invitrogen

Flowscape™ Software

USER GUIDE

Flow cytometry acquisition and analysis software

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Revision A

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1000880 A (English)

Revision	Date	Description
A	13 June 2025	New document for Flowscape™ Software.

The information in this guide is subject to change without notice.

Contents

1 Flowscape™ Software

Flowscape™ Software overview

The Flowscape™ flow cytometry acquisition and analysis software is used to control and monitor the Invitrogen™ Attune™ Xenith™ Flow Cytometer. The software is opened and maintained using the Flowscape™ Launcher, which is preinstalled on the computer workstation included with the system.

- **Computer workstation:** The computer workstation provides full access to the Flowscape™ Software. The software enables you to control and monitor the instrument, design and perform experiments, optimize data collection, check and track instrument performance, acquire and record data, manage and process recorded data, and perform instrument maintenance functions.
- **Touchscreen:** The touchscreen provides access to a limited set of features: **Startup** and **Shutdown** procedures and **Settings**, such as instrument name. The touchscreen also provides a view of the fluid levels of the containers on the Fluid Cart.

IMPORTANT! For instructions about using the Attune™ Xenith™ Flow Cytometer, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

System requirements

A PC workstation running the Flowscape™ Software on a Windows™ 11 64-bit platform is provided with the instrument.

Table 1 Minimum system requirements (offline workstation)

Processor	Intel™ Core i7 (minimum of 8 cores)
RAM	32 GB RAM
Hard drive	1 TB (SSD recommended)
Operating system	Windows™ 11
Monitor resolution	4K resolution recommended

Supported files

- The Flowscape™ Software records and outputs data in FCS (Flow Cytometry Standard) 3.1.
- The software can export data in FCS 3.1 format.
- FCS format 3.1 is supported for data analysis.

Flowscape™ Launcher

The Flowscape™ Software is opened and maintained through the Flowscape™ Launcher, which is installed and configured by support personnel. After the software is installed, you can use the Flowscape™ Launcher shortcut icon on the computer desktop to start and update the Flowscape™ Software.

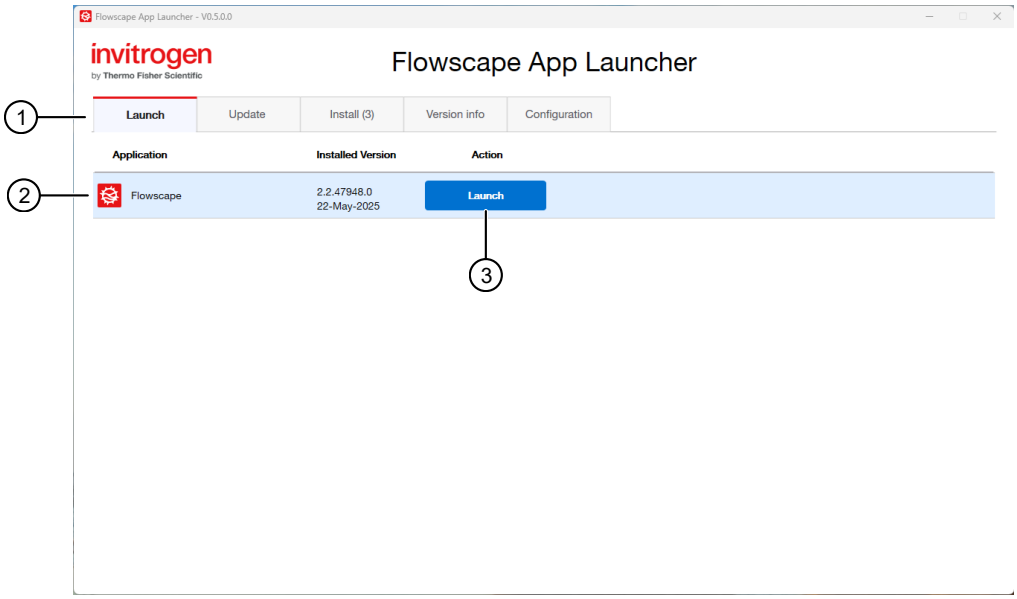


Figure 1 Flowscape™ Launcher (Launch tab selected)

① **Launch**, **Update**, **Install**, **Version info**, and **Configuration** tabs
② Application
③ **Launch button**

- If the instrument is connected to the network, the **Update** tab lists any available updates. Software updates are not required, but highly recommended to ensure current software with updated features and fixes is used.
- To update the software, click **Update All**.

Software layout

The Flowscape™ Software for the Attune™ Xenith™ Flow Cytometer has three main screens: **Sign in**, **Home**, and **Experiment Workspace**.

- **Sign in** – Used for signing into the software. The **Sign in** screen is the first screen presented after the application startup.
- **Home** – Used for running and reviewing performance tracking tests, creating new experiments, and accessing the current and stored experiments. The **Home** screen is the first screen presented after a successful sign in.
- **Experiment Workspace** – Used for controlling the instrument to run samples, generate data, and analyze results. The **Experiment Workspace** is the main application window.

Flowscape™ Software Sign In screen

The Flowscape™ Launcher shortcut is on the desktop of the computer workstation provided with the system. When you start the Flowscape™ Software using the Flowscape™ Launcher, the **Sign in** is the first screen that is displayed after the **splash** screen. Each user is required to sign in before being allowed to use the software.

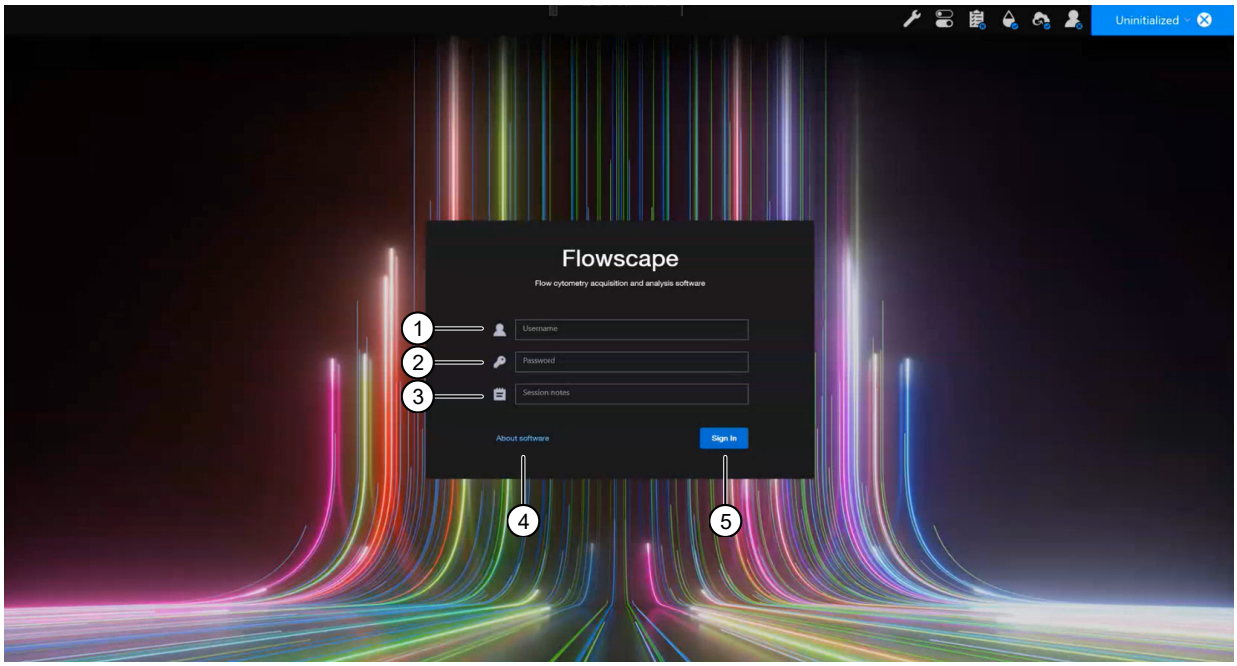


Figure 2 Software Sign In screen

① Username
② Password
③ Session notes
④ About software
⑤ Sign in button

Note: For more information about the Flowscape™ Software **Sign in** screen, see Chapter 2, “Sign in (Attune™ Xenith™ Flow Cytometer)” on page 15.

Home screen

The **Home** screen appears after you sign in to the software. This screen displays system status and provides menus and controls for using the instrument.

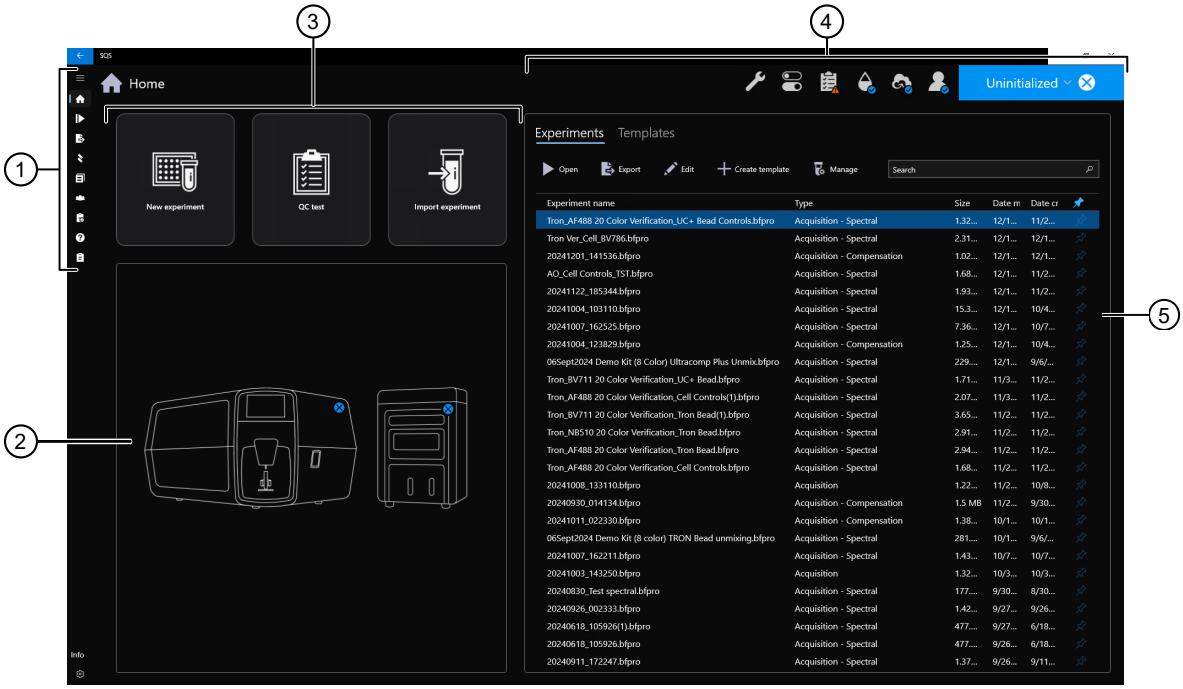


Figure 3 Home screen

1. Global navigation (page 22)
2. Instrument status (page 36)
3. Home screen buttons: New experiment, QC test, and Import experiment (page 38)
4. Status bar (page 41)
5. Experiment and Template lists (page 49)

Note: For more information about the **Home** screen, see Chapter 3, “Home screen” on page 21.

Experiment Workspace

Experiment Workspace is the main application window used for controlling the Attune™ Xenith™ Flow Cytometer to run samples, generate data, and analyze results.

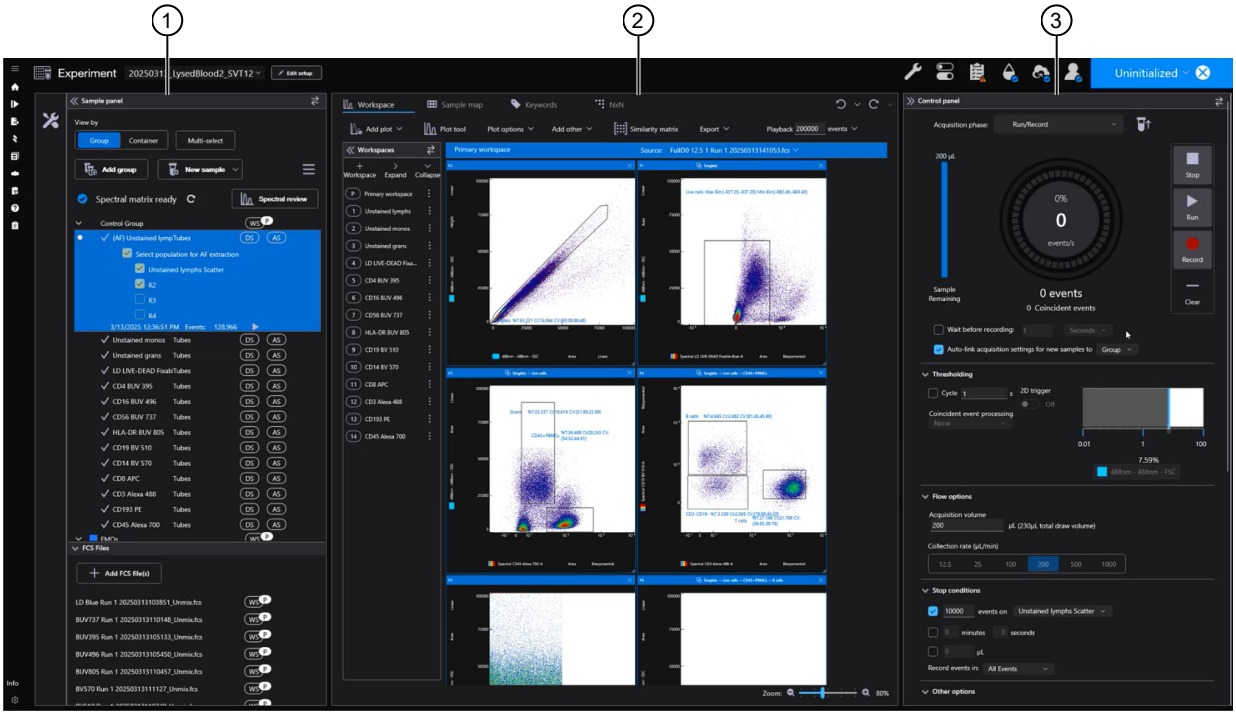


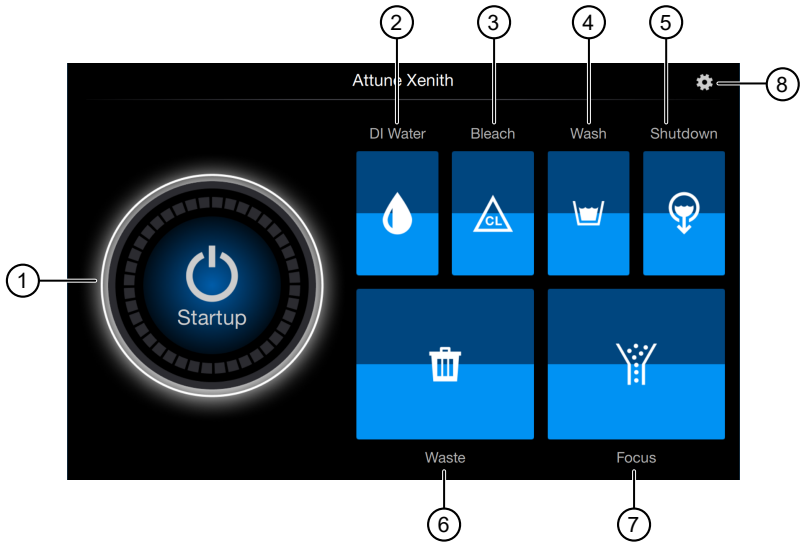
Figure 4 Experiment Workspace

1. Sample panel
2. Views (Workspace, Sample map, Keywords, NxN)
3. Control panel

Note: For more information about the **Experiment Workspace**, see Chapter 5, “Experiment Workspace” on page 71.

About the touchscreen

The touchscreen (7-inch touch display) is on the front of the instrument and provides access to instrument **Startup**, **Shutdown**, and limited **Settings** functions. The fluid levels of the containers in the fluid cart are also displayed. For more information, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).



1. Startup/Progress indicator
2. DI (deionized) water level
3. Bleach level
4. Wash solution level
5. Shutdown solution level
6. Waste level
7. Focusing fluid level
8. Settings

Note: The **Startup/Progress indicator** is contextual to what the system is running. While performing maintenance functions, the indicator only shows the progress of the procedure.

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Note: For a checklist of IT setup requirements for the Attune™ Xenith™ Flow Cytometer, see the *Attune™ Xenith™ Flow Cytometer IT Checklist* (Pub. No. MAN1000862).

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

2 Sign in (Attune™ Xenith™ Flow Cytometer)

Approve End User License Agreement (EULA) via instrument touchscreen (first use)

The first time you start the Attune™ Xenith™ Flow Cytometer, you need to read and approve the **End User License Agreement (EULA)** on the touchscreen, then set the date and time format before you start using the software and the instrument.

The EULA is displayed when:

- The instrument is powered on for the first time.
- A new version of the Flowscape™ Software is installed.

1. Power on the instrument. See the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199) for more information.
2. View the EULA on the touchscreen.

   Note: You need to approve the EULA to progress to the next screen.

3. Select the date and time format you prefer, enter the date and the time, then tap **Next**.
4. Tap **Done**. The **Home** screen appears when touchscreen setup is complete.
5. The **Home** screen on the instrument touchscreen provides access to instrument **Startup** function and the touchscreen **Settings** menu. It also shows the fluid level of each container in the Fluid Cart.

Edit touchscreen settings

1. Touch ⚙ **Settings**. The **Date and Time** format screen appears.

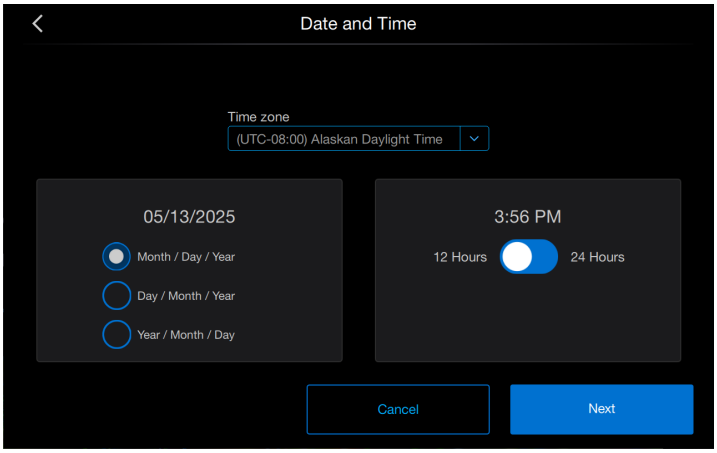


Figure 5 Format date and time

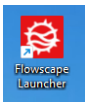
2. Select the button next to the preferred formats for the date and time.

Sign in to the software

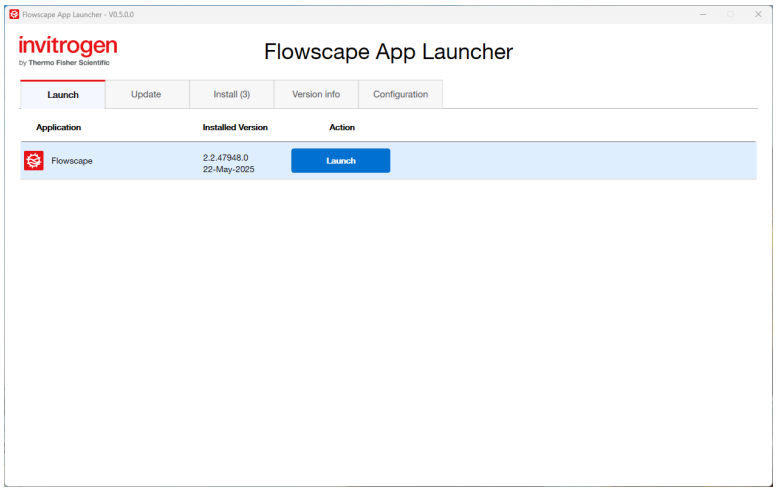
Power on the instrument, the computer, and the monitor as described in the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199). You can power on the instrument and computer in any order.

1. Sign in to Windows™. The default credentials are:
   Username: INSTR-ADMIN
   Password: INSTR-ADMIN

2. Double-click the **Flowscape™ Launcher** icon on the computer workstation desktop.



   The **Flowscape™ Launcher** opens, then checks for software versions that are installed and software versions that are available.



Note: The **Flowscape™ Launcher** has 5 tabs: **Launch**, **Update**, **Install**, **Version info**, and **Configuration**.

If the instrument is connected to the network, the **Update** tab lists any available updates. Software updates are not mandatory, but they are highly recommended.

To update the software, click **Update All**.

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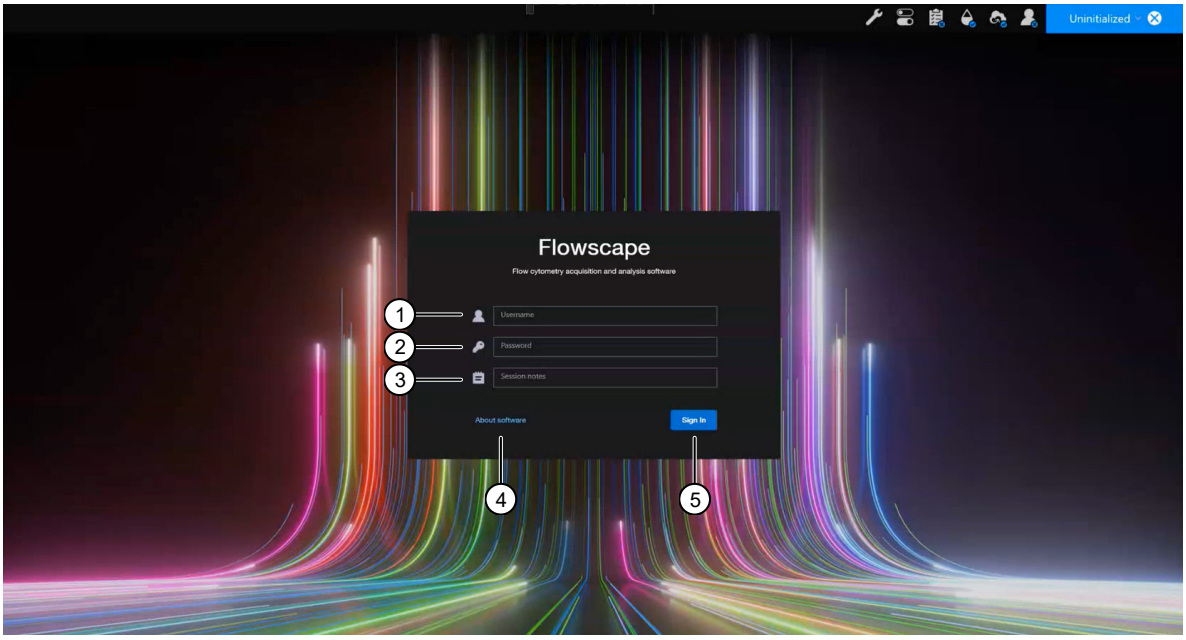
3. To start the Flowscape™ Software, select the **Launch** tab, then click **Launch**.

   The **Sign in** screen is the first screen that is displayed after the **splash** screen when you start the Flowscape™ Software. Each user is required to sign in before being allowed to use the software.



① Username
② Password
③ Session notes
④ About software
⑤ Sign in button

4. Enter **username** and **password**.

   IMPORTANT! When signing into the software for the first time after installation, you need to sign in as an **Administrator**.

   The default username and password for the Administrator are both **admin**. For more information about other available account types, see page 19.

5. *(Optional)* Enter **session notes**.

6. *(Optional)* To view system information, such as software and firmware versions, serial numbers of the instrument and the autosampler (if connected), the system model, and licensing and contact information, click **About software**.

7. Click **Sign in**.

   If the correct username and password are entered, the **Home** screen opens.

   If an invalid username or password is entered, the screen shows the “Invalid username or password” warning.

Account types

The Flowscape™ Software has three types of accounts for local users: **Administrator**, **Service**, and **Standard**.

Administrator and **Service** accounts are default accounts. The **Administrator** can create separate **Standard** accounts for each user after the first sign in. For information about how to create or delete user accounts, including additional **Administrator**-like accounts, see “User Management” on page 29.

Note: For the permissions assigned to each account type, see “Account permissions” on page 20.

Administrator

Administrator accounts have full access to the software and are granted more control and access over **Standard** accounts, including managing user accounts, resetting baseline, and changing global preferences. Assign at least two **Administrator** accounts per system. 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 to the Thermo Fisher Scientific team.

Standard

Standard accounts can access and change their own workspace and experiments, and perform QC tests, but they cannot run any advanced functions available to **Administrator** accounts. **Standard** accounts are created by the **Administrator** after the successful installation of the Flowscape™ Software and the first sign in.

For information about how to create or delete **Standard** accounts, see “User Management” on page 29.

Default usernames and passwords

For the default usernames and passwords for local users of the Flowscape™ Software, see the following table.

Account type	Default username	Default password
Administrator	admin	admin
User	Set by the **Administrator** when the account is created.[1]	Set by default to the username created by the **Administrator**.

[1] *See “User Management” on page 29.

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Note: Each account is required to reset their password after the first sign in. The new password must be 12 or more characters and cannot contain illegal words.

Account permissions

The different privileges for the **Administrator** and **Standard** accounts are shown in the following table.

Table 2 Account permissions for Administrator and Standard accounts

Permission	Administrator	Standard
Startup	Yes	Yes
Shutdown	Yes	Yes
Run QC	Yes	Yes
Reset baseline	Yes	No
Acquire data	Yes	Yes
Print analysis reports	Yes	Yes
Print user reports	Yes	No
Change user password	Yes	Yes
Change user rights	Yes	No
Delete users	Yes	No
Create users	Yes	No
Edit users	Yes	No
Reset other user passwords	Yes	No
Change global settings	Yes	No
Change user settings	Yes	Yes
Create and edit plates	Yes	No
Manage experiments for all users	Yes	No
Enable/disable Stop on bubble	Yes	No

3 Home screen

Home screen overview

The **Home** screen appears after you successfully sign in to the software. **Home** screen contains the buttons for commonly used commands (**New experiment**, **QC test**, and **Import experiment**), displays system status (**Instrument status**), shows the lists of the Experiments and Templates created by users, and provides menus and controls for using the instrument (**Global navigation** and **Status bar**).

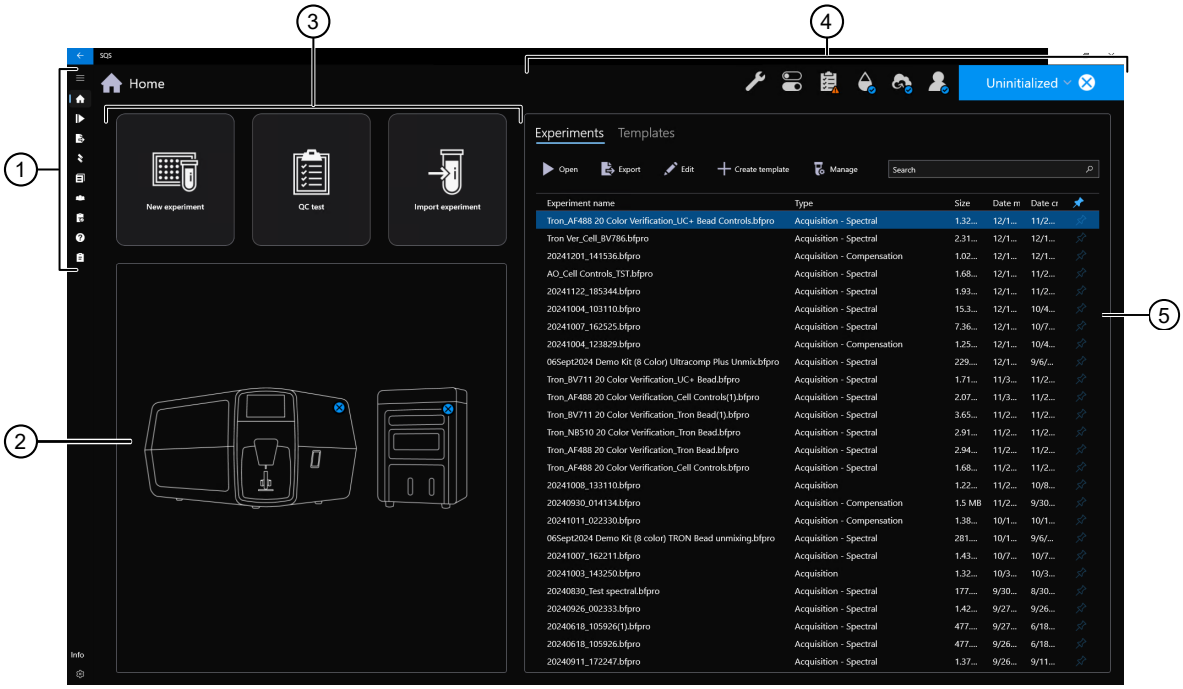


Figure 6 Home screen

1. Global navigation (page 22)
2. Instrument status (page 36)
3. Home screen buttons: New experiment, QC test, and Import experiment (page 38)
4. Status bar (page 41)
5. Experiments and Templates (page 49)

Global navigation

Global navigation is organized into a series of tabs that enable access to the basic features of the Attune™ Xenith™ Flow Cytometer. It also enables user and global level application customization.

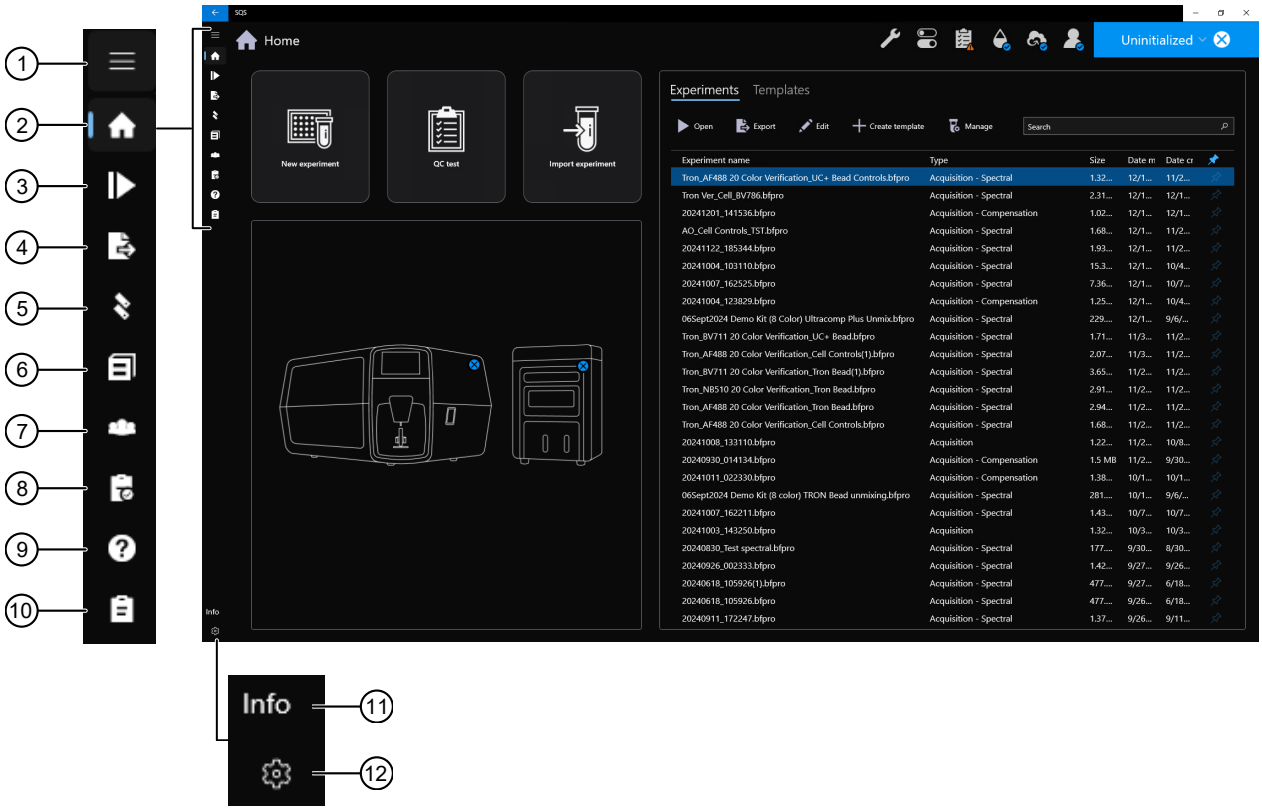


Figure 7 Global navigation menu

1. Expand/Collapse toggle (page 23)
2. Home (page 24)
3. Resume (page 25)
4. Export (page 26)
5. Filter configuration (page 26)
6. Hardware Settings Library (page 28)
7. User Management (page 29)
8. Report Management (page 31)
9. Help (page 33)
10. Release Notes (page 33)
11. Information (page 34)
12. Settings (page 35)

Expand/Collapse the Global navigation menu

To expand the **Global navigation menu**, click the **Expand/Collapse** toggle button.

To collapse the menu, click the **Expand/Collapse** button again.

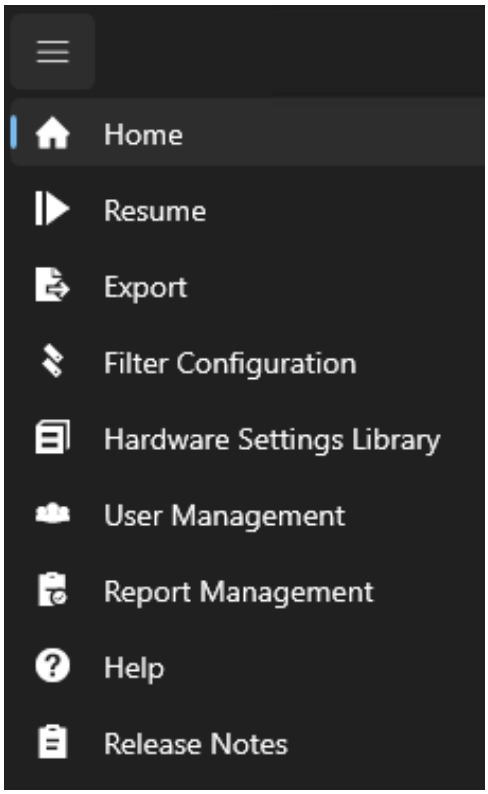


Figure 8 Global navigation menu (expanded)

Home

The **Home** tab opens the **Home screen**, where you can:

- Create new experiments
- Run QC tests
- Import experiments
- Open previously run experiments
- Manage experiments and templates

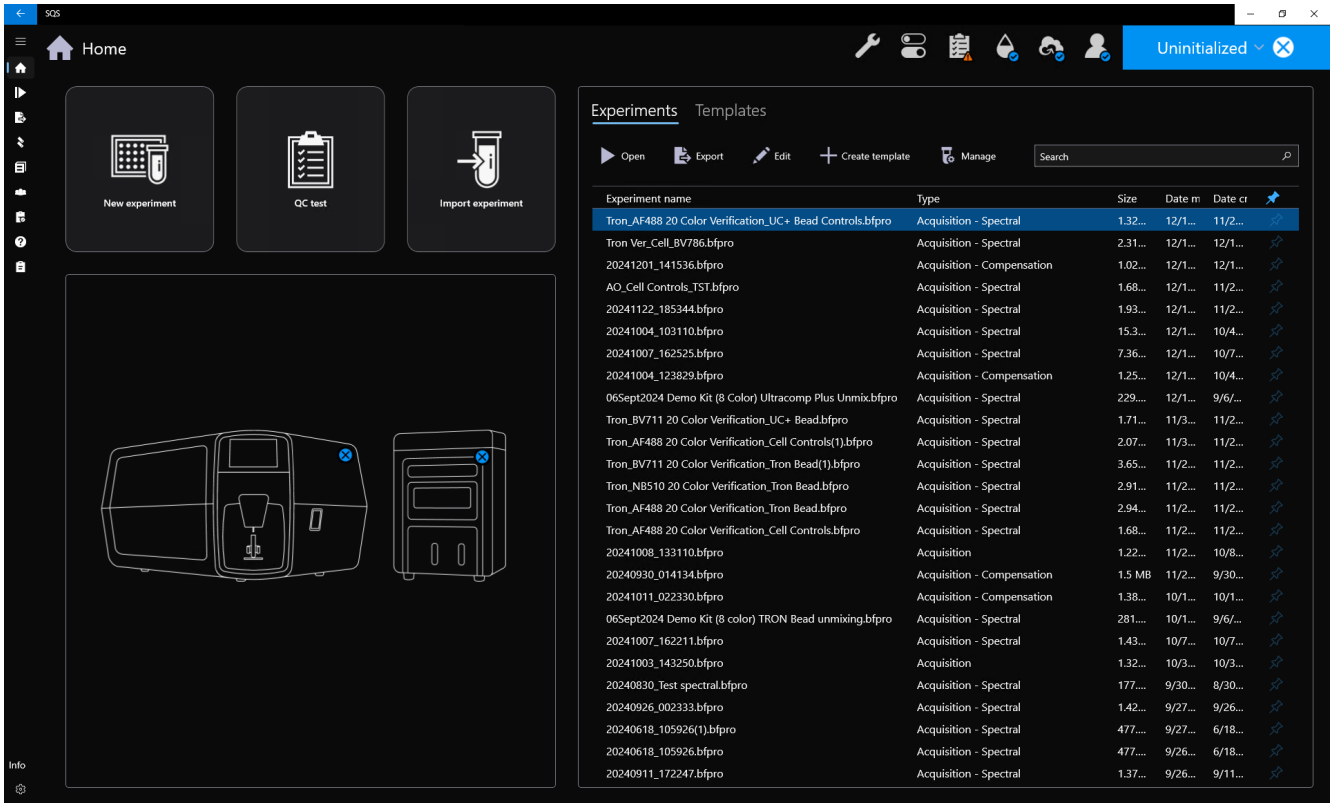


Figure 9 Home screen

Resume

The **Resume** tab opens the **Experiment Workspace** of the previously open experiment and reactivates the experiment.

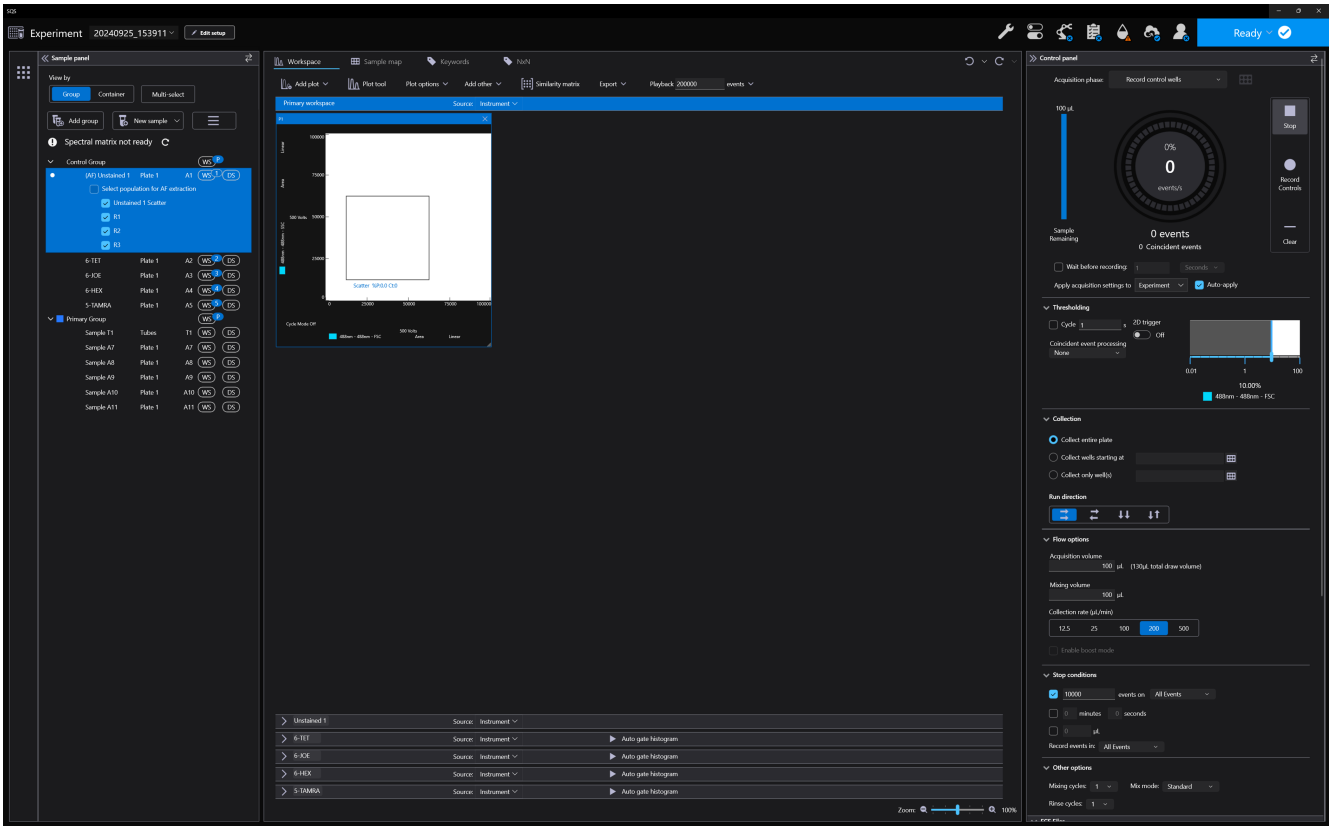


Figure 10 Reactivated experiment in the Experiment Workspace

Note: For information about the **Experiment Workspace**, see Chapter 5, “Experiment Workspace” on page 71.

Export

The **Export** tab opens the **Export FCS Files** screen, which enables you to select FCS files to export from selected experiments.

- Only experiments and FCS files for the current user are shown.
- You can change export options to select which scatter and fluorescence parameters (Height, Area, Width) to include in the exported data.
- For spectral experiments, you can select to only export unmixed parameters along with scatter parameters.
- For compensation experiments, you can select to export with the current compensation matrix.
- You can select to export with updated keywords.
- You can select the file export location and manage the export queue.
- FCS file export continues in the background. You can close the **Export FCS Files** screen anytime.

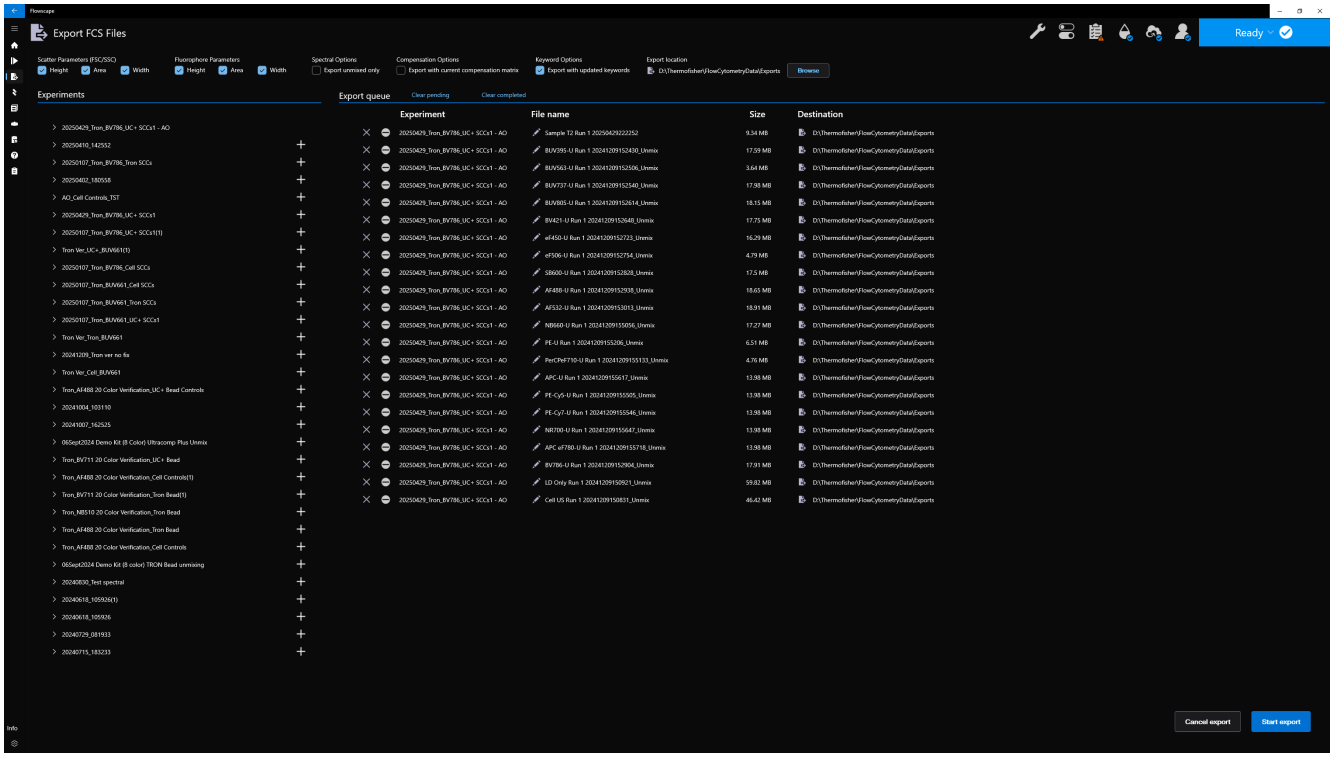


Figure 11 Export FCS Files screen

Filter configuration

The **Filter configuration** tab opens the **Filter Configuration** screen, which provides a view of the instrument optical deck with system lasers and filters. In the **Filter Configuration** screen you can:

- View the light paths for each laser through the dichroic and bandpass filters in the instrument.
- Edit filter labeling to reflect any change to the instrument filter configuration.

IMPORTANT! Target voltages and pass/fail criteria for QC have been established using instrument default filter configuration and Attune™ Xenith™ Quality Control Beads. Any changes made in the filter configuration (for example, using custom filters) can affect the validity of the QC results.

Note: Ensure the instrument is returned to the default configuration for any QC testing.

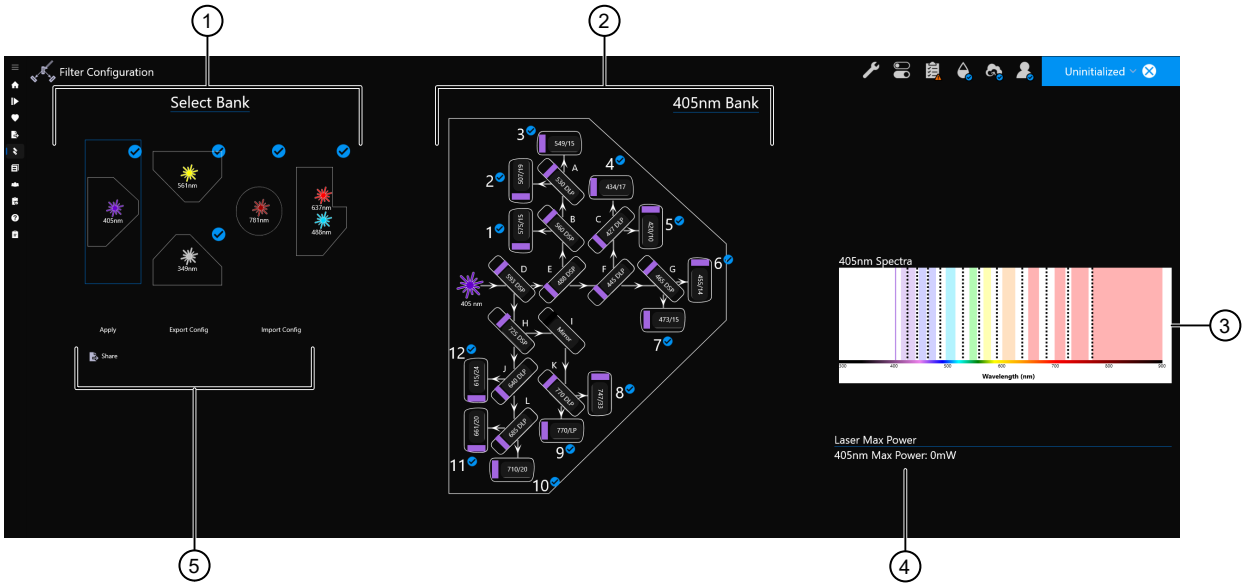


Figure 12 Filter Configuration screen

1. Select Bank
2. Selected Bank
3. Spectra
4. Laser Max Power
5. **Apply**, **Export Config**, **Import Config**, and **Share** buttons

- **Select Bank**: Enables you to select the detection bank to view. The detection bank is a collection of lasers, filters, and detectors used for acquisition.
- **Selected Bank**: Shows the light paths through the filters in the detection bank, where the numbers correspond to the detector number. To edit filter labeling, click on the filter and enter the new filter.
- **Spectra**: Shows the bandpass configuration in the selected bank and where the light will be collected.
- **Laser Max Power**: Shows the maximum power of the laser in the selected bank.
- **Apply**: Applies the edits made to the filter labeling to the instrument filter configuration.
- **Export Config**: Enables you to export the filter configuration.
- **Import Config**: Enables you to import a filter configuration.
- **Share**: Opens the **Share screen**, which enables you to print selected filter configurations as a PDF.

Hardware Settings Library

The **Hardware Settings Library** tab opens the **Hardware Settings Library screen**, which enables you to import detection settings from saved FCS files or experiment, including:

- Compensation settings
- Thresholds
- Voltages

Select the FCS file to open from the **Library** panel or click **Open** to select an FCS file from the database or a network location.

To import the detection settings from the current experiment and save it to the **Library**, click **Import Current**.

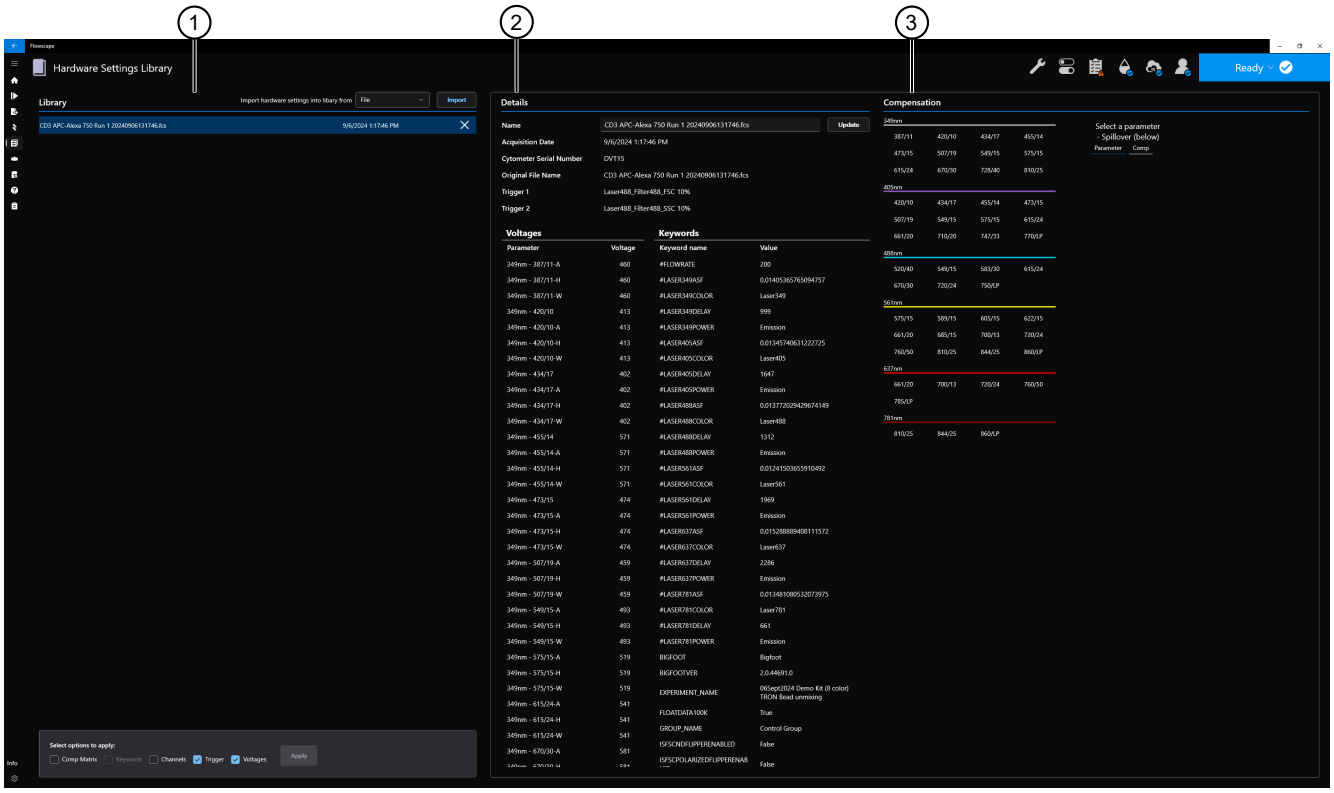


Figure 13 Hardware Settings Library screen

① Library
② Details
③ Compensation

- **Library** lists the imported FCS files that you can open.
- **Details** shows the experiment details for the selected experiment, such as **Name**, **Acquisition date**, **Trigger** thresholds, **Voltages**, **Keywords**, and **Compensation**.
- **Compensation** shows the detailed compensation information for each system laser for the selected experiment.

User Management

The **User Management** tab enables you to perform user management tasks. Depending on the account type (**Administrator** or **Standard**), it opens different screens:

- For **Administrator** accounts, **User Management** screen opens.
- For **Standard** accounts, **Reset User Password** screen opens.

User Management screen (Administrator account)

For **Administrator** accounts, the **User Management** tab opens the **User Management** screen, which enables the **Administrator** to perform the following user management tasks:

- Add new users
- Edit existing user accounts
- Set user role (Administrator vs. Standard)
- Set passwords
- Require password reset on first sign in
- Set up user profiles for each account (name, organization, contact information, billable rates)

Figure 14 User Management screen

① **Add new user**
② **Edits users**
③ **Reports**

The **User Management** screen has the following controls:

- **Add new user**: Opens the **Add New User** screen, which enables the **Administrator** to add and set up new user accounts.
- **Edit users**: Opens the **Edit Users** screen, which enables the **Administrator** to select existing user accounts to edit archive.
- **Reports**: Opens the **User Reports** screen, which enables the **Administrator** to share **Users List** and **Usage Report**. The reports are exported as ***.csv** files.

User Management – Reset User Password screen (Standard account)

For **Standard** accounts, the **User Management** tab opens the **Reset User Password** screen, which enables you to change the password for the account.

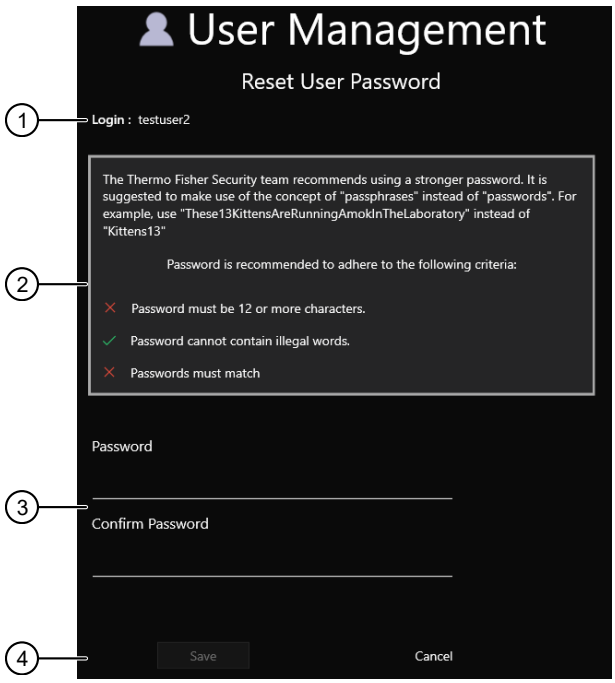


Figure 15 User Management – Reset User Password screen

① Login (current user)
② Password criteria
③ **Password** and **Confirm Password** fields
④ **Save** and **Cancel** buttons

Note: User passwords must be 12 or more characters long and cannot contain illegal words. We recommend using "passphrases" instead of "passwords". For example, use "These13KittensAreRunningAmokInTheLaboratory" instead of "Kittens13".

Report Management

The **Report Management** tab opens the **Report Management screen**, which provides access to **QC Reports**.

Figure 16 Report Management screen

① Report Management tab ② QC Reports

There are two types of **QC tests** for the Attune™ Xenith™ Flow Cytometer:

- **Baseline**: This is an in-depth quality control test that measures and sets the baseline standards for the instrument performance using Attune™ Xenith™ Flow Cytometer QC Beads. **Baseline** is performed on new instruments, when new beadlots are used, or when instruments have gone through major service or repair (see “Baseline setup” on page 171).
- **Daily QC**: After the baseline values are established, the same lot of QC beads are used to run the daily **Daily QC**. The **Daily QC** determines variation from the **Baseline** values to track the daily performance of the instrument, where deviations in performance can indicate a need for maintenance or servicing (see “Daily QC” on page 173).

The **Pass/Fail criteria** are set based on the agreement between the **Baseline** and **Daily QC** results for the following measurements:

- **dMFI** (delta of median fluorescence intensity between daily QC and baseline)
- **rCV** (robust percent coefficient of variation)

Based on the results of the **QC tests**, the Flowscape™ Software generates two types of **QC report** for the Attune™ Xenith™ Flow Cytometer:

- **Daily QC report**: Shows the high-level pass/fail results for the **Baseline** and the **Daily QC** test in the report heading. The report presents the **QC test statistics** in a table form for each laser, channel, and flow rate. The pass/fail results for the specific channel and the specific fail criteria are also indicated in the corresponding line of the channel that did not pass the test.
  - **Pass** status is indicated by the blue checkmark symbol.
  - **Fail** status is indicated by the amber warning exclamation symbol.

  By default, the most recent report is shown, but you can navigate through **Daily QC reports** using the **QC test list** on the report screen.
- **Levey-Jennings report**: The **Levey-Jennings report** tracks shifts and trends in instrument performance as indicated in **Daily QC tests** over time and provides a visual indication of system stability.

  The plots in the **Levey-Jennings report** are grouped by the excitation source. You can toggle the **Display mode** of the plots between the **Single statistic** and **Multiple statistic** modes.

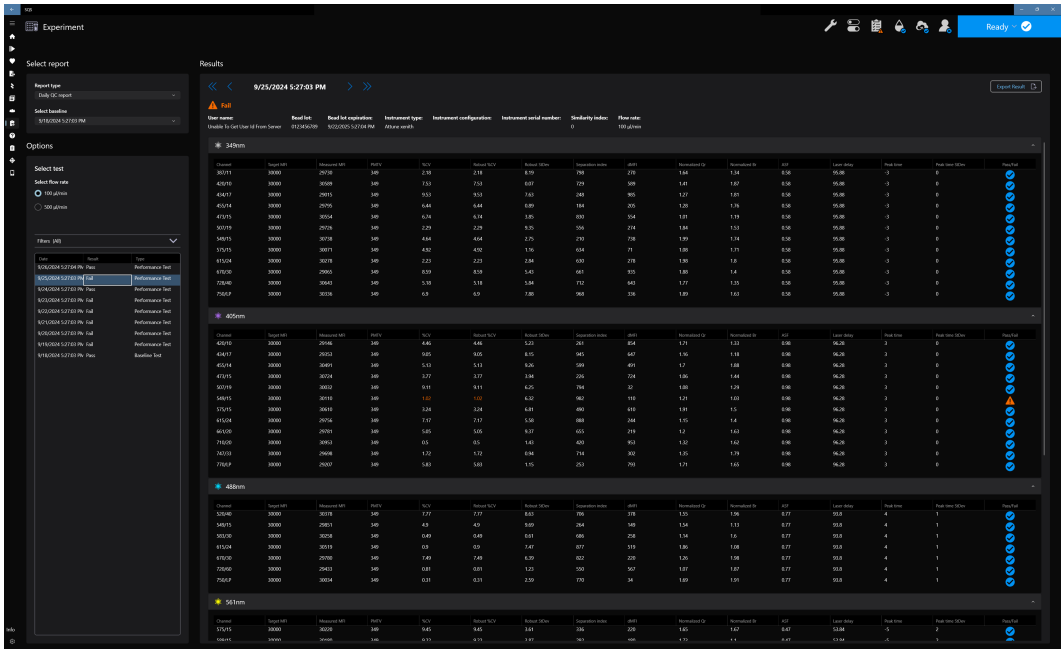


Figure 17 Daily QC report

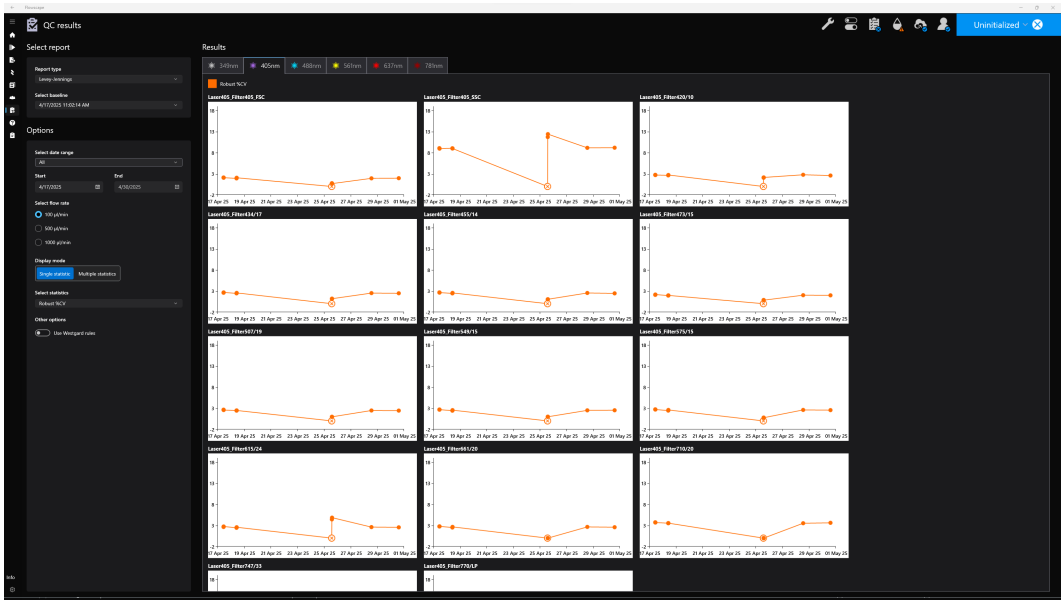
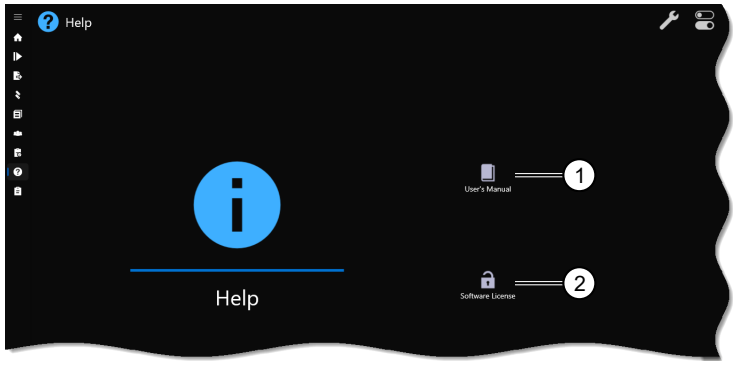


Figure 18 Levey-Jennings report

Note: For more information about the **QC tests** and **QC reports**, see the Chapter 9, “QC Test”, on page 169.

Help

The **Help** tab opens the **Help screen**, which enables you to open the *Flowscape™ Software User Guide* and the End User License Agreement (EULA).



① User's Manual
② Software License

Figure 19 Help screen

Release Notes

The **Release Notes** tab opens the **Release Notes screen**, which enables you to open the software release notes for the *Flowscape™ Software User Guide*.

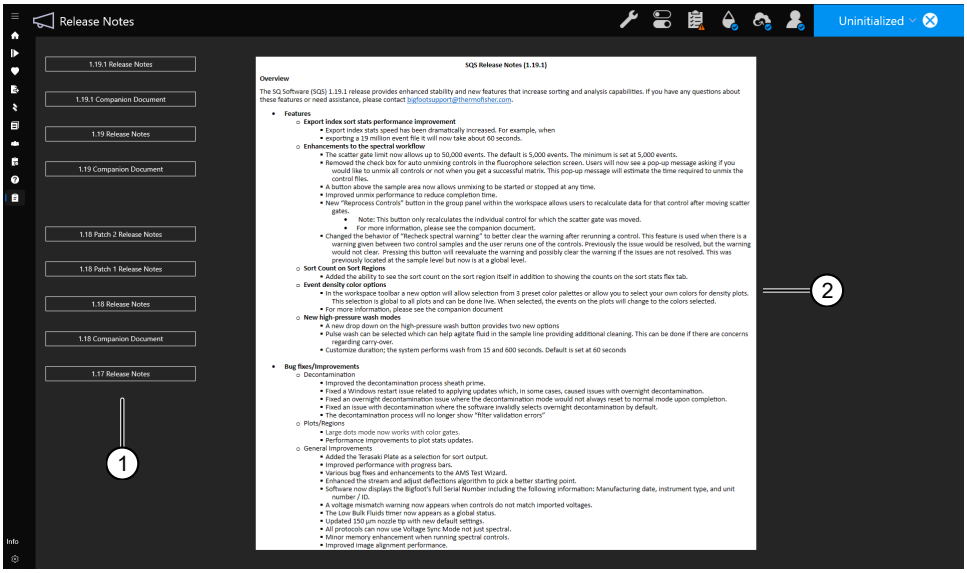


Figure 20 Release Notes screen

① Release Notes version selection

② Selected Release Notes for the software

Information

The **Info** tab opens the **Information window**, which provides information about the system, including the software version, serial numbers of the instrument and the autosampler (if connected), firmware versions of the instrument and the autosampler (if connected), the system model, and other licensing and contact information.

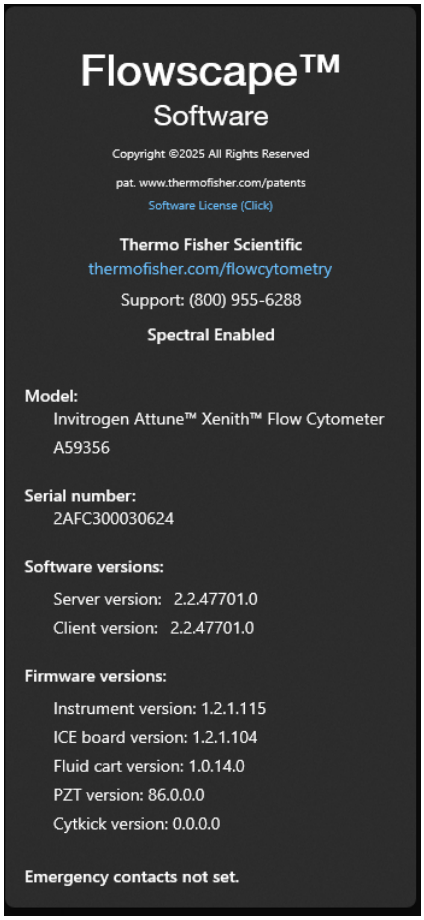


Figure 21 Information window

Settings

The **Settings** button opens the **Settings screen**, which enables you to change user and global-level application settings.

Note: Only **Administrator** accounts can change global-level application settings.

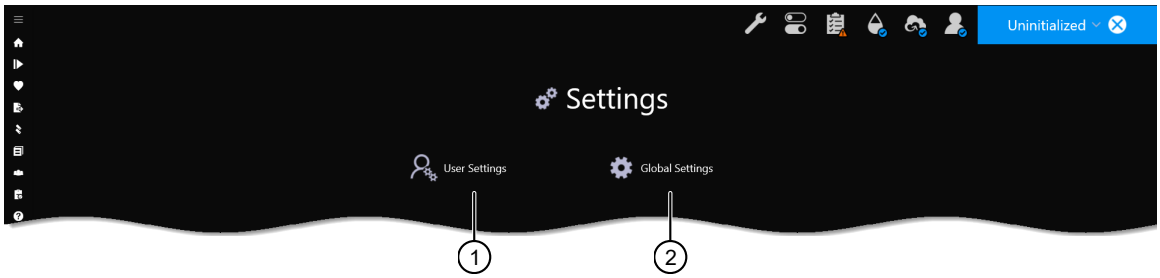


Figure 22 Settings screen

① **User Settings**
② **Global Settings**

- **User Settings** button opens the **User Settings screen**, which enables you to change the user-level application settings.
- **Global Settings** button opens the **Global Settings screen**, which enables **Administrator** accounts to change global-level appliction settings.

Note: For more information about **User Settings** and **Global Settings**, see Chapter 12, “Settings”, on page 201.

Instrument status

The **Instrument status** panel on the **Home** screen displays a picture of the instrument and the autosampler (if connected), which indicates the connection status, initialization status, and error status of the instrument and the autosampler.

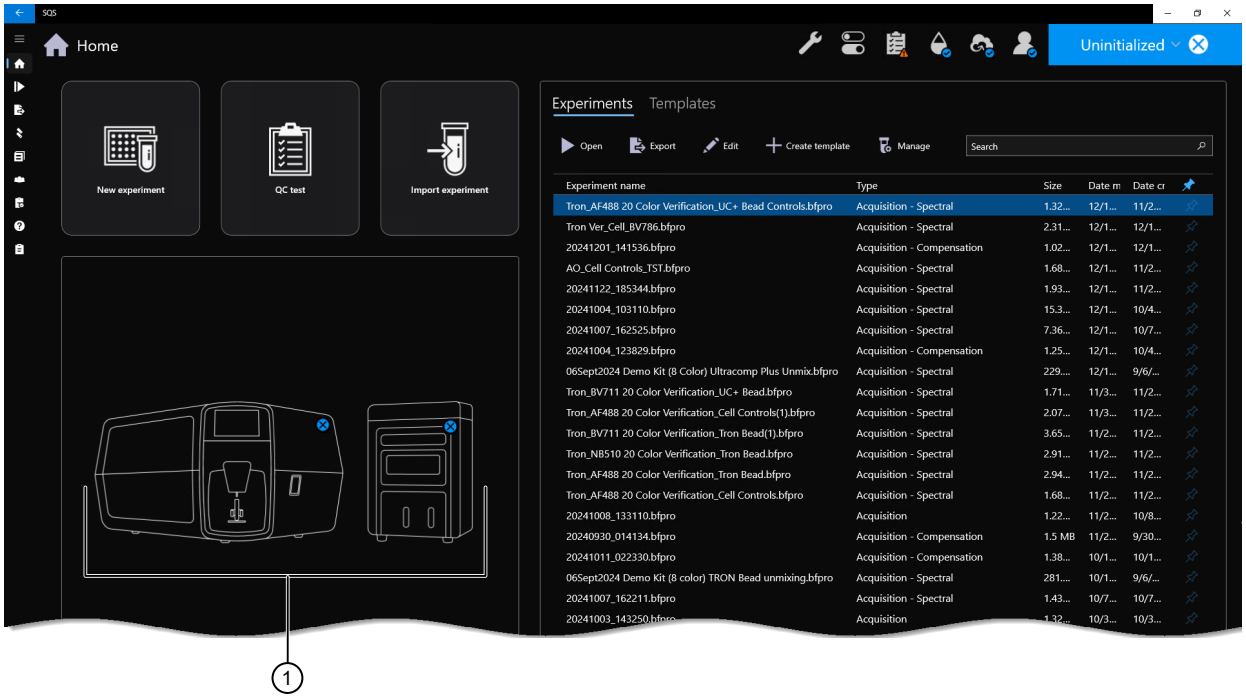
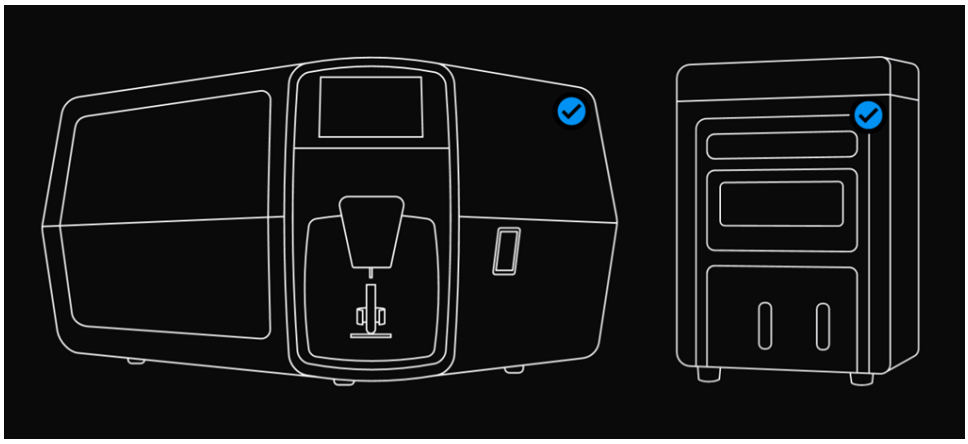


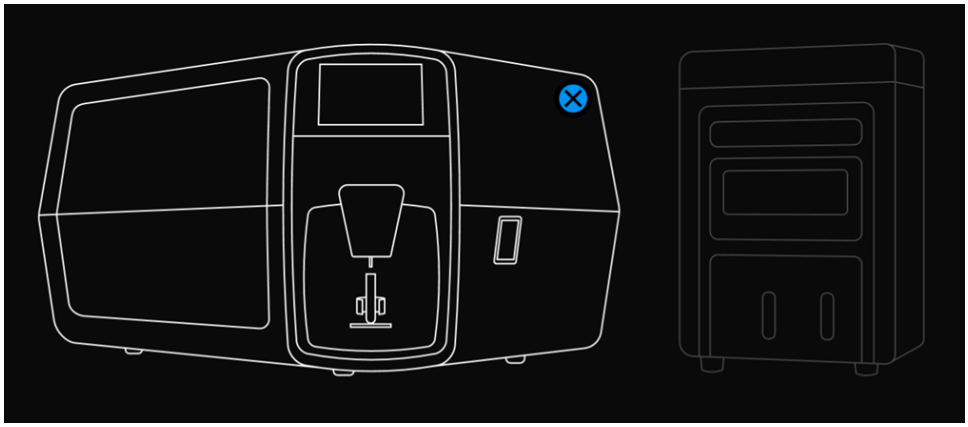
Figure 23

① Instrument status

- The connection status, initialization status, and error status of the instrument and the autosampler are indicated with the following symbols:
  - Blue checkmark symbol indicates that the instrument or the autosampler is connected and initialized. The system is ready and healthy with no issues detected.
  - Blue cross symbol indicates that the instrument or the autosampler is connected, but not initialized.
  - Amber warning exclamation symbol indicates an error state in the instrument or the autosampler.
- If the autosampler has never been connected to the instrument, it is not shown in the **Instrument status** picture.



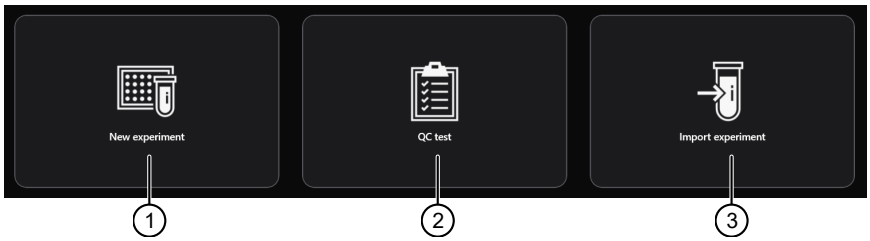
Instrument and autosampler are connected, initialized, and ready.



Instrument is connected, but not initialized. Autosampler is not connected.

Home screen buttons

The **Home** screen buttons (**New experiment**, **QC test**, and **Import experiment**) enable you to create new experiments, run baseline and daily QC tests, and import experiments.



① **New experiment**
② **QC test**
③ **Import experiment**

Figure 24 Home screen buttons

New experiment

New experiment button opens the **New Experiment Setup** screen, which enables you to create a new experiment to run on the Attune™ Xenith™ Flow Cytometer.

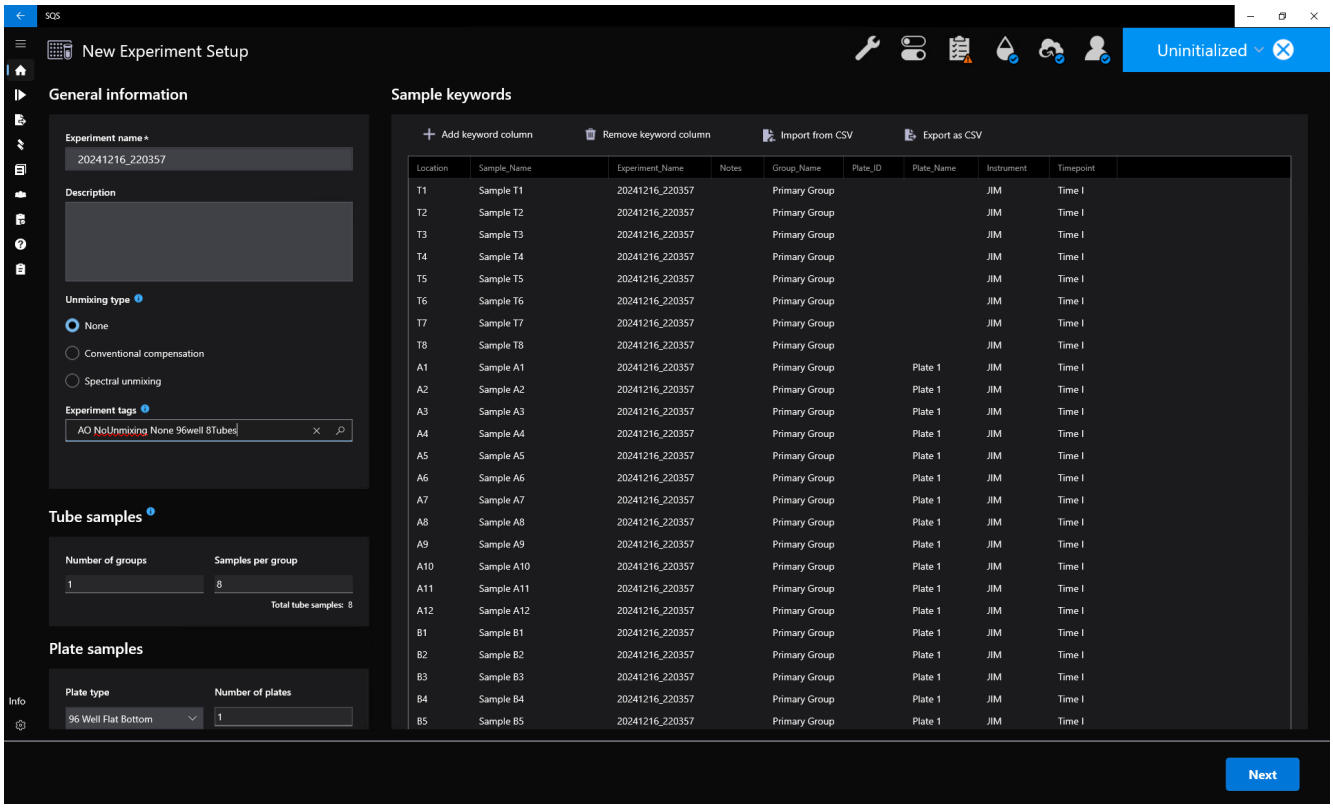


Figure 25 New Experiment Setup screen

Note: For more information about how to create new experiments using the **New Experiment Setup** screen, see Chapter 4, “New Experiment Setup”, on page 50.

QC test

QC test button opens the **QC Test** screen, which provides instructions for performing the **Baseline** or the **Daily QC** test using the Attune™ Xenith™ Flow Cytometer Quality Control Beads.

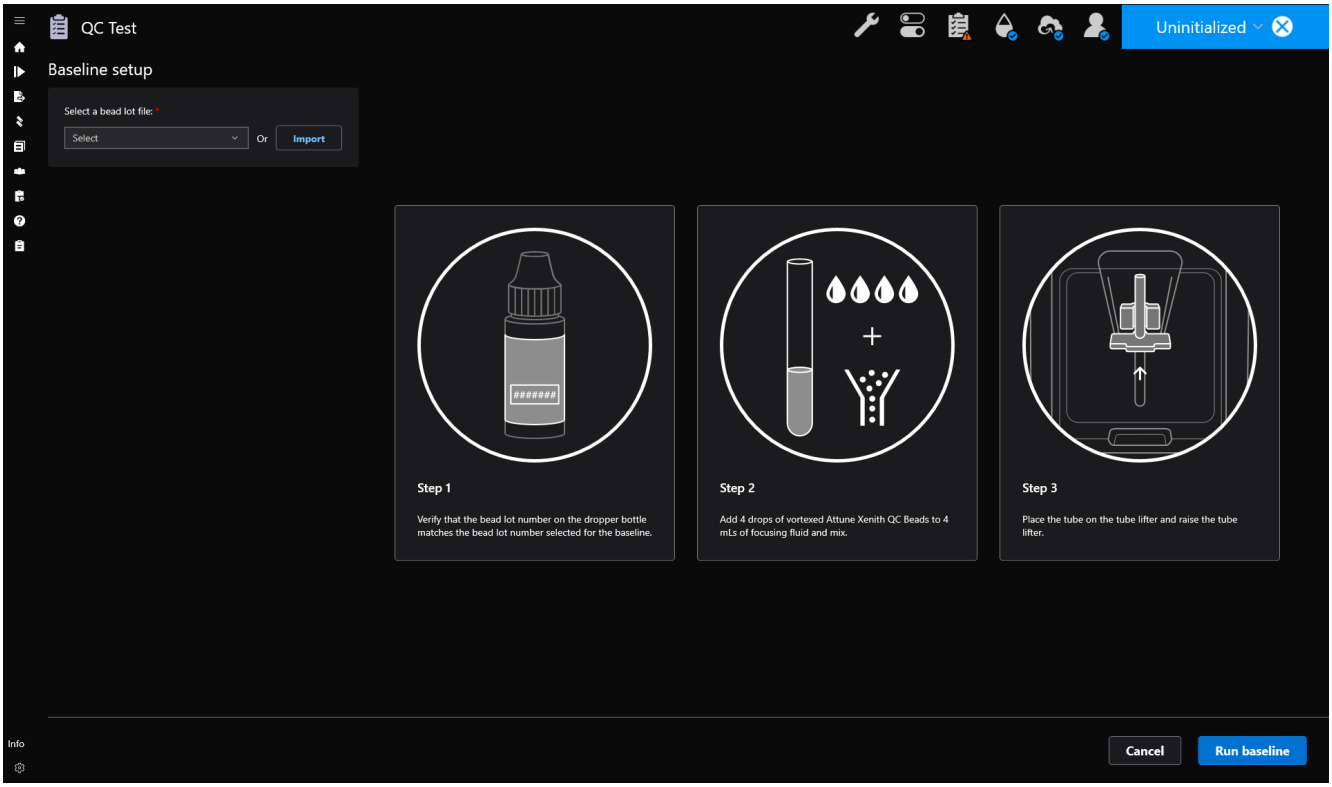


Figure 26 QC Test screen (Baseline setup)

You need to run the **Baseline** first before running the **Daily QC** test. If no **Baseline** exists, the **QC Test** screen only provides the option for the **Baseline**.

If **Startup** has not been performed, the prompt to run **Startup** before proceeding to run the **QC Test** is shown.

Note: For more information about the **QC Test** screen and how to run **Baseline** and **Daily QC** tests, see Chapter 9, “QC Test”, on page 169.
For detailed instructions about performing the QC tests on the Attune™ Xenith™ Flow Cytometer, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Import experiment

Import experiment button opens the **Open** file dialog, which enables you to import saved experiments (*bfpro files) from the database or a network location to run on the Attune™ Xenith™ Flow Cytometer.

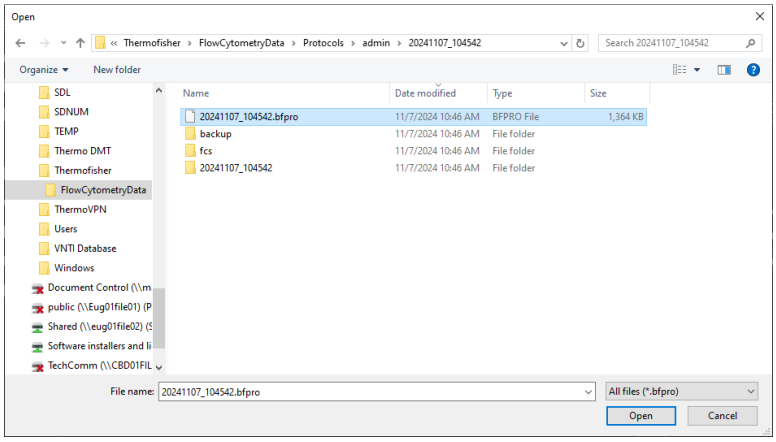


Figure 27 Open file dialog

The default save location for experiments is **D:\Thermofisher\FlowCytometryData\Protocols**, but you can save the files generated by the instrument on any location on the computer or the network.

- **Administrator** accounts can change the global default save location for experiments in the **Global Settings ▸ Files/Storage** screen.
- **Standard user** accounts can change the default save location for their own experiments in the **User Settings ▸ Files/Storage** screen.

Status bar

Status bar is organized into a series of tabs that provide status details and notifications about the system and system components, tools to perform system maintenance, and sign in options.

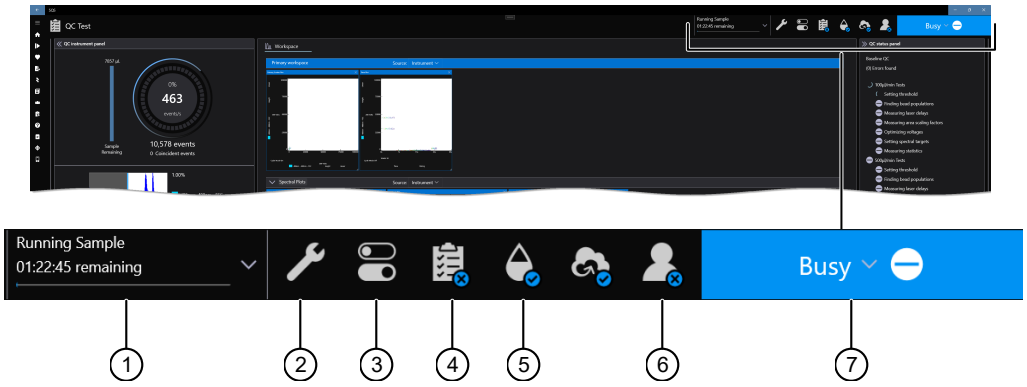


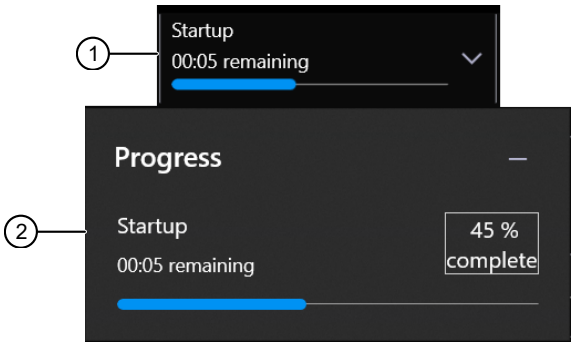
Figure 28 Status bar

1. Progress (page 41)
2. Maintenance (page 42)
3. Tools (page 43)
4. QC (page 44)
5. Fluidics (page 45)
6. User (page 46)
7. Instrument status (page 47)

Progress

Progress is visible only when acquisition or maintenance tasks are in progress. When visible, the **Progress indicator** shows the approximate time remaining for the current task to be complete.

To see the percent completion of the task, click the **down arrow** icon to open the **Progress details** window.



1. Progress indicator
2. Progress details

Figure 29 Progress indicator and Progress details

Maintenance

Maintenance tab opens the **Maintenance menu**, which enables you to perform system maintenance procedures.

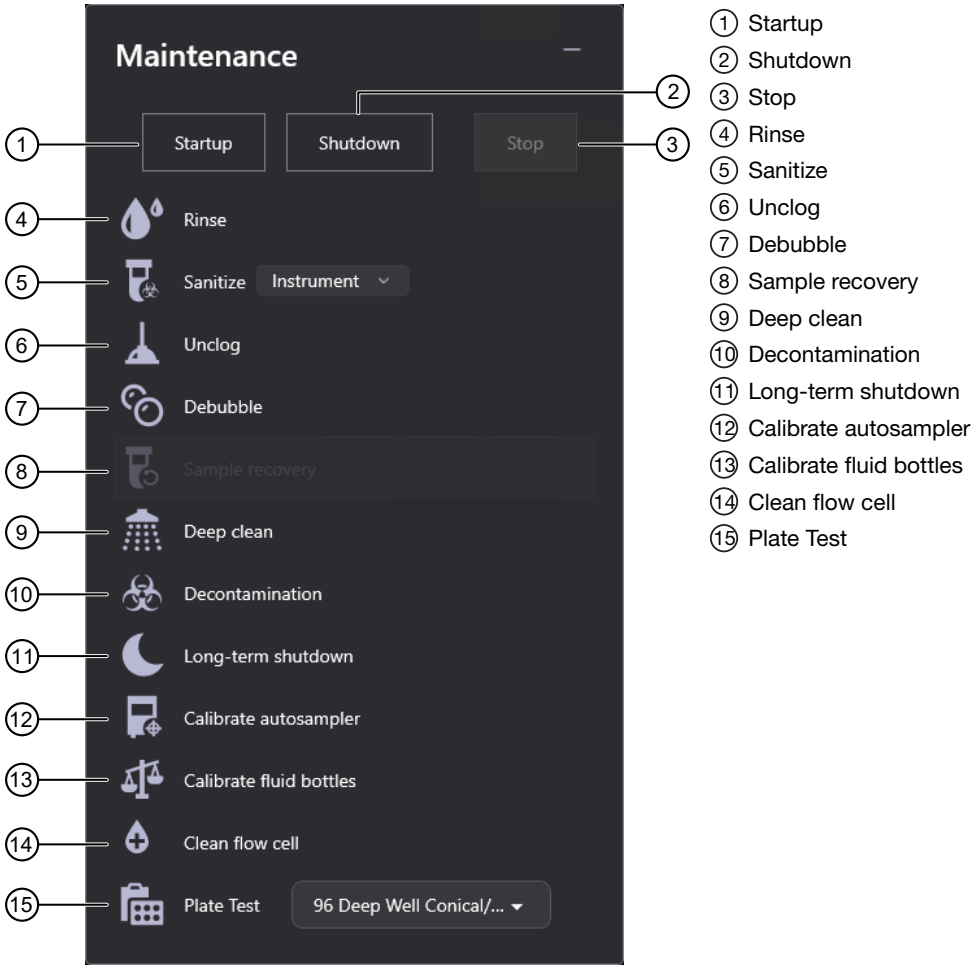


Figure 30 Maintenance menu

- Maintenance procedures are available globally from the **Maintenance menu**.
- **Startup** and **Shutdown** procedures can be performed without signing in and are also available on the instrument touchscreen.
- Maintenance procedures can require users to perform specific tasks or that the hardware is in a specific state (for example, tube lifter up).
- The system shows instructions for maintenance procedures that require users to perform tasks that cannot be automated or detected by the system. You can view the maintenance instructions in the Flowscape™ Software or the instrument touchscreen.

  Note: Many **Maintenance menu** options require that the system is in an **initialized** state and do not function when the system is shutdown or powered off.

- Specific plates can be assigned for use when performing instrument maintenance, including in the CytKick™ / CytKick™ Max Autosampler (if connected).

IMPORTANT! Stopping maintenance procedures that involve bleach use requires the **Startup** to be run to remove the bleach from the system.

Note: For more information about maintenance procedures, including descriptions of **Maintenance menu** options, see Chapter 13, “Maintenance”, on page 230.

Tools

Tools tab enables you to eject or retract the sample tray of the CytKick™ / CytKick™ Max Autosampler (if connected) directly from the software.

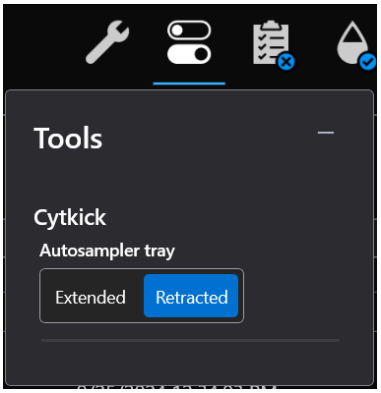


Figure 31 Tools

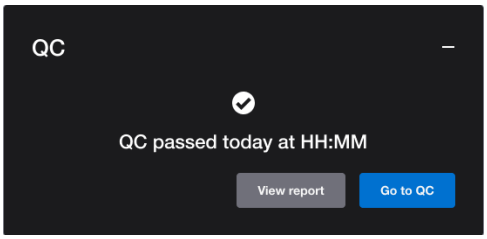
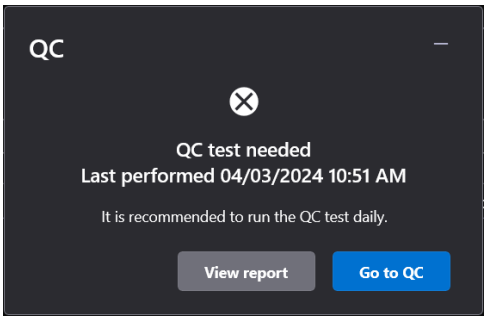
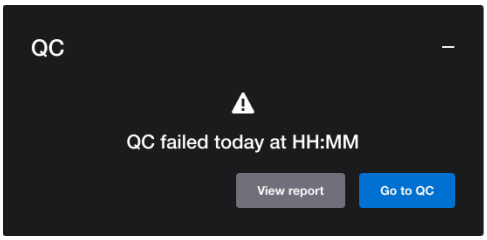
QC

QC tab indicates the **QC status** of the instrument (passed, test needed, failed) and opens the **QC dropdown**.

QC dropdown shows the details of the **QC status** and provides links to the **QC report** and the **QC screen**.

- To view the **QC report**, click **View report**.
- To go to the **QC screen**, click **Go to QC**.

Table 3 QC tab and QC status

QC status	QC tab	QC dropdown
QC test passed		
QC test needed		
QC test failed		

Fluidics

Fluidics tab indicates the instrument fluidics status and opens the **Fluidics dropdown**.

The **Fluidics dropdown** shows details (fill levels and volumes) of each fluid container in the fluid cart (**DI water**, **Bleach**, **Wash solution**, **Shutdown solution**, **Focusing fluid**, and **Waste**).

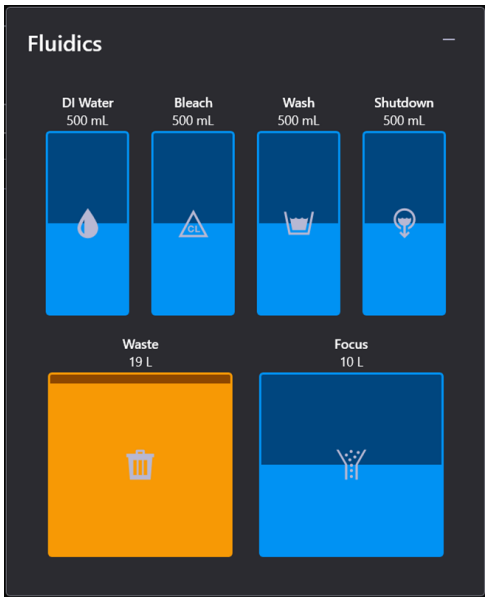


Figure 32 Fluidics dropdown showing fluid levels in each fluid container.

- The fluid containers that are sufficiently full and the waste container that has sufficient capacity are shown in blue. In this healthy state, the **Fluidics** icon on the **Status bar** shows the blue checkmark symbol.

- When the fluids reach a threshold level (too little fluid in fluid containers or too much in the waste container), the fluid containers are shown in amber color, which indicates an alarm state.

  When one or more of the fluid container are in the alarm state, the **Fluidics** icon shows amber warning exclamation symbol that indicates an alarm state.

Note: When fluids reach the critical state, the software will prompt the user to replace (or empty) the fluid when a function is run where the fluid is required.

Note: The instrument fluidics status and fluid levels in the containers are also shown on the instrument touch screen.

User

User tab indicates the user sign in status and opens the **User status dropdown**.

User status dropdown shows the name of the current user and the session time elapsed since the sign in. The dropdown also provides two controls, **Account** and **Sign out**.

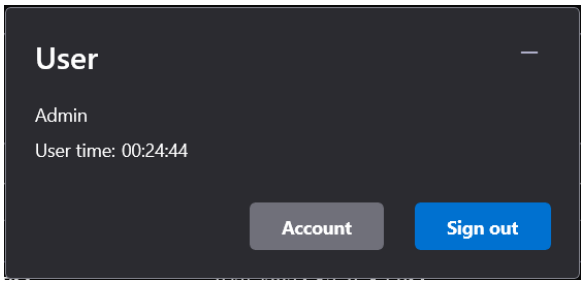


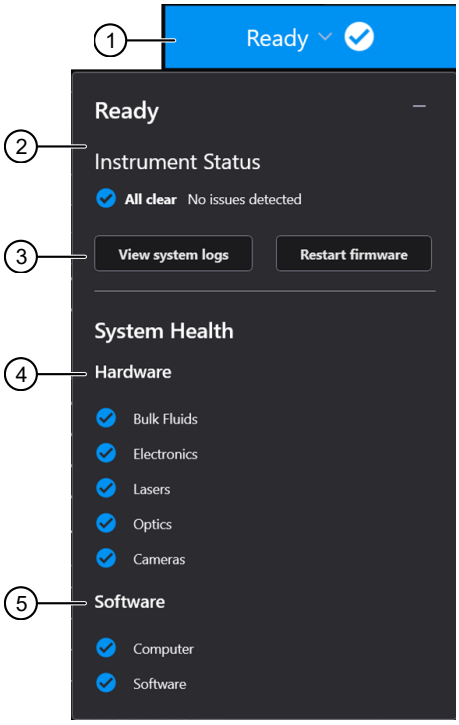
Figure 33 User status dropdown

- **Account**: For **Administrator** accounts, opens the **User Management** screen (page 29).
  For **Standard** accounts, opens the **Reset User Password** screen (page 30).
- **Sign out**: Enables the you to sign out of the software.

Note: The **User status dropdown** provides the only option to sign out of the software.

Instrument status

Instrument status indicates the instrument status (**Ready**, **Uninitialized**, **Busy**, **Asleep**, **Error**) and opens the **Instrument status dropdown**, which provides further details on the instrument status and system health.



① Instrument status icon
② Instrument Status
③ **View system logs** and **Restart firmware** buttons
④ System Health – Hardware
⑤ System Health – Software

Figure 34 Instrument status dropdown (Ready)

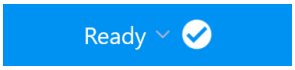
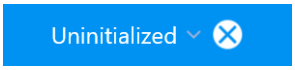
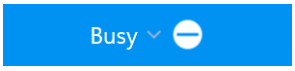
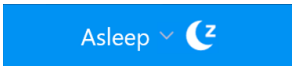
The **Instrument status dropdown ▸ System Health** indicates the health and status of the following system hardware and software components:

- **Hardware**:
  - Bulk fluids
  - Electronics
  - Lasers
  - Optics
  - Cameras
- **Software**:
  - Computer
  - Software

The dropdown also provides two controls, **View system logs** and **Restart firmware**.

- **View system logs**: Opens the **System Log Viewer**.
- **Restart firmware**: Restarts the instrument firmware.

Table 4 Instrument status indicators

Indicator	Instrument status	Details
	Ready	All clear. No issues detected.
	Uninitialized	All clear. No issues detected. System needs to be initialized before use.
	Busy	Busy. System is performing an operation.
	Busy	Busy. System is performing an operation. Warnings are located in the dropdown **Status** menu.
	Asleep	Asleep. System has been shutdown.
	Error	Error. System has one or more error states.

Experiments and Templates lists

The **Experiments** and **Templates** tabs list the experiments and templates available to the current user in table form. The lists act as an interface for creating, viewing, and managing experiments and templates.

Note: Only the experiments created or imported by the current user are available in the **Experiments** tab. **Templates** are global to all users.

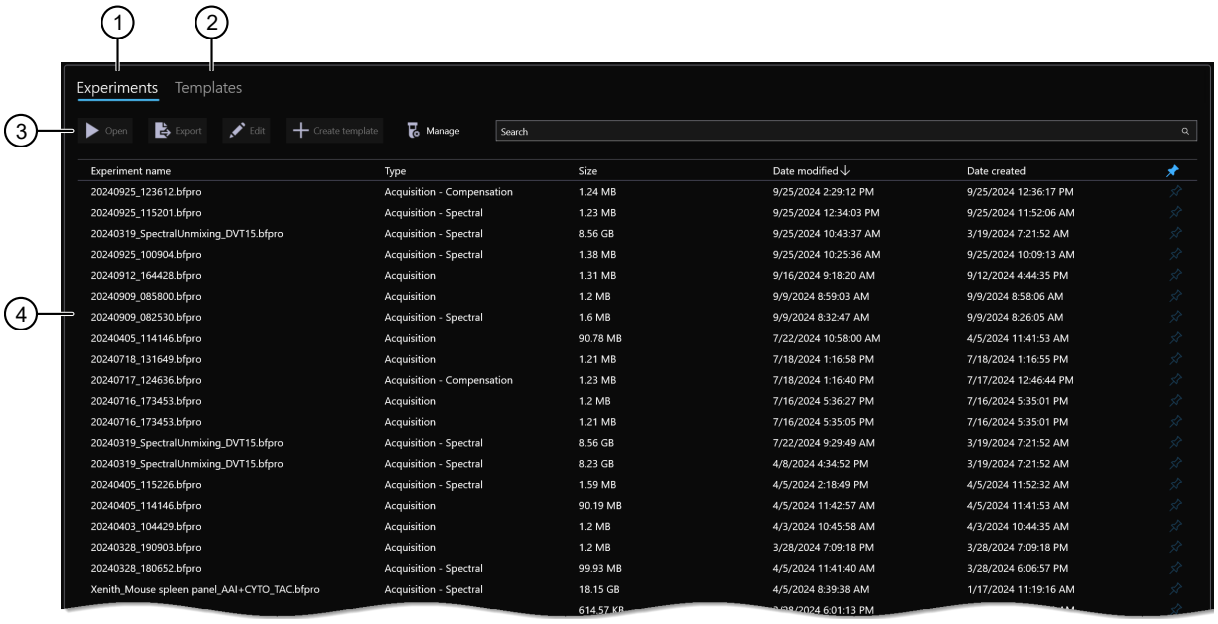


Figure 35 Experiments and Templates tabs (Experiment tab is selected)

① Experiments tab
② Templates tab
③ Experiments/Templates tools
④ Experiments/Templates table

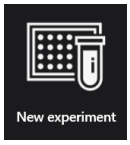
- Using the **Experiments table**, you can open existing experiments, create experiments from templates, and duplicate existing experiments.
- Using the **Experiments/Templates tools**, you can access the **Experiment management** screen, where you can export experiments and templates, delete experiments and templates, and import templates.
- The tables include the following columns:
  - Experiment name
  - Type
  - Size
  - Date modified
  - Date created
  - Pin tool

  You can sort the experiments and templates using the column headers and pin selected experiments or templates using the **pin tool** 📌.

4 New Experiment Setup

New Experiment Setup screen

New Experiment Setup screen guides through the process of creating and setting up new experiments. To open the **New Experiment Setup** screen, click the **New experiment** button in the **Home** screen.



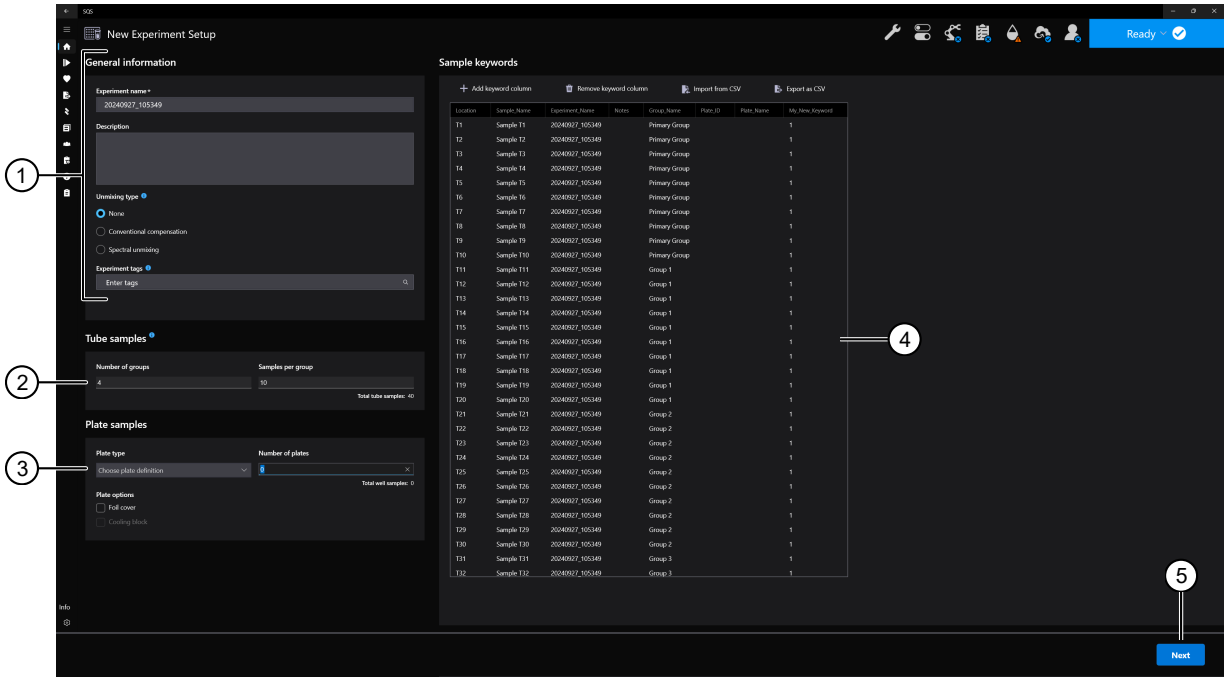


Figure 36 New Experiment Setup screen

1. General information
2. Tube samples
3. Plate samples
4. Sample keywords
5. Next button

Note: Depending on the **Unmixing type** (page 51) you select for the experiment, the **New Experiment Setup** screen provides different options after the first screen.

General information

General information section of **New Experiment Setup** enables you to name the experiment, provide a description, select an unmixing type, and enter experiment tags that can be used as search criteria to find experiments in the future.

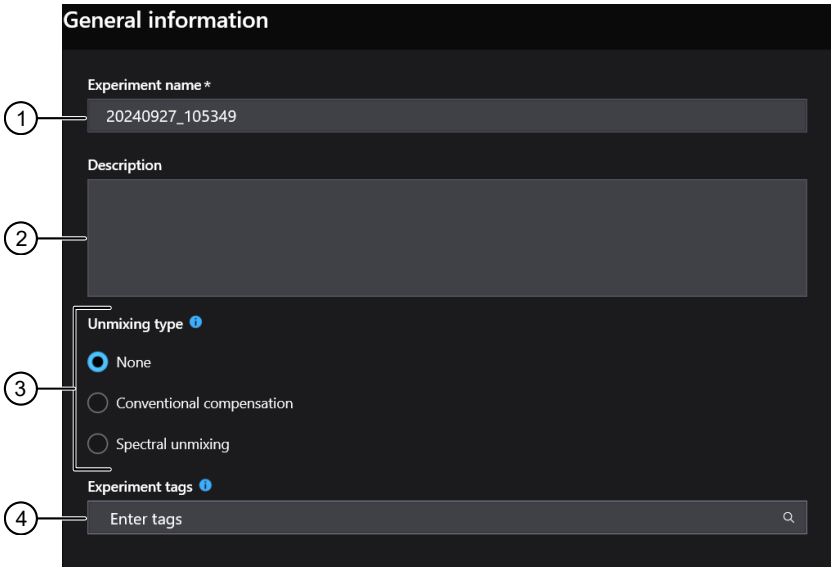


Figure 37 General information

Unmixing type

Unmixing type sets the unmixing type for the experiment. There are three options:

- **None**: Creates an experiment with all lasers and channels enabled and without single color controls, pre-populated control groups, and workspaces.

  When **None** is selected, clicking **Next** opens the **Workspace** for the new experiment.
- **Conventional compensation**: Emitted light is measured from a **single detector** and the spillover due to overlapping fluorophores is corrected via single channel-by-single channel compensation matrix.

  When **Conventional compensation** is selected, clicking **Next** opens the **Fluorophore Selection - Compensation** screen.

  For more information, see “Fluorophore Selection - Conventional compensation” on page 55.
- **Spectral unmixing**: Emitted light is measured across **all available detectors** and the similarity of fluorophores is corrected via all channels-by-all channels unmixing matrix, where the unique spectral signature of each fluorophore is distinguished by the unmixing algorithm.

  When **Spectral unmixing** is selected, clicking **Next** opens the **Fluorophore Selection - Spectral unmixing** screen.

  For more information, see “Fluorophore Selection - Spectral unmixing” on page 62.

The recommended unmixing type depends on the experiment design. When selecting the **Unmixing type** for the experiment, consider the following factors:

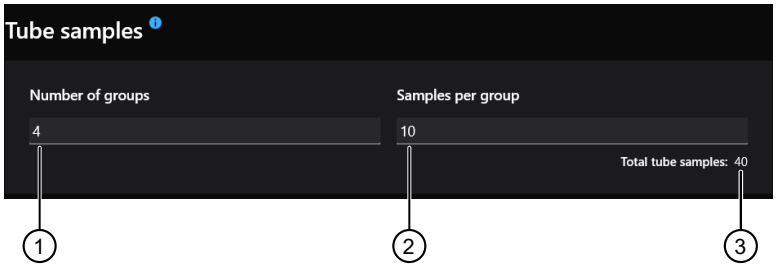
Table 5 Guidelines for unmixing type selection

Conventional compensation	Spectral unmixing
• Panels of smaller sizes or limited single channel overlap. • Panels that include functional dyes, which often require separate detector adjustments. (Adjusting the full spectra of functional dyes to keep the signal on scale in bright samples can lead to confusing spectral signatures.)	• Panels above ~15 colors (can also use for smaller panels). • Samples with autofluorescence populations. • Panels where you are not certain which best peak channel to select. • Highly beneficial when handling limited samples.

IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

Tube samples

Tube samples enables you to set the number of non-control tube samples and tube sample groups to be created when the new experiment loads.



① Number of groups
② Samples per group
③ Total tube samples

Figure 38 Tube samples

- **Number of groups**: Enables you to set the number of tube sample groups for the experiment.
- **Samples per group**: Enables you set the number of samples per group.
- **Total tube samples**: Shows the total number of tube samples (number of groups × samples per group) in the experiment.

Note: An experiment can have up to 400 tube samples.

Note: **Groups** are used to partition the workspace into functional sections, for example to show control samples such as compensation controls or the negative control. They can also be used to display different types of samples with different gating strategies simultaneously. The first group in every experiment is labeled as **Primary Group**.

Plate samples

Plate samples enables you to select the plate type, number of plates, and plate options for the experiment.

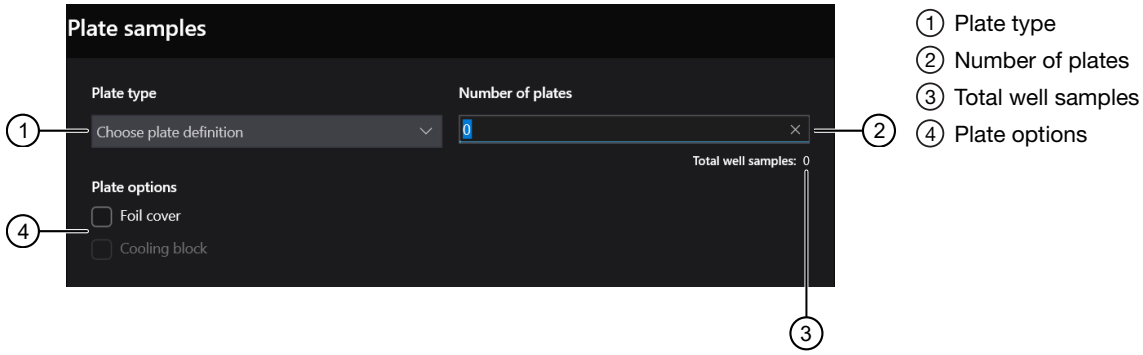


Figure 39 Plate samples

- **Plate type**: Enables you to select the plate type for the experiment (for example, 96 Well Flat Bottom).
- **Number of plates**: Enables you set the number of plates per experiment.
- **Total well samples**: Shows the total number of well samples (plate capacity × number of plates) in the experiment.

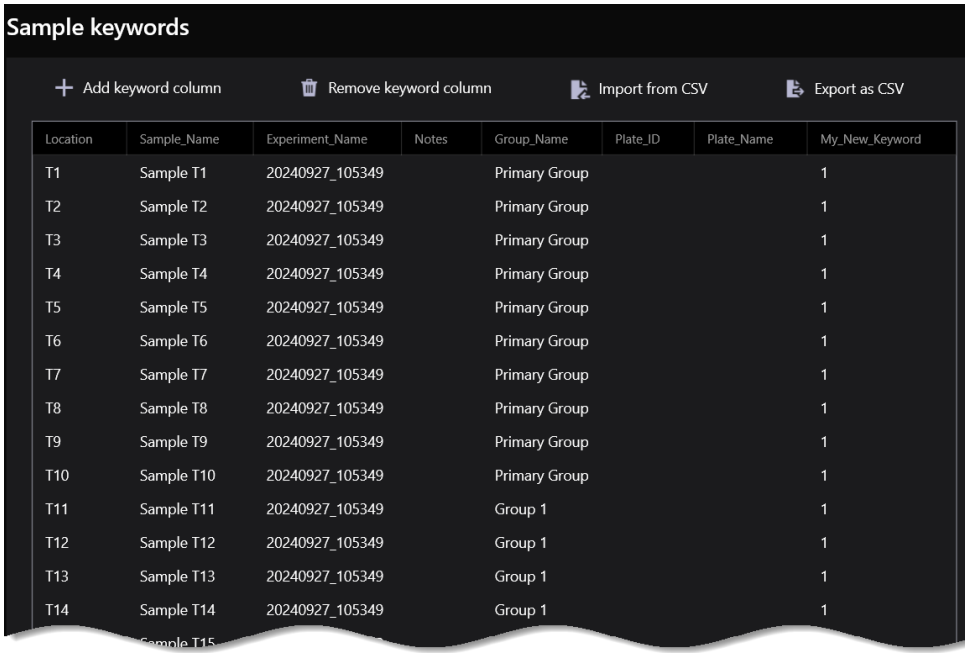
  Note: When the new experiment loads, the predefined plates are created with all the wells defined. For example, a 96 well plate will have all its 96 wells shown in the **Workspace**.

- **Plate options**: Enables you to use **Foil cover**, **Cooling block**, or both with the experiment plates.
  - When the **Foil cover** option is selected, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate to protect the sample plate from condensation or evaporation.
  - When **Cooling block** option is selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification.

Note: **Foil cover** and **Cooling block** options are available only when a CytKick™ Max Autosampler is connected to the Attune™ Xenith™ Flow Cytometer.

Sample keywords

Sample keywords enables you to update sample keywords (sample name, group name, etc.) and to create custom keywords during experiment setup. Sample keywords are saved in the FCS file metadata and can be helpful when third-party software is used for analysis.



Location	Sample_Name	Experiment_Name	Notes	Group_Name	Plate_ID	Plate_Name	My_New_Keyword
T1	Sample T1	20240927_105349		Primary Group			1
T2	Sample T2	20240927_105349		Primary Group			1
T3	Sample T3	20240927_105349		Primary Group			1
T4	Sample T4	20240927_105349		Primary Group			1
T5	Sample T5	20240927_105349		Primary Group			1
T6	Sample T6	20240927_105349		Primary Group			1
T7	Sample T7	20240927_105349		Primary Group			1
T8	Sample T8	20240927_105349		Primary Group			1
T9	Sample T9	20240927_105349		Primary Group			1
T10	Sample T10	20240927_105349		Primary Group			1
T11	Sample T11	20240927_105349		Group 1			1
T12	Sample T12	20240927_105349		Group 1			1
T13	Sample T13	20240927_105349		Group 1			1
T14	Sample T14	20240927_105349		Group 1			1

Figure 40 Sample keywords

- Default sample keywords are **Location**, **Sample_Name**, **Experiment_Name**, **Notes**, **Group_Name**, **Plate_ID**, and **Plate_Name**, which are arranged in columns for each sample.
- **Add keyword column** adds a new column to the sample keywords table for a custom keyword.
- **Remove keyword column** removes the keyword column from the table and deletes the associated keyword data from the FCS file metadata.
- **Import from CSV** enables you to import keyword data from existing CSV files.
- **Export as CSV** enables you to save sample keywords in a CSV file.
- You can copy and paste information to and from the keywords table.
- You can create custom keywords and select which keywords to use by default in experiments.

Note: Changing the tube sample and plate sample settings after keyword values are modified resets the sample keyword entries.

Fluorophore Selection - Conventional compensation

Fluorophore Selection screen enables you to select the fluorophores for the experiment.

When **Conventional compensation** is selected for **Unmixing type**, the screen is divided into four sections: **Select fluorophores** (page 56), **Check/Assign detectors** (page 58), **Compensation Options** (page 60), and **Detector Options** (page 61).

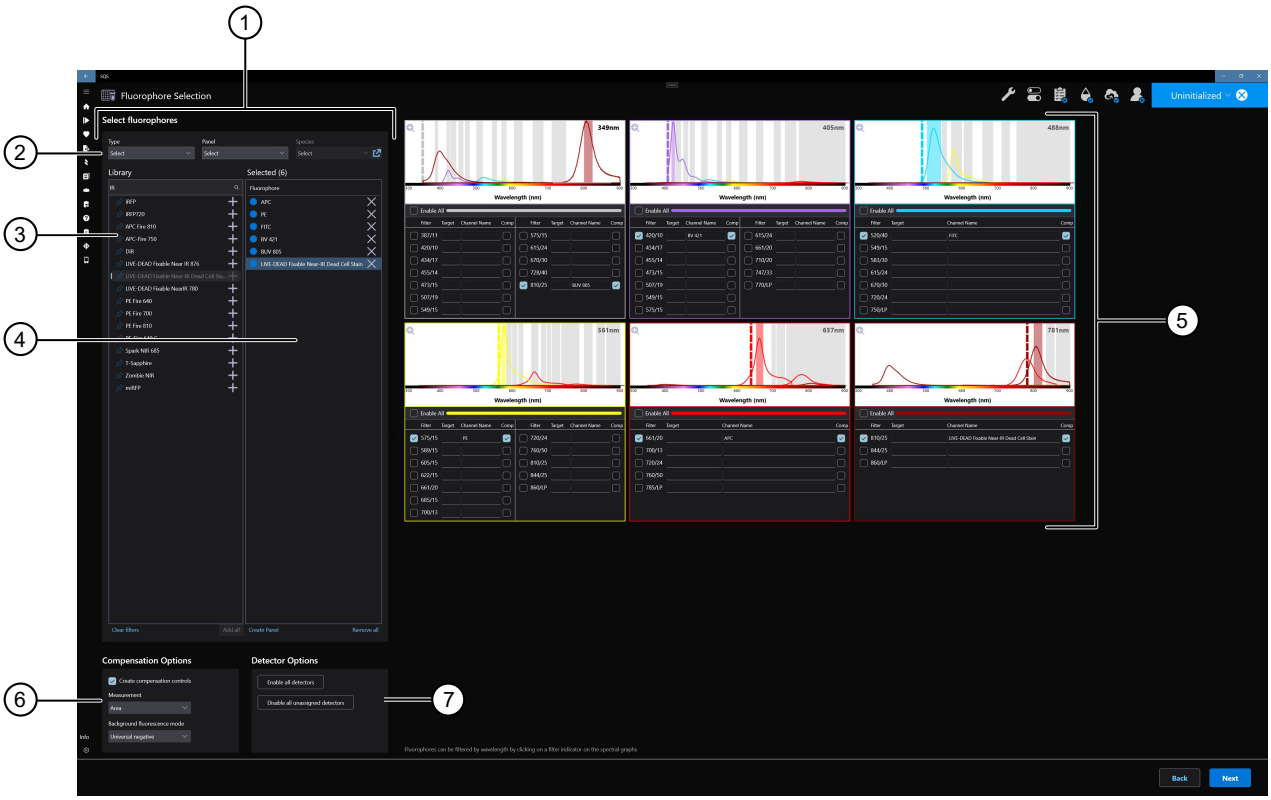


Figure 41 Fluorophore Selection screen for Conventional compensation experiments

1. Select fluorophores
2. Fluorophore filters (Type, Panel, Species)
3. Library
4. Selected fluorophores
5. Check/Assign detectors
6. Compensation Options
7. Detector Options

Select fluorophores

Select fluorophores panel enables you to select the fluorophores for the experiment from a library of available fluorophores.

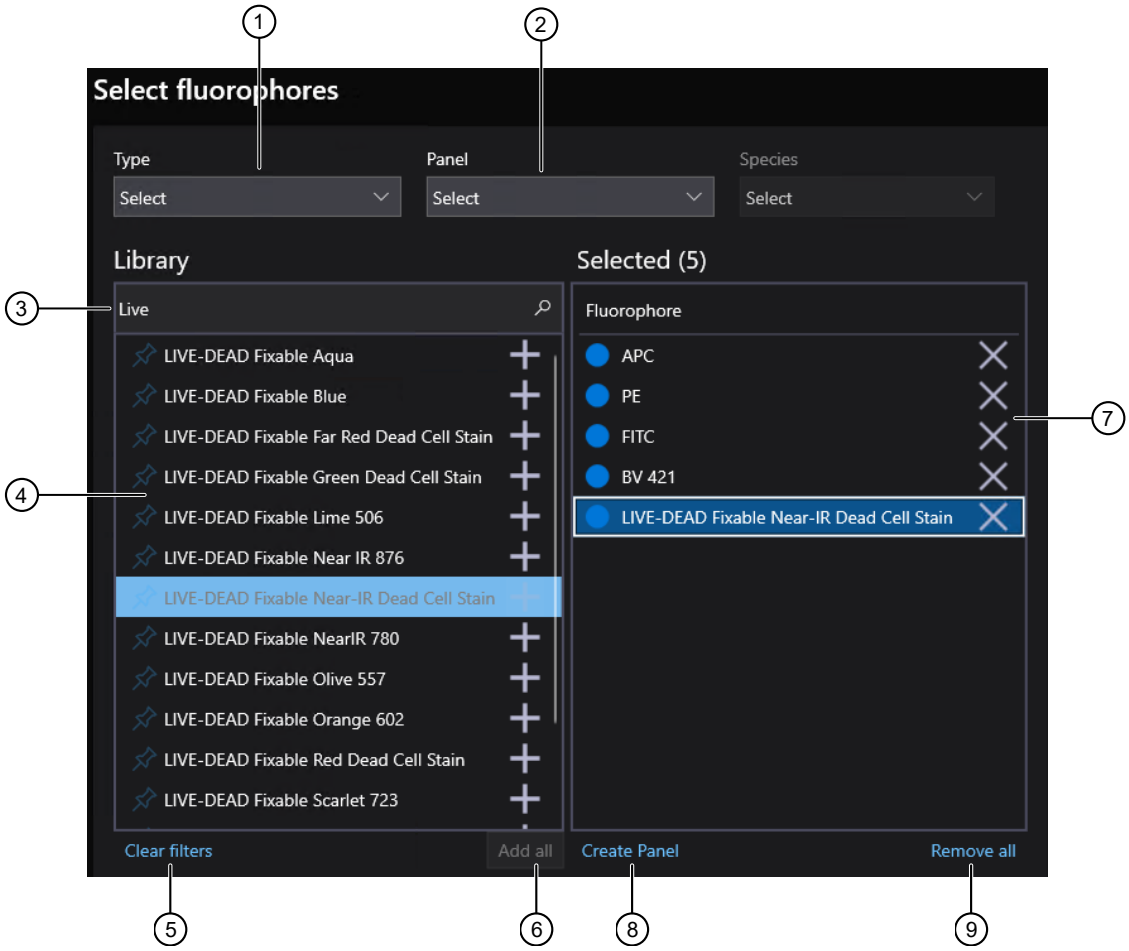


Figure 42 Select fluorophores panel for experiments with Conventional compensation

① Type filter
② Panel filter
③ Search Library
④ Library
⑤ Clear filters
⑥ Add all
⑦ Selected fluorophores
⑧ Create Panel
⑨ Remove all

- **Type**: Filters the **Library** by the fluorophore type. Available options are:
  - Reactive dye
  - DNA binding
  - Live/Dead
  - Tandem dye
  - Fluorescent protein
  - pH indicator
  - Quantum Dot
  - Custom
- **Panel**: Filters the **Library** by the panel created and saved using the **Create Panel** button.
- **Search**: Enables you search the **Library** for specific fluorophores.

- **Library**: Lists the available fluorophores.
  - To select a fluorophore, click the fluorophore name in the **Library**.
  - To add a fluorophore to the experiment, click the **+** button next to the fluorophore name. Alternatively, double-click the fluorophore.
- **Clear filters**: Clears the filters used when searching the **Library**.
- **Add all**: Adds all selected fluorophores in a panel to the experiment. This option is available only when there is a panel selected from the **Panel** dropdown menu.
- **Selected**: Lists the fluorophores selected for the experiment. The number of selected fluorophores are indicated next to the table title. Each selected fluorophore is assigned a unique color to identify it in the **Spectral energy** plot.
  - To remove a fluorophore from the experiment, click the **×** button next to the fluorophore name.
- **Create Panel**: Enables you to save the panel of selected fluorophores for use in experiments.
- **Remove all**: Removes all selected fluorophores from the **Selected** list.

Check/Assign detectors

Check/Assign detectors section shows the **emission spectra plots** of the selected fluorophores, grouped by the excitation laser. The plots enable you to verify the assigned channels and detectors for the selected fluorophores.

Figure 43 Spectral plots of the selected fluorophores, grouped by the excitation laser

The selected fluorophores are automatically placed in the channel that is expected to have the brightest signal. If desired, you can activate more channels and manually assign detectors as compensation controls using the options under each plot. In the following example, FITC (the selected fluorophore) is placed in the 488 nm blue laser line in the channel with the 520/40 nm bandpass collection filter.

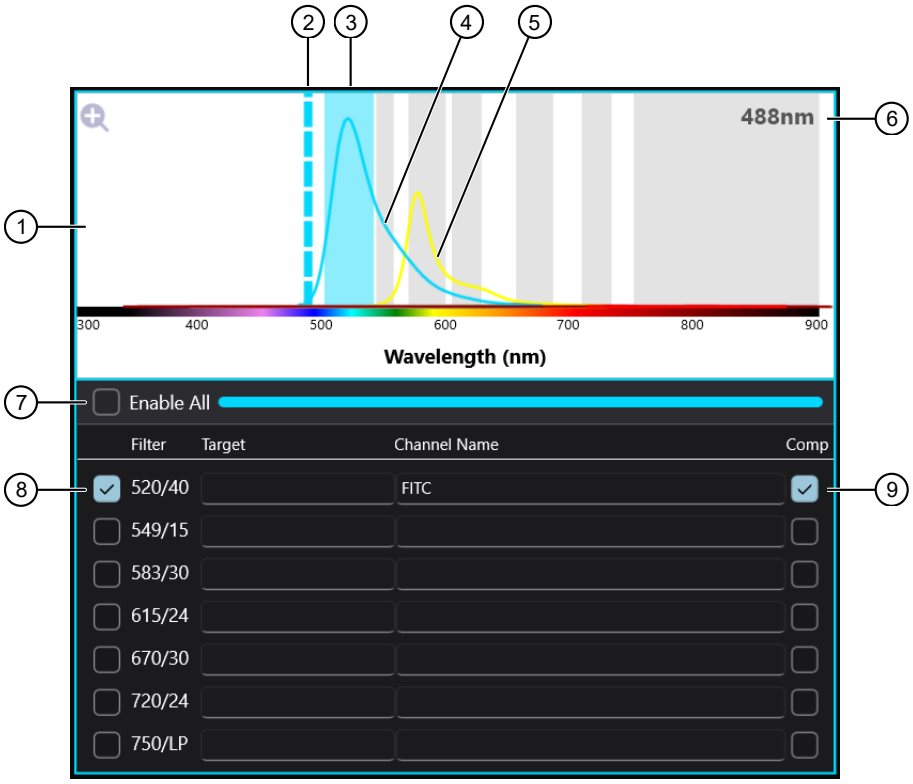


Figure 44 FITC channel in the 488 nm blue laser line

① Spectral plots in the Blue laser line (488 nm)
② Excitation wavelength (488 nm)
③ 520/40 nm bandpass filter used for collection
④ Emission spectra for FITC
⑤ Emission spectra for PE (overlap from Yellow laser line)
⑥ Excitation wavelength (488 nm)
⑦ Enable All option
⑧ Selected collection channel (520/40 nm bandpass filter)
⑨ Compensation control checkbox

- To activate more channels, check the filter under the laser you want to use.
- To enable all channels for collection for a laser, select **Enable All** under the laser you want to use.
- To manually assign a detector as a compensation control, click the **Comp** checkbox for the corresponding detector.

Compensation Options

Compensation Options enable you to create or disable compensation controls, select the measurement for calculating compensation, and select the type of unstained control to use to account for background fluorescence.

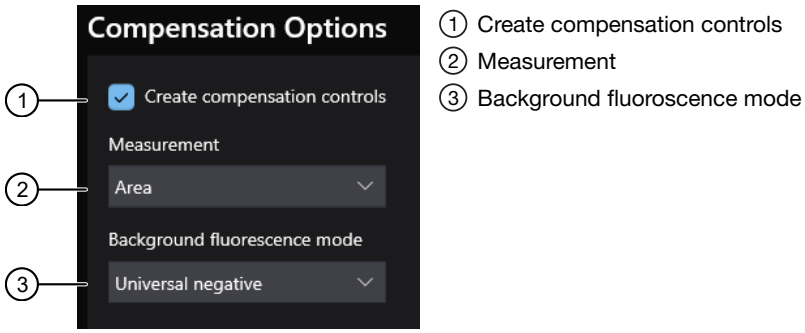


Figure 45 Compensation Options

- **Create compensation controls**: Creates the necessary compensation controls in the **Workspace**. By default, this option is selected and the compensation controls are automatically created.

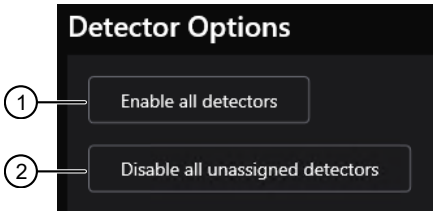
  When **Create compensation controls** is unchecked, the experiment is set up with the selected channels, but no compensation controls are created.
- **Measurement**: Enables you to select **Area** or **Height** as the parameter for calculating compensation. If the **Measurement** choice is changed, the **Workspaces** are updated to use the new **Measurement** choice and the compensation is recalculated.
- **Background fluorescence mode**: Enables you to select the source of the background fluorescence when calculating compensation. The controls are created with the **Workspaces** that reflect the **Background fluorescence mode** selected for the experiment. Available options are:
  - **Universal negative**: Adds an **Unstained** control as an extra compensation control to the **Control Group** in the **Sample panel** and creates the **Workspace** for the **Unstained** compensation control.

    Select the **Universal negative** option if you have an unstained control that can serve as universal negative. This provides an unstained control sample, but no negative gates for the controls.
  - **Internal negative**: Provides negative gates on single color controls but not a separate universal unstained control. Select this option if the controls contain a fluorescence reference internal negative.
  - **None**: No negative control is used and compensation is calculated without correcting for background autofluorescence.

Note: You can later change the **Background fluorescence mode** using the **Compensation Wizard** (see “Auto compensation” on page 85).

Detector Options

Detector Options include controls to enable and disable detectors.



① Enable all detectors
② Disable all unassigned detectors

Figure 46 Detector Options

- **Enable all detectors**: Enables all detectors.
- **Disable all unassigned detectors**: Turns off all detectors that are not assigned as compensation controls.

Note: Enabling all detectors has the following advantages:

- It ensures that the fluorescent signal from one fluorophore is detected in multiple detectors, even if it's not the intended detector. This enables the compensation process to more precisely account for spectral spillover, ensuring that the fluorescence signal detected in each channel accurately represents the quantity of the specific fluorophore.
- It allows you to import the single color controls from compensation experiments into spectral experiments, and analyze and compare the same experiment data as compensation and spectral experiments.

However, enabling all detectors increases the data footprint, which takes longer to load, transfer, and process.

Fluorophore Selection - Spectral unmixing

Fluorophore Selection screen enables you to select the fluorophores for the experiment.

Figure 47 Fluorophore Selection screen for Spectral unmixing experiments

1. Select fluorophores
2. Fluorophore filters (Type, Panel, Species)
3. Library
4. Selected fluorophores
5. Spectral energy
6. Spectral similarity matrix

When **Spectral unmixing** is selected for **Unmixing type**, the screen is organized into three sections:

- **Select fluorophores** (page 63)
- **Spectral energy plot** (page 66)
- **Spectral similarity matrix** (page 67)

Select fluorophores

Select fluorophores enables you to select the fluorophores for the panel from a library of available fluorophores, to assign **Target names** to the selected fluorophores, and to save the panel of selected fluorophores for use in future experiments.

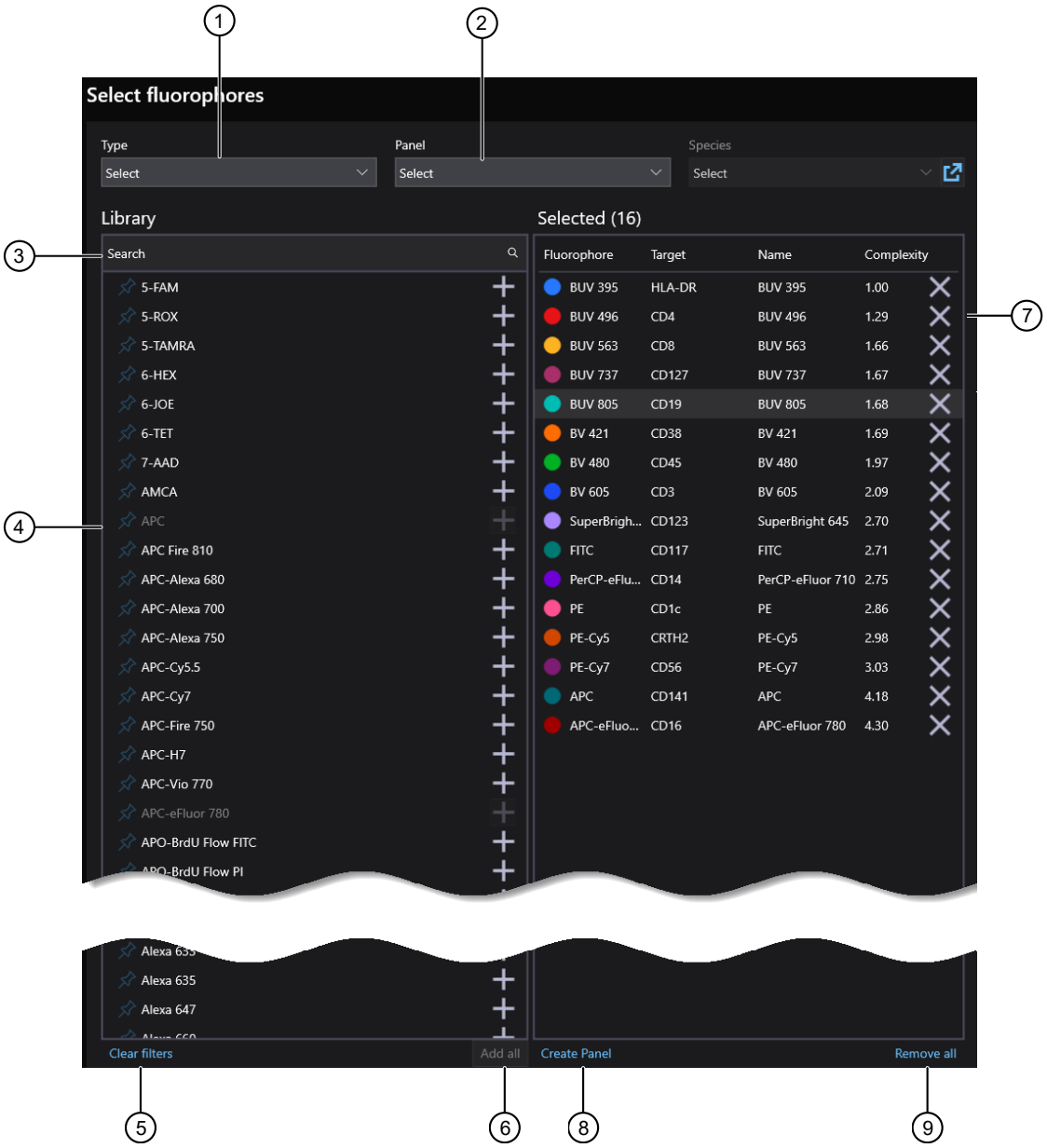


Figure 48 Select fluorophores for experiments with Spectral unmixing

1. Type filter
2. Panel filter
3. Search
4. Library
5. Clear filters
6. Add all
7. Selected fluorophores
8. Create Panel
9. Remove all

- **Type**: Filters the **Library** by the fluorophore type. Available options are:
  - Reactive dye
  - DNA binding
  - Live/Dead
  - Tandem dye
  - Fluorescent protein
  - pH indicator
  - Quantum Dot
  - Custom
- **Panel**: Filters the **Library** by the panel created and saved using the **Create Panel** button.
- **Search**: Enables you search the **Library** for specific fluorophores.
- **Library**: Lists the available fluorophores.
  - To select a fluorophore, click the fluorophore name in the **Library**.
  - To add a fluorophore to the experiment, click the **+** button next to the fluorophore name . Alternatively, double-click the fluorophore.
  - As fluorophores are added from the **Library** to the **Selected** list, the **Spectral energy** plot and the **Spectral similarity matrix** are populated with the selected fluorophores.
- **Clear filters**: Clears the filters used when searching the **Library**.
- **Add all**: Adds all selected fluorophores to the experiment. This option is available only when there is a panel selected from the **Panel** dropdown menu.
- **Selected**: Lists the fluorophores selected for the experiment. The number of selected fluorophores are indicated next to the table title.
  - Each selected fluorophore is assigned a unique color to identify it in the **Spectral energy** plot.
  - You can assign a **Target name** (for example, CD4) to selected fluorophores in the **Target** column of the **Selected** list.
  - To remove a fluorophore from the experiment, click the **×** button next to the fluorophore name.
  - When a fluorophore is removed from the panel, it is also removed from the **Spectral energy plot** and the **Spectral similarity matrix**.
  - The number in the **Complexity** column indicates the unmixing burden of the panel. This number is additive and increases a more fluorophores are added to the panel. For more information, see “Complexity number” on page 65.
- **Create Panel**: Enables you to save the panel of selected fluorophores for use in future experiments.
- **Remove all**: Removes all selected fluorophores from the **Selected** list.

Complexity number

Each fluorophore that is added to the panel triggers the calculation of a **complexity number** (also called **condition score**). The complexity number is typically defined as the ratio of the largest to the smallest singular value of the matrix (or, equivalently, the product of the matrix norm and the norm of its inverse). This number is additive and indicates how different the fluorophores are from one another.

- A **low complexity number** (close to 1) indicates a well-conditioned matrix, where small perturbations in the input data will only lead to small changes in the output.

  In spectral experiments, this means that each fluorophore is sufficiently different from the others in the panel that there is little overlap between the channels, and that the unmixing algorithm will have little difficulty of distinguishing the unique spectral signature of each fluorophore in the panel.
- A **high complexity number** indicates an ill-conditioned matrix, where small errors in the input can be amplified, leading to inaccurate solutions. A high condition number can cause issues with numerical algorithms, particularly iterative methods, as convergence can be slow or even fail.

  In spectral experiments, as more fluorophores are added to the panel, more overlaps occur between the channels, which increases the complexity number. A high complexity number indicates a heavier unmixing burden, which means that the unmixing algorithm will have more difficulty of distinguishing the unique spectral signature of each fluorophore in the panel.

The **Complexity index** is the complexity number as applied to spectral unmixing experiments. It indicates the unmixing burden of the panel, which describes the overall difficulty of distinguishing the unique spectral signature of each fluorophore in the panel by the unmixing algorithm.

Spectral energy plot

Spectral energy plot shows the expected (that is, theoretical) emission spectra of each fluorophore added to the panel. All spectral signatures in the panel are normalized so that they can be compared.

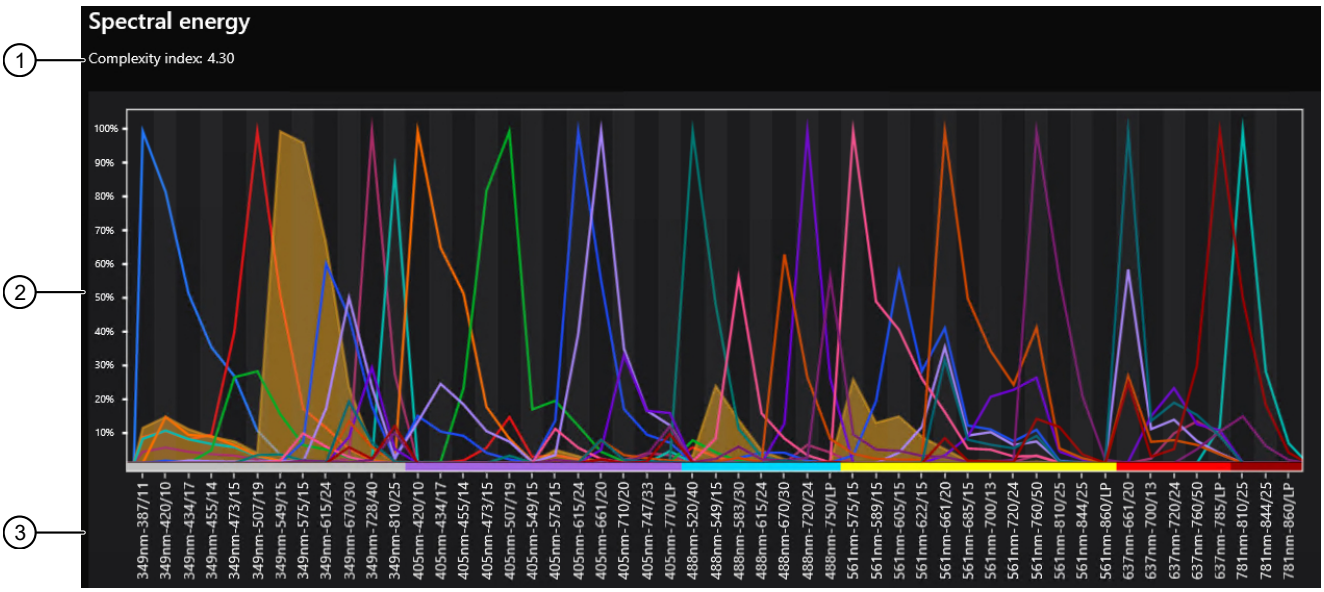
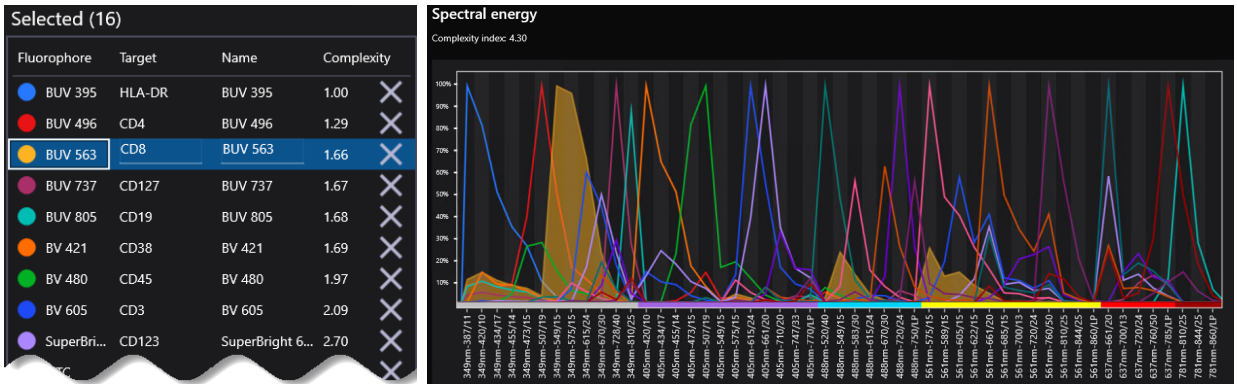


Figure 49 Spectral energy plot for a panel of 23 fluorophores

① Complexity index of the panel
② Expected emission spectra
③ Laser and filter configuration of the system

The **Complexity index** value above the plot indicates unmixing burden of the panel, which describes the overall difficulty of distinguishing the unique spectral signature of each fluorophore in the panel by the unmixing algorithm (see “Complexity number” on page 65).

When a fluorophore is highlighted in the **Selected** table, the emission spectra of the selected fluorophore is shaded in the **Spectral energy plot** with the color assigned to it.



Spectral similarity matrix

Spectral similarity matrix is used to identify combinations of fluorophores that can increase the complexity of the panel. It shows how alike the emission spectra are when comparing two fluorophores and highlights the most similar fluorophores. In the matrix, each fluorophore pair are assigned a **similarity score** on a scale of 0 to 1 (calculated as cosine similarity), where a score of 0 indicates that the spectra are entirely different and a score of 1 indicates that the fluorophores are identical.

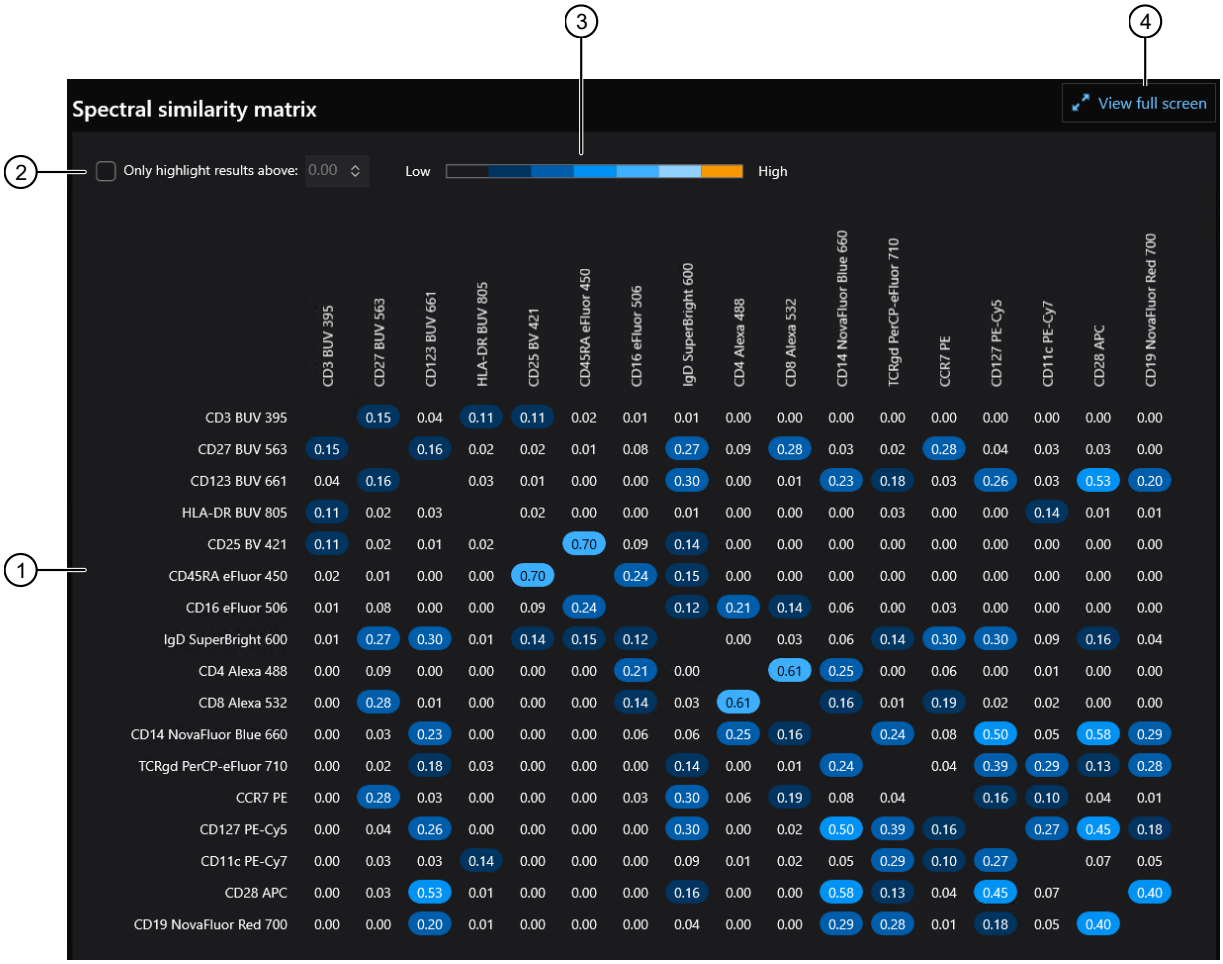


Figure 50 Spectral similarity matrix

1. Spectral similarity matrix
2. Only highlight results above
3. Similarity score legend
4. View full screen

Spectral Unstained

Spectral Unstained screen enables you to define unstained and autofluorescence controls, map fluorophores to unstained controls, and select spectral unmixing options.

Note: In experiments with conventional compensation, the controls are defined and mapped, and compensation options are selected in the **Fluorophore Selection** screen (“Fluorophore Selection - Conventional compensation” on page 55). This is different for experiments with spectral unmixing, where the equivalent tasks are performed in the **Spectral Unstained** screen.

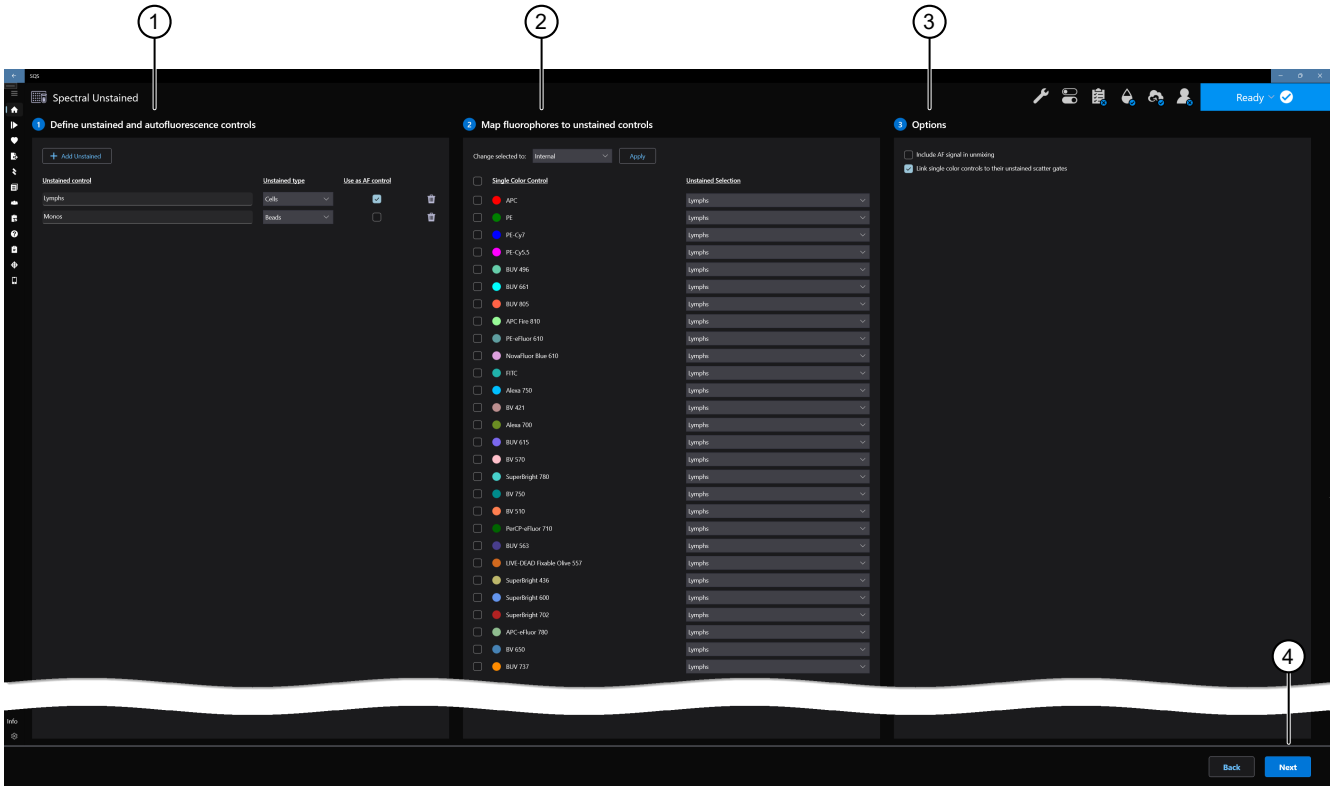


Figure 51 Spectral Unstained screen

① Define unstained and autofluorescence controls
② Map fluorophores to unstained controls
③ Options
④ Next button

Define unstained and autofluorescence controls panel enables you to add one or more unstained and autofluorescence controls to the experiment. You can select any unstained control that contains cell populations requiring autofluorescence extraction as an autofluorescence source to use in the unmixing of fully stained samples.

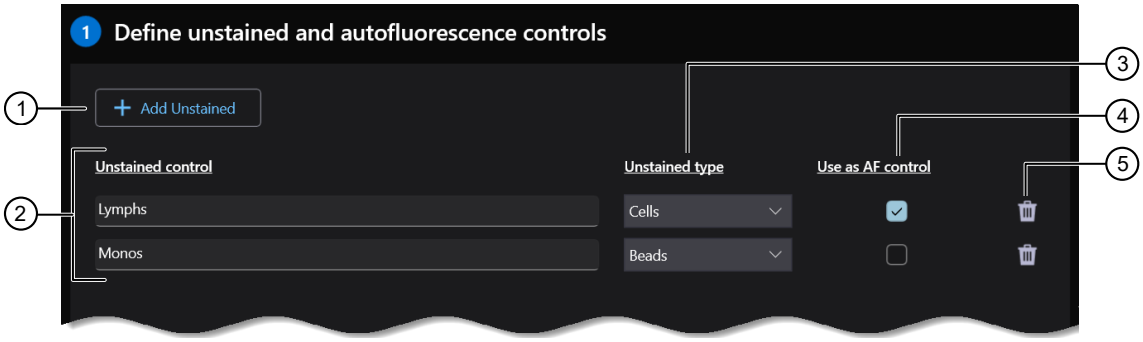


Figure 52 Define unstained and autofluorescence controls

1. Add Unstained
2. Unstained control
3. Unstained type
4. Use as AF control
5. Delete control

Map fluorophores to unstained controls panel enables you to map single color controls to unstained controls.

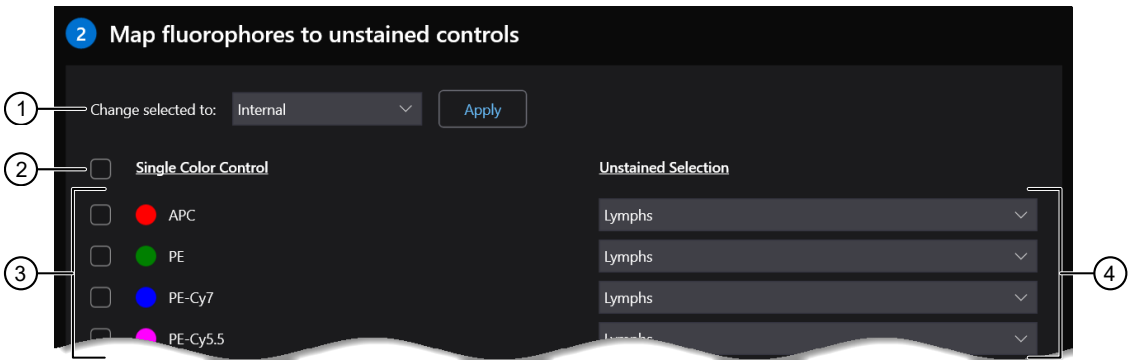


Figure 53 Map fluorophores to unstained controls

1. Change selected to
2. Select all single color controls
3. Single color controls
4. Unstained control selection

- Each single color control must be mapped to an external unstained control or, alternatively, to an internal negative control.
- When a single color control is mapped as internal negative, the single color control correction calculation relies on a negative population within the selected scatter gate.
- You can map an unstained control to multiple single color controls using the **Single Color Control** checkboxes, then clicking the **Apply** button.
- You can individually map each separate single color control to an unstained control using the **Unstained Selection** dropdown.
- The unstained control is used to subtract the dye-free cell or bead signature (autofluorescence) from the single color control to obtain the dye-only signature.

Options panel enables you select spectral options for the experiment.

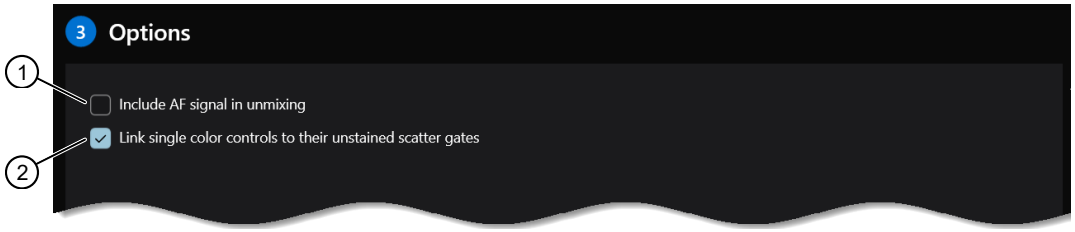


Figure 54 Options

① Include AF signal in unmixing
② Link single color controls to their unstained scatter gates

- By default, the AF (autofluoroescence) signal is not included in the unmixing. To extract the AF signal as a separate parameter, you must check the **Include AF signal in unmixing**.

  Note: You can also turn **Include AF signal in unmixing** on and off in the workspace.

- By default, the option to link single color controls to their unstained control gates is enabled.
  - When enable (checked), moving the scatter gate applies to all linked controls and its corresponding unstained control's scatter gate.
  - When inactivated (unchecked), moving gates on each control workspace is honored on a per control and per workspace basis.

5 Experiment Workspace

Experiment Workspace overview

Experiment Workspace is the main application window, and it is used for controlling the Attune™ Xenith™ Flow Cytometer to run samples, generate data, and analyze results.

You can access the **Experiment Workspace** from the **Home** screen using the following options:

- Click **New experiment** to open the **New Experiment Setup** screen to create a new experiment.
- Click **Import experiment** to open a saved experiment from the database or a network location.
- Open a previously run experiment from the **Experiment list**.
- Open a template stored in the database from the **Template list** to create a new experiment.

Note: The **Experiment list** and the **Template list** only show experiments and templates saved in the database by the current user.

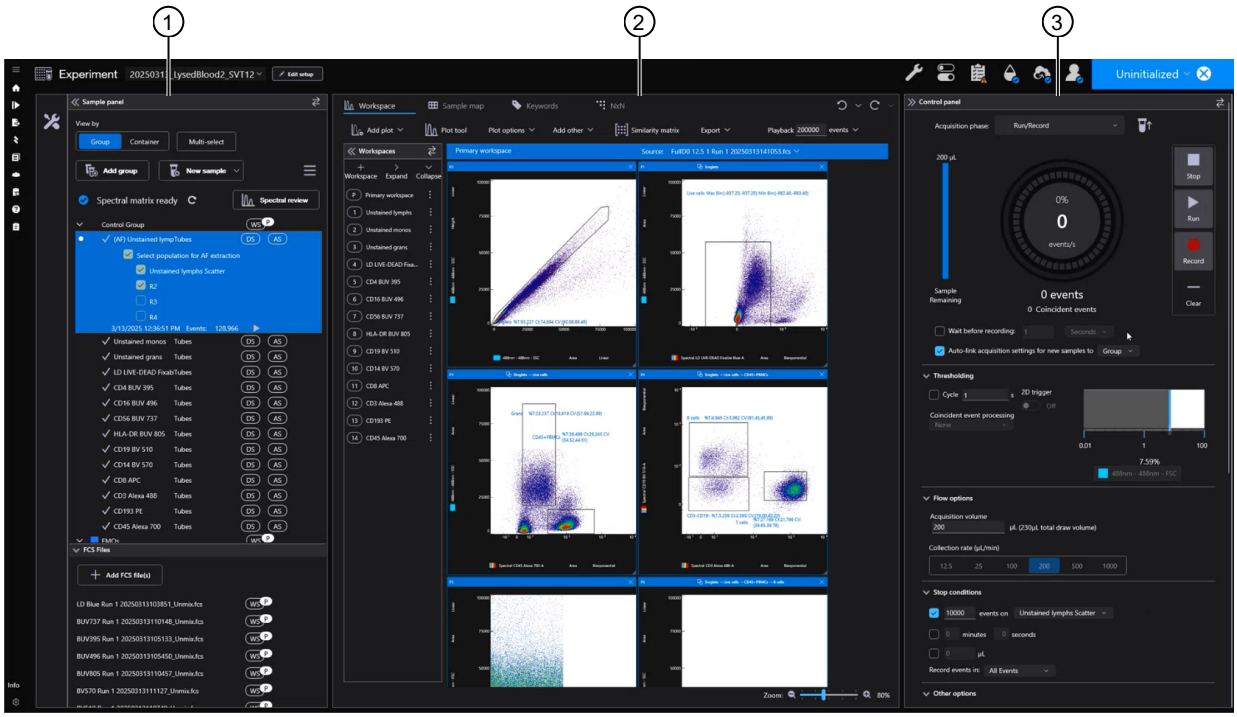


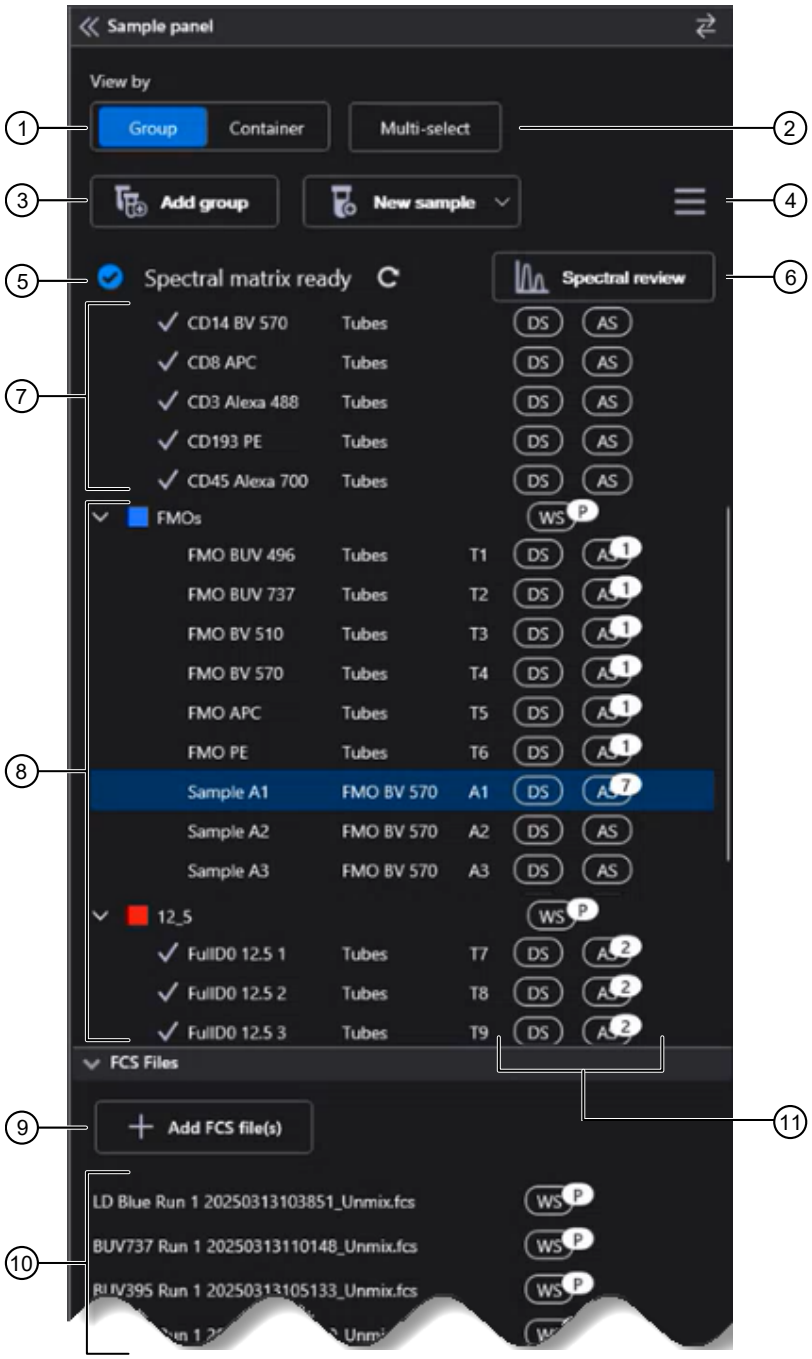
Figure 55 Experiment Workspace layout

1. Sample panel (page 72)
2. Views (Workspace, Sample map, Keywords) (page 104)
3. Control panel (page 148)

Sample panel

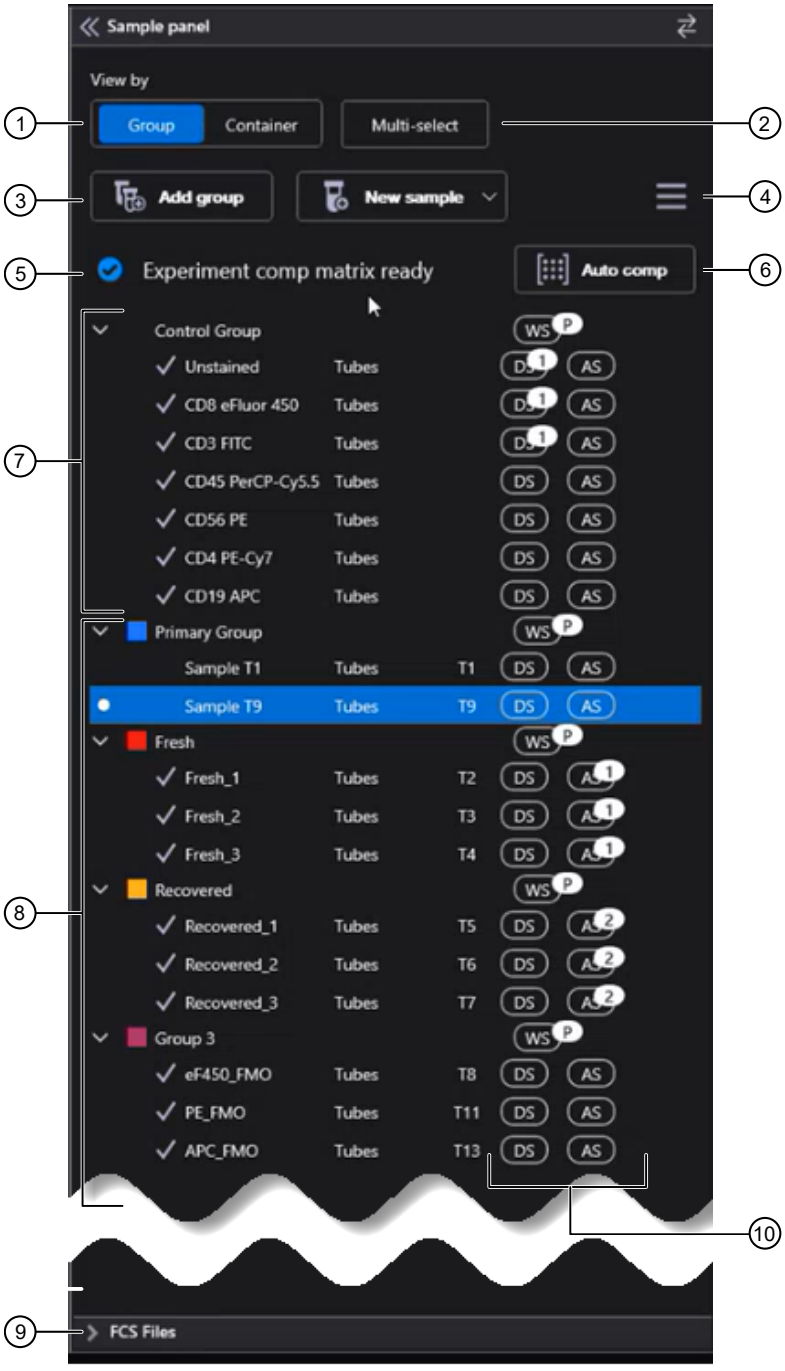
The **Sample panel** lists each control and sample in the experiment that you intend to run, and provides information such as the sample name, sample type (tube vs. plate), sample position, group–level and sample–level Workspace, Detection, and Acquisition settings, and the sample group.

Note: The controls available on the **Sample panel** depend on the type of experiment (**Spectral** or **Compensation**).



1. View by (page 74)
2. Multi-select (page 75)
3. Tool bar (page 76)
4. Sample tools dropdown (page 76)
5. Spectral matrix status (page 77)
6. Spectral review (page 78)
7. Control samples (page 91)
8. Samples (page 91)
9. Add FCS file(s) (page 103)
10. FCS files (page 103)
11. Settings badges (page 95)

Figure 56 Sample panel (Spectral)



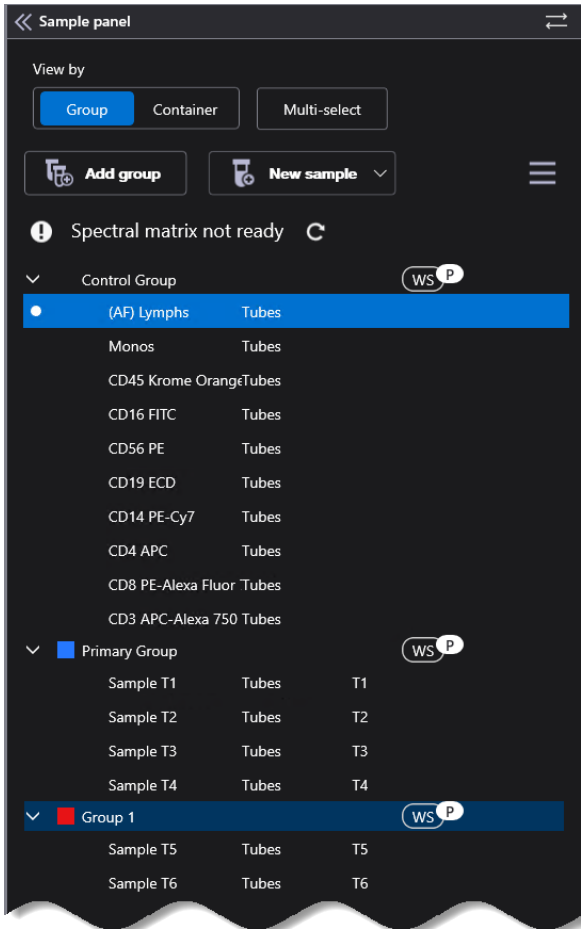
1. View by (page 74)
2. Multi-select (page 75)
3. Tool bar (page 76)
4. Sample tools dropdown (page 76)
5. Compensation matrix status (page 84)
6. Auto comp (page 85)
7. Control samples
8. Samples
9. FCS files (page 103)
10. Settings badges (page 95)

Figure 57 Sample panel (Compensation)

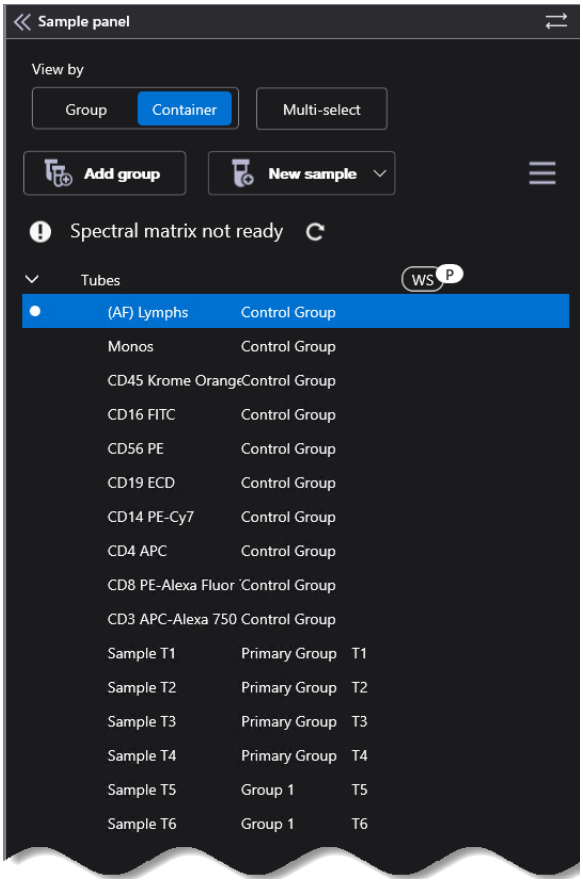
View by

You can view the samples by **Group** or by **Container**.

- **Group**: Groups enable the organization of samples by shared characteristics so that common settings (WS, DS, AS, etc.) can be applied to a group. **Group** is the default selection.
- **Container**: This option organizes samples by their respective container (tubes and plates)



View by Group



View by Container

Note: Each experiment can have multiple tube sample groups (each with multiple tube samples) and multiple plates.

Note: **Groups** are used to partition the workspace into functional sections, for example to show control samples such as compensation controls or the negative control. They can also be used to display different types of samples with different gating strategies simultaneously. The first group in every experiment is labeled as the **Primary Group**.

IMPORTANT! By default, control samples are associated with their own, automatically created groups. These should not be changed because they are used for compensation or spectral unmixing. Do **not** remove automatically created groups.

Multi-select

Multi-select enables you to toggle between **Single-select** and **Multi-select** modes. In the **Multi-select** mode, you can select multiple samples at the same time.

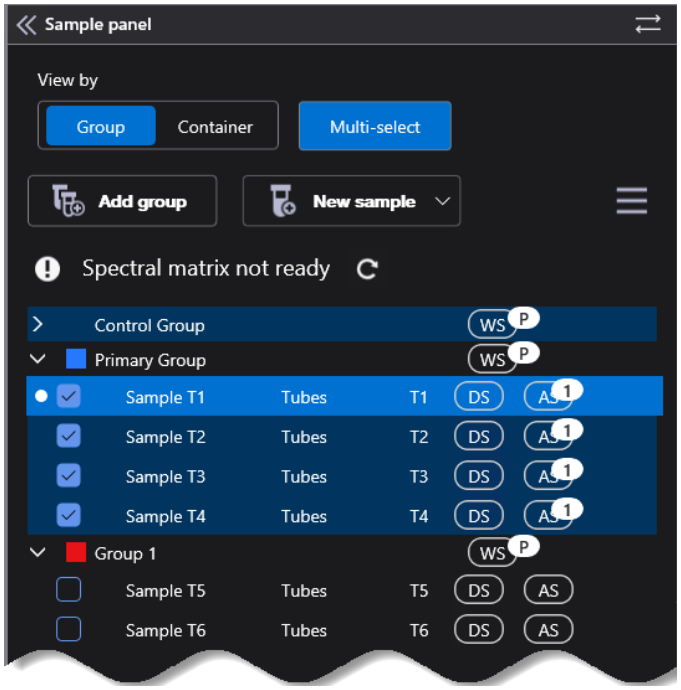


Figure 58 Sample list in Multi-select mode.

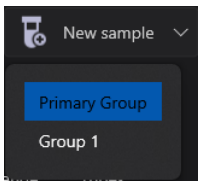
- In the **Multi-select** mode, each sample in the **sample list** has an associated **checkbox** to the left it. **Checkboxes** enable one or more samples to be selected.
- You can also **Ctrl–click** to select multiple samples that are not next to each other in the list, or **Shift–click** to select multiple samples that are next to each other in the list.
- When samples are selected in the **Multi-select** mode, changes made to **AS** (Acquisitions settings) are applied to all selected samples (if the **Auto-link acquisition settings to** is turned off).
- Multi-selected samples can be exported, deleted, and duplicated.

Toolbar

Toolbar enables you to add groups and new samples to the experiment, and to delete, duplicate, and export selected samples. Depending on the experiment type, it also enables you to set spectral options or remove compensation.

① Add group
② New sample
③ Sample tools dropdown
④ Sample tools dropdown menu
⑤ Selected sample in the Sample list

- **Add group**: Adds a new group to the experiment.
- **New sample**: Adds a sample to the **Primary group** in the experiment.
  - The **New sample dropdown** enables you to add a new sample to a selected group.



- **Sample tools dropdown** has the following options:
  - **Delete**: Deletes the selected group or sample.

    Note: The **Primary group** cannot be deleted.

  - **Duplicate sample(s)**: Makes a copy of the selected sample or group in the **Sample list**.
  - **Export**: Opens the **Export FCS Files** screen, which enables you to export the FCS file for selected sample or group.
  - **Display badges**: Shows or hides the **WS** (Workspace settings), **DS** (Detection settings), and **AS** (Acquisition settings) badges.

    Note: Badges indicate the presence of Workspace, Detection, and Acquisition settings, and enable you to quickly manage these settings from the **Sample panel**. For more information, see “Settings badges (WS, DS, AS)” on page 95.

  - **Spectral options**: Enables you to include **AF (autofluorescence) signal** in the unmixing.

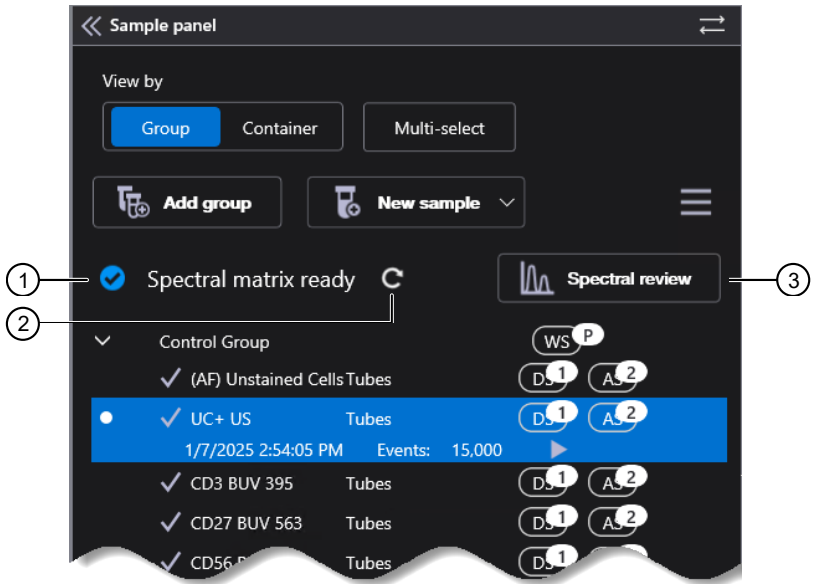
    Spectral options are only available for spectral experiments.
  - **Remove compensation**: Removes the experiment-level compensation from the experiment. When experiment-level compensation is removed, the spillover matrix gets embedded in the FCS file and compensation relies on the recorded data.

    Remove compensation is only available for compensation experiments when there is compensation applied.

Spectral matrix status

Spectral matrix status provides information about the readiness of the spectral unmixing matrix in experiments, which is used to correct the spectral overlap from similar fluorophores in the experiment. The unique spectral signature of each fluorophore is distinguished in the all channels-by-all channels unmixing matrix by the unmixing algorithm.

Note: Spectral matrix status is available only for experiments with **spectral unmixing**.



1. Spectral matrix status
2. Recalculate
3. Spectral review

Figure 59 Spectral matrix status

- **Spectral matrix status** indicates the readiness of the **spectral unmixing matrix**. It shows one of the following conditions:
  - **Spectral matrix not ready** Spectral matrix not ready
  - **Spectral matrix error** Spectral matrix error
  - **Spectral matrix calculating** Spectral matrix calculating
  - **Spectral matrix ready** Spectral matrix ready
- As spectral controls are recorded, the **spectral matrix status** and the status of individual control samples are updated in the **Sample panel**.
- **Recalculate** button enables you to recalculate the **spectral matrix** for all controls.
- **Spectral review** button opens the **Spectral Review** tool, which enables you to review the unmixed spectral data from each fluorophore in your panel based on measured data from spectral controls. For more information, see “Spectral review” on page 78.

  Note: Spectral review is only available when the spectral status indicates **Spectral matrix ready**.

Spectral review

Spectral review button opens the **Spectral Review** tool, which enables you to review the unmixed spectral data from your controls to asses the quality of your fluorophore panel before you run your samples.

Figure 60 Spectral Review tool (Nx1 review open)

① Spectral Review tool (Nx1 review open)
② Navigation sidebar
③ Nx1 review
④ Spectral energy
⑤ Spectral similarity
⑥ Zoom
⑦ Back, Next, Done buttons
(Back button not shown on Nx1 review screen)

- **Spectral Review** tool has three main screens:
  - **Nx1 review**: Enables you to review Nx1 plots for each unmixed spectral control (page 79).
  - **Spectral energy**: Enables you to review the measured spectral energy plots for each spectral control (page 80).
  - **Spectral similarity**: Enables you to review the spectral similarity matrix calculated using measured data (page 83).
- The **navigation sidebar** on the left enables you to open these screens in any order, while the **Back** and **Next** buttons at the bottom right enable you to move between the screens in sequence.

Nx1 review

Spectral Review ▸ Nx1 review screen enables you to review the **Nx1 plots** for each unmixed spectral control.

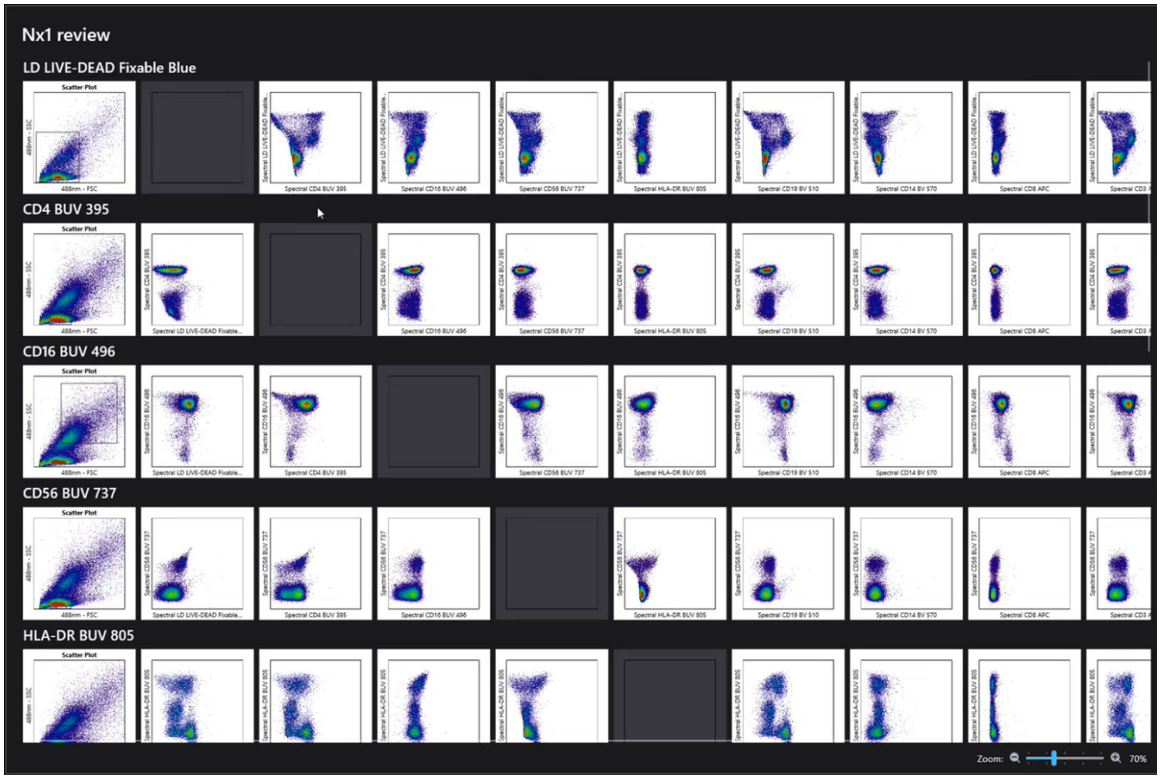


Figure 61 Spectral Review - Nx1 review

- Controls must be unmixed to display the **Nx1 plots**. If the controls are unmixed, then the **Nx1 review** screen displays a message to remind you to unmix your controls.

  Click **Unmix controls** to unmix your controls and display the **Nx1 plots**. Depending on the complexity of your fluorophore panel, the unmixing can take a few minutes to complete.
- In an **Nx1 plot**, each single-color spectral control (that is, fluorophore) is plotted against every other single-color control, creating a matrix of plots that allows you to visually inspect how well each fluorophore is separated and how they interact with each other.
- In **spectral unmixing** experiments, **Nx1 plots** are used to visualize and assess the accuracy of spectral unmixing, to help ensure that the populations are well-separated, and the data are reliable and the panel is performing as expected. Look for populations that are well-defined and easily distinguishable. Swooping positive and negative populations can indicate unmixing errors.

Spectral energy

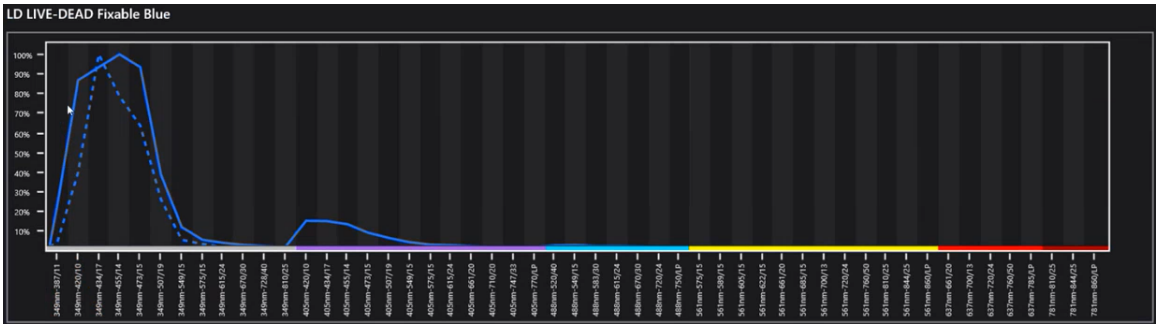
Spectral Review ▸ Spectral energy screen enables you to review the actual **spectral energy plot** for each single color control in the panel that has been measured across all detectors, then corrected by subtracting from it the unstained control signal. All resulting fluorophore-only spectral signatures in the panel are normalized so that they can be compared to other fluorophores in the panel and to the signatures in the expected spectral energy plot.

Figure 62 Spectral Review - Spectral energy

1. Show theoretical values
2. Complexity index of the panel
3. Measured spectral energy plots
4. Similarity index of the fluorophore

Note: **Spectral energy plots** in **Spectral Review** show the **measured** spectra from single color controls, whereas the spectral plots in the **New Experiment Setup ▸ Fluorophore Selection** screen show the **expected** (theoretical) spectra.

- In the **spectral energy plot**, the x–axis represents the detectors and the y-axis shows the signal intensity normalized to 100%.
- The **Complexity index** value above the plots indicates **unmixing burden** of the panel, which describes the overall difficulty of distinguishing the unique spectral signature of each fluorophore in the panel by the unmixing algorithm (see “Complexity number” on page 65).
- **Show theoretical values** enables you to display the theoretical spectral energy values superimposed on the measured values, where the dotted line represents the theoretical values and the solid line represents the measured values.



- The **Similarity index** to the right of the **Spectral energy plot** compares the measured versus theoretical spectral energy values of the fluorophore, and describes how well the measured spectral energy of the single color control corresponds with the theoretical value for the fluorophore.

  Similarity index is expressed on a scale of 0 to 1, where a score of 0 indicates that the measured and theoretical spectral energy values are entirely different, and a score of 1 indicates that the measured value corresponds perfectly with the theoretical.

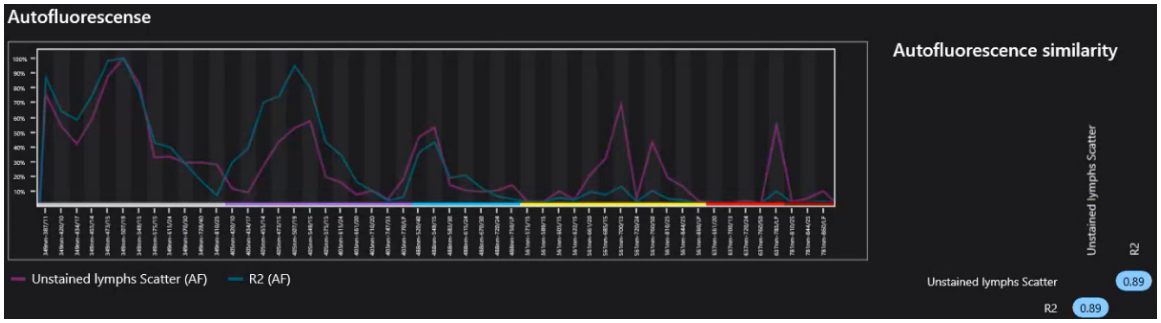
  For example, a similarity index value of 0.949 means that the measured and theoretical spectral energy values are 95% similar.

  Note: Although the **Similarity index** shown for **Spectral energy plots** and the spectral similarity depicted in the **Spectral similarity matrix** are calculated in the same way (cosine of simlarity), they differ in their application.

  - In a **Spectral energy plot**, similarity index compares the measured versus theoretical values from the **same fluorophore**.
  - In the **Spectral similarity matrix**, spectral similarity describes how alike the emission spectra are when comparing **two different fluorophores**.

- **Autofluorescence (AF) spectral energy plot** is the last plot in the **Spectral energy** screen and it is available only if you have chosen to define autofluorescence (AF) populations.

  The **AF spectral energy plot** shows the spectral signatures of each defined autofluorescence (AF) population as traces superimposed in a single spectral energy plot. This enables you to determine whether the populations defined as AF are distinct populations with unique AF signatures that should be added individually to the spectral unmixing matrix.



- **Autofluorescence (AF) similarity matrix** to the right of the **AF spectral energy plot** shows the spectral similarity matrix of the AF populations, which is generated from the actual measured spectral energy values of defined AF populations.

  It describes how alike the spectral energy are when comparing two AF populations on a scale of 0 to 1, where a score of 0 indicates that the populations are entirely different and a score of 1 indicates that the populations are identical.

Spectral similarity

Spectral Review ▸ Spectral similarity screen enables you to review the **Spectral similarity matrix** that is generated using the normalized spectral values for all spectral controls that are measured across all the detectors.

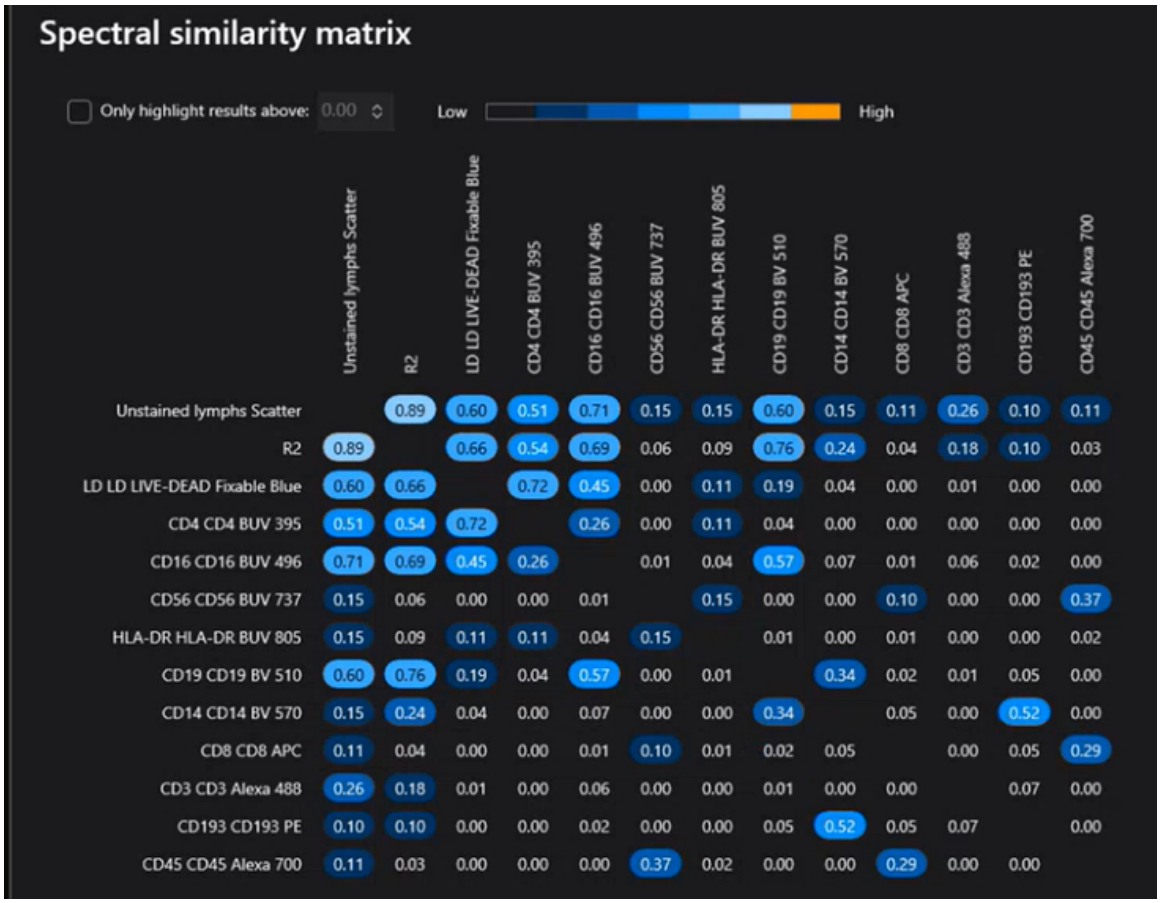


Figure 63 Spectral Review - Spectral similarity matrix

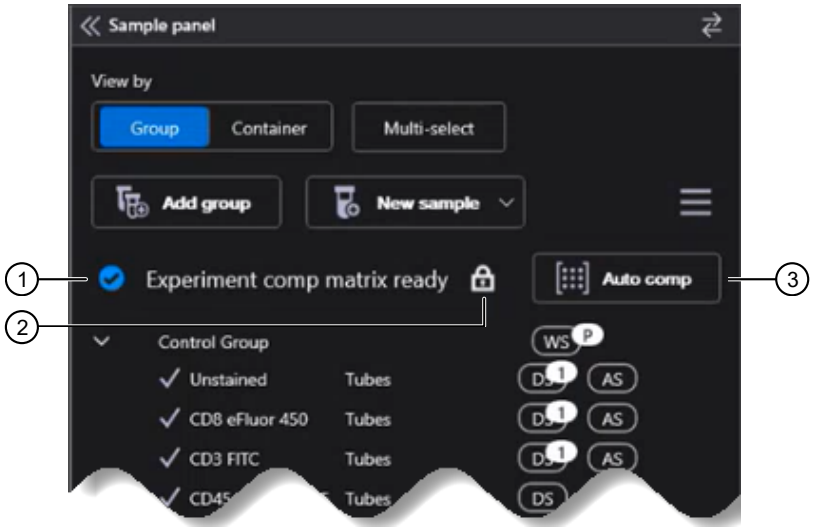
The **Spectral similarity matrix** describes how alike the **actual** emission spectra of two **different** fluorophores are on a scale of 0 to 1. Low spectral similarity values mean that the fluorophores that are compared have well separated or distinct emission peaks. High spectral similarity means that there is significant overlap between the emission spectra of the fluorophores, which increases the spectral unmixing burden of the panel and the likelihood of unmixing errors.

Note: In **Spectral Review**, the spectral similarity values represent the actual emission spectra of the fluorophores that are measured from the spectral controls, whereas the spectral similarity matrix used during fluorophore selection for experiment setup shows expected (theoretical) values.

Compensation matrix status

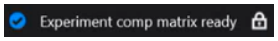
Compensation matrix status provides information about the readiness of the compensation matrix in experiments, which is used to correct the spectral overlap from similar fluorophores in the experiment. The unique spectral signature of each fluorophore is distinguished in the all channels-by-all channels unmixing matrix by the unmixing algorithm.

Note: Compensation matrix status is available only for experiments with **conventional compensation**.



1. Compensation matrix status
2. Read-only compensation indicator
3. Auto comp

Figure 64 Compensation matrix status

- **Compensation matrix status** indicates the readiness of the **compensation matrix**. It shows one of the following conditions:
  - **No experiment comp matrix** 
  - **Experiment comp matrix ready** 
  - **Experiment comp matrix ready (read-only)** 
- **Experiment comp matrix ready** status is displayed only after compensation is applied to the experiment.

  You can apply experiment-level compensation using one the following methods:
  - Use the **Compensation Wizard** to set up and acquire compensation controls (page 85).
  - Import the compensation matrix directly from the **Hardware Settings Library** (page 28).
  - Import an FCS file that has compensation embedded in it, then deselect the **Use experiment level comp** option in the **Compensation tool** (page 124).
- When compensation is imported, a **lock icon**  is displayed next to the **Experiment comp matrix ready** status, which indicates that the compensation is **read–only** and cannot be adjusted. However, you can remove experiment-level compensation from the experiment using the **Remove compensation** option in the **Sample tools** dropdown menu (page 76).
- **Auto comp** button opens the **Compensation Wizard**, which enables you to set up compensation parameters, adjust scatter, positive, and negative regions, and review compensation. For more information, see “Auto compensation” on page 85.

Auto compensation

Auto comp button opens the **Compensation Wizard**, which guides you through the compensation workflow. After you have run the compensation controls, the **Compensation Wizard** enables you to review the data, make manual changes (if needed), and apply compensation.

IMPORTANT! Until the recorded compensation controls are reviewed and verfied in **Auto comp ▸ Compensation Wizard**, the spillover or compensation calculation is not applied to the experiment.

Note: The **Auto comp** option is available on the **Sample panel** only for experiments where the **Unmixing type** is **Conventional compensation**.

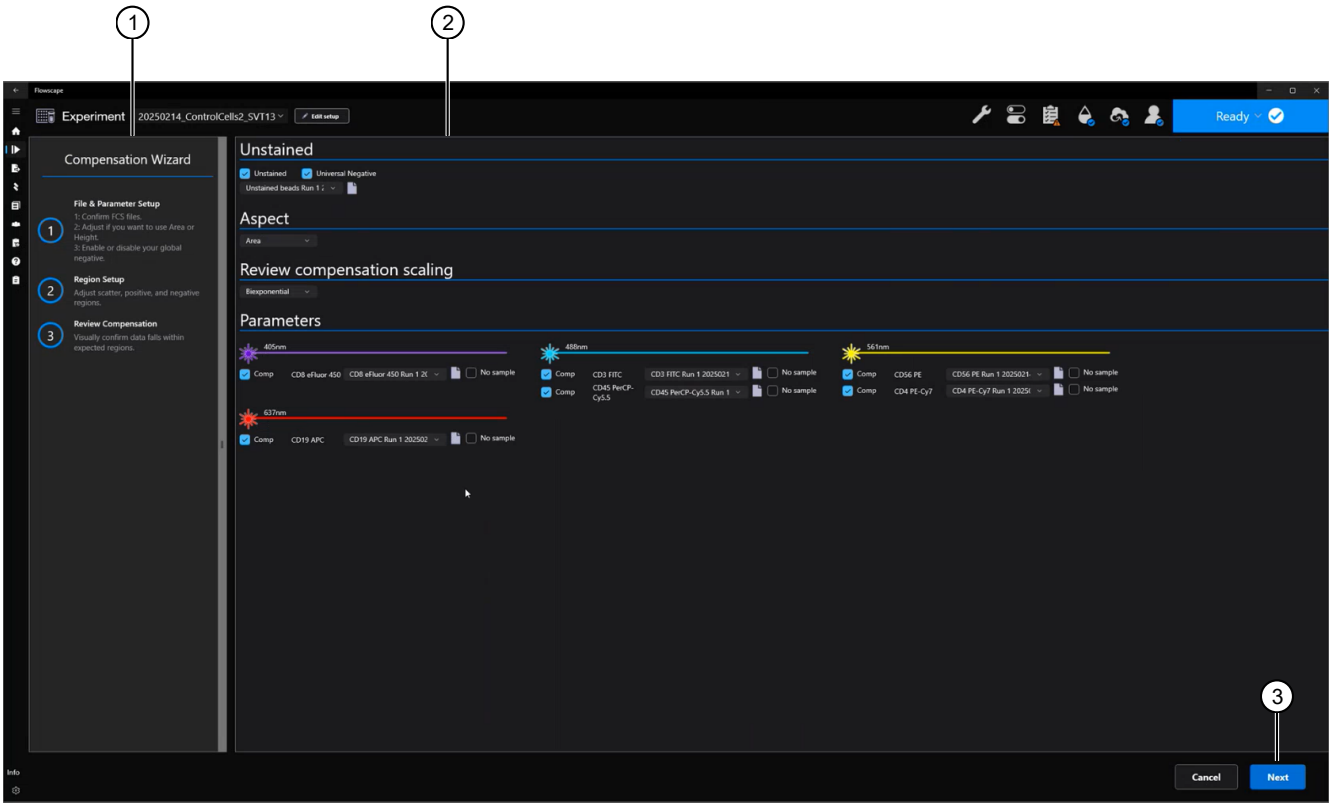


Figure 65 Compensation Wizard (File & Parameter Setup screen)

① Compensation Wizard workflow
② Compensation Wizard workspace
③ **Next** button

The **Compensation Wizard** has three screens that correspond to the Compensation workflow. After completing the tasks on a screen, click **Next** to advance to the next screen. Alternatively, select the screen you want to view from the **Compensation Wizard workflow** panel.

1. **File & Parameter Setup** (page 86)
2. **Region Setup** (page 87)
3. **Review Compensation** (page 89)

File & Parameter Setup

File & Parameter Setup screen enables you to review the setup of your compensation experiment and, if desired, change the background fluoroscence mode, measurement aspect, and compensation scaling, and reassign fluorophores to different detectors.

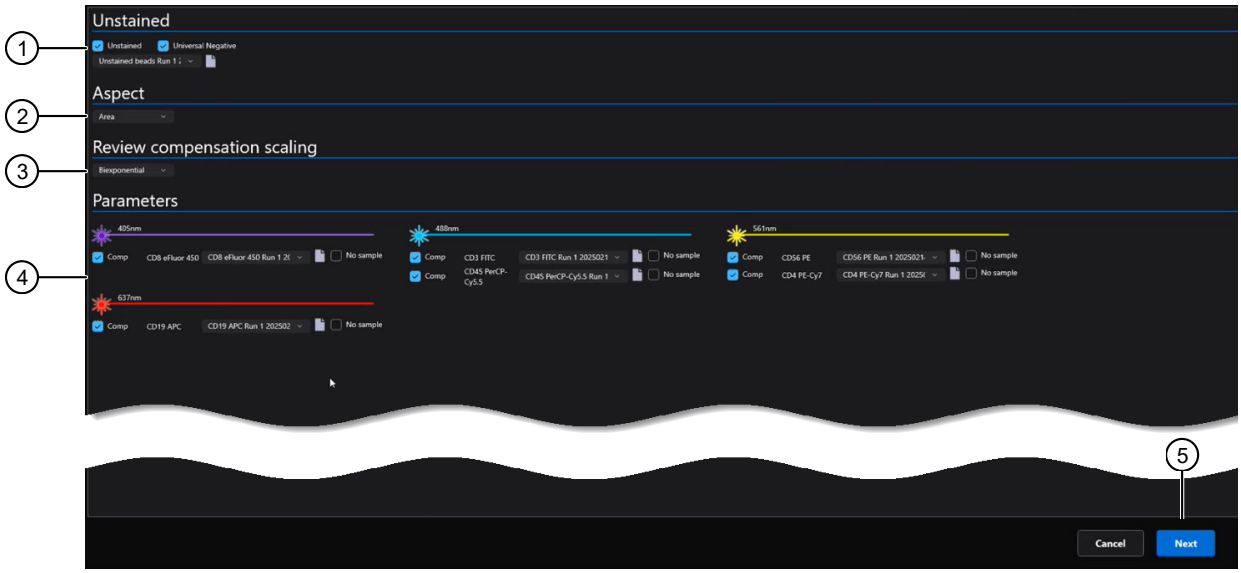


Figure 66 Compensation Wizard - File & Paramater Setup screen

① Unstained
② Aspect
③ Review compensation scaling
④ Parameters
⑤ **Cancel** and **Next** buttons

Note: When the compensation experiment is set up in **New Experiment Setup** (page 55), the selections made for compensation options and the fluorophore/detector assigments automatically carry over to the **File & Parameter Setup** screen.

- **Unstained** enables you to select the source of the background fluorescence when calculating compensation.

  When the **Unstained** option is selected, you can enable or disable the use of the unstained control as a **Universal Negative** (page 60).

  To change the source of the unstained control, select the desired source from the **dropdown menu**, which shows the recorded unstained control runs available for the experiment.

  Alternatively, click the **Import** button, then select the desired FCS file from the location it was saved in the workstation or the network.
- **Aspect** enables you to select **Area** or **Height** as the parameter for calculating compensation.
- **Review compensation scaling** enables you to select **Log** or **Biexponential** scale to use for compensation plots.
- **Parameters** enables you to verify the assigned channels and detectors for the selected fluorophores and the recorded compensation controls. The sofware automatically maps the controls to the respective fluorescence channels. If desired, you can reassign channels and detectors for the fluorophores, or use compensation from other saved FCS files by importing them.

Region Setup

The **Region Setup** screen enables you to review and, if needed, adjust scatter, positive gates, and negative gates.

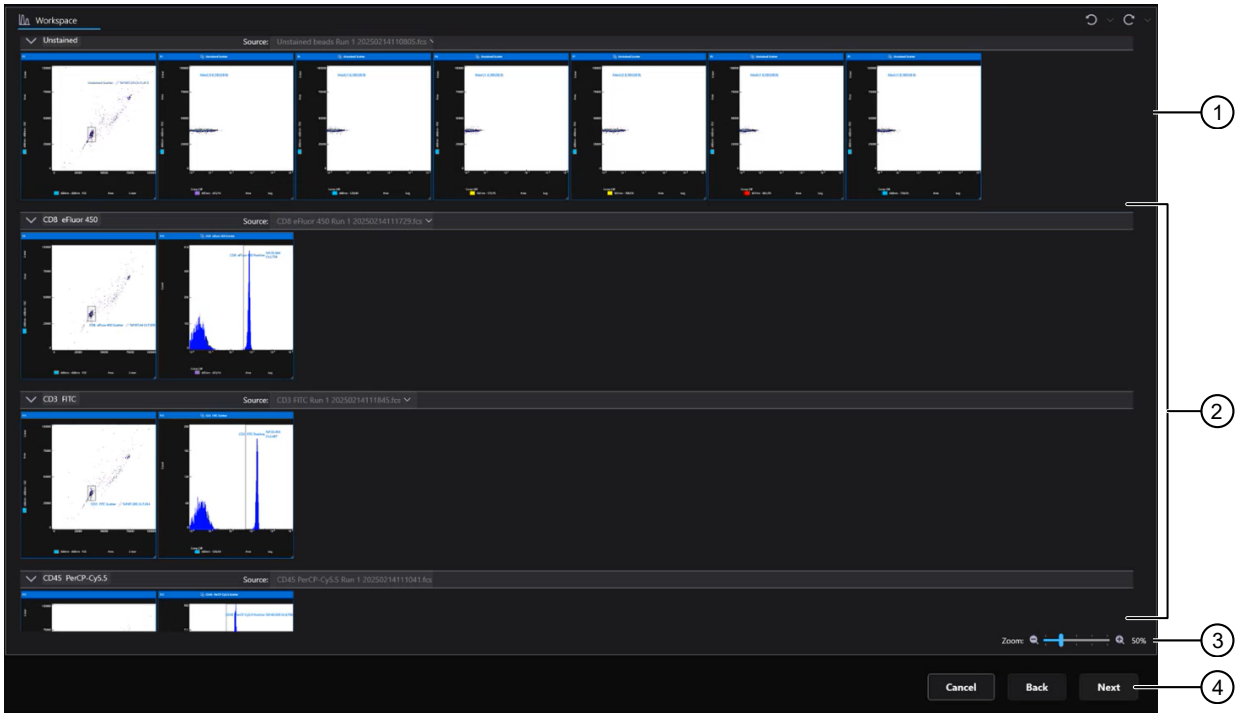


Figure 67 Compensation Wizard - Region Setup screen

① Unstained control workspace
② Single-color control workspaces
③ **Zoom**
④ **Cancel**, **Back**, and **Next** buttons

- **Unstained control workspace** contains a **Scatter plot** and **Histogram plots** for each compensation parameter.
  - The **Unstained control workspace** is available only if **Universal negative** is selected for **Background fluorescence mode** during experiment setup (“Compensation Options” on page 60).
  - The **Scatter plot** is a dual-parameter density plot used to gate the events shown on the compensation plot.

    By default, the parameters for the X- and Y-axes for the **Scatter plot** are set to **FSC** and **SSC**, respectively. The **Measurement** used for calculating compensation (**Area** or **Height**) is selected in **Compensation Options** (page 60).

    Note: If desired, you can change the **Measurement** used for calculating compensation using the **Aspect** dropdown in the **File & Parameter Setup** screen (page 86).

    By default, the **Gating plot** contains a single **Scatter gate** (also called the **Parent gate**) that cannot be deleted.
  - **Histogram plots** for compensation parameters in the **Unstained control workspace** do not display any gates. All **Histogram plots** are gated on the **Scatter gate**.

  - The background signal is calculated based on the median of the gated data for each parameter; no additional gates are required.
- Each **Single color control workspace** contains a **Scatter plot** and a **Histogram plot** for the specific single color control.
  - The **Scatter plot** for the single color control is used to gate the events shown on the **Histogram plot** for the control.
  - The **Histogram plot** for single color controls is a single-parameter daughter plot derived from the **Scatter gate** on the dual-parameter **Scatter plot**. The X-axis parameter on the plot is the single color compensation control and the Y-axis parameter is Count. This plot type cannot be changed.
  - When there is no internal negative, the **Histogram plot** for single-color controls contains a single **Histogram gate**.
  - If **Internal negative** is selected for **Background fluorescence mode** during experiment setup (page 60), the **Histogram plot** for single color controls contains an additional gate (**Negative gate**) to define the negative population.
- The **Compensation Wizard** automatically transfers the gate positions that were set up when recording the compensation controls. If the gates were not set up appropriately, you can adjust the positions of the **Scatter gates** and the **Histogram gates** so that they are correctly set on each population.
- When **Next** is clicked, the software calculates the compensation and applies it to all selected channels.

Review Compensation

Review Compensation enables you to review compensation data after the compensation calculation is complete and applied to the single-color controls, visually confirm that the data fall within the expected regions. It also provides tools (**Plot Tool**, **Quick Tool** and **Matrix**) to view and adjust the compensation values, if needed.

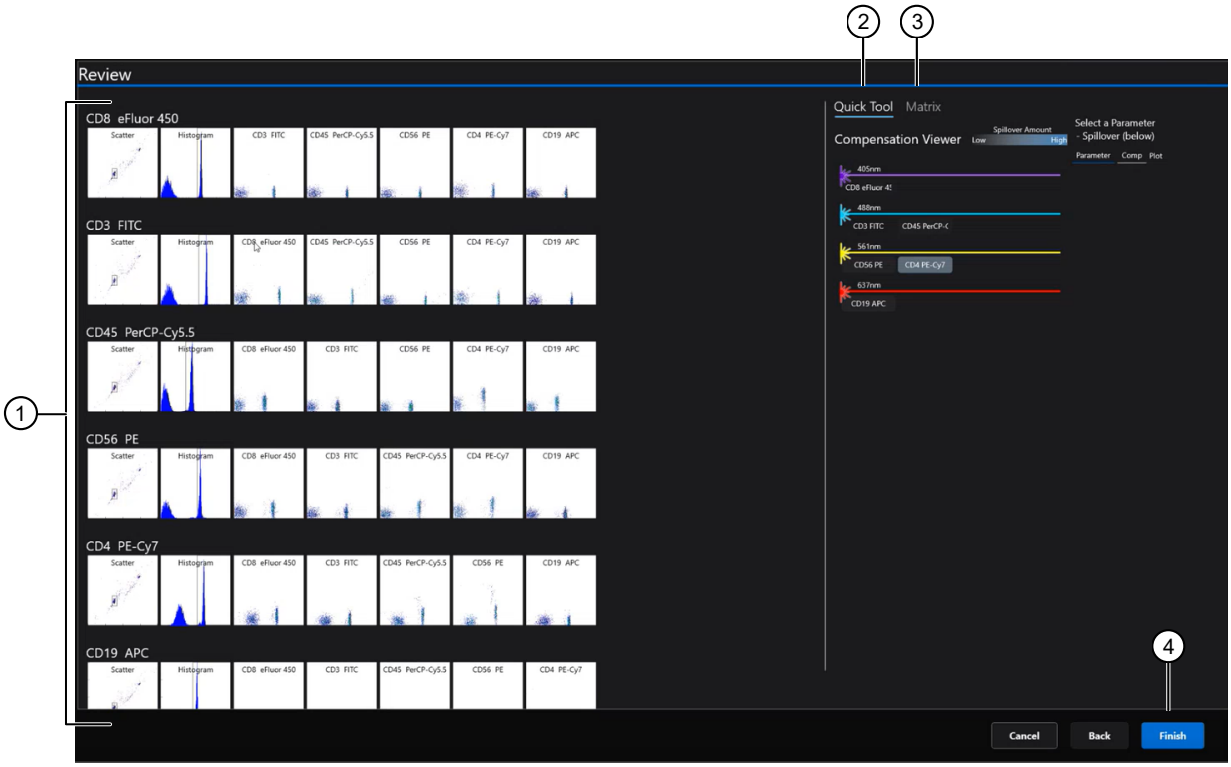


Figure 68 Compensation Wizard – Review Compensation screen

① Compensation plots for single-color controls
② Quick Tool
③ Matrix
④ **Finish** button

Clicking on a compensation plot opens it in the **Plot tool**, which enables you to visualize compensation and adjust the gating of the populations used for compenation calculation.

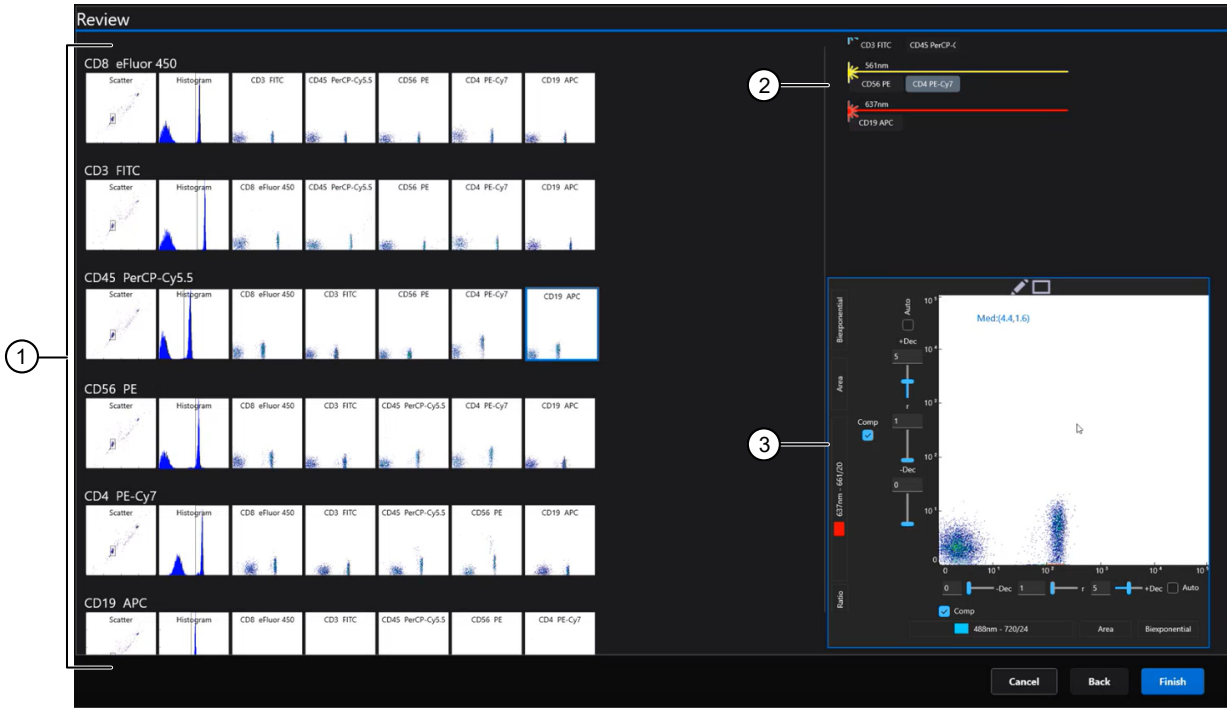


Figure 69 Compensation Wizard – Review Compensation screen with Plot tool

① Compensation plots for single-color controls
② Quick Tool
③ Plot Tool

Sample list

Sample list shows each sample in the experiment and provides sample information such as the sample name, sample type (tube vs. plate), sample position, and sample settings. The samples in the **Sample list** are organized into one or more groups.

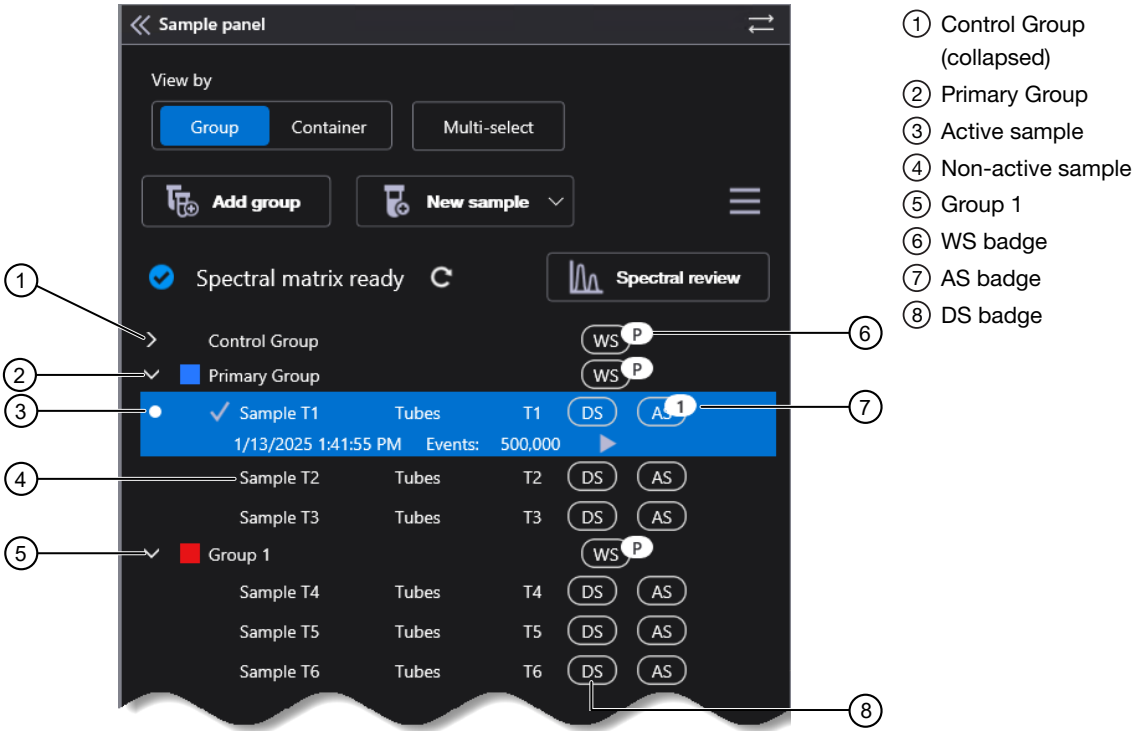


Figure 70 Sample list (Spectral)

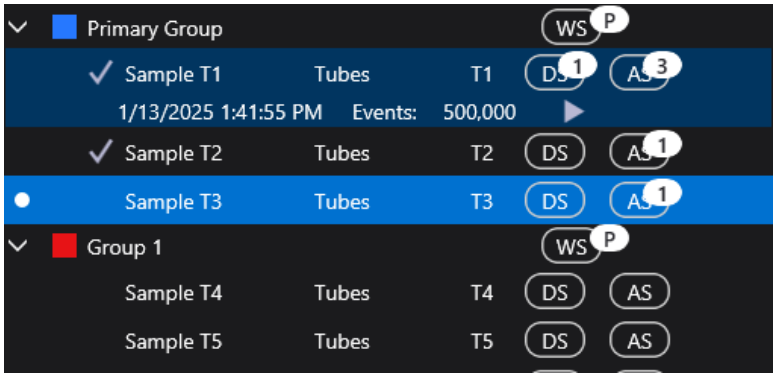
- The **active sample** is indicated by a **dot** next to the sample name and highlighted in **light blue**.

  When **non-active samples** are selected, they are highlighted in **dark blue**. You can separately export, delete, or duplicate selected non-active samples.

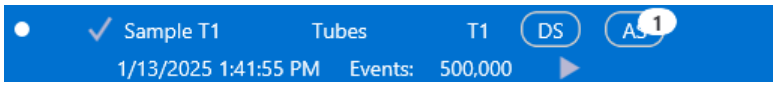
  A **checkmark** next to the sample name indicates that the sample has **recorded data**.

  In the following example:

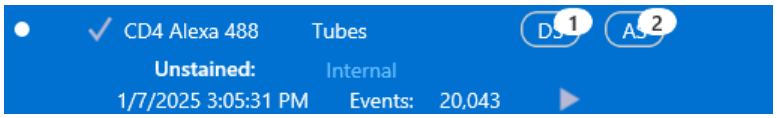
  - **Sample T1** has recorded data and is selected in the **Sample list**, but it is not the active sample.
  - **Sample T2** has recorded data.
  - **Sample T3** is the active sample in the **Workspace**, but has no recorded data.
  - **Sample T4** and **Sample T5** in **Group 1** have no recorded data.



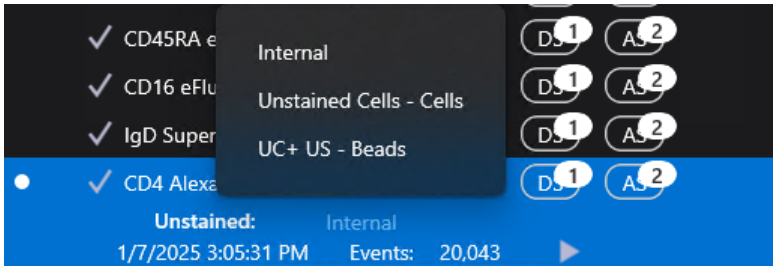
- When a **sample with recorded** data is selected, more sample information is displayed under the sample name (date and time the sample was recorded and the event count).
  To load the FCS file from a sample with recorded data, click the **Load FCS file** button .



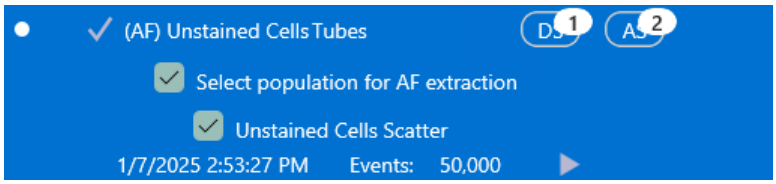
- In spectral experiments, **Single color controls** in the **Sample list** display the **Unstained control** to which they are mapped during experiment setup (see “Map fluorophores to unstained controls” on page 190).
  In the following example, **CD4 Alexa 488** was mapped to an **Internal** negative control.



To map the single color control to another unstained control, click the **Unstained** option, then select the desired unstained control from the menu.



- The **Unstained control** that is selected for use as an **AF (autofluorescence) control** during experiment setup (see “Define unstained and autofluorescence controls” on page 189) is indicated by the prefix **(AF)** in front of its name.

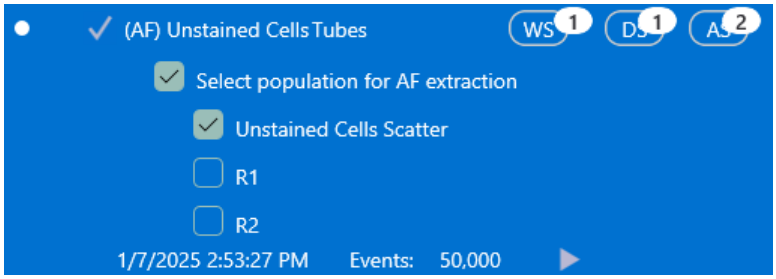


Note: You can select any unstained control that contains cell populations that require autofluorescence extraction as an autofluorescence source for use in the unmixing of fully stained samples.

- The **Select population for AF extraction** is checked to enable the selection of the population for AF extraction.

  By default, the **default scatter gate** is used as the **AF population**. However, you can gate additional populations in the **scatter plot** to use them for AF extraction or select other plots as a source for the AF population.

  The populations defined on the plot become available in the **Sample panel** and can be individually checked or unchecked to include or exclude as AF signatures.

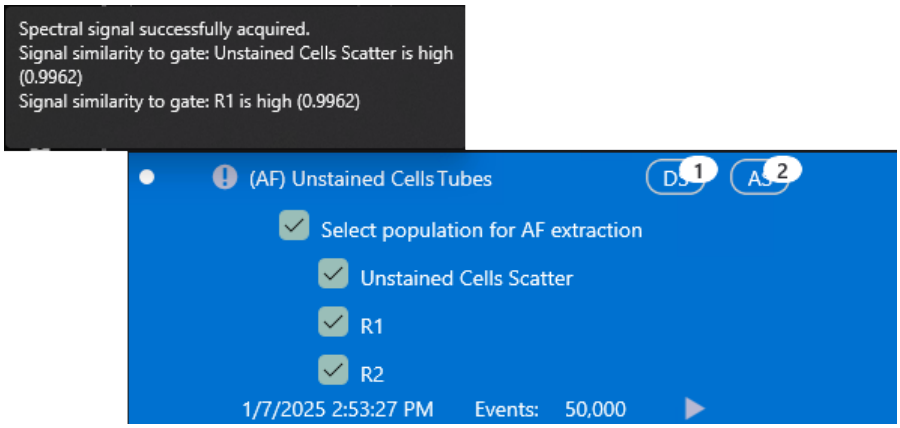


In the example above, the **Unstained Cells Scatter** population (that is, the **default scatter gate**) is selected for AF extraction, but there are two other gated populations in the plot (**R1** and **R2**) that you select as an AF source.

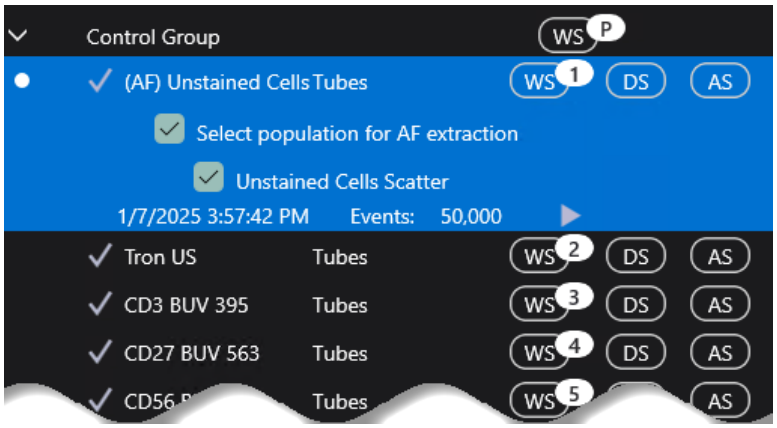
Note: The default scatter gate for the unstained control cannot be removed and it is used as the gate for all single color controls that were mapped to that unstained control.

IMPORTANT! To apply changes in AF population selection, the sample data must be re-unmixed.

- If the signal from the newly defined population selected for AF extraction is higly similar to the signal from the default scatter gate, a warning badge is displayed to the left of the **Unstained control**, indicating a high unmixing burden. When moused over, the warning badge displays a message that describes the reason for the warning.

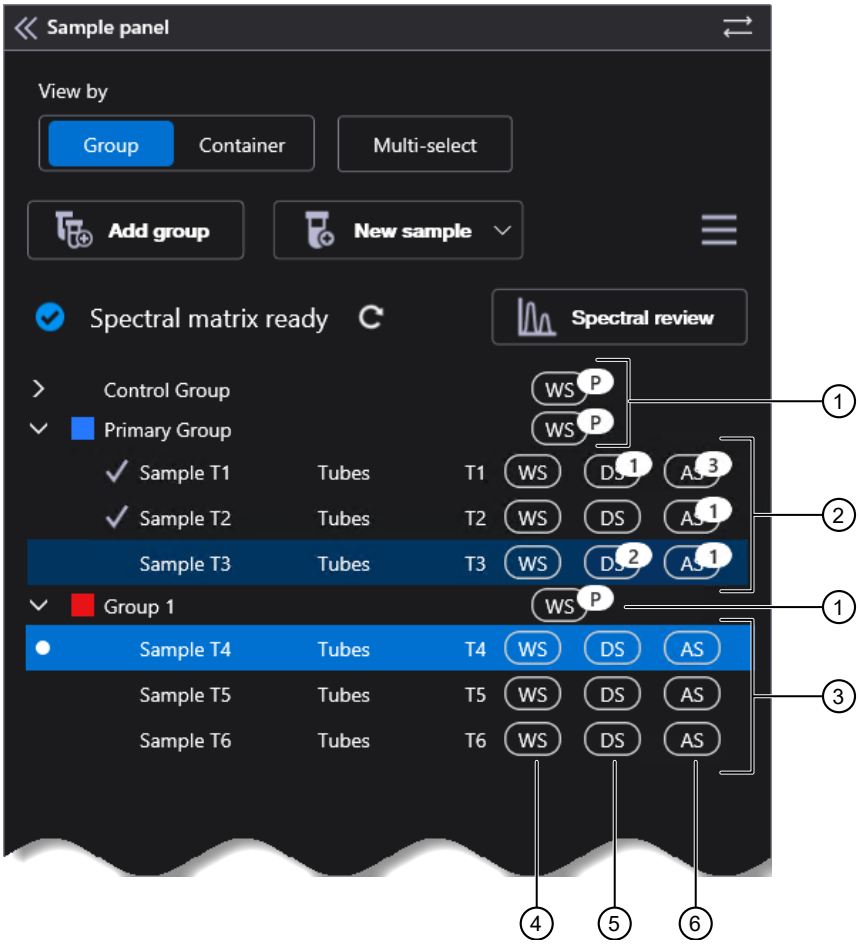


- **WS**, **DS**, and **AS** badges next to groups or samples indicate the presence of **Workspace settings**, **Detection settings**, and **Acquisition settings**, respectively. The badges indicate the specific settings that are used during the experiment and enable you to modify these settings directly from the **Sample list**. For more information, see “Settings badges (WS, DS, AS)” on page 95.



Settings badges (WS, DS, AS)

Settings badges indicate the specific Workspace (**WS**), Detection (**DS**), and Acquisition (**AS**) settings that are used during the experiment, and provide the means to change them directly from the **Sample list**.



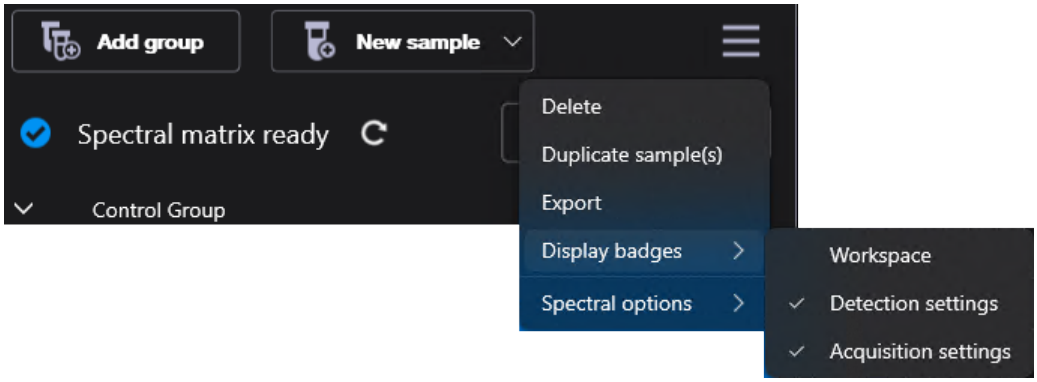
① Group-level WS
② Sample-level WS, DS, AS
③ Default WS, DS, AS
(set experiment-level by default)
④ WS (Woskspace settings)
⑤ DS (Detection settings)
⑥ AS (Acquisition settings)

Figure 71 Settings badges (WS, DS, AS)

- **WS** badge indicates the presence of **Workspace settings**. The **Workspace** can contain plots, gates, statistics, and text boxes, all of which are created using the options available in the **Workspace toolbar** (page 116). Samples with the same **Workspace settings** have the same **Workspace objects**.
- **DS** badge indicates the presence of **Detection settings**, which are lasers, channels, detectors, and optimized voltages used for acquisition.
- **AS** badge indicates the presence of **Acquisition settings**, which include all settings and threshold settings used for the acquisition of samples. **Acquisition settings** are set in the **Control panel** (page 148), and include the acquisition volume, flow rate, stop conditions, and when using plates, mixing cycles, mix mode, and rinse cycles.

- There are three levels of settings for **WS**, **DS**, and **AS**:
  - **Experiment-level settings** are common to all samples in the current experiment. These are the default settings.
  - **Group-level settings**, when created, are common to members of the group.
  - **Sample-level settings** apply to all samples sharing the same sample-level settings.
- Groups and samples can display the **WS** badge, but only samples can have **DS** and **AS** badges. However, sample-level changes made using the **DS** and **AS** badges can be applied at group- and experiment-level using the **Apply to group** and **Apply to experiment** options in the **badge menu** (see “Settings management using WS, DS, and AS badges” on page 97).
- You can select which settings badges to display or hide in the **Sample list** using the **Display badges** menu options in the **Sample tools dropdown** (see “Toolbar” on page 76).

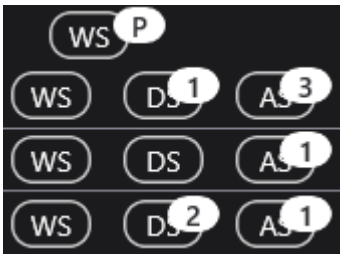
  By default, only **DS** and **AS** badges are displayed.



Settings management using WS, DS, and AS badges

- Badges without a superscript indicator represent samples that are using experiment-level settings. Any modifications made in experiment-level settings apply to all samples using experiment level settings, which automatically update to reflect those modifications.

- Badges with superscript indicators (**number** or letter **P**) represent samples with specific non-experiment-level settings, where badges with the same indicators represent identical settings.

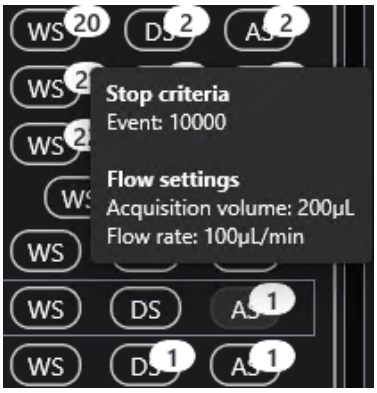


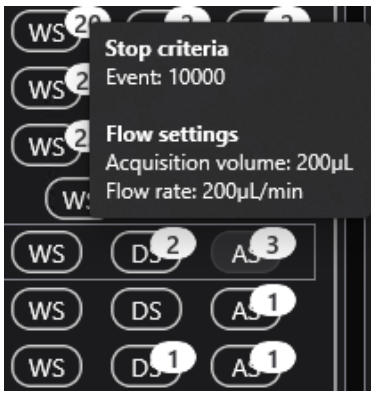
  - The superscript **P** on the badge indicates that the group-level **Workspace settings** are associated with the **Primary workspace**.
  - The superscript **numbers** indicate unique, non-experiment-level settings that are different from those of the **Primary workspace**.
- In the following example, all **AS1** badges (that is, **AS** badges with the superscript **1**) have the same **Acquisition settings**, which are different from those with the **AS3** badges.

  When you mouse over a badge, a tooltip popup provides the relevant settings information.

  For example, the tooltip for the sample with the **AS1** badge shows a **Flow rate** of **100 µL/min** (image on the left), while the tooltip for the sample with the **AS3 badge** (image on the right) shows a **Flow rate** of **200 µL/min**.

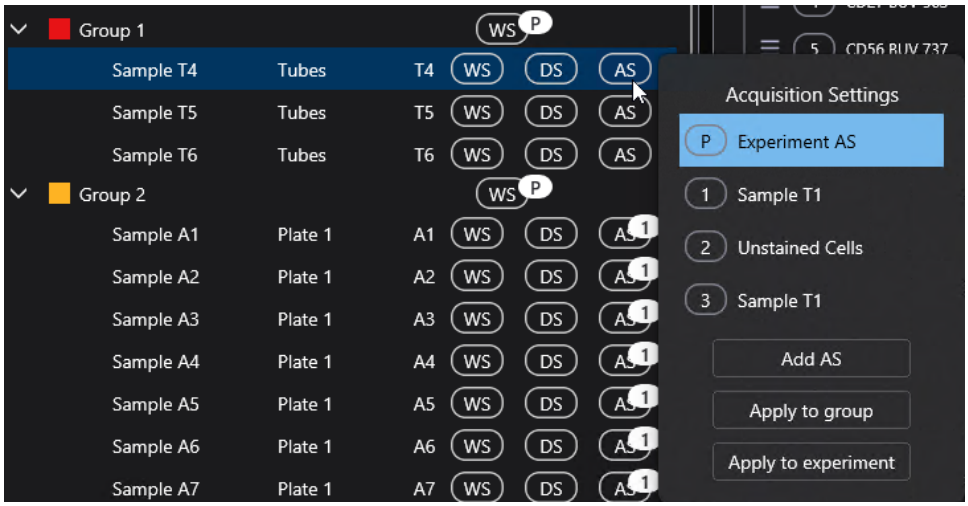
  Both **AS1** and **AS3** have **Acquisition volume** and **Stop criteria** at **200 µL** and **10000 events**, respectively.





- Clicking on a badge opens the **badge menu**, which shows the various options to modify the **Workspace settings**, **Detection settings**, or **Acquisition settings** for the sample. The **badge menu** options that are shown depend on the badge type (**WS**, **DS**, or **AS**).

Note: You can change the settings only for samples that have no recorded data. For samples with recorded data, the popup menu options are visible but disabled.



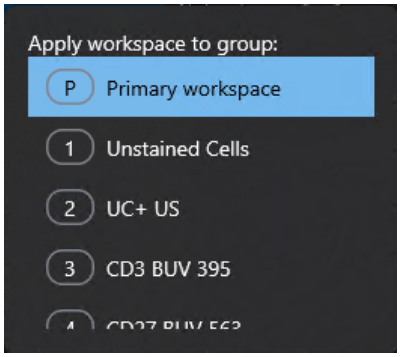
- Changes made to settings at sample-level using the **DS** and **AS** badges can be applied at group-level and experiment-level using the **Apply to group** and **Apply to experiment** options in the **badge menu**.
- Any settings changes made at experiment-level apply to all samples that do not have specific group-level or sample-level settings, which automatically update to reflect those modifications.
- **Predefined settings** are settings that have been previously applied to a sample or group in the experiment and assigned a unique badge indicator (**P**, **1**, **2**, **3** and so on). They are represented in the **badge menu** with their unique badge indicators and the sample name for which they were first defined (for example, 1 Sample T1).

Note: A sample can have different settings at different times during the course of an experiment. In the example above, two different **Acquisition Settings** were defined for **Sample T1**, **AS1** and **AS3**, which are both available in the **AS badge menu**.

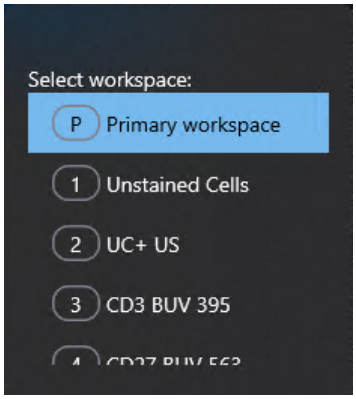
WS badge menu

WS badge menu enables you to select a **Workspace** from a list of predefined workspaces and apply it at group- or sample-level. **Workspaces** can contain plots, gates, statistics, and text boxes.

The **WS badge menu** is available from the **WS** badges for groups and samples in the **Sample list**.



WS badge menu (Group-level)



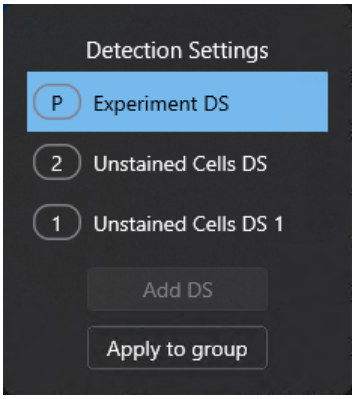
WS badge menu (Sample-level)

- To apply a predefined **Workspace** to all samples in a group, click the **WS** badge for the group, then select the desired **Workspace**. The selected **Workspace** is applied to all the samples in the group.
- To apply a predefined **Workspace** to a sample, click the **WS** badge for the sample, then select the desired **Workspace**. The selected **Workspace** is applied only the sample that the badge belongs.

DS badge menu

DS badge menu enables you to modify the **Detection settings** of samples in the **Sample list**. **Detection settings** are lasers, filters, detectors, and optimized voltages used for acquisition. Using the **DS badge menu**, you can create new settings for the selected sample or select from available **predefined settings**.

The **DS** badge is only available at the sample level, but you can apply the new or modified settings at group-level using the **Apply to group** option available in the menu.



- To apply predefined **Detection Settings** to a sample, click the **DS** badge for the sample, then select the desired option from the list of **predefined settings**.

  The selected **Detections Settings** are applied only the sample that the badge belongs and the **DS** badge displays the new superscript settings indicator (for example, DS 2).
- To apply predefined **Detections Settings** to all samples in the group, click the **DS** badge for the sample that has the settings you want to apply to the group, then click **Apply to group**.

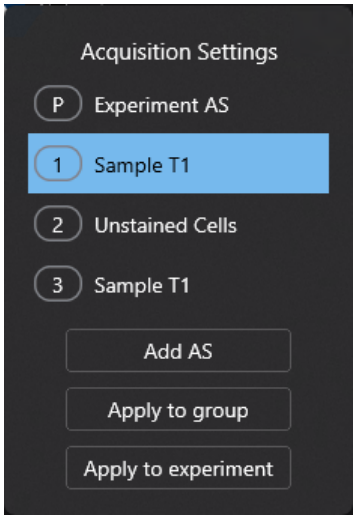
  The **Detection Settings** from the selected sample are applied to all the samples in the group that the selected sample belongs and all the samples in the group display the new superscript settings indicator.

AS badge menu

AS badge menu enables you to modify the **Acqusition Settings** of samples in the **Sample list**. Using the **AS badge menu**, you can create new settings for the selected sample or select from available **predefined settings**.

Acquisition settings include all settings used for the acquisition of samples such as the acquisition volume, flow rate, stop conditions, and when using plates, mixing cycles, mix mode, and rinse cycles.

The **AS** badge is only available at the sample level, but you can apply the new or modified settings at group-level or experiment-level using the **Apply to group** and **Apply to experiment** options available in the menu.



- You can apply **predefined acquisition settings** to other samples at the sample-, group-, or experiment-level.
  - **Sample-level**: To apply **predefined settings** to a single sample, click the **AS** badge for the sample to open the **AS badge menu**, then select the desired option from the list of **predefined settings**.

    The acquisition settings are applied to the sample and the **AS** badge displays the new superscript settings indicator (for example, AS 1).
  - **Group-level**: Click the **AS** badge for the sample with the desired acquisition settings to open the **AS badge menu**, then click **Apply to group**.

    The acquisition settings are applied to all samples in the group, which then display the corresponding settings badge.

    Note: You can use the **Apply to group** option only for samples that are in the same group as the sample with the desired **predefined settings**.

    If none of the samples in the group has the desired acquisition settings, first apply the acquisition settings to a sample in the group as described for sample-level acquisition settings, then use the **Apply to group** option to apply the new settings to the rest of the samples in the group.

  - **Experiment-level**: Click the **AS** badge for the sample with the desired predefined settings to open the **AS badge menu**, then click **Apply to experiment**.

    The settings are applied to all samples in the experiment, which then display the blank badge () that indicates the presence of experiment-level settings.

- To create **new acquisition settings** that are sample-specific, first unlink the acquisition settings of the sample from shared settings, then modify the **acquisition settings** in the **Control panel** (page 148) to create new acquisition settings for the sample.
  - To unlink the sample, click the **AS** badge to open the **AS badge menu**, then click **Add AS**.

    The acquisition settings for the selected sample are unlinked from the settings (sample-, group-, or experiment-level) shared with the samples that have the same superscript indicator. The **AS** badge for the unlinked sample displays an asterisk (for example,  or ) to indicate its unlinked status.

    Note: A blank badge with an asterisk () indicates that the acquisition settings of the sample are unlinked from the experiment-level settings that it had previously.

    A badge with a superscript number and an asterisk () indicates that the acquisition settings of the sample are unlinked from the samples that shared the same acquisition settings and displayed the **AS** badge with the same superscript number.

  - To create the new sample-specific acquisition settings, modify the acquisition settings (acquisition volume, flow rate, stop conditions, and so on) in the **Control panel**.

    The changes made to the acquisition settings only apply to the unlinked sample and are automatically saved. The sample then displays a new superscript indicator (for example, ) that represents its new acquisition settings and to distinguish it from samples with different settings.

  - The new acquisition settings can be applied to other samples at the sample-, group-, or experiment-level as described above.

FCS Files

FCS Files in the **Sample panel** enables you to import and view FCS files (*.fcs) in the **Workspace** for analysis and comparison.

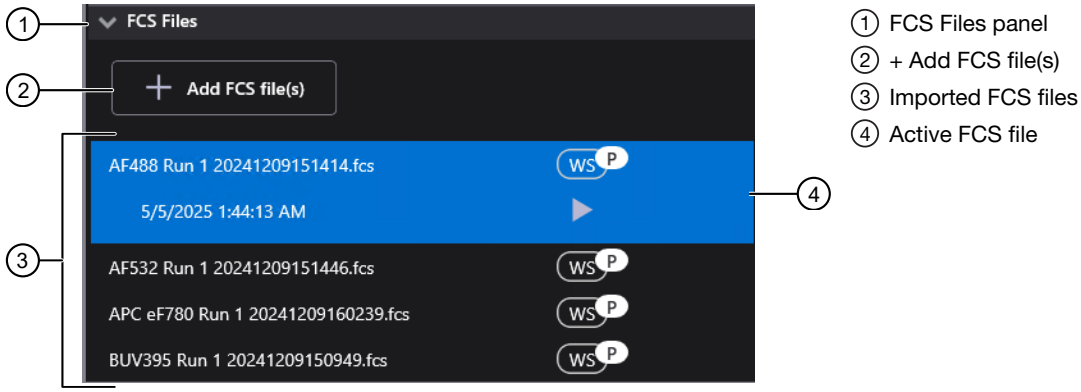
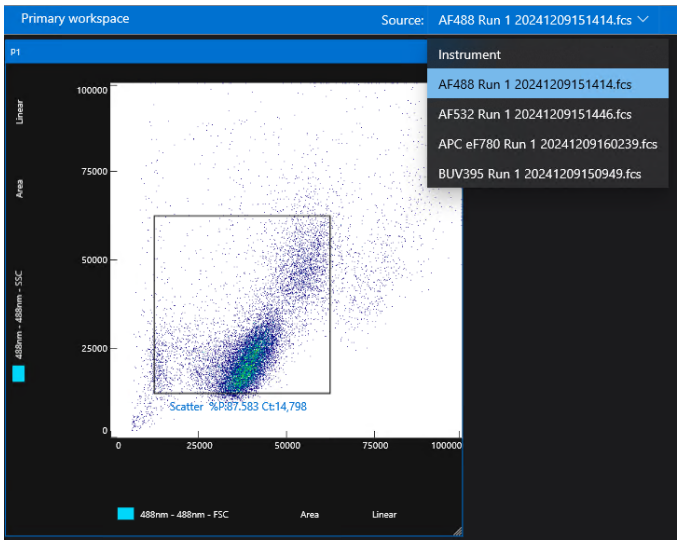


Figure 72 FCS Files (with 4 imported files)

- To import an FCS file, click **+ Add FCS File(s)**, select the desired file with an *.fcs extension, then click **Open**.

  The imported FCS file is added to the **FCS Files** panel and becomes available in the **Workspace** ▸ **Source** dropdown for all available **Workspaces**.



- To view an imported FCS file in the **Primary workspace**, double-click the file in the **FCS Files** panel. Data from the selected file is displayed in the **Primary workspace**.
- Alternatively, select the imported FCS file from the **Source** dropdown of any desired **Workspace** that is available. Data from the selected file is displayed in the selected **Workspace** with all workspace objects (plots, gates, statistics, and text boxes) applied to the imported data.
- To export an FCS file from the **FCS Files** panel, right-click the file, then select **Export FCS** file.
- To remove an FCS file from the **FCS Files** panel, right-click the file, then select **Remove FCS file**.

Note: An FCS file can only be removed if it is not being displayed in any workspace.

Views

The **Views** panel of the **Experiment Workspace** displays the various application panels grouped by functionality. They include the main application workspace with analysis objects (plots, gates, and statistics), the sample map, the keywords that are associated with the current or a saved experiment, and the NxN plots. **Views tabs** at the top of the panel enable you to toggle between the application panels.

The following example shows the **Workspace** tab of a spectral experiment.

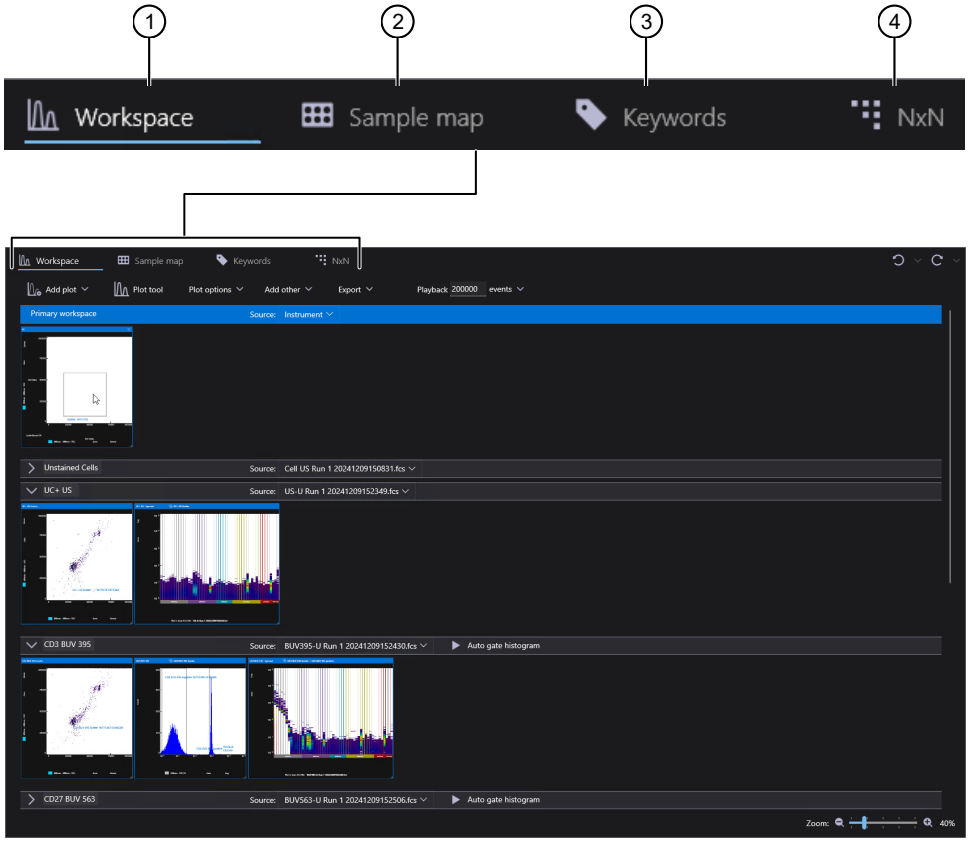


Figure 73 Views panel and Views tabs (Workspace tab selected)

1. Workspace (page 105)
2. Sample map (page 109)
3. Keywords (page 110)
4. NxN (page 113)

The **Views tabs** enable you to view the following application panels:

- **Workspace**: Enables you to add and view the plots, gates, statistics, and text boxes for data acquisition and analysis, and to perform compensation and spectral unmixing (page 105).
- **Sample map**: Displays the plate layout editor, which enables you to add one or more plates or tubes to the experiment and setup the plate layout (page 109).
- **Keywords**: Enables you to view and update the sample keywords (page 110).
- **NxN**: Enables you to generate NxN plots for up to two samples to compare data sets (page 113).

Workspace

Workspace view displays the plots, gates, statistics, and text boxes that are associated with the current or a saved experiment. It contains the **Workspace toolbar**, the **Workspace groups**, and the **Plot area**.

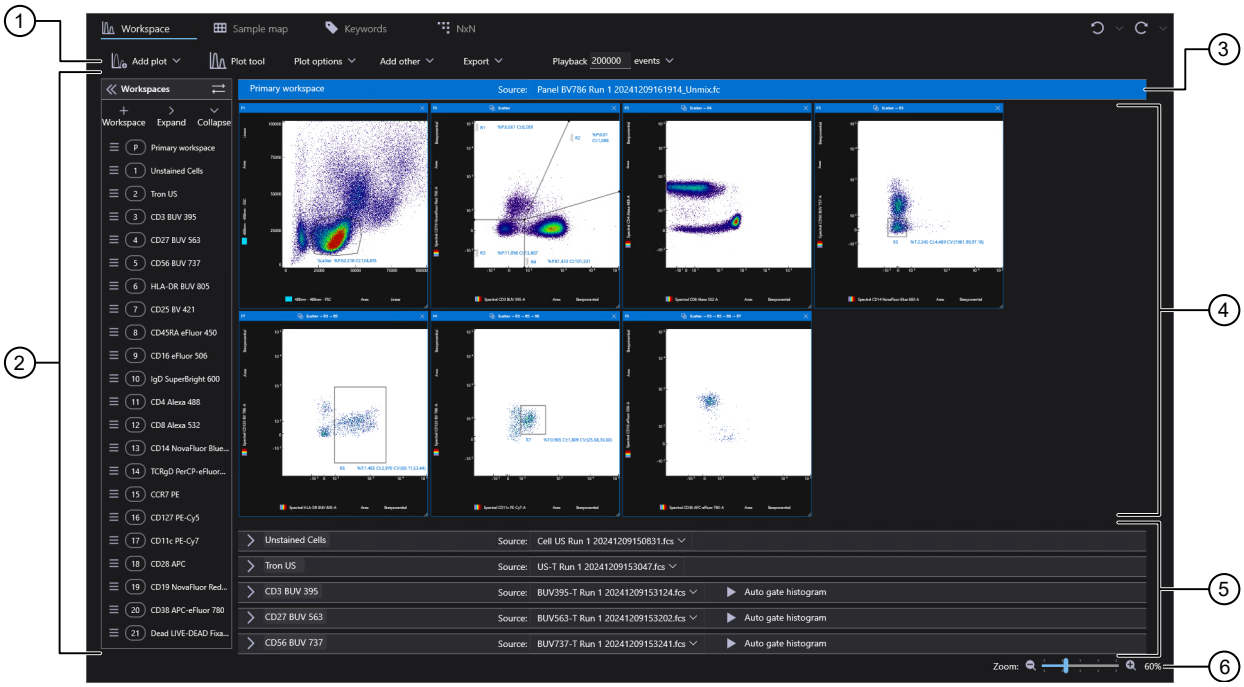


Figure 74 Workspace view

1. Workspace toolbar (page 106)
2. Workspaces panel (page 127)
3. Primary workspace (page 107)
4. Plot area (page 108)
5. Workspace groups (page 107)
6. Zoom

Note: For more information about the **Workspace** view, including detailed descriptions of the **Workspace toolbar**, **Workspaces panel**, **Workspace groups**, and the **Plot area**, see Chapter 6, “Workspace view” (page 115).

Workspace toolbar

Workspace toolbar contains the tools to build and edit plots, perform conventional compensation or spectral unmixing, and view, annotate, and share data.

Note: Depending on the **Unmixing type** selected for the experiment (**Spectral unmixing** or **Conventional compensation**), the **Workspace toolbar** provides different options.

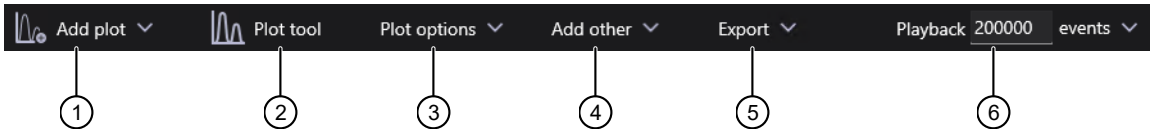


Figure 75 Workspace toolbar for an experiment with spectral unmixing

1. Add plot
2. Plot tool
3. Plot options
4. Add other
5. Export
6. Playback events

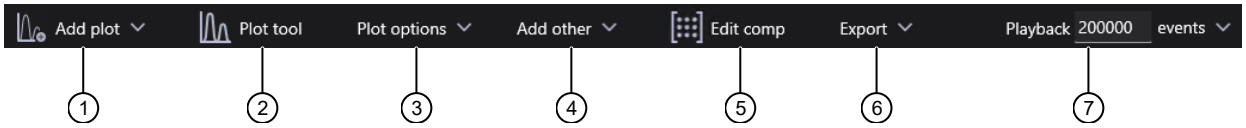


Figure 76 Workspace toolbar for an experiment with conventional compensation

1. Add plot
2. Plot tool
3. Plot options
4. Add other
5. Edit compensation
6. Export
7. Playback events

Note: For more information about the **Workspace toolbar**, including detailed descriptions of the toolbar options, see page 116.

Workspace groups

Workspace groups are collapsible groups corresponding to each workspace in the experiment, and are used to partition the workspace into functional sections. They can be used to show control samples such as compensation controls or the negative control. Groups can also display different types of samples with different gating strategies simultaneously.

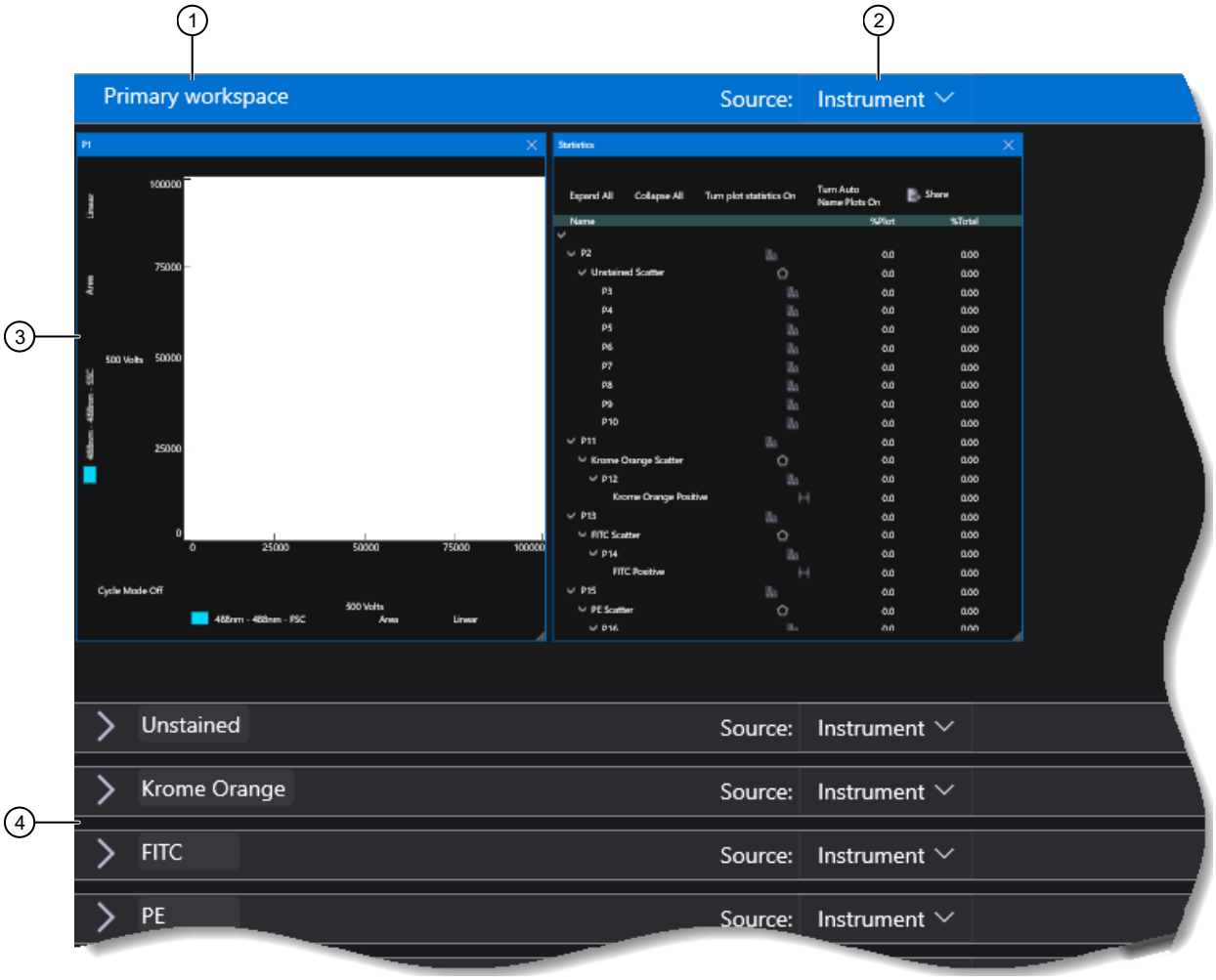


Figure 77 Workspace groups

1. Primary group workspace (expanded by default)
2. Source (Instrument)
3. Plot area for the primary group
4. Control sample groups (collapsed)

Note: For more information about **Workspace groups**, see “Workspace groups” on page 128 in Chapter 6, “Workspace view”.

Plot area

The **plot area** shows all the **Plots** for each workspace. **Statistics** and **Text** boxes, when added, are also shown here.

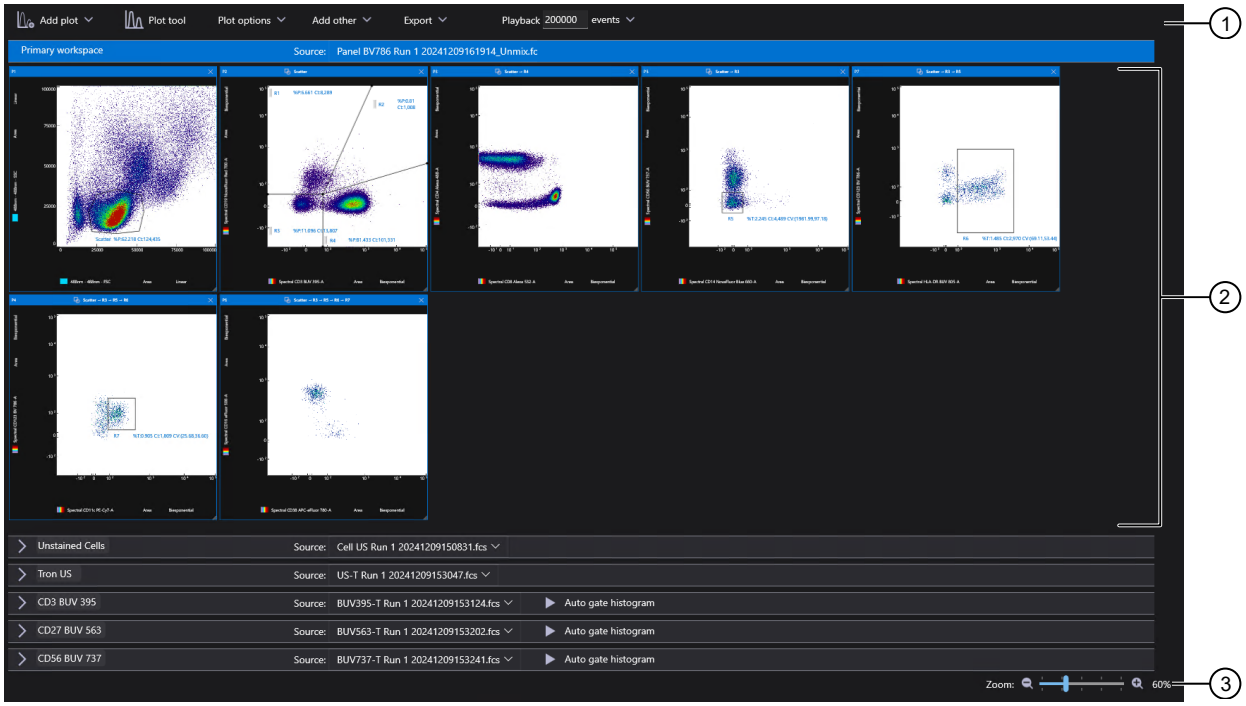


Figure 78 Plot area

① Workspace tool bar
② Plot area
③ Zoom

Note: For more information about the **Plot area**, see “Plot area” on page 130 in Chapter 6, “Workspace view”.

Sample map

The **Sample map** provides a graphical method for setting up experiments. It enables you to add one or more plates, samples, and groups to the experiment and setup the plate and tube layout. Using the **Sample map**, you can also assign samples and control wells, and designate a shutdown well.

The **Sample map** contains the **Sample map toolbar**, the **Plate manager**, and the **Layout editor**.

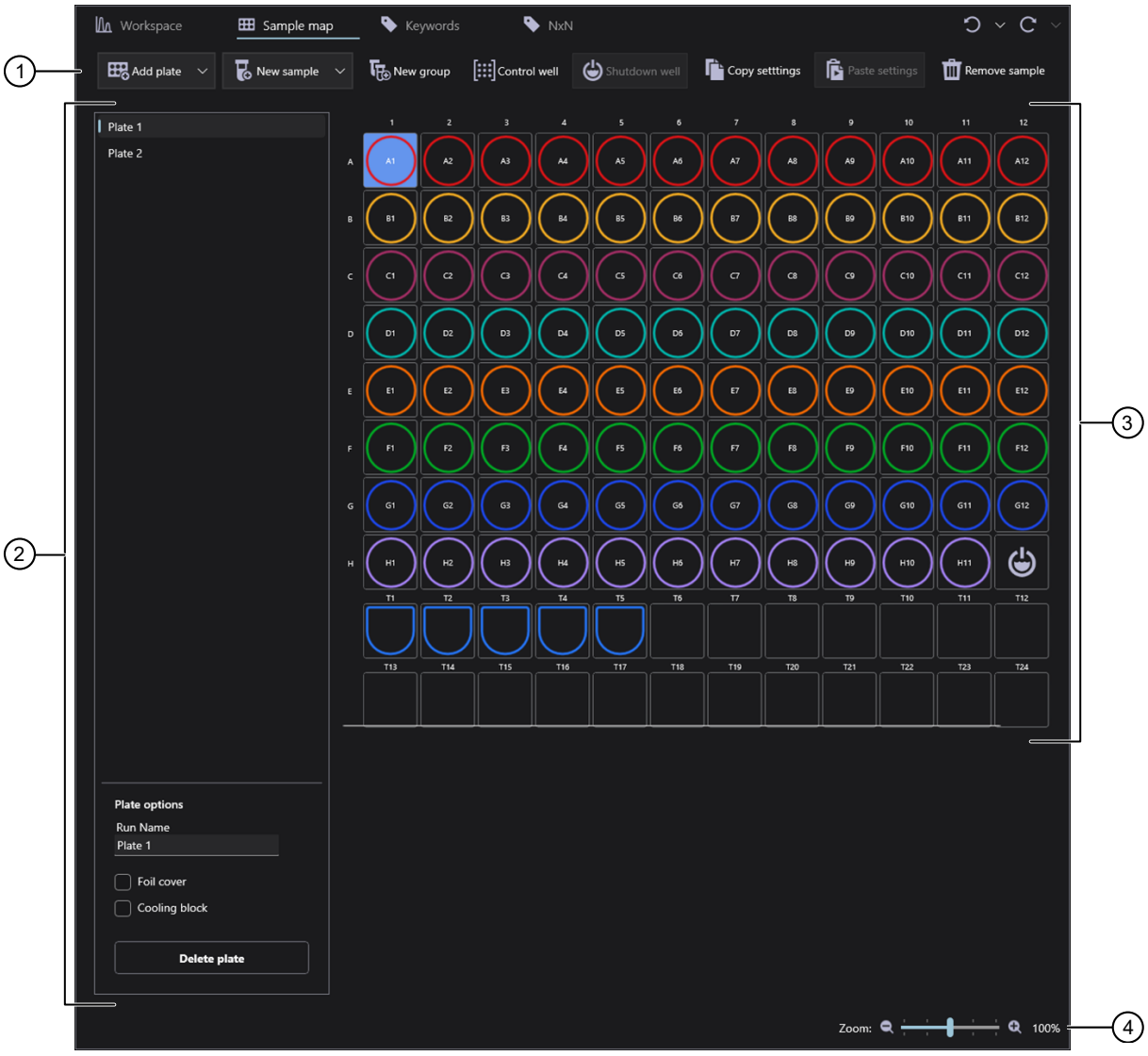


Figure 79 Sample map

1. Sample map toolbar (page 138)
2. Plate manager (page 147)
3. Layout editor (page 132)
4. Zoom

Note: For more information about the **Sample map** view, including detailed descriptions of the **Sample map toolbar**, **Plate manager**, and the **Layout editor**, see Chapter 7, “Sample map view”.

Keywords

Keywords view shows the **Keywords table**, which lists the sample keywords (Sample name, Group name etc.) for the experiment saved in the FCS file metadata, and enables you to update them after experiment setup (including adding custom keywords). Sample keywords provide more information about the sample and can be helpful when the FCS files are analyzed with different software packages.

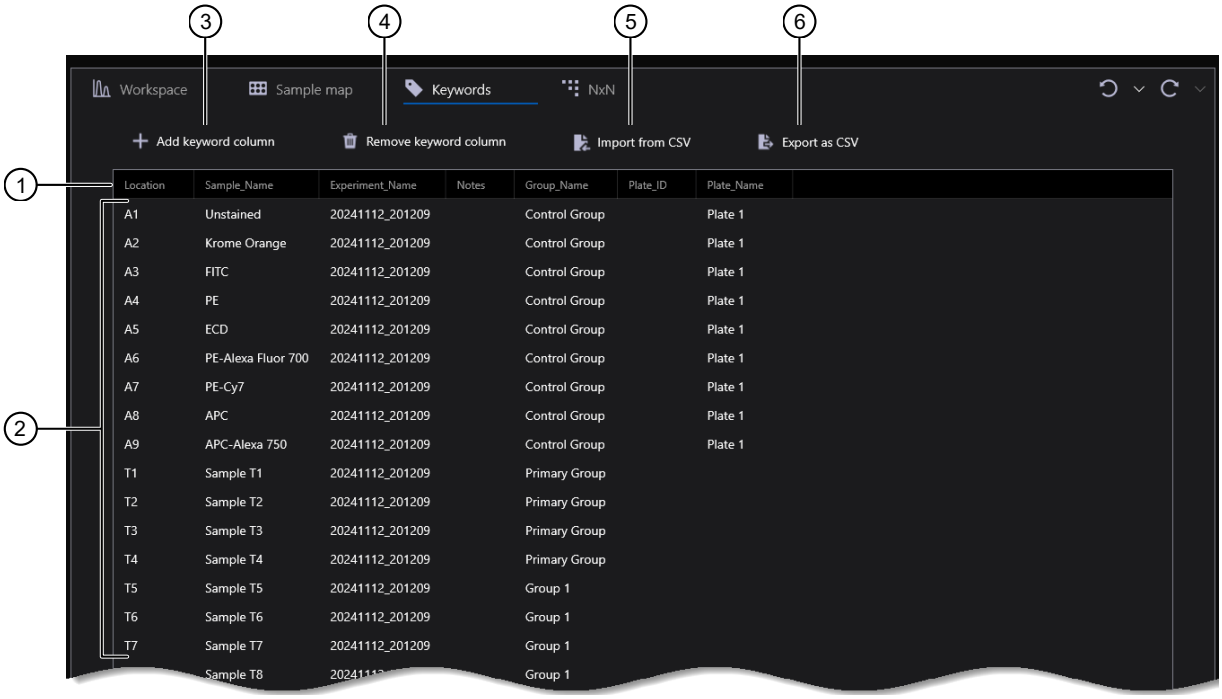


Figure 80 Keywords view

① Keywords table column headers
② Sample keywords
③ Add keyword column
④ Remove keyword column
⑤ Import from CSV
⑥ Export as CSV

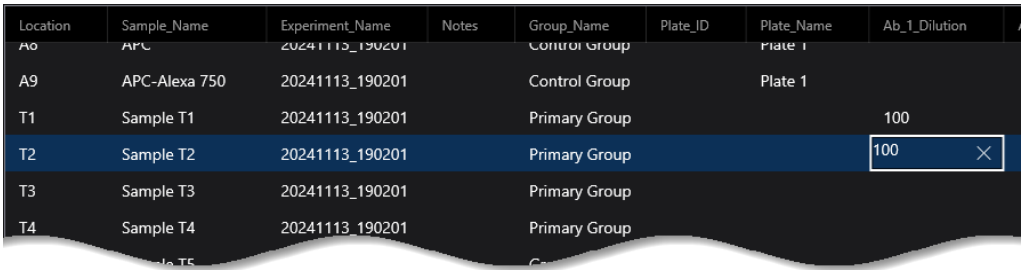
Keywords view has the following controls:

- **Add keyword column**: Enables you to add a new keyword column to the **Keywords table** from a predefined list of global keywords and to create custom keywords to add to the global keyword list. For more information, see page 111.
- **Remove keyword column**: Enables you to remove a keyword column from the **Keywords table**. Default global keyword columns cannot be removed. For more information, see page 112.
- **Import from CSV**: Opens the **Open (Import) dialog**, which enables you to import keywords from a CSV file.
- **Export as CSV**: Opens the **Save As (Export) dialog**, which enables to export the keyword as a CSV file.

Default keyword columns are **Location** ($WELLID), **Sample_Name** ($FIL), **Experiment_Name** ($PRO), **Notes**, **Group_Name** ($SMNO), **Plate_ID** ($PLATEID), and **Plate_Name** ($PLATENAME). These keywords are default at global level and cannot be removed.

You can manage the user-level and global keywords in the **Keywords Management** screen available via **Settings ▸ User Settings** or **Settings ▸ Global Settings**.

To add a sample keyword to a cell in the Keywords table or to change an existing keyword, click the cell to select, then type in the new keyword.



Add keyword column

Add keyword column + Add keyword column opens the **Add keyword column dialog**, which enables you to select from a list of predefined keywords to add to the **Keywords table**.

Note: The **keyword list** in the **Add keyword column dialog** is initially empty, but as more keywords are created using the **New keyword dialog**, the list gets populated with the custom created keywords.

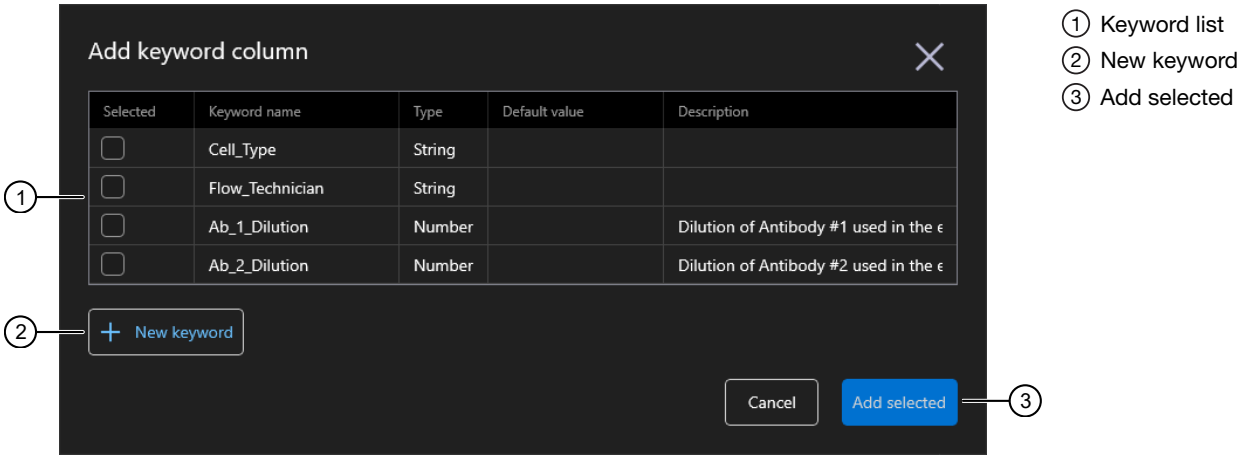


Figure 81 Add keyword column dialog (with 4 custom created keywords)

To add a keyword column to the **Keywords table**, select a keyword from the **keyword list**, then click **Add selected**.

To create a custom keyword and add it to the **keyword list**, click **New keyword**. The **New keyword dialog** opens, which enables you to create custom keywords.

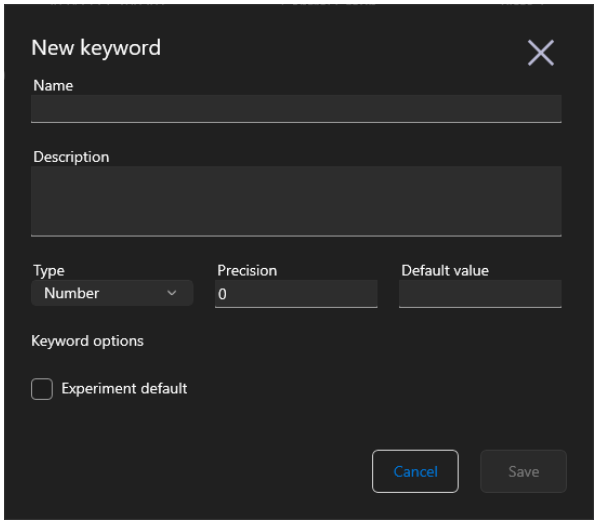


Figure 82 New keyword dialog

- Enter the **Name** and **Description** of the custom keyword you want to create.
- Select **Type**:
  - **Number**: Enables you to use numeric values for the sample keywords (for example, dilution factor). Enter **Precision** and **Default value** (if desired).
  - **String**: Enables you to use alphanumeric strings as keywords (for example, flow technician). Enter the **Default value** (if desired).

  Note: You can edit the **Default value** for the custom keyword later in the **Keywords table**.
- Click **Save** to add the new custom keyword to the **keyword list** in the **Add keyword column dialog**.

Remove keyword column

Remove keyword column Remove keyword column opens the **Remove keyword column dialog**, which enables you to delete keyword columns from the **Keywords table**.

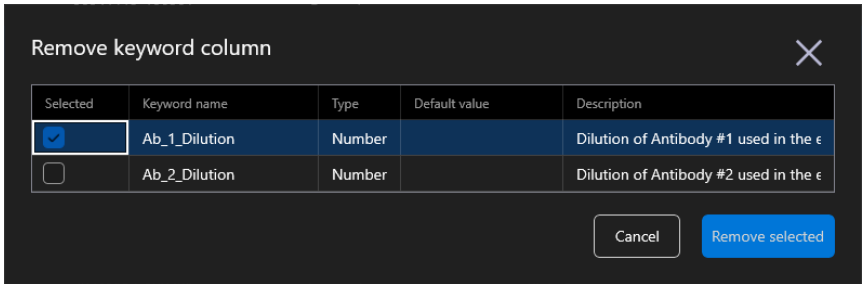


Figure 83 Remove keyword column dialog

Select the keyword columns you want to remove from the **Keywords table**, then click **Remove selected**.

Note: Only custom keywords are listed as an option for removal. Default keywords cannot be removed.

NxN

The **NxN** view enables you to generate **NxN plots** for up to two samples to compare data sets. NxN plots (also called multi-plots) are used to visualize every possible combination of parameters (markers) against each another. This allows you to quickly and efficiently evaluate the performance of your panel and identify potential problems with compensation, spectral unmixing, or other aspects of your experiment.

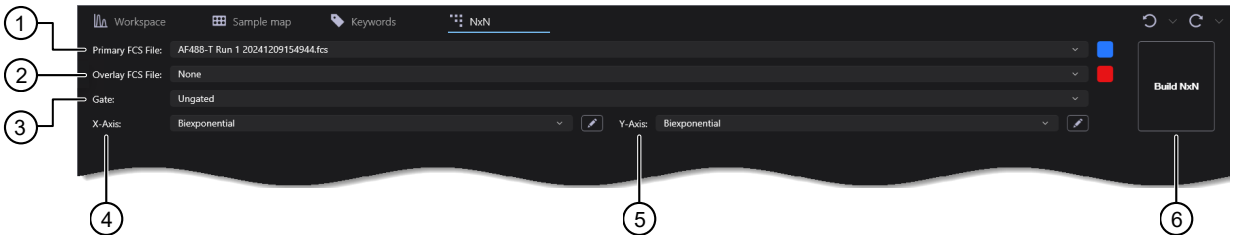


Figure 84 NxN tab

① Primary FCS File
② Overlay FCS File
③ Gate
④ X-Axis
⑤ Y-Axis
⑥ Build NxN

- **Primary FCS File**: Enables you to select the primary FCS file from the dropdown menu and view every parameter in the FCS file against every other parameter.

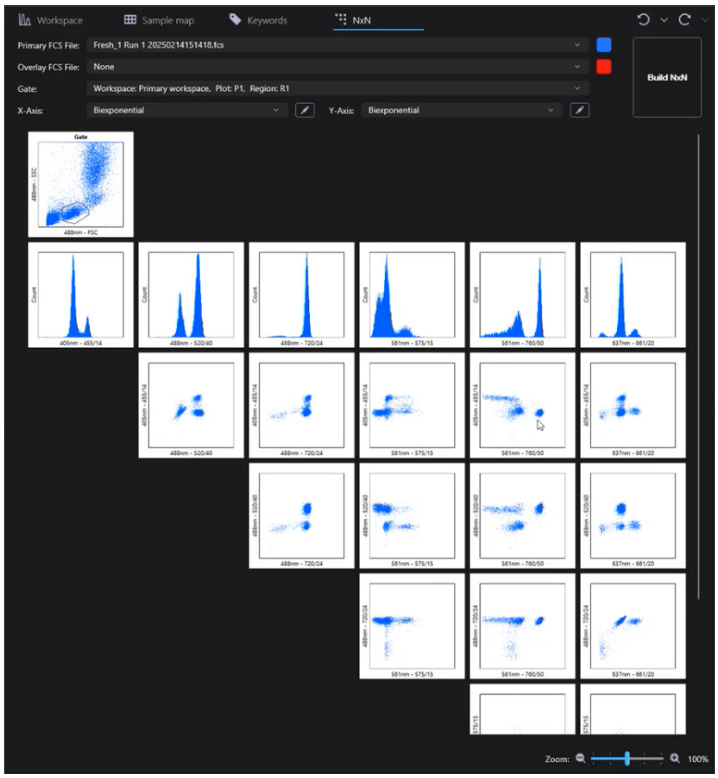
  The plot color for the primary FCS file is blue.
- **Overlay FCS File**: Enables you to select another FCS file to compare it to the primary FCS file.

  The plot color for the overlay FCS file is red.
- **Gate**: Enables you to select from the list of all gates available in the experiment.
- **X-Axis**: Enables you to select the scale for the X–axis of the plots.
- **Y-Axis**: Enables you to select the for the Y–axis of the plots.

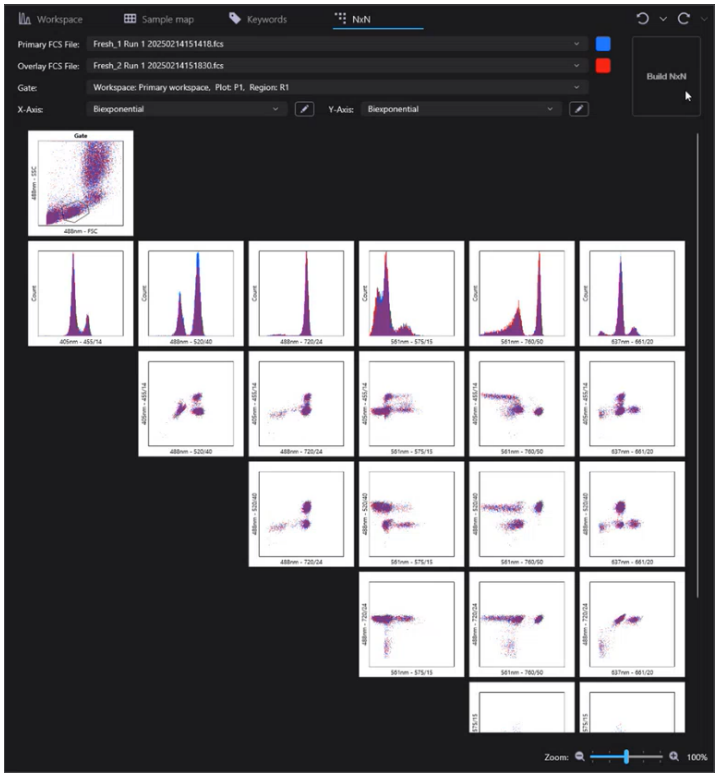
  Available scale options for the X–axis and Y–axis of the NxN plots are **Linear**, **Biexponential**, and **Log**. By default, **Biexponential** is selected.
- **Build NxN**: Generates the NxN plots from the selected FCS files and gate.

  If an overlay FCS file is selected, the data from the FCS files are shown superimposed in the plots, which enables the visualization and comparison of overlapping data.

Note: NxN plots take longer to generate as the number of parameters increases. We recommend that you create NxN plots only for spectral and compensation experiments where spectral and compensation parameters are available.



NxN plots (Primary FCS file only)



NxN plots (Primary with Overlay FCS file)

6 Workspace view

Workspace layout

Workspace displays the plots, gates, statistics, and text boxes that are associated with the current or a saved experiment. It contains the **Workspace toolbar**, **Workspaces panel**, **Workspace groups**, and the **Plot area**.

You can hide the **Workspaces panel** to provide more space for the plots by clicking the **hide icon** .

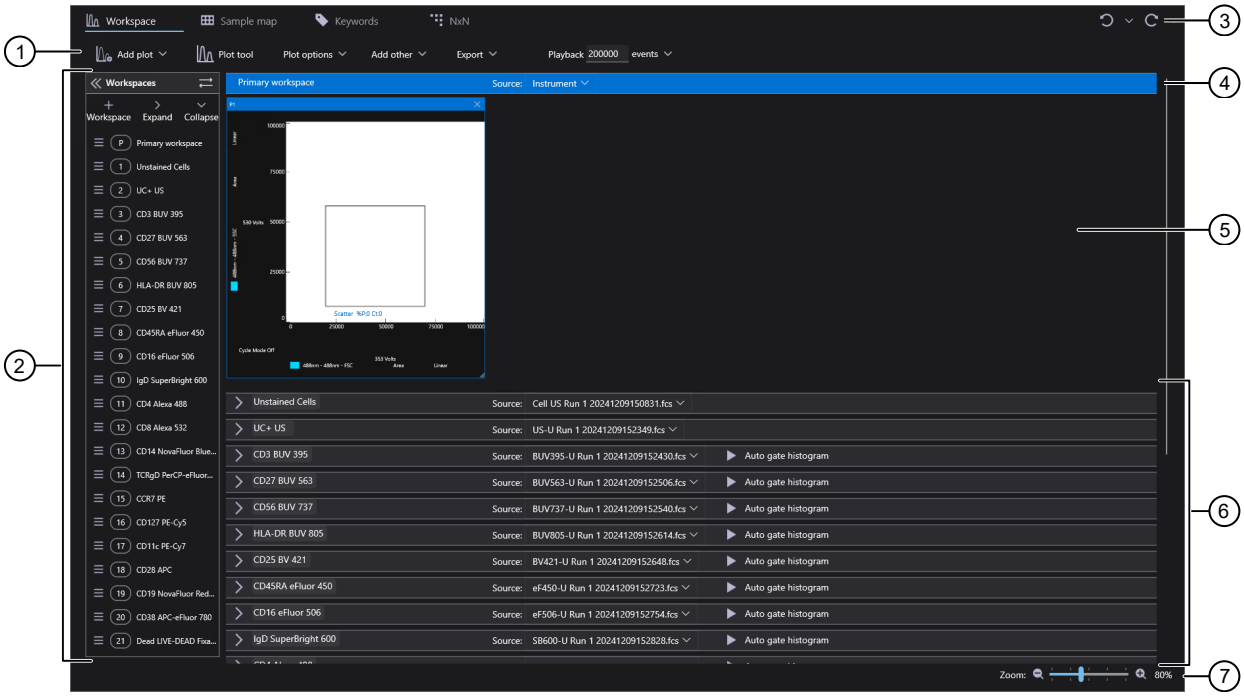


Figure 85 Workspace layout

1. Workspace toolbar (page 116)
2. Workspaces panel (page 127)
3. Undo and Redo buttons
4. Workspace groups (Primary group) (page 128)
5. Plot area (page 130)
6. Control groups (collapsed)
7. Zoom slider

Workspace toolbar

Workspace toolbar contains the tools to build and edit plots, perform conventional compensation or spectral unmixing, and view, annotate, and share data.

Note: Depending on the **Unmixing type** selected for the experiment (**Spectral unmixing** or **Conventional compensation**), the **Workspace toolbar** provides different options.

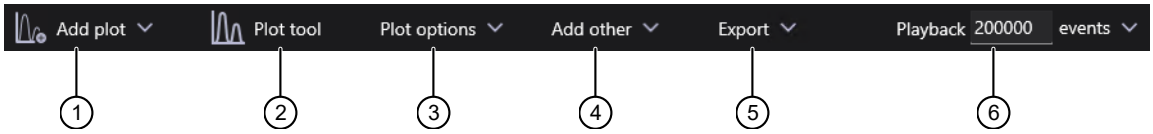


Figure 86 Workspace toolbar for an experiment with spectral unmixing

1. Add plot (page 117)
2. Plot tool (page 118)
3. Plot options (page 120)
4. Add other (page 121)
5. Export (page 126)
6. Playback events (page 126)

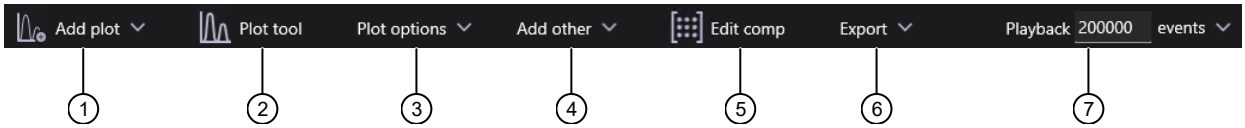


Figure 87 Workspace toolbar for an experiment with conventional compensation

1. Add plot (page 117)
2. Plot tool (page 118)
3. Plot options (page 120)
4. Add other (page 121)
5. Edit compensation (page 124)
6. Export (page 126)
7. Playback events (page 126)

Add plot

Add plot enables you to create the plot types listed below and add them to the **Plot area**.

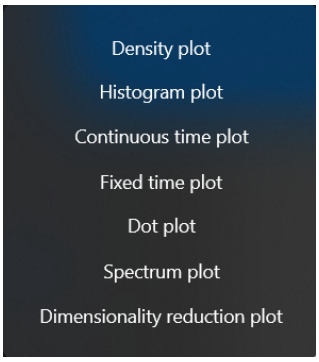


Figure 88 Add plot dropdown

- **Density Plot**: This is a density plot that is used to compare two different channels simultaneously, such as FSC vs. SSC, where each axis represents the signal intensity of one parameter.
- **Histogram Plot**: Used to analyze a specific channel and it is useful for looking at the intensity difference for a specific color in a population.
- **Continuous Time Plot**: Useful for assessing the number of events throughout time, such as Time vs. FSC to determine when the event rate is stable. Continuous plots always show all data with more time added when the maximum axis count is met.
- **Sliding Time Plot**: Useful for assessing the number of events throughout time, such as Time vs. FSC on a more immediate scale than continuous time plots. Sliding time plots always keep the same time frame shown and slide the axis values as time increases.
- **Fixed Time Plot**: Fixed time plots are useful for assessing the number of events throughout a fixed time scale. These plots show the specified time frame and do not increase as more data are collected.
- **Dot Plot**: Used to compare two different channels simultaneously, such as FSC vs. SSC, 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.
- **Spectrum Plot**: Shows the intensity of a sample in each detector. This option is enabled only for spectral systems in spectral unmixing experiments.
- **Dimensionality Reduction Plot**: Used to generate t-SNE (t-Distributed Stochastic Neighbor Embedding) or UMAP (Uniform Manifold Approximation and Projection) plots. Both t-SNE and UMAP are statistical methods for visualizing high-dimensional data by giving each datapoint a location in a two or three-dimensional map.

Plot tool

Plot tool opens the **Plot batch tool**, which enables you to create one or more plots in batch with the plot parameters, group, and gating already specified.

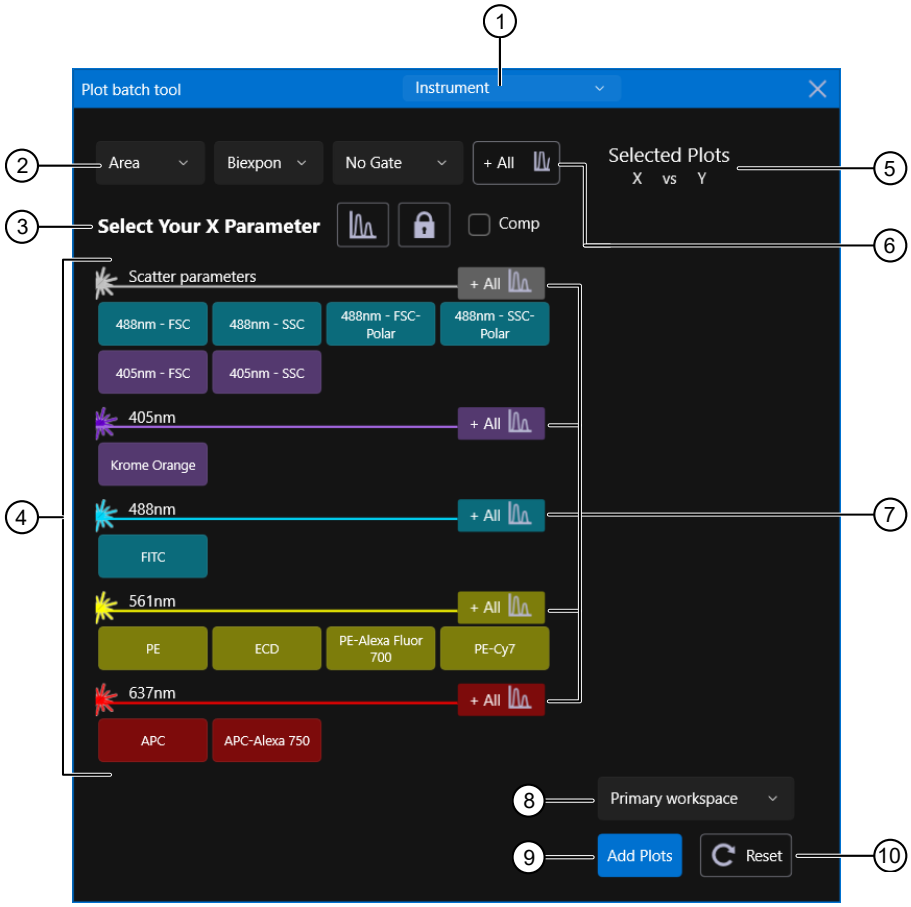


Figure 89 Plot batch tool

1. Data source
2. Data handling options
3. Select Parameter, Select Plot scale type, Select locked parameter
4. Parameter list
5. Selected Plots
6. Add All
7. Add All (for excitation source)
8. Select Group
9. Add Plots
10. Reset

- **Data source**: You can select **Instrument** or any saved FCS file for the plot data source.
- **Data handling options**: These controls enable you to select the measurement, plot scale type, and gates for the plot. The selected parameters are applied to all plots created in this batch.
  - **Measurement**: Enables you to select **Area** or **Height** measurement for calculating compensation. **Area** is selected by default.
  - **Plot scale type**: Sets all plot axes to the selected scale. Available scale types are **Logarithmic**, **Linear**, and **Biexponential**.
  - **Gate**: Enables you to select the gating for the plots in the batch.

- **Select Parameter** and **Plot scale type**: Guides you with the selection of the plot axes parameters and enables you select the plot scale type.
  - **Select Parameter**: Guides you to select plot axis parameters from the **Parameter list**. Depending on the **plot scale type** choice, you can select X–axis or X- and Y–axis parameters.
  - **Plot scale type**: Toggles between **dual-parameter** or **histogram** (single–parameter) plots.

    The default plot scale type is **dual parameter**, which enables you to select X– and Y–axis parameters.

    When the **Plot scale type button** is clicked, the plot scale type is **histogram** (single-parameter), which enables you select the X–axis parameter.
  - **Locked parameter** : Locks the selected X–axis parameter for all plots, so that several plots with the same X–axis parameter can be added.
- **Parameter list**: Enables you to select the scatter and fluorescence parameters for the plot. The parameters are grouped according to the **excitation source**. To select **X–axis** and **Y–axis parameters** when prompted, click the desired **fluorophore** from the list.
- **Selected Plots**: Lists the plots to be created based on the selections made using the **Plot batch tool**.

- **Add All**: When there is no locked parameter, adds new plots with the X–axis parameters set to each available fluorophore and the Y-axis parameter set to count.

  When there is a locked X-axis parameter, adds new plots with the locked X–axis vs. Y-axis parameters set to each available fluorophore.
- **Add All (for excitation source)**: When there is no locked parameter, adds new plots with the X–axis parameters set to each available fluorophore for the excitation source (that is, laser and filter configuration) and the Y-axis parameter set to count.

  When there is a locked X-axis parameter, adds new plots with the locked X–axis vs. Y-axis parameters set to each available fluorophore for the excitation source.
- **Group**: Selects the **group workspace** where the new plots are added.
- **Add Plots**: Creates the plots listed in the **Selected Plots** list with the plot parameters, group, and gating choices made in the tool.
- **Reset**: Clears the **Selected Plots** list.

Plot options

Plot options enables you to specify the display options for plots.

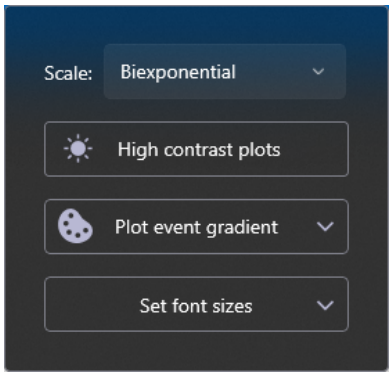
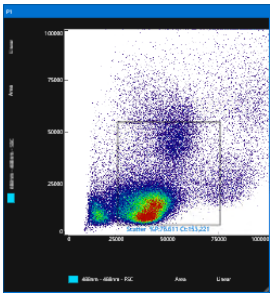


Figure 90 Plot options dropdown

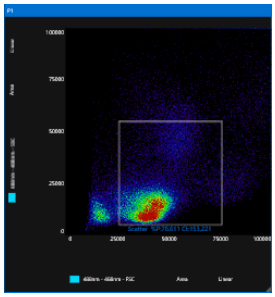
- **Scale**: Sets the transformation scale for the plot to the selected scale. Available scale types are **Logarithmic** and **Biexponential**.

  Note: Only one scale can be selected at a time to keep the scaling consistent in the experiment.

- **High contrast plots**: Toggles between the **normal contrast** and **high contrast** plot displays.



Normal contrast plot



High contrast plot

- **Plot event gradient**: Enables you to select the color gradient for the events shown on the plot. Available options are **Rainbow (Default)**, **High Contrast**, **Red Green**, **Rare Population**, and **User Defined**.
- **Set font sizes**: Enables you to set **Plot font size** and **Region font size**.

Add other

Add other provides the option to add statistics and text boxes to the group workspace.

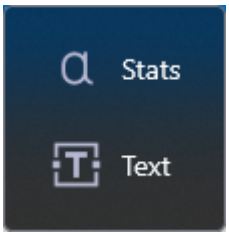


Figure 91 Add other dropdown

- **Stats**: Adds a **Statistics table** to the workspace, which shows the combined statistics from acquired events. For more information, see “Statistics” on page 121.
- **Text**: Adds an **Annotation box** to the workspace, which enables you to add text for annotation and notes. For more information, see “Text” on page 123.

Statistics

Stats button adds a **Statistics table** to the workspace, which shows the combined statistics from all the plots and regions in the workspace. Statistics can be displayed for any parameter and calculated for any defined population. As the events are acquired, statistics are updated in real time.

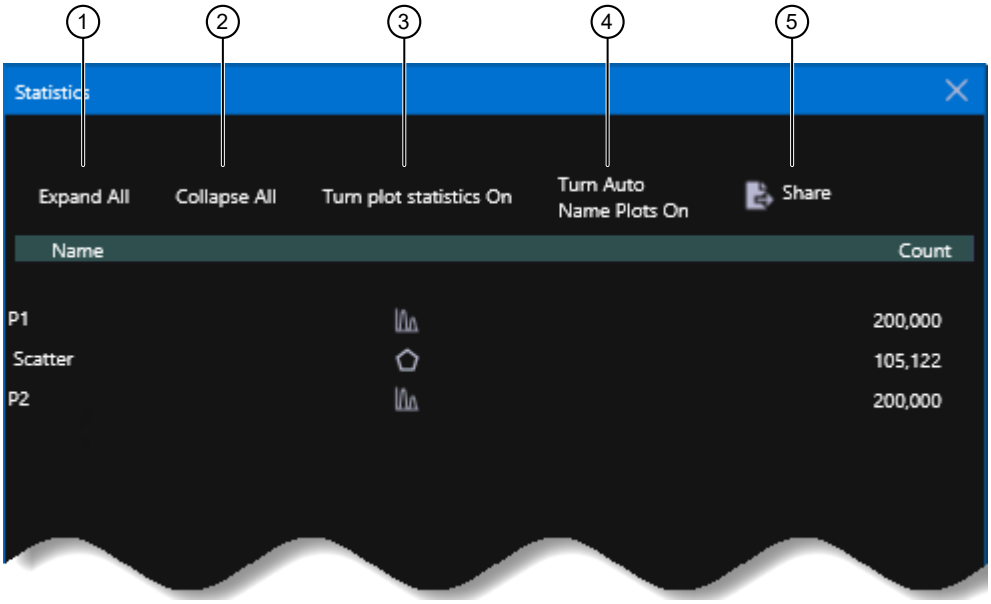


Figure 92 Statistics table (all expanded)

① Expand All
② Collapse All
③ Turn plot statistics On
④ Turn Auto Name Plots On
⑤ Share

The upper section of the **Statistics table** contains statistics from the primary group. The lower section shows any statistics from control groups that are automatically created, which are minimized by default. In each statistics section, the plot and gate hierarchy is shown.

The **Statistics table** shows the statistics selected in the **User Settings ▸ Statistics** or the **Global Settings ▸ Statistics** screens. You can select to show specific statistics for the **Plot statistics**, **Combined statistics**, and **Region statistics**.

Depending on the statistics you want to show (**Plot**, **Combined**, or **Region statistics**), you can select from the following statistics:

- **Count (Ct)**
- **%Total (%T)**
- **%Plot (%P)**
- **Voltage (V)**
- **Variance (Var)**
- **Mode**
- **Mean**
- **StdDev (SD)**
- **rStdDev (rSD)**
- **Laser Wavelength (WL)**
- **Concentration (Cn)**
- **Volume (Vm)**
- **Geometric Mean (GM)**
- **CV**
- **rCV**
- **Max**
- **Median (Med)**
- **Min**

Note: For a description of the statistics, see “Statistics” on page 217.

Pn is the name of the plot. Plots can be renamed on the plot itself. Any new names are reflected in the **Statistics table**. If no name has been entered, the plot has the default name P1, P2, and so forth.

Expand All shows the hierarchy of all the plots and regions.

Collapse All hides the hierarchy of all the plots and regions.

Turn Plot Statistics On toggles the display of plot statistics on all plots in the experiment. You can select the default statistics in **Settings ▸ User Settings ▸ Statistics** or **Settings ▸ Global Settings ▸ Statistics**.

Share opens a file save menu that enables a CSV file of the statistics to be exported to any drive available.

Text

Text button adds an **Annotation box** to the workspace, which enables you to add text for annotation and notes.

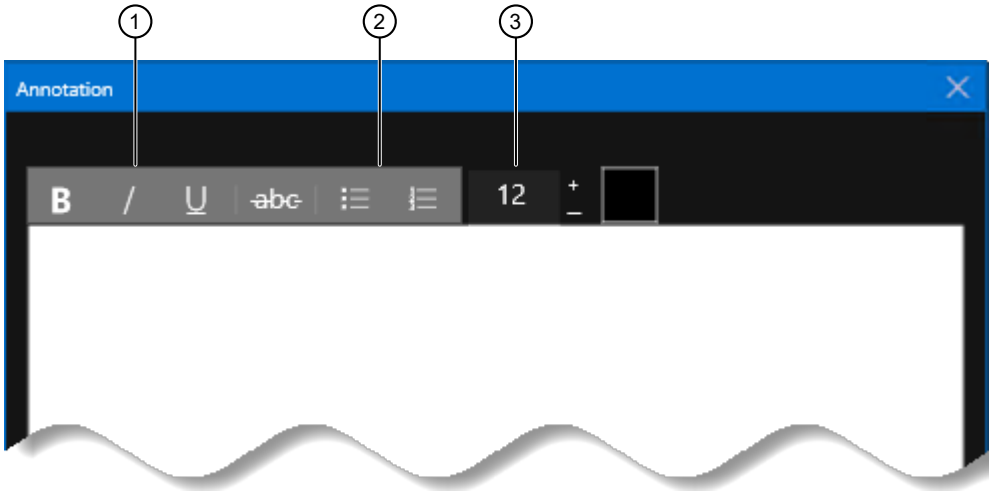


Figure 93 Annotation text box

① Font styles
② Add ordered list and Add numbered list
③ Font size

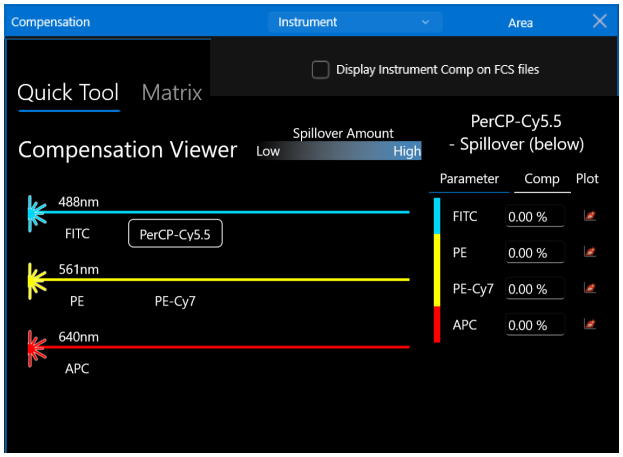
- **Font styles** enable you to select one or more of the **Bold**, **Italic**, and **Underline** options for the text font style.
- **Add ordered list** formats the selected text in bullet points.
- **Add numbered list** formats the selected text as a numbered list.
- **Font size** sets the font size for the selected text.

Edit compensation

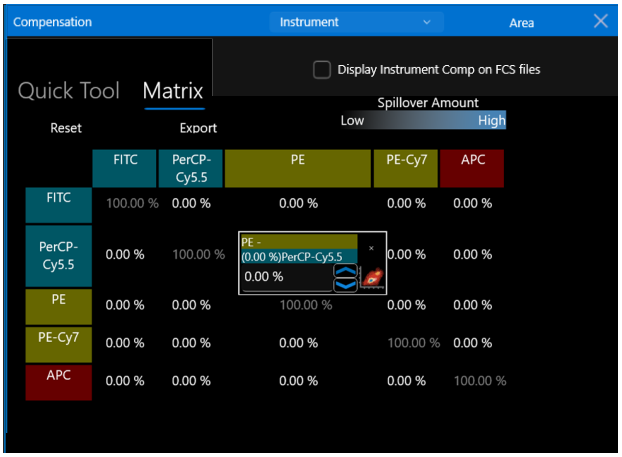
Edit comp button opens the **Compensation tool**, which enables you to verify and manually edit compensation results.

You can select to edit compensation values using the **Quick Tool** or the **Matrix**.

Note: The **Edit comp** option is available on the **Workspace toolbar** only if the **Conventional compensation** option was selected for **Unmixing type** when creating the experiment.



Quick Tool



Matrix

- **Quick Tool** shows parameters on the left in laser order and the possible combinations of each parameter on the right. The amount of compensation is shown in the **Comp** column. You can adjust each compensation value in real time to see its effect. To create new plots from each combination, click the **Plot** button to the right of each parameter.
- **Matrix** shows a table of each parameter compared against the other with the compensation value at their intersection point. Select a value to change the compensation and create density plots of the selected parameters.

Note: For more information about compensation, see the **Compensation** chapter on page xx.

Similarity matrix

Similarity matrix button opens the **Spectral similarity matrix**, which shows how alike the emission spectra are on a scale of 0 to 1 when comparing two dyes and highlights the most similar fluorophores. A score of 0 indicates that the colors are entirely different and a score of 1 indicates that the fluorophores are identical.

The **Spectral similarity matrix** is used to identify combinations of fluorophores that can increase the complexity of the panel getting built to resolve the required populations. The example below shows a similarity matrix of 23 colors.

Note: The **Similarity matrix** option is available on the **Workspace toolbar** only if the **Spectral unmixing** option was selected for **Unmixing type** when creating the experiment.

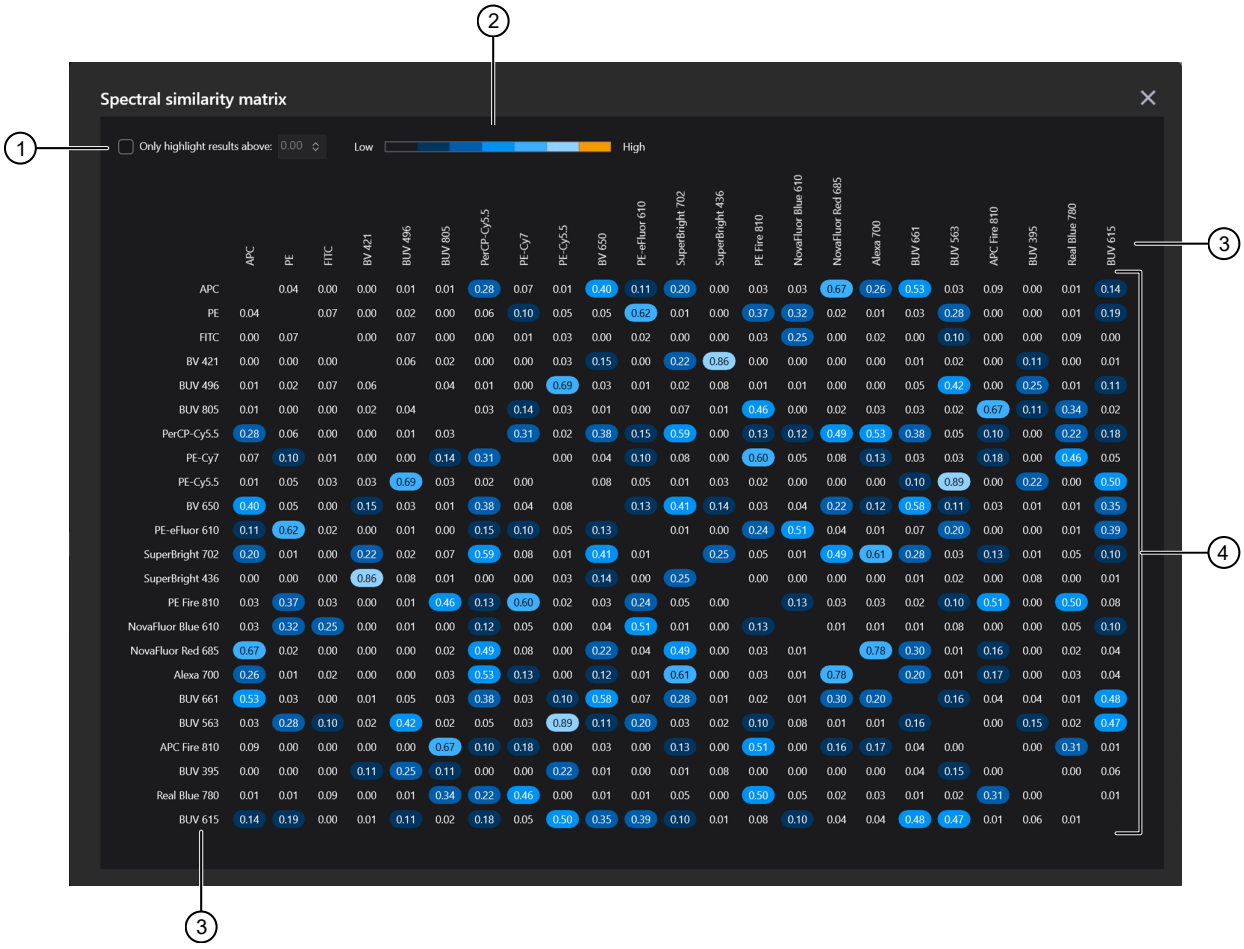


Figure 94 Spectral similarity matrix

1. Only highlight results above
2. Similarity gradient legend
3. Fluorophores
4. Spectral similarity scores

Note: For more information about the similarity matrix and setting up experiments with spectral unmixing, see the **Spectral unmixing** chapter on page xx.

Export

Export enables you to share and print the experiment workspace as a PDF and to copy the workspace as images.

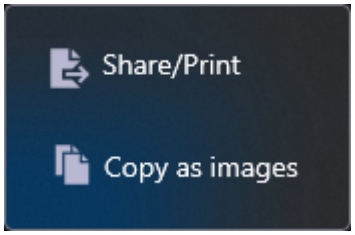


Figure 95 Export dropdown

- **Share/Print**: Opens the **Share** screen, which enables you to share the experiment workspace as a PDF. On the **Share** screen, you can select which workspace objects to include in the PDF. You can also refresh the data on the workspace before creating the PDF, if some workspace plots are blank.
- **Copy as images**: Copies the plots and workspace objects from all groups to the clipboard. You can then paste the images to another document.

Playback events

Playback events enables you to set the number of events in the FCS file to replay. You can enter the number of events to replay directly into the text field or select it from the dropdown list.

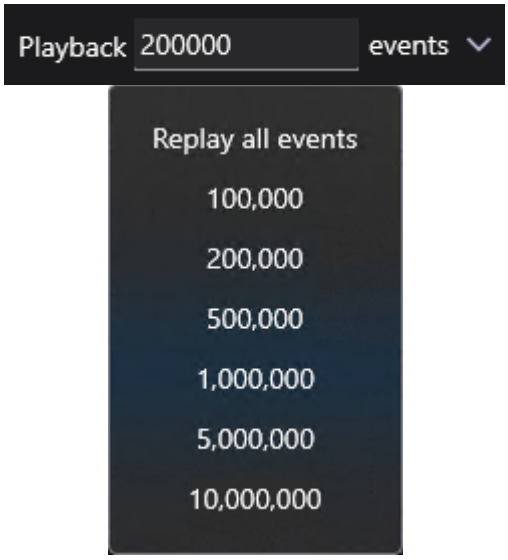
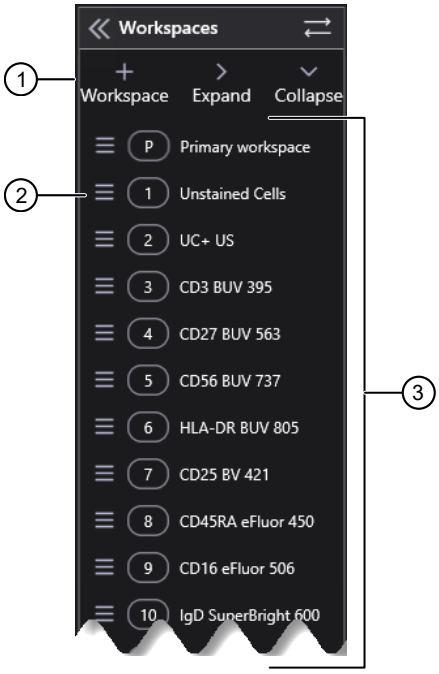


Figure 96 Playback events dropdown

Note: Replaying every event or large number of events in the FCS file can be time consuming for large FCS files, but necessary for viewing samples that include rare events.

Workspaces panel

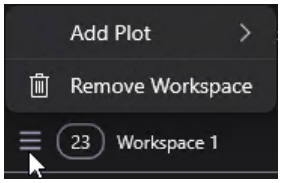
Workspaces panel lists all groups that correspond to each workspace in the experiment and enables you to add new workspaces and workspace plots to the experiment. You can also toggle the display of groups in the **Workspace view** from the **Workspaces panel**.



1. Add Workspace, Expand, and Collapse options
2. Options menu
3. Workspaces

Figure 97 Workspaces panel

- To hide the **Workspaces panel**, click the **hide** icon .
  To view the **Workspaces panel** again, click the **Expand Workspaces panel** icon .
- **Expand** expands all the groups in the **Workspace view**.
  Collapse collapses all the groups in the **Workspace view**.
- **Add Workspace** add a new group to the **Workspaces panel** and the experiment.
- **Options menu** enables you to manage the experiment workspaces, and has the following options:



  - **Add Plot**: Enables you to add a plot to the **Workspace**.
    Available plot types to add are **Density plot**, **Histogram plot**, **Continuous time plot**, **Fixed time plot**, **Dot plot**, and **Spectrum plot**.
  - **Remove Workspace**: Removes the selected group from the experiment and all plots associated with the group.

Note: You cannot remove the **Primary group** or any of the **control groups**.

Workspace groups

Workspace groups are collapsible groups that correspond to each workspace in the experiment, and are used to partition the workspace into functional sections. They can be used to show control samples such as compensation controls or the negative control. Groups can also include different types of samples with different gating strategies simultaneously.

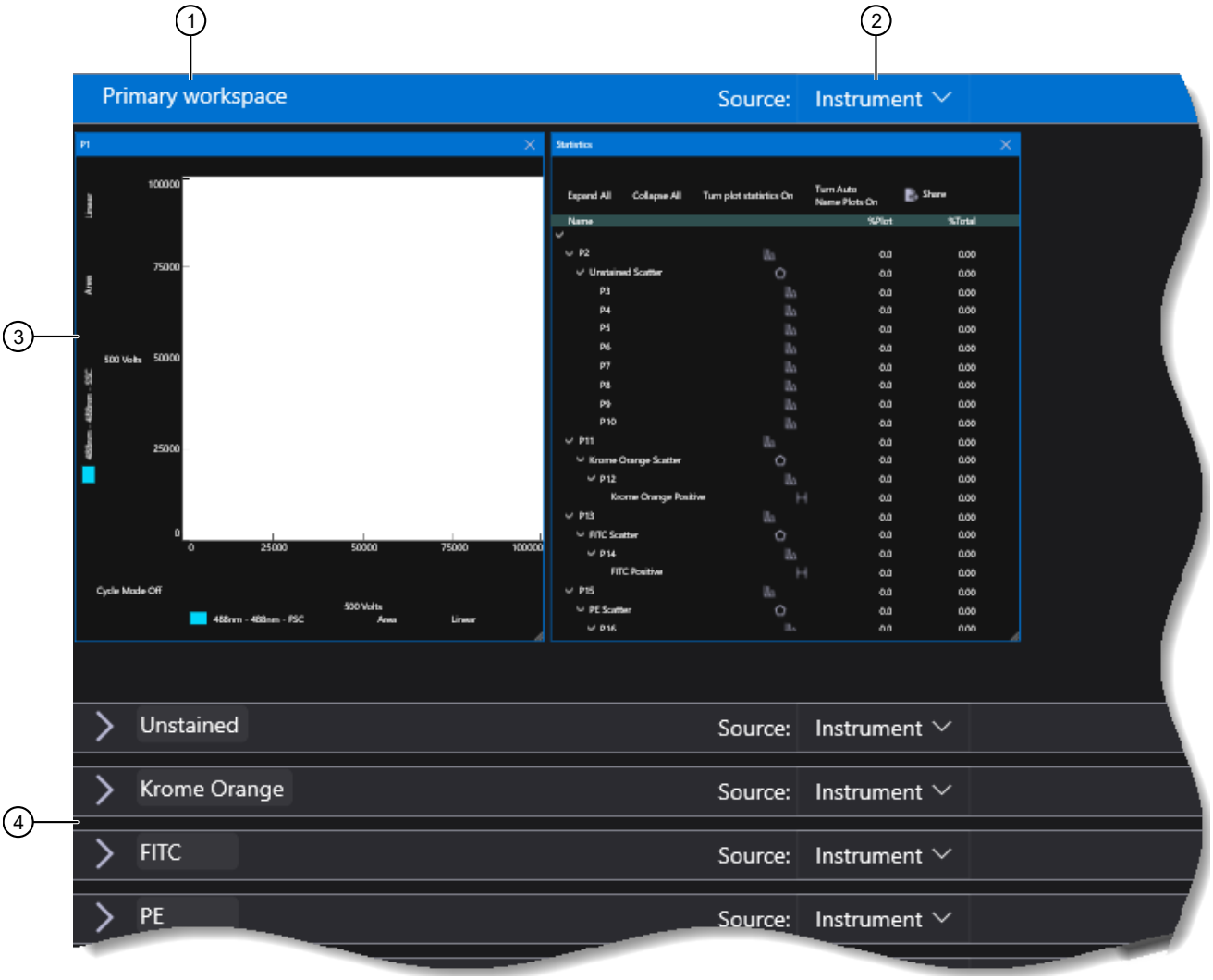


Figure 98 Workspace groups

① Primary group workspace (expanded by default)
② Source (Instrument)
③ Plot area for the primary group
④ Control sample groups (collapsed)

- The first group in every experiment is labeled **Primary group**.
- When the **Workspace** first opens, all groups are collapsed, except the **Primary group**. To expand a group, click the **gray expand/collapse arrow** next to the group name.
- The **group title bar** above each group shows the **group name** and the **data source**.

- If compensation or spectral options (such as single-color controls, unstained controls) were selected during experiment setup, then the workspaces for each control sample are automatically created and organized into their own groups. Both the auto compensation wizard and spectral unmixing algorithms interact with these groups.

  IMPORTANT! Do **not** remove automatically created groups.

- Groups are named automatically for selected controls. You can change the **group names** by directly editing the text in the **group name** field in the **title bar**.
- By default, the **data source** for each group is **Instrument**. If FCS files have been added to the sample list, a group can be set to show the data from an FCS file.
- To create more groups, click **Add group** in the **Sample panel**.

  To delete a group, click the **Toolbar dropdown** in the **Sample panel** with the group selected, then select **Delete**.

  Note: The **Primary group** cannot be deleted.

- If more than one group is present, use the workspace scrollbar to view groups that are off screen in the **Workspace**.

Plot area

The **plot area** shows all the **Plots** for each workspace. **Statistics** and **Text** boxes, when added, are also shown here.

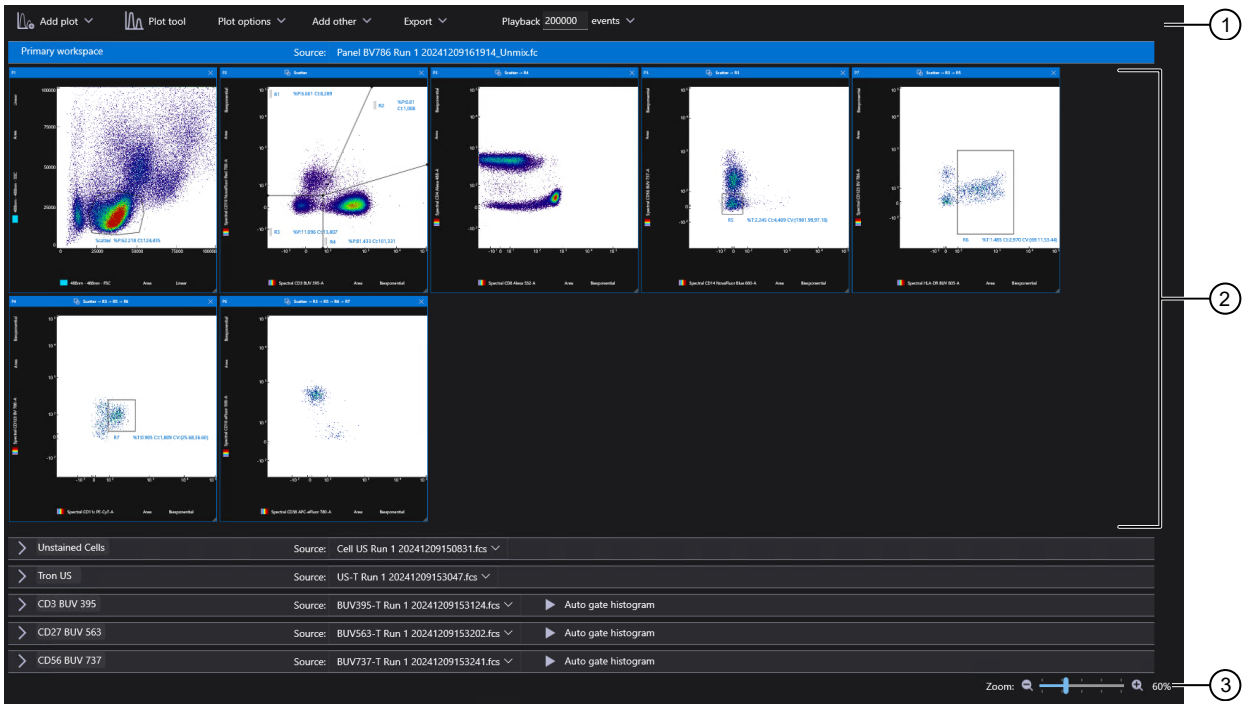


Figure 99 Plot area

① Workspace tool bar
② Plot area
③ Zoom

- To add a new plot to a group, select a group, then click **Add plot** and select a plot type to add. Alternatively, double-click a blank part of the workspace.
- To add a **Statistics** or a **Text** box, click **Add other**, then select **Stats** or **Text**.
- Use the **Zoom** tool to zoom in and out of the workspace to view more or fewer plots in the **plot area**. The default value is 100%.

  Click **–** or **+** icons on the **Zoom** tool to decrease or increase the zoom value by 10%.

  Click on the **Zoom scale** to adjust the zoom value to within 10% of the position selected.

7 Sample map view

Sample map layout

The **Sample map** provides a graphical method for setting up experiments. It enables you to add one or more plates, samples, and groups to the experiment and setup the plate layout. Using the **Sample map**, you can also assign samples and control wells, and designate a shutdown well.

The **Sample map** view is divided into the **Layout editor**, the **Sample map toolbar**, and the **Plate manager**.

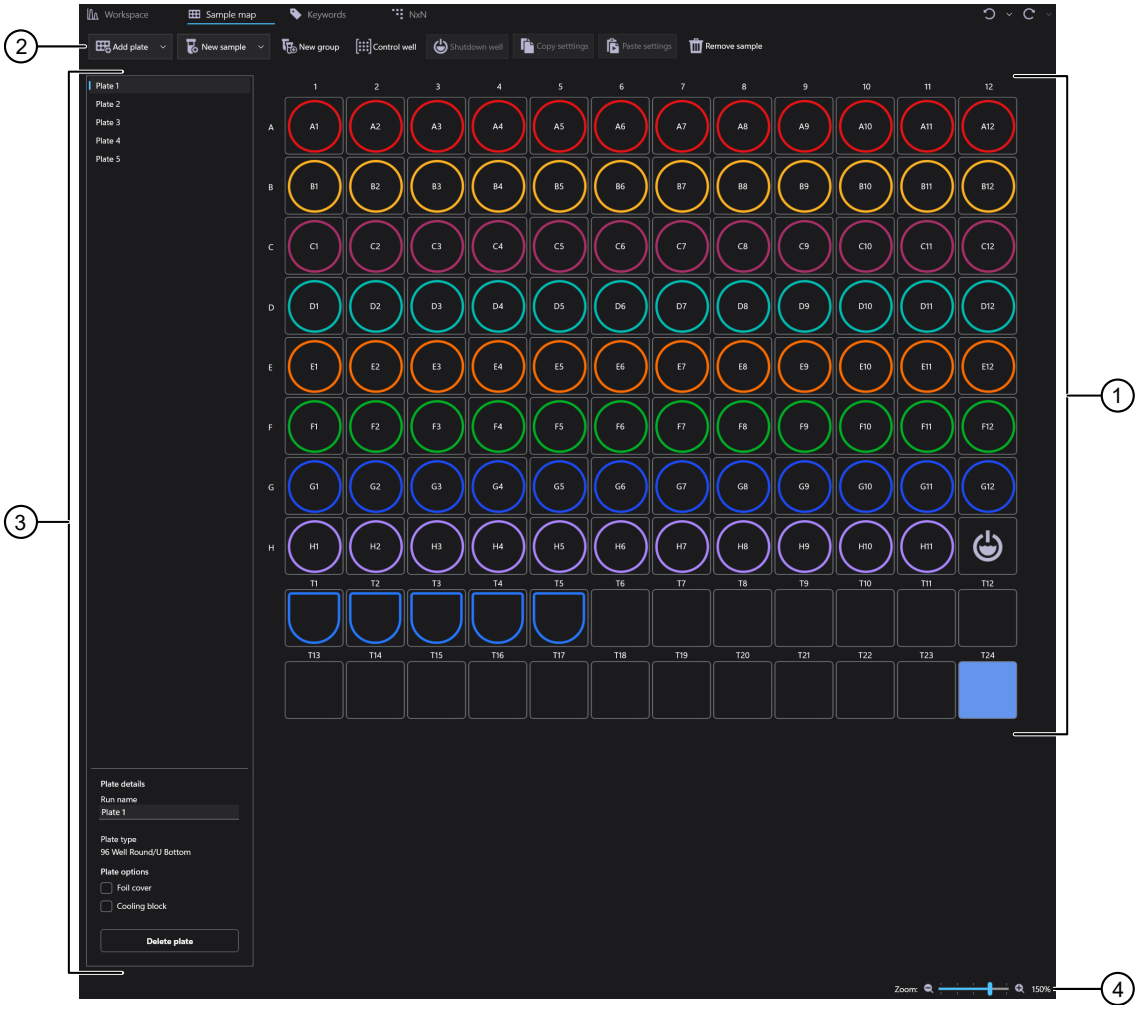


Figure 100 Sample map layout

① Layout editor (page 132)
② Sample map toolbar (page 138)
③ Plate manager (page 147)
④ Zoom

Layout editor

The **Layout editor** provides a graphical method for setting up plate- and tube-based experiments.

Display of samples

- **Plate samples** are created as a table with the number of wells based on the **Plate type** (96-well, 384-well).
- **Tube samples** are created in rows of 12 samples.
- In the following example, the experiment has both plate samples and tube samples.
  - In the 96-well plate, there are 9 controls (**A1**–**A9**) and 48 plate samples in two groups (**B1**–**C12** and **D1**–**E12**). Rows **F**–**H** in the plate are empty.
  - There are 8 tube samples in the first row, which are divided between two groups (**T1**–**T4** and **T5**–**T8**). **T9**–**T12** in the first row of tubes and the second row are empty.
  -

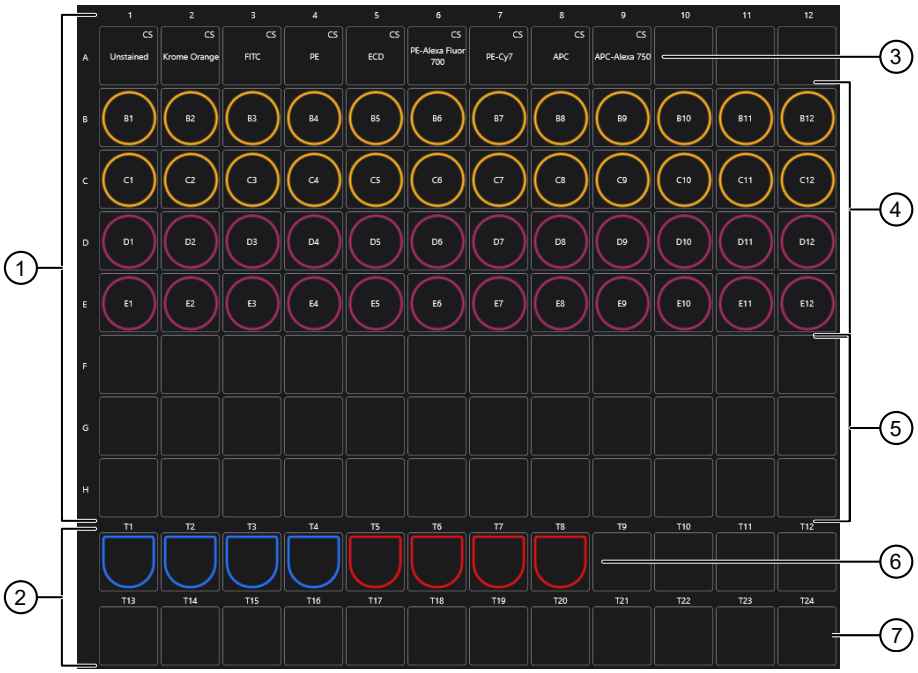
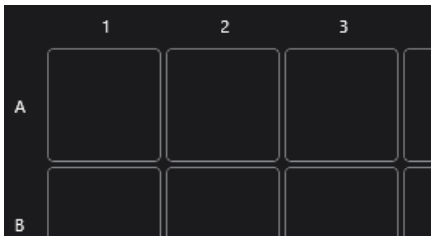


Figure 101 Layout editor with a 96-well Plate and 24 Tubes

1. 96-well plate
2. 24 tube rack
3. Control samples in 96-well plate
4. Plate samples
5. Empty wells in the 96-well plate
6. Tube samples
7. Empty row in tube rack

- Empty sample locations are shown as empty squares.

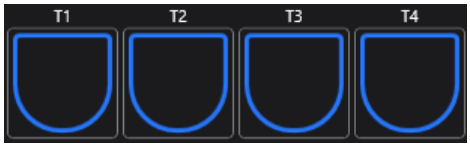


Empty Plate sample locations (A1, A2, A3)

Empty Tube sample locations (T1, T2, T3)

- **Plate samples** and **Tube samples** have different shapes to help identify them more easily.

Plate samples (Wells C1–C4 of a 96-well plate)



Tube samples (Locations T1–T8)

- Each **Group** has a unique identifying color that outlines the **Sample** that belongs to it. This color differentiates the **Sample** belonging to that **Group** from **Samples** in other **Groups**.

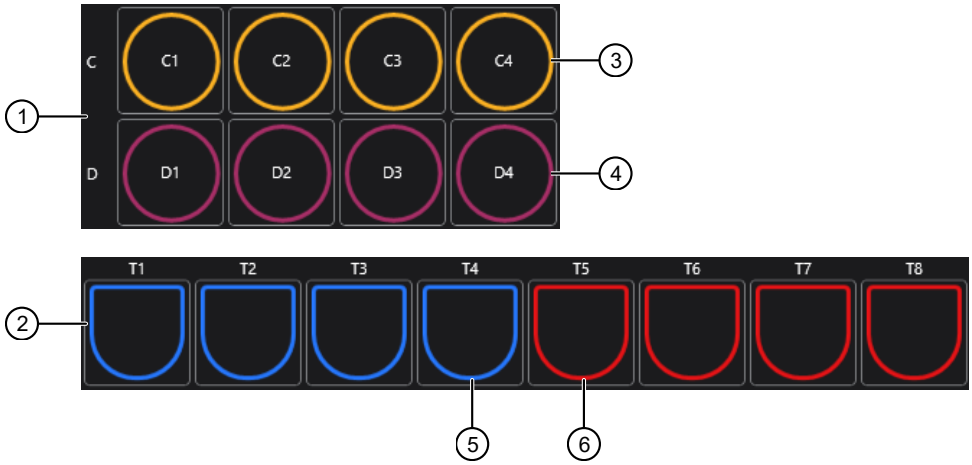
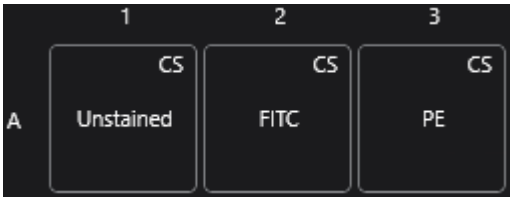


Figure 102 Plate samples and Tube samples in different Groups

① Plate samples (Wells C1–C4 and D1–D4)
② Tube samples (Locations T1–T8)
③ Group color for Plate samples C1–C4 (Group 2)
④ Group color for Plate samples D1–D4 (Group 3)
⑤ Group color for Tube samples T1–T4 (Primary Group)
⑥ Group color for Tube samples T5–T8 (Group 1)

- You can label selected wells in the **Plate layout editor** as **Control wells** for the acquisition using the **Control well** button in the **Sample map toolbar**.
- **Plate samples** that are labeled as **Control wells** are tagged with the **Compensation well badge (CS)** or the **Spectral unmixing well badge (SP)** based on the **Unmixing type** selected for the experiment. The **Control wells** also show the name of the parameter they represent.



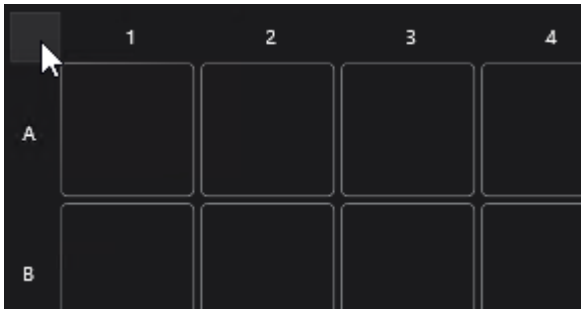
Compensation control wells (CS)

Spectral unmixing control wells (SP)

Selection in Layout editor

You can select multiple **Locations** or **Samples** simultaneously. The following methods can be used alone or in combination to make the desired selections:

- Click as you hold the **Ctrl** key to select non-adjacent **Plate samples**, **Tube samples**, or empty **Locations** on the **Layout editor**.
- Click as you hold the **Shift** key to select all **Samples** or **Locations** between the selected points.
- Click the **row header** on a **Plate** to select that entire row.
- Click the **column header** on a **Plate** to select that entire column.
- Click a **Plate sample** or **Location** to select, then use the **Ctrl+A** key combination to select the entire **Plate**.
- Click the top left corner of a **Plate** to select the entire **Plate**.



- Click a **Tube sample** or **Tube location** to select, then use the **Ctrl+A** key combination to select all **Tube samples** or **Tube locations** in the **Layout editor**.

Move samples

To move samples or controls from one location to another on the **Layout editor**, select the samples or controls, then drag-and-drop them to the new location.

Figure 103 Move controls in Layout editor

① Select the controls you want to move, then drag and...
② ...drop the controls at the new location.
③ The controls are at their new location.

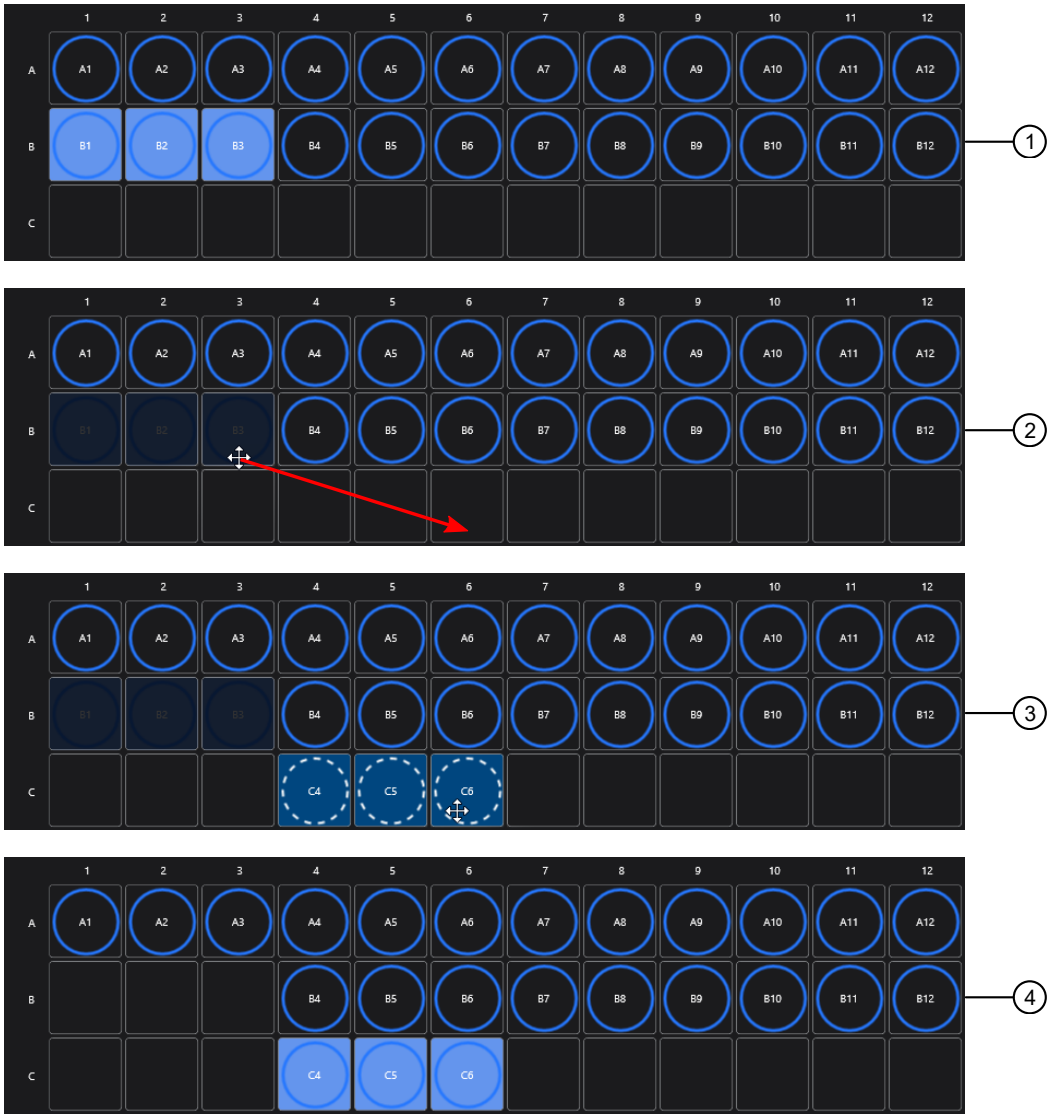


Figure 104 Move samples in Layout editor

① Select the samples you want to move, then...
② ...drag the samples to their new location.
③ Drop the samples at their new location.
④ The samples are at their new location.

Sample map toolbar

Sample map toolbar enables you to add one or more plates, samples, and groups to the experiment, setup the plate layout, desigante the control wells and the shutdown well, copy and paste settings, and remove samples from the sample map.

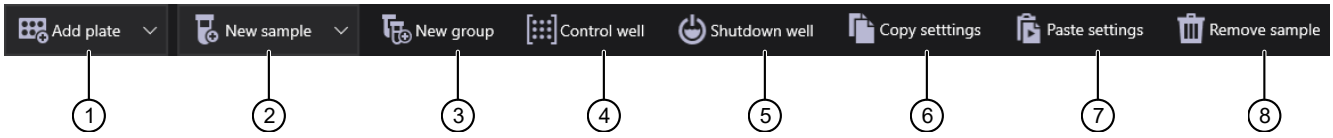


Figure 105 Sample map toolbar

① Add plate (page 138)
② New sample (page 140)
③ New group (page 142)
④ Control well (page 144)
⑤ Shutdown well (page 146)
⑥ Copy settings (page 146)
⑦ Paste settings (page 146)
⑧ Remove sample (page 146)

Add plate

Add plate adds a plate run to the workflow based on the current plate.

Clicking on the dropdown arrow opens the **Add plate dropdown**, which enables you select a plate type to add to the run.

Figure 106 Add plate dropdown

- **Add plate dropdown** shows the plate list filtered by **Favorites**.
- After the first plate is added, clicking the main part of the **Add plate** button adds the same type of plate last selected.
- The software creates a unique name for each new **Plate**, starting with **Plate 1**, continuing with **Plate 2**, **Plate 3**, and so on.
- If a plate is not available in the **Favorites** list (or recently used list), you can access the full list of available plates by selecting the **View all…** option from the dropdown.

  View all... opens the **Add Plate window**, which lists all available plates types (see Figure 107).
  - **Plate size filter** in the **Add Plate window** enables you filter the available plates by size.
  - **Plate search tool** enables you to enter the **description** or the **part number** of a plate to search for a specific plate type.

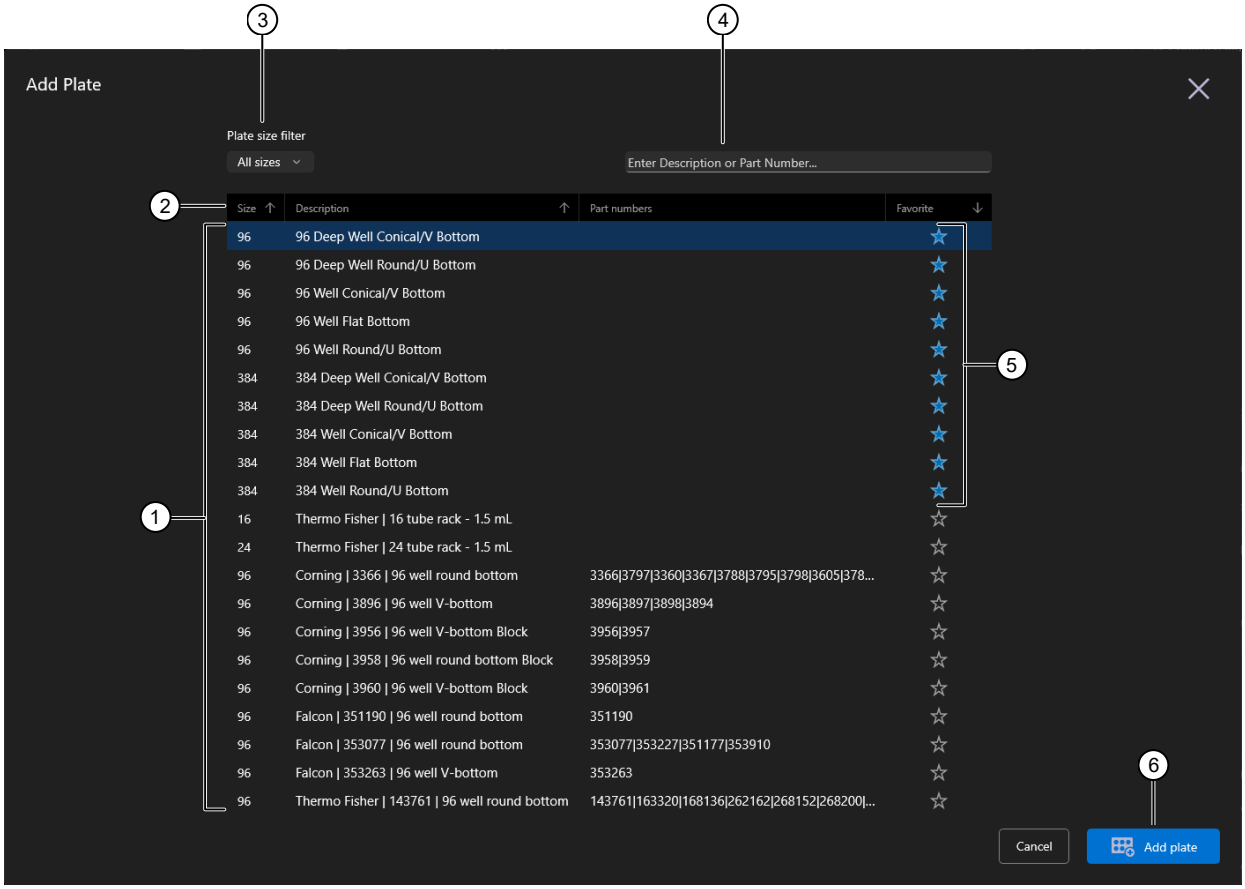


Figure 107 Add Plate window

1. Plate list
2. Plate list column headers (Size, Description, Part numbers, Favorite)
3. Plate size filter
4. Plate search tool
5. Favorites
6. Add plate

Note: If the Attune™ Xenith™ Flow Cytometer is connected to a CytKick™ Max Autosampler, you can create custom 96-well and 384-well plate definitions in **Plate Options**, available in **Global Settings** or **User Settings**, which you can access via the **Global navigation ▸ Settings** option.

New sample

New sample adds a new **Sample** to the selected tubes, wells, or both. The new **Sample** is added to the last-created **Group**.

If there are multiple **Groups** in the experiment, then the **New sample** button opens the **New sample dropdown**, which enables you to select the **Group** to add the new **Sample**.

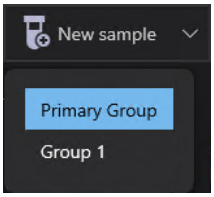


Figure 108 New sample dropdown

- By default, clicking the main part of the **New sample** button creates a new **Sample** in the **Primary group**.
- If there are multiple **Groups** in the experiment, clicking the **New sample** button opens the **New sample dropdown**.

  New sample dropdown shows the available **Groups** into which you can add new **Samples**.
- After the first new **Sample** is added to a **Group**, clicking the main part of the **New sample** button adds the new **Sample** to the last selected **Group**.
- Newly created **Samples** are visible in the **Layout editor** and the **Sample list** in the **Sample panel**.

To add a new **Sample**:

1. Click the **Location** on the **Layout editor** to select where you want to add the **Sample**. To select multiple locations to create multiple new **Samples** simultaneously:
   - Click as you hold the **Ctrl** key to select non-adjacent **Locations**.
   - Click as you hold the **Shift** key to select all **Locations** between the selected points.

   The selected **Locations** are highlighted in blue. In this example, only a single **Location** is selected (F1 on the 96-well plate).

2. To add the new **Sample**, click the main part of the **New sample** button.
   - If there are no **Groups** other than the default **Primary group**, then the new **Sample** is added to the **Primary group**.
   - If there are multiple **Groups** in the experiment, then the new **Sample** is added to the last selected **Group**.
3. To add the new **Sample** to a specific **Group**, click the **dropdown arrow** on the **New sample** button, then select the **Group** from the **New sample dropdown**.
4. The newly added **Sample** is shown in the **Layout editor** and in the **Sample panel**. The new **Sample** shows the color of the **Group** into which it is added (in this example, red for **Group 1**).

Figure 109 New Sample in the Sample panel and the Layout editor

① Group 1 (Group color: Red)
② New Sample in Group 1 in the Sample panel
③ New Sample with Group 1 color in the Layout editor

New group

New group creates a new **Group** and adds the selected **Tube samples**, **Plate samples**, or both to the new **Group**. Future **Samples** are added to the last created **Group** by default.

To create a new **Group**:

1. In the **Layout editor**, select the **Tube samples**, **Plate samples**, or both that you want in the new **Group**. To select multiple **Samples**:
   - Click as you hold the **Ctrl** key to select non-adjacent **Samples**.
   - Click as you hold the **Shift** key to select all **Samples** between the selected points.

   The selected **Samples** are highlighted in blue (in this example, **Samples** in **Row B** of the 96-well plate, B1–B12).

2. To create the new **Group** with the selected **Samples**, click **New group**.
3. The newly created **Group** containing the selected **Samples** is shown in the **Layout editor** and in the **Sample panel**.

Figure 110 New Group in the Sample panel and the Layout editor

① New Group 1 with selected Samples in the Sample panel
② New Group 1 in the Layout editor (Group color: Red)

Control well

Control well designates the selected wells in the **Layout editor** as controls for the acquisition.

Based on the **Unmixing type** selected for the experiment, the **Control wells** are tagged with the **Compensation well badge (CS)** or the **Spectral unmixing well badge (SP)**. The **Control wells** also show the name of the parameter they represent.

Note: You can add **Control wells** only in compensation or spectral unmixing type experiments, and only designate **Plate sample wells** as **Control wells**.

Compensation control wells (CS)

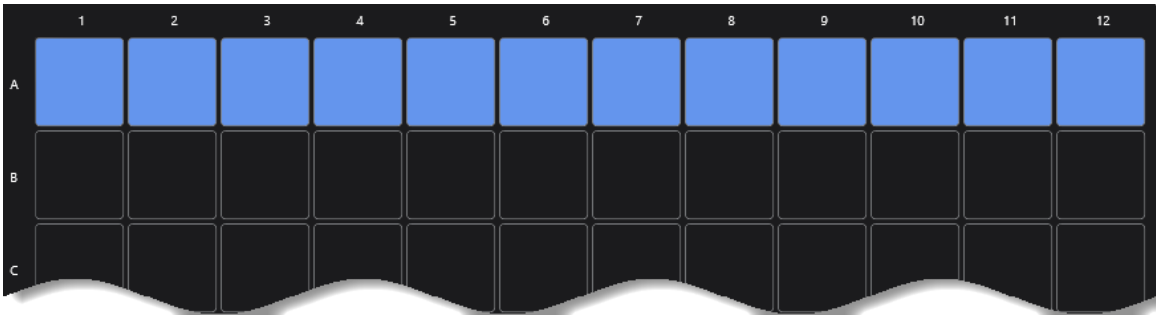
Spectral unmixing control wells (SP)

Figure 111 Control wells in the Layout editor

To add a new **Control well**:

1. Click the **Location** on the **Layout editor** to select where you want to add the **Control well**. To select multiple **Locations**:
   - Click as you hold the **Ctrl** key to select non-adjacent **Locations**.
   - Click as you hold the **Shift** key to select all **Locations** between the selected points.

   The selected **Locations** are highlighted in blue. In this example, Row A (wells A1–A12) of a 96-well plate is selected.



2. To label the selected wells as controls, click **Control well**.
3. The selected wells are designated as **Control wells** in the **Layout editor**.

   Controls wells show the name of the parameter they represent and a badge that indicates the **Unmixing type** (in this example, **SP** badge for **Spectral unmixing**).

Note: The software creates only as many **Control wells** as there are control samples in the experiment. In this example, 12 wells (A1– A12) were selected in Step 1, but there are only 8 control samples in the experiment, so only the first 8 wells (A1–A8) in the plate are designated as **Control wells**.

Shutdown well

Shutdown well designates a single selected empty well on the **Layout editor** as the **Shutdown well**, which is used for the maintenance and shotdown processes after the acquisition is complete. After the **Shutdown well** is run, the instrument shuts down automatically.

The **Shutdown well** is indicated in the **Layout editor** by the **Shutdown well icon** (in the following example, well F2).

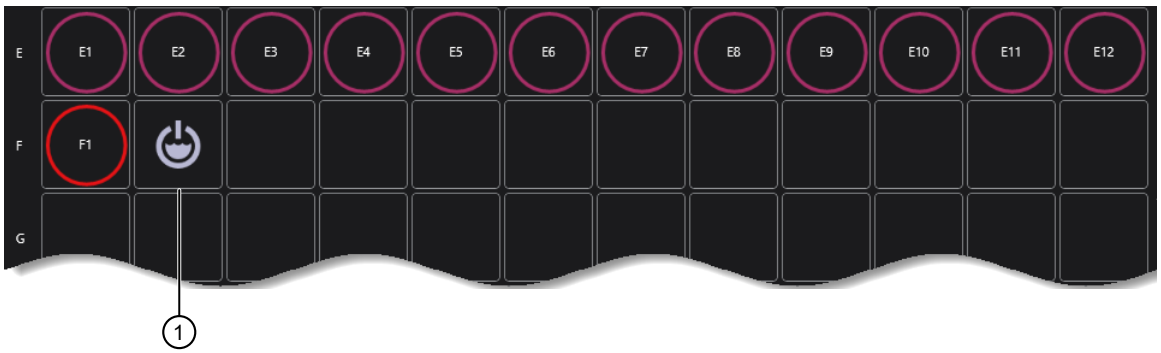


Figure 112 Shutdown well in the Layout editor

① Shutdown well

- All **Sample wells** are processed before the **Shutdown well** if they are included in the run selection.
- When the **Shutdown well** is processed, the system proceeds to run an automated shutdown procedure without having to add a tube to the SIP (Sample Injection Port).

Note: When the **Shutdown well** is processed at the end of the acquisition and the instrument is shut down, the SIP is not considered rinsed or sanitized (see Chapter 13, “Maintenance”).

Copy settings, Paste settings, and Remove sample

Copy settings copies the acquisition settings from the selected wells to the clipboard.

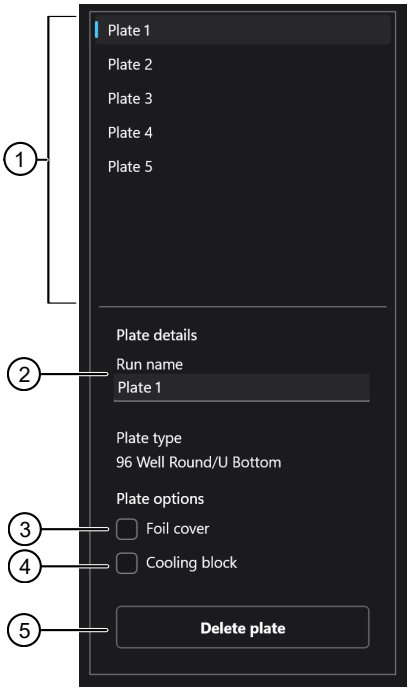
Paste settings applies the previously copied acquisition settings into the current selection of wells.

Remove sample deletes all properties from the selected samples and removes the samples from the acquistion.

Note: **Control samples** cannot be deleted.

Plate manager

Plate manager enables you to select plates to view and edit in the **Play layout editor**. Using the **Plate manager**, you can also rename plates, select **Foil cover** and **Cooling block** options, and delete selected plates.



1. Plate list
2. Run Name (Plate name)
3. Foil cover
4. Cooling block
5. Delete plate

Figure 113 Plate manager

- **Plate list** enables you to select a **Plate** from the list of plate runs created for the experiment to view in the **Plate layout editor**.

  Click a **Plate** in the **Plate list** to select it. The selected **Plate** is highlighted in blue and shown in the **Plate layout editor**.

- **Run Name** enables to rename the selected **Plate**.

  To rename the selected **Plate**, click the **Run Name text box** and type in the new name.

- When the **Foil cover** option is selected, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate to protect the sample plate from condensation or evaporation.

- When the **Cooling block** option is selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification.

  Note: Foil cover and **Cooling block** options are available only when a CytKick™ Max Autosampler is connected to the Attune™ Xenith™ Flow Cytometer.

- **Delete plate** deletes the selected **Plate** from the experiment and all non-control samples related to it. However, control samples will stay in the **Sample panel** view list and associate with tubes.

8 Control panel

Overview

The **Control panel** is on the right side of the **Workspace** and it is used for the collection of samples.

From the **Control panel**, you can start, stop and pause sample, record sample data, set cycle mode, coincident event processing and thresholding settings, adjust flow and event rate, set stop conditions, work with the samples list, and load and view FCS files.

The **Flex Controls** are at the bottom of the **Control panel** and enable you to optimize voltages for all channels and view sample-specific statistics .

The appearance of the **Control panel** is contextual to the sample type and shows different controls based on the current or selected sample type (**Tube**, **Well**, **Control well**) and **Acquisition phase**. It is organized into the following functional groups as shown in Figure 114 on page 149.

- **Acquisition workflow** (page 150)
- **Thresholding** (page 154)
- **Collection** (page 160)
- **Flow options** (page 161)
- **Stop conditions** (page 162)
- **Other options** (page 163)
- **Flex Controls** (page 165)

To expand or close the functional groups, click the group header. The **Acquisition workflow** group is always open.

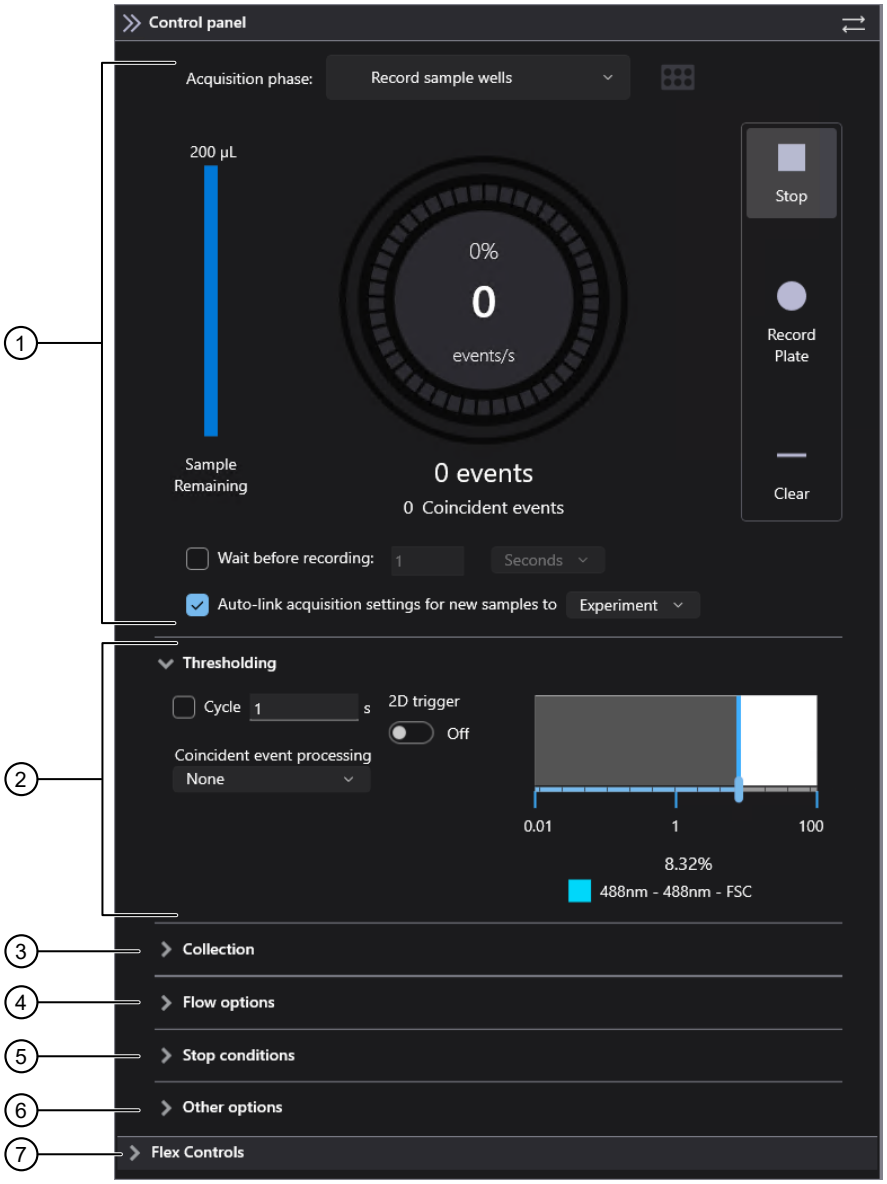


Figure 114 Control panel (Thresholding expanded)

1. Acquisition workflow (page 150)
2. Thresholding (page 154)
3. Collection (page 160)
4. Flow options (page 161)
5. Stop conditions (page 162)
6. Other options (page 163)
7. Flex Controls (page 165)

Acquisition workflow

The **Acquisition workflow** panel provides information about the current acquisition status of the instrument and contains the **Collection controls**, which enable you to run samples and record flow cytometric data.

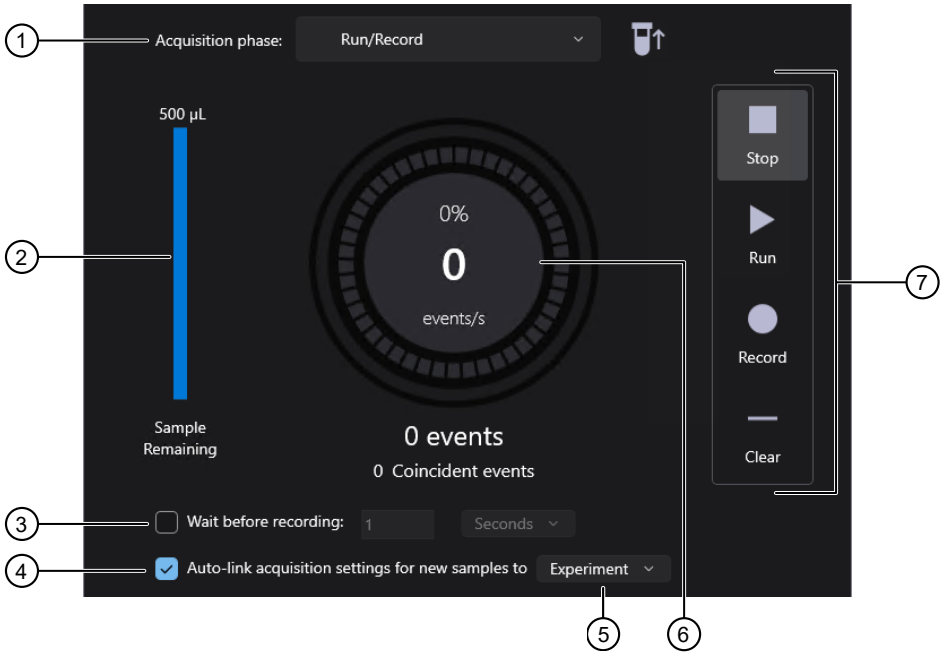


Figure 115 Acquisition workflow panel (Run/Record acquisition phase)

1. Acqusition phase
2. Sample Remaining
3. Wait before recording
4. Auto-link acquisition settings for new samples to
5. Auto-link to dropdown
6. Acquisition status indicators
7. Collection controls

Note: The appearance of the **Acquisition workflow** panel is contextual to the sample type and **Acquisition phase**.

- **Acquisition phase**: Enables you to select the **Acquisition phase** for the run. Available options are:
  - **Run/Record**: Enables samples to be run in setup mode (**Run**) or recorded (**Record**).

    Note: Any sample type can be run using the **Run/Record** phase.
  - **Record control wells**: When control wells are activated, the **Acquisition phase** is set to **Record control wells** by default, which enables you to record multiple control wells.
  - **Record sample wells**: When normal sample wells are activated, the **Acquisition phase** is set to **Record sample wells** by default, which enables you record multiple sample wells.
  - **Record all wells**: Enables you multi-record both control and sample wells.

  Note: When plates are run in multi-sample recording mode, wells can only be recorded (no option to run in setup mode is available).

- **Sample Remaining**: Shows the remaining volume of the current sample. The **progress bar** shows the amount of sample remaining relative to the acquisition volume set as part of the run protocol.
- **Wait before recording**: Sets a delay for the start of acquisition.

  When the **Wait before recording** is enabled, the data at the start of acquisition is discarded. After the delay period, data collection resumes.

  The **dropdown menu** enables you to select **Seconds**, **Events**, or **µL (Volume)** to be the deciding factor for the start of recording the data.
- **Auto-link acquisition settings for new samples to**: When enabled, current acquisition settings are applied automatically at experiment-level or at group-level (based on your selection in the **Auto–link to dropdown** menu) to all new samples and to samples with no recorded data.
  - When **Experiment** is selected, the current acquisition settings are applied at experiment-level to all new samples and to samples with no recorded data.
  - When **Group** is selected, the current acquisition settings are applied at group-level to all new samples and to samples with no recorded data in the selected **Group**.

  When the **Auto-link acquisition settings** is disabled, any changes made to the acquisition settings remain at sample-level (that is, apply only to the current sample).

  By default, **Auto-link acquisition settings** is enabled and the **Experiment** option is selected.

Note: **Auto-link acquisition settings for new samples to** option enables you to manage acquisition settings globally instead of managing them individually at sample-level.

- **Acquisition status indicators**: Provide information about the current acquisition status of the instrument (see page 152).
- **Collection controls**: Enable you to run samples and record flow cytometric data (see page 153).

Acquisition status indicators

Acquisition status indicators provide information about the current acquisition status of the instrument.

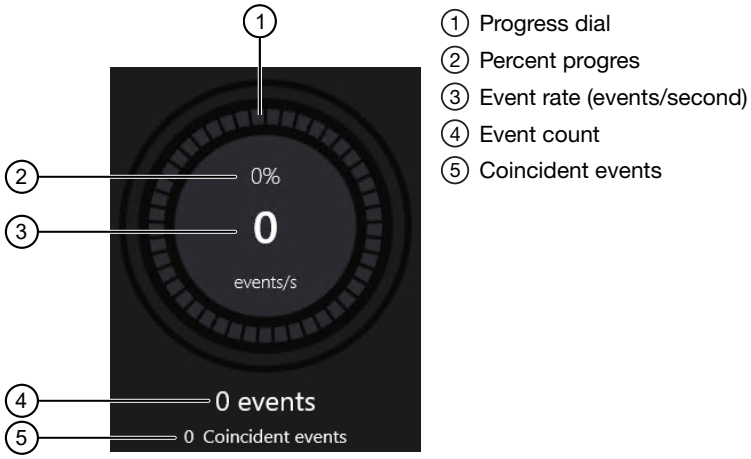


Figure 116 Acquisition status indicators

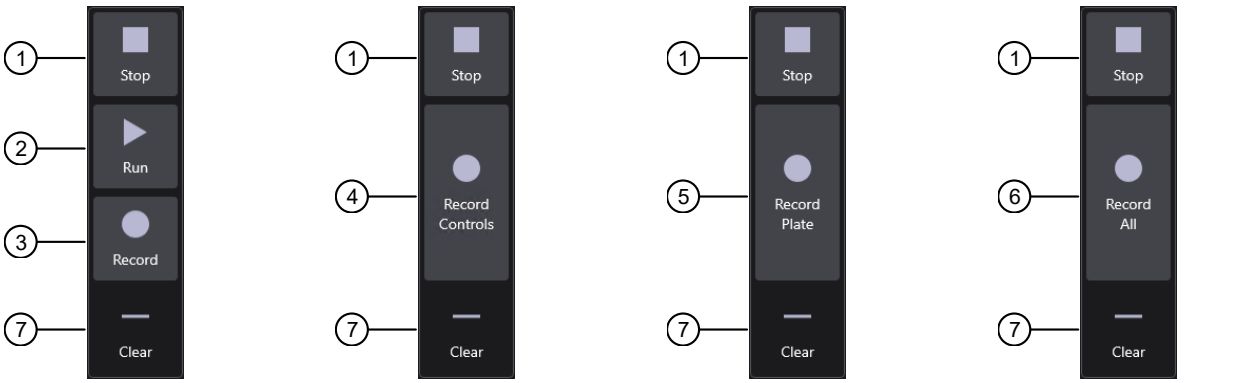
- **Progress dial** shows the progress of the current acquisition related to conditions set in the **Stop conditions** (page 162).

  If multiple **Stop conditions** are selected, the **Progress dial** shows the condition that has progressed furthest towards being met.

  When a sample is run but not recorded, the progress dial does not increase.
- **Percent progress** shows the percent progress of the current acquisition related to conditions set in the **Stop conditions**.
- **Event rate (events/s)** shows 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.
- **Event count** shows 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, when the **Clear** button is pressed, or if a gate is moved and the stop condition is set to stop on a specific number of gated events.
- **Coincident events** shows the total number of coincident events. This value is reset to zero at the start of an acquisition, at the start of recording, or when the **Clear** button is pressed. For more information about coincident events, see page 155.

Collection controls

Collection controls enable you to prompt the Attune™ Xenith™ Flow Cytometer to run samples and record flow cytometric data. The **Collection controls** are contextual and the options that are displayed depend on the **Acquisition phase**.



Run/Record | Record control wells | Record sample wells | Record all wells

① Stop
② Run
③ Record
④ Record Controls
⑤ Record Plate
⑥ Record All
⑦ Clear

- **Stop**: Stops the acquisition or recording of the wells immediately. **Stop** button is available in all phases of acquisition.
- **Run**: Starts the acquisition of the sample in the **setup mode**, which enables the optimization of instrument settings such as PMT voltage and threshold changes without recording and storing the data. **Run** button is available only when the **Acquisition phase** is **Run/Record**.
- **Record**: Starts recording data in the **setup mode**. The system records data to an FCS file until the **Stop** button is clicked, a preset limit is reached, or an error condition is detected. **Record** button is available only when the **Acquisition phase** is **Run/Record**.
- **Record Controls**: Starts recording data from all control wells defined in the **Sample map** view. The system records data to an FCS file until the **Stop** button is clicked, a preset limit is reached, or an error condition is detected. **Record Controls** button is available only when the **Acquisition phase** is **Record control wells**.
- **Record Plate**: Starts recording of data from all sample wells defined in the **Sample map** view. The system records data to an FCS file until the **Stop** button is clicked, a preset limit is reached, or an error condition is detected. **Record Plate** button is available only when the **Acquisition phase** is **Record sample wells**.
- **Record All**: Opens the first control or sample well in the current experiment, and automates the recording of all controls and sample wells in the current experiment. The system records data to an FCS file until the **Stop** button is clicked, a preset limit is reached, or an error condition is detected. **Record All** button is available only when the **Acquisition phase** is **Record all wells**.
- **Pause**: Pauses the acquisition or recording of the wells after the current well has finished. Clicking **Pause** during the acquisition of a well does not temporarily stop the acquisition of the well. The **Pause** button is available only when the system is acquiring or recording from a plate.

Thresholding

Thresholding panel enables you to:

- Set **Cycle mode** settings
- Select the **Coincident event processing mode**
- Set **Threshold settings**

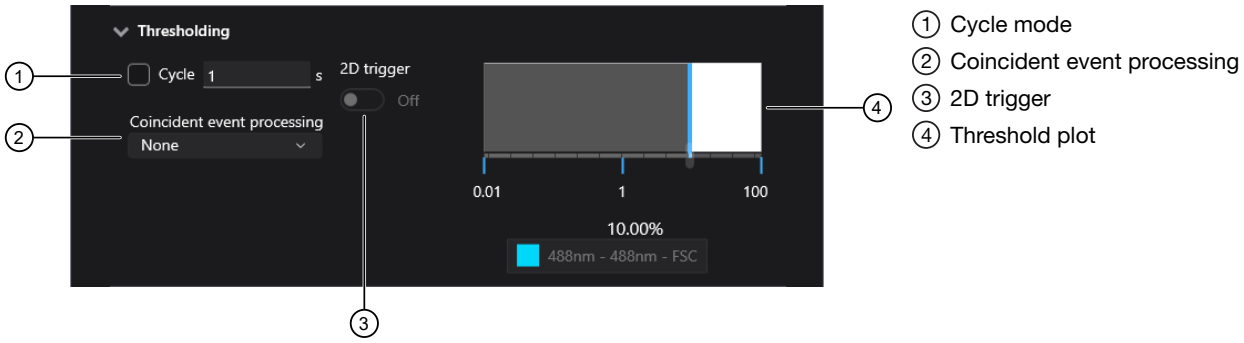
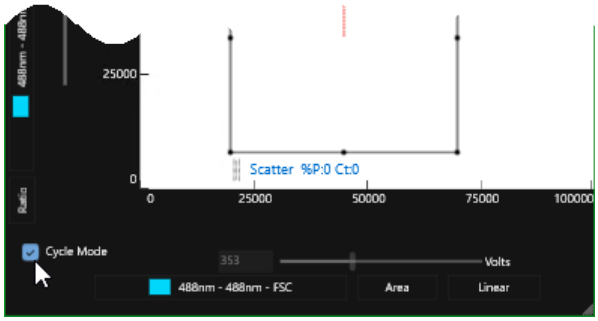


Figure 117 Thresholding panel

- **Cycle mode**: When enabled, all plots in the workspace show only the events that are captured during the time interval that is entered into cycle field. After the time interval is reached, all plots refresh their data. This is useful during setup and voltage adjustment. When recording data, **Cycle mode** refreshes the plots, but all events are still recorded and saved. By default, **Cycle mode** is unchecked.

Note: Individual plots can be cycled with the **Cycle Mode** checkbox on the plot.



- **Coincident event processing**: Enables you to select **Coincident event processing mode**, which helps the collection of high-quality flow cytometry data from single cell analysis. Available options are:
  - **Force to Singlets**
  - **None**
  - **Exclude Coincident Events**

  For more information about single events vs. coincident events and **Coincident event processing modes**, see page 155.

- **2D trigger**: Enables you to apply thresholding to one or two channels in logical combination (AND or OR logic). For more information, see page 157.
- **Threshold plot**: Enables you to set the minimum signal level to remove unwanted events and decrease noise in the data. For more information, see page 157.

Coincident event processing

Single vs. Coincident events

Coincident events occur when two particles pass through the laser interrogation point so quickly that their respective voltage pulse cannot be separated. High quality flow cytometry data are obtained from **single** cell analysis, so removal or exclusion of coincident events is highly recommended.

Events that are measured above the threshold without overlapping measurement intervals are correctly processed as single events.

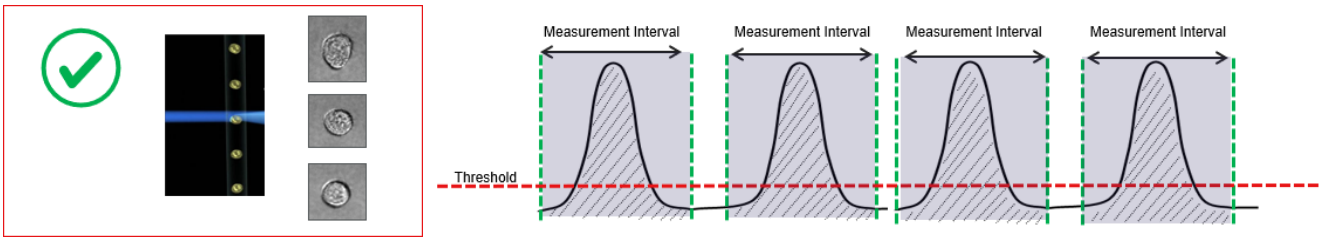


Figure 118 Single event example

If the voltage pulse is created by multiple events and the signal does **not** drop below the threshold, these events are processed as **single event**, regardless of whether the peak consists of multiple peaks and defined valleys within this window gate (**Example A** below).

If the signal **drops below the threshold**, but pulse processing windows of the coincident events overlap, the system processes the pulse as **a single event beginning with the first pulse and ending with the second pulse** (**Example B** below).

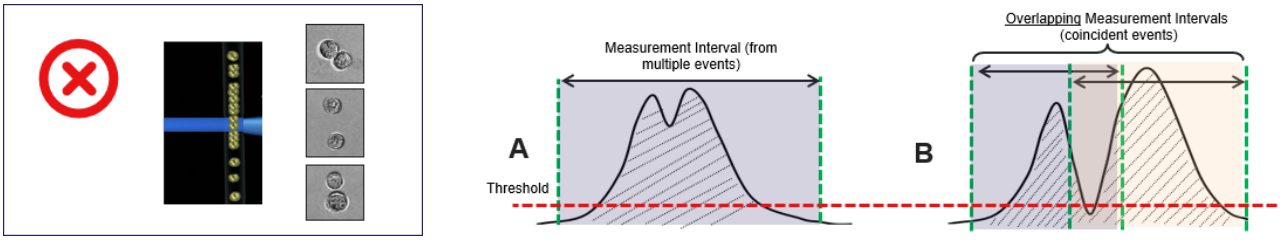
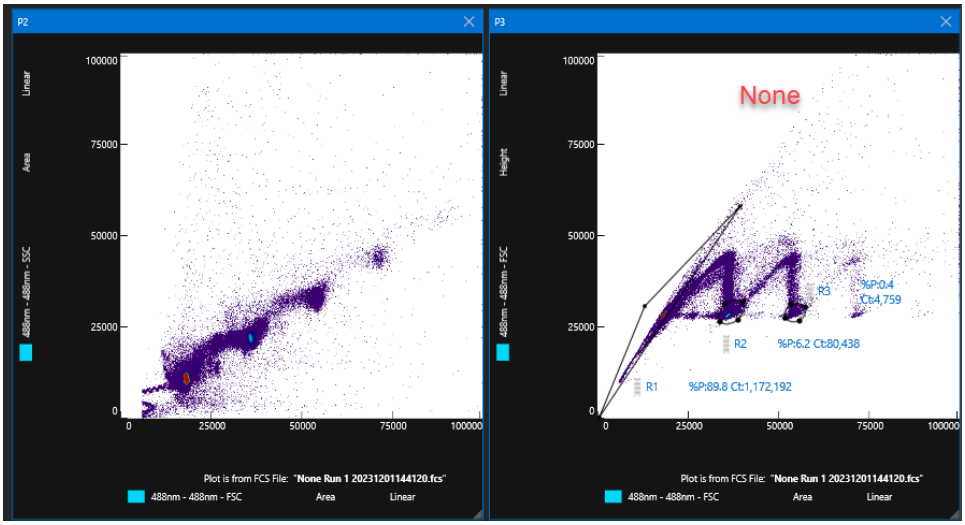


Figure 119 Coincident event examples

Coincident event processing modes

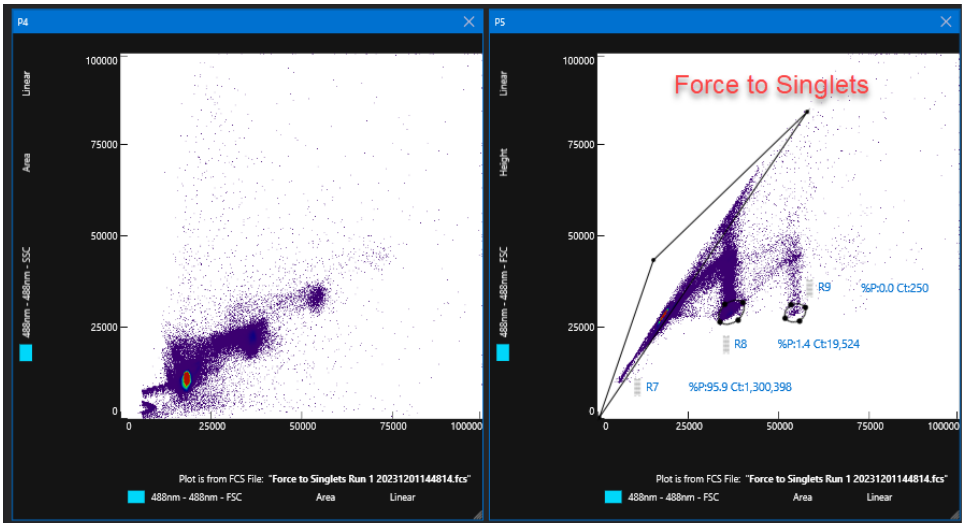
Flowscape™ Software has three **Coincident event processing modes**:

- **None**:



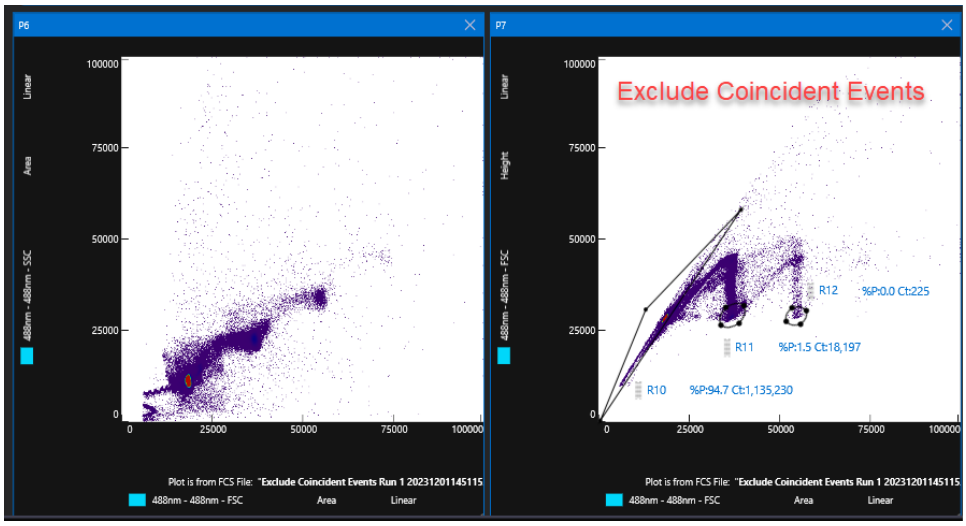
  - No special processing of the pulse peak is performed.
  - Coincident events are included in the data set.
  - At high event rates (for example, with beads), data can include singlets, doublets, triplets, and so on.

- **Force to Singlets**:



  - Pulse processing attempts to split the coincident events into separate singlet events.
  - An increase in singlet population count is observed.
  - A decrease in multimer events is observed.
  - Event rate increases.

- **Exclude Coincident Events**:



  - Pulse processing discards coincident events.
  - Singlet population count is unchanged.
  - A decrease in multimer events is observed.
  - Event rate is unchanged.

Threshold settings

Threshold settings enable you to set the minimum signal level to remove unwanted events and decrease noise in the data. When threshold is set, only events above the threshold are saved in the FCS file, and data below the threshold level is discarded. You can apply thresholding to one or two channels using the **2D trigger**.

- The default **trigger parameter** is **forward scatter (488 nm laser)**. The Flowscape™ Software enables data triggering on any available scatter or fluorescence parameter. You can apply threshold with one or two unique channels (using the **2D trigger** mode) from among available ones.
- The **threshold values** are displayed in logarithmic percentages of the signal in the trigger detector (from 0.01% to 100%) and can be adjusted using the **threshold slider** or by double clicking on the label and entering a value. The default value is 10.00%.
- By default, a single parameter **threshold value** is required for data acquisition.
- When the **2D trigger** mode is activated, the **threshold plot** changes from a single parameter histogram to a density plot. The parameter and trigger value for the Y axis can be set in addition to and independent of the X axis value.
- When the **2D trigger** mode is enabled, the thresholding can be set to **AND** or **OR** logic.

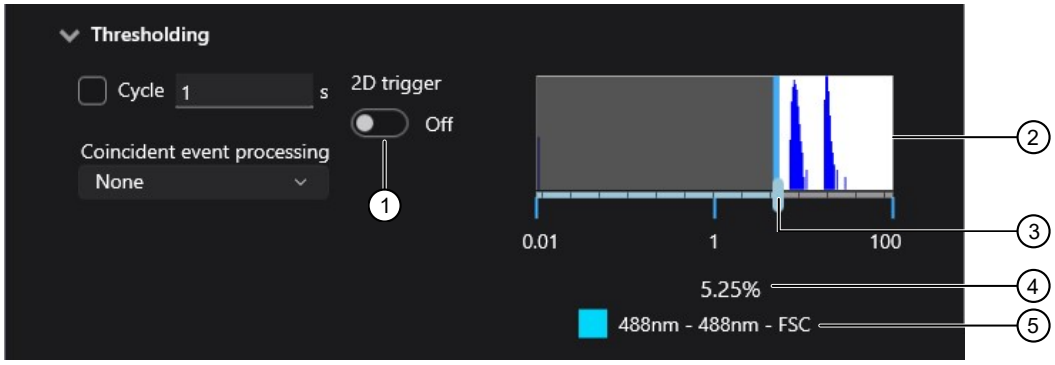


Figure 120 Threshold settings (1D)

① 2D trigger (Off)
② Threshold plot
③ Threshold slider
④ Threshold value
⑤ Trigger parameter

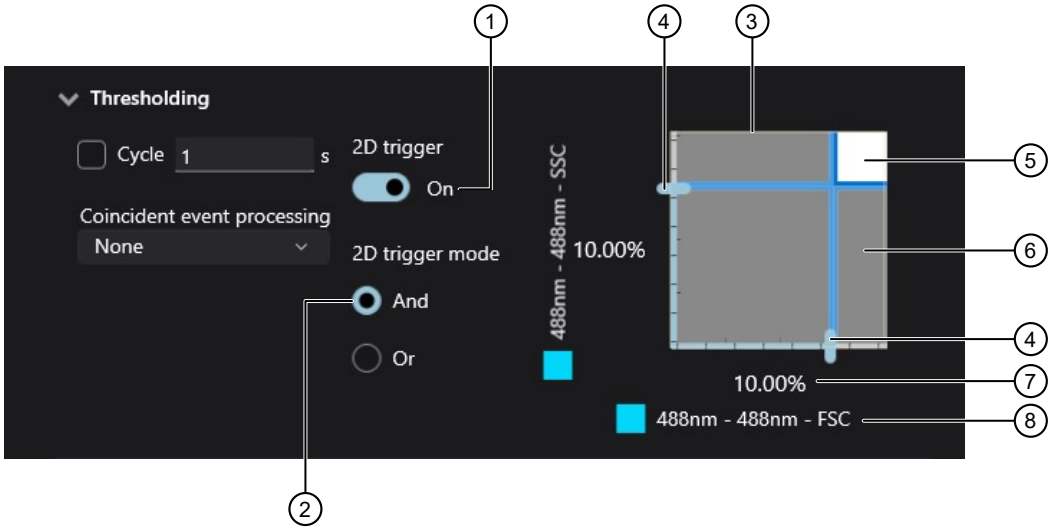


Figure 121 Threshold settings (2D AND mode)

① 2D trigger (On)
② 2D trigger mode: And
③ Threshold plot
④ Threshold sliders
⑤ Values above the threshold (white)
⑥ Values below the threshold (grey)
⑦ Threshold value
⑧ Trigger parameter

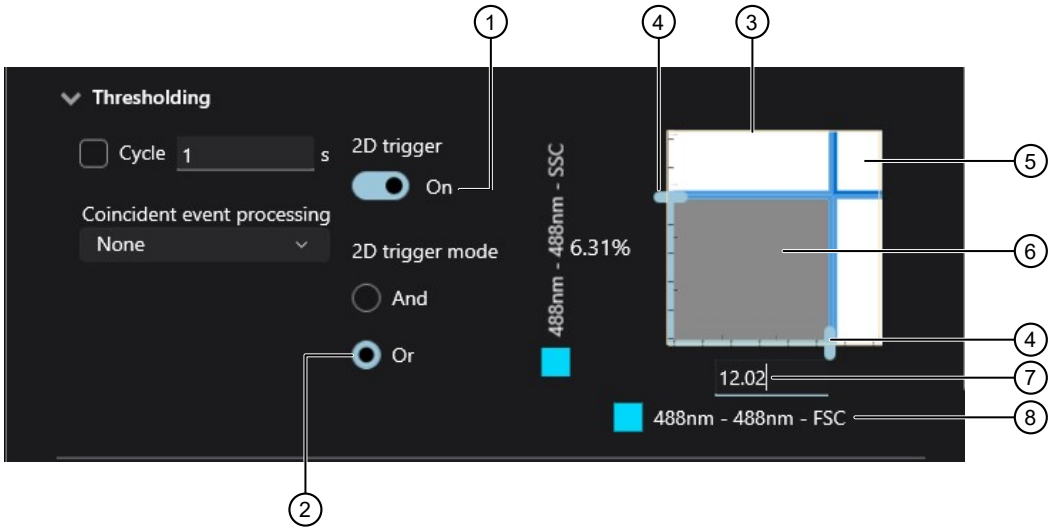


Figure 122 Threshold settings (2D OR mode)

① 2D trigger (On)
② 2D trigger mode: Or
③ Threshold plot
④ Threshold sliders
⑤ Values above the threshold (white)
⑥ Values below the threshold (grey)
⑦ Threshold value
⑧ Trigger parameter

Collection

Collection panel enables you to control which wells are collected and in which order during acquisition and recording. The options in this panel are available only for Plate experiments and are not displayed for Tube samples.

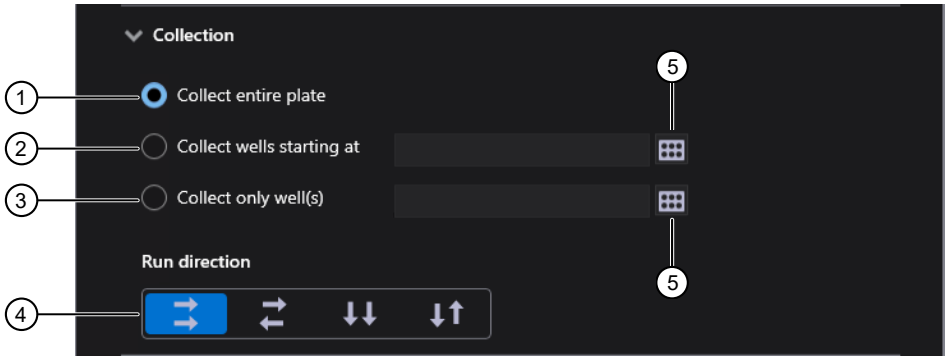


Figure 123 Collection panel

- **Collect entire plate**: Collects all samples on a plate from the beginning. This is the default option.

  If a plate layout is specified, acquisition starts at well **A1** and proceeds in the selected **Run direction**.

- **Collect wells starting at**: Collects all samples starting at the specified well.

  To specify the starting well, enter its location in the textbox field. The entry format is the row letter followed by the column number. For example: **A1**.

  Alternatively, you can specify the starting well directly from the **Sample map** by selecting the **Sample map** button .

- **Collect only well(s)**: Collects only from the specified wells.

  To specify the wells you want to collect, enter 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 contains a row letter followed by one or two digits for the column number. For example: **B11**.

  You can enter multiple wells by using a hyphen to indicate a range of wells and a comma to separate wells and ranges. White spaces are ignored. For example: **A1, B1– C12, D3**.

  Where a range spans multiple rows, it is interpreted as including all wells in between. For example: **B2–C6** on a 96-well plate is interpreted as containing the wells **B2** to **B12**, **C1** to **C6**, inclusive.

  Alternatively, you can specify the starting well directly from the **Sample map** by selecting the **Sample map** button .

  When **Collect only well(s)** is selected, the **Run direction** options are inactive and the wells are collected in the order they are specified in the edit control, where all ranges are processed left to right.

- **Run direction**: Enables you to select the order in which the wells are processed. Available options are:
  - Row by row, left to right (default)
  - Row by row, left to right, right to left (zig-zag)
  - Column by column, top to bottom
  - Column by column, top to bottom, bottom to top (zig-zag)

Flow options

Flow options depend on whether the sample is a tube sample or a well sample and include **Acquisition volume**, **Mixing volume** (wells only), **Collection rate**, and **Enable boost mode** (wells only).

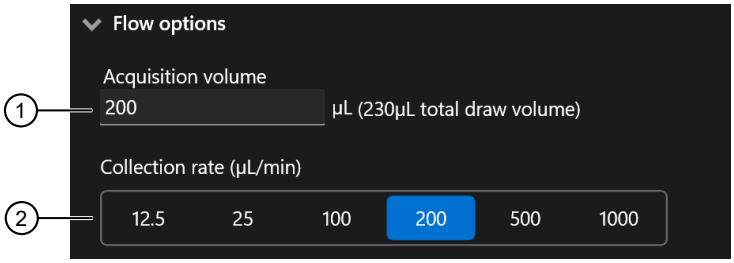


Figure 124 Flow options (Tube samples)

① Acquisition volume (µL)
② Collection rate (µL/minute)

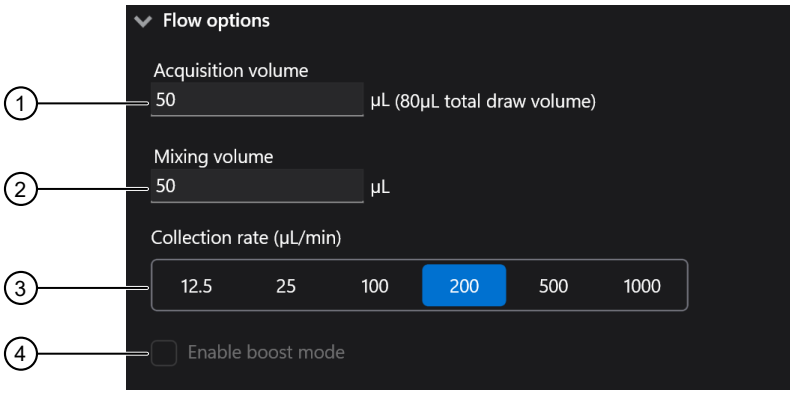


Figure 125 Flow options (Well samples)

① Acquisition volume (µL)
② Mixing volume (µL)
③ Collection rate (µL/minute)
④ Enable boost mode

- **Acquisition volume (µL)**: Specifies the volume of sample in µL that can be used during the acquisition of the well or the tube.

  The **total draw volume** (acquisition volume + dead volume) is displayed next to the acquisition volume field.
- **Mixing volume (µL)**: Used to select the optimal mixing algorithm for the sample (based on volume and plate type). It is available only for well samples.
- **Collection rate (µL/minute)**: Controls the rate of delivery of the sample during acquisition of Well samples and Tube samples in the **Record** mode. Available options for **Collection rate** are:
  - 12.5 µL/minute
  - 25 µL/minute
  - 100 µL/minute
  - 200 µL/minute
  - 500 µL/minute
  - 1,000 µL/minute

The default **Collection rate** is 200 µL/minute.

- **Enable boost mode**: Enables the processing of the samples with reduced boost volume for high-throughput acquisition, which decreases the processing time for plates.

  The **Enable boost mode** option is available only when a CytKick™ Max Autosampler is connected to the instrument and when the **Collection rate** is set to **500 µL/minute**.

  The **Enable boost mode** is not available for Tube samples.

Stop conditions

Stop conditions enable you to specify when the collection of data ends. If multiple conditions are selected, the acquisition ends when any of the selected stop conditions are met. If no option is selected, the sample continues recording until the acquisition volume is exhausted.

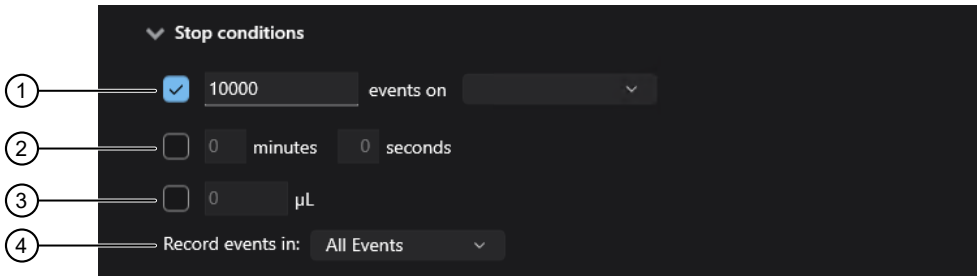


Figure 126 Stop conditions

① **Event count stop**
② **Time stop**
③ **Volume stop**
④ **Record events in**

- **Event count stop**: Stops acquisition when the specified number of events has been collected in the specified gate.

  The dropdown list displays all gates in the current workspace. The **All events** option is always available and is the default selection.

  Note: When event-based stop criteria are not reached, you can append the data using the **Append** option from the **Record** dropdown. When **Append** is selected, new data are appended to the existing data and current stop conditions apply to new data. No indication is provided that the event-based stop criteria was not reached.

- **Time stop**: Stops acquisition when the specified time has elapsed.
- **Volume stop**: Stops acquisition when the specified volume is delivered for interrogation.

  The allowable volume range depends on the sample source, plate or tube. The volume is validated on entry and must be an integer.
- **Record events in**: Enables you to set a gate where only the events that are in that gate at the end of the run are saved in the FCS file.

  The dropdown displays a list of all gates on the experiment-level workspace. By default, **All events** is selected.

  As the data are acquired, **all events are displayed**, but only the events in the specified gate are saved (data exclusion only occurs on the writing of the FCS file).

Other options

Other options depend on whether the sample is a tube sample or a well sample and include **Rinse & recovery** options for tube samples, and **Mixing cycles**, **Mixing mode**, and **Rinse cycles** for well samples. These options are available when at least one tube or well is selected.

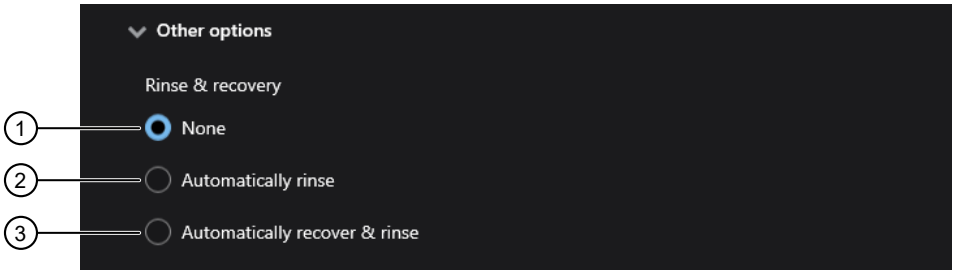


Figure 127 Other options - Rinse & recovery (Tube samples)

① None
② Automatically rinse
③ Automatically recover & rinse

Rinse & recovery options for **Tube samples** apply only when recording. The options are:

- **None**: There is no automatic rinse of the sample lines or sample recovery from the sample loop. This is the default selection.

  If needed, **Rinse** and **Sample Recovery** need to be started manually from the **Maintenance** dropdown.
- **Automatically rinse**: The system proceeds to rinse the sample lines at the completion of the stop criteria without prompting. After the rinse cycle, the tube lifter is automatically dropped to present the system as ready.
- **Automatically recover & rinse**: At the completion of the stop criteria, the system automatically recovers the unused sample from the sample loop and returns it into a tube, then proceeds to rinse the sample lines without prompting. After the rinse cycle, the tube lifter is automatically dropped to present the system as ready.

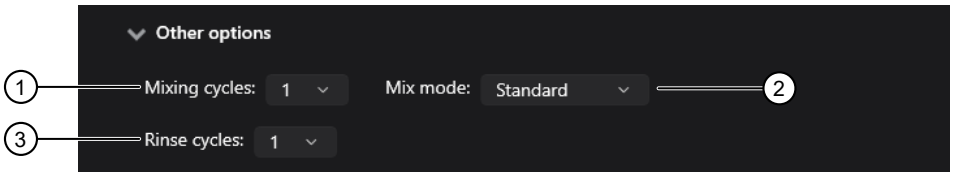


Figure 128 Other options (Well samples)

① Mixing cycle
② Mix mode
③ Rinse cycles

Other options for **Well samples** are available when at least one well is selected.

- **Mixing cycles**: Sets the number of mix cycles to perform on the selected wells before the sample is analyzed. The **dropdown** enables you to select between 0 and 10 mixing cycles. The default value is 1.

  Note: High numbers of mixing cycles can increase the potential for creating bubbles.

- **Mix mode**: Selects between **Standard** and **Gentle** mix modes. The **Mix mode** option is available only when a CytKick™ Max Autosampler is connected to the instrument.
  - **Standard**: The sample is mixed using the normal mixing speed.
  - **Gentle**: The sample is mixed and aspirated at a slower speed.

  Note: For fragile cells, viscous samples, or samples prepared in viscous buffers, use the **Gentle** mix mode.

- **Rinse cycles**: Sets the number of rinse cycles to perform between well samples. The **dropdown** enables you to select between 0 and 10 rinses. The default setting is 1.

Flex Controls

Flex Controls are at the bottom of the **Control panel** and have two functional tabs:

- **Detection**: Contains controls to optimize voltages for all channels and to import instrument settings from a previous experiment.
- **Stats**: Used to view sample-specific statistics.

Detection

Detection shows the available lasers and fluorophores and enables **Voltage** settings for each channel to be changed individually, together, or imported from the experiment metadata library. It provides three options to adjust **Voltage** settings:

- **Manual**: Enables you to adjust voltage settings for each available channel individually or to import from the experiment metadata library.
- **Sync Mode**: Enables you to adjust voltages for all channels at once or all channels for a specific laser, and to apply QC or spectral voltages to the experiment.
- **Voltage Set**: Simultaneously sets voltages on all channels to position the current media to the selected target value.

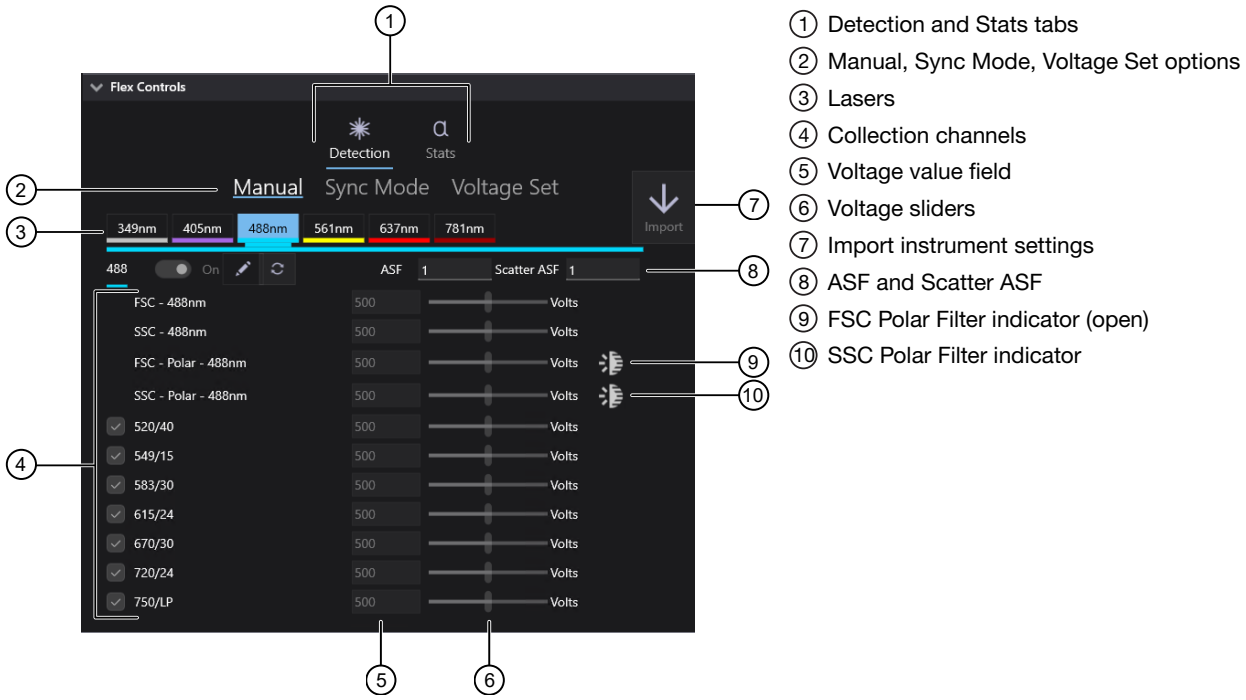


Figure 129 Detection - Manual

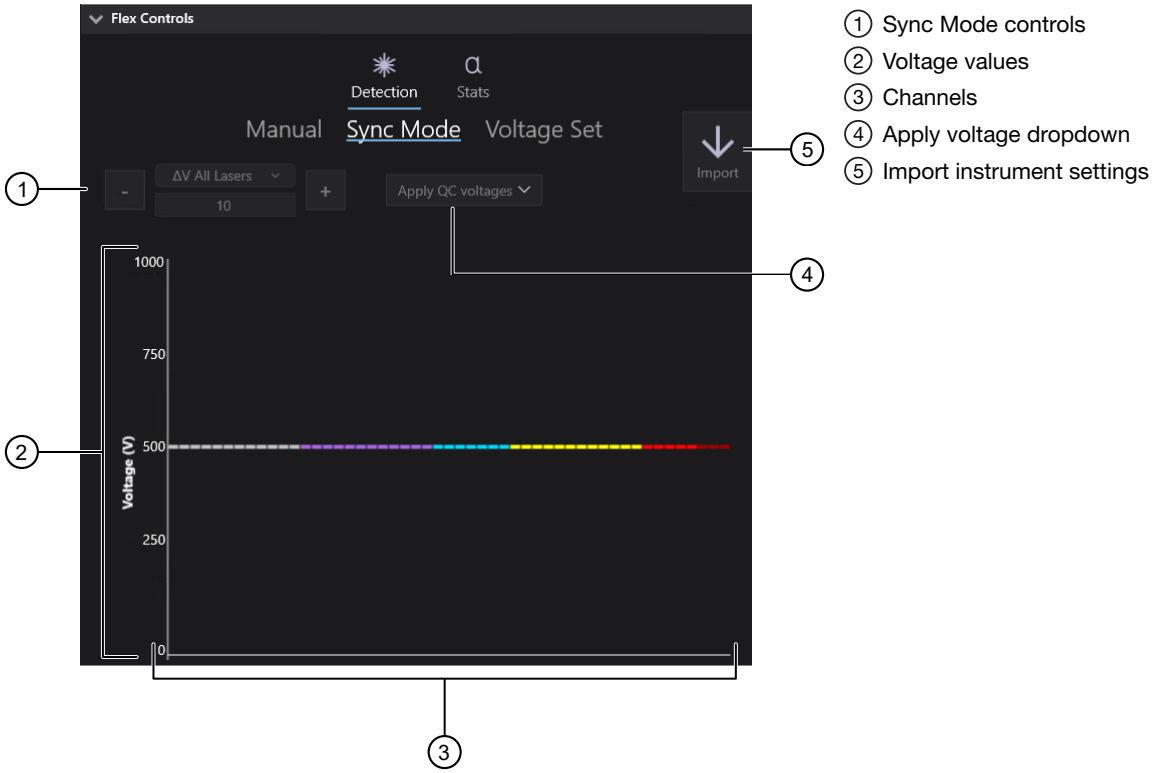


Figure 130 Detection - Sync Mode

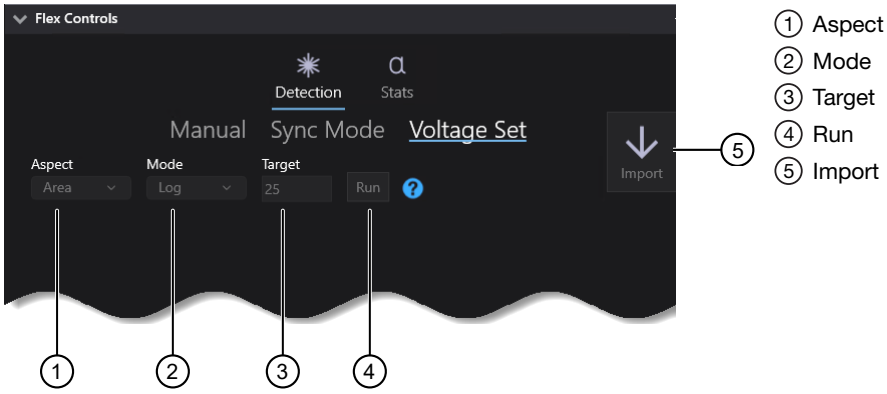
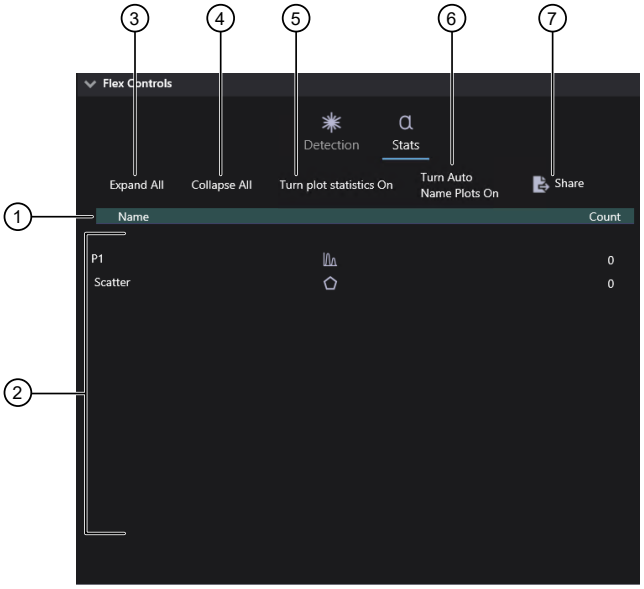


Figure 131 Detection - Voltage Set

Stats

Stats shows the combined statistics from all the plots and regions in the workspace. At first view, only the statistics from the primary group is shown and all other statistics are minimized by default. Within each statistics section, the plot and gate hierarchy are shown. Options such as **Expand all**, **Collapse all**, **Turn Plot Statistics On**, **Turn Auto Name Plots On**, and **Share** are available in the statistics window.



Stats - All collapsed

Stats - All expanded

1. Statistics table headings
2. Statistics table
3. Expand All
4. Collapse All
5. Turn Plot Statistics On
6. Turn Auto Name Plots On
7. Share

The **Statistics table** shows the statistics selected in the **User Settings ▸ Statistics** or the **Global Settings ▸ Statistics** screens. You can select to show specific statistics for the **Plot statistics**, **Combined statistics**, and **Region statistics**.

Depending on the statistics you want to show (**Plot**, **Combined**, or **Region statistics**), you can select from the following statistics:

- **Count (Ct)**
- **%Total (%T)**
- **%Plot (%P)**
- **Voltage (V)**
- **Variance (Var)**
- **Mode**
- **Mean**
- **StdDev (SD)**
- **rStdDev (rSD)**
- **Laser Wavelength (WL)**
- **Concentration (Cn)**
- **Volume (Vm)**
- **Geometric Mean (GM)**
- **CV**
- **rCV**
- **Max**
- **Median (Med)**
- **Min**

Note: For a description of the statistics, see “Statistics” on page 217.

Pn is the name of the plot. Plots can be renamed on the plot itself. Any new names are reflected in the **Statistics table**. If no name has been entered, the plot has the default name P1, P2, and so forth.

Expand All shows the hierarchy of all the plots and regions.

Collapse All closes the hierarchy of all the plots and regions.

Turn Plot Statistics On toggles the display of plot statistics on all plots in the experiment. You can select the default statistics in **Settings ▸ User Settings ▸ Statistics** or **Settings ▸ Global Settings ▸ Statistics**.

Turn Auto Name Plots On toggles the automatic naming of the plots on and off.

Share opens a file save menu that enables a CSV file of the statistics to be exported to any drive available.

9 QC Test

QC Test overview: Baseline and Daily QC

To determine whether the Attune™ Xenith™ Flow Cytometer is running in good condition, the instrument has a quality control feature referred to as **QC Test**, which has two separate but related parts, **Baseline** and **Daily QC**.

- **Baseline**: On brand-new instruments, brand-new beadlots, or instruments that have gone through major service repair, a **Baseline** test is performed. This is an in-depth quality control test that measures and sets the baseline standards for the instrument performance using the Attune™ Xenith™ Quality Control Beads (see “Baseline setup” on page 171).
- **Daily QC**: After the Baseline values are established, the same lot of Attune™ Xenith™ Flow Cytometer QC Beads are used to run the **Daily QC**. The **Daily QC** determines variation from the **Baseline** values to track the daily performance of the instrument, where deviations in performance can indicate a need for maintenance or servicing (see “Daily QC” on page 173).

The **QC Test** screen provides instructions to perform the **Baseline** test or the **Daily QC** test.

To open the **QC Test** screen, click **QC test** on the **Home** screen. Alternatively, select **QC** tab on the **Status bar**, then click **Go to QC**.

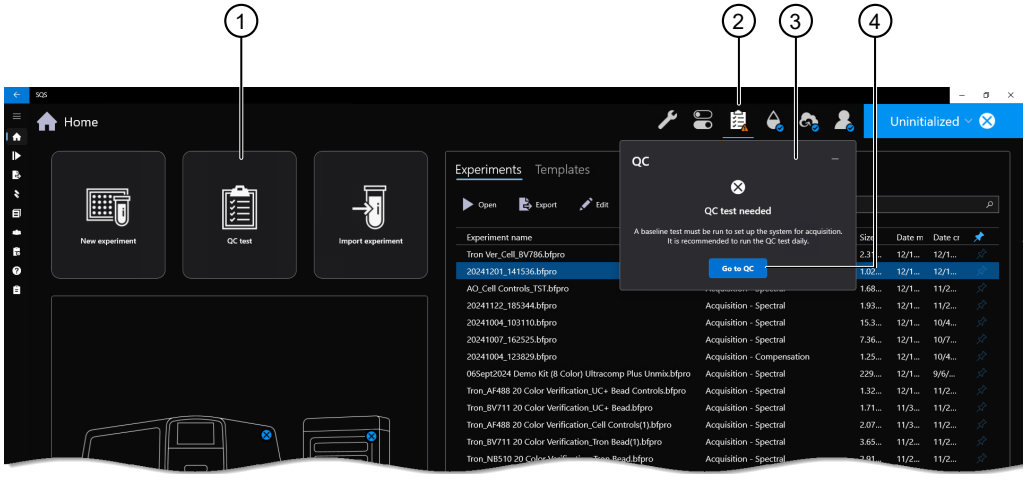


Figure 132 Open the QC Test screen from the Home screen.

① QC test
② QC tab (on Status bar)
③ QC dropdown
④ Go to QC

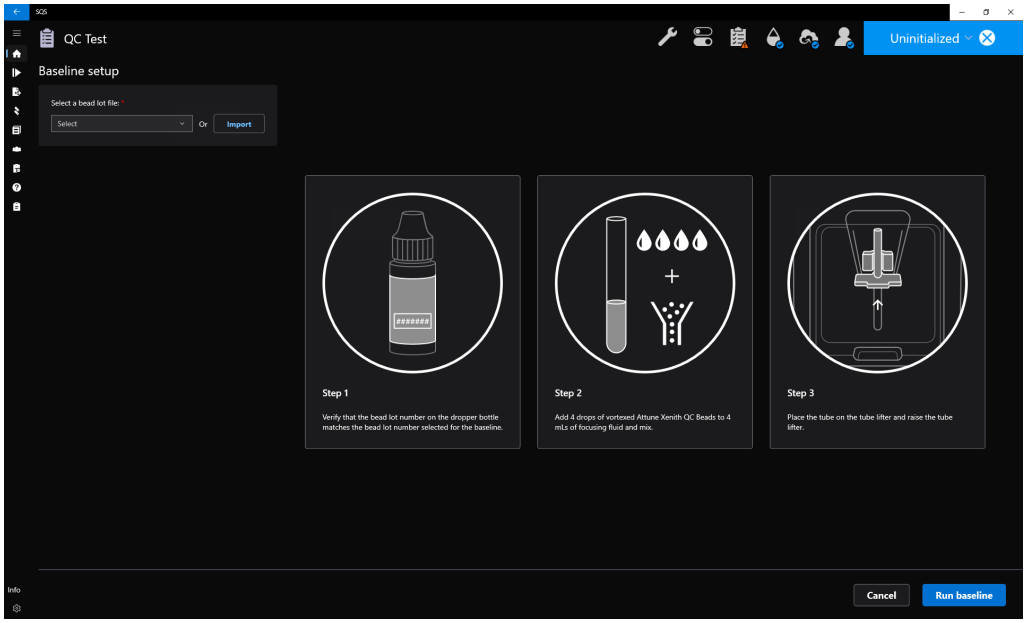


Figure 133 QC Test screen - Baseline setup

QC testing workflow (when to run)

Baseline setup is performed:

- By the Field Service Engineer (FSE) at time of installation.
- By the FSE after any major service.
- By the user every time the Attune™ Xenith™ Quality Control Beads lot changes.
- By the user when recommended by FSE or FAS.

Daily QC test is performed by the user every day samples are run or recorded.

Note: **Baseline setup** can only be performed by **Administrator** accounts.
Daily QC test can be performed by **Administrator** and **Standard** user accounts.

Baseline setup

Open the **QC Test** screen to view the **Baseline setup** screen.

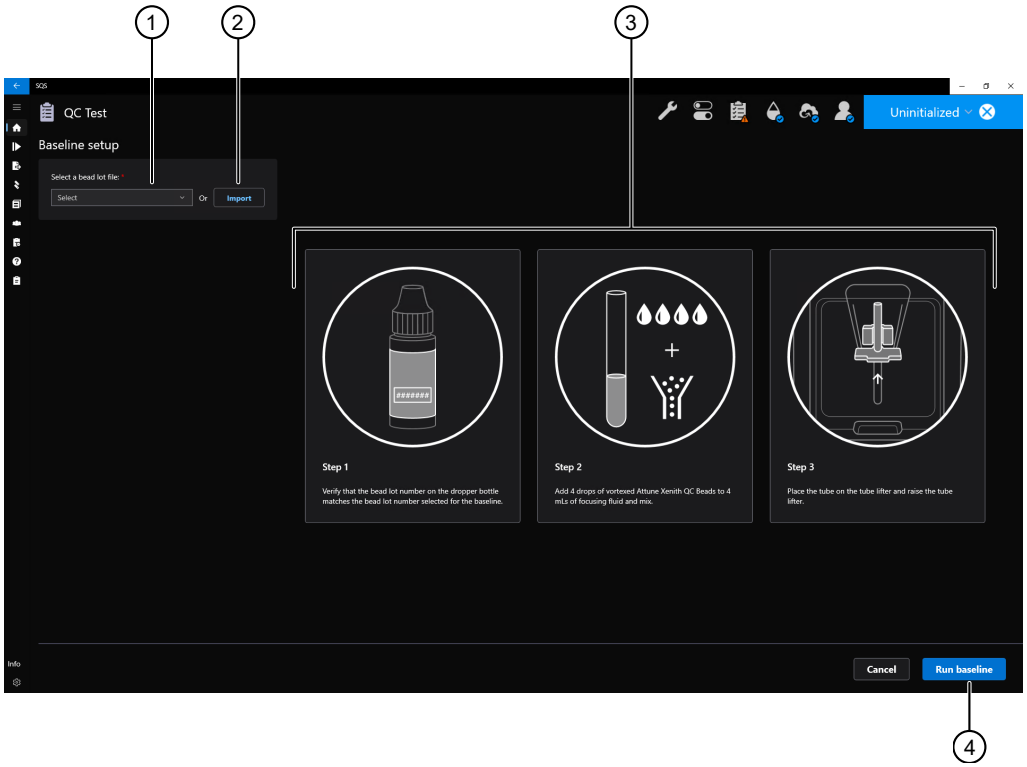


Figure 134 Baseline setup screen

1. Select bead lot file - Bead lot file dropdown
2. Select bead lot file - Import
3. Baseline setup instructions
4. Run baseline

Note: If a **Baseline** already exists, the **Daily QC** screen is displayed instead of the **Baseline setup** screen. On the **Daily QC** screen, you can run a **Daily QC** test or reset the **Baseline**.

The **Baseline setup** screen provides general instructions for setting up a **Baseline**:

1. Verify that the bead lot number on the dropper bottle matches the bead lot number selected for the baseline.
2. Add 4 drops of vortexted Attune™ Xenith™ Flow Cytometer QC Beads to 4 mL of focusig fluid and mix.
3. Place the tube on the tube lifter and raise the tube lifter.

After you have completed the steps above, click **Run baseline**.

Note: **Baseline** can only be run by **Administrator** accounts.
Daily QC test can be performed by **Administrator** and **Standard** user accounts.

The **Baseline** screen provides progress information for the **Baseline** procedure.

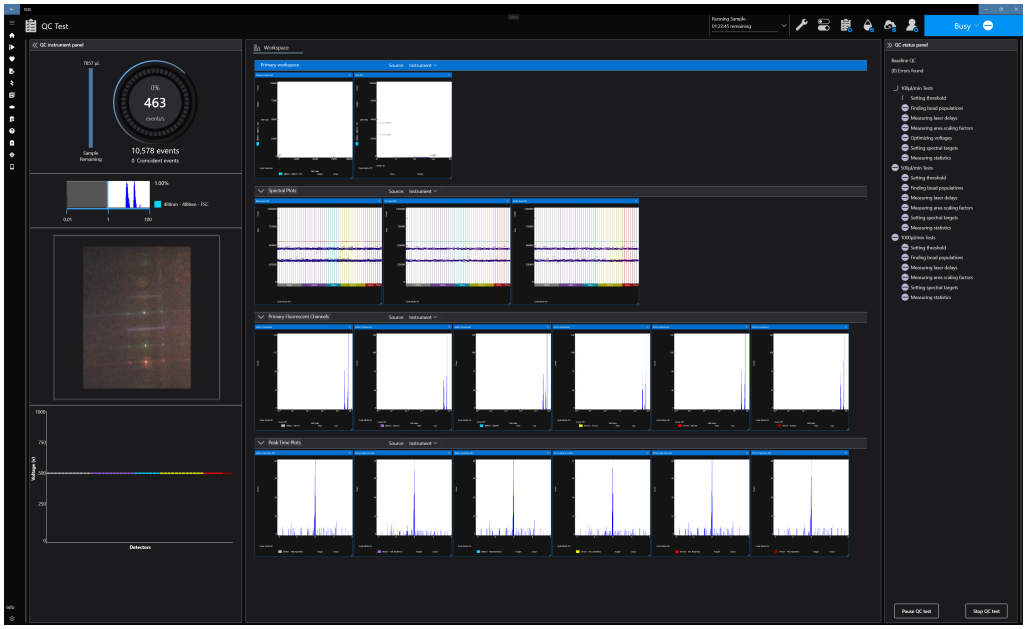


Figure 135 Baseline run in progress

During the **Baseline**, the instrument performs the following tasks:

- Initializes the system and workspace.
- Attempts to auto-tune the laser delays.
- Sets initial threshold values.
- Finds the peaks for blank, dim, and bright QC beads:
  - Performs a voltration on a single channel off of the blue laser.
  - Checks laser delays and recalculates as required using peak time.
- Performs a voltration across all fluorescence detectors to find:
  - Detector optimized voltages
  - Spectral target voltages (MFI based) (MFI: median fluorescence intensity)
  - Daily QC target voltages (MFI based)
- Measures statistics for results.

Note: During detector voltage optimization, the goal is to obtain maximum separation between positive and negative values while keeping the negative outside of the system noise, and to keep the voltages within the linear range of PMT response.
QC Test performs voltration and determines optimal voltage using rCV (robust coefficient of variation) inflection point and separation index.

Daily QC

Open the **QC Test** screen to view the **Daily QC** screen.

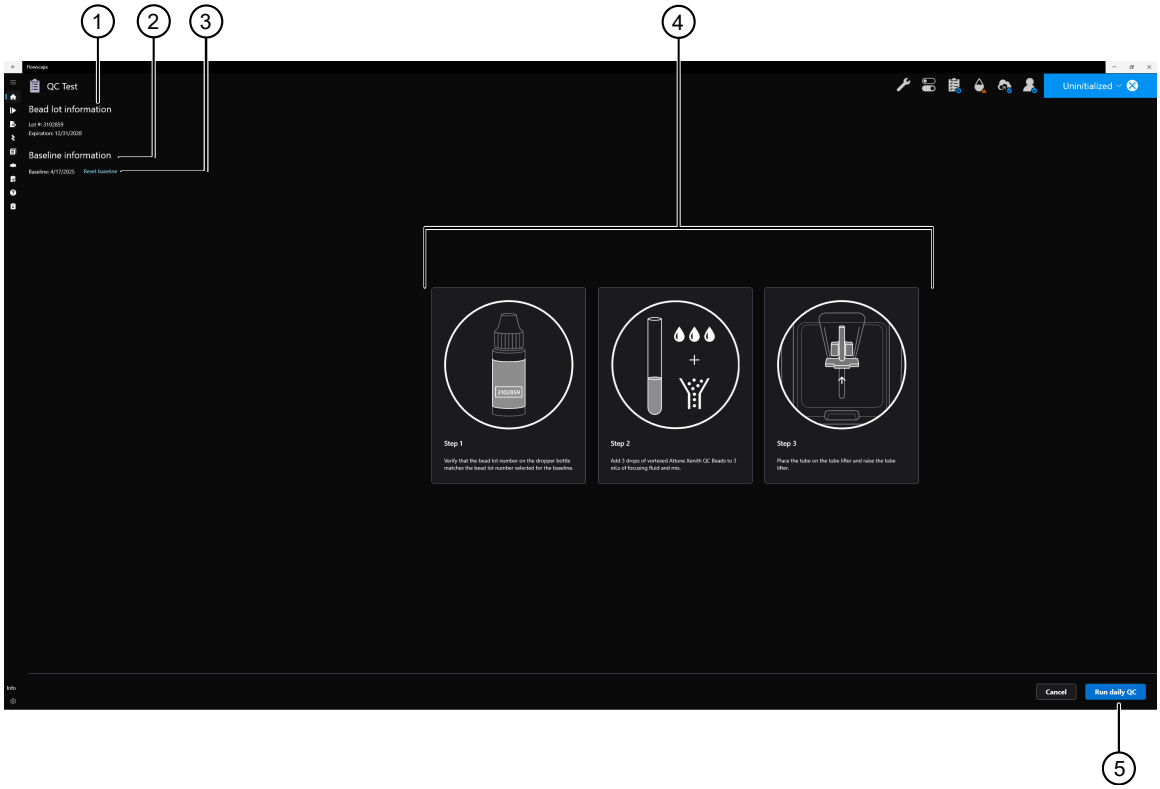


Figure 136 Daily QC screen

1. Bead lot information
2. Baseline information
3. Reset baseline
4. Daily QC setup instructions
5. Run daily QC

Note: The **Daily QC** screen is displayed only if there is already a **Baseline**; otherwise, the **Baseline setup** screen is shown. If needed, you can reset the **Baseline** in the **Daily QC** screen.

The **Daily QC** screen provides general instructions for running the **Daily QC** test:

1. Verify that the bead lot number on the dropper bottle matches the bead lot number selected for the baseline.
2. Add 3 drops of vortexted Attune™ Xenith™ Flow Cytometer QC Beads to 3 mL of focusig fluid and mix.
3. Place the tube on the tube lifter and raise the tube lifter.

After you have completed the steps above, click **Run daily QC**.

To reset the **Baseline**, click the **Reset baseline** link, then select a beadlot file.

Note: **Daily QC** test can be performed by **Administrator** and **Standard** user accounts. **Baseline** can only be run by **Administrator** accounts.

The **QC Test** screen provides progress information for the **Daily QC** procedure.

Daily QC test is run each day at a fixed PMTV determined in the **Baseline** to achieve a target MFI (~30% of maximum signal). It is run at multiple flow rates as required to set system constants and measure flow rate dependent variation.

During the **Daily QC**, the instrument performs the following tasks:

- Initializes the system and workspace.
- Attempts to auto-tune the laser delays.
- Sets initial threshold values.
- Finds the peaks for blank, dim, and bright QC beads:
  - Performs a voltration on a single channel off of the blue laser.
  - Checks laser delays and recalculates as required using peak time.
- Measures statistics for results.

The results of the **Daily QC** test are reported in the **QC Test Results** (see page 174).

QC Test Results

Based on the results of the **QC tests**, the Flowscape™ Software generates two types of **QC report** for the Attune™ Xenith™ Flow Cytometer:

- **Daily QC report**: Shows the high-level pass/fail results for the **Baseline** and the **Daily QC** tests and presents the **QC test statistics** in a table form for each laser, channel, and flow rate (see page 174).

  To view the **Daily QC report**, click **Global navigation** ▸ **Report Management** ▸ **QC Reports**, then select **Report type** ▸ **Daily QC report**.
- **Levey-Jennings report**: The **Levey-Jennings report** tracks shifts and trends in instrument performance as indicated in **Daily QC tests** over time and provides a visual indication of system stability (see page 178).

  To view the **Levey-Jennings report**, click **Global navigation** ▸ **Report Management** ▸ **QC Reports**, then select **Report type** ▸ **Levey-Jennings**.

Daily QC report

Daily QC report shows the high-level **Pass/Fail (P/F)** results for the **Baseline** and the **Daily QC** tests in the report heading and presents the **QC test statistics** in a table form for each laser, channel, and flow rate. The pass/fail results for the specific channel and the specific fail criteria are also indicated in the corresponding line of the channel that did not pass the test.

To view the **Daily QC report**, click **Global navigation** ▸ **Report Management** ▸ **QC Reports**, then select **Report type** ▸ **Daily QC report**.

By default, most recent report is shown, but you can navigate through **Daily QC reports** using the **QC test list** on the report screen.

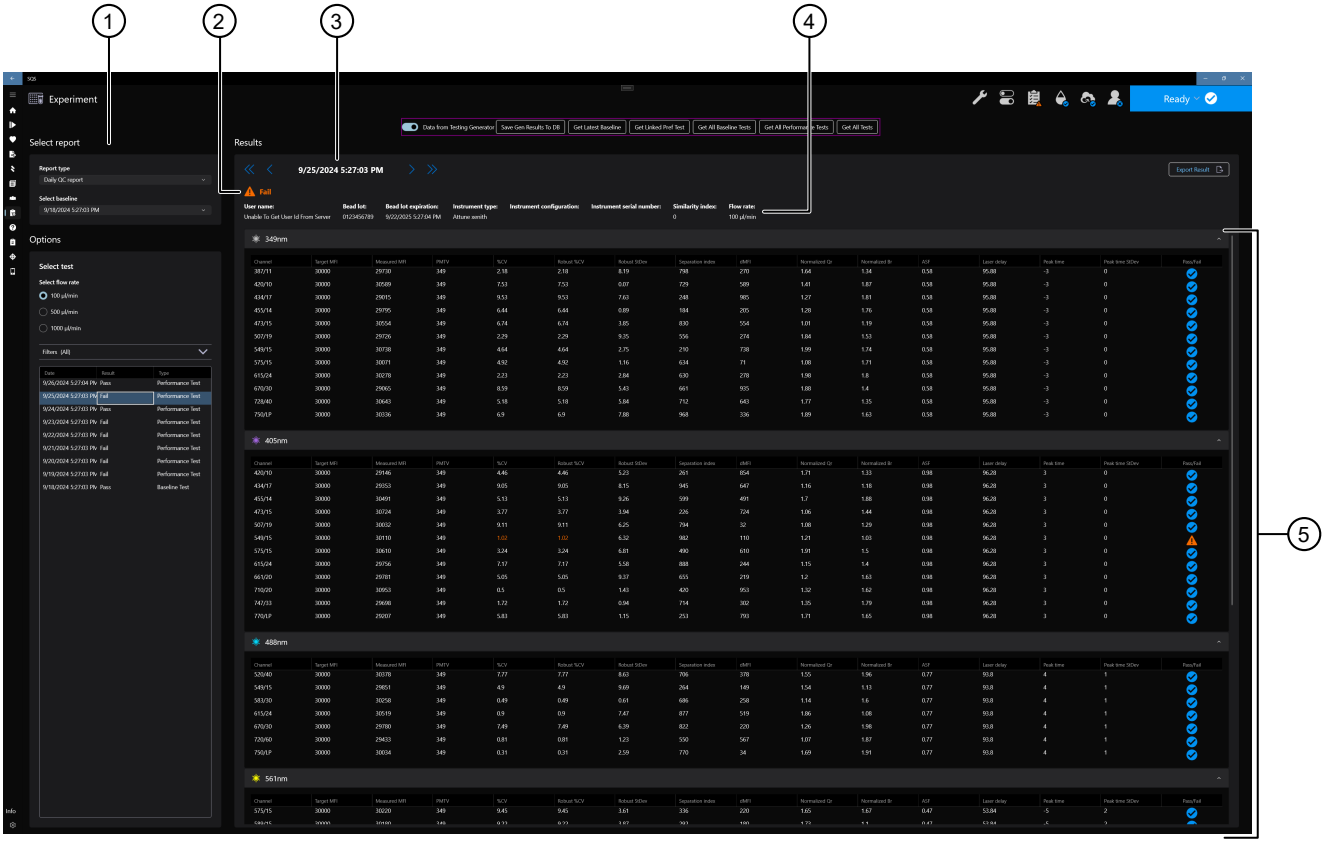
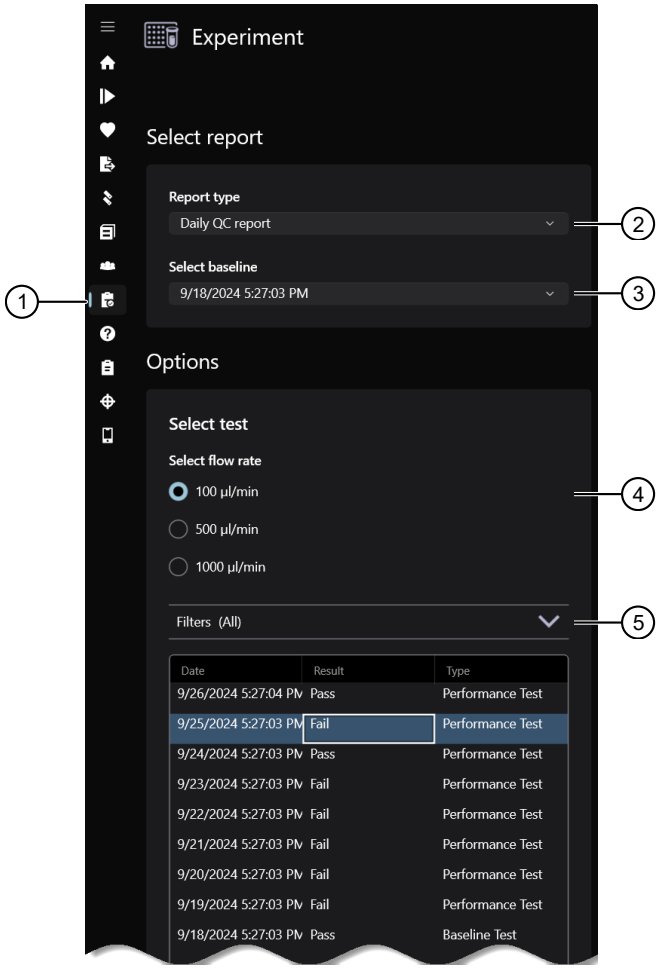


Figure 137 Daily QC Report

1. Select report and Options
2. Pass/Fail status
3. Selected test
4. QC test details
5. QC test statistics



① Report Management tab
② Select report - Report type
③ Select report - Select baseline
④ Options - Select test - Select flow rate
⑤ Options - Select test - QC list and Filters

Figure 138 Daily QC Report - Select report and Options

- **Pass** status is indicated by the blue checkmark symbol .
- **Fail** status is indicated by the amber warning exclamation symbol .
- **P/F** results for specific channel and specific failing criteria are indicated in the **QC test statistics** table in amber color.
- **P/F** criteria are based on beadlot for **dMFI** and **rCV** measurements.
- You can navigate through **Daily QC reports** using navigation buttons or the **QC list** on the left.
- You can view previous sets of results by selecting a different **Baseline**.
- You can view QC results for each flow rate.

QC Test statistics

Baseline test uses the Attune™ Xenith™ Quality Control Beads to generate data for the parameters listed below to establish the initial status of the cytometer.

Daily QC test uses the Attune™ Xenith™ Flow Cytometer QC Beads to determine the variation of the test data from the target values established by the **Baseline** calculations to track the daily performance of the instrument. The results are reported in the **Daily QC Report**.

- **dMFI**: Delta MFI (median fluorescence intensity) between **Baseline** and **Daily QC**.
- **rCV**: Robust coefficient of variation of the brightest bead.
- **Relative Qr**: Qr(DailyQC) / Qr(Baseline) of each detector, where Qr is the Relative Quantum Efficiency (this avoids the need to measure MESF of the dim bead).
- **Relative Br**: Br(DailyQC) / Br(Baseline), where Br is the Relative Background (Br) of each detector (this avoids the need to measure MESF of the dim bead).
- **ASF**: Area Scaling Factor for every laser.
- **Laser Delay**: The laser delay for the corresponding laser.
- **Separation Index**: Measured between bright and dim bead populations.
- **Similarity Index**: MFI response of all fluorescence detectors compared to **Baseline** (want 100%).
- **Peak Time** and **Peak Time rSD**: Displayed in **Levey-Jennings plots** (rSD: robust standard deviation).
- **System Pressures**: Based on sample pump and focusing fluid pump.
- **Measured gear pump RPM**: The measured RPM of the gear pump for the given flow rate.
- **Measured flow rate**: The measured flow rate of the focusing fluid at the given flow rate.
- Images are also captured as part of the **Daily QC** test and are used to evaluate image acquisition stability from day to day.

Pass/Fail criteria is set based on beadlot for **dMFI** and **rCV**.

Levey-Jennings Report

The **Levey-Jennings report** tracks shifts and trends in instrument performance as indicated in **Daily QC tests** over time and provides a visual indication of system stability.

To view the **Levey-Jennings report**, click **Global navigation** ▸ **Report Management** ▸ **QC Reports**, then select **Report type** ▸ **Levey-Jennings**.

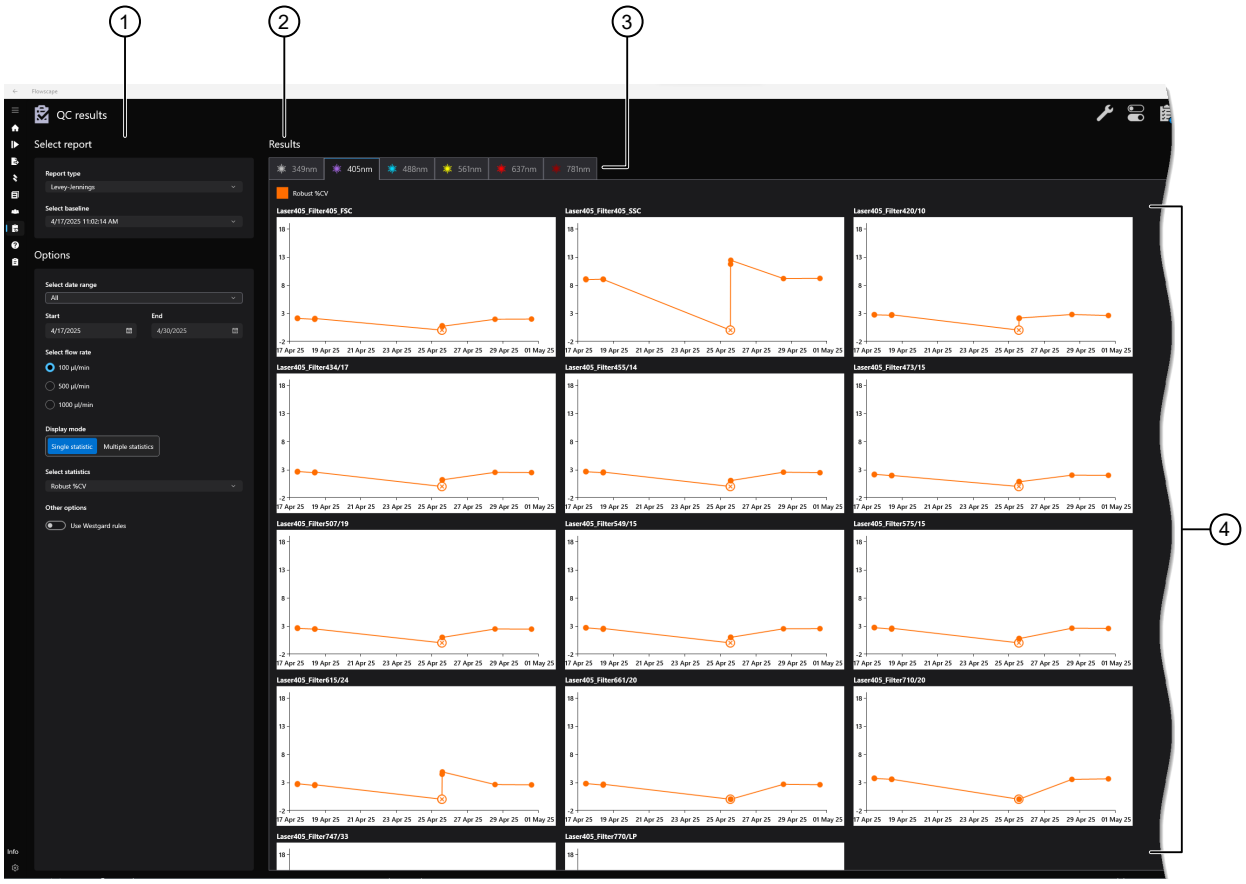
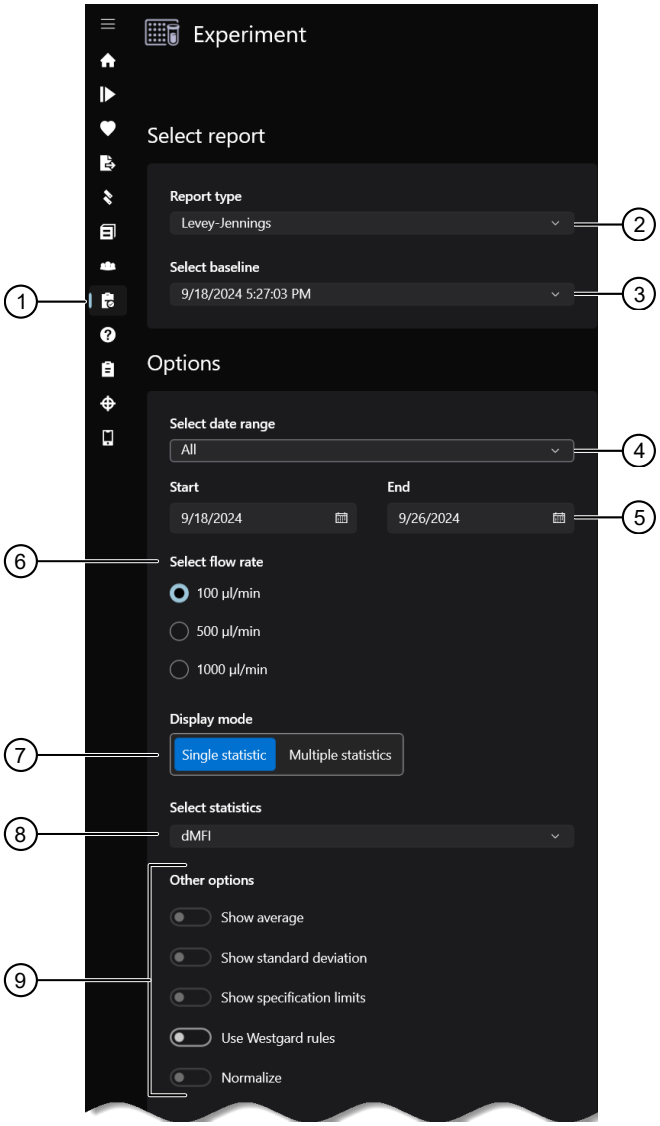


Figure 139 Levey-Jennings report

1. Select report and Options
2. Results
3. Excitation source tabs
4. Levey-Jennings plots



1. Report Management tab
2. Select report - Report type
3. Select report - Select baseline
4. Options - Select date range
5. Options - Start and End dates
6. Options - Flow rate
7. Options - Display mode
8. Options - Select statistics
9. Options - Other options

Figure 140 Levey-Jennings Report - Select report and Options

- The plots in the **Levey-Jennings report** are grouped by the excitation source.
- You can change the date range for the report or view reports from all dates.
- You can view the results specific to a flow rate.
- You can toggle the **Display mode** of the plots between the **Single statistic** and **Multiple statistic** modes.

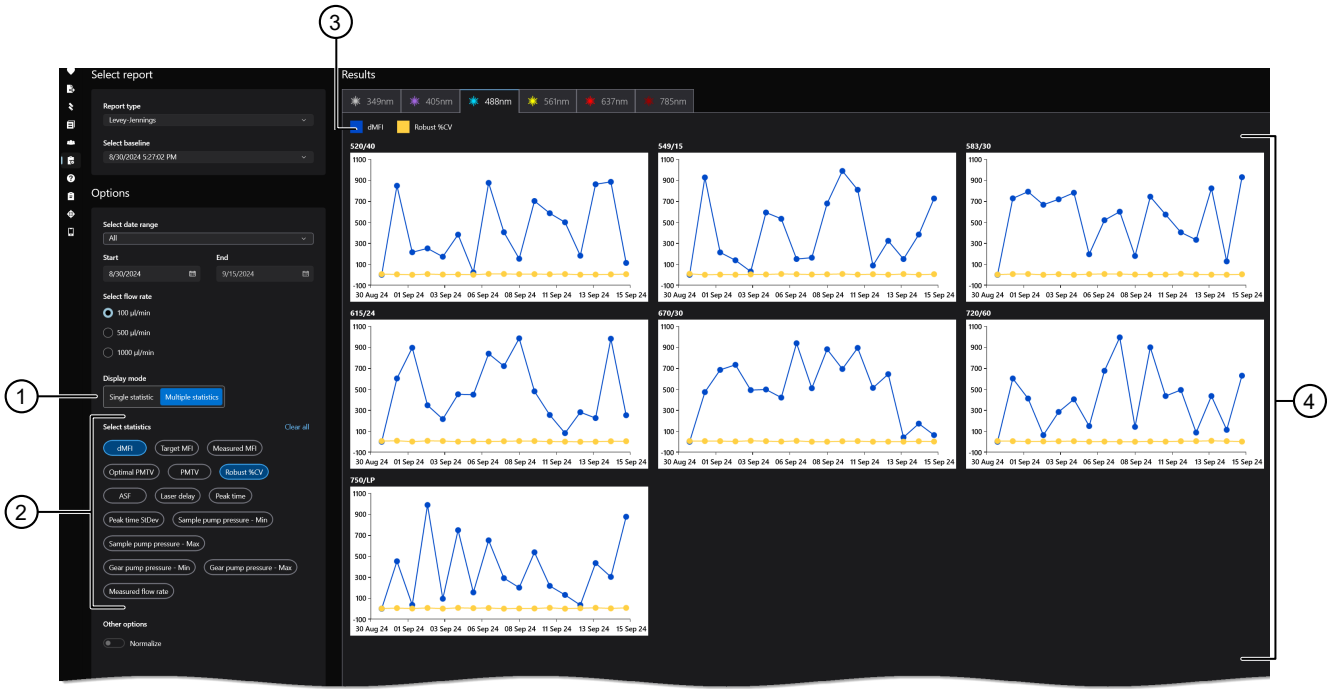


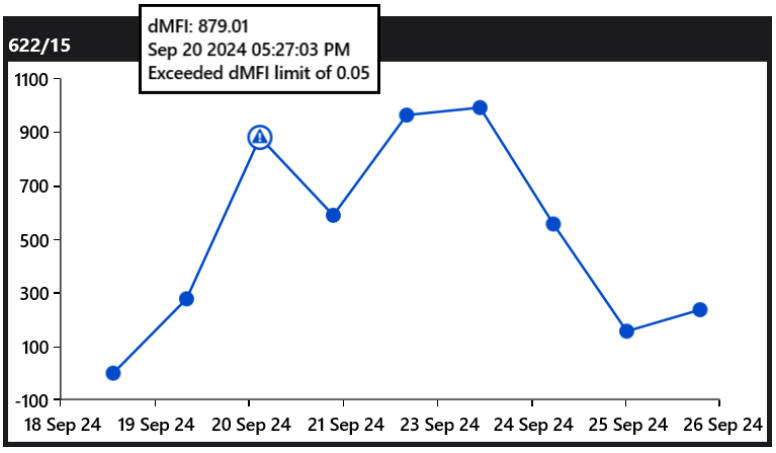
Figure 141 Levey-Jennings report in Multiple statistics mode showing dMFI (blue) and Robust %CV (yellow)

① Display mode (Single vs. Multiple statistics)
② Select statistics
③ Statistics legend
④ Levey-Jennings plots

- You can select different statistics to view in the plots.
- You can view Westgard results.

Note: Westgard rules are a statistical quality control technique to detect errors in analytical test results.

- Failures are indicated by warning exclamation symbol on the data point.
- Tooltip is provided for each data point.



10 Spectral unmixing experiment setup

Spectral unmixing overview

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.

Spectral unmixing is a mathematical method used to identify a fluorophore signature within a multitude of potentially overlapping ones and to create its own parameter.

In experiments with spectral unmixing, emitted light is measured across **all available detectors** and the similarity of fluorophores is corrected via the all channels-by-all channels unmixing matrix, where the unique spectral signature of each fluorophore distinguished by the unmixing algorithm.

The benefits of spectral unmixing over conventional compensation are:

- Increased panel sizes, which is highly beneficial when handling limited samples.
- Increased flexibility in fluorochrome choices.
- Ability to remove autofluorescence.

The recommended **Unmixing type** depends on the experiment design. We recommend **spectral unmixing** for:

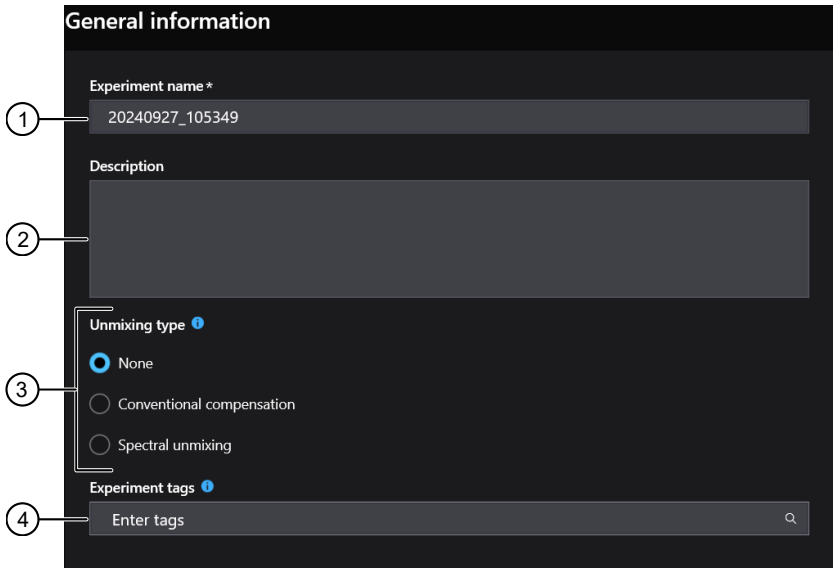
- Panels above ~15 colors (can also use for smaller panels).
- Samples with mixed autofluorescence populations.
- Panels where you are not certain which best peak channel to select.
- Highly beneficial when handling limited samples.

Note: For assays using functional reagents such as MitoTracker™ dyes and cell cycle dyes, which are very bright and have a lot of spread across channels, conventional compensation is the preferred method. These dyes often require individual detector adjustments, and adjusting the full spectra to keep these dyes on scale in bright samples can lead to confusing spectral signatures.

IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

Create a new spectral unmixing experiment

1. On the **Home** screen, click **New experiment** to open the **New Experiment Setup** screen.
2. Under **General information**, enter a new experiment name in **Experiment name** or keep the name created by the system.

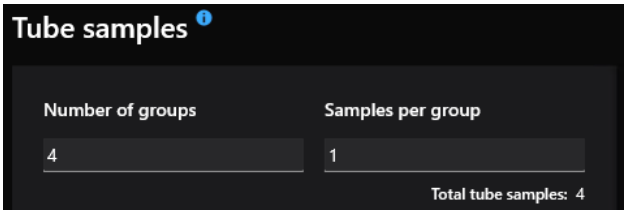


1. Experiment name
2. Description
3. Unmixing type
4. Experiment tags

3. *(Optional):* Enter experiment details in the **Description field** and keywords that can be used as search criteria in the **Experiment tags** field.
4. For **Unmixing type**, select the **Spectral Unmixing** option.

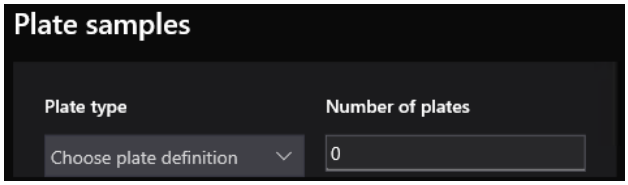
IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

5. Under **Tube samples**, enter the **Number of groups** and **Samples per group**.**Total tube samples** shows the total number of tube samples (number of groups × samples per group) in the experiment.



Note: Each experiment can have up to 400 tube samples.

6. Under **Plate samples**, select **Plate type**, then enter the **Number of plates**.

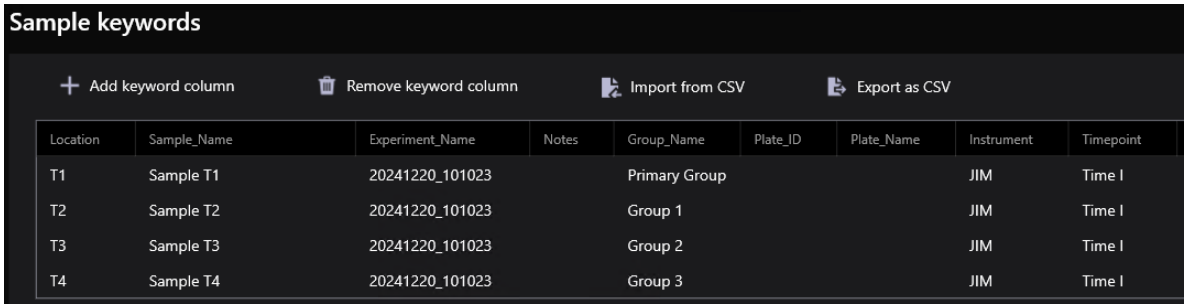


Note: There is no limit to the number of plates in an experiment.

7. If applicable, select **Foil cover**, **Cooling block**, or both.
   - When the **Foil cover** option is selected, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate to protect the sample plate from condensation or evaporation.
   - When **Cooling block** option is selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification.
   - The **Foil cover** and **Cooling block** options are available only if a CytKick™ Max Autosampler is connected to the Attune™ Xenith™ Flow Cytometer.

8. (*Optional*) If desired, update sample keywords (sample name, group name, etc.) or create custom keywords in the **Sample keywords** table.

   Sample keywords are saved in the FCS file metadata and can be helpful when third-party software is used for analysis.



9. Click **Next** to proceed to **Fluorophore Selection** screen.

Select fluorophores

Fluorophore Selection screen enables you to select the fluorophores for the experiment. For spectral unmixing, the screen is divided into three panels: **Select fluorophores**, **Spectral energy**, and **Spectral similarity matrix**.

Figure 142 Fluorophore Selection screen

1. Select fluorophores
2. Fluorophore filters (Type, Panel, Species)
3. Library
4. Selected fluorophores
5. Spectral energy
6. Spectral similarity index

Available fluorophores are listed in the **Library**. You can filter the fluorophores by **Type** (Reactive dye, DNA binding, Live/Dead, Tandem dye, Fluorescent protein, pH indicator, Quantum Dot, Custom) or search by **Name** in the **Library search box**.

Add fluorophores to the experiment

1. In the **Library**, click the fluorophore to select, then click the **+** button to add to the experiment. Alternatively, double-click the fluorophore to select and add it to the experiment.

Note: Each fluorophore added to the panel has a **complexity number** (also called **condition score**). This number is additive and indicates how different the fluorophores are from one another.

- A **complexity number** of 1 indicates that each fluorophore is entirely different from the others in the panel.
- As more overlaps occur between the channels when more fluorophores are added to the panel, the **complexity number** increases.

---

2. If desired, assign a **Target name** (for example, CD4) to each selected fluorophore in the **Target** column of the **Selected** list.

3. As fluorophores are added from the **Library** to the **Selected** list, the **Spectral energy** plot and the **Spectral similarity matrix** are populated with the selected fluorophores.

IMPORTANT! Add fluorophores with high similarity first, because the software unmixes after each single-color control.

---

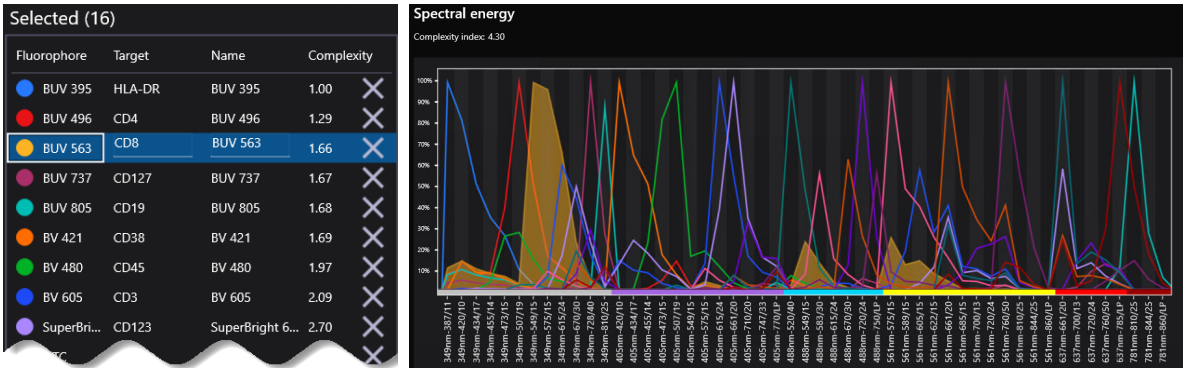
4. If desired, click **Create Panel** to save the panel of selected fluorophores for use in future experiments.

Review spectral energy and spectral similarity

Review the spectral energy and spectral similarity of the fluorophores in the experiment to understand the unmixing burden of the panel, which describes the overall difficulty of distinguishing the unique spectral signature of each fluorophore in the panel by the unmixing algorithm.

1. Review the **Spectral energy plot** of your panel, which shows each fluorophore in the experiment with its expected emission/excitation graphs.

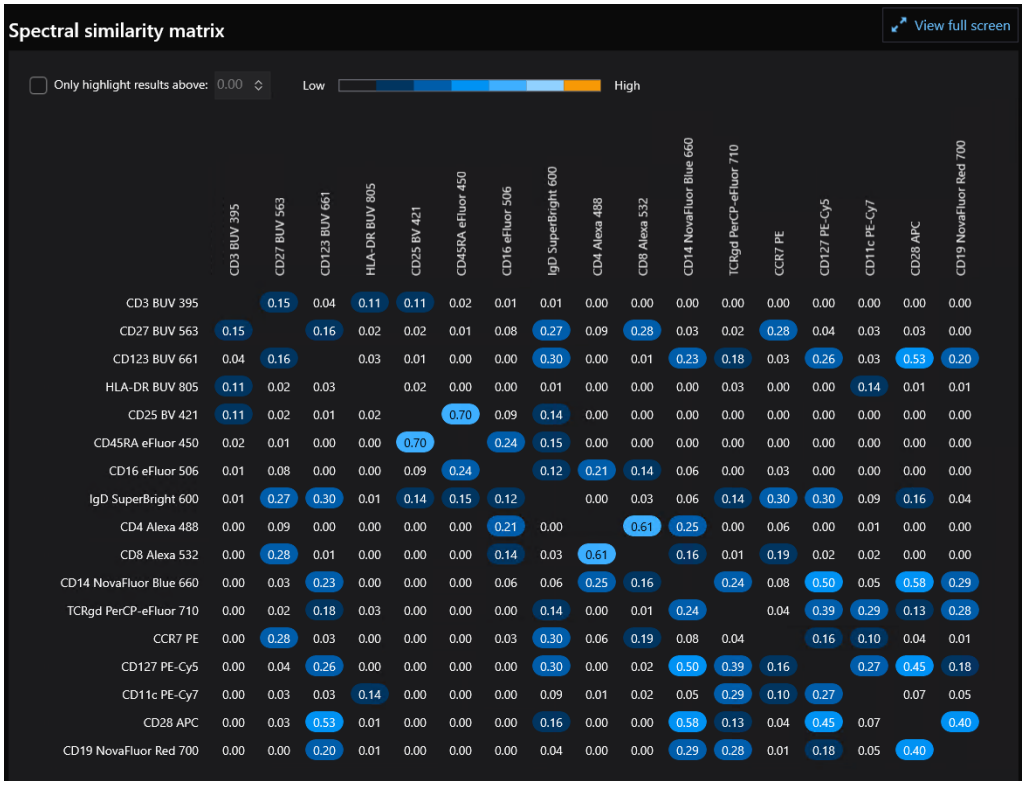
   You can identify each selected fluorophore in the **Spectral energy plot** by the unique color it is assigned in the **Selected** list. When a fluorophore is highlighted in the **Selected** table, the emission spectra of the selected fluorophore is shaded in the **Spectral energy plot** with the color assigned to it.



Note: All spectral signatures in a panel are normalized, so that they can be compared.

2. Review the **Complexity index** value above the **Spectral energy plot**, which indicates the unmixing burden of the panel.
   - A **complexity index** value of 1 indicates that each fluorophore is entirely different from the others in the panel and there is no overlap between the channels.
   - As more fluorophores are added to the panel that result in more overlap between the channels, the **complexity index** increases, indicating a heavier unmixing burden.

3. Review the **Spectral similarity matrix**, which shows how alike the emission spectra are when comparing two fluorophores. In the matrix, a score of 0 indicates that the spectra are entirely different and a score of 1 indicates that the fluorophores are identical.



IMPORTANT! Add fluorophores with high similarity to the panel first, because the software unmixes after each single-color control.

4. If satisfied with the panel and the experiment setup, click **Next** to define unstained controls.

Define and map controls

In experiments with spectral unmixing, the **Spectral Unstained** screen is used to define unstained and autofluorescence controls, map fluorophores to unstained controls, and select spectral unmixing options.

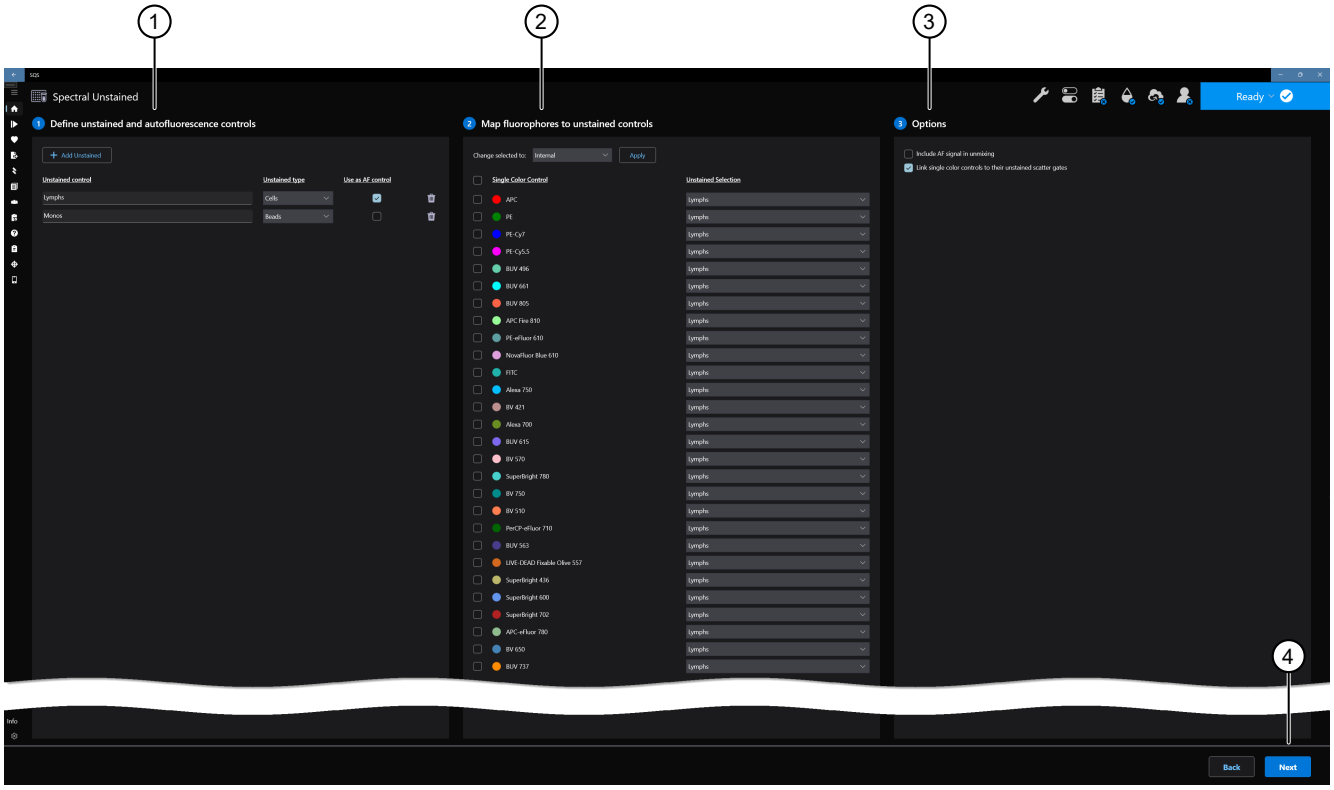


Figure 143 Spectral Unstained screen

① Define unstained and autofluorescence controls
② Map fluorophores to unstained controls
③ Options
④ Next button

When selecting controls for spectral unmixing experiments, it is important to choose both technical and biological controls.

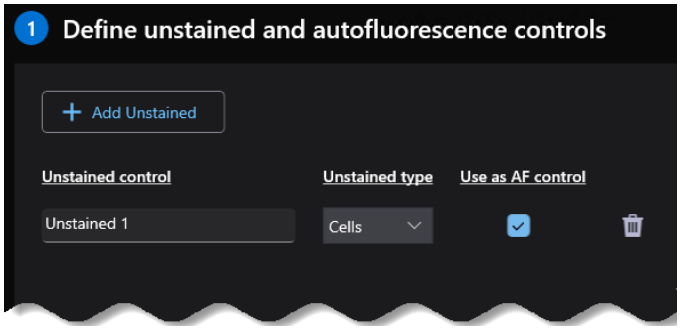
- **Technical controls** help when adjusting detector voltages and other instrument settings, to generate the unmixing matrix, and to set boundries of positive expression. They include unstained and single stained controls (for example, beads or cells) and fluorescence minus one (FMO) controls.
- **Biological controls** validate the success of the experiment. They include positive biological control (for example, treated cells known to cause response) and negative biological control (for example, untreated control cells).

Note: For more information about controls in spectral unmixing experiments, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Define unstained and autofluorescence controls

Define unstained and autofluorescence controls panel enables you to add one or more unstained and autofluorescence controls to the experiment.

1. The first **Unstained control** is automatically added by the software and is labeled **Unstained 1**. To change the name of the control, click it, then enter the desired name.



2. Select the **Unstained type**: **Cells** or **Beads**.

3. If desired, select the **Use as AF control** checkbox to use the unstained control as an autofluorescence control.

Note: You can select any unstained control that contains cell populations that require autofluorescence extraction as an autofluorescence source for use in the unmixing of fully stained samples.

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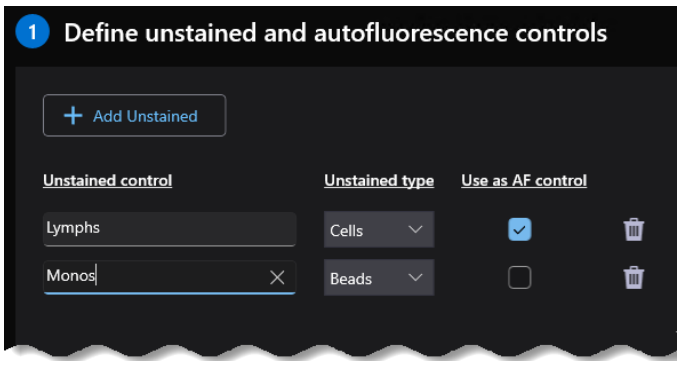
4. To add another unstained control, click **+ Add Unstained**, then repeat steps 1–3 to define the control.



5. If desired, repeat steps 1–4 to add more unstained and autofluorescence controls to the experiment.

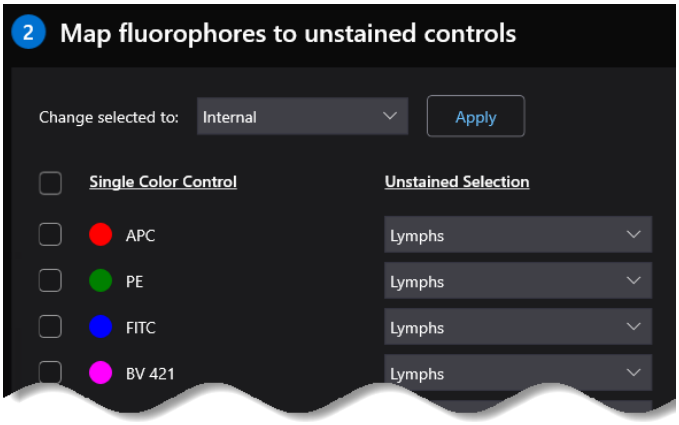
6. When you have defined all unstained controls to use in the experiment, proceed to map the fluorophores in the panel to the defined unstained controls.

Map fluorophores to unstained controls

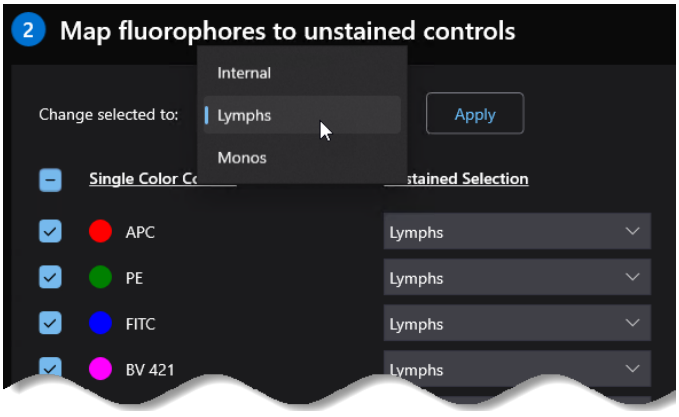
Each single color control must be mapped to an external unstained control or, alternatively, to an internal negative control. This step is performed in the **Map fluorophores to unstained controls** panel.

- The unstained control is used to subtract the dye-free cell or bead signature (autofluorescence) from the single color control to obtain the dye-only signature.
- When a single color control is mapped to an internal negative control, it will rely on a negative population within the selected scatter gate to obtain the dye-only signature.

1. In the **Map fluorophores to unstained controls** panel, for each **Single Color Control** select an unstained control from the corresponding **Unstained Selection** dropdown.

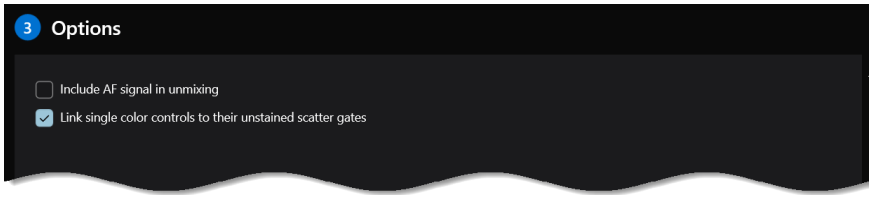


2. To map multiple single color controls to an unstained control, select the desired controls using the corresponding **Single Color Control checkboxes**, then select the desired **Unstained control** from the **Change selected to** dropdown and click **Apply**.



Select spectral options

Spectral options for the experiment are selected in the **Options** panel.



1. To subtract the autofluorescence (AF) signal from true fluorophore signatures, check the **Include AF signal in unmixing** checkbox.

   Note: By default, **Include AF signal in unmixing** is unchecked and not included in the unmixing; it must be checked to be extracted as a separate AF parameter.

   Extracting the AF signal during unmixing reduces background noise by isolating true fluorophore signal and improves the signal-to-noise ratio, which in turn enhances data accuracy and facilitates precise gating. However, including the AF signal in unmixing increases matrix complexity; this trade-off may not be valuable for all samples or panels.

   For more information about AF subtraction, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

   ---

2. By default, the **Link single color controls to their unstained scatter gates** option is enabled.
   - When enable (checked), moving the scatter gate applies to all linked controls and its corresponding unstained control's scatter gate.
   - When inactivated (unchecked), moving gates on each control workspace is honored on a per control and per workspace basis.

Next steps in spectral unmixing workflow

After the unstained and autofluorescence controls are defined, the fluorophores (single color controls) are mapped to unstained controls, and spectral unmixing options are determined, click **Next** to finish the setup of the spectral unmixing experiment. The software automatically creates all selected controls and opens the **Experiment Workspace**.

Experiment Workspace is the main application window, and it is used for controlling the Attune™ Xenith™ Flow Cytometer to run samples, generate data, and analyze results.

- The **Sample panel** lists each control and sample in the experiment and shows the spectral status of the experiment. You can also toggle the autofluorescence extraction option on and off in the **Sample panel**.
- The **Workspace view** enables you to add and view the plots, gates, statistics, and text boxes for data acquisition and analysis, and to perform compensation and spectral unmixing.
- The **Control panel** is used for the collection of samples. From the **Control panel**, you can start, stop and pause sample, record sample data, set trigger and threshold, adjust flow and event rate, set stop conditions, work with the samples list, and load and view FCS files

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Note: For more information about the **Experiment Workspace** layout and controls, see Chapter 5, “Experiment Workspace”.

In the **Experiment Workspace**, you will:

1. Add plots on the **Workspace**.
2. Optimize scatter voltages by acquiring the unstained cell sample.
3. Confirm that the fluorescent channels are on scale and, if needed, adjust the voltages from the fluorescent channels.
4. Acquire all spectral controls. We recommend acquiring both cells controls and beads controls, starting with cells controls, and using the appropriate cells/beads controls in subsequent experiments.
5. If the automatic gates are not set correctly for all controls, adjust the gates appropriately.
6. When the setup is complete, acquire all samples.

Note: For more information about how to optimize voltages, acquire spectral controls, and run samples, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

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11 Compensation experiment setup

Conventional compensation overview

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 a mathematical method used to correct the emission overlap from one fluorophore into the emission channel of another fluorophore.

In experiments with conventional compensation, emitted light is measured from a **single detector**. The similarity of fluorophores is corrected via the single channel-by-single channel compensation matrix, where the spillover from one detector into other detectors is corrected by the compensation algorithm.

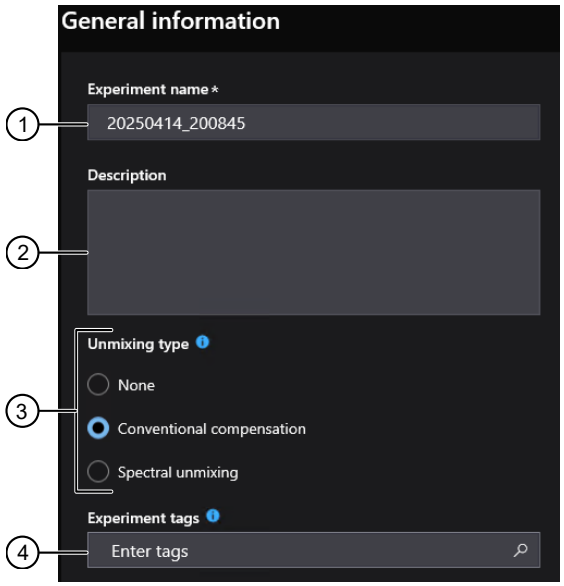
The recommended **Unmixing type** depends on the experiment design. We recommend **conventional compensation** for:

- Panels of smaller sizes.
- Panels with limited single channel overlap.
- Panels that include functional dyes (for example, MitoTracker™ dyes), which often require individual detector adjustments.

IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

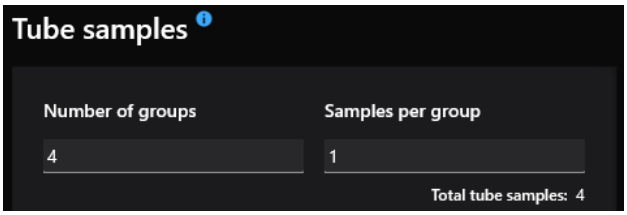
Create a new compensation experiment

1. On the **Home** screen, click **New experiment** to open the **New Experiment Setup** screen.
2. Under **General information**, enter a new experiment name in **Experiment name** or keep the name created by the system.



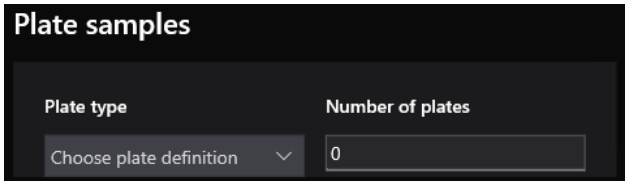
① Experiment name
② Description
③ Unmixing type
④ Experiment tags

3. *(Optional):* Enter experiment details in the **Description field** and keywords that can be used as search criteria in the **Experiment tags** field.
4. For **Unmixing type**, select the **Conventional compensation** option.
5. Under **Tube samples**, enter the **Number of groups** and **Samples per group**.**Total tube samples** shows the total number of tube samples (number of groups × samples per group) in the experiment.



Note: Each experiment can have up to 400 tube samples.

6. Under **Plate samples**, select **Plate type**, then enter the **Number of plates**.

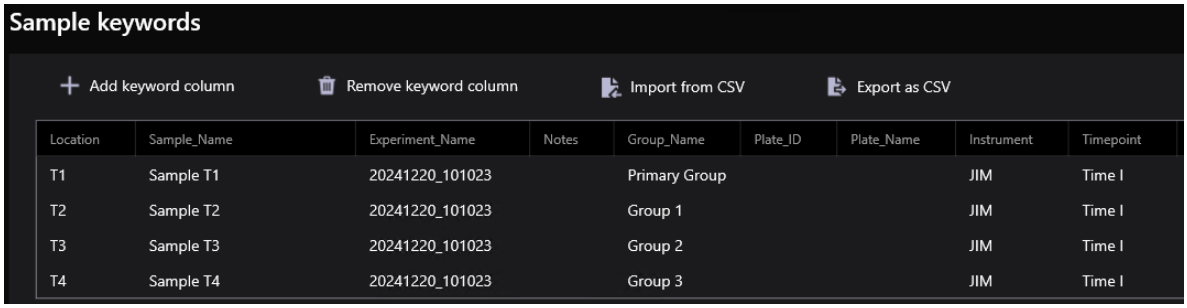


Note: There is no limit to the number of plates in an experiment.

7. If applicable, select **Foil cover**, **Cooling block**, or both.
   - When the **Foil cover** option is selected, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate to protect the sample plate from condensation or evaporation.
   - When **Cooling block** option is selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification.
   - The **Foil cover** and **Cooling block** options are available only if a CytKick™ Max Autosampler is connected to the Attune™ Xenith™ Flow Cytometer.

8. (*Optional*) If desired, update sample keywords (sample name, group name, etc.) or create custom keywords in the **Sample keywords** table.

   Sample keywords are saved in the FCS file metadata and can be helpful when third-party software is used for analysis.



9. Click **Next** to proceed to **Fluorophore Selection** screen.

Select fluorophores

Fluorophore Selection screen enables you to select the fluorophores for the experiment. For **Conventional compensation**, the screen is divided into four panels: **Select fluorophores**, **Check/Assign detectors**, **Compensation Options**, and **Detector Options**.

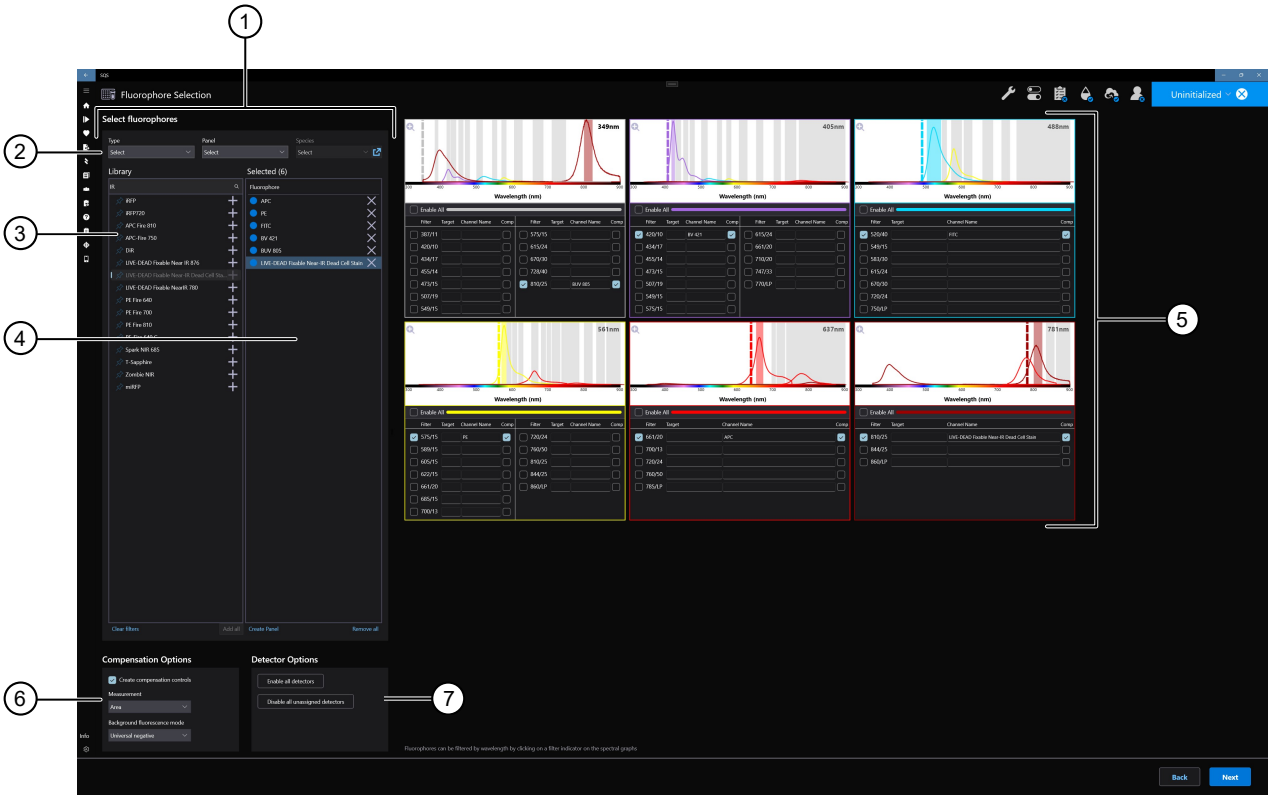


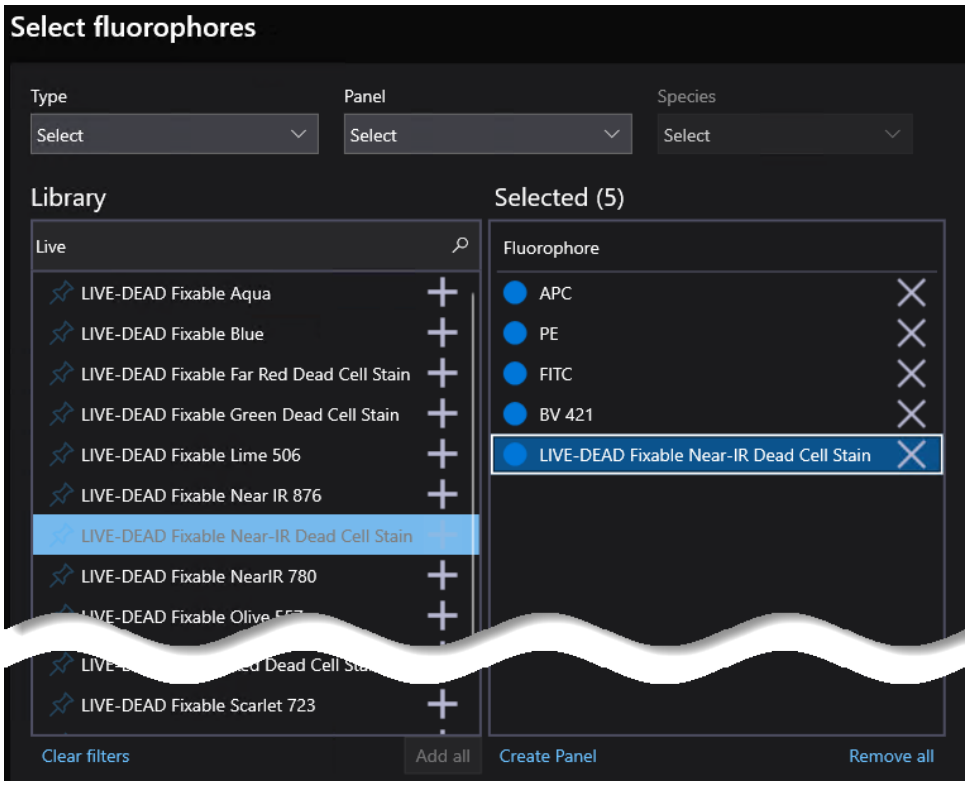
Figure 144 Fluorophore Selection screen for Conventional compensation experiments

1. Select fluorophores
2. Fluorophore filters (Type, Panel)
3. Library
4. Selected fluorophores
5. Check/Assign detectors
6. Compensation Options
7. Detector Options

Available fluorophores are listed in the **Library**. You can filter the fluorophores by **Type** (Reactive dye, DNA binding, Live/Dead, Tandem dye, Fluorescent protein, pH indicator, Quantum Dot, Custom) or search by **Name** in the **Library search box**.

Add fluorophores to the experiment

1. In the **Library**, click the fluorophore to select, then click the **+** button to add to the experiment. Alternatively, double-click the fluorophore to select and add it to the experiment.

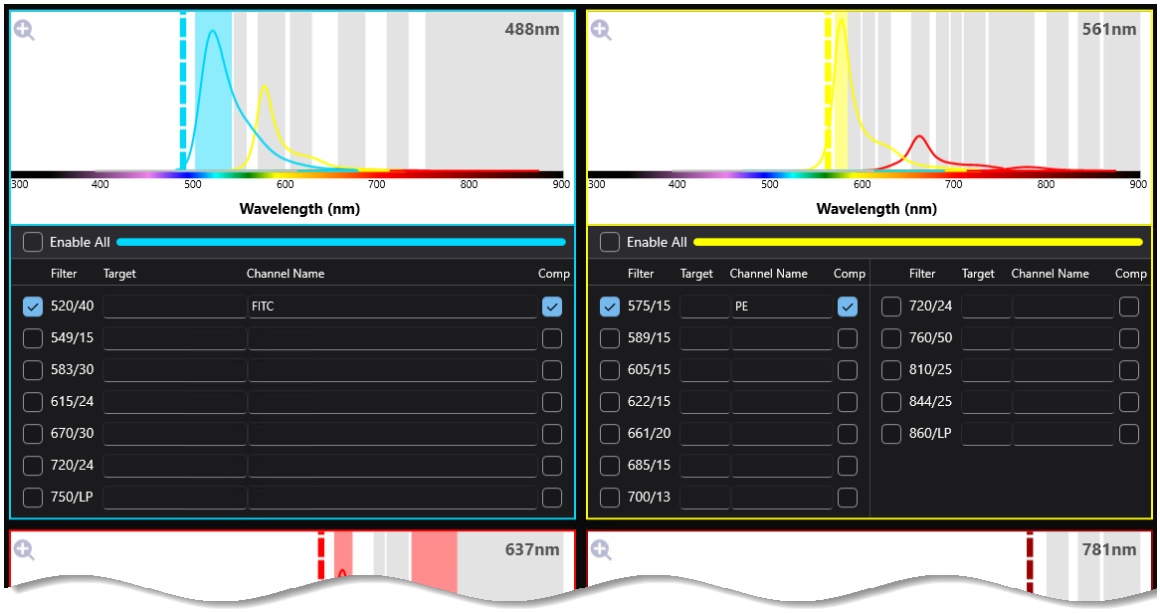


2. As fluorophores are added from the **Library** to the **Selected** list, the plots in the **Check/Assign detectors** panel are populated with the selected fluorophores.

3. If desired, click **Create Panel** to save the panel of selected fluorophores for use in future experiments.

Check/Assign detectors

Check/Assign detectors panel shows the **excitation/emission spectra** of the selected fluorophores, with the plots grouped by the excitation laser. The plots enable you to review and verify the assigned channels and detectors for the selected fluorophores.



Note: For more information about the excitation/emission plots in the Check/Assign detectors panel, see page 58.

1. Review and verify that the selected fluorophores are placed in the appropriate channels.

   Note: The selected fluorophores are automatically placed in the channel that is expected to have the brightest signal. If desired, you can activate more channels and manually assign detectors as compensation controls using the options under each plot.

2. To activate any additional channels, check the desired **filter** under the laser you want to turn on (for example, 520/40 nm bandpass filter under the 488 nm laser, as seen in the image above).

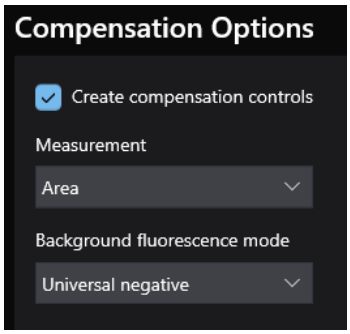
3. To manually assign detectors as compensation controls, click to select the **Comp** checkbox for the corresponding detector.

4. To enable all channels for collection for a laser, select **Enable All** under the laser you want to use.

5. To add a **Target** and **Channel Name**, click the approprite textbox and enter the desired values.

Select compensation options

Compensation Options enable you to create or disable compensation controls, select the parameter to measure for calculating compensation, and select the type of unstained control to use to account for background fluorescence.



1. Verify that the **Create compensation controls** option under **Compensation Options** is selected, which ensures that the necessary compensation controls are created in the **Workspace**.

   Note: By default, the **Create compensation controls** option is selected. When this option is unselected, the experiment is set up with the selected channels, but no compensation controls are created.

2. From the **Measurement** dropdown, select **Area**, **Height**, or **Width** as the parameter for calculating compensation.

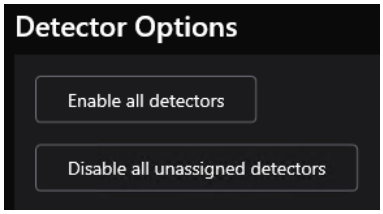
3. From the **Background fluoroscence mode** dropdown, select the source of the background fluorescence when calculating compensation. Available options are:

   - **Universal negative**: Adds an **Unstained** control as an extra compensation control to the **Control Group** in the **Sample panel** and creates the **Workspace** for the **Unstained** compensation control.

     Select the **Universal negative** option if you have an unstained control that can serve as universal negative. This provides an unstained control sample, but no negative gates for the controls.
   - **Internal negative**: Provides negative gates on single color controls, but does not create a separate universal unstained control. Select this option if the controls contain a fluorescence reference internal negative.
   - **None**: No negative control is used and compensation is calculated without correcting for background autofluorescence.

Select detector options

Detector Options panel includes controls to enable and disable detectors.



1. To enable all detectors for the experiment, click **Enable all detectors**.

   Note: When all detectors are enabled, data are collected across all detectors, which allows you to import the data from the compensation experiment into a spectral experiment. With the data imported for the same single color controls and samples, you can compare and contrast the results from compensation and spectral experiments.

   Another advantage of collecting data across all detectors in an experiment is the ability to select off-peak channels for compensation that might have less spillover from fluorophores in adjacent channels.

2. To turn off all detectors that are not assigned as compensation controls, click **Disable all unassigned detectors**.

   Note: Turning off detectors that are not assigned as compensation controls reduces the footprint of the data from the experiment.

12 Settings

Settings

The **Settings** button opens the **Settings screen**, which enables you to change user and global level application settings.

Note: Only **Administrator** accounts can change global level application settings.

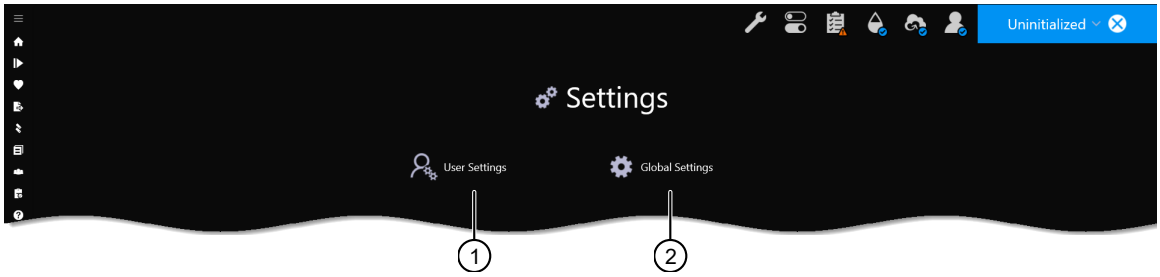


Figure 145 Settings screen

① **User Settings** ② **Global Settings**

User Settings button opens the **User Settings screen**, which enables you to change the following settings for the current user:

- **Detector Names** (page 202)
- **Files/Storage** (page 205)
- **General**
- **Plots** (page 215)
- **Statistics** (page 217)
- **Plate Options** (page 220)
- **Instrument Configuration** (page 224)
- **Keyword Management** (page 227)

Global Settings button opens the **Global Settings screen**, which enables **Administrator** accounts to change global level appliction settings:

- **Detector Names** (page 202)
- **Diagnostics** (page 202)
- **Emergency Contacts** (page 204)
- **Files/Storage** (page 205)
- **Fluorophores** (page 208)
- **General**
- **Plots** (page 215)
- **QC**
- **Statistics** (page 217)
- **Cloud Connect**
- **Plate Options** (page 220)
- **Instrument Configuration** (page 224)
- **Hardware Interface** (page 226)
- **Keyword Management** (page 227)

Detector Names

Detector Names enables you to assign custom names to the detectors on the instrument and to enable 445 nm and 785 nm lasers in spectral protocols. **Detectors Names** settings are available in both **User Setting** and **Global Settings**.

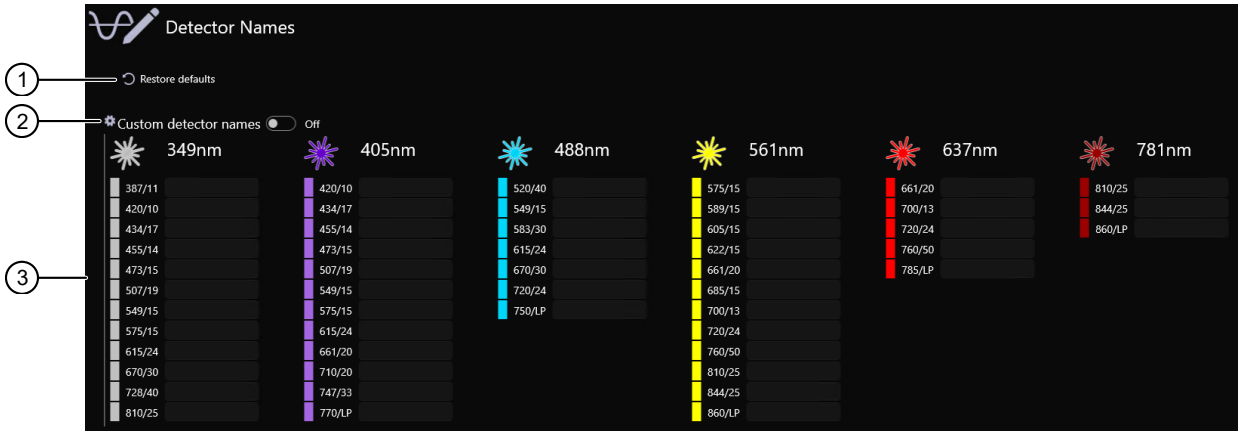


Figure 146 Detector Names screen

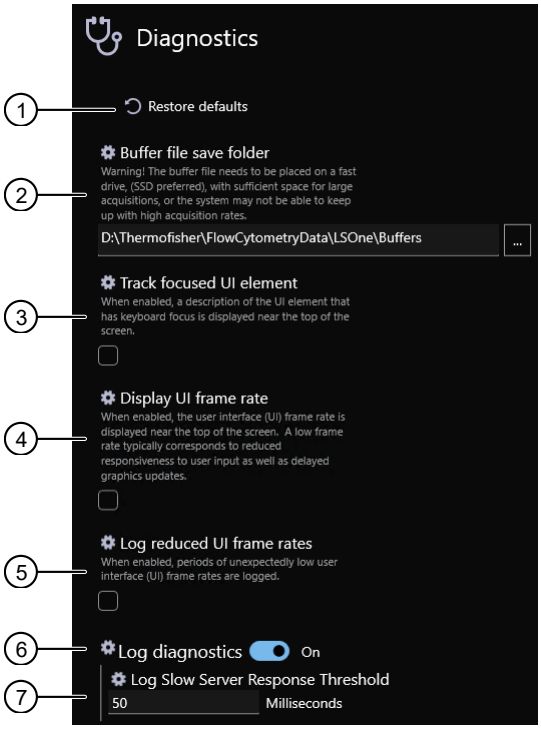
1. Restore defaults
2. Custom detector names
3. Detectors

- To assign custom names to detectors, click the **Custom detector names** switch to turn it on, then click on the desired detector to type in the new custom name.

  By default, **Custom detector names** option is off.

Diagnostics

Diagnostics screen enables users with **Administrator** accounts to set system diagnostics options.



1. Restore defaults
2. Buffer file save folder
3. Track focused UI element
4. Display UI frame rate
5. Log reduced UI frame rate
6. Log diagnostics
7. Log Slow Server Response Threshold

Figure 147 Diagnostics screen

- **Buffer file save folder**: Enables the **Administrator** to select a folder to save the buffer file.

  IMPORTANT! The buffer file needs to be placed on a fast drive (solid-state drive preferred) with sufficient space for large acquisitions. Otherwise, the system may not be able to keep up with high acquisition rates.

- **Track focused UI element**: When enabled, a description of the UI (user interface) element that has keyboard focus is displayed near the top of the screen.
- **Display UI frame rate**: When enabled, the UI frame rate is displayed near the top of the screen. A low frame rate typically corresponds to reduced responsiveness to user input and delayed graphics updates.
- **Log reduced UI frame rates**: When enabled, periods of unexpectedly low UI frame rates are logged.
- **Log diagnostics**: When enabled, the system diagnostics are saved in the **System Log**.

  Note: User with an **Administrator** account can view the **System Log** by clicking **View system logs** in the **Status bar ▸ Instrument status** dropdown (47).

- **Log Slow Server Response Threshold**: Sets the server response time in milliseconds after which an unresponsive server is logged as slow in the **System Log**.

Emergency Contacts

Emergency Contacts screen enables the **Administrator** to designate a **Primary contact** and a **Secondary contact** for emergencies, and save their **Name**, **Phone**, and **Email address** in the instrument.

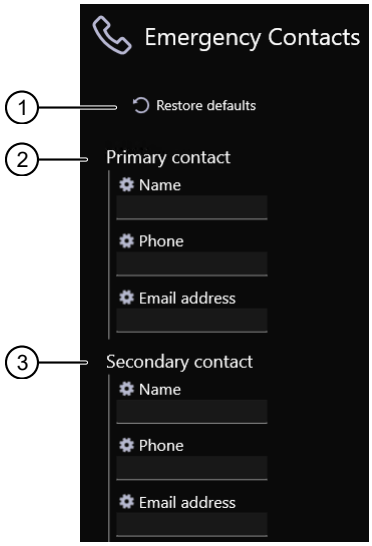
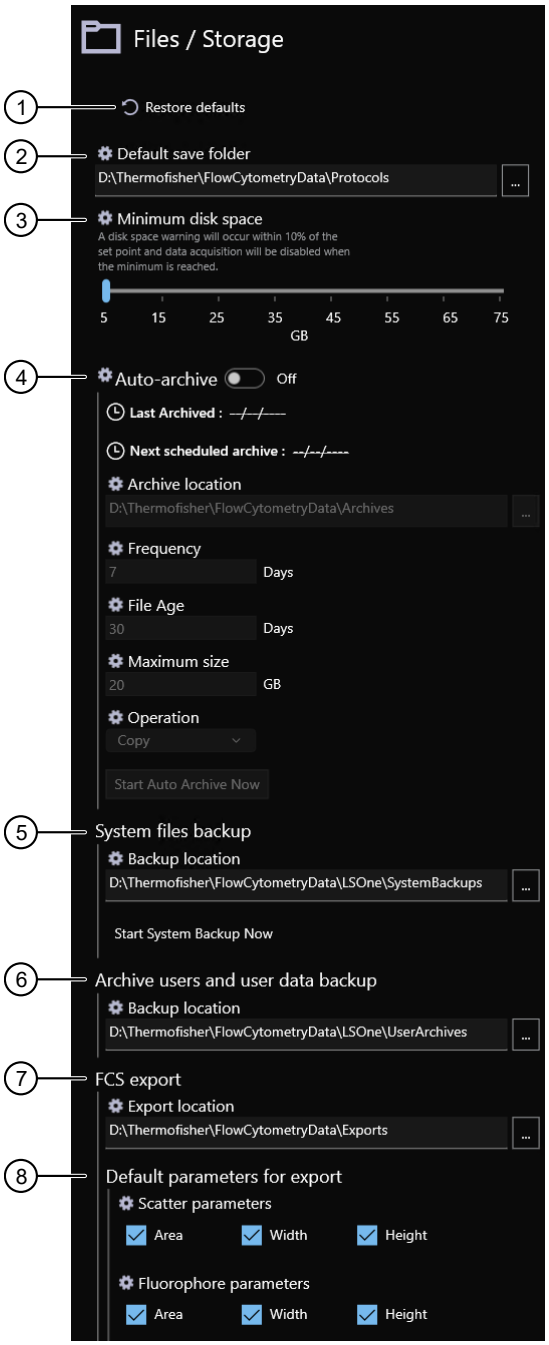


Figure 148 Emergency Contacts screen

① Restore defaults
② Primary contact
③ Secondary contact

Files/Storage

Files/Storage screen, when accessed via **Global Settings**, enables the **Administrator** to set global system and user data backup options such as default save location for flow cytometry data, auto-archive settings, and save locations for system files backup, user and user data backup, and FCS file export.



1. Restore defaults
2. Default save folder
3. Minimum disc space
4. Auto–archive
5. System files backup
6. Archive users and user data backup
7. FCS export
8. Default parameters for export

Figure 149 Files/Storage screen (Global Settings)

Files/Storage screen is also accessible to **Standard Users** via **User Settings**. When accessed via **User Settings**, the only options availableto the user are **Default save folder** and **FCS export locations** and the option to choose **Default parameters for export**.

Files/Storage options set via the **User Settings** apply only to the signed-in user.

Figure 150 Files/Storage screen (User Settings)

① Restore defaults
② Default save folder
③ FCS export
④ Default parameters for export

- **Default save folder**: Enables you to select the location for the default save folder.
  When set in **Global Settings**, the save location applies to all users.
  When set in **User Settings**, the save location applies only to the user; save location for other users remain unchanged.
- **Minimum disk space**: Enables you to set the minimum disk space in gigabytes (GB) required to continue with data acquisition. A disk space warning is displayed when the free space falls within 10% of the set point. When the set minimum is reached, data acquisition is disabled.
- **Auto–archive**: Enables the auto-archive function for flow cytometry data and displays the **Last archived** and **Next scheduled archive** dates. By default, **Auto–archive** is disabled.
  For **Auto–archive**, you can set the following options:
  - **Archive location**: Enables you to select the location for the archive save folder.
  - **Frequency**: Determines the number of days must pass before the next archive is created.
  - **File Age**: Determines the number of days an experiment must remain unchanged before it is automatically archived.
  - **Maximum size**: Determines the maximum size (in GB) of the archive.

  - **Operation**: Enables you to select **Copy** or **Move** options for the archive data.
    When **Copy** is selected, the data files are copied and saved in the selected archive folder.
    When **Move** is selected, the data files are moved to the selected archive folder and removed from the instrument.
  - **Start Auto Archive Now**: Enables you to start the auto-archive process immediately without having to wait until the **Next scheduled archive** date. This option is available only when **Auto–archive** is enabled.
- **System files backup**: Enables you to select the location to backup system files.
  Start System Backup Now starts the backup process immediately.
- **Archive users and user data backup**: Enables you to select the backup location for user archives, which includes user and user data files.
- **FCS export**: Enables you to select the export location for the FCS files.
- **Default parameters for export**: Enables you select the default **Scatter parameters** and **Fluorophore parameters** to include when exporting **FCS files**.
  Available parameters to include are **Area**, **Width**, and **Height**.

Fluorophores

Fluorophores screen is used to manage fluorophores available in the system and to add new fluorphores.

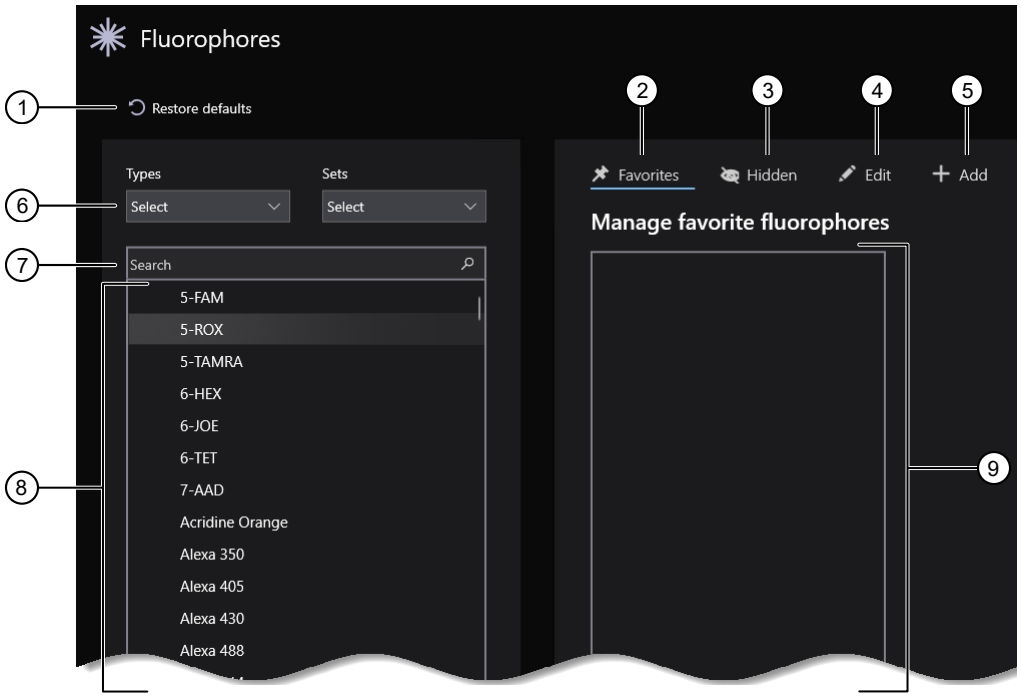


Figure 151 Fluorophores screen

1. Restore defaults
2. Favorites tab
3. Hidden tab
4. Edit tab
5. Add tab
6. Types and Sets filters
7. Search
8. Fluorophores list
9. Selected fluorophores

Fluorophores settings screen consists of two sections: the **fluorophores list** on the left, and the **management panel** on the right, which has four tabs:

- **Favorites** (page 209)
- **Hidden** (page 210)
- **Edit** (page 211)
- **Add** (page 213)

Types and Sets filters enable you to filter the **Fluorophores list** by **Types** and **Sets**.

- **Types** filter dropdown has the following options:
  - **Reactive dye**
  - **DNA**
  - **Live/Dead**
  - **Tandem Dye**
  - **Fluorescent Protein**
  - **pH Indicator**
  - **Quantum Dot**
  - **Custom**
- **Sets** filter dropdown enables you to filter the **Fluorophores list** by **Custom** sets.

Favorites

Favorites tab enables you to manage favorite fluorophores. **Favorite fluorophores** are shown with a **pin icon** to distinguish them from the rest of the fluorophores. When setting up experiments, favorite fluorophores are shown on top of the **Library** during fluorophore selection for **compensation** or **spectral controls**.

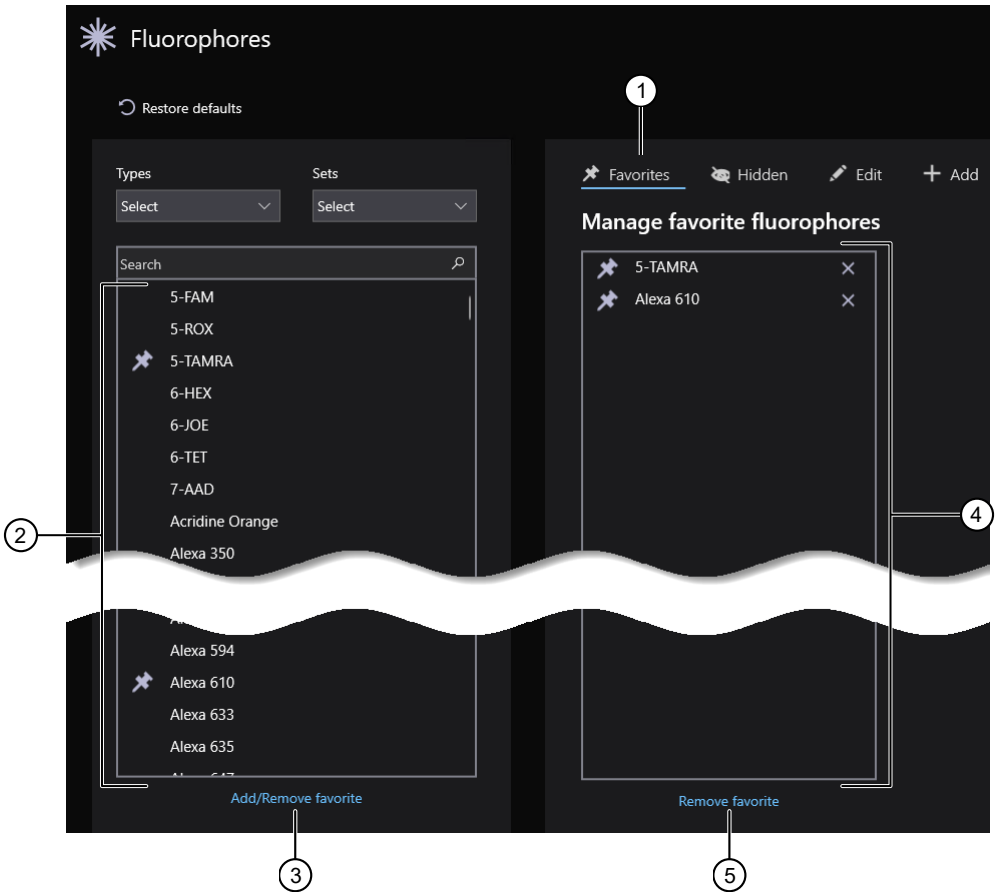


Figure 152 Fluorophores screen with Favorites tab open

1. Favorites tab
2. Fluorophore library
3. Add/Remove favorite
4. Favorite fluorophores list
5. Remove favorite

- To designate a fluorophore as **favorite**, select the fluorophore from the **Library**, then click **Add/Remove fluorophore**. Alternatively, double-click the fluorophore in the **Library**.

  The fluorophore is added to the **Favorite fluorophores list** and is shown with the **pin icon** next to its name.
- When a favorite fluorophore with the pin icon is selected in the **Library**, clicking **Add/Remove fluorophore** removes the fluorophore from the **Favorite fluorophores list**.
- To remove a fluorophore from the **Favorite fluophores list**, click to select it, then click **Remove favorite**.

Hidden

Hidden tab enables you to hide and show fluorophores. **Hidden fluorophores** are shown with a **hidden eye icon** to distinguish them from the visible fluorophores. Hidden fluorophores are not shown in the **Library** when setting up experiments and cannot be selected as **compensation** or **spectral controls**.

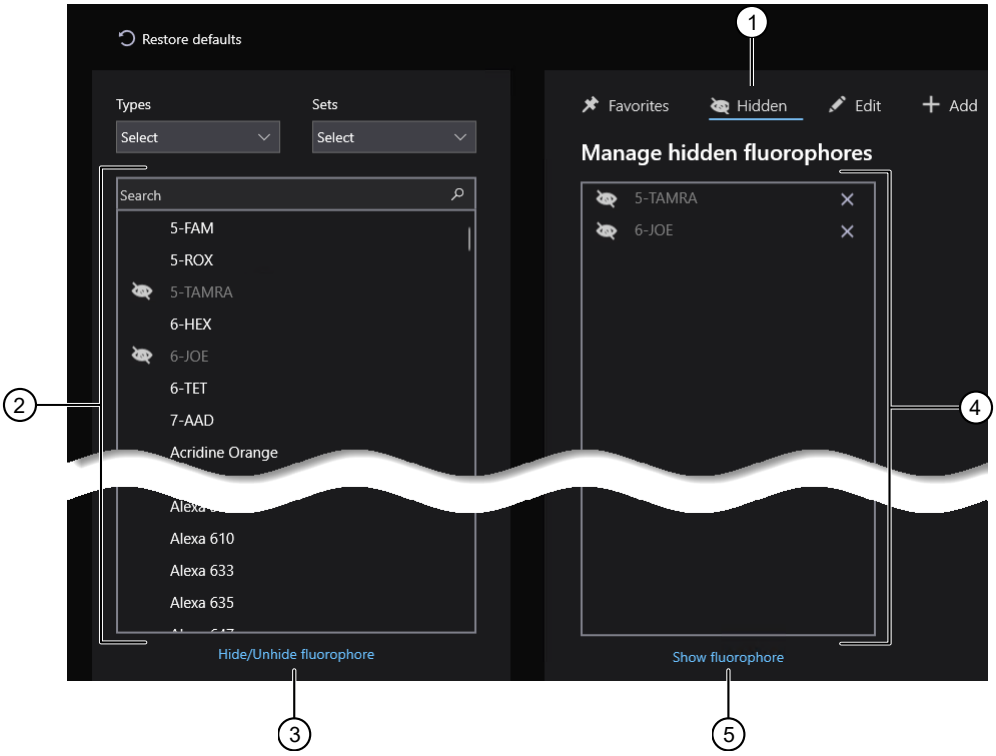


Figure 153 Fluorophores screen with Hidden tab open

① Hidden tab
② Fluorophore library
③ Hide/Unhide fluorophore
④ Hidden fluorophores list
⑤ Show fluorophore

- To hide a fluorophore, select the fluorophore from the **Library**, then click **Hide/Unhide fluorophore**. Alternatively, double-click the fluorophore in the **Library**.

  The fluorophore is added to the **Hidden fluorophores list** and is shown with the **hidden eye icon** next to its name.
- When a hidden fluorophore with the hidden eye icon is selected in the **Library**, clicking **Hide/Unhide fluorophore** removes the fluorophore from the **Hidden fluorophores list** and it becomes available in the **Library**.
- To remove a fluorophore from the **Hidden fluophores list**, click to select it, then click **Show favorite**.

Edit

Edit tab enables you to change the name and type of the selected fluorophore, and edit custom fluorophore sets in the **Library**.

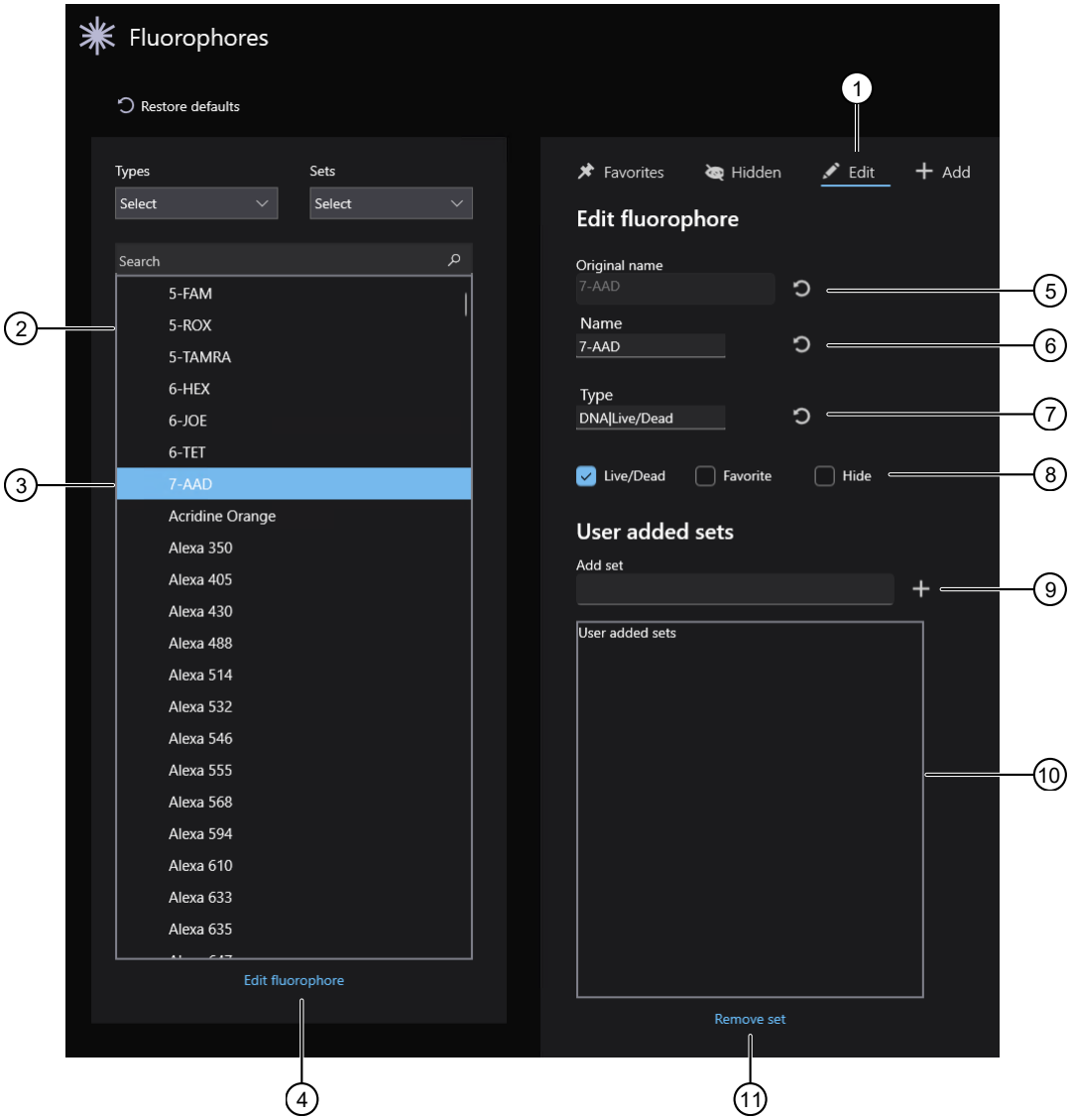


Figure 154 Fluorophores screen with Edit tab open

1. Edit tab
2. Fluorophore library
3. Selected fluorophore
4. Edit fluorophore
5. Original name
6. Name
7. Type
8. Live/Dead, Favorite, Hide options
9. Add set
10. User added sets
11. Remove set

- To edit a fluorophore, use the **Edit fluorophore** tool in the **Edit** tab.
  First, select the fluorophore from the **Library**, then click **Edit fluorophore**. Alternatively, double-click the fluorophore in the **Library**.

The fluorophore is opened in the **Edit fluorophores tool**, which shows its **Original name** and **Type**.

- To rename the fluorophore, enter the new name in the **Name** field.
- To change the fluorophore type, enter it in the **Type** field. To designate the fluorophore as a Live/Dead stain, select the **Live/Dead** checkbox.
- To add the fluorophore to the favorites list, select the **Favorite** checkbox.
- To hide the fluorophore, select the **Hide** checkbox.

Note: **Live/Dead**, **Favorite**, and **Hide** checkboxes show the original state of the fluorophore before it was first selected for editing.

- To add the selected fluorophore to a new custom set, enter the name of the set in the **Add set** field, then click the **+** button.

Add

Add tab enables you to add new fluorophores to the **Library**, and create custom fluorophore sets.

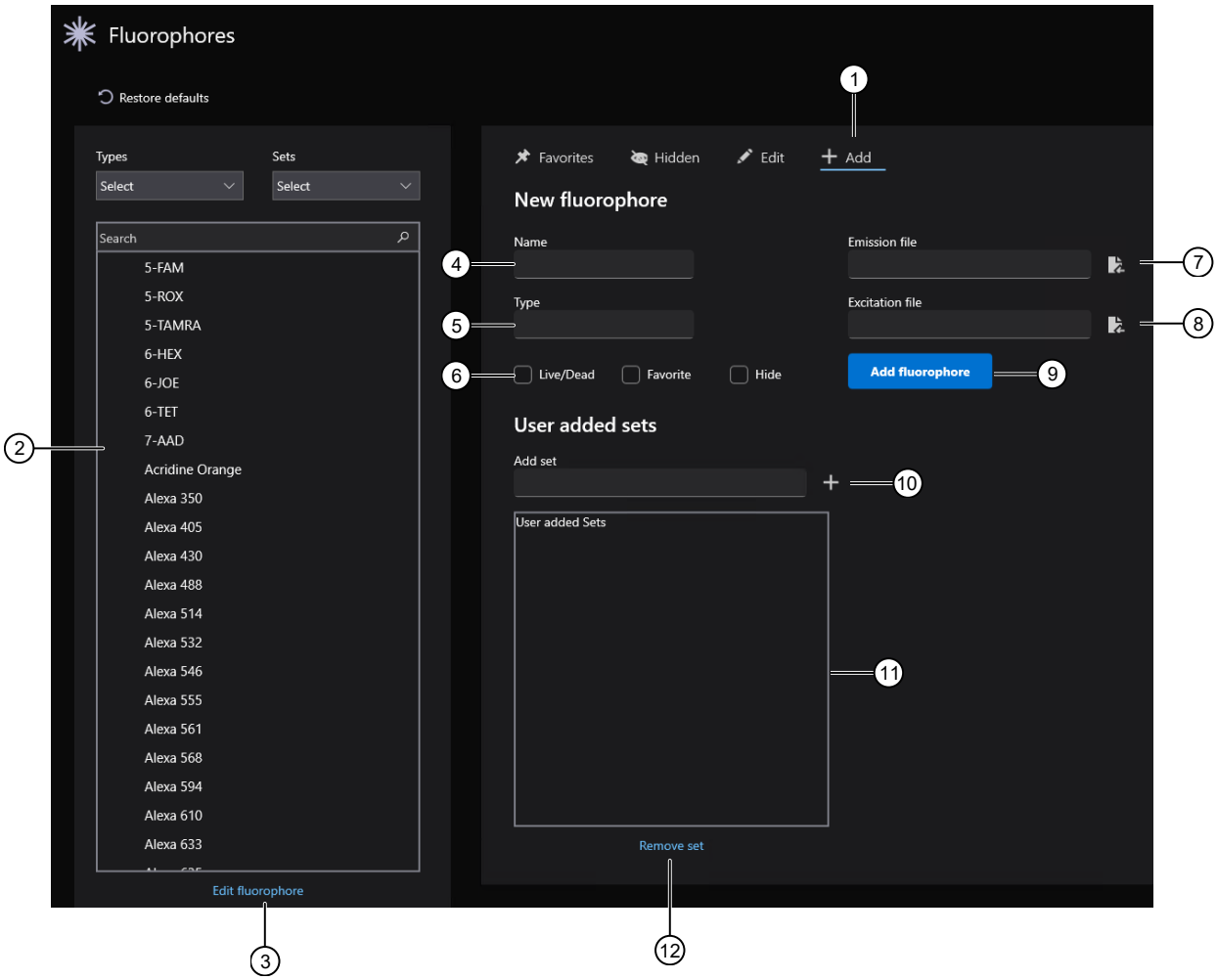


Figure 155 Fluorophores screen with Add tab open

1. Add tab
2. Fluorophore library
3. Edit fluorophore
4. Name
5. Type
6. Live/Dead, Favorite, Hide options
7. Emission file
8. Excitation file
9. Add fluorophore
10. Add set
11. User added sets
12. Remove set

- To add a new fluorophore to the **Library**, use the **New fluorophore** tool in the **Add** tab.
- Enter the name of the new fluorophore in the **Name** field and the fluorophore type in the **Type** field.
- Use the Emission file and Excitation file controls to upload the emission and excitation files for the new fluorophore. The emission and excitation files have the .csv extension.
- To designate the fluorophore as a Live/Dead stain, select the **Live/Dead** checkbox.
- To add the fluorophore to the favorites list, select the **Favorite** checkbox.

- To hide the fluorophore, select the **Hide** checkbox.

  Note: **Live/Dead**, **Favorite**, and **Hide** checkboxes show the original state of the fluorophore before it was first selected for editing.

- When you have entered the properties of the new fluorophore and uploaded emission and excitation files, click **Add fluorophore**.
- To create new custom set, use the **User added sets** tool.

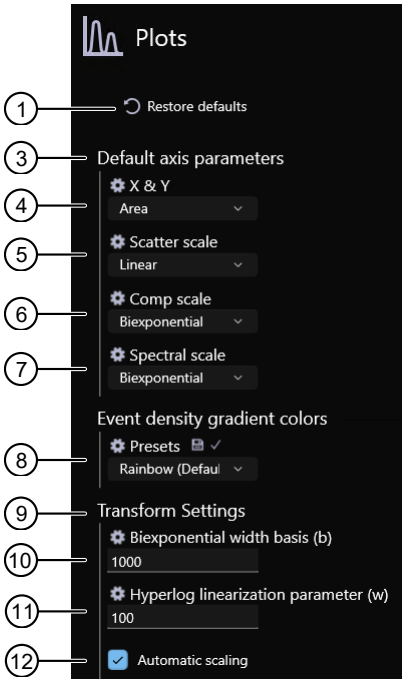
  First enter the name of the new set in the **Add set** field, then click the **+** button.

  The new set is added to the **User added sets** list, and becomes available for search with the **Sets ▸ Custom** filter.

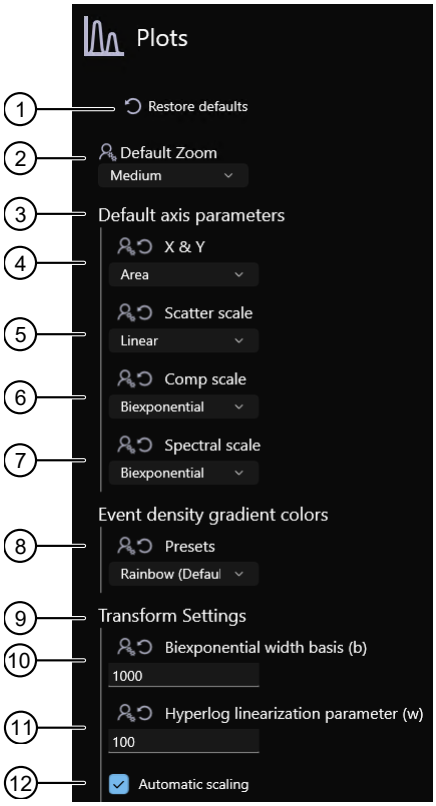
Plots

Plots screen, when accessed via **Global Settings**, enables the **Administrator** to set global default axis parameters, event density gradient colors, transform settings, and automatic scaling options. Plots screen is also available to **Standard Users** via **User Settings**, but the options set via the **User Settings** apply only to the signed-in user.

Note: Any changes made to plot settings are applied only to newly created experiments.



Plots screen (Global Settings)



Plots screen (User Settings)

Figure 156 Plots screen for Global and User settings

1. Restore defaults
2. Default Zoom
3. Default axis parameters
4. X & Y
5. Scatter scale
6. Comp scale
7. Spectral scale
8. Event density gradient colors
9. Transform Settings
10. Biexponential width basis (b)
11. Hyperlog linearization parameter (w)
12. Automatic scaling

- **Default Zoom**: Enables you to select the zoom amount for the plots, which determines the size of the plots that are created in the **Workspace**.

  Default Zoom options are: **Tiny**, **Small**, **Medium**, **Large**, and **Big**. By default, it is set to **Medium**.

  The **Default Zoom** control is available only in the **User settings** ▸ **Plots** screen.

  If desired, the zoom amount can be changed after the plots are created using the **Zoom slider** in the **Workspace** screen.

- **Default axis parameters**: Sets the default transformation scale for the axis parameters for the following plots:
  - **X & Y**: Available options are **Area**, **Width**, and **Height**. By default, it is set to **Area**.
  - **Scatter scale**: Available options are **Linear**, **Log**, **Hyperlog**, and **Biexponential**. By default, it is set to **Linear**.
  - **Comp scale**: Available options are **Log**, **Hyperlog**, and **Biexponential**. By default, it is set to **Biexponential**.
  - **Spectral scale**: Available options are **Log**, **Hyperlog**, and **Biexponential**. By default, it is set to **Biexponential**.
- **Event density gradient colors**: Enables you to select the color gradient for the events shown on the plot. Available options are **Rainbow (Default)**, **High Contrast**, **Red Green**, **Rare Population**, and **User Defined**.
- **Transform Settings**: Enables you to set the following transformation options on the plots:
  - **Biexponential width basis (b)**: The biexponential transformation compresses the data points near zero, making the visual representation of low fluorescence values more sensitive to changes in intensity. The **width basis** setting dictates how much of this compression occurs around zero.

    A larger width basis value means the linear scale is narrower, and the data points are compressed more towards the edges of the graph. Conversely, a smaller width basis value results in a wider linear scale, allowing for better visualization of low fluorescence levels.

    By default, the **biexponential width basis** is set to 1000.
  - **Hyperlog linearization parameter (w)**: Hyperlog scaling is used to display flow cytometry data with a wide dynamic range on a single plot. It is an alternative to a standard logarithmic scale, which can have limitations when dealing with negative or zero values.

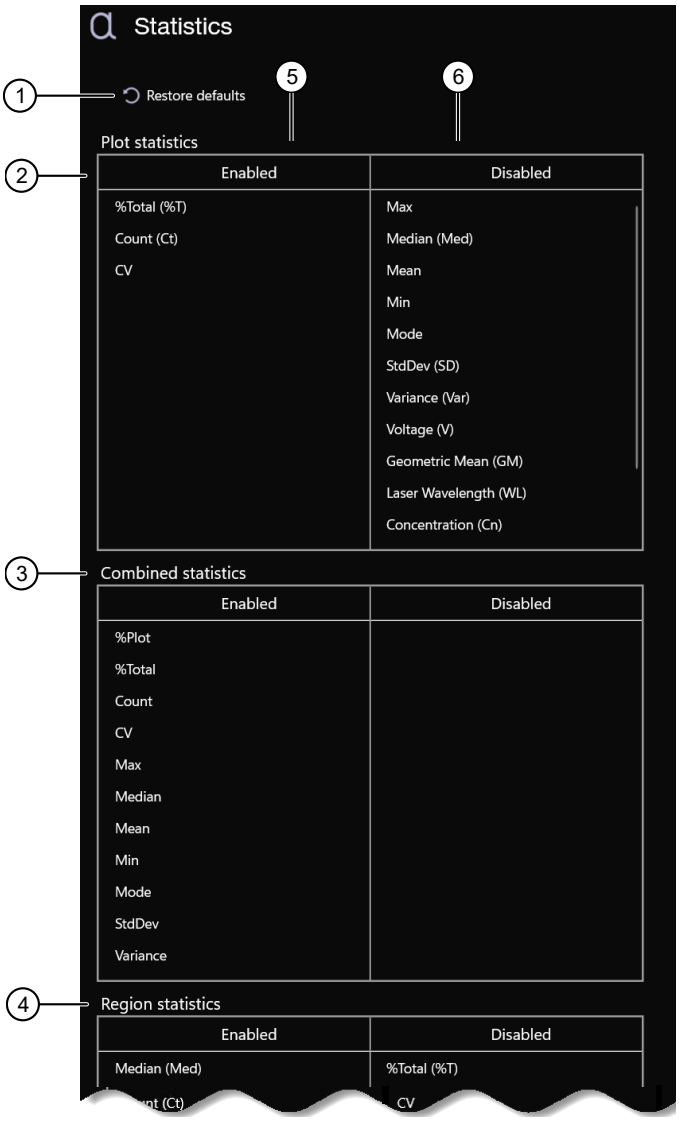
    The hyperlog linearization parameter determines the transition point between a linear and logarithmic scale in the plot. Below this value, the scaling behaves primarily linearly, while above it, it becomes more log-like.

    By default, the **hyperlog linearization parameter** is set to 100.
- **Automatic scaling**: Enables the automatic scaling function on plot axes. When enabled, the minimum and maximum values are set automatically and the upper value is adjusted to the highest channel with data.

Statistics

Statistics screen, when accessed via **Global Settings**, enables the **Administrator** to set global default display preferences for **Plot statistics**, **Combined statistics**, and **Region statistics**.

Statistics screen is also accessible to **Standard Users** via **User Settings**. When accessed via **User Settings**, changes made in **Statistics** settings apply only to the signed-in user.



1. Restore defaults
2. Plot statistics
3. Combined statistics
4. Region statistics
5. Enabled column
6. Disabled column

Figure 157 Statistics screen (Global Settings)

Plot statistics shows the statistics from the entire plot. **Region statistics** shows the statistics from the selected region on a plot. **Combined statistics** show all statistics from all the plots and regions in the workspace

- Statistics can be displayed for any parameter and calculated for any defined population. As the events are acquired, statistics are updated in real time.
- Statistics in the **Enabled** column are displayed in the **Statistics table** in the **Workspace**.
- To remove specific statistics from being displayed in the **Statistics table** in the **Workspace**, click and drag it from the **Enabled** column to the **Disabled** column.

  To enable a statistic, click and drag it from the **Disabled** column to the **Enabled** column.
- Changes made in **Statistics** settings are applied only to newly created **Statistics tables** in the **Workspace** and to newly created experiments.

Depending on the statistics you want to show (**Plot**, **Combined**, or **Region statistics**), you can select from the following statistics:

- **Count (Ct)**: Total number of events.
- **%Total (%T)**: Number of events as a percentage of all triggered data.
- **%Plot (%P)**: Number of events as a percentage of all the events in the plot.
- **Voltage (V)**: PMT voltage used for the selected parameters.
- **Variance (Var)**: The variation within the plot or region.
- **Mode**: The channel that has the most events.
- **Mean**: Average channel number in a distribution.
- **StdDev (SD)**: Standard Deviation, a measurement of data heterogeneity defined as the average distance of each point from the mean.
- **rStdDev (rSD)**: Robust Standard Deviation, a measure of the dispersion of data points in a dataset that is less affected by outliers than the standard standard deviation. It is designed to provide a more reliable estimate of the spread of data, even when the dataset contains extreme values.
- **Laser Wavelength (WL)**: The laser wavelenght for which the statistics is calculated.
- **Concentration (Cn)**: The number of events per µL.
- **Volume (Vm)**: The volume of sample in µL from which the data are derived.
- **Geometric Mean (GM)**: A statistical measure that describes the average fluorescence intensity, especially for data on a logarithmic scale.

  Note: Flow cytometry data often shows a non-normal distribution, which is better visualized on a logarithmic scale. The geometric mean (GM) is calculated by multiplying all individual fluorescence intensity values, then taking the nth root of the product, where 'n' is the number of values. The GM is preferred over the arithmetic mean in flow cytometry because it more accurately represents the central tendency of log-transformed data, such as fluorescence intensities.

- **CV**: Coefficient of Variation, a measurement of data heterogeneity independent of the position of the data on the plot that is given by Standard Deviation / Mean × 100.

- **rCV**: Robust Coefficient of Variation. rCV offers a more reliable measure of relative dispersion than CV, particularly for data that contain outliers or exhibit skewed distribution.

  Note: The Robust Coefficient of Variation (rCV) measures the relative dispersion of data with reduced sensitivity to outliers compared to the traditional Coefficient of Variation (CV). It is calculated using the interquartile range (IQR), which is the difference between the 75th and 25th percentiles, divided by the median.

- **Max**: The maximum value in that plot or region.
- **Median (Med)**: Channel number at which there are 50 percent of events on either side of the distribution.
- **Min**: The minimum value in that plot or region.

Plate Options

Plate Options screen enables you to designate selected plates in the **Plate library** as **Favorites**, to create and import new plate definitions, and to export plate definitions.

Plate Options is available for **Administrator** accounts via **Global Settings** and for **Standard User** accounts via **User Settings**.

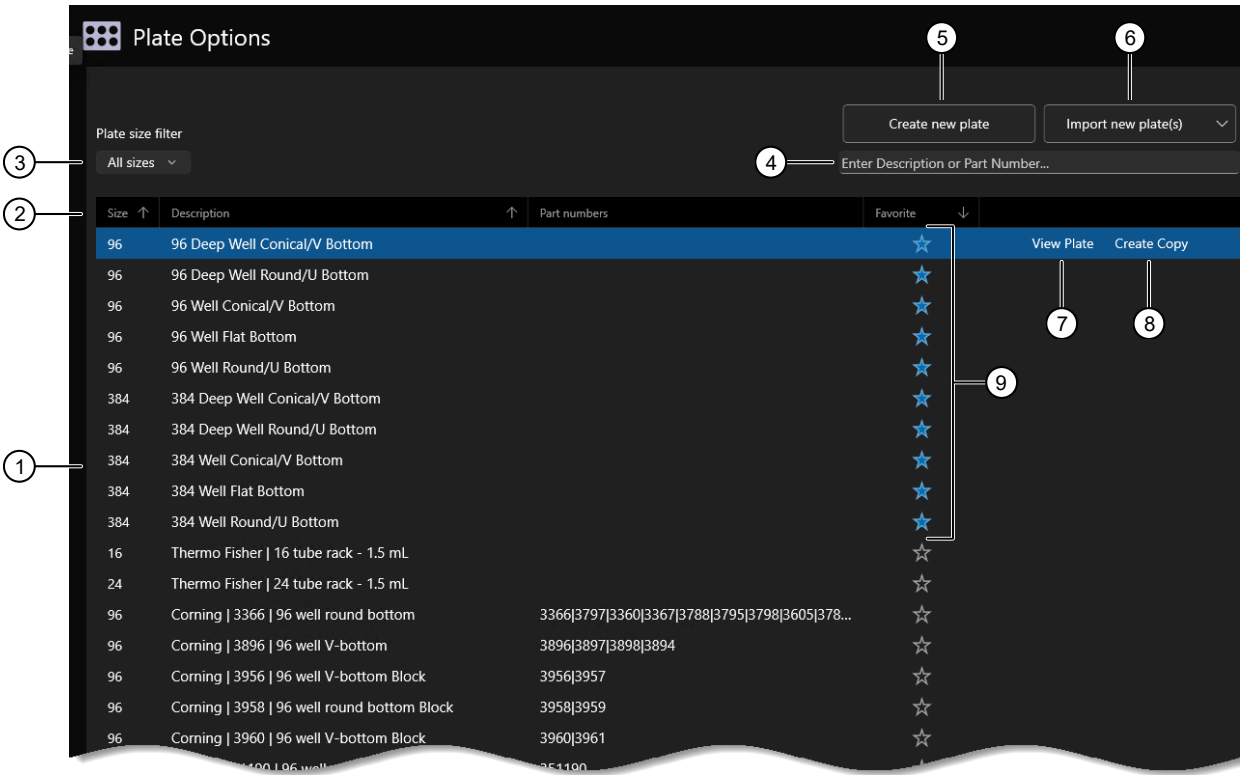


Figure 158 Plate Options

1. Plate library
2. Size, Description, Part numbers, Favorite column headers
3. Plate size filter
4. Search
5. Create new plate
6. Import new plate(s)/Export plate(s)
7. View Plate
8. Create Copy
9. Favorite plates

- **Plate library** lists the plates available in the database.
  - The plates in the library can be ordered by **Size**, **Description**, and **Favorite** status using the corresponding column headers.
  - **Plate size filter** enables you to filter the library by plate size. Available options are **16** (tube rack), **24** (tube rack), **96** (well plate), and **384** (well plate).
  - **Search** field enables you to search the library for a specific plate using plate description or part number.
- To designate a plate as a **Favorite**, select the corresponding start in the **Favorite** column. The selected plate becomes available at the top of the list in the **Plate type** dropdown in the **New Experiment Setup** screen.

- To view a plate definition, select the plate from the library, then click **View Plate**.

  The **View Plate** dialog opens, which shows the description and physical measurements of the plate.

- To duplicate a plate, select the plate from the library, then click **Create Copy**.

  The **Create Plate** dialog opens with all the fields prepopulated to agree with the selected plate definition. However, the description field is updated to have the **Copy <#>** added to create a unique name for the duplicated plate definition.

  Clicking **Save** in the dialog creates a new plate with the same plate definition in the library.

Note: The **Create Copy** function is useful when adding a new plate definition to the library, especially if the new plate is similar to the duplicated plate. When a plate copy is created, the plate definition fields are prepopulated, which you can then edit to define the new plate instead of manually entering the dimension into each empty field.

- **Create new plate** opens the **Create Plate** dialog (see “Create Plate” on page 222), which enables you to define a new plate and save it in the **Plate library**.
- **Import new plate(s)** enables you to import new plate definitions to the **Plate library**.

  Export plate(s) enables you to save plate definitions for use in third party applications.

Create Plate

Note: Only available on the CytKick™ Max Autosampler. Not available on the CytKick™ Autosampler.

Create Plate dialog enables you to create custom plates with specific dimensions (such as plate height and well volume) to add to the database.

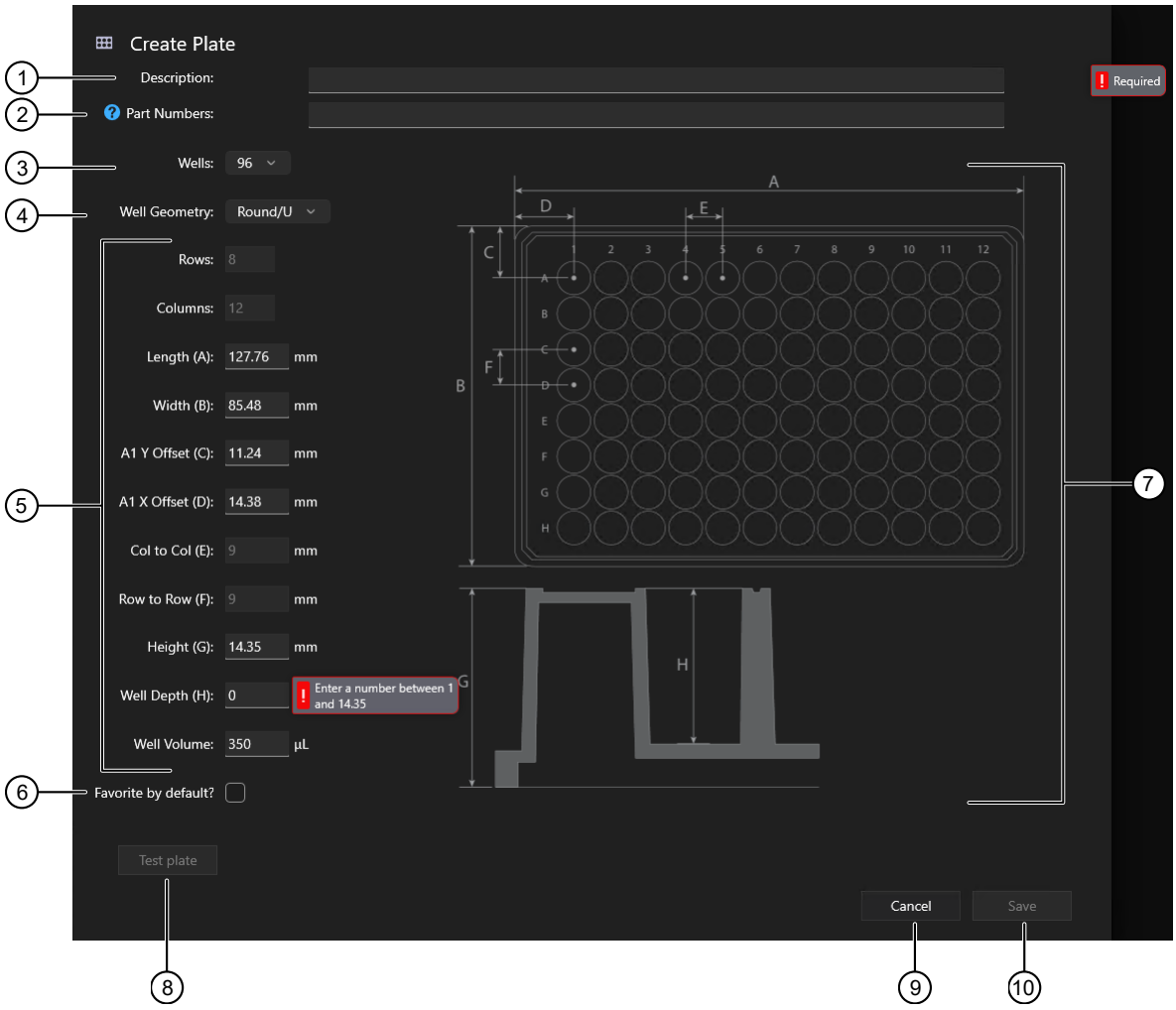


Figure 159 Create Plate dialog

1. Description
2. Part Numbers
3. Wells
4. Well Geometry
5. Plate dimensions
6. Favorite by default
7. Plate image and dimension callouts
8. Test plate
9. Cancel
10. Save

- **Description**: The **Description** field must contain a unique description of the new plate. This is a required field and it cannot be blank.
- **Part Numbers**: If known, enter the part number of the plate here. If there are multiple part numbers, separate the part numbers with the | character.
- **Wells**: Select the plate size here. Available options are **96** and **384** (well plates).

- **Well Geometry**: Describes the geometry of the wells. Avalable options are **Round/U** bottom, **Conical/V** bottom, and **Flat/F** bottom.
- **Plate dimensions**: Describe the dimensions of the plate and include following measurements:
  - **Rows** (number of rows)
  - **Columns** (number of columns)
  - **Length (A)** (in mm)
  - **Width (B)** (in mm)
  - **A1 Y Offset (C)** (in mm)
  - **A1 X Offset (D)** (in mm)
  - **Col to Col (E)** (in mm)
  - **Row to Row (F)** (in mm)
  - **Height (G)** (in mm)
  - **Well Depth (H)** (in mm)
  - **Well Volume** (in µL)

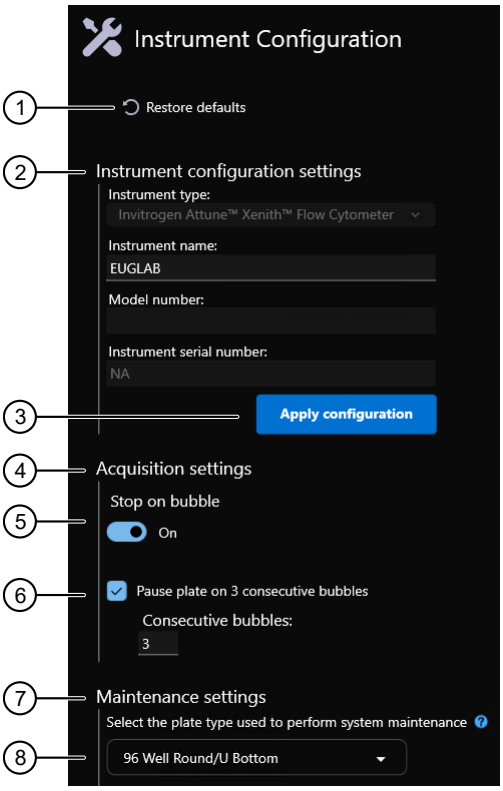
Note: The dimension fields are prepopulated with standard measurements based on the plate type selected (96-well or 384-well). Some of these measurements are fixed and cannot be changed (number of rows and columns, column to column and row to row distances), but others are editable (length, width, and height, A1 well Y and X offsets, well depth and volume) to enable you to define the plate correctly for use with the system.

- **Favorite by default**: Designates the new plate as a **Favorite**.
- **Test plate**: Enables you to validate a plate to ensure that the probe position is in the correct location in all corners for the plate.
- **Save**: Saves the new plate in the **Plate library** and closes the **Create Plate** dialog.
- **Cancel**: Closes the **Create Plate** dialog without saving the new plate.

Instrument Configuration

Instrument Configuration enables you to set offline instrument configuration when working in analysis-only conditions, and to set bubble sensor options and maintenance plate options.

Instrument Configuration is available to **Administrator** accounts via **Global Settings** and to **Standard User** accounts via **User Settings**. However, **Standard User** accounts can only modify **Acquisition settings** at user-level; other options are available only to **Administrator** accounts.



① **Restore defaults**
② **Instrument configuration settings**
③ **Apply configuration**
④ **Acquisition settings**
⑤ **Stop on bubble**
⑥ **Pause plate on consecutive bubbles**
⑦ **Maintenance settings**
⑧ **Plate type dropdown**

Figure 160 Instrument Configuration screen (Global Settings)

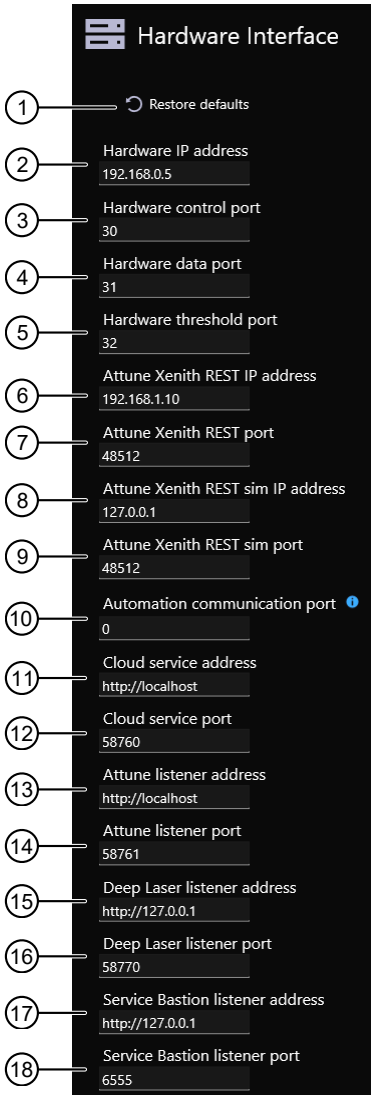
- **Instrument configuration settings** enable you to select offline instrument type and model. This selection aids in the creation of experiments when not connected to an instrument by maintaining correct instrument settings, channel mapping, and run protocol options.
  - **Instrument type** opens the instrument type dropdown list, which contains the instrument configurations listed in the database.
  - **Instrument name** can be updated by the user at any time.
  - When the instrument is connected, the **Instrument type**, **Model number**, and **Instrument serial number** fields are automatically populated based on the instrument and model in use.
  - **Apply configuration** sets the instrument configuration to the selections made in the **Instrument configuration settings**.

  - You can import an experiment created on any Attune™ Xenith™ Flow Cytometer model for analysis in the system. However, acquisition is only possible using the model that is the same as that of the system.
  - **Instrument configuration settings** are only available to **Administrator** accounts.
- **Acquisition settings** enable you to set bubble sensor options.
  - **Stop on bubble**, when enabled, stops the acquisition when a bubble is detected in the fluidics lines of the instrument. By default, **Stop on bubble** option is enabled.
  - **Pause plate on X consecutive bubbles**, when selected, pauses the acquisition of a plate when the bubble sensor detects the number consecutive bubbles set in the **Consecutive bubbles** field.
  - **Acqusition settings** are available to **Administrator** accounts via **Global Settings** and to **Standard User** accounts via **User Settings**.
- **Maintenance settings** enable you to select the **Plate type** that is used to perform system maintenance.
  - **Plate type** dropdown list shows the **Favorite plates** selected in **Plate Options** and the **See all** option.

    See all opens the **Plate library**, which lists all the plates available in the database that can be used for maintenance routines. Only **96-well** and **384-well** plate types can be used in maintenance routines.
  - The selected plate **Plate type** information is sent to the **Deep Clean**, **Shutdown**, and **Decontamination** routines, to ensure that the correct plate dimensions are used in these routines.

Hardware Interface

Hardware Interface screen enables the **Administrator** to modify the hardware interface ports and IP addresses for the system. Hardware interface ports facilitates the physical connection and communication between the instrument and peripheral devices, while the IP addresses enable communication between devices and servers over the network.



① Restore defaults
② Hardware IP address
③ Hardware control port
④ Hardware data port
⑤ Hardware threshold port
⑥ Attune Xenith REST IP address
⑦ Attune Xenith REST port
⑧ Attune Xenith REST sim IP address
⑨ Attune Xenith REST sim port
⑩ Automation communication port
⑪ Cloud service address
⑫ Cloud service port
⑬ Attune listener address
⑭ Attune listener port
⑮ Deep Laser listener address
⑯ Deep Laser listener port
⑰ Service Bastion listener address
⑱ Service Bastion listener port

Figure 161 Hardware Interface screen

IMPORTANT! Changing the system interface ports and IP addresses in the **Hardware Interface** screen can affect instrument operations. Ensure settings are changed with the assistance of Thermo Fisher Scientific technical support. Changes can result in an inoperable instrument.

Keyword Management

Keyword Management screen enables you to create, edit, and delete custom keywords, which provide more information about the sample and the experiment than the default keywords alone. You can also import and export keywords from this screen.

Keyword Management is available for **Administrator** accounts via **Global Settings** and for **Standard User** accounts via **User Settings**.

All users can create, edit, and delete their own custom keywords and add them to their user list in **Keyword Management**. However, only **Administrator** accounts can manage **Global keywords**.

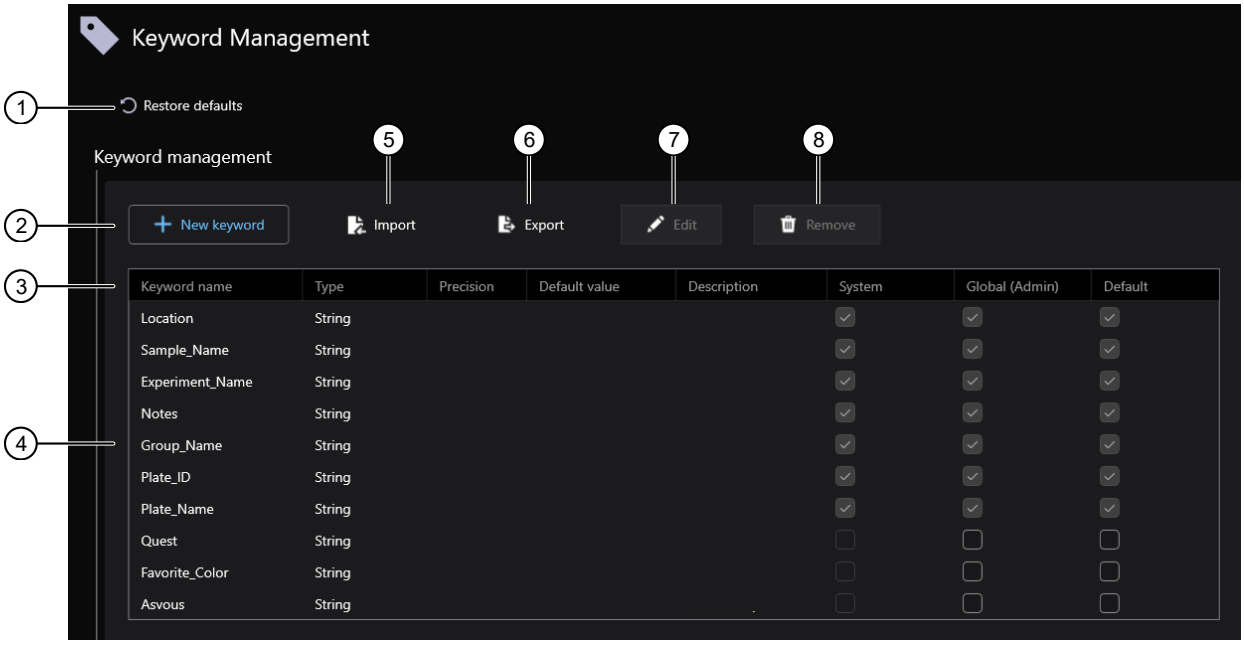


Figure 162 Keyword Management screen (Global Settings)

① Restore defaults
② + New keyword
③ Keywords table headers
④ Keywords table
⑤ Import
⑥ Export
⑦ Edit
⑧ Remove

- The **Keywords table** shows the list of all default and custom (that is, user-defined) keywords. It includes the following columns:
  - **Keyword name**
  - **Type**
  - **Precision**
  - **Default value**
  - **Description**
  - **System**
  - **Global (Admin)**
  - **Default**
- **Global (Admin)** and **Default** columns display **checkbox controls** that enable you to set the custom keywords to **Global** or **Default** or both.
- **Location**, **Sample_Name**, **Experiment_Name**, **Notes**, **Group_Name**, **Plate_ID**, and **Plate_Name** keywords are **System** keywords. They are available permanently as **Global** and **Default** keywords and cannot be removed.

- **+ New keyword** button opens the **New keyword** dialog, which enables you to create a custom keyword to add to the **Keywords table** (see).
- **Import** button enables you to import keywords files with the ***.akw** file extension into the **Keywords table**. If the imported keyword is a duplicate of an existing keyword, the duplicate keyword is appended by a numeric character.
- **Export** button enables you to export the selected keywords to a file with the ***.akw** file extension.
- **Edit** button opens the **Edit keyword** dialog, which enables you to edit an existing keyword.
- **Remove** deletes the selected keywords from the **Keywords table**.

Note: Only users with **Administrator** accounts can create, edit, or delete a **Global keyword**. **Global keywords** are displayed to all users, but the **Edit** option is disabled for non-administrator users. Edits made to a **Global keyword** apply to the keyword master lists of all users, but they do not affect keywords that are associated with existing experiments

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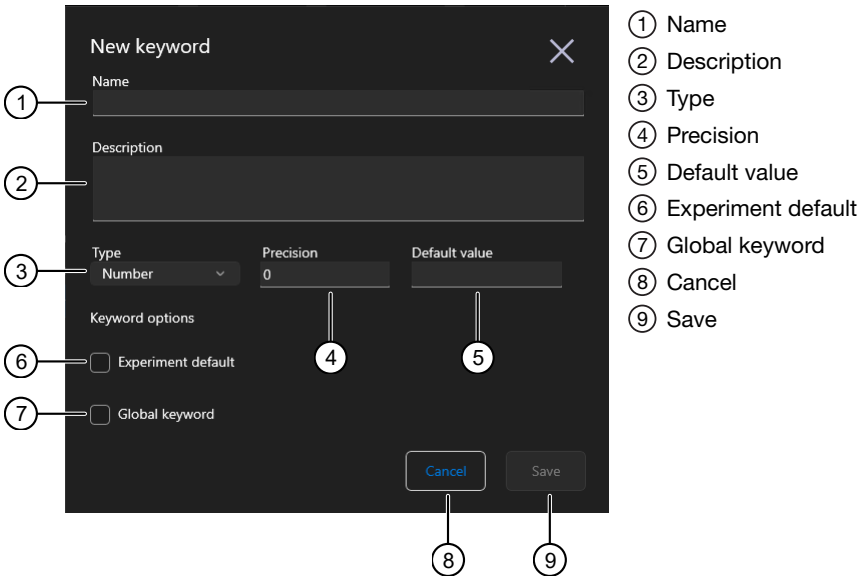
New keyword

New keyword dialog enables you to create custom keywords.



1. Name
2. Description
3. Type
4. Precision
5. Default value
6. Experiment default
7. Global keyword
8. Cancel
9. Save

Figure 163 New keyword dialog

- **Name** text field is used to assign a name to the new keyword, which is then displayed in the **Keyword name** column in the **Keywords table**.
- **Description** text field is used to describe to what the new keyword refers. For example, if the new keyword name is "Ab_1_Lot_No", the **Description** field might have "Lot number of Antibody #1" as an explanation for the new keyword.
- **Type** describes the keyword type. Available options are:
  - **Number**: The new keyword consists of numbers only. For example, 123456.
  - **String**: The new keyword consists of alphanumeric characters. For example, AB_123.
- **Precision**: Sets the number of decimal places for the **Number** type keyword. **Precision** option is not available for **String** type keywords.
- **Default value**: Sets the default value of the new keyword.
- **Keyword options**: Enables you to set the new keyword as an **Experiment default**, **Global keyword**, or both.

Note: Only users with **Administrator** accounts can create, edit, or delete a **Global keyword**.

13 Maintenance

Note: This chapter provides an overview of the maintenance workflows available on the Flowscape™ Software, which are necessary to keep the Attune™ Xenith™ Flow Cytometer in good working order. For detailed instructions about how to perform the routine maintenance procedures, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Flow cytometer maintenance

The Attune™ Xenith™ Flow Cytometer is designed to require minimum maintenance. However, for continued reliability of the cytometer perform basic preventive maintenance procedures regularly as listed in “Maintenance schedule” on page 231.

CAUTION! BIOHAZARD. All biological samples and materials that have contact with them have the potential to transmit infectious diseases and are considered biohazardous. Follow all applicable local, state, provincial, and national regulations. Wear appropriate protective eyewear, clothing, and gloves. Never pipette by mouth.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

Note that where ultra or concentrated bleach is used further dilution can be necessary to obtain to this final concentration.

Maintenance schedule

Preventive maintenance should be performed by Thermo Fisher personnel every 12 months. Regular user maintenance is not required other than regular cleaning. However, if the system will be unused for more than 14 days, perform the long-term shutdown procedure to keep the instrument in good operating condition.

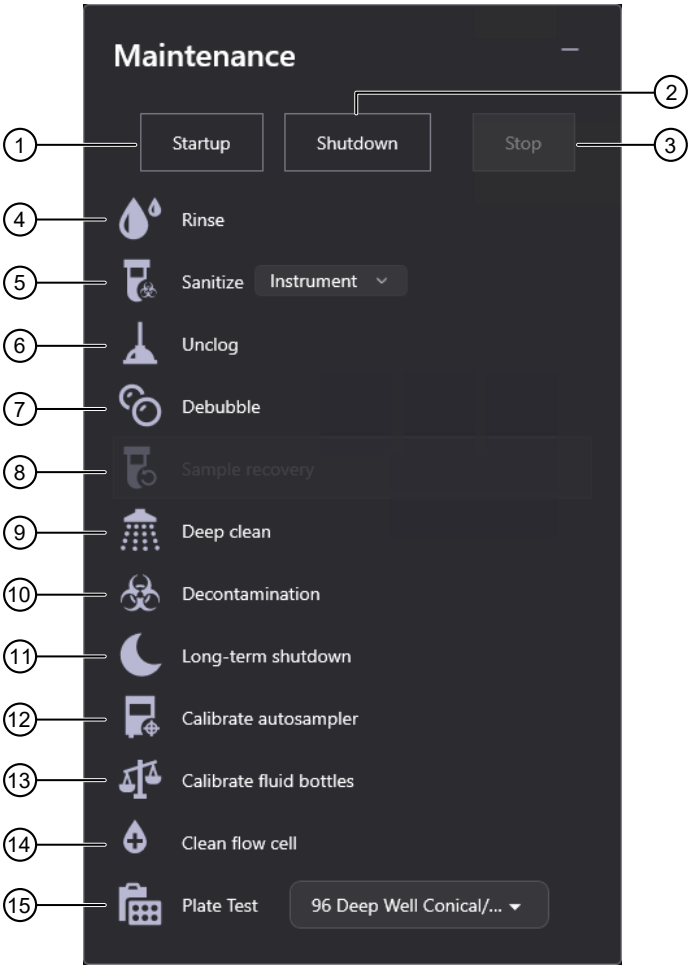
The following table lists the routine maintenance procedures that keep the flow cytometer and all its peripheral systems in good working condition.

Procedure	Frequency
Shutdown	Daily
Visual inspection of sample injection port (SIP), cove area, cove shield, fluid bottles and connections, and syringe pumps	Daily
Syringe replacement	Every 3 months (>6 hours/day), every 6 months (standard use), or if Error 1009 is repeatedly encountered
Rinse	As needed
Sanitize	Daily or between users, as needed
Unclog	As needed
Debubble	As needed
Deep clean	Weekly
Decontamination	Every 3 months (more frequently if running sticky sample types)
Long-term shutdown	When the system is not going to be used for >14 days
Flow Cell Clean	Weekly or as needed

Note: High volume use is defined as daily operation of the instrument for >6 hours/day.

Maintenance menu

In the Flowscape™ Software, the maintenance tools are grouped under the **Status bar** ▸ **Maintenance** tab, which opens the **Maintenance menu**.



1. Startup (page 233)
2. Shutdown (page 234)
3. Stop (page 235)
4. Rinse (page 235)
5. Sanitize (page 236)
6. Unclog (page 237)
7. Debubble (page 238)
8. Sample recovery (page 238)
9. Deep clean (page 239)
10. Decontamination (page 240)
11. Long-term shutdown (page 242)
12. Calibrate autosampler (page 243)
13. Calibrate fluid bottles (page 244)
14. Clean flow cell (page 245)
15. Plate Test (page 245)

Figure 164 Maintenance menu

- **Startup** and **Shutdown** tasks can be performed without signing in and are also available on the instrument touchscreen.
- **Maintenance procedures** can require users to perform specific tasks or that the hardware is in a specific state (for example, tube lifter up).
- The system shows instructions for **Maintenance procedures** that require users to perform tasks that cannot be automated or detected by the system. You can view the maintenance instructions in the Flowscape™ Software or the instrument touchscreen.
- To run the **Maintenance procedures** that require user interaction, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark as complete** checkbox to globally marked steps as complete.
- Many software **Maintenance menu** options require that the system is in an **initialized** state and do not function when the system is shutdown or powered off.

- When performing maintenance, the software shows notifications if the hardware is not in the correct state to proceed, and provides instructions with an illustration as to what is expected to continue.
- Specific plates can be asigned for use when performing instrument maintenance, including in the CytKick™ / CytKick™ Max Autosampler (if connected).

IMPORTANT! Stopping **Maintenance procedures** that involve bleach use requires the **Startup** to be run to remove the bleach from the system.

Startup

During **Startup**, the flow cytometer initializes the pumps, primes the instrument fluidics, and notifies you of the **System Status** (Ready, Attention, Clog, and so on).

Note: You can run the **Startup** procedure from the Attune™ Xenith™ instrument touchscreen without signing in to the software or after signing in from the Flowscape™ Software or the instrument touchscreen.

IMPORTANT! Before running the **Startup** procedure, ensure that you have filled the fluids tanks and emptied the waste container.

- To run the **Startup** procedure from the software, open the **Maintenance menu**, then click **Startup**.

  The system primes the fluids and checks for leaks. You are prompted with on-screen instructions if further action is needed.
- To start the instrument from the touchscreen, touch **Startup**. You are prompted with on-screen instructions if further action is needed.
- The entire **Startup** procedure takes about 6.5 minutes.

Note: For more information about how to run the **Startup** procedure, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Shutdown

The **Shutdown** procedure ensures that all sample fluid and dyes have been removed from the fluidics lines of the Attune™ Xenith™ Cytometer and the CytKick™ / CytKick™ Max Autosampler (if connected), and the pumps have been decontaminated and filled with Attune™ Shutdown Solution to prevent the formation of salt crystals. This leaves the system sanitized, protects the fluidic components, and ready for fluidics prime to be run by a subsequent **Startup**. Run the **Shutdown** procedure after the last run of the day.

During **Shutdown**, the instrument runs a dilute bleach solution through unclog, backflush, and sample/rinse lines (bleach scrub), rinses all lines with water, runs Attune™ Wash Solution through all lines and the sample pathway (wash scrub), then washes all lines again with water before running Attune™ Shutdown Solution through all lines and the SIP. However, most steps are automated and do not require monitoring. At the end of the **Shutdown** procedure, the system goes into a "sleep state".

Note: You can run the **Shutdown** procedure from the Attune™ Xenith™ instrument touchscreen without signing in to the software or after signing in from the Flowscape™ Software or the instrument touchscreen.

IMPORTANT! Perform the **Shutdown** procedure every day, even if the instrument is intended for continuous use. Proper instrument cleaning helps to obtain consistent and accurate operation.

CAUTION! Cytometer hardware can be contaminated by biohazardous material. We recommend fresh 10% bleach solution in deionized water to decontaminate the cytometer. The Attune™ Xenith™ Flow Cytometer dilutes the 100% bleach supplied on the fluid cart to the correct concentration for decontamination.

100% bleach is defined as 5.25% sodium hypochlorite in water. We recommend using laboratory-grade bleach. Avoid bleach with additives such as perfumes.

IMPORTANT! Before running the **Shutdown** procedure, ensure that the Wash and Shutdown solution tanks are at least half-full and the waste container has at least 10% capacity.

- To run the **Shutdown** procedure from the software, open the **Maintenance menu**, then click **Shutdown**.

- To run the **Shutdown** procedure from the instrument touchscreen, touch **Shutdown**. You are prompted with on-screen instructions if further action is needed.
- The **Shutdown** procedure takes about 1 hour to complete.

Note: For more information about how to run the **Shutdown** procedure, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Stop

The **Stop** button in the **Maintenance menu** stops the maintenance procedures listed below.

- **Startup**
- **Shutdown**
- **Sanitize**
- **Deep clean**
- **Decontamination**
- **Long-term shutdown**
- **Clean flow cell**
- **Plate Test**

IMPORTANT! Stopping **Maintenance procedures** that involve bleach use requires the **Startup** procedure to be run to remove the bleach from the system.

The **Stop** button is not active during the maintenance procedures listed below.

- **Rinse**
- **Unclog**
- **Debubble**
- **Calibrate autosampler**
- **Calibrate fluid bottles**

Rinse

Rinse enables you to rinse the sample lines.

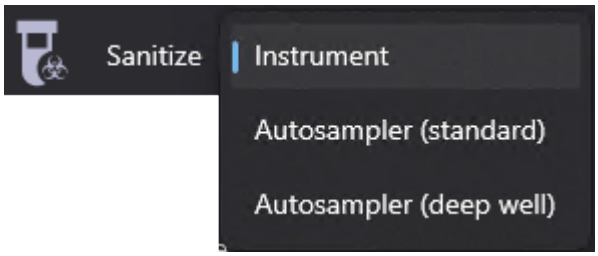
The **Rinse** procedure with or without the CytKick™ / CytKick™ Max Autosampler is intended to remove a majority of residual particles from fluidics components that have contacted sample during acquisition, to leave the cytometer in a fluidics state ready for sample acquisition, and to meet requirements for sample integrity and carryover.

The **Rinse** procedure takes <8 seconds to complete.

The **Stop** button is inactive during the **Rinse** procedure.

Sanitize

Sanitize sanitizes the instrument SIP (sample injection port) and sample lines or the autosampler SIP and sample lines. It is intended to reduce or eliminate microbes, residual dye, and particle carryover from previous acquisitions.



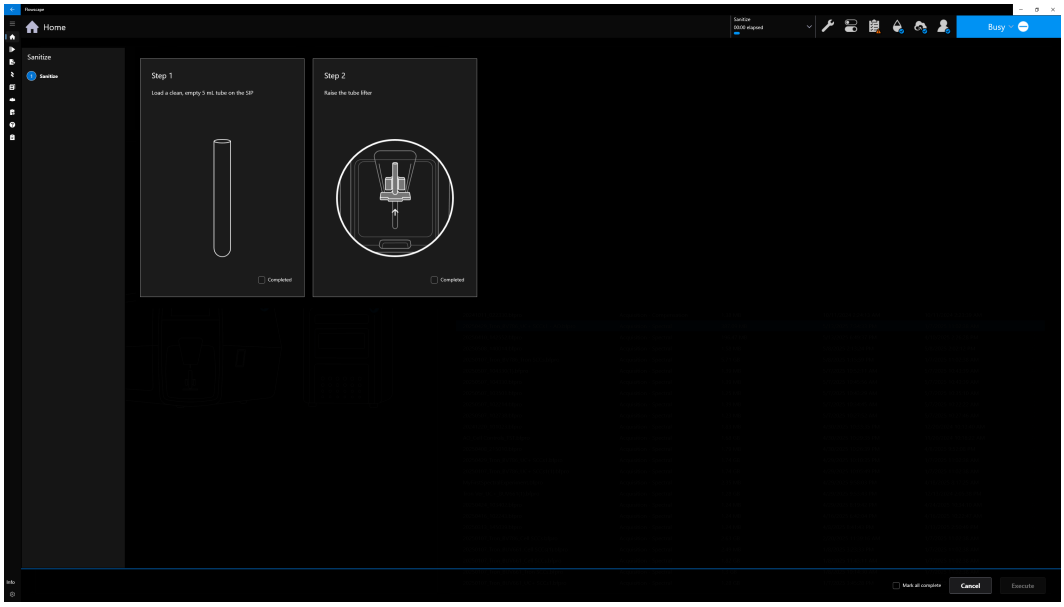
The **Sanitize menu** enables you to select the sanitize procedure to run. Available options are:

- **Instrument**
- **Autosampler (standard)**
- **Autosampler (deep well)**

The **Sanitize** procedure is typically run between experiments, or between samples, to minimize cross-contamination of sample types.

IMPORTANT! It is especially important to perform the **Sanitize** procedure between experiments when running sticky samples (such as DCs, RBCs, activated T cells, many cancer lines, bacteria, and yeast), DNA stains, or beads.

The **Sanitize screen** provides instructions to perform the steps in the sanitize procedure that cannot be automated.



To run the **Sanitize** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark as complete** checkbox to globally marked steps as complete.

After all steps are marked as **Completed**, click **Execute** to run the **Sanitize** procedure.

The **Sanitize** procedure takes about 3.5 minutes to complete.

Unclog

Unclog enables you to perform a back flush operation to remove residual particles that are partially or completely obstructing the system sample fluidics pathway.

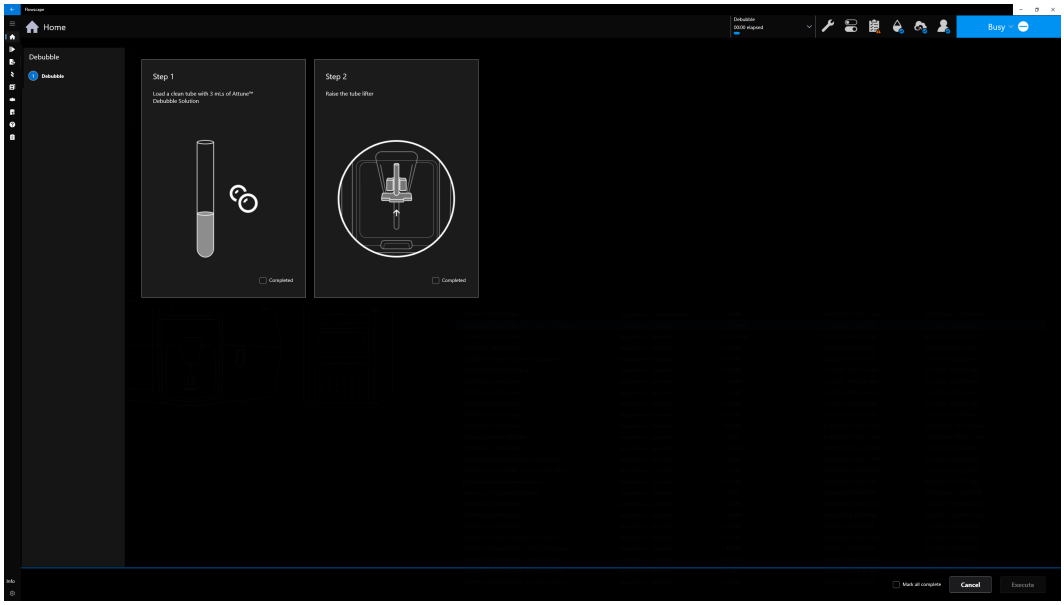
The **Unclog** procedure takes <2 minutes to complete.

The **Stop** button is inactive during the **Unclog** procedure.

Debubble

Debubble removes from the flow cell or interrogation assembly any bubbles large enough to disrupt the sample stream.

The **Debubble screen** provides instructions to perform the steps in the Debubble workflow that cannot be automated.



To run the **Debubble** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark as complete** checkbox to globally marked steps as complete.

After all steps are marked as **Completed**, click **Execute** to run the **Debubble** procedure.

The **Debubble** procedure takes <2 minutes to complete.

The **Stop** button is inactive during the **Debubble** procedure.

Sample recovery

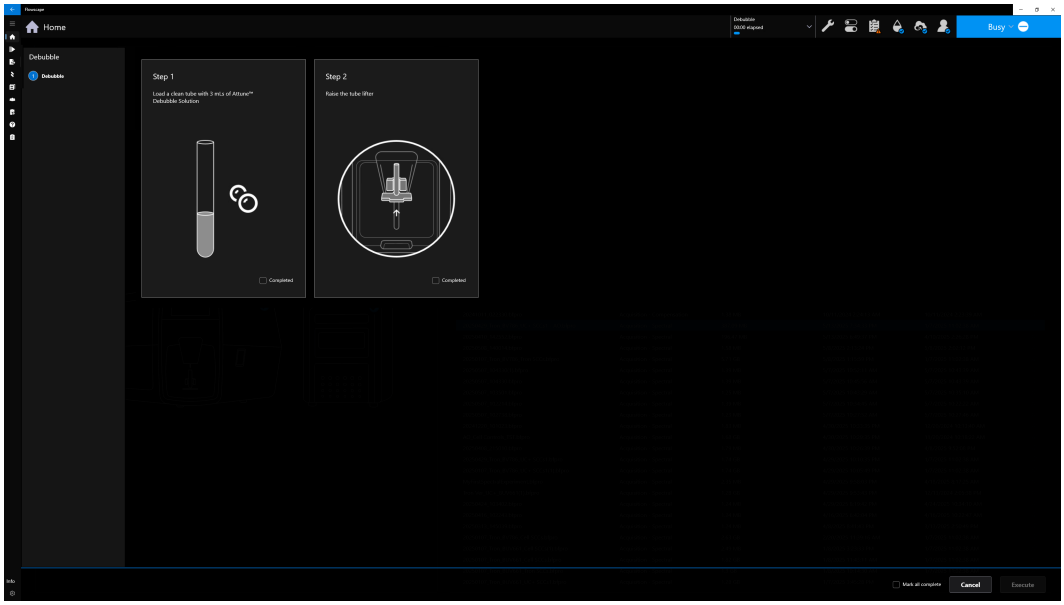
Sample recovery recovers the unused sample from the sample loop. When the button is clicked, the remaining sample is returned into a tube (from the sample loop) or back into the sample well (preloaded sample in a plate).

Sample recovery button is active when the instrument status is idle (that is, it is not currently acquiring) and there is sufficient sample in the sample loop or sufficient preloaded sample to recover.

Deep clean

Deep clean (also referred to as “Wash”) cleans, sanitizes, and rinses fluidic lines (including outside of SIP) and the sample cuvette. It removes residual dyes, proteins, and biological materials from system fluid lines, and leaves the instrument in a ready-to-use state when complete. The procedure does not sanitize the fluid bottles.

The **Deep clean screen** provides instructions to perform the steps in the procedure that cannot be automated.



To run the **Deep clean** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark as complete** checkbox to globally marked steps as complete.

After all steps are marked as **Completed**, click **Execute** to run the **Deep clean** procedure.

The **Deep clean** procedure takes about 1 hour to complete.

Decontamination

Decontamination sanitizes the system, fluid lines, and fluid bottles with bleach and Attune™ Wash Solution. The procedure ensures full system cleanliness at regular maintenance intervals to prevent build-up of contaminants in the system or fluid bottles.

If a CytKick™ / CytKick™ Max Autosampler is connected to the system and powered on, **Decontamination** starts a decontamination cycle for the cytometer and the autosampler.

CAUTION! BIOHAZARD. Cytometer hardware can be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure recommended for cytometer decontamination.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

The **Decontamination** procedure with or without the CytKick™ / CytKick™ Max Autosampler is intended to treat fluid lines exposed to samples, including external surfaces that are part of fluidics lines (for example, the tip of the SIP, tip of the CytKick™ probe), with sufficient exposure to decontamination fluid to minimize microbial growth and destroy residual dyes, to remove debris dislodged by the fluid cycles, and to leave the system safe for removal from a BSL-2 environment.

IMPORTANT! Decontamination of surfaces not intentionally exposed to samples is the responsibility of the user. The **Decontamination** procedure only covers internal components that are intentionally exposed to samples and system fluids.

Perform **Decontamination**:

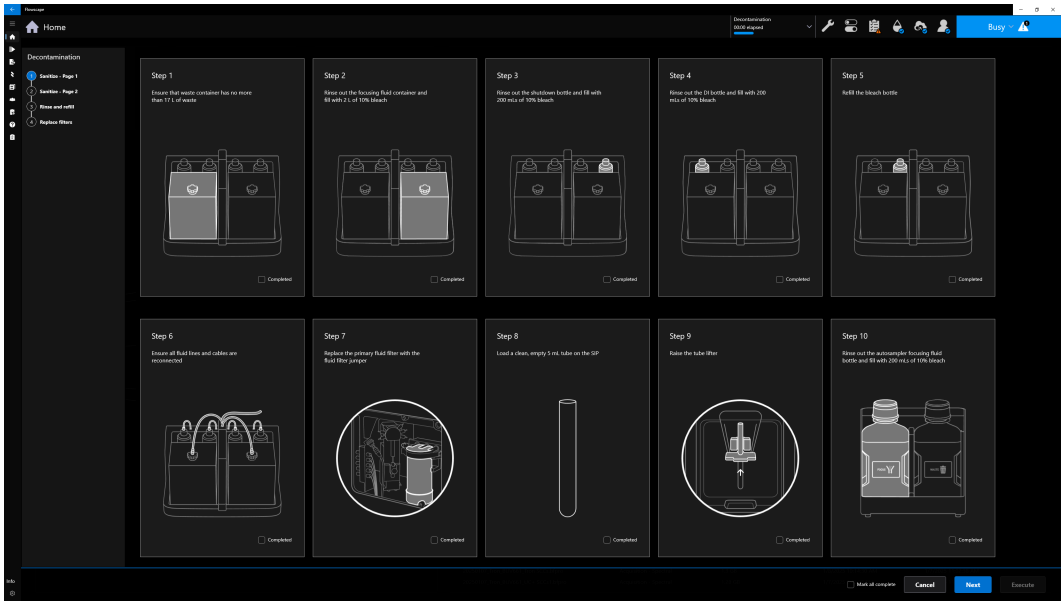
- At least every 3 months, or monthly if instrument is heavily used, to prevent and reduce microbial growth in the instrument.
- If the instrument has been idle for more than two months.
- If the instrument has been idle for more than two weeks without a decontamination run before becoming idle.

Note: If the system is likely to be idle for more than two weeks, perform the **Long-term shutdown** procedure (page 242).

The steps in the **Decontamination** procedure vary depending on whether an autosampler is connected.

The **Decontamination** procedure for the flow cytometer is broken into three phases: **Sanitize**, **Rinse and Refill**, and **Replace filters**, which are shown on the left navigation pane.

Decontamination screen provides instructions to perform the decontamination steps that cannot be automated.



To run the **Decontamination** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark as complete** checkbox to globally marked steps as complete.

After all steps are marked as **Completed**, click **Execute** to run the **Decontamination** procedure.

The **Decontamination** procedure takes about 2 hours to complete.

Note: For more information about how to run the **Decontamination** procedure, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Long-term shutdown

Long-term shutdown enables you to prepare the system for long-term storage and perform a shutdown procedure. The procedure is intended to leave the fluidics components and lines of the free of microbial growth, in a state that allows the system to subsequently be brought up, ready for use, without replacing parts (with possible exception of sheath filters) or unclogging fluidic lines.

CAUTION! BIOHAZARD. Cytometer hardware can be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure recommended for cytometer decontamination.

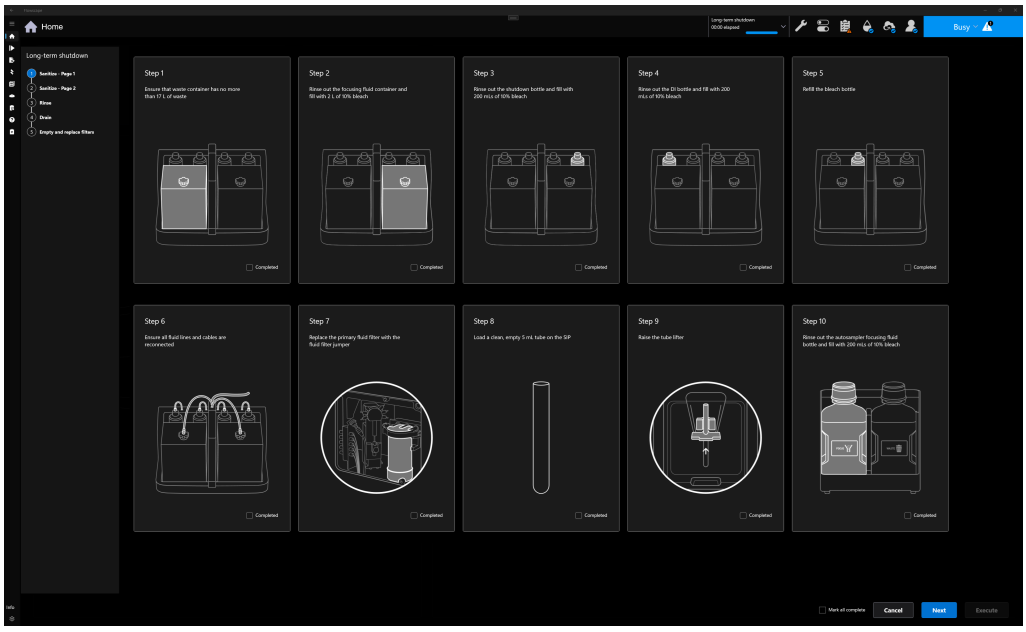
IMPORTANT! A **10% bleach** is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

Perform **Long-term shutdown** if the system is not going to be used for >14 days.

The steps in the **Long-term shutdown** procedure vary depending on whether an autosampler is connected.

The **Long-term shutdown** procedure for the flow cytometer is broken into four phases: **Sanitize**, **Rinse**, **Drain**, and **Empty and replace filters**, which are shown on the left navigation pane.

The **Long-term shutdown** screen provides instructions to perform the steps in the **Long-term shutdown** workflow that cannot be automated.



To run the **Long-term shutdown** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark as complete** checkbox to globally marked steps as complete.

After all steps are marked as **Completed**, click **Execute** to run the **Long-term shutdown** procedure.

The **Long-term shutdown** procedure takes about 4 hours to complete.

Note: For more information about how to run the **Long-term shutdown** procedure, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1001199).

Calibrate autosampler

Calibrate autosampler enables you to calibrate the CytKick™ / CytKick™ Max Autosampler line volume and motion.

Note: The autosampler is precalibrated before the unit is shipped and the instrument automatically recalibrates during **Startup** every 30 days. The **Calibrate autosampler** procedure is only needed for troubleshooting or if the autosampler was knocked out of calibration.

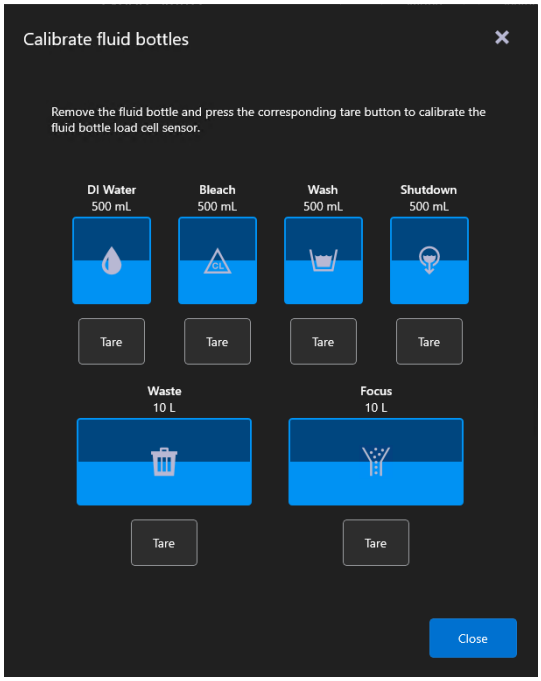
The **Calibrate autosampler** procedure takes about 1 minute to complete.

The **Stop** button is inactive during the **Rinse** procedure.

Calibrate fluid bottles

Calibrate fluid bottles is used to tare load sensors for the fluid cart bottles. The load sensors relay information about the fluid levels in each fluid container in the fluid cart, which are displayed in the **Status bar** ▸ **Fluidics** tab (page 45) and on the instrument touchscreen (page 13).

Calibrate fluid bottles opens the **Calibrate fluid bottles dialog**, which provides instructions and controls to calibrate fluid bottle load sensors.



To calibrate a fluid bottle load sensor, remove the fluid bottle from the fluid cart, then click the corresponding **Tare** button.

The fluid containers in the fluid cart are:

- DI water (deionized water, laboratory grade)
- Bleach (100% bleach, defined as 5.25% sodium hypochlorite in water)
- Wash (Attune™ Wash Solution)
- Shutdown (Attune™ Shutdown Solution)
- Waste (varies)
- Focus (Attune™ Focusing Fluid)

The **Stop** button is inactive during the **Calibrate fluid bottles** procedure.

Clean flow cell

Clean flow cell starts the flow cell cleaning procedure, during which the flow cell is soaked in Attune™ Flow Cell Cleaning Solution and periodically scrubbed to clean it.

For optimal cytometer performance, perform the **Clean flow cell** procedure every week or as needed.

Clean flow cell procedure takes about 45 minutes to complete.

Plate Test

Plate Test tests the current plate in the autosampler so that the autosampler probe position is in the correct location in all four corners of the selected plate and the probe consistently measures from the same spot in each well.

Plate Test menu enables you to select a plate type to test.

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