S3™ and S3e™ Cell Sorters

Instruction Manual

Catalog #145-1001
#145-1002
#145-1005
#145-1006
#145-1008

BIO-RAD

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Bio-Rad Laboratories Resources

Bio-Rad provides many resources for scientists. Table 1 lists available resources and how to locate what you need.

Table 1. Bio-Rad resources.

Resource	How to Contact
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad Laboratories website by selecting your country on the homepage (**www.bio-rad.com**). Find the nearest international office listed on the back of this manual
Technical support scientists	Bio-Rad's technical support scientists provide our customer with practical and expert solutions. To find local technical support on the phone, contact your nearest Bio-Rad office. For technical support in the United States and Canada, call 1-800-424-6723 (toll-free), and select the technical support option
Service support engineers	Maintenance and repairs should be carried out only by authorized service support engineers
	For service support in the United States and Canada, call 1-800-424-6723 (toll-free), and select the technical support option to request service support
Technical notes and literature	Go to the Bio-Rad website (**www.bio-rad.com**). Type a term in the Search box and select Documents tab to find links to literature

Writing Conventions Used in This Manual

This manual uses the writing conventions listed in Table 2.

Table 2. Conventions used in this manual.

Convention	Meaning
Note:	Provides helpful information and instructions, including information explained in further detail elsewhere in this manual
WARNING!	Explains very important information about something that might injure the researcher, damage the instrument, or cause data loss
X > Y	Instruction to select X and then select Y from a toolbar, menu, or software window
	Highlights area of interest on a screenshot
	IMPORTANT! Provides important information about necessary actions or common mistakes

Safety and Regulatory Compliance

For safe operation of the S3 and S3e Cell Sorter systems, we strongly recommend that you follow the safety specifications listed in this section and throughout the manual.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 3 to review the meaning of each safety warning label.

Table 3. Meaning of safety warning labels.

CAUTION: Shock hazard! This symbol draws attention to a possible injury or danger to life if the associated directions are not followed correctly

CAUTION: Risk of danger! This symbol identifies components that pose a risk of personal injury or damage to the instrument if improperly handled. Wherever this symbol appears, consult the manual for further information before proceeding

CAUTION: Laser hazard! This symbol draws attention to a possible injury or danger to life due to laser radiation if the associated directions are not followed correctly

CAUTION: Biohazard! This symbol identifies components that may become contaminated with biohazardous material

Instrument Warning Labels

The warning labels shown in Table 4 are displayed on the instrument and refer directly to the safe use of the S3 and S3e Cell Sorter systems.

Table 4. Instrument safety warning labels.

Warning about risk of shock.
Only qualified, trained technicians should carry out service work on electronic components due to potential shock hazard

Warning about electronic components.
Electronic components are sensitive to electrostatic charges and can be destroyed by a discharge

Warning about weight of the system.
Lifting should be accomplished with a minimum of two people and only by the inset handles on the instrument base. Use caution to keep instrument level and handle gently

Warning about handling biohazardous materials.
When handling biohazardous samples or the S3 or S3e System's waste container, adhere to the recommended precautions and guidelines in this manual, and comply with any local guidelines specific to your laboratory and location

Safe Use Specifications and Compliance

Laser Product Hazard Classification
The intent of the laser hazard classification is to identify hazards to users posed by the laser, and provide appropriate protective measures. The S3 or S3e laser is a Class 1 laser product that complies with 21 CFR 1040.10 and 1040.11, except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007 stating that operators are not exposed to harmful levels of laser radiation during normal operation, maintenance and/or service. During times of repair and/or major service by a trained technician, laser safety controls for Class 3B lasers must be followed.

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

Electrical Safety Information and Classification
The S3 and S3e Systems conform to international regulations encompassing the accessibility of high voltages by the user. Use all protective housings, interlocks, and shields as identified in this manual. Further information about specific electrical hazards is listed in the hardware description.

AC Fuse Requirements
Remove power cord before replacing fuses.
Fuses are 5 x 20 mm and must be rated to 250 VAC, 4 A slow blow such as Schurter 0034.3123

AC Power Cord Requirements
Power cord must be IEC 60320-1 compliant with a C13 plug on the instrument end. The power cord must be rated at minimum 250 VAC, 10 A at 60ºC minimum. In the U.S. and Canada, the power cord must be rated at minimum 125 VAC, 10 A at 60ºC minimum.

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

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

- IEC 61010-1:2010 (3rd Ed.), EN61010-1:2010 (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, Canda, 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 interference at his own expense.

Hazards
The S3 and S3e Cell Sorters are designed to operate safely when used in the manner prescribed by the manufacturer. If the S3 or S3e Cell Sorter or any of its associated components are used in a manner other than prescribed, or if modifications to the instrument are not performed by a Bio-Rad or other authorized agent, then the warranty on the system will be voided. Service of the S3 or S3e Cell Sorter should be performed only by Bio-Rad personnel.

Biohazards
The S3 and S3e Cell Sorters are laboratory products. However, if biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

General Precautions
- Always wear laboratory gloves, coats, and safety glasses with side shields or goggles

- Keep your hands away from your mouth, nose, and eyes

- Completely protect any cut or abrasion before working with potentially infectious materials

- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory

- Remove wristwatches and jewelry before working at the bench

- Store all infectious or potentially infectious material in unbreakable leak-proof containers

- Before leaving the laboratory, remove protective clothing

- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves

- Change gloves frequently. Remove gloves immediately when they are visibly contaminated

- Do not expose materials that cannot be properly decontaminated to potentially infectious material

- Upon completion of the operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach)

- No biohazardous substances are exhausted during normal operations of this instrument

Disposal of Biohazardous Material
The S3 and S3e Systems include a waste container that may potentially contain hazardous biological materials, depending on the sample used. Dispose of the following potentially contaminated materials in accordance with laboratory, local, regional, and national regulations:

- Content in waste container

- Reagents

- Used reaction vessels or other consumables that may be contaminated

Chemical Hazards
The S3 and S3e Systems include a waste container that may potentially contain hazardous chemical materials depending on the sample used.

Explosive or Flammability Hazards
The S3 and S3e Systems pose no uncommon hazard related to flammability or explosion when used in a proper manner as specified by Bio-Rad Laboratories.

Electrical Hazards
The S3 and S3e Systems pose no uncommon electrical hazard to operators if installed and operated properly without physical modification and if connected to a power source of proper specification.

Transport
Moving the S3 and S3e Systems is not recommended after installation. If the system needs to be moved, follow the decontamination procedure in this manual and remove all bulk fluidics. A QC procedure will be required after a move to ensure instrument is functioning properly.

Lifting should be performed with a minimum of two people. Lift with the inset handles on the instrument base. Use caution to keep instrument level, and handle the instrument gently.

Storage
The S3 or S3e System can be stored under the following conditions:

- Temperature range 5–35°C

- Relative humidity 20–70%

Disposal
The S3 and S3e Systems contain electronic or electrical materials; it should be disposed of as unsorted waste and must be collected separately, according to European Union Directive 2002/96/CE on waste and electronic equipment — WEEE Directive. Before disposal, contact your local Bio-Rad representative for country-specific instructions.

Warranty
The S3 and S3e Cell Sorters and associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad Laboratories office for details of the warranty.

Table of Contents

1 Introduction

The S3™ and S3e™ Cell Sorters are designed to offer affordable, dependable, and simplified cell sorting. As benchtop cell sorters, the S3 and S3e Cell Sorters are equipped with one or two lasers and up to four fluorescent detectors, plus forward and side scatters. Samples are analyzed using the traditional jet-in-air technology, and events can be sorted at high speeds while maintaining sensitivity and high purity.

Sorting is typically accomplished by breaking the stream of fluid containing particles into droplets and applying a charge to the stream when a particle passes the criteria to be sorted. The charged droplet containing the particle is then deflected by an electric field into a collection vessel. To ensure proper deflection of the correct droplets, proper instrument setup is integral to achieving high-purity sort results.

By automating the complex presort setup with a unique technology, the S3 and S3e Cell Sorters create a more efficient and consistent workflow.

Key automated features:
- Startup/shutdown
- Stream-to-laser alignment
- Drop delay calculation
- Droplet break-off monitoring and feedback
- Phase and deflection adjustments to sort stream
- Daily QC reporting and trending
- Collection volume monitoring to prevent overfilling

The S3 and S3e Cell Sorters include a complete internalized fluidics and temperature control system. The instrument uses a unique internal buffer chamber system for dilution of 8x sheath fluid with deioinized (DI) water, allowing users to swap fluids without the need to shut down the system. This process is known as a "hot swap," and allows uninterrupted sorting. For more information, refer to Section 5.3, Checking or Swapping Fluidics.

1.1 System Components

The S3 or S3e Cell Sorter complete system includes the following components:

- **S3 or S3e Cell Sorter Instrument**, 1 each
  - **S3 or S3e Installation Kit**, 1 each

S3 cap assembly, white, sheath fluid, 1 each	5 ml tubes, 12 x 75 mm, 1 pack
S3 cap assembly, blue, DI water, 1 each	S3 collection adaptor set, 1 each
S3 cap assembly, red, waste, 1 each	Bulk fluidics tray, 3 each
S3 fluidics container 4 L size, empty, 2 each	USB cord, 1 each
S3 individual filter holder, black, empty, 5 each	Ethernet cord, 1 each
S3 forward scatter (FSC) filter holder with neutral density filter, 2.0, 1 each	Power cord, 1 each
S3 filter block A with preset filter configuration, 1 each	USB drive with ProSort™ software
	Instrument and software manual
S3 filter block B with preset filter configuration, 1 each	Instrument quick guide

- **S3 Accessory Kit (optional)**, 1 each (catalog #145-1065)

S3 nozzle tip, 100 μm, 1 each	2 mm hex driver, 1 each
S3 nozzle O-rings, 2 each	S3 spanner wrench, 1 each
S3 nozzle alignment disk, 2 each	1 ml syringe, 1 each
Neutral density filter, 1.0, 2 each	Plastic box, 1 each

- **Computer CPU and 24 inch monitor**, 1 each (catalog #145-1066)
- **ProFlow™ 8x Sheath Fluid**, preservative free, 5 x 4 L, 1 case (catalog #145-1082)
- **ProLine™ Calibration Beads**, 3 x 5 ml, 1 pack (catalog #145-1081) or
  ProLine Universal Calibration Beads, 3 x 5 ml, 1 pack (catalog #145-1086)

1.2 Installation Requirements

The S3 and S3e Cell Sorters should be installed by a trained service engineer to ensure proper operation and calibration of the instrument. If any items are missing or damaged, contact your local Bio-Rad office for assistance.

Before the S3 and S3e Cell Sorters can be installed by a service engineer, a site must be chosen. The instrument should be located on a sturdy bench or table top, away from any other instruments that may interfere electrically or mechanically by causing vibration. The bench or table top must be able to accommodate 198 lb (90 kg), the weight of the instrument. The area should be free of excessive dust or moisture.

Table 5. Dimensions for instrument alone or with computer and monitor.*

Instrument only (W x D x H)	70 x 65 x 65 cm 27.5 x 25.5 x 25.5 in
Instrument wiith computer and monitor, (W x D x H)	116 x 65 x 65 cm 46 x 25.5 x 25.5 in

* An additional 61 cm (24 in) of height clearance is needed for service.

2 **Hardware Description**

This chapter describes the hardware of the S3™ and S3e™ systems. Understanding the system's hardware is essential for proper operation.

2.1 System Overview

The S3 and S3e Systems consist of fluidics, optics, electronics, and software. These can be broken down into several subsystems (Figure 1).
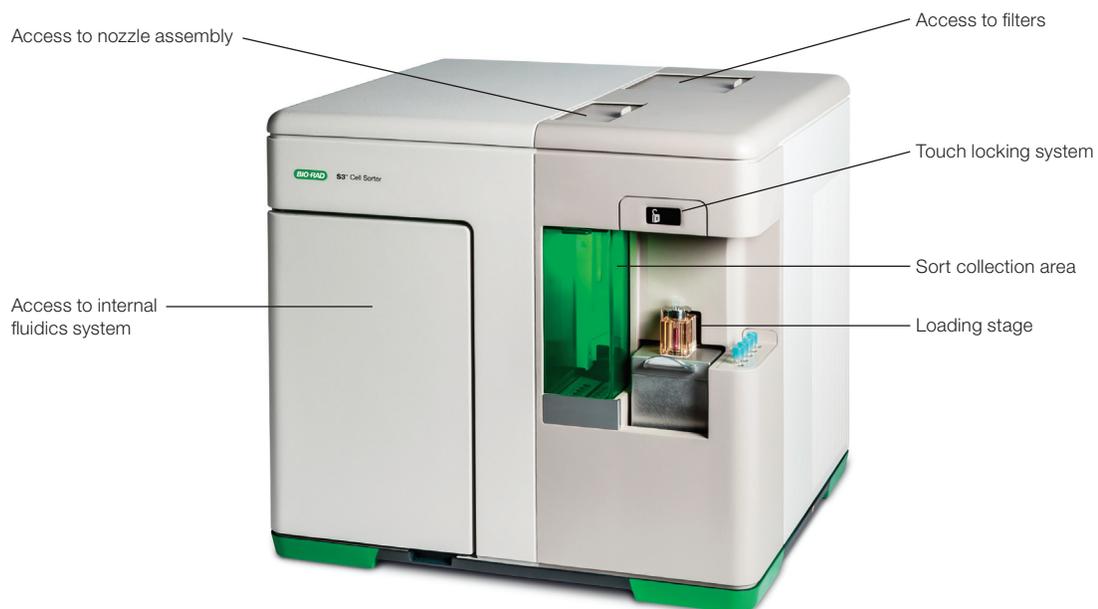

Fig. 1. Front view of the S3 system.

2.1.1 Instrument Back Panel

The rear connector panel of the S3 or S3e system includes these features:

- **Main power switch (black)** — press the main power switch to turn on power to the system. **WARNING!** The main power switch should not be used to shut down the system. Perform system shutdown from the ProSort™ software. For more information, refer to Chapter 8, Shutdown

- **Power input (black)** — plug in the power cord here. The system requires 100 or 240 VAC outlet

- **USB port (gray)** — use this port to connect the system to the computer for communication

- **Ethernet port (green)** — use this port to connect the system to the computer for communication

There is a color-coded guide for proper connection location and orientation (Figure 2).

Fig. 2. Instrument rear connector panel.

CAUTION: Three cords connect the instrument to the computer for power and communication. Be cautious when walking around the instrument, as these cords can become a tripping hazard.

2.1.2 Aerosol Evacuation Port

The S3 and S3e systems also include an aerosol evacuation port used to directly evacuate the sort collection chamber when connected to a biosafety system. This port can be covered when not in use.
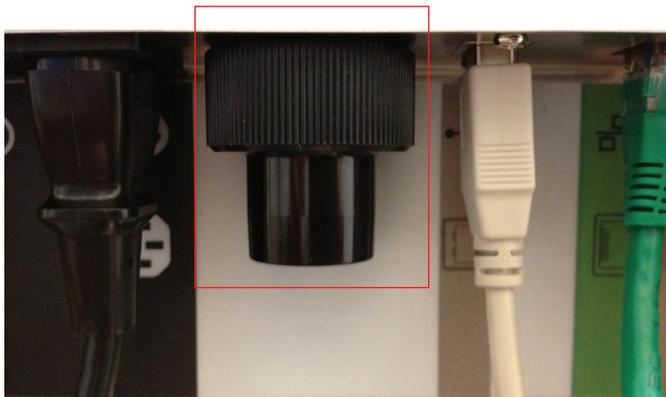

Fig. 3. Aerosol evacuation port.

2.2 Fluidics System

The S3 and S3e fluidics system consists of the bulk fluidics, loading stage, nozzle, and sort collection chamber. The fluidics system supplies sheath fluid, DI water, and sample to the nozzle, and then collects the waste for proper disposal.

> **CAUTION: Biohazard!** Biosafety is of utmost importance while operating this instrument. Consult with your local safety officer or review local, state, and federal regulations to ensure proper handling and disposal of biohazardous substances.

2.2.1 Bulk Fluidics

The S3 and S3e system includes two empty containers as part of the bulk fluidics. Table 6 describes the function of each container.

Table 6. Containers and their functions.

Label	Container	Function
	Biohazardous Waste	The waste container holds the system's fluid after it has run through the nozzle and waste lines. This container has a red cap and holds 4 liters of fluids. Approximately 9 hours of run time can be performed until the empty container is filled. The fluid collected in this container should be decontaminated as appropriate to the application and samples being run on the instrument. Please verify proper treatment and disposal with your safety officer or local health and safety bodies
	Sheath Fluid	The sheath fluid container holds the 8x sheath fluid for the system. This container has a white cap and holds 4 liters of fluid. If preferred, 1x sheath fluid may be used instead. In this case, the DI water container will not be used to dilute the sheath, but only for rinsing and cleaning. If 1x sheath fluid is used, ensure the option is checked in the Global Preferences of the software. This setting will apply globally to the system when set by an administrator. A full container of 8x sheath fluid has approximately 50 hours of run time, while a full tank of 1x sheath fluid has approximately 9 hours of run time
	Deionized water (DI water)	The DI water container holds deionized water for mixing with the 8x sheath fluid to create a 1x sheath fluid. This container has a blue cap and holds 4 liters of DI water with a run time of approximately 9 hours between refills when using 8x sheath fluid. The DI water is also used for cleaning at the end of the day and to rinse the sample probe between samples

Note: The ProFlow™ 8x sheath fluid is diluted with the DI water using an internal fluidics chamber.

Each container uses a quick disconnect system for easy swapping (Figure 4). Both the DI water and the sheath fluid containers have one connector, while the waste container has two connectors for waste fluid from the sorting chamber and the washing station. Located above the containers inside the instrument is a magnetic holder for each quick disconnect. The magnetic holder will keep the quick disconnect away from exchange area.
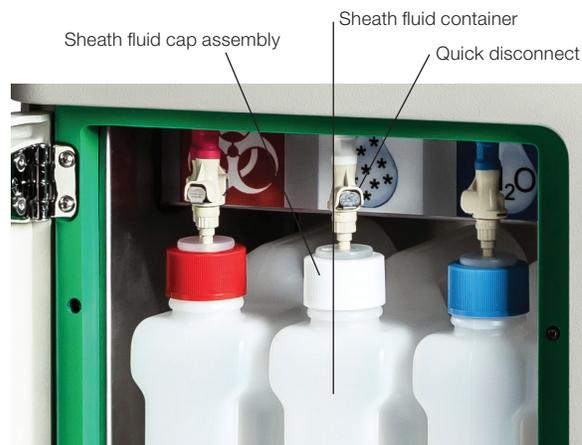

Fig. 4. Fluidics container highlighting the quick disconnect system for the sheath fluid container.

The sheath fluid and DI water are both filtered through a 0.2 μm filter to remove any particulates from the fluid before circulating through the system. Fluidic levels are monitored by the software using a weight measurement system. Below each fluidic container is a bulk fluidics tray. Each tray contains a sensor that translates volume weight into fluidic run time and helps to keep the system dry while swapping fluidics.

> ⓘ **IMPORTANT!** Replace filter cartridges on a regular basis. Filter replacement is part of the annual service preventative maintenance visit. For additional information, refer to Chapter 10, Maintenance.

2.2.2 Loading Stage

The loading stage consists of two functional stations; sample input and washing. The sample input station is located in the front position of the loading stage and it supports a 5 ml, 12 x 75 mm tube (Figure 5).

Note: Polypopylene tubes for sample acquisition are recommended, but not required.

When a sample is loaded and ready to run, the loading stage can be moved into the run position.

To move the loading stage into the run position:

1. Push the handle on the loading stage down.
2. Push the loading stage inward.
3. Gently raise the loading stage into the run position.

The locking mechanism automatically engages when the tube is in the run position (Figure 6). After the tube is locked, the entire sample chamber is pressurized and a short pressure boost is applied to push the sample to the nozzle. After the boost, the sample line is pinched to prevent sample flow until acquisition or sorting is selected in the software.

Fig. 5. Loading stage in the wash position.

Fig. 6. Loading stage in the run position.

The washing station is located in the back of the loading stage and is not accessible to users. When the sample input station is accessible, the washing station is automatically engaged. The washing station is used during startup, shutdown, and in between samples. This will help to reduce carryover since the sample line is cleared and the outside of the line is washed. The loading stage is locked in position while washing and sampling.

During washing, a new sample can be loaded into the sample input station. The status of the loading stage is displayed by a locked or unlocked padlock symbol located on the touch locking system screen (Figure 7).

Fig. 7. Touch locking system screen.

To move the loading stage from the run position to the wash position:

1. Press the touch locking system screen until the padlock displays as unlocked. This will depressurize and unlock the sample station.
2. Push the loading stage handle down, pull forward, and lift up.

WARNING! The loading stage uses a spring to push up into the run or wash position. To prevent the loading stage from over-springing into position and possibly damaging the instrument, guide the loading stage into the run or wash position by holding onto the handle for a smooth movement into position.

Note: If the loading stage is in the wash position and another wash is required, press the touch locking system screen to perform washing again.

The temperature of the sample input station and the sort tube holder can be controlled from the ProSort Software via a single Peltier solid state system. The temperature range can be set from 4–37ºC in 1ºC intervals.

The sample input station is capable of mixing the sample. Sample mixing is performed by vortexing. Users can set the vortexing speed to high, low, or off, depending on the sample type.

Note: It is recommended that resuspended adherent samples are mixed at the high speed to prevent clumping.

IMPORTANT! It is highly recommended that samples are filtered prior to running. This will minimize clogs in the sample tube and nozzle tip.

2.2.3 Nozzle

The nozzle controls many crucial aspects associated with sorting such as:

- Creating a stable vibration to generate droplets
- Hydrodynamically focusing the sample
- Removing air from the nozzle (debubbling)

The nozzle compartment (Figure 8) can be accessed through the top, front sliding door. The nozzle tip is a 100 µm orifice for centering the sheath stream to the laser interrogation point. The tip may be removed for cleaning or to remove a clog from the nozzle stage.

Note: If the nozzle door is opened, lasers will be shuttered, sample will be stopped, and the stream will be disabled.

IMPORTANT! Refer to Chapter 10, Maintenance, for a detailed procedure on removal, cleaning, and replacement of the nozzle assembly and nozzle tip. This is performed using the Swap Nozzle Tip wizard.

CAUTION! Shock hazard! The nozzle door is interlocked and will disable stream charge when opened. Only qualified and trained personnel can override this interlock.
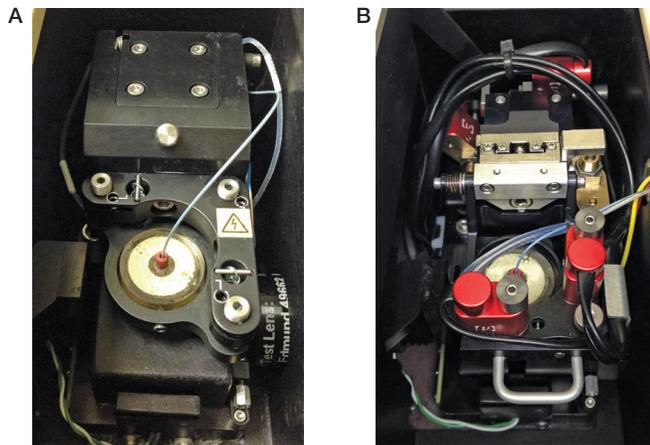
Fig. 8. Nozzle compartment containing the nozzle stage. **A**, Agilis System; **B**, AutoGimbal System.

2.2.4 Sort Collection Chamber

The deflection plates are located in the sort collection chamber (Figure 9). When these plates are electrostatically charged they direct the sorted droplets into the appropriate tubes. These plates should remain clean and dry for optimal deflection.

IMPORTANT! Both plates should be cleaned on a regular basis. If a significant amount of sheath fluid builds up on the plates due to a clog or misalignment, arcing between the plates can occur. When arcing is sensed by the system's electronics, the plates will be disabled and a message will appear in the software. Using a cotton swab, clean the plates and remove any stray fluid before attempting to sort.

Sorted samples can be collected into three collection vessels listed below.

- 1.5 ml tubes
- 5 ml tubes, 12 x 75 mm
- 8-well strips
- Microscope glass slides

Deflection plates

Fig. 9. Sort collection chamber and deflection plates.

The sort positions for each collection vessel are numbered and will correlate to the position numbering in the software when the sort logic and limits are set. When placing tubes in the sort chamber for sorting, it is recommended to add media or buffer to the tube to help prevent sorted cells from drying out and to cushion their collection. The minimum recommended volume is 0.5 ml of media or buffer for each 5 ml tube. Quick-attaching adaptors are available for the 8-well strips or microscope slides. Adaptors are part of the accessory kit and they can be kept inside the fluidics door for storage.

CAUTION! Biohazard! When running samples and sorting, hazardous aerosols may be created depending on the sample type. To prevent hazardous aerosols from spreading, keep the green containment door closed as often as possible. Consult with your local safety officer or review local, state, and federal regulations to ensure proper handling and disposal of biohazardous substances including samples, sorted fractions, and waste.

CAUTION! Shock hazard! The sort deflection plates in the sort chamber are charged while sorting. An interlock on the sort chamber door will disable the plates when opened. Only qualified personnel should override this interlock.

2.3 Optics

The S3 and S3e optics include the laser(s), mirrors, filters, and lenses, which guide the laser light to the stream of sample and collect scattered and emitted light for detection.

CAUTION! Laser hazard! Laser radiation can be hazardous. Please do not override optical interlocks or remove light shields, as they are in place for your safety. Only trained personnel should access the exposed laser beam.

2.3.1 Laser

The S3 and S3e Cell Sorters are installed with a 488 nm, 100 mW laser for cell and particle interrogation that passes through the system in the sample core. The laser power and shutter can be controlled through the software interface. An optional 561 nm or 640 nm 100 mW laser are available as a second excitation source.

2.3.2 Beam Shaping Optic (BSO)

The beam shaping optic sits in between the laser(s) and the interrogation point. This serves to shape and focus the laser beam(s) to optimize the illumination of the cell.

2.3.3 Interrogation

The interrogation point is the point at which the laser beam(s) intercepts the core stream of the sample. At this point, light is scattered around each individual particle and the particle fluoresces if any fluorophores are attached.

2.3.4 Light Collection

Light is collected from two directions, forward and side angle. The light can be categorized into two types — scattered and fluorescent. Scattered light refers to the wavelength of light coming from the laser, which is scattered when a particle is encountered. Fluorescent light refers to the light emitted by the fluorophores or dyes attached to the particle or cell after excitation from the laser. This emitted light is of a higher wavelength than the excitation light and therefore can be separated and detected using optical filters.

2.3.5 Forward Scatter

Light coming around the particle in the forward direction (same plane as the laser beam) is collected to give an indication of particle size. By default, the scattered laser light is collected, but fluorescent light could be collected by changing the filter.

2.3.6 Optical Filters

Optical filters are coated pieces of glass used to divide the spectrum of light into bands for analysis. By separating and detecting different bands of light, it is possible to understand multiple properties of each particle.

IMPORTANT! When removing or replacing filters, wear gloves to avoid depositing smudges and fingerprints on the glass surfaces. Please see Section 10.5, Optical Filter Cleaning, for specific instructions on cleaning optical filters.

2.3.7 Photomultiplier Tubes (PMTs)

The PMTs are used to detect and amplify the light signals coming from each particle. They are located behind the optical filters to detect specific bands of light based on the fluorophores that are attached to the cell.

2.3.8 Cameras

The S3 and S3e Cell Sorters contain several cameras for system alignment and calibration. The function of each of these cameras is detailed below.

Note: Cameras do not require adjustments by users.

- **Pinhole camera** — aligns the stream and laser to the optical detection path. This camera is viewable only by service personnel and during the nozzle tip swap process
- **Droplet camera** — calibrates the droplets and maintains the drop delay. The image of the break-off can be visualized in the software through the Droplet Monitor option on the home tab
- **Streams camera** — calibrates the side streams and aligns them with the collection tubes. This camera can be visualized in the software through the View Streams option on the home tab

2.4 Electronics

The S3 and S3e electronics process and deliver the emitted light signals to the software for user analysis. Also included in this subsystem are the deflection plates in the sort chamber and the safety interlocks.

⚠️ **CAUTION! Shock hazard!** Only qualified, trained technicians should carry out service work on electronic components due to potential shock hazard.

2.4.1 Interlocks

To prevent exposure to laser light and shock hazards, the S3 and S3e systems are equipped with safety interlocks (Figure 10). On the instrument control panel of the software, the instrument status box will show visual indicators if these interlocks have been disengaged.

Note: When opened, the nozzle door will close the laser shutters to prevent laser light exposure. This interlock will also turn off the drop drive and the stream.

🔴 **IMPORTANT!** Open this door only if required to clear a clog or change the nozzle tip. The QC procedure must be repeated to readjust the alignment and reset the drop delay if this door is opened.

The sort door interlock will disable the plate voltage. In addition, if sample is running it will be stopped.

Fig. 10. S3 and S3e safety interlocks.

2.4.2 Pre-Amplifier

The pre-amplifiers are used to boost the signal coming from the PMT.

2.4.3 ADCs

Analog to digital converters (ADCs) convert the electrical signal coming from the pre-amplifier into a digital signal and transfer that signal to the software for visualization of the data.

3 ProSort™ Software

This chapter describes the features of the ProSort Software. The software is the main interface for the S3™ or S3e™ Cell Sorter System, giving overall status and providing control.

To start the software, double click the ProSort icon (Figure 11) on the desktop.

Fig. 11. ProSort Software icon.

The login window will appear (Figure 12). This window will also appear when switching users. Enter your user name and password to log in. Session notes may be added and will be logged with the user session in the user report for administrative use. These notes will appear in the user report when viewed.

Note: If an automatic startup has been scheduled, the login and startup windows will appear different. Please refer to Chapter 9, Automatic Startup for details.

Fig. 12. User login window.

ProSort offers two modes of user control, administrator and user. The administrator mode provides additional control and access over user mode. Differences between the two modes are shown in Table 7. For more information on administrator privileges, refer to Section 3.3, Administrator Tab Toolbar.

Table 7. Control differences between administration and user modes.

Control	Administrator	User
Startup	●	●
Shutdown	●	●
Run QC	●	●
Print QC reports	●	●
Print QC trending reports	●	●
Edit QC criteria	●	
View droplets	●	●
View droplet settings	●	●
Edit droplet settings	●	
View streams camera	●	●
Edit stream settings	●	
Acquisition	●	●
Sort	●	●
Print analysis reports	●	●
Print sort reports	●	●
Print user reports	●	
Change user password	●	●
Change user rights	●	
Delete users	●	
Create users	●	
Edit users	●	
Reset other user passwords	●	

3.1 Main Software Window

The main software window contains tools for system operation and data analysis. Depending on user control mode, the main software window will either display more or fewer features.

Features available in the main software window for user mode are shown in Figure 13.
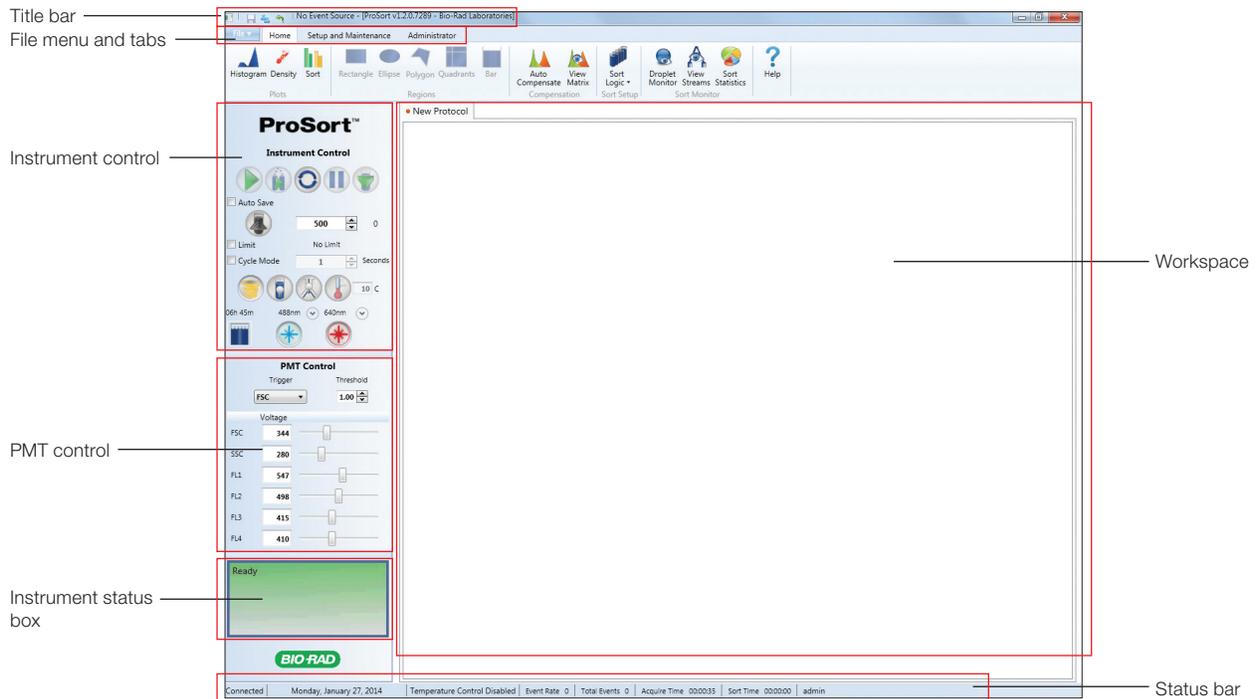
Fig. 13. Main software window for user mode.

3.1.1 Title Bar Buttons

The title bar of the main software window provides the items listed in Table 8.

Table 8. Title bar buttons in the main software window.

Button	Command	Function
	Save	Saves FCS file after acquiring sample
	Sign out/ Switch user	Signs out the current user and returns the software to the login window. If the shutdown procedure was not performed prior to sign out, the system will maintain startup and QC. The next user will need to log in to start a new run
	Undo	Certain commands can be undone and reverted to the previously existing state. Examples of functions that can be undone are: deleted region, deleted gate, deleted histogram or density plot, moved region or gate, creation of a plot or region, compensation adjustment

3.1.2 File Menu

The dropdown file menu provides the items listed in Table 9.

Table 9. File menu buttons and their function.

Button	Command	Function
	New protocol	Opens a new protocol tab in the workspace
	Open protocol	Opens an existing protocol. An FCS file may also be selected at this time. The protocol used to acquire the data will be loaded from the FCS file
	Load instrument settings	Opens an existing protocol's settings and loads them into the current protocol. The current protocol must be saved
	Save protocol	Saves the current protocol
	Open FCS with protocol	Opens FCS file and will load data into the embedded protocol. All contents of the protocol (such as plots, regions, sort logic, notes) will be displayed
	Save FCS file	Saves the current FCS file
	Save partial FCS file	Saves the last user defined number of events from the acquired sample. The number of events to be saved is entered by the user before saving the file
	Print	Opens the print window. **Note:** Use the print preview option to see where page breaks occur
	Page orientation	Changes between a printable landscape or portrait workspace
	Preview	Opens the print preview window
About...	About	Displays software information (software, hardware, firmware version number, serial number, etc.)
Recent Protocols	List of recent protocols for the user that is logged in.	Lists the recent protocols used by the logged-in user
Recent FCS Files	List of recent FCS files for the user that is logged in.	Lists the recent FCS files used by the logged-in user

3.1.3 Home Tab Toolbar

The Home tab toolbar consist of six sections for basic workspace functions and sort options (Figure 14):

- **Plots** — available plots for viewing data. Table 10 describes the plots and their functions

- **Regions** — different shapes for selecting region of interest. Table 11 describes the regions and their functions

- **Compensation** — perform fluorescence compensation. Table 12 describes the compensation buttons and their functions

- **Sort Setup** — collection vessel and sort logic criteria for a run. Table 13 describes the sort setup buttons and their functions

- **Sort Monitor** — monitor the sort of a run. Table 14 describes the sort monitor buttons and their functions

- **Help** — opens the user manual in PDF format
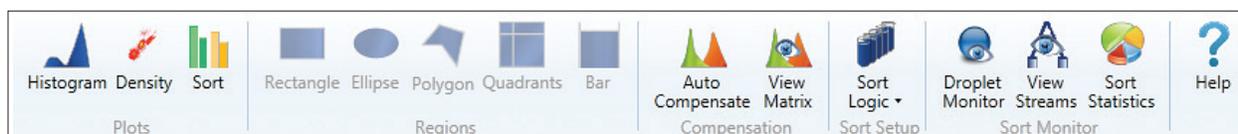
Fig. 14. Home tab toolbar.

Table 10. Plot buttons and their functions.

Button	Name	Function
	Histogram	Adds a new histogram to the current protocol. The histogram can be resized by dragging the bottom right corner
	Density	Adds a new density plot to the current protocol. The density plot can be resized by dragging the bottom right corner
	Sort	Adds a sort plot to the current protocol. The sort plot can be resized by dragging the bottom corner. The sort plot graphically shows the sorts and aborts in each sort position.

Table 11. Region buttons and their functions.

Button	Name	Function
	Rectangle	Adds a rectangular region to the selected density plot. If a density plot is not selected, this region will be grayed out. The region can be moved, resized, rotated, and/or deleted
	Ellipse	Adds an ellipse region to the selected density plot. If a density plot is not selected, this region will be grayed out. The region can be moved, resized, rotated, and/or deleted
	Polygon	Adds a polygon region to the selected density plot. If a density plot is not selected, this region will be grayed out
		Select each desired point and move the mouse cursor outside the density plot to complete. The region can be moved, resized, and/or deleted

Table 11. Region buttons and their functions (continued).

Button	Name	Function
	Quadrant	Adds a quadrant region to the selected density plot. If a density plot is not selected, this region will be grayed out. The region can be moved, resized, and/or deleted
	Bar	Adds a bar region to the selected histogram. If a histogram is not selected, this region will be grayed out. The region can be moved, resized, and/or deleted

Table 12. Compensation buttons and their functions.

Button	Name	Function
	Auto Compensation	Opens the Automatic Compensation Calculation Wizard window. This wizard will assist in establishing the proper compensation matrix coefficients. Single control FCS files must be acquired and saved prior to using this feature.
	View Matrix	Opens the Compensation Matrix window. Users can view and edit the compensation matrix in this window

Table 13. Sort Setup buttons and their functions.

Button	Command	Function
	Sort Logic	Opens the Sort Logic window. By default, 5 ml tube is selected for the Sort Logic window. Users can click on the Sort Logic dropdown arrow to select 1.5 ml tube, 8-well strip, or glass slide
	5 ml tube	Indicates that 5 ml tube is selected as the collection vessel. Individual mode and limits may be set for each 5 ml tube in each direction
	1.5 ml tube	Indicates that 1.5 ml tube is selected as the collection vessel. Individual mode and limits may be set for each 1.5 ml tube in each direction
	8-well strip	Indicates that 8-well strip is selected as the collection vessel. Sort limits or mode can be set for each well in an 8-well strip
	Glass slide	Indicates that glass slide is selected as the collection vessel. Sort limits or mode can be set for each microscope slide. A maximum of 500 events can be sorted onto each location on the slide

Table 14. Sort Monitor and help buttons and their functions.

Button	Name	Function
	Droplet Monitor	Opens the Droplet Monitor window. Droplets will be visible in this window after performing a QC procedure. The status of droplet maintenance is shown in this window. The currently set drop delay and drop drive amplitude are also shown in this window
	View Streams	Opens the View Streams window. This camera shows the sort streams at a point just above the collection vessel
	Sort Statistics	Opens the Sort Statistics window. The window displays sort count, sort rate, percentage abort count, rate and %, efficiency, etc. Sort Statistics can be added to the print window for reporting. This window will open automatically when a sort is started
	Help	Opens a PDF file of the user manual as a reference

3.1.4 Setup and Maintenance Tab Toolbar

The Setup and Maintenance tab toolbar consists of five sections (Figure 15):

- **System** — startup or shutdown the instrument from the software. Table 15 describes the system buttons and their functions

- **Quality Control (QC)** — perform or review QC runs. Table 16 describes the QC buttons and their functions

- **Fluidics** — access common fluidic functions. Table 17 describes the buttons and their functions

- **Other** — additional features. Table 18 describes the additional features and their functions

- **Publish** — features for supporting publications. Table 19 describes the publish features and their functions as well as the toolbar's remaining user buttons and their functions

Fig. 15. Setup and maintenance tab toolbar.

Table 15. System buttons and their functions.

Button	Name	Function
	Start-Up	Starts up the system by starting the sheath flow, turning on the laser(s), and backflushing the sample line
		Use this button to start up the system if auto startup has not been selected
	Shutdown	Shuts down the system. The shutdown button is not active unless the loading stage is in the wash position. The shutdown button will prompt to clean system and schedule an auto startup

Table 16. Quality control buttons and their functions.

Button	Name	Function
	Run QC	The daily QC procedure ensures optimal system performance and should be completed every day prior to running samples. The procedure consists of placing a tube of ProLine™ calibration beads on the system, setting the droplet break-off, setting up side streams, adjusting event rate, aligning nozzle, adjusting PMTs, checking the coefficient of variation (CVs) and voltages, calculating drop delay, and placing the system in droplet monitor mode. The progress of this procedure will be displayed on screen
	Trending	Daily QC data are compiled into trending reports. These reports show trending of CVs, PMT voltages, drop delays, etc. over a range of dates. This date range may be selected in the report screen. These reports may be saved and/or printed
	QC Report	This button shows and allows saving and/or printing of the daily QC report. This report includes date, user, CV/PMT/droplet info, etc

Table 17. Fluidics buttons and their functions.

Button	Name	Function
	Debubble	Removes bubbles from nozzle
	Unclog	Allows user to pull vacuum in the case of a nozzle clog. Use this option before removing the tip for sonication or replacing with a new nozzle tip
	Swap Tip	Stops the sheath flow to allow nozzle tip replacement. A wizard will appear to walk the user through a nozzle change. Once the tip has been replaced, QC needs to be rerun to verify alignment and set the drop delay
	Swap Fluidics	Allows fluidic containers to be swapped without shutting the system down. Prepare a full replacement or empty container prior to pressing this button, as a three-minute timer starts after the button is pressed
	Clean	Cleaning can be done at low or high pressure. High pressure cleaning will cause the system to stop maintaining droplets and a QC procedure must be run before sorting. If cleaning between samples, the low pressure option is recommended

Table 18. Other buttons and their functions.

Button	Name	Function
	Annotation	Adds an annotation window to the workspace. Annotations added prior to saving will be saved to the FCS file's embedded protocol
	Image	Allows an image to be added to the workspace. The image can be moved or deleted. If added prior to saving, this image will be saved to the FCS file's embedded protocol
	Basic Information	Adds basic information to the print page in the form of a header. This information includes date, time, user login, serial number, filename, event source. If added prior to saving, this information will be saved to the FCS file's embedded protocol
	Filter Configuration	Adds the optical layout to the print page showing PMT and optical filter setup. This can be modified to match the actual filters if they have been changed. If added prior to saving, this will be saved to the FCS file's embedded protocol

Table 19. Publish and user buttons and their functions.

Button	Name	Function
	Preview	Displays a preview of the page layout(s) setup prior to printing
	Page Orientation	Changes between a printable landscape or portrait workspace
	Print	Opens the print dialog to select printer, number of copies, etc. and print. **Note:** Use the print preview option to see where page breaks occur
	Print Options	Adds additional windows or instrument settings to the page layout for printing.
	Change Password	Allows the currently logged-in user to change password
	System Log	Opens window for system log. Displays date, time, error, error details. This option is helpful when working with technical support and service engineers for troubleshooting

3.2 Control Panel

System operation is performed and monitored with the control panel (Figure 16). The control panel consists of three parts:

- **Instrument control** — controls for performing a run
- **PMT control** — controls for modifying the PMTs
- **Instrument status box** — displays the status of your instrument

Instrument control buttons are described in Table 20 and PMT control buttons are described in Table 21.

Sample agitation high/low button
Sample station light on/off button
Sample collection area light on/off button
Temperature control on/off button
Temperature control option

Biosafety System serial number
Biosafety System firmware version
Biosafety System status
Time remaining on filter life
Temperature inside the system

Start/Stop acquisition button
Start/Stop sort button
Refresh data button
Pause/Restart sample acquisition button
Eject sample tube button

Auto save sample upon reaching limit
Preset sample event rate button
Enter sample event rate
Event limit option
Cycle mode option
Gate limit option

Time remaining with fluidic volume
Fluidics Status window button

Laser on/off button
Laser power options

Trigger channel
Threshold value
Alter PMT voltages
Assign values for detection channels

Instrument status box
Indicator of actions required or needing attention

Fig. 16. Overview of instrument and PMT control panel.

Table 20. Instrument controls and their functions.

Button	Name	Function
	Start Sample Acquisition	Starts sample acquisition. If a data file is loaded and it has been saved, the data will clear and acquisition will start
	Stop Sample Acquisition	Stops acquisition and sample flow. Regions, gates, and annotations may be modified before saving the FCS file
	Start Sorting	Starts sorting. If a data file is loaded and it has been saved, the data will clear and sorting will start
	Stop Sorting	Stops sorting and sample flow. Regions, gates, and annotations may be modified before saving the FCS file
	Clear Acquired Data	Clears acquired data. All plots and statistics will be refreshed.
	Pause Sample	Pauses sample acquisition and sample flow. The data at this point may be appended (by the Resume function) or saved as is. Saving after pausing will disable the Resume function. To remove sample tube, unlock the touchscreen locking system located above the loading stage after pausing acquisition
	Resume Sample	Resumes acquisition when a sample has been paused. Resuming will append the paused FCS file and the time paused can be seen on a time histogram. If you have removed the sample tube it must be returned and relocked before acquisition can be resumed
	Eject Sample Tube	Depressurizes and unlocks the sample. The loading stage can be moved into wash position to access sample. The touchscreen locking system can also be used to unlock the sample
	Adjust Sample Agitation	Starts or stops agitation of the sample. There are three levels of agitation: low, high, and off. Click this button once to set to low, twice to set to high, and three times to turn off agitation
	Auto Save	When checked, automatically prompted to save a data file after acquisition stops.
	Target Event Rate	Allows selection of target event rate for sample acquisition or use of preset sample rates low/med/high based on sample concentration
	Limit	When checked, stops data acquisition after the number of events entered has been reached
	Gate Limit	When checked, data acquisition stops after the number of events entered has been reached within the selected gate
	Cycle Mode	When checked, automatically refreshes the data displayed according to the entered time. The acquired data set continues to accumulate in the file
	Sample Chamber Light	Turns the sample chamber light on and off
	Enable/Disable Sort Chamber Light	Illuminates entire sort collection chamber for easy viewing of deflection plates, sort stream, and collection vessels
	Enable/Disable Temperature Control	Enables or disables temperature control of the sample station and sort collection vessel
	Biosafety System Status	Indicates the status of the biosafety system and gives information on filter life. If the biosafety system loses connection, cannot connect, or if the filter life is at 0% an error will occur and not allow the user to sort or acquire.
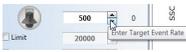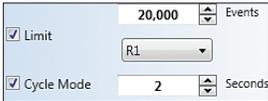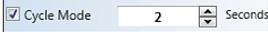
continues

Table 20. Instrument controls and their functions (continued).

Button	Name	Function
	Fluidics System Status	Indicates the status of the fluidics system and run time information. When the fluidics system is down to an hour of run time, the fluidics status window will automatically open to show which container needs attention. If the run time reaches 5 min, the fluidics will automatically shut down
	Toggle 488 nm Laser Shutter	Controls the 488 nm laser shutter. The shutter is interlocked for safety. When this button is grayed out, either the laser is off or the interlock is open
	Toggle 561 or 640 nm Laser Shutter	Controls the 561 or 640 nm laser shutter. The shutter is interlocked for safety. When this button is grayed out, either the laser is off or the interlock is open
	Adjust Laser mWatt Power	The dropdown arrows next to each laser adjust the laser power. Actual power is shown by hovering over the 488 nm, 561 nm, and 640 nm labels
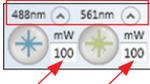
Table 21. PMT controls and their functions.
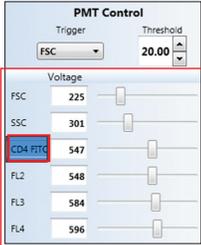
Button	Name	Function
	Editable parameter name	The name for each parameter is editable and will be saved with the acquired FCS data. The edited parameter name will be applied to all plot axes, compensation controls, and filter configurations
	PMT Voltage	Adjusts the PMT voltage for the given detector
	Select trigger parameter	The trigger parameter is used to alert the system to the presence of an event over the threshold. The default for this is Forward Scatter
	Adjust threshold setting	Threshold is a percentage of the trigger signal. The default value is 1.0%

3.3 Administrator Tab Toolbar

The administrator tab is available only for administrator users. To change a user's privileges, refer to Section 3.3.5, Managing Users.

The toolbar for the administrator tab (Figure 17) consists of:

- **System and User Control** — edit system controls and manage users

Table 22 describes the buttons in this section.

IMPORTANT! Adjusting any of these settings will impact the overall performance of the system. Only knowledgeable users should make any changes to these settings. All changes made here will apply globally to the system.
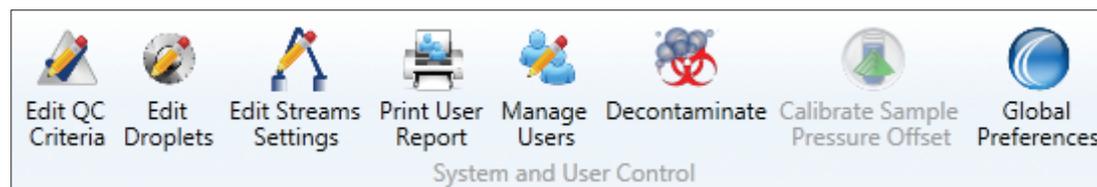
Fig. 17. Administrator tab toolbar.

Table 22. Administrator tab toolbar buttons and their functions.

Button	Name	Function
	Edit QC Criteria	View and edit the CV and PMT voltage criteria for the quality control procedure. This may be done as a result of a bead lot change
	Edit Droplets	Edit the settings for droplet creation. These settings include drop drive amplitude, drop drive frequency, charge phase, defanning, and drop delay. These settings are automatically adjusted as part of the QC procedure and will result in sort changes. These settings are grayed out while the system is maintaining
	Edit Streams Settings	View sort streams and edit the settings for stream creation. These settings include deflection, charge plate voltage, defanning, phase, and test pattern adjustment
	Print User Report	View usage reports including times, dates, and duration of system use for all users
	Manage Users	Create new users, reset passwords for existing users, set rights, and delete users
	Decontaminate	Run a decontamination wizard that will walk user through a complete fluidic system decontamination. This will take approximately 2–3 hr
	Calibrate Sample Pressure Offset	Reset the differential offset sample pressures, which returns the preset buttons (low, med, high) to their correct ranges
	Global preferences	Change global instrument options for sheath concentration used, idle shutdown time limit, and boost adjustment, plot default options and file save default location

3.3.1 Editing QC Criteria

An administrator can edit the QC criteria used to pass or fail the instrument during the daily QC. This should be performed only by an experienced user who has verified system performance. The rewind arrow on the middle bottom of Edit QC Criteria window will reset the QC criteria to the factory defaults for the system (Figure 18).

When receiving a new lot of ProLine calibration beads:

1. Download the bead lot file from the S3 or S3e Cell Sorter website, **www.bio-rad.com/cellsorter**.
2. Click the green plus button (Figure 18).
3. Navigate to the new bead lot file.
4. Select the file and click **Open**.

The software will automatically update the QC criteria for the new lot of calibration beads. Values in red indicate values that are not used for pass/fail criteria for QC. If you plan to enter the bead lot information manually:

1. Verify the acceptance criteria on the certificate of analysis.
2. Compare the old lot to the new lot.
3. Enter the new lot information into the Edit QC Criteria window.

IMPORTANT! Changes to these criteria will apply globally to the system.
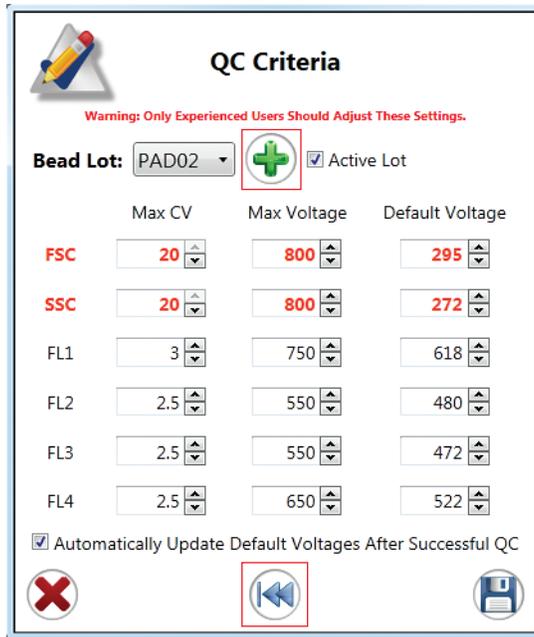
Fig. 18. Edit QC Criteria window with green plus button and rewind arrow highlighted.

3.3.2 Edit Droplets

Droplet controls are available to the administrator for troubleshooting purposes. While the system is maintaining, the drop delay, drop drive amplitude, and drop drive frequency cannot be adjusted (Figure 19). If any of these settings is adjusted, use ProLine calibration beads to recalculate the drop delay before running samples. The verify drop delay button at the bottom of this screen will sort three puddles onto a slide which can be examined under a microscope to confirm drop delay. These puddles will sort 100 events at the current drop delay – 1, the current drop delay, and the current drop delay + 1.

⚠️ **CAUTION!** Optimal settings are calculated automatically after performing a quality control procedure. Adjusting any of these values will change the settings and will affect the drop delay, including the break-off point.

3.3.3 Edit Streams

Streams controls are available to the administrator for troubleshooting purposes. While the system is maintaining, these settings can be adjusted. Side streams can also be turned on or off by checking the Enable Test Pattern box (Figure 20).

⚠️ **CAUTION!** Optimal side stream settings are calculated automatically after performing a quality control procedure. Adjusting any of these values will change the settings and will affect the side streams and sort.

Calibrate droplets
Recalculate drop delay
Verify drop delay
Close window
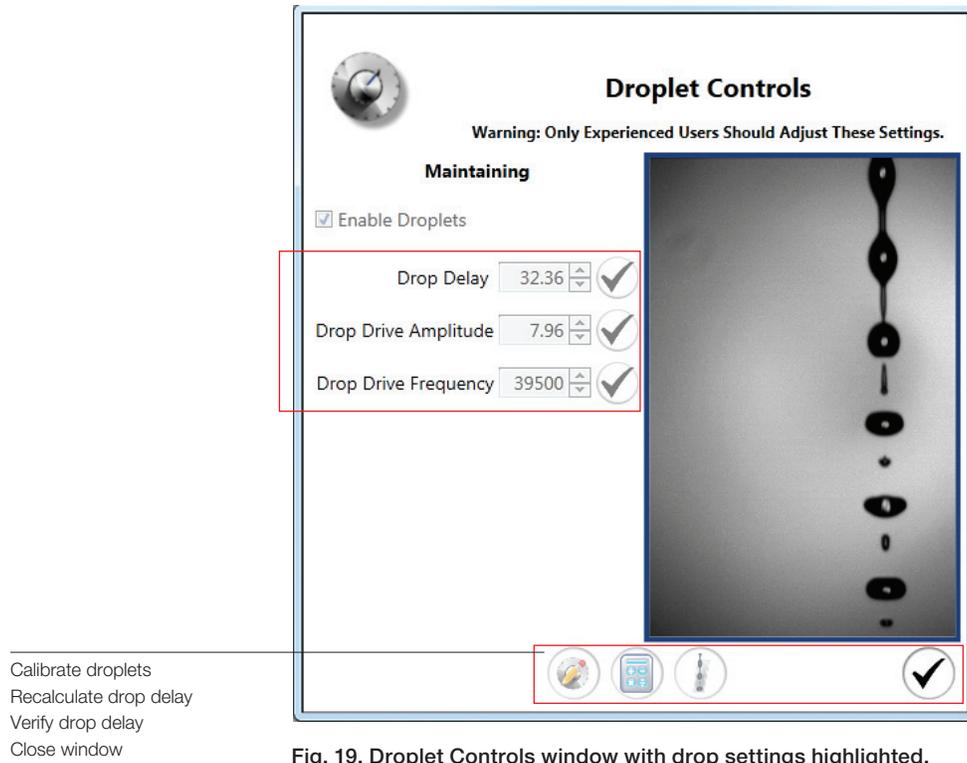
Fig. 19. Droplet Controls window with drop settings highlighted.
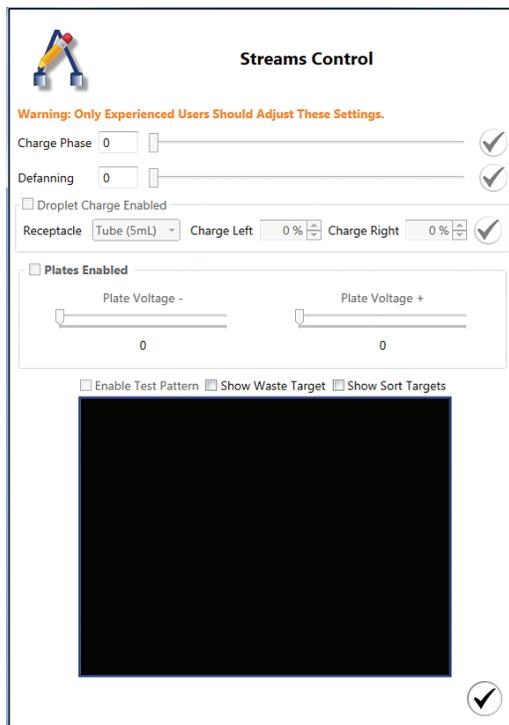
Fig. 20. Streams Control window with Enable Test Pattern highlighted.

To check for stream defanning:

1. Locate the center stream. The stream is visualized as a small bright spot that is illuminated by the stream laser.

2. Check the **Enable Test Pattern** check box the system will charge the side streams to simulate a sort.

   Fanning of the streams can be seen more easily with the center stream than the two side streams. The test pattern will make the center stream slightly broader.

3. Determine if the center stream is one solid stream.

   If the center stream spreads into several streams, the fanning should be adjusted.

4. Use the defanning slider until the center stream is as focused as possible (Figure 21).

5. Click the checkmark when completed to save the settings.
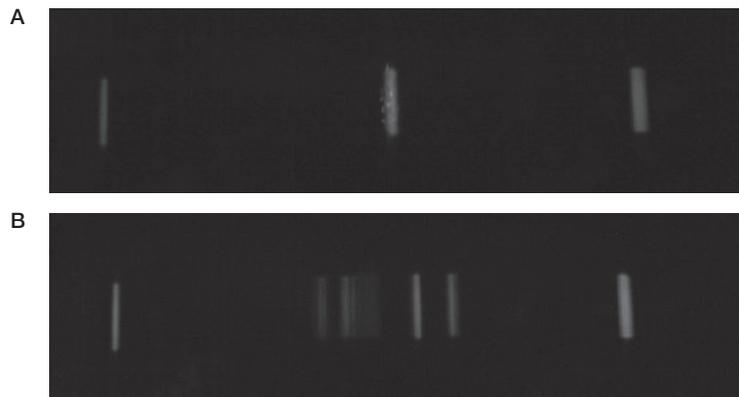
Fig. 21. Examples of a good center stream, A and a defanned center stream, B.

3.3.4 User Reports

User reports can be viewed, saved, printed, and exported for analysis of system usage and for billing purposes. To display a user's usage information, select the date range of interest and click **Update** (Figure 22).
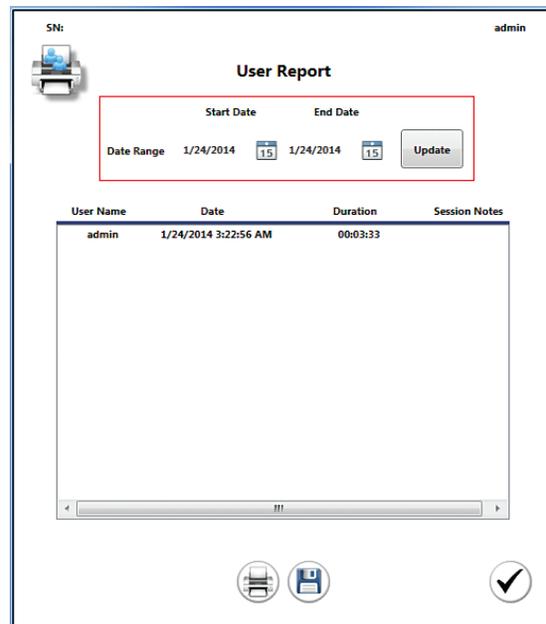
Fig. 22. User Report window with date range highlighted.

3.3.5 Managing Users

The Manage Users button opens the Manage Users window. This window allows an administrator to add, delete, or edit users (Figure 23). Passwords can be reset and users can be notified to reset their passwords from this window.
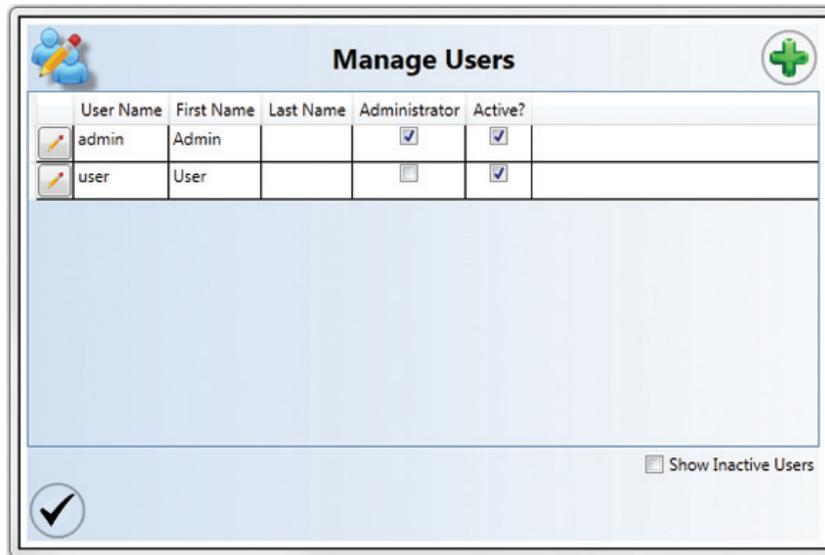
Fig. 23. Manage Users window.

To edit a user's information:

1. Click the pencil icon next to the user's name.
2. Modify user information in the Edit User window (Figure 24).
3. Click **Save**.

Note: In the event that all administrator passwords are lost or forgotten, call your local technical support team to receive a temporary password.

Fig. 24. Edit User window.

3.3.6 Decontaminate

An administrator can run the decontaminate procedure if the system shows signs of heavy background, noticeable debris when running water, or contamination. The complete internal fluidic system will be decontaminated. Follow the wizard procedure located in Section 10.7, Decontamination.

3.3.7 Calibrate Sample Pressure Offset

If the system shows an event rate of 0 when running calibration beads in the low setting, or if it shows 500+ events, the offset will need to be reset. Using this tool will reset the differential offset sample pressure, which will return the preset sample flow rates (low/med/high) back to their correct ranges.

1. Load calibration beads into the sample station and move loading stage to the run position.
2. Click **Calibrate Sample Pressure Offset**.

A warning window will appear asking if the user wants to continue (Figure 25). ProLine calibration beads must be running for this calibration process.

The system will automatically begin the calibration process (Figure 26). Once complete the system offset pressure has been calibrated.
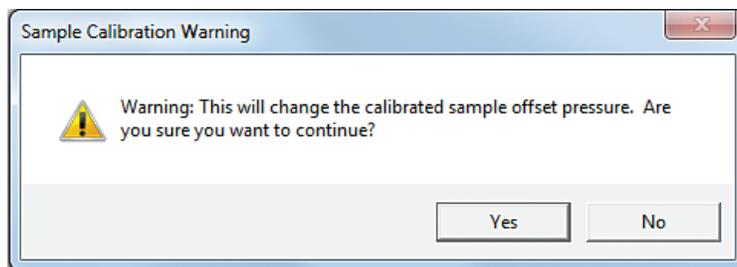
Fig. 25. Sample Calibration Warning dialog box.

Fig. 26. Calibration Sample Offset window.

3.3.8 Global Preferences

Users with administrator privileges have the ability to change global settings for the instrument. Changes to global preferences will become effective upon accepting the changes. Preferences specific to plots will be displayed when new plots are created.

CAUTION! These settings are global and may influence other users' experiments. Adjusting any of these values will change the optimal settings for the instrument.

Sheath can be used at 8x or at 1x concentration

Option to enable mandatory cleaning upon shutdown or logout

Parameter names can be preset to have user-defined names displayed

Define idle shutdown option and countdown time

- Choose to prompt user to save unsaved FCS and protocols
- Define default save file locations

Boost duration can be increased or decreased

Plot default options:
- Define which plot statistics to be displayed and number of digits after the decimal
- Choose which axis parameter will be displayed and log verses linear axis values
- Define plot display size, maintain aspect ratio of plots
- Display and highlight events that are off axis
- Display compensation sliders on plots

Define emergency contact information

Fig. 27. Global Preferences window.

Sheath Fluid Options

The system can use 8x or 1x sheath solution. The default concentration is 8x. The 8x sheath fluid will mix automatically with DI water prior to running through the system. One container of 8x sheath fluid will provide up to 50 hours of run time compared to 9 hours of run time for one container of 1x sheath solution.

If a 1x sheath solution will be used, select 1x in the sheath concentration section prior to running the system to avoid diluting the solution.

Idle Shutdown Time

The system has the ability to shut down automatically and start the shutdown procedure after sitting idle for a designated time period. The system has a default setting of 2 hours.

This option helps to preserve laser lifetime and prevent the system from running overnight.

Boost Adjustment

The default boost duration is optimized to bring the sample into position for interrogation by the laser once acquisition is started. Default values can be restored by selecting Default. If this value is adjusted, test the boost duration with a known sample to optimize the duration before running the unknown samples.

Force Cleaning

There are two methods to end a user session in the software — log out or shut down. For both methods, a Force Cleaning option is available to promote cleaning and maintenance of the system. Force cleaning can help to avoid unnecessary clogs that can happen when the system is not cleaned regularly or left overnight with sample in the lines.

Default Plot Statistics

Each plot can be displayed with statistics for all events or regions drawn on the plot. Each statistic can be displayed or can be hidden by setting the preference. In addition, the number of significant figures for each statistic can be defined. Once this parameter is changed the subsequent new plots will reflect the new statistics defaults.

Plot Defaults

In this section there are several options that can be chosen.

- Histogram and Density Plot axis parameters can be defined and set in the global preferences. The user can choose the default axis parameter type: Area, Height, or Width. In addition, the axis can be displayed in log or linear scales. Log can be selected as the default by checking the box. Once these parameters are changed, subsequent new plots will reflect the new parameter defaults

- The width and height of the plot can be defined specifically. The default number of pixels is 400 x 400. In addition, the aspect ratio can be maintained if the box is checked for this option. This option applies to both histograms and density plots. Once this parameter is changed, subsequent new plots will reflect the new size default

- Events may fall off scale and be displayed on or below the axis. A preference can be chosen to show an arrow indicator when events are off scale. This will help to raise awareness that some settings may need to be adjusted

- Auto compensation is the recommended method to compensate samples properly and in a statistically correct method. Compensation sliders can be selected to be displayed or hidden on the density plot axes when the compensate box is checked. Compensation bars can also be used to make compensation adjustments, however proper gating and match medians must be used to compensate correctly

Parameter Names

Each parameter is defaulted to its channel name (FSC, SSC, FL1, FL2, FL3, and FL4). The default parameter name can be entered by the user. This will be the parameter name for all new protocols created once the preferences have been changed.

Note: Parameter names can also be changed on the instrument panel but they will revert to default names when a new protocol is opened. Refer to Section 5.1.6, PMT Voltages, to see how to change names on the instrument panel.

Default Save Location

The software can autosave after each file has finished acquiring. Files can be saved to a default destination folder on the computer. This can be set for both protocols and for FCS files. In addition, there is an option to require a prompt prior to clearing data.

Emergency Contact Information

An emergency contact name and phone number can be entered into these fields. The information will be displayed in the **File > About** window. This will let all users of the instrument know what person within the lab or institution to contact for any emergency help with the S3 or S3e System.
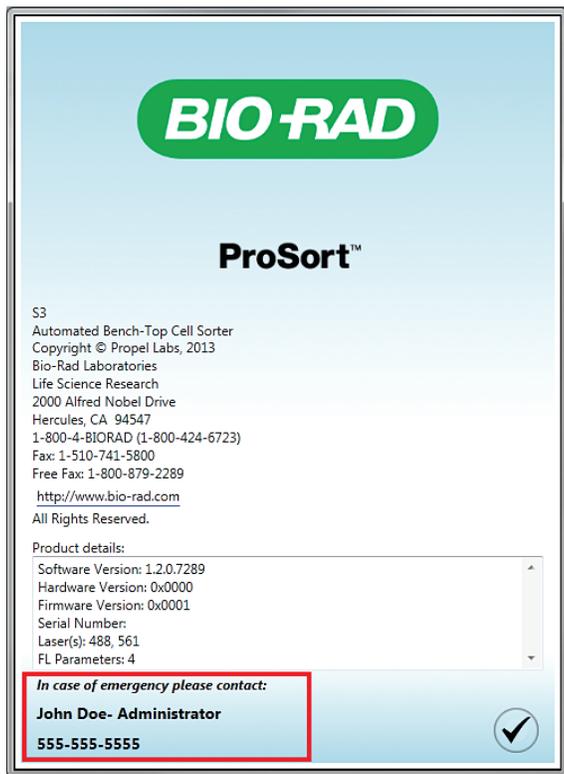
Fig. 28. About window displaying emergency contact information.

4 **Getting Started**

After system installation by a Bio-Rad certified service engineer, the S3™ or S3e™ System should not be turned off via the main power switch. The system is safe in standby mode after performing a software shutdown of the system. This will help facilitate quick or automated startup.

If the system is shut off via the main power switch, always power the instrument on before starting the ProSort™ Software. The internal computer system of the instrument must be running in order to communicate with the software.

> **IMPORTANT!** Powering the system on or off incorrectly can cause the instrument to not run properly.

4.1 Checking Bulk Fluidics

Prior to starting the ProSort Software, open the bulk fluidics door and check the volume of each container. Ensure that the waste container is empty, and the sheath fluid and DI containers are full.

Note: To change the bulk fluidics after starting the software or during a run, refer to Section 5.3, Checking or Swapping Fluidics.

To refill or change a container (Figure 29):

1. Detach the quick disconnect from the cap assembly by pushing in the metal button and lifting up the quick disconnect until it engages the magnetic holder.
2. Slightly lift and pull out the container.
3. Remove the cap assembly from the container.
4. Refill or change the container.
5. Carefully place the cap assembly onto the container and tighten the cap.
6. Place the container into position.
7. Attach the quick disconnect to the cap assembly. An audible click should be heard.

Sheath fluid cap assembly

Sheath fluid container

Quick disconnect
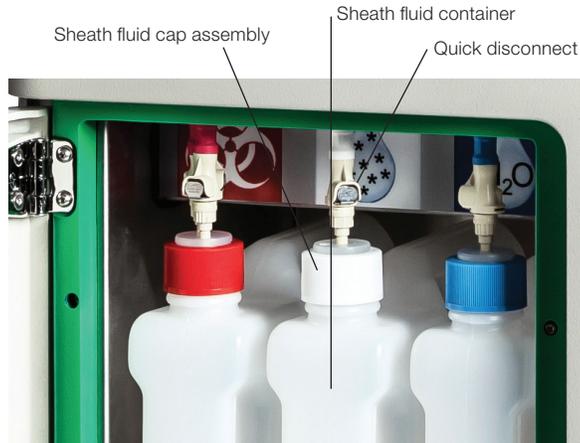
Fig. 29. Bulk fluidics with sheath fluid components noted.

IMPORTANT!
- When handling sheath fluid and DI water containers, minimize air exposure to help avoid contamination
- While transferring cap assemblies to a new container, avoid touching the assembly to the outside surfaces of the containers. If it is necessary to place the cap assembly down, only use pre-sterilized surfaces
- Treat all waste as bio-hazardous for safety

4.2 Logging In

To use the S3 or S3e system, ProSort Software is required. The software is preinstalled on the computer that came with the instrument.

1. Double click the ProSort Software icon located on the desktop.
2. Log into the ProSort Software.
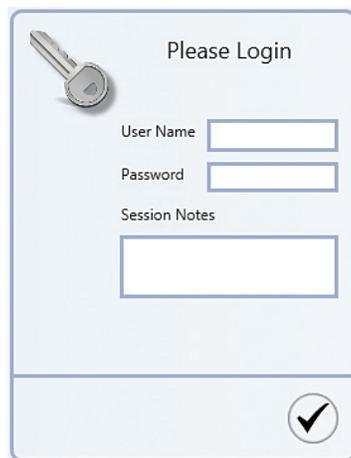
Fig. 30. Login window.

Note: If the system has been scheduled for an automatic startup, a startup counter will be visible in the Login window (Figure 31). Please refer to Chapter 9, Automatic Startup, for startup options.

Fig. 31. Automatic startup message in login window.

4.3 Daily Startup

On a daily basis, the system requires approximately 20 min of warmup time before running quality control or any samples. To warm up the system:

1. Move the loading stage into the wash position.
2. Click on the **Setup and Maintenance** tab.
3. Click **Startup**.

Automatically the Startup Settings window will pop up (Figure 32). Using the startup button, the instrument can either be started up at the moment or a scheduled startup can be designated. Startup Now is the default option.
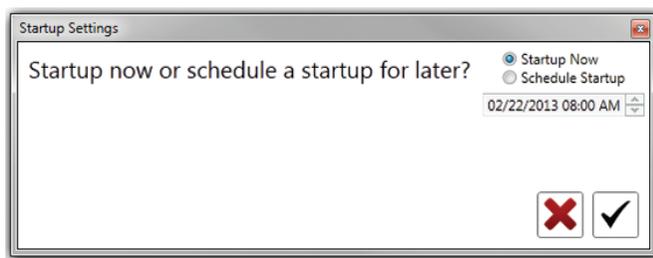
Fig. 32. Startup Settings window.

4. Choose startup option in the Startup Settings window.

5. Click the checkmark box to proceed.

Note: If scheduling an auto startup, the software will return to the main window and will not start up the system until the scheduled time.

The Startup Status window will display the status of the startup process (Figure 33). The startup process includes initializing the system, filling the fluidics lines, and warming up the lasers.
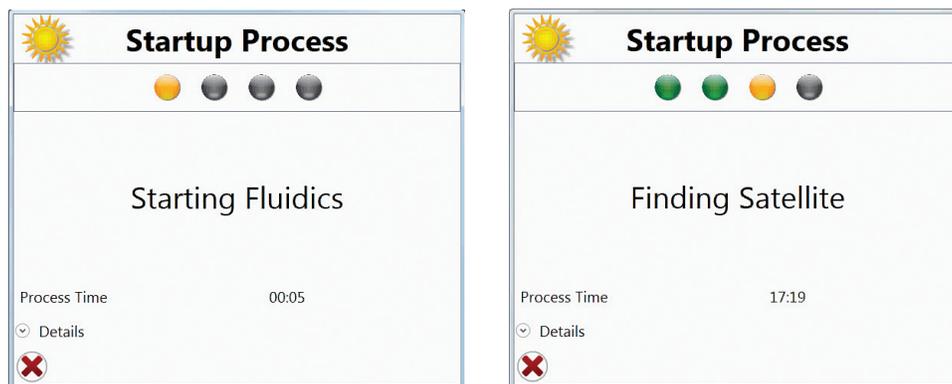
Fig. 33. Startup Process windows.

During the startup process, the fundamental alignment window will also open to initiate cameras, maintain droplets, and align streams to the lasers. The startup process also includes a warm up period (Figure 34).

Fig. 34. Startup Process window.

After startup completion, both windows will close automatically and buttons in the toolbars will become available to use.

Note: Automated startup is an option for this instrument. At shutdown, a startup time can be designated. If this option is selected, be sure to empty the waste container and fill the sheath fluid and DI containers at the end of the day.

Note: To change the fluidic containers after the software has been initiated or during a run, refer to Section 5.3, Checking or Swapping Fluidics, for instructions on performing a hot swap.

4.4 Quality Control

The quality control (QC) procedure must be run every day to ensure optimal system performance. This procedure requires the ProLine™ calibration beads to adjust alignment, verify system against the QC criteria, set up droplet creation, adjust side streams, calculate drop delay, and maintain the droplet break-off.

Note: ProLine Universal calibration beads **must** be used on a system with a 488/640 nm lasers. These beads can also be used on the 488 nm or 488/561 nm systems.

To perform the quality control procedure:

1. Vortex a bottle of ProLine calibration beads.

2. Place at least 10 drops (approximately 0.5 ml) into a 5 ml tube. Do not dilute these beads.

3. Place the tube with beads into the sample input station.

4. Move the loading stage into the run position.

5. Click **Run QC** on the Setup and Maintenance tab.

6. Choose the correct bead lot from the dropdown menu in the Event Based Alignment Settings window (Figure 35).
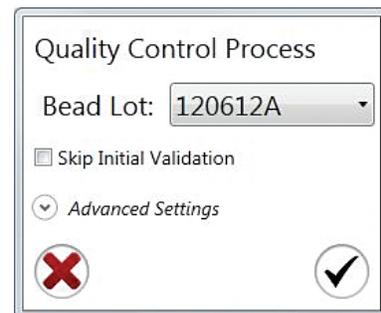
Fig. 35. Event Based Alignment Settings window.

Note: If the bead lot is not available in the dropdown menu, refer to Section 3.3.1, Editing QC Criteria.

A series of windows will open during the QC procedure to perform alignment and calculate drop delay settings. User interaction is not required for these windows. These steps are described later in this section.

7. After completion, the Drop Delay Alignment Status window will open with the optimal drop delay settings (Figure 36A).

Fig. 36. A, Drop Delay Alignment Status window and B, Daily QC Report.

8. Click the checkmark to open the QC Report.
9. Review the QC report (Figure 36B).
    - If the QC status passed, exit the window. The system is now ready for sample acquisition and sorting.
    - If the QC status failed, rerun the QC procedure up to two more times.

4.4.1 Droplet Setup

The first step in the QC process is droplet setup. Droplet setup is performed automatically and the droplets will be optimized to a stable break-off. The droplet camera image may be viewed by clicking **Droplet Monitor** on the Home toolbar. The Droplet Monitor window will display the drop delay, drop drive amplitude, and status of the drop delay (Figure 37).

Users with administrator privileges may access and modify these settings in the administrator tab. For more information, refer to Section 3.3, Administrator Tab Toolbar.

Fig. 37. Droplet Monitor window.

4.4.2 Side Streams

Following droplet setup, the side streams are adjusted to align with the sort tube positions and to ensure they are not fanning. The streams may be viewed by clicking **View Streams** on the Home tab. The View Streams window displays a charge phase and defanning slider bar (Figure 38). Users can manually adjust the charge phase and defanning values by moving the slider bar. The Enable Test Pattern button should be selected in order to apply a charge to the stream.

Users with administrator privileges may access and modify these settings in the Administrator tab. For more information, refer to Section 3.3, Administrator Tab Toolbar.

⚠️ **CAUTION!** Optimal side stream settings are calculated automatically after performing a quality control procedure. Adjusting any of these values will change the optimal settings and will affect the side streams and sort.

Fig. 38. View Streams window with charge phase and defanning slider bars highlighted.

4.4.3 Alignment

During the alignment phase, automated micro-motors sweep the fluidic stream through the laser beams to find the point of optimal illumination. This automated process requires the stream and laser(s) to be running. After alignment, the CVs and voltages are compared to the QC criteria. During the QC procedure, a calibration protocol will open and run automatically. Upon completion, the data are saved as an FCS file. This file may be opened and viewed by opening the daily QC report, selecting the desired date and time, and clicking the open FCS file button at the bottom of the report.
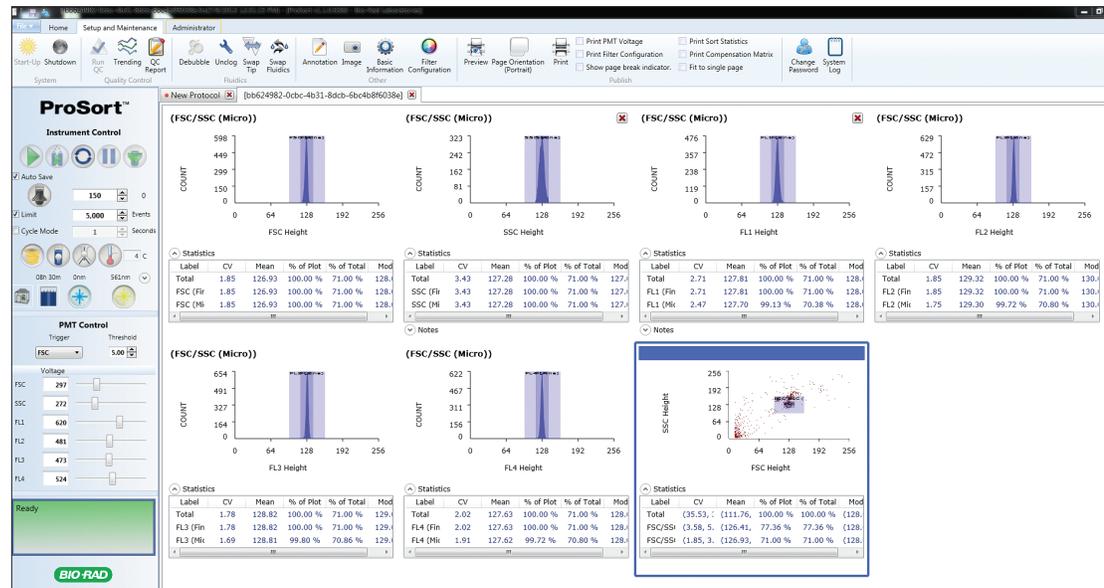
Fig. 39. QC histograms displayed in the workspace.

4.4.4 Drop Delay Determination

After the droplet and side stream formations have been optimized and the system is optically aligned, drop delay will be determined using ProDrop™ technology and set using the same ProLine Calibration Beads used for the alignment.

ProDrop technology accurately calculates the drop delay on the S3 and S3e Systems by analyzing the waste stream while running calibration particles. Events are detected normally at the nozzle and then again in the waste stream below. The drop delay setting is adjusted automatically through a set range, first coarsely then finely, while sorting is enabled. The correct drop delay value is determined when the calibration particles are deflected from the center stream and therefore no longer present in the waste stream. This technology allows accurate measurement of the drop delay value without requiring the user to count beads under a microscope.

While the ProDrop technology is running, the Drop Delay Alignment Status window will open. The yellow line indicates the best drop delay during the process and this value will be set as the drop delay once completed. The green points, light green for coarse adjustments and dark green for fine adjustments, indicate the number of beads detected in the waste stream. After completion, three buttons will appear in the lower right corner. These buttons, from left to right, recalculate drop delay, run a verify drop delay test, or confirm and close the window (Figure 40).
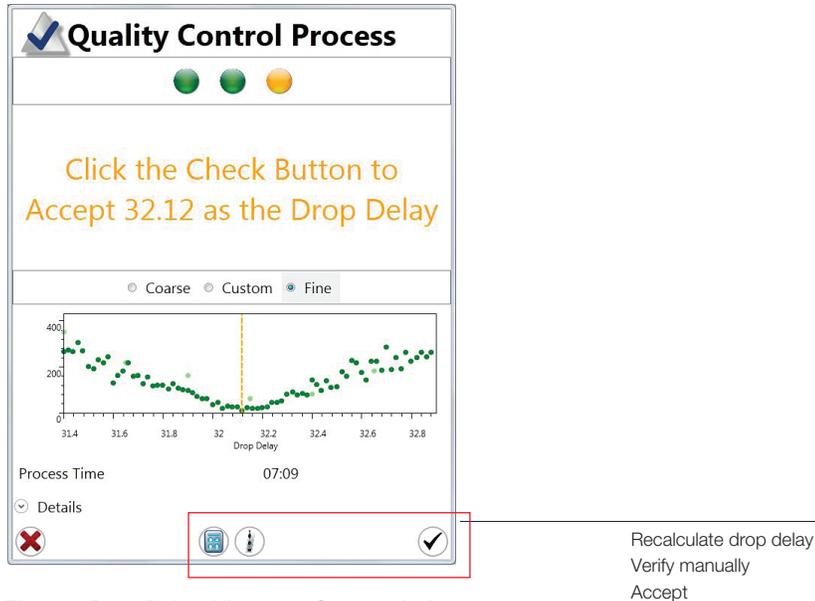
Fig. 40. Drop Delay Alignment Status window.

4.4.5 Drop Delay Maintenance

After drop delay determination, the system will be automatically placed into a droplet monitor mode to maintain the break-off during use. The changes required for this are completed automatically. To verify that the drop delay is being maintained, use the Droplet Monitor button on the tool bar. This window will note if the system is maintaining (Figure 41).

Fig. 41. Droplet Monitor window with status and recalculate button highlighted.

4.4.6 Recalculate Drop Delay

To recalculate the drop delay using ProLine calibration beads, click the **Recalculate** button (Figures 40 and 41). This button will not align the streams or verify CVs. This button will only calculate the drop delay.

During the drop delay calculation in the QC procedure, the same recalculate button on the Drop Delay Alignment Status window will be available in the Drop Monitor window. During the QC procedure, recalculation can be performed until values are acceptable, prior to clicking the checkmark box.

4.4.7 QC Criteria

Data collected during the QC procedure are checked against the QC criteria after the system has been aligned. Users with administrator privileges may access and modify these settings in the Administrator tab. For more information, refer to Section 3.3, Administrator Tab Toolbar.

⚠️ **CAUTION!** Adjusting any of these values will affect the overall system performance.

For each fluorescent parameter, two criteria may be set:

- **PMT voltage** — the PMT voltage required to bring the population to **channel** 128 must be below the set point for each parameter
- **Coefficient of variation (CV)** — the CV is measured in each parameter and must be under the set point

🛑 **IMPORTANT!** These values may need to be changed to account for lot-to-lot variation of the ProLine calibration beads.

4.4.8 QC Report

A QC Report window will open after completing the alignment and drop delay settings. This window displays the QC status and whether the system has passed or failed the QC requirements. This report can be saved or printed (Figure 42).

To view a previous QC Report, on the Setup and Maintenance tab select **QC Report** > **Date**.

Print report
Open FCS file
Save data file

Fig. 42. QC Report window with QC status highlighted.

4.5 Protocols and Workspace

Before acquiring samples, a protocol with at least one histogram or density plot is required to visualize the events. The last used protocol will automatically open with the software. A protocol consists of histograms, gates, regions, trigger, threshold, PMT settings, sort logic, compensation settings, and all other information required for a run. Protocols can be used as a template for future runs.

To add a new protocol:

1. Click **File**.

2. Select **New Protocol** from the dropdown menu. A new protocol will open as a new tab in the workspace.

3. From the Home tab, add a histogram or density plot to your workspace. Plots will automatically open in the workspace in the order they were added.

4. Click on a plot to activate the window. A blue frame will appear indicating items within the window can be changed.

5. Change the parameters; resize window; add regions, bars or quadrants; apply gates; or show statistics within this window.

6. Repeat for other plots.

To close a protocol, use the X symbol alongside the Protocol tab (Figure 43).

Fig. 43. Creating new protocols.

A protocol can be saved using the File dropdown menu. Protocols that have not been saved are marked with a dot in the title. After a protocol is prepared, samples can be acquired using the protocol or an existing FCS file can be embedded into the current protocol. Refer to Section 4.5.4, Opening FCS Files, to view existing data files.

4.5.1 Opening Protocols

Protocols can be opened from the File menu (Figure 44). When selected, the Open Protocol option will bring up a Windows prompt to locate and open the desired protocol. To the right of the file name field is a dropdown box for protocol or FCS file. By selecting the FCS file option, a protocol can be opened directly from a saved FCS file.
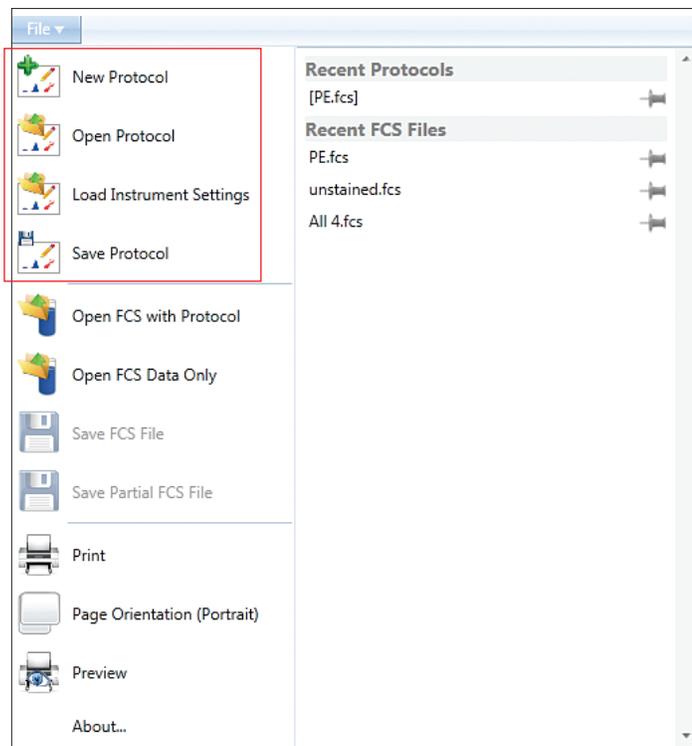
Fig. 44. File dropdown menu with New, Open, Load Instrument Settings, and Save Protocol highlighted.

4.5.2 Loading Instrument Settings

Protocols can be saved for future use. Each protocol is saved with the following information: plots, regions, gates, trigger, threshold, PMT voltages, sort logic, sort limits, sort modes, compensation settings, and filter configuration.

To load a previously saved instrument setting:

1. Open the protocol to be used or create a new protocol and save.
2. Click **File > Load Instrument Settings**.
3. Choose the protocol that contains the settings to be loaded.

The preexisting settings will be loaded into and displayed in the current protocol and will be ready to use for acquisition or sorting.

4.5.3 Saving Protocols

Protocols can be saved for future use. Each protocol is saved with the following information: plots, regions, gates, trigger, threshold, PMT voltages, sort logic, sort limits, sort modes, compensation settings, and filter configuration.

To save a protocol (Figure 44), click **File** > **Save Protocol**.

Refer to Section 3.3.8, Global Preferences, for information on how to assign default folders for saving both protocols and FCS files.

4.5.4 Displaying Multiple Protocols

Multiple protocols can be opened at the same time by opening an existing protocol or a new protocol from the file menu. When multiple protocols are opened, they will be displayed as tabs along the top of the workspace (Figure 45). Selecting between each tab will update instrument settings with the information contained in each protocol.
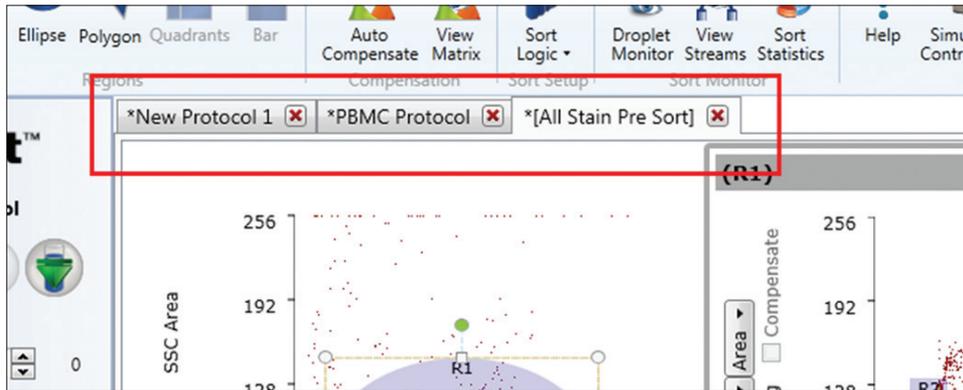
Fig. 45. Workspace with protocol tab highlighted.

4.5.5 Opening FCS Files

The ProSort Software allows an FCS file to open into two different types of protocols (Figure 46). The data can be embedded into the currently opened protocol by using the **Open FCS Data Only** option. The FCS file can also be opened into the original protocol in which it was acquired by using the **Open FCS with Protocol** option. The existing gates and plots associated with that protocol will be recalled to the workspace.
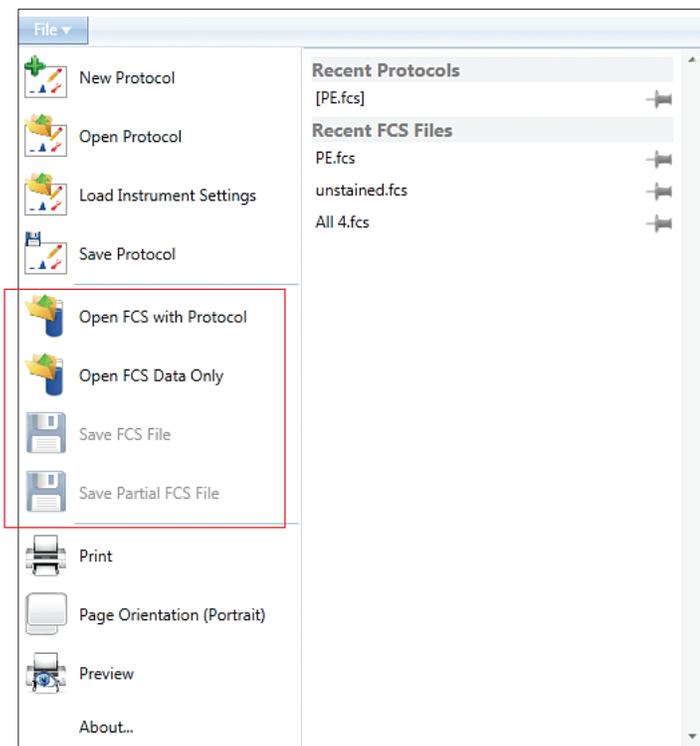
Fig. 46. Opening and saving FCS files.

4.5.6 Saving FCS Files

When the AutoSave box is not checked, the FCS file can be saved after acquisition or sort. The protocol used during the acquisition or sort is embedded in the FCS file. The file can be opened at a later time for analysis or to be used as a template for a subsequent acquisition.

4.5.7 Saving Partial FCS Files

There may be instances when a large number of events will be acquired during a sort. These FCS files can become very large and potentially can cause the software to run more slowly when reopening for analysis. In addition, it may be difficult to view populations with large numbers of events displayed at one time. It is possible to save a partial FCS file during a sort by saving only a set number of events (Figure 47). Click **File** > **Save Partial FCS File**. A window will appear to select how many events to save. The selected number of events will be saved from the end of the sort. Sort statistics from the entire sort will be saved regardless of the partial of the FCS data saved.

For example, if 500,000 events are sorted and 20,000 events are saved as a partial FCS file, then the last 20,000 events sorted (480,001–500,000) will be saved to the file.
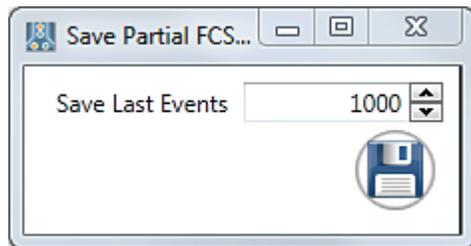
Fig. 47. Save Partial FCS File Events window.

4.5.8 Histograms and Density Plots

Histograms and density plots can be created from the Home tab. Clicking on each button will create the plot and add it to the protocol workspace. Each plot will be added to the right of the prior plot and will wrap to the next row as needed. Plots will be created with the default parameters of FSC for histograms, and FSC and SSC for density plots. Refer to Section 3.3.8, Global Preferences, for information on how to change plot defaults for axis, log vs. linear, plot size, and plot display options.

4.5.9 Resizing, Moving, and Deleting Plots

When plots are added to the workspace they are tiled automatically based on the size of the screen.

To resize histograms or density plots:

1. Hover over the desired plot.
2. Hold down the handle in the lower right corner and drag the plot to the desired size.

Plots can be moved within the workspace by grabbing the bar at the top and dragging to the desired position. The remaining plots will shift accordingly. To delete a plot, click the red X in the upper right corner. This will delete the selected plot (Figure 48).
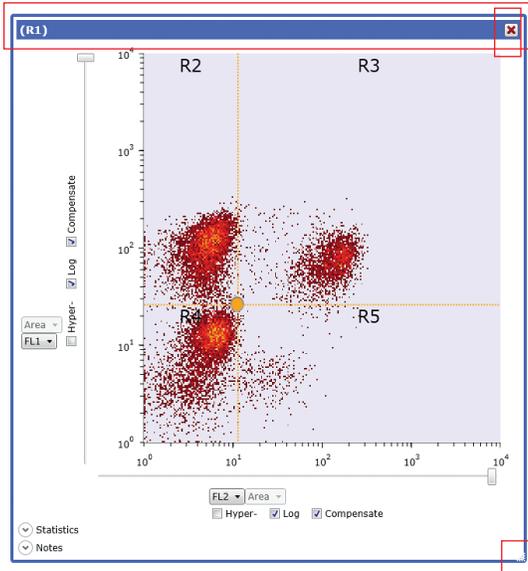
Fig. 48. Density plot with resizing, moving, and deleting functions highlighted.

4.5.10 Changing Parameters

To change the parameters displayed in a plot, click the dropdown arrow and select a parameter (Figure 49). If a log or compensated parameter is desired, check the appropriate box.
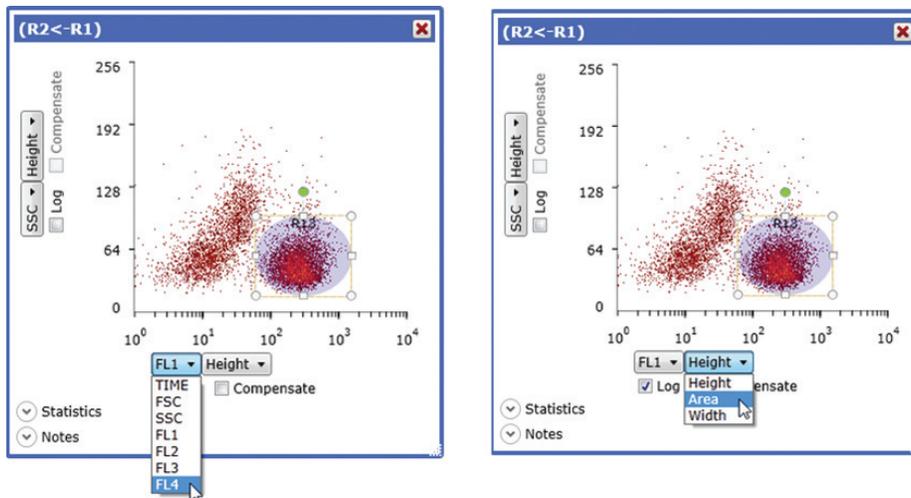
Fig. 49. Density plot with parameters displayed.

4.5.11 Viewing Statistics

To view the statistics on a plot, highlight the plot and select the **Statistics** dropdown arrow in the lower left corner (Figure 50). To hide the statistics, click the same button.
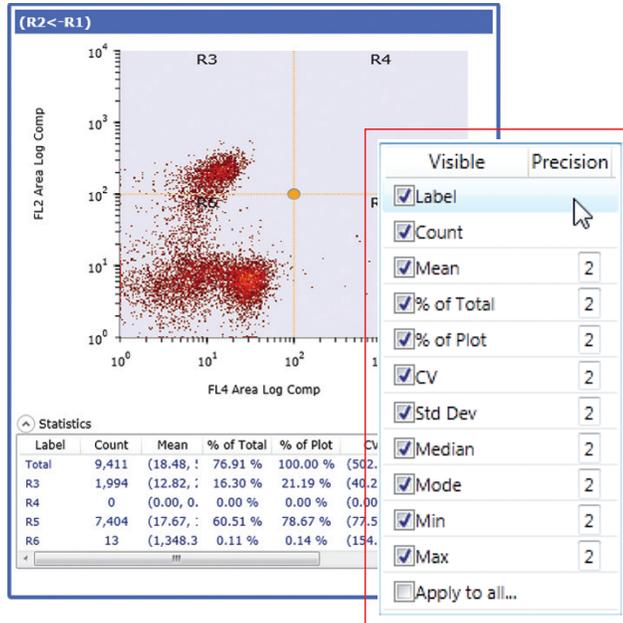
Fig. 50. Density plot with statistics.

4.5.12 Editing and Reordering Statistics

There are several options for displaying statistics. Changes in the settings will be saved with the protocol (Figure 50). Refer to Section 3.3.8, Global Preferences, for information on how to change the default statistics.

To remove statistic headings:

1. Right click in the Statistics panel.

2. Check or uncheck statistics to display in the statistics table.

3. Check **Apply to all** to apply to all plots in the protocol.

To reorder statistic headings:

1. Click the statistic name in the Statistics title bar.

2. Drag and drop to the desired location.

or

1. Right click on the Statistics panel.

2. Click on the statistic name and drag and drop to the new location.

3. Check **Apply to all** to apply to all plots in the protocol.

To alter number of digits after the decimal:

1. Right click in the Statistics panel.

2. Find statistic of interest and change the value in the Precision box.

3. Check **Apply to all** to apply to all plots in the protocol.

4.5.13 Creating Regions

Regions can be created in a histogram or density plot to further identify populations of interest. A bar region is the only option on a histogram and all other regions will be unavailable. Density plots may have ellipses, rectangles, quadrant, or polygon regions. There are two ways to create a region on the plot — by using the Home tool bar or by right clicking on the graph.

To create a region using the Home toolbar:

1.   Click on the desired plot.

2.   Select the region from the Home toolbar.

To create a region using the right click on the mouse:

1. Click on the desired plot.

2. Right click within the plot and choose a region type (Figure 51).

The selected region will appear in the desired plot. Region buttons will be grayed out if the incorrect plot is selected.
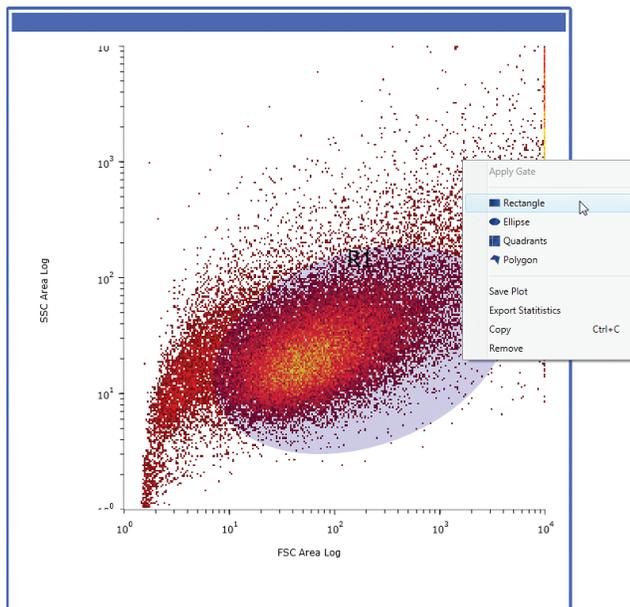
Fig. 51. Right-click option for creating regions.

A region can be moved, tilted, resized, and deleted (Figure 52).

1. Click the desired region.

2. To move region, hover till the crosshairs icon appears. Click and drag to new location.

3. To tilt, click the green circle indicator above the region and drag to change the angle.

4. To resize, click the circle or square indicators around the region and drag to the appropriate size.

5. To delete, right click within the region and choose **Remove region**.
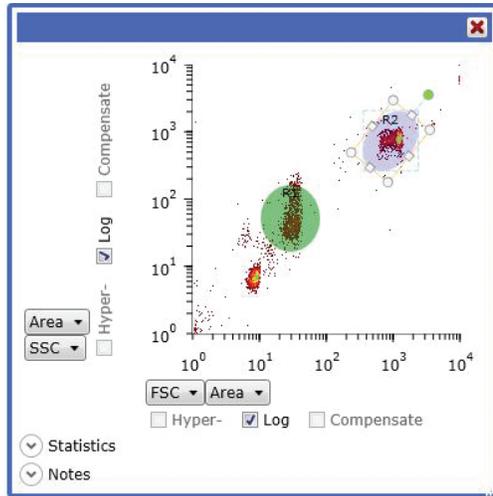
Fig. 52. Moving or tilting a region within a plot.

4.5.14 Creating Gates

Gates can be used between plots once regions have been created. The purpose of gating is to limit the events shown in one plot by a region in another plot.

To create a gate (Figure 53):

1. Select the plot to be gated.

2. Right click within the plot.

3. Select **Apply Gate** to the region or regions of interest.

The regions on the gated plot will appear in the title bar of the plot. To ungate a plot, right click in the gated plot and select **Clear Gate**.
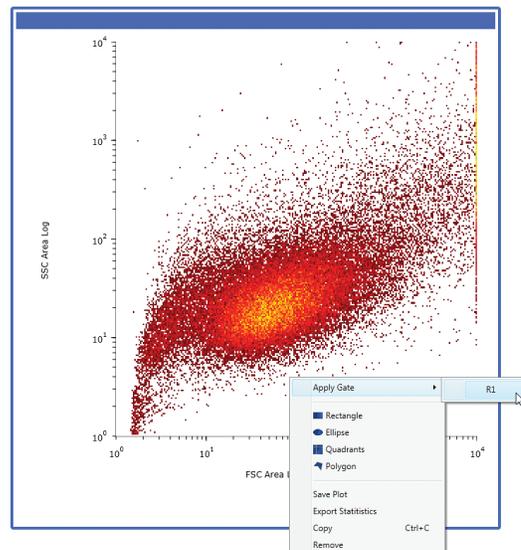
Fig. 53. Density plot with Apply Gate selected.

5 Acquisition

Sample acquisition can be done independent of sorting. As a sample is run through the system, data are gathered and can then be saved in FCS 3.1 format. These files can be loaded and analyzed using third party software compatible with FCS 3.1 files. Often, setup samples — for example, unstained samples or single-color compensation samples — are acquired prior to sorting the actual sample of interest.

5.1 Acquisition Setup

Prior to running a sample, a protocol must be loaded or created. At least one histogram or density plot is required to begin acquisition. Protocols can be created new or loaded using a protocol file or from a previously acquired FCS file. Refer to Section 4.5, Protocols and Workspace, for information on how to create or open protocols. Access all instrument controls via the ProSort™ instrument control panel.

To set up an acquisition or sort:

1. Create or open a protocol.

Note: At least one histogram or dot plot must be created to continue.

2. Load the sample onto the sample input position and move the loading stage into the run position.

3. Detectors can be renamed for each parameter based on fluorophores or markers used.

4. Check cycle mode to allow refresh of the data displayed.

5. Define target event rate, trigger parameter, and threshold value.

6. From the instrument control panel, click the **Start Sample Acquisition** button.

7. Adjust PMT voltages to place populations.

8. Adjust target event rate, the trigger parameter, and threshold value.

9. Stop sample acquisition by pressing the same button.

10. Check and define event limit. **Gate Limit** can be chosen. Cycle mode can be unchecked at this time.

Note: Data can be displayed in hyperlog if the compensation box is checked.

Optional: AutoSave can be enabled to automatically prompt to save file after event limit is reached.

11. Re-collect sample and save FCS file if AutoSave is not enabled.

12. Unlock and remove sample tube. Move the loading stage into the wash position before the next sample is run.

Note: Cycle mode can be enabled or the refresh button can be used to refresh the data displayed on the screen as adjustments are being made.

When running multicolored experiments, compensation is necessary. In order to use the Auto Compensation wizard, single stained compensation control samples must be saved in addition to the multicolored stained samples.

13. Run single stained compensation control for first parameter.
14. Set event limit high enough to visualize positive population; collect and save file.
15. Repeat and collect samples for each parameter.
16. Use the Auto Compensation wizard to set compensation for multicolored samples.

Refer to Section 5.2.1, Auto Compensation Wizard, for instructions on compensation methods.

Depending on the fluorophores used in the experiment, filters may need to be optimized in order to capture signal properly. Please refer to Section 5.4, Optical Filters, for information on how to create custom filter configurations.

Refer to Section 3.2, Control Panel, for an overview of the instrument control functions.

5.1.1 Target Event Rate

The event rate is related to the sample concentration. Generally, for every 1,000,000 cells per ml sample concentration, the event rate may be increased by 1,000 events per second. As an example, if the sample is concentrated to 10,000,000 cells per ml, the maximum event rate should be 10,000 events per second.

The target event rate is dependent on the trigger and threshold settings. A target event rate can be entered into the event rate box in the instrument control panel (Figure 54). The software will maintain the entered rate throughout the acquisition or sort if the sample concentration is appropriate for the target event rate. The actual target event rate is shown to the right of the target event rate and on the status bar at the bottom of the software screen. The maximum target event rate while acquiring is 100,000 events per second.

The button for preset sample event rate can also be used. The sample pressure is preset for low/med/high rates. The sample rate will be dependent upon the concentration of the sample.

Fig. 54. Target event rate.

5.1.2 Event Limit

An event limit may be set on the acquisition (Figure 55). This limit will be based on the total events collected. The gate default is **All**. When this event limit is reached, the acquisition will stop and the FCS file can be saved. The maximum number of events acquired in a single FCS file is 100,000,000.

Note: Large FCS files may take time to save, load, and display.

Fig. 55. Event limit and cycle mode.

A gate limit can be used to define the event limit. Each gate created on the plots will be listed on the dropdown menu. Once a gate is chosen, the acquisition will continue until the event limit is reached within the gate.

5.1.3 Cycle Mode

At the beginning of an acquisition, it may be helpful to utilize the cycle mode for a quicker refresh of the data displayed while changing the settings (Figure 55). Cycle mode is set in units of seconds and will progressively display events that fall within that period of time. Older data will be not be displayed.

Use this function when adjusting PMT voltages and/or threshold and trigger settings. After data and populations of interest appear where desired on your plots, disable this mode and restart acquisition for normal cumulative acquisition.

5.1.4 Trigger

The trigger parameter is the initial parameter of detection to signal the system that a particle of interest is present. Combined with the threshold setting, the trigger parameter defines real events which should be detected and analyzed (Figure 56). Typically, a scatter parameter is selected for the trigger, as it will identify all particles above a given size regardless of the fluorescent signal, though any parameter can be used for this function.

Fig. 56. Target event rate and threshold level.

5.1.5 Threshold

The threshold level is set using the trigger parameter with a range of 0.1–100% (Figure 56). The purpose of the threshold is to set an intensity level to eliminate any background noise and debris while still allowing real events to be detected and analyzed.

5.1.6 PMT Voltages

Each parameter is collected by a photo multiplier tube (PMT). Independent voltage control is available for each PMT. These voltages can be adjusted to bring the populations of interest on the histogram or density plot scale (Figure 57). Custom names for each parameter can be entered by clicking on the parameter of interest on the PMT control panel. Changing this name will update the names on all plot axes, in the filter configuration window, and on the compensation matrix. Refer to Section 3.3.8, Global Preferences, for information on how to set default parameter names.
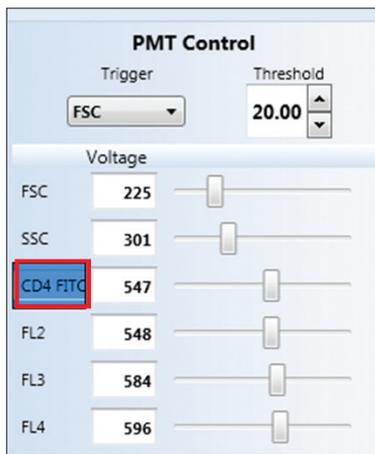
Fig. 57. PMT control panel with name changed on the FL1 parameter.

5.1.7 Auto Save

An auto save option is available as a check box on the instrument control panel. If this box is checked (Figure 58), a prompt will appear to name and save the FCS file after an acquisition or sort is completed.
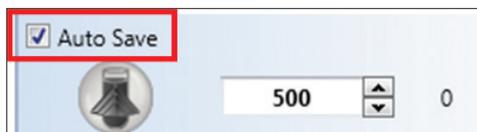
Fig. 58. Checked auto save box.

5.1.8 Pausing and Resuming

Acquisition may be paused and resumed while in progress using buttons on the instrument control panel. Pausing will stop the sample flow. During the pause, the time parameter within the data file will continue, and will show a time gap when resumed.

If you wish to replace the sample tube during the pause:

1. Press the touch locking system screen above the loading stage until the padlock is displayed as unlocked.
2. Move the loading stage into the wash position.
3. Replace the sample.
4. Move the loading stage into the run position.

To resume, click the **Resume** button on the instrument control panel.

> **IMPORTANT!** If the loading stage is not placed in the wash position after a sample is removed and before a new one is loaded, the system will not provide a pressure boost. Without washing the sample line, contamination in the new sample may occur.

5.1.9 Stopping and Saving

When the desired data have been collected, acquisition can be stopped and the data saved. The data are saved in FCS 3.1 format and will include the protocol used for acquisition. The stop button will depressurize and unlock the sample. Before running another sample, place the loading stage in the wash position to backflush and clean the sample line.

5.1.10 Backflushing and Washing

After the sample is unlocked, the loading stage can be moved to the wash position. The wash process includes a backflush and an exterior wash of the sample line. This process is crucial to reducing carryover and for overall cleanliness of the system. After washing, the loading stage will automatically unlock, and the next sample may be placed into the run position.

If an additional wash is desired, you have two options:

- Lower the loading stage for >2 sec then bring it back to the wash position. An additional wash cycle will run after returning to the wash position

- Press the touch locking system screen above the loading stage until the padlock displays as locked. This will initiate an additional wash

5.2 Compensation

Compensation is the process by which spectral overlap (spillover) is removed from other detectors. The inherent overlap of emission spectra from fluorescent labels and probes makes compensation necessary. This is of particular importance when simultaneously measuring fluorescence from several labeled antibodies or probes. During acquisition, compensation can be adjusted to eliminate this spectral overlap between fluorophores (Figure 59).

The density plot shown displays an FL1 control data file (Figure 59). The FL1 positive population is higher on the FL2 scale than the negative population, though it has no FL2 fluorescence. When compensation has been applied, the positive FL1 population has the same median value as the FL1 negative population in the FL2 parameter.
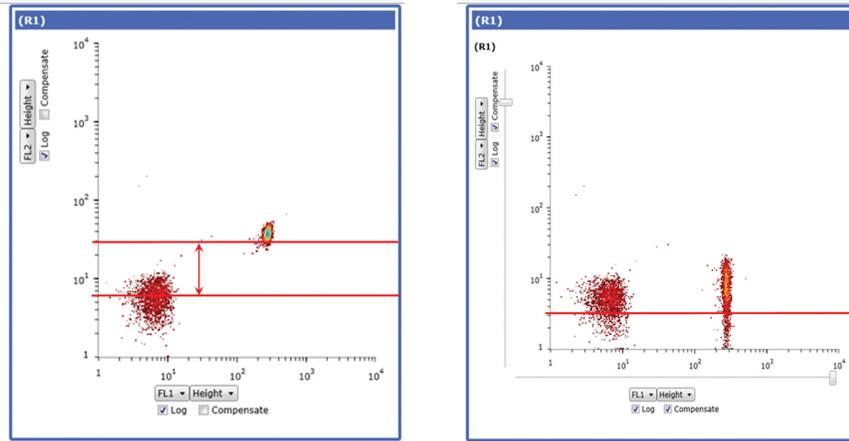
Fig. 59. Density plot showing uncompensated (left) and compensated data (right).

The same can be done for the FL2 positive control, though the compensation required is much less (Figure 60).
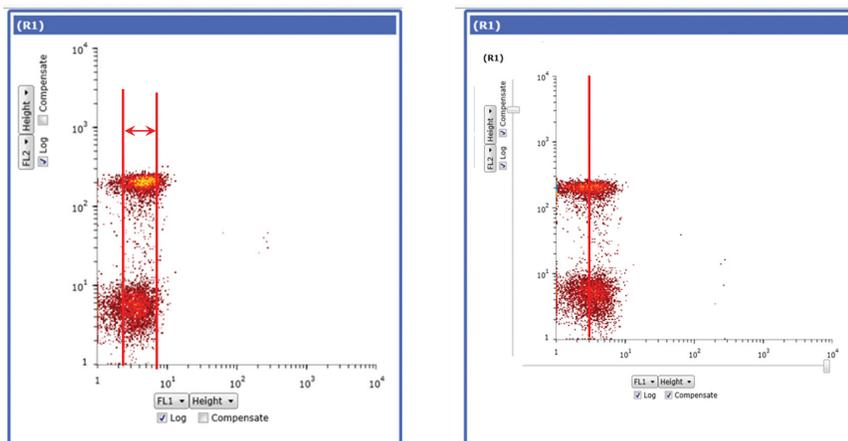
Fig. 60. Same data plots showing incorrectly compensated file (left) and correctly compensated file (right).

As compensation is adjusted, the compensation matrix will be updated. At any time the compensation matrix can be viewed using the button on the toolbar (Figure 61).
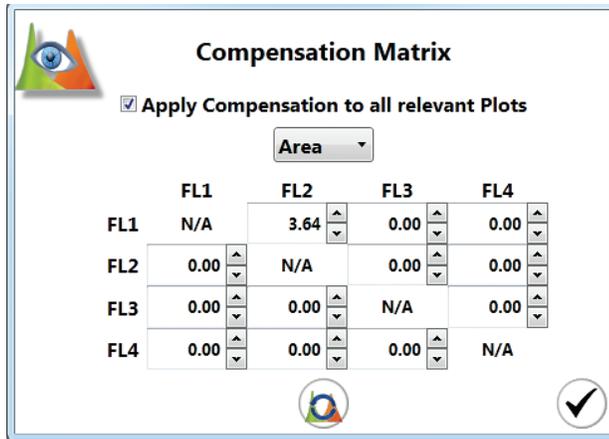
Fig. 61. Compensation matrix.

5.2.1 Auto Compensation Wizard

The compensation wizard assists with establishing the proper compensation matrix coefficient for each parameter. Single controls for each parameter and the associated FCS files must be saved prior to compensation.

To use the compensation wizard:

1. **Run single controls** for each parameter and your multi-stained sample.
2. Save FCS files.
3. Select **Home** > **Compensation Wizard**.
4. Select the parameters for compensation (Figure 62).
5. Click the file icon for each selected parameter (Figure 62).
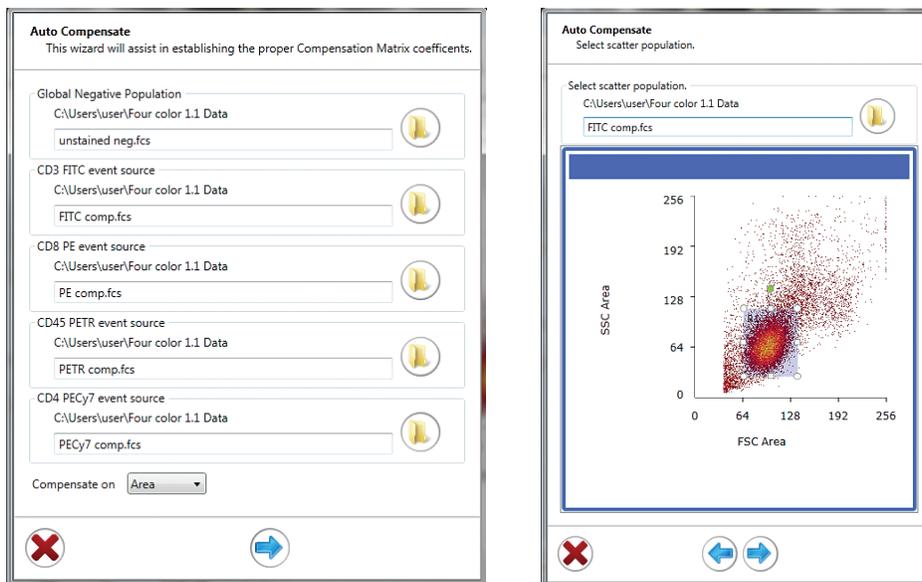
Fig. 62. Use the auto compensation wizard to select individual parameter FCS files and adjust regions.

6. Locate and select the single control FCS file for each parameter.

7. Select whether to use height or area for all parameters.

8. Click the forward blue arrow to open the Automatic Compensation Calculation Wizard window (Figure 63).

9. Adjust the scatter, negative, and positive regions as necessary.

Note: The positive and negative population gates must not overlap.
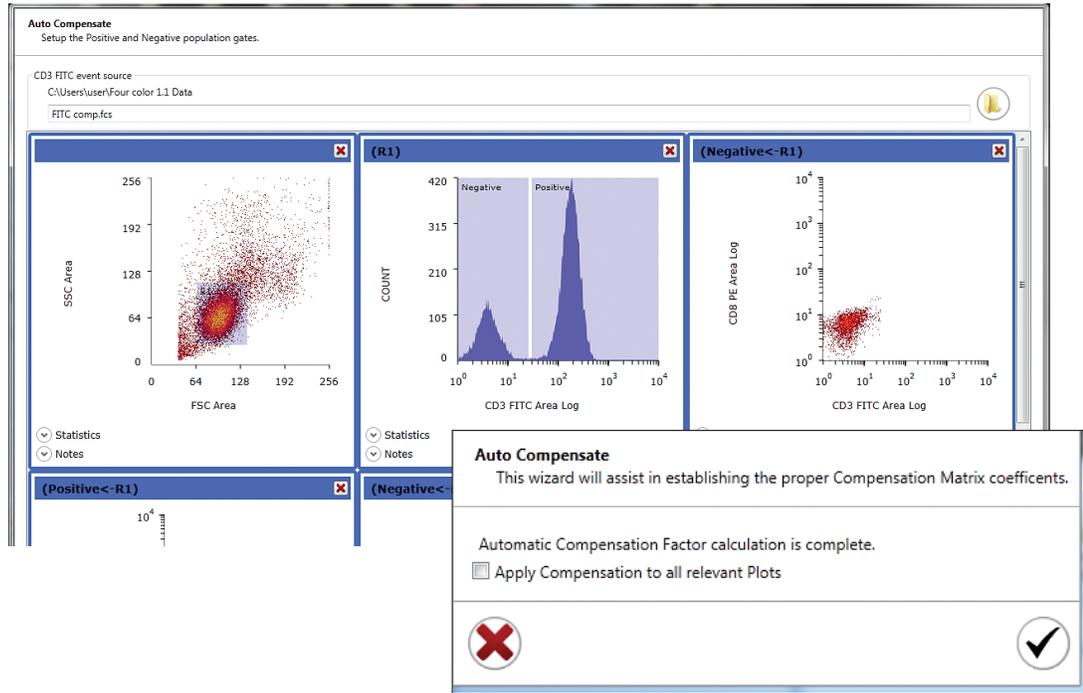
Fig. 63. Using the auto compensation wizard to adjust positive and negative gates.

10. Click the blue forward arrow to go to the next control.

11. Repeat steps 9 and 10 for remaining controls.

12. After all controls are adjusted, click the checkmark button. This will complete the automatic compensation calculation.

13. Check the compensation box on the axis of the desired histogram or density plot to display the compensated data and acquire or sort.

14. The compensation matrix may be viewed at this point if desired.

The hyperlog feature allows data that have values <0 to be correctly displayed. This data display format is used only for compensated data (Figure 64). It converts a small portion of the scale around zero to linear, and adds a small amount of negative log scaling to each access, preventing negative events from piling up on the axis. This allows better visualization of compensated data.

Note: The area parameter is grayed out and cannot be changed because compensate is checked. Compensation can only be applied to area or height depending on which selection was chosen during auto compensation.
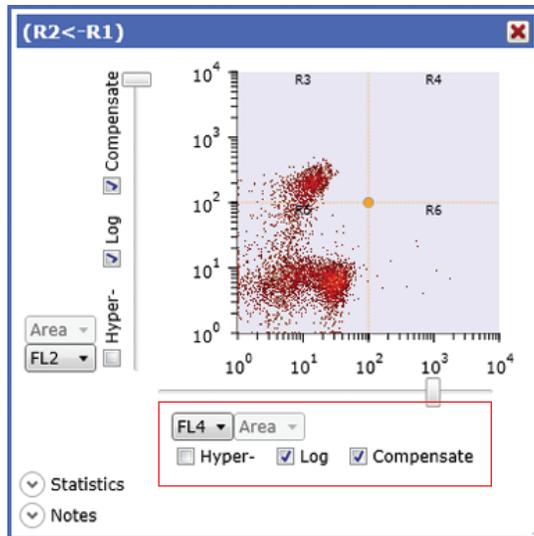
Fig. 64. Density plot showing hyperlog, log, and compensation options.

5.2.2 Manual Compensation

It is advised to use the auto compensation feature of the software, however it is possible to alter the compensation matrix manually (Figure 65). This process, although historically used, is difficult to set correctly and the process of compensation should only be performed using the wizard unless knowledgeable and statistically correct adjustments can be done by manual compensation. Compensation controls for each fluorochrome you will be using in your experiment. The process is most accurate if you have both positive and negative events in each sample, and it is advisable to acquire at least 5,000 positive events. After the sample is acquired, load each sample one by one into the software, setting the histogram axes to the parameters you would like to adjust, and checking the Comp box on the histogram axes. By applying quadrants it is possible to attempt to match the median of the negative population to the positive population (Figure 64). When the value in the matrix is changed, the populations will move. Alter until the median values are correct and matched.

Fig. 65. Compensation matrix.

5.3 Checking or Swapping Fluidics

The bulk fluidic containers are located inside the system and can be accessed by the fluidics door on the left front of the instrument. These fluidic containers require changing on a regular basis. As recommended in Chapter 4, Getting Started, fluidic containers should be changed prior to initiating the software or after shutting down the system. If the fluidic containers need to be swapped after startup or during a run, the S3™ and S3e™ Cell Sorters are designed to easily allow this without the need to shut down the system. This is considered as a hot swap of the fluidics.

The fluidics status indicator on the instrument control panel provides information regarding the levels and approximate run times remaining for these containers (Figure 66). This window will pop up when the approximate run time is down to an hour and will continue to alert every 15 min until a swap is performed. A hot swap can be done when the limiting fluid is highlighted. The alert may be snoozed at this point if desired.

WARNING! When the remaining run time is down to 5 min, the system will automatically shut down. The fluidics will need to be refilled and the startup procedure must be performed to ensure the lines contain no air and the system is properly QC'd.
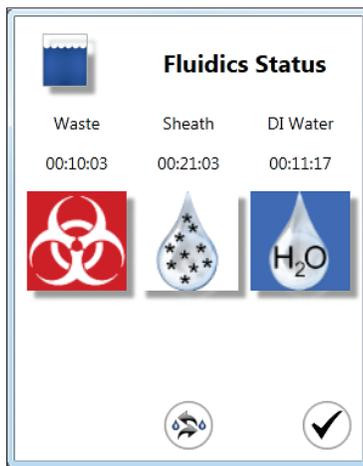
Fig. 66. Fluidics Status window.

5.3.1 Hot Swap

A hot swap is defined as the process of changing a fluidic container after initial instrument startup or during a run without the need to shut down the instrument. Unlike other cell sorters, the S3 and S3e Systems maintain the fluidics pressure internally to enable a hot swap for uninterrupted sort collection and eliminates the need for additional repressurization time.

IMPORTANT! New containers should be prepared before beginning a hot swap, as the swap time is limited to three min before the system will automatically shut down due to low fluid levels.

To perform a hot swap:

1. On the Setup and Maintenance tab select **Swap Fluidics**.
2. A countdown timer will automatically start. This is the amount of time left to swap the containers before the system will automatically shut down (Figure 67).

WARNING! The system contains a limited amount of reserve fluids to continue running the system during the swap. If the timer reaches zero, the system will automatically shut down. Work quickly and carefully during these swapping steps. If the system shuts down, perform the QC procedure prior to running a sample.
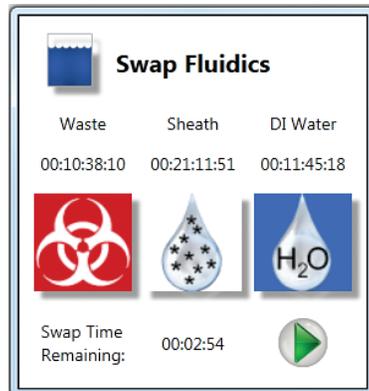
Fig. 67. Swap Fluidics window with swap time remaining highlighted.

3. Open fluidics door.
4. Detach the quick disconnect from the cap assembly by pushing the metal button.
5. Lift the quick disconnect until it engages the magnetic holder. This will hold the quick disconnect away from exchange area.

For sheath fluid or DI water:

6. Slightly lift and pull out the empty container.
7. Remove the cap assembly from the empty container.
8. Uncap and remove the seal from a new 4 L container.
9. Carefully place the cap assembly onto the new 4 L container and tighten the cap.
10. Proceed to step 11.

For Waste:

6. Slightly lift and pull out the waste container.
7. Remove the waste cap assembly.
8. Empty the waste container following lab procedures for liquid biohazardous and chemical waste removal.
9. Place the waste cap assembly into a new designated waste container or recap emptied waste container.
10. Proceed to step 11.

Acquisition

11. Place the container back into position.
12. Attach the quick disconnect to the cap assembly. An audible click should be heard.
13. Click the play button in the Swap Fluidics window to finish the swapping procedure.

> **IMPORTANT!**
> - When handling sheath fluid and DI water containers, minimizing air exposure will help to avoid contamination
> - While transferring cap assemblies to a new container, avoid touching the assembly to outside surfaces of the containers. If it is necessary to set the cap assembly down, use only sterilized surfaces
> - Treat all waste as biohazardous for safety

5.4 Optical Filters

The optical filters separate and direct the fluorescent light to the PMTs for detection. These filters are located behind a sliding panel on the top and toward the rear of the S3 or S3e System.

5.4.1 Standard Filter Configuration

The standard filter configurations for the S3 or S3e Systems are shown in Figure 68. The filter configuration can be viewed and edited in the ProSort™ Software.

To view or edit the filter configuration:

1. On the Setup and Maintenance tab select **Filter Configuration**.
2. Click inside each area to modify the filter name or wavelength.
3. Click the checkmark button to save the changes.

WARNING! Changes to the filter configuration will be set as the default.
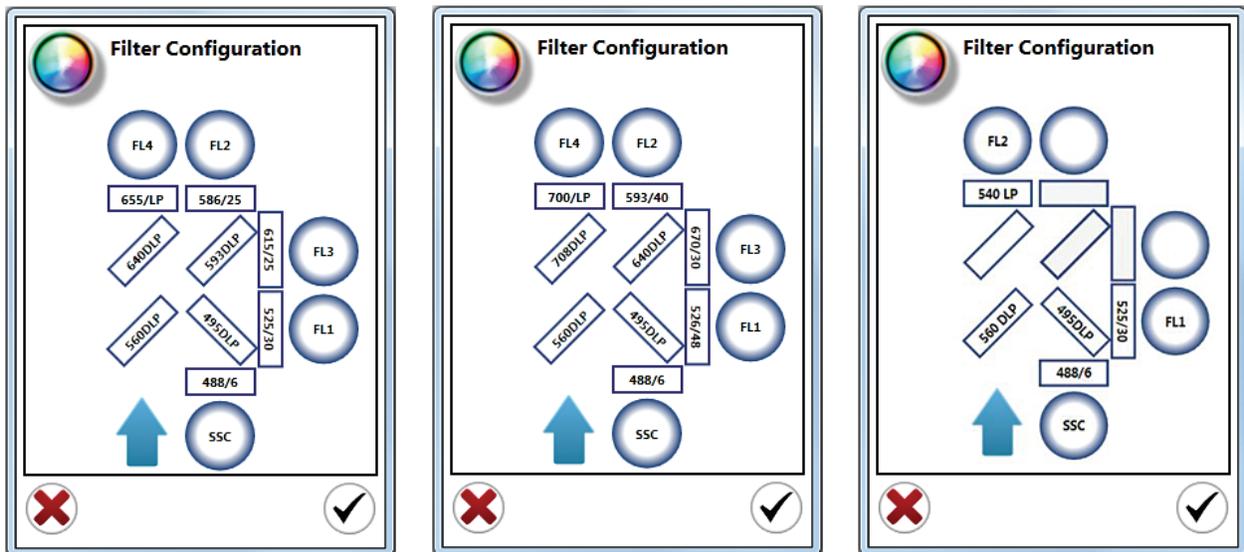
Fig. 68. Different filter configurations. A, 488/561 nm system; B, 488/640 nm system; C, 488 nm system.

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In front of each PMT is a holder for a neutral density filter or bandpass filter. Each filter holder contains a screw-in retaining ring that can be removed or replaced with the provided red S3 spanner wrench.

5.4.2 Optical Filter Blocks

The S3 and S3e Systems include two preset filter blocks. The filter set depends on the instrument configuration. The optical filter blocks can be changed to accommodate dectection of different fluorophores. These blocks can be easily removed with the silver handle (Figure 69).

Fig. 69. Lifting an optical filter block.

The filter blocks contain the dichroic and bandpass filters. The optical filters within the block are user changeable and will accommodate multiple filter sizes as described in Table 23. The dichroic filter holders are locked from the bottom of the block. Once unlocked, they can be removed and replaced from the top of the block.

Table 23. Description of filter sizes for use with optical filter blocks.

Filter	Descripiton
Normal incidence filters	25.4 mm (1 in) round, up to 2 mm thick 12.7 mm (0.5 in) round, up to 2 mm thick 11 x 11 mm square, up to 2 mm thick 12 x 25.4 mm rectangle, up to 2mm
Dichroic holders	25.4 mm (1 in) round, up to 2.7 mm thick 12 x 25.4 mm rectangle, up to 2.7 mm thick
Neutral Density (ND) holders	25.4 mm (1 in) round, up to 10 mm thick

6 Sorting

Sorting cell populations is highly dependent on several factors such as cell type, population frequency in the starting sample, cell concentration, sample preparation, and sort logic definitions. It is very important to start with a single cell suspension with minimal amount of dead cells or debris contamination.

6.1 Sort Setup

The majority of sort setup procedure is automatic and is completed during daily QC. When ready to sort, the desired populations must be selected using regions and gates. At this time sort logic definitions must be set.

To start a sort:

1. Click **File** > **New Protocol** or **Open Protocol**.
2. Click **Home** > **Histogram** or **Density** to add plots to the workspace.
3. Adjust temperature control for sample input station and sort collection area if desired.
4. Place filtered sample into the sample input station.
5. Move the loading stage into the run position.
6. Click **Start** on the instrument control panel to acquire a small amount of the sample.
7. Set sorting regions and gates on plots with populations of interest.
8. Right click on the desired region to be sorted and select sort direction.
9. Repeat with a second region if two-way sorting is desired.
10. Click the **Sort Logic** dropdown menu to select the collection vessel of choice.
11. Define the sort logic parameters in the Sort Logic window (Figure 70) by setting the sort mode (purity, enrich, or single) and event limit for each collection position.
12. Define sort collection tube volume if you want to track collected volume to prevent collection overflow.
13. Place sort collection vessel in the sort collection device at positions 1, 2, etc.

    Note: 5 ml tubes should have at least 0.5 ml media and 1.5 ml tubes should have at least 0.1 ml filtered media to collect and cushion the sorted cells.
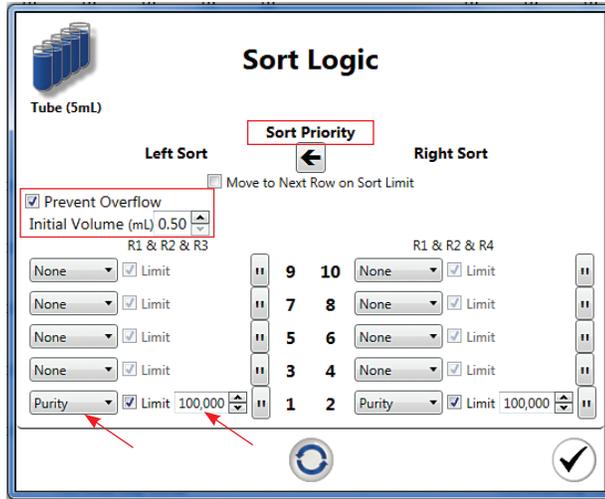
Fig. 70. Sort Logic window.

14. Close the sort chamber door.

15. Click **Start Sort** on the instrument control panel. The Sort Statistics window will open and sorting will begin (Figure 71). The Sort Statistics window can be reopened using the Sort Statistics button on the Home toolbar. Click on the arrow in the upper right corner to expand the window and show the statistics table.

16. If no limits have been set, click the same sort button to stop the sort when desired.

17. Return loading stage to the washing position.

18. Open sort chamber door and remove sorted cells.
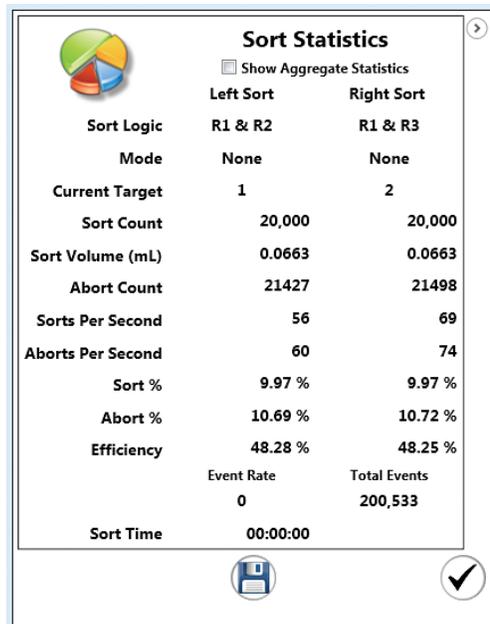
Fig. 71. Sort Statistics window.

6.1.1 Sort Collection

The S3™ and S3e™ Systems offer four types of sort collection vessel options:

- 5 ml tubes
- 1.5 ml tubes
- 8-well strips
- Microscope glass slides

Each sort direction has the capability of sorting into 5 ml tubes and 1.5 ml tubes up to a quantity of 5 tubes in each direction. Using the quick-attaching adaptors, sorted cells can be collected into 8-well strips (up to a quantity of 8) or onto a microscope slide (up to a quantity of 20). Each tube and well may have different sort modes and limits assigned.

It is highly recommended that media, buffer, or serum is added to the sort tubes or wells prior to sorting to give the cells a cushion, avoid evaporation of droplet volumes, and to help preserve cell viability during the sort collection period. The minimum recommended volume is 0.5 ml of filtered buffer or media for each 5 ml tube. The minimum starting volume for 1.5 ml tube is 0.1 ml.

Note: The sort output may be temperature controlled to the same temperature as the sample input station. This temperature can be enabled/disabled and adjusted on the instrument control panel.

6.1.2 Volume Tracker

During a sort, the system has the ability to track the volume and number of droplets deflected into each sample collection tube. Volume tracking can be enabled to automatically stop a sort or move to the next tube position before a tube overflows.

To enable this feature:

1. Click the **Prevent Overflow** checkbox in the Sort Logic window (Figure 72).
2. Enter the starting volumes for the sort collection tubes.

Note: The minimum starting volume for a 5 ml tube is 0.5 ml and the maximum is 2 ml. The minimum starting volume for 1.5 ml tube is 0.1 ml and the maximum is 0.5 ml.
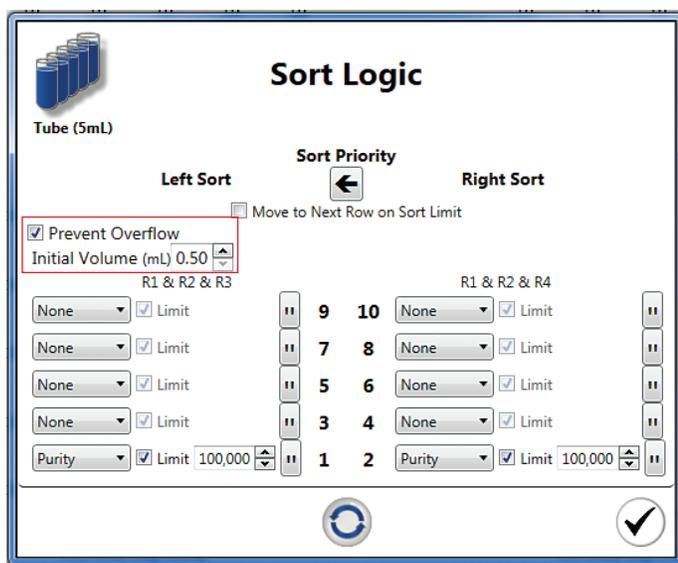
Fig. 72. Sort Logic window with Prevent Overflow highlighted.

Note: If a sort limit is selected and volume tracking enabled, whichever criterion (limit or full tube) is met first will stop the sort.

IMPORTANT! The maximum total volume for a 5 ml collection tube is considered to be 4 ml. The maximum total volume for 1.5 ml tube is 1 ml. Going above these maximum volumes will cause spraying and risk contamination.

6.1.3 Setting Regions to Sort

Regions and gates in the histograms and dot plots are used to create sort logic. Multiple regions can be used to establish the sort logic (Figure 73).

To set the sort logic:

1. Identify the population of interest with regions and gates.

2. Right click in the region to sort and select **Right Sort** or **Left Sort**. That region will change color and any regions used in the gating of that plot will change as well. Regions used in sort criteria may be confirmed in the Sort Logic window and sort statistics panel.
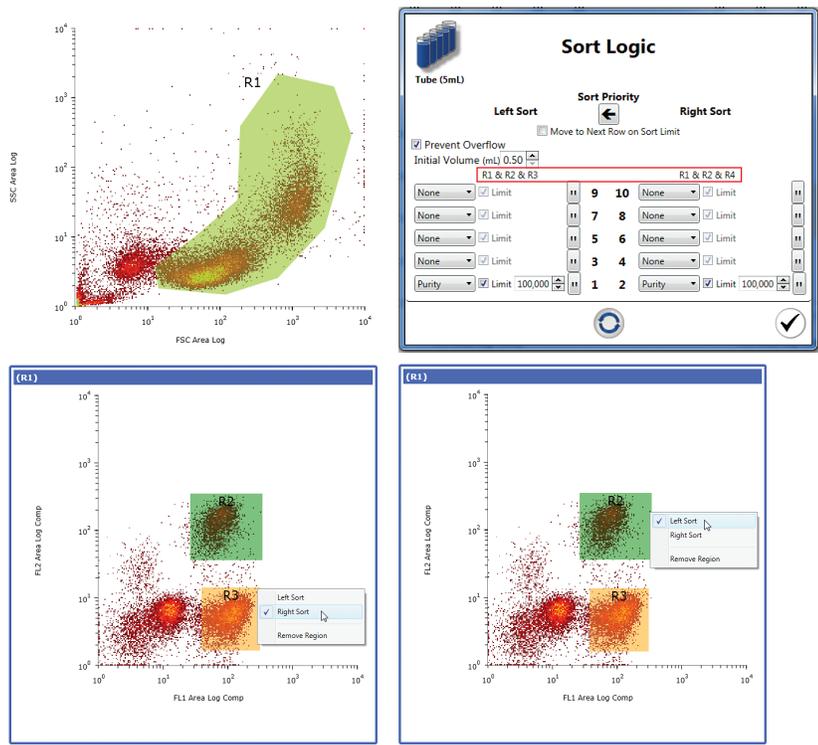
Fig. 73. Choosing sort definitions.

6.1.4 Sort Logic and Collection Vessel

After regions and gates have been assigned to sort left or right, the sort collection vessel must be selected. The Sort Logic window can be used to review sort logic, set sort modes and limits, and set priority (Figure 74). The selected collection vessel will be displayed as the icon to show the sort type selected. If there is overlap between the logic of the two streams, the left sort direction will take priority unless changed. The numbers shown in this window represent the numbers in the sort output area.
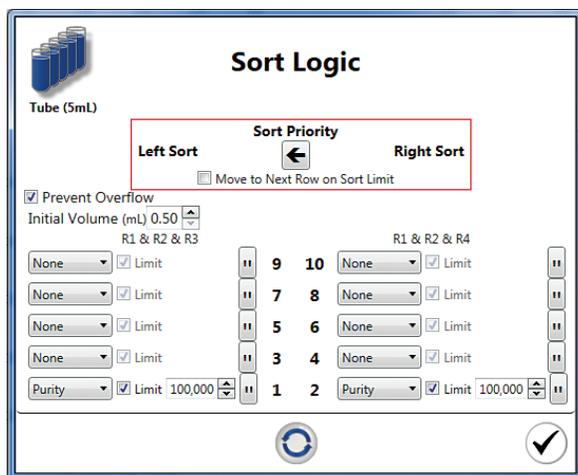
Fig. 74. Sort Logic window with sort priority and sort modes highlighted.

6.2 Sort Modes

Table 24 describes the three sort modes available when setting up a sort. Sort modes can be selected for each sort direction.

Table 24. Sort mode definitions.

Sort Mode	Definition	When to Use
Single Cell	This mode requires that one and only one positive event be in the center of the droplet without any negative events nearby. Recovery will be reduced due to the sort restrictions	Single cell deposition
Purity	This mode will sort as long as no negative events are present in the droplet. If multiple positive events are present, sorting will still occur	General sorts where both purity and recovery are important
Enrich	This mode will sort all positive events regardless if negative events are nearby. Purity will be reduced to maintain high recovery	Sorts when recovery is critical and purity is not as important

6.3 Sort Statistics

The sort statistics window can be viewed during a sort and added to a print page for reporting (Figure 75). This window will automatically open when a sort is started.

If a saved partial FCS file is opened, aggregate statistics can be shown:

1. **File** > **Open FCS File with Protocol**.
2. Click on the **Home** tab and click **Sort Statistics**.
3. Check the **Show Aggregate Statistics** box to view statistics from all sorted events.

Fig. 75. Sort Statistics window.

Table 25 describes the available sort statistics. Each statistic is sort-stream specific. The Sort Statistics window can be placed in the workspace for printing. Sort statistics are saved within the FCS file.

Table 25. Description of sort statistics.

Statistics	Definition
Sort logic	The regions used to identify the populations to sort
Mode	The sort mode used for sorting left or right
Current target	The sort target position used during the sort
Sort count	The current number of positive events sorted
Sort volume (ml)	The volume sorted into each tube/well. This does not include the media placed in the tube prior to sorting
Abort count	The current number of positive events aborted
Sorts per second	The sort count per second as a rate
Aborts per second	The abort count per second as a rate
Sort %	Number of sorted events divided by total events x 100
Abort %	Number of aborted events divided by total events x 100
Efficiency	Number of sorted events divided by the sum of sorted and aborted events x 100
Event rate	Real-time events per second during the sort
Total events	Total events acquired during the sort
Sort time	Sort time elapsed

Additional information can be shown in the Sort Statistics window by using the arrow on the upper right hand side of the window. This will expand the window to show a table listing the data for each tube during the run. There are up to ten outputs equivalent to five tubes in two directions. This information can be saved as a tab-delimited file using the save button located in the lower right corner of the window. This information is automatically stored in the FCS file.
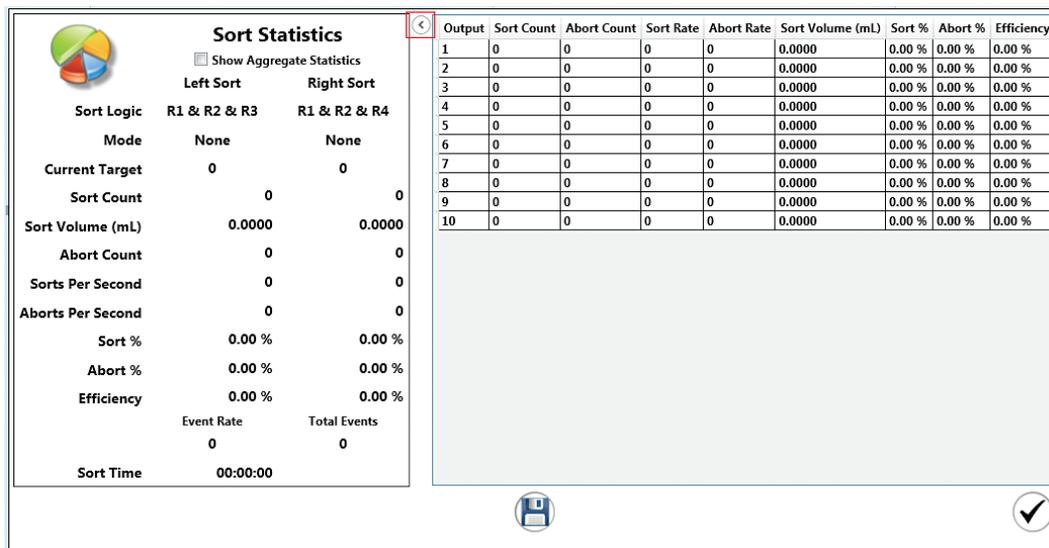
Fig. 76. Sort Statistics window expanded to reveal additional information.

6.4 Sort Plots

During a sorting experiment a sort plot can be created in the current protocol. This plot displays graphically the counts of events (sorts and aborts) per tube/well/slide spot in the sort run. The data on this sort plot (Figure 77) correspond to the Sort Statistics window (Figure 76). The numbers 1–10 are the sort position designations as assigned in the Sort Logic window (Figure 74). Regions can be applied to the sort plot. These regions can be applied to other histograms or density plots.
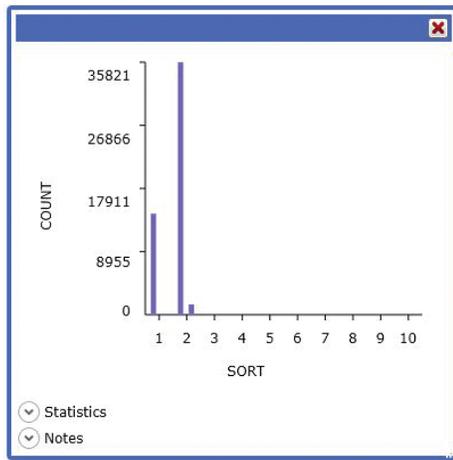
Fig. 77. Sort Plot window.

7 Additional Software Features

The ProSort™ software is an acquisition and sorting software package with the additional features described in this chapter.

7.1 Debubble

During the startup procedure, a debubble step will occur to clear the system of air. At times, a bubble may enter the nozzle and cause problems with the stream. On the Setup and Maintenance tab select **Debubble** to rinse the system and attempt to debubble the nozzle. The stream can also be monitored at this time to see any changes.

7.2 Unclog

A clog can easily be introduced and can cause multiple problems with the fluidics. When a clog occurs, the software will display an error, stop the sample, stop the sort, and eject sorted samples for protection.

Use the Unclog button on the Setup and Maintenance tab toolbar as a first-line action. The S3™ and S3e™ Systems have the ability to backflush the nozzle, potentially releasing the clog and flushing it into the waste. If successful, run a couple of debubble cycles to remove any air that may have been pulled into the nozzle body.

IMPORTANT! To avoid clogs when working with cells, resuspend cells into single cell suspension and always use a 40 µm or smaller filter prior to cell sorting.

7.3 Swap Tip

There will be occasions when the unclog function will not be able to clear a clog in the system. Use the Swap Tip button on the Setup and Maintenance tab toolbar to run the Swap Tip wizard. This wizard will walk through each step of the nozzle tip swap. Use the same Swap Tip button when nozzle adjustments are necessary or the nozzle needs cleaning.

Please refer to Section 10.4, Swap Nozzle Tip Wizard, for step-by-step instructions on swapping the nozzle tip, or contact Technical Support if any of these solutions do not resolve the problems.

7.4 Clean System

In between samples or users, cleaning can be done at low or high pressure. High pressure cleaning will cause the system to stop maintaining droplets and a QC procedure must be run before sorting. If cleaning between samples, the low pressure option is recommended. The fluidics lines will be flushed thoroughly with cleaning solution, similarly to the cleaning protocol used during the shutdown or logout procedure.

To clean the system:

1. Fill a 5 ml tube with a minimum of 3 ml of cleaning solution.
2. Load tube into the sample station and move in to run position.
3. Click the **Set Up and Maintenance** tab.
4. Click **Clean System**.

The cleaning protocol will automatically begin. A prompt will appear to warn the user that the clean function will begin and that the contents within the sample tube will be used rapidly.
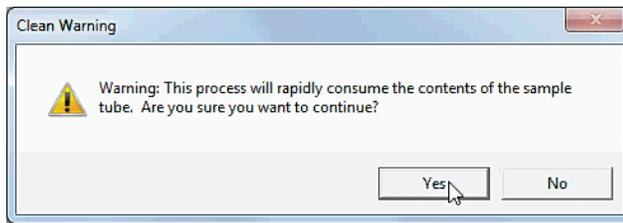
Fig. 78. Cleaning protocol warning box.

5. Select the cleaning mode to run (Figure 79).
6. Click **Yes** to proceed with mode (Figure 80).
7. Move the loading stage into wash position (Figure 81).

Fig. 79. Clean System modes.
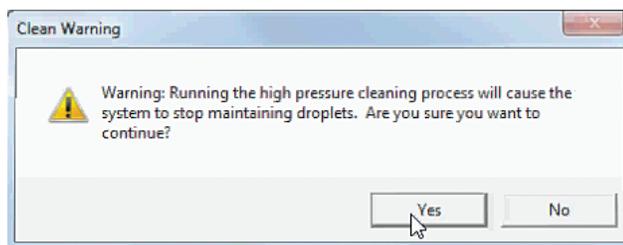
Fig. 80. Warning for high pressure cleaning protocol.

Fig. 81. Cleaning protocol final stage.

The cleaning protocol will be completed when the window disappears. If a high pressure cleaning has been chosen, run a QC using ProLine™ calibration beads before sorting.

7.5 Instrument Status Box

The instrument status box is located at the bottom of the instrument control panel. The notification and the color of the box will change depending on the state of the instrument. Table 26 describes the notifications and colors.

Table 26. Instrument Status Box notifications and colors.

Color	Notification	Action	Example
Gray	Off	Nothing	Off
Green	Ready	Can begin acquisition or sort	Acquiring sorting
Yellow	Information	Perform requested action before proceeding	Require QC Fluids Low/High
Orange	Warning	Nothing, system is running	Calibrating
Red	Error	Perform required maintenance	Not maintaining droplets

7.6 Status Bar

The status bar is located at the bottom of the ProSort Software window with nine status indicators (Figure 82). Table 27 describes the status indicators.

Fig. 82. Status bar with the status indicators numbered.

Table 27. Description of each status indicator.

Number	Description
1	Software connectivity to instrument
2	Current date
3	Temperature control status
4	Event rate (events per second)
5	Total event number
6	Acquisition time elapsed
7	Sort time elapsed
8	Current user

7.7 Printing

Print and print preview buttons are available in the File dropdown menu and on the Setup and Maintenance tab (Figure 83). Use the print preview function to move histograms and density plots in your print layout. Regions can also be modified in this view and will apply to the protocol displayed. Once arranged, click the print button to bring up the print dialog where the printer and paper may be selected. Sort statistics, filter configuration, annotations, and compensation matrix may also be printed on the page. Select the desired print options on the Setup and Maintenance tab.

Fig. 83. Printing options available.

7.8 Quality Control Reports

Two different reports are available to track the daily performance of the S3 and S3e Systems. One is the daily QC report which will show pass/fail and droplet information for the selected day (Figure 84). Past days can be viewed using the date select option. If QC has been run multiple times in a given day, select the daily QC report of interest from the Time dropdown list. To open the FCS file for the QC data shown in the report, select the **Open FCS File** button at the bottom of the report. This report may also be printed if desired.
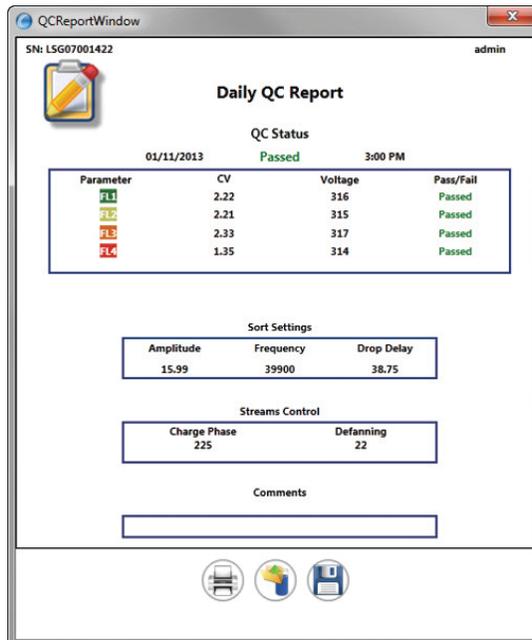
Fig. 84. Daily QC report.

The second QC report is a trending report in which a range of dates can bev viewed for PMT voltages, CVs, and drop drive amplitude and frequency (Figure 85). When viewed, comments can be added and the reports may be printed for reference.
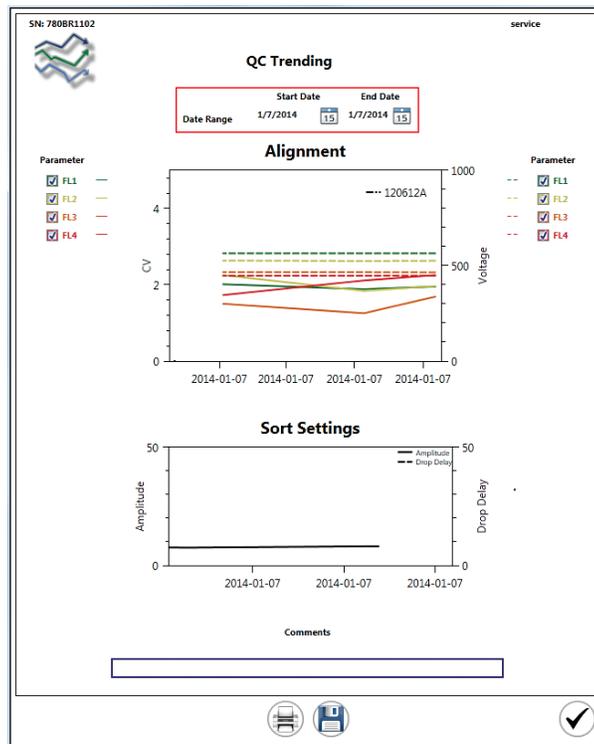

Fig. 85. QC trending report.

7.9 User Reports

User reports are accessible by administrators and can be used to track system usage over time (Figure 86). These reports can be printed for reference and will include the session notes entered when users were logged into the software.
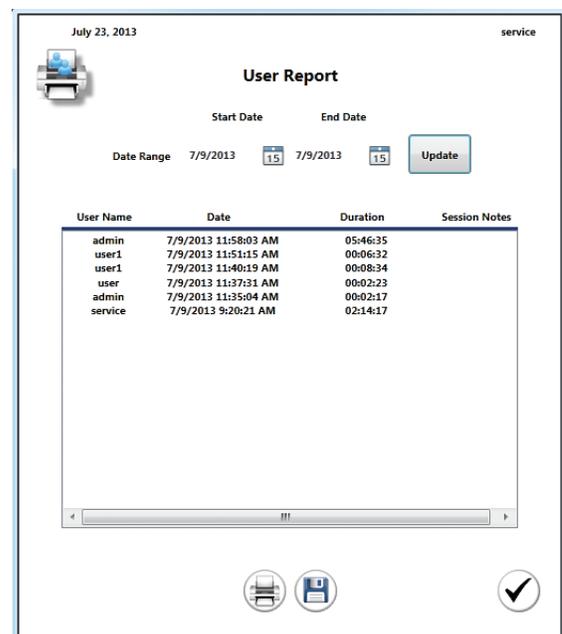
Fig. 86. User report.

7.10 Biosafety System

The S3™ Biosafety System is a Class I aerosol containment hood designed to work with the S3 and S3e Cell Sorter, which works in conjunction with ProSort™ Software, providing users and the environment protection from aerosols created during the cell sorting process.

The S3 Biosafety System is fully integrated with and monitored by the S3 and S3e Cell Sorter's ProSort Software, so users have real-time information about HEPA filter life and the temperature of the system inside. To maintain containment, the fan speed is regulated based on the cell sorter operation mode and the airflow rate through the HEPA filter.

The S3 Biosafety System functionally meets airflow requirements from the National Institutes of Health (NIH) standards for Class I biosafety and the International Society for Advancement of Cytometry (ISAC) guidelines.

For more information please see the S3 Biosafety System Class I manual.
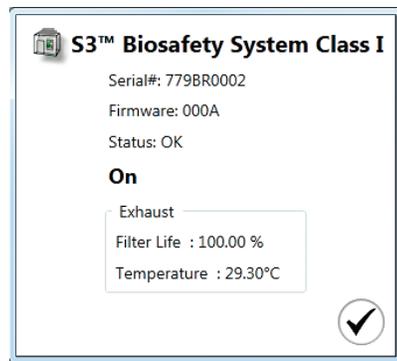
Fig. 87. S3 Biosafety System Class I status window.

IMPORTANT! The S3 Biosafety System must remain on at all times when the S3 or S3e Cell Sorter is inside the system. The cell sorter should be left with the main system power on and will enter into idle mode when not in use.

Should the S3 Biosafety System be turned off when the S3 or S3e Cell Sorter power is on inside, there could be risk of overheating and it may become a fire hazard.

If any of the following occur, the S3 and S3e Cell Sorters will stop sample acquisition or sort and alert the user:

- Airflow through HEPA filter drops below required limit
- Filter life reaches 0%
- ProSort Software loses communication with the enclosure

Until resolved, the software will not allow running samples or sorting.

When performing the swap tip procedure, the front panel may be lifted and attached via magnet to the upper filter housing to allow nozzle access. Note that in this state, the S3 Biosafety System's airflow is reduced and the system is not considered to be in Class I containment.

IMPORTANT! If the airflow through the HEPA filter falls below required limits for containment, the software will alert the user and stop the sort or acquisition immediately.

8 Shutdown

8.1 Daily Shutdown

The S3™ and S3e™ System should be shut down at the end of the day. This will turn off all droplet formation and charging, lasers, and streams. A flush will be completed with the DI water container to rinse the system.

> ⛔ **IMPORTANT!** Always follow the personal protective equipment (PPE) guidelines relevant to your laboratory's safety procedures for dealing with ethanol or bleach.

Note: At any time, the shutdown procedure can be canceled by clicking the X icon on the dialog windows.

Note: If a user chooses to log off from the software, the force cleaning procedure option can be run. Similar to the shutdown procedure, windows will appear with the exception of the automatic startup window.

To shut down:

1. Ensure that the sample loading stage is in the wash position.
2. On the Setup and Maintenance tab select **Shutdown**. The software will confirm with a dialog box that you intend to shut down the system (Figure 88).
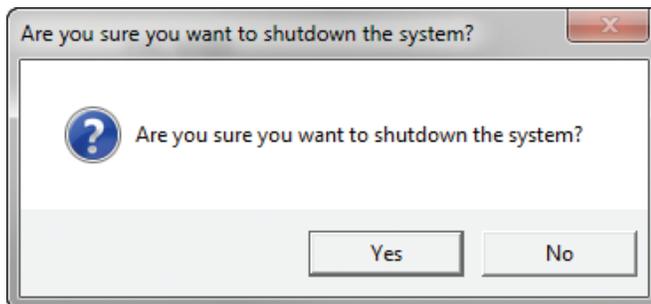
Fig. 88. System shutdown check.

3. Click **Yes** to confirm shutdown.

    Note: If there are any unsaved protocols, the software will warn you that unsaved changes will be lost (Figure 89).

4. Click **Yes** to continue or **No** to abort shutdown procedure.
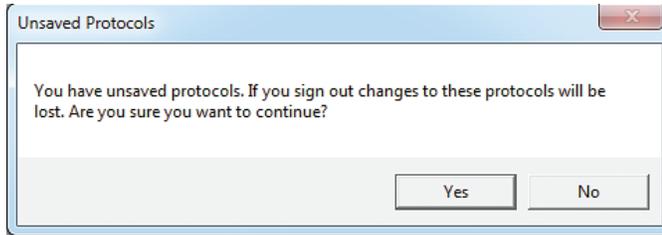
Fig. 89. Unsaved protocols check.

The shutdown procedure will display an automatic startup option. For ease of use and efficient workflow, the system can be automatically started the next day (Figure 90).

5. If using the automatic startup option, select **Yes** and set the restart date and time.
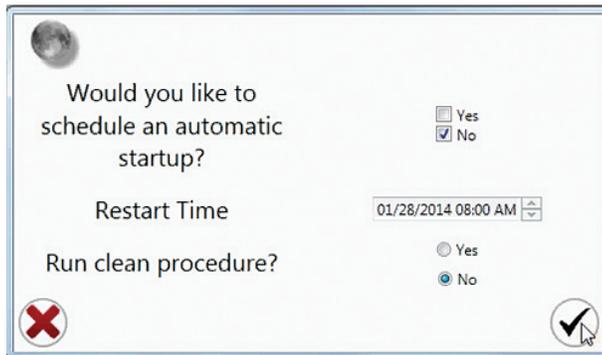
Fig. 90. Automatic Restart window with shutdown icon highlighted.

A window will open requesting a tube of cleaner to be used to clean the system (Figure 91).

6.   Load a tube of cleaning solution.

7.   Move the loading stage into the run position.
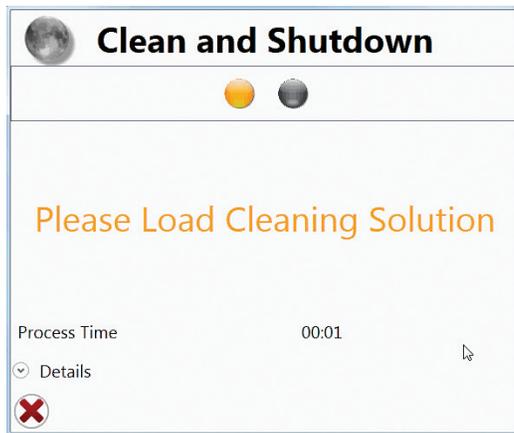
Fig. 91. Start the clean system option by following the prompt to load a tube of cleaner.

8.   The system will automatically run through the cleaning procedure, which takes approximately 2 min, while displaying status windows (Figure 92).

9.   Put the Loading Stage into the wash position.
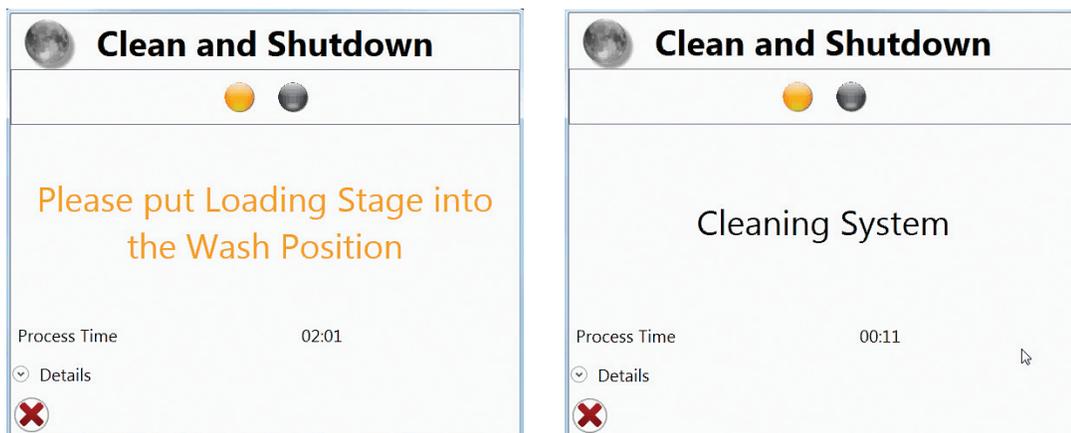
Fig. 92. Cleaning status windows.

Once the system is clean, the shutdown procedure will shutdown hardware, fluidics, air will be purged, rinsing and backflushing of the sample line, rinsing and backflushing the sample probe and lastly clearing the waste catcher (Figure 93).

The user will then be logged out and if automatic startup has been entered the countdown will begin.
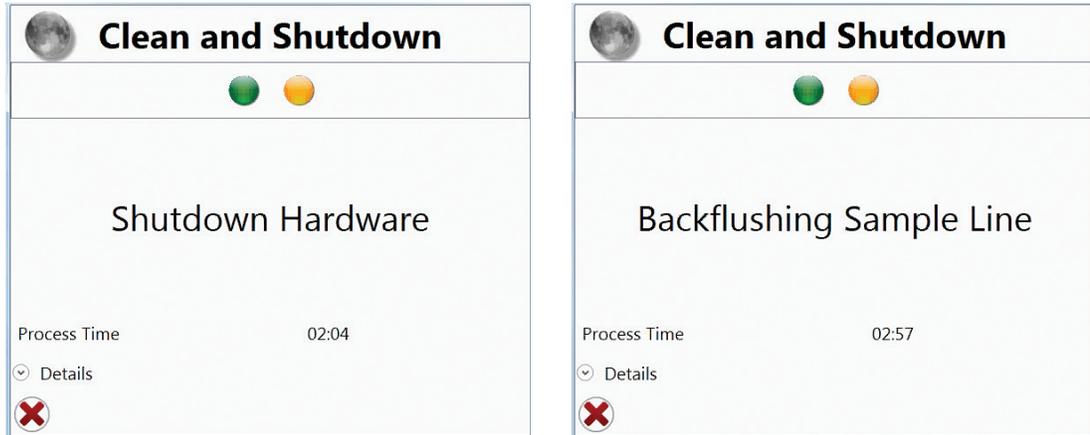
Fig. 93. Shutdown Status windows.

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IMPORTANT! It is advised to clear waste and refill fluidic containers at the end of the day's use.

For automatic startup, waste must be empty and 8x sheath fluid and DI water must have a supply sufficient for a 2 hr run time, minimum, in order to start up the system properly without errors.

Once the shutdown procedure is completed the software will return to the opening login screen.

At this time the system is fully shut down and can be left in standby mode till the next use.

9 Automatic Startup

The ProSort™ can schedule an automatic startup of the system. Upon automatic start up, the system will verify the stream is coarsely aligned and no clogs are present. If these conditions are not met, the stream will be shut off automatically to avoid spraying or flooding. A warning will appear and the system may be manually started to resolve the issue.

9.1 Scheduling an Automatic Startup

There are three ways to schedule an automatic startup.

1. Using the Startup button (provided no scheduled startup has been set). Please refer to Section 4.3, Daily Startup.
2. During the shutdown procedure. Please refer to Section 8.1, Daily Shutdown.
3. During the decontamination procedure. Please refer to Section 10.7, Decontamination.
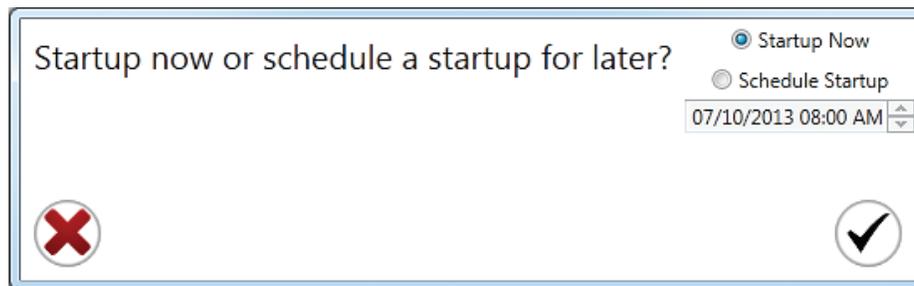

Fig. 94. Startup settings.
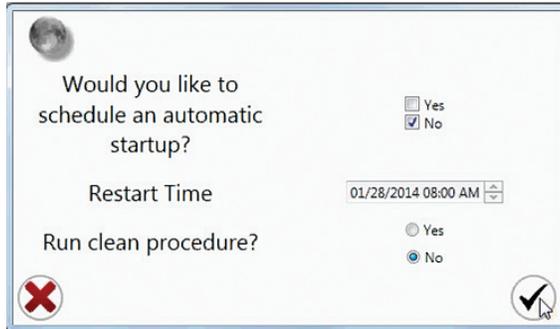
Fig. 95. Automatic Restart window during the shutdown procedure.
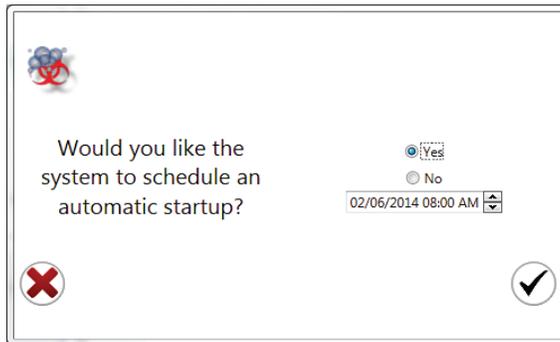
Fig. 96. Automatic restart window during the decontamination procedure.

9.2 Previously Scheduled Automatic Startups

After double clicking the ProSort Software icon on the desktop, a login window will appear. If a previous user has scheduled an automatic startup of the system, a countdown will appear at the bottom of the login window (Figure 97). This countdown indicates how much time is left before the startup procedure will begin.

Note: The software must be opened to the login screen for the auto startup to occur.
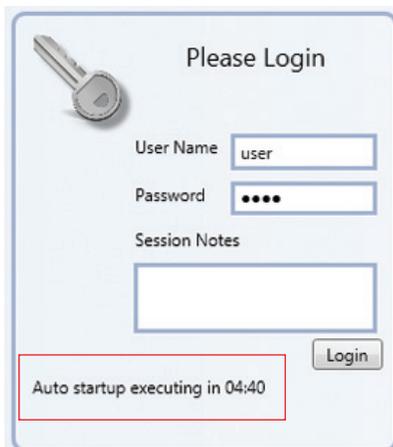
Fig. 97. User login window with auto startup countdown.

When a user logs onto the system and an automatic startup time is designated, the software will display the Auto Startup Scheduled window (Figure 98).

There are three options that can be performed at this time. These options determine whether the automatic startup will proceed as scheduled or be canceled.
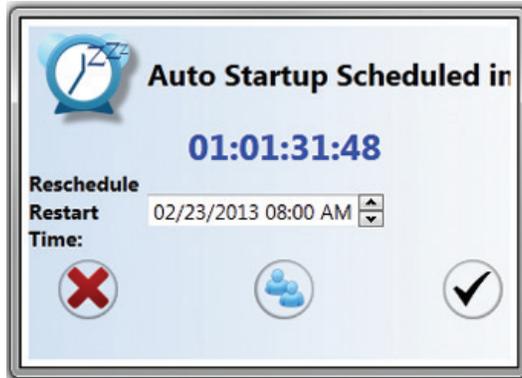
Fig. 98. Auto Startup Scheduled window.

WARNING! Canceling a previously scheduled automatic startup may influence other users' plans. Be sure to check with others when choosing this option.

Table 28. Auto startup buttons.

Button	Command	Function
	Cancel	Cancels the previously scheduled automatic startup. A message will appear in the login screen indicating that a user has cancelled the automatic starup
	Accept and sign out	Logs current user out of system and continues with scheduled automatic startup
	Allow auto startup	Keeps scheduled automatic startup time but allows the user to start up the system and run experiments in the meantime. The user can also alter the startup time

10 **Maintenance**

Always follow the PPE guidelines relevant to your laboratory's safety procedures for dealing with the chemicals recommended below.

10.1 General Maintenance

10.1.1 Daily

It is recommended that the sample line is cleaned every day prior to shutdown. See Section 10.6, Disinfectants, for details regarding approved cleaners for this tubing.

The system must be shut down through the software on a daily basis. If the system is not shut down properly, it may be prone to software-instrument communication problems, and the sample lines, nozzle, and fluidics may be prone to contamination.

If using the automated startup option, be sure to clear the waste and fill the fluidics at the end of use to ensure no errors will occur.

10.1.2 Weekly

Each week, wipe down the system with a mild disinfectant. Clean any salt crystal buildup in the sort chamber or on the deflection plates.

Inspect the camera windows for any debris or salt. Clean them with a swab and mild disinfectant as needed.

10.1.3 Yearly

It is recommended to purchase the annual preventative maintenance (PM) plan offered with the S3™ and S3e™ Cell Sorter. The PM includes but is not limited to an annual onsite visit by a service engineer to:

- Replace the inline sheath filter
- Replace the inline DI filter
- Replace the bulk fluid filters in the sheath and DI bottles
- Replace the air pump filter
- Replace the inline filters
- Replace the rinse filters
- Clean the optical filters
- Replace the nozzle assembly
- Examine tubing and connectors
- Perform system check
- Verify and adjust optical alignment

10.2 Dealing with Clogs

A clogged or partially clogged nozzle tip can occur while analyzing or sorting and it is usually a result of an unfiltered sample delivering clumps of cells to the tip. When this happens, the stream will be unable to spray straight or will be blocked completely. Several things can be done to remove this clog. These options, in order of severity are listed below. Once complete, click the startup button and allow the system to verify that the clog has been removed.

10.2.1 Unclog Option in Software

A clog can easily be introduced and can cause multiple issues with the fluidics. Resuspend cells into a single cell suspension and always use a 40 μm or smaller filter prior to cell sorting to help avoid clogs. If a clog does occur, use the Unclog button on the Setup and Maintenance tab toolbar as a first-line action. The S3 and S3e Cell Sorters have the ability to backflush the nozzle, potentially releasing the clog and flushing it into the waste.

If successful, a couple of debubble cycles are required to remove any air that may have been pulled into the nozzle body.

If unsuccessful, see Section 10.2.2, Run Sample of Cleaner, or Section 10.4, Swap Nozzle Tip Wizard.

10.2.2 Run Sample of Cleaner

Running a sample with 70% ethanol or 10% sodium hypochlorite could help free the clog. This is run through the sample input station. Cleaning solution can dislodge or loosen clogs to free up the line or the nozzle tip.

If successful, run at least two rinse cycles prior to running any test samples.

If unsuccessful, swap nozzle tip (Section 10.4).

10.3 Cleaning or Replacing Nozzle Tip

The nozzle is the main fluidics component of the system and provides stable sheath flow, focused sample introduction, and droplet creation and charging. The nozzle tip is fixed at a 100 μm tip orifice that provides centered sheath stream for laser interrogation. The tip may be removed to clean or to remove a clog. The nozzle can be accessed through the top, front sliding cover.

Note: The cover is interlocked and lasers will be turned off if it is opened.

Note: When performing any of the following instructions, use the Swap Nozzle Tip button to open the wizard, which will assist in swapping or cleaning the tip. The wizard will also walk through restarting and aligning the system.

10.4 Swap Nozzle Tip Wizard

The Swap Tip wizard will walk through each step necessary to perform a successful nozzle tip swap. Read through the wizard windows carefully and follow instructions on each screen as you proceed with the protocol.

Clear an area around the instrument to have adequate working space. At times, adjustment to the nozzle stage is necessary. Handle the nozzle with clean gloves.

First, determine which nozzle system is installed in your cell sorter (Table 29).

Table 29. Cell sorter and nozzle systems.

Catalog #	Cell Sorter	Nozzle System	Proceed to Section
145-1001, 145-1002	S3 Cell Sorter	Agilis	10.4.1
145-1001, 145-1002	S3 Cell Sorter (upgraded with AutoGimbal™ feature)	AutoGimbal	10.4.2
145-1005, 145-1006, 145-1008	S3e Cell Sorter	AutoGimbal	10.4.2

10.4.1 Nozzle Tip Wizard with the Agilis System

The Agilis system uses two axis-motorized positioners that are controlled by the software. These piezo-driven micromotors help align the stream to the pinhole and to the collinear lasers. In addition, there are three gimbaling screws that are used to make manual adjustments to the nozzle stage for nozzle height in relation to the pinhole and to the streams laser.

The nozzle tip must still be removed, cleaned, and reseated manually prior to alignment. The stream must be hitting the waste hole to ensure successful results.

To swap the nozzle tip:

1. On the Setup and Maintenance tab select **Swap Tip**. The Swap Nozzle Tip wizard will open (Figure 99). Follow the instructions indicated by the wizard.
2. Open the nozzle chamber door.
3. Press on the left side of the black metal light protection device and lift out of the way.
4. Locate the thumb screw hinge.
5. Loosen the thumbscrew until the nozzle body rises up and the tip can be seen.
6. Click the blue arrow.
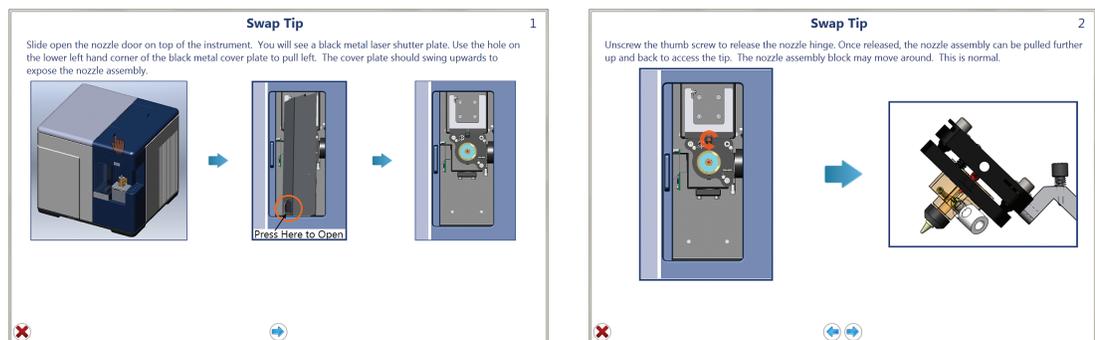
Fig. 99. Nozzle swap wizard window.
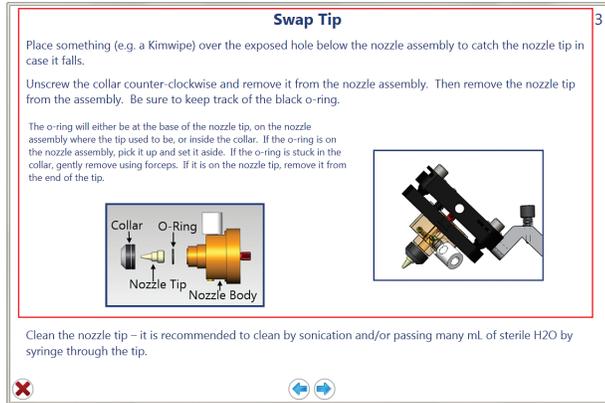
Fig. 100. Nozzle swap wizard window.

7. Place a lint-free cloth in the nozzle chamber under the nozzle tip to prevent dropping the nozzle into the instrument.

8. Rotate the black collar holding in the nozzle until it comes loose.
   Note: There is a small O-ring that will be removed as well.

9. Pull out the small white nozzle tip.

If sonication is desired or necessary:

10. Place the tip into a tube containing DI water.

11. Cap the tube.

12. Place the tube into a sonicator for several minutes.

13. Proceed to step 14.

If sonication of the tip is not performed:

10. Using a 1 ml syringe filled with ethanol, press against the top and eject ethanol through the nozzle tip, rotating between the 100 μm tip and the larger opposite end to free clog.

11. Watch the resulting stream. The fluidic stream should be coming out straight from the tip.

12. Repeat if necessary until the stream is coming out straight.

13. Proceed to step 14.

14. Place the O-ring at the point on the nozzle where the bottom of the black collar will be.

15. Place the nozzle tip back onto the nozzle assembly by pushing it up using the sides of the tip. Avoid touching the end.

16. Secure the black retaining collar until finger tight.

17. Remove the lint-free cloth from nozzle chamber.

18. Press the stage back down and tighten the thumb screw.

19. Click the blue forward arrow.
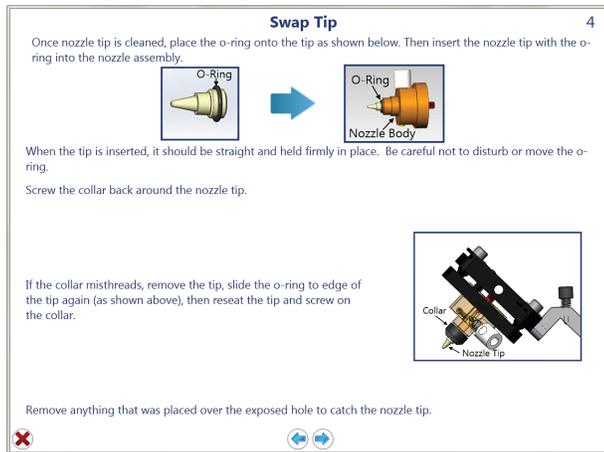
**Fig. 101. Nozzle swap wizard window.**
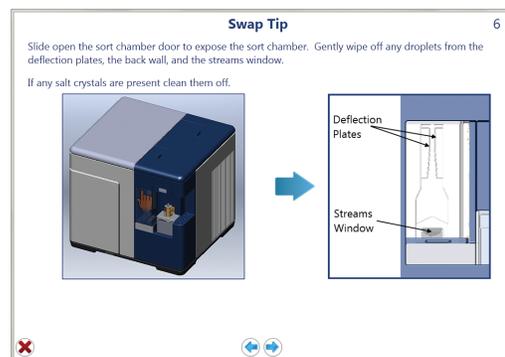

Fig. 102. Nozzle swap wizard window.
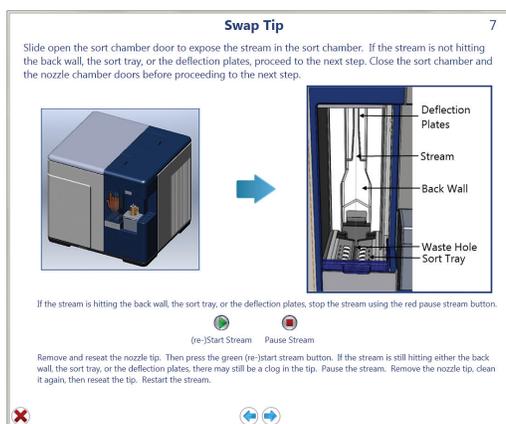
Fig. 103. Nozzle swap wizard window.

20. Check to see if the stream is hitting the waste hole in the back of the sort collection area.

21. If not, coarse adjustments can be made by physically moving the black block area at the top of the nozzle.

Note: The upper right gimbal moves the nozzle right to left. The bottom right gimbal moves the nozzle foward and back.

22. Fine movements can be made using the gimbals and a hex wrench provided in the accessory kit.
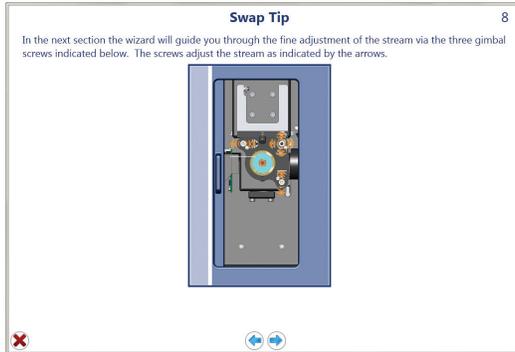
Fig. 104. Nozzle swap wizard window.

The S3 Cell Sorter uses the micromotors to align the stream to the pinhole and to the lasers. Three camera views — pinhole, droplet, and stream — will appear during the alignment. The micromotors will bring the stream into focus.
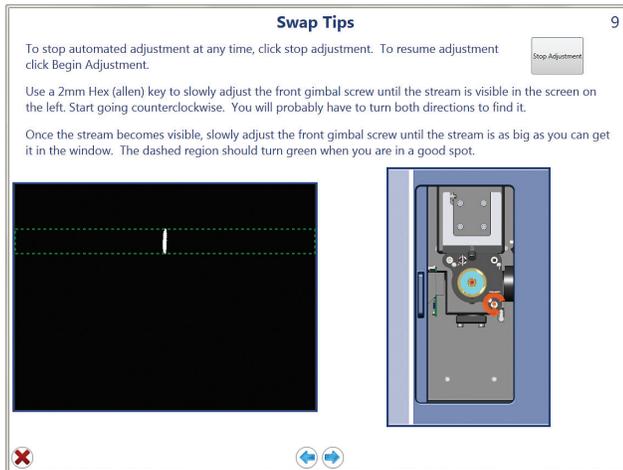

**Fig. 105. Nozzle swap wizard window.**
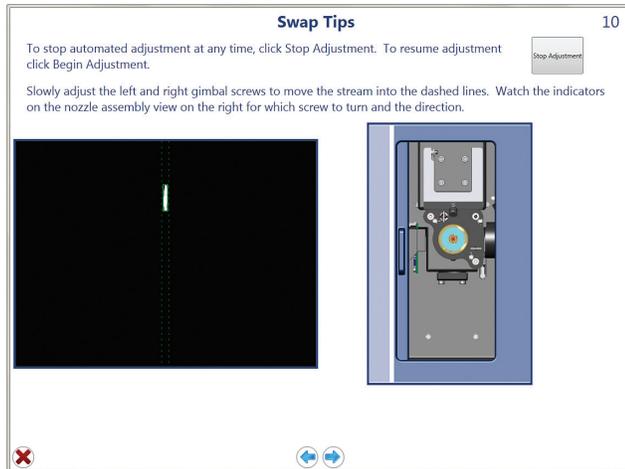
Fig. 106. Nozzle swap wizard window.

An image of the stream alignment is shown with the stream camera. It is necessary to center the stream and optimize the amount of light on the stream by scanning forward and back, or side to side.

23. Adjust the gimbals with the hex wrench as they are highlighted by the wizard.

24. Count turns and visually estimate where the stream looks the brightest.

Once optimized, the wizard arrows will disappear. If the picture does not get any brighter or the arrows do not disappear, proceed to next step. More adjustments can be made later.

25. Follow the arrows on the diagram. Arrows will disappear when the stream is properly adjusted.

Note: The box on the image will also turn from yellow to green once the stream is in proper position.
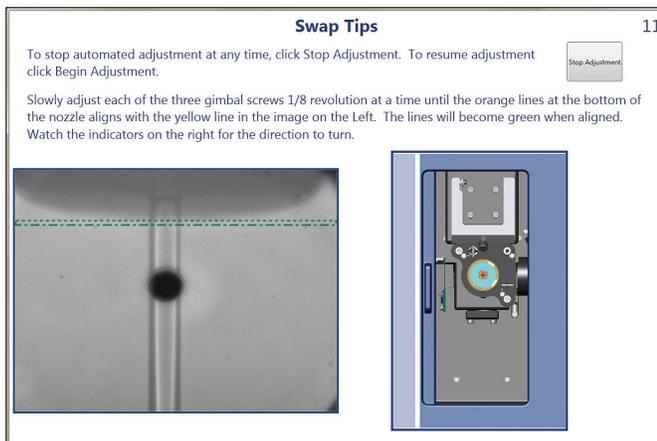

Fig. 107. Nozzle swap wizard window.

Figure 108 displays the stream image (left, center) and the nozzle camera image (right). The bottom of the nozzle will be displayed as a double dashed green line.

26. Lower or raise the nozzle, using the three gimballs, until the orange line and the yellow line meet and turn green.

The S3 Cell Sorter will again use the micromotors to align the stream to the pinhole and the lasers. The three camera views will be shown again. The micromotors will bring the stream into focus again. This second alignment process should complete the alignment.
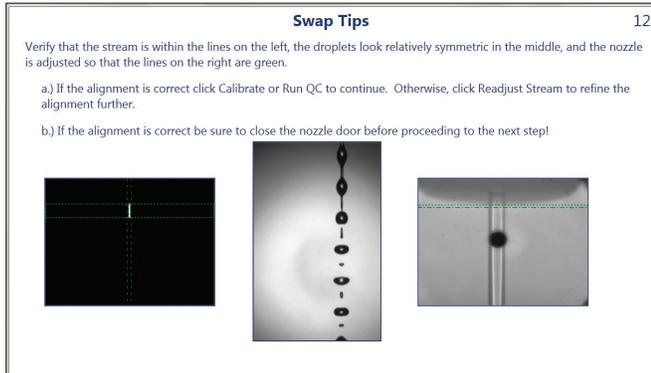
Fig. 108. Nozzle swap wizard window.

The window will give an option of repeating the gimball adjustment process or skipping and proceeding to droplet calibration. If the camera views show that the stream is centered and the nozzle line is green, droplet calibration can be done.

27. Close nozzle door.

28. Perform droplet calibration and QC to complete the tip swap process (Figure 109–112).

Prior to droplet calibration, the Adjust Stream Position window appear as a final check for positioning of the stream (Figure 109). The pinhole image can also be accessed from this window (Figure 110).
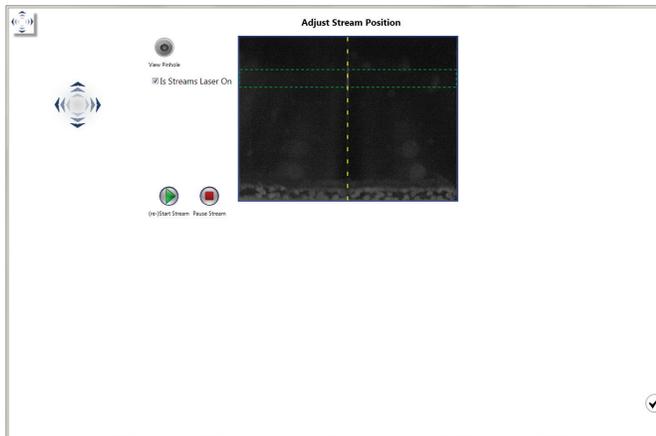

Fig. 109. Adjust Stream Position window streams view.
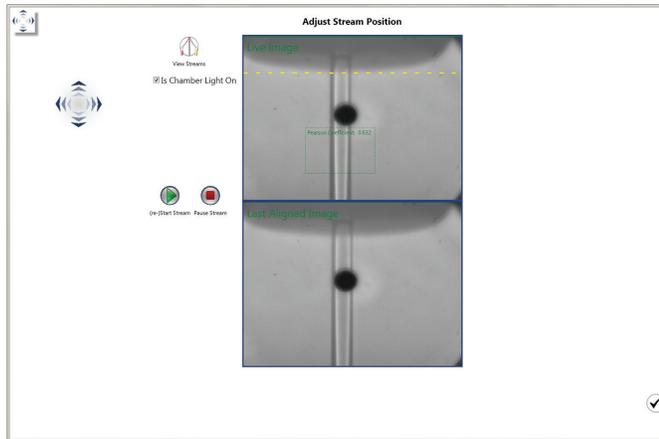
Fig. 110. Adjust Stream Position window pinhole view
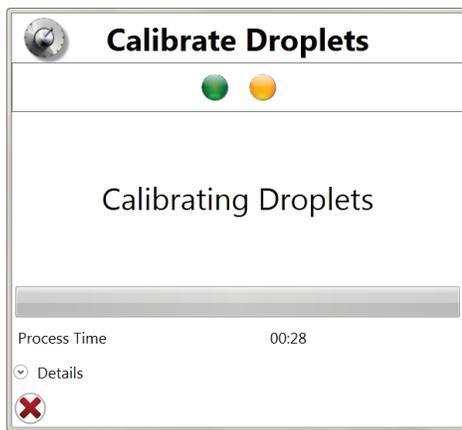

Fig. 111. Calibrating Droplets.
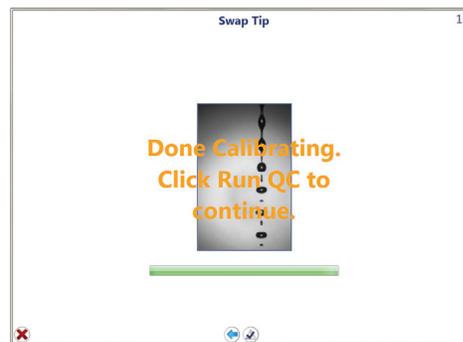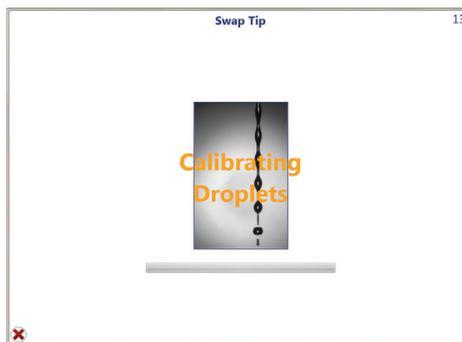
Fig. 112. Calibrating Droplets.
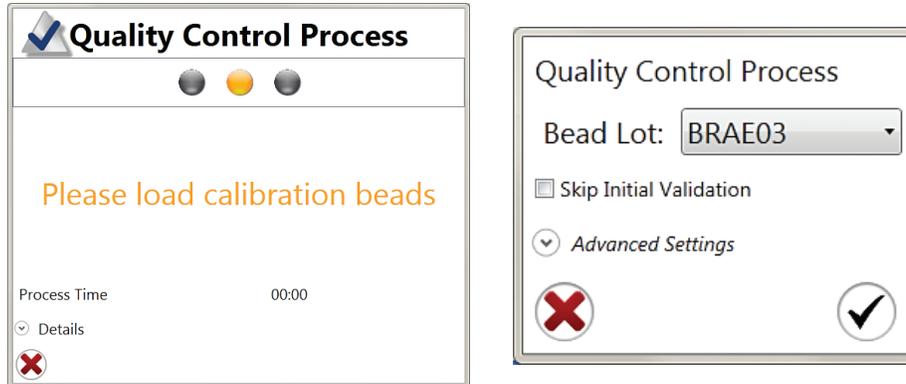
Fig. 113. Quality Control Process.

10.4.2 Nozzle Tip Wizard with the AutoGimbal System

The AutoGimbal system uses the software to enable hands-free alignment and optimization of the stream to optics. This provides increased repeatability and eliminates the need to make delicate adjustments by hand. The AutoGimbal system uses five axis picomotors to align the stream to the pinhole and to the lasers. Small micrometer movements are controlled by the software. Software algorithms, calculated from processed images, are used to determine the best position for stream and optical alignment.

The nozzle tip must still be removed, cleaned, and reseated manually prior to AutoGimbal alignment. For the AutoGimbal process to work successfully, the stream must not hit the back wall of the sort chamber, the sort tray, or the deflection plates.

To swap the nozzle tip:

1. On the Setup and Maintenance tab select **Swap Tip**. The Swap Tip wizard will open (Figure 114A). Follow the instructions indicated by the wizard.
2. Open the nozzle chamber door.
3. Press on the left side of the black metal laser shutter plate and lift out of the way.
4. Lift the nozzle stage using the small handle to expose the nozzle (Figure 114B).
5. Place a lint-free cloth in the nozzle chamber under the nozzle tip to prevent dropping the nozzle into the instrument.
6. Rotate the black collar holding in the nozzle until it comes loose.

Note: There is a small O-ring that will be removed as well — be careful to keep track of the O-ring.

7. Pull out the small white nozzle tip.

A

Swap Tip　　　　　　　　　　　　　　　　　　1

Slide open the nozzle door on top of the instrument. You will see a black metal laser shutter plate. Use the hole on the lower left hand corner of the black metal cover plate to pull left. The cover plate should swing upwards to expose the nozzle assembly.

Press here to Open

B

Swap Tip　　　　　　　　　　　　　　　　　　2

Lift up on the nozzle assembly handle to release the nozzle assembly. The nozzle assembly can be pulled further up and back to access the tip.

Fig. 114. AutoGimbal Swap Tip window.

Swap Tip　　　　　　　　　　　　　　　　　　3

Place something (e.g. a Kimwipe) over the exposed hole below the nozzle assembly to catch the nozzle tip in case it falls.

Unscrew the collar counter-clockwise and remove it from the nozzle assembly. Then remove the nozzle tip from the assembly. Be sure to keep track of the black o-ring.

The o-ring will either be at the base of the nozzle tip, on the nozzle assembly where the tip used to be, or inside the collar. If the o-ring is on the nozzle assembly, pick it up and set it aside. If the o-ring is stuck in the collar, gently remove using forceps. If it is on the nozzle tip, remove it from the end of the tip.

Collar　　O-Ring

Nozzle Tip　　Nozzle Body

Clean the nozzle tip – it is recommended to clean by sonication and/or passing many mL of sterile H2O by syringe through the tip.

Fig. 115. AutoGimbal Swap Tip window.

Cleaning the nozzle tip:

The nozzle tip may be clogged visibly, but there may also be a clog that is not visible. A syringe wash may solve the problem. Often the tip will need to be sonicated to remove micro clogs or stuck debris. In some instances the cleaning may need to be repeated several times to resolve the issue.

Sonication:

8. Place the tip into a tube of DI water.

9. Cap the tube.

10. Place the tube into a sonicator for 10–30 min.

11. Perform a syringe wash after sonication.

Syringe wash:

12. Using a 1 ml syringe filled with DI water or ethanol, press the syringe against the base of the nozzle tip and force liquid through the nozzle tip.

13. Perform the same wash by pressing the syringe tip against the top of the nozzle tip and ejecting the liquid.

14. Repeat as needed by rotating between top and base of the tip to free clog.

15. Watch the resulting stream. If the tip is clean, the fluidic stream should be coming out straight from the tip with no bend or significant spraying.

16. Repeat if necessary until the stream is coming out straight.

17. Place the O-ring at the edge of the base of the nozzle tip (Figure 116).

18. Place the nozzle tip back onto the nozzle assembly by pushing it in, touching only the sides of the tip. Avoid touching the end.

19. Secure the black retaining collar until finger tight.
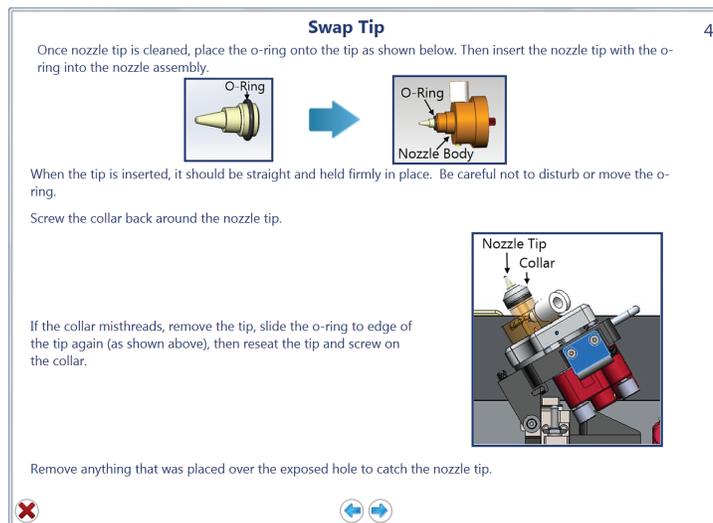
Fig. 116. AutoGimbal Swap Tip window.

20. Remove the lint-free cloth from nozzle chamber.

21. Press the nozzle stage back down. The nozzle stage should snap back into position (Figure 117).

22. Click the blue forward arrow.
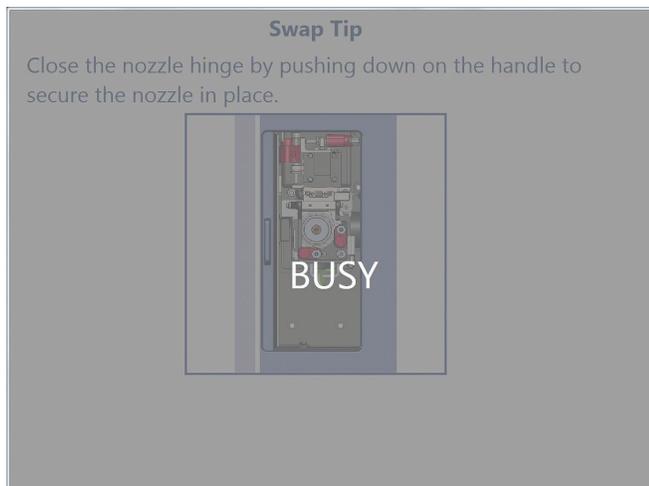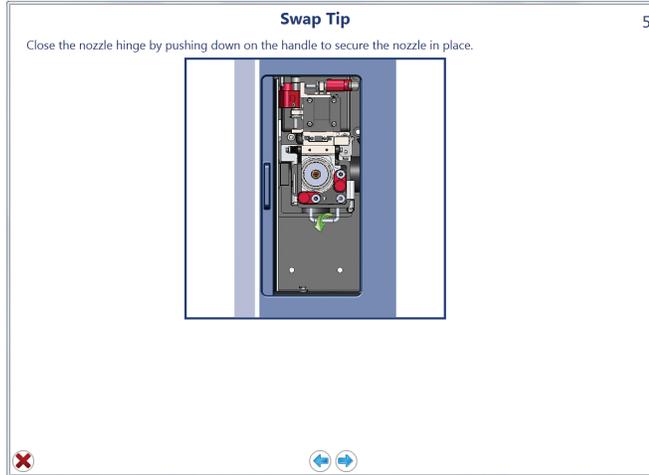
Fig. 117. AutoGimbal Swap Tip window.

23. Slide the sort chamber door open.

24. Clean the sort chamber to ensure there are no stray droplets on the back wall, sort tray, or deflection plates, and that the streams camera window is clean (Figure 118).

25. Check to see if the stream is hitting the waste hole. If it is, proceed to step 39. If the stream is hitting the back wall, sort tray, or deflection plates, remove and reseat the nozzle tip (Figure 119).

Note: The system will perform a series of debubble cycles before proceeding, which can be heard by active clicking (Figure 120).
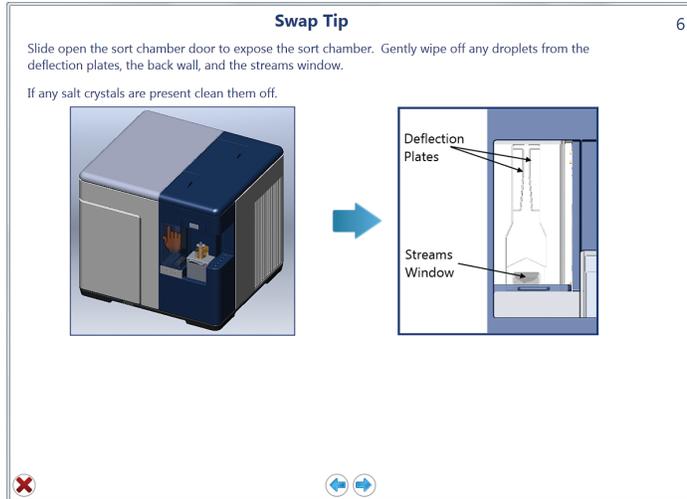
Fig. 118. AutoGimbal Swap Tip window.
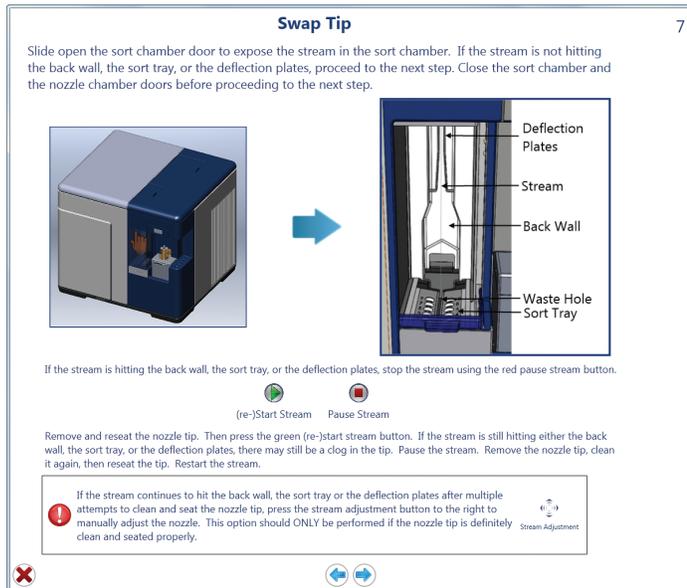
Fig. 119. AutoGimbal Swap Tip window.
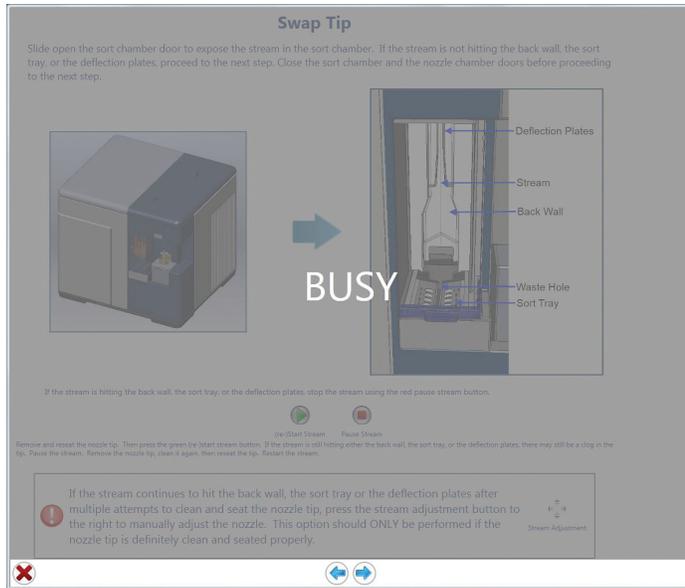
Fig. 120. AutoGimbal Swap Tip window.

26. If after reseating the nozzle tip the stream is still hitting the back wall, sort tray, or deflection plates, proceed to step 28.

27. Click the Stream Adjustment icon (see bottom of Figure 119).

28. The Adjust Stream Position window will open (Figure 121).
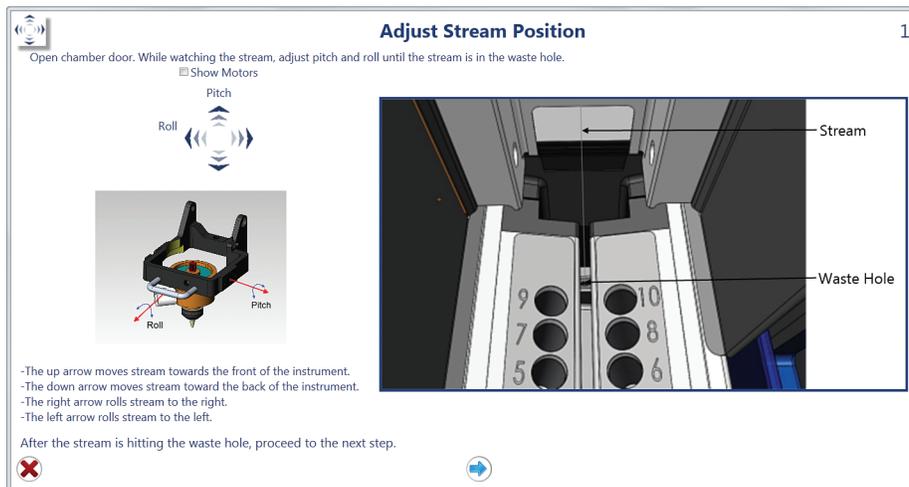
Fig. 121. Adjust Stream Position window.

29. Using the Pitch and Roll arrows, adjust the stream's position until the stream is hitting the waste hole.

30. Click the blue forward arrow.

31. Close the sort chamber door.

32. Using the Pitch and Roll arrows, move the stream until it is visible as a vertical white line in the camera image on the right of the window (Figure 12).

33. Adjust the stream so that the red dotted lines become green.

34. Click the blue forward arrow.

Note: When the stream is in line with the stream laser, the illuminated stream will become thicker and more visible.
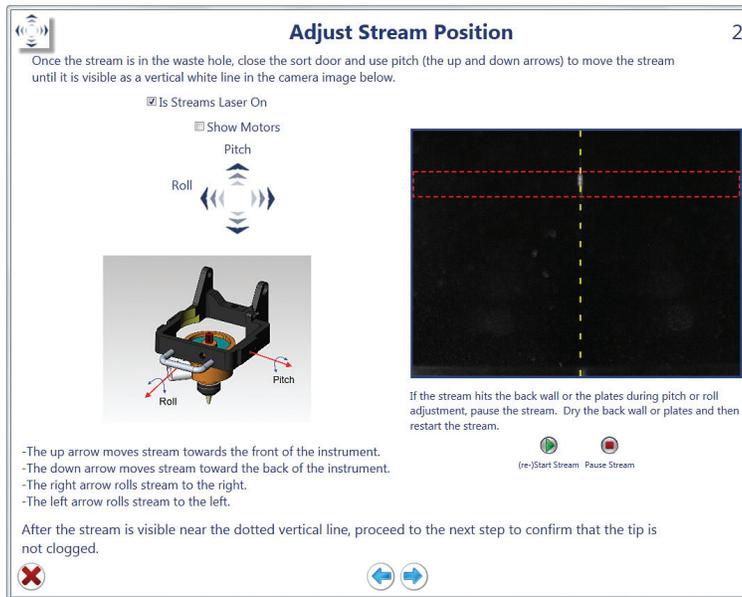

Fig. 122. Adjust Stream Position window.

35. Using the Vertical, Horizontal, and Focus arrows, adjust the nozzle tip and stream until it matches the last aligned image (Figure 123).

36. If there appears to be a clog or if the stream appears to be uneven, repeat cleaning the tip and reseating the nozzle as described starting on step 1.

37. If the nozzle tip and stream are aligned and there are no clogs, click the checkmark to proceed (Figure 123).
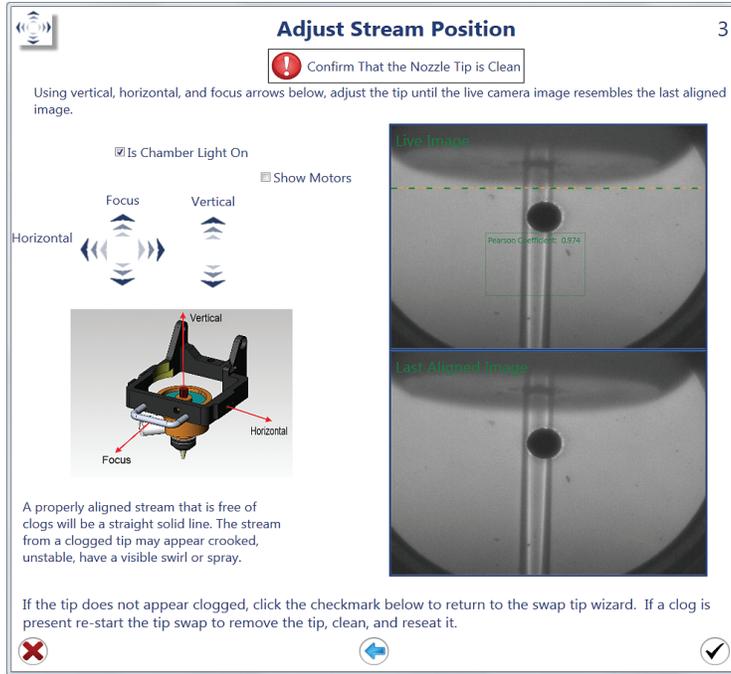
Fig. 123. Adjust Stream Position window.

38. The AutoGimbal system will commence automatic alignment of the nozzle tip and stream to the optics.

During the automatic alignment process, a series of windows will appear (Figure 124). This process can take 5–6 min to complete.
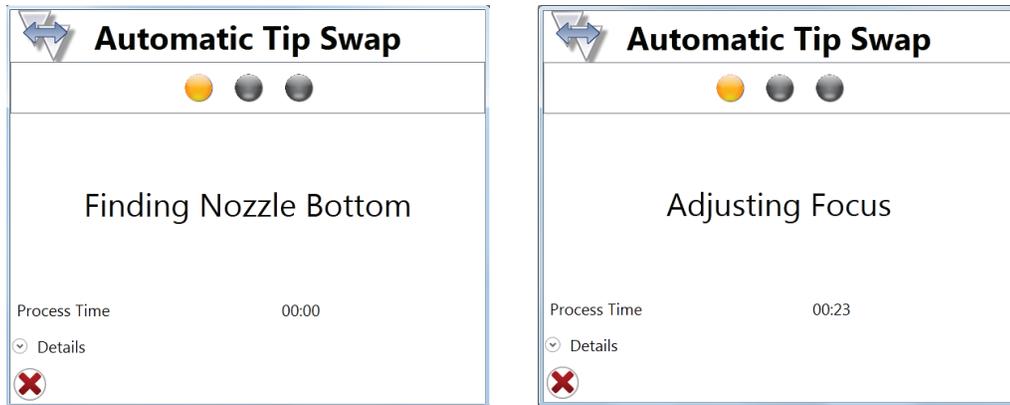
Fig. 124. Automated alignment process windows.
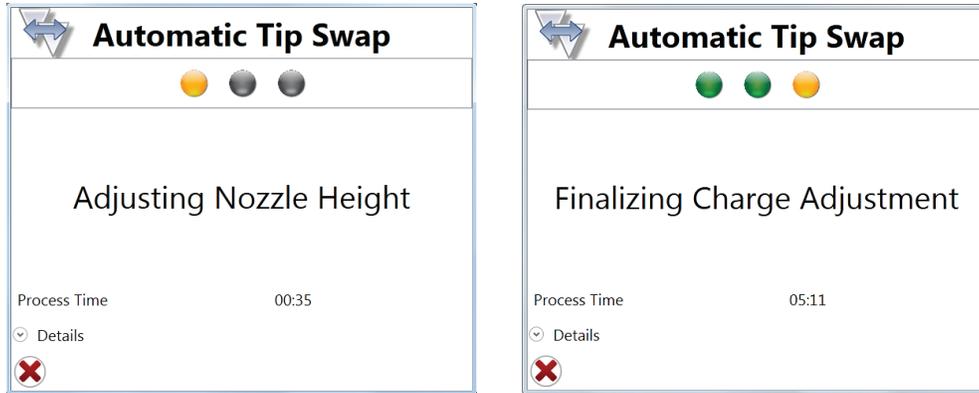
Fig. 125. Automated alignment process windows.

After the AutoGimbal system has completed its alignment and found the most optimal position, system calibration and drop delay calculation will begin automatically.

39. Select the bead lot from the dropdown menu and click the checkmark (Figure 126).

40. Load 500 μl ProLine™ Universal Calibration or ProLine Calibration Beads into a 5 ml tube and place into the sample station.

41. Move the loading stage into running position.
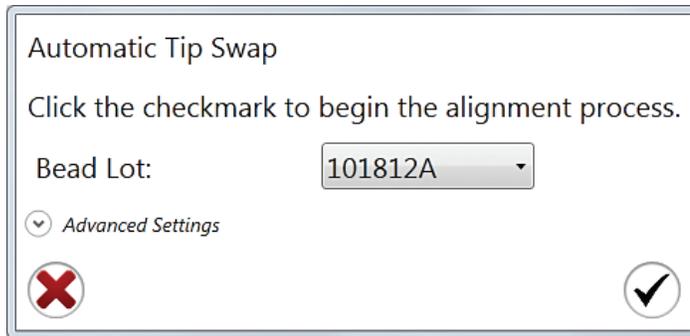
Fig. 126. Calibration Bead selection window.

Fig. 127. Automatic Tip Swap window.

10.5 Optical Filter Cleaning

The optical filters in the S3 and S3e Systems will lose performance when dirt, dust, or fingerprints are present on the glass surface. Inspection of these optical components and regular cleaning will help maintain the high performance of the system. Always remember that these coated pieces of glass are delicate and should be handled with care. Any scrape or scratch on the surface could significantly affect the light passing though.

To clean a filter, first put on gloves to avoid depositing oils and particulates on the surface. Using compressed air, gently blow off the surfaces of the filter to remove any large debris particles. Then, using a lint-free wipe (lens paper) or swab moistened with isopropyl alcohol, gently wipe the surfaces of the filter. Inspect filter by holding up to a light to ensure all debris particles have been removed. Replace filter into the S3 or S3e System.

10.6 Disinfectants

Always follow the PPE guidelines relevant to your laboratory's safety procedures for dealing with the disinfectants recommended below.

Disinfectants for Use in Sample Line

- 70% ethanol in DI water
- Bleach solution with a maximum concentration of 5,800 ppm active chlorine, a 1:10 dilution, which is roughly equivalent to 10% active chlorine. The bleach solution may be diluted further, depending on the pathogenicity of the sample

Disinfectants for Use in Sheath Line

- Bleach solution containing 580 ppm active chlorine, a 1:100 dilution, which is roughly equivalent to 1% active chlorine

Disinfectants for Use in the Waste Container

The following disinfectant types may be used in the waste container. It is imperative that the appropriate type and quantity of disinfectant is placed in the waste container to ensure effective inactivation of the biologics in the tank. Please check compatibility of combined products before use.

- 10% sodium hypochlorite or bleach

10.7 Decontamination

The Decontaminate tool is accessible by administrators and can be used to completely decontaminate the system. All materials within the system are compatible with 1% household bleach (580 ppm active chlorine).

It is recommended to run the decontaminate procedure at least once every six months but can be run as often as monthly. This tool should also be used if there is a noticeably high background level in the acquired data. The source could be within the fluidic path. Bacteria or fungus can potentially grow in the lines if samples are not handled using basic cellular sterile technique. The bulk fluidics can also contribute to contamination, despite having internal filters built into the lines.

To run the Decontaminate tool:

1. Move the loading stage into the wash position.

2. On the Administrator tab select **Decontaminate**.

A warning window will appear to confirm that a decontamination procedure should be run.
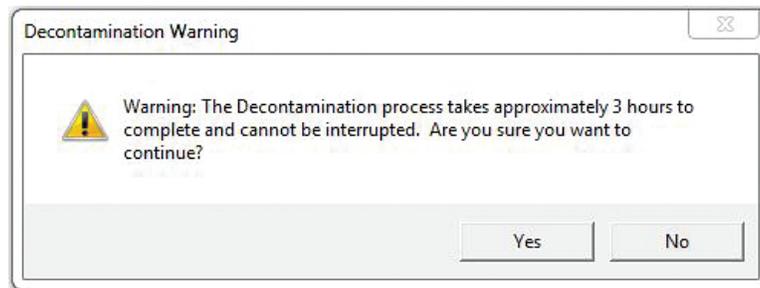
Fig. 128. Decontamination warning dialog box.

A decontamination wizard will start and walk through each procedure. When prompted follow the instructions.

3. Choose whether the system should be shut down after decontamination has completed (Figure 129).

4. Click the checkmark button to proceed.

5. Select whether or not an automatic startup should be scheduled (Figure 129).
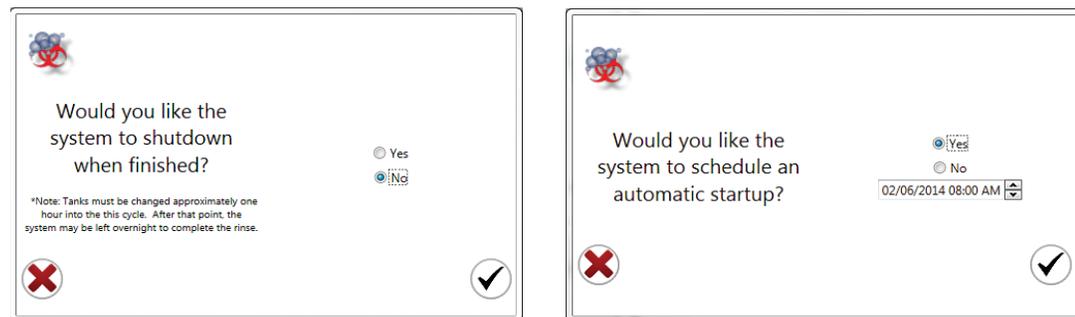
Fig. 129. Automatic shutdown and automatic startup option in the decontamination procedure.

6. If yes, assign date and time for automatic startup procedure to run.

7. Click the checkmark button to proceed.

8. Replace the DI water and sheath fluid containers with at least 1 L of decontamination solution (Figure 130).

9. Empty waste container and replace.

10. Click the checkmark button to proceed.
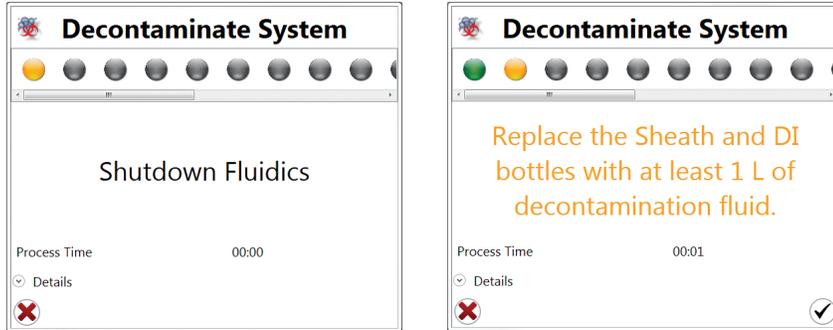
Fig. 130. Decontamination instructions to prepare the system.

The system will drain the internal sheath reservoirs into the waste container (Figure 131).

The system will directly prime the fluidics with the decontamination fluid (Figure 132).
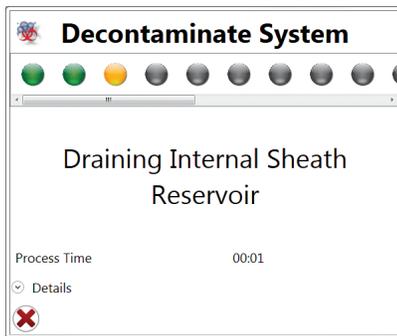
Fig. 131. Decontamination window while draining internal system.

Fig. 132. Decontamination window while priming system.
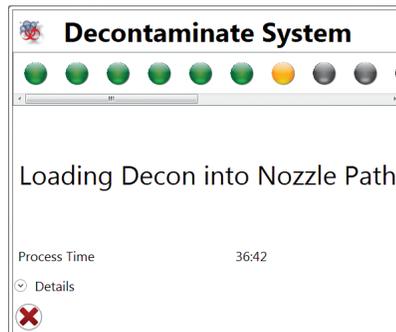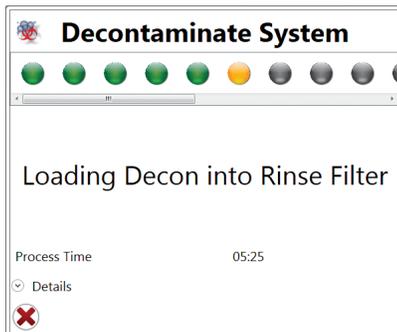
Fig. 133. Decontamination window while priming rinse filter and nozzle path.

In order to decontaminate, the system will soak the fluidic lines in the decontamination fluid and then will shut down (Figure 134).

Fig. 134. Decontamination window during the soaking period and shutdown.

After the system shuts down, the containers can be removed and the sheath and DI water cap assemblies should be rinsed with DI water. Sheath fluid and DI water containers can be returned to proper position (Figure 135). The system will drain the sheath fluid reservoir and the sheath filter before priming the system with 8x sheath fluid or DI water (Figures 136–137). After decontamination the system should be ready for running samples. Any high background should be eliminated.

Fig. 135. Decontamination window prompting to replace fluidics.
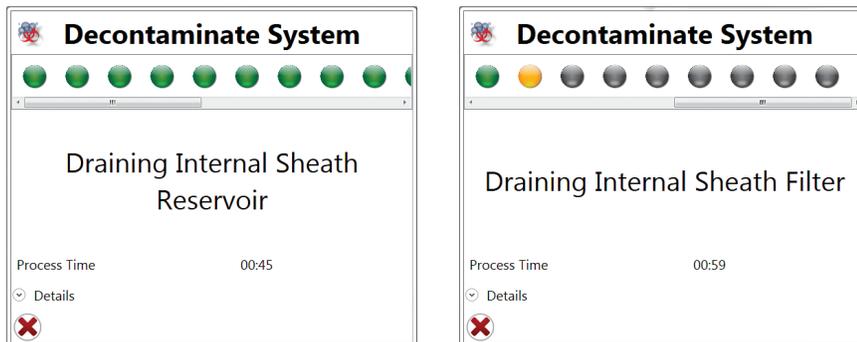
Fig. 136. Decontamination window when system is draining sheath reservoir and filter.
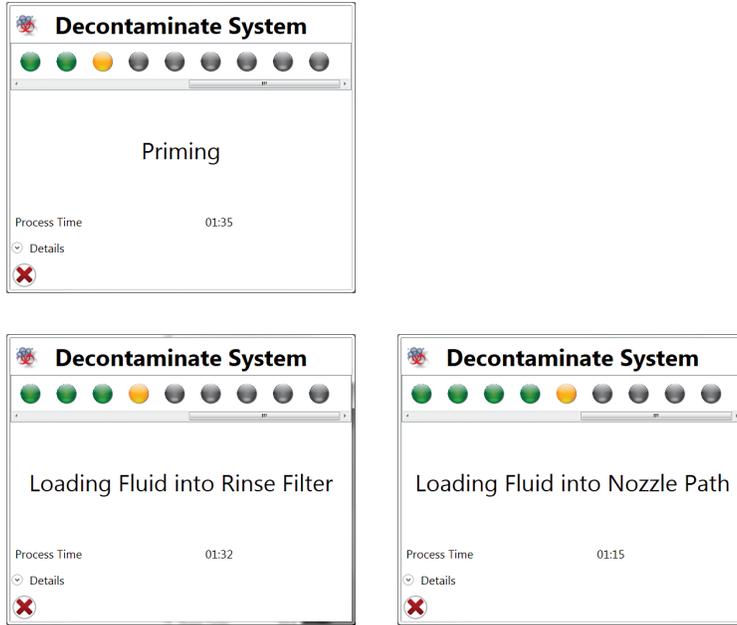
Fig. 137. Decontamination window when priming rinse filter and nozzle path.

Note: The user must be present to change the containers approximately 1 hour into the procedure. After this point the system can be left overnight to complete the rinse and shutdown if that option is chosen.

The system will soak and go through an extended warmup (Figure 138) before a system startup is commenced (Figure 139).
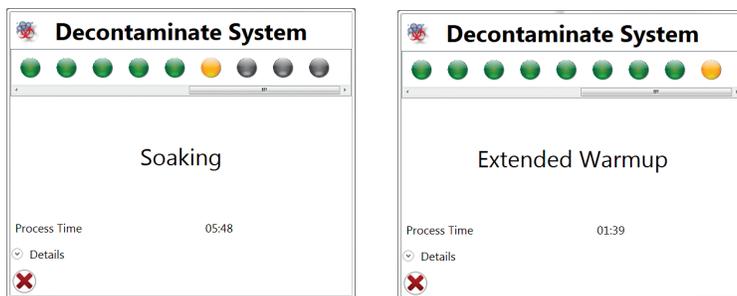
Fig. 138. Decontamination window while soaking system in proper fluidics and warming up.

After decontamination the system should be ready for running samples. Any high background should be eliminated.

Fig. 139. Startup process at end of decontamination.

11 Troubleshooting

Table 30 describes possible errors and causes with troubleshooting steps.

Table 30. Troubleshooting table.

| Error	Possible Causes	Troubleshooting Steps
Bubble detected	Bubble in sample line	Place the loading dock in the wash position to backflush the sample line and remove the bubble
Nozzle door interlock	Nozzle door is open	Close nozzle door
Sort door interlock	Sort door is open	Close sort door
Waiting for QC	QC has not been run since the system has been started up	Run QC to proceed with sorting
Not maintaining droplets	Large shift in drops	Perform Drop Delay to put the system back into maintaining droplets
Cannot unlock loading stage	System is still washing	Wait for wash to complete
	Sample is currently running	Stop sort or acquisition
QC will not pass	Calibration beads diluted	Use the undiluted ProLine™ beads directly from bottle
	Using wrong calibration beads	Use only ProLine beads
	QC criteria changed	Refer to the correct bead lot file
Fluidic error message	Quick disconnects are not connected properly	Recheck connections. An audible click must be heard for proper connection
Stream coming out at an angle	Clog in nozzle tip	Use Swap Tip Wizard and clean nozzle tip
		Reseat nozzle tip on to the nozzle
Manual compensation not working	Compensation checkbox not checked	Check and uncheck compensation box on the axis
Cannot see events labeled with fluorophores	Filter configuration may be incorrect	Check filter configuration against fluorophore combination
	Laser turned off	Check that both lasers are powered
Cannot see rare events	Total events displayed is too low	Increase cycle mode value to display more events

continues

Table 30. Troubleshooting table (continued).

Error	Possible Causes	Troubleshooting Steps
Drastic light change	Background movement in front of green sliding door during QC process	Redo QC and avoid changing light conditions in front of green sliding door during the procedure
Sheath error	The internal sheath reservoir cannot maintain proper levels	The connections to the DI water and 8x sheath fluid containers should be checked to make sure they are connected, that they are not empty, and that fluid can flow. It is possible that their filters are clogged and need to be replaced
Waste error	Waste quick disconnect not connected properly to waste container Internal waste reservoir filled	The connections to the waste container should be checked to make sure they are connected and that waste can flow. Once corrected, it will take time for the system to clear the internal reservoir. You can observe that pumping is happening by watching for splashing in the waste container
Nozzle stage cannot return to proper position after nozzle tip swap	Laser intersection cover has been moved during tip swap	Adjust laser intersection cover so it is seated correctly. Once in the proper place the nozzle stage can be lowered into position
Cannot acquire sample. Hovering on acquisition button displays "bubble detected"	Room too cold or faulty bubble detector	Warm room to >63°F. If this does not help, contact service support
Arcing	The high voltage deflection plate monitor has measured the plate voltage deviating from normal	Stream spray or droplets are on the deflection plates. Carefully clean and dry the plates
No data appear on the work-space after changing nozzle tip	Nozzle interlock has been activated	Open nozzle door all the way then close to reset the laser interlock
No events during acquisition	Nozzle door open	Close the nozzle door
	Laser shutter closed	Cycle the nozzle door open then closed, especially if cover was removed
	Laser not on	Turn on the laser in the user interface Restart instrument and computer to correct bad communication
	Threshold too high	Lower threshold
	Triggering on noise	Change the trigger parameter to FSC or SSC
	Stream not centered in laser beam	Use the electronic micromotors (Agillis) positioner while acquiring to maximize event signal — access within the system log file
	No beads in sample	Check sample tube, vortex

Table 30. Troubleshooting table (continued).

Error	Possible Causes	Troubleshooting Steps
High event rate (while trying to run QC, or running a sample at 500 eps)	Drop drive noise causing excessive event rate (>40,000 eps)	Toggle between the FSC/SSC trigger (in the service panel)
		Perform a nozzle tip swap
		Calibrate droplets (located in the Edit Droplets window when logged in as administrator)
	Too much light. Event rate (~8,000 eps)	Light leak in system, ensure all covers are closed
		Check that the filter blocks or filters have not been removed or that they are seated in proper position
		If triggering on FSC, may be misaligned or ND filter removed
		Obscuration bar may need adjustment. Contact Bio-Rad Technical Support
	Inappropriate sample pressure. Event rate (~2,000 eps)	Perform Calibrate Sample Pressure Offset
Running samples at above 2,000 eps and getting 40,000 eps	Too much light	Obscuration bar may be flat and may need adjustment. Contact Bio-Rad Technical Support
		Threshold may be set too low, raise threshold value
		Signal intensity is too bright. Add ND filter
Poor drop delay (uneven V, flat on one side)	Bad side streams or center stream	Set defanning manually until the center stream is solid and tight
		Adjust phase to ensure the side streams are tight
		Recalibrate droplets located in Droplet Control window on the admin tab or at the end of a nozzle tip swap procedure
		Side stream deflection not set properly. Adjust side stream deflection on the admin tab
Very little volume is used while performing cleaning user logout or prior to (after system shutdown) Uses 2 ml of 10% bleach/ water takes approx. 1 min Usually 2 ml of 70% ethanol takes approx. 2 min	Pinch in the sample line	If sample line is partially clogged, run clean cycle multiple times with backflushing to remove clog
		If only a very small amount of liquid (200–300 µl) is taken from the tube containing the cleaning solution during a cleaning protocol, the sample line may be pinched, and may need replacement. Sometimes massaging the pinched area in the line (if visible) may resolve the issue. Contact Bio-Rad Technical Support
Waste Trough Filling, System goes into Safe Mode	Waste trough port clogged and overfilling	Clear debris from waste trough port and rinse with bleach. Restart instrument and computer.
	Trough is empty, droplet trapped on sensor that causes sensor to trip indiscriminately	Check to see if trough has liquid or if waste trough port is clogged. If not use canned air to clear droplet form sensor as a temporary fix. Restart instrument and computer. Contact service support for repair or replacement.
Variable event rate during acquisition (changes at regular intervals)	Agitator seal may be leaky	Turn off the agitator to see if it resolves the varying event rate. If it does, contact service support for replacement.
	Possible nozzle clog	Clean nozzle tip
	Hardware issue	Contact Bio-Rad Technical Support
Waste container is abnormally expanded	Hydrophobic filter is wet	Contact service support for replacement.

Table 30. Troubleshooting table (continued).

Error	Possible Causes	Troubleshooting Steps
Decontamination procedure freezes during priming	Software error	Contact technical support for help on the phone.
Stream is spraying everywhere	No tip in place	Run nozzle tip swap wizard and install nozzle.
	No O-ring in place	Run nozzle tip swap wizard to reseat nozzle with O-ring.
	Tip is still clogged	Run nozzle tip swap wizard to clean nozzle using sonication and syringe wash. Reseat nozzle.
Stream is spraying at an angle with spray.	Tip was not seated straight	Run nozzle tip swap wizard to clean nozzle and then reseat nozzle correctly.
	No O-ring in place	Run nozzle tip swap wizard to reseat nozzle with O-ring.
	Wagon wheel has fallen out, extra wagon wheel installed, or wagon wheel installed incorrectly.	Run nozzle tip swap wizard to check if wagon wheel is installed properly. Reseat nozzle.
	Tip is still clogged	Run nozzle tip swap wizard to clean nozzle using sonication and syringe wash. Reseat nozzle.
Stream is moving around, droplet camera shows droplets are moving inconsistently	No O-ring in place	Run nozzle tip swap wizard to reseat nozzle with O-ring.
	Wagon wheel has fallen out, extra wagon wheel installed, or wagon wheel installed incorrectly.	Run nozzle tip swap wizard to check if wagon wheel is installed properly. Reseat nozzle.
	Tip is still clogged	Run nozzle tip swap wizard to clean nozzle using sonication and syringe wash. Reseat nozzle.
Stream is hitting the back wall, deflection plates or sort tray	Tip was not seated straight	Run nozzle tip swap wizard to clean nozzle and then reseat nozzle correctly.
	No O-ring in place	Run nozzle tip swap wizard to reseat nozzle with O-ring.
	Wagon wheel has fallen out, extra wagon wheel, or wagon wheel installed incorrectly.	Run nozzle tip swap wizard to check check if wagon wheel is installed properly. Reseat nozzle.
	Tip is still clogged	Run nozzle tip swap wizard to clean nozzle using sonication and syringe wash. Reseat nozzle.

12 References

The following references and resources are useful for learning more about flow cytometry and its applications.

Shapiro HM (2003). Practical Flow Cytometry: Fourth Edition (Hoboken: John Wiley & Sons).

Cytometry: Part A; Journal of the International Society for the Advancement of Cytometry. Wiley. http://onlinelibrary.wiley.com/journal/10.1002/%28ISSN%291552-4930

Purdue University Cytometry Laboratories: cytometry and confocal microscopy education and research material, cytometry email archive, and links to cytometry web sites and suppliers worldwide.
http://www.cyto.purdue.edu/

13 S3™ and S3e™ Cell Sorter Specifications

The specifications for the S3 and S3e Cell Sorters are shown in Table 31.

Table 31. S3 and S3e Cell Sorter specifications.

Performance	Droplet frequency	37–43 kHz
	Sort type	True jet-in-air, high performance sorting
	Sort rate	No hardware limitations, limited only by droplet frequency and application
	Sorting purity	>99% pure
	Sensitivity	<125 MESF for FITC and PE
	Temperature control	Sample and collection temperature control from 4–37°C
Optics	Lasers	Single laser: 488 nm 100 mW
		Single laser upgrade: 561 nm 100 mW or 640 nm 100 mW
		Dual laser: 488 nm 100 mW and 561 nm 100 mW
	Detection	Forward scatter (FSC) with PMT
		Side scatter (SSC) with PMT
		4 fluorescent detectors with PMT
Fluidics	Nozzle	100 µm fixed
	Pressure	30 psi fixed
	Sheath	Onboard dilution of 8x sheath or direct use of 1x sheath
	Automation	Auto alignment
		Auto drop delay calibration
		Auto droplet monitoring
		Auto stream to laser alignment
Sorting	Directions	Two way
	Modes	Single, purity, enrich
	Collection	Up to 5 x 5 ml or 1.5 ml sample tube each direction
		Microscope slides
		2 x 8 micro well strip each direction
Software	Program	ProSort™
Workstation	Computer requirements monitor	5 Quad Core; 8 GB RAM; 250 GB HDD, Windows 7 Pro x64
		Recommended 24" LCD; 1920 x 1080 resolution
Physical requirements	Dimensions (W x D x H)	70 x 65 x 65 cm deep (instrument only)
	Weight	90 kg (instrument only)
	Electrical	120–230 VAC; 50–60 Hz
	Temperature	Ambient temperature range of 18–25°C
	Noise	<60 dBA while in operation
Options	Biosafety cabinet	S3 Biosafety System Class I
Regulatory status	Electrical	Electrical Safety for Europe
		IEC 61010-1 CB Scheme
		UL/CSA Standard for Safety Electrical Equipment for Electrical Safety (USA, Canada, NRTL)
		IEC61010-2-081, EN 61010-2-081
		EN/IEC 60825-1, EMC EN 61326-1
		CE Declaration of Certification
	Laser	Class I (1) laser product per CDRH regulations and EN/IEC 60825-1

Ordering Information

Catalog #	Description
Instrumentation	
145-1001	**S3 Cell Sorter (488 nm)**
145-1002	**S3 Cell Sorter (488/561 nm)**
145-1005	**S3e Cell Sorter (488 nm)**
145-1006	**S3e Cell Sorter (488/561 nm)**
145-1078	**S3 Biosafety System Class I**
145-1021	**S3 Cell Sorter (488 nm)**, with S3 Biosafety System Class I
145-1022	**S3 Cell Sorter (488/561 nm)**, with S3 Biosafety System Class I
145-1029	**S3e Cell Sorter (488 nm)**, with S3 Biosafety System Class I
145-1030	**S3e Cell Sorter (488/561 nm)**, with S3 Biosafety System Class I
Consumables	
145-1081	**ProLine Calibration Beads**, 3 x 5 ml
145-1082	**ProFlow 8x Sheath Fluid**, 5 x 4 L
145-1083	**ProFlow Sort Grade Water**, 5 x 4 L
145-1085	**ProLine Rainbow Beads**, 1 x 5 ml
145-1086	**ProLine Universal Calibration Beads**, 3 x 5 ml
Accessory Parts	
145-1065	**S3 Accessory Kit**
145-1084	**S3 Empty Fluidic Containers**, 3 x 4 L
144-1001	**S3 Sheath Cap Assembly**, white
144-1002	**S3 DI Water Cap Assembly**, blue
144-1003	**S3 Waste Cap Assembly**, red
144-1005	**S3 Filter Block A**, empty with 2 dichroic filter holders
144-1006	**S3 Filter Block B**, empty with 2 dichroic filter holders
144-1007	**S3 Individual Filter Holder**, black, empty
144-1008	**Neutral Density Filters**, 1.0
144-1009	**S3 Nozzle**, 100 µm tip
144-1010	**S3 Nozzle O-Rings with Alignment Disk**, 2-pack
144-1011	**S3 Collection Adaptor Set**
144-1012	**Hex Driver**, 2 mm
144-1013	**S3 Individual Dichroic Filter Holder**
145-1076	**S3 Power Conditioner**, International
145-1077	**S3 Power Conditioner**, US

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Laboratories, Inc.**