invitrogen

iSort™ Automated Cell Sorter

Catalog Number A33041

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

ThermoFisher SCIENTIFIC

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Revision history: MAN0016718

Revision	Date	Description
A.0	19 October 2018	New user guide

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Contents

About this guide

Audience

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Invitrogen™ iSort™ Automated Cell Sorter.

User attention words

Two user attention words appear in this document. Each word implies a particular level of observation or action as described below.

Note: Provides information that may be of interest or help but is not critical to the use of the product.

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

Safety alert words

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

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

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

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

1. Product information

Product description

iSort™ Automated Cell Sorter

The Invitrogen™ iSort™ Automated Cell Sorter (Cat. No. A33041) is a compact, automated cell sorter designed for simplicity and affordability. The system contains a 488-nm laser and is optimized for sorting cells or particles expressing GFP.

The iSort™ Automated Cell Sorter features an easy-to-use touch-screen interface that requires minimal training. By automating the complex presort setup, the iSort™ Automated Cell Sorter creates a more efficient and consistent workflow.

The small footprint allows the self-contained iSort™ Automated Cell Sorter to fit within a standard biosafety cabinet or on a basic laboratory cart for portability.

Product use

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

Standard items included

The iSort™ Automated Cell Sorter is shipped with the system components listed below. All components are shipped at ambient temperature.

- iSort™ Automated Cell Sorter
- 1 sheath fluid bottle
- 1 waste bottle
- 1 accessory box (located in the instrument box), containing:
  - Power cables
  - Power supply
  - 1 replacement nozzle tip and cover
  - 1 torque wrench
  - 6 O-rings

Note: For a list of replacement parts for the iSort™ Automated Cell Sorter, see "Appendix C: Ordering information", page 128.

Required materials not supplied

The following table lists the materials recommended for use with the iSort™ Automated Cell Sorter. The sample and collection tubes listed have been tested with the system. Unless otherwise indicated, all materials are available through **thermofisher.com**.

MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Purpose	Supplier	Catalog No.
50-mL Falcon™ tube	Collection tube	Fisher Scientific	14-432-22
5-mL Falcon™ Round-Bottom Polypropylene tubes	Sample and Collection tube	Fisher Scientific	14-959-11A
1.5-mL Tube (non-sterile)	Collection tube	VWR	89000-288
1.5-mL Tube (sterile)	Collection tube	VWR	89000-290
Falcon™ Test Tube with Cell Strainer Snap Cap	Sample preparation	Fisher Scientific	08-771-23
Flowmi™ Cell Strainers for 1000-µL Pipette Tips	Sample preparation	Bel-Art™ SP Scienceware™	H136800040
		Fisher Scientific	14-100-150
Partec CellTrics™ Filter 30 µm	Sample preparation	Sysmex	04-0042-2316
Partec CellTrics™ Filter 50 µm	Sample preparation	Sysmex	04-0042-2317
PBS (1X), pH 7.4 (flow cytometry grade)	Sheath fluid, sample preparation	Thermo Fisher Scientific	A1286301
PBS (10X), pH 7.4	Calibration and QC	Thermo Fisher Scientific	70011044
Tween™ 20 (50% solution)	Calibration and QC	Thermo Fisher Scientific	003005
Attune™ Performance Tracking Beads	Calibration and QC	Thermo Fisher Scientific	4449754
Attune™ Wash Solution	Daily Shut Down	Thermo Fisher Scientific	A24974
Decon™ Contrad™ 70 Liquid Detergent	Nozzle tip cleaning and storage	Fisher Scientific	04-355-01
Distilled water	Preparation of solutions	Thermo Fisher Scientific	15230162
Deionized water, reagent grade	Instrument cleaning	Thermo Fisher Scientific	751610
Bleach (without additives and perfumes)	Fluidics deep clean and decontamination	MLS	MLS
Texwipe™ TX761MD Microdenier Swabs	Cleaning of optical elements	Thermo Fisher Scientific	A36701
Porta PlumeSafe™ 604 Smoke Evacuation System *(optional)*	Biosafety and aerosol management when sorting cells under BSL-2 conditions	Buffalo Filter	PPS604
Ultrasonic cleaning bath	Nozzle tip cleaning	MLS	MLS
Analytical scale (with 4 decimal places)	Sort performance verification during installation	MLS	MLS
Pipettes (10-µL, 200-µL, 1000-µL) and pipette tips	Sample preparation	MLS	MLS

Instrument exterior components

Front view

1. Touch-screen monitor
2. Power button
3. USB port, Type A
4. Sorting compartment
5. Fluidics compartment
6. Sample pump compartment

Front view with sorting compartment door open

Front view with fluidics compartment door open

Rear view

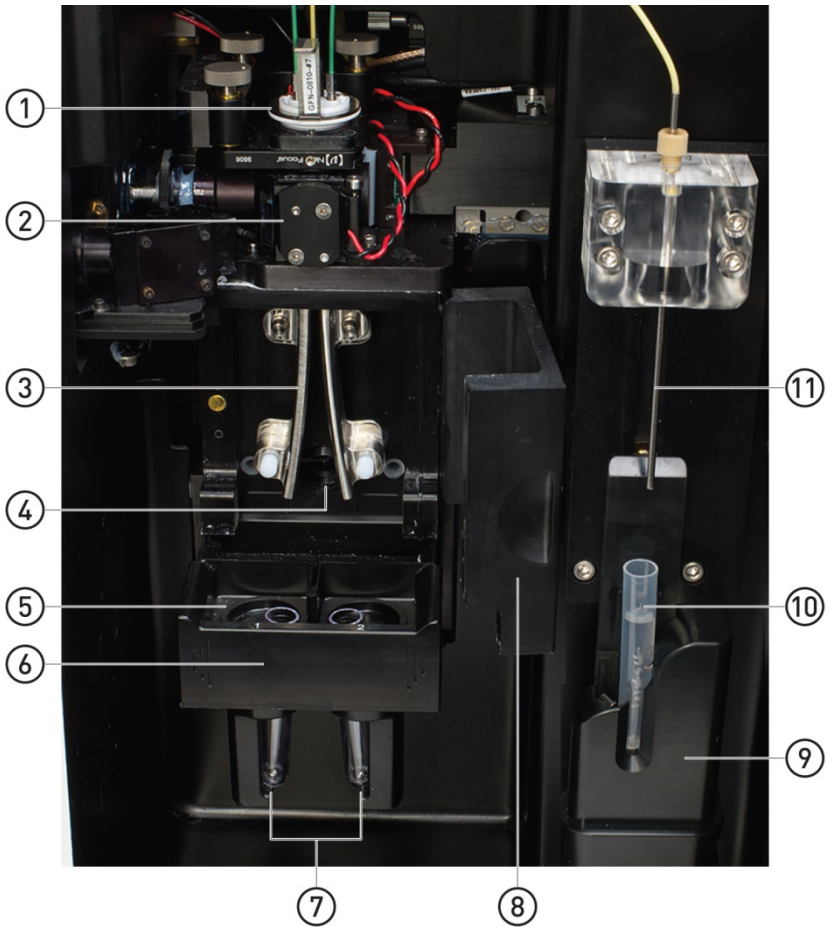
1. HDMI port (see Important note below)
2. USB ports, Type A (2×)
3. USB port, Type B
4. Ethernet connection port
5. Power input port
6. Air exhausts

IMPORTANT! The HDMI port is used by Technical Service representatives to monitor system performance with diagnostic tools. We do not recommend attaching an external monitor to the iSort™ Automated Cell Sorter via the HDMI port as this might compromise instrument performance.

Rear view with fluidics compartment door partially open

Rear view with fluidics compartment door fully open

Sorting compartment

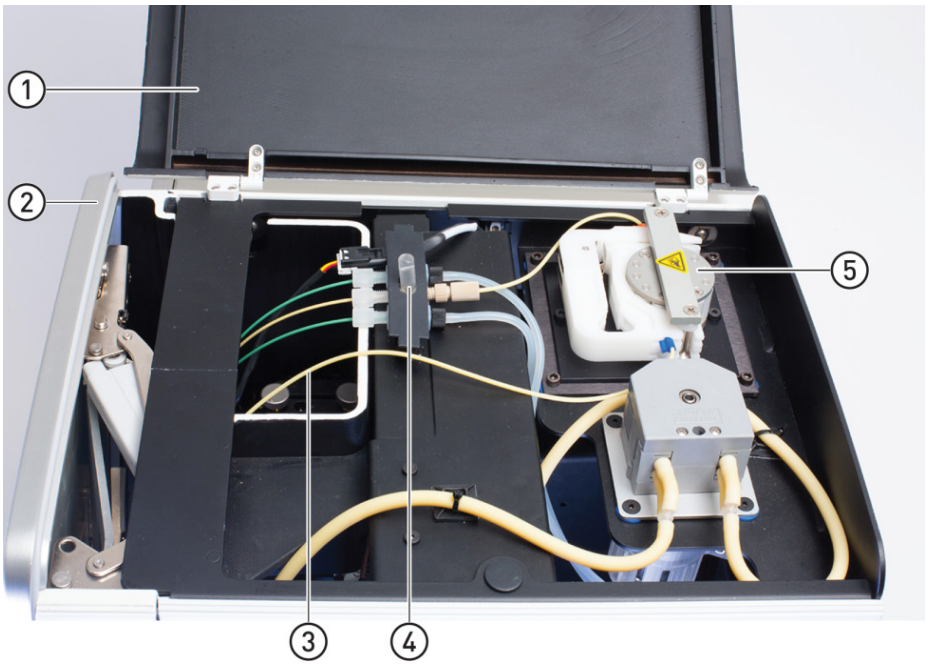


1. Nozzle assembly
2. Laser interrogation chamber
3. Deflection plates
4. Streamwatch camera window
5. Collection tray (shown in out position)
6. Collection tube holder (shown with two 12 × 75-mm tubes with 5-mL capacity; can hold any combination of 1.5-mL, 5-mL, or 50-mL tubes)
7. Collection tubes (5-mL tubes shown)
8. Sort chamber door (shown open)
9. Sample tube holder (shown in down position)
10. Sample tube (12 × 75-mm tube with 5-mL sample capacity)
11. Sample input port (SIP)

Note: The sample input port (SIP) is used for loading samples for Analysis or Sort modes, and for injecting solutions during maintenance and cleaning cycles.

For instructions on how to load your samples to the iSort™ Automated Cell Sorter for Analysis or Sort, see page 35.

Sample pump compartment

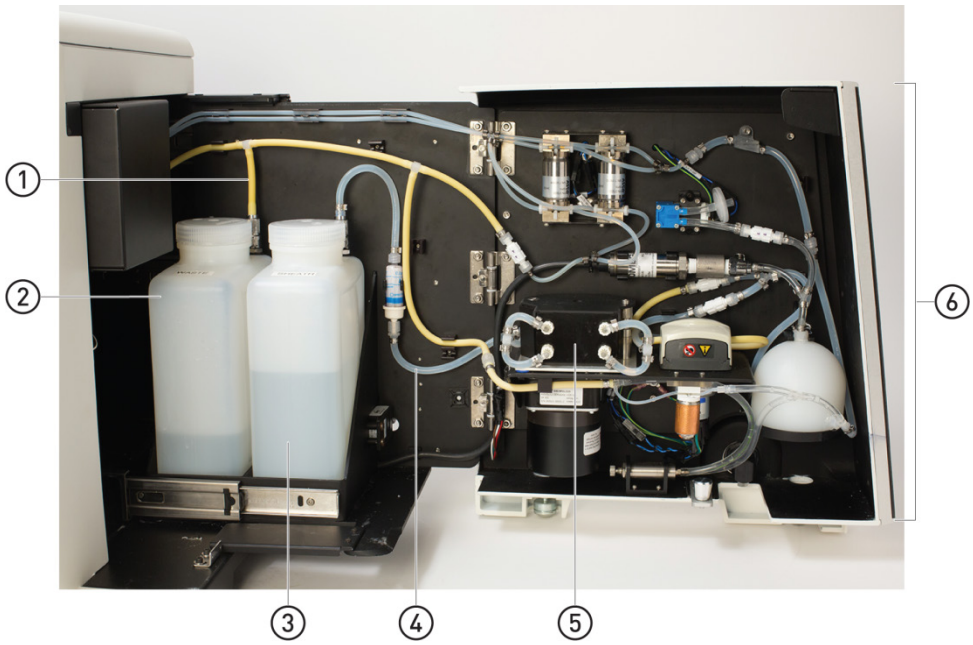


Note: The front of the instrument is facing to the left.

1. Top cover
2. Sorting compartment door
3. Sample line
4. Sample pump dampener
5. Sample pump

Rear view of instrument with the top cover open, showing the sample pump compartment

Fluidics compartment



1. Waste fluid lines (yellow)
2. Waste container
3. Sheath fluid container
4. Sheath fluid lines (clear)
5. Sheath fluid pump assembly
6. Fluidics compartment door (contains fluidics lines, filters, and the sheath pump

Fluid containers (detail)

1. Waste container (2 L capacity)
2. Waste fluid line with quick release connector
3. Sheath fluid container (2 L capacity)
4. Sheath fluid line with quick release connector

Note: For instructions on how to refill or empty the fluid containers, see page 23.

Graphical user interface (GUI)

The iSort™ Automated Cell Sorter utilizes a graphical user interface (GUI) accessed by a touch-screen monitor. The integrated iSort software is pre-installed on the instrument.

GUI layout and Home Screen

Key functions of the software (Sort, Analyze, Files, Maintenance, and Settings) are displayed on the home screen. These functions contain all menus and controls necessary to execute the selected function.

1. **Sort:** Allows you to perform a one-way or two-way sort. For a detailed description of the Sort workflow, see page 31.
2. **Analyze:** Analysis (or non-sorting experiment) is designed for quick verification of sample content and can be performed independently of the Sort workflow. For a detailed description of the Analyze workflow, see page 31.
3. **Files:** Allows you to manage data files, which are saved in the FCS 3.0 format after a sort or analysis run. For a detailed description of the Files screen and how to save and manage data files, see page 46.
4. **Maintenance:** Allows you to execute various instrument maintenance functions, including cleaning cycles, instrument calibration, and QC analysis. For a detailed description of the Maintenance functions, see page 51.
5. **Settings:** Allows you to start and stop fluid flow to the instrument, to set stop criteria (i.e., number of events) for analysis runs, and to review calibration and system information. For a detailed description of the Settings menu, see page 92.

2. Prepare site for installation

Site preparation workflow

IMPORTANT! Do not install the iSort™ Automated Cell Sorter yourself. A Thermo Fisher Scientific representative will contact you to schedule the installation.

When the installation is scheduled:

1. Receive and inspect the system (page 19).
2. Move the crated instrument to the installation site (page 19).
3. Complete the site preparation checklist before the installation date (page 15).

Timeline and training

Installation of the iSort™ Automated Cell Sorter is performed by a Thermo Fisher Scientific Field Service Engineer. Once successfully installed, the engineer performs the necessary installation tests. This entire process takes approximately two days.

During and/or after installation, the Thermo Fisher Scientific Service representative will walk through basic instrument maintenance functions. Additional training is provided separately by a Thermo Fisher Scientific Applications specialist.

Site preparation checklist

IMPORTANT! Complete all items in the checklist before the scheduled installation date. If the site preparation checklist is not complete when the Thermo Fisher Scientific service representative arrives, the scheduled installation may be delayed.

✓	Site preparation requirement	Page
	Customer responsibilities have been reviewed and personnel assigned.	16
	The installation site is identified and meets requirements: ☐ Space and clearance ☐ Environmental ☐ Electrical ☐ Network ☐ Safety	17
	Materials that are needed for installation and operation are available.	19
	The system was received and inspected: ☐ All items on the shipping list are the same items that were ordered at the time of purchase. ☐ Any damage to shipping containers was recorded on the shipping containers	19
	The installation site is cleared and ready for instrument installation.	19
	The crated instrument and other shipping containers are moved to the installation site.	19

Pre-installation requirements

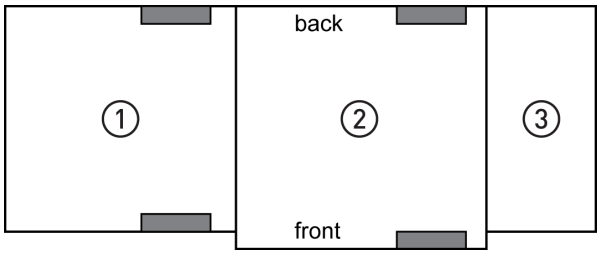
Personnel	Responsibilities
Instrument maintenance personnel	• Reviews the site preparation guide for safety information and system requirements. • Coordinates personnel and tasks. • Orders required materials. • Selects the site. • Reviews checklists with appropriate personnel, then with the Thermo Fisher Scientific service representative to ensure that the site is properly prepared. • Receives and inspects the system. • Schedules the installation and notifies personnel of the installation date. • Ensures that the site is clear of unnecessary material on the installation day. • Is available to help the service representative throughout installation. • Ensures that installation requirements are met for: – Space at the installation site – Building clearances – Temperature and humidity – Waste collection – Electrical supply – Computer – Required safety equipment (page 18) and installation materials (page 19) • If possible, move the crated system to the site before the installation date. • Available to help service representative and laboratory personnel throughout installation. • Review safety information. • Ensure that all customer-provided materials for installation are present at the site. • Primary users (responsible for training other users) are available during the installation, so that they can receive initial training on the instrument. • Reviews the user guide for safety information. • Ensures that the required safety practices and equipment are in place. • Is in the vicinity and available to the Thermo Fisher Scientific service representative when the service representative is at your facility.
Network or IT specialist (if the system will be connected to a network)	• Ensures that one active, tested wireless local area network (WLAN) is in place for Wi-Fi connection before the scheduled installation date. • Is available during installation to connect the system to the network. The Wi-Fi connections will also be used for remote troubleshooting if a problem arises. **Note:** The Thermo Fisher Scientific Field Service Engineer will connect the Wireless Wi-Fi Dongle – Linksys™ AE3000 N900 Dual-Band Wireless-N USB Adapter during installation.

Operating environment and site requirements

- The iSort™ Automated Cell Sorter has a footprint (W × D × H) of approximately 67 cm × 41 cm × 49 cm (26.5 in × 16 in × 19 in) with the fluidics compartment door and the top cover closed.

  With the top cover open, the height of the instrument is 74 cm (29 in).

  With the fluidics compartment door open, the entire system requires a total bench width of ~135 cm (53.2 in).
- The setup for the iSort™ Automated Cell Sorter is shown below (top down view). The gray rectangles in the diagram represent the four handholds in the base that are used when lifting the instrument out of the box.



① Touch-screen monitor
② Sorting compartment
③ Fluidics compartment

IMPORTANT! Do **not** lift the iSort™ Automated Cell Sorter from the fluidics compartment. Lifting from the fluidics compartment can damage the instrument. Lift the instrument using the designated handholds.

- The bench or table top must be able to accommodate 43 kg (95 lbs), the weight of the instrument plus the weight of the full 2-liter fluid bottles (sheath fluid and waste).
- The iSort™ Automated Cell Sorter should be placed on a level surface away from vibrations from other pieces of equipment. Tabletop centrifuges, vortex mixers, and other laboratory equipment can vibrate the instrument during a run and impact instrument performance.
- The area should be free of excessive dust or moisture. Position the instrument so that the power button and main power supply can be reached easily.
- Allow at least 6.5 cm (2.5 in) free space at the back of the instrument to allow for proper ventilation and prevent overheating of electronic components.
- Ambient temperature range: 18°–25°C (64°–77°F)
- Storage temperature range: 5°–35°C (41°–95°F)
- Relative humidity range: 20–70%
- Electrical input: 100–240 VAC, 4.0 A
- Frequency: 50–60 Hz

IMPORTANT! Cell sorting creates aerosols. Exposure to and inhalation of aerosols can potentially be hazardous depending on the cell types you are using. Consult your institution's safety officer for guidelines related to your individual samples. The iSort™ Automated Cell Sorter may need to be placed in a biosafety cabinet, depending on your institutions' safety requirements. The use of a filtration system (such as the Porta PlumeSafe™ 604 system from Buffalo Filter; page 8) may also be required to meet BSL-2 safety requirements of your institution.

Safety requirements

Safety practices

A safety representative from your facility must ensure that:

- Personnel establish and follow all appropriate safety practices and policies to protect laboratory personnel from potential hazards.
- All appropriate safety devices and equipment are always available.

Required safety equipment

The following safety protection and equipment must be available at the installation site:

- Protection from any sources of hazardous chemicals, radiation (for example, lasers, radioisotopes, radioactive wastes, and contaminated equipment), and potentially infectious biological material that may be present in the area where the Thermo Fisher Scientific service representative will work.
- Appropriate fire extinguisher appropriate for use on electrical and chemical fires as specified in current codes, regulations, and/or standards, and with approval of the Fire Marshall or other authority having jurisdiction.
- Eyewash
- Safety shower
- Eye and hand protection
- Sufficient ventilation, including vent line/fume hood, if applicable
- Biohazard waste container, if applicable
- First-aid equipment
- Spill cleanup equipment
- Applicable Safety Data Sheets (SDSs)

WARNING! BIOHAZARD. Cell sorting creates aerosols. Exposure to and inhalation of aerosols can potentially be hazardous depending on the cell types you are using. Consult your institution's safety officer for guidelines related to your individual samples.

The iSort™ Automated Cell Sorter may need to be placed in a biosafety cabinet and/or connected to an evacuator filter/pre-filter or liquid disinfectant trap, depending on your institutions safety requirements.

We recommend that you follow the International Society for the Advancement of Cytometry (ISAC) guidelines for cell sorter biosafety standards (**www.ncbi.nlm.nih.gov/pmc/articles/PMC4117398/**).

Materials required for installation

- Safety glasses, lab coats, and chemical-resistant, disposable gloves (powder-free)
- Lint-free tissues
- Isopropanol, HPLC-grade or better
- 10% bleach solution
- Distilled water
- 5-mL and 50-mL Polypropylene Falcon™ tubes
- 1.5-mL centrifuge tubes
- PBS (1X), pH 7.4 (flow cytometry grade)
- PBS (10X), pH 7.4
- Tween™ 20 (50% solution)
- Attune™ Performance Tracking Beads
- Attune™ Wash Solution
- Decon™ Contrad™ 70 Liquid Detergent
- Texwipe™ TX761MD Microdenier Swabs
- Pipettes (10-µL, 200-µL, 1000-µL) and pipette tips
- Analytical scale (with 3 to 4 decimal places)
- Ultrasonic cleaning bath (such as Fisherbrand™ CPX Series Digital Ultrasonic Cleaning Bath or similar)

Prepare for installation

Receive and inspect the shipment

Carefully inspect the shipping containers and report any damage to the Thermo Fisher Scientific service representative. Record any damage or mishandling on the shipping documents.

Move the instrument to the installation site

1. Clear the installation site.
2. If possible, move the crated instrument and other shipping containers to the installation site. Do not uncrate.

CAUTION! PHYSICAL INJURY HAZARD. The weight of the iSort™ Automated Cell Sorter is 41 kg (89 lbs). Do not attempt to lift or move the instrument without the help of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons

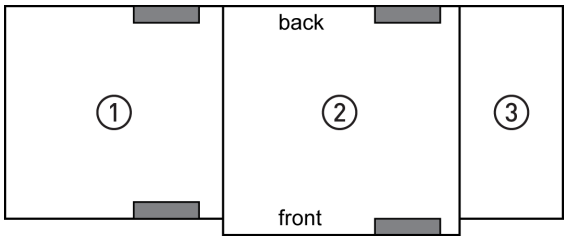
IMPORTANT! Do not subject the iSort™ Automated Cell Sorter to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

Set up

IMPORTANT! The following set-up instructions are provided for informational purposes only. **Do not attempt the initial set-up of the instrument on your own.** Thermo Fisher Scientific service representative will install the iSort™ Automated Cell Sorter and all of its components and provide some basic operator training.

Place the instrument at the installation site

1. After the instrument has been uncrated, verify all parts are present. See page 7 for the list of standard items included in the shipment.
2. Carefully lift the instrument out of the box with the help of at least one other person, holding it by the four handholds in the base (gray rectangles in the diagram).



① Touch-screen monitor
② Sorting compartment
③ Fluidics compartment

CAUTION! PHYSICAL INJURY HAZARD. The weight of the iSort Automated Cell Sorter is 41 kg (89 lbs). Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons

IMPORTANT! Do **not** lift the iSort™ Automated Cell Sorter from the fluidics compartment. Lifting from the fluidics compartment can cause damage to the instrument. Lift the instrument only using the designated handholds.

IMPORTANT! Do not subject the iSort™ Automated Cell Sorter to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

3. Examine the instrument carefully for any damage that may have incurred during transit. Contact your distributor if anything is missing. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage. If you do not have your distributor information, contact Technical Support (page 139).
4. Place the instrument on the installation site and leave enough room around it for the hinged doors of the sorting and fluidics compartments to move and open freely.

Connect the instrument

1. Confirm that the power is switched off.
2. Plug the power cable into the power supply, then plug the power cable to the wall outlet. Check for the light on the power supply.
3. Plug the power supply connector into the instrument.

Note: At this point, everything should be plugged in and OFF. Save the packaging for future shipping/storage of the instrument.

3. Startup

Before you begin

Required solutions

- **Sheath fluid:** We recommend using sterile, flow cytometry grade 1X PBS as the carrier for transporting particles through the optical cell.
- **100% bleach solution:** 5.25% sodium hypochlorite in water. We recommend using laboratory-grade bleach without additives and perfumes. Add 5 mL of the 100% bleach solution to the Waste container to help prevent microbial contamination.
- **10% bleach solution in deionized water:** Used for decontaminating the fluidics lines. Prepare this solution fresh daily and use during the Shut Down procedure.
- **Detergent solution:** Different detergent solutions are recommended for different cleaning and maintenance functions.
  - We recommend using the Attune™ Wash Solution (Cat. No. A24974) when running the cleaning cycles (page 56) or performing the system Shut Down (page 88).
  - For the storage of the nozzle tip after daily system Shut Down (page 88), we recommend using 5% Decon™ Contrad™ 70 (Decon Laboratories, Inc.) liquid detergent solution in deionized water.
  - We recommend using 5% Tween™ 20 in deionized water when preparing the Attune™ Performance Tracking Beads for use in the daily performance test (page 28).
- **Deionized water**: Used in various cleaning and maintenance procedures, for overnight storage of the instrument when not in use, and for preparing various solutions.

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

IMPORTANT! The use of the proper sheath fluid is essential for successful results. Components such as sodium fluoride and phenoxyethanol can reduce cell viability significantly and endotoxins, RNase, DNase, and proteases can hinder further research procedures.

The use of non-sterilized sheath fluid is likely to result in contaminated cell cultures. To reduce the risk of sheath contamination, make sure that the sheath fluid container has been cleaned before adding sheath fluid.

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

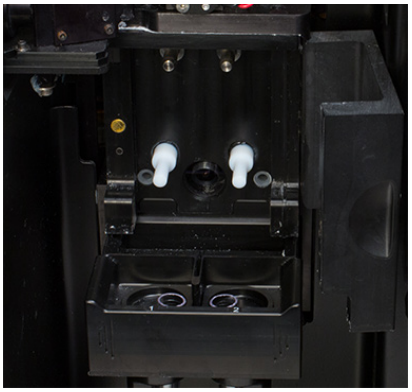
Install the nozzle tip

Before starting up the iSort™ Automated Cell Sorter, you must reinstall the nozzle tip that has been removed from the nozzle assembly after the last Shut Down procedure (page 98). For instructions on how to install the nozzle tip on the nozzle assembly, see page 101.

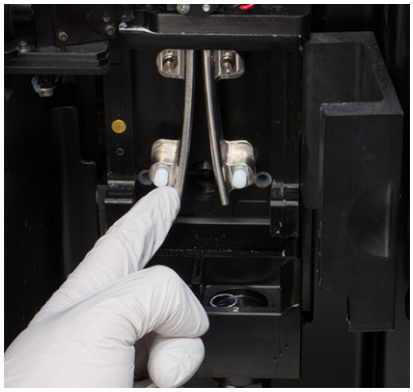
Install the deflection plates

If the deflection plates have been removed from the sort chamber after the last Shut Down (page 97), you must reinstall them before Startup.

1. Open the sort chamber door in the sorting compartment to access the location of the deflection plates (page 11).
2. With the side engraved with "TOP" facing out and on top, place one of the deflection plates into position so that the white plastic post in the sort chamber fits through the bottom hole of the deflection plate. The deflection plates are directional and can only be installed in one way.
3. Align the top hole of the deflection plate with the top metal post in the sort chamber, then push the deflection plate firmly until it clicks securely into place.
4. Repeat the installation process for the other deflection plate.



Deflection plates removed



Deflection plates reinstalled

IMPORTANT! The deflection plates must be removed and cleaned on a regular basis to prevent any buildup of deposit (page 97). Significant buildup of sheath fluid deposits due to clogs or misalignment of the nozzle tip can cause arcing between the deflection plates, which can damage the instrument.

Clean the sort chamber and the sample input port

Before running a sort or analysis, clean the sample input port and the sort chamber, including the sort drawer, door, sort window, and deflection plates (which can be removed). To clean the instrument surfaces, you can use Kimwipes™ laboratory tissues (or other soft, lint free cloth). Do **not** use abrasive cloths or sponges and harsh or corrosive cleansers.

IMPORTANT! Do **not** attempt to clean the lenses inside the laser interrogation chamber. This should only be done by trained service professionals.

1. Wipe the sort drawer, the door, the stream watch camera window, and the deflection plates with sterile deionized water to remove dried salt residue, then wipe dry with Kimwipes™ tissues.

   Note: The deflection plates must remain clean and dry for optimal deflection.

2. Blot any liquid from the nozzle tip, the floor of the sort chamber, and the sample input port with Kimwipes™ tissues.

Check the system fluidics

- Check the levels in the fluid containers in the fluidics compartment to ensure that the sheath container is full and the waste container is empty.
  - If empty, fill the sheath container with sheath fluid.
  - If full, empty the waste container. Before replacing, add 200 mL of full strength bleach solution (5.25% sodium hypochlorite in water) into the emptied waste container to help prevent microbial growth.

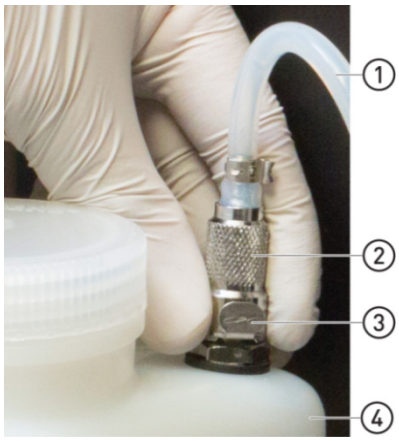
IMPORTANT! The iSort™ Automated Cell Sorter must be in the idle state (i.e., not sorting or performing instrument functions) before refilling or emptying the fluid containers.

- Visually inspect the sample input port, fluidics tanks and connections, and the pumps for any leakage. If you notice any leaks in the fluidics lines, contact your service representative (see page 139). Decontaminate any spills of biological origin by wiping the area with 10% bleach solution.

Refill or empty the fluid containers

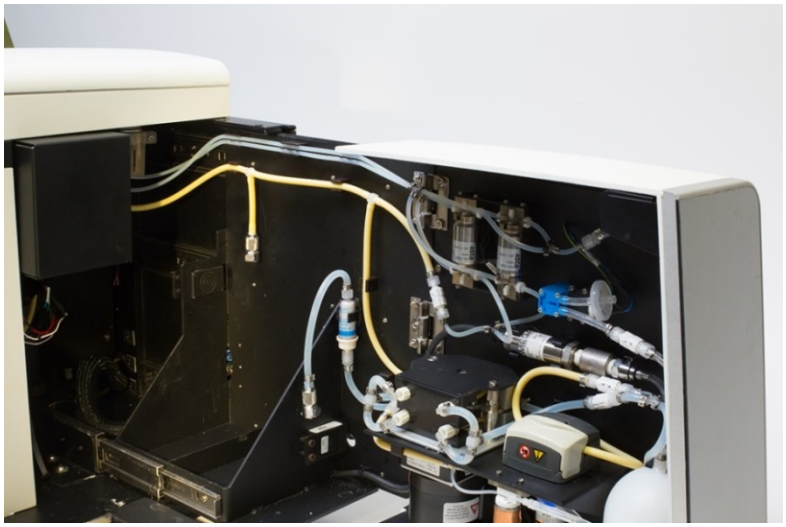
IMPORTANT! Always remove the sheath fluid and waste containers from the instrument to refill or empty them. The iSort™ Automated Cell Sorter must be in the idle state (i.e., not sorting or performing instrument functions) before refilling or emptying the fluid containers.

1. To detach the fluid line from a container, press the metal quick release button on the connector (page 13) and lift the connector up.



① Fluid line
② Quick release connector
③ Quick release button
④ Fluid container

2. After detaching the fluid lines from the containers, slightly lift and pull out the containers from the fluidics compartment.



3. Unscrew the lid and fill the sheath fluid container with the appropriate solution. The sheath fluid container has 2 L capacity, sufficient to process 5 mL of sample. Do not overfill past the 2 L line on the bottle.

> **Note:** To reduce the risk of sheath contamination, make sure that the sheath fluid container has been cleaned with bleach before adding sheath fluid. Minimize air exposure to sheath when handling to minimize the chance of contamination. We recommend that you have at least two sheath fluid containers so that you can wash and dry one container while using the other.

4. Screw the lid back on the sheath fluid container without over-tightening it.
5. Unscrew the lid from the waste container and empty it. Treat all waste as biohazardous.

> **Note:** Empty the waste container whenever you refill the sheath fluid to prevent the overfilling of the waste container during a run.

6. Add 200 mL of full strength bleach solution into the emptied waste container to help prevent microbial growth, then screw the lid back on without over-tightening it.
7. Replace the containers by sliding them into the appropriate slots in the fluidics compartment and reconnect the fluid lines.

> **IMPORTANT!** Do not let the filters (0.2-µm PTFE) on top of the sheath and waste fluid containers get wet when emptying the containers. Replace the filters if they get wet.

8. Before use, recheck the quick connectors to ensure that they are properly in place. If the sheath fluid container is not connected, a prompt is displayed indicating that the instrument will not be able to build pressure. **If the waste container is not connected, fluid will leak into the interior of the instrument**.

> **Note:** The iSort™ Automated Cell Sorter actively monitors the fluid levels in all fluid containers. When the sheath fluid level is low or the waste container is full, the instrument displays the appropriate warning message. To resume the run, follow the displayed instructions.

Start up the system

Power on the instrument

1. To start up the iSort™ Automated Cell Sorter, press the illuminated, blue power button located at the bottom-left side of the instrument (page 9)
2. When the Home Screen is displayed, the iSort™ Automated Cell Sorter is ready to use.

Run Initial Cleaning

Overview

Initial Cleaning must be performed before running the first analysis or sort of the day. The initial cleaning process takes approximately 10 minutes. It is not necessary to repeat the initial cleaning cycle before subsequent analysis or sorts. However, if the instrument remains idle for more than 30 minutes, it will prompt you to perform the initial cleaning procedure again to prepare the sample line for further analysis or sort runs. This repeat process is optional, but recommended for improved sort performance and to minimize clogs.

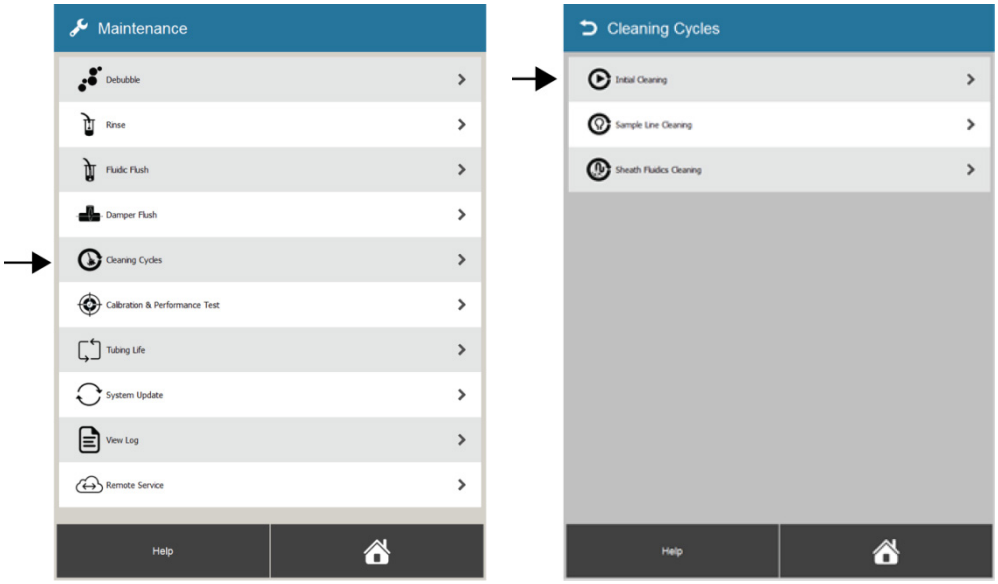
Required solutions

- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water

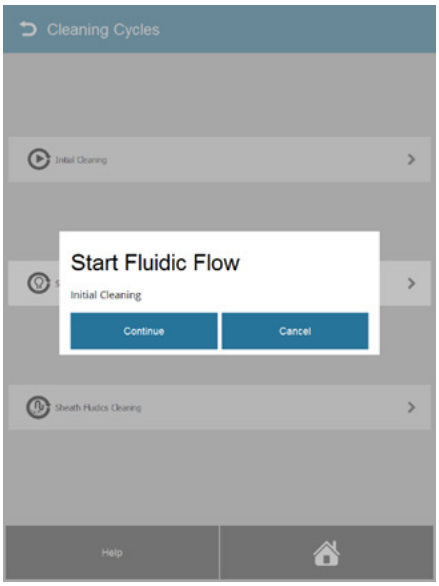
Run Initial Cleaning

1. On the Home Screen, press the **Maintenance** button to open the Maintenance screen.

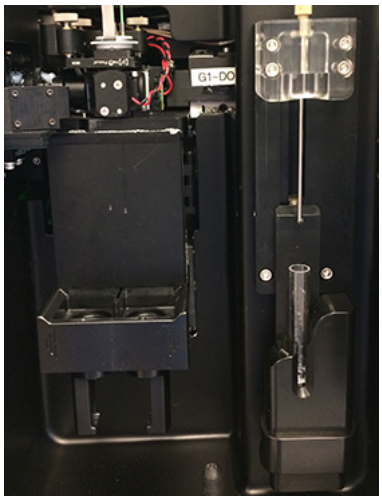
2. On the Maintenance screen, press **Cleaning Cycles**, then select **Initial Cleaning**.



3. When prompted, press **Continue** to start the fluidic flow and begin the initial cleaning process. Some noise will be generated as the fluidics flush begins.

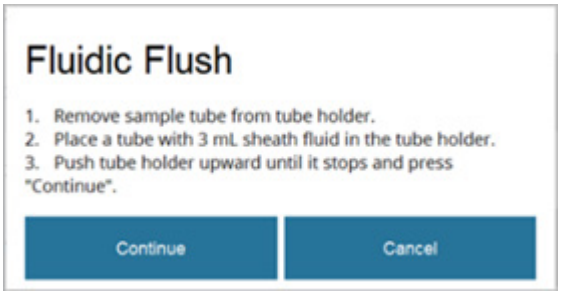


4. When prompted, place an empty 12 × 75-mm sample tube into the sample tube holder in the down position.



5. Press **Continue** to begin the back flush component of the initial cleaning cycle, which removes clogs from the sample lines.

6. When prompted, place a fresh 12 × 75-mm sample tube with 3 mL of sheath fluid into the sample tube holder, then move the sample tube holder to the up position.



7. Press **Continue** to begin the fluidic flush component of the initial cleaning cycle, which washes the sample lines with sheath fluid and is designed to prevent carryover between samples.
8. At the completion of the initial cleaning, a prompt will confirm "Initial Cleaning Complete". Proceed to Performance Test (page 28) or Analyze and Sort (page 31).

Run Performance Test

Overview

The Performance Test procedure allows you to track the performance of the iSort™ Automated Cell Sorter and verify performance against the QC criteria. The procedure requires the use of the Attune™ Performance Tracking Beads.

We recommend that you run the procedure every day to ensure that the system is performing optimally before starting your experiments.

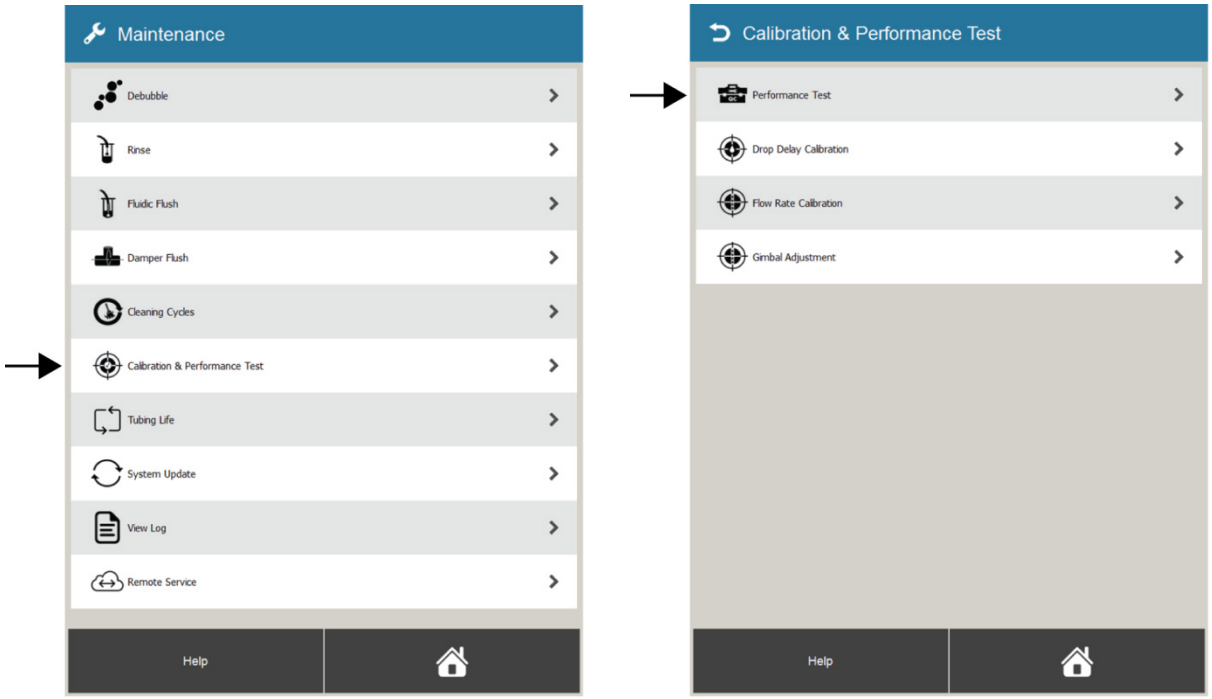
Required materials

- Attune™ Performance Tracking Beads (Cat. No. 4449754). Before use, briefly vortex the beads to mix.
- 5% Tween™ 20 in deionized water (Cat. No. 003005). Before use, dilute the 50% Tween™ 20 solution to 5% in deionized water.
- 10X PBS
- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water

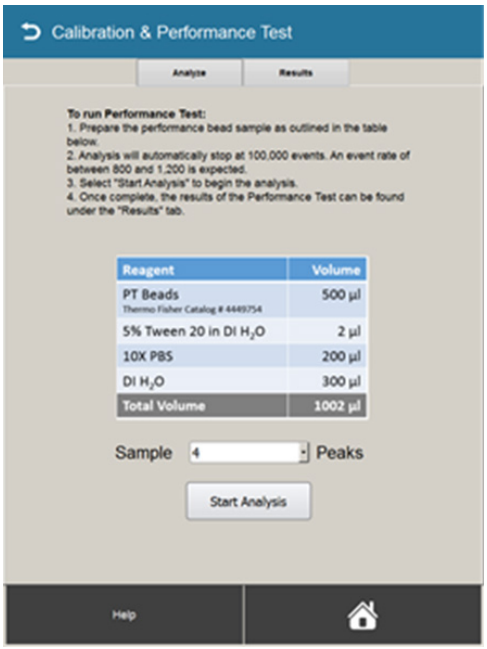
CAUTION! The Attune™ Performance Tracking Beads contain 0.05% sodium azide as a preservative. Sodium azide is an extremely toxic and dangerous compound, particularly when combined with acids or metals. Properly dispose of solutions containing sodium azide.

Run Performance Test

1. On the **Maintenance** screen, press **Calibration & Performance Test**, then select **Performance Test**.



2. On the **Calibration & Performance Test** screen, select the **Sample 4 Peaks** option.

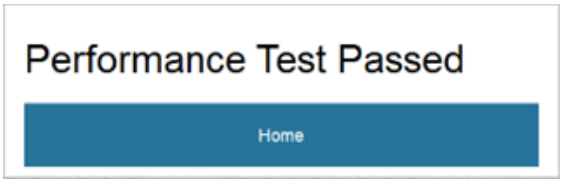


3. Briefly vortex the vial of Attune™ Performance Tracking Beads to mix, then prepare the performance tracking (PT) bead sample by adding the following components into a 12 × 75-mm disposable test tube.

Attune™ Performance Tracking Beads	500 µL
5% Tween™ 20 in deionized water	2 µL
10X PBS	200 µL
Deionized water	300 µL
Total volume:	1002 µL

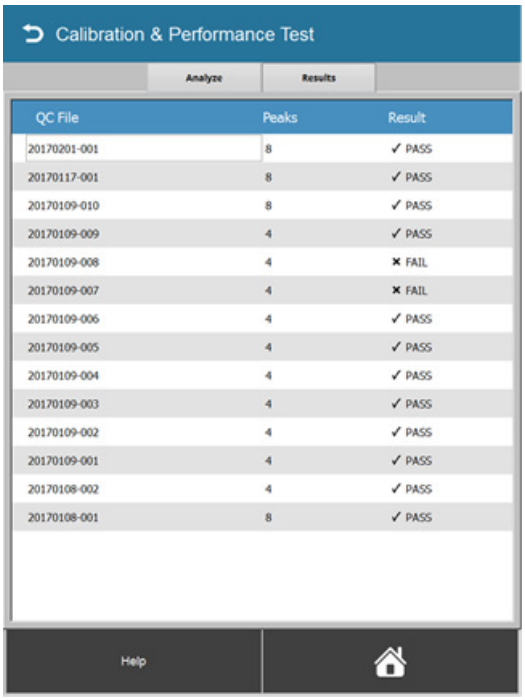
Note: Attune™ Performance Tracking Beads consist of equal concentrations of beads of four fluorescence emission intensities.

4. Place the sample tube with the PT beads into the sample tube holder, then move the sample tube holder to the up position.
5. Press **Start Analysis** to begin the analysis and follow the instructions provided on the touchscreen. The analysis automatically stops at 100,000 events. An event rate of between 800 and 1,200 events per second is expected.
6. After completion, the touchscreen displays the performance test result.



- If the performance test result is **Pass**, press **Home**, then proceed with your Analysis or Sort run (page 31).
- If the performance test result is **Fail**, make sure that the bead sample was correctly prepared, then rerun the performance test. If the test fails again, contact Technical Support (page 139).

7. To review the results of previous performance tests, go to the **Calibration & Performance Test** screen, then press the **Results** tab.



4. Analyze and Sort

Overview

The Analysis and Sort applications of the iSort™ Automated Cell Sorter have similar workflows that contain many identical steps. Therefore, they are described together in this section.

- **Analysis** mode (or non-sorting experiment) is designed for quick verification of sample content and can be performed independently of the Sort workflow. You can use the Analysis workflow to verify proper sample preparation, to determine the purity of a sorted cell population, and to verify a successful sorting process.
- **Sort** mode allows you to designate up to two specific cell populations within a sample, then isolate and collect them. When sorting, the desired populations are selected by defining sort regions on the density plots that are displayed on the touchscreen. You can also define a debris gate to exclude a population from the final sort count.
- Every time an Analysis or a Sort is completed, the data acquired during the run are saved in FCS 3.0 format. These files contain the raw data that can be imported into any standard flow cytometry analysis software (see "Manage files", page 46).

Required materials

- Sample (for sample preparation guidelines, see page 32)
- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water
- Sample tubes, 12 × 75-mm (5-mL capacity)
- Polypropylene collection tubes, 1.5-mL, 12 × 75-mm (5-mL), or 50-mL tubes in any combination

WARNING! BIOHAZARD. When running biological samples for analysis or sorting, hazardous aerosols may be created depending on the sample type. To prevent hazardous aerosols from spreading, keep the sorting compartment door closed as much as possible. Follow all applicable local, state/provincial, and/or national regulations for the handling and disposal of all biohazardous substances, including samples, sorted fractions, and waste. Wear appropriate protective eyewear, clothing, and gloves.

Prepare samples

Guidelines for sample preparation

- To maximize success, it is essential that samples are prepared in a single-cell suspension at a density of 10^5–10^7 cells/mL and are free of aggregates. The ideal cell concentration is cell type dependent.
- To prepare single-cell suspensions, use gentle mechanical dissociation, non-enzymatic methods, or trypsin alternatives (e.g., TrypLE™ Cell Dissociation Reagent).
- Cell clumps and aggregates affect detection and purity and can cause blockages in the fluidic pathway. Late passaged and contaminated (primary) cell cultures, and cell cultures that have been allowed to grow beyond confluence are particularly likely to contain cell clumps and aggregates as a result of DNA and debris from dead and dying cells.
- To remove clumps and aggregates, filter the cell sample through cell strainer before loading it on the instrument.

  We recommend using the Corning™ Falcon™ Test Tube with Cell Strainer Snap Cap (35-µm mesh size), available from Fisher Scientific (Cat. No. 08-771-23).

  Flowmi™ Cell Strainers (40-µm mesh size) and Partec CellTrics™ Filters (30-µm or 50-µm) also produce single-cell suspensions appropriate for sorting with the iSort™ Automated Cell Sorter.
- If your cells are sticky and filtering does not adequately remove clumps and aggregates, use EDTA to reduce clumps.
- Avoid frequent temperature changes to minimize the debris from dead and dying cells in the sample.
- Do not run samples containing more than 30% dead cells. We recommend determining the cell viability of the sample just before running it on the iSort™ Automated Cell Sorter.
- The maximum recommended cell sorting rate is 12,000 cells per second, which also includes the debris detected by the instrument. Sort rates higher than the recommended maximum can result in lower yield or recovery, and can increase the likelihood of clogs in the fluidic system.

  Note: Because the flow rate of the iSort™ Automated Cell Sorter is fixed, the sorting rate depends solely on the sample concentration.
- Phosphate buffered saline (PBS) is a common suspension buffer. For best results, we recommend using flow cytometry grade PBS (Cat. No. A1286301).
- Place your sample into a 12 × 75-mm sample tube to load it into the instrument (page 33). The sample tube must contain at least 250 µL of sample volume to begin sorting or analysis.

System preparation

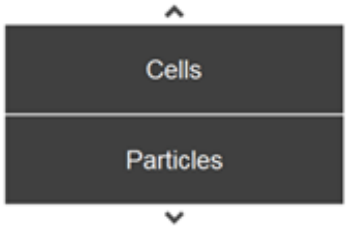
Before starting

- Make sure that the collection tray is pulled out and there are no collection tubes present.
- Place an empty 12 × 75-mm tube into the sample tube holder in the down position.

Choose application

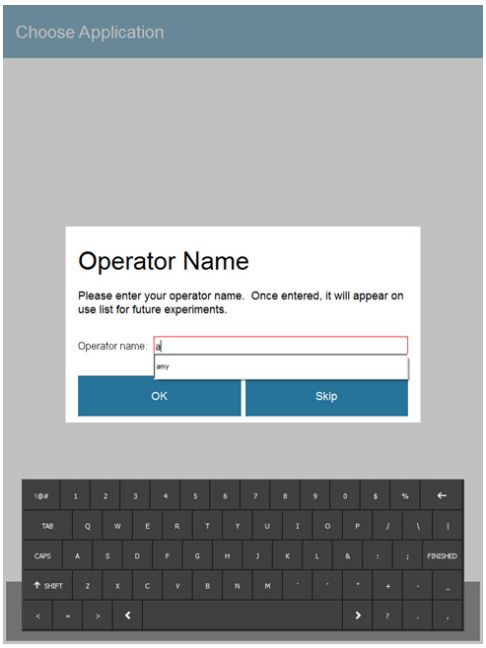
1. On the **Home Screen**, press **Sort** or **Analyze** to select the corresponding application.

2. On the first screen of the selected application, choose **Cells** or **Particles**.
   - Select **Cells** for cells that express green fluorescent proteins (GFP), are labeled with green extra cellular labels (FITC or AlexaFluor™ 488 dye), or have been stained with green dyes (e.g., Sytox™ Green dye)
   - Select **Particles** for fluorescent beads (e.g., Attune™ Performance Tracking Beads)



3. When prompted, enter your name in the **Operator Name** text box using the alpha-numeric keyboard, then press **OK**.

   The name you enter will appear as the **File Owner** next to the name and size of the saved FCS file in the **File Viewer** screen (see "Manage files", page 46).

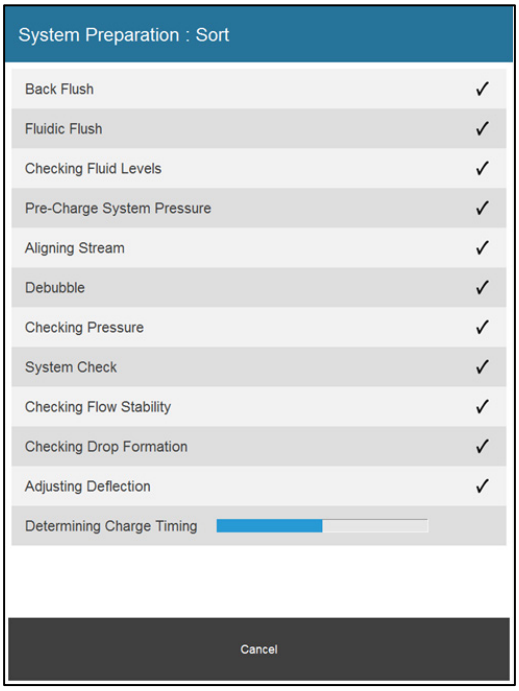


4. After you have entered the operator name, the system automatically initiates the **System Preparation** (page 34).

System Preparation

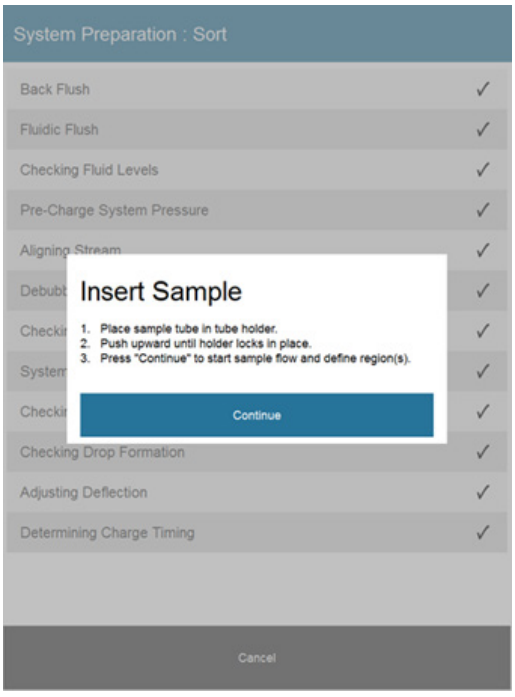
System Preparation is a set of procedures that prepares the iSort™ Automated Cell Sorter for the Analysis or Sort run and ensures optimal instrument performance.

- System Preparation is initiated automatically after you have entered the operator name for the Analysis or Sort (page 33) and it takes 10–15 minutes to complete with minimal user input.
- If you have not already done so, a dialog box will remind you to pull out the collection tray and ensure that no collection tubes are present.
- During System Preparation, the instrument performs the following functions in the order listed and shows the progress of each step in real time.
  - Check fluid levels
  - Pre-charge system pressure
  - Align stream
  - Debubble
  - Check pressure
  - System check
  - Check flow stability
  - Check drop formation
  - Adjust deflection
  - Determine charge timing



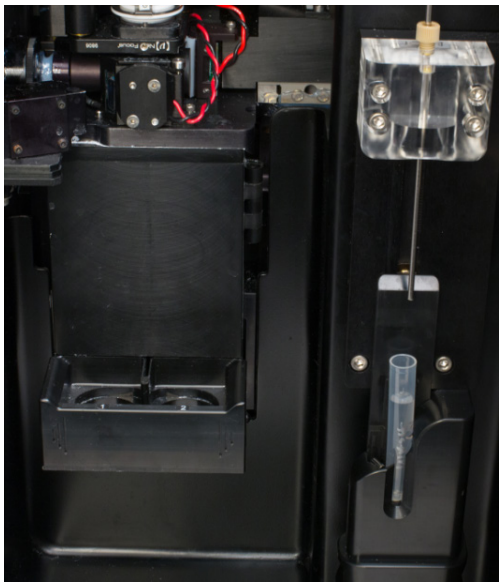
- When the system preparation is completed, the **Insert Sample** dialog box appears and prompts you to load your sample to initiate sample flow and define regions on the dot plot for Analysis or Sort.

  For detailed instructions on how to load your sample to start your Analysis or Sort, see "Load sample", page 35.



Load sample

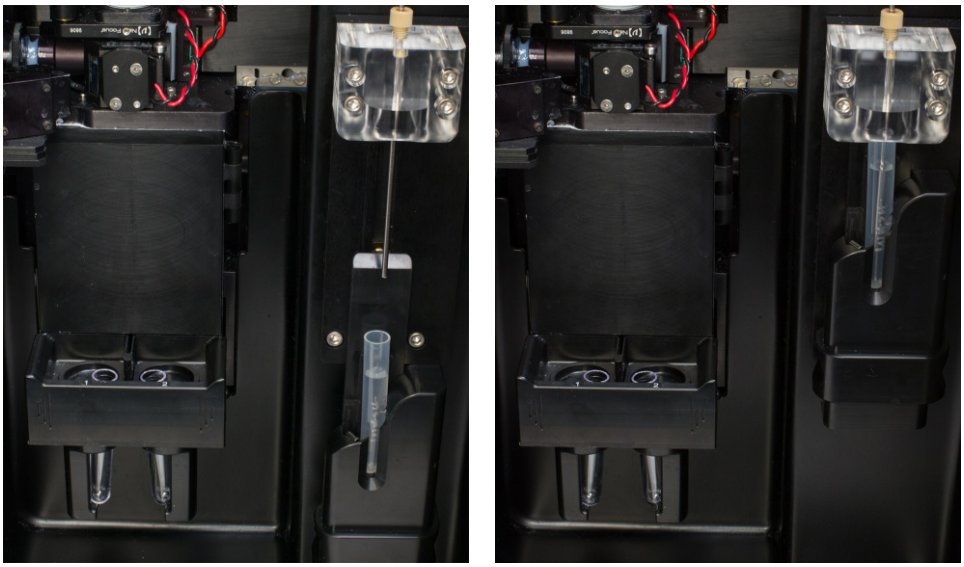
1. When prompted by the **Insert Sample** dialog (page 34), open the sort compartment door to access the sample input port and the collection area.
2. Make sure that the sample tube holder is in the down position and the collection tray is pulled out.
3. Place the 12 × 75-mm tube containing your sample into the sample tube holder.



Note: The sample tube must contain at least 250 µL of sample volume to begin Analysis or Sort. For guidelines on sample preparation, see page 32.

4. If sorting, insert the desired collection tubes into Position 1 and Position 2 of the collection tube holder. The collection tube holder can hold two 1.5-mL, 5-mL (12 × 75-mm), or 50-mL tubes in any combination.
   - If you wish to sort into 1.5-mL or 5-mL tubes, insert a tube adapter into the desired position on the collection tray to hold your collection tubes.
   - If you wish to sort into 50-mL tubes, remove the tube adapter and insert the 50-mL tube directly into the desired position on the collection tray.

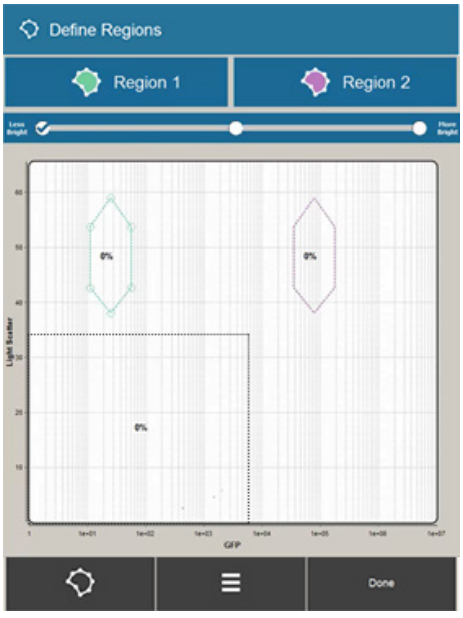
5. To initiate sample loading, move the sample tube holder up until it clicks into place, close the sorting compartment door, then press **Continue**.



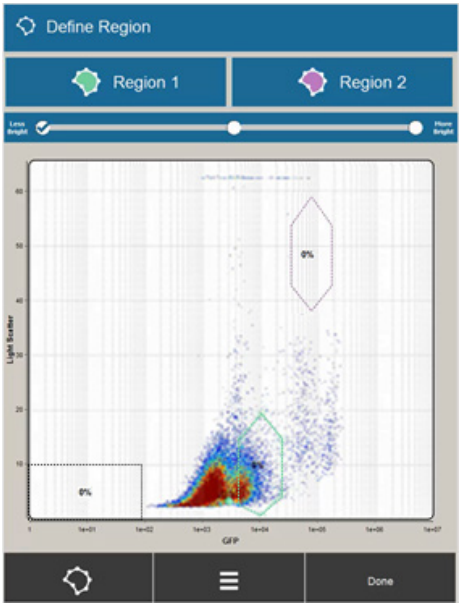
IMPORTANT! Cell sorting creates aerosols. Exposure to and inhalation of aerosols can potentially be hazardous depending on the cell types you are using. Always keep the door to the sorting compartment shut to minimize the risk, but understand that this does not eliminate the risk entirely.

We recommend placing the iSort™ Automated Cell Sorter in a biosafety cabinet when sorting human primary cells and cells known to be infected with viruses or other agents. Consult your institution's safety officer for guidelines related to your individual samples.

6. When sample loading is initiated, the **Define Region** screen opens and the fluidic system temporarily increases the sample flow rate to quickly transfer the cells to the optical detection area.



7. Within 30 seconds of sample loading, the fluorescence scatter plot on the Define Region screen starts displaying the data from your sample.

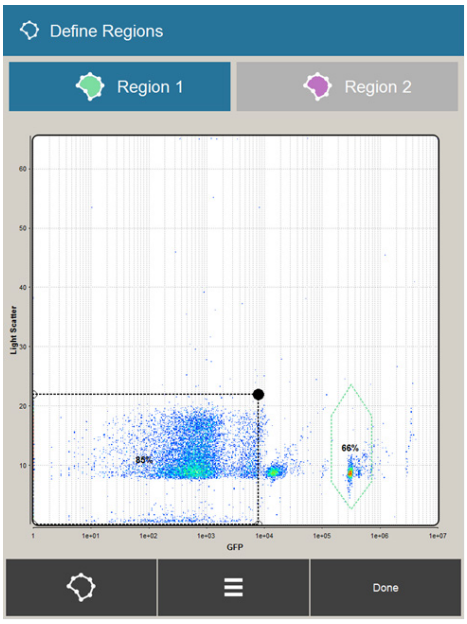


8. When you observe data displayed on the screen, you are ready to proceed with your Analysis or Sort (see "Analyze or Sort sample", page 38).

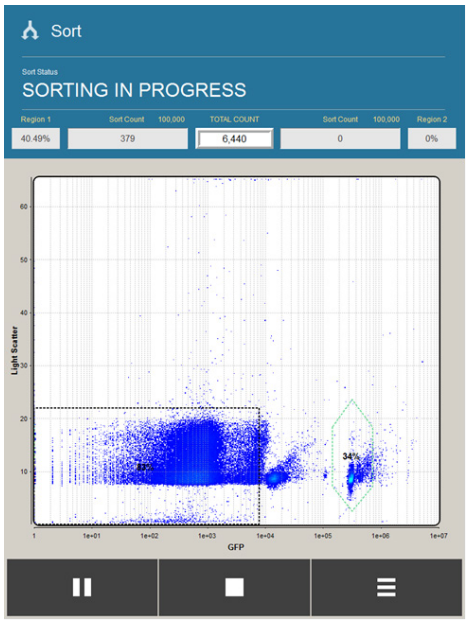
Analyze or Sort sample

Overview

As the cells or particles in the sample pass through the optical detection area, they are interrogated by a 488-nm laser, which generates scattered light and fluorescence signals that are displayed in the fluorescence scatter plot on the screen.

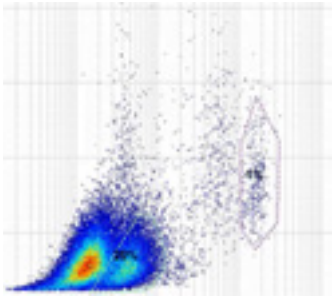


Scatter plot on Define Regions screen



Scatter plot on Sort screen

- Each data point (i.e., event) displayed on the fluorescence scatter plot represents a single cell or particle measured.
- The position of an event in the plot is determined by the light scatter (y-axis) and fluorescence (x-axis) measurements. The light scatter value reflects the cell size and granularity, while the fluorescence value represents the expression level of the GFP reporter gene or the brightness of the label or dye.
- The accumulation of events in the scatter plot allows you to discern specific clusters that can represent discrete populations in your sample.
- The number of events registered for specific populations in the sample are indicated by different colors in a density color gradient, where light blue, green, yellow, and red hot spots indicate increasing number of the events (blue represents the lowest and red represents the highest density).



Note: The presence and position of discrete clusters in the plot are entirely sample dependent.

- You can draw gates around the target populations to define them as Region 1, Region 2, or debris for Analysis or Sort.
- When sorting (page 41), the target population defined by Region 1 is sorted into the collection tube placed in Position 1 (left) in the collection tube holder, while the target population defined by Region 2 is sorted into the collection tube placed in Position 2 (right).

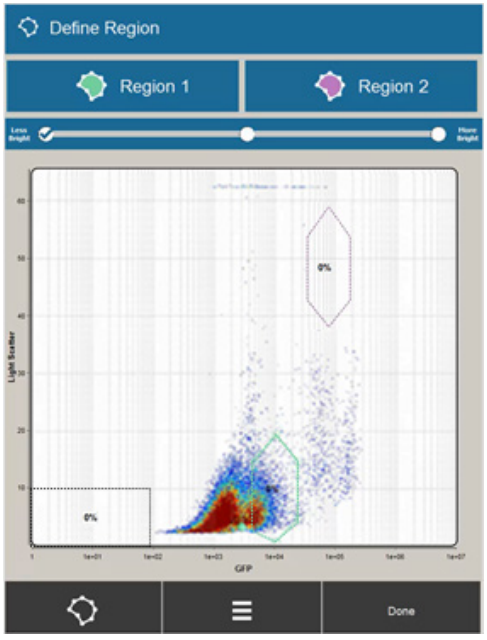
Define regions

1. When you observe events appearing on the scatter plot on the Define Regions screen, press the **Region 1** or **Region 2** button to display the corresponding region on the plot. You can select either or both of the regions to display. In the example below, Region 1 is selected.

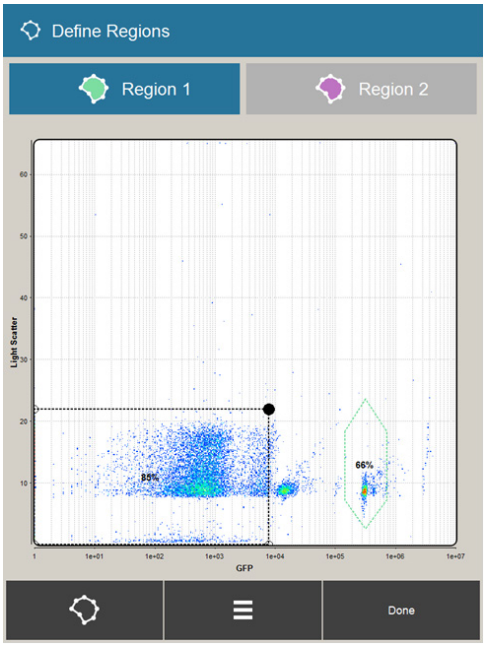
   - Region 1 is shown in green, Region 2 in purple, and the debris region in black. The numbers inside the regions indicate the percentage of events bounded by the region.
   - If you have previously run an Analysis or a Sort, the regions are displayed in the shape and location used in the previous sort.

     If you have not previously defined any regions, the regions are displayed in the default locations.

     If you shut down the instrument, the next time the instrument is powered on, the regions will appear in the default locations.
   - You can use the regions as they are shown or you can change the position and shape of the regions to define new target populations (Step 3, page 40).
   - If there is overlap between Region 1 and Region 2, the cells or particles that fall within the overlap are not counted or sorted.



Define Regions screen for Analyze Cells

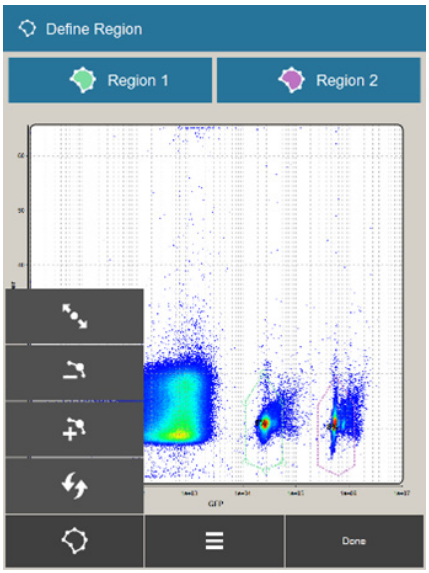


Define Regions screen for Analyze Particles

2. If you have selected **Cells** for analysis or sorting (page 33), select **Less Bright** (left), **Normal Bright** (middle) or **More Bright** (right) using the radio buttons above the plot. This allows you to optimize the display based on the cells' level of fluorescence expression.

   For example, if your cells typically express low levels of GFP, select **Less Bright** to display your populations more clearly. If the cells express very high levels of GFP, select **More Bright**. For cells expressing normal levels of GFP, select **Normal Bright**.

3. Change the position and shape of the regions as necessary so that they enclose the populations of interest on the plot.
   - To move a region, touch anywhere inside the region, then drag it to the desired location on the plot.
   - To move the nodes of a region, touch outside of the region closest to the node you want to move.
   - To display the region editing tools, press the **Edit Region** button.

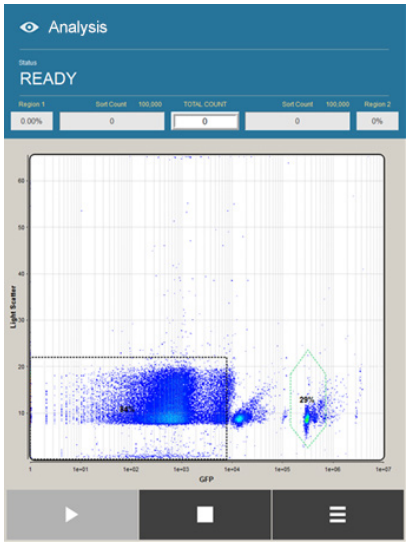


   - To change the shape of a region, press the **Region Nodes** button, then touch and drag the desired node on a region.

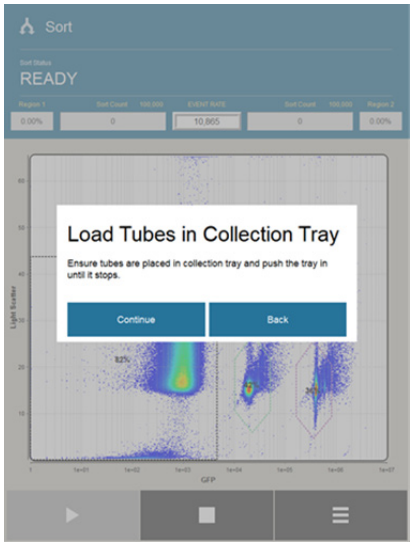
   - To add a node to a region, press the desired region to select it, then press the **Add Node** button.

   - To remove a node from a region, press the region to select it, then press the **Remove Node** button.

4. When finished defining the regions, press **Done** to proceed with your Analysis or Sort (page 41). Depending on your workflow, the Analysis Ready or the Sort Ready (Load Tubes) screen opens.



Analysis Ready screen



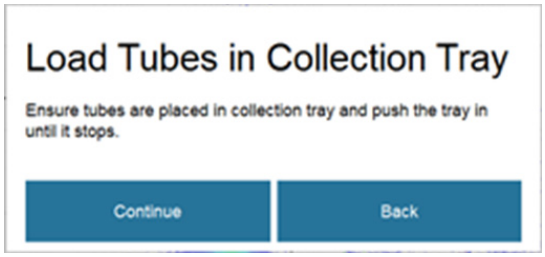
Sort Ready (Load Tubes) screen

Perform Analysis/Sort

The majority of the steps in the Analysis and Sort workflows are identical. Therefore, the workflows are described together.

- If you are running a Sort, start with Step 1.
- If you are running an Analysis, directly go to Step 4 (page 42).

1. When prompted, ensure that the collection tubes are placed in the collection tube holder (page 35), push the collection tray inward until it clicks into place.



2. Close the sorting compartment door to minimize interfering light and exposure to aerosols, then press **Continue**.

3. When prompted, select the collection tube size for Region 1 and Region 2.

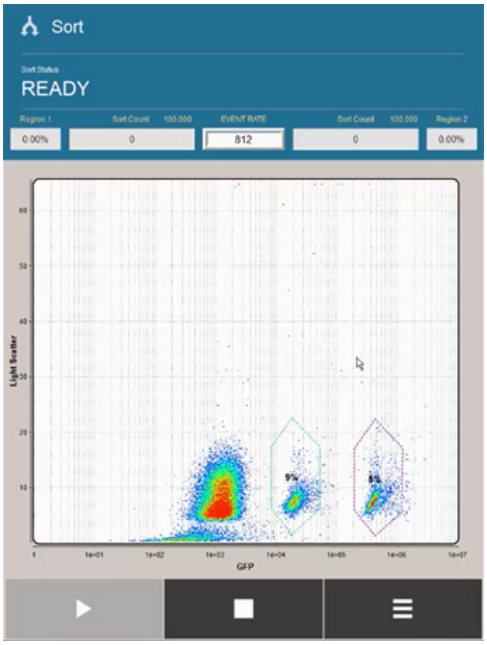
Note: The Sort Tube Size prompt is displayed only if there is a collection tube adapter for the 1.5-mL and 5-mL tubes in the collection tray. Otherwise, the instrument assumes that 50-mL collection tubes are being used.

The Sort Tube Size prompt is displayed only for active regions.

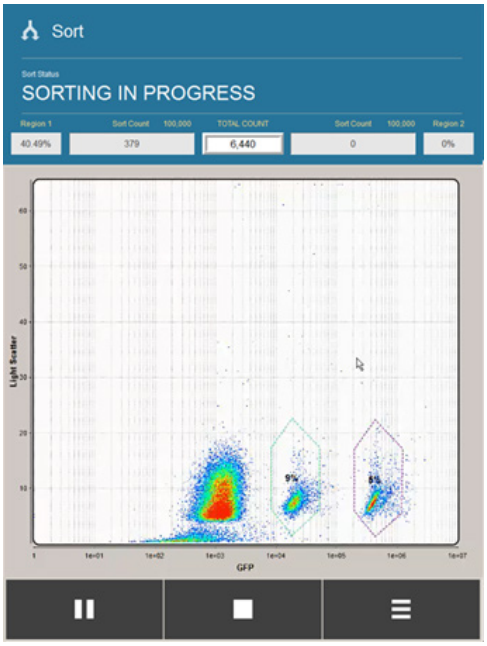
Note: When sorting, the target population defined by Region 1 is sorted into the collection tube placed in Position 1 (left) in the collection tube holder, while the target population defined by Region 2 is sorted into the collection tube placed in Position 2 (right).

4. To start the Analysis/Sort, press the flashing **Play** button.

The Play button changes to the Pause button, and the counters above the plot display the current Analysis/Sort statistics (see Table 1).



Sort screen - Ready



Sort screen – Sorting in progress

Note: The percentage that appears in a region defined on the plot is not the same as the percentage that appears in the status bar. The percentage that appears in the region is the real time percentage of events in the region compared to the total event rate. This measure excludes the events in the debris gate.

Table 1. Description of Analysis/Sort statistics. Statistics are updated in real time when the Analysis/Sort is in progress, and they are saved within the FCS file when it is completed. Note that all the numbers displayed in the statistics exclude the events in the debris gate.

Statistic	Description
Region 1	Percentage of the total accumulated events that fall within Region 1, calculated as (Region 1 accumulation/total accumulation) × 100.
Count/Sort Count (left)	Total number of accumulated events that fall within Region 1 (Analysis/Sort) and sorted into Position 1 (Sort only).
Total Count	Real-time number of events that have been counted.
Event Rate	Real-time events per second during the Analysis or Sort run.
Abort Rate	Real-time events per second that are not sorted in a given region.
Region 2	Percentage of the total accumulated events that fall within Region 2, calculated as (Region 2 accumulation/total accumulation) × 100.
Count/Sort Count (right)	Total number of accumulated events that fall within Region 2 (Analysis/Sort) and sorted into Position 2 (Sort only).

5. To view the statistics for the **Total Count**, **Event Rate**, or **Abort Rate**, press on the center counter window until the desired statistic is displayed (Abort Rate is available only in the Sort workflow).

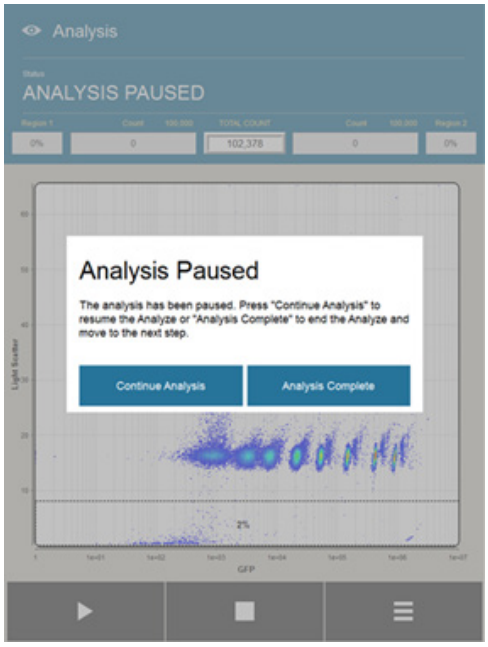
6. To pause the sample flow during the Analysis/Sort, press the **Pause** button.

Pausing the sample flow allows you to load a new aliquot of your sample, agitate your sample to resuspend settled cells or particles, or to replace full collection tubes with empty ones.

- If running Analysis, the sample flow stops and the counters stop registering further events. The screen displays the Analysis Paused dialog.

  To resume the sample flow and data acquisition, press **Continue Analysis**.

  To stop sample flow and complete your analysis, press **Analysis Complete** and go to Step 7 (page 44).



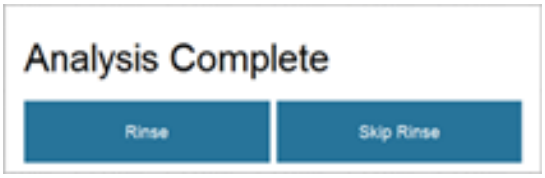
- If running Sort, the sample flow and sorting stops, and the screen displays the Sorting Paused dialog.

  To resume the sample flow and sorting, press **Continue Sorting**.

  To stop sample flow and complete the Sort, press **Sorting Complete** and go to Step 7 (page 44).

7. When you are finished with your Analysis or Sort, press the **Stop** button. 

   - If running Analysis, the sample flow stops and the screen displays the Analysis Complete dialog.



   - If running Sort, the sample flow and sorting stops, and the screen displays the Sorting Complete dialog.

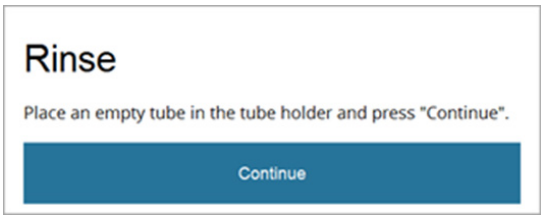
> **Note:** Pressing the **Analysis Complete** or the **Sorting Complete** button also stops the sample flow and displays the Analysis/Sorting Complete dialog.
>
> When the Analysis/Sort is complete, the instrument saves the data acquired during the Analysis/Sort in FCS 3.0 format in the specified location.

8. Press **Rinse** to rinse the fluidic lines of the system (page 45) or press **Skip Rinse** to add another sample for Analysis or Sort.

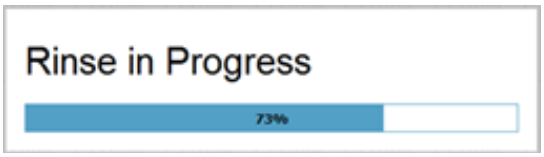
> **Note:** We recommend that you rinse the fluidic lines if your next sample is different from the one that you have just run. The rinse procedure clears the sample line by back-flushing it with sheath fluid to reduce carryover and decrease the risk of clogging.
>
> If you decide to skip the rinse, the only other option is to add another sample and sort again. You will not be able to go back to the Home screen without rinsing.

Rinse

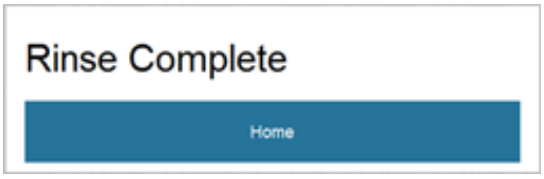
1. When prompted, remove the sample tube from the tube holder, place an empty tube in the sample tube holder, then press **Continue**.



The screen shows the progress of the rinse as the instrument flushes the fluidic lines with sheath fluid.



2. When you are finished with the rinse, press **Home** to go to the Home screen.



On the Home screen, you can choose to:

- Review and manage the FCS files saved from your Analysis or Sort (page 46)
- Perform instrument maintenance functions (page 51)
- Execute the daily Shut Down (page 88)

IMPORTANT! If you are finished using the iSort™ Automated Cell Sorter for the day, you must complete the daily Shut Down procedure (page 88) and remove the nozzle tip for cleaning and overnight storage (page 98).

---

5. Manage files

Select files

Select files on File Viewer

Every time an Analysis or a Sort is completed, the data acquired during the run are saved in FCS 3.0 format. These files contain the raw data that can be imported into any standard flow cytometry analysis software.

The File Viewer screen allows you to select and manage the FCS files.

1. To access the File Viewer screen, press the **File Viewer** button on the Home screen.

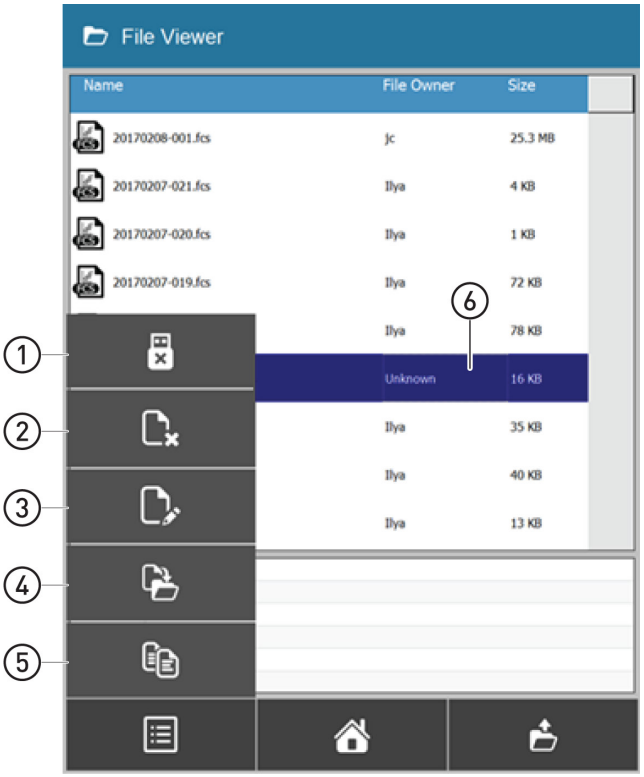
   

   The File Viewer lists the FCS files that are saved in the instrument and displays the name, owner, and size of each file.

2. To manage the FCS files, press the desired file on the list to select it, then press the **Manage Files button** to open the Manage Files menu.

   The Manage Files menu allows you to delete a selected file, to rename it, or to move or copy it to a USB flash drive, and to eject a connected USB drive.

File Viewer screen



Manage Files menu

① Eject USB (page 47)
② Delete FCS File (page 47)
③ Rename FCS File (page 48)
④ Move FCS File to USB (page 66)
⑤ Copy FCS File to USB (page 82)
⑥ Selected FCS File

Manage files

Eject USB

1. To eject the USB drive connected the instrument, press the **Eject USB** button on the Manage Files menu.

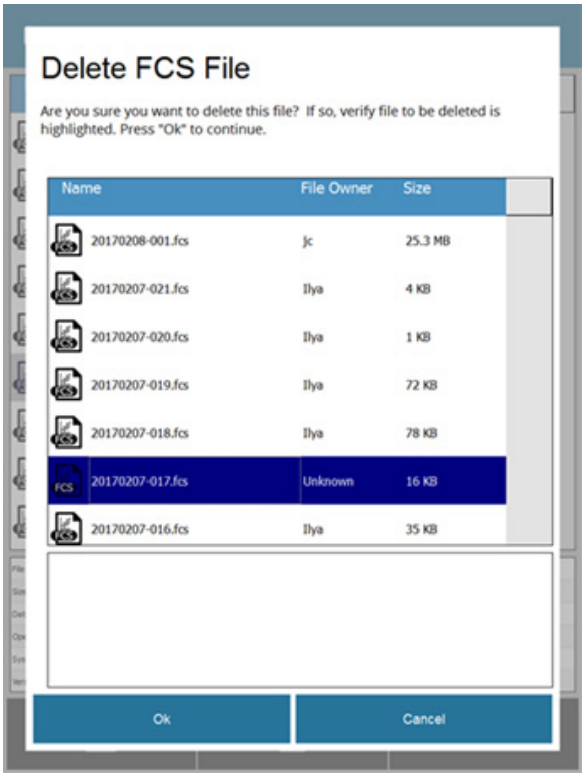
2. When prompted, then remove the USB drive from the USB port.

Delete FCS File

1. To delete an FCS file, select the desired file from the File Viewer, then press the **Delete FCS File** button on the Manage Files menu.

2. When prompted, verify that the file to be deleted is highlighted on the list, then click **OK**.

   To return to the File Viewer without deleting the file, press **Cancel**.



IMPORTANT! Once you have deleted an FCS file, you cannot reload it to the iSort™ Automated Cell Sorter and generate a Report (see page xx)

Rename FCS File

The file naming convention of the iSort™ Automated Cell Sorter is based on the date and the number of the runs completed on that date.

The default file name follows the YYYYMMDD-### format, where YYYY is the year, MM is the month, and DD is the day of the run. ### is the run number starting with 001 for the first run of the day, and increasing by one for each subsequent run.

1. To rename an FCS file, select the desired file from the File Viewer, then press the **Rename FCS File** button on the Manage Files menu.

   You can select multiple files to rename.

2. When prompted, select each file to be renamed from the list on the Rename screen one by one, then enter the new name using the alpha-numeric keyboard on the screen.

3. Click **OK** to accept the new names.

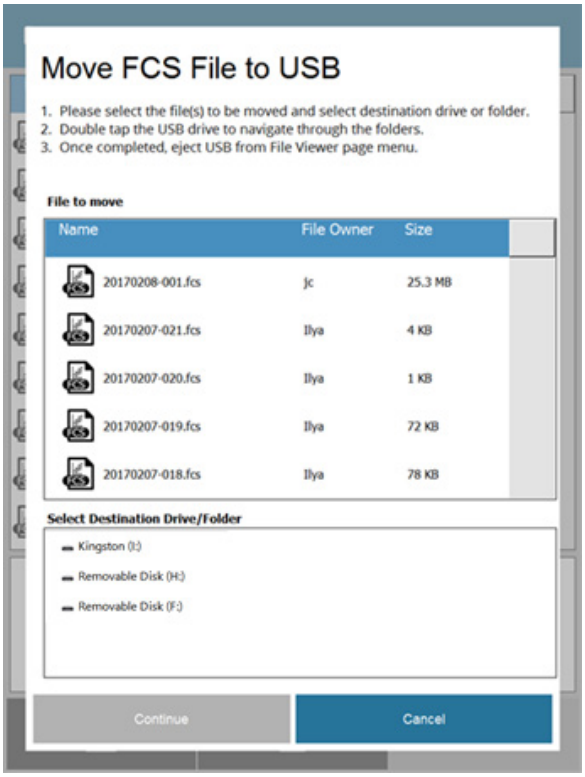
   To return to the File Viewer without renaming the files, press **Cancel**.

Move FCS File to USB

Moving FCS files to a USB drive copies the files to the selected destination folder on the USB drive and deletes them from the instrument.

1. To move FCS files save from the instrument to a USB drive, press the **Move FCS File** button on the Manage Files menu.
2. When prompted, press to select the files on the list that you wish to move to the USB drive. Selected files will be highlighted in blue.

   Note: You can select multiple files. To unselect a file, press the file again.



3. Double-tap the USB drive that appears on **Destination Drive/Folder** list to navigate through its folders and select the desired destination folder.
4. Click **Continue** to move the selected FCS files to the destination folder on the USB drive. Once the files are copied to the USB drive, they are deleted from the instrument.

   To return to the File Viewer without moving the selected files, press **Cancel**.

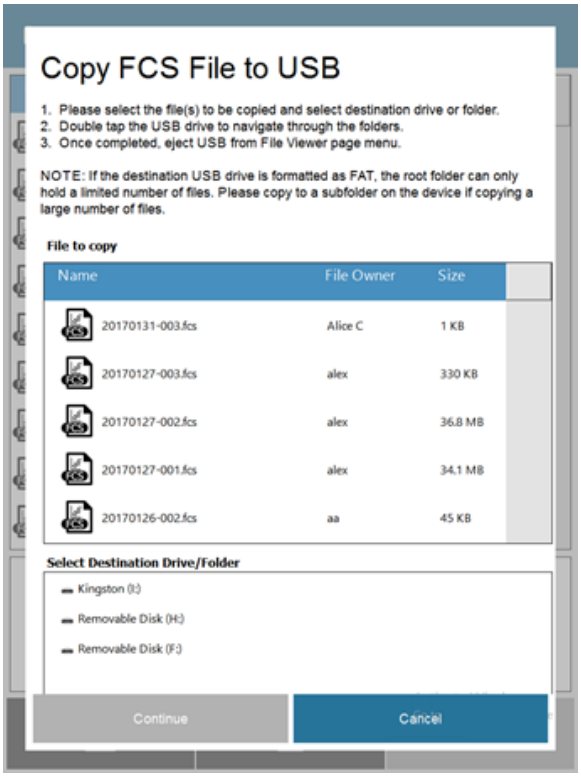
IMPORTANT! Moving an FCS file to a USB drive deletes that file from the iSort™ Automated Cell Sorter. Once deleted, you cannot reload an FCS file to the instrument and generate a Report (see page xx)

Copy FCS File to USB

Copying FCS files to a USB drive places a copy of the each selected file in the destination folder on the USB drive without deleting them on the instrument.

1. To copy FCS files save from the instrument to a USB drive, press the **Copy FCS File** button on the Manage Files menu.
2. When prompted, press to select the files on the list that you wish to move to the USB drive. Selected files will be highlighted in blue.

   Note: You can select multiple files. To unselect a file, press the file again.



3. Double-tap the USB drive that appears on **Destination Drive/Folder** list to navigate through its folders and select the desired destination folder.
4. Click **Continue** to copy the selected FCS files to the destination folder on the USB drive. The copied files will not be deleted from the instrument.

   To return to the File Viewer without copying the selected files, press **Cancel**.

Warnings for data storage

The data storage capacity of the iSort™ Automated Cell Sorter is 400 gigabytes. If your data storage is getting low, the system displays a warning message. The system displays a warning message again when the data storage is full.

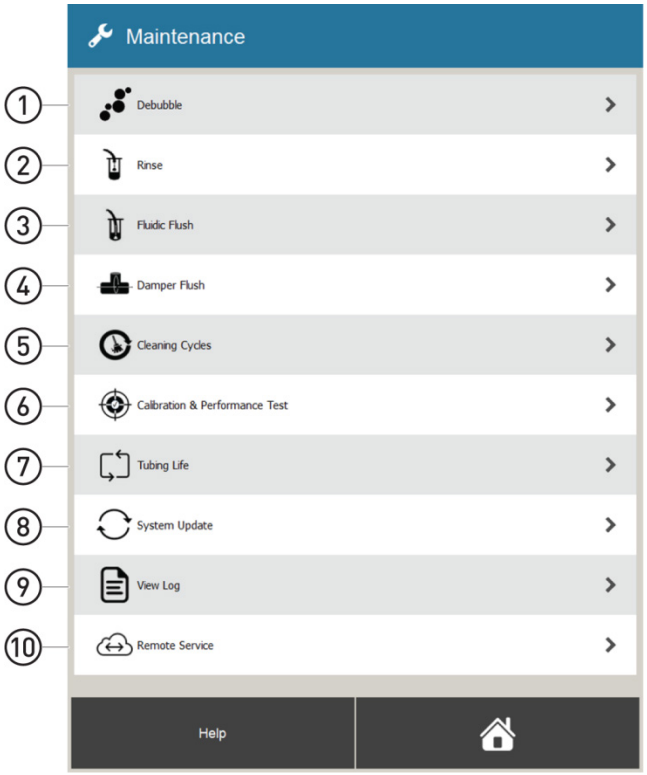
In such cases, either delete the FCS files stored on the instrument or move them to a USB drive to free up storage space in the instrument.

6. Run maintenance functions

Maintenance screen

The Maintenance screen allows you to execute various instrument maintenance, cleaning, and calibration functions.

To access the Maintenance screen, press the **Maintenance** button on the Home screen.



1. Debubble (page 52)
2. Rinse (page 52)
3. Fluidic Flush (page 54)
4. Damper Flush (page 55)
5. Cleaning Cycles (page 56)
6. Calibration and Performance Test (page 66)
7. Tubing Life (page 82)
8. System Update (page 84)
9. View Log (page 86)
10. Remote Service (page 87)

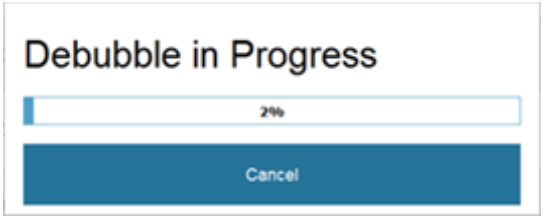
Debubble

The Debubble function is a user-initiated function for clearing air bubbles from the fluidics lines of the system. Air bubbles in the fluidics lines may cause fluctuations in the system pressure, which can result in problems with the stream from the nozzle and adversely affect droplet formation.

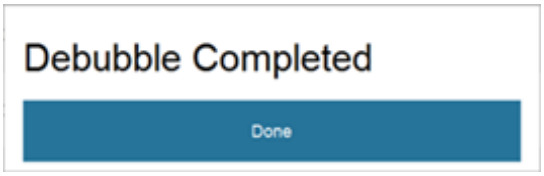
Run Debubble

1. Ensure that the sample tube holder contains an empty sample tube, then select **Debubble** from the Maintenance screen to initiate the Debubble procedure.

The instrument performs the Debubble procedure automatically, which takes approximately 2 minutes to complete.



2. When completed, press **Done** to return to the Maintenance screen.



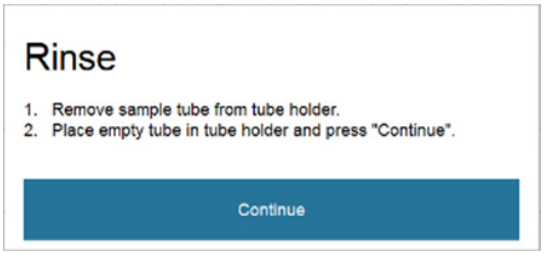
Rinse

The Rinse function removes clogs from the sample and probe lines by back flushing the lines, and can be used to prevent sample carryover between runs. Clogs in the system can affect sample flow, stream alignment, and droplet formation.

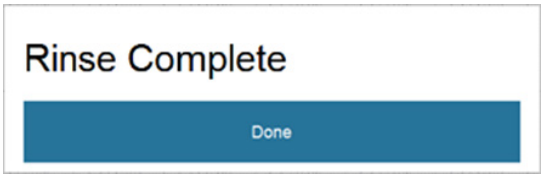
Run Rinse

1. Select **Rinse** from the **Maintenance** screen.

2. Replace the sample tube in the sample tube holder with an empty tube, then press **Continue**. The instrument performs the rinse automatically, which takes approximately 30 seconds to complete.



3. When completed, press **Done** to return to the Maintenance screen.



Note: To reduce the likelihood of clog formation when working with cells, do not exceed the maximum cell density of ~3.0 × 10^7 cells/mL and filter the sample through a cell strainer before loading it on the instrument (see "Guidelines for sample preparation", page 32).

Fluidic Flush

The Fluidic Flush function washes the fluidic lines of the system with buffer or sheath fluid to prevent sample carryover between runs.

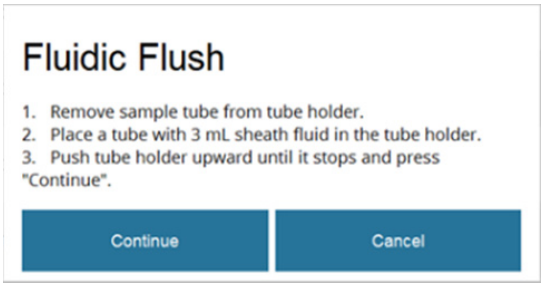
Required solutions

Sheath fluid (1X PBS, flow cytometry grade)

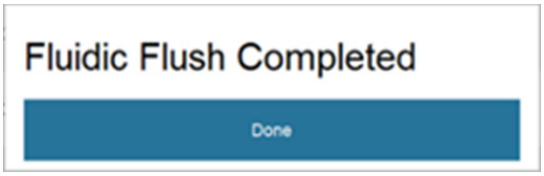
Run Fluidic Flush

1. Select **Fluidic Flush** from the **Maintenance** screen.

2. Place a new tube containing 3 mL of sheath fluid or buffer in the sample tube holder.
3. Move the sample tube holder up until it clicks into place, then press **Continue**. The instrument performs the fluidic flush automatically, which takes approximately 30 seconds to complete.



4. When completed, press **Done** to return to the Maintenance screen.



Damper Flush

The Damper Flush function removes contamination from the pulse damper in the sample line.

Required solutions

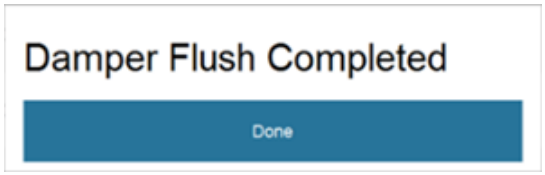
Sheath fluid (1X PBS, flow cytometry grade)

Run Damper Flush

1. Select **Damper Flush** from the **Maintenance** screen.

2. Place a new tube containing 3 mL of sheath fluid in the sample tube holder.
3. Move the sample tube holder up until it clicks into place, then press **Continue**. The instrument performs the damper flush automatically, which takes approximately 30 seconds to complete.

4. When completed, press **Done** to go to the Maintenance screen.



Cleaning Cycles

Cleaning Cycles screen

Cleaning Cycles screen allows you to run Initial Cleaning, Sample Line Cleaning, and Sheath Fluidics Cleaning (decontamination) functions.

1. Initial Cleaning (page 57)
2. Sample Line Cleaning (page 58)
3. Sheath Fluidics Cleaning (page 61)

To access the Cleaning Cycles screen, press **Cleaning Cycles** on the Maintenance screen.

Initial Cleaning

Overview

Initial Cleaning is required before the first analysis or sort of the day. It is not necessary to repeat the initial cleaning before the subsequent analysis or sort runs.

However, if the instrument remains idle for more than 30 minutes, you will be prompted to perform the initial cleaning again to prepare the sample line for further runs.

Required solutions

- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water

Run Initial Cleaning

For detailed instructions on how to run the initial cleaning cycle, see “Run Initial Cleaning”, page 26.

Sample Line Cleaning

Overview

Sample Line Cleaning is a four step cleaning sequence that sanitizes and rinses the sample lines of the system to prevent cross-contamination and carryover between samples. The procedure is similar to the cleaning procedure used in the Shut Down procedure (page 88), but the instrument does not power off when the cycle is completed.

We recommend that you run the sample line cleaning cycle in between samples or users. The entire procedure takes approximately 20 minutes to complete.

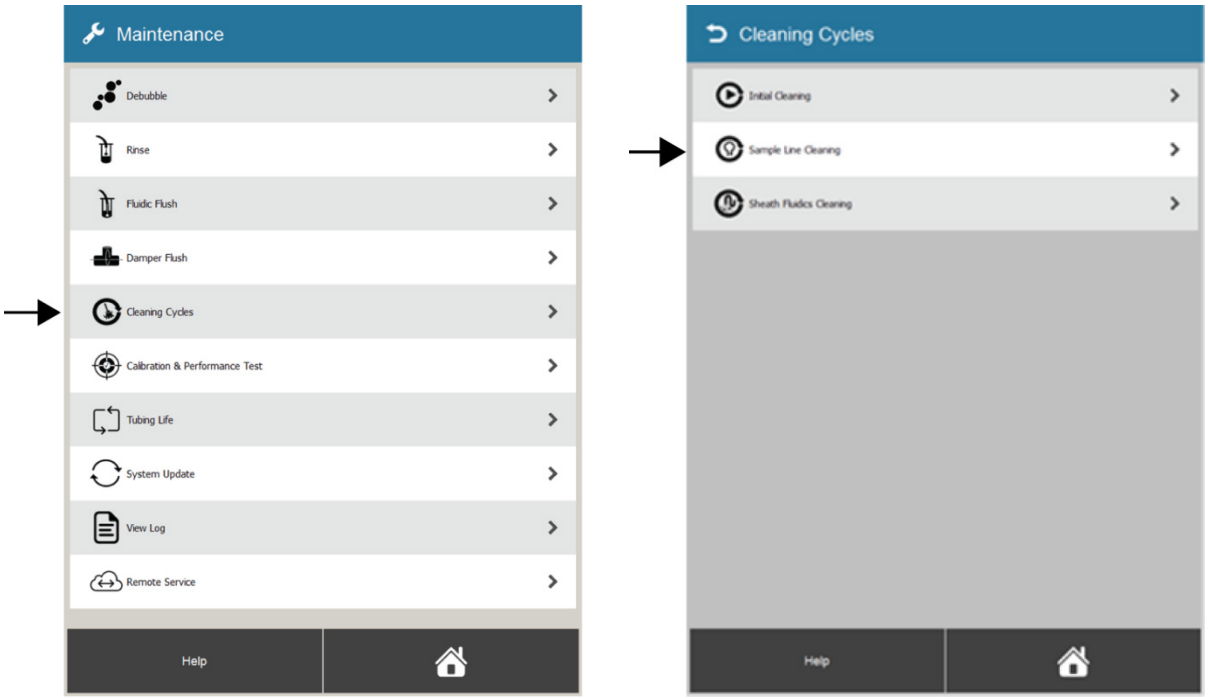
Required solutions

- 10% bleach solution
- Attune™ Wash Solution (Cat. No. A24974) (detergent solution used in Step 5 of the protocol, page 59)
- Deionized water

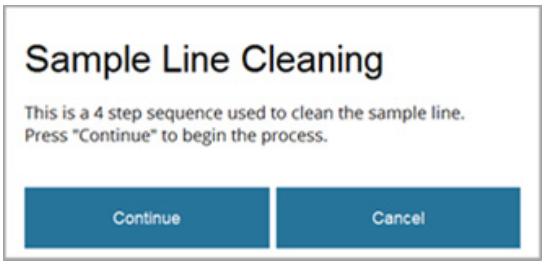
IMPORTANT! 10% bleach is defined as a 1:10 dilution of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5000 ppm of available chlorine. We recommend using laboratory-grade bleach. Avoid bleach with additives (such as perfumes).

Run Sample Line Cleaning

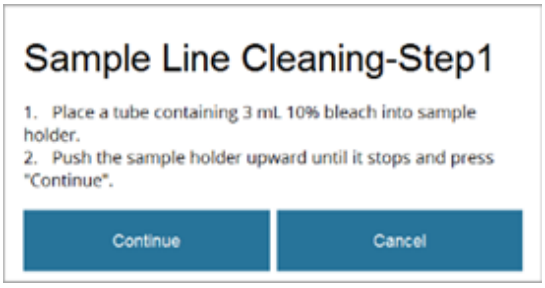
1. On the Maintenance screen, press **Cleaning Cycles**, then select **Sample Line Cleaning**.



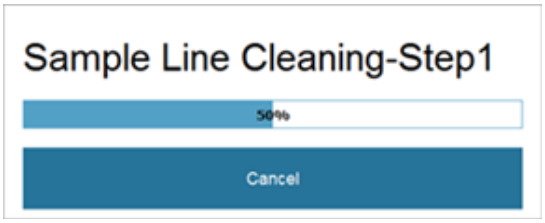
2. When prompted, press **Continue** to begin the sample line cleaning.



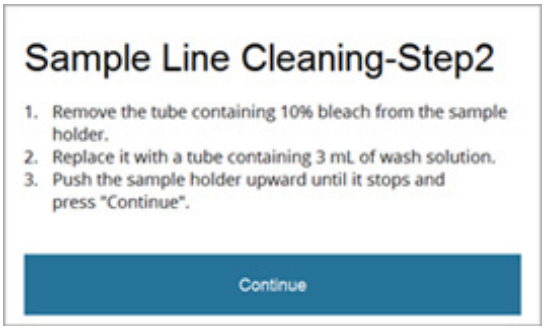
3. When prompted, place a tube containing 3 mL of 10% bleach solution into the sample tube holder.



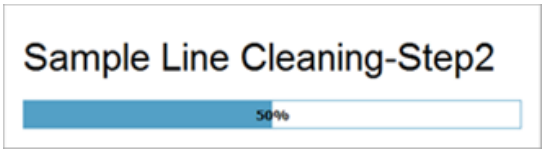
4. Move the sample tube holder up until it stops, then press **Continue** to initiate the first step of the cleaning process.



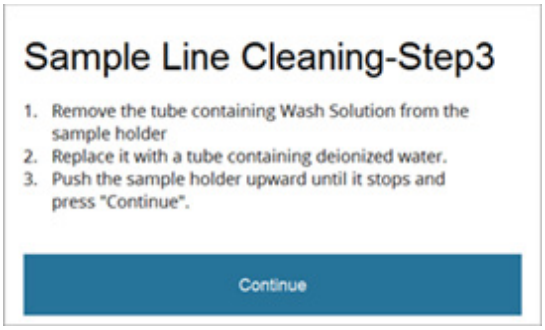
5. When prompted, remove the tube containing the 10% bleach solution from the sample tube holder, and replace it with a tube containing Attune™ Wash Solution.



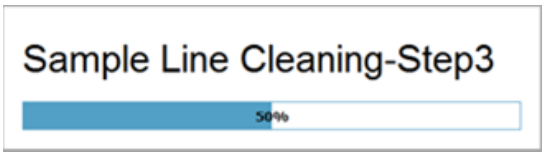
6. Move the sample tube holder up until it stops, then press **Continue** to initiate the second step of the cleaning process.



7. When prompted, remove the tube containing the Attune™ Wash Solution from the sample tube holder, and replace with a tube containing deionized water.

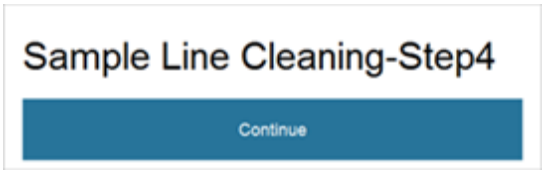


8. Move the sample tube holder up until it stops, then press **Continue** to initiate the third step of the cleaning process.



9. When prompted at the completion of the third step of the cleaning process, click **Continue** to initiate the fourth step.

   The instrument performs this step automatically and you do not have to change solutions.



10. At the successful completion of the sample line cleaning cycle, the instrument goes back to the Cleaning Cycles screen.

Sheath Fluidics Cleaning

Overview

Sheath Fluidics Cleaning is used for decontaminating the fluidics lines of the iSort™ Automated Cell Sorter. Running the sheath fluidics cleaning also requires you to change the 0.2-µm sheath fluid filter. The entire procedure takes approximately 2 hours to complete.

Note: We recommend that you run the sheath fluidics cleaning procedure at least once every six months or as needed to decontaminate the fluidics lines. If you plan to ship your instrument for service, you must also perform the decontamination procedure.

A noticeably high background in the acquired data can indicate bacterial or fungal contamination in the fluidics lines, which can result from not following sterile technique when handling cell samples. Another source of contamination may be the bulk fluidics despite the presence of filters in the fluidics lines of the system.

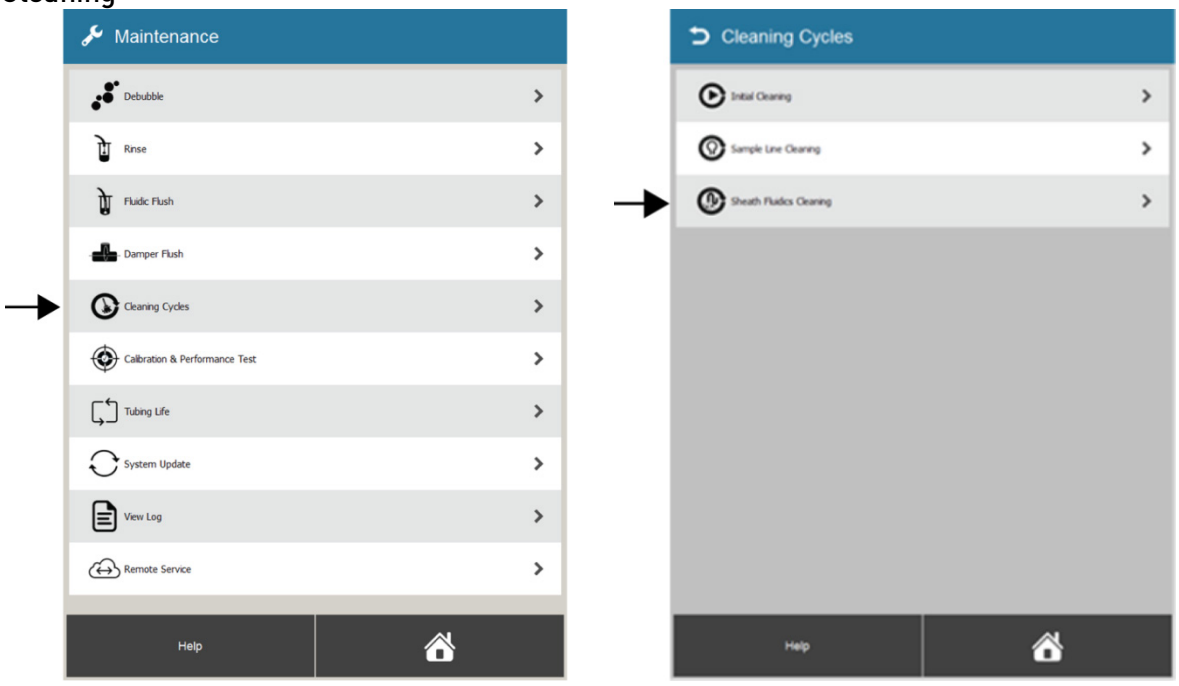
Required materials

- 1 L of 10% bleach solution (cleaning solution used in Step 8 of the protocol, page 63)
- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water
- 0.2-µm sheath inlet filter (Cat. No. A33307)
- Sheath strainer (Cat. No. A33311)

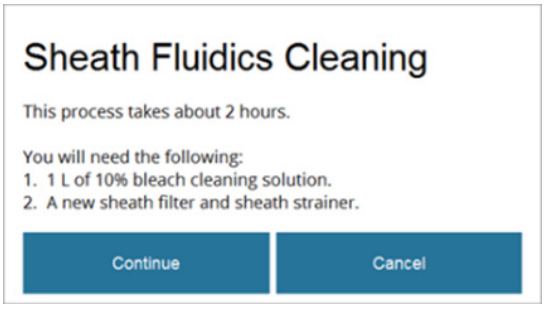
IMPORTANT! 10% bleach is defined as a 1:10 dilution of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5000 ppm of available chlorine. We recommend using laboratory-grade bleach. Avoid bleach with additives (such as perfumes).

Run Sheath Fluidics Cleaning

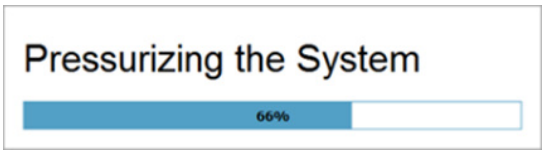
1. On the Maintenance screen, press **Cleaning Cycles**, then select **Sheath Fluidics Cleaning**.



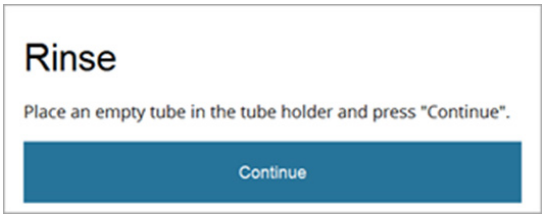
2. When prompted, ensure that you have all of the required materials at hand to perform the sheath fluidics cleaning, then press **Continue**.



The instrument will pressurize the fluidics system and initiate the sheath fluidics cleaning.

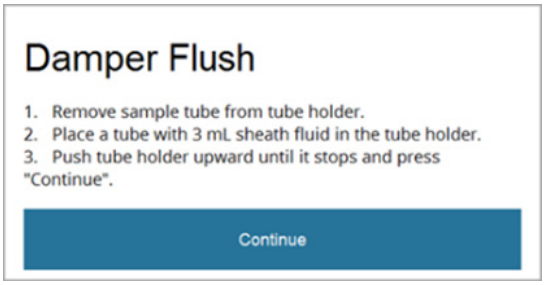


3. When prompted, remove the sample tube (if present) from the sample tube holder and replace it with an empty tube.

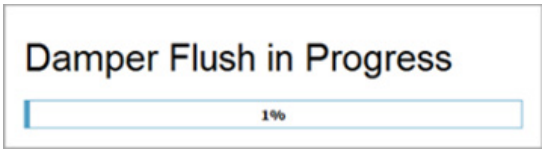


4. Press **Continue** to rinse the sample line.

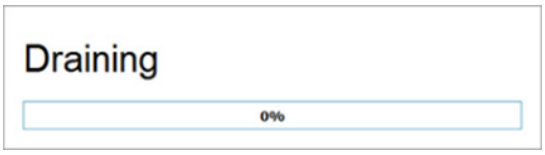
5. When prompted, remove the empty sample tube from the sample tube holder and replace it with a tube containing 3 mL of sheath fluid.



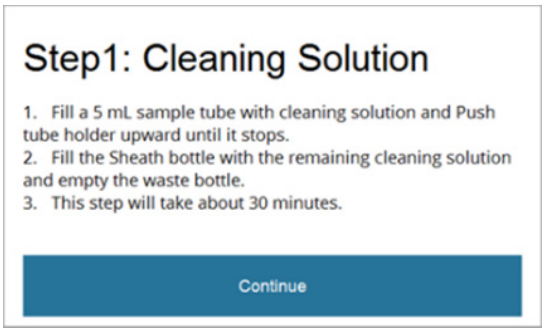
6. Move the sample tube holder up until it stops, then press **Continue** to begin the damper flush.



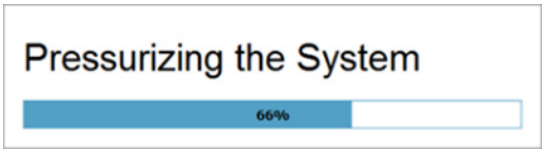
7. When the damper flush is completed, the instrument drains the sheath fluid from the fluidics lines and internal reservoirs into the waste container.

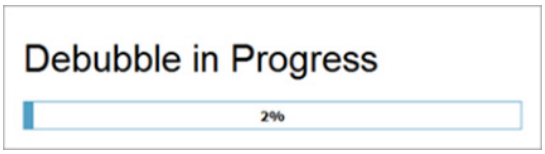


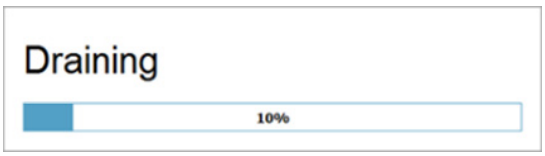
8. When prompted, fill a new sample tube with 5 mL of cleaning solution (10% bleach solution), place it in the sample tube holder, then move the sample tube holder in the up position.



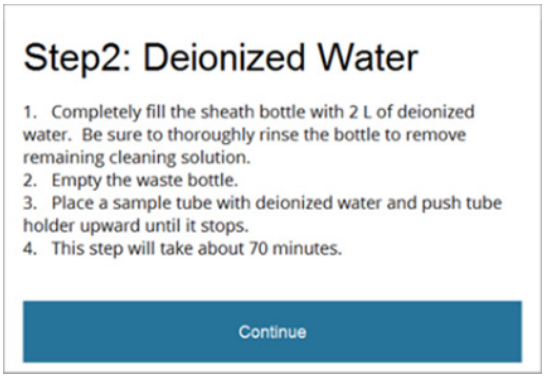
9. Empty the sheath fluid and waste containers, then fill the sheath fluid container with the remaining cleaning solution (~995 mL of 10% bleach solution).
10. Press **Continue** to begin the first step (decontamination) of the sheath fluidics cleaning. This step takes about 30 minutes to complete.
11. The instrument pressurizes the fluidics system, performs one cycle of debubble and one cycle of fluidic flush, then drains the 10% bleach solution from the fluidics lines and internal reservoirs into the waste container.



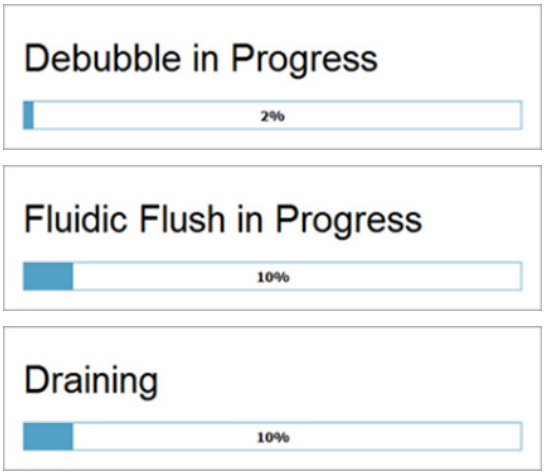




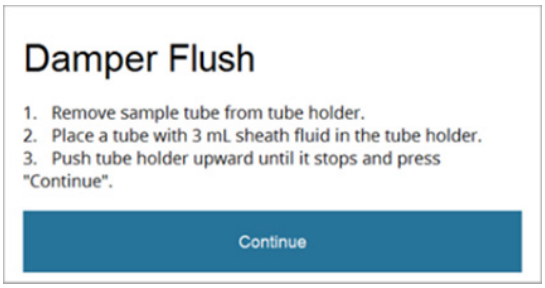
12. When prompted, empty the waste container and the sheath fluid container holding the remaining cleaning solution.



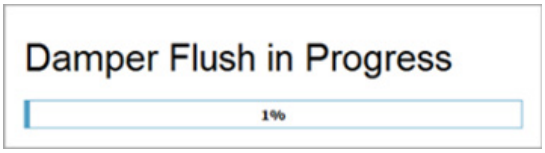
13. Thoroughly rinse the sheath fluid container to remove any traces of the cleaning solution, then completely fill it up with deionized water (~ 2 L).
14. Fill a new sample tube with 5 mL of deionized water, place it in the sample tube holder, then move the sample tube holder in the up position.
15. Press **Continue** to begin the second step of the sheath fluidics cleaning. This step takes about 70 minutes to complete.
16. The instrument performs three consecutive debubble-fluidic flush cycles and drains the fluidics lines and internal reservoirs into the waste container at the completion of each debubble-fluidic flush cycle.



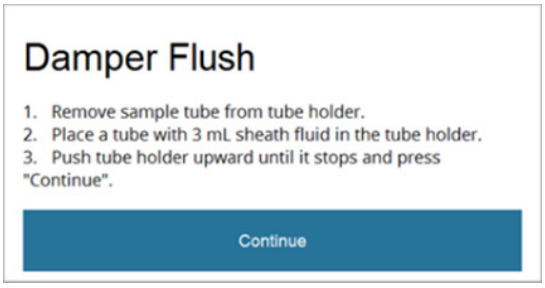
17. When prompted, remove the sample tube with the deionized water from the sample tube holder, and replace it with a new sample tube containing 3 mL of sheath fluid.



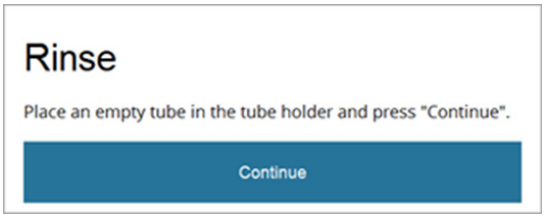
18. Move the sample tube holder in the up position, then press **Continue** to begin the damper flush cycles. The instrument performs three cycles of damper flush.



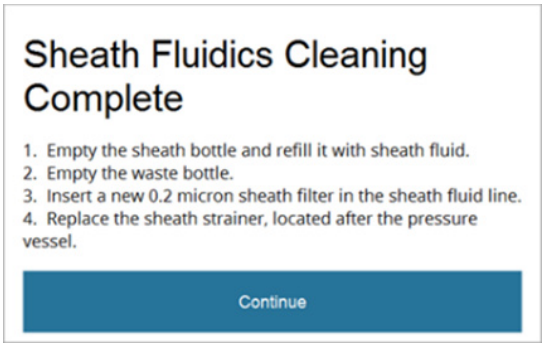
19. At the completion of the first and second damper flush cycles, the instrument prompts you to place a new sample tube containing 3 mL of sheath fluid in the sample tube holder in the up position.



20. After the completion of the third damper flush, the instrument prompts you to perform a rinse. When prompted, remove the sample tube containing the sheath fluid and replace it with an empty sample tube.



21. With the sample tube holder in the down position, press **Continue** to initiate the final rinse. At the completion of the rinse, he instrument drains the fluidics lines and internal reservoirs into the waste container.
22. When prompted, empty the sheath fluid and waste containers, then refill the sheath container with sheath fluid.

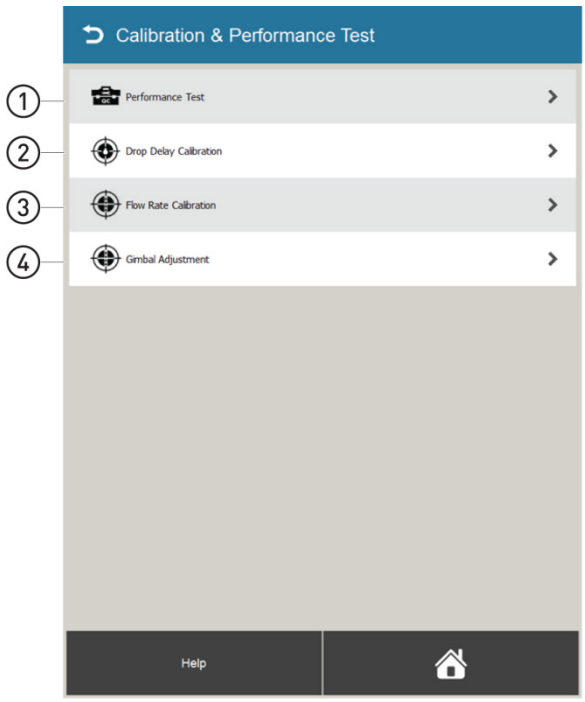


23. To complete the sheath fluidics cleaning procedure, replace the 0.2-µm sheath inlet filter in the sheath fluid line with a new filter (see page 117), then press **Continue** to return to the Cleaning cycles menu.

> **IMPORTANT!** After the performing Sheath Fluidics Cleaning, you must install a new 0.2-µm sheath inlet filter in the sheath fluid line. For instructions on how to replace the 0.2-µm sheath inlet filter, see page 117.

Calibration and Performance Test screen

The Calibration and Performance Test screen contains the controls to run the daily performance test and perform various calibration functions.



① Performance Test (page 67)
② Drop Delay Calibration (page 68)
③ Flow Rate Calibration (page 74)
④ Gimbal Adjustment (page 78)

To access the Calibration and Performance Test screen, select the **Calibration & Performance Test** option on the Maintenance screen.

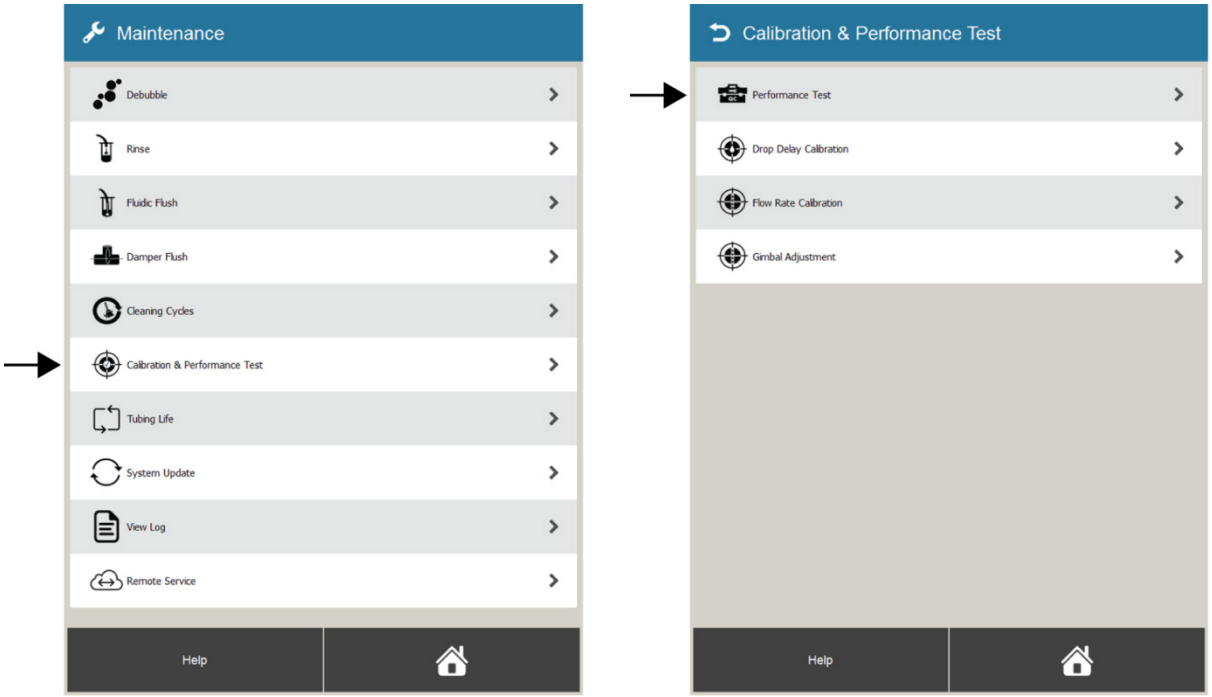
Performance Test

Overview

The Performance Test procedure allows you to track the performance of the iSort™ Automated Cell Sorter and verify it against the QC criteria. The procedure requires the use of the Attune™ Performance Tracking Beads.

IMPORTANT! We recommend that you run the procedure every day to ensure that the system is performing optimally before starting your experiments.

To access the Performance Test screen, press **Calibration & Performance Test** on the Maintenance screen, then select **Performance Test**.



Required materials

- Attune™ Performance Tracking Beads (Cat. No. 4449754)
- 5% Tween™ 20 in deionized water
- 10X PBS, flow cytometry grade
- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water

Run Performance Test

For detailed instructions on how to run the daily performance test, see "Run Performance Test", page 28.

Drop Delay Calibration

Overview

The drop delay value represents the distance between the point of interrogation of the sample and the exact point where the sample stream breaks off into droplets that contain the particle of interest. Therefore, the stability of the break-off dictates the accuracy of the sorting.

Under typical operation, the iSort™ Automated Cell Sorter automatically calibrates the optimal drop delay value during system preparation before an Analysis or Sort (page 34). To do this, the instrument checks flow stability and drop formation, and determines charge timing automatically without any user input.

The Drop Delay Calibration function allows authorized users and service personnel to troubleshoot and fine tune the drop delay value.

IMPORTANT! The Drop Delay Calibration function is only available to authorized users and service personnel for troubleshooting and verification purposes. Do not run the Drop Delay Calibration unless you are authorized to do so.

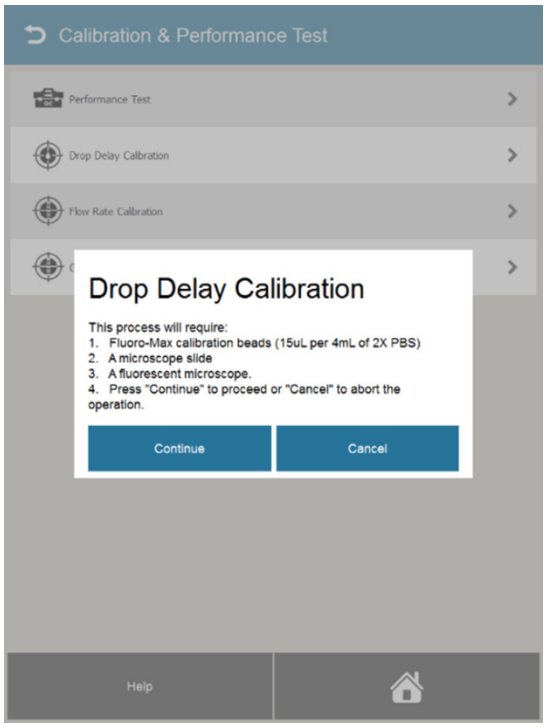
Required materials

- Attune™ Performance Tracking Beads
- Microscope slide
- Fluorescent microscope
- 10X PBS, flow cytometry grade
- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water

Run Drop Delay Calibration

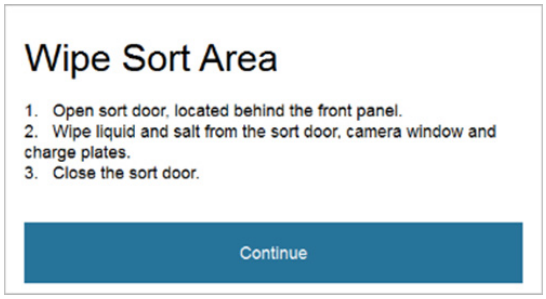
1. On the **Calibration & Performance Test** screen, press **Drop Delay Calibration.**

2. When prompted, press **Continue** to initiate the calibration process.
   If you are not authorized to run the Drop Delay Calibration, press **Cancel** to abort the operation.



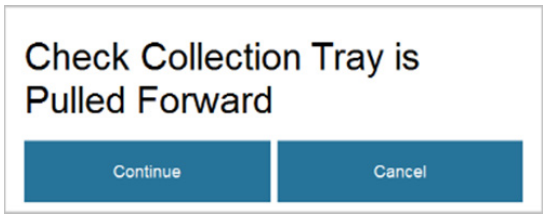
3. If needed, the instrument will prompt you to prepare the sample line. To prepare the sample line, press **Continue**.
   To proceed with Drop Delay Calibration without preparing the sample line, press **Skip**.

4. When prompted, open the sort chamber door (located behind the front panel; see page 11), then wipe the liquid and salt from the sort chamber door, camera window, and the deflection plates as described on page 96.

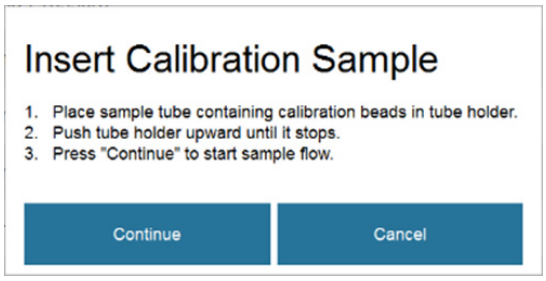


5. Close the sort chamber door, then press **Continue**.

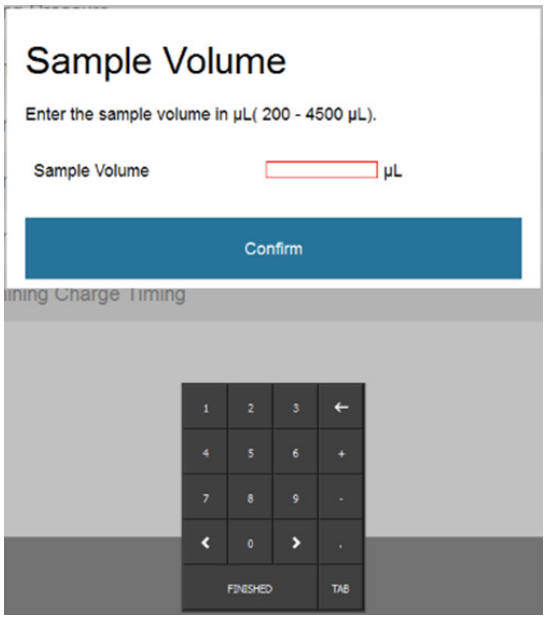
6. Make sure that the collection tray is pulled out, then press **Continue**.



7. When prompted, add Attune™ Performance Tracking Beadst in a sample tube, then place the sample tube with the calibration beads in the tube holder.

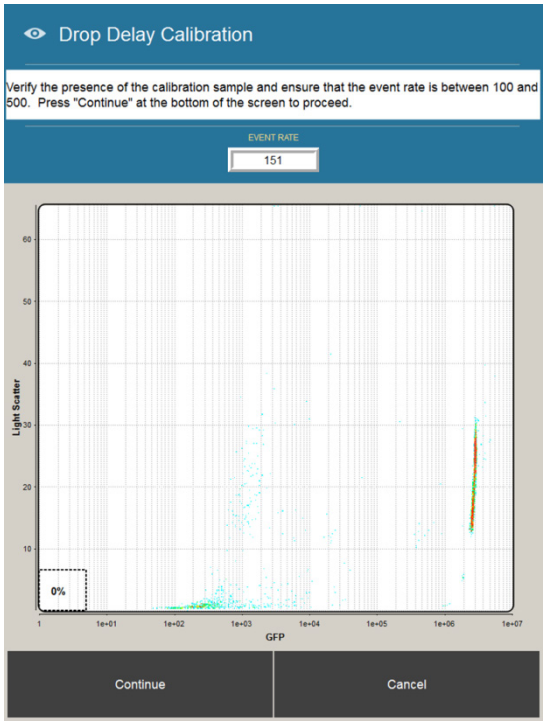


8. Push the tube holder upward until it stops, then press **Continue** to start the sample flow.
9. When prompted, enter the calibration sample volume (200–4500 µL) using the numeric keypad on the touchscreen, then press **Finished**.

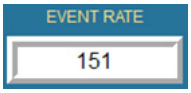


10. Confirm that the sample volume is entered correctly, then press **Confirm** to proceed to the next step.

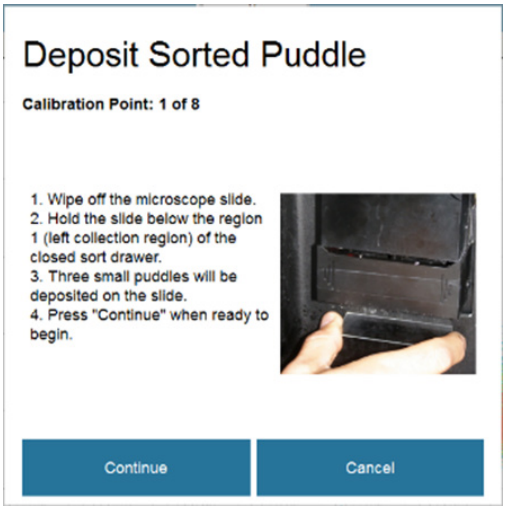
11. On the Drop Delay Calibration screen, verify the presence of the calibration beads in the fluorescence scatter plot.



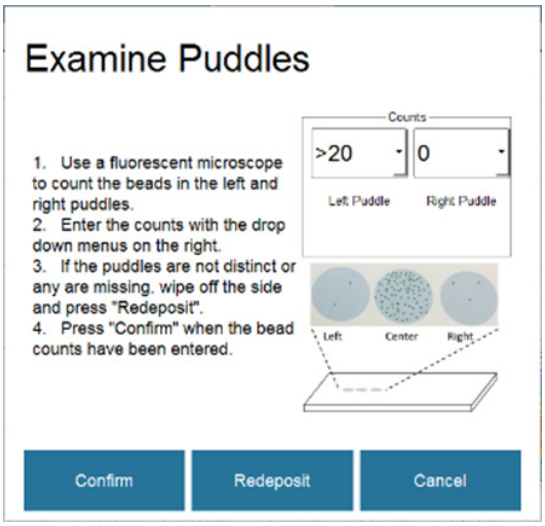
12. Ensure that the event rate counter located above the scatter plot registers an event rate of between 100 and 500, then press **Continue**.



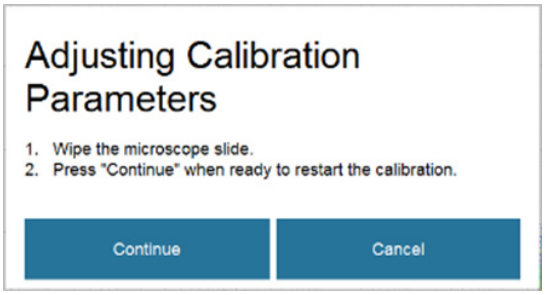
13. When prompted, collect the sorted puddles for the first calibration point (you will collect a total of eight calibration points):
    a. Wipe off a microscope slide with a laboratory wipe to make sure it is clean and free of dust and other particulate matter.
    b. Hold the slide below Region 1 (left collection region) of the closed sort drawer until the instrument deposits three small puddles on the slide.
    c. Press **Continue** to proceed with examining the puddles for the presence of the calibration beads.



14. When prompted, examine the puddles deposited on the slide under a fluorescent microscope for the first calibration point:
    a. Using a fluorescent microscope, count the calibration beads in the left and right puddles.
    b. Enter the number of the beads in the left and right puddles using the dropdown menus on the touchscreen.
    c. If the puddles are not distinct or any of them are missing, wipe off the slide with a laboratory wipe, hold it below Region 1, then press **Redeposit**.
    d. After you have counted and entered the calibration bead counts, press **Confirm**.



15. As the instrument adjusts the calibration parameters based on the first calibration point, wipe the microscope slide, then press **Continue** to restart the calibration for the second calibration point.



16. When prompted, collect the second calibration point as described in Steps 13–15 (pages 71–72), then continue to collect the remaining calibration points.

Note: You will collect a total of eight calibration points. The instrument will adjust the calibration parameters based on the calibration bead counts after the collection of each calibration point.

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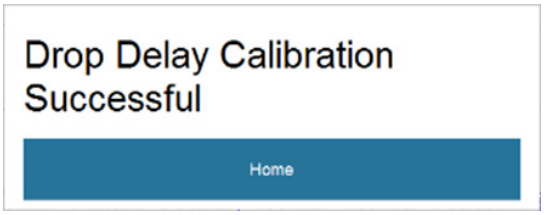
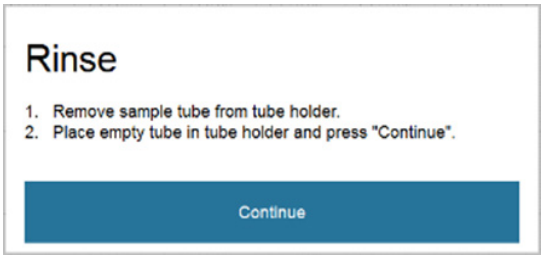
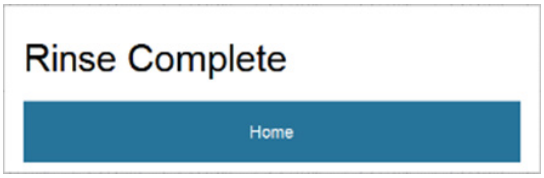
17. After the instrument adjusts the calibration parameters based on the final calibration point, the instrument displays the “Drop Delay Calibration Successful” message.



18. Press **Home** to complete the Drop Delay Calibration procedure.
19. When prompted, replace the sample tube with the calibration beads with an empty tube in the tube holder, then press **Continue** to start the final rinse procedure.



20. After the rinse is completed, press **Home** to return to the Home screen.



Flow Rate Calibration

Overview

The Flow Rate Calibration function allows authorized users and service personnel to troubleshoot and fine tune the flow rate value.

The sample flow rate of the iSort™ Automated Cell Sorter is fixed at 1.4 mL per hour and cannot be adjusted. However, under certain circumstances, authorized users and service personnel may need to use the Flow Rate Calibration function when troubleshooting hardware issues. This function is not available to users for typical daily use.

IMPORTANT! The Flow Rate Calibration function is only available to authorized users and service personnel for troubleshooting and verification purposes. Do not run the Flow Rate Calibration unless you are authorized to do so.

Required materials

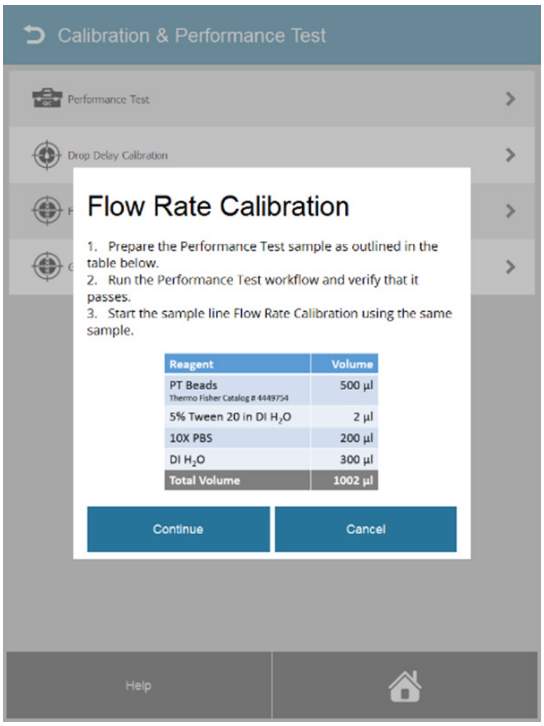
- Attune™ Performance Tracking Beads
- 5% Tween™ 20 in deionized water
- 10X PBS, flow cytometry grade
- Sheath fluid (1X PBS, flow cytometry grade)
- Deionized water

CAUTION! The Attune™ Performance Tracking Beads contain 0.05% sodium azide as a preservative. Sodium azide is an extremely toxic and dangerous compound, particularly when combined with acids or metals. Properly dispose of solutions containing sodium azide.

Run Flow Rate Calibration

1. On the **Calibration & Performance Test** screen, press **Flow Rate Calibration.**

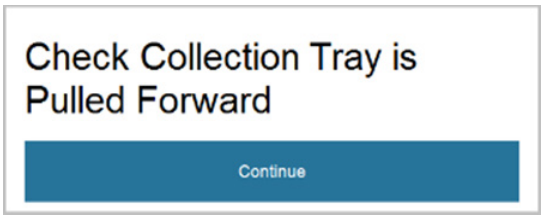
2. When prompted to prepare the Performance Test sample, briefly vortex the vial of Attune™ Performance Tracking Beads to mix, then add the following components into a 12 × 75-mm disposable test tube.



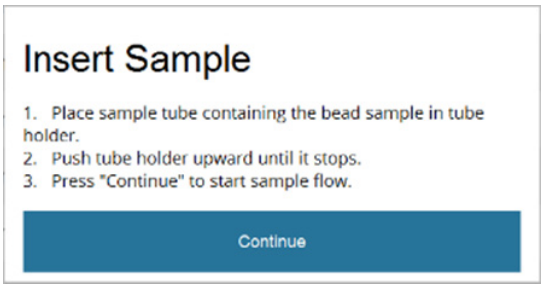
Attune™ Performance Tracking Beads	500 µL
5% Tween™ 20 in deionized water	2 µL
10X PBS	200 µL
Deionized water	300 µL
Total volume:	1002 µL

3. Press **Continue** to initiate the calibration process.
   If you are not authorized to run the Flow Rate Calibration, press **Cancel** to abort the operation.
4. If needed, the instrument will prompt you to prepare the sample line. To prepare the sample line, press **Continue**.
   To proceed with Flow Rate Calibration without preparing the sample line, press **Skip**.

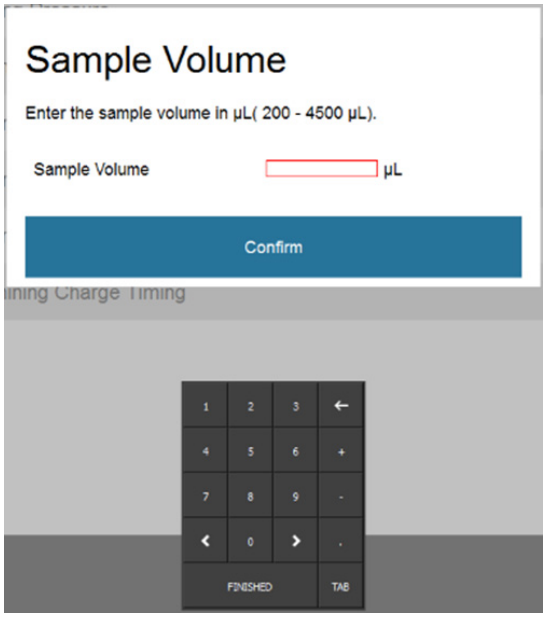
5. Make sure that the collection tray is pulled out, then press **Continue**.



6. When prompted, place the sample tube with the performance tracking beads in the tube holder, push the tube holder upward until it stops, then press **Continue** to start the sample flow

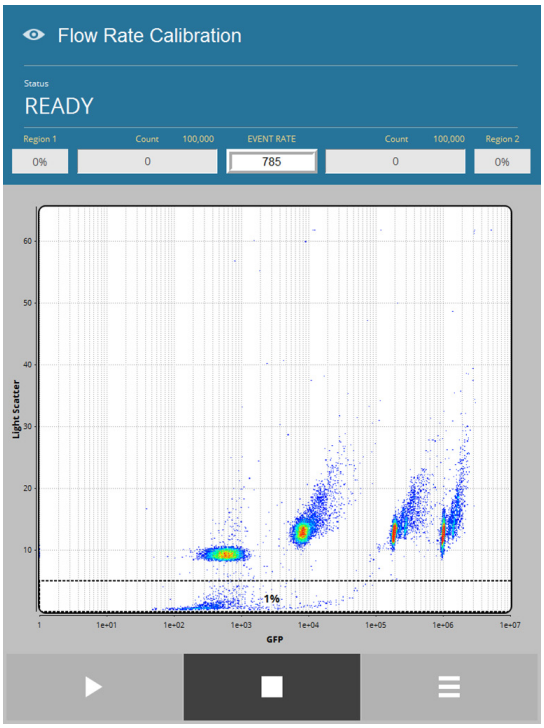


7. When prompted, enter the calibration sample volume (200–4500 µL) using the numeric keypad on the touchscreen, then press **Finished**.

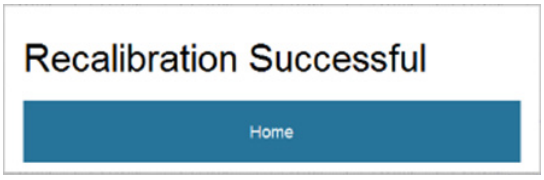


8. Confirm that the sample volume is entered correctly, then press **Confirm** to proceed to the next step.

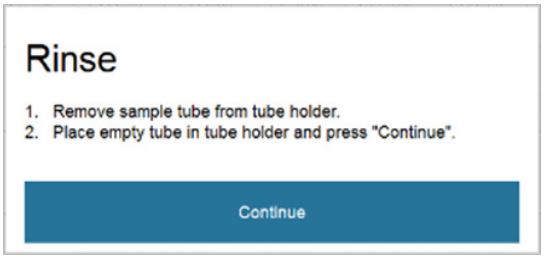
9. On the Flow Rate Calibration screen, verify the presence of the performance tracking beads in the fluorescence scatter plot, which consist of equal concentrations of beads of four fluorescence emission intensities.



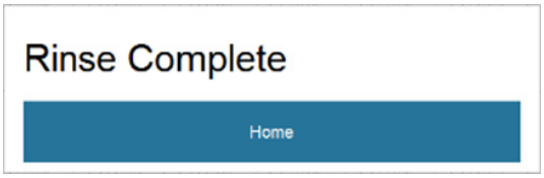
10. After the instrument automatically adjusts the flow rate calibration parameters, it displays the "Recalibration Successful" message.



11. Press **Home** to complete the Flow Rate Calibration procedure.
12. When prompted, replace the sample tube with the calibration beads with an empty tube in the tube holder, then press **Continue** to start the final rinse procedure.



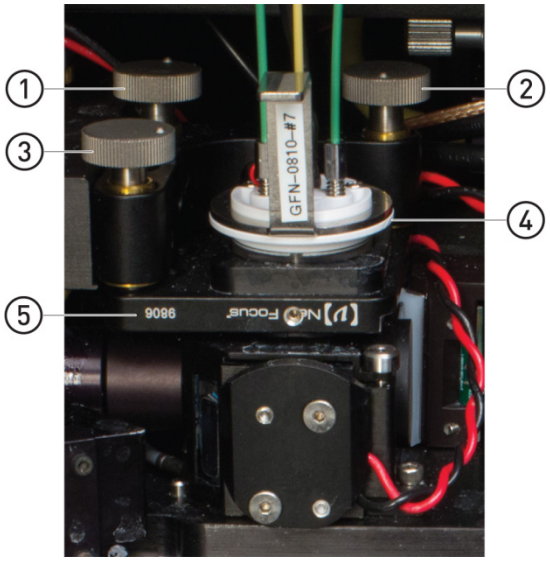
13. After the rinse is completed, press **Home** to return to the Home screen.



Gimbal Adjustment

Overview

The Gimbal Adjustment function allows you to manually adjust the position and the tilt of the nozzle stage so that the sample stream is properly aligned with the system optics. The alignment is accomplished by means of the three gimbal knobs located on the nozzle stage. When the nozzle is aligned properly, unsorted sample stream should hit the waste hole in the sort chamber.



1. Gimbal knob "C"
2. Gimbal knob "B"
3. Gimbal knob "A"
4. Nozzle assembly
5. Nozzle stage

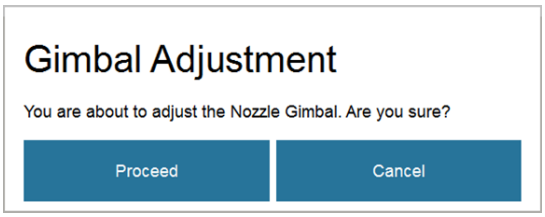
IMPORTANT! The nozzle tip should be removed, cleaned, and reinstalled to prior to gimbal adjustment ensure a focused and stable stream.

Perform gimbal adjustment

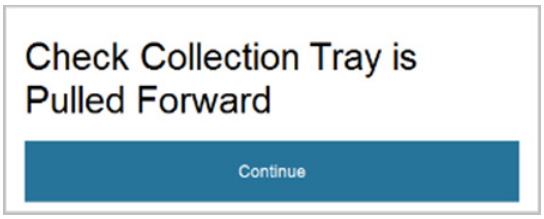
1. On the **Calibration & Performance Test** screen, press **Gimbal Adjustment.**

2. When prompted, press **Proceed** to begin the Gimbal Adjustment process.

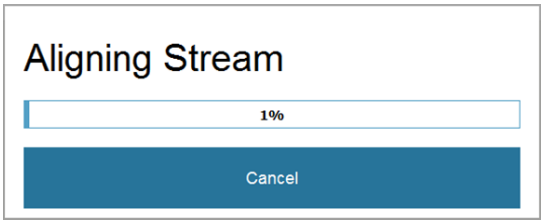
   If you are not authorized to adjust the nozzle gimbal, press **Cancel** to abort the operation.



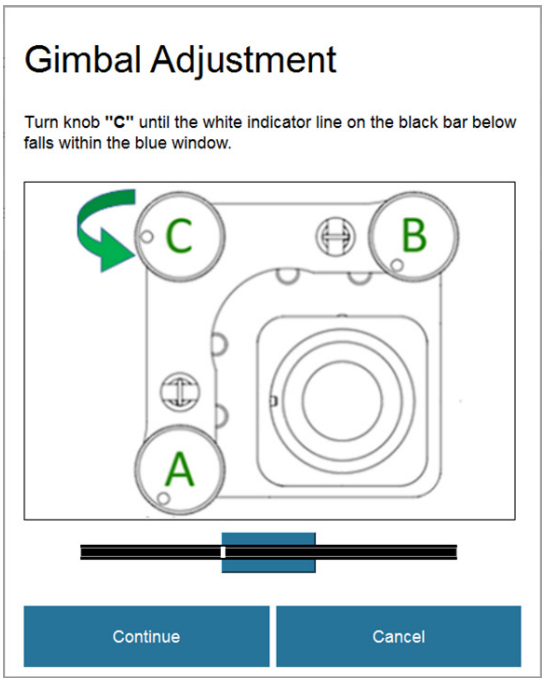
3. Make sure that the collection tray is pulled out, then press **Continue**.



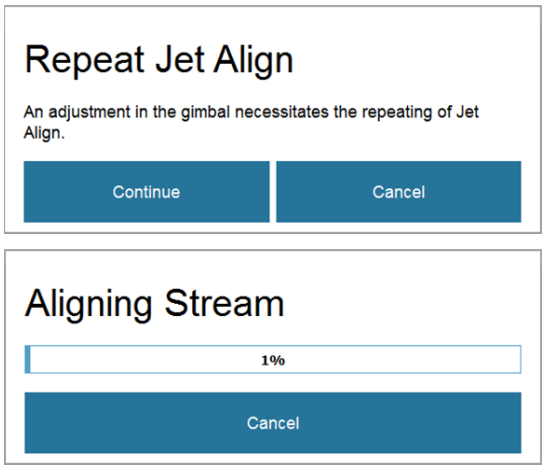
4. The instrument automatically aligns the stream before guiding you through the gimbal adjustment process.



5. When prompted, turn the **Gimbal knob C** until the white indicator line on the screen falls within the blue target window, then press **Continue**.

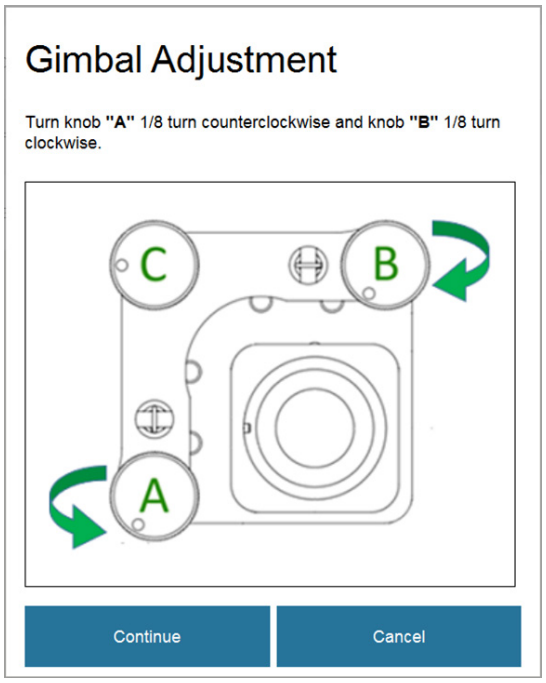


6. When prompted, press **Continue** to repeat the automatic Jet Align procedure.



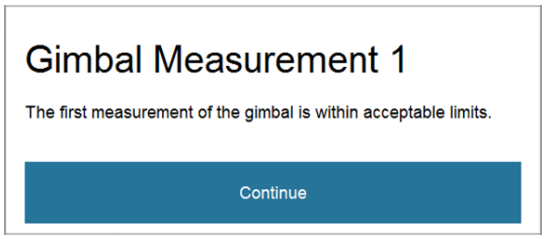
Note: Any adjustment made to the nozzle gimbal requires the repeat of the Jet Align procedure.

7. When prompted, turn the **Gimbal knobs A** and **B** as instructed on the screen, then press **Continue**.



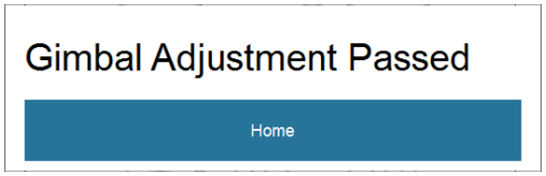
8. When prompted, press **Continue** to repeat the automatic Jet Align procedure.

9. After completing the alignment, the instrument automatically measures the nozzle tilt to ensure that it is within specifications, then measures the gimbal angle.



10. If further adjustments are required, the instrument provides you with the necessary instructions, and rechecks the nozzle tilt for proper alignment.

    Follow the instructions provided until the instrument displays the "Gimbal Adjustment Passed" dialog, then press **Home** on the dialog to return to the Home screen.

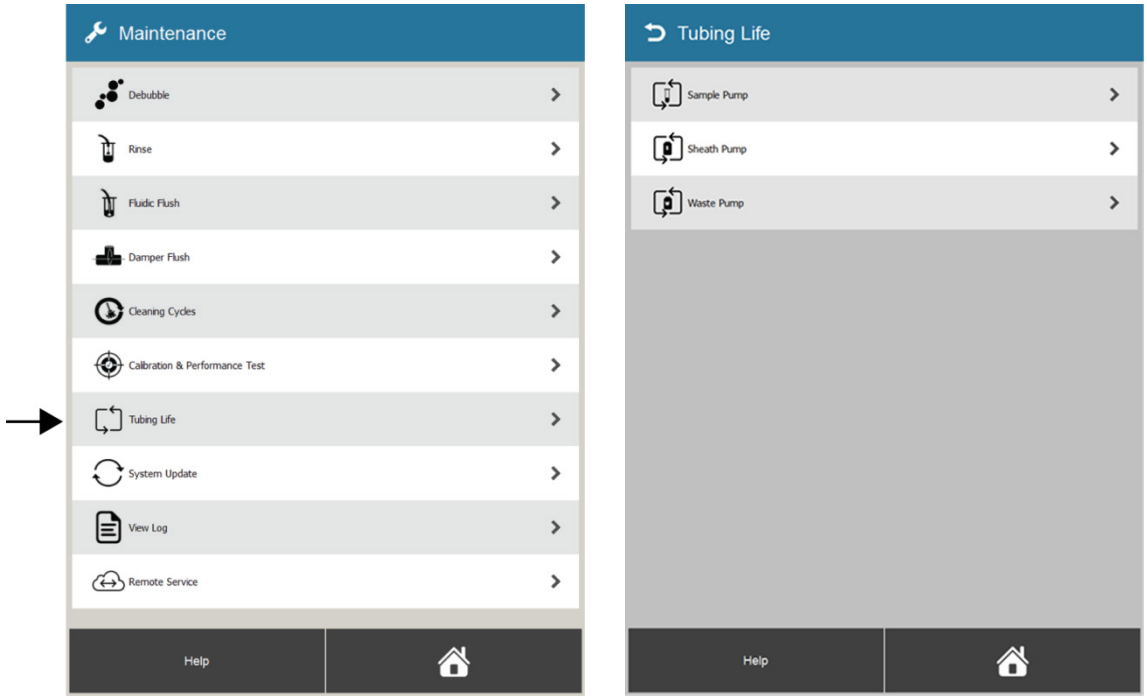


Tubing Life

Tubing Life screen

The Tubing Life screen allows you to check the remaining tubing life for the sample, sheath, and waste pumps, and to reset the tubing life counter after replacement.

To access the Tubing Life screen, press **Tubing Life** on the Maintenance screen.

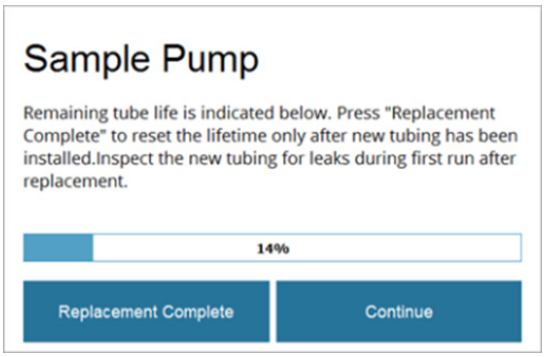


Check remaining tubing life

1. On the Tubing Life screen, select **Sample Pump**, **Sheath Pump**, or **Waste Pump** to check the remaining tubing life for the corresponding pump.

   In the following example, Sample Pump has been selected.

2. The instrument displays the remaining tubing life for the selected pump.

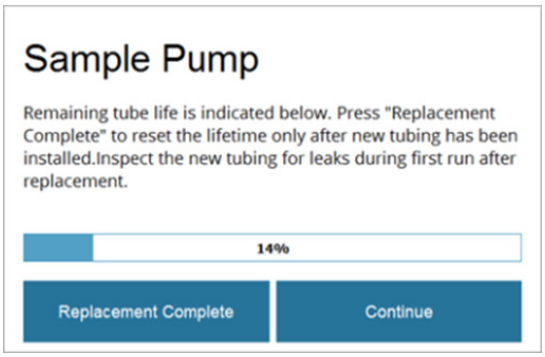


3. Click **Continue** to return to the Tubing Life screen.

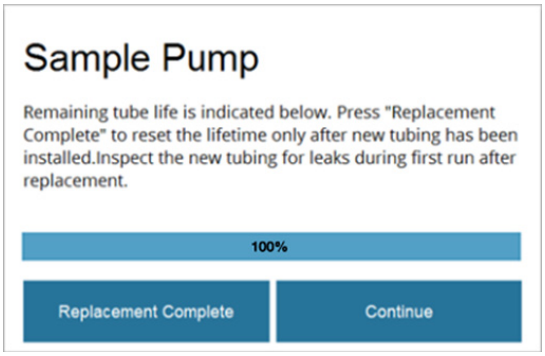
Reset tubing life counter

IMPORTANT! Make sure that you reset the tubing life counter after replacing the tubing for the sample, sheath, or the waste pump. Reset the counter only after you have replaced the existing tubing.

1. On the Tubing Life screen, select **Sample Pump**, **Sheath Pump**, or **Waste Pump** to open the remaining tubing life dialog for the corresponding pump.



2. Click **Replacement Complete** to reset the tubing life counter to 100% and return to the Tubing Life screen.

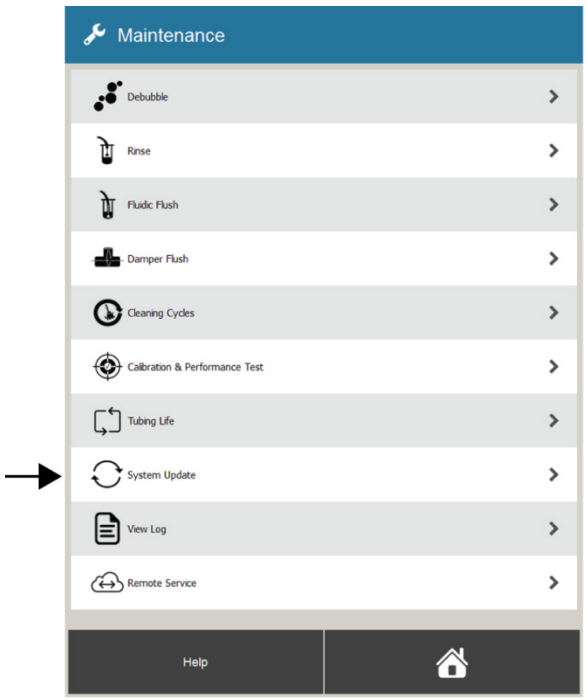


System Update

System Update screen

The System Update screen allows you to update the firmware and software for the iSort™ Automated Cell Sorter.

To access the System Update screen, press **System Update** on the Maintenance screen.



Firmware and software updates

Periodically, Thermo Fisher Scientific makes improvements to the iSort™ Automated Cell Sorter. We recommend keeping your iSort™ Automated Cell Sorter up to date with the latest iSort™ firmware and software.

The latest firmware and software updates are available from the iSort™ Automated Cell Sorter product page at **thermofisher.com/iSort**. If you have any questions about firmware or software updates, contact Technical Support (page 139).

Download latest update

Go to the iSort™ Automated Cell Sorter product page at **thermofisher.com/iSort** and download the firmware or the software update directly to the top level of a USB flash drive with at least 100 MB available.

Do not open or rename the file on your computer; the iSort™ Automated Cell Sorter will verify and install it during the update process.

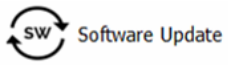
Update firmware

1. Insert the USB flash drive containing the latest iSort™ firmware version into the USB port located at the front of the instrument (see page 9).
2. On the System Update screen, select **Firmware Update** to open the Firmware Update screen.

3. Select the latest iSort™ firmware file from the USB list on the Firmware Update screen, then click **Continue**. The system will go through a partial Shut Down before upgrading the firmware automatically. When the firmware is upgraded, the instrument will complete the Shut Down operation.

Update software

1. Insert the USB flash drive containing the latest iSort™ software version into the USB port located at the front of the instrument (see page 9).
2. On the System Update screen, select **Software Update** to open the Software Update screen.



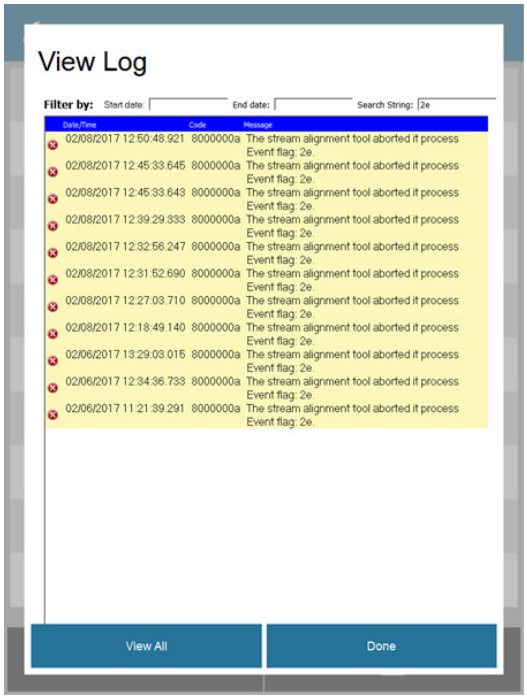
3. Select the latest iSort™ software file from the USB list on the Software Update screen, then click **Continue**. The system will automatically restart after the update is complete.

View Log

View Log screen

The iSort™ Automated Cell Sorter keeps track of actions and errors that occur in the software and saves them as a searchable list accessible through the View Log screen. You can use the log for troubleshooting and, if necessary, reference it when consulting Technical Support.

1. On the Maintenance screen, press **View Log** to access the View Log screen.



2. To filter the log entries by **Start date**, **End date**, **Search String**, or a combination thereof, enter the desired query in the appropriate search box.

3. To view all log entries, press **View All**.
4. To return to the Maintenance screen, press **Done**.

Remote Service

Overview

Remote Service screen allows you to download and launch the Remote Service application.

Remote Service application is a real-time remote instrument diagnostics tool that allows our service engineers and the Remote Service Center support team to help get a failed instrument up and running as quickly as possible using remote monitoring and diagnostic tools. If a field service engineer must be dispatched to your site, this service helps to ensure that they arrive with the right parts to get your instrument repaired quickly.

For more information, go to **thermofisher.com/instrumentservices**.

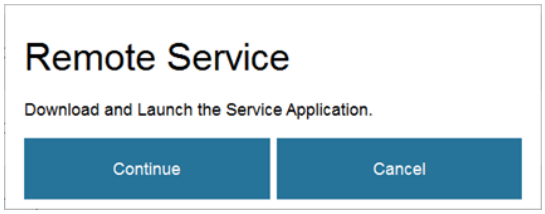
Note: To use the Remote Service application, the iSort™ Automated Cell Sorter must be connected to a network.

Launch Remote Service application

1. On the Maintenance screen, press **Remote Service** to access the Remote Service screen.

2. When prompted, press **Continue** and follow the on-screen instructions to download and launch the Remote Service application.

   Press **Cancel** to return to the Maintenance screen without downloading the application.



7. Shut Down

Daily Shut Down

You must perform the Shut Down procedure after the last Analysis or Sort of the day, then remove the nozzle tip for cleaning and storage. The Shut Down procedure sanitizes the system fluidics with 10% bleach solution, flushes it with a detergent solution, then rinses it with deionized water to complete the cleaning process. It then turns off the fluid stream, charging plates, and the laser. The entire procedure takes approximately 30 minutes to complete.

Required solutions

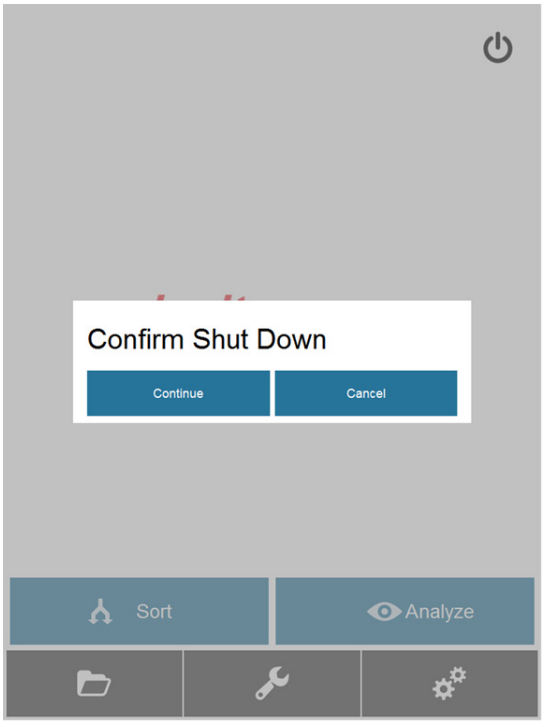
- 10% bleach solution
- Attune™ Wash Solution (Cat. No. A24974) (wash solution in Step 5)
- Deionized water

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

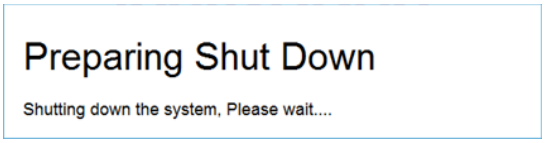
Run the Shut Down procedure

1. On the Home screen, press the **Shut Down** button.

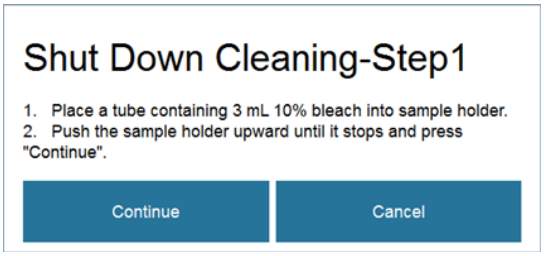
The instrument displays the Confirm Shut Down dialog.



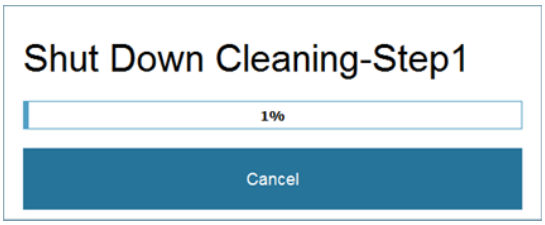
2. Press **Continue** to confirm the Shut Down. The instrument initiates the Shut Down procedure.



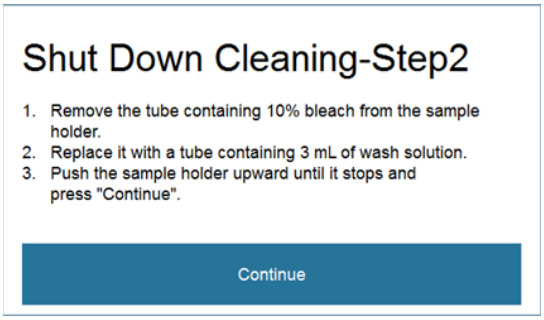
3. When prompted, place a tube containing 3 mL of 10% bleach solution into the sample tube holder.



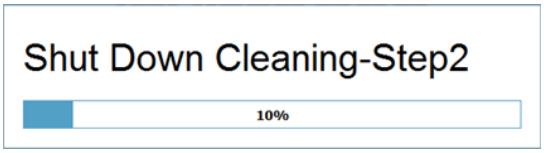
4. Move the sample tube holder up until it stops, then press **Continue** to start the Shut Down Cleaning – Step 1.



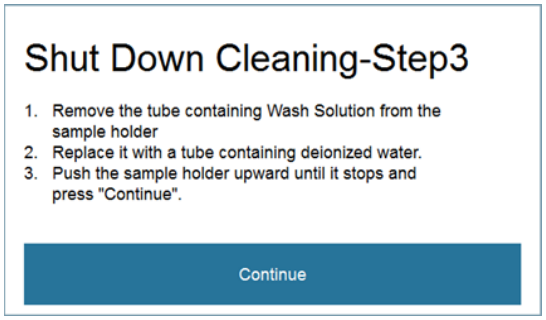
5. When prompted, remove the tube containing the 10% bleach solution from the sample tube holder, and replace with a tube containing Attune™ Wash Solution.



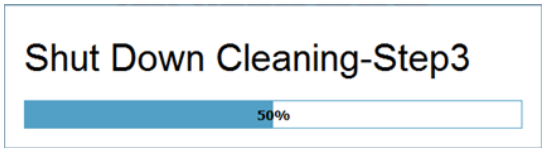
6. Move the sample tube holder up until it stops, then press **Continue** to initiate the Shut Down Cleaning – Step 2.



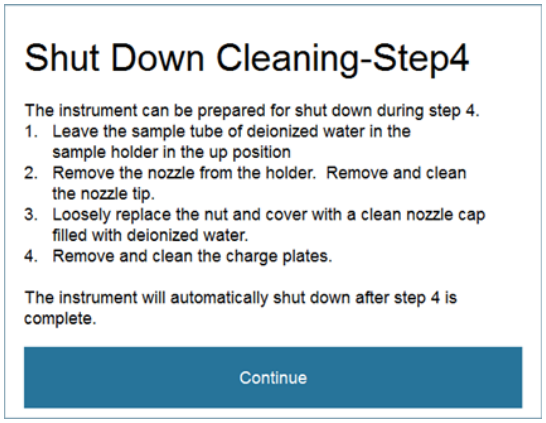
7. When prompted, remove the tube containing the Wash Solution from the sample tube holder, and replace with a tube containing deionized water.



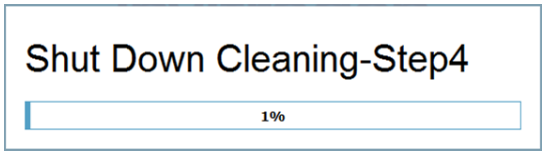
8. Move the sample tube holder up until it stops, then press **Continue** to initiate the Shut Down Cleaning – Step 3.



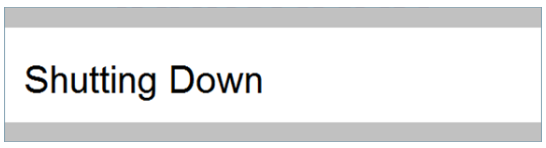
9. After completing the Shut Down Cleaning – Step 3, the instrument displays the prompt for Shut Down Cleaning – Step 4, which describes the subsequent steps to prepare the instrument for shut down.



10. Leave the sample tube of deionized water in the sample tube holder in the up position, then press **Continue** to initiate the Shut Down Cleaning – Step 4.



11. After completing the Shut Down Cleaning – Step 4, the instrument turns off the fluid stream, the deflection plates, and the laser, then powers itself off.



IMPORTANT! When the Shut Down procedure is completed and the system is powered off, remove the nozzle tip and the deflection plates for cleaning and storage (see "Prepare instrument for Shut Down, page 91).

Prepare instrument for Shut Down

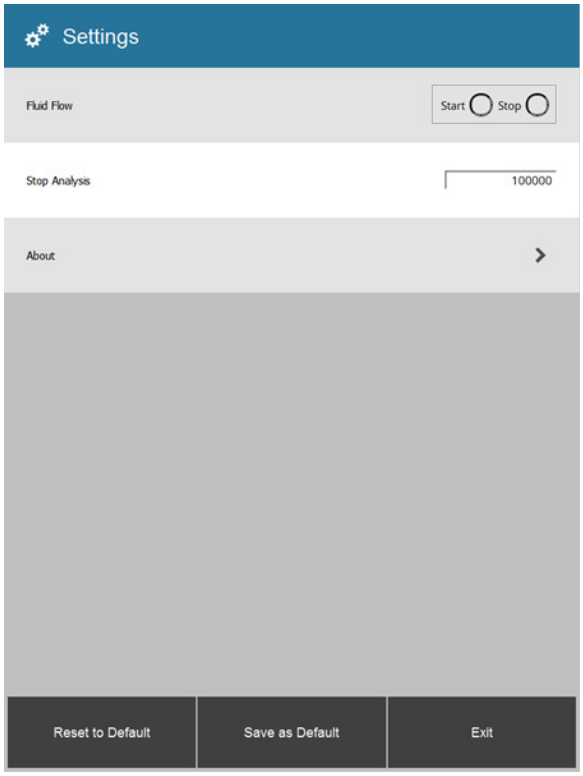
1. When the Shut Down procedure is completed and the system is powered off, leave the sample tube of deionized water in the sample tube holder in the up position.
2. Remove the nozzle from the holder, then remove the nozzle tip from the nozzle assembly, and clean it in an ultrasonic bath (sonicator) as described on page 98.
3. While the nozzle tip is in the ultrasonic bath, loosely screw the small black nut back on the nozzle assembly, then cover it with the black rubber protector containing deionized water.
4. Remove and clean the deflection plates as described on page 97.

8. Settings

Settings screen

The Settings screen allows you to start and stop fluid flow, set Stop Analysis criteria, and review system version information.

To access the Settings screen, press the **Settings** button on the Home screen.



- To save the changes that you have made in the Settings screen as default, press **Save as Default**.
- To reset to the factory settings, press **Reset to Default**.
- To exit the Settings screen without saving any changes, press **Exit**.

Fluid Flow

Allows you to start and stop sheath fluid flow without running Analysis/Sort experiments or the cleaning procedures. This feature is useful when checking fluid flow from nozzle tip or when troubleshooting other instrument functions.

1. To start fluid flow, press the **Start** radio button.
2. To stop fluid flow, press the **Stop** radio button.

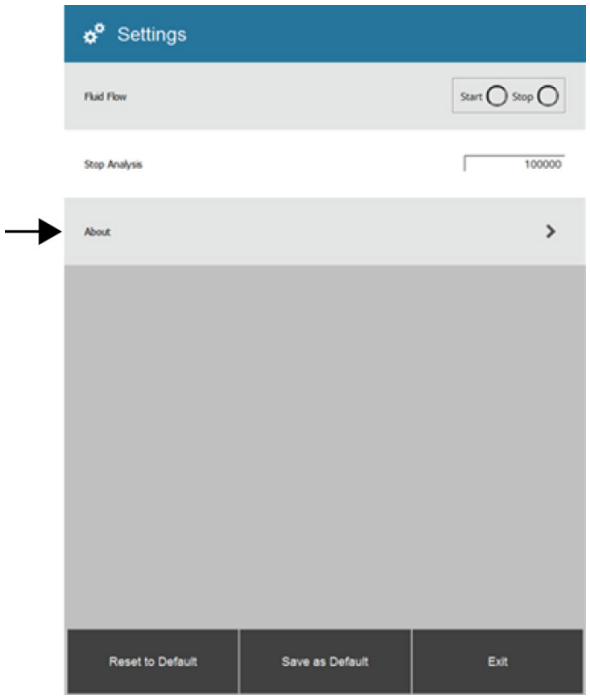
Stop Analysis

Allows you to set event limit for Analysis. The event limit is based on total number of events collected (i.e., total number of events that fall within the regions set). When the event limit is reached, the acquisition stops, and the FCS file is saved. The default event limit is 100,000 events.

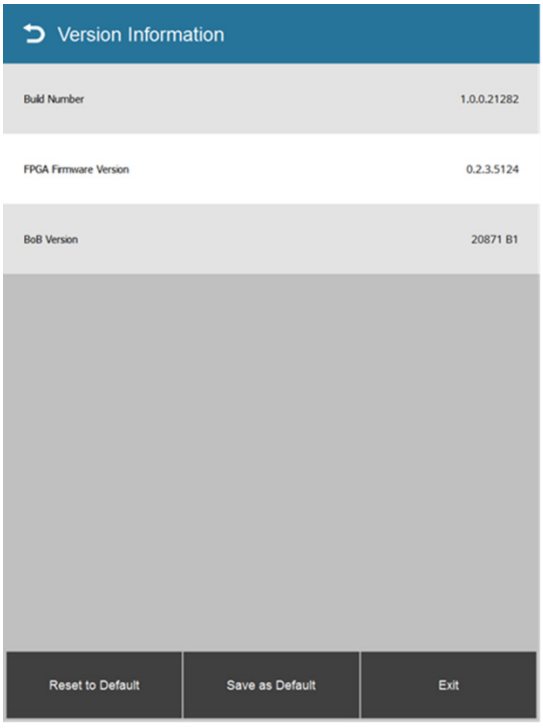
1. To change the event limit, enter the desired value in the Stop Analysis text box, then press **Save as Default**.
2. To reset the event limit, press **Reset to Default**.

About (Version Information)

1. To view system version information about the iSort™ Automated Cell Sorter, press **About** on the Settings Screen.



2. The Version Information screen opens and shows the Build Number, FPGA Firmware Version, and BoB (Break Out Board) Version information for the system.



9. Instrument care and maintenance

Planned maintenance schedule

To ensure reliability of the iSort™ Automated Cell Sorter, you must perform basic preventative maintenance procedures on a regular basis. In addition, certain maintenance and parts replacement procedures must be performed by the Thermo Fisher Scientific field service engineers (FSE) annually. The following table lists the routine maintenance procedures that keep the iSort™ Automated Cell Sorter in good working condition. For detailed instructions on how to perform a specific maintenance function, see pages 96–118.

Procedure	Frequency	Materials required	User	FSE
Inspect and wipe down bottle and fluidics compartment area for drips	Daily	Kimwipes™ laboratory tissues (or other soft, lint free cloth)	✓	
Perform initial cleaning	Daily and when prompted	Sheath fluid (1X PBS, flow cytometry grade) (Cat. No. A1286301), deionized water	✓	
Shut down the system	Daily	10% bleach solution, Attune™ Wash Solution (Cat. No. A24974), deionized water	✓	
Remove and clean the nozzle tip, store it in cleaning solution	Daily	5% Contrad™ 70 detergent solution (cleaning solution), deionized water, ultrasonic bath (sonicator), iSort™ Nozzle Tip cover (Cat. No. A33411)	✓	
Remove and clean the deflection plates	Daily	Kimwipes™ laboratory tissues (or other soft, lint free cloth), isopropyl alcohol or ethanol	✓	
Clean the sort chamber	Between sorts	Kimwipes™ laboratory tissues (or other soft, lint free cloth), deionized water	✓	
Clean the SIP (sample input port)	Between sorts	Kimwipes™ laboratory tissues (or other soft, lint free cloth)	✓	
Perform sample line cleaning	Between sorts	10% bleach solution, Attune™ Wash Solution (Cat. No. A24974), deionized water	✓	
Perform flow rate calibration	As needed	Attune™ Performance Tracking Beads (Cat. No. 4449754), 5% Tween™ 20 in deionized water, 10X PBS (Cat. No. 70011044), Sheath fluid (1X PBS, flow cytometry grade) (Cat. No. A1286301), deionized water	✓	
Perform drop delay calibration	As needed	Attune™ Performance Tracking Beads (Cat. No. 4449754), 10X PBS (Cat. No. 70011044), Sheath fluid (1X PBS, flow cytometry grade) (Cat. No. A1286301), deionized water, microscope slide (Cat. No. A36699), fluorescent microscope	✓	✓
Perform gimbal adjustment	As needed	Sheath fluid (1X PBS, flow cytometry grade) (Cat. No. A1286301)	✓	

Procedure	Frequency	Materials required	User	FSE
Clean or replace waste and sheath bottles	As needed	Full strength bleach solution (5.25% sodium hypochlorite), 10% bleach solution, distilled water, iSort™ Waste Bottle (Cat. No. A33412), iSort™ Sheath Bottle (Cat. No. A33413)	✓	
Perform computer maintenance and back up files before auto-delete	When prompted	USB flash drive	✓	
Perform sheath fluidics cleaning, replace sheath inlet filter and sheath strainer	Monthly and for long term shut down	10% bleach solution, Sheath fluid (1X PBS, flow cytometry grade) (Cat. No. A1286301), deionized water, 0.2-µm sheath inlet filter (Cat. No. A33307), sheath strainer (Cat. No. A33311)	✓	
Replace iSort™ Nozzle Tip O-ring	Monthly (with sheath fluidics cleaning)	iSort™ Nozzle Tip O-ring (Cat. No. A33314)	✓	
Replace sample line peristaltic pump tubing	When prompted (based on timer)	Peristaltic pump tubing (Sample line) (Cat. No. A33303)	✓	✓*
Replace sheath line peristaltic pump tubing	When prompted (based on timer)	Peristaltic pump tubing (Sheath line) (Cat. No. A33279)	✓	✓*
Replace all fluidics lines (Sheath, Waste, and Helper pump tubing)	Annually	Peristaltic pump tubing (Sheath line) (Cat. No. A33279), Peristaltic pump tubing (Waste line) (Cat. No. A33305), Helper pump tubing (Cat. No. A33304)		✓
Clean instrument optics	Annually	0.02 M sodium hydroxide, 95% ethanol, clear ammonia, TexWipe™ TX761MD Microdenier Swabs (Cat. No. A36701)		✓
Replace air pump inlet filter	Annually	Air pump inlet filter (Cat. No. A33306)		✓
Clean fan grids and replace if needed	Annually	Rear fan filter (Cat. No. A33308)		✓
Replace check valves	Annually	Check valves (Cat. No. A33315)		✓
Inspect and re-apply hydrophobic coating in sort area	Annually	Apizon grease		✓
Remove and reapply grease to nozzle tip retaining nut threads and mating surface	Annually	Vacuum grease, DOW (Cat. No. A36181)		✓

* If not already performed by the user.

General care

- When cleaning optical elements, use only optical-grade materials to avoid scratching soft lens coatings. We recommend using TexWipe™ TX761MD Microdenier Swabs (Cat. No. A36701) and deionized water.
- Use the appropriate cleaning solutions for each component, as indicated in the instructions for cleaning and decontamination.

IMPORTANT! Always use the correct power supply. The power adapter specifications appear on the serial number label and in the Electrical safety section (page 136). Damage due to an incompatible power adapter is not covered by warranty.

CAUTION! Never disassemble or service the instrument yourself. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation. Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local distributor to arrange for service.

IMPORTANT! If you have any doubt about the compatibility of decontamination or cleaning agents with parts of the equipment or with material contained in it, contact Technical Support (page 139) or your local iSort™ Automated Cell Sorter distributor for information.

Cleaning procedures

Clean the sort chamber and the sample input port

Before running a sort or analysis, clean the sample input port and the sort chamber, including the sort drawer, door, deflection plates (which can be removed), and stream watch camera window (located between the deflection plates). To clean the instrument surfaces and the stream watch camera window, you can use Kimwipes™ laboratory tissues (or other soft, lint free cloth).

IMPORTANT! Do **not** use abrasive cloths or sponges and harsh or corrosive cleansers as they can damage the lenses.

1. Wipe the sort drawer, the door, the stream watch camera window, and the deflection plates with sterile deionized water to remove dried salt residue, then wipe dry with Kimwipes™ tissues.

   Note: The deflection plates must remain clean and dry for optimal deflection.

2. Blot any liquid from the nozzle tip, the floor of the sort chamber, and the sample input port with Kimwipes™ tissues.

Remove and clean the deflection plates

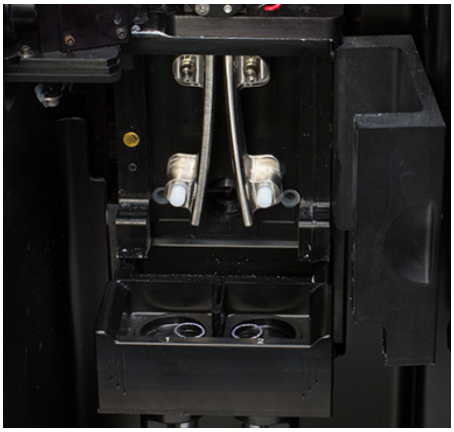
The deflection plates must be removed and cleaned on a regular basis to prevent any buildup of deposits. Significant buildup of sheath fluid deposits due to clogs or misalignment of the nozzle tip can cause arcing between the deflection plates, which can damage the instrument.

We recommend removing and cleaning the deflection plates every day after the daily Shut Down procedure.

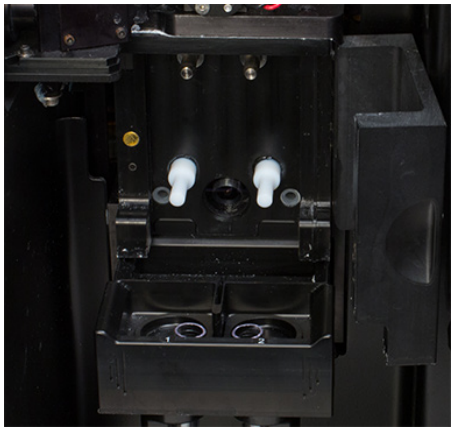
DANGER! ELECTRICAL SHOCK HAZARD. The deflection plates in the sort chamber are charged when sorting. A safety interlock on the sort chamber door disables the deflection plates when the sort chamber door is opened. Do not disable the safety interlock on the sort chamber door.

1. Make sure that the instrument is powered off, then open the sort chamber door to access the deflection plates (see page 11).
2. Wearing gloves, firmly grab one of the deflection plates and pull it out at a right angle to remove it from the sort chamber.

Note: Each deflection plate is secured to the sort chamber by two posts (one metal, one white plastic) that fit snugly into the holes on the plates. You may have to apply some force and wiggle the plates as you pull them out.



Sort chamber with deflection plates



Deflection plates removed

3. Wipe the deflection plates with sterile deionized water to remove dried salt residue, then wipe dry with Kimwipes™ laboratory tissues (or other soft, lint free cloth).
4. To clean contamination from biological spills, wipe the deflection plates with Kimwipes™ tissues moistened with isopropyl alcohol or ethanol, then air dry.

   Alternatively, spray the deflection plates with isopropyl alcohol or ethanol from a spray bottle, then wipe dry with Kimwipes™ tissues.

IMPORTANT! The deflection plates must remain clean and dry for optimal deflection. Before using the iSort™ Automated Cell Sorter, reinstall the deflection plates as described on page 22).

Decontaminate the fluidics lines

To decontaminate the fluidics lines, run the **Sheath Fluidics Cleaning** function. We recommend that you run the sheath fluidics cleaning procedure at least once every six months or as needed to decontaminate the fluidics lines. If you plan to ship your instrument for service, you must also perform the decontamination procedure. For instructions on how to run Sheath Fluidics Cleaning, see page 60.

Remove and clean the nozzle tip

Overview

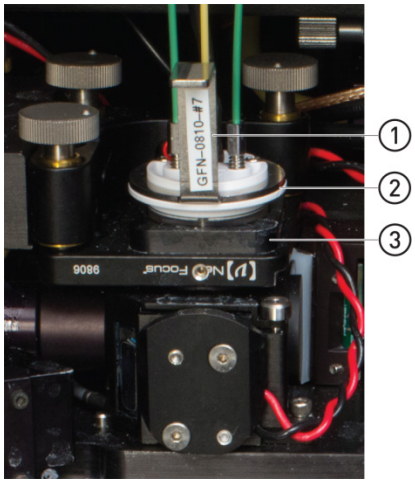
The nozzle is responsible for accepting sample and sheath flows, for producing a focused and stable stream through the 85-µm nozzle tip, and for creating and charging droplets.

For optimal performance, the nozzle tip must be removed and cleaned in an ultrasonic cleaning bath following instrument Shut Down at the end of the day. It is also possible for the nozzle tip to become clogged during normal operation, which might require removal and manual cleaning. In such cases, the instrument displays a warning dialog.

IMPORTANT! Always use clean gloves when handling the ceramic nozzle tip. Never handle the nozzle tip with bare hands, because the oils from your skin can affect fluid flow and instrument performance.

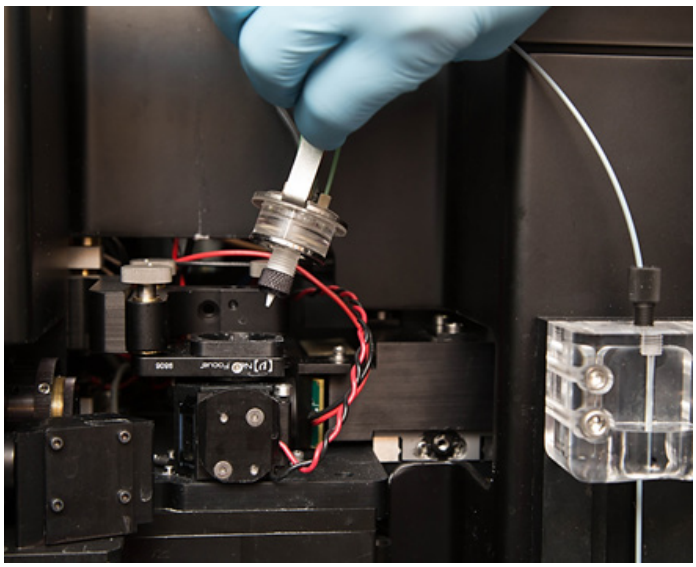
Remove and clean the nozzle tip

The nozzle assembly is located in the sorting area and is secured to its housing via magnetic fasteners. Before removing the nozzle assembly, make sure that the sample and sheath flows have stopped.



① Handle
② Nozzle assembly
③ Nozzle stage

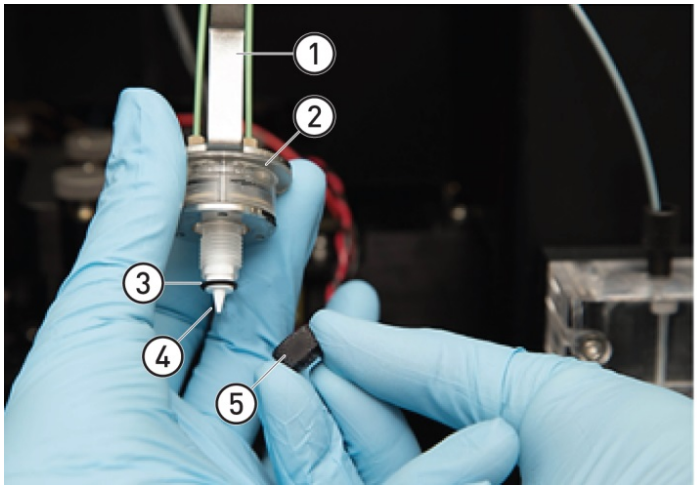
1. Grab the nozzle assembly by the handle and lift it up to remove it from its housing. Note that it may require some force to overcome the magnetic fastener.



2. Unscrew the small black cap that secures the nozzle tip to the nozzle assembly using the torque wrench provided with the instrument.

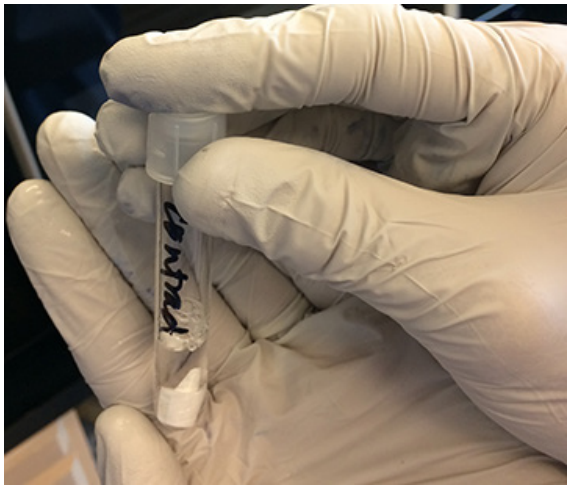
3. Remove the ceramic nozzle tip and the O-ring.

IMPORTANT! Be very careful when handling the nozzle tip. Hold the nozzle tip only by its base and never handle it with bare hands. Always use clean gloves when handling the nozzle tip.



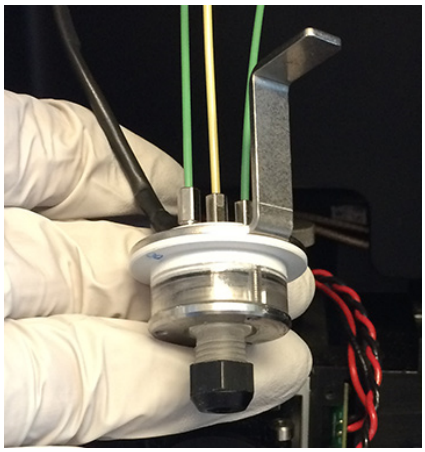
① Handle
② Nozzle assembly
③ O-ring
④ 85-µm nozzle tip
⑤ Black cap

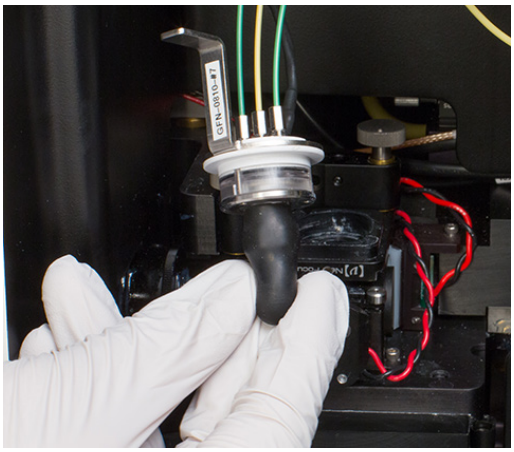
4. Place the nozzle tip in a tube containing 5% Contrad™ 70 detergent solution in deionized water with the nozzle tip facing up.



5. Cap the tube and place it in an ultrasonic cleaning bath (sonicator) for 30 minutes.

6. While the nozzle tip is in the ultrasonic cleaning bath, loosely screw the small black cup back on the nozzle assembly, then cover it with the black rubber protector containing deionized water.





7. If you have removed the nozzle tip after performing the Shut Down operation (page 88), store the nozzle tip in the detergent solution at room temperature until the next time you need to use the instrument.

   If you have removed the nozzle tip to clear a clog and wish to resume your Analysis or Sort, go to Step 8.

8. Remove the nozzle tip from the detergent solution, then rinse it thoroughly with deionized water before reinstalling it on the nozzle assembly (page 101).

9. *Optional:* Use a 1-mL syringe to force deionized water backward through the 85-µm nozzle tip to ensure that the nozzle tip is free of obstructions. Repeat as necessary, switching between the nozzle tip and the larger opposite end to remove any deposits or clogs.

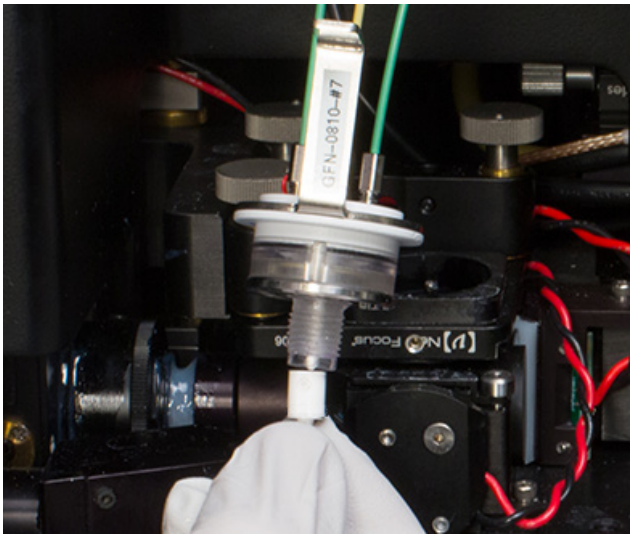
   Note: Watch the nozzle tip as you force the fluid through it. If clean, the fluid stream should come out straight from the tip.

10. When all the deposits or clogs have been removed from the nozzle tip and the nozzle tip is free of all obstructions, reinstall the nozzle tip (page 101).

Reinstall the nozzle tip

IMPORTANT! Hold the nozzle tip only by its base and never handle it with bare hands. Always use clean gloves when handling the nozzle tip.

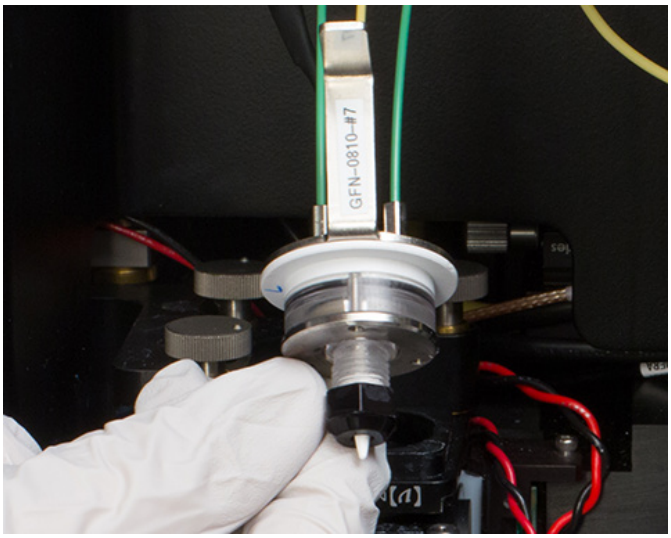
1. Rinse the nozzle tip with deionized water, then reinsert it into the nozzle assembly. The nozzle tip is keyed and can only be inserted in two orientations.



2. Place the O-ring around the nozzle tip, then screw the small black cap back onto the nozzle assembly to secure the tip. It is crucial that the O-ring is removed from the cap and placed around the nozzle tip before the cap is screwed back on.

IMPORTANT! Cross-threading the black cap on the nozzle assembly is a serious concern and may result in alignment failure of the system. We recommend turning the black cap counter-clockwise until the threads engage before beginning to tighten it by turning it clockwise.

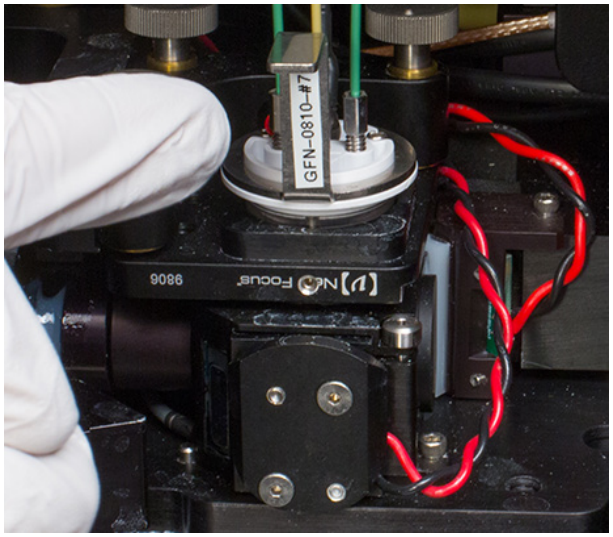
3. Tighten the black cap with the torque wrench (provided with the instrument) until you hear two clicks. Do not overtighten.



Note: Loose coupling of the nozzle tip to the nozzle assembly can result in decreased system performance.

4. Reinsert the nozzle assembly into the Nozzle stage. The nozzle assembly should snap into place by the magnetic fastener.

   When the nozzle is placed into its housing correctly, you will hear a click as it snaps into place. Once you hear the click, you should not be able to rotate the nozzle.



Note: To manually adjust the positioning of the nozzle assembly so that the sample stream is properly aligned with the system optics, see “Gimbal Adjustment”, page 77.

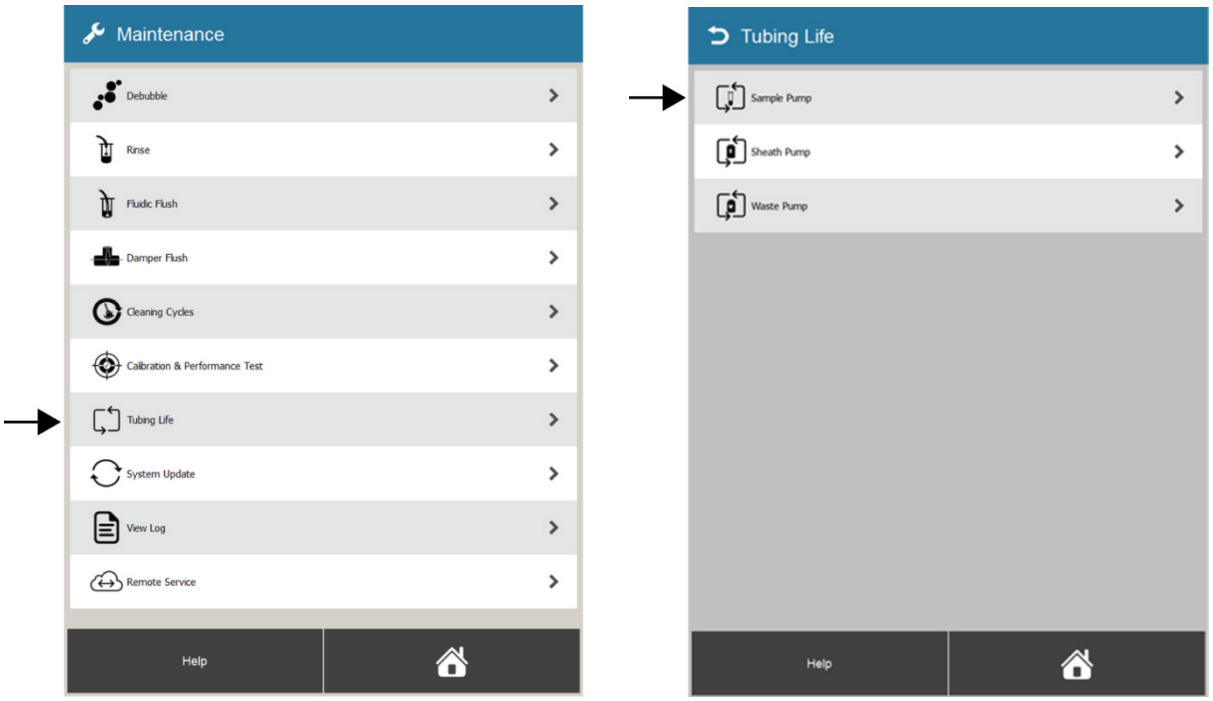
Replace peristaltic pump tubing

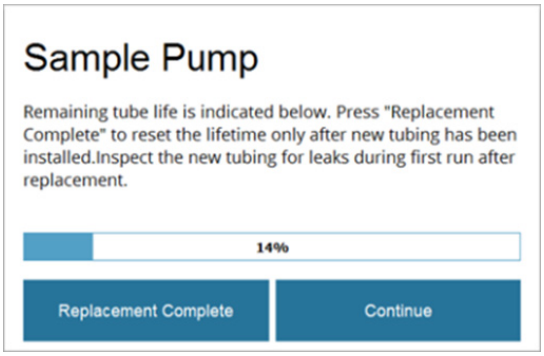
The peristaltic pump tubing for the sample and sheath pumps is a consumable part and requires regular replacement for the instrument to continue normal operation. Additionally, all other tubing in the system can be replaced by a trained technician, if there are concerns relating to the cleanliness of the tubing or contamination.

WARNING! Before replacing any tubing, make sure that the iSort™ Automated Cell Sorter is powered off and unplugged.

Replacement schedule for peristaltic pump tubing

- When the sample, sheath, or waste tubing is nearing the end of its life, the instrument displays a warning message to remind you to replace the tubing.
- You can also check the remaining tubing life for the sample, sheath, and waste pumps by selecting the corresponding option in the **Maintenance ▶ Tubing Life** screen (page 82).

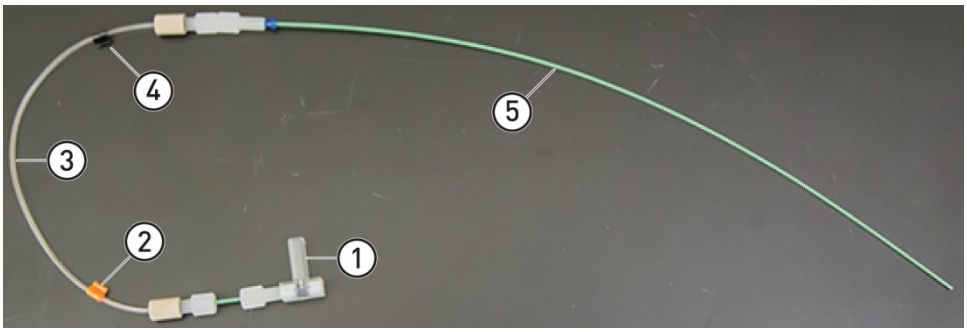




Replace sample pump tubing

In general, we recommend that you replace peristaltic pump tubing for the sample line (Cat. No. A33303) when prompted by the instrument. Normally, the sample pump tubing needs to be replaced every 10 weeks based on 20 hours per week average run time (or every 200 hours of run time).

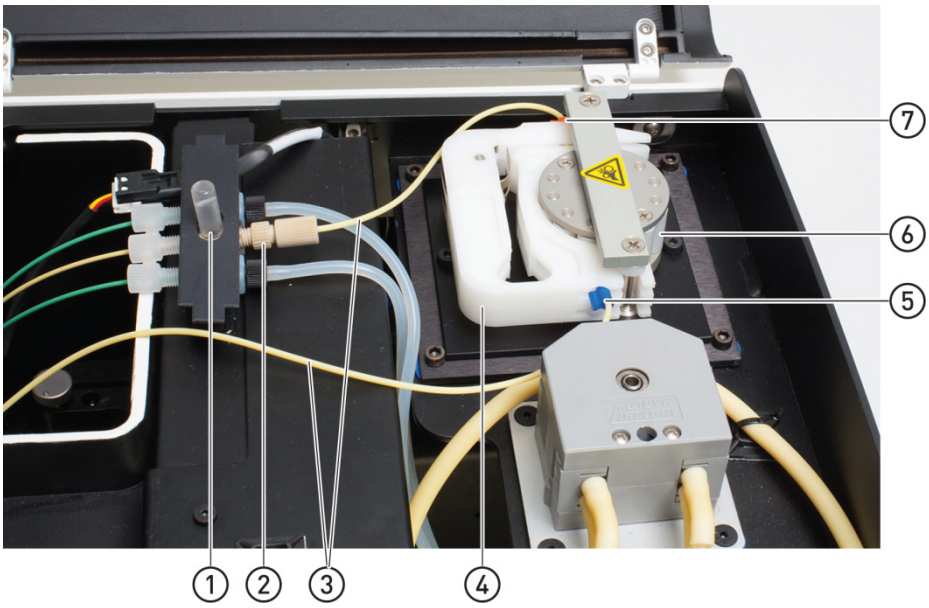
IMPORTANT! When handling the tubing, make sure that it does not become twisted or kinked.



① Sample tube dampener
② Orange stopper
③ Sample pump tubing
④ Blue stopper
⑤ Sample line

Remove sample pump tubing

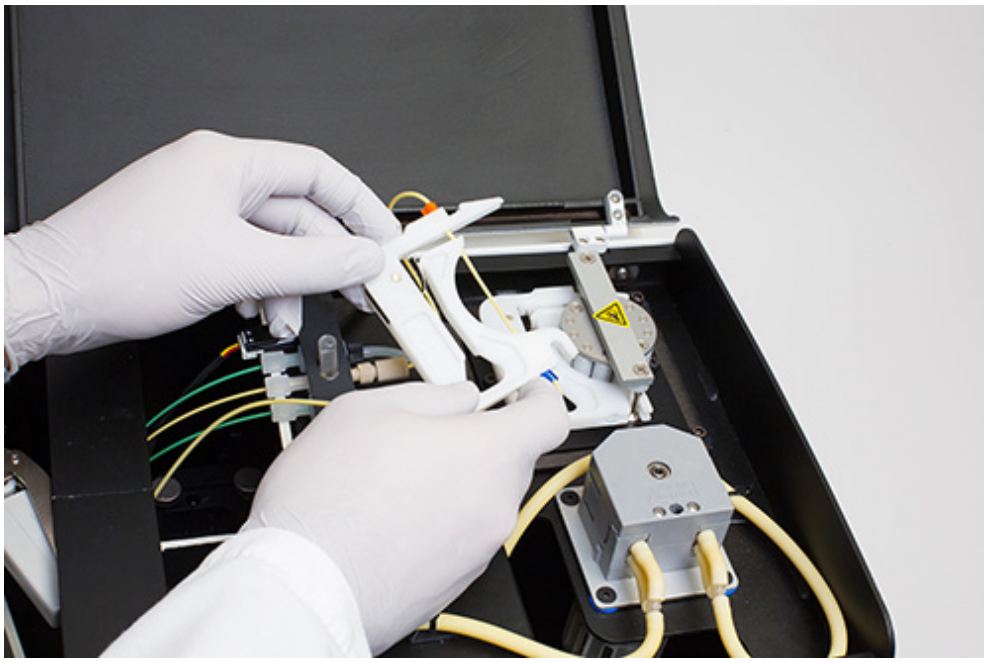
1. Shut down the instrument and unplug before replacing any tubing. Once the Shut Down is complete, open the top cover of the instrument and the sorting compartment door.



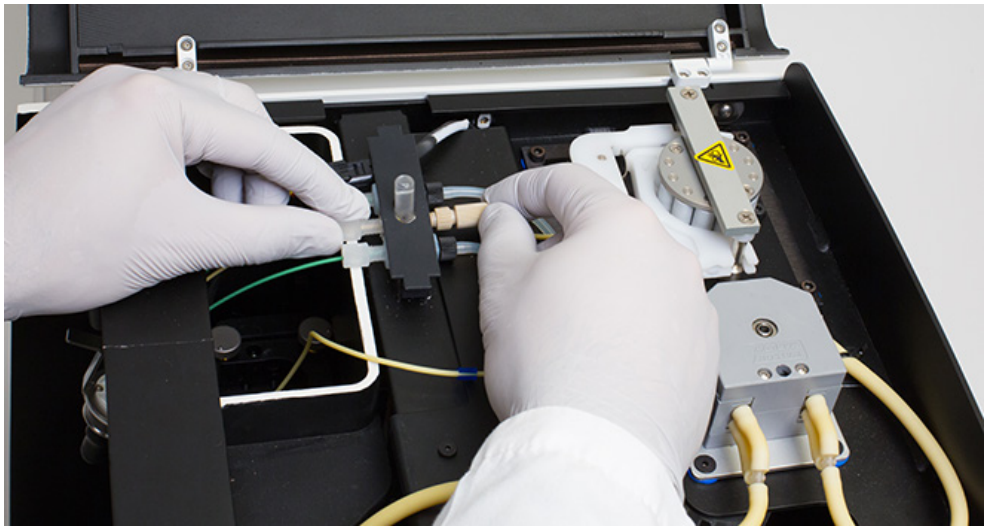
① Sample tube dampener
② Screw
③ Sample pump tubing
④ Shoe
⑤ Blue stopper
⑥ Rollers
⑦ Orange stopper

2. Remove the shoe that secures the sample line to the rollers on the back of the sample pump.

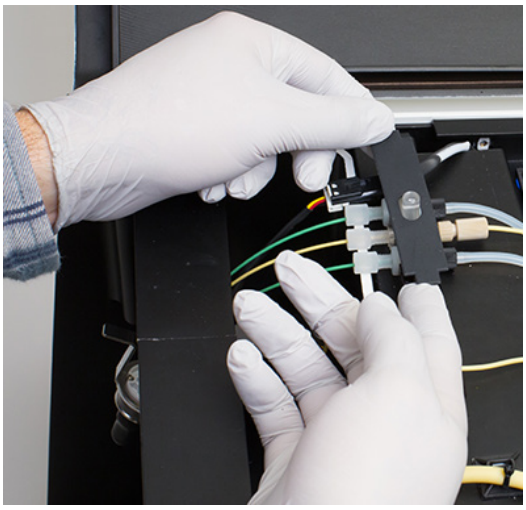
   To do this, push the left arm of the shoe inward to release the notch that secures it to the pump assembly and rotate the shoe away from the pump, then pull the shoe away from the rollers.

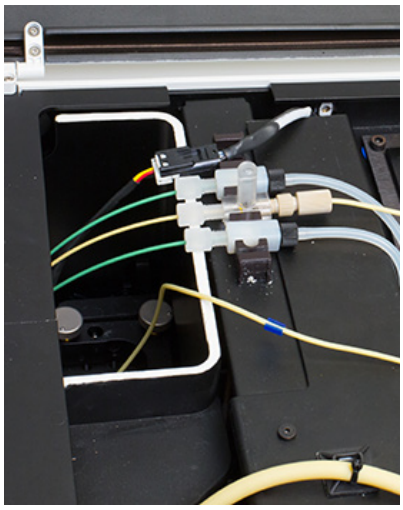


3. Remove the tubing from the shoe by pulling on the blue and orange stoppers attached to the tubing line and moving the line away from the shoe.
4. Loosen the screws that hold the sample pump dampener, but do not yet detach the dampener from the remaining tubing.

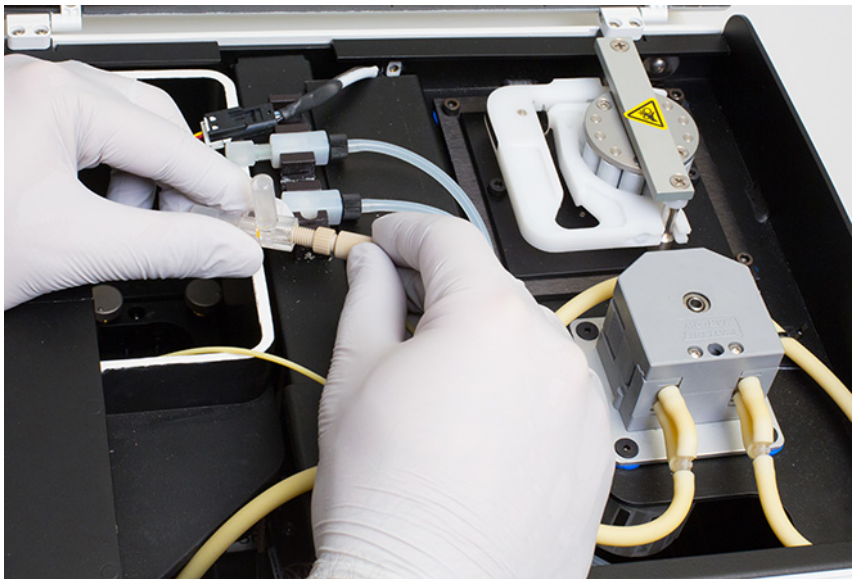


5. Remove the bracket that secures the sample tube dampener and the fluidic lines to the instrument.

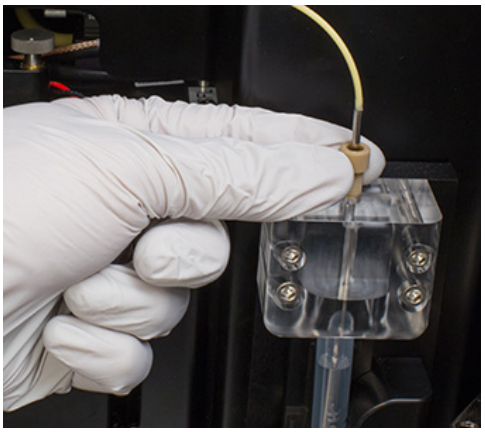




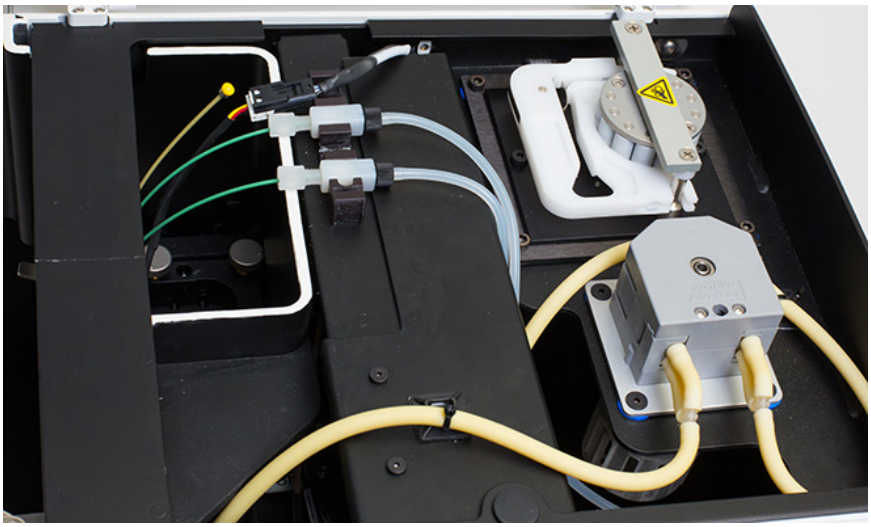
6. Unscrew the sample pump dampener and detach from the instrument.



7. Unscrew the screw above the Lucite block that secures the sample line to the instrument.

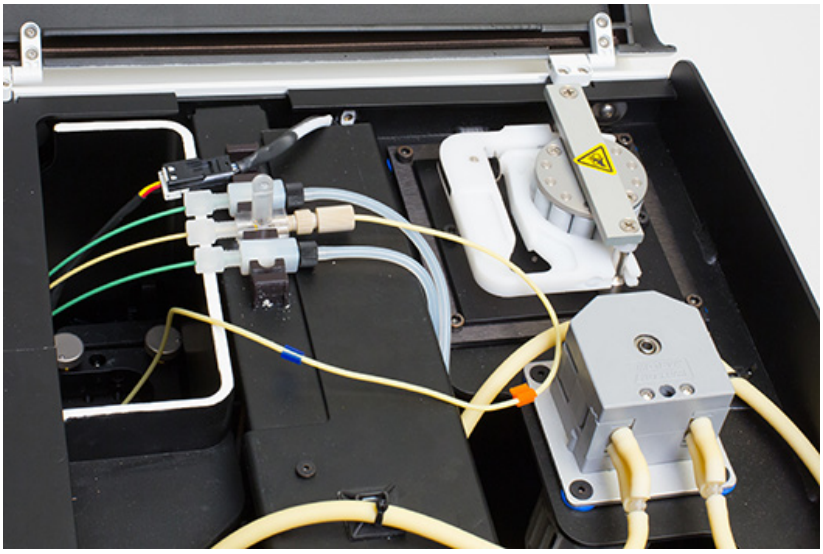


8. Remove the dampener and the sample pump tubing from the instrument.

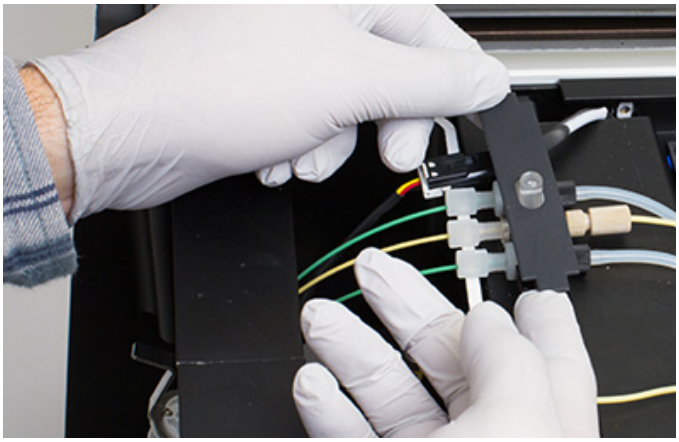


Install new sample pump tubing

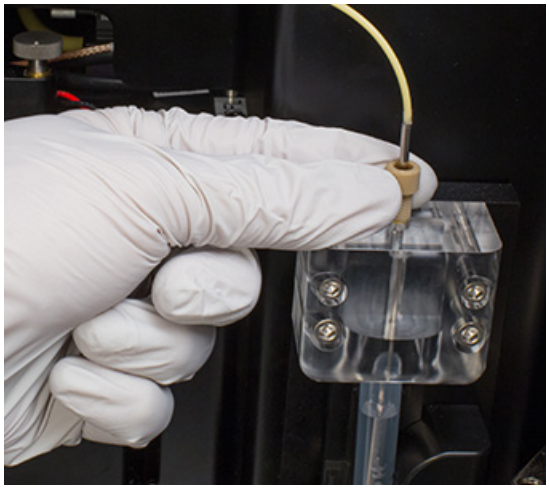
1. Attach the new sample pulse dampener into the remaining tubing, then place the sample pulse damper back into its holder.



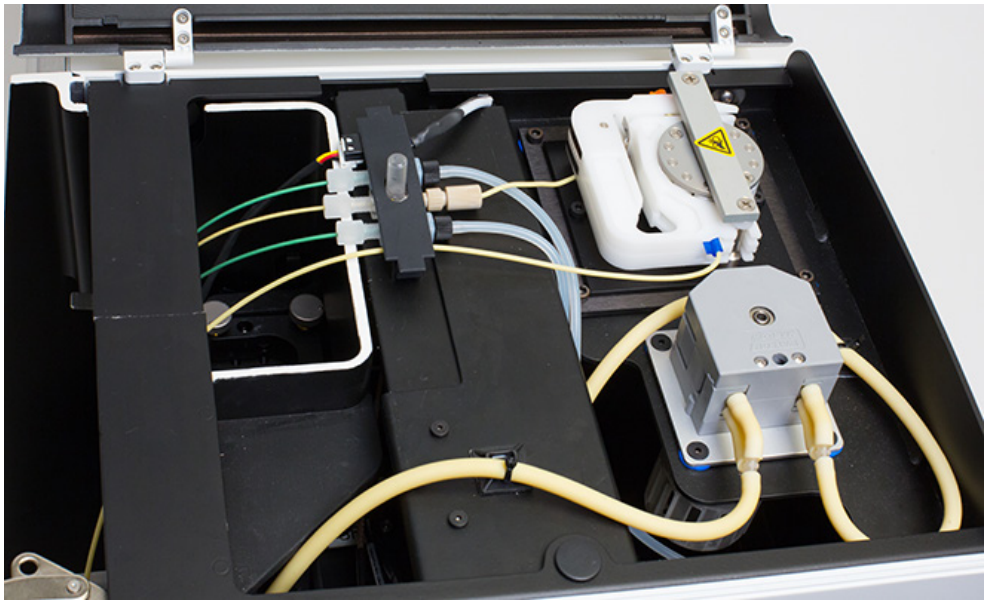
2. Make sure that the sample pulse dampener is upright, then attach the bracket that secures the sample tube dampener and the fluidic lines to the instrument.



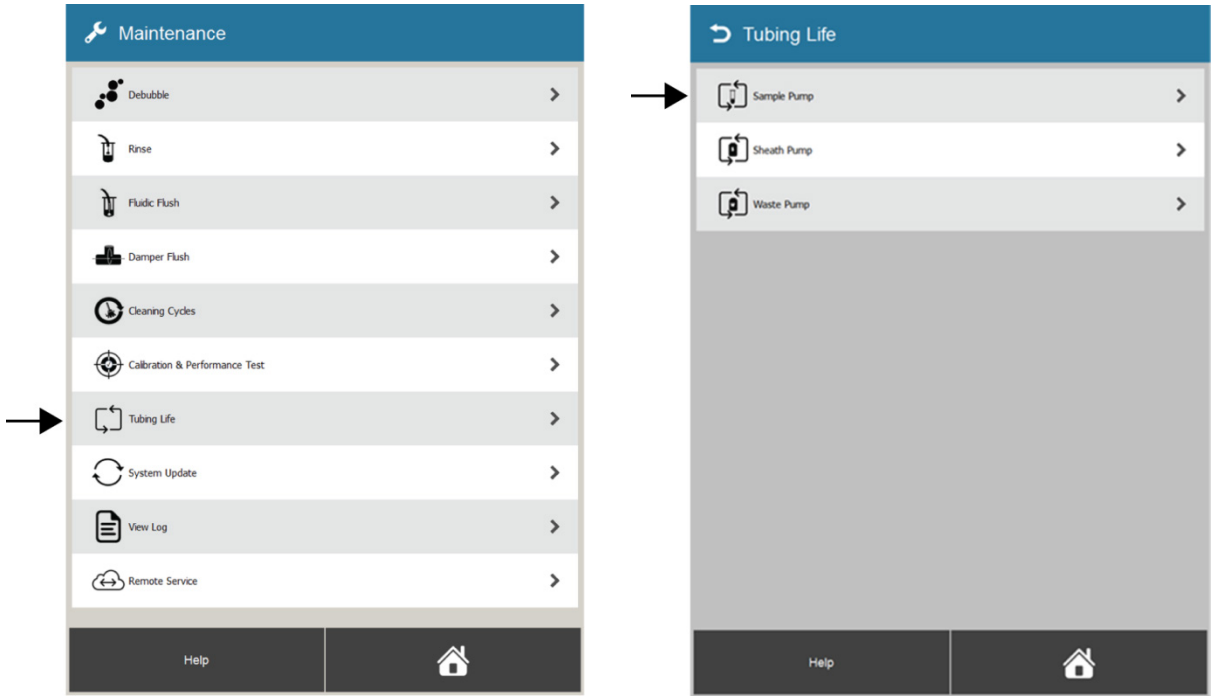
3. Insert the sample line through the screw and the clear Lucite block that secures the sample injection port to the instrument. When the sample line is positioned correctly, tighten the screw.



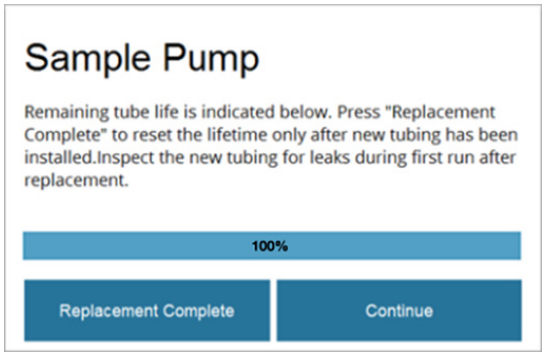
4. Insert the sample pump tubing into the shoe, making sure that the tubing remains slack.
5. Insert the sample pump shoe into its slot in the sample pump assembly, making sure that the sample pump tubing is pressed against the pump rollers.
6. Tighten the sample pump tubing by pulling the tubing outwards and sliding the blue and orange stoppers flush against the sample pump shoe.



7. When you are finished replacing the sample pump tubing, close the top the cover and the sorting compartment door, then power on the instrument.
8. In the **Maintenance** ▶ **Tubing Life** screen, select **Sample Pump.**



9. In the Sample Pump Life dialog, press **Replacement Complete** to reset the sample tube life counter to 100%.



IMPORTANT! Inspect the new tubing for leaks during the first run after replacement.

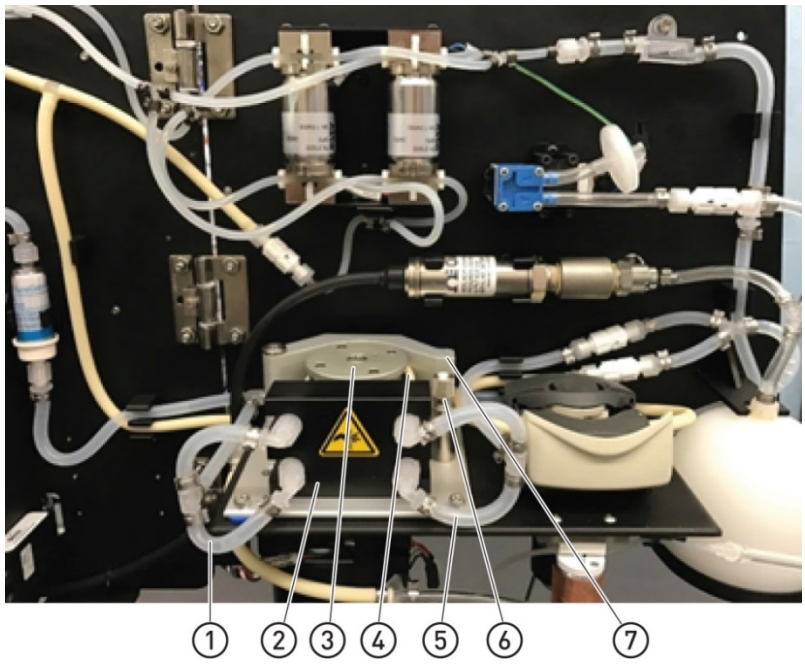
Replace sheath pump tubing

In general, we recommend that you replace peristaltic pump tubing for the sheath line (Cat. No. A33297) when prompted by the instrument. Normally, the sample pump tubing needs to be replaced every 10 weeks based on 20 hours per week average run time (or every 200 hours of run time).

IMPORTANT! When handling the tubing, make sure that it does not become twisted or kinked.

Remove sheath pump tubing

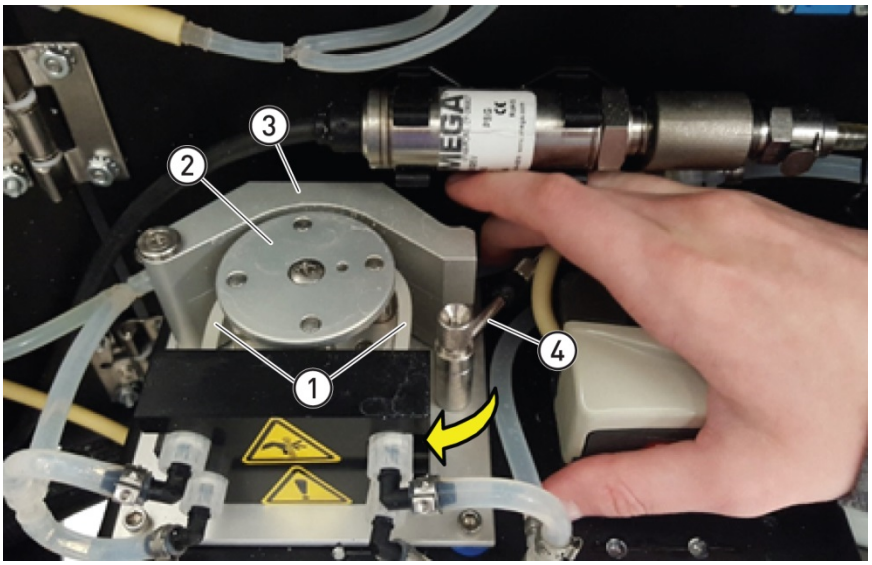
1. Shut down the instrument and unplug before replacing any tubing. Once the Shut Down is complete, open the fluidics compartment door and slide the compartment out for easy access.
2. The sheath pump is located next to the helper pump in the fluidics door. It has two tubes requiring replacement, both located in the same compartment and pushed against the pump roller assembly by a clamping bracket.



1. Sheath fluid lines (clear)
2. Sheath fluid pump assembly
3. Pump roller assembly
4. Sheath line pump tubing
5. Sheath fluid lines (clear)
6. Locking lever
7. Clamping bracket

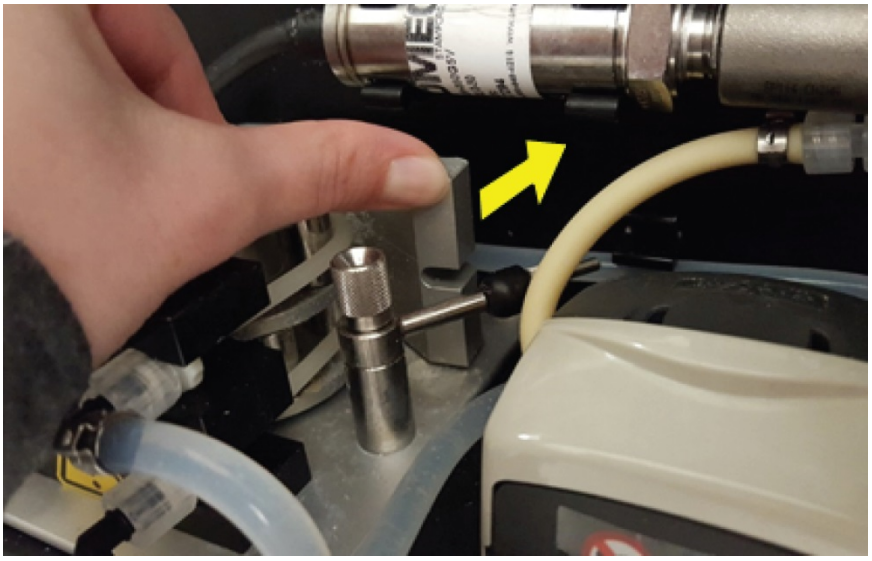
3. Before removing the sheath pump tubing, clamp the incoming and outgoing sheath fluid lines using hemostats. This will stop any potential leaks or pressure drops in the system.

4. To release the sheath pump tubing from the pump roller assembly, swing the locking lever clockwise and out of the slot in the clamping bracket, as indicated by the yellow arrow.

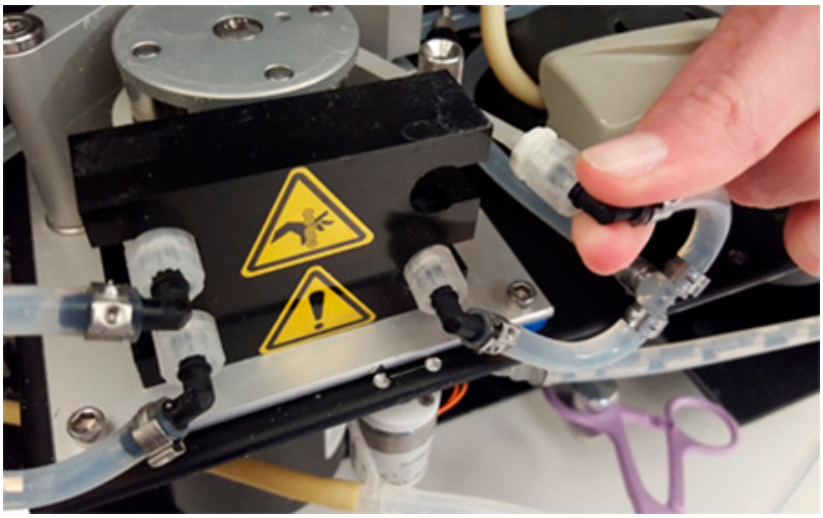


① Sheath line pump tubing
② Pump roller assembly
③ Clamping bracket
④ Locking lever

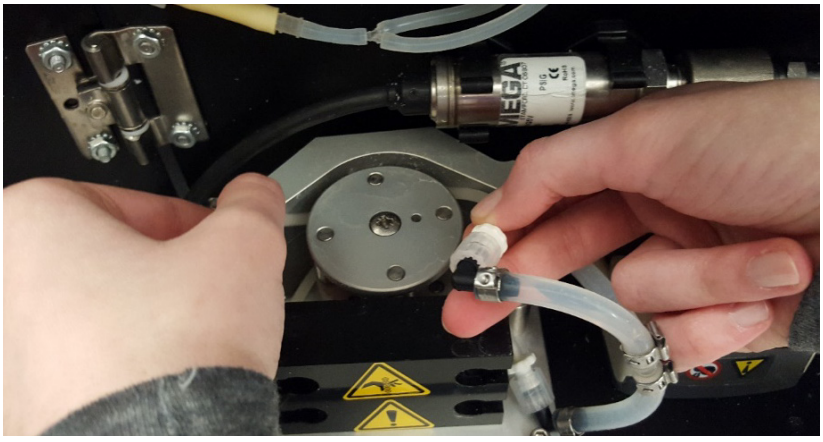
5. Swing the clamping bracket away from the pump roller assembly, as indicated by the yellow arrow.

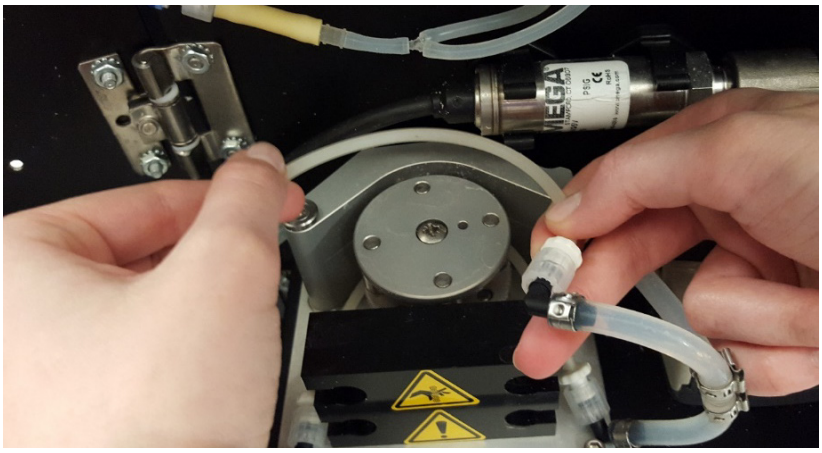


6. Gently pull the fittings that connect the tubing to the sheath fluid lines towards you, then out to the side until all four fittings are freed from the pump assembly.



7. Gently lift the sheath pump tubing and remove it from the pump roller assembly.

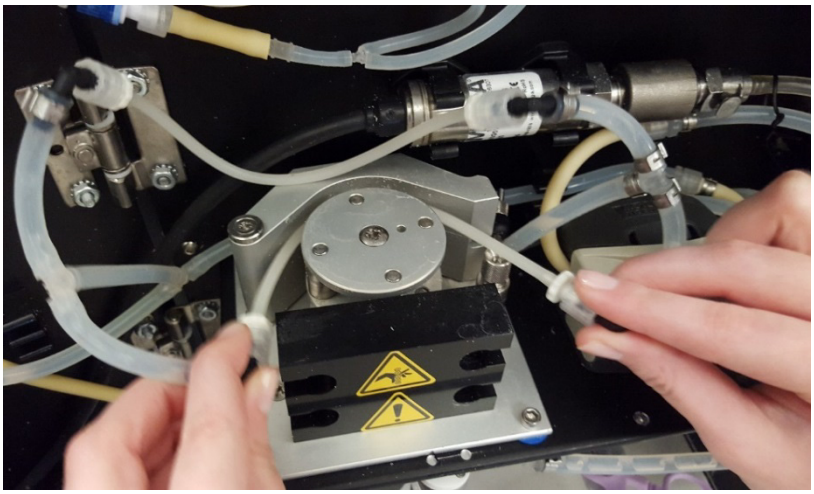




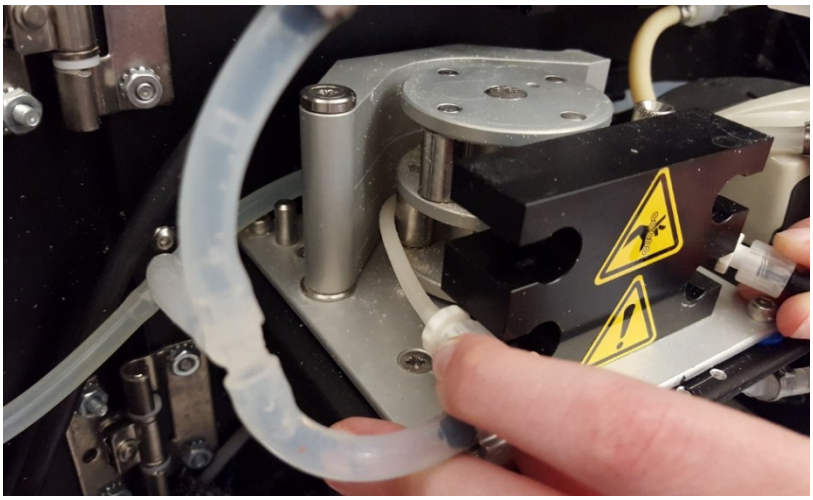
8. After you have removed both sheath pump tubes from the pump roller assembly, twist and pull apart the fittings to disconnect the sheath pump tubes from the fluid lines (clear lines). Discard the used sheath pump tubing.

Install new sheath pump tubing

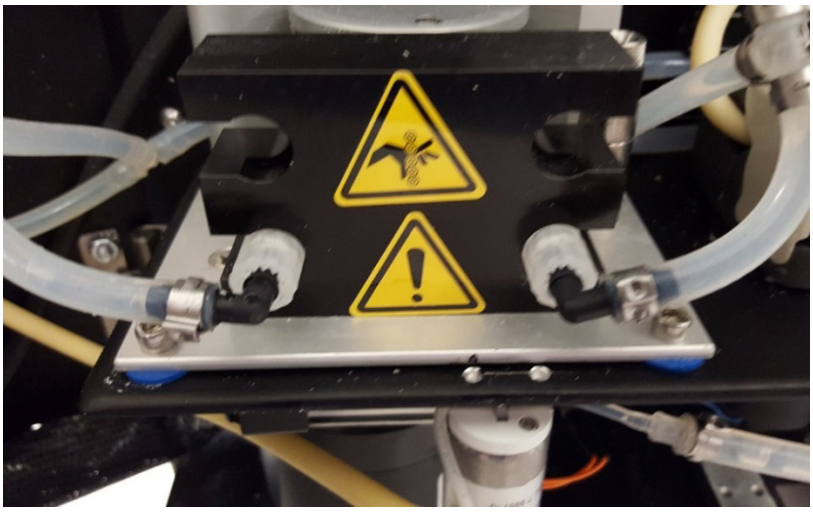
1. To connect the new sheath pump tubing (Cat. No. A33297) to the sheath fluid lines, twist together the fittings while keeping the sheath pump tubing with the same curvature as the pump rollers.



2. Gently slide the bottom set of the sheath pump tubing into position between the pump rollers and the clamping bracket.

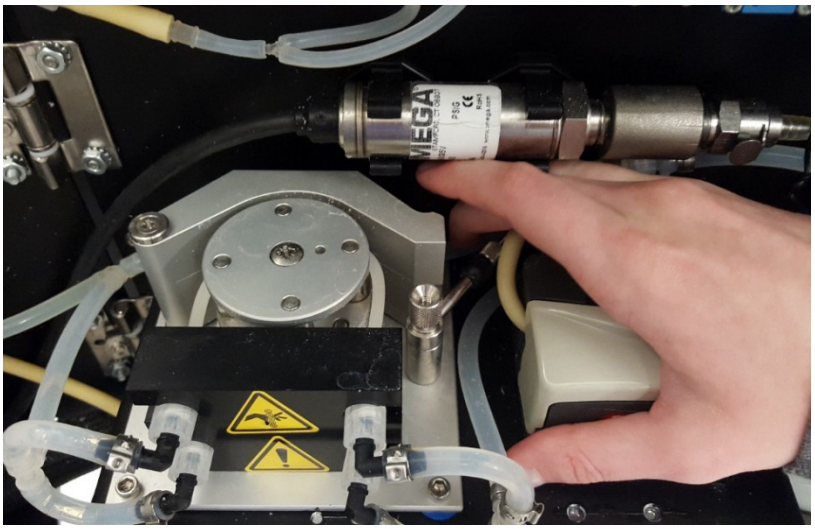


3. Insert the fittings into the bottom slots in the pump assembly to secure them.

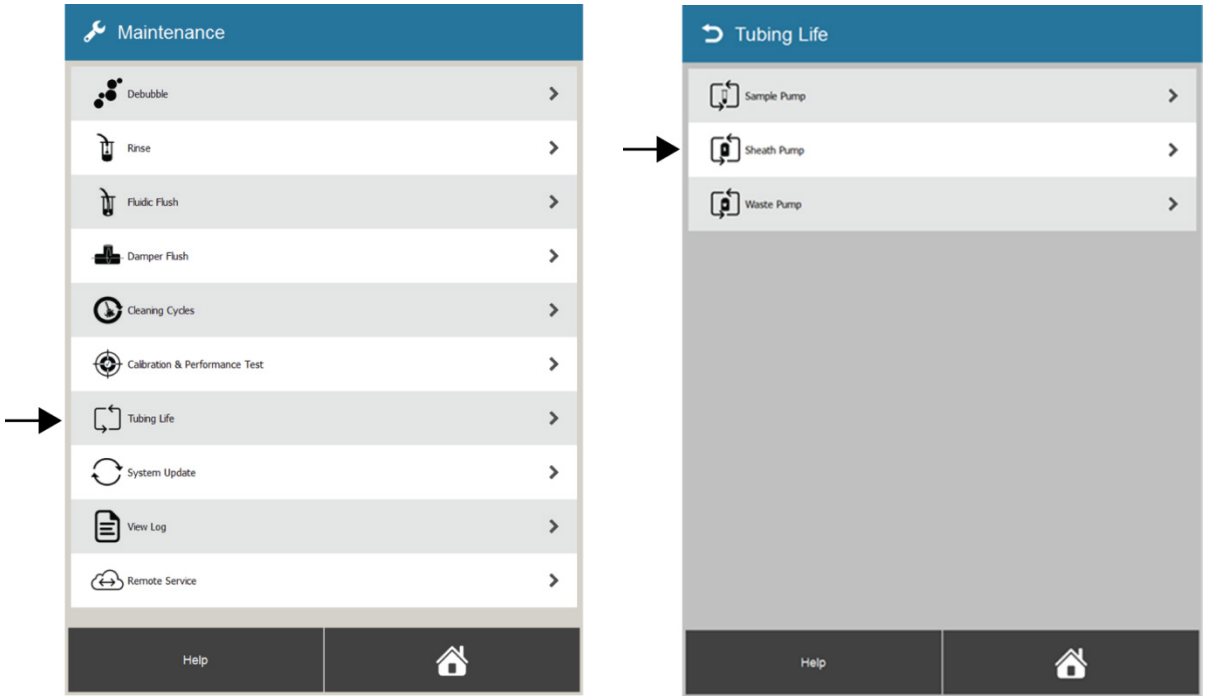


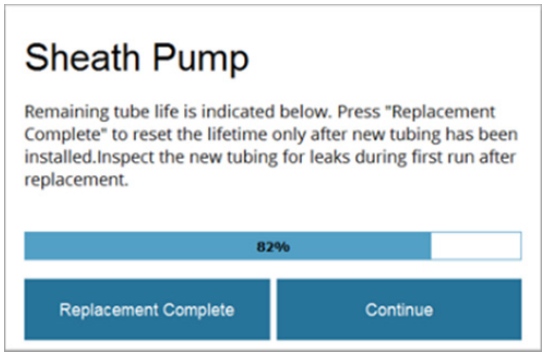
4. Align the sheath pump tubing to the center of bottom rollers, keeping it away from the edges where it could possibly get pinched or caught in a gap.

5. Repeat the process for the top set of the sheath pump tubing, gently sliding it into position and centering it within the top rollers.
6. After you have installed both sets of sheath pump tubing and aligned them into position, pull the clamping bracket towards you to engage the new tubing.

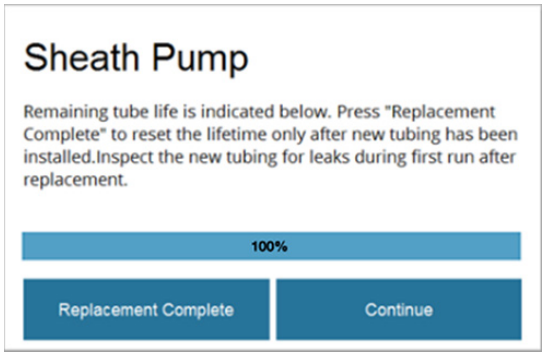


7. Swing the locking lever counterclockwise and engage it with the slot in the clamping bracket to secure the sheath pump tubing within the pump roller assembly.
8. When you are finished replacing the sheath pump tubing, close the fluidics compartment door, then power on the instrument.
9. In the **Maintenance ▶ Tubing Life** screen, select **Sheath Pump.**

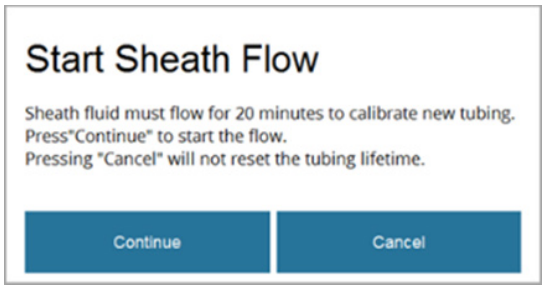




10. In the Sheath Pump Life dialog, press **Replacement Complete** to reset the sheath pump tube life counter to 100%.

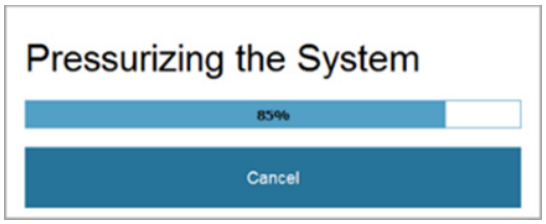


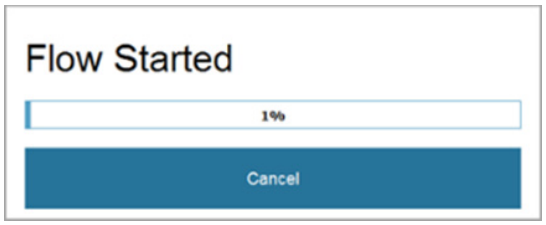
11. After replacing the sheath pump and sheath tubing, sheath fluid must flow for 20 minutes to calibrate the new tubing. When prompted, prompted, press **Continue** to start the flow.



Note: If you press **Cancel**, the tubing lifetime counter will not be reset.

12. The instrument will pressurize the system and start the sheath fluid flow for calibration.

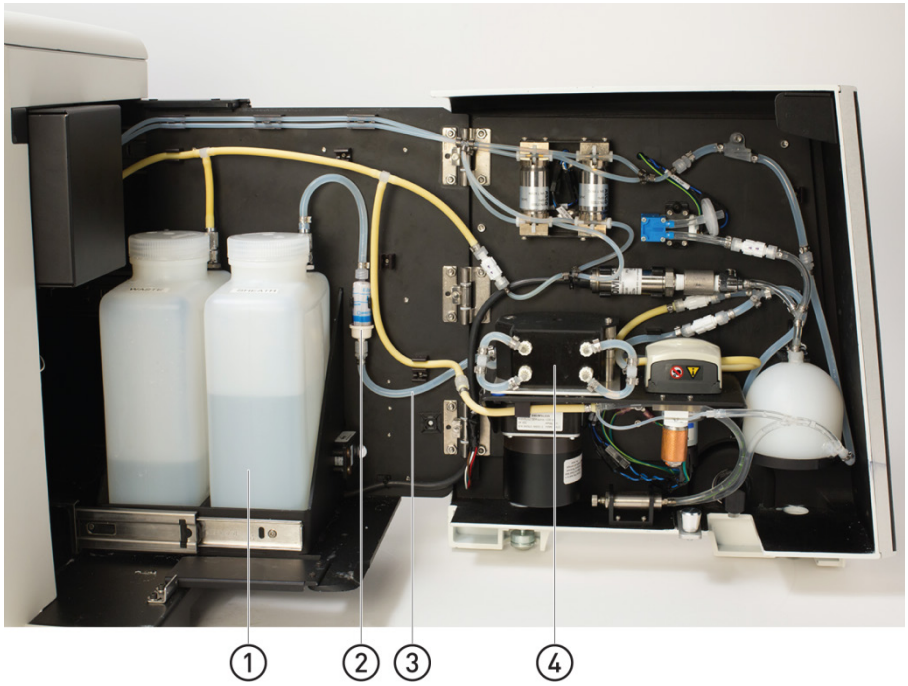




Replace fluidics filters

Replace sheath inlet filter

The 0.2-µm sheath inlet filter (Cat. No. A33307) is located in the sheath fluid line between the sheath fluid container and the sheath pump. It should be replaced each time sheath fluidics cleaning is performed (page 60).



1. Sheath fluid container
2. 0.2-µm sheath inlet filter
3. Sheath fluid line
4. Sheath pump

1. Detach the 0.2-µm sheath inlet filter from the bracket that secures it to the instrument. To do this, grab the Luer lock fittings that attach the filter to the sheath fluid line and pull it directly out.

2. Remove the sheath inlet filter from the sheath line by disconnecting the Luer fittings on each end.

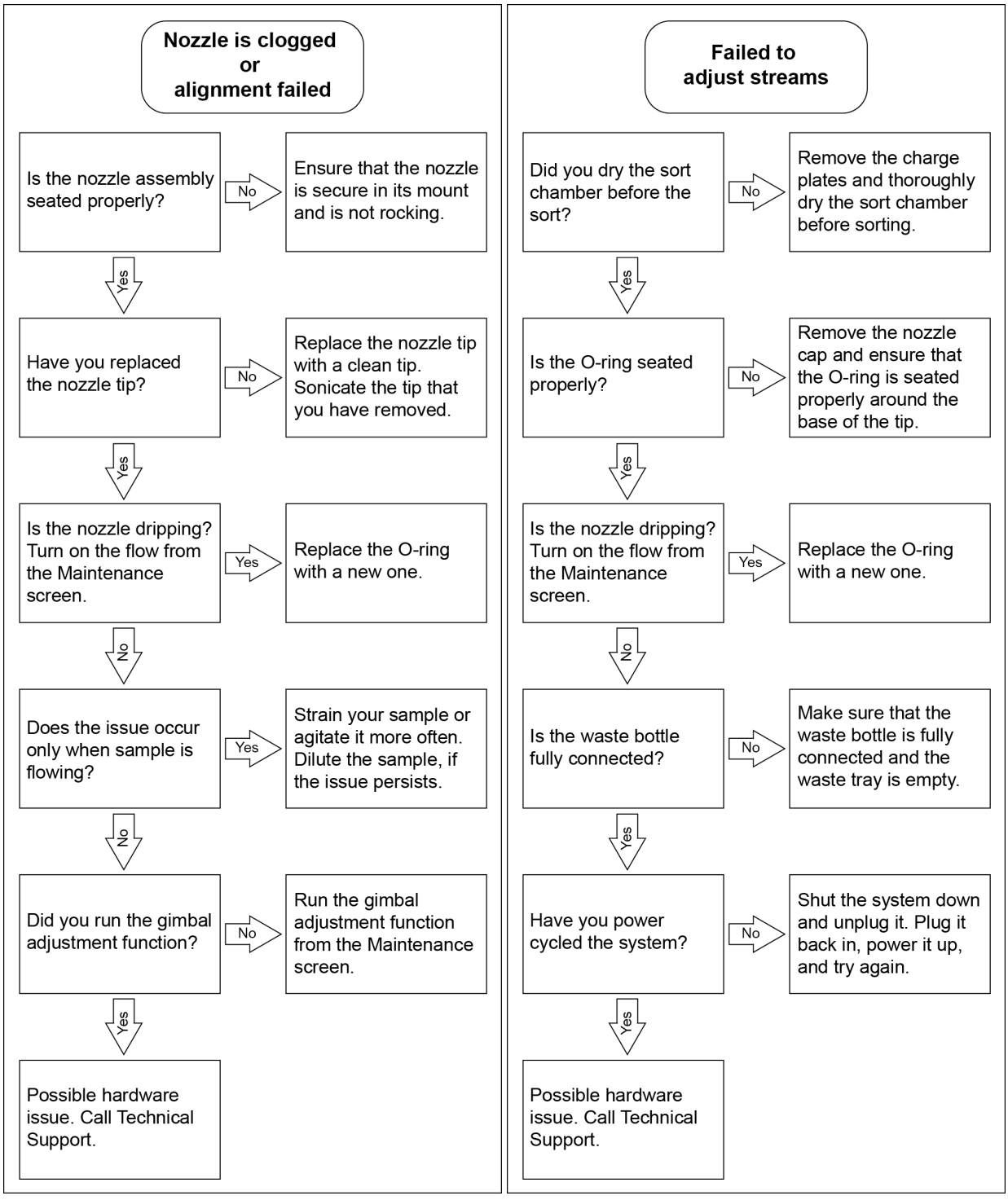
3. Attach the replacement 0.2-µm sheath inlet filter (Cat. No. A33307) by connecting the Luer fittings to the sheath lines on each end.

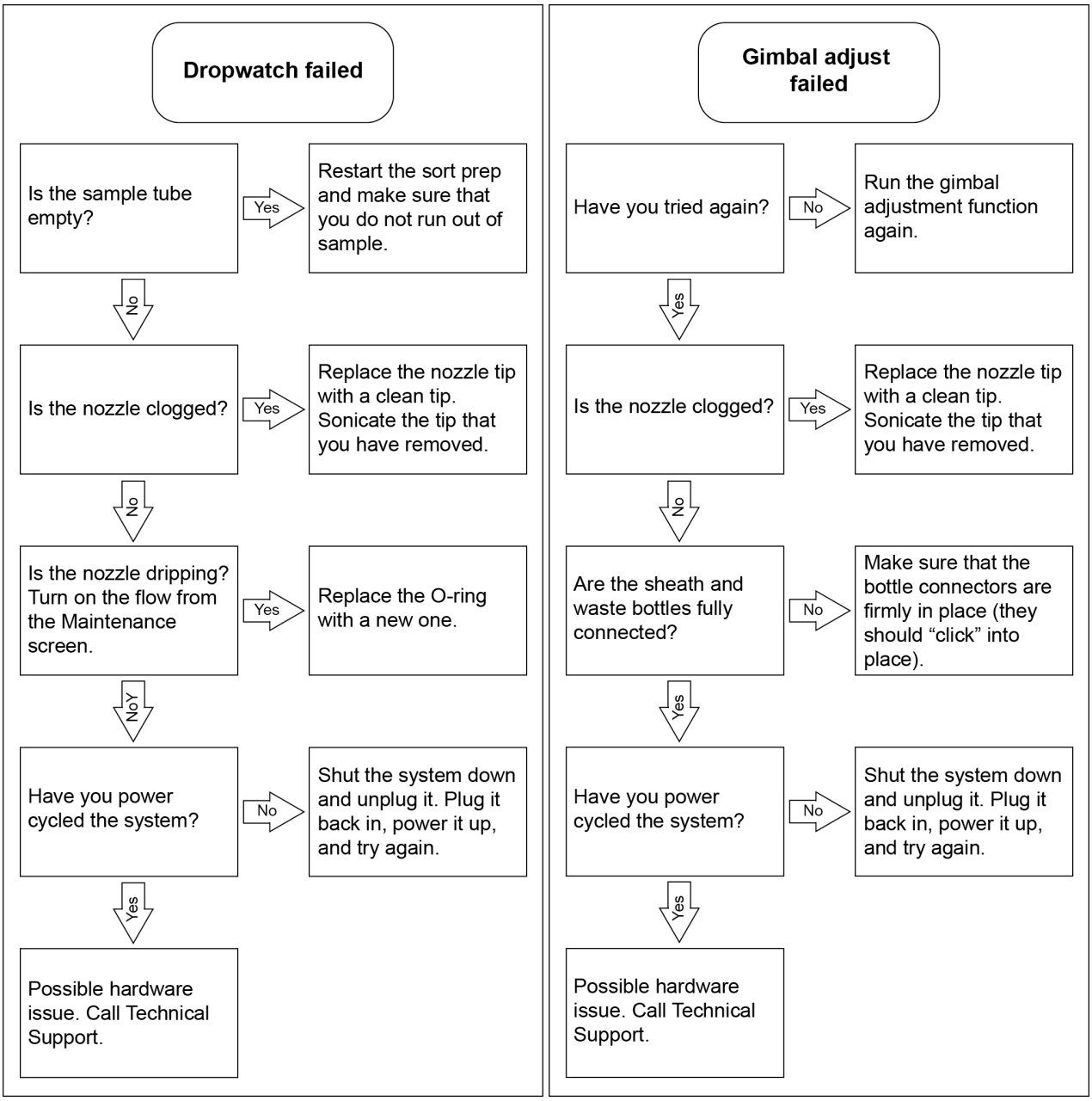
Note: The 0.2-µm sheath inlet filter is directional. Make sure that the blue arrow on the filter is aligned with the fluid flow and it is pointing to the sheath pump.

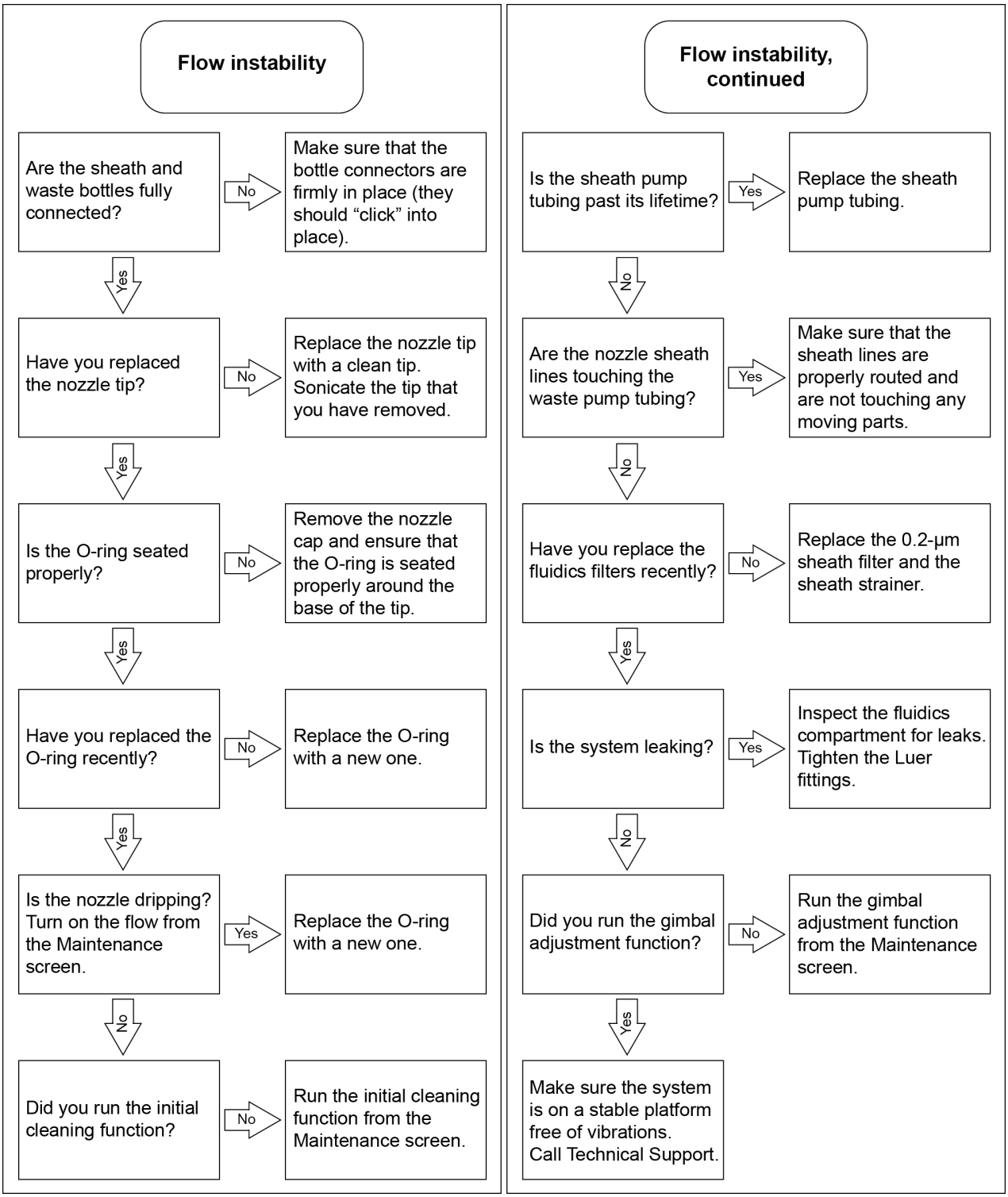
4. Snap the filter connected into its bracket to secure it to the instrument.

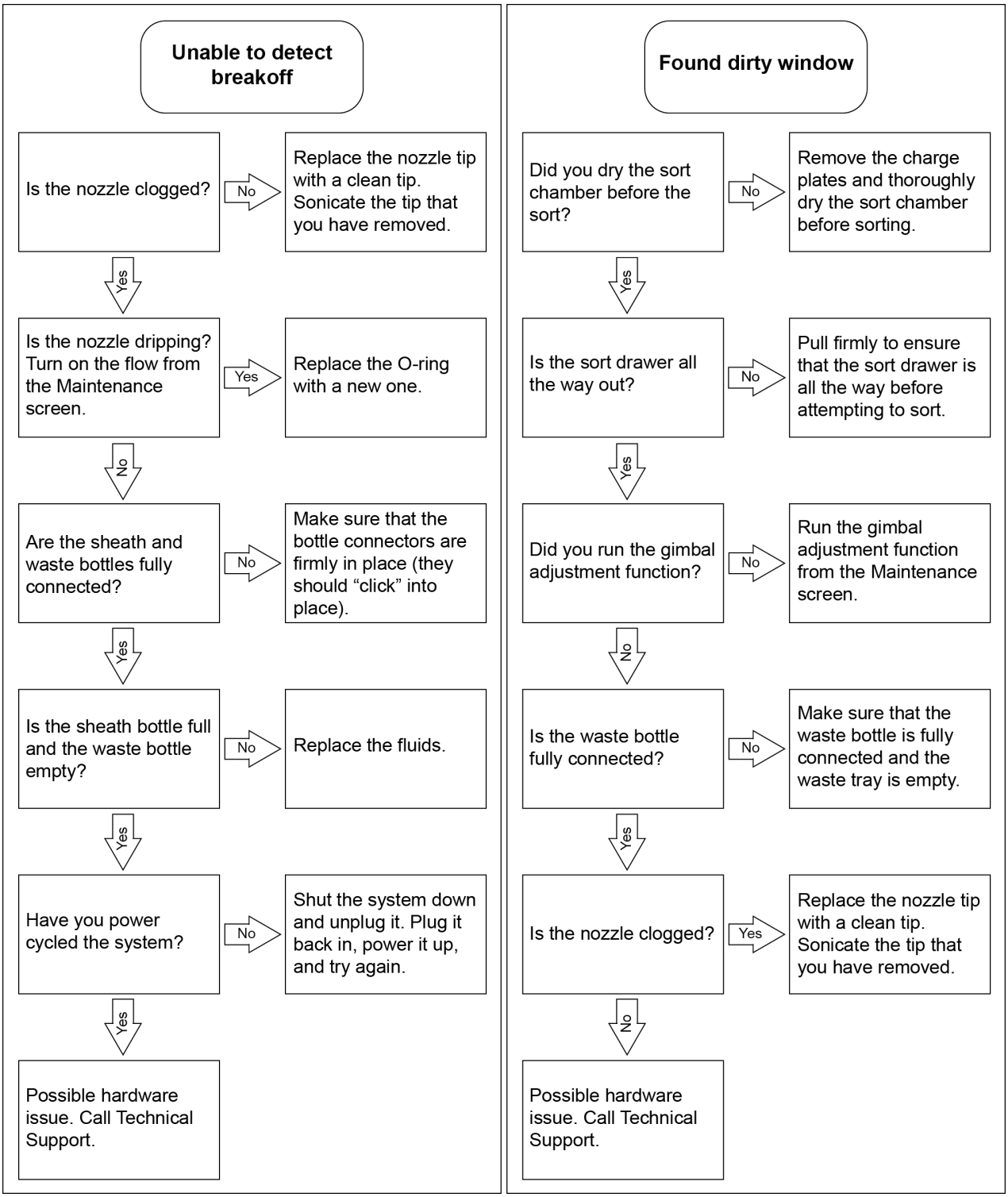
Appendix A: Troubleshooting

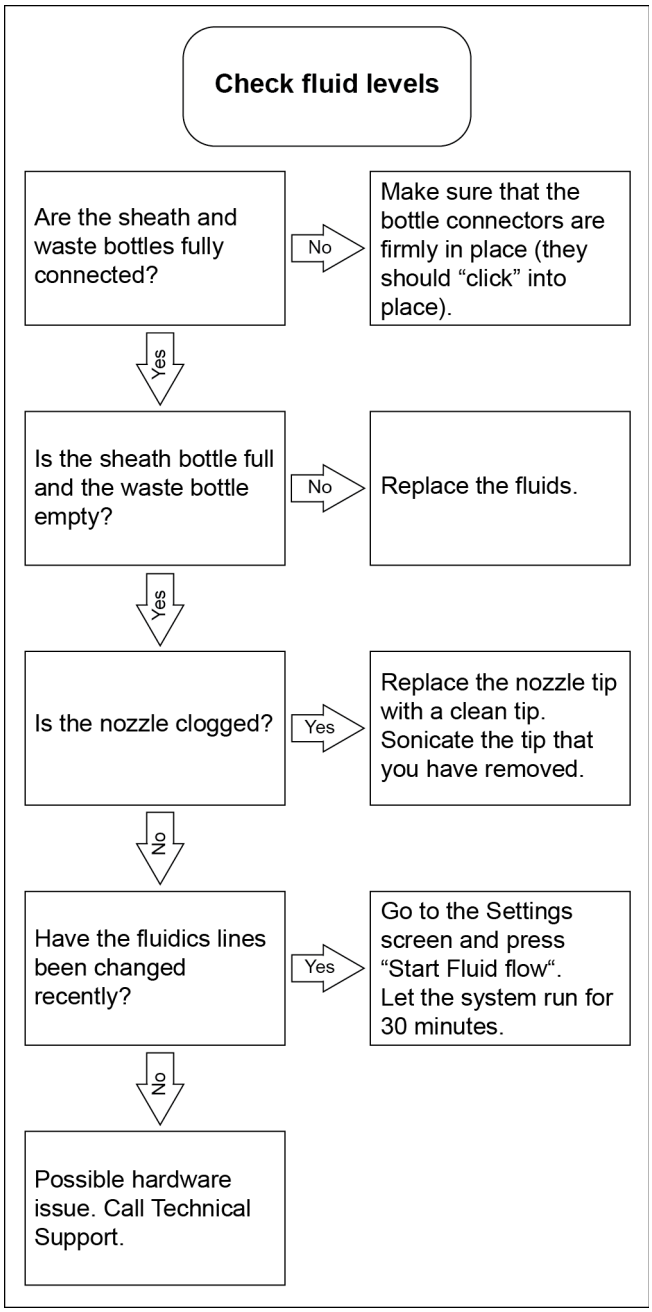
Use the following flowcharts to troubleshoot issues that may be encountered when operating the iSort™ Automated Cell Sorter. For additional information, refer to the tables that follow the flowcharts (pages 124–125). If you are unable to resolve the issue with the information provided or there are any other questions or concerns, contact Technical Support (page 139).




Dropwatch failed
Is the sample tube empty?
Yes
Restart the sort prep and make sure that you do not run out of sample.
No
Is the nozzle clogged?
Yes
Replace the nozzle tip with a clean tip. Sonicate the tip that you have removed.
No
Is the nozzle dripping? Turn on the flow from the Maintenance screen.
Yes
Replace the O-ring with a new one.
NoY
Have you power cycled the system?
No
Shut the system down and unplug it. Plug it back in, power it up, and try again.
Yes
Possible hardware issue. Call Technical Support.
Gimbal adjust failed
Have you tried again?
No
Run the gimbal adjustment function again.
Yes
Is the nozzle clogged?
Yes
Replace the nozzle tip with a clean tip. Sonicate the tip that you have removed.
No
Are the sheath and waste bottles fully connected?
No
Make sure that the bottle connectors are firmly in place (they should “click” into place).
Yes
Have you power cycled the system?
No
Shut the system down and unplug it. Plug it back in, power it up, and try again.
Yes
Possible hardware issue. Call Technical Support.


Flow instability
Are the sheath and waste bottles fully connected?
No
Make sure that the bottle connectors are firmly in place (they should “click” into place).
Yes
Have you replaced the nozzle tip?
No
Replace the nozzle tip with a clean tip. Sonicate the tip that you have removed.
Yes
Is the O-ring seated properly?
No
Remove the nozzle cap and ensure that the O-ring is seated properly around the base of the tip.
Yes
Have you replaced the O-ring recently?
No
Replace the O-ring with a new one.
Yes
Is the nozzle dripping? Turn on the flow from the Maintenance screen.
Yes
Replace the O-ring with a new one.
No
Did you run the initial cleaning function?
No
Run the initial cleaning function from the Maintenance screen.
Flow instability, continued
Is the sheath pump tubing past its lifetime?
Yes
Replace the sheath pump tubing.
No
Are the nozzle sheath lines touching the waste pump tubing?
Yes
Make sure that the sheath lines are properly routed and are not touching any moving parts.
No
Have you replace the fluidics filters recently?
No
Replace the 0.2-µm sheath filter and the sheath strainer.
Yes
Is the system leaking?
Yes
Inspect the fluidics compartment for leaks. Tighten the Luer fittings.
No
Did you run the gimbal adjustment function?
No
Run the gimbal adjustment function from the Maintenance screen.
Yes
Make sure the system is on a stable platform free of vibrations. Call Technical Support.


Unable to detect breakoff
Is the nozzle clogged?
No
Replace the nozzle tip with a clean tip. Sonicate the tip that you have removed.
Yes
Is the nozzle dripping? Turn on the flow from the Maintenance screen.
Yes
Replace the O-ring with a new one.
No
Are the sheath and waste bottles fully connected?
No
Make sure that the bottle connectors are firmly in place (they should “click” into place).
Yes
Is the sheath bottle full and the waste bottle empty?
No
Replace the fluids.
Yes
Have you power cycled the system?
No
Shut the system down and unplug it. Plug it back in, power it up, and try again.
Yes
Possible hardware issue. Call Technical Support.
Found dirty window
Did you dry the sort chamber before the sort?
No
Remove the charge plates and thoroughly dry the sort chamber before sorting.
Yes
Is the sort drawer all the way out?
No
Pull firmly to ensure that the sort drawer is all the way before attempting to sort.
Yes
Did you run the gimbal adjustment function?
No
Run the gimbal adjustment function from the Maintenance screen.
No
Is the waste bottle fully connected?
No
Make sure that the waste bottle is fully connected and the waste tray is empty.
Yes
Is the nozzle clogged?
Yes
Replace the nozzle tip with a clean tip. Sonicate the tip that you have removed.
No
Possible hardware issue. Call Technical Support.


Check fluid levels
Are the sheath and waste bottles fully connected?
No
Make sure that the bottle connectors are firmly in place (they should “click” into place).
Yes
Is the sheath bottle full and the waste bottle empty?
No
Replace the fluids.
Yes
Is the nozzle clogged?
Yes
Replace the nozzle tip with a clean tip. Sonicate the tip that you have removed.
No
Have the fluidics lines been changed recently?
Yes
Go to the Settings screen and press “Start Fluid flow“. Let the system run for 30 minutes.
No
Possible hardware issue. Call Technical Support.

The following tables list a number of issues that may be encountered when operating the iSort™ Automated Cell Sorter. Symptoms, possible causes, and recommended actions are provided. If you are unable to resolve the issue with the information provided or there are any other questions or concerns, contact Technical Support (page 139).

Error messages and warnings

Error message	Possible cause	Recommended action
HW Failure Camera	Electrical malfunction	Reboot system. If problem persists, contact Technical Support (page 139).
HW Failure Laser	Laser has failed and can no longer communicate.	Reboot system. If problem persists, contact Technical Support (page 139).
HW Failure SACNet	Hardware failure	Contact Technical Support (page 139).
HW Failure FPGA::Not Found	Hardware failure	Contact Technical Support (page 139).
HW Failure FPGA ::Load	The FPGA software is corrupt or the board is defective.	Contact Technical Support (page 139).
HW Failure Automation::Timeout	The Automation module did not report in the required time.	Reboot system. If problem persists, contact Technical Support (page 139).
HW Failure SacLogger Load Error	Non-fatal error	No action required. If the message persists, contact Technical Support (page 139).
HW Failure Overcurrent	System detected a component that was drawing excessive current.	Reboot system. If problem persists, contact Technical Support (page 139).
HW Failure Stage Motors	One of the motor controllers is not reporting correctly to the system.	Reboot system. If problem persists, contact Technical Support (page 139).

Nozzle clogs

Error message	Possible cause	Recommended action
Nozzle is clogged.	Full or partial nozzle clog.	Remove nozzle tip, clean and replace tip, or replace with spare nozzle tip. Nozzle clogs can be reduced in frequency by ensuring sample and sheath lines are kept clean as part of routine maintenance.
	Nozzle is not seated properly in the gimbal.	Rotate the nozzle very slightly back and forth; the nozzle should sit securely in the gimbal and the alignment pin should be secure in the nozzle notch.
Automation Failure: The automation software cannot detect a stable aligned stream.	Full or partial nozzle clog.	Remove nozzle tip, clean and replace tip, or replace with spare nozzle tip.
	No sheath fluid.	Fill the sheath fluid container.

Fluidics issues

Observation	Possible cause	Recommended action
System preparation fails before completing the checking pressure step.	Sheath pump tubing is worn out.	Replace the Sheath pump tubing.
Error Message: Flow instability detected.	Air bubbles in the system, worn tubing on the Sheath peristaltic pump.	The System will attempt another Debubble cycle on the restart of the workflow. Check and replace the worn tubing.
Warning Message: Sheath fluid level low.	Low sheath fluid level.	Refill the sheath fluid.
Warning Message: Waste Level High.	Waste bottle is nearly full.	Empty the contents of the waste bottle.
Warning Message: Sample Level Low.	Low sample level.	Pause sort and add buffer, or stop sort.

Automation issues

Observation	Possible cause	Recommended action
System Preparation is unable to complete.	Liquid droplets in sort output.	Clean sort chamber including sort drawer, door, camera lens, and deflection plates.
Error Message: Automation failure.	Poor flow, sheath level low, air bubbles.	Restart automation after verifying sheath flow.
Error Message: Found Dirty lens.	Moisture or debris in the sorting area.	Open the sort door and clean the sort chamber, including the lens.
Error Message: Failed to Adjust Stream.	Moisture or debris in the sorting area. Air Bubbles in the system	Restart the sort preparation. If the problem persists, contact Technical Support (page 139).
Error Message: The automation software could not obtain an aligned stable stream.	Clogged nozzle, no sheath fluid.	Verify that the nozzle is not clogged and that you have sheath flow.

Other issues

Observation	Possible cause	Recommended action
Event rate drops over time	Cells are settling out of suspension	Pause the sort or analysis and mix cells

Appendix B: Technical specifications

Note: Technical specifications of the iSort™ Automated Cell Sorter are subject to change without notice. For the latest product information, see the product page at **thermofisher.com/iSort**.

Physical characteristics	**Dimensions (H × D × W):** 49 × 41 × 67 cm (19 × 16 × 26.5 inches) **Weight:** 41 kg (89 lb) **Footprint:** Approximately 92 cm × 92 cm (36 in × 36 in). With the fluidics compartment door completely open, the entire system requires a total bench width of 135 cm (53.2 in). **Operating temperature range:** 18–25°C (64–77°F) **Storage temperature range:** 5–35°C (41–95°F) **Operating humidity:** 20–70% non-condensing **Operating power:** 100–240 VAC, 4.0 A **Frequency:** 50–60 Hz
Optics	**Laser:** 488-nm (blue), 165 mW **Optical detection:** SSC (488/10) and GFP (525/50BP), Photomultipliers (PMT)
Fluidics	**Nozzle:** Ceramic tip, 85-µm orifice **Sample sort rate:** Up to 12,000 events/second **Sample flow rate:** 23 µL/minute (fixed) **Maximum run time:** ~4 hours (limited by sheath volume)
Hardware	**Biohazard containment:** Fits in any standard >4-foot biosafety cabinet. Additional modifications/filters may be required to meet individual institutional safety requirements. **Output ports:** 3 ports, USB 2.0 **Networking capability:** Connection through Windows/SMB network via an Ethernet cable connection. **Power supply:** AC adapter with country-specific power cords.
Sample	**Cell type:** Mammalian **Sample sort direction:** One- or Two-way **Sample input vessel:** 12 × 75-mm tube (5 mL) **Sort collection vessel:** 1.5 mL, 5 mL, 50-mL tubes **Minimum sample volume**: 200 µL **Separation limit**: ~2–20 µM diameter using a 85 µM nozzle

Sort performance

Automation: Auto alignment (auto drop delay calibration, auto droplet monitoring, auto stream to laser alignment)

Sort technology: Jet-in air

Sort purity: >99% (sample dependent; see Note below)

Sensitivity: <250 molecules of equivalent fluorescein

Signal dynamic range: 1 to 10,000 (4 orders of magnitude)

Note: Sort purity is dependent on sample preparation and experimental protocol. >99% purities were achieved with HEK293 GripTite™ cells stably expressing GFP, using the Attune™ NxT Acoustic Focusing Cytometer to assess purity.

Data management

Data files: PDF reports and FCS 3.0

Software: iSort™ Automated Cell Sorter Software

Appendix C: Ordering information

Accessory products and consumables

The following accessory products and consumables for use with the iSort™ Automated Cell Sorter are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 139).

Product	Amount	Cat. No.
Attune™ Performance Tracking Beads	3 mL	4449754
Attune™ Wash Solution	250 mL	A24974
PBS (1X), pH 7.4 (flow cytometry grade)	10 L	A1286301
PBS (10X), pH 7.4	500 mL 10 × 500 mL	70011044 70011069
Tween™ 20 (50% solution)	20 mL	003005
Distilled water	500 mL	15230162
Deionized water, reagent grade	1 gal	751610
Texwipe™ TX761MD Microdenier Swabs	Pack of 100	A36701
Decon™ Contrad™ 70 Liquid Detergent	1.33 gal	Fisher Scientific, 04-355-01
Falcon™ Test Tube with Cell Strainer Snap Cap	Case of 500	Fisher Scientific, 08-771-23
Bell-Art™ SP Scienceware™ Flowmi™ Cell Strainers for 1000-µL Pipette Tips	Pack of 50	Fisher Scientific, 14-100-150

Replacement parts

The following replacement parts for the iSort™ Automated Cell Sorter are available separately from Thermo Fisher Scientific. For more information, go to **thermofisher.com** or contact Technical Support (page 139).

Product	Amount	Cat. No.
Cap for iSort™ Nozzle Tip storage	1 each	A33411
Peristaltic pump tubing (Sample line) replacement	1 each	A33303
Peristaltic pump tubing (Sheath line) replacement	1 each	A33297
Sheath inlet filter	1 each	A33307
Sheath strainer	1 each	A33311
iSort™ Waste Bottle	1 each	A33412
iSort™ Sheath Bottle	1 each	A33413
iSort™ Nozzle Tip	1 each	A33312
iSort™ Nozzle Tip O-ring	1 each	A33314
iSort™ Nozzle Tip cover	1 each	A33411
iSort™ Nozzle Nut	1 each	P/N?

Appendix D: Safety

Safety conventions used in this document

Safety alert words

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

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

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

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

Symbols on instruments

Electrical symbols on instruments

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

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

Safety symbols

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

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

Environmental symbols on instruments

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

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

Safety labels on instruments

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

Hazard Symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! HAZARDOUS WASTE. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to 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.
	**DANGER!** Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	**DANGER!** Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

General instrument safety

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

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by trained personnel. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons. Lift the instrument only by holding it at the specific lifting points (the four handholds in the base; see page 20).

Moving and lifting stand-alone computers and monitors

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

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

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

Operating the instrument

Ensure that anyone who operates the instrument has:

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

Cleaning or decontaminating the instrument

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

Removing covers or parts of the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be serviced only by trained personnel or vendor specified in the user guide. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation.

Chemical safety

Chemical hazard warning

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

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

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

General safety guidelines

To minimize the hazards of chemicals:

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

Chemical waste safety

Chemical waste hazard

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

Waste disposal

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

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

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

Electrical safety

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the iSort™ Automated Cell Sorter without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

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

AC fuse requirements

The fuses used in the iSort™ Automated Cell Sorter are 5 × 20 mm and must be rated to 250 VAC, 3.15 A, FA.

Remove the power cord before replacing fuses.

Power

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

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

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

AC power cord requirements

The power cord for the iSort™ Automated Cell Sorter must be IEC 60320-1 compliant with C13 plug on the instrument end.

The power cord must be rated at minimum 250 VA, 10A at 60°C minimum.

In the U.S. and Canada, the power cord must be rated at minimum 125 VAC, 10A at 60°C minimum.

Position the instrument for easy access to the power switch and the power cord.

Overvoltage rating

The iSort™ Automated Cell Sorter has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical hazard safety

Moving parts

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

Biological hazard safety

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

ATTENTION! BIOHAZARD. Les échantillons biologiques tels que les tissus, les fluides corporels et le sang des humains et d'autres animaux ont la possibilité de transmettre des maladies infectieuses. Suivre tous les règlements municipaux, provinciaux/provincial et / ou nationales en vigueur. Porter des lunettes de protection approprié, des vêtements et des gants.

In the U.S.:

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

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

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

  www.cdc.gov

In the EU:

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

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

Safety and electromagnetic compatibility (EMC) standards

This instrument has been tested and founded to be in compliance with applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd Ed.), EN 61010-1:2010 (3rd Ed.). Electrical Equipment for Measurement, Control, and Laboratory Use – Part 1: General Requirements.
- UL/CSA 61010-1:2012 (3rd Ed.), Standard for Safety Electrical Equipment for Electrical Safety (USA, Canada, NRTL)
- IEC 60825-1:2007 (2nd Ed.), EN 60825-1:2007 (2nd Ed.). Safety of laser products – Part 1: Equipment classification and requirements
- Class 1 laser product per CDRH requirements and regulations
- IEC 61010-2-081:2001+A1, EN61010-2-081:2002+A1. 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 (includes Amendment 1)
- EN 61326-1:2006 (Class A) Electrical equipment for measurement, control and laboratory use. EMC requirements, Part 1: General requirements.

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the inference at his or her expense.

Documentation and support

Obtaining support

Technical support

For the latest services and support information for all locations, visit **www.thermofisher.com**.

At the website, you can:

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

Safety Data Sheets (SDS)

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

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

IMPORTANT! Wiping the iSort™ Automated Cell Sorter computer (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.