USER GUIDE

invitrogen

Attune™ NxT Acoustic Focusing Cytometer

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Contents

About this guide

Purpose of this guide

This user guide describes how to operate the Invitrogen™ Attune™ NxT Acoustic Focusing Cytometer to acquire and analyze data.

IMPORTANT! For a detailed description of the Attune™ NxT Software, refer to the *Attune™ NxT Software User Guide* (Pub. no. 100024236).

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

CAUTION! Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Conventions

Text and keyboard conventions

Text and keyboard conventions used in the *Attune™ NxT Acoustic Focusing Cytometer User Guide* are listed below. For safety alert words and symbols used in the user guide, see page 8.

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

Clicking

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

User attention symbols

User attention symbols used in the *Attune™ NxT Acoustic Focusing Cytometer User Guide* are listed below. For safety alert words and symbols used in guide, see page 8.

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

Acronyms

The following table explains the acronyms used in the *Attune™ NxT Acoustic Focusing Cytometer User Guide*.

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

Other Attune™ NxT user guides

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

Guide	Pub. no.
Attune™ NxT Acoustic Focusing Cytometer Quick Reference Guide	100024233
Attune™ NxT Acoustic Focusing Cytometer User Guide	100024235
Attune™ NxT Software User Guide	100024236
Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide	100024234
Attune™ NxT Acoustic Focusing Cytometer Site Preparation Guide	100024428
Attune™ NxT External Fluid Supply User Guide	100038577
Attune™ NxT External Fluid Supply Quick Reference Guide	100037944
Attune™ NxT Auto Sampler User Guide	100032905

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

Safety information

Note: See "**Appendix D: Safety**" for the complete the chemical or instrument safety information.

Safety alert words

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

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

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

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

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

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

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Life Technologies are available to you free 24 hours a day. For instructions on obtaining SDSs, see "**Obtaining SDSs**".

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies contact the chemical manufacturer.

1. Product information

Product description

Attune™ NxT Acoustic Focusing Cytometer

The Invitrogen™ Attune™ NxT Acoustic Focusing 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.

Product contents

The Attune™ NxT Acoustic Focusing Cytometer is shipped with the system components listed below. All components are shipped at ambient temperature.

Component	Quantity
Attune™ NxT Acoustic Focusing Cytometer, in one of the following configurations: Blue/Violet/Red/Yellow configuration Blue/Violet/Red configuration Blue/Violet/Yellow configuration Blue/Red/Yellow configuration Blue/Violet configuration Blue/Red configuration Blue/Yellow configuration Blue configuration	1
Power cord kit, universal voltage C13 2.5 m RC	3
Cable, USB 3.0 A-B M/M, 2 m RC	1
Cable, network RJ45 M/M CAT6 STP, 7 ft BLUE RC	1
23-inch monitor	1
Dell™ computer (including mouse and keyboard)	1
Attune™ Performance Tracking Beads, 3 mL	1
Attune™ Wash Solution, 250 mL	1
Attune™ Focusing Fluid, 6 × 1 L	2
Attune™ 1X Shutdown Solution, 250 mL	1
Attune™ NxT Software license	1
Attune™ NxT Quick Reference Card	1

Product use

For Research Use Only. Not for use in diagnostic procedures.

Upon receiving the instrument

Examine the instrument carefully for damage incurred during transit. Ensure that all parts of the instrument, including accessories listed above, are included with the product. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

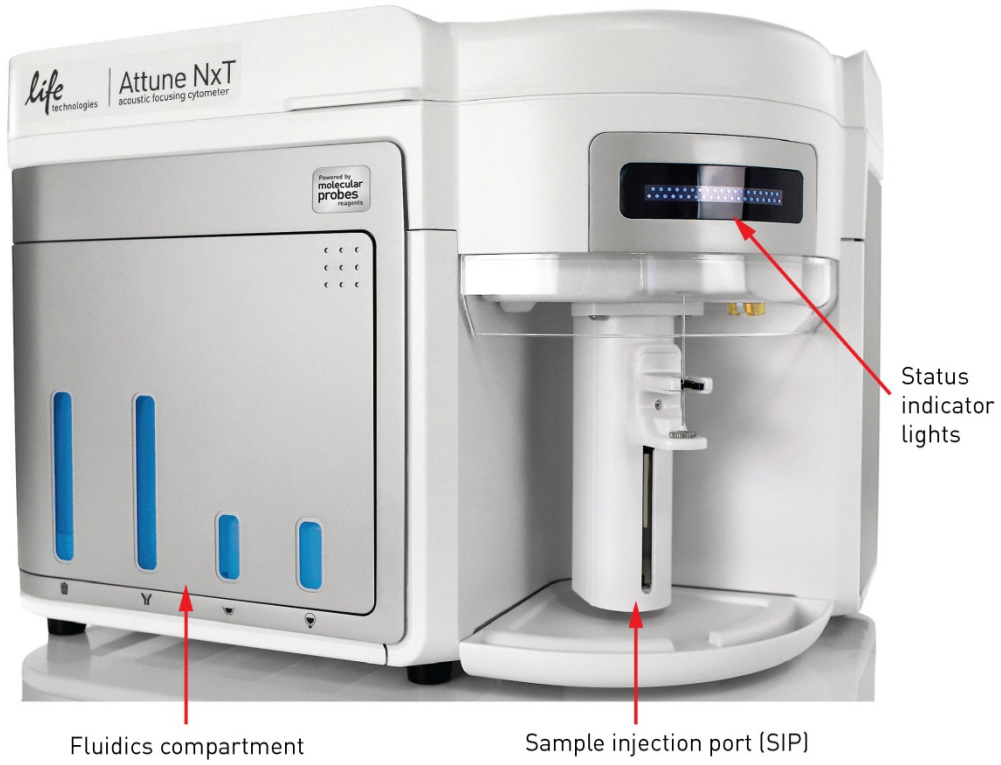
IMPORTANT! Refer to the *Attune™ NxT Acoustic Focusing Cytometer Site Preparation Guide* (Part. no. 100024428) for instructions on installing the Attune™ NxT Acoustic Focusing Cytometer.

Register your instrument

Visit **www.thermofisher.com** to register your instrument. You will be asked to supply the serial number, your name, and your contact details. Registering your instrument ensures that you will receive notifications of software upgrades and information on new assays for use with the Attune™ NxT Acoustic Focusing Cytometer.

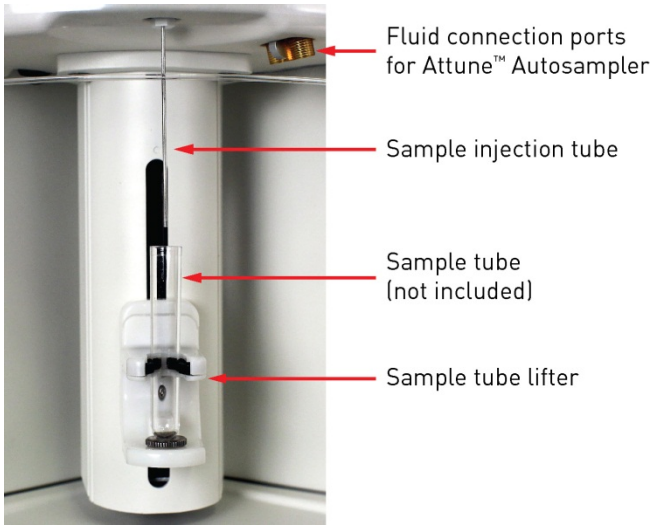
Instrument exterior components

Front view of instrument



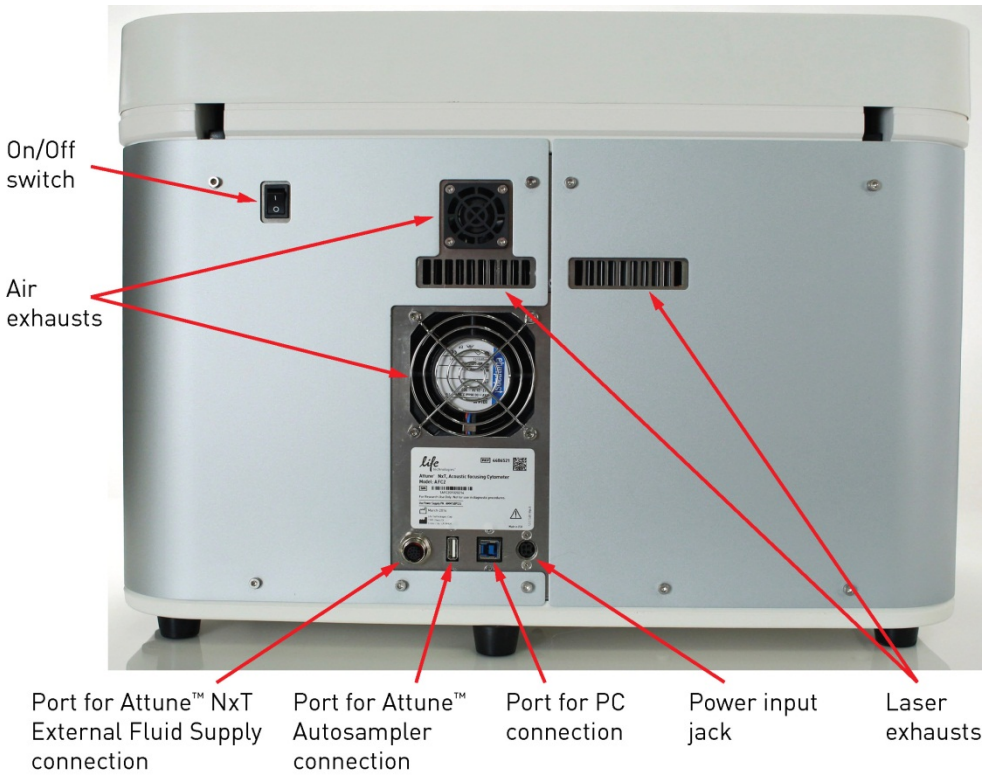
Note: For a detailed view of the fluidics compartment, see page 13.

Sample Injection Port (SIP)

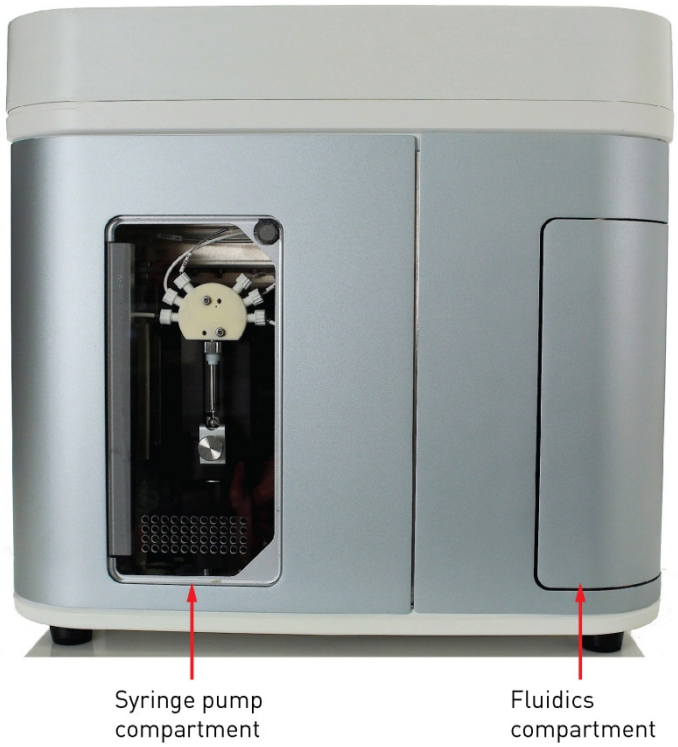


Note: The fluid lines that connect the Attune™ NxT Acoustic Focusing Cytometer to the Attune™ Auto Sampler can be attached to either fluid connection port.

Rear view of instrument



Side view of instrument

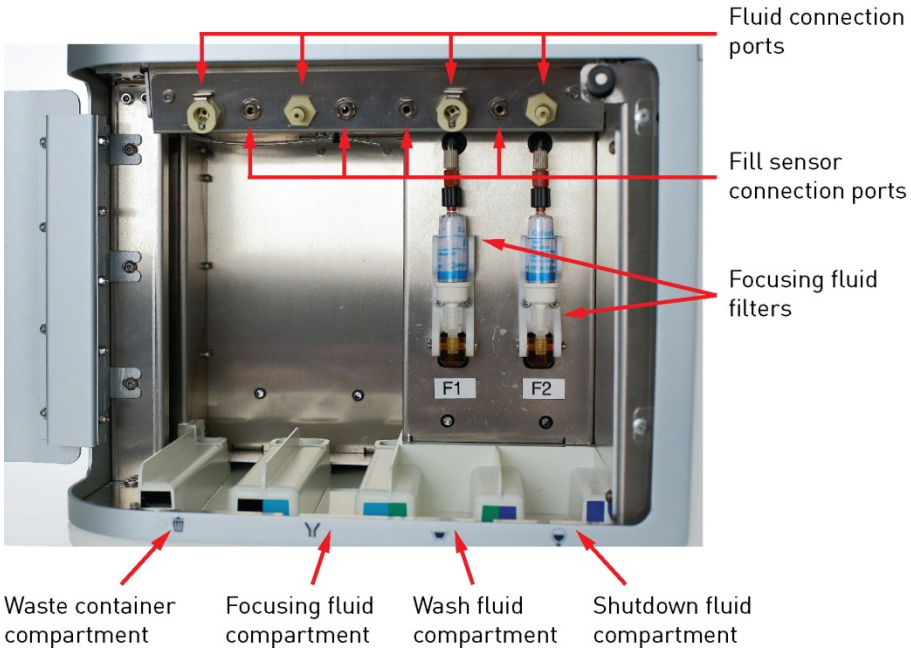


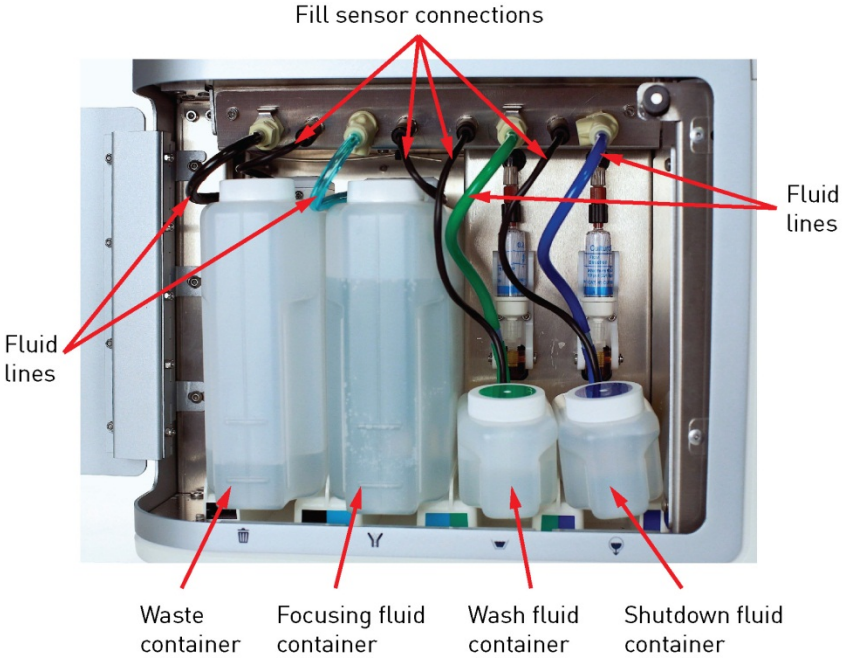
Note: For a detailed view of the fluidics compartments, see page 13; for the syringe pump compartment, see pages 14.

Instrument interior components

Fluidics compartment

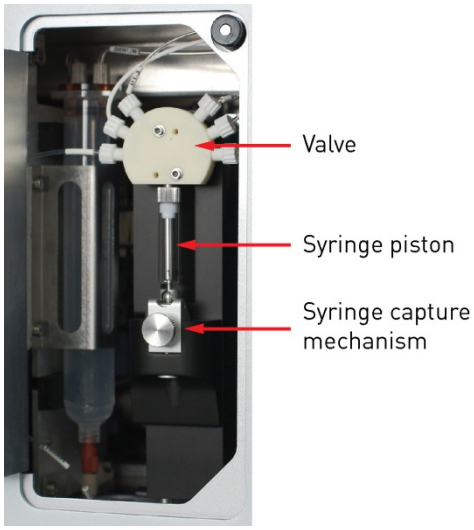
The images below show the fluidics compartment of the Attune™ NxT Acoustic Focusing Cytometer with and without the fluid containers and connections.





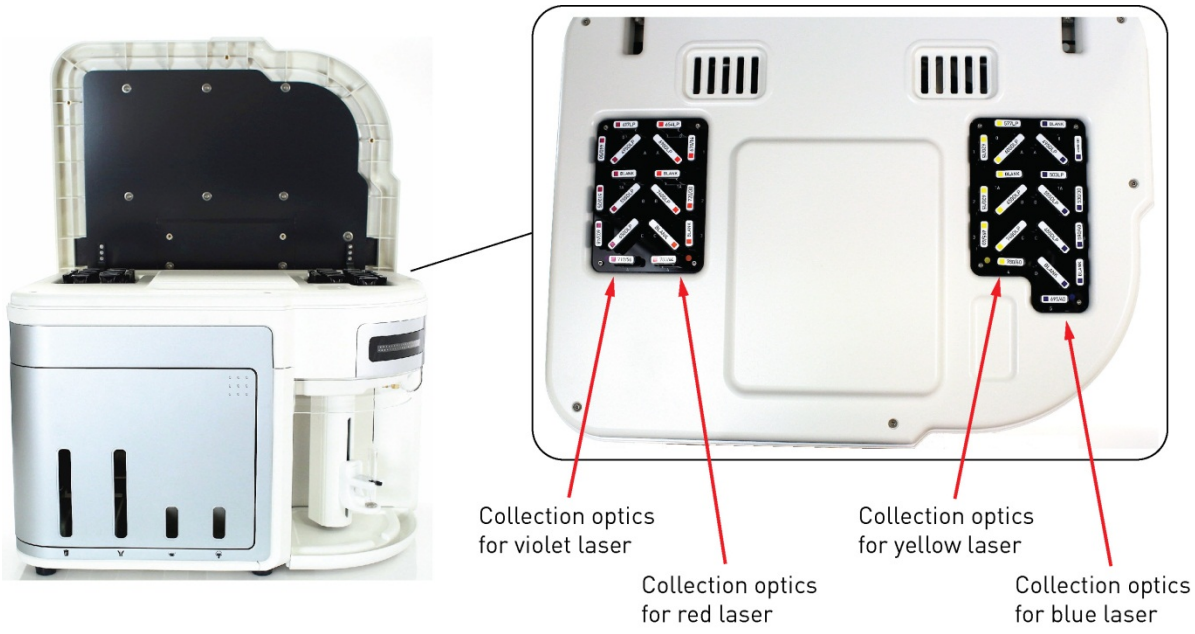
Syringe pump compartment

The image below shows the syringe pump compartment of the Attune™ NxT Acoustic Focusing Cytometer.



Optics compartment

The image below shows the optics compartment of the Attune™ NxT Acoustic Focusing Cytometer. The optics compartment houses the collection optics (i.e., optical filters and mirrors) for the violet and red lasers on the left, and for the yellow and blue lasers on the right.



Note: For a schematic depiction of the overall optical layout of the Attune™ NxT Acoustic Focusing Cytometer, see page 53.

Status indicator lights

The *Status Indicator Lights* above the sample injection port identify the status of the cytometer.

Instrument cycle	Status indicator lights
Startup and all other instrument functions (except Rinse)	Flashing blue
Startup complete	Green solid
Idle	Green solid
Warm up	Blue fade
Warm up complete	Blue solid
Acquiring data/Run	Flashing green
Run complete	Green solid
Wash/Unclog/De-bubble	Green solid
Rinse	Green solid
Clog detected	Amber blink
Focusing fluid container empty	Amber blink
Waste container full	Amber blink
Wash container empty	Amber blink
Shutdown fluid container empty	Amber blink
Shutdown	Green solid
Shutdown complete	Blue fade
Error	Amber blink

Attune™ NxT Software

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

About the software

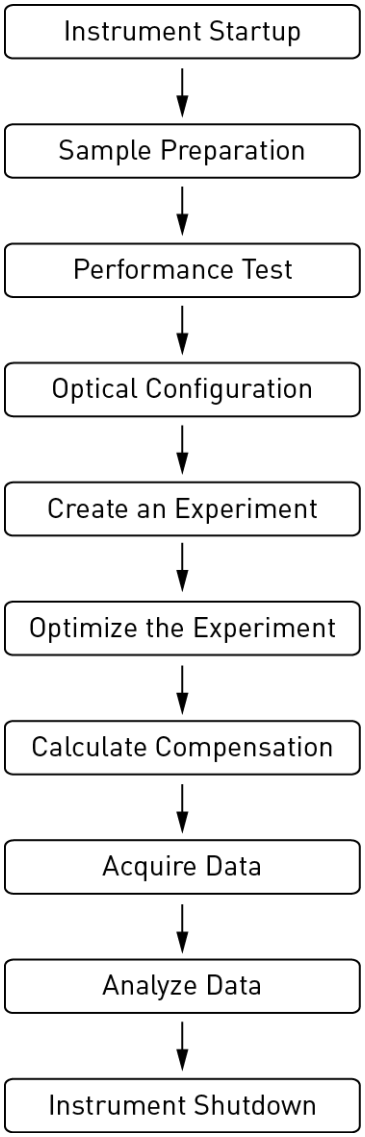
The Attune™ NxT Software is a flexible data acquisition and analysis tool that uses a browser view to:

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

Note: For detailed information on the Attune™ NxT Software, refer to the *Attune™ NxT Software User Guide* (Part no. 100024236), which provided with the product. You can also download the software user guide from **www.thermofisher.com/attune**.

Instrument workflow

The diagram below summarizes the workflow of Attune™ NxT Acoustic Focusing Cytometer. For detailed instructions on each step of the workflow, refer to the appropriate chapter in this user guide. For detailed instructions on using the Attune™ NxT Software, refer to the *Attune™ NxT Software User Guide* (Part no. 100024236) provided with the product and also available for download at **www.thermofisher.com/attune**.



2. Startup

Workflow

Before you begin

Startup procedure

- **Check fluid and waste levels**
- **Fill the fluid tanks**
- **Power on the cytometer and computer**
- **Launch the software and sign in**
- **Run Startup function**

IMPORTANT! Although the daily Startup procedure is automated and requires minimal user input, we recommend that you familiarize yourself with the instrument and its operating principles by reading "Appendix A: System Overview" (page 50) and "Appendix B: Technical Overview" (page 63) before starting your experiments. For a detailed description of the software user interface, refer to the *Attune™ NxT Software User Guide* (Part no. 100024236).

Before you begin

Required solutions

- **Attune™ Focusing Fluid** – 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.
- **10% bleach solution in deionized water** – decontaminates the fluidics lines. Prepare this solution fresh daily and use during the shutdown procedure.
- **Deionized water** – used for diluting bleach, as well as for long-term storage of the instrument.
- **Debubble solution –** a solution optimized for removing bubbles from the Attune™ NxT system.

IMPORTANT! **10% bleach** is defined as a 1 in 10 dilution (1 part bleach to 9 parts water) of 5.25% sodium hypochlorite in water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5000 ppm of available chlorine. We recommend using laboratory-grade bleach. Avoid bleach with additives (such as perfumes).

IMPORTANT! 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.

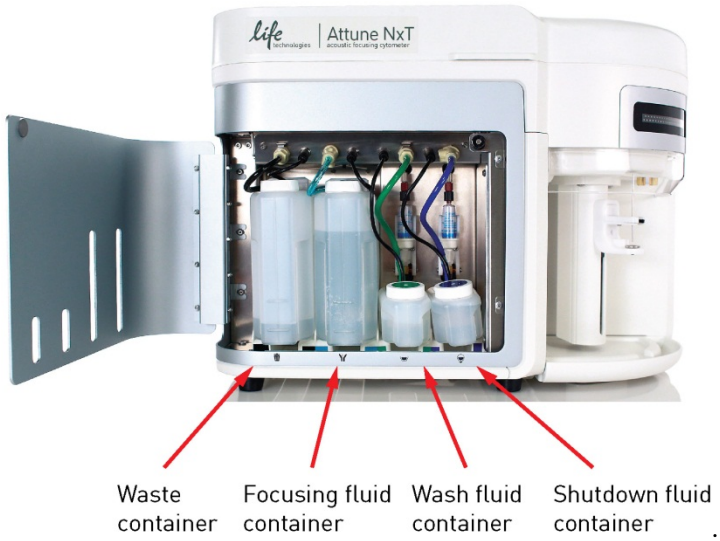
Startup procedure

During Startup, the Attune™ NxT Acoustic Focusing Cytometer:

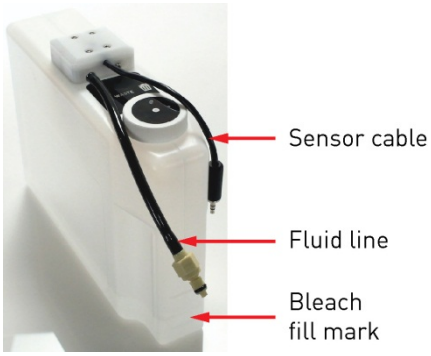
- Warms the lasers to operating temperature
- Initializes the pumps
- Primes the instrument fluidics
- Informs the user of System Status (Ready, Attention, Clog, etc.)

Check fluid and waste levels

1. Check the levels in the fluid containers (see image below).



2. Fill the focusing fluid, wash solution, and shutdown solution containers (see page 21).
3. Empty the waste container. Fill the emptied waste container with full strength bleach solution up to the bleach fill mark on the bottle (see image below).

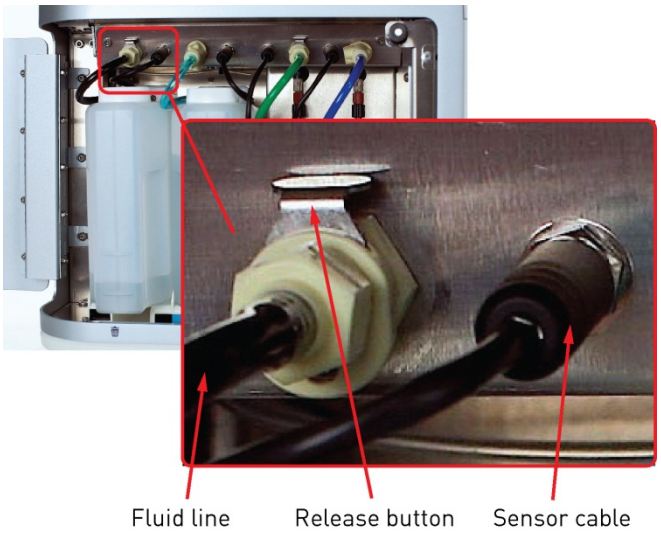


IMPORTANT! The bleach fill mark is the bottom line on the waste container. Be sure to fill the waste container to the middle of the bottom line.

IMPORTANT! The Attune™ NxT Acoustic Focusing Cytometer must be in the idle state (i.e., not acquiring or performing instrument functions) before refilling the fluidics containers.

Fill the fluid tanks

1. Press the metal release buttons to free the tubing and remove the sensor cable from the instrument.



2. Remove the container from the instrument, unscrew the lid, and fill the container with the appropriate solution.

   Note: The focusing fluid container has 1.8 L capacity, and the shutdown and wash solution containers each have 175 mL capacity. Do not overfill the fluid containers.

3. Screw the lid back on without over-tightening it.
4. Replace the container by sliding it into the appropriate slot. Plug the fluid line and the sensor cable back into instrument.

IMPORTANT! For all fluid containers, always connect the fluid line first. Connecting the sensor cable while leaving the fluid line disconnected may result in increased back pressure and introduction of air into the system.

IMPORTANT! Make sure that all fluid containers are placed in the correct orientation with the lids of the containers away from the back of the instrument. If the containers are placed in the wrong orientation, the fluidics lines can become kinked, which will obstruct the flow.

Note: The fluid levels are monitored via floating sensors in all fluidics containers. When the fluid level is low, the waste container is full, or the bottle level sensor is unplugged, the software displays the appropriate warning message and the blue LED on the affected fluid container will pulse. To resume the run, follow the displayed instructions and click **OK**.

Power on the cytometer and computer

1. Flip the rocker switch located on the back of the instrument (page 12) to the ON (up) position to power on the Attune™ NxT Acoustic Focusing Cytometer.
2. Power on the computer and monitor, and wait for the computer to boot.
3. Log in to Windows. The default credentials are:
   - User name: INSTR-ADMIN
   - Password: INSTR-ADMIN

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

Launch the software and sign in

1. Launch the Attune™ NxT Software by double-clicking the Attune™ NxT Software shortcut icon on the desktop.

   Alternatively, select **Start ▶ All Programs ▶ Attune™ NxT Software**.

   The *Login* screen is the first screen that is displayed after the splash screen when you start the Attune™ NxT Software.

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

   The default credentials are:

   - User name: admin
   - Password: admin

   After you log successfully in to the Attune™ NxT Software, the *Main Menu* is displayed. For more information on the Main Menu screen, see "Main Menu" in the *Attune™ NxT Software User Guide*.

Note: After logging in, create a new administrator account and assign a password to it as soon as possible. For more information on user management, refer to the *Attune™ NxT Software User Guide*.

IMPORTANT! A license control mechanism in the form of a DESkey device is required for the operation of the Attune™ NxT Software. If a valid DESkey device is not present, the software will display a warning message. For more information, see "Main Application Startup" in the *Attune™ NxT Software User Guide*.

Run Startup function

The Startup function primes the system fluidics. The Attune™ NxT Software guides you through the Startup function. Make sure to follow all the instructions provided by the software during the procedure. For more information, refer to the *Attune™ NxT Software User Guide*.

1. To initiate the Startup function, click the **Startup** button on the Instrument ribbon tab or the Collection panel. 

   The *Startup dialog* opens and provides instructions to perform the Startup operation.

2. If the tube lifter is raised, lower the tube lifter.

   If your system includes the optional Attune™ Auto Sampler and a plate is loaded in the Auto Sampler, remove the plate.

3. Click **Next** to run the Startup function.

   During Startup, the Attune™ NxT Software automatically turns on the lasers and instrument systems, initializes the pumps, and primes the fluidics lines. The status window displays the Startup operation being performed.

    After the Startup function is completed and no system errors are encountered, the Status bar displays the *Ready* icon.

    If any system errors are encountered during the Startup, the status bar displays the *Alarm* icon.

Note: A fading blue status indicator light above the sample injection port (see page 15) indicates that Startup is under way, and a continuous green light indicates that the instrument is ready.

IMPORTANT! When you power on the instrument, always allow at least 5 minutes for the lasers to reach operating temperature before you run samples.

3. Performance tracking

Note: Results of instrument performance tracking tests are available for all users, but only users authorized by the administrator can perform baseline calculations and Performance tests.

Overview

Performance tracking

Performance tracking is a comprehensive set of procedures to monitor the daily performance of the Attune™ NxT Acoustic Focusing Cytometer. The performance tracking process involves:

- Establishing the cytometer's initial *Baseline status* by running the Attune™ Performance Tracking Beads
- Running the same performance tracking bead particle set to perform the *Performance test*
- Monitoring the changes in the coefficient of variation and the changes in PMT voltages
- Tracking the linearity of the cytometer
- Evaluating the detector quantum efficiency (Q) and optical background (B)

Performance tracking is critical to ensure the accuracy and sensitivity of the cytometer and it provides information about the lasers and detection channels available on the Attune™ NxT Acoustic Focusing Cytometer.

Note: For optimal cytometer performance, follow the periodic and scheduled maintenance procedures as described in the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide* (Part no. 100024234).

Baseline and Performance test (BL/PT) module

The Attune™ NxT Software provides automated *Baseline Calculations* and *Performance test* functions with minimal user interaction and facilitates performance tracking through its *Reports* feature. These functions are controlled by the *Baseline and Performance test (BL/PT) module* of the software.

You can access the BL/PT module by clicking **Performance test** on the Main Menu.

Alternatively, you can click **Performance History** on the Instrument ribbon tab, when the instrument is connected.

For more information on the BL/PT module of the Attune™ NxT Software, refer to the *Attune™ NxT Software User Guide*.

Attune™ Performance Tracking Beads

The Attune™ Performance Tracking Beads (Cat. no. 4449754) are used to define a baseline for cytometer performance and conduct daily performance measurements of the cytometer. They are stained with a combination of fluorophores that can be excited by the lasers used in the Attune™ NxT Acoustic Focusing Cytometers and emit fluorescence signals at designed levels to all the channels in the cytometer.

Each vial of performance tracking beads contains a mixture of four beads at an equal concentration that differ in relative fluorescence emission intensity: blank, dim, medium, and bright. The blank beads in Attune™ Performance Tracking Beads have a nominal diameter of 2.4 µm. The dim, medium, and bright intensity beads have a nominal diameter of 3.2 µm.

IMPORTANT! Prepare the Attune™ Performance Tracking Bead suspension immediately before use. Attune™ Performance Tracking Beads are non-hazardous and may be disposed according to local regulations.

Baseline setup

The *Baseline setup* workflow of the Attune™ NxT Software uses Attune™ Performance Tracking Beads to define the cytometer's initial baseline status. During this process, the median fluorescence intensity of each bead and the r%CV (robust percent coefficient of variation) are automatically measured in all fluorescence detectors. Software algorithms use this information to determine cytometer settings and provide target values for subsequent application specific settings.

How it works

The software guides you through this process to measure the following values for each fluorescent bead using assigned MESF (molecule of equivalent soluble fluorophore) values:

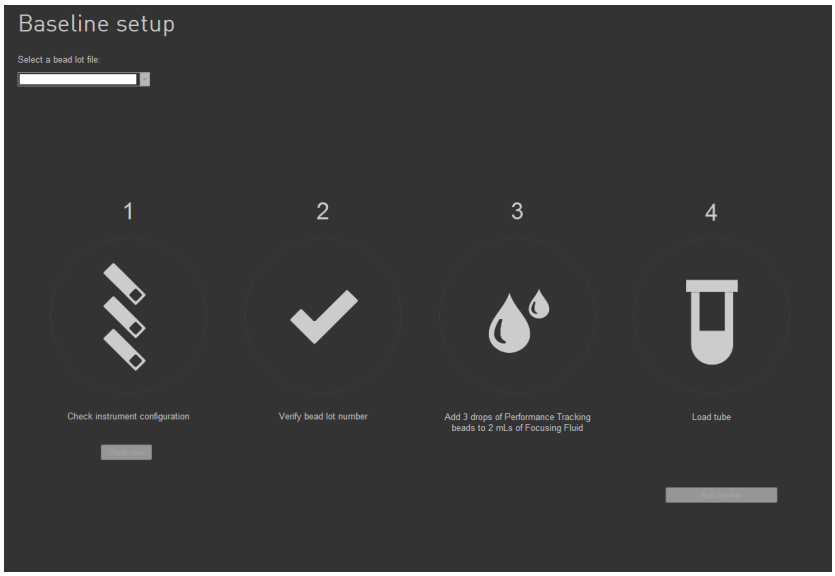
- PMTV (photomultiplier tube voltage)
- Delta PMTV
- Target MFI (target median fluorescence intensity)
- Measured MFI
- r%CV (robust percent coefficient of variation)
- Quantum Efficiency (Q)
- Background (B)
- Linearity
- Area Scaling Factor
- Laser Delay

The Attune™ NxT Software then automatically adjusts the PMT voltages to maximize population resolution in each detector, and creates *Baseline Calculations Report*. For more information on the Baseline Calculations Report, refer to the *Attune™ NxT Software User Guide*.

IMPORTANT! Make sure to perform baseline calculations after any major troubleshooting or cytometer service.

Baseline setup procedure

1. Open the **BL/PT module** (see page 25) to view the *Baseline setup* screen, which provides general instructions for setting up a Baseline.



Note: If a Baseline already exists, the *Performance test setup* screen is displayed instead of the Baseline setup screen. On the Performance test setup screen, you can run a Performance test or reset the Baseline (see page 29).

2. Select the appropriate bead lot file from the Select bead lot file dropdown menu.

   If needed, import a new bead lot file by selecting **Import** at the end of the dropdown menu.

3. Click **Check now** under the filter block image to verify the instrument configuration. The software displays the current instrument configuration.

   Note: This function is not available in early access instruments; it will be available with the next software update.

   If you need to select a different optical configuration, see "Instrument Configuration" in the *Attune™ NxT Software User Guide*.

4. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.

   The bead lot number is the **first six digits** printed on the label (disregard the alpha numeric characters).

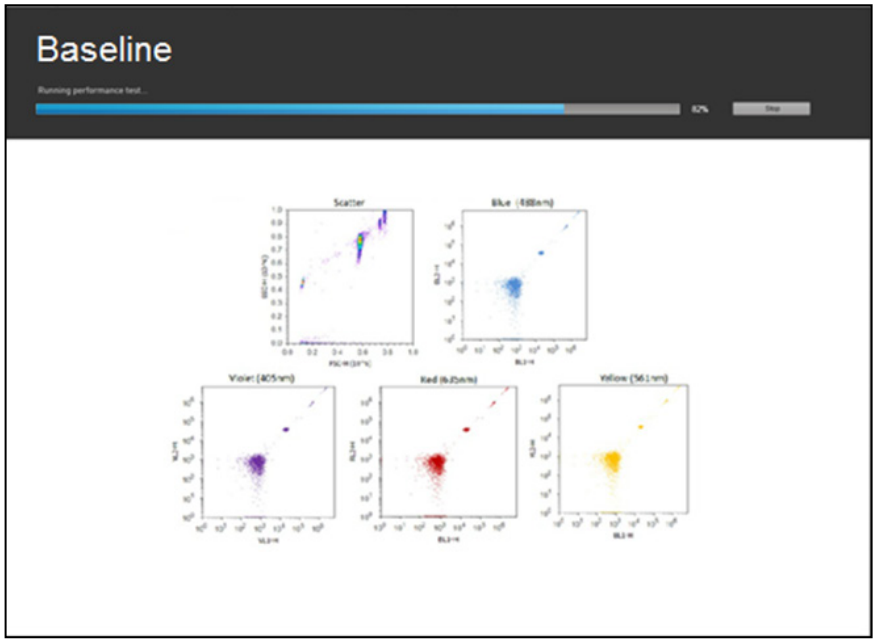
5. Shake the Attune™ Performance Tracking Bead bottle to resuspend the beads, and then add 3 drops of the bead suspension to 2 mL of focusing fluid in a 12 × 75-mm tube. Mix the bead suspension by gentle inversion or vortexing.

6. Load the tube by placing it in the sample tube lifter (see page 11).

7. Click **Run Baseline** to initiate the automated baseline calculations.

   Note: If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Baseline**. You must run the Startup procedure before proceeding. For more information, refer to the *Attune™ NxT Software User Guide*.

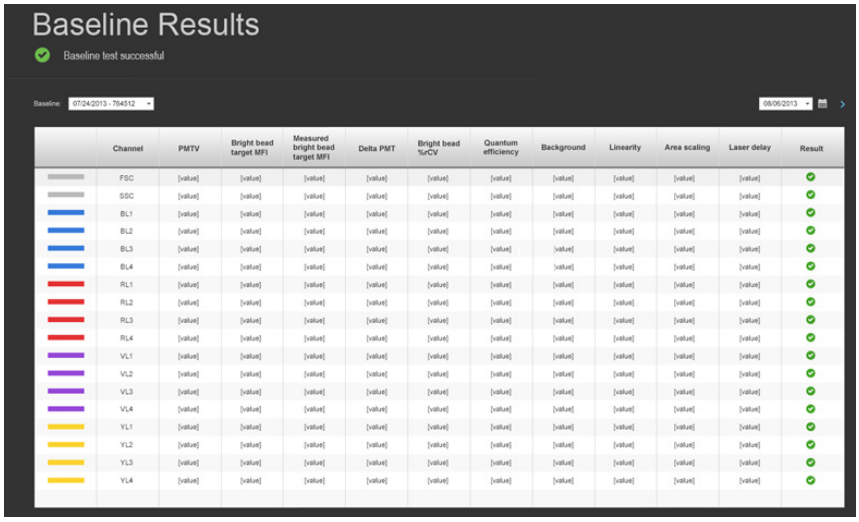
8. The *Baseline screen* provides progress information for the Baseline procedure.



Baseline completion

If the baseline passes, the software:

- Displays the *Baseline Results* screen. For details on how to interpret the Baseline results, refer to the *Attune™ NxT Software User Guide*.



- Calculates the system area scaling factor constant based on the results for each configured laser.
- Updates the default system values for the area scaling factor in the Instrument Settings panel.
- Applies the measured laser delays to the system settings in the Instrument Settings panel.

If the Baseline fails, the software displays a dialog, which provides a list of the channel statistics that failed and recommended actions to remedy the errors.

Note: For detailed information on the Baseline setup function of the Attune™ NxT Software, refer to the *Attune™ NxT Software User Guide*.

Performance test

After the Baseline has been run and the baseline values are defined, the same lot of Attune™ Performance Tracking Beads is used to run the *Performance tests* to measure variation from those baseline measurements to track the daily performance of the cytometer.

During this process, the software measures and records the following observables in all fluorescence detectors:

- Voltage required to place the bright bead in the target channel
- Change in PMT voltage (ΔPMT)
- r%CV (robust percent coefficient of variation) of the bright bead

Using assigned MESF (molecule of equivalent soluble fluorophore) values for each fluorescent bead, the software calculates the following for each channel:

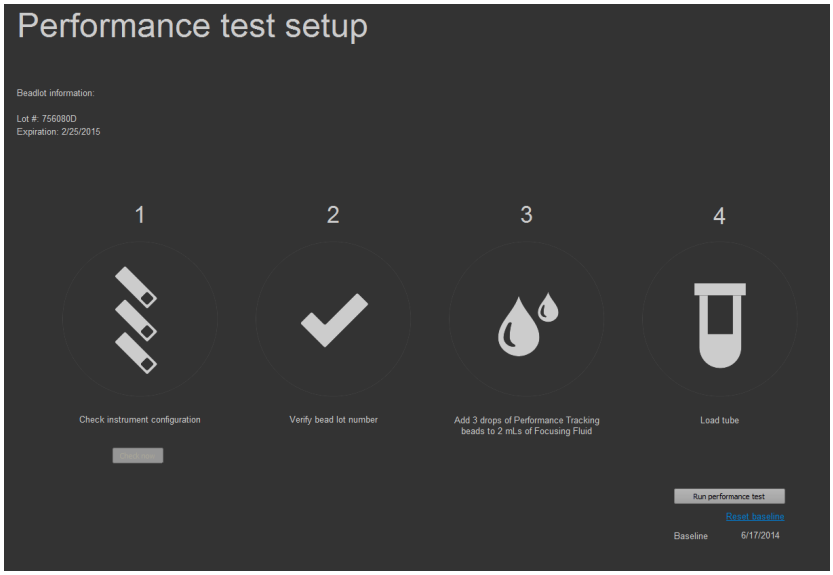
- Detector quantum efficiency (Qr)
- Background level (Br)
- Linear regression
- Laser delay setting

The results of the Performance test can also be viewed as Levey-Jennings charts, which provide a visual to track the r%CV and changes in PMT voltage to check for shifts and trends in cytometer performance.

IMPORTANT! We recommend that you run the Performance test at least once per day when the cytometer is in use.

Performance test setup procedure

1. Open the **BL/PT module** (see page 25) to view the *Performance test setup* screen, which provides general instructions for setting up a Performance test.



Note: If no Baseline exists, the Baseline setup screen is displayed instead of the Performance test setup screen. You must run the Baseline first; see "Baseline setup procedure", page 27.

2. Select the appropriate bead lot file from the **Select bead lot file** dropdown menu. If needed, import a new bead lot file by selecting **Import** at the end of the dropdown menu.

Note: If needed, you can first select a different Baseline against which to run the Performance test, and then click **Reset Baseline** to navigate to the Baseline setup screen.

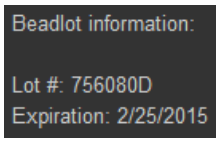
3. Click **Check now** under the filter block image to verify the instrument configuration. The software displays the current instrument configuration.

   Note: This function is not available in early access instruments; it will be available with the next software update.

4. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.

   Make sure that the bead lot number of the performance tracking beads you are using is identical to the bead lot number used in current baseline.

   The bead lot number is the **first six digits** printed on the label (disregard the alpha numeric characters).



5. Shake the Attune™ Performance Tracking Bead bottle to resuspend the beads, and then add 3 drops of the bead suspension to 2 mL of focusing fluid in a 12 × 75-mm tube. Mix the bead suspension by gentle inversion or vortexing.
6. Load the tube by placing it in the sample tube lifter (see page 11).
7. Click **Run Performance test** to initiate the automated Performance test.

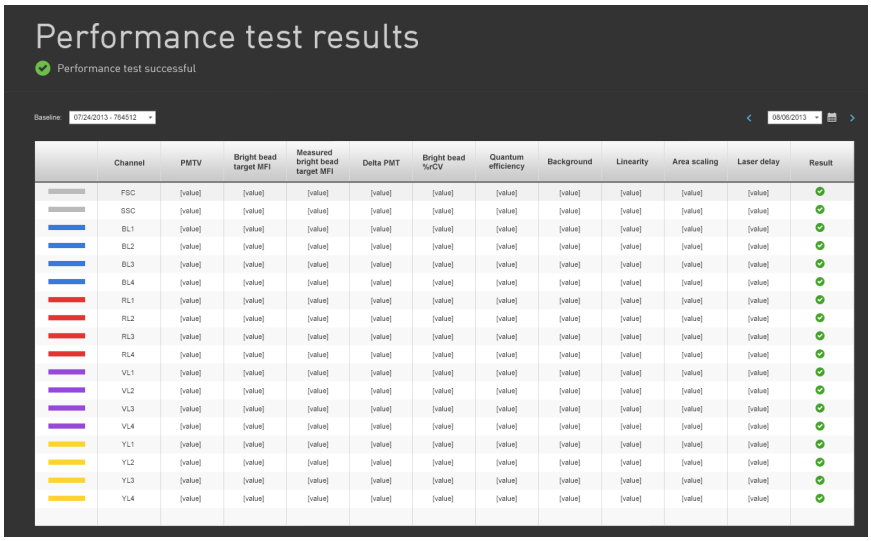
   The *Performance test screen* provides progress information for the Performance test procedure, which takes about 5 minutes to complete.

Note: If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Performance test**. You must run the Startup function before proceeding. For more information, see "Startup" on page 18.

Performance test completion

If the Performance test completes without errors, the software:

- Displays the *Performance test results* screen. For details on how to interpret the Performance test results, refer to the *Attune™ NxT Software User Guide*.



- Calculates the system area scaling factor constant based on the Performance test results for each configured laser.
- Updates the default system values for the area scaling factor in the Instrument Settings panel.
- Applies the measured laser delays to the system settings in the Instrument Settings panel.

If the Baseline completes with errors, the software displays the *Errors Detected* dialog, which provides a list of the channel statistics that failed and recommended actions to remedy the errors.

Note: You can also access the most recent Performance test report by clicking **Current PT result** on the Performance Test ribbon tab of the BL/PT module.

For more information, refer to the *Attune™ NxT Software User Guide*.

Performance test reports

After running the Performance tests, you can generate the following reports in the Attune™ NxT Software:

- Performance history
- Current PT results
- Levey-Jennings report
- Baseline results

For more information on viewing and interpreting the Performance Test reports, see "Performance test reports" in the *Attune™ NxT Software User Guide*.

4. Running samples

Workflow

Before you begin

Recommended flow rates

The Attune™ NxT Acoustic Focusing Cytometer has a range of flow rates from 12.5 µL/minute to 1000 µL/minute.

- At low sample rates (e.g., 12.5 µL/minute and 25 µL/minute), the instrument operates predominantly as a hydrodynamic focusing instrument. These rates are recommended for small particles (diameter <2 µm) and for dim expressing assays relative to an unbound, fluorescent background contributor.
- 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.
- At higher flow rates some loss of sensitivity may occur.

For more information, see "Flow rate" on page 65.

Sample requirements

- The Attune™ NxT Acoustic Focusing Cytometer is designed to handle samples in tubes ranging from 17 × 100 mm to 8.8 × 45 mm.
- The method used to prepare a specimen depends on the sample type and the assay desired.

Startup

If not yet performed, execute the **Startup** procedure as described on page 18–23.

Performance test

If not yet performed, execute the **Performance test** procedures as described on pages 24–31, and verify that the Attune™ NxT Acoustic Focusing Cytometer is in good working order.

Wash procedure between users

We recommend that you sanitize the system between users:

1. Run **SIP sanitize** function with 1 mL of 10% bleach solution (1 in 10 dilution of 5.25% sodium hypochlorite in water).
2. Run **Rinse** function after the SIP sanitize function is complete.

   For more information on SIP sanitize and Rinse functions of the instrument, refer to the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide* available at **www.thermofisher.com/attune**.

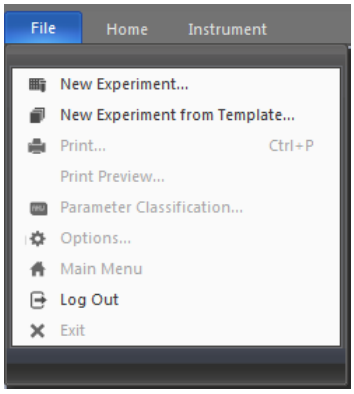
Create an Experiment

To run your samples and collect cytometry data, you need to create an *Experiment* in the Workspace. This section explains how to create an Experiment using the *New Experiment* dialog.

Open the New Experiment dialog

There are several different ways to access the New Experiment dialog. To open the dialog, you can perform one of the following:

- click the **New Experiment** icon on Main Menu or the Home tab
- select **File** ▶ **New Experiment** on the Ribbon bar

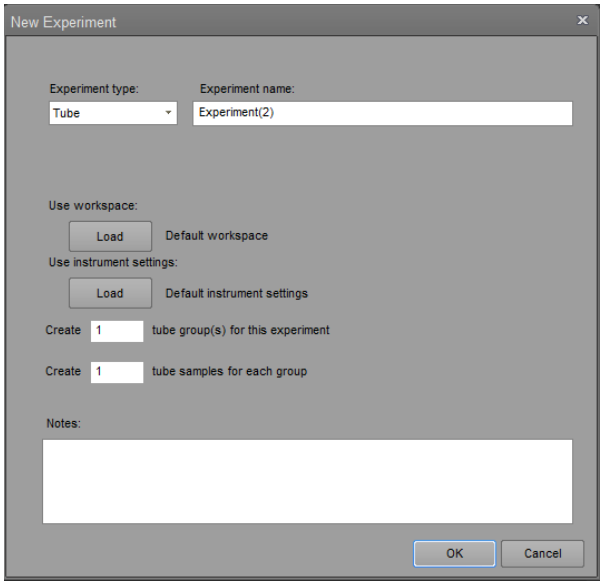


- right-click on the **User Folder heading** in Experiment Explorer, and then select **New Experiment** from the context menu

The New Experiment dialog allows you to create the following experiment types (see page 35):

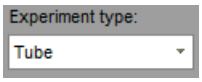
- Tube-only Experiment
- Plate Experiment
- Analysis Experiment using imported FCS files

The contents of the *New Experiment* dialog changes, depending on the experiment type you select. For more information on the New Experiment dialog, refer to the *Attune™ NxT Software User Guide*.



Note: The New Experiment option is enabled only when the instrument is not acquiring.

Create a Tube-only Experiment

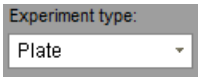


1. From the Experiment type dropdown menu on the New Experiment dialog, select **Tube** (default selection).
2. Accept the default experiment name, or enter a new name.
3. Accept the default workspace and default instrument settings.
4. Enter the number of tube groups to create for the Experiment.
5. Enter the number of tube samples to create for each tube group.
6. Click **OK** to create the new experiment and close the *New Experiment* dialog.

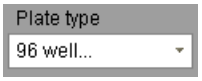
   The software opens the first sample in the Experiment and the Experiment Workspace.

Create a Plate Experiment

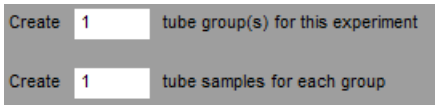
A *Plate Experiment* includes one plate, and can also include tube groups and tube samples.



1. From the Experiment type dropdown menu on the New Experiment dialog, select **Plate**.
2. Accept the default plate name, or enter a new name.



3. From the Plate type dropdown menu, select the type of plate that you are using for the experiment and enter the plate ID.
4. Accept the default workspace and default instrument settings.



5. If you are including tubes, enter the number of tube groups to create for the experiment.
6. If you are including tubes, enter the number of tube samples to create for each tube group.
7. Click **OK** to create the new experiment and close the *New Experiment* dialog.

Note: Plate Experiment function is not available in early access instruments; it will be available with the next software update.

Optimize instrument settings

Before you can record data for a sample, you need optimize instrument settings. Optimizing instrument settings involves fine-tuning the PMT voltages, compensation, and threshold settings for each dye and sample used in the experiment, which allows you to adjust the positions of populations of interest on scale for the scatter and fluorescence parameters.

Note: We recommend that you optimize each individual experiment prior to collecting data. If compensation is to be applied, ensure that all Voltage settings (except for FSC and SSC) have been finalized for all samples prior to recording any data. Compensation applied to samples that have voltages that are different from those used in the compensation setup may produce erroneous results.

Before you begin

- If the experiment requires compensation, prepare the necessary compensation controls. You will need single-stained controls (i.e., compensation beads or cells) for each fluorophore you are using for compensation. Unless you select to use a negative gate or none, you will also need an unstained or isotype-labeled control.
- We recommend that you optimize the instrument settings for compensation controls in the *Compensation Workspace*.

  To optimize instrument settings for compensation control samples, open the *Compensation Setup dialog* as described on page 37 and select the necessary parameters for compensation.
- You need to use Tubes for your compensation controls. The Compensation Setup dialog provides different options for setup based on your selection of the compensation source. See pages 38–39 for details about optimizing compensation controls.
- If no compensation is necessary, you can optimize the instrument settings within the Sample Workspace in the Experiment Explorer. The procedure for optimizing samples is similar to that described for compensation control samples.
- Adjust all voltages to put the population of interest on scale in all necessary channels. Voltages should be set to maximize the signal-to-noise ratio.
- You can adjust the threshold and voltages using the *Instrument Configuration* tab on the Collection Panel. Alternatively, you can use *Instrument Settings* in the View tab, which gives you the additional ability to rename parameters.
- For more information on the Instrument Settings tab, refer to the Attune™ NxT Software User Guide, available for download at **www.thermofisher.com**.

Define control samples

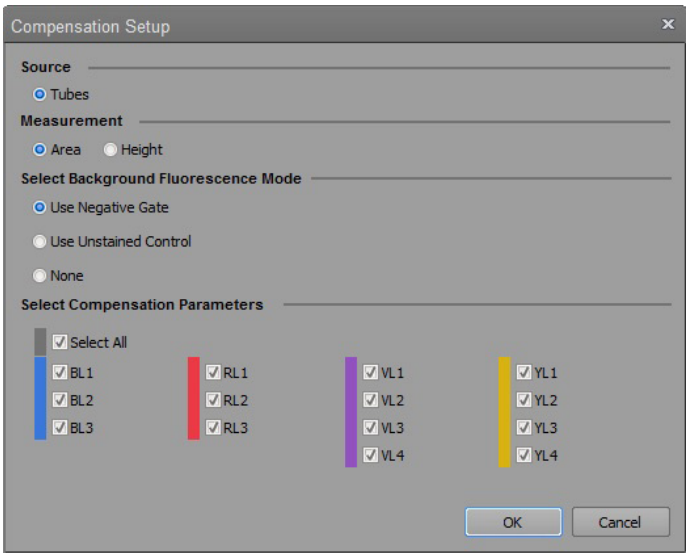
1. To define the control samples, open the Compensation Setup dialog by clicking the **Compensation Setup** button on the *Compensation ribbon tab*.

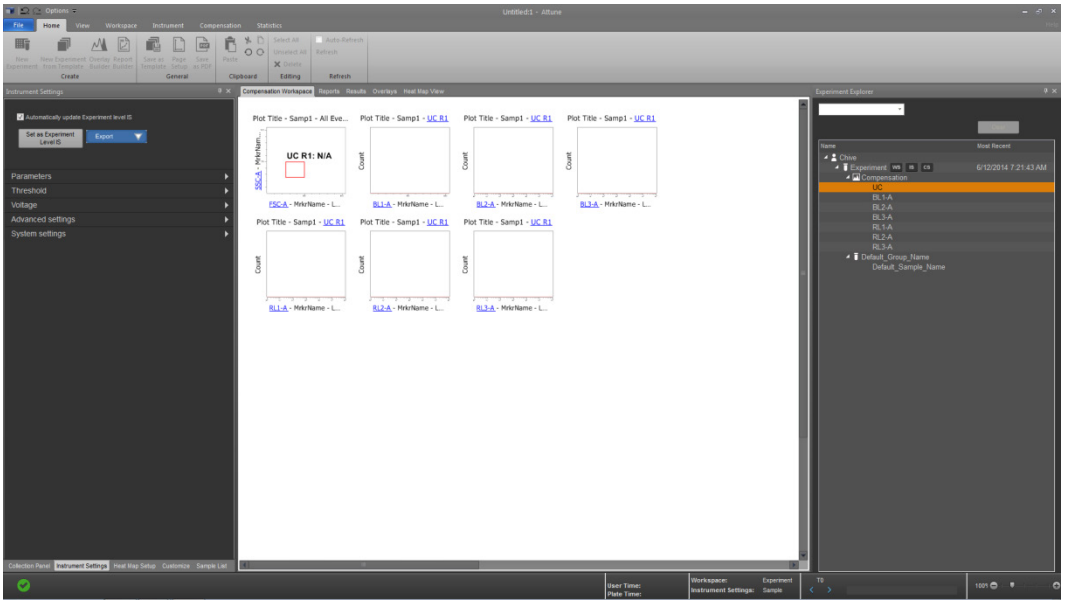
   Alternatively, double-click on the **Compensation node** within an Experiment on the *Experiment Explorer* when there are no compensation controls present.

   Each of these methods launches the *Compensation Setup dialog*.

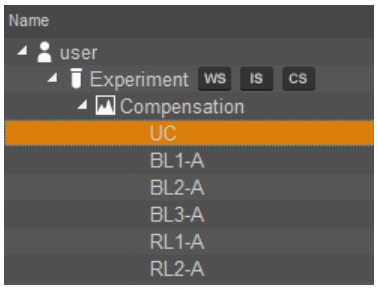


2. On the Compensation Setup dialog, select the source of compensation controls. The Compensation Setup dialog provides different options for setup based on your selection of the compensation source.
3. Next, select the measurement parameter, background fluorescence mode, and the compensation parameters. For more information on each option, refer to the "Compensation" chapter in the *Attune™ NxT Software User Guide*.
4. Click **OK**. The dialog box closes and the software creates or updates the compensation control for each selected parameter in the Experiment Explorer. The Compensation Workspace for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically.

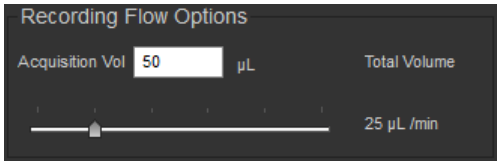


Optimize instrument settings for unstained control

1. Double-click **UC** (i.e., unstained control) under Compensation in the Experiment Explorer.

   The Compensation Workspace for the unstained control contains one SSC vs. FSC plot with a polygon gate, and histogram plots for each of the fluorescent parameters selected during the compensation setup.
2. Install the unstained control on the tube lifter.
3. In the *Collection Panel tab*, enter the **Acquisition Volume**, and then set the **Flow Rate** by adjusting the slider bar.

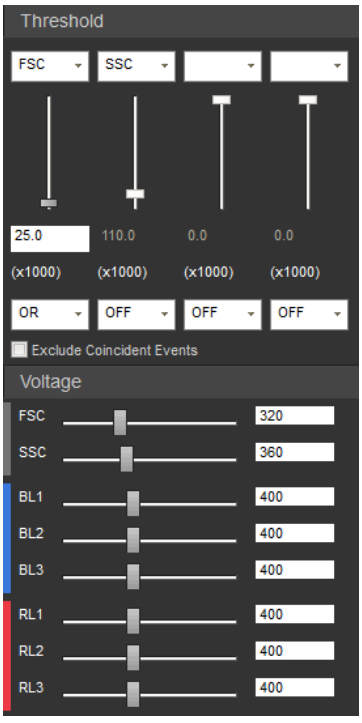
   

   For setup, you can conserve your sample by running the cytometer at a 25 µL per minute collection rate.
4. Click **Run**. Events will appear in the FSC vs. SSC plot. You can obtain data in real-time without saving them to a file.

> **IMPORTANT!** DO NOT click **Record** at this point. Once you click **Record** on any of the compensation controls within the Compensation Setup, the instrument settings for all fluorescent channels will be grayed out and cannot be changed. It is critical that you optimize voltages prior to recording any sample or any compensation controls.

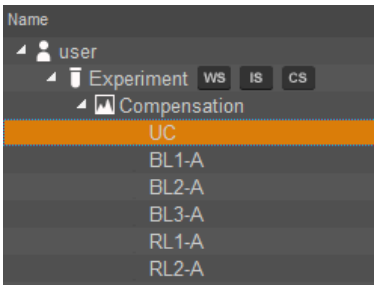
5. Select the *Instrument Settings tab*, and adjust the **FSC voltage** to place the population on scale by sliding the **FSC slider bar** up or down.

   Alternatively, you can type a specific numerical value in the settings window above each channel.
6. Adjust the **SSC voltag***e* to place the population on scale by sliding the **SSC slider bar** up or down.
7. Adjust **Threshold** on instrument control panel to remove unwanted events and background.
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 your voltages.
9. Adjust the **Fluorescence Channels** to place your unlabeled sample in the appropriate area in the plot (generally around 10^3 for unstained control).
10. Remove the unstained control from the sample injection port.

Optimize instrument settings for single-stained controls

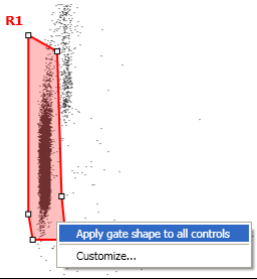
After you have optimized the instrument settings for the unstained control (if applicable), optimize the instrument controls for each of the single-stained controls.



1. Stay on the **Unstained** compensation control.
2. Install the first single-stained control on the tube lifter.
3. Using the same optimization procedure, adjust the instrument settings and set the scatter gate on the population of interest.
4. For each compensation control sample (i.e., fluorophore), observe the corresponding histogram to optimize the voltages.
5. Perform the optimization procedure for all single-stained controls.
6. After you have optimized instrument settings for each single-stained control, proceed to "Calculate Compensation", page 40.

IMPORTANT! Once you click **Record** on any of the compensation controls within the Compensation Setup, the instrument settings for all fluorescent channels will be grayed out and cannot be changed. It is critical that you optimize voltages prior to recording any sample or any compensation controls.

Note: The Scatter Gate defined in the **Unstained** control can be applied to all other compensation controls by right clicking the gate (R1) and selecting **Apply gate shape to all controls…**



Note: The Attune™ NxT Software automatically executes the Rinse function each time the tube lifter is pushed down to remove the sample from the sample injection port. This ensures that the fluidics system of the instrument is flushed and any remaining sample is removed to minimize carryover.

Calculate compensation

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

The Attune™ NxT Software calculates the compensation settings automatically as it guides you through the process.

IMPORTANT! Once you click **Record** on any of the compensation controls within the compensation setup, the Instrument Settings for all fluorescent channels will be shaded gray and cannot be changed. It is critical that you optimize voltages prior to recording any sample or any compensation controls.

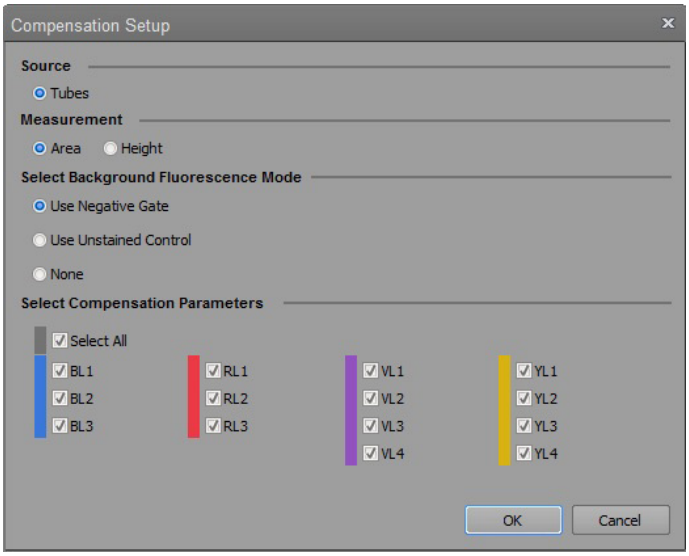
Compensation setup

1. Open the Compensation setup dialog by clicking the **Compensation setup** button on the *Compensation ribbon tab*.

   Alternatively, double-click on the **Compensation node** within your optimized Experiment on the *Experiment Explorer*.

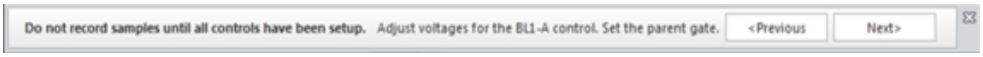
   

2. On the Compensation setup dialog, select the source of compensation controls. The Compensation setup dialog provides different options for setup based on your selection of the compensation source.
3. Next, select the measurement parameter, background fluorescence mode, and the compensation parameters. For more information on each option, refer to the "Compensation" chapter in the *Attune™ NxT Software User Guide*.
4. Click **OK**. The dialog box closes and the software creates or updates the compensation control for each selected parameter in the Experiment Explorer. The Compensation Workspace for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically.

Compensation acquisition workflow

Once compensation controls have been defined and compensation is set up, compensation controls can be acquired. The expected workflow is to go through a round of optimization to correctly set the voltages, thresholds, and gates. This is followed by a round of recording at which point the recorded compensation controls are factored into the compensation calculation.

Step-by-step instructions for acquiring compensation are provided in the *Status Notification Bar*, located below the below the Ribbon bar and above the Main Application Workspace. The specific instructions provided depend on the action being performed.



- Compensation messages contain two action buttons, *Previous* and *Next*.
- Clicking **Next** moves to the next available Compensation control.

  Clicking **Previous** moves to the previous Compensation control.

  These buttons are visible but disabled when the instrument is acquiring.
- The message is removed from the display when the action is completed on notification bar or the **X** button is clicked.

IMPORTANT! Once you have recorded all compensation controls and calculated and applied the Compensation Matrix, you cannot adjust the PMT voltages for experimental data.

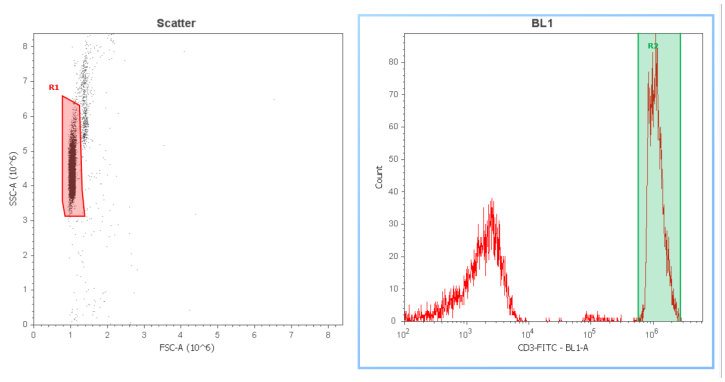
Compensation acquisition from tubes

Compensation setup and acquisition from tubes is performed manually.

1. Select the type of compensation you want to perform and the desired channels as described on page 40, and click **OK.**

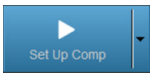
   Compensation Workspace opens and is automatically populated with the plots necessary to calculate compensation.

   Instructions for running the Compensation functions are also provided in the Status Notification Display.
2. Install the tube containing the unstained control beads/cells on the sample injection port as prompted by the software.
3. Push up the tube loader to the active position in the sample injection port and click **Run** on the Collection Panel.
4. Wait until the sample equilibrates, and click **Record**. The Compensation matrix is calculated as new Compensation controls are recorded. The calculation will only include the controls that have been recorded.
5. Repeat the process for each of the single-stained controls, making sure that the positive signal for all samples is on scale.

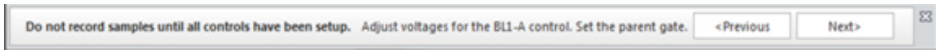


Compensation acquisition from wells

Compensation setup and acquisition from wells is initiated using the **Set Up Comp** button on the *Collection panel*. This button is displayed by default if the plate contains compensation wells and the system is ready for acquisition setup.

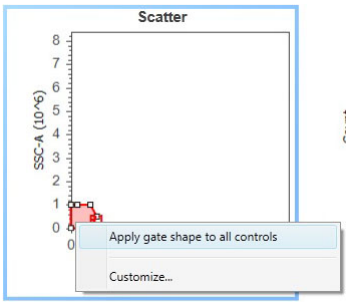


Step-by-step instructions for acquiring compensation are provided in the *Status Notification Bar* as described on page 41. The specific instructions provided depend on the action being performed.

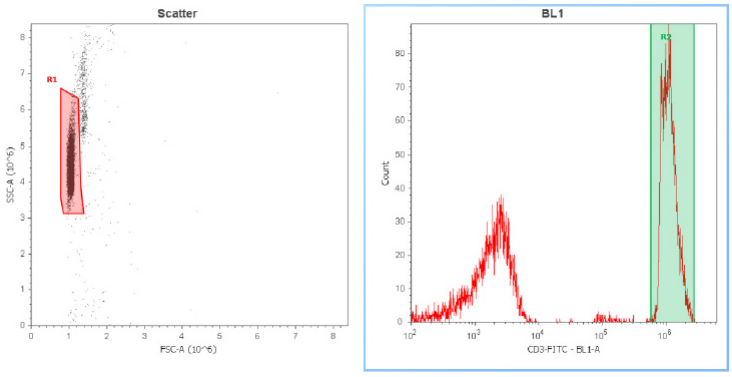


1. Select the type of compensation you want to perform and the desired channels as described on page 40, and click **OK.**

   Compensation Workspace opens and is automatically populated with the plots necessary to calculate compensation.
2. Ensure that the sample plate is loaded containing the compensation control beads into the Auto Sampler as prompted by the software.
3. Click **Set Up Comp** to initiate the optimization of compensation control samples in the *Set Up mode*, starting with the unstained control.
4. While unstained control is running, use the **voltage slider bars** on the Instrument Configuration tab to optimize the voltages for the scatter parameters for FSC and SSC.
5. Set the scatter gate on the population of interest so that the fluorescence histograms are reflective of the population for which you are optimizing your voltages.
6. Right-click on the gate and select **Apply gate shape to all controls**.



7. Click **Stop** on the Collection Panel when you are satisfied with the data and then click **Next** to move onto the next compensation control.
8. Again, using the voltage slider bars on the Instrument Configuration tab, optimize the voltage for the selected compensation control.
9. Set the **R2 gate** on the peak of interest on the histogram plot.



10. Repeat setup steps for the remaining compensation controls and ensure that the gates are set appropriately on the population of interest.

    Clicking the **Next** navigation button on the last compensation control sample within the compensation setup task advances the task button to **Record Comp**.

11. Once the optimization phase is completed, click the **Record Comp** button on the Collection panel to initiate the recording of the compensation control wells.

    The Attune™ NxT Software acquires and records compensation controls starting with the first defined control using the Run Protocol applied to each individual well.

    While the compensation controls are being recorded, ensure that gates created during compensation setup are still set on the populations of interest.

Note: The parameter order for compensation controls in plates is unstained control, blue laser controls, red laser controls, violet laser controls, and yellow laser controls, and it is independent of the well location on the plate.

Compensation acquisition from files

- Compensation setup from files is performed by selecting the **File** option as the compensation source.

  Files can also be imported into each compensation control created using the **Tube** option. Importing files behaves like recording and will force the matrix to recalculate.

- The Compensation matrix is calculated as each control workspace is loaded and the gates are adjusted.

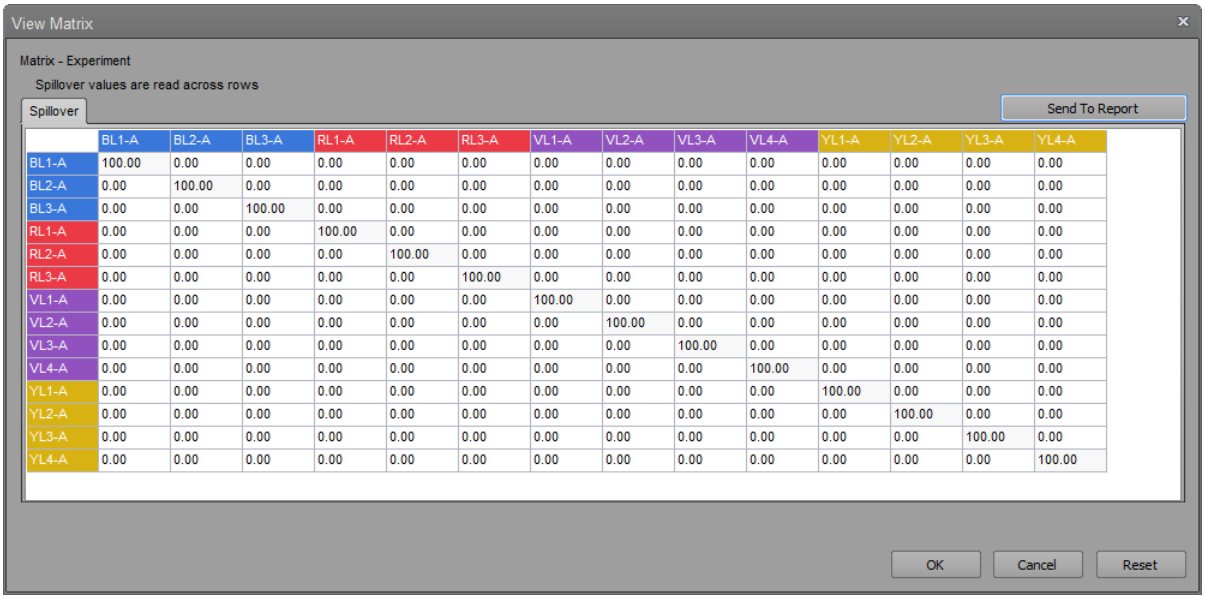
IMPORTANT! Once you have recorded all compensation controls and calculated and applied the compensation matrix, you cannot adjust the PMT voltages for experimental data.

If you need to adjust voltages after recording any of the compensation controls, you need to remove the data from all compensation controls containing recorded data by right-clicking on the control sample and selecting *Clear Control Sample Data* or right-clicking on **Compensation** in the Experiment Explorer and selecting *Remove Compensation.* If you opt for the latter, you have to re-run **Compensation setup** to recreate all compensation control samples.

Compensation/ Spillover matrix

When the last compensation control is recorded, *Matrix dialog* automatically opens for your review. The matrix dialog displays either the *Compensation matrix* or the *Spillover matrix*, depending on the selection made for the Matrix option in the *Options dialog*.

You can also launch the Matrix dialog by clicking the **View Matrix** button on the *Compensation ribbon tab* or by double-clicking the **Compensation node** on the Experiment Explorer when Compensation controls are present.



	BL1-A	BL2-A	BL3-A	RL1-A	RL2-A	RL3-A	VL1-A	VL2-A	VL3-A	VL4-A	YL1-A	YL2-A	YL3-A	YL4-A
BL1-A	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL2-A	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL3-A	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL1-A	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL2-A	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL3-A	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL1-A	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
VL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
VL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
YL1-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
YL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
YL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
YL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Click **OK** to accept any changes made to the matrix and apply the updated compensation values to the dataset. You can also manually edit or reset the compensation matrix values (not recommended).

For more information about the Compensation or the Spillover Matrix, refer to the "Compensation" chapter in the *Attune™ NxT Software User Guide*.

Note: *Spillover matrix* shows the amount of spillover from each fluorophore into each of the other fluorescent channels. Data compensation is calculated using the *Compensation matrix*, which is the inverse of the Spillover matrix.

Run samples and collect data

After you have calculated the compensation settings, you are ready to run your samples to acquire and record data.

The Attune™ NxT Software has two modes for data collection, the *Run* mode and the *Record* mode.

Run mode

The Run mode is initiated when **Run** on the Collection Panel is selected.

In this mode, the software starts data acquisition but no FCS file containing the data is created.

- This mode is primarily used for adjusting the various parameters for cytometer and experiment optimization and customizing the charts for recording.
- The software actively stores only 20,000 events, and once that limit is reached, it recycles the events.
- During the run, the events are displayed on the plots as the graphs are being populated.

Note: If compensation is available and turned ON, data will be displayed compensated during acquisition.

Record mode

The Record mode is initiated when **Record** on the Collection Panel is selected.

During recording, the events are displayed on the plots as the graphs are being populated.

- In the Record mode, the Attune™ NxT Software automatically creates an FCS file when **Stop** is clicked.
- The software creates a unique name for each FCS file so that an existing file is not overwritten.
- If the Sample already has an FCS file, the software displays a warning that the FCS file is going to be overwritten. You can choose to append the existing FCS file or overwrite it (see "*Optional:* Append Data," page 46).

Prepare the Workspace

Prepare your Workspace and customize your plots by selecting each individual plot one at a time. Change the axis labels and other properties appropriate to your experiment.

Acquire data

1. Select the Sample of interest from the Experiment Explorer panel. Double-click **Sample** to activate it.

   Workspace displays the default sample plots set up for the Experiment. If desired, you may delete or modify these plots.
2. Enter the collection criteria in the Collection Panel. Set limits to collection by the number of events for specified gates, total sample volume analyzed, or by elapsed time (see "Collection Panel" in the *Attune™ NxT Software User Guide*.).
3. Install the tube containing the sample on the sample injection port and lift up the tube loader to the active position.
4. Click **Run**. The events are displayed on the plots as the graphs are being populated. Wait a short time for the sample to equilibrate.

Record data

1. While still in the Run mode, adjust the PMT voltages and Threshold values for the appropriate channels using the Instrument Configuration tab (see "Instrument Configuration" in the *Attune™ NxT Software User Guide*). Make sure that the events are on scale.
2. Click **Record** to start data collection.

   Data is recorded until any of the conditions set in Collection Criteria is satisfied.

   Note: Click **Pause** to temporarily halt the data collection. You can click **Run** to resume the data collection from that point on.
3. Click **Stop** to stop the data collection. The Attune™ NxT Software automatically saves the data in a unique FCS file.

Optional: Append data

After recording data, you can append the data file with additional data. If you wish to append a data file for a Sample, you must not modify the Instrument Settings or collection rate, or change the Sample (e.g., go to the next Sample). However, you can execute a Rinse function prior to appending data.

To append data:

1. Click **Run** button, and then click **Record**.

   A dialog box opens and prompts you to select **Append**, **Overwrite**, or **Cancel**.
2. Click **Append** if you wish to add the new data to the existing FCS.

Note: When appending data, the stop criteria is based on the individual record cycle and not the total resulting FCS file.

For example, a file containing 10,000 events appended with a run that has stop criteria of 20,000 events will result in a file with 30,000 total events.

5. Shutdown

Workflow

Shutdown procedure

Check fluid and waste levels

Run Shutdown function

Optional: **Perform system flush**

IMPORTANT! Although the daily Shutdown procedure is automated and requires minimal user input, we recommend that you familiarize yourself with the instrument and its operating principles by reading "Appendix A: System Overview" (page 50) and "Appendix B: Technical Overview" (page 63) before starting your experiments.

For a detailed description of the software user interface, refer to the *Attune™ NxT Software User Guide* (Part no. 100024236) provided with the product.

Shutdown procedure

The *Shutdown* function of the Attune™ NxT Software facilitates the automated shutdown of the instrument. The function removes all sample fluid and dyes from the system, decontaminates the fluidics lines and sample pumps, and fills them with Attune™ Shutdown Solution to prevent the formation of salt crystals.

The automated shutdown procedure takes at least 40 minutes to complete.

IMPORTANT! Perform the following shutdown procedures at least once a day, even if the instrument is intended for continuous use. Proper cleaning of the instrument ensures its consistent and accurate operation.

CAUTION! BIOHAZARD. Cytometer hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure we recommend for decontaminating the cytometer.

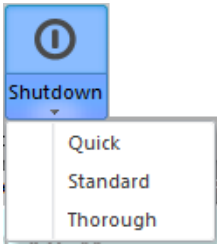
Check fluid and waste levels

1. Check the levels in the fluid tanks (see page 13 for the location of the fluidics compartment).
2. Ensure that the Wash and Shutdown solution tanks are at least half-full.

Shutdown options

There are three options available for the Shutdown function:

- **Quick** –Quick option uses 15 bleach scrubs and 10 wash scrubs through sample pathway lines.
- **Standard** –Standard option uses 25 bleach scrubs and 20 wash scrubs through sample pathway lines.
- **Thorough** –Thorough option uses 35 bleach scrubs and 30 wash scrubs through sample pathway lines.



For daily use, we recommend the Standard Shutdown function.

Run Shutdown function

1. Before running the Shutdown function, empty the Waste container(s) and refill the fluid tanks with the appropriate solutions.
2. On the Instrument ribbon tab, click the **Shutdown** button, and select the **Quick**, **Standard**, or **Thorough** option from the dropdown menu.

   The *Shutdown dialog* opens and provides step-by-step instructions to perform the Shutdown operation. The steps of the Shutdown operation differ depending on whether or not an Attune™ Auto Sampler is connected to the cytometer.
3. The Attune™ NxT Software automatically performs the shutdown operation and the shutdown status window displays the steps of the shutdown function as they are being executed.
4. After initiating the Shutdown operation, you can turn off the computer.
5. At the end of the Shutdown operation, the Attune™ NxT Software automatically puts the Attune™ NxT Acoustic Focusing Cytometer in a low power state.

IMPORTANT! If you cancel the shutdown, allow at least 3 minutes for the lasers to reach operating temperature before running any samples. You will also need to re-run Startup.

IMPORTANT! If you intend to leave the Attune™ NxT Acoustic Focusing Cytometer in the shutdown state for longer than two weeks, perform system flush and leave the instrument in deionized water to prevent salt crystals from clogging the fluidics system.

Optional: Perform system flush

Perform system flush if you intend to leave the cytometer shut down for longer than two weeks.

1. Replace all fluidic containers (focusing fluid, waste, wash, and shutdown tanks) with deionized water.
2. Run the **Shutdown** function (page 48) using deionized water on the SIP instead of bleach.
3. Empty all fluidic tanks and decontaminate with 10% bleach.
4. Allow empty fluidics tanks to dry.

Appendix A: System overview

Technical specifications

Physical characteristics

Footprint (H × W × D): Approximately 16″/40 cm × 23″/58 cm × 17″/43 cm
Weight: Approximately 64 lb/29 kg
Operating temperature: 15–30°C
Operating humidity: 10–90% non-condensing
Electrical requirements: 100–240VAC, 50/60 Hz, <150 W

Optics

Excitation lasers:

Blue laser: 488 nm, 50 mW
Violet laser: 405 nm, 50 mW
Red laser: 637 nm, 100 mW
Yellow laser: 561 nm, 50 mW

Alignment: Fixed alignment, no customer maintenance required

Fluidics

Sample input rates: ~1 sample/minute
Sample rates: 12.5–1,000 µL/minute
Sample delivery: Calibrated delivery volumes for volumetric analysis
Sample analysis volume: 20 µL–4 mL
Fluid storage: Within instrument with level sensing
Nominal fluid consumption: 1.8 L/day
Sample tubes: Accommodates from 17 × 100 mm to 8.5 × 45 mm tubes
Particle size range: 0.5 µm to 50 µm

Electronics

Data acquisition: Up to 35,000 events/second
Resolution: 20 bits
PMT voltage: User-adjustable

Computer

- Minitower running Windows™ 7 SP1
- 24-inch flat panel monitor

User interaction with instrument

- Visual display of system status on instrument
- Instrument startup ≤ 35 minutes and automated shutdown
- User-changeable, keyed filters
- Fully automated and manual compensation modes
- Audible noise <65 dBA at 1.0 m

Attune™ NxT Software

- Software allows data acquisition and analysis (width, height, and area measurements) and controls the instrument
- Output file format FCS (Flow Cytometry Standard) format 3.0 or 3.1
- Live gating with automatic saving
- Operator and administrator log in

Operation principles

The Attune™ NxT Acoustic Focusing 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 Attune™ NxT Acoustic Focusing Cytometer measures scattered light and fluorescence as cells pass through the laser beam.

Sample loading

The sample is loaded into the Attune™ NxT Acoustic Focusing Cytometer via the sample injection tube, which automatically delivers the sample to the flow cell after the tube lifter is engaged and the user defines collection criteria.

The sample is pushed through a capillary assembly and wrapped in a sheath of focusing fluid before it is intercepted by the laser beam 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. The Attune™ NxT Acoustic Focusing Cytometer allows a tight control on particle positioning, which enables high sensitivity and precision at high sample input rates.

Sample interrogation

As the sample traverses the interrogation point, the Attune™ NxT Acoustic Focusing Cytometer uses multiple lasers to illuminate the particles or cells in the sample, which scatter the laser light and emit fluorescent light from fluorescent dyes attached to them. The optical filters and mirrors route specified wavelengths of the resulting light scatter and fluorescence signals to the designated optical detectors.

Signal processing

The Attune™ NxT Acoustic Focusing Cytometer uses PMT detectors and a diode detector (FSC) for converting the fluorescence signals and collected light scatter into electrical signals (i.e., voltage pulses), which are proportional to the intensity of the light received by the detectors. The PMTs are dedicated to fluorescence and SSC detection, and the FSC is obtained from the diode detector. The analog and digital electronics of the cytometer amplify and analyze these pulses, and transfer them to the workstation computer for further processing by the Attune™ NxT Software.

Results

Results are saved as FCS 3.0 or 3.1 files in the appropriate user folders.

Fluidics

The fluidics system of the Attune™ NxT Acoustic Focusing Cytometer handles the flow of fluids required for acoustic focusing cytometer operation. This includes the fluid functions during the main data collection operation as well as during the Startup, Deep Clean, Unclog, Sanitize SIP, System Decontamination, Rinse, Run, De-bubble, Stop, and 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 pressure 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 line (i.e., the 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 arrives at the waste container.

Fluidics functions

The fluidics functions of the Attune™ NxT Acoustic Focusing Cytometer are controlled by the Attune™ NxT Software. These functions are:

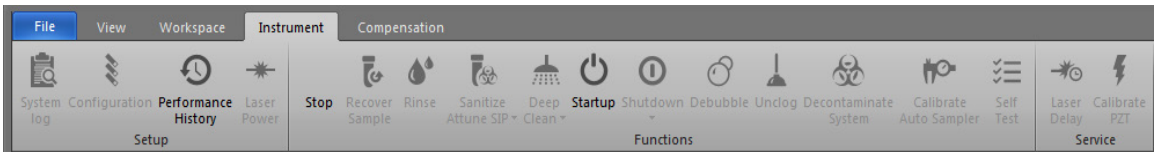
- **Sanitize SIP –** Quickly washes and sanitizes the SIP and sample lines. It is especially important to perform the Sanitize SIP function when running sticky samples, DNA stains, or beads. This function requires user-supplied bleach or detergent.
- **System Decontamination** – Sanitizes the system and fluidics bottles with bleach and Wash solutions for a proscribed period of time. Ensures full system cleanliness at regular maintenance intervals to prevent buildup of contaminants in the system or fluidics bottles.
- **De-bubble** is a user-initiated function for clearing bubbles in the fluidics lines of the cytometer.
- **Deep Clean** is a user-initiated system cleaning between sticky samples. This function requires user-supplied 10% bleach solution.

  IMPORTANT! We recommend that you sanitize the system between users. See “Wash procedure between users” on page 33.

- **Unclog** function is a user-initiated back flush operation to remove clogs from the sample probe and flow cell.
- **Rinse** automatically flushes the system between samples to minimize carryover. This function can also be user-initiated. During the rinse, the Red Status Indicator Light will stop blinking when it is okay to load a new sample onto the SIP.
- **Shutdown** is an automated function that initiates the cleaning cycle and post-cleaning rinse. This mode requires user supplied bleach, Attune™ Wash Solution, and Attune™ Shutdown Fluid.

- **Startup** is an automated function that starts the fluidics, optics, and electronics of the Attune™ NxT Acoustic Focusing Cytometer. The Startup functions include priming the instrument fluidics and allowing the laser time to warm.
- **Stop** is used to end all data collection.
- **Clear** is used to delete the data from the screen. It refreshes the Workspace while the instrument is in Run or Record mode.

You can initiate the available fluidics functions by selecting it from the Instrument ribbon tab. For more information, refer to the *Attune™ NxT Software User Guide.*



IMPORTANT! The lasers are powered down during the Deep Clean and Shutdown cycles. The lasers must warm up for at least 5 minutes before running additional samples.

Optics

Filters and fluorophores

The table below lists the filters and fluorophores for each configuration of the Attune™ NxT Acoustic Focusing Cytometer.

Excitation laser	Channel color	Instrument config.*	Default filter (nm)	Filter range (nm)	Primary fluorophores	Other reagents
Violet (405 nm)	VL1 blue	BVRY, BVR, BVY, BV	440/50	415–465	Pacific Blue™ Alexa Fluor™ 405	PO-PRO™-1 DyeCycle™ Violet Fixable Violet Dead Cell Stain CellTrace™ Violet Calcein Violet SYTOX™ Blue FxCycle™ Violet Click-iT™ Pacific Blue™
	VL2 green	BVRY, BVR, BVY, BV	512/25	500–525	Pacific Green™ Qdot™ 525	Fixable Aqua Dead Cell Stain F2N12S (apoptotic)
	VL3 orange	BVRY, BVR, BVY, BV	603/48	579–627	Pacific Orange™ Qdot™ 605	Fixable Yellow Dead Cell Stain F2N12S (live)
	VL4 near IR	BVRY, BVR, BVY, BV	710/50	685–735	Qdot™ 705	
Blue (488 nm)	FSC blue scatter	BVRY, BVR, BVY, BR, BV, BY, B	488/10	483–493	NA	NA
	SSC blue scatter	BVRY, BVR, BVY, BR, BV, BY, B	488/10	483–493	NA	NA
	BL1 green	BVRY, BVR, BVY, BR, BV, BY, B	530/30	515–545	Alexa Fluor™ 488 Fluorescein GFP YFP	Calcein Fluo-3/Fluo-4 TO-PRO™-1 iodide CFSE JC-1/DiOC2(3) SYTOX™ Green DyeCycle™ Green Rhodamine 123 YO-PRO™-1 iodide Fixable Green Dead Cell Stain Click-iT™ Alexa Fluor™ 488
	BL2 orange	BVR, BR,BV, B	574/26	561–587	PE	Fura Red™ AM Cell Permeant DyeCycle™ Orange JC-1/DiOC2(3) pHrodo™ Phagocytosis Particle Labeling Kit SNARF™ (low pH) SYTOX™ Orange Dead Cell Stain
	BL2 orange	BVRY, BVY, BY,	590/40	570–610	PI	PE
	BL3 far red	BVRY, BVR, BVY, BR, BV, BY, B	695/40	675–715	PerCP-PE-Cy®5.5 PerCP-Cy®5.5	
	BL4 near IR	BVR, BR, BV, B	780/60	750-810	PE-Alexa Fluor™ 750 PE-Cy®7	Qdot™ 800

***Instrument configuration:** B = Blue laser, V = Violet laser, R = Red laser, Y = Yellow laser

Excitation laser	Channel color	Instrument config.*	Default filter (nm)	Filter range (nm)	Primary fluorophores	Other reagents
Yellow (561 nm)	YL1 orange	BVRY, BVY, BY	585/16	577–593	PE	Fura Red™ AM Cell Permeant DyeCycle™ Orange JC-1/DiOC2(3) pHrodo™ Phagocytosis Particle Labeling Kit SNARF™ (low pH) SYTOX™ Orange Dead Cell Stain
	YL2 red	BVRY, BVY, BY	615/20	605–625	Alexa Fluor™ 594 PE-Alexa Fluor™ 610 m-Cherry	
	YL3 far red	BVRY, BVY, BY	695/40		TRI-COLOR™ (TC, PE-Cy®5) PE-Cy®5.5	Vybrant™ DyeCycle™ Ruby
	YL4 near IR	BVRY, BVY, BY	780/60	750–810	PE-Alexa Fluor™ 750 PE-Cy®7	Qdot™ 800
Red (638nm)	RL1 far red	BVRY, BVR, BR	670/14	650–670	Allophycocyanin (APC) Alexa Fluor™ 647	Fixable Far Red Dead Cell Stain Click-iT™ Alexa Fluor™ 647 FxCycle™ Far Red SYTOX™ Red Dead Cell Stain
	RL2 near IR	BVRY, BVR, BR	720/30	705–735	Alexa Fluor™ 700	Vybrant™ DyeCycle™ Ruby
	RL3 near IR	BVRY, BVR, BR	780/60	750–810	APC-Alexa Fluor™ 750 APC-Cy®7 APC-H7	Fixable Near-IR Dead Cell Stain Vybrant™ DyeCycle™ Ruby

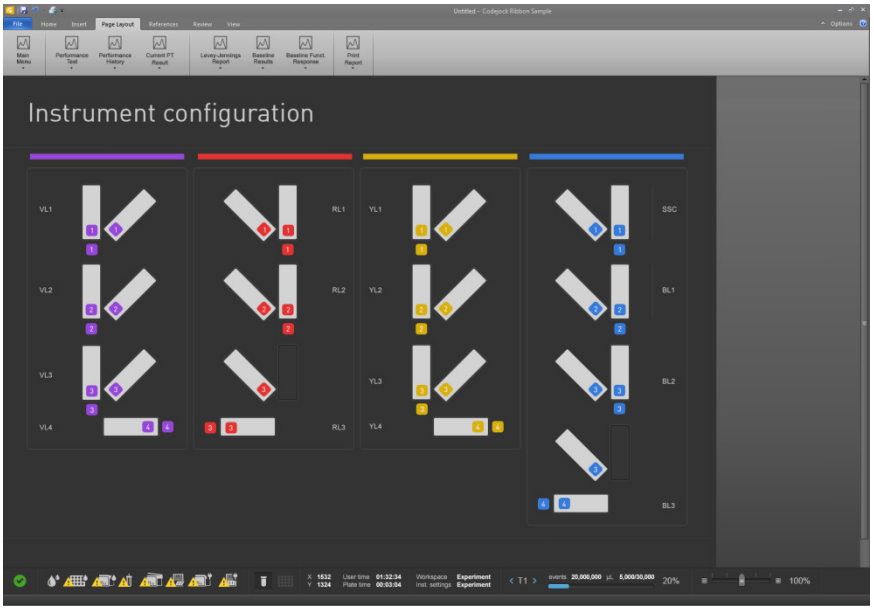
***Instrument configuration:** B = Blue laser, V = Violet laser, R = Red laser, Y = Yellow laser

Instrument configurations

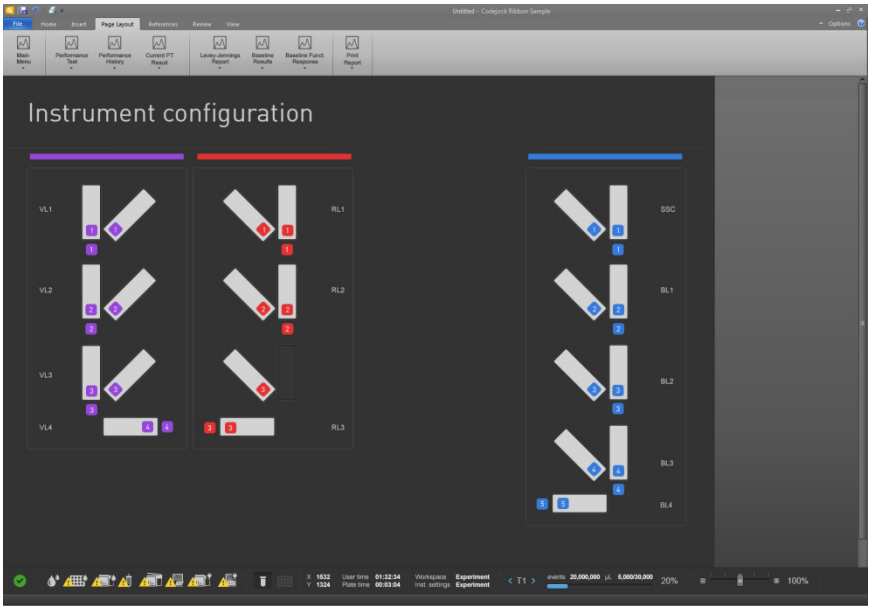
Default instrument configurations

The following images show the Instrument Configuration panel for each laser configuration. For ordering information, see page 73.

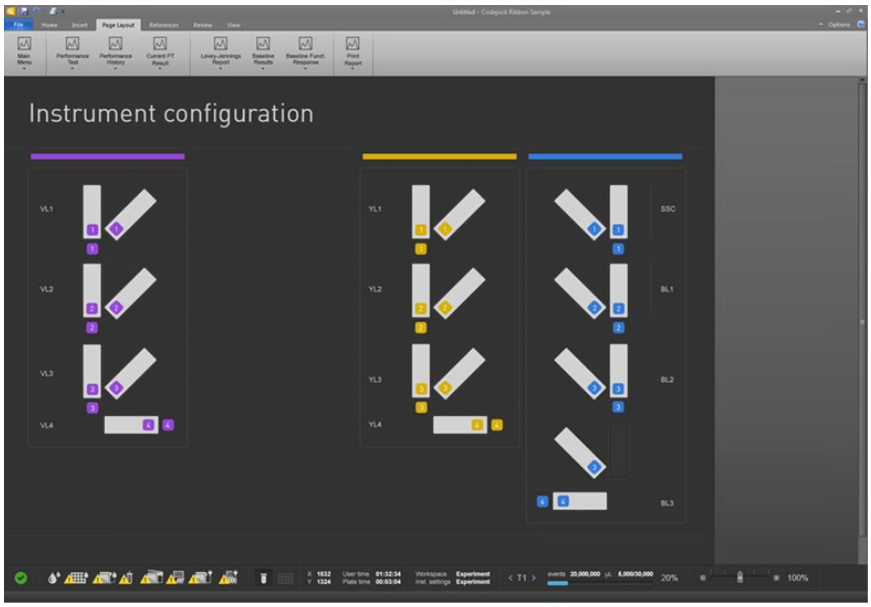
BRVY (Blue, Red, Violet, Yellow)



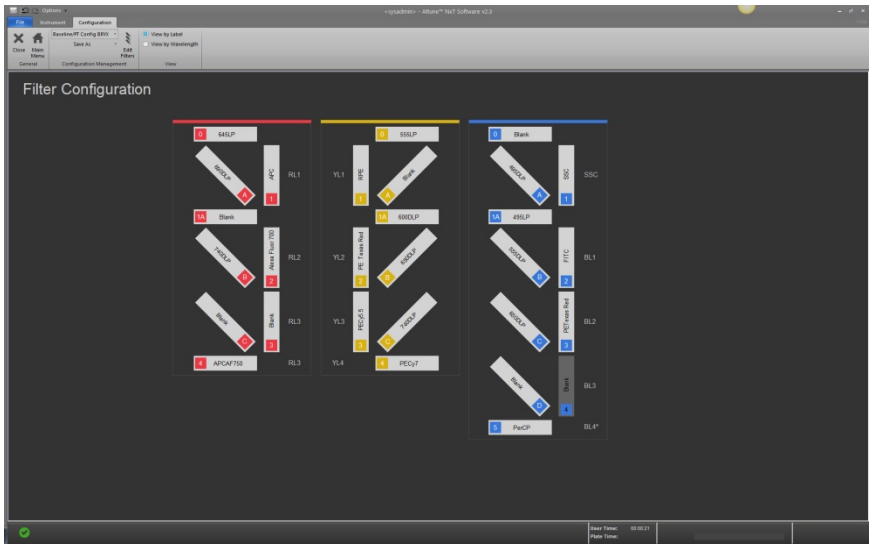
BRV (Blue, Red, Violet)



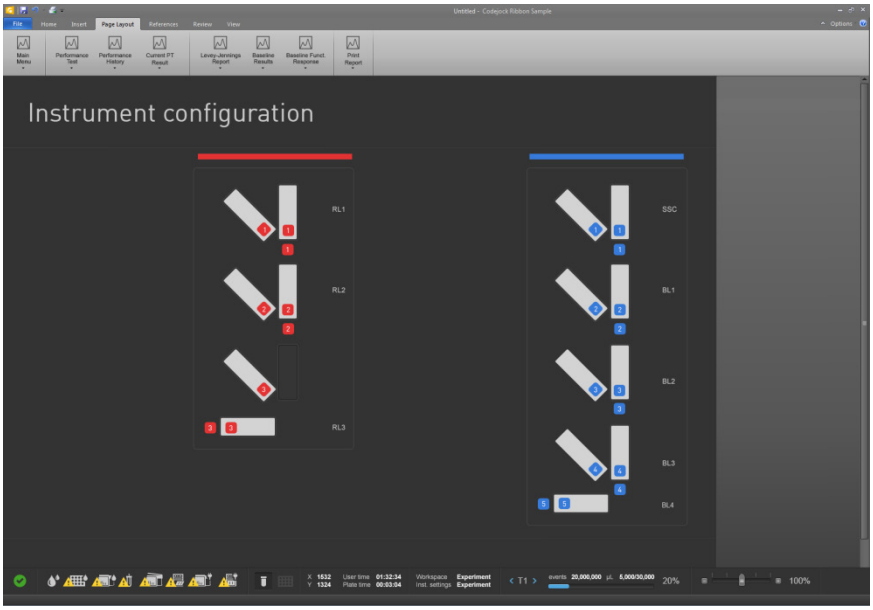
BVY (Blue, Violet, Yellow)



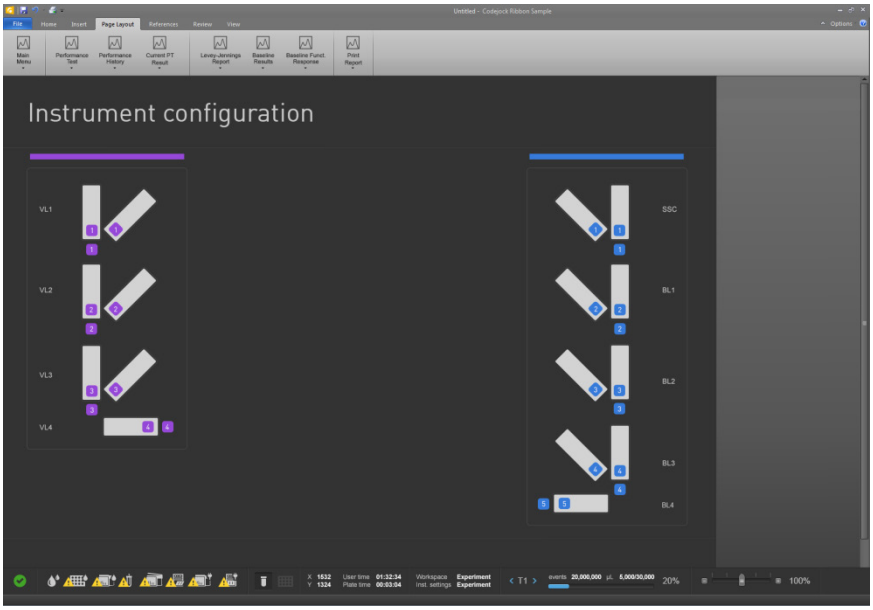
BRY (Blue, Red, Yellow)



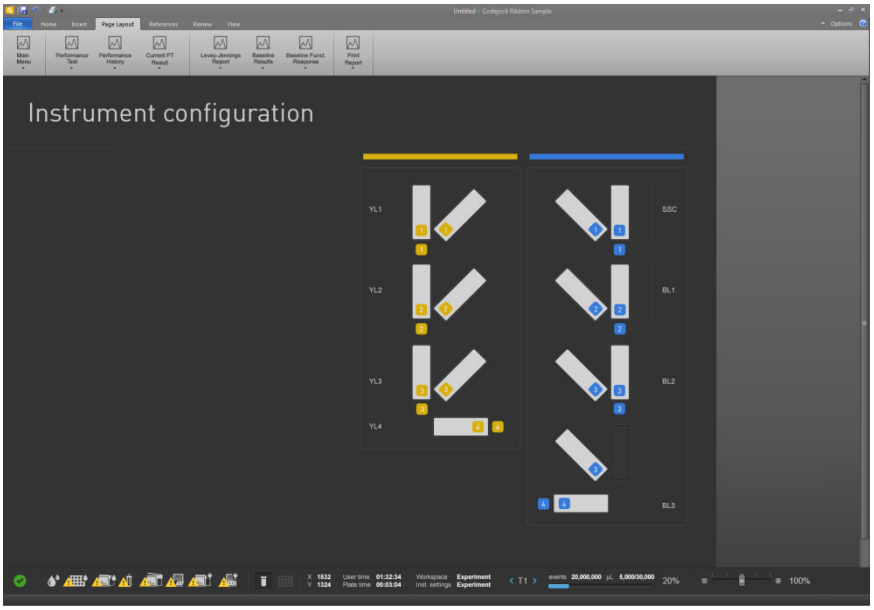
BR (Blue, Red)



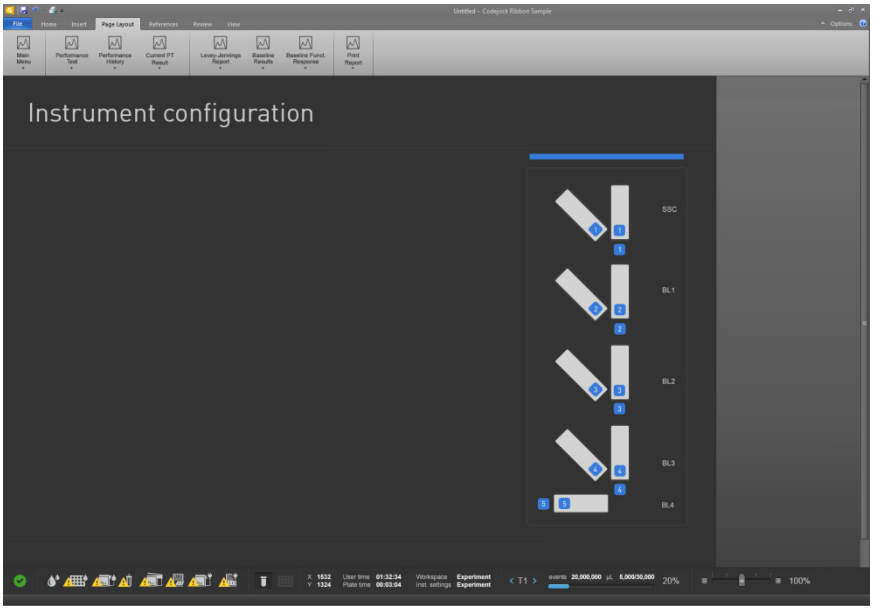
BV (Blue, Violet)



BY (Blue, Yellow)



B (Blue)



Default filter label list

The table below lists the filter labels that are displayed for each channel for various system default configurations available.

The naming convention for the system default instrument configuration is as follows:

Baseline/PT Config <*CCCC*>,

where **<C>** is the first letter of each laser color (**B**lue, **R**ed, **V**iolet, or **Y**ellow) and an **X** in the name indicates that one of the lasers is not present.

An **X** in the Instrument Configuration columns indicates that the filters listed in the table apply to that specific instrument configuration.

					Instrument Configuration						
Laser Line	**Channel**	**Filter Type**	**Wavelength**	**Labels**	**BXXX**	**BRXX**	**BVXX**	**BYXX**	**BRVX**	**BVYX**	**BRVY**
Blue	**BL1**	**Band Pass**	**530/30**	FITC, Alexa Fluor 488, GFP, cFSE, SYTOX Green	X	X	X	X	X	X	X
	BL2	**Band Pass**	**574/26**	RPE, Alexa Fluor 568, PI, SYTOX Orange, DyeCycle Orange	X	X	X		X		
	BL2(Y)	**Band Pass**	**590/40**	PE- Texas Red, PE- AF 610, PI, Fixable Red				X		X	X
	BL3	**Band Pass**	**695/40**	PE-Cy5.5, PE-AF700, PerCP-Cy5.5, PerCP, SYTOX AADVanced	X	X	X		X		
	BL3(Y)	**Band Pass**	**695/40**	PerCP, PerCP-Cy5.5, PE-AF 700				X		X	X
	BL4	**Band Pass**	**780/60**	PE–Cy7, Qdot 800, DyeCycle Ruby	X	X	X		X		
Red	**RL1**	**Band Pass**	**670/14**	APC, Alexa Fluor 647, Fixable Far Red, SYTOX Red		X			X		X
	RL2	**Band Pass**	**720/30**	Alexa Fluor 700, Qdot 700, Alexa Fluor 680		X			X		X
	RL3	**Band Pass**	**780/60**	APC-AF750, Fixable Near IR, APC Cy7, APC H7 DyeCycle Ruby		X			X		X
Violet	**VL1**	**Band Pass**	**440/50**	Pacific Blue, Alexa Fluor 405,Fixable Violet, DyeCycle Violet, SYTOX Blue, CellTrace			X		X	X	X
	VL2	**Band Pass**	**512/25**	Pacific Green, Fixable Aqua, CFP			X		X	X	X
	VL3	**Band Pass**	**603/48**	Pacific Orange, Qdot 605, Fixable Yellow			X		X	X	X
	VL4	**Band Pass**	**710/50**	Qdot 700			X		X	X	X
Yellow	**YL1**	**Band Pass**	**585/16**	RPE, Alexa Fluor 568,, PI, SYTOX Orange, DyeCycle Orange				X		X	X
	YL2	**Band Pass**	**620/15**	PE- Texas Red, PE- Alexa Fluor 610, mCherry, SYTOX AADvanced				X		X	X
	YL3	**Band Pass**	**695/40**	PE-Cy5.5, PE-AF700, PerCP-Cy5.5				X		X	X
	YL4	**Band Pass**	**780/60**	PE–Cy7, Qdot 800				X		X	X

Instrument reagents and consumables

The following reagents are approved for use on this system. For ordering information, see page 73.

IMPORTANT! Reagents may be stored at colder temperatures, but running the cytometer with cold reagents (<15°C) will affect the data quality. Before you run the cytometer, ensure that all reagent temperatures are at least 15°C.

Attune™ Focusing Fluid

The Attune™ Focusing Fluid is a buffered, azide-free support/carrier reagent for transporting the samples through the Attune™ NxT Acoustic Focusing Cytometer. It contains a surfactant and a preservative.

For best results, store the Attune™ Focusing Fluid at 15–30°C.

Attune™ Wash Solution

The Attune™ Wash Solution is a ready-to-use solution that has been formulated to remove cellular debris and dyes from the fluidics system of the Attune™ NxT Acoustic Focusing Cytometer.

Store the Attune™ Wash Solution at 15–30°C. The wash solution is stable on the cytometer for 30 days after the bottle has been opened.

Attune™ Shutdown Solution

The Attune™ Shutdown Solution is a ready-to-use solution that prevents the formation of air bubbles and salt deposits in the fluidics system of the cytometer. Trapped air bubbles in the fluidics lines can dislodge and result in inaccurate data as they pass through the flow cell, and salt deposits can clog the lines.

Store the Attune™ Shutdown Solution at 15–30°C.

Attune™ Performance Tracking Beads

The Attune™ Performance Tracking Beads are designed for use with Attune™ NxT Software to automatically characterize, track, and report performance measurements of the Attune™ NxT Acoustic Focusing Cytometer. The beads are used to define a baseline and conduct daily performance tracking of the cytometer. Each vial contains enough beads for 50 measurements.

Store the Attune™ Performance Tracking Beads at 2–8°C.

Note: Prior to use, you need to download the appropriate lot specific file into the Attune™ NxT Software for each new lot of Attune™ Performance Tracking Beads.

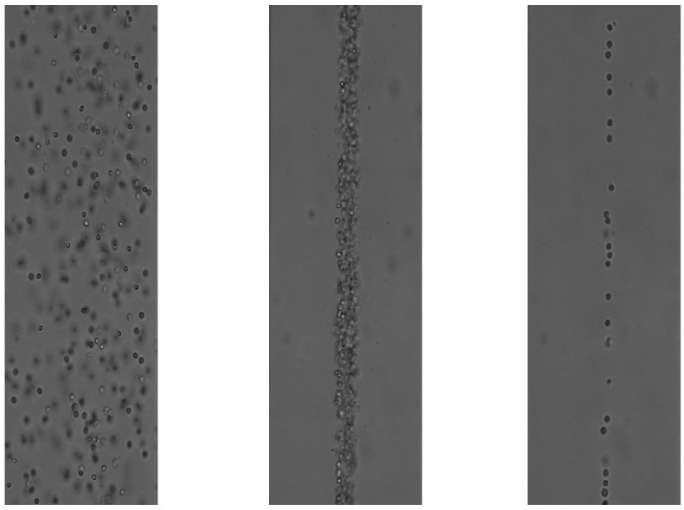
Appendix B: Technical overview

Acoustic focusing

The objective 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™ NxT Acoustic Focusing Cytometer uses acoustic radiation pressure in addition to hydrodynamic focusing to allow for more sample input options, enabling the user to choose higher throughput, shorter run time, higher sensitivity, or a combination of them all depending upon their individual needs.

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



Acoustic focusing off | Acoustic focusing on | Acoustic focusing on (dilute sample)

Effects of acoustic focusing on cell viability

Acoustic focusing differs fundamentally from ultrasonic lysis of cells and is generally 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™ NxT Acoustic Focusing 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 very high sample flow rates and the design of the acoustically-driven capillary spreads this energy over the entire length of the capillary, significantly 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™ NxT Acoustic Focusing 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™ NxT Acoustic Focusing 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. While increasing the sample concentration results in a higher sample throughput, it also increases the probability of a coincident event, defined as more than one cell present in the interrogating laser beam.

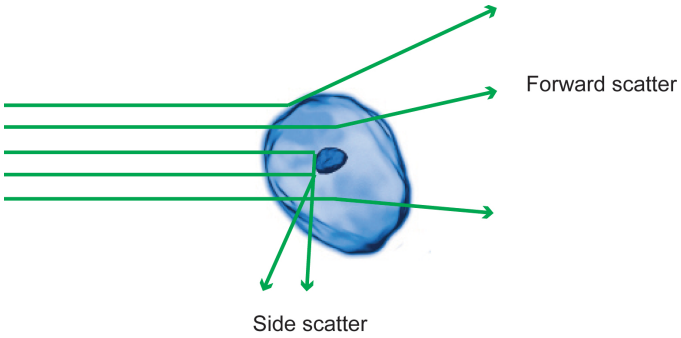
The Attune™ NxT Acoustic Focusing Cytometer can maintain its maximum particle analysis rate over a large range of initial sample concentrations without the need to concentrate using centrifugation or filtration. The ability to analyze dilute samples has the added benefits of reduced background fluorescence from free fluorophores in the sample and capability to analyze very small initial samples sizes.

Optics

Light scatter

When a cell or particle passes through a focused laser beam, it refracts or 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 roughly proportional to the size of the cell or particle, and this 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 the cell or particle.



Measuring light scatter

Forward-scattered light is quantified by a detector that converts 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 around 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

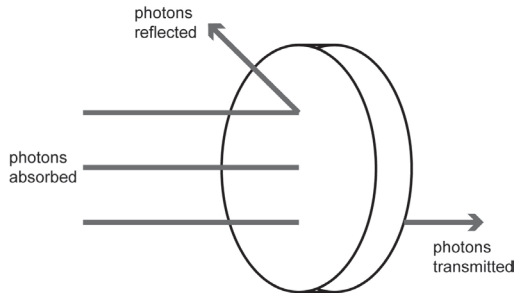
Fluorescence is the emission of light that occurs when an emitting particle such as a fluorophore-labeled antibody absorbs light from another source such as the intercepting laser beam. When the particle absorbs the intercepting light, it is elevated to an excited electronic state, and as it returns to its ground state, the absorbed energy is radiated where most of it is emitted 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 involves the 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 is collected in generally the same way as side scatter data. In a population of labeled cells, some will be brighter than others. As each cell crosses the path of the laser, a fluorescence signal is generated. The fluorescent light is then directed to the appropriate detector where it is translated into a voltage pulse proportional to the amount of fluorescence emitted. All of the 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 upon the number of detectors available in the cytometer. The different colors are collected using select optical filters that direct the light to the right detector and capture the peak fluorescent signals.

Optical filters

Optical Filters separate the light scatter and fluorescence directed to detectors by wavelength, which is measured in nanometers (nm). They selectively transmit light having a particular range of wavelengths, while absorbing or reflecting the remainder.



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

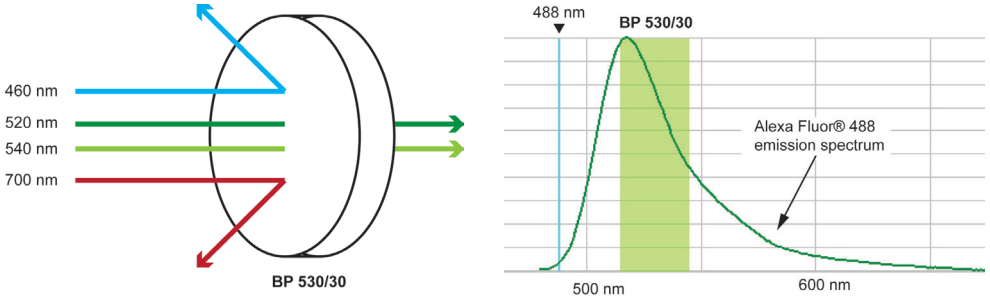
- Bandpass filter (BP)
- Longpass filter (LP)
- Shortpass filter (SP)
- Dichroic mirror (DM)
- Neutral density filter (ND)

Bandpass filter

Bandpass Filter (BP) is a device that passes wavelengths within a certain range and attenuates (i.e., rejects) wavelengths outside that range. Combining an LP filter and an SP filter produces a bandpass (BP) filter. These filters usually have lower transmittance values than SP and LP filters, and block all wavelengths outside the selected interval, which can be wide or narrow depending on the number of layers of the filter.

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

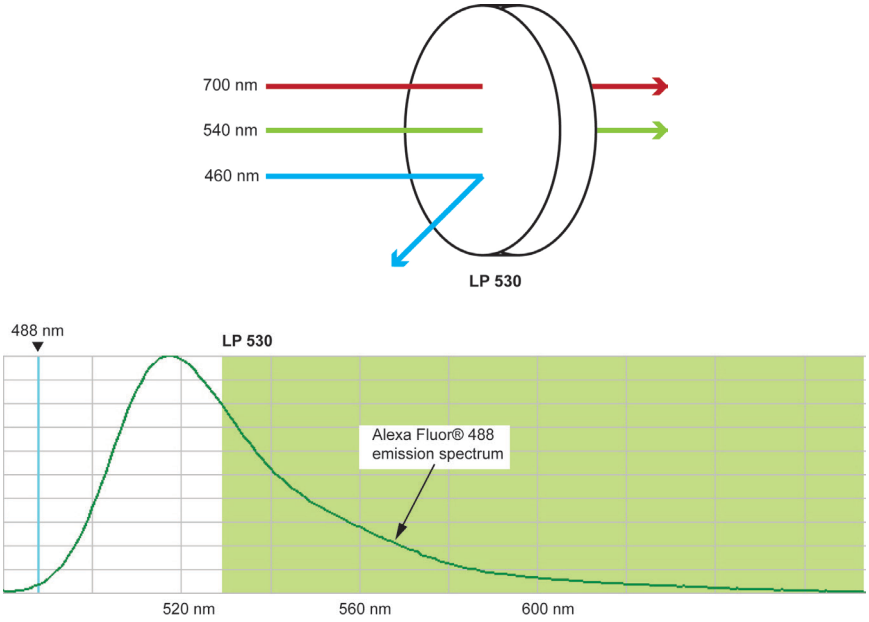
For example, a filter that would detect Alexa Fluor™ 488 dye would be 530/30, which would allow wavelengths in the 515–545 nm range to pass.



Longpass filter

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

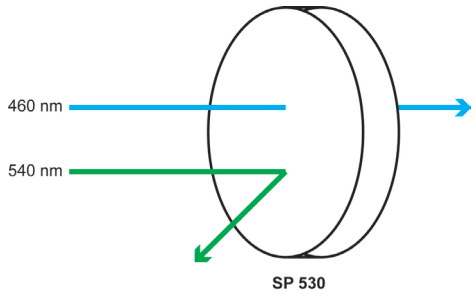
For example, an LP 530 filter permits wavelengths longer than 530 nm to pass, while reflecting or absorbing wavelengths shorter than 530 nm.



Shortpass filter

Shortpass filter (SP) is an optical interference or colored glass filter that attenuates longer wavelengths and transmits shorter wavelengths over the active range of the target spectrum (usually the ultraviolet and visible region).

For example, an SP 530 filter permits wavelengths shorter than 530 nm to pass, while reflecting or absorbing wavelengths longer than 530 nm.

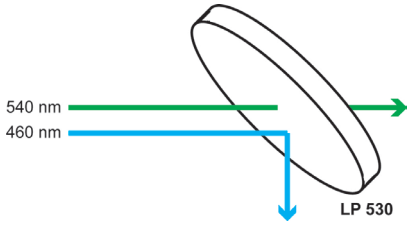


Dichroic mirror

Dichroic mirrors, also called "reflective," "thin film," or "interference" filters, are produced by coating a glass substrate with a series of optical coatings. Dichroic filters usually reflect the unwanted portion of the light and transmit the remainder.

A *dichroic filter* is a very accurate color filter used to selectively pass light of a small range of colors while reflecting other colors. By comparison, dichroic mirrors and dichroic reflectors tend to be characterized by the color(s) of light that they reflect, rather than the color(s) they pass.

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



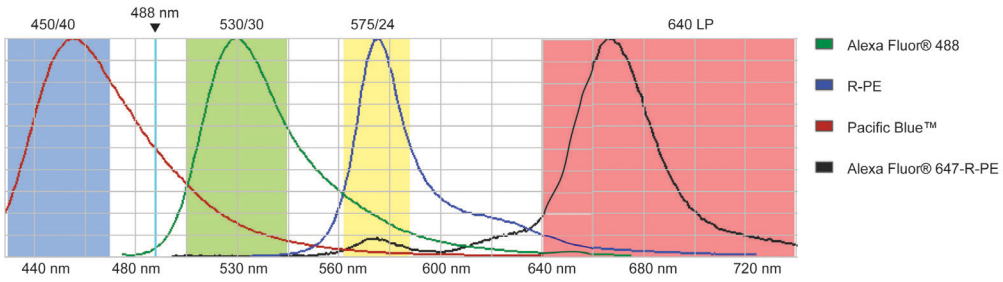
Neutral density filter

Neutral density filters (ND) have constant attenuation across the range of visible wavelengths, and are used to reduce the intensity of light by reflecting or absorbing a portion of it.

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 particular spectrum unique to that molecule. These emission spectra overlap, in some cases very significantly. The example below shows the emission spectra of Pacific Blue™, Alexa Fluor™ 488, R-phycoerythrin (R-PE), and Alexa Fluor™ 647-R-PE dyes.



Each dye emits with a characteristic emission spectrum that is specific for the fluorophore: Alexa Fluor™ 488 dye has a maximum around 520 nm, R-PE at about 575 nm, Pacific Blue™ dye at about 454 nm, and Alexa Fluor™ 647-R-PE dye at about 666 nm. The teal line represents the laser excitation wavelength of an argon ion laser (488 nm).

In this example, the emission spectra of each dye is backlit with a shaded area indicating the emission filter where the specific light is captured on the Attune™ NxT Cytometer. In general, filters are chosen which collect the emitted light near the emission maximum. For example, to capture the emission from Alexa Fluor™ 488 dye, a BP 530/30 filter is used (i.e., the filter has a pass-band centered at 530 nm, and the width of the pass-band is 30 nm).

However, it is impossible to choose filters that measure only one dye. For instance, the Alexa Fluor™ 488 dye has a significant emission in the region that RP-E is measured (575 nm). Therefore, the emission from Alexa Fluor™ 488 dye will register in 530 nm and 575 nm bands. If R-PE is also present, it will contribute to the 575 nm band. Compensation is the mathematical process for correcting for the amount of the Alexa Fluor™ 488 dye fluorescence in the 575 nm band so that R-PE fluorescence can be accurately measured. Performing multicolor analyses can complicate this process further, because fluorophores are not usually spectrally separated very well.

Fluorescence spill over can be estimated by running single fluorescence controls specific for a certain dye and then subtracting out the fluorescence in the other detection channels, thus leaving 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 above, the Pacific Blue™ conjugate has some fluorescence in the 530/30 filter and very little in the 575/24 filter; therefore, the amount of compensation required in the 530/30 filter will be more than in the 575/24 filter.

Electronics

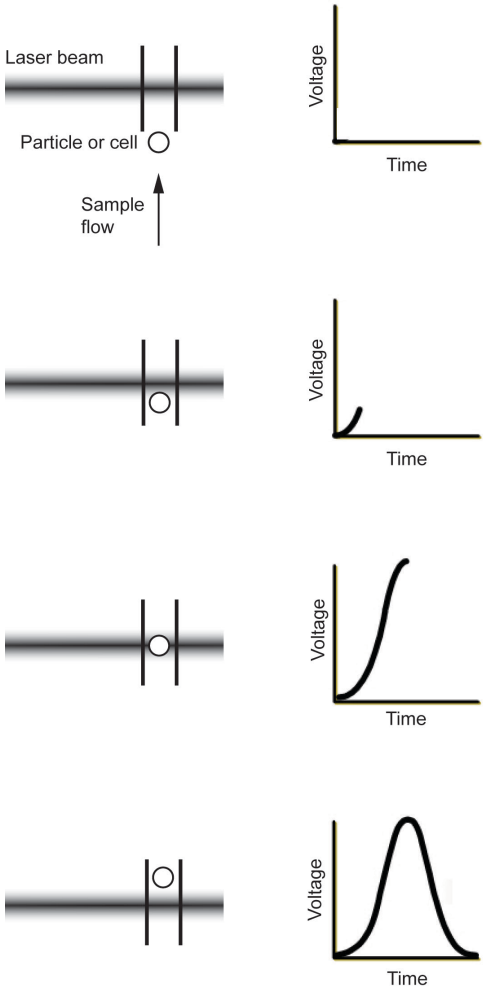
Voltage pulse

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 very 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 portion 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, thus causing more light to scatter off of the particle.

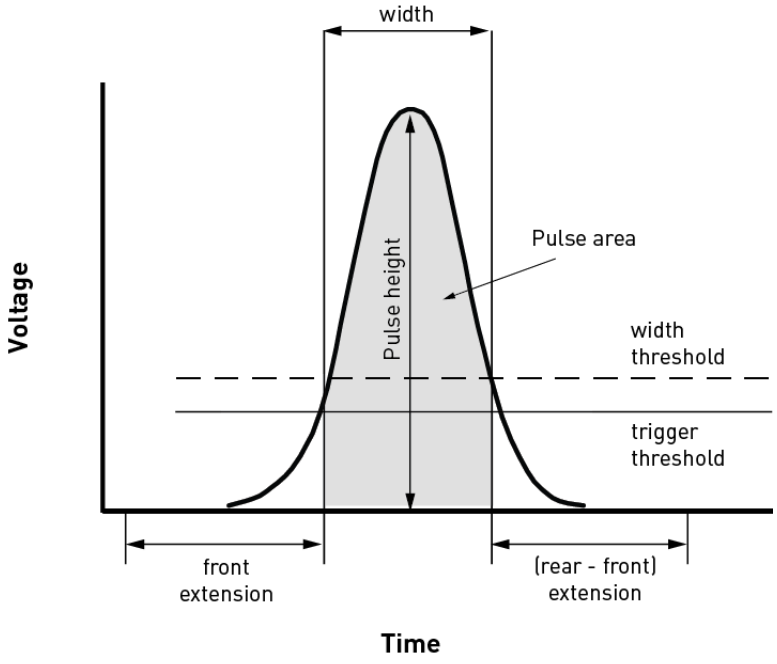
As the cell or particle exits the beam, the signal starts decreasing and eventually trails off below the threshold.



Pulse measurement

The analog signal from the detectors are amplified and relayed to the 16-bit analog-to-digital converter (ADC), which samples the signals at a rate of up to 20 MHz, converting the continuous signal into digital data up to 35,000 events per second.

The data is 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.



Appendix C: Ordering information

Attune™ NxT Acoustic Focusing Cytometer

The Attune™ NxT Acoustic Focusing Cytometer is available with the following laser configuration options from Thermo Fisher Scientific. For more information, go to **www.thermofisher.com** or contact Technical Support (page 88).

Product	Amount	Cat. no.
Attune™ NxT Acoustic Focusing Cytometer, blue	1 each	A24864
Attune™ NxT Acoustic Focusing Cytometer, blue/yellow	1 each	A24861
Attune™ NxT Acoustic Focusing Cytometer, blue/violet	1 each	A24862
Attune™ NxT Acoustic Focusing Cytometer, blue/red	1 each	A24863
Attune™ NxT Acoustic Focusing Cytometer, blue/violet/yellow	1 each	A24859
Attune™ NxT Acoustic Focusing Cytometer, blue/red/violet	1 each	A24860
Attune™ NxT Acoustic Focusing Cytometer, blue/red/yellow	1 each	A28993
Attune™ NxT Acoustic Focusing Cytometer, blue/red/yellow/violet	1 each	A24858

Attune™ NxT Acoustic Focusing Cytometer accessory products

The following products and replacement parts for the Attune™ NxT Acoustic Focusing Cytometer are available separately from Thermo Fisher Scientific. For more information, go to **www.thermofisher.com** or contact Technical Support (page 88).

Product	Amount	Cat. no.
Attune™ Focusing Fluid, 1X Solution	1 × 1 L 6 × 1 L 1 × 10 L	4488621 4449791 A24904
Attune™ Wash Solution	250 mL	A24274
Attune™ 1X Shutdown Solution	250 mL	A24975
Attune™ Performance Tracking Beads	3 mL	4449754
Attune™ NxT Wash Bottle, 175 mL	1 each	100022151
Attune™ NxT Shutdown Bottle, 175 mL	1 each	100022154
Attune™ NxT Waste Bottle, 1.9 L	1 each	100022156
Attune™ NxT Focusing Fluid Bottle, 1.9 L	1 each	100022155
Attune™ NxT Sample Syringe, 1 mL	1 each	100022591
Attune™ NxT Focusing Fluid Filter	1 each	100022587
Attune™ Custom Filter Holder Kit	1 each	A27784
Attune™ NxT No-Wash No-Lyse Filter Kit	1 kit	100022776
Attune™ NxT Fluorescent Protein Filter Kit	1 kit	100022775
Attune™ 96-well Plate	1 each	4477131

Attune™ NxT filters

The following replacement filters used in the optics path of the Attune™ NxT Acoustic Focusing Cytometer are available separately from Thermo Fisher Scientific. For more information, go to **www.thermofisher.com** or contact Technical Support (page 88).

Product	Amount	Cat. no.
Long Pass filters		
Attune™ NxT Filter, 413LP	1 filter	100022752
Attune™ NxT Filter, 496LP	1 filter	100022753
Attune™ NxT Filter, 569LP	1 filter	100022754
Attune™ NxT Filter, 646LP	1 filter	100022755
Dichroic Long Pass filters		
Attune™ NxT Dichroic Filter, 495DLP	1 filter	100022769
Attune™ NxT Dichroic Filter, 555DLP	1 filter	100022776
Attune™ NxT Dichroic Filter, 600DLP	1 filter	100022771
Attune™ NxT Dichroic Filter, 650DLP	1 filter	100022772
Attune™ NxT Dichroic Filter, 690DLP	1 filter	100022773
Attune™ NxT Dichroic Filter, 740DLP	1 filter	100022774
Band Pass filters		
Attune™ NxT Emission Filter, 440/50BP	1 filter	100022756
Attune™ NxT Emission Filter, 512/25BP	1 filter	100022757
Attune™ NxT Emission Filter, 530/30BP	1 filter	100022758
Attune™ NxT Emission Filter, 585/16BP	1 filter	100022759
Attune™ NxT Emission Filter, 590/40BP	1 filter	100022760
Attune™ NxT Emission Filter, 603/48BP	1 filter	100022761
Attune™ NxT Emission Filter, 620/15BP	1 filter	100022762
Attune™ NxT Emission Filter, 670/14BP	1 filter	100022763
Attune™ NxT Emission Filter, 695/20BP	1 filter	100022764
Attune™ NxT Emission Filter, 710/50BP	1 filter	100022765
Attune™ NxT Emission Filter, 720/30BP	1 filter	100022767
Attune™ NxT Emission Filter, 780/60BP	1 filter	100022766

Attune™ Auto Sampler and accessory products

The following products and replacement parts for the Attune™ Auto Sampler are available separately from Thermo Fisher Scientific. For more information, go to **www.thermofisher.com** or contact Technical Support (page 88).

Product	Amount	Cat. no.
Attune™ Acoustic Focusing Cytometer Auto Sampler	1 each	4473928
Attune™ Auto Sampler Focusing Fluid Bottle Assembly	1 each	4477847
Attune™ Auto Sampler Waste Bottle Assembly	1 each	4477850
Attune™ Auto Sampler Syringe, 1 mL	1 each	4478686

Attune™ NxT External Fluid Supply and accessory products

The following products and replacement parts for the Attune™ NxT External Fluid Supply are available separately from Thermo Fisher Scientific. For more information, go to **www.thermofisher.com** or contact Technical Support (page 88).

Product	Amount	Cat. no.
Attune™ NxT External Fluid Supply	1 each	A28006
Attune™ Focusing Fluid, 1X Solution, 10 L Cubetainer	1 × 10 L	A24904
Attune™ NxT External Fluid Supply Waste Container, 20 L	1 each	100027470
Attune™ NxT External Fluid Supply Waste Bottle Interface Assembly	1 each	100028800
Attune™ NxT External Fluid Supply Cubetainer Interface Assembly	1 each	100027471
Attune™ NxT External Fluid Supply Bottle Connections Assembly	1 each	100027251
Attune™ NxT External Fluid Supply Tube/Cable Harness Assembly	1 each	100027250
Attune™ NxT External Fluid Supply Cable, Cytometer to EFS	1 each	100026482

Appendix D: Safety

This section includes the following topics:

- Safety conventions used in this document
- Symbols on instruments
- Safety labels on instruments
- General instrument safety
- Chemical safety
- Chemical waste safety
- Electrical safety
- Physical hazard safety
- Biological hazard safety
- Laser safety
- Workstation safety
- Safety and electromagnetic compatibility (EMC) standards

Safety conventions used in this document

Safety alert words

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

Definitions

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

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

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

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

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

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on the instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on the instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "**Safety labels on instruments**"). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.

Environmental symbols on instruments

The following symbol applies to all electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your Customer Service representative for equipment pick-up and recycling. See **www.thermofisher.com** for a list of customer service offices in the European Union.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on the instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Life Technologies qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Life Technologies.
	**DANGER!** Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	**DANGER!** Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Thermo Fisher Scientific may result in personal injury or damage to the instrument.

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs) (see "**Obtaining SDSs**").

Cleaning or decontaminating the instrument

CAUTION! Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

General safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials (see "**Obtaining SDSs**").
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical waste safety

Chemical waste hazard

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste (see "**Obtaining SDSs**").
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical safety

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Attune™ NxT Acoustic Focusing Cytometer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER! ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

DANGER! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The Attune™ NxT Acoustic Focusing Cytometer has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical hazard safety

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories*

  (stock no. 017-040-00547-4; **www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens

  (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at:

  www.cdc.gov

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition

  www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Laser safety

Laser classification

The Attune™ NxT Acoustic Focusing Cytometer has seven different laser configurations, using one or more of the following excitation lasers: blue 488 nm, 50 mW laser; violet 405 nm, 50 mW laser; red 637 nm, 100mW laser; and yellow 561 nm, 50 mW laser. Under normal operating conditions, the Attune™ NxT Acoustic Focusing Cytometer is categorized as a Class 1 Laser Product. When safety interlocks are disabled during certain servicing procedures and/or input/output optics covers are removed, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

Laser safety requirements

To ensure safe laser operation:

- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, you may be exposed to laser emissions in excess of the Class 3B rating.
- Do not remove safety labels.

Additional laser safety information

Refer to the user documentation provided with the laser for additional information on government and industry safety regulations.

WARNING! LASER HAZARD. Lasers can burn the retina, causing permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Do not remove the instrument top or front panels. Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the top or front panels are removed for service.

WARNING! LASER HAZARD. An overheated laser can cause severe burns if it comes in contact with the skin. DO NOT operate the laser when it cannot be cooled by its cooling fan. Always wear appropriate laser safety goggles.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- European safety and EMC standards
- Australian EMC standards

U.S. and Canadian safety standards

The Attune™ NxT Acoustic Focusing Cytometer has been tested to and complies with standard:

UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

FDA "Radiation Control for Health and Safety Act of 1968 Performance Standard 21 CFR 1040.10 and 1040.11," as applicable.

Canadian EMC standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European safety and EMC standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards:

IEC 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

IEC 60825-1: Ed. 2 (2007), "Radiation Safety of Laser Products - Equipment Classification and Requirements."

IEC 61010-2-081:2003, "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"

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard IEC 61326 (Group 1, Class A), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Documentation and support

Obtaining support

Technical support

Visit **www.thermofisher.com/support** for the latest in services and support, including:

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

Note: When contacting customer support for instrument troubleshooting, provide the instrument model and the instrument serial number. Convey to the technical support any error messages that were displayed on your instrument and any troubleshooting that you have already performed (refer to *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide*; Pub. no. 100024234).

Obtaining SDSs

Safety Data Sheets (SDSs) are available at **www.thermofisher.com/sds**.

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**.

If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

IMPORTANT! Wiping the computer supplied with the Attune™ NxT Acoustic Focusing Cytometer (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.

Life Technologies I Carlsbad, CA 92008 USA I Toll free in USA 1.800.955.6288

For support visit thermofisher.com/support

thermofisher.com

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