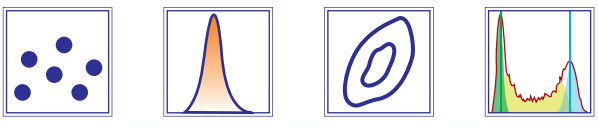
ACEA

NovoExpress® Software Guide

Software Version 1.3.0



1. Introduction

1.1 Revision History

Version	Revision Date
1.0	2014.07
1.1	2014.11
1.2	2015.04
1.3	2015.10
1.4	2016.08
1.5	2017.05
1.6	2018.08

1.2 About the NovoExpress Software

The NovoExpress® Software provides users with the ability to control data collection and analysis on the NovoCyte® and NovoCyte Quanteon™ Flow Cytometer. The software contains features to control Quality Control (QC) test, sample acquisition, data analysis, and report generation. The NovoExpress Software is the property of ACEA Biosciences, Inc. and cannot be copied or modified in any way without the written consent of ACEA Biosciences, Inc.

1.3 Conventions

Text Conventions

To impart information that is consistent and easy-to-read, the following text conventions are used in this guide:

Format	Description
Numbered Listing 1 2	Describes the steps in a procedure that must be performed in the listed order.
Italic Font, gold	Points to a different chapter in this guide, which should be referred to for better understanding.

Format	Description
Italic Font	Describes buttons, icons or functions when operating the NovoExpress software. In addition, important notes and information notes are also shown in italic font.
→	Indicates the sequence of the menu operation in NovoExpress software. For example, *File* → *Print* means to select the *Print* function from the *File* menu.
Ctrl+X	When used with keyboard characters, + means to press two keys simultaneously. For example, Ctrl + C means to hold down the Control key while pressing the letter C key.

Symbols

The following table lists the symbols used in this guide:

Symbol	Meaning	Description
	IMPORTANT NOTE	This symbol indicates information which is critical to the success of the procedure or use of the product.
	ADDITIONAL INFORMATION	This symbol provides additional information about the current topic or procedure.
▶▶▶		Table continues on the next page.
■		End of a table.

2. Installation

This chapter will introduce the installation and un-installation of the NovoExpress Software and the software user management feature.

2.1 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: 2 GB free space
- Screen Resolution: 1024×768 pixels or higher

Software:

- Operating System: Windows XP SP3/Windows Vista SP2/Windows 7 SP1/Windows 8/Windows 10
- PDF Reader Software

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

2.2 Installing the NovoExpress Software

Install the NovoExpress Software using the following instructions:

1. Download the NovoExpress Software installation package from website http://www.ace-abio.com/novoexpress and unzip it. Double-click the *SetupEn.exe* file in the NovoExpress installation directory to start the installation process.

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

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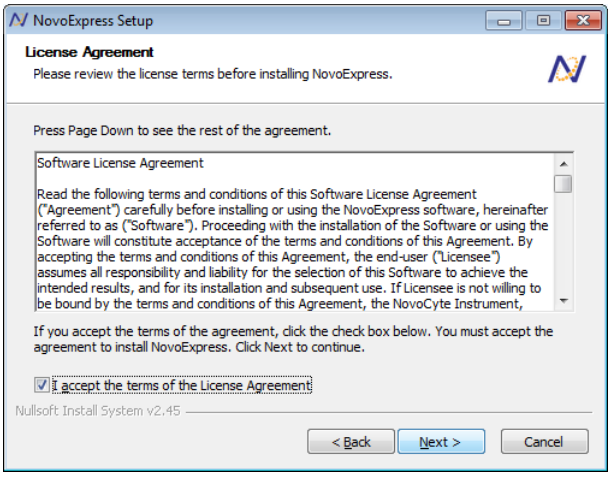
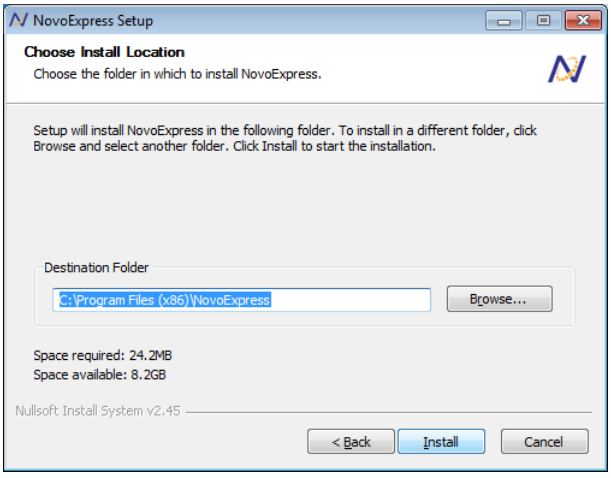
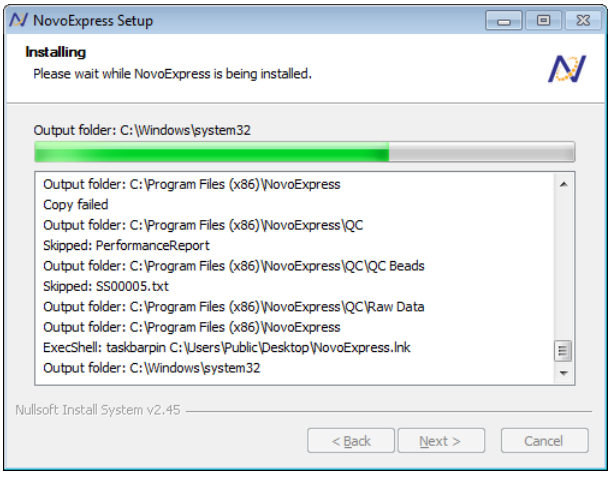
3 Please read the license agreement and accept by selecting the check box and clicking *Next*.



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.





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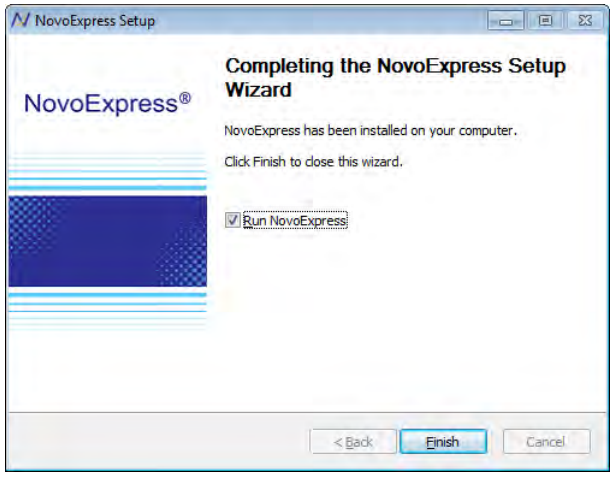
5 After the installation is complete, click *Finish* to finish the installation and start the software. If you would not like to immediately start the software, uncheck the *Run NovoExpress* box, and click *Finish*.

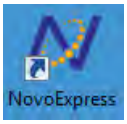


The installation of PDFCreator 1.7.1 is included with the NovoExpress Software installation. Please do not uninstall or update PDFCreator, as doing so may cause exporting to PDF files through the NovoExpress Software not working properly.

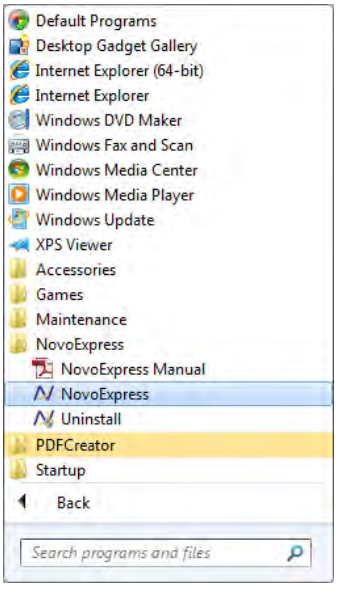
2.3 Starting the NovoExpress Software

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

- Desktop Shortcut



- Start Menu



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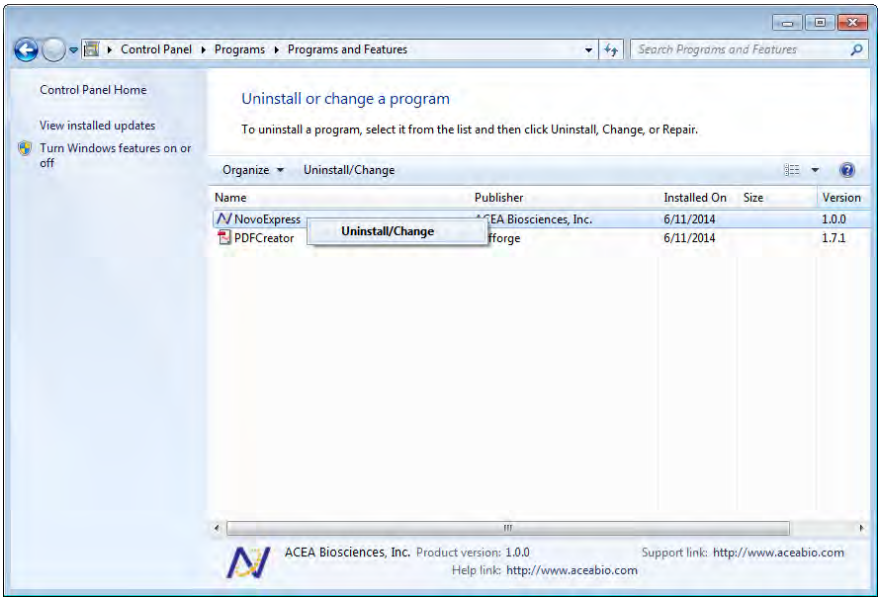
2.4 Uninstalling the NovoExpress Software

The NovoExpress Software can be uninstalled by the following methods:

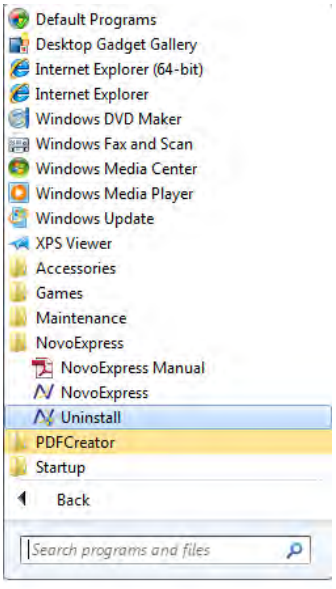
- Control Panel

  Start → Control Panel → Program and Features

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



- Start Menu



2.5 NovoExpress License

NovoExpress is available for a 30-day free trial. Registration will be needed to use the software after the trial expiration. NovoExpress Software License Key comes with NovoCyte Flow Cytometer, and can also be purchased separately from ACEA.

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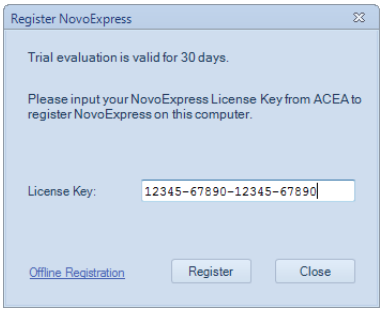
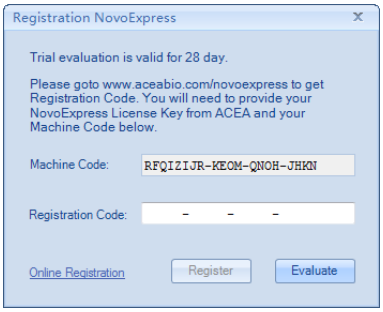
2.5.1 NovoExpress Registration

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

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



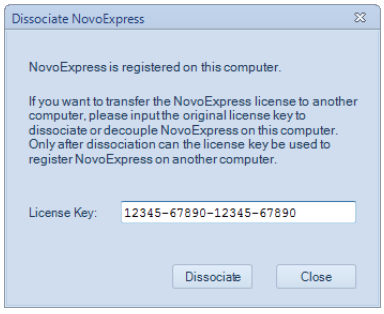
- If the computer is not connected to the internet, click the *Offline Registration* button to switch to offline registration mode. Write down the *Machine Code* displayed in the window, and go to another computer that is connected to the internet and open the *Get Registration Code* web page (http://www.aceabio.com/novoexpress). On the web page, enter the *Machine Code* into the specified textbox, type a valid ACEA Biosciences 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.



A license key is required for NovoExpress software registration or license transfer. Keep a safe record of license key assigned to each computer or user.

2.5.2 NovoExpress Dissociation

NovoExpress supports license transfer to another computer. Each license may be transferred up to 5 times. If you want to transfer the NovoExpress license to another computer, click *File* → *About* → *Dissociate NovoExpress* to open the *Dissociate NovoExpress* window.



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Enter the original license key in the text box and 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.

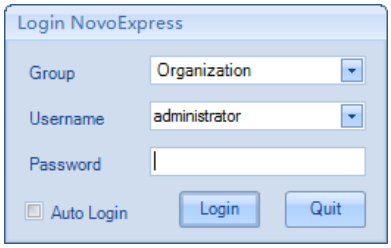
Please connect the workstation to the internet when dissociating the license. Contact ACEA technical support for how to dissociate the license if no connection to the internet is available.

2.6 User Management

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

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

The NovoExpress Software initially includes a system administrator account with username as "administrator". The default password for this account is "administrator". This system administrator account has the highest privilege and the username "administrator" cannot be changed. This system administrator and users with the *Administrator* privilege can add, delete, and modify information for all the other users and user groups. There is no limit to the number of user groups or user accounts. Each user belongs to a specific user group. The methods for adding, modifying and deleting a user group are described in *Section 2.6.1*. The methods for adding, modifying, and deleting a user account are described in *Section 2.6.2*. A user can directly enter the username and password to log in the software, or select the specific user group first, select the username, and enter the correct password to log in the software.



2.6.1 User Groups

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

2.6.1.1 Adding a User Group

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

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

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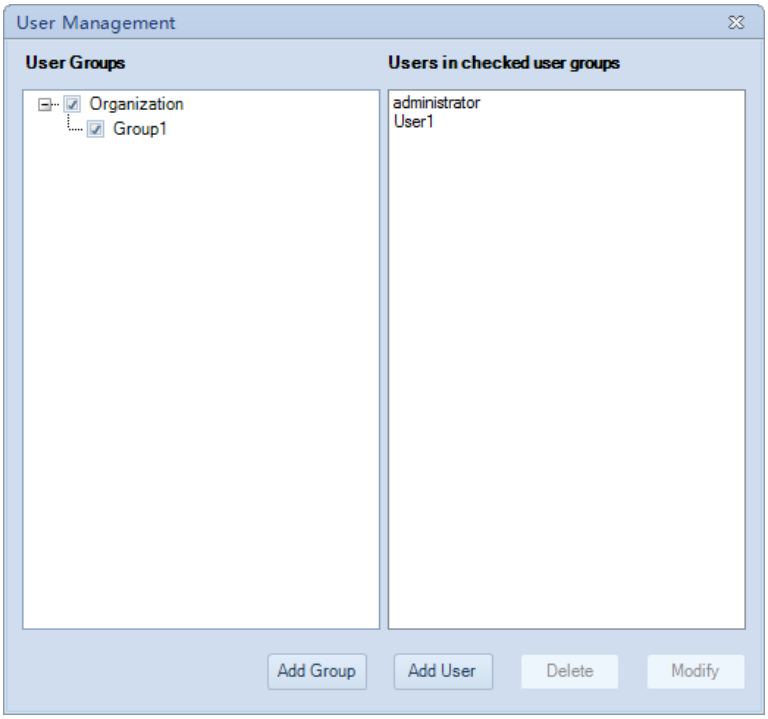
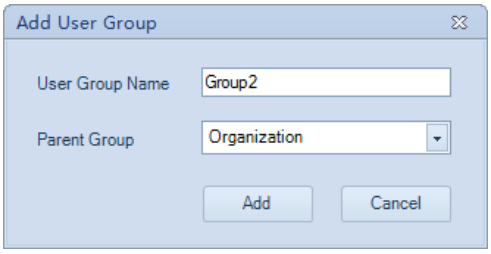
2. Select the *Setting* tab.

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



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



5. Enter the group name and select the desired parent group for the created user group in the prompted window.

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

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2.6.1.2 Modifying a User Group

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

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

2. Select the *Setting* tab.

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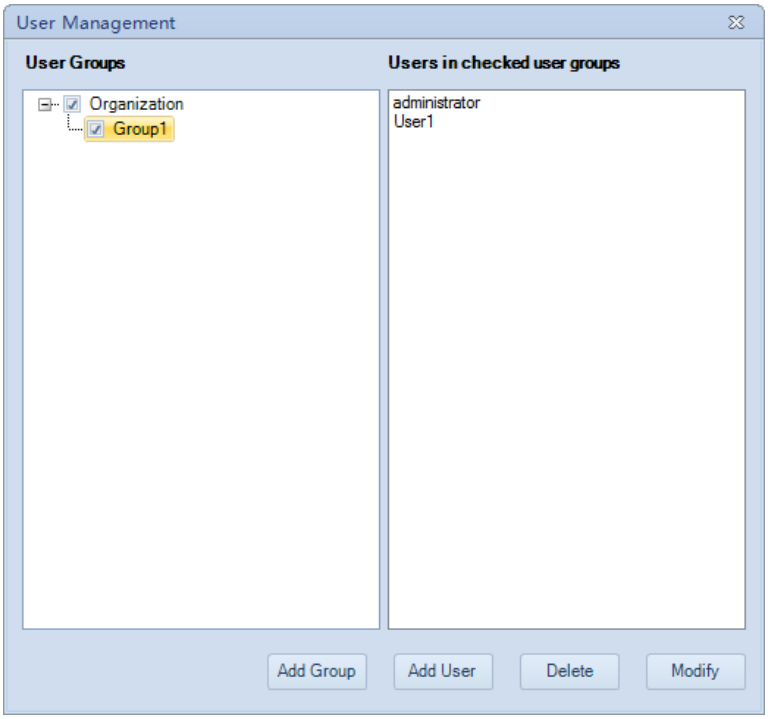
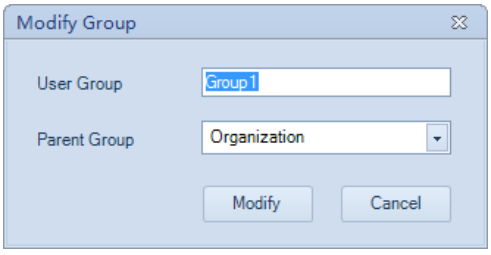
3 Click *User* → *Management*. The *User Management* window will appear.



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



5 Modify the group name and select the desired parent group for the modified user group in the prompted window.

6 Click *Modify* and the user group is modified.

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2.6.1.3 Deleting a User Group

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

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

2 Select the *Setting* tab.

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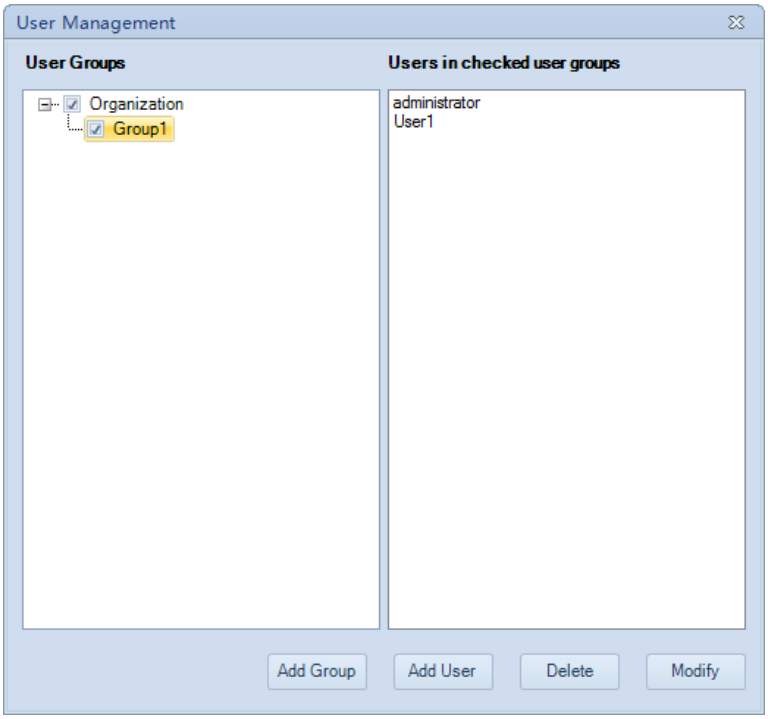
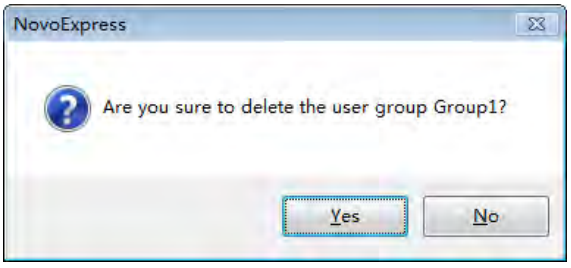
3 Click *User* → *Management*. The *User Management* window will appear. Select the group to be deleted. Click *Delete*.



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

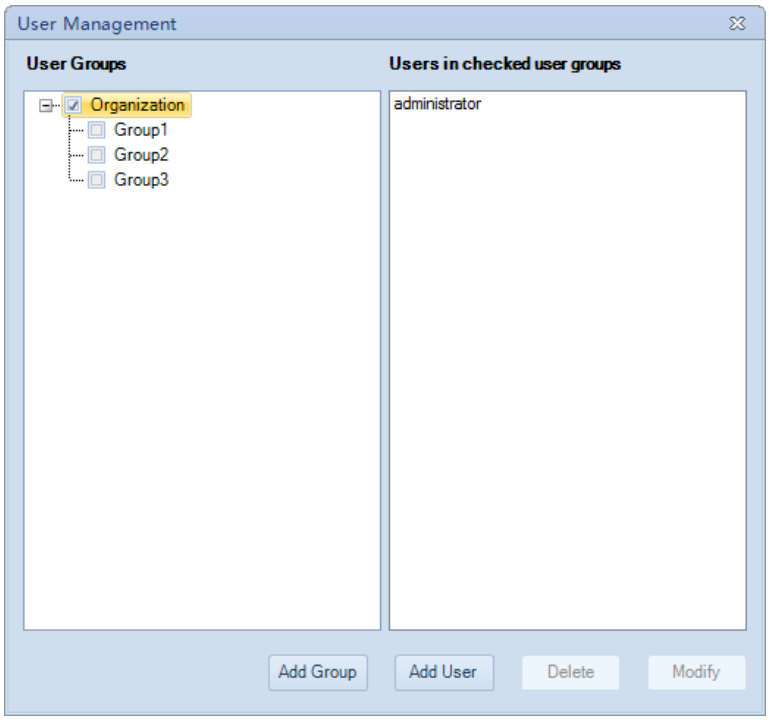


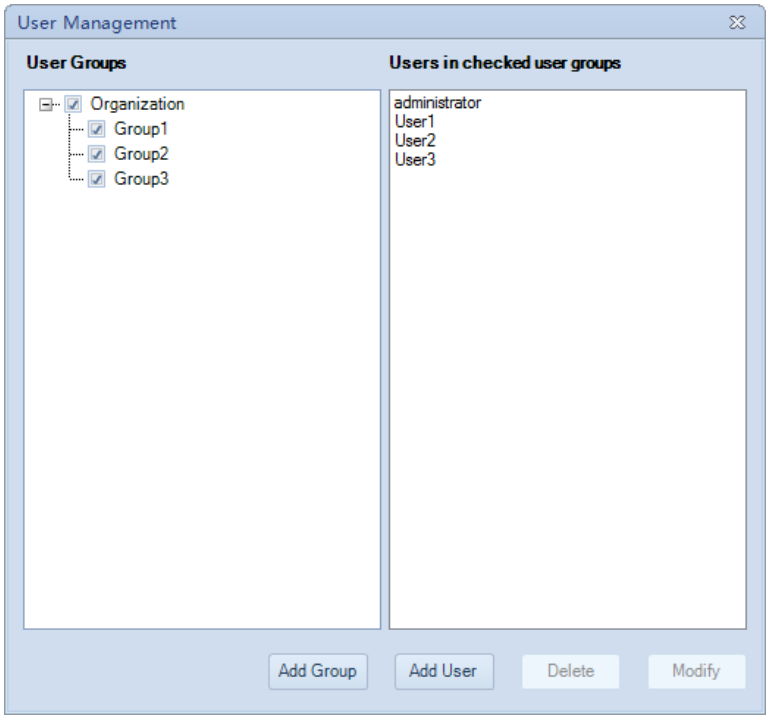
When the selected user group is deleted, the user accounts in the group will automatically be moved to the deleted group's parent group.

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

2.6.1.4 Display the Users in a User Group

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





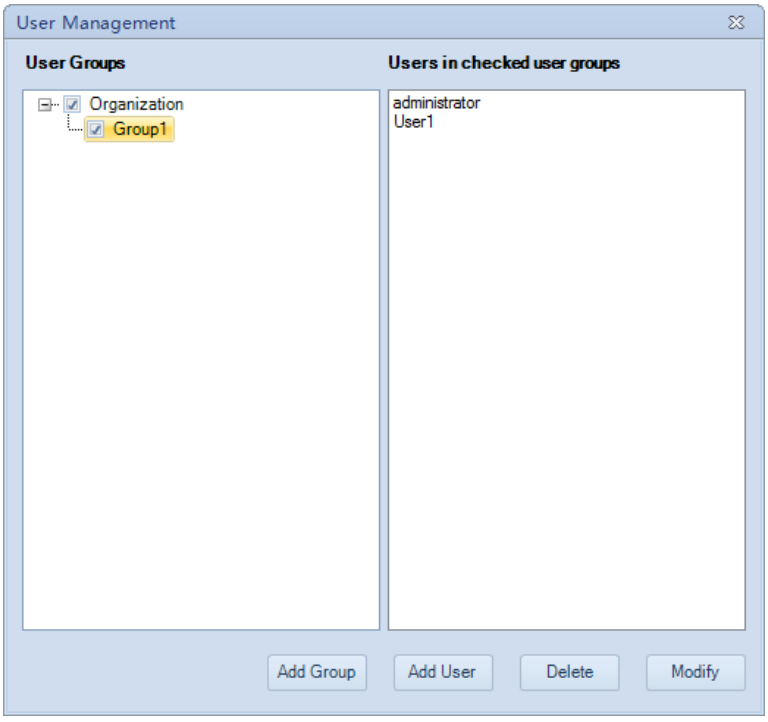
2.6.2 Users

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

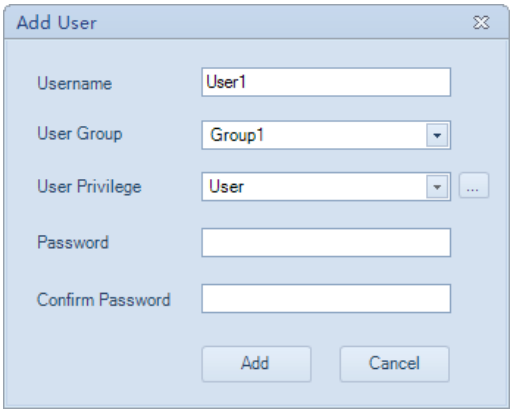
2.6.2.1 Adding a New User

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

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



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



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

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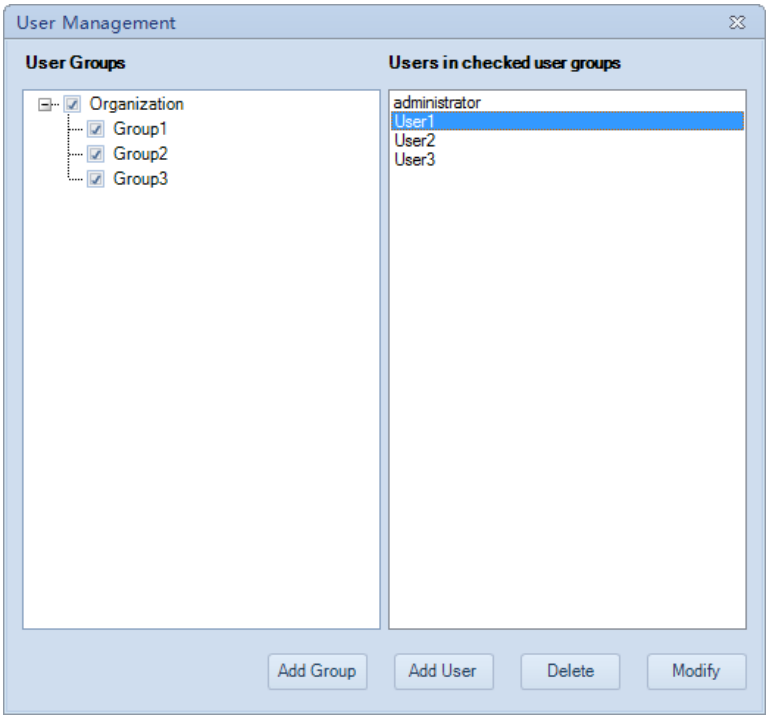
2.6.2.2 Modifying User Information

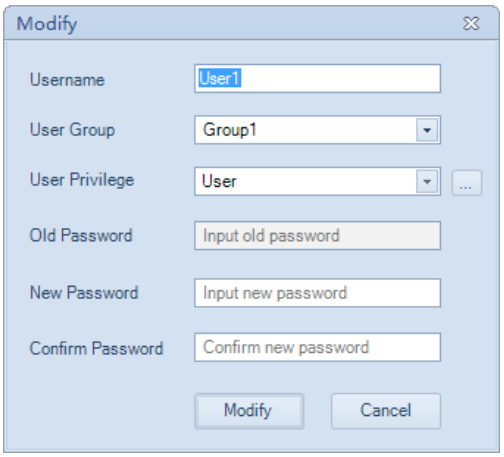
Accounts with *Administrator* privilege can modify username, user privilege, access privilege, and password for each user, while other accounts can only modify its own username, user group name, and password.

► From the administrator account or an account with *Administrator* privilege:

1. After logging in, select the *Setting* tab.
2. Click User → Management. The *User Management* window will appear. Select the user group, and then the user account you would like to modify.



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



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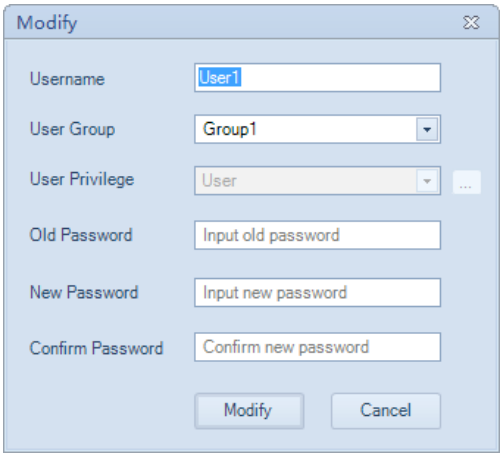
- From individual user accounts:

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

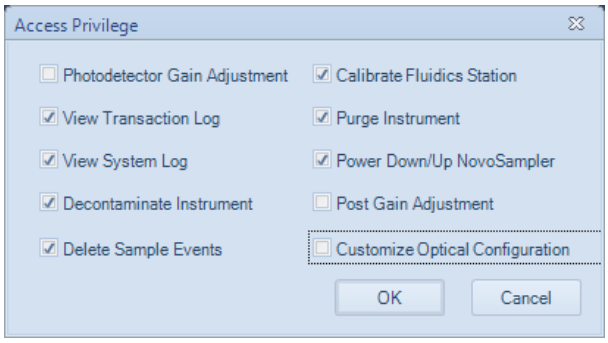


2.6.2.3 Access Privilege

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

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

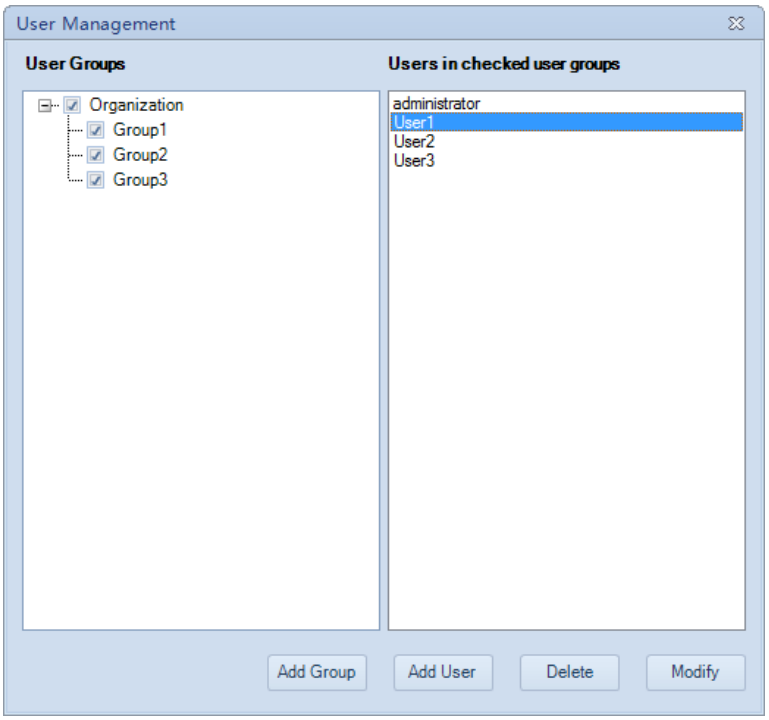
To modify the *Access Privilege* for users, click the ... button in the *Modify* window and then select or unselect checkboxes in the popup *Access Privilege* window. Click *OK* to confirm changes.



2.6.2.4 Deleting a User

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

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

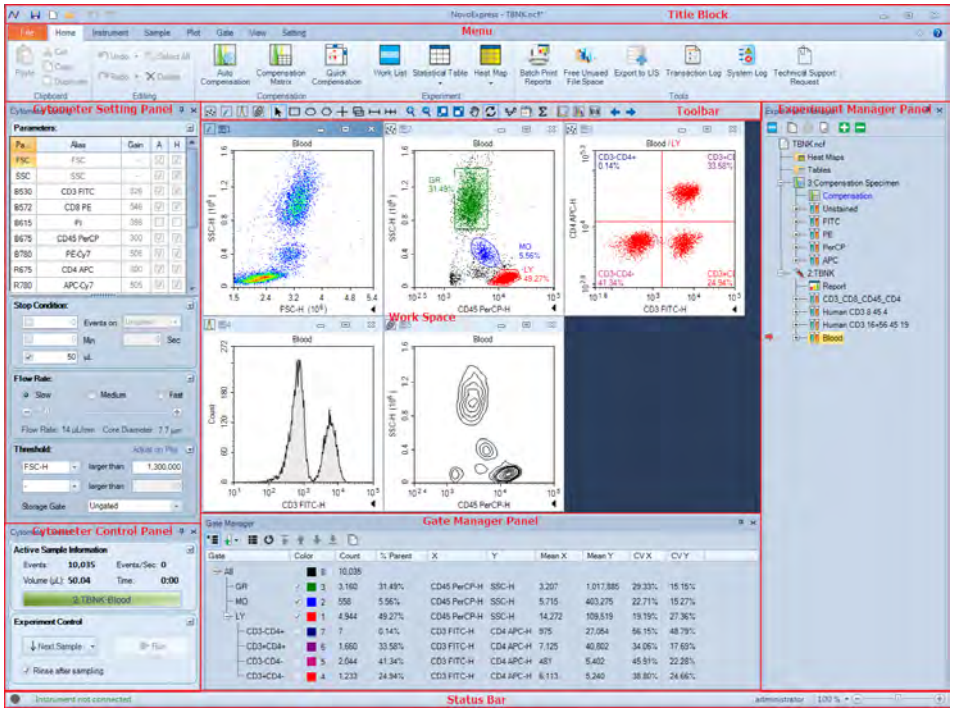


4. Select the user account that you would like to delete.
5. Click *Delete*.
6. In the confirmation window, click *Yes*.

3. Using the NovoExpress Software

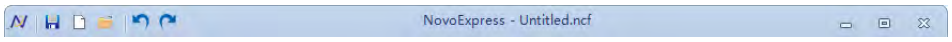
3.1 NovoExpress Software Interface

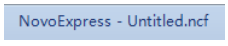
After starting the NovoExpress Software, the initial interface will include a *Title Block*, *Menu*, *Cytometer Setting Panel*, *Toolbar*, *Experiment Manager Panel*, *Cytometer Control Panel*, *Work Space*, and *Status Bar*.



3.2 Title Block

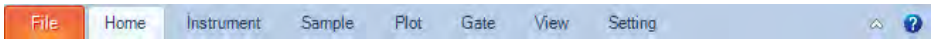
The *Title Block* displays the data file name in the center. It also provides options for opening, saving, and closing an experiment file.



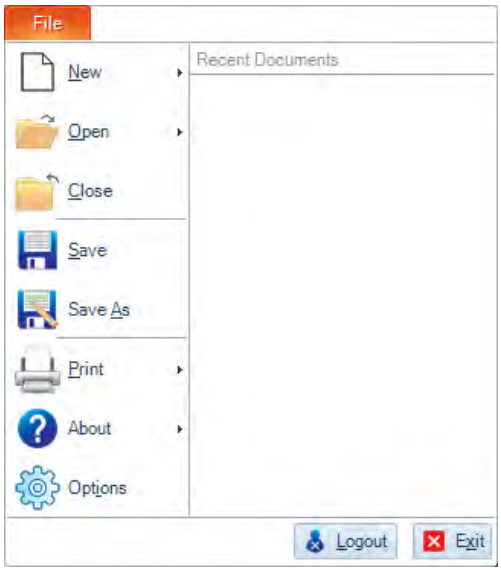
Icon	Description
	Clicking this button displays a drop-down menu with options to resize the display window and close the software.
	Saves the experiment file.
	Creates a new experiment file.
	Opens an experiment file.
	Undo drawing gates, deleting gates, zooming in or zooming out on a plot, etc. This 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.
	Displays the data file name (*.ncf).
	Minimizes window.
	Maximizes window.
	Restores window.
	Closes window.

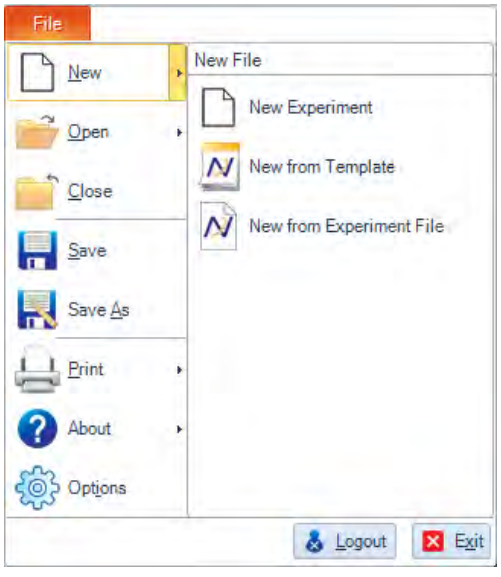
3.3 Menu

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



3.3.1 File



Icon	Description
	New experiment file  ▶ *New Experiment*: Creates a new experiment file. ▶ *New from Template*: Imports a template (*.nct) for the new experiment. ▶ *New from Experiment File*: Creates a new experiment file with the experiment setting and data analysis method inherited from an existing experiment file.

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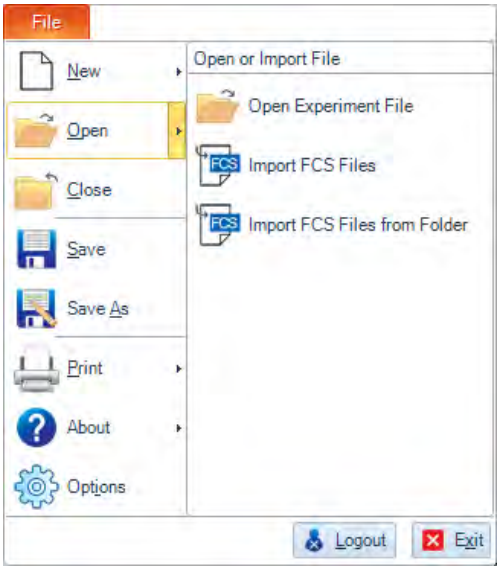
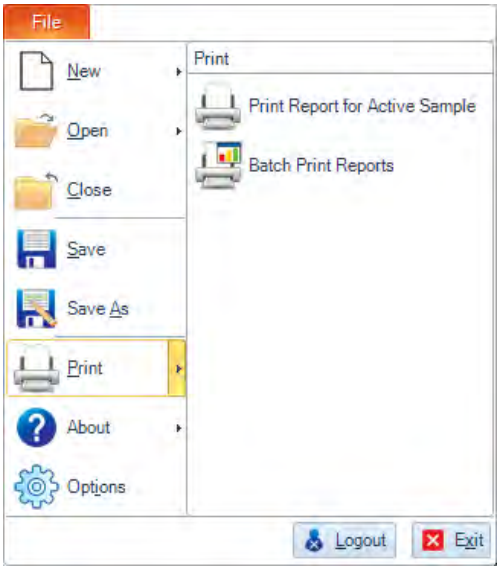
Icon	Description
	Open experiment (*.ncf) file  ► *Open Experiment File*: Opens an experiment file. ► *Import FCS files*: Imports selected file to the current specimen. ► *Import FCS Files from Folder*: Imports all FCS files in a selected folder to the current FCS specimen.
	Closes the current experiment file and creates a new experiment file.
	Saves the experiment data, experiment setup, and data analysis of the current experiment file.
	Saves the current experiment file to a specified location.
	Prints sample report/batch print reports  ► *Print Report for Active Sample*: Prints the report for the current sample. ► *Batch Print Reports*: Opens the *Batch Print Reports* window.

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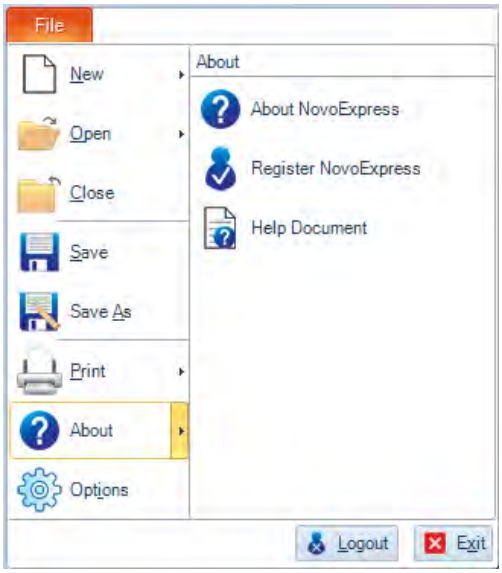
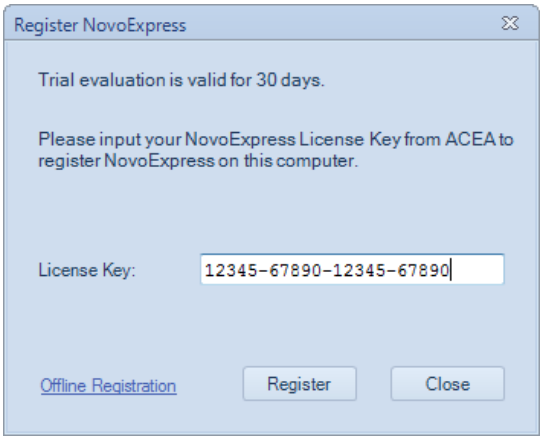
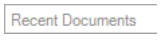
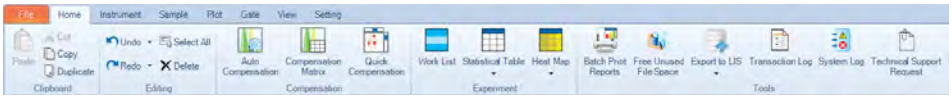
Icon	Description
	*About*: Contains the software version, copyright information, help files, and registration information.  ▶ *About NovoExpress*: Displays the software version and copyright information. ▶ *Register NovoExpress*: Registers the NovoExpress software.  ▶ *Help Document*: Opens the help file.
	*Options*: Opens the *Options* window. See *Section 3.3.8 Setting* 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*: Exits and logs out of the current account. The login window will appear.
	*Exit*: Exits the software.

3.3.2 Home



Icon	Description
Clipboard	
	*Copy*: Copies the selected gate or selected data.
	*Paste*: Pastes the copied gate to other plots, or pastes the copied item in the *Experiment Manager*.
	*Duplicate*: Creates a duplicate of the plot (the gates will not be included.), or creates a duplicate sample or specimen.
Editor	
	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.
	*Select All*: Selects all the gates in the current plot.
	*Delete*: Deletes the selected gate.
Compensation Settings	
	*Auto Compensation*: Opens the *Automatic Compensation Settings* window.
	*Compensation Matrix*: Displays the current compensation matrix for the selected sample.
	*Quick Compensation*: Displays the Compensation scrollbars on the active plot for quick compensation.
Experiments	
	*Work List*: Users may view and edit the work list.
	*Statistical Table*: Creates a statistical analysis table.
	*Heat Map*: Creates a heat map for the defined parameter in a plate layout format.
Tools	
	*Batch Print Reports*: Prints or creates PDF of multiple test reports.

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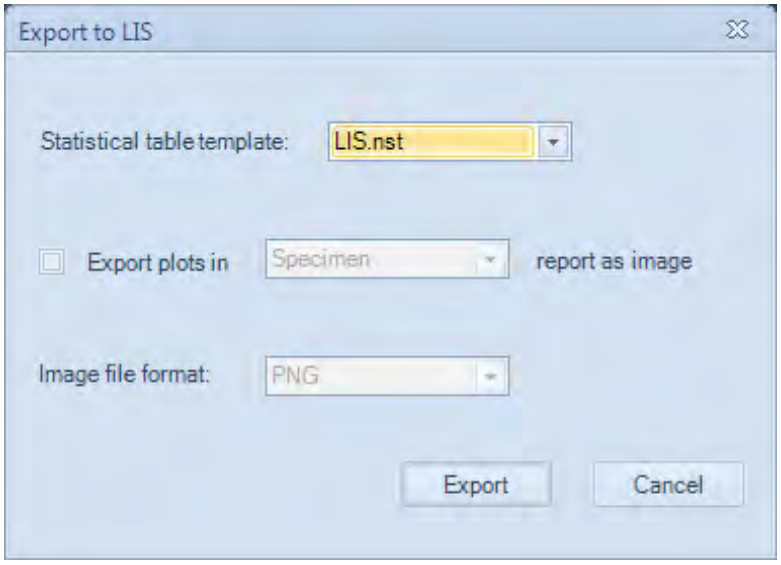
Icon	Description
	*Free Unused File Space*: When events of a sample or entire samples are deleted, the file space is not automatically released. Click on this icon to free the file space. The file space can also be released by saving the file to the hard drive. Large files may take longer to release.
	*Export to LIS*:  This function allows a user to export data analysis results in the designated format to CSV file and such CSV file can be parsed by a Laboratory Information System (LIS) or read by other programs. ► *Statistical table template*: Sets the proper statistical table template to export the data. To add a statistical table template, export the statistical table as a template to the default folder "*User data root folder*\Statistical Table Templates".Refer to *Section 5.7.6 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 *3.3.8 Setting* for details of changing the default user data root folder.

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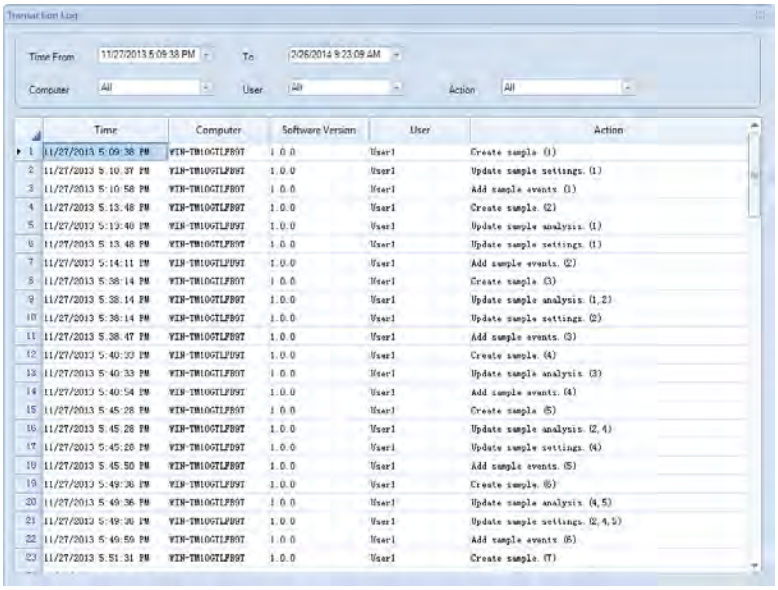
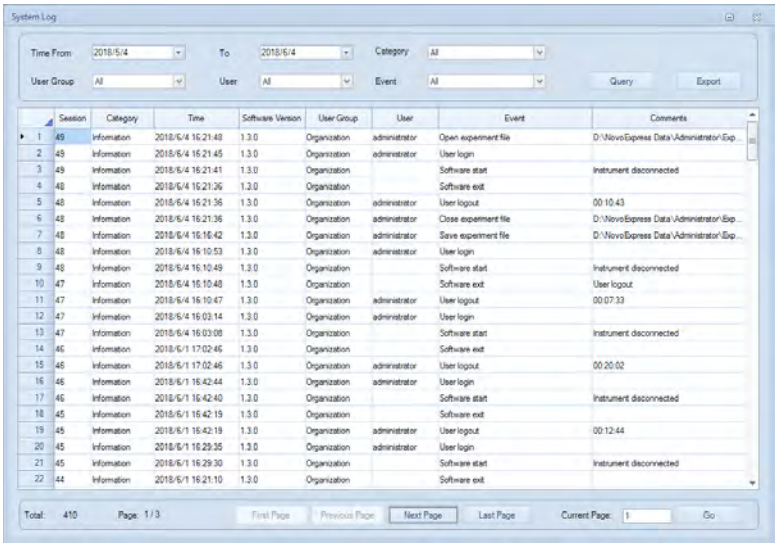
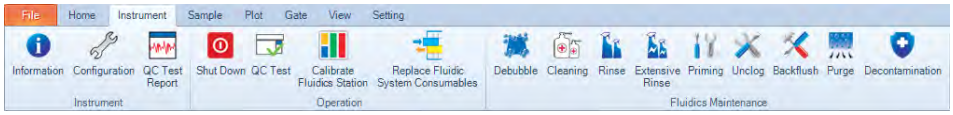
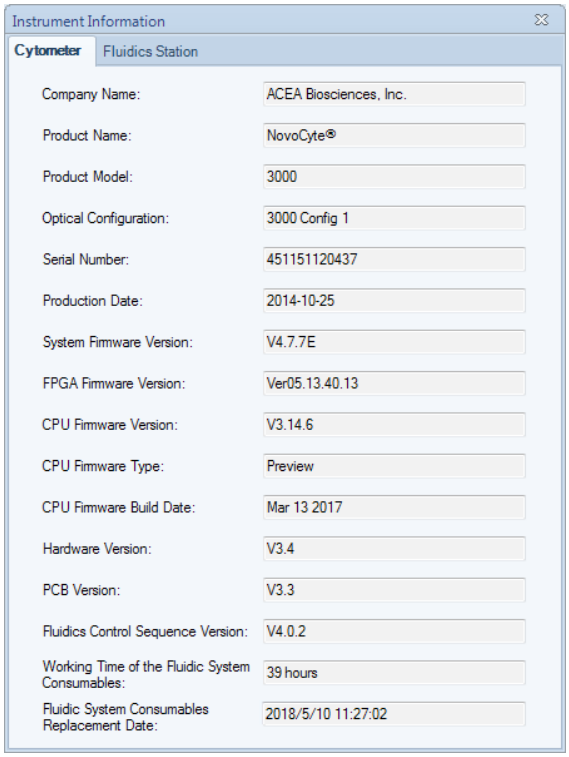
Icon	Description
	*Transaction Log*: Displays the *Transaction Log* window. Records can be filtered by Time, Computer, User, and Action. Only accounts with the *View Transaction Log* privilege can access the *Transaction Log*. 
	*System Log*: Displays the *System Log* window. Only accounts which have the *View System Log* privilege can access the *System Log*. The *System Log* window records information including user login and log out, and instrument operating activities including data acquisition, fluidic maintenance, etc. 
	*Technical Support Request: Technical Support Request Creation Wizard* automatically collects NovoCyte configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of NovoCyte instrument. You can also attach any other files using this function. Refer to *Section 9 Troubleshooting* for details.

3.3.3 Instrument

3.3.3.1 NovoCyte Instrument



Icon	Description
Instrument	
	*Information*: Displays the instrument information. 
	*Configuration*: Displays and modifies the NovoCyte Flow Cytometer excitation laser and detection channel and options for the NovoSampler and NovoSampler Pro. See *Section 4.4 Instrument Configuration* for details.
	*QC Test Report*: Displays results from QC tests. The results can be viewed individually or plotted over a time interval in a Levey-Jennings chart.
Operation	
	*Shut Down*: Starts the shutdown process. After completion, the NovoCyte instrument will automatically turn off. To clean sample injection probe while shutting down NovoCyte, see *NovoCyte® Flow Cytometer Operator's Guide* for more information.
	*QC Test*: Runs the instrument QC test.

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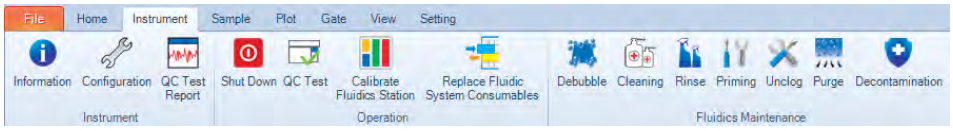
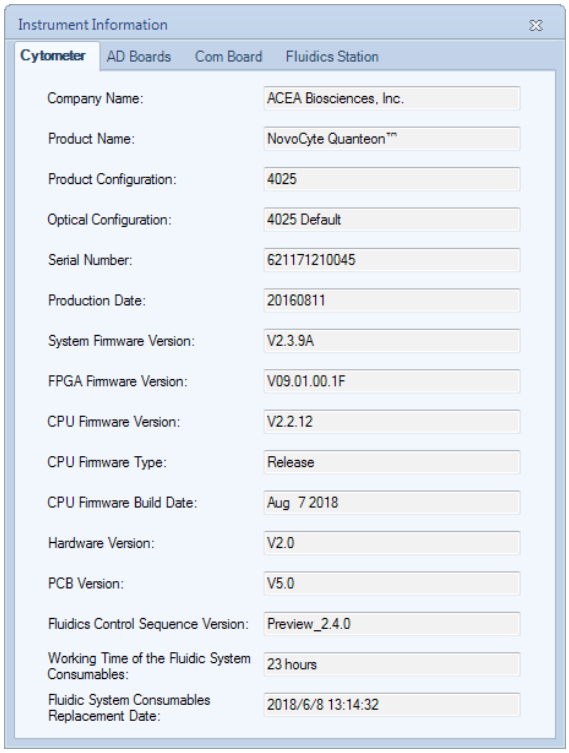
Icon	Description
	Calibrate Fluidics Station: Long distance transportation, movement and other reasons may cause the fluidics station system malfunction. Use this function to re-calibrate the fluidics station. After clicking the button, it will ask to remove the instrument reagent containers from the fluidics station before calibration. Only accounts with the *Calibrate Fluidics Station* privilege can calibrate the fluidics station.
	Replace Fluidic System Consumables: Click the button to open the *Replace Fluidic System Consumables* window, and follow the instructions to replace fluidic system consumables. See *NovoCyte® Flow Cytometer Operator's Guide* for more information. *NovoCyte flow cytometer monitors the accumulated running time of the fluidic system consumables to ensure the consumables are changed in a timely manner for optimal flow cytometry results. When the accumulated running time is reached, NovoExpress software will prompt a message to remind the users to replace the consumables.*
Fluidics Maintenance	
	Debubble: Removes the air bubbles from the fluidic system.
	Cleaning: Uses a cleaning solution to decontaminate the biohazards that may exist in the fluidic system.
	Rinse: Rinses the fluidic system using a rinsing solution.
	Extensive Rinse: Performs an extensive rinse of the fluidic system.
	Priming: After the instrument has not been in use for a period of time, this function clears the air bubbles and primes the fluidic system with fresh sheath fluid.
	Unclog: Clears blockage from the flow cell.
	Backflush: Clears blockage from the sample injection probe.
	Purge: If the NovoCyte flow cytometer needs to be shipped, click this button and follow the procedure shown on the popup window to purge the fluidic system before packaging and shipment. Only accounts with the *Purge Instrument* privilege can perform the purge operation. Refer to *Section 2.7 Purge Fluidic System before Shipment* in the *NovoCyte® Flow Cytometer Maintenance Guide* for detailed procedure.
	Decontamination: If the NovoCyte flow cytometer is known to have bacterial contamination or to prevent the occurrence of bacterial contamination, click this button and follow the instruction shown in the popup window to decontaminate the instrument. Only accounts with the *Decontaminate Instrument* privilege can perform this decontamination operation. Refer to *Section 2.5 Decontamination* in the *NovoCyte® Flow Cytometer Maintenance Guide* for detailed procedure.

3.3.3.2 NovoCyte Quanteon Instrument



Icon	Description
Instrument	
	*Information*: Displays the instrument information. 
	*Configuration*: Displays and modifies the NovoCyte Quanteon Flow Cytometer excitation laser and detection channel and options for the NovoSampler Q. See *Section 4.4 Instrument Configuration* for details.
	*QC Test Report*: Displays results from QC tests. The results can be viewed individually or plotted over a time interval in a Levey-Jennings chart.
Operation	
	*Shut Down*: Starts the shutdown process. After completion, the NovoCyte Quanteon instrument will automatically turn off. To clean sample injection probe while shutting down NovoCyte Quanteon, see *NovoCyte Quanteon™ Flow Cytometer Operator's Guide* for more information.
	*QC Test*: Runs the instrument QC test.
	*Calibrate Fluidics Station*: Long distance transportation, movement and other reasons may cause the fluidics station system malfunction. Use this function to re-calibrate the fluidics station. After clicking the button, it will ask to remove the instrument reagent containers from the fluidics station before calibration. Only accounts with the *Calibrate Fluidics Station* privilege can calibrate the fluidics station.

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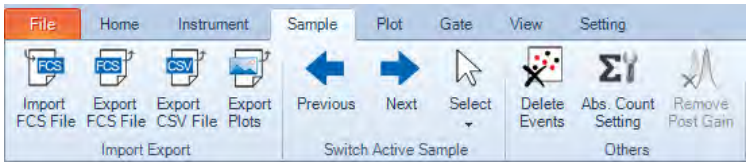
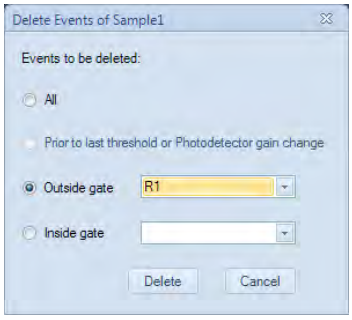
Icon	Description
	*Replace Fluidic System Consumables*: Click the button to open the *Replace Fluidic System Consumables* window, and follow the instructions to replace fluidic system consumables. See *NovoCyte Quanteon™ Flow Cytometer Operator's Guide* for more information.  *NovoCyte flow cytometer monitors the accumulated running time of the fluidic system consumables to ensure the consumables are changed in a timely manner for optimal flow cytometry results. When the accumulated running time is reached, NovoExpress software will prompt a message to remind the users to replace the consumables.*
Fluidics Maintenance	
	*Debubble*: Removes the air bubbles from the fluidic system.
	*Cleaning*: Uses a cleaning solution to decontaminate the biohazards that may exist in the fluidic system.
	*Rinse*: Rinses the fluidic system using a rinsing solution.
	*Priming*: After the instrument has not been in use for a period of time, this function clears the air bubbles and primes the fluidic system with fresh sheath fluid.
	*Unclog*: Clears blockage from the flow cell.
	*Purge*: If the NovoCyte Quanteon flow cytometer needs to be shipped, click this button and follow the procedure shown on the popup window to purge the fluidic system before packaging and shipment. Only accounts with the *Purge Instrument* privilege can perform the purge operation. Refer to *Section 2.5 Purge Fluidic System before Shipment* in the *NovoCyte Quanteon™ Flow Cytometer Maintenance Guide* for detailed procedure.
	*Decontamination:* If the NovoCyte Quanteon flow cytometer is known to have bacterial contamination or to prevent the occurrence of bacterial contamination, click this button and follow the instruction shown in the popup window to decontaminate the instrument. Only accounts with the *Decontaminate Instrument* privilege can perform this decontamination operation. Refer to *Section 2.3 Decontamination* in the *NovoCyte Quanteon™ Flow Cytometer Maintenance Guide* for detailed procedure.

3.3.4 Sample



Icon	Description
Import and Export	
	*Import FCS File*: Users may select FCS format files to import.
	*Export FCS File*: Exports the current data into a FCS file.
	*Export CSV File*: Exports the current data into a CSV file.
	*Export Plots*: Exports the plots of the active sample into files in PNG, JPEG, Bitmap, GIF, or TIFF format.
Switch Active Sample	
	*Previous*: Switches the Active Sample to the previous sample.
	*Next*: Switches the Active Sample to the next sample.
	*Select*: Switches to an Active Sample from the prompted drop-down menu.
Other	
	*Delete Events*: Deletes events from a sample. Users may select events inside a gate, outside a gate, or all events to delete. If the threshold or the photodetector gain has been adjusted during data acquisition, the Prior to last threshold or *photodetector gain change radio* button will be available to allow event deletion before the last adjustment. Only accounts with the *Delete Sample Events* privilege can perform this operation.  *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 Section 3.3.2 for details.*

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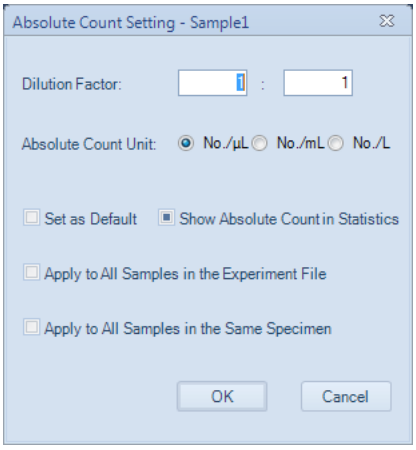
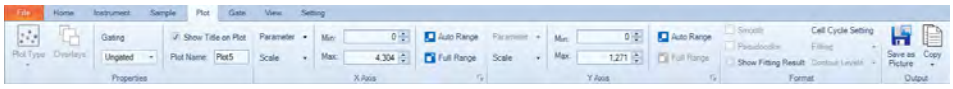
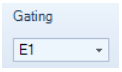
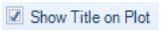
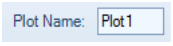
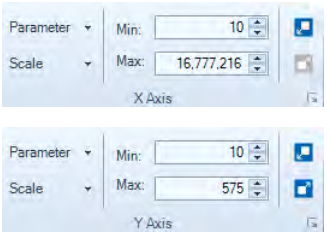
Icon	Description
	*Absolute Count Setting*: Sets up absolute counting conditions for active sample.  ▶ *Dilution Factor*: The *Dilution Factor* is conversion coefficient used to calculate the absolute counting results for the original sample. For instance, if the original sample is diluted 10 times and is run on NovoCyte flow cytometer, 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 NovoCyte 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. *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. *There are three states of the checkbox:* • : *Show **Absolute Count** column for all plots of the sample.* • : *No change.* • : *Hide **Absolute Count** column for all plots of the sample.* ▶ *Apply to All Samples in the Experiment File*: Set *Absolute Count Setting* for all samples in the experiment file. ▶ *Apply to All Samples in the Same Specimen*: Set *Absolute Count Setting* for all samples belonging to the same specimen as the active sample. Further information on absolute count calculations are described in *Section 5.3.2*.
	*Remove Post Gain*: Click this button to remove *Post Gain* of all parameters of the Active Sample. Only accounts with the *Post Gain Adjustment* privilege can perform this operation. To adjust *Post Gain*, refer to *Section 5.9*.

3.3.5 Plot



Icon	Description
Properties	
	*Plot Type*: Changes the plot type for the selected plot.
	*Overlays*: Opens the *Edit Overlays* window. Overlay is only available for dot plots and histograms.
	*Gating*: The gate and its events are selected as the current data source for plotting.
	*Show Title on Plot*: Toggle on and off the display of a title on plots. This is set for all data analysis plots in the *Workspace*.
	*Plot Name*: Sets the name of a plot.
X -Axis, Y –Axis	
	► Sets plot properties for the X and Y-axes including the plotted parameter, scale (linear, logarithmic, or biexponential), and the display range. ► *Custom Range*: Uses the input boxes to set the minimum and maximum displayed values for the selected parameter. ► *Automatic Range* : Automatically sets the range of the X and Y-Axes according to the range of the dataset. ► *Full Range* : Sets the full range of the X and Y-Axes. The default range is 2^{24}=16,777,216.
Format	

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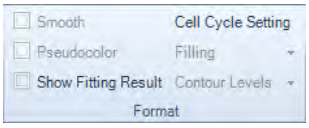
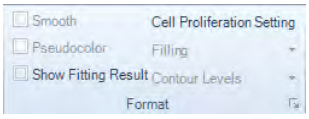
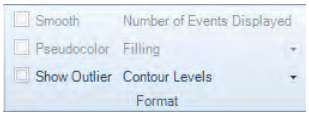
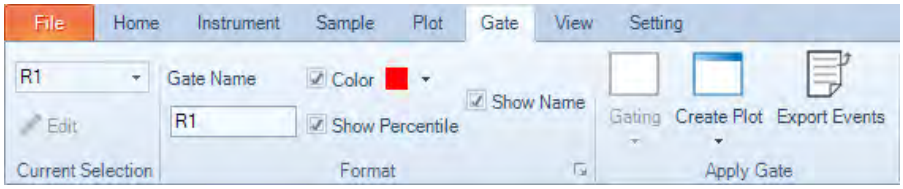
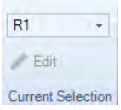
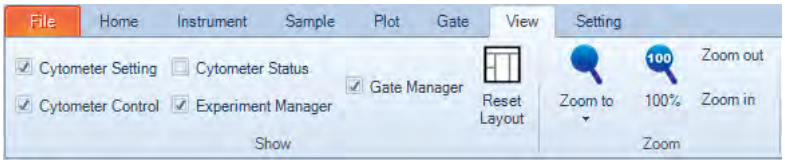
Icon	Description
 Format the cell cycle plot  Format the cell proliferation plot  Format dot plots, density plots, histograms, contour plots	*Format*: Controls format settings for the plots. Available settings depend on the type of the selected plots. ▶ *Smooth*: Smooths the data in histogram and density plots. ▶ *Pseudocolor*: Density plots are displayed in pseudo-color to visualize event density. ▶ *Cell Cycle Setting*: Opens the *Cell Cycle Setting* window to set the parameters for cell cycle analysis. ▶ *Cell Proliferation Setting*: Opens the *Cell Proliferation Setting* window to set the parameters for cell proliferation analysis. ▶ *Number of Events Displayed*: Opens a window to set the number of events to be displayed in the plot. ▶ *Show Fitting Result*: Displays the results for the cell cycle analysis. ▶ *Filling*: Sets the histogram fill mode: *None*, *Filled*, or Tinted. ▶ *Contour Level*: Sets the contour levels in contour plots. The three levels are: *10%, 5%*, and *2%*. ▶ *Show Outlier*: Show outlier events as dots on contour plot.
Output	
	*Save As Image*: The plot can be exported in JPEG, BMP, PNG, GIF, TIFF, and EMF formats. EMF format is a vector format which, when exporting, uses 600dpi resolution TIFF format.
	*Copy*: Selects from the drop-down menu to copy to the clipboard. The plots can then be pasted into Microsoft® Word, PowerPoint, Excel, and other documents. Pull-down options include: ▶ *Copy Plot (Bitmap)*: Copies the selected plot as a Bitmap. ▶ *Copy Plot with Statistics (Bitmap)*: Copies the selected plot and associated statistical information as a Bitmap. ▶ *Copy Vector Graphics (EMF)*: Copies the selected plot as an EMF. ▶ *Copy Statistics (Text)*: Copies the statistics for the selected plot in text format.

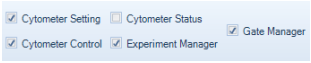
3.3.6 Gate



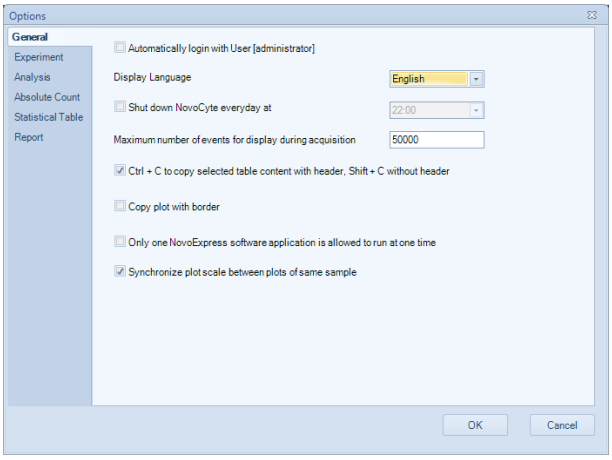
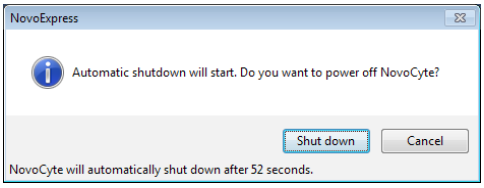
Icon	Description
Current Selection	
	Provides a drop-down menu of all available gates. A gate can be selected from this list. The *Edit* button is available only when a logic gate is selected. Click the *Edit* button to open the *Edit Logic Gate* window for modifying the setting of a logic gate.
Format	
	Formats the selected gate. *Gate Name*: Sets the name for the selected gate. *Color*: Sets the color for the selected gate. *Show Percentile*: Shows the percentage of the gated events relative to the total number of events on the plot. If the *Show population percentile in gate label* in *Setting* → *Analysis* is not checked, the *Show Percentile* option here will be disabled. *Show Name:* Shows the gate name in gate label on plot. If the *Show gate name in gate label* option in *Setting* → *Analysis* is not checked, the *Show Name* option here will be disabled.
Apply Gate	
	*Gating*: Applies the current gate to the selected plot.
	*Create Plot*: Creates a new plot and applies the current gate to the new plot.
	*Export Events*: Exports data for the events inside the current gate in either FCS or CVS format.

3.3.7 View

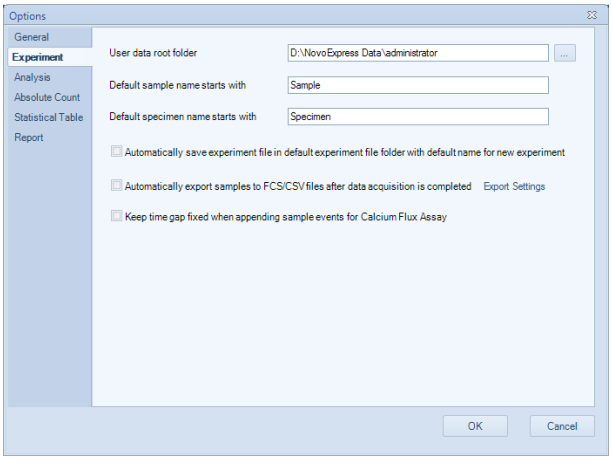


Icon	Description
Show	
	Shows or hides the corresponding panel.
	*Reset Layout*: Resets the layout of the panels to the default layout.
Zoom	
	*Zoom to*: Selects the scaling of the size of the plots inside the workspace in the drop-down menu (50%-150%). This function is equivalent to adjusting the sliding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

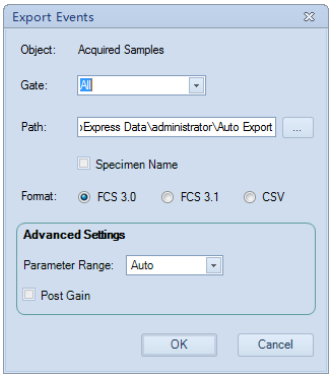
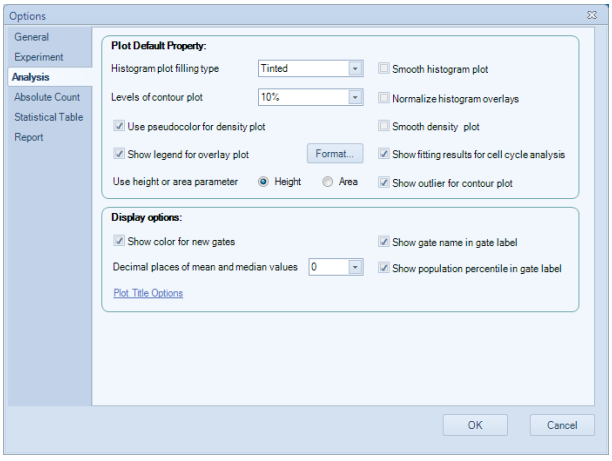
3.3.8 Setting

Icon	Description
User	
	Management: Use this function to manage all user groups and user accounts. It allows for adding, deleting, and modifying user groups and user accounts. This function is only available on the accounts with the *Administrator* privilege.
	Modify: Use this function to change the username and password of the currently logged in account.
Options	
	General  ▶ *Automatically login with User*: If this box is checked, the current user will automatically be logged in when the software starts and the login window will not appear. ▶ *Display Language*: Sets the display language. ▶ *Shut down NovoCyte every day at:* If this box is checked, the NovoCyte will automatically shut down at the selected time. For example, if the time is set to 22:00, the software will prompt a message window as shown below at 22:00 o'clock every day. The system will automatically shut down in 1 min if there is no operation from the user.  Only user with *Administrator* privilege can enable this function. If the instrument is not in the "Ready" status at the set time (i.e. the instrument is performing fluidic maintenance or data acquisition etc.), the software will wait until the instrument enters the "Ready" status before shutting down the instrument. If the instrument cannot enter the "Ready" status 30 mins after the set time, automatic shutdown function will be cancelled automatically.

▶▶▶

Icon	Description
	The NovoExpress software should be opened at the selected time to perform this function. *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.* ▶ *Maximum number of events for display during acquisition*: Sets a limit to the number of events displayed on plots during sample acquisition. For example, if this is set to 20,000 events, only the last 20,000 events collected will be displayed. The maximum setting is 50,000 events. ▶ *Ctrl+C to copy selected table content with header, Shift+C without header:* When selected, statistical data copied from either the *Statistical Table* or the table below the plots will include a header when copied using Ctrl+C and will not include a header when selected using Shift+C. ▶ *Copy plot with border*: When selected, plots copied will include a dotted line border. ▶ *Only one NovoExpress software application is allowed to run at one time:* When selected, only one instance of NovoExpress is allowed to run at a time. An error message will be displayed when user tries to run a second instance of NovoExpress. ▶ *Synchronize plot scale between plots of same sample:* When selected, the axis range and scale of same parameter on different plots of same sample will be automatically synchronized when user change axis range or scale. Click *Synchronize Plot Scale* button on workspace toolbar to quick switch this setting. Refer to *Section 3.4* for more information.
	Experiment:  ▶ *User data root folder:* Sets the default folder directive for saving the experiment file. ▶ *Default sample name starts with*: Sets the prefix for the name of the sample. ▶ *Default specimen name starts with*: Sets the prefix for the name of the specimen.

▶▶▶

Icon	Description
	▶ *Automatically save experiment file in default experiment file in default experiment file folder with default name for new experiment:* This setting determines how to save a current experiment file when a new experiment is started. If selected, the current file is automatically saved when a new experiment is started. The file is saved at the default experiment file folder with the name based on the current time in the format of *YYMMDD_hhmm.ncf*. If not selected, a *Save As* window appears when a new experiment is started. The user then has the option to set a name and location to save the current experiment file. ▶ *Automatically export samples to FCS/CSV files after data acquisition 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.  ▶ *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*:  **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.

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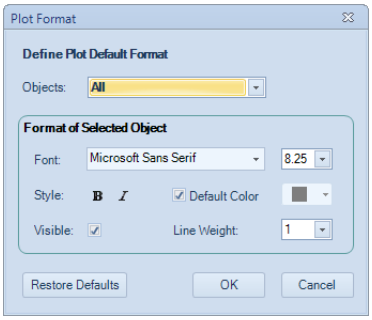
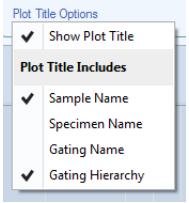
Icon	Description

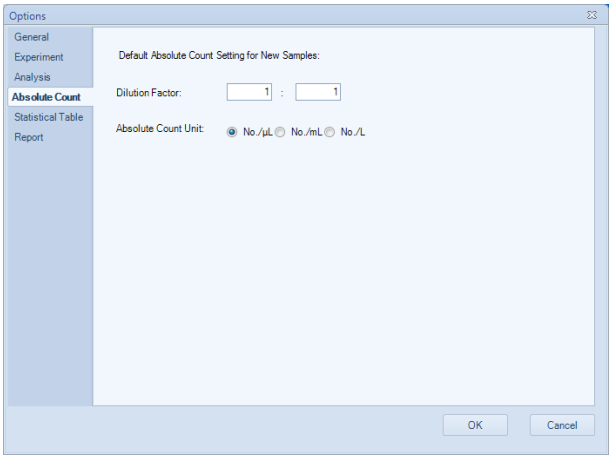
- *Levels of contour plot*:
  Sets the contour plot level (*2%, 5%*, and *10%*).
- *Normalize histogram overlays*:
  If selected, the histogram overlays are normalized to a 100% scale.
- *Use pseudocolor for density plot*:
  If selected, density plots are displayed in pseudocolor.
- *Smooth density plot*:
  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 *Section 5.1.8.5* for more details about plot format.



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:



Icon	Description
	• *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:*  Set Default Absolute Count setting for new samples. ► *Dilution Factor:* Sets the default dilution factor for new samples. The *Dilution Factor* is a conversion coefficient used to calculate the absolute counting results for the original sample. For instance, if the original sample is diluted 10 times and is run on NovoCyte flow cytometer, 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 NovoCyte 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 *Section 5.3.2*.

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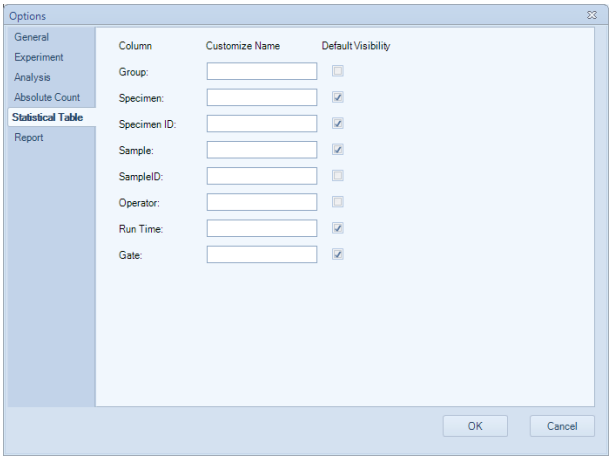
Icon	Description
	*Statistical Table*:  ▶ *Group* *Customize Name* allows the user to re-name the *Group* column header in the statistical table. If *Default Visibility* is selected, the *Group* column will appear in the statistical table by default. ▶ *Specimen ID:* *Customize Name* allows the user to re-name the *Specimen ID* column header in the statistical table. If *Default Visibility* is selected, the *Specimen ID* column will appear in the statistical table by default. ▶ *Specimen:* *Customize Name* allows the user to re-name the *Specimen* column header in the statistical table. If *Default Visibility* is selected, the *Specimen* column will appear in the statistical table by default. ▶ *Sample:* *Customize Name* allows the user to re-name the *Sample* column header in the statistical table. If *Default Visibility* is selected, the *Sample* column will appear in the statistical table by default. ▶ *Sample ID:* *Customize Name* allows the user to re-name the *Sample ID* column header in the statistical table. If *Default Visibility* is selected, the *Sample ID* column will appear in the statistical table by default. ▶ *Operator:* *Customize Name* allows the user to re-name the *Operator* column header in the statistical table. If *Default Visibility* is selected, the *Operator* column will appear in the statistical table by default. ▶ *Run Time:* *Customize Name* allows the user to re-name the *Run Time* column header in the statistical table. If *Default Visibility* is selected, the *Run Time* column will appear in the statistical table by default. ▶ *Gate:* *Customize Name* allows the user to re-name the *Gate* column header in the statistical table. If *Default Visibility* is selected, the *Gate* column will appear in the statistical table by default.

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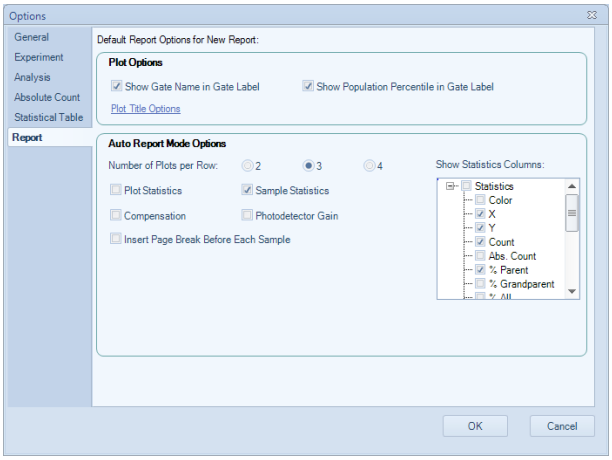
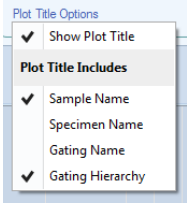
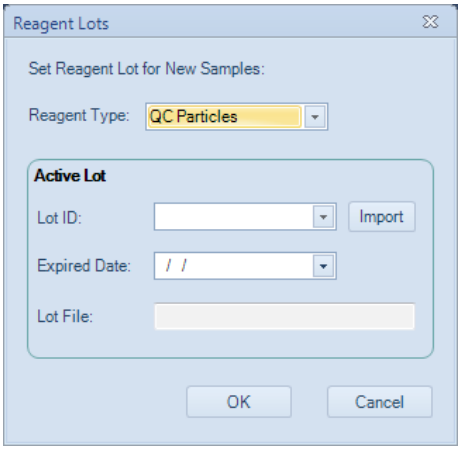
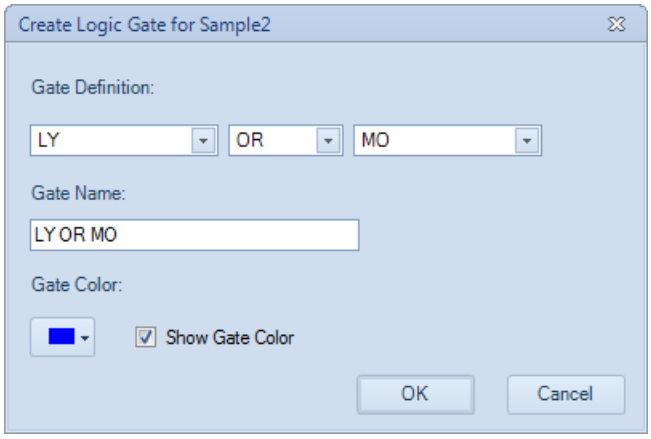
Icon	Description
	*Report Options:*  *Set Default Report Options for New Report.* The settings in *Plot Options* panel are used for customizing plots inside report. They are effective for both auto and manual report mode. ▶ *Show Gate Name in Gate Label:* If selected, gate name is displayed in gate label on the plot. ▶ *Show Population Percentile in Gate Label:* If selected, gate label is displayed with the percentage of the population within the gate. ▶ *Plot Title Options:* If clicked, a drop down menu will show as below:  ● *Show Plot Title:* If selected, plot titles are displayed on report plots. ● *Sample Name:* If selected, the sample name is displayed in the report plot title. ● *Specimen Name:* If selected, the specimen name is displayed in the report plot title. ● *Gating Name:* If selected, the gating name is displayed in the report plot title. ● *Gating Hierarchy:* If selected, the gating hierarchy is displayed in the report plot title. The settings in *Auto Report Mode Options* panel are used for customizing auto report. They are only effective for auto report mode. ▶ *Number of Plots per Row*: Sets how many plots are shown in one row. ▶ *Plot Statistics*: If selected, shows gate statistics of plot. ▶ *Sample Statistics*: If selected, shows gate statistics of sample. ▶ *Compensation*: If selected, shows compensation matrix. ▶ *Photodetector Gain*: If selected, shows photodetector gain of parameters. ▶ *Insert Page Break Before Each Sample:* Only available for specimen report. If selected, a page break will be inserted before each sample. ▶ *Show Statistics Columns:* Selects statistical items to display.

▶▶▶

Icon	Description
	*Reagent Lots:*  ▶ *Reagent Type:* Sets the type of the reagent. ▶ *Lot ID:* Sets the ID of the active lot. ▶ *Import:* Import the lot file download from http://www.aceabio.com/novocyte/qc-particles. 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.

3.4 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.

Icon	Description
	*Polygon Gates*: 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.
	*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 *Section 4.1.4 Threshold Settings* for detailed procedures.

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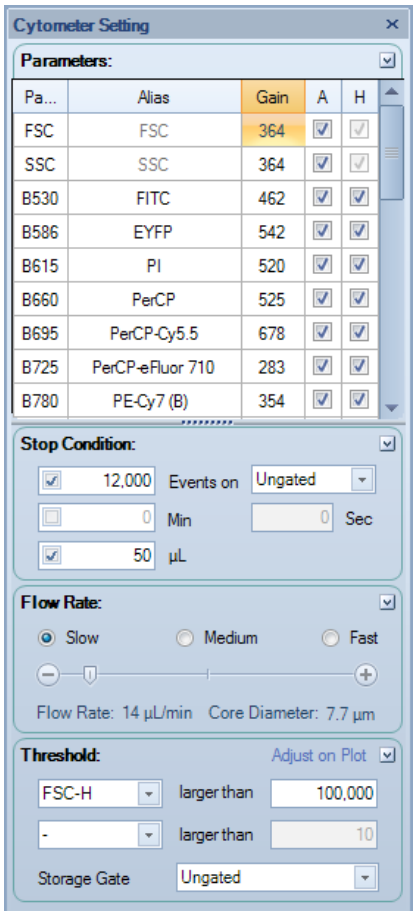
Icon	Description
	*Adjust Post Gain*: Use this tool to adjust *Post Gain*. Only accounts with the *Post Gain Adjustment* privilege can perform this operation. Refer to *Section 5.7.1 Adjust Post Gain* for detailed procedures.
	*Quick Compensation*: Allows for quick fluorescence compensation through scrollbars. 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.

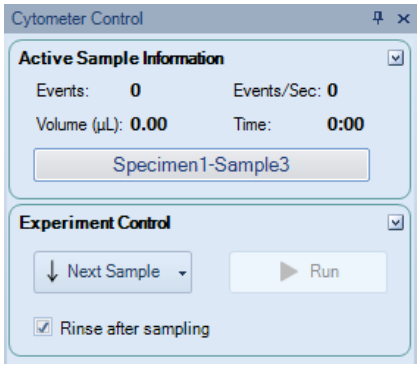
3.5 Cytometer Setting Panel

The *Cytometer Setting* panel sets the data collection parameters, stop conditions, flow rate, and threshold. Please refer to *Section 4.1* for cytometer settings.



3.6 Cytometer Control Panel

The *Cytometer Control* panel contains the *Active Sample Information* and the *Experiment Control* panel. Please refer to *Section 4.3* for cytometer controls.



- Active Sample Information

  The *Active Sample Information* panel provides information regarding sample collection during acquisition. Information includes the number of events collected, the number of events collected per second, the sample volume collected, and the sample collection time.

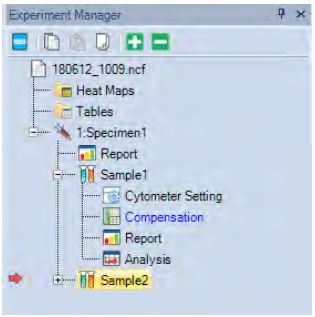
- Experiment Control

  The *Experiment Control* panel contains the *Next Sample* and *Run* buttons and *Rinse* after sampling check box. Clicking *Next* Sample switches the active sample to the next

sample. If a next sample has not already been created, clicking *Next Sample* automatically creates a new sample. Clicking *Run* begins sample acquisition. The *Run* button changes to a *Stop* button after sample acquisition begins. Clicking the *Stop* button stops sample acquisition. Refer to *Section 4.3* for a detailed description of *Cytometer Control*.

3.7 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 *Section 6* for experiment management.



3.8 Cytometer Status Panel

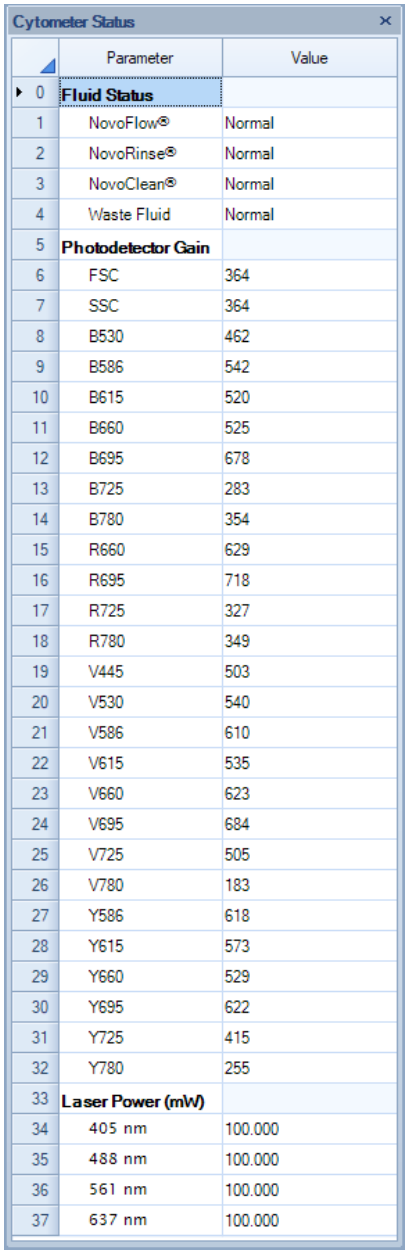
The *Cytometer Status* panel displays the fluidic component status, photodetector gains, and laser powers. This panel is hidden by default. To show this panel, click on the *Cytometer Status* box in the *Show* group of the *View* tab.

3.8.1 Cytometer Status Panel for NovoCyte Instrument

Cytometer Status

	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

3.8.2 Cytometer Status Panel for NovoCyte Quanteon Instrument

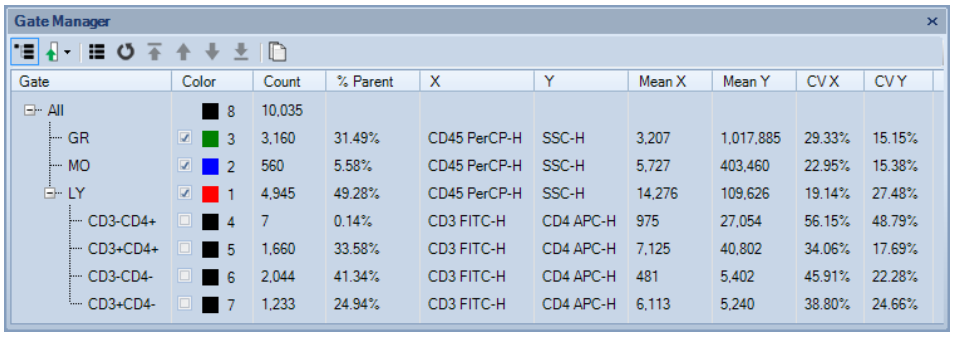


Cytometer Status

	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

3.9 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.



3.10 Status Bar

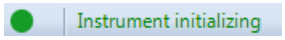
The status bar located at the bottom of the monitor displays the instrument's status through color indicators. If the instrument is connected, the indicator can be green, red, or yellow, indicating a normal condition, an error, or a warning, respectively. If the instrument is not connected to the computer,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

3.10.1 Green Indicator

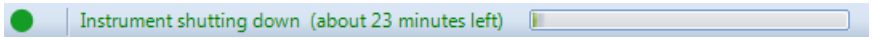
The indicator light is green if the instrument is connected and without warnings or errors.

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

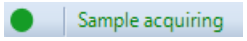
The status bar displays *Instrument initializing* after powering on the instrument. The initializing sequence flushes the fluidic lines in the instrument to prepare for sample acquisition.



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.

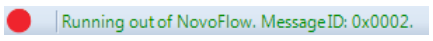


The status bar displays *Sample acquiring* during sample acquisition.

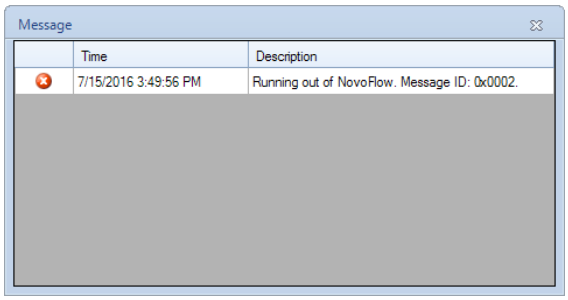


3.10.2 Flashing Red Indicator

If an error occurs, the indicator flashes red. An error message is displayed with the cause and possible solutions. Please see *Section 9 Troubleshooting* for details.

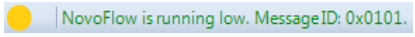


Clicking on the indicator displays the message box.

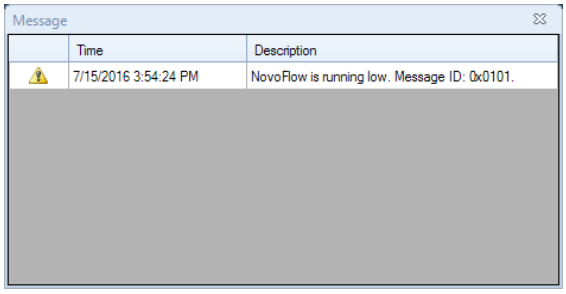


3.10.3 Flashing Yellow Indicator

If a warning occurs, the indicator flashes yellow. A warning message displays the cause of the problem and possible solutions. Please see *Section 9 Troubleshooting* for details.

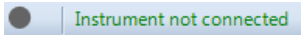


Clicking on the indicator displays the message box.



3.10.4 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.

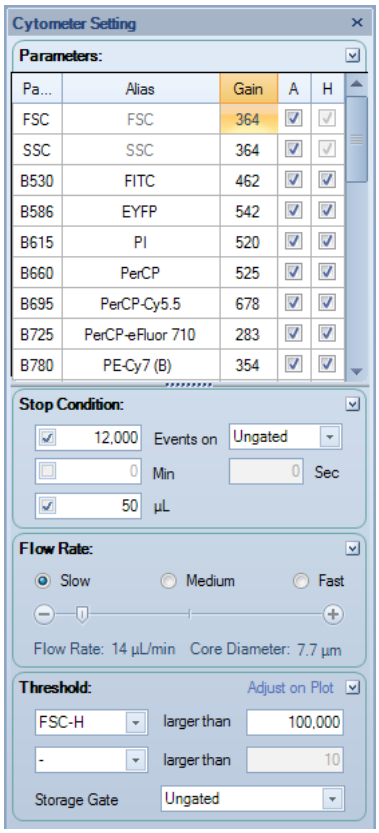


4. Sample Acquisition

This chapter will cover how to set the sample acquisition conditions using the *Cytometer Setting* panel and *Work List*, how to begin sample acquisition, and how to monitor the sample acquisition status.

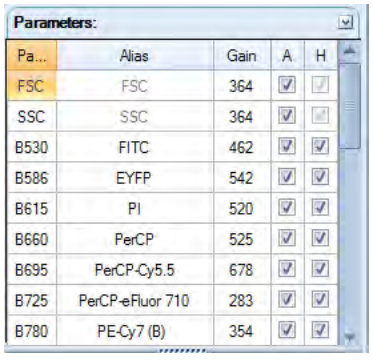
4.1 Cytometer Setting

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



4.1.1 Parameters Settings

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



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

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

Drag the slider bar or directly enter the target value to change the photodetector gain. Click the *Reset* link button to set as default value. If currently logged in user does not have *photodetector Gain Adjustment* privilege, only *Reset* button will be available. To grant *photodetector Gain Adjustment* privilege, refer to *Section 2.6.2.3*.

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.

*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.*

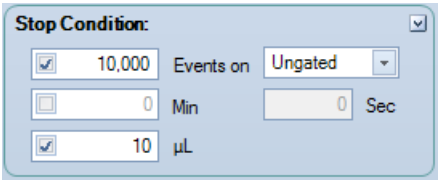
For NovoCyte instrument, different parameters may share the same photodetector, for example B675 and R675. If the photodetector gain of one parameter is changed, the other one will be changed too. For NovoCyte Quanteon instrument, the photodetector gain for each parameter can be changed independently.

*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 Section 4.3.2 for the **Restart** function. You can also delete the previous events after the acquisition is completed by using the **Delete Events** function (refer to Section 3.3.4).*

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

4.1.2 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 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.

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.

The number of events displayed in a plot during sample acquisition can be set in Settings. See Section 3.3.8. The maximum number of events displayed is 50,000 events.

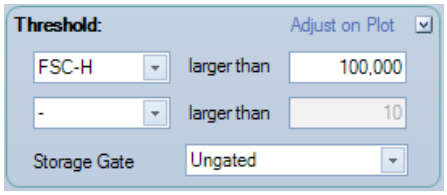
File size can be excessively large if you acquire a large number of events, i.e., 1,000,000 events. Therefore, it is always important to consider disabling unnecessary parameters (Section 4.1.1) before acquisition in order to reduce the file size. If events have already been acquired or collected, you can delete events (See Section 3.3.4) to discard parts of unnecessary events in the sample.

4.1.3 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.

4.1.4 Threshold Settings

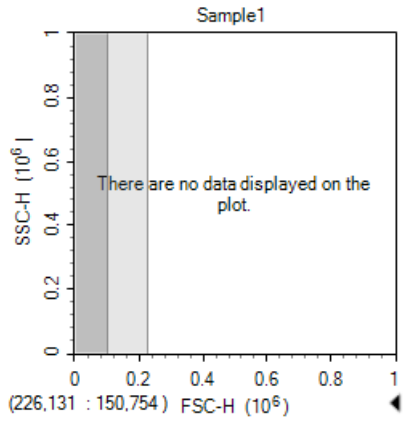
The threshold settings determine which events are recorded during sample acquisition. Only events that exceed the set threshold values are recorded.



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.

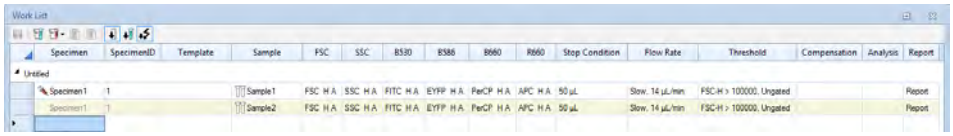


Threshold channels cannot be changed after data acquisition begins. Threshold values and ***Storage Gate*** *can be changed during acquisition, but the events already acquired will not be processed. When changing threshold values during acquisition, the plot will only show events after threshold adjustment. 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 Section 4.3.2 for* ***Restart*** *function. You can also delete the previous events after the acquisition is completed by using the* ***Delete Events*** *function (refer to Section 3.3.4).*

4

4.2 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, import specimen information from a CSV file, and copy and paste sample information. Please see *Section 6 Experiment Manager* for additional details.



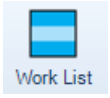
The *Work List* contains all specimens and samples listed in rows. The columns include the specimen name, specimen ID, template, sample name, acquisition parameters, stop conditions, threshold settings, compensation settings, and analysis and report information.

4.2.1 Opening the Work List

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

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

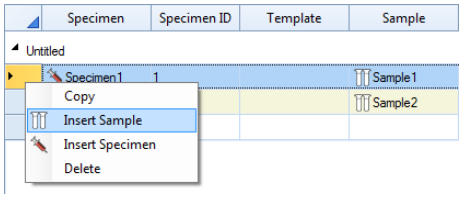
- From the *Home* tab in the menu bar, click on the *Work List* icon in the *Experiment* group.



4.2.2 Work List Management

4.2.2.1 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.

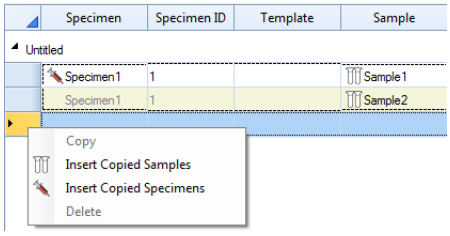


4.2.2.2 Copy and Insert the Copied Specimen or Sample

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.

■



4.2.2.3 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.

■

4.2.2.4 Importing 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.

4.2.2.5 Creating 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.

4.2.3 Editing a Work List Cell

4.2.3.1 Editing Specimen, Specimen ID and Sample Names

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

Specimen	Specimen ID	Template	Sample
Untitled			
Specimen1	1		Sample1
Specimen1	1		Sample2

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

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

4.2.3.2 Editing Template

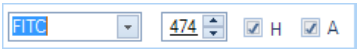
To modify a template, double-click on the *Template* cell in the *Work List*. After a new template is selected, the template is applied into the current specimen. See *Section 6.3 Templates* for additional details.

Specimen	Specimen ID	Template	Sample
Untitled			
Specimen1	1	Template1.nc	Sample1
Specimen1	1	Template1.nct	Sample2
Specimen2	2	Template2.nct	Sample1
Specimen2	2	Browse Template...	Sample2

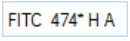
To edit template in an empty row, the template's first specimen would be imported into Work List after the edit is done.

4.2.3.3 Editing 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.

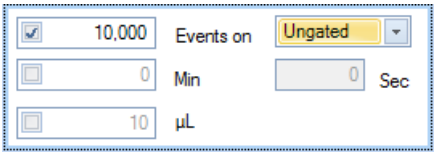


When in non-edit mode, the character * after photodetector gain text indicate the voltage is not default value. For more information about photodetector gain, refer to *Section 4.1.1*.

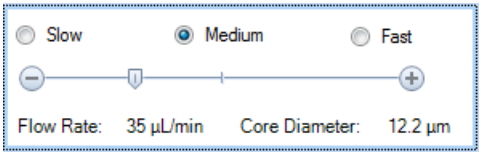


4.2.3.4 Editing Stop Conditions, Sample Flow Rates, and Threshold Settings

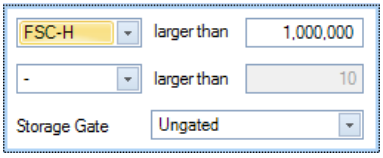
To set the sample stop conditions, double-click the stop condition cell to enter edit mode.



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.



4.2.3.5 Copying and Pasting 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

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****.*

4.2.4 Other Tools Buttons

The *Apply Modification* icon can be used to save the changes made to the *Work List*. After applying the modification, the *Experiment Manager* panel updates to reflect the changes.

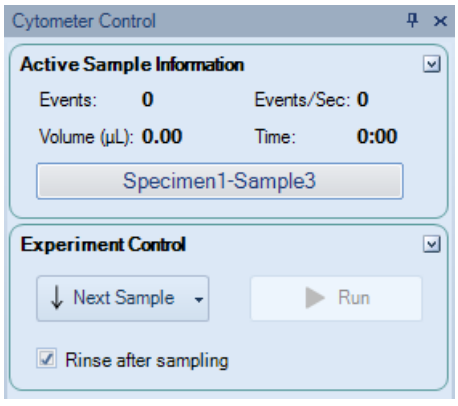
The *Hide Disabled Parameters* icon is used to hide the acquisition parameters that are currently disabled.

The *Hide Samples Containing Events* icon is used to hide the samples that contain events.

The *Hide Photodetector Gain* icon is used to hide the photodetector gain value in parameters columns.

4.3 Cytometer Control

The *Cytometer Control* panel contains the *Active Sample Information* and the *Experiment Control* panel.

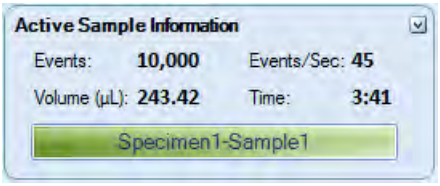


4.3.1 Active Sample Information

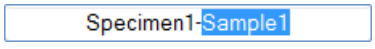
In the *Experiment Manager* panel, the active sample is indicated by the red arrow. The active sample can be switched by double-clicking on a new sample or by using the keyboard

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. To rename the sample from this box, double-click the name to enter edit mode. The specimen name cannot be edited from this box.

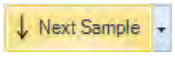


4.3.2 Experiment Control

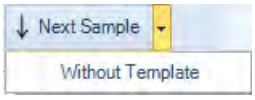
The *Experiment Control* panel includes the *Next Sample* and the *Run* buttons.

- *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*.

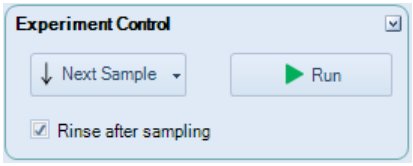
  To create a new sample without the template settings, click on the arrow on the right side of the *Next Sample* button and select *Without Template*. The new sample contains the same cytometer settings as the previous samples, but analysis, report and compensation settings are not transferred.

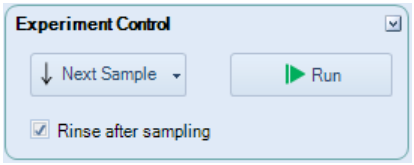
- *Run*

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

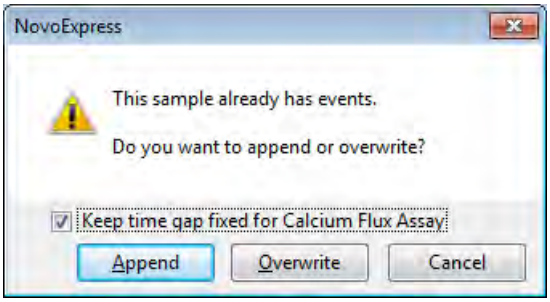
  If the active sample does not contain event data, the *Run* button appears with a solid green triangle. Click the *Run* button to begin sample acquisition.

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



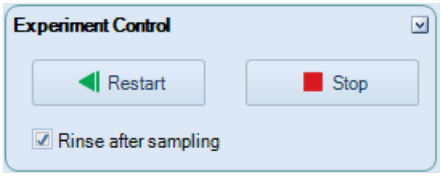
Clicking the *Run* button causes a dialog window to appear. Click *Append* to add additional events to the existing events. Click *Overwrite* to delete the existing events and collect new events. If the *"Keep fixed time gap when appending sample events for Calcium Flux Assay"* function is enabled in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



- *Restart*

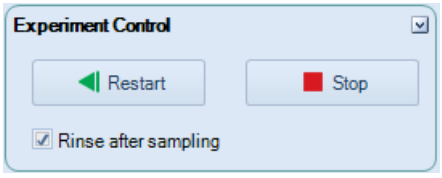
The *Restart* button is used to restart sample acquisition while sample acquisition is in process and the previously acquired events are desired to be deleted. *Restart* button is particularly useful when user wants to adjust the photodetector gain or threshold first to a proper value and then collect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



*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.



Checking the *Rinse after sampling* checkbox enables SIP rinse function after each sample acquisition.

4.4 Instrument Configuration

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

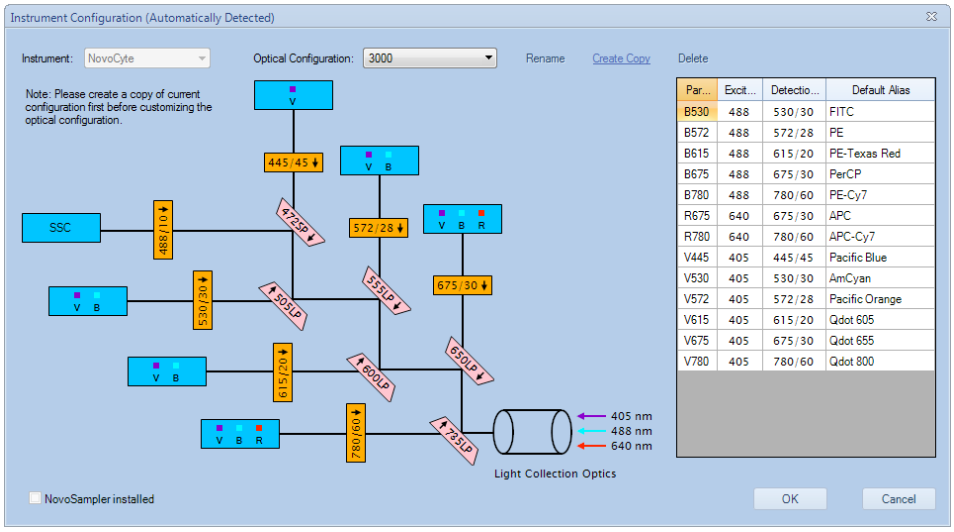
NovoExpress Software automatically detects the fluorescence parameters, excitation lasers, and detection channels of a connected NovoCyte instrument or NovoCyte Quanteon instrument. The software also detects if the NovoSampler or NovoSampler Pro is connected while connecting NovoCyte instrument, and detects if the NovoSampler Q is connected while connecting NovoCyte Quanteon instrument. Users are allowed to switch to other optical configurations provided by the software or to customize user-defined optical configurations in this window.

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.

4.4.1 Instrument Configuration with the NovoCyte Connected

The *Instrument Configuration* window displays the instrument type, the name and schematic of the current optical configuration, parameter window, and status of NovoSampler (Pro). When the NovoCyte 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, photodetectors and the excitation laser. User can switch to other available configurations provided by the software or define their own customized configuration in this window. Please refer to *Section 4.4.4* for more details on this function. 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 (Pro) is displayed in the lower left corner of this window. When the workstation is connected to the NovoCyte instrument and the instrument is powered on, it can automatically detect the installed NovoSampler (Pro) and this box will be automatically checked.



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

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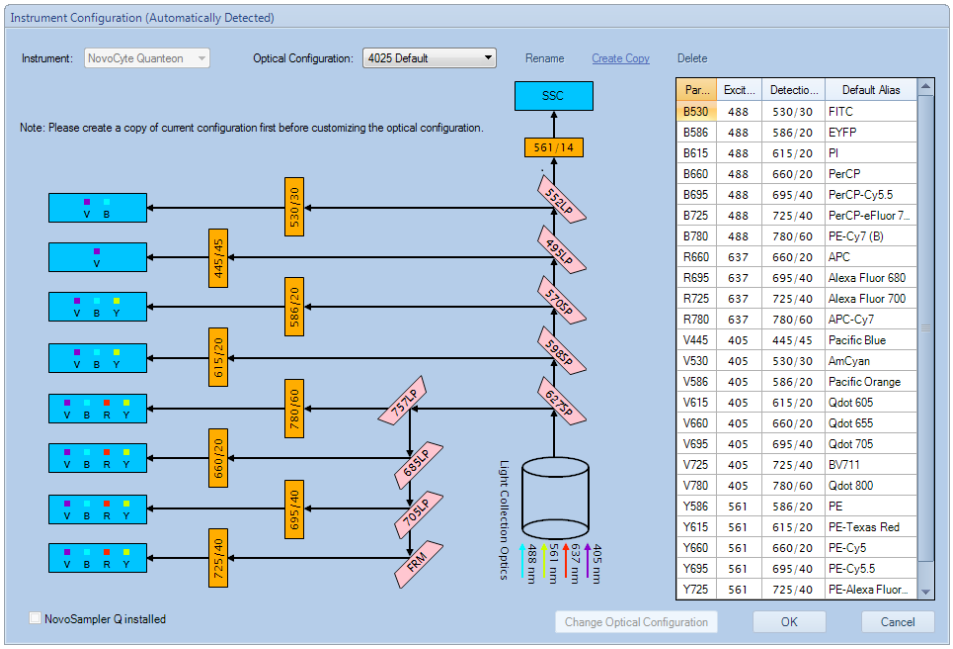
4.4.2 Instrument Configuration with the NovoCyte Quanteon Connected



The *Instrument Configuration* window displays the instrument type, the name and schematic of the current optical configuration, parameter window, and status of NovoSampler Q. When the NovoCyte Quanteon 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, and the excitation laser. User can define their own customized configuration in this window. Please refer to *Section 4.4.5* for more details on this function. 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 Q is displayed in the lower left corner of this window. When the workstation is connected to the NovoCyte Quanteon instrument and the instrument is powered on, it can automatically detect the installed NovoSampler Q and this box will be automatically checked.

4.4.3 Instrument Configuration with the NovoCyte (Quanteon) Disconnected

When there is no instrument connected to the workstation or the instrument is powered off, user can select NovoCyte or NovoCyte Quanteon 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 NovoCyte or NovoCyte Quanteon is connected to the workstation and powered on, the software will automatically detect the current hardware setting, and restore the correct optical configuration.

4.4.4 Modifying NovoCyte Instrument Optical Configuration

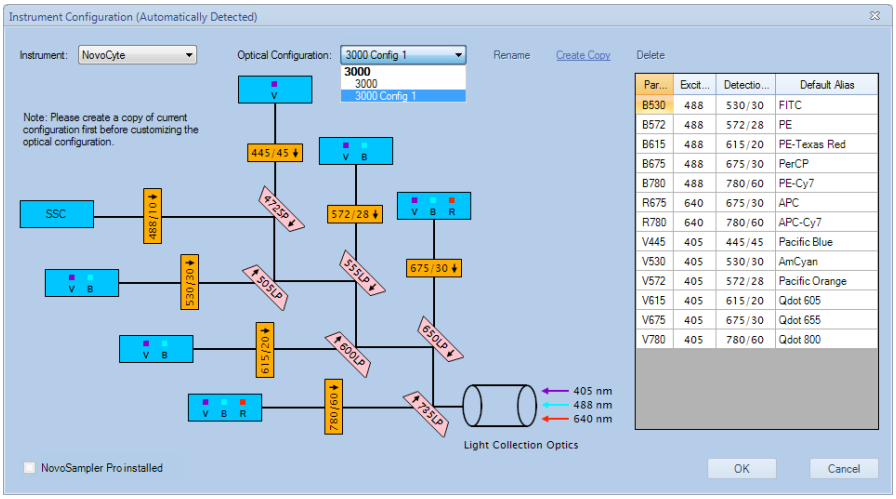
Users can modify the existing optical configuration to the alternative configurations provided by the software, or customize their own optical configuration. Users need to replace the appropriate optical filters and dichroic mirrors of the system and enter the changes into the *Instrument Configuration* window. To make the new optical configuration effective, a QC Test with automatic adjustment of photodetector gain will be performed to optimize instrument performance. This function provides user with more flexibility and convenience to match NovoCyte optical configuration with expanded fluorochrome panel.

To modify the instrument optical configuration:

1. Ensure the instrument is properly connected and powered on. Click *Instrument* → *Configuration* to open the *Instrument Configuration* window.

2. Click the *Optical Configuration* field. Click and select the desired optical configuration, and click *OK* to continue.

   By default, only the standard configurations (i.e. recommended by ACEA) will be displayed in this window. If configurations other than the ones listed in this window are needed, follow the instructions in Step 3.



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

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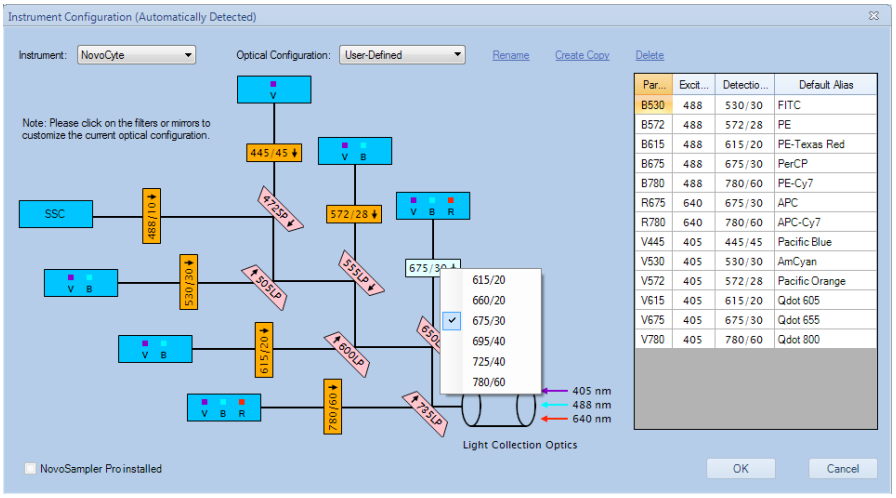
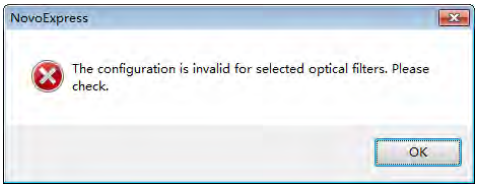
3 If optical configurations other than the ones listed by the software are needed (i.e. users need create their own customized configuration), the *Customize Optical Configuration* in the *Access Privilege* window for the current user account needs to be enabled first as described in *Section 2.6.2.3* in *NovoExpress® Software Guide*. To make the customized optical configuration, click *Create Copy* in the *Instrument Configuration* window to generate an editable copy of the configuration. Click *Rename* to rename the configuration if needed. Click the bandpass filter or dichroic mirror to be replaced and select the appropriate filter or mirror from the pop-up list. Once all the filters and mirrors desired to be replaced have been edited, click *OK* to continue. A customized optical configuration can also be deleted by clicking *Delete*.

Only the user-defined optical configurations can be deleted.



The software will automatically check the validity of user-defined configuration. The following error message will pop up if the user-defined optical configuration is not valid.



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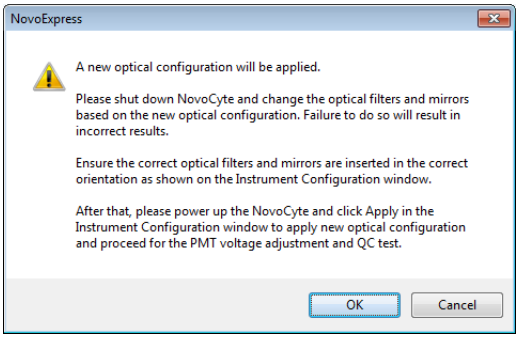
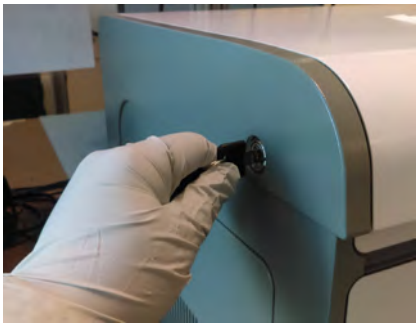
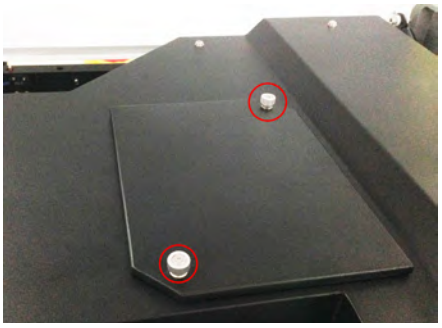
4 Ensure to read the instructions from the prompted window as below. To change the optical filters, press the power button on the front panel of NovoCyte to turn off the instrument first. Insert the NovoCyte key into the keyhole on the left side of the instrument to open the top cover of the instrument.



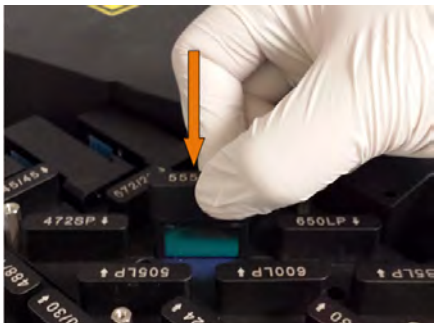
Remove the two mounting screws and open the cover of the filter module as shown below.





5 Gently hold the selected optical filter or dichroic mirror and pull it upward to remove it from the slot. Insert the new optical filter or dichroic mirror into the slot as shown in following figure. Record the position, wavelength and arrow direction for each optical filter and dichroic mirrors. Install the filter module cover by screwing in the two mounting screws. Close the top cover of the instrument. Press the power button on the front panel of NovoCyte to turn on the instrument.

Ensure all the new optical filters and mirrors are fully inserted in the correct filter slot and in the correct orientation as indicated by the arrows (i.e. the arrow of each optical filter and dichroic mirror should point away from the corresponding photodetector).



6 Wait until the instrument initialization process is completed. Click *OK* in the prompted window shown in step 4 to continue.

7 Ensure that the recorded optical configuration (i.e. the position, wavelength, and the orientation of each bandpass filter and dichroic mirror) matches the schematic of the selected optical configuration. Click *Apply* to continue.

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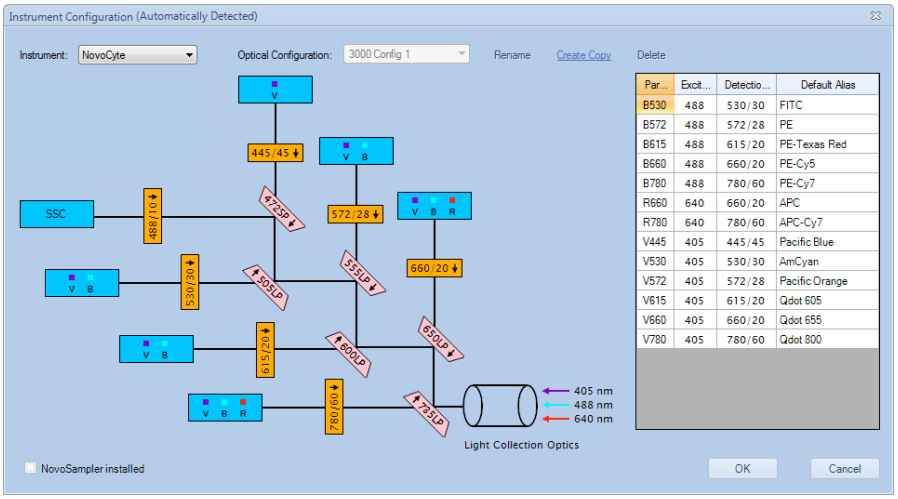
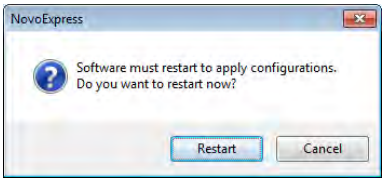
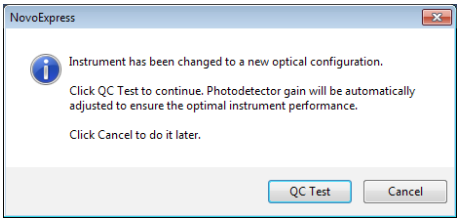


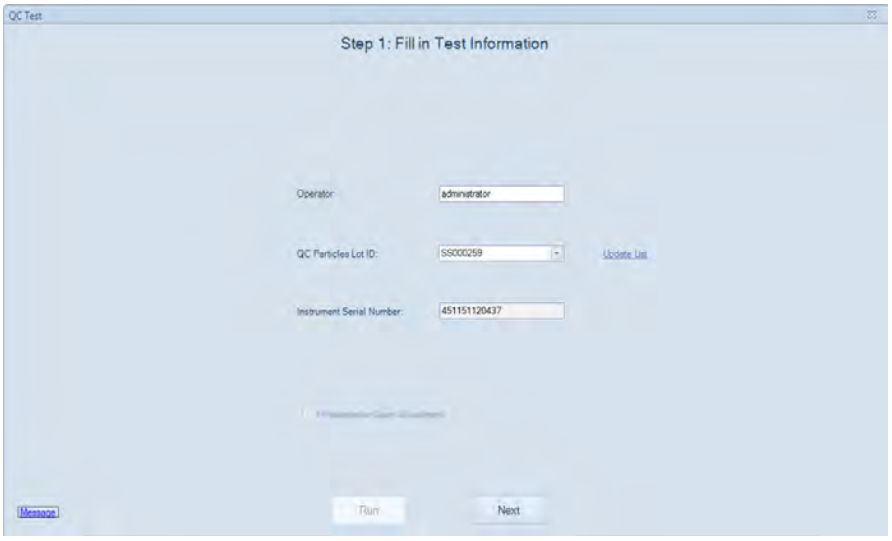
8 Click *Restart* in the next window to restart the software to apply the new optical configuration setting.



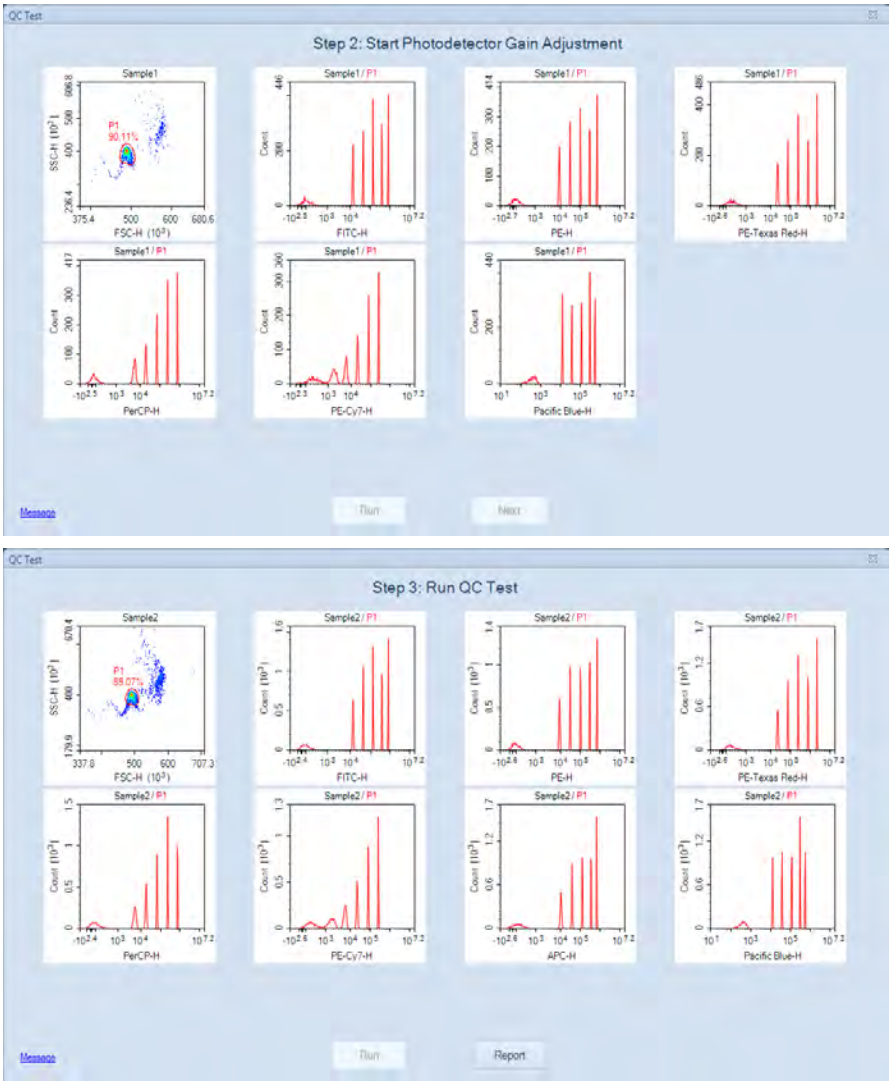
9 After NovoExpress is restarted, the following window will appear. Click *QC Test* to continue.



10 Properly prepare 1 mL ACEA QC particles sample as described in the *NovoCyte® Flow Cytometer Operator's Guide*. Place the sample tube in the tube holder or NovoSampler (Pro). Fill in the test information in the pop-up window. Click *Next* to continue.



11 Ensure all the test information is correct and the sample tube is properly installed on the tube holder, click *Run* to start. The software will automatically adjust the photodetector gain by running the QC particles. When the adjustment is completed, the software will automatically perform the QC Test. Click *Report* when the QC test is completed.



QC Test: Step 2 and Sep 3

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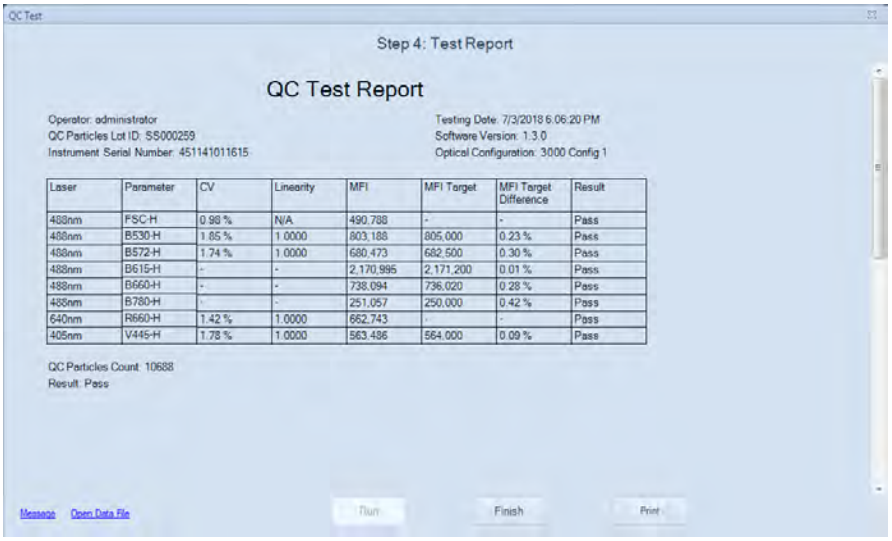
12 Ensure the result shows *Pass* for all the channels. Click *Finish* to close the window and complete the optical configuration modification.

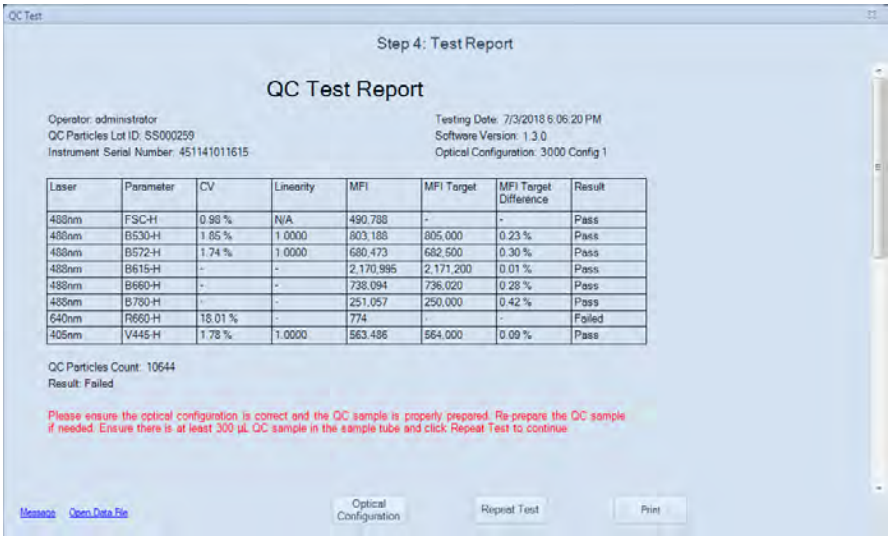


Laser	Parameter	CV	Linearity	MFI	MFI Target	MFI Target Difference	Result
488nm	FSC-H	0.98 %	N/A	490,788	-	-	Pass
488nm	B530-H	1.85 %	1.0000	803,188	805,000	0.23 %	Pass
488nm	B572-H	1.74 %	1.0000	680,473	682,500	0.30 %	Pass
488nm	B615-H	-	-	2,170,995	2,171,200	0.01 %	Pass
488nm	B660-H	-	-	738,094	736,020	0.28 %	Pass
488nm	B780-H	-	-	251,057	250,000	0.42 %	Pass
640nm	R660-H	1.42 %	1.0000	662,743	-	-	Pass
405nm	V445-H	1.78 %	1.0000	563,486	564,000	0.09 %	Pass

QC Test: Step 4

*If the result of certain detection channels shows **Acceptable** or **Failed** as below, make sure the optical filters configured in the hardware matches with the selected optical configuration in the software and the QC particles are properly prepared. Run the QC particles once again after the correct actions have been taken. Ensure there is at least 300 µL sample remaining in the sample tube. Click **Repeat Test** to repeat the QC test. The photodetector gain will be automatically re-adjusted.*

Please contact ACEA technical support if the QC test failed for three times in a row.



Laser	Parameter	CV	Linearity	MFI	MFI Target	MFI Target Difference	Result
488nm	FSC-H	0.98 %	N/A	490,788	-	-	Pass
488nm	B530-H	1.85 %	1.0000	803,188	805,000	0.23 %	Pass
488nm	B572-H	1.74 %	1.0000	680,473	682,500	0.30 %	Pass
488nm	B615-H	-	-	2,170,995	2,171,200	0.01 %	Pass
488nm	B660-H	-	-	738,094	736,020	0.28 %	Pass
488nm	B780-H	-	-	251,057	250,000	0.42 %	Pass
640nm	R660-H	18.01 %	-	774	-	-	Failed
405nm	V445-H	1.78 %	1.0000	563,486	564,000	0.09 %	Pass

QC Report Indicating Failed QC Test

4.4.5 Modifying NovoCyte Quanteon Instrument Optical Configuration

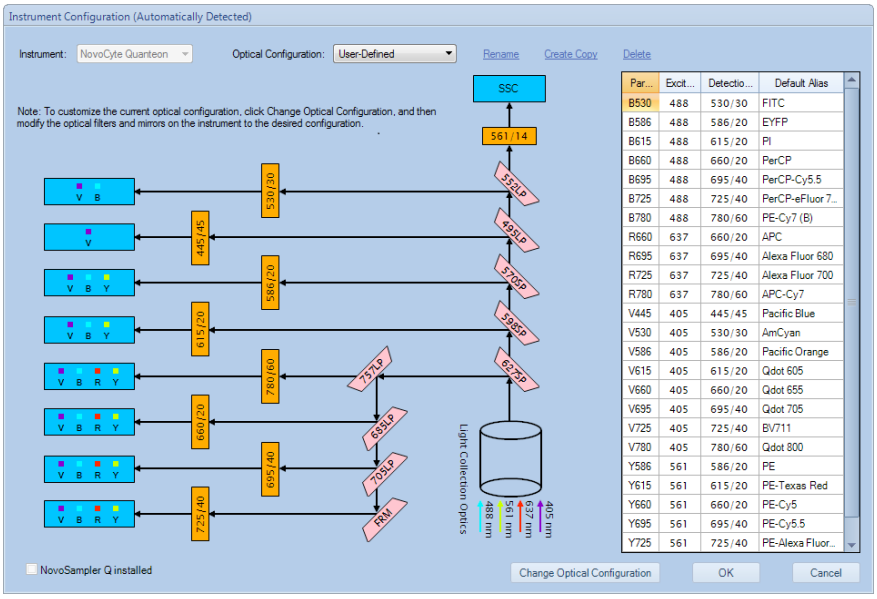
Users can customize the optical configuration on NovoCyte Quanteon. NovoCyte Quanteon has a sensor on each optical filter, and the instrument can directly read the information of each optical filter and automatically updates the optical configuration. A QC Test with automatic adjustment of photodetector gain will be conducted to optimize the instrument performance. This function on NovoCyte Quanteon provides a flexible and convenient way to reconfigure the optical detection channel to match a specific fluorochrome panel.

To modify the instrument optical configuration:

1. Ensure the instrument is properly connected and powered on. Click *Instrument* → *Configuration* to open the *Instrument Configuration* window.

2. The *Customize Optical Configuration* in the *Access Privilege* window for the current user account must be enabled first as described in *Section 2.6.2.3* in *NovoExpress® Software Guide*. To make the customized optical configuration, click *Create Copy* in the *Instrument Configuration* window to generate an editable copy of the configuration. Click *Rename* to rename the configuration if needed. Click *Change Optical Configuration* to continue. A customized optical configuration can also be deleted by clicking *Delete*.

   Only the user-defined optical configurations can be deleted.

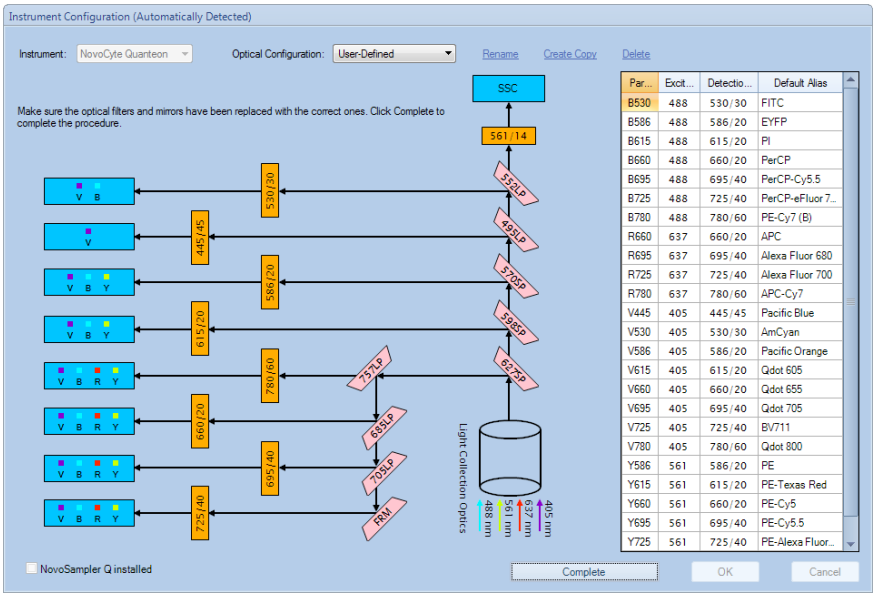


Par...	Excit...	Detectio...	Default Alias
B530	488	530/30	FITC
B586	488	586/20	EYFP
B615	488	615/20	PI
B660	488	660/20	PerCP
B695	488	695/40	PerCP-Cy5.5
B725	488	725/40	PerCP-eFluor 7...
B780	488	780/60	PE-Cy7 (B)
R660	637	660/20	APC
R695	637	695/40	Alexa Fluor 680
R725	637	725/40	Alexa Fluor 700
R780	637	780/60	APC-Cy7
V445	405	445/45	Pacific Blue
V530	405	530/30	AmCyan
V586	405	586/20	Pacific Orange
V615	405	615/20	Qdot 605
V660	405	660/20	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
Y660	561	660/20	PE-Cy5
Y695	561	695/40	PE-Cy5.5
Y725	561	725/40	PE-Alexa Fluor...

3. Open the top cover of the instrument. Insert one end of the Allen wrench through the hole. Gently hold the proper optical filter or dichroic mirror and pull it upward to remove it from the slot. Insert the new optical filter or dichroic mirror into the slot as shown in following figure. Close the top cover of the instrument.

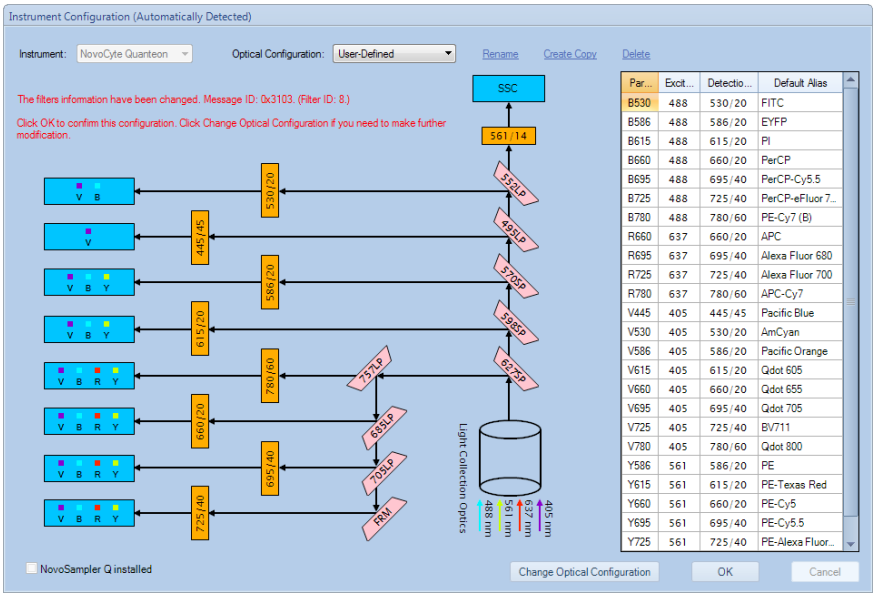
4

4 Click *Complete* to read new optical configuration.



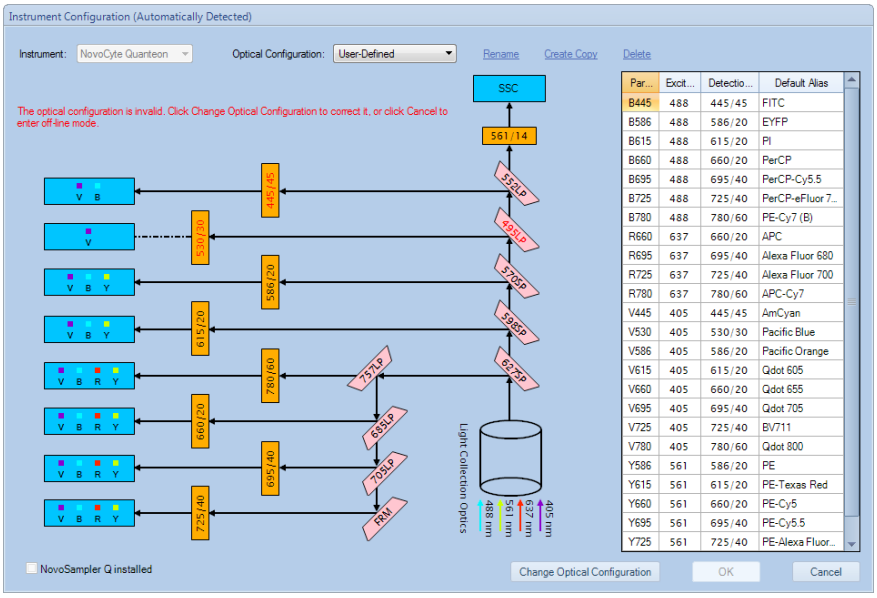
Par...	Excit...	Detectio...	Default Alias
B530	488	530/30	FITC
B586	488	586/20	EYFP
B615	488	615/20	PI
B660	488	660/20	PerCP
B695	488	695/40	PerCP-Cy5.5
B725	488	725/40	PerCP-eFluor 7...
B780	488	780/60	PE-Cy7 (B)
R660	637	660/20	APC
R695	637	695/40	Alexa Fluor 680
R725	637	725/40	Alexa Fluor 700
R780	637	780/60	APC-Cy7
V445	405	445/45	Pacific Blue
V530	405	530/30	AmCyan
V586	405	586/20	Pacific Orange
V615	405	615/20	Qdot 605
V660	405	660/20	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
Y660	561	660/20	PE-Cy5
Y695	561	695/40	PE-Cy5.5
Y725	561	725/40	PE-Alexa Fluor...

5 Click *OK* to confirm the new configuration.

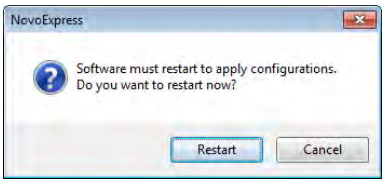


Par...	Excit...	Detectio...	Default Alias
B530	488	530/20	FITC
B586	488	586/20	EYFP
B615	488	615/20	PI
B660	488	660/20	PerCP
B695	488	695/40	PerCP-Cy5.5
B725	488	725/40	PerCP-eFluor 7...
B780	488	780/60	PE-Cy7 (B)
R660	637	660/20	APC
R695	637	695/40	Alexa Fluor 680
R725	637	725/40	Alexa Fluor 700
R780	637	780/60	APC-Cy7
V445	405	445/45	Pacific Blue
V530	405	530/20	AmCyan
V586	405	586/20	Pacific Orange
V615	405	615/20	Qdot 605
V660	405	660/20	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
Y660	561	660/20	PE-Cy5
Y695	561	695/40	PE-Cy5.5
Y725	561	725/40	PE-Alexa Fluor...

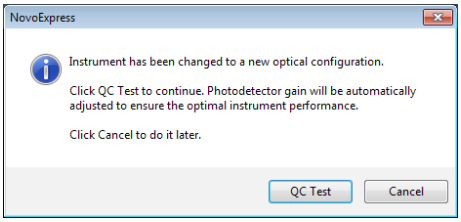
If the new optical configuration is invalid, there will be message in Instrument Configuration window.



6 Click *Restart* in the next window to restart the software to apply the new optical configuration setting.



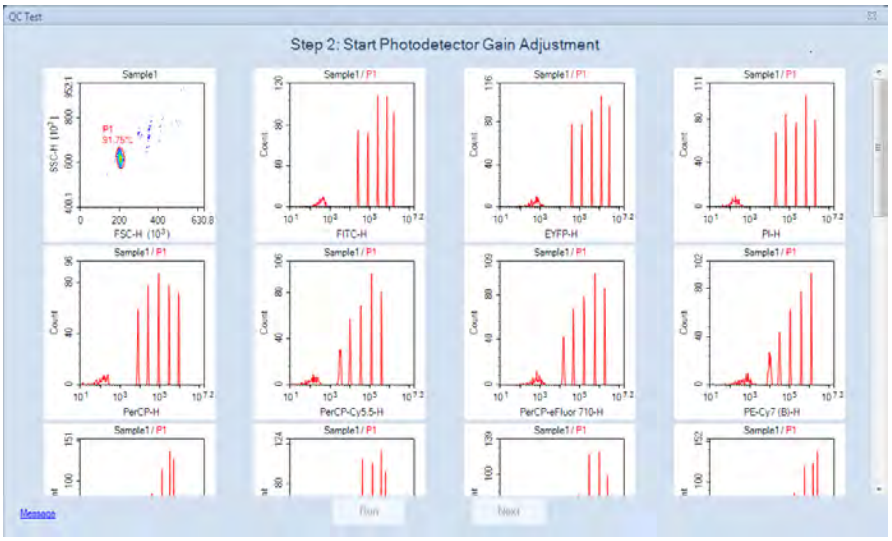
7 After NovoExpress is restarted, the following window will appear. Click *QC Test* to continue.

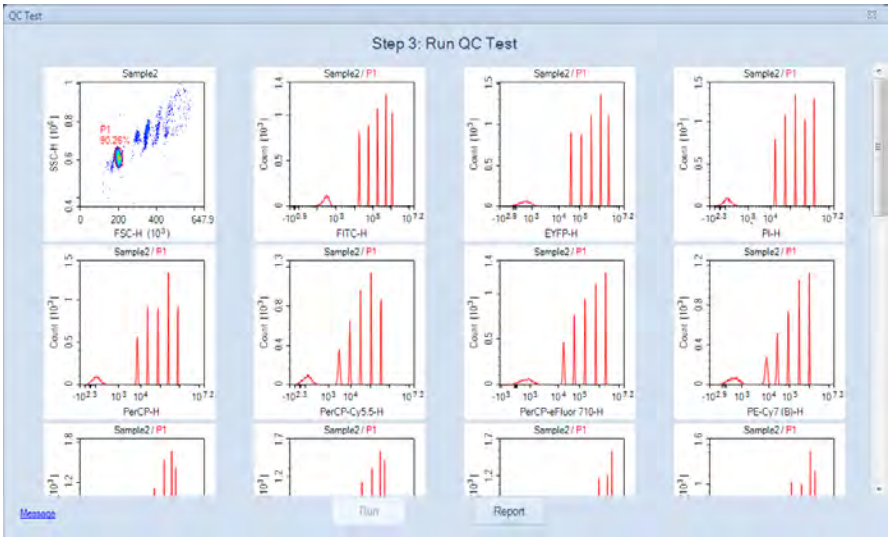


8 Properly prepare 1 mL ACEA QC particles sample as described in the *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*. Place the sample tube in the tube holder or NovoSampler Q. Fill in the test information in the pop-up window. Click *Next* to continue.

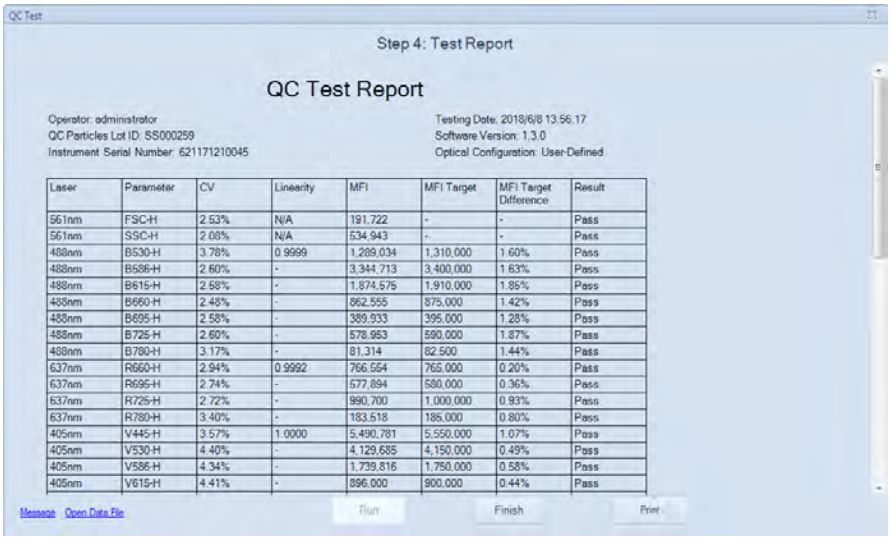
4

9 Ensure all the test information is correct and the sample tube is properly installed on the tube holder, click *Run* to start. The software will automatically adjust the photodetector voltage by running the QC particles. When the adjustment is completed, the software will automatically conduct the QC Test. Click *Report* when the QC test is completed.

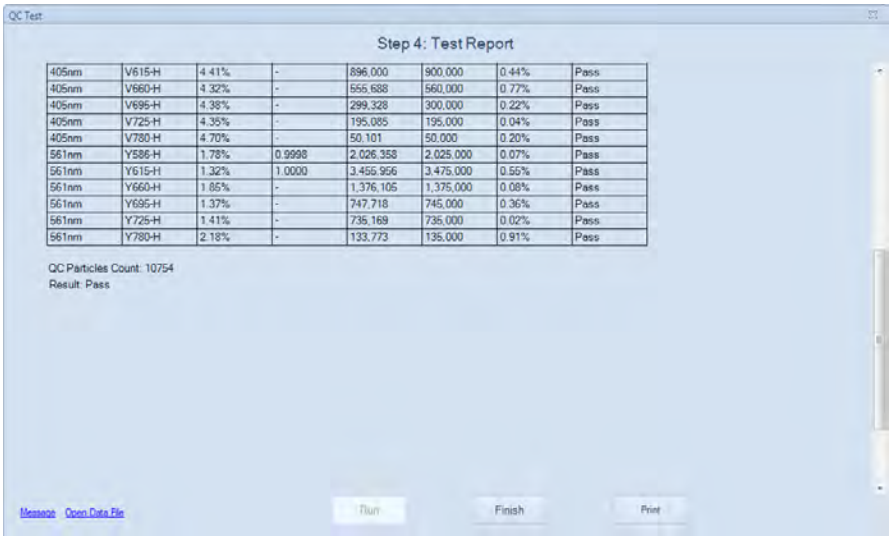




10 Ensure the result shows *Pass* for all the channels. Click *Finish* to close the window and complete the optical configuration modification.

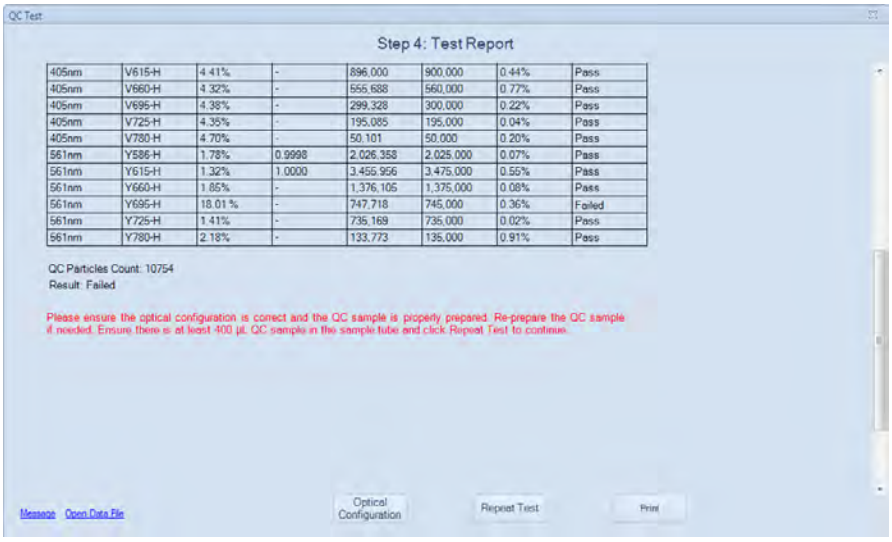


Laser	Parameter	CV	Linearity	MFI	MFI Target	MFI Target Difference	Result
561nm	FSC-H	2.53%	N/A	191,722	-	-	Pass
561nm	SSC-H	2.08%	N/A	534,943	-	-	Pass
488nm	B530-H	3.78%	0.9999	1,289,034	1,310,000	1.60%	Pass
488nm	B586-H	2.60%	-	3,344,713	3,400,000	1.63%	Pass
488nm	B615-H	2.58%	-	1,874,575	1,910,000	1.85%	Pass
488nm	B660-H	2.48%	-	862,555	875,000	1.42%	Pass
488nm	B695-H	2.58%	-	389,933	395,000	1.28%	Pass
488nm	B725-H	2.60%	-	578,953	590,000	1.87%	Pass
488nm	B780-H	3.17%	-	81,314	82,500	1.44%	Pass
637nm	R660-H	2.94%	0.9992	766,554	765,000	0.20%	Pass
637nm	R695-H	2.74%	-	577,894	580,000	0.36%	Pass
637nm	R725-H	2.72%	-	990,700	1,000,000	0.93%	Pass
637nm	R780-H	3.40%	-	183,518	185,000	0.80%	Pass
405nm	V445-H	3.57%	1.0000	5,490,781	5,550,000	1.07%	Pass
405nm	V530-H	4.40%	-	4,129,685	4,150,000	0.49%	Pass
405nm	V586-H	4.34%	-	1,739,816	1,750,000	0.58%	Pass
405nm	V615-H	4.41%	-	896,000	900,000	0.44%	Pass



*If the result of certain detection channels shows **Acceptable** or **Failed** as below, make sure the QC particles are properly prepared. Run the QC particles once again after the correct action has been taken. Ensure there is at least 400 µL sample remaining in the sample tube. Click **Repeat Test** to repeat the QC test. The photodetector voltage will be automatically re-adjusted.*

Please contact ACEA technical support if the QC test failed for three times in a row.

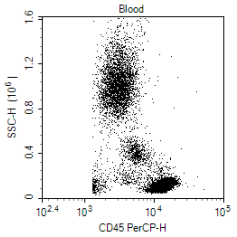
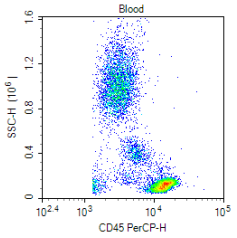
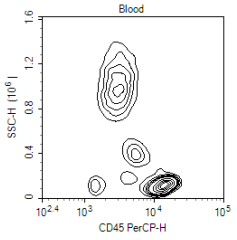
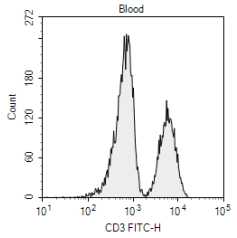


5. Data Analysis

Data analysis tools in the NovoExpress Software include plots, gates, and statistical analysis functions. The plots enable users to visualize events based on measured parameters, and gates allow for separation of subpopulations for further statistical analysis.

5.1 Plots

The NovoExpress Software includes dot plots, density plots, contour plots, histograms, and the option for cells cycle diagrams for cell cycle analysis.

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.	

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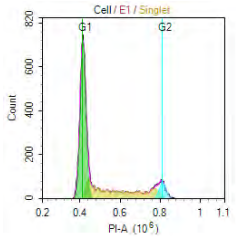
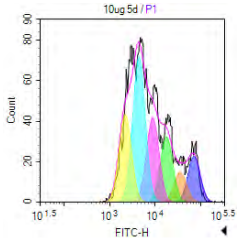
Plot Type	Icon	Display Parameters	Description	Example
Cell Cycle Analysis		Single-parameter	DNA content is represented along the horizontal axis, and the number of events at each value is represented along the vertical axis. The cell cycle fitting algorithm is used to separate the population into G1, S, and G2 phases of the cell cycle. See *Section 5.5* for more information.	
Cell Proliferation Analysis		Single-parameter	Cell Proliferation Analysis can be used to analyze the samples containing cell proliferation information and show the fitting results. See *Section 5.6* for more information.	

5.1.1 Creating a Plot

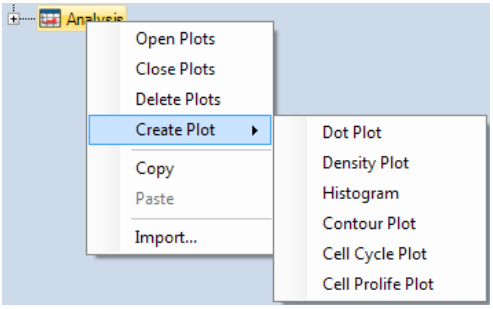
In the NovoExpress Software, plots can be created through the toolbar, the *Experiment Manager*, and the *Gate Manager*. In addition, plots can be duplicated in the *Workspace*, copied in the *Experiment Manager*, and imported from templates.

5.1.1.1 Creating 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.

5.1.1.2 Creating 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.



5.1.1.3 Creating a Plot from a Gate

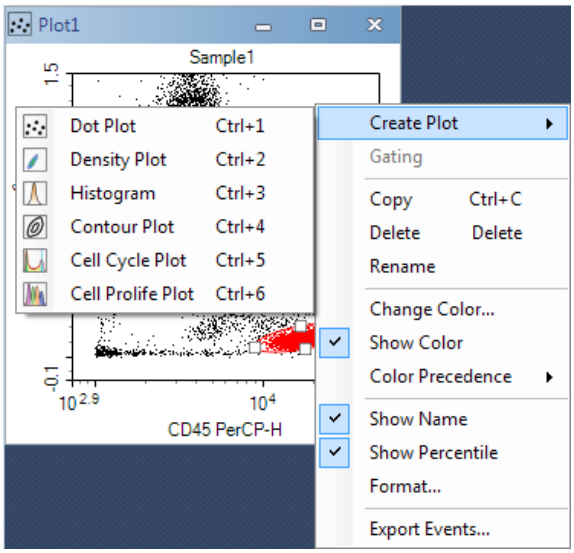
In the *Workspace*, new plots can be created using gates from previously created plots. New plots created through gates will only display events within the gate. There are multiple methods for creating a new plot through a gate:

- 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.

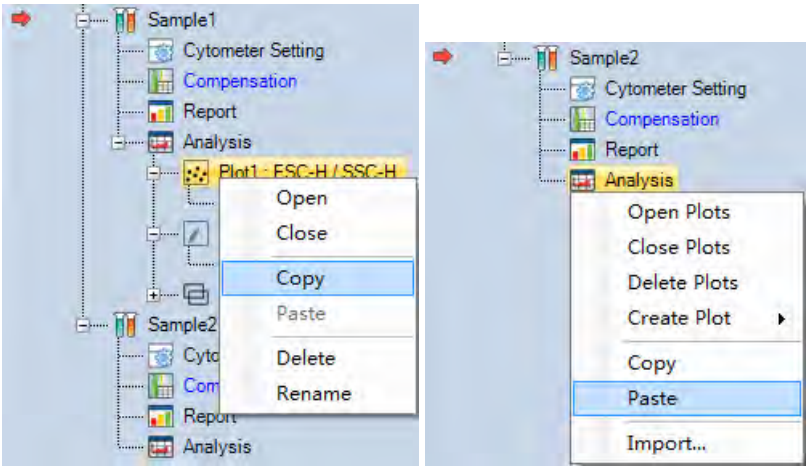
5.1.1.4 Creating a Duplicate Plot

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.

5.1.1.5 Copying and Pasting 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.



*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.*

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

5.1.1.6 Importing 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.

5.1.2 Opening and Closing 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.

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

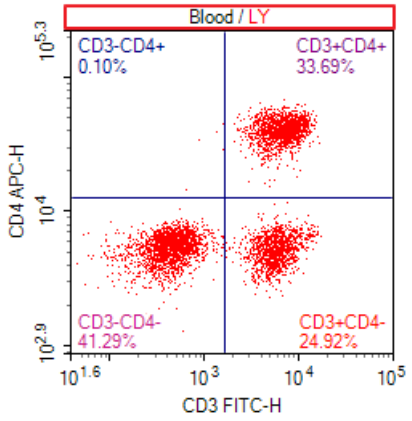
5

5.1.3 Editing Plots

5.1.3.1 Plot Gating

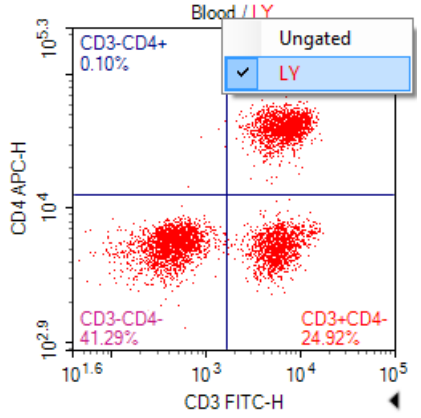
To analyze subpopulations, plots can be set to only display events from within a specific gate. For this method, a gate from a previous plot will be applied to a newer plot. The new plot can then be used to analyze the subpopulation or to further gate for more specific populations.

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:

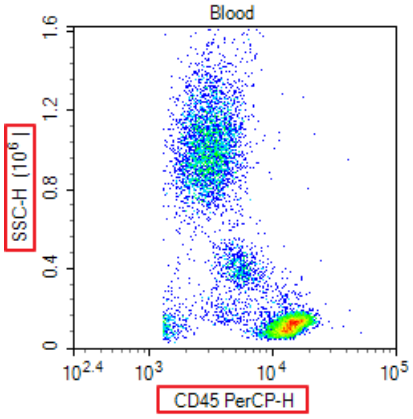
- In the plot header, right-click to display a drop-down menu. In the drop-down menu, select the gate. If *Ungated* is selected, the plot is not gated and all events are displayed in the plot.



- 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.

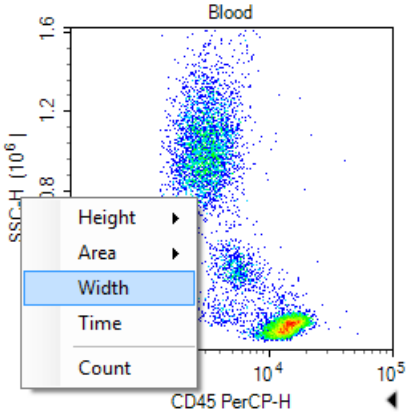
5.1.3.2 Parameter Plot Settings

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.

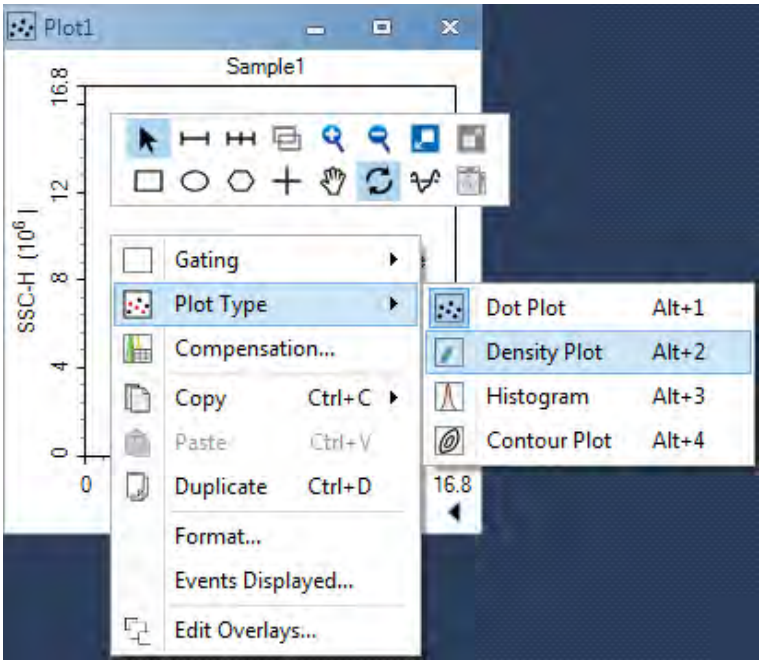


5

5.1.3.3 Setting 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.

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.

5.1.3.4 Renaming 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.

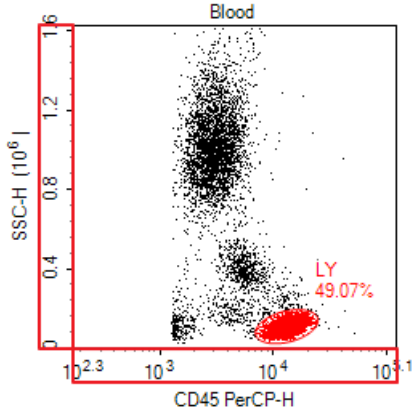
5.1.3.5 Deleting Plots

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.

5.1.4 Setting the Coordinates of the Axis

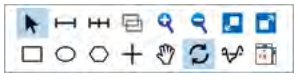
As shown in the figure below, the coordinates of an axis are labeled next to the axis. The axis multiplier is labeled within parentheses in the axis label.



5.1.4.1 Setting 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.

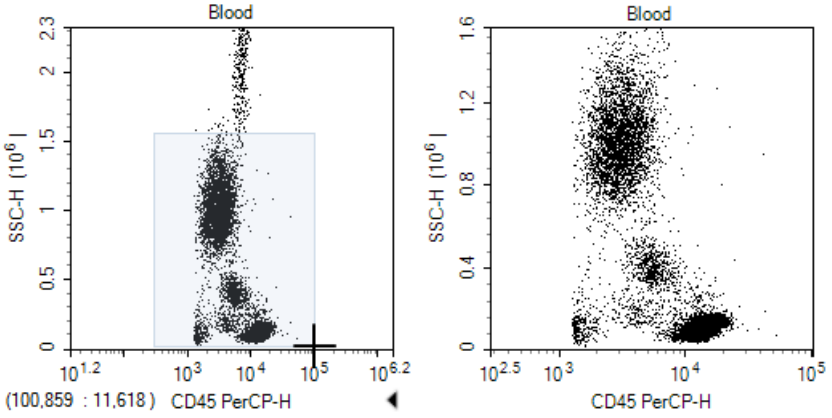
  Move the cursor to the maximum or minimum position of the X or Y coordinate. The cursor will change to ⇔ for X coordinate or ⇕ for Y coordinate. Click and move the cursor to change the maximum or minimum value of the corresponding value. Double clicking the arrow will set the axis to *Auto Range* on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



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.

  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.

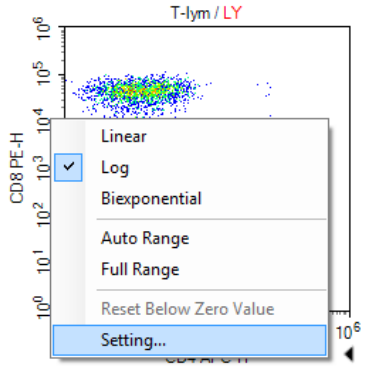
  There are multiple methods to access this tool. This tool can be activated by clicking on the icon in the *Workspace* toolbar, using the keyboard shortcut Ctrl+M, or right-clicking on a plot and selecting *Move*.

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

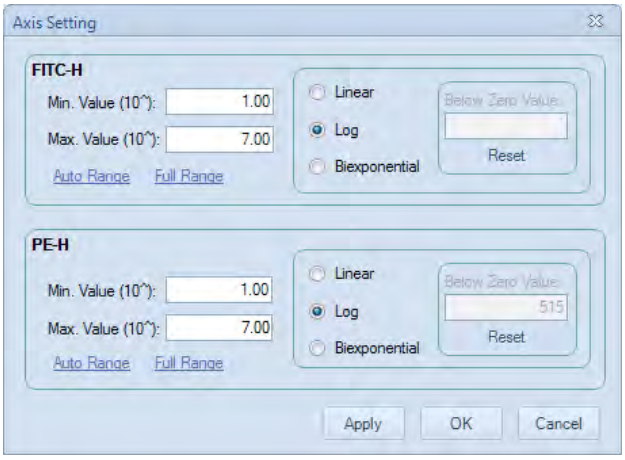
To pan the plot only along one parameter, click and drag along the parameter's coordinate label. This method causes the plot to pan along the selected parameter, while the second parameter's coordinate range will remain the same.

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

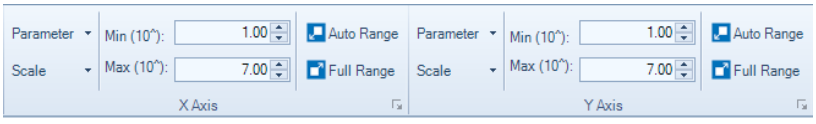
  To access the *Axis Setting* window, right-click on the coordinate label of a plot and select *Setting*.



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



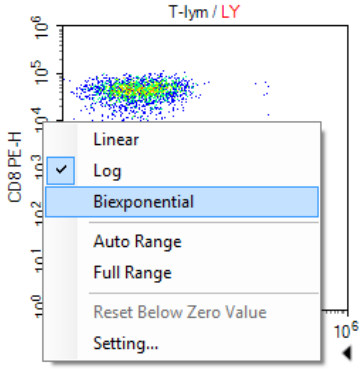
- Manually Setting the Coordinate Range in the *Plot* tab of menu bar.



5.1.4.2 Setting 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.

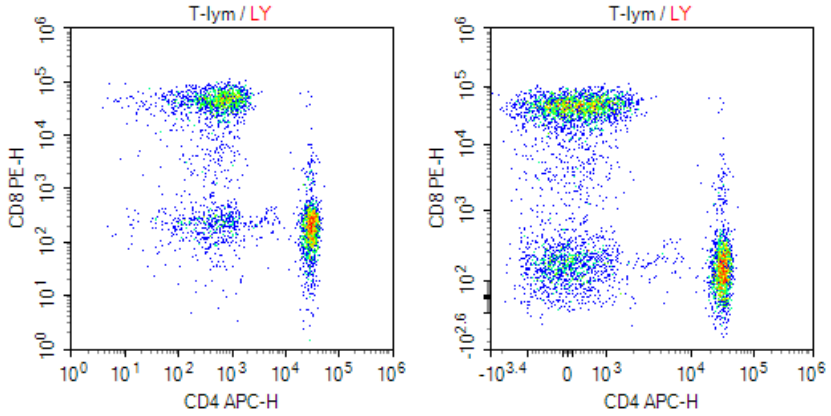
To set the coordinate scaling, right-click on the coordinate label and select the axis scaling.



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

5.1.4.3 Displaying a Biexponential Plot

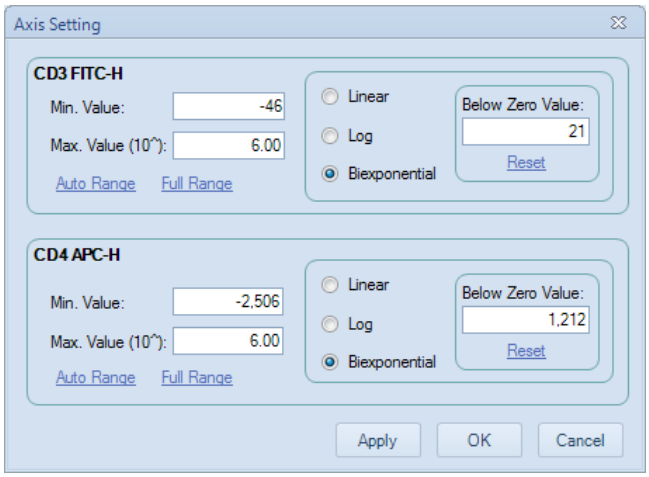
Biexponential display uses biexponential scale to transform data, especially for those where cells become piled up in the first decade at the axis. This is displayed as fluorescence values <0 even for uncompensated data. Biexponential transformation incorporates linear scaling for low values together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



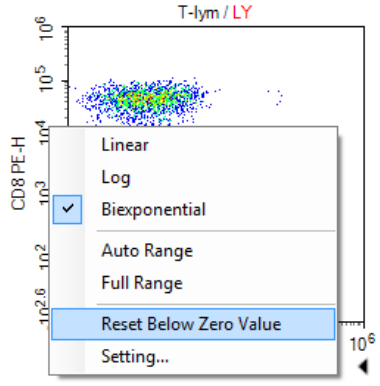
Below Zero Value of biexponential scale

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.

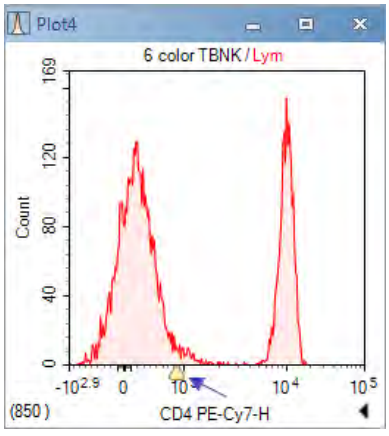
Manually enter the Below Zero Value in *Axis Setting* dialog or click *Reset* button to let NovoExpress software calculate the value automatically. When resetting, software calculates the Below Zero Value according to the events data in current gating of plot. The minimum value of the axis will be automatically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

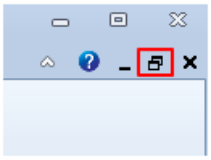


5.1.5 Adjusting the Size of Plots

- 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*.

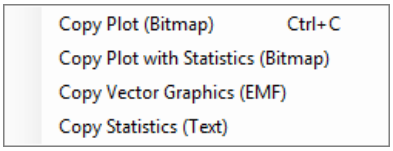
Adjusting the size of the plot window does not affect the coordinate range of the plots. To adjust the coordinate ranges, see *Section 5.1.4.1*.

5.1.6 Copy or Save Plots

Plots from the NovoExpress Software can be copied and saved.

- To copy a plot to the clipboard

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

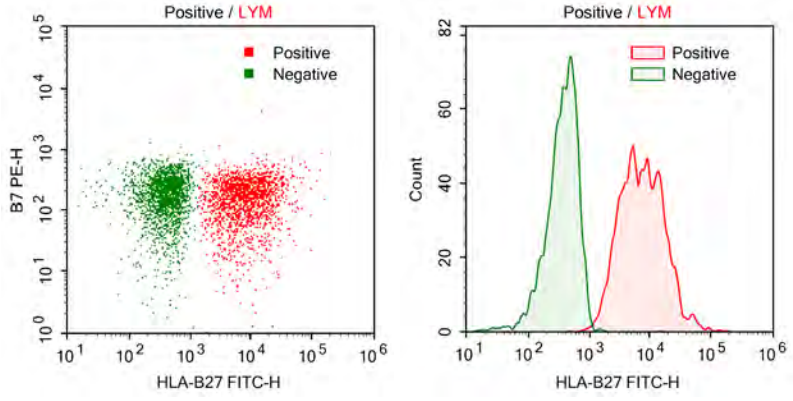


- To save a plot

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

5.1.7 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.

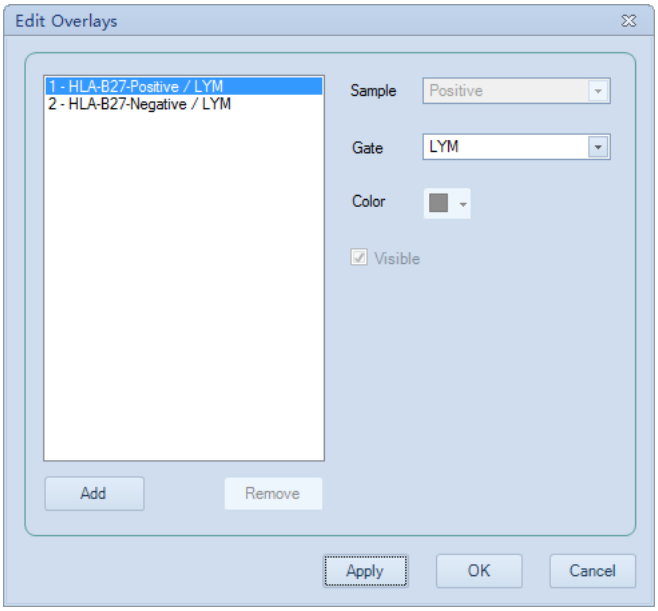


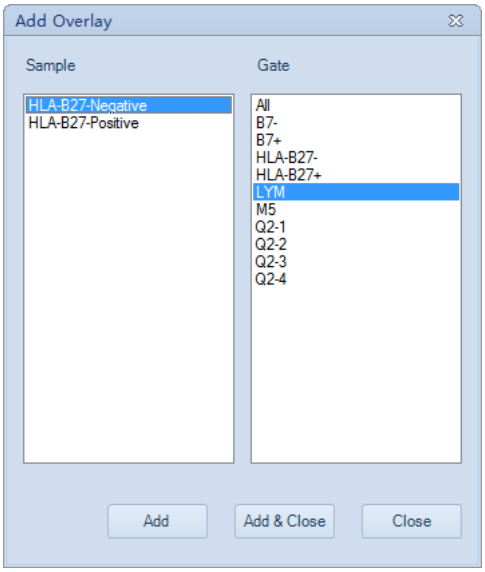
- Add overlays by using the drag and drop method:

  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 take the same name as the gate of plot. It will be the *All* gate, if no gate with the name is found in the sample of the overlay.

- Edit overlays:

  Right-click a plot to access the shortcut menu, select *Edit Overlays* to generate the *Edit Overlays* dialog window as shown below. In the dialog, all overlays of the plot are listed. One can select an overlay, set the overlay's sample, gate or color, and make a choice to show or hide the selected overlay on the plot. Adding new overlays or deleting overlays can also be done here. Click *Add* button to open the *Add Overlay* window, press Ctrl or Shift key while clicking the selected sample (s), click *Add* or *Add & Close* in this window to add selected sample (s) to the *Edit Overlays* window. Click *Apply* and *OK* to complete adding the new overlays to the current plot.

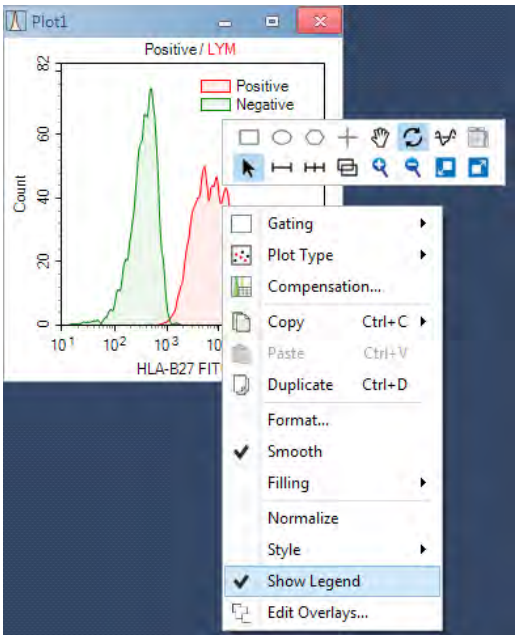




5

- 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.

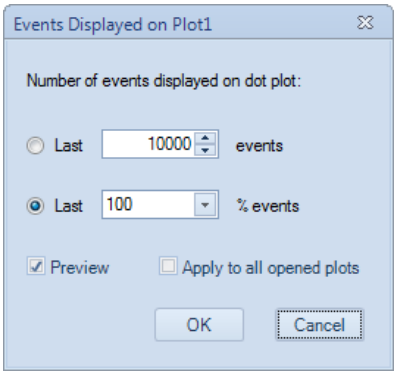


5.1.8 Plot Formatting

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.

5.1.8.1 Dot Plot Formatting

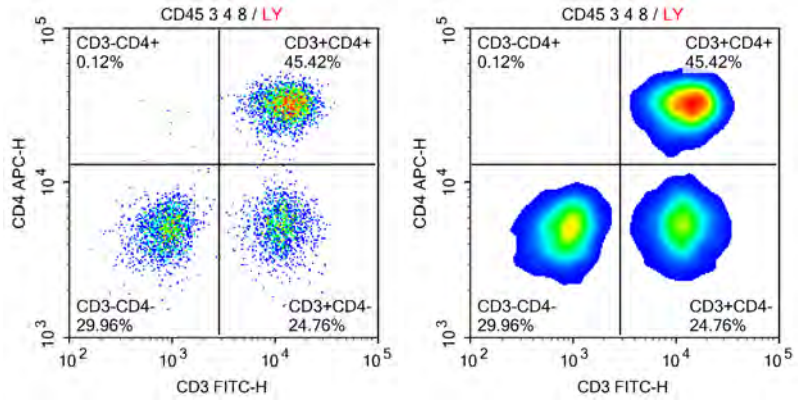
With dot plots, there is an option to only display the most recently collected events. This option allows the user to set a number or percentage of the most recently collected events to display. To open the *Events Displayed* window, right click on the dot plot and select *Events Displayed…*.



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.

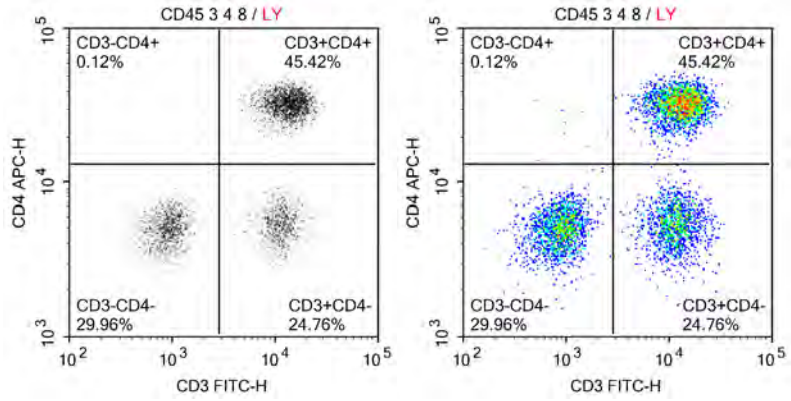
5.1.8.2 Density Plot Formatting

- 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.



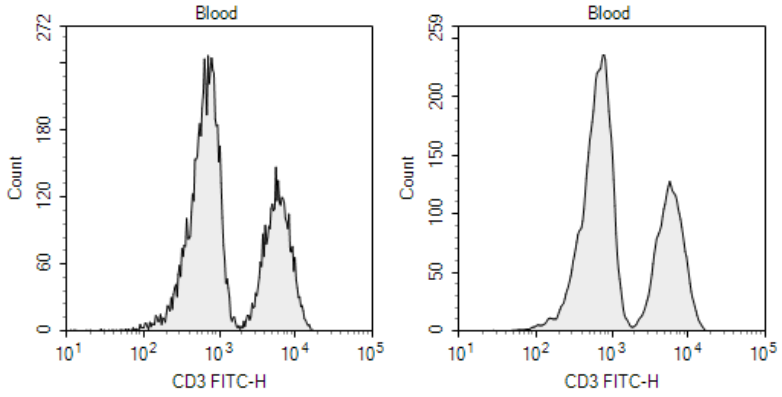
- Pseudocolor density plot: By default, density plots are displayed in pseudocolor. In this view, areas of the plot with a higher density of events are shown in warmer colors (colors toward the right of the color bar below) and areas of the plot with a lower density of events are shown in cooler colors (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.

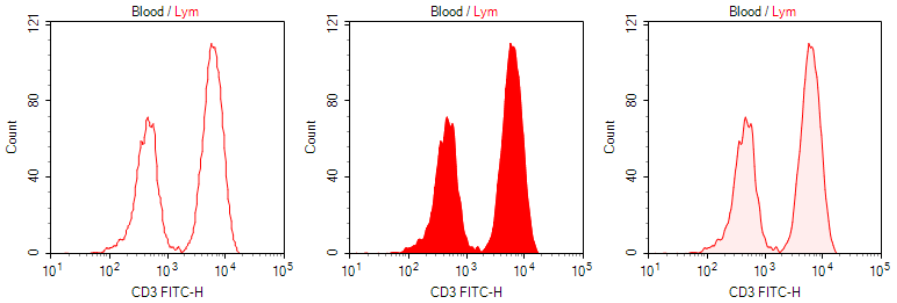


5.1.8.3 Histogram Plot Formatting

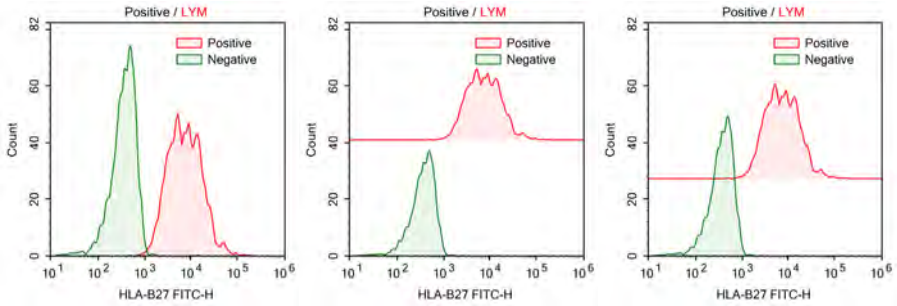
- Smooth histogram: To smooth the edges of a histogram, right-click on a histogram plot and select *Smooth* or select *Smooth* from the *Plot* tab of the *Menu Bar*. A comparison of a standard histogram plot (left) and a smooth histogram plot (right) is shown below.



- 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).

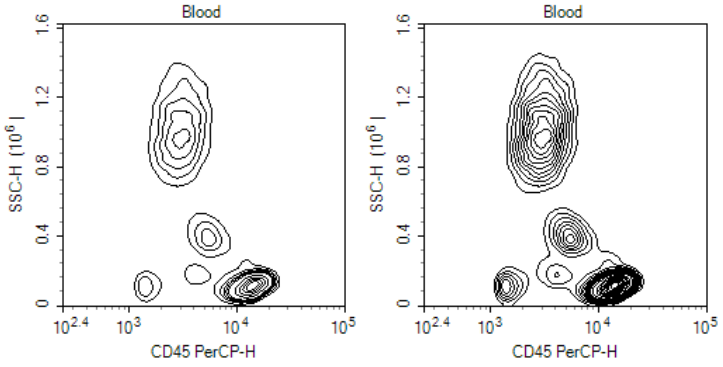


- Histogram layering: When overlaying histogram plots, different overlay styles can be selected. To select an overlay style, right-click in a histogram plot with layers and select *Style* to select the overlay style. The overlay style options, as shown below, include *Overlaid* (left), *Offset* (middle),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

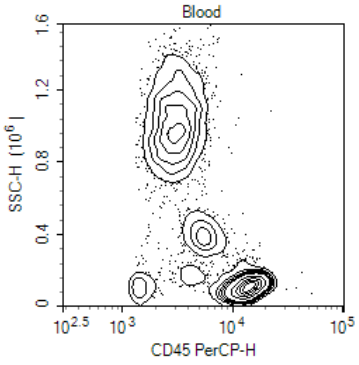


5.1.8.4 Contour Plot Formatting

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).

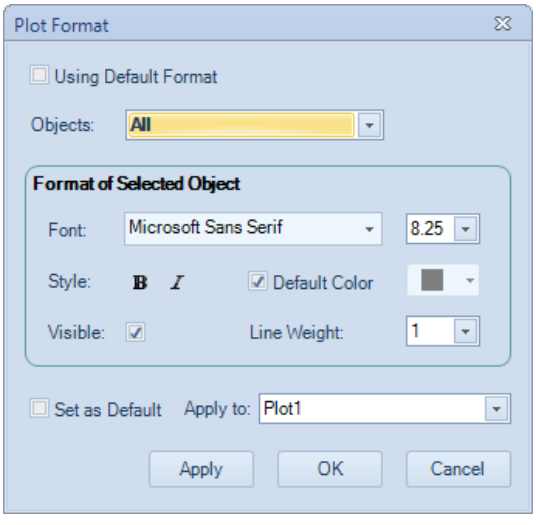


Show Outlier: If selected, outlier events are shown as dots on contour plots. A contour plot is shown below with *Show Outlier* selected.



5.1.8.5 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*:

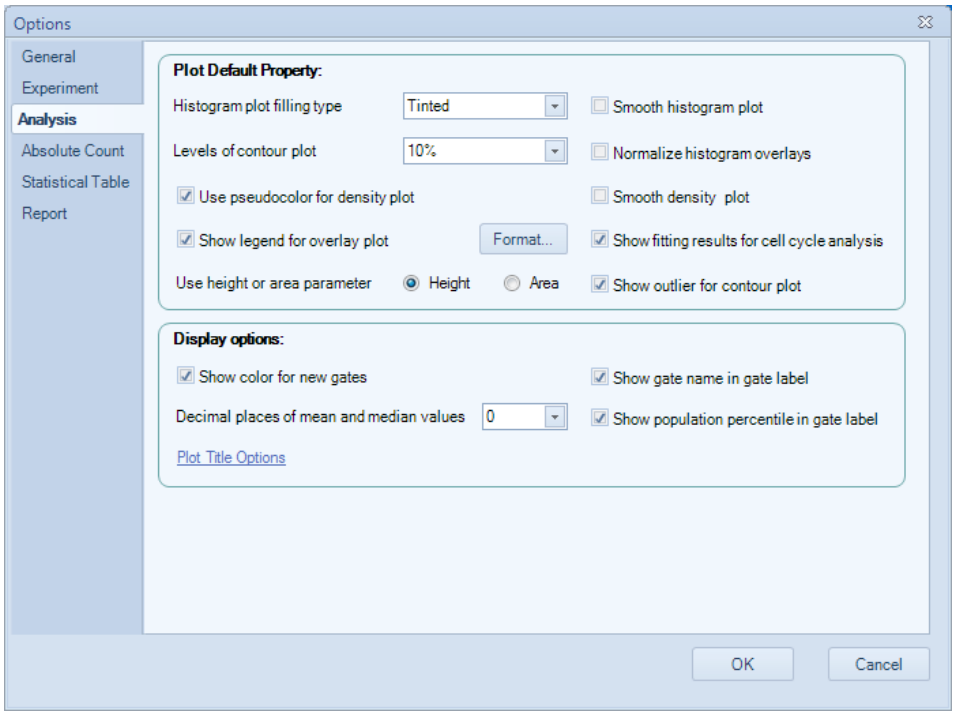
  Click to apply changes and close this window.

- *Cancel*:

  Click to close this window without applying any changes.

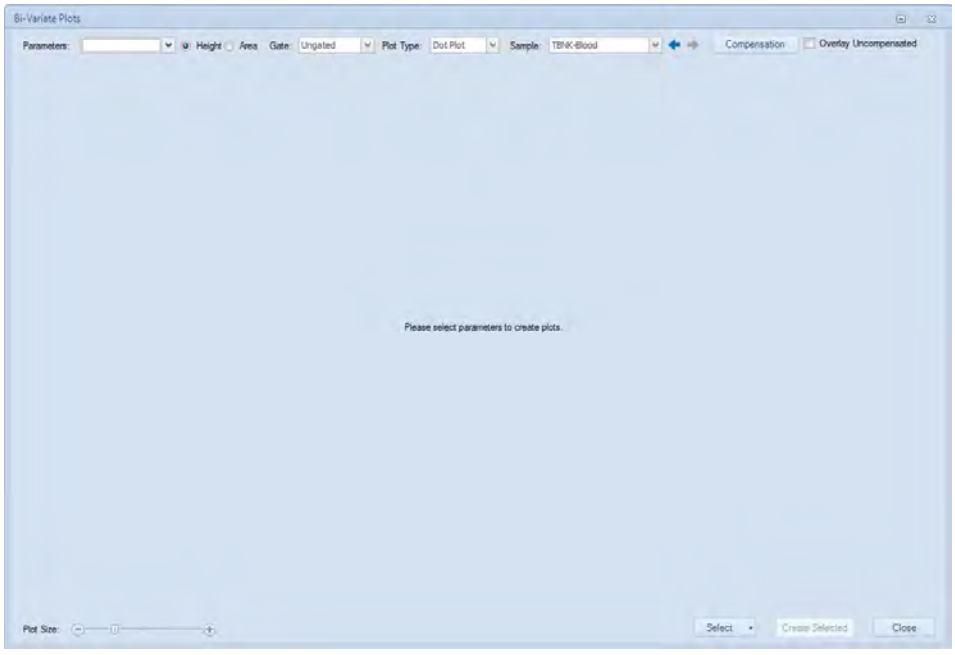
5.1.8.6 Default Plot Settings

To change the default settings for plots, go to *File* → *Options* and select the *Analysis* tab. See *Section 3.3.8 Setting* for details.



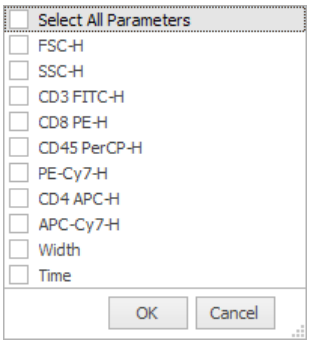
5.1.9 Bi-Variate Plots

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 N×N 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:*

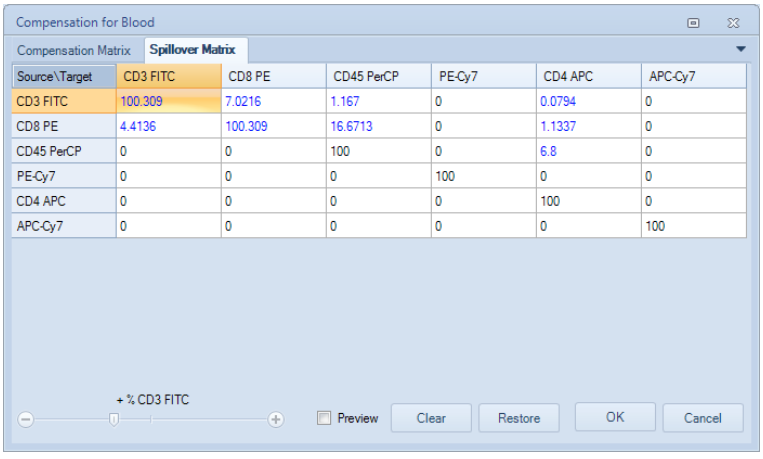
  Select plot type for the created bi-variate plots, including *Dot Plot, Density Plot,* and *Contour Plot*.

- *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.

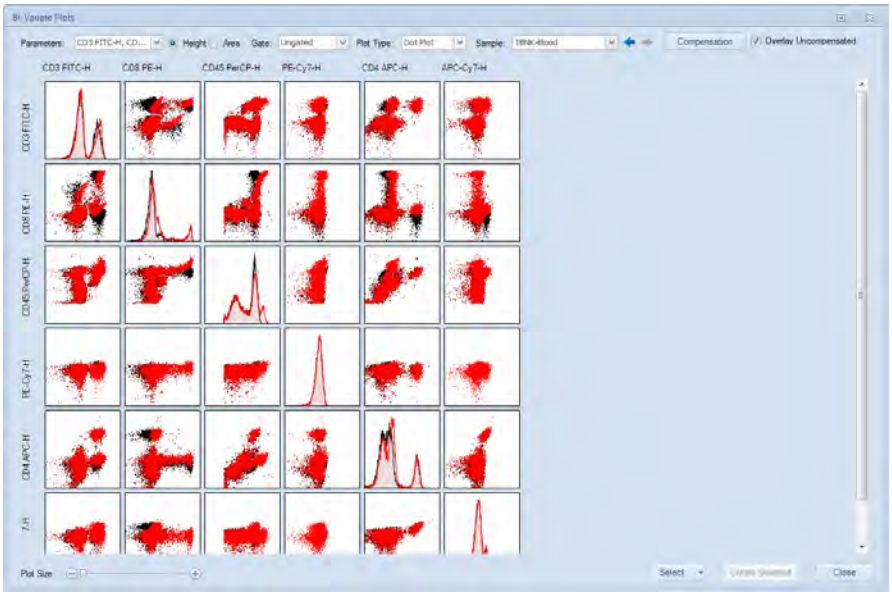
- *Compensation:*

  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 *Section 5.4.2* for detailed description.



- *Overlay Uncompensated:*

  Select to overlay uncompensated data on the created bi-variate plots.

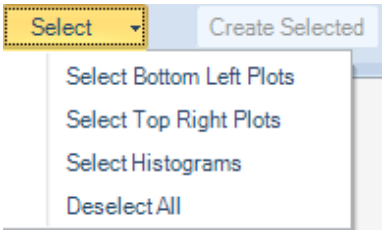


- *Plot Size:*

  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:*

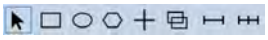
  Click to close *Bi-Variate Plots* window.

5.2 Gates

Gates allow for the analysis of subpopulations from the total population collected. As described in *Section 5.1.3.1* 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 , quadrant gates , logic gates , range gates , and bi-range gates .



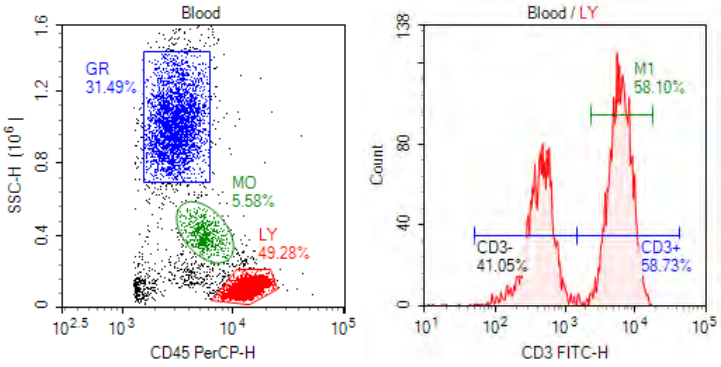
The gating tools can be also selected from theright-click popup mini toolbar on plots.

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.

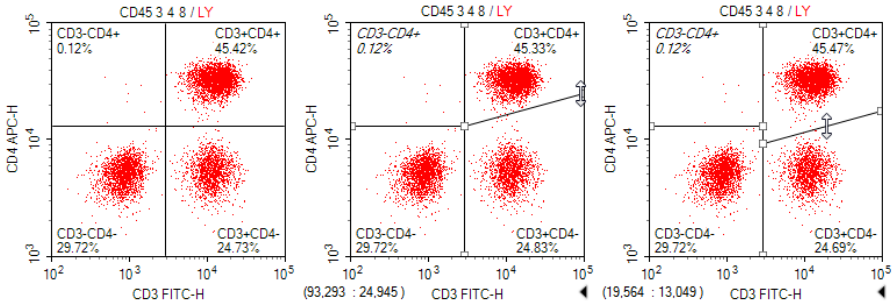
5.2.1 Creating Gates

- 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.

  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.

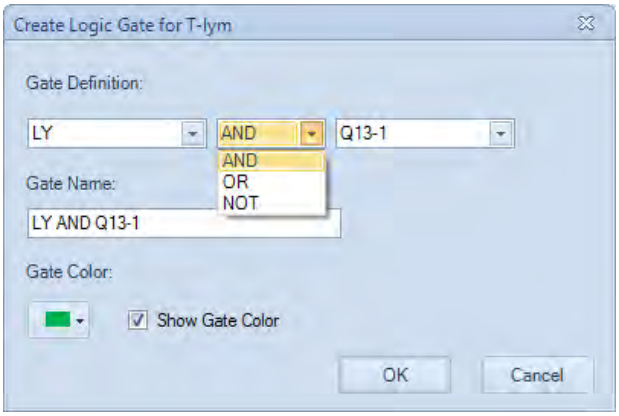


- To create a logic gate: Click the logic gate icon , in the *Workspace Toolbar* to open the *Create Logic Gate* window. In this window, the user can create a logic gate for the sample in the selected plot.

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

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



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

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.*

5.2.2 Editing 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.

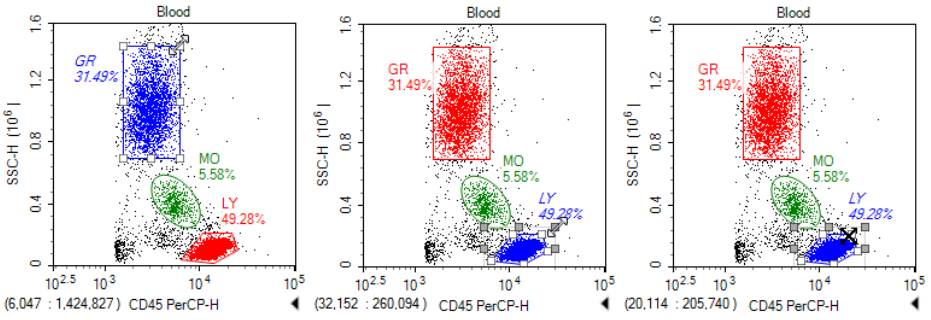
There are multiple methods to select a gate for editing. Options include:

- 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.

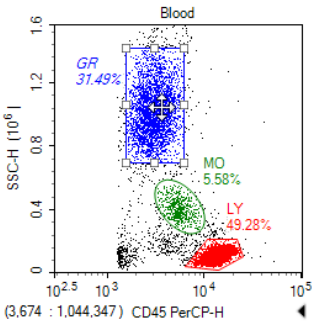
After the gate is selected, the gate's control points are displayed (as white boxes). To change

the size of a gate, click and drag the control points to modify the gate.

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.

5.2.3 Gate Display Format

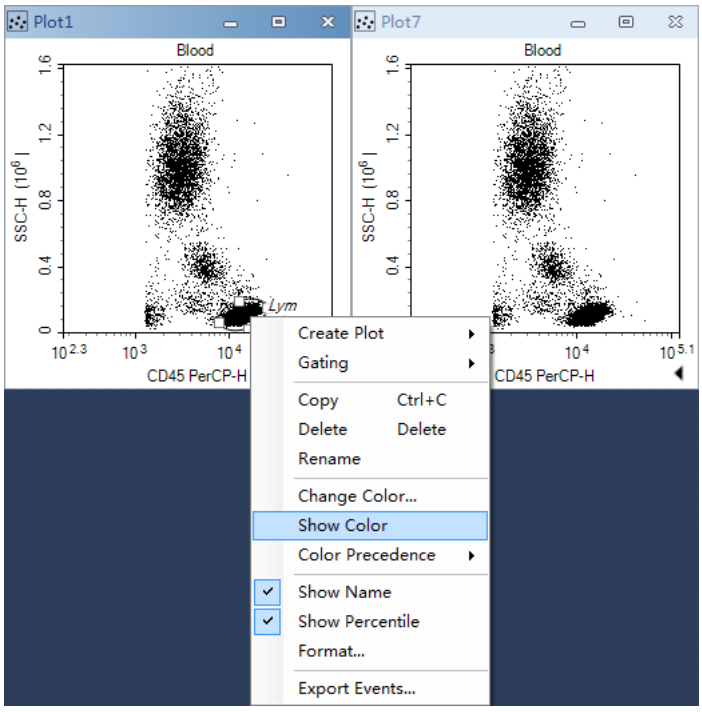
The NovoExpress Software allows users to format the color and labels of gates. Gate color determines the color of events displayed on the dot plot, as well as the color of the histogram when the gate is applied to a histogram.

5.2.3.1 Set the Color of the Gate

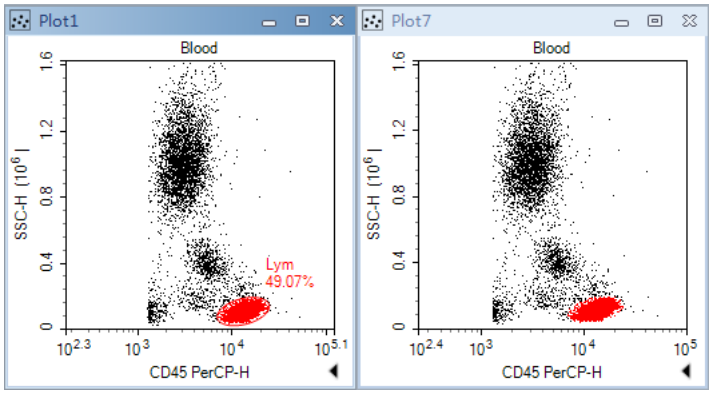
The color of each gate can be set. In dot plots, the events included in the gates are displayed in the chosen color. If additional dot plots are created, these events are displayed in the same color for easy identification.

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.

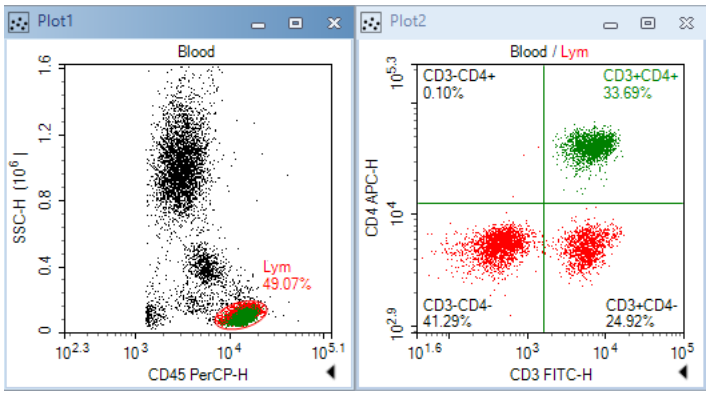


The plots below have a gate with the color selected.



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 *Section 5.2.7*.



5

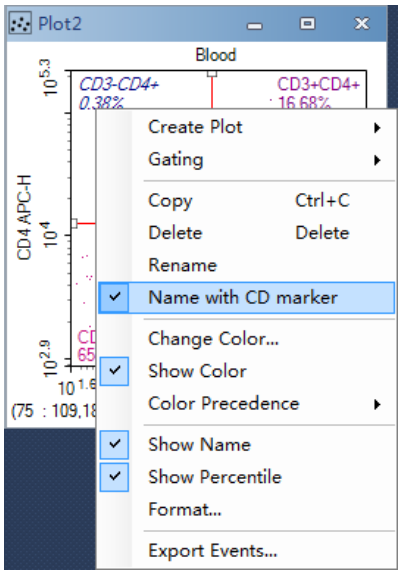
5.2.3.2 Gate Labels

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.



5.2.4 Applying 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.

5.2.5 Copying and Pasting Gates

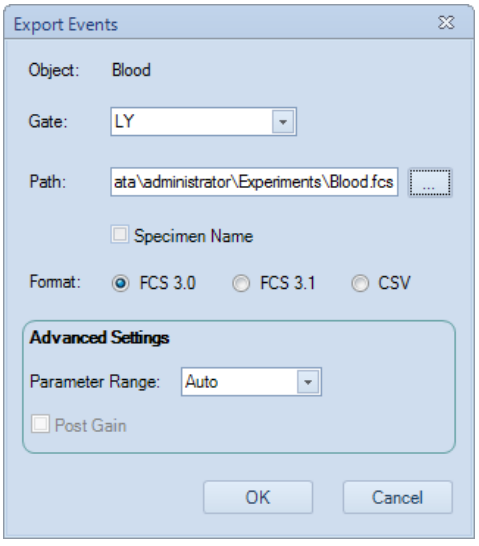
There are multiple methods to copy and paste a gate:

- 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.

5.2.6 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.

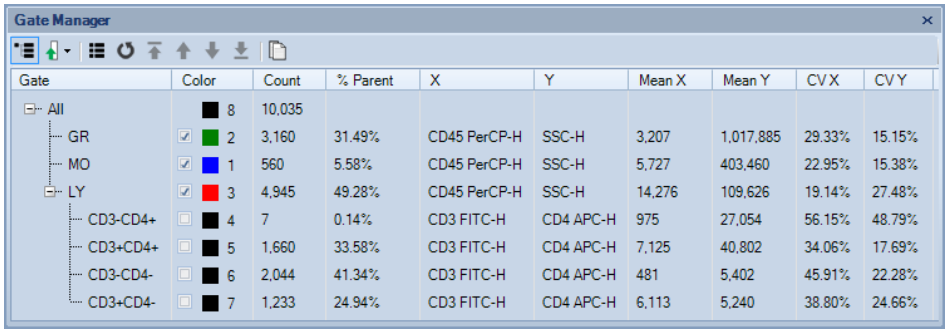


3. In the window, set the export path, file format, parameter range and post gain. Click *OK* to export the data.

For more information regarding this window, see Section 6.4.2.

5.2.7 Gate Manager

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



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

5.2.7.1 Toolbar of Gate Manager

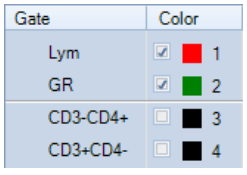
Icon	Description
	*Show Gate Hierarchy*: When checked, the table displays output in tree mode. Child gates are indented.
	*Show Columns*: Choose which statistical columns to display in the table. Refer to *Section 5.3.2* for further information on calculation of gate statistics.
	*Modify Color Precedence*: 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.

5.2.7.2 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 *Section 5.2.3.1*.

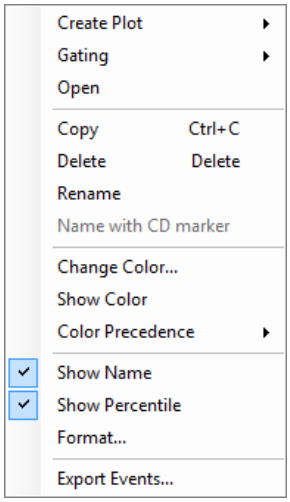
To modify gate color precedence, check the *Modify Color Precedence* tool in the toolbar. The color column in *Gate Manager* is shown below:



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.

5.2.7.3 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.

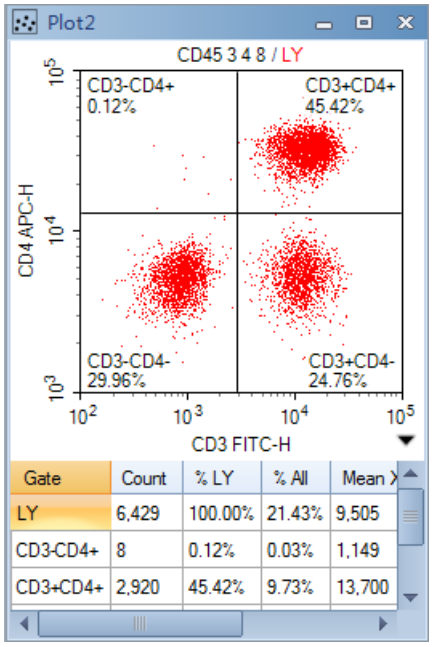
*When multiple gates are selected in **Gate Manager**, only **Delete**, **Show Color**, **Color Precedence**, **Show Name** and **Show Percentile** are available.*

5.3 Statistics

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

5.3.1 Display Statistical Information

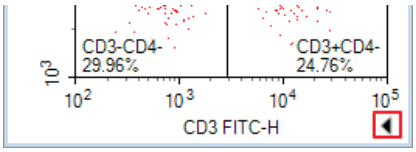
In the following figure, the statistical information chart is displayed below the plot.



5.3.1.1 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.



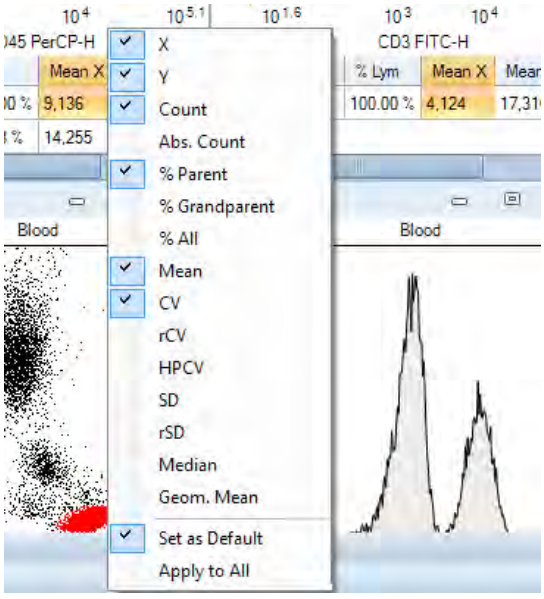
- From the *Workspace Toolbar*, click the *Show Statistics* button Σ, to show/hide the statistics chart of a plot.

5.3.1.2 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.



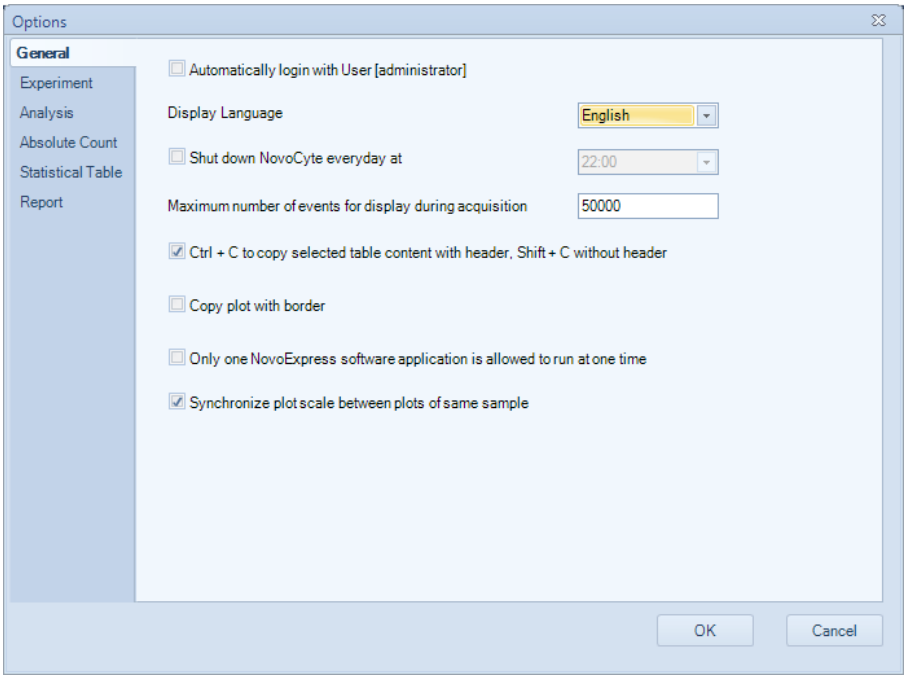
5.3.1.3 Copy Statistical Information to Clipboard

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.

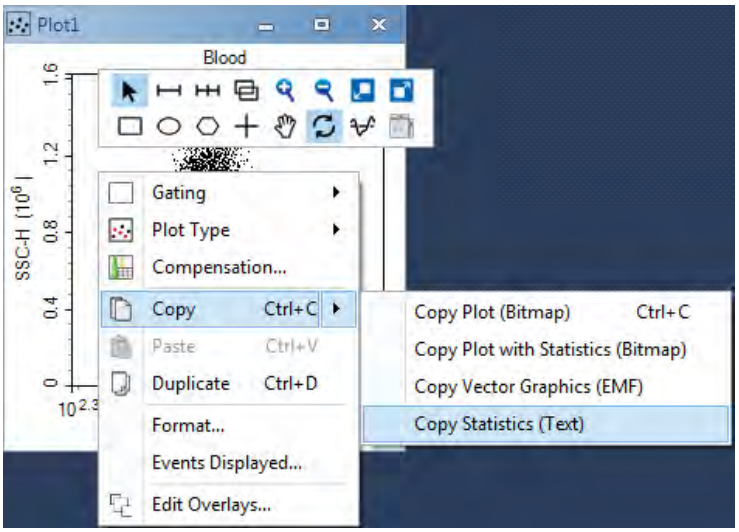
There are two methods to copy statistical information to the clipboard:

- 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)*.



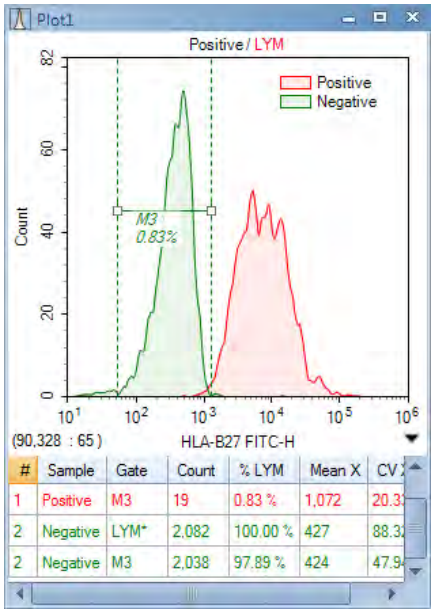
5.3.1.4 Statistic Layers

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 statistic 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

5.3.2 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

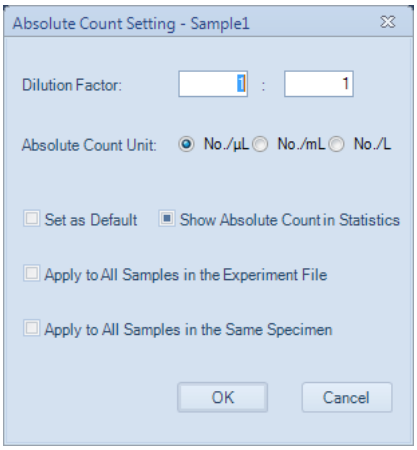
  The number of events collected in the specified gate.

- Absolute Count

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

  Absolute Count = *Count / Ve / DF / 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 $\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$,

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

- SD

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

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

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

- rSD (Robust SD)

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

  The RSD is defined as

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

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

- CV

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

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

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

- rCV (Robust CV)

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

  $$RCV = RSD / Median$$

- HPCV

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

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

  Where *FWHM* is the full width at half maximum of the peak and $\overline{X}$ 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

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

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

- Stain Index

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

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

  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.

5.4 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.

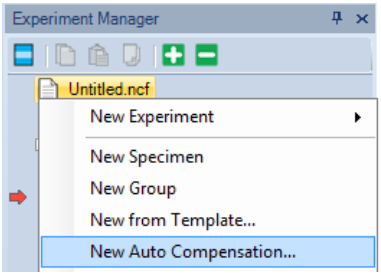
The NovoExpress Software provides three methods for the user to adjust fluorescence compensation. These methods include an automatic method, a compensation matrix adjustment method, and a quick compensation method.

5.4.1 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 NovoCyte instrument and from samples imported with FCS files. *Section 5.4.1.5* describes the details on how to generate automatic compensation matrix using imported FCS files.

5.4.1.1 Set Automatic Compensation

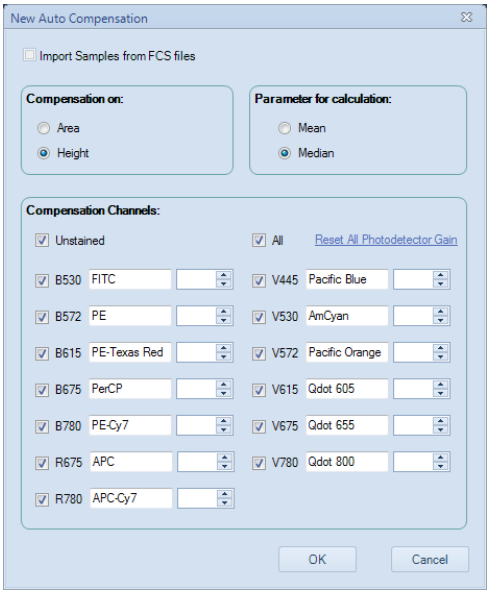
In the *Home* tab of the *Menu Bar*, click the *Auto Compensation* button, or in the *Experiment Manager* panel, right click on the file name or a group and select *New Auto Compensation…*. The *New Auto Compensation* window appears.



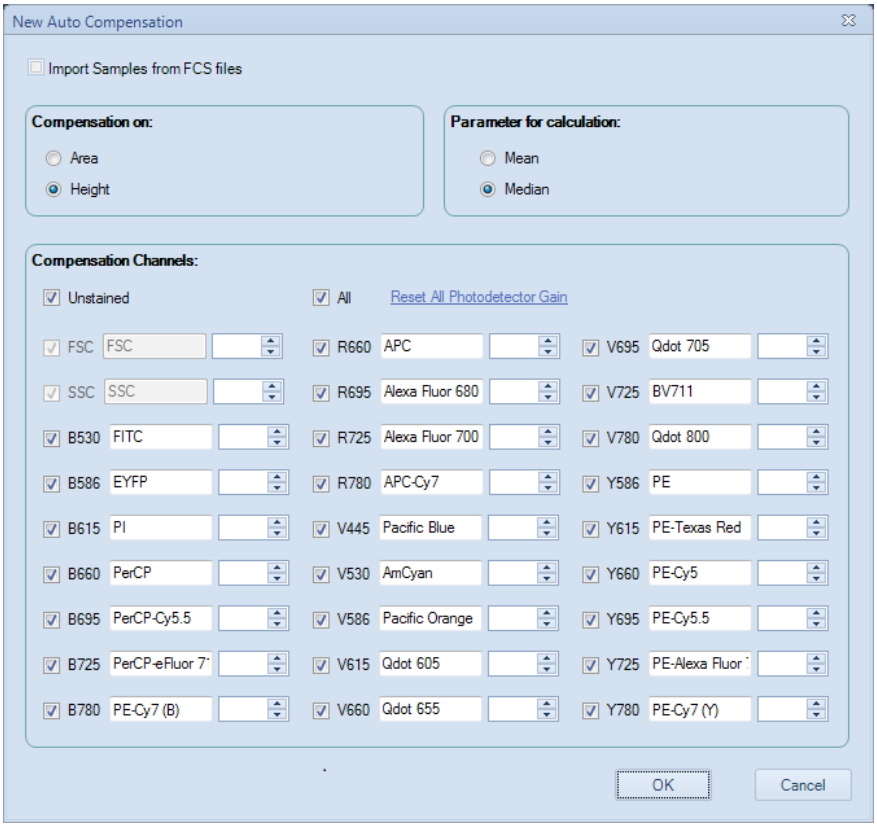
In the *New Auto Compensation* window, the user can select the channels for compensation, whether to compensate using area or height measurements, and whether to calculate compensation based on the measured mean or median.

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.



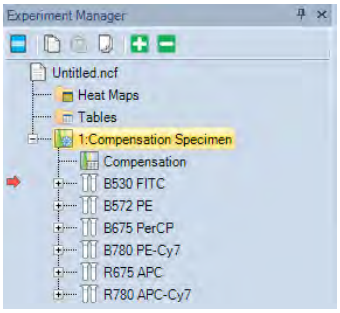
New Auto Compensation window for NovoCyte instrument



New Auto Compensation window for NovoCyte Quanteon instrument

4. Edit photodetector gain of each channel. Click *Reset All photodetector Gain* to reset photodetector gains of all channels to default values. Every NovoCyte instrument has the default photodetector gain setting. An underlined photodetector gain text as shown above for the B530 channel indicates it has been modified and is not the default value. A non-underlined photodetector gain indicates that it is the default setting.

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



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.

5.4.1.2 Preparing Samples for Automatic Compensation

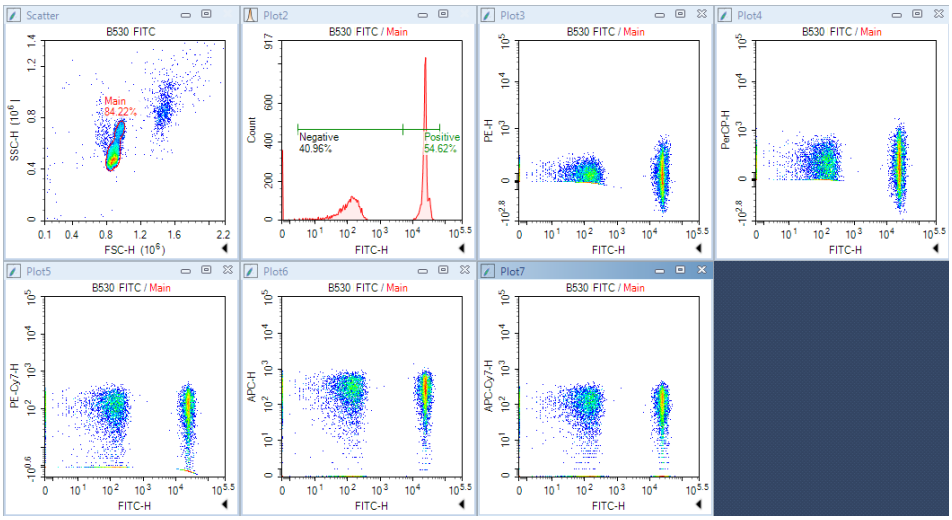
To calculate the compensation, experimental data will need to be collected for each of the control samples in the compensation specimen node. For the unstained sample, the sample should be prepared without any fluorophores added. For the other control samples in the compensation specimen node, the samples 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.

5.4.1.3 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.



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

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

- Open the scatter plot for the sample and modify the position or size of the polygonal *Main* gate to enclose the correct main population.
- Another option is to create a new gate in the scatter plot to enclose the correct main population, and then apply the new gate to the histogram containing the *Positive* and *Negative* gates. See *Section 5.2.4* for more information on applying a gate to a plot.
- Adjust the *Positive* and *Negative* gates in the histogram to enclose the correct population.

*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.*

*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.

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

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 9.4007% into PE, 2.2213% into PerCP, 0.0067% into PE-Cy7 and 0.0025% into APC.

Compensation for BL1 FITC

Compensation Matrix | **Spillover Matrix**

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

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

Compensation for Compensation Specimen

Compensation Matrix | **Spillover Matrix**

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

5.4.1.4 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.

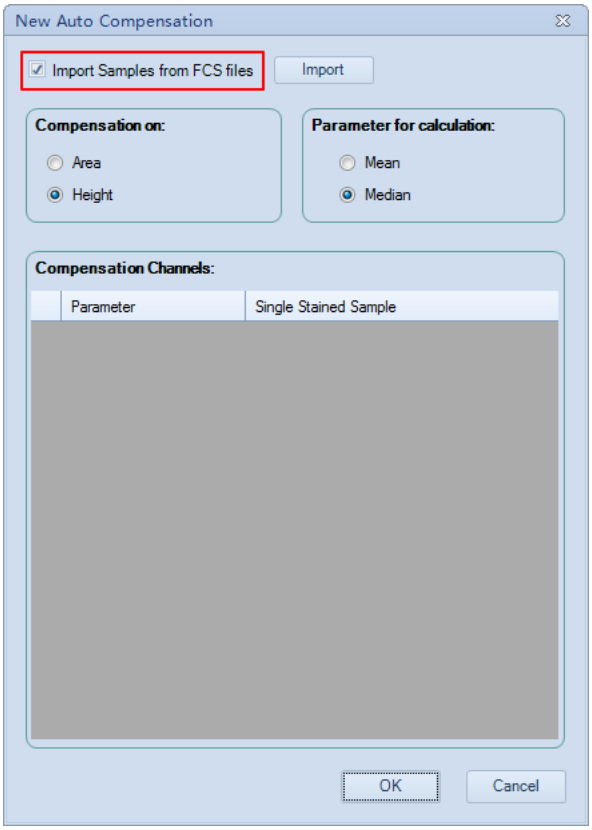
The compensation matrix is calculated at specified photodetector gains. It should only be applied to samples that were acquired with the same photodetector gains.

5.4.1.5 Conduct Automatic Compensation from Imported FCS Files

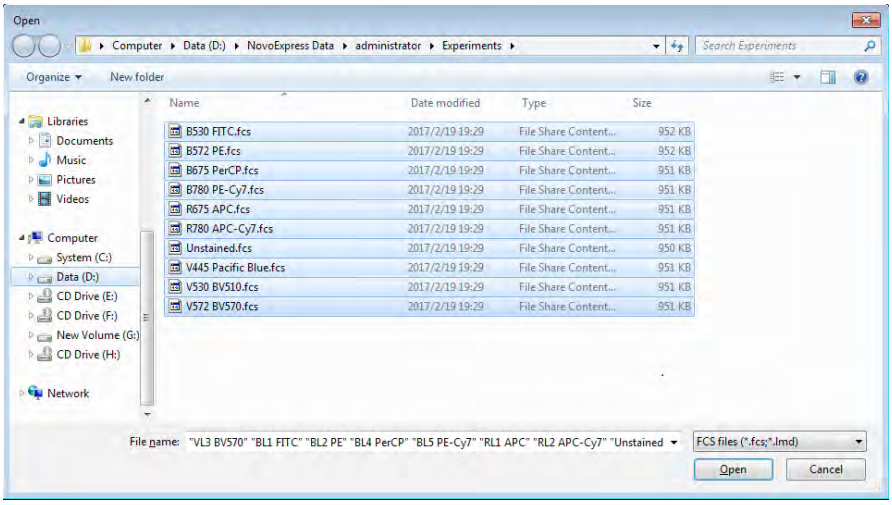
To perform auto-compensation directly from the imported FCS file:

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

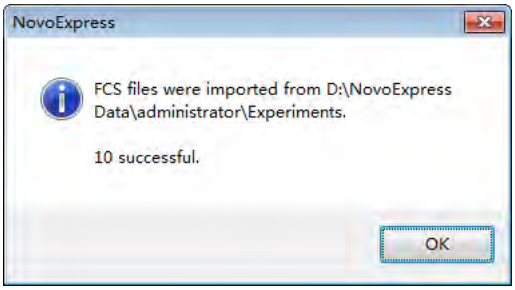
   *This window can also be accessed by right clicking the experiment file and selecting **New Auto Compensation** in the **Experiment Manager** window.*



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.



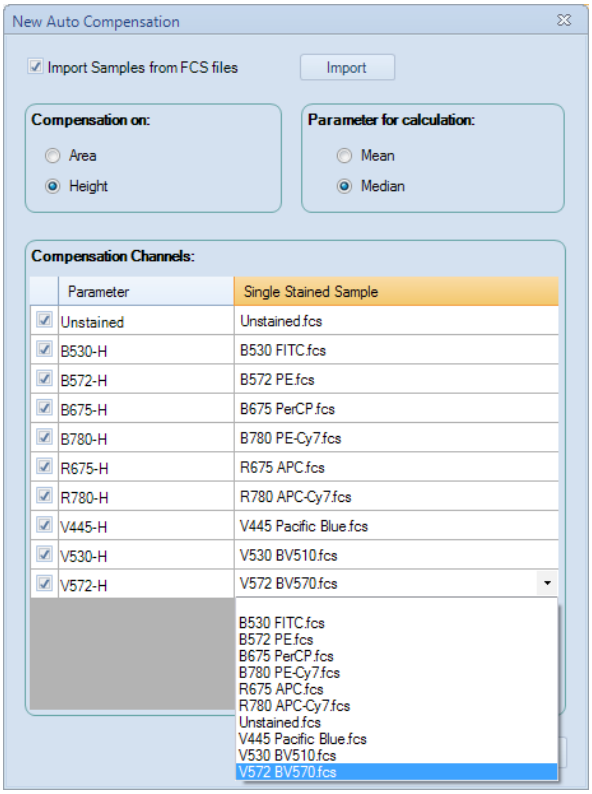
3 Ensure all the selected FCS files are successfully imported to the software. Click OK to continue.



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.

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

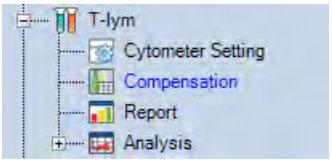
▶▶▶



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.

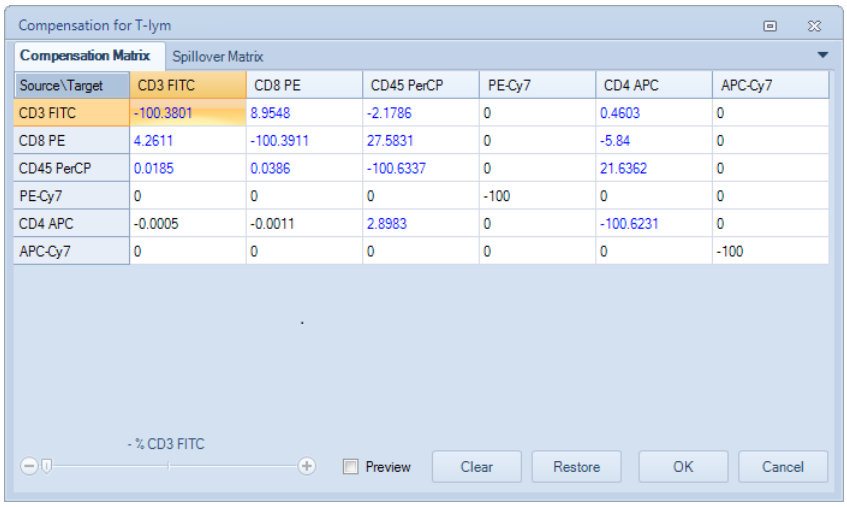
5.4.2 Active Compensation

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.



*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.



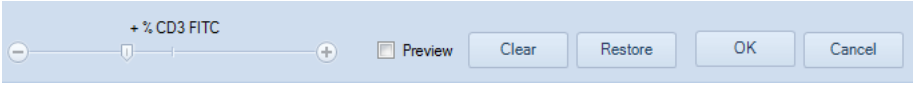
5.4.2.1 Relationship between the Spillover and Compensation Matrix

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

5.4.2.2 Editing 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 300 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 300 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.

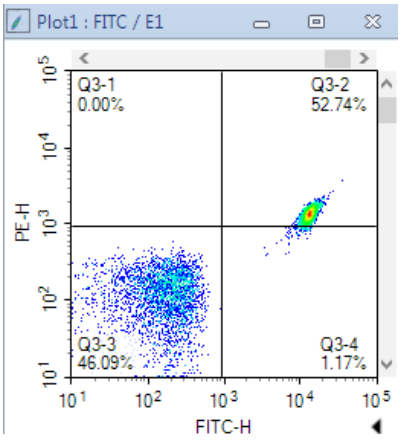
5.4.3 Quick Compensation Adjustment

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

5

5.4.3.1 Opening 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.



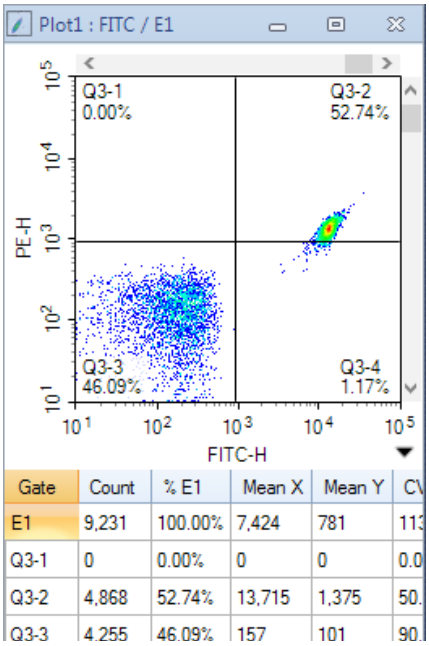
To hide the quick compensation scrollbars, click again on the *Quick Compensation* button.

5.4.3.2 Using 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.

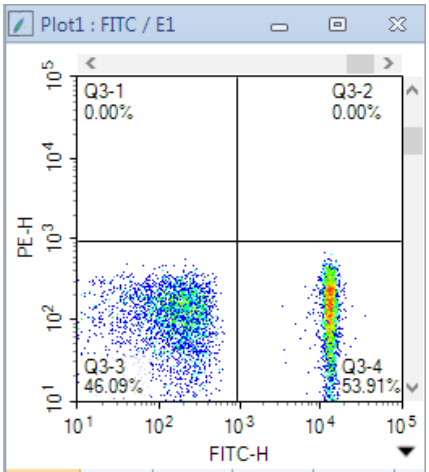
2. Create a quadrant gate to gate the positive and negative populations as shown below.



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.

▶▶▶

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.

■

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.

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

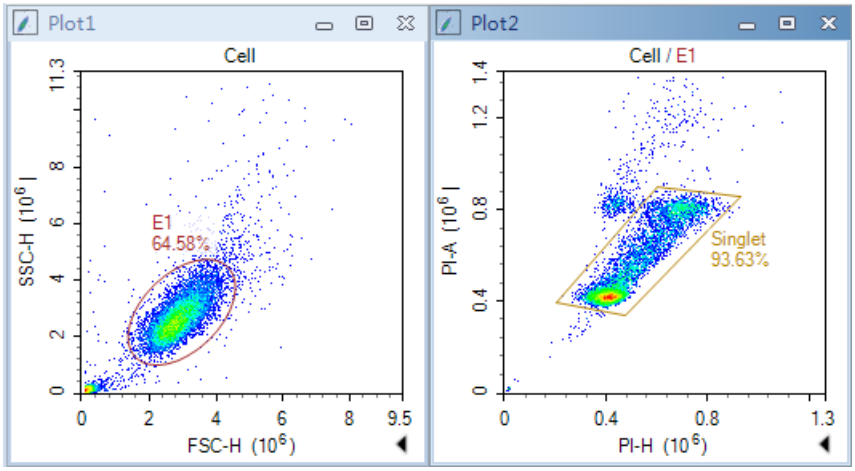
5.5 Cell Cycle Analysis

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.

5.5.1 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.



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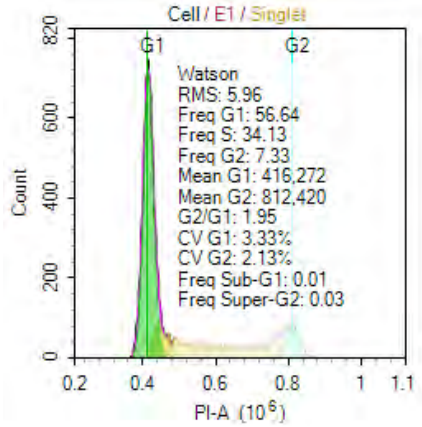
Cell Cycle Analysis

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



Fitting results:

Item	Description
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

▶▶▶

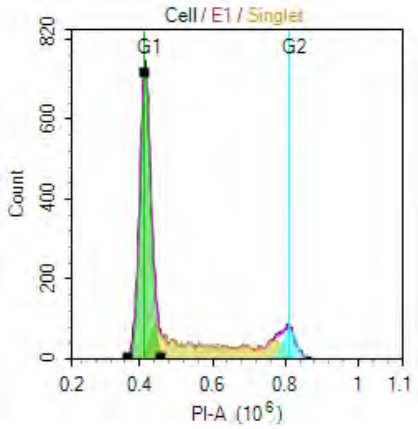
Item	Description
CV G1	The coefficient of variation of the G1 phase
CV G2	The coefficient of variation of the G2 phase
Freq Sub-G1	Percentage in Sub-G1
Freq Super-G2	Percentage in Super-G2

5.5.2 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.

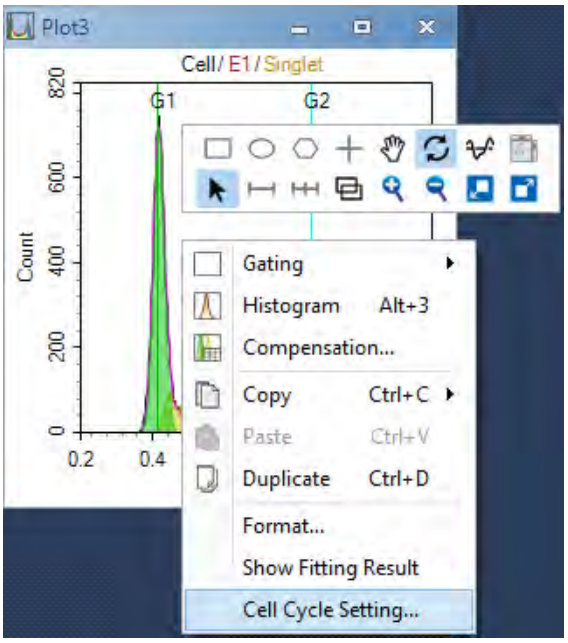
- Constrain G1 and G2 peaks

  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.

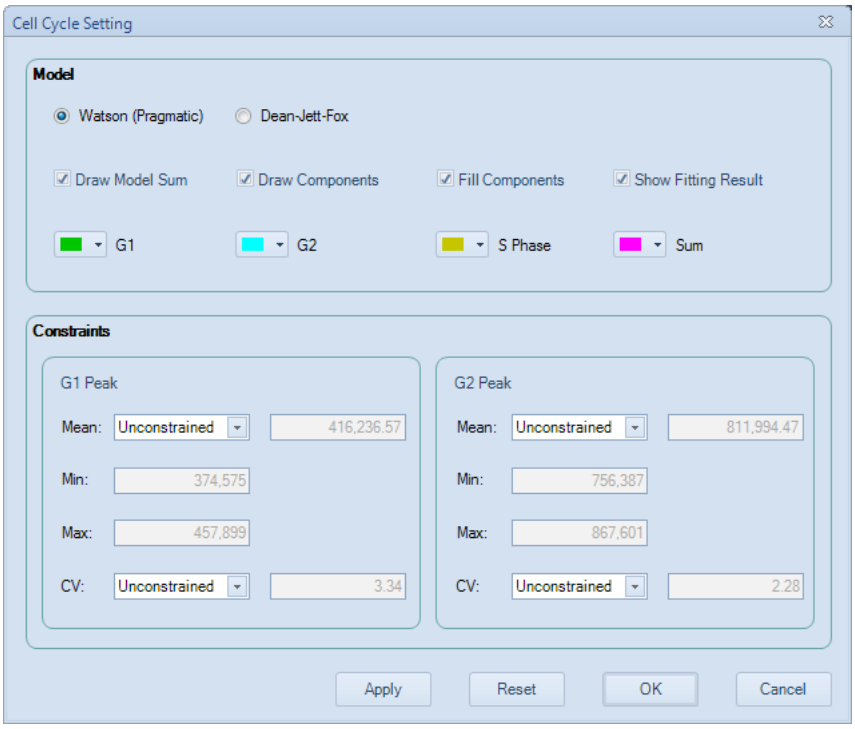


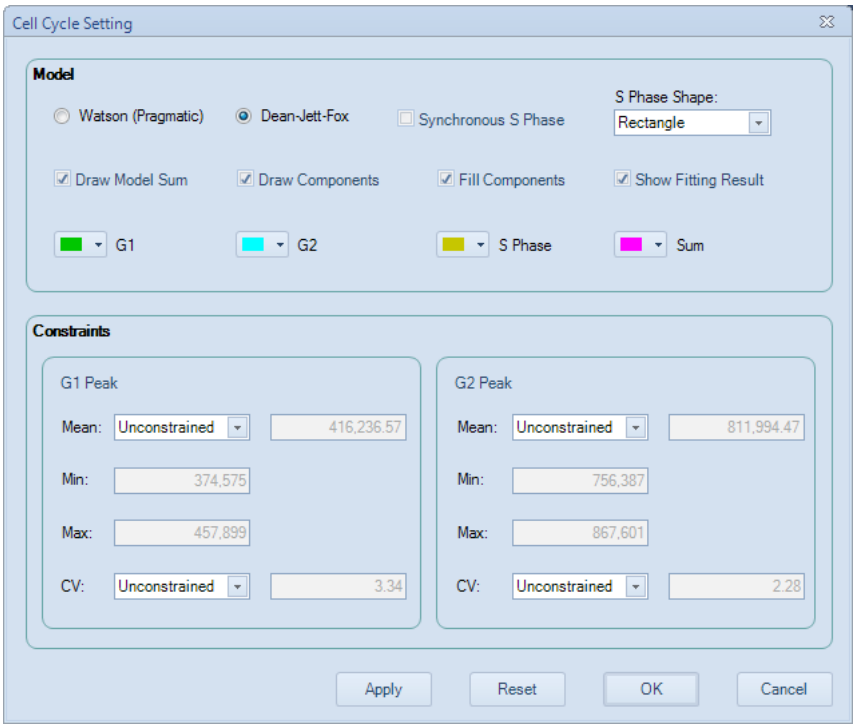
- Cell Cycle Setting Window

  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.



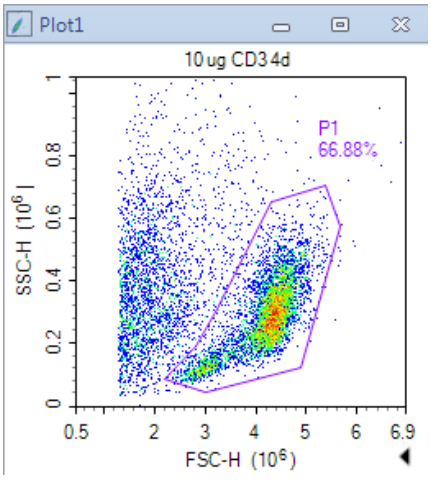


5.6 Cell Proliferation Analysis

5.6.1 Automated Cell Proliferation Analysis

- Gating Target Cell Population

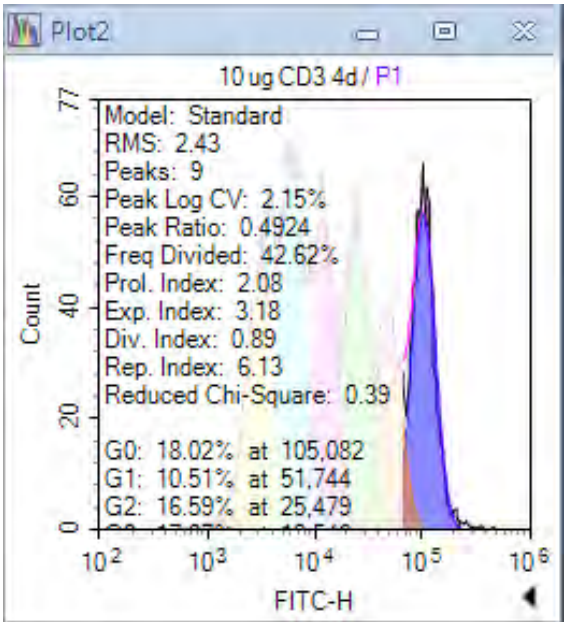
  On the density plot of FSC-H/SSC-H, create a gate which includes the target cell population you are interested in, for example P1 as show below.



- 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 is within the plot scale. The

software automatically attempts to fit the data using the modeling algorithm and calculates the results. To display the statistics, right click the resulting cell proliferation histogram and select *Show Fitting Result*.



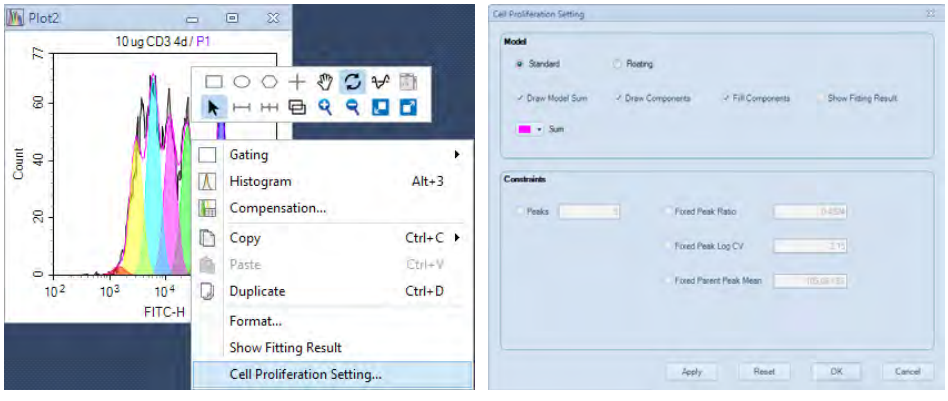
Item	Description
Model	The name of model used for analysis and generates results, including Standard and Floating models.
RMS	Root Mean Square error. It is an estimate of the "goodness of fit" of the model.
Peaks	The count of Peaks.
Peak Log CV	Log CV of peak
Peak Ratio	The average ratio of all the peak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

▶▶▶

Item	Description
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 i^{th} 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 i^{th} generation.

5.6.2 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.

5.7 Statistical Tables

The statistical tables provide a summary from multiple samples, gates, and parameters enabling batch analysis and data comparison.

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

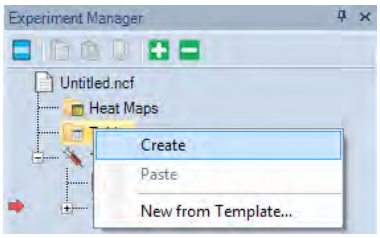
Icon	Description
	Sets the type of statistical table
	Adds a column to the table
	Edits a column
	Duplicates columns
	Deletes columns
	Hides columns
	Selects columns to show
	Selects samples to show
	Deletes rows
	Exports table as a CSV file
	Opens the *Options* → *Statistical Table* window

5.7.1 Creating Different Types of Statistical Tables

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

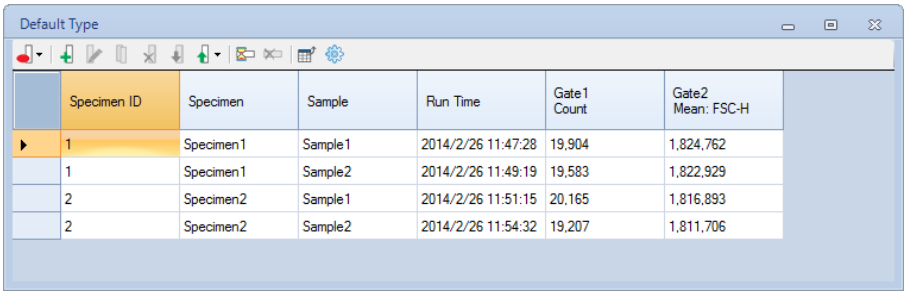
- Creating a New Statistical Table

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



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

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



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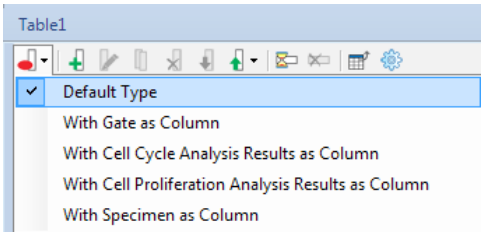
- Creating a New Statistical Table from Template

  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.

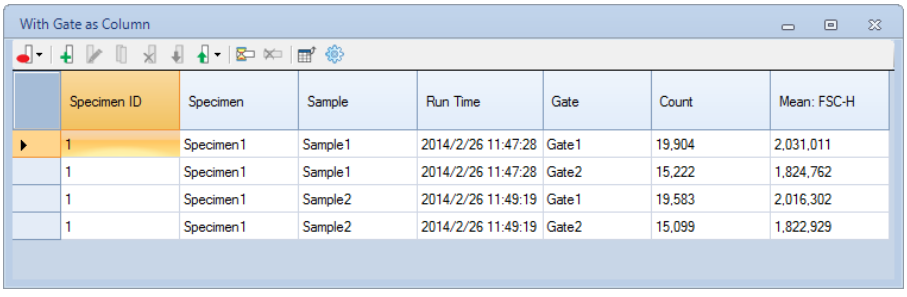
- Selecting the Type of Statistical Table

  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 *Specimen as Column*.

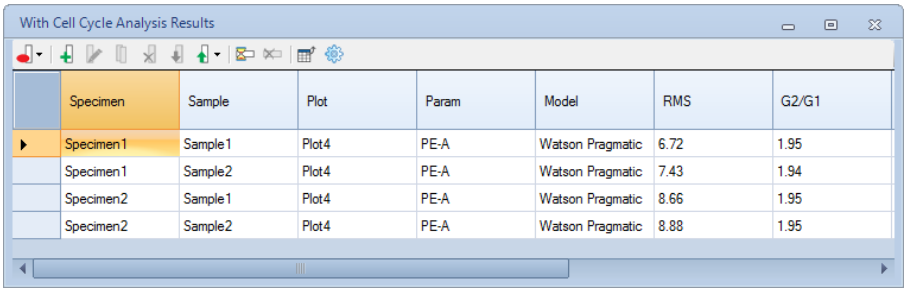
  New tables are created as *The Default Type*. To change the table type, click the *Table Type* button from the toolbar and select the new table type from the drop-down menu.



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*.


With Cell Cycle Analysis Results

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

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

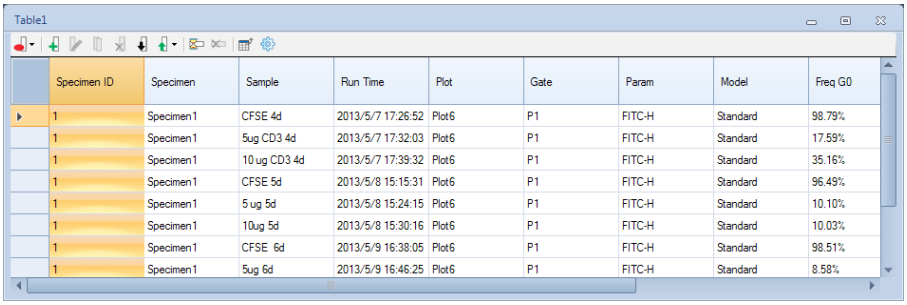
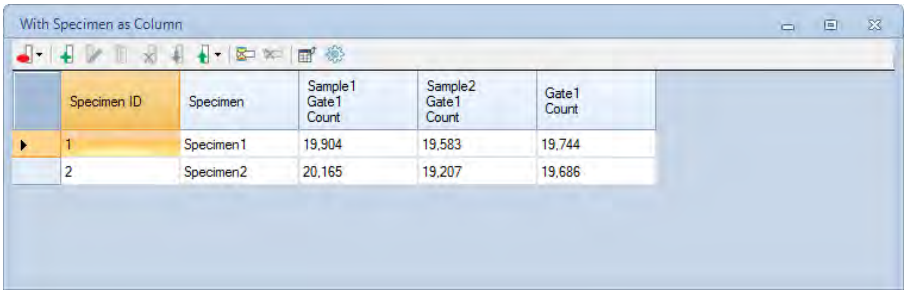


Table1

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

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


With Specimen as Column

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

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

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

5.7.2 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.

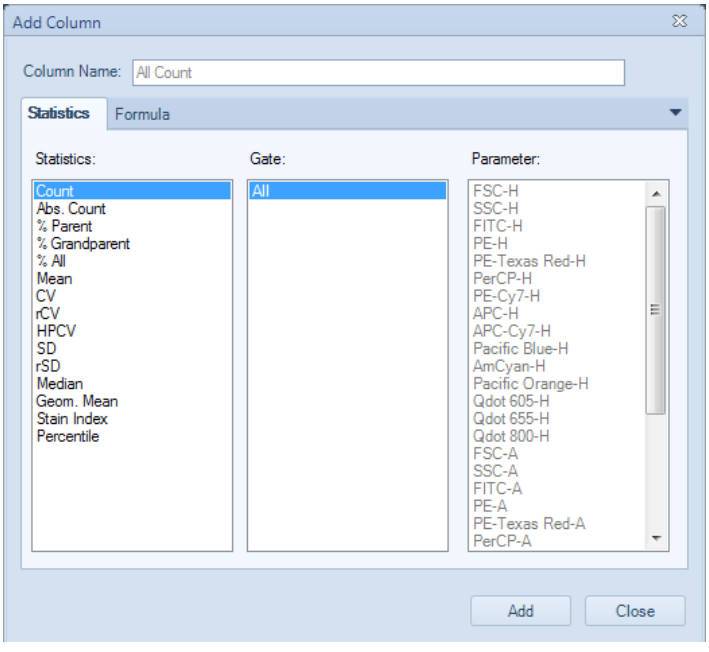
5.7.2.1 Add and Edit Columns

- 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

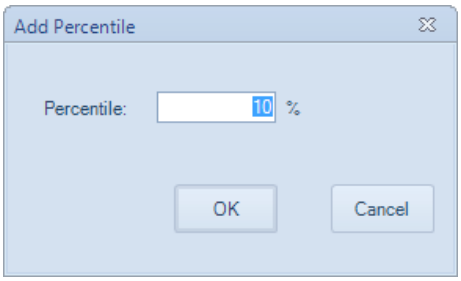
  Click on the *Add Column* button in the toolbar . The *Add Column* window will appear. Select the statistical value, the gate, and the parameter. Click the *Add* button to add the column to the table.

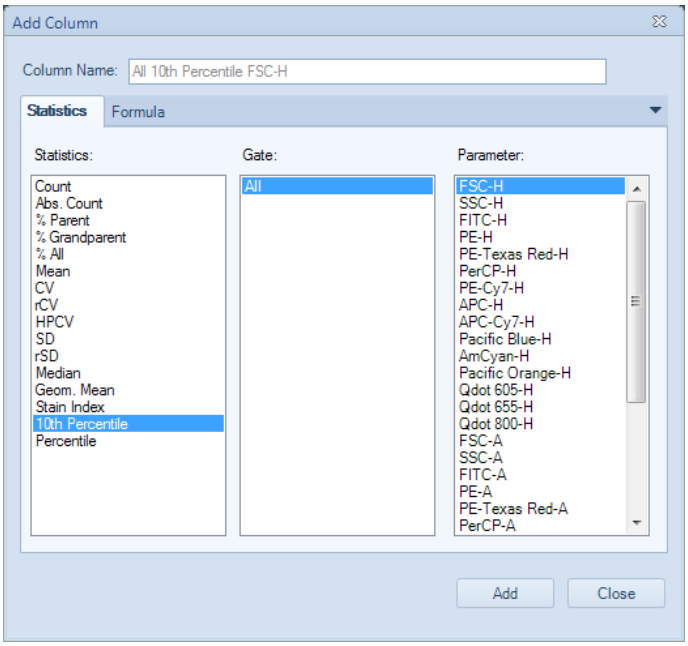


*For a table with Specimen as Column, the Sample Name should be specified when adding columns. If selecting "**All**" in the sample list box, the statistical results will be the average of all samples in each specimen. For Absolute Count calculation, if Absolute Count Unit defined for samples in the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

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 Section 5.3.2 for detailed description of Stain Index.

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





- Edit a Column

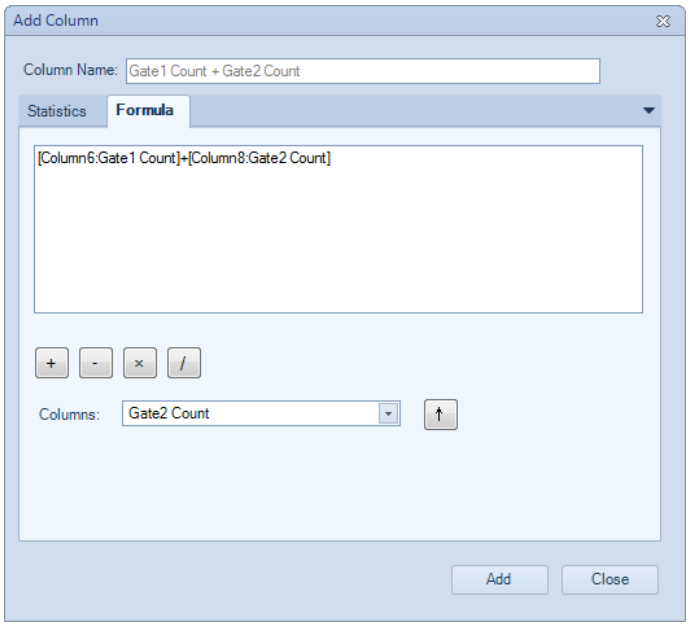
  Select the column in the statistical table window and click the *Edit Column* button, in the toolbar. The *Edit Column* window opens. Select the modifications and click *OK* to edit the column.

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

5.7.2.2 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.

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



5.7.2.3 Select Multiple Columns

In the header row of the table, click and drag in the top half of the cell to select multiple columns.

Click and drag in the lower half of the cell to move the column.

5.7.2.4 Duplicate Columns

Select the column, and click the *Duplicate Column* button , in the toolbar to duplicate the selected column.

5.7.2.5 Delete Columns

Select the column, and click the *Delete Column* button , in the toolbar to delete the selected column.

5.7.2.6 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.

5.7.2.7 Move Columns

In the header row of the table, click and drag in the lower half of the cell to move columns.

Click and drag in the upper half of the cell to select multiple columns.

5.7.2.8 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.

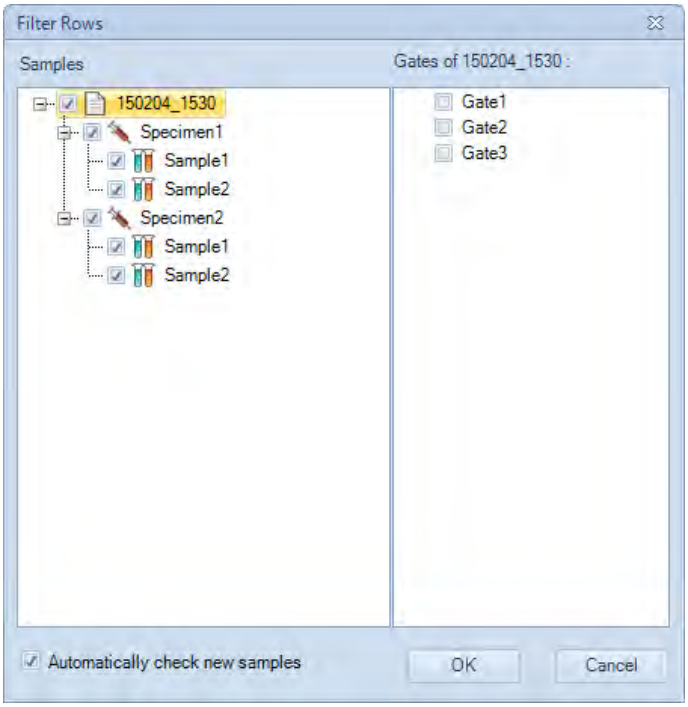
5.7.3 Statistical Table Rows

In the statistical table, the rows list separate populations for analysis.

5.7.3.1 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.

5.7.3.2 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.

5.7.3.3 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.

5.7.4 Statistical Tables Export or Copy Text

Statistical table results can be exported to CSV file or copied to clipboard as text.

5.7.4.1 Exporting Statistical Tables as CSV File

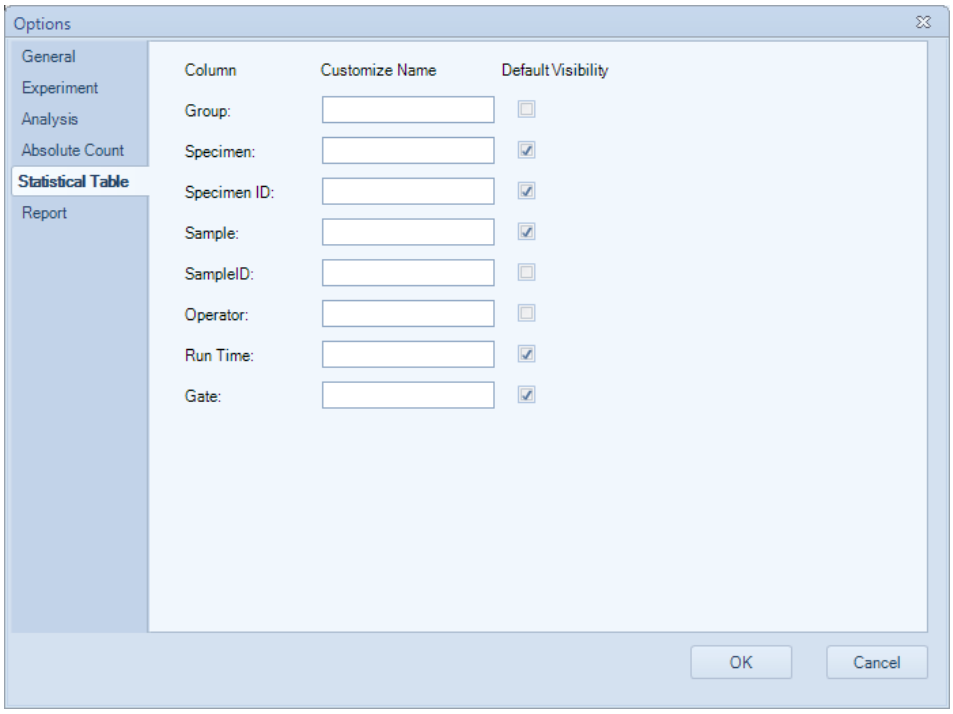
In the *Sample* tab toolbar, click the *Export CSV File* button . Enter the file path and click *Save* to export the file.

5.7.4.2 Copying 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.

5.7.5 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*, *Sample*, *Run Time* and *Gate* columns.



5.7.6 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

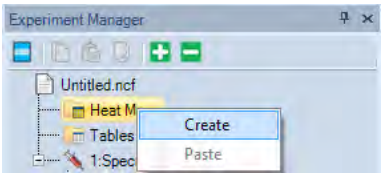
  In the *Experiment Manager* panel, select the table. Right-click and select *Export Template...* Enter the name for the template in the prompted window and click *Save* to create a template. The template will be saved as a *.nst file.

5.8 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.

5.8.1 Creating 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.

5.8.2 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.

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

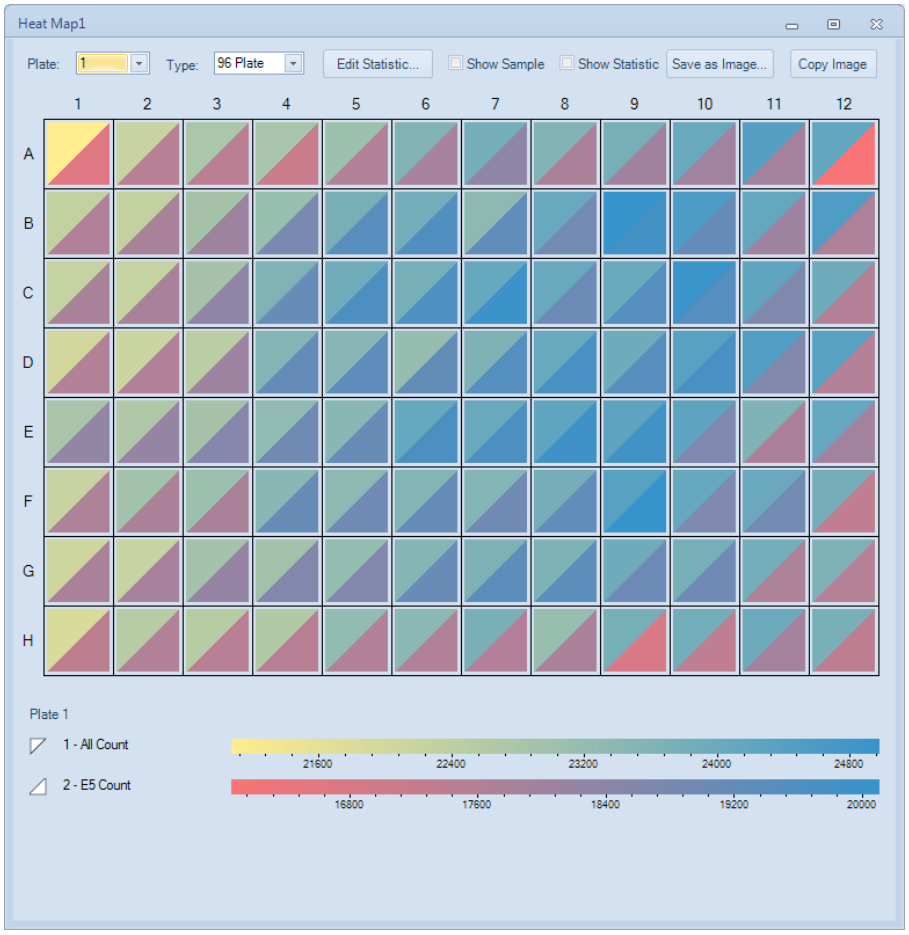
If there are samples outside the current plate type, "" will be displayed in the upper left corner of the heat map.*

5.8.2.1 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.

5.8.2.2 Heat Map for Multiple Statistics

The following figure shows a heat map with two statistics.



The gate, color scale, and color scale range of each statistic parameter can be set separately.

5.8.2.3 Add Statistic

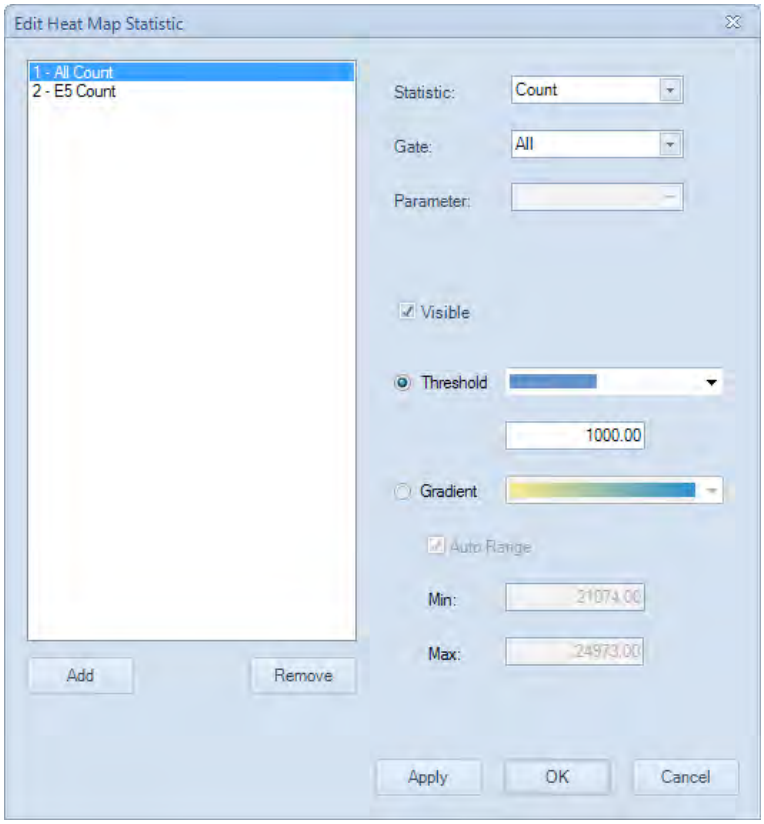
- Drag and Drop to Add Statistic Parameter

  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 param-

eter 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.

5.8.3 Edit Heat Map Statistic Window

You can add, edit, and remove statistics in *Edit Heat Map Statistic* window. To do so, click the *Edit Statistic…* button in the heat map. The *Edit Heat Map Statistic* window will appear.



- *Add:* Click to add a new statistic to the list. Up to 4 statistic parameters can be added into the list.
- *Remove:* Click to delete the selected statistic parameter.
- *Edit Statistics properties:* When select a statistic, you can select the statistic type, gate, parameter for the statistic, and set whether the statistic is visible in the heat map or not.
- *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 statistic 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 statistic parameter in a color gradient. Select different color scheme from the drop-down list in the Gradient option. The scale of the statistic parameter can be defined as Auto Range (software identifies the minimum and maxi-

mum 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.

5.8.4 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.

5.8.5 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.

5.9 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.

***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**.*

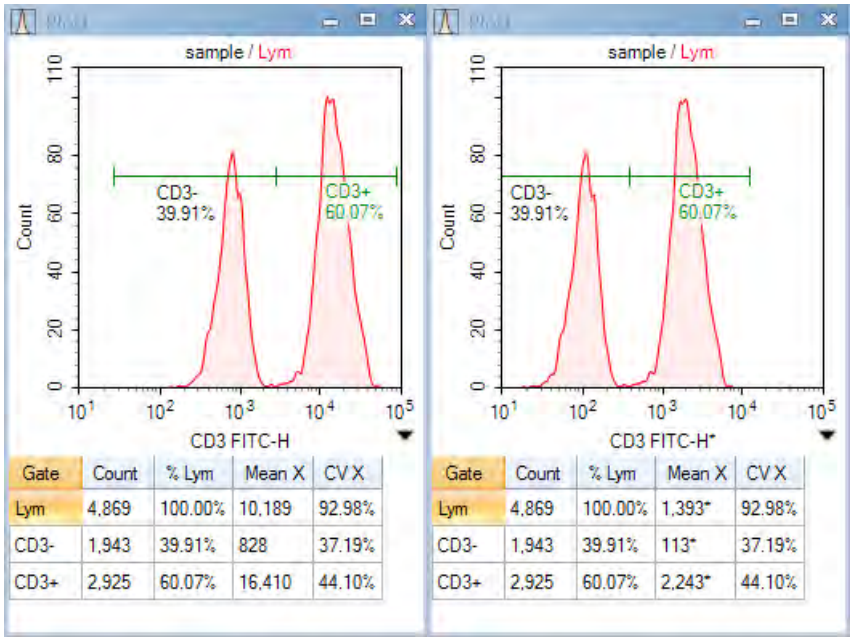
*Only accounts with the **Post Gain Adjustment** privilege can adjust **Post Gain**.*

5.9.1 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

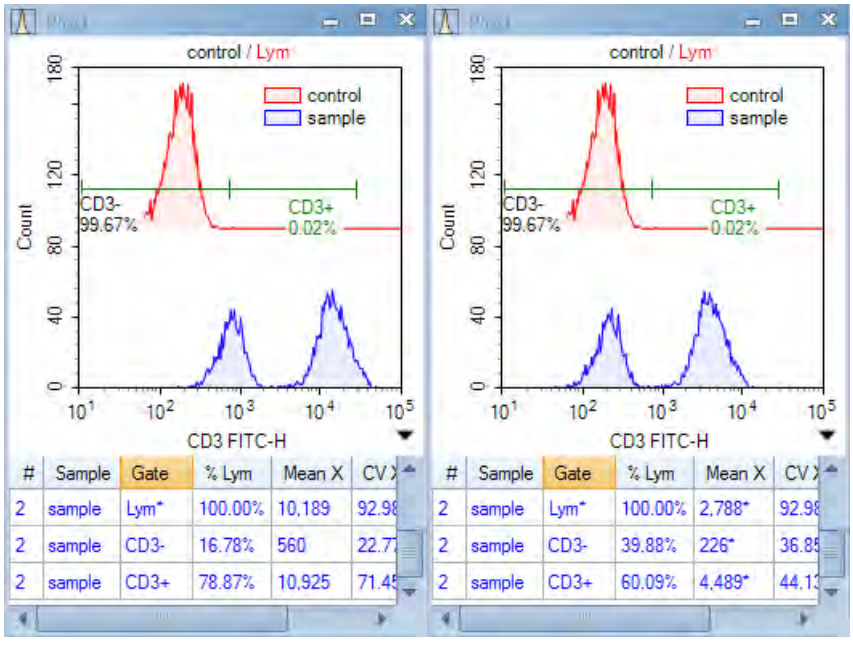
of the mouse and drag the histogram curve, then drag and drop the peak of interest to the target location. In the example below the left plot is prior to the post gain adjustment and the right plot is after the post gain adjustment.



Gate	Count	% Lym	Mean X	CV X
Lym	4,869	100.00%	10,189	92.98%
CD3-	1,943	39.91%	828	37.19%
CD3+	2,925	60.07%	16,410	44.10%

Gate	Count	% Lym	Mean X	CV X
Lym	4,869	100.00%	1,393*	92.98%
CD3-	1,943	39.91%	113*	37.19%
CD3+	2,925	60.07%	2,243*	44.10%

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.



#	Sample	Gate	% Lym	Mean X	CV X
2	sample	Lym*	100.00%	10,189	92.98
2	sample	CD3-	16.78%	560	22.77
2	sample	CD3+	78.87%	10,925	71.45

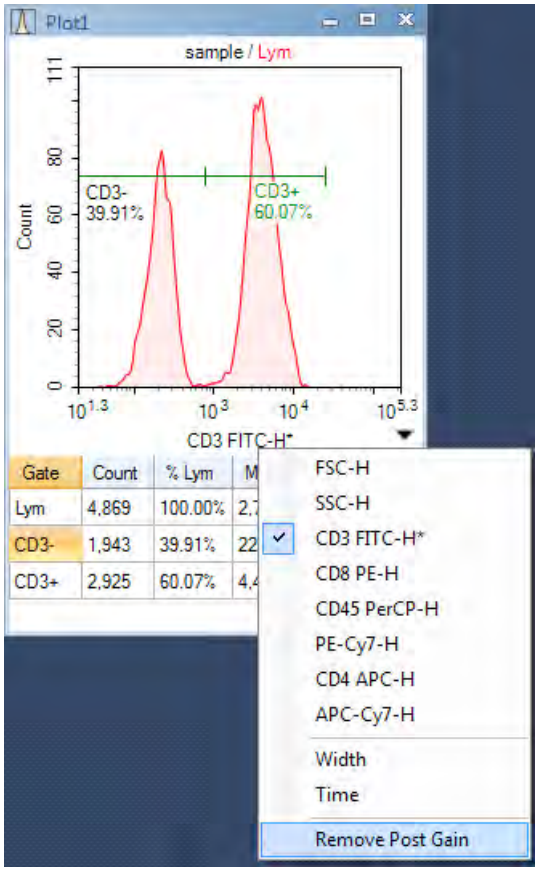
#	Sample	Gate	% Lym	Mean X	CV X
2	sample	Lym*	100.00%	2,788*	92.98
2	sample	CD3-	39.88%	226*	36.85
2	sample	CD3+	60.09%	4,489*	44.13

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 statistic with post gain will be shown with a * mark.

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.

5.9.2 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.

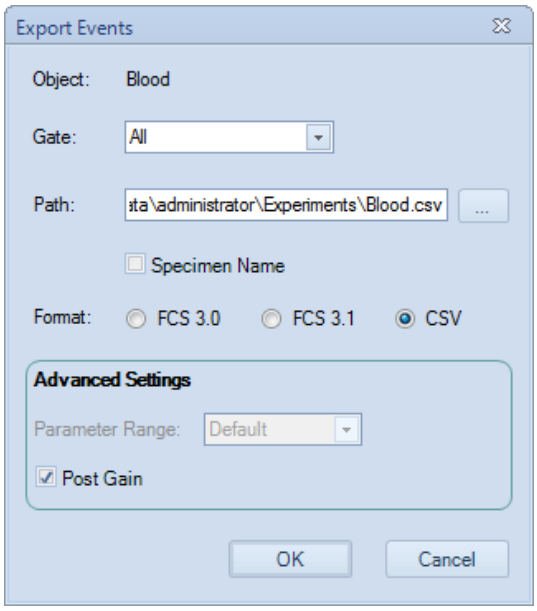
5.9.3 Apply Post Gain

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 an .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.

5.9.4 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.

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

6. Experiment Manager

The NovoExpress Software uses a hierarchy structure including groups, specimen, and samples to organize and manage experimental data. This section describes how the *Experiment Manager* panel sets up a hierarchy structure to organize the samples, the use of templates, and importing and exporting data.

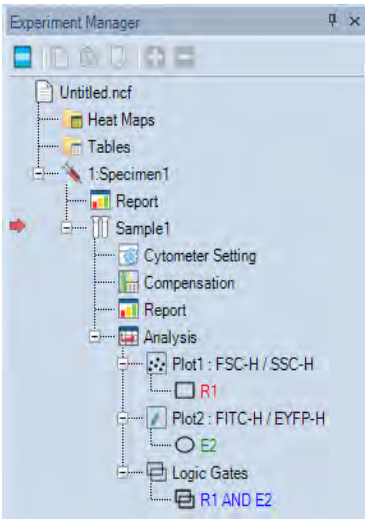
6.1 Experiment Manager Toolbar

Icon	Description
	Work List: View and edit the work list. Contains information on the sample names and collection parameters. Refer to *Section 4.2 Work List* for more information.
	Copy the selected node.
	Paste the copied content.
	Create a copy of the currently selected sample or specimen.
	Expand all child nodes of the current node.
	Collapse all child nodes of the current node.

6.2 Hierarchy

6.2.1 Description

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



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

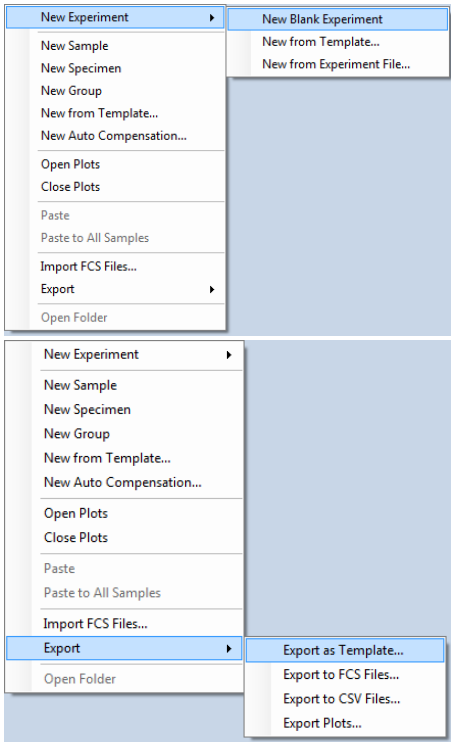
Icon	Description
File	The experiment file (*.ncf file format)
Heat Maps	This node contains Heat Maps for the experiment file. Right-clicking this node allows for creating new heat map.
Statistical tables	This node contains statistical tables for the experiment file. Right-clicking this node allows for the creation of new statistical tables
Statistical table	Statistical analysis table
Group	This node represents a Group in the organizational hierarchy. A group can contain multiple specimens and sub groups, and specimens will always be placed in front of sub groups.
Specimen	This node represents a Specimen in the organizational hierarchy. A specimen can contain multiple samples. Each specimen contains a specimen report. In the node text "1:Specimen 1", the former number is Specimen ID, the latter text is Specimen Name.

▶▶▶

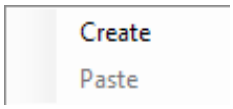
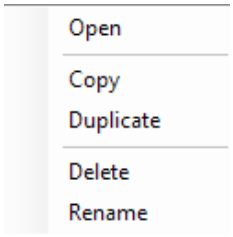
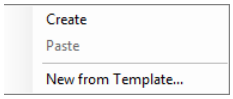
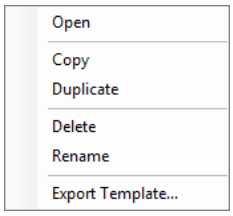
Icon	Description
Sample	This node represents a Sample in the organizational hierarchy. The sample is the most basic organizational unit and contains sample data collection parameters, instrument settings, fluorescence compensation settings, reports, analysis, and data. The sample icon will display differently depending on the status of the sample. A blank sample without any data collected will appear as A sample listed for sample acquisition and during acquisition will appear as A sample with data collected will appear as
Cytometer settings	Cytometer settings contain the sample parameters, the acquisition stop conditions, and the sample flow rate and threshold settings. See *Section 4.1 Cytometer Setting* for additional information.
Fluorescence compensation	Fluorescence compensation matrix for the sample. If the matrix is empty, the node is displayed in black. If the matrix is filled, the matrix is displayed in blue. See *Section 5.4 Fluorescence Compensation* for additional information.
Report	Contains a report of the data analysis and is found under both specimen and sample nodes. Reports under specimen nodes can include plots and statistical analysis for all samples under the specimen. Reports under the sample nodes can include plots and statistical analysis only for the sample. See *Section 7 Report* for additional information.
Analysis	Analysis contains the plots and gates for a sample. Under the *Analysis* node, there are plot nodes and a logic gate node. *Plot* nodes are listed for individual plots of the sample, and each plot node contains the gates created for the plot. A separate logic gate node contains all of the logic gates created for the sample.
Plot	A plot created for the analysis of a sample
Gate	A gate created within a plot
Logic gate group	Contains all logic gates for a sample
Logic gate	Logic gates
Red arrow	The red arrow indicates the sample is the active sample.
/ Flashing green and dark green arrows	Flashing green and dark green arrows indicate the sample is being collected.
/ Alternatively flashing red and green arrows	Flashing red and green arrows indicate the active sample is being collected.

6.2.2 Right-Click Menu

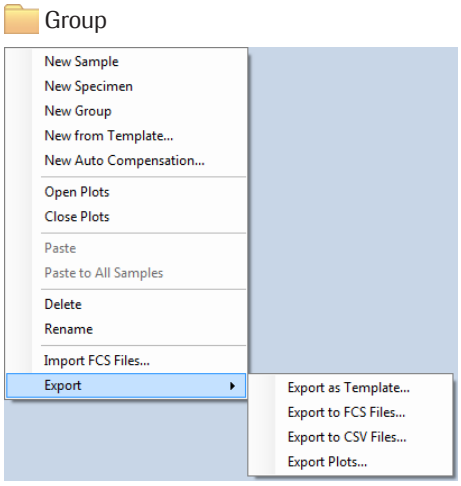
From the *Experiment Manager* panel, right-clicking each node will bring up a menu of functions. The table below lists the specific functions available by right-clicking each node type.

Icon	Description
File 	*New Experiment:* ▶ *New Blank Experiment: Create a blank experiment file.* ▶ *New from Template: Create an experiment file from a template.* ▶ *New from Experiment File: Create an experiment file from an existing experiment file.* *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 Template*: Exports the file as a template file. ▶ *Export to FCS Files*: Exports all samples as FCS files. ▶ *Export to CSV Files*: Exports all samples as CSV files. ▶ *Export Plots*: Exports all plots from all samples as image files. *Open Folder*: Opens the folder containing the experiment file.

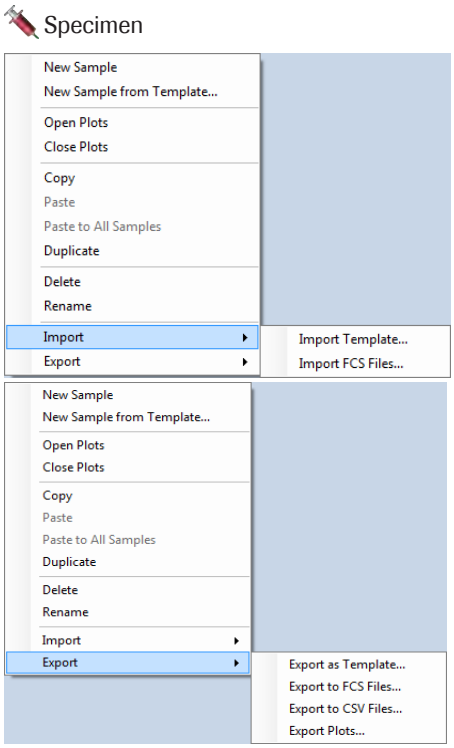
▶▶▶

Icon	Description
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.

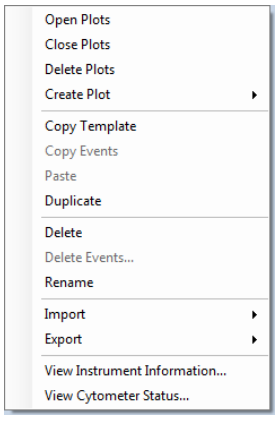
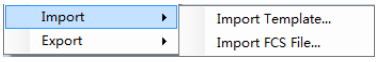
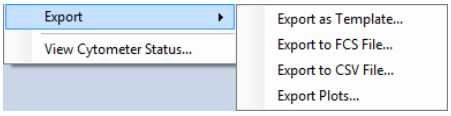
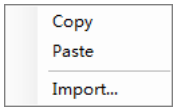
▶▶▶

Icon	Description
	*New Sample:* Creates a new specimen with a new sample included. *New Specimen*: Creates a new specimen. *New Group*: Creates a new group. *New from Template*: Imports selected group, specimen, and samples from a template. *New Auto Compensation*: Creates a compensation specimen containing samples to compute a compensation matrix. *Open Plots*: Opens all plots from all of the samples within the group. *Close Plots*: Closes all plots from all of the samples within the group. *Paste*: Creates a new specimen with the copied specimen template. *Paste to All Specimens*: Pastes the copied specimen template to all specimens in the group. *Paste to All Samples*: Pastes the copied sample template to all samples in the group. *Delete*: Deletes the group. *Rename*: Renames the group. *Import FCS Files*: Selects a folder to import all FCS files within the folder or subfolders as samples. Files up to 10 subfolders deep from the selected folder will be added and organized according to the folder structure. *Export*: ▶ *Export as Template*: Exports the group as a template file. ▶ *Export to FCS Files*: Exports all samples as FCS files. ▶ *Export to CSV Files*: Exports all samples as CSV files. ▶ *Export Plots:* Exports all plots in current group as image files.

▶▶▶

Icon	Description
	*New Sample*: Creates a new sample in the specimen. *New Sample from Template*: Imports the samples of the first specimen from a selected template. *Open Plots*: Opens all plots from all of the samples within the specimen. *Close Plots*: Closes all plots from all of the samples within the specimen. *Copy*: Copies the template of the specimen. *Paste*: Pastes the template of a copied specimen, or creates a new sample with the copied sample template. *Paste to All Samples*: Pastes the copied sample template to all samples in the specimen. *Duplicate*: Creates a duplicate of the specimen. *Delete*: Deletes the specimen. *Rename*: Renames the specimen. *Import*: ▶ *Import Template:* Imports template and apply to the selected specimen. ▶ *Import FCS Files*: Selects one or more FCS files imported as samples. *Export*: ▶ *Export as Template*: Exports the specimen as a template file. ▶ *Export to FCS Files*: Exports all samples as FCS files. ▶ *Export to CSV Files*: Exports all samples as CSV files. ▶ *Export Plots:* Exports all plots in current specimen as image files.

▶▶▶

Icon	Description
Sample 	*Open Plots*: Opens all plots from the sample. *Close Plots*: Closes all plots from the sample. *Delete Plots*: Deletes all plots from the sample. *Create Plot*: Creates a new plot for the sample. *Copy Template*: Copies the template of the sample. *Copy Events*: Copies the events of the sample. *Paste*: Pastes the copied template or events of a sample. *Duplicate*: Creates a duplicate of the sample. *Delete*: Deletes the sample. *Delete Events*: Deletes all or part of the sample's events. Only accounts with the *Delete Sample Events* privilege can perform this operation (Refer to *Section 3.3.4*). *Rename*: Renames the sample. *Import*: Imports a sample template or FCS file, as shown below:  *Export*: Exports the sample as a template, FCS file, CSV file, or exports all plots of the sample as image files.  *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.
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.

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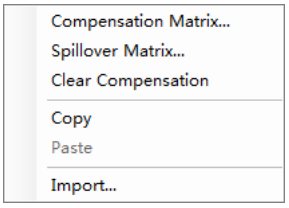
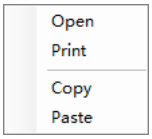
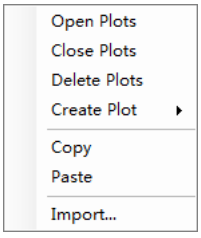
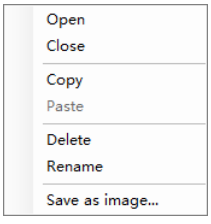
Icon	Description
Fluorescence compensation 	*Compensation Matrix*: Opens the sample's compensation matrix. *Spillover Matrix*: Opens the sample's spillover matrix. *Clear Compensation*: Clears fluorescence compensation information of the sample. *Copy*: Copies the sample's fluorescence compensation. *Paste*: Pastes the copied fluorescence compensation to the sample. *Import*: Imports fluorescence compensation from a selected sample template.
Report Specimen Report:  Sample Report: 	*Open*: Opens the report. *Print*: Prints the report. *Copy*: Copies the report template. *Paste*: Pastes the copied report template to the sample. Specimen reports and sample reports are not able to copy and paste each other. *Import*: Imports a report template to the sample from a selected template file.
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.

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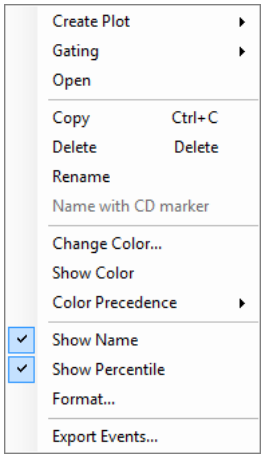
Icon	Description
Gate 	*Create Plot:* Creates a new plot including the events from the selected gate. *Gating:* Selects plots to apply the gate. *Open*: Opens the plot containing the gate. *Copy*: Copies the gate. *Delete*: Deletes the gate. *Rename*: Renames the gate. *Name with CD Marker*: If a fluorescence 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.
Logic gate group 	*Create*: Creates a logic gate. *Delete*: Deletes all logic gates.

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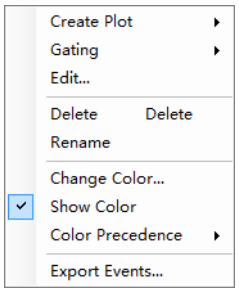
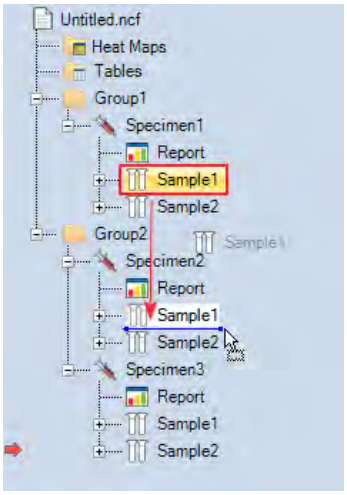
Icon	Description
Logic gate 	*Create Plot:* Creates a new plot including the events from the selected gate. *Gating:* Selects plots to apply the gate. *Edit*: Opens the logic gate editing window. *Delete*: Deletes the logic gate. *Rename*: Renames the logic gate. *Change Color*: Modifies the color of the logic gate. *Show Color*: Sets to display the logic gates in color. *Gating*: Selects plots to apply the gate. *Create Plot*: Creates a new plot with the gate applied to the plot.

6.2.3 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, see *Section 6.3.2 Drag and Drop the Template*.



During the drag, press the ESC key to cancel the drag operation.

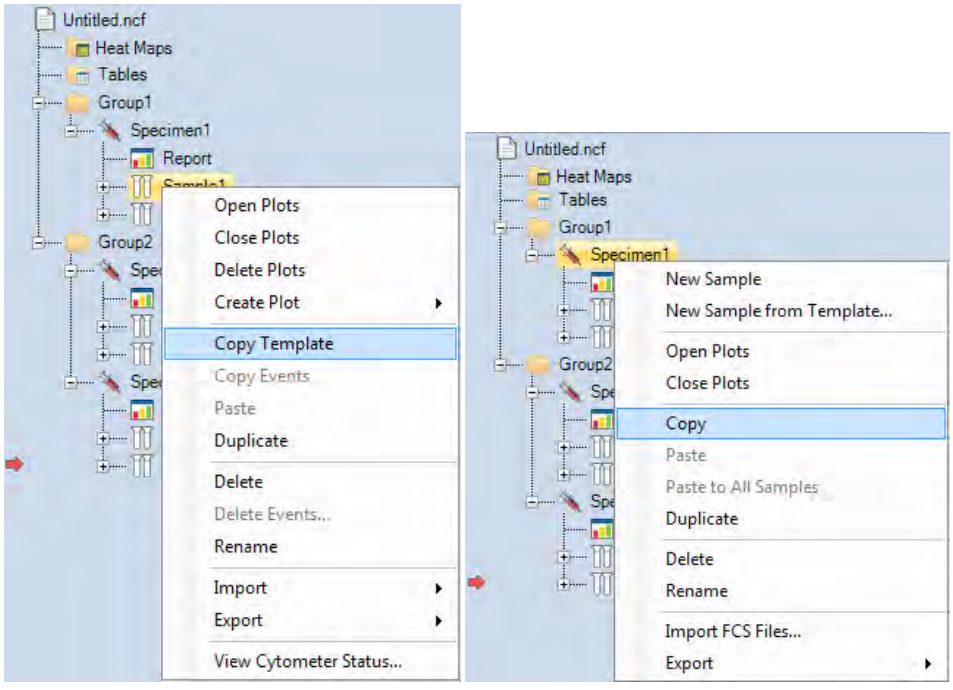
Experiment Manager supports making multiple selections within tree node to allow batch operation of multiple objects. Use Shift key to select continuous nodes on the tree and use ***Ctrl*** *key to select discontinuous nodes on the tree, just like selecting multiple files on Windows Explorer. Only nodes of same type can be selected simultaneously. The selection menu may have less items available when in multiple selection mode.*

6.3 Templates

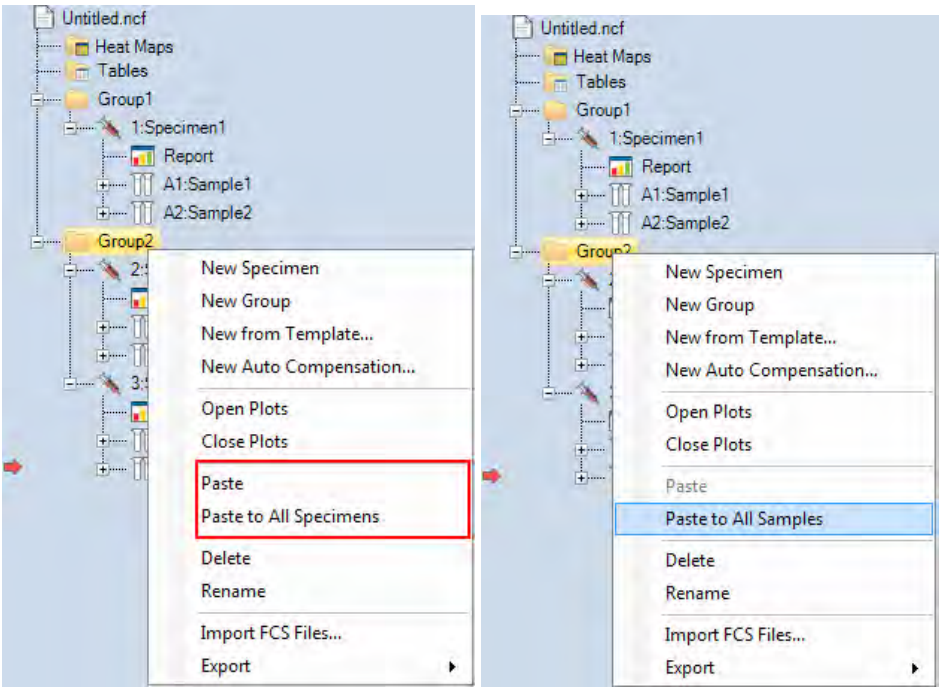
The NovoExpress Software allows for the use of templates to quickly set up experiment settings. These files can contain the settings for groups, specimens, or samples. There are multiple methods for a template to be applied including: copying and pasting, dragging and dropping, adding through the toolbar, and importing and exporting templates.

6.3.1 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 (*Section 6.3.2*) and using the toolbar (*Section 6.3.3*) except for one exception described in *Section 6.3.2*.

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.

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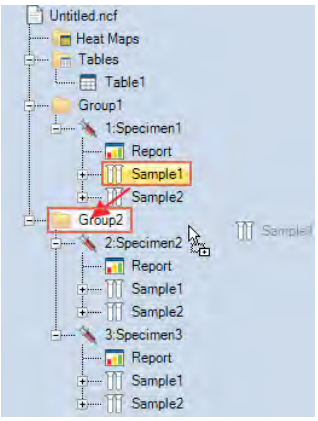
The source node	Target node
Cytometer Setting	*Cytometer Setting, Sample*: The cytometer settings template is replaced. If the target sample node contains previously collected events, only the parameter aliases are replaced. *Specimen, Groups, Experiment File*: The cytometer settings templates for all 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.

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

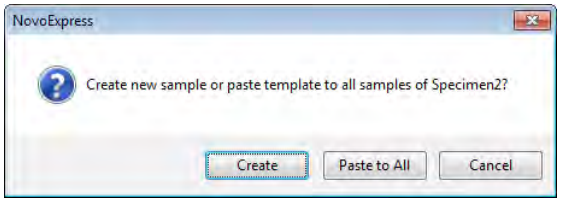
6.3.2 Drag and Drop the Templates

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 *Section 6.2.3 Move Items*.

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.



During the drag, press the ESC key to cancel the drag operation.

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.

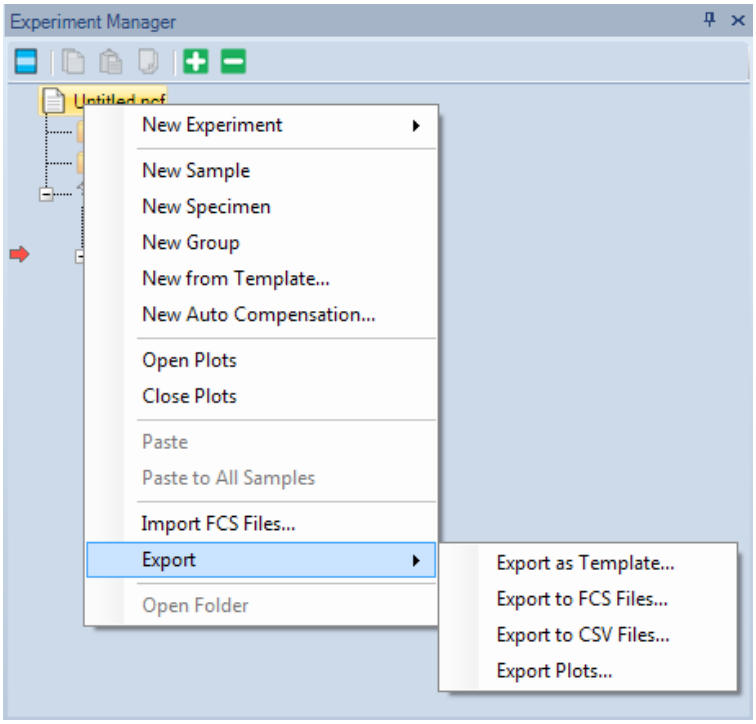
6.3.3 Using the Toolbar

In the *Experiment Manager* panel, select the source node to be copied. The node will be highlighted in yellow (*e.g.* Specimen1). To copy the selected node, click the *Copy* button , from the *Experiment Manager* panel toolbar. To paste the copied node, select the target node in the *Experiment Manager* panel, and click the *Paste* button , from the toolbar.

6.3.4 Import and Export Templates

Export Template

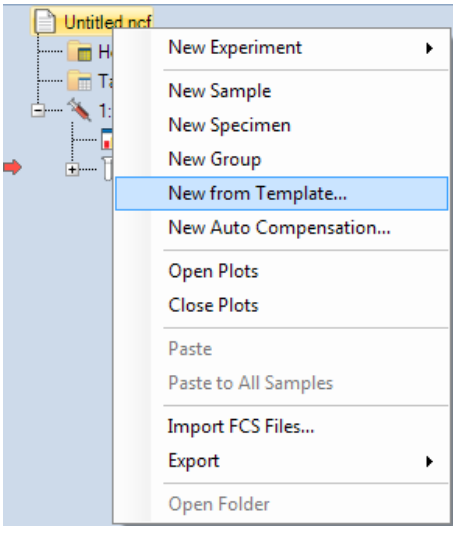
In the *Experiment Manager* panel, select the sample, specimen, group, or experiment file to export. Right-click the selected node and select *Export* → *Export as Template…*. The template will be exported as a *.nct template file.



Import Template

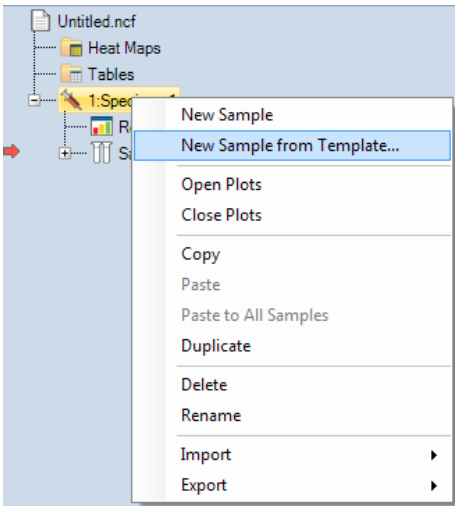
- Import to Experiment File

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



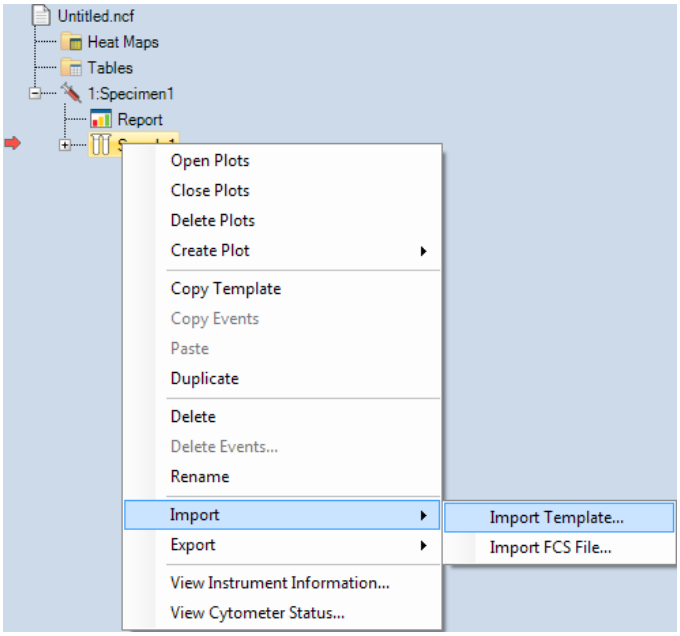
- Import to Specimen

  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*…

6.4 Importing and Exporting 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.

6.4.1 Importing Data

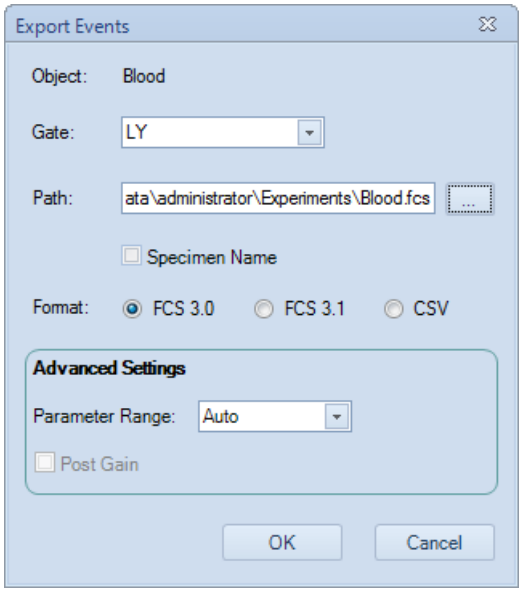
There are multiple methods for importing FCS files through the *Experiment Manager* panel:

- 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.

  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*.

6.4.2 Exporting 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.



The *Export Events* window has the following settings:

- *Object:* This is the node to be exported. If the object is a sample, only the sample will be exported. If the object is a specimen, group, or experiment file, all samples within the object will be exported.
- *Gate:* The default gate setting is All. In this setting, all events are exported for each of the exported samples. A specific gate can be selected using the drop-down menu. When a gate is selected, only events within the gate are exported. If an exported sample does not contain the selected gate, all events within the sample are exported. Also, if an exported sample contains the gate but the gate does not include any events, all of

the sample's events are exported.

- *Path:* The path specifies the location to save the exported files. The user can type in the textbox or use the button , to change the path. When the object is a single sample, the exported data file is saved directly at the path. When the object is a specimen, group, or experiment file, the exported data files is saved in subfolders representative of the sample hierarchy organization in the experiment manager.
- *Specimen Name:* Check this box to include specimen name in the name of the exported file.
- *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 NovoCyte System (10 to 2^{24}). 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.

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

*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.*

6.4.3 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*.

*This is useful when using the **Auto Compensation** feature during analysis to apply single stained compensation controls to the generated Auto Compensation samples.*

7. Reports

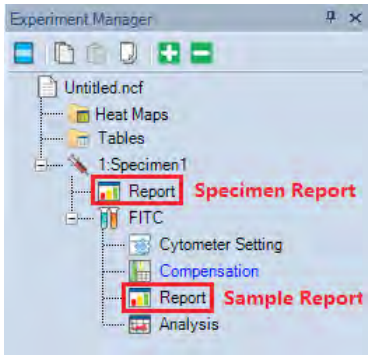
The NovoExpress Software's *Report* function enables the user to quickly generate customizable summaries of analyzed data. This section describes the creation and editing of reports using the NovoExpress Software.

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.

Real-time changes made to a plot within the ***Report*** *are also applied to sample's* ***Analysis*** *back in the main interface. Statistics are automatically adjusted.*

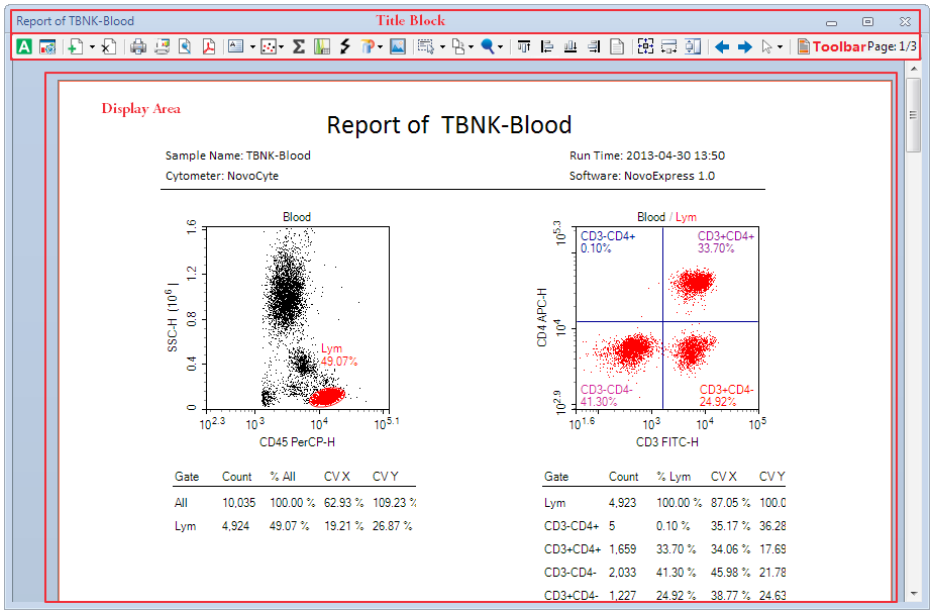
From the *Experiment Manager* panel, the report can either be a sample report or a specimen report. A sample report can contain plots, statistical information, compensation matrices, and collection information for the sample. A specimen report contains such information for all of its samples and basic information of its own. Double clicking on *Report* in the *Experiment Manager* allows the user to view the report interface window.

After the reports are created, a batch print function (*Section 7.6*) can be used to generate PDF files.



7.1 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  .  *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 *Section 7.3* for detail information of Report Options.

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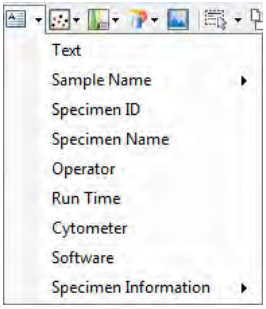
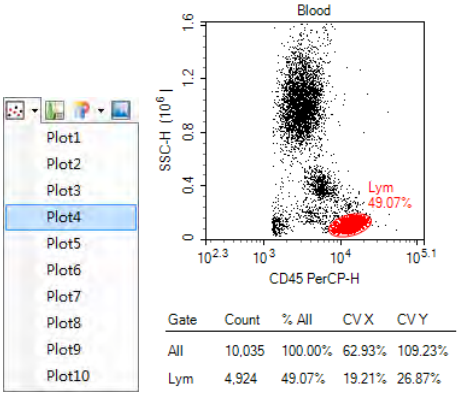
Icon	Description
	*Insert Page*: This function inserts a page into the report. By default, clicking the button inserts a blank page after the current page being viewed. In the display window, the current page being viewed has a red border. From the arrow to the right of the button, a drop-down menu allows 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 *Section 7.6*.
	*Print Preview*: Displays a print preview of the report.
	*PDF*: Generates a PDF file of the report.
	*Insert Text*: Inserts a textbox. The user can edit and format the text. Text types include: text, sample name, specimen name, operator, run time, cytometer, and software. In addition, specimen reports contain specimen information text type. 
	*Insert Plot*: Click the icon to list the plots for the sample, including dot plots, density plots, histogram plots, contour plots, and cell cycle plots. Select the plot to insert. The plot is inserted with the statistics as shown below. 

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Icon	Description
	*Insert Sample Statistics*: Inserts a table of gate statistics for a sample. In a sample report, click the button to insert a gate statistics table for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a gate statistics table for the selected sample.
	*Insert Compensation*: Inserts a spillover matrix. In a sample report, click the button to insert the spillover matrix for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert the spillover matrix for the selected sample. The spillover matrix cannot be edited from within the report. If the spillover matrix is modified in the main interface, the matrix in the report window will update.
	*Insert Photodetector Gain*: Inserts a table of photodetector gain setting for a sample. In a sample report, click the button to insert a table of photodetector gain setting for the sample. In a specimen report, click the button and select a sample from the dropdown menu to insert a table of photodetector gain setting for the selected sample.
	*Insert Shape*: Click to insert a horizontal line, vertical line, or rectangle.
	*Insert Picture*: Click open the *Open* window, select a picture to insert. The picture will be resized to an appropriate size with the original aspect ratio and inserted in the report.
	*Select*: The default is *Select All*. From the arrow on the right of the button, *Select Similar Objects* is also available.
	*Page Rotation*: Switches the page layout orientation between portrait and landscape.
	*Zoom*: Sets the zoom percentage of the displayed page. When report displays is zoomed in or zoomed out (zoom percentage is not 100%), user cannot double-click report item to enter into edit mode.
	*Align*: Select two or more objects in a page to activate these features. When selecting multiple objects, the first object has white squares on the border, while additional objects have black squares. From the four alignment options (align top, left, bottom, or right), select the edge to be aligned. The objects will be moved so that the selected edge of all selected objects aligns with the object first selected.
	Lock Position: Check this item to forbid moving any object of the report. *Align Top, Align Left, Align Bottom, Align Right* buttons will be disabled when *Lock Position* is checked.
	*Make Same Size / Width / Height*: Select two or more objects in a page to activate these features. When selecting multiple objects, the first object has white squares on the border, while additional objects will have black squares. From the three resize options (make same size, width, or height), select the desired resize dimension. The selected objects will be resized to match the selected dimension of the object first selected.

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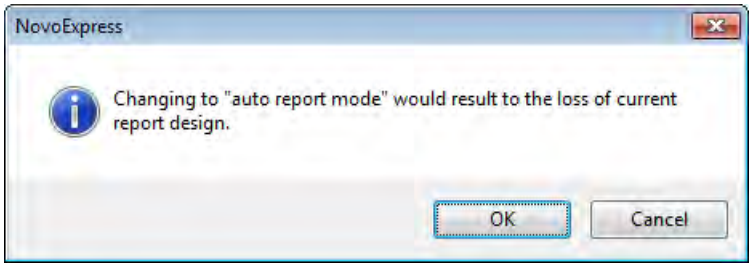
Icon	Description
	*Previous*: In a sample report, click to switch to the previous sample's report. In a specimen report, click to switch to the previous specimen's report.
	*Next*: In a sample report, click to switch to the next sample's report. In a specimen report, click to switch to the next specimen's 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.

7.2 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.

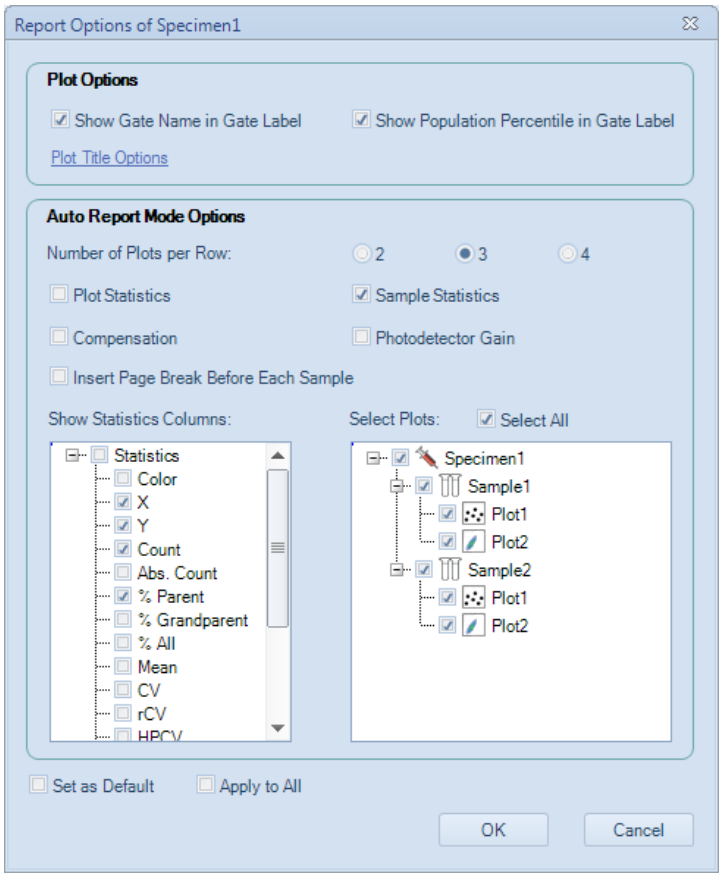


To switch the mode from automated to manual, click on *Auto Report Mode* button in the toolbar . The user can now make modifications to the report.

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.

7.3 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:*

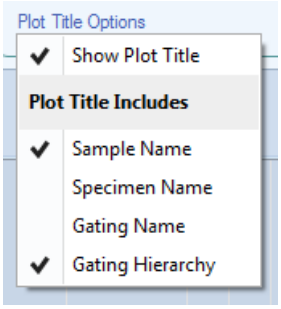
  If selected, gate name is displayed in gate label on the plot.

- *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.

- *Plot Statistics:*

  If selected, shows gate statistics of plot.

- *Sample Statistics:*

  If selected, shows gate statistics of sample.

- *Compensation:*

  If selected, shows compensation matrix.

- *Photodetector Gain:*

  If selected, shows the photodetector gain setting.

- *Insert Page Break Before Each Sample:*

  Only available for specimen report. If selected, a page break will be inserted before each sample.

- *Show Statistics Columns:*

  Selects statistical items to display.

- *Select Plots:*

  Selects plots to display on report.

- *Select All:*

  Selects all plots to display on report.

- *Set as Default:*

  Sets above settings as default setting for new reports.

- *Apply to All:*

  Applies above settings to all report in the experiment.

7.4 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

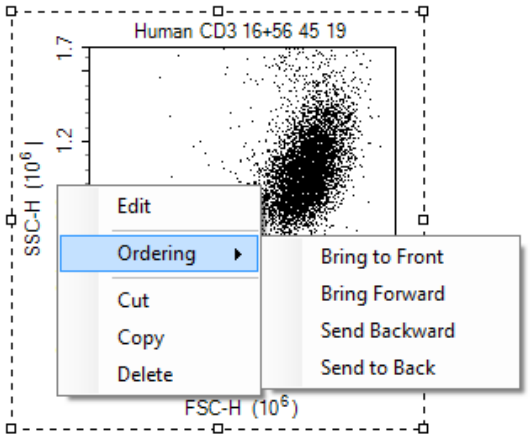
7.4.1 Add Report Objects

Objects can be added to the report through the toolbar, *Work Space*, and *Experiment Manager* panel. To add objects using the toolbar, use the insert functions described in *Section 7.1 Report Interface*. From both the *Work Space* and *Experiment Manager* panel, objects corresponding to the sample can be dragged and dropped into the report.

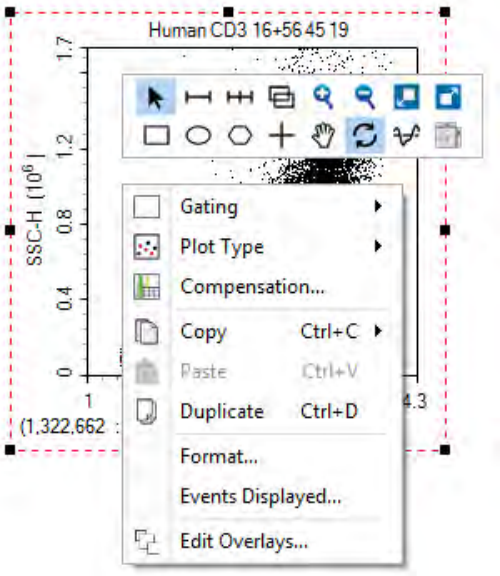
7.4.2 Select Report Objects

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.

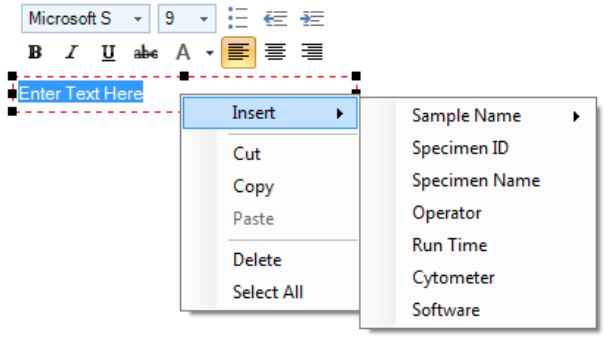
When multiple items are selected, the first item is bordered by a black dashed line with white control points and additional items are bordered by black dashed lines with black control points.

7.4.3 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.

7.4.3.1 Edit Text

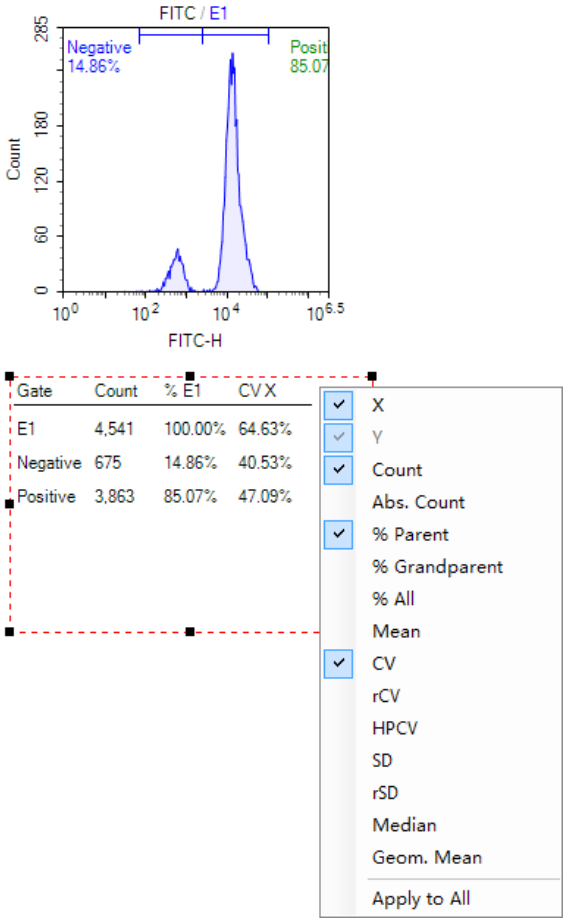
Double-click on the textbox or right-click on the textbox and select *Edit* to enter editing mode. In this mode, text formatting tools will appear. Right-click on the textbox and select *Insert* to insert sample information including sample name, specimen ID, specimen name, operator, run time, cytometer and software information, as shown below. This can also be accessed by clicking the *Insert Text* icon's drop-down menu in the tool bar.



7.4.3.2 Edit Plots and Statistics

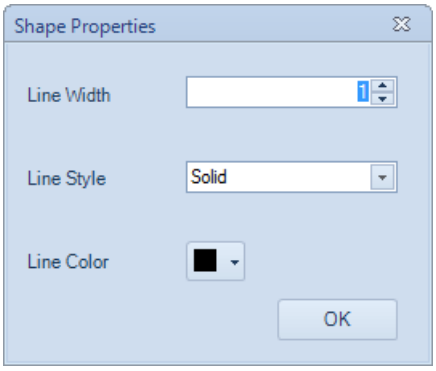
Double-click on the plot or right-click on the plot and select *Edit* to enter editing mode. Plots can be edited in the report by right-clicking in the plot to access the plot tools. Modifications made to the plot will also be updated to the plot in the main interface. In addition, if the plots are modified 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.



7.4.3.3 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.



7.4.3.4 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.

7.4.4 Aligning 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.)

7.4.5 Resizing 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.)

7.4.6 Ordering 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 movement for the object. Objects toward the front are displayed over objects further back.

7.4.7 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.

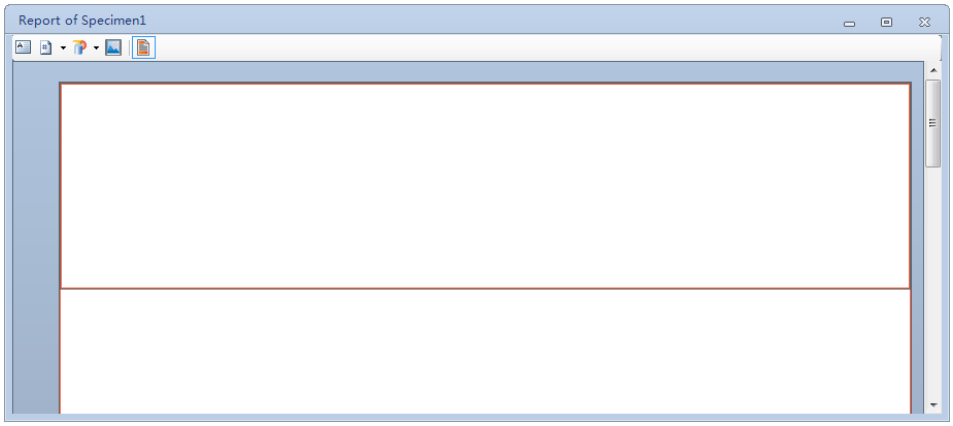
7.4.8 Insert or Delete Pages

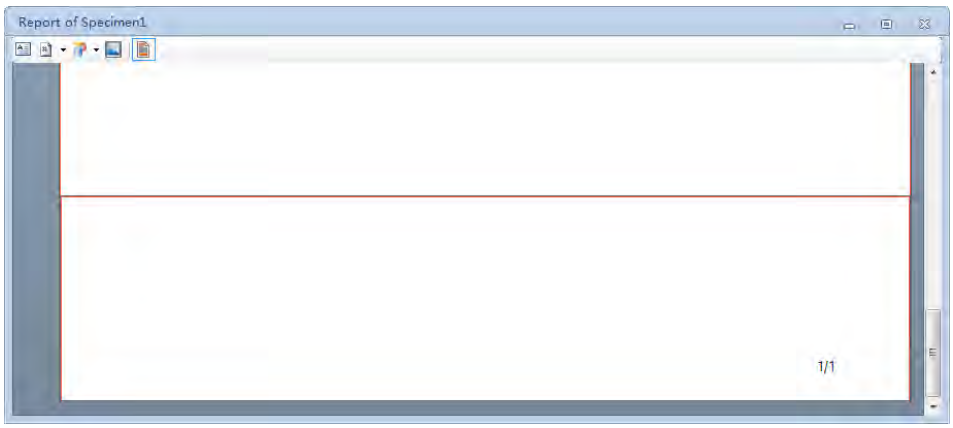
To insert or delete pages, use the *Insert Page* and *Delete Page* button from the toolbar. For more information, see *Section 7.1 Report Interface*.

7.4.9 Header and Footers

7.4.9.1 Headers and Footers Working Interface

The header and footer displays 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.

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Icon	Description
	*Insert Picture:* Inserts a picture.
	*Set Page Header and Footer:* Click to exit header and footer editing mode.

7.4.9.2 Edit Headers and Footers

To edit the header, click in the header region at the top of the page. Once selected, the region is bordered by a red rectangle. The toolbar can now be used to add objects to the header.

To edit the footer, click in the footer region at the bottom of the page. After selected, the region is bordered by a red rectangle. The toolbar can now be used to add objects to the footer. The default footer includes an object for page number.

The methods for editing the header and footer are consistent with the rest of the report interface with the following exceptions.

- The level of the objects in the header and footer cannot be ordered. Newer created objects are automatically created more towards the top. Objects in the header and footer are behind objects created in the main report interface.
- Smart guides are not available to help align objects in the header and footer.
- Copying and pasting objects is unavailable in the header and footer.
- Variables such as sample name, specimen ID. Specimen name, operator, run time, and cytometer and software information cannot be inserted into textboxes in the header or footer.

After editing the header and footer is complete, click the *Set Page Header and Footer* button  , to return to the main report interface. The header and footer will display on all pages of the report.

7.4.9.3 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 *Section 6.3 Templates*.

7.5 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.

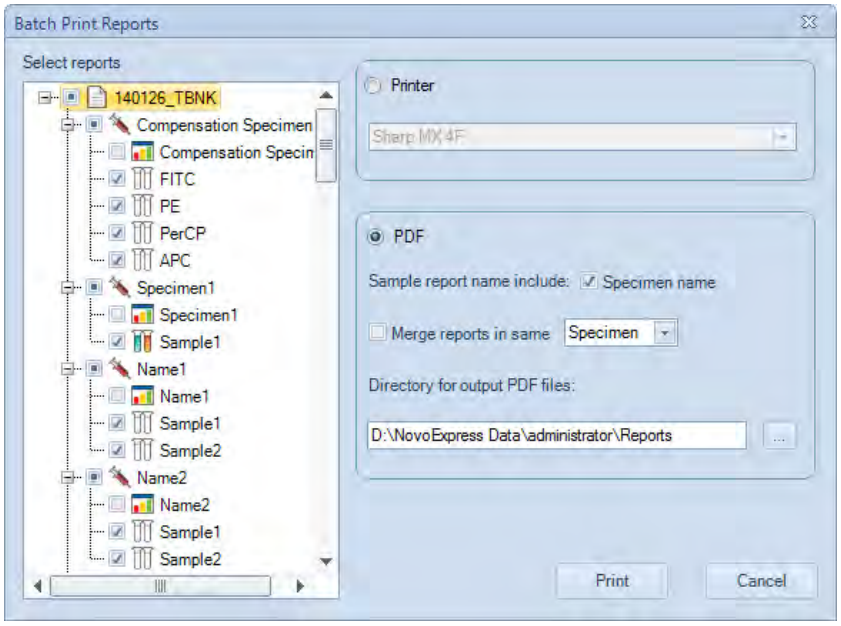
- To Convert to PDF:

  Click the toolbar *PDF* button  . The *Save As* window will appear to save the report as a PDF file.

7.6 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.



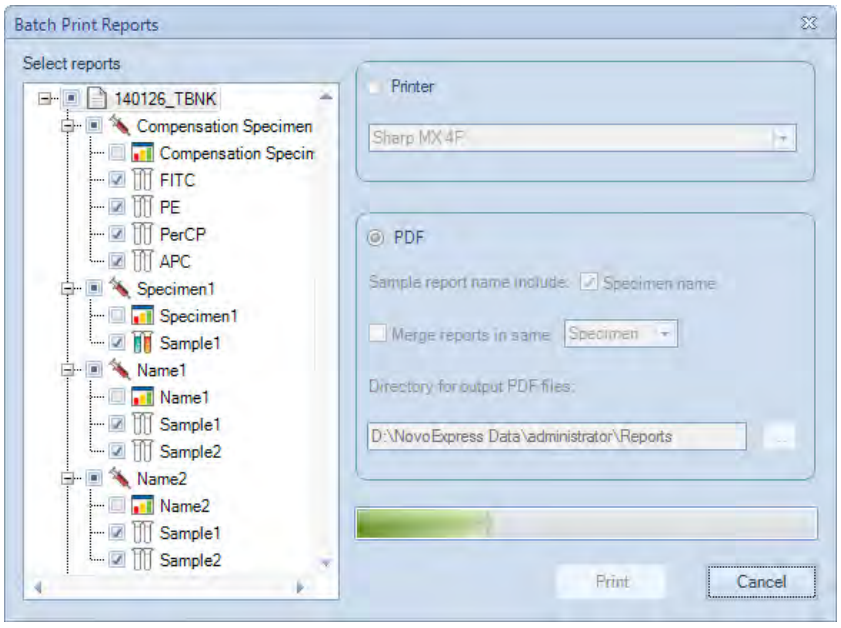
The reports are listed on the left side of the *Batch Print Reports* window. Use the check-boxes to select the reports to be printed. On the right side of the window, select either to print the report or generate PDF files for the report.

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_YYYYM-MDD_hhmmss*. If the box is unchecked, the PDF files will be saved in the format *sample name_YYYYMMDD_hhmmss*. Click *Print* to begin generating the PDF files.

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.

8. QC Test

Quality control (QC) test is an essential part of the maintenance of the NovoCyte and NovoCyte Quanteon system. In QC test, NovoCyte QC particles are used to check the instrument performance. Measured data are used to determine if the performance of NovoCyte or NovoCyte Quanteon system fall into the standard range to ensure stable and reliable operation of the instrument. This section covers running the QC test and reviewing the QC test report.

8.1 Run the QC Test for NovoCyte Instrument

To run the QC test:

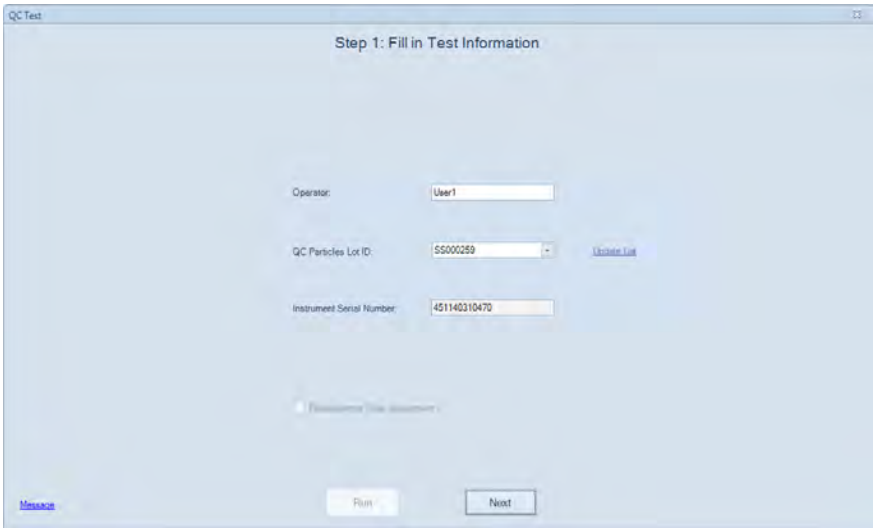
1. Save the NovoCyte QC particles Lot file.

   Download the Lot File for the specific batch of NovoCyte QC particles from http://www.aceabio.com/novocyte/qc-particles. Save the Lot File in a specific directory in the NovoExpress Software's installation directory. (Save the file in *C:\Program Files (x86)\NovoExpress\QC \QC Beads*, if the installation directory is *C:\Program Files (x86)\NovoExpress*). Saving the Lot file is only done once for each new batch of NovoCyte QC particles. After saving the Lot File to the directory, the lot ID for the specific batch will be listed when running the QC test.

2. Prepare the QC particles by diluting the beads with dilution buffer.

   First thoroughly mix the NovoCyte QC particles. In a test tube, add 2 drops of NovoCyte QC Particles (Generation 2, Cat. # 8000004) to 1 mL of dilution buffer (0.8 mL PBS and 0.2 mL ACEA NovoRinse solution). Vortex the microsphere suspension. Place the prepared QC particles sample in the sample holder of the instrument.

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.



QC Test: Step 1 Fill in Test Information

   In the window, enter the name of the operator, select the *QC particles lot ID*, and click *Next*. If the correct QC particles lot ID is not listed, please refer to the first step to download and save the QC particles Lot File.

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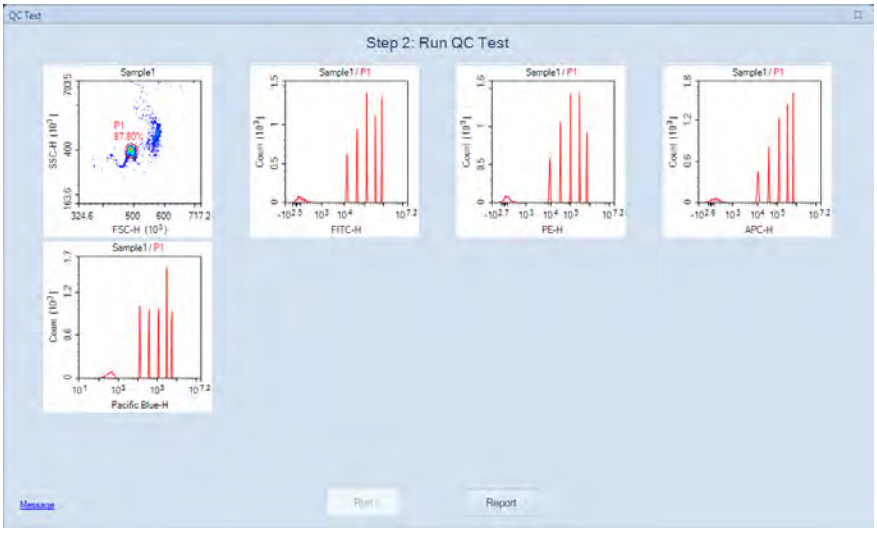
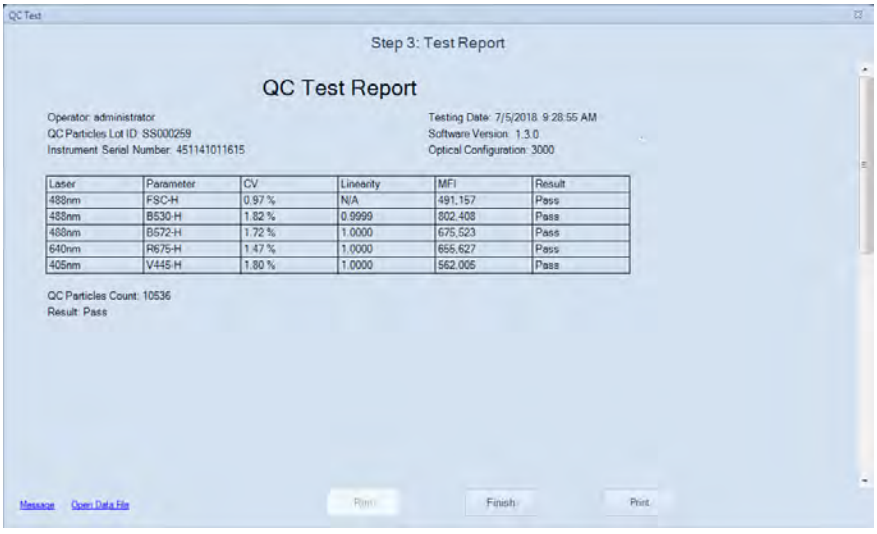
4 Click *Run* to begin the test. The instrument will begin collecting events and the results will be displayed in real-time on the *QC Test* window.



QC Test: Step 2 Run QC Test

5 After data collection is complete, click the *Report* button to view the test results.



QC Test: Step 3 Test Report

From the figure, the QC test will provide results for various parameters. Click *Print* to print the test report.

To view the history of the resulting QC data, click ***QC Test Report*** *from the* ***Instrument*** *tab of the* ***Menu Bar*** *(described in Section 8.3). Highlight the date to be viewed and select the* ***QC Test Report*** *tab.*

The QC test provides a result for each tested parameter. There are three possible results.

- *Pass*: The parameter meets performance requirements.
- *Failed*: The parameter does not meet the performance requirements.
- *Acceptable*: The parameter does not meet the factory calibration requirements, but the use of the instrument does not affect experimental results.

The QC test provides a result of the test, *Pass* or *Failed* or *Acceptable*. If failed, a label in red will show on report to indicate the reason of failure.

8.2 Run the QC Test for NovoCyte Quanteon Instrument

To run the QC test:

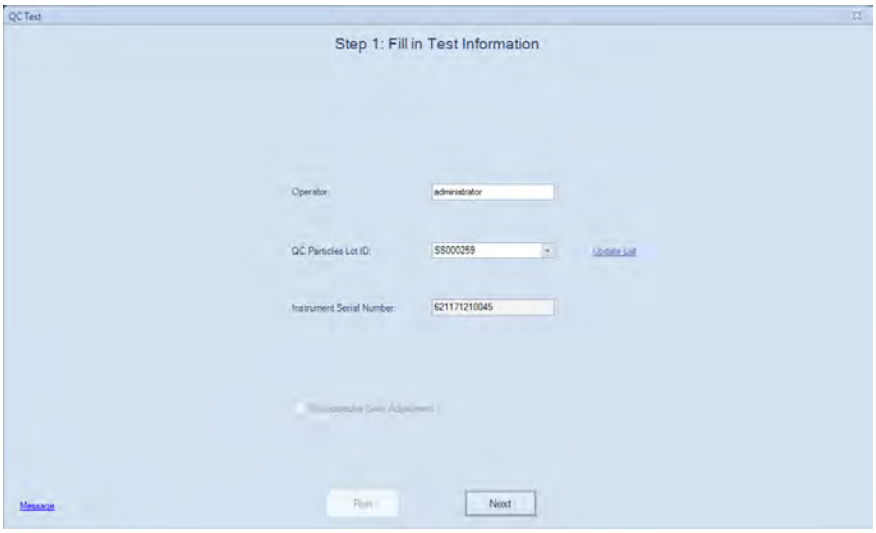
1. Save the NovoCyte QC particles Lot file.

   Download the Lot File for the specific batch of NovoCyte QC particles from http://www.aceabio.com/novocyte/qc-particles. Save the Lot File in a specific directory in the NovoExpress Software's installation directory. (Save the file in *C:\Program Files (x86)\NovoExpress\QC \QC Beads*, if the installation directory is *C:\Program Files (x86)\NovoExpress)*. Saving the Lot file is only done once for each new batch of NovoCyte QC particles. After saving the Lot File to the directory, the lot ID for the specific batch will be listed when running the QC test.

2. Prepare the QC particles by diluting the beads with dilution buffer.

   First thoroughly mix the NovoCyte QC particles. In a test tube, add 2 drops of NovoCyte QC Particles (Generation 2, Cat. # 8000004) to 1 mL of dilution buffer (0.8 mL PBS and 0.2 mL ACEA NovoRinse solution). Vortex the microsphere suspension. Place the prepared QC particles sample in the sample holder of the instrument.

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.



QC Test: Step 1 Fill in Test Information

   In the window, enter the name of the operator, select the *QC particles lot ID*, and click *Next*. If the correct QC particles lot ID is not listed, please refer to the first step to download and save the QC particles Lot File.

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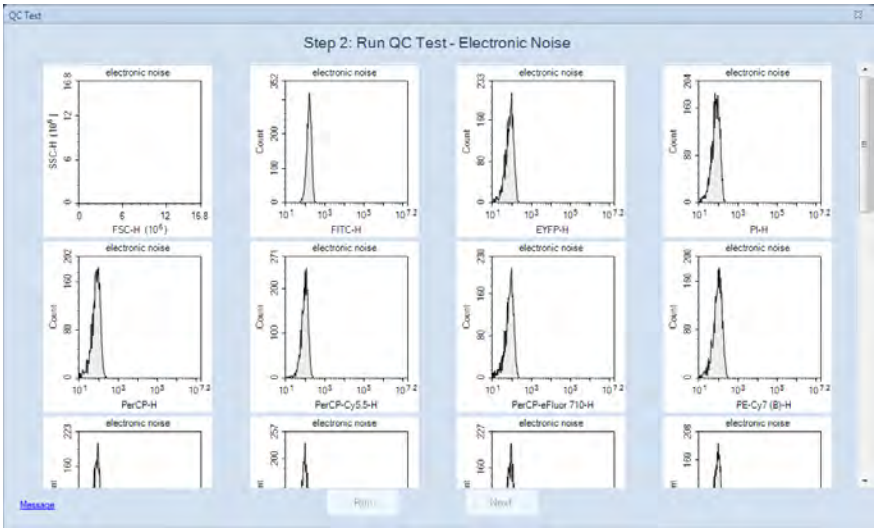
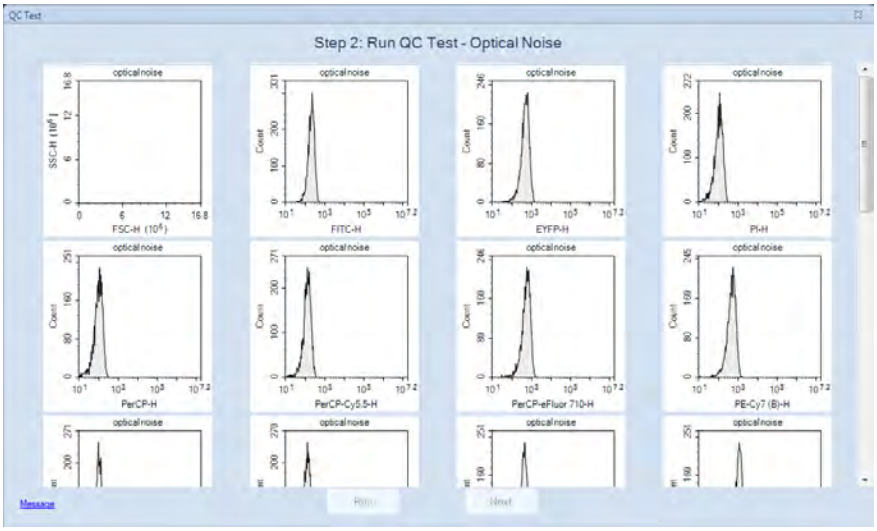
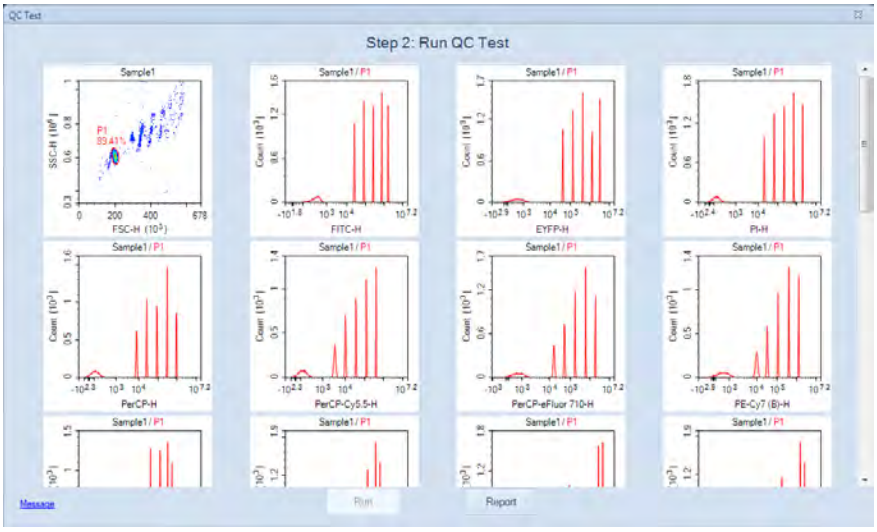
4 Click *Run* to begin the test. The instrument will begin collecting electronic noise, optical noise and events, the results will be displayed in real-time on the *QC Test* window.



QC Test: Step 2 Collect Electronic Noise

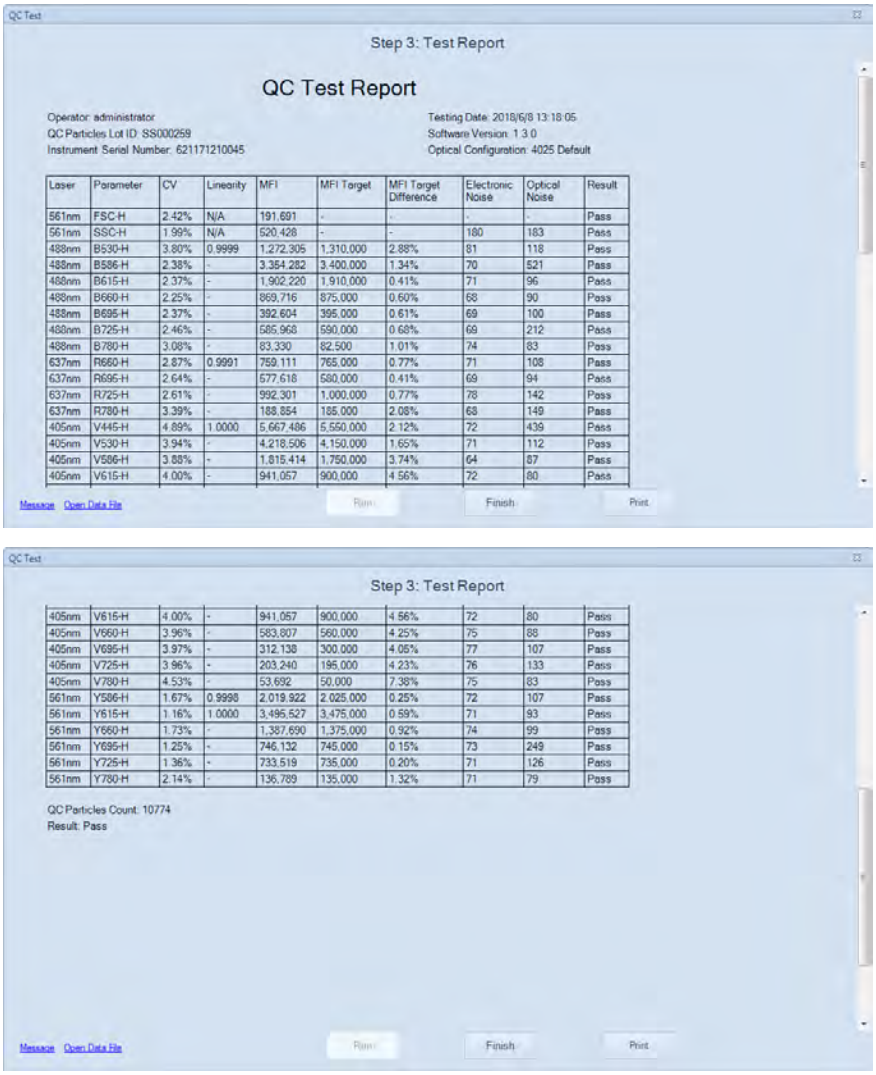


QC Test: Step 2 Collect Optical Noise



QC Test: Step 2 Collect Events

5 After data collection is complete, click the *Report* button to view the test results.



QC Test: Step 3 Test Report

The QC Test Report will provide results for various parameters. Click *Print* to print the test report. Click *Open Data File* to open the QC file.

To view the history of the resulting QC data, click ***QC Test Report*** *from the* ***Instrument*** *tab of the* ***Menu Bar*** *(described in Section 8.3). Highlight the date to be viewed and select the* ***QC Test Report*** *tab.*

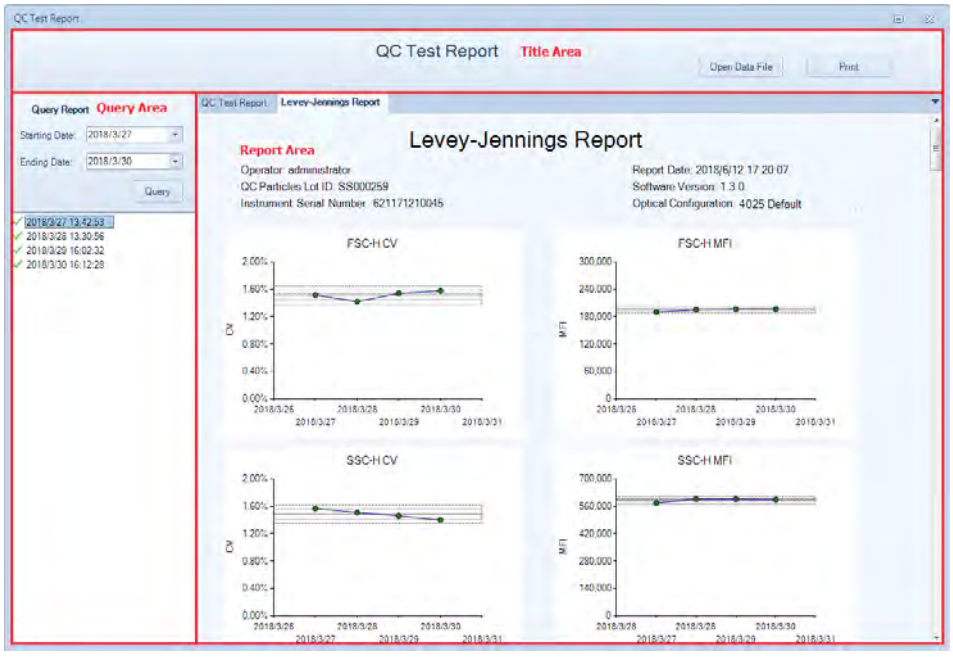
The QC test provides a result for each tested parameter. There are three possible results.

- *Pass*: The parameter meets performance requirements.
- *Failed*: The parameter does not meet the performance requirements.
- *Acceptable*: The parameter does not meet the factory calibration requirements, but the use of the instrument does not affect experimental results.

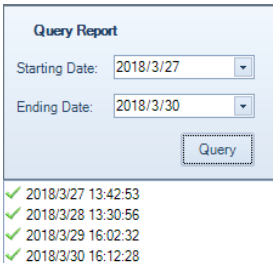
The QC test provides a result of the test, *Pass* or *Failed* or *Acceptable*. If failed, a text in red will show on report to indicate the reason of failure.

8.3 View QC Test Report

The QC test report function stores previous QC test results and provides a data analysis feature to track instrument performance changes over a period time. To open the *QC Test Report* window, click *QC Test Report* from the *Instrument* tab of the *Menu Bar*. The window is shown below.



The *QC Test Report* window contains the following sections.

Interface	Description
Title Area	Displays the report name and the *Print* button. Click the *Print* button to print the currently displayed QC Test Report or Levey-Jennings Report page.
Query Area	Query a time interval for QC test reports. As shown: 

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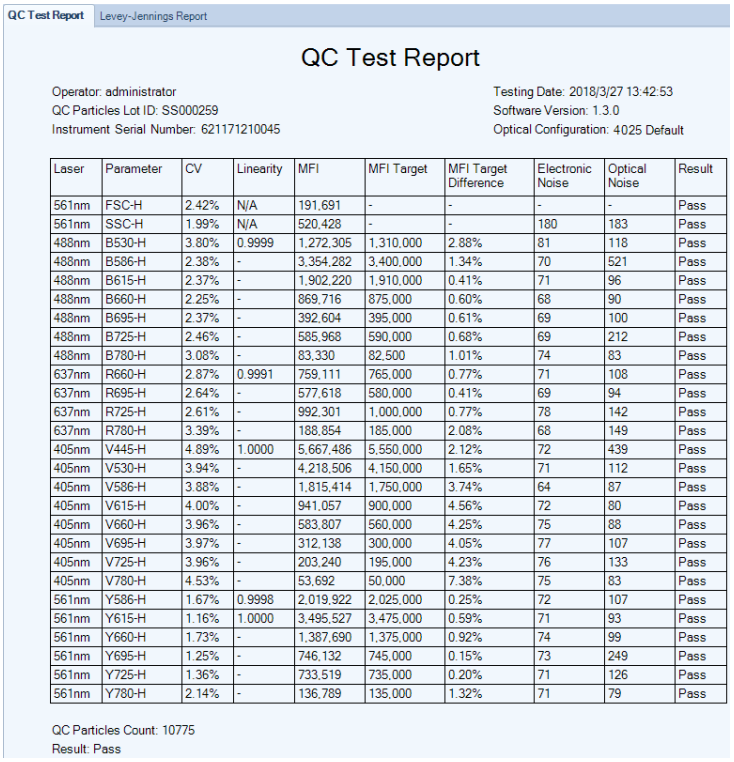
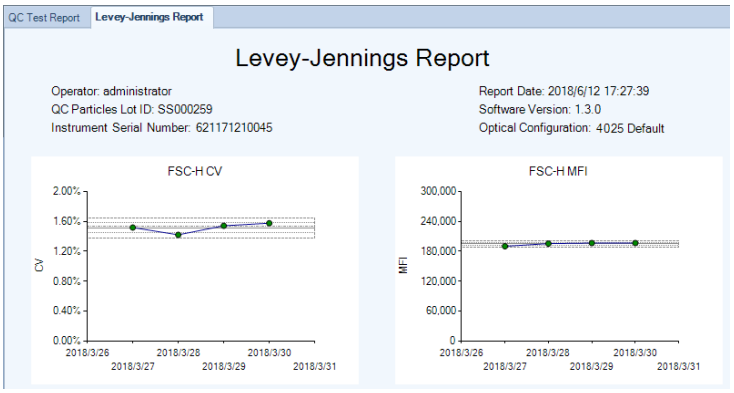
Interface	Description
QC Test Report	Displays the QC test report for a selected report from the query results. As shown: 
Levey-Jennings Report	Displays the QC test data over time to observe trends in instrument performance. As shown: 
Open Data File	Open the QC data file of the selected report.
Print	Click the *Print* button in the top right corner of the window to print QC test reports or Levey-Jennings reports.

Laser	Parameter	CV	Linearity	MFI	MFI Target	MFI Target Difference	Electronic Noise	Optical Noise	Result
561nm	FSC-H	2.42%	N/A	191,691	-	-	-	-	Pass
561nm	SSC-H	1.99%	N/A	520,428	-	-	180	183	Pass
488nm	B530-H	3.80%	0.9999	1,272,305	1,310,000	2.88%	81	118	Pass
488nm	B586-H	2.38%	-	3,354,282	3,400,000	1.34%	70	521	Pass
488nm	B615-H	2.37%	-	1,902,220	1,910,000	0.41%	71	96	Pass
488nm	B660-H	2.25%	-	869,716	875,000	0.60%	68	90	Pass
488nm	B695-H	2.37%	-	392,604	395,000	0.61%	69	100	Pass
488nm	B725-H	2.46%	-	585,968	590,000	0.68%	69	212	Pass
488nm	B780-H	3.08%	-	83,330	82,500	1.01%	74	83	Pass
637nm	R660-H	2.87%	0.9991	759,111	765,000	0.77%	71	108	Pass
637nm	R695-H	2.64%	-	577,618	580,000	0.41%	69	94	Pass
637nm	R725-H	2.61%	-	992,301	1,000,000	0.77%	78	142	Pass
637nm	R780-H	3.39%	-	188,854	185,000	2.08%	68	149	Pass
405nm	V445-H	4.89%	1.0000	5,667,486	5,550,000	2.12%	72	439	Pass
405nm	V530-H	3.94%	-	4,218,506	4,150,000	1.65%	71	112	Pass
405nm	V586-H	3.88%	-	1,815,414	1,750,000	3.74%	64	87	Pass
405nm	V615-H	4.00%	-	941,057	900,000	4.56%	72	80	Pass
405nm	V660-H	3.96%	-	583,807	560,000	4.25%	75	88	Pass
405nm	V695-H	3.97%	-	312,138	300,000	4.05%	77	107	Pass
405nm	V725-H	3.96%	-	203,240	195,000	4.23%	76	133	Pass
405nm	V780-H	4.53%	-	53,692	50,000	7.38%	75	83	Pass
561nm	Y586-H	1.67%	0.9998	2,019,922	2,025,000	0.25%	72	107	Pass
561nm	Y615-H	1.16%	1.0000	3,495,527	3,475,000	0.59%	71	93	Pass
561nm	Y660-H	1.73%	-	1,387,690	1,375,000	0.92%	74	99	Pass
561nm	Y695-H	1.25%	-	746,132	745,000	0.15%	73	249	Pass
561nm	Y725-H	1.36%	-	733,519	735,000	0.20%	71	126	Pass
561nm	Y780-H	2.14%	-	136,789	135,000	1.32%	71	79	Pass

9. Troubleshooting

9.1 Troubleshooting for NovoCyte Instrument

The following table lists possible causes for warning and error prompts from the status bar when connecting with NovoCyte instrument.

Message ID	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. Click the *OK* button in the prompted dialog box or wait 10 seconds for automatic error handling. The instrument will move the SIP to the home position. This procedure will take about 3 minutes.
		Incorrect plate selected in plate manager	Select the correct plate in the plate manager window.
		Incorrect positioning of plate in NovoSampler Pro	► Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps. ► Re-calibrate NovoSampler Pro.
		Dirty SIP or SIP wash apparatus	Clean the SIP or SIP wash apparatus following the procedures described in *Section 1.1 Preventative Maintenance* in *NovoCyte® Maintenance 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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.
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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.
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 *Section 2.1.3 Empty Waste* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.
0x0006	*System voltage is out of range*	System Error	Restart the NovoCyte instrument.
0x0007	*System electric current is out of range*	System Error	Restart the NovoCyte instrument.
0x0008	*Firmware configuration error*	System Error	Restart the NovoCyte instrument.
0x0009 *0x000A* *0x000B*	..... *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*	..... *laser is not connected*	Specified laser is not detected	Restart the NovoCyte instrument.
0x000F	*NovoSampler communication lost*	The cable between the NovoSampler (Pro) and NovoCyte Flow Cytometer is not securely connected	Reconnect the cable between the NovoSampler (Pro) and NovoCyte Flow Cytometer.
		NovoSampler (Pro) is not communicating with NovoCyte	Restart NovoCyte instrument.
0x0010	*NovoSampler (Pro) has not been calibrated*	NovoSampler (Pro) is newly installed or re-connected.	Follow the prompted instructions to calibrate the NovoSampler (Pro).

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Message ID	Software Messages	Possible Causes	Recommended Solutions
0x0011	*NovoSampler (Pro) calibration failed……*	NovoSampler (Pro) is not installed properly	Re-install and calibrate the NovoSampler (Pro) following the procedures described in *Section 1.2 Installation* in *NovoSampler® Operator's Guide* or *NovoSampler® Pro Operator's Guide*.
		The NovoSampler (Pro) cover was opened during calibration.	Close the NovoSampler (Pro) cover and redo the calibration.
0x0012	*The movement of plate is out of range*	The movement of the orbital shaker is blocked.	▶ Check the path of the orbital shaker to make sure there are no objects blocking the movement. Clear the block if there is any. ▶ Click *Instrument* → *NovoSampler Pro* → *Calibrate* in NovoExpress Software to re-calibrate the NovoSampler (Pro).
		NovoSampler (Pro) is not installed properly	Re-install and calibrate the NovoSampler (Pro) following the procedures described in *Section 1.2 Installation* in *NovoSampler® Operator's Guide* or *NovoSampler® Pro Operator's Guide*.
0x0013	*Cover of NovoSampler (Pro) is opened during moving plate*	The cover of NovoSampler (Pro) is open during moving plate	Close the NovoSampler (Pro) cover. NovoSampler (Pro) will automatically reset and be ready for operation.
0x0014	*Pressure is out of limit*	Waste container is not correctly connected to the instrument	Check the quick coupling connectors to ensure the waste container is correctly connected to the instrument.
		Sheath in-line filter is clogged	Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Maintenance Guide*.
		Flow cell is clogged	Conduct Unclog from NovoExpress.
0x0015	*NovoSampler firmware error*	NovoSampler or NovoSampler Pro firmware is not working properly	Re-install or upgrade the NovoSampler or NovoSampler Pro firmware.
0x0016 *0x0017* *0x0018*	*…… laser does not emit*	Specified laser is not connected properly or laser is not working properly	▶ Reconnect the laser cable properly. ▶ Restart the instrument.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
0x0019 *0x001A* *0x001B*	*…… laser communication error*	Interference or bad connection or bad laser	▶ Reconnect the laser cable properly. ▶ Restart the instrument.
0x001C	*Sample injection probe reset failed*	Bad connection or optocoupler damaged	Click the *OK* button in the prompted dialog box or wait 10 seconds for automatically error handling. Restart the instrument.
0x001D	*Sampling Pump reset failed*	Bad connection or optocoupler damaged	Restart the instrument.
0x001E	*The SIP module needs to be upgraded to support NovoSampler Pro*	SIP module is not compatible with connected NovoSampler Pro	Contact ACEA technical support for service.
0x0020	*System initialization is paused*	Liquid level in the reagent containers is not within normal range when NovoCyte is powered up.	▶ Make sure that the instrument reagent containers are placed correctly and the liquid level in each of 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 damaged	Restart the instrument.
0x0022	*Resetting NovoSampler Pro to zero position failed*	NovoSampler Pro is not working properly.	Click *Reset*. Restart the NovoSampler Pro.
0x0023	*Resetting NovoSampler Pro to home position failed*	NovoSampler Pro is not working properly.	Click *Reset*. Restart the NovoSampler Pro.
0x0100	*Instrument cover opened*	Instrument cover is open 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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station not working properly	Replace the fluidic station.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
0x0102	*NovoRinse running low*	NovoRinse solution is below the pre-set volume limit	Refill the NovoRinse container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station not working properly	Replace fluidic station.
0x0103	*NovoClean running low*	NovoClean solution is below the pre-set volume limit	Refill the NovoClean container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station not working properly	Replace fluidic station.
0x0104	*Waste container is close to full*	Waste is above the pre-set volume limit	Empty the waste container following the procedures described in *Section 2.1.3 Empty Waste* in *NovoCyte® Flow Cytometer Operator's Guide*.
		Fluidic station not working properly	Replace fluidic station.
0x0105	*Cover of NovoSampler (Pro) opened*	Cover of NovoSampler (Pro) is opened	Close the cover.
0x0106	*NovoSampler (Pro) is disconnected when powered up*	The NovoSampler (Pro) is disconnected when powered up	▶ Shut down NovoCyte Flow Cytometer. ▶ Reconnect the cable of the NovoSampler (Pro) to NovoCyte flow cytometer. ▶ Turn on NovoCyte and follow the prompts to calibrate the NovoSampler (Pro).
0x0109	*Fluidics station is not connected*	Cable between the fluidics station and the NovoCyte instrument is not properly connected	▶ Power down the instrument. ▶ Reconnect the cable between the fluidics station and the NovoCyte instrument. ▶ Power up the instrument.
0x010A *0x010B* *0x010C* *0x010D*	*…… liquid level sensor failure*	Specified liquid level sensor in the fluidics station is not working properly	▶ Reconnect the fluidics station cable. ▶ Restart the NovoCyte instrument. ▶ Replace the fluidics station.
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 *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Maintenance Guide*.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
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 Pro is not working properly	No action is needed. The NovoSampler Pro can be used normally.
	Communication error (code: xx, xx). Please restart NovoCyte and NovoExpress	USB cable between the NovoCyte instrument and workstation is not connected properly	▶ Reconnect the USB cable between the NovoCyte instrument and the workstation. ▶ Restart the instrument and NovoExpress software.

9.2 Troubleshooting for NovoCyte Quanteon Instrument

The following table lists possible causes for warning and error prompts from the status bar when connecting with NovoCyte Quanteon instrument.

Message ID	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. This procedure will take about 10 seconds.
		Incorrect plate selected in plate manager	Select the correct plate in the plate manager window.
		Incorrect positioning of plate in NovoSampler Q	▶ Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps. ▶ Re-calibrate NovoSampler Q.
		Dirty SIP or SIP Cleaning apparatus	Clean the SIP or SIP wash apparatus following the procedures described in *Section 1.1 Preventative Maintenance* in *NovoCyte Quanteon™ Maintenance 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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.

Message ID	Software Messages	Possible Causes	Recommended Solutions
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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.
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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.
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 *Section 2.1.3 Empty Waste* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.
0x0006	*System voltage is out of range*	System Error	Restart the NovoCyte Quanteon instrument.
0x0007	*System electric current is out of range*	System Error	Restart the NovoCyte Quanteon instrument.
0x0008	*Firmware configuration error*	System Error	Restart the NovoCyte Quanteon instrument.
0x0009 *0x000A* *0x000B* *0x4009*	*…… 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*	*….laser is not connected*	Specified laser is not detected	Restart the NovoCyte Quanteon instrument.
0x000F	*NovoSampler communication lost*	The cable between the NovoSampler Q and NovoCyte Quanteon Flow Cytometer is not securely connected	Reconnect the cable between the NovoSampler Q and NovoCyte Quanteon Flow Cytometer.
		NovoSampler Q is not communicating with NovoCyte Quanteon	Restart NovoCyte Quanteon instrument.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
0x0010	*NovoSampler has not been calibrated*	NovoSampler Q is newly installed or re-connected.	Follow the prompted instructions to calibrate the NovoSampler Q.
0x0011	*NovoSampler Q calibration failed……*	NovoSampler Q is not installed properly	Re-install and calibrate the NovoSampler Q following the procedures described in *Section 1.2 Installation* in *NovoSampler® Q Operator's Guide*.
		The NovoSampler Q cover is opened during calibration.	Close the NovoSampler Q cover and redo the calibration.
		The optocoupler of NovoSampler Q is not working properly	Restart NovoCyte Quanteon instrument and redo the calibration.
		The motor of NovoSampler Q is not working properly.	Restart NovoCyte Quanteon instrument and redo the calibration.
0x0012	*The movement of plate is out of range*	The movement of the orbital shaker is blocked.	▶ Check the path of the orbital shaker to make sure there are no objects blocking the movement. Clear the block if there is any. ▶ Click *Instrument* → *NovoSampler Pro* → *Calibrate* in NovoExpress Software to re-calibrate the NovoSampler Q.
		NovoSampler Q is not installed properly	Re-install and calibrate the NovoSampler Q following the procedures described in *Section 1.2 Installation* in *NovoSampler® Q Operator's Guide*.
0x0013	*Cover of NovoSampler is opened during moving plate*	The cover of Novo Sampler Q is opened during moving plate	Close the NovoSampler Q cover. NovoSampler Q will automatically reset and be ready for operation.
0x0014	*Pressure is out of limit*	▶ Sample injection probe or flow cell is clogged ▶ Sheath fluid in-line filter is clogged ▶ Waste container is not correctly connected to the instrument ▶ Other fluidic components are not working properly	Follow the prompted instructions from NovoExpress to clear the error.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
0x0015	*NovoSampler firmware error*	NovoSampler Q firmware is not working properly	Re-install or upgrade the NovoSampler Q firmware.
0x0016 *0x0017* *0x0018* *0x400B*	..... *laser does not emit*	Specified laser is not connected properly or laser is not working properly	▶ Turn off the instrument. ▶ Reconnect the laser cable properly. ▶ Restart the instrument.
0x0019 *0x001A* *0x001B* *0x400C*	..... *laser communication error*	Specified laser is not communicating with the instrument properly or the laser is not working properly	▶ Turn off the instrument. ▶ Reconnect the laser cable properly. ▶ Restart the instrument.
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 the instrument to automatically start the error handling. ▶ Restart the instrument.
0x001D	*Sampling Pump reset failed*	Bad connection or optocoupler is not working properly	Restart the instrument.
0x0020	*System initialization is paused*	Liquid level in the reagent containers is not within normal range when NovoCyte Quanteon 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.
0x0023	*Resetting NovoSampler to home position failed*	NovoSampler Q is not working properly.	Click Reset. Restart the 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 *Section 2.1.2 Add Instrument Reagents* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace the fluidic station.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
0x0102	*NovoRinse running low*	NovoRinse solution is below the pre-set volume limit	Refill the NovoRinse container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace fluidic station.
0x0103	*NovoClean running low*	NovoClean solution is below the pre-set volume limit	Refill the NovoClean container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace fluidic station.
0x0104	*Waste container is close to full*	Waste is above the pre-set volume limit	Empty the waste container following the procedures described in *Section 2.1.3 Empty Waste* in *NovoCyte Quanteon™ Flow Cytometer Operator's Guide*.
		Fluidic station is not working properly	Replace fluidic station.
0x0105	*Cover of NovoSampler is opened*	Cover of NovoSampler Q is opened	Close the cover.
0x0106	*NovoSampler is disconnected when powered up*	The NovoSampler Q is disconnected when powered up	▶ Shut down NovoCyte Flow Cytometer. ▶ Reconnect the cable of the NovoSampler Q to NovoCyte flow cytometer. ▶ Turn on NovoCyte Quanteon and follow the prompts to calibrate the NovoSampler Q.
0x0109	*Fluidics station is not connected*	Cable between the fluidics station and the NovoCyte Quanteon instrument is not properly connected	▶ Power down the instrument. ▶ Reconnect the cable between the fluidics station and the NovoCyte Quanteon instrument. ▶ Power up the instrument.
0x010A *0x010B* *0x010C* *0x010D*	*… liquid level sensor failure*	Specified liquid level sensor in the fluidics station is not working properly	▶ Reconnect the fluidics station cable. ▶ Restart the NovoCyte Quanteon instrument. ▶ Replace the fluidics station.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
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 *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte Quanteon™ Maintenance 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 Q is not working properly	No action is needed. The NovoSampler Q can be used normally.
0x1001	*Fluidics procedure run error*	Fluidics procedure file is damaged	Restart the instrument.
0x3100	*Failed to read optical filter information*	Optical filter sensor is not working properly	Replace the appropriate filter following the procedures described in *Section 2.1.6 Verify and Modify Instrument Configuration* in *NovoCyte Quanteon™ Maintenance Guide*.
0x3101	*Failed to read dichroic mirror information*	Dichroic mirror sensor is not working properly	Replace the appropriate mirror following the procedures described in *Section 2.1.6 Verify and Modify Instrument Configuration* in *NovoCyte Quanteon™ Maintenance Guide*.
0x3102	*Failed to read photodetector information*	Photodetector sensor is not working properly	Restart the instrument.
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*	Bad connection between NovoSampler Q and shaker or NovoSampler Q is not working properly.	Restart NovoCyte Quanteon instrument.

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Message ID	Software Messages	Possible Causes	Recommended Solutions
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 NovoCyte Quanteon instrument
		More than four temperature sensors of the photodetector module are not working properly	Restart NovoCyte Quanteon instrument
0xA100	*The temperature of the photodetectors is abnormal*	One to three temperature sensor of the photodetector module is not working properly	No action is needed. The NovoCyte Quanteon instrument can be used normally.
	Communication error (code: xx, xx). Please restart NovoCyte and NovoExpress.	USB cable between the NovoCyte Quanteon instrument and workstation is not connected properly	▶ Turn off the instrument. ▶ Reconnect the USB cable between the NovoCyte Quanteon instrument and the workstation. ▶ Restart the instrument and NovoExpress software.

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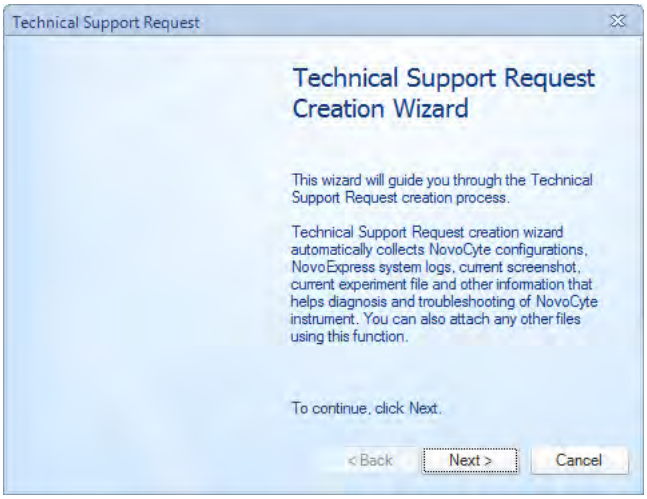
9.3 Technical Support Request

In case you need to contact ACEA technical supports, use the *Technical Support Request* from *Home* menu to create a request. *Technical Support Request Creation Wizard* automatically collects NovoCyte configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps diagnosis and troubleshooting of NovoCyte instrument. You can also attach any other files using this function.

1 Click *Home* → *Technical Support Request* to open the *Technical Support Request Creation Wizard*.



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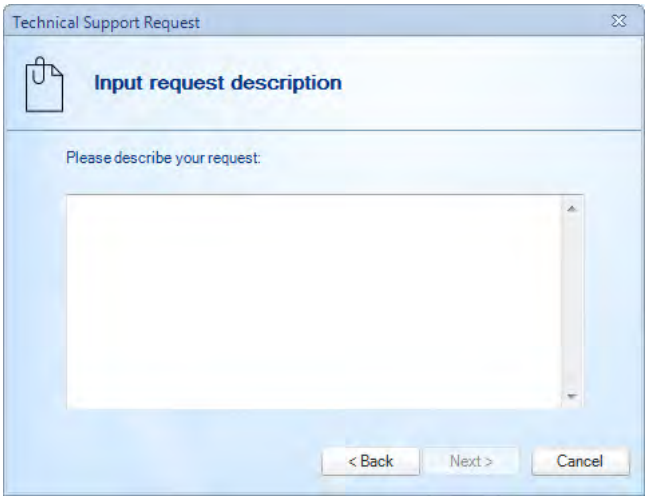
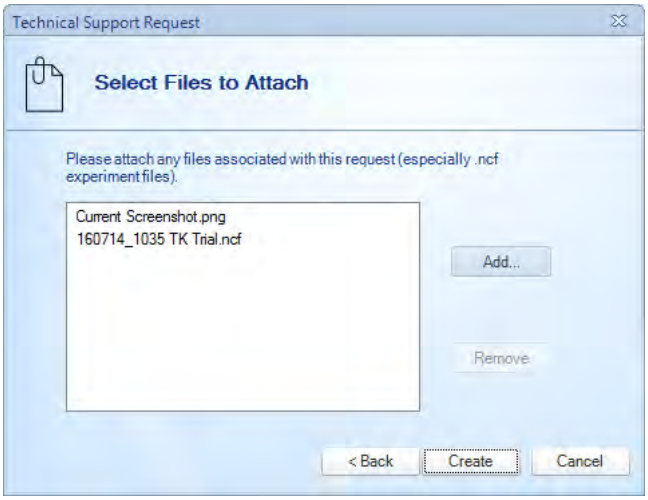
2 Click *Next* button and input request description.

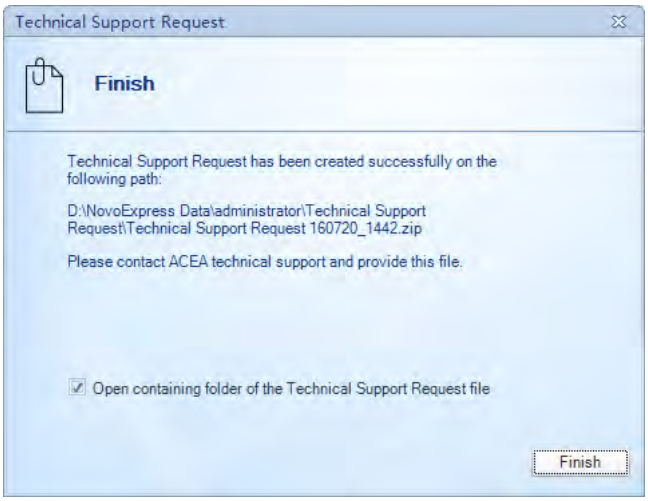


3 Click *Next* button and select the files to be attached. Any related files including experiment files (*.ncf) can be attached.



4 Click *Create* button to start creating technical support request.

5



After the request creating process is completed, send the created request files to ACEA technical support through email (techsupport@aceabio.com). Click *Finish* to complete this process.

Appendix 1 Keyboard Shortcuts

Shortcuts	Command
Overall Situation	
Ctrl+S	Save the file
Ctrl+W	Close the file
Ctrl+O	Open the file
Ctrl+N	New file
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
F5	Run / Stop
F4	Next sample
F3	Next sample without template
F8	Restart
Plot	
Ctrl+Z	Undo
Ctrl+Y or Ctrl+Shift+Z	Redo
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
Ctrl++	Zoom In
Ctrl+-	Zoom Out
Ctrl+A	Auto Range
Ctrl+F	Full Range

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Shortcuts	Command
Ctrl+C	Copy the plot or gate
Ctrl+V	Paste gate
Ctrl+D	Duplicate a plot or gate
Alt+Q	Quick compensation
Ctrl+R	Rectangular gate
Ctrl+E	Elliptical gate
Ctrl+P	Polygonal gate
Ctrl+Q	Quadrant gate
Ctrl+L	Logic gates
Ctrl+H	Range gates
Ctrl+B	Bi-Range gates
Ctrl+M	Move
Ctrl+T	Adjust threshold
Experiment Manager	
Ctrl+C	Copy
Ctrl+V	Paste
Ctrl+D	Duplicate
F2	Rename

Appendix 2 Glossary

Workspace: In the main interface of the NovoExpress Software, the middle area where plots are displayed.

Experiment File: The NovoExpress Software saved experimental data files. A file can store experimental data from multiple samples.

Experiment Management: 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.

Group: A part of the NovoExpress Software's hierarchy structure. The group can contain multiple specimens.

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.

Sample: The basic unit of experimental data organization. Samples contain the information from sample data collection, instrument settings, fluorescence compensation, reporting, analysis and data.

Blank samples: Samples without collected event data. Blank samples only contain instrument settings. 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 *Section 6.2*.

Active sample: The sample currently displayed and being analyzed. The cytometer control and settings 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 keyboard shortcut Ctrl+[and Ctrl+].

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.

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.

Instrument settings: The settings include the sample, the stop condition, the sample flow rate, and the threshold settings.

Parameters: Refers to fluorescent or scattering intensity measurements. Parameters can be differentiated by the specific light channel or measurement type (height or area).

Stop condition: A defined number of events, length of time, or volume of collection, where sample collection is stopped immediately after reaching the condition.

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.

Sample flow rate: The flow rate of the sample can be used to control the number of events collected per second.

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 of its samples.

Analysis: The process of plotting, gating, and comparing statistical information on collected parameters of samples.

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.

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.

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.

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.

Histogram: Single-parameter plot. The X-axis will plot a parameter, and the Y-axis will plot the number of events.

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.

Cell cycle diagram: Uses a histogram of DNA content to derive cell cycle phase populations based on curve fitting to the histogram.

Cell proliferation diagram: Generates c modeling results to analyze different cell generations during the cell proliferation procedure.

Gate: Used to select a specific population of events. Gate types include rectangular, elliptical, polygonal, range, and bi-range gates. Gates can also be combined to create logic gates. Gates are used to further analyze specific populations.

Logic gates: The combination of individual gates using the logic operators AND, OR, or NOT to create logic gates.

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.

Quick compensation: Use scrollbars in two-parameter plots to quickly adjust the fluorescence compensation of a sample.

Automatic compensation: Use a specimen containing an unstained sample and single stained samples to automatically calculate a compensation matrix.

Absolute count: Number of cells or particles per unit volume. NovoCyte 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.

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.

Templates: Set contains the group, instrument settings, specimens and samples, fluorescence compensation, reporting, analysis, etc., can be saved as *.nct file format.

Statistical tables: A customizable table of statistical information for batch data analysis. It can contain multiple samples, multiple gates, and statistical information for multiple parameters.

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.

QC test: In QC test, NovoCyte QC particles are used to check the instrument performance. Measured data are used to determine if the NovoCyte System's parameters fall within a standard range to ensure stable and reliable operation of the instrument.

QC test reports include: 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.

Debubble: Clear bubbles present in the sample line.

Cleaning: Clear biological hazards that may exist in the pipeline.

Rinse: Rinse tubing.

Extensive Rinse: Extensively rinse tubing.

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.

Unclog: Clear blockages in the flow cell.

Backflush: Clear blockages in the sample pipeline.

FCS: Data file standard for flow cytometry. NovoExpress is compatible with FCS 3.0 and 3.1.

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.

LIS (Laboratory Information System): Result of a statistical table and plots can be exported and parsed by LIS.

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