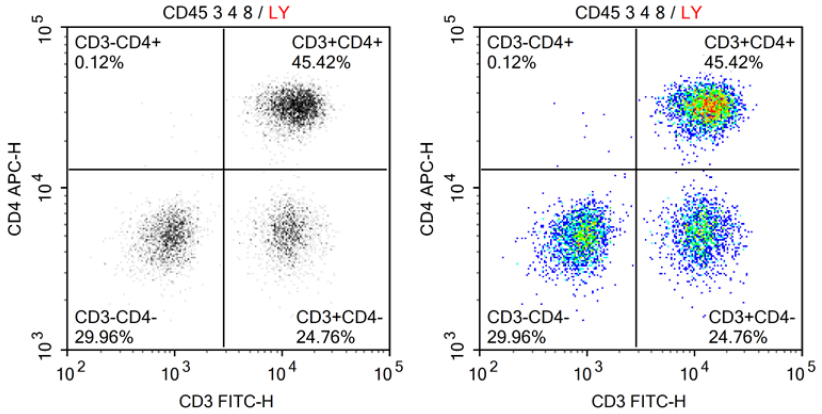
- **Smooth** density plot: In this view, the density plot data are smoothed. To use this view, right-click on the density plot and select **Smooth** or select **Smooth** from the **Plot** tab of the **Menu Bar**. A comparison of a standard pseudocolor density plot (left) and a smooth pseudocolor density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

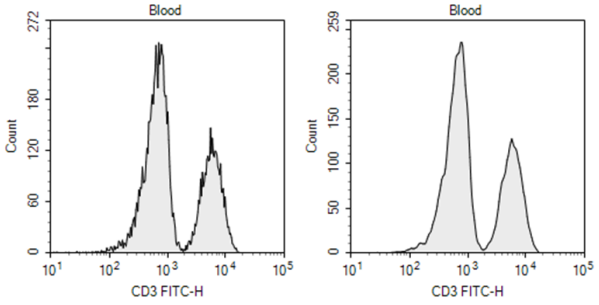
shown in warmer colors (colors toward the right of the color bar below) and areas of the plot with a lower density of events are shown in cooler colors (colors toward the left of the color bar below).

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.



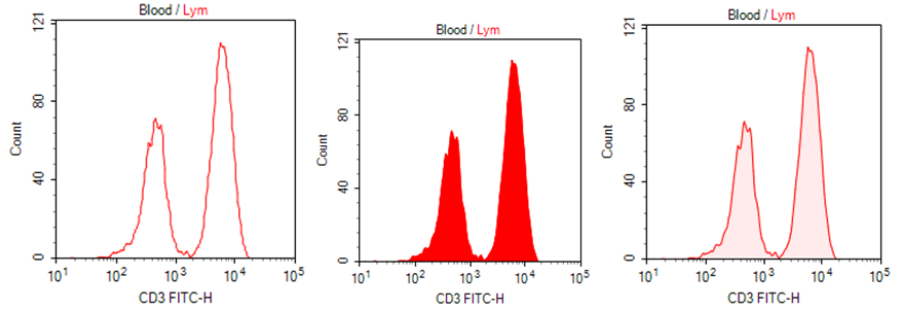
Histogram Plot Formatting

- **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.

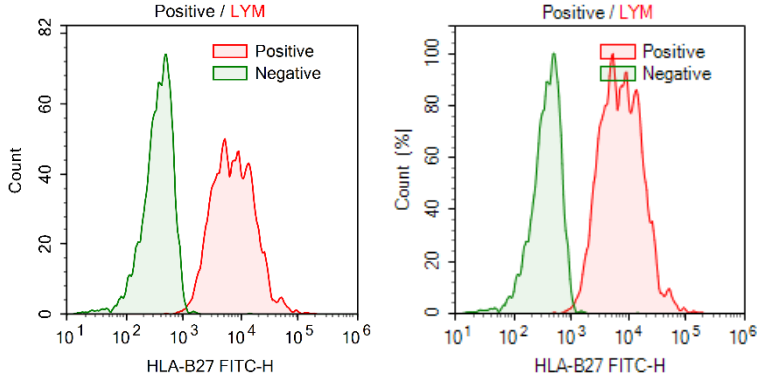


- Histogram fill type: Histogram plots can be viewed with different fill types. To select a fill type, right-click a histogram plot and select **Filling** and select the fill

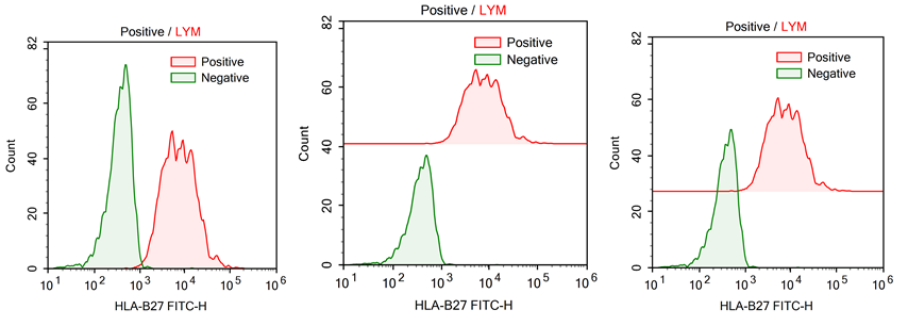
type or select the fill type from the **Filling** drop-down menu in the **Plot** tab of the **Menu Bar**. A comparison of the **filling** types is shown below. The options include **None** (left), **Filled** (middle), and **Tinted** (right).



- 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.



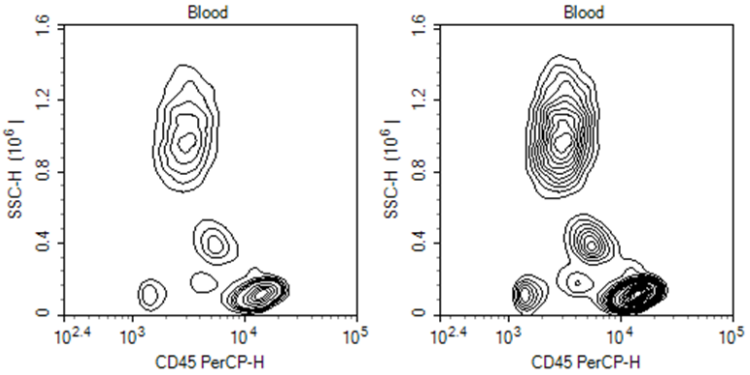
- Histogram layering: When overlaying histogram plots, different overlay styles can be selected. To select an overlay style, right-click in a histogram plot with layers and select Style to select the overlay style. The overlay style options, as shown below, include **Overlaid** (left), **Offset** (middle),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



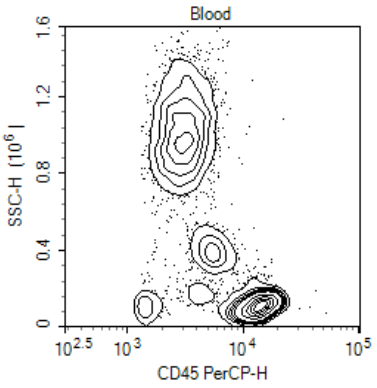
Contour Plot Formatting

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).

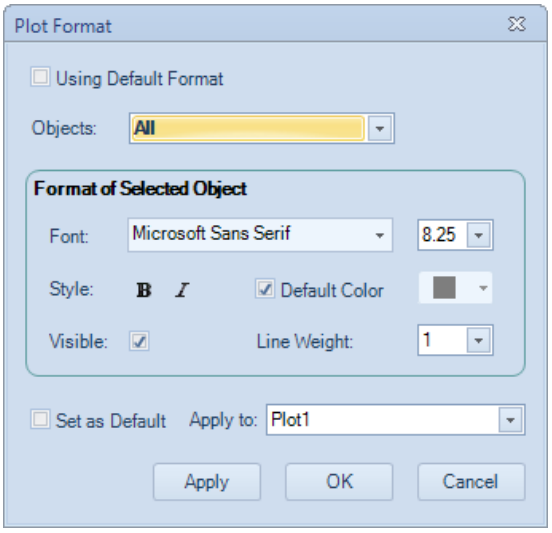


- **Show Outlier**: If selected, outlier events are shown as dots on contour plots. A contour plot is shown below with **Show Outlier** selected.



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**:

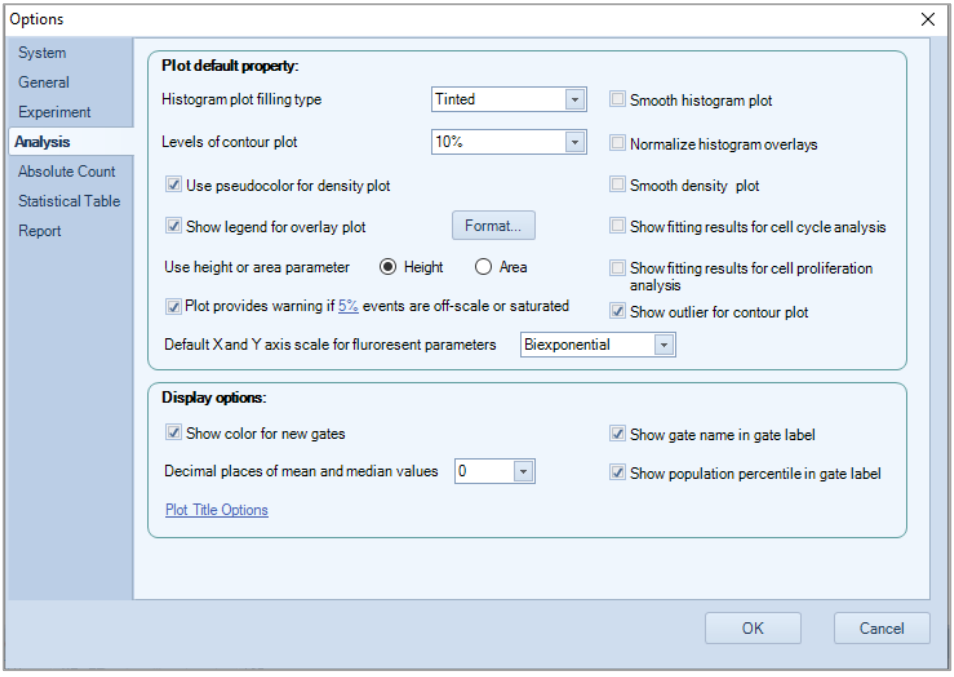
  Click to apply changes and close this window.

- **Cancel**:

  Click to close this window without applying any changes.

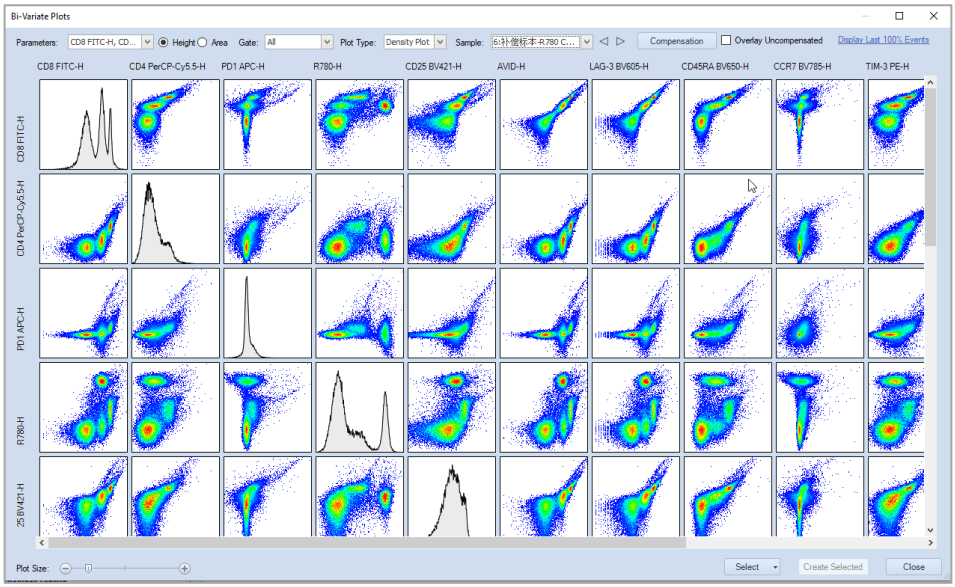
Default Plot Settings

To change the default settings for plots, go to **File** > **Options** and select the **Analysis** tab. Refer to Setting in this guide for details.

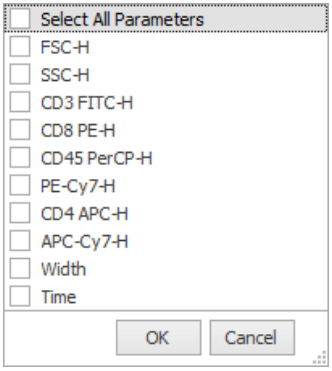


Bi-Variate Plots

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

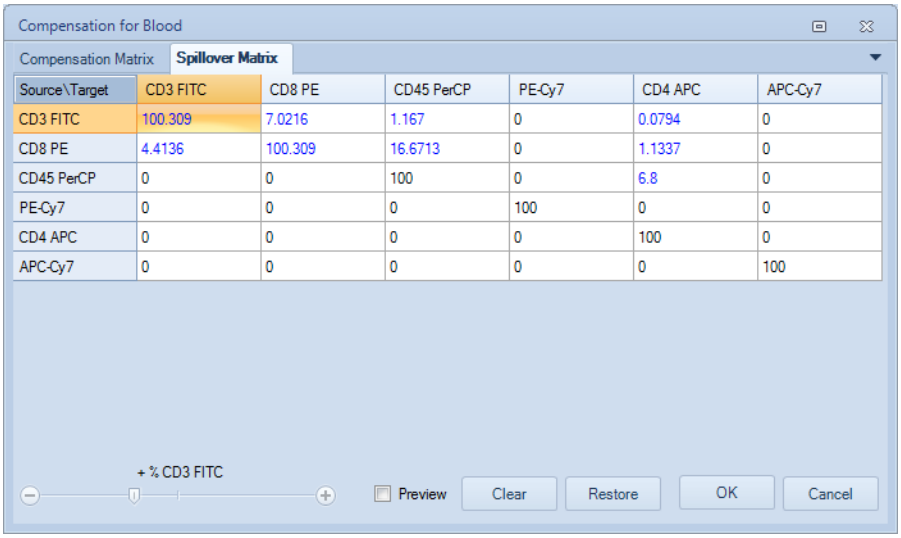


- **Parameters**: List all the parameters of the selected sample in the **Sample** drop-down box. Clicking the **OK** button after selecting the parameters in the drop-down list will create the N×N plots of the selected parameters. Check **Select all parameters** to check all the parameters.

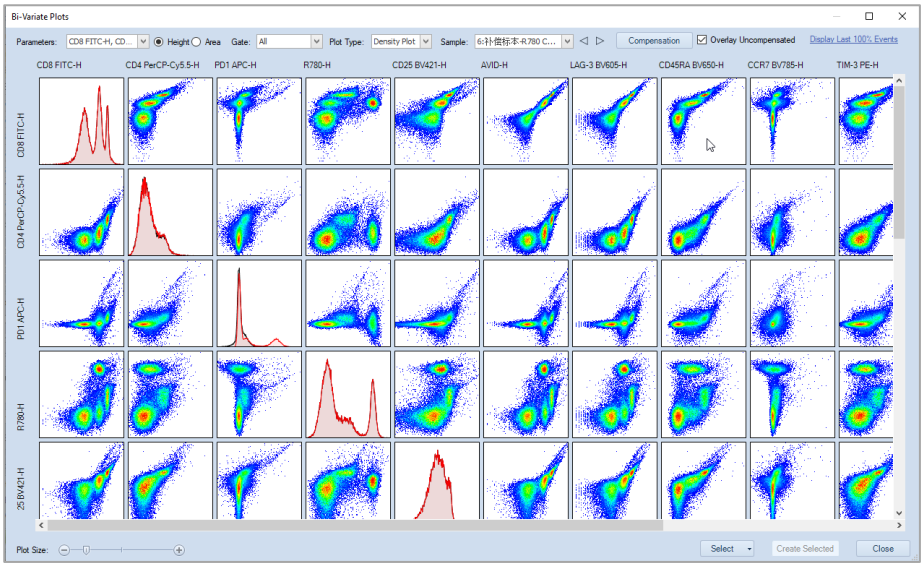


- **Height**: Select to set the parameter of the plots to Height.
- **Area**: Select to set the parameter of the plots to Area.
- **Gate**: List all the gates of the selected sample in the **Sample** drop-down box. The first item is **Ungated**. When select a gate, only the data in the gate will be displayed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

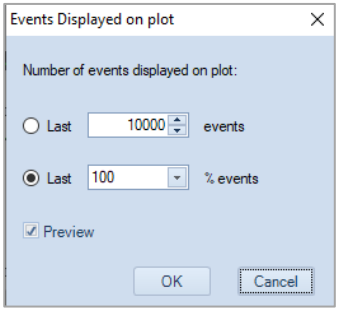
- **Sample**: Select which sample in the experiment file to be plotted. Be default, the current active sample will be selected when the window is opened. Used icon to switch samples forward and backward.
- **Compensation**: Click to open **Compensation** window to change the compensation settings. The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



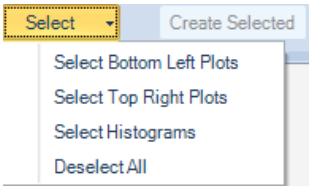
- **Overlay Uncompensated**: Select to overlay uncompensated data on the created bi-variate plots.



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



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



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

NOTE

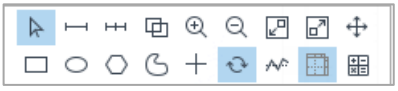
The settings user defined in Bi-variate Plots window, including Parameter, Height, Area, Gate, Plot Type, Sample, Compensation, Overlay, and Events to be displayed will be automatically recorded by the software. When user re-open this window or restart the software, these settings will be automatically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

Gates

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

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

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

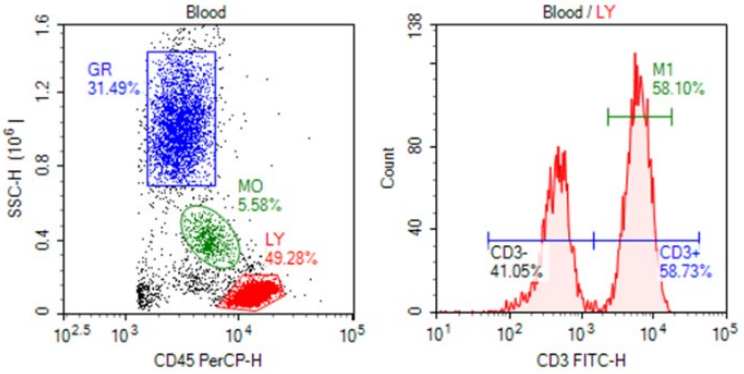


All gate types can be created on dot plots and density plots. Range gates and bi-range gates can be created on histograms. Gates can also be combined to create a logic gate.

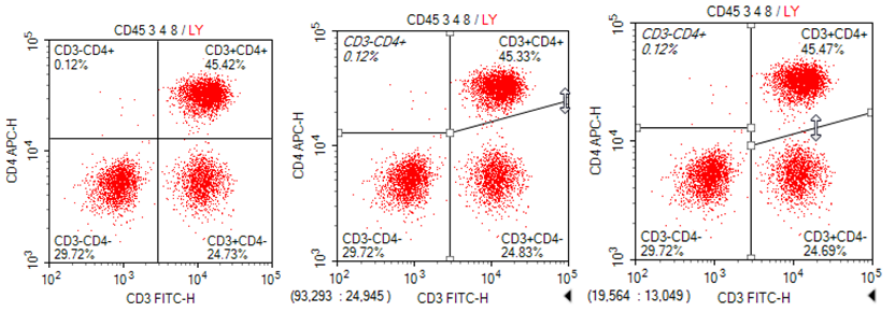
Create Gates

- To create a rectangular gate: Click the rectangular gate icon , in the Workspace Toolbar. Click and drag in the plot to enclose the target population within the rectangle. Release the mouse button to create the gate.
- To create an elliptical , range , or bi-range gate : Follow similar procedures as for creating the rectangular gate.
- To create a polygonal gate: Click the polygonal gate icon , in the Workspace Toolbar. Left click in the plot to create the first vertex of the polygon. Click in a new location to create the second vertex of the polygon. Continue moving around the target population and creating vertices until the target population is enclosed. On the last vertex, double-click to complete the polygon and create the gate.
- To create a Freehand Gate: Click the **Freehand Gate** icon , in the Workspace Toolbar. Left click start point in the plot to begin creating the Freehand Gate. Move the mouse cursor inside the plot to draw curve. Double-click the end point to complete and create the gate.

  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.

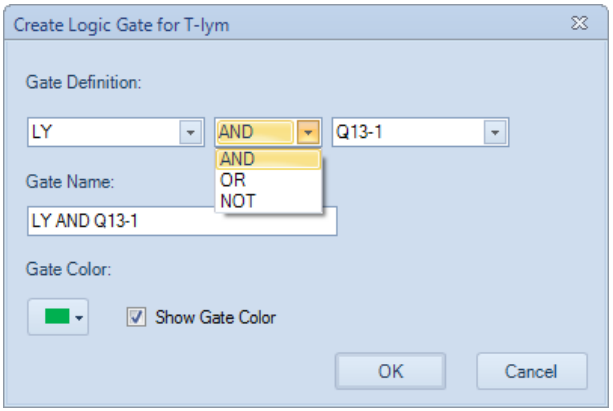


- To create a logic gate: Click the logic gate icon, in the Workspace Toolbar to open the **Create Logic Gate** window. In this window, the user can create a logic gate for the sample in the selected plot.

  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 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 the events excluded from the selected gate.



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

NOTE

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

Edit Gates

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

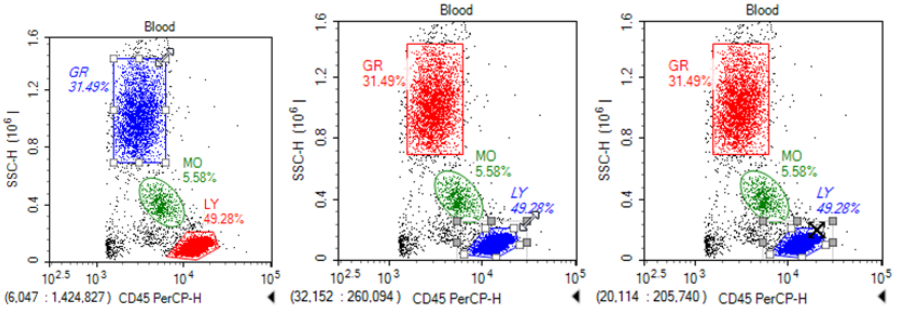
There are multiple methods to select a gate for editing. Options include:

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

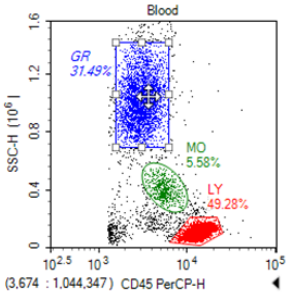
After the gate is selected, the gate's control points are displayed (as white boxes). To change the size of a gate, click and drag the control points to modify the gate.

NOTE

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



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



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

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

Gate Display Format

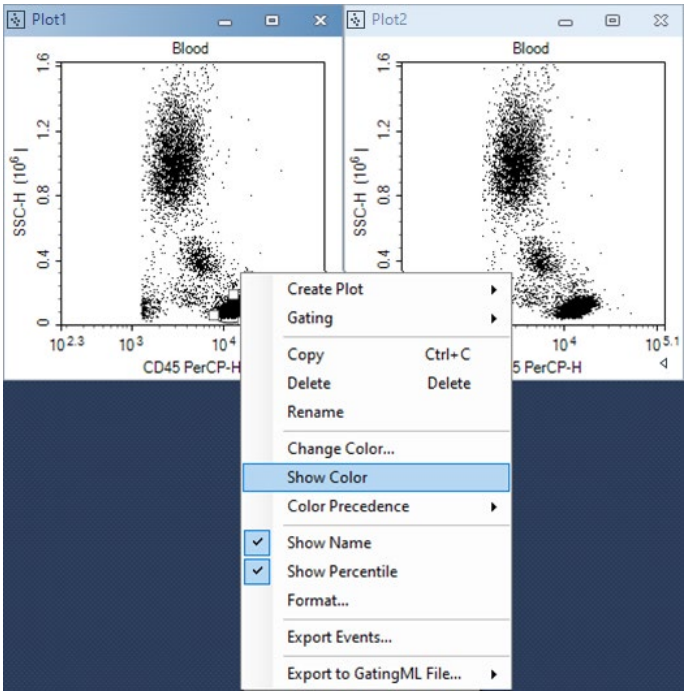
The NovoExpress Software allows users to format the color and labels of gates. Gate color determines the color of events displayed on the dot plot, as well as the color of the histogram when the gate is applied to a histogram.

Set the Color of the Gate

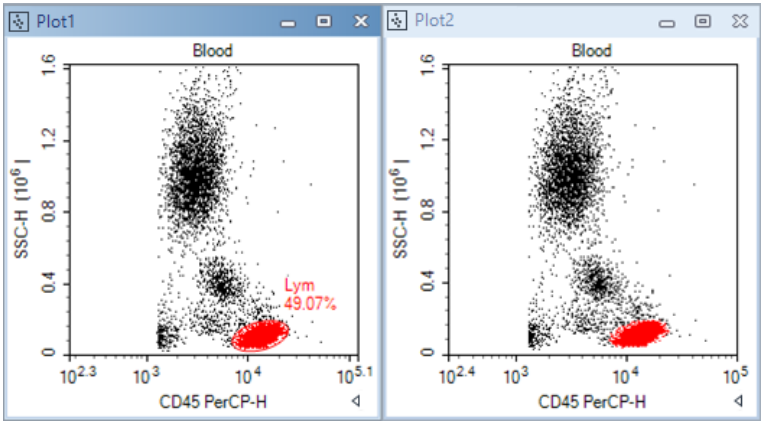
The color of each gate can be set. In dot plots, the events included in the gates are displayed in the chosen color. If additional dot plots are created, these events are displayed in the same color for easy identification.

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

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

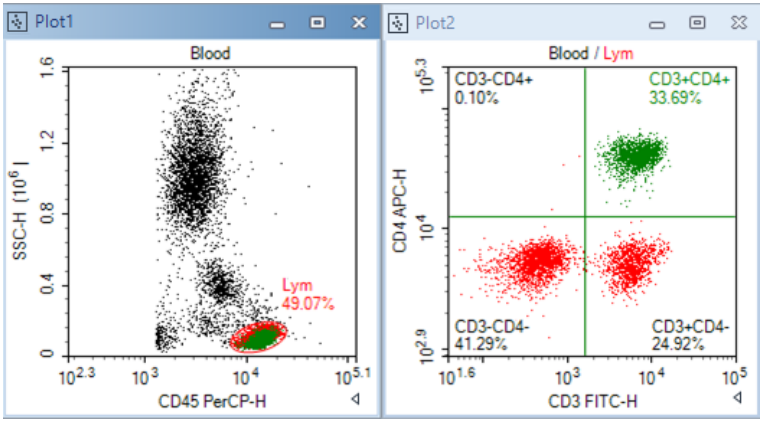


The plots below have a gate with the color selected.



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.



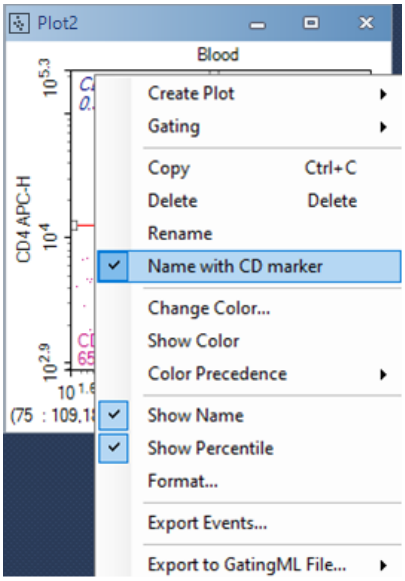
Gate Labels

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



Apply a Gate to a Plot

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

To apply a gate to a plot:

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

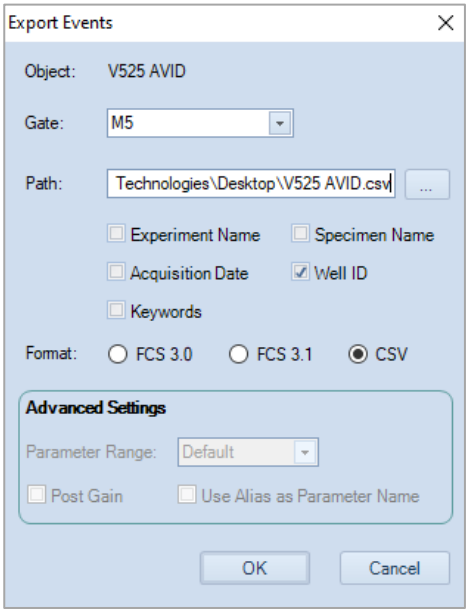
- Select a gate. Drag and drop the gate into a different plot. The dragged gates are pasted into the plot where it was dropped.
- To duplicate a gate within the same plot, select a gate and use the keyboard shortcut Ctrl D or select the gate and use the **Duplicate** button from the **Home** tab of the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

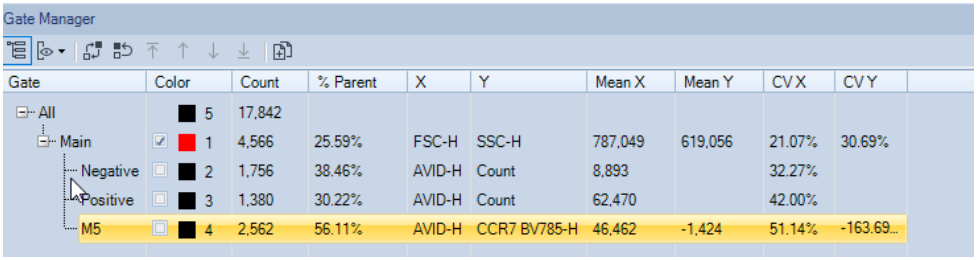


3 In the window, set the export path, file format, parameter range and post gain. Click OK to export the data.

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 shows gate hierarchy and gate statistics.



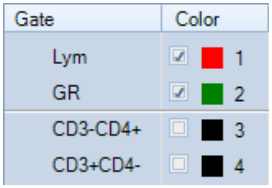
Toolbar of Gate Manager

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.
	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.

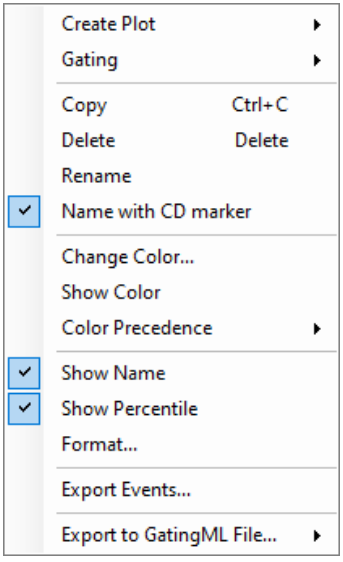
To modify gate color precedence, check the **Modify Color Precedence** tool in the toolbar. The color column in **Gate Manager** is shown below:



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 check box to set whether to show gate color (black color indicates no color is shown). Click the color square box to change gate color in a pop-up tool window.

Context menu

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



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

Gating: Selects plots to apply the gate.

Open: Opens the plot containing the gate.

Copy: Copies the gate.

Delete: Deletes the gate.

Rename: Renames the gate.

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

Change Color: Modifies the color of the gate.

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

Color Precedence: Modifies color precedence of the gate.

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

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

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

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

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

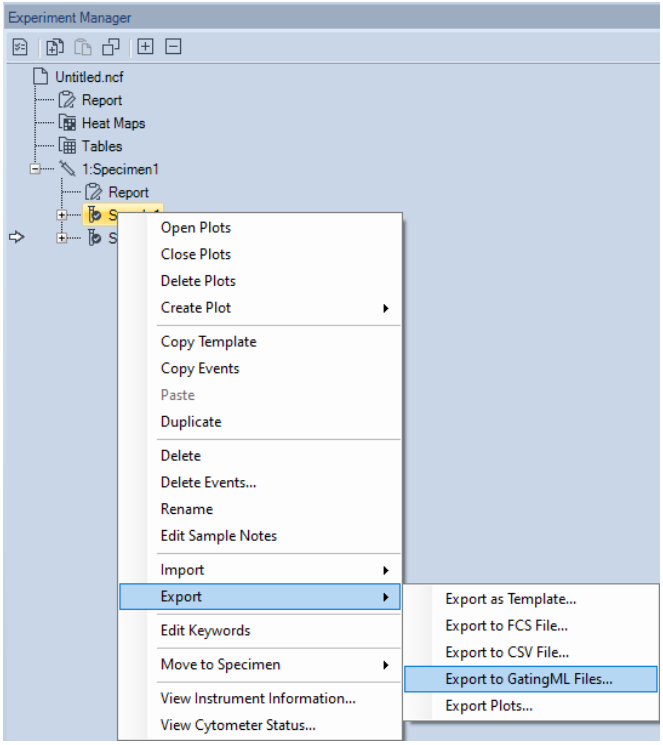
When multiple gates are selected in **Gate Manager**, only **Delete, Show Color, Color Precedence, Show Name** and **Show Percentile** are available.

Export to Gating-ML File

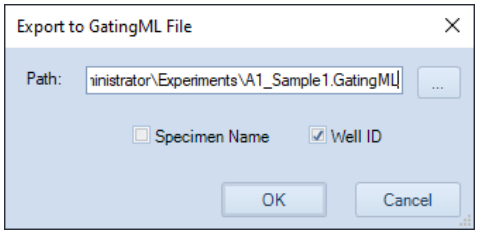
The gating information of the selected gates can be exported to a Gating-ML file. The exported Gating-ML file can be further analyzed by a third-party software which also supports the Gating-ML standard, using the **Export to GatingML File** function.

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

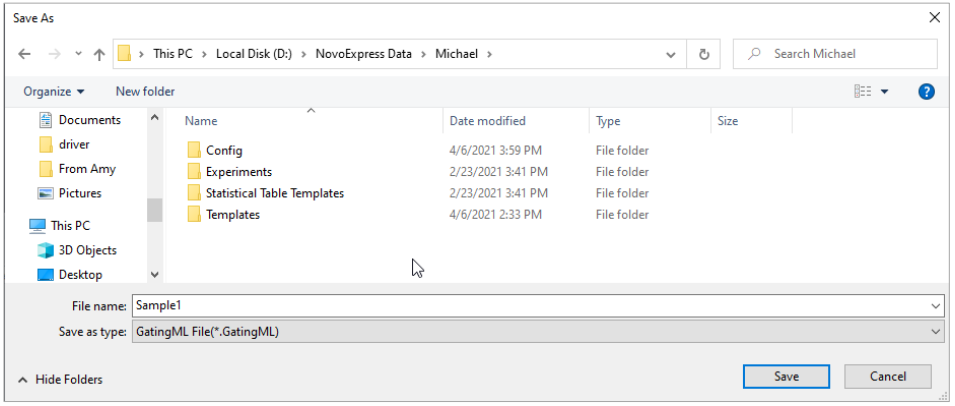
- From **Experiment Manager** panel: Right click the selected sample, click **Export→ Export to GatingML Files…** to export gating information associated with the selected sample.



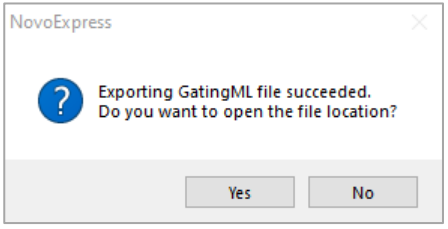
In the prompted window, the **Path** field will be automatically filled with the default target folder (i.e., the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously) and the default file name (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, or NovoSampler Pro, or NovoSampler Q is connected) to include Well ID as part of the name.



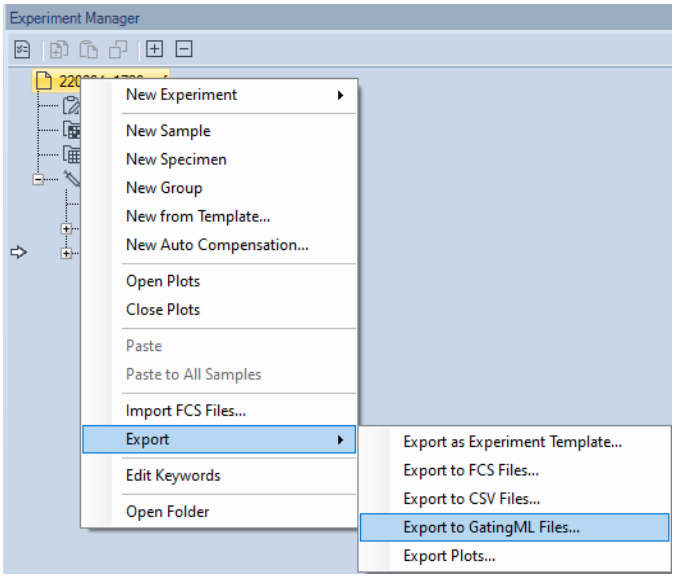
To use different folder and file name, user can directly edit the **Path** field, or click ... to the right of the **Path** text box to open the window below, select the appropriate path and edit the file name, and click **Save**. The updated folder and file name will be automatically filled into **Path**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



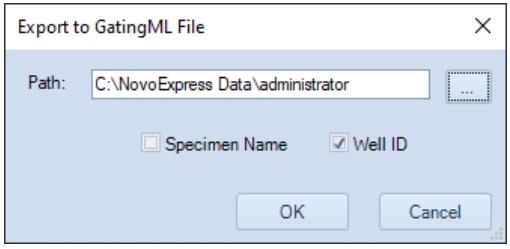
Click **OK** in **Export to GatingML File** window to export Gating-ML file, the file contains all the gating information of the sample. Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.



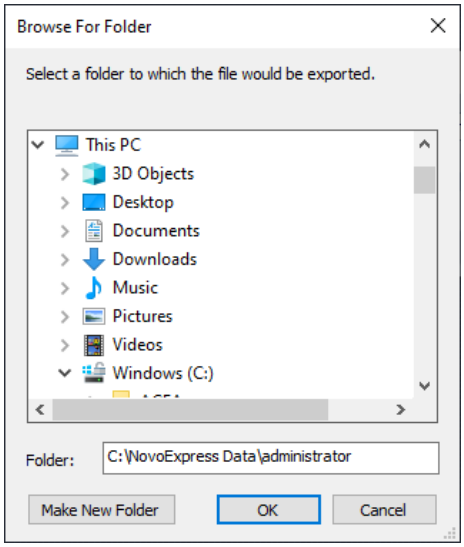
- From **Experiment Manager** panel: Right click an experiment file, a group, or a specimen, and click **Export→ Export to GatingML Files…** to export gating information of all samples associated with the selected experiment, group or specimen correspondingly. Each sample corresponds to a Gating-ML file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



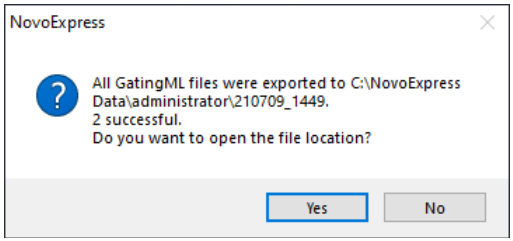
In the prompted window, the **Path** field will be automatically filled with the default folder (i.e., the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously). The default name of exported file 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, or NovoSampler Pro, or NovoSampler Q is connected) to include Well ID as part of the name.



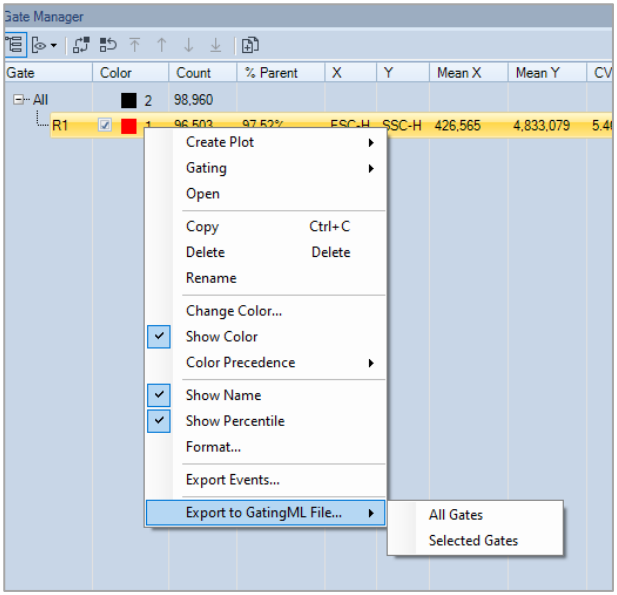
To use a different folder, user can directly edit the **Path** field, or click ... to the right of the **Path** text box, select the appropriate path, and click **OK**. The updated folder will be automatically filled into **Path** field in **Export to GatingML File** window.



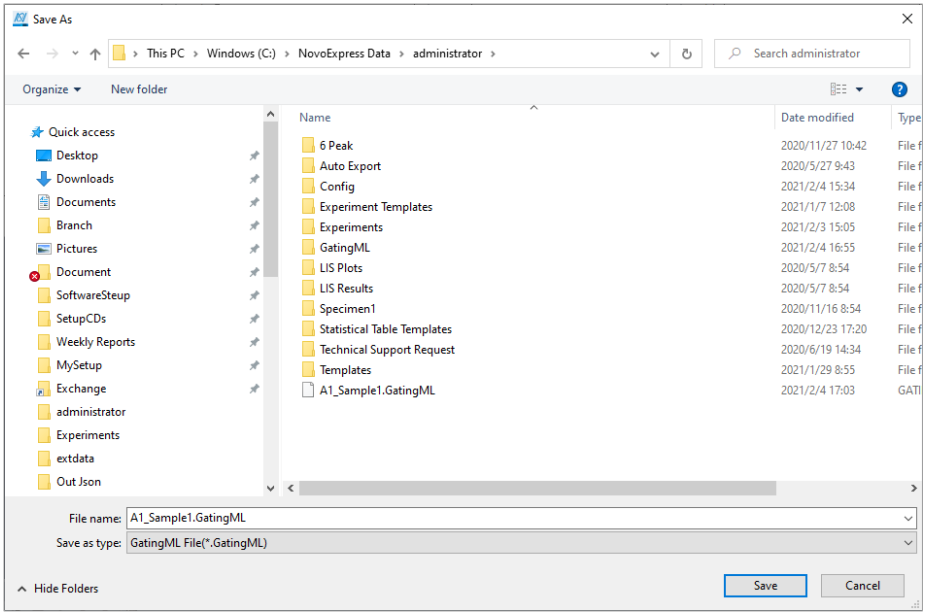
Click **OK** in **Export to GatingML File** window to export Gating-ML file. The file contains all gates of the sample. Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.



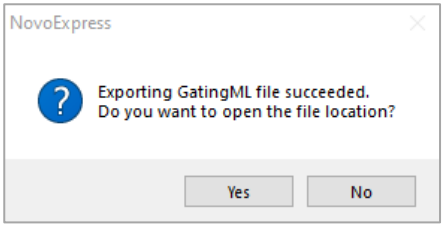
- From **Gate Manager** panel: Right click the selected gates, click **Export to GatingML File…** and select **All Gates** to export all gates of the selected sample to the Gating-ML file, or select **Selected Gates** to export the selected gates and their associated parent gates to the Gating-ML file.



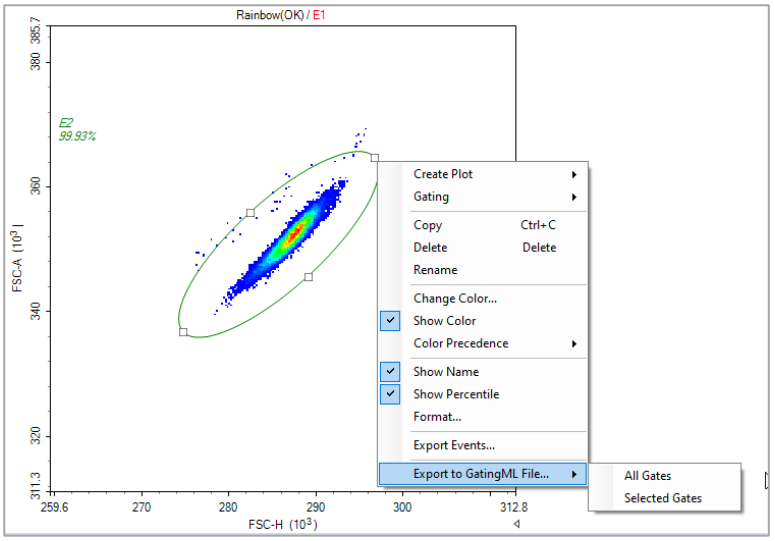
In prompted window as shown below, user can select the target folder and edit the file name. The default folder is the folder where the experiment file is stored when exporting the file for the first time, or the folder that user has defined when the file was exported previously. The default file name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



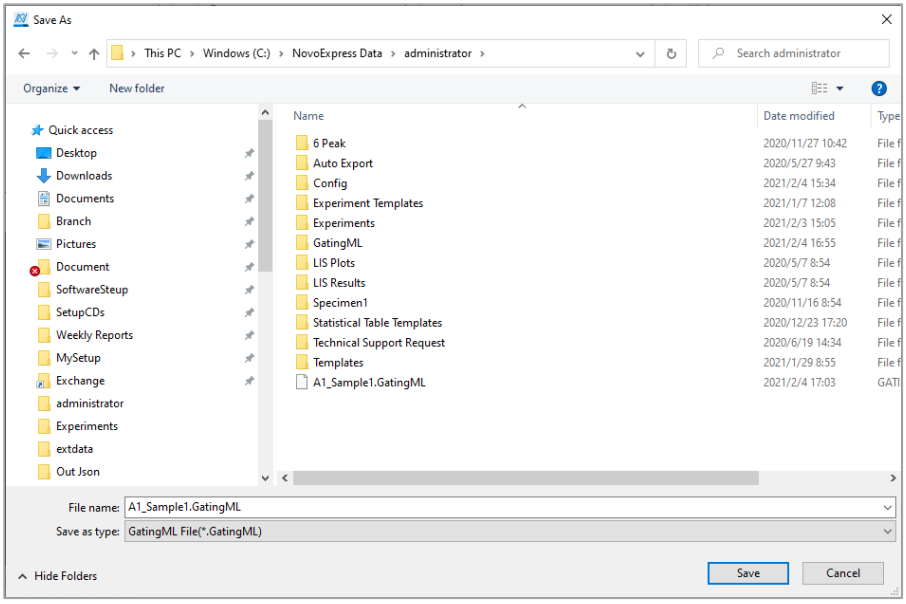
Following window will be prompted. Click **Yes** to open the file location or click **No** to close this window and complete the exporting process.



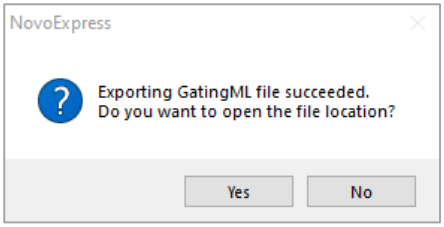
- From **Plot** area: Right click the selected gate, click **Export to GatingML File…** and select **All Gates** to export all gates of the active sample to the **Gating-ML** file, or select **Selected Gates** to export the selected gates and their associated parent gates to the **Gating-ML** file.



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.

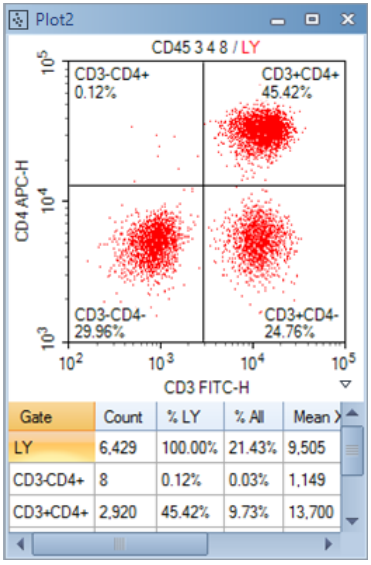


Statistics

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

Display Statistical Information

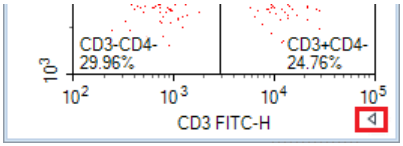
In the following figure, the statistical information chart is displayed below the plot.



Open the Statistics Chart

There are two methods to open the statistical information chart.

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



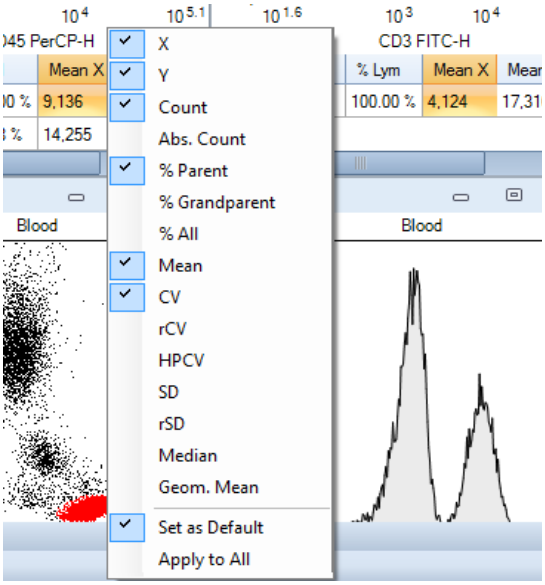
- From the Workspace Toolbar, click the **Show Statistics** button to show/hide the statistics chart of a plot.

Statistics Layout

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

Gate	Count	% All	Mean X	Mean Y
All	30,000	100.00%	14.412	497,847
MO	1,681	5.60%	12,411	260,983
GR	18,163	60.54%	6,696	703,977

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.

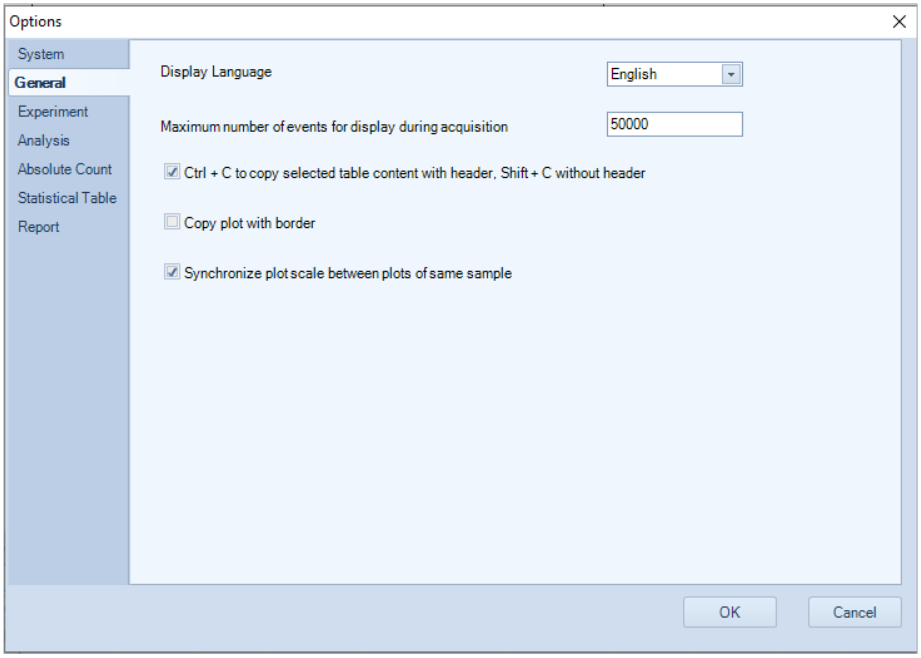


Copy Statistical Information to Clipboard

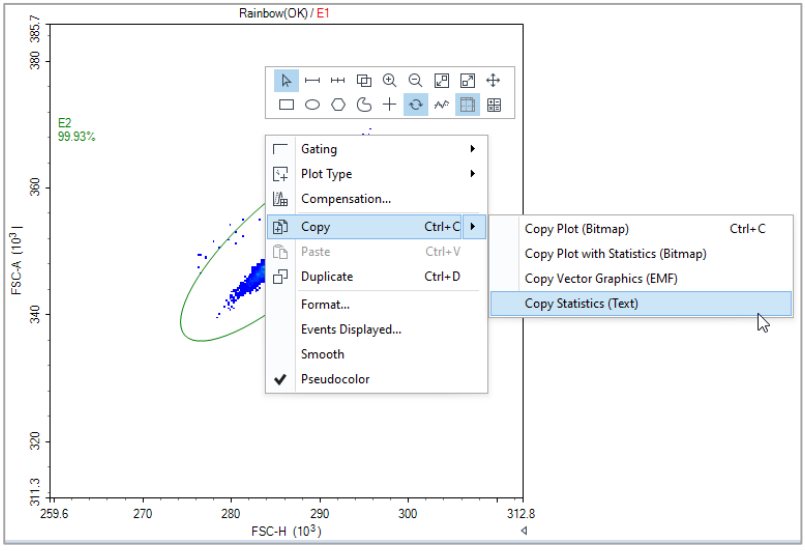
Data from the statistics chart can be copied to the clipboard. The copied data can by pasted to a spreadsheet program, such as Microsoft Excel, for further analysis.

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)**.



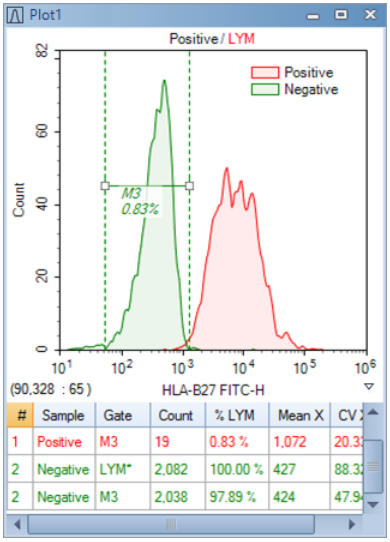
Statistics Layers

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	% LYM	Mean X	CV
1	Positive	M3	19	0.83 %	1,072	20.3
2	Negative	LYM*	2,082	100.00 %	427	88.3
2	Negative	M3	2,038	97.89 %	424	47.9

Calculation of Statistics

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

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

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

- Count

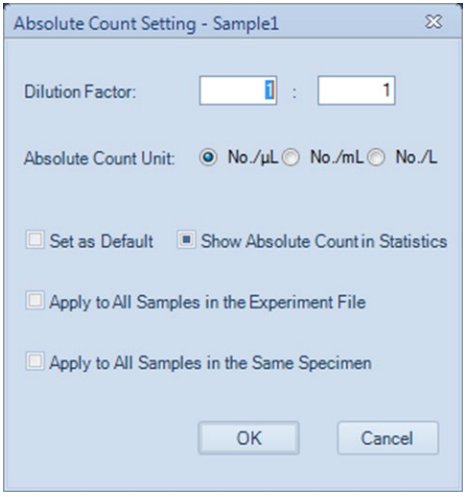
  The number of events collected in the specified gate.

- Abs. Count

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

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

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



- %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

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

  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 $\bar{X}$ 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

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

  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.

Fluorescence Compensation

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

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

Automatic Compensation

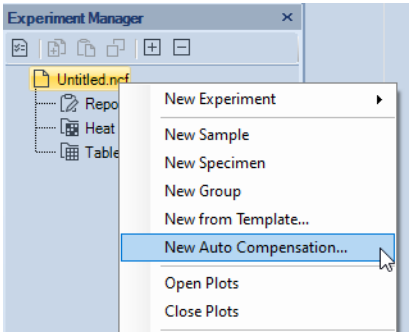
The automatic compensation method automatically calculates the compensation matrix and also allows for the compensation matrix to be applied to additional samples. Automatic Compensation matrix can be generated from samples acquired on instrument and from samples imported with FCS files. Conduct Automatic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

NOTE

Please refer to Importing Data in this guide for more information on importing a FCS file.

Set Automatic Compensation

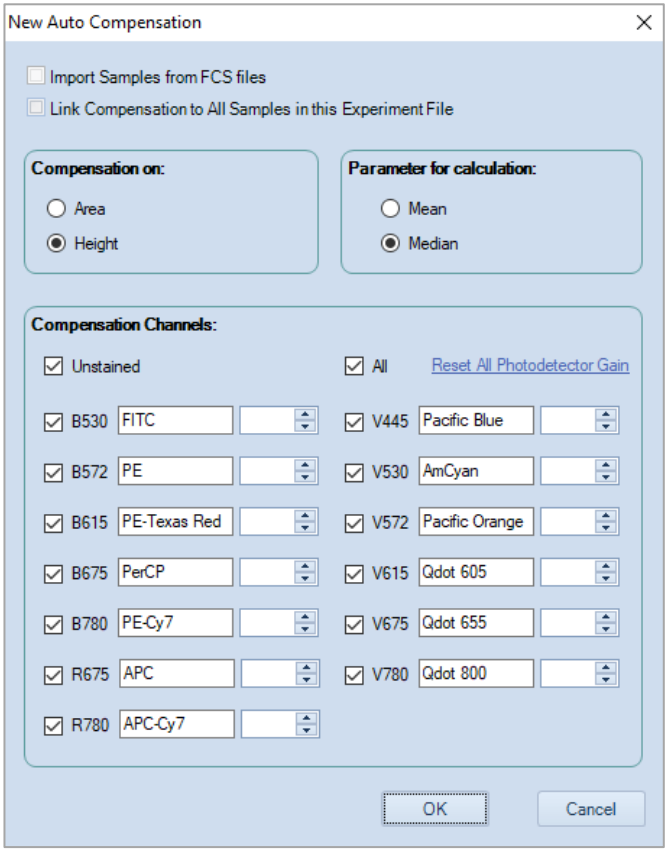
In the **Home** tab of the **Menu Bar**, click the **Auto Compensation** button, or in the **Experiment Manager** panel, right click on the file name or a group and select **New Auto Compensation…**. The **New Auto Compensation** window appears.



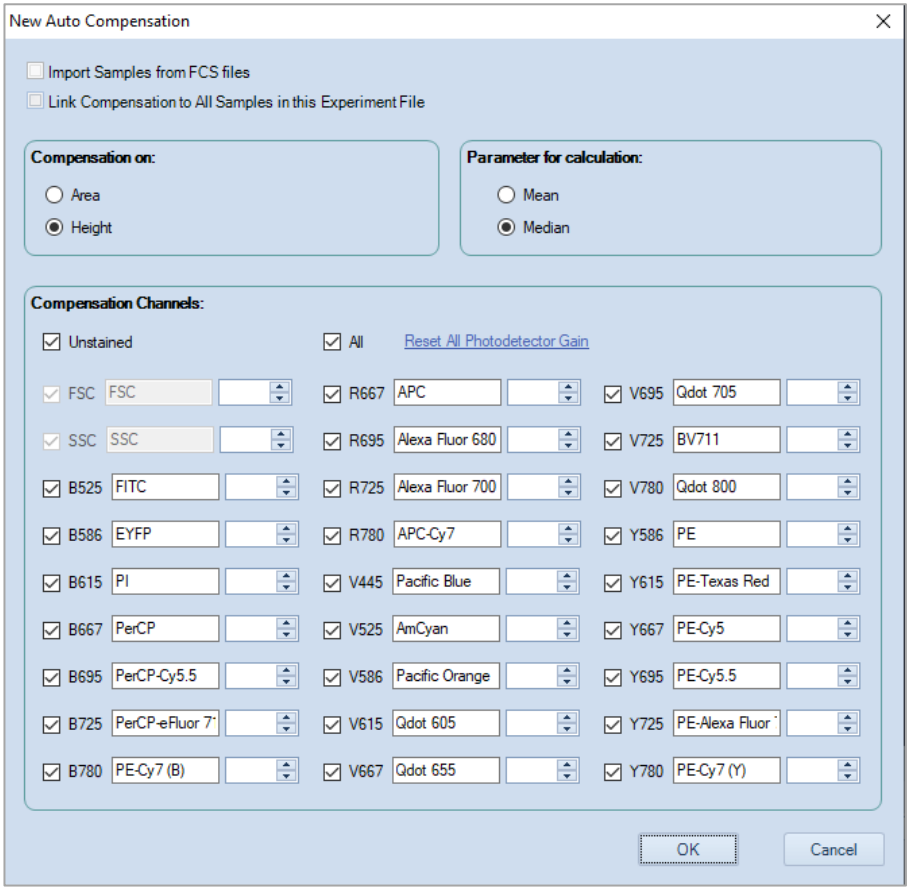
In the **New Auto Compensation** window, the user can select the channels for compensation, whether to compensate using area or height measurements, and whether to calculate compensation based on the measured mean or median.

To set up the automatic compensation:

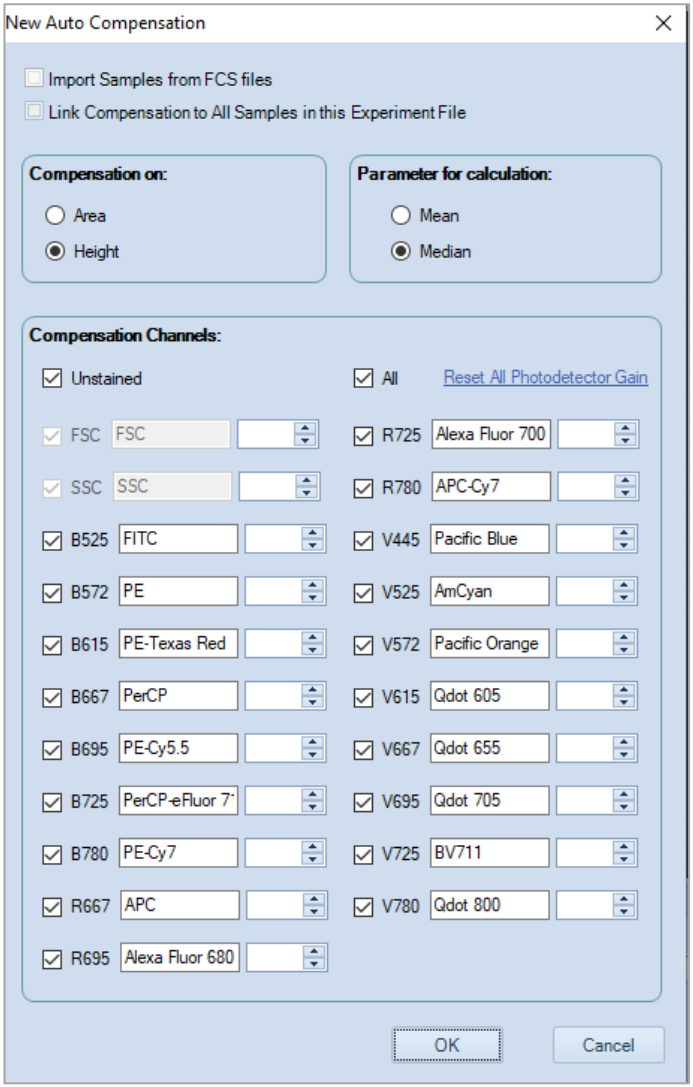
1. Select the channels to compensate: Use the checkboxes to select the channels to compensate. If an unstained sample is to be used to assist in compensation, select the **Unstained** box.
2. Choose to compensate either using the Area or Height measurements.
3. Choose to compensate based on either mean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



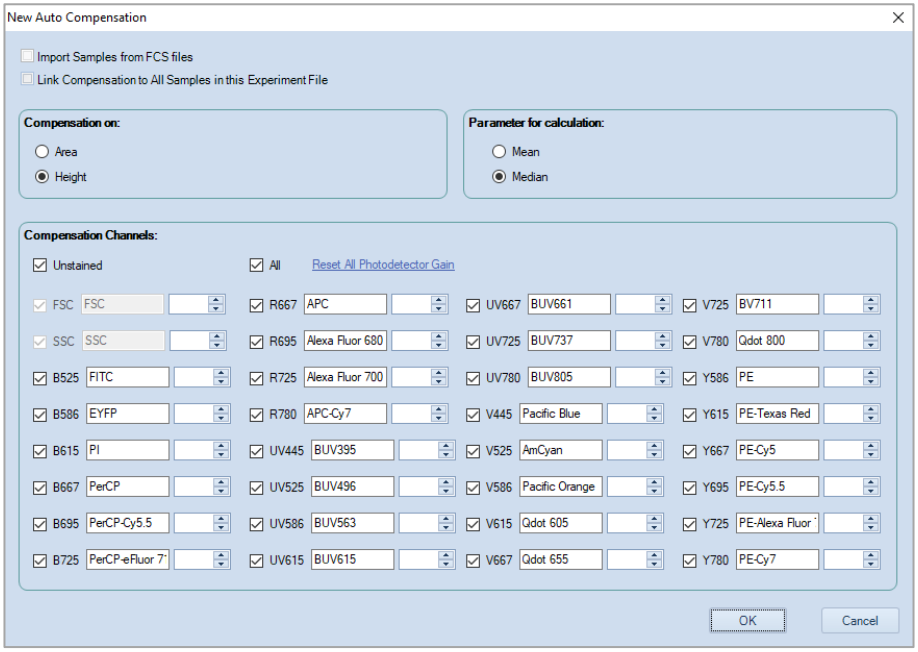
New Auto Compensation window for NovoCyte instrument (e.g., 3000 System)



New Auto Compensation window for NovoCyte Quanteon instrument (e.g., V8B7Y6R4 System)



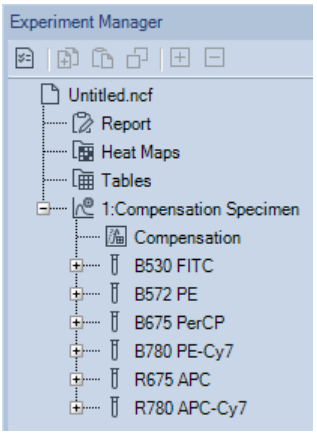
New Auto Compensation window for NovoCyte Advanteon instrument (e.g., V8B7R4 System)



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

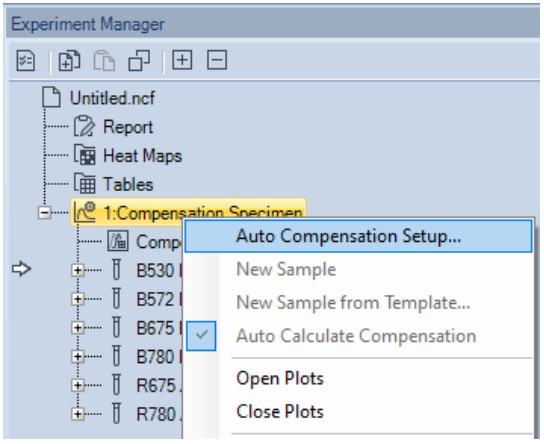
4 Edit photodetector gain of each channel. Click **Reset All Photodetector Gain** to reset photodetector gains of all channels to default values. Every instrument has the default photodetector gain setting. An underlined photodetector gain text as shown above for the B525 channel indicates it has been modified and is not the default value. A non-underlined photodetector gain indicates that it is the default setting.

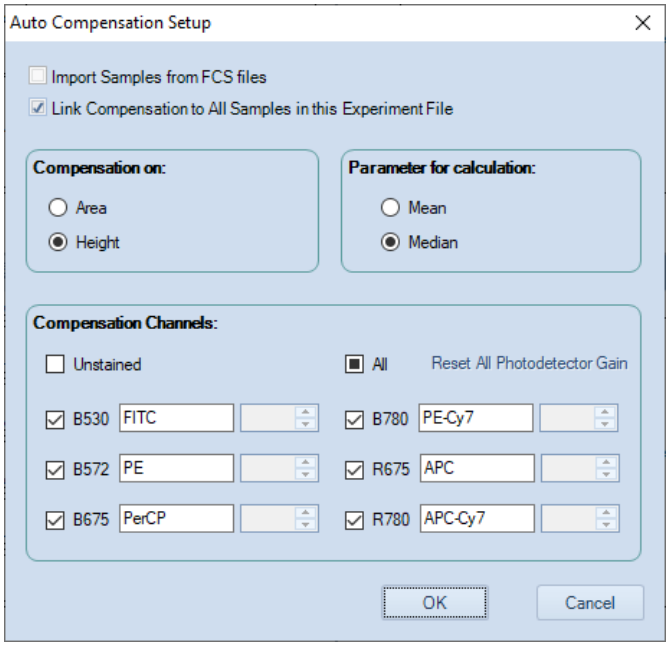
5 Click **OK**. In the **Experiment Manager** panel, a **Compensation Specimen** node gets created with blank control samples created for the compensation calculation. The samples include the channels selected in the **New Auto Compensation** window.



Modify Automatic Compensation Settings

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





Prepare Samples for Automatic Compensation

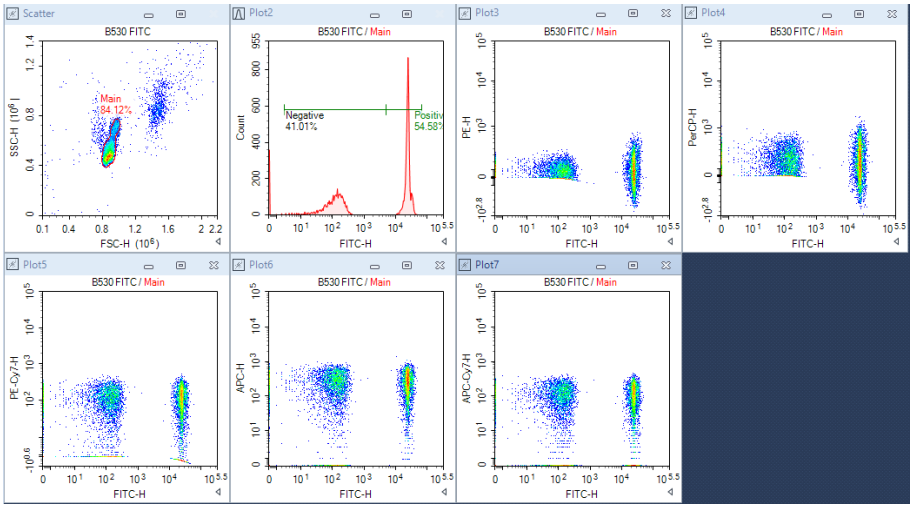
To calculate the compensation, experimental data will need to be collected for each of the control samples in the **Compensation Specimen** node. For the unstained sample, the sample should be prepared without any fluorophores added. For the other control samples in the **Compensation Specimen** node,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

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

Automatic Calculation of the Compensation Matrix

- Automatic Gating

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



NOTE

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

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

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

NOTE

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

NOTE

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

- Automatic Calculation of the Spillover Values

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

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

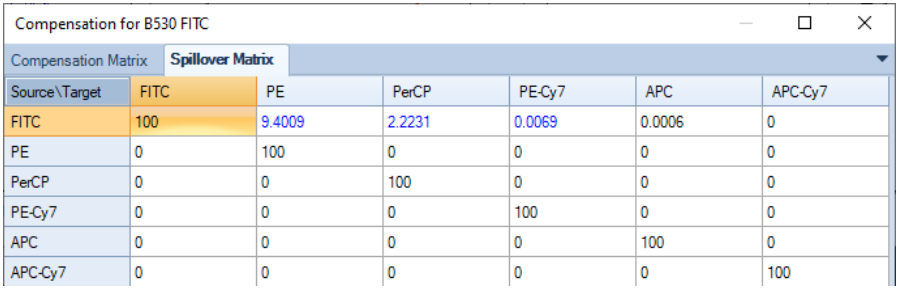
$$\text{Spillover of FITC into PE} = \frac{(X_{PE,positive} - X_{PE,negative})}{(X_{FITC,positive} - X_{FITC,negative})}$$

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

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

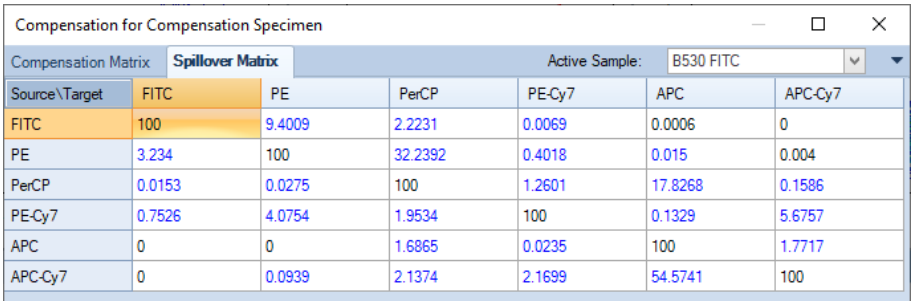
- Automatic Calculation of the Spillover Matrix

  In the following figure, the spillover of the single stained FITC sample is automatically calculated. In the matrix, the fluorophore is listed in the row and the spillover channel is listed in the column. In this figure, the FITC fluorophore spillover is 9.4009% into PE, 2.2231% into PerCP, 0.0069% into PE-Cy7.



Source\Target	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7
FITC	100	9.4009	2.2231	0.0069	0.0006	0
PE	0	100	0	0	0	0
PerCP	0	0	100	0	0	0
PE-Cy7	0	0	0	100	0	0
APC	0	0	0	0	100	0
APC-Cy7	0	0	0	0	0	100

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

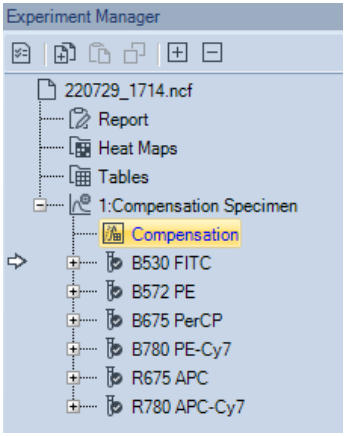


Source\Target	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7
FITC	100	9.4009	2.2231	0.0069	0.0006	0
PE	3.234	100	32.2392	0.4018	0.015	0.004
PerCP	0.0153	0.0275	100	1.2601	17.8268	0.1586
PE-Cy7	0.7526	4.0754	1.9534	100	0.1329	5.6757
APC	0	0	1.6865	0.0235	100	1.7717
APC-Cy7	0	0.0939	2.1374	2.1699	54.5741	100

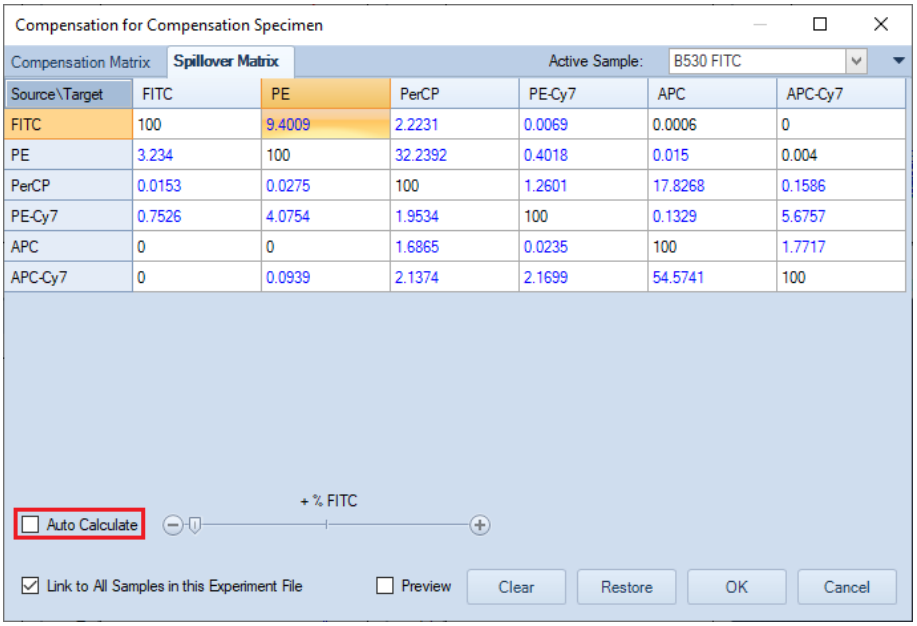
Edit Automatic Compensation Coefficients

User can edit the Spillover Matrix for compensation specimen or experiment sample by following the instructions below.

1 Double click the **Compensation** node of Compensation Specimen or experiment sample.



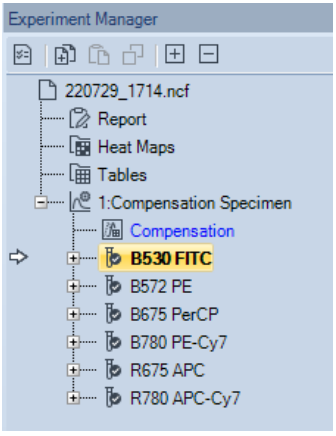
2 In prompted **Compensation** window, ensure to select **Spillover Matrix** tab, uncheck **Auto Calculate**, double click designated cell to enter the value or use the slider bar to modify the value. Values can range between 0 and 800.



3 Click **OK** to complete.

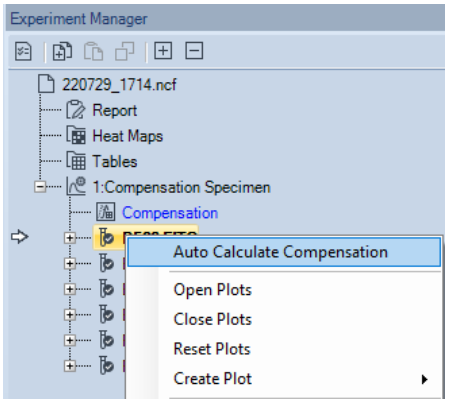
NOTE

After the Spillover Matrix is modified, the associated sample name in **Experiment Manager** window will be displayed in bold.

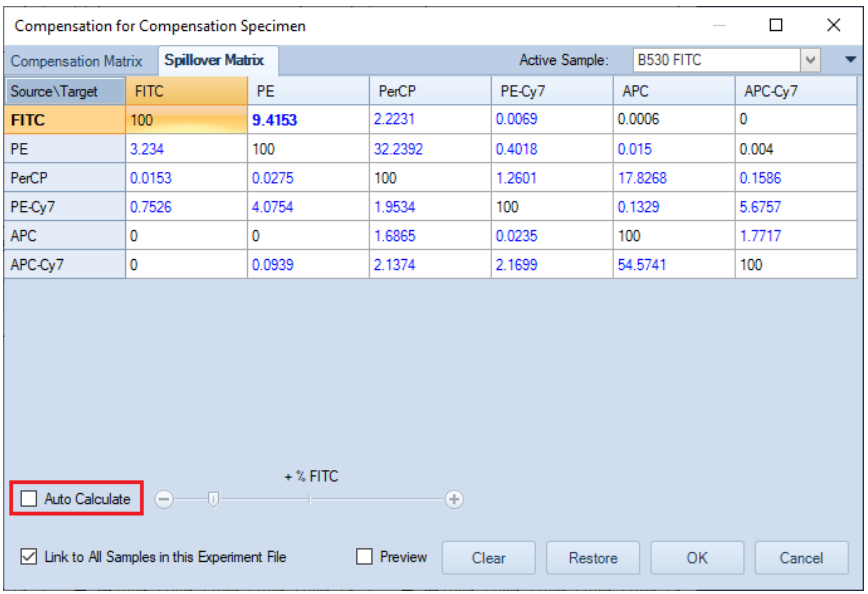


After manually editing the compensation of Compensation Specimen or Compensation Sample, user can re-do the auto compensation again using one of the methods below.

- Right click the Compensation Specimen or Compensation Sample node, click **Auto Calculate Compensation** from the right click menu.



- Double click **Compensation** node under the **Compensation Specimen** node or Compensation Sample node, check **Auto Calculate** in prompted **Compensation** window, and click **OK**.



Application of Automatic Compensation Results

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

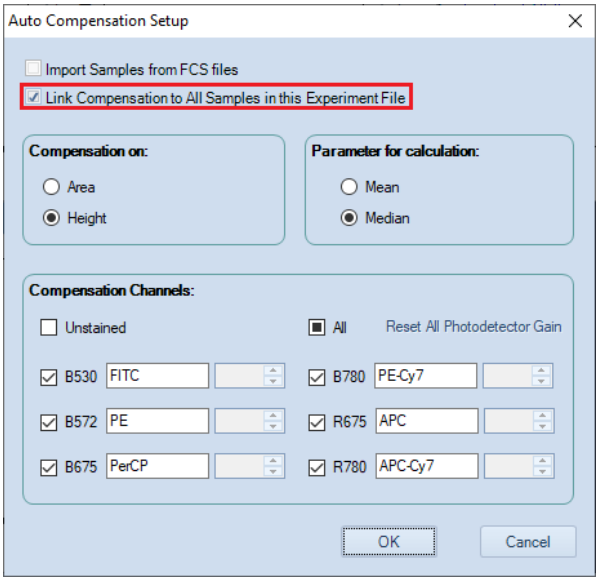
WARNING **The compensation matrix is calculated at specified photodetector gains. It should only be applied to samples that were acquired with the same photodetector gains.**

Link Automatic Compensation to Samples

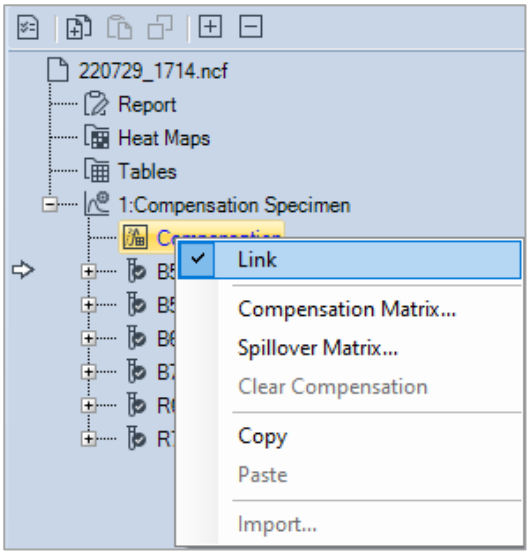
In the case of automatic applying the generated Compensation Matrix to other samples in the experiment is needed, Link Automatic Compensation to Samples function can be used. Use one of the methods below to do so.

- Click **Home** > **Auto Compensation** to open the **New Auto Compensation** window. Select the **Link Compensation to All Samples in this Experiment File** and click **OK.**

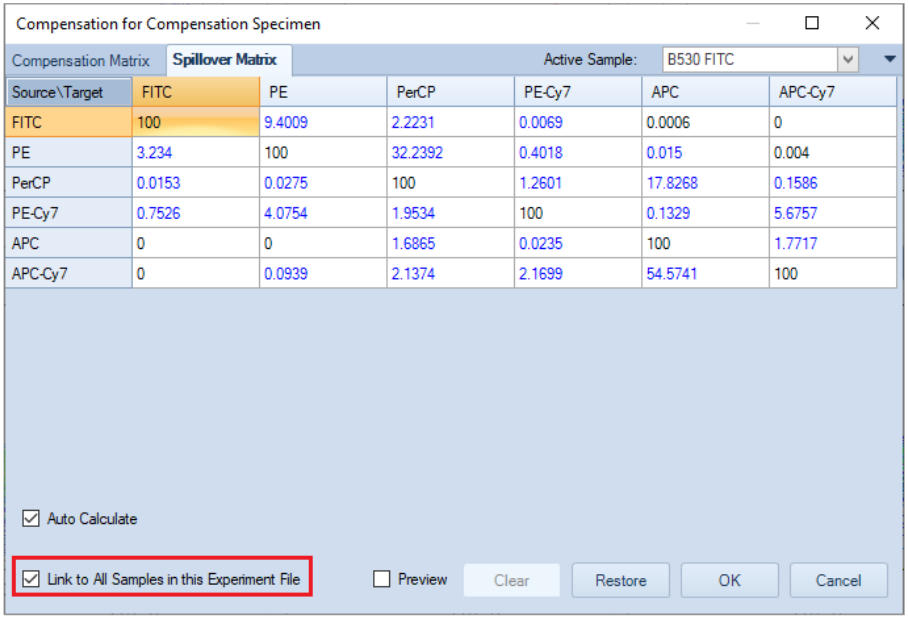
NOTE This window can also be accessed by right clicking the experiment file and selecting **New Auto Compensation** in the **Experiment Manager** window.



- In **Experiment Manager**, right click **Compensation** under the **Compensation Specimen** node, and click **Link**.



- Double click **Compensation** under the **Compensation Specimen** node, select the **Link to All Samples in this Experiment File** in prompted window and click **OK.**

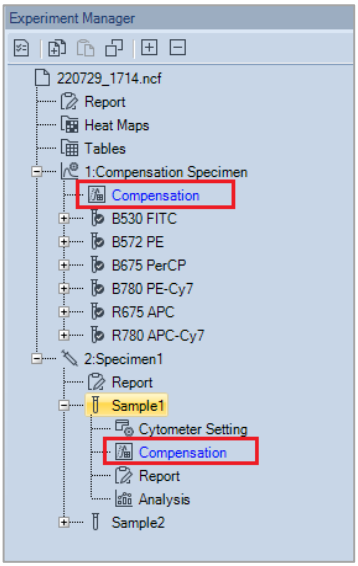


Source\Target	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7
FITC	100	9.4009	2.2231	0.0069	0.0006	0
PE	3.234	100	32.2392	0.4018	0.015	0.004
PerCP	0.0153	0.0275	100	1.2601	17.8268	0.1586
PE-Cy7	0.7526	4.0754	1.9534	100	0.1329	5.6757
APC	0	0	1.6865	0.0235	100	1.7717
APC-Cy7	0	0.0939	2.1374	2.1699	54.5741	100

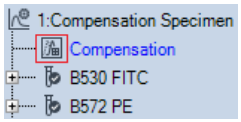
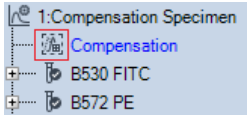
NOTE

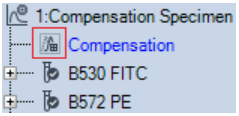
This window can also be accessed by right clicking **Compensation** under the **Compensation Specimen** node and selecting **Compensation Matrix** or **Spillover Matrix** in the **Experiment Manager** window.

After linking auto-compensation to all samples, when user creates new sample(s) in **Experiment Manager**, the compensation matrix will be automatically applied to all samples. The icon in front of each sample compensation node will be displayed as indicating the sample compensation is linked to compensation specimen.



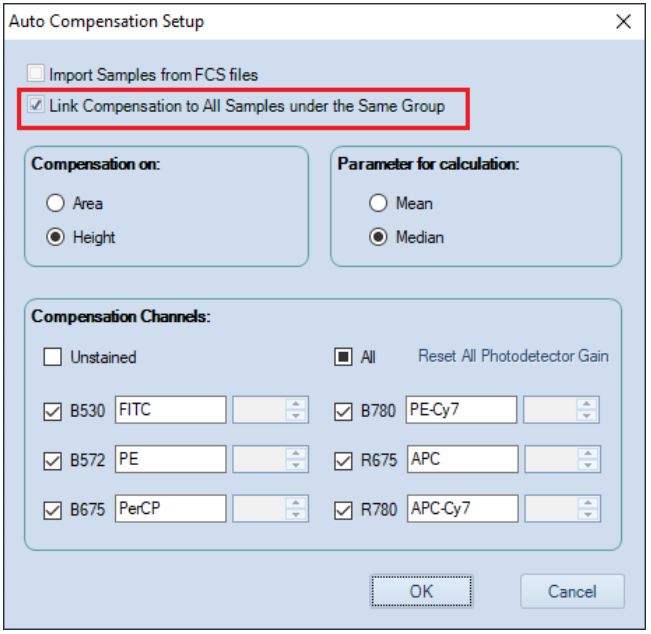
The icon associated with Compensation under the Compensation Specimen node may change depending on how the compensation is linked to sample (s). Refer to table below for more details.

Icon	Description
	This icon indicates that compensation of all samples in the experiment file are linked with the Compensation Specimen.
	This icon is displayed only under Compensation Specimen node and indicates that not all the samples in the same group as the Compensation Specimen are linked with the Compensation Specimen.

	This icon is displayed only under Compensation Specimen node and indicates that no samples in the same group as the Compensation Specimen are linked with the Compensation Specimen

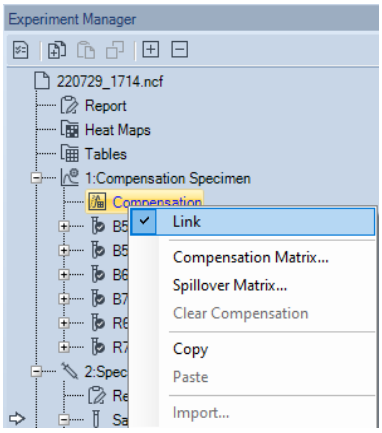
NOTE

User can only link the auto compensation to all samples under the same group.

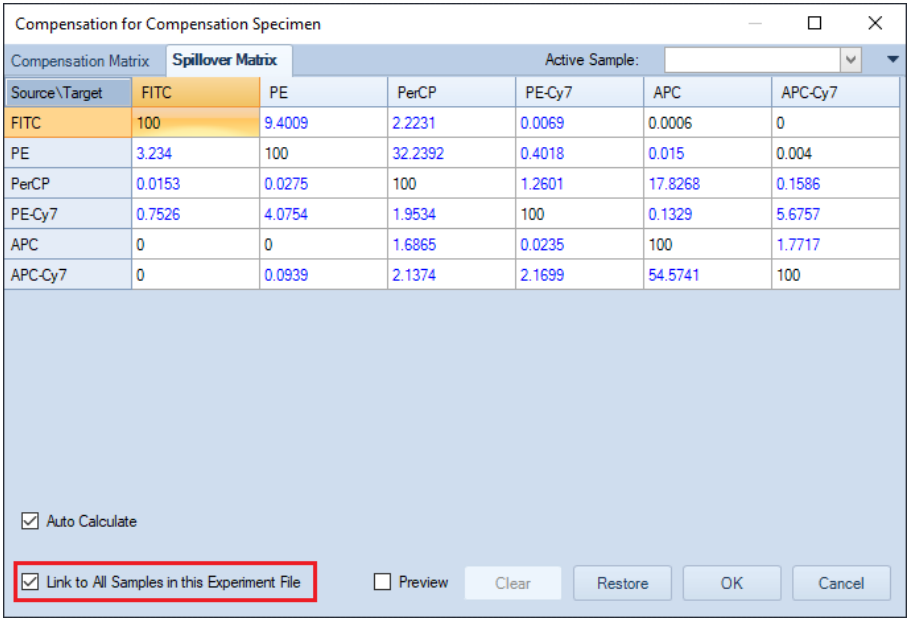


After compensation is linked to samples, user can manually turn it off or back on for all the samples or selected samples in the experiment file by using one of the methods below.

- Right click the **Compensation** node under the **Compensation Specimen** node (when link for all samples needs to be turned on or off), or selected sample node (when link for selected sample needs to be turned on or off) and check/uncheck **Link**.



- Double click **Compensation** node under the **Compensation Specimen** node (when link for all samples needs to be turned on or off), or selected sample node (when link for selected sample needs to be turned on or off) to open following window, check or uncheck **Link to Auto Compensation Specimen**.



Source\Target	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7
FITC	100	9.4009	2.2231	0.0069	0.0006	0
PE	3.234	100	32.2392	0.4018	0.015	0.004
PerCP	0.0153	0.0275	100	1.2601	17.8268	0.1586
PE-Cy7	0.7526	4.0754	1.9534	100	0.1329	5.6757
APC	0	0	1.6865	0.0235	100	1.7717
APC-Cy7	0	0.0939	2.1374	2.1699	54.5741	100

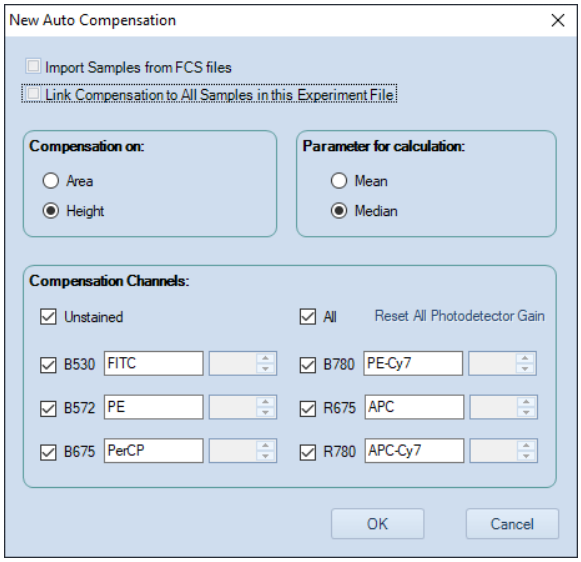
Conduct Automatic Compensation from Imported FCS Files

This function is not available if the **Compliance Supporting Mode** is enabled. To perform auto-compensation directly from the imported FCS file:

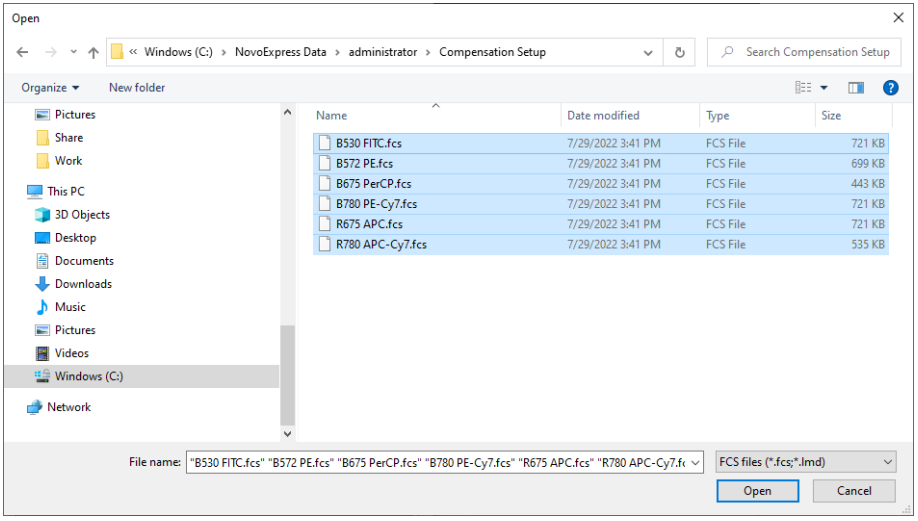
1 Click **Home** > **Auto Compensation** to open the **New Auto Compensation** window. Select the **Import Samples from FCS files**.

NOTE

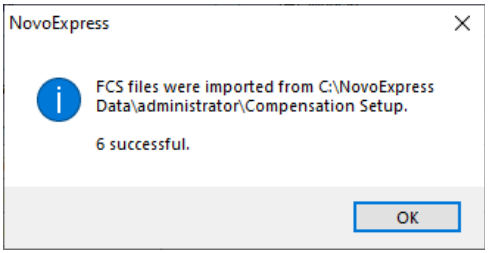
This window can also be accessed by right clicking the experiment file and selecting **New Auto Compensation** in the **Experiment Manager** window.



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

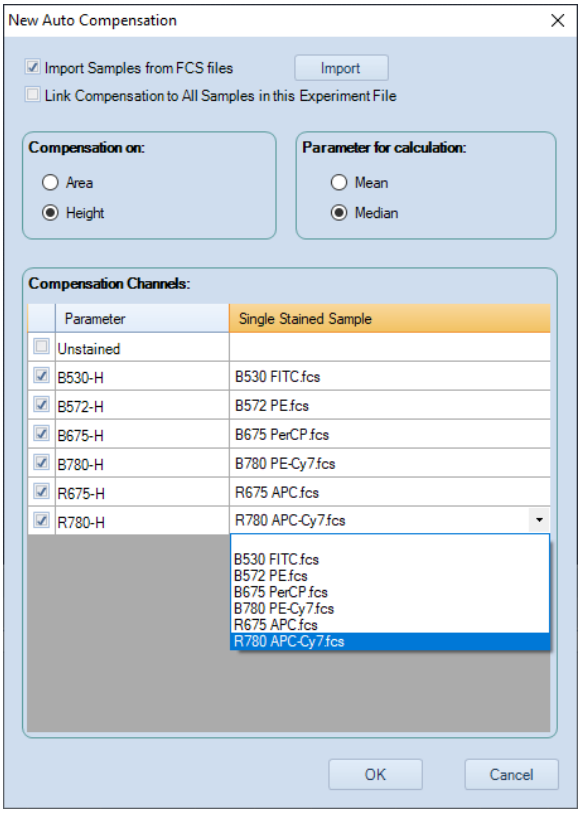


3 Ensure all the selected FCS files are successfully imported to the software. Click **OK** to continue.



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

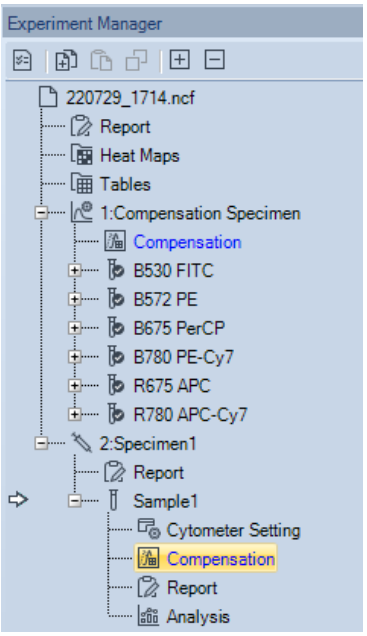
WARNING **The software will automatically associate the imported FCS file with the selected channel parameter based on the keyword of the file name (e.g., B525). Users need to verify and select the correct FCS file for each fluorescence detection channel.**



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

Active Compensation

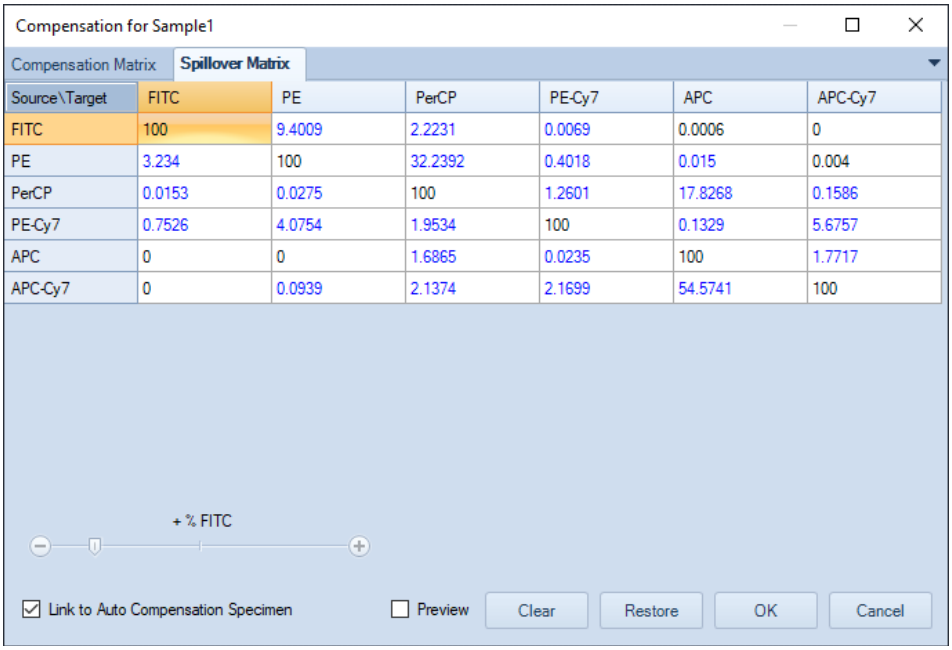
The compensation matrix for each sample can be set to correct for fluorescence spillover in each channel. To open the compensation matrix for a specific sample, in the **Experiment Manager** panel, double-click on **Compensation** under the sample.



NOTE

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

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



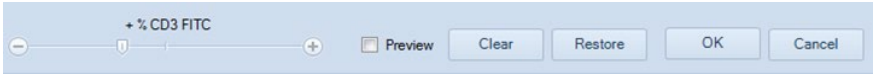
Relationship between the Spillover and Compensation Matrix

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

Edit the Spillover Matrix

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

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



Additional options in the matrix window:

- **Preview**:

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

- **Clear**:

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

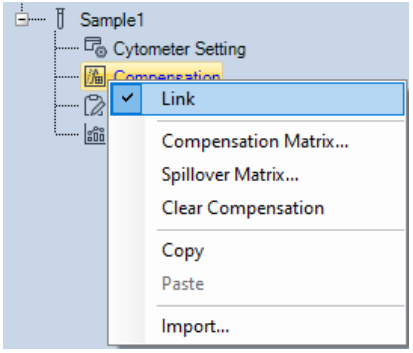
- **Restore**:

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

Link and Unlink Sample Compensation

After the auto-compensation is linked to a sample in the same group, user can manually unlink or relink the compensation using one of the methods below.

- Right click **Compensation** under the sample node, uncheck **Link** to unlink the auto-compensation. User can also re-check **Link** to relink the auto-compensation.



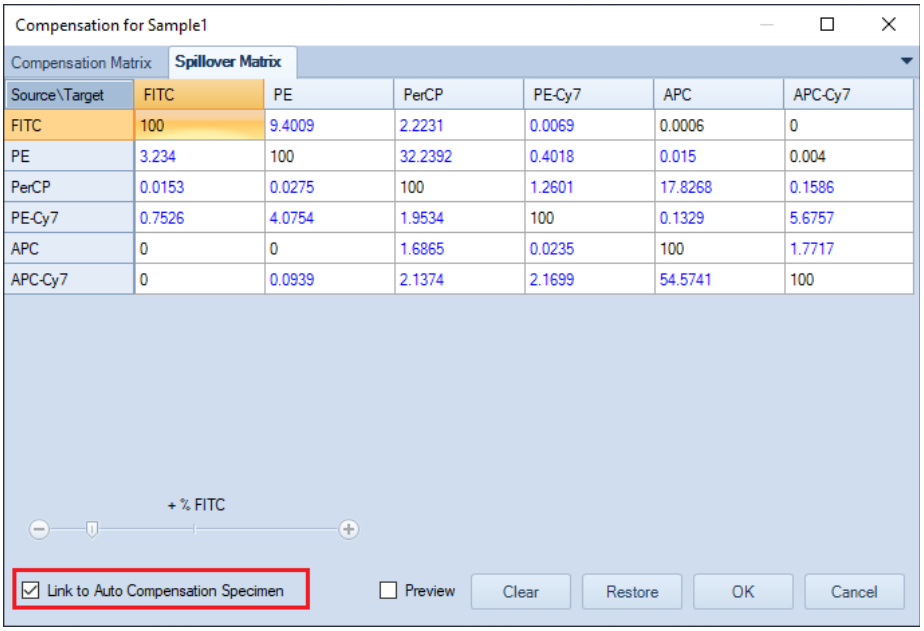
- Double click **Compensation** under the **Sample** node, uncheck the **Link to Auto Compensation Specimen** in prompted window and click **OK.** User can also re-check this option to relink the auto-compensation.

NOTE

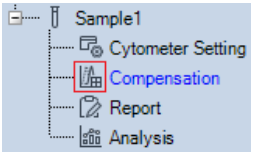
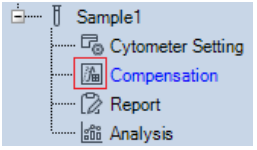
This window can also be accessed by right clicking **Compensation** under the **Sample** node and selecting **Compensation Matrix** or **Spillover Matrix** in the **Experiment Manager** window.

NOTE

The auto-compensation will be automatically unlinked if the Spillover Matrix of the sample is manually edited. When user check **Link to Auto Compensation Specimen** in this window again, the previous manual edits will be automatically voided.



The icon associated with Compensation under the Sample node may change depending on how the compensation is linked to sample. Refer to table below for more details

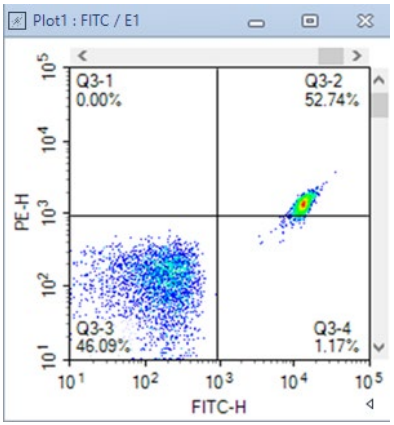
Icon	Description
	This icon indicates that compensation of the sample is not linked with the Compensation Specimen.
	This icon indicates that compensation of the sample is linked with the Compensation Specimen

Quick Compensation Adjustment

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

Open the Quick Compensation Adjustment

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

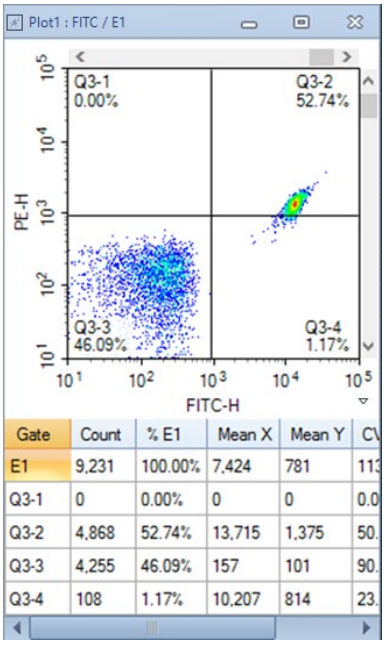


To hide the quick compensation scrollbars, click again on the **Quick Compensation** button.

Use the Quick Compensation Adjustment

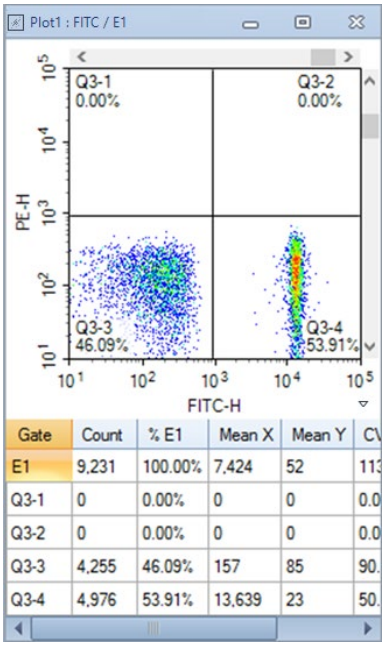
To use the quick compensation:

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



Gate	Count	% E1	Mean X	Mean Y	CV
E1	9,231	100.00%	7,424	781	113
Q3-1	0	0.00%	0	0	0.0
Q3-2	4,868	52.74%	13,715	1,375	50.
Q3-3	4,255	46.09%	157	101	90.
Q3-4	108	1.17%	10,207	814	23.

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



Gate	Count	% E1	Mean X	Mean Y	CV
E1	9,231	100.00%	7.424	52	113
Q3-1	0	0.00%	0	0	0.0
Q3-2	0	0.00%	0	0	0.0
Q3-3	4,255	46.09%	157	85	90.
Q3-4	4,976	53.91%	13,639	23	50.

NOTE

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

NOTE

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

Cell Cycle Analysis

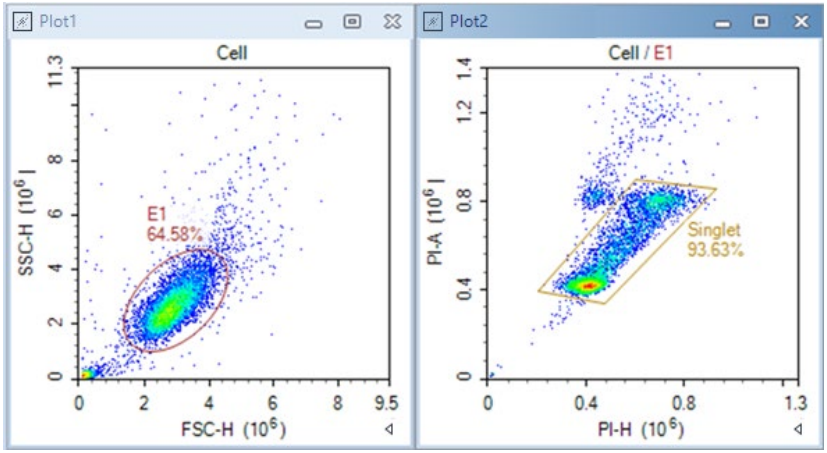
The NovoExpress Software includes a cell cycle analysis feature that allows for the quantification of cells in each phase of the cell cycle based on DNA content.

Automated Cell Cycle Analysis

- Gating Single Cell Population

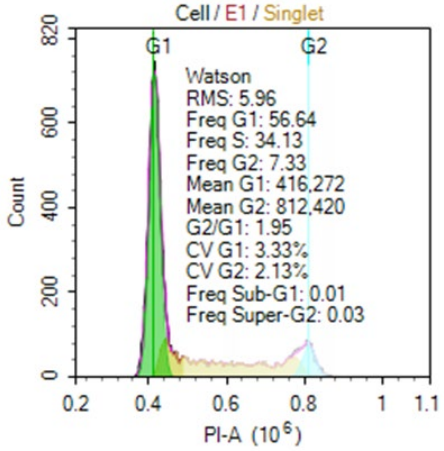
  After collecting the DNA-stained cells, use a FSC-H /SSC-H density plot to get the target population and exclude cell debris. From the target population,

create a Height versus Area density plot on the fluorescent channel corresponding to the DNA stain, and gate for the single cell population and exclude cell aggregates. This is shown in the figure below.



- Cell Cycle Analysis

  Click on the **Cell Cycle Plot** button in the Workspace Toolbar to create a cell cycle plot. Set the X-axis of the plot to a channel for the DNA content stain, such as PI-A. Apply the previously created single cell gate to the plot and make sure all events inside the single cell gate 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:

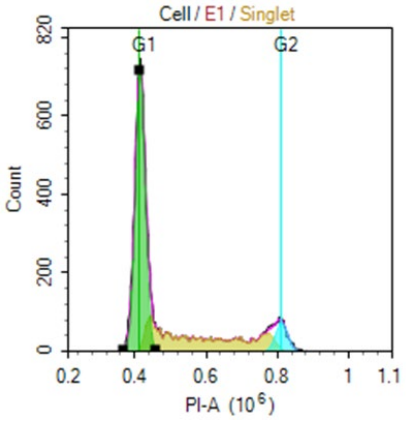
Item	Description
Watson	The model used for cell cycle fitting
RMS	The root mean square error of the fit of the G1, S, and G2 phases. A smaller value indicates a better fit.
Freq G1	Percentage in G1 phase
Freq S	Percentage in S phase
Freq G2	Percentage in G2 phase
Mean G1	The mean fluorescence intensity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

Manual Cell Cycle Analysis

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

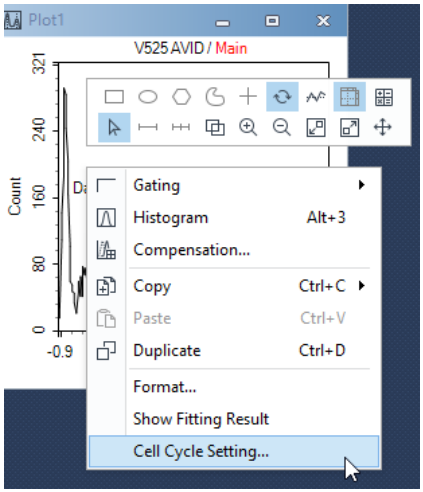
- Constrain G1 and G2 peaks

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

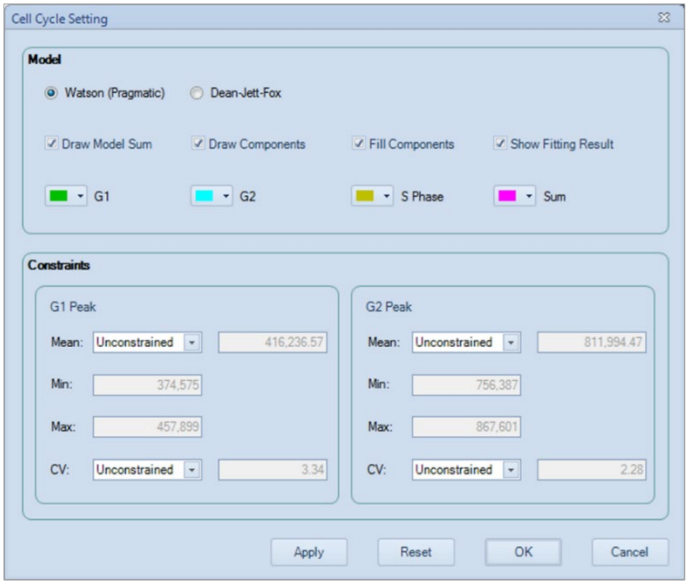


- **Cell Cycle Setting** window

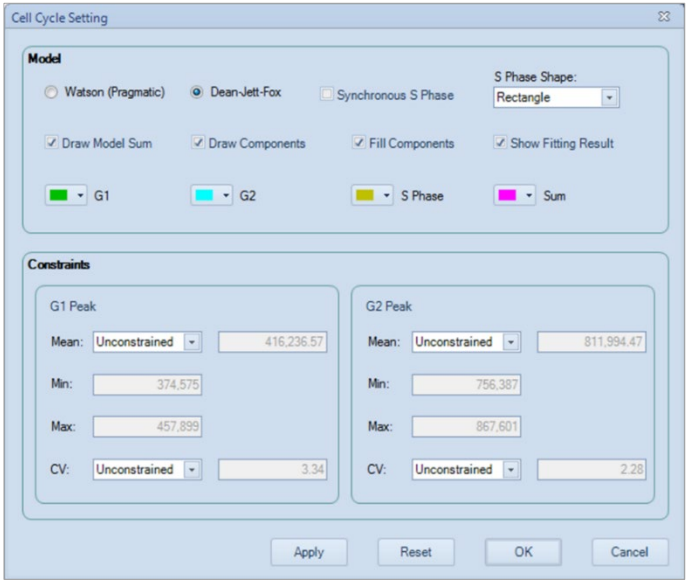
  Right-click on the cell cycle plot and select **Cell Cycle Setting** to open the **Cell Cycle Setting** window.



  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



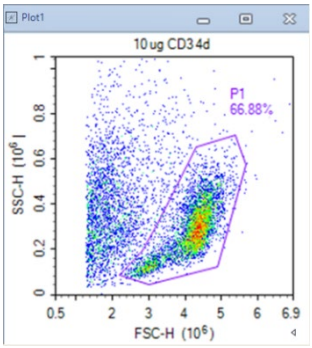
When Dean-Jett-Fox model is selected

Cell Proliferation Analysis

Automated Cell Proliferation Analysis

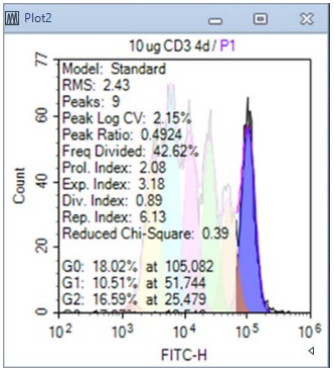
- Gating Target Cell Population

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



- Cell Proliferation Analysis

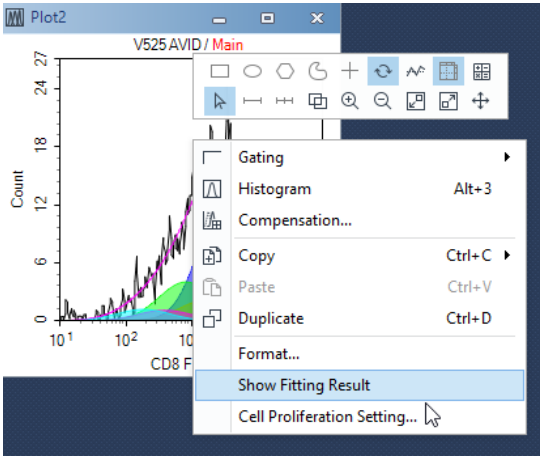
  Click on the **Cell Proliferation Plot** icon in the Workspace Toolbar to create a cell Proliferation plot. Set the X-axis of the plot to a detection channel that relates to the proliferation staining dye, such as CFSE-H. Apply the previously created cell gate to the plot and make sure all the events inside this gate 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**.

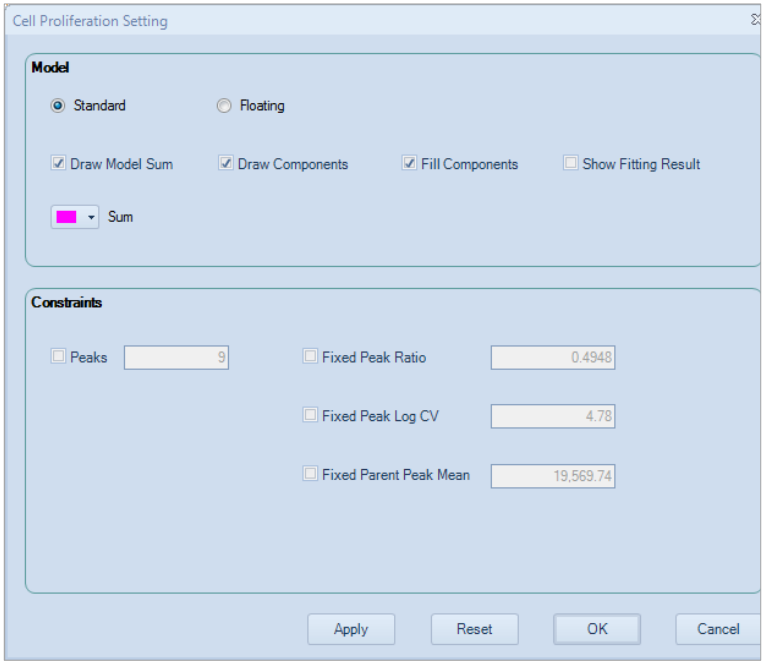
Item	Description
	
Model	The name of model used for analysis and generates results, including **Standard** and **Floating** models.
RMS	Root Mean Square error. It is an estimate of the "goodness of fit" of the model.
Peaks	The count of Peaks.
Peak Log CV	Log CV of peak
Peak Ratio	The average ratio of 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 ith 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. $\text{Prol. Index} = \frac{\sum_{i=1}^{n-1} i\frac{N_i}{2^i}}{\sum_{i=1}^{n-1}\frac{N_i}{2^i}}$
Exp. Index	Expansion Index. It is the number of cells divided by the number of original cells. $\text{Exp. Index} = \frac{\sum_{i=0}^{n-1} N_i}{\sum_{i=0}^{n-1}\frac{N_i}{2^i}}$

Div. Index	Division Index. It is the sum of the number of divisions in each generation divided by the number of original cells. $\text{Div. Index} = \frac{\sum_{i=0}^{n-1} i\frac{N_i}{2^i}}{\sum_{i=0}^{n-1}\frac{N_i}{2^i}}$
Rep. Index	Replication Index. It is the number of non-original cells divided by the number of original cells that divided. $\text{Rep. Index} = \frac{\sum_{i=1}^{n-1} N_i}{\sum_{i=1}^{n-1}\frac{N_i}{2^i}}$
Reduced Chi-Square	Reduced Chi-Square equals the Chi-Square value divided by the free degree. It is an estimate of the "goodness of fit" of the model.
Freq Gi	The percentage of the ith generation. It equals the number of cells of ith generation divided by the number of cells.
Mean Gi	The mean of the peak of the ith 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 (or NovoSampler Pro), additional columns including **Plate ID**, **Well ID and Plate Description** are available. For data generated using NovoSampler Q, additional columns including **Plate ID**, **Well ID**, **Plate Barcode** and **Plate Description** are available.

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

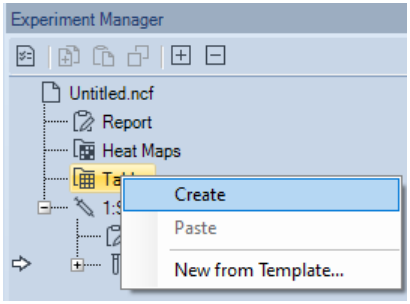
Icon	Description
	Sets the type of statistical table
	Adds a column to the table
	Edits a column
	Duplicates columns
	Deletes columns
	Hides columns
	Selects columns to show
	Selects samples to show
	Deletes rows
	Exports table as a CSV file
	Opens the **Options** > **Statistical Table** window

Create Different Types of Statistical Tables

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

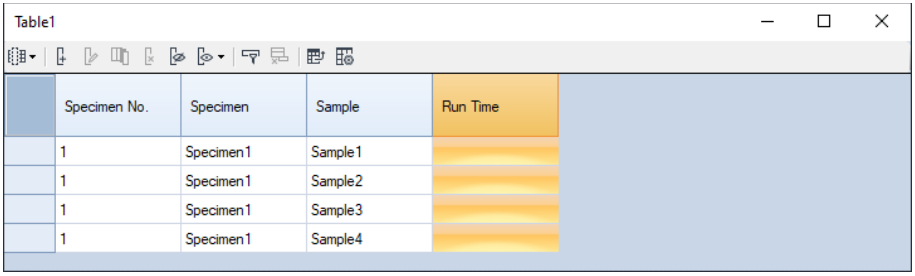
- Creating a New Statistical Table

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



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

  The new statistical table will contain **Specimen No, Specimen, Sample**, and **Run Time** columns, and lists all of the samples in the experiment file.



- Creating a New Statistical Table from Template

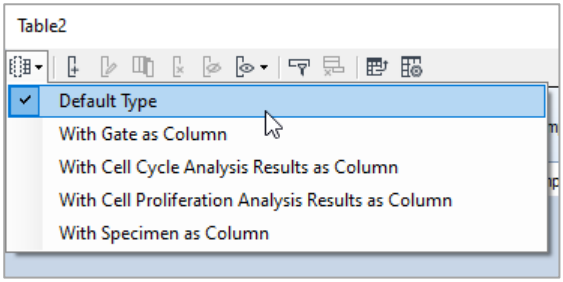
  In the **Experiment Manager** panel, under the experiment filename, right-click on the **Tables** node and select **New from Template**, a new table is created.

- Selecting the Type of Statistical Table

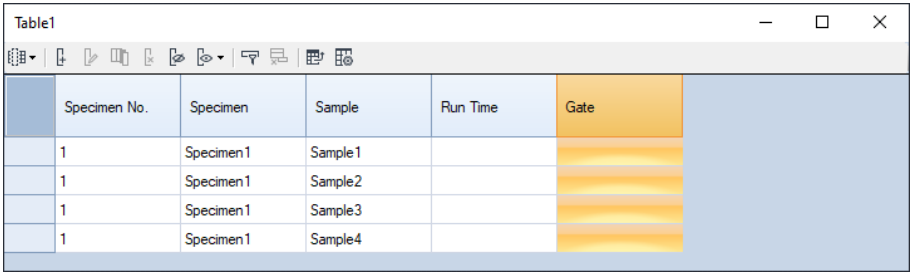
  There are five types of statistical tables to choose from: **Default Type**, a table **With Gate as Column**, a table **With Cell Cycle Analysis Results as Column**, a table **With Cell Proliferation Analysis Results as Column**, and a table **With Specimen as Column**.

  New tables are created as The Default Type. To change the table type, click

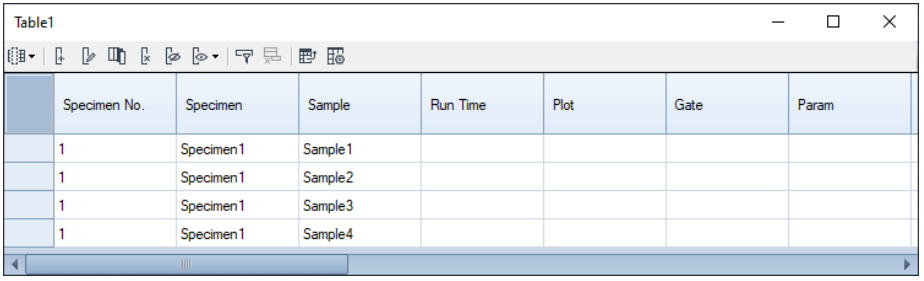
the **Table Type** button from the toolbar and select the new table type from the drop-down menu.



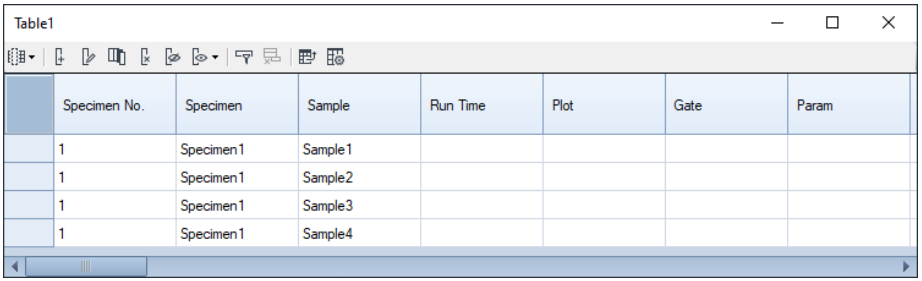
Shown below is an example for a table **With Gate as Column**.



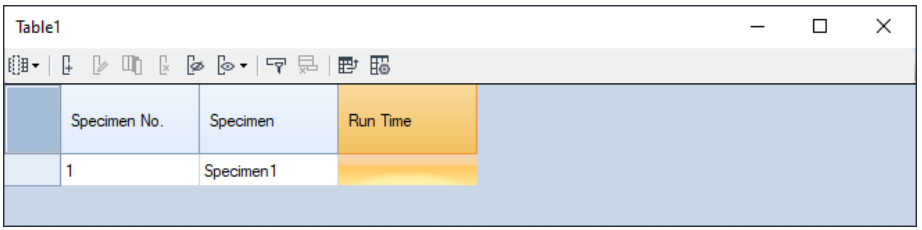
Shown below is an example for a table **With Cell Cycle Analysis Results as Column**.



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

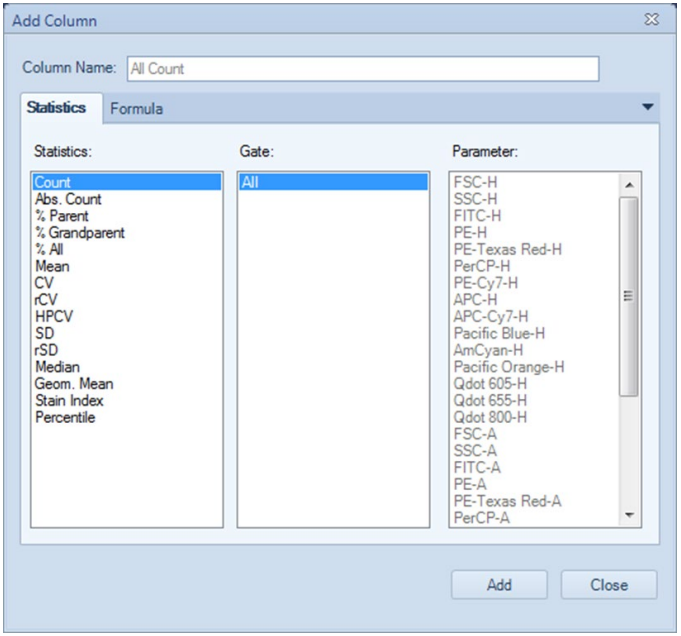
- Drag and Drop to Add Columns

  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

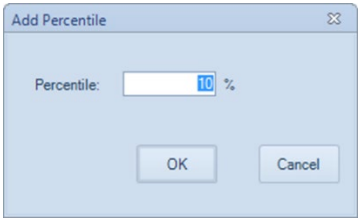
For a table **With Specimen as Column**, the **Sample Name** should be specified when adding columns. If selecting "**All**" in the sample list box, the statistical results will be the average of all samples in each specimen. For Abs. Count calculation, if Abs. Count Unit defined for samples in the specimen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

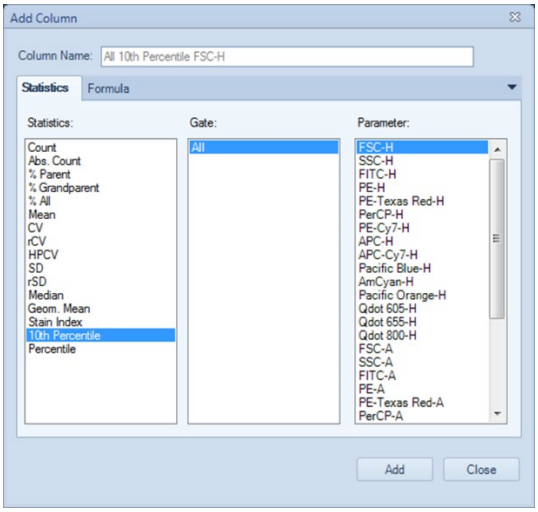
NOTE

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.

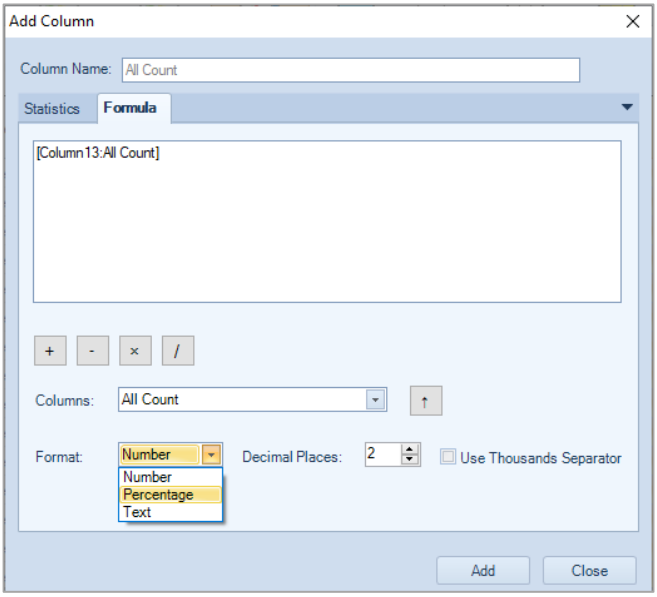
  By default, the column name is automatically generated. The name can be modified by the **Column Name** box at the top of the **Edit Column** window.

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.

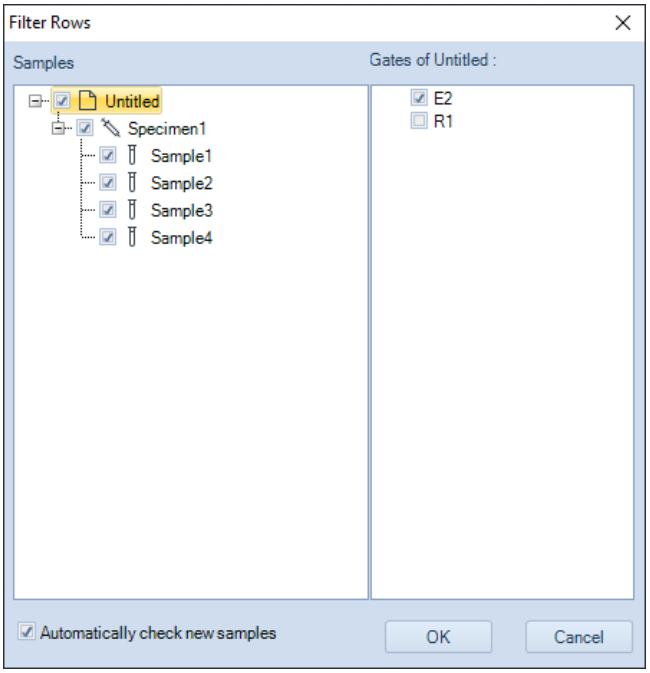
Statistical Table Rows

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

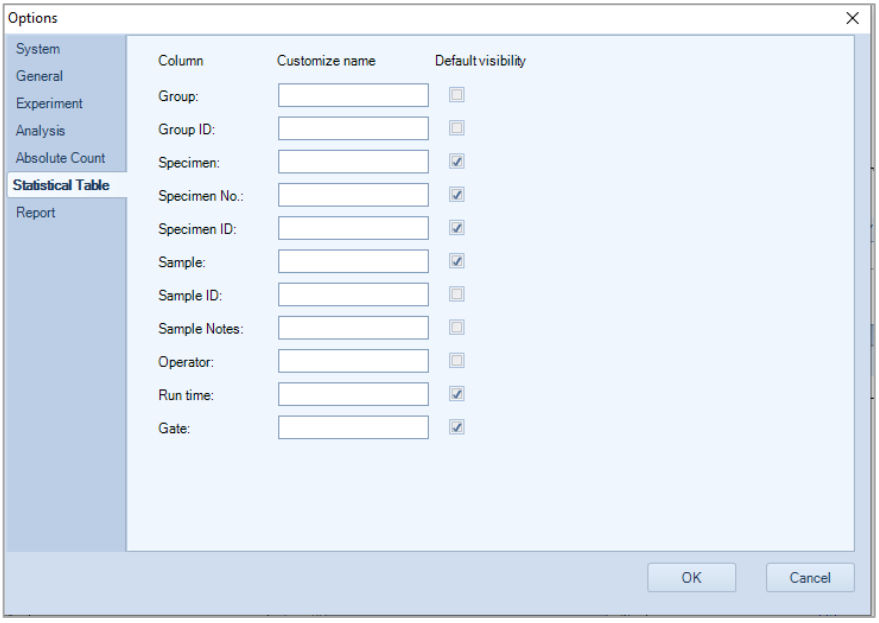
In the Sample tab toolbar, click the **Export CSV File** button . Enter the file path and click **Save** to export the file.

Copy Statistical Table as Text to the Clipboard

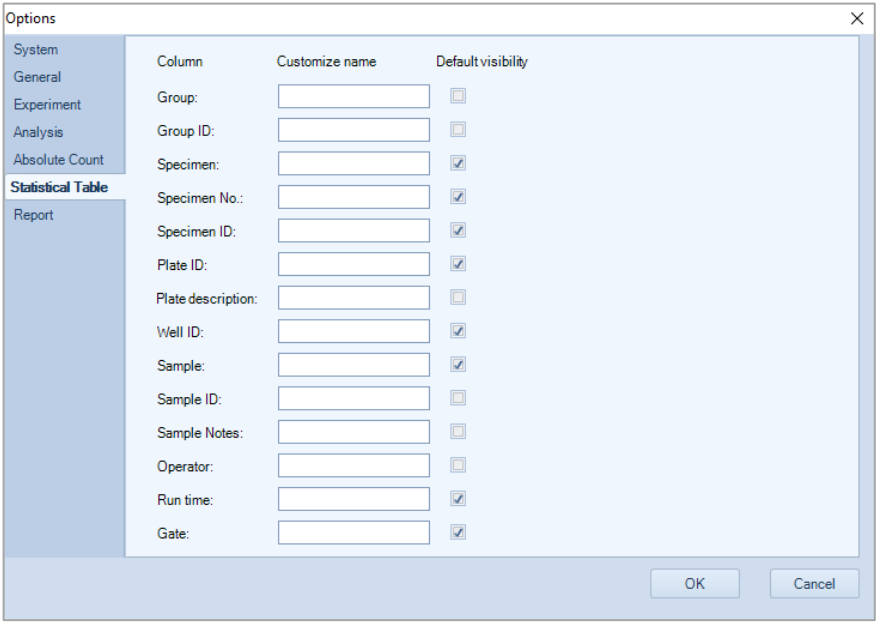
Select the cells to be copied by clicking and dragging with the mouse or select all using the keyboard shortcut Ctrl A. Use the keyboard shortcut Ctrl C to copy the selected cells to the clipboard. The copied table can be pasted to a program, such as Microsoft Excel, for further analysis.

Statistical Table Options

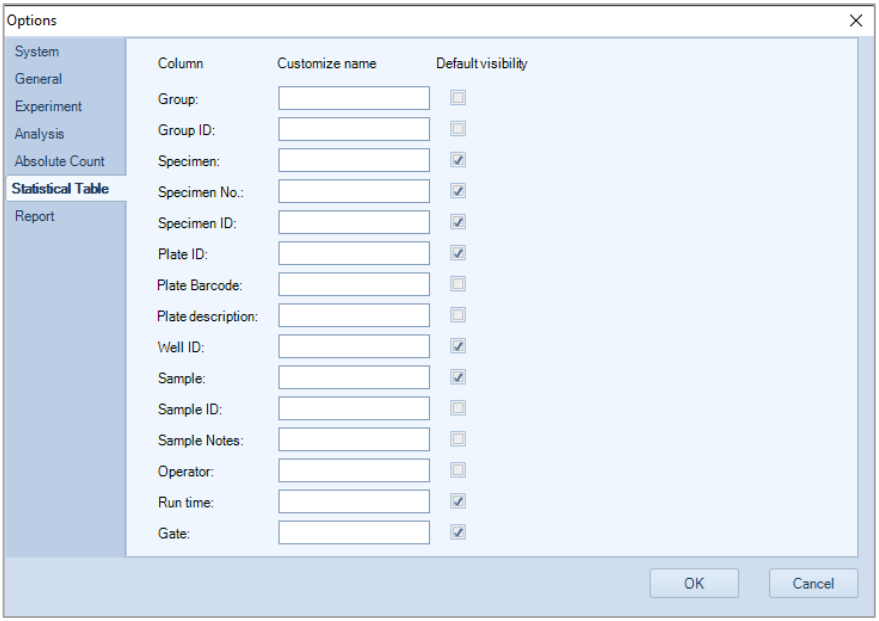
In the toolbar, click the **Statistical Table Options** button to open the **Statistical Table** tab of **Options** window, set **Customize Name** and **Default Visibility** of **Specimen**, **Specimen No**, **Case Number**, **Sample**, **Sample ID**, **Operator**, **Run Time** and **Gate** columns. When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, user can set **Customize Name** and **Default Visibility** of **Plate ID, Plate Barcode** (for NovoSampler Q only), and **Well ID** column.



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



When NovoSampler or NovoSampler Pro is connected



When NovoSampler Q is connected

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

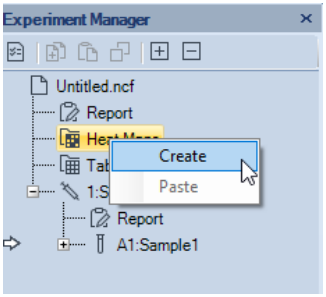
In the **Experiment Manager** panel, select the table. Right-click and select **Export Template..**. Enter the name for the template in the prompted window and click **Save** to create a template. The template will be saved as a *.nst file.

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

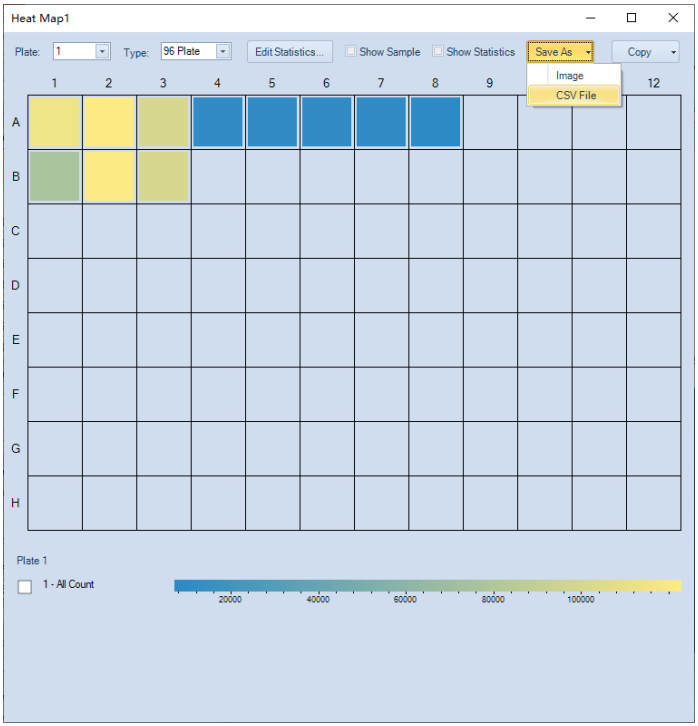
In the **Experiment Manager** panel, under the experiment file name, right-click on the **Heat Maps** node and select **Create**.



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

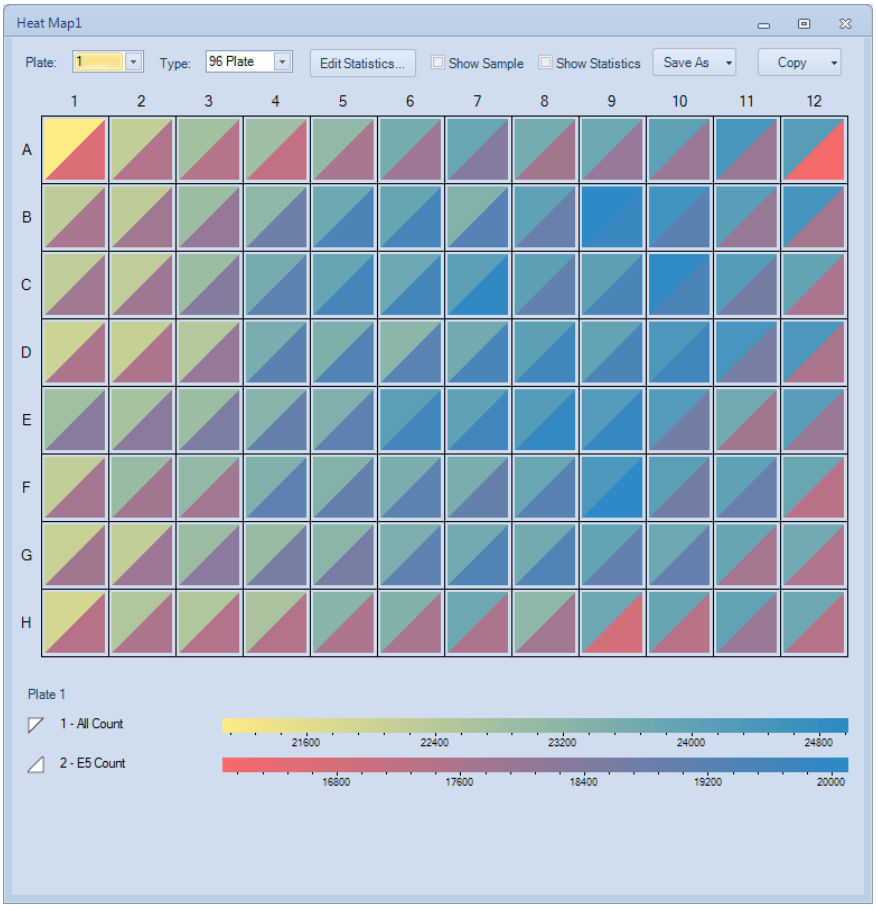
If there are samples outside the current plate type, "*" will be displayed in the upper left corner of the heat map.

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

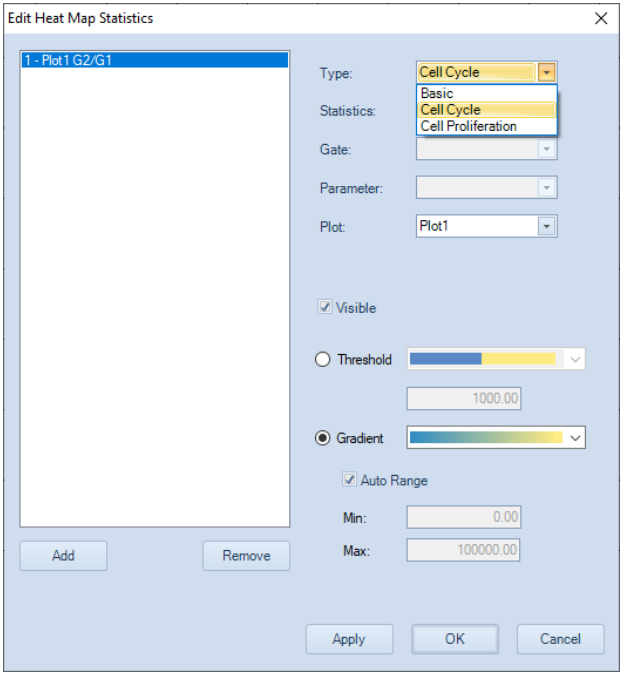
- Drag and Drop to Add Statistic Parameter

  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.

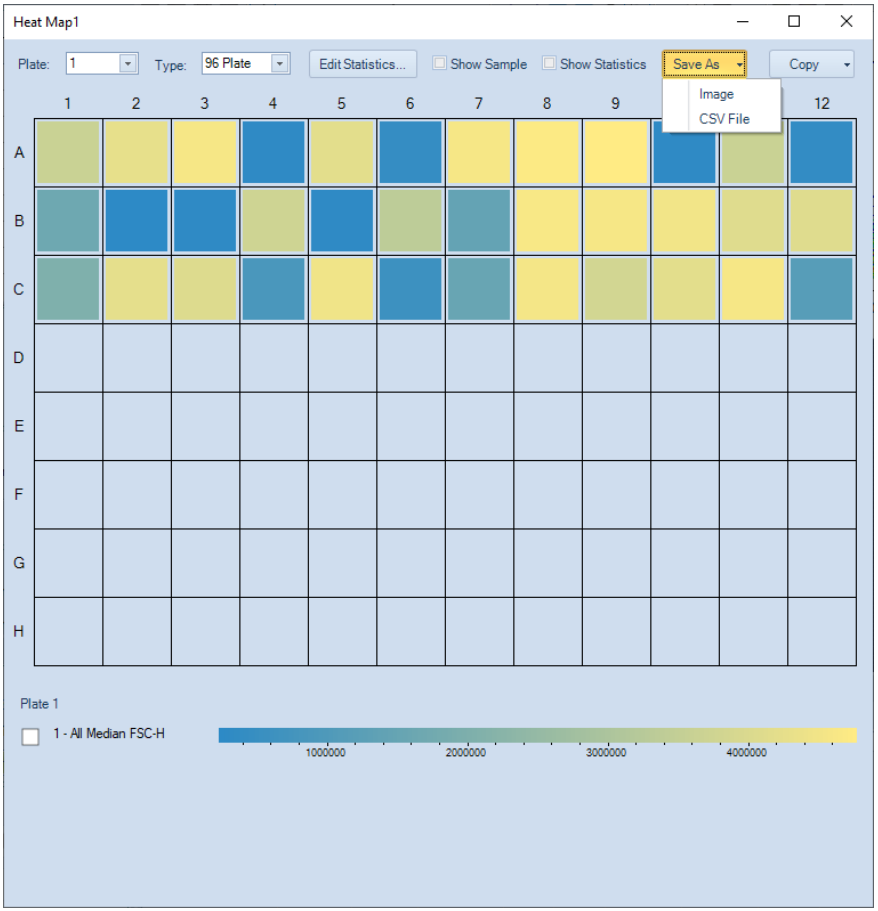
Update Heat Map

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.

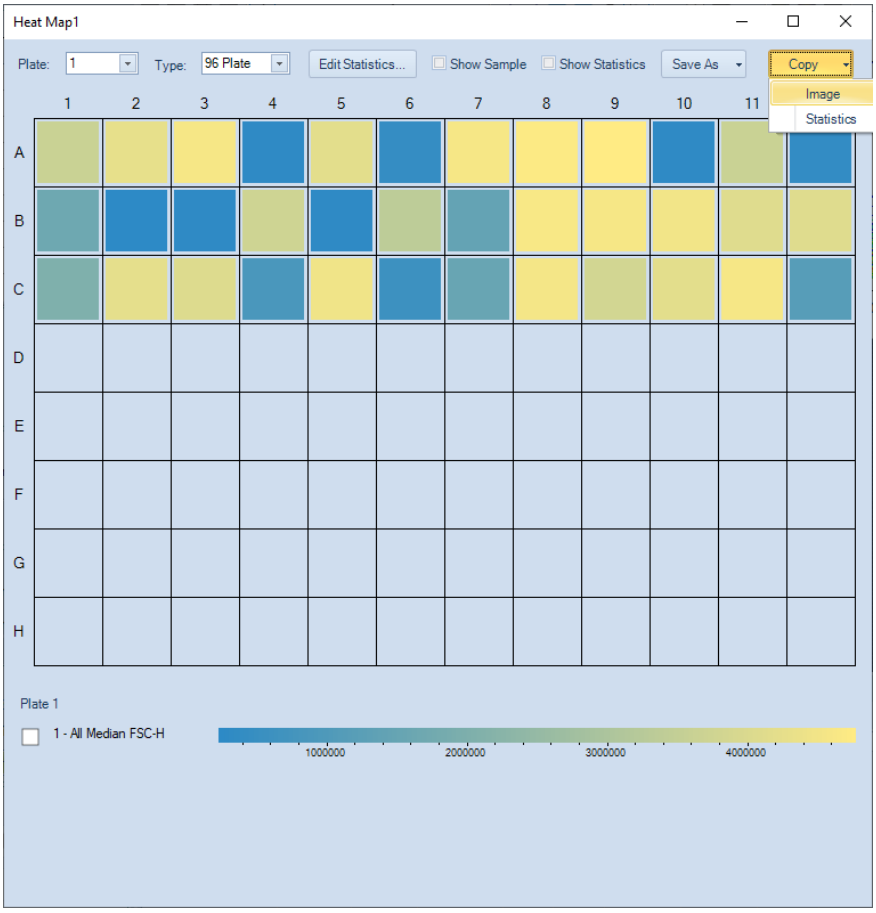
- Select **Image** or **CSV File** from the drop-down menu of **Save As.**



- 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.

Post Gain

In certain situations, user may want to align a particular peak on different samples on the same plot. Post Gain function in NovoExpress software allows such adjustment to be done after data acquisition.

NOTE

Post Gain does not affect data acquisition. The threshold value entered in the **Cytometer Setting** panel is based on event data value before Post Gain.

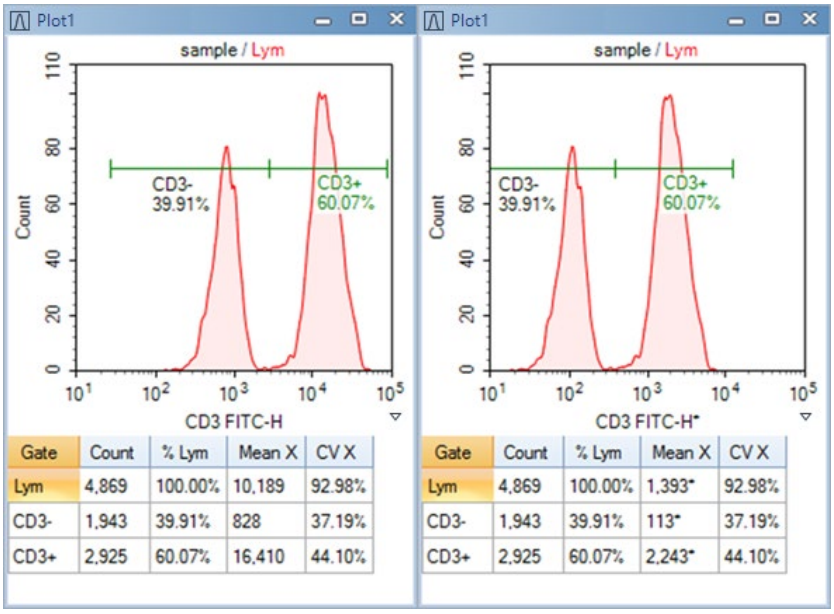
NOTE

Only user accounts with the **Adjust Post Gain** permission can adjust Post Gain.

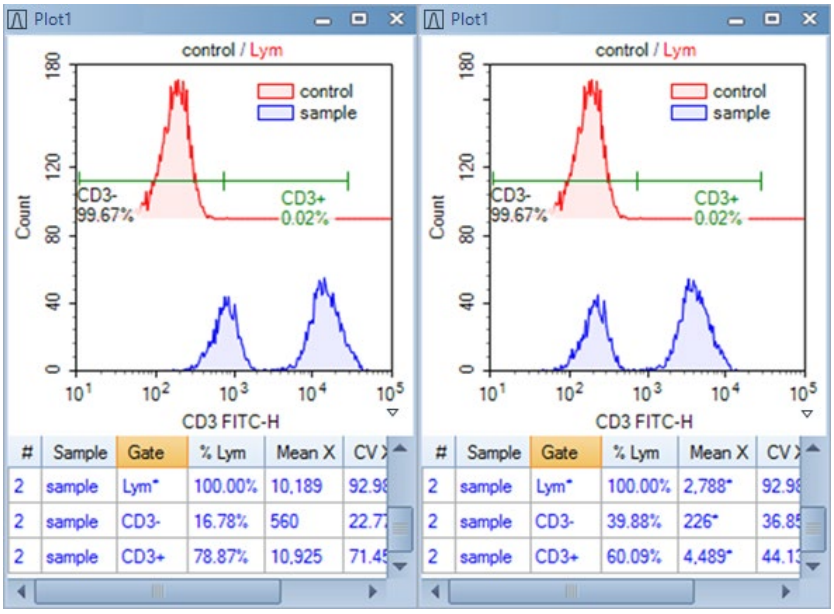
Adjust Post Gain

You can adjust the post gain value for each parameter of a sample.

First create a histogram of the desired parameter and click **Adjust Post Gain** button , on the workspace toolbar. Move mouse to the histogram plot area, hold down the left button of the mouse and drag the histogram curve, then drag and drop the peak of interest to the target location. In the example below the left plot is prior to the post gain adjustment and the right plot is after the post gain adjustment.



If you want to align peaks of different samples, first create a histogram plot of the parameter with multiple overlays of these samples. Set the style of the histogram plot as offset, and then use the mouse to drag each histogram curve to align them. In the examples below the left plot is prior to alignment and the right plot after alignment using the post gain function.



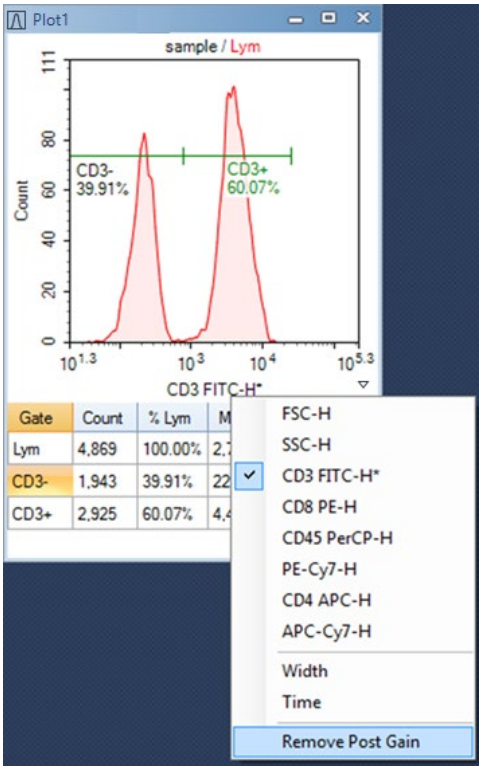
After a parameter is set using post gain, a * mark will be shown with parameter name in the statistics information of a plot or in the analysis table, and the value of any statistics with post gain will be shown with a * mark.

NOTE

Post gain will have no effect on the calculation of compensation, such that, compensated data will be calculated from the original data first, and the post gain will then be applied to the data. Control samples in an auto compensation specimen cannot be set using post gain.

Clear Post Gain

If you want to clear the post gain of a parameter, on area of axis parameter of any plot which contains the parameter, click the **Clear Post Gain** in the shortcut menu. If the menu item is not in the menu, it means that no post gain is set to the parameter.



If you want to clear post gain of all parameters of a sample, click **Clear Post Gain** menu under the main menu Sample, the post gain of active sample will be clear. If the menu item is gray, it means that no post gain is set to the parameters of the active sample.

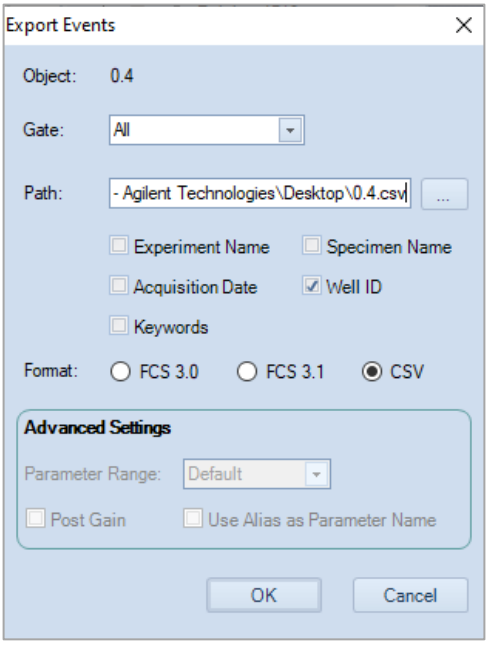
Apply Post Gain

When pasting an **Analysis** node of a sample to other sample on the experiment management tree, post gain will be pasted to the target sample too.

When exporting a sample to a .nct template file in the experiment management tree, post gain is contained in that template file too. When importing a template file to an **Analysis** node of a sample, post gain will also be imported and applied to the target sample.

Export Post Gained Data

In **Export Events** dialog, check the **Post Gain** option to enable exporting post gained data to FCS or CSV file. If the **Post Gain** option is unchecked, data without post gain will be exported.



If **Post Gain** option is checked, the sample data exported to FCS file are post gained data, and if there is compensation matrix in the sample, the data are first compensated and then post gained. If the FCS file is again imported in NovoExpress, post gain can be readjusted or cleared, as well as the compensation matrix. If the FCS file is imported into a third-party software, the third-party software will treat the data like an original data and does not know about the original compensation matrix and Post Gain information.

NOTE

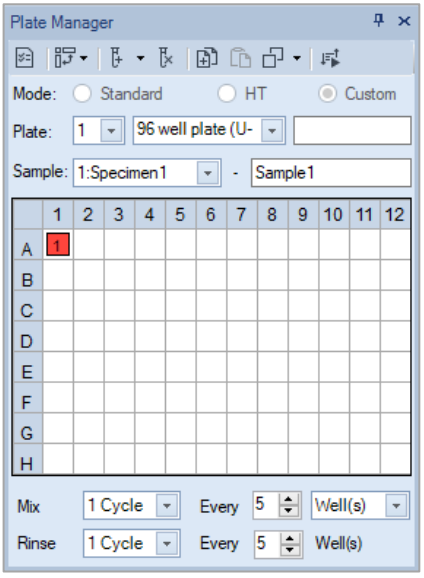
When export post gained data to CSV file, the name of parameters with post gain will be followed with a * mark.

6 Plate Manager

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

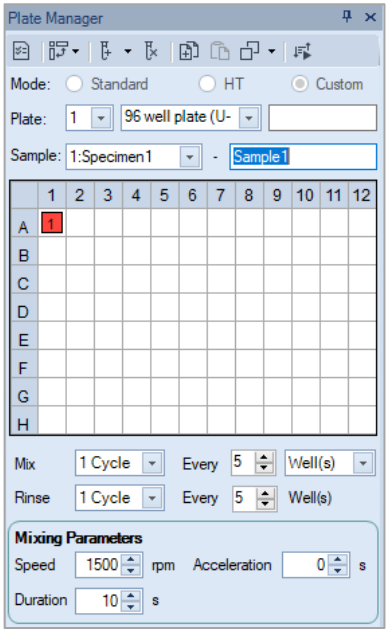
Overview of Plate Manager Panel

The **Plate Manager** panel will only be available when the NovoSampler (or NovoSampler Pro or NovoSampler Q) is installed or the **NovoSampler (or NovoSampler Pro or NovoSampler Q) installed** checkbox is checked in the **Instrument Configuration** window in the NovoExpress software offline mode. This management tool provides users with an intuitive and convenient way to manage samples on a multi-well plate or tube rack. The **Plate Manager** also allows user to set up the working mode, plate type, mixing and rinsing conditions.

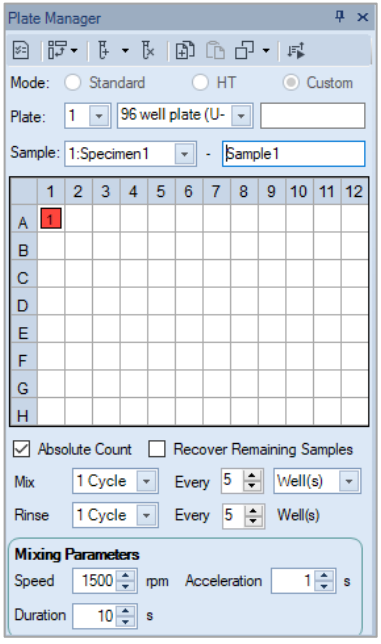


When NovoSampler is connected

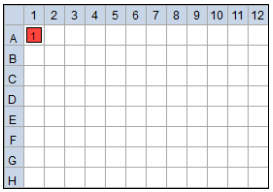
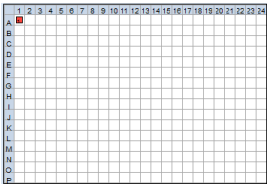
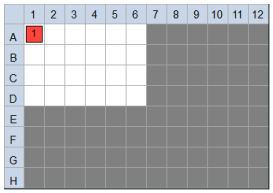
Plate Manager

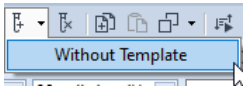
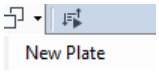


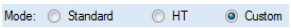
When NovoSampler Pro is connected



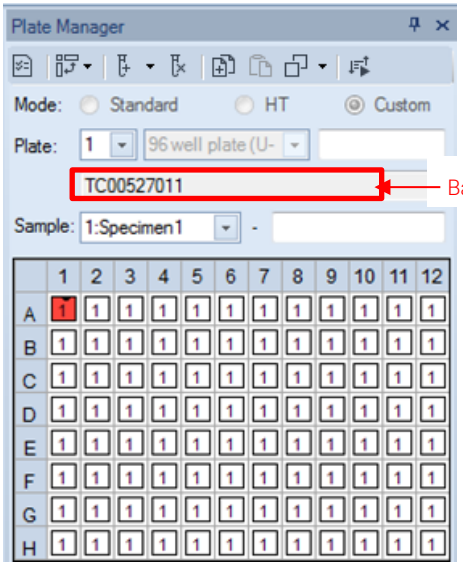
When NovoSampler Q is connected

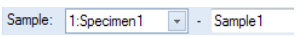
Icon	Description
  	The **Plate Manager** panel displays a 16 x 24 grid indicating a 384-well plate map (for NovoSampler Q only) and an 8 x 12 grid indicating a 96-well plate map. In settings for a 24 or 40 tube-rack or a plate type other than 384-well and 96-well plate, an 8 x12 grid will be displayed while invalid wells will be grayed out. 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 No 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 (or NovoSampler Pro or NovoSampler Q) in this area. Default mixing condition, rinse condition, stop condition and flow rate will be automatically applied when **Standard** or **HT** mode is selected. Please refer to associated operator's guide for more details. User can only customize these settings when **Custom** mode is selected. NOTE Selecting the working mode is only available before the sample is created.
	This area is designed to input plate information. The left drop-down box is the **Plate ID**. The **Plate ID** is a positive integer and can be modified by highlighting the area and entering a new number. The middle drop-down box is the **Plate Type**. The drop-down box lists all the available plate type defined in the **Manage Plate Types** window. Pull down the list and select the corresponding plate type for the experiment. The right box is the Plate Name. Input plate name in this box to help identify the plate. NOTE The plate type cannot be modified once the sample acquisition has begun.

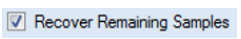
NOTE When NovoSampler Q is connected and the barcode reading function is enabled, user can view the scanned barcode information from the row below this area (see example below). Please refer to NovoSampler Q Operator's Guide for more details.





This area is designed to input the specimen and sample information. The left drop-down box is the **Specimen No** 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** ....

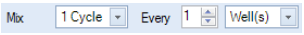
This function is available only when NovoSampler Q is connected. With **Absolute Count** is selected, user can analyze the exact number of cells or particles per unit volume. This function is disabled by default when NovoSampler Q works in Standard or High-Throughput (HT) mode. When Custom mode is selected, user can select to disable or enable this function.



This function is available only when NovoSampler Q is connected. With Recover Remaining Samples selected, user can recover the remaining sample aspirated into the sampling tube back to the sample tube or plate well after sample acquisition. This function is disabled by default when Standard or High-Throughput (HT) are selected. When Custom mode is selected, user can select to disable or enable this function.

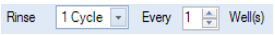
NOTE The Recover Remaining Samples function is disabled by default. To enable this function, user need to check the check box in the front.

NOTE Any changes to this setting (i.e., disable or enable) during the sample collection will only be effective from the next well forward.



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.

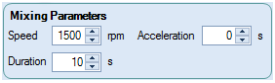
NOTE Default mixing conditions (i.e., Mix 1 Cycle Every 1 Well) will be automatically applied and cannot be modified by user when Standard or HT mode is selected.



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: This panel is available only when NovoSampler Pro or NovoSampler Q is connected. User can view or set the mixing parameters for the orbital shaker in this panel. When NovoSampler Pro or Q works in **Custom** mode, these mixing parameters for each plate type are defined in the **Manage Plate Type** window and can be modified in this area for the current experiment. When NovoSampler Pro or NovoSampler Q works in **Standard** or **HT** mode, default mixing parameters will be automatically applied and cannot be modified by user.

Speed: The mix speed of the orbital shaker. Please refer to table below for the default mix speed when NovoSampler Pro or NovoSampler Q works in different mode.

Plate Type		Default Mix Speed (rpm)		
		Standard Mode	HT Mode	Custom Mode
NovoSampler Pro	96 well plate (U, V and Flat bottom)	1500	1500	1500
	24 well plate	2500	2500	2500

	24 tube rack (12 X 75 mm tubes)	1500	1500	1500
	48 well plate	1500	1500	1500
NovoSampler Q	96 well plate (U, V and Flat bottom)	1500	1500	1500
	24 well plate	2500	2500	2500
	48 well plate	1500	1500	1500
	40 tube rack (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 0 second for NovoSampler Pro, and 1 second for NovoSampler Q.
Duration: The mixing time for the orbital shaker to mix the sample in one cycle. Please refer to table below for the default mix duration when NovoSampler Pro and NovoSampler Q works in different mode.

Plate Type		Default Mix Duration (s)		
		Standard Mode	HT Mode	Custom Mode
NovoSampler Pro	96 well plate (U, V and Flat bottom)	10	10	10
	24 well plate	10	10	10
	24 tube rack (12 X 75 mm tubes)	10	10	10
	48 well plate	10	10	10
NovoSampler Q	96 well plate (U, V and Flat bottom)	8	3	10
	24 well plate	10	4	10
	48 well plate	10	5	10

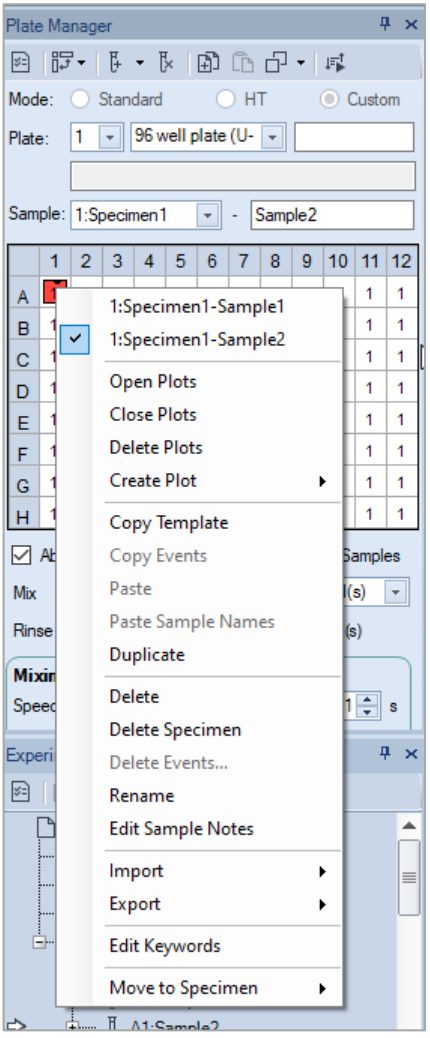
40 tube rack (12 X 75 mm tubes)	10	5	10
384 well plate	8	2	10
24 tube cooling rack (12 X 75 mm tubes)	10	5	10
96 well plate (U, V and Flat bottom) with cooling box	8	3	10
40 tube rack (1.5/2.0 mL tubes)	10	5	10

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.

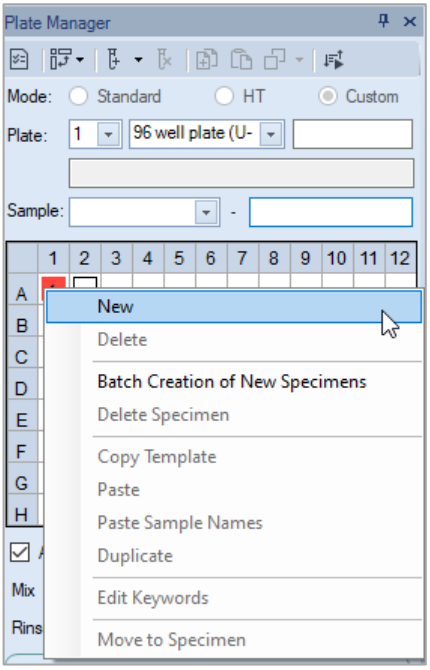


If the selected well has no sample defined, the right-click menu generally has two items available, **New** and **Batch Creation of New Specimens**. **New** will create new sample without a template. Batch Creation of New Specimens allows user to create new specimen(s) with customized specimen and sample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

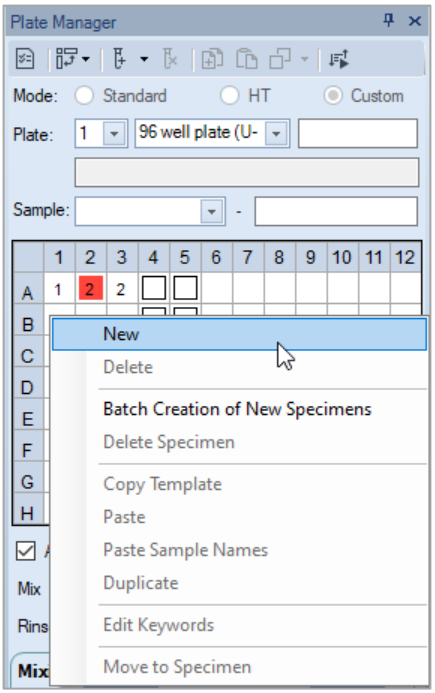
NOTE

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

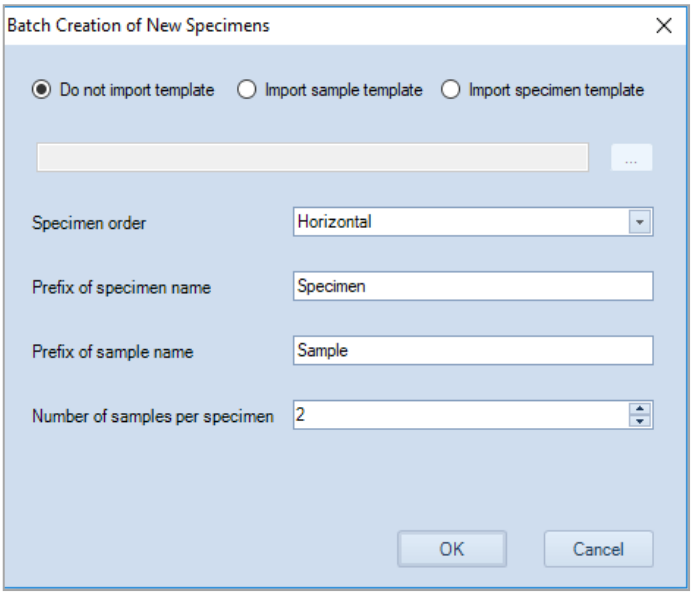
Plate Manager



Context menu when multiple wells are selected



- **New**: Same as the **New Sample(s) on Selected Well(s)** tool in the toolbar.
- **Delete**: Same as the **Delete Sample(s) on Selected Well(s)** tool in the toolbar.
- **Batch Creation of New Specimens**: Create new specimen(s) with customized specimen and sample organization. When clicked, software will prompt following window. User 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 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**.

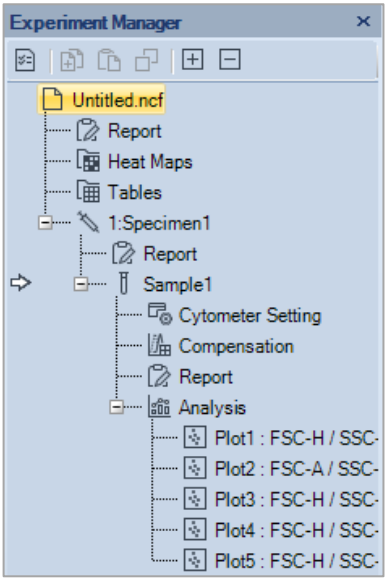
Experiment Manager Toolbar

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

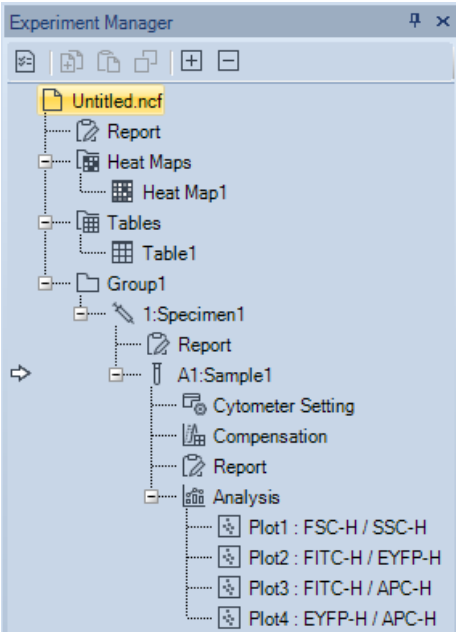
In the NovoExpress Software, the hierarchy structure from high to low is groups, specimen, and samples.



In the figure above, the arrow indicates the active sample. In the NovoExpress Software, the active sample collection parameters are displayed in the **Cytometer Setting** and **Cytometer Control** panels. In the **Experiment Manager** panel, double-clicking on a sample node will make it the active sample. When switching to a new active sample, the **Cytometer Setting** and **Cytometer Control** panels will update with the new active samples' information, and the plots in the Workspace will be replaced with the new active sample's plots.

When NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected, the samples listed in the **Experiment Manager** panel include the Well ID and the sample name, separated by a colon (see figure below). Click the sample node to select the sample, right-click and select Rename to rename the sample. When renaming the sample, both the Well ID and the sample name can be modified. Specimen is also associated with a Specimen No. The Specimen No is the number before the specimen name, separated by a colon. The Specimen No is used to identify specimen and can be modified. For additional information regarding the Specimen No, refer to Plate Manager Panel in this guide.

Experiment Manager



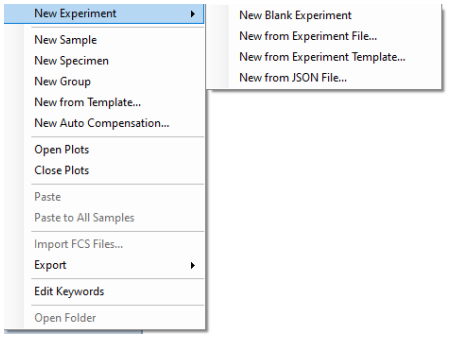
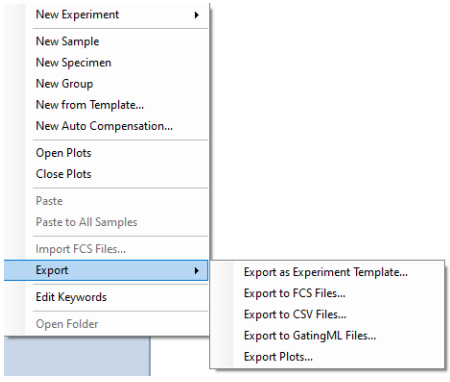
Icon	Description
File	The experiment file (*.ncf file format)
Report	Contains a report of the data analysis. There is a Report node under experiment file, specimen and sample. • Reports under Experiment File can include plots and statistical analysis for all specimen and samples in this experiment file. • 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.
Heat Maps	This node contains Heat Maps for the experiment file. Right clicking this node allows for creating new heat map.
Heat Map	Heat map.
Statistical tables	This node contains statistical tables for the experiment file. Right clicking this node allows for the creation of new statistical tables.

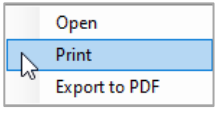
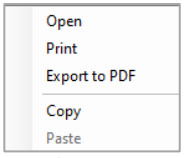
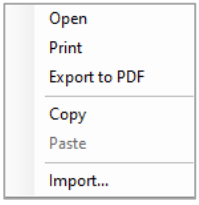
Statistical table	Statistical analysis table
Group	This node represents a Group in the organizational hierarchy. A group can contain multiple specimens and subgroups, and specimens will always be placed in front of subgroups.
Specimen	This node represents a Specimen in the organizational hierarchy. A specimen can contain multiple samples. Each specimen contains a specimen report. In the node text "**1:Specimen 1**", the former number is Specimen No, the latter text is Specimen Name.
Sample	This node represents a Sample in the organizational hierarchy. The sample is the most basic organizational unit and contains sample data collection parameters, instrument settings, fluorescence compensation settings, reports, analysis, and data. The sample icon will display differently depending on the status of the sample. A blank sample without any data collected will appear as A sample listed for sample acquisition and during acquisition will appear as A sample with data collected will appear as
Cytometer settings	Cytometer settings contain the sample parameters, the acquisition stop conditions, and the sample flow rate and threshold settings. Refer to Cytometer Setting in this guide for additional information.
Fluorescence compensation	Fluorescence compensation matrix for the sample. If the matrix is empty, the node is displayed in black. If the matrix is filled, the matrix is displayed in blue. Refer to Fluorescence Compensation 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 red arrow indicates the sample is the active sample.
	Flashing green arrow indicate the sample is being collected.

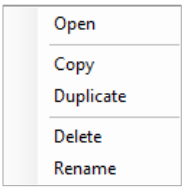
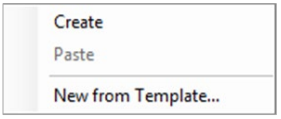
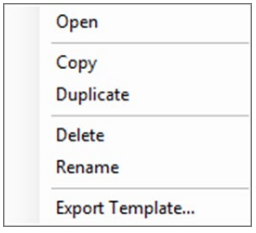
Flashing green arrow	
Alternatively flashing white and green arrows	Flashing white and green arrows indicate the active sample is being collected.

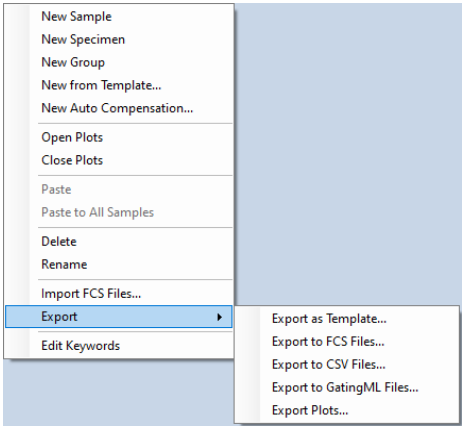
Right-Click Menu

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**: • **New Blank Experiment**: Create a blank experiment file. • **New from Experiment File**: Create an experiment file from an existing experiment file. When NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon is connected, 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 from Experiment Template**: Create an experiment from an experiment template file. • **New from JSON File**: Create an experiment file from an existing JSON file. This option is available when NovoCyte Quanteon, NovoCyte Advanteon or NovoCyte Penteon is connected. **New Sample**: Creates a new specimen with a new sample included. **New Specimen**: Creates a new specimen. **New Group**: Creates a new group. **New from Template**: Imports selected group, specimen, and samples from a template. **New Auto Compensation**: Creates a compensation specimen containing samples to compute a compensation matrix. **Open Plots**: Opens all plots from all of the samples. **Close Plots**: Closes all plots from all of the samples. **Paste**: Creates a new specimen with the copied specimen template. **Paste to All Specimens**: Pastes the copied specimen template to all specimens.

	Paste to All Samples: Pastes the copied sample template to all samples. **Import FCS Files**: Selects a folder to import all FCS files within the folder or subfolders as samples. Files up to 10 subfolders deep from the selected folder will be added and organized according to the folder structure. **Export**: • **Export as Experiment Template**: Exports the file as an experiment 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.
 Report File Report  Specimen Report:  Sample Report:  	**Open**: Opens the report. **Print**: Prints the report. **Export to PDF**: Exports the report to PDF. **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.

 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.
 Group	**New Sample**: Creates a new specimen with a new sample included. **New Specimen**: Creates a new specimen. **New Group**: Creates a new group. **New from Template**: Imports selected group, specimen, and samples from a template. **New Auto Compensation**: Creates a compensation specimen containing samples to compute a compensation matrix.



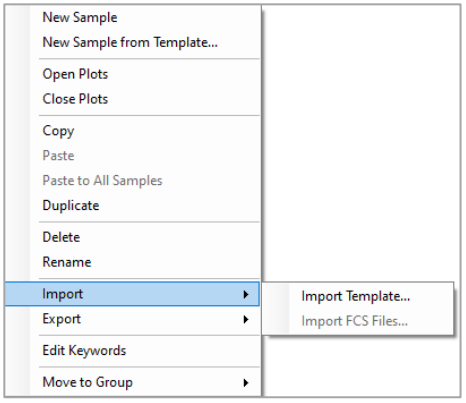
Open Plots: Opens all plots from all of the samples within the group.
Close Plots: Closes all plots from all of the samples within the group.
Paste: Creates a new specimen with the copied specimen template.
Paste to All Specimens: Pastes the copied specimen template to all specimens in the group.
Paste to All Samples: Pastes the copied sample template to all samples in the group.
Delete: Deletes the group.
Rename: Renames the group.
Import FCS Files: Selects a folder to import all FCS files within the folder or subfolders as samples. Files up to 10 subfolders deep from the selected folder will be added and organized according to the folder structure.
Export:

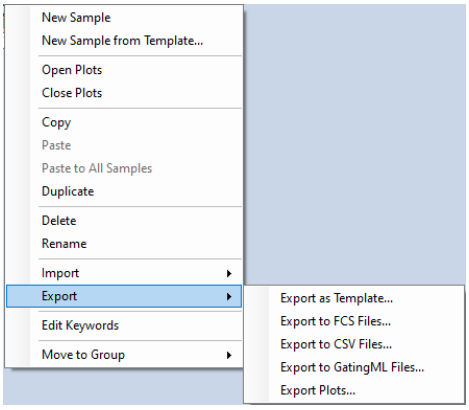
- **Export as Template**: Exports the group as a template file.
- **Export to FCS Files**: Exports all samples as FCS files.
- **Export to CSV Files**: Exports all samples as CSV files.
- **Export to GatingML Files:** Exports all gates in each sample as Gating-ML files.
- **Export Plots**: Exports all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

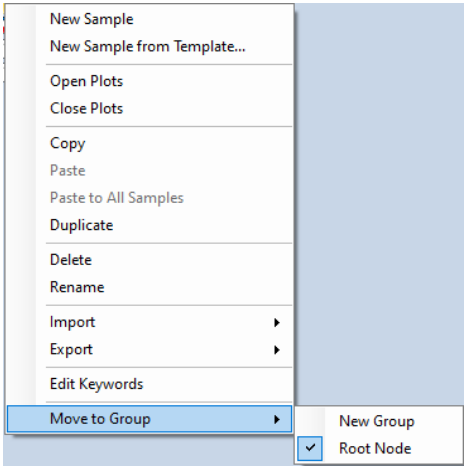
Edit Keywords: Opens **Edit Keywords** window**.** The **Edit Keywords** window will list **the** keyword**s** information for all samples in **the** selected group. Please refer to Edit Keywords Window in this guide for more details**.**

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 the samples within the specimen.
Close Plots: Closes all plots from all the samples within the specimen.
Copy: Copies the template of the specimen.
Paste: Pastes the template of a copied specimen, or creates a new sample with the copied sample template.
Paste to All Samples: Pastes the copied sample template to all samples in the specimen.
Duplicate: Creates a duplicate of the specimen.
Delete: Deletes the specimen.
Rename: Renames the specimen.







Import:

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

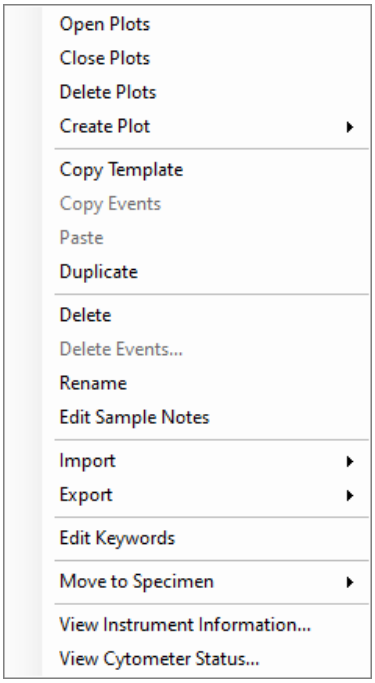
Export:

- **Export as Template**: Exports the specimen as a template file.
- **Export to FCS Files**: Exports all samples as FCS files.
- **Export to CSV Files**: Exports all samples as CSV files.
- **Export 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**.**

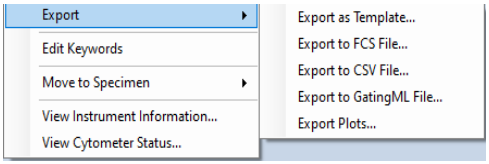
Move to Group: Move the specimen to the existing group or new group.

Sample

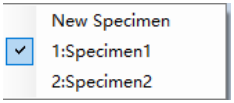


Open Plots: Opens all plots from the sample.
Close Plots: Closes all plots from the sample.
Delete Plots: Deletes all plots from the sample.
Create Plot: Creates a new plot for the sample.
Copy Template: Copies the template of the sample.
Copy Events: Copies the events of the sample.
Paste: Pastes the copied template or events of a sample.
Duplicate: Creates a duplicate of the sample.
Delete: Deletes the sample.
Delete Events: Deletes all or part of the sample's events. Only accounts with the permission of **Delete Sample Events** can perform this operation (Refer to Sample in this guide).
Rename: Renames the sample.
Edit Sample Notes: Edits the sample notes.
Import: Imports a sample template or FCS file, as shown below:

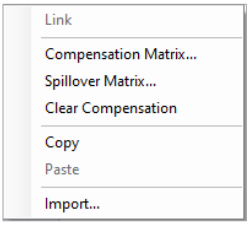
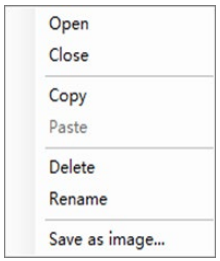
Export: Exports the sample as a template, FCS file, CSV file, exports all gates as a Gating-ML file or exports all plots of the sample as image files.

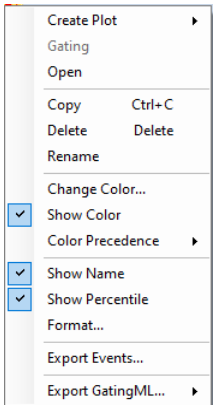
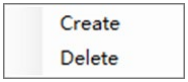


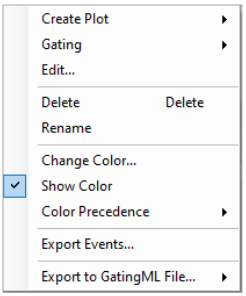
Edit Keywords: Opens **Edit Keywords** window. The **Edit Keywords** window will list the keywords information for the sample. Please refer to Edit Keywords Window in this guide for more details.
Move to Specimen: Move the sample to the selected specimen or new specimen.



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.
 Fluorescence compensation 	**Link**: Links the sample's compensation to Compensation Specimen. **Compensation Matrix**: Opens the sample's Compensation Matrix. **Spillover Matrix**: Opens the sample's Spillover Matrix. **Clear Compensation**: Clears fluorescence compensation information of the sample. **Copy**: Copies the sample's fluorescence compensation. **Paste**: Pastes the copied fluorescence compensation to the sample. **Import**: Imports fluorescence compensation from a selected sample template.
 Analysis 	**Open Plots**: Opens all plots from the sample. **Close Plots**: Closes all plots from the sample. **Delete Plots**: Deletes all plots from the sample. **Create Plot**: Creates a new plot for the sample. **Copy**: Copies the sample analysis template. **Paste**: Pastes the copied analysis template to the sample. **Import**: Imports an analysis template to the sample from a selected template file.
Plot 	**Open**: Opens the plot. **Close**: Closes the plot. **Copy**: Copies the plot. **Paste**: Pastes a copied gate to the plot. **Delete**: Deletes the plot. **Rename**: Renames the plot. **Save as image...**: Save plot as an image file.

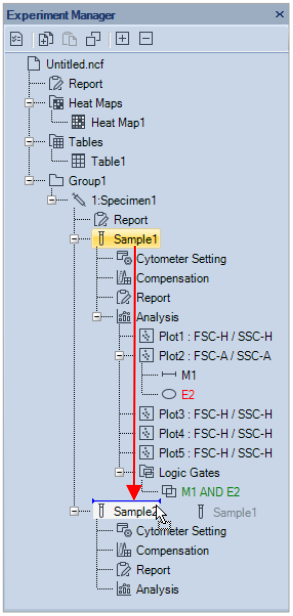
Gate 	**Create Plot**: Creates a new plot including the events from the selected gate. **Gating**: Selects plots to apply the gate. **Open**: Opens the plot containing the gate. **Copy**: Copies the gate. **Delete**: Deletes the gate. **Rename**: Renames the gate. **Name with CD Marker**: If a fluorescence parameter is labeled as a CD (Cluster of Differentiation) marker in the **Parameter** panel by setting the **Alias** as CD and a number, this labels the gate using the CD markers. **Change Color**: Modifies the color of the gate. **Show Color**: Sets whether to display the gates in color. **Color Precedence**: Modifies color precedence of the gate. **Show Name**: Shows the gate name in gate label on plot. If the **Show gate name in gate label** option in **Setting** > **Analysis** is not checked, the Show Name menu item here will be disabled. **Show Percentile**: Shows the percentage of the gated events relative to the total number of events on the plot. If the **Show population percentile in gate label** option in **Setting** > **Analysis** is not checked, the Show Percentile menu item here will be disabled. **Format**: Opens **Plot Format** dialog to define gate format. **Export Events**: Exports data for the events inside the current gate in either FCS or CVS format. **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,

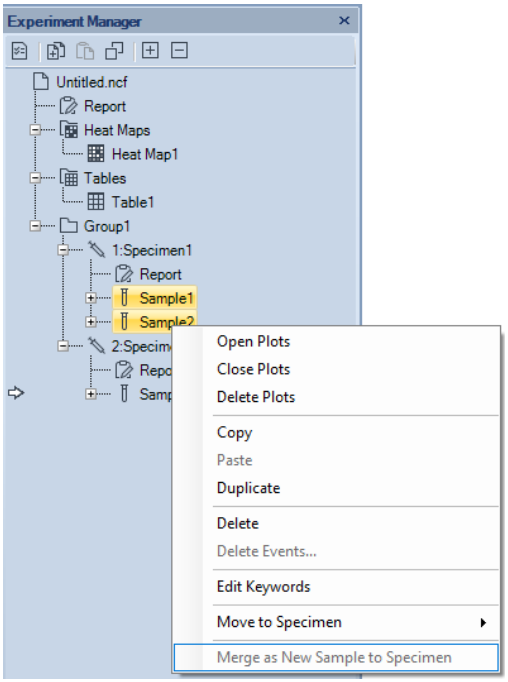
1 Ensure to log into the software with permission of **Merge Sample**. Refer to Access Control in this guide for more details.

NOTE

When user logs into the software with Administrator account, the Merge Samples permission is automatically enabled by default.

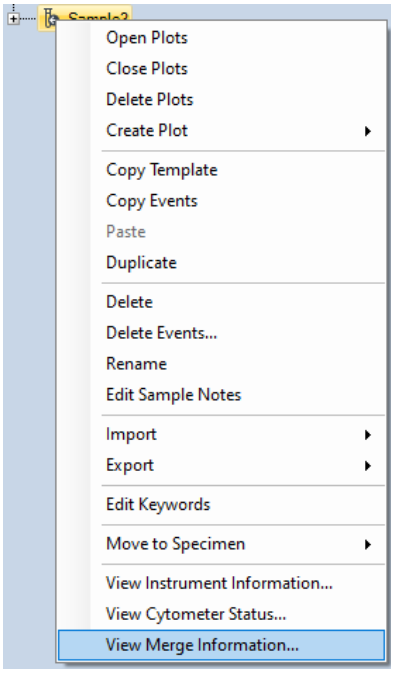
2 Select the samples from the **Experiment Manager** panel or **Plate Manager** panel (if NovoSampler, NovoSampler Pro or NovoSampler Q is connected) while holding the **Ctrl** or **Shift** key on the keyboard

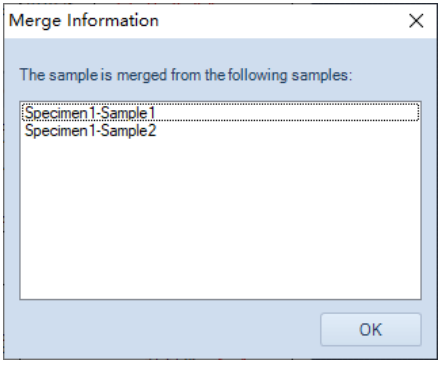
3 Right click any sample, click **Merge as New Sample to Specimen**. User can select either **New Specimen** to have the merged samples placed under a newly created specimen or select **Specimen X** to have the merged sample placed under the selected existing specimen.



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).





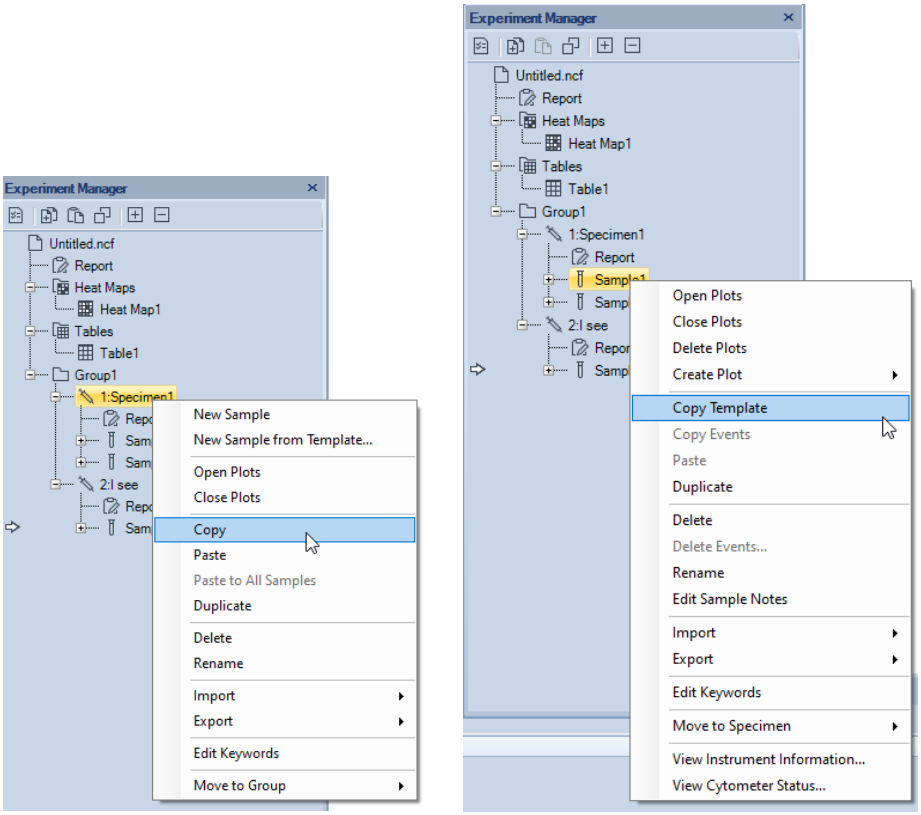
Templates

The NovoExpress 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:

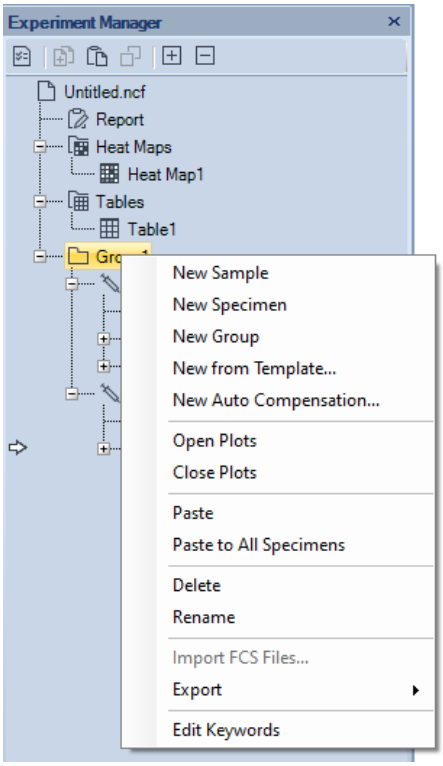
copying and pasting, dragging and dropping, adding through the toolbar, and importing and exporting templates.

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.

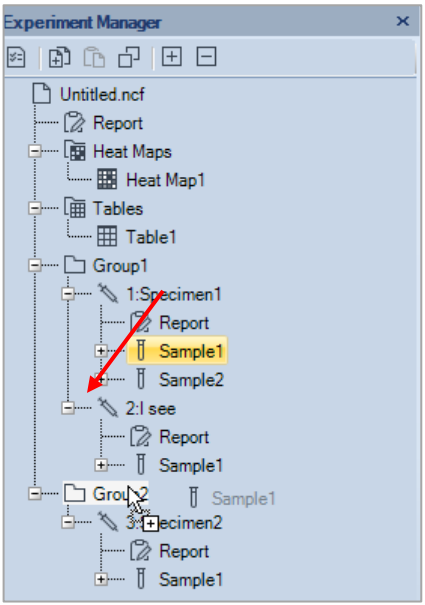
The source node	Target node
Specimen	**Specimen**: The target specimen report is replaced by the template of source specimen report and samples in the target specimen are replaced by copying samples in the source specimen and pasting to them. Group, Experiment File: Creates the same specimen as the source 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.
Compensation	**Compensation, Sample**: The compensation of target sample is replaced. Specimen, Group, Experiment File: The compensations of all samples within the target node are replaced.
Sample Report	**Sample Report, Sample**: The report template is replaced by the report template of source sample. **Specimen, Group, Experiment File**: The sample report templates of all samples within the target node are replaced.
Analysis	**Analysis, Sample**: The analysis template of target sample including plots and gates is replaced by the analysis template of source sample. **Specimen, Group, Experiment File**: The analysis templates of all samples within the target node are replaced.
Plot	**Analysis, Plot**: If the target node contains a plot with the same name as the source node, the plot will be replaced. Otherwise, the plot will be created. **Specimen, Group, Experiment File**: Either replace or create the plot in all of the samples of the target node.
Gate (does not include logic gates)	**Plot** (does not include cell cycle plots): If the target node contains a gate with the same name as the source node, the gate will be replaced. Otherwise, the gate is created. Only range and bi-range gates can be drawn into a one-dimensional histogram. i.e., a rectangular gate cannot be applied to a histogram. Also, changing a two-dimensional gate (such as FITC vs. PE, that has a rectangular gate drawn in it) to a histogram will delete rectangular gates. **Analysis, Sample**: Source gate will be pasted to the plot with the same plot name as source gate's plot in the target sample if it exists. **Specimen, Group, Experiment File**: Source gate will be pasted to all samples in the target node.

NOTE Specimen reports and sample reports cannot copy and paste each other.

Drag and Drop the Templates

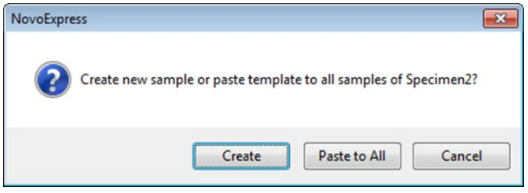
The template from source nodes can also be applied to target nodes by dragging and dropping, as shown below:



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.

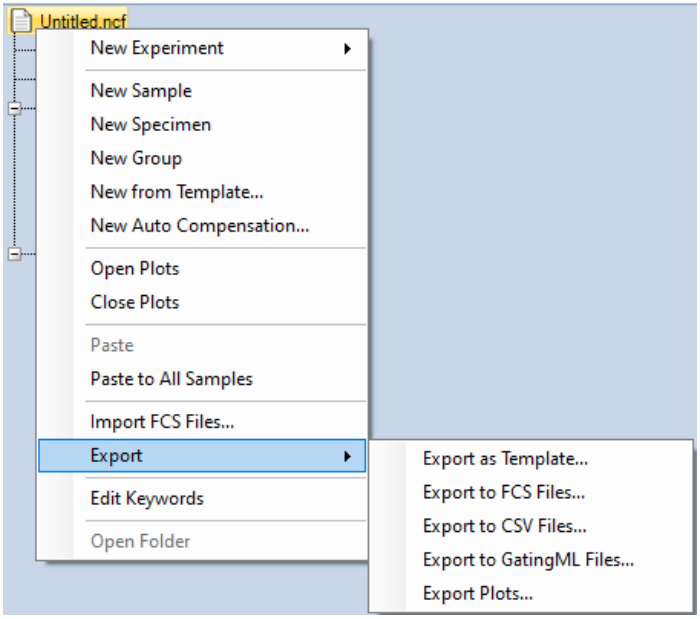
Use the Toolbar

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.



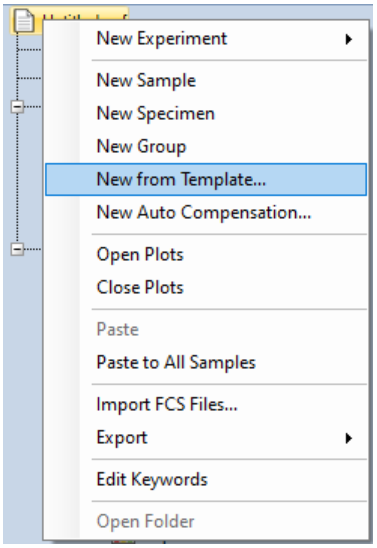
NOTE

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

Import Template

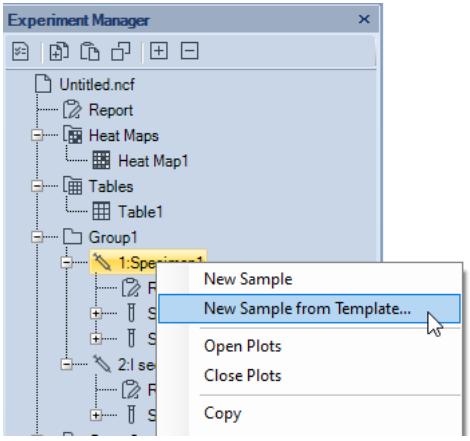
- Import to Experiment File

  If importing to an experiment file, right-click the experiment file node and select **New from Template…**.



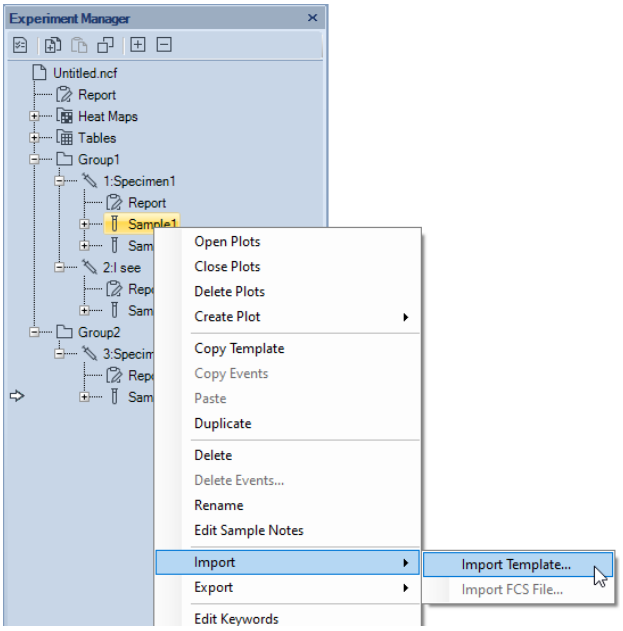
- Import to Specimen

  If importing to a specimen, right-click the specimen node and select **New Sample from Template…**.



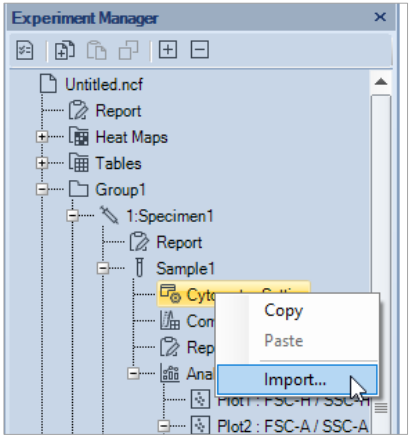
- Import to Sample

  If importing to a sample, right-click the sample node and select **Import**, then **Import Template…**.



- Import to **Cytometer Setting**, **Compensation**, **Report**, or **Analysis**

  If importing to **Cytometer Setting**, **Compensation**, **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 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…**.

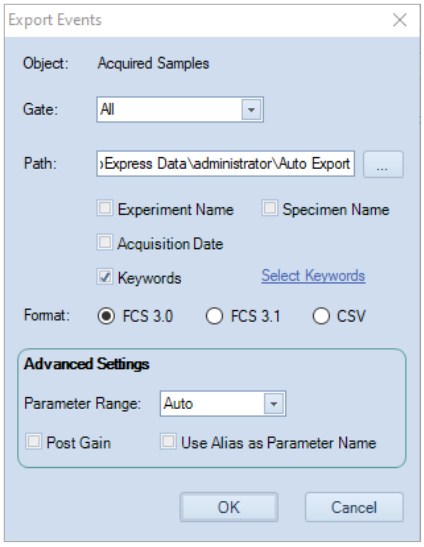
  Select the FCS file, and data from the file will be imported to the blank sample. It is not possible to import data to a sample already containing collected data. To import data to a sample already containing data, first clear the sample of any events by right clicking the sample and selecting **Delete Events**.

NOTE

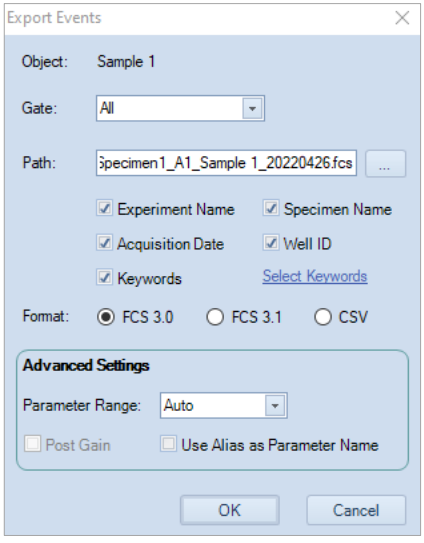
When importing a FCS file, all the **FCS Standard Keywords** will be automatically included. Other keywords (if any) will be imported as **User-Defined Keywords**. If the imported keyword has the same name as the existing keyword, software will automatically add **(1)** behind the imported keyword. For 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.



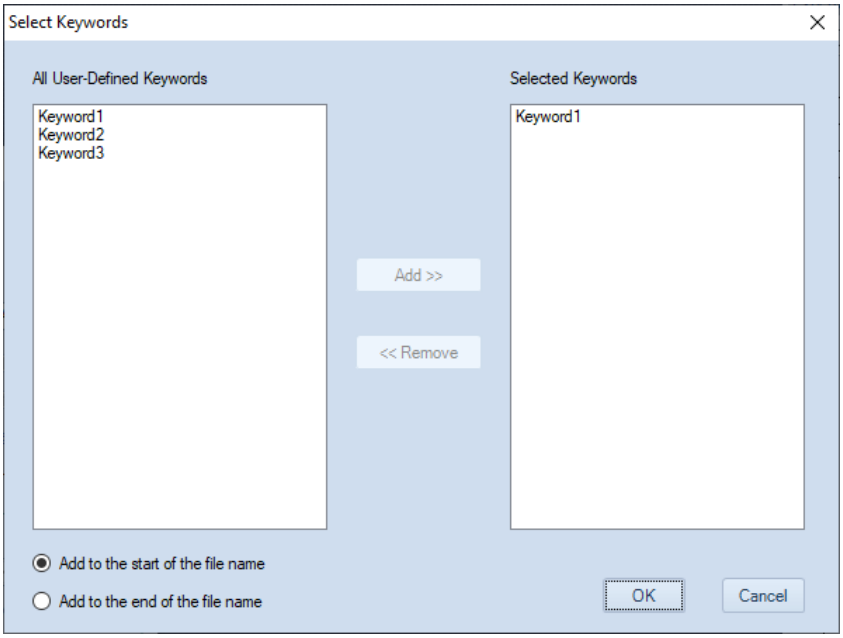
When NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected



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

The **Export Events** window has the following settings:

- **Object**: This is the node to be exported. If the object is a sample, only the sample will be exported. If the object is a specimen, group, or experiment file, all samples within the object will be exported.
- **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**.
- **Experiment Name**: Check this box to include experiment name in the name of the exported file.
- **Specimen Name**: Check this box to include specimen name in the name of the exported file.
- **Acquisition Date**: Check this box to include acquisition date in the name of the exported file.
- **Well ID**: Check this box to include **Well ID** in the name of the exported file. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
- **Keywords**: Allows user to include selected keyword(s) in the name of the exported file. When checked, the **Select Keywords** option will be enabled next to this option. User can click **Select Keywords** to open **Select Keywords** window as shown below.



- **All User-Defined Keywords:** Displays all **User-Defined Keywords** available for selected samples.
- **Selected Keywords**: Displays the keyword(s) user selected to be included into the exported file.
- **Add**: Click to add highlighted keyword(s) from **All User-Defined Keywords** area to **Selected Keywords** area. When adding more than one keywords at the same time, user can also press **Ctrl** or **Shift** key while highlighting the keywords from **All User-Defined Keywords** area and click **Add**. User can also double click the selected keyword from **All User-Defined Keywords** area and software will automatically add it to the **Selected Keywords** area. This **Add** function is automatically disabled if no keyword is listed in **All User-Defined Keywords** area.
- **Remove**: Click to remove highlighted keyword(s) from **Selected Keywords** area. When removing more than one keywords at the same time, user can also press **Ctrl** or **Shift** key while highlighting the keywords from **Selected Keywords** area and click **Remove**. User can also double click the selected keyword from **Selected Keywords** area and software will automatically remove it. This **Remove** function is automatically disabled if no keyword is listed in **Selected Keywords** area.

  - **Add to the start of the file name**: When checked, the selected keyword(s) will be added to the start of the exported file name.
  - **Add to the end of the file name**: When checked, the selected keyword(s) will be added to the end of the exported file name.
- **Format**: This specifies the exported data file format. The default setting depends on whether the window was opened using **Export** > **Export to FCS Files…** or **Export** > **Export to CSV Files…**. However, the format can be changed post selection using this setting. If you want to import the FCS files to FlowJo with version below v10, please select **FCS 3.0**.
- **Parameter Range**: When exporting as a FCS formatted files, there is the option to set the recommends visualization parameter range. The three options are **Default**, **Auto**, and **Plots**. When **Default** is selected, the parameter range is the full range of the instrument (10 to 224). When **Auto** is selected, the software automatically calculates the best range based on the distribution of the sample data. When **Plots** is selected, the parameter range is determined from parameter ranges used in plots.
- **Post Gain**: This option is enabled only when the samples specified to export have Post Gain defined. When checked, exported event value is the value after Post Gain adjusted.
- **Use Alias as Parameter Name**: Check this option to use the parameter alias as keyword when exporting FCS file.

After setting the above options, click **OK** to begin exporting the data.

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.

NOTE

When exporting the file with more than one selected keyword, software will automatically add "_" between each keyword and other characters. Any keyword without value will be automatically ignored. Any invalid characters of keyword will be automatically replaced by "_".

NOTE

The exported file path and name cannot exceed 255 characters in total. Software will automatically cut off the file name accordingly.

NOTE

When user reopen the **Export Events** window, the software will automatically apply the effective settings user defined last time (i.e., all settings user applied after clicking **OK** in this window).

Copy and Paste Events

In the **Experiment Manager** panel, data of collected events can be copied and pasted to blank samples.

- Select the sample containing the events to be copied. Right-click and select **Copy Events**.
- Select the empty sample. Right-click and select **Paste**, or select the **Paste** button, from the toolbar. Data from all of the collected events in the original sample is pasted into the blank sample. Note that only events and cytometer settings are pasted. To copy and paste Analysis, select the sample containing the Analysis to be copied. Right click and select **Copy Template**. Select the sample to paste the Analysis into. Right click and select **Paste**. Alternatively, dragging and dropping Analysis from one sample to another will also copy and paste the Analysis. Similarly, this can be performed with Compensation and Report.

NOTE

This is useful when using the **Auto Compensation** feature during analysis to apply single stained compensation controls to the generated Auto Compensation samples.

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**.

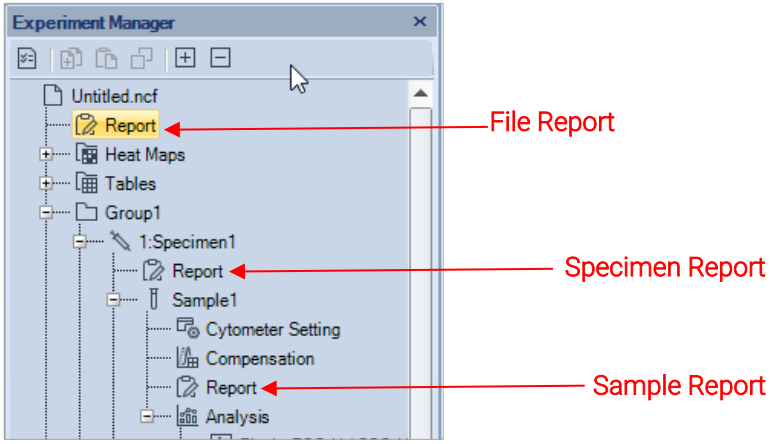
Reports can be created using an auto mode and a manual mode. In the auto mode, the software generates the report using a fixed format to include user created plots, statistics (**Gate, Count, % of Parent, Mean X and Mean Y**), and basic information (**Sample Name, Run Time, Cytometer, and Software**). 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.

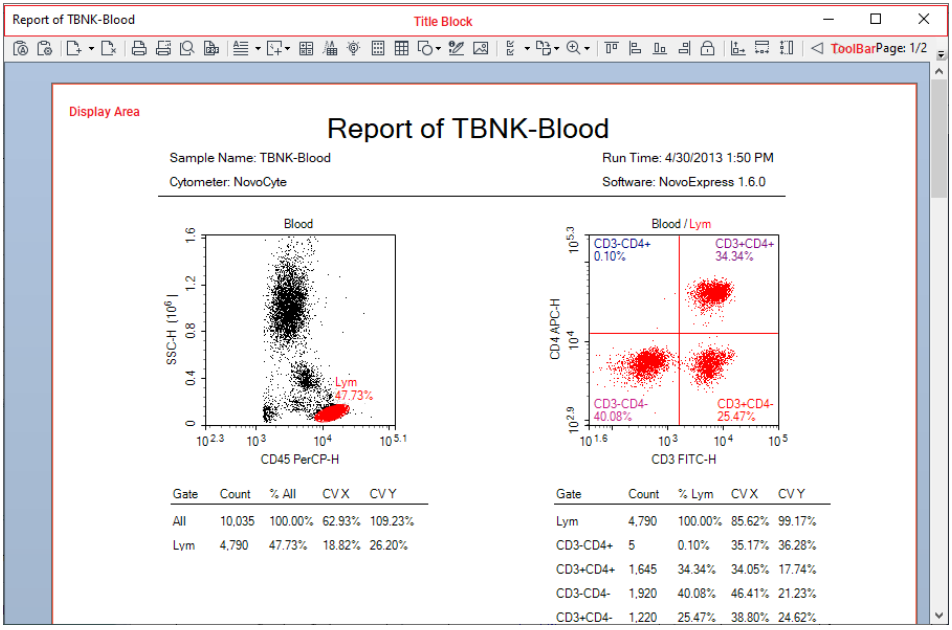
From the **Experiment Manager** panel, the report can either be a file report, or sample report or a specimen report. A sample report can contain plots, statistical information, compensation matrices, and collection information for the sample. A specimen report contains such information for all of its samples and basic information of its own. A file report contains all the sample and specimen information within experiment file. Double clicking on **Report** in the **Experiment Manager** allows the user to view the report interface window.

After the reports are created, a batch print function (Batch Print Reports) can be used to generate PDF files.



Report Interface

The report interface window is shown below. It can be opened by double-clicking on the report node in the **Experiment Manager** panel.



The interface window is divided into three main sections: Title Block, Toolbar, and Display Area.

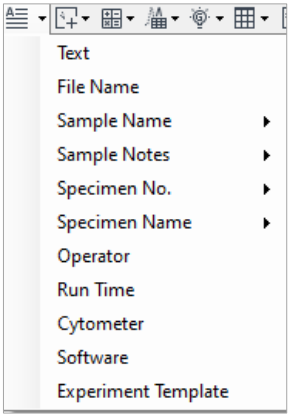
The title bar contains the name of the current report in the window.

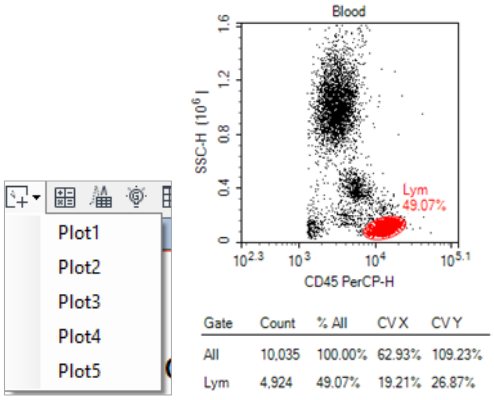
The toolbar contains the functions available to generate and edit the report.

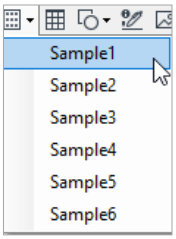
The display area is the main area of the window. Using this area, the user is able to edit the objects displayed and add and delete pages. The objects here forth are referred to as report items and include text, graphics, statistical information, fluorescence compensation, and plots.

The toolbar functions are described below:

Icon	Description
	Auto Report Mode: Clicking on this button switches between the automatic report generation mode and the manual mode. When selected and in automatic

	mode, the icon appears with a blue border. When unselected and in manual mode, the icon appears without the blue border. NOTE The original automatic report can be restored after making manually changes in manual mode by re-selecting auto report mode.
	Report Options: Click to open **Report Options** dialog. Refer to Report Options 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, file name, sample name, sample notes, specimen No, specimen name, operator, run time, cytometer, software, and experiment template. In addition, specimen reports contain specimen information text type. 

	Insert Plot: Click the icon to list the plots for the sample, including dot plots, density plots, histogram plots, contour plots, and cell cycle plots. Select the plot to insert. The plot is inserted with the statistics as shown below. 
	Insert Sample Statistics: Inserts a table of gate statistics for a sample. In a sample report, click the button to insert a gate statistics table for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a gate statistics table for the selected sample.
	Insert Compensation: Inserts a Spillover Matrix. In an experiment file report, click this button and select a compensation sample or sample from the drop-down menu to insert the associated Spillover Matrix. In a sample report, click the button to insert the Spillover Matrix for the selected sample. In a specimen report, click the button and select a sample from the dropdown menu to insert the Spillover Matrix for the selected sample. The Spillover Matrix cannot be edited within the report. If the spillover matrix is modified in the main interface, the matrix in the report window will be updated automatically.
	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.
	Statistical Table: In an experiment file report, click the button and select **Report Table** to create and insert a report table into the report, or click **Linked Table** and select the table that is already created in **Experiment Manager** to insert a table linked with the original table. The inserted linked table will be automatically updated when the original table is modified. In a sample or specimen report, click this button to insert the table.
	Plate Information: Inserts plate information for selected sample, including plate ID, barcode of the plate (if scanned), and description. It is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.

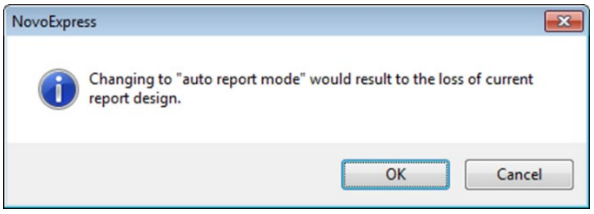
	
	File Audit Trail： Click to insert **File Audit Trail** information. This function is only available in the experiment file report.
	Insert Shape: Click to insert a horizontal line, vertical line, or rectangle.
	Insert Signature Information: Click to insert signature information.
	Insert Picture: Click open the **Open** window, select a picture to insert. The picture will be resized to an appropriate size with the original aspect ratio and inserted in the report.
	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.



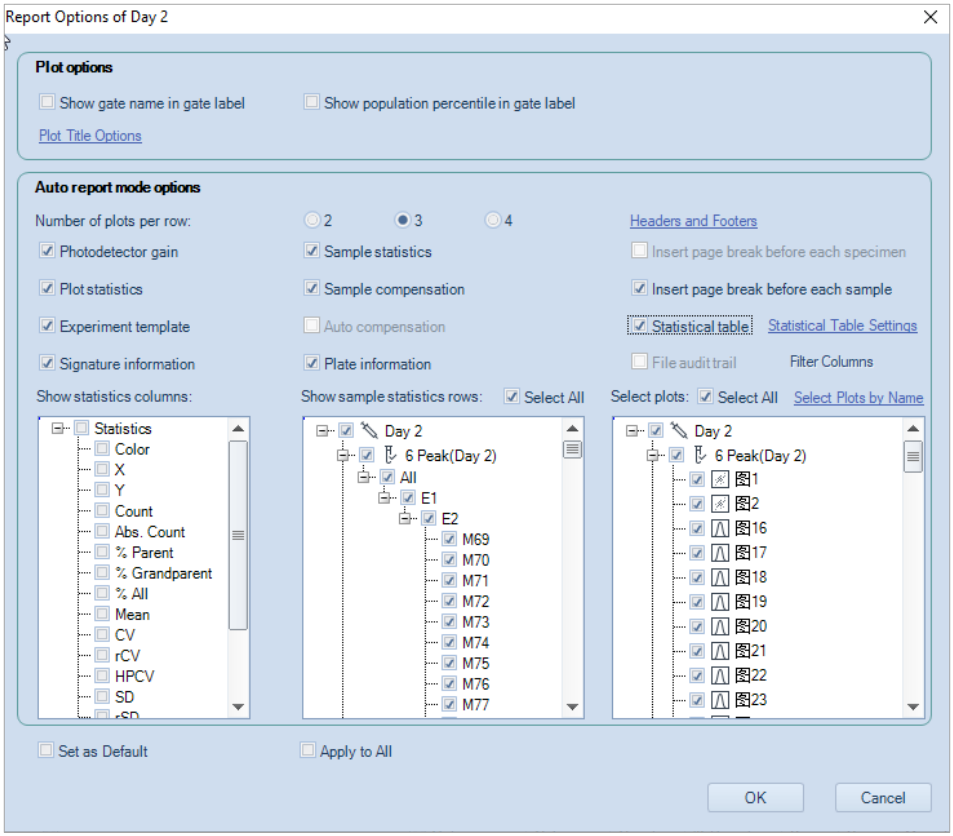
To switch the mode from automated to manual, click on **Auto Report Mode** button in the toolbar . The user can now make modifications to the report.

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**:

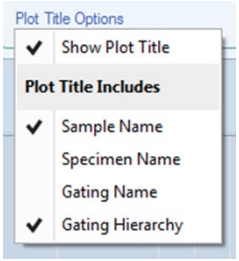
  If selected, gate name is displayed in gate label on the plot.

- **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.
- **Photodetector Gain**:

  If selected, it shows the photodetector gain setting.
- **Plot Statistics**:

  If selected, it shows gate statistics of plot.
- **Experiment template**:

  If selected, it shows experiment template information.
- **Signature Information**:

  If selected, it shows the e-signature information.

- **Sample Statistics**:

  If selected, it shows gate statistics of sample.
- **Sample Compensation**:

  If selected, it shows compensation matrix.
- **Auto Compensation**:

  If selected, it shows Spillover Matrix information.
- **Plate Information**:

  If selected, it shows the plate information. This option is available only when NovoSampler, NovoSampler Pro or NovoSampler Q is connected.
- **Headers and Footers**:

  If clicked, user can customize the header and footer in prompted window.
- **Insert Page Break Before Each Specimen**:

  Only available for experiment file report. If selected, a page break will be inserted before each specimen.
- **Insert Page Break Before Each Sample**:

  Only available for specimen report. If selected, a page break will be inserted before each sample.
- **Statistical Table**:

  If selected, it shows statistical table.
- **Statistical Table Settings**:

  if clicked, user can edit the statistical table in prompted window.
- **File Audit Trail**:

  If selected, it shows the File Audit Trail information.
- **Filter Columns**:

  If clicked, user can select the designated File Audit Trail items to display.
- **Show Statistics Columns**:

  Selects statistical items to display.
- **Show Sample Statistics Rows**:

  Selects sample statistical items to display.
- **Select Plots**:

  Selects plots to display on report.
- **Select All**:

Selects all associated items to display on report.

- **Select Plots by Name**:

  Selects plots to display on report based on its name. It is not available for sample report.

- **Set as Default**:

  Sets above settings as default setting for new reports.

- **Apply to All**:

  Applies above settings to all report in the experiment.

Report Editor

In the manual mode, the user is able to freely edit the report. Options include adding, removing, and editing objects in the report

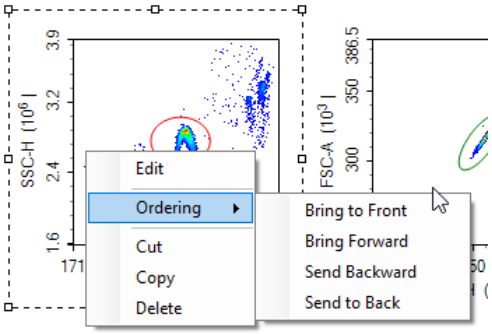
Add Report Objects

Objects can be added to the report through the toolbar, Workspace, and **Experiment Manager** panel. To add objects using the toolbar, use the insert functions described in Report Interface. From both the Workspace and **Experiment Manager** panel, objects corresponding to the sample can be dragged and dropped into the report.

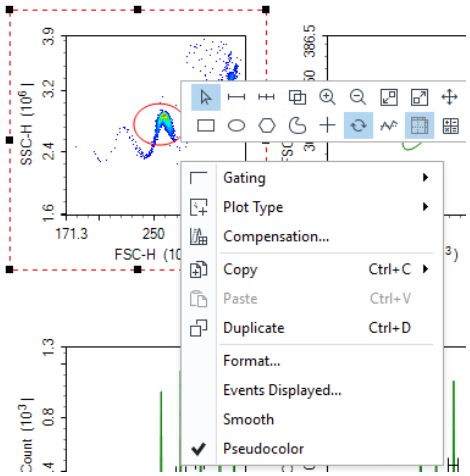
Select Report Objects

Click on an object to select it in the report. An object can have one of two selected states. After clicking on an object, the object will be highlighted. In this state, the object is bordered by a black dashed line with white control points. If the object is then double-clicked, it is in the edit state, and the object is bordered by a red dashed line with black control points. Different operations can be performed on the object depending on the selected state. The two states are shown below.

This is the object after being selected:



This is the object selecting and in the editing state:



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.

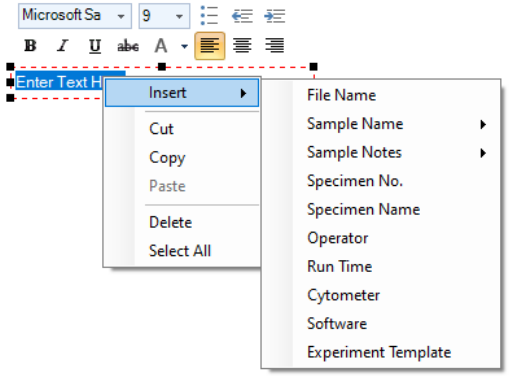
When multiple items are selected, the first item is bordered by a black dashed line with white control points and additional items are bordered by black dashed lines with black control points.

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

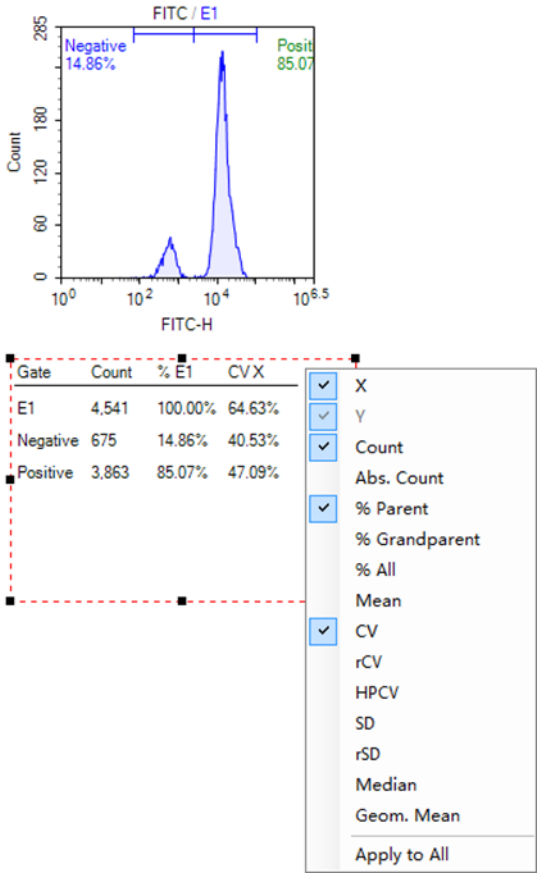
Double-click on the textbox or right-click on the textbox and select **Edit** to enter editing mode. In this mode, text formatting tools will appear. Right-click on the textbox and select **Insert** to insert sample information including sample name, sample notes, specimen No, specimen name, operator, run time, cytometer, software and experiment template 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

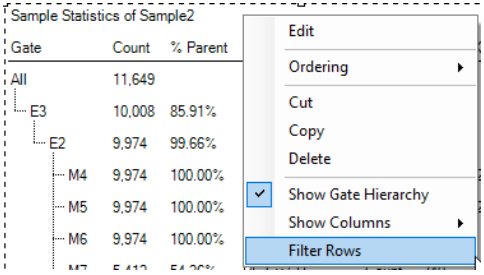
Double-click on the plot or right-click on the plot and select **Edit** to enter editing mode. Plots can be edited in the report by right-clicking in the plot to access the plot tools. Modifications made to the plot will also be updated to the plot in the main interface. In addition, if the plots are 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.

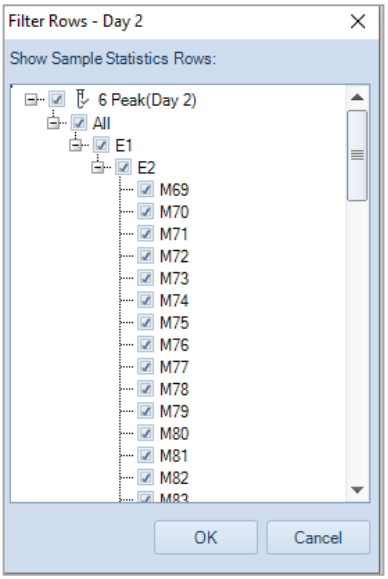


Filter Statistics

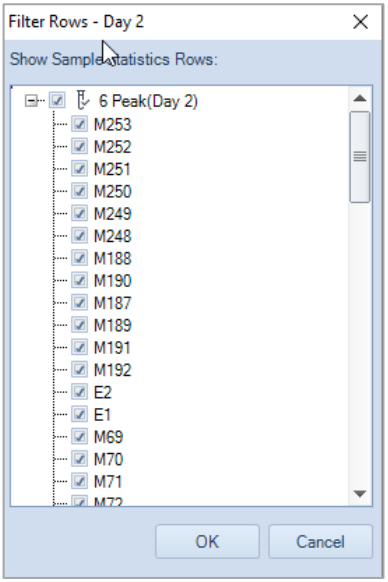
User can right click the statistics box of the report, click **Filter Rows** to open the **Filter Rows** window. Check to select the gate(s) to display associated sample statistical information in the report and click **OK** to confirm.



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.

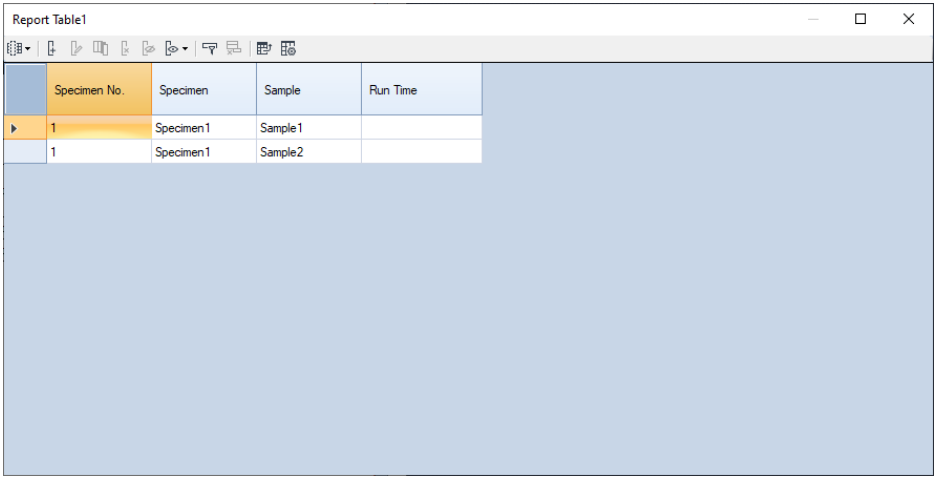


When the statistics box does not show the gate hierarchy, the **Filter Rows** window does not display the gate hierarchy either.



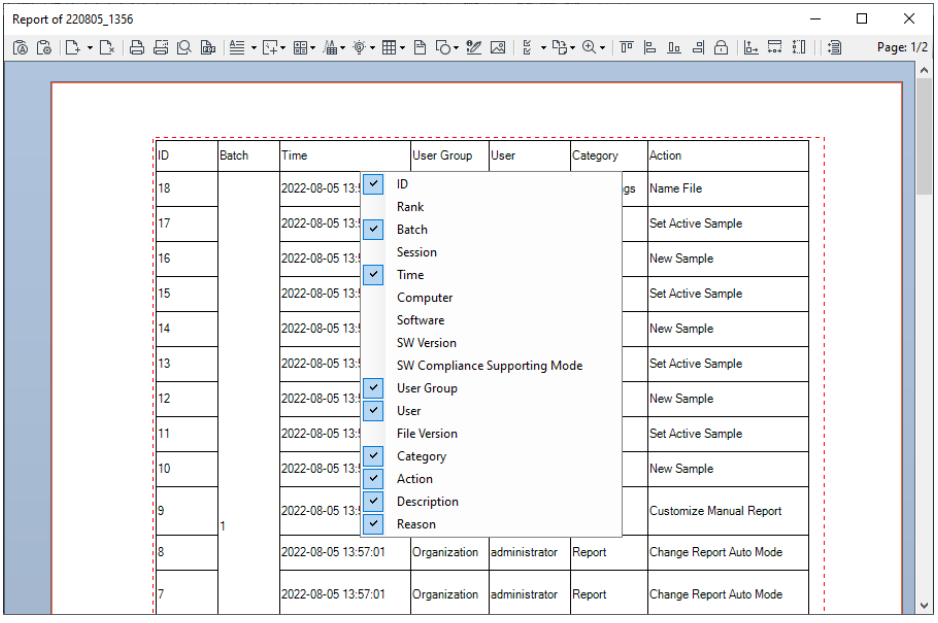
Edit Statistical Table

User can double click a statistical table in the report and edit the statistical table. Please refer to Statistical Tables in this guide for more details.



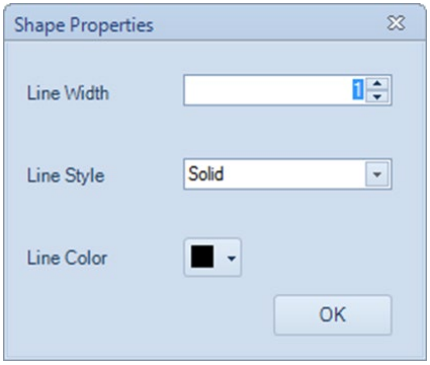
Filter File Audit Trail Table

User can double click and right click the File Audit Trail table to select the designated column to show the corresponding information.



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.

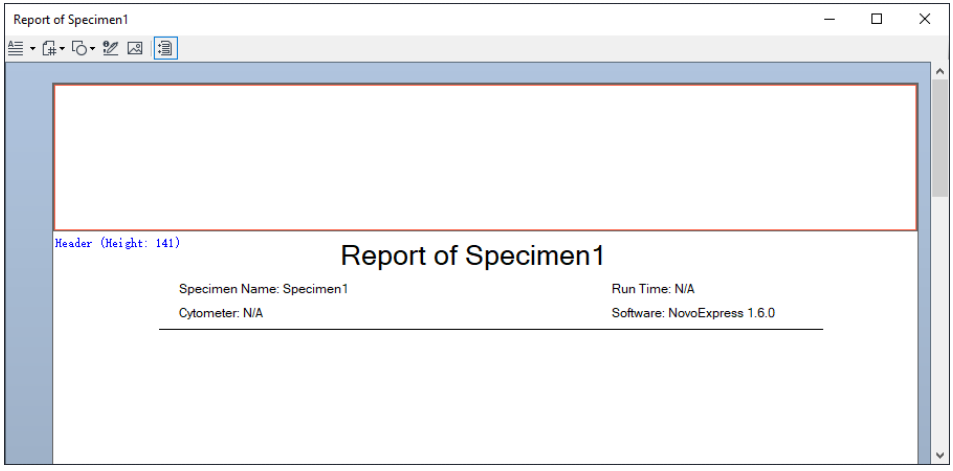
Insert or Delete Pages

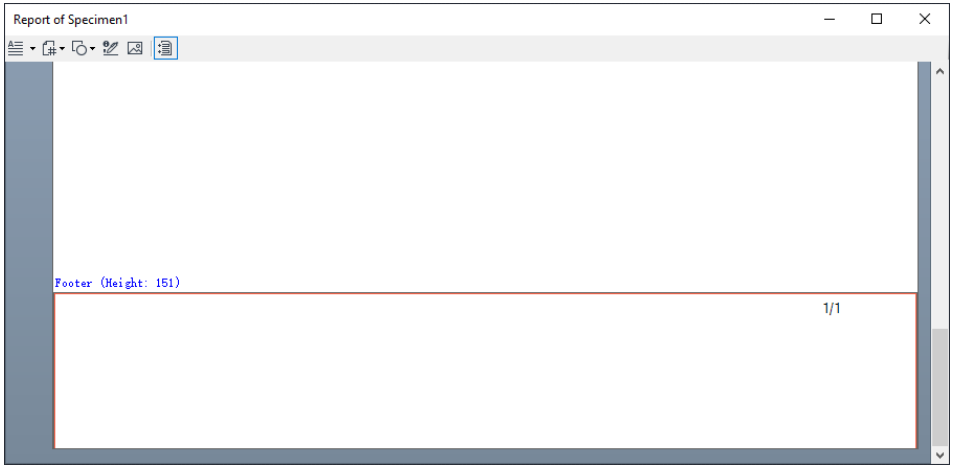
To insert or delete pages, use the **Insert Page** and **Delete Page** button from the toolbar. For more information, Refer to Report Interface in this guide.

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 Signature Information: Click to insert the e-signature information.
	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.

- 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.

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.

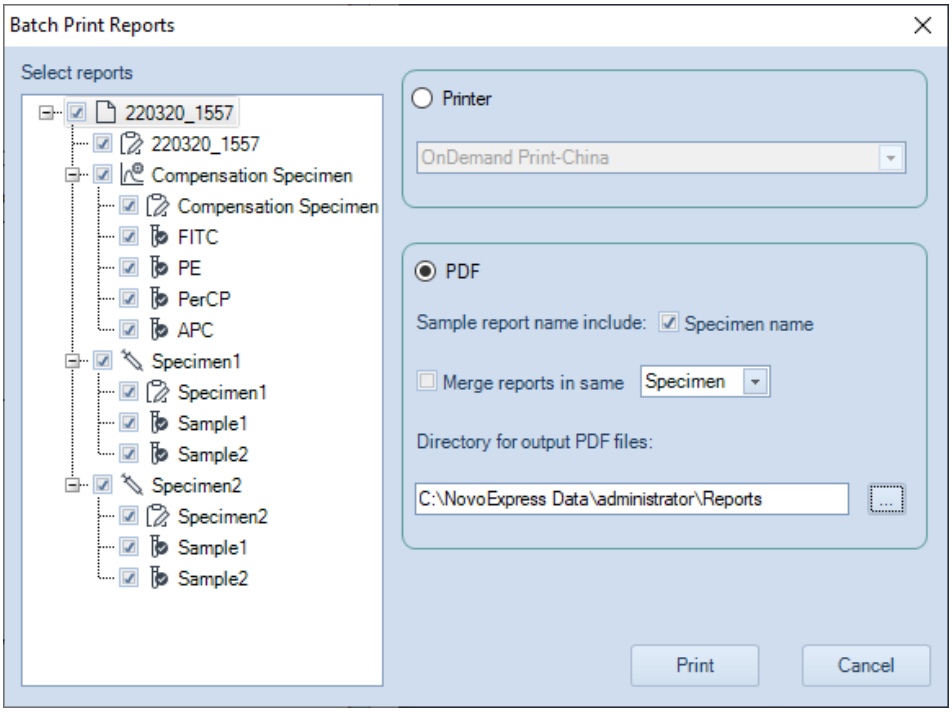
- To Convert to PDF:

  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.

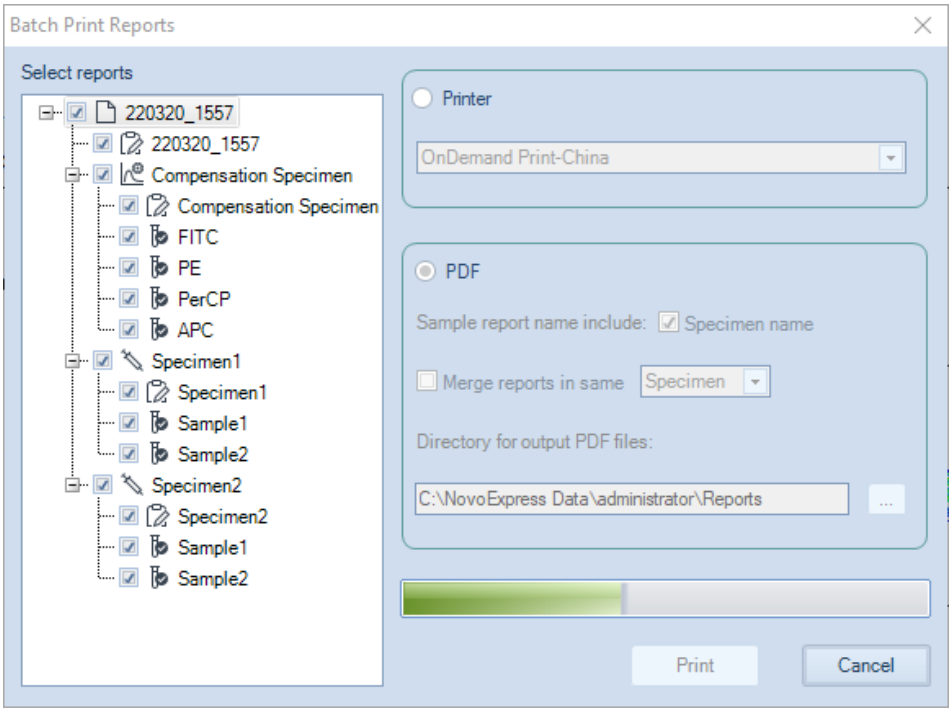
If printing, select a printer and click **Print**.

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**. User can also select to merge reports in the same specimen, group or experiment. 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 software **QC Test** function automatically conducts the QC procedure using Agilent flow cytometer QC particles. Click **Instrument** > **QC Test** from the NovoExpress software to initialize the QC test. The **QC Test Report** tracks the QC test results over time and can be reviewed through **Instrument** > **QC Test Report** from the NovoExpress software.

Agilent Flow Cytometer QC particles are polystyrene microspheres with uniform size (average diameter of 3 µm). The QC particles contain 5 kinds of microspheres with differing fluorescence intensities and one blank microsphere. These fluorescent microspheres are internally stained with fluorescent dye, which can be excited by all the lasers with known MESF (Molecules of Equivalent Soluble Fluorochrome) values for each fluorescent peak, from which the linearity can be calculated.

There are three test results:

- **Pass**

  Indicating that all the performance parameters meet the requirements.

- **Acceptable**

  Indicating that the performance parameters are not optimal compared to the manufacturing QC criteria. However, continued use of the instrument is still allowed and the experiment results should not be impacted.

- **Fail**

  Indicating that the performance parameters do not meet the requirements. A label in red will show on report to indicate the reason of failure. Please contact your local Agilent Technologies representative when the QC test fails.

The QC Test procedure generally includes three steps:

- Prepare QC particles sample
- Run QC test
- View QC reports and Levey-Jennings plot

NOTE

The software prompted windows displayed in this chapter is for illustration purpose only. User may observe different windows depending on the Agilent flow cytometer models.

Prepare QC Particle Sample

1. Label a 12 × 75 mm tube for the QC test. Add 1 mL dilution buffer (0.8 mL PBS and 0.2 mL NovoRinse solution) into the tube.
2. Take one bottle of QC particles from storage. Invert the bottle to mix the particles thoroughly.
3. Add two drops of QC Particles into the test tube.
4. Vortex the test tube to mix the QC particles thoroughly.
5. Place the tube onto the instrument and start the QC test. If NovoSampler or NovoSampler Pro or NovoSampler Q is used, transfer 150 µL of the QC particles sample from the test tube to the A1 well of a 96-well plate and run the QC Test immediately. If the prepared QC particle sample is not intended to be run immediately, store the sample in a 2-6 ℃ refrigerator away from light. The prepared QC particle sample should be used within four hours after preparation.

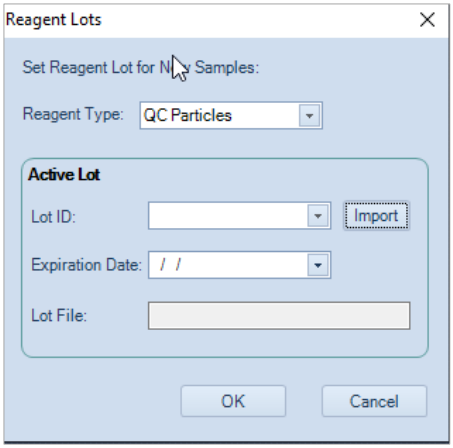
NOTE

Make sure the QC particle sample is prepared properly following the above procedure. Properly prepared QC particle sample would give enough QC particle counts (~10,000 QC particles) during the QC Test.

Run QC Test

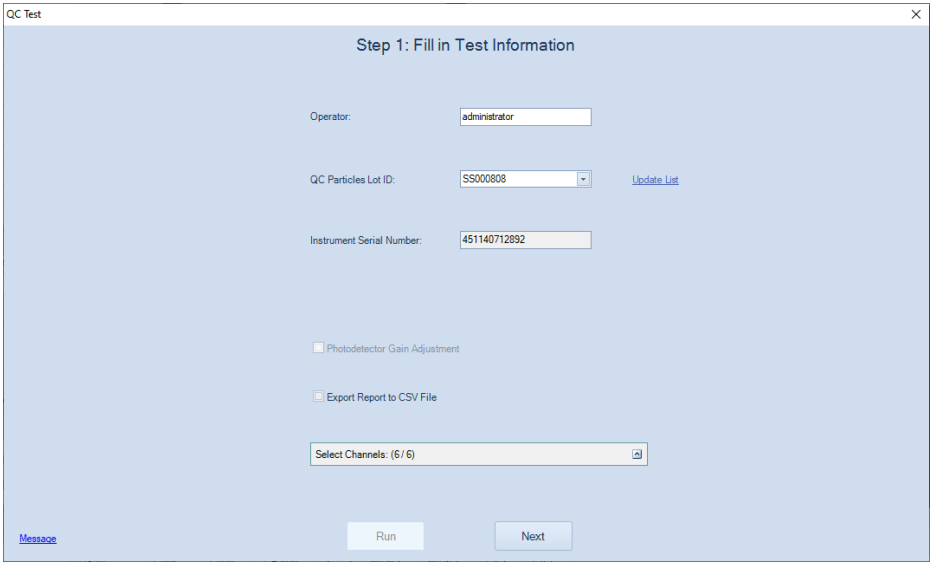
1. Import the Agilent Flow Cytometer QC Particles Lot file.

   Download the Lot File for the specific batch of QC Particles from https://www.agilent.com/chem/novocyte-qc-lot-files. Launch and log into NovoExpress 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.

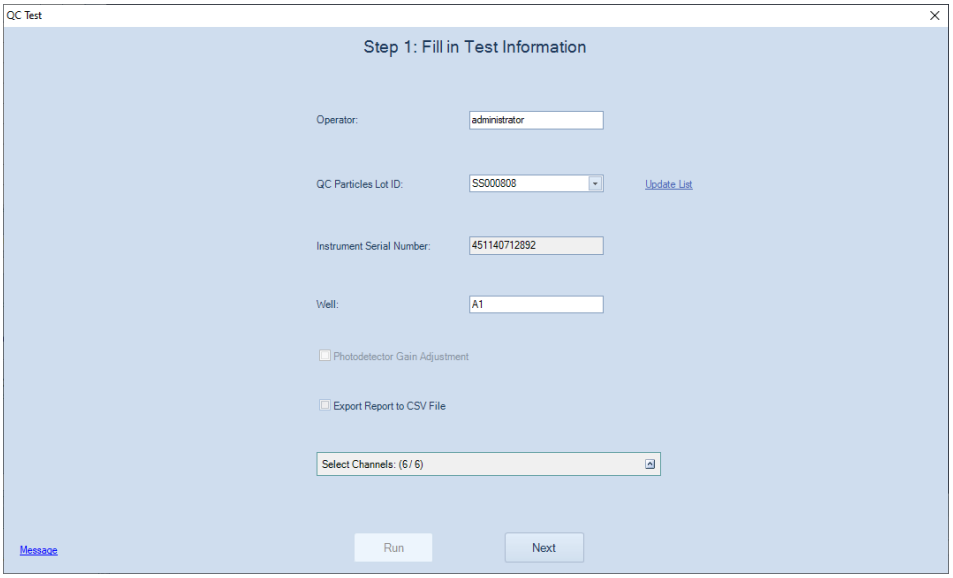


2 Ensure the instrument is in **Ready** status. If NovoSampler (or NovoSampler Pro or NovoSampler Q) is used, open the cover of NovoSampler (or NovoSampler Pro or NovoSampler Q) and place the sample plate on the tray. Ensure the plate is fully seated with the correct orientation onto the shaker.

3 In the NovoExpress Software, click the **QC Test** button in the **Instrument** tab of the **Menu Bar** to open the **QC Test** window. The window is shown below.



NovoExpress QC Test Window when NovoSampler (or NovoSampler Pro or NovoSampler Q) is not connected



NovoExpress QC Test Window when NovoSampler (or NovoSampler Pro or NovoSampler Q) is connected

In the window, enter the name of the **Operator** and select the **QC particles Lot ID** from the drop-down list. If the correct QC particles Lot ID is not listed, please refer to the first step to download and save the QC particles Lot File. Check **Export Report to CSV File** if user want to export report as a CSV file after QC test is completed. If checked, the exported CSV file (in the format of YYMMDD_hhmmss.csv) will be automatically saved in **C:\Program Files (x86)\NovoExpress\QC \QC Report Files** by default. Select the channels to be included in the QC Test by clicking **Select Channels** and selecting fluorescence channels from the drop-down list. Select the **Well ID** (if NovoSampler or NovoSampler Pro or NovoSampler Q is used). Click Next to proceed.

NOTE

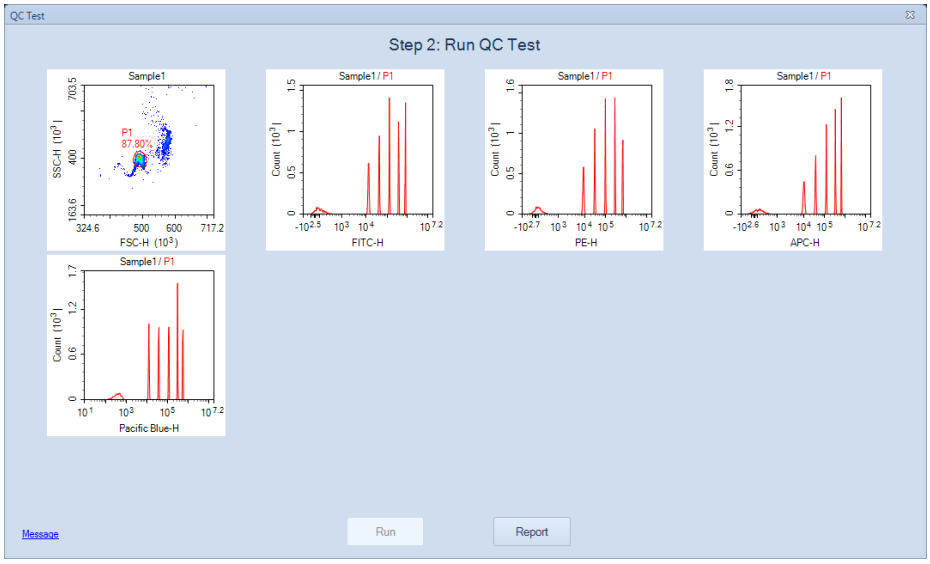
The default name of the exported CSV file is in the format of QC test date and time. For example, if the QC test is performed at 10:30:40 am on January 18, 2019, the default name of the exported CSV file will be 190118_103040.csv.

NOTE

If user does not select specific channels to be tested, software will automatically test all available channels by default.

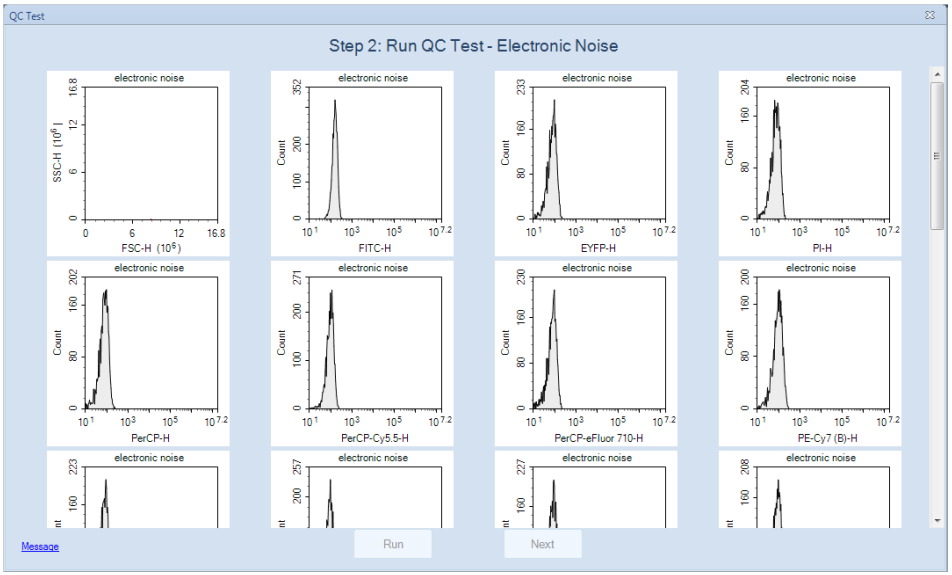
4 Click **Run** to begin the test. The instrument will begin collecting events and the results will be displayed in real-time on the **QC Test** window.

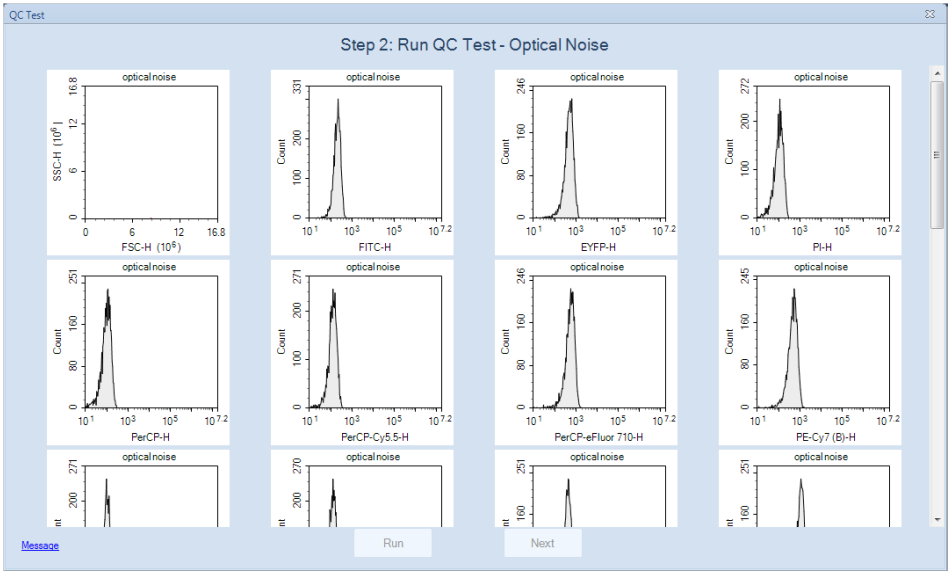
QC Test


QC Test
Step 2: Run QC Test
Sample1
P1
87.80%
SSC-H (10³)
FSC-H (10³)
Sample1 / P1
Count (10³)
FITC-H
PE-H
APC-H
Pacific Blue-H
Message
Run
Report

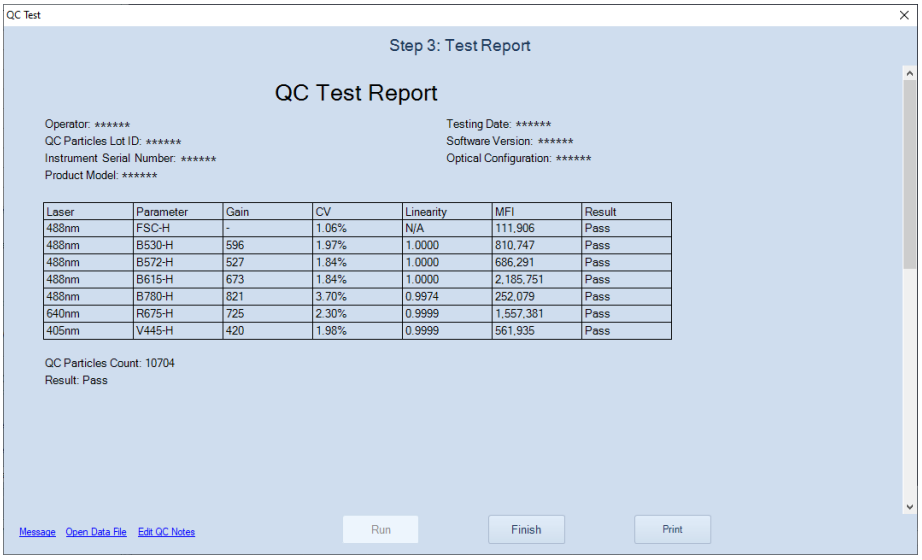
NOTE

When NovoCyte Quanteon, Advanteon or Penteon is used, the software will collect electronic and optical noise (see figure below) before collecting the events.

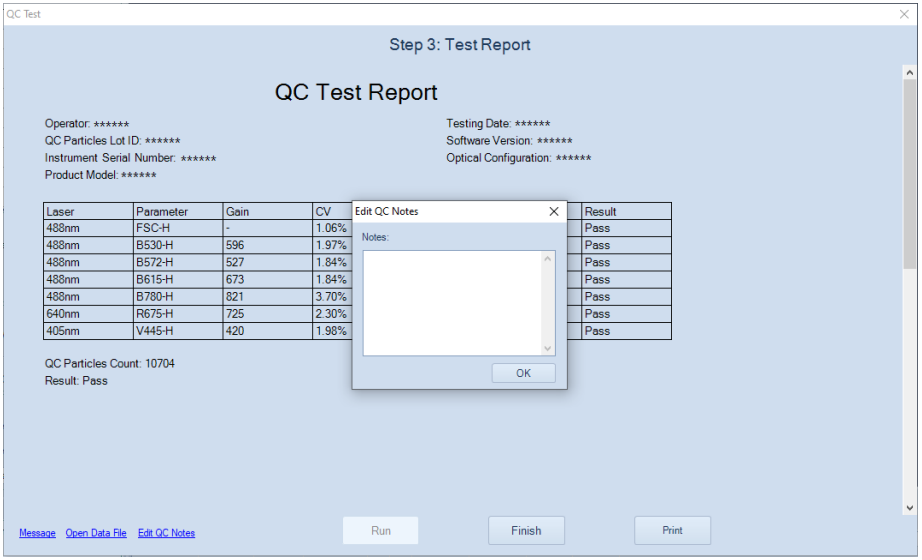




5 After data collection is complete, click the **Report** button to view the test results as shown below.



User can click **Edit QC Notes** from lower left side of this window, enter the notes into the prompted window and click **OK**. The notes will be automatically added to the end of the QC test report. This function is available only when user log in the software with permission of **Edit QC Notes.**



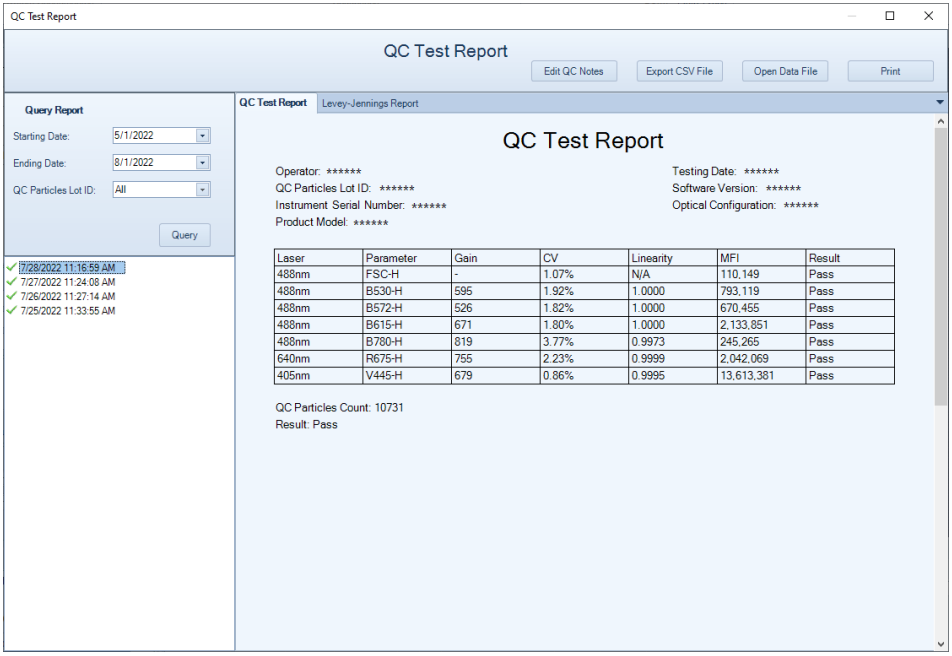
User can also click **Message** to view the message or click **Open Data File** to open the QC data file or click **Print** to print the test report. To complete the QC test, click **Finish** to close the **QC Test Report** window.

NOTE

To view the history of the resulting QC data, click **QC Test Report** from the **Instrument** tab of the **Menu Bar** (Refer to View QC Test Report in this guide). Highlight the date to be viewed and select the **QC Test Report** tab.

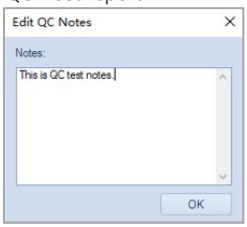
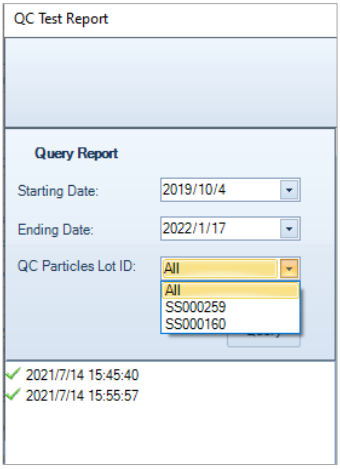
View QC Test Report

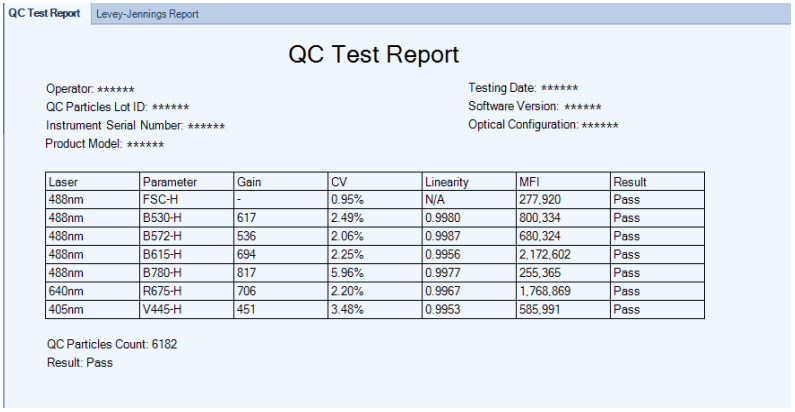
The QC test report function stores previous QC test results and provides a data analysis feature to track instrument performance changes over a period time. To open the **QC Test Report** window, click **QC Test Report** from the **Instrument** tab of the **Menu Bar**. The window is shown below.



The **QC Test Report** window contains the following sections.

Interface	Description
Title Area	Displays the report name and **Edit QC Notes, Export CSV File**, **Open Data File** and **Print** button. • **Edit QC Notes:** Allows user to edit the QC Notes for selected QC report. This function is available only when user log in the software with permission of **Edit**

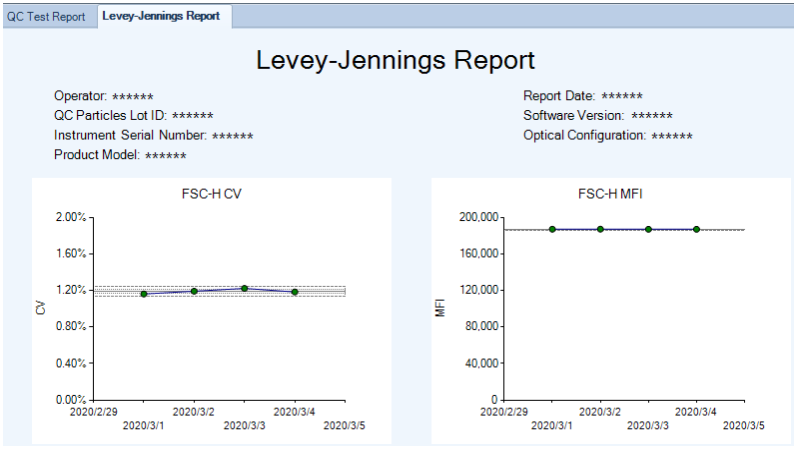
	QC Notes. When clicked, following window will be prompted. User can edit the notes in this window and click **OK** to display the updated notes in the end of the QC Test report.  • **Export CSV File:** Export the QC result as a csv file. • **Open Data File:** Open the QC data file of the selected report. • **Print:** Click to print QC test reports or Levey-Jennings reports.
Query Area	Query a time interval and QC Particles Lot ID for QC test reports. If more than one Lot ID was used on connected NovoCyte workstation, **QC Particle Lot ID** will be available, and user can select the designated Lot ID from the associated drop-down list to display the filtered result. By default, **All** is selected and all results will be displayed. 
QC Test Report	Displays the QC test report for a selected report from the query results.



Laser	Parameter	Gain	CV	Linearity	MFI	Result
488nm	FSC-H	-	0.95%	N/A	277,920	Pass
488nm	B530-H	617	2.49%	0.9980	800,334	Pass
488nm	B572-H	536	2.06%	0.9987	680,324	Pass
488nm	B615-H	694	2.25%	0.9956	2,172,602	Pass
488nm	B780-H	817	5.96%	0.9977	255,365	Pass
640nm	R675-H	706	2.20%	0.9967	1,768,869	Pass
405nm	V445-H	451	3.48%	0.9953	585,991	Pass

Levey-Jennings Report

Displays the QC test data over time to observe trends in instrument performance. As shown:



10 Troubleshoot

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

Troubleshoot Instrument

The following table lists possible causes for warning and error prompts from the status bar when connecting with Agilent flow cytometer instrument.

Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0001	**Collision of SIP**	The movement of the SIP is blocked by some obstacles.	Locate and clear the obstacles. The instrument will automatically start error handling and move the SIP to the home position.
		Incorrect plate selected in plate manager.	Select the correct plate in the plate manager window.
		Incorrect positioning of plate in NovoSampler, NovoSampler Pro or NovoSampler Q.	Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps.
		NovoSampler (or NovoSampler Pro or NovoSampler Q) is not calibrated.	Re-calibrate NovoSampler (or NovoSampler Pro or NovoSampler Q).
		Dirty SIP or SIP Cleaning apparatus.	Clean the SIP or SIP cleaning apparatus following the procedures described in Preventative Maintenance in associated instrument operator's guide.
0x0002	**Running out of NovoFlow**	NovoFlow solution is not sufficient to continue to run any samples.	Refill the NovoFlow container following the procedures described in Add Instrument Reagents in

			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 (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 (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 (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 (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(or NovoSampler Pro or NovoSampler Q) and instrument is not securely connected.	Reconnect the cable between the NovoSampler (or NovoSampler Pro or NovoSampler Q) and instrument.
		NovoSampler (or NovoSampler Pro or NovoSampler Q) is not communicating with instrument.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x0010	**NovoSampler has not been calibrated**	NovoSampler (or NovoSampler Pro or NovoSampler Q) is newly installed or re-connected.	Follow the prompted instructions to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q).
0x0011	**NovoSampler calibration failed……**	NovoSampler (or NovoSampler Pro or NovoSampler Q) is not installed properly.	Re-install and calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q) following the procedures described in Installation in associated operator's guide. Ensure the NovoSampler is seated flat on the stage and tightly close to the instrument.
		The NovoSampler (or NovoSampler Pro or NovoSampler Q) cover is opened during calibration.	Close the NovoSampler (or NovoSampler Pro or NovoSampler Q) cover and redo the calibration.
		The optocoupler or motor of NovoSampler Q is not working properly.	• Restart the instrument and redo the calibration. • Contact Agilent technical support if the error persists.
0x0012		The movement of the orbital shaker is blocked.	• Check the path of the orbital shaker to make sure there are no objects blocking the

	The movement of plate is out of range		movement. Clear the block if there is anything. • Re-calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q).
		NovoSampler (or NovoSampler Pro or NovoSampler Q) is not installed properly.	Re-install and calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q) following the procedures described in Installation in associated operator's guide.
0x0013	**Cover of NovoSampler is opened during moving plate**	The cover of NovoSampler (or NovoSampler Pro or NovoSampler Q) is open during moving plate. Or the cover is not closed well.	Close the cover. NovoSampler (or NovoSampler Pro or NovoSampler Q) will automatically reset and be ready for operation.
0x0014	**Pressure is out of limit**	Waste container is not correctly connected to the instrument. Or waste tubings are bending and clogged.	Follow the prompted instructions from NovoExpress to clear the error. If NovoCyte Flow Cytometer is connected, check the quick coupling connectors to ensure the waste container is correctly connected to the instrument. Ensure the waste tubings are not bending and clogged.
		Sheath in-line filter is clogged.	Follow the prompted instructions from NovoExpress to clear the error. If NovoCyte Flow Cytometer is connected, replace the sheath in-line filter following the procedures described in Replacing Fluidic System Consumables in associated instrument operator's guide.
		Sample injection probe or flow cell is clogged.	Follow the prompted instructions from NovoExpress to clear the error.
0x0015	**NovoSampler firmware error**	NovoSampler (or NovoSampler Pro or NovoSampler Q) firmware is not working properly.	Re-install or upgrade the NovoSampler (or NovoSampler Pro or NovoSampler Q) firmware.
0x0016 0x0017 0x0018 0x400B 0X400F	**…… laser does not emit**	Specified laser is not connected properly or laser is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x0019 0x001A 0x001B 0x400C	**…… 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.

0x4010			
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.
0x001E	**The SIP module needs to be upgraded to support NovoSampler Pro**	SIP module is not compatible with connected NovoSampler Pro.	• 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.
0x0022	**Resetting NovoSampler Pro to zero position failed**	NovoSampler Pro is not working properly.	• Click **Instrument** → **NovoSampler Pro**→ **Reset** in NovoExpress software to reset NovoSampler Pro. • Restart the NovoSampler Pro.
0x0023	**Resetting NovoSampler to home position failed**	NovoSampler (or NovoSampler Pro or NovoSampler Q) is not working properly.	• Click **Instrument** → **NovoSampler (or NovoSampler Pro or NovoSampler Q)** → **Reset** in NovoExpress software to reset NovoSampler (or NovoSampler Pro or NovoSampler Q). • Restart the NovoSampler (or NovoSampler Pro or NovoSampler Q).
0x0100	**Instrument cover opened**	Instrument cover is opened or not tightly closed.	Close the instrument cover.

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 (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 (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 (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 (or Cart) following the procedures described in associated instrument operator's guide. • Contact Agilent technical support if the error persists.
0x0105	**Cover of NovoSampler is opened**	Cover of NovoSampler (or NovoSampler Pro or	Close the cover.

		NovoSampler Q) is opened. Or the cover is not closed well.	
0x0106	**NovoSampler is disconnected when powered up**	The NovoSampler (or NovoSampler Pro or NovoSampler Q) is disconnected when powered up	• Shut down instrument. • Reconnect the cable of the NovoSampler (or NovoSampler Pro or NovoSampler Q) to instrument. • Turn on instrument and follow the prompts to calibrate the NovoSampler (or NovoSampler Pro or NovoSampler Q). • Contact Agilent technical support if the error persists.
0x0109	**Fluidics Station is not connected**	Cable between the fluidics station and the instrument is not properly connected.	• Power down the instrument. • Reconnect the cable between the fluidics station and the instrument. • Power up the instrument. • Contact Agilent technical support if the error persists.
0x010A 0x010B 0x010C 0x010D	**… liquid level sensor failure**	Specified liquid level sensor in the fluidics station (or Cart) is not working properly.	• Reconnect the fluidics station (or Cart) cable. • Restart the instrument. • Contact Agilent technical support if the error persists.
0x010F	**Sheath filter is clogged. Please replace the sheath filter and run the Priming procedure**	Sheath in-line filter is clogged.	Replace the sheath in-line filter following the procedures described in Replacing Fluidics System Consumables in associated instrument operator's guide.
0x0110	**Recovering collision error**	Plate stops at an incorrect position.	No action is needed. Instrument will automatically recover the error.
0x0111	**Orbital shaker homing position reset failure**	Orbital shaker of NovoSampler (or NovoSampler Pro or NovoSampler Q) is not working properly.	No action is needed. The NovoSampler (or NovoSampler Pro or NovoSampler Q) can be used normally.
0x0112	**0.05 µm sheath fluid ultrafiltration (UF) filter is clogged**	0.05 µm sheath fluid ultrafiltration (UF) filter is clogged.	Replace the 0.05 µm sheath fluid ultrafiltration (UF) filter following the procedures described in Replacing Fluidics System Consumables in associated instrument operator's guide.
0x1001	**Fluidics procedure run error**	Fluidics procedure file is damaged.	• Restart the instrument. • Contact Agilent technical support if the error persists.

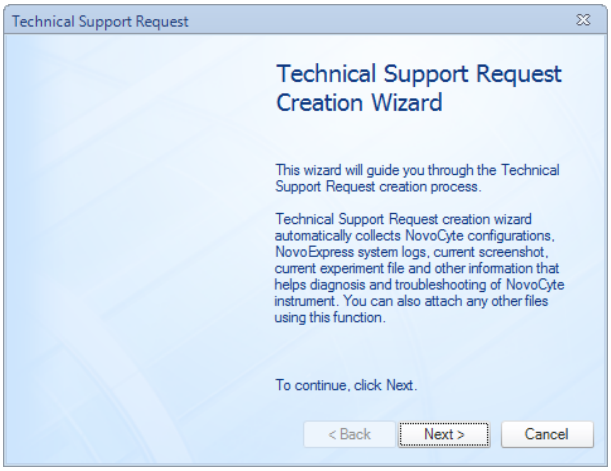
0x300A	**Calibrating DA coefficient failed**	AD board is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x3100	**Failed to read optical filter information**	Optical filter sensor is not working properly.	• Restart NovoExpress software. • Reinstall the optical filter. • Replace an optical filter. • Restart the instrument. • Contact Agilent technical support if the error persists.
0x3101	**Failed to read dichroic mirror information**	Dichroic mirror sensor is not working properly.	• Restart NovoExpress software. • Reinstall the dichroic mirror. • Replace a dichroic mirror. • Restart the instrument. • Contact Agilent technical support if the error persists.
0x3102	**Failed to read photodetector information**	Photodetector sensor is not working properly.	• Restart the instrument. • Contact Agilent technical support if the error persists.
0x3103	**The optical filter information has been changed**	The optical filter has been replaced.	Follow the software prompted instructions to perform the appropriate operation.
0x3104	**The dichroic mirror information has been changed**	The dichroic mirror has been replaced.	Follow the software prompted instructions to perform the appropriate operation.
0x3105	**The photodetector information has been changed**	The photodetector has been replaced.	Follow the software prompted instructions to perform the appropriate operation.
0x6100	**Communication error between NovoSampler and the orbital shaker**	The orbital shaker is not communicating to NovoSampler Pro (or NovoSampler Q) is not working properly.	• Restart NovoSampler Pro (or NovoSampler Q) through NovoExpress. • Restart the instrument. • Contact Agilent technical support if the error persists.
0x7000	**Fluidics cart firmware error**	NovoCyte Fluidics Cart firmware is not working properly.	• 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.
0xA003	**The temperature of the photodetectors is abnormal**	Ambient temperature outside the operating range.	• Ensure to have the instrument working at normal ambient temperatures. • Restart the instrument. • Contact Agilent technical support if the error persists.
		More than four temperature sensors of the photodetector module are not working properly.	• 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 photodetectors is abnormal**	One to three temperature sensors of the photodetector module may not work properly.	No action is needed. The instrument can use the temperature from other sensors and ensure the data is processed normally.
	Communication error (code: xx, xx). Please restart Instrument and NovoExpress.	USB cable between the instrument and workstation is not connected properly.	• Reconnect the USB cable between the instrument and the workstation. • Restart the instrument, workstation and NovoExpress software. • Contact Agilent technical support if the error persists.

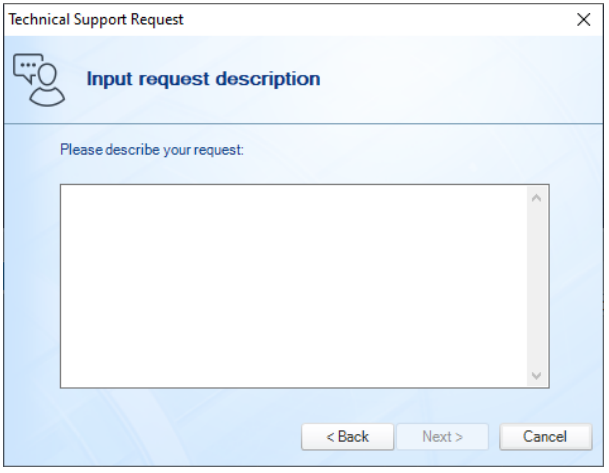
Technical Support Request

In case you need to contact Agilent technical supports, use the **Technical Support Request** from **Home** menu to create a request. **Technical Support Request Creation Wizard** automatically collects instrument configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of Agilent flow cytometer. You can also attach any other files using this function.

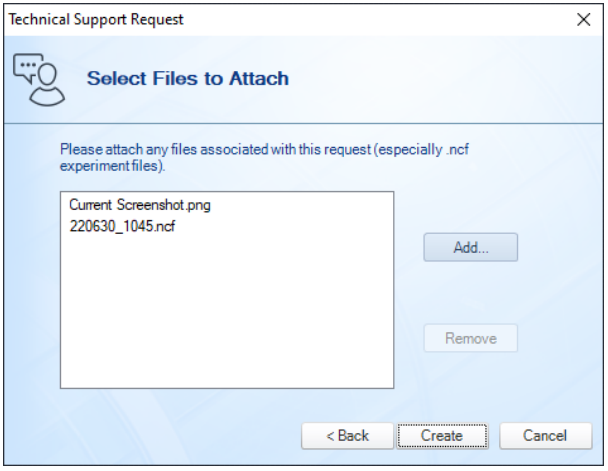
1 Click **Home** > **Technical Support Request** to open the **Technical Support Request Creation Wizard**.



2 Click **Next** button and input request description.

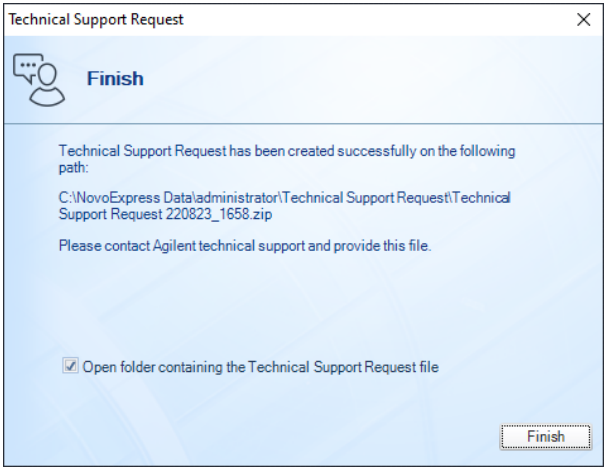


3 Click **Next** button and select the files to be attached. Any related files including experiment files (*. ncf) can be attached.



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/Version	Changed by	Description
10/30/2020		Initial release.
10/15/2021		Second Edition
08/31/2022		Third Edition
11/15/2022		Edition 3.1: Update software snapshot
07/07/2023		Edition 3.2: Update " Register NovoExpress Compliance License", "Access Control", "Setting"

Appendix A: Permission List

Permission			Administrator	Developer	Analyst
Instrument Operation	Run Experiment		X	X	X
	Overwrite Sample Events		X	X	
	Append Sample Events		X	X	
	Modify Instrument Configuration		X	X	
	Customize Optical Configuration				
	QC Test	Run QC Test	X	X	X
		Select Channels for QC Test	X	X	
		View QC Test Report	X	X	X
		Print QC Test Report	X	X	X
		Edit QC Notes	X	X	X
	Shut Down instrument		X	X	X
	Schedule Power On & Off		X	X	X
	Calibrate Fluidics Station/Cart		X	X	
	Replace Fluidic System Consumables		X	X	
	Fluidics Maintenance	Debubble	X	X	X
		Cleaning	X	X	X
		Rinse	X	X	X
		Extensive Rinse (Applicable to NovoCyte)	X	X	X
		Priming	X	X	X
		Unclog	X	X	X
		Backflush (Applicable to NovoCyte)	X	X	X
		Fluidics Maintenance Sequences	X	X	X
		Purge			
		Decontaminate			
	NovoSampler	Power Down/Up NovoSampler	X	X	
		Manual Mix	X	X	X
		Calibrate NovoSampler	X	X	
		Add Plate Type	X	X	

		Remove Plate Type	X	X	
		Edit Plate Type	X	X	
		Reset NovoSampler	X	X	X
		Calibrate Plate	X	X	
Experiment Control	Cytometer Setting	Paste Cytometer Setting	X	X	
		Import Cytometer Setting	X	X	
		Adjust Photodetector Gain			
		Change Parameter Alias	X	X	
		Enable/Disable Parameters	X	X	
		Change Events Stop Condition	X	X	
		Change Time Stop Condition	X	X	
		Change Volume Stop Condition	X	X	
		Change Flow Rate	X	X	
		Change Threshold	X	X	
		Change Storage Gate	X	X	
		Enable/Disable Absolute Count	X	X	
		Enable/Disable Sample Recovery	X	X	
		Enable/Disable Rinse After Sampling	X	X	
	Plate Manager	Change Plate Run Mode	X	X	
		Change Plate Type	X	X	
		Change Plate Description	X	X	
		Modify Mix Settings	X	X	
		Modify Rinse Setting	X	X	
		Modify Mixing Parameters	X	X	
Experiment Manager	Experiment	New from Experiment File	X	X	X
		New from Experiment Template	X	X	X
		New from JSON File	X	X	X
	Group	New Empty Group	X	X	
		Rename Group	X	X	
		Move Group	X	X	
		Delete Group	X	X	

	Specimen	New Specimen	X	X	
		New Specimen from Template File	X	X	
		New Specimen from Existing Specimen	X	X	
		Rename Specimen	X	X	
		Paste Specimen	X	X	
		Move Specimen	X	X	
		Delete Specimen	X	X	
		Import Template File and apply to Specimen	X	X	
		Change Specimen No.	X	X	
	Sample	New Sample Without Template	X	X	
		New Sample from Template File	X	X	
		New Sample from Existing Sample	X	X	
		Delete Sample Without Events	X	X	
		Delete Sample with Events	X	X	
		Delete Sample Events	X	X	
		Rename Sample Without Events	X	X	
		Rename Sample with Events	X	X	
		Paste Sample Template	X	X	
		Paste Sample Analysis	X	X	
		Paste Events	X	X	
		Edit Sample Notes	X	X	X
		Move Sample	X	X	
		Merge Sample	X	X	
		Change Well ID of Sample Without Events	X	X	
		Change Well ID of Sample with Events	X	X	
		Change Plate ID of Sample Without Events	X	X	
		Change Plate ID of Sample with Events	X	X	
		Import Template File to Sample	X	X	
		Import Template File to Sample Analysis	X	X	
		Modify Absolute Count Setting	X	X	
	Plot	New Plot	X	X	

	Paste Plot	X	X
	Delete Plot	X	X
	Edit Overlays	X	X
	Change Events Displayed	X	X
	Change Plot Type	X	X
	Change Plot Name	X	X
	Change Plot Gating	X	X
	Change Axis Parameter	X	X
	Change Axis Scale	X	X
	Change Axis Range	X	X
	Enable/Disable Synchronize Plot Scale	X	X
	Modify Plot Format	X	X
	Enable/Disable Show Title on Plot	X	X
	Adjust Post Gain		
	Modify Cell Cycle Setting	X	X
	Modify Cell Proliferation Setting	X	X
Gate	New Gate	X	X
	Paste Gate	X	X
	Delete Gate	X	X
	Change Gate Name	X	X
	Change Gate Definition	X	X
	Change Gate Color	X	X
	Change Gate Color Precedence	X	X
	Enable/Disable Show Color	X	X
	Enable/Disable Show Name	X	X
	Enable/Disable Show Percentile	X	X
	Modify Gate Format	X	X
Compensation	New Auto Compensation	X	X
	Edit Auto Compensation	X	X
	Paste Compensation	X	X
	Import Template File to Compensation	X	X

		Modify Compensation	X	X	
		Enable/Disable Synchronize Compensation	X	X	
		Reset Plots of Compensation Sample	X	X	
	Statistical Table	New Table	X	X	
		Edit Table	X	X	
		Delete Table	X	X	
		New Table from Existing Table	X	X	
		New Table from Template	X	X	
		Rename Statistical Table	X	X	
	Heat Map	New Heat Map	X	X	
		Edit Heat Map	X	X	
		Delete Heat Map	X	X	
		New Heat Map from Existing Heat Map	X	X	
		Rename Heat Map	X	X	
	Keywords	Create Keywords	X	X	
		Modify Statistical Keywords	X	X	
		Read FCS Standard Keywords	X	X	
		Read User-Defined Keywords	X	X	
		Delete Keywords	X	X	
		Modify Keywords Library	X	X	
		Rename Keywords	X	X	
		Edit User-Defined Keywords Value	X	X	X
File Explorer (for Secure data folder)	New Folder		X	X	
	Rename File/Folder		X	X	
	Move File/Folder		X	X	
	Delete Folder		X	X	
	Delete Experiment File		X	X	
	Delete Experiment Template File		X	X	
	Delete Template File		X	X	
	Delete Statistical Table Template File		X	X	

Data Import/Export	Export	Export Sample/Specimen/Group/Experiment as Template File	X	X	
		Export Sample as FCS File	X	X	X
		Export Sample Events as CSV File	X	X	X
		Export Sample Plots	X	X	X
		Export GatingML File	X	X	X
		Export Statistical Table to CSV File	X	X	X
		Export Statistical Table Template	X	X	X
		Export Heat Map	X	X	X
		Export Heat Map to CSV File	X	X	X
		Export Keywords to CSV File	X	X	X
		Export QC Test Report to CSV File	X	X	X
		Export System Audit Trail	X	X	X
		Export File Audit Trail	X	X	X
		Export to LIS	X	X	X
	Import	Import FCS File	X	X	
Data Read/Write	Unlock File Locked by Others		X	X	
	Lock/Unlock Experiment File		X	X	
	Lock/Unlock Template File		X	X	
	Data Read	Open Experiment File	X	X	X
		Open Experiment Template	X	X	X
	Data Write	Save Experiment File	X	X	X
		Save Experiment Template	X	X	
Report	File Report	Change File Report Auto Mode	X	X	
		Customize Auto File Report	X	X	
		Customize Manual File Report	X	X	
	Specimen Report	Change Specimen Report Auto Mode	X	X	
		Customize Auto Specimen Report	X	X	
		Customize Manual Specimen Report	X	X	
		Paste Specimen Report	X	X	
	Sample Report	Change Sample Report Auto Mode	X	X	

		Customize Auto Sample Report	X	X	
		Customize Manual Sample Report	X	X	
		Paste Sample Report	X	X	
		Import Template File to Sample Report	X	X	
	Print Report or Save as PDF File		X	X	X
Setting	Modify System Options		X		
	Modify User Options		X	X	X
	Manage Reagent Lots		X	X	X
Security Setting	Modify Access Control Settings		X		
	Modify Data Security Settings		X		
	Modify E-Signature Settings		X		
	Modify Audit Trail Settings		X		
Miscellaneous	Register/Dissociate NovoExpress		X		
	Sign Experiment File		X	X	X
	Sign Experiment Template		X	X	X
	View File Audit Trail		X	X	X
	View System Audit Trail		X	X	X
	View Signature Log		X	X	X

In This Guide

This guide describes the following:

- Prologue
- Installation
- Use NovoExpress Software
- Sample Acquisition
- Data Analysis
- Plate Manager
- Experiment Manager
- Reports
- QC Test
- Troubleshoot
- Version History
- Permission List

www.agilent.com

© Agilent Technologies, Inc. 2023

Edition 3.02 July 2023

XA150013

Agilent