invitrogen

Attune™ Flow Cytometry Maintenance and Troubleshooting Guide

For the maintenance and troubleshooting of Attune™ NxT and Attune™ CytPix™ Flow Cytometers, Attune™ NxT Auto Sampler, and Attune™ External Fluid Supply

Publication Number 100024234
Revision C.0

ThermoFisher SCIENTIFIC

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

Information in this document is subject to change without notice.

DISCLAIMER
TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 100024234

Revision	Date	Description
C.0	15 September 2021	Add Attune™ CytPix™ information, add instructions to replace the Auto Sampler syringe, add new instrument filter configurations, add Attune™ NxT EFS maintenance and troubleshooting sections, add CytKick™/CytKick™ Max Autosampler syringe replacement instructions and Appendix C: Parts of the CytKick™/CytKick™ Max Autosampler, add Appendix F: Minimizing background in Attune™ NxT Cytometer, revise the maintenance schedule, rebrand the user guide.
B.0	23 April 2015	Basis for this revision

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Contents

About this guide

This user guide describes how to perform basic preventive maintenance procedures to ensure reliability of Invitrogen™ Attune™ NxT and Attune™ CytPix™ Flow Cytometers, Attune™ NxT Auto Sampler, CytKick™ and CytKick™ Max Autosamplers, and the Attune™ External Fluid Supply. The guide also provides tips to help you troubleshoot your experiments.

IMPORTANT! For workflows and instructions on using the Attune™ NxT Flow Cytometer, see the *Attune™ NxT Flow Cytometer User Guide* (Pub. No. 100024235) and the *Attune™ NxT Flow Cytometer Quick Reference Guide* (Pub. No. 100024233).

For workflows and instructions on using the Attune™ CytPix™ Flow Cytometer, see the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440).

For workflows and instructions on using the CytKick™ and CytKick™ Max Autosamplers, see the *CytKick™ and CytKick™ Max Autosampler User Guide* (Pub. No. MAN0018351).

For a detailed description of the Attune™ Cytometric Software, see the *Attune™ Cytometric Software User Guide* (Pub. No. 100024236).

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

Safety information

See "**Appendix H: 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 specific 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, could result in minor or moderate injury. It is also 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 this instrument").

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 "Customer and technical support".

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

Flow Cytometer maintenance

The Attune™ NxT and Attune™ CytPix™ Flow Cytometers are designed to require minimum maintenance. However, to ensure reliability of the cytometer, you must perform basic preventive maintenance procedures regularly, as listed in "Maintenance schedule", page 7.

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

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

Maintenance schedule

The following table lists the routine maintenance procedures that keep the flow cytometer and all its peripheral systems in good working condition.

Procedure	Frequency
Shutdown	Daily
Visual inspection of sample injection port (SIP), fluid bottles and connections, and syringe pumps	Daily
Fluidics maintenance	Daily or as needed
Sanitize between uses	Daily or as needed
Computer maintenance	Weekly
Fluidics decontamination	Monthly
Optical filter and mirror inspection	Monthly
Replace focusing fluid filters	At least every 3 months (more frequently for high volume use) and after each system decontamination
System decontamination	At least every 3 months (more frequently for high volume use)
Syringe replacement	3 months (high volume use at >6 hours/day) or 6 months (standard use)
Rinse	As needed
Sanitize SIP	As needed
Unclog	As needed
De-bubble	As needed
Deep Clean	As needed

Note: High volume use is defined as daily operation of the instrument for >6 hours/day.

Daily maintenance

Visual inspection

- Visually inspect the sample injection port, fluid bottles and connections, and the syringe pumps for any leakage. If you notice any leaks in the fluidics lines, contact your service representative. Decontaminate any spills by wiping the area with 10% bleach solution.
- Check the fluid levels. Empty waste and refill fluids, if needed.
- Ensure that fluid lines and probe lines for all bottles are firmly connected. An audible click indicates full engagement.
- Inspect the floor of the fluid bottle compartment. Clean up any spilled liquid, remove any salt or dust deposits with a damp paper towel. If needed, decontaminate by wiping area with 10% bleach solution. Monitor the instrument as liquid in the bottle compartment can indicate a leak.
- Inspect sample injection port (SIP) and ensure that it is clean and straight.
- Inspect sample syringe (glass syringe in left side compartment of flow cytometer) and ensure that top and bottom metal fittings are tightened. Check for signs of leaks (salt deposits or rusty metal plunger). If there is any indication of a potential leak, replace the syringe.
- Visually inspect the area behind the instrument to ensure that cords are plugged in and connections not stressed. Verify that at least 6 inches of free space behind the instrument is maintained and that all four exhaust ports are free of blockage.

Important guidelines to power on the system

- After the system has been visually inspected, it must be powered on and the software launched. The first time the flow cytometer is used, it will be powered off and must be powered on in the correct order.
- If the system is used daily and put through daily **Shutdown** (page 11), it will be left in a "sleep state". The sleep state is not a full power off state and the instrument can be "woken" from the sleep state using the Attune™ Cytometric Software.

  When the system is powered off, the status indicator lights (page 79) do not display any color. When the system is in the sleep state, the indicator lights will be in multi-color fade mode.
- The power switches are located on the back of the instrument towards the right (when facing the instrument) (page 72).

  Note: If an Attune™ NxT External Fluid Supply (Cat. No. A28006) is used with the flow cytometry system, it is automatically powered on when the flow cytometer is powered on.
- If an Attune™ Auto Sampler or a CytKick™/CytKick™Max Autosampler is part of the flow cytometry system, power on the auto sampler before powering on the flow cytometer.
- Launch the Attune™ Cytometric Software after powering on the auto sampler and the flow cytometer.

- If the autosampler is not going to be used, it is best practice to power on the autosampler at the same time as the flow cytometer to ensure that the autosampler is cleaned at the same frequency as the cytometer.
- Do not leave the autosampler in a shutdown state for more than 2 weeks, even if the flow cytometer is in use. If the autosampler is not being used, ensure that it is put into long term shutdown.

IMPORTANT! To conserve energy, you can power off your instrument daily. The order of powering the system off does not matter; however, the system must not be powered off using the physical switch at the back of the instruments until the shutdown cycle is completed.

Startup

- Execute the **Startup** function (page 46). During Startup, the flow cytometer warms the lasers to operating temperature, initializes the pumps, primes the instrument fluidics, and notifies you of the System Status (Ready, Attention, Clog, and so on).
- During the Startup procedure, observe the movement of the sample syringe on the flow cytometer and the autosampler.
  - Listen for any sounds that are out of the ordinary, such as loud grinding sound or the absence of normal sounds during start up. If possible, record any unusual sound for support reference.
  - The flow cytometer sample syringe is in the left side compartment of the instrument. Observe the syringe for smooth movement on the up and down strokes. There should be no bubbles being delivered on the upstroke of the syringe and no drops should escape past the plunger. Any large air bubbles on the up or down strokes might indicate a large leak or a worn syringe.
  - The autosampler syringe is in the fluid bottle compartment, positioned on the left side. Observe the autosampler syringe for the same potential problems as the flow cytometer sample syringe.
  - If the flow cytometer or the autosampler syringe is not moving or appears to be sticking (non-fluid motion), we recommend that you replace the syringe before contacting Technical Support.

Performance tracking

Performance tracking is a comprehensive set of procedures to monitor the daily performance of the flow cytometer. It is critical to ensure the accuracy and sensitivity of the cytometer, and it provides information about the lasers and detection channels available on the instrument.

The performance tracking process involves:

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

For the detailed procedure, see the *Attune™ NxT Flow Cytometer User Guide* (Pub. No. 100024235) or the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440), which are available for download at **thermofisher.com/attune**.

IMPORTANT! Run the **Sanitize SIP** function (page 47) before running your samples after completing the Performance tracking procedure. This prevents bead carryover and ensures consistent and accurate results.

Fluidics maintenance

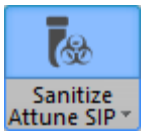
Fluidics maintenance includes the following user-performed functions. Perform each function daily or as needed.

- **De-bubble**– Clears bubbles in the fluidics lines of the cytometer. For instructions, see page 49.
- **Unclog**– Removes clogs from the sample probe and flow cell (back-flush operation). For instructions, see page 48.
- **Sanitize SIP**– 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. The procedure requires user-supplied bleach or detergent. For instructions, see page 47.
- **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 46.
- **Deep Clean**– Sanitizes the system with bleach and Wash solutions for a user-selected time. The procedure ensures system cleanliness while allowing you to continue using the instrument after the cycle is complete. The procedure does not sanitize the fluid bottles. For instructions, see page 53.
- **Decontaminate System**– Sanitizes the system and fluid bottles with bleach and Wash solutions for a proscribed time. The procedure ensures full system cleanliness at regular maintenance intervals to prevent build-up of contaminants in the system or fluid bottles. For instructions, see page 24.

Sanitize between uses

Sanitize the flow cytometer between uses to minimize cross-contamination. Note that these procedures are for fast cleaning of the instrument to minimize cross-contamination. For a more thorough decontamination, perform the system decontamination procedure (page 24).

- For non-sticky cells, such as B and non-activated T cells, run the **Sanitize SIP** procedure (page 47) 2 times (~3–4 minutes total).
  - First iteration: Use 3 mL of 10% bleach.
  - Second iteration: Use 3 mL of Attune™ Wash Solution (Cat. No. A24974)



- For sticky cells, such as DCs, RBCs, activated T cells, many cancer lines, bacteria, and yeast, run the **Quick Deep Clean** cycle (page 53) (~20–30 minutes total).

Shutdown

After the last run of the day, execute the **Shutdown** function (page 50). The Shutdown procedure 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.

Depending on the Shutdown option selected, the procedure takes 25 to 70 minutes to complete; however, most steps are automated and do not require monitoring. At the end of the Shutdown procedure, the goes into a “sleep state”.

Weekly maintenance

Computer maintenance

Periodic maintenance of the computer running the Attune™ Cytometric 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 regularly to a secondary storage device.
- When planning the experiments, remember to turn off the channels and parameters that you do not need (that is, only collect parameters in either area or height, but not both, unless you need both parameters for a specific application such as cell cycle). This decreases file size and facilitates analysis in third party software.
- Data is saved to the D: drive of the computer. Ensure that that there is adequate space available on the D: drive of the computer. If there is less than 50 GB of free space, export, save externally, and delete your experiments from the Experiment Explorer.
- To determine which accounts are using the most space on the D: drive, perform one of the following:
  - **Check User log:** Sign in as an **Admin**, go to **Instrument tab ▶ System log ▶ User log tab**. Filter the User log data by [install date] to current date, All Users, and Sample Count (instead of User Time).
  - **Check User folder size in D: drive:** D:\\User\public\public documents\life technologies\AttuneNxT\Userdata

IMPORTANT! Wiping the computer that is supplied with flow cytometer (that is, erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.

Monthly maintenance

Contamination symptoms

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

- Bacterial growth in the bottle, indicated by cloudy fluid, globs or strings in the fluid, or discoloration of the fluid.
- A high number of events that do not correspond to the sample (that is, sample dilution has no effect). You can confirm this by running Attune™ Performance Tracking Beads and observing a high event rate (over 1,000 events/second compared to the expected 200–300 events/second).
- If you observe bacterial growth in the bottles, replace the focusing fluid filters when the bottles are decontaminated (page 18).

Decontaminate fluid bottles

1. Disconnect all fluid 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 then discard the fluid.
6. Pour at least 20 mL of deionized water in each bottle, replace cap, and invert or gently shake to rinse out any remaining particulates.
7. Invert each bottle and allow to air dry.
8. Fill each bottle with the appropriate fluid (fresh Attune™ Focusing Fluid, Attune™ Wash Solution, or Attune™ Shutdown Fluid).
9. Place all fluid bottles back in the instrument. Be sure to attach the fluidics cable before attaching the probe cable.
10. Run **Startup** function (page 46).

Inspect optical filters and mirrors

The optical filters and mirrors are housed in optical holders, which are in the optics compartment. For optimal cytometer performance, inspect the optical filters and mirrors monthly and **clean only if necessary**. For detailed instructions, see "Clean optical filters and mirrors", page 14.

CAUTION! LASER HAZARD. Clean or change optical filters and mirrors only when the instrument is powered off or when not analyzing sample. For laser safety precautions, see "Laser safety" on page 113.

Clean optical filters and mirrors

Clean the optical filters and mirrors **only if necessary**.

1. Lift the cytometer lid. The following photograph shows the configuration of the optical filters and mirrors for a 4 laser (blue, red, violet, yellow) Attune™ NxT Flow Cytometer. The four violet and three red parameters are on the left, and the four yellow and three blue parameters are on the right.

Note: The optical layout will appear different depending on the instrument (Attune™ NxT or Attune™ CytPix™ Flow Cytometer) and instrument configuration (1, 2, 3, or 4 laser configuration). For illustrations of default instrument configurations, see page 80.

2. Remove the optical holder containing the appropriate filter or mirror. Do **not** touch the filter or lens surface.

3. Gently remove any dust from the surface of the filter or mirror with a bulb-blower, 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.

Decontaminate system

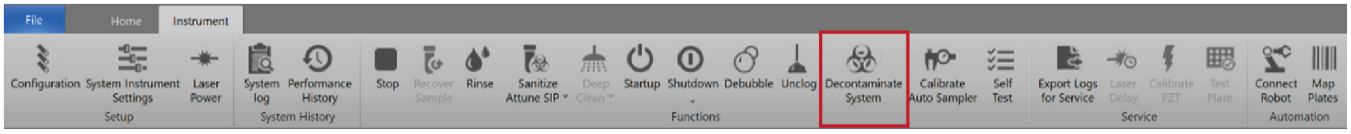
CAUTION! BIOHAZARD. Cytometer hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure that we recommend for decontaminating the cytometer.

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

Decontaminate System function

The *Decontaminate System* function is used for the automated decontamination of the flow cytometer and autosampler 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 instead 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 a decontamination run before becoming idle
- The **Decontaminate System** button on the Instrument tab of the Ribbon bar opens the *Decontaminate* dialog, which provides instructions to perform the Decontaminate System function.



- The steps in the Decontaminate System function vary depending on whether an autosampler is connected. See page 24 for the decontamination procedure for the flow cytometer equipped with an autosampler.
- The Decontaminate System function for the flow cytometer is broken into three phases. It can take up to 45 minutes to complete each phase of the procedure; however, most of the operation is performed automatically.
- Each step of the Decontaminate System procedure is displayed at the top of the dialog box and the procedure step is highlighted.
- The Decontaminate System function is only available to Administrator accounts.

Prepare for system decontamination

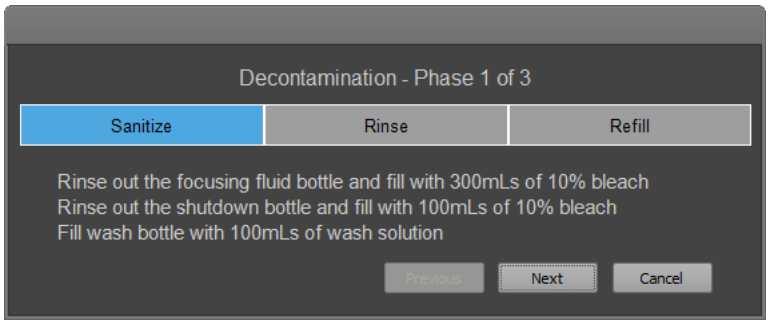
1. Rinse out all fluid containers with deionized water.
2. Ensure that all fluid lines and probe cables are connected.

Note: For the location of the fluidics compartment and instructions on filling the fluid bottles, see the *Attune™ NxT Flow Cytometer User Guide* (Pub. No. 100024235) or the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440), which are available for download at **thermofisher.com/attune**.

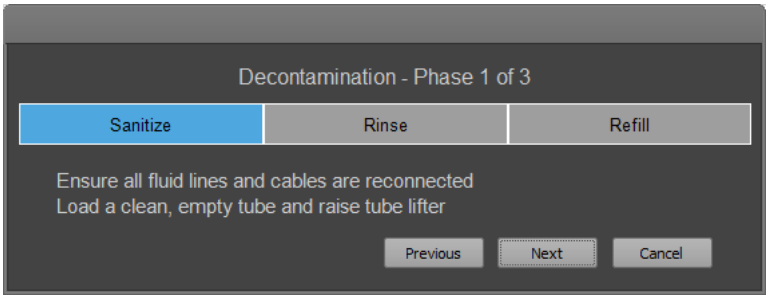
Run Decontaminate System function (flow cytometer only)

1. Click the **Decontaminate System** button 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:

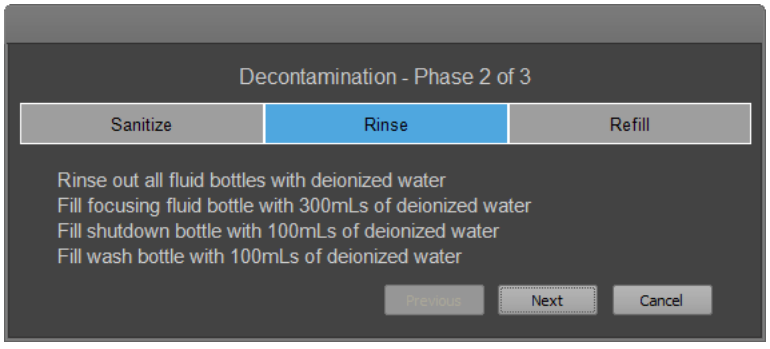


   a. Rinse all fluid bottles, except the wash bottle, with 10% bleach.
   b. Fill the focusing fluid bottle with 300 mL of 10% bleach and the shutdown bottle with 100 mL of 10% bleach.
   c. Fill the wash bottle with 100 mL of Attune™ Wash solution, then click **Next**.

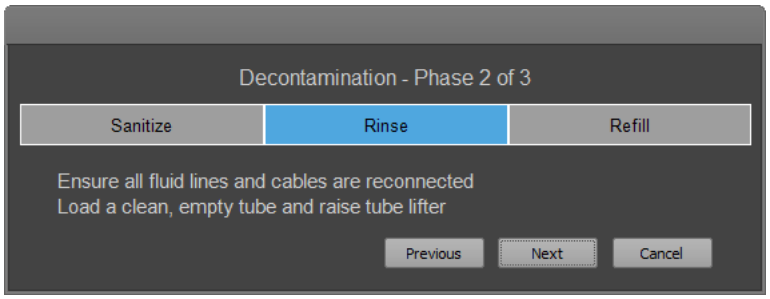


   d. Reconnect all fluid lines and bottle cables.
   e. Load a clean, empty tube on the SIP, raise the tube lifter.

3. Click **Next** to start Decontamination Phase 2. When prompted:



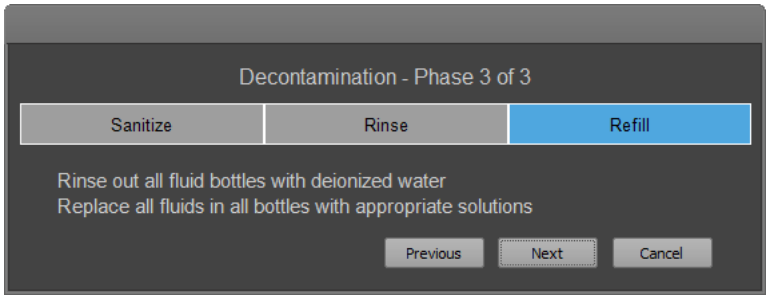
   a. Rinse all fluid bottles, except the wash bottle, with deionized water.
   b. Fill the focusing fluid bottle with 300 mL of deionized water and the shutdown and wash bottles with 100 mL of deionized water.



c. Reconnect all fluid lines and bottle cables.

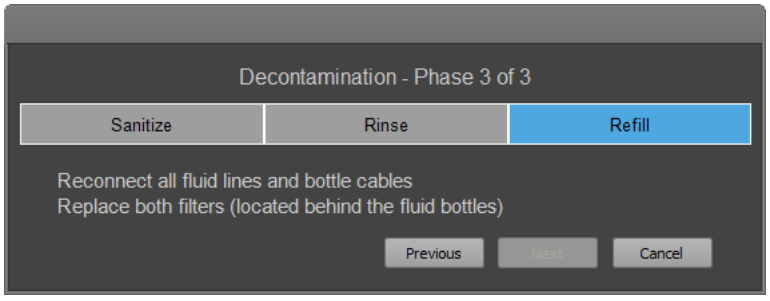
d. Load a clean, empty tube on the SIP, then raise the tube lifter.

4. Click **Next** to start Decontamination Phase 3. When prompted:



a. Rinse all fluid bottles with deionized water.

b. Replace all fluids in all fluid bottles with the appropriate solutions.



c. Reconnect all fluid lines and bottle cables.

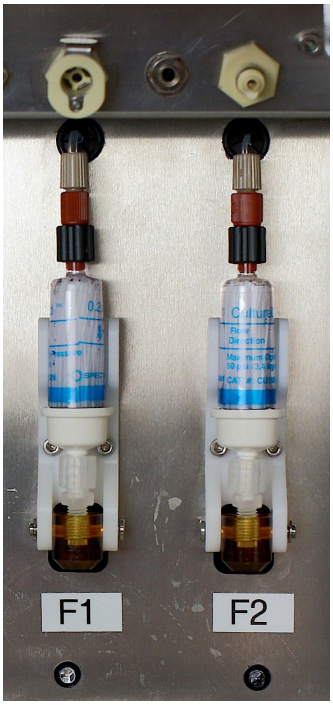
d. Replace the focusing fluid filters with new filters (page 18).

IMPORTANT! After System Decontamination is complete, replace the focusing fluid filters (page 18).

Replace focusing fluid filters

Replace focusing fluid filters

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



1. Disconnect the Velcro strap that holds the filter in place (not shown in the image), grab the top tubing with your thumb and forefinger, then gently pull the filter to a slight angle away from the instrument.
2. Disconnect the top of the filter by rotating the black Luer lock nut counterclockwise, then loosen the top connection from the fitting. There may be a small amount of fluid at the top of the fitting that comes out.
3. Grab the filter body by the middle with your thumb and forefinger, then using your other hand to hold the lower Luer lock fitting in place, twist the filter body counterclockwise to remove the filter from the bottom Luer lock seat.
4. Unscrew the bottom Luer lock fitting in a counterclockwise fashion to remove it from the filter. If the Luer lock fittings rotate and come loose from their sockets, retighten them.
5. With the filter removed, do the following:
   a. Unscrew the black section of the female-to-male Luer lock adapter.
   b. Unscrew the bottom male-to-female Luer lock adapter. Some liquid will exit the filter.
   c. Discard the used filter
6. Remove the new filter from its packaging, then orient the filter with the arrow on the filter body pointing down.
7. Screw in the bottom male-to-female Luer lock adapter to the bottom of the new filter, then reconnect the filter with the bottom Luer lock (insert and turn clockwise). Ensure that the tubing from either end of the filter is not kinked or bent.
8. Screw in the black section of the female-to-male Luer lock adapter to the top of the filter, then reconnect the top Luer lock fitting (insert and turn the nut clockwise to engage the Luer lock). Ensure that the tubing from either end of the filter is not kinked or bent.
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 female fitting into the top of the tubing until a click is felt or heard.
11. Check for leaks. If either fitting came loose during operation, and a small amount of fluid is leaking out, tighten the fittings gently by hand.

12. Run **Startup** function (page 46) and carefully observe filter for leaks.
13. Prime the system using the following fluidics priming sequence: Run 3 startup cycles, 2 De-bubble cycles, and 2 Rinse procedures.

IMPORTANT! After replacing the focusing fluid filters, the fluidics priming sequence is required to purge air from system. Failure to purge air from the system and saturate the filters with Attune™ Focusing fluid will result in instrument issues.

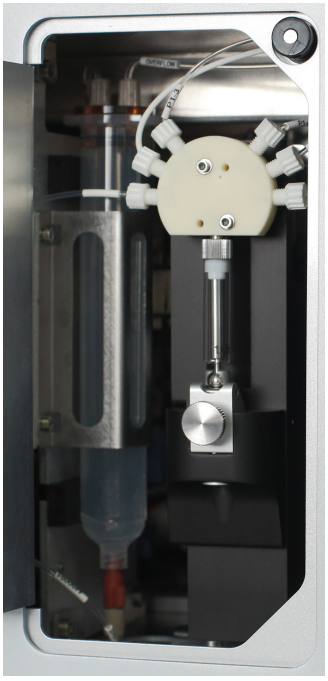
Replace the cytometer sample syringe

Replace sample syringe

Visually inspect the syringe pump compartment daily for leaks. Replace the sample syringe if you observe leaks from the syringe assembly, or if there is erratic or no fluid drawn up from the fluid bottles or the sample injection port. Evidence of leaks includes liquid or dried salt crystals in the syringe compartment area.

For periodic maintenance, replace the flow cytometer syringe every 3 months (for high volume instrument use, defined as >6 hours/day of operation) or every 6 months (for standard use).

1. Execute the **Shutdown** function with 10% bleach (page 50), which automatically lowers the plunger drive and powers off the cytometer.
2. Open the Syringe Pump door on the left side of the cytometer.



3. Open the syringe retention clasp and carefully remove the ball bearing while supporting the syringe piston.
4. After you have removed the ball, unscrew the syringe from the valve by rotating it counterclockwise.
5. To install a new syringe, insert the ball end of the syringe carefully into the capture mechanism.

6. Lift the capture mechanisms and syringe barrel, align the syringe with the syringe port of the valve, then rotate it clockwise until the syringe end cap seal hits the bottom of the valve.

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

> **IMPORTANT!** Failure to align the syringe properly when engaging the valve can lead to cross-threading. Do **not** use any tools for fastening and tightening the syringe to the valve. Over-tightening the syringe beyond the above recommendation can result in damage to the syringe and the valve.

8. Tighten the knurled thumbscrew to secure the ball end of the syringe within the syringe capture mechanism. Ensure that the ball is fully fastened when tightened.

> **Note:** Only hand-tighten this connection. Do **not** overtighten. Excessive tightening can adversely affect syringe performance.

9. Close the syringe pump door.
10. Prime the system using the following priming sequence: Run 3 startup cycles, 2 De-bubble cycles, and 2 Rinse procedures.

> **Note:** Proper syringe-to-valve seal is crucial for the operation of the cytometer, when fluids are cycling through the system. If a seal is not properly attained, cavitation can occur in the fluidics system.

> **IMPORTANT!** After replacing the syringe, all surfaces of the fluidics system, including the new syringe, must be wet. Failure to prime the system following syringe replacement will result in instrument issues.

Replace the SIP tube

Replace SIP tube

The SIP tube (Attune™ NxT Tubing DIBA 517-022; see page 104 for ordering information) has a click-and-seal fitting, which allows customers to replace damaged, bent, clogged, or leaking SIP tubes. Perform the following procedure to replace the SIP tube with a click-and-seal fitting.

1. Position the flow cytometer such that the SIP tube overhangs the edge of the counter.
2. Lower the tube lifter, then unscrew the fitting by turning it counterclockwise and remove the old SIP tube.
3. Insert the click-and-seal fitting over the sleeve around the new SIP tube.

4. Install the new SIP tube into the bottom port of the rotary valve and hand tighten until the fitting clicks.
5. Run the **SIP sanitize** procedure (page 47).
6. Prime the system using the following fluidics priming sequence: Run 3 startup cycles, 2 De-bubble cycles, and 2 Rinse procedures.
7. Load a test tube with 500 µL of Attune™ Focusing Fluid and run acquisition at 500 µL/minute.

   Ensure that the sample is drawn from the tube, that no liquid is leaking from the SIP, and that no bubble errors are issued in the software. Bubble error warnings after SIP tube replacement can indicate a loose SIP.

IMPORTANT! Failure to run a SIP sanitize procedure and full priming sequence after SIP replacement will result in instrument issues.

Autosampler maintenance

The Attune™ Auto Sampler and the CytKick™/CytKick™ Max Autosamplers are designed to require minimum maintenance. However, to ensure reliability of the autosampler, you must perform basic preventive maintenance procedures regularly, as listed in this guide.

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

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

Maintenance schedule

The following table lists the routine maintenance procedures that keep the autosampler in good working condition.

Procedure	Frequency
Shutdown	Daily
Visual inspection of sample injection port (SIP), fluid bottles and connections, and syringe pumps	Daily
Fluidics maintenance	Daily or as needed
Sanitize between uses	Daily or as needed
Fluidics decontamination	Monthly
Calibrate Attune™ Auto Sampler	Monthly (completed automatically when using Attune™ Cytometric Software version 2.5 or greater)
System decontamination	At least every 3 months (more frequently for high volume use)
Syringe replacement	3 months (high volume use at >6 hours/day) or 6 months (standard use)
Rinse	As needed
Sanitize SIP	As needed
Unclog	As needed
De-bubble	As needed
Deep Clean	As needed

Note: High volume use is defined as daily operation of the instrument for >6 hours/day.

Daily maintenance – Autosampler

Visual inspection

- Visually inspect the sample injection port, fluid bottles and connections, and the syringe pumps for any leakage. If you notice any leaks in the fluidics lines, contact your service representative. Decontaminate any spills by wiping the area with 10% bleach solution.
- Check fluid levels in the waste and Attune™ Focusing Fluid bottles. Empty waste and refill fluids if needed.
- Ensure that the fluid lines and fluid level sensor probe lines for all bottles are firmly connected. An audible click indicates full engagement.
- Inspect the floor of the fluid bottle compartment. Clean up any spilled liquid, remove any salt or dust deposits with a damp paper towel. If needed, decontaminate by wiping area with 10% bleach solution. Monitor the instrument as liquid in the bottle compartment may indicate a leak.
- Visually inspect the area behind the instrument to ensure that cords are plugged in and connections not stressed. Verify that at least 6 inches of free space behind the instrument is maintained and that all three exhaust ports are free of blockage. Ensure that the USB cable that connects the flow cytometer and the autosampler is firmly inserted into each instrument and is not stretched or strained unnecessarily.

Startup

- Execute the **Startup** function (page 46).
- During the Startup procedure, observe the movement of the sample syringe on the flow cytometer and the autosampler. The autosampler syringe is in the fluid bottle compartment, positioned on the left side of the compartment (page 91).
  - Listen for any sounds that are out of the ordinary, such as loud grinding sound or the absence of normal sounds during start up. If possible, record any unusual sound for support reference.
  - Observe the syringe for smooth movement on the up and down strokes. There should be no bubbles being delivered on the upstroke of the syringe and no drops should escape past the plunger. Any large air bubbles on the up or down strokes might indicate a large leak or a worn syringe.
  - If the autosampler syringe is not moving or appears to be sticking (non-fluid motion), we recommend that you replace the syringe before contacting Technical Support.

Fluidics maintenance

Fluidics maintenance includes the following user-performed functions. Perform each function daily or if needed.

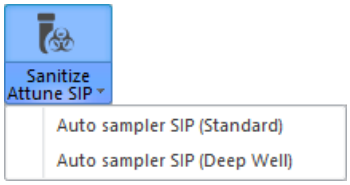
- **De-bubble** – Clears bubbles in the fluidics lines of the cytometer. For instructions, see page 49.
- **Unclog** – Removes clogs from the sample probe and flow cell (back-flush operation). For instructions, see page 48.
- **Sanitize SIP**– Quickly washes and sanitizes the SIP and sample lines between uses. It is especially important to perform the Sanitize SIP procedure when running sticky samples, DNA stains, or beads. The Sanitize SIP procedure requires user-supplied bleach or detergent. For instructions, see page 47.
- **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 46.
- **Deep Clean** – Sanitizes the system with bleach and Wash solutions for a user-selected time. The procedure ensures system cleanliness while allowing you to continue using the instrument after the cycle is complete. The Deep Clean procedure does not sanitize the fluid bottles. For instructions, see page 53.
- **Decontaminate System** – Sanitizes the system and fluid bottles with bleach and wash solutions for a proscribed time. The procedure ensures full system cleanliness at regular maintenance intervals to prevent build-up of contaminants in the system or fluid bottles. For instructions, see page 24.

Sanitize between uses

Sanitize the autosampler between uses to minimize cross-contamination. Note that the following procedures are for fast cleaning of the instrument to minimize cross-contamination. For a more thorough decontamination, perform the **System Decontamination** procedure (page 24).

- For non-sticky cells, such as B and non-activated T cells, run the **Sanitize SIP** procedure (page 47) 2 times (~3–4 minutes total).

  On the Instrument ribbon tab, click **Sanitize Attune SIP**, then select **Auto sampler SIP (Standard)** or **Auto sampler SIP (Deep Well)**.

  - First iteration: Use 3 mL of 10% bleach.
  - Second iteration: Use 3 mL of Attune™ Wash Solution.
- For sticky cells, such as DCs, RBCs, activated T cells, many cancer lines, bacteria, and yeast, run the **Quick Deep Clean** cycle (page 53) (~20–30 minutes total).

Daily Shutdown

Daily shutdown involves executing the **Shutdown** function (page 50). The Shutdown procedure 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.

Depending on the cycle selected, the Shutdown procedure takes at least 40 minutes to complete, but most steps are automated and do not require monitoring. At the end of the Shutdown procedure, the cytometer goes into a "sleep state". For instructions on how to perform the Shutdown function, see page 50.

Decontaminate system

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

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

Decontaminate System function

The *Decontaminate System* function is used for the automated decontamination of the flow cytometer and the autosampler 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 instead 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 a decontamination run before becoming idle
- The **Decontaminate System** button on the Instrument tab of the Ribbon bar opens the *Decontaminate* dialog, which provides instructions to perform the Decontaminate System function.

- The steps in the Decontaminate System function vary depending on whether an autosampler is connected. See page 15 for the decontamination procedure for the flow cytometer without the autosampler.
- The Decontaminate System function for the system is broken into three phases. It can take up to 45 minutes to complete the procedure; however, most of the operation is performed automatically.
- Each step of the Decontaminate System procedure is displayed at the top of the dialog box and the procedure step is highlighted.
- The Decontaminate System function is only available to Administrator accounts.

Prepare for system decontamination

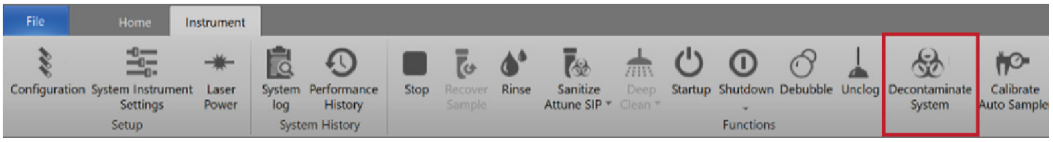
1. Rinse out all fluid containers with deionized water.
2. Ensure that all fluid lines and probe cables are connected.

Note: For the location of the fluidics compartment and instructions on filling the fluid bottles, see the *Attune™ NxT Flow Cytometer User Guide* (Pub. No. 100024235) or the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440), which are available for download at **thermofisher.com/attune**.

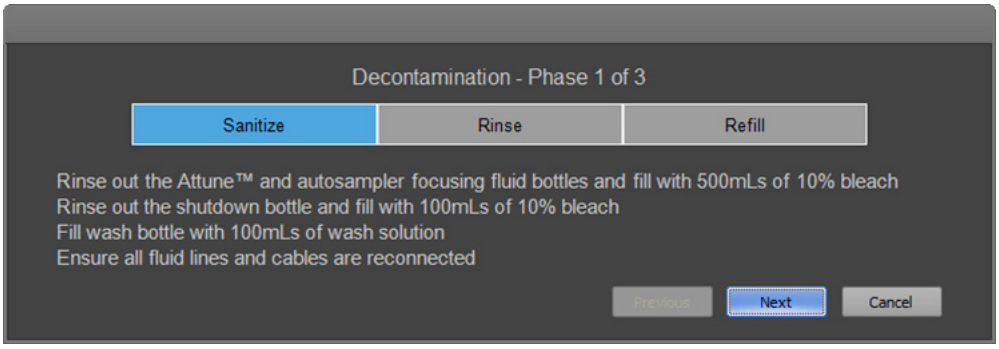
Run Decontaminate System function (flow cytometer and autosampler)

Follow all the instructions that are 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.

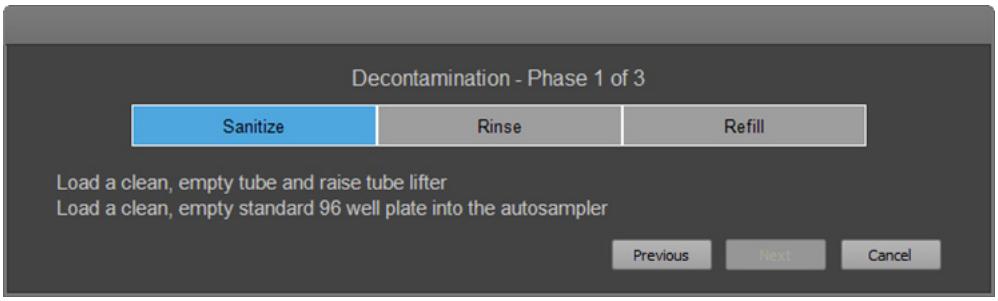
1. Click the **Decontaminate System** button on the Instrument tab of the Ribbon bar and follow the prompts in the *Decontamination dialog*.



2. Click **Next** to start Decontamination Phase 1. When prompted:

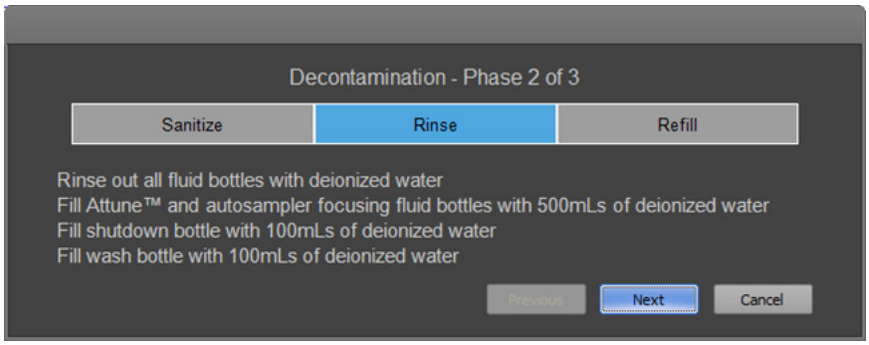


   a. Rinse all fluid bottles with deionized water.
   b. Fill the flow cytometer and autosampler focusing fluid bottles with 500 mL of 10% bleach.
   c. Rinse the shutdown fluid bottle with 100 mL of 10% bleach.
   d. Fill the wash bottle with 100 mL of Attune™ wash solution (Cat. No. A24974).
   e. Reconnect all fluid lines and bottle cables, then click **Next**.



   f. Load a clean, empty tube on the SIP of the flow cytometer, then raise the tube lifter.
   g. Load a clean, empty standard 96-well plate into the autosampler.

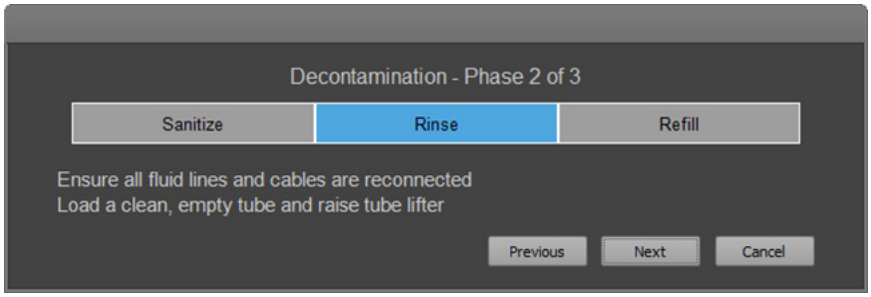
3. Click **Next** to start Decontamination Phase 2. When prompted:



   a. Rinse all fluid bottles with deionized water.

   b. Fill the flow cytometer and autosampler focusing fluid bottles with 500 mL of deionized water.

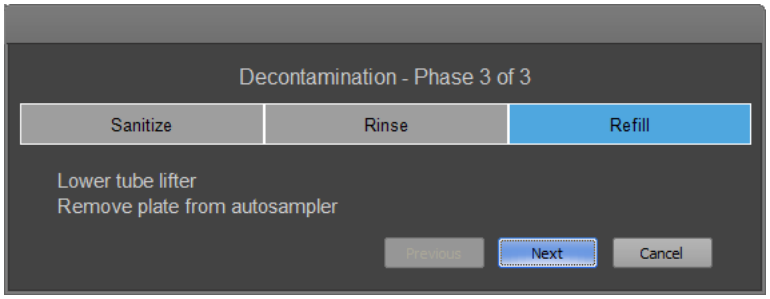
   c. Fill the shutdown and wash bottles with 100 mL of deionized water, then click **Next**.



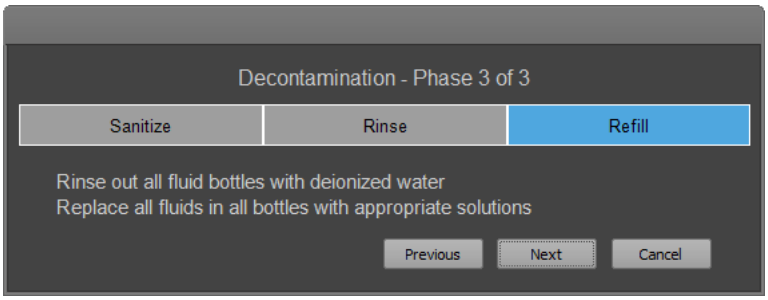
   d. Reconnect all fluid lines and bottle cables.

   e. Load a clean, empty tube on the SIP of the flow cytometer, then raise the tube lifter.

4. Click **Next** to start Decontamination Phase 3. When prompted:

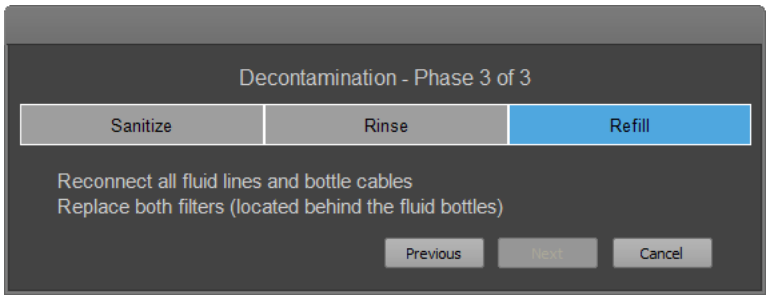


   a. Lower the tube lifter and remove the plate from the autosampler, then click **Next**.



b. Rinse all fluid bottles with deionized water.

c. Replace all fluids in all fluid bottles with the appropriate solutions, then click **Next**.



d. Reconnect all fluid lines and bottle cables.

e. Replace the focusing fluid filters with new filters (page 18).

IMPORTANT! After replacing the focusing fluid filters, run 3 Startup, 2 De-bubble, and 2 Rinse procedures to saturate the new filters with Attune™ Focusing Fluid and purge air from system.

Replace the Attune™ Auto Sampler syringe

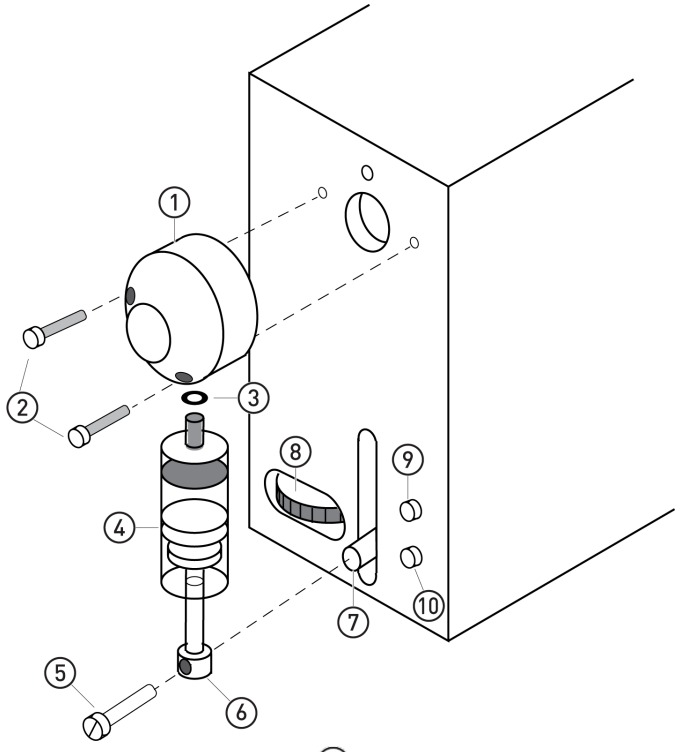
Visually inspect the syringe pump of the autosampler daily for leaks. Replace the syringe if you observe leaks from the syringe assembly, or if there is erratic or no fluid drawn up from the fluid bottles or the sample injection port.

For periodic maintenance, replace the autosampler syringe every 3 months (for high volume instrument use, defined as >6 hours/day of operation) or every 6 months (for standard use).

Attune™ Auto Sampler syringe replacement requires two steps to complete: installation (page 30) and calibration (page 32). For the names and locations of the parts that make up the syringe pump assembly, see the following schematic.

A flat blade screwdriver or a coin that fits in the slot on the syringe screw is required during calibration. For older Attune™ Auto Sampler systems that have a locking screw on the syringe threads, a 0.050-inch Allen (or hex key) wrench is required.

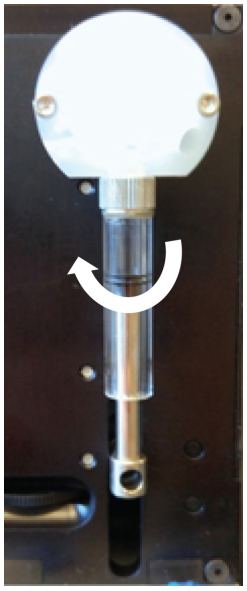
Attune™ Auto Sampler syringe pump assembly



1. Valve
2. Valve screws
3. Teflon washer
4. Syringe
5. Syringe screw
6. Plunger button
7. Pump actuator (drive pin)
8. Thumbwheel
9. Set Home button
10. Initialize button

Replace the syringe

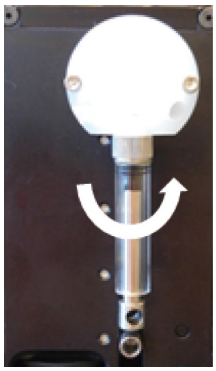
1. Power off the autosampler and the flow cytometer.
2. Open the fluidics compartment door of the auto sampler. Remove the focusing fluid and waste bottle to allow access to the syringe pump assembly.
3. If the syringe plunger is not in the down position, turn the thumbwheel (8, as shown in the schematic on page 29) until the plunger is down approximately 1 inch (25 mm) from the top of stroke.
4. Examine the front of the valve (1, as shown in the schematic). If there is a small brass-colored screw in the valve that is located just above the syringe, it must be loosened before the syringe is removed.
5. Remove the syringe screw (5, as shown in the schematic) and set it aside. The syringe screw is used to connect the plunger to the pump actuator (that is, the drive pin) after the new syringe is installed.
6. Turn the syringe body (4, as shown in the schematic) in a counterclockwise direction to loosen it, then remove it from the valve body.



   a. In older systems, you may see a small white washer come out as the syringe is removed from the valve body. If the replacement syringe includes a washer, then discard old washer. If the new syringe does not contain a washer in the kit, then retain the existing washer and install it with the new syringe.
   b. For newer systems, the washer is built into the valve. If no washer comes out with the syringe, install the new syringe without the washer.
7. Install the new syringe. If a washer came out of the valve assembly during syringe removal, replace the washer. If no washer came out of the valve assembly, proceed with installation without the washer.

> **Note:** If required for installation, place the washer to the tip of the syringe as you screw the new syringe assembly into place.

8. Turn the syringe body clockwise until it is fastened in its place.



Note: Do not use pliers or other tools to grip the syringe body to tighten it. Over tightening will damage the syringe, threads, or the syringe seat, which require valve replacement to repair.

9. Pull the plunger button (6, as shown in the schematic on page 29) down until the hole on the plunger lines up with the hole on the pump actuator (7, as shown in the schematic).

10. Re-install the syringe screw (5, as shown in the schematic) to connect the plunger to the pump actuator and tighten. Do not over tighten the syringe screw; it must only be finger tightened.

11. Power on the autosampler first, then power on the flow cytometer. When the system is powered on, proceed to syringe calibration (page 32).

Calibrate the syringe

After the installation of a new syringe, valve, or syringe washer, you must calibrate the syringe zero position before using the Attune™ Auto Sampler. The autosampler must be powered on before proceeding with syringe calibration. The following syringe calibration procedure applies only to the Attune™ Auto Sampler.

1. With the syringe and the valve installed to the pump assembly, press the **Initialize** button (10, as shown in the schematic on page 29), which is the lower of the two front panel buttons on the autosampler (page 29).

   The syringe moves to a position a small distance below the top-of-stroke. This position is internally fixed and is called the soft limit.

2. When the initialize move completes, rotate the thumbwheel at the lower left corner to move the syringe piston upward until it barely contacts the top of the syringe. This is the zero position (that is, home position).

3. Press **Set home** button (9, as shown in the schematic), which is the upper of the two front panel buttons on the autosampler (page 29).

   The syringe first moves downward to the initialize position, then return to the zero position. When this step is completed, the location of the zero position is stored in the pump memory.

Note: If you observe any leakage from the threads of the syringe assembly, tighten syringe barrel. If leakage continues, the plastic washer is missing from the tip of the syringe. Locate the missing washer and re-install the syringe with the washer.

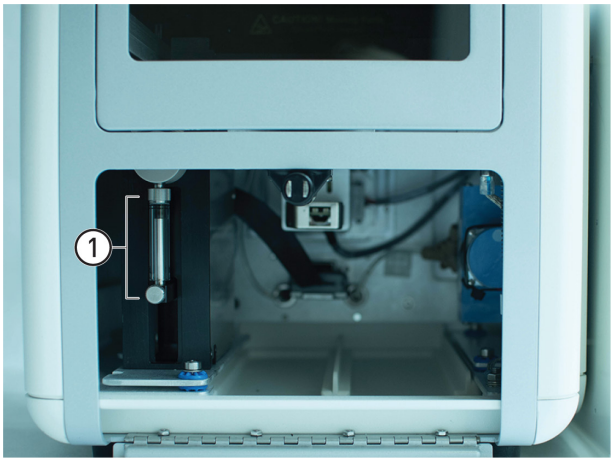
Replace the CytKick™/CytKick™ Max Autosampler syringe

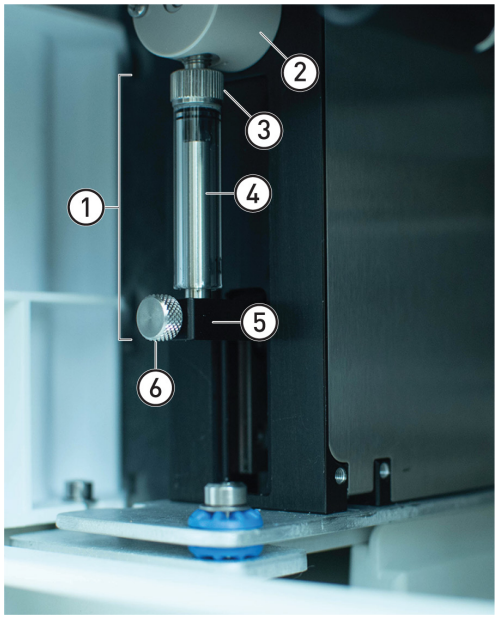
Visually inspect the syringe pump of the CytKick™/CytKick™ Max Autosampler daily for leaks. Replace the syringe if you observe leaks from the syringe assembly, or if there is erratic or no fluid drawn up from the fluid bottles or the sample injection port.

For periodic maintenance, replace the CytKick™/CytKick™ Max Autosampler syringe every 3 months (for high volume instrument use, defined as >6 hours/day of operation) or every 6 months (for standard use).

CytKick™/CytKick™ Max Autosampler syringe pump assembly

The CytKick™/CytKick™ Max Autosampler syringe pump and syringe assembly are housed in the syringe pump compartment. For the names and locations of the parts that make up the syringe pump assembly, see the following image. The replacement syringe (Cat. No. 100054593) is available separately from Thermo Fisher Scientific (page 105).





① Syringe assembly
② Valve
③ Top syringe fitting
④ Plunger (inside the syringe assembly)
⑤ Syringe holder (connects the plunger and drive pin)
⑥ Bottom thumb screw)

Replace the syringe

1. Power off the autosampler and the flow cytometer.
2. Open the syringe pump compartment door of the autosampler. The syringe pump assembly is on the left side of the compartment (1, in the image on page 33).
3. Loosen the top syringe fitting (3, in the image on page 33) to break the seal on the syringe assembly.
4. If the syringe plunger is not in the down position, move the plunger down by hand until it is approximately 1 inch (25 mm) from the top of stroke.
5. Remove the bottom thumb screw (6, in the image on page 33) and set it aside. The syringe thumb screw is used to connect the plunger to the pump actuator (that is, the drive pin) after the new syringe is installed.
6. Completely loosen the top fitting, then remove the syringe from the valve body and the pump assembly.
7. Place the bottom of the syringe in the syringe holder (5, in the image on page 33), then install the thumb screw to secure the syringe in place.

   Note: Do not over-tighten the syringe screw; it must only be finger tightened.

8. Move the plunger until the top of the syringe touches the valve, then finger-tighten the top screw to secure the syringe assembly.

   Note: Do not use pliers or other tools to grip the syringe body to tighten it. Over tightening will damage the syringe, threads, or the syringe seat, which require valve replacement to repair.

9. Power on the autosampler first, then power on the flow cytometer.
10. Run the **Startup** function (page 46) to prime the system fluidics.

Note: You do not need to calibrate the CytKick™ and CytKick™ Max Autosampler syringe after replacing it.

Calibrate the autosampler

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 calibration takes approximately 1 minute to complete. The following autosampler calibration procedure applies to both the Attune™ Auto Sampler and the CytKick™/CytKick™ Max Autosamplers.

Note: The autosampler is pre-calibrated before the unit is shipped and the instrument auto recalibrates during Startup every 30 days. The Auto Sampler Calibration function is only needed for troubleshooting and if the autosampler 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 autosampler, remove the plate.
3. Click **Next** to run the Auto Sampler Calibration function and follow the instructions that are provided by the Calibrate Auto Sampler dialog.

Prepare the autosampler for shipment

Perform the following steps to decontaminate a defective autosampler and prepare it for shipment to the Thermo Fisher Scientific Repair Center.

Note that the decontamination procedure used preparing the autosampler for shipment is different from the System Decontamination procedure that is described on page 24.

For instructions to install a replacement autosampler, see the *Attune™ Auto Sampler User Guide*, available for download at **thermofisher.com**.

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

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

Decontaminate the Attune™ Auto Sampler

1. Remove the sample plate, if present, and run the **Decontaminate System** function as described on page 24.
2. Power off the autosampler.
3. Disconnect the power supply cord and the USB cable from the autosampler.
4. Disconnect and remove the bottles from the autosampler.
5. Disconnect the fluidics lines from the two ports on the flow cytometer.
6. Apply a freshly made solution of 10% bleach to accessible external surfaces of the autosampler. A spray dispenser can 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 arrives without a power supply cord, USB cable, or bottles.

Note: After you unpack and install the replacement autosampler, you will use that instrument's packaging materials to return the defective unit.

Pack and ship the autosampler

1. Connect the two external fluidics lines with the union fitting, and bag and tape them to the left side of the autosampler.
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 autosampler in the plastic bag supplied with the replacement unit, then 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 autosampler to match the cutout in the foam, then place the instrument in the inner box.
7. Orient the top foam to match the autosampler 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.
    a. Tape one copy to the outer box.
    b. Fax the second copy to (760) 930-2300.
10. Complete and print the FedEx shipment form, 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 that the package handlers are not handling harmful substances.

Attune™ External Fluid Supply (EFS) maintenance

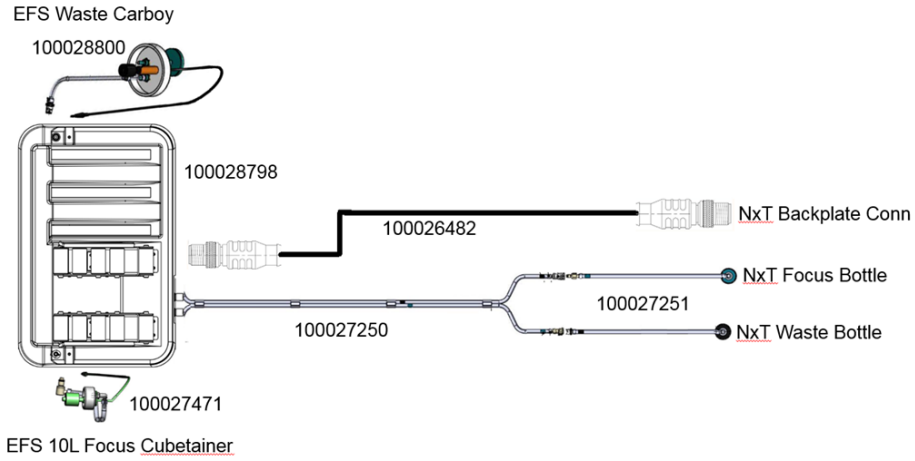
The Attune™ External Fluid Supply (EFS) is designed to require minimum maintenance. However, to ensure reliability of the EFS, you must perform basic preventive maintenance procedures regularly, as listed in this user guide.

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

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

Attune™ NxT EFS fluidics and electrical connections

The following image shows the Attune™ NxT EFS fluidics and electrical connections and part numbers.



General care

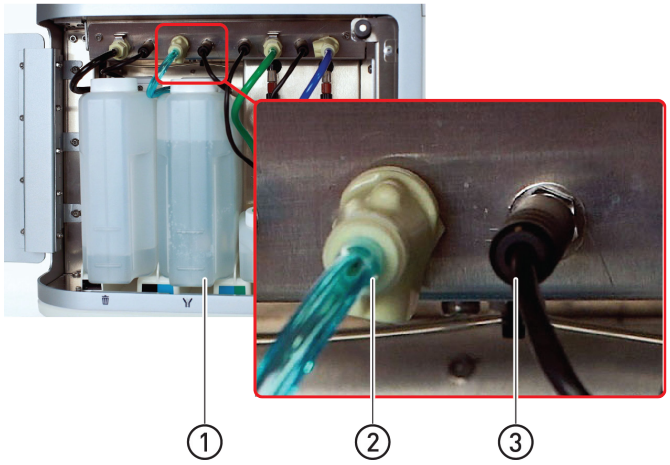
- Each time you replace the focusing fluid cubetainer, inspect the fluid lines for kinks or obstructions.
- Make sure that the electrical connections are fully engaged. The system will not function properly if any of the level sensor plugs are not fully seated.
- Wipe up any spills that might occur during installation and when replacing the fluidics containers.
- Perform the cleaning procedure once a month or if putting the device into storage.

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

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

Clean the focusing fluid lines

1. Power off the flow cytometer.
2. Unplug the 1.8 L Attune™ focusing fluid bottle (teal) and empty.
3. Place the Attune™ focusing fluid bottle back into the fluidics compartment of the cytometer and plug the fluid line and the level sensor cable.



① Focusing fluid bottle
② Fluid line
③ Sensor cable

4. Place the Attune™ focusing fluid bottle insert (i.e., the modified lid with the focusing fluid supply tube from the EFS) into a 2 L bottle or beaker to capture the bleach solution to avoid pumping bleach solution into the 1.8 L Attune™ focusing fluid bottle. Secure the tube so that it does not fall out when fluid transfer starts.
5. Pour 2 L of 10% bleach solution into an empty cubetainer.
6. Attach the cubetainer with the bleach solution to Attune™ NxT EFS. Ensure that both electrical and fluidics connections are securely fastened.
7. Power on the flow cytometer. The system will automatically sense the empty 1.8 L Attune™ focusing fluid bottle, which will trigger the EFS to start fluid transfer. The EFS lines will fill with the bleach solution, pumping the fluid through the focusing fluid bottle insert into the collection beaker.
8. When the fluid transfer stops, turn off power to the flow cytometer and let the system rest for 20 minutes to allow complete decontamination.
9. At the end of the decontamination wait time, empty collection beaker.
10. To repeat the decontamination process, power on the flow cytometer to allow more bleach solution to be transferred from the cubetainer to the beaker.
11. To rinse, repeat steps 1–7 with E-pure water instead of 10% bleach solution. 20 minute wait time is not required for the rinse procedure.
12. After rinse, purge the system with air as described in “Purge the focusing fluid lines with air” (page 40).

Note: Air purge is required to prevent E-pure water from diluting the system fluids. Running the flow cytometer with E-pure water will cause invalid experiment results.

Purge the focusing fluid lines with air

1. Empty the Attune™ focusing fluid bottle and re-install it in the fluidics compartment.
2. Unplug and remove the focusing fluid cubetainer. Disconnect and remove the cubetainer umbilical from cubetainer.
3. Plug the fluidic and level sensor umbilical back into the Attune™ NxT EFS.
4. Hold the umbilical assembly with the float sensor down to activate the Attune™ NxT EFS. Allow the Attune™ focusing fluid bottle to fill as the lines on the EFS are pumped dry.

   Note: To prevent contamination of the Attune™ focusing fluid bottle, you can place the Attune™ focusing fluid bottle insert into a separate collection beaker.
5. If needed, repeat the procedure to empty all fluidics lines on the Attune™ NxT EFS.

Clean the waste lines

1. Empty the waste containers of both the Attune™ NxT EFS and the cytometer.
2. Fill the Attune™ NxT cytometer waste bottle to the brim with 10% bleach solution.
3. Reconnect the Attune™ NxT EFS waste container.
4. Reconnect the cytometer waste bottle.
5. Attune™ NxT EFS will automatically activate and transfer the bleach solution to the EFS waste container.
6. Refill the cytometer waste bottle with 10% bleach solution and re-connect to the instrument. The Attune™ NxT EFS will transfer the solution to the EFS waste container.
7. Allow the bleach solution to remain in the waste container for 20 minutes to decontaminate the container and the fluidics lines.
8. Remove the EFS waste container and dispose of the bleach solution.
9. Fill the cytometer waste bottle with E-pure water and repeat steps 3–8 to rinse the bleach solution from the fluidics system.
10. If the system lines need to be purged of fluid and dried (recommended for long term storage), see "Purge the waste lines with air" below.

    If the system is going to be used after cleaning, empty the EFS waste container and verify that all fluidics lines are connected properly.

Note: This process only decontaminates the waste fluidics system and the flow cytometer waste bottle. The 20-L EFS waste bottle requires additional cleaning to ensure that the entire system has been decontaminated.

Purge the waste lines with air

1. Empty the flow cytometer waste bottle until only droplets of water remain.
2. Unplug the flow cytometer waste fluid connection but keep the sensor connection plugged in.
3. Connect the Attune™ EFS waste container to the EFS.
4. Hold the empty flow cytometer waste bottle upside-down; this will trigger the EFS waste pump to activate. After the pump activates, you can place the cytometer waste bottle back in the fluidics compartment of the cytometer.
5. Allow the fluid lines to empty. If there is any fluid left in the lines, invert the cytometer waste bottle to activate the pump again.
6. When the pump stops (noise from the EFS stops), disconnect and drain the EFS waste container. You can also clean the waste container at this step.

Reset the safety interlock

The EFS is controlled by the Attune™ firmware, and there are several built-in safeguards to minimize the possibility of overflow. These safeguards are triggered and timeouts are activated when the focusing fluid or the waste bottle is in danger of overflowing.

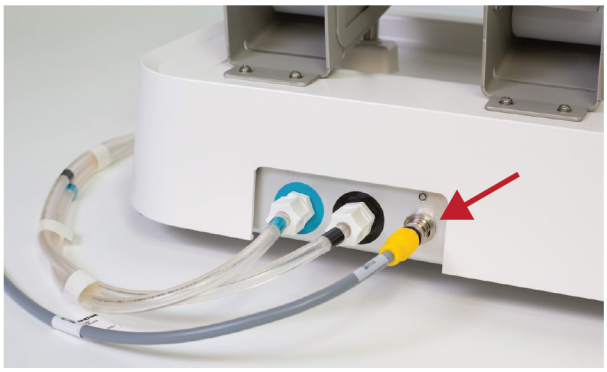
The interlock protection is also triggered if any of the fluid bottles is unplugged while the fluid transfer process is in progress. Unplugging the focusing fluid bottle in the cytometer or the EFS stops the focusing fluid transfer. Similarly, unplugging the waste bottle stops the waste transfer.

When the interlock protection is triggered, the EFS will have to be reset to resume normal operation. Note that there will be no indication that an interlock has been triggered.

1. Ensure that all fluid bottles in the flow cytometer and the EFS are fully connected (electrical and fluidic connections).
2. Power off the flow cytometer, then power it back on.

> **Note:** Before you power on the flow cytometer to check EFS function, double-check all electrical (3.5-mm jacks) bottle connections to ensure that they are fully seated. If any of the bottle electrical connectors are not fully seated, the fluidics pumps will not activate (safety interlock).

3. Unscrew the EFS 12-pin power/control cable from the flow cytometer or from the back of the EFS (indicated by red arrows), then reattach it.



Relieve back pressure in fluid lines and pumps

If there is blockage in fluid lines that increases the back pressure in fluid lines and pumps, the interlock protection is triggered to stop the fluid transfer. When the interlock protection is triggered due to increased backpressure, the backpressure must be relieved and the EFS reset to resume normal operation. Note that there will be no indication that an interlock has been triggered.

1. Leave the flow cytometer and the EFS fluid lines attached to their respective focusing fluid and waste bottles.
2. Unscrew the lids of the bottles in the flow cytometer so that they are completely loose, but leave the lines inserted into their respective bottles.
3. Unscrew the rear EFS focusing fluid and waste lines. Leave the lines disconnected for at least 5 minutes to relieve backpressure that might have built up in the lines and pumps. Wipe up any spills with a towel, then re-attach the tubing.
4. Empty the flow cytometer focusing fluid bottle (it will cause the flow cytometer focus LED to flash) into an empty container to trigger the EFS fill command. When EFS fill is triggered, the pump pushes focusing fluid into the focusing fluid inlet line. If the pump does not activate, repeat step 3 waiting 10 minutes instead.
5. Fill the flow cytometer waste bottle (it will cause the flow cytometer waste LED to flash) with water to trigger the EFS empty command. When EFS empty is triggered, the pump pushes fluid into the waste inlet line. If the pump does not activate, repeat step 3 waiting 10 minutes instead.

Attune™ NxT EFS top focusing fluid (left) and rear waste (right) inlets

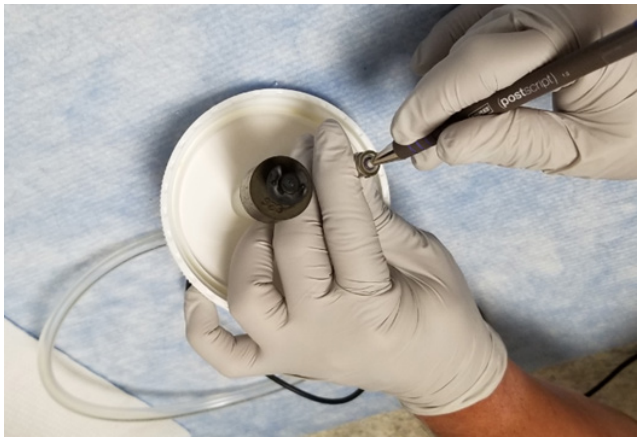
6. After you have confirmed that the EFS pumps are functional, refill the flow cytometer focusing fluid bottle and empty the waste bottle, then replace their lids.

Clean check valves and dip tubes

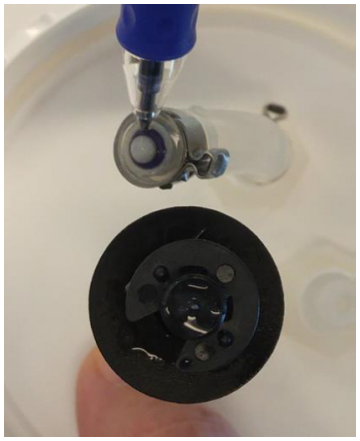
Check valves on the Attune™ NxT EFS lids and dip tubes may become sticky if left in storage for extended periods, which can prevent fluid transferring to and from the cytometer. We also recommend that you inspect and clean the dip tubes with 10% bleach solution if you find them to have biomaterial on them.

1. Locate the check valves in the Attune™ NxT EFS focusing fluid bottle lid and dip tube.

2. Using gloves and a small tip screwdriver, activate the check valve by levering the internal poppet a small amount. Do not overwork or push too hard to prevent damage to the check valve. This step is to simply move the poppet a small amount and clear any residue that has built up over time.



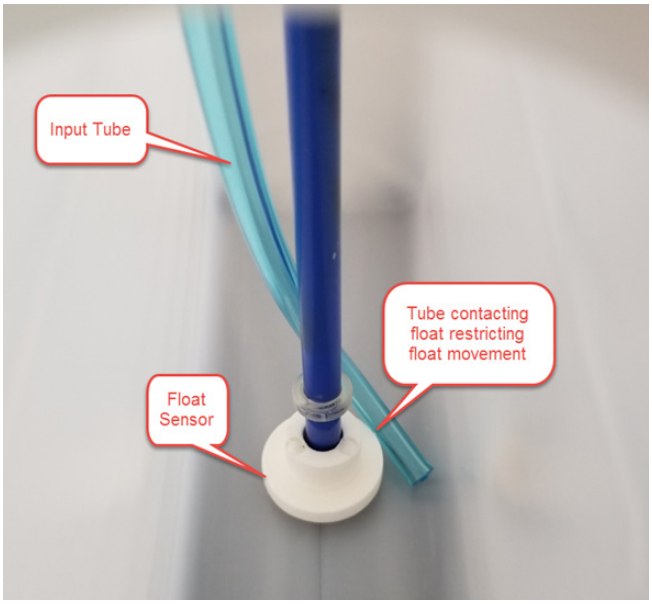
3. Clean the check valve with 10% bleach, then rinse thoroughly with E-pure water.
4. Repeat the process for the check valve in the Attune™ NxT EFS waste connection.



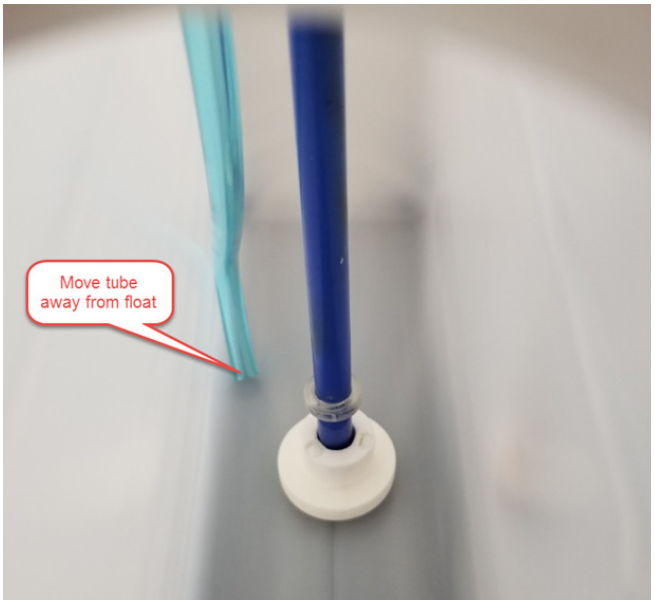
Free the float sensor from the input tube

When the EFS refills the focusing fluid bottle in the flow cytometer, the float sensor sends a "full" signal when it rises to the top of the bottle. However, if the float sensor is stuck at the bottom of the focusing fluid bottle sending a false "bottle empty" signal to the EFS, the focusing fluid bottle can potentially overflow.

1. To prevent the focusing fluid bottle from overflowing, relocate the tube inside the focusing fluid bottle away from the float sensor to the rear of the focusing fluid bottle. You can use a tool like a long screwdriver to push the tube back away from the float.



Float stuck at the bottom of the bottle



Float free from obsruction

Instrument functions

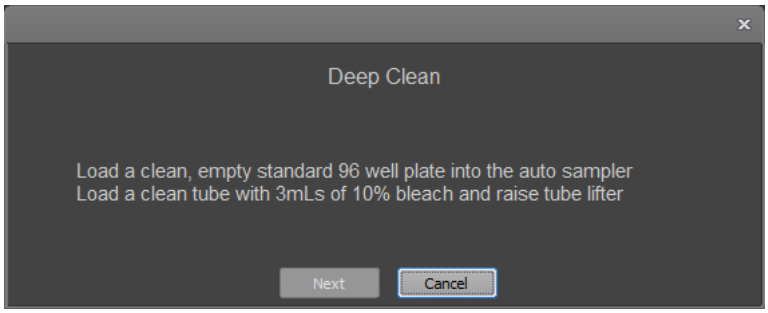
The following instrument functions are used during maintenance and troubleshooting of the flow cytometer and the autosampler:

- Startup (page 46)
- Rinse (page 46)
- Sanitize SIP (page 47)
- Unclog (page 48)
- De-bubble (page 49)
- Shutdown (page 50)
- Deep Clean (page 53)

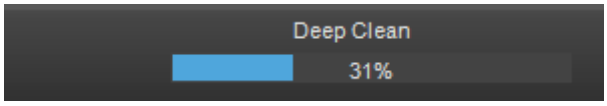
The Attune™ Cytometric Software provides instructions to perform each function. Follow all the instructions that are provided by the software during the procedure.

Monitoring progress

- Instrument Function dialogs appear when the instrument requires user interaction to perform an instrument function.



- After an instrument function is initiated, the progress of the instrument function is displayed in the status bar.



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

> **Note:** If you click **Cancel** before the instrument function is completed, the function may need to be re-initiated.
>
> If the Shutdown function is cancelled, Startup must be completed before you can re-initiate the Shutdown procedure.

- If necessary, you can stop the instrument function by clicking the **Stop** button in the Instrument ribbon tab.
- If any system errors are encountered during an instrument function, the status bar displays the *Alarm* icon and the relevant indicator icons that describe the nature of the error.

- The following image shows the fixed positions of Instrument Status and Alerts indicator icons on the status bar. If an indicator icon is not displayed, then a gap is left in its position. For more information, see the *Attune™ Cytometric Software User Guide*.

Startup

During *Startup*, the flow cytometer performs the following functions:

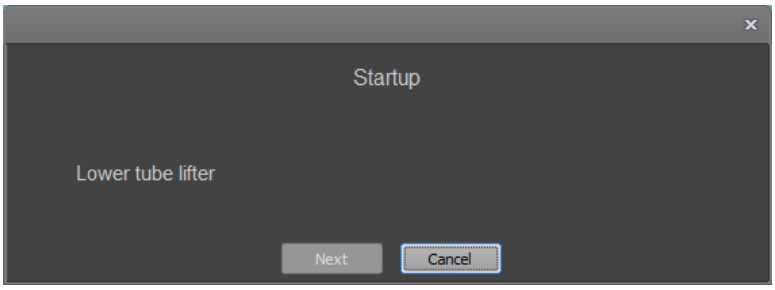
- Warms the lasers to operating temperature
- Initializes the pumps
- Primes the instrument fluidics
- Notifies you of the System Status (Ready, Attention, Clog, and so on)

Perform Startup

The Attune™ Cytometric Software guides you through the Startup function. Follow all the instructions that are provided by the software during the procedure. For more information, see the *Attune™ Cytometric Software User Guide* (Pub. No. 100024236), which is available for download at **thermofisher.com/attune**.

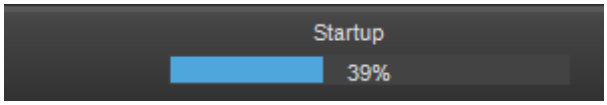
1. To start the Startup function, click the **Startup** button on the Instrument ribbon tab or the Collection panel. 

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



2. If the tube lifter is raised, lower the tube lifter.
3. If your system includes the optional autosampler and a plate is loaded in the autosampler, remove the plate.
4. Click **Next** to proceed with the Startup procedure.

   During Startup, the status window displays the progress of the Startup operation.



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 and the relevant indicator icons describing the nature of the error.

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.

Rinse

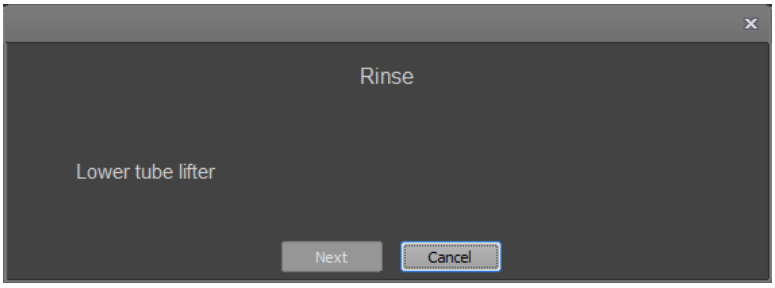
Perform Rinse

The *Rinse* function rinses the sample lines.

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

   The *Rinse dialog* box appears (if any user action is required, such as lowering the tube lifter) and provides instructions to perform the Rinse procedure.



2. If the tube lifter is raised, lower the tube lifter.
3. Click **Next** to start the Rinse procedure.

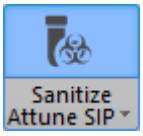
Sanitize SIP

The *Sanitize SIP* function is a user-performed function that washes and sanitizes the Instrument Sample Injection Port (SIP) and sample lines or the Auto Sampler SIP and sample lines. You can run the SIP sanitize function after an experiment or after a certain number of samples (as needed).

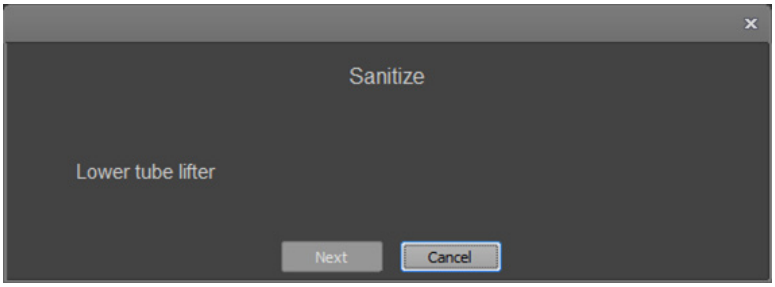
IMPORTANT! It is especially important to perform the Sanitize SIP function between experiments when running sticky samples, DNA stains, or beads.

Perform Sanitize SIP for the flow cytometer

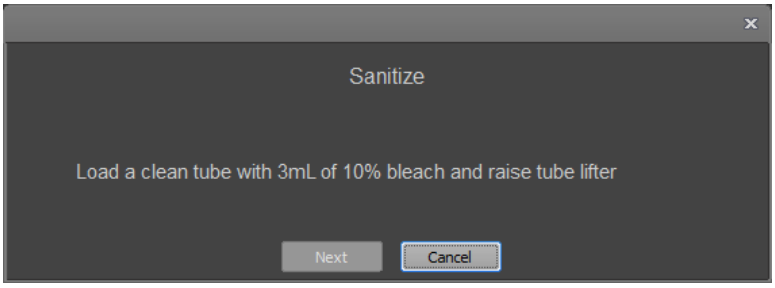
1. On the Instrument ribbon, click **Sanitize**.

   The *Sanitize dialog* box appears and provides instructions to perform the Sanitize Attune™ SIP procedure.



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

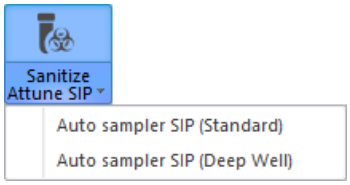


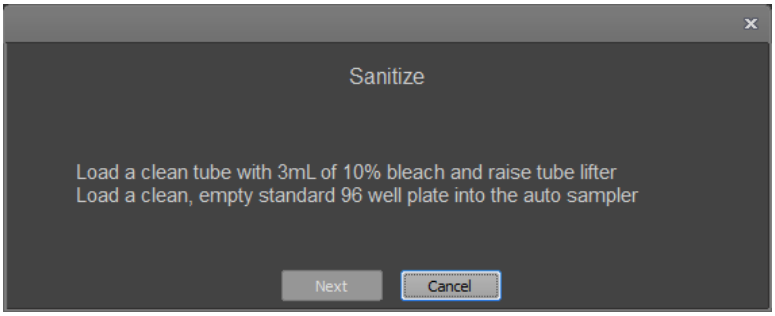
3. Fill a clean tube with 3 mL of 10% bleach, load the tube into the instrument, then raise the tube lifter.
4. Click **Next** to start the Sanitize SIP procedure.

Perform Sanitize SIP for the autosampler

1. On the Instrument ribbon, click **Sanitize**.
2. From the dropdown list, select **Auto sampler SIP (Standard)** or **Auto sampler SIP (Deep Well)**, depending on the type of plate you are using.

   The *Sanitize dialog* box appears and provides instructions to perform the Sanitize Auto Sampler SIP procedure.





3. Fill a clean tube with 3 mL of 10% bleach, load the tube into the instrument, then raise the tube lifter.
4. Load a clean, empty standard 96-well plate into the Auto Sampler.
3. Click **Next** to start the Sanitize SIP procedure.

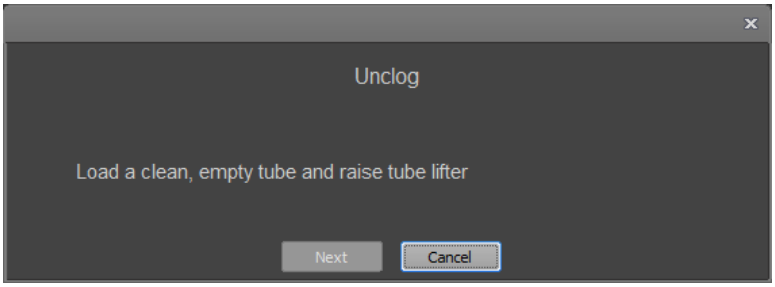
Unclog

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

Perform Unclog

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 start 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

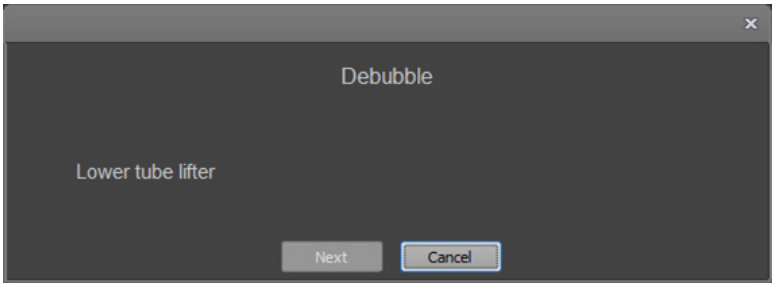
The *De-bubble* function is a user-started function to clear bubbles in the fluidics lines of the cytometer and flow cell.

Perform De-bubble

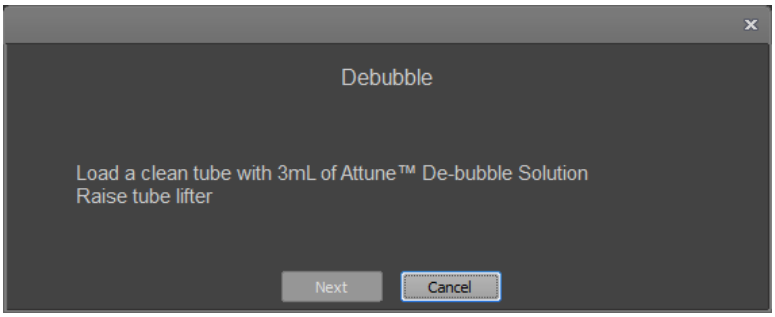
1. On the Instrument ribbon, click **De-bubble**.

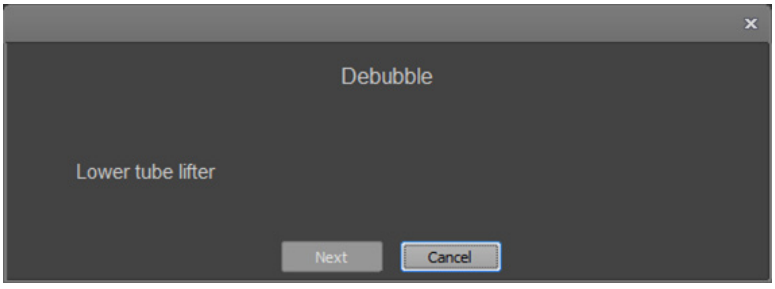
   The *De-bubble dialog* box appears and provides instructions to perform the De-bubble procedure.



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 3 mL of Attune™ De-bubble Solution.



5. Load the tube into the instrument, then raise the tube lifter.
6. Click **Next** to start the De-bubble procedure.
7. When the procedure is complete, lower the tube lifter.



8. Click **Next** to close the dialog box and automatically start a Rinse procedure.

Shutdown

The *Shutdown* function sanitizes and shuts down the instrument. The Shutdown function varies depending on whether an autosampler 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), then washes all lines again with water before running Attune™ Shutdown Solution through all lines and the SIP.

Depending on the Shutdown option selected, the procedure takes 25 to 70 minutes to complete; however, most steps are automated and do not require monitoring. At the end of the Shutdown procedure, the cytometer is automatically powered down.

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

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

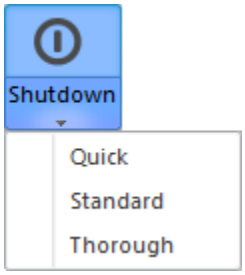
Check fluid and waste levels

1. Check the levels in the fluid tanks (see page 77 for the location of the fluidics compartment).
2. Ensure that the Wash and Shutdown solution tanks are at least half-full.

Shutdown options

There are three options available for the Shutdown function:

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



For daily use, we recommend the Standard Shutdown function.

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.

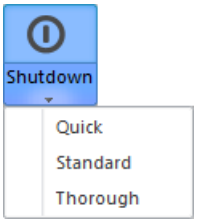
Perform Shutdown (flow cytometer only)

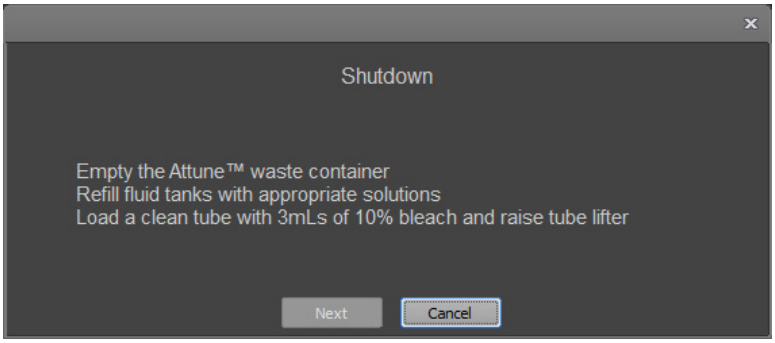
1. On the Instrument ribbon, click **Shutdown**.

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

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

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



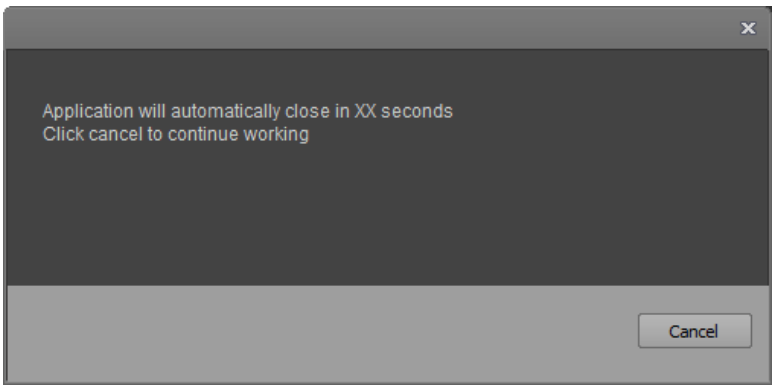


3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to automatically perform a Rinse procedure.

Note: Steps 3 and 4 are only required if the tube lifter is raised at the start of the Shutdown procedure.

5. When the rinse is complete, empty the instrument and autosampler waste containers.
6. Refill the fluid bottles with the appropriate solutions.
7. Fill a clean tube with 3 mL of 10% bleach.
8. Load the tube into the instrument, then raise the tube lifter.
9. Click **Next** to start the Shutdown procedure.
10. When the Shutdown procedure is complete, the system will log you out after 60 seconds, shutdown the computer, and power off the system.

    To remain signed in to perform non-instrument functions, click **Cancel**.



IMPORTANT! If you intend to leave the flow cytometer in the shutdown state for longer than two weeks, perform system flush and leave the instrument in deionized water to prevent salt crystals from clogging the fluidics system.

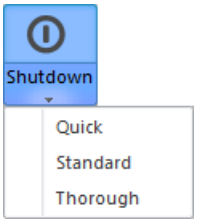
Perform Shutdown (flow cytometer and autosampler)

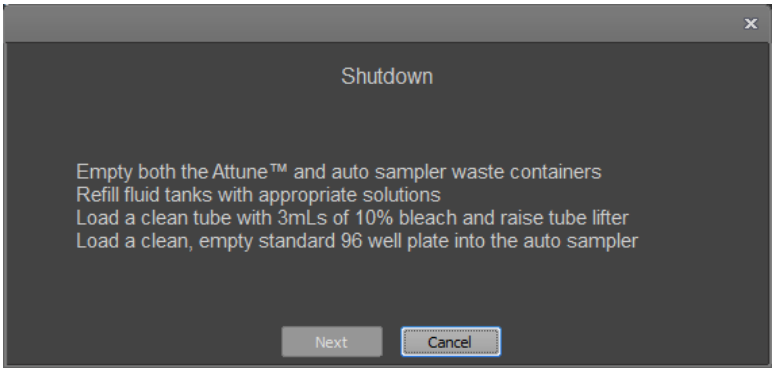
1. On the Instrument ribbon, click **Shutdown**.

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

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

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



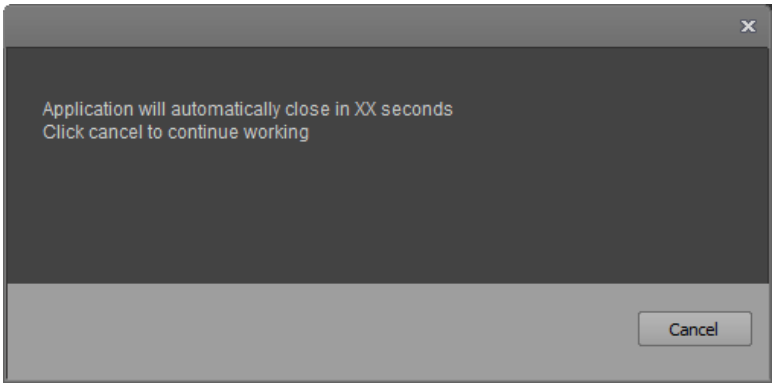


3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to automatically perform a Rinse procedure.

Note: Steps 3 and 4 are only required if the tube lifter is raised at the start of the Shutdown procedure.

5. When the rinse is complete, empty the instrument and autosampler waste containers.
6. Refill the fluid 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 autosampler.
9. Click **Next** to start the Shutdown procedure.
10. When the Shutdown procedure is complete, the system will log you out after 60 seconds, shutdown the computer, and power off the system.

    To remain signed in to perform non-instrument functions, click **Cancel**.



IMPORTANT! If you intend to leave the flow cytometer in the shutdown state for longer than two weeks, perform system flush and leave the instrument in deionized water to prevent salt crystals from clogging the fluidics system.

Deep Clean

The *Deep Clean* function thoroughly washes the sample lines and flow cell of the flow cytometer. The Deep Clean procedure varies depending on whether an autosampler is connected to the cytometer.

Deep Clean options

There are three options available for the Deep Clean procedure:

- **Quick** –Quick option uses 5 wash cycles and takes about 20 minutes to complete.
- **Standard** –Standard option uses 15 wash cycles and takes about 45 minutes to complete.
- **Thorough** –Thorough option uses 25 wash cycles and takes about 65 minutes to complete.

For normal use, we recommend performing the Deep Clean procedure in the Standard mode.

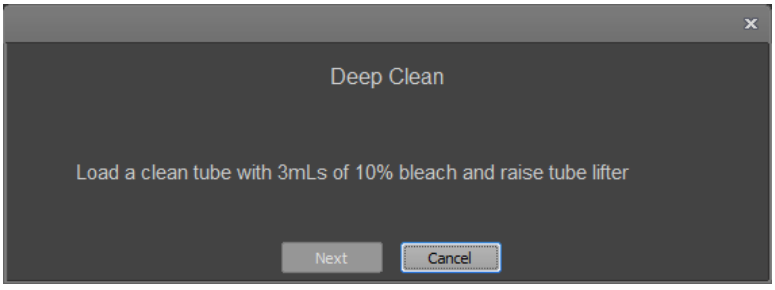
Perform Deep Clean (flow cytometer only)

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

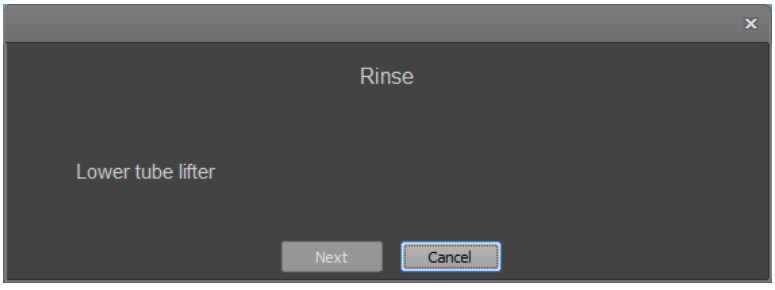
   The *Deep Clean dialog* box appears and provides instructions to perform the Deep Clean procedure.
3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to start a Rinse procedure.

Note: Steps 3 and 4 are only required if the tube lifter is raised at the start of the Shutdown 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 start the Deep Clean procedure.
8. At the end of the wash, lower the tube lifter when prompted, then click **Next** to start the final Rinse procedure.



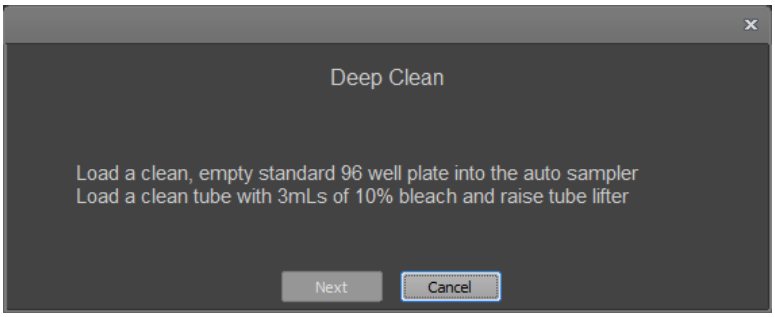
Perform Deep Clean (flow cytometer and autosampler)

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

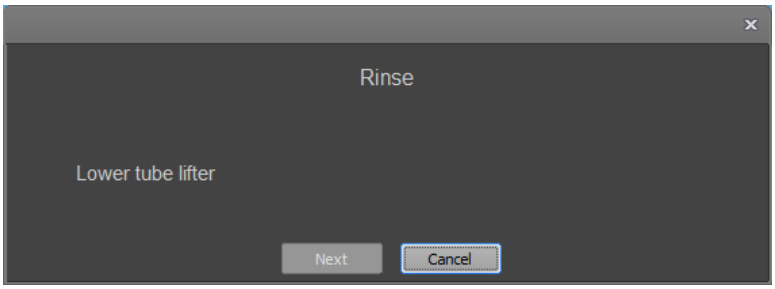
   The *Deep Clean dialog* box appears and provides instructions to perform the Deep Clean procedure.
3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to start a Rinse procedure.

Note: Steps 3 and 4 are only required if the tube lifter is raised at the start of the Shutdown procedure.

5. When the rinse is complete, load a clean, empty standard 96-well plate into the autosampler.



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 start the Deep Clean procedure.
8. At the end of the wash, lower the tube lifter when prompted, then click **Next** to start the final Rinse procedure.



Troubleshooting

This section includes the following topics:

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

Note: For Software Troubleshooting, see the Attune™ Cytometric Software Release Notes and the *Attune™ Cytometric Software User Guide* (Pub. No. 100024236), 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. • Ensure that the syringe is moving smoothly during aspiration. • If syringe problems can be seen, change the syringe. • If syringe problems cannot be seen, contact Technical Support.
	Threshold is not set correctly	• Ensure that the threshold is: - Set to the correct Boolean trigger logic: **And** or **Or** (not **Ignore**). - Not set to 0 (zero). - Not set too high. - Set for the correct parameter on the instrument settings panel. • Lower the threshold 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. • 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. • Reinstall the SIP tube.
	Loose sample syringe	Check for liquid dripping from the syringe and tighten the syringe.
	Incorrect filter in detection channel	Verify that the configuration is correct.

Observation	Possible causes	Recommended solutions
No events are displayed in the Workspace *(continued)*	Laser is not functional	• Verify laser function by rerunning the Performance Test. • Check laser function tied to threshold. • 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. • If the problem persists, contact Technical Support.
	Sample may be too dilute	Increase the sample flow rate or concentrate the sample.
	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. • 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. • Adjust the voltage to get the population on scale. • If events are detected in the collection panel display counter, ensure that the axis is set correctly • 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 might not be on scale but will still be within the gate and therefore counted.
	Gating problem	Verify that the plots are set to all events and the gate logic is correct.
	Threshold set too high	• Lower the threshold. • 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 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 or computer has no power	Power supply not plugged into the appropriate outlet	Ensure that the instrument and the computer are plugged into the appropriate outlet.
	No power at the outlet	• Ensure that the outlet is functional and the circuit breaker is not tripped. • Consider using a Universal Power Supply to guarantee a stable current.
	Faulty power supply	Contact Technical Support.
Getting events on scatter plots and 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 functional	• Ensure that the laser is powered on. • Run Performance Test to verify laser function. If laser is not functional, contact Technical Support.
	Area scaling factor set too low	Adjust the area scaling factor
	Incorrect fluorophore	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. • 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. • Dilute the sample.
	Sample flow rate is too high	Lower the sample flow rate.
	Bacterial contamination	• Ensure that the sample is not contaminated. • 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. • Run deionized water or distilled water as a sample to see if the event rate stays high. • Run the monthly decontamination procedure recommended for the fluid bottles and replace the focusing fluid filter. • Run the Deep Clean function using Wash solution instead of bleach to see if this decreases the background signal. • 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. • Ensure that the 1-mL syringe is tightly sealed. If needed, tighten or replace the syringe. • If the problem persists, contact Technical Support.
	Large density differences between buffer and focusing fluid	Calculate the density of the sample buffer and reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 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. 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. • Inspect the sample loop and sample syringe for bubbles.
Erratic event rate	Partial clog in the flow cell	• Run Unclog function. • Run Deep Clean function. • If clogs occur because of sample clumping, consider: - Filtering the samples to remove any clumps. - Adding 1 mM of EDTA to the buffer to prevent clumping. - Diluting the sample and running at a higher flow rate. • 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 before loading to the instrument. • Add 1 mM of EDTA to the buffer to prevent clumping. • 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 or is operating at inaccurate speed	• Run the Unclog and Deep Clean functions to ensure that there is not a blockage. • Ensure that the syringes are properly tightened and that they have been changed in the previous 12 months. • If the problem persists, contact Technical Support.
	Contaminated sample	Prepare new sample using clean tubes.
	Bubble in fluidics lines	• Run De-bubble function. • 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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 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. • Run Self Test function. • 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. • Ensure that the axes have the same scale.
	Cells were fixed	Some reagents that are used for fixing and permeabilizing the cells alter scatter patterns: • Prepare new sample using different reagents for fixing and permeabilizing the cells. • 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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See Appendix D (page 95) for more information.
Signal drift during run		• Run Self Test function. • 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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 for more information.
High %CV with sample	Poor sample preparation	• Repeat the sample staining procedure. • Optimize reagent stain conditions.
	Flow cell may be dirty	• Run Deep Clean function. • If problem persists, run Decontaminate System function.
	Air bubble in flow cell	Run De-bubble function, then rerun Performance Test.
	Dirty filters	Clean the filters, then rerun Performance Test.
	Incorrect laser delay	Rerun 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: - Filtering the samples to remove any clumps. - Adding 1 mM of EDTA to the buffer to prevent clumping. - Diluting the sample and running at a higher flow rate. • Run Unclog function. • Run Deep Clean function. • 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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 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 probe is malfunctioning	• Perform Stop function. • Power off the instrument and the computer and unplug the connectors. Wait for 1 minute, then reconnect and start up as normal. • 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. • 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. • Clean the optical filters with a lens cloth or compressed air. In severe cases, use a lens cleaner such as methanol. • If the problem persists, run the PT beads as a sample, save the *.fcs files, then contact Technical Support.
	Incorrect performance tracking bead lot used	• Verify the lot number and download the correct lot information. • 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. • 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.
	Particles 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 (that is, 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 that is used. • 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, and so on
	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	Rerun the Performance Test; if it fails, contact Service.
High nonspecific 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 before 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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 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 include only 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. • Dilute the sample to reduce coincidence.
	Nonspecific staining due to dead cells	Use an appropriate dead cell stain to eliminate dead or dying cells.

Autosampler troubleshooting

Observation	Possible causes	Recommended solutions
No sample being analyzed	No power to autosampler	Attach the power plug and turn the autosampler 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 autosampler	Place a plate in the autosampler.
	Incompatible plate type	Contact Technical Support for a list of verified 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 flow cytometer or the autosampler. • Ensure that the fill lines and fluid level detectors are plugged in completely.
	Autosampler is powered off	Power on the autosampler.
	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 that is displayed in the SW is the **minimum** sample volume required. Any deviation to less than this volume in a well (e.g. pipetting error) can lead to bubbles drawn into system.
Red light blinks	Error occurred in system	• Power the instrument off and on. • Perform Auto Sampler Calibration routine.
After the first 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 autosampler	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
Autosampler or computer has no power	Power supply not plugged into the appropriate outlet	Ensure that the autosampler, flow cytometer, and computer are plugged into the appropriate outlet.
	No power at the outlet	Ensure sure that the outlet is functional 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 flow cytometer and the autosampler for leaks and tighten the syringes if necessary. Do not over tighten.
	Defective sample syringe on the autosampler	Replace sample syringe on the autosampler.
	Defective sample syringe on the flow cytometer	Replace sample syringe on the flow cytometer.
	Fluidic valve or tubing failure within the flow cytometer	Verify that the sample can be properly analyzed on the flow cytometer in tube mode and contact Technical Support.
Sample aspirated, then backfilled into sample well	Clog in the sample line	• Run Unclog function. Contact Technical Support if problem persists. • If persistent, designate rinse wells throughout plate between samples, increase rinses between wells, or both. • Ensure that the sample size is within system specification (< 50 µm).
	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 verified 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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 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 probe is malfunctioning	Turn off the Auto Sampler and contact Technical Support.
Rinse fluid pump does not shut off	Rinse (Waste) fluid reservoir level probe 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 that is loaded into each well is not adequate	Total Draw Volume that is displayed in SW is the **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 that sample preparation and well loading is consistent across the plate.
	Sample concentration exceeds system specifications	Verify that sample concentration is not more than system requirements.
Large amount of debris is seen in data	Auto Sampler has been idle for an extended time	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 reformulate the focusing fluid to balance the densities of the buffer and the focusing fluid. See page 92 for more information.
No events at low sample flow rates		
Pulsing of data at medium to high sample flow rates		
Abnormal, grinding sounds at power-up or during operation	Tray is loose or disconnected from magnet	Power off the instrument, pull open the tray door, and push tray all the way back until it stops moving and is coupled with magnet.

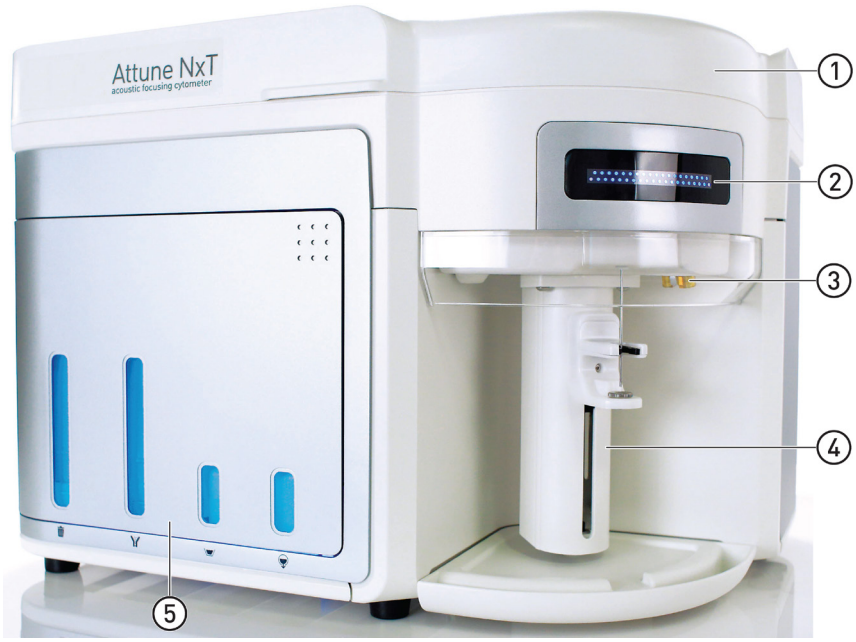
Attune™ External Fluid Supply (EFS) troubleshooting

Observation	Possible causes	Recommended solutions
Fluid stopped transferring to/from Focusing fluid and Waste bottles	No power to the EFS	Attach the 12-pin power/control cable to the EFS and the cytometer.
	Fluidics are not connected	Connect fluidic lines to the Attune™ System and the EFS.
	Fluidics are leaking	Check for leaks at the connectors at the Attune™ System and the EFS.
	Safety interlock that stops fluid transfer has been triggered due to overflow or unplugged fluid bottles.	Determine and address the cause of the activated interlock, then reset the safety interlock (page 41).
	Safety interlock triggered and fluid pumps inactivated due to increased backpressure	Relieve backpressure in the fluid lines and pumps (page 42).
EFS pumps are running, but fluid is not transferred to/from the cytometer	Check valve on the focusing fluid and/or waste bottle lid and/or dip tube has become sticky	Clean check valves on the focusing fluid and/or waste bottle lids and dip tubes (page 43).
	Dip tube on the focusing fluid and/or waste umbilical is blocked due to accumulated biomaterials	Clean dip tubes on the focusing fluid and/or waste umbilicals with 10% bleach solution (page 43).
Focusing fluid bottle in the cytometer is overflowing	Float sensor is stuck at the bottom of the focusing fluid bottle	Relocate the tube inside the focusing fluid bottle away from the float sensor to the rear of the focusing fluid bottle (page 44).
Computer is not communicating with the EFS	12-pin power/control cable not fully plugged in	Verify that the 12-pin power/control cable connection is in place in the back of the EFS and the computer.
	Faulty 12-pin power/control cable	Replace 12-pin power/control cable. Contact Technical Support if problem persists.

Appendix A: Parts of Attune™ NxT and Attune™ CytPix™ Flow Cytometers

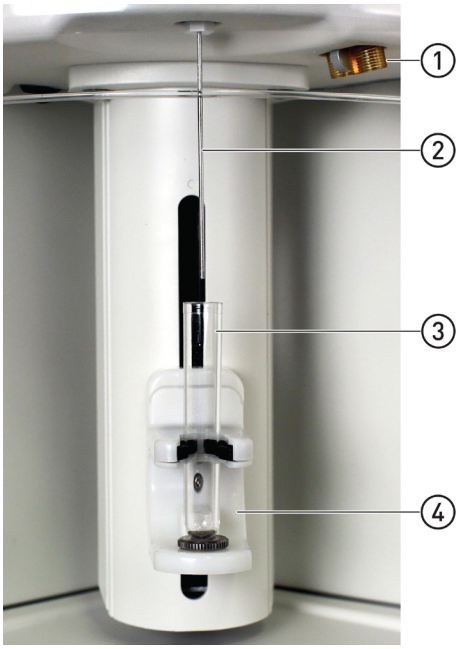
Attune™ NxT Flow Cytometer components

Front view of Attune™ NxT Flow Cytometer



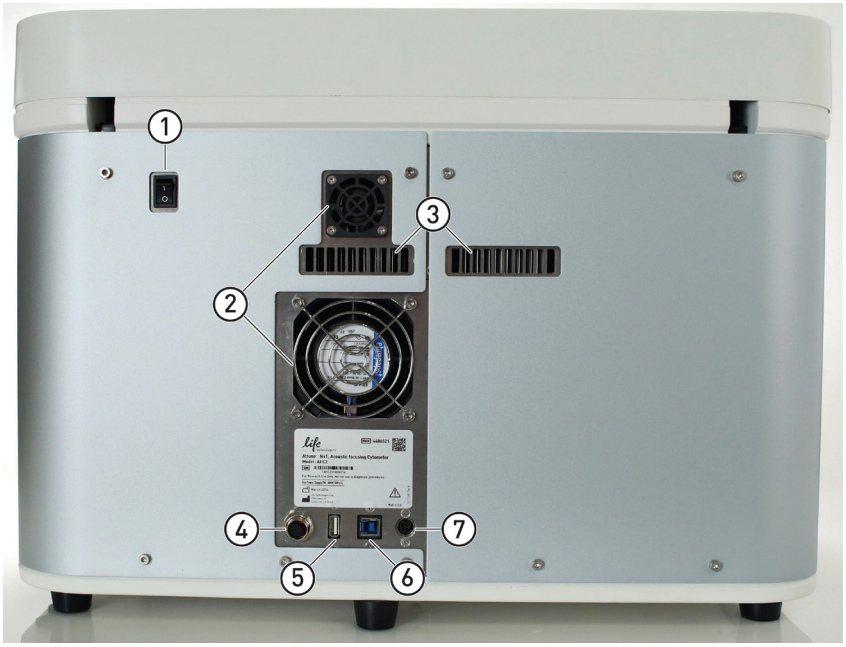
1. Optics compartment
2. Status indicator lights
3. Fluidics connections to the autosampler
4. Sample injection port (SIP)
5. Fluidics compartment

Sample injection port (SIP)



1. Fluid connection ports for autosampler
2. Sample injection tube
3. Sample tube (not included)
4. Sample tube lifter

Rear view of Attune™ NxT Flow Cytometer

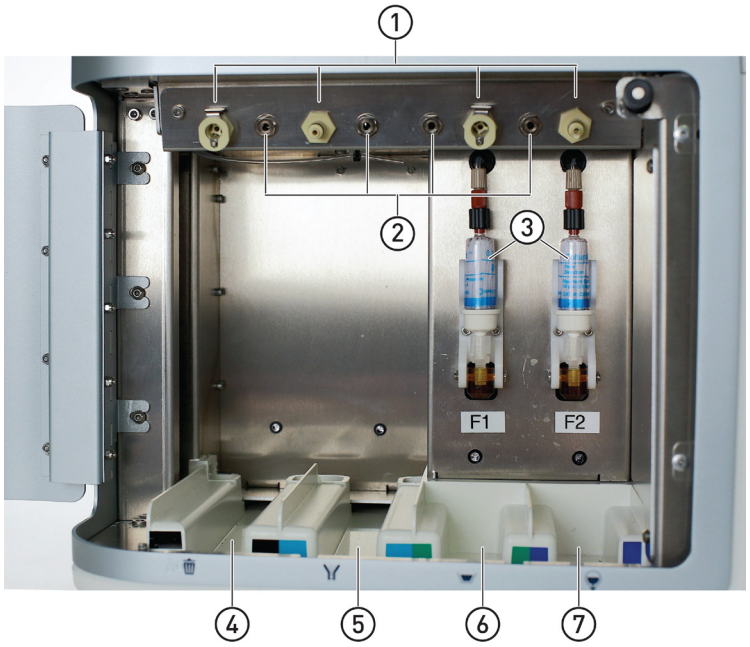


1. On/Off switch
2. Air exhausts
3. Laser exhausts
4. Port for Attune™ NxT External Fluid Supply connection
5. Port for autosampler connection
6. Port for computer connection
7. Power input jack

Side view of Attune™ NxT Flow Cytometer

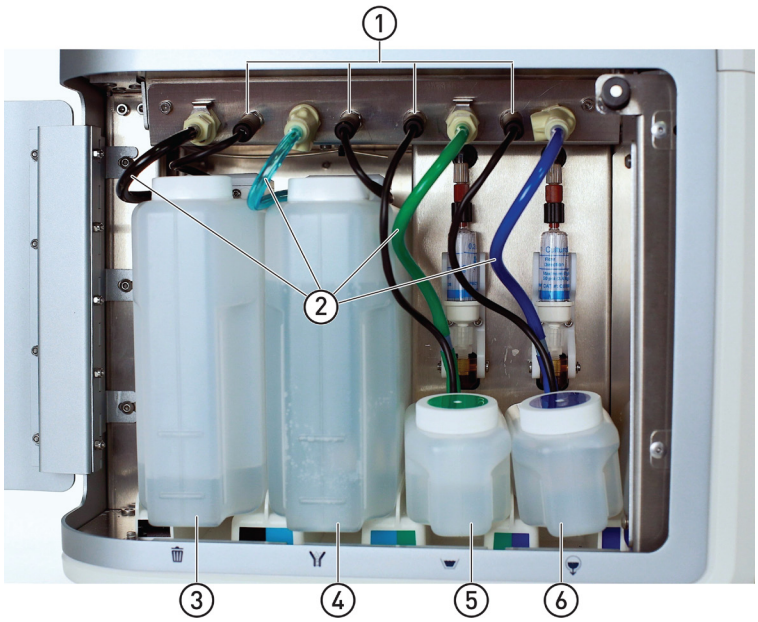
1. Syringe pump compartment
2. Fluidics compartment

Fluidics compartment (without containers)



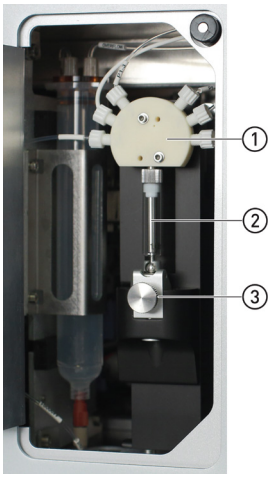
1. Fluid connection ports
2. Fill probe connection ports
3. Focusing fluid filters
4. Waste container compartment
5. Focusing fluid compartment
6. Wash fluid compartment
7. Shutdown fluid compartment

Fluidics compartment (with containers and connections)



1. Fill probe connections
2. Fluid lines
3. Waste container
4. Focusing fluid container
5. Wash fluid container
6. Shutdown fluid container

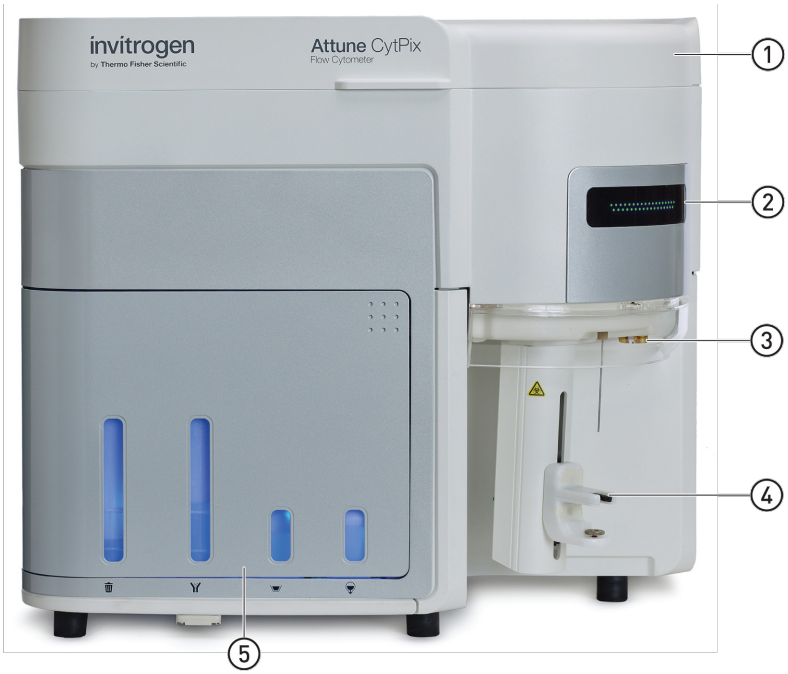
Syringe pump compartment



1. Valve
2. Syringe piston
3. Syringe capture mechanism

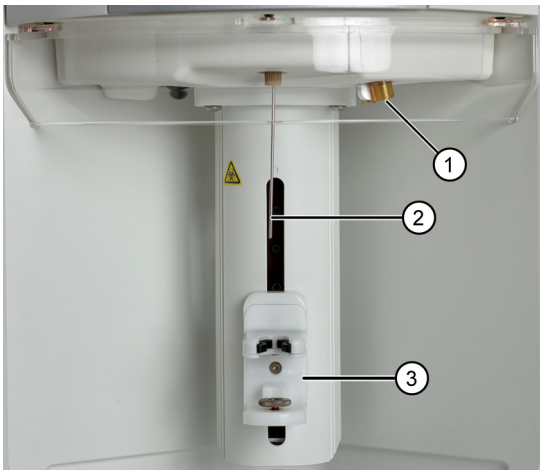
Attune™ CytPix™ Flow Cytometer components

Front view of Attune™ CytPix™ Flow Cytometer



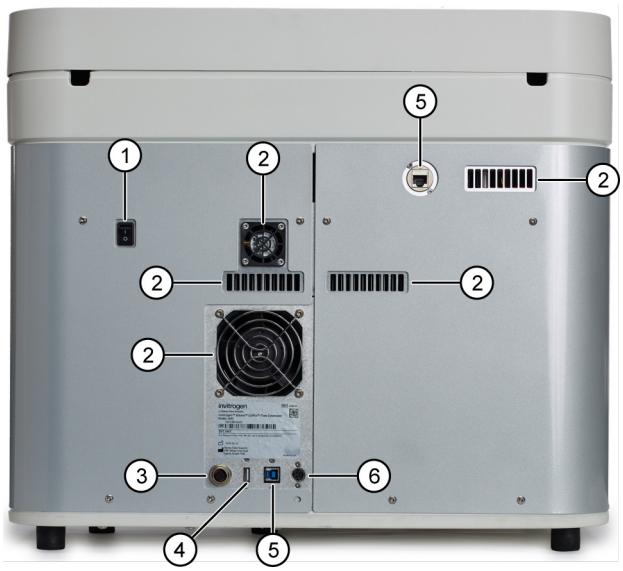
1. Optics compartment
2. Status indicator lights
3. Fluidics connections to the autosampler
4. Sample injection port (SIP)
5. Fluidics compartment

Sample injection port (SIP)



1. Fluid connection ports for autosampler
2. Sample injection tube
3. Sample tube lifter

Rear view of Attune™ CytPix™ Flow Cytometer

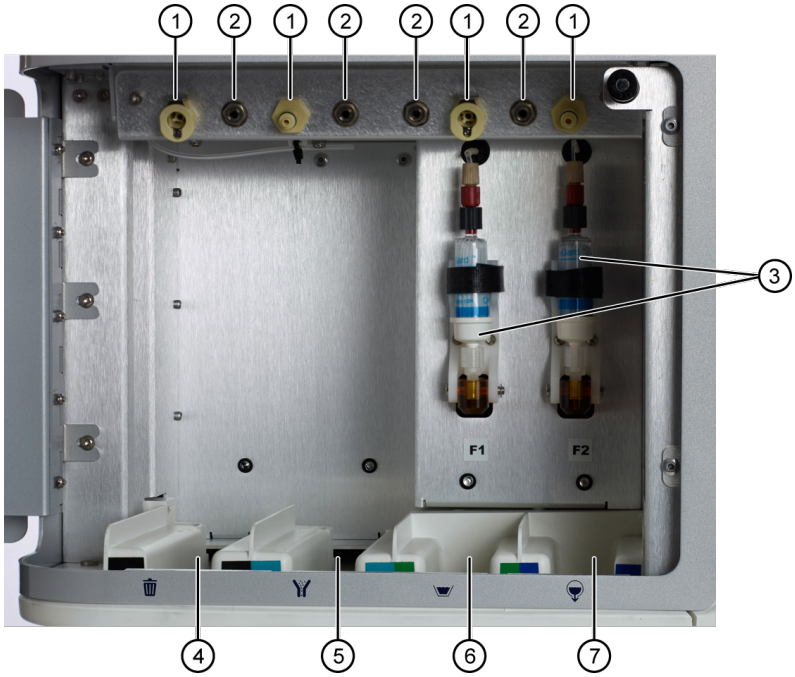


1. On/Off switch
2. Air exhausts
3. Port for Attune™ NxT External Fluid Supply connection
4. Port for autosampler connection
5. Port for computer connection
6. Power input jack

Side view of Attune™ CytPix™ Flow Cytometer

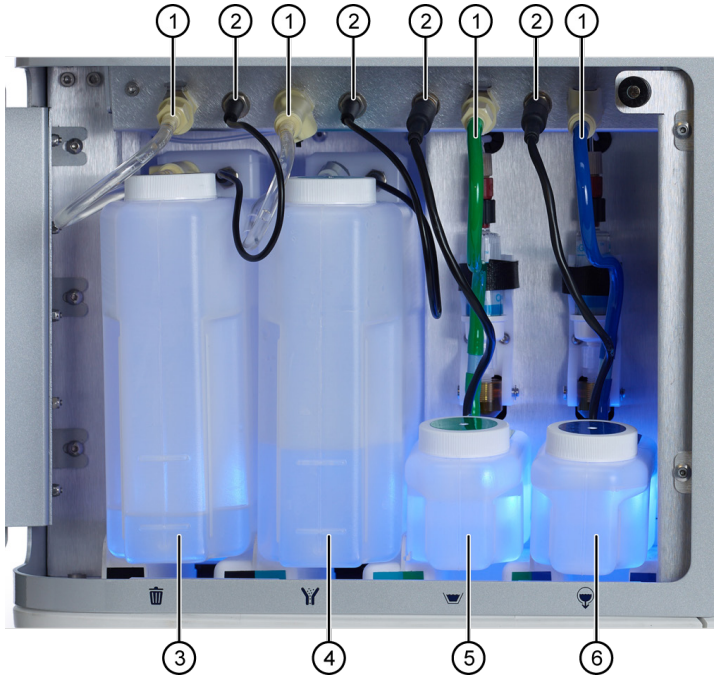
1. Syringe pump compartment
2. Fluidics compartment

Fluidics compartment (without containers)



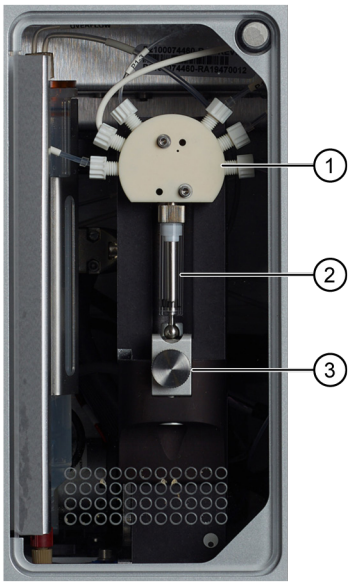
1. Fluid connection ports
2. Fill sensor connection ports
3. Focusing fluid filters
4. Waste container compartment
5. Focusing fluid compartment
6. Wash fluid compartment
7. Shutdown fluid compartment

Fluidics compartment (with containers and connections)



1. Fluid lines
2. Fill sensor connections
3. Waste container
4. Focusing fluid container
5. Wash fluid container
6. Shutdown fluid container

Syringe pump compartment

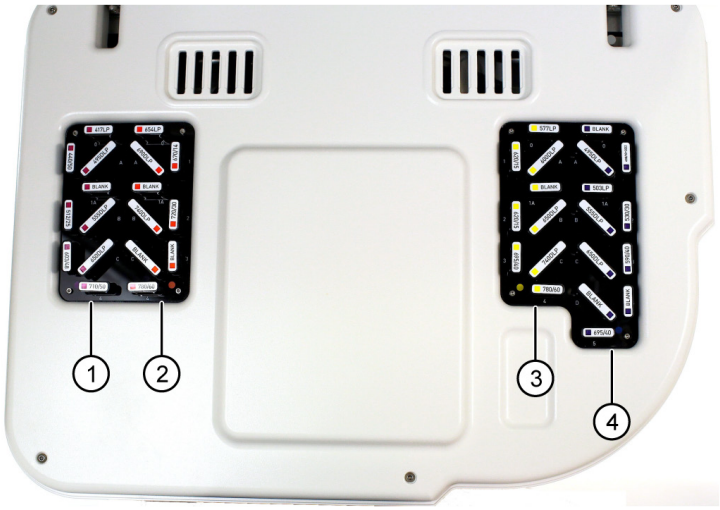


1. Valve
2. Syringe piston
3. Syringe capture mechanism

Optics compartment

The following image shows the optics compartment of the Attune™ NxT and Attune™ CytPix™ Flow Cytometers. The optics compartment houses the collection optics (that is, optical filters and mirrors). The locations of the optical filters and mirrors depend on the specific instrument configuration.

The following example shows the BYRV4 (Blue, Yellow, Red, Violet) instrument configuration, where the collection optics for the violet and red lasers are on the left, and for the yellow and blue lasers on the right. For default instrument configurations, see page 80.



1. Collection optics for violet laser
2. Collection optics for red laser
3. Collection optics for yellow laser
4. Collection optics for blue laser

Status indicator lights

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

Instrument configurations

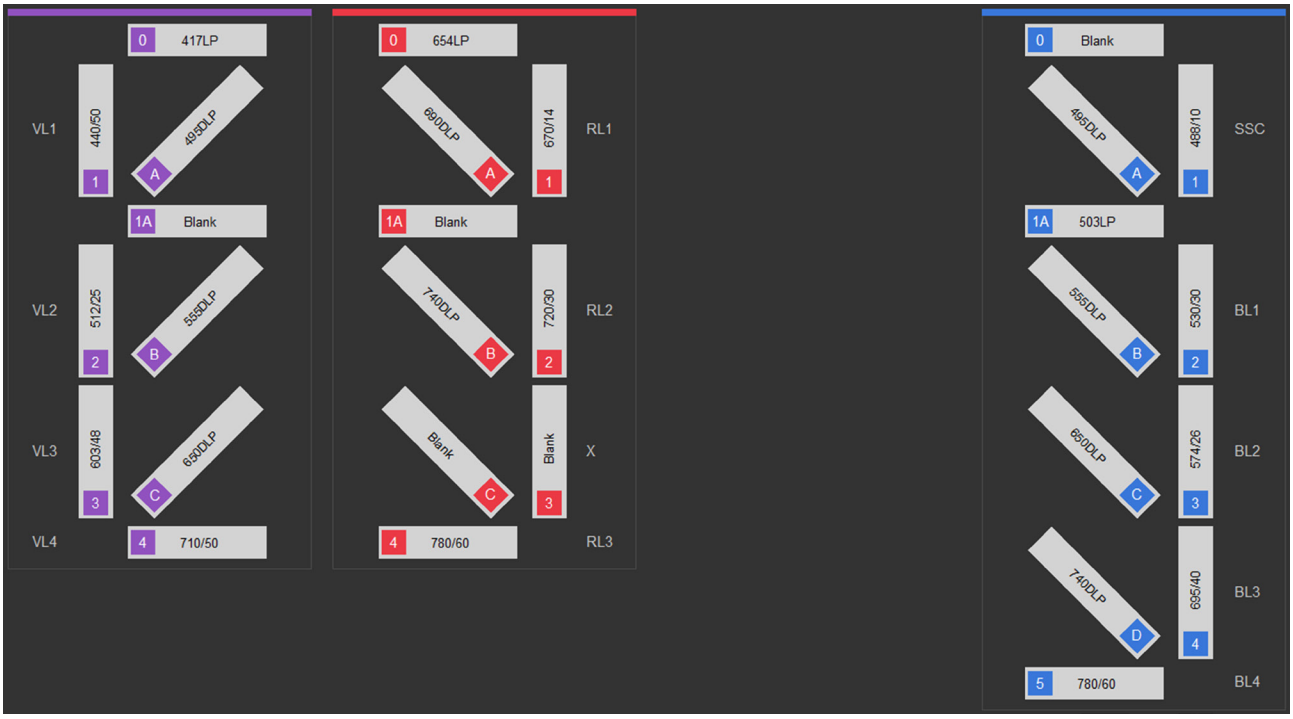
Default instrument configurations

The following images show each laser configuration that is available for Attune™ NxT and Attune™ CytPix™ Flow Cytometers (page 80), for Attune™ NxT Flow Cytometer only (page 84), and for Attune™ CytPix™ Flow Cytometer only (page 88).

Available for Attune™ NxT and Attune™ CytPix™ Flow Cytometers

BYRV4 (Blue, Yellow, Red, Violet)

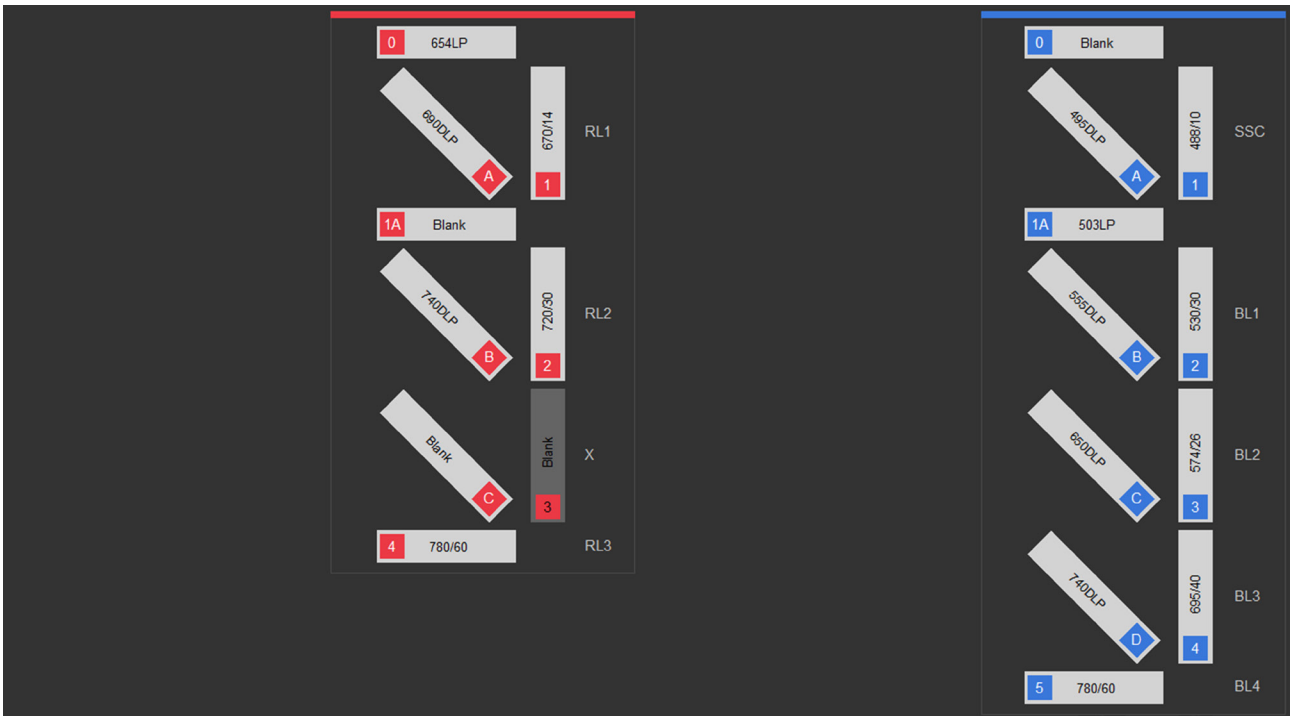
BRV4 (Blue, Red, Violet)



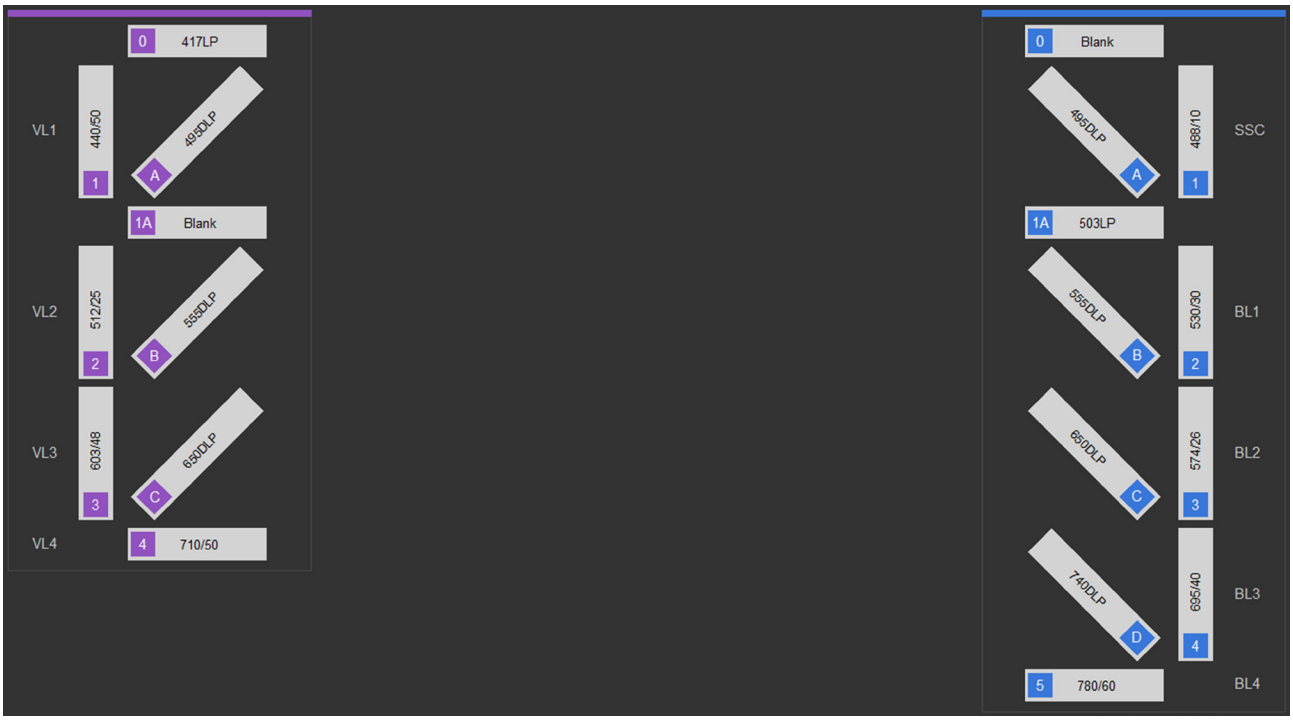
BYV4 (Blue, Yellow, Violet)

BYR (Blue, Yellow, Red)

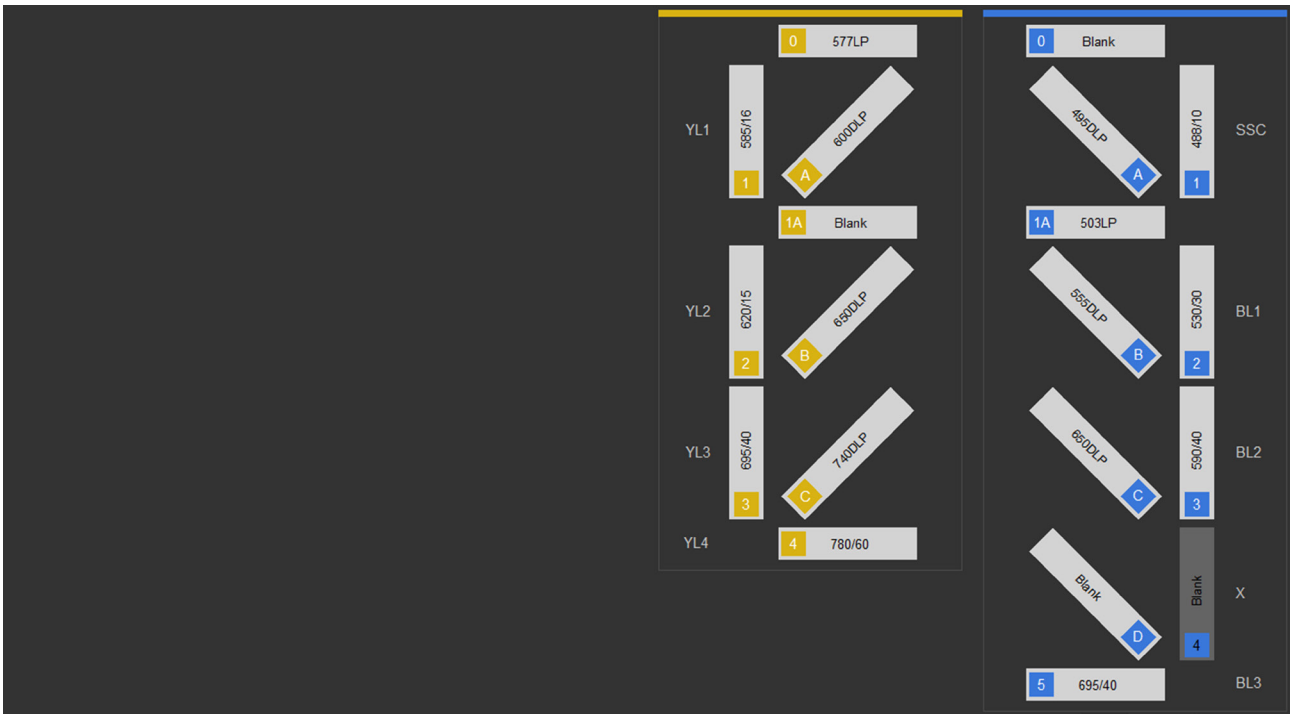
BR (Blue, Red)



BV4 (Blue, Violet)

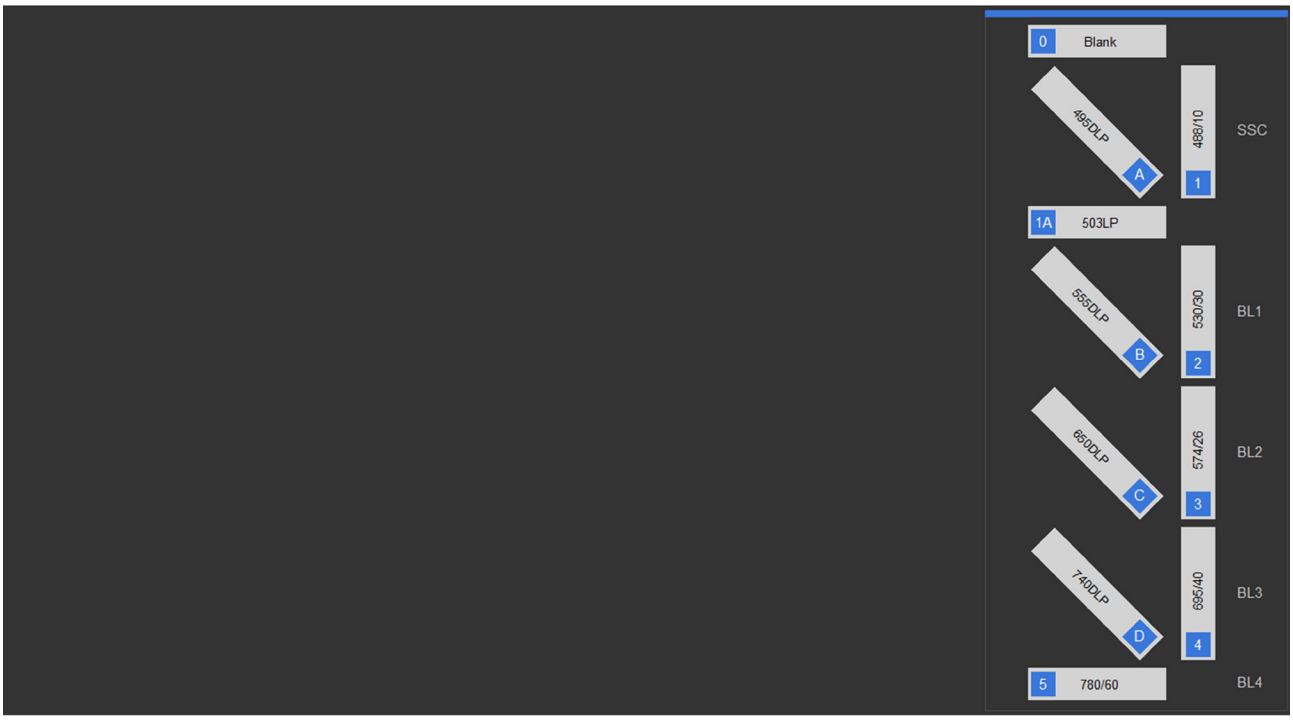


BY (Blue, Yellow)

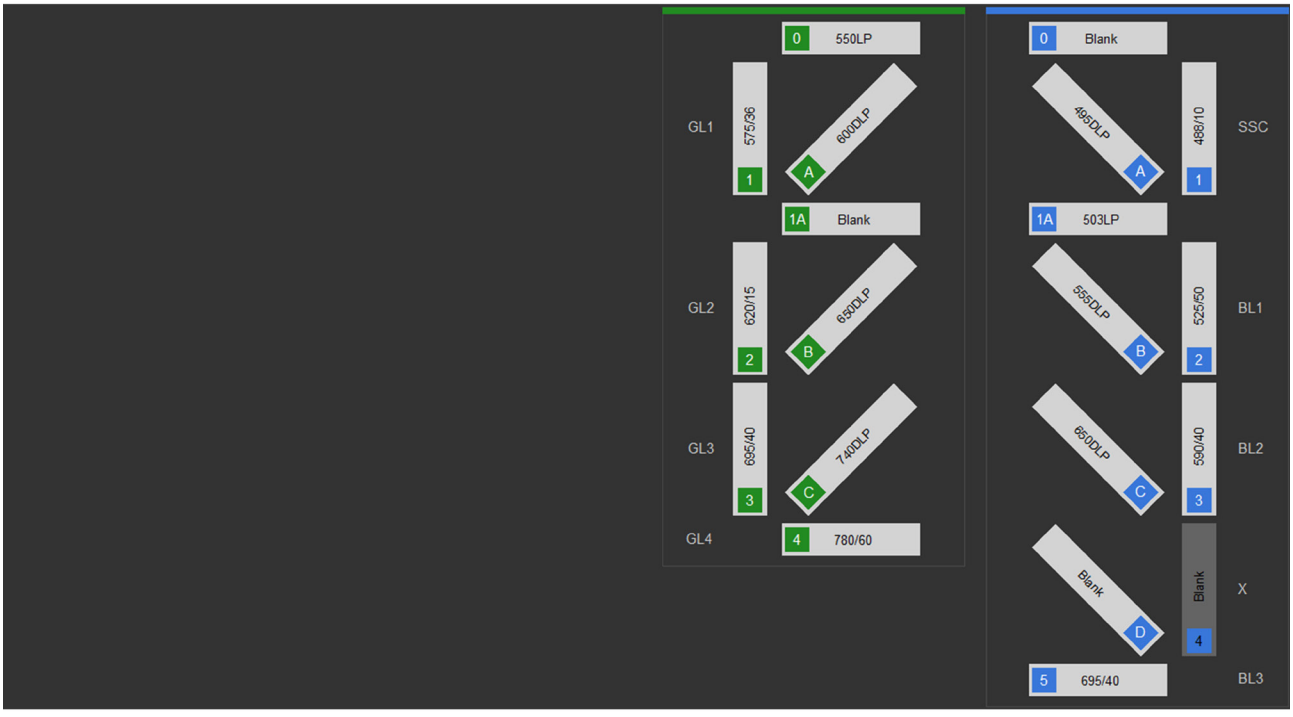


Available for the Attune™ NxT Flow Cytometer only

B (Blue)

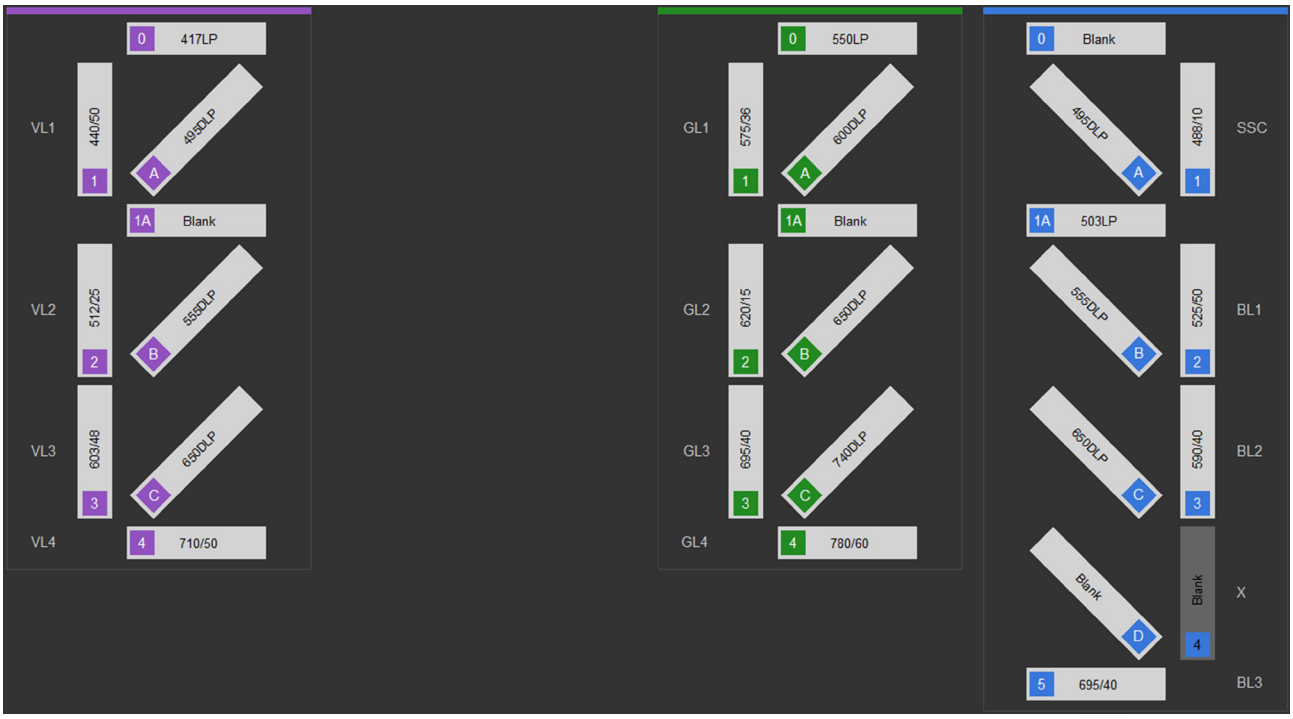


BG (Blue, Green)



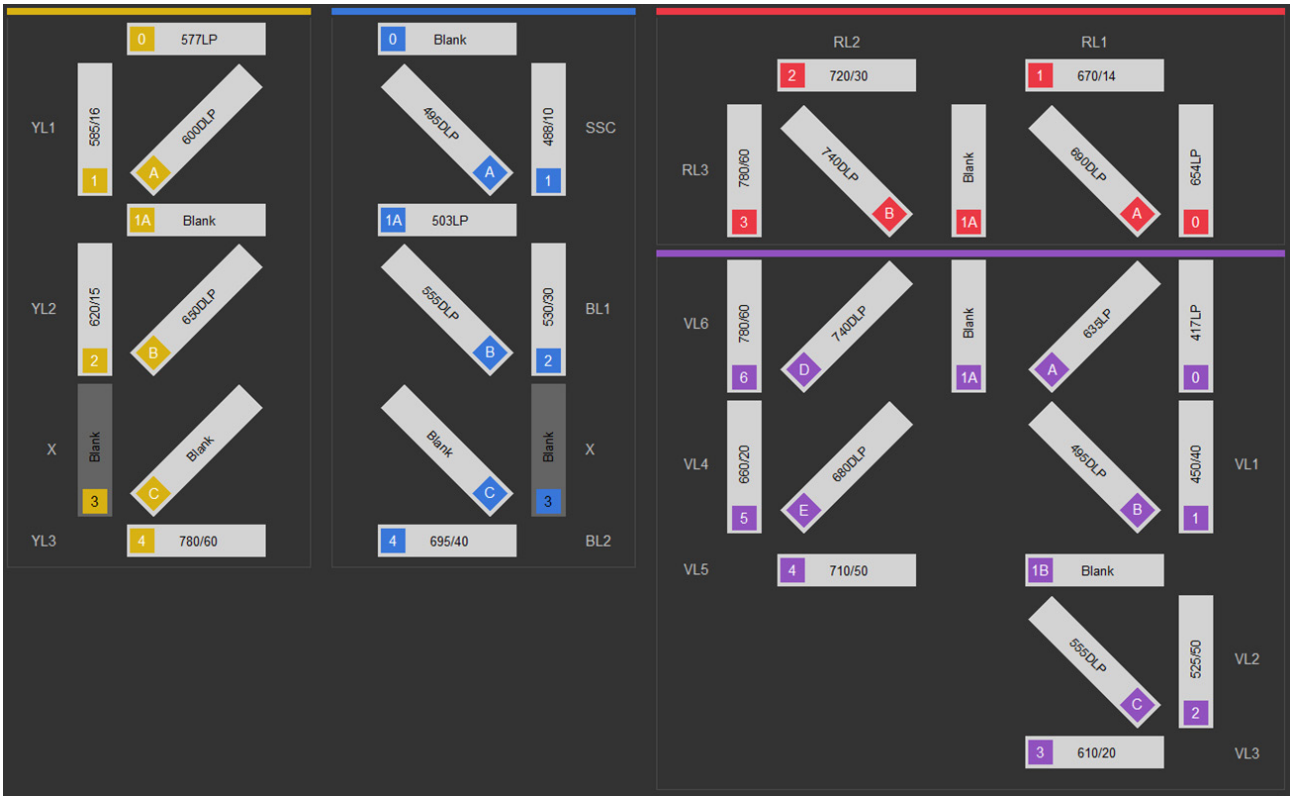
BGR (Blue, Green, Red)

BGV4 (Blue, Green, Violet)

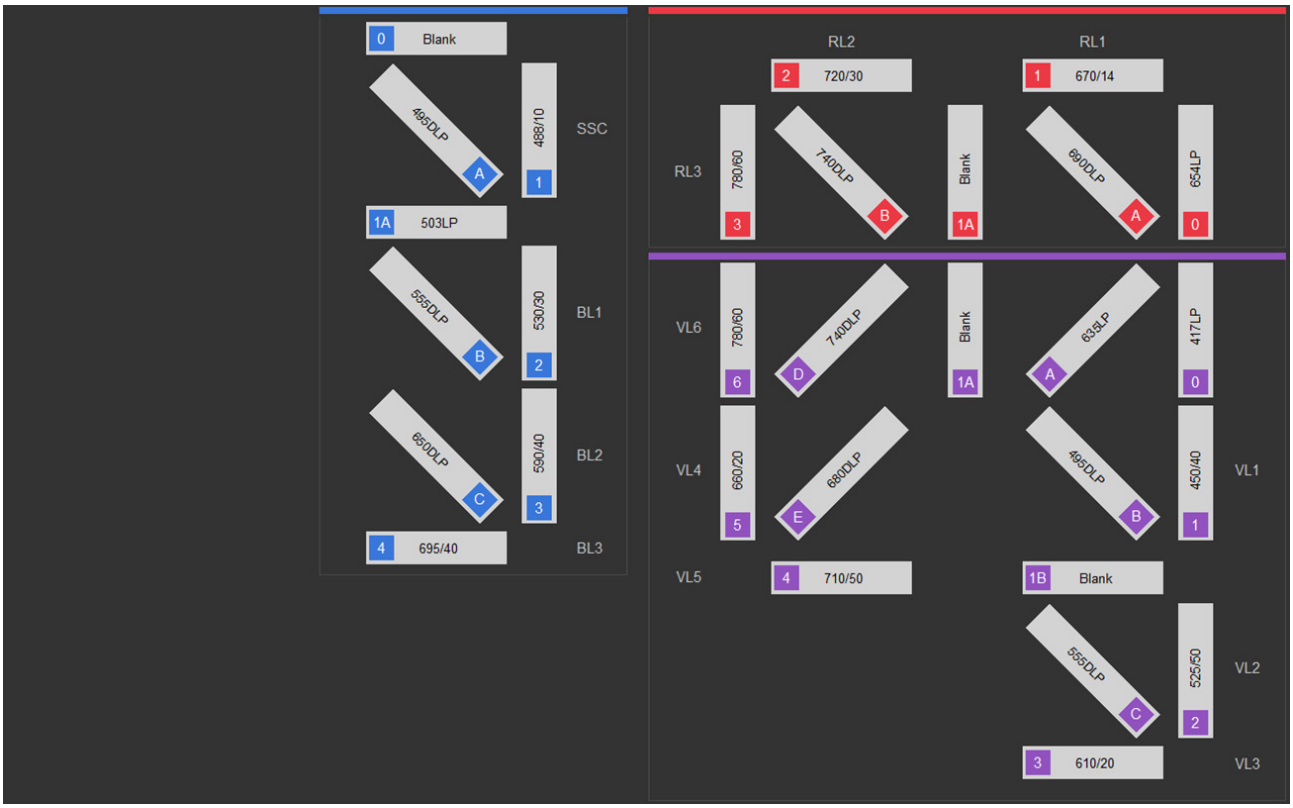


BGRV4 (Blue, Green, Red, Violet)

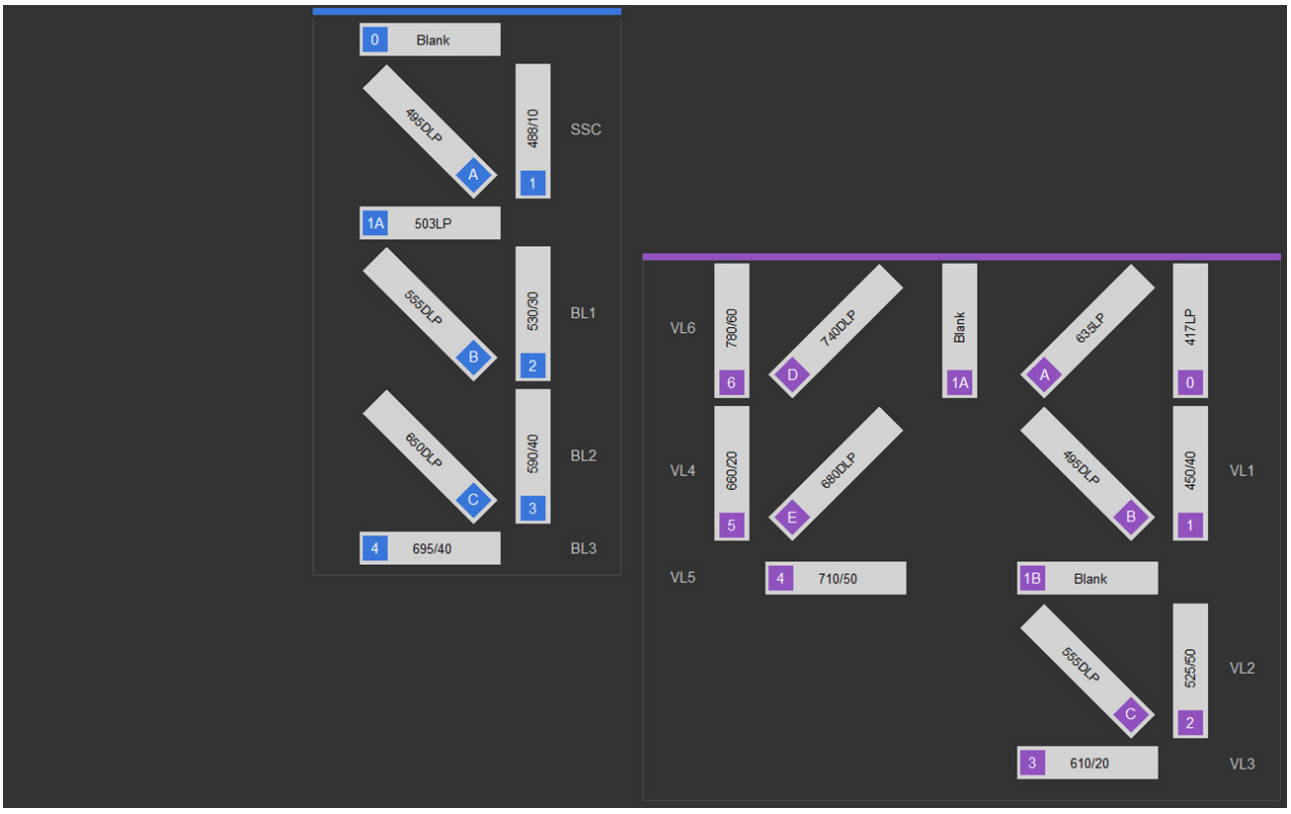
BYRV6 (Blue, Yellow, Red, Violet 6)



BRV6 (Blue, Red, Violet 6)

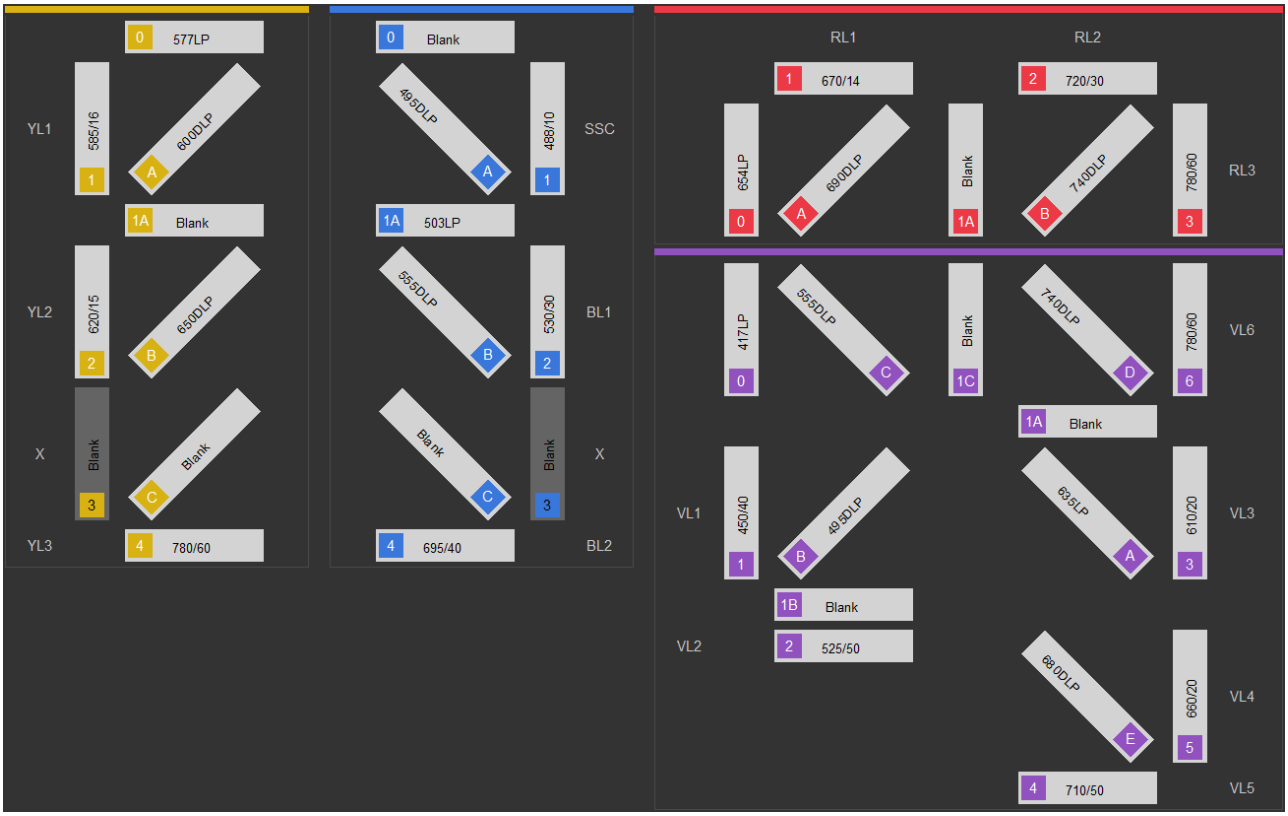


BV6 (Blue, Violet 6)

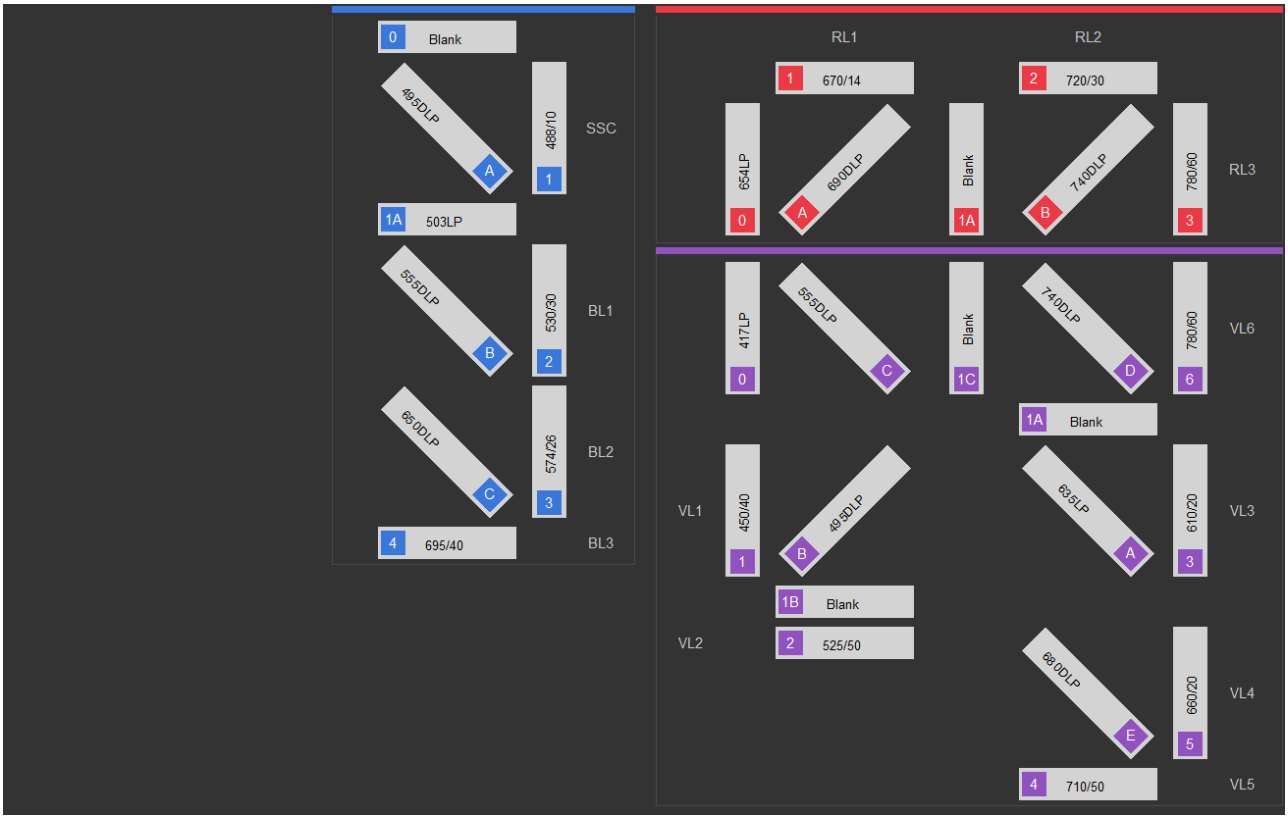


Available for the Attune™ CytPix™ Flow Cytometer only

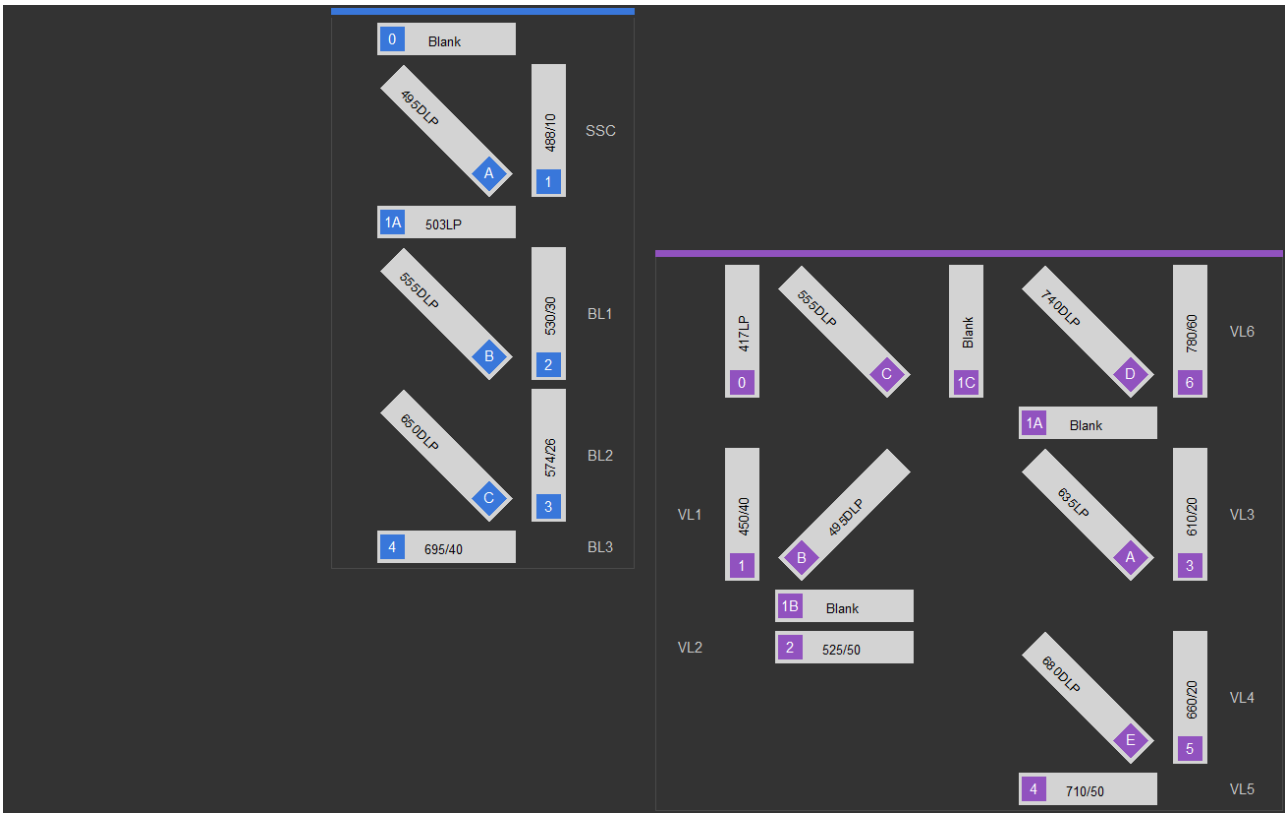
BYRV6 (Blue, Yellow, Red, Violet 6)



BRV6 (Blue, Red, Violet 6)



BV6 (Blue, Violet 6)



Note: In Attune™ CytPix™ Flow Cytometers, the orientation of the Violet 6 laser configuration is reversed to accommodate the image collection optics.

Default filter configurations

Default filter label list

The following tables list the filter labels that are displayed for each channel for the available system default configurations. The naming convention for the system default filter configuration is as follows:

Baseline/PT Config <*CCCC*#>, where **<C>** is the first letter of each laser color (**B**lue, **G**reen, **Y**ellow, **R**ed, or **V**iolet) and **#** corresponds to 4 or 6 channels that are detected off the Violet laser. An **X** for laser color indicates that one of the lasers is not present.

Configuration		BXXX	BGXX	BYXX	BRXX	BV4XX	BV6XX	BGRX	BRV4X	BGV4X	BYRX	BYV4X	BRV6X	BYRV6	BGRV4	BYRV4
No. of detectors		4	7	7	7	7	9	10	10	11	11	11	12	14	14	14
Laser	Channel	Emission filter (nm)														
Blue	BL1	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30
	BL2	574/26	590/40	590/40	574/26	574/26	574/26	590/40	574/26	590/40	574/26	590/40	574/26	695/40	590/40	590/40
	BL3	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40		695/40	695/40
	BL4	780/60			780/60				780/60		780/60					
Green	GL1		575/36					575/36		575/36					575/36	
	GL2		620/15					620/15		620/15					620/15	
	GL3		695/40					695/40		695/40					695/40	
	GL4		780/60					780/60		780/60					780/60	
Yellow	YL1			585/16							585/16	585/16		585/16		585/16
	YL2			620/15							620/15	620/15		620/15		620/15
	YL3			695/40							695/40	695/40		780/60		695/40
	YL4			780/60							780/60	780/60				780/60
Red	RL1				670/14			670/14	670/14		670/14		670/14	670/14	670/14	670/14
	RL2				720/30			720/30	720/30		720/30		720/30	720/30	720/30	720/30
	RL3				780/60			780/60	780/60		780/60		780/60	780/60	780/60	780/60
Violet	VL1					440/50	450/40		440/50	440/50		440/50	450/40	450/40	440/50	440/50
	VL2					512/25	525/50		512/25	512/25		512/25	525/50	525/50	512/25	512/25
	VL3					603/48	610/20		603/48	603/48		603/48	610/20	610/20	603/48	603/48
	VL4					710/50	660/20		710/50	710/50		710/50	660/20	660/20	710/50	710/50
	VL5						710/50						710/50	710/50		
	VL6						780/60						780/60	780/60		

Appendix B: Parts of the Attune™ Auto Sampler

① Fluidics connections to the autosampler	④ Eject button
② Light button	⑤ Sample tray door
③ Status indicators	⑥ Autosampler fluidics compartment

Note: The fluid lines that connect the Attune™ NxT Flow Cytometer or the Attune™ CytPix™ Flow Cytometer to the autosampler can be attached to either fluid connection port.

Appendix C: Parts of the CytKick™/CytKick™ Max Autosampler

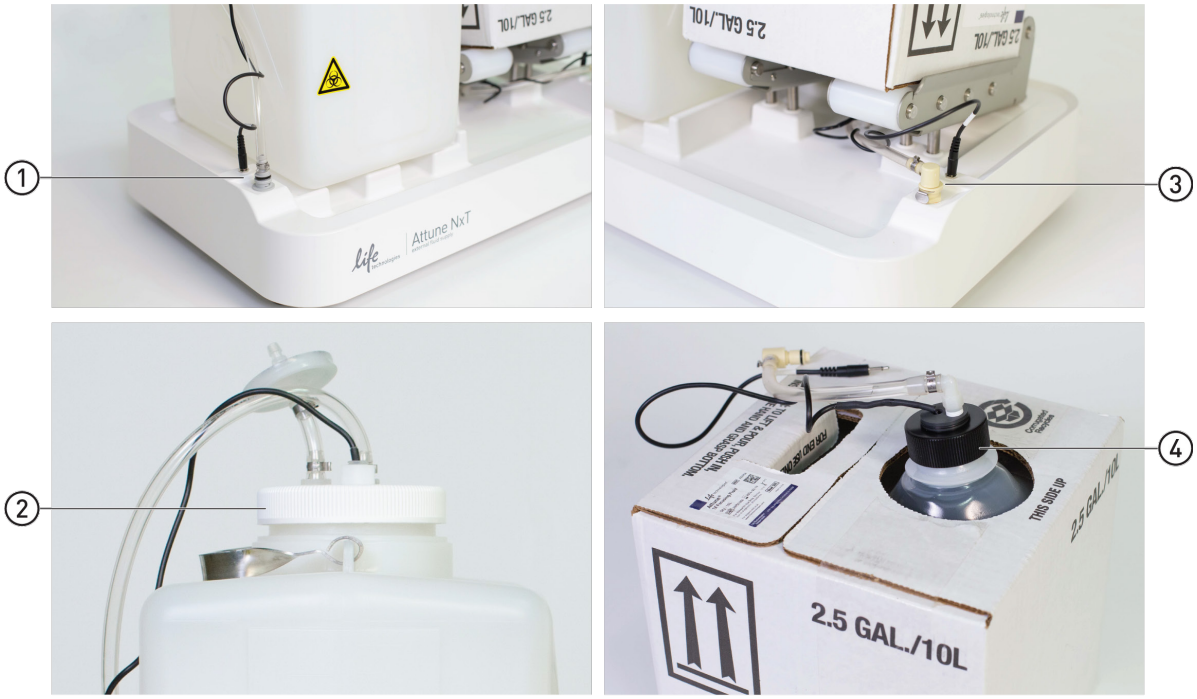
1. Sample tray door
2. Syringe pump compartment
3. Light button
4. Status indicators
5. Eject button
6. Waste bottle
7. Focusing Fluid bottle

1. Fluidics connections (from the cytometer)
2. Sample tray door (open)

Appendix D: Parts of the Attune™ NxT External Fluid Supply (EFS)

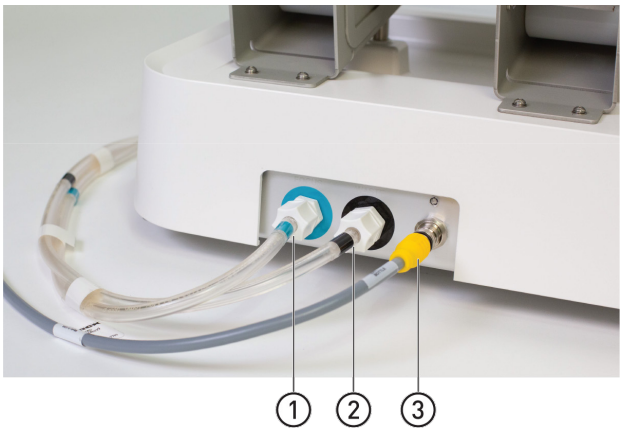
Front view of Attune™ NxT EFS

1. Waste container
2. EFS base assembly
3. Focusing fluid cubetainer



1. Waste umbilical (connects to Waste container lid)
2. Waste container lid (with waste umbilical attached)
3. Fosucing fluid umbilical (connects to focusing fluid cubetainer)
4. Focusing fluid cubetainer (with focusing fluid umbilical attached)

Rear view of Attune™ NxT EFS



1. Focusing fluid tubing
2. Waste tubing
3. 12-pin power cable (connects to Attune™ NxT Flow Cytometer)

Appendix E: Attune™ Focusing Fluid density adjustment

Sample buffers with 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 the start 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, adjust the concentration of the Attune™ Focusing Fluid to balance the densities of the sample buffer and the focusing fluid, and to 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.

   See the following table 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 F: Minimizing background in Attune™ Flow Cytometers

Overview

One of the main advantages of the Attune™ NxT and Attune™ CytPix™ Flow Cytometers is that it achieves sample throughput rates of up to 1,000 µL/minute. Using the acoustic focusing technology, we can minimize signal variation for higher flow rates that are not feasible with hydrodynamic focusing alone. Acoustic focusing minimizes variation of particle speed and location in the sample core relative to laser alignment, but it does not overcome variation due to photon counting statistics or the effects of sample core size on background. These effects are described in this chapter.

Photon counting statistics

As we process samples at higher speeds, the velocity of the sample increases from 4 m/second (12.5–200 µL/minute) to 8 m/second (500–1,000 µL/minute) and the interrogation time in the laser decreases by a factor of 2. This two-fold decrease in interrogation time translates to less total photons and an increase in the spread (%CV) of the data as governed by photon counting statistics. The standard deviation as determined by photon counting statistics is based on the square root of the photon count (SD = $\sqrt{(Count)}$). By doubling the velocity, we hypothetically decrease the photon count by a factor of 2, which decreases the standard deviation by $\sqrt{2}\times$ or 1.4× but with an effective increase in the error or %CV by the same fold (1.4×). This increase in error has a much more pronounced effect on dim signals as illustrated in Table 1:

Table 1. Error and corresponding %CV as a function of photon count.

Photon count	SD	%CV
10	3.16	31.62%
100	10.00	10.00%
1,000	31.62	3.16%
1,0000	100.00	1.00%

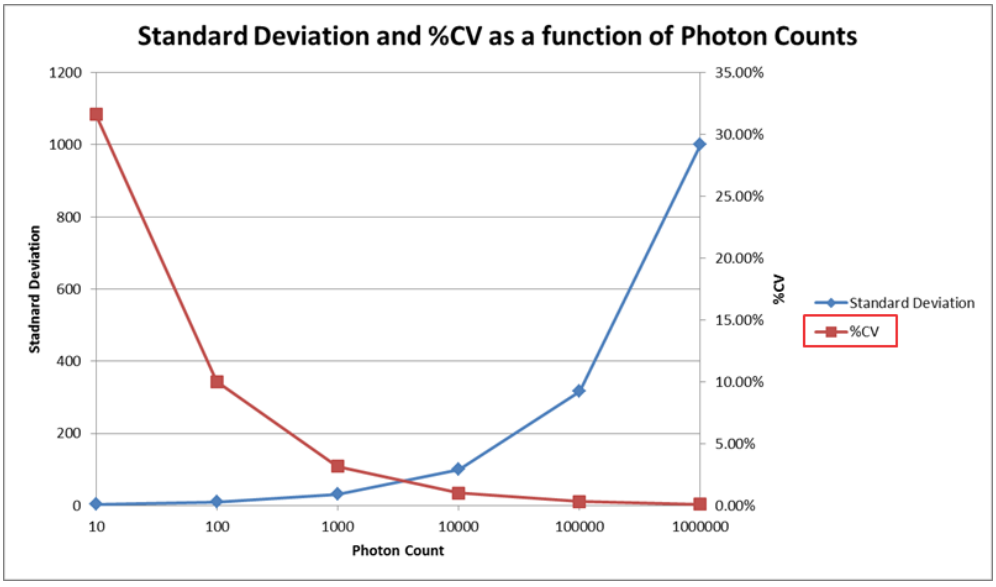


Figure 1. The relationship of standard deviation and percent coefficient of variation (%CV) as a function of photon counts. As the photon count increases, the standard deviation also increases, but the %CV decreases. Dim signals (that is the negative population) exhibit higher CVs due to the intrinsic nature of photon counting statistics.

Background subtraction/ Baseline restoration

Photon counting statistics are only one part of the equation. A more significant factor that can have a large effect on the spread and MFI of a population is the process of background subtraction or baseline restoration. The system is continuously processing the electronic signals in all channels. Signal that is measured below the trigger threshold (the signal at which we decide to collect data) is continuously averaged and processed as background and subtracted from the pulse that is then generated above the trigger threshold. In the presence of unbound dye or buffer components that exhibit autofluorescence, the background that is subtracted from the desired pulse peak (signals above threshold) includes the signal from these fluorescence contributions. The degree of this fluorescence contribution changes as a function of sample core size. As we change our flow rates, the core of the sample buffer changes as shown in Table 2:

Table 1. Sample core size at each flow rate.

Flow rate (µL/minute)	Core size (µM)
12.5	6
25	9
100	20
200	28
500	33
1,000	45

The increase in core size means that at different flow rates, there is a different volume of unbound dye or buffer containing autofluorescent components, which effectively changes the degree of background fluorescence as a function of core size. The volume of unbound dye in the interrogation region should scale approximately as the ratio of the squares of the core radii. The ratio of unbound dye between the 25 µL/minute and 1,000 µL/minute flow rates should be a factor of (22.5 × 22.5/(4.5 × 4.5) = ~ 25×. Therefore, the increase in background due to unbound dye increases by a factor of 25. This can lessen the distance between stained and unstained populations, particularly when there is a high level of unbound dye due to inadequate washing, poor antibody titration, or use of antibodies or ligands with low binding affinity.

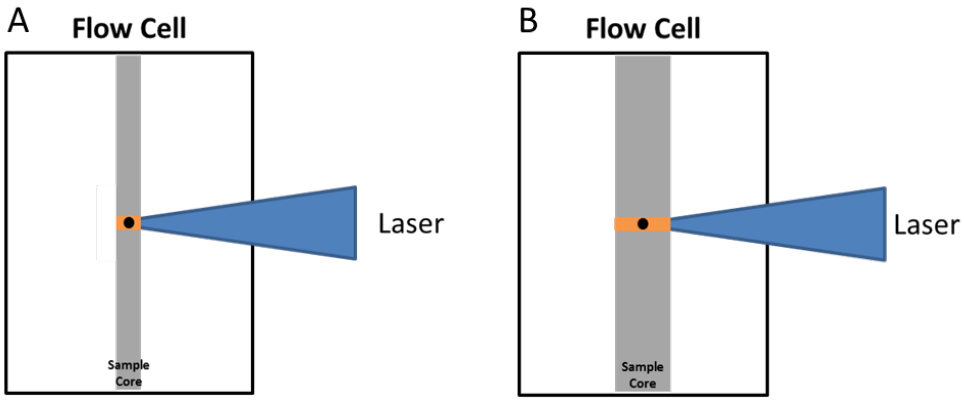


Figure 2. Schematic of flow cell containing the sample core (gray), a particle (black), and the background fluorescence (orange). The two images illustrate the idea that as the core size increases, more of the sample buffer is excited and has the potential to contribute to background signal. The two figures represent the sample core size at **(A)** 100 µL/minute and **(B)** 1,000 µL/minute.

This background is factored into a background subtraction algorithm, which constantly averages the signal in each channel that is below the trigger threshold. In the example above, the 25-fold increase in the background signal is then subtracted from the signal of a cell or particle. It is important to note that the distribution of signal (that is the standard deviation) that is subtracted from our desired signal is dictated by photon counting statistics as described earlier. This means that, as the background increases, the standard deviation increases and persists throughout the subtraction process. Consequently, we now have a higher standard deviation, which further increases the spread (%CV) of our negative/dim populations.

This effect was demonstrated by running blank Antibody Capture Beads (AbC™ beads) in the presence of a CD4-PE and looking at the effect on the MFI at different flow rates and concentrations of the PE conjugate.

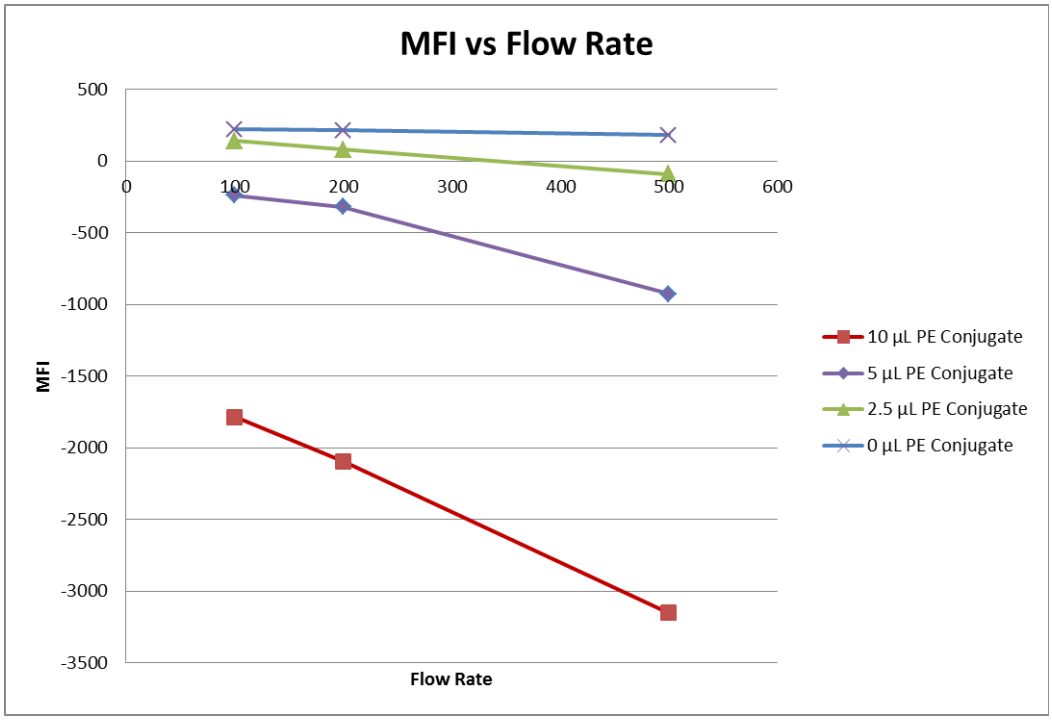


Figure 3. At low concentrations of dye, the effect of flow rate on the signal of the negative bead population is minimal. As we increase the free dye concentration, the impact on the negative population becomes quite apparent.

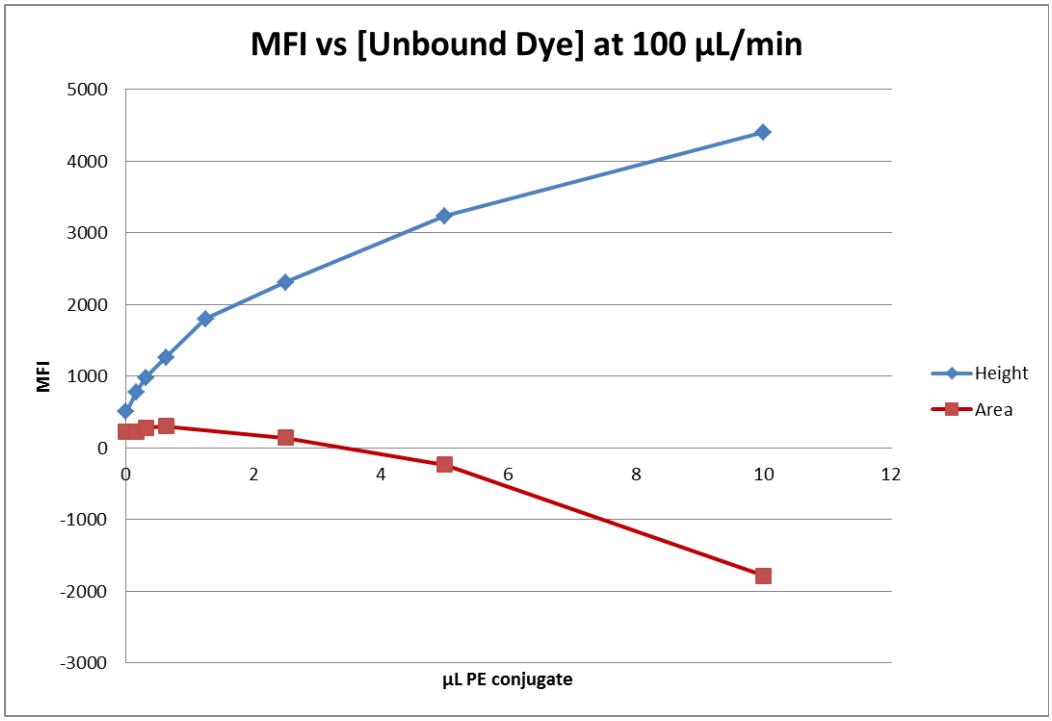


Figure 4. The impact on unbound dye is clearly demonstrated here at a fixed flow rate (100 µL/minute). **Area:** as the concentration of unbound dye increases, the signal of the bead population appears to decrease for the area measurement due to the subtraction of signal in the process of baseline restoration. **Height:** The height measurement appears to increase as there is no negative measurement associated with height, but we see an increase in the pile up of data with a signal value of 0 MFI. This skews the signal to the right which results in the apparent increase in signal.

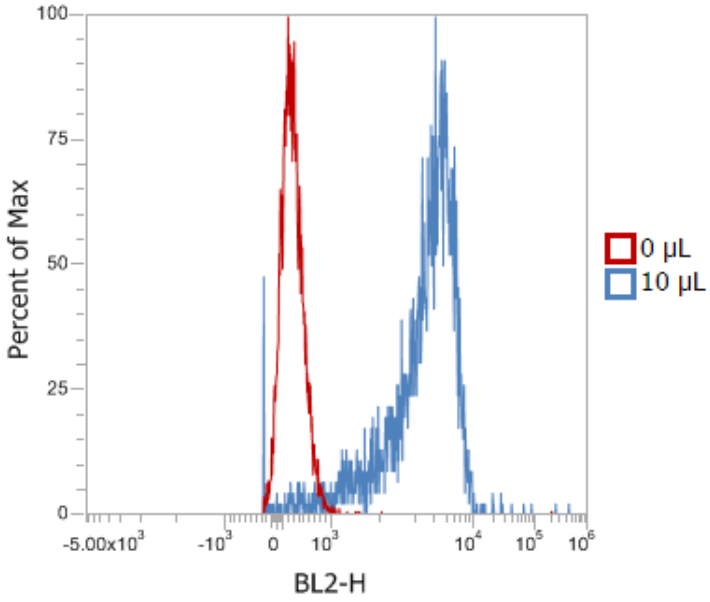


Figure 5. Figure showing the pile up of data with a signal of 0 at high concentrations of unbound dye.

To mitigate the effect of unbound dye on the background subtraction algorithm (baseline restoration), the samples should be washed or diluted to a level where the extent of free or unbound dye is low enough to not significantly contribute to the background. This will ensure that the background subtraction algorithm is mainly considering system noise (that is electronic noise/shot noise) and not including significant fluorescence contribution from the sample buffer.

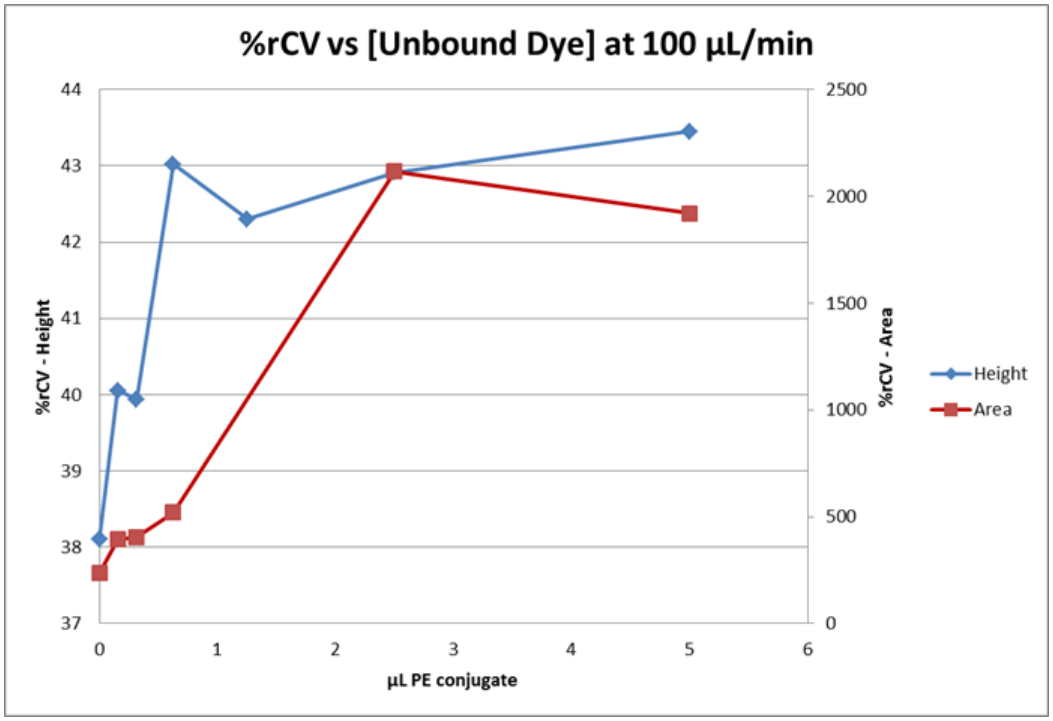


Figure 6. This chart shows the relationship of %CV to the presence of unbound dye. As the concentration of unbound dye increases, the spread (%CV) of the data also increases. Since the standard deviation is based on the photon count, higher levels of background result in a higher standard deviation. The standard deviation of the measurement persists after the digital processing subtracts this background from the measure signal which results in higher CVs.

System optimization

As described in Background subtraction/ Baseline restoration (page 97), unbound dye or buffers containing autofluorescent components can obfuscate our ability to resolve dim populations from noise. To minimize these effects, it is important to reduce the amount of free dye by diluting or thoroughly washing the samples. In addition, we should select sample buffers that do not exhibit autofluorescence in the fluorescence channels used in the flow cytometry assay.

Instrument settings optimization is also critical for resolving dim populations from noise. The goal is to set the voltages such that the signal we are measuring is set at a level where the contribution of electronic noise is negligible (Maecker & Trotter, 2006). The optimal voltage settings cannot be set using an unstained control alone as it may not be possible to resolve the autofluorescence from the electronic noise.

To determine the optimal voltage settings, a control should be run that ideally has both a positive and negative population and the voltage should be walked from low to high to determine the setting where we maximize the separation of the populations while minimizing the noise. The stain index (SI) is a quantitative way to determine the optimal setting, which is the PMT voltage that maximizes the SI:

$$\textbf{Stain Index (SI):} \quad \frac{\text{Mean (pos)} - \text{Mean (neg)}}{2\text{xSD of Neg}}$$

Figure 7. Equation for staining index used to determine optimal voltage where the control includes both the positive and negative populations.

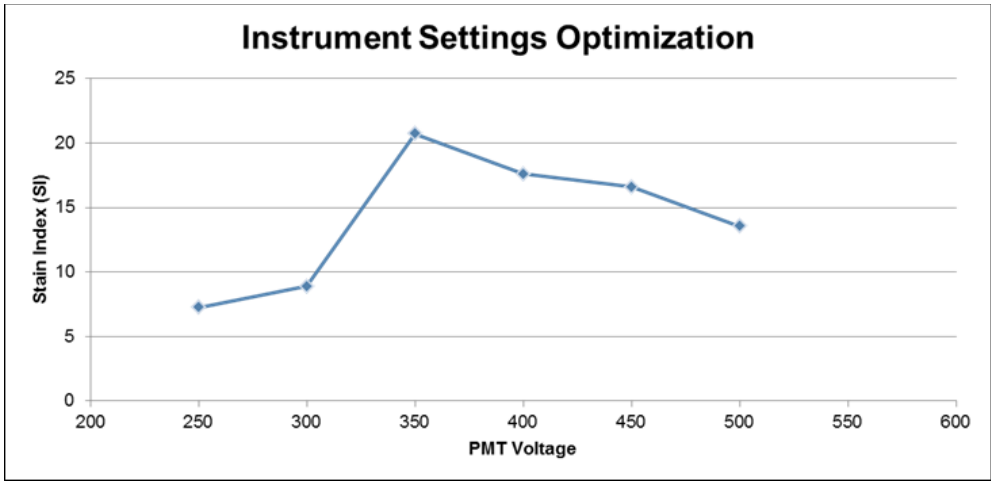


Figure 8. The optimal PMT voltage is the voltage that maximizes the Stain Index (SI). In this example, AbC™ beads were incubated with CD4-PE and the SI was measured at PMT voltages from 250 to 500.

If a control is not available that includes both a negative and positive population, the minimum voltage can be ascertained by setting the voltage based on a particle simulating dim fluorescence and walking the voltage and measuring the %CV of the signal at each PMT setting. The voltage at which the CV stabilizes is the minimum voltage setting that should be used for the specific channel.

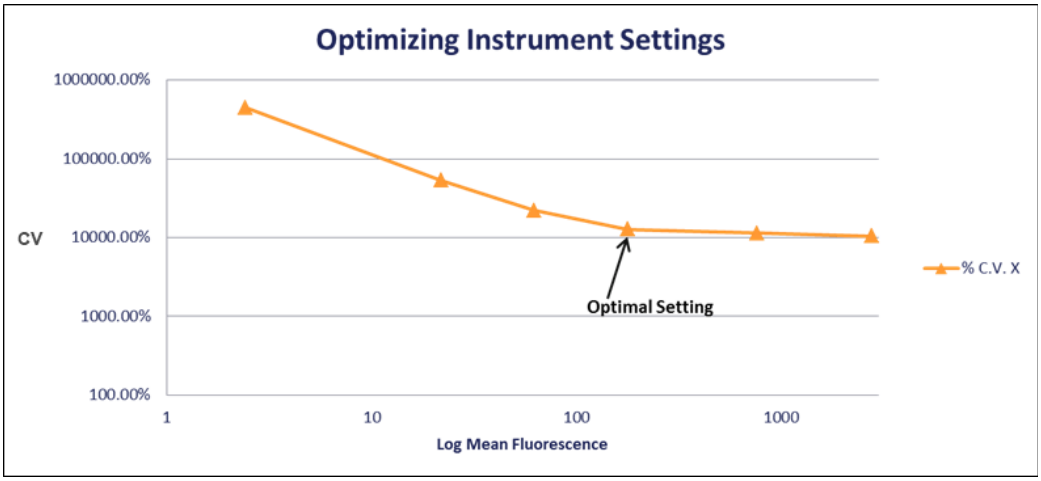


Figure 9. Determining the minimum PMT voltage for a detector can be achieved by measuring the CV at different voltage settings and plotting CV vs. Log (PMTV). The inflection point of this curve represents the minimum voltage that should be used for that detector or channel (Maecker & Trotter, 2006).

In addition to voltage settings, threshold settings should also be optimized. Since the baseline subtraction algorithm is based on the signal below the threshold, the threshold can have a large impact on the fluorescence signal of dim or negative populations when there is significant fluorescence due to unbound dye or autofluorescent components in the buffer. The threshold should be set high enough to eliminate noise or debris from the data, but low enough to minimize the impact the sample buffer on the background subtraction. In the case where diluting the sample or choosing a different buffer is not possible, the threshold value should be optimized in a similar fashion to how voltages are optimized. The voltages should be optimized first, and then the threshold setting should be adjusted to optimize the signal or SI for the fluorescence channels.

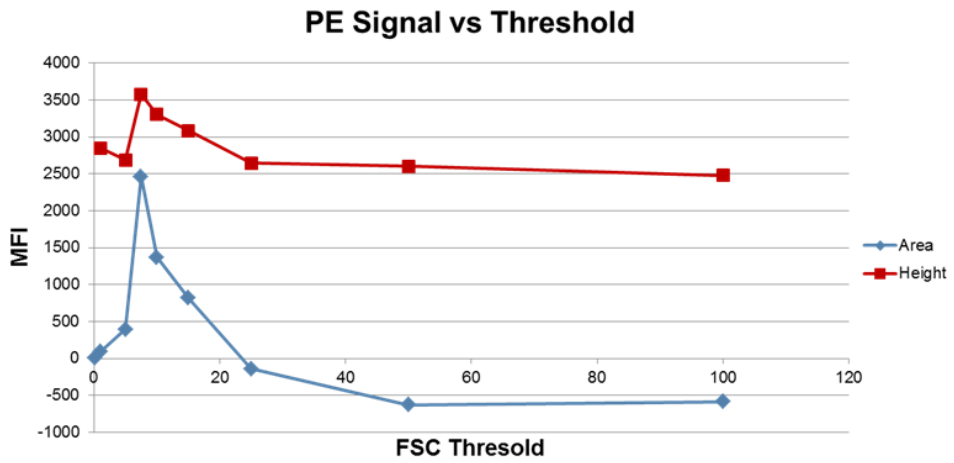


Figure 10. Threshold was optimized by measuring the signal in the PE (BL2) channel and adjusting the threshold value in the FSC channel. 5 µL/mL of CD4-PE conjugate was incubated with negative AbC™ beads. When the threshold is set too low, the signal is dominated by electronic noise; when the threshold value is set too high, the unbound dye adversely affects the background subtraction algorithm.

Summary

Although the Attune™ NxT and Attune™ CytPix™ Flow Cytometers minimize variation at higher flow rates due to particle focusing that are not feasible with hydrodynamic focusing alone, it is still subject to variation and error due to photon counting statistics and factors that contribute to noise in the measurement just like any other cytometer.

Electronic noise and photon counting statistics are a significant component to the standard deviation and error for dim and negative populations that should be considered when setting up the system. Care should be taken to properly adjust instrument settings including PMT voltages and thresholds to minimize the contribution of electronic noise and photon counting statistics to the signal we are trying to measure.

In addition to optimizing the instrument settings, sample preparation is important to ensure the sample itself does not contribute to noise and obfuscate our ability to resolve dim populations. The degree of unbound dye or autofluorescence components in the buffer should be minimized to mitigate the effects the buffer has on the digital signal processing that takes places when acquiring a sample. These effects are magnified at higher flow rates due to the increased sample core size, which will result in increased subtraction of this baseline fluorescence resulting in potential over correction of the baseline and increased spread (CV) of the data that is more pronounced for negative or dim signals.

Reference

Maecker, HT & Trotter, J. (2006) Flow Cytometry Controls, Instrument Setup, and the Determination of Positivity. Cytometry 69A:1037–1045.

Appendix G: Ordering information for consumable and spare parts

Consumables and spare parts

The following products and replacement parts for the Attune™ NxT and Attune™ CytPix™ Flow Cytometers are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 118).

Product	Amount	Cat. No.
Attune™ Performance Tracking Beads	3 mL	4449754
Attune™ Focusing Fluid, 1X Solution	1 × 1 L 6 × 1 L 1 × 10 L	4488621 4449791 A24904
Attune™ Wash Solution	250 mL	A24974
Attune™ 1X Shutdown Solution	250 mL	A24975
Attune™ Debubble Solution (1X)	50 mL	A10496
Attune™ NxT Flow Cell Cleaning Solution	30 mL	A43635
Attune™ NxT Wash Bottle, 175 mL	1 each	100022151
Attune™ NxT Shutdown Bottle, 175 mL	1 each	100022154
Attune™ NxT Waste Bottle, 1.9 L	1 each	100022156
Attune™ focusing fluid Bottle, 1.9 L	1 each	100022155
Attune™ NxT Sample Syringe, 1 mL	1 each	100022591
Attune™ focusing fluid Filter	1 each	100022587
Attune™ Custom Filter Holder Kit	1 each	A27784
Attune™ NxT No-Wash No-Lyse Filter Kit	1 kit	100022776
Attune™ NxT Fluorescent Protein Filter Kit	1 kit	100022775
Attune™ NxT Maintenance Kit	1 kit	A43038
Attune™ NxT Blocker Bar Conversion Kit	1 kit	A35966
Attune™ NxT Tubing DIBA 517-022 (SIP tube replacement)	1 each	4490099

Attune™ Auto Sampler spare parts

The following products and replacement parts for the Attune™ Auto Sampler are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 118).

Product	Amount	Cat. No.
Attune™ Auto Sampler Focusing Fluid Bottle	1 each	4477847
Attune™ Auto Sampler Waste Bottle	1 each	4477850
Attune™ Auto Sampler Syringe, 1 mL	1 each	4478686
Attune™ NxT Auto Sampler Plug, 1/4-28 Teflon	1 each	4476990

CytKick™/CytKick™ Max Autosampler spare parts

The following products and replacement parts for the CytKick™ and CytKick™ Max Autosamplers are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 118).

Product	Amount	Cat. No.
CytKick™ Autosampler PLUG,1/4-28 Teflon	1 each	4476990
CytKick™ Autosampler Bottle Assembly – Focusing Fluid	1 each	100054670
CytKick™ Autosampler Bottle Assembly – Waste	1 each	100054669
CytKick™ Autosampler 1 mL Syringe	1 each	100054593
CytKick™ Autosampler Tubing Assembly	1 each	100054481

Attune™ NxT External Fluid Supply spare parts

The following products and replacement parts for the Attune™ NxT External Fluid Supply are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 118).

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

Attune™ filters

The following replacement filters used in the optics path of the Attune™ NxT and Attune™ CytPix™ Flow Cytometers are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 118).

Product	Amount	Cat. No.
Long Pass filters		
Attune™ NxT Filter, 413LP	1 filter	100022752
Attune™ NxT Filter, 496LP	1 filter	100022753
Attune™ NxT Filter, 569LP	1 filter	100022754
Attune™ NxT Filter, 646LP	1 filter	100022755
Dichroic Long Pass filters		
Attune™ NxT Dichroic Filter, 495DLP	1 filter	100022769
Attune™ NxT Dichroic Filter, 555DLP	1 filter	100022776
Attune™ NxT Dichroic Filter, 600DLP	1 filter	100022771
Attune™ NxT Dichroic Filter, 650DLP	1 filter	100022772
Attune™ NxT Dichroic Filter, 690DLP	1 filter	100022773
Attune™ NxT Dichroic Filter, 740DLP	1 filter	100022774
Band Pass filters		
Attune™ NxT Emission Filter, 440/50BP	1 filter	100022756
Attune™ NxT Emission Filter, 512/25BP	1 filter	100022757
Attune™ NxT Emission Filter, 530/30BP	1 filter	100022758
Attune™ NxT Emission Filter, 585/16BP	1 filter	100022759
Attune™ NxT Emission Filter, 590/40BP	1 filter	100022760
Attune™ NxT Emission Filter, 603/48BP	1 filter	100022761
Attune™ NxT Emission Filter, 620/15BP	1 filter	100022762
Attune™ NxT Emission Filter, 670/14BP	1 filter	100022763
Attune™ NxT Emission Filter, 695/40BP	1 filter	100022764
Attune™ NxT Emission Filter, 710/50BP	1 filter	100022765
Attune™ NxT Emission Filter, 720/30BP	1 filter	100022767
Attune™ NxT Emission Filter, 780/60BP	1 filter	100022766
Other filters		
Attune™ NxT Small Particle Side-Scatter Filter	1 filter	100083194
Attune™ NxT OD2-488/10 Filter	1 filter	100022768

Appendix H: Safety

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

- Before using an instrument or device, read and understand the safety information in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

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

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

Standard safety symbols

Symbol	English	Français
	**CAUTION!** Risk of danger. Consult the manual for further safety information.	**MISE EN GARDE !** Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
	**CAUTION!** Risk of electrical shock.	**MISE EN GARDE !** Risque de choc électrique.
	**CAUTION!** Hot surface.	**MISE EN GARDE !** Surface chaude.
	**CAUTION!** Laser radiation.	**MISE EN GARDE !** Rayonnement laser.
	**CAUTION!** Moving parts.	**MISE EN GARDE !** Parties mobiles.
	**CAUTION!** Piercing hazard.	**MISE EN GARDE !** Danger de perforation.
	**CAUTION!** Potential biohazard.	**MISE EN GARDE !** Danger biologique potential.
	**CAUTION!** Ultraviolet light.	**MISE EN GARDE !** Rayonnement ultraviolet.

Safety alerts on this instrument

Symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**MISE EN GARDE !** Produits chimiques dangereux. Lire les fiches signalétiques (FS) avant de manipuler les produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**MISE EN GARDE !** Déchets dangereux. Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à 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 Thermo Fisher Scientific 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 Thermo Fisher Scientific.
	**CAUTION!** Class 3B (III) visible and/or invisible laser and/or LED radiation present when open. Avoid exposure to beam.	**MISE EN GARDE !** Rayonnement laser ou DEL visible ou invisible de classe 3B, (III) présent en position ouverte. Éviter toute exposition au faisceau.

Location of safety labels

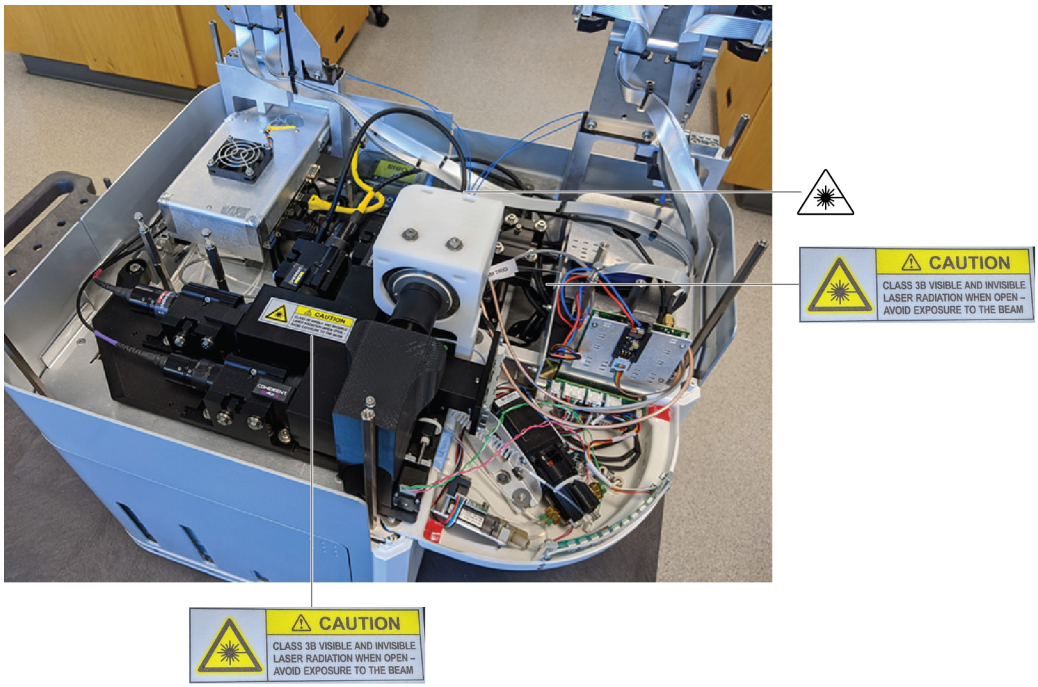
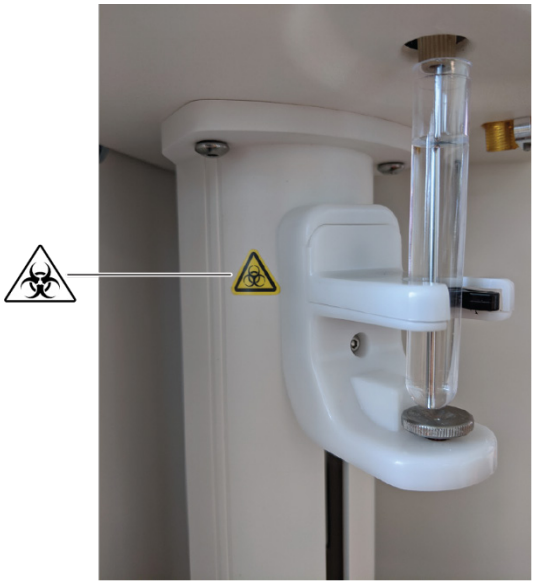
Label and location


Figure 1. Optics compartment top view (cover removed)

Label and location	
 Figure 2. Sample injection port	 Figure 3. Waste fluid bottle in the fluidics compartment (fluidics compartment door open)
 Figure 4. Location of the label with regulatory symbols	

Control and connection symbols

Symbol	English	Français
	On	On (marche)
	Off	Off (arrêt)
	Standby	En attente
	On/Off	On/Off (marche/arrêt)
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif

Environmental symbols

Symbol	English	Français
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. **CAUTION!** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Instrument safety

General

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

Physical injury

CAUTION! **Moving and Lifting Injury.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide.

Improper lifting can cause painful and permanent back injury.

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

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

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

Electrical safety

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

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

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

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

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

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

WARNING! **Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

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

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

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

Overvoltage rating

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

Cleaning and decontamination

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

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

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

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

Instrument component and accessory disposal

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

Laser safety

WARNING! LASER HAZARD. Under normal operating conditions, the Attune™ NxT Flow Cytometer is categorized as a Class 1 laser product. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class 3B (III) laser. Lasers can burn the retina, causing permanent blind spots. To ensure safe laser operation:

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

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

Alert
 **DANGER!** Class 3B (III) visible and/or invisible laser and/or LED radiation present when open. Avoid exposure to beam.

Alerte
 **DANGER !** Rayonnement laser ou DEL visible ou invisible de classe 3B, (III) présent en position ouverte. Éviter toute exposition au faisceau.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture comply with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union "Low Voltage Directive"
EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 60825-1 EN 60825-1	*Safety of laser products – Part 1: Equipment classification and requirements*
21 CFR 1040.10 and 1040.11 as applicable	U.S. FDA Health and Human Services (HHS) "Radiological health performance standards for laser products" and "Radiological health performance standards for specific purpose laser products"

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive"—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive"—Restriction of hazardous substances in electrical and electronic equipment

Chemical safety

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

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

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

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du recipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)

- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

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

Biological hazard safety

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

WARNING! **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at:
  www.cdc.gov/biosafety
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at:
  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 local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at:
  www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Documentation and support

Related documentation

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

Guide	Pub. No.
Attune™ Cytometric Software User Guide	100024236
Attune™ NxT Flow Cytometer Quick Reference Guide	100024233
Attune™ NxT Flow Cytometer User Guide	100024235
Attune™ NxT Flow Cytometer Site Preparation Guide	100024428
Attune™ CytPix™ Flow Cytometer User Guide	MAN0019440
Attune™ CytPix™ Flow Cytometer Site Preparation Guide	MAN0019443
Attune™ NxT External Fluid Supply User Guide	100038577
Attune™ NxT External Fluid Supply Quick Reference Guide	100037944
Attune™ Auto Sampler User Guide	100032905
CytKick™ Autosampler and CytKick™ Max Autosampler User Guide	MAN0018351
Attune™ Cytometric Software Network DK2 DESkey Installation Guide	100070568
Attune™ NxT SAE Administrator Console User Guide	MAN0019099

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

Customer and technical support

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

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

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

Note: When contacting customer support for instrument troubleshooting, provide the instrument model and the instrument serial number. Convey to the technical support any error messages that were displayed on your instrument and any troubleshooting that you have already performed.

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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

For support visit **thermofisher.com/support**

thermofisher.com

15 September 2021