USER GUIDE

Attune™ NxT Auto Sampler

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ThermoFisher SCIENTIFIC

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Contents

About this guide

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Attune™ NxT Acoustic Focusing Cytometer equipped with the Attune™ NxT Auto Sampler sample loading device.

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) and the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Pub. no. 100024235).

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 Auto Sampler 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 Auto Sampler User Guide* are listed below. For safety alert words and symbols used in the user 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 Auto Sampler 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™ Auto Sampler Quick Reference Card	4479066

Additional resources are available on the Flow Cytometry Technical Resources page. Go to **www.lifetechnologies.com**, and then search for "Flow Cytometry" to open this page. 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 at points in this 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 Thermo Fisher Scientific are available to you free 24 hours a day. For instructions on obtaining SDSs, see "**Obtaining SDSs**".

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

1. Product information

System components

The Attune™ NxT Auto Sampler (Cat. no. 4473928) is shipped with the system components listed below. All components are shipped at ambient temperature.

Component	Quantity
Attune™ NxT Auto Sampler	1
Attune™ Auto Sampler Starter Kit	1
Attune™ Auto Sampler Quick Reference Card	1

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.

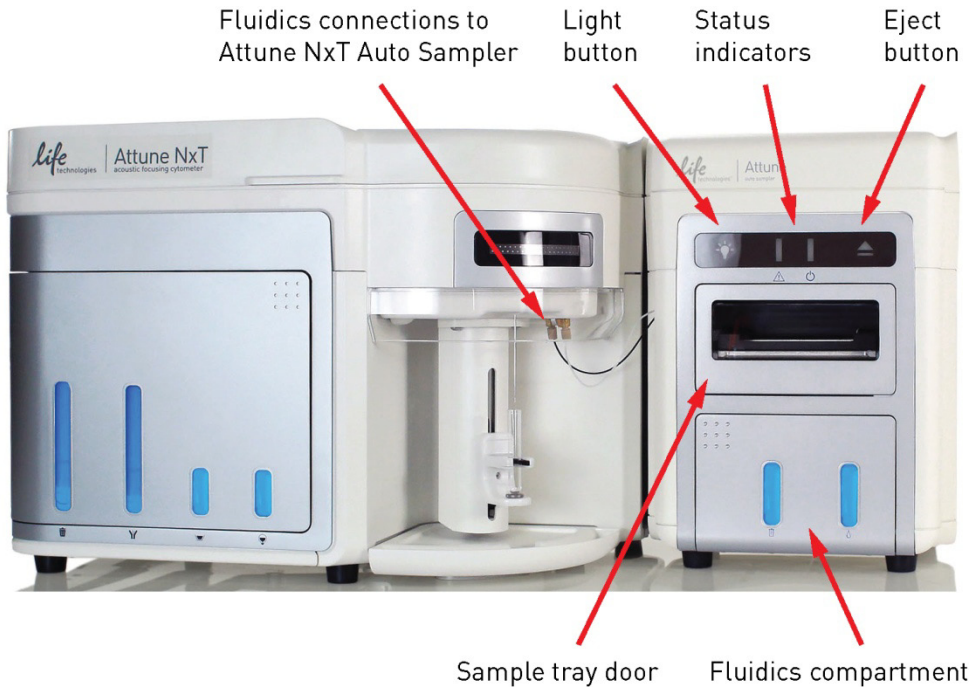
Registering your instrument

Visit **www.lifetechnologies.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 and the Attune™ NxT Auto Sampler.

Product use

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

Instrument exterior components



Installation

IMPORTANT! For instructions on installing the Attune™ NxT Acoustic Focusing Cytometer, refer to the *Attune™ NxT Acoustic Focusing Cytometer Site Preparation Guide* (Pub. no. 100024428), available for download at **www.lifetechnologies.com**.

Install the Attune™ NxT Auto Sampler

1. Uncrate the Attune™ NxT Auto Sampler and place it on the bench next to the Attune™ NxT Acoustic Focusing Cytometer.

   Do not attach the cast bracket (if included) to hold the cytometer and the auto sampler together as it is not designed to work with the Attune™ NxT Acoustic Focusing Cytometer.
2. Remove the shrink wrap from the Attune™ NxT Auto Sampler and inspect the instrument for any sign of damage. Remove the tape and bag from the two external fluidics lines and disconnect the lines from the union fitting.
3. Connect the fluidics lines from the Attune™ NxT Auto Sampler into the two ports on the Attune™ NxT Cytometer; it does not matter which line goes into which port on the cytometer. Make sure to tighten the fittings on the fluidics lines connections until you hear or feel them click.

   Note: Fittings that connect the fluidics lines to the ports on the cytometer are auto-torque style. Tighten the fitting until you hear the first click. Because of the size and location, you might not be able to get them to the catch point; if the fittings are as tight as you can reasonably tighten and they have not clicked, they are likely in tightly enough. Watch for leaks at the connector when the instrument is first run to verify proper seal at the fittings.
4. From the fluidics compartment, remove bag and tape from the two bottle fluidics lines and connect the Waste and Focus bottles to the Attune™ NxT Auto Sampler.
5. Open the sample tray door and remove the protective shipping foam from the plate tray compartment. The sample tray door is spring loaded, but it is easy to open from either the left or right tray door corners.
6. Connect the power supply cord to the Attune™ NxT Auto Sampler and then plug it in to the electrical outlet. Make sure to follow the labels on the back of the auto sampler to get the plug into the correct orientation.
7. Connect the USB cable into the back of the Attune™ NxT Auto Sampler and then plug the other end of the USB cable into the back of the Attune™ NxT Acoustic Focusing Cytometer.
8. Fill all of the fluidics bottles and ensure that the waste tank is empty for both the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler.
9. Power on the Attune™ NxT Auto Sampler, and then the Attune™ NxT Acoustic Focusing Cytometer.
10. Launch the Attune™ NxT Software and login.

Prime the system fluidics

Priming the system fluidics is critical to proper instrument performance. After completing the auto sampler installation, follow the priming procedure below.

1. Run the **Shutdown** procedure using the **Quick** option (see page 40).

   Note: After the shutdown script has completed, the Attune™ NxT Acoustic Focusing Cytometer will automatically power off, and the Attune™ NxT Auto Sampler will enter into an idle mode.

2. Power-cycle both the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler.
3. Run the **Startup** procedure (see page 15).
4. Run the **De-bubble** procedure (see page 45).
5. *Optional*: Run the **Auto Sampler Calibration** function (see page 48).

Note: For instructions on decontaminating the Attune™ NxT Auto Sampler and preparing it for shipment, refer to the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide* (Pub. no. 100024234), available for download at **www.lifetechnologies.com**.

2. Running samples

Workflow

Before you begin
Startup
Create an Experiment
Set up a Workspace
Define Run Protocol
Optimize the Experiment
Calculate compensation
Run Samples and collect data
Analyze and process data
Shutdown

IMPORTANT! Although the daily Startup and Shutdown procedures are automated and require minimal user input, we recommend that you familiarize yourself with the Attune™ Acoustic NxT Focusing Cytometer, its operating principles, and the software user interface by reading the Attune™ NxT Acoustic Focusing Cytometer User Guide before starting your experiments.

The Attune™ NxT Acoustic Focusing Cytometer User Guide is available for download at **www.lifetechnologies.com**.

Before you begin

Required solutions

- **Attune™ Focusing Fluid** – is a buffered, azide-free support/carrier reagent for transporting particles through the capillary assembly. 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 1X salt-free solution that prevents the formation of bubbles and the accumulation of salt in the fluidics system of the instrument when the instrument is powered off.
- **10% bleach solution in deionized water** – decontaminates the fluidics lines. Prepare this solution fresh daily and use during the shutdown procedure.
- **Attune™ 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 deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

IMPORTANT! Reagents may be stored at colder temperatures, but running the Attune™ NxT Acoustic Focusing Cytometer with cold reagents (<15°C) will affect the data quality. Before you run the instrument, ensure that all fluids have equilibrated to room temperature.

Sample requirements

- The Attune™ NxT Acoustic Focusing Cytometer with the Attune™ NxT Auto Sampler is designed to handle samples in 96-well or 384-well standard or deep well plates with round (U), flat, or conical (V) bottoms.

  Note: We strongly recommend using round bottom plates for any assay in which homogeneous sampling and consistency of concentration is essential.

- The method used to prepare a specimen depends on the sample type and the assay desired.
- In general, the maximum recommended sample concentration for analysis is 1×10^6 cells/mL. If the concentration of your sample is $>1 \times 10^6$ cells/mL, dilute it down prior to running it on the Attune™ NxT Cytometer.
- The maximum recommended sample concentration for 500 µL/minute and 1,000 µL/minute flow rates is 5×10^5 cells/mL.

IMPORTANT! Although running a full 384-well plate in the standard mode requires only 1.6 L of Attune™ Focusing Fluid, it is necessary to have at least 1.8 liters of focusing fluid in the focusing fluid tank to ensure that the fluid sensor in the tank detects the correct liquid level.

Startup

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.)

The Startup function ensures that all fluidic lines are clean, the fluidic lines and the system's two pumps are filled with fresh focusing fluid, and the lasers are warmed to operating temperature.

Before you begin

1. Check the levels in the fluidics containers located in the fluidics compartments of both the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler.

   If empty, fill the focusing fluid, wash solution, and shutdown solution containers.

   If full, empty the waste container. For more information, refer to the Attune™ NxT Acoustic Focusing Cytometer User Guide.

   Note: If you are running a full 384-well plate in the standard mode, make sure that the focusing fluid tank contains at least 1.8 liters of Attune™ Focusing Fluid.

2. Power on the Attune™ NxT Acoustic Focusing Cytometer, the Attune™ NxT Auto Sampler, and the computer.
3. Open the lid to the optical compartment and verify that the optical filters are appropriate for your experiment on the Attune™ NxT Acoustic Focusing Cytometer.
4. Start up the computer and log in to Windows.
5. Launch the Attune™ NxT Software.

Run Startup function

1. Login to the Attune™ NxT Software.
2. Click the **Startup** button located in the center of the Instrument Ribbon.

   Alternatively, you can click the **Startup** button from the Collection Panel menu, underneath the progress dial.

   The *Startup dialog* opens and provides instructions to perform the Startup operation.
3. If the tube lifter is raised, lower the tube lifter. If a plate is loaded in the Auto Sampler, remove the plate.
4. Click **Next** and follow the instructions on the Startup prompt screen to perform the Startup operation, which takes less than 5 minutes to complete.

   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.

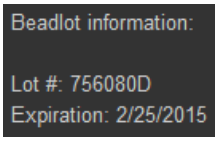
Note: If the daily Startup function has already been performed, proceed to the daily Performance Test.

Run Performance test

1. Click **Performance test** on the Main Menu to view the *Performance test setup* screen, which provides general instructions for setting up a Performance test.

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. Verify that the lot number of the Attune™ Performance Tracking Beads you are using is identical to the bead lot number used in the current baseline (If not, download the bead lot file information at **www.lifetechnologies.com** and perform baseline calculations).



4. Briefly vortex 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 brief vortexing.
5. Load the tube by placing it in the sample tube lifter.
6. 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: For more information on Performance Tracking functions (i.e., Baseline Calculations and Performance Test), downloading bead lot information, and preparing the Attune™ Performance Tracking Beads suspension, refer to the Attune™ NxT Acoustic Focusing Cytometer User Guide, available for download at **www.lifetechnologies.com**.

Create an Experiment

To run your samples and collect cytometric data using the Attune™ NxT Auto Sampler, you need to create a *Plate Experiment*. The Attune™ NxT Software allows you to:

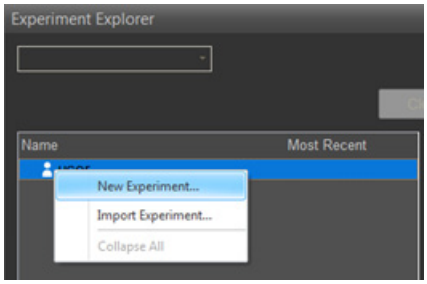
- Create a Plate Experiment using a blank template
- Create a Plate Experiment from the Experiment Explorer using the default Workspace
- Duplicate a saved Plate Experiment in the Experiment Explorer

You can perform these functions from the *Main Menu* or by using the *Experiment Explorer*.

Create a Plate Experiment

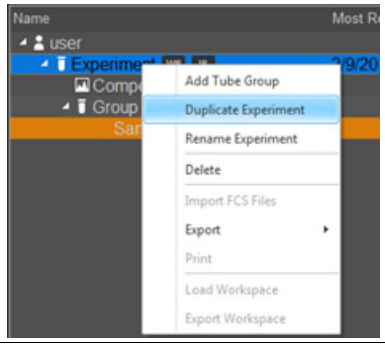
1. From the Main Menu screen, click **New Experiment** or one of the pre-populated Plate Experiment templates (if available).

Alternatively, right-click on the **User** in Experiment Explorer and select **New Experiment**.

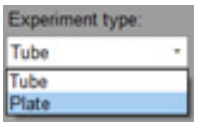


You can also click **New Experiment** in the Home tab of the Ribbon bar to create a new Plate Experiment.

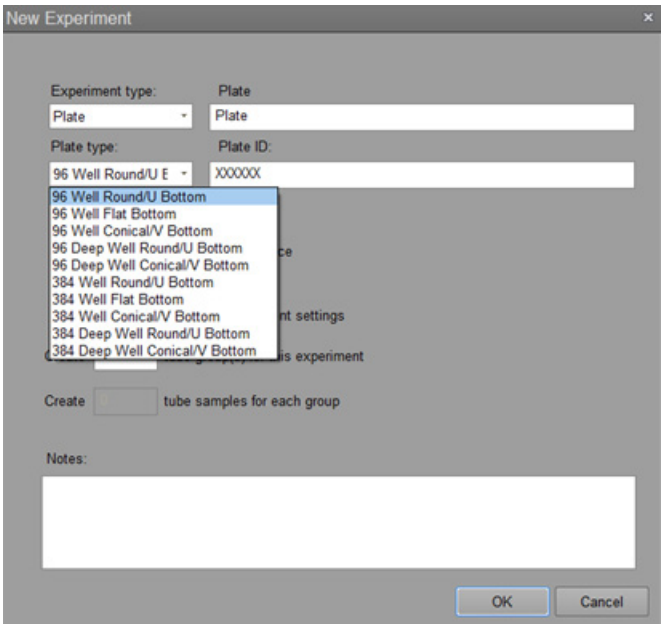
Note: To duplicate a saved Plate Experiment, right-click on an existing plate in the Experiment Explorer and select **Duplicate Experiment**.



2. In the New Experiment dialog, select **Plate** as the Experiment type.

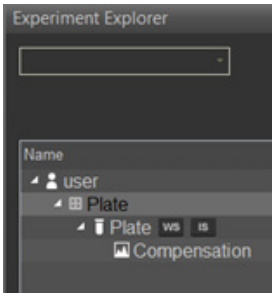


3. Enter the **Plate Name** and **Plate ID**, and select the appropriate **Plate Type** from the drop-down menu.



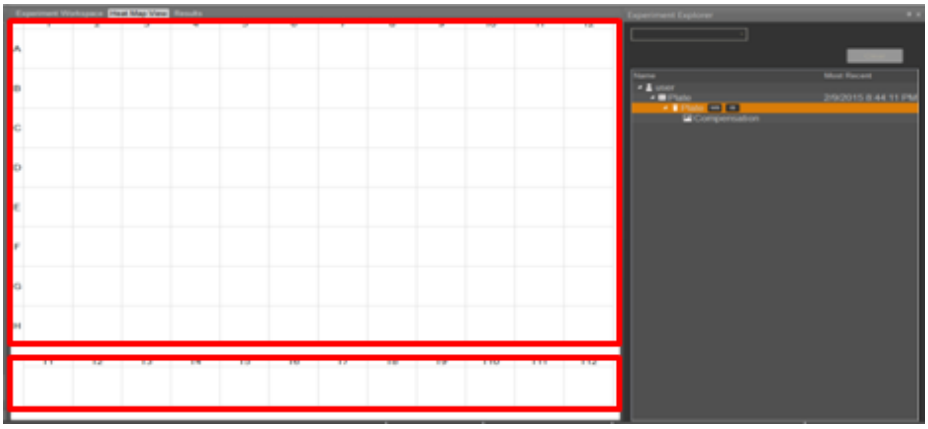
4. Click **OK** to create the New Experiment and close the New Experiment dialog.

   The Experiment Explorer displays the newly created Plate Experiment.

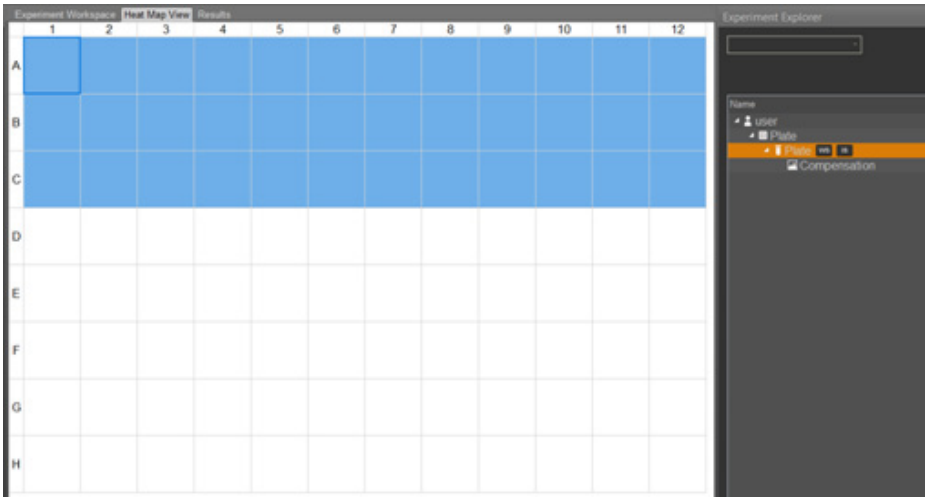


5. Double-click on the **Plate Experiment node** representing the newly created experiment to activate it and open the associated *Heat Map view*.

6. The Heat Map view shows a virtual plate layout that represents the wells available on the plate (96 or 384 wells, depending on the plate type selected). By default, the virtual plate layout also displays additional tube samples that may be incorporated into the experiment.

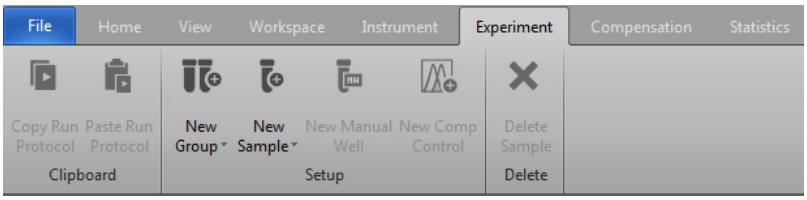


7. In the virtual plate layout, select the wells you want to include in your *Experiment*.
   - To select the entire plate, click on the upper left hand corner of the virtual plate.
   - To select whole columns or rows, click on the number or letter of the column or row you would like to select.
   - To multi-select wells, columns, or rows hold down the **CTRL** key while selecting.



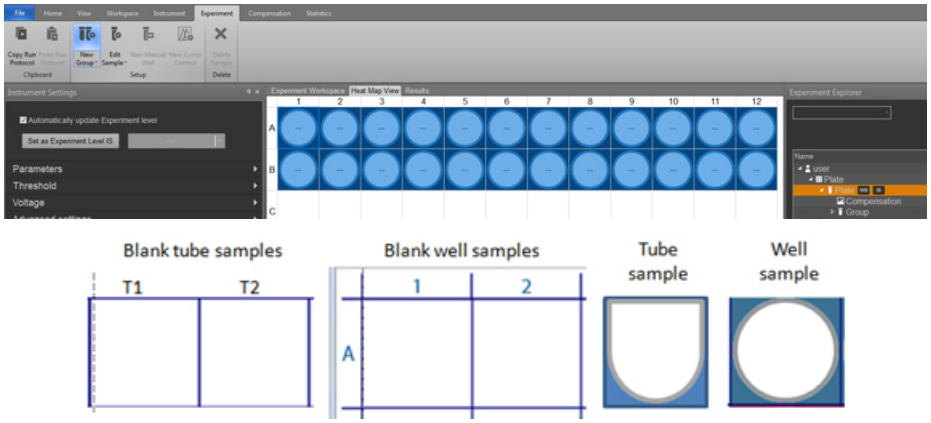
Note: The exact number of wells to select depends on how many fluorescent parameters you are using in your experiment and how many samples you will run.

8. After selecting the appropriate number of wells, click **New Group** or **New Sample** on the Experiment tab of the Ribbon bar.



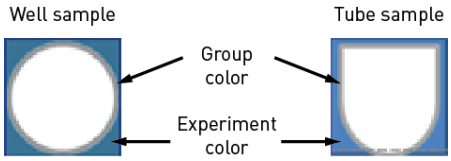
- If the Plate Experiment will include multiple groups on the same plate, select **New Group**.
- If the Plate Experiment will include samples belonging to the same group, select **New Sample**.

9. The virtual plate in the Heat Map view displays the newly created Samples. Blank spaces without assigned Samples are displayed as white squares.

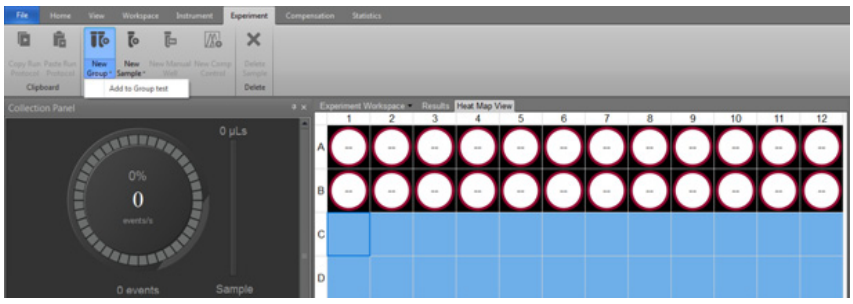


Note: Samples marked as part of an Experiment are displayed using a unique identifying color that fills the area around the Sample. This color differentiates the sample belonging to that Experiment from Samples in other Experiments.

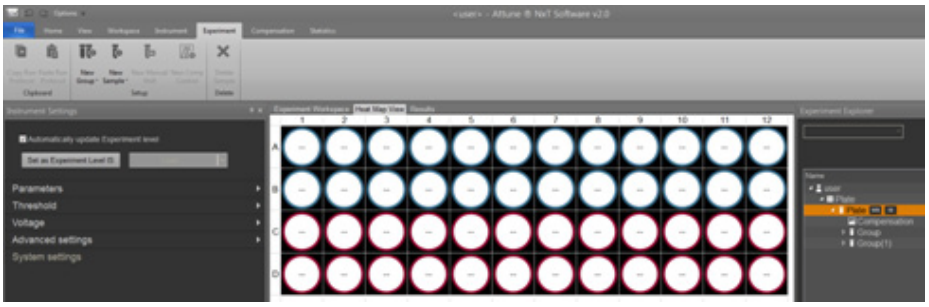
Similarly, each Group has a unique identifying color that is marked around the inside of the Experiment color identifier.



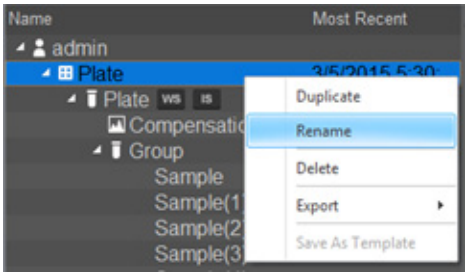
10. To change the size of the Experiment, select additional wells on the virtual plate layout, select **Add to Group** from the **New Group** or **New Sample** drop-down menu on the Experiment tab of the Ribbon bar.



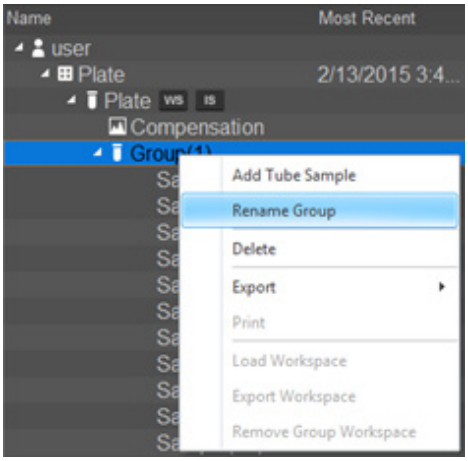
11. To add a new Group to the Experiment, select additional wells in the virtual plate layout and click **New Group** or **New Sample** on the Experiment tab of the Ribbon bar.



Note: To rename a new Plate Experiment, right-click the **Plate node** (the default Experiment name is Plate) in the Experiment Explorer and select **Rename** from the drop-down menu. Type in the new Plate Experiment name and press **Enter** on the keyboard.



To rename a Group, right-click on the **Group node** and select **Rename Group** from the drop-down menu. Type in the new Group name and press **Enter** on the keyboard.

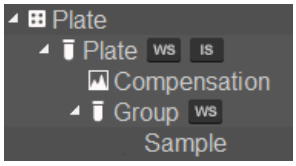


Set up a Workspace

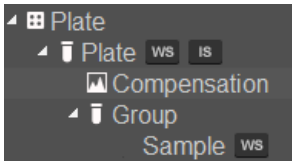
Workspace

Workspace displays analysis objects (plots, gates, and statistics) associated with the current sample, as well as text boxes and image files. There are three Workspace types:

- *Experiment Workspace* is common to all Samples in an Experiment.
- *Group Workspace*, when created, is common only to Samples of the same Group in the Experiment. The Workspace indicator icon (WS) to the right of the Group name indicates the presence of a Group-level Workspace.



- *Sample Workspace* is unique to the selected Sample. The Workspace indicator icon (WS) to the right of the Sample name indicate the presence of a Sample-level Workspace.

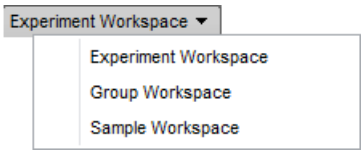


Note: For more information on working with Workspace objects and customizing the Workspace, refer to the Attune™ NxT Software User Guide, available for download at **www.lifetechnologies.com**.

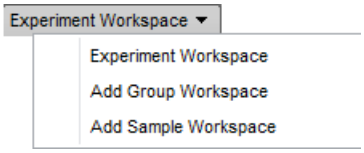
Workspace selection

The Experiment Workspace is the default workspace on the Attune™ Desktop.

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



- If the Group or Sample Workspaces do not exist, the Workspace selection dropdown menu displays the "Add Group Workspace" or the "Add Sample Workspace" options.

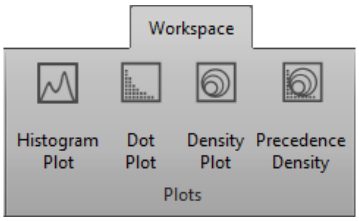


- To add a Group or Sample Workspace, select **Add Group Workspace** or **Add Sample Workspace**, as appropriate.
- When a new Group Workspace is created, the Experiment Workspace is used as a template, and all plots, gates, and statistics are reproduced.
- When a new Sample Workspace is created, the Group Workspace is used as a template. If the Group Workspace does not exist, then the Sample Workspace will use the Experiment Workspace as a template.

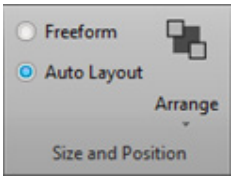
Add analysis objects to Workspace

Follow the instructions below to add analysis objects (*Plots*, *Gates*, and *Statistics*) to your Workspace. For more information on working with Workspace objects and customizing the Workspace, refer to the Attune™ NxT Software User Guide, available for download at **www.lifetechnologies.com**.

Plots



- To insert a *Histogram*, *Dot*, *Density*, or *Precedence Density Plot* to your Workspace, click **Histogram Plot, Dot Plot, Density Plot,** or **Precedence Density Plot** on the Workspace tab of the Ribbon bar.
- To duplicate an existing plot, hold the **CTRL** key, and click and drag the desired plot. Alternatively, use **CTRL +C** and **CTRL +V** or the **Copy** and **Paste** options from the right mouse-click drop down menu to copy and paste the desired plot.



- When the Workspace is in the *Auto Layout* mode, plots are inserted into the Workspace as identically sized objects in a 3 × 4 block grid. Objects cannot be resized when the Workspace is in the Auto Layout mode.
- When the Workspace is in the *Freeform* mode, plots are inserted into the Workspace as identically sized objects, but may be resized by dragging the corner of the plot.
- To change the Parameter and/or the Scale, right-click directly on the plot axis and select from the drop-down menu.

Gates

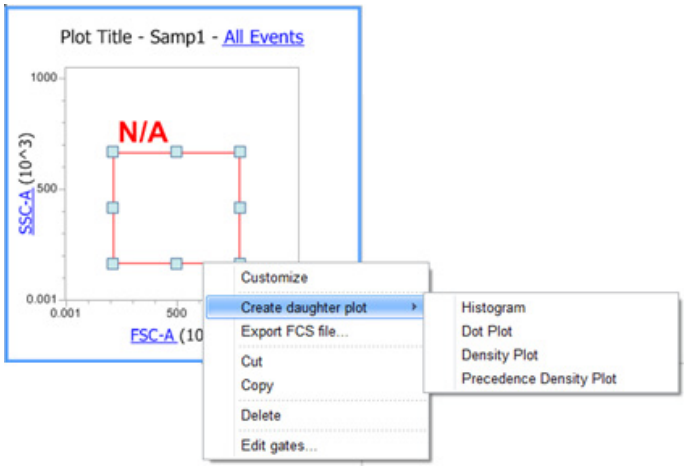
Gating Tools allow you to isolate a region in a selected plot for analysis.

- To insert a *Gate* in a plot, click the plot to select it, and then select the desired **Gate** from the *Gating Tools* on the Workspace tab of the Ribbon bar.
- Dual-parameter gate types are enabled when the current Workspace contains at least one dual-parameter plot (i.e., Dot plot, Density plot, Precedence Density plot).
- Single-parameter gate types are enabled when the Workspace contains at least one Histogram plot.

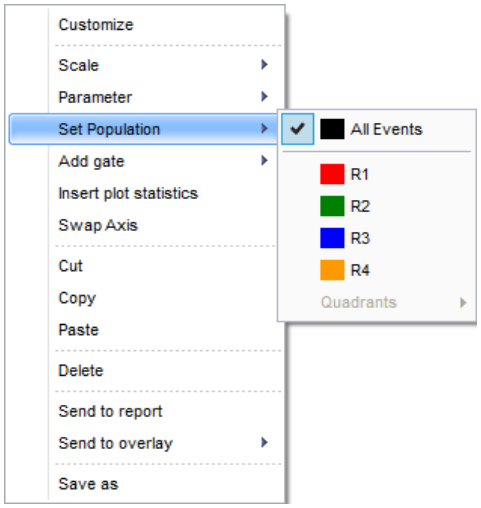
Note: *Rectangular*, *Oval*, and *Polygon Gates* can be inserted into a *Dot Plot*, *Density Plot*, or a *Precedence Density Plot* while *Histogram* and *Bi-Marker Gates* can only be inserted into a *Histogram Plot*. A *Derived Gate* can be created as long as there is at least one gate present in the Workspace. You can create a derived gate without selecting a plot.

Daughter Plots

- To create a *Daughter Plot* from a gate, right-click the gate of interest and then select **Create daughter plot** to choose the type of plot to display (Histogram, Dot Plot, Density Plot or Precedence Density Plot).



- Alternatively, you can right-click an existing plot, select **Set Population** from the drop-down menu, and choose the population for which to create a daughter plot.

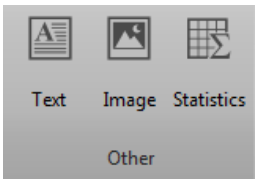


Note: The *Set population* option allows you to choose to limit the data displayed on a plot to a given gate. It makes the plot a daughter of the selected upstream gate.

Statistics

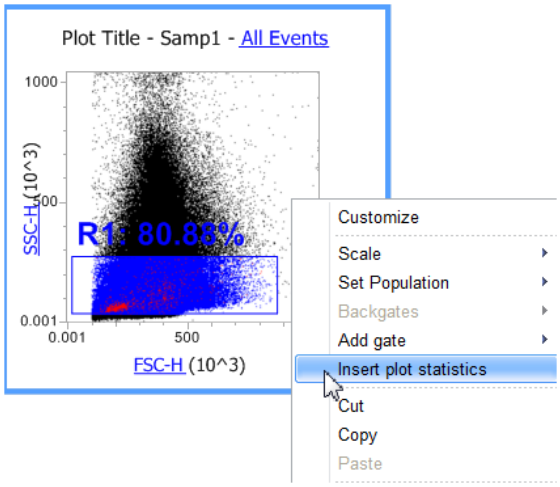
Statistics fall into two categories: *Global Statistics* show statistics for the full gating hierarchy from *All Events* and descendent gates, while *Local Statistics* show statistics for a particular branch of the gating hierarchy and are plot-specific.

- To insert an Experiment-level Statistics box, click **Statistics** on the Workspace tab of the Ribbon bar without selecting any plots.



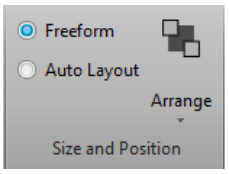
- To insert a Plot-specific Statistics box, select the desired plot, and then click **Statistics** on the Workspace tab of the Ribbon bar.

  Alternatively, right-click the plot and then select **Insert plot statistics** from the drop-down menu.

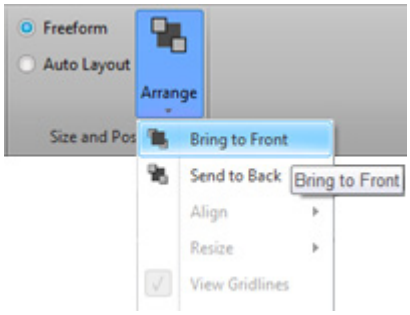


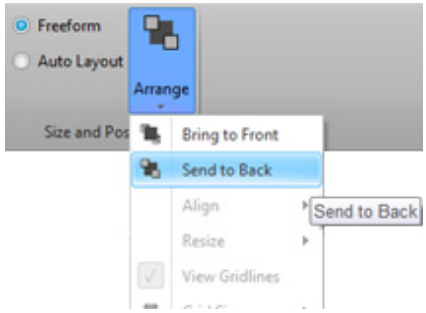
Position groups of objects in the Workspace

Size and Positions tools are used to reposition and resize selected analysis objects (Plots and Statistics boxes) in the Workspace while the Workspace is in the Freeform mode. These tools can be selected from the *Arrange* drop-down menu on the Workspace tab of the Ribbon bar.



- To reposition selected workspace analysis objects in front of or behind other objects, click the analysis object of interest and select the desired option from the **Arrange** dropdown menu on the Workspace tab of the Ribbon bar.





- To select multiple objects, hold the **CTRL** key and click on the desired object(s).

  Alternatively, click and hold while dragging the cursor over multiple objects to select them as group (i.e., lasso tool).

Customize the Workspace

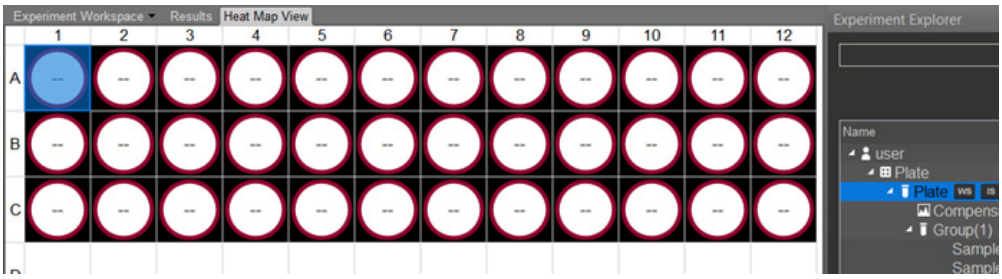
For more information on customizing the Workspace and working with Workspace objects (Plots, Gates, and Statistics), refer to the Attune™ NxT Software User Guide, available for download at **www.lifetechnologies.com**.

Define Run Protocol

Before processing a plate for sample collection, you need to define the *Run Protocol* for Compensation Controls, Manual Well(s), and Sample Well(s) using the options available from the *Collection Panel*. The Run Protocol allows you to define the collection criteria, collection mode, acquisition volume, recording, mixing, and rinse options.

Define Run Protocol for Sample Wells

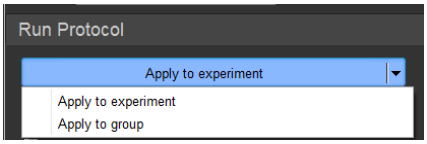
1. Click on the first **Sample Well** (previously defined; see "Create a Plate Experiment", page 17) on the virtual plate display to select it.



2. Define the **Run Protocol** for the Sample Well by setting the following criteria on the *Collection Panel*:

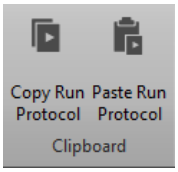
   - *Stop Options:* Set the conditions to stop recording based on the number of **Events**, elapsed **Time**, and/or sample **Volume** analyzed.
   - *Recording Flow Options:* Define the **Acquisition Volume**, **Total Sample Volume**, and **Flow Rate** (you can set the flow rate to 12.5–1000 µL/min; the default flow rate is 200 µL/min).
   - *Recording Options*: Set the **Time** (seconds) or **Events** (number) to elapse before the recording commences.
   - *Mixing Options*: Set the number of **Mixing Cycles** (0–10).
   - *Rinse Options:* Set the number of **Rinse cycles** between Samples (0–10).

3. Click **Apply to experiment** or select **Apply to group** from the dropdown menu.
   - When **Apply to experiment** is selected, the Run Protocol is applied to all Samples on the Plate.
   - When **Apply to group** is selected, the Run Protocol is applied to all Samples within the Group to which the selected well belongs.



4. Alternatively, you can apply the Run Protocol from a specific well to other Samples and/or Groups.

   To do this, click **Copy Run Protocol** from the Experiment tab on the Ribbon bar, select the desired Samples in the Plate by clicking on the wells in the virtual plate layout, and then click **Paste Run Protocol** to apply the copied Run Protocol to the selected wells.



5. To modify specific Run Protocol parameters for a selection of wells, select the wells, change the desired parameters, and then click **Apply to group** (if all wells belong to the same group) or **Copy Run Protocol** and **Paste the Run Protocol** as described above.

Optimize the Experiment

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 30 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 31–32 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 Settings* tab on the Collection Panel.
- For more information on the Instrument Settings tab, refer to the Attune™ NxT Software User Guide, available for download at **www.lifetechnologies.com**.

Define control samples

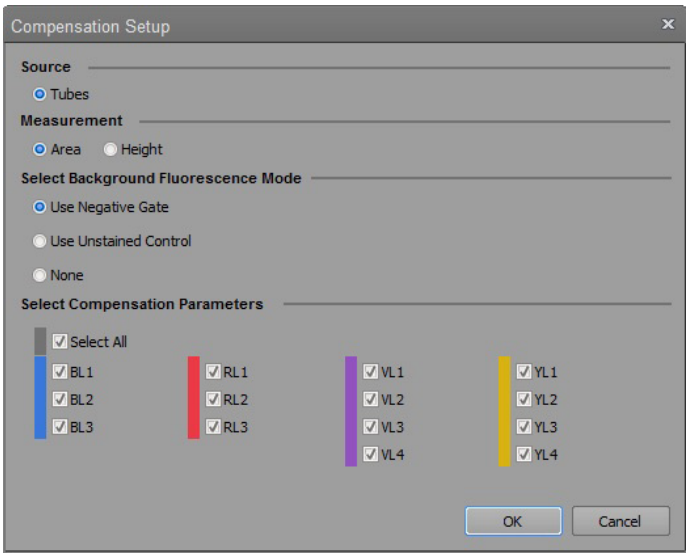
1. To define the control samples, open the Compensation Setup dialog by clicking the **Compensation Setup** button on the *Compensation ribbon tab*.

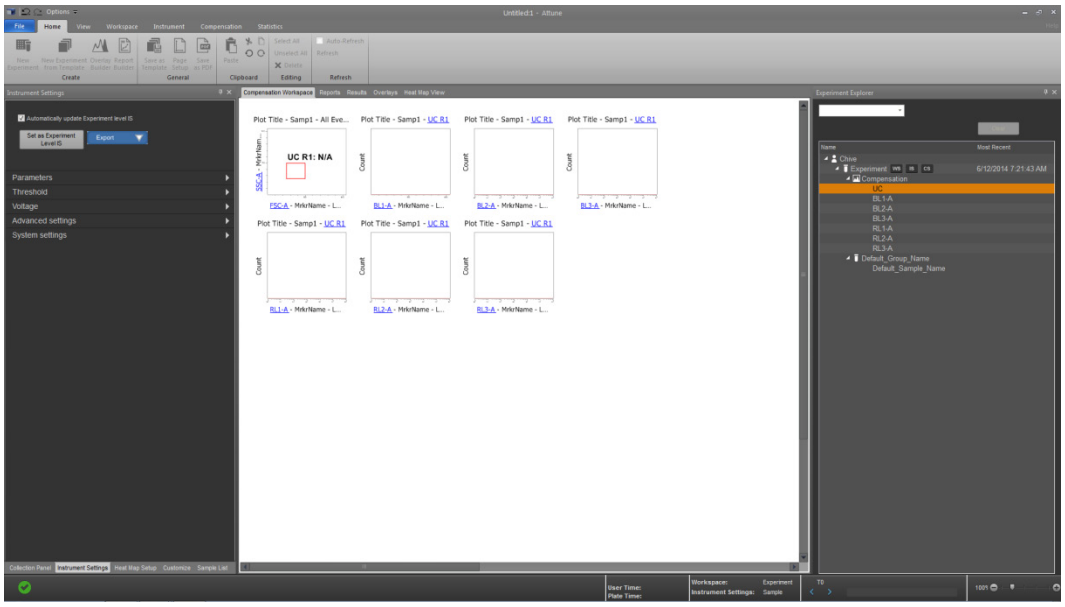
   Alternatively, double-click on the **Compensation node** within an Experiment on the *Experiment Explorer* when there are no compensation controls present.

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

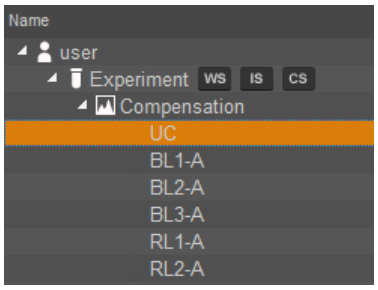


2. On the Compensation Setup dialog, select the source of compensation controls. The Compensation Setup dialog provides different options for setup based on your selection of the compensation source.
3. Next, select the measurement parameter, background fluorescence mode, and the compensation parameters. 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.

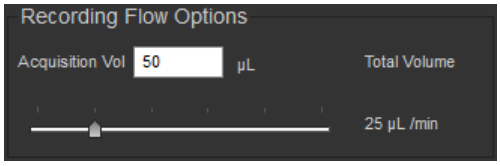


Optimize instrument settings for unstained control

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.

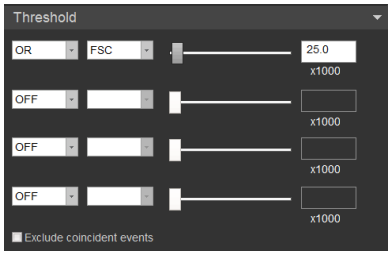
   

   For setup, you can conserve your sample by running the cytometer at a 25 µL per minute collection rate.
4. Click **Run**. Events will appear in the FSC vs. SSC plot. You can obtain data in real-time without saving them to a file.

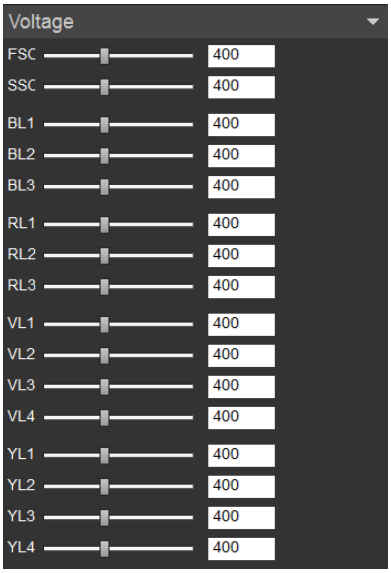
   

> **IMPORTANT!** DO NOT click **Record** at this point. Once you click **Record** on any of the compensation controls within the Compensation Setup, the instrument settings for all fluorescent channels will be grayed out and cannot be changed. It is critical that you optimize voltages prior to recording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

5. Select the *Instrument Settings tab*, and adjust the **FSC voltage** to place the population on scale using the **FSC slider bar**.

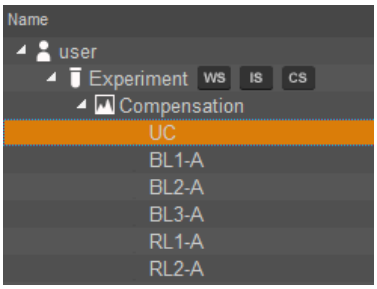
   Alternatively, you can type a specific numerical value in the settings window above each channel.
6. Adjust the **SSC voltage** to place the population on scale by sliding the **SSC slider bar** up or down.
7. Adjust **Threshold** on instrument control panel to remove unwanted events and background.
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 33.

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 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.

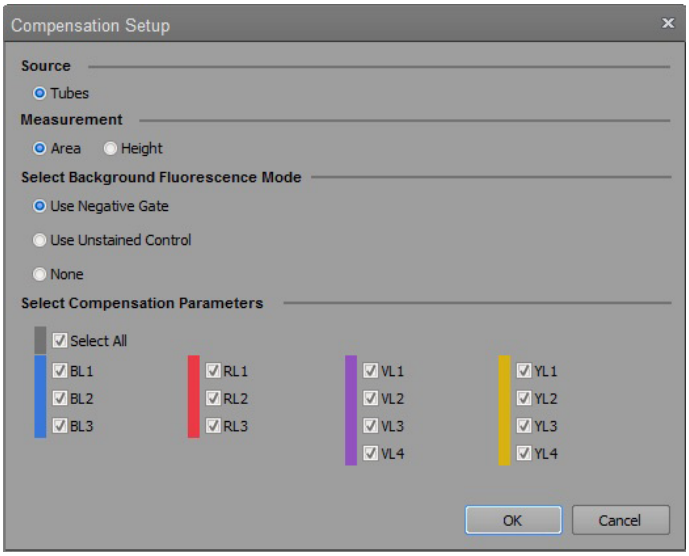
Compensation setup

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 by the software. The specific instructions depend on the action being performed.

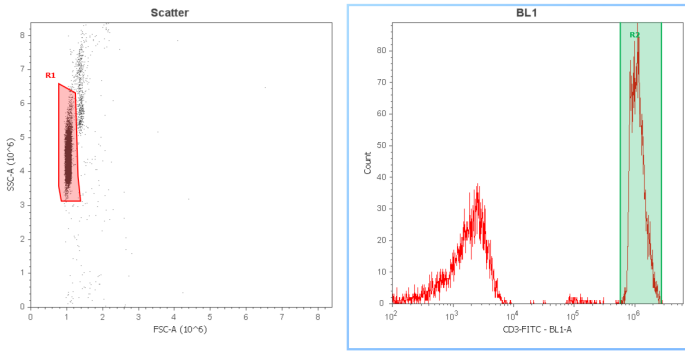
IMPORTANT! Once you have recorded all compensation controls and calculated and applied the Compensation Matrix, you cannot adjust the PMT voltages for experimental data.

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 33, and click **OK.**

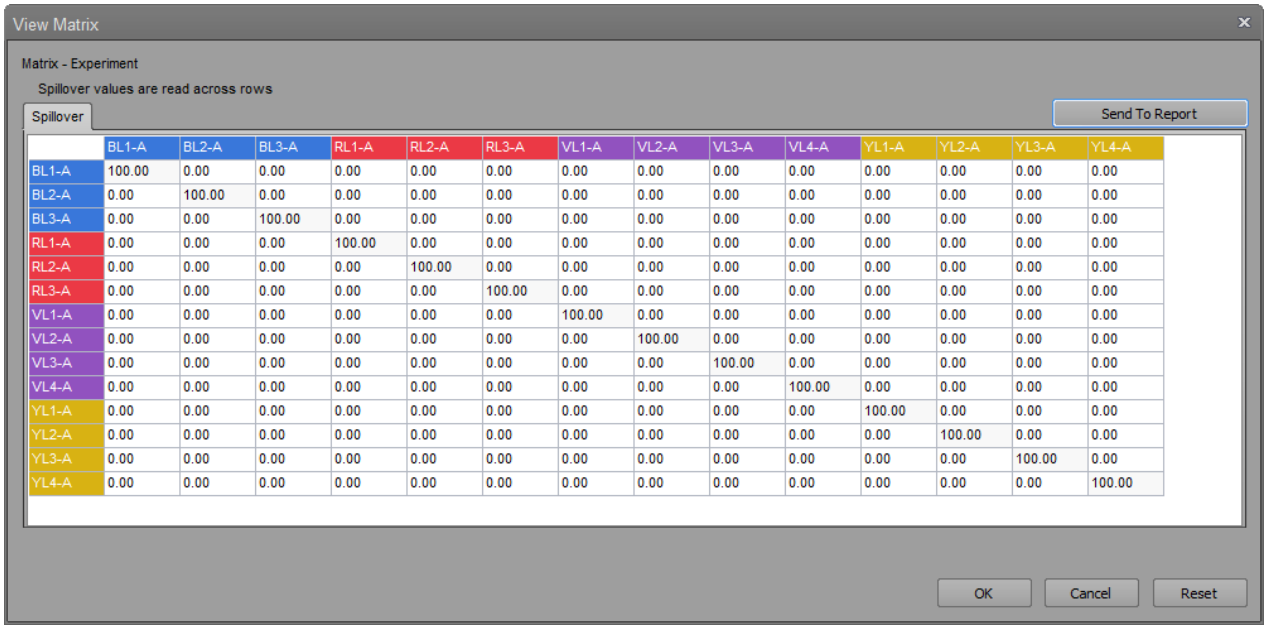
   Compensation Workspace opens and is automatically populated with the plots necessary to calculate compensation.
2. Install the tube containing the unstained control beads/cells on the sample injection port as prompted by the software.
3. Push up the tube loader to the active position in the sample injection 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.



Matrix dialog

When the last compensation control is recorded, *Matrix dialog* automatically opens for your review. The *Matrix dialog* shows the active compensation associated with the current Experiment or FCS file, and allows you to manually edit or reset the *Spillover matrix* values.

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 matrix values (not recommended).

For more information about the Spillover matrix, refer to the "Compensation" chapter in the Attune™ NxT Software User Guide, available for download at **www.lifetechnologies.com**.

Note: *Spillover matrix* shows the amount of spillover from each fluorophore into each of the other fluorescent channels.

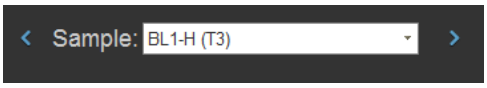
Run Samples and collect data

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

The following example protocols are specific for running Samples from a Plate Experiment. For information on running Tube Samples, refer to the Attune™ NxT Acoustic Cytometer and the Attune™ NxT Software User Guides, available for download at **www.lifetechnologies.com**.

Collect Samples (Plate Collection Mode)

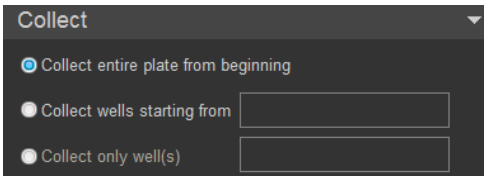
1. After you have completed the procedures for optimizing instrument settings and calculating compensation, click the **right arrow** next to the Sample box in the Collection Panel to move onto the Sample wells.



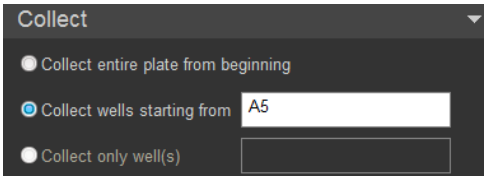
   Alternatively, double-click on the first **Sample well** in the virtual plate layout to select it.

2. Designate the collection method:

   - To collect all wells defined during Experiment set up, select **Collect entire plate from beginning** under the Collect settings in the Collection Panel.

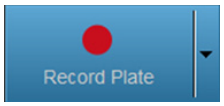


   - If you wish to collect all wells starting after a particular well, select **Collect wells starting from** and type in the well number in the text box next to the selection.



3. Click Record Plate to start the automated Sample collection procedure.

   The Attune™ NxT Cytometric Software acquires and records Samples starting with the first designated Sample well using the appropriate Run Protocol applied to that well.



Note: While collecting Samples, you can create plots, daughter plots, and statistics, and insert and adjust gates. However, once collection of a Sample well has begun you cannot adjust the PMT voltages and Threshold values.

4. Click **Pause** to temporarily halt Sample collection, if needed.
   The instrument completes the collection of the current well and then pauses the collection procedure.

   When the instrument is paused between wells, you can make changes to the Run Protocols and Workspace objects for the remaining wells.

   Click **Clear** to erase all collected data from that well without pausing the collection procedure.

   Click **Restart** to restart collection from the first well and overwrite all collected data including compensation controls and Instrument Settings wells.

   Note: If you click on another Plate or Tube Sample within the Plate Experiment, or adjust collection criteria when sample collection is paused, the software will warn you that the sample will be discarded if this action is completed. To avoid losing your sample, you can choose to run the *Sample Recovery* function from the Functions menu of the lower taskbar.

5. Sample collection automatically stops after all the selected samples in the plate are collected and the Attune™ NxT Software automatically saves the data in a unique FCS file.

Note: For more information on data collection using the Attune™ Acoustic Cytometer, refer to the Attune™ NxT Acoustic Cytometer User Guide, available at **www.lifetechnologies.com**.

Analyze and process data

Data analysis and processing

1. To analyze and process data globally, double-click any **Sample well** in the Heat Map View to open the Experiment Workspace.

   Alternatively, double-click the desired Experiment in the Experiment Explorer.

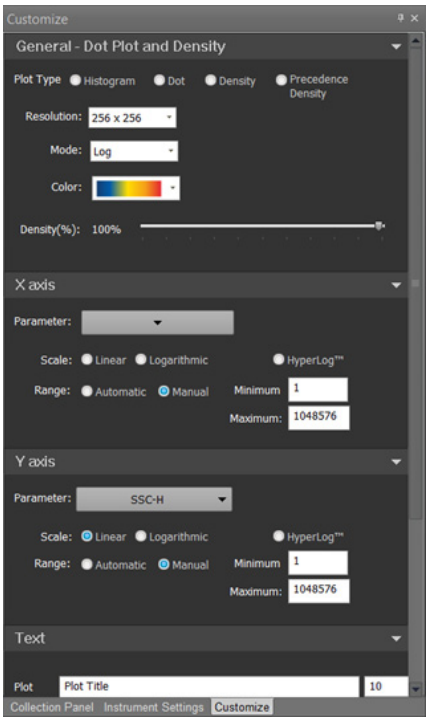
2. Adjust gates and add or remove plots as desired.

   To customize plots, gates, and other analysis objects, select the **Customize Panel**.

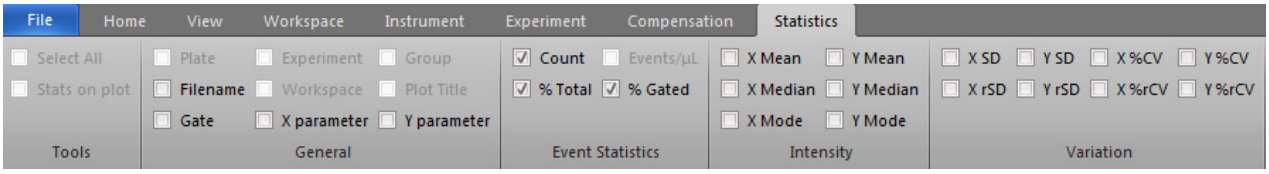
   The options available in the Customize Panel depend upon the analysis object selected.

   You can make adjustments to Plot type, Resolution, Mode, Axis parameters, and Range, as well as plot headings and text when a plot is selected.

   You can adjust Gate type, Name, Opacity, and Coordinates when a gate is selected.

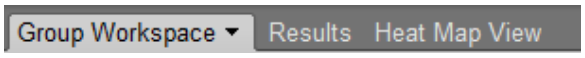


   Use the parameters available in the **Statistics tab** of the Ribbon bar to make adjustments to the statistics box.

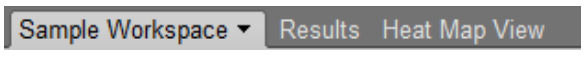


   All plots containing modified parameters will be automatically updated after each change and for all subsequent Samples in the Experiment workspace.

3. To analyze and process data locally (i.e., only for the selected Group), click the **Group Workspace** tab. Any changes made within the Group Workspace will be propagated to other sample workspaces to samples in the same group but not to workspaces of samples in different groups.

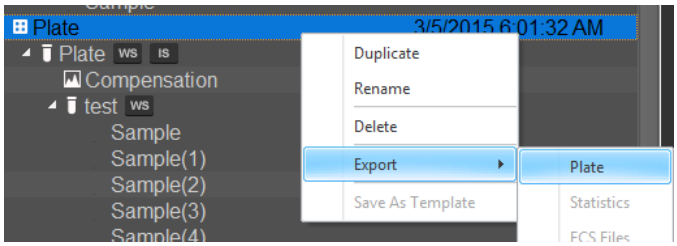


4. To analyze and process data locally for individual samples (i.e., only for selected wells), click the **Sample Workspace** tab. Any changes made to the Sample Workspace are specific for that workspace alone.



Exporting Plates

1. Right-click the **Plate** you want to export in the Experiment Explorer.
2. From the drop-down menu, select **Export Plate**.



3. Select the storage location and click **Save**.

   All the Experiments in the Plate, along with their associated Workspaces, Instrument Settings, Compensation Controls, and all Sample-specific information will be exported.

Note: For more information on data analysis and processing using the Attune™ NxT Software, refer to the Attune™ NxT Software User Guide, available at **www.lifetechnologies.com**.

Shutdown

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.

If the Attune™ NxT Auto Sampler is not powered on, the system will perform a tube based shutdown.

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.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

Check fluid and waste levels

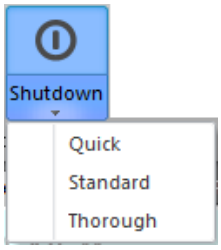
1. Check the levels in the fluidics tanks.
2. Ensure that the wash and shutdown solution tanks are full. If empty, fill the appropriate tanks with Attune™ Wash or Attune™ Shutdown Solution.
3. Empty the waste tank on both the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler.

Note: For the location of the fluidics compartment and instructions on filling the fluidics tanks refer to the Attune™ NxT Acoustic Cytometer User Guide, available for download at **www.lifetechnologies.com**.

Shutdown options

There are three options available for the Shutdown function:

- **Quick** –Quick option uses 5 wash cycles and takes 10 minutes to complete.
- **Standard** –Standard option uses 15 wash cycles and takes 40 minutes to complete.
- **Thorough** –Thorough option uses 25 wash cycles and takes 60 minutes to complete.



For daily use, we recommend the Standard Shutdown function.

IMPORTANT! The Shutdown function powers off the laser and the instrument automatically. If you cancel the Shutdown function while it is running, you will need to exit and restart the Attune™ NxT Software, complete the Startup function, and then restart the Shutdown function and let it run to completion.

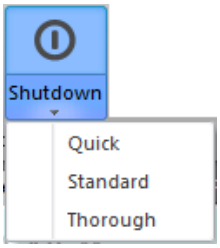
Run Shutdown function

The Attune™ NxT Software provides instructions to perform the *Shutdown* operation. The Shutdown operation is broken into three phases in systems with the Attune™ NxT Auto Sampler, and it can take more than 40 minutes to complete; however, most of the operation is performed automatically.

Make sure to follow all the instructions provided by the instrument during the Shutdown cycle. During the operation, the software provides real-time updates on the shutdown function being executed.

1. Click the **Shutdown** button located on the Instrument tab of the Ribbon bar.

   The dropdown menu provides three options for Shutdown (**Quick**, **Standard**, and **Thorough**). Select the appropriate option. For daily use, we recommend the Standard Shutdown option.



2. The *Shutdown* prompt screen appears.
3. When prompted, place 3 mL of 10% bleach solution in a tube on the sample injection port (SIP) and raise the tube lifter.
4. When prompted, place 3 mL of deionized water in a tube on the SIP, lift the tube filter, and click **Next**.
5. When prompted, place an empty 96-well round bottom plate in the Auto Sampler and close the door.
6. Move the manual valve to **plate mode** and click **Next**.
7. At the end of the Shutdown operation, the Attune™ NxT Cytometric Software automatically powers down the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler.

   The Attune™ NxT Auto Sampler remains in a low power standby mode and will automatically power on if the software is started and then the Attune™ NxT Acoustic Focusing Cytometer is powered on.

   The eject button of the Attune™ NxT Auto Sampler blinks in the standby mode until the Attune™ Nxt Auto Sampler is turned off using power switch located at the back of the instrument.

IMPORTANT! If you cancel the Shutdown function while it is running, you will need to exit and restart the Attune™ NxT Software, complete the Startup function, and then restart the Shutdown function and let it run to completion.

IMPORTANT! If you intend to leave the Attune™ NxT Acoustic Focusing Cytometer in the shutdown state for longer than two weeks, perform *System Decontamination* (page 46) instead of the Shutdown. System Decontamination cleans the fluidics systems of the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler and leaves the fluidics systems in deionized water to prevent salt crystals from clogging them.

3. System Maintenance

The Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler are designed to require minimum maintenance. However, to ensure reliability of the cytometer all its peripheral systems, you must perform basic preventative maintenance procedures on a regular basis, as listed below.

CAUTION! BIOHAZARD. All biological samples and materials that come into contact with them have the potential to transmit infectious diseases and are considered biohazardous. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Never pipette by mouth.

Maintenance Schedule

The table below lists the routine maintenance procedures that keep the Attune™ NxT Acoustic Focusing Cytometer and all its peripheral systems in good working condition.

Procedure	Frequency
Shutdown	Daily
Visual inspection of sample injection port (SIP)	Daily
Visual inspection of fluidics bottles and connections	Daily
Visual inspection of syringe pumps	Daily
Fluidics maintenance (i.e., Rinse, Unclog, and De-bubble functions)	Daily and as needed
Computer maintenance	Monthly
Optical filter cleaning	Monthly and as needed
Fluidics decontamination	Monthly
System decontamination	Monthly and as needed
Changing focusing fluid filter	After each system decontamination or every 6 months
Replacing syringes	As needed*

*The frequency of maintenance depends on how often you run the cytometer. On average, syringes last about 6 month.

Daily maintenance

Daily Shutdown

Daily shutdown involves executing the **Shutdown** function. This function ensures that all sample fluid and dyes have been removed from the fluidics lines and the two pumps have been decontaminated and filled with Attune™ Shutdown solution to prevent the formation salt crystals.

The shutdown procedure can take up to 30 minutes, but most of the steps are automated and under computer control. At the end of the shutdown procedure, the cytometer is automatically powered down.

Visual inspection

Visually inspect the sample injection port, fluidics bottles and connections, and the syringe pumps for any leakage. If you notice any leaks in the fluidics lines, contact your service representative. Decontaminate any spills by wiping the area with 10% bleach solution.

Fluidics maintenance

Daily fluidics cleaning involves executing the **Unclog**, **Rinse,** and **De-bubble** functions as needed.

- *Unclog* function is a user-initiated back flush operation to remove clogs from the sample probe and flow cell (see page 44).
- *Rinse* is a user-initiated system cleaning between sticky samples. This function requires user supplied bleach or detergent (see page 44).
- *De-bubble* is a user-initiated function for clearing bubbles in the fluidics lines of the cytometer (see page 45).

Sanitize between uses

Run the **Sanitize SIP** procedure (page 44) to sanitize the Attune™ NxT Auto Sampler between uses. Note that this procedure is intended for a quick cleaning of the instrument to minimize cross-contamination. For a more thorough decontamination, perform the Decontaminate System procedure (see page 46).

Note: For monthly and periodic maintenance of the system, refer to the Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide, available for download at **www.lifetechnologies.com**.

Sanitize SIP

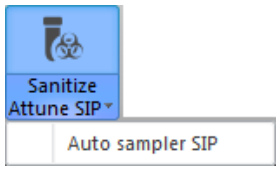
The Sanitize SIP function is a user-initiated function that quickly washes and sanitizes the:

- Instrument Sample Injection Port (SIP) and sample lines
  OR
- Auto Sampler SIP and sample lines

Note: It is especially important to perform the Sanitize SIP function when running sticky samples, DNA stains, or beads.

Perform the Sanitize SIP function for the Auto Sampler

1. On the Instrument ribbon, click **Sanitize**.
2. From the dropdown menu, select **Auto Sampler SIP**.
   The *Sanitize dialog* box appears and provides instructions to perform the Sanitize Auto Sampler SIP procedure.
3. Click **Next** to run the Sanitize SIP function.



Rinse

Perform the Rinse function

The Rinse function rinses the sample lines.

1. On the Instrument ribbon, click **Rinse**.
   The *Rinse dialog* box appears and provides instructions to perform the Rinse procedure.
2. If the tube lifter is raised, lower the tube lifter.
3. Click **Next** to run the Rinse function.

Unclog

The Unclog function is a user-initiated back flush operation to remove clogs from the sample probe and flow cell.

Perform the Unclog function

1. On the Instrument ribbon, click **Unclog**.
   The *Unclog dialog* box appears and provides instructions to perform the Unclog procedure.
2. Load a clean, empty tube into the instrument, then raise the tube lifter.
3. Click **Next** to run the Unclog function.
4. When the function completes, lower the tube lifter.
5. Click **Next** to close the dialog box and automatically perform a Rinse.

De-bubble

The De-bubble function is a user-initiated function for clearing bubbles in the fluidics lines of the cytometer and flow cell.

Perform the De-bubble function

1. On the Instrument ribbon, click **De-bubble**.

   The *De-bubble dialog* box appears and provides instructions to perform the De-bubble procedure.
2. If the tube lifter is raised, lower the tube lifter.
3. Click **Next** to automatically perform a Rinse.
4. When the rinse is complete, fill a clean tube with at least 1.5 mL of Attune™ Debubble Solution.
5. Load the tube into the instrument, then raise the tube lifter.
6. Click **Next** to start the De-bubble function.
7. When the function completes, lower the tube lifter.
8. Click **Next** to close the dialog box and automatically begin a Rinse.

Decontaminate system

The *Decontaminate System* function of the Attune™ NxT Software facilitates the automated decontamination of the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler fluidics.

Perform the Decontaminate System operation:

- as a monthly maintenance routine to prevent and reduce microbial growth within the instrument
- if the system is likely to be idle for more than two weeks (run it in place of the Shutdown function)
- if the instrument has been idle for more than two months
- if the instrument has been idle for more than two weeks without decontamination run prior to it becoming idle

The system decontamination procedure is broken into four phases (mostly automated) and takes approximately 45 minutes. Each step of the process is displayed at the top of the dialog box and the step in progress is highlighted. Make sure to follow all the instructions provided by the instrument during system decontamination. Note that this function is only available to system administrators.

IMPORTANT! Perform the following decontamination procedure on a monthly basis, or if you intend to leave the Attune™ NxT Acoustic Focusing Cytometer in the shutdown state for longer than two weeks or if you plan to ship the instrument for service. Proper decontamination of the instrument ensures its consistent and accurate operation, and reduces potential health hazards.

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.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

Prepare for System Decontamination

1. Rinse out all fluidics containers with deionized water.
2. Make sure that all fluidics lines and sensor cables are connected.

Note: For the location of the fluidics compartment and instructions on filling the fluidics tanks refer to the Attune™ NxT Acoustic Cytometer User Guide, available for download at **www.lifetechnologies.com**.

Run Decontaminate System function

The Attune™ NxT Software provides instructions to perform the *Decontaminate System* function. The Decontaminate System function for the Attune™ NxT Auto Sampler is broken into four phases and it can take up to 45 minutes; however, most of the operation is performed automatically.

Make sure to follow all the instructions provided by the instrument and to click **Next** between each phase of the System Decontamination function. During the operation, the software provides real-time updates on the Decontaminate System function being executed.

1. Click the **Decontaminate System** button located on the Instrument tab of the Ribbon bar and follow the prompts in the *Decontamination dialog* box.

2. Click **Next** to start Decontamination Phase 1. When prompted:
   a. Rinse all fluidics bottles with deionized water.
   b. Fill the Attune™ NxT Cytometer and Auto Sampler focusing fluid bottles with 500 mL of 10% bleach.

      Leave all other bottles empty.
   c. Reconnect all fluidics lines and bottle cables.
3. Click **Next** to start Decontamination Phase 2. When prompted:
   a. Load a clean, empty standard 96-well plate into the Auto Sampler.
   b. Load a clean, empty tube on the SIP of the Attune™ NxT Cytometer, and then raise the tube lifter.
4. Click **Next** to start Decontamination Phase 3. When prompted:
   a. Rinse the Attune™ NxT Cytometer and Auto Sampler focusing fluid bottles with deionized water
   b. Fill both focusing fluid bottles with 500 mL of deionized water.
   c. Reconnect all fluidics lines and bottle cables.
   d. Load a clean, empty tube on the SIP of the Attune™ NxT Cytometer, and then raise the tube lifter.
5. Click **Next** to start Decontamination Phase 4. When prompted:
   a. Replace the focusing fluid filters with fresh filters.
   b. Rinse all fluidics bottles with deionized water.
   c. Replace all fluids in all fluidics bottles with the appropriate solutions.
   d. Reconnect all fluidics lines and bottle cables.
   e. Lower the tube lifter and remove the plate from the Attune™ NxT Auto Sampler.

Auto Sampler Calibration

The Auto Sampler Calibration function sets the plate tray position to ensure that the probe consistently measures from the same spot in each well. The Attune™ NxT Auto Sampler calibration operation takes approximately 1 minute to complete.

Note: The Attune™ NxT Auto Sampler is pre-calibrated before the unit is shipped and the instrument auto re-calibrates every 3 months. The Auto Sampler Calibration function is only needed for troubleshooting and if the Auto Sampler was knocked out of calibration for some reason.

Run the Auto Sampler Calibration function

1. On the Instrument ribbon, click **Calibrate Auto Sampler**.
   The *Calibrate Auto Sampler dialog* box appears and provides instructions to perform the Calibrate Auto Sampler procedure.
2. If a plate is loaded in the Auto Sampler, remove the plate.
3. Click **Next** to run the Auto Sampler Calibration function and follow the instructions provided by the Calibrate Auto Sampler dialog.

Appendix A: Troubleshooting

This section includes the following topics:

- Tips to help you troubleshoot issues with Attune™ NxT Auto Sampler
- Technical Assistance Information

Note: For troubleshooting issues encountered with the Attune™ NxT Cytometer, including cytometer operation, performance tracking, and sample preparation, refer to the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide*, available for download at **www.lifetechnologies.com**.

Attune™ NxT Auto Sampler troubleshooting

Observation	Possible causes	Recommended solutions
No sample being analyzed	No power to Auto Sampler	Attach the power plug and turn the Auto Sampler ON.
	Fluidics are not connected	Connect fluidic connectors to the Attune™ System.
	Fluidics are leaking	Check for leaks at the connectors at the Attune™ System.
	No plate in Auto Sampler	Place a plate in the Auto Sampler.
	Incompatible plate type	Contact Technical Support for a list of validated plates.
	Instrument is clogged	Run Unclog function. Contact Technical Support if problem persists.
	Empty fluid container	• Check for empty fluid tank (Focusing fluid, Wash, or Shutdown solution) on the Attune™ NxT Cytometer or the Auto Sampler. • Ensure that the fill lines and fluid level detectors are plugged in completely.
	Auto Sampler is powered OFF	Power ON the Auto Sampler.
	USB cable not connected	Ensure that the USB cable is plugged into the instrument and the computer.
	Sample plate is not selected	Select the sample plate.
	Sample volume is less than specified for the system	Total Draw Volume displayed in the SW is the **absolute minimum** sample volume required. Any deviation to less than this volume in a well (e.g. pipetting error) can lead to bubbles drawn into system.
Red light blinks	Error occurred in system	• Power the instrument OFF and ON. • Perform Auto Sampler Calibration routine.
After initial power ON, the tray is ejected with the well plate	Well plate is present during power ON of the instrument	Remove the well plate from the tray during the power ON cycle.
Computer is not communicating with the Auto Sampler	USB cable not fully plugged in	Verify that the USB cable connection is in place in the back of the Auto Sampler and the computer.
	Faulty USB cable	Replace USB cable. Contact Technical Support if problem persists.
	USB port changed from the original port	Try a different USB port on the computer. If the problem persists, reinstall the USB drivers.

Observation	Possible causes	Recommended solutions
Auto Sampler and/or computer has no power	Power supply not plugged into the appropriate outlet	Ensure that the Auto Sampler, Attune™ NxT Cytometer, and the computer are plugged into the appropriate outlet.
	No power at the outlet	Ensure sure that the outlet is functioning properly and the circuit breaker is not tripped.
	Faulty power supply	Contact Technical Support.
Sample is not aspirating	Loose sample syringe	Check the sample syringes on the Attune™ NxT Cytometer and the Auto Sampler for leaks and tighten the syringes if necessary. Be careful not to over tighten.
	Defective sample syringe on the Auto Sampler	Replace sample syringe on the Auto Sampler.
	Defective sample syringe on the Attune™ NxT Cytometer	Replace sample syringe on the Attune™ NxT Cytometer.
	Fluidic valve or tubing failure within the Attune™ NxT Cytometer	Verify that the sample can be properly analyzed on the Attune™ NxT Cytometer in tube mode and contact Technical Support.
Sample aspirated, then backfilled into sample well	Clog in the sample line	• Run Unclog function. Contact Technical Support if problem persists. • If persistent, designate rinse wells throughout plate between samples and/or increase rinses between wells. • Ensure sample size is within system specification (< 50 microns).
	Fluidic system failure in the Attune™ System	Contact Technical Support.
Long delay between sample aspiration and events appearing on screen (normally events appear in ~10 seconds)	Sample syringe is leaking	Ensure that the sample syringe is sealed properly in the Attune™ NxT Cytometer and the Auto Sampler.
	Incompatible plate type	Contact Technical Support for a list of validated plates.
	Partial clog in the fluidics system	Run Unclog function. Contact Technical Support if problem persists.

Observation	Possible causes	Recommended solutions
Sample probe is not centered in the sample well	SIP tube is bent or faulty	Replace SIP tube.
	Incorrect plate type selected	Select appropriate plate type.
Focusing fluid pump does not shut off	Focusing fluid reservoir level sensor is malfunctioning	Turn OFF the Auto Sampler and contact Technical Support.
Rinse fluid pump does not shut off	Rinse (Waste) fluid reservoir level sensor is malfunctioning	Turn OFF the Auto Sampler and contact Technical Support.
Fluid is leaking from the base of the Auto Sampler or into the bottle bay drip tray	Crack in fluidics container	Replace the damaged fluidics container.
	Snap fitting is broken or dripping	Contact Technical Support.
	1-mL syringe seal is broken	Replace sample syringe on the Auto Sampler.
Inconsistent results experienced between wells	Sample volume loaded into each well is not adequate	Total Draw Volume displayed in SW is the **absolute minimum** sample volume required. Any deviation to less than this volume in a well (e.g. pipetting error) can lead to bubbles drawn into system and inconsistent results.
	Inconsistent sample preparation	Verify sample preparation and well loading is consistent across the plate.
	Sample concentration exceeds system specifications	Verify sample concentration is not in excess of system requirements.
Large amount of debris is seen in data	Auto Sampler has been idle for an extended time period	Run the Startup function on the Attune™ system three times. Run the De-bubble function two times with Attune™ Debubble solution. Run the Rinse function two times.
	Recent replacement of a fluidics line component	

Appendix B: Technical overview

System specifications

Physical characteristics

Footprint (H × W × D): Approximately 16″/40 cm × 11.5″/29 cm × 11.5″/29 cm

Space requirements (H × W × D): 29″/74 cm × 13.8″/35 cm × 23.1″/58.5 cm above the mounting surface

Weight: Approximately 35 lb/15.9 kg

Operating temperature: 15–30°C (50–95°F)

Operating humidity: <80% non-condensing

Electrical requirements: 100–240VAC, 50/60 Hz, <300 W

Fluidics

Fluid storage: Within instrument with level sensing

Total fluid volume: 800 mL per container; capable of running four (4) 96-well plates in standard mode with 2 washes/well

Sample analysis

Compatible plate types: 96-well, standard depth (flat, round, V-bottom)
96-well, deep-well (flat, round, and V-bottom)
384-well, standard depth (flat, round, and V-bottom)
384-well, deep-well (round and V-bottom)

Processing time: <45 minutes for 96-well plate, using high-throughput mode
<70 minutes for 96-well plate, using standard mode, 2 wash cycles
<260 minutes for 384-well plate, using standard mode, 2 wash cycles

Carry-over: <0.5% in standard mode using 2 wash cycles

Mixing Cycles: Each well is mixed via aspiration of sample (not shaking).

Wash Cycles: User defined number of wash cycles (up to 10 wash cycles)

Minimum Sample Volume: Does not exceed 50 µL for 96-well plates

Minimum Dead Volume: Does not exceed 30 µL

Sample Acquisition Volume: 20 µL

Software

Attune™ NxT Software Version 2.0.1 or higher required.

Operation principles and technical overview

The Attune™ NxT Auto Sampler is a sample loading device for use with the Attune™ NxT Acoustic Focusing Cytometer. The Attune™ NxT Software controls the auto sampler and facilitates the acquisition of samples from multi-well plates, subsequent analysis, and batch processing for a high-throughput capability.

Instrument description

The Attune™ NxT Auto Sampler is a detachable instrument accessory that allows for the quick and easy processing of 96- and 384-well microtiter plates (both standard and deep well depth) with the Attune™ NxT Acoustic Focusing Cytometer. The Attune™ NxT Auto Sampler, which comes with easy-to-use software for high-throughput environments, includes its own on-board fluidics that can run four 96-well plates or one 384-well plate without requiring fluid replacement, and can process a 96-well microtiter plate in less than 60 minutes in the standard mode with less than 1% carryover.

The Attune™ NxT Auto Sampler is intended to operate in conjunction with the Attune™ NxT Acoustic Focusing Cytometer. Both the cytometer and the auto sampler connect are operated by the Attune™ NxT Software installed on the connected PC. The software provides the user interface to control the instrument, and to collect, analyze, and save data. The software is capable of analysis only (without instrument and sampler connections) on any PC that meets the minimum system requirements for post-acquisition data analysis. Note that a software license found on a USB dongle is required to use the software on any PC.

Key features

- Acquires samples from 96-well and 384-well plates
- Includes two modes of operation (High-throughput and Standard)
- Allows the customization of the plate assay parameter, including mixing, sample aspiration, and sensitivity mode
- Minimizes well-to-well carry over
- Supports multiple experiments on a single plate
- Allows easy switching between tubes and plates using software alone (no manual adjustments required)
- Features Heat Map View Analysis
- Contains on-board fluidics

Modes

The Attune™ NxT Auto Sampler can process a 96-well plate in approximately 70 minutes in standard mode using 2 wash cycles and 45 minutes in high-throughput mode using pre-defined settings. The table below provides a comparison of the two modes.

	High-throughput	Standard
Sample Volume	40 µL	50 µL-well volume
Number of Mixes	0–1	0–10
Number of Rinses	0–1	0–10
Rinse volume	200 µL	200 µL

Volumes

There are many different volumes that need to be considered when using the auto sampler.

Well Volume: Total volume a well can hold when completely full

Draw Volume: Volume drawn from the well that is necessary to provide the user-defined acquisition volume

Dead Volume: Volume aspirated to fill the fluidics lines up to the analysis point

Total Sample Volume: Total sample volume in each well necessary for efficient mixing of the sample

Minimum Volume: 40 µL for high-throughput mode and 50 µL for standard mode

Available Acquisition Volume: Well volume minus dead volume

Mixing

Mixing of the sample is done by sample aspiration and dispensing. Mixing effectiveness depends on the amount of sample aspirated and the viscosity of the sample. The number of mixing cycles can be defined by the user (10 cycles maximum). The system determines the optimal volume of sample to mix based on the total sample volume and plate type selected. The number of mixes defined or the amount of sample mixed will affect the time to process the plates. In the high-throughput mode, mixing has been optimized to enable maximal sample throughput.

Note: Mixing efficiency can vary depending upon the type of plate used. We strongly recommend using round bottom plates for any assay in which homogeneous sampling and consistency of concentration is essential.

Carry over

The number of rinses between samples can be defined to help minimize carryover. In general, large number of wash cycles between samples results in the less carryover.

Appendix C: Ordering information

The following reagents and consumables have been specifically formulated for use with the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler, and are available separately from Thermo Fisher Scientific. Ordering information is provided below. For more information, go to **www.lifetechnologies.com** or contact Technical Support.

Product	Amount	Cat. no.
Attune™ Focusing Fluid, 1X Solution	1 × 1 L 6 × 1 L	4488621 4449791
Attune™ Focusing Fluid, 1X Solution	1 × 10 L	A24904
Attune™ Wash Solution	500 mL	A24974
Attune™ 1X Shutdown Solution	250 mL	A24975
Attune™ Performance Tracking Beads	3 mL	4449754
Attune™ 10 mL syringe	1 each	4452819
Attune™ 1 mL syringe	1 each	4452079
Attune™ NxT Focusing Fluid Filter	1 each	100022587
Attune™ NxT Auto Sampler PLUG,1/4-28 Teflon	1 each	4476990
Attune™ NxT Auto Sampler Bottle Assembly Focusing Fluid	1 each	4477847
Attun™ NxT Auto Sampler Bottle Assembly Waste	1 each	4477850
Attune™ NxT Auto Sampler 10 mL Syringe	1 each	4478686
Attune™ Emission Filter Holder Blade	1 each	4465834
Attune™ NxT Blank Dichroic Filter Holder	1 each	100022651
Attune™ Bottle assembly, Wash, 250 mL	1 each	90032053
Attune™ Bottle assembly, Waste, 1 L	1 each	90032053
Attune™ Bottle assembly, Sheath, 1 L	1 each	90039273
Attune™ Bottle assembly, Shutdown, 250 mL	1 each	90039274

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
- SDSs

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.lifetechnologies.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, 20 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

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Thermo Fisher Scientific Technical Support and Sales facilities.

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.lifetechnologies.com/sds**.

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

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website.

Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

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.