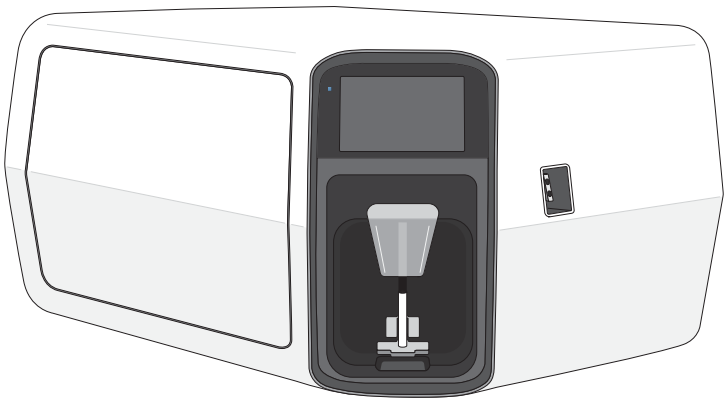
invitrogen

Attune™ Xenith™ Flow Cytometer

USER GUIDE

Publication Number MAN1001199

Revision A



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ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured at this site:

- Invitrogen™ Attune™ Xenith™ Flow Cytometer
- Invitrogen™ Attune™ Xenith™ Flow Cytometer Fluid Cart
- CytKick™ / CytKick™ Max Autosampler

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

Revision history: MAN1001199 A.0 (English)

Revision	Date	Description
A	5 August 2025	New document for Attune™ Xenith™ Flow Cytometer.

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

Contents

About this guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

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1 Product information

Product description

The Invitrogen™ Attune™ Xenith™ Flow Cytometer is an acoustic focusing spectral flow cytometer that uses multiple detectors to measure the full spectrum emission of every fluorophore across multiple lasers used in the system to create a more detailed signature for each fluorophore. Acoustic focusing technology enables samples to be run at flow rates from 12.5 µL to 1,000 µL per minute and up to 35,000 events per second. The optical configuration includes 6 lasers and 57 detectors (51 fluorescent and 6 scatter) that can detect an expansive array of fluorophores with high sensitivity. The resulting data are visualized in the software, which is capable of both spectral unmixing and conventional color compensation.

The standard system includes the following components:

- Attune™ Xenith™ Flow Cytometer
- Fluid Cart
- Power cord kit, universal voltage C13 2.5 m RC
- Cable, network
- 32-inch monitor
- Invitrogen™ Flowscape™ Software for instrument control, data collection, quality control, analysis and reports
- Dell™ computer (including mouse and keyboard)
- Attune™ Xenith™ Quality Control Beads, 3 mL
- Attune™ Wash Solution, 1 L
- Attune™ Focusing Fluid, 20 L
- Waste container, 20 L
- Attune™ Shutdown Solution, 1 L
- Attune™ Flow Cell Cleaning Solution, 30 mL

- Primary Focusing Fluid Filter
- Attune™ NxT Focusing Fluid Filter

IMPORTANT! The protection provided by the equipment can be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer (Thermo Fisher Scientific).

IMPORTANT! Observe current good laboratory practices when using this instrument.

System components

Part	Function
Instrument	
Optics bay	Houses the optical filters, mirrors, and detectors for each laser.
Sample tube lifter	Slides the sample tube into position on the sample injection port.
Safety shield	Provides a barier between the user and the sample during sample acquisition.
Touchscreen	Enables user interaction with the instrument.
Sample syringe	Primary pump used to move samples and fluids through the instrument.
Primary focusing fluid filter	First stage focusing fluid filter to prevent large particles from entering the system.
Secondary focusing fluid filter	Second stage focusing fluid filter to prevent large particles from entering the flow cell.
Wash pump syringe	Auxiliary pump to move fluids through the instrument for rinsing and cleaning.
Fluidics bay	Houses the instrument pumps and primary focus fluid filter.
Power switch	Enables the user to power on/off the instrument.
Fluid cart	
Waste container	The 20 L cube receives fluid waste from the instrument.
Focus fluid container	The 20 L cube provides premixed focus fluid to the instrument.
Deionized water bottle	The 1 L bottle contains deionized water for mixing with bleach for sanitizing, and for rinsing.
Bleach bottle	The 1 L bottle contains bleach solution for sanitizing.
Wash fluid bottle	The 1 L bottle contains wash solution for cleaning.
Shutdown fluid bottle	The 1 L bottle contains shutdown solution for cleaning and bubble reduction.

(continued)

Part	Function
Optional	
(Optional) CytKick™ / CytKick™ Max Autosampler connection	The optional CytKick™ / CytKick™ Max Autosampler enables rapid processing of multiple samples from 96- and 384-well plates (standard and deep), and some custom plate types and microcentrifuge tube racks.

Exterior components

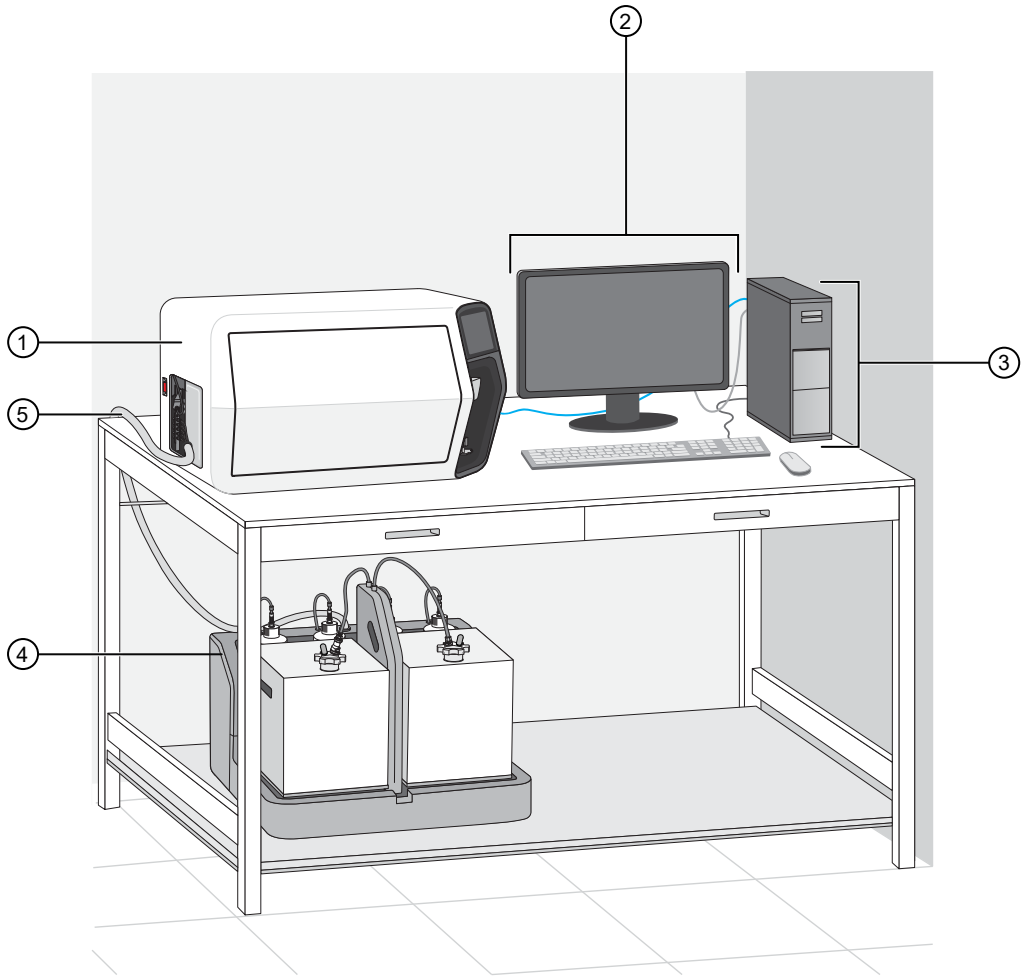


Figure 1 Typical system

1. Monitor
2. Computer
3. Fluid Cart
4. Umbilical from Fluid Cart to instrument
5. Instrument

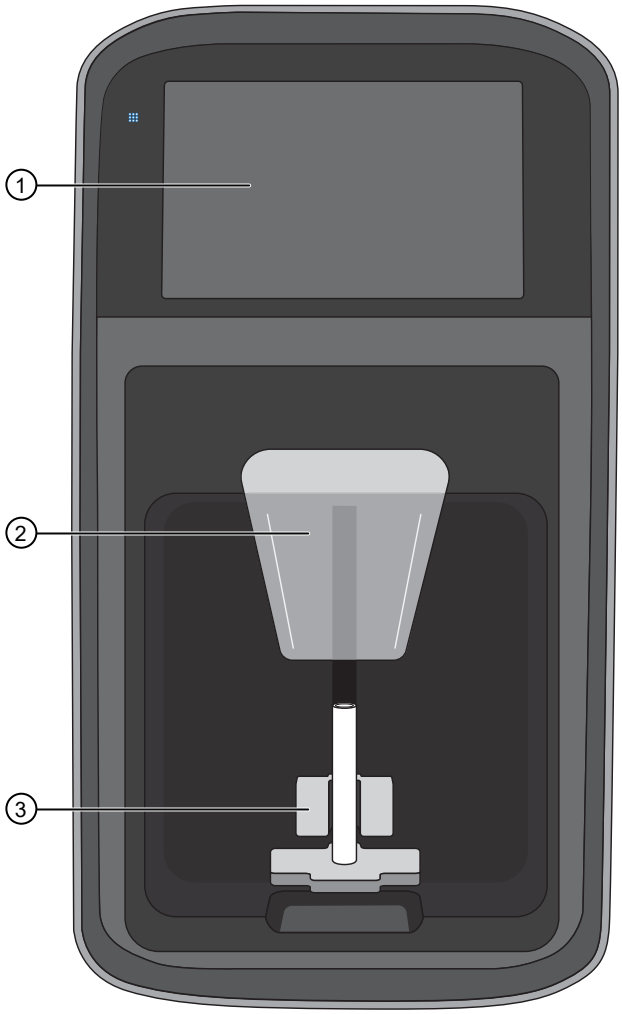


Figure 2 Sample injection port (SIP)

1. Touchscreen
2. Safety shield
3. Sample tube lifter

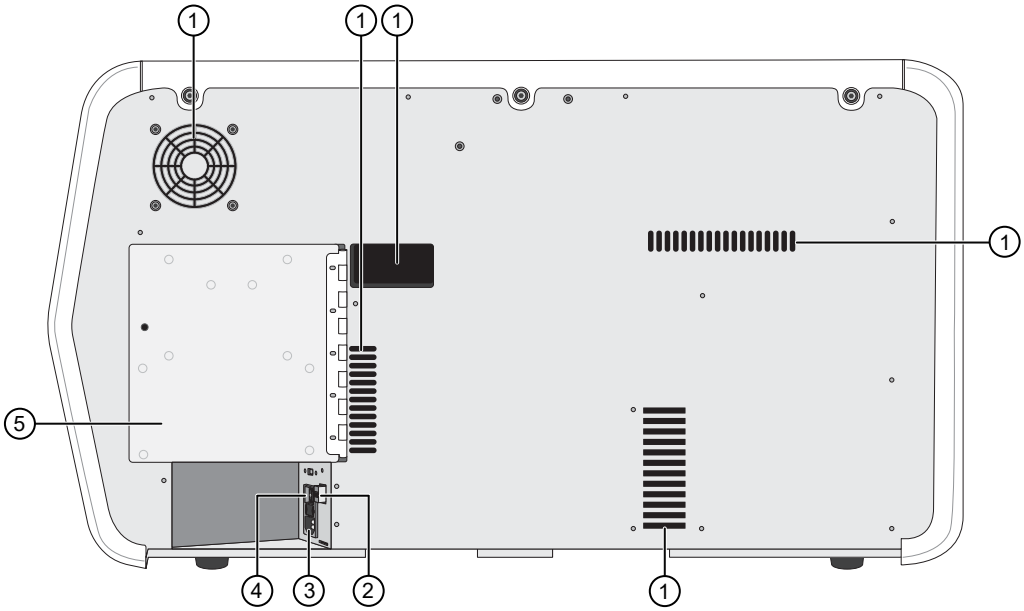


Figure 3 Instrument back panel

1. Air exhaust
2. Fiber ethernet connector
3. Power connector
4. Primary On/Off switch
5. Service panel

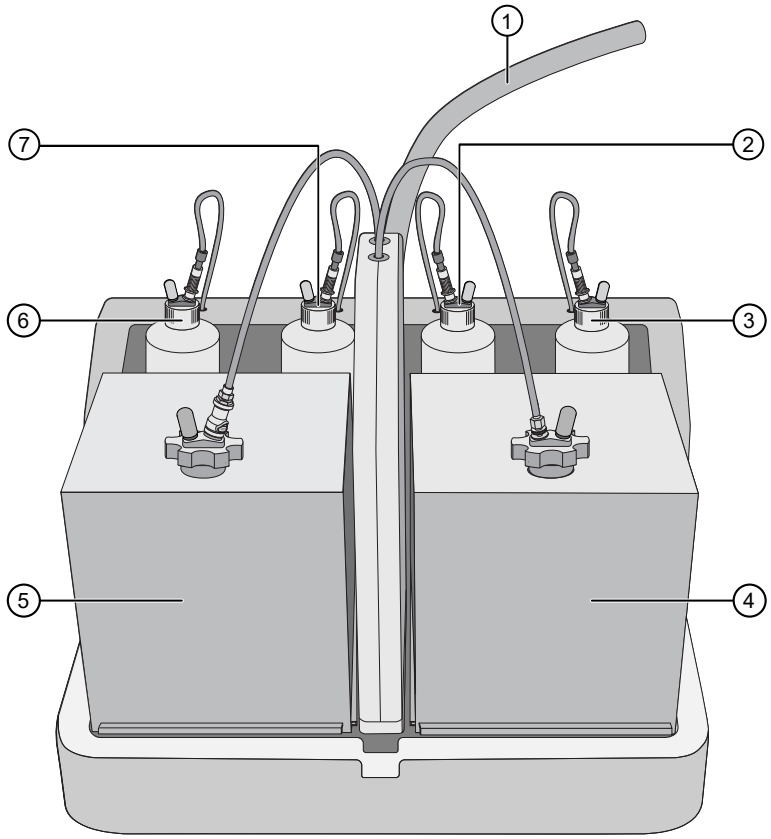


Figure 4 Fluid Cart (front)

1. Umbilical (contains fluids tubing, power, and communication cables)
2. Wash bottle
3. Shutdown fluid bottle
4. Focusing fluid container
5. Waste container
6. Deionized water bottle
7. Bleach bottle

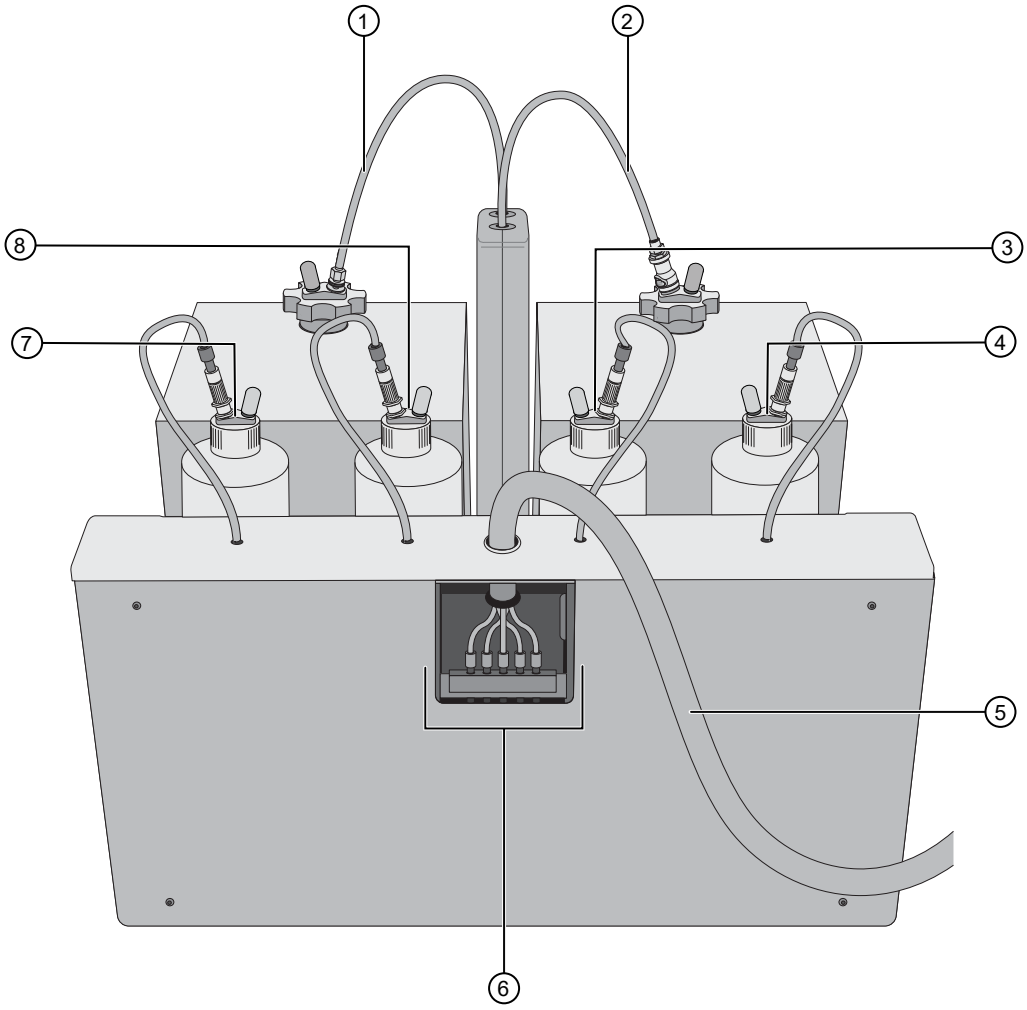


Figure 5 Fluid Cart (back)

1. Tubing from the focusing fluid container to the manifold and instrument
2. Tubing from the instrument and manifold to the waste container
3. Bleach bottle
4. Deionized water bottle
5. Umbilical (contains fluids tubing, power, and communication cables)
6. Umbilical attachment
7. Shutdown fluid bottle
8. Wash fluid bottle

Interior components

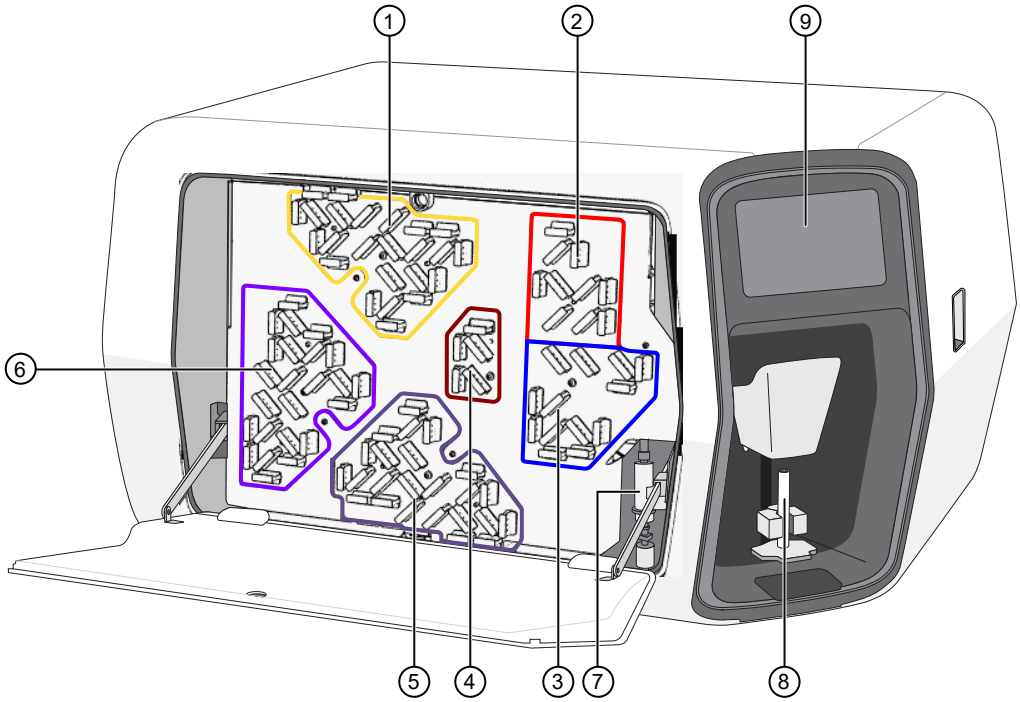


Figure 6 Attune™ Xenith™ Flow Cytometer Front view (optics door open)

1. Filters and mirrors for 561 nm laser (yellow)
2. Filters and mirrors for 637 nm laser (red)
3. Filters and mirrors for 488 nm laser (blue)
4. Filters and mirrors for 781 nm laser (infrared)
5. Filters and mirrors for 349 nm laser (ultra violet)
6. Filters and mirrors for 405 nm laser (violet)
7. Secondary focus fluid filter
8. Tube lifter and sample tube (exterior)
9. Touchscreen (exterior)

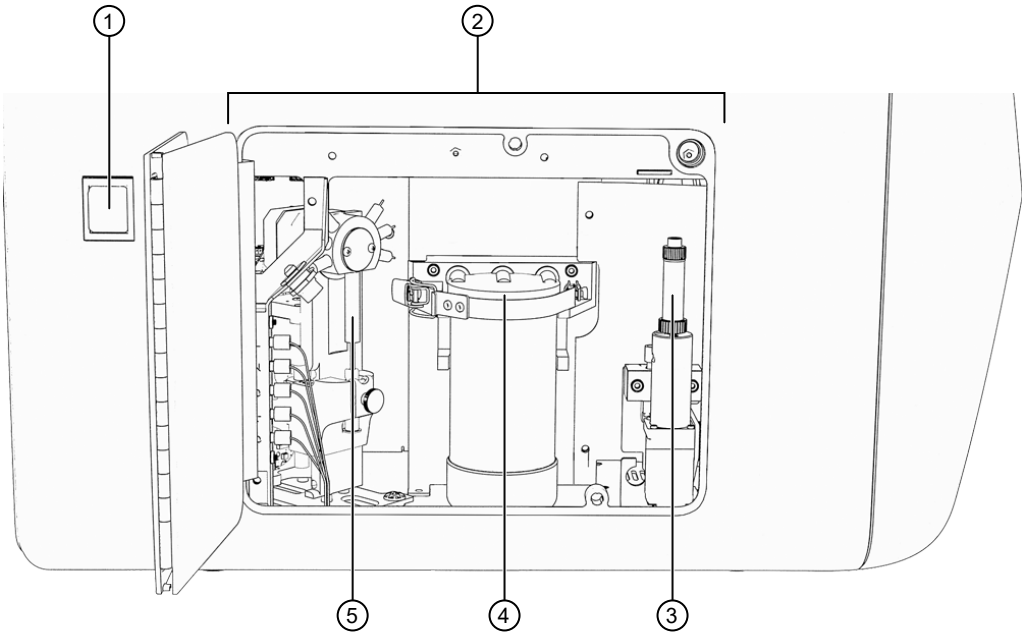


Figure 7 Attune™ Xenith™ Flow Cytometer (left side)

① User facing On/Off switch
② Fluidics bay
③ Wash pump syringe
④ Primary focus fluid filter
⑤ Sample syringe

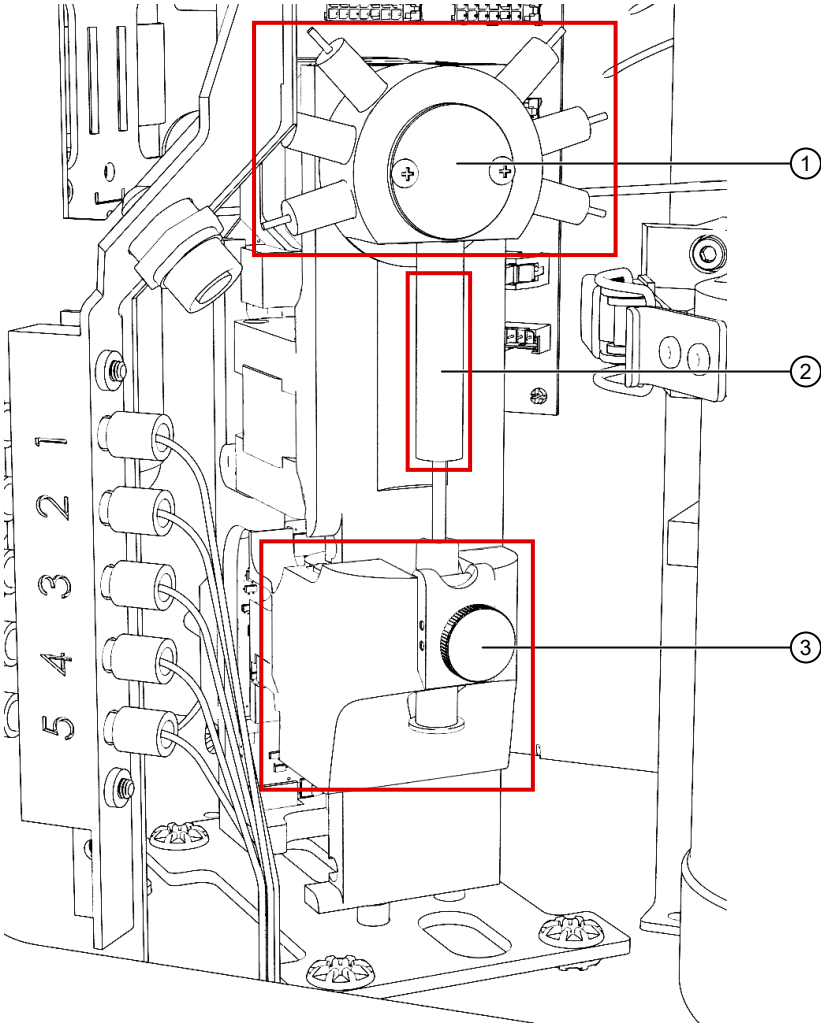


Figure 8 Syringe pump compartment

① Valve
② Syringe piston
③ Syringe capture mechanism

Power and communication connections

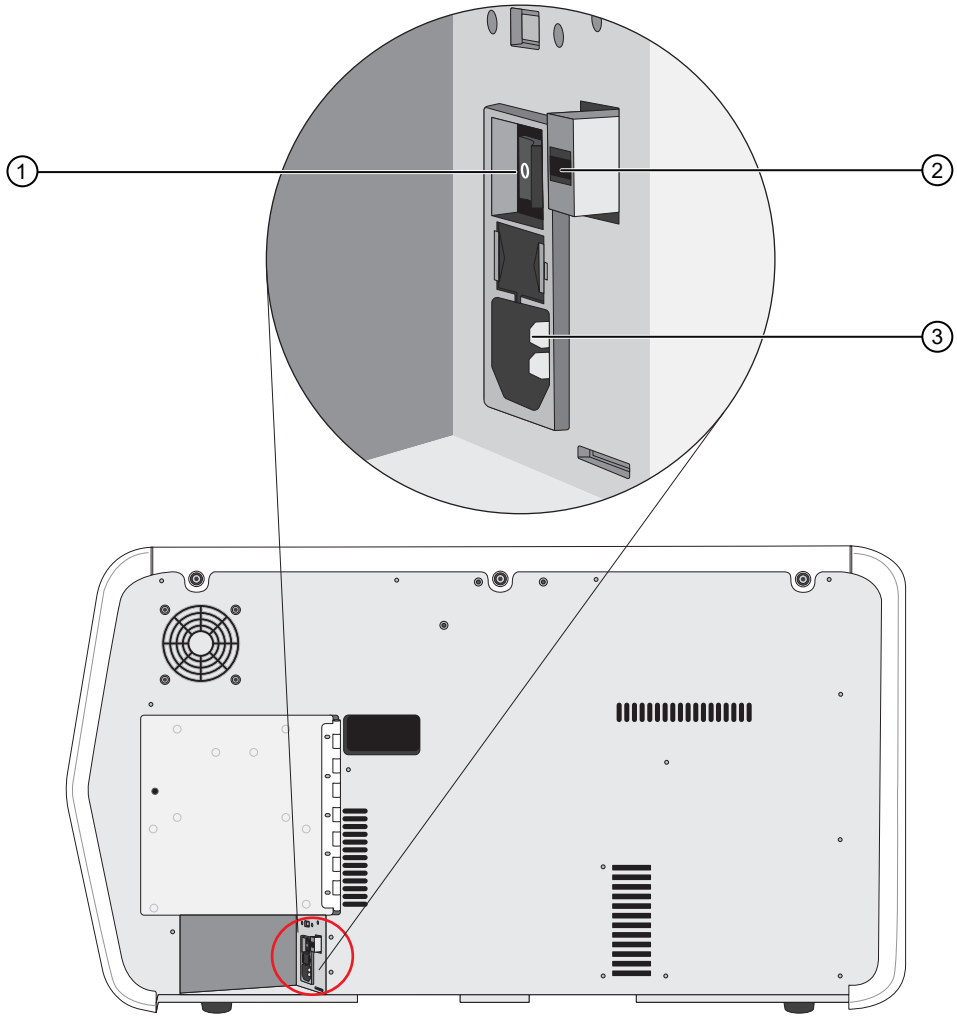


Figure 9 Instrument rear panel connections

① Primary On/Off switch
② Fiber ethernet connector
③ Power connector

Fluid tubing connections

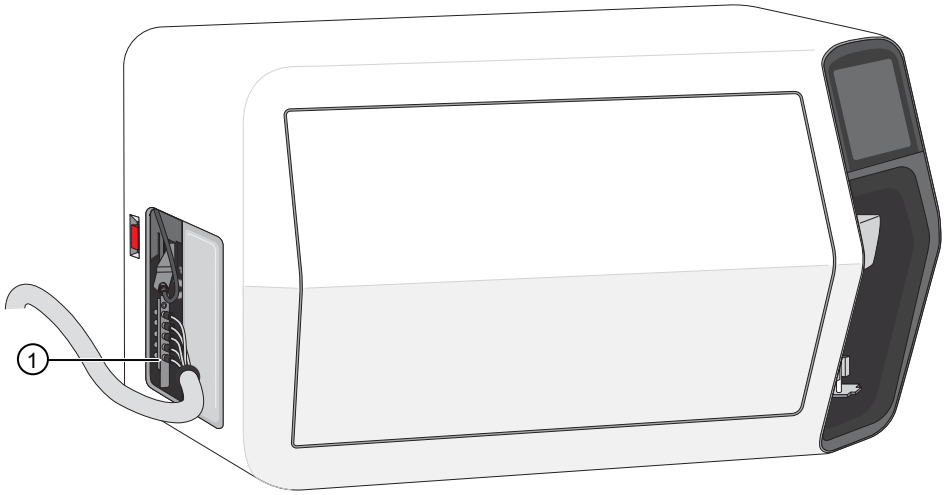


Figure 10 Fluid cart connections to the instrument

① Fluid tubing and electrical and communication cable connections

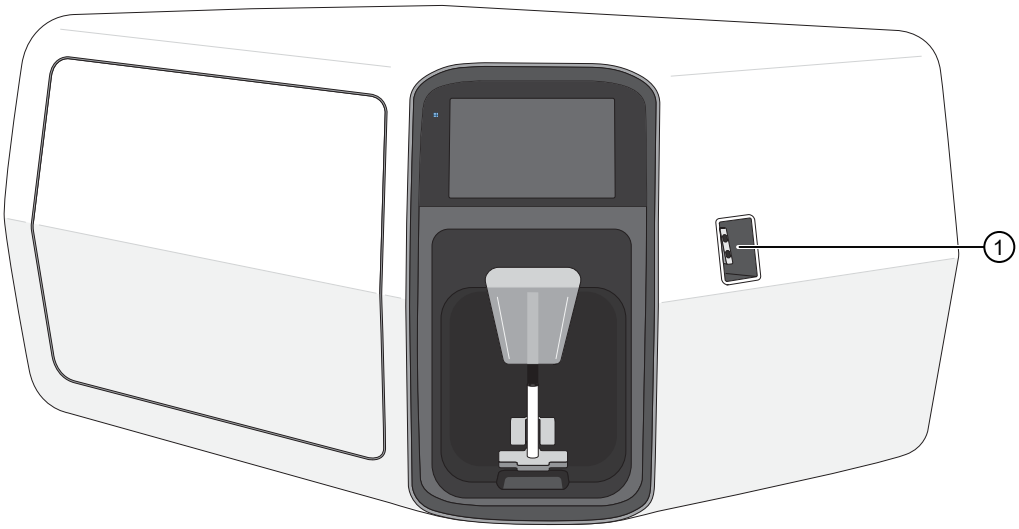


Figure 11 Attune™ Xenith™ Flow Cytometer and the CytKick™ / CytKick™ Max Autosampler connection ports

① Connection for the optional CytKick™ / CytKick™ Max Autosampler

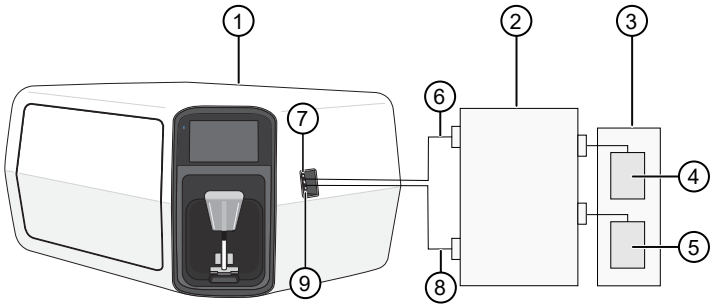


Figure 12 CytKick™ / CytKick™ Max Autosampler fluid line attachments

1. Attune™ Xenith™ Flow Cytometer
2. CytKick™ / CytKick™ Max Autosampler
3. CytKick™ fluids
4. CytKick™ waste receptacle
5. CytKick™ focus fluid receptacle
6. Output from the autosampler (sample)
7. Top port on instrument
8. Input to the autosampler (waste)
9. Bottom port on instrument

Note: The fluidic tubing that connects the Attune™ Xenith™ Flow Cytometer and the CytKick™ / CytKick™ Max Autosampler must be attached correctly. The top port on the Attune™ Xenith™ Flow Cytometer connects to the top port on the CytKick™ / CytKick™ Max Autosampler. The bottom port on the Attune™ Xenith™ Flow Cytometer connects to the bottom port on CytKick™ / CytKick™ Max Autosampler.

About the Flowscape™ Software

The Attune™ Xenith™ Flow Cytometer is controlled by the touchscreen and the Invitrogen™ Flowscape™ Software. The software is installed on the computer workstation that is supplied with the system and a shortcut icon is placed on the computer desktop.

The software is a flexible data acquisition and analysis tool that enables users to perform the following tasks.

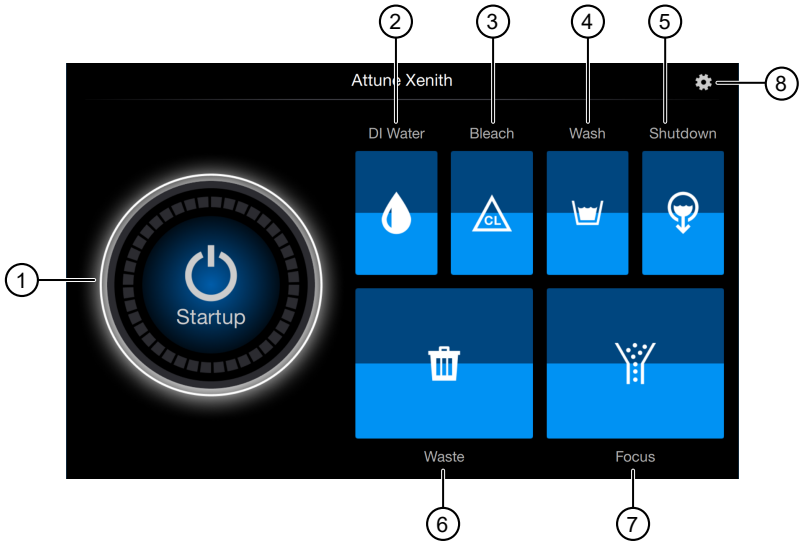
- Design and perform experiments
- Define independent instrument settings and optimize data collection
- Check and track instrument performance
- Acquire and record data
- Manage and process recorded data

Note: For more information about the Flowscape™ Software, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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.

The touchscreen can also be used to view and perform instrument maintenance functions after they are started from the **Maintenance menu** (“Maintenance menu” on page 80), while the **Startup** and **Shutdown** procedures can directly be started and run from the touchscreen.



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.

2 Workflow

Set up and run the instrument

Maintain fluids

Empty the waste container and fill the bulk fluid bottles on the Fluid Cart.

Run Startup

Start up the instrument and sign in to software.

Daily QC

Run the Daily QC test.

Prepare single-color controls and samples.

Prepare single-color controls and samples.

Set up an experiment

Set up a spectral unmixing experiment or a compensation experiment.

Run samples

Run samples one at a time using the tube lifter or in bulk using the autosampler.

Run run spectral controls or color compensation controls.

Acquire sample and set up regions and gates. Optimize experiment.

Analyze data

Analyze data

Run and record sample. View and analyze results.

Set up and run the instrument

Cleanup

Cleanup

Perform cleanup and maintenance between users.

When finished for the day, shut down the instrument to remove sample fluid and dyes from the system, sanitize the fluid lines and sample pumps, and fill them with shutdown solution.

3 Set up the instrument

Sign in (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.

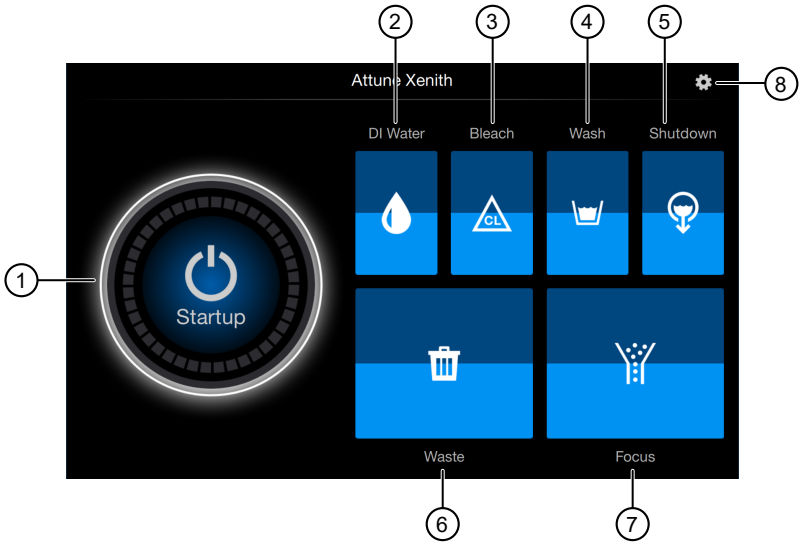
The EULA is displayed when:

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

1. Power on the instrument. See “Power on the instrument” on page 31.
2. View the EULA on the touchscreen.

   Note: You must 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**.



1. Startup
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

Edit touchscreen settings

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

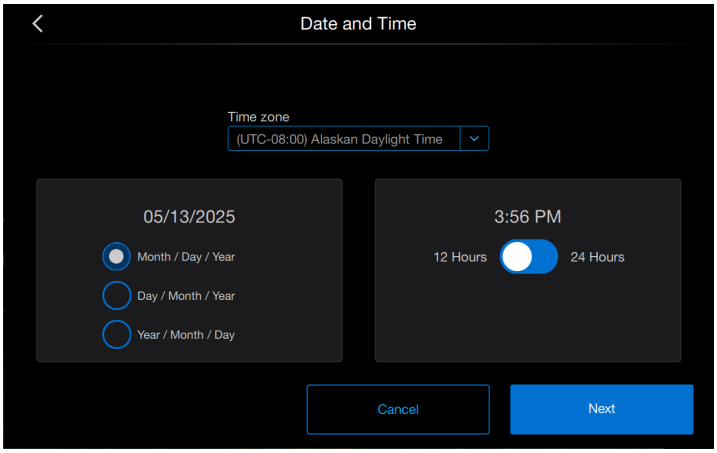


Figure 13 Format date and time

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

4 Instrument Operation

Before you begin

Review the Technical overview (page 103). Obtain the solutions that are needed for operation and allow them to come to ambient temperature.

IMPORTANT! The recommended storage temperature for reagents and solutions is room temperature (15–30°C), but they can also be stored at colder temperatures. However, running the instrument with cold reagents (<15°C) will affect the data quality. Before you run the instrument, ensure that all fluid temperatures are at least 15°C.

Required solutions

- Attune™ Focusing Fluid (1X) is a buffered, azide-free support/carrier reagent for transporting particles through the optical cell. It contains a preservative and detergent designed to minimize bubble formation.
- Attune™ Wash Solution is a ready-to-use solution for removing cellular debris and dyes from the fluidics system of the instrument.
- Attune™ Shutdown Solution is a ready-to-use solution added to the Shutdown container to prevent bubble formation in the fluidics system of the instrument.
- Attune™ Flow Cell Cleaning Solution is an alkaline liquid concentrate that, when mixed with water, removes bubbles and contaminants from the flow cell without damaging the quartz glass used in the optical system of the instrument.
- Bleach (5.25% Sodium Hypochlorite) with no additives is mixed with water on board the instrument to decontaminate the fluidics lines.

  IMPORTANT! Only use laboratory-grade bleach. Avoid bleach with additives (such as perfumes or surfactants).

- Proclin™-treated (at 15 ppm concentration) deionized water is used for diluting bleach and for long-term storage of the instrument. 1 mL of Proclin™ 150 added to 1 L of deionized water results in a 15 ppm concentration.

Maintain fluids

Weight sensors continuously monitor the fluid containers. Fluid levels can be observed on the instrument touchscreen or in the software. When the fluid level is low, or the waste container is full, the software displays a warning message and the amber LED on the affected fluid container blinks. On-screen instructions help you maintain the fluids and resume the run.

IMPORTANT! The waste container and the focusing fluid container can be changed during instrument operation. However, the fluids in the 1 L bottles can be replaced only when the instrument is idle.

1. Check all containers on the Fluid Cart before instrument Startup.

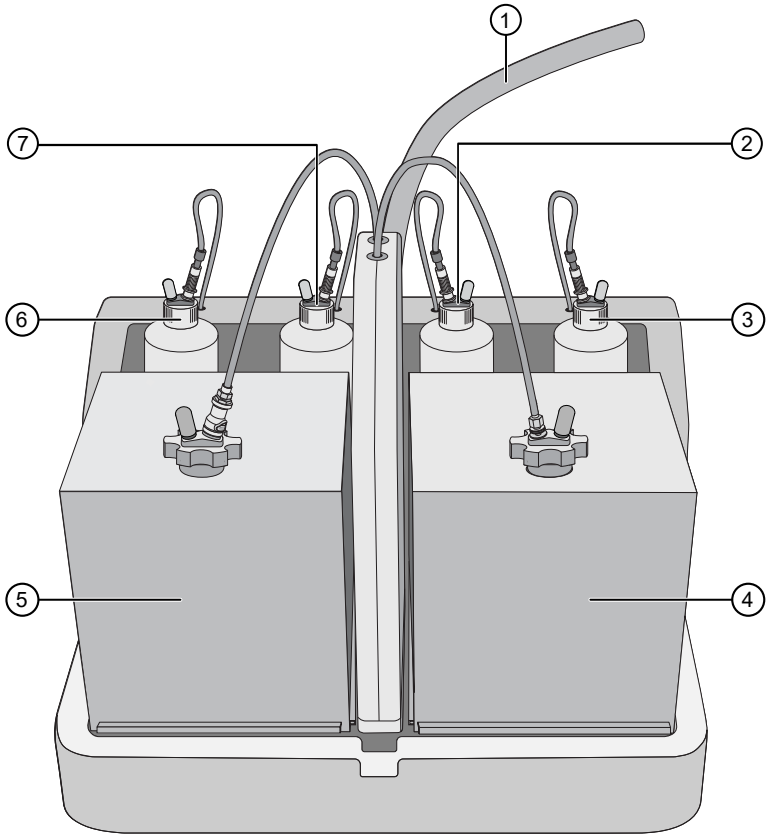


Figure 14 Fluid Cart, container locations

1. Bleach bottle, 1 L
2. Wash solution, 1 L
3. Shutdown solution, 1 L
4. Focusing fluid, 20 L
5. Waste container, 20 L
6. Deionized water, 1 L

2. Empty the waste container and fill the bulk fluids containers. For more information see “Remove and replace the waste container” on page 29, “Remove and replace the focusing fluid” on page 29 and “Remove and replace the 1 L bottles” on page 30.

Remove and replace the waste container

1. Unfasten the connector from the Fluid Cart to the waste container ⑤.
2. Remove the container from the cart.

   IMPORTANT! Empty the waste container in accordance with laboratory safety practices.

3. Put an empty container on the cart in the appropriate position ⑤

   IMPORTANT! We recommend using a waste container with a 20 L volume. A smaller container can lead to waste overflow.

4. Reconnect the tubing.

Remove and replace the focusing fluid

The Attune™ Focusing Fluid comes in a premixed 20 L container. Use each focusing fluid container until it is reported as empty by the instrument. You can save the small remaining volume in a separate storage container to top up another focusing fluid container.

IMPORTANT! DO NOT fill any focusing fluid container to more than the original volume delivered.

1. Unfasten the tubing from the Fluid Cart to the focusing fluid container ④.
2. Remove the container from the cart.
3. Put a full container of focusing fluid on the cart in the appropriate position ④.
4. Reconnect the tubing.

Remove and replace the 1 L bottles

IMPORTANT! Ensure the instrument is idle before removing the 1 L bottles from the Fluid Cart.

1. Press the release button on the connector to release the tubing from the bottle cap.

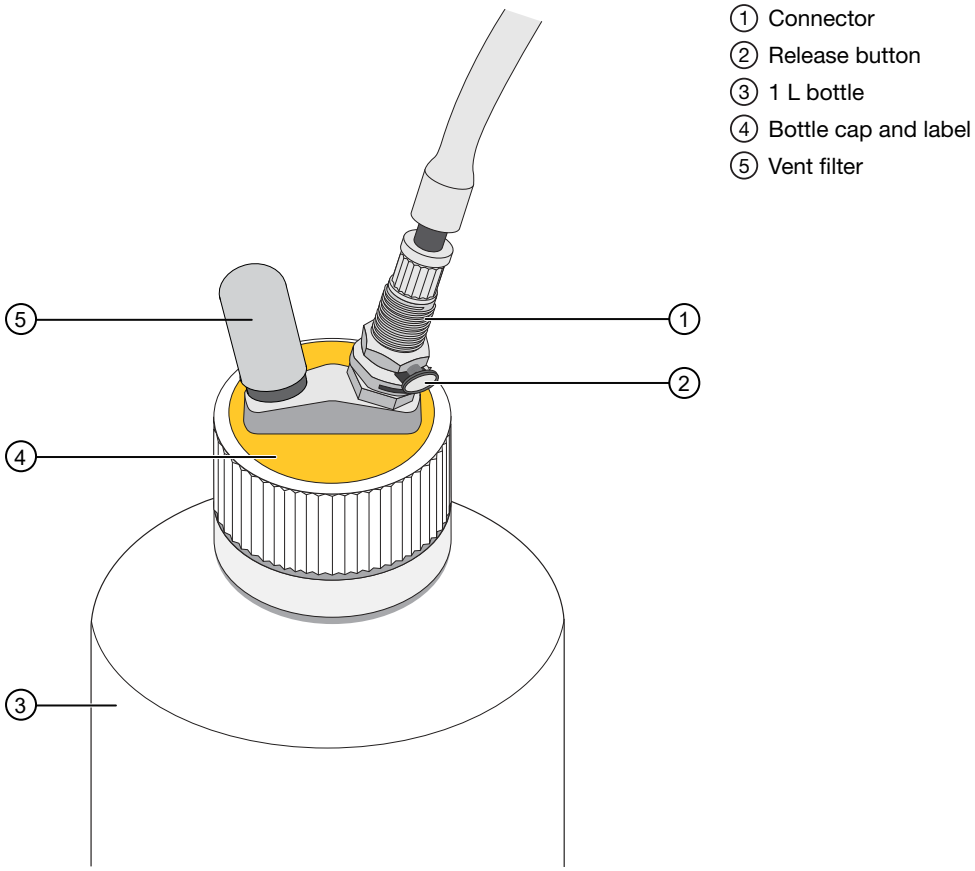


Figure 15 1 L bottle connector

2. Remove the bottle from the fluids cart.
3. Remove the bottle cap and fill the bottle with the solution shown on the bottle cap label.
4. Replace the bottle cap and tighten until it is snug.
5. Put the bottle in the appropriate position on the Fluid Cart. See Figure 14 on page 28.
6. Reconnect the appropriate fluid cart tubing to the bottle connector.

Power on the instrument

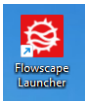
Note: You can power on the instrument and computer in any order.

1. Press the rocker switch on the back of the instrument to the ON position. This enables electric current into the internal power supply. See Figure 9 on page 19.
2. Press the rocker switch on the side of the instrument to the ON position to power the Attune™ Xenith™ Flow Cytometer. See Figure 7 on page 17.
3. Power on the computer and monitor.
4. Sign in to Windows. The default credentials are:
   User name: INSTR-ADMIN
   Password: INSTR-ADMIN

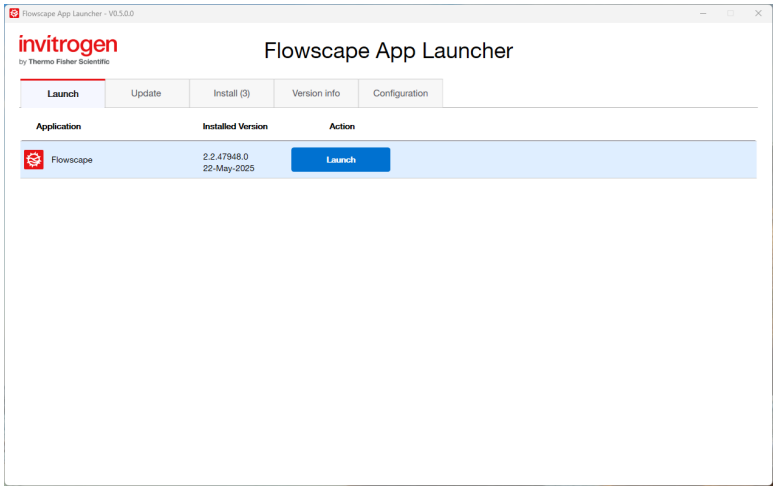
Sign in to the software

After you have powered on the instrument, the computer, and the monitor, and signed in to Windows, open the Flowscape™ Software and sign in as described below.

1. 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.



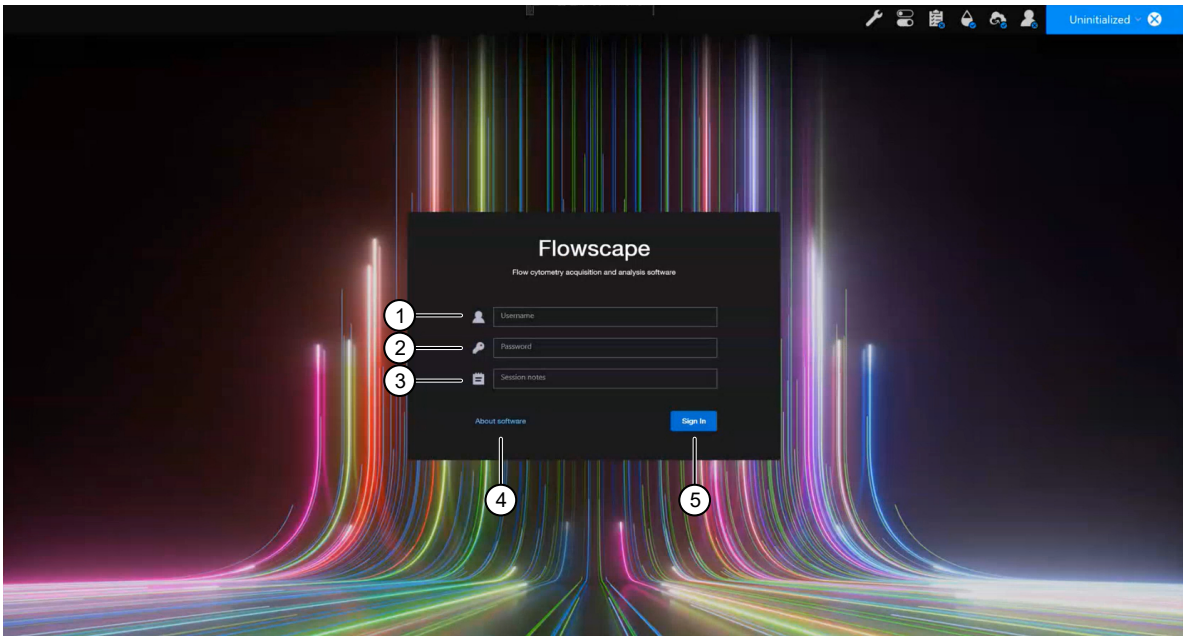
Note: The **Flowscape™ Launcher** has 5 tabs: **Launch**, **Update**, **Install**, **Version info**, and **Configuration**.

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**.

2. 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

3. 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 “Account types” on page 33.

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

5. *(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**.

6. 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 more information about how to create or delete user accounts, including additional **Administrator**-like accounts, see "User Management" in *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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" in *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

Note: Each account is required to reset their password after the first sign in. User passwords must be 12 or more characters long and cannot contain illegal characters. We recommend using "passphrases" instead of "passwords". For example, use "These13KittensAreRunningAmokInTheLaboratory" instead of "Kittens13".

Run Startup

Instrument **Startup** primes the system fluidics. The software guides you through the Startup function. Ensure that you follow all instructions provided by the software during the procedure.

Ensure that you have filled the fluids and emptied the waste. See “Maintain fluids” on page 28. The instrument must be powered on. See “Power on the instrument” on page 31.

Note: The instrument can be started up from the touchscreen or from the software.

1. To start the instrument from the touchscreen, touch **Startup**. The system primes the fluids and checks for leaks. You are prompted with on-screen instructions if further action is needed.

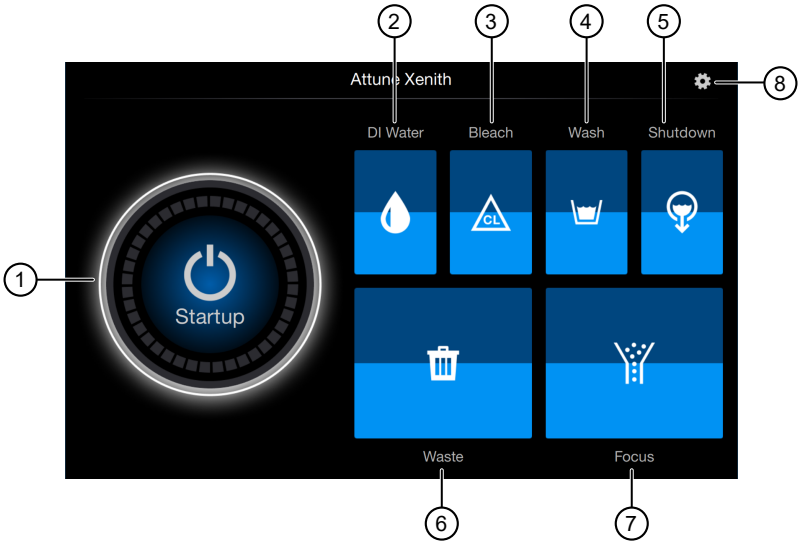


Figure 16 Touchscreen

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

2. To start the instrument from the software, open the **Maintenance** menu and select **Startup**. You are prompted with on-screen instructions if further action is needed.

5 Instrument quality control (QC)

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 38).
- **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 40).

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**.

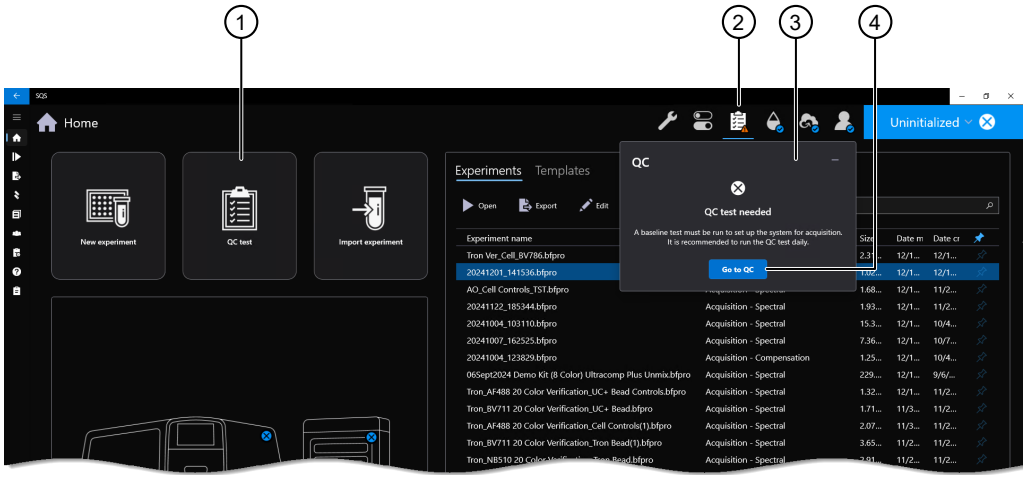


Figure 17 Open the QC Test screen from the Home screen.

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

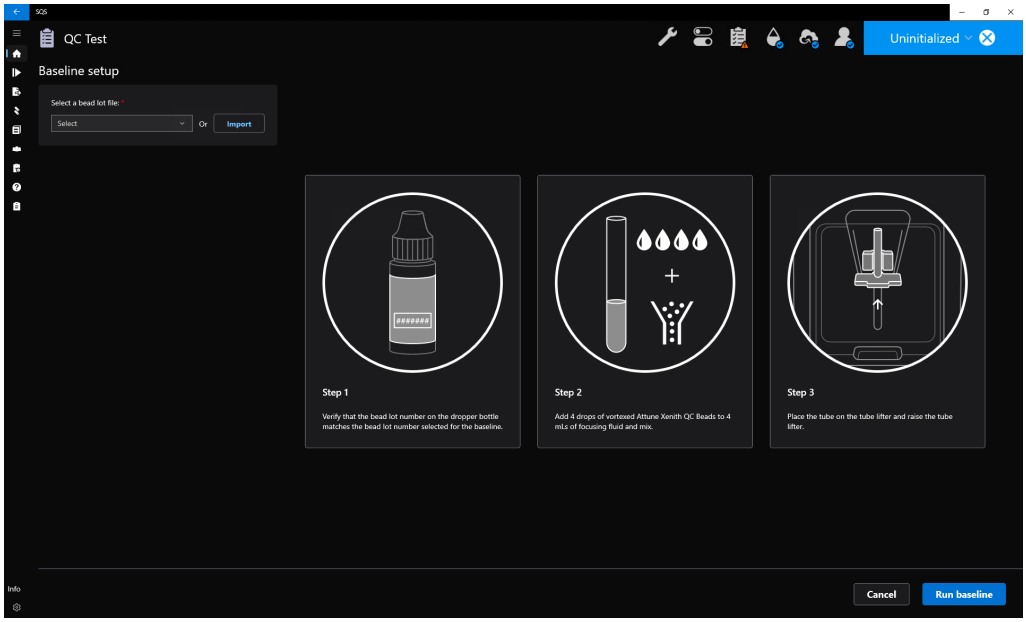


Figure 18 QC Test screen - Baseline setup

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.

Attune™ Xenith™ Quality Control Beads

The Attune™ Xenith™ Quality Control Beads (Cat. No. X20000) are used to determine instrument baseline performance and perform daily performance measurements. The beads are stained with a combination of fluorophores that, when excited by the lasers in the Attune™ Xenith™ Flow Cytometer, emit fluorescence signals at designed levels to all the channels in the cytometer.

Each vial of performance tracking beads contains a mixture of three beads of equal concentration that differ in relative fluorescence emission intensity (blank, dim, and bright). The nominal diameter of the blank beads in Attune™ Xenith™ Quality Control Beads is 2.4 µm. The nominal diameter of the dim and bright intensity beads is 3.7 µm.

IMPORTANT! Prepare the Attune™ Xenith™ Quality Control Beads suspension immediately before use. The beads are non-hazardous and can be disposed according to local regulations.

Baseline setup

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

Note: Before you can run the **Baseline** procedure, the instrument must successfully complete the **Startup** procedure.

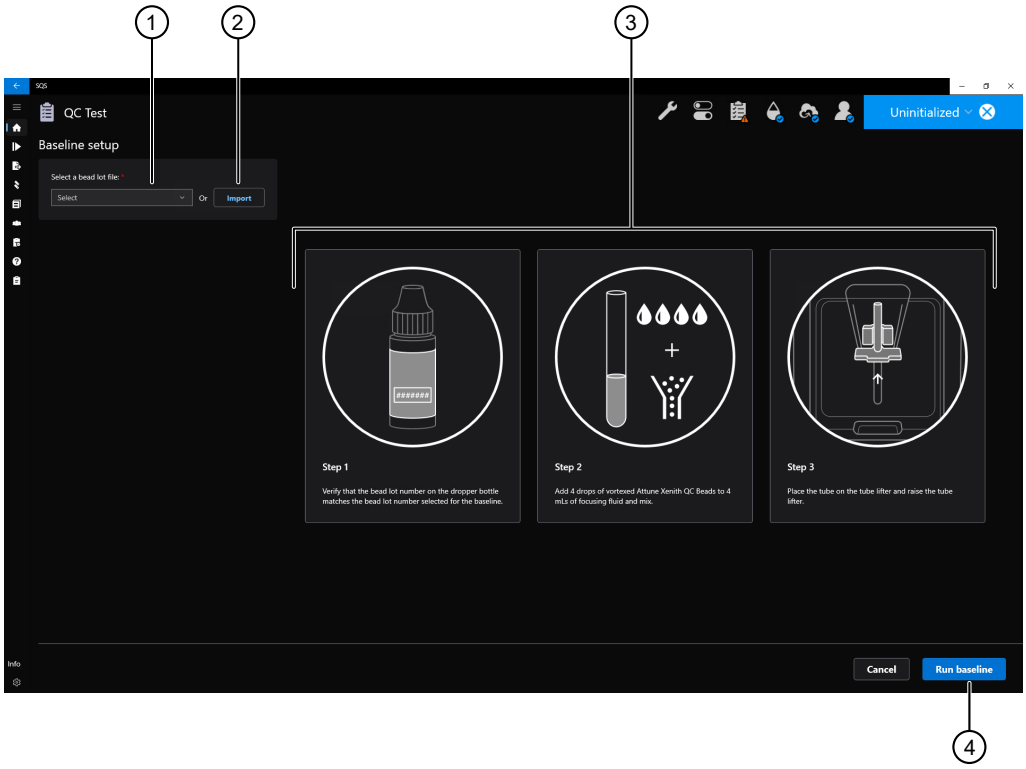


Figure 19 Baseline setup screen

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 vortexed Attune™ Xenith™ Quality Control Beads to 4 mL of focusing fluid and mix.
3. Place the tube on the tube lifter, then 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.

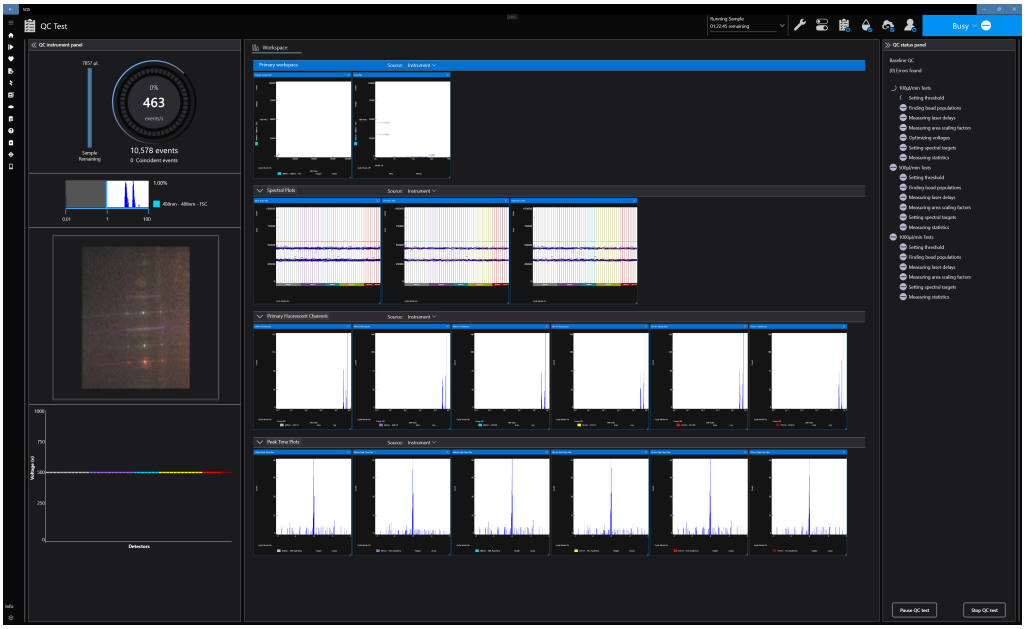


Figure 20 Baseline run in progress

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.

Daily QC

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

Note: Before you can run **QC Test**, the instrument must successfully complete the **Startup** procedure.

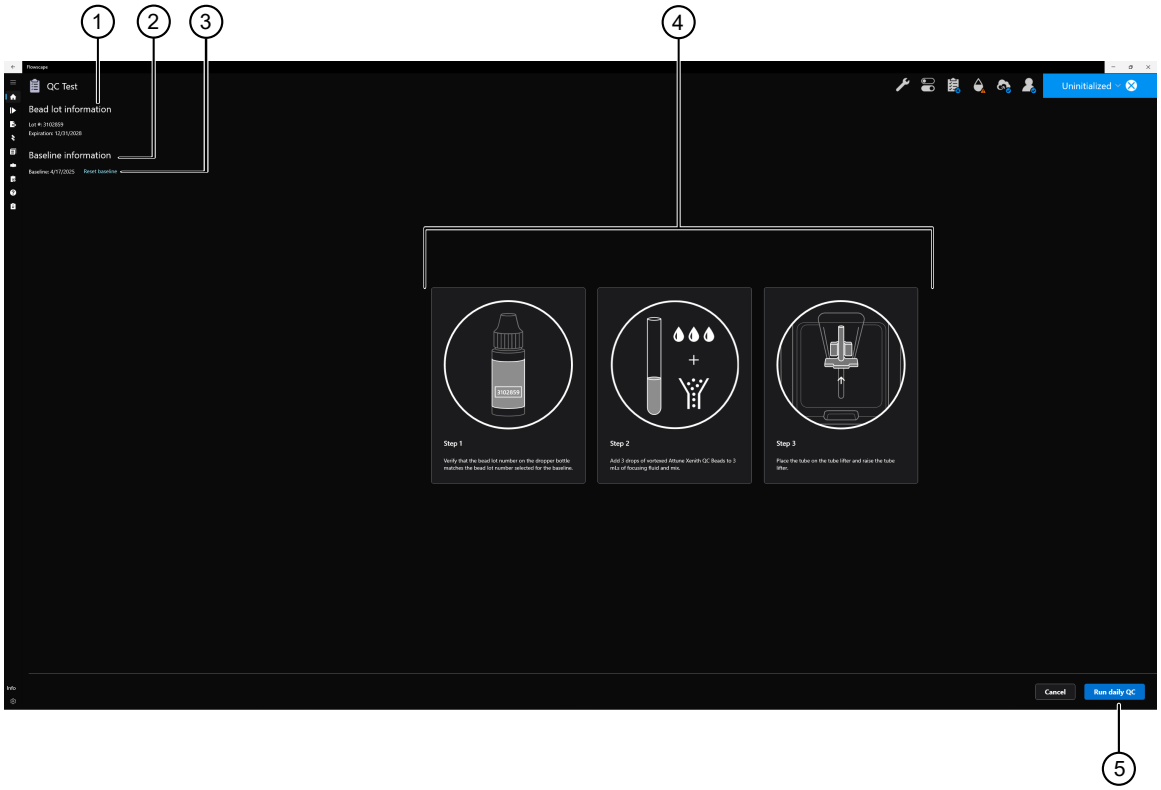


Figure 21 Daily QC screen

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 vortexed Attune™ Xenith™ Quality Control Beads to 3 mL of focusing 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 “QC Test Results” on page 41).

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.

  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.

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

Note: For more information about the **Daily QC report** and the **Levey-Jennings report**, see the *Attune™ Xenith™ Flow Cytometer User Guide* (Pub. No. MAN1000880).

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6 Run samples

Before you begin

Recommended flow rates

During experiment optimization, the user sets the flow rate at which sample is delivered to the flow cell for interrogation. The flow rate on the Attune™ Xenith™ Flow Cytometer ranges from 12.5 µL/minute to 1000 µL/minute. To set the optimal flow rate, consider the characteristics of the sample in relation to these guidelines.

- 12.5 µL/minute and 25 µL/minute is the recommended flow rate for small particles <2 µm.
- 100 µL/minute, 200 µL/minute, 500 µL/minute, and 1000 µL/minute flow rates are not recommended for particles <2 µm in size.
- The highest flow rate is not recommended for particles <4 µm.
- At greater flow rates some loss of sensitivity can occur.

For more information, see “Flow rate” on page 105.

Sample requirements

The Attune™ Xenith™ Flow Cytometer is designed to handle samples in 1.2 mL, 1.5 mL, and 2.0 mL microcentrifuge tubes, 2.0 mL bullet tubes, and 12 × 75 mm 5.0 mL flow tubes.

The method used for sample preparation depends on the sample type and the assay desired.

Guidelines for control samples

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).

When choosing between cells or beads as controls, consider the following:

Table 1 When to select cells vs. beads for unmixing controls?

Cells	Beads
• Good if you have plenty of sample. • Good if the antibody will stain a large, distinct positive population. • Good if there will be an internal negative.	• Good if your sample is limited. • Good if the antibody will stain a very small subset of cells. • Good if the antibody does not stain a distinct population of cells. • Good if the spectral characteristics are maintained on beads.

Follow these rules when selecting controls for spectral unmixing experiments:

- For accurate unmixing, the reference spectra should match the spectra in the multi-color sample.
- The same fluorophore must be used in the control as in the fully stained sample.
- When using tandem dyes, use the same lot for your control as in the fully stained sample.
- Prepare samples and controls under identical conditions, because buffer, fixatives, light, and heat can affect fluorescence.
- The positive peak in the single color control must have the same brightness or be brighter than the signal in the fully stained sample. However, bead controls can still be dimmer than bright cells.

Fluorescence minus one (FMO) control consists of a sample of cells stained with all the fluorescent reagents in the panel *except for one*. FMO controls are important in spectral unmixing for the following reasons:

- **Accurate gating:** By providing a baseline fluorescence level for cells stained with all but one fluorophore, FMO controls help to distinguish true positive signals from background noise.
- **Spillover compensation:** It helps you account for the spillover of fluorescence from other channels into the channel of interest, and identify the true positive signals from background noise.
- **Standardization:** It ensures that the gating strategy is consistent across different samples and experiments.

- **Minimizing false positives:** It enables to define the boundaries of negative and positive populations more precisely, which helps to minimize false-positive events, leading to more reliable and interpretable data. This is especially critical for populations where dim expression or rare events are expected.

To optimize the control samples, do the following:

- When establishing a panel, prepare a full set of single stain controls on beads and cells.
- After recording controls, check the spectral signature of beads versus cells.
  - If the spectral signatures differ, use cells for controls.
  - If cells are brighter than beads, use cells controls.

Perform Startup

If not yet performed, run **Startup**. See “Run Startup” on page 34

Run daily QC Test

If not yet performed, run **Daily QC Test**. See “Daily QC” on page 40.

The system is automatically rinsed when the test is complete.

Set up an experiment

The **New Experiment Setup** screen of the Flowscape™ Software enables you to set up the following experiment types:

- Experiment without single-color controls
- Spectral unmixing experiment
- Experiment with single-color compensation controls using auto compensation

1. From the **Home** screen, click **New Experiment**.
2. Enter a unique name for the experiment. It is optional to enter a description or tags.
3. Select the **Unmixing type** for the experiment.

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

- **None**: Creates an experiment without single color controls and all lasers and channels enabled.
- **Conventional compensation**: Emitted light is measured from a **single detector** and the spillover due to the similarity of fluorophores is corrected via single channel-by-single channel compensation matrix.
- **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 distinguished by the unmixing algorithm.

Table 2 When to select Conventional compensation vs. Spectral unmixing?

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 mixed autofluorescence populations. • Panels where you are not certain which best peak channel to select. • Highly beneficial when handling limited samples.

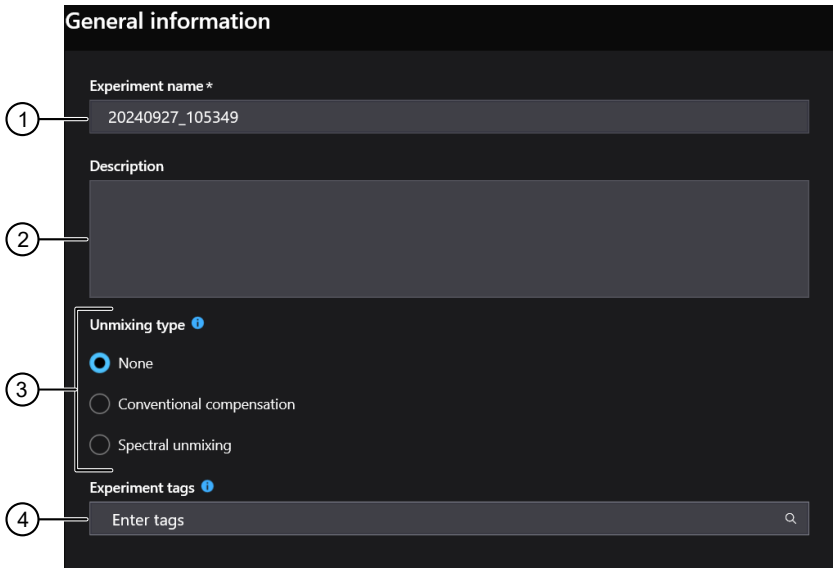
IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

Note: For more information about **New Experiment Setup**, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

Create a new spectral unmixing experiment

Note: For detailed information about **New Experiment Setup** and how to create spectral unmixing experiment, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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.

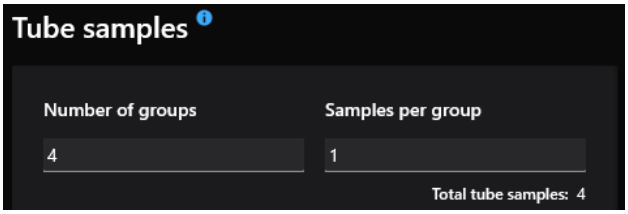


1. Experiment name
2. Description
3. Unmixing type
4. Experiment tags

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.

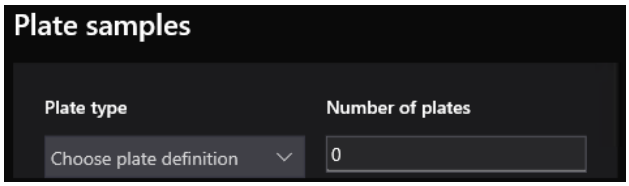
IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

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.

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

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

10. To select the fluorophores that you have chosen for the experiment, scroll through the fluorophores list on the left of the screen, or enter the first few letters or numbers of the fluorophore name in the search box until you see the correct item. Double-click the fluorophores to add them to the experiment.

    Selected fluorophores appear in the **Selected Fluorophores** list and the emission spectra appear in the plots in the middle of the screen. As fluorophores are added, ensure that the **Complexity Index** meets the criteria for the experiment.

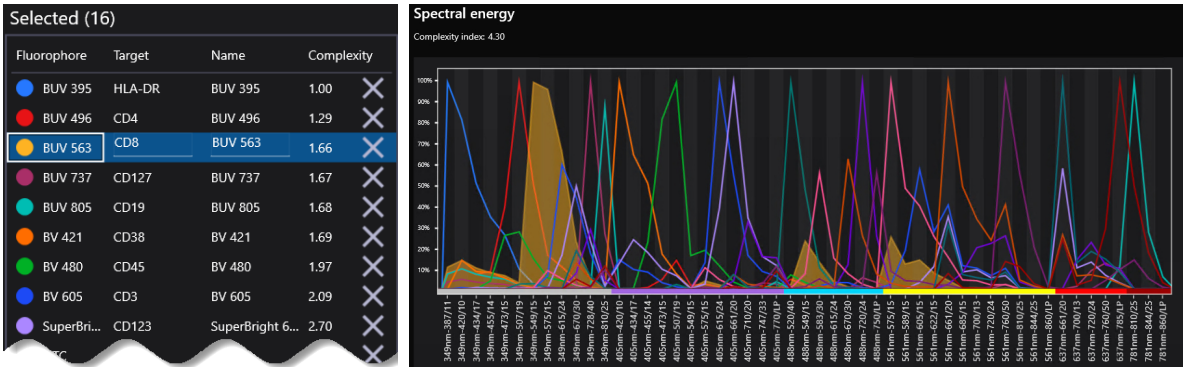
Note: **Spectral similarity matrix**, which shows how alike the emission spectra are on a scale of 0 to 1 when comparing two dyes and highlights the most similar fluorophores. A score of 0 indicates that the colors are entirely different and a score of 1 indicates that the fluorophores are identical. For more information, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

11. *(Optional):* The default parameter name is the name of the fluorophore in the fluorophores list. If desired, change the **Channel Name** field to include more descriptive information for each detector. You can also add a prefix to the parameter name.

12. *(Optional):* If using beads for single-color controls, select a secondary unstained control.

13. Review the **Spectral energy plot** and the **Complexity index** of your panel.

    The **Spectral energy plot** shows each fluorophore in the experiment with its expected emission/excitation graphs. The **Complexity index** value above the **Spectral energy plot** indicates the unmixing burden of the panel.



14. After you have selected the fluorophores for the experiment, click **Next** to define unstained and autofluorescence controls, and map them to fluorophores.

Note: Each single color control must be mapped to an external unstained control or, alternatively, to an internal negative control.

- 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.

For more information, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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15. Select **Spectral Options**:

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

    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.

    b. 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.

16. The software adds the controls to the sample list and builds the workspaces needed to run the negative and single-color controls you have prepared.

17. Default detector voltages are set from a successfully run QC process. You can set detector voltages in real time when you run the single-color controls.

Set voltages

You can set voltages in real time during experiment set up or you can import voltages from a previous run protocol.

1. To set voltages in real time, right-click the scatter plot region and click the **Spectral Voltage Target** field.

2. Click **Run**. The system automatically sets the voltages.

Run spectral controls

1. Click the first sample in the list, normally the negative control.

2. Set the **Flow Rate** and enter acquisition volume.

3. Click **Run**.

4. Adjust the PMT voltages on the FSC and SSC plot to provide the correct view of the sample, adjust the threshold if needed, check scatter gate positions and adjust them if needed.

5. Click **Stop**.

6. When voltages have been set, click **Record** to acquire sample.

7. It is optional to adjust the bar regions in the histograms to show the positive events in the fluorophore graphs. This is for visualization; the spectral process automatically detects and records all positive events for spectral unmixing. A green checkmark appears next to each sample when it has been recorded and the data have been unmixed.

8. Repeat the process for all controls.

9. After all samples have been acquired, a Spectral Matrices Success message appears.

   Note: Warning messages can appear regarding the quality of the unmixing process. It is optional to address the warning messages, or to continue. However, errors are handled differently.

10. If there were errors during the unmixing process, they must be handled before you can continue.

11. If there are no errors and you see the success message, click **Ok**. You are now ready to create new plots, set regions and run sample.

Run samples and collect data

After you have set up the experiment and performed color compensation or spectral unmixing, the system is ready to acquire test sample data. Plots, regions and gates can be built before or during acquisition.

1. Double-click the first sample in the **Sample panel ▸ Primary group**.

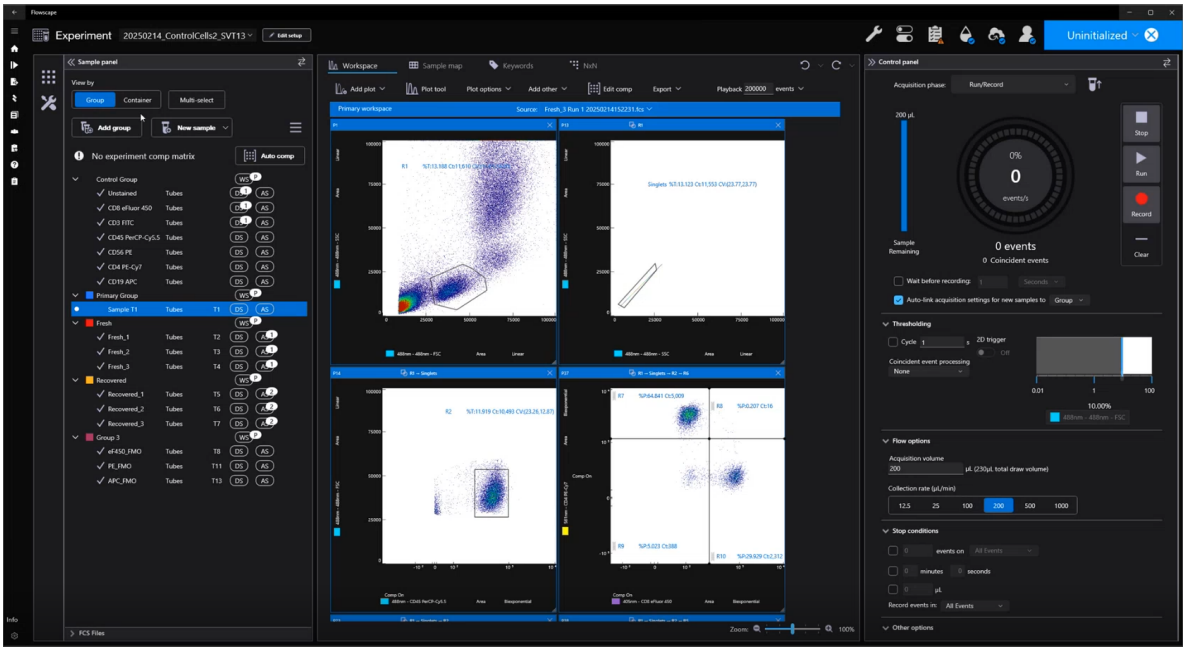
   Primary workspace displays the default FSC Area vs. SSC Area scatter plot, which can be modified. You can add more plots, gates, statistics, and text boxes to the workspace for data acquisition and analysis.

2. In the **Control panel**, set the stop conditions, flow rate and acquisition volume.

3. Load the tube containing the sample on the sample injection port, then lift up the tube loader to the active position.

4. Click **Run** to acquire sample. In this mode data are shown but not stored.

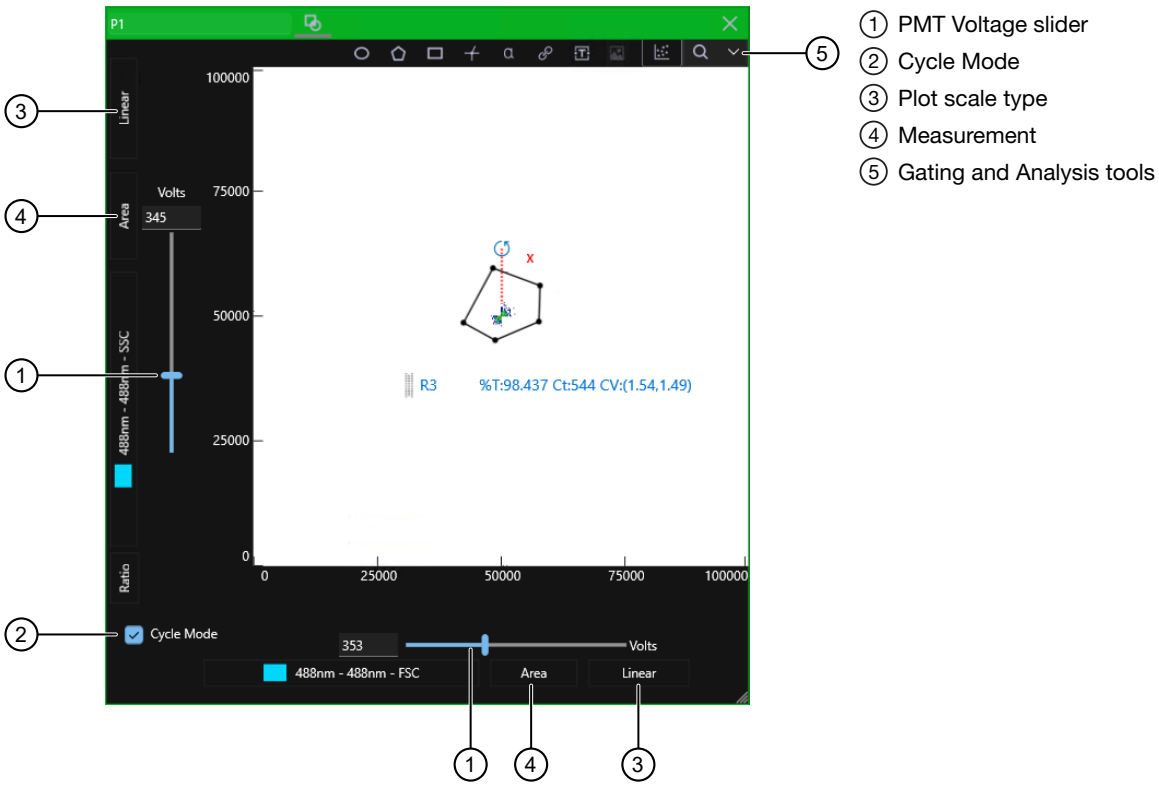
   The events are displayed on the plots as the graphs are being populated. Wait a short time for the sample to equilibrate.



5. Enable the **Cycle Mode** to quickly see any changes in the data. Remember to disable **Cycle Mode** when you want to record data.

6. View the data plots and make adjustments to the PMT voltages and threshold values for the appropriate channels. Ensure that the events are on scale.

7. From the **Gating and Analysis tools** on the plots, select the desired gates to add to the plots to isolate and analyze selected populations.



8. Disable **Cycle Mode**, then click **Record**.

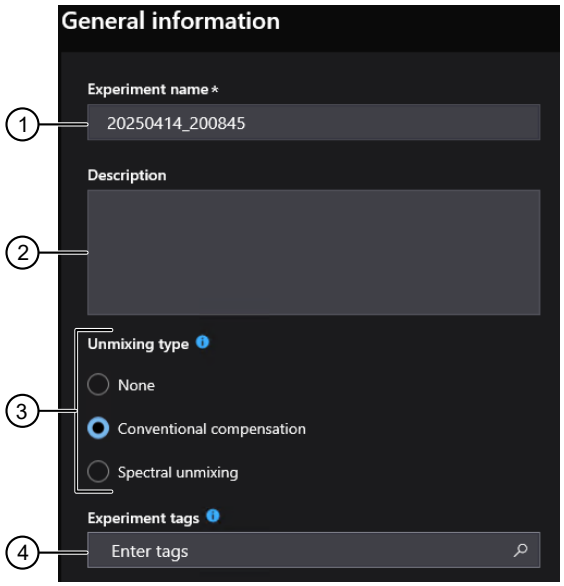
9. Click **Record**.

   If stop criteria is set, sample data are recorded until that limit is reached. If a limit is not set, stop the sample when you have recorded the desired number of events.

Create a new compensation experiment

Note: For detailed information about **New Experiment Setup** and how to create conventional compensation experiment, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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.

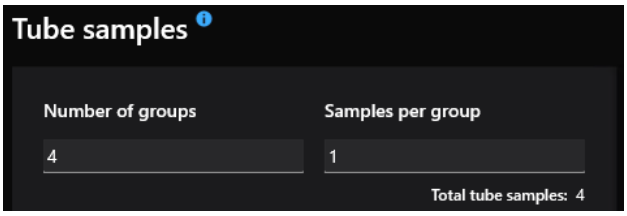


1. Experiment name
2. Description
3. Unmixing type
4. Experiment tags

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.

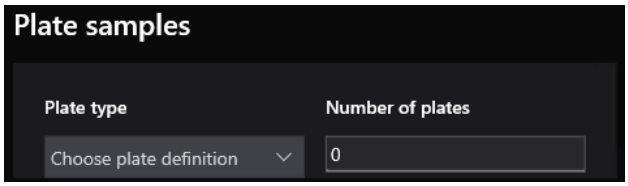
IMPORTANT! The **Unmixing type** cannot be modified after an experiment is created.

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**.



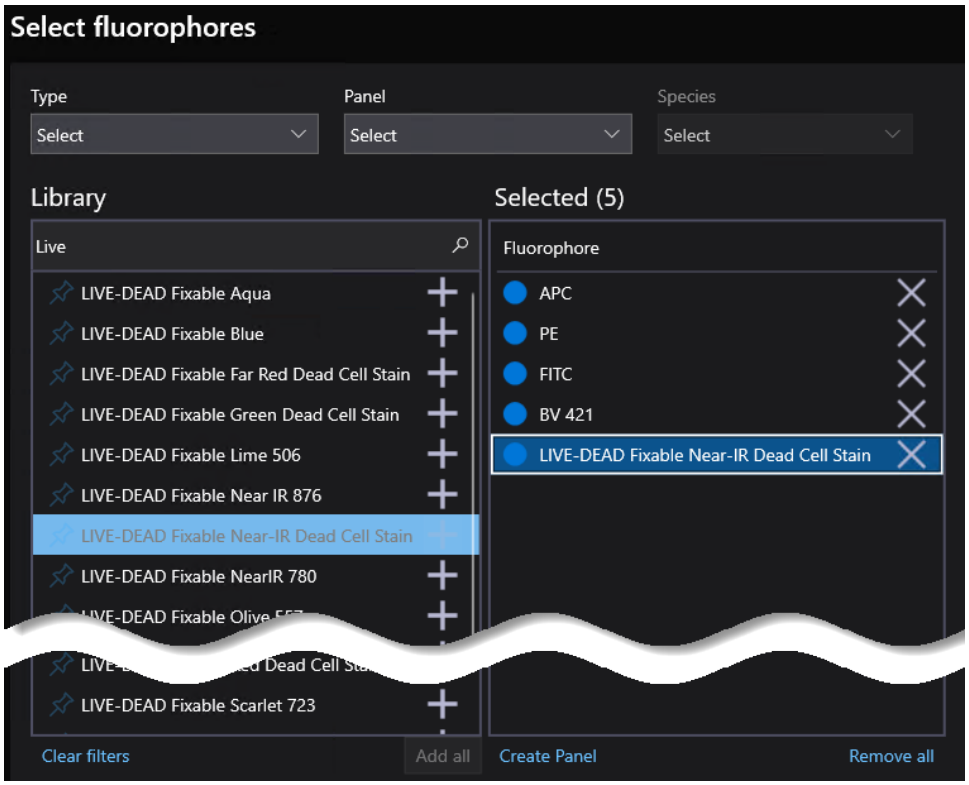
Note: There is no limit to the number of plates in an experiment.

7. If applicable, select **Foil cover**, **Cooling block**, or both.

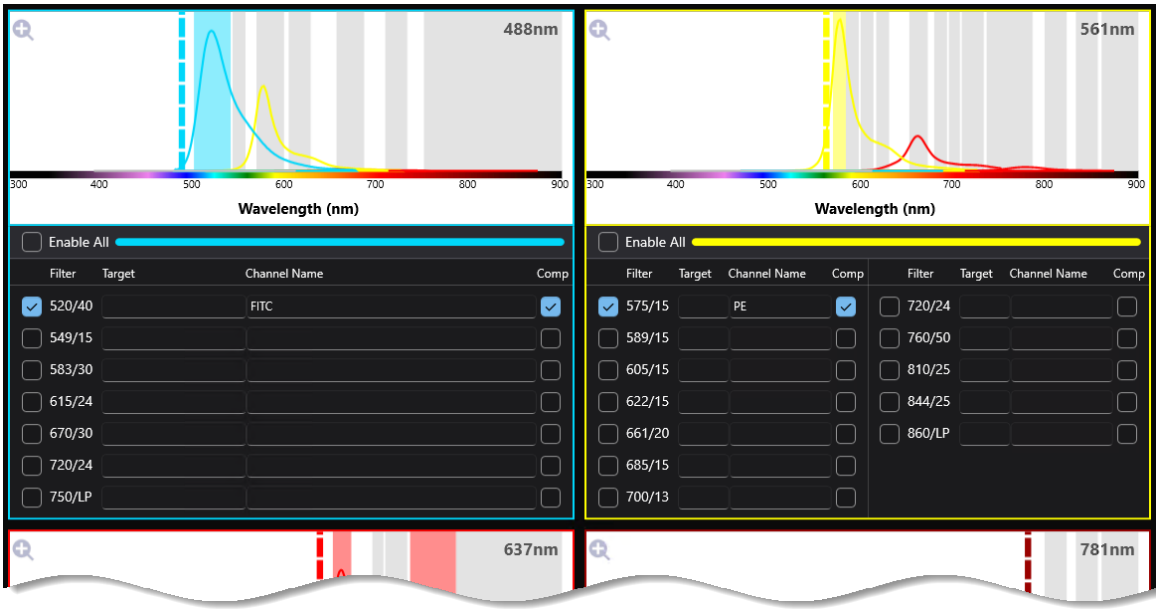
8. *(Optional):* If desired, update sample keywords (sample name, group name, etc.) or create custom keywords in the **Sample keywords** table.

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

10. To select the fluorophores that you have chosen for the experiment, scroll through the fluorophores list on the left of the screen, or enter the first few letters or numbers of the fluorophore name in the search box until you see the correct item. Double-click the fluorophores to add them to the experiment.



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.



11. Review and verify that the selected fluorophores are placed in the appropriate channels. 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).

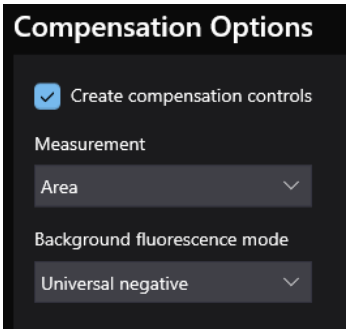
Note: The selected fluorophores are automatically placed in the channel that is expected to have the brightest signal. If desired, you can activate more channels and manually assign detectors as compensation controls using the options under each plot.

---

12. To manually assign detectors as compensation controls, click to select the **Comp** checkbox for the corresponding detector.

13. *(Optional):* The default parameter name is the name of the fluorophore in the fluorophores list. If desired, change the **Channel Name** field to include more descriptive information for each detector. You can also add a prefix to the parameter name.

14. Select **Compensation Options**:



a. Verify that the **Create compensation controls** option under **Compensation Options** is selected, which ensures that the necessary compensation controls are created in the **Workspace**.

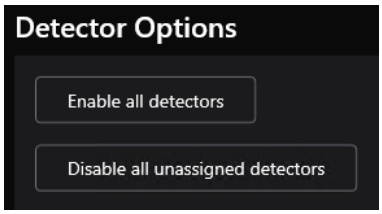
b. From the **Measurement** dropdown, select **Area**, **Height**, or **Width** as the parameter for calculating compensation.

c. 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 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.

15. Select **Detector Options**:



a. To enable all detectors for the experiment, click **Enable all detectors**.

b. To turn off all detectors that are not assigned as compensation controls, click **Disable all unassigned detectors**.

Note: For more information about compensation and detector options, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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16. When finished with fluorophore selection, and compensation and detector options, click **Next** to finish the setup of the compensation experiment.

    The software automatically creates all selected controls and samples, and opens the **Experiment Workspace**. The **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.

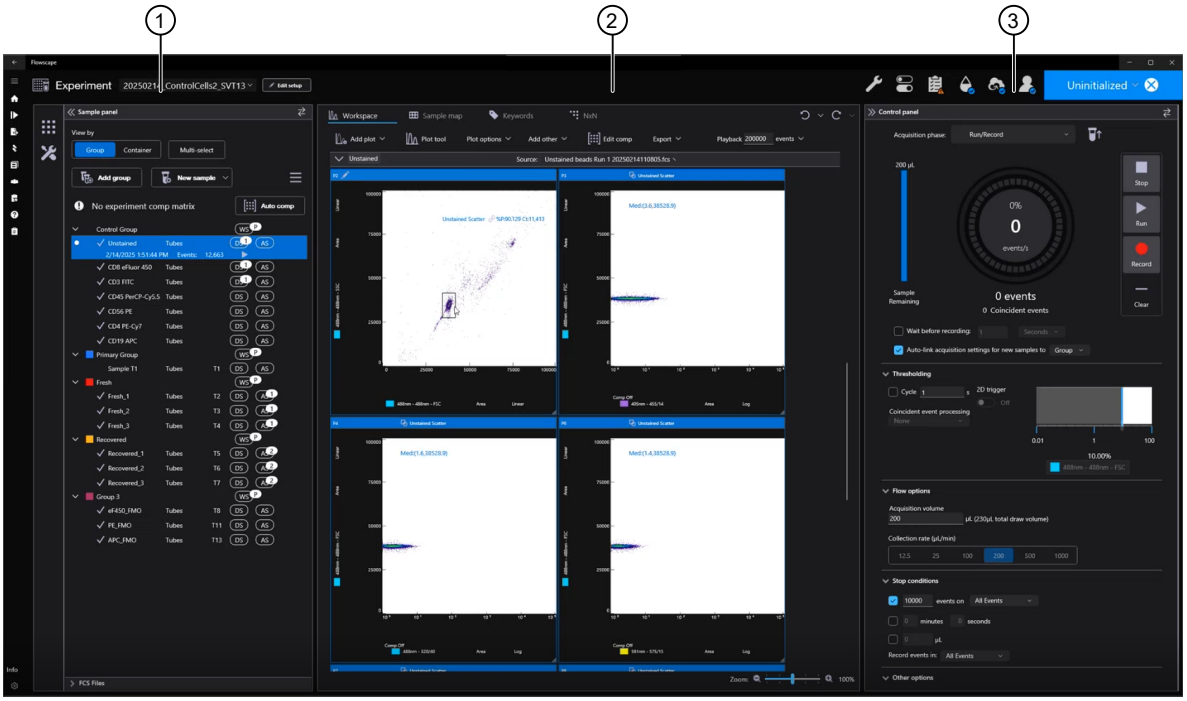


Figure 22 Experiment Workspace for a compensation experiment

① Sample panel
② Views (showing Workspace view)
③ Control panel

- The **Sample panel** lists each control and sample in the experiment and shows the **compensation matrix status** of the experiment.

  The control samples are placed in the **Control Group**. The **Primary Group** is designated for the sample or samples that are to be acquired. If other tube sample groups and plates were created in the **New Experiment Setup**, they are also placed in their own group in the **Sample panel**.
- The **Workspace view** contains the dedicated workspaces for each control and sample in the experiment. It 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.

Note: For more information about the **Experiment Workspace**, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

Optimize instrument settings

Before you can record data for a sample, you need optimize instrument settings. This involves running single-color controls with populations of negative and positive cells or beads. These controls help fine-tune PMT voltages, compensation, and threshold settings for each fluorophore and sample used in the experiment, and put the populations of interest on scale for the scatter and fluorescence parameters. If the controls do not contain a fluorescence reference **internal negative**, you can use a **universal negative** (that is, an **unstained** control).

After you have recorded the compensation controls, you need to use the **Compensation Wizard** to review the data, make manual changes (if needed), and apply compensation.

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

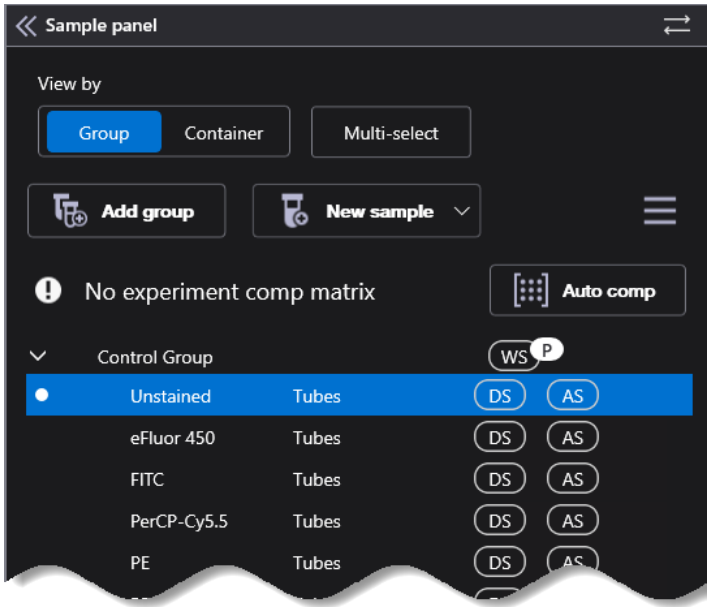
Note: If no compensation is necessary (that is, if **None** is selected for **Background fluorescence mode**), you can optimize the instrument settings in the **Primary workspace** of the **Experiment Workspace**. The procedure for optimizing samples is similar to that described for compensation control samples.

Record unstained control

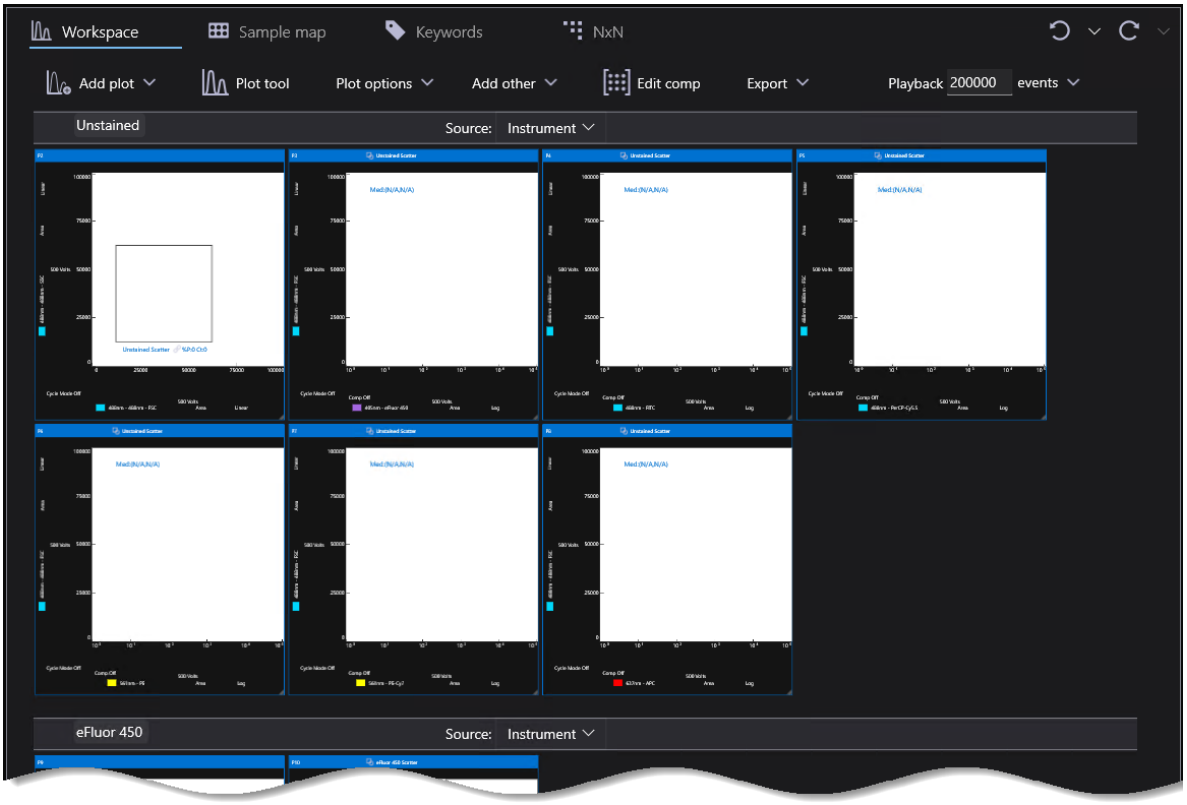
An unstained control is a sample that is processed in the same way as the experimental samples, but without the addition of fluorescent antibodies.

Unstained controls are crucial for setting up the instrument, determining background fluorescence (autofluorescence), and establishing negative populations for gating. When calculating compensation, the software compares the fluorescence of single-color controls to the unstained control, calculates the spillover between detectors, then corrects for it.

1. Double-click the **Unstained** control in the **Control Group** of the **Sample panel**.

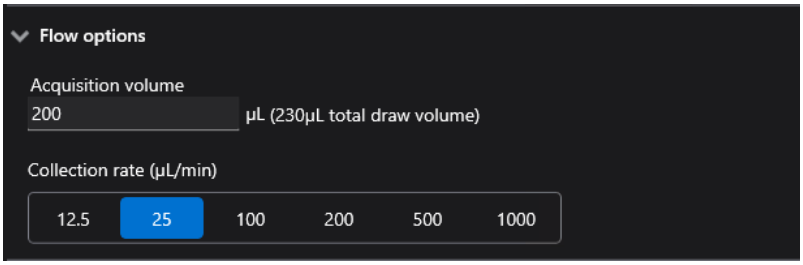


The **Workspace** for the unstained control contains one **SSC vs. FSC scatter plot** with a polygon gate, and **Histogram plots** for each fluorescent parameter selected during the compensation setup.



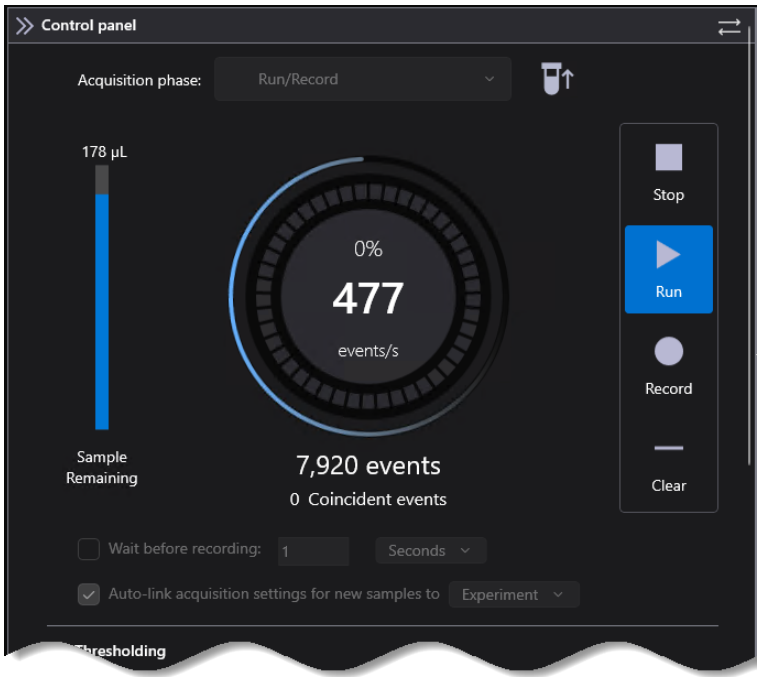
2. Load the unstained control on the tube lifter.

3. In the **Control panel** ▸ **Flow options**, enter the **Acquisition volume**, and then select the **Collection rate**.

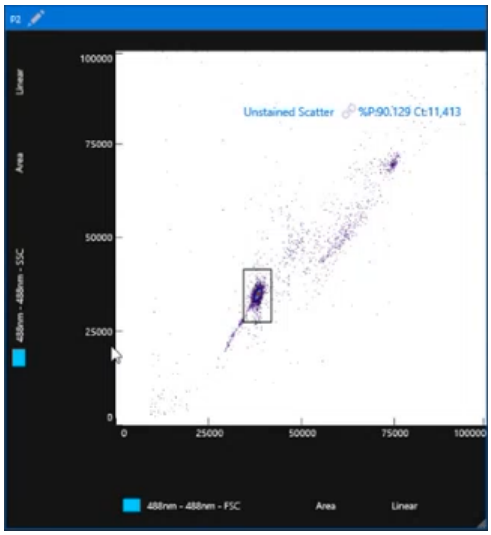


Note: For setup, you can conserve the sample by running the cytometer at a 25 µL/minute collection rate.

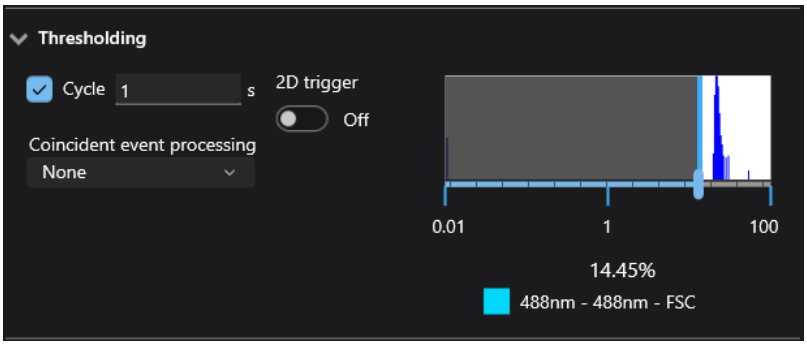
4. Click **Run**.



Events appear in the **FSC vs. SSC plot**. You can obtain data in real-time without saving to a file.

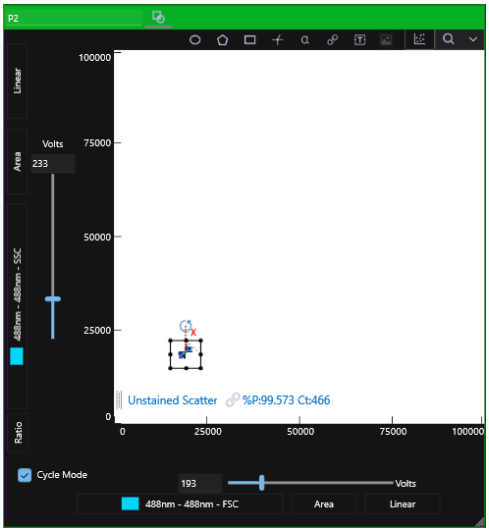


5. Enable **Cycle mode** to quickly see any changes in the data. Remember to disable **Cycle mode** when you want to record data later.



6. View the plots and adjust the **Threshold level plot** to remove unwanted events and background.

7. Click on the **FSC vs. SSC plot**, then adjust the PMT voltages using the **PMT sliders** on each axis to place the target population on scale.



8. Set the scatter gate on the population of interest so that the fluorescence histograms are reflective of the population for which you are optimizing the voltages.

9. Adjust the PMT voltage for **Fluorescence Channels** until 95% of negative cells are on scale (that is, just to the right of 10^0 in the log plot).

Note: View uncompensated data in **log mode** (instead of **hyperlog** or **biexponential**), because it is easier to see if most events are on scale. To do Auto voltage adjustment, by right-click in the scatter gate on the **FSC vs. SSC plot**, select **Region properties**, then use the **Voltage Set** function. Ensure that most if not all negative populations are above 10^0.

---

10. After making sure that the PMT voltage are correct and set to maximize the signal-to-noise ratio, disable the **Cycle mode**, then click **Record**.

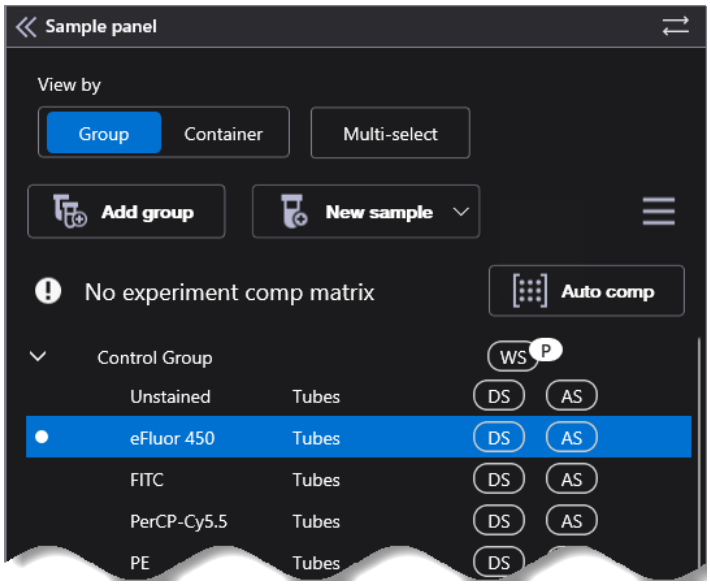
    If an event limit is set, sample data are recorded until that limit is reached. If an event limit is not set, stop the sample when you have recorded sufficient events.

11. When finished recording, remove the unstained control from the sample injection port, then proceed to "Record the single color controls" on page 61.

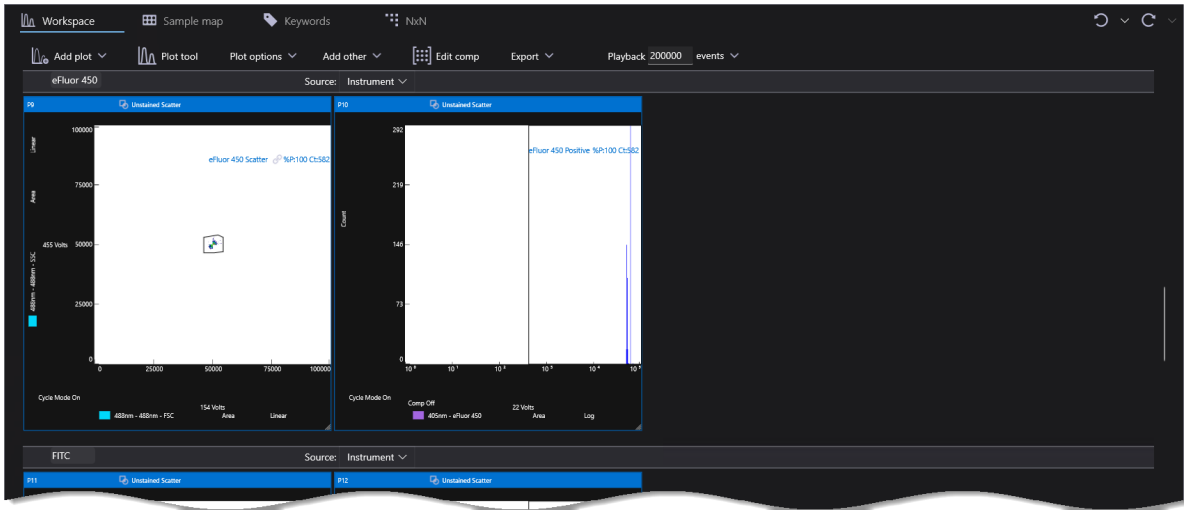
Record the single color controls

After you have optimized the **Instrument Settings** for the unstained control (if applicable), optimize the **Instrument Settings** for each single-stained control.

1. Double-click the first **single color control** in the **Control Group** of the **Sample panel**.



The **Workspace** for the single color control contains a **SSC vs. FSC scatter plot** with a polygon gate and a **Histogram plot** for the fluorescent parameter.



2. Load the first single color control on the tube lifter.
3. Using the same optimization procedure as the unstained control (page 57), adjust the **Instrument Settings** and set the scatter gate on the population of interest.
4. For each compensation control sample (that is, fluorophore), view the corresponding histogram to optimize the voltages.
5. Perform the optimization procedure for all single color controls.
6. After you have optimized **Instrument Settings** for each single color control and finished recording, proceed to “Apply Auto compensation” on page 63.

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

Apply Auto compensation

Auto comp button Auto comp in the **Sample panel** 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.

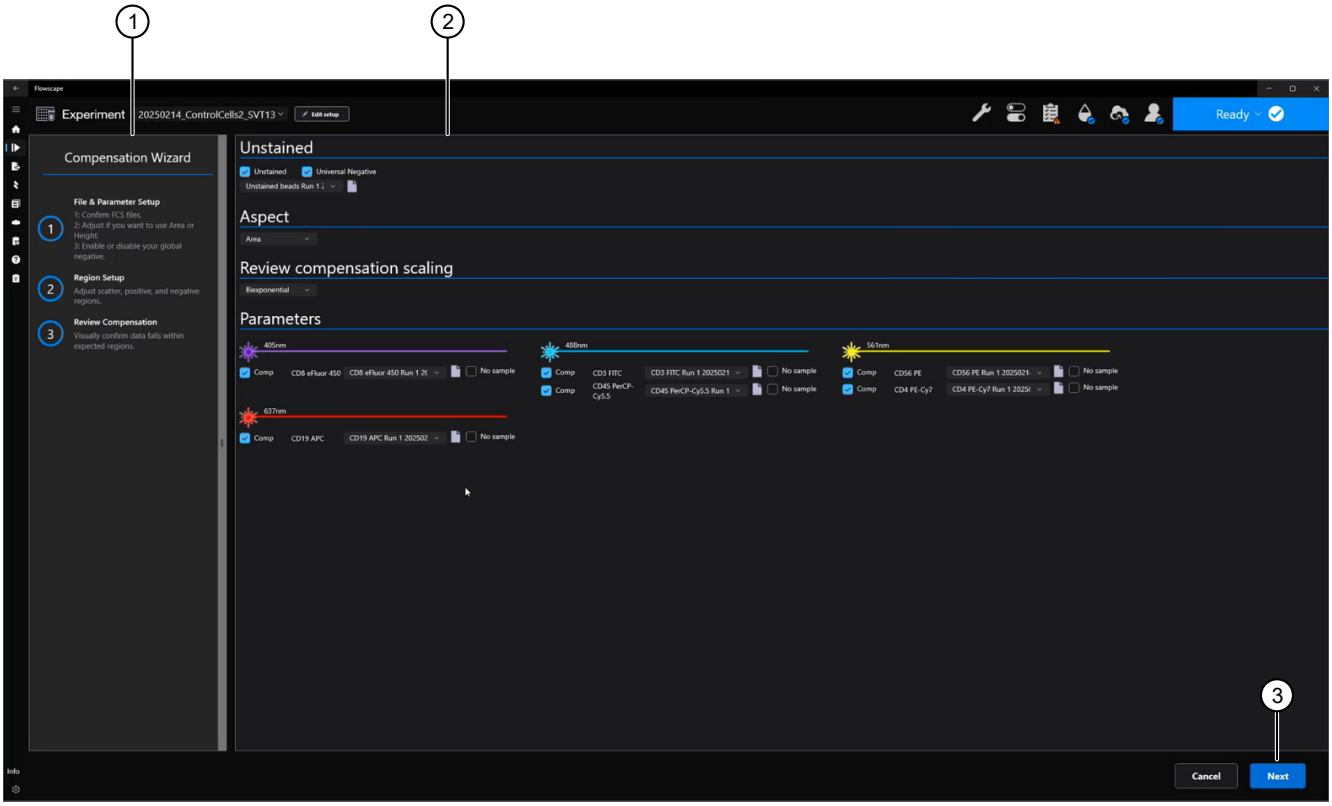


Figure 23 Compensation Wizard (File & Parameter Setup screen)

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 64)
2. **Region Setup** (page 66)
3. **Review Compensation** (page 68)

Note: For more information about the **Compensation Wizard**, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

File and 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.

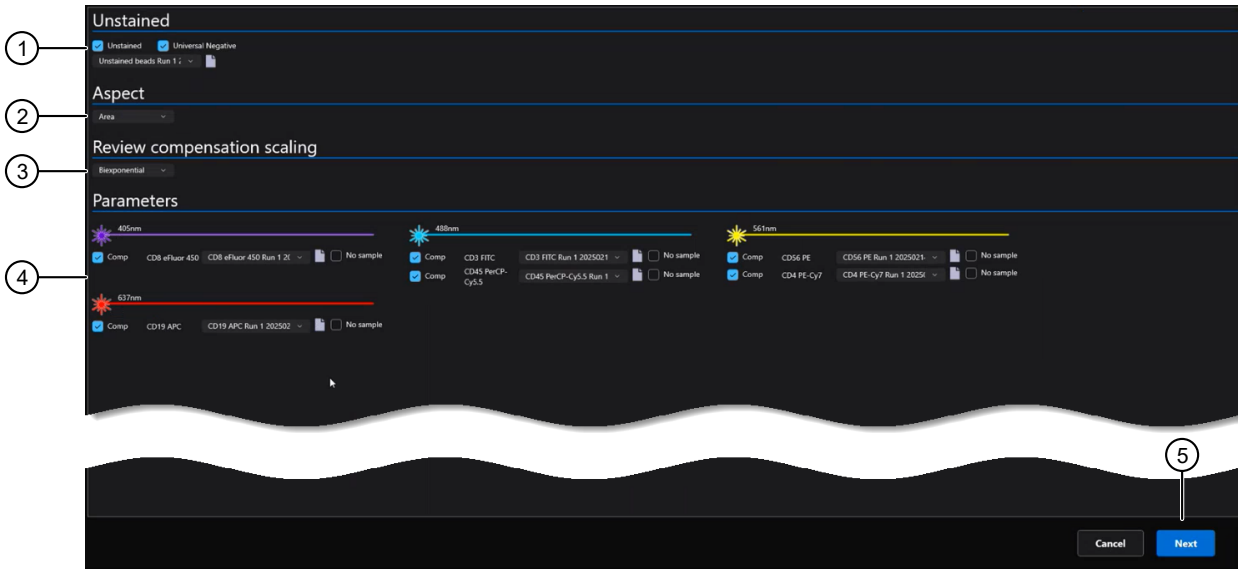
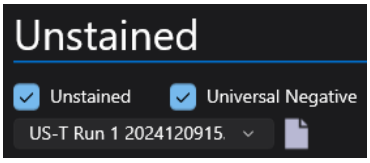


Figure 24 Compensation Wizard - File & Paramater Setup screen

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

Note: When the compensation experiment is set up using the **New Experiment Setup** screen (page 52), the selections made for compensation options and the fluorophore/detector assigments automatically carry over to the **File & Parameter Setup** screen.

1. Under **Unstained**, review and ensure that the correct controls are selected as the source of the background fluorescence when calculating compensation.

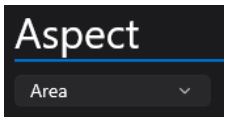


   a. When the **Unstained** option is selected, you can enable or disable the use of the unstained control as a **Universal Negative**.

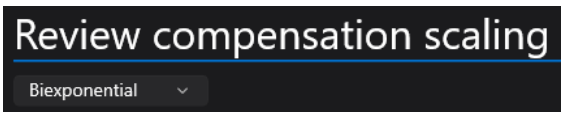
   b. To change the source of the unstained control, select the desired source from the **dropdown menu**, which shows the recorded unstained control runs available for the experiment.

   c. Alternatively, click the **Import** button, then select the desired FCS file from the location it was saved in the workstation or the network.

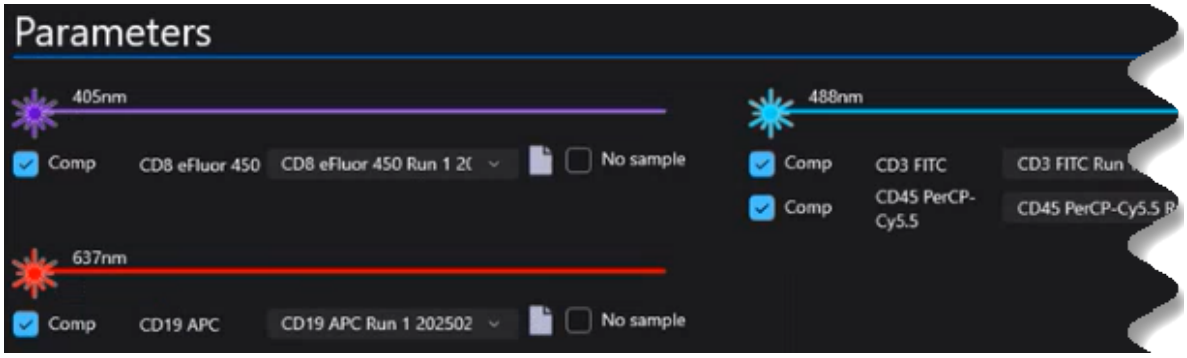
2. Under **Aspect**, select **Area** or **Height** as the parameter for calculating compensation.



3. Under **Review compensation scaling**, select **Log** or **Biexponential** scale for reviewing compensation plots.



4. Under **Parameters**, verify the assigned channels and detectors for the selected fluorophores and the recorded compensation controls.



   a. The sofware automatically maps the controls to the respective fluorescence channels. If desired, reassign channels and detectors for the fluorophores, or use compensation from other saved FCS files by importing them.

   b. Select the **Comp** checkbox to include this parameter in the compensation calculation. All parameters are included by default.

   c. Select the **No sample** checkbox to exclude the parameter from affecting the compensation of other parameters, while still allowing other parameters to be compensated into it. This is useful for live/dead markers that are gated out anyway.

5. Click **Next** to go to the **Region Setup** screen in the wizard (page 66).
   To exit the auto compensation workflow and return to the workspace, click **Cancel**.

Region Setup

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

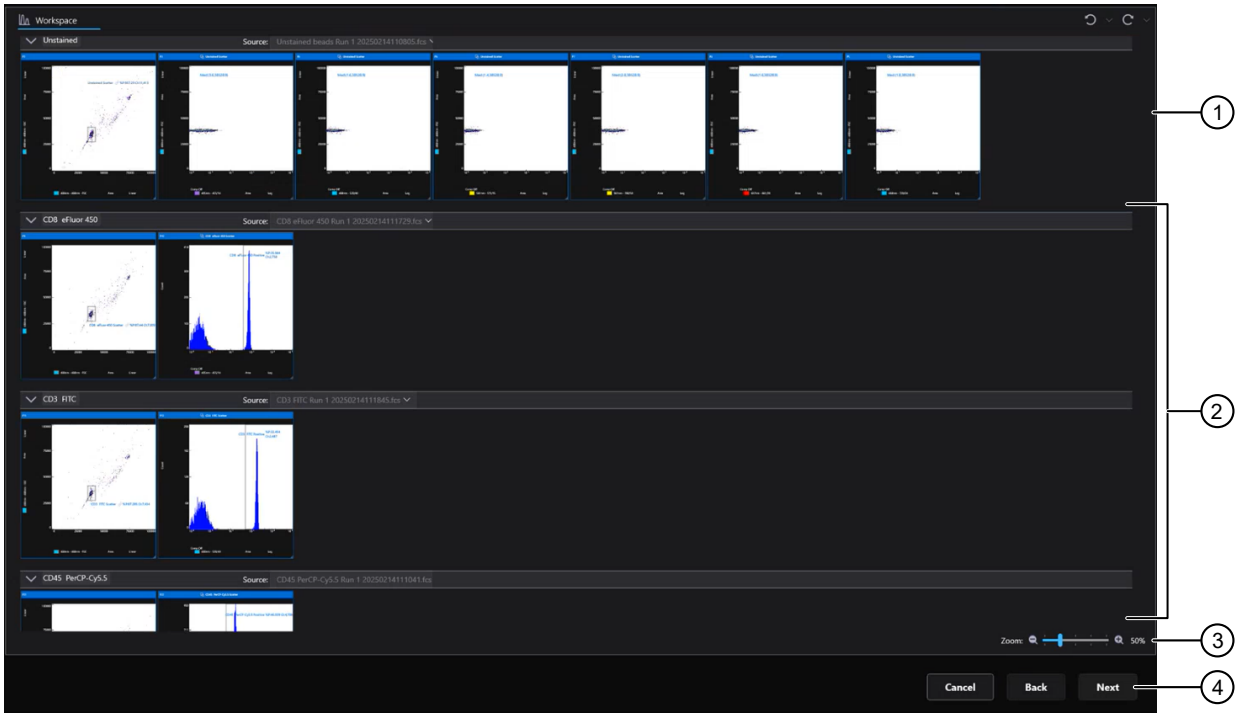


Figure 25 Compensation Wizard - Region Setup screen

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

Region Setup screen shows the plots and groups from the experiment with the FCS files loaded into each.

- **Unstained control workspace** contains a dual-parameter **Scatter plot** (also called the **Gating plot**) and **Histogram plots** for each compensation parameter.

  By default, the **Gating plot** contains a single **Scatter gate** (also called the **Parent gate**) that cannot be deleted.
- Each **Single color control workspace** contains a **Scatter plot** and a **Histogram plot** for the specific single color control.
- The **Compensation Wizard** automatically transfers the gate positions that were set up when recording the compensation controls.

Note: For more information about the **Unstained control workspace** and the **Single color control workspaces** shown in the **Region Setup** screen, see the *Flowscape™ Software User Guide* (Pub. No. MAN1000880).

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1. Review the **Scatter plot** in the **Unstained control workspace**. If needed, adjust the **Parent gate** in the plot, so that the gate encompasses the population of interest.

   Note: The **Parent gate** is automatically linked throughout the groups. Therefore, all scatter gates move to the same location when adjusted. If individual control of a scatter gate in another plot is needed, such as when bead and cell controls are used, right-click the gate and toggle the **Link** button to **Off**.

2. Review the **Histogram plots** in the **Single color control workspaces**. If needed, adjust the fluorescence positive and negative bar regions to encompass the positive and negative populations in each single color control.

3. When satisfied with the gate positions, click **Next** to calculate the compensation and go to the **Review Compensation** screen of the wizard (page 68).

Review Compensation

Review Compensation enables you to review compensation data after the compensation calculation is complete and adjust the compensation values, if needed.

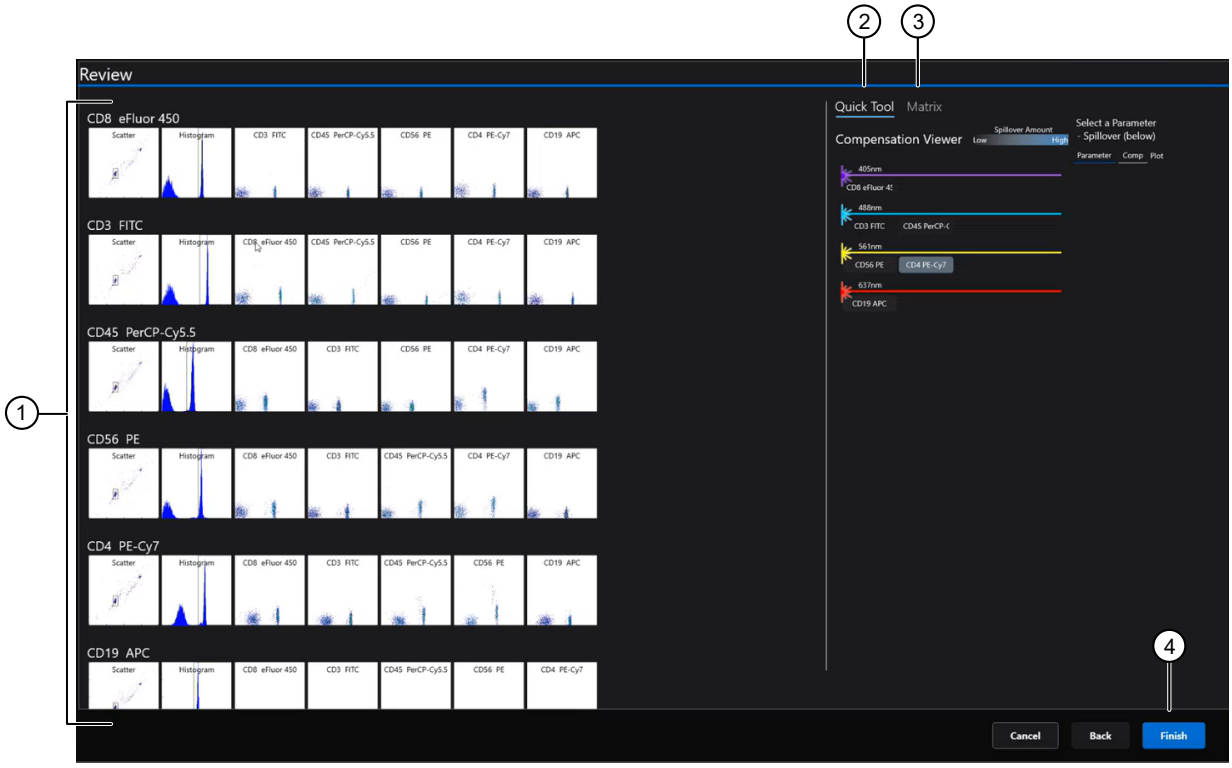
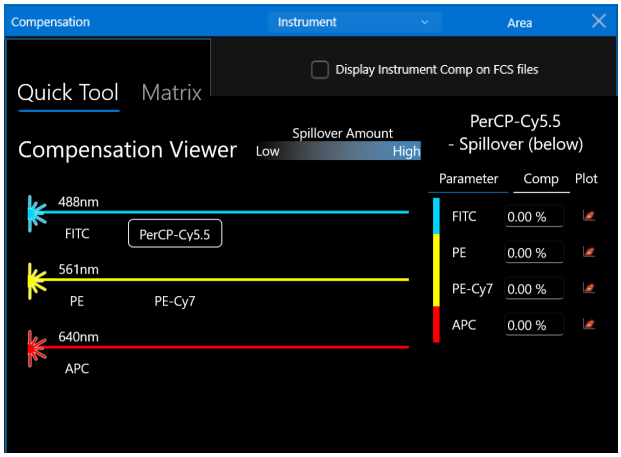


Figure 26 Compensation Wizard – Review Compensation screen

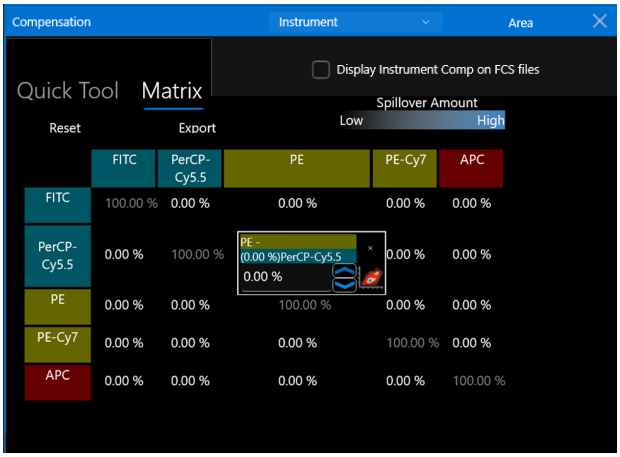
1. Compensation plots for single-color controls
2. Quick Tool
3. Matrix
4. **Finish** button

1. Review the **compensation plots** and visually confirm that the compensation data falls within the expected regions.

2. If manual changes need to be made to the compensation, use the **Quick Tool** or the **Matrix** to the right of the plots to view and edit the **compensation matrix**.



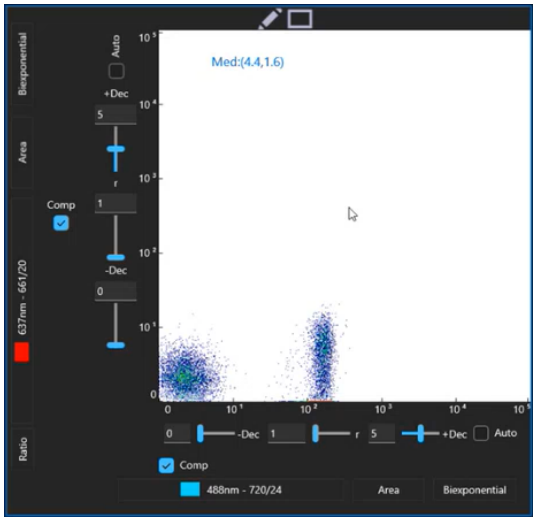
Quick Tool



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.
- **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.

3. To create new plots from each parameter combination, click the **Plot** button to the right of the parameter in the **Quick Tool**, which opens the **Plot Tool** below the **Quick Tool**.



The **Plot Tool** enables you to visualize compensation and adjust the gating of the populations used for compenation calculation.

4. When all plots are adjusted, click **Finish**. Compensation is applied to all plots where the **Comp** checkbox is selected (step 4 on page 65).

   When the compensation is applied to the experiment, the **Compensation matrix status** shows the **Experiment comp matrix ready** condition.

Run samples and collect data

After you have set up the experiment and performed color compensation or spectral unmixing, the system is ready to acquire test sample data. Plots, regions and gates can be built before or during acquisition.

1. Double-click the first sample in the **Sample panel** ▸ **Primary group**.

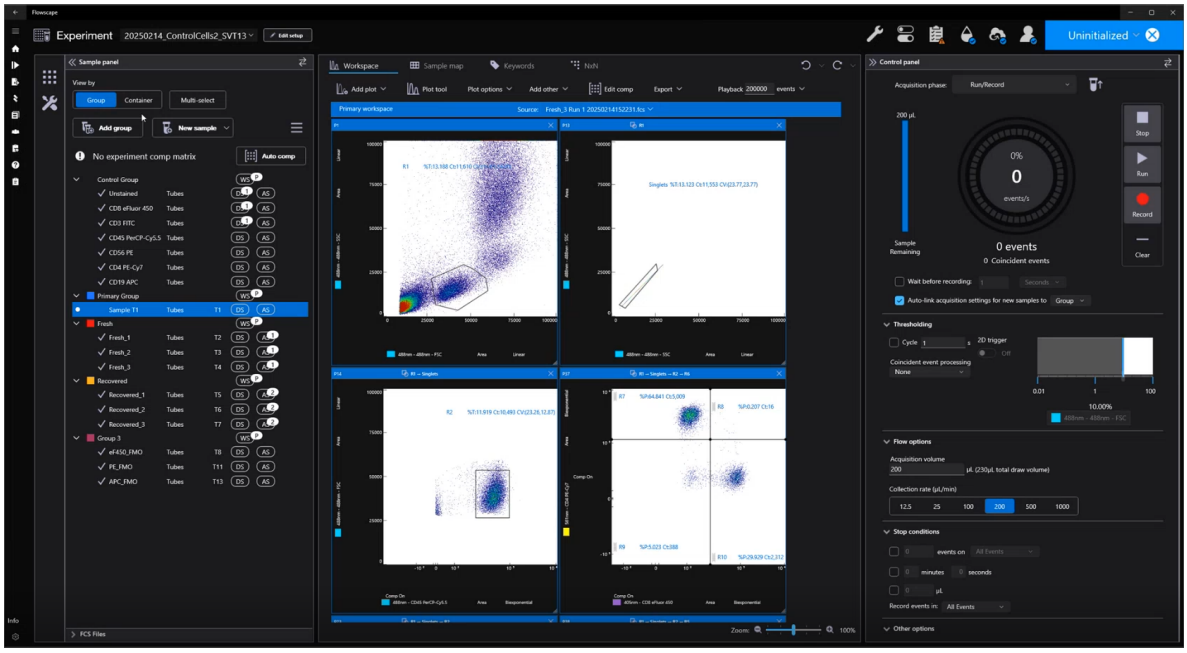
   Primary workspace displays the default FSC Area vs. SSC Area scatter plot, which can be modified. You can add more plots, gates, statistics, and text boxes to the workspace for data acquisition and analysis.

2. In the **Control panel**, set the stop conditions, flow rate and acquisition volume.

3. Load the tube containing the sample on the sample injection port, then lift up the tube loader to the active position.

4. Click **Run** to acquire sample. In this mode data are shown but not stored.

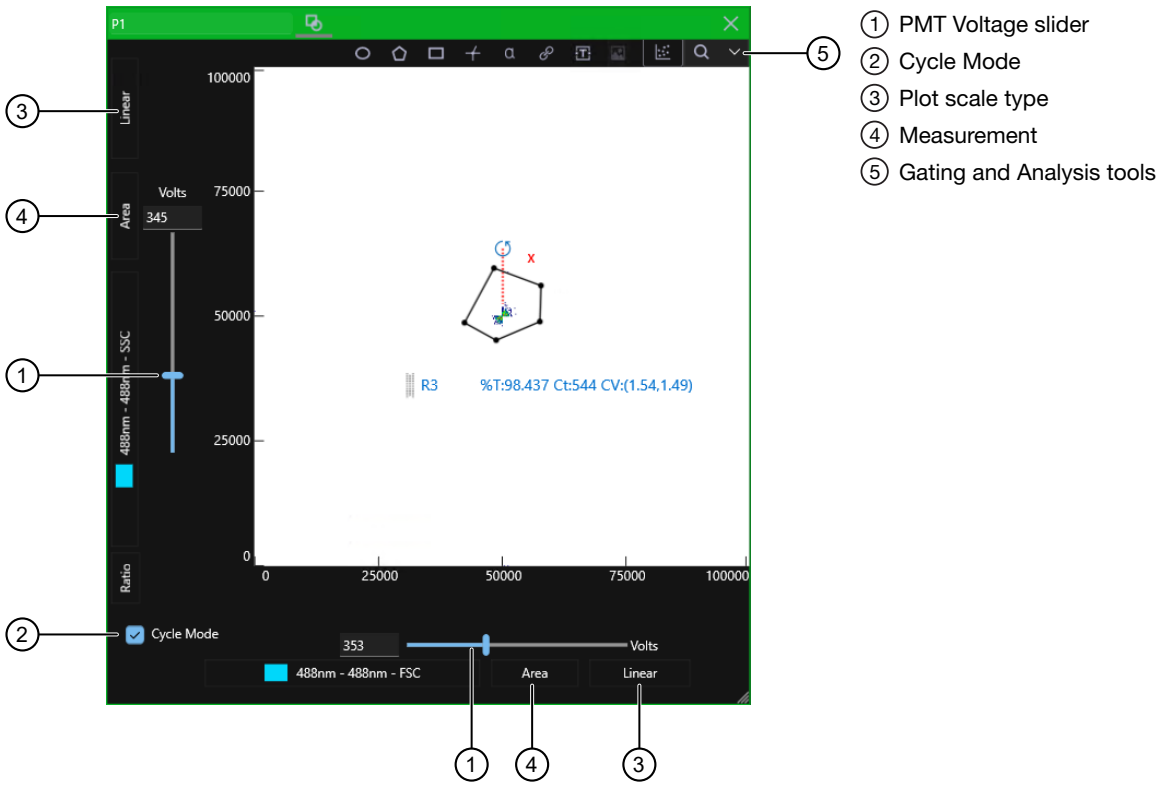
   The events are displayed on the plots as the graphs are being populated. Wait a short time for the sample to equilibrate.



5. Enable the **Cycle Mode** to quickly see any changes in the data. Remember to disable **Cycle Mode** when you want to record data.

6. View the data plots and make adjustments to the PMT voltages and threshold values for the appropriate channels. Ensure that the events are on scale.

7. From the **Gating and Analysis tools** on the plots, select the desired gates to add to the plots to isolate and analyze selected populations.



8. Disable **Cycle Mode**, then click **Record**.

9. Click **Record**.

   If stop criteria is set, sample data are recorded until that limit is reached. If a limit is not set, stop the sample when you have recorded the desired number of events.

7 Shutdown

The software facilitates the automated shutdown of the instrument. Shutdown removes sample fluid and dyes from the system, sanitizes the fluids lines and sample pumps, and fills them with Attune™ Shutdown Solution to prevent the formation of salt crystals. The automated shutdown procedure takes about 1 hour to complete.

IMPORTANT! Perform the shutdown procedure every day, even if the instrument is intended for continuous use. Proper instrument cleaning helps to ensure consistent and accurate operation.

CAUTION! Cytometer hardware can be contaminated by biohazardous material. We recommend fresh 10% bleach solution in deionized water to sanitize the surface areas of the cytometer. The Attune™ Xenith™ Flow Cytometer dilutes the 100% bleach supplied on the fluid cart to the correct concentration to sanitize the fluid lines and sample pumps.

100% bleach is defined as 5.25% sodium hypochlorite in water. We recommend using laboratory-grade bleach. Avoid bleach with additives such as perfumes.

Note: For more information about the Shutdown procedure, see “Shutdown” on page 82.

Check fluid and waste levels

1. Empty the waste container if needed. See “Remove and replace the waste container” on page 29.

   Note: The waste container must be less than 90% full before you initiate **Shutdown**.

2. Fill any Fluid Cart containers that appear low.
   - Bleach, deionized water, wash solution, and shutdown solution bottles must be at least 20% full.
   - Focusing fluid must be at least 10% full.

Run shutdown

1. You can shut down the instrument from either the software or the touchscreen.
2. From the software, click **Maintenance**, then click **Shutdown**.

3. Or from the touchscreen, touch **Shutdown**.

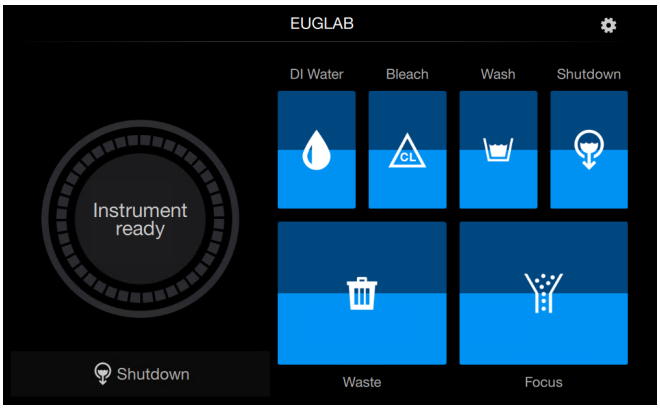


Figure 27 Shutdown from the touchscreen

4. Follow the on-screen prompts.

Sign out

To sign out from the software click **User Information**, then select **Sign out**.

A Maintenance and cleaning

IMPORTANT! Read all of the Maintenance and cleaning sections before attempting these functions.

- To keep the instrument in proper working order, perform the Startup and Shutdown procedures every day the instrument is in use.
- 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 laboratory manager must determine the appropriate cleaning protocol depending on instrument usage. Daily, weekly, monthy and yearly examples can be used as guidelines.

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 76.

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 (high volumes use at >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: For maintenance procedures performed from the **Maintenance menu** on the Flowscape™ Software or the Attune™ Xenith™ Flow Cytometer instrument touchscreen, see “Maintenance menu” on page 80 .

Fluids inspection — daily

1. Ensure that the waste receptacle is empty and the bulk fluids containers on the Fluid Cart are full. Inspect the instrument and fluid cart for leaks or salt residue. Clean any existing spills to enable proper monitoring of potential leaks during operation.

2. Start up the instrument. During **Startup** observe the movements of the instrument syringes and the autosampler syringe.
   - Listen for sounds that are out of the ordinary such as loud grinding or the absence of normal sounds during Startup. Record if possible for support reference.
   - The sample and wash syringes are in the left side compartment of the cytometer. Observe for smooth movement on up/down strokes. Observe for excessive bubbles in the sample upstroke or drops that escape past the plunger. The upstroke of a syringe should not deliver bubbles. Observe for any large air bubbles on the down stroke that might indicate a large leak or worn syringe.
   - The autosampler syringe is in the syringe pump compartment, on the left side. Observe for the same items as listed above. If the syringe is not moving or appears to move in a non-fluid motion, replace the syringe before contacting technical support.

Computer maintenance — monthly

1. Check the space on the D: drive. If less than 50 GB are free, request that users export their data and save it externally.

2. Delete the externally saved experiments from the software.

3. To determine the accounts that use the most data:
   a. Sign in as Administrator.

   b. In **Global navigation ▸ User Management**, click **Reports**.

   c. Click **Usage report**, then click **Export**.

      To filter the **Usage report** by date, turn the **Date Range** option **On**, then select the date range for which to create the report.

   The **Usage report** is saved in the selected file folder on the computer or the network location as a CSV file.

Replace primary focusing fluid filter (200-nm standard filter)

The primary focusing fluid filter is housed in the fluids bay. The standard Attune™ Xenith™ 200-nm Primary Focusing Fluid Filter (Cat. No. 100114360S) reduces background by filtering large particles and contaminants over 200 nm in size out of the focusing fluid as it enters the instrument. We recommend changing the focusing fluid filter every 3 to 6 months, depending on usage.

1. Open the fluids bay door on the left side of the instrument. See Figure 7 on page 17.
2. Unfasten the bracket that secures the filter.
3. Pull the filter straight out of the instrument.
4. Insert the filter and ensure it is fully seated.
5. Fasten the bracket.
6. Discard the used filter in accordance with local regulatory requirements.

Replace primary focusing fluid filter (optional 50-nm filter kit)

The optional Attune™ Xenith™ 50-nm Filter Kit (Cat. No. X20050) contains one 50-nm primary focusing fluid filter and one set of filter caps. This filter can be used instead of the standard 200-nm primary fluid filter provided with the Attune™ Xenith™ Flow Cytometer to filter particulates larger than 50 nm in size out of the focusing fluid as it enters the instrument. This further reduces background on the system that can be caused by debris and particulates, and enables analysis of smaller particles of 160-nm by scatter alone. The filter caps provided can be used to keep the filter wet when not in use on the instrument.

If the optional 50-nm filter is used to improve small particle performance, follow these steps to rinse larger particles from the system and improve small particle performance of the instrument:

1. Follow the instructions for replacing the focusing fluid filter (see “Replace primary focusing fluid filter (200-nm standard filter)”).
2. After installation, run ultra-pure water filtered to 100 nm or below as a sample.
3. Run a 1200 µL sample at 12.5 µL/minute.

   This will turn on the fluidics of the instrument and run sheath for approximately 90 minutes to prime the newly installed filter and rinse out the system with more finely filtered focus fluid.

Replace fluids tubing

The fluids tubing umbilical connects the Fluid Cart with the instrument. The umbilical connection is a numbered manifold inside the fluids bay and the tubing is labeled with numbers for the correct port on the manifold. The umbilical tubing is sold as a kit and can be replaced, if needed.

1. Open the fluids bay. See Figure 7 on page 17.

2. Unfasten the fittings and remove the existing tubing.

3. Install the tubing by matching the tubing ends labeled (FBAY) to the fluids bay and the tubing ends labeled (FCART) to the fluids cart. Tighten the fittings until they snap into place.

Cleaning

The laboratory manager must determine the appropriate cleaning protocol depending on instrument usage. Daily, weekly, monthy and yearly examples can be used as guidelines.

Clean the Sample Injection Port (SIP)

It is important to clean the SIP daily between users. The appropriate cleaning protocol depends on the type of the sample that has been run.

- After the instrument is used to analyze non-adherent cells, such as B and non-activated T cells, perform the **Sanitize** protocol. The **Sanitize** protocol takes about 3–4 minutes.
- After the instrument is used to analyze adherent cells, such as dendritic cells (DCs), red blood cells (RBCs), activated T cells, many cancer lines, bacteria or yeast, run the **Deep Clean** protocol. The **Deep Clean** protocol takes about 60 minutes.
- After the last run of the day run **Shutdown**, which cleans the instrument.

Clean the SIP after last run of the day

Clean the SIP after the last run of each day.
Perform instrument **Shutdown**.

For more information, see “Shutdown” on page 82.

Clean optical filters

If there is dust on the filter, use compressed air or a bulb blower to gently remove the dust.

System decontamination

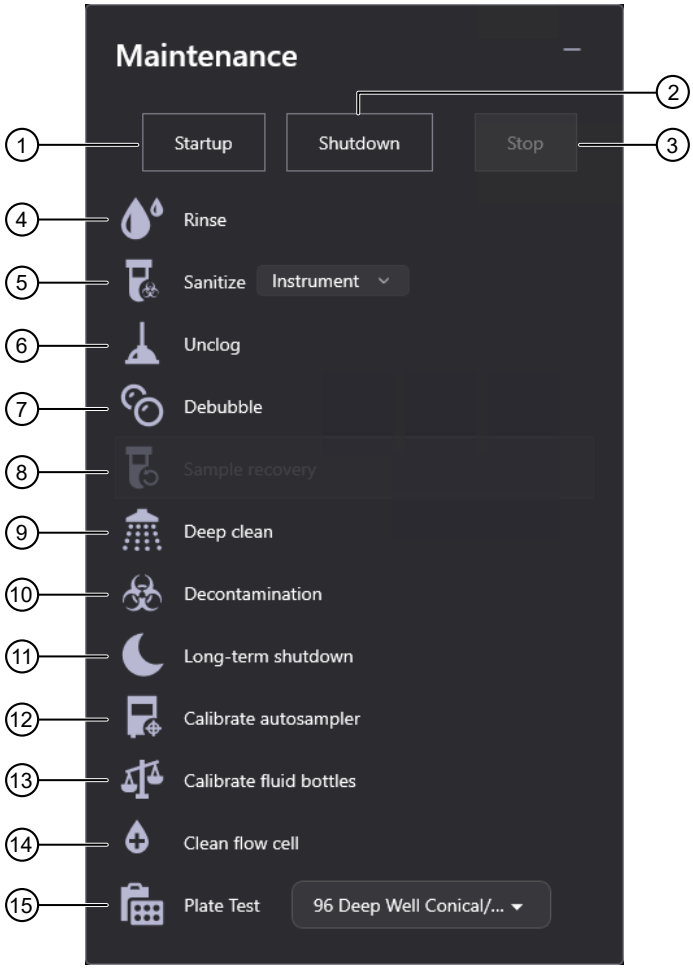
System decontamination fully decontaminates the fluidics system and can be performed every 3–6 months depending on instrument usage and performance.

1. Perform the **Decontamination** protocol.

2. After the **Decontamination** process is complete, follow the on-screen prompts to replace both focusing fluid filters (Cat. No. 100022587 and Cat. No. 100114360S).

Maintenance menu

Maintenance tools are grouped under the **Status bar ▸ Maintenance** tab, which opens the **Maintenance menu**.



1. Startup (page 81)
2. Shutdown (page 82)
3. Stop (page 83)
4. Rinse (page 84)
5. Sanitize (page 84)
6. Unclog (page 85)
7. Debubble (page 85)
8. Sample recovery (page 87)
9. Deep clean (page 88)
10. Decontamination (page 89)
11. Long-term shutdown (page 92)
12. Calibrate autosampler (page 95)
13. Calibrate fluid bottles (page 95)
14. Clean flow cell (page 97)
15. Plate Test (page 98)

Figure 28 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 show 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.

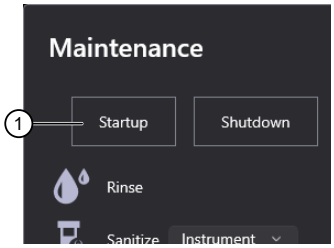
Startup

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.

Shutdown

The **Shutdown** procedure ensures that all sample fluid and dyes have been removed from the fluidics lines of the Attune™ Xenith™ Flow Cytometer and the CytKick™ / CytKick™ Max Autosampler (if connected), and the pumps have been sanitized and filled with Attune™ Shutdown Solution to prevent the formation of salt crystals. This protects the fluidic components, and leaves the system ready for fluidics prime to be run by a subsequent **Startup**. Perform 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. After the initial user-performed tasks, **Shutdown** is automated and does 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.

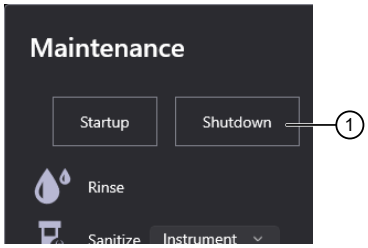
IMPORTANT! Perform the **Shutdown** procedure every day, even if the instrument is intended for continuous use. Proper instrument cleaning helps to obtain consistent and accurate operation.

CAUTION! Cytometer hardware can be contaminated by biohazardous material. We recommend fresh 10% bleach solution in deionized water to sanitize the surface areas of the cytometer. The Attune™ Xenith™ Flow Cytometer dilutes the 100% bleach supplied on the fluid cart to the correct concentration to sanitize the fluid lines and sample pumps.

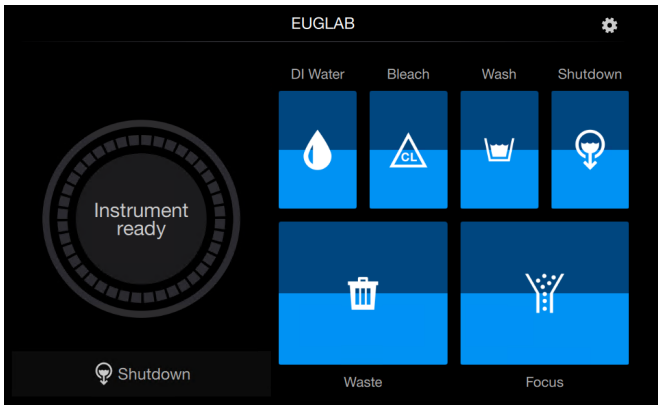
100% bleach is defined as 5.25% sodium hypochlorite in water. We recommend using laboratory-grade bleach. Avoid bleach with additives such as perfumes.

IMPORTANT! Before running the **Shutdown** procedure, ensure that the Wash and Shutdown solution tanks are at least half-full and the waste container is less than 90% full (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.

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.

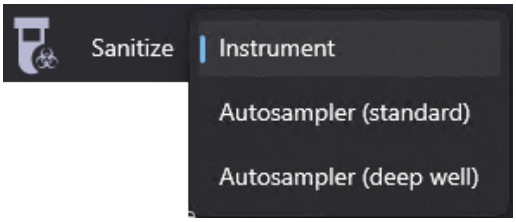
The **Rinse** procedure with or without the CytKick™ / CytKick™ Max Autosampler is intended to remove a majority of residual particles from fluidics components that have contacted sample during acquisition, to leave the cytometer in a fluidics state ready for sample acquisition, and to meet requirements 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.



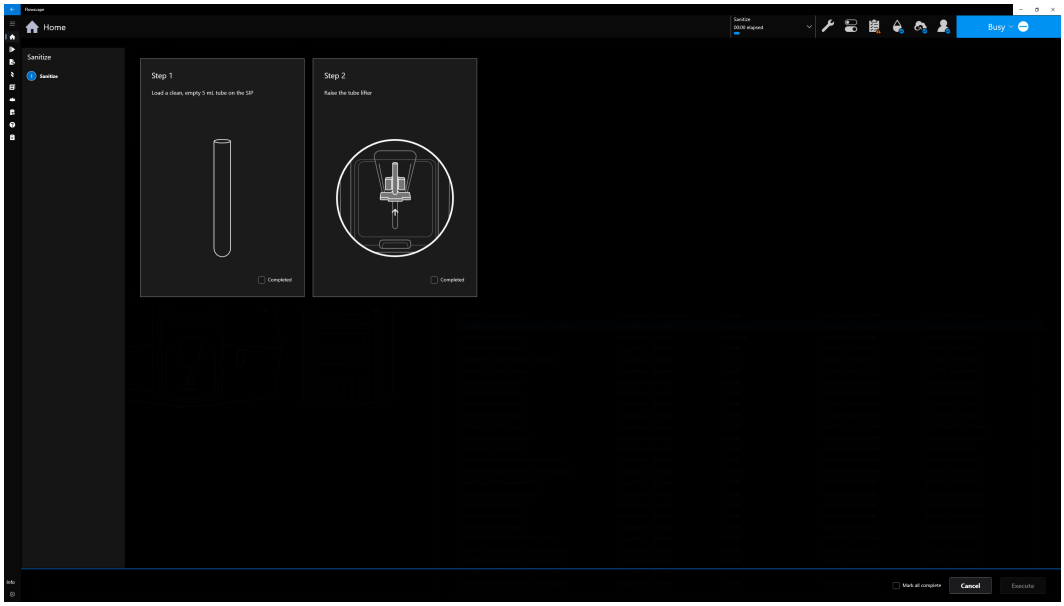
The **Sanitize** menu enables you to select the sanitize procedure to run. Available options are:

- **Instrument**
- **Autosampler (standard)**
- **Autosampler (deep well)**

The **Sanitize** procedure is typically run between experiments, or between samples, to minimize cross-contamination of sample types. It takes about 3.5 minutes to complete.

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.



1. Load a clean, empty 5 mL tube on the SIP. When done, mark the **Completed** checkbox on the Step 1 card.
2. Raise the tube filter. When done, mark the **Completed** checkbox on the Step 2 card.

   Note: To run the **Sanitize** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps in the workflow.
3. Click **Execute** to run the **Sanitize** procedure.

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.

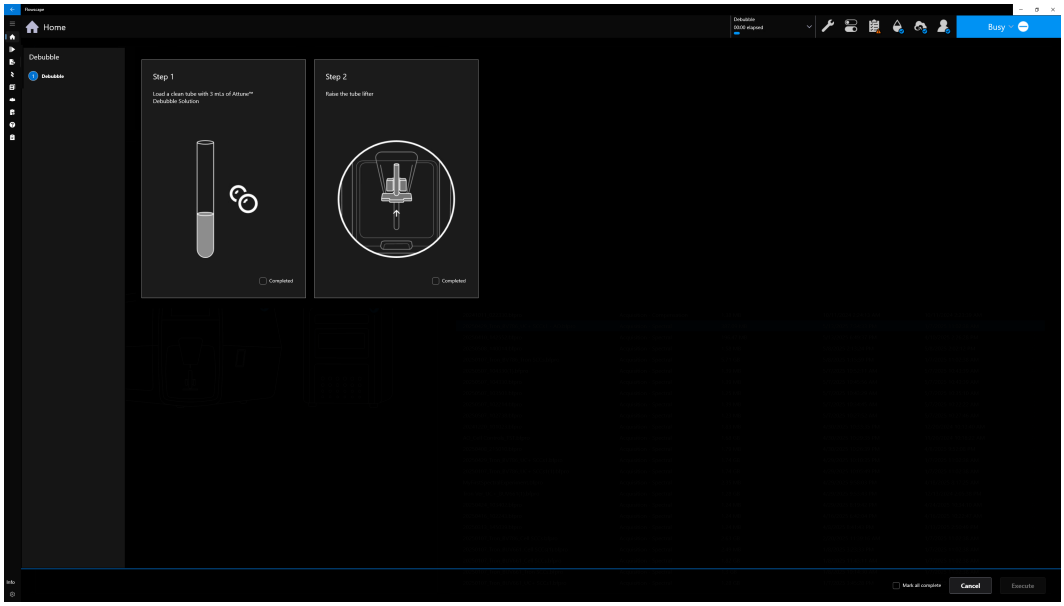
The **Stop** button is inactive during the **Unclog** procedure.

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.

The **Debubble** procedure takes <2 minutes to complete.



1. Load a clean tube with 3 mL of Attune™ Debubble Solution.
2. Raise the tube lifter.

Note: To run the **Debubble** procedure, all steps in the workflow must have their **Completed** checkbox checked.

Alternatively, you can check the **Mark all complete** checkbox in the bottom ribbon bar after you have completed all of the steps in the workflow.

---

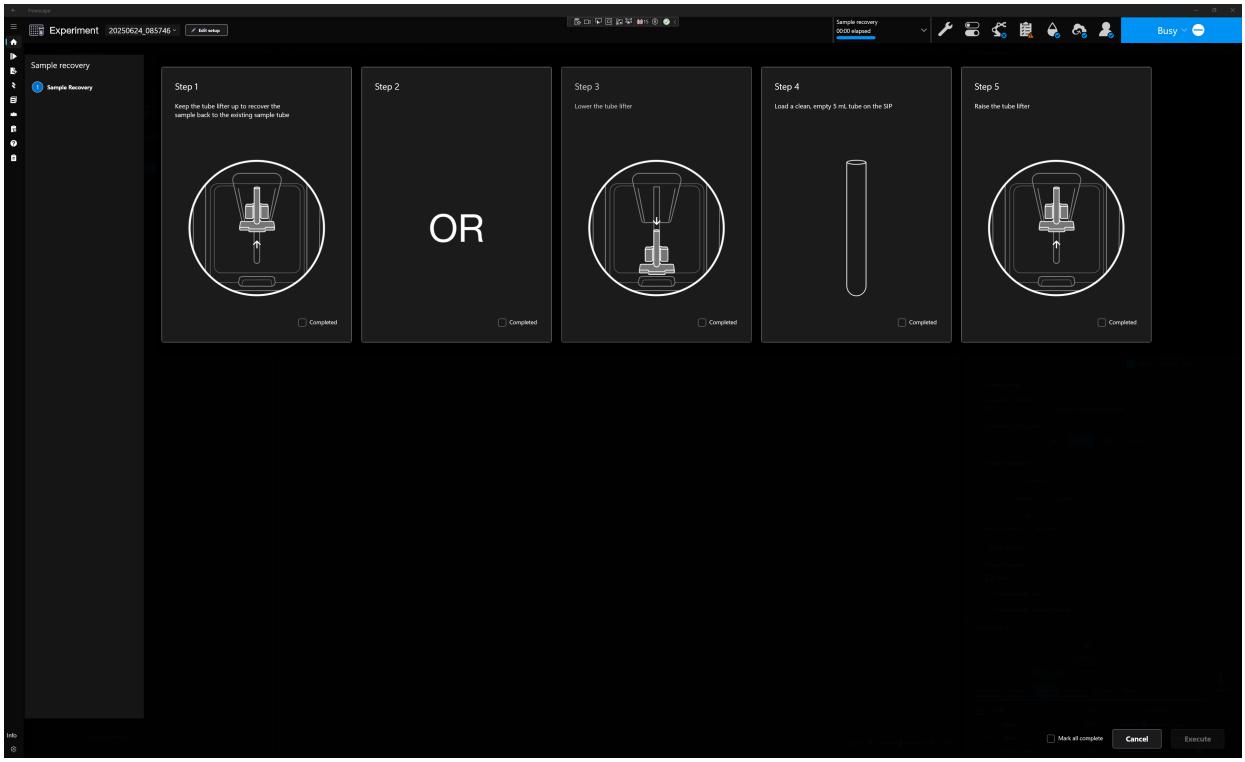
3. After all steps are marked as **Completed**, click **Execute** to run the **Debubble** procedure.

Note: 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 from the sample loop into a tube or back into the sample well (preloaded sample in a plate).

Sample Recovery can be done using 2 different step sequences:



1. **Follow the Step 1 card:** Keep the tube lifter up to recover the sample back to the existing sample tube.

   Note: The **Step 2 OR** card indicates that **Step 1** or **Steps 3–5** can be used to accomplish **Sample Recovery**.

   ---

2. **Follow the Steps 3–5 cards:**

   a. Lower the lifter tube.

   b. Load a clean, empty 5 mL tube on the SIP.

   c. Raise the tube filter.

   Note: To run the **Sample Recovery** procedure, all steps in the workflow (including the **OR** card in **Step 2**) must have their **Completed** checkbox checked.

   Alternatively, you can check the **Mark all complete** checkbox in the bottom ribbon bar after you have completed all of the steps in the workflow.

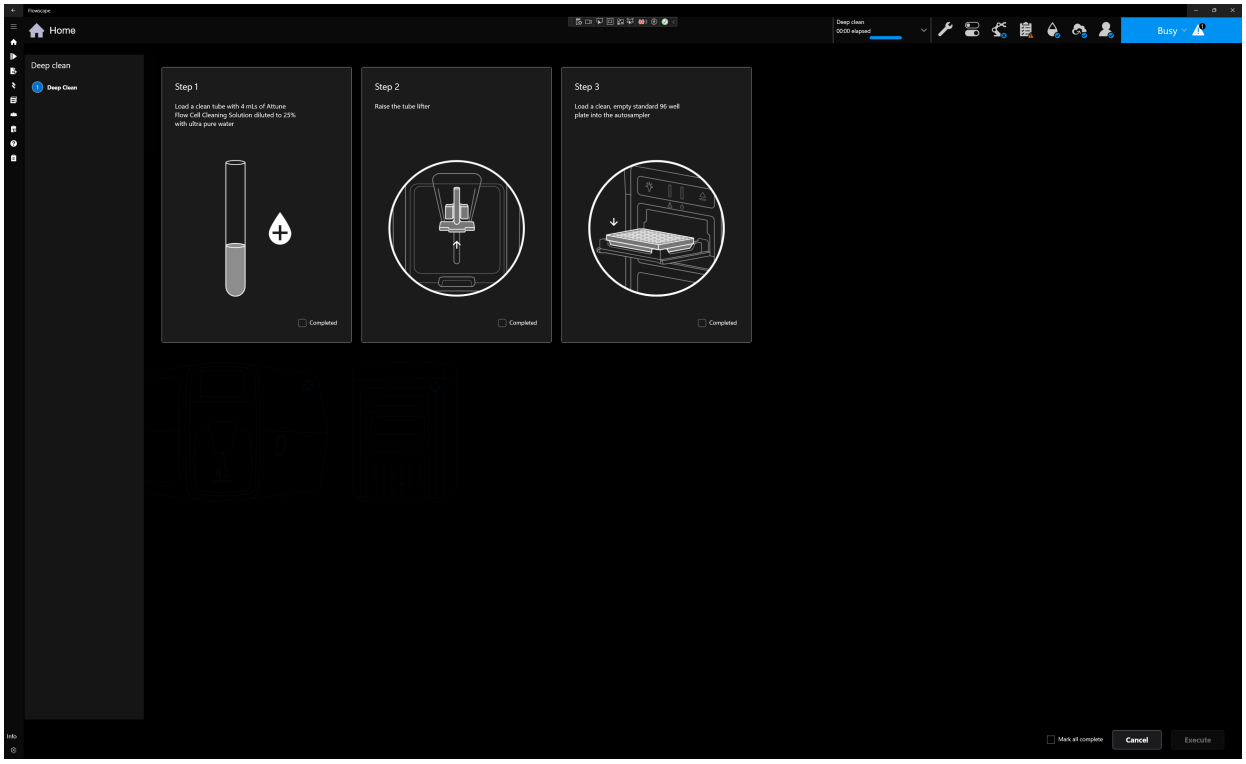
   ---

3. After all steps are marked as **Completed**, click **Execute** to run the **Sample Recovery** procedure.

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. The procedure takes about 1 hour to complete.



1. Load a clean tube with 4 mL of Attune™ Flow Cell Cleaning Solution diluted to 25% with ultrapure water.
2. Raise the tube lifter.

   Note: To run the **Deep Clean** procedure, all steps in the workflow must have their **Completed** checkbox checked.

   Alternatively, you can check the **Mark all complete** checkbox in the bottom ribbon bar after you have completed all of the steps in the workflow.

3. After all steps are marked as **Completed**, click **Execute** to run the **Deep Clean** procedure.

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! A solution of **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**:

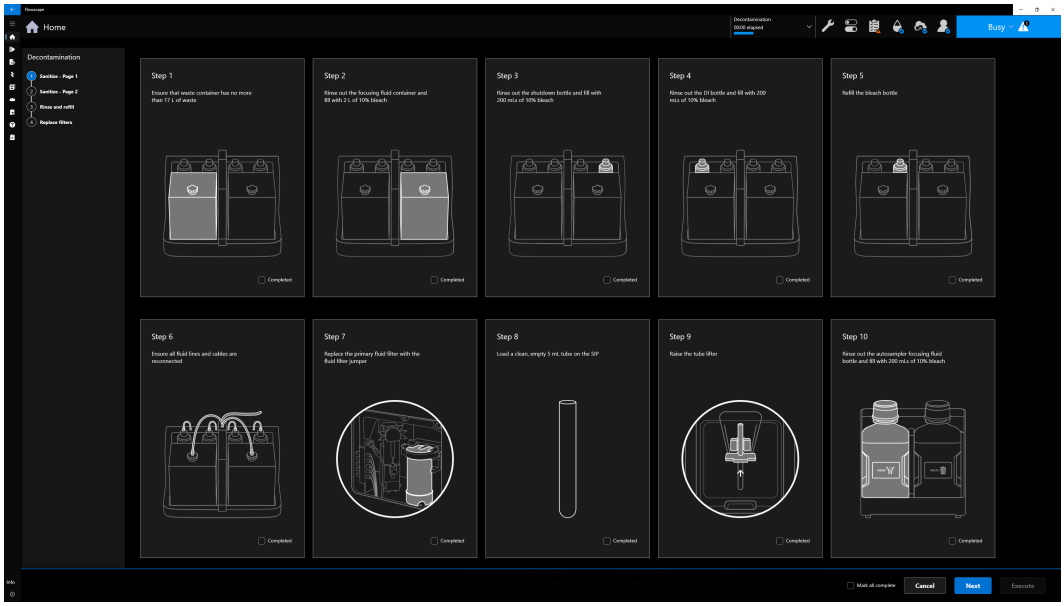
- At least every 3 months, or monthly if instrument is heavily used, to prevent and reduce microbial growth in the instrument.
- If the instrument has been idle for more than two months.
- If the instrument has been idle for more than two weeks without a **Long-term shutdown** run before becoming idle.

Note: If the system is likely to be idle for more than two weeks, perform the **Long-term shutdown** procedure (“Long-term shutdown” on page 92).

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. The entire procedure takes about 2 hours to complete.



1. **Sanitize – Page 1**

    a. Ensure that waste container has no more than 17 L of waste.
    b. Rinse out the focusing fluid container and fill with 2 L of 10% bleach.
    c. Rinse out the shutdown bottle and fill with 200 mL of 10% bleach.
    d. Rinse out the deionized water (DI) bottle and fill with 200 mL of 10% bleach.
    e. Refill the bleach bottle.
    f. Ensure all fluid lines and cables are reconnected.
    g. Replace the primary fluid filter with the fluid filter jumper.
    h. Load a clean, empty 5 mL tube on the SIP.
    i. Raise the tube lifter.
    j. Rinse out the autosampler focusing fluid bottle and fill with 200 mL of 10% bleach.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

2. **Sanitize – Page 2**

    a. Rinse out the autosampler waste bottle and fill with 200 mL of 10% bleach.
    b. Load a clean, empty standard 96-well plate into the autosampler.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

3. **Rinse and refill**

    a. Replace the focusing fluid tank with a new cubetainer.
    b. Rinse out the shutdown bottle with deionized (DI) water and refill with shutdown solution.
    c. Rinse out the DI bottle and refill with proclin-treated DI water.
    d. Rinse out the autosampler focusing fluid bottle with deionized water and refill with focusing fluid.
    e. Keep the tube lifter raised.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

4. **Replace filters**

    a. Keep the tube lifter raised.
    b. Replace the primary fluid filter.
    c. Replace the secondary fluid filter.

Note: Check each **Completed** checkbox once the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

5. After all steps are marked as **Completed**, click **Execute** to run the **Decontamination** procedure.

Long-term shutdown

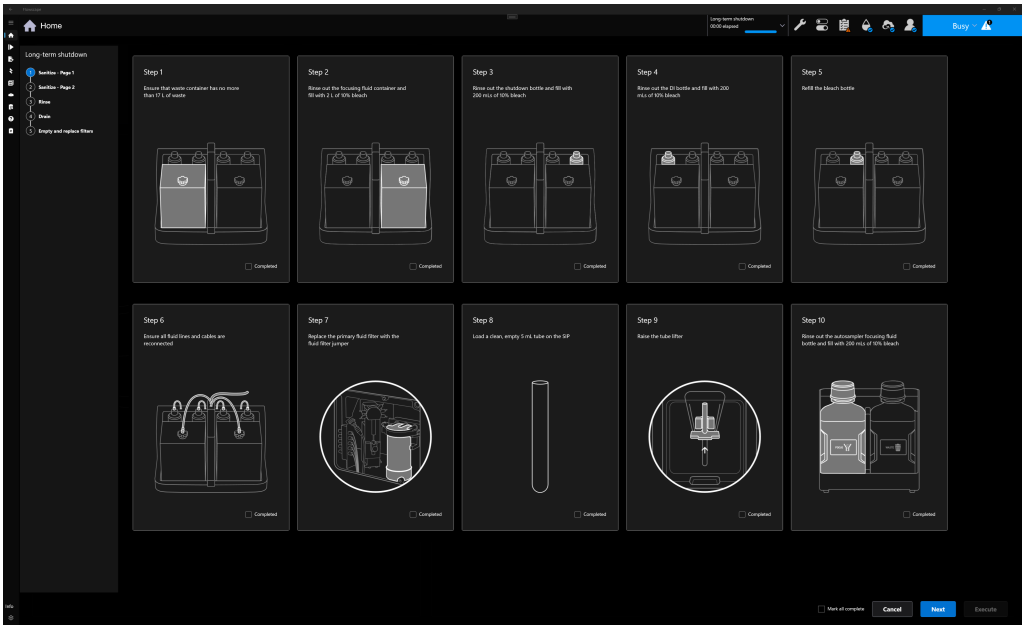
Long-term shutdown enables you to prepare the system for long-term storage and perform a shutdown procedure. The procedure is intended to leave the fluidics components and lines free of microbial growth, in a state that allows the system to subsequently be brought up, ready for use, without replacing 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** screens provide instructions to perform the steps in the **Long-term shutdown** workflow that cannot be automated. The entire procedure takes about 4 hours to complete.



1. **Sanitize – Page 1**

    a. Ensure that waste container has no more than 17 L of waste.
    b. Rinse out the focusing fluid container and fill with 2 L of 10% bleach.
    c. Rinse out the shutdown bottle and fill with 200 mL of 10% bleach.
    d. Rinse out the deionized water (DI) bottle and fill with 200 mL of 10% bleach.
    e. Refill the bleach bottle.
    f. Ensure all fluid lines and cables are reconnected.
    g. Replace the primary fluid filter with the fluid filter jumper.
    h. Load a clean, empty 5 mL tube on the SIP.
    i. Raise the tube lifter.
    j. Rinse out the autosampler focusing fluid bottle and fill with 200 mL of 10% bleach.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

2. **Sanitize – Page 2**

    a. Rinse out the autosampler waste bottle and fill with 200 mL of 10% bleach.
    b. Load a clean, empty standard 96-well plate into the autosampler.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

3. **Rinse**

    a. Rinse out all fluid bottles with deionized water except the waste bottle.
    b. Fill focusing fluid container with 3 L of proclin-treated deionized water.
    c. Fill the deionized water bottle with 500 mL of proclin-treated deionized water.
    d. Fill the bleach bottle with 500 mL of proclin-treated deionized water.

e. Fill the wash bottle with 500 mL of proclin-treated deionized water.
f. Fill the shutdown bottle with 500 mL of proclin-treated deionized water.
g. Rinse out the autosampler focusing fluid bottle with deionized water and fill with 500 mL deionized water.
h. Rinse out the autosampler waste bottle with deionized water and fill with 500 mL of proclin-treated deionized water.
i. Keep the tube lifter raised.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

4. **Drain**

   a. Keep the tube lifter raised.
   b. Empty all fluid bottles and ensure they are dry.
   c. Empty the autosampler focusing fluid bottle and ensure it is dry.
   d. Reconnect all fluid lines and bottle cables.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

5. **Empty and replace filters.**

   a. Empty waste tank and reinstall.
   b. Empty the autosampler waste bottle and reinstall.
   c. Remove the fluid filter jumper, pour contents out to waste.
   d. Replace the primary fluid filter.
   e. Replace the secondary fluid filter.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps on the screen.

---

6. After all steps are marked as **Completed**, click **Execute** to run the **Long-term shutdown** procedure.

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.

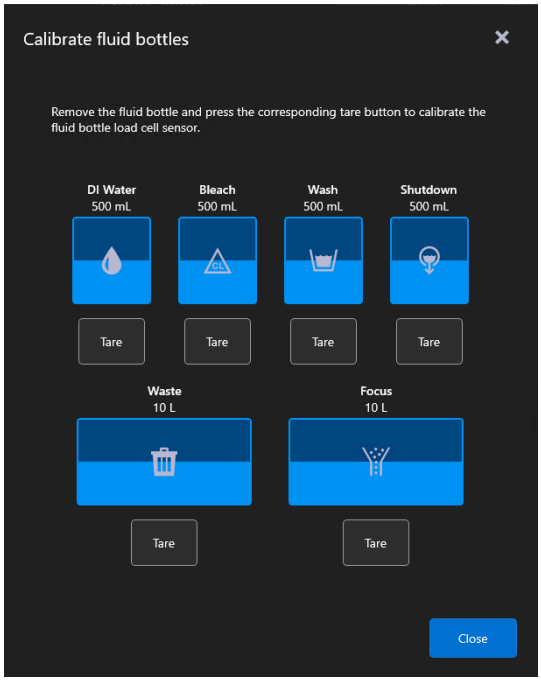
The **Calibrate autosampler** procedure takes about 1 minute to complete.

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 ("Fluidics system" on page 106) and on the instrument touchscreen ("About the touchscreen" on page 22).

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)

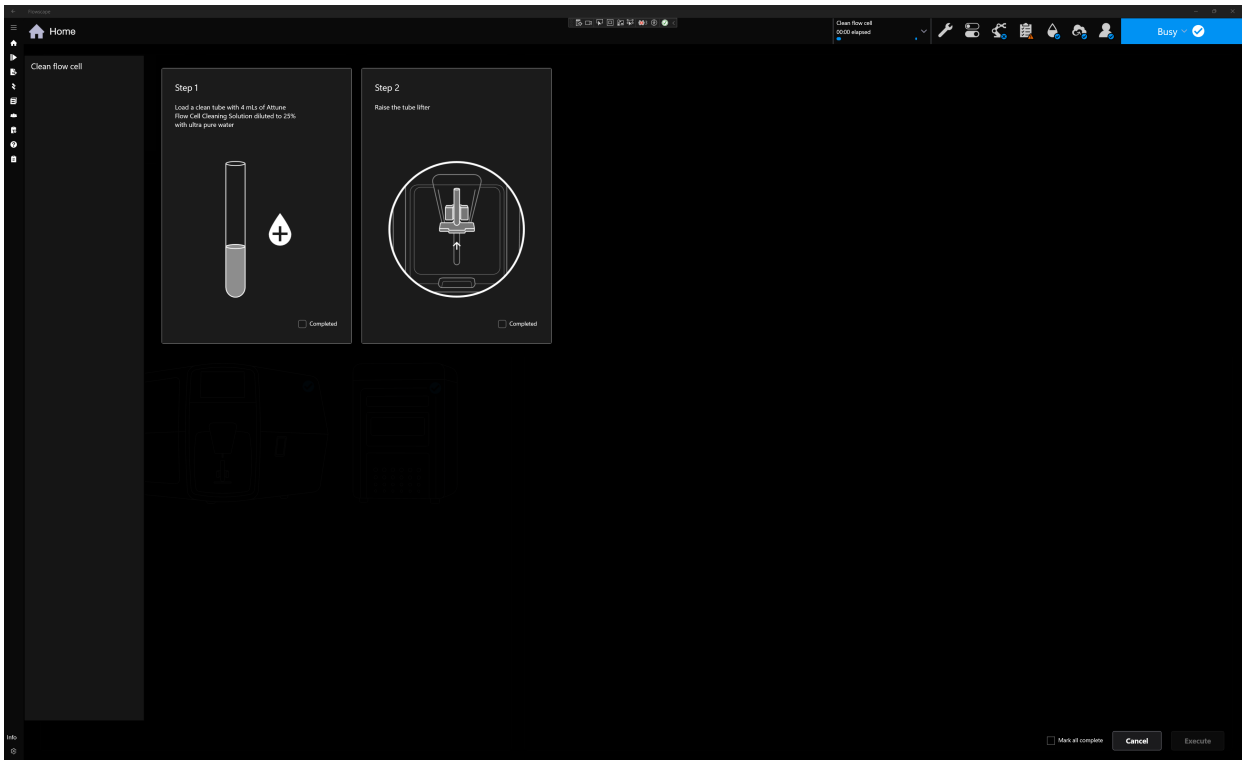
The **Stop** button is inactive during the **Calibrate fluid bottles** procedure.

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.

The **Clean flow cell** screens provide instructions to perform the steps in the **Clean flow cell** workflow that cannot be automated. The entire procedure takes about 45 minutes to complete.



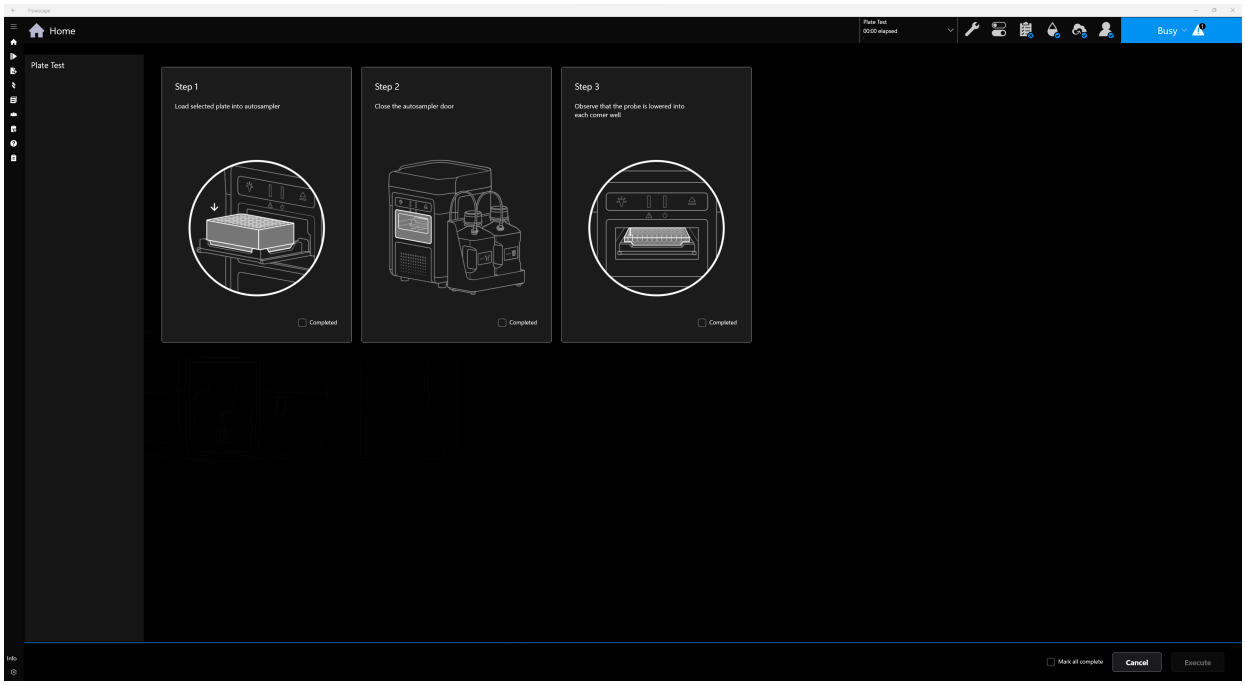
1. Load a clean tube with 4 mL of Attune™ Flow Cell Cleaning Solution diluted to 25% with ultrapure water.
2. Raise the tube lifter.
3. After all steps are marked as **Completed**, click **Execute** to run the **Clean flow cell** procedure.

Note: Check each **Completed** checkbox after the step is done. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps in the workflow.

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.



1. Load the selected plate into autosampler.
2. Close the autosampler door.
3. Observe that the probe is lowered into each corner well.

Note: To run the **Plate Test** procedure, all steps in the workflow must have their **Completed** checkbox checked. Alternatively, you can check the **Mark all complete** checkbox after you have completed all of the steps in the workflow.

---

4. After all steps are marked as **Completed**, click **Execute** to run the **Plate Test** procedure.

B

Specifications

Performance specifications

Table 3 Performance specifications

Alignment	Fixed alignment, no user maintenance required
Sample input rate	12.5 µL – 1,000 µL per minute
Sample delivery	Calibrated delivery volumes for volumetric analysis
Sample analysis volume	20 µL – 4 mL
Fluid storage	Instrument with level sensing
Nominal fluid consumption	3.6 L per day
Sample tubes	Accommodates 1.2 mL, 1.5 mL, and 2.0 mL microcentrifuge tubes, 2.0 mL bullet tubes, and 12 × 75 mm 5.0 mL flow tubes
Particle size detection	0.16 µm to 50 µm
Data acquisition	Up to 35,000 events per second
Resolution	24 bits
PMT voltage	User adjustable
Filters	User changeable, keyed
Software	• Software enables data acquisition and analysis (height, width and area measurements) and controls the instrument. • Automated and manual color compensation modes • Spectral data acquisition • Output format Flow Cytometry Standard (FCS) 3.1 • Live gating with automatic saving • Operator and administrator log-in accounts

Instrument specifications

Table 4 Instrument specifications

Operating conditions (indoor use only)	Ambient temperature range between +15°C (59°F) and +30°C (86°F) Ambient humidity range between 20% and 80% non-condensing.
Transportation conditions	Temperature range between –10°C (14° F) and +40°C (104°F), packed in transport packaging Humidity range between 10% and 90% non-condensing.
Storage conditions	Temperature range between –10°C (14° F) and +40°C (104°F), packed in transport packaging Humidity range between 10% and 90% non-condensing.
Mains power supply	100–240 Vac, 50/60 Hz (nominal), <1500 W
Power consumption	≤1500 W
Heat dissipation	750 BTU/h max.
Audible noise	≤71 dBA at 1 m
Data connectivity	Network cable RJ45
Display/Touch screen	7-inch touch display
Monitor	32-inch flat panel
Computer	Dell 3660 running Windows™ 11 IoT

Physical dimensions

Components (unpackaged)

Ensure that the installation site bench space can accommodate the dimensions and support the weights of the components.

Component	Height	Length (depth)	Width	Weight
Instrument	49 cm (19.5 in.)	61 cm (24 in.)	86 cm (34 in.)	Approximately 65 kg (143 lbs)
Fluid Cart	65 cm (25.5 in.)	76.2 cm (30 in.)	60 cm (23.6 in.)	30 kg (66 lbs)
Computer	36.9 cm (14.52 in.)	42.0 cm (16.53 in.)	17.3 cm (6.81 in.)	~14 kg (30.9 lbs)
Monitor	61.87 cm (24.36 in.)	23.3 cm (9.18 in.)	71.24 cm (28.05 in.)	~14 kg (30.9 lbs)
Keyboard	5 cm (2 in.)	15.25 cm (6 in.)	44.7 cm (17.5 in.)	~0.09 kg (0.2 lbs)
(*Optional*) CytKick™ / CytKick™ Max Autosampler[1]	Autosampler: 40.6 cm (16.0 in.) Side Car: 29.0 cm (11.5 in.)	Autosampler: 28.5 cm (11.25 in.) Side Car: 28.0 cm (11.0 in.)	Autosampler: 29.0 cm (11.5 in.) Side Car: 15.0 cm (5.9 in.)	16.9 kg (37.2 lbs) empty 20.9 kg (46 lbs) with focus and waste bottles at full capacity

[1] If using the optional Orbitor™ RS2 Microplate Mover, the Side Car bottle module must be placed in the back of the autosampler to allow automation access to the tray area.

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more people.

C Reagents and supplies

The Attune™ Xenith™ Flow Cytometer starter kit contains the following items.

Reagent	Cat. No.	Storage conditions	Usage conditions	Stability
Attune™ Focusing Fluid (1X), 1 × 20 L	J106627	15°C to 30°C	15°C to 30°C [1]	The focusing fluid is stable on the instrument for 30 days after the bottle has been opened.
Attune™ Wash Solution	J24974	15°C to 30°C		The wash solution is stable on the instrument for 30 days after the bottle has been opened.
Attune™ Shutdown Solution	J106628	15°C to 30°C		The shutdown solution is stable on the instrument for 30 days after the bottle has been opened.
Attune™ Xenith™ Quality Control Beads	X20000	2°C to 8°C		The beads are stable for 1 year, when stored as directed.

[1] Reagents can be stored at colder temperatures, but ensure that all reagents are at 15°C to 30°C before running the instrument.

WARNING! CHEMICAL HAZARD Some chemicals used with Thermo Fisher Scientific instruments are potentially hazardous and can cause injury, illness, or death. Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, work with, or dispose of any chemicals or hazardous materials.

D

Technical overview

Operation principles

The Attune™ Xenith™ Flow Cytometer is a benchtop cytometer that uses acoustic pressure to confine the injected particles to a tight central line as the sample passes through the optical cell for interrogation.

This section explains how the system detects and measures scattered light and fluorescence as cells pass through the laser beams.

Sample loading

The sample is delivered to the instrument via the sample injection port. The user defines the collection criteria and engages the tube lifter, which then delivers the sample to the flow cell. The sample is pushed through a capillary assembly where it is sheathed in focusing fluid and sent past the laser beams for interrogation. The capillary assembly is an acoustic resonant device that focuses cells or particles into a single, tight line using a capillary coupled to a piezoelectric transducer.

Acoustic focusing

Acoustic focusing exploits the size, density, and compressibility differences between cells or particles relative to the background carrier medium to position the particles or cells into a single, focused line along the central axis of a flow channel. Tightly controlled particle positioning enables high sensitivity and precision at high sample flow rates.

Sample interrogation

As the sample traverses the laser interrogation point, multiple lasers illuminate the particles or cells in the sample, one at a time. Each event scatters and emits light from fluorescent dyes attached to the particle or cell. Optical filters and mirrors route specified wavelengths of the resulting light scatter and fluorescence signals to the designated optical detectors.

Signal processing and results

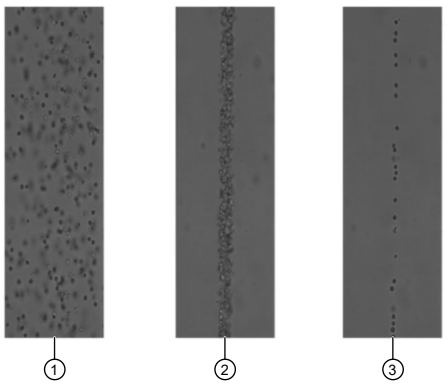
The instrument uses PMT detectors for converting the fluorescence signals and collected light scatter into voltage pulses, which are proportional to the intensity of the light received by the detectors. The analog and digital electronics of the cytometer amplify and analyze these pulses, then transfer them to the workstation computer for further processing by the software.

Acoustic focusing

The goal in flow cytometry is to measure the properties of individual particles as they move through the laser beam. When a sample in solution is injected into a flow cytometer, the cells or particles are randomly distributed in three-dimensional space and must be ordered into a stream of single particles.

Acoustic focusing exploits the physical differences between cells or particles relative to the background carrier medium to position the particles or cells into a single, focused line along the central axis of a flow channel independent of sample fluid flow. In contrast to a conventional hydrodynamic sheath-focused cytometer, the Attune™ Xenith™ Flow Cytometer uses acoustic radiation pressure in addition to hydrodynamic focusing to enable more sample input options, enabling you to select a greater throughput rate, shorter run time, greater sensitivity, or a combination of them all depending on their individual needs.

The images below show the alignment and concentration effects of acoustic focusing on a whole-blood sample.



1. Acoustic focusing off
2. Acoustic focusing on
3. Dilute sample with acoustic focusing on

Figure 29 Acoustic focusing examples

Effects of acoustic focusing on cell viability

Acoustic focusing differs fundamentally from ultrasonic lysis of cells and is usually gentler on cells than the forces occurring in hydrodynamic focusing.

Ultrasonic lysis of cells relies on cavitation produced at sub-megahertz frequencies where tiny gas bubbles form and collapse with immense local shear and heating in the solution containing the sample. In contrast, the acoustic focusing capillary of the Attune™ Xenith™ Flow Cytometer operates at a frequency well above 1 MHz, where the possibility of cavitation is greatly reduced.

Further, acoustic cytometry is performed with relatively low energy levels at high sample flow rates and the design of the acoustically-driven capillary spreads this energy over the entire length of the capillary, greatly reducing the probability of cellular damage.

Flow rate

In a conventional cytometer, the diameter of the sample core is varied by the pressure difference between the sample stream and the sheath fluid stream. Increasing the sample flow rate enlarges the core diameter, which allows faster data acquisition but lower resolution because the cells are distributed across the sample core stream and may pass through the laser spot off center.

In contrast, the alignment of cells in the Attune™ Xenith™ Flow Cytometer is independent of the total fluid flow through the cytometer. While large changes in the amount of sample injected or the total fluid flow may alter the diameter of the sample core, acoustic focusing arranges the particles in a very small region within the core, which ensures that the cells remain in the optimal position for interrogation by the lasers. This feature of the cytometer allows even dilute samples to be analyzed at a much higher sample input rate and saves the user valuable time.

The Attune™ Xenith™ Flow Cytometer has a range of flow rates from 12.5 µL/minute to 1000 µL/minute, representing sample delivery that is predominantly hydrodynamically focused (low flow rate) to sample delivery that is predominantly acoustically focused (high flow rate).

- At low sample flow rates (e.g., 12.5 µL/minute and 25 µL/minute), the instrument operates predominantly as a hydrodynamic focusing instrument. At these lower rates, the core diameter is the smallest, which yields the best resolution for dim expressing assays relative to an unbound, fluorescent background contributor. These rates are also recommended for small particles (diameter <2 µm).
- At higher flow rates, acoustic focusing is implemented at greater degrees to keep the particles in a tight stream as they enter the laser interrogation region. These rates give the user the flexibility to choose a rate to match their sample concentration. As the sample becomes more and more dilute, the user can move to greater sample input rates. Sample input rates 100 µL/minute, 200 µL/minute, and 500 µL/minute are not recommended for particles <2 µm in size. The highest rate is not recommended for particles <4 µm in size.

Sample concentration

All cytometers are governed by Poisson statistics, which predict the probability of a given number of cells or particles being intercepted by the interrogating laser beam. Although increasing the sample concentration results in greater sample throughput, it also increases the probability of a coincident event, defined as more than one cell present in the interrogating laser beam.

The instrument can maintain its maximum particle analysis rate over a large range of sample concentrations, and it is not necessary to concentrate sample using centrifugation or filtration. The ability of the instrument to analyze dilute sample provides the added benefit of reduced background fluorescence from free fluorophores as well as the capability to analyze remarkably small sample sizes.

Fluidics system

The fluidics system of the Attune™ Xenith™ Flow Cytometer handles the flow of fluids required for acoustic focusing cytometer operation. This includes the fluid flow during sample data collection and for the Startup, Rinse, Sanitize, Unclog, De-Bubble, Sample Recovery, Deep Clean, System Decontamination, Shutdown, and Long-Term Shutdown operations.

The sample to be analyzed is driven by a syringe displacement pump and passes through a bubble sensor along the path of the sample loop before arriving at the capillary assembly. A separate continuous flow pump controls the focusing fluid through the focusing fluid filter and combines it with the sample fluid to allow for particle hydrodynamic focusing. The capillary assembly is an acoustic resonant device that focuses cells or particles in the sample fluid into a single tight sample core using a capillary coupled to a single piezoelectric transducer. The capillary carries the sample core upward through the center of the optical cell, where the particles to be analyzed are intercepted by a tightly focused laser beam for interrogation. After passing through the optical cell, the stream is deposited in the waste container.

Electronics

Voltage pulse

When a cell or particle passes through a focused laser beam, it refracts or scatters light in all directions and can emit fluorescence. The scatter and the fluorescence last only a few microseconds, because the cells or particles are moving rapidly through the focused laser beam. The detectors convert the momentary flash of light into an electrical signal called a *voltage pulse*.

When the cell or particle begins to enter the intercepting laser beam, the signal intensity is low, because only a small part of the particle scatters the light.

The pulse reaches its maximum when the cell or particle is in the middle of the laser beam, and the whole particle scatters the light. Further, the laser beam is brightest in the middle, which causes more light to scatter off of the particle.

As the cell or particle exits the beam, the signal starts decreasing and eventually trails off below the threshold.

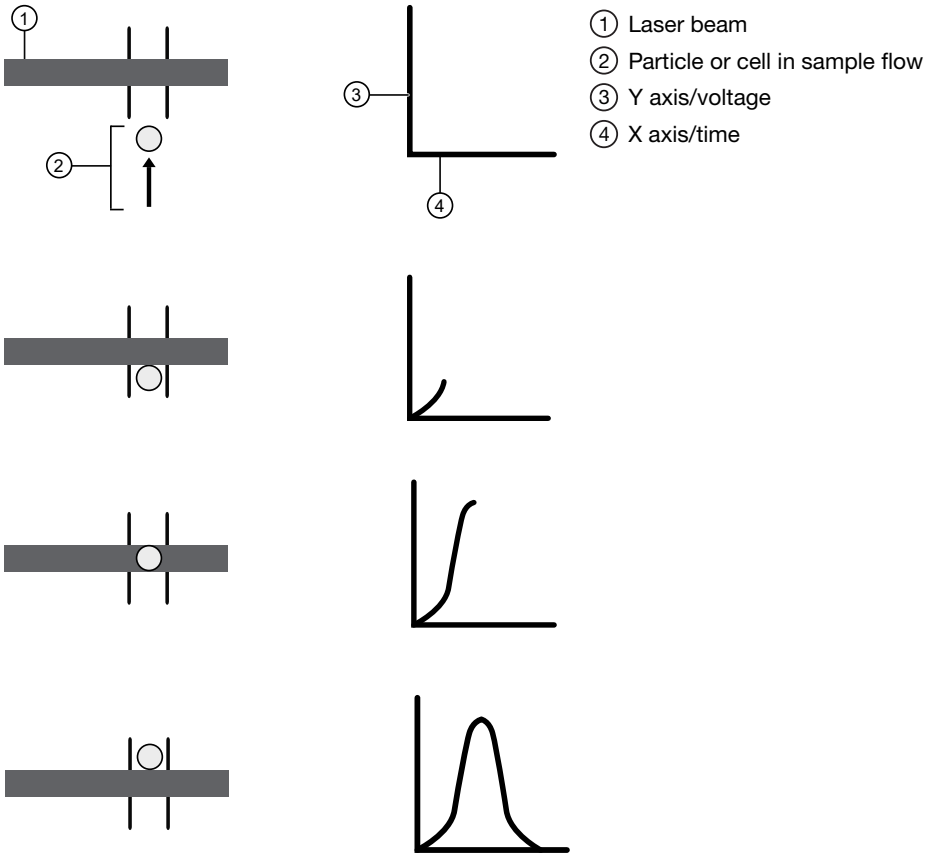
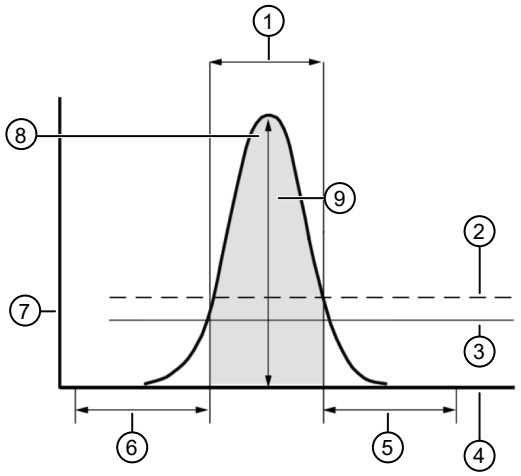


Figure 30 Voltage pulse diagram

Pulse measurement

The analog signal from each detector is amplified and relayed to the 24-bit analog-to-digital converter (ADC), which samples the signals at a rate of up to 25 MHz, converting the continuous signal into digital data up to 100,000 events per second.

The data are further processed by the field programmable gate array (FPGA), which simultaneously calculates pulse height, area, and width when the pulse exceeds the user-specified threshold values. The height is defined by the peak voltage of the pulse, the area is the integrated value of the pulse extending into the front and rear extensions, and the width of the pulse (in units of ADC points) is measured at the user-specified width threshold value.



1. Width
2. Width threshold
3. Trigger threshold
4. X axis/time
5. Rear-front extension
6. Front extension
7. Y axis/voltage
8. Pulse height
9. Pulse area

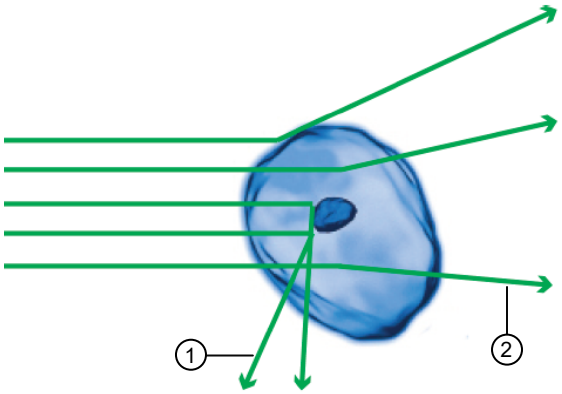
Figure 31 Pulse measurement diagram

Optics

Light scatter

When a cell or particle passes through a focused laser beam, it refracts a scatters light in all directions.

- Forward scatter, or low-angle light scatter, is the light that is scattered in the forward direction as laser light strikes the cell. The magnitude of forward scatter is proportional to the size of the cell or particle, and these data can be used to quantify particle size.
- Side scatter is defined as the light that is scattered at larger angles. Side scatter is indicative of the granularity and structural complexity inside and on the surface of the cell or particle.



1. Side scatter
2. Forward scatter

Figure 32 Light scatter diagram

Polarizing filters

The forward scatter and side scatter detection paths on the instrument are both equipped with polarizing filters and added detectors to discriminate between polarized and non-polarized emissions from the 488 nm laser. Polarization of light is sometimes changed due to interaction with the intercepted cell, and the amount of change is specific to various cell types, such as eosinophil granulocytes. The instrument includes a 50:50 beam-splitting optic that sends half the light from the forward scatter (or side scatter) path to the polar detector. The polarizing filter is placed in front of the polar detector and is precisely oriented such that it blocks the maximum of vertically polarized laser light. In addition, the polarizing filter is on an actuator that can be moved in and out of the light path so the operator can see the effect, with and without the polarizing filter, on the data plot.

Measuring light scatter

Forward-scattered light is quantified by a detector that converts light intensity into voltage. In most cytometers, a blocking bar (called an obscuration bar) is placed in front of the forward scatter detector. The obscuration bar prevents intense laser light from reaching the detector. As a cell crosses the laser, light is scattered past the obscuration bar and is collected by the detector.

Side-scattered light is focused through a lens system and is collected by a separate detector, usually located 90° from the laser's path.

Fluorescence

Fluorescence is the emission of light that occurs when an emitting particle such as a fluorophore-labeled antibody absorbs light from another source such as the intercepting laser beam. When the particle absorbs the intercepting light, it is elevated to an excited electronic state. As it returns to its ground state, the absorbed energy is emitted mostly as light. The emitted light is always a longer wavelength (i.e., less energetic) than the absorbed light. The difference between the excitation and emission wavelengths is known as the Stokes shift.

Flow cytometry uses fluorescence detectors to identify different aspects of cells including functional assays and subset identification. One of the most common ways to study cellular characteristics using flow cytometry makes use of fluorescent molecules such as fluorophore-labeled antibodies. In these experiments, a fluorescently-labeled antibody is added to the cell sample. The antibody then binds to a specific molecule on the cell surface or inside the cell. When laser light of the right wavelength strikes the fluorophore, a fluorescent signal is emitted and detected by the flow cytometer, indicating a specific binding event.

Fluorescence data are collected similar to side scatter data. In a population of labeled cells, some are brighter than others. As each cell crosses the path of the laser, a fluorescence signal is generated. The signal is then directed to the appropriate detector where it is translated into a voltage pulse proportional to the amount of fluorescence emitted. All voltage pulses are recorded and can be presented graphically. Multiple colors can be used on a flow cytometer and the number of colors that can be detected depends on the number of detectors available in the cytometer. The different colors are collected using select optical filters that direct the light to the correct detector.

Optical filters

Optical Filters separate the light scatter and fluorescence by wavelength, which is measured in nanometers (nm). They selectively allow light in a particular wavelength range to pass to the appropriate detectors.

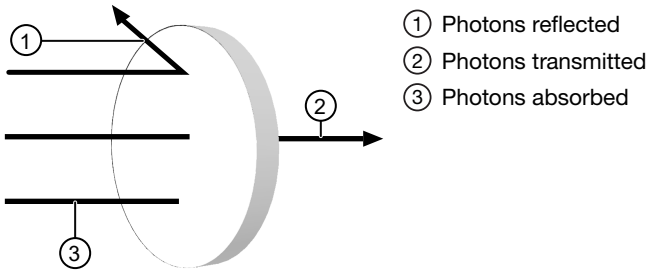


Figure 33 Optical filter

There are several types of optical filters used in flow cytometry:

- Bandpass filter (BP)
- Longpass filter (LP)
- Dichroic mirror (DM)
- Neutral density filter (ND)

Bandpass filter

Bandpass Filter (BP) is an optical interference filter that passes wavelengths in a specific range and reflects wavelengths outside that range. Bandpass filters block all wavelengths outside the selected interval, which can be wide or narrow depending on the thickness and number of layers of the filter.

The bandwidth of the filter is the difference between the upper- and lower-cutoff wavelengths. Common bandpass filter nomenclature is the peak emission/bandwidth.

For example, a filter that detects FITC 488 dye labeled 520/40, allows wavelengths in the 500–540 nm range to pass.

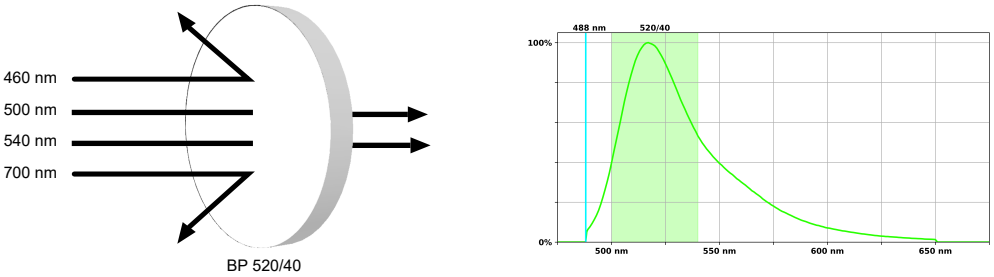


Figure 34 520/40 bandpass filter, 488 nm laser excitation, FITC fluorophore emission

Longpass filter

Longpass filter (LP) is an optical interference filter that reflects shorter wavelengths and transmits longer wavelengths over the active range of the target spectrum (ultraviolet, visible, or infrared). Longpass filters, which can have a sharp slope (referred to as edge filters), are described by the cut-on wavelength at 50% of peak transmission.

For example, an 785/LP filter permits wavelengths longer than 785 nm to pass, and reflects wavelengths shorter than 785 nm.

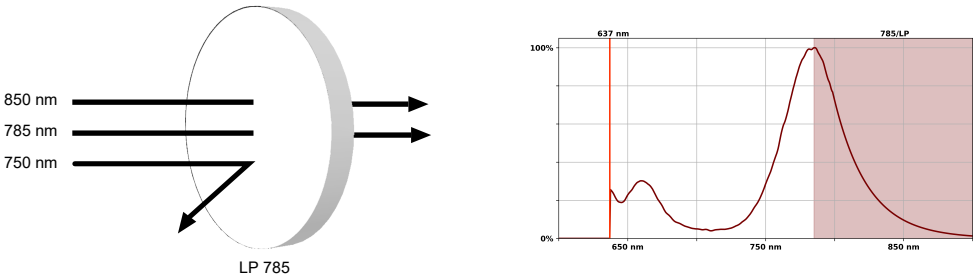


Figure 35 Longpass filter and emission graph (placeholder)

Dichroic mirror

Dichroic mirrors, also called “reflective,” “thin film,” or “interference” filters, are produced by coating a glass substrate with specific optical coatings. Dichroic filters are typically placed at a 45° angle to the light path, where they usually reflect the unwanted portion of the light and transmit the remainder.

Dichroic mirrors are essential to the optical path of a flow cytometer because they direct the emission light to the appropriate photomultiplier tube for detection.

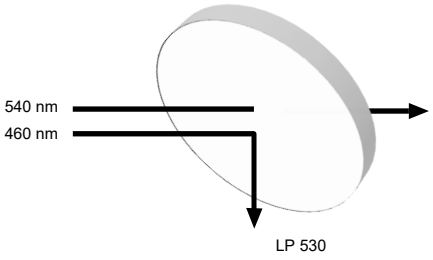


Figure 36 Dichroic mirror

Neutral density filter

Neutral density filters (ND) have constant attenuation across the range of visible wavelengths and absorb light to reduce intensity.

Compensation

Fluorophores emit light over a range of wavelengths. Although optical filters limit the range of frequencies measured by a given detector, when two or more fluorophores are used in an experiment, there is often an overlap between the wavelength ranges. Compensation is the mathematical method used to correct for the overlap of one fluorophore's emission into another fluorophore's emission measurements.

Every fluorescent molecule emits light with a spectrum unique to that molecule. These emission spectra overlap, and in some cases the overlap is significant. The example below shows the emission spectra of Pacific Blue™, Alexa Fluor™ 488, PE, and Alexa Fluor™ 647 dyes.

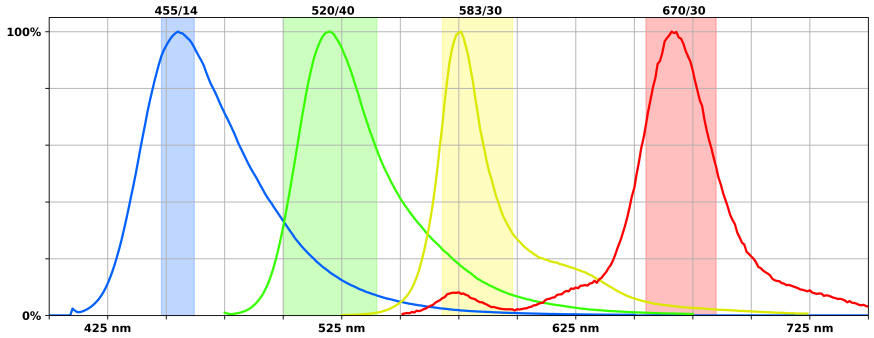


Figure 37 Compensation plot shows fluorophores excited by 405 nm, 488 nm, 561 nm and 637 nm lasers; laser lines not marked in plot. Fluorophores are listed from left to right: Pacific Blue™, Alexa Fluor™ 488, PE and Alexa Fluor™ 647. Bandpass filters are listed from left to right: 455/14, 520/40, 583/30, and 670/30.

Each dye emits with a characteristic emission spectrum that is specific for the fluorophore.

In this example, the emission spectra of each dye is highlighted with an area indicating the emission filter where the specific light is captured by the cytometer. In general, filters are chosen to collect the emitted light near the emission maximum. For example, to capture the emission from Alexa Fluor™ 488 dye, a BP 520/40 filter is used (that is, the filter has a pass-band centered at 520 nm, and the width of the pass-band is 40 nm).

However, it is impossible to select filters that measure only one dye. For instance, the Alexa Fluor™ 488 dye has a significant emission in the region that PE is measured (561 nm). Therefore, the emission from Alexa Fluor™ 488 dye registers in 520 nm and 583 nm bands. If PE is also present, it also contributes to the 583 nm band. Compensation is the mathematical process for correcting for the amount of the Alexa Fluor™ 488 dye fluorescence in the 583 nm band so that PE fluorescence can be accurately measured. Performing multicolor analyses can complicate this process further, because fluorophores usually overlap.

Fluorescence spillover can be estimated by running single fluorescence controls specific for a given dye and then subtracting out the fluorescence in the other detection channels. This leaves the true signal of the other fluorophores. If a fluorescent dye emission is collected through three different filters, then the amount of fluorescence captured through the first filter can be estimated based on how much spillover or contaminating signal is present in the second or the third filters. In the example, the Pacific Blue™ conjugate has some fluorescence in the 520/40 filter and little in the 583/30 filter; therefore, the amount of compensation needed in the 520/40 filter is more than in the 583/30 filter.

Spectral flow cytometry

In conventional flow cytometry, each fluorophore present is measured in a single target detector with a portion of the full emission collected using band-pass or long-pass optical filters and PMTs. Spillover from other fluorophores that may have emission in that detector is corrected using compensation.

In contrast, spectral flow cytometry uses multiple detectors to measure the full spectrum emission of every fluorophore across multiple lasers used in the system to create a more detailed signature for each fluorophore. The spectrum detected by each group or array forms a spectral signature. Although conventional flow cytometry uses compensation to correct for fluorescence spillover, spectral flow cytometry uses a process called unmixing to identify each fluorophore. Spectral unmixing uses a mathematical algorithm that distinguishes the many fluorophore signatures within a multicolor sample, based on the unique spectral signature of each fluorophore. Through this approach, fluorophores with near-identical peak emissions but different off-peak emissions maybe distinguished and used together in a panel. Finally, cellular autofluorescence can be extracted from the fluorescence signal to improve signal resolution. This provides greater flexibility and capability in panel design.

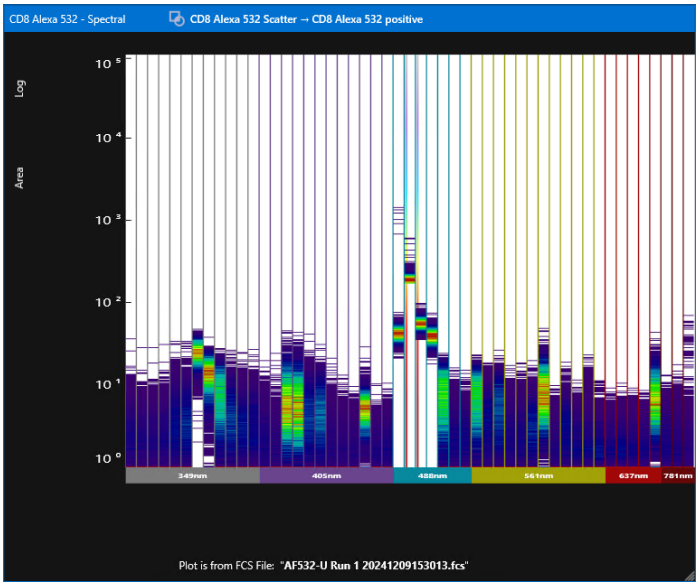


Figure 38 Spectral signature of Alexa Fluor™ 532 dye is shown using the Attune™ Xenith™ Flow Cytometer equipped with six lasers.

With well-designed panels, both spectral and conventional flow cytometers can generate high-resolution data. However, as researchers want to evaluate more parameters, spectral flow cytometry can resolve more individual fluorophores by collecting and processing the data as full spectra. This enables the use of more existing fluorophores that would otherwise be incompatible on a conventional flow cytometer and the expansion of immunophenotyping panels beyond 40 fluorescent parameters.

Comparing spectral and conventional flow cytometry

Conventional and spectral flow cytometers differ in the bandwidth of emitted light delivered to the photodetectors, the number of detectors used per fluorophore, the algorithms employed to separate one fluorophore from another and the ability to differentiate autofluorescence from fluorophore emission.

	Color compensation flow cytometry	Spectral flow cytometry
Wavelength range of detection for a given fluorophore	Near emission maxima	~350–900 nm
Number of detectors per fluorophore	One	Multiple
Spillover correction method	Compensation	Unmixing
Fluorophore selection	Limited by optical configuration	Limited by fluorophore spectral signature uniqueness
Autofluorescence extraction	No	Yes

E

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.
CAUTION! Hot surface.
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Additional safety symbols

Symbol and description
 **CAUTION!** Moving parts.
 **WARNING!** All instrument panels must be in place on the instrument during operation. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, you can be exposed to laser emissions.

Symbole et description
 **MISE EN GARDE !** Parties mobiles.
 **AVERTISSEMENT !** Tous les panneaux de l'instrument doivent être en place pendant son fonctionnement. Une fois tous les panneaux installés, aucun rayonnement n'est détectable. Si un panneau est retiré pendant le fonctionnement du laser, vous risquez d'être exposé à des émissions laser.

Location of safety labels

Label and location

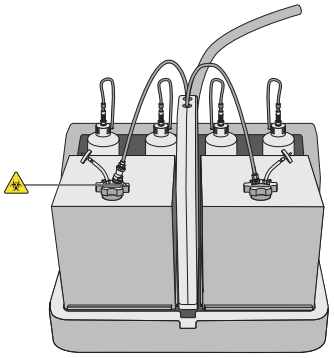



Figure 39 Waste receptacle, below bench left side

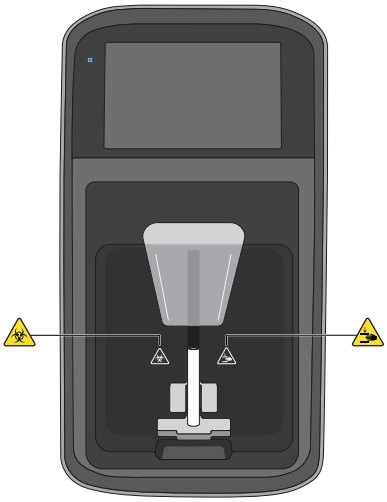



Figure 40 Sample injection port, instrument front

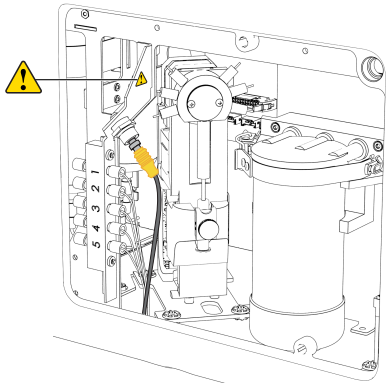



Figure 41 Fluids bay, instrument left side

(continued)

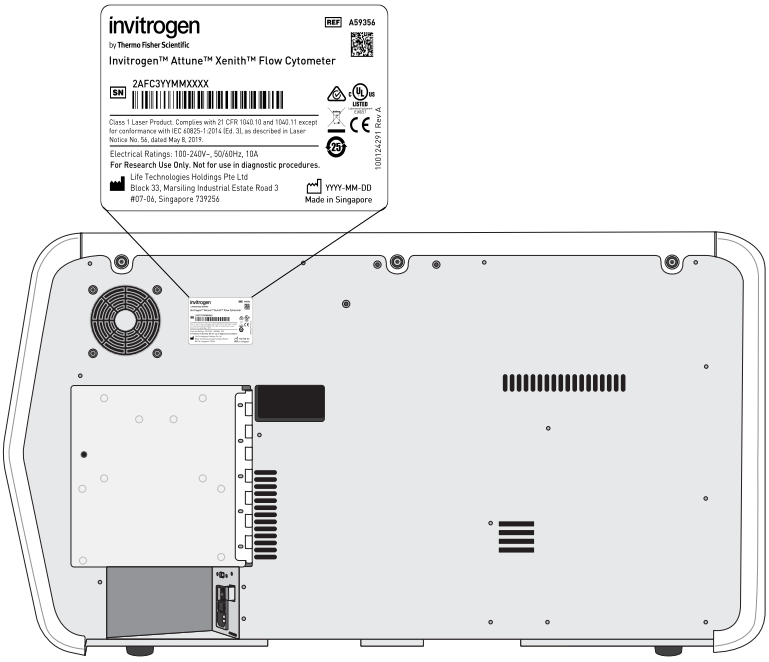
Label and location


Figure 42 Instrument information label, back panel

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Protective conductor terminal (main ground)
	Alternating current

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with China RoHS requirements.
	Indicates conformity with European Union requirements.
	INDICATES CONFORMITY WITH UNITED KINGDOM REQUIREMENTS
	Indicates conformity with Australian standards for electromagnetic compatibility.
	INDICATES CONFORMITY WITH THE WEEE DIRECTIVE 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

If covers are removed, do not use the instrument. Contact Technical Support.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the *Attune™ Xenith™ Flow Cytometer Site Preparation Guide*. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

AVERTISSEMENT ! Installation des fusibles. Avant d'installer l'instrument, vérifier que les fusibles sont correctement insérés et que leur tension correspond à celle fournie par le circuit d'alimentation. Ne remplacer les fusibles que par des modèles du type et de la puissance spécifiés pour l'appareil. L'utilisation de fusibles inadaptés peut endommager le circuit électrique de l'instrument et provoquer un incendie.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Laser safety

WARNING! LASER HAZARD. Under normal operating conditions, the Attune™ Xenith™ Flow Cytometer is categorized as a Class 1 laser product. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class 4 laser. Lasers can burn the retina, causing permanent blind spots. To ensure safe laser operation:

- Never look directly into the laser beam.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others.
- Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing.
- DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.
- Use of controls or adjustments or performance of procedures other than those provided in this guide may result in hazardous radiation exposure.

The following table lists laser safety symbols and alerts that may be present on the instrument.

Alert
 **DANGER!** Class 4(IV) visible and/or invisible laser radiation present when open. Avoid eye or skin exposure to direct or scattered radiation.

Alerte
 **DANGER !** Rayonnement visible ou invisible de classe 4 présent en position ouverte Éviter toute exposition au faisceau.

Laser product hazard classification

The intent of the laser hazard classification is to provide clear distinction of the laser, or laser product properties, and the hazards to users so that appropriate protective measures can be taken. Attune™ Xenith™ Flow Cytometer is a Class 1 laser product meaning operators are not exposed to harmful levels of laser radiation during normal operation, maintenance and/or service.

There six laser sources in the instrument for sample excitation. These lasers vary in wavelength from the UV (349 nm) through visible and into the IR range.

Wavelength	Maximum Power (CW)
349 nm	54 mW
405 nm	100 mW
488 nm	50 mW
561 nm	50 mW
637 nm	90 mW
781 nm	90 mW

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1 CAN/CSA C22.2 No. 61010-2	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 60825-1 EN 60826-1:2014/All:2021	*Safety of laser products – Part 1: Equipment classification and requirements*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with the proper operation.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Radio compliance standards

Reference	Description
Directive 2014/53/EU	European Union “RE Directive”—Radio equipment
RFID	FCC Notice (for U.S. Customers): This device complies with Part 15 of the FCC Rules: Operation is subject to the following conditions: 1. This device may not cause harmful interference, and 2. This device must accept any interference received, Including interference that may cause undesired operation. Changes and modifications not expressly approved by Thermo Fisher Scientific can void your authority to operate this equipment under Federal Communications Commissions rules.
RFID	Canada: This device complies with Industry Canada licence-exempt RSS standard(s). Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device.
RFID	Canada (Français québécois): Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes : (1) l'appareil ne doit pas produire de brouillage, et (2) l'utilisateur de l'appareil doit accepter tout brouillage adioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.