USER GUIDE

# Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide

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# Contents

# About this guide

This user guide describes how to perform basic preventative maintenance procedures to ensure reliability of the Attune™ NxT Acoustic Focusing Cytometer and the Attune™ NxT Auto Sampler, and provides tips to help you troubleshoot your experiments.

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

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

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

# Conventions

## Text and keyboard conventions

Text and keyboard conventions used in the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide* are listed below. For safety alert words and symbols used in this document, see page 7.

| 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:<br>*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:<br>Click **Run**. |
| ▶ | Right arrow symbol (▶) indicates a menu choice, and separates successive commands you select from a drop-down or shortcut menu. For example:<br>Select **Show Events**▶**All Events**. |
| Ctrl+X | When used with key names, a plus sign means to press two keys simultaneously. For example:<br>Click **Ctrl+P**. |

## Clicking

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

### User attention symbols

User attention symbols used in the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide* are listed below. For safety alert words and symbols used in this document, see page 7.

| 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. |

## 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 Maintenance Log* | 100025061 |
| *Attune™ NxT Acoustic Focusing Cytometer Site Preparation Guide* | 100024428 |
| *Attune™ NxT Auto Sampler User Guide* | 100032905 |

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

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

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

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

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

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

## SDSs

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

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

# 1. Attune™ NxT Cytometer maintenance

The Attune™ NxT Acoustic Focusing Cytometer is designed to require minimum maintenance. However, to ensure reliability of the cytometer, 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), fluidics tanks and connections, and syringe pumps | Daily |
| Fluidics maintenance | Daily |
| Computer maintenance | Monthly |
| Optical filter and mirror inspection | Monthly |
| System decontamination | 3 months |
| Replace focusing fluid filters | After each system decontamination or every 6 months |
| Fluidics decontamination | 6 months |
| Syringe replacement | 6 months |
| Rinse | As needed |
| Sanitize SIP | As needed |
| Unclog | As needed |
| De-bubble | As needed |
| Deep Clean | As needed |

# 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 of salt crystals.

The shutdown procedure takes approximately 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 tanks 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.
- Visually inspect the area behind the instrument to make sure cords are plugged in and connections not stressed. Verify that a minimum of 6 inches of free space behind the instrument is maintained and that all four exhaust ports are free of blockage.

## Fluidics maintenance

Fluidics maintenance includes the user-initiated functions listed below. Initiate each function on a daily basis or as needed.

- **De-bubble** – Clears bubbles in the fluidics lines of the cytometer. For instructions, see page 29.
- **Unclog** – Removes clogs from the sample probe and flow cell (back-flush operation). For instructions, see page 29.
- **Sanitize SIP –** Quickly washes and sanitizes the SIP and sample lines. It is especially important to perform the Sanitize SIP function when running sticky samples, DNA stains, or beads. This function requires user-supplied bleach or detergent. For instructions, see page 28.
- **Rinse –** Clears sample volume with excess sheath in the SIP, rotary valve, sample line, capillary, and flow cell. The entire sample goes to waste. For instructions, see page 27.
- **Deep Clean** – Sanitizes the system with bleach and Wash solutions for a user-selected period of time. Ensures system cleanliness while allowing you to continue using the instrument after the cycle is complete. The Deep Clean function does not sanitize the fluidics bottles. For instructions, see page 32.
- **Decontaminate System** – Sanitizes the system and fluidics bottles with bleach and Wash solutions for a proscribed period of time. Ensures full system cleanliness at regular maintenance intervals to prevent build up of contaminants in the system or fluidics bottles. For instructions, see page 21.

# Monthly maintenance

## Computer maintenance

Periodically maintaining the computer running the Attune™ NxT Software is an important component of a comprehensive maintenance strategy. To preserve the integrity of your data, observe the following precautions:

- De-fragment the hard drive of the computer monthly.
- Back up your experiments on a regular basis to a secondary storage device.
- When planning the experiments, remember to delete parameters that you do not need (i.e., only collect parameters in either area or height, but not both, unless you need both parameters for a certain application such as cell cycle).

If an experiment contains several samples, consider collecting some of the samples under one experiment and then collecting the rest under a second experiment.

## Optical filter and mirror maintenance

The optical filters and mirrors are housed in optical holders, which are located in the optics compartment. To clean optical filters and lenses, follow the instructions below.

**CAUTION! LASER HAZARD.** Follow the precautions outlined in "Laser safety" on page 72 while changing optical filters and mirrors.

- Lift the cytometer lid. The photograph here shows configuration of the optical filters and mirrors for the violet and red lasers on the left, and for the yellow and blue lasers on the right.

**Note:** The Attune™ NxT Acoustic Focusing Cytometer can accommodate up to four lasers in seven instrument configurations. If there is no yellow laser, the channel positions may vary. For illustrations of all instrument configurations, see page 55.

2. Remove the optical holder containing the appropriate filter or mirror.


3. Gently remove any dust from the surface of the filter or mirror with a blower (bulb-blower or compressed gas), lens cleaning tissue, or a soft brush.
4. If necessary, *gently* clean the surface of the filter or mirror using a *lint free* lens cloth dipped in dish soap and water. Do not wipe dry.
5. Return the optical holder back to its slot and close the cover of the cytometer.

**Note:** The optical holders fit into the slots only one way.

# System decontamination

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 system decontamination:

- as a quarterly 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

**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 5000 ppm of available chlorine.

## Decontaminate System function

- The Attune™ NxT Software provides instructions to perform the *Decontaminate System* function. Each step of the procedure is displayed at the top of the dialog box and the step in progress is highlighted.
- The steps in the Decontaminate System function vary depending on whether an Auto Sampler is connected. See page 21 for the decontamination procedure for the Attune™ NxT Acoustic Focusing Cytometer equipped with the Attune™ NxT Auto Sampler.
- The Decontaminate System function for the Attune™ NxT Cytometer is broken into three phases. It can take up to 45 minutes to complete the procedure; however, most of the operation is performed automatically.
- The Decontaminate System function is only available to administrators.

## Prepare for system decontamination

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

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

## Run Decontaminate System function (Attune™ NxT Cytometer only)

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


2. Click **Next** to start Decontamination Phase 1. When prompted:
   a. Rinse all fluidics bottles, except the wash bottle, with 10% bleach.
   b. Fill the focusing fluid bottle with at least 500 mL of 10% bleach and the shutdown bottle with at least 125 mL of 10% bleach.
   c. Fill the wash bottle with at least 125 mL of Attune™ Wash solution.
   d. Reconnect all fluid lines and bottle cables.
   e. Load a clean, empty tube on the SIP, and then raise the tube lifter.
3. Click **Next** to start Decontamination Phase 2. When prompted:
   a. Rinse all fluidics bottles, except the wash bottle, with deionized water.
   b. Fill the focusing fluid bottle with at least 500 mL of deionized water and the shutdown and wash bottles with at least 125 mL of deionized water.
   c. Reconnect all fluid lines and bottle cables.
   d. Load a clean, empty tube on the SIP, and then raise the tube lifter.
4. Click **Next** to start Decontamination Phase 3. When prompted:
   a. Replace the focusing fluid filters with new filters (see page 15).
   b. Rinse all fluidics bottles with deionized water.
   c. Replace all fluids in all fluidics bottles with the appropriate solutions.
   d. Reconnect all fluid lines and bottle cables.

# Periodic maintenance

## Fluidics decontamination

To ensure reliability of the instrument, we recommend a monthly decontamination of the fluidics bottles to prevent any bacterial contamination in the bottles. Contamination symptoms include:

- Bacteria growth in the bottle, indicated by cloudy fluid, globs or strings in the fluid, or discoloration of the fluid.
- A very high number of events that do not correspond to the sample (i.e., sample dilution has no effect). You can confirm by running Attune™ Performance Tracking Beads and seeing the high event rate (over 1000 events/sec compared to the expected 200–300 events/sec).
- If bacteria growth is seen in bottles, focus fluid filters should be replaced when the bottles are decontaminated.

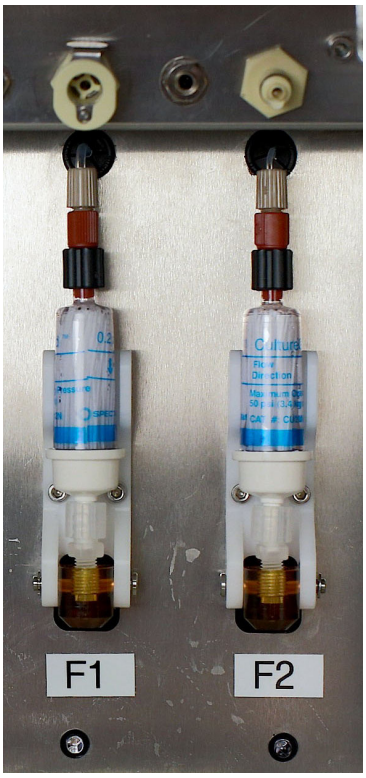
To decontaminate the fluidics bottles:

1. Disconnect all fluidics bottles from the instrument.
2. Discard all unused fluids.
3. Pour at least 20 mL of deionized water in each bottle, replace cap, and invert or gently shake to coat all internal surfaces. Discard the deionized water.
4. Pour at least 20 mL of 70% isopropanol or 100 mL of 10% bleach in each bottle, replace cap, and invert or gently shake to coat all internal surfaces.
5. Leave the isopropanol or the bleach solution in the bottle for 10 minutes and then discard the fluid.
6. Invert each bottle and allow to air dry.
7. Place fresh Attune™ Focusing Fluid, Attune™ Wash Solution, and Attune™ Shutdown Fluid in the corresponding bottle.
8. Replace all fluidics bottles on the instrument. Be sure to attach the fluidics cable before attaching the sensor cable.
9. Run **Startup** function.

# Replacement of spare parts

## Focusing fluid filter replacement

Replace focusing fluid filters every 3 months or after you perform System Decontamination.




1. Gently pull the filter by placing thumb and forefinger around the top tubing to a slight angle from the instrument.
2. Unscrew the ¼-28″ tubing at the top of the filter in a counter clockwise fashion.

   There may be a small amount of fluid at the top of the fitting that comes out.
3. Place the thumb and forefinger at the middle of the body of the filter and unscrew the bottom fitting in a counter clockwise fashion.
4. With the filter removed do the following:
   a. Unscrew the black section of the ¼-28″ female-to-male luer lock adapter.
   b. Unscrew the bottom ¼-28″ male-to-female luer lock adapter.

      Some liquid will exit the filter.
5. Discard the used filter
6. Open the new filter and orient the filter where the arrow on the filter body is pointing down.
7. Screw in the bottom ¼-28″ male-to-female luer lock adapter to the bottom of the new filter.
8. Screw in the black section of the ¼-28″ female-to-male luer lock adapter to the top of the filter.
9. Screw in the bottom portion of the filter to the amber portion of the threaded adapter in the instrument.
10. Carefully screw in the top of the ¼-28 female fitting into the top of the tubing until a click is felt and heard.
11. Check for leaks.
12. Run **Startup** function and carefully observe filter for leaks.

**IMPORTANT!** After replacing the focusing fluid filters, we recommend running a full priming sequence, 3 Startup procedures, 2 De-bubble procedures (if solution is available), and 2 Rinse procedures.

## Syringe replacement

Visually inspect the syringe pump daily for leaks. Replace the syringe if you observe leaks from the syringe assembly and/or there is erratic or no fluid draws up from the fluidics tanks or the sample injection port.

1. Execute the **Shutdown** function with 10% bleach (see page 30). The plunger drive will be lowered and the cytometer powered off automatically.
2. Open the Syringe Pump door located on the left side of the cytometer (see "Syringe Pump Compartment", page 50).

3. Open the syringe retention clasp and carefully remove the ball bearing while supporting the syringe piston. After the ball is removed, unscrew the syringe from the valve by rotating it counter-clockwise.
4. To install a new syringe, insert the ball end of the syringe carefully into the capture mechanism. Lift the capture mechanisms and syringe barrel, then align the syringe with the syringe port of the valve and rotate clockwise until the syringe end cap seal hits the bottom of the valve.

5. After bottoming out, rotate clockwise ¼-turn to ensure complete seal without over-tightening.

**IMPORTANT!** Failure to properly align the syringe when engaging the valve may lead to cross-threading. No tools should be used for tightening and securing the syringe to the valve. Over-tightening the syringe beyond the above recommendation could result in damage to the syringe and the valve.

6. Tighten the knurled thumbscrew to secure the ball end of the syringe within the syringe capture mechanism. Be sure that the ball is fully secured when tightened.
7. Close the syringe pump door.

**Note:** Proper syringe-to-valve seal is crucial for the operation of the cytometer, when fluids are cycling through the system. Cavitations may occur if a seal is not properly attained.

**IMPORTANT!** After replacing the syringe, we recommend running a full priming sequence, 3 Startup procedures, 2 De-bubble procedures (if solution is available), and 2 Rinse procedures.

## SIP tube replacement

The SIP tube has a click-and-seal fitting, which allows customers to replace damaged, bent, clogged, or leaking SIP tubes. Follow the procedure below to replace the SIP tube with a click-and-seal fitting.

1. Position the Attune™ NxT Cytometer so that the SIP tube overhangs the edge of the counter.
2. Lower the tube lifter, then unscrew and remove the old SIP tube.
3. Insert the click-and-seal fitting over the sleeve.

4. Install the SIP tube into the bottom port of the rotary valve and hand tighten until the fitting clicks.
5. Run the **Startup** function (see page 27).
6. Load a test tube with 500 µL of Attune™ Focusing Fluid and run acquisition at 500 µL/minute. Observe the sample loop (use a 4× eye-loop, if available) during aspiration to verify that no air bubbles are entering the sample loop.

**IMPORTANT!** After replacing the SIP, we recommend running a SIP sanitize procedure, as well as a full priming sequence, 3 Startup procedures, 2 De-bubble procedures (if solution is available), and 2 Rinse procedures.

# 2. Attune™ NxT Auto Sampler maintenance

The Attune™ NxT Auto Sampler is designed to require minimum maintenance. However, to ensure reliability of the auto sampler, 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 Auto Sampler in good working condition.

| Procedure | Frequency |
|---|---|
| Shutdown | Daily |
| Visual inspection of sample injection port (SIP), fluidics tanks and connections, and syringe pumps | Daily |
| Fluidics maintenance | Daily and as needed |
| Sanitize between uses | Daily |
| Fluidics decontamination | Monthly |
| System decontamination | Every 3 months and as needed |
| Replace syringes | Every 6 months |
| Replace focusing fluid filters | After each system decontamination or every 6 months |
| Rinse | As needed |
| Sanitize SIP | As needed |
| Unclog | As needed |
| De-bubble | As needed |
| Deep Clean | As needed |

# 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 of 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

1. Visually inspect the sample injection port, fluidics tanks 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.
2. Visually inspect the area behind the instrument to make sure cords are plugged in and connections not stressed. Verify that a minimum of 6 inches of free space behind the instrument is maintained and that all three exhaust ports are free of blockage.

## Fluidics maintenance

Fluidics maintenance includes the user-initiated functions listed below. Initiate each function on a daily basis or as needed.

- **De-bubble** – Clears bubbles in the fluidics lines of the cytometer. For instructions, see page 29.
- **Unclog** – Removes clogs from the sample probe and flow cell (back-flush operation). For instructions, see page 29.
- **Sanitize SIP**– Quickly washes and sanitizes the SIP and sample lines between uses. It is especially important to perform the Sanitize SIP procedure when running sticky samples, DNA stains, or beads. This function requires user-supplied bleach or detergent. For instructions, see page 28.
- **Rinse** – Clears sample volume with excess sheath in the SIP, rotary valve, sample line, capillary, and flow cell. All of the sample goes to waste. For instructions, see page 27.
- **Deep Clean** – Sanitizes the system with bleach and Wash solutions for a user-selected period of time. Ensures system cleanliness while allowing you to continue using the instrument after the cycle is complete. The Deep Clean function does not sanitize the fluidics bottles. For instructions, see page 32.
- **Decontaminate System** – Sanitizes the system and fluidics bottles with bleach and wash solutions for a proscribed period of time. Ensures full system cleanliness at regular maintenance intervals to prevent build up of contaminants in the system or fluidics bottles. For instructions, see page 21.

## Sanitize between uses

Run the **Sanitize SIP** procedure (page 28) 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 system decontamination procedure (see page 21).

# System decontamination

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 system decontamination:

- as a quarterly 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

**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 5000 ppm of available chlorine.

## Decontaminate System function

- The Attune™ NxT Software provides instructions to perform the *Decontaminate System* function. Each step of the procedure is displayed at the top of the dialog box and the step in progress is highlighted.
- The steps in the Decontaminate System function vary depending on whether an Auto Sampler is connected. See page 12 for the decontamination procedure for the Attune™ NxT Acoustic Focusing Cytometer without the auto sampler.
- The Decontaminate System function for the Attune™ NxT Cytometer equipped with the Attune™ NxT Auto Sampler is broken into four phases. It can take up to 45 minutes to complete the procedure; however, most of the operation is performed automatically.
- The Decontaminate System function is only available to administrators.

## Prepare for system decontamination

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

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

## Run Decontaminate System function (Attune™ NxT Cytometer and Auto Sampler)

Make sure to follow all the instructions provided by the instrument and to click **Next** between each phase of the Decontaminate System 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*.


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 fluidics bottles with 500 mL of 10% bleach.

      Leave all other bottles empty.
   c. Reconnect all fluid 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 fluidics bottles with deionized water
   b. Fill both focusing fluidics bottles with 500 mL of deionized water.
   c. Reconnect all fluid 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 new filters (see page 15).
   b. Rinse all fluidics bottles with deionized water.
   c. Replace all fluids in all fluidics bottles with the appropriate solutions.
   d. Reconnect all fluid 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.

# Prepare Attune™ NxT Auto Sampler for shipment

Follow the instructions below to decontaminate a defective Attune™ NxT Auto Sampler and prepare it for shipment to the Thermo Fisher Scientific Repair Center.

Note that the decontamination procedure used preparing the Attune™ NxT Auto Sampler for shipment is different from the System Decontamination procedure described on page 21.

For instructions to install a replacement Attune™ NxT Auto Sampler, refer to the *Attune™ NxT Auto Sampler User Guide*, available for download at **www.lifetechnologies.com**.

**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.

**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.

## Decontaminate the Attune™ NxT Auto Sampler

1. Remove the sample plate, if present, and run the **Decontaminate System** function as described on page 21.
2. Power off the Attune™ NxT Auto Sampler.
3. Disconnect the power supply cord and the USB cable from the Attune™ NxT Auto Sampler.
4. Disconnect and remove the bottles from the Attune™ NxT Auto Sampler.
5. Disconnect the fluidics lines from the two ports on the Attune™ NxT Cytometer.
6. Apply a freshly made solution of 10% Bleach to accessible external surfaces of the auto sampler. A spray dispenser may help to ensure complete coverage.
7. Keep surfaces wet for at least 15 minutes, then wipe dry.

**Note:** Do not ship the power supply cord, USB cable, and the bottles with the instrument to the repair center. The replacement unit will arrive without a power supply cord, USB cable, or bottles.

**Note:** After you unpack and install the replacement Attune™ NxT Auto Sampler, you will use that instrument's packaging materials to return the defective unit.

## Pack and ship the Attune™ NxT Auto Sampler

1. Connect the two external fluidics lines with the union fitting, and bag and tape them to the left side of the Attune™ NxT Auto Sampler.
2. Inside the fluidics compartment, bag and tape the two bottle fluidics lines to the instrument base.
3. To prevent any Y-axis movement during shipping, place the protective shipping foam into the plate tray compartment. The tray door is spring loaded, but it is easy to open from either the left or right tray door corners.
4. Wrap the Attune™ NxT Auto Sampler and seal with tape.
5. Place the four foam corners in the outer box and insert the inner box in the four foam corners within the outer box. Ensure that the base foam is well placed in the inner box. Note that the base foam has cutouts for instrument feet.
6. Orient the Attune™ NxT Auto Sampler to match the cutout in the foam, and then place the instrument in the inner box.
7. Orient the top foam to match the Attune™ NxT Auto Sampler and insert it into the box.
8. Position the four foam corners, close and tape the outer box.
9. Complete the Decontamination Form and print 2 copies.
    - Tape one copy to the outer box.
    - Fax the second copy to (760) 930-2300.
10. Complete and print the FedEx shipment form, and then schedule pick up with FedEx.

**IMPORTANT!** The return must be shipped within 2 weeks of receipt of the instrument replacement.

**IMPORTANT!** FedEx will not take the package unless the decontamination form is attached to the outer box. This form is required by the US Government to ensure package handlers are not handling harmful substances.

# 3. Instrument functions

The following instrument functions are used during maintenance and troubleshooting of the Attune™ NxT Acoustic Focusing Cytometer and/or the Attune™ NxT Auto Sampler:

- Startup (page 27)
- Rinse (page 27)
- Sanitize SIP (page 28)
- Unclog (page 29)
- De-bubble (page 29)
- Shutdown (page 30)
- Deep Clean (page 32)

The Attune™ NxT Software provides instructions to perform each function. Make sure to follow all the instructions provided by the software during the procedure.

## Monitoring progress

- After an instrument function begins running, the *Instrument Function dialog* box is minimized to the lower status bar.


- To maximize the dialog box and monitor the progress of the instrument function, double-click the instrument function progress bar in the lower status bar.


- To stop the instrument function and close the dialog box, click **Cancel**.

# 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.

## Run Startup function

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

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

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

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

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

3. Click **Next** to proceed with the Startup procedure.

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

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

   If any system errors are encountered during the Startup, the status bar displays the *Alarm* icon as well as the relevant indicator icon(s) describing the nature of the error.

   The image below shows the fixed positions of Instrument Status and Alerts indicator icons on the status bar. If a particular indicator icon is not displayed, then a gap is left in its position. For more information, refer to the *Attune™ NxT Software User Guide*.

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

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

# 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 initiate the Rinse procedure.

# 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

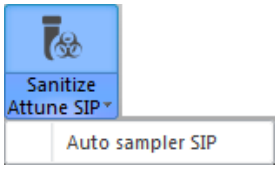
**Note:** It is especially important to perform the Sanitize SIP function when running sticky samples, DNA stains, or beads.

## Perform the Sanitize SIP function for the instrument


1. On the Instrument ribbon, click **Sanitize**.
2. From the dropdown menu, select **Sanitize Attune™ SIP**.

   The *Sanitize dialog* box appears and provides instructions to perform the Sanitize Attune™ SIP procedure.
3. If the tube lifter is raised, lower the tube lifter.
4. Fill a clean tube with 3 mL of 10% bleach.
5. Load the tube into the instrument, and then raise the tube lifter.
6. Click **Next** to initiate the Sanitize SIP procedure.

## 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 initiate the Sanitize SIP procedure.

# 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 initiate the Unclog procedure.
4. When the procedure is complete, lower the tube lifter.
5. Click **Next** to close the dialog box and automatically perform a Rinse procedure.

# 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 procedure.
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 initiate the De-bubble procedure.
7. When the procedure is complete, lower the tube lifter.
8. Click **Next** to close the dialog box and automatically begin a Rinse procedure.

# Shutdown

The Shutdown function sanitizes and shuts down the instrument. The Shutdown function varies depending on whether or not an Auto Sampler is connected to the instrument.

During Shutdown, the instrument runs a dilute bleach solution through unclog, backflush, and sample/rinse lines (bleach scrub), rinses all lines with water, runs Attune™ Wash Solution through all lines and the sample pathway (wash scrub), and then washes all lines again with water before running Attune™ Shutdown Solution through all lines and the SIP.

## Shutdown options

There are three options available for the Shutdown function:

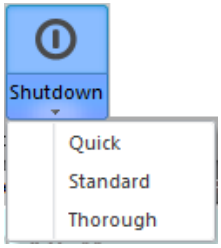
- **Quick** –Quick option uses 5 wash cycles and takes 10 minutes to complete.
- **Standard** –Standard option uses 15 wash cycles and takes 40 minutes to complete.
- **Thorough** –Thorough option uses 25 wash cycles and takes 60 minutes to complete.

For daily use, we recommend the Standard Shutdown function.

## Shutdown (Attune™ NxT Cytometer only)

1. On the Instrument ribbon, click **Shutdown**.

   Alternatively, you can click **Shutdown** on the Main menu.




2. From the dropdown menu, select **Quick**, **Standard**, or **Thorough** option.

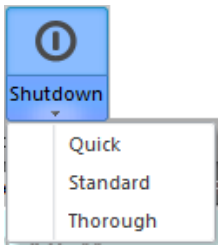
   The *Shutdown dialog* box appears and provides instructions to perform the Shutdown procedure.
3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to automatically perform a Rinse procedure.
5. When the rinse is complete, empty the waste container.
6. Refill the fluidics bottles with the appropriate solutions.
7. Fill a clean tube with 3 mL of 10% bleach.
8. Load the tube into the instrument, and then raise the tube lifter.
9. Click **Next** to initiate the Shutdown procedure.
10. When the Shutdown procedure is complete, you can log out, shutdown the computer, and power the system off.

**Note:** After the Shutdown procedure is complete, a small amount of Attune™ Shutdown Solution remains in the SIP tube. This ensures that the instrument fluidics system does not dry out.

## Shutdown (Attune™ NxT Cytometer and Auto Sampler)

1. On the Instrument ribbon, click **Shutdown**.

   Alternatively, you can click **Shutdown** on the Main menu.




2. From the dropdown menu, select **Quick**, **Standard**, or **Thorough** option.

   The *Shutdown dialog* box appears and provides instructions to perform the Shutdown procedure.

3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to automatically perform a Rinse procedure.
5. When the rinse is complete, empty the instrument and Auto Sampler waste containers.
6. Refill the fluidics bottles with the appropriate solutions.
7. Fill a clean tube with 3 mL of 10% bleach, load the tube into the instrument, then raise the tube lifter.
8. Load a clean, empty standard 96-well plate into the Auto Sampler.
9. Click **Next** to initiate the Shutdown procedure.
10. When the Shutdown procedure is complete, you can log out, shutdown the computer, and power the system off.

**Note:** After the Shutdown procedure is complete, a small amount of Attune™ Shutdown Solution remains in the SIP tube. This ensures that the instrument fluidics system does not dry out.

# Deep Clean

The Deep Clean function thoroughly washes the sample lines and flow cell. The Deep Clean function varies depending on whether or not an Auto Sampler is connected to the instrument.

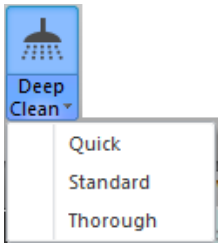
## Deep Clean options

There are three options available for the Deep Clean 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 normal use, we recommend performing the Deep Clean function in the Standard mode.

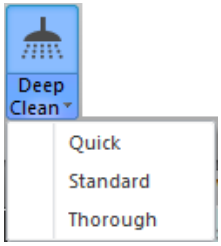
## Deep Clean (Attune™ NxT Cytometer only)




1. On the Instrument ribbon, click **Deep Clean**.
2. From the dropdown menu, select the **Quick**, **Standard**, or **Thorough** option.

   The *Deep Clean dialog* box appears and provides instructions to perform the Deep Clean procedure.
3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to initiate a Rinse procedure.
5. When the rinse is complete, fill a clean tube with 3 mL of 10% bleach.
6. Load the tube into the instrument, then raise the tube lifter.
7. Click **Next** to initiate the Deep Clean procedure.

## Deep Clean (Attune™ NxT Cytometer and Auto Sampler)




1. On the Instrument ribbon, click **Deep Clean**.
2. From the dropdown menu, select the **Quick**, **Standard**, or **Thorough** option.

   The *Deep Clean dialog* box appears and provides instructions to perform the Deep Clean procedure.
3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to initiate a Rinse procedure.
5. When the rinse is complete, load a clean, empty standard 96-well plate into the Auto Sampler.
6. Fill a clean tube with 3 mL of 10% bleach, load the tube into the instrument, then raise the tube lifter.
7. Click **Next** to initiate the Deep Clean procedure.

# 4. Troubleshooting

This section includes the following topics:

- Tips to help you troubleshoot your experiments
- Technical Assistance Information

**Note:** For Software Troubleshooting, refer to the Attune™ NxT Software Release Notes and the Attune™ NxT Software User Guide, or contact Technical Support.

# Cytometer troubleshooting

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| No events are displayed in the Workspace | Sample is not aspirated or only partially aspirated | • Ensure that the 1-mL sample syringe is sealed properly, with no signs of leaks and no loose connection at the top.<br>• Ensure that the syringe is moving smoothly during aspiration.<br>• If syringe problems can be seen, change the syringe.<br>• If syringe problems cannot be seen, contact Technical Support. |
| | Threshold is not set correctly | • Ensure that the threshold is:<br>- Set to the correct Boolean trigger logic: **And** or **Or** (not **Ignore**).<br>- Not set to 0 (zero).<br>- Set for the correct parameter on the instrument settings panel.<br>• Lower the trigger point. |
| | Threshold level is too high | Lower the threshold using the slider bars on the instrument configuration panel. Bring the threshold down to a low number (for example, 10 on FSC or SSC, 1 to 2 on the fluorescent parameter); see if the event rate goes up and the required population becomes visible. |
| | PMT voltages are set too low | Optimize PMT setting by increasing the voltages. |
| | Clog in the system | • Run Unclog function.<br>• Run 4 mL of Attune™ Debubble Solution at a 500 µL/minute flow rate. |
| | Loose Sample Injection Port (SIP) tube | • Remove the SIP tube and clean; observe sample aspirating.<br>• Reinstall the SIP tube. |
| | Loose sample syringe | Check for liquid dripping from the syringe, and tighten the syringe. |
| | Incorrect filter in detection channel | Verify the correct configuration. |
| | Laser is not functioning | • Verify laser function by re-running the Performance Test.<br>• Check laser function tied to threshold.<br>• Run an experiment using PT beads as a sample. Using the PMT voltages from the last Baseline or Performance Test, see if a positive signal can be seen from any parameters on the suspected laser.<br>• If the problem persists, contact Technical Support. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| No events are displayed in the Workspace (continued) | Sample may be too dilute | Increase the sample flow rate. |
| | No sample in tube | Add sample or install new sample tube. |
| | Cells have been lysed | • Ensure that the cells have not been lysed or broken up.<br>• Ensure that your sample contains cells. |
| | Bubbles in the fluidics system | Run De-bubble function. |
| | Plots on the Workspace do not match the enabled parameters | Check the instrument settings and confirm that the correct parameters have been applied to the Workspace plots. |
| No events are registered in the collection panel display | Population is off scale | • Adjust the axis to view the population.<br>• If events are detected in the collection panel display counter, ensure that the axis is set correctly<br>• If populations are not visible, make a bi-parameter dot plot of the fluorescent channels of interest and create a quadrant gate. Look at the statistics for each quadrant and check the event count to see if events are present in each quadrant. A population may not be on scale but will still be within the gate and therefore counted. |
| | Gating issue | Verify that the plots are set to all events and/or the gate logic is correct. |
| | Threshold set too high | • Lower the threshold.<br>• Increase the PMT voltages. |
| Run button is not visible | Instrument is powered off | Power on the instrument. |
| | Startup not completed | Run Startup function. |
| Run button is not enabled | The system is acquiring samples or busy performing another function | Wait for the active function to complete. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Computer is not communicating with the instrument | USB cable not fully plugged into USB 3.0 port | Examine the USB plug in the back of the instrument and the computer. |
| | Faulty USB cable | Contact Technical Support. |
| | USB port changed from the original port | Try different USB ports until communication is restored. If the problem persists, reinstall the USB drivers. |
| | | If you have checked all of the above, power off everything and disconnect the cables from the back of the computer. Wait a few moments, then reconnect and power on as usual. If the problem persists, contact Technical Support. |
| Instrument and/or computer has no power | Power supply not plugged into the appropriate outlet | Ensure that the instrument and/or computer are plugged into the appropriate outlet. |
| | No power at the outlet | • Make sure that the outlet is functioning properly and the circuit breaker is not tripped.<br>• Consider using a Universal Power Supply to guarantee a stable current. |
| | Faulty power supply | Contact Technical Support. |
| Getting events on scatter plots and/or LED is flashing, but no fluorescent signal | Incorrect parameter is selected | Ensure that the correct parameter is selected. |
| | Incorrect filter is installed | Verify that appropriate filters are installed for each detection channel. |
| | Laser is not functioning | • Ensure that the laser is powered on.<br>• Run Performance Test to verify laser function. If laser is not functioning properly, contact Technical Support. |
| | Area scaling factor set too low | Adjust the area scaling factor |
| | Incorrect fluorochrome | Verify that the reagent excitation/emission spectra match the collection filter set. |
| | Workspace gating logic is incorrect | Set the plot hyperlink to **All events**. |
| | Voltages are set too low | Increase the voltage settings. |
| | Reagent has degraded | Restain your sample with fresh reagents. |
| | Laser delay is incorrect | Run Performance Test. |
| | Threshold wrong if set to fluoresce | If you are using a fluorescent threshold, adjust the threshold level down. |
| | Incorrect filter | Check the optical layout. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Event rate is too high | Air bubble in flow cell | • Run De-bubble function.<br>• Look at the sample syringe and the sample loop to see if there are any bubbles present. |
| | Threshold is set too low | Increase the threshold level to reduce noise. |
| | PMT voltage for threshold is too high | Lower the PMT voltage for threshold parameters. |
| | Sample may be too concentrated | • Lower the sample flow rate.<br>• Dilute the sample. |
| | Sample flow rate is too high | Lower the sample flow rate. |
| | Bacterial contamination | • Ensure that the sample is not contaminated.<br>• Run the sample to see if there is a high number of background events, which usually appear small on the Scatter plots. Check the background levels on the Performance Test for signs of instrument contamination.<br>• Run deionized water or distilled water as a sample to see if the event rate stays high.<br>• Run the monthly decontamination procedure recommended for the fluidics tanks and replace the focusing fluid filter.<br>• Run the Deep Clean function using Wash solution instead of bleach to see if this decreases the background signal.<br>• If contamination persists, contact Technical Support. |
| Event rate is too low | Threshold level is too high | Lower the threshold level. |
| | PMT voltage for the threshold parameter is set too low | Set the PMT voltage higher for threshold. |
| | System may be clogged | • Run Unclog function or Deep Clean function.<br>• Ensure that the 1-mL syringe is tightly sealed. If needed, tighten or replace the syringe.<br>• If the problem persists, contact Technical Support. |
| | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Event rate is too low (continued) | Sample is not adequately mixed | Mix the sample to suspend the cells. |
| | Sample is too dilute | Increase the sample flow rate. |
| | Loose sample syringe | Check the sample syringe for leaks and tighten if necessary.<br>Note from reviewer: "Can also cause no events (sample not aspirating)." |
| Low event rate with high %CV | Bubbles in flow cell | • Run De-bubble function.<br>• Inspect the sample loop and sample syringe for bubbles. |
| Erratic event rate | Partial clog in the flow cell | • Run Unclog function.<br>• Run Deep Clean function.<br>• If clogs occur because of sample clumping, consider:<br>- Filtering the samples to remove any clumps.<br>- Adding 1 mM of EDTA to the buffer to prevent clumping.<br>- Diluting the sample and running at a higher flow rate.<br>• If the problem persists, run PT tracking beads as a sample at all the flow rates; record the data, then contact Technical Support. |
| | Sample has large clumps | • Filter the sample prior to loading to the instrument.<br>• Add 1 mM of EDTA to the buffer to prevent clumping.<br>• If sample clumping causes clogging, dilute the sample and run at a higher flow rate. |
| | Loose syringe | Check syringes for leaks, tighten if necessary. |
| | Focusing Fluid pump or Sample pump is not delivering the correct volume and/or is operating at inaccurate speed | • Run the Unclog and Deep Clean functions to ensure that there is not a blockage.<br>• Ensure that the syringes are properly tightened and that they have been changed in the previous 12 months.<br>• If the problem persists, contact Technical Support. |
| | Contaminated sample | Prepare new sample using clean tubes. |
| | Bubble in fluidics lines | • Run De-bubble function.<br>• Inspect the sample loop and sample syringe for bubbles. |
| | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Sample is not aspirating | Clog in SIP tube | Run Unclog function. |
| | Loose sample syringe | Check the sample syringe for leaks and tighten if necessary. |
| | Faulty valve | • Ensure that the 1-mL sample syringe is not leaking or drawing erratically.<br>• Run Self Test function.<br>• If the problem persists, contact Technical Support. |
| | Defective sample syringe | Replace sample syringe. |
| Scatter pattern is unclear | Instrument settings are not optimized | • Optimize experiment parameters for cell type.<br>• Ensure that the axes have the same scale. |
| | Cells were fixed | Some reagents used for fixing and permeabilizing the cells alter scatter patterns:<br>• Prepare new sample using different reagents for fixing and permeabilizing the cells.<br>• Visualize cells on a fluorescent microscope. |
| | Problems with sample preparation | |
| | Bubbles in the fluidics system | Run De-bubble function. |
| | Filters are not in the correct place | Ensure that filter is in the correction position for side scatter. |
| | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See Appendix C (page 61) for more information. |
| Signal drift during run | | • Run Self Test function.<br>• Contact Technical Support. |
| Pulsing of data at medium to high sample flow rates | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |
| High %CV with sample | Poor sample preparation | • Repeat the sample staining procedure.<br>• Optimize reagent stain conditions. |
| | Flow cell may be dirty | • Run Deep Clean function.<br>• If problem persists, run Decontaminate System function. |
| | Air bubble in flow cell | Run De-bubble function, then re-run Performance Test. |
| | Dirty filters | Clean the filters, then re-run Performance Test. |
| | Incorrect laser delay | Re-run Performance Test. |
| Sample aspirated, then backfilled into sample tube | Valve failure | Contact Technical Support. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Long delay between sample aspiration and events appearing on screen | Sample syringe is leaking | Ensure that the sample syringe is sealed properly. |
| | Partial clog in the fluidics system | • If clogs occur because of sample clumping, consider:<br>- Filtering the samples to remove any clumps.<br>- Adding 1 mM of EDTA to the buffer to prevent clumping.<br>- Diluting the sample and running at a higher flow rate.<br>• Run Unclog function.<br>• Run Deep Clean function.<br>• If the problem persists, contact Technical Support. |
| | Loose syringe | Tighten the syringe. |
| | Air in sample syringe | Run Rinse function, refill the sample tube, then run De-bubble function. |
| | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |
| Sample probe is not centered in the sample tube | SIP tube is bent | Carefully bend the SIP tube in place. |
| | SIP tube is faulty | Replace the SIP tube. |
| | Sample tube is not aligned vertically on tube lifter | Readjust the sample tube on the tube lifter. |
| Focusing fluid pump does not shut off | Focusing fluid filter is clogged | Replace the focusing fluid filter |
| | Focusing fluid reservoir level sensor is malfunctioning | • Perform Stop function.<br>• Power off the instrument and the computer and unplug the connectors. Wait for 1 minute, then reconnect and start up as normal.<br>• If the problem persists, shut off the instrument and contact Technical Support. |
| Fluid is leaking from the base of the instrument or into the drip tray | Crack in a fluidics container | Replace the damaged fluidics container. |
| | Snap fitting is broken or dripping | Contact Technical Support. |
| | Fluidics valve failure | Turn off the instrument and contact Technical Support. |
| | Syringe seal is broken | Change the syringe, or contact Technical Support. |
| | Focusing fluid filter is leaking | Replace the filter. |
| | Connection is loose | Contact Technical Support. |

# Performance Tracking troubleshooting

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| High ΔPMT in a single channel | Improper bandpass filter in channel | Check the optical configuration. |
| | Improper dichroic mirror placement | Check the optical configuration. |
| | Scratched or defective bandpass filter or dichroic mirror | • Clean filters; if problem persists, contact Technical Support.<br>• If there is an obvious scratch or break in the bandpass filter or dichroic mirror, order a replacement. |
| | PMT malfunction | • If the Performance Test failure is limited to one channel, confirm that the optical filters are in the correct configuration.<br>• Clean the optical filters with a lens cloth or compressed air. In severe cases, use a lens cleaner such as methanol.<br>• If the problem persists, run the PT beads as a sample, save the *.fcs files, then contact Technical Support. |
| | Incorrect performance tracking beadlot used | • Verify the lot number and download the correct lot information.<br>• Ensure that the current Baseline is using the correct lot number. |
| | If the high ΔPMT is seen in the FSC channel: Contamination of the flow cell | • Run the Deep Clean function using 3 mL of Wash solution instead of 10% bleach.<br>• If problem persists, contact Technical Support. |
| | Incorrect lot of PT beads used | Check the bead lot file. |
| High ΔPMT in all channels | Incorrect bead sample is used | Prepare a new bead sample. |
| | Clog or partial clog in the flow cell | Run Unclog function. |
| | Particle(s) stuck in the flow cell | Run Deep Clean function. |
| | Bubbles in the system | Run De-bubble function. |
| –ΔPMT in all channels for a single laser | Low or no laser power | Contact Technical Support. |
| | Wrong optical configuration for a single laser | Check the optical configuration. |
| | Laser is misaligned | Contact Technical Support. |
| | Bubbles in the system | Contact Technical Support. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| High %HPCV in a single channel for a single laser line | Dirty emission filter | Inspect and clean filter. |
| | Incorrect emission filter | Check the optical configuration. |
| | PMT malfunction | Contact Technical Support. |
| | Fluidics system is dirty | Run Deep Clean function. If the problem is in the FSC channel, run the Wash function with Wash solution instead of bleach. |
| High %HPCV in all channels for a single laser line | Improper filter placement | Check the optical configuration. |
| | Laser is misaligned | Contact Technical Support. |
| | Laser delay calculated incorrectly | Contact Technical Support. |
| | Bubbles in the system | Run De-bubble function. |
| High %HPCV in two channels for a single laser line | Improper dichroic mirror placement | Check the optical configuration. |
| | Emission filters swapped | Check the optical configuration. |

# Sample troubleshooting

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Weak or no fluorescence from the sample | Insufficient antibody present in sample | Ensure adequate antibody concentration for the total number of cells stained by titration. |
| | Target may not be accessible to the antibody (i.e., intracellular target) | Ensure that the fixation and permeabilization conditions are optimized for the target. |
| | Incorrect choice of fluorophore | • Ensure that the fluorophore matches the channel used.<br>• Use bright fluorophores for dim markers |
| | Incorrect compensation | Ensure that the positive single color control is set up correctly on the flow cytometer and gated/compensated correctly to capture all the events. |
| | Target not present or expressed poorly | Ensure that the sample expresses the target protein and allows its detection. |
| | Experiment is not optimized correctly | Use positive control to set PMT voltage, threshold, etc. |
| | Reagent has degraded | Restain sample with fresh reagent. |
| | Primary antibody is not compatible with the secondary antibody | Ensure that the secondary antibody was raised against the species in which the primary antibody was raised. |
| | Lasers are powered OFF | Power ON the lasers. |
| | Lasers are not aligned properly | Re-run the Performance Test; if it fails, contact Service. |
| High non-specific binding of label or high fluorescence from the sample | Antibody concentration is too high | Reduce the amount of antibody added to the sample. |
| | Excess antibody is trapped inside the cell | Ensure adequate washing of the sample with Wash solution containing permeabilization reagent. |
| | Inadequate blocking of the sample | Perform the blocking step prior to staining the cells. |
| | Experiment is not optimized correctly | Readjust PMT settings to ensure that all populations are on scale. |
| | Cells have high auto-fluorescence | |
| Unclear scatter data | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Two or more cell populations are observed when there should be only one | Inaccurate gating | Revise gate to only include the population of interest. |
| | Target protein is expressed on multiple cells | Verify the expression level and ensure adequate cell identification and separation. |
| | Cell doublets | • Filter cells to remove clumps.<br>• Dilute the sample to reduce coincidence. |
| | Non-specific staining due to dead cells | Use an appropriate dead cell stain to eliminate dead or dying cells. |

# 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.<br>• 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.<br>• 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. |

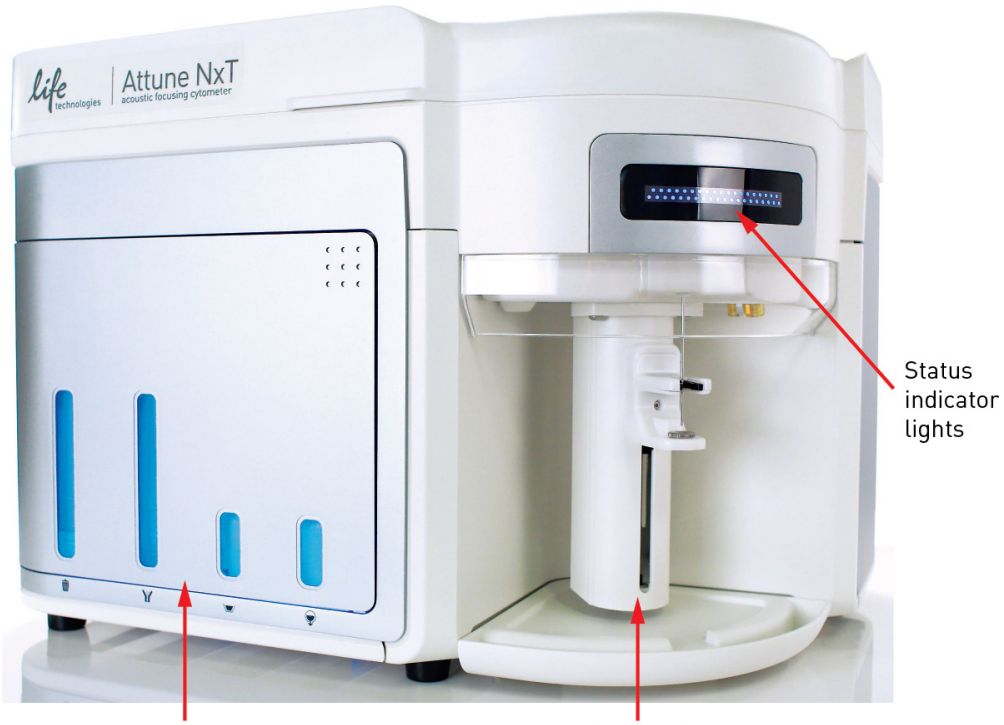
| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Auto Sampler and/or computer has no power | Power supply not plugged into the appropriate outlet | Ensure that the Auto Sampler, Attune™ NxT Cytometer and computer are plugged into the appropriate outlet. |
| | No power at the 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.<br>• If persistant, designate rinse wells throughout plate between samples and/or increase rinses between wells.<br>• 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. |
| | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |

| Observation | Possible causes | Recommended solutions |
|---|---|---|
| Sample probe is not centered in the sample well | SIP tube is bent or faulty | Contact Technical Support. |
| | 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 | |
| Event rate is too low | Large density differences between buffer and focusing fluid | Calculate the density of the sample buffer and re-formulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 61 for more information. |
| No events at low sample flow rates | | |
| Pulsing of data at medium to high sample flow rates | | |

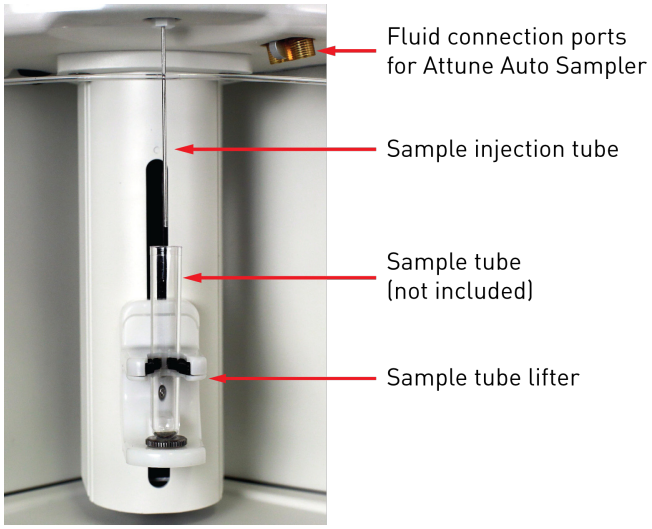
# Appendix A: Attune™ NxT Cytometer components

## Instrument exterior components

### Front view of instrument

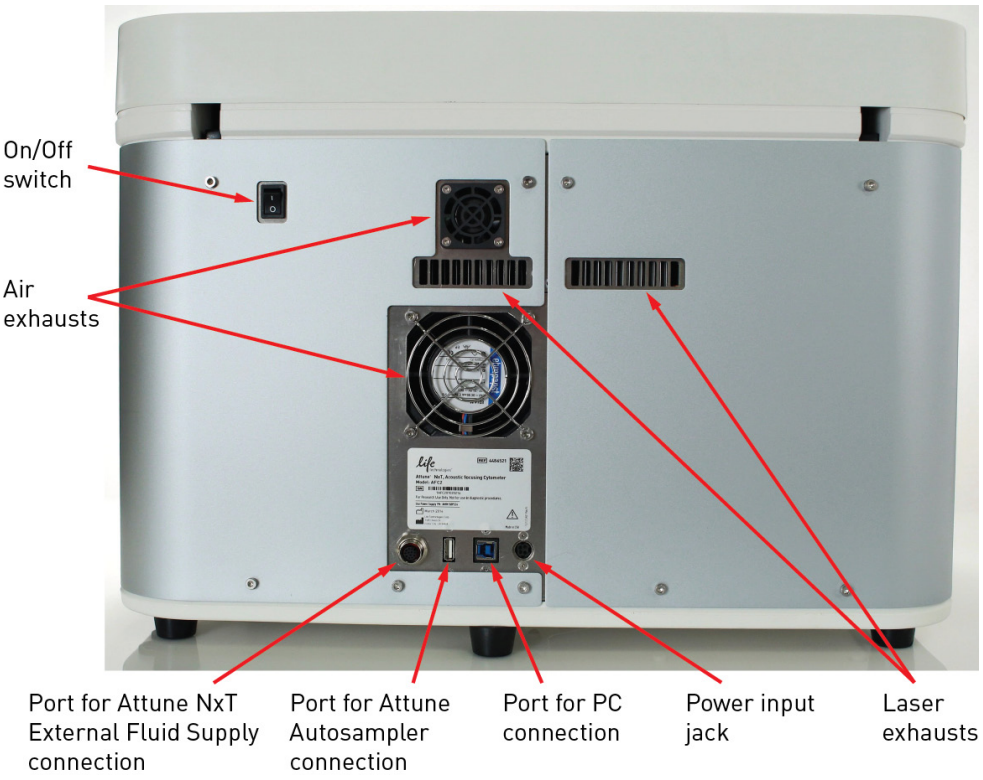



### Sample Injection Port (SIP)

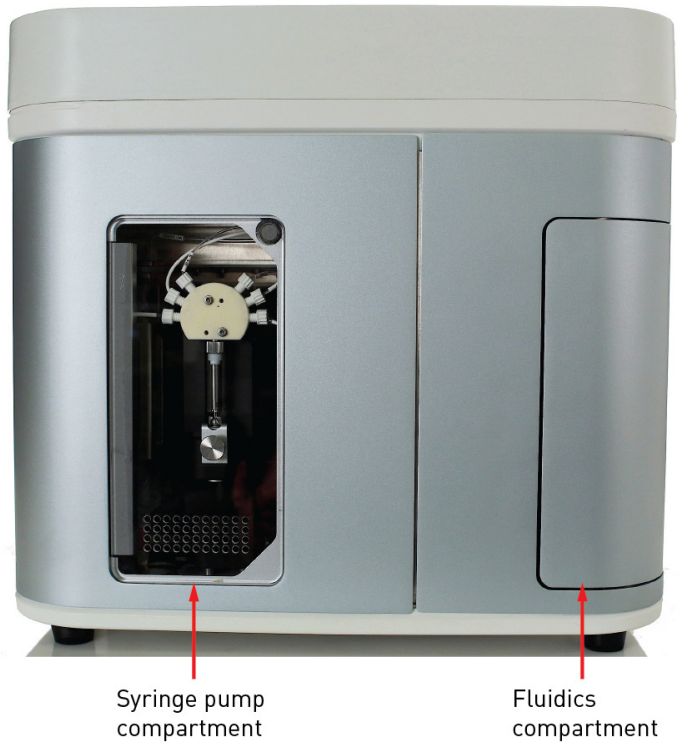



**Note:** The fluid lines that connect the Attune™ NxT Acoustic Focusing Cytometer to the Attune™ NxT Auto Sampler can be attached to either fluid connection port.

## Rear view of instrument



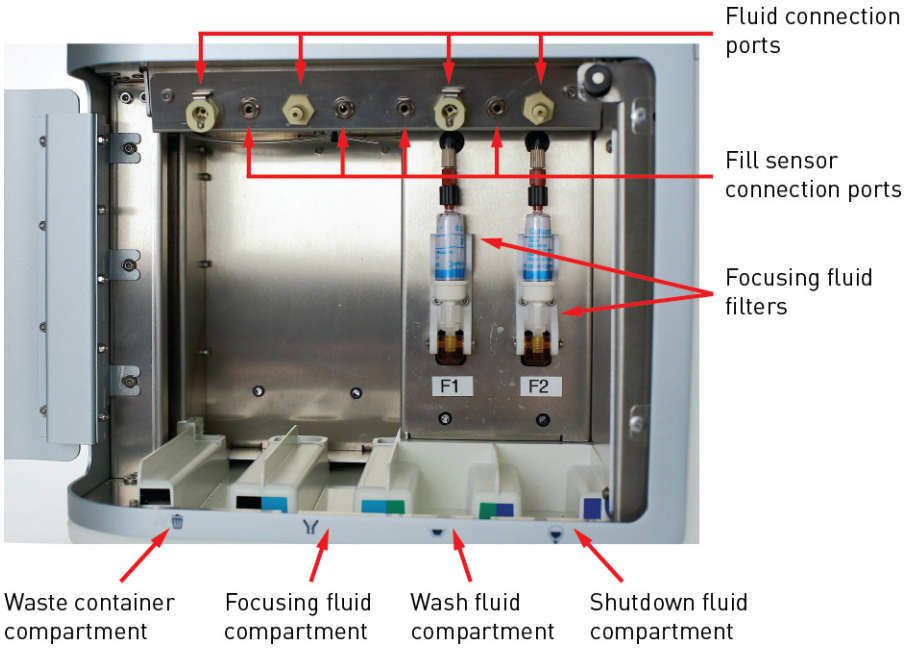

## Side view of instrument

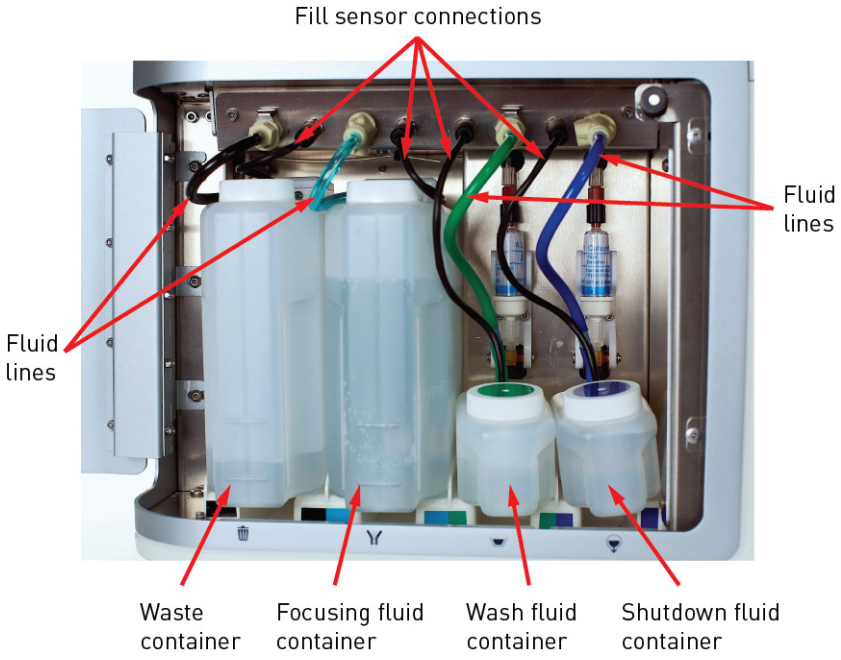


# Instrument interior components

## Fluidics compartment

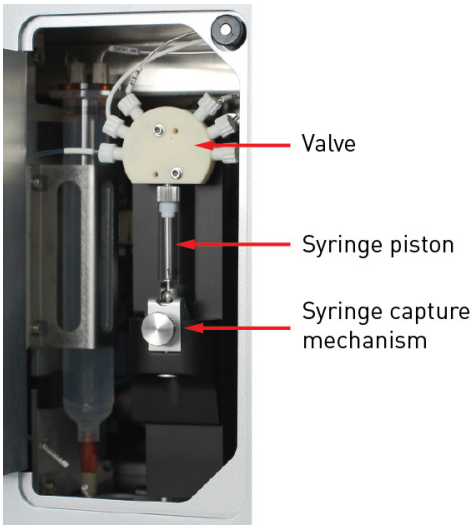
The images below show the fluidics compartment of the Attune™ NxT Acoustic Focusing Cytometer with and without the fluid containers and connections.



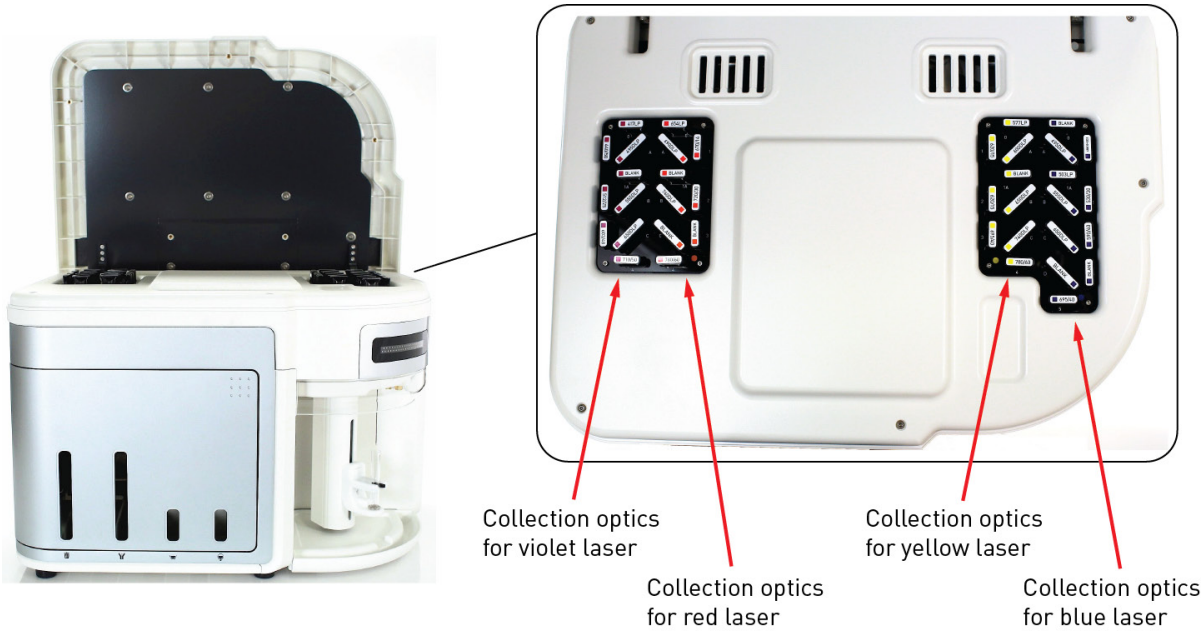
## Syringe pump compartment

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



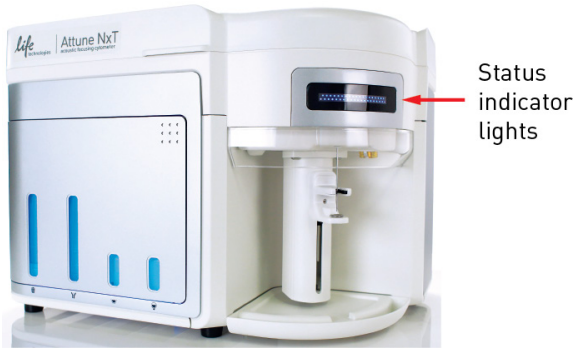

## Optics compartment

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



# Status indicator lights

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




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

# Optics

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

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

| Excitation laser | Channel color | Instrument config.* | Default filter (nm) | Filter range (nm) | Primary fluorophores | Other reagents |
|---|---|---|---|---|---|---|
| Yellow (561 nm) | YL1 orange | BVRY, BVY, BY | 582/22 | 571–593 | PE | Fura Red™ AM Cell Permeant<br>DyeCycle™ Orange<br>JC-1/DiOC2(3)<br>pHrodo™ Phagocytosis Particle Labeling Kit<br>SNARF™ (low pH)<br>SYTOX™ Orange Dead Cell Stain |
| | YL2 red | BVRY, BVY, BY | 615/20 | 605–625 | Alexa Fluor™ 594<br>PE-Alexa Fluor™ 610<br>m-Cherry | |
| | YL3 far red | BVRY, BVY, BY | 695/40 | | TRI-COLOR™ (TC, PE-Cy™5)<br>PE-Cy™5.5 | Vybrant™ Dye Cycle™ Ruby |
| | YL4 near IR | BVRY, BVY, BY | 780/60 | 750–810 | PE-Alexa Fluor™ 750<br>PE-Cy™7 | Qdot™ 800 |
| Red (638nm) | RL1 far red | BVRY, BVR, BR | 660/20 | 650–670 | Allophycocyanin (APC)<br>Alexa Fluor™ 647 | Fixable Far Red Dead Cell Stain<br>Click-iT™ Alexa Fluor™ 647<br>FxCycle™ Far Red<br>SYTOX™ Red Dead Cell Stain |
| | RL2 near IR | BVRY, BVR, BR | 720/30 | 705–735 | Alexa Fluor™ 700 | Vybrant™ Dye Cycle™ Ruby |
| | RL3 near IR | BVRY, BVR, BR | 780/60 | 750–810 | APC-Alexa Fluor™ 750<br>APC-Cy™7<br>APC-H7 | Fixable Near-IR Dead Cell Stain<br>Vybrant™ Dye Cycle™ Ruby |

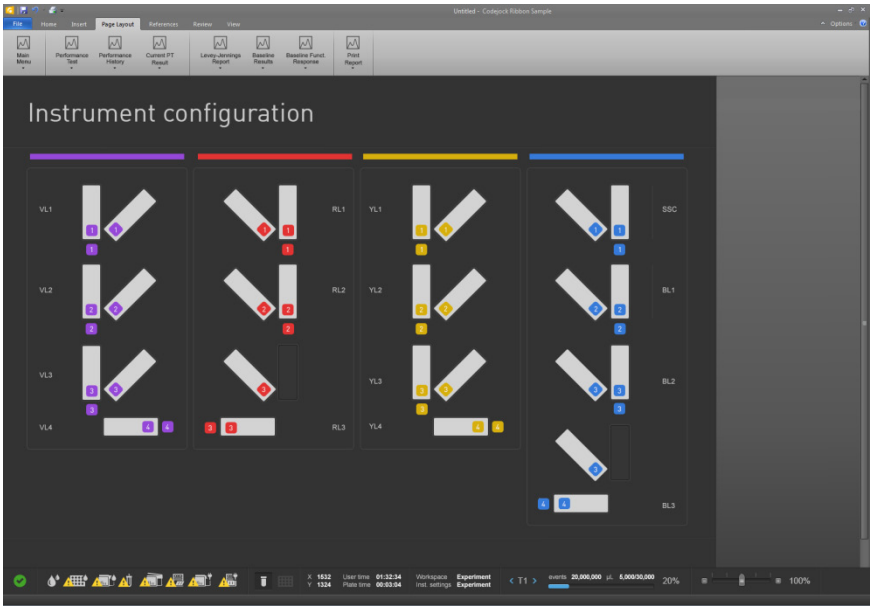
***Instrument configuration:** B = Blue laser, V = Violet laser, R = Red laser, Y = Yellow laser

# Instrument configurations

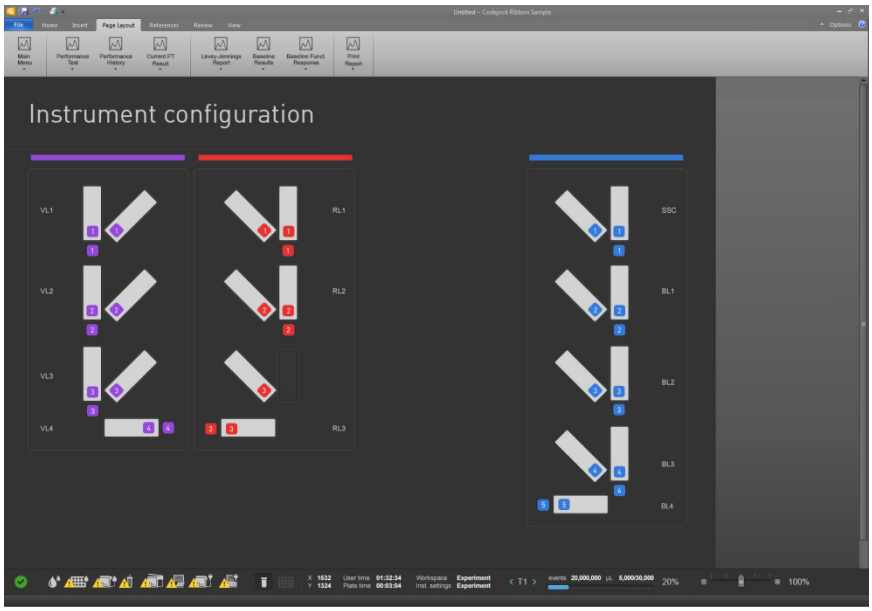
## Default instrument configurations

The following images show the Instrument Configuration panel for each laser configuration.

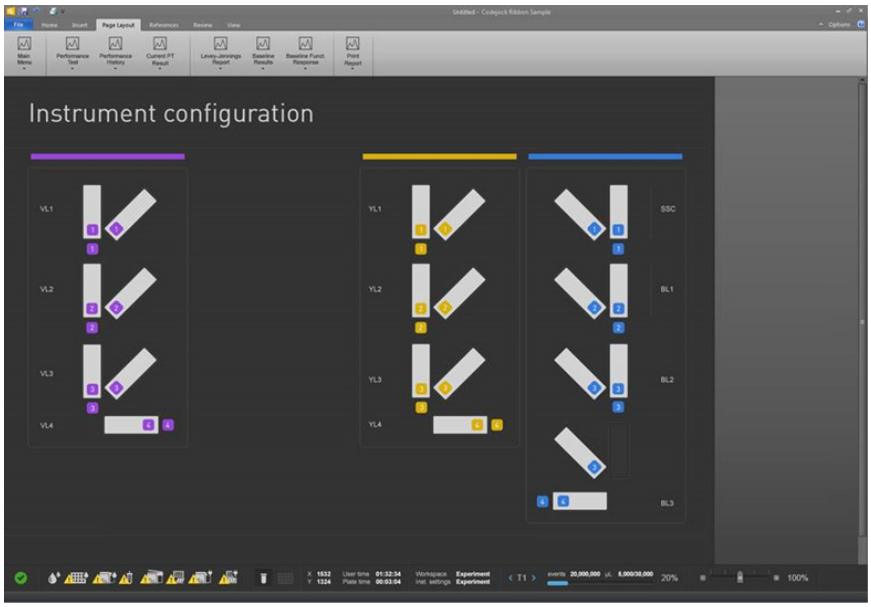
### BRVY (Blue, Red, Violet, Yellow)



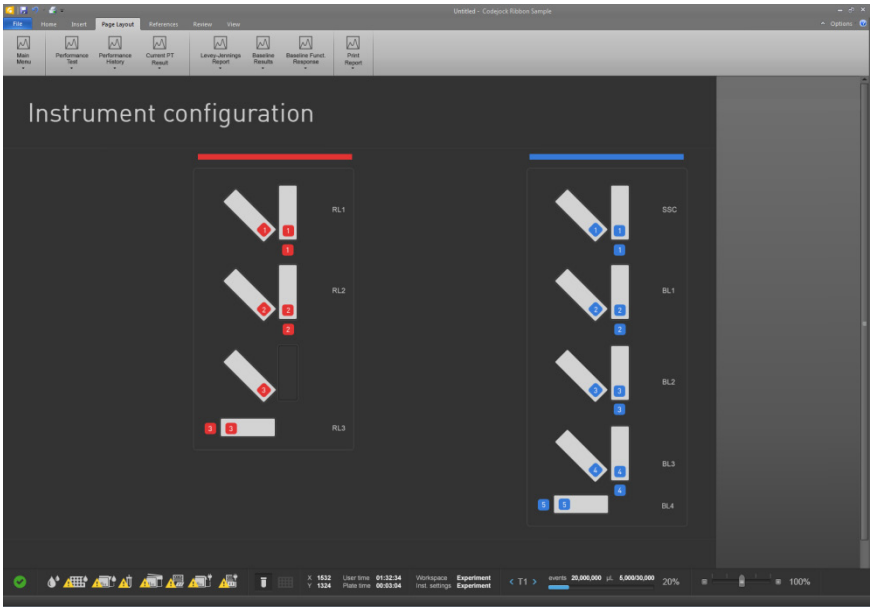

### BRV (Blue, Red, Violet)



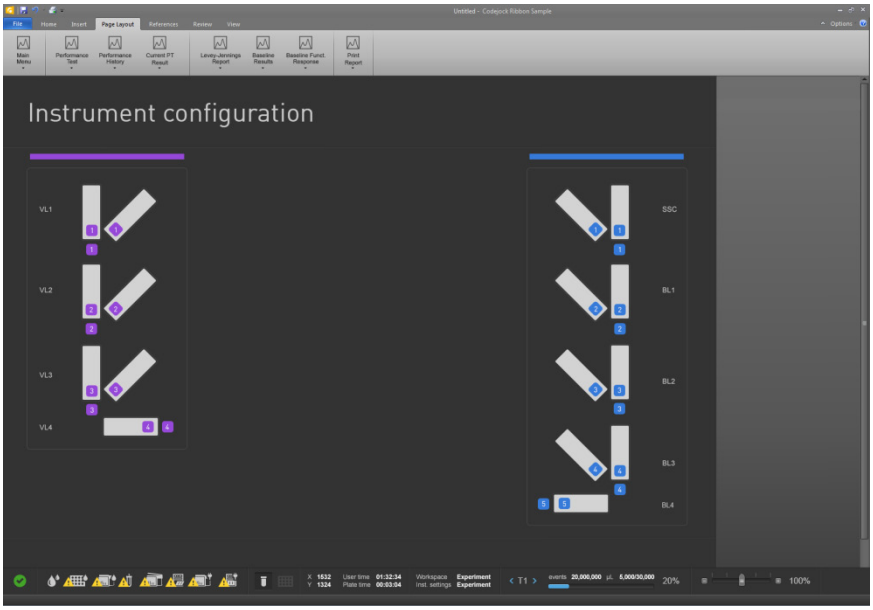
## BVY (Blue, Violet, Yellow)



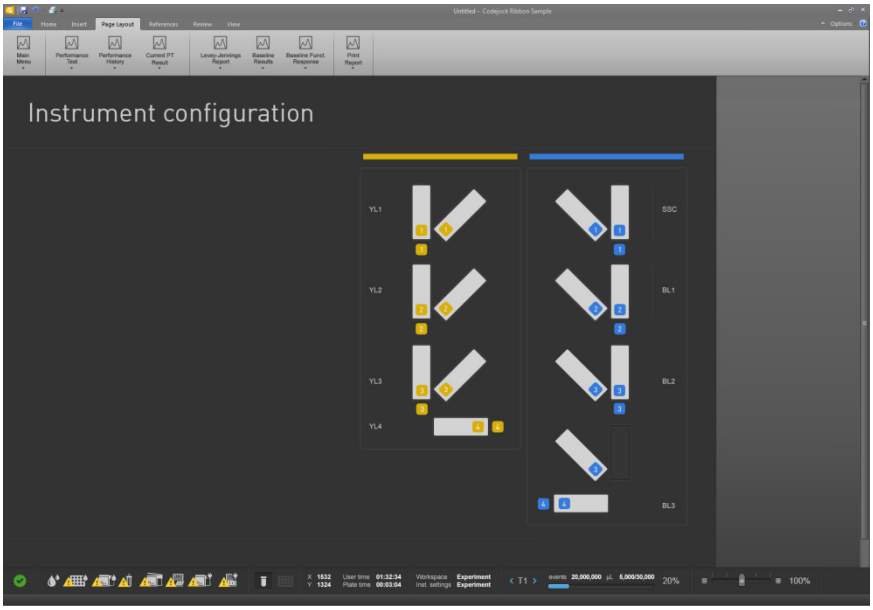

## BR (Blue, Red)



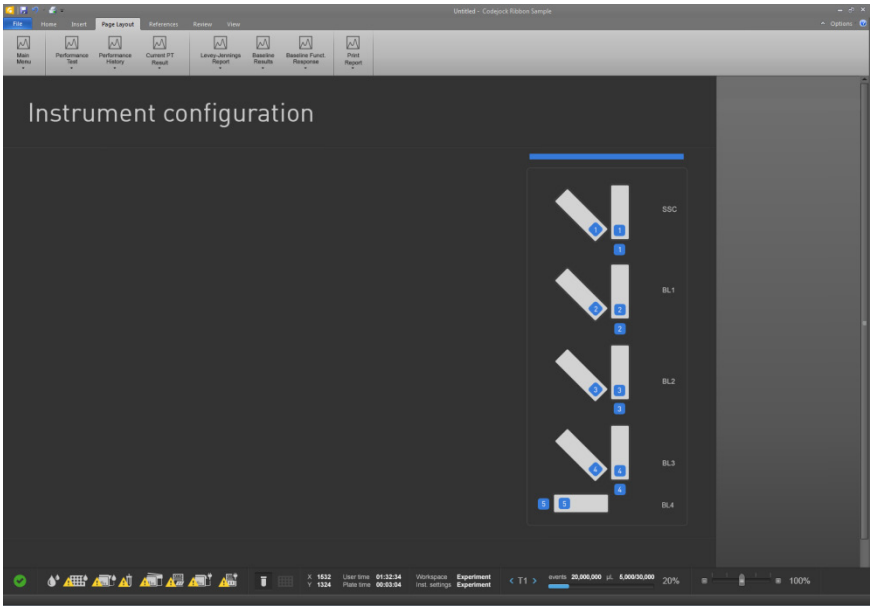
## BV (Blue, Violet)




## BY (Blue, Yellow)



## B (Blue)



## Default filter label list

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

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

**Baseline/PT Config <*CCCC*>,**

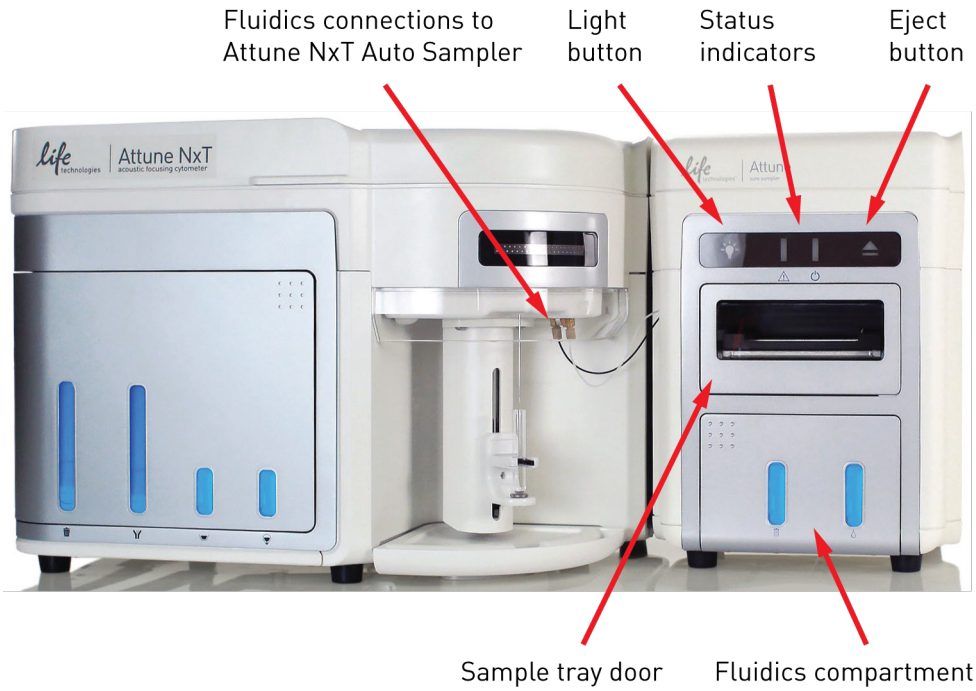
where **<C>** is the first letter of each laser color (**B**lue, **R**ed, **V**iolet, or **Y**ellow) and an **X** in the name indicates that one of the lasers is not present.

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

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

# Appendix B: Attune™ NxT Auto Sampler components

## Instrument exterior components



# Appendix C: Attune™ Focusing Fluid density adjustment

Sample buffers with very high salt concentrations create a large density difference between the buffer and the focusing fluid, which causes artifacts in the data that can include:

- delay in events at beginning of data streaming
- absence of events at low sample flow rates
- pulsing of data at medium to high sample flow rates
- low event counts

If your sample requires a buffer with a high salt concentration, you need to adjust the concentration of the Attune™ Focusing Fluid to balance densities of the sample buffer and the focusing fluid and improve data streaming.

## Adjust the focusing fluid density

1. Calculate the density of your sample buffer or use a hydrometer to measure it.
2. Add the appropriate amount of NaCl to Attune™ Focusing Fluid (1X) to adjust its density so that it matches the density of the sample buffer.

   Refer to the table below for the amount of NaCl to add to the focusing fluid.

| Attune™ Focusing Fluid | NaCl to add (g) | Concentration (mM) | Density (g/cm$^3$) |
|---|---|---|---|
| 1X | NA | 155 | 1.00 |
| 2X | 9 | 310 | 1.08 |
| 3X | 18 | 465 | 1.16 |
| 4X | 27 | 620 | 1.24 |
| 5X | 36 | 775 | 1.32 |
| 6X | 45 | 930 | 1.40 |

# Appendix D: Safety

This section includes the following topics:

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

# Safety conventions used in this document

## Safety alert words

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

### Definitions

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

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

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

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

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

# Symbols on instruments

## Electrical symbols on instruments

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

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

## Safety symbols

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

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

## Environmental symbols on instruments

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

| Symbol | Description |
|---|---|
|  | **Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).<br>**European Union customers:**<br>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 Material Safety Data Sheets and local regulations for handling and disposal.

## Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

## Waste disposal

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

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

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

# Electrical safety

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

## Fuses

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

## Power

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

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

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

## Overvoltage rating

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

# Physical hazard safety

## Moving parts

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

# Biological hazard safety

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

In the U.S.:

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

  (stock no. 017-040-00547-4;
  **www.cdc.gov/OD/ohs/biosfty/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 I laser product. When safety interlocks are disabled during certain servicing procedures and/or input/output optics covers are removed, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

## Laser safety requirements

To ensure safe laser operation:

- The system must be installed and maintained by a Life Technologies 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.

## 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.