

Attune™ NxT Software User Guide

For data acquisition and analysis using the Attune™ NxT Acoustic Focusing Cytometer

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E.0	20 May 2016	Add new instrument functions and filter configurations.
D.0	13 November 2015	Basis for this revision

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About this guide

This user guide describes how to use the Attune™ NxT Software to acquire and analyze data using the Attune™ NxT Acoustic Focusing Cytometer, and assumes that you have a working knowledge of basic Microsoft™ Windows™ operation.



IMPORTANT! For instructions on using the Attune™ NxT Acoustic Focusing Cytometer, refer to the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Pub. no. 100024235) and the *Attune™ NxT Acoustic Focusing Cytometer Quick Reference Guide* (Pub. no. 100024233).

Conventions

Text and keyboard conventions

Text and keyboard conventions used in the *Attune™ NxT Software User Guide* are listed below.

For safety alert words and symbols used in this user guide, see page 11.

Convention	Use
<i>Italics</i>	<i>Italic</i> text highlights new or important terms on their first appearance in the user guide. It is also used for emphasis and for user guide or reference titles. For example: <i>Experiment Explorer</i> lists <i>Experiments</i> in a hierarchal view and functions as an interface for creating new Experiments and recording data.
Bold	Bold text indicates user action. For example: Click Run .
▶	Right arrow symbol (▶) indicates a menu choice, and separates successive commands you select from a drop-down or shortcut menu. For example: Select Show Events ▶ All Events .
Ctrl+X	When used with key names, a plus sign means to press two keys simultaneously. For example: Click Ctrl+P .

Clicking

Unless explicitly stated, clicks are left mouse button clicks. If you have transposed the mouse buttons, the primary click is considered to be the left click, even though it may be physically swapped.

User attention symbols

User attention symbols used in the *Attune™ NxT Software User Guide* are listed below. For safety alert words and symbols used in this user guide, see page 11.

Symbol	Use
	Note: Describes important features or instructions, and highlights tips that can save time and prevent difficulties.
	IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

Acronyms

The following table explains the acronyms used in the *Attune™ NxT Software User Guide*.

Acronym	Definition
ADC	Analog-to-Digital Converter.
Br	Relative background level of detection channel.
BL1–BL4	Detectors that measure the output from the 488-nm laser (blue).
%CV	Percent coefficient of variation = standard deviation/mean × 100%. It is a measure of variation in signal intensity generated as particles pass repeatedly through the laser beam, and is expressed as a percentage of average signal intensity.
FSC	Forward scatter.
%rCV	Percent robust coefficient of variation.
MESF	Molecule of equivalent soluble fluorophore.
MFI	Mean Fluorescence Intensity as described by the mean ADC value for a given bead intensity population.
PMT	Photomultiplier tube.
PMTV	PMT voltage setting.
RL1–RL3	Detectors that measure the output from the 638-nm laser (red).
SD	Standard deviation.
SIP	Sample injection port.
SSC	Side scatter.
VL1–VL4	Detectors that measure the output from the 405-nm laser (violet).
YL1–YL4	Detectors that measure the output from the 561-nm laser (yellow).

Other Attune™ NxT user guides

The guides listed below are available with the Attune™ NxT Acoustic Focusing Cytometer.

Guide	Pub. no.
<i>Attune™ NxT Acoustic Focusing Cytometer Quick Reference Guide</i>	100024233
<i>Attune™ NxT Acoustic Focusing Cytometer User Guide</i>	100024235
<i>Attune™ NxT Software User Guide</i>	100024236
<i>Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide</i>	100024234
<i>Attune™ NxT Auto Sampler User Guide</i>	100032905

Additional resources are available on the Flow Cytometry Technical Resources page at www.thermofisher.com/flowresources. There you can find protocols, application notes, and tutorials.

Safety information



Note: See “

Appendix C: Safety” for the complete the chemical or instrument safety information.

Safety alert words

Four safety alert words appear in this user guide at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:



IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.



CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.



WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.



DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT!** safety alerts, each safety alert word in a this document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instruments (see “**Symbols on instruments**”).

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Thermo Fisher Scientific are available to you free 24 hours a day. For instructions on obtaining SDSs, see “**Obtaining SDSs**”.



IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

1. Attune™ NxT Software

Attune™ NxT Software overview

The functions of the Attune™ NxT Acoustic Focusing Cytometer are controlled by the Attune™ NxT Software. The software is pre-installed to the computer workstation supplied with the Attune™ NxT Acoustic Focusing Cytometer, and the Attune™ NxT Software icon (i.e., shortcut) is placed on the computer desktop and under **Start ▶ ThermoFisher ▶ Attune NxT Software**.



Note: If you need to reinstall the Attune™ NxT Software for recovery purposes, you must have *Administrator* access (see page 18).

About the software

The Attune™ NxT Software is a flexible data acquisition and analysis tool that uses a browser view to:

- Design and perform experiments
- Define independent instrument settings and optimize data collection
- Carry out instrument performance checks and track instrument performance
- Acquire and record data
- Manage and process recorded data

System requirements

A PC workstation running the Attune™ NxT Software on a Windows® 7 (Service Pack 1) 64-bit platform is provided with the Attune™ NxT Acoustic Focusing Cytometer.



Note: The text must be set to small (100%) in the Windows display options (96 dpi) for proper display of this software application.

Supported files

- The Attune™ NxT Software records and outputs data in FCS (Flow Cytometry Standard) 3.1.
- The Attune™ NxT Software can export data in FCS 3.0 and 3.1 formats.
- FCS formats 3.0 and 3.1 are supported for data analysis.

Software layout and Ribbon bar menu system

- Software layout** The Attune™ NxT Software has three main screens, *Login*, *Main Menu*, and *Attune™ Desktop*.
- *Login* – Used for logging into the software. The Login screen is the first screen presented after the main application startup. For more information, see page 15.
 - *Main Menu* – Used for running and reviewing performance tracking tests, creating new experiments, and accessing the current and stored experiments. Main Menu is the first screen presented after a successful login. For more information, see page 20.
 - *Attune™ Desktop* – Used for controlling the Attune™ NxT Acoustic Focusing Cytometer to run samples, generate data, and analyze results. Attune™ Desktop is the main application window. For more information, see page 23.

Ribbon bar menu system The Attune™ NxT Software uses the Microsoft™ Ribbon interface. The *Ribbon bar* consolidates related functionality by organizing control elements in logical groups under contextual tabs. Each *Ribbon tab* relates to a type of activity.

The availability of the Ribbon tabs in the Ribbon bar is dependent on the context of the software, i.e., they are contextual tabs that are displayed only when they are needed by the user. Objects that are not available as a function in a particular context are shaded gray in the tab.

For more information on the Ribbon bar, see page 27.



Note: Only a limited set of Ribbon tabs are available on the Main Menu (page 20) when running Performance Tests (page 260).
On the Login screen (page 15), only the File tab (page 39) is available.

Context menus The Attune™ NxT Software interface also allows the use of *context menus* (also called contextual, shortcut, and pop-up menu), which appear upon user interaction with a right-click mouse operation. A context menu offers a limited set of choices that are available in the current state, or context, of the software and the available choices are actions related to the selected object.

2. Startup, Login, and Main Menu

Main application startup

Main application startup

1. To launch the Attune™ NxT Software, double-click the Attune™ NxT Software shortcut icon on the desktop.
Alternatively, select **Start ▶ ThermoFisher ▶ Attune NxT Software**.



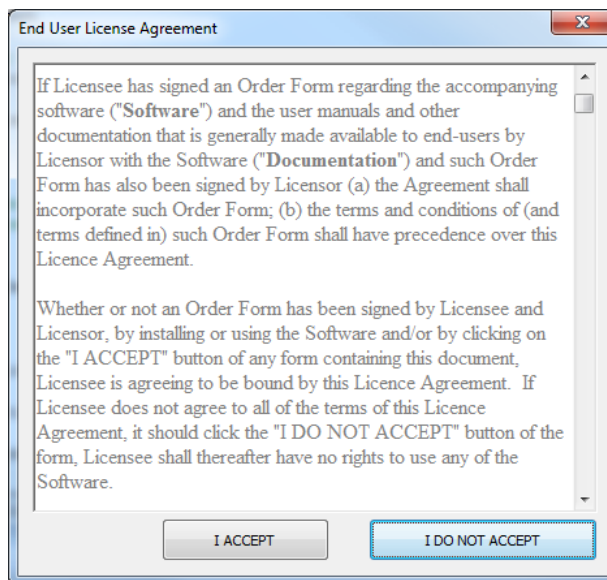
IMPORTANT! A license control mechanism in the form of a DESkey device is required for the operation of the Attune™ NxT Software.

- If a valid license mechanism is detected when the software is started, the End User License Agreement (EULA) will be displayed on first use of the software (see page 14).
- If a valid DESkey device is not present, the software will display the appropriate warning message.

End User License Agreement (EULA)

The End User License Agreement (EULA) is displayed when:

- The Attune™ NxT Software is first installed.
- A new version of the Attune™ NxT Software is installed.
- A new Windows user is attempting to open the Attune™ NxT Software.

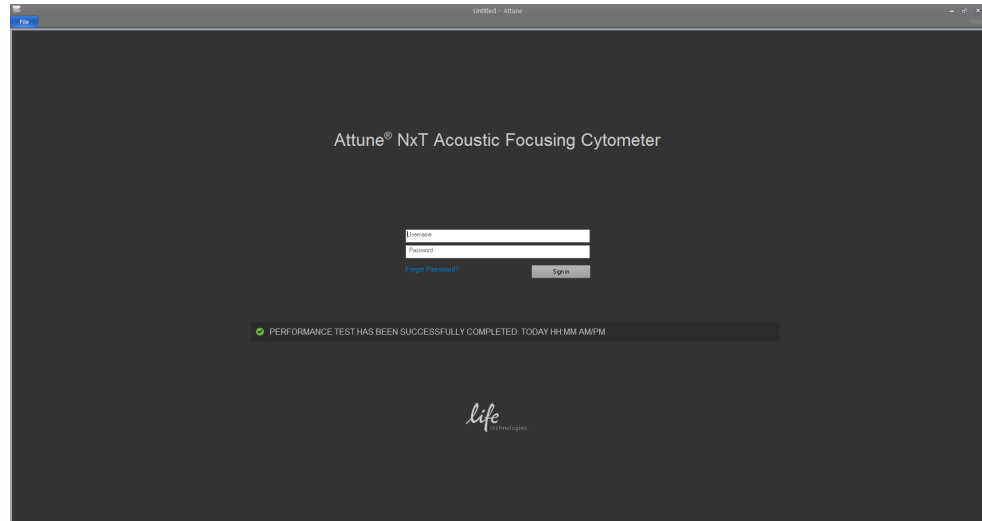


Click **I ACCEPT** to accept the terms of the agreement and launch the software.

Click **I DO NOT ACCEPT** to reject the terms of agreement. The EULA dialog closes and the software is not opened.

Login screen

The *Login* screen is the first screen that is displayed after the splash screen when you start the Attune™ NxT Software. Each user is required to log in before being allowed to use the software. The Login screen also displays the current Performance Test status (see “Performance Test Status”, page 16).



User sign in

1. To sign in the Attune™ NxT Software, type a valid username and password in the appropriate text box fields and click **Sign In**.

Alternatively, You can press the **Enter** key on the computer keyboard after typing the username and password.

2. If the correct username and password are entered, the user login is completed and the *Main Menu* is displayed (see page 20).

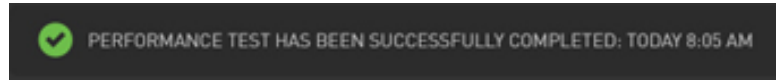
If an invalid username or password is entered, the warning banner displays “Invalid username or password” (see page 16).






IMPORTANT! When logging into the Attune™ NxT Software for the first time after installation, you must sign in as an *Administrator*. The default username and password for the Administrator are both admin. For more information on other available account types, see “Account types”, page 18.

Performance test status

Performance test is a comprehensive set of procedures to monitor the daily performance of the cytometer. The *Performance test status* for the day is displayed below the Sign in text fields on the Login screen.



- The Performance test status includes a message and an icon that indicate the date and the results of the last performance test (see below). If no baseline has ever been run, no message is displayed.

Icon	Message
	Performance test has been completed successfully today.
	Performance test has not been completed today.
	Performance test has not been completed successfully today.

- For more information on Performance test procedures, see “Performance Testing”, page 260.

For more information on reports detailing the performance test results, see “Performance Test Reports”, page 270.



Note: Performance test status is also displayed in the Main Menu, below the Main Menu buttons. For more information, see “Main Menu”, page 20.

Invalid username or password

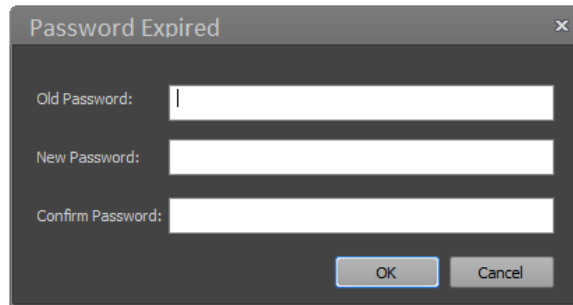
If you enter an invalid username or password, the warning banner displays “Invalid username or password”. You must then enter the username or the password correctly or the process described above is repeated.

- If you attempt to login to an account that has been disabled (i.e., set to inactive), the warning banner displays “Your account has been disabled. Contact an administrator.”

Expired password

If your password has expired, the Attune™ NxT Software will prompt you to change your password.

You must then enter your old password, your new password, and a confirmation of the new password.

A screenshot of a dialog box titled "Password Expired" with a close button (X) in the top right corner. The dialog contains three input fields: "Old Password:", "New Password:", and "Confirm Password:". At the bottom right, there are two buttons: "OK" and "Cancel".

Note: The *Password Expired dialog* will only be displayed if you successfully log in with the correct credentials. If any updates to the password are required, you must complete them before the Main Menu is displayed.

- If the new password and confirmation password do not match, you will be prompted to enter the new password again.
- If the new password that does not meet password complexity requirements, you must choose another password. The new password must contain at least one uppercase, one lowercase, one numeric, and one non-alpha-numeric character, and must be at least 3 characters long.
- If you enter a password that has been previously reused within the disallowed time frame as specified by the System Administrator, you will be prompted to choose another password.
- For more information on user passwords, see "User Management", page 356.

Account types

Default accounts Upon installation, the Attune™ NxT Software has three types of default accounts, *System Administrator*, *Administrator*, and *Service*.

System Administrator

System Administrator is the highest level account and can define the system security policy for username length, password length, password expiration and lock-out, and the auto lock out time due to system inactivity. The default system administrator account cannot be deleted.

Administrator

Administrators have full access to the software and can perform a variety of tasks, including managing user accounts, running system diagnostic tests, system decontamination, performance tests and baseline calculations. However, they cannot set system security policy. The default administrator account cannot be deleted.

Service

Service is a special type of account that gives access to service-only features of the software. The service account cannot be deleted and is only accessible by Thermo Fisher Scientific service team.

For more information on default accounts, including how to create additional accounts or delete existing ones, see “User Management”, page 356.

User accounts Users can access and modify their own Workspace and Experiments, and run Performance tests, but they cannot run any advanced functions available to other account types. User accounts must be created by the System Administrator or Administrator upon successful installation of the Attune™ NxT Software and the initial sign in.

For more information on how to create additional User accounts or delete existing ones, see “User Management”, page 356.

Default usernames and passwords

System Administrator

- The default username for the system administrator account is sysadmin
- The default password for the system administrator account is sysadmin
- Upon initial login, the system administrator will be forced to reset the password

Administrator

- The default username for the admin account is admin.
- The default password for the admin account is admin.
- Upon initial login, the administrator will be forced to reset the password

User

- Upon initial account creation, the username is set by the System Administrator or Administrator (see “User Management”, page 356).
- The password is set by default to the username set up the by the System Administrator or Administrator.
- Upon initial login, each user will be forced to reset the password

Account permissions

The permissions assigned to each account type are provided in the table below.

Permission	Description	User	Advanced User	Administrator	System Administrator
Run Performance Test	Allows a user to run the Performance Test and view the Performance Test reports	X	X	X	X
Run Baseline calculations	Allows a user to run and set new Performance Test Baseline calculations		X	X	
Run system decontamination	Allows a user to run the Decontaminate System function		X	X	
Run System Tests	Allows a user to run the system tests		X	X	
Manage User accounts	Allows a user to create user accounts, edit user accounts, reset passwords, change passwords, view login/logout times for all users, and view the length of all user sessions			X	X
Set security policy	Allows a user to set system security settings for username length, password length, password expiration and lock-out, and the auto lock out time due to system inactivity				X

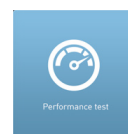
Main Menu

After you log successfully in to the Attune™ NxT Software, the *Main Menu* is displayed. The Main Menu contains the buttons for the most frequently used commands (Performance Test, New Experiment, Import Experiment, and Templates), the *Experiment Explorer* panel, the *Performance test status* bar, and the *Log Out* hyperlink control.



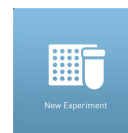
Performance test button

Performance test button initializes the *Performance Test setup* screen within the Performance Test module, which allows you to perform the baseline and performance test and view the baseline and performance test reports (see “Performance Testing”, page 260).



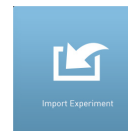
New Experiment button

New Experiment button launches the *New Experiment dialog*, which allows you to create a tube-only experiment, a Plate Experiment, or an experiment using imported files (see “New Experiment dialog”, page 303).



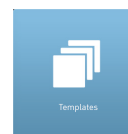
Import Experiment button

Import Experiment button launches the *Import Experiment file selection dialog*, which allows you to import an Experiment that was previously exported.



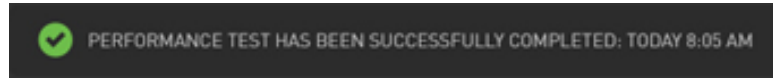
Templates button

Templates button launches the *New Experiment from Template dialog*, which allows you to create a new experiment form an existing template stored in the database within your account (see “New Experiment from Template dialog”, page 307).



Performance test status

Performance test is a comprehensive set of procedures to monitor the daily performance of the cytometer. The *Performance test status* for the day is displayed below the sign in text fields in the Login screen.



The performance test status includes a message and an icon that indicate the date and the results of the last performance test (see below). If no baseline has ever been run, no message is displayed.

Icon	Message
	Performance test has been completed successfully today
	Performance test has not been completed today
	Performance test has not been completed successfully today
	Bead lot has expired (see Note below)

For more information on performance test procedures, see “Performance Testing”, page 260.

For more information on reports detailing the performance test results, see “Performance Test Reports”, page 270.



Note: If the baseline calculations have expired, the performance test status bar displays the “Bead lot has expired” message. For more information on baseline calculations, see “Setting up and running a Baseline”, page 262, and “Baseline results”, page 265.

Log Out

Clicking **Log Out** logs out the current user and the software returns to the Login screen (page 15).



Note: The Log Out link is only enabled when the instrument is not acquiring (see “Acquisition Status Indicators”, page 160).

Main Menu ribbon bar

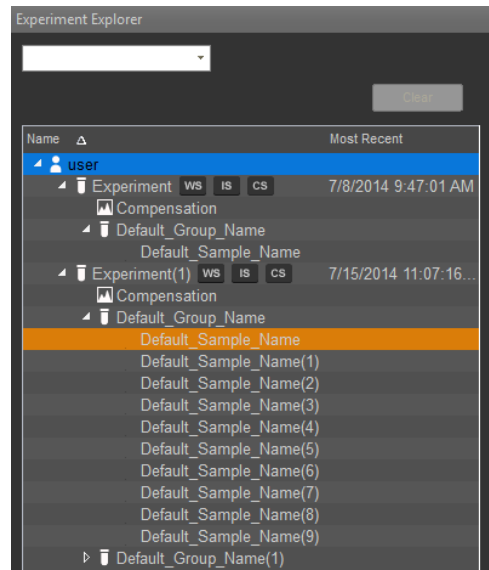
Main Menu provides a limited set of options for the user to perform, and contains only the File (page 39), Home (page 40), and Instrument ribbon tabs (page 48).

- The Main Menu ribbon bar is minimized by default. The minimized or expanded state of the Main Menu ribbon bar persists on a per user basis.
- When the Main Menu ribbon is expanded, the Home tab is selected by default.

Experiment Explorer

Experiment Explorer is used to create, view, and manage *Experiments*, and it is described in detail in “Experiment Explorer”, page 140.

The Experiment Explorer panel cannot be undocked or resized.

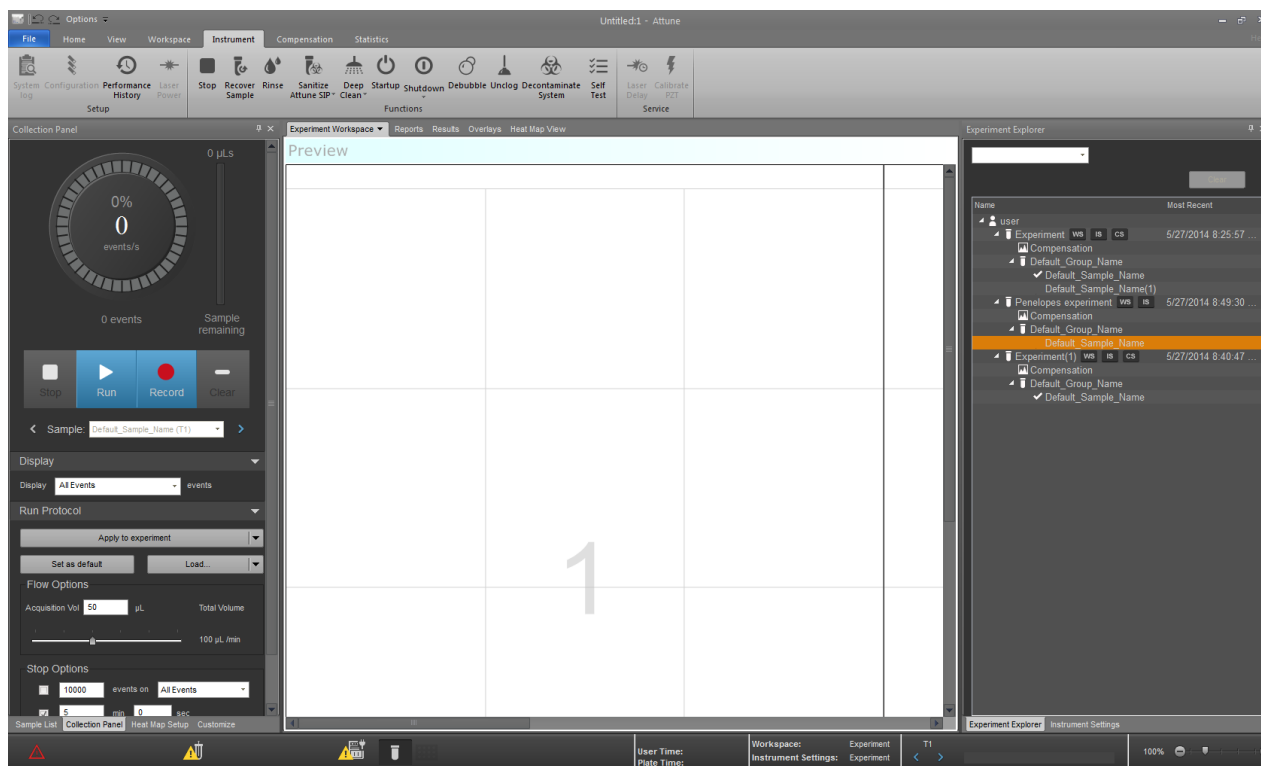


3. Attune™ Desktop

Attune™ Desktop overview

Attune™ Desktop is the main application window, and it is used for controlling the Attune™ NxT Acoustic Focusing Cytometer to run samples, generate data, and analyze results. It consists of the sections listed below.

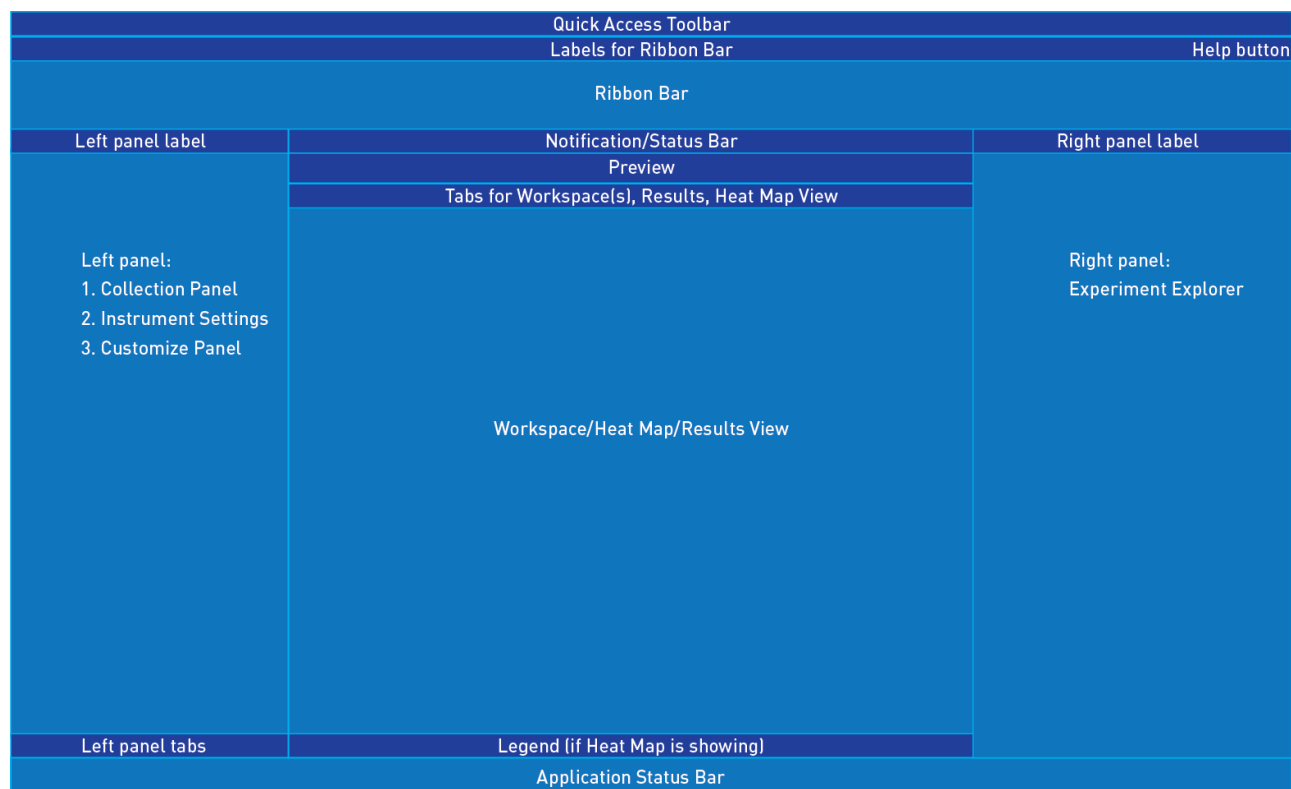
- *Application title bar* (page 25) contains the Application button, Quick Access toolbar, Application name, and Window Size buttons.
- *Ribbon bar* (page 27) is organized into a series of tabs that allow access to the main functions of the Attune™ NxT Software.
- *Main application workspace* (page 28) accommodates the Application area, a number of adjustable docking panels that contain controls grouped by functionality, the Experiment Explorer, and the Status notification display.
- *Application status bar* (page 33) displays various icons depicting the status of the instrument and the experiment, any alerts relating to the instrument, as well as the progress bar for instrument functions, loading and refreshing. It also contains the Size slider (page 37).



Attune™ Desktop layout

The layout of the Attune™ Desktop follows Microsoft™ Windows 7 and Microsoft™ Office 2010 styles, and uses the ribbon menu system and adjustable docking panels that contain controls grouped by functionality.

The schematic below represents the default layout of the Attune™ Desktop, showing the default locations of the various panels and toolbars. Note that the contents of the Attune™ Desktop changes depending on the context of the software.

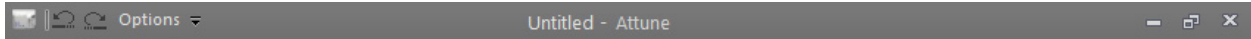



Note: *Application panels* are dockable panels that contain controls grouped by functionality. For more information, see “Application panels”, page 28.

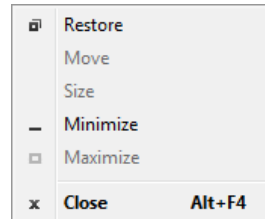
Application title bar

Application title bar layout

The *Application title bar* is displayed at the top of the application window, and contains the Application button, Quick Access toolbar, Application name, and Window Size buttons.



- *Application button*  opens the standard Windows application size menu.



You can also open the application size menu by right-clicking anywhere on the application title bar.

- *Quick Access toolbar* provides easy access to frequently used commands and can be customized to include any commands within the ribbon menu system.



- *Application name* shows the version number of the Attune™ NxT Software and the currently logged in username. If these names are too long, they are truncated and ellipses are used.
- *Window Size buttons* are the standard buttons used in Microsoft™ Windows applications for minimizing, maximizing, and restoring the open application window.



Quick Access toolbar






The *Quick Access toolbar* is a customizable toolbar containing frequently used commands that are independent of the tab currently displayed.




By default, the Quick Access Toolbar is located above the Ribbon bar. However, you can also choose to display it below the Ribbon bar using the Customize Quick Access Toolbar drop-down list (see below).

Default buttons

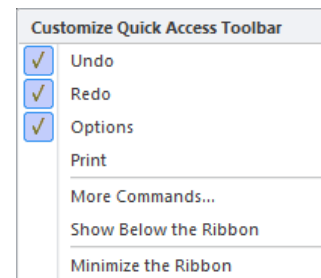
By default, the Quick Access Toolbar contains the Undo and Redo commands, a link to the Main Menu, a link to the Options menu, and the Quick Print command.

-  **Undo:** Negates the last undoable action performed and brings the application to its previous state. The Undo button is enabled only if there is an undoable action available.
-  **Redo:** Reverses the Undo or advances the application to a more current state. The Redo button is enabled only if there is a redo action available.
-  **Main Menu:** Navigates to the Main Menu (page 20). The Main Menu button is enabled only if a user is logged in, and the instrument is not acquiring.
-  **Options:** Opens the Options dialog (page 326). The Options button is enabled only if a user is logged in.
-  **Customize Quick Access Toolbar drop-down menu:** Opens the *Customize Quick Access Toolbar drop down menu* (see below)

Customize Quick Access toolbar menu

The arrow next to the Quick Access buttons  opens the *Customize Quick Access Toolbar drop down menu*. The dropdown list includes the following options:

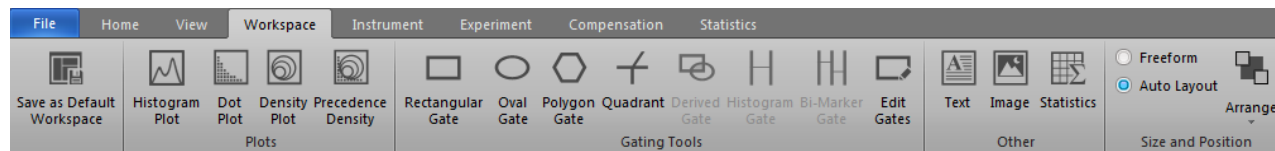
- **Show Quick Access Toolbar Below the Ribbon/Above the Ribbon:** Toggles the Quick Access Toolbar to show below or above the Ribbon bar; the text on the menu item toggles between *Show Below the Ribbon* and *Show Above the Ribbon* depending on its location.
- **Minimize the Ribbon:** Checking and un-checking this menu item toggles the Ribbon bar between minimized and restored. When the Ribbon bar is minimized, the tabs are still active. Changing to another tab displays the Ribbon bar.



Note: Options to customize the Quick Access Toolbar, to show the toolbar above or below the ribbon, and to minimize the ribbon are also available through the Ribbon context menu. For more information, see “Ribbon context menu”, page 27.

Ribbon bar

The *Ribbon bar* is organized into a series of tabs which represent the main functions of the Attune™ NxT Software.



Ribbon tabs

The availability of the tabs in the Ribbon bar is dependent on the context of the software; they are displayed only when the user needs them. Objects that are not available as a function in a particular context are shaded gray in the tab. Depending on the context of the application, the Ribbon bar contains one or more of the following tabs (see Note below):

- File tab (page 39)
- Home tab (page 40)
- View tab (page 42)
- Workspace tab (page 44)
- Instrument tab (page 48)
- Compensation tab (page 50)
- Statistics tab (page 58)



Note: Only a limited set of tabs are available on the Main Menu (page 20) when performing the Performance Test (page 260).

On the Login screen (page 15), only the File tab (page 39) is available.

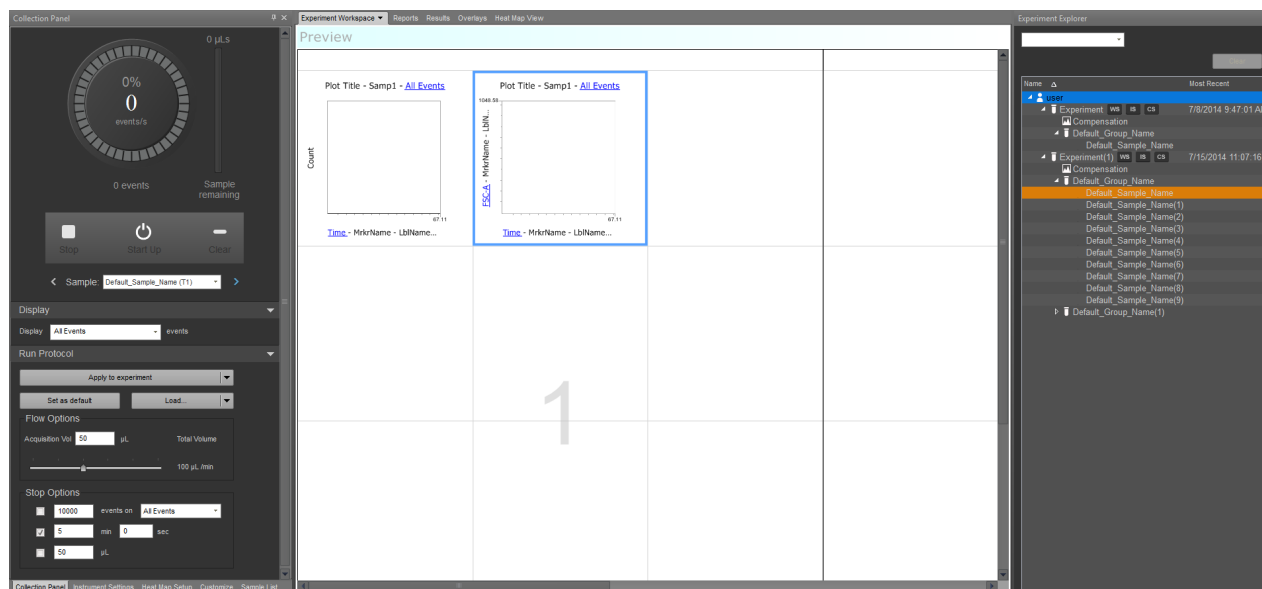
Ribbon context menu

Right-clicking on a control in the Ribbon bar brings up the *Ribbon context menu*, which contains the options listed below.

- **Show Quick Access Toolbar Below the Ribbon/Above the Ribbon:** Toggles the Quick Access Toolbar to show below or above the Ribbon bar; the text on the menu item toggles between *Show Below the Ribbon* and *Show Above the Ribbon* depending on its location.
- **Minimize the Ribbon:** Checking and un-checking this menu item toggles the Ribbon bar between minimized and restored. When the Ribbon bar is minimized, the tabs are still active. Changing to another tab displays the Ribbon bar.

Main application workspace

The *Main application workspace* accommodates the *Application area*, a number of *Application panels*, the *Experiment Explorer*, and the *Status notification display*. The contents of this area change depending on the context of the software.



Application area

The *Application area* displays the analysis objects (plots, gates, and statistics) that are associated with the current or a saved Sample or Experiment. Text boxes and image files can also be displayed in the Application area. The Application area contains the following *Application tabs*:

- Workspace view (page 63)
- Heat Map view (page 105)
- Results (page 112)

Application panels

Application panels are adjustable docking panels that contain instrument and software controls that are grouped by functionality. The Main Application Workspace contains the following Application panels:

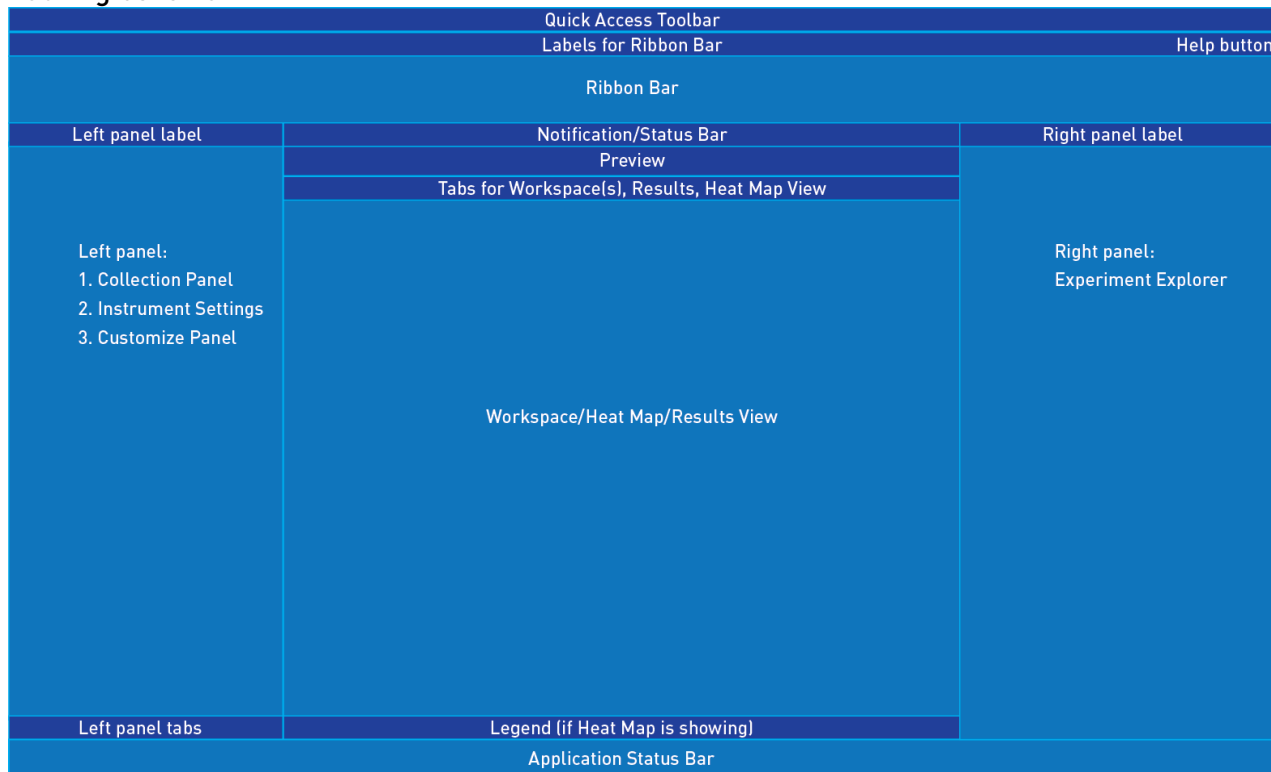
- Experiment Explorer (140)
- Collection (page 159)
- Instrument Settings (page 177)
- Customize (page 200)
- FCS Information (page 239)



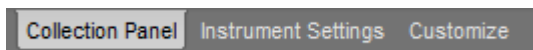
Note: The Application tabs and the Application panels are displayed in the Main Application Workspace as described above, except in the following application-specific modules:

- Login screen – All panels are hidden.
- Main Menu – All panels are hidden except for Experiment Explorer, which cannot be undocked or resized.
- Performance Test/Baseline Test – All panels are hidden.

Docking behavior



- By default, the Collection, Instrument Settings, and Customize panels are docked to the left of the Application area in the order listed.
 - The Experiment Explorer is by default docked to the right of the Application area (see the schematic above).
 - The FCS Information panel is set as a floating panel by default (i.e., with its “Dockable” attribute deselected; see page 32).
 - The Workspace, Results, and Heat Map view tabs are docked to the Application area. By default, they appear in the order listed when docked.
 - You can re-order all tabs except the Workspace tab, which always appears first in the list. To reorder the tabs, click a tab and drag it to different position.
 - When multiple panels are docked together to the left or the right of the Application area, the name of the currently displayed panel appears in the *container title bar* (i.e., the title bar immediately above the panel). When docked to the Application area, no title bar is displayed.
 - When docked to the Application area, tabs for each docked panel appear on the top edge of the Application area.
- When docked to the left or right of the Application area, docked panels are displayed as tabs on the bottom of the panel, unless only one panel is located within the *panel container*.
- The tab containing the currently open panel is highlighted.



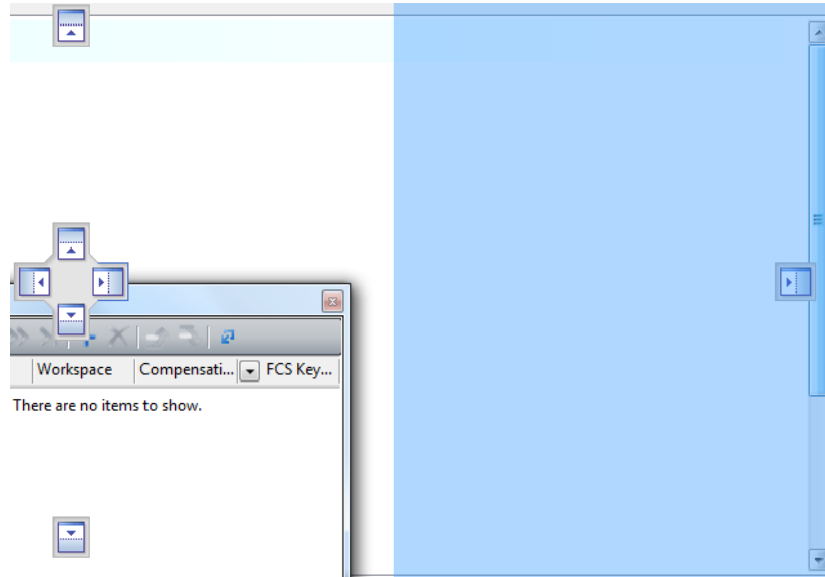
- Application panels that are docked to the Application area will display as tabs.
- The layout of dockable panels persists for each user.

Docking and undocking panels

1. To undock an Application Panel, click and drag the title bar of the panel container. While dragging, docking icons will be displayed in the center, left, and right of the Application area to show where the panel can be docked.



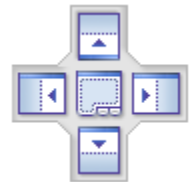
2. Hover over one of the docking icons to display the indicator showing where the panel will be docked (see image below).



3. Release the panel on the desired docking icon to dock it to the area indicated.

Docking and undocking tabs

1. To undock an Application tab, select and drag the individual tab.
2. Hover over an existing panel to display the docking icon, which allows you to dock the selected tab to any of four sides of the panel or to add it as a tab to the full size panel.
3. Release the tab on the desired docking icon to dock it to the left or right of the Main Application Workspace or to any of the four docking points of another panel.



You can also drop a selected panel onto the title bar of an existing panel to dock it as a full size panel, which will be added as a tab.



Note: Heat Map and Results tabs can be docked anywhere on the Main Application Workspace. All other tabs and panels are limited to left and right only docking rules.

Floating panels

- Double-clicking on the title bar of a docked panel or tab results in panel to be floated to its default position.
- If a panel has been docked from a floating position, it returns to the same floating position when re-floated.
- If multiple floating panels are docked together before docking the entire container to the left or right of the Main Application Workspace, clicking the title bar of the docked container re-floats the container in its entirety to its previous position.
- Double clicking on floating container title bar or tab results in that container or panel to be re-docked to its last docked position.

Auto Hide

Auto Hide option reduces a panel to a tab icon when the focus is lost by clicking anywhere outside the menu.

Push Pin icon is used for turning the Auto Hide option on and off, and it is available only when a panel is docked. The Push Pin icon changes to display the current state of the panel.

Alternatively, you can turn the Auto Hide option on and off using the *Title bar context menu* (see page 32).

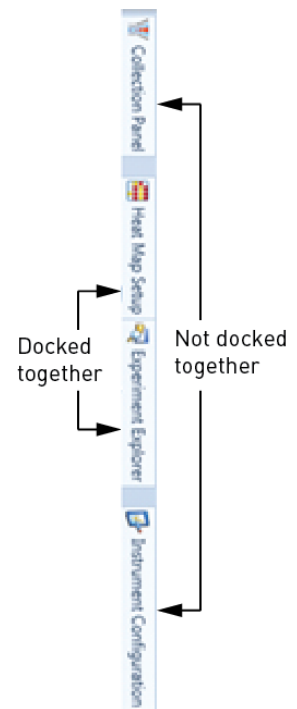


Panel pinned – Auto Hide is turned off.



Panel unpinned – Auto Hide is turned on

- When hidden, the panel name appears as a tab on the side of the Main Application Workspace where the panel was docked.
- When multiple panels that are docked together, hiding one panel also hides the others with which it was docked. All hidden panels then appear together as tabs on the side of the Main Application Workspace where they were docked.
- Clicking on a tab only displays the selected panel.
- Clicking on the Push Pin of an open panel restores all panels with which it was docked.
- When hiding multiple panels on the same side of the Main Application Workspace, panels that were docked together will be grouped together and be separated from other panels as shown on the right.



Close

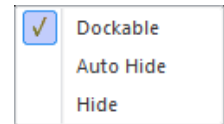
Clicking the **Close icon (X)** closes the current panel. This deselects the current panel in the *Show Panels* group of the *View ribbon tab* (see page 43).

Alternatively, you can select the Hide option using the *Title bar context menu* (see below).

- The only way do re-display a closed panel is to reselect the relevant check box in the Show Panels group of the View ribbon bar (page 43).
- If multiple panels are docked together and the panel is docked, the close icon only hides the current panel. If the panel is undocked the entire panel is closed.

Title bar context menu

Right-clicking on the title bar of a panel displays the *Title bar context menu*, which contains the options listed below.



The current state of the panel is indicated in the context menu by a check mark.

- **Dockable:** Allows the docking of a panel. A panel cannot be docked unless this option is checked.
Unchecking the Dockable option for a docked panel floats that panel, which cannot be re-docked until the Dockable option is re-selected.
Dockable option is not available when the Auto Hide option is selected, because a panel must be docked to allow auto-hiding.
- **Auto Hide:** Reduces a panel to a tab icon when the focus is lost by clicking anywhere outside the menu. This has the same action as selecting the *Push Pin icon* in the panel header (see page 31).

The *Auto Hide* option is only available when the panel is docked, not floating.

- **Hide:** Hides the current panel. When hidden, the panel name appears as a tab on the side of the Main Application Workspace where the panel was docked.
Selecting the Hide option from the context menu deselects the current panel in the *Show Panels* group of the *View ribbon bar* (see page 43).

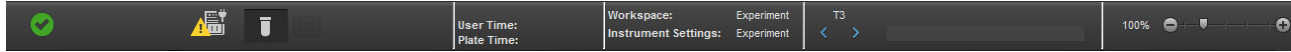
The only way do re-display a hidden panel is to reselect the relevant check box in the Show Panels group of the View ribbon bar.

If multiple panels are docked together, the Hide option only hides the current panel.

Application status bar


The *Application status bar* indicates the status of the Attune™ NxT Acoustic Focusing Cytometer and provides feedback on the system using indicator icons. It also contains additional controls to navigate between samples.

The configuration of the Application status bar depends on whether the instrument is acquiring or whether it used for analysis.



The Application status bar is described in parts as listed below:

- Instrument status and alerts (below)
- Time and coordinates (page 35)
- Workspace and Instrument Settings (page 35)
- Acquisition (page 36)
- Analysis (page 36)
- Size slider (page 37)

 **Note:** Clicking on indicator icons does not have an effect on the instrument as they are only used for providing feedback on the status of the system.

Instrument status and alerts

The image below shows the fixed positions of *Instrument Status and Alerts* indicator icons on the status bar. If a particular indicator icon is not displayed, then a gap is left in its position.



Instrument status icons



Instrument Idle: The Instrument Idle icon is displayed when the instrument is connected and there are no alarms, or if an instrument has never been connected. The tooltip displays “Idle”.



Instrument Busy: The Instrument Busy icon is displayed when the instrument is connected and it is not idle. The tooltip displays “Instrument busy”.



Alarm: The Instrument Status Alarm icon is displayed when there is an instrument error state. The tooltip displays the relevant error message. If multiple error messages are present, each error message is separated by a comma.

Instrument alert icons



Instrument Fluid: The Instrument Fluid icon is displayed when a fluid level warning is given. The tooltip may contain more than one message concerning the fluidics (see below), using commas between messages.

- “Shutdown solution tank low”
- “Wash tank low”
- “Focusing fluid tank low”
- “Waste tank full”
- “Auto sampler focusing fluid tank low”
- “Auto sampler waste tank full”



Plate Leak Detected: The Plate Leak Detected icon is displayed when a leak in the Auto Sampler has been detected. Tooltip displays “Auto Sampler leak detected”.



Instrument Leak Detected: The Instrument Leak Detected icon is displayed when a leak in the instrument has been detected. Tooltip displays “Instrument Leak Detected”.

If a leak is also detected in the Auto Sampler, the Tooltip displays “Instrument Leak and Auto Sampler Leak Detected”.



Tube Lifter Down: The Instrument Tube Lifter icon is displayed when the tube lift is not in the up position. If the tube lifter is down, the Tooltip displays “Tube lifter is down”.

If the tube lifter is in the intermediate state, the Tooltip displays “Tube lifter is intermediate”.



Instrument Cover Open: The Instrument Top Cover icon is displayed when the top cover of the cytometer is not properly closed. Tooltip displays “Top cover is open”.



Auto Sampler Door Open: The Auto Sampler Door Open icon is displayed when the Auto Sampler door is not properly closed. Tooltip displays “Auto Sampler door is open”.

Connection status icons



Cytometer Not Connected: The Cytometer Not Connected icon is displayed if the Attune™ NxT Acoustic Focusing Cytometer is not connected. The tooltip displays “Attune™ not connected”.

If both the cytometer and the Auto Sampler are not connected, the Tooltip displays “Attune™ and Auto Sampler are not connected”.











Auto Sampler Not Connected: The Auto Sampler Not Connected icon is displayed if the Auto Sampler is not connected. The Tooltip displays “Auto Sampler is not connected”.



Note: The connection status icons are only displayed if the software has previously been connected to an instrument as indicated by the database flag, and the conditions described above are met.

Valve position icons

-   **Tube mode – Plate present:** The Valve Tube icon is displayed when the valve is set to Tube mode and the Auto Sampler contains a plate. The Tooltip displays “Tube mode”.
-   **Tube mode – Plate absent:** The Valve Tube icon is displayed when the valve is set to Tube mode and the Auto Sampler does not contain a plate. The Tooltip displays “Tube mode”.
-   **Plate mode – Plate present:** The Valve Plate icon is displayed when the valve is set to Plate mode and the Auto Sampler contains a plate. The Tooltip displays “Plate mode, plate present in Auto Sampler”.
-   **Plate mode – Plate absent:** The Valve Plate icon is displayed when the valve is set to Plate mode and the Auto Sampler does not contain a plate. The Tooltip displays “Plate mode no plate in the Auto Sampler”.



Note: When connected to an instrument, one of the valve position icons described above is always displayed.

Time and coordinates

The *Time and coordinates* panel displays the *User* and *Plate* or *Sample times*. The X-Y coordinates of a selected gate are also displayed here.



X	1532	User time	01:32:34
Y	1324	Plate time	00:03:04

- **User time:** User time shows the duration a user has been logged on to the instrument in the HH:MM:SS format. The time resets after the user logs out.
- **Plate time/Sample time:** Plate time shows the time taken to complete a *Record Plate* operation in the HH:MM:SS format. Similarly, Sample time shows the time taken to complete a *Record Tube* operation in the HH:MM:SS format.

If the operation is manually stopped or terminated automatically due to a fault condition, Plate/Sample time displays the time at which the operation was stopped or paused. When and if **Resume** is clicked, the Plate/Sample time is reset.

During *Compensation Setup* or *Record Compensation* operations using wells, the Plate/Sample time will be blank.

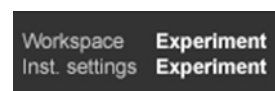
- **Coordinates:** Coordinates panel shows the X and Y coordinates of the selected gate as described in “Moving and Resizing Gates”, page 79.



Note: If the timer reaches 99:59:59 for the User time or the Plate/Sample time, the displayed timer will stop and remain at this value. However, the time recorded in the log file will display the correct time.

Workspace and Instrument Settings

The *Workspace and Instrument Settings* panel displays the open Workspace and the Instrument settings for the open sample.

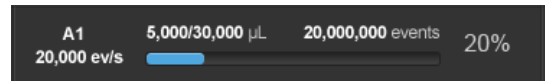


Workspace	Experiment
Inst. settings	Experiment

- **Workspace:** Workspace displays the Experiment-level workspace name during acquisition. Depending on which Workspace is selected from the Workspace dropdown menu (page 64) for the active Sample, this will be “Experiment”, “Group”, or “Sample”.
- **Instrument Settings:** Instrument settings displays the lowest level instrument settings, either “Experiment” or “Sample”.

Acquisition

The *Acquisition status* panel is visible only during acquisition and provides information and feedback on the parameters listed below.



- Well location for a Plate or the Tube ID **A1**
- Calculated event rate (number of events/acquisition time) **20,000 ev/s**
- Current acquired volume and the total available volume **5,000/30,000 µL**
- Number of events acquired at the current time **20,000,000 events**
- Acquisition progress bar showing the progress towards the *Stop condition*, where 100% activates the stop condition **20%**

Analysis

The *Analysis status* panel is visible only when an active Experiment is open for analysis and the instrument is not acquiring. It consists of sample location (well location or Tube ID), navigation buttons, and the Analysis progress bar.



- Well location for a Plate or the Tube ID is displayed for active Experiments. **< T1 >**
 - The navigation buttons allow navigation between Samples for the current Experiment.
 - The *Left navigation button* is disabled when the first Sample in an Experiment is active.
 - If the last Tube sample in an Experiment is active, and the 400 Sample limit has not been reached, the *Right navigation button* will be active and add a new Tube sample, if clicked. The Tooltip for the button will display “Create new Sample”.
- In all other cases when the navigation buttons are enabled, the Tooltip displays either the previous or the next sample name as appropriate.
- If the 400 Sample limit has been reached, the right button will be disabled.
 - The Analysis progress bar is displayed during instrument functions and while loading or refreshing data.
 - If multiple operations requiring a progress bar occur simultaneously, the progress bar displays the operation requiring the longest time to be completed.
 - In the collapsed state, the priority for display is: instrument function, refreshing, loading. As operations are completed, remaining operations are brought to the foreground.
 - The Analysis Progress bar is not displayed for tasks that take less than 3 seconds to complete.

Size slider

The *Size slider* is used for adjusting the size of a selected panel on the Main Application Window.



- The Size slider is contextual for the current view. If a panel that is not zoomable is in focus, the Size slider is disabled. The views for which this is applicable are:
 - Workspace
 - Preview
- The zoom settings persist per Experiment.
- Size adjustments apply only to the screen display and do not affect the print view.
- The scale of the slider runs from 10% to 400% magnification, with tick marks shown at 10%, 100%, and 400% magnification.
- The scale shows the current scale value rounded to the nearest percent.
- Clicking the **+** **button** increases the magnification to the nearest whole 10%. For example, from 93% to 100%.
- Clicking the **-** **button** decreases the magnification to the nearest whole 10%. For example, from 93% to 90%.
- Dragging the slider caret adjusts the plot size according to its position. The minimum drag rate is a single percent.
- A left mouse click on the scale, lower to upper tick, will set the caret to match the horizontal position.



Note: The Size slider can also be adjusted by holding the **Ctrl** key down and turning the mouse wheel. This will adjust the view that is currently in focus or underneath the mouse cursor at the time the adjustment is made.

4. Ribbon tabs

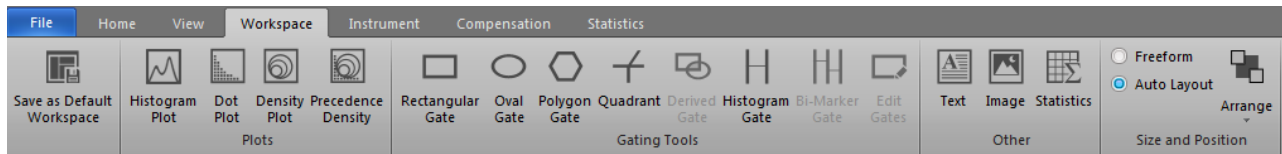
Overview

Ribbon tabs

Ribbon tabs are contextual tabs that consolidate related software and instrument functionality in the Ribbon bar.

The availability of the Ribbon tabs in the Ribbon bar is dependent on the context of the software, i.e., the tabs are displayed only when the user needs them.

Similarly, objects and controls that are not available as a function in a particular context are shaded gray in the tab.



Depending on the context of the application, the Ribbon bar contains one or more of the following tabs:

- *File* (page 39)
- *Home* (page 40)
- *View* (page 42)
- *Workspace* (page 44)
- *Instrument* (page 48)
- *Experiment* (page 50)
- *Compensation* (page 54)
- *Statistics* (page 58)



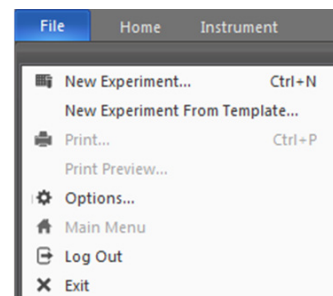
Note: Only a limited set of tabs are available on the Main Menu (page 20) when performing Performance Testing (page 260).









On the Login screen (page 15), only the File tab (page 39) is available.

File tab

File menu

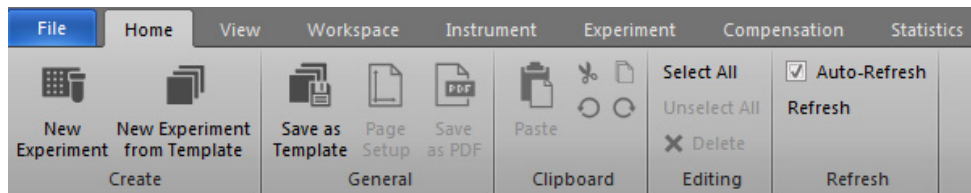
The *File* tab is unique amongst Ribbon bar tabs in that it functions as a dropdown menu. Clicking **File** on the Ribbon bar opens the *File menu*, which provides the following options:



-  **New Experiment:** Opens the *New Experiment* dialog (page 303), which allows you to create a tube only Experiment, a Plate Experiment, or an Experiment using imported files. The New Experiment option is enabled only when the instrument is not acquiring.
-  **New Experiment from Template:** Opens the *New Experiment from Template* dialog (page 307), which allows you to create a new Experiment from an existing template with pre-defined settings. This option is enabled only when the instrument is not acquiring.
-  **Print:** Opens the *Print* dialog (page 315), which is a modified Windows® Print dialog containing the standard Windows® print commands as well as the option to select Workspace, Heat Map, Results, Overlays, and Reports for printing. The Print dialog option is enabled only when the instrument is not acquiring. It is not enabled on the Main Menu (page 20).
-  **Print Preview:** Opens the *Print Preview* dialog (page 315), which displays the document as it will appear when printed and contains the controls for various printing options. The Print Preview option is enabled only when the instrument is not acquiring.
-  **Options:** Opens the *Options* dialog (page 326), which is used to customize the Attune™ NxT Software. Some options are user-specific, while others are application-specific (i.e., global to all users) and customizable only by an authorized user. This option is enabled only when the instrument is not acquiring.
-  **Main Menu:** Navigates to the starting Main Menu (page 20). This option is enabled only when the instrument is not acquiring.
-  **Log Out:** Closes the software and logs out the current user, and navigates to the *Login* screen (page 15). During Log Out, all FCS files that have not been backed up are backed up automatically. The Log Out option is enabled only when the instrument is not acquiring.
-  **Exit:** Closes the Attune™ NxT Software. This option is enabled only if the instrument is idle.


Home tab


The *Home* tab provides Experiment setup and Output options, and it is organized into five functional groups: *Create*, *General*, *Clipboard*, *Edit*, and *Refresh*.



Create group


The *Create* group allows you to create a new Experiment.


 **New Experiment:** Opens the *New Experiment* dialog (page 303), which allows you to create a Tube-only Experiment, a Plate Experiment, or an Experiment using imported files. This button is always visible and enabled except during acquisition.

 **New Experiment from Template:** Opens the *New Experiment from Template* dialog (page 307), which allows you to create a new Experiment from an existing template with pre-defined settings. This button is always visible and enabled except during acquisition.

General group


The *General* group allows you to save an active Experiment as a template.


 **Save as Template:** Opens the *Save as Template* dialog, which allows you to save the current Experiment as a template so that the Experiment Workspace, Experiment Instrument settings, Run Protocols, Heat Map settings, and Experiment hierarchy are preserved for future Experiments. This button is disabled on the Main Menu, Performance Test, and Instrument Configuration screens.


 **Page Setup:** Opens the *Page Setup* dialog, which allows you to customize the page layout (page size and orientation). This button is only available when Workspace, Results, Heat Map, or Overlay tab is active.



Clipboard group

The *Clipboard* group allows you to cut, copy, and paste selected objects and text in the work area, and to Undo and Redo previous actions.

 **Paste:** Pastes the clipboard item into the current Workspace or Run Protocol. This option is only available when an item has previously been copied or cut into clipboard memory.

 **Cut:** Removes the selected item from the Workspace and retains it in memory until another item is cut or copied into memory. This option is only available when a Workspace item is selected.

 **Copy:** Retains the selected item in memory for pasting until another item is cut or copied in to memory. Selecting Copy does not remove the item from the Workspace. Workspace objects, Run Protocols, and Results can be copied into memory for pasting.


-  **Undo:** Negates the last undoable action in the order performed until the undo stack is empty. The Undo button is enabled only if there is an undoable action available. External functions including Save, Import, Export, or Print commands cannot be undone.
-  **Redo:** Reverses the Undo action in the order performed until all available tasks have been carried out. This option is only available when an Undo action has been successfully performed.

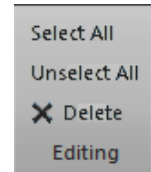
Edit group

The *Edit group* contains shortcuts for selecting, unselecting, and deleting any of the objects in the work area.

Select All: Selects and highlights all objects on the current Workspace, Results, or Heat Map. This button is available if a Workspace, Results, or Heat Map is open and one or more items are present.

Unselect All: Deselects all previously selected objects on the current Workspace. This button is available if a Workspace is open and one or more items are currently selected.

 **Delete:** Deletes the selected item. This button is available when a Workspace object or a Heat Map location is selected.



Refresh group

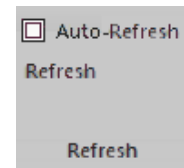
The *Refresh group* contains options that allow the *Auto-Refresh* function to be turned off for inactive Workspaces, Heat Maps, and Results, thus requiring them to be manually refreshed.

Auto-Refresh checkbox: When **Auto-Refresh** is selected, all statistics in the Results tab are updated automatically in the background as the Workspace is modified. The Heat Map for all wells and tubes are also updated automatically.

Un-checking the Auto-Refresh checkbox turns off the automatic updates. However, printing and exporting statistics force an automatic refresh of all out-of-date items.

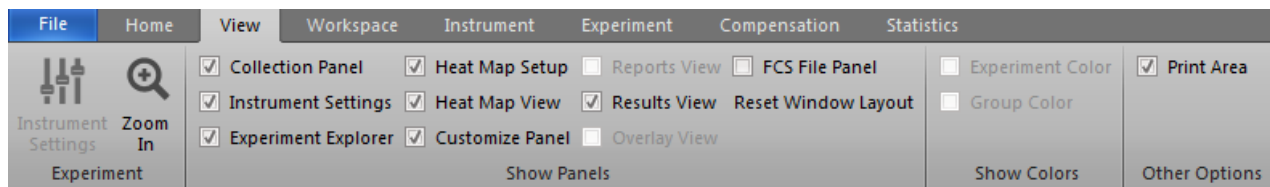
The Auto-Refresh checkbox is enabled and checked by default.

Refresh: Deselects all previously selected objects on the current Workspace. This button is available if a Workspace is open and one or more items are currently selected. The Refresh button is always enabled.



View tab

The *View* tab provides options to adjust the instrument settings, and control the overall look of the software. It is organized into four functional groups: *Experiment*, *Show Panels*, *Show Colors*, and *Other Options*.



Experiment group

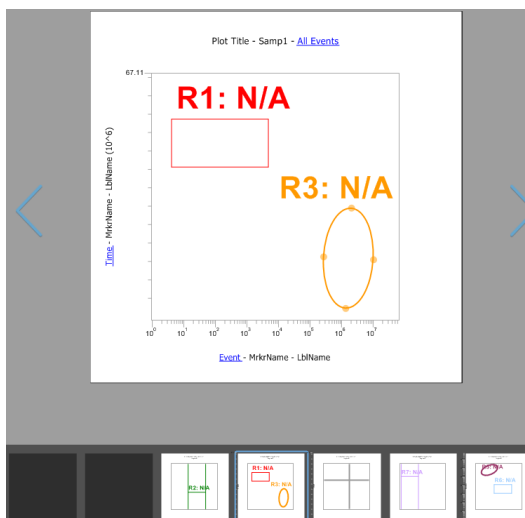
The *Experiment* group allows you to view and adjust instrument settings for an Experiment, and zoom in/out.



Zoom In: Displays all plots present in the *filmstrip* area of the Workspace view (page 71), and allows you to zoom in (maximize) each plot. This is a toggle button (see “Zoom Out” below) and is always visible; it is enabled when the Workspace view is active and contains plots.

The plots are displayed in the filmstrip area in the order that they were created. When you press the Zoom In button, the plots are maximized as follows:

- If no plots are selected, the first plot is maximized.
- If one plot is selected, the selected plot is maximized.
- If more than one plot is selected, the first plot created is maximized.



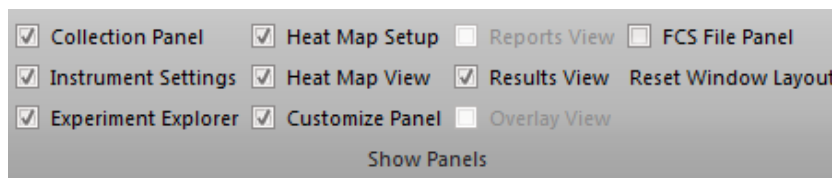
Zoom Out: Closes the Zoom In function and restores the normal Workspace view. This is a toggle button (see “Zoom In” above) and is always visible; it is enabled when the Workspace view is in the zoomed-in state.



Note: Alternatively, click the Close icon () to restore the normal Workspace view.

Show Panels group

The *Show Panels group* allows you to open or close the following panels and views in the Main Application Workspace (page 28).



- Collection Panel (page 159)
- Instrument Settings (page 177)
- Experiment Explorer (page 140)
- Heat Map Setup (page 191)
- Heat Map View (page 105)
- Customize Panel (page 200)
- Results View (page 112)
- FCS File Panel (page 239)

All of the Show Panels options are always visible and enabled. For the default positions and display status of the panels and views, see “Docking behavior” under “Main Application Workspace” on page 29.

- To close a panel or view, deselect its checkbox.
- To open a panel or view, select its checkbox. Selecting a checkbox restores the panel or view to the state it was in when it was last closed.
- If a panel or view is hidden (see page 31), but not closed, its checkbox remains selected.
- By default, the FCS File panel is deselected, and all other panels and views are selected.
- Click **Reset Windows Layout** to restore all panels and views to their default states.



Note: When hidden, the panel or view name appears as a tab on the side of the Main Application Workspace where the panel was docked.

Other Options group

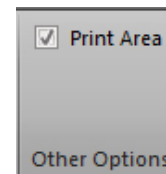
The *Other Options group* contains the display options for the print area and for events.

The Print Area option is always visible and enabled.

Print Area

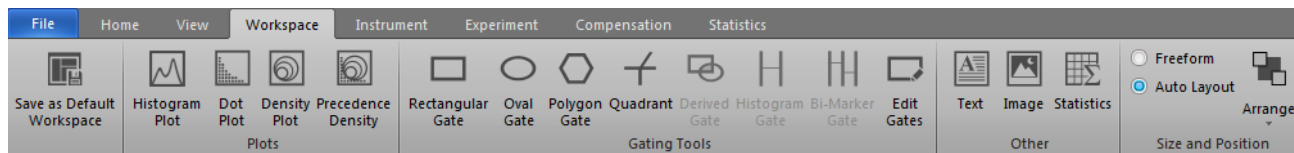
Allows you to display the Workspace view (page 63) as it will be printed on a page.

- Select the Print Area checkbox to display the page boundaries as gray lines drawn under an object.
- Deselect the Print Area checkbox to restore the normal Workspace view.



Workspace tab

The *Workspace* tab provides tools for adding objects to a Workspace and aligning objects on the Workspace.



The Workspace tab is organized into six functional groups: *Plots* (below), *Gating Tools* (page 45), *Other* (page 46), and *Size and Position* (page 47). It also contains the *Save as Default Workspace* option (below).



Note: All options on the Workspace tab are disabled when a *Compensation Control Sample* is being analyzed.

Save as Default Workspace option



Save as Default Workspace: Saves the active Workspace as the default for the current user. Any new Experiments and Plates will use this Workspace as the default Experiment Workspace. The Save as Default Workspace option is disabled if the Workspace view (page 63) is not in focus.

Plots group

The Plots group is used for creating plots of various types. The buttons within the Plots group are enabled when the Workspace view (page 63) is in focus and at least two parameters are active, except for the Histogram plot button, which requires only one active parameter.

The Plots group contains the buttons listed below. For more information on plot types, see “Plots” under “Workspace view” (page 75).



Histogram Plot: Adds a new single-parameter *Histogram plot* to the current Workspace. The Histogram plot displays the default parameter plotted against count (page 75).



Dot Plot: Adds a new *Dot plot* to the current Workspace. The Dot plot displays two-parameter data where each axis represents the signal intensity of one parameter. Different colors are used to represent the parent gate of events that fall within bins on the plot (page 76).



Density Plot: Adds a new *Density plot* to the current Workspace. The Density plot displays two-parameter data where the colors represent the collection of events with the same intensity and each axis represents the signal intensity of one parameter (page 76).



Precedence Density Plot: Adds a new *Precedence Density plot* to the current Workspace. The Precedence Density plot is a combination of Dot and Density plots, where a gradient is used to indicate the number of events within each of the plot bins and color is used to display the parent gate of events present (page 76).








Note: The buttons within the Plots group are highlighted when selected, and revert to their standard color when you click in the Workspace area to add the selected plot, select another button, or press the **ESC** key.

Gating Tools group



The *Gating Tools* allow you to isolate a region in a selected plot for analysis. The Gating Tools are available when the Workspace view (page 63) is displayed, unless otherwise specified.

- Dual-parameter gate types are enabled when the current Workspace contains at least one dual-parameter plot (i.e., Dot plot, Density plot, Precedence Density plot).
- Single-parameter gate types are enabled when the Workspace contains at least one Histogram plot.
- A maximum of 128 gates may be created on plots on a Workspace, except for Quadrant gates, of which at least 1000 may be created. The Gating Tools buttons become disabled when the Workspace reaches the maximum number of gates allowed.

The Gating Tools group contains the buttons listed below. For more information on gates, including instructions for creating and deleting gates, see “Gates” under “Workspace view” (page 77).

-  **Rectangular Gate:** Allows you to draw a *Rectangular gate* on a dual-parameter plot as described on page 80.
-  **Oval Gate:** Allows you to draw an *Oval gate* on a dual-parameter plot as described on page 81.
-  **Polygon Gate:** Allows you to draw a *Polygon gate* on a dual-parameter plot as described on page 82.
-  **Quadrant Gate:** Allows you to draw a *Quadrant gate* on a dual-parameter plot as described on page 83.
-  **Derived Gate:** Displays the *Derived Gate dialog*, which allows you to create gates based on Boolean operators (i.e., *Derived gates*) as described on page 319.

The Derived Gate button is enabled when the Workspace view is displayed and the current Workspace has at least one gate present.

-  **Histogram Gate:** Allows you to draw a *Histogram gate* on a Histogram plot as described on page 83.
-  **Edit Gates:** Displays the *Edit Gates dialog*, which allows the editing of gate color, parent gate, gate math expression, the z-order in which gates are painted as described on page 322.

The Edit Gates button is available when the Workspace view is displayed and the current Workspace has at least one gate present.



Note: The buttons within the Gating Tools group are highlighted when selected, and revert to their standard color when you click in the Workspace plot to create the gate, select another button, or press the **ESC** key.

Other group

The *Other group* is used to insert text and images into the Workspace, and to display the statistics corresponding to a selected plot in the Workspace.

The Other group contains the buttons listed below, which are available whenever the Workspace view is in focus.



Text: Adds a new *Text box* to the current Workspace.

- The Text box displays the default text “New Text Box”, which can be edited in place without opening a separate dialog box.
- The style and border of the Text box can be customized as described in “Customize Text Box Options” on page 217.



Image: Opens a standard Windows file selection dialog for selecting an image file to add to the Workspace.

- The image file cannot be larger than one page and will be scaled to fit on the page preserving the aspect ratio, if necessary.
- The image types available for insertion include jpg, gif, bmp, png, tif and windows enhanced metafile.



Statistics: Adds a new *Statistics box* to the current Workspace.

- If no plot is selected, clicking on the Statistics button inserts a *Workspace statistics table* in the default Workspace location.
- If one or more plots are selected, then one *Plot Statistics box* is added to each selected plot immediately below the relevant plots as described in “Workspace statistics”, page 87.



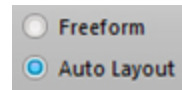
Note: The buttons in the Other group are highlighted when selected, and revert to their standard color when you click in the Workspace plot to add a Text box, an image, or a Statistics box, select another button, or press the ESC key.

Size and Position group

The *Size and Position group* provides tools that assist in creation and customization of Workspaces, and contains the buttons listed below.

Layout radio buttons

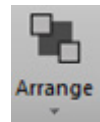
Layout Radio Buttons consist of two radio buttons, which control the view mode of the Workspace layout as described in “Workspace view modes” on page 68.



- **Auto Layout:** In this mode, objects are placed into a number of grid slots. The number of available slots per page is defined by the *Grid Size* options (page 47). Auto Layout is the default mode.
- **Freeform:** In this mode, objects can be freely resized and moved anywhere on the Workspace. There is no automatic arrangement or alignment of objects on the page.

Arrange tools

Clicking on **Arrange** opens the *Arrange Tools* dropdown menu, which contains the tools to arrange, align, and size multiple Workspace objects (see below).

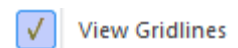


- The Arrange Tools commands are available only when multiple items are selected on the Workspace, unless otherwise stated.
- The View Gridlines and Grid Size options are only enabled in the Auto Layout mode; all other options are enabled only in the Freeform mode.

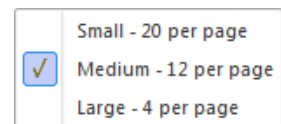
Grid options

Grid options are enabled only when the Auto Layout mode is selected using the Layout Radio Buttons (page 47).

View Gridlines: Selecting this option displays the lines that form the grid onto which the Workspace objects are snapped. By default, the gridlines are displayed.



Grid Size: Opens the *Grid Size dropdown* menu, which contains the options to define the number of grid slots available per page. A check mark appears next to the currently selected size.

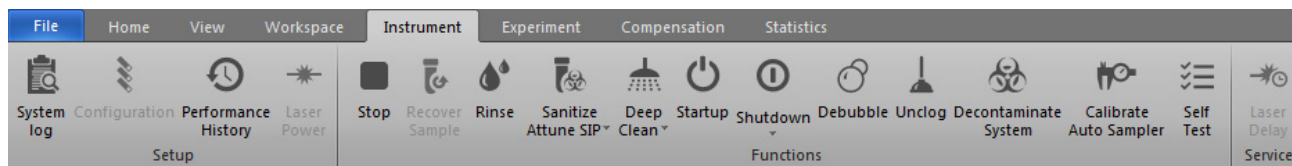


The default selection is 12 grid slots per page.

You can select a larger grid size allowing only 4 items per page, or a smaller grid size allowing up to 20 items per page at the default sizes.

Instrument tab

The *Instrument* tab provides tools for viewing instrument data and performing instrument functions. It is organized into three functional groups: *Setup*, *Functions*, and *Service*.



Setup group

The *Setup* group allows you to view instrument data and manage configuration files.



System Log: Opens the *System Log* dialog, which allows you to view system transactions. The information displayed in the System Log depends on the type of account that you have: service, administrator, system administrator, or general user account. This button is always visible and enabled.



Configuration: Opens the *Configuration* module (page 244), which allows you to manage instrument configuration files and filters. This button is always visible and enabled except during acquisition.



Performance History: Opens the *Performance History* report (page 271), which allows you to view the pass/fail status of all Performance Tests. This button is always visible and enabled except during acquisition.

Functions group

The *Functions* group allows you to initiate the instrument functions.



Stop: Allows you to stop any acquisition or instrument function, except the *Calibrate* and *Self Test* instrument functions. This button is always visible and enabled.

- If you click **Stop** during the following instrument functions, you must run **Startup** before running the instrument again:
 - **Startup** (page 49)
 - **Unclog** (page 49)
 - **Shutdown** (page 49)
 - **Deep Clean** (page 49)
 - **Decontaminate System** (page 49)
- If you click **Stop** during the **Rinse** function, the instrument stops and performs an automatic Rinse on the next instrument action.
- If you click **Stop** during all other instrument functions, the instrument performs an automatic rinse before stopping.



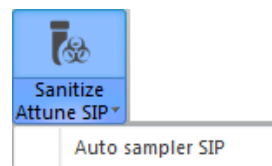
Recover Sample: Allows you to recover unused sample. The button is enabled when the instrument status is idle (i.e., not actively acquiring) and there is enough sample within the sample loop or enough preloaded sample to recover. When the button is pressed, the remaining sample is returned into a tube (from the sample loop) or back into the sample well (preloaded sample in a plate).



Rinse: Opens the *Rinse* dialog, which allows you to rinse the Sample lines. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring a plate.



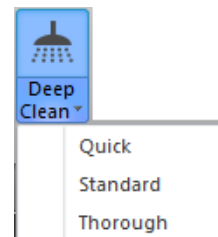
Sanitize Attune™ SIP: Opens the *Sanitize* dialog, which allows you to sanitize the instrument SIP (sample injection port) and sample lines, or the Auto Sampler SIP and Sample lines. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring.



This is a split button: Click **Sanitize Attune™ SIP**, or select **Auto Sampler SIP** from the dropdown menu.



Deep Clean: Opens the *Deep Clean* dialog, which allows you to thoroughly wash the system sample lines and flow cell. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring.



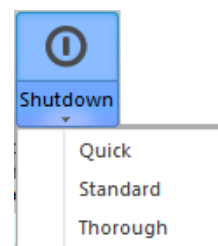
This is a split button: Click **Deep Clean** to perform a standard clean cycle, or select **Quick**, **Standard**, or **Thorough** from the dropdown menu.



Startup: Opens the *Startup* dialog, which allows you to prime the system fluidics. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring, or when the instrument is in the “sleep” state.



Shutdown: Opens the *Shutdown* dialog, which allows you to sanitize and shut down the instrument. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring.



This is a split button: Click **Shutdown** to perform a standard shutdown cycle, or select **Quick**, **Standard**, or **Thorough** from the dropdown menu.



De-bubble: Opens the *De-bubble* dialog, which allows you to clear the sample lines and flow cell of bubbles. This button is always visible, and is enabled when the instrument status is idle.



Unclog: Opens the *Unclog* dialog, which allows you to unclog the Sample lines. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring.



Decontaminate System: Opens the *Decontaminate* dialog, which allows you to decontaminate all system fluid lines. This button is always visible, and is enabled when the instrument status is idle and not actively acquiring.



Calibrate Auto Sampler: Opens the *Auto Sampler Calibration* dialog, which allows you to calibrate the Attune™ Auto Sampler. This button is visible only when there is an Auto Sampler connected to the instrument; it is enabled when the instrument status is idle and not actively acquiring.

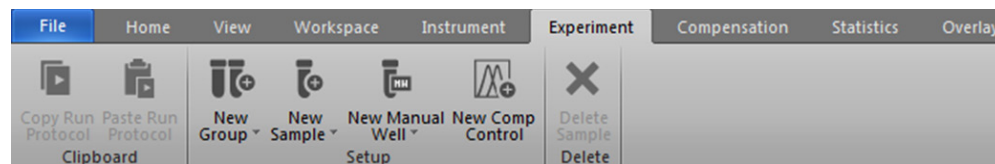


Self Test: Opens the *System Test* dialog, which allows you to run system diagnostics. This button is visible only to service accounts; it is enabled when the instrument status is idle and not actively acquiring.

Experiment tab

The *Experiment* tab provides tools to define the Experimental layout for tubes and plates. The Experiment tab is visible only when the *Heat Map view* (page 105) is active.

Any changes that you make to the Experiment layout in the Experiment tab are automatically reflected in real-time in the *Experiment Explorer* (page 140).



Experiment limits

Plate-based Experiments can contain Samples in wells and/or tubes. Unless otherwise specified, in this section *Sample* is used to mean Samples in tubes and in wells.

All Samples on a Plate Experiment must total ≤ 400 wells and tubes. This limits the number of tube samples per plate to:

- ≤ 304 tube samples for 96-well plates
- ≤ 16 tube samples for 384-well plates

Changes made to the Experiment within the Heat Map view will be automatically reflected within the Experiment Explorer.

Experiment tab controls



Copy Run Protocol: Copies the Run Protocol for the selected samples to the clipboard. This button is always visible, but it is enabled only when samples are selected.

- If you select multiple samples that have different Run Protocols, the software copies all of the protocols and maintains the relative positions of the selected samples.



Paste Run Protocol: Pastes Run Protocol from the clipboard into the selected samples. This button is always visible, but it is enabled only when one or more Run Protocols are copied to the clipboard and samples are selected.

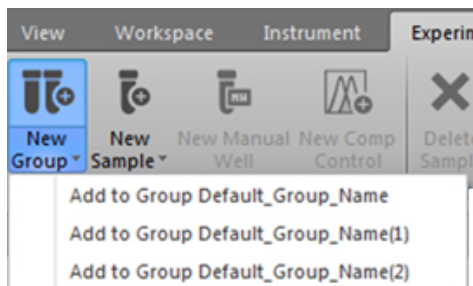
- If you copy only one Run Protocol to the clipboard, the software pastes that Run Protocol into all selected samples.
- If you copy multiple Run Protocols to the clipboard, this button is active only when the same array of samples is selected. The software pastes each unique Run Protocol into the selected sample of the same position in the array.
- If the Run Protocol is copied from and pasted into plates of different types, the software checks all entries for validity following rules set in “Run Protocol Options” (page 172).

For example, if the pasted well volume is greater than allowed in the selected well, the software defaults to the maximum volume allowed.



New Group: Adds the selected Samples to a new or existing *Group*. This button is always visible, but it is enabled only when samples are selected. The selected samples can be un-programmed or marked as part of the Experiment.

The New Group button is a split button that includes a dropdown menu option.



- To create a new Group and add the selected Samples to it, click the main portion of the **New Group** button.
- To add the selected Samples to an existing Group, select the Group from the **New Group dropdown** menu.

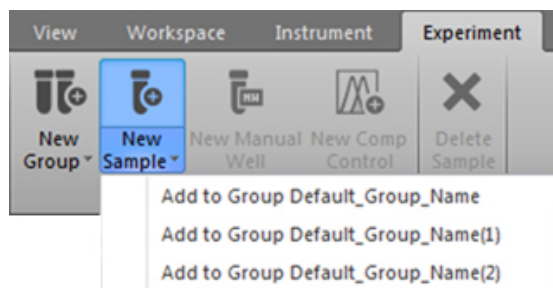


Note: Each Experiment that has Samples assigned consists of one or more Groups. Each group may contain up to 400 Samples, each of which may be derived from a well within a plate or from a tube.



New Sample: Marks selected blank tubes or wells as Samples and adds these new Samples to a Group. This button is always visible, but it is enabled only when blank tubes and/or wells are selected.


The New Sample button is a split button that includes a dropdown menu option.



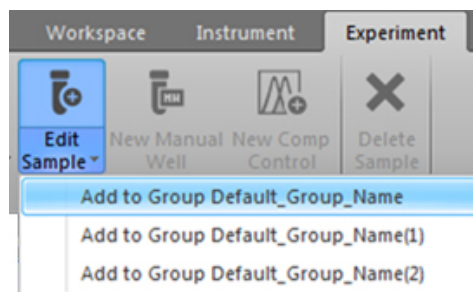
- To add the new Samples to the most recently created Group, click the main portion of the **New Sample** button. If no groups exist, the software creates a new Group.
- To add the new Samples to an existing Group, select the Group from the **New Sample dropdown** menu.




Note: The New Sample button is a toggle button, which changes to “Edit Sample” when one or more wells and/or tubes already marked as Samples are selected (see “Edit Sample”, page 52).

 **Edit Sample:** Moves selected Samples to a different Group. This is a toggle button (see “New Sample” above) and it is always visible, but enabled only when Samples are selected.

The Edit Sample button is a split button that includes a dropdown menu option.



- To move the selected Samples to a different Group, select the Group from the **Edit Sample** dropdown menu.
- If your selection also includes new Samples, the button remains **Edit Sample**. New Samples are added to the same Group selected for the existing Samples.

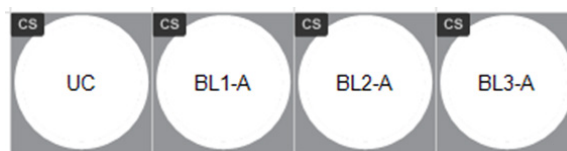
 **Manual well:** Allows you to acquire Samples from a well in a Plate while adjusting Instrument Settings. This button is always visible, but it is enabled only when one or more wells are selected. The button is disabled for Tube-based Experiments or if Tube locations are selected on the Heatmap in a Plate-based Experiment.

- The software marks Manual wells with a “MW” icon in the top left corner of the well.
 - The tooltip for the Manual well button reads “Defines a well that has both ‘Run’ and ‘Record’ modes.”



New Comp Control: Opens the *Compensation Setup dialog* (page 284).

- Clicking on the “New Comp Control” button in the ribbon bar will open the Compensation Setup dialog. You can select the compensation source from wells or tubes. If blank wells are selected before clicking **New Comp Control**, the compensation dialog box automatically selects wells as the compensation source and the selected wells are pre-populated with the compensation controls. By default, all possible compensation controls are selected. Note that each parameter selected for compensation needs to have an associated well location before the dialogue box will close.
- Compensation wells are indicated within the software by the “CS” icon in the top left corner of the well (shown below). The well is also labeled with the fluorescence channel or unstained control mapped to the compensation well.



Delete Sample: Deletes the selected Samples, which returns the Samples to the undefined state and removes all associated sample data (Workspaces, Run Protocols, and Listmode files). This button is always visible, but it is enabled only when Samples marked as part of an Experiment are selected.

Hot keys

The following hot keys may be used alone or in combination to copy and paste, Run Protocols and delete selected samples.

- **Ctrl+C** combination on the keyboard copies the selected Run Protocols to the clipboard as described in “Copy Run Protocol” (page 50).
- **Ctrl+V** combination on the keyboard pastes the Run Protocol in the clipboard to the selected sample as described in “Paste Run Protocol” (page 50).
- **Delete** key on the keyboard deletes selected samples as described in “Delete sample”, page 52.

Compensation tab

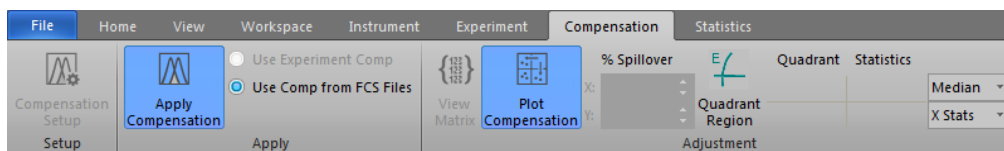
The *Compensation* tab contains controls for setting compensation, selecting the compensation source, and changing the view of Compensation samples.

The contents of the Compensation ribbon tab vary depending on whether a *Sample Workspace* or a *Compensation Workspace* is displayed (see below).

The Compensation tab contains the the *Setup* (see below), *Apply* (page 55), and *Adjustment* (page 56) groups, depending on the context.

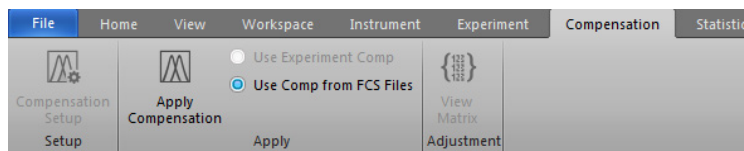
Compensation tab on Compensation Workspace

The Compensation tab fully expanded on a Compensation Workspace is shown below.



Compensation tab on Sample Workspace

The Compensation tab fully expanded on a Sample Workspace is shown below.



Note: For more information on the compensation functions of the Attune™ NxT Software, see “Compensation” on page 283.

Setup group

The *Setup* group contains the *Compensation Setup* button. When the Compensation Workspace is active, additional controls are visible to allow the selection of *Histogram View*. The Setup group is always visible.



Compensation Setup: Opens the *Compensation Setup dialog* as described on page 284. The Compensation Setup button is always visible, but it is enabled only when a Plate or Tube Experiment is active.

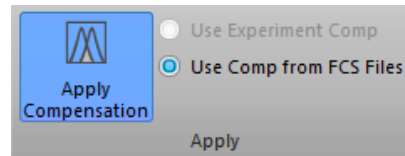
Apply group

The *Apply group* includes controls to turn compensation on and off for the selected Workspace and to choose whether to use Experiment Compensation or compensation loaded from the FCS headers. The Apply group is only visible on a Sample Workspace.



Apply Compensation: When selected, all data shown on the Workspace and used for calculating statistics is compensated. When deselected, all data shown on the Workspace and used for calculating statistics is the raw uncompensated data.

- The Apply Compensation button is ON by default, if compensation is available. The button state persists on a per Experiment basis.



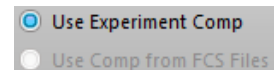
- If neither Experiment-level compensation nor FCS file compensation exists, **Apply Compensation** button opens the *Compensation Setup dialog* as described on page 284.



Note: The Apply Compensation button is a state button; it remains ON until turned OFF. When active, the selected compensation option is applied. If an alternative radio button is selected, the settings are updated automatically.

Use Experiment Comp/Use Comp From FCS Files

These radio controls are only visible and enabled when a Sample is active. These options are disabled on the Compensation Workspace and when compensation is not available from either the Experiment or the selected FCS file.

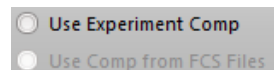


The button state persists on a per Experiment basis except for Samples where the selected compensation is invalid.

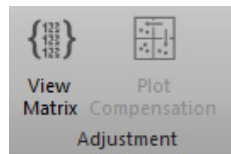
Use Experiment Comp: The *Use Experiment Compensation* option uses the *Compensation Matrix* defined using compensation controls or an imported compensation settings file. This is the default selection when Experiment-level compensation exists.

Use Comp From FCS Files: The *Use Compensation From FCS Files* option uses the Compensation Matrix values stored within the FCS header. This is the default when Experiment compensation does not exist

- If an FCS file does not contain a Compensation Matrix and the Use Comp From FCS Files selection is set, then Experiment Compensation option will be used for that Sample, if available.
- If an Experiment-level compensation is incompatible with an FCS file in the Experiment (due to importing an FCS file), then Use Compensation From FCS Files option will be used for that Sample, if available.
- In these cases, the invalid option will be disabled. If the selected option is disparate to the compensation used for a selected Sample (due to the Sample-level override), the buttons will display the indeterminate state.



Adjustment group



The *Adjustment* group includes controls to view the *Compensation Matrix* and to enable the *Plot Compensation* function.



View Matrix: Opens the *Matrix* dialog (page 298), which shows the active compensation associated with the current Experiment or file. The View Matrix button is always visible, but it is only enabled when a Plate or Tube Experiment is active and either Experiment-level compensation settings exist or there is an active Sample containing an FCS file.

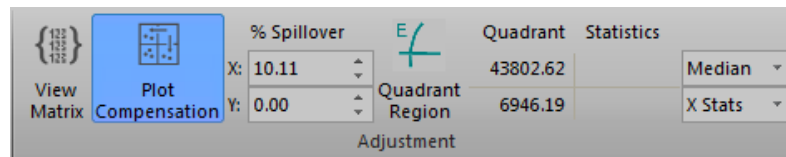


Plot Compensation: Enables *On-Plot Compensation Adjustment* (page 301), and controls the visibility of the *Compensation Adjustment Tools* (below).

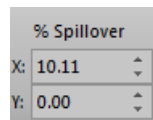
- The Plot Compensation button is deselected (OFF) by default.
- The button is enabled and visible only when **Apply Compensation** is selected. It is not visible in the Compensation Workspace, unless the **View Results** button is selected.
- Any changes made to compensation persist for both the Experiment-level compensation and the FCS compensation.
- The Plot Compensation button is a state button; it remains ON until it is turned OFF or if the selection of Workspace or the Sample changes.

Compensation adjustment tools

- When **Plot Compensation** is turned ON, a collection of compensation adjustment tools are displayed on the Compensation ribbon tab.




- The Compensation Adjustment tools allow the manual adjustment of a selected plot, the ability to add a Quadrant gate to a plot, and the display of Quadrant statistics as described below.
- When **Plot Compensation** is turned OFF, these controls are not visible.



% Spillover Adjustment: These tools consist of two spin box controls that allow adjustment of the compensation or the spillover matrix.

- These controls are only enabled when a dual-parameter plot with two fluorescence parameters is selected in a Sample Workspace or on the Compensation Workspace when “View Results” is turned ON.
- The top control corresponds to the X-axis and the bottom control corresponds to the Y-axis. The up/down arrows of the control or the keyboard move the spillover matrix value by increments of 1 with each click or continuously by maintaining the click.
- Pressing the **Shift** while clicking on the arrows or when using the keyboard up/down arrow buttons moves the value by increments of 0.1.
- The textbox accepts numbers from 0 to 100 with 2 decimal places displayed at all times.

 **Quadrant Gate:** Allows the insertion of a Quadrant gate on a dual-parameter plot as described on page 45.

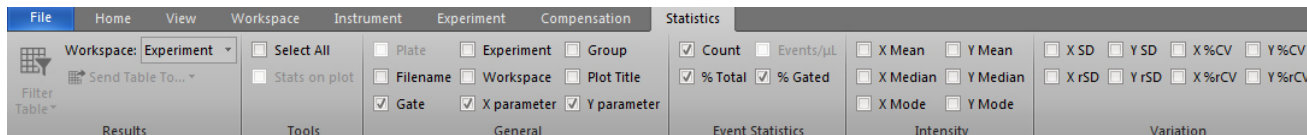
Quadrant	Statistics
43802.62	Median ▾
6946.19	X Stats ▾

Quadrant Statistics: Allows the selection of the mean or the median values and the Y-axis or X-axis statistics.

- By default, the median and the X-axis statistics are selected.
- The statistics values are displayed within a representation of a quadrant gate. When selected, the X-axis statistics are shown in the lower left and lower right quadrants, and the Y-axis statistics are shown in the lower left and upper left quadrants.
- The values shown are based on the quadrant set on the currently selected plot.
- For the statistics to be displayed, a single dual-parameter plot containing only fluorescence parameters (not scatter) must be selected, and the plot must display a quadrant. In addition, a compensatable FCS file must be present. If these requirements are not met, the control will not show any statistics.
- If a statistics value cannot be calculated, the respective quadrant will display "N/A" (i.e., not applicable).

Statistics tab

The *Statistics* ribbon allows you to select which statistic is displayed on the Plots, Workspace(s), and the Results view. The statistics are grouped within the ribbon according to their statistical type (*general*, *event statistics*, *intensity*, *variation*, and *custom statistics*).



The Statistics tab displays the following ribbon groups:

- *Results* (page 59)
- *Tools* (page 59)
- *General* (page 60)
- *Event Statistics* (page 60)
- *Intensity* (page 60)
- *Variation* (page 61)

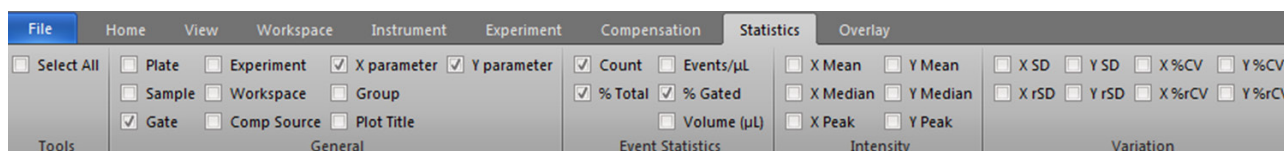


Note: The Statistics tab behaves differently (as explained below) depending on which Application tab is in focus or which object within a Workspace is selected. The entire Statistics tab is disabled when the *Heat Map view* is in focus (page 105).

Statistics tab behavior

Behavior in Workspace view

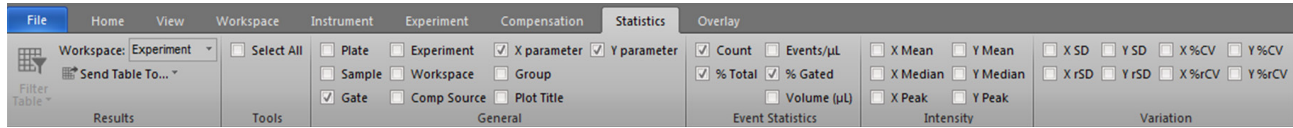
When the *Workspace view* (page 63) is in focus and a statistics table is **not** selected, only a single statistic can be selected at a time. This selection applies to the statistic displayed on all plots. In the Workspace view, the Results group (page 59) is not visible and the General group (page 60) is not enabled.



- **Plot statistics:** By default, all gates show the *% Gated* statistic. The selection persists on a per Workspace basis.
When a statistic is checked (i.e., selected), all plots with gates are automatically updated to display it. Selecting a new statistic automatically un-checks the previously selected one.
- **Statistics tables:** Both individual Plot statistics and Workspace statistics can be selected at the same time. When multiple statistics tables are selected, the selection and deselection of statistics in the ribbon applies to all tables.
Where a statistic is selected in one statistics table and not in another, the checkbox will be in the indeterminate state.
The selection or deselection of the Experiment name, Group name, Sample name, and plot title will also update the selections in the *Customize statistics* panel.

Behavior in Results view

- When the *Results view* (page 112) is in focus, all statistics can be checked and unchecked. The Results group (below) is only available when the Results view is in focus.



- A statistical value is displayed in the Results table when the associated checkbox is checked in the Statistics ribbon. Unchecking the checkbox removes the entire column from the Results table.

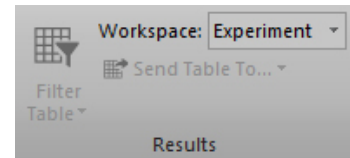
Persistence of selections

The persistence of selections in the Statistics tab is context dependent.

- For Workspace selections, the selections for on-plot statistics and for each statistics box persist on a Workspace level.
- For the Results view, all selections (including the filter selection) persist on an Experiment-level.

Results group

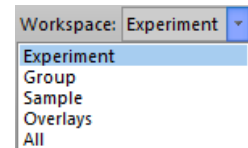
The *Results group* contains the controls for turning the *Results table filter* ON and OFF, editing the filter criteria, selecting where to send the *Results table* (page 113), and selecting the Workspace on which the Results table is based. The Results group is only visible when the Results view is in focus.



Workspace filter dropdown

The *Workspace filter* dropdown is used for selecting the Workspace on which the Results table will be based.

The options include **Experiment**, **Group**, **Sample**, and **All**. When **All** is selected, all options are displayed. By default the **Experiment** is selected.



Tools group

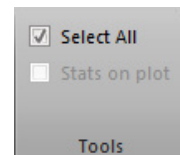
The *Tools group* allows all statistics to be enabled with a single click.

Select All: Enables all statistics. The Select All checkbox is enabled only when the Results view is in focus or if a Statistics box is selected on the Reports view or the Workspace view. The checkbox is disabled for all other instances.

When **Select All** is checked, then all statistics are checked.

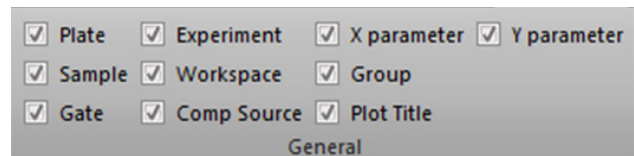
If any statistics are unchecked, Select All checkbox becomes unchecked.

Clicking the checkbox again will reselect all statistics. Deselecting the checkbox will uncheck all statistics.



General group

The statistics in the *General group*, when checked, are displayed on plots and in the Results table.

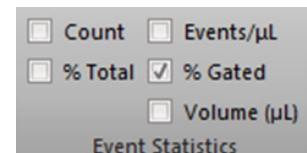


If a statistics table is not selected, this group is disabled on the Workspace view.

- **Plate:** Displays the Plate name (enabled for plate experiments only)
- **Experiment:** Displays the Experiment name.
- **Group:** Displays the Group name.
- **Sample:** Displays the Sample name.
- **Workspace:** Displays the Workspace from where the statistics is calculated.
- **Comp Source:** Displays the Compensation Source for the Results, either calculated in the Experiment or embedded with FCS files
- **Plot Title:** Displays the plot title for all gates.
- **Gate:** Displays the name of the gate for which the statistics is calculated.
- **X Parameter:** Displays the X parameter name as shown on the plot containing the gate.
- **Y Parameter:** Displays the Y parameter name as shown on the plot containing the gate. If the Y parameter is from a Histogram plot, *Count* or *% of max* is displayed as the parameter name, depending on what is selected for that plot.

Event Statistics group

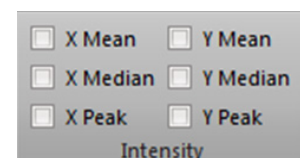
The statistics in the *Event Statistics group*, when checked, are displayed on plots and in the Results table.



- **Count:** Displays the number of events in gates.
- **% Total:** Displays the percentage of total events.
- **Events/μL (Concentration):** Displays the number of events per μL.
- **% Gated:** Displays the percentage of events within the gate.
- **Volume (μL):** Displays the volume of sample that the data is derived from.

Intensity group

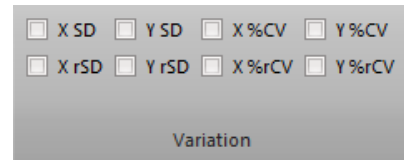
The statistics in the *Intensity group*, when checked, are displayed on plots and in the Results table.



- **X Mean:** Displays the arithmetic mean for the X-axis of a gate.
- **Y Mean:** Displays the arithmetic mean for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X Median:** Displays the median for the X-axis of the gate.
- **Y Median:** Displays the median for the Y-axis of the gate. When the gate is on a single-parameter plot the entry on the Results table is "N/A".
- **X Peak:** Displays the peak channel (mode) for the X-axis of the gate.
- **Y Peak:** Displays the peak channel (mode) for the Y-axis of the gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".

Variation group

The statistics in the *Variation group*, when checked, are displayed on plots and in the Results table. The Variation group contains the checkboxes listed below.



- **X SD:** Displays the standard deviation for the X-axis of a gate.
- **Y SD:** Displays the standard deviation for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X %CV:** Displays the %CV (percentage coefficient of variation) statistics for the X-axis of a gate.
- **Y %CV:** Displays the %CV (percentage coefficient of variation) statistics for the Y-axis of a gate. When the gate is on a single-parameter plot the entry on the Results table is "N/A".
- **X rSD:** Displays the robust standard deviation statistics for the X-axis of a gate.
- **Y rSD:** Displays the robust standard deviation statistics for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X %rCV:** Displays the percentage robust CV (coefficient of variation) statistics for the X-axis of a gate.
- **Y %rCV:** Displays the percentage robust CV (coefficient of variation) statistics for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".

Overlay tab

The *Overlay* ribbon tab contains the tools used for creating *Overlays*, which are used for comparing data by superimposing selected plots. Each *Overlay* consists of an *Overlay plot* and an associated *Overlay gallery* (page 117).



The *Overlay* tab contains three ribbon groups:

- *Create group*
- *Gating Tools group*
- *Galleries View group*



Note: The *Overlay* plots and the associated *Overlay* galleries are displayed in the *Overlay view* (page 117).

Create group

The *Create group* contains the *Overlay Builder* button.



- The **Overlay Builder** button launches the *Overlay Builder* (page 133), which allows you to select the samples and plots for inclusion in an *Overlay* plot.
- The *Overlay Builder* button is only enabled when at least one plot exists on the *Experiment Workspace* and the instrument is not acquiring.

Gating Tools group

The *Gating Tools group* contains the buttons used for inserting *Rectangle*, *Oval*, *Polygon*, *Quadrant*, and *Histogram* gates into *Overlay* plots. These buttons are only enabled if at least one *Overlay* plot exists.



Rectangular Gate: Allows you to draw a *Rectangular gate* on a dual-parameter plot.



Oval Gate: Allows you to draw an *Oval gate* on a dual-parameter.



Polygon Gate: Allows you to draw a *Polygon gate* on a dual-parameter.



Quadrant Gate: Allows you to draw a *Quadrant gate* on a dual-parameter.



Histogram Gate: Allows you to draw a *Histogram gate* on a *Histogram* plot.



Note: The buttons within the *Gating Tools* group are highlighted when selected, and revert to their standard color when you click in the *Overlay* plot to create the gate, select another button, or press the **ESC** key.

Galleries View group

The *Galleries View* group contains the *Wrap Galleries* button.

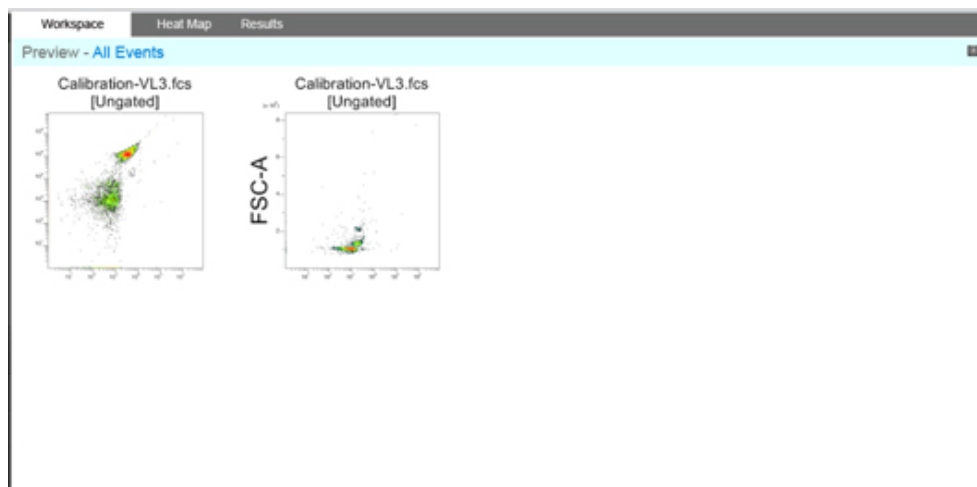
When **Wrap Galleries** is selected, the plots within the view all fit within the width of the gallery view area. If there are too many plots to be shown vertically, vertical scroll bars are shown.

When the gallery is wrapped, the *Wrap Galleries* button is highlighted in blue. The button is only enabled if the gallery plot view or the overlay view area is in focus.

5. Workspace view

Overview

The *Workspace view* displays the plots, gates, and statistics associated with the current sample, as well as text boxes and image files. It also contains the *Previews panel* (page 71), which is displayed by default as a minimized bar.



Workspace behavior

- The Workspace view is always the first tab of the *Main Application area* (page 28). It cannot be undocked from the main application and the associated ribbon bar (see “Docking behavior”, page 29).
- The Workspace view can display one of three independent workspaces, the *Sample Workspace*, *Group Workspace*, or the *Experiment Workspace* (page 64)
- The Workspace can be displayed in two different modes, *Freeform* or *Auto Layout* (page 68). These allow automatic or user-specified placement of objects on the Workspace depending on the mode selected.
- The Workspace acts as a single worksheet that is split into “pages” when printed. The page breaks can be visualized by selecting **Show print area** in the *View tab* (page 42).
- Changes to the Workspace are saved automatically if the active Workspace changes, if the program is exited, if the active sample changes, prior to printing, or if one minute has elapsed since the last Workspace save action.
- When a Compensation sample is active, all Workspace functionality is disabled except as specified in “Compensation”, page 283.
- The Workspace view will always move to the front of any docked panels at the beginning of acquisition for each sample. The Experiment Workspace will become active and the dropdown menu items will become disabled.



Note: The Previews panel automatically displays previews of all possible permutations of the Histogram and Precedence Density plots based on the parameters selected in the Workspace tab, and allows you to quickly add these plots to the Workspace as described in “Previews” on page 71.

Workspace selection

Workspace levels

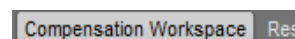
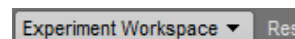
The Workspace view can display one of three independent workspaces, the *Sample Workspace*, *Group Workspace*, or the *Experiment Workspace*.

- Experiment Workspace is a Workspace that is common to all members of the current Experiment.
- Group Workspace, when created, is common to all members of the current Group.
- Sample Workspace is unique to each Sample.

Workspace view label

The Workspace view tab on the Main Application area is labeled according to the level of Workspace displayed: Sample Workspace, Group Workspace, or Experiment Workspace.

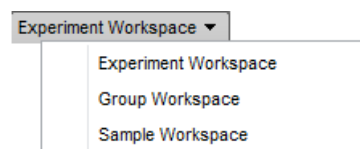
- When a Compensation sample is open, the Workspace is labeled “Compensation Workspace”, and the dropdown selection menu is not present.
- By default, the Experiment Workspace is displayed when the Sample is first displayed. Group and Sample-level Workspaces are created upon first use.



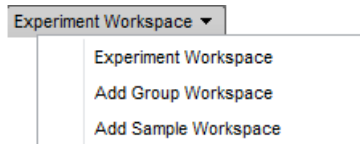
Workspace selection menu

- The *Workspace selection dropdown* menu on the Workspace view tab allows you to select level of Workspace displayed:

- Experiment Workspace
- Group Workspace
- Sample Workspace



- If the Group or Sample Workspaces do not exist, the workspace label is displayed as “Add Group Workspace” or “Add Sample Workspace”.



- If you select a Group Workspace, that Workspace is displayed. If a Group Workspace does not exist for the current Group, a default group-level Workspace is created.
- If you select the Sample Workspace option, the Sample Workspace for that sample is displayed. If a Sample Workspace does not exist for the current Sample, a default Sample-level Workspace is created.
- If you select the Experiment Workspace, the current Experiment Workspace is displayed.
- When a new Group Workspace is created, the Experiment Workspace is used as a template, and all plots, gates, and statistics are reproduced.
- When a new Sample Workspace is created, the Group Workspace is used as a template. If the Group Workspace does not exist, then the Sample Workspace will use the Experiment Workspace as a template.
- When a new Group or Sample Workspace is created, an indicator is displayed in the Experiment Explorer as described in “Experiment Explorer”, page 140.

Workspace objects

Creating Workspace objects

- The Workspace can display plots, gates, statistics, text boxes, and images (i.e., Workspace objects), all of which can be created using the options available on the *Workspace ribbon tab* (page 42).
You can also use the *Workspace view context menu* (page 90) to add the Workspace objects onto the Workspace.
- The *Plot context menu* (page 93) allows you to add plot-specific statistics.
- The *Previews panel* allows you to add plots to the Workspace as described on page 73.
- In the *Freeform* mode (page 68), you can add Workspace objects (i.e., plots, text boxes, images, or statistics) of any size to the Workspace. These objects can be freely resized and moved on the Workspace. There is no automatic arrangement or alignment of objects.
- In the *Auto Layout* mode (page 68), the Workspace is split into a number of grid slots. By default, new Workspace objects are placed into the next available grid space and are sized to one grid square.
- For single plots that are added using the Previews option (page 73) or the daughter plot option (page 96), plots are inserted at next available space of sufficient size after the last workspace object. Objects are created using the default size.

Selecting Workspace objects

- You can select Workspace objects by clicking within the **gray bounding rectangle** that is displayed when you move the mouse pointer over the object.

When you select a Workspace object by clicking within the gray bounding rectangle, it is displayed with a **light blue selection rectangle**.

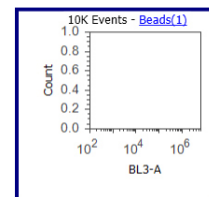
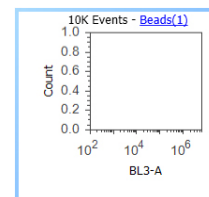
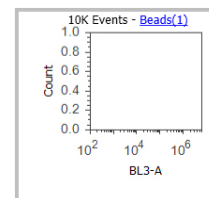
Non-selected Workspace objects do not have a selection rectangle displayed.

- You can select multiple objects within the Workspace by clicking on the desired Workspace objects while holding down the **Ctrl** key.

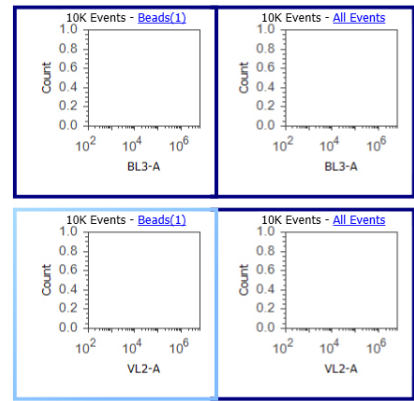
Alternatively, you can drag a selection rectangle to select several objects with the mouse.

Using the **Ctrl+A** key combination on the keyboard selects all Workspace objects on the current Workspace.

Objects which are part of the multi-select are bounded by **dark blue multi-select rectangles**.

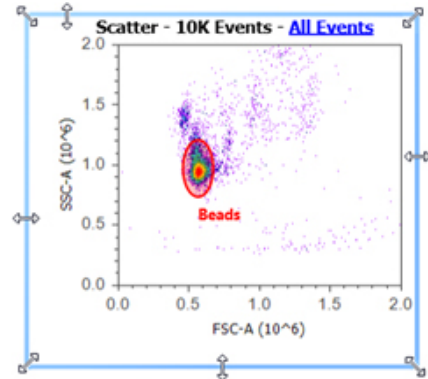


- When multiple objects are selected (either individually clicked or selected by dragging the selection rectangle), the last selected object remains light blue to designate it as the *reference item for align options*.
- The reference plot can be changed by pressing **Shift** key while selecting a plot. The selected plot shows the light blue colored bounding box.



Resizing Workspace objects

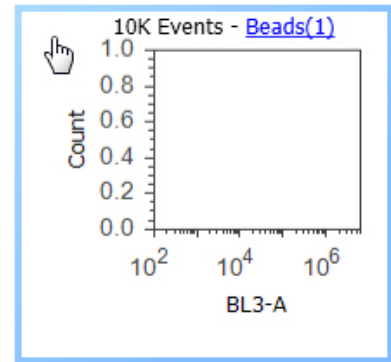
- You can resize single or multiple Workspace objects by selecting the object and then moving the mouse pointer over the edge of the bounding rectangle.
Vertical, horizontal, and diagonal resize cursors are displayed as you move the mouse pointer over the appropriate area.
The resize cursors are also displayed when the mouse pointer is hovered over the bounding rectangle of non-selected objects; when clicked, this selects the object and begins resizing.



- Once the resize cursor is displayed, the object can be dragged to resize in the direction indicated by the arrow cursor. All selected objects are resized in the same dimension and magnitude.
- Dual parameter plots and images always maintain their aspect ratio when resized.
Histograms can be stretched horizontally. They can be stretched vertically, but the width of the object must always be greater than or equal to its height.
Text boxes and statistics boxes can be stretched in any direction and any ratio.
- Pressing the **Shift** key while dragging diagonal resizing cursors resizes the selected object and maintains its aspect ratio.
- When multiple Workspace objects are selected, resizing options are determined by the object being manipulated. All other objects in the selection are bound by the resizing constraints described above.
- If mixed object types are selected when manipulating an object whose aspect ratio is not limited, histogram and dual parameter plots are resized within the aspect ratio constraints described above.
Where necessary, requirements for width take precedence over height to ensure that the resizing constraints are met.

Moving Workspace objects

- Moving the pointer over a Workspace object displays the *select cursor* in the shape of a pointing finger, except when the pointer is over a line or the select point of a gate. In such cases, *resize*, *move*, or *rotate cursors* are displayed as described on page 79.
- When the select cursor is displayed, you can click and drag single or multiple selected Workspace objects to a new position.



Deleting, formatting, and duplicating Workspace objects

Deleting Workspace objects:

- You can delete selected Workspace objects by pressing the **Delete** key on the keyboard.
- Alternatively, you can right-click on the object to open the *object context menu* and selecting the **Delete** option.

Formatting Workspace objects

- You can format the selected Workspace object using the *Customize panel options* (page 200).
- Alternatively, you can right-click on the object to open the object context menu and selecting the desired formatting option.

Duplicating Workspace objects

- You can duplicate Workspace objects by selecting one or more objects and dragging them while pressing the **Ctrl** key on the keyboard.
- Objects can also be duplicated using the keyboard combinations **Ctrl+C** (copy) followed by **Ctrl+V** (paste).
- Alternatively, you can copy the object using the **Copy** command on the object's context menu and then pasting it using the keyboard combination **Ctrl+V**.

Pasting Workspace objects

- Selecting the **Ctrl+V** keyboard combination pastes the object at same location as the original. As each new object is pasted, it is inserted cascading downwards and to the right of the original location.
- Objects can be cut or copied from any Workspace and pasted to any other Workspace.
- Plots are pasted with any gates present. If a gated plot is copied to a new location where the parent gate does not exist, the pasted plot will not be gated and will display all events.
- If a Plot statistics box is pasted to a new Workspace and the target Workspace does not contain a copy of the original plot, the statistics box will update to show all gates present in the current Workspace. The Plot axis and Gate fields will be removed from the statistics box header (if originally present).
- Pasted objects are selected by default after the paste action has completed.

Workspace view layout

Resizing the Workspace view

There are two ways to resize the display of the Workspace view:

- Turning the **mouse scroll wheel** while holding down the **Ctrl** key zooms in or out of the page when the mouse cursor is hovered over the Workspace or when the Workspace is in focus.
- The **Size slider** on the *Application status bar* (page 37) can be used to resize the Workspace view when the Workspace is in focus.

Workspace view modes

The Workspace can be displayed in two different modes, *Freeform* or *Auto Layout*. These allow automatic or user-specified placement of objects on the Workspace depending on the mode selected.

The Workspace view mode is controlled by the **Layout radio buttons** available on the Workspace ribbon tab (page 47).

Freeform mode

Freeform mode allows you to add plots, text boxes, images, or statistics of any size anywhere on the Workspace. These objects can be freely resized and moved on the Workspace. There is no automatic arrangement or alignment of objects.

- To add a custom-size object to the Workspace, select the desired object type from the Workspace ribbon tab (page 42) and then draw the area in the Workspace where you want to insert the selected object. When you let go of the mouse button, the object is inserted into the area drawn.
- Selecting an object type and then single-clicking on the Workspace places the default-sized object at the position clicked.

The default size for new plots, statistics boxes, and text boxes in the Freeform mode is 240 × 240 pixels with objects arranged in 3 columns × 4 rows per page.

- In this mode, objects can be moved and positioned anywhere within the Workspace, and they can be positioned across multiple pages.
- You cannot print a Workspace in the Freeform mode.

Auto Layout mode

In the Auto Layout mode, the Workspace is split into a number of *grid slots*, which contain the Workspace objects of fixed size and placement. Auto Layout is the default option.

Layout behavior

- Only one object can appear in each grid slot. By default, new Workspace objects are placed into the next available grid space and are sized to one grid square.
- When switching from Freeform to Auto Layout mode, any objects present on the Workspace are placed into grid positions in the following order:
 - Plots based in Workspace insertion order
 - Plots statistics based on Plot insertion order
 - Workspace statistics
 - Text boxes
 - Images
- When switching from Auto Layout to Freeform mode, objects remain in their current position.
- The Workspace is initially setup as one page view. Additional pages are added automatically as existing pages are filled and as the Workspace zoom is changed.

Grid size

- The number of available grid slots per Workspace page is defined by **Grid Size** options on the Workspace ribbon tab (page 47).
- By default the grid size is set to 12 grid slots per page.
- You can select a larger grid size allowing only 4 items per page, or a smaller grid size allowing up to 20 items per page at the default sizes.

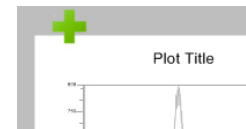
1	2	3
4	5	6
7	8	9
10	11	12

Object size

- By default, objects on Workspace are sized to one grid square when the Workspace view is in the Auto Layout mode.
- Objects on the Workspace may be resized, but they have limited resize options. When an object is resized, the top left corner of the object is pinned to its current position. Objects cannot be resized across pages or resized to occupy more than one page
- The resize options available for various Workspace objects are listed below, where the sizes are defined as (Rows × Columns).
 - **Dual parameter plots** are constrained to remain square and can be resized to take occupy 1, 4 (2 × 2), 9 (3 × 3) or 16 (4 × 4) squares.
 - **Histogram plots** are either constrained to remain square and occupy 1, 4 (2 × 2), 9 (3 × 3), or 16 (4 × 4) grid spaces, or they can be made rectangular and occupy 2 (1 × 2), 3 (1 × 3), 4 (1 × 4 or 2 × 2), 6 (2 × 3), 8 (2 × 4), 12 (3 × 4), or 16 (4 × 4) squares.
 - **Statistics boxes and images** may be resized up to the maximum number of grids (5 × 4) per page. For images, the aspect ratio will be maintained within the grid container(s).
- When increasing grid sizes (from more slots per page to less slots per page) or changing page orientation, any object that exceed the allotted slots in the horizontal and/or vertical direction(s) is resized to 1 × 1.

Moving objects in Auto Layout mode

- Object arrangement precedence is dictated by grid position and page number. The position with the highest precedence is the top left corner of the first page, and the precedence decreases from left to right, top to bottom.
- The grid position of objects larger than 1 × 1 is dictated by the top, leftmost position occupied by that object.
- When moving an object, a gray box indicates the grid location into which the object will be inserted or expanded.
- When multiple objects are selected, only the object with the highest precedence is displayed, and a plus icon indicates that multiple objects have been selected.
- If the objects have been selected using the Ctrl+click method, then the first selected object is displayed while dragging.
- If the objects have been selected by dragging a selection area, then the object with the highest precedence based on Workspace location (top to bottom, left to right) is displayed.
- Once objects have been resized or moved, the selection indicators are maintained on the selected objects.



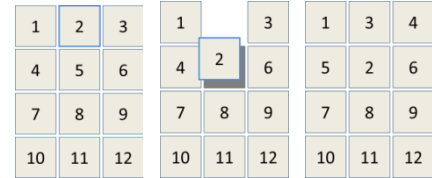
Rules for rearranging objects in Auto Layout mode

When a resized object causes another object to be displaced or a deleted object results in a blank grid slot, the objects on the Workspace are rearranged according to the rules listed below.

1. The displacing object(s) are always inserted in the position indicated by the gray box.
2. Displaced objects are always placed in the highest precedence available grid position as defined above.
3. The size of the object cannot span more than one page
4. Objects will always fill blank positions whenever possible.

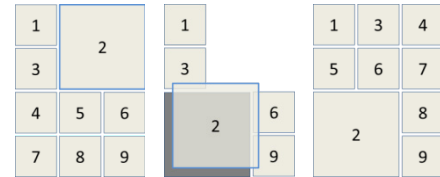
- **Example 1:** Plot 2 is a dot plot and it is dragged over Plot 5.

Plot 3 moves to fill position 2, Plot 4 moves to fill position 3, and Plot 5 moves to fill position 4. Plot 2 takes the position of Plot 5.



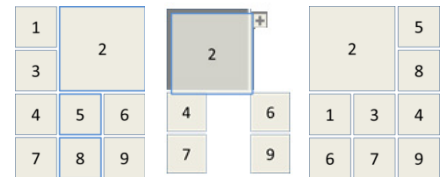
- **Example 2:** Plot 2 is a dot plot occupying 4 grid positions and it is dragged over Plot 4.

Plots 3, 4, 5, 6, 7, 8, and 9 move to refill the displaced space.



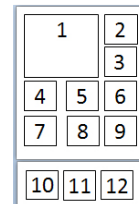
- **Example 3:** Plot 2 (a dot plot occupying 4 grid positions) along with Plots 5 and 8 are dragged over position 1.

Plots 1, 3, 4, 6, 7, and 9 move to refill the displaced spaces.



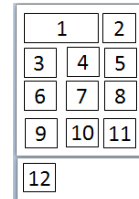
- **Example 4:** Plot 1 is a dot plot and it is resized to be 4 grid slots.

Plots 2 and 3 automatically re-arrange to optimally fill the space. Plots 10-12 are pushed onto the next page, which appears below the current page.



- **Example 5:** Plot 1 is a histogram and it is resized to 2 grid slots.

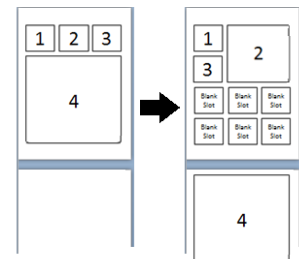
Plots automatically re-arrange as required, and Plot 12 is pushed onto the next page, which appears below the current page.



- **Example 6:** Plot 2 is dot plot and it is resized to be 4 grid slots.

Plots automatically re-arrange as required, and Plot 4 is pushed onto the next page, which appears below the current page.

Because there are no other plots available to occupy them, blank slot(s) is left on the original page.



Previews



Overview

The *Previews panel* of the Workspace displays all permutations of Histogram (page 75) and Precedence Density plots (page 76) based on the parameters selected in the *Workspace ribbon tab* (page 44). This provides an easy way of adding plots to the current Workspace.

Accessing the Previews panel

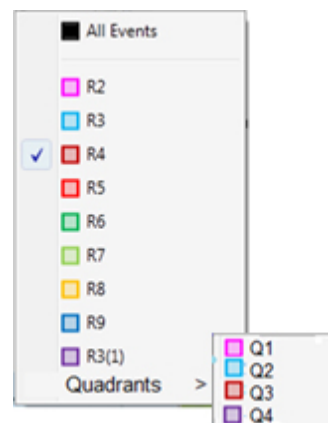
The Previews panel is located at the top portion of the Workspace view. By default, the panel is displayed as a minimized bar.



- When opened, the Previews panel occupies 50% of the vertical space of the Workspace view. The size of the panel cannot be changed.
- The Previews panel cannot be opened during acquisition. The panel automatically closes when **Tube** or **Plate acquisition** buttons are selected on the *Collection panel* (page 159).
- The Previews panel is disabled in the Compensation Workspace.
- When the Previews panel is minimized, the *Expand button* is shown in the collapsed panel. The panel can be expanded by clicking anywhere, except for the *gate hyperlink* on the title bar of the collapsed Previews panel. The expanded setting of the Previews panel does not persist between user sessions. 
- When the Previews panel is expanded, the *Collapse button* is shown on the panel. The panel can be collapsed by clicking anywhere on the title bar, except for the gate hyperlink of the expanded Preview panel or when the panel is closed automatically as described above. 
- When the Previews panel is disabled during acquisition or in the Compensation Workspace, the hyperlink control and the Expand/Collapse buttons are also disabled.

Previews panel title bar

- The Previews panel title bar displays “Previews – <Gate Name> <Gate Color>” where <Gate Name> is the name of the selected gate used to filter the Preview plots. The gate name is a hyperlink control.
- Clicking on the **Gate Name** and/or **Gate Color** on the Previews panel title bar opens a menu of all available gates and an option for **All Events** at the top of the list. Quadrant gates are shown in a submenu under the title **Quadrants**.



Preview plots

Layout of Preview plots

- The preview plots are arranged in a grid organized such that all plots share the same X parameter within a column and the same Y parameter within a row.



- The single-parameter Histogram plots are displayed in the first row.
- Dual-parameter plots show all possible combinations of available parameters.
- The plot order is based on parameter order, and starts with the first parameter in Column 1, Row 1 and ends with the Nth parameter in Column N, Row N.
- Self vs. Self dual parameter plots are not shown in the Preview panel, and are represented by white space that forms a diagonal from the top left to the bottom right.
- The default scale for Preview plots is set to logarithmic

Resizing Preview plots

- The **Size slider** on the Application status bar (page 37) can be used to resize the Preview plots when the Previews panel is in focus.
- Alternatively, turning the **mouse scroll wheel** while holding down the **Ctrl** key resizes the Preview.

Adding plots to Workspace – single plot selection

- Plots are added sequentially to the currently displayed Workspace and their inserted position depends on the Workspace view mode (page 68).
- Plots may be added to the Workspace multiple times.
- The indicator for plot addition persists with the Workspace.
- Single clicking a plot adds it to the Workspace in the order described in “Creating Workspace objects”, page 65.

Adding plots to Workspace – group selection

Column header selection buttons

- Each column in the Previews area has an *Add column* button labeled with the parameter name positioned at the top of the column as a column header.
- By default, the parameter name is specified in the *Options dialog* (page 328).
- Clicking **Add column** adds the entire column of plots (excluding Histograms) to the Workspace area as described in “Creating Workspace objects”, page 65.

Row header selection buttons

- Each column in the Previews area has an *Add row* button labeled with the parameter name positioned at the beginning of the row as a row header.
- By default, the parameter name is specified in the *Options dialog* (page 328).
- Clicking **Add row** adds the entire row of plots (both Histograms and dual-parameter plots) to the Workspace area as described in “Creating Workspace objects”, page 65.

Select All split button

- Positioned at the top left corner of the Previews area is a split button, which can add the plots in either the upper right triangle or lower left triangle of the Previews area to the Workspace.
- Clicking the **top right** button adds the plots in the upper right triangle.
- Clicking the **lower left** button adds the plots in the lower right triangle.
- Plots are added to the Workspace as described in “Creating Workspace objects”, page 65.

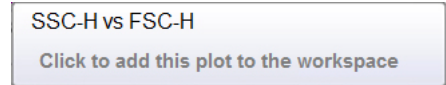
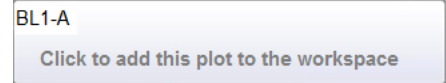
Mouse over behavior

Mouse over plot

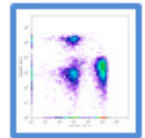
- When the mouse pointer is hovered over a plot, a tooltip is displayed with the plot name and required action to add the plot to the Workspace.

For single-parameter plots, the tooltip includes the X-parameter label.

For dual parameter plots the tooltip includes the X-parameter and Y-parameter labels in the format <Y Parameter> vs <X Parameter>.

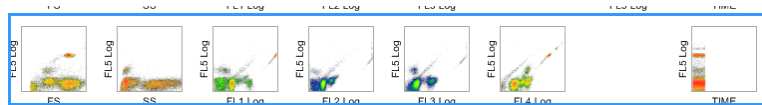


- When the mouse pointer is hovered over a plot, a bounding rectangle as described in "Selecting Workspace objects" on page 65 is shown around the plot.

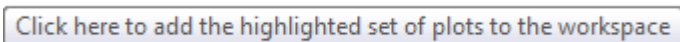


Mouse over buttons

- When the mouse pointer is hovered over *group selection* buttons (i.e., column header selection, row header selection, or the *Select All* split buttons; page 73), the plots that will be added when the button is clicked are highlighted in an outline as described in "Selecting Workspace objects" on page 65.



- All group selection buttons display the following tooltip when the mouse pointer is hovered over them:



Plots

Overview

The Attune™ NxT Software can display *Histogram*, *Dot*, *Density*, and *Precedence Density plots* on the Workspace.

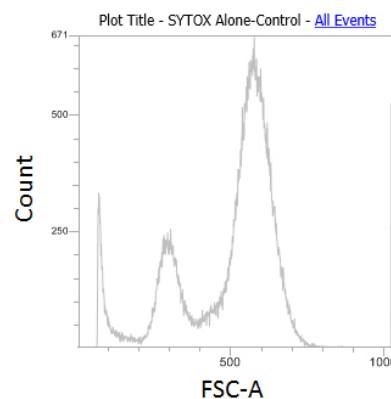
- Plots are created using the Plots group options present on the *Workspace ribbon tab* (page 44). The buttons within the Plots group are enabled when the Workspace view is in focus and at least two parameters are active, except for the Histogram plot button, which requires only one active parameter.
- Plot options such as titles, axis labels, axis scaling, resolution, and colors used to display data points can be customized using the *Customize panel* (page 201). Available customization options depend on the plot type selected.
- Default plot options are set using the *Options dialog* (page 331).
- Dual-parameter plots (i.e., Dot, Density, and Precedence Density plots) display FSC values on the x-axis and SSC values on the y-axis by default. Histogram plots display FSC values on the x-axis by default.
- If FSC and/or SSC parameters are not selected for collection, the next parameter selected in the *Parameters* section of the *Instrument Settings panel* (page 180) is used. The *Time* and *Event* parameters are only used if no other parameters are being collected.
- If the parameters that exist in the Workspace are disabled prior to recording data, a watermark on the plot states “Parameter(s) not available in dataset”. Any daughter plot also displays the watermark. The watermark will not appear until data is displayed/collected on the Workspace.
- Gates can be manipulated on top of the watermark. The plot scales are set to the last seen valid scale. Gate statistics from plots that do not have available parameters are displayed as “N/A”.

Histogram plot

A Histogram plot is a graphical representation of single-parameter data and shows the relative number and distribution of events.

In a Histogram plot, the horizontal axis corresponds to the signal intensity of the selected parameter while the vertical axis represents the number of events (count).

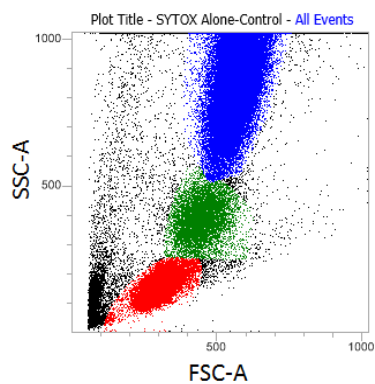
- Plot customization options available for Histograms are Resolution, Normalize Count, Use Shading, and Line Width.
- The maximum plot resolution is 1024 channels, which is also the default resolution.
- The default line color for an ungated plot is set according to the selection in *Plot Options* in the *Options dialog* (page 331).
- The line color for a histogram plot that is based on a parent gate is determined by the parent gate’s color.



Dot plot

A Dot plot is a graphical representation of two-parameter data where each axis represents the signal intensity of one parameter. Each dot in the plot corresponds to one or more events detected above the threshold. Different colors are used to represent the parent gate of events that fall within bins on the plots.

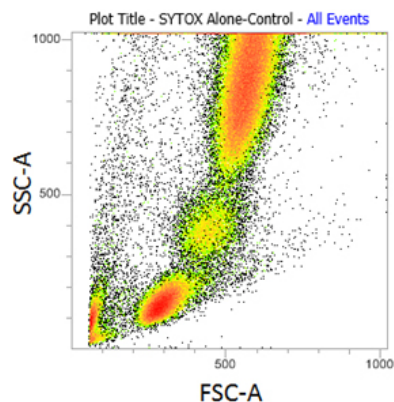
- Plot customization options available for Dot plots are Resolution and % of Events displayed.
- The maximum plot resolution is 1024 × 1024 channels. The default plot resolution is set at 256 × 256 channels.
- Where an event may be in multiple gates, the precedence order determines which color is displayed. Where events from multiple gates fall within a single bin, the highest precedence gate color is displayed.
- Ungated events are painted black by default. The default dot color for ungated events can be modified in *Plot Options* in the *Options dialog* (page 331).



Density plot

A Density plot is a graphical representation of two-parameter data where the colors represent the collection of events with the same intensity and each axis represents the signal intensity of one parameter.

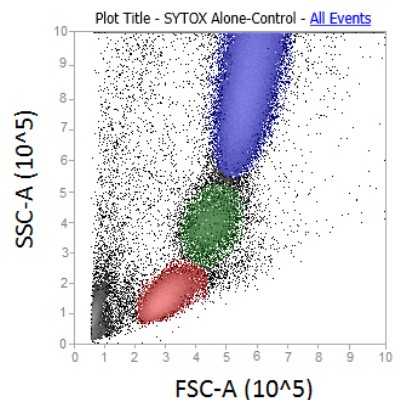
- Plot customization options available for Density plots are Resolution, Color, Color Mode, and % of Events displayed.
- The maximum plot resolution is 1024 × 1024 channels. The default plot resolution is set at 256 × 256 channels.



Precedence Density plot

A Precedence Density plot uses a combination of Dot and Density display, where a gradient indicates the number of events within each of the plot bins and color is used to display the parent gate of events present.

- Plot customization options available for Precedence Density plots are Resolution, Color Mode, and % of Events displayed.
- The maximum dot plot resolution is 1024 × 1024 channels. The default plot resolution is set at 256 × 256 channels.



Gates

Overview

Gates allow you to identify and analyze subsets of data (i.e., populations) by isolating a region in a selected plot. They can also be used to limit the number of events collected or stored.

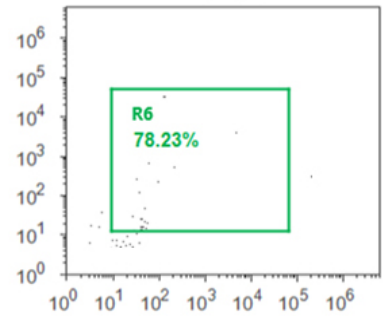
After defining gates, you can combine them to create gates based on Boolean operators (i.e., *Derived gates*). You can use gated populations to generate statistics, display them in a hierarchal view, and create subsets within defined populations (i.e., *daughter plots* and associated statistics).

Creating and deleting gates

- You can create gates on a selected plot using the *Gating Tools* available on the Workspace ribbon tab (page 45).
Alternatively, you can use the *Plot context menu* (page 94) to create gates.
- The following types of gates can be added on plots on a Workspace:
 - **Rectangular gate** (page 80)
 - **Oval gate** (page 81)
 - **Polygon gate** (page 82)
 - **Quadrant gate** (page 83)
 - **Histogram gate** (page 83)
 - **Derived gate** (page 84)
- Gate creation defaults are set in *Gate Options* in the *Options dialog* (page 329).
- You can create a maximum of 128 gates on plots on a Workspace, except for Quadrant gates, of which at least 1000 may be created.
- You can create a maximum of 128 *gating equations*. A gating equation is generated when a new rectangle, polygonal, oval, or histogram gate is created, or when the Derived Gate option is used to create a compound gate equation.
- The maximum number of gates present in an individual gate equation is 28.
- When the Workspace reaches the maximum number of gates allowed, the relevant gate creation buttons on the Workspace ribbon tab and the options on the Plot context menu become disabled.
- To delete a gate, select it, and press the **Delete** key on the keyboard or the **Delete** button on the *Home* tab (page 41).
- When a gate is deleted, any Derived Gate that includes the deleted gate is modified to exclude the deleted gate.
If the gate equation includes only the deleted gate, then the entire gate equation is deleted.
- Gate name, appearance, and position can be customized using the *Customize panel* as described in “Customize Gate options” (page 220).

Gate name

- By default, each new gate is automatically assigned a unique name, which consists of the letter R followed a number. The number increases incrementally for each new gate created, ensuring that each gate has a unique name (e.g., R1, R2,...).
- For Quadrant gates, the default gate name consists of the letter Q followed by an incrementally increasing number until a unique gate name is achieved (e.g., Q1, Q2,...).
- The gate name and the associated statistic (optional; see below) are displayed adjacent to the gate on the relevant plot on separate lines. The font size optionally auto-adjusts to optimally display the text.
- The display of a statistic is selected on the *Statistics ribbon tab* (page 58) along with the desired statistic. Only one statistic can be shown and this applies to all plots. If a statistical value cannot be calculated, "N/A" will be displayed instead of a numerical value.
- You can change the automatically assigned gate name by manually entering a name in the *Name* field in the *Customize* panel or selecting one of the pre-defined names from the **Quick Select** dropdown menu (page 223).
Alternatively, you can double-click the name of a selected gate to enter the name on plot edit mode
- You can reposition the gate name by first selecting the gate, and then placing the cursor over the name. When the cursor changes to the standard precision select pointer (i.e., cross-hair), you can click and drag the gate name to a new position. You cannot drag the gate name outside the plot.
The gate name can be moved independently of the gate, but moving the gate keeps the gate name in the same relative position.



Gate color

- When a gate is created, the gate color is automatically assigned.
- There are ten default colors defined for gates. As a new gate is created, the next available color is used. When an eleventh gate is created, the first color will be re-used. This process continues as each new gate is created.
- The gate color and the opacity of the fill color can be customized using the *Customize panel* as described in "Customize Gate options" (page 220).

Moving and resizing gates

You can move and resize any gate. When a gate is moved or resized, and all populations derived from that gate is affected and statistics are automatically updated.

- Clicking on a gate to select it displays the *control points*, which are used for resizing or rotating the gate.
- When you move the mouse over the gate or the handle, the cursor will change its shape to indicate its action mode (move, resize, or rotate) as shown below.



Move: Used to move the selected object in any direction.



Resize: Used to resize a single dimension (vertical or horizontal).



Diagonal resize: Used to resize two dimensions simultaneously.



Rotate: Used for freely rotating the selected object around a pivot point.


- To move a selected gate, grab it between the handles as indicated by the Move cursor, and then drag it to a new position. For opaque gates, you can also grab the center area of the gate to move it.
- A Quadrant gate (page 83) displays a move cursor over the *intercept point*. For opaque quadrants, the center is not selectable for moving the gate.
- You cannot move a gate or quadrant intercept point outside of the plot axes, although a gate may exist outside the visible plot area, if an area that does not include the location of the gate was zoomed on the plot.



Note: It is also possible for a gate to exist outside of the plot limits, if the scale type or scale range of a plot has been modified after the gate has been defined (see “Off-plot Gates”, page 85).

- When the rotate icon is displayed on a selected gate, you can rotate the gate by dragging the indicated rotate control point.
- When a control point of a selected gate is being moved, the following X and Y coordinates are displayed in the *Application status bar* (page 35):
 - **Polygon gate:** Coordinates of the point being moved are displayed.
 - **Histogram gate:** Coordinates of the point being moved are displayed.
 - **Rectangular gate:** Coordinates of the point being moved are displayed.
If moving the top or right boundary line, the coordinates for the upper right control point are displayed.
If moving the lower or left boundary line, the coordinates of the lower left control point are displayed.
 - **Oval gate:** Coordinates of the center point are displayed.
 - **Quadrant gate:** Coordinates of the intercept are displayed.
- When moving an entire Rectangular, Polygon, or Oval gate, or a broken quadrant, no coordinates are displayed.

Rectangular gate

You can insert a *Rectangular gate* into a selected dual parameter plot using the **Rectangular gate** button on the Workspace ribbon tab (page 45). 

Alternatively, you can select **Add Gate ► Rectangular** from the Plot context menu (page 94).

- To insert the gate, click the **Rectangular gate** button to select and then click on the desired dual parameter plot, which defines a corner of the rectangle.

To draw the gate, drag out a rectangle from this point.

- When creating a Rectangular gate, the Application status bar displays the X and Y coordinates of the point being dragged.
- Selecting the **Rectangular gate** button and clicking on a dual parameter plot inserts a default sized Rectangular gate centered on the point where the plot was clicked.

The width of the Rectangular gate is 40% of the plot width and its default height is 20% of the plot width.

- On a single parameter plot, a *no entry cursor* (⊘) indicates that a Rectangular gate cannot be created.
- Rectangular gates have eight control points.
- The center of each line forming the rectangle may be dragged perpendicular to the line, allowing resizing of the gate.
- The four corners of the rectangle may be dragged, with the opposite corner being the anchored point. Its new position is determined by the rectangle formed by the anchored point and the final position where the mouse button is released.
- Dragging on the rectangle, but not on a control point, allows the whole gate to be dragged while maintaining its shape. For opaque gates, the center area is also selectable for moving the gate.



Oval gate

You can insert an *Oval gate* into a selected dual parameter plot using the **Oval gate** button on the Workspace ribbon tab (page 45).



Alternatively, you can select **Add Gate ► Oval** from the Plot context menu (page 94).

- To insert the gate, click the **Oval gate** button to select and then click on the desired dual parameter plot, which defines a corner of the bounding box for the ellipse. To draw the gate, drag out an ellipse from this point.
- When an Oval gate is being created, the Application status bar displays the X and Y coordinates of the upper right corner of the bounding rectangle.
- Selecting the **Oval gate** button and clicking on a dual parameter plot inserts a default sized Oval gate centered on the point where the plot was clicked. The default width of the Oval gate is 40% of the plot width and its default height is 20% of the plot height.

The default Oval gate is created with the major axis horizontal.

- On a single parameter plot, a no entry cursor (⊘) indicates that an Oval gate cannot be created.
- Oval gates have four control points. These represent the major and minor axis of the ellipse.
- Dragging the control point in the direction of an axis resizes that axis.
- Dragging the rotation indicator point rotates the gate.
- Dragging on the ellipse, but not on a control point, allows the whole gate to be dragged while maintaining its shape.



For opaque gates, the center area is also selectable for moving the gate.

Polygon gate

You can insert a *Polygon gate* into a selected dual parameter plot using the **Polygon gate** button on the Workspace ribbon tab (page 45).

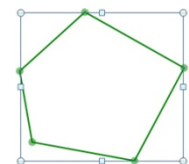


Alternatively, you can select **Add Gate ► Polygon** from the Plot context menu (page 94).

- To insert the gate, click the **Polygon gate** button to select and then click on the desired dual parameter plot, which determines where the first point of the gate is placed. Continue to click on the plot to add additional points to the polygon to draw the gate around the population of interest. To complete the process, click on the original point or double-click when inserting the last point.
- If there are at least three points, you can also complete the polygon by right clicking, which adds a line from the last to first point.

If you right click when only two points have been defined, the gate is removed from the plot.

- The maximum allowable number of defined points on a Polygon gate is 40.
- When setting the initial point of the polygon, a no-entry cursor (⊘) is displayed when hovering outside a plot window. On a single parameter plot, a no entry cursor indicates that a Polygon gate cannot be created.
- When a Polygon gate is being drawn, the Application status bar displays the X and Y coordinates of the cursor as each point is created.
- When creating a point of the polygon (other than the initial point), if you move the cursor outside of the plot but still remaining within the application window (or child windows), a precision select pointer (i.e., cross-hair) continues to be displayed. If you click while the cursor is outside the plot, a control point is created at the coordinate closest to the clicked point.
- If you click outside the application window (or application child windows), the current gate being created is removed and the create mode is cancelled.
- Clicking when the no-entry cursor is displayed to create the first point of the polygon cancels the create mode without creating the point.
- Each vertex of the polygon is a control point. A bounding rectangle is displayed when the completed Polygon gate is selected.
- Dragging a vertex moves that vertex only within the polygon.
- Dragging on a line within the polygon or the bounding rectangle moves the gate while maintaining the shape of the gate.
- Double clicking on a line within the polygon inserts a new vertex.
- The line forming the bounding rectangle may be dragged perpendicular to the line, allowing resizing of the gate.
- The four corners of the bounding rectangle may be dragged, with the opposite corner being the anchored point.
- Dragging the bounding rectangle, but not on a vertex control point, or dragging the line between vertex control points allows the whole gate to be moved while maintaining its shape. For opaque gates, the center of the Polygon gate is also selectable for moving the gate
- The default Polygon gate is defined by a bounding rectangle with 5 control points in the center of the plot. The width of the default bounding rectangle is 40% of the plot width and its default height is 20% of the plot height.



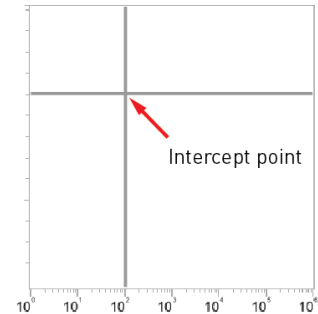
Quadrant gate

You can insert a Quadrant gate into a selected dual parameter plot using the **Quadrant gate** button on the Workspace ribbon tab (page 45).



Alternatively, you can select **Add Gate ► Quadrant** from the Plot context menu (page 94).

- Only a single Quadrant gate can be created on a plot.
- Quadrant gates are formed from two divider lines (one vertical, one horizontal). To insert the gate, click the **Quadrant gate** button, then click on the desired dual parameter plot, which determines where the quadrant intercept point is placed.
- On a single parameter plot, a no entry cursor (⊘) indicates that a Quadrant gate cannot be created.
- Once you have created the gate, you can edit the placement of the gate using the control point displayed on the quadrant intercept.
- The quadrant intercept cannot be moved outside of the plot axes.
- While a control point is being moved, the X and Y coordinates of the intercept point are displayed in the status bar.



Histogram gate

You can insert a Histogram gate into a selected single parameter plot using the **Histogram gate** button on the Workspace ribbon tab (page 45).

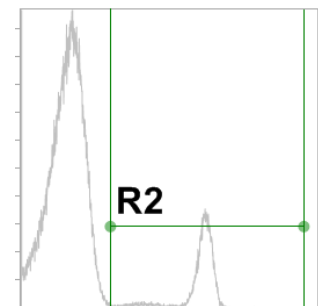


Alternatively, you can select **Add Gate ► Histogram** from the Plot context menu (page 94).

- To insert the gate, click the **Histogram gate** button, then click on the desired single parameter plot and drag out a line to determine the full gate size.
- When a Histogram gate is being created, the Application status bar displays the X and Y coordinates of the point being dragged.
- Selecting the Histogram gate button and clicking on a single parameter plot inserts a default sized Histogram gate centered on the point where the plot was clicked.

The default width of the Histogram gate is 40% of the plot width. Full height vertical lines are used to mark the upper and lower limits of the gate.

- On a dual parameter plot, a no entry cursor (⊘) indicates that a Histogram gate cannot be created.
 - Histogram gates have control points situated at each end of the gate. When the gate is selected, control points are displayed on each boundary.
 - Dragging the control points or the vertical boundary line moves the end points in the direction of the appropriate axis.
- Dragging the horizontal line, but not on a control point, allows the whole gate to be moved.



- The Histogram gate cannot be moved outside the plot axes. For opaque gates, the center is also selectable for moving the gate.
- When required, the default position of the Histogram gate is the center point of the gate set to the center of the plot.

Derived gate

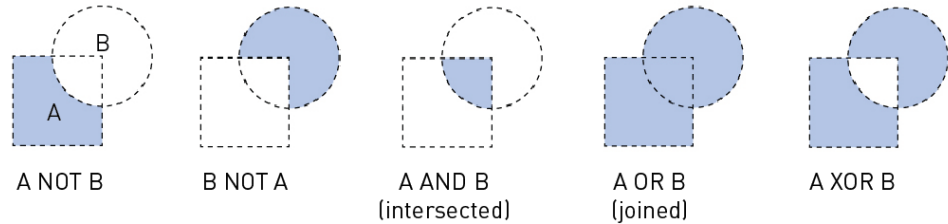
Derived gates are gates based on Boolean operators applied to existing gates. You can create a derived gate as long as there is at least one gate present in the Workspace, with any or no plot selected.

- You can create a Derived gate through the *Derived Gate dialog* (page 319). To open the Derived Gate dialog, click the **Derived Gate** button on the Workspace ribbon tab (page 45).



Alternatively, you can select **Add Gate ► Derived Gate** from the Plot context menu (page 94).

- The Boolean operators available for creating Derived gates are blank, AND, OR, XOR, AND NOT, and OR NOT.

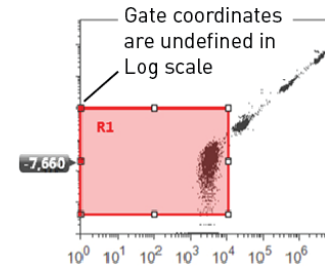
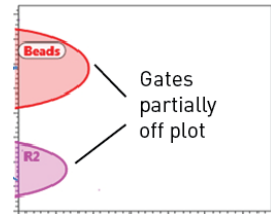


- For more information on creating Derived gates, see “Derived Gate dialog” on page 319.

Off-plot gates

It is also possible for a gate to exist outside of the plot limits, if the scale type or scale range of a plot has been modified after the gate has been defined.

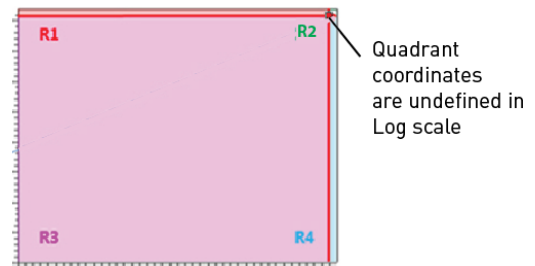
- If the gate is partially off the visible plot scale range and the gate coordinates exist within the plot scale type, the displayed gate is clipped at the plot axis or plot boundary.
- If the gate is partially off the visible plot scale range and the gate coordinates do not exist within the plot scale type (e.g, changing from Linear scale to Log scale with negative coordinates), the displayed gate is shown with the undefined gate coordinates and the vertices depicting the undefined coordinates are filled with red.



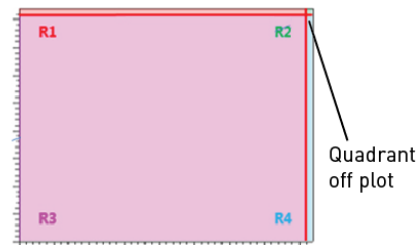
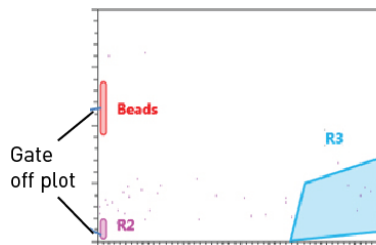
Hovering over the vertices depicting undefined coordinates displays a tooltip that states

“Coordinate exists beyond the limits of the selected scale type”. These vertices are always shown until the gate is moved so that all vertices exist within the defined space or the scale type is changed to allow these points to be displayed in the plot.

- For a Quadrant gate, the quadrant is displayed away from the plot axis so that the presence of the off-plot gate can clearly be seen and a single intercept point depicting the undefined coordinates is filled with red.



- Dragging the gate away from plot axis moves the gate into the defined space.
- If the gate is completely off plot, the gate is displayed adjacent to the plot axis with a default size for the off plot parameter, so that the presence of the off-plot gate can be ascertained. A tooltip for the off-plot gate will display “Double click to expand range.”



- Double clicking a gate that is either partially or completely off-scale expands the plot scale range as required, so that the gate is displayed on scale.
- If the gate is off-plot because the gate coordinates do not exist within the scale type, double clicking the gate sets the gate to the default position (center of the gate, set to the center of the plot) within the selected scale type. For a Quadrant gate, the intercept point will be set to the center of the plot. A broken quadrant will be reset to a standard quadrant set to the center of the plot.

Gate behavior

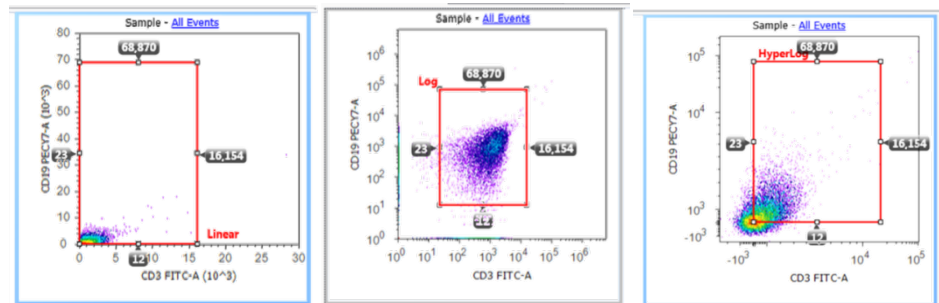
When changing plot parameter number or range, the gate is resized or deleted according to the rules described below. When the plot scale range, scale type, axes' parameters, or plot type is changed, the gates are resized or deleted as described below.

Changing Plot Scale Range

- If the plot scale range is changed on any axis, all gates remain fixed in their specified locations.
- If the change in scale range results in a gate being off plot, the gate behaves as described above in "Off-plot gates", page 85.

Changing Plot Scale Type

- If the plot scale type is changed (linear, log, HyperLog™) on any axis, the coordinates of the vertices for all gates (excluding oval gates) remain fixed and their connecting lines are redrawn as necessary.



- For oval gates, the coordinates of the bounding rectangle remain fixed and the perimeter ellipse and center position are redrawn as necessary.
- If the change in scale type results in a gate being off plot, the gate behaves as described above in "Off-plot gates", page 85.

Changing Plot Parameter

- When the parameter of any axis is changed, including using the *Swap Axes* option in the Plot context menu (page 95), all gates are maintained on the plot in their specified locations.
- If the change in parameter results in a change in scale type or scale range, the gates will conform to the rules defined for changing the plot scale or scale range as described above.

Changing Plot Type

- When a plot type is changed, a gate may or may not be deleted.
- When changing from a single parameter to a dual parameter plot or vice versa, all gates are removed from the plots.
- When changing from any dual parameter plot another type of dual parameter plot, the gates are maintained at their specified locations.

Workspace statistics

Overview

The Attune™ NxT Software generates statistics from acquired events. Statistics can be displayed for any parameter and calculated for any defined population. As the events are acquired, statistics are updated in real time.

- You can add a new *Statistics box* to the current Workspace by selecting the **Statistics** button on the Workspace ribbon tab (page 46).



Alternatively, you can select **Insert ► Statistics** from the *Workspace view context menu* (page 90) or the *Plot context menu* (page 93).

Parameters: SSC-A vs FSC-A
Gate: All Events
Experiment: Experiment
Group: Default_Group_Name
Sample: NOT-IN_STATS

Name	Count	%Gated	%Total
All Events	0	N/A	N/A
R1	0	N/A	N/A
R2	0	N/A	N/A
R3	0	N/A	N/A
R4	0	N/A	N/A

- The statistics available are as follows: Plate, Experiment, Group, Sample, Workspace, Plot Name, Gate, X parameter, Y parameter, event count, concentration, % Total, % Gated, X mean, Y mean, X median, Y median, X mode, Y mode, X SD, Y SD X % CV, Y % CV, X rSD, Y rSD, X r% CV, and Y r% CV.
- You can customize the Statistics box using the *Customize Statistics Box Options* available in the *Customize panel* as described on page 218.
- When the statistics container is not large enough to hold all columns and rows of the Statistics table, indicators reveal that more data are present to the right or below the viewable area. To view any non-visible results, you must expand the results area.

Parameters: SSC-A vs FSC-A
Gate: All Events
Experiment: Experiment
Group: Default_Group_Name
Sample: NOT-IN_STATS

Name	Count	%Gated	%Total
All Events	0	N/A	N/A
R1	0	N/A	N/A
R2	0	N/A	N/A
R3	0	N/A	N/A
R4	0	N/A	N/A

- Double-clicking the Statistics table allows you to scroll to see the contents that are outside the visible area. In this mode, the right-click context menu also includes an option to export the statistics of the selected table.

Workspace statistics

- If no Workspace object is selected, clicking on the **Statistics** button on the Workspace ribbon tab or selecting **Insert ► Statistics** from the Workspace view context menu inserts a *Workspace statistics table* displaying results from all gates.



Experiment: concentration				
Group: LYSED WHOLE BLOOD				
Sample: NOT-IN_STATS				
Time Recorded: 15:51:50				
	Name	Count	%Gated	%Total
■	All Events	23086	100.00	100.00
■	R1	3781	16.38	16.38
■	R3	2836	75.01	12.28
■	R4	2588	91.26	11.21
■	R2	1761	7.63	7.63
■	R5	3781	16.38	16.38
■	R6	1761	7.63	7.63

- The gates in the Workspace statistics table are displayed in the order specified by the *gate hierarchy*. Gates at the same hierarchical level are displayed in the order they are created. The color of the gate is displayed as a colored square to the left of the gate name.
- The *All Events statistic node* is displayed at the top of the hierarchy. The All Events node in the Statistics table only shows results for the following statistics: %gated, %Total, and Event Count. All other statistics for the All Events node display “N/A”.

Individual plot statistics

- If a single plot is selected, clicking the **Statistics** button on the Workspace ribbon tab or selecting **Insert ► Statistics** from the Plot context menu creates a *Plot statistics box*, which displays the results from the gates present on the selected plot.
- The statistics box for the selected plot also displays the All Events statistic node as well as the results from any gate present on the plot.
- The individual plot statistics box displays the results for the gates on the current plot and any upstream gates.



Multiple plot statistics

- If multiple plots are selected, clicking the **Statistics** button on the Workspace ribbon tab or selecting **Insert ► Statistics** from the Plot context menu creates a Plot statistic box for each plot selected.
- Each statistic box contains the All Events statistics row, and only the statistics for the gates present on the selected plots as well as their parent gates.

Workspace images and text

Workspace images You can add an image to the Workspace by selecting the *Image button* on the Workspace ribbon tab (page 46).



- The image types available for insertion include jpg, gif, bmp, png, tif and windows enhanced metafile. The image file cannot be larger than one page and will be scaled to fit on the page preserving the aspect ratio, if necessary.
- Workspace images can be resized as described in “Resizing Workspace objects”, page 66.

Workspace text You can add a Text box to the Workspace by selecting the *Text box button* on the Workspace ribbon tab (page 46).



- The Text box displays the default text “New Text Box”, which can be edited in place without opening a separate dialog box. The Text box can contain at up to 500 characters.
- The style and border of the Text box can be customized as described in “Customize Text Box Options” on page 217.
- If the Text box container is not large enough to hold all text, indicators reveal that more text is present to the right or below the viewable area. To view any non-visible text, you must expand the Text box.

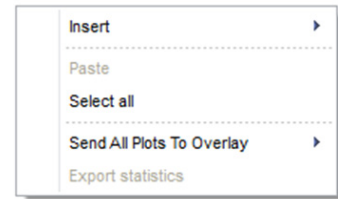
This is a new text box with text This is a new text box with text
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Workspace view context menu

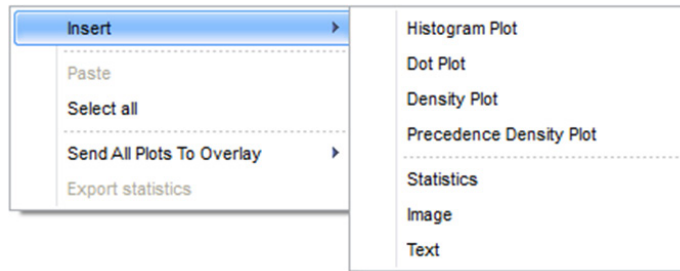
Overview

The *Workspace view context menu* is displayed when you right-click on any empty area (i.e., white space) within the Workspace view. It contains the tools to insert plots, statistics, images, and text to the Workspace, to paste and select Workspace objects, and to export statistics.



Insert

Selecting the **Insert** option displays a sub-menu, allowing you to add Histogram Plot, Dot Plot, Density Plot, Precedence Density Plot, Statistics, Image, and Text objects to the current Workspace.



These menu options have the same effect as pressing the respective buttons on the Workspace ribbon tab as described on page 44.

Paste and Select all

Paste

- **Paste** inserts the object currently in the clipboard to the Workspace at the location clicked. Paste option is only available if a Workspace object has been copied or cut to the clipboard.
- Clicking the **Ctrl+V** keyboard combination pastes the object at same location as the original. As each new object is pasted, it is inserted cascading downwards and to the right of the original location.
- Objects can be cut or copied from any Workspace and pasted to any other Workspace.
- Plots are pasted with any gates present. If a gated plot is copied to a new location where the parent gate does not exist, the pasted plot will not be gated and will display all events.
- If a Plot statistics box is pasted to a new Workspace and the target Workspace does not contain a copy of the original plot, the statistics box will update to show all gates present in the current Workspace. The Plot Axis and Gate fields will be removed from the statistics box header (if originally present).
- Pasted objects are selected by default after the paste action has completed.

Select all

- **Select all** selects all objects on the Workspace.

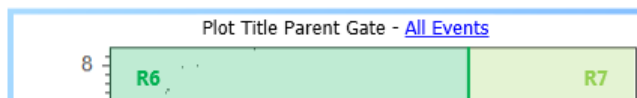
Send All Plots to Overlay

- **Send All Plots to Overlay** sends all Workspace plots to a selected *Overlay* (page 117).
The options depend on the type of plot present on the Workspace and the Overlay plots already present in the Overlay view.
- The *New Overlay* option is always present and enabled unless the maximum number of Overlays have already been created.
- The names of any Overlay plots already present in the Overlay application are also displayed.
- If both Histogram and dual parameter plots (Dot plot or Density) are present on the Workspace, the only option is to send plots to a new Overlay. This will send histogram plots to a new Overlay and dual parameter plots to another new Overlay.
- If only Histogram plots or dual parameter plots are present on the Workspace, you can either create a new Overlay or send the plots to an existing Overlay that contains plots with the same number of parameters.
Overlay plot names in the context menu will be disabled for Overlay plots with non-matching number of parameters.
- The Overlay name in the context menu will also be disabled if the number of plots to be sent exceeds the allowable number of plots in the specific Overlay.



Plot title hyperlink

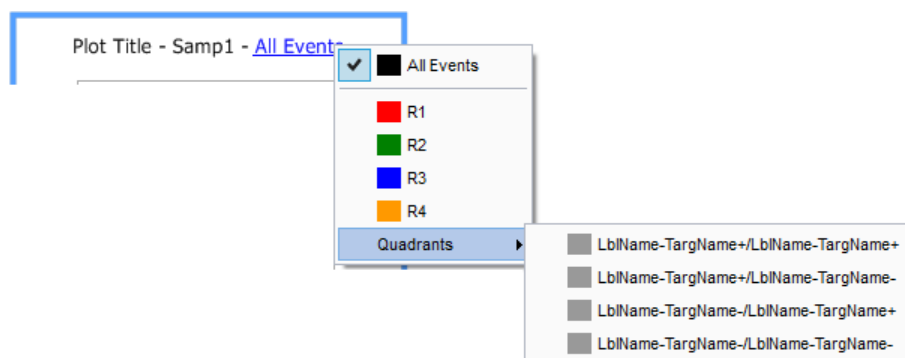
If **Include** option is checked in the *Customize Plot* options (page 201), the plot title displays the current parent gate of the plot as a hyperlink. This option is checked by default.



- Clicking the **plot title hyperlink** displays a dropdown menu that contains a list of all available gates, including any derived gates.

Any gates present on the currently selected plot and their daughter gates are not included in the list of gates.

Quadrant gates are displayed in a submenu.



- You can select one gate from the dropdown menu to be used as the parent gate of the current plot. The hyperlink then updates to display the currently selected gate name.

Plot context menu

Overview

The *Plot context menu* is displayed when you right-click on any empty area (i.e., white space) within the plot boundary, but not on another active area (gate, plot title, or plot axis).

The Plot context menu contains the tools for gate creation and customization, to cut, copy, paste, and delete selected items, and save all selected items as a new graphics file.



Customize

Selecting **Customize** from the dropdown menu displays the *Plot Customize panel*, which allows you to format different properties of the selected plot as described in “Customize Plot Options” on page 201.

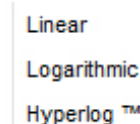
- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Scale

Scale sets all parameter axes to the selected scale. Available scale types are Linear, Logarithmic, and HyperLog™.

- The Scale option is available only when single or multiple plots are selected. If images or statistics objects are also selected, this option is not available.
- On dual-parameter plots (Dot or Density plots) the X and Y-axes are modified.

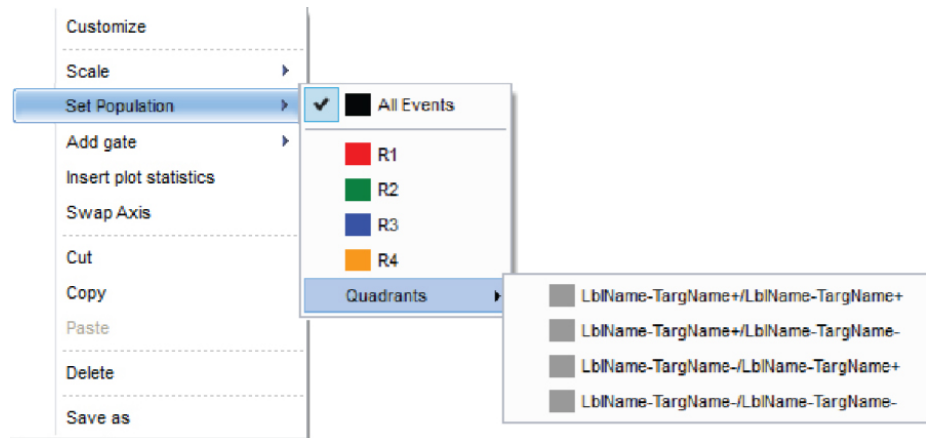
On single parameter plots (i.e., Histograms), the selection is only applied to the X-axis.

A screenshot of a dropdown menu for the Scale option. The menu items are: Linear, Logarithmic, and Hyperlog™.

Note: The Hyperlog™ scale uses log-linear hybrid transformations to display compensated flow cytometry data that frequently contain negative values due to compensation. Logarithmic transformations cannot properly handle negative values, and poorly display normally distributed cell types.

Set population

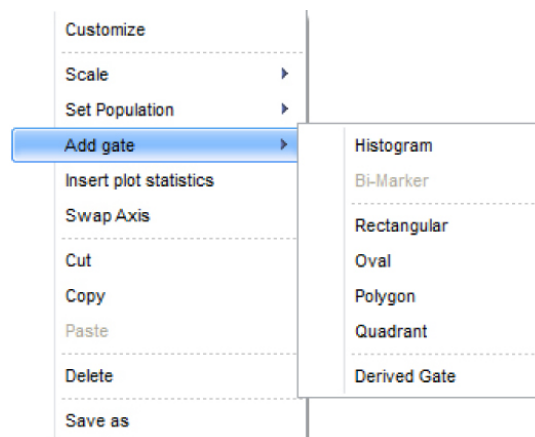
Set population allows you to choose to limit the data displayed on a plot to a given gate. It makes the plot a daughter of the selected upstream gate.



- A single gate can be selected from the list of available gates to be used as a parent gate for the selected plot. The selected gate is indicated by a tick mark in the list. It makes the plot a daughter of the upstream gate.
- The list of gates includes the All Events option and any available derived gates.
- The list does not include any gates present on the selected plot or any gates derived from those gates.
- The Set population option is also available when multiple plots are selected. In such cases, the available gate list excludes the gates that are present on any of the selected plots or any gates derived from those gates. The selected gate is used as a parent gate for all selected plots.

Add gate

Add gate allows you to create a new gate or a new derived gate in the default position on the currently selected plot. This option is available only when a single plot is selected.



- The Histogram option is only available for Histogram plots; Rectangular, Oval, Polygon, and Quadrant options are available only for dual parameter plots. Selecting these options creates the selected gate type in the default position described in “Creating and deleting gates” on page 77.
- The Derived Gate option opens the Derived Gate dialog, which is described on page 319.

Insert plot statistics **Insert plot statistics** inserts a *Plot statistics box* for each selected plot as described on page 88. This option is available when single or multiple plots are selected.

Cut, Copy, and Delete

Cut

Selecting **Cut** copies the selected items to the clipboard and removes them from the Workspace.

Copy

Selecting **Copy** copies the selected items to the clipboard, but keeps the original items on the Workspace.

Delete

Selecting **Delete** permanently removes all selected items from the Workspace.

Send to Overlay

Send to Overlay sends all selected plots to a selected *Overlay* (page 117). The available options depend on the type of plots selected and the Overlay plots already present in the Overlay view.



- The **New Overlay** option is always present and enabled unless the maximum number of Overlays have already been created.
- The names of any Overlay plots already present in the Overlay application are also displayed.
- If both Histogram and dual parameter plots (Dot plot or Density) are selected, the only option is to send plots to a new Overlay. This will send histogram plots to a new Overlay and dual parameter plots to another new Overlay.
- If only Histogram plots are present or only dual parameter plots are selected, you can either create a new Overlay or send the plots to an existing Overlay that contains plots with the same number of parameters.
- Overlay plot names in the context menu will be disabled for Overlay plots with non-matching number of parameters. The Overlay name in the context menu will also be disabled if the number of plots to be sent exceeds the allowable number of plots in the specific Overlay.

Save As

Selecting **Save As** displays the *File Save (Export) dialog* as described on page 363, which allows you to send all selected items to a new graphics file.

- The layout of the graphics file will replicate the layout of the selected items on the current Workspace.
- The selection area for saving may only cover one page.
- The available image file formats are PDF, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile. The default format is JPG.

Swap Axes

Selecting **Swap Axes** flips the axes of the selected plot, so that the X parameter becomes the Y parameter, and the Y parameter becomes the X parameter.

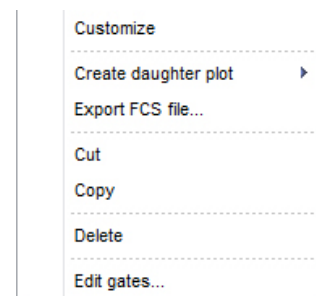
The Swap Axes option is only visible if at least one dual parameter plot is selected.

Gate context menu

Overview

The *Gate context menu* is displayed when you right-click on any part of a gate, on the gate boundary line, or a control point.

The Gate context menu contains the tools for customizing and editing gates, creating daughter plots, exporting FCS file, as well as for cutting, copying, and deleting selected gates.



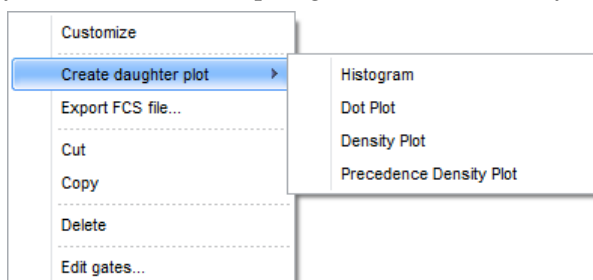
Customize

Customize displays *Gate Customize panel*, which allows you to format different properties of the selected gate as described in “Customize Gate options” on page 220.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Create daughter plot

Create daughter plot displays a sub-menu, which allows you to choose the type of plot to display. This creates a new plot gated on the currently selected gate.



- Properties of the newly created daughter plot are based on the defaults set in the *Plot Options dialog* (page 331) and not those of the parent plot.
- If you select a dual parameter plot type (Dot plot, Density plot, or Precedence Density plot), the new plot displays parameter 1 on the X-axis (normally FSC) and parameter 2 (normally SSC) on the Y-axis. If FSC and/or SSC are not being collected, the appropriate parameter 1 and 2 will be used as described in “Plots” on page 71.
- If you select the Histogram option, the new plot displays parameter 1 (normally FSC) on the X-axis and Count on the Y-axis.
- If the Workspace is in Freeform mode (page 68), the new plot is created in the default position as described “Creating Workspace objects”, page 65.
- If the Workspace is in Auto Layout mode (page 68), a new plot of the same size as the parent is inserted in the next free grid location available on the Workspace.

Cut, Copy, and Delete

Cut

Cut copies the selected gate to the clipboard and removes it from the plot.

When a gate is removed from a plot, any Derived Gate equations that include the removed gate are modified to exclude that gate. If the Derived gate includes only the gate that was removed, then the entire Derived Gate equation is deleted.

The cut gate may be pasted as described on page 67.

Copy

Copy copies the selected gate to the clipboard, but keeps the original gate on the plot.

Delete

Delete permanently deletes the currently selected gate and removes the gate from any Derived Gate equations and plot gates. If the gate equation includes only the deleted gate, then the entire gate equation is deleted.

Edit gates

Edit gates displays the *Edit gates dialog* as described on page 322.

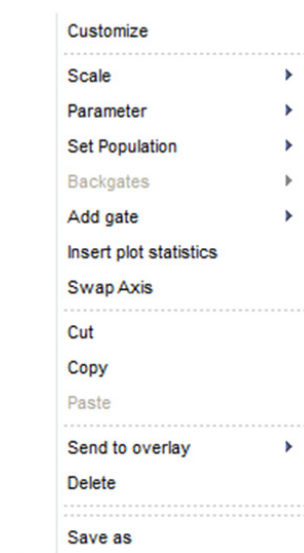
This dialog provides a list of all available gates on the active workspace and allows editing of gate color, parent gate, gate math expression, the z-order in which gates are painted, as well as toggle of backgate all plots and deletion of gates.

Plot axis context menu

Overview

The *Plot axis context menu* is displayed when you right click on a plot scale or parameter name.

The Plot axis context menu contains the tools for creating and customizing plots and gates, to cut, copy, paste, and delete selected items, and save all selected items as a new graphics file.



Customize

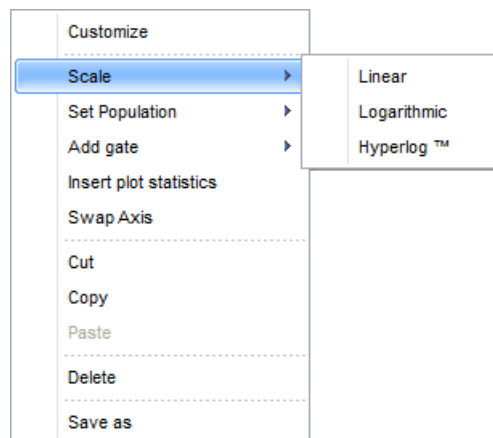
Selecting the *Customize* option displays the *Plot Customize panel*, which allows you to format different properties of the selected plot as described in “Customize Plot Options” on page 201.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Scale

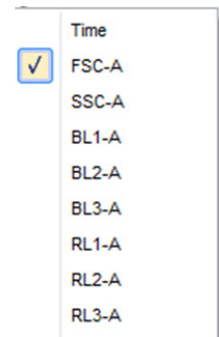
The *Scale* option sets all parameter axes to the selected scale. Available scale types are Linear, Logarithmic, and HyperLog™.

- The Scale option is available only when single or multiple plots are selected. If images or statistics objects are also selected, this option is not available.
- On dual-parameter plots (Dot or Density plots) the X and Y-axes are modified.
On single parameter plots (i.e., Histograms), the selection is only applied to the X-axis.



Parameter

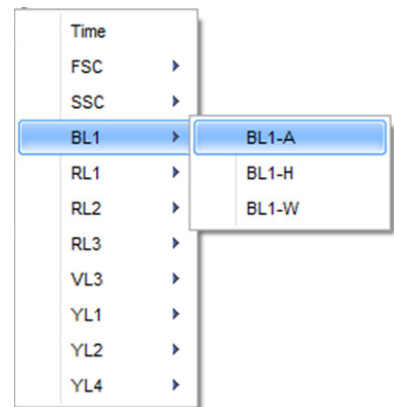
The *Parameter* option allows you to choose the parameters to display on a selected plot axis. This option is visible and enabled when single or multiple plots are selected. If images or statistics objects are also selected, this option is not visible.



- The options displayed in the Parameter submenu depend on the number of parameters present in the current sample.

If no dataset is present (i.e., the sample is empty), then the parameter list is based on the available and enabled parameters within the *Instrument Settings* (page 180).

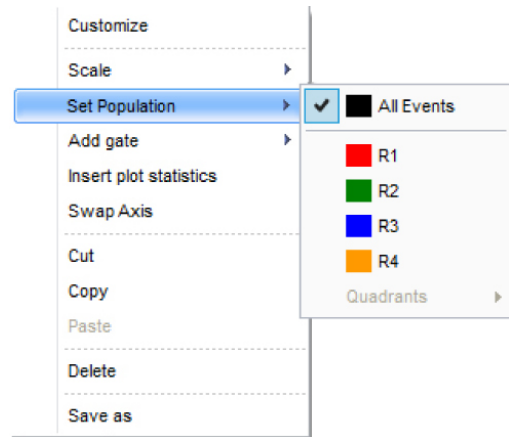
- If 18 or less parameters are selected in the current sample, the Parameter submenu displays all the parameters.
- The currently displayed parameter appears checked in the menu, and you can select any of the available parameters to display. The appropriate plot axis will be updated on all selected plots to display the chosen parameter.
- If more than 18 parameters are selected in the current sample, the context menu displays the parameter names with the parameter type displayed as a sub-menu.



Set population

The *Set population* option allows you to choose to limit the data displayed on a plot to a given gate. It makes the plot a daughter of the selected upstream gate.

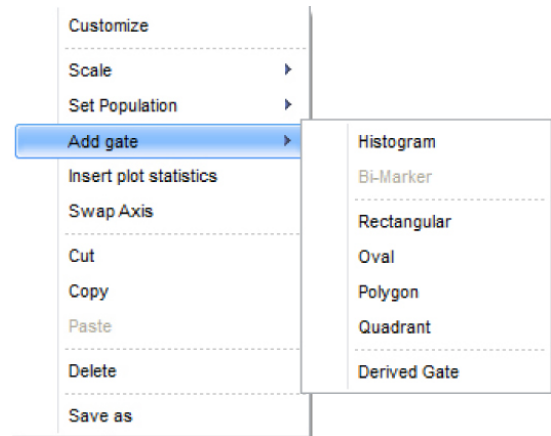
- A single gate can be selected from the list of available gates to be used as a parent gate for the selected plot. The selected gate is indicated by a tick mark in the list. It makes the plot a daughter of the upstream gate.
- The list of gates includes the All Events option and any available derived gates.
- The list does not include any gates present on the selected plot or any gates derived from those gates.
- The Set population option is also available when multiple plots are selected. In such cases, the available gate list excludes the gates that are present on any of the selected plots or any gates derived from those gates. The selected gate is used as a parent gate for all selected plots.



Add gate

The *Add gate* option allows you to create a new gate or a new derived gate in the default position on the currently selected plot. This option is available only when a single plot is selected.

- The Histogram option is only available for Histogram plots; Rectangular, Oval, Polygon, and Quadrant options are available only for dual parameter plots. Selecting these options creates the selected gate type in the default position described in “Creating and deleting gates” on page 77.
- The Derived Gate option opens the Derived Gate dialog described on page 319.



Insert plot statistics *Insert plot statistics* inserts a *Plot statistics* box for each of the selected plots as described on page 88. This option is available when single or multiple plots are selected.

Swap axis

Selecting the *Swap axis* option flips the axes of the selected plot, so that the X parameter becomes the Y parameter, and the Y parameter becomes the X parameter.

The Swap Axis option is only visible if at least one dual parameter plot is selected.

Cut and Copy

Cut

Selecting *Cut* copies the selected items to the clipboard and removes them from the Workspace.

Copy

Selecting *Copy* copies the selected items to the clipboard, but keeps the original items on the Workspace.

Paste and Delete

Paste

Selecting *Paste* inserts the gate currently in the clipboard to the Workspace. The new gate will be assigned the next available gate name as described in “Gate name” on page 78.

The size and position of the pasted gate will be identical to the location and size of the original gate when it was copied to the clipboard, even if the location is outside the current plot area.

The Paste option is available only if a single plot is selected and if a gate type relevant to the current plot type has previously been copied into the clipboard.

If *Smart gating* is enabled, the new gate name will be created as described on page 223.

Delete

Selecting *Delete* permanently removes all selected items from the Workspace.

Send to Overlay

Send to Overlay sends all selected plots to a selected *Overlay* (page 117).

The options available in the dropdown depend on the type of plots selected and the Overlay plots already present in the Overlay view.



- The **New Overlay** option is always present and enabled unless the maximum number of Overlays have already been created.
- The names of any Overlay plots already present in the Overlay application are also displayed.
- If both Histogram and dual parameter plots (Dot plot or Density) are selected, the only available option is **New Overlay**. This sends histogram plots to a New Overlay and dual parameter plots to another New Overlay.
- If only Histogram plots are present or only dual parameter plots are selected, you can either create a New Overlay or send the plots to an existing Overlay that contains plots with the same number of parameters.
- Overlay plot names in the context menu are disabled for Overlay plots with non-matching number of parameters. The Overlay name in the context menu is also disabled if the number of plots to be sent exceeds the allowable number of plots in the specific Overlay.

Save As

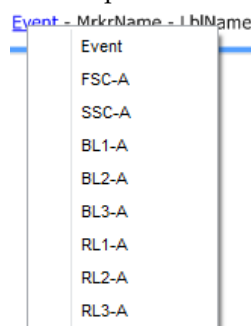
Save As displays the *File Save (Export) dialog* as described on page 363, which allows you to send all selected items to a new graphics file.

- The layout of the graphics file will replicate the layout of the selected items on the current Workspace.
- The selection area for saving may only cover one page.
- The available image file formats are PDF, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile. The default format is JPG.

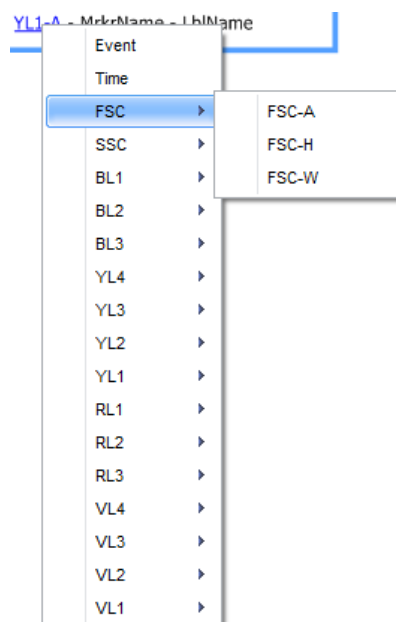
Plot parameter hyperlink

The plot parameter name, the X- and Y-axes of a dual parameter plot, or the X-axis of a Histogram plot are hyperlinks.

- Clicking on a parameter name displays the list of available parameters and calculated parameters. You select a parameter to apply to the selected axis from this list.
- The options displayed in the parameter name context menu depend on the number of parameters present in the current sample. Only parameters that are part of the dataset and selected in the *Workspace filters* (page 59) are listed. If no dataset is present (i.e., the sample is empty), then the parameter list is based on the available parameters within the *Instrument Settings* (page 180).
- If 18 or less parameters are selected in the current sample, the Parameter submenu displays all the parameters present in the current sample.



- The currently displayed parameter appears checked in the menu, and you can select any of the available parameters to display. The appropriate plot axis will be updated on all selected plots to display the chosen parameter.
- If more than 18 parameters are selected in the current sample, the parameter name context menu displays the parameter names with the parameter type displayed as a sub-menu.
- If only one parameter type exists, the parameter selection will remain in the main menu.
- If multiple plots are selected, the chosen parameter is applied to the selected axis of all selected plots. Any Y-parameter selection is not applied to any Histogram plots that are part of the multi-select.

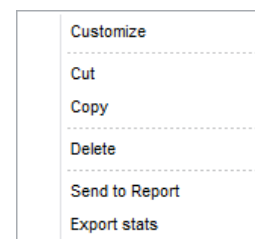


Statistics context menu

Overview

The *Statistics context menu* is displayed when you right-click a Statistics box on the Workspace (page 87).

The Statistics context menu contains the tools for customizing the Statistics box, to cut, copy, and delete the selected Statistics box, and to Export the selected Statistics as a single file.



Customize

Customize option displays the *Statistics Box Customize panel*, which allows you to customize the statistic style and formatting of the selected Statistics box as described in “Customize Statistics Box Options” on page 218.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Cut, Copy, and Delete

Cut

Cut copies the selected items to the clipboard and removes them from the Workspace. Cut items may be pasted as described on page 67.

Copy

Copy copies the selected items to the clipboard, but keeps the original items on the Workspace. Copied items may be pasted as described on page 67.

Delete

Delete permanently removes all selected items from the Workspace.

Export stats

Selecting **Export stats** opens the *Save As* which allows you to specify a file name and location to export the selected statistics table to a CSV file.

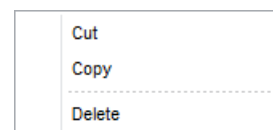
This option is only enabled if the statistics table is activated by double clicking the table.

Image context menu

Overview

The *Image context menu* is displayed when you right-click on an image on the Workspace (page 87).

The Image context menu contains the tools to cut, copy, and delete the selected image.



Cut, Copy, and Delete

Cut

Selecting **Cut** copies the selected images to the clipboard and removes them from the Workspace. Cut images may be pasted as described on page 67.

Copy

Selecting **Copy** copies the selected images to the clipboard, but keeps the original images on the Workspace. Copied images may be pasted as described on page 67.

Delete

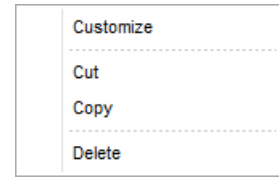
Selecting **Delete** permanently removes all selected images from the Workspace.

Text box context menu

Overview

The *Text box context menu* is displayed when you right-click on a Text box on the Workspace (page 87).

The Text box context menu contains the tools to cut, copy, and delete the selected image.



Cut, Copy, and Delete

Cut

Cut copies the selected Text box to the clipboard and removes it from the Workspace. Cut Text box may be pasted as described on page 67.

Copy

Copy copies the selected Text box to the clipboard, but keeps the original Text box on the Workspace. Copied Text box may be pasted as described on page 67.

Delete

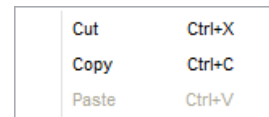
Delete permanently removes all selected Text boxes from the Workspace.

Text box text context menu

Overview

The *Text box text context menu* is displayed when you right-click on text within the edit area of a text box while the Text box is being edited or text is selected.

The Text box text context menu contains the tools to cut, copy, and delete the selected text.



Cut, Copy, and Delete

Cut

Cut copies the selected text to the clipboard and removes it from the Text box. Cut text may be pasted as described on page 67.

Copy

Copy copies the selected text to the clipboard, but keeps the original text in the Text box. Copied text may be pasted as described on page 67.

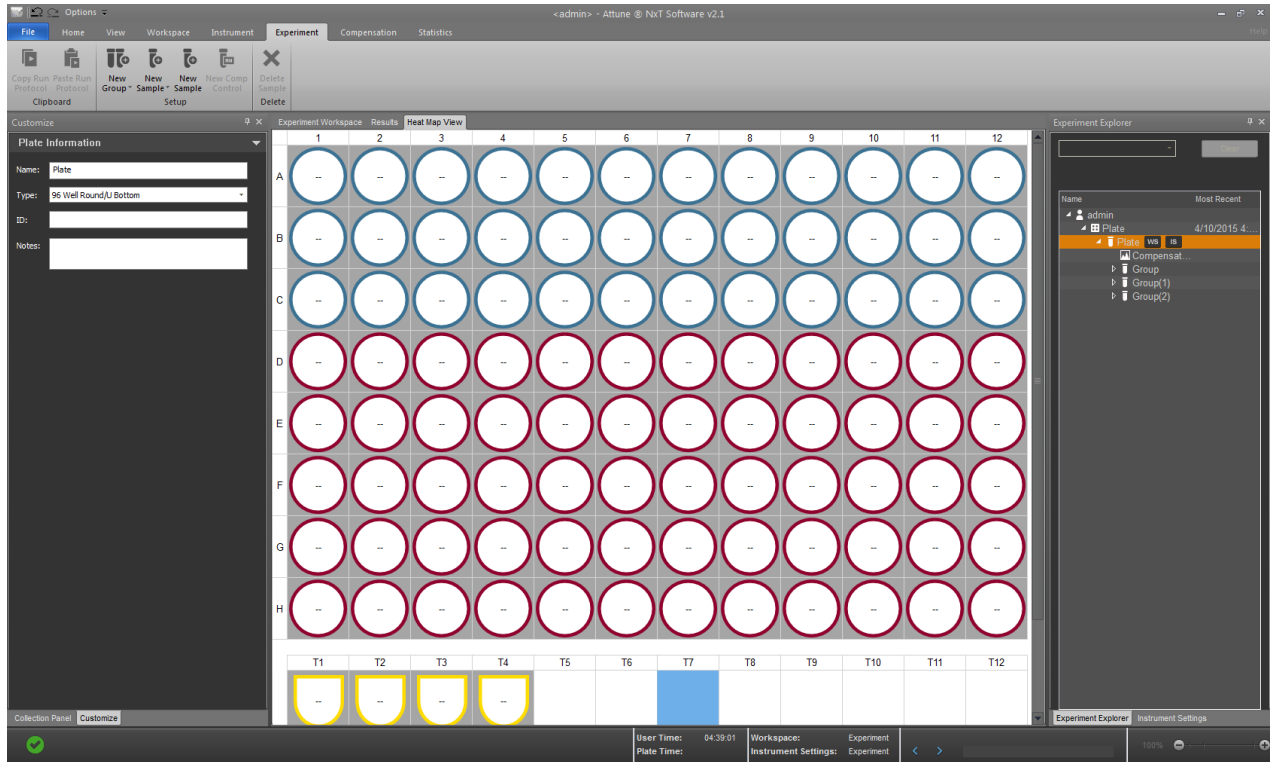
Delete

Delete permanently removes all selected text from the Text box.

6. Heat Map view

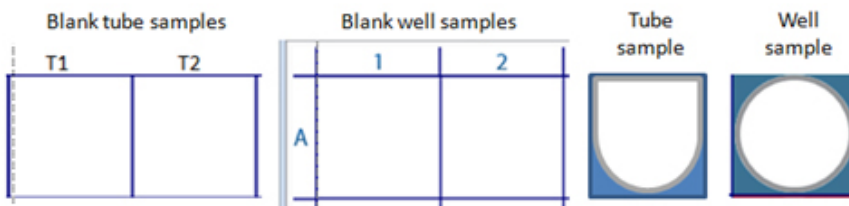
Overview

The *Heat Map view* provides a graphical method (i.e., *Heat Map*) for setting up and analyzing plate- and tube-based experiments. By default, the Heat Map view is docked to the *Main Application Workspace* (page 28).



Display of Samples

- The Heat Map displays both *Well* and *Tube samples*.
- A tube- or plate-based experiment may contain a total of 400 Samples.
- Well samples are created in a table as part of a 96- or 384-well plate and Tube samples are created by row.
- Blank sample spaces are displayed as white squares and Samples marked as part of an experiment show the experiment and group color as described in “Experiment/Group labels” on page 109.



Tube Experiment

- Tube samples for a new tube experiment are displayed in rows of 12 until 205 Samples are reached, at which point the view will switch to 24 Samples per row.
- Each Tube sample is labeled with its location on the Heat Map, beginning with T1 and continuing to T400, which is the limit to the number of Samples a tube- or plate-based experiment may contain.



- The Heat Map for a tube experiment contains the number of Sample tubes that were originally created using the *New Experiment dialog* (page 303). Any remaining tube spaces in a Sample tube row and the entire row below it is left as *blank Tube samples*.
- As new Tube samples are generated after the initial experiment creation, the Heat Map continues to retain a complete row of blank Tube samples until the total Sample limit is reached.

Plate Experiment

- Heat Map for a new Plate Experiment is displayed with an empty table of 96 or 384 wells (depending on the plate type selected), plus the number of Tube samples created in the New Experiment dialog (page 303).

	1	2	3	4	5	6	7	8	9	10	11	12
A	--	--	--	--	--	--	--	--	--	--	--	--
B	--	--	--	--	--	--	--	--	--	--	--	--
C	--	--	--	--	--	--	--	--	--	--	--	--
D	--	--	--	--	--	--	--	--	--	--	--	--
E	--	--	--	--	--	--	--	--	--	--	--	--
F	--	--	--	--	--	--	--	--	--	--	--	--
G	--	--	--	--	--	--	--	--	--	--	--	--
H	--	--	--	--	--	--	--	--	--	--	--	--
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
	--	--	--	--								

- 96-well Plate Experiments can contain up to 304 Tube samples (for a total of 400 Samples per experiment). Any remaining Samples are shown as blank Tube samples.
- 384-well Plate Experiments can contain up to 16 Tube samples (for a total of 400 Samples per experiment). Any remaining Samples are shown as blank Tube samples.
- Any Tube samples displayed in the Heat Map for a Plate Experiment are followed by the required number of blank Tube samples to complete the row, plus an entire row of Blank samples.
- If the Plate Experiment contains no Tube samples, then the Heat Map displays one complete row of blank Tube samples following Well samples.
- As new Tube samples are generated after the initial experiment creation, the Heat Map continues to retain a complete row of blank Tube samples until the total Sample limit is reached.



Note: Compensation samples in tubes are not shown in the Heat Map. Therefore, they do not count towards the 400 Sample limit.

Navigation and selection

- Double-clicking a Sample in the Heat Map view loads the Sample using the *Experiment-level Workspace*.
- Multiple Samples may be selected at once. The following methods may be used alone or in combination to select the desired Tube and/or Well samples.
 - Use left-click and drag to lasso a selection of Tube and/or Well samples.
 - Click while holding **Ctrl** key to select non-contiguous Tube and/or Well samples.
 - Click while holding **Shift** key to select all wells between the selected points, by row order.
 - Click the header of a row to select that entire row.
Alternatively, you can use the **Shift+Space** combination to select the entire row, if the row contains at least one selected Sample.
 - Click the header of a column to select that entire column.
Alternatively, you can use the **Ctrl+Space** combination to select the entire column, if the column contains at least one selected Sample.
 - Click the top left corner of a plate diagram to select the entire plate.
Alternatively, you can use the **Ctrl+A** combination to select the entire plate, if the plate contains at least one selected Sample.

Setting up an Experiment

Experiment/Group labels

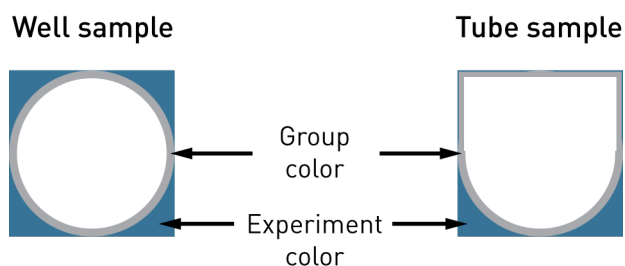
Use the **New Group** (🗑️), **New Sample** (🗑️), and **Edit Sample** (🗑️) buttons to create Samples and organize them into groups.

- Tube- and Well-based Samples have different shape to help identify them more easily.
- Each Experiment has a unique identifying color that fills the area around the Sample that belongs to it. This color differentiates the Sample belonging to that Experiment from Samples in other Experiments.

You can change the Experiment color using the *Experiment information* options in the *Customize panel* (page 229) while in the Heat Map view.

- Each Group has a unique identifying color that is marked around the inside of the Experiment color identifier.

You can change the Group color from the *Group information* options in the *Customize panel* (page 230) while in the Heat Map view.



Run Protocol

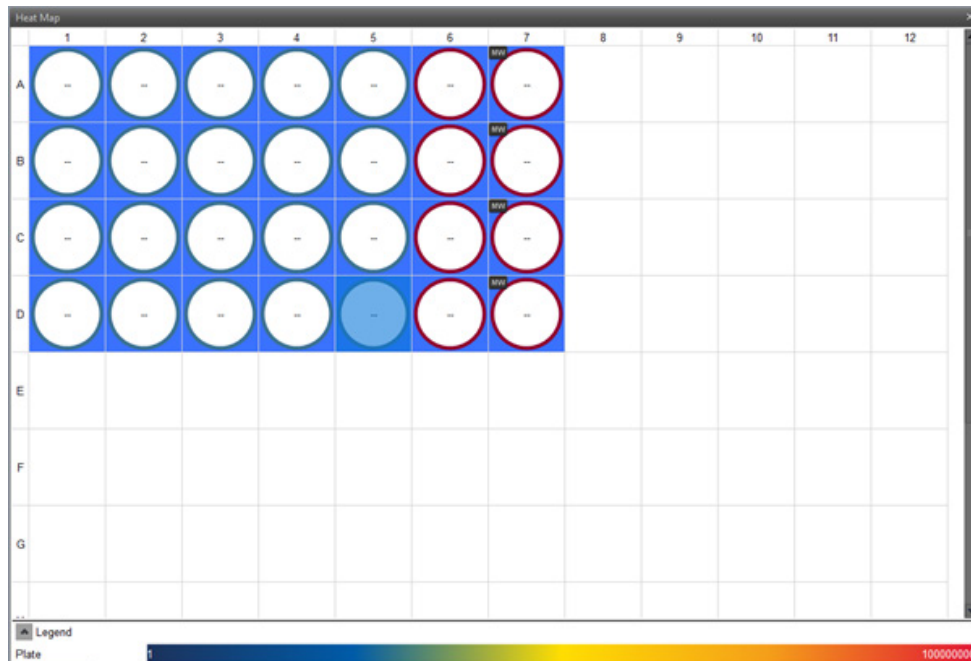
- Run Protocols are set up via the *Collection panel* (page 159). Each Sample may have a unique Run Protocol.
- Run Protocols may be copied and pasted using the Copy Run Protocol (📄) and Paste Run Protocol (📄) buttons.

Alternatively, you can use the hot keys to copy and paste the selected Run Protocols.

- If multiple wells with different Run Protocols settings are selected, individual fields may be edited in common without altering other settings.
- Fields with all values in common will display the selected value. Fields with differing values will display as blank but active.

Legend

The legend for the Experiment shows the display mode, color key, and the range that is specified during Heat Map setup (page 191).



- The legend is attached to the bottom of the Heat Map panel and may be minimized but not closed. It is automatically resized horizontally if the Heat Map panel size is changed.
- The legend displays the Experiment name in a column on the left, and the Heat Map or Threshold colors on the right.
- In the Threshold display mode (page 111), transition values are shown just to the right side of the transition point.
- In Gradient display (i.e., Heat Map display) mode (page 111), minimum and maximum values are always displayed on the legend.
- The value and location of any transition points that have been added by the user will be displayed on the legend just to the right side of the transition point.



Minimized



Gradient mode



Threshold mode

Analyzing results

Display of results Based on the Heat Map settings specified in the *Heat Map Setup panel* (page 191), each Sample with saved data displays a color and, if it is set, the numerical value of the statistic selected for that Sample.

Threshold display mode

If the **Threshold display mode** is selected on the Heat Map Setup panel, each Sample in the Heat Map view displays the appropriate color depending on whether the signal from the Sample falls above or below the set threshold value.

Heat Map display mode (i.e., Gradient mode)

If the **Heat Map display mode** (i.e., Gradient mode) is selected on the Heat Map Setup panel, each Sample in the Heat Map view displays the color that corresponds to the appropriate signal range set during Heat Map setup.

- If the values fall below the minimum range set for the sample, the Heat Map shows the color representing the minimum value and the '<' symbol.
- If the values exceed the maximum range set for the sample, the Heat Map shows the color representing the maximum value and a '>' symbol.
- If statistics are selected for display on the Heat Map, the < and > symbols are hidden by the displayed statistic.



Tooltips

- Hovering the mouse pointer over a well or a tube displays a tooltip with the following information:
 - Gate
 - Statistic
 - Sample
 - Group
 - Time recorded
 - Location
- Hovering the mouse pointer over Blank sample spaces displays a tooltip that shows the Heat Map location (i.e., plate row and column or tube number).
- Hovering the mouse pointer over Compensation samples displays a tooltip that shows the compensation parameter and the Heat Map location (i.e., plate row and column or tube number).
- Any fields on the tooltip which are not applicable is left blank.

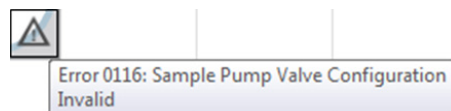
Indicators

- If an error causes the Sample run to terminate prematurely, the *error icon* will be displayed in the Heat Map location of the Sample.

On mouse-over, a tooltip is displayed providing more information about the error that was encountered.

- If a bubble is detected during acquisition, the *Bubble detected icon* is displayed in the Heat Map location of the Sample. 
- If an error occurs during acquisition, the *Generic Error icon* is displayed in the Heat Map location of the Sample. 

A tooltip is displayed providing details of the error when you hover the mouse pointer over the error icon.



7. Results view

Overview

The *Results view* displays results from all samples consolidated into a single table (i.e., the *Results table*).

Results							
Drag a column header here to group by that column.							
X Parameter	Y Parameter	Gate	Region	Count	%Gated	X Var	Y SD
SS	FS	Ungated	A	2618	55.7614	5187.3415	62.3188
SS	FS	Ungated	B	2618	55.7614	5187.3415	62.3188
SS	FS	Ungated	C	2618	55.7614	5187.3415	62.3188
SS	FS	Ungated	D	2618	55.7614	5187.3415	62.3188
SS	FS	D	E1	659	25.1719	4136.2788	43.8315
SS	FS	D	E2	55	2.1008	451.3657	41.1433
SS	FS	D	E3	1887	72.0779	3059.0024	34.1520
SS	FS	D	E4	17	0.6494	577.0956	37.4281
SS	FS	D	F1	938	35.8289	4501.1147	46.5650
SS	FS	D	F2	54	2.0626	424.3498	44.6279
SS	FS	D	F3	1618	61.8029	2907.6162	29.6078
SS	FS	D	F4	8	0.3056	427.3571	33.6611
SS		Ungated	G	3459	73.6741	8670.1276	
SS	FS	Ungated	A	577	12.2897	4968.5392	103.8612
SS	FS	Ungated	B	577	12.2897	4968.5392	103.8612
SS	FS	Ungated	C	577	12.2897	4968.5392	103.8612
SS	FS	Ungated	D	577	12.2897	4968.5392	103.8612

Results table

Results table

The Results table shows the statistics associated with gates from the selected Workspaces for all Samples within an Experiment.

- Results table displays only the gates for the Workspace. Each gate created in the Workspace generates a *results row* in the Results table.
- If *Auto-Refresh* is enabled, then results for all files in the Experiment are displayed; otherwise, only results for the processed files are displayed.

Primary table

- Primary table only displays the statistics selected in the Statistics tab (page 58), with each statistic arranged in a separate column in the table.
- By default, columns automatically adjust their width to fit the widest text in the column. The width of a column can be also manually adjusted by dragging the right column divider (i.e., the divider on the right of the column).

Once a column width has been manually adjusted, it does not automatically adjust to fit any new text in the column. Double-clicking the right column divider will set that column back to auto-adjust its width.

- By default, the columns for the primary table are added in the following order: Plate, Experiment, Group, Sample, Workspace, Plot Title, Gate, X parameter, Y parameter, Count, Concentration, % Total, % Gated, X mean, Y mean, X median, Y median, X mode, Y mode, X SD, Y SD, X %CV, Y %CV, X rSD, Y rSD, X %rCV, and Y %rCV.

Plate, Experiment, Group, and Sample columns display their respective names, and the Workspace column displays the source.

- All columns are added to the table in the order the checkboxes are arranged on the Statistics tab, left to right then top to bottom within a Ribbon group. You can re-order the columns by dragging and dropping their headers.
- When a new checkbox is selected, the respective column is inserted in the correct position in the table.
- You can remove the columns from the table by un-selecting them in the Statistics tab or by dragging a column header outside of the column header area. When dragging a column header, an “X” indicates that it will be removed.
- If a statistical value cannot be calculated, “N/A” will be displayed instead of a numerical value.
- If a value is not applicable to the row (e.g., Y values for a histogram gate), it is left blank.
- The table can be sorted by any column in an ascending or descending order (case insensitive) by clicking the column title.

Selecting results rows

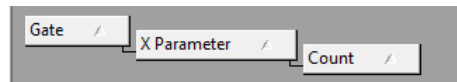
- Left or right mouse click on a results row selects the entire row.
- Holding down the **Ctrl** or **Shift** keys allows multiple rows to be selected.
- **Ctrl+A** combination selects all the rows in the table.
- **Ctrl+Shift+End** combination selects all rows from the currently selected row to the end of the table.
- **Ctrl+Shift+Home** combination selects all rows from the currently selected row to the top of the table.



Grouping results

- Grouping results by column headers**
- Results can be grouped by the values displayed in the column headers by dragging a column header from the Results table onto the *group control* labeled “Drag a column header here to group by that column”.

Results				
Drag a column header here to group by that column.				
X Parameter	Y Parameter	Gate	Region	Count
SS	FS	Ungated	A	2618
SS	FS	Ungated	B	2618

- You can place more than one header in the “Drag a column header ...” group control, which will group results by all the headers placed there. The headers will be linked by arrows denoting the sort order:



- You can change the sort order by dragging a header in a different position on the group control.
- Clicking on a header arrow (/) toggles the sorting order between ascending and descending within the column.
- You can collapse the information displayed in the groups by clicking the  button and expand it by clicking the  button.

Adding and removing column headers

- When a header is dragged, two hot divider arrows indicate where the column header will be placed on the “Drag a column header ...” group control.
- You can remove a header from the grouping by dragging it back to its position in the Results table. When a column header is dragged back to the Results table, two hot divider arrows indicate where the header will be placed.



Alternatively, you can drag the header off the table to remove it from the group control.

Moving versus copying headers

- Dragging a header onto the “Drag a column header ...” group control removes the header from the table and places it in the group control. Dragging the header back to the table removes it from the group control and places it back in the table.
- If you hold down the **Ctrl** key while dragging a header, the header is copied rather than moved.

Examples of grouped results

Example of results grouped by Gate

X Parameter	Y Parameter	Region	Count	%Gated	X Peak	Y Peak	X Mean	Y Mean	X Var	Y Var
Gate: A										
FL1 Log	FS	B	8121	68.3528	1.2593	753.0...	2.0437	680.08...	10...	611...
Gate: C										
FL3 Log	FS	D	4820	46.1376	6.0233	642.0...	3.1244	472.12...	7.4...	121...
FL5 Log	FS	E	4486	42.9406	0.4599	450.0...	0.5214	471.10...	1.7...	125...
Gate: Ungated										
SS	FS	A	11881	43.4533	1023....	710.0...	580.61...	686.79...	536...	134...
FL2 Log	FS	C	10447	38.2086	0.6531	753.0...	1.1581	574.76...	0.9...	255...

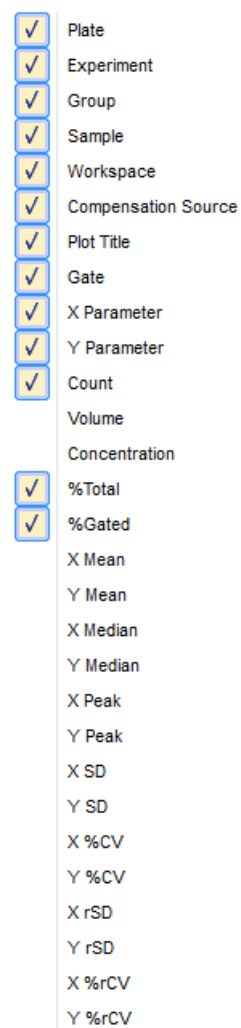
Example of results grouped by Gate and X Parameter

Y Parameter	Region	Count	%Gated	X Peak	Y Peak	X Mean	Y Mean	X Var	Y Var
Gate: A									
X Parameter: FL1 Log									
FS	B	8121	68.3528	1.2593	753.0...	2.0437	680.08...	10...	611...
Gate: C									
X Parameter: FL3 Log									
FS	D	4820	46.1376	6.0233	642.0...	3.1244	472.12...	7.4...	121...
X Parameter: FL5 Log									
FS	E	4486	42.9406	0.4599	450.0...	0.5214	471.10...	1.7...	125...
Gate: Ungated									
X Parameter: FL2 Log									
FS	C	10447	38.2086	0.6531	753.0...	1.1581	574.76...	0.9...	255...
X Parameter: SS									
FS	A	11881	43.4533	1023....	710.0...	580.61...	686.79...	536...	134...

Column header context menu

Right-clicking on a column header displays the *column header context menu*.

- The column header context menu contains all the statistics that can possibly be displayed in the Results table.
- The statistics that are already present in the table are checked in the context menu.
- You can add new columns to the Results table by checking the corresponding item in the column header context menu.
- You can remove columns from the Results table by un-checking the corresponding item in the column header context menu.
- The column header context menu can be displayed when there are no columns in the table or when clicking on a blank area of the header.



8. Overlay view

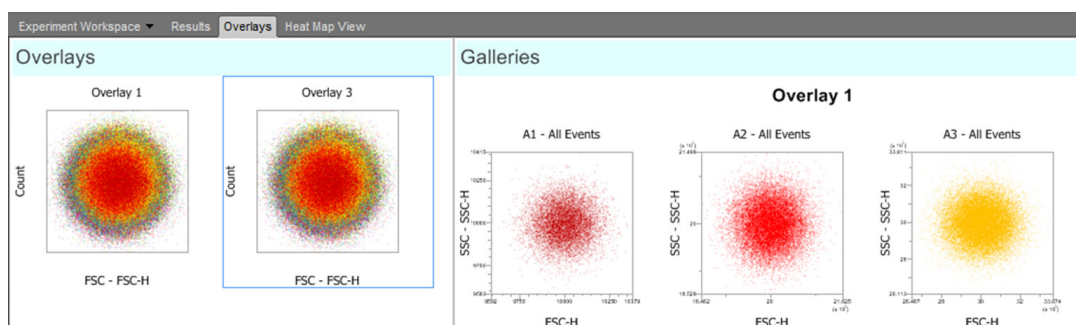
Overview

Overlays provide a graphical method for comparing Experiment data, in which selected plots are superimposed for a direct visual comparison of overlapping data.

- Each Overlay consists of an *Overlay plot* (page 119) and an associated *Gallery* containing the Source plots for the Overlay (i.e., *Gallery plots*; page 122).
- Overlays are viewed and modified on the *Overlay view tab* (below).

Overlay view tab

Overlay view tab is displayed in the Application Area on the Main Application Workspace (page 28) and allows comparison of Histogram or dual-parameter plots.

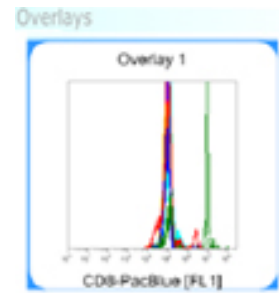


- The *Overlay view* is divided vertically into two windows by a splitter bar.
 - The left window of the Overlay view is the *Overlays area* (page 118)
 - The right window is the *Galleries area* (page 118)
- The docking behavior of the Overlay view tab is described on page 62.
- There is no limit to the number of Overlays that can be added to the Overlay view.
- When the Customize Overlay panel (page 232) is visible, the panel contents are updated based on the selected plots. The customize panel allows you to customize the appearance of Overlay and Gallery plots.

Overlays area

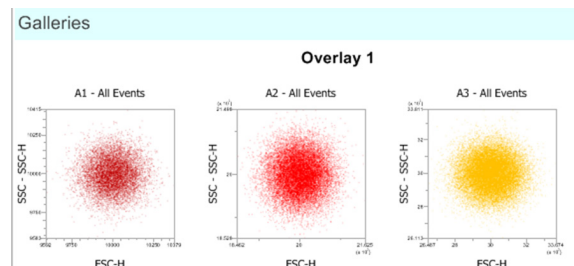
The *Overlays area* displays the Overlay plots (page 119).

- The Overlays area can be resized by sliding the splitter bar to the left or to the right.
- Plots within the Overlays area automatically wrap, when needed. By default, the divider is set so that only one Overlay plot is shown per row.
When required, vertical and horizontal scroll bars become available on the Overlays area to allow the viewing of all existing plots.
- When new plots are added to an existing Overlay or if a new Overlay is created by sending plots from the Workspace, the most recently created and/or modified Overlay(s) are selected when viewing the Overlay view tab.
- When one or more Overlay plots are selected, the titles for the corresponding galleries are highlighted in blue.
- Overlay plots are displayed 240 × 240 pixels. If legends are enabled, the Overlay plots are resized to 240 × 480 pixels (H × W).



Galleries area

The *Galleries area* contains the *Galleries* of Source plots (i.e., *Gallery plots*; page 122) for each Overlay plot displayed in the Overlays area.



- Each Gallery starts on a new line and has the same title as the Overlay whose Source plots it contains. The Gallery title is displayed above the plots in a central position and is always visible, even when the plots are horizontally scrolled.
- Horizontal and vertical scroll bars are available, when needed.
- The Galleries area can be resized by sliding the splitter bar to the left or to the right. The minimum width of the Galleries area is such that the Gallery title is always visible.
- Selecting any plot within a Gallery highlights the Gallery title in blue.
- You can resize the Plots within the Galleries area using the **Size Slider** in the bottom right corner of the on the *Application Status Bar* (page 37) or by holding down the **Ctrl** key on the keyboard and turning the **mouse scroll wheel**.
- Gallery plots are displayed in 240 × 240 pixels. If legends are enabled, the Gallery plots are resized to 240 × 480 pixels (H × W).

Overlay plots

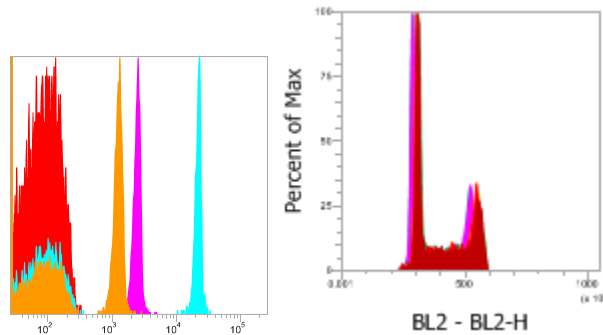
Overlay plots allow the visualization and comparison of overlapping data by superimposing the selected Histogram or of dual-parameter plots. The Overlay plots are displayed in the Overlays area (page 118).

Overlay plot modes Overlays plots have two display modes: *Overlay mode* and *3D mode*.

To switch between Overlay and 3D modes, select or deselect the **View 3D Mode** checkbox within the *Customize Overlay panel* (page 232). The selected mode only applies to the currently selected Overlay.

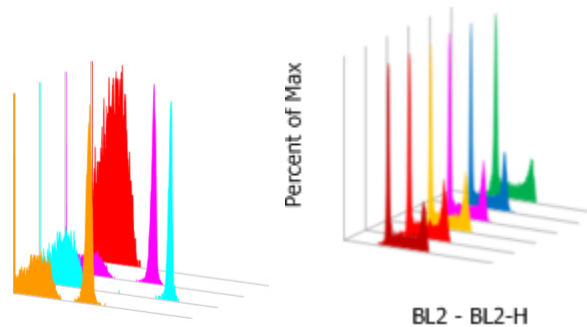
Overlay mode

- The Overlay mode is the default display mode, where the overlay plots are shown superimposed over one another.

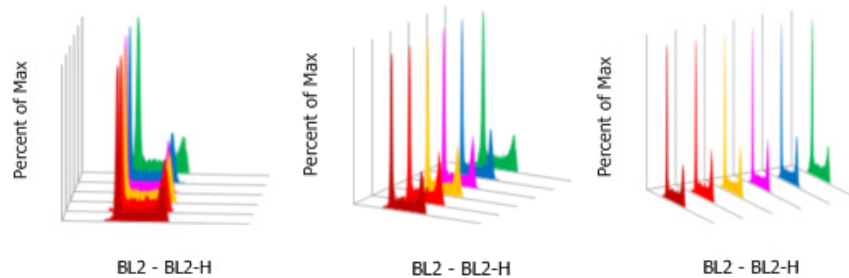


3D mode

- In the 3D mode, plots are displayed three dimensionally at a user-specified angle and are separated by a set distance. The distance between the plots is calculated based on the number of plots being overlaid.



- In this mode, you can rotate the plot by selecting the desired angle from the **Angle (deg)** dropdown control on the *Customize Overlay panel* (page 236).



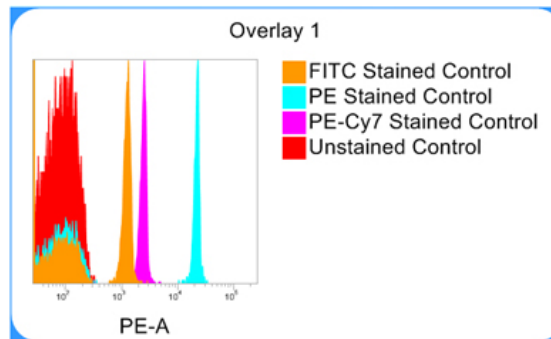
Overlay plot characteristics

Plot title

- Overlay plot titles are defined using the **Customize Text** controls on the Customize Overlay panel (page 232).

Legend

- By default, the legend is not displayed. However, you may choose to display the legend by checking the **Show Legend** checkbox in the Customize Overlay panel (page 233). The option to display the legend may also be set using the Overlay Builder described on page 139.
- By default, the legend displays the name of each sample in the overlay. The Parameter name may also be displayed in the legend with or without the Sample name using the Customize Overlay panel (page 233).



- The color associated with each sample in the legend corresponds to the data color of that sample on each plot.

Name labels of dual parameter plots in the legend are represented by solid colored rectangles.

Name labels of single parameter plots in the legend are represented by unfilled rectangles with colored borders unless the single parameter plot is itself filled. In that case, the legend is also displayed as filled.

- If all the plots in the Overlay cannot be represented on the legend due to insufficient space, a hyperlink titled "See All" is displayed below the last legend item shown.

Clicking the **See All** hyperlink displays a floating list that shows all the plots on the Overlay.

Clicking the **close button (X)** on the floating list closes it.



Axis labels

- In the Overlay mode, an axis label is displayed only if it is the same for all the plots in the Overlay. If an overlay is created from plots where a parameter is different for one of the axes, no axis label will be displayed for that axis.
- Axis labels of the Overlay plot may be edited using the **Customize Text** controls on the Customize Overlay panel (page 238).

Axis tick marks

- For the selected Overlay plots, you can specify the presence of X- and/or Y-axis tick marks by checking the **Show X-Axis Ticks** and **Show Y-Axis Ticks** checkboxes on the Customize Overlay panel (page 233).
- By default, both the X-axis and Y-axis tick mark display options are enabled.
- Changing tick mark display options for an Overlay plot updates all the plots in the associated gallery.
- By default, the tick marks are displayed on Overlay plots when all the Source plots have the same scale and range. If the scale ranges are different, the tick marks are not displayed, even if they are enabled on the Customize Overlay panel.

Gates

- You can add Gates to Overlay plots in Overlay mode using the **Gating Tools** on the Overlay ribbon tab (page 62).
- When a gate is drawn on an Overlay plot, the same gate is drawn on all the plots in the associated Gallery.

Re-ordering Plots

- You can reorder Plots in the Overlay mode by dragging and dropping selected plots.
When you reorder Overlay plots, their respective galleries are also re-ordered to match the order of the Overlay plots.
- When dragging plots, a blue vertical line indicates the insertion location of the plots.
- When you select and drag multiple plots, they are grouped together and can be moved and inserted as a group.
- If more than 3 plots are selected, a plus (+) icon is shown next to the plot images indicating the presence of additional plots being dragged.
- Plots can only be moved when and where the *insert indicator* appears; otherwise they return to their original positions when released.

Gallery plots

Gallery plots are the Source plots that are overlaid to generate the Overlay plot. The Gallery plots are displayed in the Galleries area (page 118).

Gallery plot characteristics

Plot title

- Gallery plot titles are defined using the **Customize Text** controls on the Customize Overlay panel (page 238).

Axes labels

- Gallery plot axis labels may be edited using the **Customize Text** controls on the Customize Overlay panel (page 238).

Axis tick marks

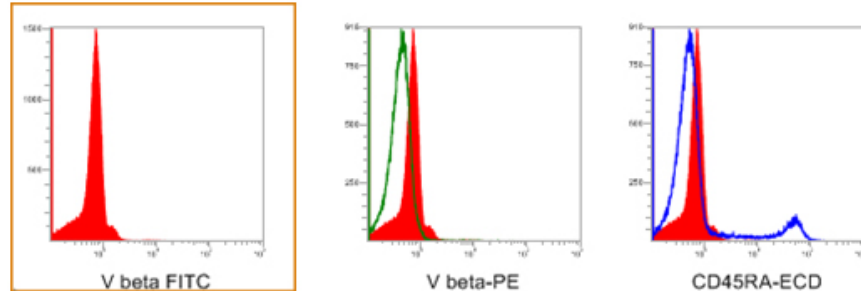
- By default, both the X-axis and Y-axis tick mark display options are enabled.
- Tick mark visibility for the selected Gallery plots can be changed using the **Show X-Axis Ticks** and **Show Y-Axis Ticks** checkboxes on the Customize Overlay panel (page 233).

Order of plots in the Galleries area

- The order of the plots within a Gallery (left to right) determines the z-order (front to back) of plots in its corresponding Overlay plot.
- Plots can be rearranged within each Gallery by dragging and dropping. When plots are re-arranged in a Gallery, they are re-arranged in the respective Overlay plot as well.
- When dragging plots, a vertical blue line highlights the insertion location of the plots.
- When multiple plots are selected and dragged, they are grouped together and moved and inserted as a group.
- If more than 3 plots are selected, a plus (+) icon is shown next to the plot images indicating the presence of additional plots being dragged.
- Plots can only be moved when and where the *insert indicator* appears; otherwise they return to their original positions when released.

Control plot

- Within the Galleries view, one gallery plot for each single parameter overlay may be selected to be a *Control plot*.
- The Control plot is displayed as an overlay on all single parameter plots present for a particular overlay in the gallery. The specified control plot is shown with an orange dashed line around its perimeter.
- By default, a Control plot is not defined.



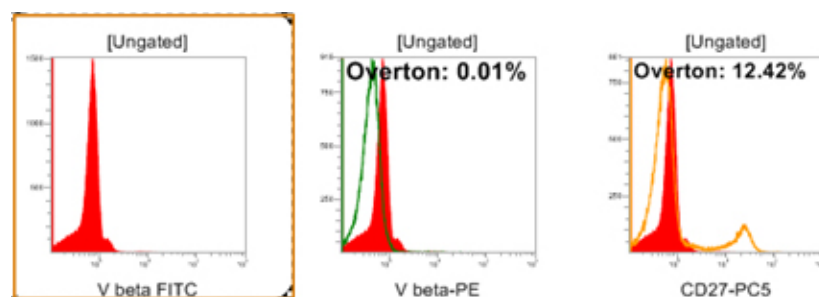
- The Control plot is selected by clicking the **Set as Control** button on the Customize Overlay panel (page 237).
- If there is already a Control plot, defining a different plot as a new Control plot replaces the previous plot as the control.
- Selecting the **Move to Front** option on the Customize Overlay panel (page 237) displays the Control plot on each Gallery plot in front of the data plot. By default this option is not selected and the data plots are displayed in front of the Control plot.
- When a Control plot has been defined, the software allows comparison statistics to be calculated (see “Overlay Calculations”, below).

Overlay calculations

- When a Control plot has been defined, the data sets can be compared statistically through selection of the desired **Calculation method** radio buttons on the Customize Overlay panel (page 238).
The available options are *Overton* (below) and the *Kolmogorov-Smirnov (K-S)* (page 124) methods. Both methods are used to determine the statistical difference between two samples.

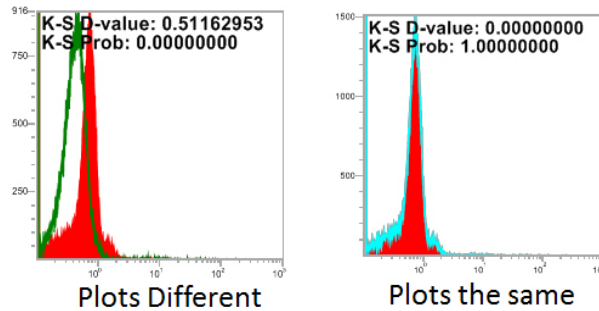
Overton method

- Selecting the **Overton** option compares the control sample and each Gallery plot using the Overton’s cumulative statistics calculation (page 380).
- The resulting value indicates the percent difference between the test sample and the control sample and is displayed on each test plot as shown below. The calculations are not shown on the plot set as the Control plot.



Kolmogorov-Smirnov (K-S) method

- **KS** option compares the control and test sample using the Kolmogorov-Smirnov test (page 380) to determine the probability that the two plots are the same. Results are displayed on the plot.



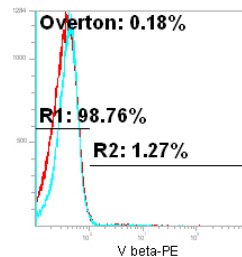
- The lower the calculated *D-value*, the more likely that the plots are the same. The lowest possible *D-value* is 0, in which case the plots are identical.
- The *Probability value* is a value between 0 and 1. The higher the Probability value, the greater is the probability that the plots are the same.



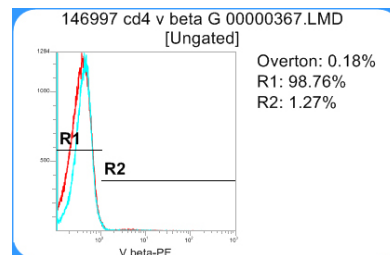
Note: For more information on the *Overton cumulative statistics calculation* and the *Kolmogorov-Smirnov (K-S) test*, see “Appendix B: Technical Reference”, page 380).

Display of statistics

- The Overlay calculation statistics (page 123) and the Overlay gate statistics (page 232) can be displayed on the plot or to the side of the plot. By default, they are displayed on the plot.



- When the **Show legend** option (page 233) is selected, statistics are displayed on the side of the plot as a legend.



- If all the plots and statistics cannot be shown on the legend due to insufficient space, a hyperlink titled “See All” is displayed below the last legend item shown. Clicking the **See All** hyperlink displays a floating list that shows all the plots on the Overlay. Clicking the **close button (X)** on the floating list closes it.
- Overton statistics always display two decimal places on the plot. All other statistics show decimal places as specified in the *Options dialog* (page 342).

Gates

- Gates are created on a selected plot using the *Gating Tools* available on the *Workspace ribbon tab* (page 45).
- Gates created on a Gallery plot are displayed on all the plots in the Gallery and on the related Overlay plot, if the Overlay plot is in Overlay mode and not in 3D mode (page 119).
- Gates are not displayed on Overlay plots that are in the 3D mode (page 119).
- Gates created on an Overlay plot in the Overlay mode are reflected on all the plots in its Gallery.
- The maximum number of gates for an Overlay is 5.
If you attempt to add a gate after the maximum number of gates on that plot has already been reached, a *no entry symbol* (⊘) is displayed, indicating that the additional gate cannot be created.
- Only one Quadrant gate can be drawn per Overlay. A Quadrant counts as a single gate, thus allowing 4 additional, non-Quadrant gates on the same plot.
- Gates are drawn in black with no fill, and are assigned default names in the order of their creation.
- When a gate is moved or resized on any plot in the Overlay, it is updated accordingly in all the rest of the plots in the same Overlay.
- Statistics are shown on the Gallery plot as specified in “Display of statistics”, page 124; they are not shown on the Overlay plot.
- The default statistic displayed is *%Gated* (page 60). You can change the statistics displayed by selecting the desired one from the *Statistics ribbon tab* (page 58).
- The decimal places for the statistics are as specified in the Options dialog (page 342). The displayed statistics persist on a per Experiment basis.
- If the statistic cannot be calculated, N/A is displayed.
- Gates on the Overlay tab do not affect the Source plots on the Workspace and do not count towards the maximum numbers of gates.

Overlay Properties

Overlay settings

Overlay title

- The *Overlay title* is unique for each Overlay, and it distinguishes the Overlay plot and its respective Gallery from other existing Overlays.
- The default title for a new Overlay is “*Overlay n*”, where n is a numerical suffix (first available integer) to ensure that the title is unique. If an Overlay is deleted or renamed, the number designation is re-used.

Overlay plot colors

- By default, all dual-parameter plots (i.e., Dot, Density, and Precedence Density plots) in an Overlay are displayed in a solid color.
- By default, all single-parameter plots are displayed as a solid line, without fill.
- The color of a plot in an Overlay is determined by the defaults specified in the *Color and Themes* tab of the *Options dialog* (page 330).

Each new plot in the Overlay is assigned the next available color listed in the *Gates and sample Overlays* group.

Range

You cannot change the range of individual plots on the Overlays tab.


Parameter

You cannot change parameters of plots on the Overlays tab.

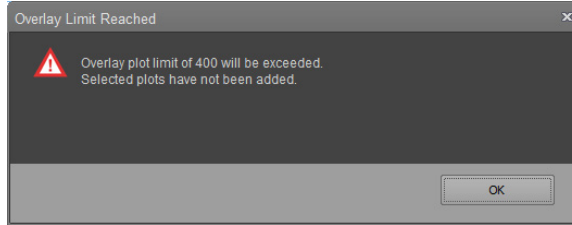
Persistence of Overlay settings

All plots, options, and settings defined on the Overlays tab persist at the Experiment level.

Adding plots to Overlays

- Source plots from a Workspace can be sent to a new or existing Overlay in several ways:
 - Right-click on any empty area (i.e., white space) within the Workspace view to open the *Workspace view context menu*.
From here, select **Send All Plots to Overlay** (page 90) and all plots within the Workspace of the single Sample will be overlaid on top of each other.
 - Right-click on any empty area within the boundary of a single plot to open the *Plot context menu*.
From here, select **Send to Overlay** (page 93) and select the appropriate overlay from the dropdown menu. The selected plot will be added to the selected overlay.
 - Alternatively, click the **Overlay Builder** button on the Overlay ribbon tab to create an overlay using *Overlay Builder* (page 133). 
- Each plot is added to the selected Overlay plot and to its respective Gallery.
- By default, when plots are added to a new Overlay, they are arranged in the order of addition.
When new plots are added to an existing Overlay, they are added to the end of the Gallery.
- By default, New Overlay plots are displayed in the Overlay mode (page 119).
- Gates present on the Source plots in the Workspace are not added to the Overlay.

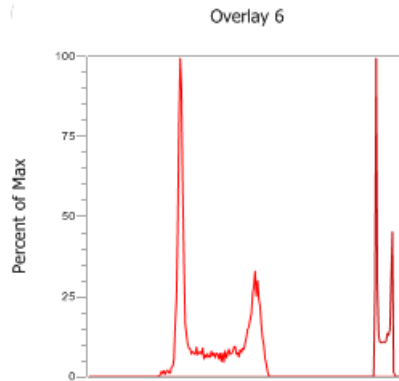
- Range and scale settings of the Source plots are applied to the Overlay plots. The scale setting of the newly added Source plot always takes precedence over any existing scaling set in the Overlay.
- An Overlay can contain a maximum of 400 Source plots. If the addition of new plots would cause the Overlay to exceed the 400 plot limit, the software displays a warning dialog.



Clicking **OK** closes the dialog without adding the new plots to the Overlay.

Comparing plots with different scale and range settings

- Plots with different scale settings (i.e., linear, log, Logicle, or HyperLog™) or different scales of the same type may be compared on the same Overlay.



- Plots with different range settings may be compared on the same Overlay. When comparing plots with different range settings, axis labels behave as described on page 127.



Note: The comparing plots with different scale and range, the settings of individual plots remain unchanged.

Selecting plots

Selecting a single plot

- Single Overlay or Gallery plots are selected by clicking on a plot.
The selected plot is surrounded by a **light blue selection rectangle**.

Selecting multiple plots

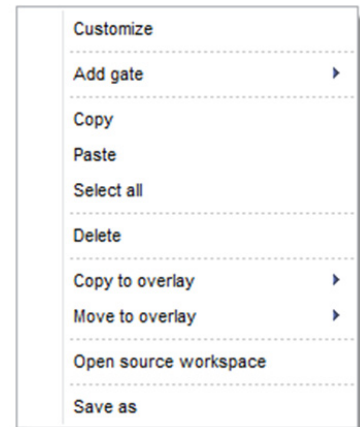
- To select multiple plots, click on the desired plots while holding down the **Ctrl** key.
Objects which are part of the multi-select are bounded by **dark blue multi-selection rectangles**.
- Alternatively, drag a selection rectangle using the mouse to select several plots within the same area.
When drawing a selection rectangle, the selection process must be initiated outside a currently selected plot to avoid dragging the current plot to a new position.
- Clicking on a selected plot while holding down the **Ctrl** key de-selects the plot.

Selecting all plots

- Pressing the **Ctrl+A** key combination when an Overlay plot is selected, selects all of the plots in the Overlays area.
- Pressing the **Ctrl+A** key combination when a Gallery plot is selected, selects all of the Gallery plots for the selected Overlays.

Moving and copying plots between Overlays

- Plots may be moved by right-clicking the plot in the Gallery to open the *Gallery plot context menu* and selecting **Move To Overlay** (page 131).
Alternatively, plots may be moved from one Overlay to another by the cutting and pasting, using the standard **Ctrl+X** (cut) and **Ctrl+P** (paste) key combinations.
- You can copy a plot from one Overlay to another by using **Copy** and **Paste** options or the **Copy to Overlay** option on the *Overlay plot context menu* (page 130) or by using the standard **Ctrl+C** (copy) and **Ctrl+P** (paste) key combinations..



Copying plot images

You can copy images of the selected plots in the Overlay view to the clipboard for pasting in other applications. This is accomplished by using the **Copy** option on the *Home ribbon tab* (page 40) or using the standard **Ctrl+C** key combination.

Resizing plots

- All Overlay plots within the Overlay area and Gallery plots within the Gallery area have a uniform size. You can resize Overlay and Gallery plots independently.
- Turning the **mouse scroll wheel** while holding down the **Ctrl** key resizes the Overlay or Gallery plots depending on where the mouse pointer is situated, provided that dragging is not currently taking place.
- If either an Overlay plot or a Gallery plot is selected, you can use the **Size Slider found on the bottom right** on the *Application Status Bar* (page 37) to resize the currently selected plot type.

Overlay plot links to Source plot

Link to Live Data of the Source Plot

- Overlay plots are automatically updated to reflect the changes made to them in the Workspace, except for the gates created on Source plots in the Workspace area, which are not displayed on the Overlay plots.
- If a single-parameter plot is changed to a dual-parameter plot or vice versa, the plot is removed from its respective Overlays.

Source Plot Not Available

- When Source plots are deleted from the Workspace or a sample is deleted, their corresponding plots are removed from the Gallery and Overlay plots.

Empty Overlays

- If all plots in the same Gallery are deleted, the Overlay itself is automatically deleted.

Auto-Refresh

- When **Auto-Refresh** is selected on the *Home ribbon tab* (page 40), all plots and statistics in the Overlays tabs are updated automatically in the background as the Workspace is modified.
- If Auto-Refresh is disabled or the system has not yet retrieved the image, the Overlays only show data from source locations that are up-to-date.
- When Auto-Refresh is disabled, the Gallery plots for the source locations that need to be refreshed display the *out-of-date watermark* stating “Source plot out of date” on the plot.



The Gallery plots for the source locations that do not contain data are left blank.



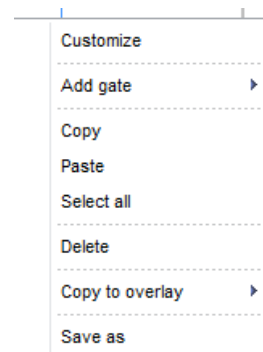
- Only Gallery plots containing data (no watermark) are shown in the Overlay plot.

Overlay Plot Context Menu

Overview

The *Overlay Plot context menu* is displayed when an area within the plot boundary is right clicked.

The Overlay Plot context menu contains the commands used for customizing the Overlay, adding gates to Overlay plots, for cutting, copying, pasting, and deleting selected plots, for sending selected plots to a selected Overlay, and saving all selected items as a new graphics file.



Overlay Plot context menu options

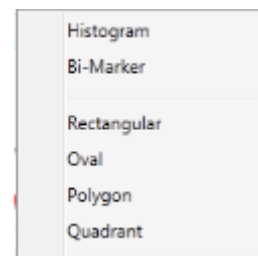
Customize

- **Customize** opens the *Customize panel*, which allows you to modify the appearance of the selected Overlay plot as described in “Customize Overlay Options”, page 232.
- If the panel is closed, it will be opened and brought to the front of other open panels.

Add gate

- Selecting **Add gate** allows creation of a new gate in the default position on the currently selected plot. If multiple plots are selected, this option adds a gate to a single plot.

The Histogram option is the only available for Histogram plots; Rectangular, Oval, Polygon, and Quadrant options are available only for dual-parameter plots.



- Selecting these options creates the selected gate type in the default position described in “Creating and deleting gates” on page 77.
- The maximum number of gates allowed is 5, as described on page 121.
- The Quadrant option is unavailable if a Quadrant gate has been created on the current plot; all other options are unavailable once the gate limit has been reached.
- The gate set on the selected Overlay plot appears on all Gallery plots for the current Overlay and also appears on the Overlay plot.

Copy

Copy copies the selected objects to the clipboard, keeping the original objects on the Workspace.

Paste

Paste inserts the objects currently in the clipboard onto the current Overlay.

Select all

Select all option selects all Overlay plots.

Delete

Delete removes all selected Overlay plots and deletes all of the associated Gallery plots.

Save As

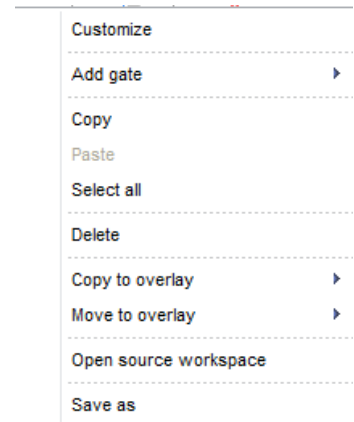
- Selecting **Save As** displays the *File Save (Export) dialog* as described on page 363, which allows you to send all selected items to a new graphics file.
- The layout of the graphics file will replicate the layout of the selected items on the current Workspace, and the plot contents will be based on the options selected in the *Export Options* menu as described on page 340.
- The selection area for saving may only cover one page.
- The available image file formats are, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile (EMF). The default format is JPG.

Gallery Plot Context Menu

Overview

The *Gallery Plot context menu* is displayed when any within the Gallery plot boundary is right clicked.

The Gallery Plot context menu contains the commands used for customizing the Overlay, adding gates to Overlay plots, for cutting, copying, pasting, and deleting selected plots, for sending selected plots to a selected Overlay, and saving all selected items as a new graphics file.



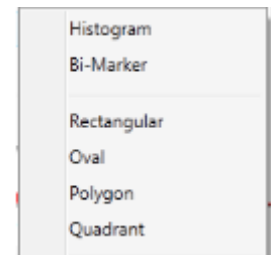
Gallery Plot context menu options

Customize

- Selecting **Customize** displays the *Customize Overlay options panel*, which allows you to customize the appearance of the selected Gallery plot as described on page 232.
- If the panel is closed, it will be opened and brought to the front of other open panels.

Add gate

- Selecting **Add gate** allows a new gate to be created in the default position in all gallery plots as described on page 77.
- The Histogram option is only available for Histogram plots; Rectangular, Oval, Polygon, and Quadrant options are available only for dual-parameter plots.
- The maximum number of gates allowed is 5, as described on page 121.
- The Quadrant option is unavailable if a Quadrant gate has been created on the current plot; all other options are unavailable once the gate limit has been reached.



Copy

Selecting **Copy** copies the selected plots to the clipboard, while keeping the original plots on the Workspace.

Paste

Selecting **Paste** inserts the previously copied Gallery plots onto the current Overlay plot. The plots are appended at the end of the existing plots.

Select all


Select all option selects all Gallery plots for the current Overlay plots.

Delete

Selecting **Delete** removes selected Gallery plots and removes them from the Overlay.

Copy to Overlay


- **Copy to Overlay** sends a copy of selected plots within a Gallery to a selected Overlay, while retaining the original plots in the Gallery.
- A new Overlay may be created by selection of **New Overlay** or send the plots to an existing Overlay that contains plots with the same number of parameters.
- Overlays with non-matching number of parameters are listed in the context menu, but they cannot be selected.
- Similarly, if the selected number of plots would cause an Overlay to exceed the allowable number of plots, that Overlay cannot be selected from the menu.



New Overlay
Overlay1
Overlay2
Overlay3

Move to Overlay

- **Move to Overlay** moves the selected plots within a Gallery to a selected Overlay. The Gallery does not retain the plot that is moved.
- A plot may be moved to either a **New Overlay** or an existing overlay.
- Overlays with non-matching number of parameters are listed in the context menu, but they cannot be selected.
- Similarly, if the selected number of plots would cause an Overlay to exceed the allowable number of plots, that Overlay cannot be selected from the menu.



New Overlay
Overlay1
Overlay2
Overlay3

Save As

- Selecting **Save As** displays the *File Save (Export) dialog* (page 363), which allows you to send all selected items to a new graphics file.
- The layout of the graphics file replicates the layout of the selected items on the current Workspace, and the plot contents are based on the options selected in the *Export Options* menu as described on page 340.
- The selection area for saving may only cover one page.
- The available image file formats are, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile (EMF). The default format is PNG.

Overlay Builder

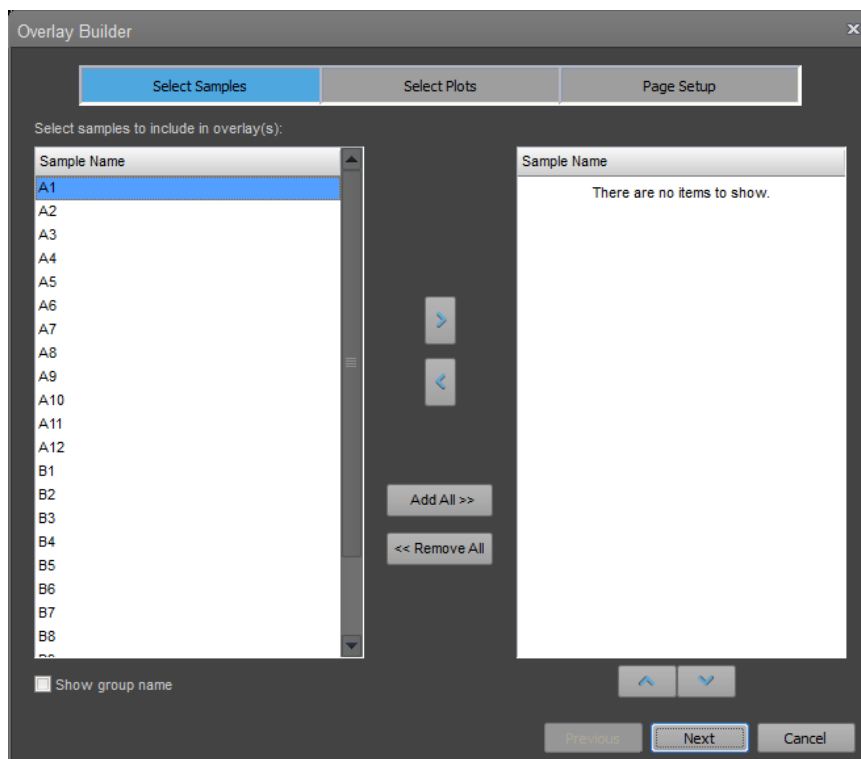
Overview

The *Overlay Builder* tool guides you through the process of creating new Overlay plots within a single experiment. The Overlay builder allows the selection of samples and plots to include into a new Overlay and determines how the selected plots are displayed.

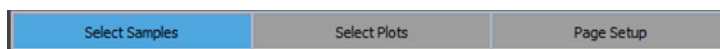


Note: Overlay of plots from different experiments is not permitted.

- To launch the Overlay Builder, click the **Overlay Builder** button on the Overlay ribbon tab (page 62). This button is only enabled when at least one plot exists on the Experiment Workspace and the instrument is not acquiring.



- The Overlay Builder consists of three tab, listed below, that are displayed sequentially to guide you through the process of building a New Overlay.
 - **Select Samples** (page 134)
 - **Select Plots** (page 136)
 - **Page Setup** (page 137)
- The top portion of the Overlay Builder contains the *progress indicator*, which shows the current page highlighted.

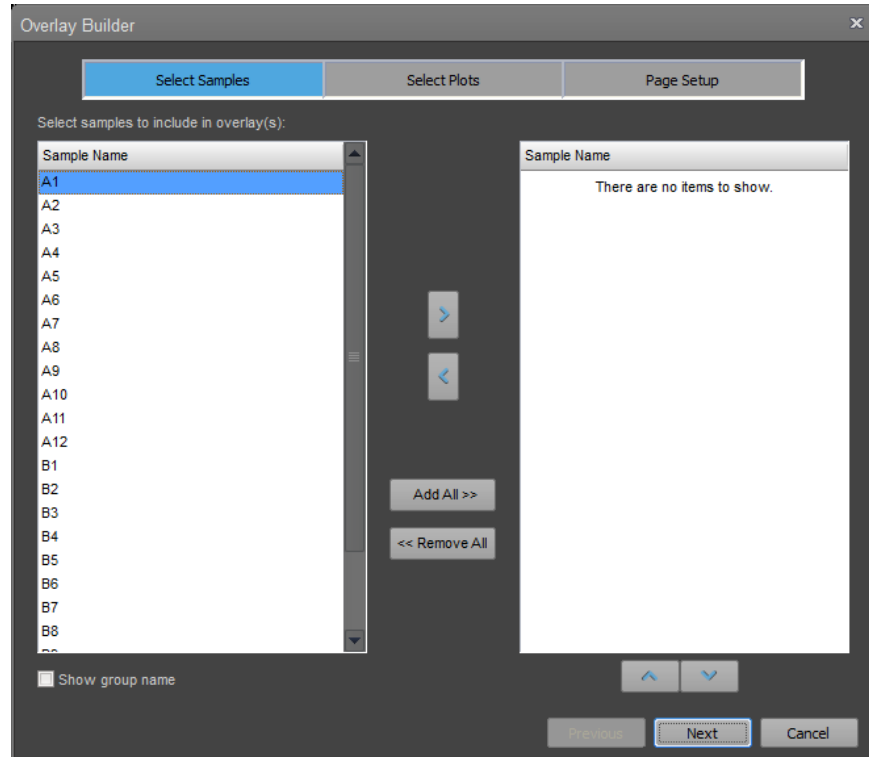


- Clicking **Next** moves to the next tab of the Overlay Builder. This button is not displayed on the last tab of the builder (Page Setup tab), where it is replaced by the Finish button.
- Clicking **Previous** returns to the previous tab of the builder. This button is not active on the first tab of the builder (Select Samples tab).

- Clicking **Finish** closes the Overlay Builder and creates a new Overlay based on the selections made in the Overlay Builder.
- Clicking **Cancel** closes the Overlay Builder without creating Overlay plots. Any changes made with the builder are discarded.
This button is available on all pages of the builder.
- The default title for a new Overlay is “*Overlay n*”, where n is a numerical suffix (first available integer) that ensures that the title is unique.
Gallery plot titles display the sample name by default.

Select and Order samples

The **Select Samples** tab of the Overlay Builder allows you to select of sample for inclusion in the New Overlay and reorder the selected samples, if desired.



Sample lists

- The *Available Samples panel* on the left contains a list of all samples in the current Experiment based on the Experiment hierarchy.
By default, this list only displays the sample name.
Checking the **Show group name** checklist enables the display of the group name in front of the sample name (e.g. group1\sample1).
- The *Selected Samples panel* on the right contains a list of the samples that are selected to be displayed in the Overlay plots. By default this list is empty.
- Sample names only appear in one list at a time.
- You can transfer Samples between the two lists using the **arrow** buttons (page 135) located between the two panels.

- You can select multiple samples in a list simultaneously using one of the following methods:
 - To select non-consecutive samples in a list, press and hold down the **Ctrl** key, then click each **sample** that you want to select.
 - To select a consecutive group of samples in a list, click the **first sample**, press and hold down the **Shift** key, then click the **last sample**.
 - Use the **Ctrl+A** key combination to select all samples in the currently active list.
- Vertical and horizontal scroll bars will appear in each list as needed.

Arrow buttons

- Click the **right arrow** to transfer all selected samples from the Available Samples panel to the Selected Samples panel.
The right arrow button is only enabled when one or more samples are selected in the Available Samples list.
- Click the **left arrow** to transfer all selected samples from the Selected Samples panel to the Available Samples panel.
The left arrow button is only enabled when one or more samples are selected in the Selected Samples list.

Add All button

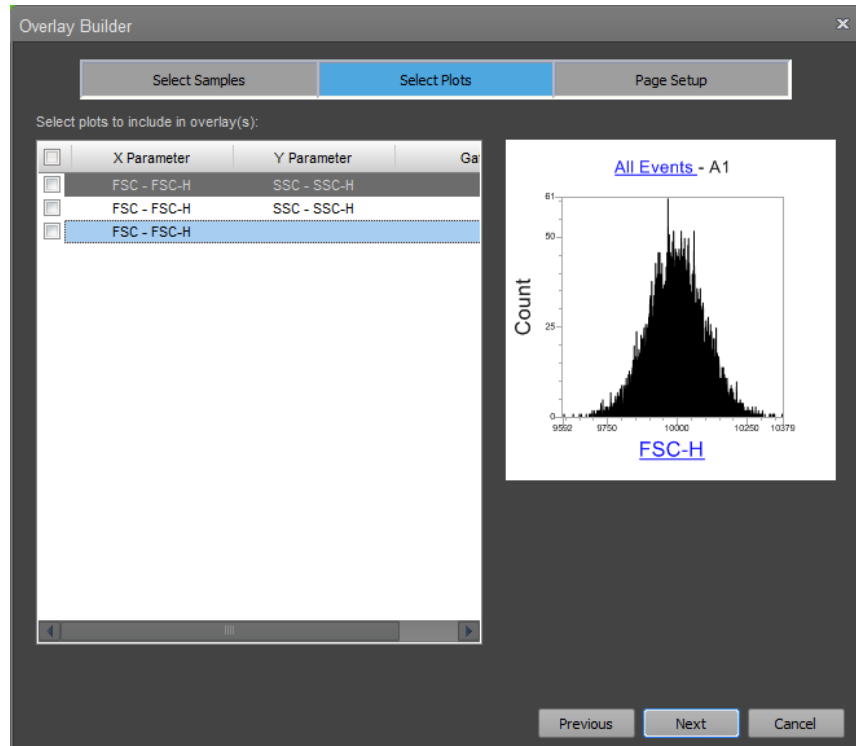
- Click **Add All** to transfer all samples from the Available Samples panel to the Selected Samples panel.

Remove All button

- Click **Remove All** to transfer all samples from the Selected Samples list to the Available Samples panel.

Select Plots

The Select Plots tab allows you to select plots to display them on an Overlay plot.



- The select plots tab contains the Plot Selection Table (left panel) and a preview image of the currently selected plot (right panel).

Plot Selection Table

- The Plot Selection Table lists all of the plots present on the Experiment-level Workspace of the current Experiment in the order they were created.

<input type="checkbox"/>	X Parameter	Y Parameter	Gate
<input type="checkbox"/>	FSC-A	SSC-A	
<input type="checkbox"/>	FSC-A		
<input type="checkbox"/>	FSC-A		

The Plot Selection Table consists of X parameter, Y parameter name, and parent gate name columns, and an additional checkbox column to the left of the plot title used for selecting the plots of interest.

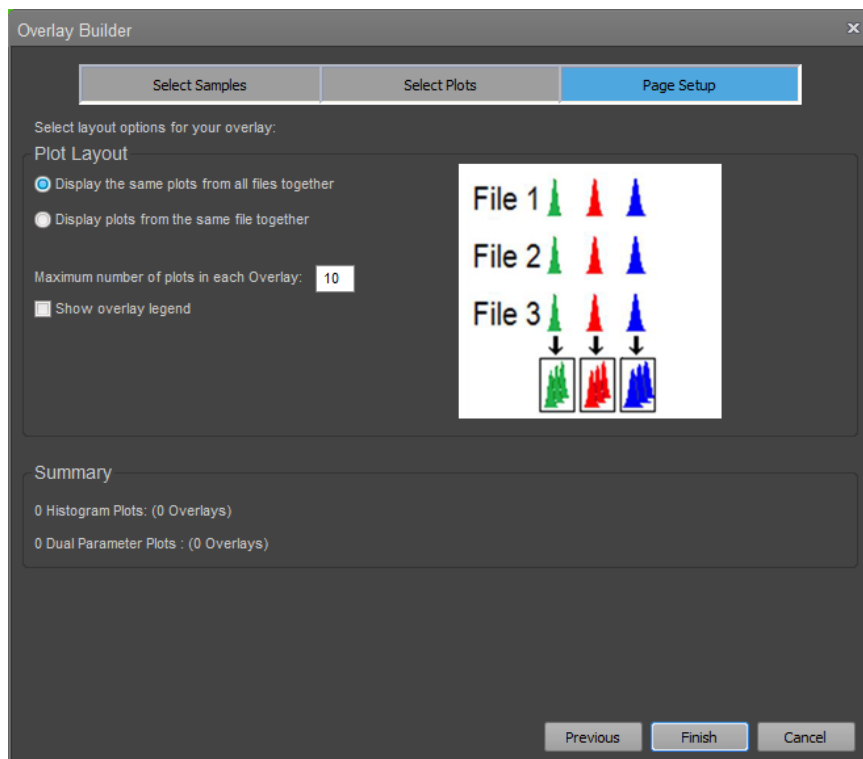
- To display one or more plots on an Overlay plot, select the relevant **checkboxes** next to the plot of interest.
- To display all of the plots listed in the plot selection list, click the **column heading checkbox**.
- Uncheck the **column heading checkbox** to deselect all individual plots.
- If one or more of the individual plot checkboxes are deselected, the column heading checkbox is also unchecked.
- By default, no plots are selected for overlay and all checkboxes are deselected.
- Vertical and horizontal scroll bars will appear in the list as needed.

Plot Preview

- Click on a single row in the plot selection list to highlight the selected row and display a preview of the selected plot in the preview area.
- The preview displays the selected plot with data from the first selected sample together with any gates and labels that are present on the plot.
- You cannot format plots or modify gate positions on the preview image.

Page Setup

Page Setup allows you to set the display options for the selected plots.



- The Plot Layout section contains options to control how selected plots from selected files are added to new Overlay plots.

Display the same plots from all files together

- The **Display the same plots from all files together** option is the default option. It creates overlay plots that include data from all samples (multiple files) within an experiment as shown below.

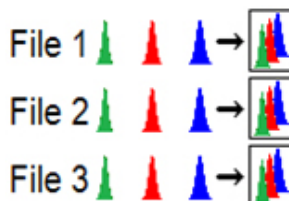


- When the **Display the same plots from all files together** option is selected, Overlay plots created are generated according to the rules listed below:
 - Selected Histogram plots are placed in an Overlay plot based on the X-axis parameter. Each parameter is contained in a different Overlay plot. If only one histogram plot is selected for overlay, only one histogram overlay plot is generated. If multiple plots are selected, multiple histogram overlay plots are generated.
 - If the number of plots set in the **Maximum number of plots** (page 139) is exceeded at any time, additional Overlay plots are created to contain the remaining single-parameter Histogram plots from each file.

- All selected dual-parameter plots are contained in an Overlay plot based on like X- and Y-axis parameters. Each parameter combination is placed in a different Overlay plot.
If only one dual parameter plot is selected for overlay, only one overlay plot of dual parameter data is generated.
If multiple plots are selected, multiple dual parameter overlay plots are generated.
- If the number of plots set in the **Maximum number of plots** (page 139) is exceeded at any time, additional Overlay plots are created to contain the remaining dual-parameter Histogram plots from each file.

Display plots from the same file together

- When the **Display plots from the same file together** option selected, each Overlay plot generated contains plots from a single Sample (one FCS file) within the Experiment and not multiple Samples as shown below. This option is useful if fluorescence emission is compared between parameters, for example spillover of FITC into PE.



- When the **Display plots from the same file together** is selected, Overlay plots are created according to the rules listed below:
 - All single-parameter Histograms specific for the Sample are placed in a single Overlay plot.
 - If the number of plots set in the **Maximum number of plots** (page 139) is exceeded at any time, additional Overlay plots are created to contain the remaining single-parameter Histogram plots from the same file.
 - All selected dual-parameter plots specific for the Sample are placed in a single Overlay plot.
 - If the number of plots set in the **Maximum number of plots** (page 139) is exceeded at any time, additional Overlay plots are created to contain the remaining dual-parameter Histogram plots from the same file.

Maximum number of plots

- The **Maximum number of plots** edit box allows you to specify the maximum number of plots to be added to an Overlay plot by the Overlay Builder. If the requested plots exceed this number, new Overlay plots are created as required.
- The default number of plots in an Overlay is 10 plots.
The minimum number of plots is 1.
The maximum number of plots is 400.
If you attempt to enter a number outside this range, the number is automatically adjusted to the closest limit.
- Only numeric characters are allowed to be entered.
- If the number entered is greater than the number of samples or plots, then the Overlay is created with the number of samples or plots.

Show Overlay legend

- If the **Show Overlay legend** option is checked, Overlay plots are created with the legend panel displayed as described on page 120.

Number of plots indicator

- The **Number of plots indicator** shows the number of Source plots and Overlay plots created for both Histogram and dual parameter plots.

9. Experiment Explorer

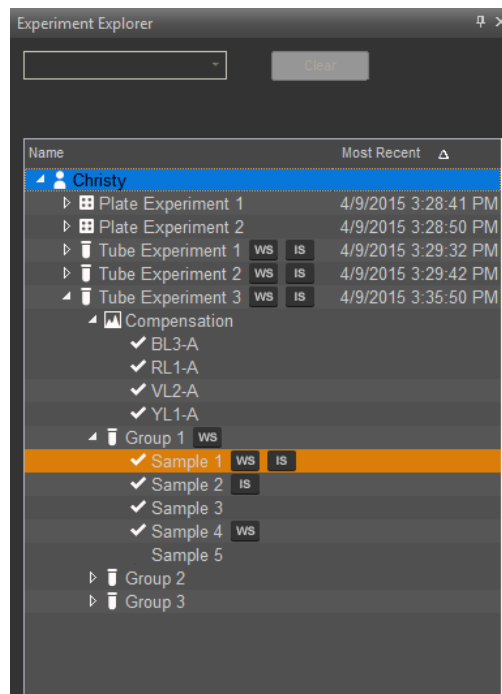
Overview

Experiment Explorer functions as an interface for creating, viewing, and managing *Experiments*.

Experiment Explorer is displayed both on the Main Menu (page 20) and the Attune™ Desktop (page 23). By default, it is docked on the right side of the application window.

- Files view section (page 141) displays the User folders, which contain all Experiment files in a hierarchal view.
- The Files view section can contain multiple folders, each with multiple Experiments.
- Each Experiment can have more than one *Group*, each with one or more *Samples*.

Each Sample is associated with its own FCS file.



Experiment Explorer icons

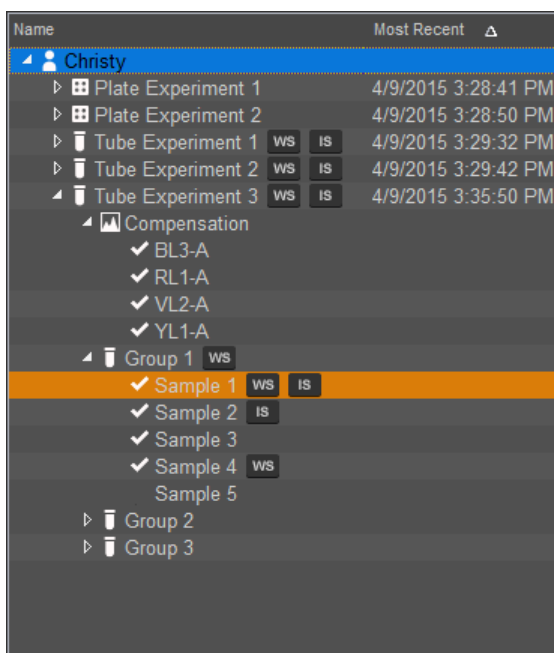
Experiment Explorer uses the following icons to indicate the various elements and their conditions within the Files view.

Icon	Indication	Icon	Indication
	User		Compensation
	Plate Experiment node		Read-only compensation
	Tube Experiment node		Workspace
	Group node		Instrument Settings
	Sample with recorded data		Compensation Settings

Files view

Organization

Files view shows all Experiments and Samples generated by the current user or the Samples selected as a result of a filter being applied.

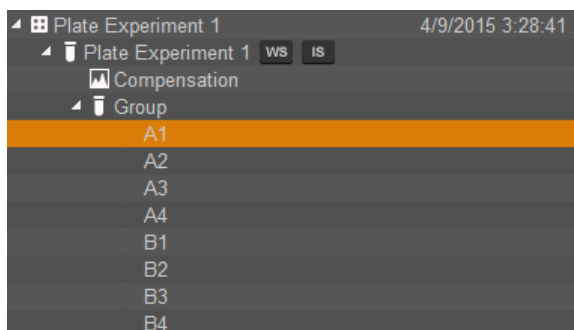


- By default, the *User folder* is displayed on top of the files list and bears the name of the user currently logged in.
- The files in the folders are arranged in a tree structure, which displays the hierarchy of Plates, Experiments, Groups, and Samples.
The tree structure varies for Plate and Tube-based Experiments (see “Experiment hierarchy”, page 142).
- Indicator icons next to individual nodes or files in the hierarchy indicate the presence of Workspaces (**WS**) and Instrument Settings (**IS**).
- You can use the *folder context menu* (page 148) to add a new Plate or Tube Experiment to any folder.
- When you create a new Tube or Plate Experiment, the Experiment hierarchy is expanded by default.
- When items are selected in the Experiment Explorer, the entire row is highlighted as specified.

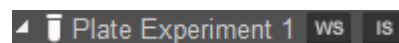
Experiment hierarchy

Plate hierarchy

The Experiment Explorer displays the Plate Experiments in a defined hierarchy with a tree structure.

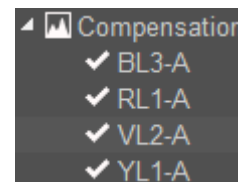


- When you create a new Plate, the software automatically creates a Plate Experiment containing a Compensation node. However, the software does not create any Groups or Samples for new Experiments, unless specified in the *New Experiment dialog* (page 303).
- A Plate can contain one Plate Experiment, which has an associated Workspace, as well as Instrument and Compensation settings.



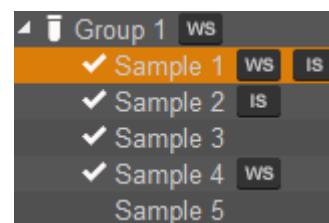
Indicator icons to the right of the Experiment name indicate the presence of Experiment-level Workspace (WS) and Instrument (IS) settings.

- The Experiment-level compensation settings may have multiple associated Compensation samples, which are displayed in laser and detector order.
- When compensation is recorded for a Compensation sample, the check mark icon (✓) appears to the left of the Compensation sample name, indicating that the Sample has recorded data.



If Compensation has not been recorded for a Sample, then no icon will appear.

- Each Experiment that has assigned Samples consists of one or more Groups. A Workspace indicator icon to the right of the Group name indicates the presence of Group-level Workspace.

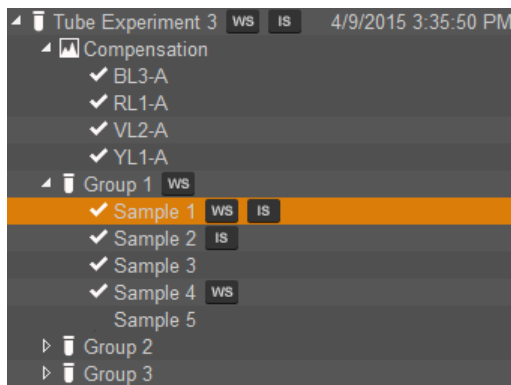


Each group may contain up to 400 Samples. Samples may be derived from a well within a Plate or a Tube.

- Each Sample may have a Sample-level Workspace. A Sample may also have Sample-level Instrument settings used instead of the Experiment defaults. Indicator icons to the right of the Sample name indicate the presence of Sample-level Workspace (WS) and Instrument (IS) settings.
- When the Sample is recorded, the check mark icon (✓) appears to the left of the Sample name, indicating that the Sample has recorded data. If the Sample has not been recorded, then no icon will appear.

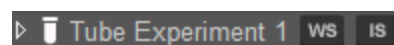
Tube hierarchy

The Experiment Explorer displays the Tube Experiments in a defined hierarchy with a tree structure.



- When you create a new Tube Experiment, the software automatically creates a Compensation node in the hierarchy. However, the software does not create any Groups or Samples for new Experiments, unless specified in the *New Experiment dialog* (page 303).

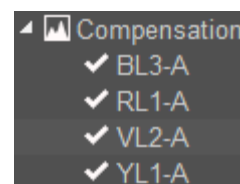
- Each Tube Experiment has an associated Workspace, as well as Instrument and Compensation settings.



Indicator icons to the right of the Experiment name indicate the presence of Experiment-level Workspace (WS) and Instrument (IS) settings.

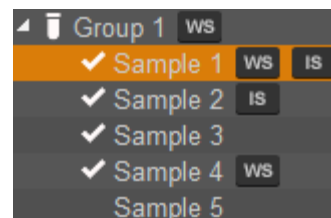
- The Experiment-level compensation settings may have multiple associated Compensation samples, which are displayed in laser and detector order.

- When compensation is recorded for a Compensation sample, the check mark icon (✓) appears to the left of the Compensation sample name, indicating that the Sample has recorded data.



If compensation has not been recorded for a Sample, then no icon will appear.

- Each Experiment that has assigned Samples consists of one or more Groups. A Workspace indicator icon to the right of the Group name indicates the presence of Group-level Workspace.



Each group may contain up to 400 Samples. Samples may be derived from a well within a Plate or a Tube.





- Each Sample may have a Sample-level Workspace. A Sample may also have Sample-level Instrument settings used instead of the Experiment defaults. Indicator icons to the right of the Sample name indicate the presence of Sample-level Workspace (WS) and Instrument (IS) settings.

- When the Sample is recorded, the check mark icon (✓) appears to the left of the Sample name, indicating that the Sample has recorded data.


If the Sample has not been recorded, then no icon will appear.

Experiment Explorer behavior

Selection indicators Colors are used to indicate various states of selection within the Experiment Explorer. When items are selected, the entire selected row in the Files view, including the name and date columns, is highlighted as specified below.

- **Mouse over:** When the mouse is moved over an item in the Experiment Explorer, the item is highlighted in gray. 
- **Selected item:** When an item is selected in the Experiment Explorer, the item is highlighted in dark blue. 
- You can select multiple items at once by holding the **Ctrl** or the **Shift** key while clicking. Items which are multi-selected are highlighted in light blue, except the last selected item, which is highlighted in dark blue. 
- **Active item:** When an item is loaded into the current Workspace, the Sample name in Experiment Explorer is highlighted in orange. 

Selecting

- Double clicking on the Plate node, Plate or Tube Experiment node, or the Group node sets the focus to the *Heat Map view* (page 105) for that Experiment. If another Experiment was already open, this action opens the first Sample within the selected Experiment.
If no Sample exists within the selected Experiment, then double-clicking the node opens the Experiment-level Workspace and sets the Experiment node as active. The Workspace dropdown menu will be disabled.
- Double-clicking on the Compensation node or on any Compensation sample sets the focus to the Compensation Workspace for the selected Sample and the *Compensation tab* become available in the ribbon bar (page 50).
- If the main Compensation node is double-clicked and *Compensation Controls* exist, the first Compensation Control Sample is selected and the focus is set to the *Workspace view* (page 63).
If no Compensation Controls exist, double-clicking on the Compensation node opens the *Compensation Setup dialog* described on page 284.
Double-clicking the Compensation node showing the “read-only” icon () opens the *Compensation Matrix* (page 298) for that Experiment.
- Double-clicking on a Sample opens the Experiment-level Workspace for that Sample and loads the data, if present. This sets the focus to the Workspace view for the selected Sample.
- During acquisition, the active Sample may not be changed
- During acquisition, double-clicking on the active Sample’s Plate node, Plate or Tube Experiment node, or the Group node sets the focus to the Heat Map view for the active Experiment.
- The double-clicking on any other node or object will not have an effect.


Drag and drop

Workspace

A Workspace () icon can be dragged from an Experiment, Group, or Sample, and dropped to another Experiment, Group, or Sample.

- Dragging and dropping a Workspace within the Experiment Explorer creates a copy of the Workspace at the dropped location. The copy of the Workspace will have contents identical to the original, but it will not be linked to it.
- Experiment-level Workspace icon can be dragged to another Experiment, Group, or Sample, copying the Workspace to the Experiment, Group, or Sample, as appropriate.
- Group-level Workspace icon can be dragged to another Experiment, Group, or Sample, copying the Workspace to that respective level.
- A Sample-level Workspace can be dragged to another Experiment, Group, or Sample, copying the Workspace to that respective level.

Instrument Settings (IS)

An Instrument Settings () icon can be dragged from an Experiment or Sample to another Experiment, Group, or Sample.

- Experiment-level IS icon can be dragged to another Experiment or empty Samples, updating the IS for the Experiment or the Samples, as appropriate. If the Experiment contains acquired Samples that have Experiment-level IS, the Samples will be updated to display the Sample IS indicator.
- Experiment-level IS icon can also be dragged to a Group folder to apply the Experiment IS to all unrecorded Samples within that Group. If this is done within the same Experiment, any Sample-level IS that that previously existed within that Group is cleared.
- Experiment-level IS cannot be dragged to an Experiment that has existing compensation.
- If the Experiment contains acquired Samples that already have Sample-level IS, there will be no effect on these Samples.
- Sample-level IS icon can be dragged to other Experiments or empty Samples.
- Sample-level IS icon can be dragged to a Group folder to apply the IS to all unrecorded Samples within that Group.
- Dragging a Sample-level IS icon to an Experiment updates the Experiment IS. When this is done, the Sample-level IS icon is removed from the Sample, which is set to use the Experiment-level IS. All other Sample-level IS icons remain regardless of their similarity to the current Experiment-level IS settings.
- Sample-level IS cannot be dragged to a Sample with acquired data.
- The Instrument Settings icon cannot be dropped on an Experiment, Group, or Sample where the instrument configuration does not match. In this case, the drop action will result in the warning dialog being displayed with the message "The instrument settings are not supported by the current instrument configuration and cannot be imported".

Compensation Settings (CS)

A Compensation Settings (CS) icon can be dragged from one Experiment to another.

- The Compensation Settings icon is only shown next to an Experiment if the Experiment has compensation defined.
- The Compensation Settings icon can be dragged from one Experiment to another, which copies the Compensation Settings and the corresponding Instrument Settings to that Experiment.
- When Compensation Settings are applied to another Experiment via drag and drop, they are applied as read-only compensation based on an XML file. This is indicated in the software by a padlock icon adjacent to the compensation control.
- The Compensation Settings can only be applied to another Experiment if the instrument models match and the target Experiment does not already have compensation defined.
- The rules for drag and drop of the Compensation Settings icon follow the same guidelines described for dragging an IS icon to an Experiment-level IS.

Experiment Explorer context menus

Overview

Experiment Explorer has context menus associated with different levels of the hierarchy in the tree structure. You can access each context menu by right-clicking the appropriate object in the tree structure.

- The Experiment Explorer context menus include the following:
 - User context menu (page 148)
 - Plate context menu (page 149)
 - Experiment context menu (page 150)
 - Group context menu (page 152)
 - Sample context menu (page 154)
 - Compensation node context menu (page 156)
 - Compensation sample context menu (page 157)
- Unless indicated otherwise, all Experiment Explorer context menu items are always visible and enabled.
- When a combination of different objects is selected in the Experiment Explorer (e.g. Experiment, Group, and Samples), the right-click context menus are not available.

Validation of name fields

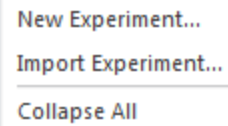
Context menu options that allow you to create or edit names of items follow the validation rules outlined below:

- **Duplicate** context menu option duplicates the selected object and displays the name in a textbox in the Edit mode.
- **Rename** context menu option displays the current name of the selected object in a textbox in the Edit mode.
- You can rename a node by pressing the **F2** key to enter the Edit mode.
- Clicking anywhere outside the edit textbox or pressing **Enter** validates the entered name and closes the Edit mode.
- If a valid name is present in the textbox, the name is updated.
- Leading and trailing spaces are removed on validation. Consecutive spaces are converted to single spaces on validation.
- If you attempt to enter invalid characters into the edit textbox, a warning pop-up dialog indicates the error condition and the invalid characters do not appear in the textbox.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- If the entered name already exists, when focus is lost a warning pop-up dialog indicates that the entered name already exists and the textbox remains in Edit mode.

User context menu

Right-clicking on the **User folder heading** displays the *User folder context menu*, which allows you to create New Experiments from scratch or using a template, or to import pre-defined Experiments.

During acquisition, only the *Collapse All* option is enabled (see below).



New Experiment...
Import Experiment...
Collapse All

New Experiment

- **New Experiment** opens the *New Experiment dialog* (page 303), which allows you to create a new Plate or Tube Experiment or to import FCS files into a new Analysis Experiment.
- The New Experiment option is not available on any Shared Folder context menus or during acquisition.

Import Experiment

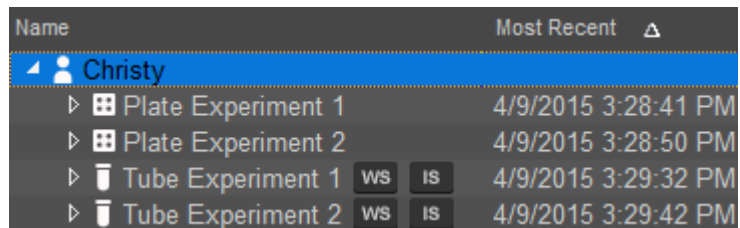
- **Import Experiment** opens the *File Open (Import) dialog* (page 365), which allows you to import pre-existing Plate- or Tube-based Experiments.
- The Import Experiment option is not available during acquisition.

New Experiment Using Template

- **New Experiment Using Template** opens the *New Experiment from Template Dialog* (page 307), which allows you to select a Plate- or Tube-based Experiment as a template for a new Experiment.
- The New Experiment Using Template option is not available during acquisition.

Collapse All

- **Collapse All** collapses all expanded nodes down to the Plate or Tube Experiment-levels for the current Folder.



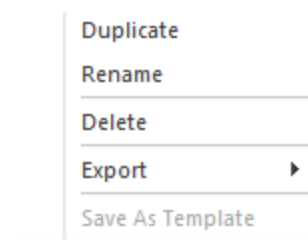
Name	Most Recent
Christy	
▶ Plate Experiment 1	4/9/2015 3:28:41 PM
▶ Plate Experiment 2	4/9/2015 3:28:50 PM
▶ Tube Experiment 1 WS IS	4/9/2015 3:29:32 PM
▶ Tube Experiment 2 WS IS	4/9/2015 3:29:42 PM

- The Collapse All option is always available.

Plate context menu

Right-clicking on a **Plate icon** or **name** in the User folder displays the *Plate context menu* for the User folder. The Plate context menu contains the *Duplicate*, *Rename*, *Delete*, *Export*, and *Save as Template* commands.

During acquisition, all options in the Plate context menus are disabled.



Duplicate

- **Duplicate** allows you to duplicate the selected plates and their full hierarchy (i.e., Experiment, Compensation, Groups, and Samples).
- The Duplicate option is enabled when single or multiple plates are selected. This option is disabled if the Plate Experiment Instrument settings do not match the system configuration
- All Workspaces (Experiment-, Group-, and Sample-level), Run Protocols, and annotations are preserved when a Plate Experiment is duplicated. Only the Experiment-level Instrument settings are preserved; Sample-level Instrument settings are not duplicated. FCS files are not duplicated.
- If Compensation settings are available, the newly created Plate contains Experiments with empty Compensation wells or tubes as defined in the source Experiment.
- The newly created Plate must have a unique name. Once a Plate is duplicated, it is automatically assigned a name, which consists of the same name as the current Plate plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.
- The Plate name appears in an edit textbox ready for editing. The newly entered name is validated as described on page 147.

Rename Plate

- **Rename** opens an edit textbox containing the name of the current Plate in the edit mode.
- The Rename option is enabled when a single plate is selected.
- Clicking anywhere outside the edit textbox validates the Plate name as described on page 147.
- You can also rename a selected Plate by pressing **F2** to enter the editing mode.

Delete Plate

- **Delete** opens the *Deleted Items dialog* (page 158), which allows you to delete the selected Plate(s).
- The Delete option is enabled when single or multiple Plates are selected.

Export Plate

- **Export ► Plate** option opens the *File Save (Export) dialog* (page 363), which allows you to save the selected Plates in the desired location.
- The Export option is enabled when single or multiple Plates are selected.

Save As Template

Save As Template opens the *Save As Template dialog*, which allows you to name the current Plate and save it as a template in database for future re-use.

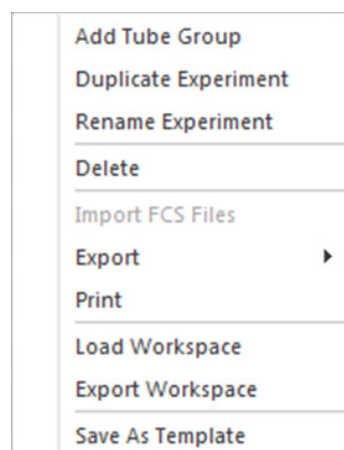
Experiment context menu

Right-clicking on a **Tube Experiment** in the User folder displays the *Tube Experiment context menu* for the User folder.

Right-clicking on a **Plate Experiment node** in the User folder displays the *Plate Experiment context menu* for the User folder.

Add Tube Group

- **Add Tube Group** opens the *New Group dialog* (page 307), which allows you to add a Group to the end of the currently selected Experiment.
- You can create up to 100 Groups for each Experiment.
- The Add Tube Group option is only enabled when a single Experiment is selected.



Duplicate Experiment

- **Duplicate** allows you to duplicate the selected Experiments(s) with their associated Groups and Samples.
- The Duplicate option is visible only for Tube Experiments, and it is enabled only when single or multiple Experiments are selected.
This option is disabled if the Experiment Instrument settings do not match the system configuration
- All Workspaces (Experiment-, Group-, and Sample-level), Run Protocols, and annotations are preserved when an Experiment is duplicated.
Only the Experiment-level Instrument settings are preserved; Sample-level Instrument settings are not duplicated. FCS files are not duplicated.
- If Compensation settings are available, the newly created Experiment contains a Compensation node with empty Samples as defined in the source Experiment.
- The newly created Experiment must have a unique name. Once an Experiment is duplicated, it is automatically assigned a name, which consists of the same name as the current Experiment plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.
- The Experiment name appears in an edit textbox ready for editing. The newly entered name is validated as described on page 147.

Rename Experiment

- **Rename** opens an edit textbox containing the name of the current Experiment in the edit mode.
- The Rename option is enabled when a single Experiment is selected.
- Clicking anywhere outside the edit textbox validates the Experiment name as described on page 147.
- You can also rename an Experiment by pressing **F2** to enter the editing mode.

Delete Experiment

- **Delete** opens the *Deleted Items dialog* (page 158), which allows you to delete the selected Experiment(s).
- The Delete option is enabled when single or multiple Experiments are selected.

Save As Template

Save As Template opens the *Save As Template dialog*, which allows you to name the current Experiment and save it as a template in database for future re-use.

Export Experiment

- **Export** displays a sub-menu containing *Experiment*, *Statistics*, and *FCS Files* as options.
- The Export option is enabled when single or multiple Plates are selected.
- **Export ► Experiment** option opens the *File Save (Export) dialog* (page 363), which allows you to save the selected Experiment(s) in the desired location.



Experiment
Statistics
FCS Files

Load Workspace

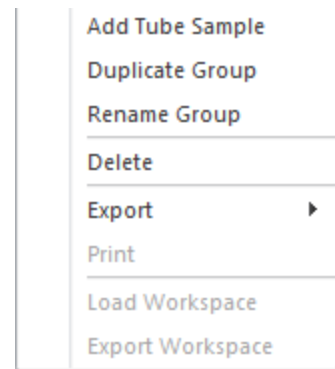
- **Load Workspace** option opens the *File Open (Import) dialog* (page 365), which allows you to select a Workspace to import.
- The selected Workspace will replace the existing Experiment-level Workspace.
- The Load Workspace option is only enable when a single Experiment is selected.

Export Workspace

- **Export Workspace** option opens the *File Save (Export) dialog* (page 363) which allows you to select a name and location to save the Workspace file.
- The Export Workspace option is only enable when a single Experiment is selected.

Group context menu

Right-clicking on a **Group node** in the User folder displays the *Group context menu* for the User folder.



Add Tube sample

- **Add Tube sample** adds a Tube sample to the selected Group.
- You can create up to 400 Samples for each Experiment. The option is disabled if there are already 400 Samples within the Experiment.
- This option is only enabled when a single Group is selected.

Duplicate Group

- **Duplicate** allows you to duplicate the current Group within the current Experiment, which creates an exact duplicate with the same number of Tubes and Sample-level Workspaces.

The FCS files and Sample-level Instrument settings are not duplicated.

- If duplicating a Group results in the creation of over 400 Samples for that Experiment, the new Group will be created with as many Samples as possible until the 400 Sample limit is reached.
- The Duplicate option is available for Tube Experiments and for Groups within Plate Experiments containing only Tube samples. It is enabled only when a single Group is selected.
- The newly created Group must have a unique name. Once a Group is duplicated, it is automatically assigned a name, which consists of the same name as the current Group plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.
- The Group name appears in an edit textbox ready for editing. The newly entered name is validated as described on page 147.

Rename Group

- **Rename** opens an edit textbox containing the name of the current Group in the edit mode.
- The Rename option is enabled when a single Group is selected.
- Clicking anywhere outside the edit textbox validates the Group name as described on page 147.
- You can also rename a selected Group by pressing **F2** to enter the editing mode.

Delete Group

- **Delete** opens the *Deleted Items dialog* (page 158), which allows you to delete the selected Groups.
- The Delete option is not available for Shared Folders. It is only enabled when single or multiple Groups are selected in the User folder.

Remove Group Workspace

- **Remove Group Workspace** removes the Group-level Workspace of the selected Groups. This option is only available if a Group-level Workspace is present in at least one of the selected Groups.

Load Workspace

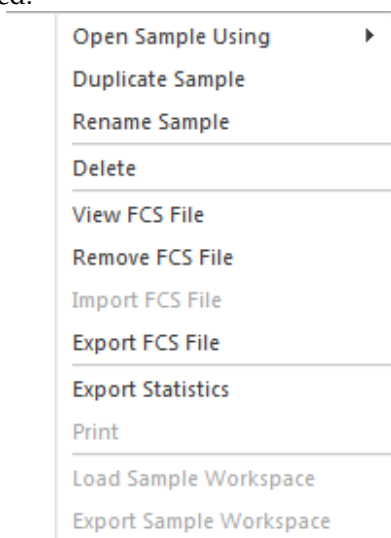
- **Load Workspace** option opens the *File Open (Import) dialog* (page 365), which allows you to select a Workspace to import.
- The selected Workspace will replace the existing Group-level Workspace.
- The Load Workspace option is only enabled when a single Group is selected.

Export Workspace

- **Export Workspace** option opens the *File Save (Export) dialog* (page 363) which allows you to select a name and location to save the Workspace file
- This option is only enabled if a Group-level Workspace exists for the selected Group.
- The Export Workspace option is only enabled when a single Group is selected.

Sample context menu

Right-clicking on a **Tube sample** or **Plate sample** in the User folder displays the *Sample context menu*. During acquisition, all options in the Sample context menu are disabled.



Tube sample context menu

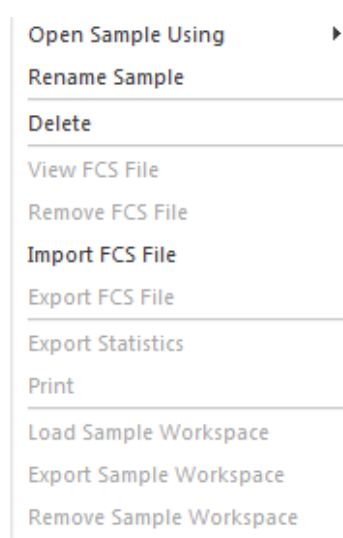
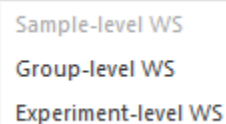


Plate sample context menu

Open Sample Using

- **Open Sample Using** opens a secondary menu with the choice to open the selected data file using the Sample, Group, or the Experiment Workspace.
- This option enabled only when a single Sample is selected.
- The Group and Sample options are enabled only if the selected Sample has a Group or Sample Workspace, respectively.



Duplicate Sample

- **Duplicate** creates a duplicate of the current Tube within the current Group. The Sample-level Workspace is copied, if present. The Sample-level Instrument settings and the FCS file are not duplicated.
- The Duplicate option is available only for Tube samples, and it is enabled only when a single Sample is selected.
- You can create up to 400 Samples for each Experiment. If the 400 Sample limit has been reached for the Experiment, this option is disabled.
- Each new Sample is created at the end of the current Group and must have a unique name. Once a Sample is duplicated, it is automatically assigned a name, which consists of the same name as the current Sample plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.
- The Group name appears in an edit textbox ready for editing. The newly entered name is validated as described on page 147.

Rename Sample

- **Rename** opens an Edit textbox with the name of the current Sample in edit mode.
- The Rename option is enabled when a single Sample is selected.
- Clicking anywhere outside the edit textbox validates the Sample name as described on page 147.

Delete Sample

- **Delete** opens the *Deleted Items dialog* (page 158), which allows you to delete the selected Samples.
- It is only enabled when single or multiple Samples are selected.

Remove FCS File

- **Remove FCS File** opens the *Deleted Items dialog* (page 158).
- Confirming the Delete command removes the data file from the selected Samples and sends the files to the recycle bin.
Empty Sample entries are retained in the hierarchy if files are removed.
- This option is visible and enabled if one or more of the selected Samples have data present.

Import FCS File

- **Import FCS File** opens the *File Open (Import) dialog* (page 365), which allows you to select a data file to import.
- This option is visible but disabled if the selected Sample contains FCS data. It is not available when multiple Samples are selected.

Export FCS File

- **Export FCS File** allows you to save the selected FCS file in the desired location.
- If a single Sample is selected for export, the Export FCS File option opens the *File Save (Export) dialog* (page 363), which allows you to save the FCS file associated with the selected Sample using a custom name.
- If multiple Samples are selected for export, this option opens the *File Browser dialog* on page 366, which allows you to create or to select a destination folder for exporting multiple FCS files.
- When exporting FCS files, you have the option to choose the FCS file name and the FCS version. Alternatively, you can save the FCS files in the form of a text file (tab delimited).
- If the Experiment Compensation settings are different from the Compensation settings contained within the FCS file, a prompt to update the compensation keyword is displayed.

Load Workspace

- **Load Workspace** option opens the *File Open (Import) dialog* (page 365), which allows you to select a Workspace to import.
- The selected Workspace will replace the existing Sample-level Workspace.
- The Load Workspace option is only enabled when a single Sample is selected.

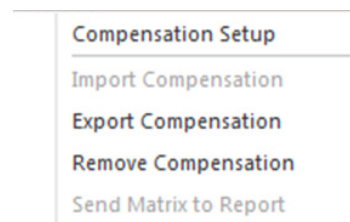
Export Workspace

- **Export Workspace** option opens the *File Save (Export) dialog* (page 363) which allows you to select a name and location to save the Workspace file
- This option is only enabled if a Sample-level Workspace exists for the selected Group.
- The Export Workspace option is only enabled when a single Sample is selected.

Compensation node context menu

Right-clicking on the main **Compensation node** displays the *Compensation node context menu*.

During acquisition, all options in this context menu are disabled.



Compensation Setup

- **Compensation Setup** opens the *Compensation Setup dialog* (page 284), which allows you to choose the Compensation Source, Compensation Measurement, Background Fluorescence Mode, and the required compensation parameters.
- This dialog also allows you to make modifications to an existing compensation setup.

Export Compensation

- **Export Compensation** opens the *File Save (Export) dialog* (page 363), which allows you to select a name and location to save the compensation file (*.ACS).
- This option is enabled only when all compensation samples have been acquired.

Import Compensation

- **Import Compensation** opens the *File Open (Import) dialog* (page 365), which allows you to select a compensation file (*.ACS) to import.
- Importing compensation creates Compensation samples with a read only attribute (🔒).

Remove Compensation

- **Remove Compensation** removes the compensation settings and existing Compensation samples, and opens the *Deleted Items dialog* (page 158).

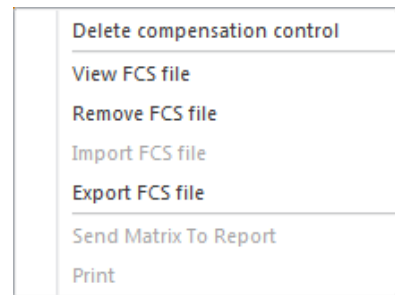
Compensation sample context menu

Right-clicking on a **Compensation sample** displays the *Compensation sample context menu*.

During acquisition, all options in this context menu are disabled.

Delete Compensation Control

- **Delete Compensation Control** opens the *Deleted Items dialog* (page 158), which allows you to delete the selected Compensation sample.



View FCS File

- **View FCS File** opens the *FCS sample Information Panel* (page 239) and brings it to the foreground. The FCS sample Information panel displays the FCS metadata (the text segment portion) for the selected Compensation sample.
- This option is enabled only for the active Compensation sample containing data.

Remove FCS File

- **Remove FCS File** opens the *Deleted Items dialog* (page 158).
- Confirming the Delete command removes the data file from the selected Compensation samples and sends the files to the recycle bin.
Empty Compensation sample entries are retained in the hierarchy, if the files are removed.
- This option is visible and enabled if one or more of the selected Compensation samples have data present.

Import FCS File

- **Import FCS File** opens the *File Open (Import) dialog* (page 365), which allows you to select a data file to import.
- This option is visible but disabled if the selected Compensation sample contains FCS data. It is not available when multiple Compensation samples are selected.

Export FCS File

- **Export FCS File** allows you to save the selected FCS file in the desired location.
- If a single Compensation sample is selected for export, the Export FCS File option opens the *File Save (Export) dialog* (page 363), which allows you to save the FCS file associated with the selected Sample using a custom name.
- If multiple Compensation samples are selected for export, this option opens the *File Browser dialog* (page 366), which allows you to create or to select a destination folder for exporting multiple FCS files.

In this case, the FCS files saved are automatically named with Experiment Name and Sample Name with an FCS extension.

Deleted items dialog

The *Deleted items dialog* appears when you select **Delete** from the context menus on the Experiment Explorer or the Heat Map view, or press the **Delete key** on the keyboard to delete Tube samples, Samples, Compensation samples, Groups, Experiments, or Plates.

- All relevant prompts occur before the deletion process begins.
- Click **Yes** to move the selected FCS files to the recycle bin and remove the items from the Experiment Explorer.
- Click **Cancel** or **X** to close the dialog box (if the last item in the list) without performing an action.

Do this for all other cases (x found) checkbox

- This checkbox appears only if multiple Experiments, multiple Groups, or multiple Samples are selected. It is unchecked by default.
- The warning displayed by the checkbox states “Do this for all other selected items (x found)”, where x is the number of selections.
- If checked, all of the other selected items selected are subjected to the same command.
- If left unchecked, the dialog reappears for the each selected item in the queue.

10. Collection panel

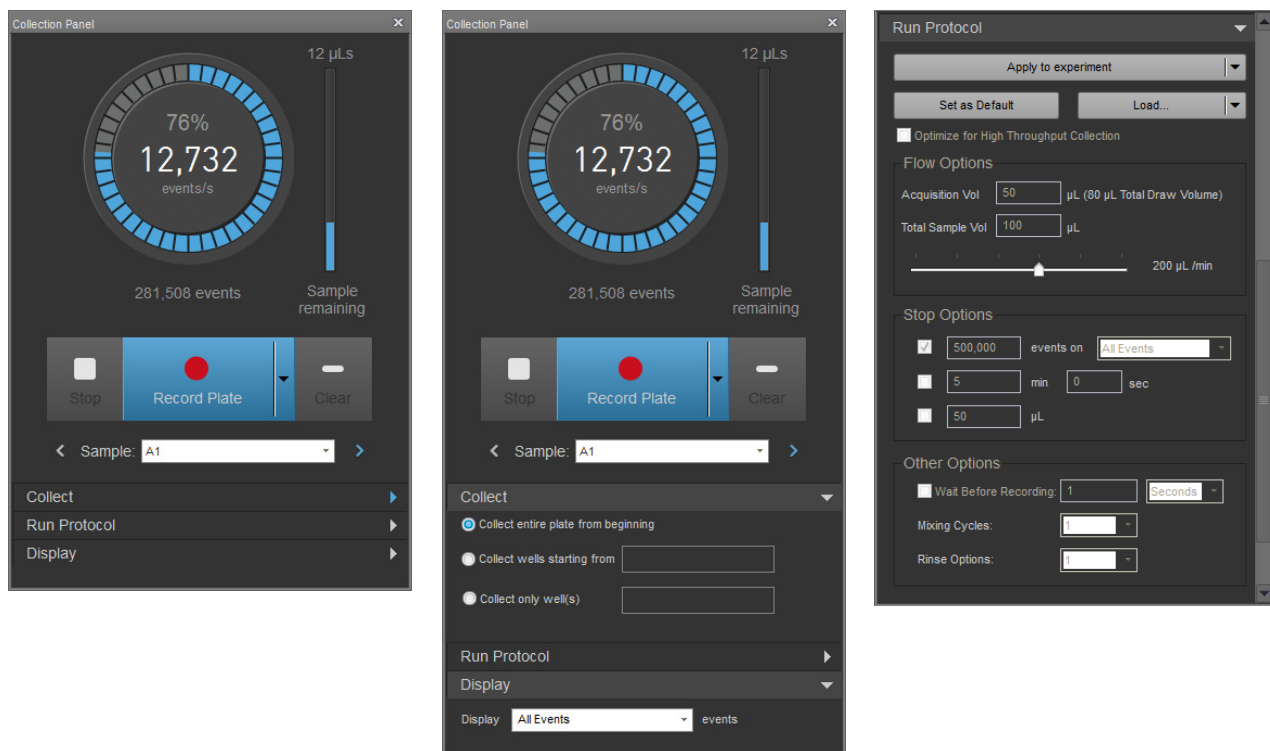
Overview

The Collection panel contains controls and indicators used for acquisition of samples. The appearance of the Collection panel varies depending on the sample source (i.e., Plate or Tube) and the setup of the Experiment. By default, it is docked to the left of the Main Application area (page 28).

- The Collection panel is organized into four functional groups:
 - *Acquisition status* (page 160),
 - *Collection controls* (page 161),
 - *Collect and Display options* (page 164)
 - *Run Protocol* (page 172).

By default, only the Run Protocol group is expanded.

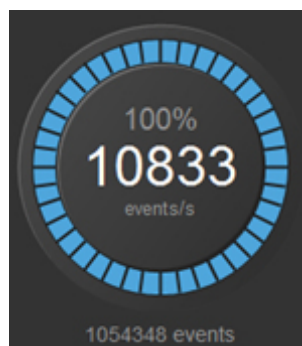
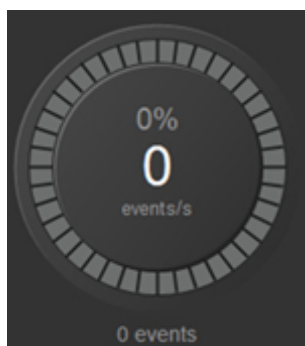
- The groups can be expanded or collapsed by clicking on the group header.
- If the Collection panel is resized, the acquisition status indicators (progress dial and sample remaining progress bar) and the collection controls (collection and sample navigation buttons) will remain horizontally centered in the panel.
- The images below show the Collection panel with all the groups collapsed (left), with the Collect and Display groups expanded (middle), and the Run Protocol group expanded (right).



Acquisition status

Acquisition status indicators

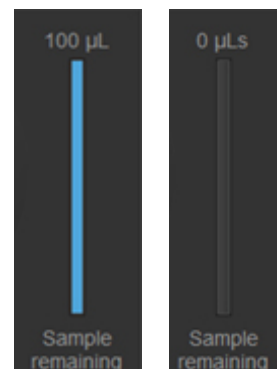
The *acquisition status indicators* display information about the current acquisition status of the instrument.



- *Events per second (events/s)* field displays the number of events currently being collected per second. This field is set to zero and does not change unless the system is acquiring data.
- *Events* field displays the total number of events collected. This value is reset to zero at the start of an acquisition, the start of recording the current sample, or when the **Clear** button is clicked.
- *Progress dial* displays the progress of the current acquisition related to stop conditions set in the *Stop options* as described below (page 175). The *percent progress* is displayed inside the dial above the events per second field.
- If **Complete Stop Condition** is selected (page 171), the progress dial and the events field will resume from where they left off.
- If multiple stop conditions are selected, the progress dial displays the condition that has progressed furthest towards being met.
- When a sample is being run but not recorded, the progress dial does not increment.

Sample remaining indicator

- The *sample remaining indicator* displays the remaining volume of the current sample that is being acquired.
- The *progress bar* shows the amount of sample remaining in relation to the acquisition volume set as part of the Run Protocol (page 173).
- The sample remaining volume cannot exceed the acquisition volume.



Collection controls

Overview

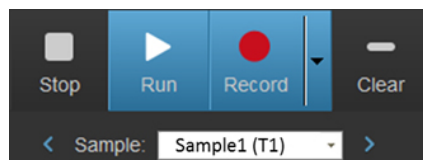
The *collection controls* allow you to prompt the Attune™ NxT cytometer to run your samples and record flow cytometric data.

- The collection controls are contextual; the options that are displayed depend on the tube or the type of well that is being acquired. Controls that are not available as a function in a particular context are not shown or shaded gray on the panel.
- If a plate is being acquired, the default controls are the *plate collection controls* (see “Collection Controls – Plate”, page 164).
- If a tube is being acquired, only the *tube collection controls* are available as described in “Collection Controls – Tube and Manual wells”, below.

Collection controls – Tube and Manual wells

Overview of Tube collection controls

The collection controls for Tube and Manual well samples are similar to the controls used for Plate samples; however, the middle button is a split button by default. The Sample navigation and Sample dropdown buttons are arranged below the basic controls.



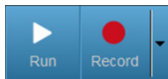
- Run, Record, Stop, and Clear buttons are the base set of buttons.
- Run/Record and Run/Save split buttons have an additional dropdown control that allows the navigation through acquisition tasks. The acquisition task dropdown is not available for Tube samples.
- When any button within the *Functions group* of the *Instrument tab* (page 48) is clicked, all plate collection control buttons are disabled and grayed out until the process is completed, except the navigation buttons, the acquisition task dropdown, and the Save button.
- Run and Record buttons are disabled for any active sample where the sample instrument settings do not match the system instrument configuration and for any experiment where the experiment instrument settings do not match the system instrument configuration (e.g., RB vs. RBVY).
- During processing of single or multi-draw, the collection panel state does not change if the tube lifter is dropped.

Buttons displayed

Collection controls for Tube samples are contextual; the buttons that are displayed depend on the acquisition task and the type of Sample being acquired. The collection control buttons that are available are described below.

Middle button options

The buttons that occupy the middle section of the collection controls are used for running and recording Sample, and saving data.



Run/Record: Clicking **Run** (on the left) starts the acquisition of the selected Sample; clicking **Record** (on the right) initiates the recording of the selected Sample.

- Run/Record split button is displayed by default when the system is ready for acquisition.
- The acquisition task dropdown is displayed only for Samples.

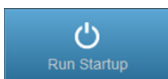


Run/Save: Clicking **Run** re-runs the selected Sample; clicking **Save** saves the data buffer to the FCS file for the selected Sample. If the Sample has existing data, you can append or overwrite the existing data.

- Run/Save split button is displayed when a Run mode acquisition has been completed (i.e., end of Sample reached or **Stop** is clicked).
- The acquisition task dropdown is displayed only for Samples.



Note: The buffer size is determined by the number of events set in the Display Events group. By default this is set to "All Events".



Run Startup: Initiates the *Startup* function.

- Run Startup button is displayed if Startup function has not been run prior to running samples or an error state requires Startup to be performed.

Left button options

The buttons that appear on the left side of the collection controls are used for stopping sample acquisition and for recovering unused samples.



Stop: Stops the acquisition or recording of the current sample immediately.

- Stop button is displayed by default when the system is in acquisition state (i.e., running or recording Sample) and while running the Startup function.



Recover: Recovers the unused sample to a tube.

- **Recover** button is displayed when the instrument is idle and there is a sample available to recover.
- Clicking **Recover** returns the unused sample to a tube.

Right button options

The right side of the collection controls displays the Clear button.



Clear: Clears the most recent data in the memory (see below).

- Clear button is displayed by default when the system is in acquisition state. It is also available post-acquisition when an existing sample has been overwritten in the Run mode.
- Clicking **Clear** during acquisition clears the data in memory and displays fresh data.
- Clicking **Clear** post-acquisition discards the most recent data and retains the original data.

Navigation buttons and Sample dropdown



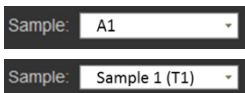
Previous: Allows navigation through previous tasks and through Samples pre- and post-acquisition.

- This navigation button is available for both well and Tube samples, and can navigate from well to Tube samples, and from tube to Well samples.
- The Previous button is active when the current sample is not the first sample in the task list. It is disabled during acquisition.
- The button tooltip for Well samples before recording has the following form: “<phase> (sample location)”, where <phase> is Record Plate.
- Once the plate is recorded, the tooltip for the wells is: “<well location>”. For example, “A1”.
- The tooltip for Tube samples has the form: “<sample name> (tube location)”, where (tube location) is T1 through T399. For example, “Sample 1 (T1)”.



Next: Allows navigation through samples pre- and post-acquisition, and through steps within each acquisition task.

- This navigation button is available for both well and Tube samples, and can navigate from well to Tube samples, and from tube to Well samples.
- The Next button is active when the current sample is not the last well of the plate. However, it is active for last Tube sample and creates a new Tube sample, if clicked. The button is disabled during acquisition.
- The button tooltip for Well samples before recording has the format: “<phase> (sample location)”, where <phase> is Record Plate.
- Once the plate is recorded, the tooltip for the wells is: “<well location>”. For example: “A1”
- The tooltip for Tube samples has the form: “<sample name> (tube location)”, where (tube location) is T1 through T400. For example, “Sample 1 (T1)”.
- If a tube new sample will be created by clicking **Next**, then the tooltip states: “New Sample”.



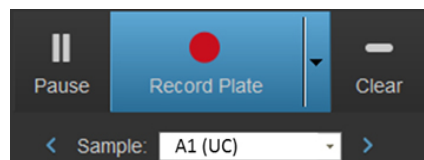
Sample dropdown: Allows the selection of any sample within the current acquisition task. The dropdown is disabled during acquisition.

- For Sample wells, only the well location is displayed. For example, “A2”.
- For Tube samples, the name will be: “<sample name> (tube location)”. For example, “Sample 1 (T1)”.

Collection controls – Plate

Overview of plate collection controls

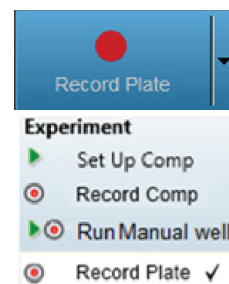
Plate collection controls are displayed in an array of three buttons that execute the basic commands for acquisition. The Sample navigation and Sample dropdown buttons are arranged below the basic controls.



- The state and the label of the buttons depend on the setup of the Plate, specifically the presence of Compensation Samples in addition to Well Samples.
- The *Setup*, *Run*, and *Record* buttons are disabled for:
 - any active Sample where the Sample-level Instrument Settings do not match the System Instrument Configuration.
 - any Experiment where the Experiment-level instrument settings do not match the System Instrument Configuration (e.g. RB vs RBVY).
- The dropdown arrow adjacent to the center button opens the *acquisition task list*, which allows you to select an acquisition task to perform. The dropdown arrow is available only for Plate samples.

The available tasks are:

- Set-up compensation
 - Record compensation for the current Experiment
 - Run and/or Record manual wells for the current Experiment
 - Record plate
- The tasks in the list have the same color as the Experiment.

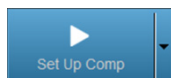


Buttons displayed

Plate collection control buttons and their functions are described below. The plate collection controls are contextual; the buttons that are displayed depend on the acquisition task and the type of well that is being acquired. Buttons not needed in a particular context are either not shown or disabled and shaded gray.

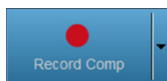
Middle button options

The buttons that occupy the middle section of the collection controls are used for running and recording samples, and saving data. The labels and the visibility and functionality of the buttons depend on the plate setup and acquisition context, as described below.



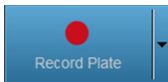
Set Up Comp: Initiates acquisition of compensation wells in the run mode.

- **Set Up Comp** button is displayed by default if the plate contains Compensation wells and a Compensation well is active. It is also displayed when **Set Up Comp** is selected from acquisition task menu.
- When the mouse pointer is hovered over the button, a tooltip states: “Allows optimization of Compensation control voltages”.



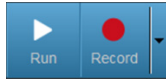
Record Comp: Initiates recording of data from all compensation wells defined in the “Collect options” (page 168).

- Record Comp button is displayed when **Record Comp** is selected from the acquisition task menu or after completing the *Set Up Comp* phase described above.



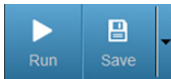
Record Plate: Initiates recording of data from all Sample wells defined in the “Collect options” (page 168).

- Record Plate button is visible and enabled by default when the system is ready for recording wells and the plate contains only Well samples.
- It is also displayed when **Record Plate** is selected from the acquisition task menu or after any compensation tasks have been completed.



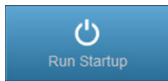
Run/Record: Clicking Run (on the left) runs the selected Sample; clicking Record (on the right) records the selected Sample.

- Run/Record split button is displayed by default if the plate contains only Well samples (no compensation), when Run is selected from the acquisition task menu or after the completion of any compensation tasks (if present).



Run/Save: Clicking Run re-runs the same Sample; clicking Save saves the data buffer to the FCS file for the sample that has just been run. If the Sample has existing data, you can append or overwrite the existing data.

- Run/Save split button is displayed at the end of running a Tube sample.



Run Startup: Initiates the *Startup* function.

- Run Startup button is displayed if Startup function has not been run prior to running samples or an error state requires Startup to be performed.

Left button options

The buttons that appear on the left side of the collection controls are used for stopping and pausing sample acquisition, and for recovering pre-loaded samples. The visibility and functionality of the buttons depend on the acquisition context, as described below.



Stop: Stops the acquisition or recording of Compensation wells immediately.

- Stop button is displayed by default during compensation setup and while recording compensation. It is also available during Startup.



Recover: Recovers the unused sample to a tube.

- Recover button is displayed when the instrument is idle and there is sample available to recover.
- Clicking **Recover** returns a preloaded sample back to a well; otherwise, the unused sample to a tube.

Right button options

The right side of the collection controls only displays the Clear button.



Clear: Clears the data of the current sample and displays fresh data.

- Clear button is always displayed. It is enabled during running or recording of all samples and Compensation wells.



Recover: Recovers the unused sample to a tube.

- Recover button is displayed when the instrument is idle and there is sample available to recover.
- Clicking **Recover** returns a preloaded sample back to a well; otherwise, the unused sample to a tube.

Navigation buttons and Sample dropdown



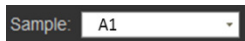
Previous: Allows navigation through previous tasks and through samples pre- and post-acquisition.

- This navigation button is available for both well and Tube samples, and can navigate from well to Tube samples, and from tube to Well samples.
- The Previous button is active when the current sample is not the first sample in the task list. It is disabled during acquisition.
- The tooltip for Tube samples has the form: “<sample name> (tube location)”, where (tube location) is T1 through T399. For example, “Sample 1 (T1)”.



Next: Allows navigation through samples pre- and post-acquisition, and through steps within each acquisition task.

- This navigation button is available for both well and Tube samples, and can navigate from well to Tube samples, and from tube to Well samples.
- The Next button is active when the current sample is not the last well of the plate. However, it is active for last Tube sample and creates a new Tube sample, if clicked. The button is disabled during acquisition.
- Once the plate is recorded, the tooltip for the wells is: “<well location>”. For example: “A1”
- The tooltip for Tube samples has the form: “<sample name> (tube location)”, where (tube location) is T1 through T400. For example, “Sample 1 (T1)”.
- If a tube new sample will be created by clicking **Next**, then the tooltip states: “New Sample”.



Sample dropdown: Allows the selection of any sample within the current acquisition task. The dropdown is disabled during acquisition.

- For Sample wells, only the well location is displayed. For example, “A2”.
- For Tube samples, the name will be: “<sample name> (tube location)”. For example, “Sample 1 (T1)”.

Button availability

- The plate collection control buttons are contextual; the buttons that are displayed depend on the acquisition task and the type of well that is being acquired.
- Record Plate, Stop, and Clear buttons are the base set of buttons for plates.
- Buttons are grayed out when they are not available in a particular context.
- All plate collection control buttons are disabled if the instrument is not ready for acquisition, between wells, and after **Stop** has been clicked but the acquisition of the current sample has not been completed.
- When any button within the *Functions group* of the *Instrument tab* (page 48) is clicked, all plate collection control buttons are disabled and grayed out until the process is completed, except the navigation buttons, the acquisition task dropdown, and the Save button.
- A “Bubble detected error” does not change the enabled/disabled state of a button.

Current well indicator

- The location of the current well and the sample type that is being analyzed is displayed above the collection control buttons.
- The indicator has the following format:
<well location> (Compensation designation)
- For example:
 - A1 = sample in well A1
 - A1 (M) = sample in Manual well A1
 - A1 (UC) = Unstained Control sample in well A1
 - A1 (BL1) = BL1 Compensation sample in well A1

Set Up Compensation workflow

Clicking the **Set Up Comp** button initiates the acquisition of the current Compensation well as indicated by the Current well indicator.

- Acquisition of the Compensation control continues until either **Stop** is clicked or the acquisition volume as defined by the Run Protocol (page 172) is exhausted.
- The current well remains active until the navigation buttons are used to move onto the next well within the compensation setup task or a different task is selected from the acquisition task dropdown list.
- To initiate the acquisition of the next Compensation control, **Set Up Comp** must be clicked again.
- Clicking the **Next** navigation button on the last sample within the compensation setup task advances the task button to **Record Comp**.

The order in which Compensation controls are acquired from a plate is in the same order they are listed in the Experiment Explorer:

Unstained control, Blue laser controls, Red laser controls, Violet laser controls, and Yellow laser controls.

Collect and Display options

Collect group overview

Collect group allows you to control which wells are collected during acquisition. The options in this group are available only for Plate Experiments and are not displayed for Tube samples.

- Collect group contains two options:
 - Collect entire plate from beginning
 - Collect wells starting from
- Collect group is collapsed by default and its display state persists for the user between sessions.
- If a combination of Tube and Well samples are selected in the Heat Map or in the Experiment Explorer, the options in the Collect group are grayed out and not available for changing.
- Wells can be selected in the Heat Map to populate the “Collect wells starting from” text box.
- If the Collection panel is in the Resume state and the Collect options are modified, the Collection panel will be reset to the pre-acquisition state.
- The order of collection is left to right, then top to bottom.



Collect options

Collect entire plate from beginning

Selecting **Collect entire plate from beginning** collects all samples on a plate.

- This option is only available for Plate Experiments, and it is the default option.
- If a plate layout is specified, acquisition starts at well A1.
- The buttons that occupy the middle section of the collection controls are used for running and recording samples, and saving data (see page 164).

Collect wells starting from

Selecting **Collect wells starting from** collects all samples, with acquisition starting at the specified well.

- The starting well is specified by entering its location in the textbox field. The entry format is the row letter followed by the column number (e.g., A1). Any letter typed will be automatically capitalized.
- The well location entered must be a valid location and entered in the correct format. If an invalid entry is made, a warning balloon describing the invalid entry is shown, and the acquisition control buttons are disabled until the invalid entry is corrected. The acquisition button status indicator updates according to the well type selected.
- Invalid entries include using incorrect format (e.g., 1A), specifying a location not mapped to the selected well, entering a location not on the selected plate type (e.g., H13 on a 96-well plate), using a character that is not alphanumeric.
- If a collect plate sequence is stopped, the location will be automatically updated to the next well to run.

Collect only well(s)

The **Collect only well(s)** option allows collection from the specified wells.

- Wells to be collected are specified by entering their location in the textbox field. The entry format is one or more well locations separated by a comma or a hyphen. A well location comprises a row letter followed by one or two digits for the column number (e.g., B11). Any letter typed will be automatically capitalized. Commas and hyphens may be used for multiple entries (see below), but all other non-alphanumeric characters are invalid.
- Multiple wells may be entered using a hyphen to indicate a range of wells and a comma to separate individual wells and ranges. White space will be ignored. For example: A1, B1– C12, D3.
- Where a range spans multiple rows, it will be interpreted as including all wells in between. For example: B2– C6 on a 96-well plate would be interpreted as comprising the wells B2 to B12, C1 to C6, inclusive.
- The well location entered must be a valid location and entered in the correct format. If an invalid entry is made, a warning balloon describing the invalid entry is shown, and the acquisition control buttons are disabled until the invalid entry is corrected. The acquisition button status indicator updates according to the well type selected.
- Invalid entries include using incorrect format (e.g., 1A), specifying a location not mapped to the selected well, entering a location not on the selected plate type (e.g., H13 on a 96-well plate), using a character that is not alphanumeric.
- The order of collection is based on the order of entry of well locations. All ranges are processed left to right, then top to bottom.
- Alternatively, wells may be selected directly from the Heatmap by pressing the “plate” indicator icon next to the textbox
- After the plate icon button is pressed, wells in the Heatmap may be selected by dragging the cursor over the desired wells. The Collect Only Wells textbox will be updated to reflect the selected wells as described above.

Heatmap Interaction

When the **Collect well starting from** or the **Collect Only Well(s)** option is selected, a Heatmap interaction control becomes visible.



- When the **Heatmap interaction** button is clicked, the Heatmap is brought to the foreground.
- The textbox will update the selected wells based on the wells that are selected on the Heatmap.
- For the “Collect wells starting from” option, only a single well can be selected. Each time the Heatmap is clicked, the selected well will be updated.
- For the “Collect only well(s)” option, only multiple wells can be selected by clicking and dragging them on the Heatmap or by pressing the **Ctrl** key while clicking to select non-adjacent wells.

Display option

Display option allows you control how many events are displayed during acquisition. It consists of a single dropdown control, which is available for both plate and tube experiments.

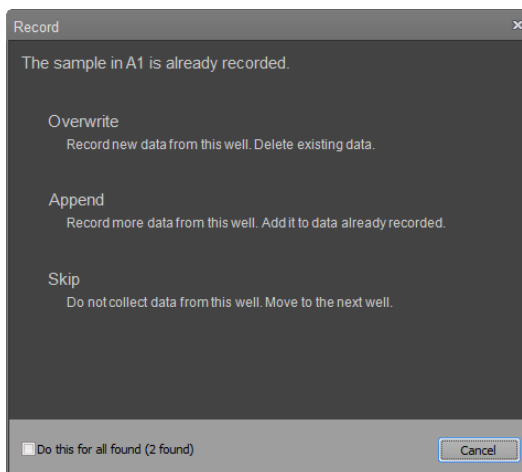


- The group is collapsed by default and its display state persists for the user between sessions.
- The default option is to display all events. The combo box dropdown contains the following choices: All Events, 10 000, 20 000, 50 000, 100 000, 250 000, 500 000, 1 000 000, 5 000 000. It also allows you to type in any value from 1 to a maximum of 20 000 000. Only integers are allowed.
- The selected number dictates the size of the rolling buffer of displayed events as they are acquired on a first-in, first-out basis. In the Run mode, events that are removed from the buffer are discarded. In the Record mode, events that are removed from the buffer are retained and saved.
- The size of the rolling buffer dictates the maximum number of events that may be saved **Save** is clicked at the end of an acquisition in the Run mode.

Record and Save dialogs

Plate Record dialog

When **Record** is selected for a Sample well that has already been collected, then the *Plate Record dialog* is displayed.



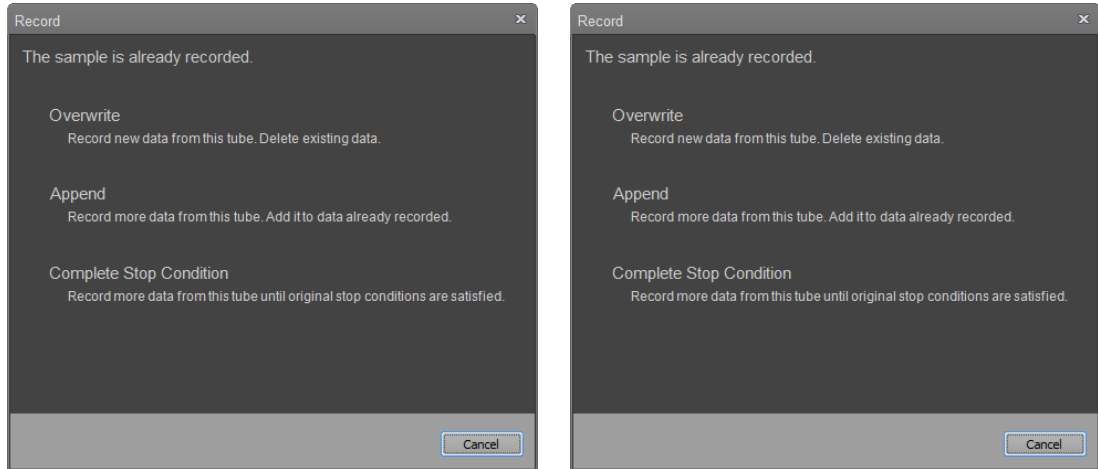
The dialog heading indicates the well location and the dialog offers the following clickable options:

- **Overwrite:** Discards the data already stored in the FCS file and collects new data. Only new data will be used for determining the stop condition.
- **Append:** Appends the new data to the data already stored in the FCS file. Only the new data is included in stop condition determination.
This option is not available if the existing data file was generated in another instrument or using a different laser configuration.
- **Skip:** Skips the well without recording.
- **Do this for all found:** Applies the selection to the indicated well and all the subsequent wells in the list that have already been collected. Otherwise the selection only applies to the well indicated in the dialog header. If there are not further wells for selection, then the next selection closes this dialog.
- **Cancel:** Closes the dialog with no action taken and the collection will not occur.

Tube sample Record/Save dialogs

When **Record** or **Save** is selected for a Tube sample that already has saved data associated with it, then one of the two dialogs is displayed with options to overwrite, append, complete stop condition, new sample, or cancel.

The dialog displayed depends on the user's actions and the stop conditions. If the stop condition has already been met, the dialog on the left is displayed. If the stop condition has not yet been met, then the dialog on the right is displayed.



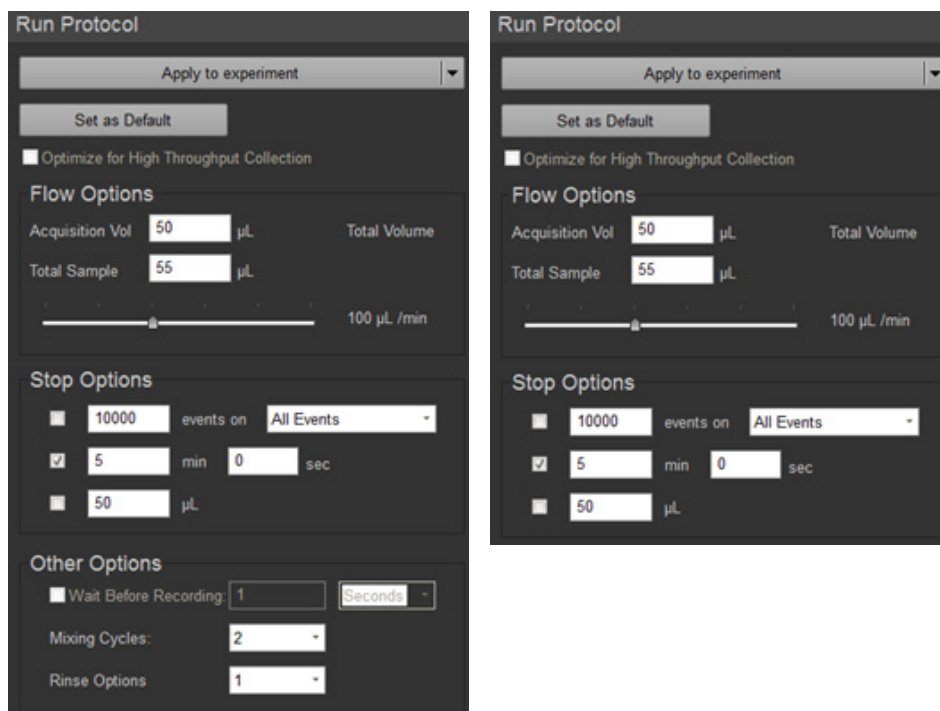
- **Overwrite:** Discards the data already stored in the FCS file and collects new data. Only new data will be used for determining the stop condition.
- **Append:** Appends the new data to the data already stored in the FCS file. Only the new data will be included in stop condition determination.
This option is not available if the existing data file was generated in another instrument or using a different laser configuration.
- **Complete Stop Condition:** Appends the new data to the data already stored in the FCS file. All data will be included in stop condition determination.
This option is available only if the stop condition has not already been met, the recording was not manually stopped, and the same sample is still being run (i.e., samples have not been switched).
- **Cancel:** Closes the dialog and leaves the operation in the Run mode.

Run Protocol

Overview

Run Protocol group allows you to define the collection criteria, including the Recording and Stop options. It is also used for determining Compensation Setup options, when available. By default, the Run Protocol group is expanded.

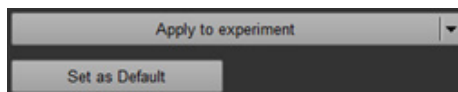
The options displayed in this group depend on the type of sample being run and the mode of collection. The images below show the Run Protocol options for Sample wells (left) and Tube Samples (right).



- Any changes made in the Run Protocol are automatically applied to all selected samples.
- When multiple selected samples have different settings, the following conditions apply:
 - Different values are displayed as blank, and a user-entered value applies to all.
 - Different check states are shown in the indeterminate state (i.e., the check box is filled solid). Clicking a checkbox in the indeterminate state results in the option being checked for all. Clicking it again toggles it to unchecked for all.

Settings buttons

Settings buttons consist of *Apply to* and *Set as default* buttons. When Run Protocol settings are modified, the changes are applied to selected wells automatically. These buttons allow you apply the settings to other members of the current Group or Experiment.



Apply to Experiment/Group split button

The main part of the split button contains the *Apply to Experiment* option. Clicking the dropdown arrow adjacent to the main button displays the *Apply to Group* option.

- Clicking **Apply to Experiment** on the main button applies the current Run Protocol to all Samples in the current Experiment.
- Selecting **Apply to Group** from the dropdown applies the current Run Protocol to all Samples of the selected Group.

Set as Default

Selecting this option sets the current Run Protocol to be used by default on all future plate or tube experiments run by the user currently signed in.

Set Up Flow options

Set Up Flow options are displayed only when a Compensation well is selected. The criteria defined here are used only during the acquisition of Compensation wells.



Acquisition volume

- *Acquisition volume* option allows you to specify the volume of sample that may be used during acquisition of a Compensation well in the Setup mode.
- The number entered into the Acquisition volume field is validated on entry and must be an integer. If the number entered is outside the allowed range, it will be automatically adjusted to the nearest allowable number.
- The default, minimum and maximum values are dependent on the type of plate being used.
- The total draw volume (acquisition volume + dead volume) is displayed next to the acquisition volume field.

Sample flow rate

The *Sample Flow Rate* option controls the rate of delivery of the sample during the acquisition of Compensation samples in the Run mode.

- The flow rate values are set using the slider bar, and the selected flow rate is displayed to the right of the slider bar.
- You can set the following values: 1000 µL/minute, 500 µL/minute, 200 µL/minute, 100 µL/minute, 25 µL/minute, 12.5 µL/minute.
- The default flow rate is 200 µL/minute.

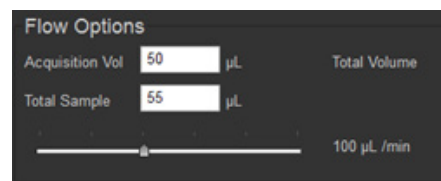


Note: The sample dead volume from wells is 30 µL for sample flow rates of up to 500 µL/minute.

Flow/Recording Flow options

Flow/Recording Flow options are displayed for all sample types (Sample well or Tube sample).

These settings are used when collecting data from these samples.



Note: For Tube samples, the group title displays *Flow options*.

For Sample wells, the group title displays *Recording Flow options*.

Acquisition volume

- *Acquisition volume* option allows you to specify the volume of sample that may be used during the acquisition of the well or the tube.
- The number is validated on entry for all fields, and must be an integer.
- The total draw volume (acquisition Volume + dead volume) is displayed next to the acquisition volume field.

Note: The sample dead volume from wells is 30 µL for sample flow rates of up to 500 µL/minute.

- The default, minimum, maximum values are dependent on the type of plate or tube in use. These values are provided by the Instrument Interface:

Plate Type – <text> or Tube for tube

Default Total Sample Volume – <integer> (µL)

Minimum Total Sample Volume – <integer> (µL) (not applicable for tube)

Maximum Total Sample Volume – <integer> (µL) (not applicable for tube)

Default Acquisition Volume – <integer> (µL)

Minimum Acquisition Volume – <integer> (µL)

Maximum Acquisition Volume – <integer> (µL)

Total sample volume

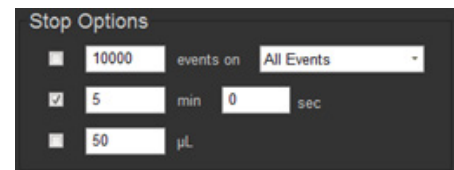
- *Total sample volume* field is only displayed for Plate samples. It allows you to specify the amount of sample present in the selected well of a plate.
- The total draw volume auto-populates the total sample volume field, but you can edit the volume to be any number greater than the total draw volume.
- If the total sample volume field already contains a value greater than the total draw volume, the total draw volume field does not update this field.
- The sum of the volumes entered into the Acquisition volume text boxes in Setup Flow options (page 173) and Record Flow options (above) cannot exceed the total allowable acquisition volume for the well. These limits are calculated by the software.

Sample flow rate

- This option controls the rate of delivery of the sample during acquisition of Sample wells and Tube samples in the Record mode.
- You can set the following values: 1000 µL/minute, 500 µL/minute, 200 µL/minute, 100 µL/minute, 25 µL/minute, 12.5 µL/minute.
- The default flow rate is 200 µL/minute.

Stop options

Stop options allow you to specify when the collection of data will end. If multiple conditions are selected, the acquisition ends when any one of the selected stop conditions are met. If no option is selected, the sample will continue recording until the acquisition volume is exhausted.



Event count stop

- *Event count stop* is used for stopping acquisition when the specified number of events has been collected within the specified gate.
- The number field allows you to enter a number between 1 and 5,000,000 for a plate well and between 1 and 20,000,000 for a Tube sample. The default value is 10,000. The number is validated on entry and must be an integer.
- The dropdown list displays all gates in the currently active Workspace for tubes or the Experiment Workspace for plates. An additional option, *All Events*, is always available and is the default selection.
- The following actions can result in recounting the data or changes in Event count stop.
 - If the stop gate or a dependent gate (i.e., a gate hierarchically upstream of the stop gate) is moved during acquisition, then all data is recounted. This does not occur during actual gate movement but on release of the gate to a new position.
 - If the combination of gates in the hierarchy on which the stop gate is dependent is changed during acquisition, then all data is recounted.
 - Renaming the stop gate or a dependent gate does not alter the event count or the stop condition. If the stop gate is renamed, the Events dropdown is updated to show the new name when the new name is validated.
 - If the stop gate is deleted, then “All Events” option is selected and the stop option remains checked. If a dependent gate is deleted, then the stop option remains unchanged. In both cases, the data will be recounted.
 - If a stop gate is un-deleted as part of an undo operation, then the previous state of the Event count stop option is restored. If a stop gate or a dependent gate is un-deleted, then the data is recounted.
- If recounting the data results in the stop condition being met, then the recording is stopped and all data displays and statistics are back-tracked to the stop event that caused the stop condition to be reached.

Time stop

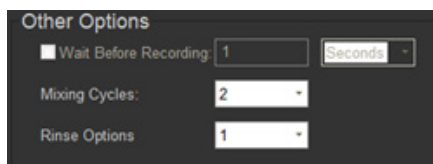
- *Time stop* is used for stopping acquisition when the specified period of time has elapsed. The default stop time is 5 minutes 0 seconds.
- A number between 0 and 59 may be entered into the **minutes** and **seconds** text fields. The number is validated on entry and must be an integer. If a number higher than 59 is entered, it is automatically adjusted to 59.

Volume stop

- *Volume stop* allows you to specify a delivered volume at which acquisition will terminate.
- The allowable range depends on the sample source, plate or tube. The number is validated on entry and must be an integer.
- If a number higher than the allowable maximum is entered, the entry is automatically adjusted. The minimum value is 1.
- For a plate, the maximum value is the entered acquisition volume.
- For a tube the maximum value is 4,000 μL .
- The default stop volume is 50 μL .

Other options

Other options contain *Mixing Cycles* and *Rinse* options. These options are visible when at least one well is selected.



Mixing Cycles options

- **Mixing Cycles** option allows you to select the number of mix cycles to be performed on the selected wells. This option is not displayed for tubes.
- The dropdown list allows you to select between 0 and 10 mixing cycles. The default value is 1.

Rinse options

- **Rinse between samples** option allows you to select the number of rinse cycles to be performed between Well samples. This option is not displayed for tubes.
- The dropdown list allows you to select between 0 and 10 rinses. The default setting is 1.

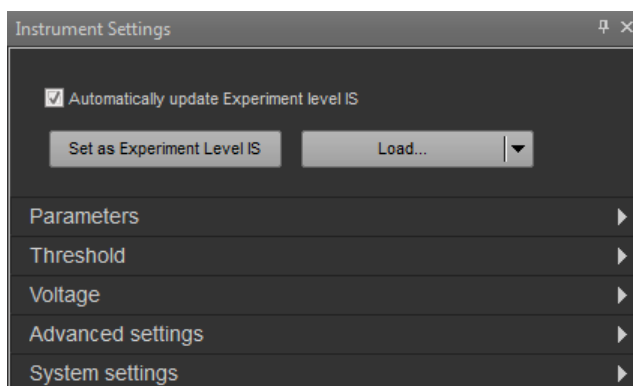
11. Instrument Settings panel

Overview

Instrument Settings panel allows you to edit acquisition parameters, create custom parameters, define threshold and voltage settings, and edit advanced instrument settings. By default, it is docked to the left of the Main Application area (page 28).

The Instrument Settings panel is organized into six functional groups in the following order:

- *Default and Load options* (page 178)
- *Parameters* (page 180)
- *Threshold* (page 183)
- *Voltage* (page 185)
- *Advanced settings* (page 187)
- *System settings* (page 189)



The Instrument Settings options are contextual; controls that are not available in a particular context are not shown or shaded gray on the panel.

General panel properties

- The docking properties of the Instrument Settings panel are detailed on page 29.
- If the instrument settings for the active sample do not match the system instrument configuration, the instrument settings panel displays the following message:
"Instrument settings cannot be displayed for the selected sample. The instrument configuration is not supported".
- The same warning message is displayed for any Experiment where the instrument settings for the Experiment do not match the system instrument configuration (e.g., RB vs. RBVY). Contents of other instrument settings panels are not displayed.
- Typing into the textbox and pressing **Enter** updates the value in the textbox. Pressing **Tab** updates and moves to the next item specified.



Default and Load options

Overview

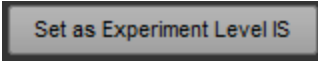


Default and Load options consist of the three controls that lie at the top of the Instrument Settings panel. They allow automatic updating of Experiment-level instrument settings, one-time updating of Experiment-level instrument settings, and saving of instrument settings as default.

Automatic update option

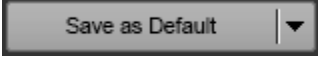
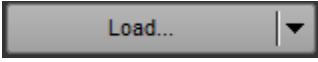
Automatically update experiment instrument settings checkbox enables automatic update of Experiment-level instrument settings to the previously recorded Sample-level instrument settings for each Sample when instrument settings are changed.

- The checkbox works for Tube samples only, and it is checked by default.
- Compensation samples (Tube or Well) always update the Experiment-level instrument settings. When Compensation samples are active, the checkbox is automatically checked and disabled (only when running compensation).
- Behavior if the checkbox is checked:
 - If the instrument settings are modified, the Experiment-level instrument settings are automatically updated.
 - If the instrument settings are modified on subsequent Samples, the Experiment-level instrument settings are automatically updated.
 - If any Sample has recorded data, modification of the Experiment-level instrument settings will result in the recorded Samples displaying the Instrument Settings icon (), which indicates that the Sample now has Sample-level instrument settings independent of the Experiment. The Sample-level instrument settings will reflect the instrument settings used at the time the Sample was recorded
- Behavior if the checkbox is unchecked:
 - If the active Sample's instrument settings are modified, the modified settings will not update the Experiment-level IS. Any additional changes to the instrument settings will only apply to the active Sample.
 - The Sample will display the instrument settings icon (), which indicates that a Sample-level instrument settings has been created independent of the Experiment-level instrument settings.
 - When the checkbox is unchecked, any modification of the instrument settings of the active Sample will only affect the active Sample.
 - If a Sample-level instrument settings is created for a Sample, the checkbox will be unchecked and disabled until the Sample's settings are applied to the Experiment either by using the "Set as Experiment Level IS" button or by dragging the IS icon to the Experiment level.

Set as Experiment IS

- Clicking **Set as Experiment IS** for Tubes saves the instrument settings of the current active Sample as the Experiment-level instrument settings. 
- Experiment-level instrument settings are indicated by the IS indicator icon () displayed to the right of the Experiment name within the Experiment Explorer.
- Subsequent Samples in this Experiment will have these instrument settings as default.
- If the Experiment contains Samples with recorded data, those Samples will now display the IS icon ().

Save as Default split button

- The *Save as Default button* allows the instrument settings to be saved as a default. 
- The split button is disabled during tube acquisition, well acquisition, or while processing a plate.
- **Save as Default:** Saves all changes to the instrument settings as default instrument settings to be used for all new Experiments. This includes all enabled and selected parameters and measurements, targets, fluorophores, thresholds, voltages, custom parameters, and advanced settings.
- **Load:** Opens the *File browser* dialog (page 366). 

Using the Database browser dialog, you can select an instrument settings file from within any experiment in your folder, from available shared folders, or from available default instrument settings files.

The Load button is available only when the Experiment does not contain data.
- **Export:** Opens the *File Save (Export) dialog* (page 363).

Using the File Save (Export) dialog, you can save the current instrument settings with a user-defined name in your own folder or any shared folders in the database.

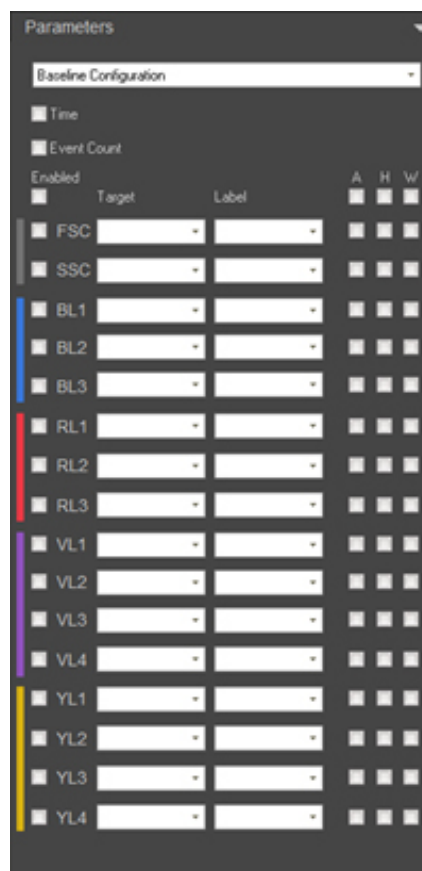
You will be prompted for confirmation before overwriting an existing instrument settings file.

Parameters

Overview

Parameters group allows you to select the parameters to be recorded within the Experiment, based on the selected instrument configuration.

- Parameters group contains the following controls:
 - Configuration dropdown (page 181)
 - Parameter selector checkboxes (page 181)
 - Target combo box (page 182)
 - Fluorophore combo box (page 182)
- Parameters are listed in the following order:
 - Scatter channels (FSC, SSC)
 - Blue laser channels (BL)
 - Red laser channels (RL)
 - Violet laser channels (VL)
 - Yellow laser channels (YL)



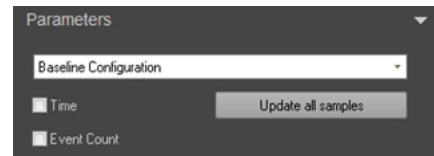
- Only lasers and channels within the installed laser configuration are available in the Parameters options. The list for possible configurations and their respective laser and channel order is shown in the table below.

B	BR	BV	BY	BRV	BVY	BRVY
FSC off B	FSC off B	FSC off B	FSC off B	FSC off B	FSC off B	FSC off B
SSC off B	SSC off B	SSC off B	SSC off B	SSC off B	SSC off B	SSC off B
BL1	BL1	BL1	BL1	BL1	BL1	BL1
BL2	BL2	BL2	BL2	BL2	BL2	BL2
BL3	BL3	BL3	BL3	BL3	BL3	BL3
BL4	BL4	BL4	YL1	BL4	VL1	RL1
	RL1	VL1	YL2	RL1	VL2	RL2
	RL2	VL2	YL3	RL2	VL3	RL3
	RL3	VL3	YL4	RL3	VL4	VL1
		VL4		VL1	YL1	VL2
				VL2	YL2	VL3
				VL3	YL3	VL4
				VL4	YL4	YL1
						YL2
						YL3
						YL4

- If you modify the instrument configuration, target, or fluorophore for any sample post-recording, a *notification button* (⚠) is displayed adjacent to the affected parameter or the instrument configuration dropdown (see page 182), and a notification key becomes visible at the bottom of the panel. Clicking the notification button reverts the change to the value stored within the FCS file.

Configuration dropdown

Configuration dropdown allows you to select a filter configuration and update the choice of fluorophore for each enabled parameter for the selected configuration.



- The configuration dropdown list is linked to the filter configuration manager and contains all available configurations saved by the current user.
- The configuration selected from the dropdown list updates the choice of fluorophores for each enabled parameter. The configuration dropdown is always active, even after a sample from the Experiment has been recorded.
- If the instrument configuration selection is changed post-recording, the **Update all samples** button becomes visible, which allows you apply the instrument configuration selection to all samples within the Experiment.
- The Update all samples button is displayed only if changes are made to the instrument configuration selection, or to any target or fluorophore name.

Parameter selector

Parameter selector checkboxes allow you to select parameters (including *Time* and *Event count*) and their respective measurements for any channel available in the selected configuration.

- By default, all parameters are selected except for the event parameter.
- Selecting the **Enable** checkbox enables all available channels with a single click.
- **Area**, **Height**, and **Width** measurements can be enabled for all channels with a single click by selecting the respective single-click checkbox (**A**, **H**, **W**).
- If all parameters or measurements are enabled using a single-click checkbox, then all related checkboxes are selected in that column.

If any of those checkboxes are deselected, then the respective single-click checkbox is unchecked.



- Deselecting a **channel selector** checkbox unchecks all of the measurement checkboxes for that channel and disables the *Target* and *Fluorophore* fields. The channel selector checkbox is selected when at least one measurement is checked for that channel. If all measurement checkboxes for a channel are unchecked, the channel selector checkbox is deselected and the Target and Fluorophore fields are disabled.
- Parameters cannot be enabled or disabled in the active sample (wells or tubes) after that sample has been recorded or contains an FCS file.
- Parameters can be enabled or disabled during acquisition and prior to recording a sample. When parameters are enabled or disabled, the data will be cleared.
- Any parameter that is part of a custom parameter or is used as a compensation control is disabled.

Target textbox

- **Target** textbox allows you to enter the target name of the fluorophore in the text field or leave it blank. The Target field is available for scatter and fluorescence parameters only.
- You can update the target name pre- or post-recording.
- Any changes made in the Target field updates the parameter name used in the Workspace.
- Entries made in the Target textbox are used by the *Smart Gate Naming* tool as described in “Customize Gate options”, page 223.
- You can enter up to 50 characters into the Target textbox. Characters exceeding this limit cannot be entered. Any ASCII character is valid.



The tooltip for Target textbox reads “Example: CD3”.

- Any changes made to the target name post-recording only applies to the active sample, unless **Update all samples** button is pressed, which applies the target name to be applied to all samples within the Experiment.
- The Update all samples button is displayed only if changes are made to the instrument configuration selection, or to any target or fluorophore name. Once it is clicked, the button is no longer visible.

The tooltip for the Update all samples button reads “Updates Target and Fluorophore names for all samples within experiment.”

Fluorophore textbox

- The **Fluorophore** textbox allows you to enter the fluorophore name (i.e., label name) in the text field or leave it blank. The Fluorophore field is available for scatter and fluorescence parameters only.
- You can update the fluorophore name pre- or post-recording.
- You can enter up to 50 characters into the textbox. Characters exceeding this limit cannot be entered. Any ASCII character is valid.



The tooltip for Fluorophore textbox reads “Example: FITC”.

- Any changes made to the fluorophore name post-recording only applies to the active sample, unless **Update all samples** button is pressed, which applies the fluorophore name to all Samples within the Experiment.

Notification button

If you modify the instrument configuration selection, target, or fluorophore for any sample post-recording, a *notification button* (▲) is displayed adjacent to the affected parameter or the instrument configuration dropdown (if modified).

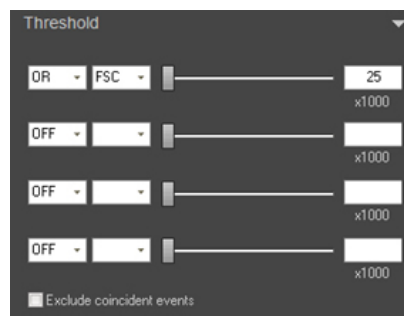


- Clicking the notification button restores the target or fluorophore to the original value stored within the FCS file.
- Clicking the button next to the instrument configuration combo box restores the selection to the configuration used at the time the file was recorded.

Threshold

Overview

Threshold settings allow you to set the minimum signal level for up to four channels (i.e., detector) to eliminate unwanted events and reduce noise. You can also combine the thresholds using Boolean operators (see *Logic selector*, below).



- Threshold settings contain the following controls in the order listed:
 - Logic selector combo box (below)
 - Channel selector combo box (page 184)
 - Threshold adjustment slider (page 184)
 - Threshold signal textbox (page 184)
 - Exclude coincident events checkbox (page 185)
- A new value for the threshold signal is applied when the slider bar is moved, if the threshold logic or channel is changed, when the focus is lost from the threshold textbox, or when the **Enter** key is pressed.
- When a new threshold value is applied, existing data are cleared, and the software records and analyzes only the events with parameter values above the set threshold.
- Adjustments made to threshold with the slider bars dynamically refresh the data on the Workspace.

Logic selector

Logic selector allows you to select up to four channels to apply threshold(s) and to combine the thresholds using Boolean operators.



- Each logic selector dropdown includes three options: **AND**, **OR**, and **OFF**.
- If the **OFF** option is selected for a channel, the detector is ignored for threshold purposes, and the channel selector, slider, and textbox are grayed out.
- The **AND** and **OR** selections follow Boolean logic rules.
 - If the **AND** operator is selected, all the set thresholds have to be met before the data is collected.
 - If the **OR** operator is selected, data collection begins when at least one of the thresholds is met.
- Since the selected logic operator determines the enabled/disabled state for the rest of the controls, the Logic selector combo boxes are available before the other threshold controls.

Channel selector

Channel selector allows you to select the channels to apply threshold.



- The first dropdown list includes all of the enabled channels selected in the Parameters group (excluding time and event) (page 181).
- Each subsequent dropdown list shows only the remaining available parameters. For example, if FSC is used as the first threshold, then all other dropdowns will not have FSC as an option.
- Threshold is only applied to parameters that are visible, allowing up to four threshold permutations.
- Default Threshold settings are: **OR** (selected in the first Logic combo box), **FSC** with a value of 25 ($\times 1000$), and **OFF** on the second, third, and fourth logic dropdowns (i.e., not used for threshold).
- When other threshold settings are added, their default value will be 1 ($\times 1000$).

Threshold adjustment slider bar

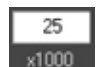
Threshold adjustment slider bar allows you to adjust the numerical threshold setting.



- Adjusting this slider dynamically updates the value in the threshold textbox accordingly. While adjusting the slider bar, a tooltip displays the full threshold value (i.e., the textbox value $\times 1000$).
- The lower limit is 0.1. The upper limit is 1/1000 of the range set for the height measurement of the selected channel.
- Moving the slider bar adjusts the threshold setting by increments of 10. When starting at the lower limit of 0.1, the next level will be 10, after which the threshold will increase in steps of 10.
- You can use the **up/right** and **down/left arrow** keys can also be used to adjust the slider bar in 1 unit ($\times 1000$) increments. The up/right arrow keys increase the value, and the down/left arrow keys decrease the value.
- When the textbox or slider bar is in focus, you can use the **mouse scroll wheel** to adjust the slider bar position.

Threshold textbox

Threshold textbox allows you to type in the numeric value for a threshold setting. The software automatically multiplies the input value by 1000.



- Only numeric values are allowed in the textbox. One decimal place is displayed at all times. If you do not enter a decimal place, it will be automatically added with a zero in the tenths place.
- Changing the value in the textbox automatically updates the position of the slider after the textbox loses focus, the **Enter** key is pressed, the **up/right** and **down/left arrow** keys are pressed, or when the value is changed using the **mouse scroll wheel**.
- The accepted range of values is 0.1 for the lower limit, and 1/1000 of the allowed maximum for the height measurement for the upper limit (2^{20} by default). The resolution of entry is 0.1.
- When a number is entered that is greater than the maximum allowed value for the parameter, the focus is set to the entire text to allow editing (instead of defaulting to maximum).
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.

Exclude coincident events check box

Exclude coincident events check box allows you to enable an instrument-based algorithm to remove coincident events.



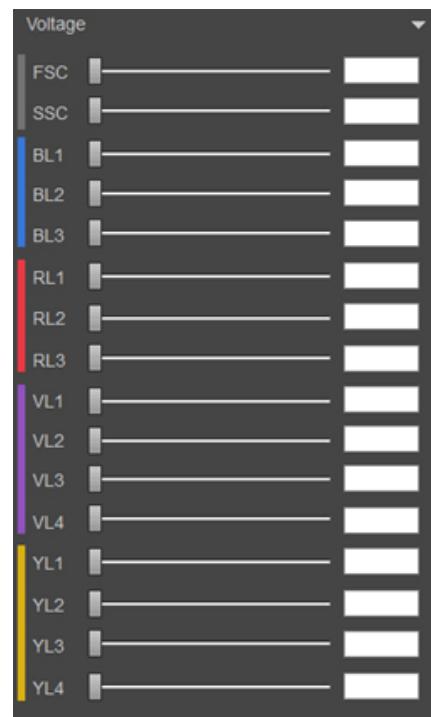
- If checked, the coincident events are removed from the data stream, and the number of aborted coincident events is counted in the FCS file.
- By default the checkbox is unchecked.

Voltage

Overview

Voltage settings allow you to adjust voltages for each channel. The number of channels shown depends on the configuration of the instrument.

- Voltage settings consist of the following controls:
 - Voltage adjustment slider bar (page 186)
 - Voltage value textbox (page 186)
- The installation default voltages are set to zero, which are automatically updated to the voltages from the first successful Baseline Performance Test results.
- You can adjust the PMT voltage for an available channel using the slider bar or the textbox for that channel.
If a parameter is deselected within the Parameters group (page 181), then the deselected channel is grayed out.



- Adjustments made to voltages with the slider bars dynamically refresh the data on the Workspace.
- Adjustments made using the textboxes automatically refresh the data on the Workspace once the focus is lost or when the **Enter** key is pressed.
- When Compensation samples are run, the respective channel is highlighted with a light blue color.
- When any compensation channel is recorded or the Compensation sample contains an FCS file, all controls associated with the enabled fluorescence channels are disabled and grayed out for the remaining samples in the Experiment.



Note: This ensures that the voltages used in the compensation calculation are not modified and the parameters are not disabled.

- If the compensation settings are removed from the Experiment, the parameter and voltage controls for the fluorescence channels are re-enabled.

Voltage adjustment slider bar

Voltage adjustment slider bar allows you to adjust the PMT voltages for available channels.



- Adjusting the voltage slider bar dynamically updates the value in the voltage textbox accordingly.
- The range of the voltage slider bar is from 1 to 1000 (left to right).
- Moving the voltage slider bar adjusts the PMT voltage by increments of 20.
- When the voltage slider or textbox is in focus, you can use the **mouse scroll wheel** or the **up/right** and **down/left arrow** keys to adjust the slider bar position by increments of 10. The up/right arrow keys increase the value, and the down/left arrow keys decrease the value.

Voltage value textbox

Voltage value textbox allows you to directly enter the desired voltage setting for a particular channel.

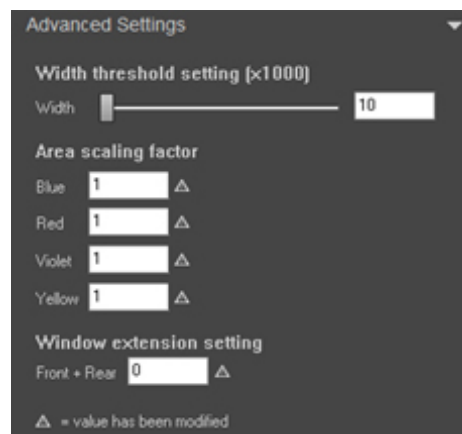


- Only numeric values are allowed in the textbox. Non-numeric characters are not registered.
- The accepted range of values is 1–1000 mV for all channels. The resolution of entry is 1 mV.
- Changing the value in the textbox automatically updates the position of the slider after the textbox loses focus, the **Enter** key is pressed, the **up/right** and **down/left arrow** keys are pressed, or when the value is changed using the mouse scroll wheel.
- Adjustments to the textbox refresh the data on the Workspace once focus is lost or **Enter** is pressed.
- When a number is entered that is greater than the maximum allowed value for the parameter, the focus is set to the entire text to allow editing (instead of defaulting to maximum).
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.
- During compensation, the fluorescence channels are highlighted with a light blue color.

Advanced settings

Overview

Advanced Instrument Settings section allows you to adjust the **Width threshold setting**, the **Area scaling factors (ASF)**, and the **Window extension setting**, provided that you have the appropriate permissions (see Note below).



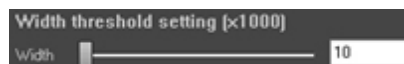
- If you do not have permissions to any of the functions within this section, the Advanced settings section is not visible.
- Adjustments to the slider bar dynamically refresh the data on the Workspace. Adjustments to the textboxes update the data once focus is lost or **Enter** is pressed.
- If the values are reset to default, the data will be cleared and new values will take effect.
- If you modify the area scaling factor (ASF) value for any laser or change the window extension setting, a *notification button* (page 188) is displayed adjacent to the affected setting, and a notification key becomes visible at the bottom of the panel.



Note: The Advanced settings options are available only to users with Advanced User and Administrator level permissions. For more information, see “Account permissions”, page 19.

Width threshold setting

Width threshold setting sets the peak threshold at which width measurement is determined. It consists of a slider and a textbox.



- The width threshold setting is set per Experiment. When it is saved as default instrument settings, it is used for new Experiments.
- The range of the width threshold setting is from 01 to 1048.5 (left to right), with a resolution of 0.1. The entered values are in thousands. The default value is 1.
- Moving the voltage slider bar adjusts the value by increments of 10.
- Only numeric values are allowed in the textbox. One decimal place is displayed at all times. If you do not enter a decimal place, it is automatically added with a zero in the tenths place.
- When the slider or text box is in focus, the **mouse slider wheel** or **up/right** and **down/left arrow** keys can be used to adjust the slider bar position by increments of 5.
- Changing the value in the textbox automatically updates the position of the slider after the textbox loses focus, the **Enter** key is pressed, the **up/right** and **down/left arrow** keys are pressed, or when the value is changed using the mouse scroll wheel.
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.

Area scaling factor (ASF)

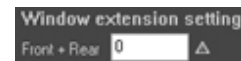
Area scaling factor (ASF) option sets the value to scale height and area to equivalent values. Area scaling is calculated during the Performance Test.



- The ASF can be set per Experiment or it can be used for all new Experiments when saved as default Instrument Settings.
- The ASF can be set for each configured laser and only configured lasers are listed.
- The ASF values are updated after each successful Performance Test. The default values are set by the most recent, successful Performance Test. If no Performance Test or Baseline has been run, the default values are set to 1.0
- The ASF values range from 0.1 to 10.
- Only numeric values are allowed in the textbox. Non-numeric characters are not registered.
- If the System Settings update the Area scaling factor settings, the ASF values are updated to the System Settings.
- If the ASF value is modified in the text box for a specific laser, the System Setting is overridden such that the ASF value for that modified laser will not automatically update based on each successful Performance Test.
- If the ASF values are deleted from the textbox and the textbox loses focus, the previous value is restored to the textbox.
- If you do not have permission to access the Advanced Instrument Settings, the ASF value used is set by the System Instrument Settings.

Window extension setting

The **Front + Rear window extensions** can be configured for an Experiment and saved as part of the default Instrument Settings.



- The value ranges from 40 to 75. The default value is 0.
- Only integer values are allowed in this text box.
- If the value is deleted from the text box, and the text box loses focus then the previous value will be restored to the text box.
- If the value is modified, the system window extension setting is overridden such that the value will not automatically update based on each successful Performance Test.

Notification button

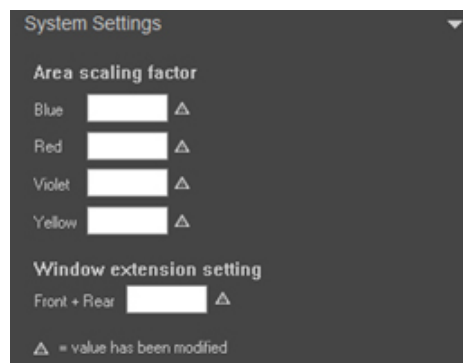
If you modify the area scaling factor (ASF) value for any laser or change the window extension setting, a *notification button* (🔺) is displayed adjacent to the affected setting.

- Clicking the notification button next to the area scaling factor (ASF) restores the affected setting to the System Setting value for the corresponding laser.
- Clicking the notification button next to the window extension setting restores the setting to the System Setting value.

System settings

Overview

System Instrument Settings section allows you to adjust the **Area scaling factors (ASF)** and the **Window extension setting** for the system, provided that you have the appropriate permissions (see Note below).



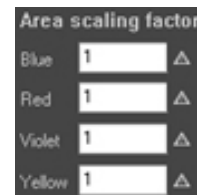
- If you do not have permissions to any of the functions within this section, the System Instrument Settings section is not visible.
- If the values are reset to default, the data will be cleared and new values will take effect.
- If you modify the Area scaling factor (ASF) value for any laser or change the window extension setting, a *notification button* (page 190) is displayed adjacent to the affected setting, and a notification key becomes visible at the bottom of the panel.
- The System settings are updated after each successful Performance Test.



Note: The System Instrument Settings options are available only to users with Administrator level permissions. For more information, see “Account permissions”, page 19.

Area scaling factor (ASF)

Area scaling factor (ASF) option sets the value to scale height and area to equivalent values. Area scaling is calculated during the Performance Test.



- The ASF setting applies to all Experiments and Users, where the Advanced Setting ASF value has not been set (see page 188).
- The ASF can be set for each configured laser and only configured lasers are listed.
- The ASF values are updated after each successful Performance Test. The default values are set by the most recent, successful Performance Test.
- If no Performance Test or Baseline has been run, the default values are set to 1.
- The values range from 0.1 to 10.
- Only numeric values are allowed in the textbox. Non-numeric characters are not registered.
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.


Window extension setting

The **Front + Rear window extensions** can be configured for an Experiment and saved as part of the default instrument settings.



- The value ranges from -40 to 75. The default value is 0.
- Only integer values will be allowed in this text box.
- If the value is deleted from the text box, and the text box loses focus then the previous value will be restored to the text box.
- The window extension value is reset to 0 after each successful Performance Test.

Notification button

If you modify the Area scaling factor (ASF) value for any laser or change the Window extension setting, a *notification button* () is displayed adjacent to the affected setting.

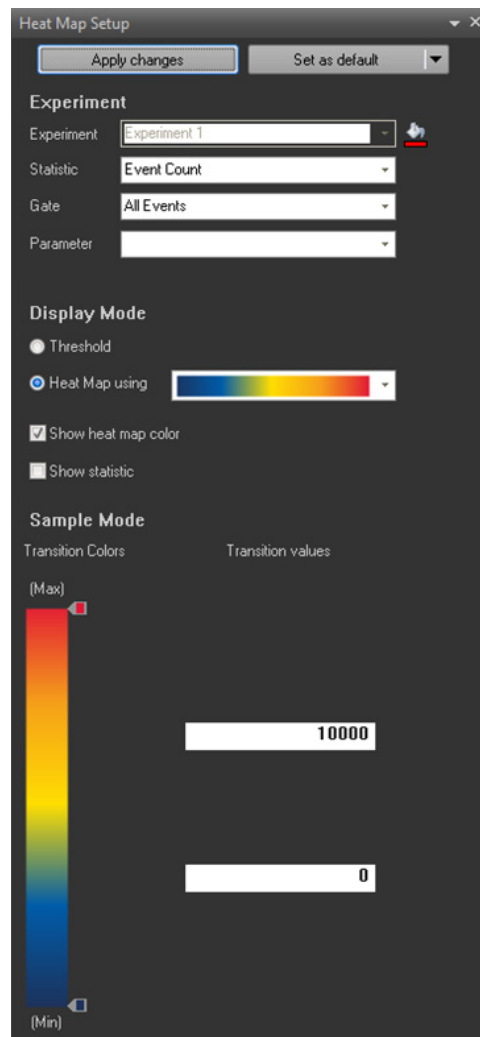
- Clicking the notification button next to the Area scaling factor (ASF) restores the affected setting to the most recent successful Performance Test value.
- Clicking the notification button next to the Window extension setting restores the setting to its default value of 0.

11. Heat Map Setup panel

Overview

Heat Map Setup panel allows you to select the statistic, gate, and parameter for visualizing the data from the current Experiment in the *Heat Map view* (page 105), and to specify the *display mode* and *transition values* to help you analyze the data at a glance.

- In the *Threshold display mode*, each well and/or tube is assigned a color based on whether the selected statistic for that sample falls above or below the set threshold value.
- In the *Heat Map display mode*, individual values in each well and/or tube are represented as colors on a gradient.
- By default, the Heat Map Setup panel is docked to the left of the Main Application Area. The docking properties of the panel are detailed on page 29.
- The Heat Map Setup panel is organized into four functional groups in the following order:
 - Apply, Save, and Load options (page 192)
 - Experiment (page 193)
 - Display mode (page 195)
 - Sample value (page 196)
- Sample value options on the Heat Map Setup panel are contextual; the controls displayed depend on whether you select Threshold or the Heat Map in the Display mode options (page 195).
- By default, the Heat Map Setup panel displays the sample value controls for the Threshold mode.



Note: For information on the Heat Map view, including Sample display and Experiment Setup, refer to “Heat Map View” on page 105.

Apply, Save, and Load options

Overview

Apply, Save, and Load options consist of the two controls that lie at the top of the Heat Map Setup panel.



Apply changes

- Clicking **Apply changes** applies the current Heat Map settings to all samples in the current Experiment.
- This option is active if the Heat Map settings of the current Experiment have been changed.
- If the Heat Map Setup panel loses focus after a change has been made, the new settings are automatically applied to the Heat Map.

The Status Notification Display located above the Main Application Workspace (page 28) then displays “The Heat Map settings have been updated” and provides an **Undo** option.

Set as default split button

Set as default button is a split button that allows you to apply the Heat Map settings to the current Experiment, set them as default, or save (export) and retrieve them for other Experiments.

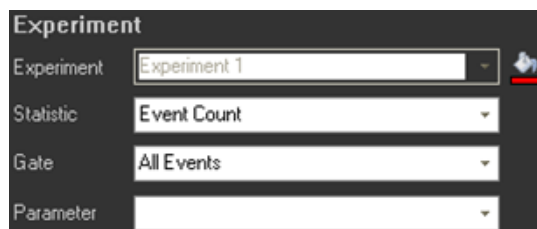
The main button on the left is the **Set as default** command and the right side is a dropdown containing **Load** and **Export** options.

- **Set as default:** Saves the current Heat Map setting as the default Heat Map settings for all new Experiments.
- **Load:** Opens the *File Browser dialog* (page 366).
Using the File Browser dialog, you can select a Heat Map settings file from within any experiment in your folder, from available shared folders, or from available default instrument settings files.
- **Export:** Opens the *File Save (Export) dialog* (page 363).
Using the File Save (Export) dialog, you can save the current Heat Map settings with a user-defined name in your own folder or any shared folders in the database.
You will be prompted for confirmation before overwriting an existing Heat Map settings file.

Experiment

Overview

Experiment options allow you to select the statistics, populations (all events or specific gated populations), and the parameter for the current Experiment to display in the Heat Map view. It also contains the control for assigning a unique indicator color for the current Experiment.



Experiment dropdown

Experiment dropdown list displays the name of the Experiment defined within the current plate and any associated tubes.



- The Experiment dropdown control is disabled as each plate is limited to a single Experiment.
- The control is labeled with the name of the currently open Experiment. The default label is **Experiment**.
- Default settings are loaded the first time the Experiment Heat Map is selected.

Experiment color button

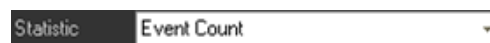
Experiment color button opens the *color picker dialog*, which allows you to select the indicator color for the current Experiment.



- By default, the Experiment color dropdown button shows the currently selected color for the Experiment.
- When applied, the selected color is reflected in the Heat Map view (page 105).

Statistic dropdown

Statistic dropdown displays the list of available statistics and allows you to select the statistic of interest to display in the Heat Map view.

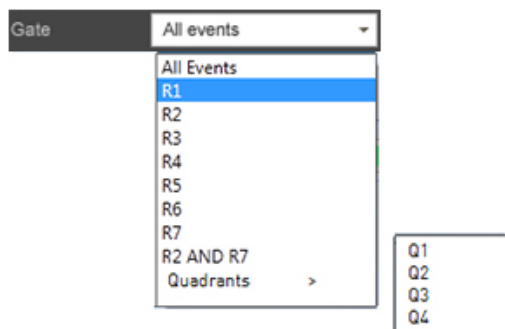


- The statistic dropdown control is labeled with the name of the currently selected statistic. The default statistic is **Event Count**.
- The dropdown list includes the following statistics:
 - Event Count
 - Median
 - Mean
 - Event Percentage of Parent
 - Event Percentage of Total
 - Standard Deviation
 - Robust Standard Deviation
 - Coefficient of Variation
 - Robust Coefficient of Variation
 - Concentration
 - Sample Volume
 - Any Derived Statistic name

Gate dropdown

Gate dropdown list allows you to select the populations of interest (all events or specific gated populations).

- The dropdown list contains all gates in the Experiment-level Workspace (page 64), including quadrant and derived gates.
- The default population is **All Events**.
- Only gates from the Experiment-level Workspace are displayed.
- Selecting a gate sets the parent gate for the Heat Map display.
- If the default Heat Map or loaded Heat Map refer to a gate that is not available on the Experiment workspace, the gate selection defaults to All Events.



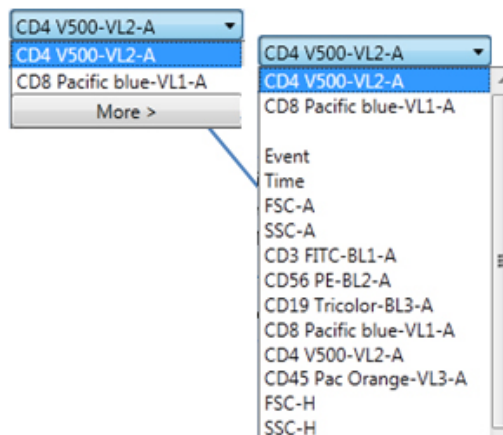
Parameter dropdown

Parameter dropdown allows you to select the parameter to display in the Heat Map view for the current Experiment.

- The Parameter dropdown is available only for intensity related statistics, Mean, Median, Standard Deviation (SD), Robust SD, Coefficient of Variation (CV), and Robust CV.

It is hidden when Event Count, Percentage of Parent, Percentage of Total, or Concentration is selected in the Statistic dropdown list (page 193).

- The drop down list contains only the parameters on the plot for the gate selected in the Gate dropdown. Selecting **More** from the Parameter dropdown list expands it to contain all the parameters selected for acquisition in the Instrument Settings panel (page 177).
- The parameters are listed in parameter order when the list is expanded. The first parameter in the parameter list is displayed as the default parameter, when available.
- If **All Events** is selected in the Gate dropdown, the parameters are not filtered and all enabled parameters are displayed by default.
- If the gate is derived from a Histogram plot (i.e., a single parameter plot), then the parameter list only contains the parameter that is on the selected plot. Selecting the **More** displays all available parameters.
- For a dual-parameter plot, the parameter list is filtered to show the two parameters that are on the plot from which the gate is derived. Selecting the **More** option shows all parameters.

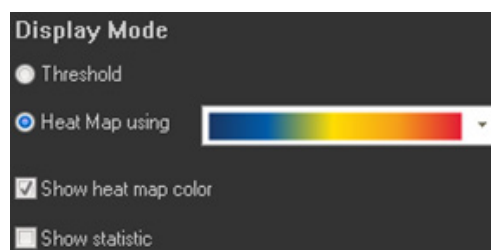


Display mode

Overview

Display mode options allow you to choose between the **Threshold** and **Heat Map** display modes and control the display of the colors used in the Heat Map view.

You can select the Threshold or the Heat Map mode by clicking the appropriate radio button. The default option is Threshold mode.



Note: Threshold display mode is a visual aid to determine at a glance whether the signal from a well falls above or below a set value; it does not affect data collection. As such, it is different from the *Threshold settings*, which sets the minimum signal level for each detector to eliminate unwanted events and reduce noise. The threshold value set in Threshold settings instructs the software to record and analyze only the events with parameter values above the set threshold.

Display mode options

Threshold mode

- Clicking the **Threshold** radio button selects the Threshold mode and displays each well in user-defined colors based on whether the signal from the well and/or tube falls above or below the set threshold value.
- By default, only a single threshold is defined. However, you can create up to 10 signal threshold levels using the **Sample value** options (page 196).
- When **Threshold** mode is selected, choosing a color gradient from the dropdown switches the radio button to the **Heat Map** mode.

Heat Map mode

- Clicking the **Heat Map** radio button allows you to represent the data from each sample well as colors on a gradient selected from the **color gradient dropdown** list.
- There are six gradients available in the color gradient dropdown list, which are not user-editable. The default option is the first color range in the list.
- By default, only the minimum and maximum transition points are defined in Heat Map. However, you can create up to 10 transition points using the **Sample value** options (page 198).

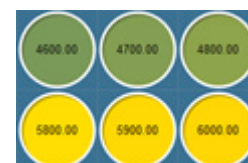


Show heat map color

- **Show heat map color** toggles the heat map color displayed on the Heat Map on and off.
- This option is selected by default (i.e., the Heat Map color is turned on).

Show statistic

- When selected, the **Show statistic** option overlays the selected statistic centered in the wells and/or tubes.
- The background color includes the Experiment color (if selected) and the Heat Map color (if selected).

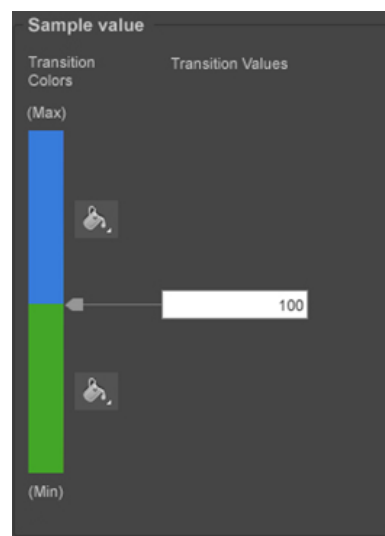


Sample value – Threshold mode


Overview

Sample value options allow you to add and remove color transition points and set the data level and the associated color.


- Clicking the **Threshold** radio button in Display mode options (page 195) displays the sample value controls for the Threshold mode.
- In the Threshold mode, each well and/or tube is assigned a color based on whether the selected statistic for that sample falls above or below the set threshold value.
- Threshold mode is the default option for the sample value controls.



Transition colors

- The color of the well in the Heat Map view (page 105) changes, if the threshold signal level is exceeded. It will not change if the value is the same or less than the transition value.
- By default, if the selected statistic is above the threshold, the well appears green; if it is below this level, the well appears blue.
- Clicking the **color picker button** next to a color block opens the standard color picker dialog, which allows you to define a different color for that block. 

Transition values

- By default, a single transition point (i.e., threshold) is set in the Threshold display mode. The default transition value is 10.
- You can enter the data value for a transition point directly in the **transition value** edit box. 
- Transition value edit boxes accept only numerical characters. Decimal values are shown only if you enter them.
- Each transition value edit box allows manual entry of a number based on the statistic being displayed.
 - Percentage of count values: 0 to 100%.
 - Percent CV values: $\pm 1000\%$
 - Events: 1 to 100,000,000
 - Intensity: minimum to maximum allowed for the selected parameter
- If the entry for the transition point exceeds the maximum value allowed, it will default to the maximum; if the value is lower than the minimum value allowed, then it will default to the minimum.
- If the statistic is changed, then the threshold returns to the default transition point set at 10.

Creating additional transition points

- You can add additional transition points by clicking on any given colored block sector. This inserts a new threshold bisecting the selected sector and adds an additional color block, which adopts the next color in the automatic color sequence.
- A transition value edit box is automatically created for each new level, and a color picker is associated with each new color block.
- You can create up to 10 levels. If you attempt to create more than 10 transition points, a warning message is displayed below the color bar.
- Clicking to create an additional transition point of a higher value creates a transition point at double the value of the first point.
For example, if the first transition point was set at 100, the new one will be set to 200.
- Clicking to create an additional transition point of a lower value creates a transition point at half the value of the first point.
For example, if the first transition point was set at 100, the new one will be set to 50.
- Clicking to create an additional transition point between two existing points creates a transition point half way between the two existing values.
For example, if you click between the transition points set at 100 and 200, the new transition point value will be set to 150.
- Clicking and dragging an indicator arrow off of the color bar by more than 20 pixels displays a red X icon, and when the mouse button is released, the selected transition point and the associated color picker and transition value edit box are removed.



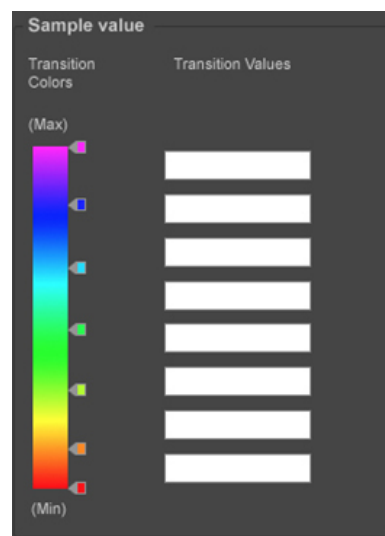
- There must always be one transition point defined. The last point cannot be removed.

Sample value – Heat Map mode

Overview

Sample value options allow you to add and remove color transition points and set the data level and the associated color.

- Clicking the **Heat Map** radio button in Display mode options and selecting the transition colors from the **color gradient dropdown** list (page 195) displays the sample value controls for the Heat Map display mode (i.e., gradient mode).
- In the Heat Map mode, each well and/or tube displays the appropriate color from the selected gradient depending on whether the signal from the well falls within the set boundaries.



Transition colors

- The color of the well in the Heat Map view (page 105) changes depending on where the result is in comparison to the selected color gradient.
- The Transition color gradient is selected using the **color gradient** dropdown list as described in “Display mode options”, page 195.
- Colored indicator arrows are displayed at the minimum and maximum points by default and any added transition points in between. The color displayed indicates the transition point color.
- Transition points can be moved up and down to adjust the color assigned to that transition point by clicking and dragging the indicator arrows with the mouse.



The associated textbox will be highlighted in light blue to distinguish which transition point is being adjusted.

Transition values

- By default, minimum and maximum transition points are defined in the Heat Map display mode.
- The default minimum transition point is 0 and the default maximum transition point is 10,000, from bottom to top respectively.
The default maximum for statistics containing a percentage is 100%.
- You can enter the data value for a transition point directly in the **transition value** edit box.
- Transition value edit boxes accept only numerical characters. Decimal values are shown only if you enter them.

- Each transition value edit box allows manual entry of a number based on the statistic being displayed.
 - Percentage of count values: 0 to 100%.
 - Percent CV values: $\pm 1000\%$
 - Events: 1 to 100,000,000
 - Intensity: minimum to maximum allowed for the selected parameter
- If the entry for the transition point exceeds the maximum value allowed, it will default to the maximum; if the value is lower than the minimum value allowed, then it will default to the minimum.
- If the statistic is changed, the minimum and maximum values revert to the appropriate default based on that statistic. Any middle transition points that existed are also removed.

Creating additional transition points

- You can add additional transition points by clicking on the color bar. This inserts a new transition point at the selected point and adds an additional indicator arrow, which adopts the color of the point selected.
- A transition value edit box is automatically created for each new transition point.
- You can create up to 10 levels. If you attempt to create more than 10 transition points, a warning message is displayed below the color bar.
- Clicking to create an additional transition point between two existing points creates a transition point half way between the two existing values.
For example, if you click between the transition points set at 100 and 200, the new transition point value will be set to 150.
- The transition value entered must be in ascending order. If an incorrect value is entered, a warning balloon is displayed.
- Clicking and dragging an indicator arrow off of the color bar by more than 20 pixels displays a red X icon, and when the mouse button is released, the selected transition point and the associated transition value edit box are removed.



- The minimum and maximum points cannot be removed.

12. Customize panel

Overview

The *Customize panel* displays context sensitive formatting options relevant to objects selected on the Workspace or the Heat Map view.

Using the Customize panel, you can format the following object(s):

- Plots (page 201)
- Text boxes (page 217)
- Statistics boxes (page 218)
- Gate styles and labeling (page 220)

Customize panel behavior

- To display the Customize panel, right-click an object to open its context menu, then select **Customize**.
- The formatting options in the Customize panel are context-sensitive and dynamically update depending on the currently selected object(s).
- When a Compensation sample is active, all customize functionality is disabled except those specified in “Compensation”, page 283.
- You can select multiple objects of the same type (e.g., plots), but not multiple objects of different types (e.g., plots and text boxes).

If you select multiple objects of different types, the Customize panel displays a warning message stating:

“Customize options are available only when similar objects are selected.”

Customize Plot options

Overview

Customize Plot options allow you to customize properties for the following plot types: *Histogram*, *Dot*, *Density*, and *Precedence Density*.



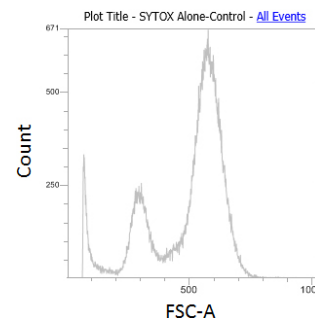
Note: To set default options for plots, use the *Options dialog* (page 331).

Plot types

Histogram plot

A *Histogram plot* is a graphical representation of single-parameter data and shows the relative number and distribution of events.

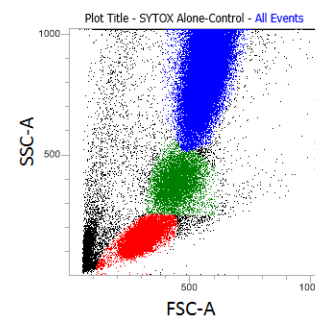
In a Histogram plot, the horizontal axis corresponds to the signal intensity of the selected parameter while the vertical axis represents the number of events (count).



Dot plot

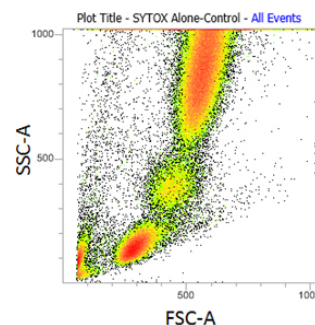
A *Dot plot* is a graphical representation of dual-parameter data, where each axis represents the signal intensity of one parameter.

Each dot in the plot corresponds to one or more events detected above the threshold. Different colors are used to represent the parent gate of events that fall within bins on the plots.



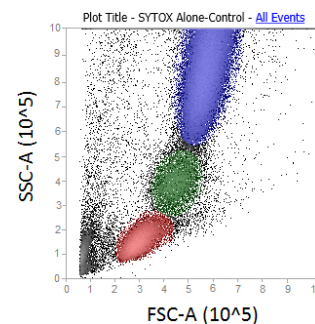
Density plot

A *Density plot* is a graphical representation of dual-parameter data, where the colors represent the collection of events with the same intensity and each axis represents the signal intensity of one parameter.



Precedence Density plot

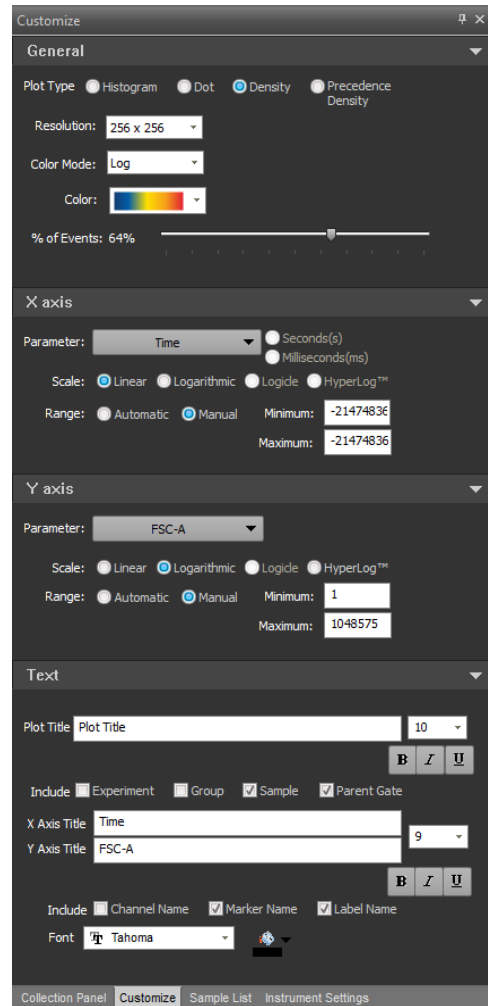
A *Precedence Density plot* is a combination of Dot and Density display, where a gradient is used to indicate the number of events within each of the plot bins and color is used to display the parent gate of events present.



Customize panel behavior for plots

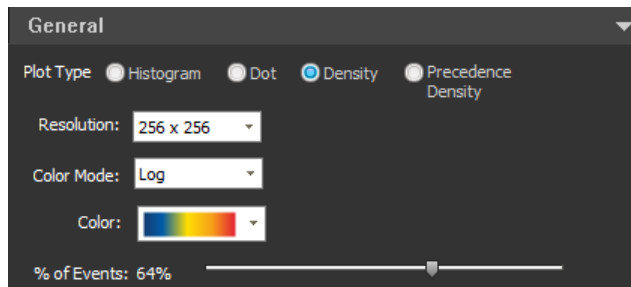
Customize Plot options are displayed only when one or more plots are selected. The example image below shows the options available for Density plots.

- The Customize Plot options are arranged into four groups:
 - *General* (page 203)
 - *X-axis* (page 211)
 - *Y-axis* (page 214)
 - *Text* (page 215)
- Each group is contained in a group box that is collapsible.
- The options displayed in each group vary, depending on the plot type(s) and options selected.
- The values displayed in any field are the current settings for the selected plot. If multiple plots with differing values are selected, the value fields show the indeterminate state.
- If you select a plot that is part of a Compensation Workspace, additional restrictions apply as described on the sections below.



General group

General group controls the plot type and general plot characteristics (such as plot resolution).



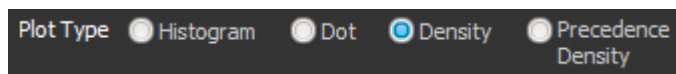
General group for Density plots



Note: The customization options that appear in the General group vary depending on the plot type(s) selected. This section describes all possible options available in the General group. For illustrations of the plot-specific options, see page 207.

Plot Type radio buttons

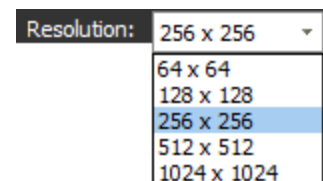
Plot Type radio buttons allow you to assign a plot type for the selected plots.



- This option applies to all plot types, and is displayed when any plot is selected.
- If one or more plots of the same type are selected, the radio button for that plot type is selected.
- If multiple plot types are selected, all radio buttons are deselected.
- Selecting a radio button changes all selected plots to the selected plot type.
- If the current Workspace is a Compensation Workspace, the Histogram radio button is disabled when Density, Dot, or Precedence Density plots are selected. Similarly, if Histogram plots are selected, the Density, Dot, and Precedence Density radio buttons are disabled.

Resolution dropdown menu

Resolution dropdown menu allows you to select a channel resolution for the selected plots.



- This option applies to all plot types, and is displayed when any plot is selected.
- If a single plot is selected, or if multiple plots with the same resolution (including a mixture of plot types) are selected, the Resolution dropdown menu displays the resolution of the currently selected plots.
- If multiple plots of any type with different resolutions are selected, the Resolution field is empty (blank).
- The maximum channel resolution is 1024 × 1024 channels for Dot, Density, and Precedence Density plots, and 1024 channels for Histogram plots.

- If a Histogram plot is selected along with two- parameter plots (Dot, Density, and/or Precedence Density plots), the Resolution dropdown menu for dual-parameter plots is displayed.
- If a Histogram plot is among the selected plots, the resolution is applied to the X-axis of the Histogram plot.
- The default resolutions are:
 - Histogram – 1024 channels
 - Dot – 256 × 256 channels
 - Density – 256 × 256 channels
 - Precedence Density – 256 × 256 channels

The defaults can be modified in *Plot Options* in the *Options dialog* (page 331).

Color dropdown menu

The **Color** dropdown menu allows you to select a color scheme for Density plots.



- This option applies is displayed when one or more Density plots are selected.
- If a single Density plot is selected, or if multiple Density plots with the same color scheme are selected, the Color field displays the density color scheme of the currently selected plots.
- If multiple Density plots with different color schemes are selected, the Color field is empty (blank).
- You can select a different color scheme from the dropdown menu. The selected color is applied to all selected Density plots.
- The default color scheme can be modified in *Plot Options* in the *Options dialog* (page 331).

% of Events slider

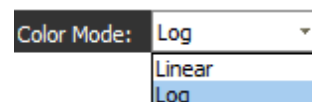
The **% of Events** slider allows you to adjust the percentage of events displayed on the selected plots. The sample data is taken from the entire data file.



- This option applies only to dual-parameter plots (Dot, Density, and Precedence Density plots), and is displayed when one or more dual-parameter plots are selected.
- This option is not displayed when only Histogram plots are selected. If Histograms plots are selected along with two- parameter plots, adjusting the slider has no effect on the Histogram plots.
- The current value for the slider setting is shown to the left of the slider and it is updated as the slider is moved.
- When using the mouse, adjustments can be made in 1-unit increments. When using the arrow keys, adjustments can be made in 5-unit increments. Clicking the bar to the left or right of the slider moves the slider by 25 units.

Color Mode dropdown menu

Color Mode dropdown menu lets you choose **Linear** or **Log** binning of the data to the selected color palette for Density and Precedence Density plots.



- This option applies only to Density and Precedence Density plots, and is displayed when one or more Density and/or Precedence Density plots are selected.
- If a single Density or Precedence Density plot is selected, or if multiple Density and/or Precedence Density plots with the same color mode are selected, the Mode field displays the color mode of the currently selected plots.
- If multiple Density and/or Precedence Density plots with different color modes are selected, the Color Mode field is empty (blank).
- You can select a different color mode from the dropdown menu. The selected mode is applied to all selected Density and/or Precedence Density plots.



Note: The *linear mode* bins data by assigning a color index for each density pixel linearly such that each increment is determined by dividing the range ($Z_{\max}-Z_{\min}$) by the number of color steps.

The *log mode* bins data by assigning a color index for each density pixel logarithmically such that each increment is determined by dividing the logarithmic range ($\log Z_{\max}-\log Z_{\min}$) by the number of color steps. The index is then determined as $\text{int}((\log Z_{\text{val}} - \log Z_{\min})/\text{increment})$.

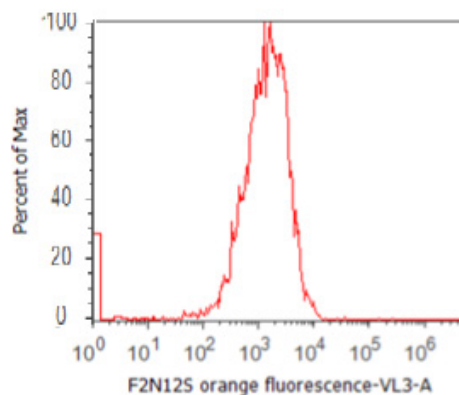
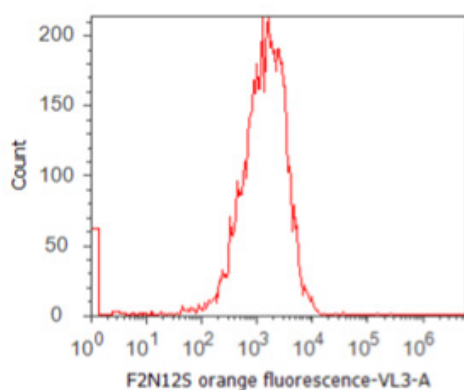
Normalize Count

Normalize Count checkbox controls scaling for the selected Histogram plots.

- This option applies only to Histogram plots, and is displayed when one or more Histogram plots are selected.
- When the checkbox is unchecked, the scale label is **Count**.
- When the checkbox is checked, the Y-axis scaling is changed to scale the plot to the Histogram peak value.

The scale displayed is a percentage scale from 0 to 100%, where 100% is the Histogram peak value.

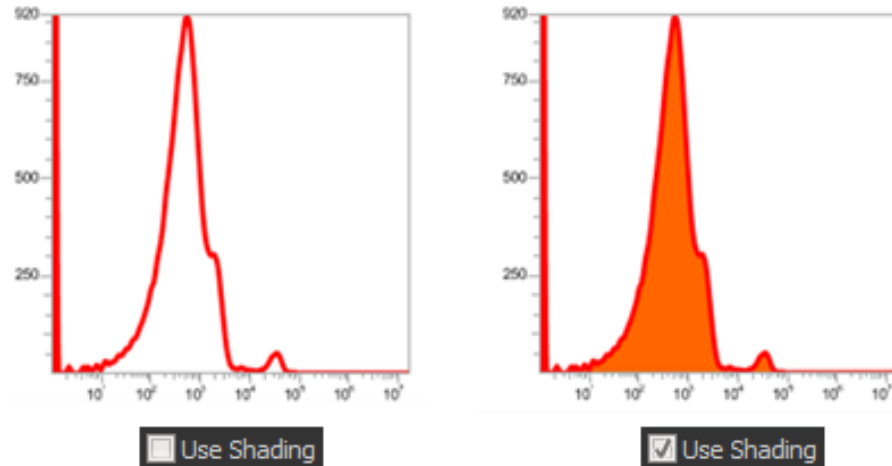
The scale label is **Percent of Max**.



Use Shading

Use Shading checkbox controls shading for the selected Histogram plots.

- This option applies only to Histogram plots, and is displayed when one or more Histogram plots are selected.
- The color used for shading is the same as that used for the line color (at 64% opacity). The color and the opacity cannot be adjusted.
- When the checkbox is unchecked, shading is removed.
- When the checkbox is checked, shading is added.



Line Width

Line Width menu controls line thickness for the selected Histogram plots.

- This option applies only to Histogram plots, and is displayed when one or more Histogram plots are selected.
- You can select or enter a number from 1 to 5.
- If you enter an out-of-range number, the software corrects it to the nearest valid number. Non-numeric characters are not permitted.

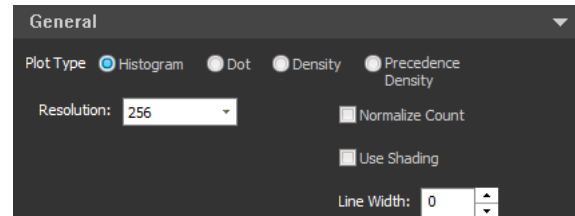
General group – plot-specific options

This section illustrates the customization options available in the General group when single or multiple plot types are selected.

The values displayed in any field are the current settings for the selected plot. If multiple plots with differing values are selected, the value fields will show the indeterminate state.

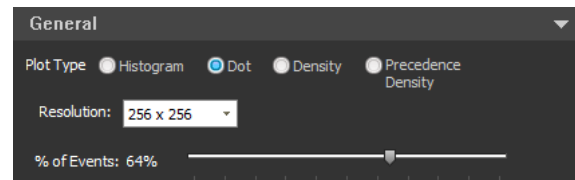
Histogram only

- Plot Type (page 203)
- Resolution (page 203)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)



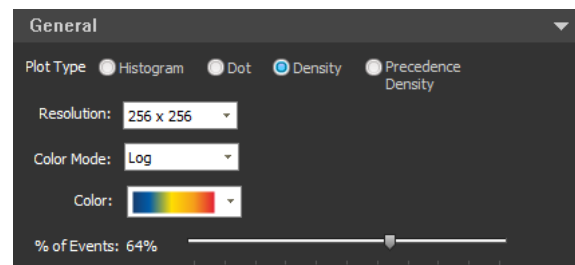
Dot only

- Plot Type (page 203)
- Resolution (page 203)
- % of Events (page 204)



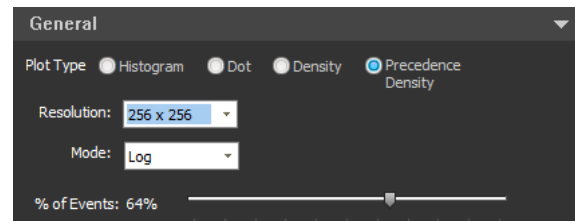
Density only

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- % of Events (page 204)



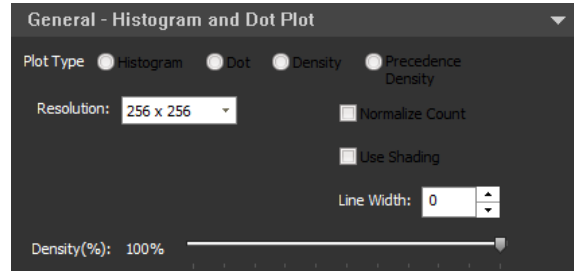
Precedence Density only

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- % of Events (page 204)



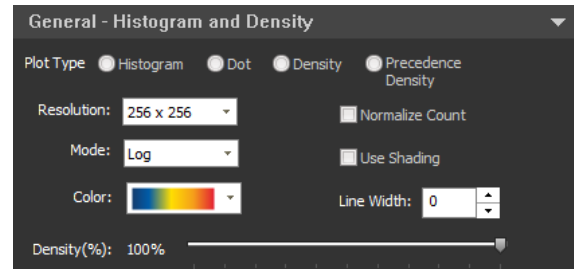
Histogram and Dot

- Plot Type (page 203)
- Resolution (page 203)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



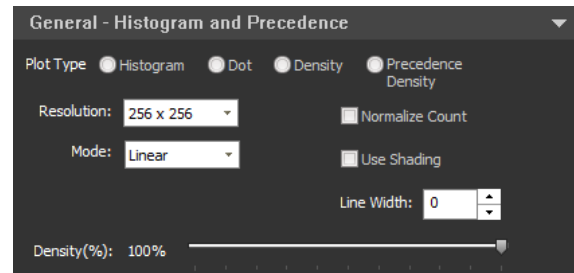
Histogram and Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



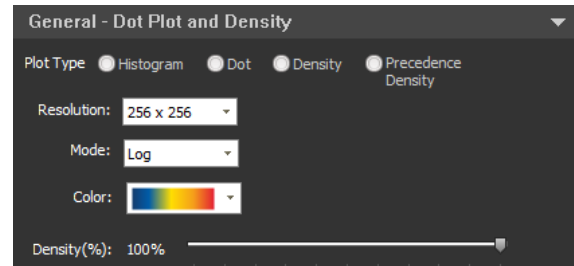
Histogram and Precedence Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



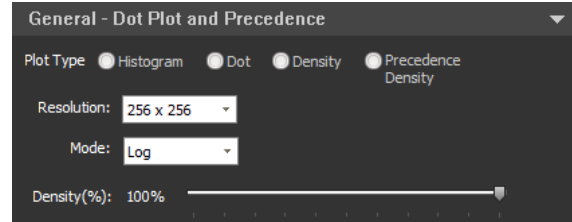
Dot and Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- % of Events (page 204)



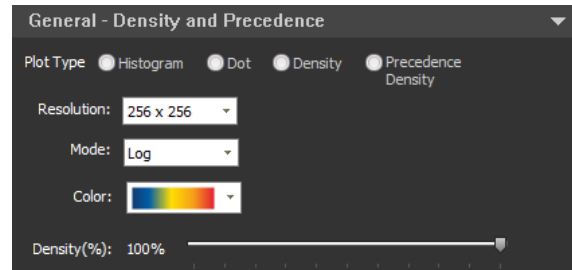
Dot and Precedence Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- % of Events (page 204)



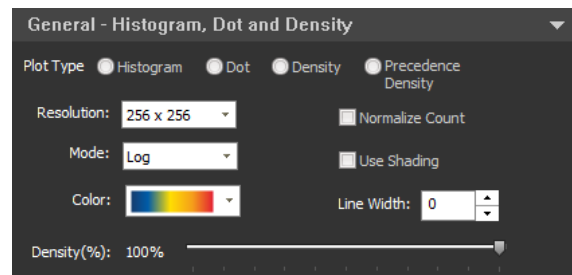
Density and Precedence Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- % of Events (page 204)



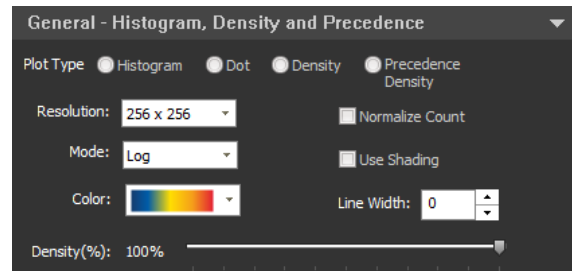
Histogram, Dot, and Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



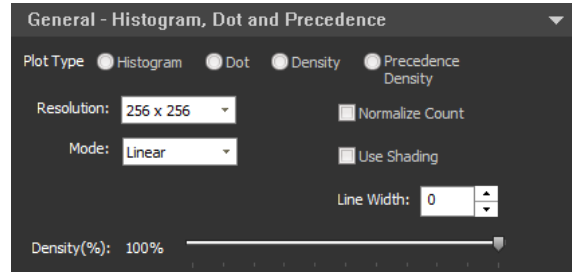
Histogram, Density, and Precedence Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



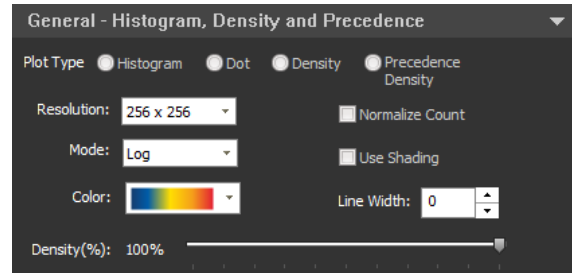
Histogram, Dot, and Precedence Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



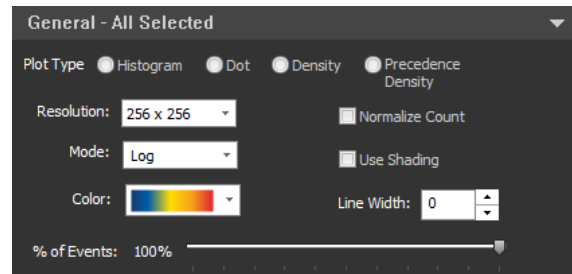
Dot, Density, and Precedence Density

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- % of Events (page 204)



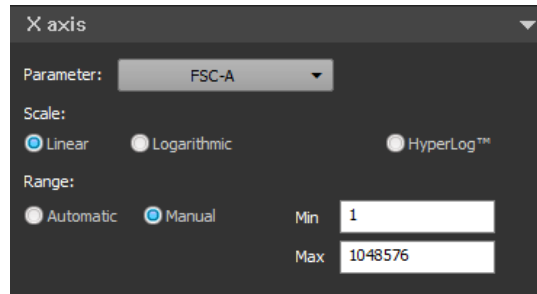
All plot types

- Plot Type (page 203)
- Resolution (page 203)
- Mode (page 205)
- Color (page 204)
- Normalize Count (page 205)
- Use Shading (page 205)
- Line Width (page 206)
- % of Events (page 204)



X-axis group

The *X-axis group* allows you to customize the X axes of selected plots.

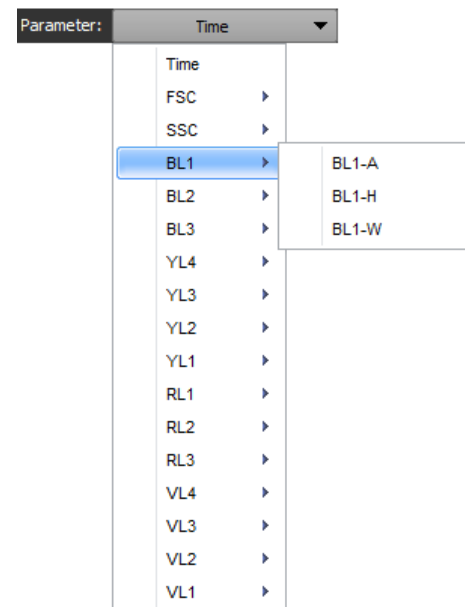
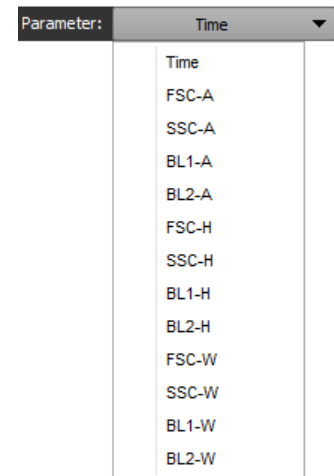


- All customization options in the X-axis group apply to all plot types, and are displayed when any plot is selected.
- The options that appear in the X-axis group vary depending on the scale selected (page 212).

Parameter dropdown menu

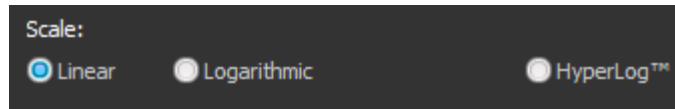
Parameter dropdown menu allows you to select any enabled parameter for the X-axis. All parameters in the FCS file.

- If a single plot is selected, or if multiple plots with the same X-axis parameter (including a mixture of plot types) are selected, the Parameter dropdown menu displays the parameters of the currently selected plots.
- If multiple plots of any type with different X-axis parameters are selected, the Parameter field is empty (blank).
- If the current Workspace is a Compensation Workspace, the Parameter dropdown menu is not available for dual-parameter or Histogram plots.
- To enable the Parameter dropdown menu, you can change the parameters of the gating plot within a Compensation Workspace.
- If more than 18 parameters are selected in the current Sample and if two or more measurements are enabled for the parameter, the dropdown menu includes a submenu of parameter types. Calculated parameters are always displayed in the primary menu.
- Selected plots are updated to display the selected parameter on the X-axis.



Scale options

- **Scale** options allow you to select the X-axis scaling method for the selected plots: **Linear**, **Logarithmic**, and **HyperLog™**.



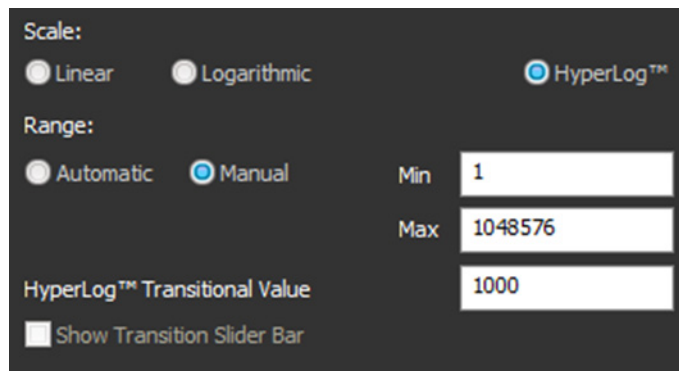
- Scale options are not available when a low-resolution (FCS2) file is the current file.



Note: The Hyperlog™ scale uses log-linear hybrid transformations to display compensated flow cytometry data that frequently contain negative values due to compensation. Logarithmic transformations cannot properly handle negative values, and poorly display normally distributed cell types.

HyperLog™ scale:

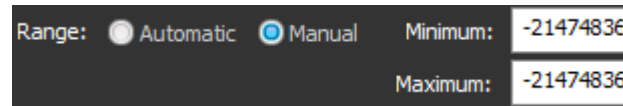
HyperLog™ is an alternative bi-exponential scaling. When the **HyperLog™** scale is selected, an additional **Transition Value** control is available.



- You can adjust the width of the linear area of the plot by entering a numeric value in the **HyperLog™ Transitional Value** field. Enter a value from 0 to 1,000,000; decimal places are not permitted.
- The Transition Value is applied to the selected parameter.

Range options

Range options allow you to adjust the scale range of a plot's axis.



Range:	<input type="radio"/> Automatic	<input checked="" type="radio"/> Manual	Minimum:	-21474836
			Maximum:	-21474836

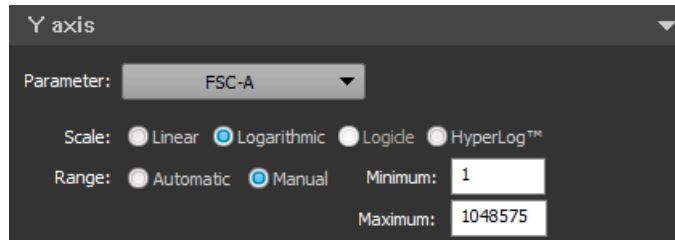
- **Automatic:** Sets the minimum and maximum values automatically and adjusts the upper value to the highest channel with data present.
 - For axes set to Log scaling, the minimum value is set at 1. For axes in Linear or HyperLog™ scaling, the minimum value is set to the lowest value in the data set.
 - Data ranges are limited by the parameter range set in the FCS file (\$PnR) (see Note below).
- **Manual:** Allows you can enter the minimum and maximum values manually. The allowable range depends on the type of scale selected.
 - For Linear and HyperLog™ scaling: The default manual range is \pm the maximum range set in the FCS file (\$PnR) (see Note below).
The allowable minimum range is -2^{32} to $2^{32}-1$.
The allowable maximum range is $-2^{32}+1$ to 2^{32} .
 - For Log scaling: The default manual range is 1 to the maximum value set in the FCS file (\$PnR) (see Note below).
The allowable minimum range is 1 to $2^{32}-1$.
The allowable maximum range is 2 to 2^{32} .
 - If **Time** is selected from the **Parameter** dropdown menu (page 211), the Range fields are based on seconds.
 - If you enter an out-of-range number in the Minimum or Maximum fields, the software adjusts it to the minimum or maximum value, respectively. Non-numeric characters are not permitted.



Note: \$PnR is the range for the selected parameter n (page 376). By default, this is set to 2^{26} for Event count and Time parameters, 2^{20} for all Height and Area measurements, and 2^{10} for all Width measurements.

Y-axis group

The *Y-axis group* allows you to customize the Y-axes of selected plots.



- All customization options in the Y-axis group apply to all plot types, and are displayed when any plot is selected.
- The options that appear in the Y-axis group vary depending on the scale selected (see below).

Parameter dropdown menu

Parameter dropdown menu allows you to select any enabled parameter for the Y-axis. All parameters in the FCS file, including any calculated parameters, are available.

The Parameter dropdown menu for the Y-axis behaves the same as the dropdown menu for the X-axis (page 211), except when a Histogram plot is selected.

- If a Histogram plot is selected, the Y-axis parameter is fixed as **Count** and cannot be changed unless the plot is changed to a dual-parameter plot type.
- If a Histogram plot is selected and the Normalize Count checkbox (page 205) is checked, the Y-axis parameter is fixed as **Percent of Max**.
- If Histograms plots are selected along with dual-parameter plots, the Parameter dropdown menu selection has no effect on the Histogram plots.

Scale options

Scale options allow you to select the Y-axis scaling method for the selected plots: **Linear**, **Logarithmic**, and **HyperLog™**.

- Scale options are not available when a low-resolution (FCS2) file is the current file.
- **Scale** radio buttons for the Y-axis behave the same as the options for the X-axis (page 212), except when a Histogram plot is selected:
 - If a Histogram plot is selected, the HyperLog™ scaling option is disabled.
 - If Histograms plots are selected along with two-parameter plots, all scale options are enabled; any changes to the HyperLog™ scaling option has no effect on the Histogram plots.

Range options

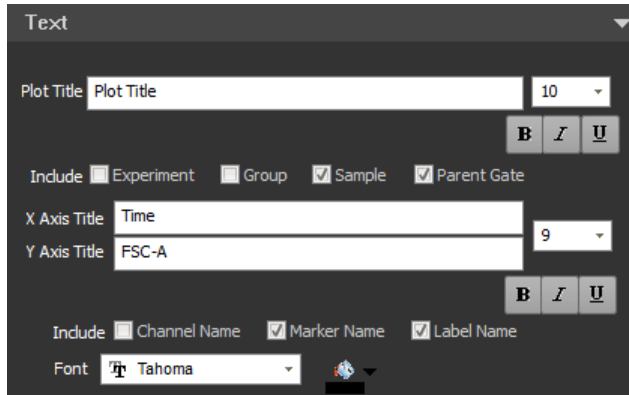
Range options allow you to adjust the scale range of a plot's axis. **Range** options for the Y-axis behave the same as the options for the X-axis (page 213), except when a Histogram plot is selected:

- If a Histogram plot is selected, the Y-axis is set to **Count**, the minimum value can be 0 to $2^{32}-1$, and the maximum value can be 1 to 2^{32} .
- For **Percent of Max**, the minimum value can be 1 to 99 and the maximum value can be 2 to 100.

Text group (plots only)

The *Text group* allows you to customize the plot text boxes. All customization options in this group apply to all plot types, and are displayed when any plot is selected.

The options in the plot Text group are disabled when items other than plots are selected in the Workspace or a Compensation Workspace is displayed.



The screenshot shows the 'Text' configuration panel for a plot. It includes the following fields and options:

- Plot Title:** A text input field containing 'Plot Title' and a font size dropdown menu set to '10'. To the right are buttons for Bold (B), Italic (I), and Underline (U).
- Include:** Checkboxes for 'Experiment', 'Group', 'Sample', and 'Parent Gate'. 'Sample' and 'Parent Gate' are checked.
- X Axis Title:** A text input field containing 'Time' and a font size dropdown menu set to '9'. To the right are buttons for Bold (B), Italic (I), and Underline (U).
- Y Axis Title:** A text input field containing 'FSC-A'.
- Include:** Checkboxes for 'Channel Name', 'Marker Name', and 'Label Name'. 'Marker Name' and 'Label Name' are checked.
- Font:** A dropdown menu set to 'Tahoma'.

Plot title options

Plot title options allow you to enter text and select font formats for the plot title. The formatted text appears at the top of all selected plots.



- You can enter up to 50 characters in the **Plot title** field; any character is permitted. If the plot title is too large to fit above the selected plots, the software truncates the title.
- In the **font size** field, you can select a font size from the dropdown menu, or enter a number from **6** to **72**.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can include the Experiment name, Group name, Sample name, and/or parent gate by checking the appropriate **Include** checkboxes. The software appends your selections to the plot title, separated by hyphens.



X-axis and Y-axis title options

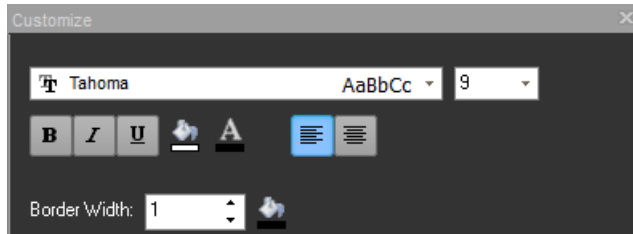
The **X-** and **Y-axis title** options allow you to enter text and select font formats for the X- and Y-axis titles. The formatted text appears as the X- and Y-axis labels for all selected plots.

- You can enter up to 50 characters in the X-axis and Y-axis title fields; any character is permitted. If an axis title is too large to fit in the selected plots, the software truncates the title.
- For the X-axis:
 - The tick channel labels change from displaying the full channel number to thousands or millions, as appropriate.
 - The tick channel labels display exponential numbers if the channel number entered is >10,000.
 - Parameter names are appended with (10^3) or (10^6) to indicate channel scaling.
- For the Y-axis:
 - If Histogram plots are selected, the Y-axis title defaults to **Count** if all Histogram plots are the same and none are normalized.
If Histogram plots are all set to Normalize Count (page 205), the title defaults to **Percent of Max**.
 - The tick channel labels display exponential numbers if the channel number entered is >10,000.
 - Parameter names are appended with (10^n) to indicate channel scaling.
- In the X-axis and Y-axis font size fields, you can select a **font size** from the dropdown menu, or enter a number from 6 to 72.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can include the wavelength, channel name, target name, and/or fluorophore name by checking the appropriate **Include** checkboxes. The software appends your selections to the axis labels, separated by hyphens.
 - If you do not check any Include checkboxes, the axis label defaults to the parameter name specified in the *Parameter Properties* dialog on the *Instrument Settings Panel* (page 180).
 - If the target and fluorophore names are selected but unavailable (i.e., the names are from third party support/imported FCS files), the default parameter or channel name are used.
 - Include options are not applied to the Y-axis of Histogram plots when the name is set to **Count** or **Percent of Max** as described above.

Customize Text box options

Text box options

Customize Text box group allows you to customize the font style and borders of text boxes. These options are displayed when one or more text boxes are selected in a Workspace.



Style options

Style options allow you to select the font type, size, and formatting for all selected text boxes.

- You can select a **font type** from the dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- You can select a **font size** from the dropdown menu, or enter a number from **6 to 72**.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can select to **left-align** or **center** the text.
- You can select **colors** for the text and for the Text box fill. The software uses the standard color picker dialog.

Border options

Border options allow you to select the border width and color for all selected text boxes.

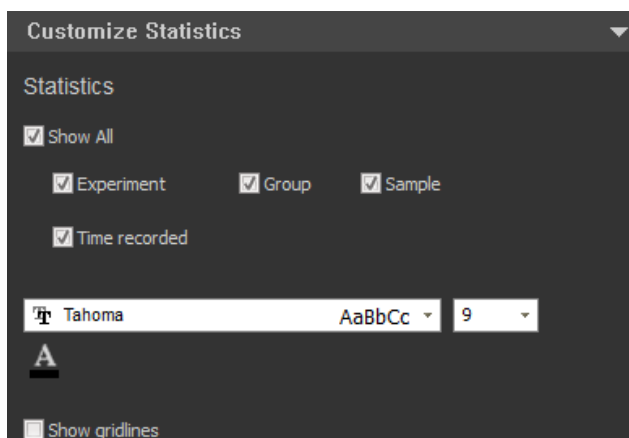
- You can select a **border width**, or enter an integer from **1 to 5**.
- You can select a **color** for the border. The software uses the standard color picker dialog.

Customize Statistics box options

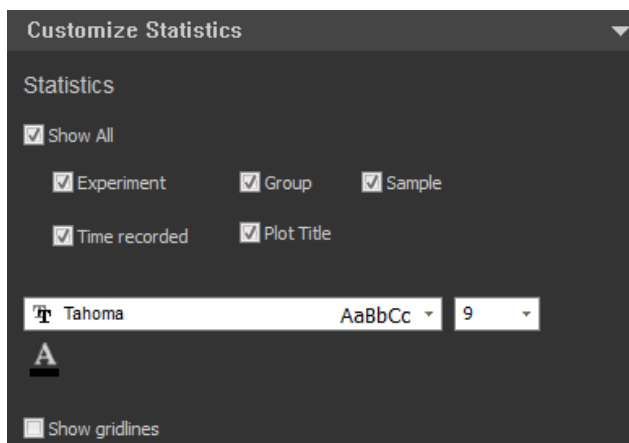
Statistics box options

Customize Statistics box group allows you to customize the formatting of statistics boxes and the statistics they displayed.

- The customization options are displayed when one or more Sample-level or Plot-level statistics boxes are selected in a Workspace. The options vary slightly between sample- and Plot-level statistics boxes.
- The following option is displayed when one or more **Sample-level** statistics boxes are selected:



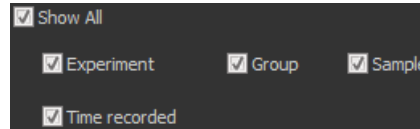
- The following option is displayed when one or more **Plot-level** statistics boxes are selected:



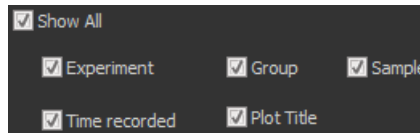
Header options

Header options allow you to select which elements are automatically added to the headers of all selected statistic boxes.

- For Sample-level statistics boxes, you can include the **Experiment**, **Specimen**, and **Sample names**, and the **Time Recorded** by checking the appropriate checkboxes.



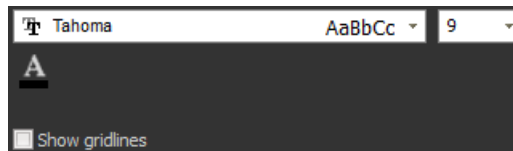
- For Plot-level statistics boxes, you can include the **Experiment**, **Group**, and **Sample** names, the **Time Recorded**, and the **Plot title** by checking the appropriate checkboxes.



- Checking the **Show All** checkbox selects all available elements.
- The software includes your selections in the statistics box header.

Formatting options

Formatting options allow you to select the font type, size, and formatting for all selected statistics boxes.



- You can select a **font type** from the dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- You can select a **font size** from the dropdown menu, or enter a number from 6 to 72.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can select a **color** for the text. The software uses the standard color picker dialog.
- You can display a border around the box and gridlines inside the box by checking the **Show Gridlines** checkbox. The border and gridline colors and widths cannot be adjusted.

Customize Gate options

Overview

Customize Gate options allow you to customize the properties of the following gate types:

- Rectangular, Oval, or Polygon
- Histogram
- Quadrant



Note: To set default options for gates, use the *Options dialog* (page 329).

Customize panel behavior for gates

- The Customize Gate options are displayed only when a gate is selected.
- The Customize Gate options are arranged into the following groups:
 - *Gate Type* (page 221)
 - *Coordinates* (page 224)
- Each group is contained in a group box that is collapsible.
- The options displayed in each group vary, depending on the gate types and options selected.
- The values displayed in any field are the current settings for the selected gate.
- When a Compensation sample is active, all gate customization options are disabled, except for the options specified in “Compensation”, page 283.

Gate Type group

Gate Type group controls the gate type and general gate characteristics (such as opacity and color).

The customization options that appear in the Gate Type group vary, depending on the gate types selected. This section describes all possible options available in the Gate Type group.

Rectangular gates

The screenshot shows the 'Gate Type' group for a rectangular gate. The 'Gate Type' dropdown is set to 'Rectangle'. Three icons are visible: a blue rectangle (selected), an oval, and a polygon. Below the icons, the 'Name' field contains 'R5' and the 'Show Name' checkbox is checked. The 'Opacity (%)' field is set to 0. The 'Coordinates' section is expanded, showing 'X Minimum: 257025', 'Y Minimum: 238593', 'X Maximum: 780268', and 'Y Maximum: 877568'.

Oval gates

The screenshot shows the 'Gate Type' group for an oval gate. The 'Gate Type' dropdown is set to 'Oval'. Three icons are visible: a rectangle, a blue oval (selected), and a polygon. Below the icons, the 'Name' field contains 'R3' and the 'Show Name' checkbox is checked. The 'Opacity (%)' field is set to 13. The 'Coordinates' section is expanded, showing 'X Center: 41247', 'Y Center: 168960', 'Height', 'Width', and 'Rotation: 0 degrees'.

Polygon gates

The screenshot shows the 'Gate Type' group for a polygon gate. The 'Gate Type' dropdown is set to 'Polygon'. Three icons are visible: a rectangle, an oval, and a blue polygon (selected). Below the icons, the 'Name' field contains 'R6' and the 'Show Name' checkbox is checked. The 'Opacity (%)' field is set to 0.

Quadrant gates

The screenshot shows the 'Gate Type' group for a quadrant gate. The 'Gate Type' dropdown is set to 'Quad'. A blue icon with a crosshair is selected. Below the icon, the 'Use smart gate naming' checkbox is checked. The 'UL Name' field contains 'Q1', 'UR Name' contains 'Q2', 'LL Name' contains 'Q3', and 'LR Name' contains 'Q4'. Each name field has a 'Show name' checkbox checked. The 'Opacity %' field is set to 0 for each quadrant. The 'Coordinates' section is expanded, showing 'X Center: 2865' and 'Y Center: 4242'.

Histogram gates

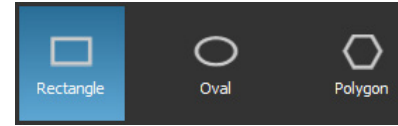
The screenshot shows the 'Gate Type' group for a histogram gate. The 'Gate Type' dropdown is set to 'Histogram'. A blue icon with the letter 'H' is selected. Below the icon, the 'Name' field contains 'R4' and the 'Show Name' checkbox is checked. The 'Opacity (%)' field is set to 0. The 'Coordinates' section is expanded, showing 'Minimum: 596' and 'Maximum: 49047'.

Gate Type buttons

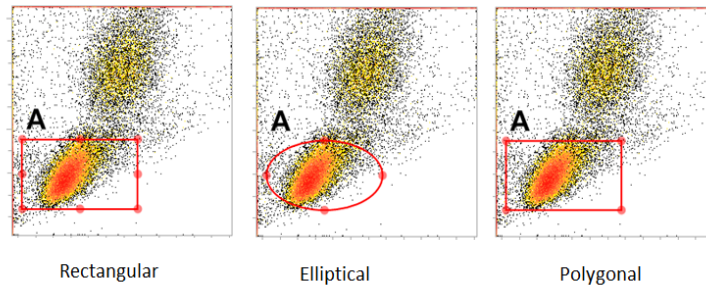
The Gate Type buttons allow you to select the gate type. The button for the currently selected gate type is highlighted.

Rectangular, Oval, or Polygon gates

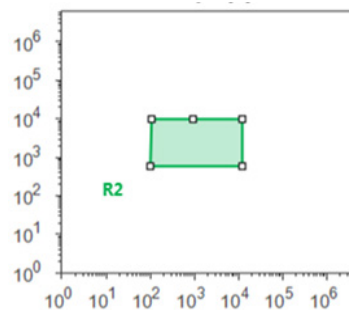
When a **Rectangular**, **Oval**, or **Polygon** gate is selected, all three gate type buttons are displayed together. The button for the currently selected gate type is highlighted.



- To change the gate type, click the **Gate Type button** of interest. The figure below shows the same area of the plot with a different gate type.

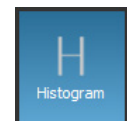


- When you select a different gate type, the software:
 - Creates a new gate with the same boundaries as the original gate (X minimum, X maximum, Y minimum, and Y maximum)
 - Sizes the new gate according to the original gate's size and position.
- When switching to a Polygon gate, the software creates a 5-point polygon in the shape of the bounding rectangle as shown below.



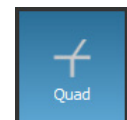
Histogram gates

When a Histogram gate is selected, only the Histogram button is displayed. You cannot change a Histogram gate to a different gate type.



Quadrant gates

When a Quadrant gate is selected, only the Quadrant button is displayed. You cannot change a Quadrant gate to a different gate type.



Name options

Name field

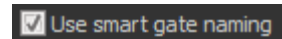
Name field displays the name of the currently selected gate. The Name options allow you to assign a gate name and show or hide the gate name on the plot.



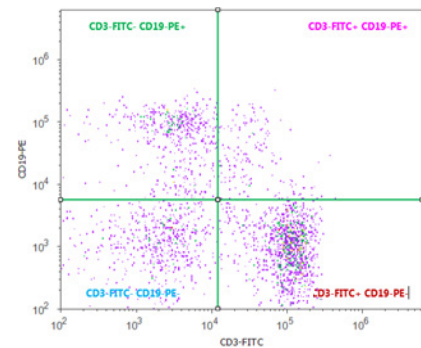
- You can enter up to 50 characters in the Name field; any character is permitted. Alternatively, you can select a gate name from the **Quick Select** dropdown menu. The dropdown menu options are set in *Gate Options* in the *Options dialog* (page 329).
- The gate name must be unique. If you enter a duplicate name, the software appends a numerical suffix in parentheses; for example, **GateName (2)**.
- You can show or hide the gate name on the plot by checking or unchecking the **Show Name** check box.
- For Quadrant gates, four Name fields are displayed (one for each quadrant).
- For Quadrant gates, you can select the **Use smart gate naming** option (see below) instead of entering the gate names.

Smart Gate Naming

Smart Gate Naming option allows you to select and deselect automatic gate naming. This option is available only for Quadrant gates.



- If you check the **Use smart gate naming** check box, the software automatically names the Quadrant gates, using the options specified in *Gate Options* in the *Options dialog* (page 329).
- For Quadrant gates, the four gate names are generated (one for each quadrant).
- Smart names can be up to 50 characters; any character is permitted.
- Smart names must be unique. If a duplicate name is generated, the software appends a numerical suffix in parentheses; for example, **GateName (2)**.
- If you edit a smart name, **Use smart gate naming** is automatically unchecked. You must enter the gate names manually, as described in **Name field** above.
- If you uncheck **Use smart gate naming**, you must enter the gate names manually, as described in **Name field** above.



Coordinates group

The customization options that appear in the Coordinates group vary depending on the gate type selected. This section describes all possible options available in the Coordinates group.



Note: Coordinate options are not available for Polygon gates.

Coordinates group for Rectangular gates

For a Rectangular gate, you can manually enter the **X** and **Y** **minimum** and **maximum** coordinates.

Coordinates	
X Minimum:	257025
Y Minimum:	238593
X Maximum:	780288
Y Maximum:	877568

- Non-numeric characters are not permitted.
- The allowable X and Y coordinates depend on the type of scale selected.
 - Linear and HyperLog™ scaling:
The allowable minimum range is -2^{32} to $2^{32}-1$.
The allowable maximum range is $-2^{32}+1$ to 2^{32} .
 - Log scaling:
The allowable minimum range is 1 to $2^{32}-1$.
The allowable maximum range is 2 to 2^{32} .
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Size and Coordinates group for Oval gates

For an Oval gate, you can manually enter the **coordinates of the center point**, and the **height**, **width**, and **rotation** values.

Coordinates	
X Center:	41247
Y Center:	168960
Height:	
Width:	
Rotation:	0 degrees

- Non-numeric characters are not permitted.
- The allowable X and Y coordinates depend on the type of scale selected:
 - Linear and HyperLog™ scaling: The allowable range is -2^{32} to 2^{32} .
 - Log scaling: The allowable range is 1 to 2^{32} .
- The allowable height range is -2^{32} to 2^{32} .
- The allowable width range is -2^{32} to 2^{32} .
- The allowable rotation range is -360 to 360 .
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Coordinates group for Histogram gates

For a Histogram gate, you can manually enter the **coordinates for the left and right boundaries**.

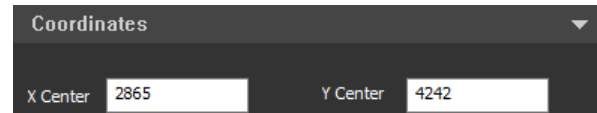


The screenshot shows a dark-themed dialog box titled "Coordinates" with a dropdown arrow on the right. Inside, there are two input fields. The first is labeled "Minimum:" and contains the number "596". The second is labeled "Maximum:" and contains the number "49047".

- Non-numeric characters are not permitted.
- The allowable coordinates depend on the type of scale selected:
 - Linear and HyperLog™ scaling:
The allowable minimum range is -2^{32} to $2^{32}-1$.
The allowable maximum range is $-2^{32}+1$ to 2^{32} .
 - Log scaling:
The allowable minimum range is 1 to $2^{32}-1$.
The allowable maximum range is 2 to 2^{32} .
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Coordinates group for Quadrant gates

For a Quadrant gate, you can manually enter the **coordinates of the center point**.



The screenshot shows a dark-themed dialog box titled "Coordinates" with a dropdown arrow on the right. Inside, there are two input fields. The first is labeled "X Center" and contains the number "2865". The second is labeled "Y Center" and contains the number "4242".

- Non-numeric characters are not permitted.
- The allowable X and Y coordinates depend on the type of scale selected:
 - Linear and HyperLog™ scaling: The allowable range is -2^{32} to 2^{32} .
 - Log scaling: The allowable range is 1 to 2^{32} .
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Customize Experiment (Plate or Tube) options

Overview

Customize Plate Experiment and *Customize Tube Experiment* options allow you to customize the following:

- Plate information (available only for Plate Experiments)
- Experiment information
- Group information
- Sample information

Customize panel for Plate Experiments

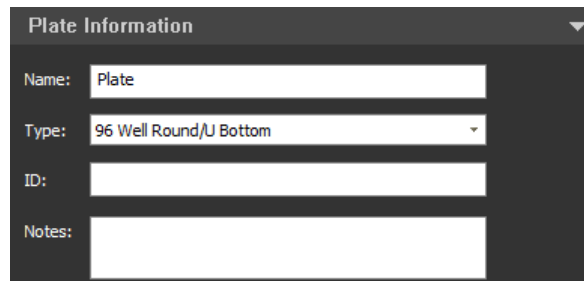
- Customize Plate options are available only when a Plate Experiment is displayed and the *Heat Map view* (page 105) is in focus.
- The Customize Plate options are arranged into the following groups:
 - *Plate information* (page 227)
 - *Experiment information* (page 229)
 - *Group information* (page 230)
 - *Sample information* (page 231)
- Each group is contained in a group box that is collapsible.
- The values displayed in any field are the current settings.
- Plate information is automatically saved and applied to all Samples in the Plate, including any Tubes added to the Plate Experiment, once the focus is lost from the selected field.
- Experiment information is applied to the selected Experiment once the focus is lost from the selected field.
- Group information is automatically saved in the selected Group once the focus is lost from the selected field.
- Sample information is automatically saved and applied to all selected Samples once the focus is lost from the selected field.

Customize panel behavior for Tubes

When a Tube Experiment is loaded, the customizations options are the same as the options available for Plate Experiments, but the Plate information options (page 227) are not displayed.

Plate information

Plate information options are available only when a Plate Experiment is displayed in the application and the Heat Map view (page 105) is in focus.



Note: Plate information options are not available when a Tube Experiment is loaded.

Name

- The **Name** field displays the current name of the Plate.
- You can modify the name of the selected Plate by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the Plate once the focus is lost from the Name field.
- The Plate Name field in the Experiment Explorer (page 142) is automatically updated when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field.

The following characters are not allowed: \ / : * ? " < > | %.

- If an invalid name is present in the Name field when focus is lost, the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Plate name when the focus is lost; for example, **Plate(2)**.

If the addition of the suffix causes the Plate name to exceed the 50-character limit, the entered name is truncated.

Plate Type

- The Plate Type dropdown list allows you to select the type of micro-titer plate (U-, V-, or flat-bottom) that is being used for the current Plate (96- or 384-well). The following options are available:
 - 96 Well Round/U Bottom
 - 96 Well Flat Bottom
 - 96 Well Conical/V Bottom
 - 96 Deep Well Round/U Bottom
 - 96 Deep Well Conical/V Bottom
 - 384 Well Round/U Bottom
 - 384 Well Flat Bottom
 - 384 Well Conical/V Bottom
 - 384 Deep Well Round/U Bottom
 - 384 Deep Well Conical/V Bottom
- You cannot switch between 96- and 384-well plates.
The dropdown list is limited to 96- or 384-well plate options based on the original Plate selected when creating the new Experiment as described in “Creating a Plate Experiment”, page 305.

Plate ID

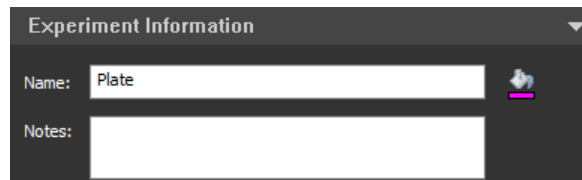
- The Plate ID field allows 50 characters to be entered to identify the selected Plate. Any printable character may be entered.
- The Plate ID is applied to the Plate and is used by any Tubes added to the Experiment.

Notes

- You can enter up to 500 characters in the Notes field to provide relevant information about the Plate; any printable character is permitted.
- The notes are saved with all Samples in the Plate, including any Tube Experiments added to the Plate.
- Two lines of space are given by default. If you enter more text than can fit in the available space, a vertical scroll bar and the “...” button is displayed adjacent to the text field.
- Clicking the ... **button** opens the *Notes dialog*, which contains an editable text field that allows up to 500 characters to be displayed and edited. You can enter any printable character.

Experiment information

Experiment information options are available when the Heat Map view (page 105) is in focus and any Sample is selected.



Name

- The **Name** field displays the name of the currently selected Experiment.
- You can modify the name of the selected Experiment by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the Experiment once the focus is lost from the Name field.
- The Experiment Name field in the Experiment Explorer (page 141) is updated automatically when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field. Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field. The following characters are not allowed: \ / : * ? " < > | %.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Experiment name when the focus is lost; for example, **Experiment(2)**.

If the addition of the suffix causes the name to exceed the 50-character limit, the entered name is truncated.

Experiment color picker

- Clicking the **color icon** opens a standard color picker dialog, which allows you to customize the color for the current Experiment.
- The Experiment color appears on the Heat Map view.



Notes

- The Notes field for the Experiment information works the same way as the Notes field for the Plate information (page 228).

Group information

Group information options are available when the Heat Map view is in focus and any Samples belonging to the same Group are selected.




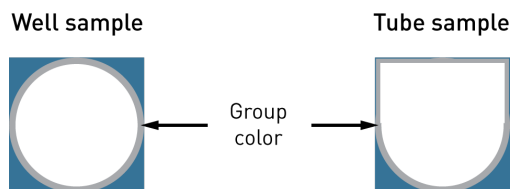
Name

- The **Name** field displays the name of the currently selected Group.
- You can modify the name of the selected Group by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the selected Group once the focus is lost from the Name field.
- The Group Name field in the Experiment Explorer (page 141) is updated automatically when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field. Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field. The following characters are not allowed: \ / : * ? " < > | %.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Group name when the focus is lost; for example, **Group(2)**.

If the addition of the suffix causes the name to exceed the 50-character limit, the entered name is truncated.

Group color picker

- Clicking the **color icon** opens a standard color picker dialog, which allows you to customize the color for the current Group. 
- The Group color appears on the Heat Map view.



- This option is disabled when the selected Well or Tube is a Compensation control (page 283). When multiple Wells are selected, the Group color is not applied to Compensation controls.

Notes

- The Notes field for the Group information works the same way as the Notes field for the Plate information (page 228).

Sample information *Sample information* options are available when the Heat Map view is in focus and a Tube or a Well sample is selected.



- The Name field is hidden if multiple Samples are selected in the Heat Map view.
- The Notes section is enabled and visible when multiple Tube or Well samples are selected.

Name

- The **Name** field displays the name of the currently selected Sample.
- You can modify the name of the selected Sample by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the Sample once the focus is lost from the Name field.
- The Sample Name field in the Experiment Explorer (page 141) is updated automatically when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field. Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field. The following characters are not allowed: \ / : * ? " < > | %.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Sample name when the focus is lost; for example, **Sample(2)**.

If the addition of the suffix causes the name to exceed the 50-character limit, the entered name is truncated.

Notes

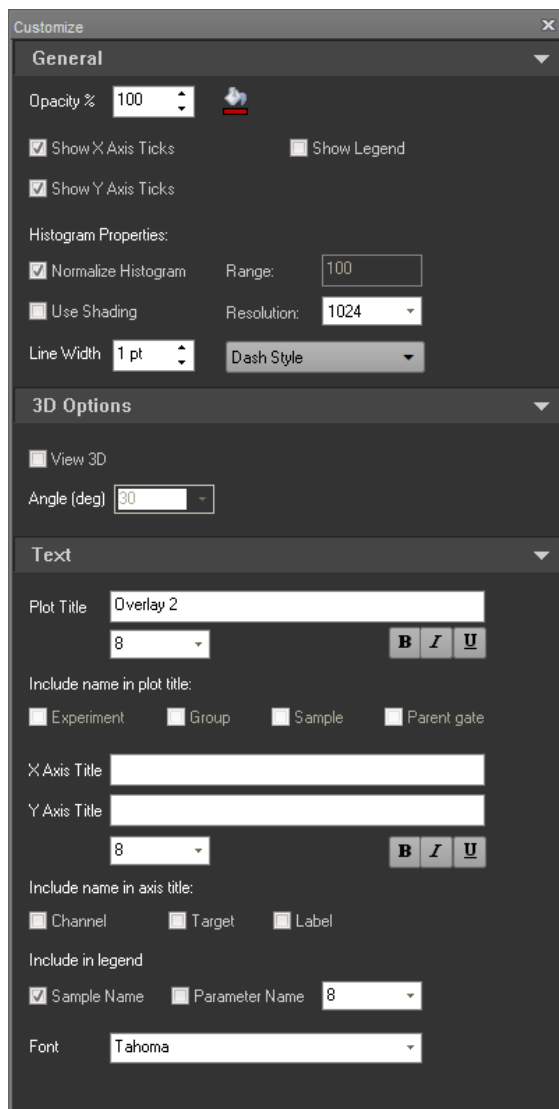
- The Notes field for the Sample information works the same way as the Notes field for the Plate information (page 228).

Customize Overlay options

Overview

The *Customize* menu displays options for editing overlay plots when Overlay or Gallery plots(s) are selected. The options displayed depend on whether Overlay plots or Gallery plots are selected.

You can customize the appearance of Overlay and Gallery plots in an Overlay using options available in the *Customize* menu.



Overlay plots selected

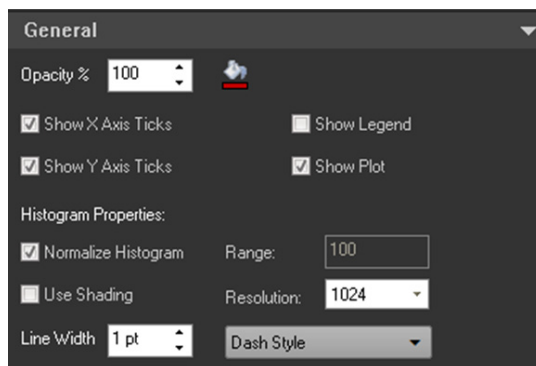


Gallery plots selected

- The *Customize* options are arranged into the following functional groups:
 - *General options* (page 233)
 - *3D options* (available only for Overlay plots) (page 236)
 - *Calculations* (available only for Gallery plots) (page 237)
 - *Text* (page 238)
- Each functional group is contained in a collapsible group box.

General options

General options control line style and plot display options. They are available for both Overlay plot and Gallery plots.



Opacity

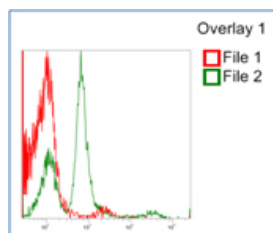
- The **Opacity** option controls the opacity of the line color for Gallery or Overlay Histograms or the data color for dual-parameter plots.
- To change the **Opacity**, enter a number between **20** and **100** into the **Opacity text field**.
- If Histograms are filled, the opacity option applies to both the histogram line and the fill of the histogram.

Data Color

- Click **Data Color** to open the color picker dialog, which allows you to choose the line color for the selected Histograms or the data color for selected dual-parameter plots.
- This option is available when any individual or a combination of gallery plots is selected.
- The selected color is applied to all selected plots.

Show Legend

- Select the **Show Legend** option to display the legend for an Overlay plot or displays the plot statistics as a legend for a Gallery plot.
- The legend appears to the right of the plot.



Show X-Axis and Show Y-Axis Ticks

- By default, **Show X-Axis Ticks** and **Show Y-Axis Ticks** options are checked.
- When the **Show X-Axis Ticks** option is checked, the X-axis tick marks are displayed on selected plots.
- When the **Show Y-Axis Ticks** option is checked, the X-axis tick marks are displayed on selected plots.
- When the options are unchecked, the corresponding tick marks are not shown on the selected plots.

Line Width

- The **Line Width** option is only active when Histogram plots in the Gallery or Histogram-based Overlay plots are selected.

This option is unavailable when only dual-parameter plots are selected.

If a mixture of plot types are selected (Histograms and dual-parameter plots), this option is available, but will only apply to Histogram plots.

- The **Line Width text box** allows adjustment of the line width of selected histogram plots. You can directly enter an integer between 1 and 5 in the textbox.

Alternatively, you can use the **up** and **down arrows adjacent to the text box** to increase or decrease the line width between **1** and **5**.

- If you enter a line width value that is outside the allowed range, the number is automatically adjusted to the nearest limit.
- If you select different line widths for individual gallery plots, the line width textbox initially appears blank with the edit box active.
- Click the **up arrow** to increase the line width in steps of 1 for each gallery plot and the overlay plot.
- Click the **down arrow** to decrease the line width in steps of 1 for each gallery plot and the overlay plot.
- The up and down arrow buttons have no effect if the edit field is blank.

Dash Style

- The **Dash Style** option is only available when Histogram Gallery plots or Histogram Overlay plots are selected.

- The option is not visible when only dual-parameter plots are selected.

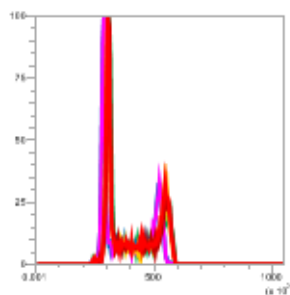
- If you select a mixture of plot types (Histograms and dual-parameter plots), this option is visible, but only applies to the selected histogram plots.



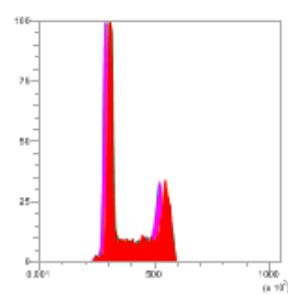
- The **Dash Style** dropdown displays a list of available dash styles (solid, dash, dots, dash dot, dash dot dot)
- The dash style selected from the dropdown list applies to all selected Gallery plots or individual members of Overlay plots.
- The default dash type is a solid line.

Use Shading

- **Use Shading** checkbox controls the shading for the selected Histogram plots.
- This option applies only to Histogram Overlay plots or Gallery plots.



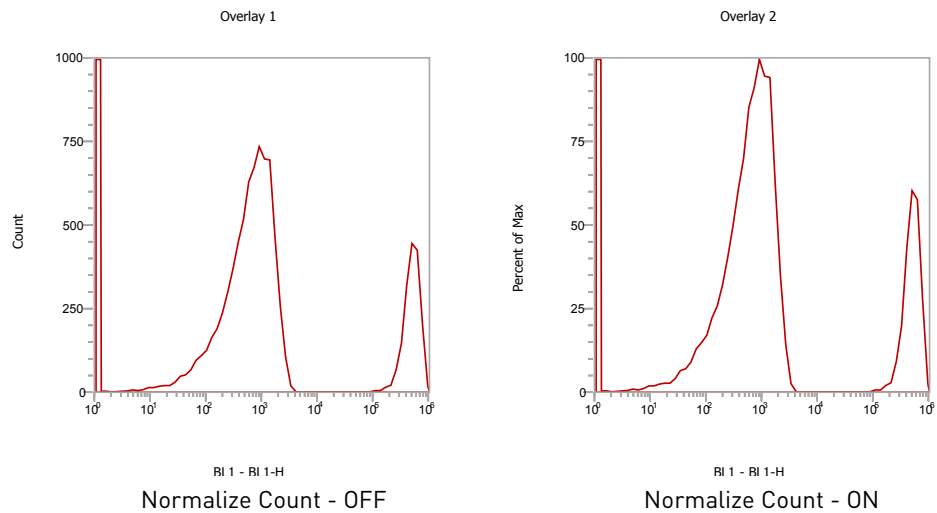
Use Shading - OFF



Use Shading - ON

Normalize Count

- **Normalize Count** checkbox controls the scaling for the selected Histogram plots.
- This option applies only to Histogram plots, and it is only displayed when one or more Histogram plots are selected.
- When the **Normalize Count** is unchecked, the scale label is **Count**.
- When the **Normalize Count** is checked, the Y-axis scaling is changed to scale the plot to the Histogram peak value.
- The scale displayed is a percentage scale from 0 to 100%, where 100% is the Histogram peak value.
- The scale label is **Percent of Max**.

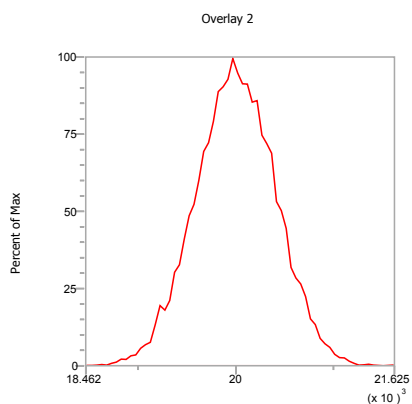


Range

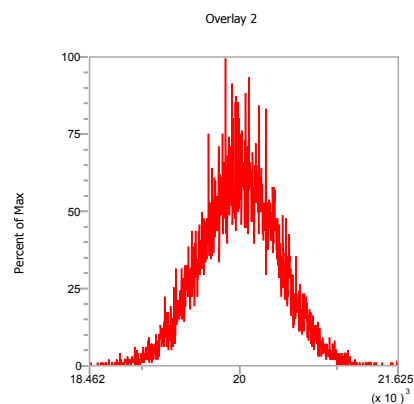
- **Range** sets the upper y-axis range for the selected histograms. The lower end of the range is always set to 0.
- To change the **Range**, enter the desired value in the **Range textbox**. Range will not be automatically set and must be manually adjusted.
- The **Range** option control is only enabled when the normalize histogram checkbox is unchecked.

Resolution

- **Resolution** determines the resolution for the selected histograms and sets the bin size for drawing the histogram data.
- The available resolutions are **64, 128, 256, 512, and 1024**.
- The default resolution is 1024.



FSC - FSC-H
Resolution: **64**

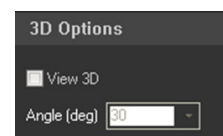


FSC - FSC-H
Resolution: **1024**

3D Options

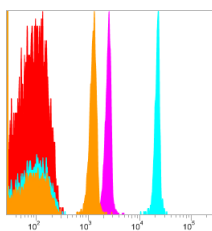
3D Options allow you to display Overlay plots three dimensionally and set the view option for 3D plots.

3D options are available only when one or more Overlay plots are selected. You cannot view Gallery plots in the 3D mode.

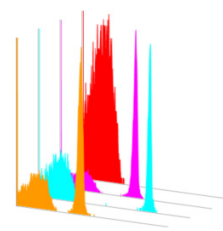


View 3D

- Select **View 3D** to display selected Overlay plots in the *3D mode*.



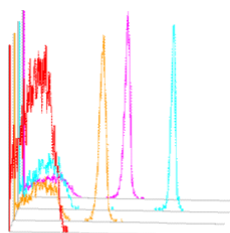
Overlay mode



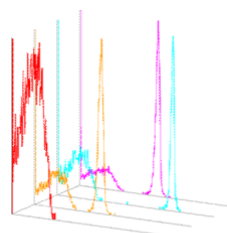
3D mode

Angle(deg)

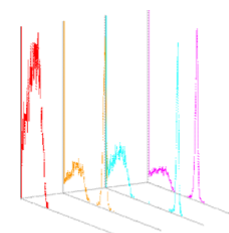
- **Angle(deg)** determines the viewing angle of the Overlay plot in the 3D mode.
- The **Angle (Deg) dropdown** becomes active only when **View 3D** is selected.
- **Angle(deg)** dropdown displays a list of angles (5, 15, 30, 45, 60, 75 degrees). Increasing the angle rotates the 3D plot clockwise.



5 Degrees



30 Degrees



60 Degrees

Calculations

The **Calculations** functional group is displayed only for Gallery plots.

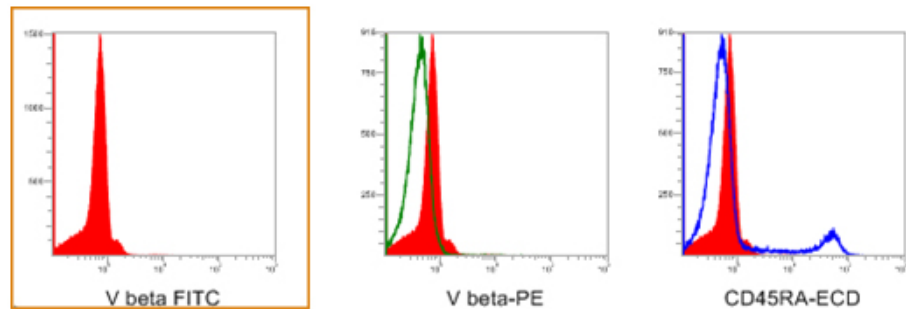
The Calculations options are available only for single-parameter plots; they are not displayed if a dual-parameter Gallery plot is selected.



Set as Control

- **Set as Control** option designates the selected plot as the “control plot” and overlays all Gallery plots with it to allow direct visual comparison of several “test plots” to one “control plot”.

The Set as Control button is available only when a single Gallery plot is selected. By default, Set as Control is deselected.



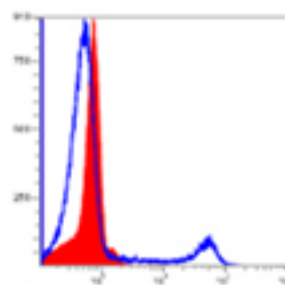
- The plot set as a control is always moved to the first position in the Gallery and highlighted in a dashed orange rectangle.
- To remove a control plot, click **Remove control**.

Move to Front

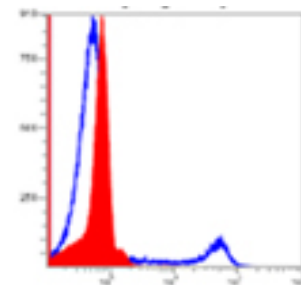
- Click **Move to Front** to display the control plot overlay on each Gallery plot in front of the data plot.

Click the button again to move the control plot behind the selected plot in the Gallery view.

- Move to Front button is available only when the Set as Control option has been selected.
- By default, this option is not selected and the data plot is displayed in front of the control plot.



Move to Front - OFF



Move to Front - ON

Calculation Method Radio Buttons

- **Calculation Method** radio buttons are only available once a plot has been set as a control.
- By default, **None** is selected and no calculations are applied.
- The **Overton** option compares the control sample and each Gallery plot using Overton's cumulative statistics calculation (page 380).
- The **KS** option compares the control and test samples using the Kolmogorov-Smirnov test (page 380) to determine the probability that the two plots are the same.

Text

Text options allow you to customize the plot text on Overlay or Gallery plots.

Text options consist of the Text group and the Legend group controls.

- The *Text group options* are the same as those described for the "Text group" in "Customize Plot Options", page 215.
- The *Legend group options* contain the sample name and Parameter name checkboxes.
- Select the **sample name** and/or the **Parameter name** checkbox to display the corresponding legend on the plot. You can select either one or both options for display
- You can edit the legend font size using the **Font size** control. Font size is selected from the Font size **dropdown menu**, or by entering a number between **6** and **72** into the **font size text field**.

The screenshot shows the 'Text' configuration panel with the following settings:

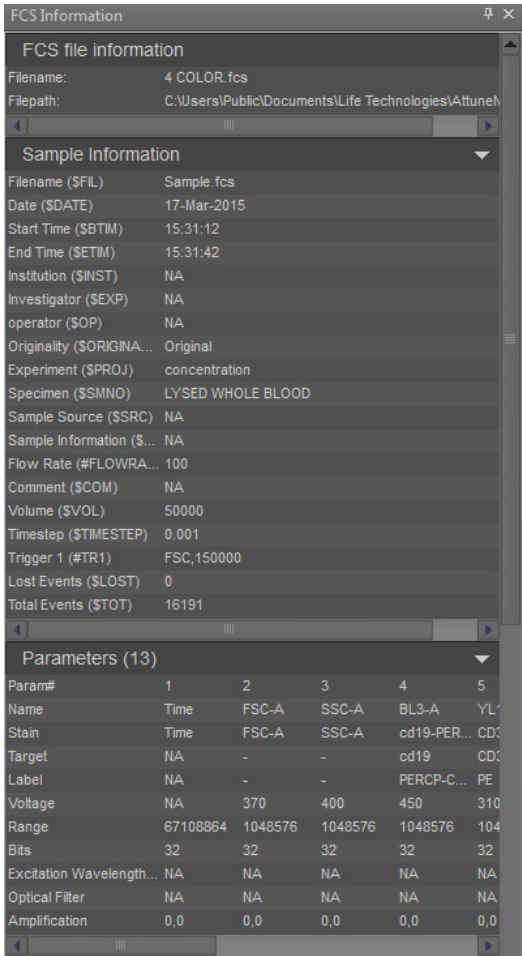
- Plot Title: Overlay 2
- Font size: 8
- Formatting: Bold (B), Italic (I), Underline (U) buttons are visible.
- Include name in plot title: Experiment, Group, Sample, Parent gate (all unchecked).
- X Axis Title: (empty)
- Y Axis Title: (empty)
- Font size: 8
- Formatting: Bold (B), Italic (I), Underline (U) buttons are visible.
- Include name in axis title: Channel, Target, Label (all unchecked).
- Include in legend: Sample Name (checked), Parameter Name (unchecked), Font size: 8
- Font: Tahoma

13. FCS Information panel

Overview

FCS Information panel displays the FCS metadata (the text segment portion) of the selected Sample.

- The panel is set as a floating panel by default (i.e., its “Dockable” attribute deselected), but it can be set as a dockable panel as described on page 32.
- The panel header displays the name of the selected Sample, and the complete file path is shown under the panel header.
- The panel contents are organized into the following groups:
 - *Sample information* (page 240)
 - *Parameters* (page 241)
 - *Compensation* (page 241)
 - *System information* (page 242)
 - *Data format* (page 243)
 - *Other* (page 243)
- Each group can be collapsed and expanded. By default, the groups are all expanded, unless otherwise specified below. The expanded or collapsed state persists on a per user basis.
- The metadata are displayed in a table where the first column is the FCS keyword and the second column is the keyword value.
- Only keywords and keyword values that are present within the FCS file are shown.
- If the panel contents exceed the visible area, vertical and horizontal scroll bars are shown as needed.



Note: The FCS keyword and keyword values are as described in “Appendix A: FCS File Reference”, page 375.

FCS file information

Sample information group

The *Sample information group* contains keywords that describe the FCS file.

The keywords displayed in the Sample information group are listed in the following table and are shown in the sort order indicated in the table.

Sample Information	
Filename (\$FIL)	Sample.fcs
Date (\$DATE)	17-Mar-2015
Start Time (\$BTIM)	15:31:12
End Time (\$ETIM)	15:31:42
Institution (\$INST)	NA
Investigator (\$EXP)	NA
operator (\$OP)	NA
Originality (\$ORIGINA...)	Original
Experiment (\$PROJ)	concentration
Specimen (\$SMNO)	LYSED WHOLE BLOOD
Sample Source (\$SRC)	NA
Sample Information (\$...)	NA
Flow Rate (#FLOWRA...)	100
Comment (\$COM)	NA
Volume (\$VOL)	50000
Timestep (\$Timestep)	0.001
Trigger 1 (#TR1)	FSC,150000
Lost Events (\$LOST)	0
Total Events (\$TOT)	16191

Sort	Keyword	Description
1	\$FIL	Filename
2	GUID – BD Keyword	GUID
3	\$DATE	Date
4	\$BTIM	Start Time
5	\$ETIM	End Time
6	\$INST	Institution
7	\$EXP	Investigator
8	\$OP	Operator
9	\$LAST_MODIFIER	Last Modified By
10	\$LAST_MODIFIED	Last Modified
11	\$ORIGINALITY	Originality
12	\$PLATENAME	Plate Name
13	\$PLATEID	Plate ID
14	\$WELLID	Well Location

Sort	Keyword	Description
15	\$PROJ	Experiment
16	\$SMNO	Specimen
17	\$SRC	Sample Source
18	\$CELLS	Sample Information:
18	#FLOWRATE	Flow Rate
19	\$COM	Comment
19	\$VOL	Sample Volume
20	#TOTALVOLUME	Sample Volume
21	\$Timestep	Timestep
22	\$TR	Trigger
23	\$ABRT	Aborted Coincident Events
24	\$LOST	Lost Events
25	\$TOT	Total Events

Parameters group

The *Parameters group* contains the parameter description keywords (e.g., \$PnN, \$PnS, etc.) for each parameter.

The number of parameters (Parameters (\$PAR)) is indicated above the table displaying the Parameter keywords as shown below.

Param#	1	2	3	4	5
Name	Time	FSC-A	SSC-A	BL3-A	YL1-A
Stain	Time	FSC-A	SSC-A	cd19-PER...	CD3
Target	NA	-	-	cd19	CD3
Label	NA	-	-	PERCP-C...	PE
Voltage	NA	370	400	450	310
Range	67108864	1048576	1048576	1048576	104
Bits	32	32	32	32	32
Excitation Wavelength...	NA	NA	NA	NA	NA
Optical Filter	NA	NA	NA	NA	NA
Amplification	0,0	0,0	0,0	0,0	0,0

The column headers indicate the parameter number and the row labels correspond to the Parameter description keywords as described in the following table below.

Sort	Keyword	Description
1	\$PnN	Channel Name
2	\$PnS	Stain
3	#PnTarget	Target
4	#PnLabel	Label
5	\$PnV	Voltage
6	\$PnR	Range
7	\$PnG	Gain
8	\$PnB	Bits

Sort	Keyword	Description
9	\$PnL	Excitation Wavelength
10	\$PnO	Excitation Power
11	\$PnF	Optical Filter
13	\$PnE	Amplification
14	\$PnP	% Emitted Light
14	\$PnT	Detector Type
15	\$PnD	Visualization Scale
16	\$PnCALIBRATION	Calibration

Compensation group

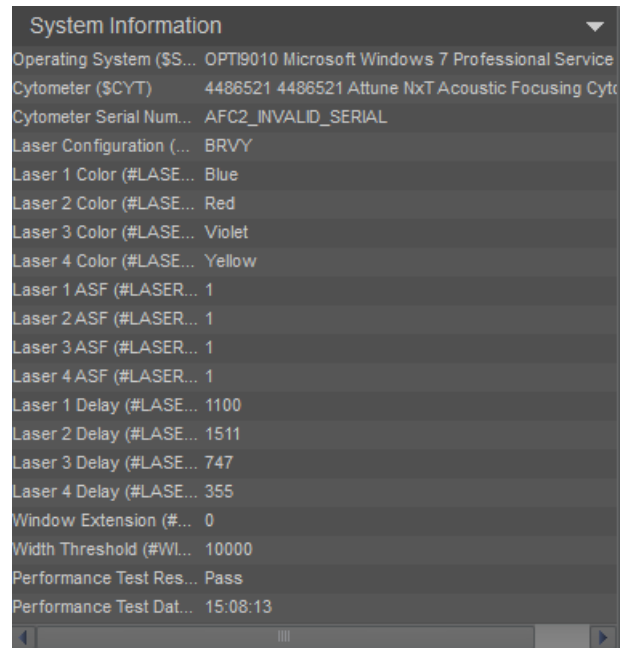
The *Compensation group* displays the spillover values contained within the FCS file in a matrix view where the row and column headers are named using the \$PnN values (i.e., Channel name).

The keywords used to establish compensation (\$Spillover, \$Comp, Spill, Spillover, None) are shown next to the compensation group name.

	BL3-A	YL1-A	RL1-A	VL2-A	BL3-H	YL1-H
BL3-A	100.00	0.04	3.20	0.02	0.00	0.00
YL1-A	63.74	100.00	0.07	0.04	0.00	0.00
RL1-A	2.48	0.01	100.00	0.01	0.00	0.00
VL2-A	0.30	0.01	0.08	100.00	0.00	0.00
BL3-H	0.00	0.00	0.00	0.00	100.00	0.00
YL1-H	0.00	0.00	0.00	0.00	63.74	100.00
RL1-H	0.00	0.00	0.00	0.00	2.48	0.00
VL2-H	0.00	0.00	0.00	0.00	0.30	0.00

System information group

The *System information group* contains keywords that that pertain to the system. For all custom keywords, keywords without the “#” sign will also be recognized.

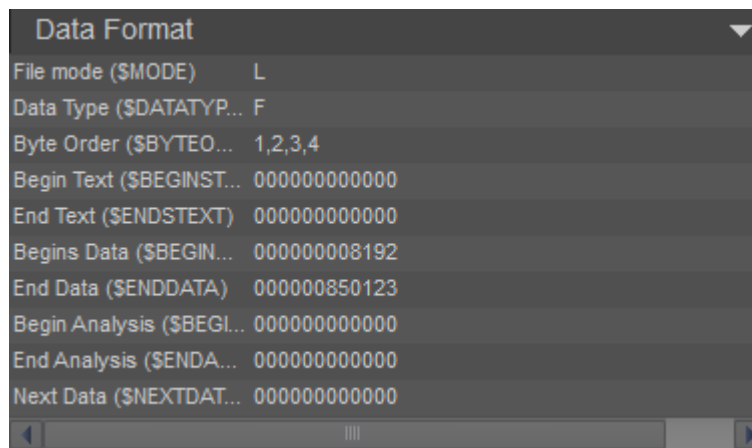


Sort	Keyword	Description
1	\$SYS	Operating System
2	\$CYT	Cytometer
3	\$CYTSN	Cytometer Serial Number
4	#LASERCONFIG	Laser Configuration
5	#LASER1COLOR	Laser 1 Color
6	#LASER2COLOR	Laser 2 Color
7	#LASER3COLOR	Laser 3 Color
8	#LASER4COLOR	Laser 4 Color
9	#LASER1ASF	Laser 1 ASF
10	#LASER2ASF	Laser 2 ASF

Sort	Keyword	Description
15	#LASER3ASF	Laser 3 ASF
16	#LASER4ASF	Laser 4 ASF
17	#LASER1DELAY	Laser 1 Delay
18	#LASER2DELAY	Laser 2 Delay
18	#LASER3DELAY	Laser 3 Delay
19	#LASER4DELAY	Laser 4 Delay
19	#WINEXT	Window Extension
20	#WIDTHTHRESHOLD	Width Threshold
21	#PTRESULT	Performance Test Result
22	#PTDATE	Performance Test Date

Data format group

The *Data format group* contains keywords that describe the instrument. This group is collapsed by default.



Sort	Keyword	Description
	FCS Standard	FCS Version
1	\$MODE	File Mode
2	\$DATATYPE	Data Type
3	\$BYTEORD	Byte Order
4	\$BEGINSTEXT	Begin Text
5	\$ENDSTEXT	End Text

Sort	Keyword	Description
6	\$BEGINDATA	Begin Data
7	\$ENDDATA	End Data
8	\$BEGINANALYSIS	Begin Analysis
9	\$ENDANALYSIS	End Analysis
10	\$NEXTDATA	Next Data
6	\$BEGINDATA	Begin Data

Other group

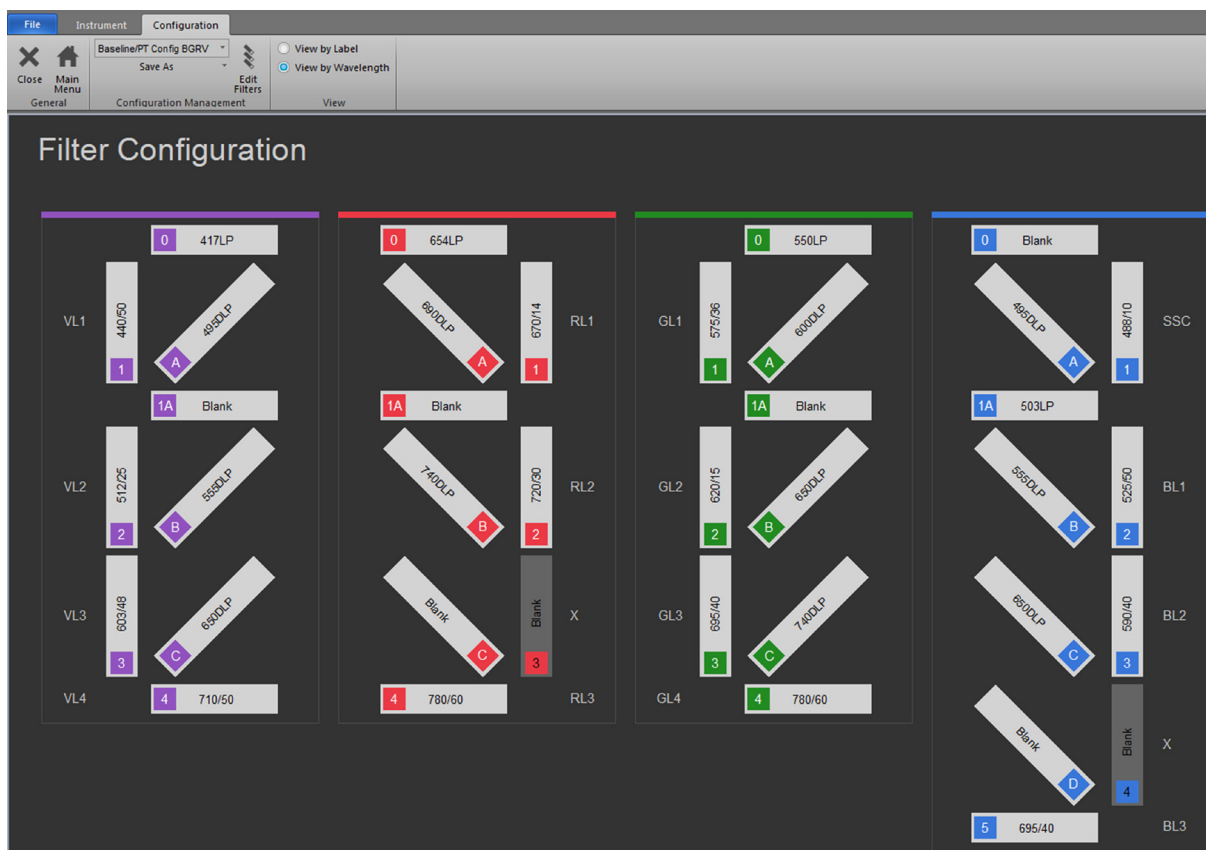
The *Other group* contains all other keywords not captured within the other groups. This group is collapsed by default.

14. Filter configuration

Filter Configuration (FC) module

Overview

The *Filter Configuration (FC) module* of the Attune™ NxT Software consists of the *File* (page 39) and *Configuration ribbon tab* (page 245), and the *Filter Configuration View* (page 252).



The FC module is used to:

- Select view options for the filters (page 251)
- Manage filter labels (page 250)
- Create, edit, and delete filters (page 248)
- Select instrument configuration files (page 245)
- Save, load, export, and delete instrument configuration files (page 246)

Launching the FC module

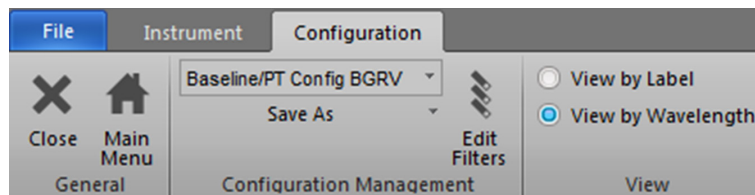
On the *Instrument ribbon tab* (page 48), click the **Configuration** button to launch the *Filter Configuration (FC) module*.

The Configuration button is always visible and enabled except during acquisition.

- When the FC module is opened, all other panels are hidden. The FC module is not resizable or floatable.
- The FC module contains only the File (page 39), Configuration (page 245), and Instrument (page 48) ribbon tabs. All other ribbon tabs are hidden.

Configuration ribbon tab



The *Configuration ribbon tab* allows you to manage the filter configuration and to select view options for the filters. It is visible only when the FC module is open.



The Configuration ribbon tab is organized into three functional groups: *General*, *Configuration Management*, and *View*, which are described below.

General group

General group allows you to close the IC module and to return to the Main Menu.

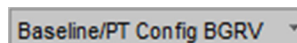
-  **Close:** Closes the IC module and returns to the previous view before the Instrument Configuration panel was opened.
If you have unsaved configuration changes, the *Save As* dialog (page 246) appears.
-  **Main Menu:** Closes the IC module and returns to the Main Menu.
If you have unsaved configuration changes, the *Save As* dialog (page 246) appears.

Configuration Management group

Configuration Management group allows you to select instrument configuration, to save, load, export, and delete filter configuration files, and edit the table of filters to be saved as a user-defined configuration.

Select configuration

Select configuration dropdown menu displays the currently selected instrument configuration.



- The default filter configuration is the baseline configuration provided by the instrument manufacturer and is called **Baseline/PT Config <CCCC>**, where <C> is the first letter of each laser color (**B**lue, **R**ed, **V**iolet, or **Y**ellow) and an **X** indicates that one of the lasers is not present.
 - The file containing the default filter configuration cannot be overwritten or deleted.
 - To change the filter configuration, select the file for the configuration of interest from the **Select configuration** dropdown menu.
1. The Filter Configuration View (page 252) is automatically updated to display the filter information for the selected configuration.

Only filter configurations that match the **Hardware/Virtual Laser Configuration** as defined in the *Options dialog* (page 259) are displayed.

Save As

The Save As button is a split button used for performing the **Save As**, **Load**, **Export**, and **Delete** functions described below.

Save As ▾

Save As: If you made changes to the current filter configuration file, selecting **Save As** allows you to save the changes to a new file, or overwrite an existing file.

To save changes to an filter configuration file:

1. Click the main portion of the **Save As** button to open the *Save As* dialog.



2. To save the changes to:
 - A new file – Enter a file name in the text field.
 - An existing file – Select the file from the dropdown menu, or enter the name of the existing file in the text field. You can enter up to 50 characters; the following characters are not permitted: \ / : * < > | ?.



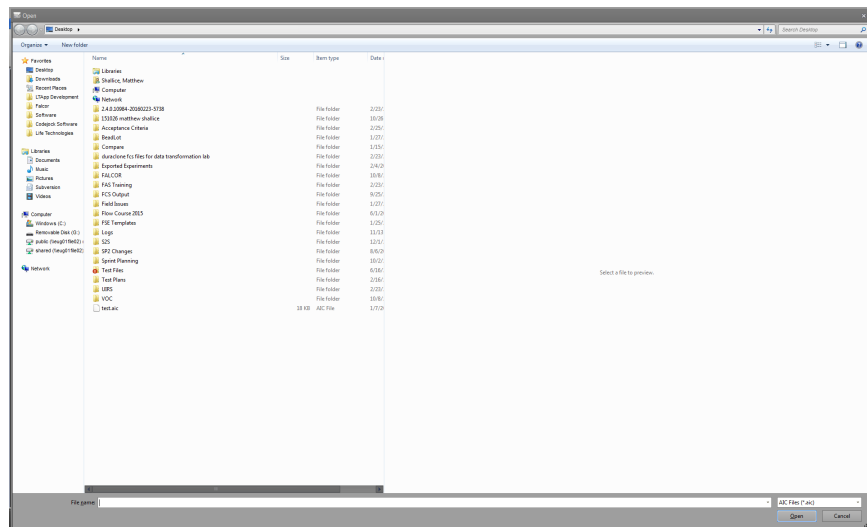
Note: If you select the default filter configuration file, the **Save** button is disabled.

3. Click **Save** (for a new file) or click **Overwrite** (for an existing file) to save the changes.
Alternatively, click **Discard** or **X** to close the *Save As* dialog without saving the changes.

Load: Selecting **Load** allows you to import a saved filter configuration.

To load (import) an filter configuration file:

1. Click the **Save As** dropdown menu, then select **Load**. The *File Open (Import)* dialog opens. The default location is the location of the last viewed folder.



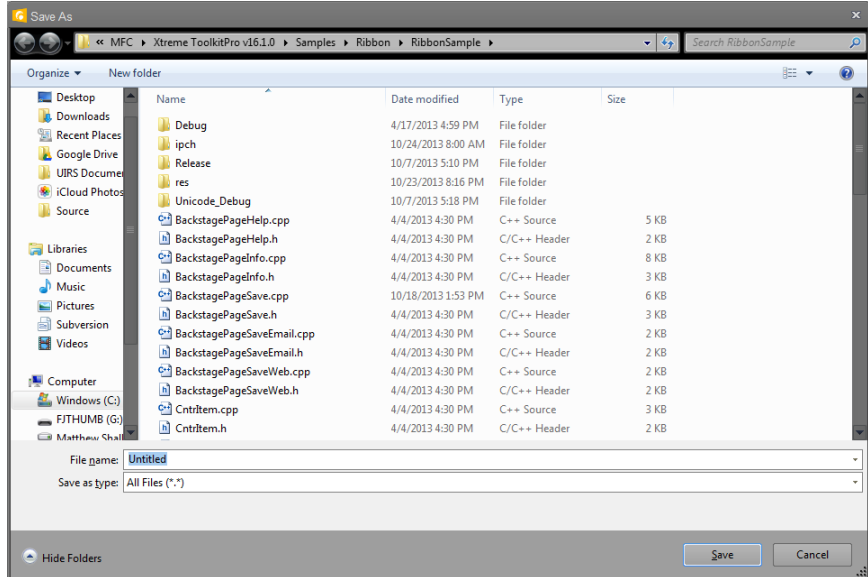
2. Select a file and then click **Import**.

If you select a file that has the same name as a file in your own user list, the software appends an integer onto the file name upon loading.

Export: Selecting **Export** allows you to export a saved filter configuration file.

To export an filter configuration file:

1. Click the **Save As** dropdown menu, then select **Export** to open the *Save As* dialog. The *Save As* dialog automatically opens the location where you last saved files.

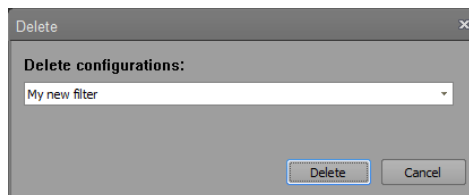


2. Browse to a save location, enter a file name, select the ***.ais** file type, then click **Save**.

Delete: The *Delete* function allows you to delete a filter configuration file.

To delete an filter configuration file:

1. Click the **Save As** dropdown menu, then select **Delete** to open the *Delete* dialog.



2. By default, the currently loaded filter configuration file is selected. Accept the default, or select another file from the dropdown menu.

The dropdown menu displays all available filter configuration files, except for the default filter configuration file.

3. Click **Delete** to delete the selected file and close the dialog box.

Alternatively, click **Cancel** or **X** to close the *Delete* dialog without deleting the file.



Note: If you delete the currently loaded filter configuration file, the software updates the view to the default filter configuration file.

Edit Filters

Edit Filters button opens the *Edit Filters dialog* (see below). The dialog contains the *Filters table*, the contents of which are based on the filter configuration selected from the **Select configuration** dropdown menu (page 245).



Using the Edit Filters dialog, you can:

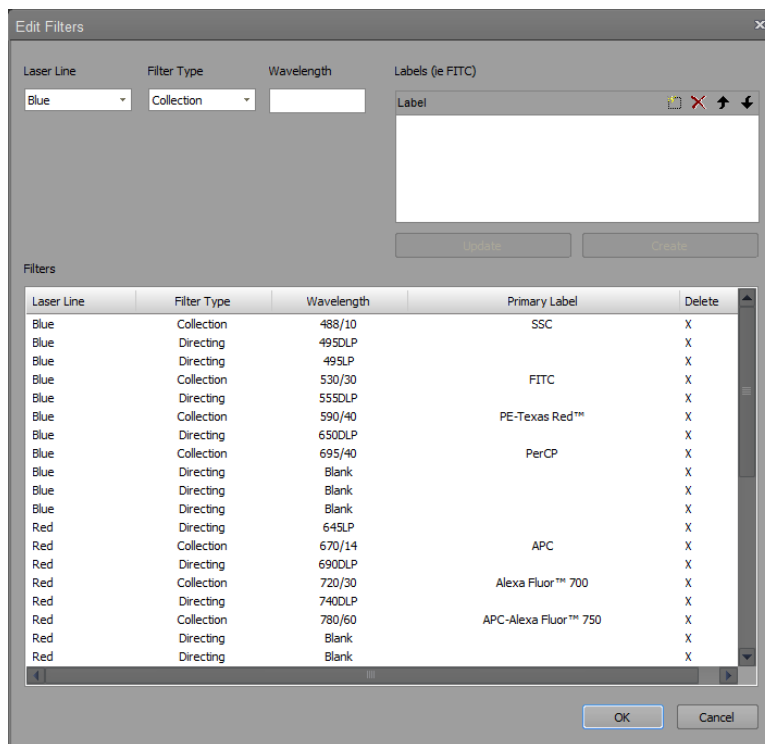
- Define a new filter (page 249)
- Edit an existing filter (page 250)
- Delete an existing filter (page 250)
- Manage filter labels (page 250)



Note: Only *Advanced Users* and *Administrators* (page 19) can perform the tasks in the *Edit Filters* dialog.

Edit Filters dialog

Using the tools available in the Edit Filters dialog, authorized users can define new filter sets, change the filters that can be applied to each instrument channel, and manage filter labels.



Filters table

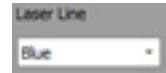
The Filters table displays the filters for the currently selected filter configuration, with each filter taking one row of the table.

- The columns in the table are listed from left to right as follows:
Laser Line, Filter Type, Wavelength, Primary Label, and Delete button.
- By default, the filters are sorted by the laser line group, and the secondary sort order is wavelength.
- Authorized users can edit the contents of this table as described below. Changes to the table are saved to the user-defined filter configuration.

Edit Filters tools

The upper portion of the Edit Filters dialog contains the tools for defining a new filter or editing an existing filter. It contains the following options:

- **Laser Line dropdown:** Allows you to select the laser to which the filter will be assigned. The default is the first laser in the configuration file.

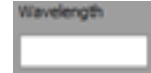


When an Attune™ system is detected or selected within the virtual mode, only the lasers which are installed in that instrument are available.

- **Filter Type dropdown:** Allows you to select the filter type. You can choose between **Band Pass**, **Long Pass**, **Short Pass**, and **Dichroic**. By default, Band Pass is selected.



- **Wavelength:** Allows you to type in the filter specifications. You can enter up to 10 characters to the text field. By default, this field is blank.



- **Labels table:** Allows you to assign and manage filter labels using the tools available in the table header (see “Managing filter labels”, page 250). You can enter up to 50 characters per line to save as a label for the selected filter. By default, this table is blank.

Update and **Create** buttons are contextual; they are enabled only when the appropriate action has been taken in the Edit Filters dialog.



Defining a new filter

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Laser Line** dropdown menu, select the laser to which you want to assign the new filter.
3. In the **Filter Type** dropdown menu, select the desired filter type.
4. In the **Wavelength** field, enter the filter specifications. You can enter up to 10 characters.
5. Assign the *primary label* (i.e., the default display name) for the filter by selecting the label of interest in the **Labels** table, and then using the up arrow in the header to move it to the top of the list.
If needed, you can edit the label, or create a new label (see “Managing filter labels”, page 250).
6. Click **Create**. The software adds the new filter to the **Filters** table.
7. Click **OK** to apply the changes and close the *Edit Filters* dialog. The software saves the changes to the currently selected instrument configuration file.

Alternatively, click **Cancel** to close the *Edit Filters* dialog without applying the changes.

Editing an existing filter

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Filters** table, select the filter to edit. The software populates the fields with the selected filter's information.
3. For user-defined filters, you can make the following changes:
 - In the **Laser Line** dropdown menu, select the laser to assign the filter to.
 - In the **Filter Type** dropdown menu, select the desired filter type.
 - In the **Wavelength** field, enter the filter specifications. You can enter up to 10 characters.



Note: The Laser Line, Filter Type, and Wavelength fields are disabled for the filters in the system default configuration.

4. Assign the primary label for the selected filter by selecting the label of interest in the **Labels** table, and then using the up arrow in the header to move it to the top of the list.

If needed, you can edit the label, or create a new label (see “Managing filter labels”, below).

5. Click **Update**.
6. Click **OK** to apply the changes and close the *Edit Filters* dialog. The software saves the changes to the currently selected instrument configuration file.
To close the *Edit Filters* dialog without applying the changes, click **Cancel**.

Deleting a filter

You can only delete filters that were created by a user; filters installed with the system cannot be deleted.

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Filters** table, select the filter to delete and click **X** in the Delete column.
3. Click **OK** to apply the changes and close the *Edit Filters* dialog. The software saves the changes to the currently selected instrument configuration file.

To close the *Edit Filters* dialog without applying the changes, click **Cancel**.

Managing filter labels

You can arrange, create, edit, and delete filter labels in the **Labels** table.

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Labels** table, perform the following tasks as needed:
 - Click on a label to select it. To select multiple labels at a time, press **Ctrl** or **Ctrl+Shift** when selecting the labels.
 - To arrange labels in the table, select a label, then use the up or down arrows in the header (⬆️ ⬆️) to move the selected label.
The label at the top of the list is considered the *primary label* and will be used as the default display name for the selected filter.
 - To create a new label, click **Add** (➕) in the header or double-click the white space within the table, and then enter a label name. You can enter up to 50 characters; the following characters are not permitted: \ / : * < > | ?.
 - To edit an existing label, double-click the label and enter changes.
 - To delete a label, select it, and then click **Delete** (✖️) in the header.

View group

View group is used for displaying the filters in the filter configuration by primary label or by assigned wavelength. It consists of **View by label** and **View by wavelength** radio buttons. Selecting one option deselects the other.



- The default selection is View by wavelength.
- The selection of View by label or View by wavelength is remembered as a user setting and persists on a per user basis.

Default filter label list

The table below lists the filter labels that are displayed for each channel for the available system default configurations.

The naming convention for the system default filter configuration is as follows:

Baseline/PT Config <CCCC>,

where <C> is the first letter of each laser color (**Blue**, **Red**, **Violet**, or **Yellow**) and an **X** in the name indicates that one of the lasers is not present.

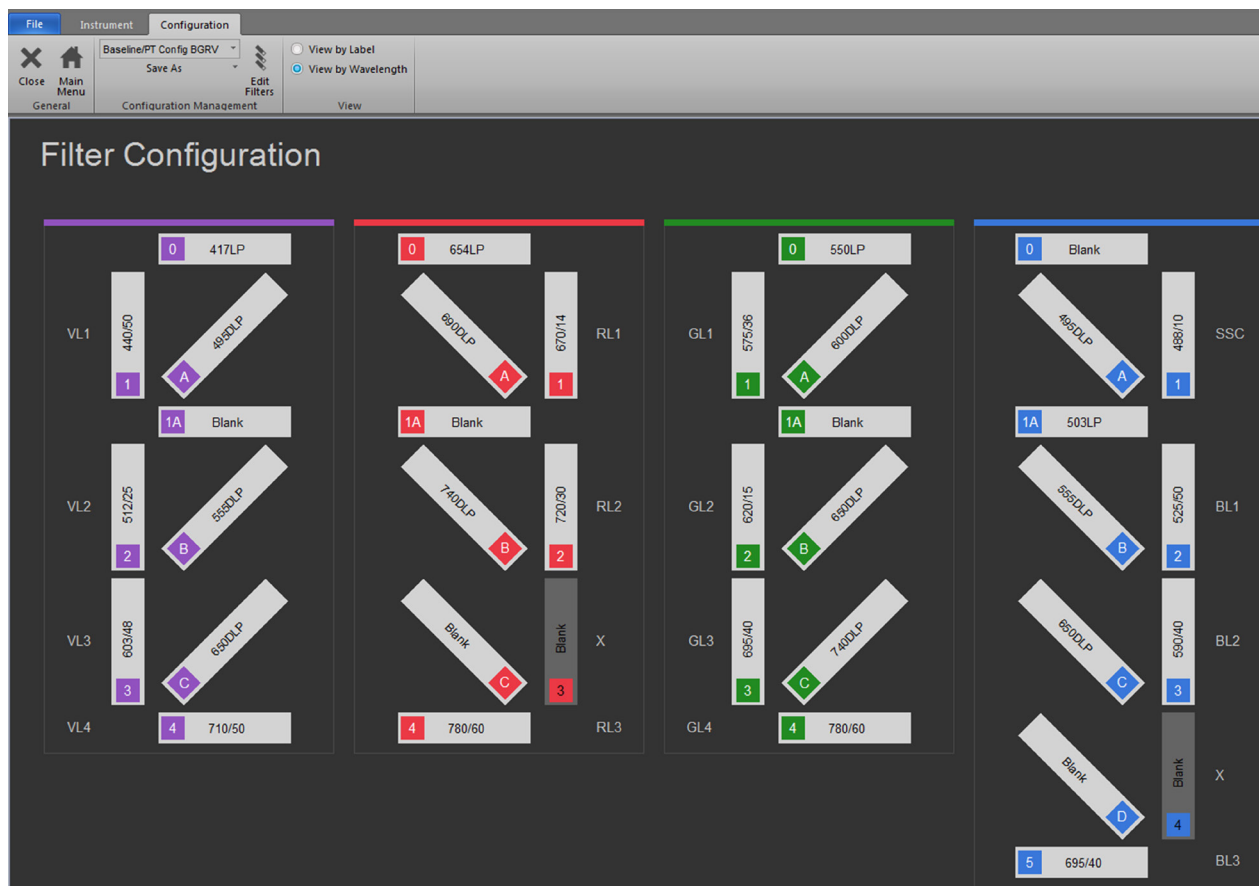
An **X** in the Filter Configuration columns indicates that the filters listed in the table apply to that specific instrument configuration.

Laser Line	Channel	Filter Type	Wavelength	Labels	Instrument Configuration						
					BXXX	BRXX	BVXX	BYXX	BRVX	BVYX	BRVY
Blue	BL1	Band Pass	530/30	FITC, Alexa Fluor 488, GFP, cFSE, SYTOX Green	X	X	X	X	X	X	X
	BL2	Band Pass	574/26	RPE, Alexa Fluor 568, PI, SYTOX Orange, DyeCycle Orange	X	X	X		X		
	BL2(Y)	Band Pass	590/40	PE- Texas Red, PE- AF 610, PI, Fixable Red				X		X	X
	BL3	Band Pass	695/40	PE-Cy5.5, PE-AF700, PerCP-Cy5.5, PerCP, SYTOX AADvanced	X	X	X		X		
	BL3(Y)	Band Pass	695/40	PerCP, PerCP-Cy5.5, PE-AF 700				X		X	X
BL4	Band Pass	780/60	PE-Cy7, Qdot 800, DyeCycle Ruby	X	X	X		X			
Red	RL1	Band Pass	670/14	APC, Alexa Fluor 647, Fixable Far Red, SYTOX Red		X			X		X
	RL2	Band Pass	720/30	Alexa Fluor 700, Qdot 700, Alexa Fluor 680		X			X		X
	RL3	Band Pass	780/60	APC-AF750, Fixable Near IR, APC Cy7, APC H7 DyeCycle Ruby		X			X		X
Violet	VL1	Band Pass	440/50	Pacific Blue, Alexa Fluor 405, Fixable Violet, DyeCycle Violet, SYTOX Blue, CellTrace			X		X	X	X
	VL2	Band Pass	512/25	Pacific Green, Fixable Aqua, CFP			X		X	X	X
	VL3	Band Pass	603/48	Pacific Orange, Qdot 605, Fixable Yellow			X		X	X	X
	VL4	Band Pass	710/50	Qdot 700			X		X	X	X
Yellow	YL1	Band Pass	585/16	RPE, Alexa Fluor 568,, PI, SYTOX Orange, DyeCycle Orange				X		X	X
	YL2	Band Pass	620/15	PE- Texas Red, PE- Alexa Fluor 610, mCherry, SYTOX AADvanced				X		X	X
	YL3	Band Pass	695/40	PE-Cy5.5, PE-AF700, PerCP-Cy5.5				X		X	X
	YL4	Band Pass	780/60	PE-Cy7, Qdot 800				X		X	X

Filter Configuration View

Overview

The *Filter Configuration View* displays an interactive diagram of the filter array for the current instrument model, which allows you to assign appropriate filters to specific filter locations.

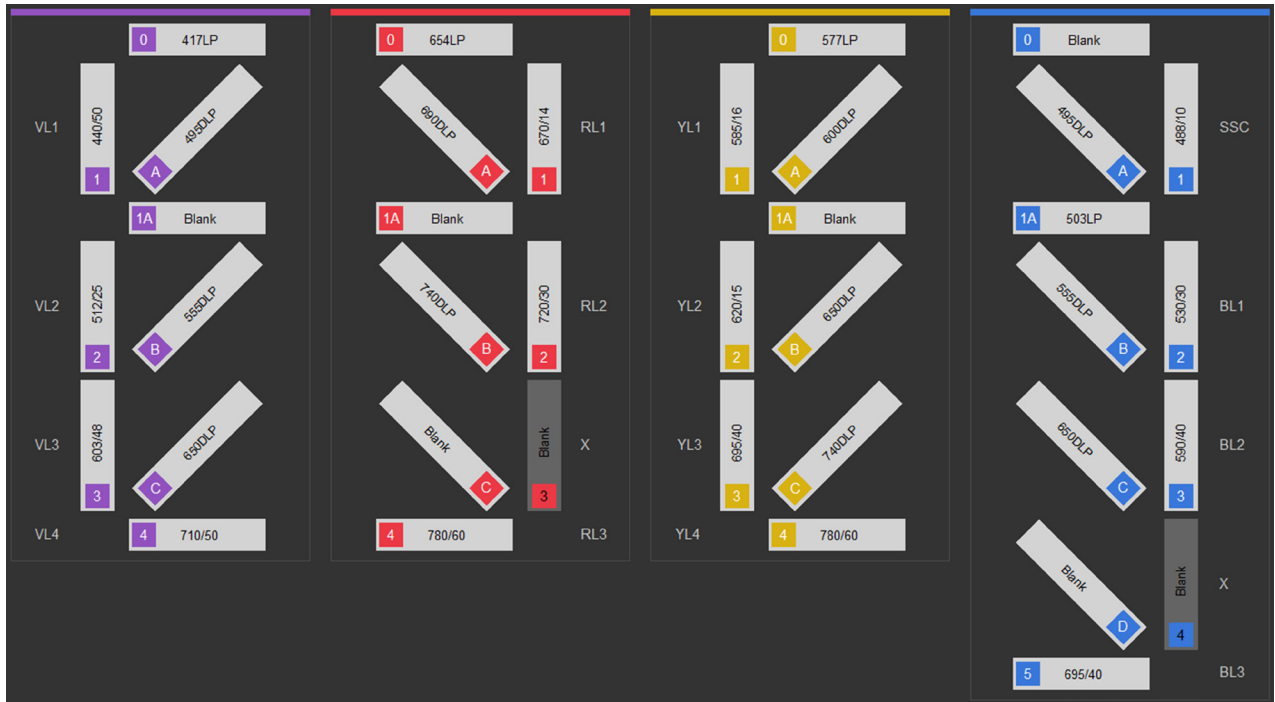


- Only detectors that are part of the active instrument model are displayed.
- The filter array reflects the **Hardware/Virtual Laser Configuration** as defined in the Configuration *Options dialog* (page 259) and can be used as a quick reference.
- Clicking one of the filter locations opens a dropdown menu, which contains all unassigned filters for that laser line and type. You can select any of these to assign it to that filter location.
Filters marked as **BP** (Band Pass) and **LP** (Long Pass) can be selected at the detector output.
- Filters marked as **Dichroic** are only available in the positions that accept dichroic filters.
- Default Instrument Configuration panels for each laser configuration are shown on pages 253–256.

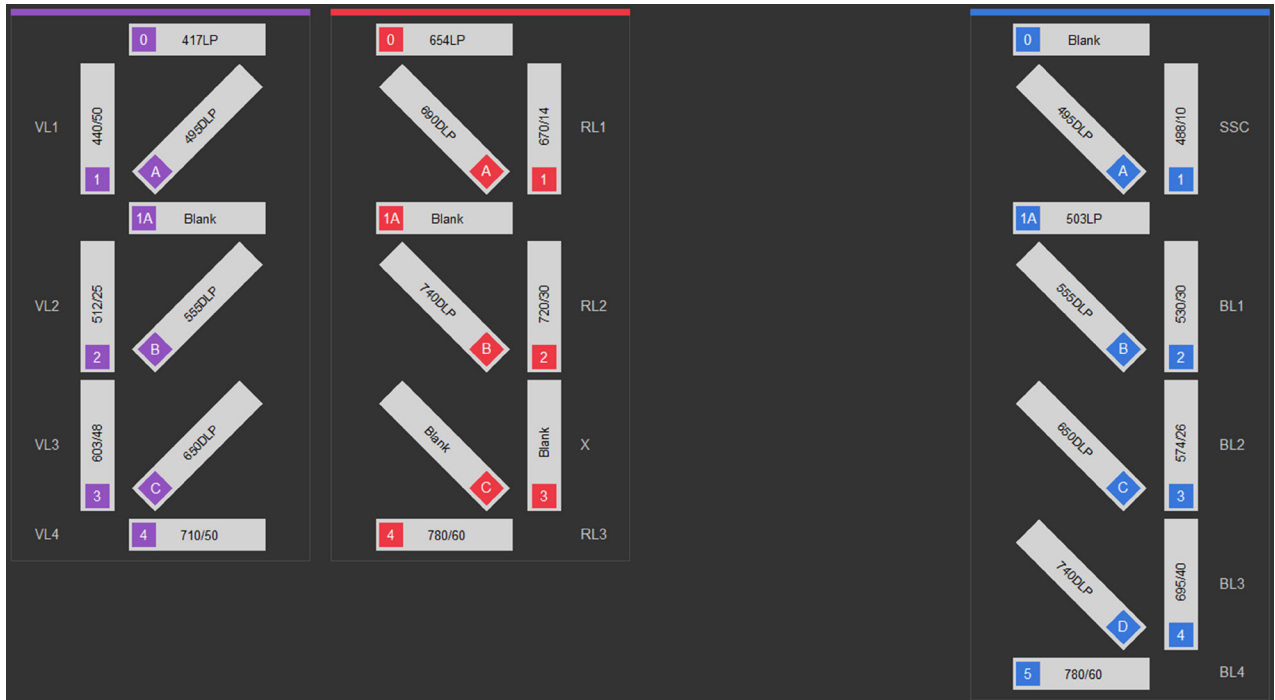
Default instrument configurations

The following images show the Instrument Configuration panel for each laser configuration.

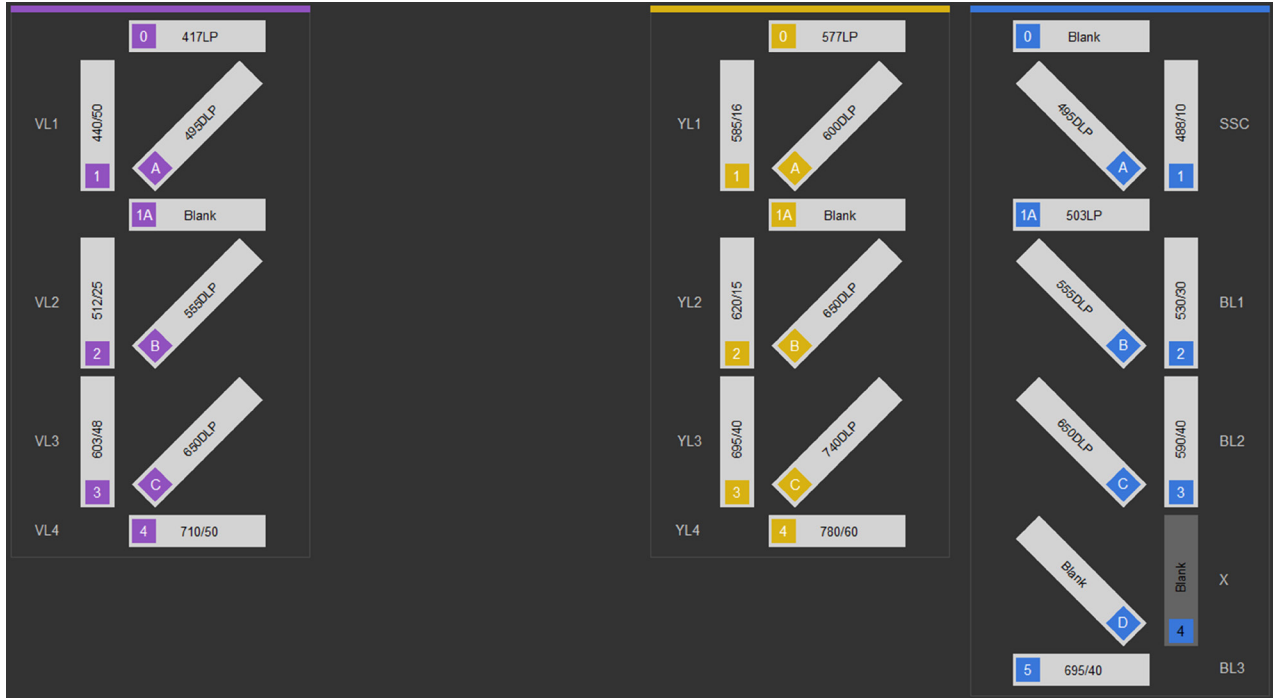
BRVY (Blue, Red, Violet, Yellow)



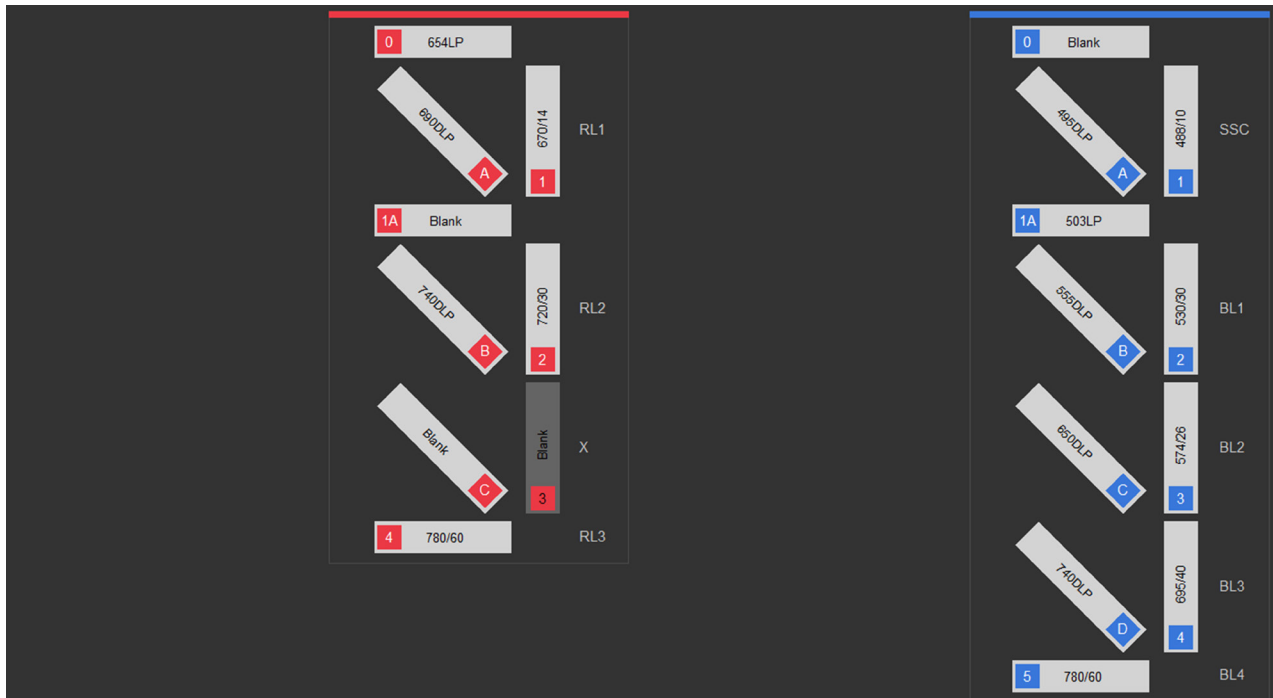
BRV (Blue, Red, Violet)



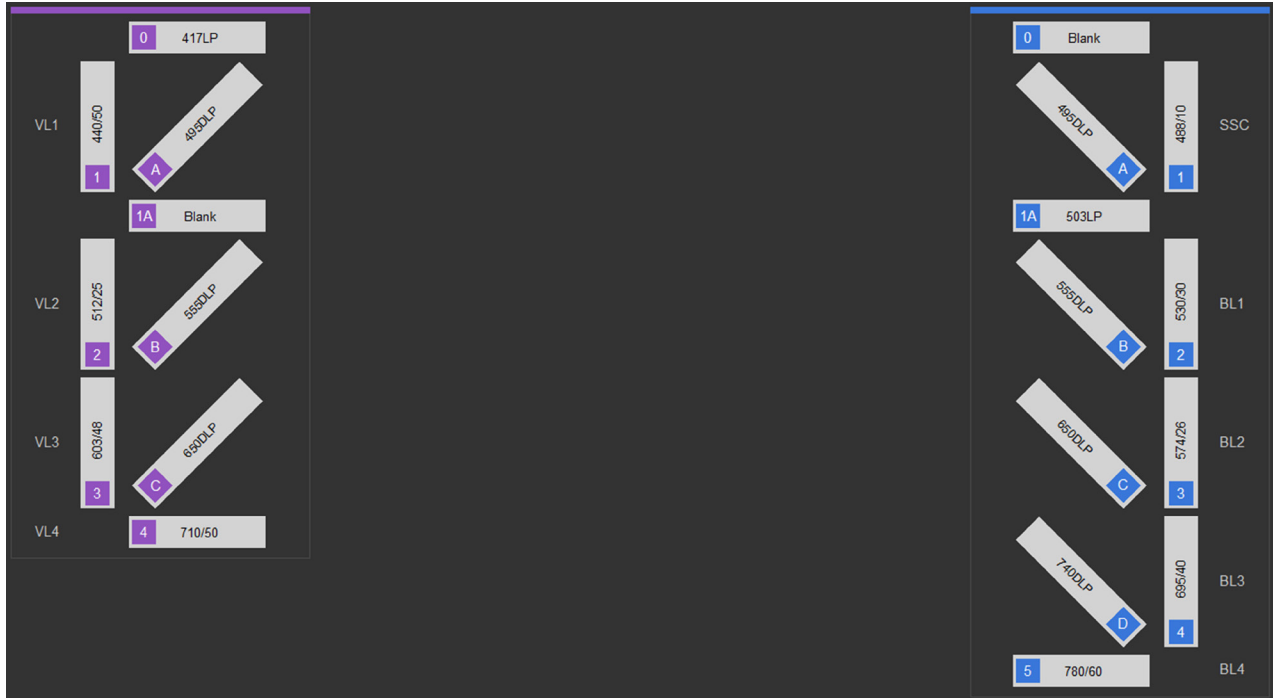
BVY (Blue, Violet, Yellow)



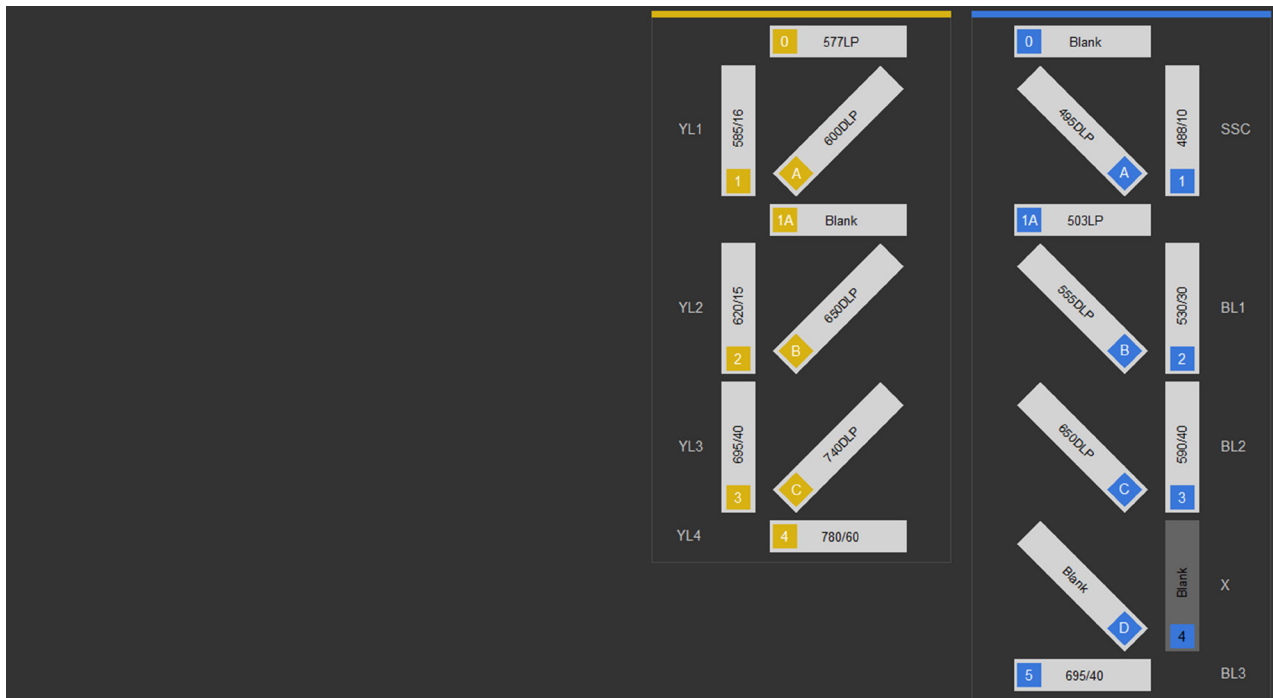
BR (Blue, Red)



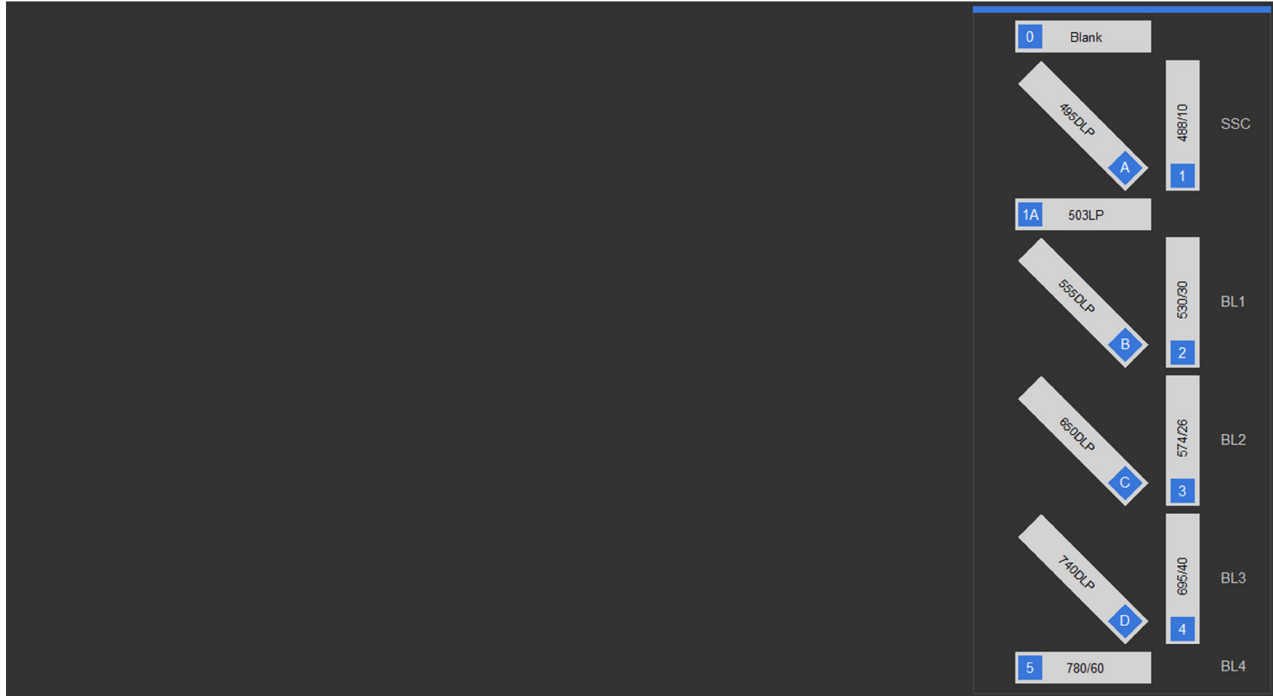
BV (Blue, Violet)



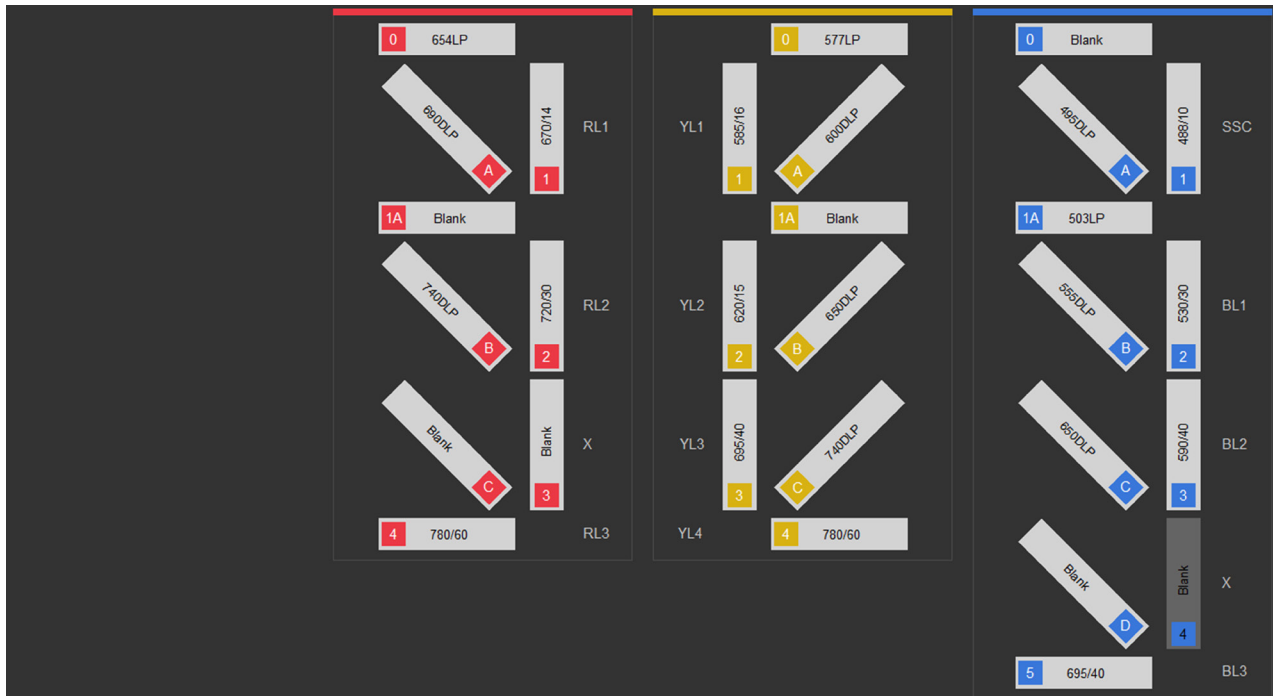
BY (Blue, Yellow)



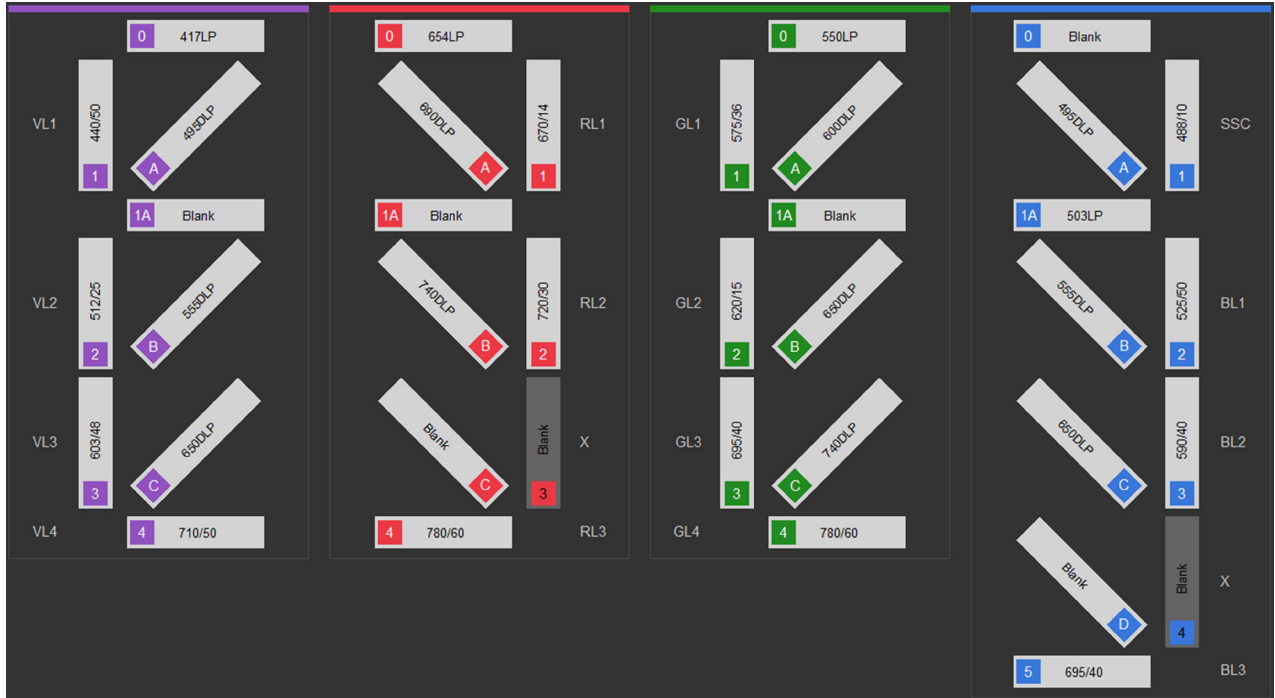
B (Blue)



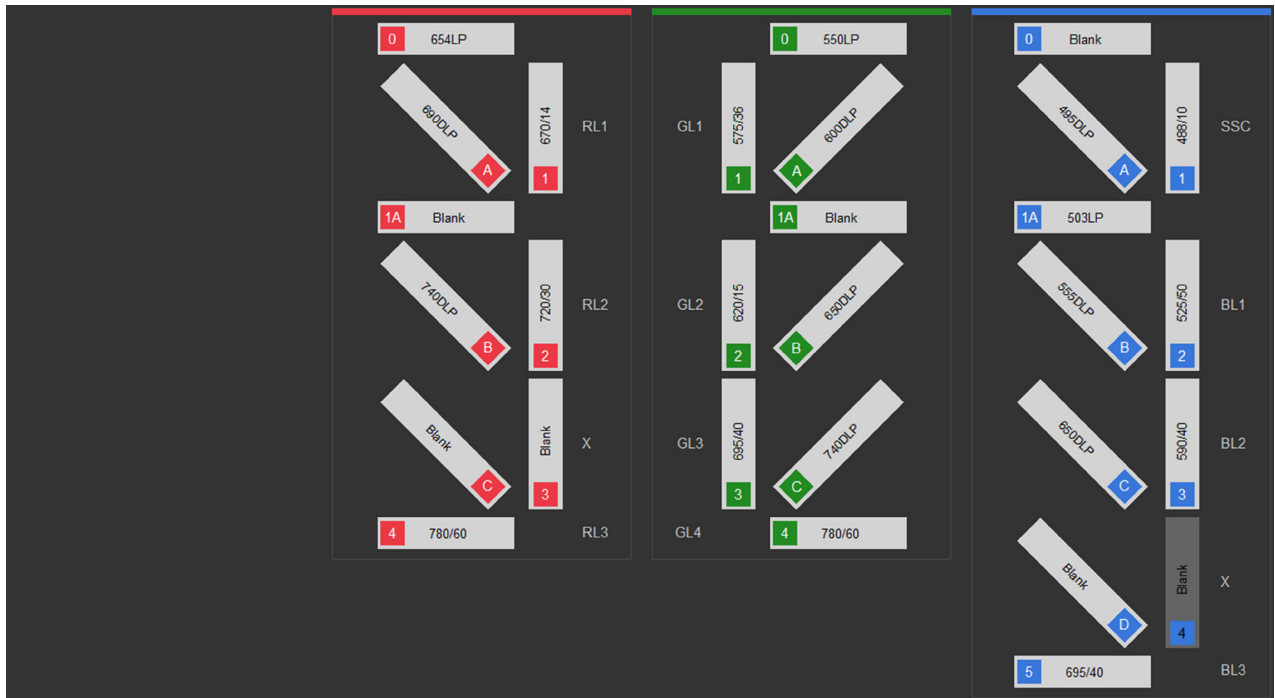
BRY (Blue, Red, Yellow)



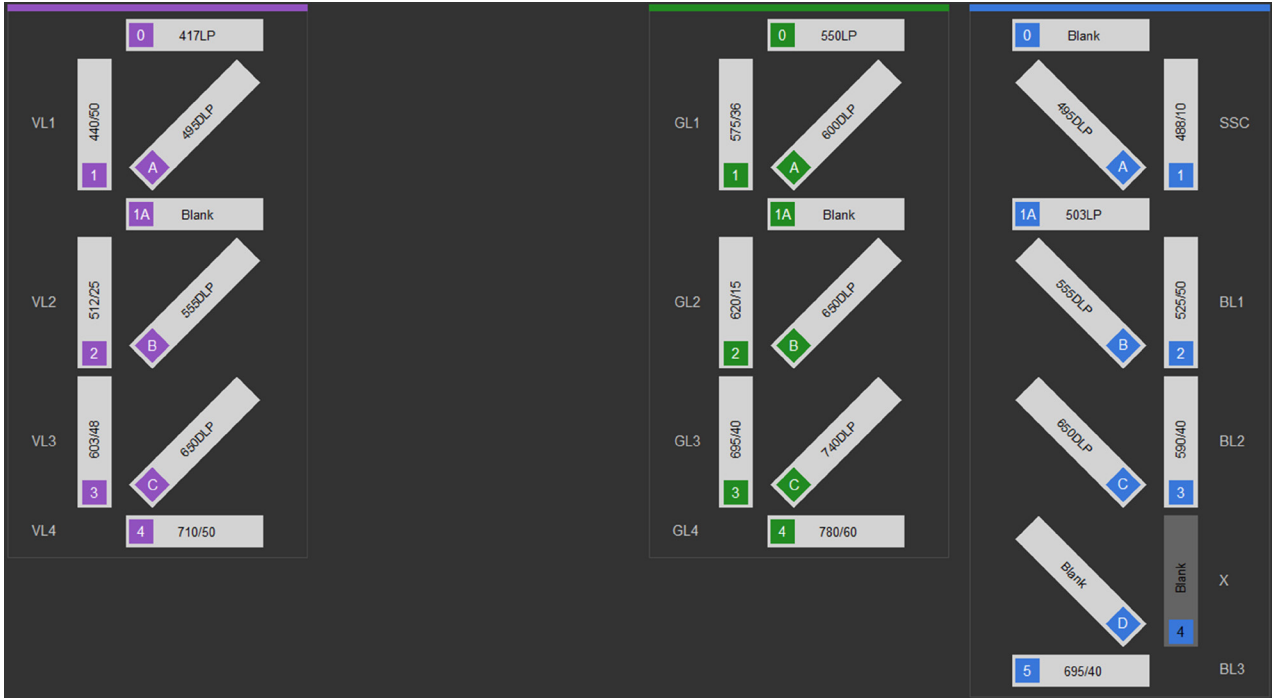
BGRV (Blue, Green, Red, Violet)



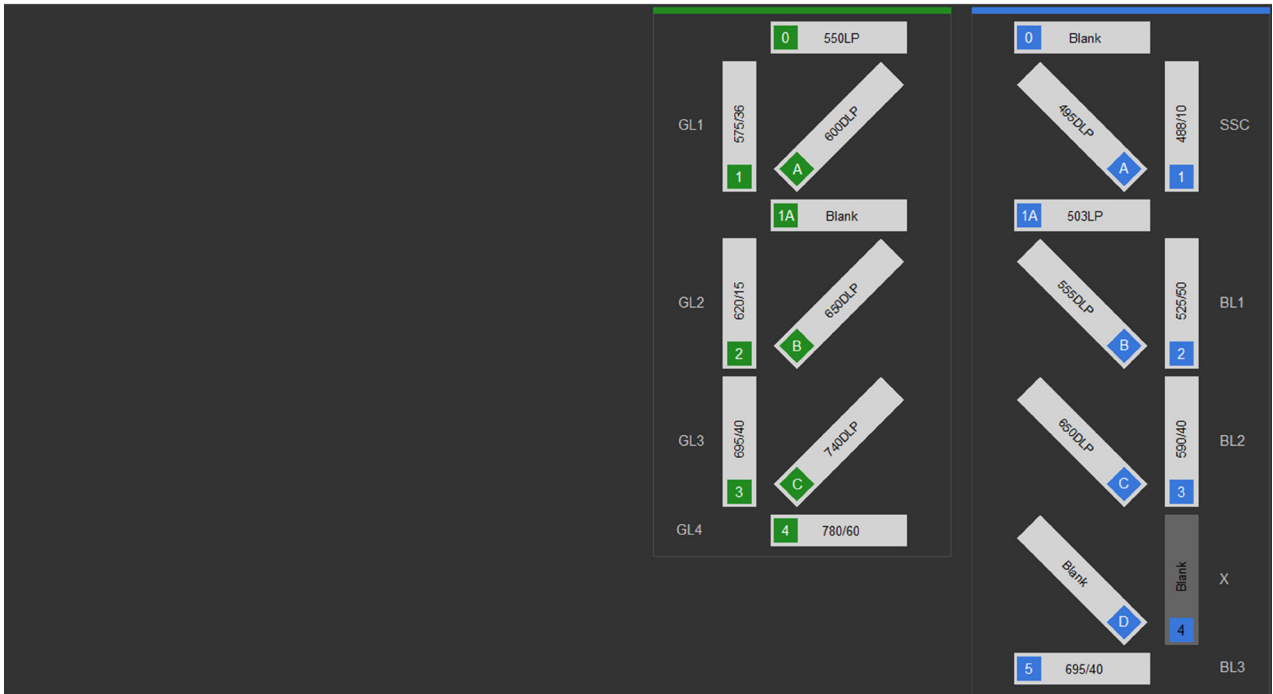
BGR (Blue, Green, Red)



BGV (Blue, Green, Violet)



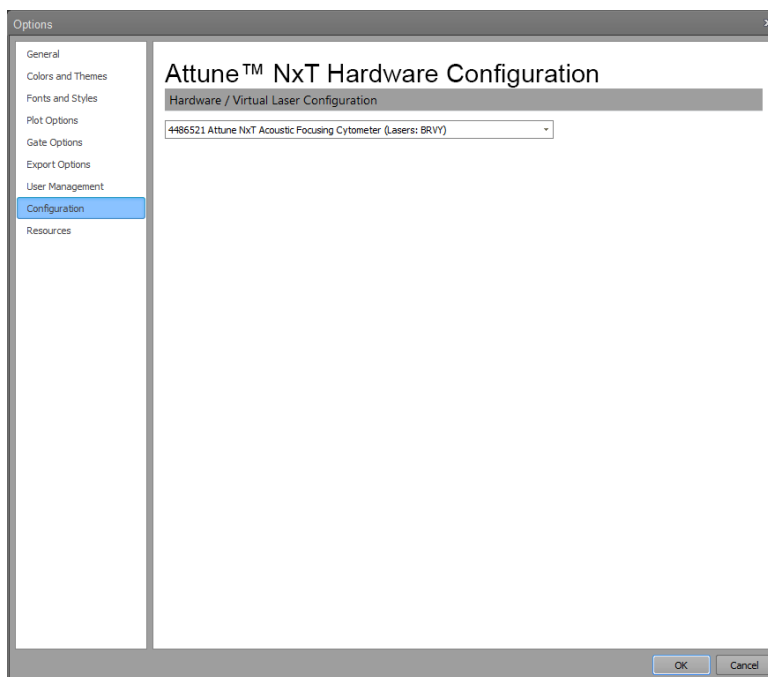
BG (Blue, Green)



Configuration options

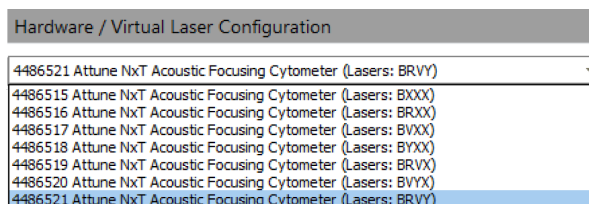
Overview

Configuration options allow you to create virtual configuration settings for working in analysis-only conditions, and to manage the bubble sensor and set the sample conservation mode property.



Hardware/ Virtual Laser Configuration

Hardware/Virtual Laser Configuration section allows you select a virtual instrument configuration from the dropdown menu. This aids in the creation of Experiments when not connected to an Attune™ NxT instrument by maintaining correct instrument settings and channel mapping.



- Clicking the **Hardware/Virtual Laser Configuration** combo box control opens the instrument configuration dropdown list, which is populated from the Attune™ NxT instrument configurations enumerated in the database.
- The available configurations are described in “Instrument Configuration” (page 253).

15. Performance testing

Overview

Performance Tests The Attune™ NxT Software uses Attune™ Performance Tracking Beads to define the cytometer's initial *Baseline status*. During this process, the median fluorescence intensity of each bead and the r%CV (robust percent coefficient of variation) are automatically measured in all fluorescence detectors. Software algorithms use this information to determine cytometer settings and provide target values for subsequent application-specific settings (see "Performance Test data", below).

After the Baseline values are established, you use the same lot of Attune™ Performance Tracking Beads to run the *Performance Tests*. The Performance Tests measure variation from the Baseline values to track the daily performance of the cytometer.

Performance Test data The Attune™ NxT Software generates data for the parameters listed below to establish the initial Baseline status of the cytometer and measures their variation from the target values established by the Baseline calculations to track the daily performance of the instrument.

- PMTV (photomultiplier tube voltage)
- Delta PMTV
- Target MFI (target median fluorescence intensity)
- Measured MFI
- r%CV (robust percent coefficient of variation)
- Quantum Efficiency (Q)
- Background (B)
- Linearity
- Area Scaling Factor
- Laser Delay

Baseline and Performance Test (BL/PT) module


The *Baseline and Performance Test (BL/PT) module* of the Attune™ NxT Software is used to:

- Set up and run the Baseline, page 262
- Run a Performance Test, page 266
- View Baseline and Performance Test reports, page 270



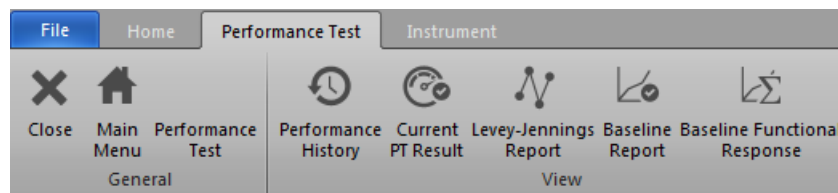
Note: For information on viewing the Baseline and Performance Test reports, see “Performance Test Reports”, page 270.

Opening the BL/PT module



On the Main Menu (page 20), click **Performance Test** to open the BL/PT module, which contains the *Performance Test ribbon tab*. 

Alternatively, click **Performance History** on the Instrument ribbon tab (page 48), when the instrument is connected. 

- In the BL/PT module, only the File, Home, Performance Test, and Instrument ribbon tabs are available. All other tabs are hidden.



Note: The Performance Test ribbon tab is only visible when the BL/PT module is active.

0. If no Baseline has previously been run, the Baseline setup screen is displayed; see “Setting up and running a Baseline”, page 262.
- If a Baseline already exists, the Performance Test setup screen is displayed; see “Running a Performance Test”, page 266.
- To close the BL/PT module and return to the previous view, click **Close**. 
- To return to the Main Menu, click **Main Menu**. 

Setting up and running a Baseline

Baseline setup screen

Open the BL/PT module (page 261) to view the *Baseline setup* screen.

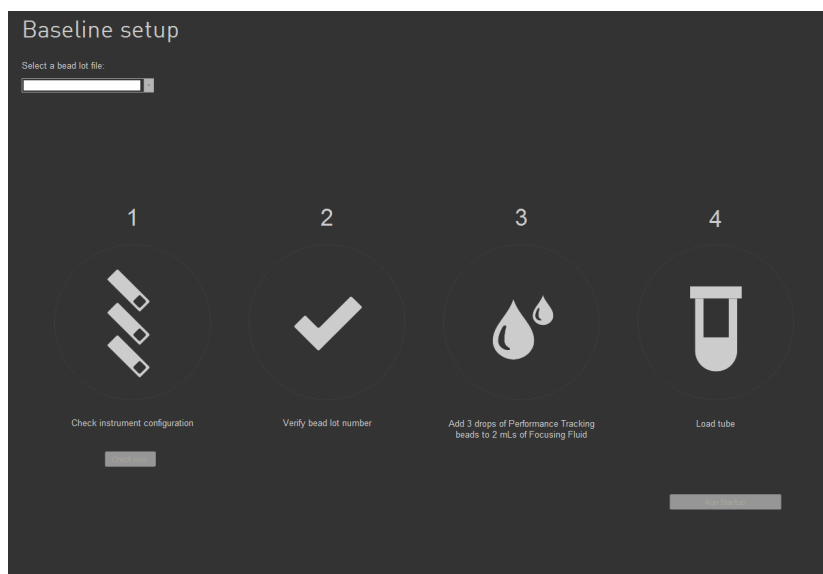


Note: If a Baseline already exists, the *Performance Test setup* screen is displayed instead of the Baseline setup screen. On the Performance Test setup screen (page 266), you can run a Performance Test or reset the Baseline.

The Baseline setup screen provides general instructions for setting up a Baseline:

- 1) Check instrument configuration
- 2) Verify bead lot number
- 3) Add 3 drops bead solutions to 2 mL of focusing fluid
- 4) Load tube

For detailed procedures, see “Set up and run a Baseline”, page 263



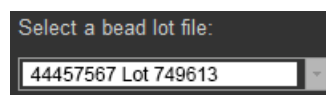
1. The Select bead lot file dropdown menu lists all bead lot files that have been imported
2. Each file in the dropdown menu is listed in the following format:

L/N: ### = Bead lot number

DD/MM/YYYY = Expiration date

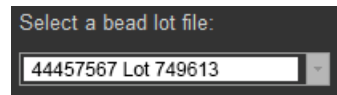
The bead lot number is the first six digits printed on the label (disregard the alpha numeric characters).

3. If no files have been imported, the dropdown menu lists only the bead lot file that is installed with the software.
- To import a bead lot file, select Import at the end of the dropdown menu. Selecting this option opens the File Open (Import) dialog described on page 365.
 - To set up a Baseline for another bead lot, select the bead lot file of interest from the dropdown menu.
 - The software displays the bead lot number and expiration date for the currently selected bead lot.



Set up and run a Baseline

1. Select the bead lot file of interest from the **Select bead lot file** dropdown menu.
If needed, import a new bead lot file by selecting **Import** at the end of the dropdown menu.



2. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.
The beadlot number is the **first six digits** printed on the label (disregard the alpha numeric characters).
3. Prepare the Focusing Fluid by adding 3 drops of bead solution to 2 mL of Focusing Fluid.
4. Load the tube as described in the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Part. no. 100024235).
5. Click **Run Baseline**.

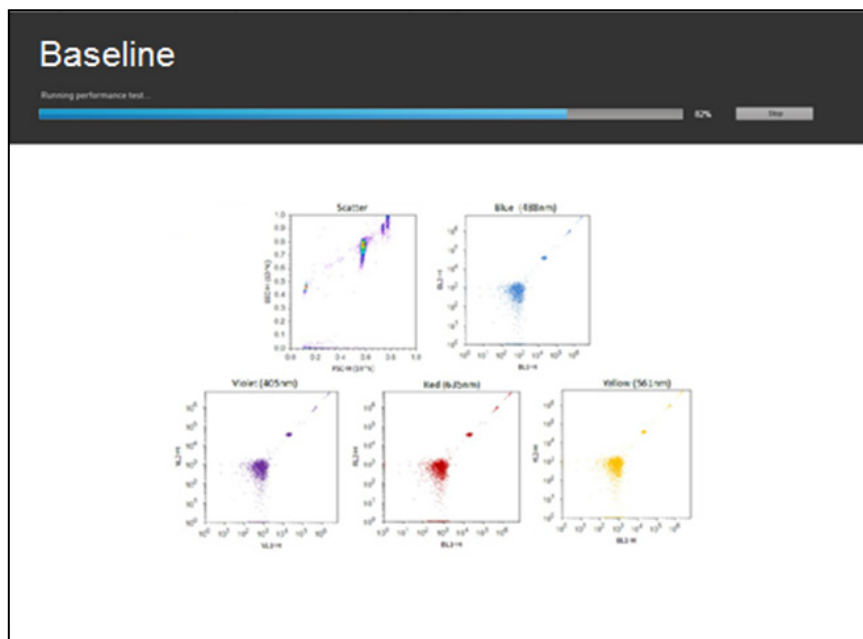


Note: If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Baseline**. You must run the Startup procedure before proceeding.

6. If the bead lot is expired, a warning dialog appears. Click **Yes** to continue, or click **No** to cancel the Baseline run.
If they are not already powered on, all lasers are powered on and will remain on at the completion of the test process.

Baseline screen

The *Baseline screen* provides progress information for the Baseline procedure.



- Four high-level baseline steps are displayed above the progress bar as they occur:
 - “Step 1 of 4: Initializing voltages”
 - “Step 2 of 4: Measuring laser delays”
 - “Step 3 of 4: Measuring system functional response”
 - “Step 4 of 4: Collecting data for analysis”
- The progress bar displays the percent completion (0–100%).



Note: The Baseline steps and progress bar are updated by the Attune™ NxT Software in real time.

- Depending on the number of configured channels, the screen displays:
 - One scatter density plot (SSC-H vs. FSC-H)
 - Bivariate dot plots for each laser (for example, BL2-H vs. BL1-H)For more information on the plots, see “Plots” (page 75).
- To zoom on a plot, use the **Size slider** on the Application status bar (page 37). You cannot resize objects on the workspace. The plots do not get resized if the application window is resized.
- To stop the Baseline run, click **Stop**. After canceling the Baseline run, the software returns to the Baseline setup screen (page 262).



Baseline results

Baseline completion without errors

If the Baseline completes without errors, the software:

- Displays the *Baseline Results* screen (page 274).
- Calculates the system area scaling factor constant based on the results for each configured laser.

Baseline completion with errors

If the Baseline completes with errors, the software displays the *Errors Detected* dialog, which provides the following information:

- A list of the channel statistics that failed
- To go to the Baseline Test Results screen (page 274), click **Continue to results**.



Note: Once you navigate away from the Errors Detected dialog, it cannot be displayed again.

Running a Performance Test

Performance Test setup screen

Launch the **BL/PT module** (page 261) to view the *Performance Test setup* screen.

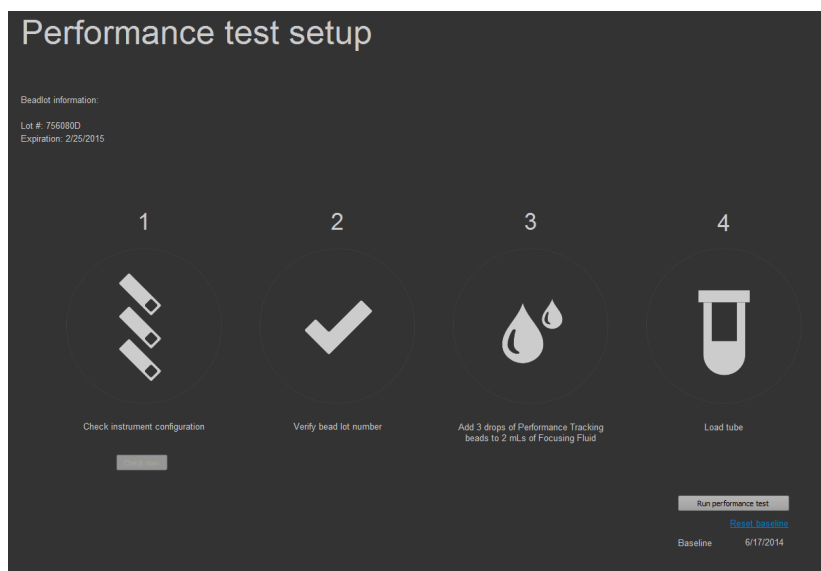


Note: If no Baseline exists, the Baseline setup screen is displayed instead of the Performance Test setup screen. You must run the Baseline first; see “Setting up and running a Baseline”, page 262.

The Performance Test setup screen provides general instructions for setting up a Performance Test:

- 1) Check instrument configuration
- 2) Verify bead lot number
- 3) Add 3 drops bead solution to 2 mL Focusing Fluid
- 4) Load tube

For detailed procedures, see “Set up and run a Performance Test”, page 267.



- The software displays the bead lot number and expiration date for the most recent bead lot used to set up a Baseline in the following format:

L/N: ### = Bead lot number

DD/MM/YYYY = Expiration date

The bead lot number is the **first six digits** printed on the label (disregard the alpha numeric characters).

- If the bead lot has expired, the lot number and expiration date are in contrasting color.

- The software displays the current Baseline information in the lower right of the screen.

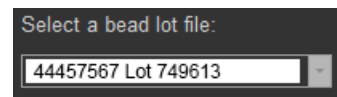
To reset the current Baseline, click **Reset Baseline**, which navigates to the Baseline setup screen (page 262).



Note: The **Reset Baseline** hyperlink is enabled only if you are assigned the privileges to run the Baseline (see page 19).

Set up and run a Performance Test

1. Select the bead lot file of interest from the **Select bead lot file** dropdown menu. For detailed procedures, see "Set up and run a Baseline", page 263.

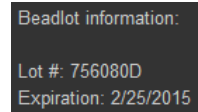


If needed, you can first select a different Baseline to run the Performance Test against, and then click **Reset Baseline** to navigate to the Baseline setup screen.



Note: The **Reset Baseline** hyperlink is enabled only if you are assigned the privileges to run the Baseline (see page 19).

2. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.



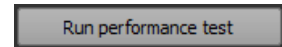
The beadlot number is the **first six digits** printed on the label (disregard the alpha numeric characters).

3. Prepare the Focusing Fluid by adding 3 drops of bead solution to 2 mL of Focusing Fluid.

For more information, refer to the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Part. no. 100024235).

4. Load the tube as described in the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Part. no. 100024235).

5. Click **Run Performance Test**.



Note: If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Performance Test**. You must run the Startup procedure before proceeding.

6. If the bead lot is expired, a warning dialog appears. Click **Yes** to continue, or click **No** to cancel the Performance Test run.

If they are not already powered on, all lasers are powered on and will remain on at the completion of the test process.

7. After the Performance Test is completed, click **Sanitize Attune™ SIP** (page 49) to run the SIP sanitize function before proceeding with your experiments.

Performance Test screen

The *Performance Test screen* provides progress information for the Performance Test procedure.



- Three high-level Performance Test steps are displayed above the progress bar as they occur:
 - “Step 1 of 3 Measuring laser delay”
 - “Step 2 of 3 Adjusting voltages”
 - “Step 3 of 3 Collecting data for analysis”
- The progress bar displays the percent completion (0–100%).



Note: The Performance Test steps and progress bar are updated by the Attune™ NxT Software in real time.

- Depending on the number of configured channels, the screen displays:
 - One scatter density plot (SSC-H vs. FSC-H)
 - Bivariate dot plots for each laser (for example, BL2-H vs. BL1-H)For more information on the plots, see “Plots” (page 75).
- To zoom on a plot, use the **Size slider** on the Application status bar (page 37). You cannot resize objects on the workspace. The plots do not get resized if the application window is resized.
- To stop the Baseline run, click **Stop**. After canceling the Baseline run, the software returns to the Baseline setup screen (page 262).



Performance Test results

Performance Test completion without errors

If the Performance Test completes without errors, the software:

- Displays the *Performance Test Results* screen (page 274).
- Calculates the system area scaling factor constant based on the Performance Test results for each configured laser.
- Updates the default system values for the area scaling factor in the Instrument Settings panel (page 188).
- Updates the laser delay.



Note: Voltage settings are not updated or modified as a result of daily performance testing. For best results, optimize voltage settings for each experiment.



Note: Laser delays for each flow rate are also set based on the performance test results. These values cannot be modified.

Performance Test completion with errors

If the Performance Test completes with errors, the software displays the *Errors Detected* dialog, which provides the following information:

- A list of the channel statistics that failed
- To go to the Performance Test Results screen (page 274), click **Continue to results**.



Note: Once you navigate away from the Errors Detected dialog, it cannot be displayed again.

Performance Test reports

After running the Performance Tests, you can generate the following reports in the Attune™ NxT Software:

- Performance History
- Current PT Results
- Levey-Jennings report
- Baseline Results



Note: For information on viewing the Performance Test reports, see “Performance Test Reports”, page 270.

16. Performance Test reports

Overview

Performance Tests The Attune™ NxT Software uses Attune™ Performance Tracking Beads to define the cytometer's initial *Baseline status*. During this process, the median fluorescence intensity of each bead and the r%CV (robust percent coefficient of variation) are automatically measured in all fluorescence detectors. Software algorithms use this information to determine cytometer settings and provide target values for subsequent application specific settings (see "Performance Test data", below).

After the Baseline values are established, you use the same lot of Attune™ Performance Tracking Beads to run the *Performance Tests*. The Performance Tests measure variation from the Baseline values to track the daily performance of the cytometer.



Note: For information on running Performance Tests, see "Performance Testing", page 260.

Performance Test data

The Attune™ NxT Software generates data for the parameters listed below to establish the initial Baseline status of the cytometer and measures their variation from the target values established by the Baseline calculations to track the daily performance of the instrument.

- PMTV (photomultiplier tube voltage)
- Delta PMTV
- Target MFI (target median fluorescence intensity)
- Measured MFI
- r%CV (robust percent coefficient of variation)
- Quantum Efficiency (Q)
- Background (B)
- Linearity
- Area Scaling Factor
- Laser Delay

Performance Test Reports


After running the Performance Tests, you can generate the following reports in the Attune™ NxT Software:

- Performance History (page 271)
- Baseline Results (page 274)
- Current PT Results (page 277)
- Levey-Jennings report (page 280)

Performance History report

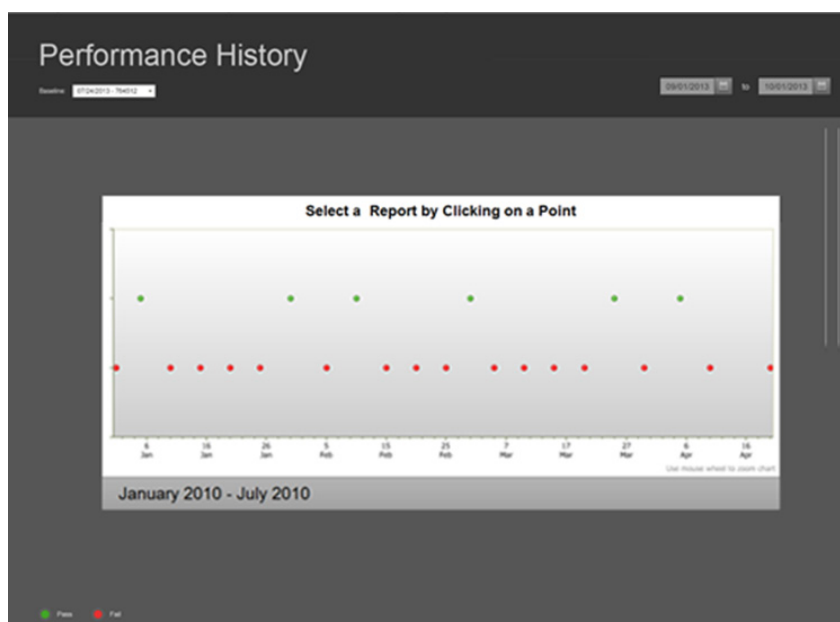
The Performance History report shows the pass/fail status of all Performance Tests run against a selected Baseline test during a selected time period. The Performance History report is useful for gauging the overall “health” of the instrument over the selected time period.

Viewing the Performance History report

On the Performance Test ribbon (available on the BL/PT module; page 261), click **Performance History**. 

Alternatively, click **Performance History** on the Instrument ribbon tab (page 48) or on the Main Menu (page 21).

4. By default, the Performance History Report is displayed for the most recent Baseline, which shows the *Pass/Fail status* of Performance Tests for the most recent 180-day period. For more information, see “Results plot”, page 272.

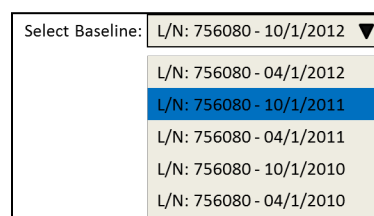


- To view the report for Performance Tests associated with another Baseline and Beadlot, select the Baseline of interest from the **Baseline** dropdown menu.

Each Baseline in the dropdown menu is listed in the following format:

L/N: ### = Performance Test bead lot number

DD/MM/YYYY = date Baseline status was established



Note: The Baseline dropdown menu is enabled only if more than one Baseline test has been run. The Baselines are ordered chronologically, with the most recent displayed first.

- By default, the Performance History Report shows the most recent 6 month data for the Performance Test series within the selected Baseline.
To view the Performance History Report for a different data range, click the **calendar icon** on the **Date Range** tool to select the desired beginning and end dates.



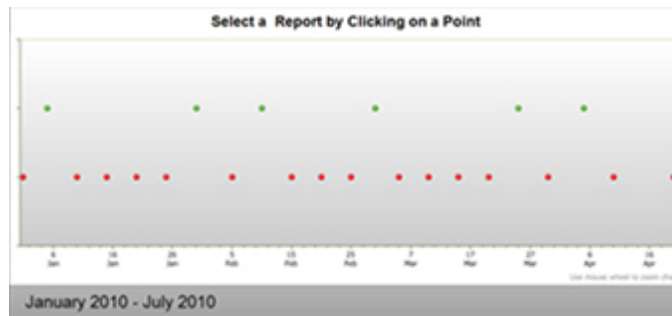
Note: By default, the range of the Performance History Report begins with the first Baseline and ends with the most recent Performance Test available for the selected Baseline.

If the default range exceeds 180 days, then the report shows data for the most recent 180-day time period.

Using the Date Range tool, you can select a date range of up to 180 days.

Results plot

The *Results plot* graphically summarizes the Pass/Fail status of the selected Baseline and associated Performance Tests within the selected date range.



- Each data point on the Results plot corresponds to a single date and the color of the data point indicates whether the result was a pass or fail.

Icon Pass/Fail status

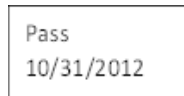


Pass - All Performance Tests run on that date are within the limits set by the Baseline calculation.



Fail - One or more Performance Tests run on that date deviate significantly from the target set by the Baseline calculation.

- If more than one result is available on a single date and the range is set such that the results overlap, the most recent result is displayed first.
- Hover over a data point to display the Pass/Fail status and the date of the Performance Test.



- Click on a data point within the plot to open the Baseline or Performance Test result for that date, depending on whether the data point represents the Baseline or the Performance Test.
- Use the mouse wheel to zoom in on the X-axis, which displays a scroll bar when the zoom function is applied. The Y-axis cannot be zoomed.
- If the date range is changed, the zoom is reset to the default.



Note: The Pass/Fail status is based on the overall result of the Performance Tests for that date and the Baseline.

- If all Performance Tests run on that date meet the target values as determined by the Baseline test, the status is *Pass*.
 - If one or more Performance Tests run on that date do not meet the Baseline test values, the status is *Fail*.
-

Printing the Performance History report

Select **File ► Print** to print the Performance History report.

In addition to the Results plot, the printout will include a header containing the date range of the report, as well as the instrument information as shown below.

Cytometer:	Attune Next Generation	User:	User name
Instrument Model Number:	SDFLSDLFN-123	Institution:	Institution name
Instrument Serial Number:	ASDFLGKNSDFG-234	Software:	Attune Next Gen Cytometric V 3.0

Baseline Test Results

The *Baseline Test Result* displays the results of a selected Baseline.

When establishing a Baseline, the Attune™ NxT Software generates data for the parameters listed in “Performance Test data”, page 270, to determine the initial status of the cytometer. These values are then used as a benchmark when tracking the daily performance of the instrument with Performance Tests.

Viewing the Baseline Test Results

The Baseline Result report automatically appears after a Baseline is completed.

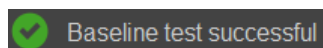
Alternatively, click **Baseline Results** on the Performance Test ribbon tab of the BL/PT module (page 261).



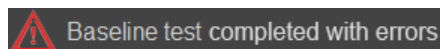
Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	591	300000	299368	1.50 %	0.000	0	0.000	0.79	1100	✓
SSC	330	300000	312932	3.02 %	0.000	0	0.000	0.79	1100	✓
BL1	428	300000	309464	1.18 %	0.036	49	1.000	0.79	1100	✓
BL2	337	300000	288903	1.03 %	0.050	73	0.965	0.79	1100	✓
BL3	386	300000	306699	1.68 %	0.045	14	1.000	0.79	1100	✓
RL1	371	300000	302229	3.73 %	0.047	33	0.997	0.90	1609	✓
RL2	400	300000	313139	3.66 %	0.006	95	1.000	0.90	1609	✓
RL3	465	300000	309675	3.98 %	0.035	84	0.999	0.90	1609	✓
VL1	297	300000	291541	1.03 %	0.008	2375	1.000	1.07	658	✓
VL2	348	300000	314794	1.27 %	0.011	215	0.998	1.07	658	✓
VL3	346	300000	307157	1.50 %	0.017	49	0.995	1.07	658	✓
VL4	414	300000	299723	2.29 %	0.004	168	0.986	1.07	658	✓
YL1	425	300000	306990	1.42 %	0.062	13	1.000	0.79	193	✓
YL2	390	300000	313169	1.51 %	0.028	19	0.971	0.79	193	✓
YL3	435	300000	310555	2.22 %	0.028	59	0.999	0.79	193	✓
YL4	456	300000	299706	2.94 %	0.006	74	1.000	0.79	193	✓

Baseline Results report

- The overall Pass/Fail status of the current Baseline is displayed by the *Pass/Fail indicator*, which includes a message and an indicator icon.

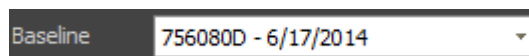


Pass – Data from all channels meet the criteria specified by the instrument.



Fail – Data from one or more of the channels fail to meet the criteria specified by the instrument.

- To view the report for another Baseline run and Beadlot, select the Baseline of interest from the **Baseline history** dropdown menu.



Each Baseline in the dropdown menu is listed in the following format:

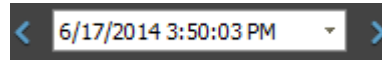
L/N: ### = Performance Test bead lot number

DD/MM/YYYY = date Baseline status was established



Note: The Baseline dropdown menu is enabled only if more than one Baseline has been established. The Baselines are ordered chronologically, with the most recent displayed first.

- To view results from other Baseline calculations, select the desired test date from the **Baseline history** dropdown, or click the blue **arrows** to navigate through the Baseline reports.








Note: If there are multiple reports for a single day, the reports are displayed chronologically, with the most recent displayed first.



Baseline Results table

The Baseline Results table displays all statistics and calculations for all configured channels on the instrument.

Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	591	300000	299368	1.50 %	0.000	0	0.000	0.79	1100	✓
SSC	330	300000	312932	3.02 %	0.000	0	0.000	0.79	1100	✓
BL1	428	300000	309464	1.18 %	0.036	49	1.000	0.79	1100	✓
BL2	337	300000	288903	1.03 %	0.050	73	0.965	0.79	1100	✓
BL3	386	300000	306699	1.68 %	0.045	14	1.000	0.79	1100	✓
RL1	371	300000	302229	3.73 %	0.047	33	0.997	0.90	1609	✓
RL2	400	300000	313139	3.66 %	0.006	95	1.000	0.90	1609	✓
RL3	465	300000	309675	3.98 %	0.035	84	0.999	0.90	1609	✓
VL1	297	300000	291541	1.03 %	0.008	2375	1.000	1.07	658	✓
VL2	348	300000	314794	1.27 %	0.011	215	0.998	1.07	658	✓
VL3	346	300000	307157	1.50 %	0.017	49	0.995	1.07	658	✓
VL4	414	300000	299723	2.29 %	0.004	168	0.986	1.07	658	✓
YL1	425	300000	306990	1.42 %	0.062	13	1.000	0.79	193	✓
YL2	390	300000	313169	1.51 %	0.028	19	0.971	0.79	193	✓
YL3	435	300000	310555	2.22 %	0.028	59	0.999	0.79	193	✓
YL4	456	300000	299706	2.94 %	0.006	74	1.000	0.79	193	✓

- The configured channels are listed in the following order and color-coded based on the laser color as shown below.

-  Scatter channels (FSC and SSC)
-  Blue channels
-  Red channels
-  Violet channels
-  Yellow channels

- The data for the following parameters are displayed for all configured channels in the order specified below.
 - PMTV (photomultiplier tube voltage)
 - Delta PMTV
 - Target MFI (target median fluorescence intensity)
 - Measured MFI
 - r%CV (robust percent coefficient of variation)
 - Quantum Efficiency (Q)
 - Background (B)
 - Linearity
 - Area Scaling Factor
 - Laser Delay
- The decimal places are only shown for r%CV, Q, Linearity, and Area Scaling Factor (2 decimal places for r%CV and Area Scaling Factor, and 3 decimal places for Q and Linearity).
- The Pass/Fail status for each configured channel is indicated by the status indicator icons shown below.
 -  **Pass** - All statistics and calculations meet the criteria set by the instrument.
 -  **Fail** - One or more of the calculations or statistics deviate significantly from the criteria set by the instrument.

Printing the Baseline Results report

Select **File** ► **Print** to print the PT Results Report.

In addition to the Baseline Results table, the printout includes a header containing the date range of the report, as well as the instrument information as shown below.

Cytometer:	Attune Next Generation	User:	<i>User name</i>
Instrument Model Number:	SDFLSDFLN-123	Institution:	<i>Institution name</i>
Instrument Serial Number:	ASDFLGKNSDFG-234	Software:	Attune Next Gen Cytometric V 3.0

Current PT Results

Current PT Results displays the results of the current Performance Test and includes all of the statistics and calculations for all configured channels on the instrument.

Viewing the Current PT Results

Current PT Results report appears automatically after a Performance Test is completed.

You can also access the most recent Performance Test report by clicking **Current PT Result** on the Performance Test ribbon tab of the BL/PT module (page 261).



Performance Test Results

Performance test successful

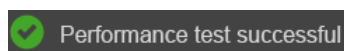
Baseline: 756080D - 6/17/2014

6/17/2014 3:50:03 PM

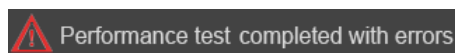
Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	591	300000	299368	1.50 %	0.000	0	0.000	0.79	1100	✓
SSC	330	300000	312932	3.02 %	0.000	0	0.000	0.79	1100	✓
BL1	428	300000	309464	1.18 %	0.036	49	1.000	0.79	1100	✓
BL2	337	300000	288903	1.03 %	0.050	73	0.965	0.79	1100	✓
BL3	386	300000	306699	1.68 %	0.045	14	1.000	0.79	1100	✓
RL1	371	300000	302229	3.73 %	0.047	33	0.997	0.90	1609	✓
RL2	400	300000	313139	3.66 %	0.006	95	1.000	0.90	1609	✓
RL3	465	300000	309675	3.98 %	0.035	84	0.999	0.90	1609	✓
VL1	297	300000	291541	1.03 %	0.008	2375	1.000	1.07	658	✓
VL2	348	300000	314794	1.27 %	0.011	215	0.998	1.07	658	✓
VL3	346	300000	307157	1.50 %	0.017	49	0.995	1.07	658	✓
VL4	414	300000	299723	2.29 %	0.004	168	0.986	1.07	658	✓
YL1	425	300000	306990	1.42 %	0.062	13	1.000	0.79	193	✓
YL2	390	300000	313169	1.51 %	0.028	19	0.971	0.79	193	✓
YL3	435	300000	310555	2.22 %	0.028	59	0.999	0.79	193	✓
YL4	456	300000	299706	2.94 %	0.006	74	1.000	0.79	193	✓

Current PT Results report

- The overall Pass/Fail status of the current Performance Test is displayed by the *Pass/Fail indicator*, which includes a message and an indicator icon.

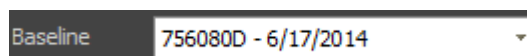


Pass - All channels meet the criteria set by the Baseline calculation.



Fail - One or more of the channels fail to meet the criteria set by the Baseline calculation.

- To view the report for Performance Tests associated with another Baseline and Beadlot, select the Baseline of interest from the **Baseline** dropdown menu.



Each Baseline in the dropdown menu is listed in the following format:

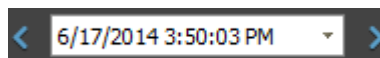
L/N: ### = Performance Test bead lot number

DD/MM/YYYY = date Baseline status was established



Note: The Baseline dropdown menu is enabled only if more than one Baseline has been established. The Baselines are ordered chronologically, with the most recent displayed first.

- To view results from other Performance tests, select the desired test date from the **Performance test history** dropdown, or click the blue **arrows** to navigate through the Baseline reports and Performance Test reports.








Note: If there are multiple reports for a single day, the reports are displayed chronologically, with the most recent displayed first.

PT Results table



The PT Results table displays all statistics and calculations for all configured channels on the instrument.

Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	591	300000	299368	1.50 %	0.000	0	0.000	0.79	1100	✓
SSC	330	300000	312932	3.02 %	0.000	0	0.000	0.79	1100	✓
BL1	428	300000	309464	1.18 %	0.036	49	1.000	0.79	1100	✓
BL2	337	300000	288903	1.03 %	0.050	73	0.965	0.79	1100	✓
BL3	386	300000	306699	1.68 %	0.045	14	1.000	0.79	1100	✓
RL1	371	300000	302229	3.73 %	0.047	33	0.997	0.90	1609	✓
RL2	400	300000	313139	3.66 %	0.006	95	1.000	0.90	1609	✓
RL3	465	300000	309675	3.98 %	0.035	84	0.999	0.90	1609	✓
VL1	297	300000	291541	1.03 %	0.008	2375	1.000	1.07	658	✓
VL2	348	300000	314794	1.27 %	0.011	215	0.998	1.07	658	✓
VL3	346	300000	307157	1.50 %	0.017	49	0.995	1.07	658	✓
VL4	414	300000	299723	2.29 %	0.004	168	0.986	1.07	658	✓
YL1	425	300000	306990	1.42 %	0.062	13	1.000	0.79	193	✓
YL2	390	300000	313169	1.51 %	0.028	19	0.971	0.79	193	✓
YL3	435	300000	310555	2.22 %	0.028	59	0.999	0.79	193	✓
YL4	456	300000	299706	2.94 %	0.006	74	1.000	0.79	193	✓

- The configured channels are listed in the following order and color-coded based on the laser color as shown below.

	Scatter channels (FSC and SSC)
	Blue channels
	Red channels
	Violet channels
	Yellow channels

- The data for the following parameters are displayed for all configured channels in the order specified below.
 - PMTV (photomultiplier tube voltage)
 - Delta PMTV
 - Target MFI (target median fluorescence intensity)
 - Measured MFI
 - r%CV (robust percent coefficient of variation)
 - Quantum Efficiency (Q)
 - Background (B)
 - Linearity
 - Area Scaling Factor
 - Laser Delay

- The decimal places are only shown for r%CV, Q, Linearity, and Area Scaling Factor (2 decimal places for r%CV and Area Scaling Factor, and 3 decimal places for Q and Linearity).
- The Pass/Fail status for each configured channel is indicated by the status indicator icons shown below.
 -  **Pass** - All statistics and calculations for the channel meet the criteria set by the Baseline calculation.
 -  **Fail** - One or more of the statistics or calculations for the channel deviate significantly from the target set by the Baseline calculation.

Printing the PT Results report

Select **File ► Print** to print the PT Results report.

In addition to the PT Results table, the printout includes a header containing the date range of the report, as well as the instrument information as shown below.

Cytometer:	Attune Next Generation	User:	User name
Instrument Model Number:	SDFLSDLFN-123	Institution:	Institution name
Instrument Serial Number:	ASDFLGKNSDFG-234	Software:	Attune Next Gen Cytometric V 3.0

Levey-Jennings Report

The Levey-Jennings report tracks the parameters listed in “Performance Test data”, page 270, for all configured channels to check for shifts and trends in cytometer performance and provides a visual indication of the cytometer performance over time.

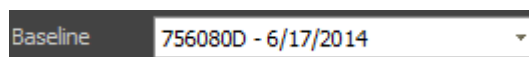
Viewing the Levey-Jennings report

To view the Levey-Jennings report, click **Levey-Jennings Report** on the Performance Test ribbon tab of the BL/PT module (page 261).

The Levey-Jennings report is displayed for the most recent Baseline, showing the data for the most recent 180-day time period.



- To view the Levey-Jennings report for Performance Tests associated with another Baseline and Beadlot, select the Baseline of interest from the **Baseline** dropdown menu.



Each Baseline in the dropdown menu is listed in the following format:

L/N: ### = Performance Test bead lot number

DD/MM/YYYY = date Baseline status was established



Note: The Baseline dropdown menu is enabled only if more than one Baseline has been established. The Baselines are ordered chronologically, with the most recent displayed first.

- To view the Levey-Jennings report for a different data range, click the **calendar icon** on the **Date Range** tool to select the desired beginning and end dates.





Note: By default, the range of the Levey-Jennings report begins with the first Baseline and ends with the most recent Performance Test available for the selected Baseline.

If the default range exceeds 180 days, then the report shows data for the most recent 180-day time period.

Using the Date Range tool, you can select a date range of up to 180 days.

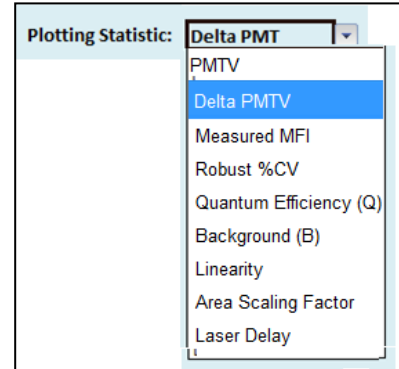
If more than one result is available on a single date and the range is set such that the results overlap, the most recent result is displayed first.

Levey-Jennings plots

- The Attune™ NxT Software generates Levey-Jennings plots for the parameters listed in “Performance Test data”, page 270.

Select the parameter of interest from the **Plotting Statistic** dropdown menu to view the Levey-Jennings plot for that parameter.

- The **Laser** selection tool on the Levey-Jennings report screen allows data to be shown for the scatter channels, blue channels, red channels, violet channels, and yellow channels.



The buttons on the Laser selection tool are color-coded based on the laser color as shown below. To display the Levey-Jennings plot for the scatter/laser channel of interest, click the button for the desired channel.



Note: The software displays only the channels that were configured at the time that the Performance Test was run.

- The Levey-Jennings plots display the Pass/Fail status for the selected parameter within the selected date range. Each data point on the plot corresponds to a single date and the color of the data point indicates its status (see below).

If more than one result is available on a single date and the range is set such that the results overlap, the most recent result is displayed first.

Icon Pass/Fail status



Pass – Performance of the selected channel for the selected parameter is within the limits set by the Baseline calculation.



Fail - Performance of the selected channel for the selected parameter deviates significantly from the target set by the Baseline calculation.



No data - There is no data for the selected channel or the parameter on that date.

- Hover over a data point to display the corresponding data and the date. The decimal places are only shown for r%CV, Q, Linearity, and Area Scaling Factor (2 decimal places for r%CV and Area Scaling Factor, and 3 decimal places for Q and Linearity).

200mV
10/31/2012

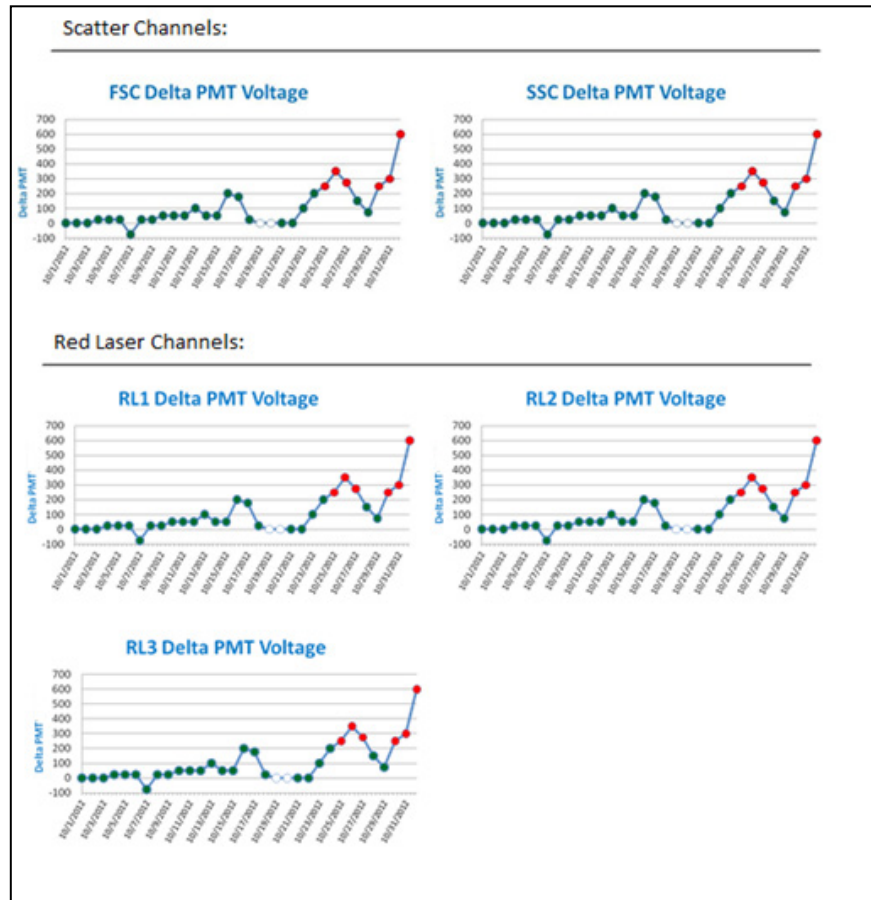
- Click on a data point within the Levey-Jennings plot to open the Baseline or Performance Test result for that date, depending on whether the data point represents the Baseline or the Performance Test.

- Use the mouse wheel to zoom in on the X-axis, which displays a scroll bar when the zoom function is applied. The Y-axis cannot be zoomed.
- If the date range is changed, the zoom is reset to the default.
- Select the **Show average** check box below the Levey-Jennings plot to display the average for the selected parameter and channel within the selected date range. The average appears as a dashed line.
- Select the **Show standard deviation lines** check box to display the standard deviation for the data. Two standard deviations appear as dotted lines above and below the center line.

Print the report

Select **File ► Print** to print the report.

The printout of the Levey-Jennings Report includes a header containing the date range of the report and the instrument information, as well as the Levey-Jennings plots for all the configured lasers. The first page of the printout contains both scatter channels and the first laser channel. The remaining channels are printed individually on the subsequent pages.



17. Compensation

Overview

Compensation

Fluorophores emit light over a range of wavelengths. Although optical filters limit the range of frequencies measured by a given detector, when two or more fluorophores are used in an experiment, there is often an overlap between the wavelength ranges. *Compensation* is the mathematical method used to correct the emission overlap from one fluorophore into the emission channel of another fluorophore.

The Attune™ NxT Cytometric Software calculates the compensation settings automatically as it guides you through the process. This chapter details:

- process of setting up compensation (page 284)
- working with the Compensation Workspaces (page 288)
- modifying compensation (page 298)

Compensation controls

- If the experiment requires compensation, prepare the necessary compensation controls. You will need single-stained controls (i.e., compensation beads or cells) for each fluorophore you are using for compensation. Unless you select to use a negative gate or none, you will also need an unstained or isotype-labeled control.
- You can use Tubes as the source of your Compensation controls. The Compensation Setup dialog provides different options for setup based on your selection of the Compensation Source. For more information, see “Compensation Setup Dialog”, page 284.



IMPORTANT! Once you click **Record** on any of the Compensation controls within the Compensation Setup, the Instrument Settings for all fluorescent channels will be shaded gray and cannot be changed. It is critical that you optimize voltages prior to recording any Sample or any Compensation controls.

Compensation Setup

Initiating Compensation Setup

To initiate Compensation Setup, click the **Compensation Setup** button on the *Compensation ribbon tab* (page 42).



Alternatively,

- double-click on the **Compensation node** within an Experiment on the *Experiment Explorer* (page 140) when there are no Compensation controls present,



or

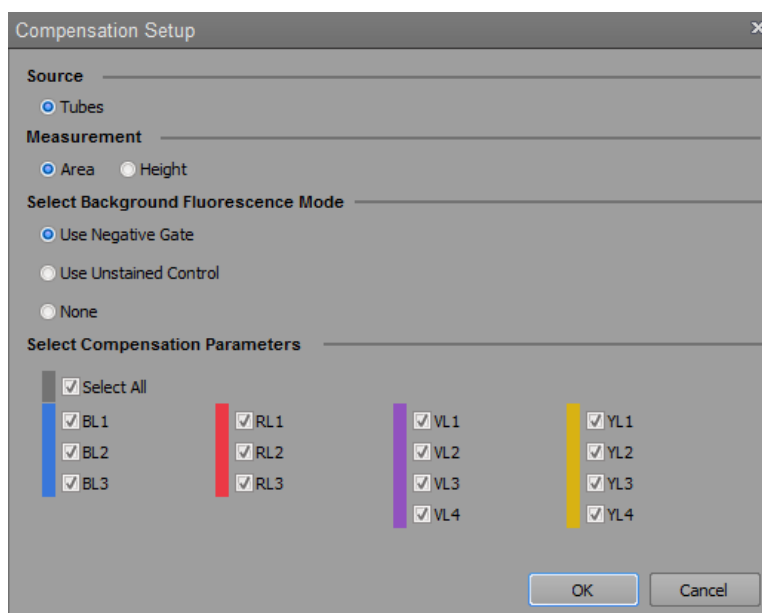
- click on the **Compensation well** button on the *Experiment ribbon tab* to define the Compensation samples.



Each of these methods launches the *Compensation Setup dialog* (see below).

Compensation Setup dialog

The *Compensation Setup dialog* contains sections that provide options to choose the compensation source, the compensation measurement, the background fluorescence mode, and the required compensation parameters. This dialog also allows you to make modifications to an existing Compensation Setup.

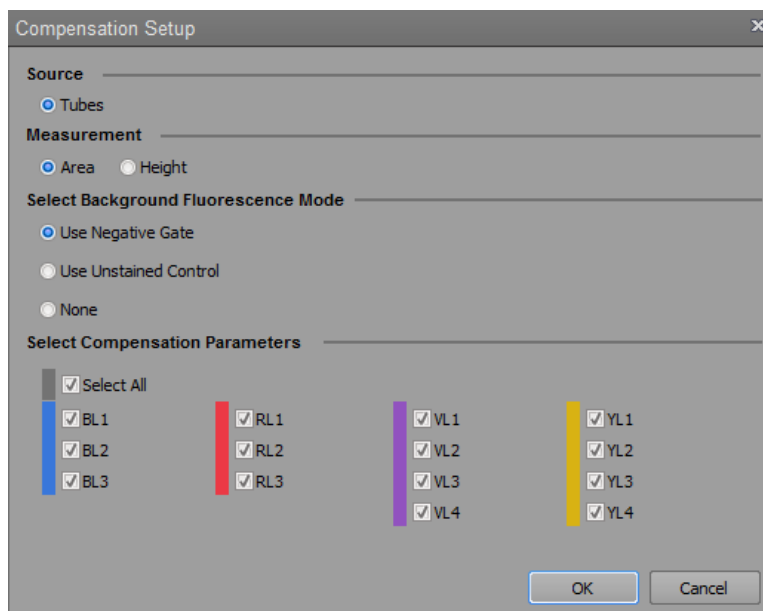


- The *Source* section shows the option for the source of Compensation controls, which is **Tubes** (page 285).
- The options and controls available in the remaining sections of the dialog (*Measurement*, *Background Fluorescence Mode*, and *Compensation Parameters*) are based on the Source selection. Once Compensation controls have been created and recorded, the Source selection cannot be changed and this option becomes disabled.

Compensation Control Source - Tubes

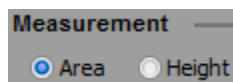
When **Tubes** are selected as the Compensation control source, the Compensation Setup dialog appears as shown below and contains the following controls:

- Measurement radio buttons (below)
- Select Background Fluorescence Mode radio buttons (page 286)
- Select Compensation Parameters check boxes (page 286)



Measurement

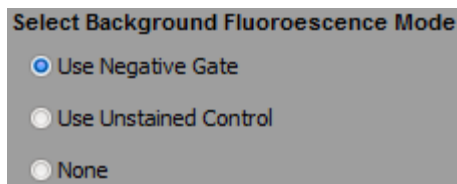
The *Measurement* section allows you select *Area* or *Height* as the parameter for calculating the compensation.



- By default, **Area** is selected. If no area measurements are selected in the Experiment Instrument Settings, the selection defaults to **Height**.
- When **Area** is selected, the resulting Compensation Workspace and controls use the area measurement as the parameter for calculating the compensation. When **Height** is selected, the resulting Compensation Workspace and controls use the height measurement as the parameter for calculating the compensation.
- If all the channels have both the height and area measurements enabled, the Measurement selection can be changed after the Compensation controls have been recorded; otherwise this option is disabled.
- If the Measurement selection is changed, the Workspaces update to use the new Measurement parameter (if the parameters are enabled) and the Compensation is recalculated.

Select Background Fluorescence Mode

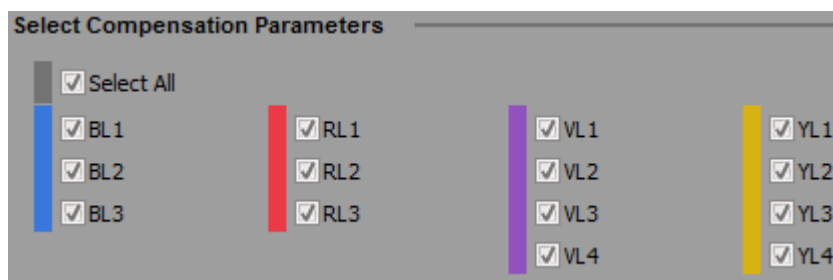
Select Background Fluorescence Mode section allows you to choose the source of the background fluorescence when calculating compensation.



- The available options are *Use Negative Gate*, *Use Unstained Control*, or *None*.
- By default, the **Use Negative Gate** radio button is selected.
- Selecting **Use Unstained Control** adds *Unstained Control* as an additional Compensation control to the Compensation node in the Experiment.
- Selecting **Use Negative Gate** provides an additional Histogram gate or Rectangle gate on the Compensation Workspace for defining the negative population.
- When **None** is selected, compensation is calculated without correcting for background auto-fluorescence.
- If you change the Background Fluorescence Mode before recording any Compensation controls, the Compensation Workspaces are refreshed with the new background option and any changes are reset to the defaults.
- The Background Fluorescence Mode options are disabled once Compensation controls have been created and any Compensation controls have data recorded. If there is no recorded data, changing the Background Fluorescence Mode option updates the Compensation controls and Workspaces accordingly.

Select Compensation Parameters

Select Compensation Parameters section contains checkbox controls that determine the parameters used for calculating compensation.



- The Compensation Parameter checkbox controls are visible and enabled only for the parameters that are selected in the Parameters section of the Instrument Settings Panel, as described on page 180.
- The checkboxes are arranged in columns grouped by laser color (Blue, Red, Violet, and Yellow) with a checkbox for each fluorescence detector assigned to each laser.
- By default, all enabled checkboxes are selected.
- When the **Select All** checkbox is selected, all enabled parameters are checked. When the **Select All** checkbox is deselected, all checkboxes are unchecked. Deselecting one or more of the parameters also deselects the **Select All** checkbox.

- At least two Compensation Parameters must be selected.

If less than two parameters are checked, the OK button is grayed out and a warning stating “At least two fluorescence parameters must be selected.” is shown under the buttons.



- Selection of Compensation Parameters can be modified after controls have been created and recorded.

Any controls that do not have data recorded can be deselected, which removes them from the Experiment Explorer.

Additional controls that have not been assigned can be selected, which adds them to the Experiment Explorer.

- The checkboxes become disabled and remain checked for any controls that have recorded data.

OK and Cancel buttons

- Clicking **OK** closes the dialog box and creates or updates the Compensation control for each selected parameter within the Compensation node of the Experiment in the Experiment Explorer.

The Compensation Workspace for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically.

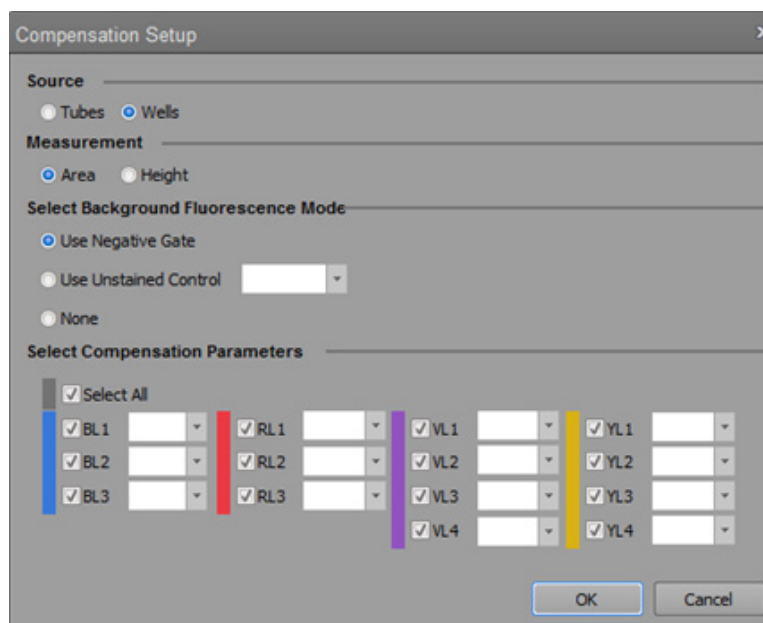
An *Identity Compensation matrix*, a matrix of $N \times N$ dimensions with ones on the main diagonal and zeros elsewhere, is created, where N is the number of compensation parameters.

- Clicking **Cancel** closes the dialog and any changes are lost.

Compensation Control Source - Wells

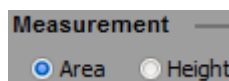
When **Wells** option is selected as the Compensation control source, the Compensation Setup dialog appears as shown below and contains the following controls:

- Measurement radio buttons: Height, Area (below)
- Select Background Fluorescence Mode radio buttons: Negative gate, Unstained control, None (page 289)
- Select Compensation Parameters combo boxes (page 289)



Measurement

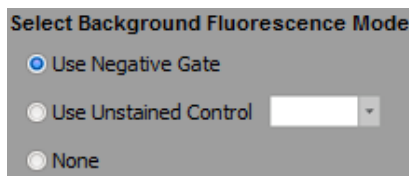
The *Measurement* section allows you select *Area* or *Height* as the parameter for calculating the compensation.



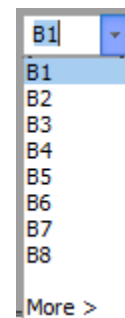
- By default, the Area is selected. If no area measurements are selected in the Experiment Instrument Settings, the selection defaults to Height.
- When **Area** is selected, the resulting Compensation Workspace and controls use the area measurement as the parameter for calculating the compensation. When **Height** is selected, the resulting Compensation Workspace and controls use the height measurement as the parameter for calculating the compensation.
- If all the channels have both the height and area measurements enabled, the Measurement selection can be changed after the Compensation controls have been recorded; otherwise this option is disabled.
- If the Measurement selection is changed, the Workspaces update to use the new Measurement parameter (if the parameters are enabled) and the Compensation is recalculated.

Select Background Fluorescence Mode

Select Background Fluorescence Mode section allows you to choose the source of the background fluorescence when calculating compensation.

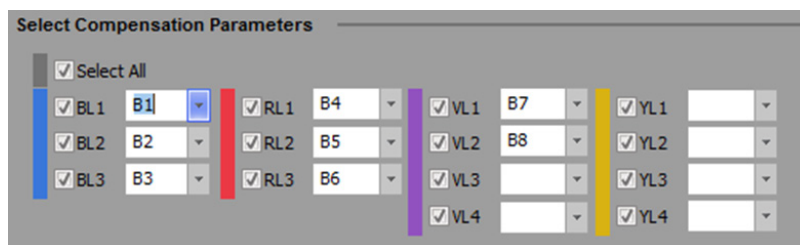


- The available options are *Use Negative Gate*, *Use Unstained Control*, or *None*. By default, the Use Negative Gate radio button is selected.
- Selecting **Use Unstained Control** adds *Unstained Control* as an additional Compensation control to the Compensation Node in the Experiment.
- The **Unstained Control** combo box in the Select Compensation Parameters section (see below) displays a list of all selected wells on the Heat Map and a More option to expand the list to include all wells in the plate that do not contain data.
- The wells in the combo box dropdown are listed in the same order as the Plate run order (A1–A12, B1–B12, etc.). By default, the first selected well is set as the Unstained Control
- Selecting **Use Negative Gate** creates an additional Histogram gate or Rectangle gate on the Compensation Workspace for defining the negative population (see “Compensation Workspace”, page 292).
- When **None** is selected, compensation is calculated without correcting for background auto-fluorescence.
- The Background Fluorescence Mode options are disabled once Compensation controls have been created and any Compensation controls have data recorded. If data has not been recorded, changing the Background Fluorescence Mode option updates the Compensation controls and compensation Workspaces accordingly.



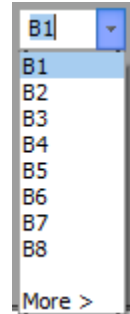
Select Compensation Parameters

Select Compensation Parameters section contains checkbox controls that determine the parameters used for calculating compensation.



- The **Compensation Parameter checkbox** controls are visible and enabled only for the parameters that are selected in the Parameters section of the Instrument Settings Panel, as described on page 180.
- The checkboxes are arranged in columns grouped by laser color (Blue, Red, Violet, and Yellow) with a checkbox for each fluorescence detector assigned to each laser.
- The checkbox labels use the display name set in the Options dialog (page 328). The display name can be modified to display the Parameter name (e.g., BL1-A), the Fluorophore name (e.g., FITC), or both (e.g., BL1-A – FITC) as described on page 328.

- When wells are selected prior to opening the Compensation Setup dialog, the corresponding Compensation control parameters are pre-selected on the Compensation Setup dialog and mapped to the wells in the order of well location (top to bottom, column by column).
- When wells are not preselected prior to opening the compensation setup dialog, the selected Compensation control parameters are mapped to the wells in the order of well location (top to bottom, column by column).
- To manually map a Compensation Parameter to a particular well, select the well location from the dropdown menu next to the selected parameter
- The Compensation Parameter combo boxes contain a list of all selected wells and a **More** option to expand the list to include all wells in the plate that are unmapped.



The wells in the combo box dropdown are listed in the same order as the Plate run order (A1–A12, B1–B12, etc.). If the list shows more than 25 well locations, a scroll bar is displayed, allowing you to view the entire list.

- If no wells are selected, the combo box list includes all unmapped wells.
- When the **Select All** checkbox is selected, all enabled parameters are checked. When the **Select All** checkbox is deselected, all checkboxes are unchecked. Deselecting one or more of the parameters also deselects the Select All checkbox.
- At least two Compensation Parameters must be selected for compensation to be calculated and applied to an experiment..
If less than two parameters are checked, the OK button is grayed out and a warning stating “At least two fluorescence parameters must be selected.” is shown under the buttons.



- Selection of Compensation Parameters can be modified after controls have been created and recorded.
To remove compensation controls from the experiment, open the Compensation Setup dialog and deselect the parameter by unchecking the checkbox next to the parameter name. Only controls that do not have data may be removed.
To add additional controls to the compensation matrix, select the appropriate parameter from the compensation setup dialogue and choose the appropriate well from the drop down menu next to the parameter name. Only unmapped wells may be designated as compensation control wells. The additional compensation controls will be listed in the Experiment Explorer and the Heat Map after clicking **OK** and closing the compensation setup dialog.
- The checkboxes become disabled and remain checked for any controls that have recorded data. If the FCS file is removed, these controls will become enabled for deselection.

OK and Cancel buttons

- Clicking **OK** closes the dialog box and creates or updates the Compensation control for each selected parameter within the Compensation Node of the Experiment in the Experiment Explorer.

The Compensation Workspace for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically following compensation setup.

An *Identity Compensation matrix* is created before compensation controls are recorded. This matrix is composed of all selected compensation parameters and has values of 0%, because compensation has not yet been measured and calculated.

- Clicking **Cancel** closes the dialog and any changes are reverted.

Compensation Workspace



Overview

Compensation Workspace is a special Workspace that contains all the necessary plots and gates to optimize Instrument Settings for the Compensation controls, to record Compensation controls, and to define the populations used when calculating compensation.

- The *Histogram View* in the Compensation Workspace is used for setting up and calculating compensation values.
- You can select to display the Histogram View using the **Workspace view** radio button on the *Compensation ribbon tab* (page 42). This button is only available when a Compensation Workspace is active.
- In the Histogram View (page 294), compensation is not applied and cannot be turned on.
- The gates on the *Gating plots* are linked, as are the negative gates and positive gates on the *Fluorescence plots*.

Customizing the Compensation Workspace

The Compensation Workspace has a fixed landscape view where plot layout or size cannot be adjusted. The default plot attributes are as described in the *Options dialog* (page 331).

- The **Workspace Zoom** tool on the *View Ribbon tab* (page 42) is available for the Compensation Workspace. This tool displays all plots present in the filmstrip area of the Workspace view (page 71), and allows you to zoom in and out of each plot.  

- Plot scale ranges for all parameters are set to **manual**, which allows you to enter the minimum and maximum values manually. The allowable range depends on the type of scale selected.

The default maximum value for each parameter is determined by the range set in the FCS file. By default, the maximum is set to 2^{26} for Event and Time counts, to 2^{20} for Height and Area measurements, and to 2^{10} for Width measurements.

The minimum value for logarithmic parameters is set to 1, and the minimum for linear parameters is set to 0.

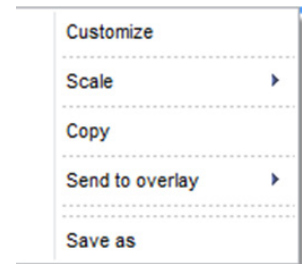
- The plot attributes listed below can be customized as described in “Customize Plot Options” (page 201) and in “Workspace view” (page 63).
 - Resolution
 - X and Y parameters on gating plots and where specified below
 - Scale type and range
- The plot and axis titles cannot be modified.
- The gates used on the Compensation Workspace plots use the gate style and gate label style default settings as described in the Options dialog (page 329).
- Gates cannot be customized and their names cannot be modified.

Plot context menu

Plot context menu is displayed when you right-click on any empty area (i.e., white space) within the plot boundary, but not on another active area (gate, plot title, or plot axis).

- Plot context menu contains the following options:

- Customize
- Scale
- Copy
- Send to Overlay
- Save As



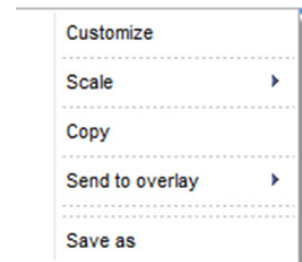
- All options listed function as described in “Plot Context Menu”, page 93.

Plot axis context menu

Plot axis context menu is displayed when you right-click on a plot scale or parameter name. The plot axis context menu options displayed depend on the plot axis selected.

- The X-axis parameter of fluorescence plots cannot be changed, and only the following options are displayed:

- Customize
- Scale
- Copy
- Send to Overlay
- Save As



- For the Scatter plots and Y-axis of dual-parameter fluorescence plots, the parameter can be changed, and the following options are displayed:

- Customize
- Scale
- Parameter
- Copy
- Send to Overlay
- Save As



- All options listed function as described in “Plot Axis Context Menu”, page 98.

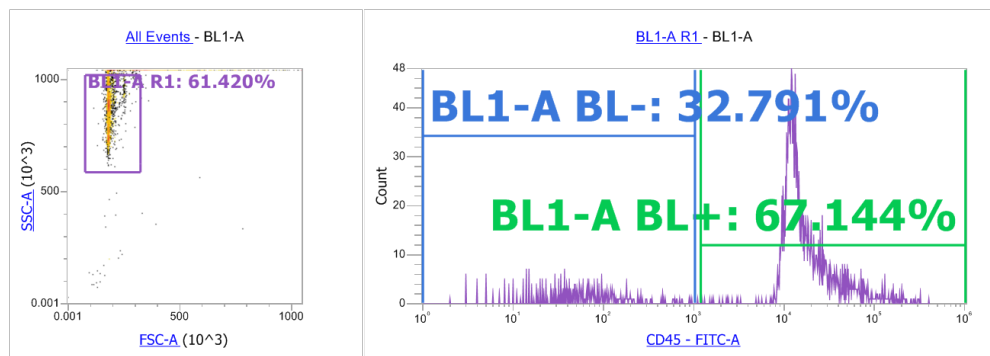
Gate context menu

- The Gate context menu in the Compensation Workspace is available only for gates on dual-parameter scatter plots.
- Right-clicking on any part of a gate, on the gate boundary line, or a control point displays the Gate context menu.
- The context menu for dual-parameter scatter plot gates consists of a single option: Apply gate shape to all controls.
- This option applies the current scatter gate type, size, and location to the scatter gate on all Compensation controls within the current Experiment.
- Apart from the scatter gate described above, there is no gate context menu for compensation plots.

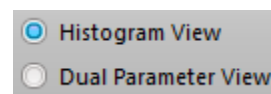
Compensation Workspace – Histogram view

Overview

The Workspace for the *Histogram View* consists of a *Gating plot* and a *Histogram Compensation plot* corresponding to the selected Compensation Parameter.



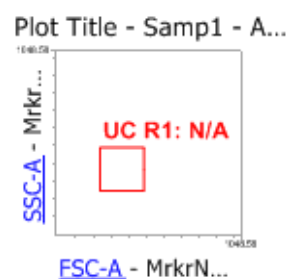
- **Histogram View** radio button on the Compensation ribbon tab (page 42) selects the Histogram View. Histogram View is the default selection on the Compensation Workspace.



Gating plot

The *Gating plot* is an ungated dual-parameter density plot used to gate the events shown on the Compensation plot.

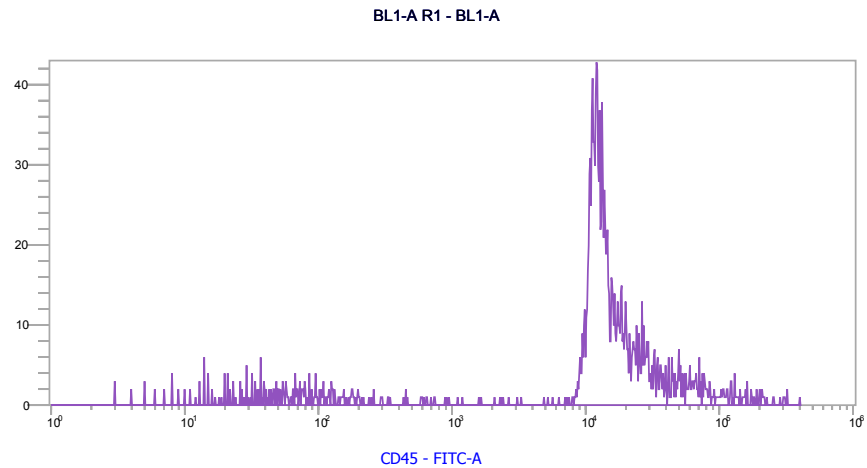
- By default, the parameters for the X- and Y-axes are set to FSC and SSC, respectively, and use the measurement specified in the Measurement section of the Compensation Setup dialog (page 284).
If the FSC and SSC parameters are not available, then parameter numbers 1 and 2 are used for X- and Y-axis parameters, respectively.



- The Gating plot type cannot be changed to a histogram type plot.
- The Gating plot can only be set to display *All Events*. The Plot title hyperlink, if shown, is disabled.
- The Parameter names on the Gating plot are hyperlink controls that link to the Parameter selection dropdown list as described in “Plot Parameter Hyperlink”, page 102. You can change the selected parameters for both the X- and Y-axes.
- The Gating plot contains a single 5-point polygon gate (drawn in a rectangle) named *Parent*. This gate cannot be deleted.
- The default position of the Parent gate has the following (X, Y) coordinates:
 - lower left corner coordinates (250000, 250000)
 - upper right corner coordinates (500000, 500000)
- You can move and resize the Parent gate as needed.
- Any adjustment made to the Parent gate automatically updates the Histogram plots and the calculated Compensation matrix (page 298).
- Initially, when the Gating plot gate position is moved on the Workspace of the first Compensation control, it automatically applies to all Compensation control samples. Once a gate is moved on subsequent Compensation control samples, moving the gate on the Workspace of the first control has no effect.

Histogram Compensation plot without Negative gate

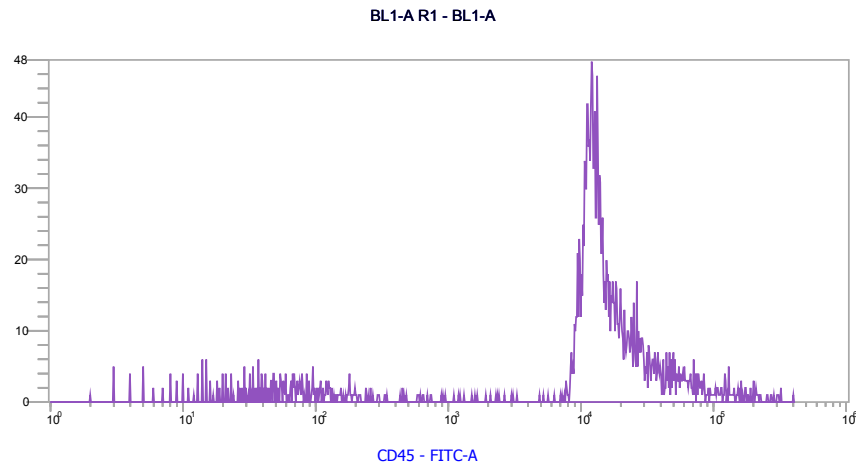
The *Histogram Compensation plot* is a single-parameter daughter plot derived from the Parent gate on the dual-parameter Gating plot (page 294). This plot type cannot be changed.



- The X-axis parameter on the plot is the currently selected Compensation control on the Experiment Explorer. This parameter cannot be changed.
- The Gate name hyperlink on the Plot title, if shown, is disabled.
- The Compensation plot contains a single Histogram gate, which is named in the following format: "<parameter channel name> +". For example, BL1+.
- The default position of the Histogram gate has the following (X, Y) coordinates:
 - left coordinate (500,000)
 - right coordinate (750,000)
- You can move and resize the gate as needed.
- The Compensation Histogram gate cannot be deleted.
- Any adjustment made to the Histogram gate automatically updates the calculated Compensation matrix (page 298).

Histogram Compensation plot with Negative gate

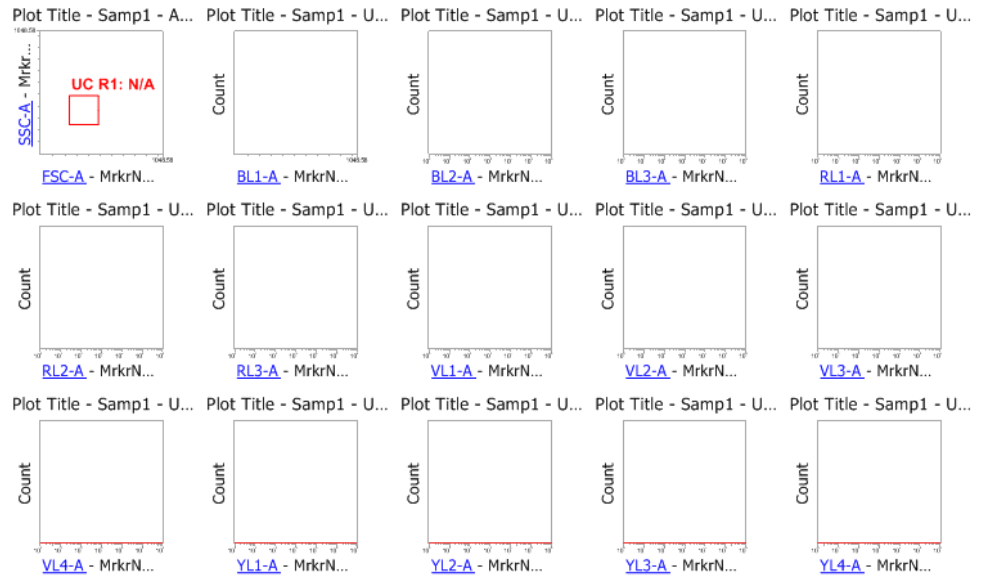
This Compensation plot is identical to the Histogram Compensation plot (page 295) with the exception that it contains an additional gate defining a negative population.



- When **Use Negative Gate** is selected in the Compensation Setup dialog (page 284), the Histogram plot contains an additional gate to define a negative population.
- The negative gate is named in the following format: "<parameter channel name> -". For example, BL1-.
- The default position of the Negative gate has the following (X, Y) coordinates:
 - left coordinate (1)
 - right coordinate (1000)

Unstained Control Workspace

When **Use Unstained Control** is selected in the Compensation Setup dialog (page 284), an additional Control Workspace for the Unstained control is created.



- The Unstained Control Workspace contains a Gating plot as described on page 294 and Histogram plots for each Compensation parameter.
- The Histogram plots for Compensation parameters on the Unstained Control Workspace do not display any gates. All Histogram plots are gated on the Parent gate.
- The background signal is calculated based on the median of the gated data for each parameter; no additional gates are required.

Matrix dialog

Overview

The *Matrix dialog* shows the active compensation associated with the current Experiment or FCS file, and allows you to manually edit or reset the *Spillover matrix* values.

- Click the **View Matrix** button on the *Compensation ribbon tab* (page 42) to launch the Matrix dialog.



Alternatively, double-click the **Compensation node** on the Experiment Explorer when Compensation controls are present.



Note: *Spillover matrix* shows the amount of spillover from each fluorophore into each of the other fluorescent channels.

View Matrix

Matrix - Plate

Spillover values are read across rows

Spillover

	BL1-A	BL2-A	BL3-A	RL1-A	RL2-A	RL3-A	VL1-A	VL2-A	VL3-A	VL4-A	YL1-A	YL2-A	YL3-A	YL4-A
BL1-A	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL2-A	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL3-A	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL1-A	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL2-A	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL3-A	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL1-A	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
VL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
VL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
YL1-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
YL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
YL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
YL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

OK Cancel Reset

- The Matrix title indicates the source of the active compensation, which is the Experiment or the Sample name based on the selection made in the *Apply group* of the Compensation ribbon tab (page 55) for the current Sample.
- If the Matrix is opened by double-clicking the **Compensation node**, the compensation source is always the Experiment.

Matrix dialog table

The *Matrix dialog table* shows the Spillover values for the current compensation in editable textboxes that are arranged in a table.

- The row and column labels use the Channel name (\$PnN) by default. The display name can be modified using the Options dialog to show Parameter name (\$PnS) and/or the Channel name (\$PnN) as described in "Naming Options", page 328.
- The row and column labels have a background color based on the laser color associated with the channel. If a channel color is not available for a parameter (i.e., non-Attune™ file), the default fill is white and the font color is black.
- When a textbox is selected, the column and row is highlighted at the point of their intersection in blue. The diagonal values of the matrix are shaded in light gray.
- In the Spillover matrix, the textbox controls accepts numbers from 0% to 100%, and allow the use of two decimal places. The diagonal values cannot be adjusted in the Spillover matrix is shown.
- You can enter up to 7 total characters into the textboxes in the Spillover matrix, but all entered values will be rounded to 2 decimal places upon loss of focus of the entry field or when **Enter** is pressed. Only numbers, decimals, and + and – signs may be entered into the textboxes.
- The Spillover values in the table are written across rows as shown below. For example, the spillover of FL1 into FL2 is shown in row 1, column 2.

	BL1-A	BL2-A	BL3-A
BL1-A	100.00	0.00	0.00
BL2-A	0.00	100.00	0.00
BL3-A	0.00	0.00	100.00
RL1-A	0.00	0.00	

Spillover	FL1	FL2	FL3	FL4	FL5	FL6
FL1	→					
FL2		→				
FL3			→			
FL4				→		
FL5					→	
FL6						→

In the example below, the spillover from FL1 (BL1-A) into FL2 (BL2-A) is 38.10%.

	BL1-A	BL2-A	BL3-A	RL1-A
BL1-A	100.00	38.10	5.86	0.01
BL2-A	0.51	100.00	24.18	0.00
BL3-A	0.22	25.40	100.00	13.92
RL1-A	0.06	0.04	0.01	100.00

- When the cursor is hovered over the table textboxes, tooltips are displayed. When the Spillover matrix is displayed, the tooltip reads: "%<Row Label> in <Column Label>". For example, %BL1 in BL2.

OK, Reset, and Cancel buttons

OK button

- Clicking **OK** accepts any changes made to the matrix and applies the updated compensation values to the dataset.
- If the matrix values are adjusted during recording a Sample and a stop gate is set on a population, the stop gate condition recounts the events once **OK** has been clicked.

Reset


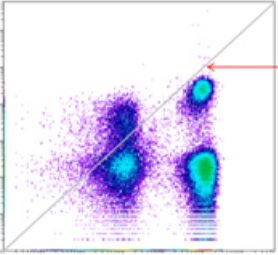
- Clicking **Reset** resets the compensation values to their default states as described below.
- If Experiment-level compensation is modified and Compensation controls are available, clicking **Reset** recalculates the compensation values based on the current Histogram gate positions on the Compensation Workspace.
- If any existing Experiment-level compensation settings are modified and Compensation controls are not available (for example, if the Experiment has been duplicated), clicking **Reset** reverts the Compensation matrix to the original Experiment-level calculated values.
- If FCS file compensation is modified, clicking **Reset** restores the compensation values to the values specified within the FCS data file.

Cancel

- Clicking **Cancel** undoes any changes made to the Spillover matrix or the Compensation matrix, and retains the existing values.

On-Plot Compensation Adjustment

Overview

- When **Plot Compensation** state button on the Compensation ribbon tab (page 42) is turned **ON**, event populations displayed on dual-parameter fluorescence plots can be dragged to new positions to change the associated Compensation matrix values. 
- When **Plot Compensation** is **ON**, hovering over a dual-parameter fluorescence plot displays a tooltip showing the compensation coefficients. The coefficients displayed are the spillover values.
- For the selected plot, a diagonal line from the lower left to the upper right distinguishes the two coefficient sections. Dragging a selected population above the line in a horizontal direction adjusts one coefficient, dragging below the line in a vertical direction adjusts the other coefficient. 
- If the plot does not contain parameters that can be compensated (e.g., Scatter or Time), then the diagonal line is not displayed and the populations cannot be dragged.
- For Spillover values, the compensation coefficients can be dragged from 0% to 100%.

Manual on-plot compensation adjustment

- The user clicks on the selected population and drags the population to the desired location on the plot. The sensitivity of the dragging action is dependent on where the plot is clicked. This is correlated to the scale of plot and the range in which a population is moved.
- Gate labels are not visible during the drag operation.
- Only the current plot is updated during dragging. When the dragging is complete, all plots and preview plots affected by the change are updated. The compensation adjustment is only registered into the undo stack once the dragging is complete.
- If compensation values are adjusted during recording a Sample and a stop gate is set on a population, the stop gate condition will recount the events once the mouse button has been released
- You can also adjust the compensation for the selected plot using the arrow keys. The **up** and **down arrow** keys adjust the Y-axis coefficient, and the **left** and **right arrow** keys adjust the X-axis coefficient. Each arrow key changes the compensation value by 1 unit. Holding down the arrow keys allows continual adjustment while the key is pressed.
- Holding **Shift+arrow** keys change the compensation value by 0.1 units.

Compensation acquisition workflow

Overview

Once the Compensation controls have been defined, they can be acquired. The expected workflow is to go through a round of optimization to correctly set the voltages, thresholds, and gates, followed by a round of recording. At this point, the recorded Compensation controls are factored into the compensation calculation.



IMPORTANT! Once you have recorded all Compensation controls and calculated and applied the Compensation matrix, you cannot adjust the PMT voltages for experimental data.

Compensation acquisition from Tubes

- Compensation setup and acquisition from Tubes is performed manually.
- Each Compensation control can either be optimized using the Unstained Control Workspace or the Workspace associated with the Compensation control sample.
- This optimization process is repeated for all Compensation controls.
- Once the optimization phase is completed, Compensation controls are recorded by loading a Compensation control sample and its corresponding Workspace. The Samples are recorded using the Collection Panel controls described on page 161.
- While recording, gates can be moved.
- The Compensation matrix is calculated as new Compensation controls are recorded. The calculation will only include the controls that have been recorded.

Compensation of instrument data

For Samples acquired from the instrument, the Compensation matrix is applied to both the Area and Height measurements, if available, regardless of whether the compensation was calculated using Area or Height measurements.

18. New Experiment and Group dialogs

New Experiment dialog

The *New Experiment* dialog allows you to create New Experiments of the following types:

- Tube-only Experiment (page 304)
- Plate Experiment (page 305)

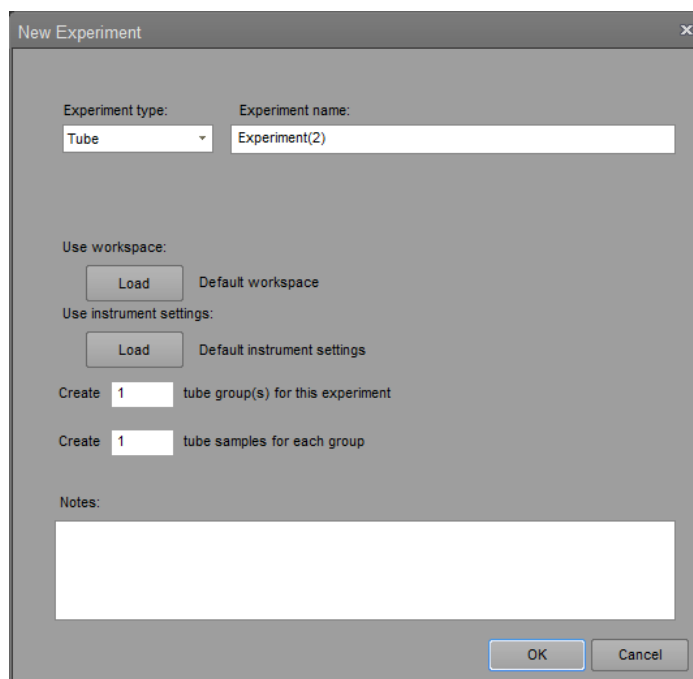
Access the New Experiment dialog

On the *Ribbon* bar (page 38), click **File** to open the *File* menu (page 39), then select **New Experiment**.

Alternatively, click the **New Experiment** icon in the *Home* tab (page 40) or use the keyboard shortcut **Ctrl+N**.



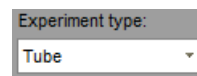
Note: The New Experiment option is enabled only when the instrument is not acquiring.

A screenshot of the 'New Experiment' dialog box. The dialog has a title bar with 'New Experiment' and a close button. It contains several sections: 'Experiment type:' with a dropdown menu set to 'Tube'; 'Experiment name:' with a text field containing 'Experiment(2)'; 'Use workspace:' with a 'Load' button and 'Default workspace' text; 'Use instrument settings:' with a 'Load' button and 'Default instrument settings' text; 'Create' section with two input fields, both containing '1', followed by 'tube group(s) for this experiment' and 'tube samples for each group' respectively; and a 'Notes:' section with a large empty text area. At the bottom right are 'OK' and 'Cancel' buttons.

Note: The contents of the *New Experiment* dialog changes, depending on the experiment type you select.

Create a Tube-only Experiment

1. From the Experiment type dropdown menu, select **Tube** (default selection).



Experiment type:
Tube

2. Accept the default Experiment name, or enter a new name.

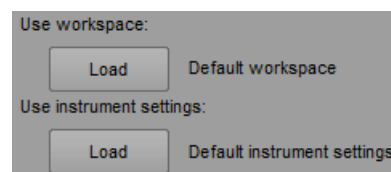


Experiment name:
Experiment

- You can enter up to 50 characters; the following characters are not permitted: \ / : * ? " < > | % . The software automatically removes trailing or leading spaces.
- Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK\$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
- Duplicate names are not permitted.
- The default name for a Tube experiment is **Experiment**. If an Experiment using the default name already exists, a numerical suffix is added in parentheses; for example, **Experiment (2)**.

3. Accept the default Workspace.

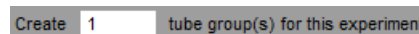
4. Accept the default instrument settings.



Use workspace:
Load Default workspace

Use instrument settings:
Load Default instrument settings

5. Enter the number of Groups to create for the Experiment.



Create 1 tube group(s) for this experiment

- You can enter a number from **1** to **25**. The software adds the number of Groups to the Experiment Explorer (page 141).
 - The software creates a unique name for each Group by using the default Group name set in the Options dialog ► General tab (page 328) and appending it with a numerical suffix; for example, **Group (2)**.
6. Enter the number of Tube samples to create for each Tube group.
 - You can enter a number from **1** to **400**. If the number of Groups times the number of Samples is >400, the number of Samples within a Group will revert to a number that is ≤400.
 - The software adds the number of Samples to Experiment Explorer (page 141).
 - The software creates a unique name for each Sample by using the default Sample name set in the Options dialog ► General tab (page 328) and appending it with a numerical suffix; for example, **Sample (2)**.
 7. *Optional*: Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.



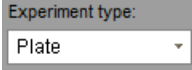

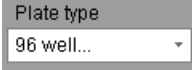

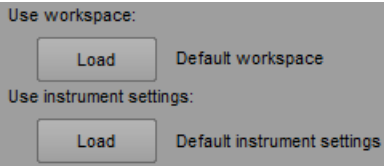
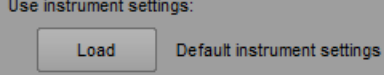
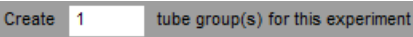
Notes:

8. Click **OK** to create the New Experiment and close the New Experiment dialog. The software opens the first Sample in the Experiment and the Experiment Workspace (page 64).

To close the dialog without creating an Experiment, click **Cancel**.

Create a Plate Experiment

A *Plate Experiment* includes one Plate, and but it can also include Tube groups and Tube samples.

1. From the Experiment type dropdown menu, select **Plate**.

2. Accept the default plate name, or enter a new name.
 - You can enter up to 50 characters; the following characters are not permitted: \ / : * ? " < > | %. The software automatically removes trailing or leading spaces.

 - Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK\$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
 - Duplicate names are not permitted.
 - The default name for a Plate Experiment is **Plate**. If an Experiment using the default name already exists, a numerical suffix is added in parentheses to ensure a unique name; for example, **Plate (2)**.
3. From the **Plate type dropdown** menu, select the type of plate that you are using for the Experiment.

4. Enter the plate ID.
 - You can enter up to 50 characters; all characters are permitted. The software automatically removes trailing or leading spaces.

5. Accept the default workspace.

6. Accept the default instrument settings.

7. If you are including Tubes, enter the number of Tube groups to create for the Experiment.

 - You can enter a number from 1 to 5, depending on the plate type you selected in step 3.
 - The software adds the number of Groups to Experiment Explorer (page 141).
 - The software creates a unique name for each Group by using the default Group name set in the Options dialog ► General tab (page 328), and appending it with a numerical suffix; for example, **Group (2)**.

8. If you are including tubes, enter the number of Tube samples to create for each tube Group.

Create tube samples for each group

- For a 96-well plate, you can enter a number from **1** to **304**. If the number of Groups times the number of Samples is >304 , the number of Samples within a group will revert to a number that is ≤ 304 .
 - For a 384-well plate, you can enter a number from **1** to **16**. If the number of Groups times the number of Samples is >16 , the number of Samples within a Group will revert to a number that is ≤ 16 .
 - The software adds the number of Samples to Experiment Explorer (page 141).
 - The software creates a unique name for each Sample by using the default Sample name set in the Options dialog ► General tab (page 328) and appending it with a numerical suffix; for example, **Sample (2)**.
9. *Optional:* Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.

Notes:

10. Click **OK** to create the New Experiment and close the New Experiment dialog. The software opens the Heat Map view (page 105).
To close the dialog without creating an Experiment, click **Cancel**.

New Experiment from Template dialog

The *New Experiment from Template* dialog allows you to create a new Experiment from an existing template with pre-defined settings.



Note: For Experiments created from a template, no FCS files are attached to any of the Samples.

Access the New Experiment from Template dialog

On the Ribbon bar (page 38), click **File** to open the File menu (page 39), then select **New Experiment from Template**.

Alternatively, click the **New Experiment from Template** icon in the *Home* tab (page 40).



Note: The New Experiment from Template option is enabled only when the instrument is not acquiring.

New Experiment from Template

Experiment Type: Tube Experiment Name: Experiment(1)

Select Template:

Notes:

OK Cancel

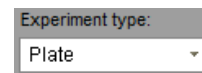


Note: The contents of the New Experiment from Template dialog changes depending on the Experiment type you select.

Create an Experiment from a template

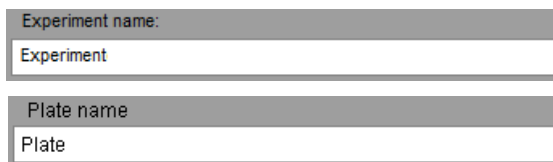
When you use an existing template to create an Experiment, the following settings are already defined: Workspace, Instrument settings, Run Protocols, Heat Map settings, Compensation, Overlays, Hierarchy, and the number of mapped Groups and Samples.

1. From the Experiment type dropdown menu, select **Tube** or **Plate**.



Experiment type:
Plate

2. Accept the default Experiment or Plate name, or enter a new name.



Experiment name:
Experiment

Plate name:
Plate

- You can enter up to 50 characters; the following characters are not permitted: \ / : * ? " < > | %.
 - The software automatically removes trailing or leading spaces.
 - Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK\$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
 - Duplicate names are not permitted.
 - The default name for a tube experiment is **Experiment**. If an experiment using the default name already exists, a numerical suffix is added in parentheses; for example, **Experiment (2)**.
 - The default name for a Plate Experiment is **Plate**. If an Experiment using the default name already exists, a numerical suffix is added in parentheses; for example, **Plate (2)**.
3. Select a template from the **Template Selection dropdown**.



Select Template:

The template files displayed in the *Template Selection* dropdown depend on the type of Experiment you selected in step 1.

4. *Optional*: Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.



Notes:

5. Click **OK** to create the New Experiment and close the New Experiment from Template dialog.
 - For tube-only Experiments, the software opens the first Sample in the Experiment and displays the Experiment Workspace (page 64).
 - For Plate Experiment, the software displays the Heat Map view (page 105).To close the dialog without creating an Experiment, click **Cancel**.

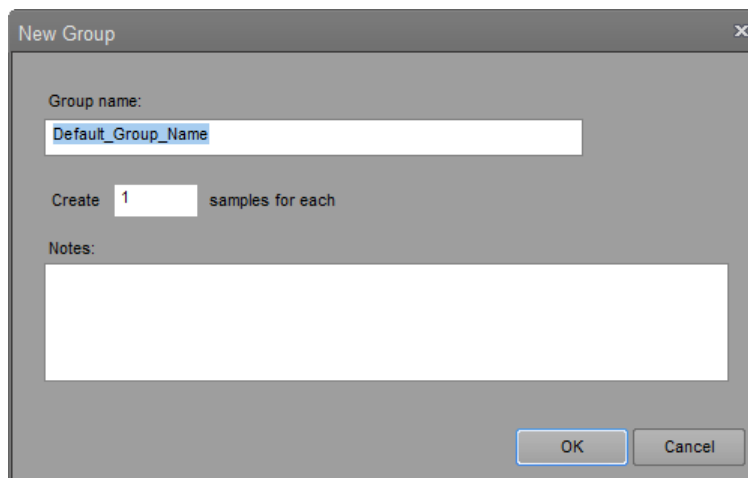
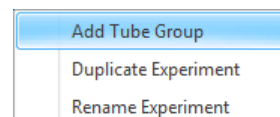
New Group dialog

The *New Group* dialog allows you to create a new Tube group and specify the number of Samples to create for that Group.

Access the New Group dialog

On the *Experiment Explorer* (page 140), right-click on an Experiment and select **Add Tube Group**.

New Group dialog opens.



Create a Group

1. Accept the default Group name, or enter a new name.
 - You can enter up to 50 characters; the following characters are not permitted: \ / : * ? " < > | %.
 - The software automatically removes trailing or leading spaces.
 - Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK\$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
 - Duplicate names are not permitted.
 - The default name for a Group is **Group**. If a Group using the default name already exists, a numerical suffix is added in parentheses to ensure a unique name; for example, **Group (2)**.
2. Enter the number of Tube samples to create for the Group.
 - For a Tube-only Experiment, you can enter a number from **1** to **400**. If the number of Groups times the number of Samples is >400, the number of Samples within a Group will revert to a number that is ≤400.
 - For a 96-well plate, you can enter a number from **1** to **304**. If the number of Groups times the number of Samples is >304, the number of Samples within a Group will revert to a number that is ≤304.
 - For a 384-well plate, you can enter a number from **1** to **16**. If the number of Groups times the number of Samples is >16, the number of Samples within a Group will revert to a number that is ≤16.
 - The software adds the number of Samples to Experiment Explorer.



3. The software creates a unique name for each Sample by using the default Sample name set in the Options dialog ►General tab (page 328) and appending it with a numerical suffix; for example, **Sample (2)**.
4. *Optional:* Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.



Notes:

5. Click **OK** to create the new Group and close the *New Group* dialog. To close the dialog without creating an Group, click **Cancel**.

19. Dialogs

Overview

This chapter describes the non-instrument dialogs that are displayed throughout the Attune™ NxT Software.

All dialogs are *modal*, unless otherwise specified.



Note: Modal dialogs appear when you are performing tasks in the main software windows. You must interact with the modal dialog before you can return to the main window.

Non-instrument dialogs

- Page Setup dialog (page 312)
- Print dialog (page 313)
- Print Preview dialog (page 315)
- Batch Print dialog – Experiment Explorer context menu (page 316)
- Export Statistics dialog – Experiment Explorer context menu (page 318)
- Derived Gate dialog (page 319)
- Edit Gates dialog (page 322)
- Confirm File Save dialog (page 325)

Page Setup dialog

The *Page Setup* dialog allows you to customize the paper size and orientation.

Open the dialog

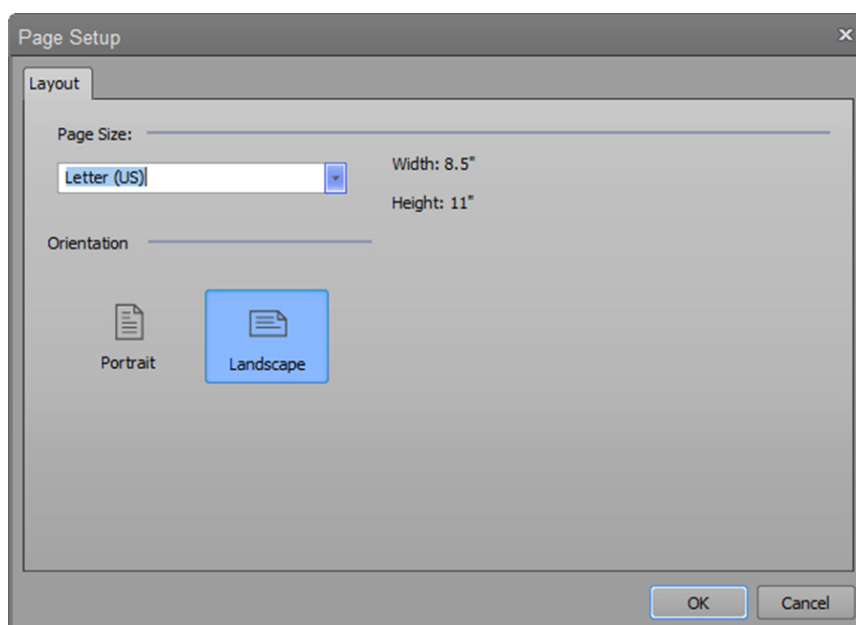
On the **Home ribbon tab** (page 40), click **Page Setup**.

The Page Setup button is only available when the Workspace, Results, Heat Map, or Overlay views are active.

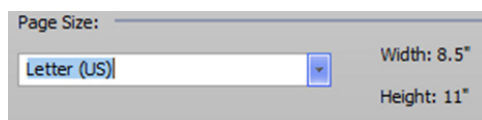


Layout tab

The Layout tab allows you to customize the page size and orientation.

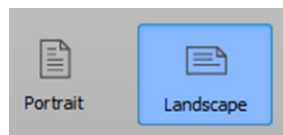


1. To select a page size for the printed document, select a size from the **Page Size** dropdown menu.



The software displays the width and height dimensions for the selected size.

2. To select the orientation of the printed document, click the **Portrait** or **Landscape** icon.



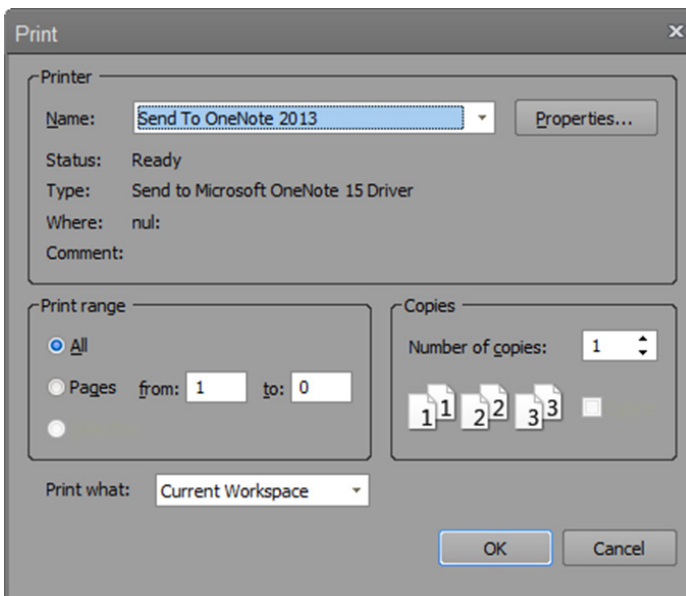
3. To save the changes and close the dialog, click **OK**.
To close the dialog without saving the changes, click **Cancel** or **X**.

Print dialog

The *Print* dialog is a modified Windows® Print dialog. All the controls on this dialog, except the *Print what* combo box and the print range, are standard Windows® functions and are not described here.

Open the dialog

On the **File Ribbon tab** (page 39), select **File ► Print** or press **Ctrl+P** on the keyboard.



Print range

This is the standard print range control. However, the Print range options are disabled when the Print dialog is opened from the Experiment Explorer context menu ► Batch Print dialog (page 316). In this case, **All** is selected and the entire print queue is processed.

Print what options

- The **Print what options** are not displayed when the Print dialog is opened from the Experiment Explorer context menu ► Batch Print dialog (page 316).
- If the Print dialog is opened from within any of the standard application views (Workspace, Heat Map, Results, or Overlays), the **Print what dropdown** list displays the following options:
 - **Current Workspace:** Prints the currently active Workspace in the order specified in the Page setup dialog (page 312).
 - **Heat Map:** Prints the Heat Map and its legend scaled to one page width.
 - **Results:** Prints Results tables (both primary and secondary, if available) scaled to one page width.
 - **Overlays:** Prints Overlay plots and their corresponding legends (if enabled) with one plot per row. The Overlay galleries cannot be printed.

- If the Print dialog is opened from the Performance Test module (page 270), the **Print what dropdown** list displays the following options:
 - **Selected PT Report:** Prints the currently active and visible Performance Test report (page 277). If no PT report is visible, the most recent PT report is printed.
 - **Levey-Jennings:** Prints the currently selected Levey-Jennings report for all installed detectors. Only the selected date range and statistics are printed. If no Levey-Jennings is selected, the most recent Levey-Jennings plot is printed using the specified date criteria.
 - **Performance History:** Prints the performance history for the currently selected baseline is printed. If no baseline is selected, the most recent performance history is printed.
- Selecting one of the options changes the view to the selected view and sends the selected option to the print queue when the **OK** button is clicked.
- If any selection is not present or does not contain data, nothing will print.
- The default selection for the Print what dropdown is determined by the view currently in focus.

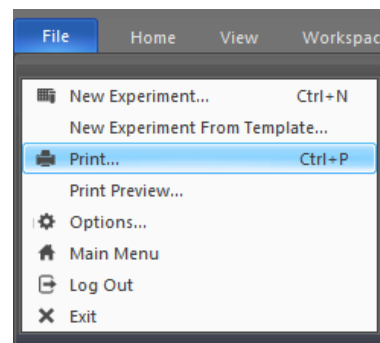
Print Preview dialog

The *Print Preview* dialog displays the document as it will appear when printed, and allows you to set various printing options.

Open the dialog

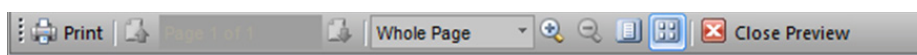
On the **File Ribbon tab** (page 39), select **File ► Print Preview**. The Print Preview option is enabled only when the instrument is not acquiring.

The Print Preview opens as a full screen modal dialog that displays the document as it will appear when printed.



Print Preview dialog

The controls to close the print preview, print the document, select portrait vs. landscape, open the page setup dialog, view the document full page width, view a whole page or multiple pages, and navigate are displayed in a command bar at the top of the dialog.

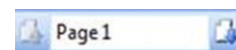


- To print the document, click **Print**.

The Print button opens a *Print* dialog that contains standard Windows® print functions.



- To navigate through the preview pages click the **up** and **down** arrows.



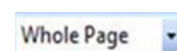
The current page number is displayed in the **Page <n>** text box.

Alternatively, navigate directly to a specific page by entering the page number in the **Page <n>** text box and press the **Enter** key.

- To resize the page view, you can select any of the following options:

- Select a size from the **Page View** dropdown menu.

By default, whole page is selected.



- **Zoom In** or **Zoom Out** buttons allow the page view to zoom in and out.



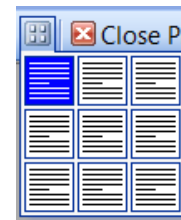
When the page zoom is at 500%, the Zoom In button is disabled.

When the page zoom is at 10%, the Zoom Out button is disabled.

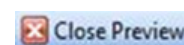
- Click the **Page Size quick selection** button to set the view to the whole page.



- To view up to 9 pages at one time, click the **View Multiple Page** icon to open a popup, then mouse-over the pages to view.



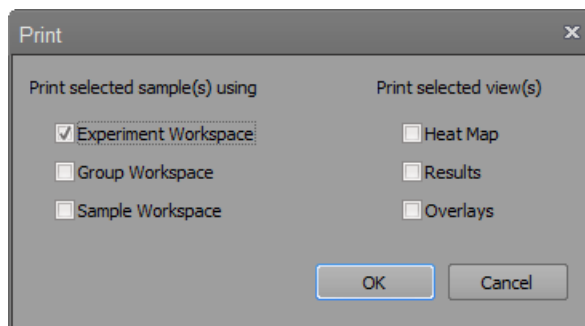
- To close the dialog, click **Close Preview**.



Batch Print dialog – Experiment Explorer Context Menu

Open the dialog

The *Print* dialog is displayed when the **Print** option is selected from the Experiment, Group, or Sample context menu in the Experiment Explorer or from the Heatmap context menu.



Print dialog options

Print selected sample(s) using

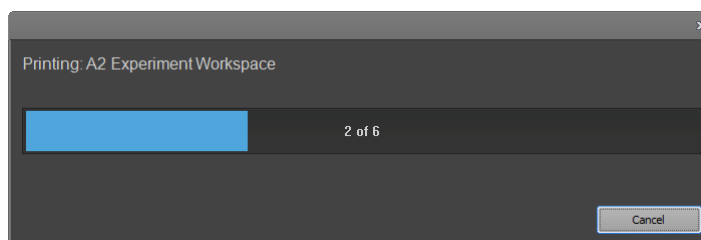
- The options in this group allow you to select the workspace(s) to use when printing the selected samples. Available options are:
 - Experiment Workspace
 - Group Workspace
 - Sample Workspace
- You can select multiple Workspaces. By default, the Experiment Workspace is selected.

Print selected view(s)

- The options in this group allow you to select the view to print. Available options are:
 - Heat Map
 - Overlay
 - Results

By default, none of these options are selected.

- To begin printing, click **OK**, which opens the Print dialog (page 313).
- Before printing, the Heatmap, Overlays, and Results view are automatically refreshed ensuring that all statistics are up to date.
- During the batch print process, the progress of the print job is indicated on a dialog pop-up. The number of items in the print queue is the sum of Workspaces printed for each Sample and views selected to print.



- To cancel the print job, click **Cancel** on the progress dialog.

Print order

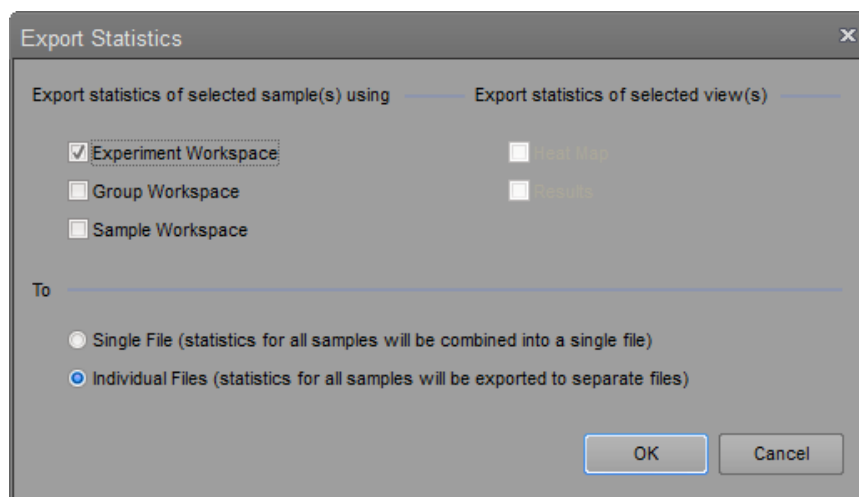
- The print order is:
 - Experiment Workspace (<n>)
 - Group Workspace (<n>)
 - Sample Workspace (<n>)
 - Results
 - Heat Map
 - Overlayswhere <n> is the sample index
- A page break is added between each new Workspace and each new Sample, and between the Heat Map, Results, and Overlays.
- Items are printed using the current page layout settings.
- The Heat Maps legend is scaled to the available print width.
- Result tables are scaled to the page width.
- The Results view is based on the currently selected filters and statistics.
- Overlays are printed in the order that they appear on the Overlay view (page 117). Gallery plots are not printed.

Export Statistics dialog – Experiment Explorer context menu

The *Export Statistics* dialog is displayed when **Export Statistics** is selected from the Sample, Group, or Experiment context menu of the Experiment Explorer (page 145).

Open the dialog

- In the *Experiment Explorer* (page 140), right click on the desired Experiment, Group, or Sample icon to open the associated context menu, then select **Export Statistics**.



Export Statistics dialog

Export statistics of selected samples using

The options in this group allow you to select the Workspaces to use when exporting the selected Samples. You can select multiple Workspaces. Available options are:

- Experiment Workspace
- Group Workspace
- Sample Workspace

By default, the Experiment Workspace is selected. For Workspace statistics, the individual plot statistics are not exported.

To

The options in this group allow you to select the output. Available options are:

- **Single File:** Exports the statistics as a single CSV file. Results are collated into a single table for the Workspace statistics and the Results tab's primary table.
- **Individual Files:** Exports the statistics as multiple CSV files. The software creates a CSV file for each workspace and each selected sample. If the Heat Map and Results views are selected, the software creates additional files.
- To begin printing, click **OK**. When prompted, complete the:
 - *Save* dialog (page 363) for the **Single File** option.
 - *Open* dialog (page 365) for the **Individual Files** option.
- To close the dialog without printing, click **Cancel** or **X**

Derived Gate dialog

The *Derived Gate dialog* allows you to create gates based on Boolean operators (i.e., *Derived gates*).

Opening the dialog

In the Workspace tab (page 45), click **Derived Gate** to open the Derived Gate dialog.



The Derived Gate button is enabled when the Workspace view is displayed and the current Workspace has at least one gate present.

Collapsed view

Gate Logic

Gate Name: LG2

Gate Color: [Black]

Gate Definiton: NOT Lymphs

AND R1

...

OK Cancel

Expanded view

Gate Logic

Gate Name: LG2

Gate Color: [Black]

Gate Definiton:

Expression

Edit using only: AND, OR, XOR, NOT, (), Gate names

NOT Lymphs AND R1

✓

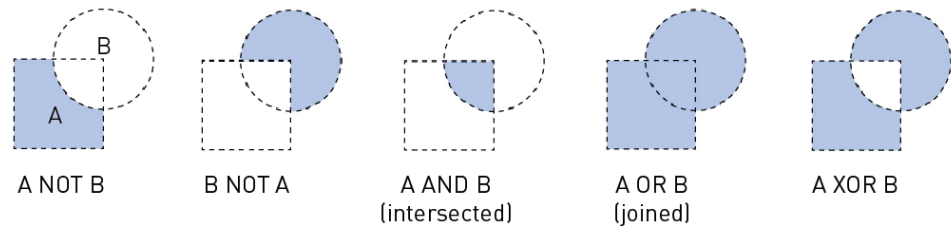
OK Cancel



Note: The contents of the Derived Gate dialog changes depending on the context of the active Workspace.

Boolean operators

The Boolean operators available for creating Derived gates are blank, AND, OR, XOR, AND NOT, and OR NOT.



Derived Gate dialog

Gate name

Allows you to accept the default gate name, or enter a new name.

Gate Name:

- You can enter up to 50 characters.
- Duplicate names and names that are only a whole number are not permitted.
- The default name for a gate is **LG1**. If a gate using the default name already exists, the next available integer is automatically used; for example, **LG2**.

Gate color

Allows you to select a gate color from the dropdown menu.

Gate Color:

Gate definition

Allows you to create gate math expressions using Boolean operators.



Gate Definition:

- Select a gate from each of the two dropdown menus. The menus contain alphabetized lists of all gates on the active Workspace.
- From the first Boolean dropdown menu, select **<blank>** or **NOT**.
- From the second Boolean dropdown menu, select another Boolean operator **<blank>**, **AND**, **OR**, **XOR**, **AND NOT**, or **NOT**.

Expression editor

- Click the **expander button** to expand the *Derived Gate dialog* and display the *Expression Editor*. If you made selections in the Gate Definition dropdown menus (above), they appear in the text box.



- Edit the text using only: **AND, OR, XOR, NOT**, *<blank>*, and gate names. You can enter at least 1500 characters.
- All gate names must be entered in quotation marks (i.e., enter R1 as "R1").
- As you enter text, the software validates the expression and indicates the status:
 -  = valid
 -  = invalid
- To save the changes and close the dialog, click **OK**.
- To close the dialog without creating gates, click **Cancel** or **X**.

Edit Gates dialog

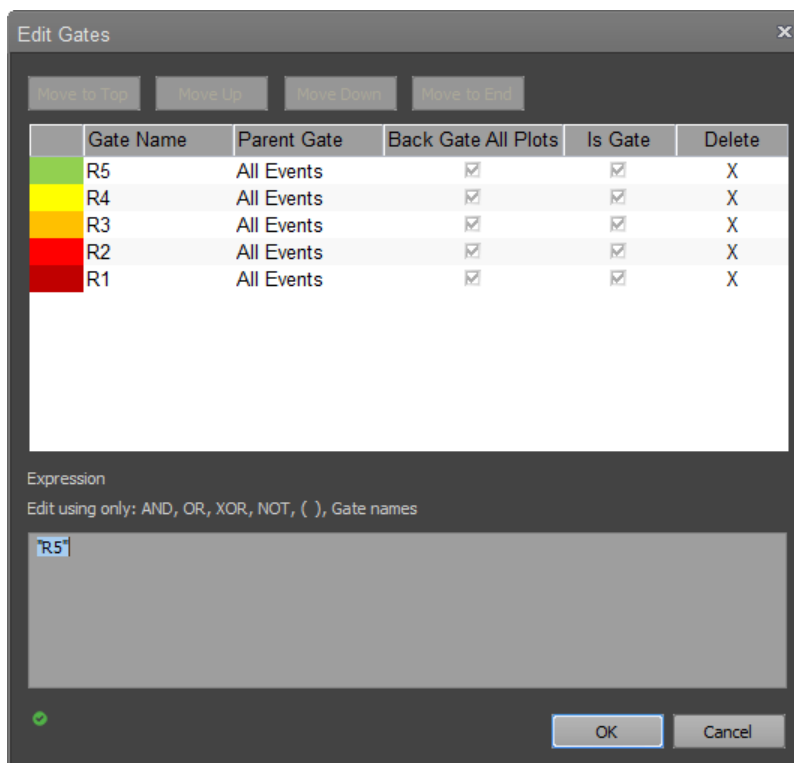
The *Edit Gates* dialog provides a list of all available gates in the active Workspace, and allows you to:

- Edit the gate color, gate math expression, and the order in which the gates are displayed in the dialog
- Delete gates

Opening the dialog

In the Workspace tab (page 42), click **Edit Gates**.

The Edit Gates button is available when the Workspace view is displayed and the current Workspace has at least one gate present.



Note: The contents of the *Edit Gates* dialog changes, depending on the context of the active workspace.

Edit Gates dialog

General properties

- By default, gates are added to the top of the list.
- Changes are applied to all gates after **OK** is clicked.
- To remove the gate name from the list and delete the gate from the plot, click **Delete**.
- You cannot sort the table or rearrange the columns.
- You can resize columns by selecting and dragging the column divider or by double clicking on the divider to auto-fit the column width to the contents.
- Vertical and horizontal scrollbars are displayed as necessary.

Reorder gates

- To reorder the gate list, select a gate, then drag and drop it to a different location within the list.
- Alternatively, select a gate and then click **Move to Top**, **Move Up**, **Move Down**, or **Move to End**, as necessary.



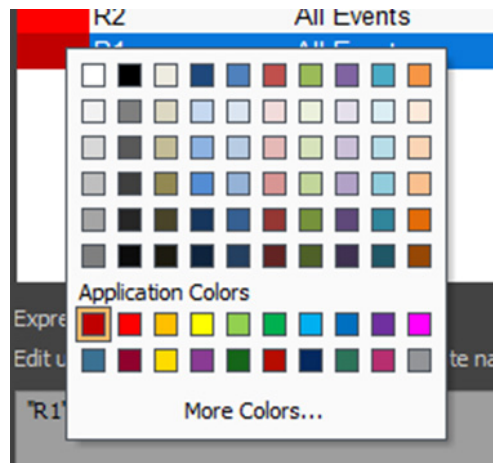
- To select multiple gates at one time, press **Ctrl** or **Shift** when selecting the gates. The order of the selected gates is maintained when they are moved.

Resize columns

- To resize a column, drag the column divider, or double-click the divider to auto-fit the column width to the contents.

Change gate color

- To change the gate color, select the **Color** cell to open the color picker.



Edit gate names

- To edit a gate name, select a name from the dropdown menu in the **Gate Name** cell, or double-click the gate name and enter new text.
- You can enter up to 50 characters.
- Duplicate names and names that are only a whole number are not permitted.
- The default name for a gate is LG1. If a gate using the default name already exists, the next available integer is automatically used; for example, LG2.

Edit gate equation





Note: Only derived gate equations may be edited; otherwise this feature is disabled.

- To edit a gate equation, select a derived gate, and then enter the equation in the *Expression Editor*.



- If you select a non-derived gate, the Expression Editor is disabled.
- Edit the text using only: **AND**, **OR**, **XOR**, **NOT**, *<blank>*, and gate names. You can enter at least 1500 characters.
- As you enter text, the software validates the expression and indicates the status:

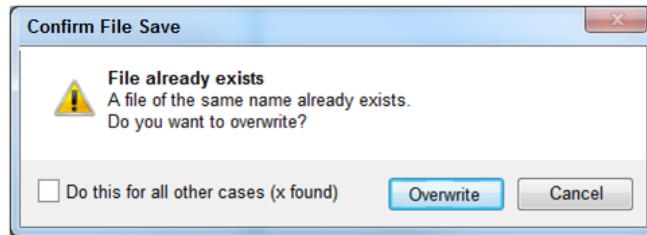
 = valid

 = invalid

- To save the changes and close the dialog, click **OK**.

Confirm File Save dialog

The *Confirm File Save* dialog helps to prevent exported files from being accidentally overwritten. The dialog is automatically displayed when you attempt to export a file to a selected export location that already contains a file of the same name.




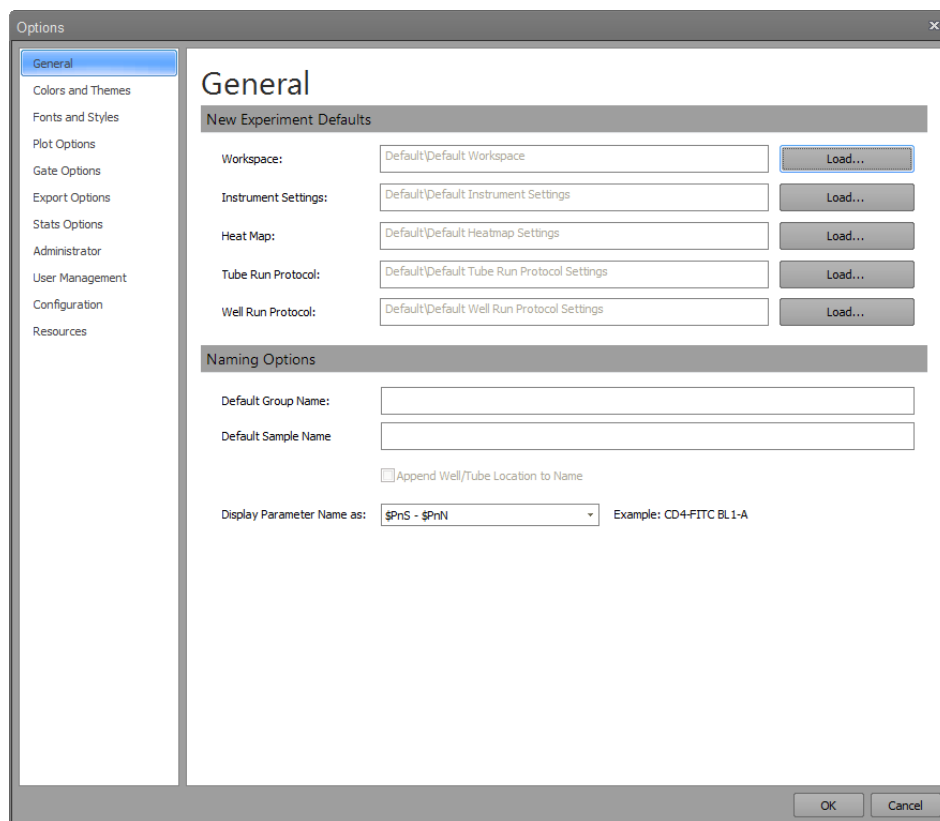
- To replace an existing file with the current file, click **Overwrite**.
- If you are exporting a batch of files, and multiple duplicate file names exist in the export location, the **Do this for all other cases (x found)** check box is enabled, where **x** is the number of existing files.
- To overwrite all duplicate files in the export location, check the **Do this for all other cases (x found)** check box.
- To decide on a case-by-case basis for each file, leave the check box unchecked.
- The checked state applies only to the current export batch and is not persisted.
- To close the dialog without exporting any files, click **Cancel** or **X**.

20. Options dialog

Overview

The *Options dialog* allows you to customize the Attune™ NxT software by configuring your personal settings and changing the default options. Some options are user-specific, while others are application-specific (i.e., global to all users) and customizable only by an authorized user.

- To launch the Options dialog, click the **Options** button on the *Quick Access toolbar* (page 26). This option is enabled only when the instrument is not acquiring. 



- The Options dialog is divided into tabs, which are listed on the left side of the window. Selecting a tab opens the tab content on the right side of the window.

- The Options dialog contains the tabs listed below.
By default, the General tab is displayed upon opening the Options dialog.
 - *General options* (page 328)
 - *Colors and Themes* (page 329)
 - *Fonts and Styles* (page 331)
 - *Plot options* (page 334)
 - *Gate options* (page 337)
 - *Export options* (page 340)
 - *Statistics options* (page 342)
 - *Administrator options* (page 345)
 - *User Management options* (page 347)
 - *Configuration* (page 352)
 - *Resources* (page 354)
- Clicking **OK** saves the changes and closes the Options dialog.
Clicking **Cancel** or the **X** button in the top right corner closes the dialog and reverts to the previous selections without applying the changes.
- When you switch between tabs or click **OK**, all fields are validated.
Any invalid fields within the currently selected tab view are indicated on a field-by-field basis. You cannot switch views or complete the OK action until all errors are corrected.

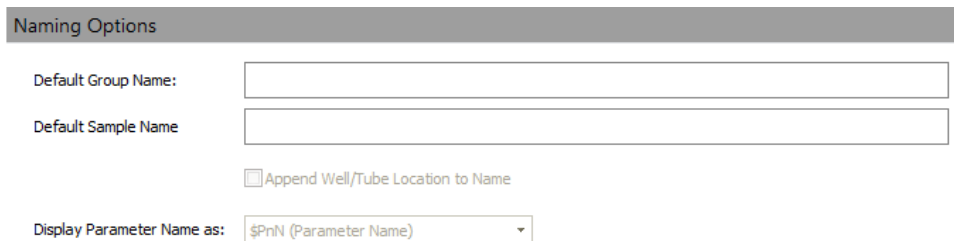
General options

Overview

General options tab is selected by default when the Options dialog is first opened. It allows you to change the default naming options for Groups and Samples. The other options are not available.

Naming options

Naming Options allows you to define the default Group and Sample names.



The screenshot shows a dialog box titled "Naming Options". It contains the following elements:

- A text input field for "Default Group Name".
- A text input field for "Default Sample Name".
- A checkbox labeled "Append Well/Tube Location to Name".
- A dropdown menu for "Display Parameter Name as:" with the selected option "\$PnN (Parameter Name)".

- Changes made to the *Default Group Name* and *Default Sample Name* options are only applied to newly created Groups or Samples.

Default Group Name

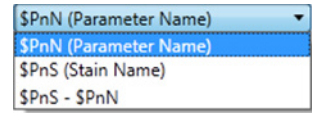
- By default, the Default Group Name text field is blank, which sets the default Group name to *Group* when creating new Groups.
- You can enter a new name in the **Default Group Name** text field, which then becomes the default Group name for all new Groups.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is automatically added to the Group name; for example, **Group(2)**.
- You can enter up to 50 alpha-numeric characters in the text field.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning dialog indicates the error condition, and the invalid characters do not appear in the text field.
The following characters are not allowed: \ / : * < > | ?.

Default Sample Name

- By default, the Default Sample Name text field is blank, which sets the default Sample name to *Sample* when creating new Tube samples and to the Well location (e.g., A1) when creating new Well samples.
- You can enter a new name in the **Default Sample Name** text field, which then becomes the default Sample name for all new Samples.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is automatically added to the Sample name; for example, **Sample(2)**.
- You can enter up to 50 alpha-numeric characters in the text field.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning dialog indicates the error condition, and the invalid characters do not appear in the text field.
The following characters are not allowed: \ / : * < > | ?.

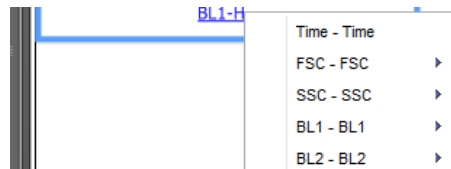
Display Parameter Name As:

- By default, the “Display Parameter Name as” is set to \$PnN (Parameter Name).
- Clicking on the **Display Parameter Name As** control opens a dropdown list that includes \$PnN (Parameter Name), \$PnS (Stain Name), and \$PnS-\$PnN options.



Option	Example label
\$PnN (Parameter Name)	“Example: BL1-A”
\$PnS (Stain Name)	“Example: FITC”
\$PnS-\$PnN	“Example: FITC-BL1-A”

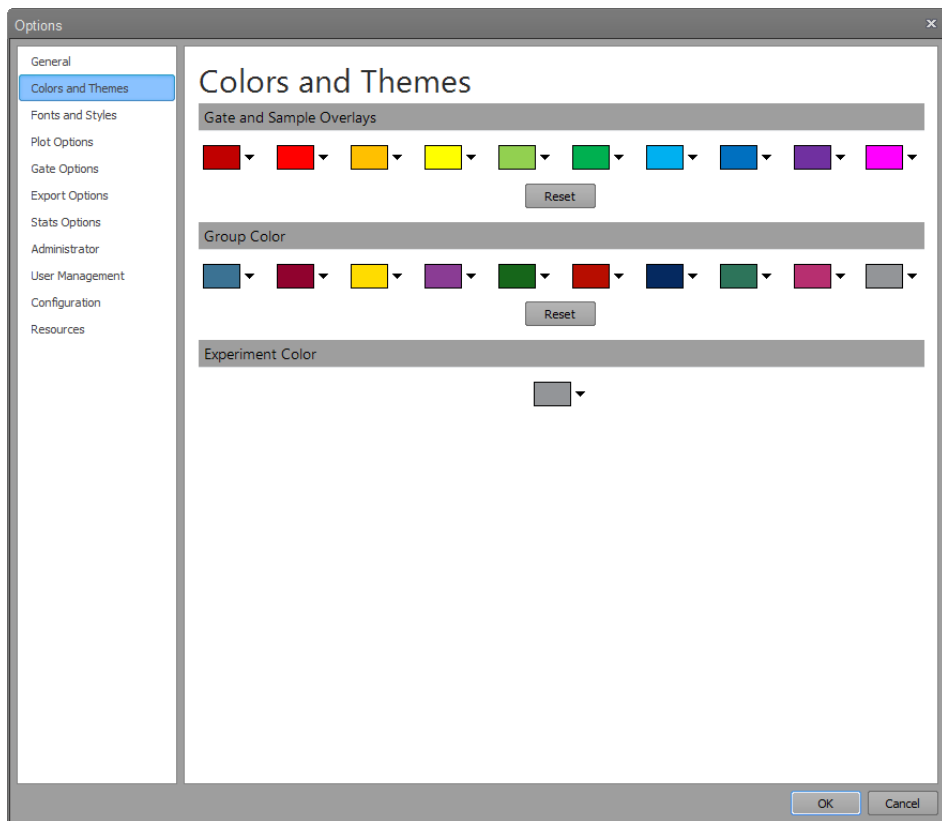
- This setting applies to all Experiments and affects the parameter name displayed in the parameter drop down menu for all plots, and the name displayed when the axis label checkboxes are all unchecked and no custom label has been entered.




Colors and Themes

Overview

Colors and Themes tab allows you to define the default colors for gates, Overlays, and Experiment and group labels.



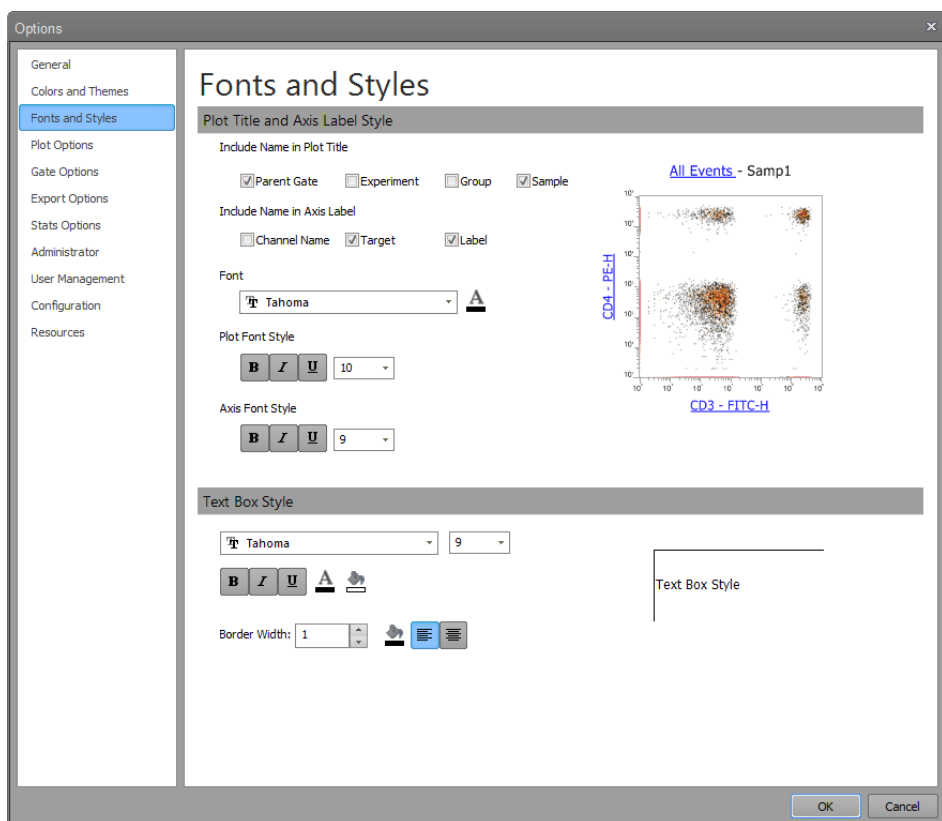
Colors and Themes options

- The Colors and Themes tabs contains three groups of controls titled “Gates and Sample Overlays”, “Group Color”, and “Experiment Color”.
- Gates and Overlays share the defaults, but the defaults for Experiment and Group labels are controlled separately.
- Each set of defaults contains 10 standard colors. You can modify each color by clicking on the **color**, which opens the standard color picker dialog. 
- When a color is selected from the color picker dialog, the color displayed in the color dropdown is updated and the color picker dialog is closed.
- The Experiment color section contains a single color dropdown with the same behavior as described above.
- Changes made to the default colors only apply to newly created Gates, Groups, and Experiments after the change is accepted.
- Clicking the **Reset** button restores the colors to the original default application colors. This option is not available on the Experiment Color section.

Fonts and Styles

Overview

Fonts and Styles tab allows you to define default display options for the text in plot labels and text boxes. The tab is divided into four sections: *Plot title*, *Axis label*, *Tick mark label*, and *Text box*.



- The current settings are displayed in the Previews area for plot and text objects on the right side of the dialog.
- Changes made in this section are applied only to newly created plots except the axis tick mark label. The axis tick label style is applied immediately upon clicking **OK** and affects all past, present, and future instances.

Plot title style

Plot title style section allows you to modify the default settings for the naming format, font, and style of the plot title.

Include Name in Plot Title

Parent Gate Experiment Group Sample

Plot Font Style

B *I* U 10

- Selecting the relevant **Include Name in Title** checkboxes allows you to include the Parent gate name, Experiment name, Group name, and/or Sample name in the default plot title. The software appends your selections to the default plot title, separated by hyphens.
- In the **font size** field, you can select a font size from the dropdown menu, or enter a number from 6 to 72. The default size is 10 pt.
- You can select the **bold**, **italics**, and/or **underline** options to format the font. Selecting one choice deselects the other.

Axis label style

Axis label style section allows you to modify the default settings for the naming format, font, and style of plot axis labels.

Include Name in Axis Label

Channel Name Target Label

Axis Font Style

B *I* U 9

- Selecting the relevant **Include Name in Label** checkboxes allows you to include the Channel name, Target name, and/or Label name in the default axis labels. The software appends your selections to the default axis labels, separated by hyphens. By default, the target name and label name are selected.
- In the **font size** field, you can select a font size from the dropdown menu, or directly enter a number. The default size is 9 pt.
- You can select the **bold**, **italics**, and/or **underline** options to format the font. Selecting one choice deselects the other.

Font

Font style section allows you to set the font and font color for the plot title and axes labels.

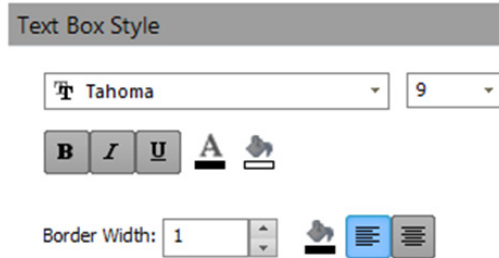
Font

Tahoma 

- You can select a **font type** from the font dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- Using the **font color** picker, you can define the default font color of the plot title. The software uses the standard color picker dialog. The default color is black.

Text box style

Text box style section allows you to modify the default settings for text boxes.

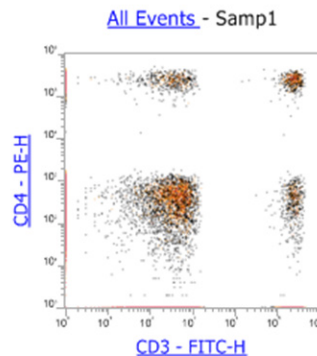


- You can select a **font type** from the font dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- In the **font size** field, you can select a font size from the dropdown menu, or directly enter a number. The default size is 9 pt.
- You can select the **bold**, **italics**, and/or **underline** options to format the font. Selecting one choice deselects the other.
- Using the **font color** picker, you can define the default font color of the plot title. The software uses the standard color picker dialog. The default color is black.
- You can select to **left-align** or **center** the text. By default, text is left-aligned.
- You can select a **border width** for the text box, or enter an integer from 1 to 5.
- You can select a **border color** and **text box fill color** using the appropriate color picker control. By default, border color is black and the text box is not filled.

Previews

Previews section displays an example plot and an example text box reflecting the current selections made in the Fonts and Styles tab.

The previews are updated as the selections are changed.

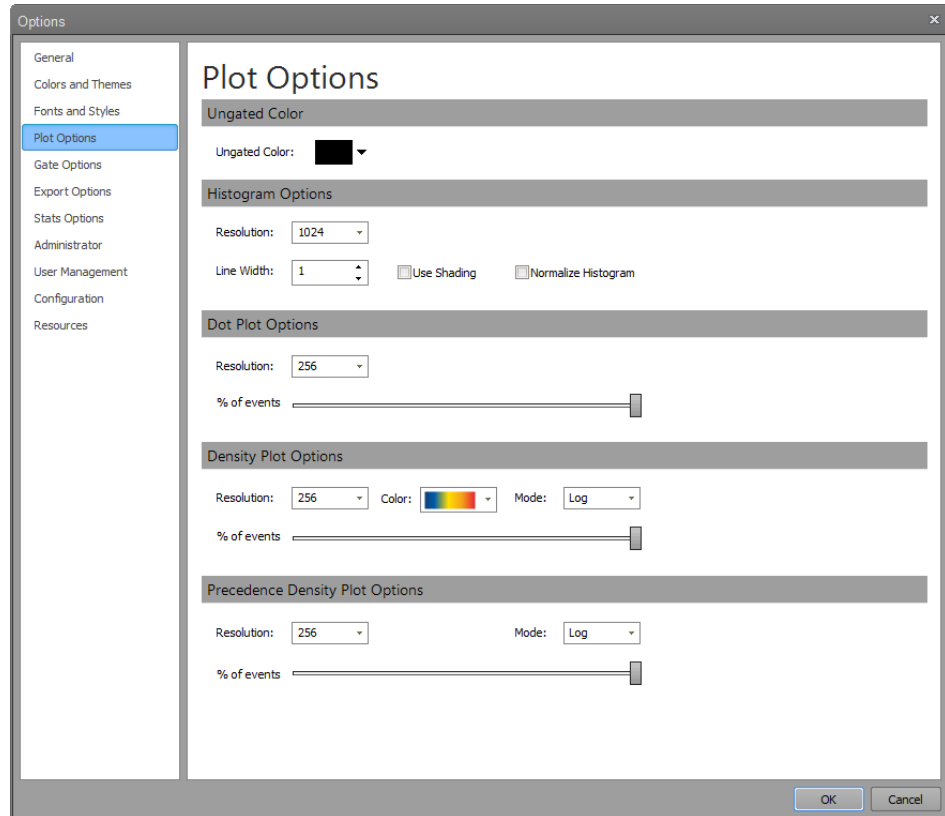


Text Box Style

Plot options

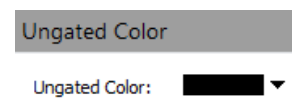
Overview

Plot Options tab is used to define display options for ungated plots (*Ungated color*) and for each type of plot: Histogram plot, Dot plot, Density plot, and Precedence Density plot. Changes made in the Histogram plot, Dot plot, Density plot, and Precedence Density plot sections are only applied to newly created plots.



Ungated color

- By default, Ungated color for all plot types is black.
- You can modify the default color for ungated plots by clicking on the **Ungated color** dropdown button, which opens the standard color picker dialog.
- When a color is selected from the color picker dialog, the color displayed in the Ungated color dropdown is updated and the color picker dialog is closed.
- Changes made to Ungated color are applied immediately upon clicking **OK** and affect all past, present and future instances.



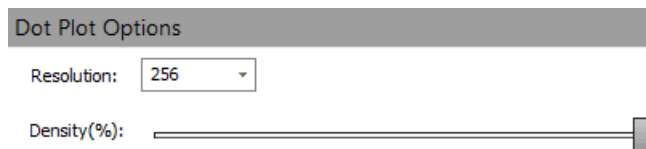
Histogram



The screenshot shows the 'Histogram Options' panel. It includes a 'Resolution' dropdown menu set to '256', a 'Line Width' input field set to '1', and two checkboxes: 'Use Shading' and 'Normalize Histogram', both of which are currently unchecked.

- The default resolution for Histograms is 1024. You can modify the resolution using the **Resolution** dropdown menu, which offers the following choices: 64, 128, 256, 512, and 1024.
- The default line thickness for Histograms is set at 1 pixel. You can modify the line thickness using the **Line Width** control. The minimum line thickness is 1 pixel and the maximum is 5 pixels.
- Selecting the **Use Shading** checkbox shades the area under the curve in the Histogram using the same color as the line color at 64% opacity. By default, Use Shading is deselected.
- Selecting the **Normalize Histogram** checkbox normalizes all new Histograms to the maximum peak. By default, Normalize Histogram is deselected.

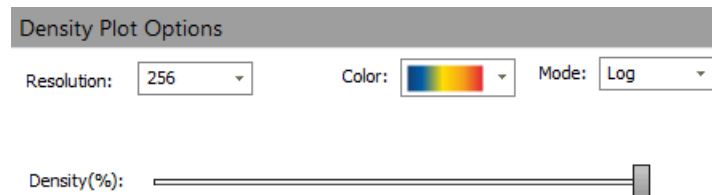
Dot plot



The screenshot shows the 'Dot Plot Options' panel. It includes a 'Resolution' dropdown menu set to '256' and a 'Density(%)' slider bar that is currently positioned at the 100% mark on the right.

- The default resolution for Dot plots is 256×256 . You can modify the resolution using the **Resolution** dropdown menu, which offers the following choices: 64×64 , 128×128 , 256×256 , 512×512 , and 1024×1024 .
- You can modify the percentage of events displayed in a Dot plot from 0% to 100% (left to right) by using **% of Events** slider bar. The sample data is taken from the entire data file. By default, Dot plots display 100% of events.

Density plot



The screenshot shows the 'Density Plot Options' panel. It includes a 'Resolution' dropdown menu set to '256', a 'Color' dropdown menu with a color gradient preview, a 'Mode' dropdown menu set to 'Log', and a 'Density(%)' slider bar that is currently positioned at the 100% mark on the right.

- The default resolution for Density plots is 256×256 .
- You can modify the resolution by using the **Resolution** dropdown menu, which offers the following choices: 64×64 , 128×128 , 256×256 , 512×512 , and 1024×1024 .
- Density plots do not use the Ungated color setting. The default color scheme for a Density plot is set to the first gradient available in the **Color** dropdown menu. You can modify the default color setting by selecting one of the gradient choices within the **Color** dropdown list.
- You can change the binning mode for Density plots by using the **Mode** dropdown menu, which offers a choice between **Log** and **Linear** modes. By default, the binning mode is set to Log.
- You can modify the percentage of events displayed in a Density plot from 0% to 100% (left to right) by using **% of Events** slider bar. The sample data is taken from the entire data file. By default, Density plots display 100% of events.

Precedence Density plot

Precedence Density Plot Options

Resolution: Mode:

Density(%):

- The default resolution for Precedence Density plots is 256×256 .
- You can modify the resolution by using the **Resolution** dropdown menu, which offers the following choices: 64×64 , 128×128 , 256×256 , 512×512 , and 1024×1024 .
- You can change the binning mode for Precedence Density plots by using the **Mode** dropdown menu, which offers a choice between **Log** and **Linear** modes. By default, the binning mode is set to Log.
- You can modify the percentage of events displayed in a Precedence Density plot from 0% to 100% (left to right) by using **% of Events** slider bar. The sample data is taken from the entire data file. By default, Precedence Density plots display 100% of events.



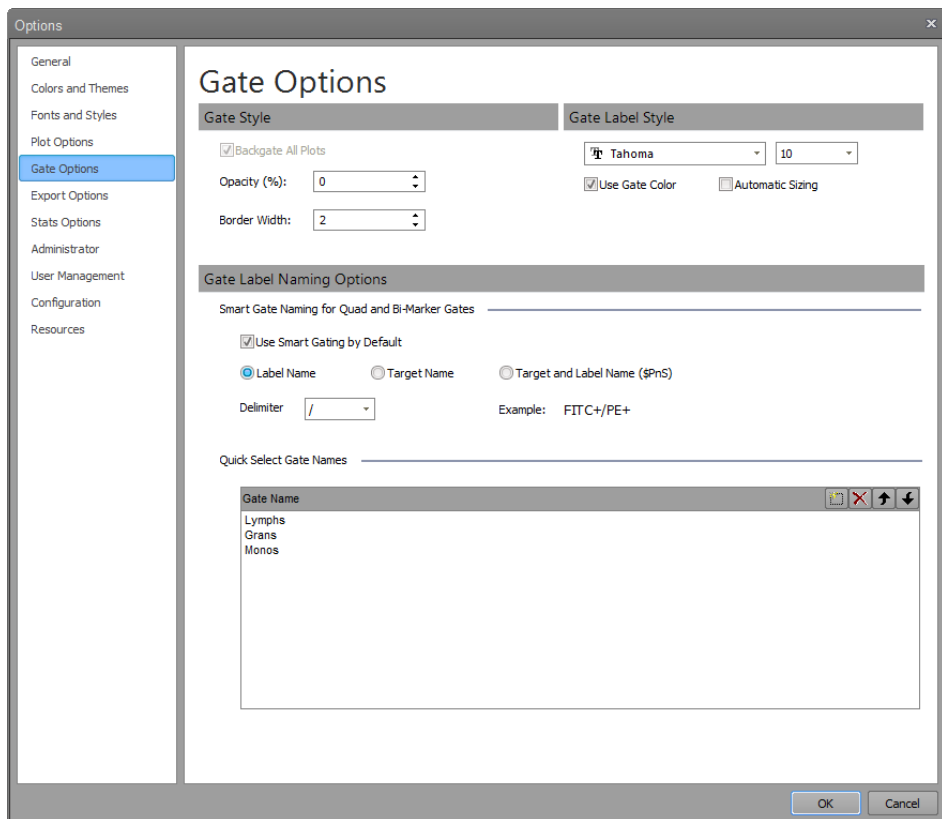
Note: The **Linear mode** bins data by assigning a color index for each density pixel linearly such that each increment is determined by dividing the range ($Z_{\max} - Z_{\min}$) by the number of color steps.

The **Log mode** bins data by assigning a color index for each density pixel logarithmically such that each increment is determined by dividing the logarithmic range ($\log Z_{\max} - \log Z_{\min}$) by the number of color steps. The index is then determined as $\text{int}((\log Z_{\text{val}} - \log Z_{\min}) / \text{increment})$.

Gate options

Overview

Gate Options tab allows you to define the default settings for gate style and gate label style, and for *Smart Gate Naming* and *Quick Select Gate Names* options.



Gate style

Gate style options include settings for gate opacity, gate border width, and an option to automatically add backgates on all plots as described below.

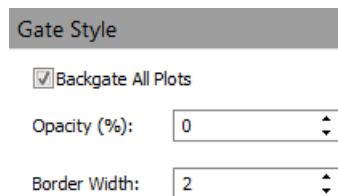
- By default, gate opacity is set at 0% and gate border width is set at 2 pixels.
- Using the **Opacity** control, you can set the gate opacity between 0% and 75%.

Alternatively, you can directly type in the desired value in the text field.

- Using the **Border Width** control, you can set the gate border width between 1 and 5 pixels.

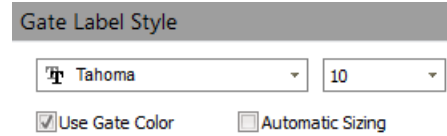
Alternatively, you can directly type in an integer from 1 to 5 in the text field.

- Clicking **OK** after changing the gate opacity and gate border width options defines the new default settings for these options.
- Only newly created gates use the new default settings.



Gate label style

Gate label style options include controls for selecting the gate label font and font size.

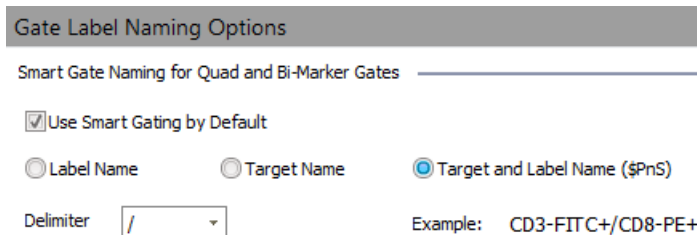


- You can select a font type from the **font** dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- You can select a font size from the **font size** dropdown menu, or enter a number from **6** to **72**.
- Selecting the **Use Gate Color** checkbox sets the gate label color to match the gate color. By default, the checkbox is selected.
When the checkbox deselected, the gate label color is black.
- Selecting the **Automatic Sizing** checkbox enables the software to adjust the text size automatically to fit within the plot. By default, the checkbox is not selected.
- Any changes made within this section are applied immediately upon closing the dialog and affect all past, present and future instances.

Gate naming

Gate naming options section includes settings for *Smart gate naming* (below) and *Quick Select Gate Names* (page 339).

Smart gate naming for Quad gates



- Selecting the **Use Smart Gate Naming** checkbox enables the software to automatically generate names for Quadrant gates. By default, the checkbox is selected.
- You can choose the format for smart gate naming by clicking the appropriate radio control button. Available options for are **Target Name**, **Label Name**, and **Target and Label Name (\$PnS)**.
By default, smart gate naming format is set to **Target and Label name (\$PnS)**.
- For Quadrant gates, the four gate names are generated (one for each quadrant).
- The **Delimiter** dropdown allows you to select a character to separate the names created for each quadrant of a Quadrant gate.
- An example of the concatenation of names and the delimiter is shown based on the selections made.
- Any changes made in this section are only applied to newly created gates.

Quick select gate names

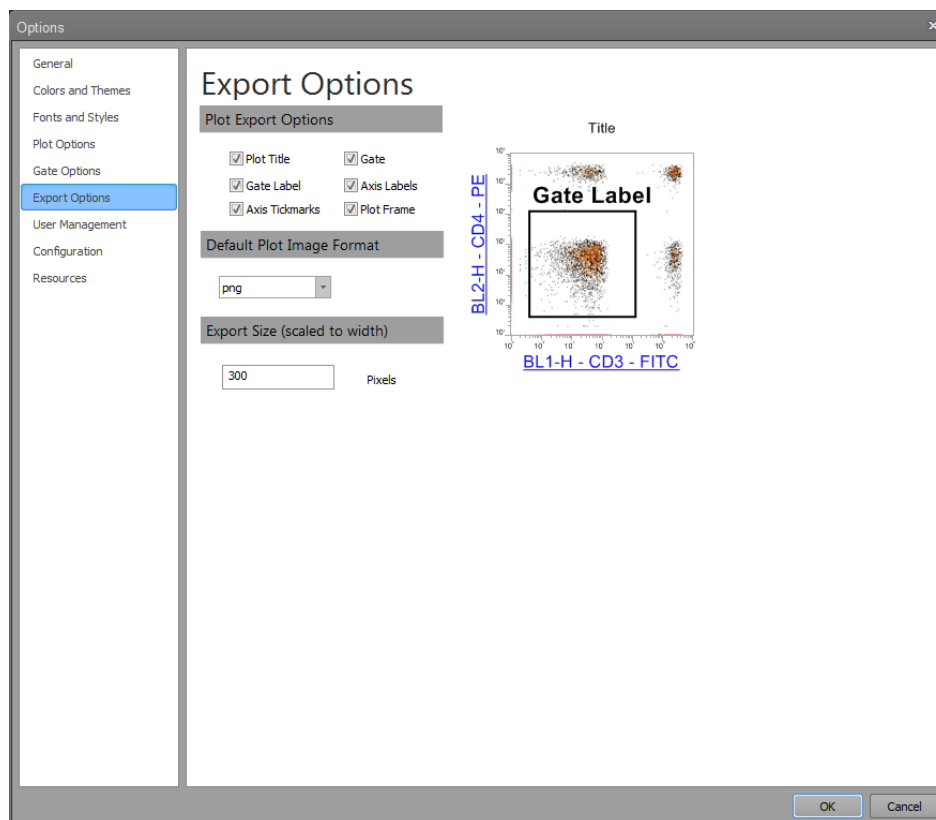


- *Quick Select Gate Names* allows you define the gate names available in the *Name* dropdown menu on the *Customize Gate panel* (page 223)
- By default, the Quick Select Gate Names table contains the **Lymphs**, **Monos**, and **Grans** options.
- You can add additional names to the table by double-clicking on the whitespace of the list view or by clicking the **New** button in the table header.
- You can edit the names in the table by double-clicking on the name you wish to edit.
- Gate names can be up to 50 characters long and can include any character except the following: \ / : * < > | ?.
- Duplicate entries are not allowed.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning dialog indicates the error condition, and the invalid characters do not appear in the text field.
- You can modify the sort order of the names in the table by selecting a row and using the **Up** and **Down** arrows located in the table header.
- You can delete names from the table by selecting a row and using the **Delete** button located in the table header.
- Changes made to entries in the table are available immediately. Gate names are not affected on established Workspaces.

Export options

Overview

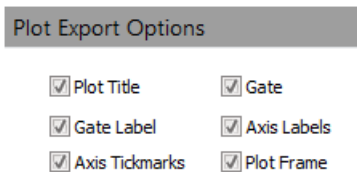
Export options tab allows you to define the default plot content that is copied to the Windows clipboard using the **Copy** command (when a plot is pasted outside the application) or when the plot is saved using the **Save As** command. In addition, it allows you to specify the default plot image format used when saving plots.



- Changes made in the Export Options section take effect immediately and applied to newly exported objects.

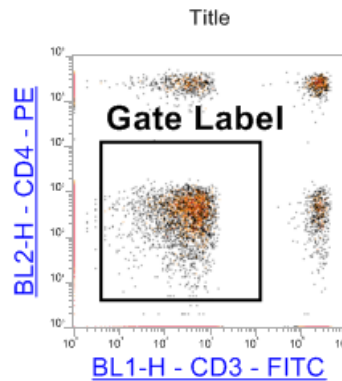
Plot export options

Plot export options allow you to define the default plot contents that are included when a plot is copied or saved. It consists of six checkbox controls, all of which are selected by default.

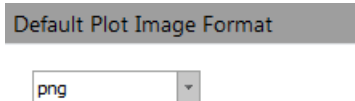


- When the Plot Export Option checkboxes are selected or deselected, a plot preview shows what plot content will be included when copying or saving a plot.

Plot export preview When the Plot export option checkboxes are selected, the *Plot export preview* displays an example plot showing the plot content to be included when copying or saving a plot.

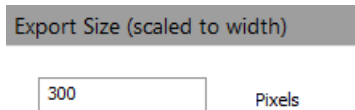


Default plot image format *Default plot image format* option sets the default format used when saving a plot.



- The combo box control for plot image format contains a list of available image formats. Available options are PNG, GIF, JPG, TIF, BMP, and EMF.
- By default, plot image format is set to PNG.

Export size (scaled to width) *Export size (scaled to width)* option allows you to set the X-axis pixel size when exporting or copying objects.

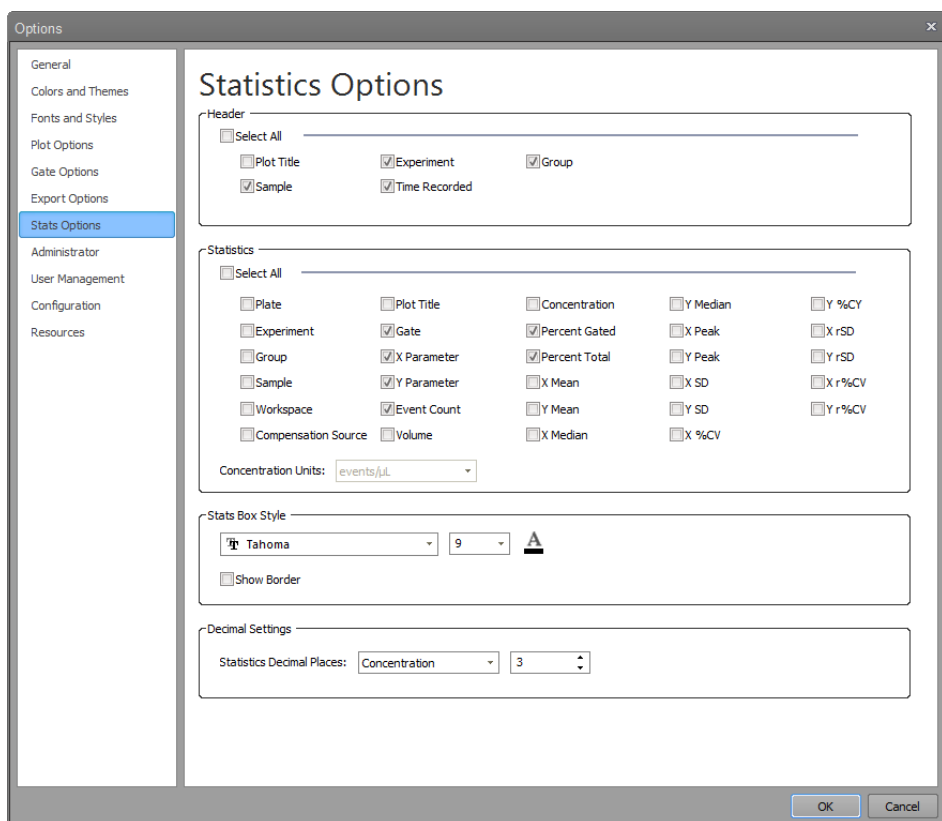


- The x-axis pixel range for the export size is 100–5000. The default pixel size is 400.
- When a pixel size value greater than 5000 is entered, the value defaults to 5000. When a value less than 100 is entered, the value defaults to 100.

Statistics options

Overview

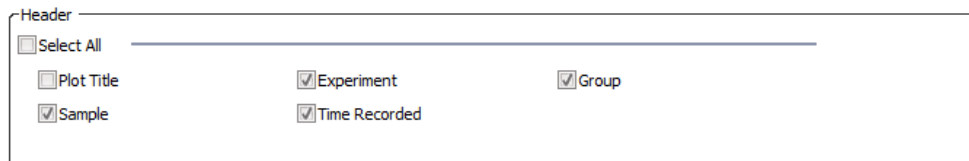
Fonts and Styles tab allows you to define default display preferences for statistics boxes in the Workspace and Results views. The tab is divided into four sections: *Header*, *Statistics*, *Font Style*, and *Decimal Settings*.



- Changes made in this section are applied only to newly created statistics tables in the Workspace and newly created Experiments in the Results view, except Decimal Settings.
- Changes made to Decimal Settings are applied immediately upon clicking **OK** to close the dialog and affects all past, present, and future instances.

Header

Header options allow you to define the default format for the statistics box headers.



- You can select to display **Plot Title**, **Experiment**, **Group**, **Sample**, and **Time Recorded** in the header area of statistics boxes on the Workspace. By default, all options are selected.
- Selecting/deselecting the **Select All** checkbox selects/deselects all other header checkboxes.
If any of the individual header checkboxes is deselected, the Select All checkbox is also deselected.

Statistics

Statistics allow you to determine which default statistics are displayed in statistics boxes on the Workspace.

Statistics

Select All

<input type="checkbox"/> Plate	<input type="checkbox"/> Plot Title	<input type="checkbox"/> Concentration	<input type="checkbox"/> Y Median	<input type="checkbox"/> Y %CY
<input type="checkbox"/> Experiment	<input checked="" type="checkbox"/> Gate	<input checked="" type="checkbox"/> Percent Gated	<input type="checkbox"/> X Peak	<input type="checkbox"/> X rSD
<input type="checkbox"/> Group	<input checked="" type="checkbox"/> X Parameter	<input checked="" type="checkbox"/> Percent Total	<input type="checkbox"/> Y Peak	<input type="checkbox"/> Y rSD
<input type="checkbox"/> Sample	<input checked="" type="checkbox"/> Y Parameter	<input type="checkbox"/> X Mean	<input type="checkbox"/> X SD	<input type="checkbox"/> X r%CV
<input type="checkbox"/> Workspace	<input checked="" type="checkbox"/> Event Count	<input type="checkbox"/> Y Mean	<input type="checkbox"/> Y SD	<input type="checkbox"/> Y r%CV
<input type="checkbox"/> Compensation Source	<input type="checkbox"/> Volume	<input type="checkbox"/> X Median	<input type="checkbox"/> X %CV	

Concentration Units:

- You can select the options you wish to display in the statistics boxes by selecting the relevant **Statistics** checkbox. The following options are available: Plate, Experiment, Group, Sample, Workspace, Compensation Source, Plot Title, Gate, X Parameter, Y Parameter, Event Count, Volume, Concentration, % Total, % Gated, X Mean, Y Mean, X Median, Y Median, X Mode, Y Mode, X SD, Y SD X % CV, Y % CV, X rSD, Y rSD, X r% CV, Y r% CV.
For more information on each option, see “Statistics tab”, page 58.
- By default, Gate, X Parameter, Y Parameter, Event Count, % Gated, and % Total are selected.
- Selecting/deselecting the **Select All** checkbox selects/deselects all statistics checkboxes.
If any of the statistics checkboxes is deselected, the Select All checkbox is also deselected.

Stats Box Style

Stats Box Style section contains the formatting tools for the display of statistics boxes.

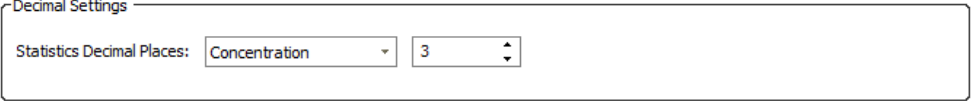
Stats Box Style

Show Border

- You can select a **font type** from the font dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- In the **font size** field, you can select a font size from the dropdown menu, or directly enter a number. The default size is 9 pt.
- Using the **font color** picker, you can define the default font color of the plot title. The software uses the standard color picker dialog. The default color is black.

Decimal Settings

Decimal Settings options define the default decimal places displayed for each statistic. It contains a combo box control with a list of statistics and a control to select the number of decimal places for each statistic.



- Clicking the **Statistics** combo box opens the **Statistics dropdown** list, which contains the following options:
All, Concentration, % Total, % Gated, Mean, Median, SD, %CV, rSD, r%CV, and Volume.
Event Count cannot be adjusted as it does not show any decimal places.
- When a statistic is selected from the list, the **Decimal Places** control updates to the default value assigned to the selected statistic.

The default number of decimals for each statistic is as follows:

Statistic	Decimals
Concentration	3
% Total	3
% Gated	3
Mean	0
Median	0
SD	2
% CV	2
rSD	2
r%CV	2
Volume	0

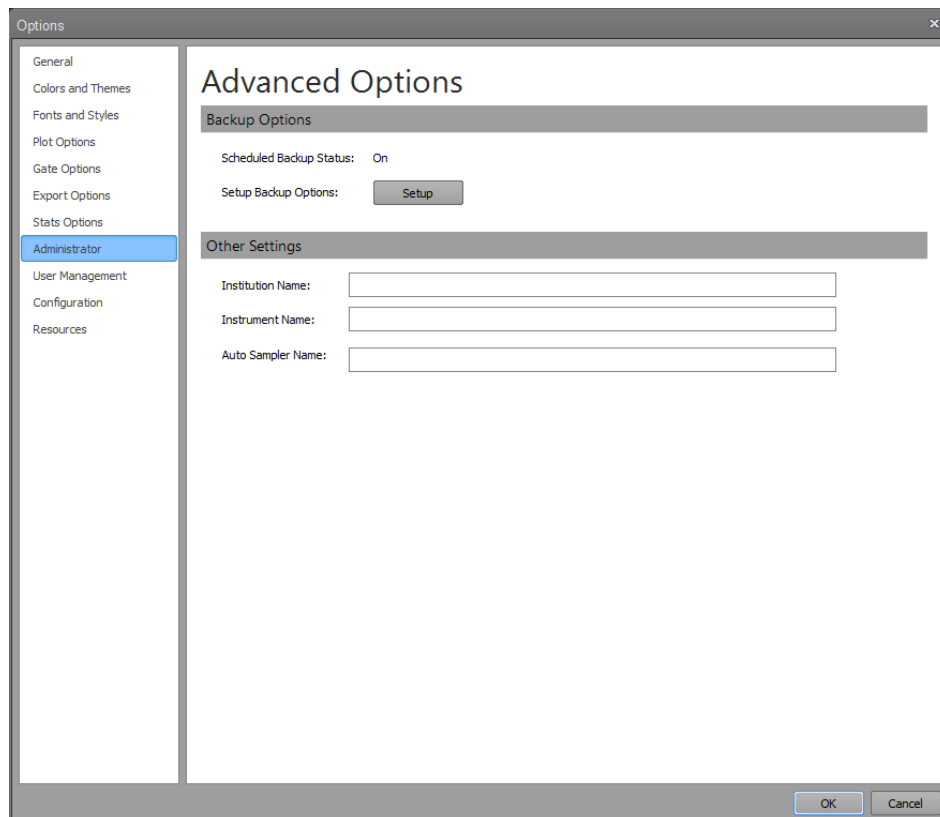
- You can modify the number of decimal places displayed using the **Decimal Places** spin box control. You can directly enter a number from **0** to **7** in the text field, or use the **up** and **down** buttons on the spin box control to modify the decimal places.
Alternatively, you can use the **up** and **down keyboard arrows**, or the **mouse scroll wheel**.
- The text field only allows integer values. Entering a value greater than 7 defaults the value to 7.
- The decimal places assigned to each statistic are used throughout the software, including the Workspace statistics tables, the Results tab values, the Report statistics tables, on-plot statistics for gates, and on the Heat Map legend.
- When **All** is selected from the Statistic dropdown list, the value entered into the spin box control applies to all statistics within the list.
- If the decimal places are different for each statistic, the spin box will be blank when **All** is selected.
- The number of decimal places specified here applies to all past, present, and future Experiments.
- The Count and Mode statistics only display integers without a decimal place.

Administrator options

Overview

Administrator options allow a user with administrator privileges, to access the backup options, and to define institution and instrument names.

- The Administrator tab is only visible to System Administrators and Administrators, and Service accounts.



Backup Options

Backup Options section allows you to see the status of a scheduled backup and to setup backup options.

Status

- When the scheduled backup is turned on, the status displays **On**.
- When the scheduled backup is turned off, the status displays **Off**.

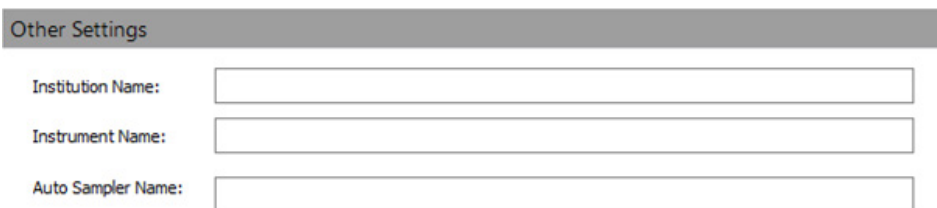
Scheduling Backups

Click the **Setup** button to launch the *Attune™ NxT Database Utility* (page 367).

The database utility allows you to back up the data and database, to restore data and database, to schedule automated backups, or to reinstall the database.

Other Settings

The *Other Settings* section includes settings for the Institution Name, Instrument Name, and Auto Sampler Name.



The screenshot shows a dialog box titled "Other Settings" with a grey header. Below the header are three text input fields, each with a label to its left: "Institution Name:", "Instrument Name:", and "Auto Sampler Name:". Each field is currently empty.

- You can enter up to 50 alpha-numeric characters in the Institution Name, Instrument Name, and Auto Sampler Name textboxes. The following characters are not allowed: \ / : * < > |.
- If invalid characters are entered, a warning dialog indicates the error condition and the invalid characters will not appear in the text field.
- Upon validation, the software automatically removes leading and trailing spaces.

Institution Name

- To define the Institution Name for the system, type in the name into the **Institution Name** textbox. The Institution Name setting is used to populate the \$INST keyword in the FCS file TEXT segment (page 377). The institution name will be saved to each FCS file when entered in the textbox.
- By default, the Institution Name is blank.

Instrument Name

- To define the Instrument Name for the system, type in the name into the **Instrument Name** textbox.
- By default, the Instrument Name is blank.

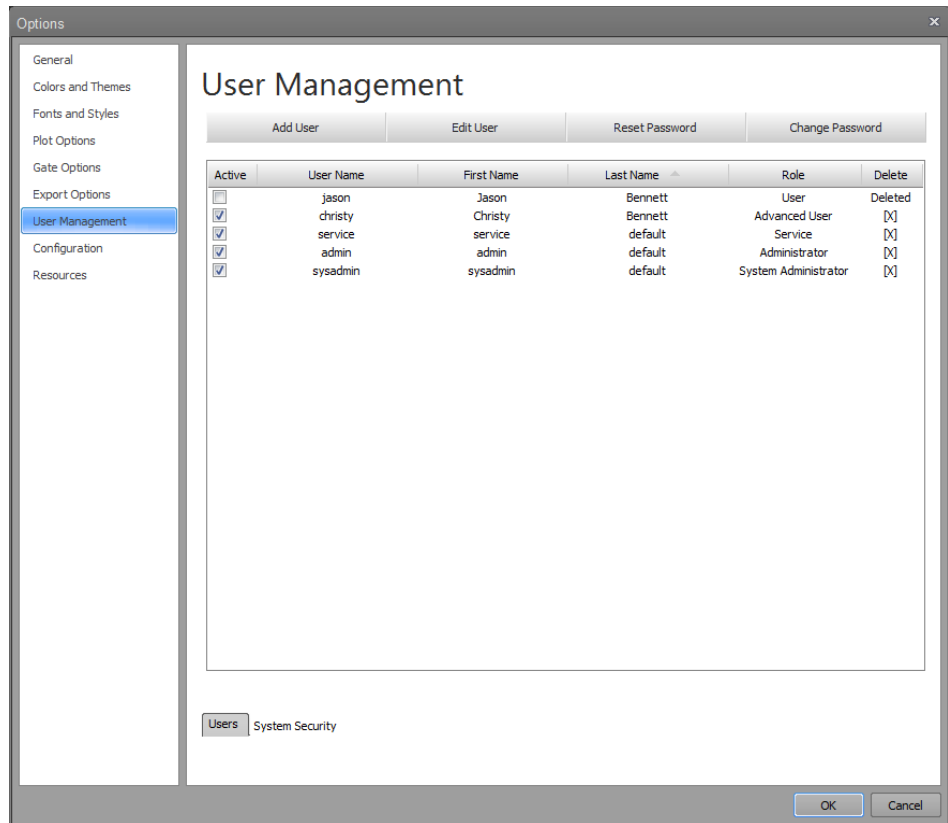
Auto Sampler Name

- To define the Auto Sampler Name for the system, type in the name into the **Auto Sampler Name** textbox.
- The Auto Sampler Name is blank by default.

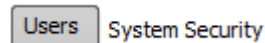
User Management options

Overview

User Management tab allows authorized users to add, edit, and view user accounts and manage user account passwords.



- User Management tab consists of an embedded tab control arranged horizontally along the bottom with the tab contents displayed above. The horizontal tab controls contain the *Users* and *System Security* tabs.



- Users tab (page 348) is selected by default and contains the controls for adding, editing, and viewing user accounts, and resetting or changing user account passwords.
- User privileges for various accounts are described in “Account permissions”, page 19.
- The settings defined in the User Management tab are global and stored in and retrieved from the database.

Users tab options

Users tab options consists of buttons across the top that allow authorized users to add and edit users, and reset or change passwords. The User Accounts table below these buttons displays the database-derived information for all users.

Add User		Edit User		Reset Password		Change Password	
Active	User Name	First Name	Last Name	Role	Delete		
<input type="checkbox"/>	jason	Jason	Bennett	User	Deleted		
<input checked="" type="checkbox"/>	christy	Christy	Bennett	Advanced User	[X]		
<input checked="" type="checkbox"/>	service	service	default	Service	[X]		
<input checked="" type="checkbox"/>	admin	admin	default	Administrator	[X]		
<input checked="" type="checkbox"/>	sysadmin	sysadmin	default	System Administrator	[X]		

- Any changes made in the Users tab take effect immediately and are not reverted if **Cancel** is clicked on the Options dialog.

Add User

- The Add User button allows an authorized user to create a new user account. This button is enabled only for Administrators and System Administrators.
- Clicking **Add User** opens the *Add User dialog* as described on page 357.

Edit User

- The Edit User button allows an authorized user to modify a user account profile or the current user currently to modify their user profile. This button is enabled for all users.
- Administrators and System Administrators can edit any user account as described on page 358.
- Users can edit their own account as described on page 358.
- Clicking **Edit User** opens the *Edit User dialog* as described on page 358.

Reset Password

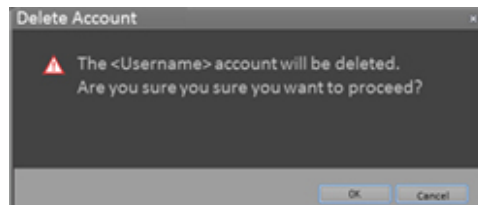
- The Reset Password button allows an authorized user to reset the password for any user account. This button is enabled only for Administrators and System Administrators.
- Clicking **Reset Password** opens the *Reset Password dialog* as described on page 360.

Change Password

- The Change Password button allows the current users to change their own passwords. This button is enabled for all users.
- Clicking **Change Password** opens the *Change Password dialog* as described on page 361.

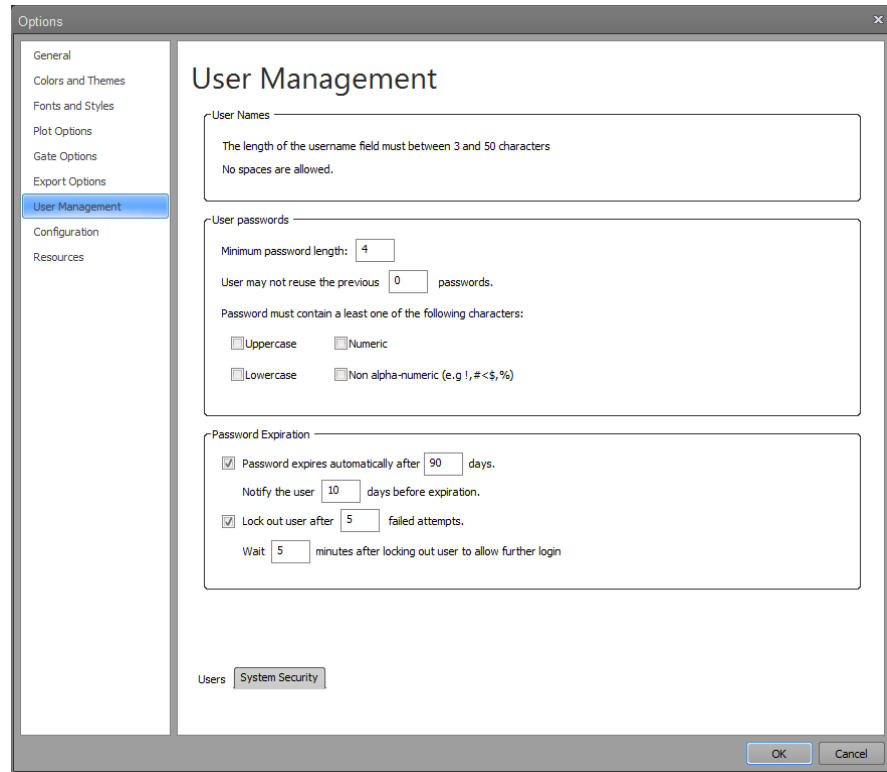
User Accounts Table

- The User Accounts table displays a list of all user accounts. It consists of columns for **Account Status**, **User Name**, **First Name**, **Last Name**, **Roles**, and a column that includes a **Delete User (X)** button to remove specific user accounts.
- All selections in this table are single select. No multi selection is allowed.
- The table can be sorted by clicking on the column headers. When clicked, a sort indicator in the column heading specifies both the active sort column and the sort direction. Any single column can be used for sorting. Clicking on the same header reverses the sort direction.
- The default sort order is ascending by Last Name.
- Any user can see their own account listed in the User Accounts table.
Only users with Administrator and System Administrator accounts can view other user accounts within the table.
- The **Account Status** (i.e., Active) column is only enabled for users with Administrator and System Administrator accounts to allow enabling and disabling of accounts. All other users can view the column, but the checkbox is disabled.
- When the **Account Status** checkbox is selected, the relevant account is enabled. When unchecked, the account is disabled.
- Disabling an account prevents a user from logging in. If a user with a disabled account attempts to log in, the software displays a warning message to inform that the account has been disabled.
- The delete user option is only enabled for System Administrators. For other users, the column is visible, but disabled.
- When the **Delete User (X)** button is clicked, the *Delete Account dialog* prompts the user to confirm the deletion. Only a single user can be selected for deletion at a time.



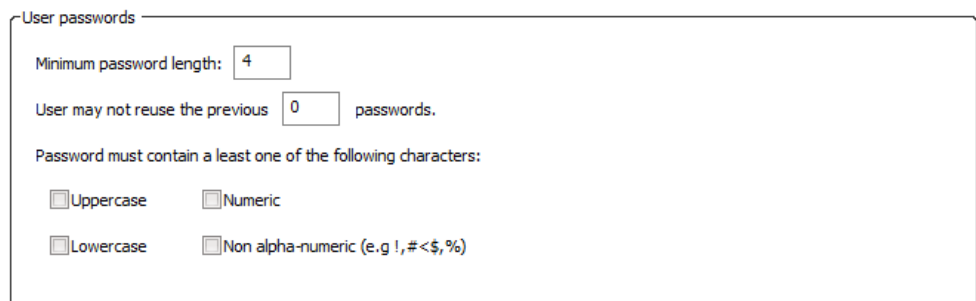
- Clicking **OK** deletes the account, closes the Delete Account dialog, and updates the User Accounts table.
Clicking **Cancel** closes the Delete Account dialog without deleting the account.
- When an account is deleted, it is removed from the list of user accounts visible to Administrator accounts.
- Only System Administrators can still view the full list of all users, where deleted user accounts are listed with the **Active** checkbox set to unchecked and disabled, and the **Roles** set to **Deleted**.
- When an account is deleted, the account is also set to a **Deleted** status, and the Active checkbox is unselected and disabled. The account remains in the list.
- A deleted account relinquishes the username, allowing the username to be reused.

System Security tab *System Security* tab allows the System Administrator to define the system security policy for user passwords and password expiration criteria. This tab is only accessible to System Administrator accounts. This tab provides controls to set requirements around.



User Passwords

User Passwords section provides options to set the minimum password length, to limit the reuse of passwords, and to set password complexity requirements.



- The default **minimum password length** is 4 characters.
- The default for not allowing **reuse of previous passwords** is 0, which allows users to reuse their last password.
- The maximum allowed value is 10, which prohibits the use of the previous 10 passwords. If a value greater than 10 is entered, the value will default to 10.
- The **password complexity requirements** are unchecked by default. When selected, passwords are required to have at least one character from the selected complexity options.

- **Uppercase** selection requires that at least one uppercase alphabetic character is used in a password. Uppercase characters are defined from European languages (A through Z, with diacritic marks, Greek and Cyrillic characters).
- **Lowercase** selection requires that at least one lowercase alphabetic character is used in a password. Lowercase characters are defined from European languages (a through z, sharp-s, with diacritic marks, Greek and Cyrillic characters).
- **Numeric** selection requires that at least one numeric character is used in a password. A numeric character is defined as a base 10 digit (0 through 9).
- **Non-alpha-numeric** selection requires that at least one non-alpha-numeric character is used in a password. The non-alpha-numeric characters are:
~ ! @ # \$ % ^ & * _ - + = ` | \ () { } [] ; : ' ' < > , . ? / .
- Any Unicode character that is categorized as an alphabetic character but is not uppercase or lowercase may also be used. This includes Unicode characters from Asian languages.

Password Expiration

Password Expiration section is used to set a period to automatically force users to change their passwords, to notify users before their password expires, and to lock out users after a defined number of failed login attempts.

Password Expiration

Password expires automatically after days.

Notify the user days before expiration.

Lock out user after failed attempts.

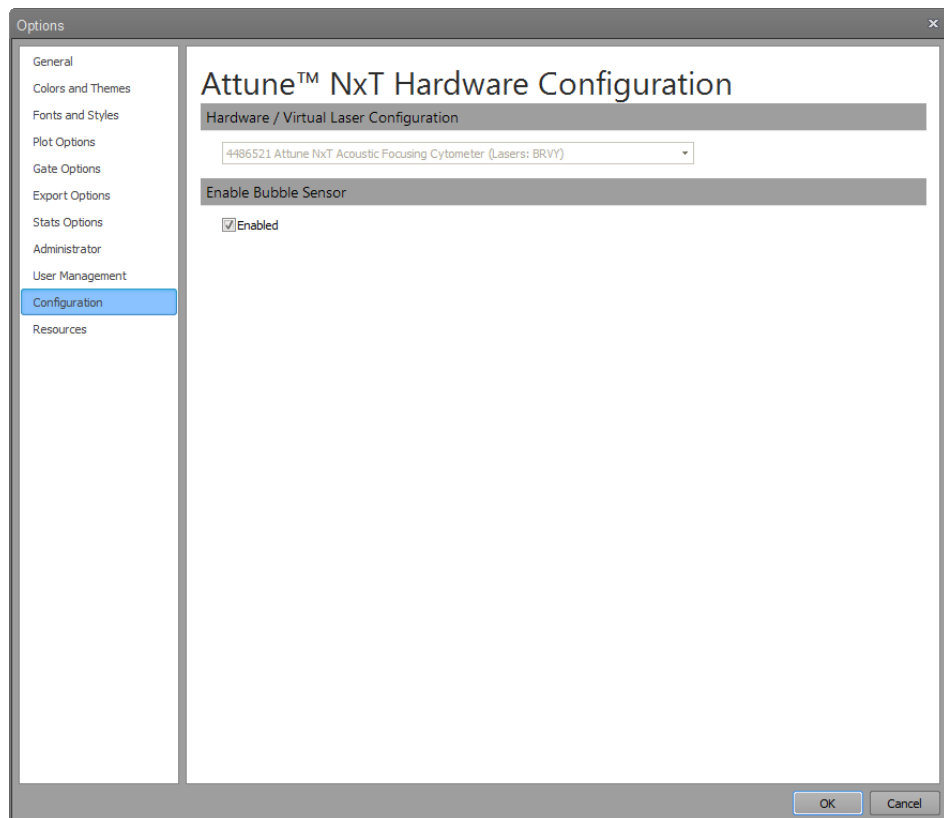
Wait minutes after locking out user to allow further login

- For all number fields, if a number lower than the minimum is entered, the number is adjusted to the minimum upon loss of focus. If a number higher than the maximum is entered, the number is adjusted to the maximum upon loss of focus. Only integers are allowed.
- The default setting for password expiration is 90 days and deselected.
- The minimum value for password expiration is 30 and the maximum is 365 days.
- The default setting for password reset notification is 10 days prior to expiration and deselected.
- The minimum value for notification is 1 and the maximum is 10 days prior to password expiration.
- The default setting for password lockout for failed login attempts is 5 and selected.
- The minimum value for password lockout is 3 and the maximum is 10 failed attempts.
- The default setting for wait between failed login attempts is 5 minutes and selected.
- The minimum value for wait between failed login attempts is 1 minute and the maximum is 15 minutes.

Configuration options

Overview

Configuration options allow you to create virtual configuration settings for working in analysis-only conditions.

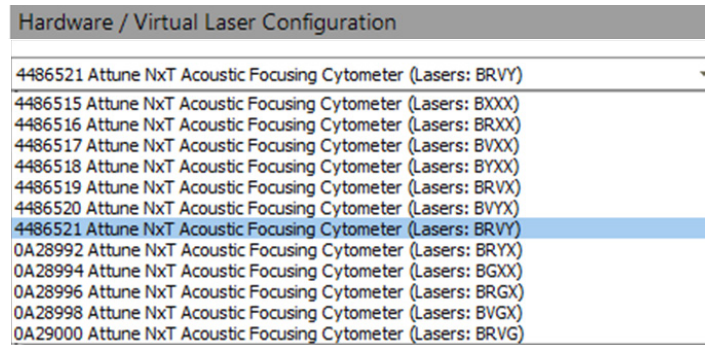


Configuration options tab contains the following controls:

- *Hardware/Virtual Laser Configuration* (page 353)
- *Enable Bubble Sensor* (page 353)

Hardware/ Virtual Laser Configuration

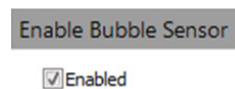
Hardware/Virtual Laser Configuration allows you select a virtual instrument configuration. This aids in the creation of Experiments when not connected to an Attune™ NxT instrument by maintaining correct instrument settings and channel mapping.



- Clicking the **Hardware/Virtual Laser Configuration** combo box opens the instrument configuration dropdown list, which is populated from the Attune™ NxT instrument configurations enumerated in the database.
- When the software connects to an Attune™ NxT instrument, it automatically detects the instrument configuration and sets the correct configuration from the dropdown. The dropdown is then disabled except for the Service and System Administrator accounts, but remains visible to all accounts.
- Service and System Administrator accounts are able to modify/set the configuration when the Attune™ NxT instrument is offline. This resets the state of the dropdown so any user can modify the configuration (i.e., if either the Service or the System Administrator modifies this, then the dropdown list will be re-enabled for all users).

Enable Bubble Sensor

- Selection of the **Enable Bubble Sensor** checkbox enables the bubble sensor for the system.
- By default, the bubble sensor option is enabled. This setting is applied to all users.
- Only an Administrator, System Administrator, or Service account can access the Enable Bubble Sensor option. For all other user types, this option is visible but disabled.



Resources

Overview	<p><i>Resources</i> tab provides information about the system including the software version, the instrument and auto sampler serial numbers, the instrument and auto sampler firmware versions, the system model, and other licensing and contact information.</p>
Attune™ NxT Software	<p>This section displays Attune™ NxT Software and database version information.</p> <p>Attune™ NxT Software</p> <p>The current Attune™ NxT Software version is shown including the software name and software revision number in the format A.B.C.D where A is the major build, B is the minor build, C is the build number and D is the revision.</p> <p>Attune™ Database Version</p> <p>The current Attune™ database version is read from the database and includes the MySQL version in the format MySQL #.#.# Database #.#.#.#####.</p>
Attune™ NxT Acoustic Focusing Cytometer	<p>This section contains the instrument serial number, the instrument model and model number, the instrument firmware, the auto sampler serial number, the auto sampler firmware, and the last calibrated date and time of the auto sampler.</p> <p>Instrument Serial Number</p> <p>The instrument serial number is automatically retrieved from the instrument.</p> <p>Instrument Model</p> <ul style="list-style-type: none">• The content for instrument model is automatically populated upon connection to an instrument.• If no instrument has been connected, the model selected in the <i>Configuration tab</i> (page 352) is shown.• The instrument model field is updated based on the selection made for instrument model number. <p>Instrument Model Number</p> <ul style="list-style-type: none">• The instrument model number combo box options are automatically populated from the database.• Upon connection to an instrument, the appropriate model number is automatically selected from the instrument model number combo box options.• The combo box is disabled by default. It is enabled only for users with Service accounts (page 19). <p>Instrument Firmware</p> <ul style="list-style-type: none">• The content for instrument firmware is automatically populated upon connection to an instrument. If no instrument is connected, this field is left blank.• The instrument firmware field displays the major, minor, and build revision number in the format #.#.#.#####, which is retrieved from the instrument interface API.

Auto Sampler Serial Number

- The auto sampler serial number field is blank by default. A user with a service account can enter an auto sampler serial number.
- The maximum length for a serial number is 50 characters. Any ASCII characters can be entered except / \ ? % * : | " < > .
Spaces are not permitted and cannot be entered.
- If a user attempts to enter an illegal character, a warning dialog indicates the error condition, and the invalid characters are not entered.

Auto Sampler Last Calibrated

- The section is automatically updated to show the date and time of the last auto sampler calibration. This value is read from the instrument.
- The data and time are displayed in the local date and time format.

Trademarks

This section contains information on any trademarks used within the software.

Contact Us

This section contains hyperlinks to Thermo Fisher Scientific websites.

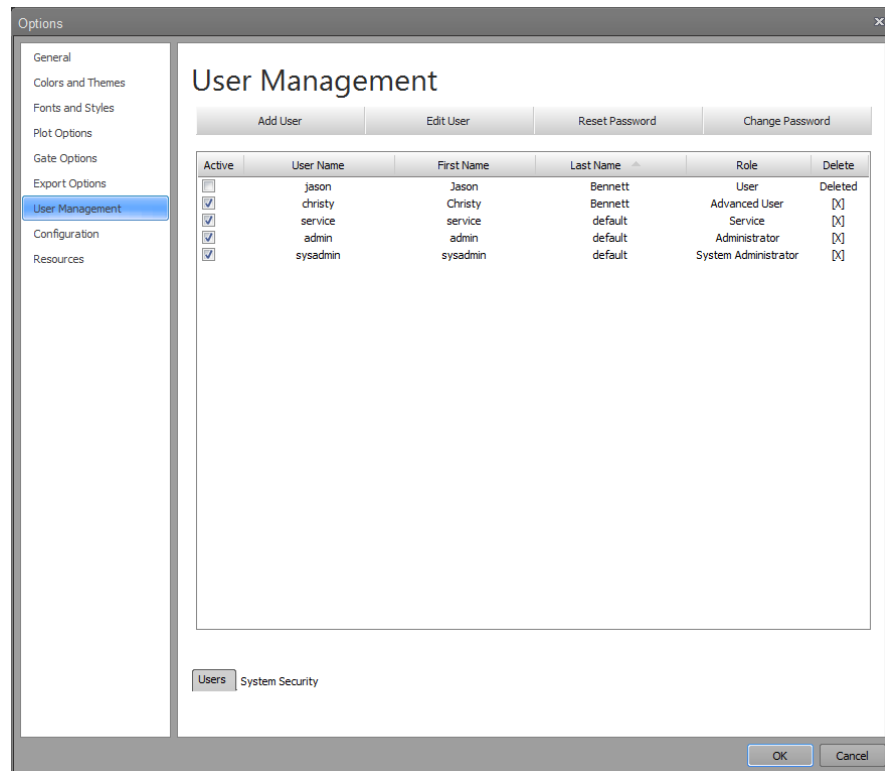
21. User Management

The *User Management tab* of the Options dialog (page 342) allows authorized users to perform the following user management functions:

- Add or edit an account (below)
- Reset a password (page 360)
- Change a password (page 361)

Access the User Management tab

1. In the *Quick Access* toolbar (page 26), click **Options Menu** button to open the Options dialog.
2. In the left pane, select the **User Management** tab.



Add or edit an account

Add an account

Only *System Administrator* or *Administrator* accounts (page 18) can add new accounts to the software. To create a user profile to add a new account, see page 357.

Edit an account

System Administrator or *Administrator* accounts can edit all accounts (except the *Service* account, which cannot be edited). *Users* can only edit their own accounts. To edit an existing user account, see page 358.

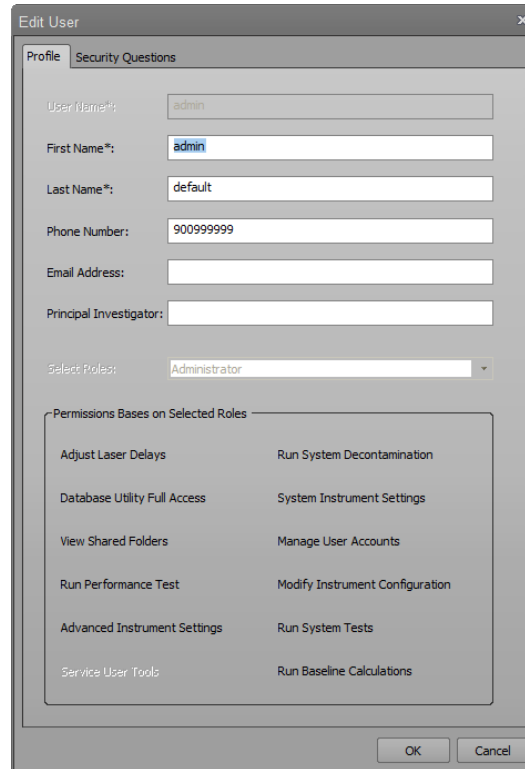
Create a user profile

Only System Administrator or Administrator accounts can create a user profile.

1. In the User Management tab of the Options dialog (page 348), click **Add User** to open the *Add User* dialog.



2. Click the **Profile** tab.

A screenshot of the 'Edit User' dialog box, showing the 'Profile' tab. The dialog has a title bar 'Edit User' and a close button. Below the title bar are two tabs: 'Profile' (selected) and 'Security Questions'. The 'Profile' tab contains several input fields: 'User Name*' (with 'admin' entered), 'First Name*' (with 'admin' entered), 'Last Name*' (with 'default' entered), 'Phone Number*' (with '900999999' entered), 'Email Address', 'Principal Investigator', and 'Select Roles' (a dropdown menu with 'Administrator' selected). Below these fields is a section titled 'Permissions Bases on Selected Roles' containing a list of permissions: 'Adjust Laser Delays', 'Database Utility Full Access', 'View Shared Folders', 'Run Performance Test', 'Advanced Instrument Settings', 'Service User Tools', 'Run System Decontamination', 'System Instrument Settings', 'Manage User Accounts', 'Modify Instrument Configuration', 'Run System Tests', and 'Run Baseline Calculations'. At the bottom right are 'OK' and 'Cancel' buttons.

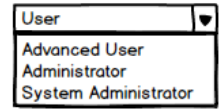
3. Enter the requested profile information:

- **User Name***: Required field. Enter a unique user name between 3 and 50 characters. Duplicate user names are not permitted. Spaces and the following characters are not permitted: \ / : * ? " < > |
- **First Name***: Required field. Enter up to 50 characters; the following characters are not permitted: \ / : * ? " < > |
- **Last Name***: Required field. Enter up to 50 characters; the following characters are not permitted: \ / : * ? " < > | %
- **Phone Number**: Optional field. Enter up to 50 characters. The following characters are permitted: +, and the numerals 0 through 9
The + character can only be in the first position; for example: **+0123456789**
- **Email Address**: Optional field. Enter up to 100 characters; the following characters are not permitted: \ / : * ? " < > | %
The email address must contain the @ character and a domain name; for example, **email@address.com**.
- **Principal Investigator**: Optional field. Enter up to 100 characters; the following characters are not permitted: \ / : * ? " < > | %



Note: For all fields, the software automatically removes trailing, leading, or consecutive spaces.

4. Select an account type from the **Select Roles** dropdown menu: **User**, **Advanced User**, **Administrator**.



- You cannot add a new Service account to the system.
- Permissions assigned to the selected account are displayed in black text; permissions not assigned to the account are grey. None of the user account permissions can be modified.
- For a list of account permissions, see “Account Types”, on page 18.

5. Click **OK** to create the user account and close the dialog.

Alternatively, click **Cancel** or **X** to close the dialog without creating an account.

Edit a user profile

System Administrator or Administrator accounts can edit all accounts (except the Service account, which cannot be edited). Users can only edit their own accounts.

1. In the User Management tab of the Options dialog (page 348), click **Edit User** to open the *Edit User* dialog.



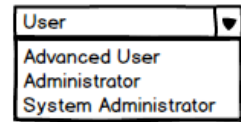
2. Click the **Profile** tab.

3. Edit the desired profile information fields as described in “Create a user profile” on page 357.



Note: For all fields, the software automatically removes trailing, leading, or consecutive spaces.

4. Select an account type from the **Select Roles** dropdown menu: **User**, **Advanced User**, or **Administrator**.



- Only System Administrator or Administrator accounts can edit the account type.

The **Select Roles** dropdown menu is disabled for all other users.

- Permissions assigned to the selected account are displayed in black text; permissions not assigned to the account are grey. None of the user account permissions can be modified.
 - For a list of user account permissions, see "Account Types", page 18.
5. Click **OK** to save the changes and close the dialog.
Alternatively, click **Cancel** or **X** to close the dialog without saving the changes.

Set password reminders

You can set password reminders only for your own account.

1. If it is not open already, click **Add User** or **Edit User** in the User Management tab (page 348) to open the User dialog.

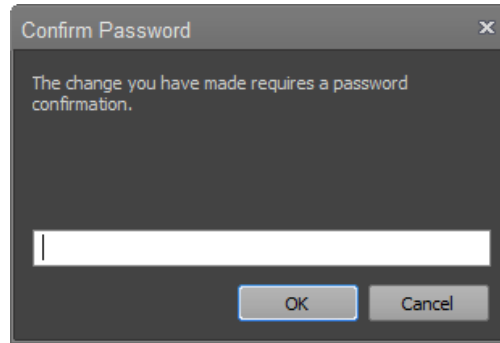


2. Click the **Password Reminder** tab.

A screenshot of the "Edit User" dialog box, specifically the "Security Questions" tab. The dialog has two tabs: "Profile" and "Security Questions". Under "Reset Password Questions:", there are three sets of input fields. Each set consists of a "Question" field and an "Answer" field. The first set has "Question 1: My first question" and "Answer: My first answer". The second set has "Question 2: My second question" and "Answer: My second answer". The third set has "Question 3: My third question" and "Answer: My third answer". At the bottom right, there are "OK" and "Cancel" buttons.

3. Enter up to three password reminder questions and answers, then click **OK**.
 - For each question and answer, you can enter up to 100 characters; any character is permitted.
 - To prevent unauthorized access to a user's account, the answers are displayed as bullet points.
 - A user can reset a forgotten password by correctly answering his or her password reminder questions.

- At the prompt, enter your password to confirm the password reminder changes, then click **OK**.

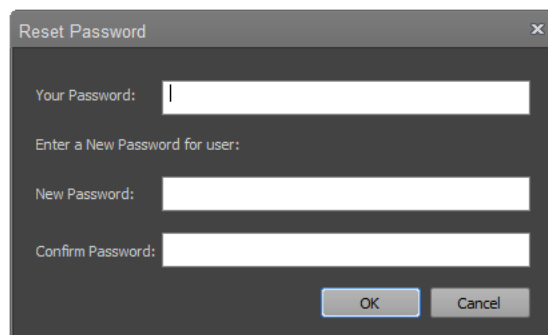


- In the User dialog, click **OK** to save the changes and close the dialog. To close the dialog without saving the changes, click **Cancel** or **X**.

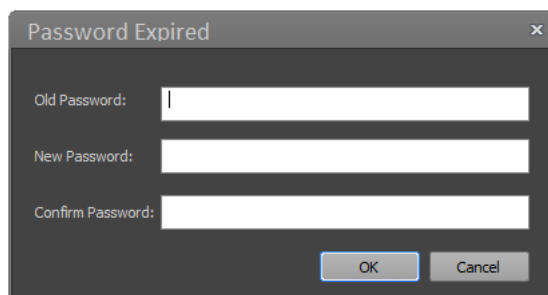
Reset a password

Only System Administrator or Administrator accounts can reset a password. Users cannot reset their own passwords.

- In the User Management tab (page 348), click **Reset Password** to open the *Reset Password* dialog.

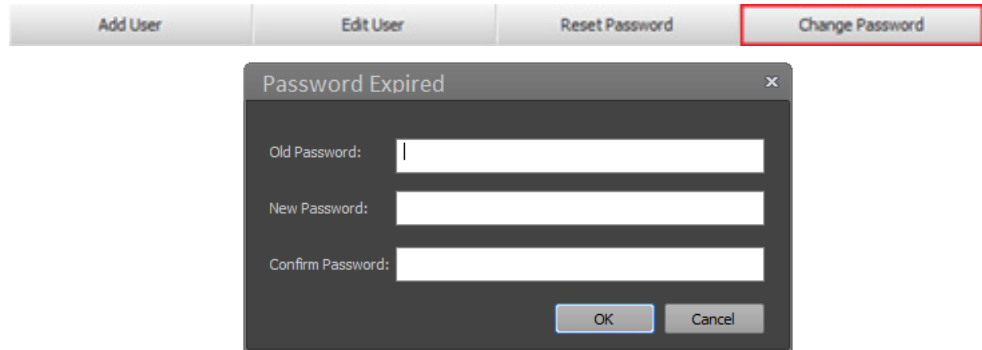


- Enter your System Administrator or Administrator password.
- Enter a new password for "Username", which is the account whose password is to be reset.
- Confirm the new password.
- Click **OK** to save the changes and close the dialog. To close the dialog without saving the changes, click **Cancel** or **X**.
- Provide the user with his or her new password. At next login, the user will be prompted to change the password.



Change a password Any user can change their own password.

1. In the User Management tab (page 348), click **Change Password** to open the *Change Password* dialog.



2. Enter your existing password.
3. Enter a new password, then confirm the new password.
4. Click **OK** to save the changes and close the dialog.
To close the dialog without saving the changes, click **Cancel** or **X**.

22. File dialogs

Overview

This chapter describes the dialogs used for opening and saving files and folders, loading configuration files (Workspace, Instrument Settings, Heat Map, Run Protocols, Compensation Settings) stored within the database, and dialogs that are displayed when importing or exporting FCS files or invalid configuration files.

All dialogs are modal, unless otherwise specified.



Note: Modal dialogs appear when you are performing tasks in the main software windows. You must interact with the modal dialog before you can return to the main window.

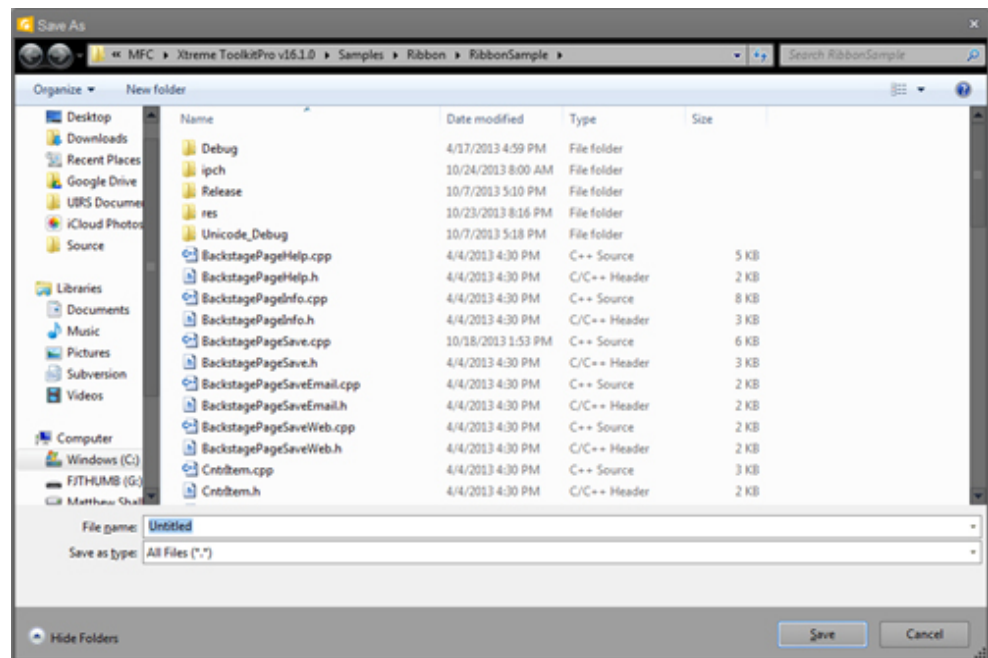
File dialogs

- File Save (Export) dialog (page 363)
- File Open (Import) dialog (page 365)
- Folder Browser dialog (page 366)

File Save (Export) dialog

Overview

File Save (Export) dialog uses the standard Microsoft™ Windows browser, which allows you to save the selected files in the desired location.



Dialog behavior

- The File Save (Export) dialog opens to the last visited directory where files were saved.
- The default filter for FCS files is set to the FCS 3.1 version.
- When saving, the file extension is automatically added to each file type and the *Confirm File Save* dialog (page 325) is displayed, if required.
- If a name with invalid characters is entered or a name that results in a path length over 260 characters, a warning dialog indicating the error condition is displayed.
- Clicking **OK** on the warning dialog returns to the File Save (Export) dialog and the file is not saved.

File filters and default names

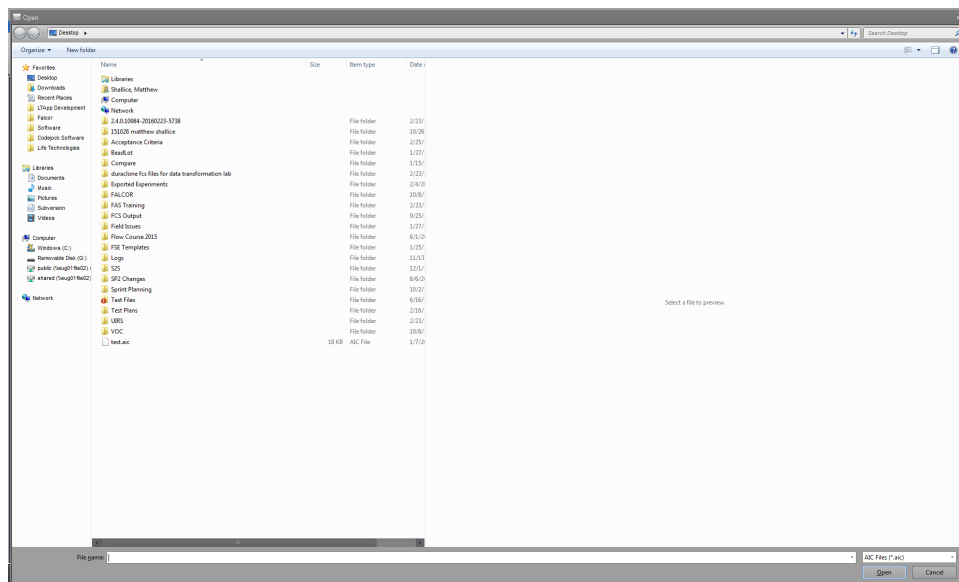
The file filters and default names depend on the source of the File Save (Export) dialog and are described in the table below.

Action/Source	Default Extension	Default File Name	Filter
Export FCS File	FCS	Sample	FCS 3.1 (*.fcs)
Export Plate Experiment	APX	Plate	Plate Experiment (*.apx)
Export Tube Experiment	ATX	Experiment	Tube Experiment (*.atx)
Export Statistics (Single File Output)	CSV	Sample Level: Plate_Experiment_Group_Sample Multiple Files: "Blank"	CSV (*.csv)
Save System Log	ALF	Long Date in system locale format	Log File (*.afl)
Save as (to image)	PNG	Sample	PNG (*.png) GIF (*.gif) JPG (*.jpg) TIF (*.tif) BMP (*.bmp)
Export Workspace	AWS	Experiment	AWS (*.aws)
Export Instrument Settings	AIS	Sample	AIS (*.ais)
Export Compensation	ACS	Experiment	ACS (*.acs)

File Open (Import) dialog

Overview

File Open (Import) dialog uses the standard Microsoft™ Windows File Open browser and is displayed when **Import** is selected from within the software.



Dialog behavior

- The File Open (Import) dialog opens to the last visited directory where files were open.
- Multiselect is only enabled for FCS files when one of the following is selected:
 - **Import FCS Files** is selected from the Experiment context menu on the Experiment Explorer (page 151)
 - **Files** source is selected on the Compensation Setup dialog (page 284)
- Multiselect is also available for Plate and Tube Experiments; it is not enabled for the other file types.
- If the Instrument settings of the imported FCS file do not match the current Instrument configuration, no option to update the Experiment voltages or experiment compensation is provided.
- When multiple FCS files are selected, no option to update the Experiment voltages or Experiment compensation is provided.

File filters

The file filters depend on the source of the File Open (Import) dialog command and are described in the table below.

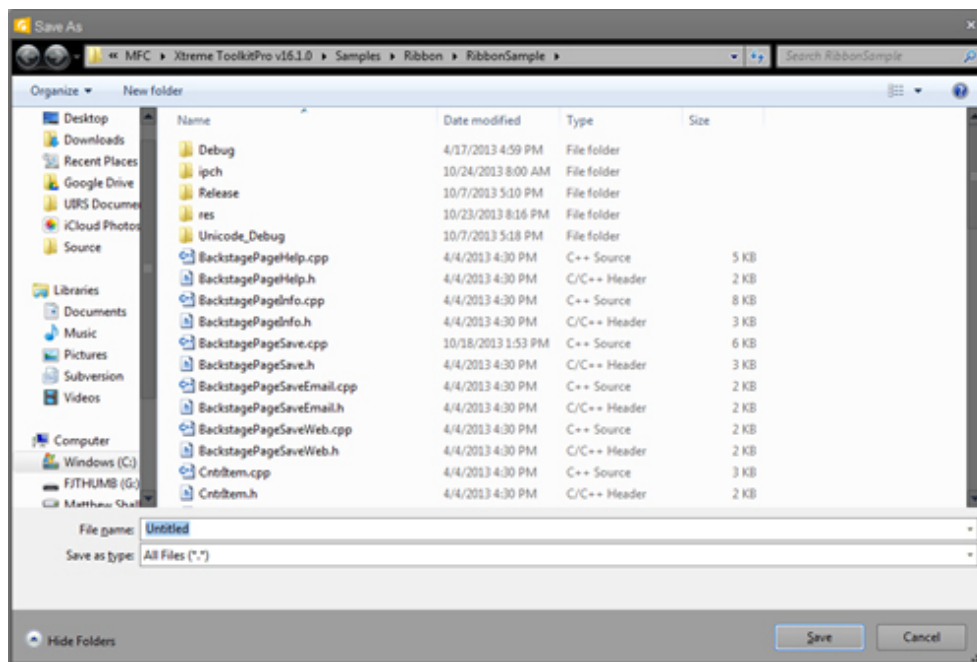
Action	Filter
Import FCS File	All (*.fcs,*.lmd)
Import Plate Experiment	Plate Experiment (*.apx)
Import Tube Experiment	Tube Experiment (*.atx)
Import Workspace	AWS (*.aws)
Import Compensation	ACS (*.acs)
Import Instrument Settings	AIS (*.ais)

Folder Browser dialog

Overview

Folder Browser dialog is displayed when multiple files can be opened by selecting a folder or multi-selecting several files within a folder. Additionally, this dialog is used when a **Save** command requires the selection of a folder.

The style of the Folder Browser is the same as those used for the File Save (Export) and File Open (Import) browsers.



Dialog behavior

- The Folder Browser dialog opens to the last visited directory where files were open.
- When a folder is selected for importing FCS files, all FCS files contained within the folder are selected.
- When exporting, the file extension is automatically added to each file type and the *Confirm File Save* dialog (page 325) is displayed for each save action.
- When multiple FCS files are selected for import, no option to update the experiment voltages or experiment compensation is provided.

File filters and default names

When exporting, the file filters and default names are as described in the table below.

Action/Source	Default Extension	Default File Name	Filter
Import FCS Files	FCS		All (*.fcs,*.lmd)
Export FCS Files	FCS	Plate_Experiment_Group_Sample	FCS 3.1 (*.fcs)
Export Statistics (Multiple File Output)	CSV	Sample-level: Plate_Experiment_Group_Sample	CSV (*.csv)

23. Attune™ NxT Database Utility

Overview

The Attune™ NxT Database Utility is used for the following procedures:

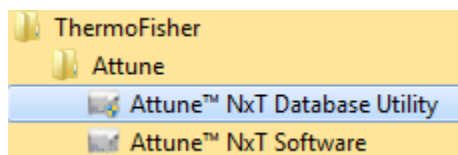
- **Backup** existing data and the database.
- **Restore** backed up data and database.
- **Schedule** an automate backup of the data and database.
- **Re-install** the database.

Startup

Main application startup

The Attune™ NxT Database Utility can be launched via the Windows “Start” menu or directly from the Attune™ NxT Software.

1. Select **Start ▶ All Programs ▶ ThermoFisher ▶ Attune ▶ Attune™ NxT Database Utility**.



Alternatively, launch the Attune™ NxT Database Utility from within the Attune™ NxT software as described on page 345.

2. The application opens to the Login screen (page 368).

Startup Errors

PostgreSQL Error

If the database application is missing (PostgreSQL), the utility will not start and will display an error dialog with the following message:

"PostgreSQL 9.3.5 was not found. Please install the PostgreSQL using the Attune™ NxT installer."

Database Missing

If the Attune™ database is not present when starting the Attune™ NxT Database Utility, the application launches in Recovery mode.

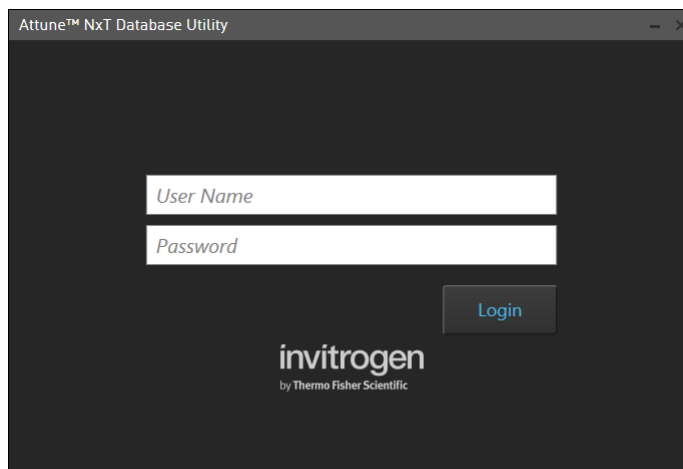
In Recovery mode, the application launches to the Restore page, which allows you to run the Restore or Reinstall database options. In this case, no login is required.

Login screen

Overview

The *Attune™ NxT Database Utility Login* screen is the first screen that is displayed after the launch of the utility. Each user is required to log in before being allowed to use the utility.

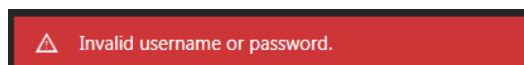
Depending on the type of user (i.e., Administrator, System Administrator, User, etc.) certain features will not be available in the utility.



User sign in

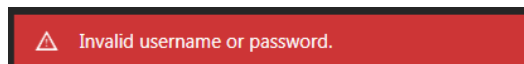
1. To sign into the Attune™ NxT Database Utility, type a valid username and password in the corresponding text box field and click **Login**.
Alternatively, press the **Enter** key on the computer keyboard after typing the username and password.
2. If the correct username and password are entered, the user login is completed and the *Attune™ NxT Database Utility* opens.

If an invalid username or password is entered, the warning banner displays “Invalid username or password” (see “Login Warnings”).

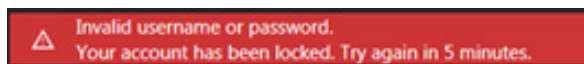


Login warnings

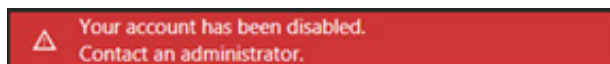
- If an invalid username or password is entered, the warning banner displays “Invalid username or password”. A valid username and password must be entered to proceed.



- If the security settings (page 350) have been set to lockout a user after a certain number of failed attempts the warning banner displays “Invalid username or password. Your account has been locked. Try again in X minutes.”



- If an attempt is made to login to an account that has been disabled, the warning banner displays “Your account has been disabled. Contact an administrator.”



Main Application

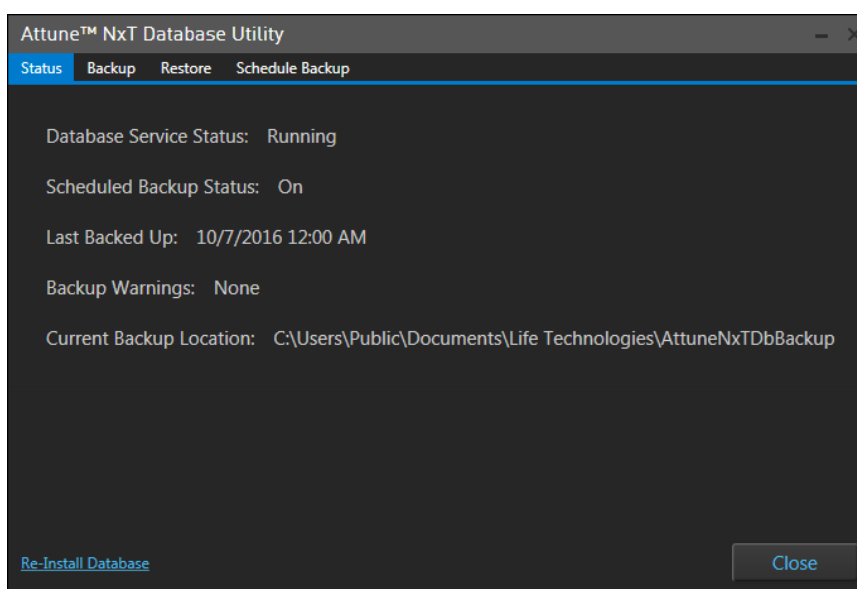
Overview

The main application of the Attune™ NxT Database Utility consists of a dialog box that contains the following controls:

- *Status tab* (page 370)
- *Backup tab* (page 371)
- *Restore tab* (page 372)
- *Schedule Backup tab* (page 373)
- *Re-Install Database hyperlink* (page 374).

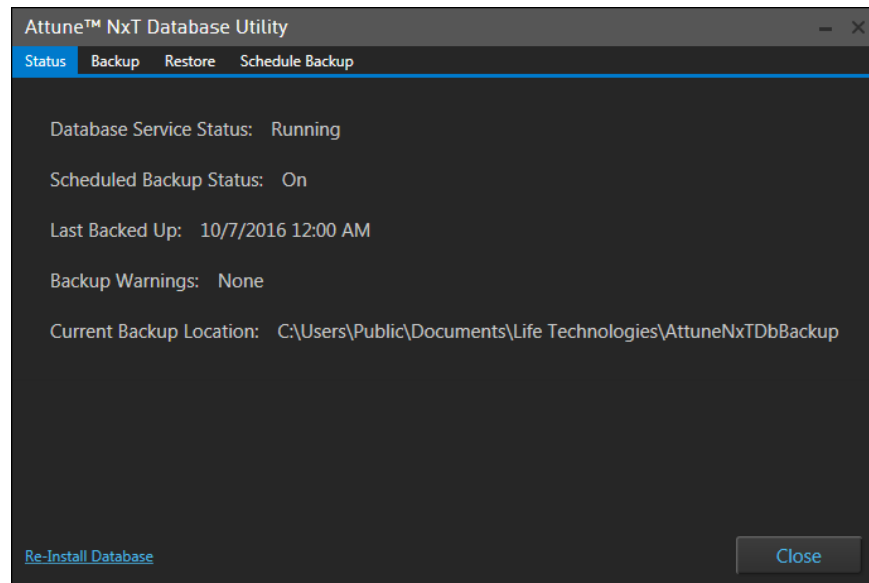
The visibility of the tabs is dependent on the user role. All users can see the Status tab and Backup/Restore tab. Only a System Administrator or Administrator can see the scheduled backup and restore tabs.

By default, the “Status” tab is selected upon logging into the application.



Status tab

The Status tab displays the current status of the scheduled backup, the last backup time and date, the backup path, and any errors reported by the backup.



Database Service Status

- The *Database Service Status* checks to see if the database service is running in the background. Typically, this will display the status of “Running”.
- If the backup service is “Stopped”, an option to start the backup service appears as the Start Service button next to the text.
Click the **Start Service** button to start the backup.
- Successful start changes the status to “Running” and hides the Start Service button.

Scheduled Backup Status

The *Scheduled Backup Status* shows the current ON/OFF state of the scheduled backup.

Last Backed Up

- The *Last Backed Up* displays the date and time of the last backup in system locale format with the time shown in 24 hour format.
- If no backup has occurred, the status displays NA.

Backup Warnings

The *Backup Warnings* displays any warnings or errors that have occurred while performing a scheduled backup.

If there are no warnings or errors, the status displays “None”.

Possible Errors include:

- **Insufficient Disk Space** warns that the attempted backup or database restore would consume >90% of the free disk space.
- **Security Error** indicates that the user does not have permission to write files or folders at the destination.
- **Directory Not Found** indicates that the destination directories have been removed or renamed during backup or restore process.

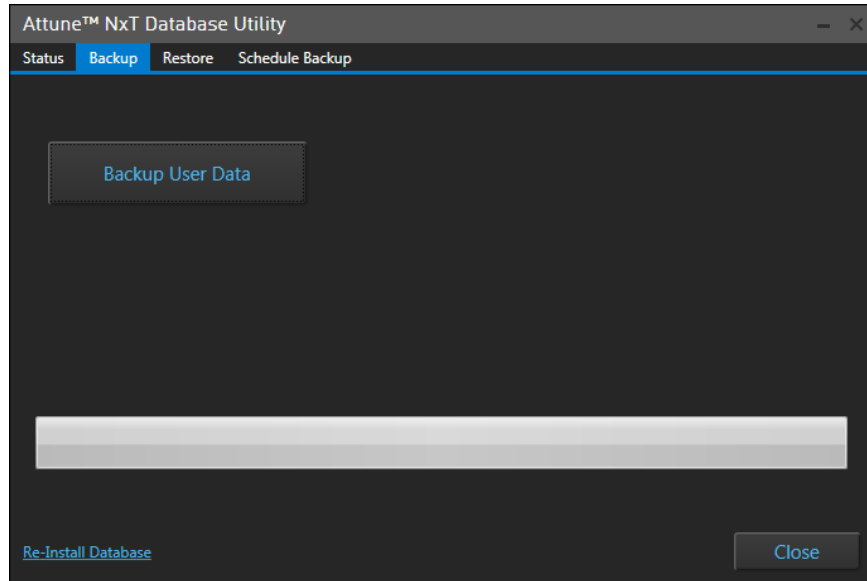
- **Path Too Long** indicates that the file path name exceeds the number of characters allowed by the system.
- **Other exceptions** lists the last of the backup fails due to unforeseen problems.

Current Backup Location

The *Current Backup Location* displays the current file path of the scheduled backup. If no path is specified, the status displays “NA”.

Backup tab

The *Backup tab* allows the logged in user to backup all user data and the database.



Backup User Data

Clicking the **Backup User Data** button opens the file browser to select a location to back up the database and corresponding data.

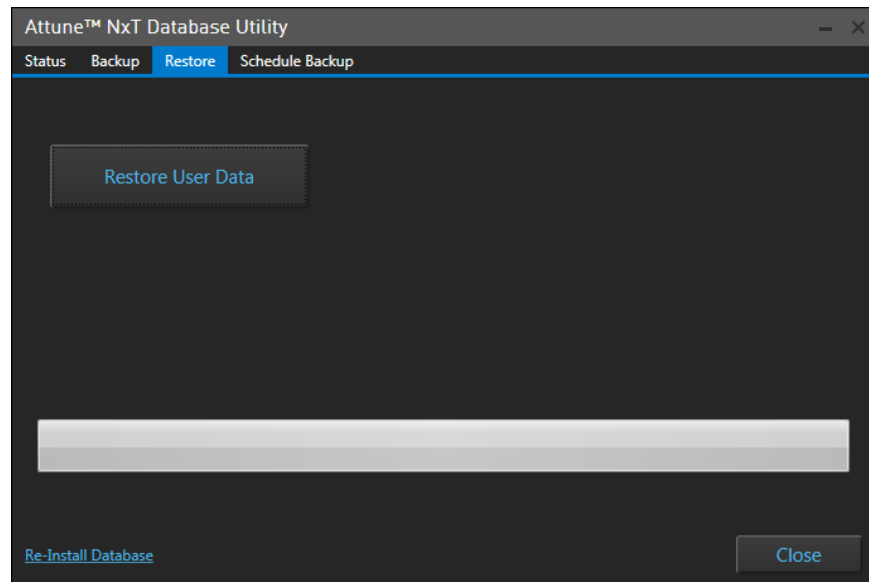
After confirming a location for the data backup, the database utility copies all user data located in the “\Users\Public\Public Documents\Thermo Fisher\AttuneNxt\Userdata” and exports the database to the specified location.

Progress

A progress bar shows the progress of the backup operation as a percentage towards completion. The progress bar shows the current status as “Copying data” or “Backing up database”.

Restore tab

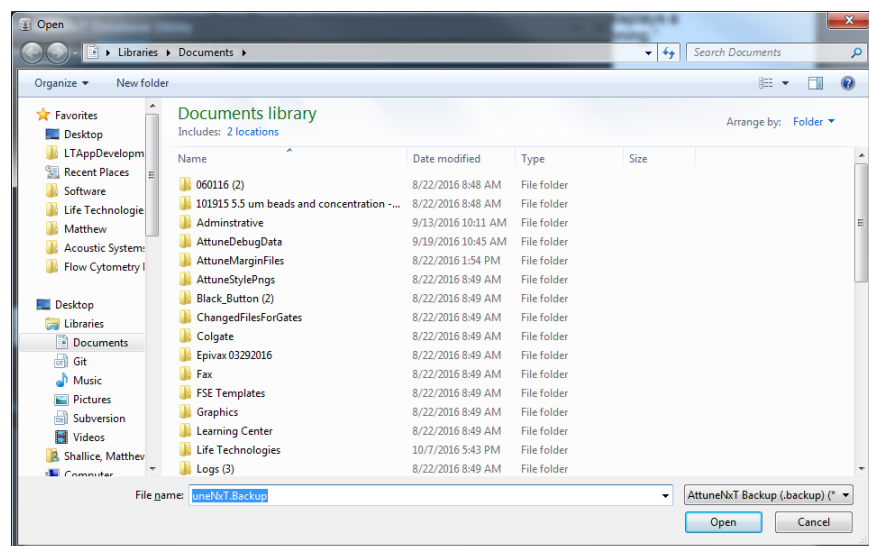
The *Restore tab* allows the logged in user to restore all user data and the database.



Restore User Data

Restore user data may not be completed if the Attune™ NxT software is open. If the application is running, this option is disabled and displays a tooltip “This operation cannot be performed while the application is running.”

Clicking the **Restore User Data** button opens up the Open File dialog:



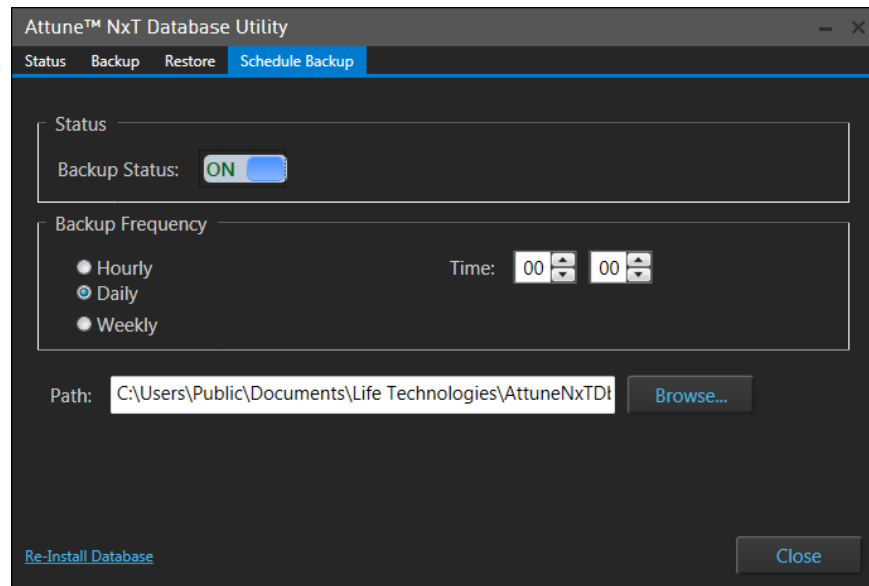
Selecting a backup file and clicking the **Open** button initiates the restore operation.

Progress

A progress bar shows the progress of the backup operation as a percentage towards completion. The progress bar shows the current status as “Copying data” or “Restoring database”.

Schedule Backup tab

The *Schedule Backup* tab allows an authorized user to set the backup policy and schedule. This tab is only visible to the System Administrator or Administrators.



Status

The *Status* group includes a toggle control to turn the backup on or off.

- By default the **Backup Status** option is set to OFF
- When turned ON, the backup will attempt to backup to the specified path and frequency.
- If an error occurs while performing a scheduled backup, the error is displayed on the Status tab (page 370).
- When turned OFF, no automatic backup will occur.
- If a valid path is not entered, a warning balloon is displayed if the user tries to turn the status ON: "Valid path is required to schedule an automatic backup."

Backup Frequency

The *Backup Frequency* sets the frequency of the scheduled backup. You can set the scheduled backup to run **Hourly**, **Daily**, or **Weekly**.

- By default the frequency is set to **Daily**.
- The **start time** must be set to specify the time to run the backup. The time format is in 24 hour time as HH:MM.
- By default, the backup is scheduled to run at 00:00.
- If the **Hourly** option is selected, the time entry will not be visible and backup will run at the start of the hour (:00).
- If the **Weekly** option is selected, the day must be specified. By default, the day is set to Sunday.

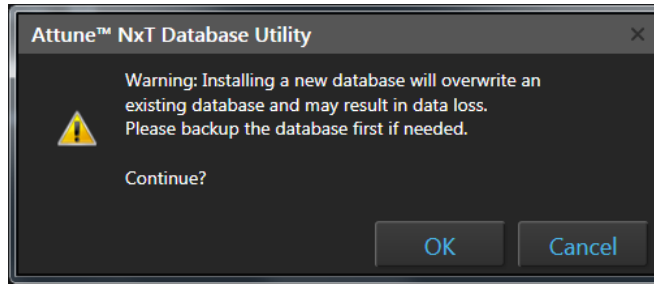
Path

You can type the File Path of the backup directory directly into the textbox or click Browse to select it from the File path dialog.

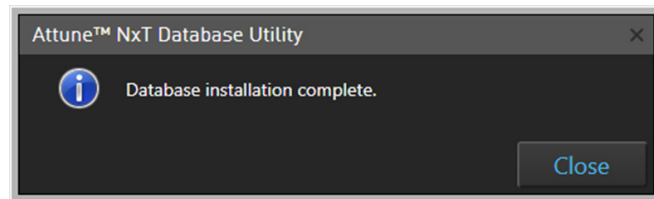
If the File Path does not exist, the directory is automatically created when the application is closed.


Re-Install Database The Re-Install Database hyperlink is only visible to the System Administrator and Administrators.

1. Click the **Re-Install Database** hyperlink. A confirmation dialog opens.



2. Click **OK** to reinstall the Attune™ NxT Database. Upon completion of the reinstall, the following confirmation dialog appears:

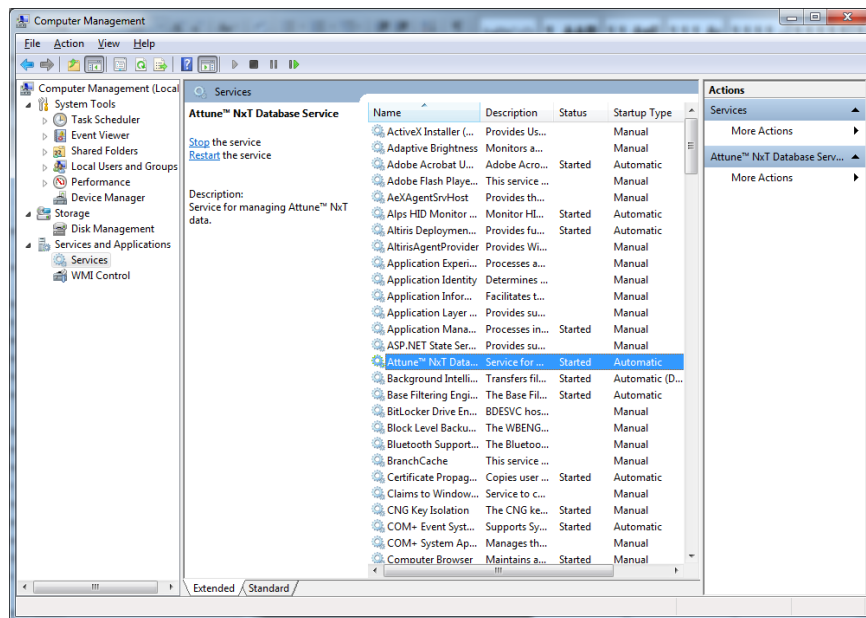


 **Note:** If the Attune™ NxT application is running, the Reinstall Database hyperlink is disabled and displays a tooltip "This operation cannot be performed while the application is running."

Attune™ NxT Database Service

The Attune™ NxT Database Service is a Windows® data backup service that runs in the background and performs the backup at the scheduled time. You can manage the service via the Windows® Management Console.

1. To access the Window Management Console, select **Start**, then right-click **Computer ▶ Manage**.



You can start or stop the service from here, or double-click the service to get more options.

Appendix A: FCS file reference

Overview

This chapter describes the FCS keywords that are used when generating FCS files from data acquired from the Attune™ NxT Acoustic focusing Cytometer.

- When in the Record mode, the FCS is recorded regardless of whether the stop criteria are met.
- For appended data sets, the time and event offsets (elapsed time from \$BTIM in ms and \$TOT of previous dataset; see below) are added to the time and event parameter values (when enabled) in the new data stream.
- The FCS files generated by the Attune™ NxT Software when recording or exporting conform to the FCS standard as described by the Data File Standards Committee of the International Society for Analytical Cytology (ISAC) for FCS 3.1.

FCS HEADER format

The *FCS HEADER* conforms to the FCS standard as described by the Data File Standards Committee of the International Society for Analytical Cytology (ISAC). An example table is displayed below.

Contents	Start by position	End byte position
FCS 3.1	00	05
ASCII(32) – space characters	06	09
ASCII-encoded offset to first byte of TEXT segment	10	17
ASCII-encoded offset to last byte of TEXT segment	18	25
ASCII-encoded offset to first byte of DATA segment	26	33
ASCII-encoded offset to last byte of DATA segment	34	41
ASCII-encoded offset to first byte of ANALYSIS segment	42	49
ASCII-encoded offset to last byte of ANALYSIS segment	50	57
ASCII-encoded offset to user-defined OTHER segment	58	beginning of next segment

FCS TEXT segment

- The delimiter used in the TEXT segment of the FCS file is / (ASCII 47). If the delimiter appears in a keyword or keyword value, it is immediately followed by a second delimiter //.
- For custom keywords, the # symbol precedes the keyword name instead of the \$ symbol.
- The FCS Keywords in the FCS TEXT segment are listed in alphabetical order.
- The parameter description keywords (\$PnN, \$PnR, etc.) in the FCS TEXT segment are numbered consecutively and are listed in the order the parameters are written to the file.

Required keywords

Keyword	Description	Source of Value
\$BEGINANALYSIS	Byte-offset to the beginning of the ANALYSIS segment	This is set to "0"
\$BEGINDATA	Byte-offset to the beginning of the DATA segment	By default, this is set at 8192. If the TEXT segment exceeds this byte offset, the value will be set to ensure correct alignment of the DATA segment. This byte-offset is a duplicate of the offset contained in the HEADER. If the DATA segment exceeds the first 99,999,999 bytes, the offset in the HEADER will be set to 0 and this value will take precedence.
\$BEGINSTEXT	Byte-offset to the beginning of a supplemental TEXT segment	This is set to "0"
\$BYTEORD	Byte order for data acquisition computer	This is set to "1,2,3,4"
\$DATATYPE	Type of data in DATA segment (ASCII, integer, floating point)	This is set to "F"
\$ENDANALYSIS	Byte-offset to the end of the ANALYSIS segment	This is set to "0"
\$ENDDATA	Byte-offset to the end of the DATA segment	This is dynamically updated based on the offset of the last DATA byte.
\$ENDSTEXT	Byte-offset to the end of a supplemental TEXT segment	This is set to "0"
\$MODE	Data mode (list mode, histogram)	This is set to "L"
\$NEXTDATA	Byte offset to next data set in the file	This is set to "0"
\$PAR	Number of parameters in an event	From the total number of enabled parameters, including custom parameters, recorded in to the FCS file.
\$PnB	Number of bits reserved for parameter number n	This is set to "32"
\$PnE	Amplification type for parameter n	This is set to 0,0
\$PnR	Range for parameter number n	By default this is set to 2^{26} for Event.* By default this is set to 2^{26} for Time.* By default this is set to 2^{20} for all Height measurements.* This is set to 2^{20} for all Area measurements.* By default this is set to 2^{10} for all Width measurements.*
\$TOT	Total number of events in the data set	Number of events in the file (updated when appended).

* Data values transferred from the instrument are constrained to be within the \$PnR limits.

Optional keywords

Keyword	Description	Source of Value
\$ABRT	Events lost due to data acquisition electronic coincidence	From datastream when "Exclude coincident events" is selected in the Instrument Settings Panel as described on page 185.
\$BTIM	Clock time at beginning of data acquisition	The system clock time at the time the recorded data stream commences.
\$CELLS	Description of objects measured	From Sample information Notes field as described on page 231.
\$COM	Comment	
\$CYT	Type of flow cytometer	From the Attune™ model name and number as described on page 354.
\$CYTSN	Flow cytometer serial number	From the cytometer serial number as described on page 354.
\$DATE	Date of data set acquisition	The system clock date at the time the recorded data stream commences.
\$ETIM	Clock time at end of data acquisition	The system clock time at the time the recorded data stream ends. For appended data, this value is based on the time of the last addition to the data file.
\$EXP	Name of investigator initiating the experiment	From the name of the PI, if specified, for the currently logged in user as specified on page 357.
\$FIL	Name of the data file containing the data set	From the sample name at the time the data is recorded. This is the pathless filename and extension.
\$INST	Institution at which data was acquired	From the institution name field as specified on page 357.
\$LAST_MODIFIED	Timestamp of the last modification of the data set	The time a data file is appended or updated based on exporting the dataset. This is only added when the \$ORIGINALITY is not set to Original.
\$LAST_MODIFIER	Name of the person performing last modification of a data set	The name of the currently logged in user when a data file is appended, or updated based on exporting the dataset. This is only added when the \$ORIGINALITY is not set to Original.
\$LOST	Number of events lost due to computer busy	From the data stream
\$OP	Name of flow cytometry operator	From the name (first and last) of the currently logged in user
\$ORIGINALITY	Information whether the FCS data set has been modified (any part of it)	For appended datasets, the keyword value is set to "Appended". For exported data where the TEXT segment or data segment is modified, the keyword value is set to "DataModified". When the dataset has not been modified, this is set to "Original".
\$PLATEID	Plate identifier	From the "Plate ID" field s. If it is left blank, the keyword is the plate name.
\$PLATENAME	Plate name	From the "Plate Name" field.
\$PnF	Name of optical filter for parameter n	From the instrument configuration information for the specified detector.
\$PnL	Excitation wavelength(s) for parameter n	From the instrument configuration information for the specified detector.
\$PnS	Name used for parameter n	From the concatenation of the "target and label". When absent, the \$PnS is equivalent to the \$PnN.
\$PnV	Detector voltage for parameter n	From the voltage for each detector at the time the file was recorded.
\$PnN	Channel name for parameter n	From the channel name for the specified detector.
\$PROJ	Name of the experiment project	From the experiment name.
\$SMNO	Specimen (e.g., tube) label	From the group name.
\$SPILLOVER	Fluorescence spillover matrix	From the Experiment-level spillover matrix state at the time the file is recorded or exported.
\$SRC	Source of the specimen (patient name, cell types)	

Keyword	Description	Source of Value
\$SYS	Type of computer and its operating system	From the system attributes including computer type and operating system.
\$TIMESTEP	Time step for time parameter	This will be set to 0.001 but should be provided by the instrument API.
\$TR	Trigger parameter and its threshold	From the threshold settings set at the time a file was recorded in the format {Boolean_Channel, Boolean_Channel....}.
\$VOL	Volume of sample run during data acquisition	From the instrument API. Volume is updated to reflect total volume for all draws of a multidraw, and, if appended, the volume is totaled.
\$WELLID	Well identifier	From the well location (i.e., A1, A2,...) for a Plate sample. For tubes recorded in a Plate Experiment, the tube location for the Tube sample is recorded (i.e., T1, T2...).

Custom keywords

Keyword	Description	Source of Value
#TOTALVOLUME	Volume of sample run during data acquisition	From the instrument API. Volume is updated to reflect total volume for all draws of a multidraw, and, if appended, the volume is totaled.
#PnTarget	The target name for parameter n	From the target field for each parameter within the Instrument Settings Panel.
#PnLabel	The label name for parameter n	From the label field for each parameter within the Instrument Settings Panel.
#FLOWRATE	The flow rate of the sample during data acquisition	From the instrument API or collection panel state.
#LASER1ASF	Area Scaling Factor used for channels recorded off of laser 1	Area scaling factor for detectors off of laser 1 at time of recording.
#LASER2ASF	Area Scaling Factor used for channels recorded off of laser 2	Area scaling factor for detectors off of laser 2 at time of recording.
#LASER3ASF	Area Scaling Factor used for channels recorded off of laser 3	Area scaling factor for detectors off of laser 3 at time of recording.
#LASER4ASF	Area Scaling Factor used for channels recorded off of laser 4	Area scaling factor for detectors off of laser 4 at time of recording.
#LASER1DELAY	Laser Delay of Laser 1	The laser delay setting for laser 1 at time of recording. Only recorded if laser 1 is present and turned on.
#LASER2DELAY	Laser Delay of Laser 2	The laser delay setting for laser 2 at time of recording. Only recorded if laser 2 is present and turned on.
#LASER3DELAY	Laser Delay of Laser 3	The laser delay setting for laser 3 at time of recording. Only recorded if laser 3 is present and turned on.
#LASER4DELAY	Laser Delay of Laser 4	The laser delay setting for laser 4 at time of recording. Only recorded if laser 4 is present and turned on.
#WINEXT	Window Extension	The window extension setting at time file is recorded
#PTRESULT	Performance Test Result	Performance Test status at time of recording (Pass/Fail)
#PTDATE	Date of most recent performance test	Date of most recent performance test at time of recording.
#WIDTH THRESHOLD	Width Threshold	The width threshold setting at the time of recording. Only needed, if width is a recorded measurement.
#LASER1COLOR	Laser 1 Color	Laser 1 Color at time of recording. Specified in terms of wavelength in units of μm .
#LASER2COLOR	Laser 2 Color	Laser 2 Color at time of recording. Specified in terms of wavelength in units of μm .
#LASER3COLOR	Laser 3 Color	Laser 3 Color at time of recording. Specified in terms of wavelength in units of μm .

Keyword	Description	Source of Value
#LASER4COLOR	Laser 4 Color	Laser 4 Color at time of recording. Specified in terms of wavelength in units of μm .
#LASERCONFIG	The laser configuration of the Attune™ Instrument	Laser configuration at time of file recording.
#DRAWnVOLUME	Acquisition volume of the n th draw in a multi-draw	Instrument API will provide this value upon completion of n th draw. Only recorded if the TIME acquisition parameter is enabled.
#DRAWnSTART	Acquisition start time in terms of the TIME parameter	Instrument API will provide this value upon start of n th draw. Only recorded if the TIME acquisition parameter is enabled.
#DRAWnSTOP	Acquisition stop time in terms of the TIME parameter	Instrument API will provide this value upon completion of n th draw. Only recorded if the TIME acquisition parameter is enabled.

Storage of compensation values in the FCS file

- If an Experiment Compensation exists prior to recording a sample, the spillover values are recorded into the FCS file using the \$Spillover keyword.
- Only the parameters that are included in the compensation calculation are included in the \$Spillover keyword value.
- Compensation controls only contain the identity matrix.

Appendix B: Technical reference

Overton's cumulative statistics

The Overton Subtraction method determines the positive result by subtracting a control histogram from the sample histogram on a channel-by-channel basis. Any negative differences (where the control has a greater number of cells in a given channel compared to the sample) are added to the positive differences in the lower channels.

The percent positive is then calculated as the sum of the positive differences for all channels divided by the total number of events.

For a complete description of the Overton Subtraction method, refer to "Modified Histogram Subtraction Technique for Analysis of Flow Cytometry Data W. Roy Overton Cytometry 9:619-626 (1988)".

Kolmogorov-Smirnov test

In statistics, the Kolmogorov-Smirnov test (K-S test) is a nonparametric test for the equality of continuous, one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (one-sample K-S test) or to compare two samples (two-sample K-S test).

The Kolmogorov-Smirnov statistic quantifies a distance between the empirical distribution function of the sample and the cumulative distribution function of the reference distribution, or between the empirical distribution functions of two samples. The null distribution of this statistic is calculated under the null hypothesis that the samples are drawn from the same distribution (in the two-sample case) or that the sample is drawn from the reference distribution (in the one-sample case). In each case, the distributions considered under the null hypothesis are continuous distributions but are otherwise unrestricted.

The two-sample KS test is one of the most useful and general nonparametric methods for comparing two samples, as it is sensitive to differences in both location and shape of the empirical cumulative distribution functions of the two samples.

HyperLog™

Compensated flow cytometry data frequently contains negative values due to compensation, and cell populations do occur which have low means and normal distributions. Logarithmic transformations cannot properly handle negative values, and poorly display normally distributed cell types. Alternative transformations addressing this issue include the log-linear hybrid transformations and HyperLog™.

The HyperLog™ transform is a log-like transform that admits negative, zero, and positive values. The transform is a hybrid type of transform specifically designed for compensated data. One of its parameters allows it to smoothly transition from a logarithmic to linear type of transform that is ideal for compensated data (Cytometry A., 2005, 64:34-42).

Linear and log color binning reference

The **Linear mode** bins data by assigning a color index for each density pixel linearly such that each increment is determined by dividing the range ($Z_{\max}-Z_{\min}$) by the number of color steps.

The **Log mode** bins data by assigning a color index for each density pixel logarithmically such that each increment is determined by dividing the logarithmic range ($\log Z_{\max}-\log Z_{\min}$) by the number of color steps. The index is then determined as $\text{int}((\log Z_{\text{val}} - \log Z_{\min})/\text{increment})$.

Appendix C: Safety

This section includes the following topics:

- Safety conventions used in this document
- Symbols on instruments
- Safety labels on instruments
- General instrument safety
- Chemical safety
- Chemical waste safety
- Electrical safety
- Physical hazard safety
- Biological hazard safety
- Laser safety
- Workstation safety
- Safety and electromagnetic compatibility (EMC) standards

Safety conventions used in this document

Safety alert words Four safety alert words appear in this user guide at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action:

Definitions



IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.



CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.



WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.











DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT!** safety alerts, each safety alert word in a this document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard icons that are affixed to the instruments (see “**Safety symbols**”).

Symbols on instruments








Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on the instruments.

Symbol	Description
	Indicates the On position of the main power switch.
	Indicates the Off position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the Standby condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the On/Off position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.


Safety symbols

The following table describes the safety symbols that may be displayed on the instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see “**Safety labels on instruments**”). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.





Environmental symbols on instruments

The following symbol applies to all of our electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	<p>Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).</p> <p>European Union customers: Call your Customer Service representative for equipment pick-up and recycling. See www.lifetechnologies.com for a list of customer service offices in the European Union.</p>

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on the instruments in combination with the safety symbols described in the preceding section.

Hazard Symbol	English	Français
	CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	ATTENTION! Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	ATTENTION! Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la réglementation locale associées à la manipulation et l'élimination des déchets.
	DANGER! High voltage.	DANGER! Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel.	AVERTISSEMENT! Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Thermo Fisher Scientific.
	DANGER! Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	DANGER! Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	CAUTION! Moving parts. Crush/pinch hazard.	ATTENTION! Pièces en mouvement, risque de pincement et/ou d'écrasement.

General instrument safety



WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Thermo Fisher Scientific may result in personal injury or damage to the instrument.

Moving and lifting the instrument



CAUTION! PHYSICAL INJURY HAZARD The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors



WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs) (see “**Obtaining SDSs**”).

Cleaning or decontaminating the instrument



CAUTION! Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Chemical safety

Chemical hazard warning



WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.



WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.



WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

General safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials (see “**Obtaining SDSs**”).
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical waste safety

Chemical waste hazard



CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste (see “**Obtaining SDSs**”).
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical safety



DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Attune™ NxT Acoustic Focusing Cytometer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses



WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power



DANGER! ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.



DANGER! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.



DANGER! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The Attune™ NxT Acoustic Focusing Cytometer has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical hazard safety

Moving parts



WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; www.cdc.gov/OD/ohs/biosfty/bmb14/bmb14toc.htm)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: www.cdc.gov

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Laser safety

Laser classification The Attune™ NxT Acoustic Focusing Cytometer has seven different laser configurations, using one or more of the following excitation lasers:
Blue 488 nm, 20 mW laser; Violet 405 nm, 50 mW laser; Red 637 nm, 100 mW laser; Yellow 561 nm, 50 mW laser, and Green 532 nm, 50 mW laser.

Under normal operating conditions, the Attune™ NxT Acoustic Focusing Cytometer is categorized as a Class I laser product. When safety interlocks are disabled during certain servicing procedures and/or input/output optics covers are removed, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

Laser safety requirements

To ensure safe laser operation:

- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, you can be exposed to laser emissions in excess of the Class 3B rating.
- Do not remove safety labels.

Additional laser safety information

Refer to the user documentation provided with the laser for additional information on government and industry safety regulations.



WARNING! LASER HAZARD. Lasers can burn the retina, causing permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Do not remove the instrument top or front panels. Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the top or front panels are removed for service.



WARNING! LASER HAZARD. An overheated laser can cause severe burns if it comes in contact with the skin. DO NOT operate the laser when it cannot be cooled by its cooling fan. Always wear appropriate laser safety goggles.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- European safety and EMC standards
- Australian EMC standards

U.S. and Canadian Safety Standards



The **CSA C/US Mark** signifies that the product meets applicable U.S. and Canadian standards, including those from CSA, CSA America, ANSI, ASME, ASSE, ASTM, NSF and UL.

European Safety and EMC Standards



The **CE Mark** symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the instrument is subject to the conditions described in this manual.

The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by Thermo Fisher Scientific.

Australian EMC standards



The **C-Tick Mark** indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.

24. Documentation and support

Obtaining support

Visit www.thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
 - Product FAQs
 - Software, patches, and updates
- Order and web support
- Product documentation, including:
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: When contacting customer support for instrument troubleshooting, provide the instrument model and the instrument serial number. Convey to the technical support any error messages that were displayed on your instrument and any troubleshooting that you have already performed (refer to *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide*; Pub. no. 100024234).

Obtaining SDSs

Safety Data Sheets (SDSs) are available at www.thermofisher.com/sds.



IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions.

If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.



IMPORTANT! Wiping the computer supplied with the Attune™ NxT Acoustic Focusing Cytometer (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.

For support visit thermofisher.com/support or email techsupport@lifetech.com
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