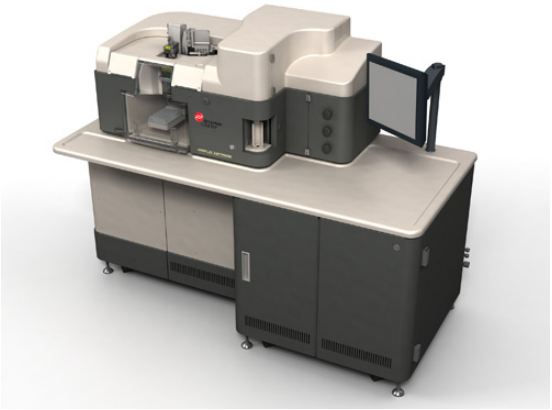
Instructions for Use

MoFlo Astrios

High-Speed Cell Sorter



PN A99481Rev B
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BECKMAN COULTER

Astrios High-speed Cell Sorter
Instructions for Use
PN A99481

Find us on the World Wide Web at: **www.coulterflow.com**

Revision History

Initial Issue
EC#0005758
PN A99481
Document Number 0005288
Software Version: Summit 6.0

Revision B
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Software Version: Summit 6.0

Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter representative.

Beckman Coulter, Inc. urges its customers to comply with all national health and safety standards such as the use of barrier protection. This may include, but is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

Alerts for Warning and Caution

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

⚠ WARNING

Risk of operator injury if:

- **All doors covers and panels are not closed and secured in place prior to and during instrument operation.**
- **The integrity of safety interlocks and sensors is compromised.**
- **Instrument alarms and error messages are not acknowledged and acted upon.**
- **You contact moving parts.**
- **You place your fingers between the bottom of the nozzle stage and the instrument frame when lowering the stage.**
- **You place your hand in the SmartSampler sample chamber when the door begins to close after a sample run is initiated.**
- **You mishandle broken parts.**
- **Doors, covers and panels are not opened, closed, removed and/or replaced with care.**
- **Improper tools are used for troubleshooting.**

To avoid injury:

- **Keep doors, covers and panels closed and secured in place while the instrument is in use.**
- **Take full advantage of the safety features of the instrument.**
- **Acknowledge and act upon instrument alarms and error messages.**
- **Keep away from moving parts.**
- **Lower the nozzle stage using the upper portion of the stage to avoid pinching points.**
- **Do not place your hand in the SmartSampler sample chamber after the door begins to close once a sample run is initiated.**
- **Report any broken parts to your Beckman Coulter Representative.**
- **Open/remove and close/replace doors, covers and panels with care.**
- **Use the proper tools when troubleshooting.**

⚠ CAUTION

System integrity could be compromised and operational failures could occur if:

- **This equipment is used in a manner other than specified. Operate the instrument as instructed in the product manuals.**
- **You introduce software that is not authorized by Beckman Coulter into your computer. Only operate your system's software with software authorized by Beckman Coulter.**
- **You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.**

⚠ CAUTION

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

⚠ CAUTION

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. If you purchased this product from a third party and would like further information concerning this topic, call your Beckman Coulter Representative.

Instrument Safety Precautions

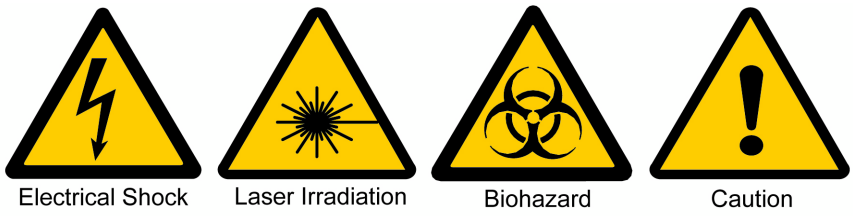
The MoFlo Astrios High-speed Sorter has been engineered with safety as one of its primary features. Safety of the operator, field service personnel, bystanders, and of valuable samples, is paramount to Beckman Coulter's commitment to high performance design and engineering.

This section explains some general safety and hazard symbols and necessary precautions operators of the MoFlo Astrios should follow during operation. Engineering controls have been put in place to protect the operator, and deliberate misuse of the instrument or its instructions may result in unintentional harm. Please follow all safety and hazard instructions as directed in this manual.

Symbols

Below are the symbols used and their corresponding meanings, which can be found on the instrument and throughout this manual.

Safety Symbols



Electrical Shock — Risk of Electric Shock

Laser Irradiation — Avoid looking directly into laser, as it may cause permanent eye damage

Biohazard — Biological Hazard/Risk

Caution — Important; Attention; Refer to Accompanying Documentation

General Safety

To protect the health, environment, and safety of MoFlo Astrios sites and their users, the following information should be reviewed by all operators.

- The MoFlo Astrios is intended for Professional Use Only. All operators should be trained on the proper use and limitations of the instrument prior to its operation.
- Be aware that the Sort Chamber, Illumination Chamber, and cabinet doors on the MoFlo Astrios present possible pinch points. While the doors are light-weight and do not have sharp edges, care should be taken when opening and closing doors.
- The sash on the biosafety cabinet can be moved manually up and down thereby creating a possible pinch point. Position hands appropriately when moving the sash on the biosafety cabinet.
- Be aware of the edges of the biosafety cabinet and instrument base.
- Familiarize yourself with the sample station. The SmartSampler has electronically controlled moving parts. When a sample run is initiated, do not insert your hand in the sample chamber.
- The sample probe on the SmartSampler poses a possible puncture hazard. Use caution when working around the sample probe.
- The input air pressure to the system should never exceed 125 psi. Over pressurization of the SmartSampler chamber can cause an O-ring to blow out causing a very loud, but short pop sound.
- Wear appropriately sized gloves providing good manual dexterity to reduce the likelihood of skin pinches and abrasions.
- Be aware that fluidic and electrical lines are secured with zip ties that can cause skin abrasion if they are contacted with force.
- The nozzle injection tube is exposed when the interior of the nozzle is cleaned. It may cause skin abrasion if it is contacted with force.
- Carefully replace tanks in the fluidics cabinet as to avoid pinching between a heavy tank and the metal supports and quick connect fittings inside the lower enclosure.
- Change the sheath and waste tanks daily and inspect the catch basin for fluid leakage. The catch basin is located below the tanks in the fluidics cabinet. If leakage occurs underneath the instrument covers, it should drain to this location.
- Use proper lifting techniques or seek assistance when handling the UPS, instrument covers, or full tanks. To reduce the likelihood of back injury, empty waste at least once per day.
- Condensation or leakage from the refrigerated water bath can drip on the floor and cause a slipping hazard. The Water Bath Auxiliary cart has the capacity to contain some amount of liquid per EN61010.
- Protect the skin and eyes whenever handling chemicals of any kind, regardless of how benign they may appear.
- Summit workstations include a keyboard interface. Evaluate the ergonomic suitability of the location of the keyboard and the user to avoid injury.
- Cords and cables may be located on the floor around the unit. Drawers and detector assemblies can be positioned by the user. Be aware that these things can cause a tripping hazard.
- Check with the site safety officer for correct disposal of waste products and for spill clean-up protocols.

- The MoFlo Astrios is capable of pressures up to 100 psi (689 kPa). Check sample and sheath pressures when changing nozzle size.
- The user should rest appropriately to avoid strain due to repetitive use, awkward movements, or sitting too long.

Electrical Safety

The MoFlo product line conforms to international regulations encompassing the accessibility of high voltages by the user (IEC 61010-1) and exposure to laser emission: IEC 60825-1:2007 Safety of Laser Products - Part 1: Equipment Classification and Requirements; 21 CFR 1040 FDA/CDRH Laser Product Performance Standard. Please familiarize yourself with the following features of MoFlo Astrios and their corresponding potential hazards:

Safety Interlocks

Under normal operating conditions the MoFlo Astrios protects the user from exposure to high voltages and is considered a Class 1 laser product. The MoFlo Astrios is equipped with three safety interlocks designed to protect the operator from inadvertent exposure to high voltage and laser radiation.

- When the Sort Chamber door opens, the safety interlock disables the voltage to the deflection plates, SortRescue moves into place, sample flow halts, and CyClone movement is stopped.
- When the Illumination Chamber door opens, the safety interlock closes the laser shutters.
- When the latch for the nozzle stage is unfastened and the nozzle stage is raised, the safety interlock closes the laser shutters, stops sample flow, and disables the electrical charge to the nozzle.

DO NOT attempt to defeat these interlocks except when this document specifically instructs you to do so. Ensure that you have the proper laser safety training prior to defeating safety interlocks.

Safety Interlock Override Key

When the Safety Interlock Override Key is used to defeat the safety interlock, there is potential for Class 4 laser exposure up to 700 mW in the 400-700 nm range and up to 100 mW at 355 nm. Do not use this key to override the safety interlock unless you have received laser safety training. Consult your organization's laser safety guidelines for appropriate precautions and personal protective equipment. Consult ANSI publication Z136.1, "Standard for the Safe Use of Lasers."

Illumination (Interrogation) Chamber Safety Interlock Override Key

Stream Charge

- When the sheath stream is charged and individual droplets are formed, the droplets retain the charge present on the stream.
- Do not defeat the safety interlock and insert any object into the charged stream.
- The steel nut connecting the sample line to the nozzle is covered with a protective cap. Do not remove the cap or touch the exposed nut when the stream is charged.

Drop Drive Voltage

This ranges from 0-140 Vac and is used to drive the piezoelectric crystal mounted in the nozzle. The frequency can be set either by IntelliSort or by the operator.

Sort Deflection Plates

The range of voltage applied to these plates is 0-5000 Vdc. This high voltage is present only when the plate voltage is turned on and the interlock is closed. High voltage is accessible only if the interlock is defeated, and only if the operator inserts an object between the charged plates. Once high voltage is enabled by the operator, it is constant until changed by the operator.

⚠ CAUTION

Do not touch the charged plates when power is applied.

Deflection Plate Arcing

Arcing may occur due to build up of sheath solution on the sort deflection plates. If arcing occurs, follow the procedure below to return the instrument to proper working order.

1. Turn off the Plate Voltage.
2. Open the Sort Chamber door. The safety interlock will open.
3. Remove the sort plates and completely dry them using an absorbent material. Alcohol can be used as a final rinse to rid the plates of any water.
4. Wipe off any wet areas of the Sort Chamber.
5. Allow the plates to completely dry.
6. Reattach the sort deflection plates to the instrument, and close the chamber door.
7. Turn on the voltage to the sort deflection plates.
8. Enable the test pattern to assess if an adjustment is required. Adjust the Charge Phase setting if necessary to prevent fanning of the side streams and wetting the plates.

Laser Power Supplies

Laser power supplies have dangerous amounts of energy and could be a hazard to the operator. Contact a Beckman Coulter representative if power supplies require service.

Disposal of Electrical Instrumentation

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that the device:

- Was put on the European Market after August 13, 2005.
- Is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of device.

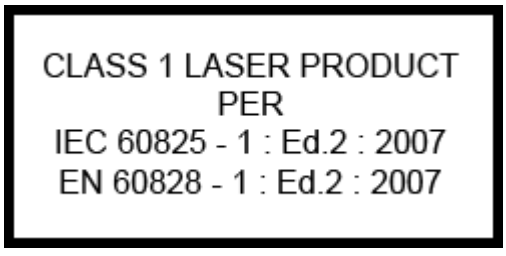
Optical/Laser Safety

Laser Product Hazard Classification

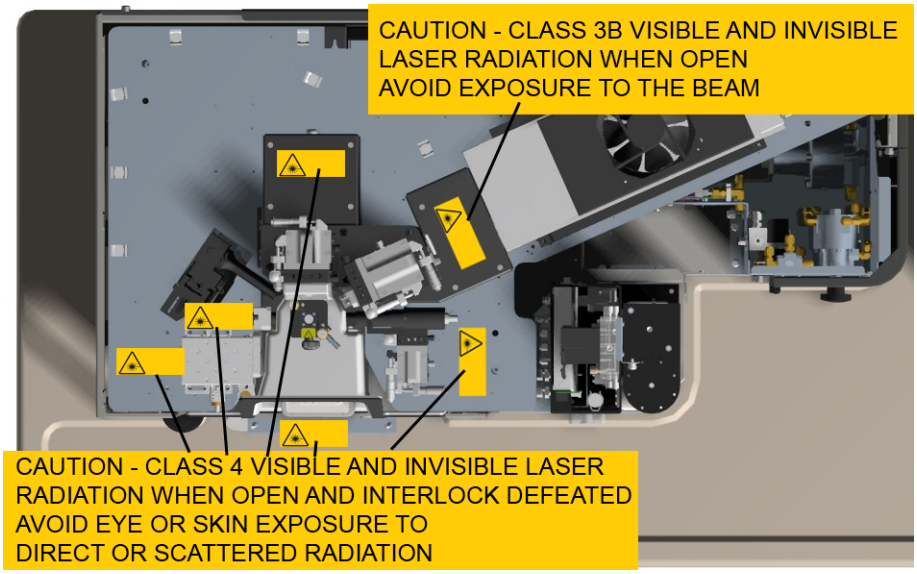
The intent of laser hazard classification is to provide clear distinction of the laser, or laser product properties, and the hazards to users so appropriate protective measures can be taken. MoFlo Astrios is a Class 1 laser product per 21 CFR 1040 and EN60825; meaning operators are not exposed to harmful levels of laser irradiation during normal operation. During times of service and/or repair, laser safety control measures for Class 3B and/or 4 lasers shall be followed.

Remove all jewelry when working with an open beam and do not place shiny or reflective objects into the path of the laser beam as to prevent reflection of the beam in unprotected directions. Use all protective housings, Safety Interlocks, and shields as identified in this manual.

Class 1 Laser Product Label



Location of Laser Safety Warning Labels (Top view with covers off.)



Biological Effects of Laser Irradiation

Eye Injury

Eye exposure to a direct laser beam can cause permanent eye damage including blindness. Laser wavelengths between 400-1400 nm are the most hazardous for retinal eye injury. UV-A lasers (315-390 nm) can cause damage to the lens of the eye contributing to cataracts. Protective eyewear should always be worn when potential exposures to direct laser beams exist, as well as exposure to diffuse UV laser light.

- Do not expose your eyes to the horizontal plane of the laser beam (direct or diffuse).
- Laser safety eyewear should always be available for the corresponding wavelengths and powers of lasers in use.
- Laser safety eyewear shall be worn during laser repair, alignment, or installation, or at any time when direct exposure to the laser beam is possible.

Skin Injury

Skin exposure to direct and diffuse laser light can cause damage. Lasers in the UV-A range (315-390 nm) can cause erythema (sunburn). Exposure in the UV-B range (280-315 nm) can cause the most severe effects, such as sunburn, skin cancer and accelerated skin aging.

- Skin burns caused by lasers can happen quite fast and with great intensity. Protective clothing should be worn when potential exposure to direct and diffuse UV laser beams exists.
- Wear protective clothing (lab coat, long-sleeves) when using UV lasers and when potential exposures to direct laser beams exist.

Biohazard Safety

IMPORTANT If any hazardous organism, material, or agent is used in the instrument, the site operator or Principal Investigator is responsible for informing Beckman Coulter in writing of those hazards before receiving service or repair. This includes a list of all pathogenic cell lines, hazardous reagents, radioactive material, or agents with a BSL Level II or higher. This information will be kept confidential and will be used to inform Beckman Coulter Field Service Representatives of any hazards prior to visiting any MoFlo site. Failure to report this information may delay service on an instrument. Safety of the user as well as safety of Beckman Coulter employees is of overriding importance. Proper decontamination procedures must be followed for all applicable returned parts

- Gloves, a laboratory coat, and eye protection should be worn whenever handling samples including insertion and removal of sample tubes from the sample station.
- If the system loses vacuum or the waste tube becomes clogged, waste fluid could spill into the sort chamber. Immediately turn off sheath and sample flow, wear proper personal protective equipment and attend to the spill.
- Waste fluid may contain hazardous levels of biological and chemical contamination. Gloves, a laboratory coat and eye protection should be worn whenever exposure to waste fluid exists. See APPENDIX A, *Disinfectants for Use in the Waste Tank*.
- To ensure inactivation of biological organisms in the waste tank, an appropriate type and quantity of an EPA registered disinfectant should be placed in the tank initially upon use, and every time the waste tank is emptied and reinstalled.
- The Aerosol Containment Shield, also known as the Sort Chamber door, is part of a passive aerosol containment assembly that isolates the contents of a sort from the rest of the instrument, the operator, and the laboratory. When closed, the door prevents movement of air into and out of the Sort Chamber. It is optional to purchase an Aerosol Evacuation system for additional protection from aerosols. See CHAPTER 2, *Aerosol Evacuation System*.

For additional information on laboratory biosafety, please review the U.S. Department of Health and Human Services, Centers for Disease Control document, *Biosafety in Microbiological and Biomedical Laboratories*. Contact the safety officer at your site and discuss proper waste disposal precautions and practices. Consult the Original Equipment Manufacturer (OEM) manuals for the biosafety cabinet and the Aerosol Evacuation System for additional information.

Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of hazardous waste.

Electromagnetic Information

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in its installation. This equipment generates, uses, and can radiate radio frequency energy. If not installed and used in accordance with the instruction manual this equipment may cause harmful interference to radio communications. If this equipment does cause harmful interference the user will be required to correct the interference. This Class A digital apparatus complies with Canadian ICES-003.

Contents

Illustrations

Tables

Introduction

How to Use Your Manual

Scope

The MoFlo Astrios manual contains basic information regarding the use and operation of the MoFlo Astrios High-speed Sorter and assumes you have received basic training on the instrument. Please contact your Beckman Coulter Representative for information not provided in this manual. This manual does not provide instructions for the installation or upgrade of hardware because such actions must be provided by a Beckman Coulter Representative.

Intended Use

This instrument is intended for research use only.

Use the Instructions for Use manual for the day-to-day running of your instrument and workstation. Go through the detailed step-by-step procedures of startup, quality control (QC), running samples, analyzing data, printing reports, and shutdown before operating the instrument. This manual contains safety and troubleshooting information, as well as procedures for cleaning the instrument and replacing some components.

About this Manual

The information in your Instructions for Use manual is organized as follows:

Chapter 1, Installation
Provides system specifications, lab environment requirements, and the instrument installation recommendations.

Chapter 2, System Overview
Provides an overview of MoFlo Astrios features, system architecture, and subsystems.

Chapter 3, Touch Screen Control Panel
Provides definitions of the screen elements on the instrument control panel.

Chapter 4, Summit Software Overview
Provides basic information regarding the features in Summit software.

Chapter 5, Startup and Shutdown Procedures
Provides the instructions to start and start the MoFlo Astrios.

Chapter 6, Instrument Alignment
Provides information on stream and laser alignment, and laser spot determination.

Chapter 7, Quality Control
Provides instructions on how to follow the automatic Quality Control procedure.

Chapter 8, Sorting and IntelliSort
Provides instructions on how to define a Sort Output Type, set up deflection, verify CyClone positions, perform automatic drop delay determination, enable IntelliSort monitoring, acquire data to set regions and gates, set sort decisions, and configure sorting to a slide, plate or tubes.

Chapter 9, Cleaning and Maintenance
Provides the daily decontamination procedure as well as a yearly fluidics decontamination procedure. Information regarding changing the sheath filter and annual preventative maintenance by a Beckman Coulter representative is also included.

Chapter 10, Troubleshooting and Replacement Procedures
Provides a basic troubleshooting matrix and procedures for replacing customer-replaceable parts. This section also provides instructions on how to coarsely align the lasers, align the Forward Scatter optics, background image subtraction, PMT alignment, and filter layouts.

Appendix A, Approved Cleaners and Disinfectants
Contains a list of cleaners and disinfectants that can be used on the MoFlo Astrios.

Appendix B, Consumables
Contains a list of consumables to be used with the MoFlo Astrios.

Appendix C, Compensation Background Information
Provides information on how to resolve actual intensities from each antibody conjugate in a multicolored sample.

Appendix D, CytoCalc Table
The CytoCalc Table provides suggested starting values for operating pressure, frequency, amplitude, and drop delay that can be used when you are adjusting settings.

Appendix E, Symbols
Defines the symbols used on MoFlo Astrios labels.

CHAPTER 1

Installation

General Laboratory Information

IMPORTANT Your Beckman Coulter Representative is responsible for uncrating, installing, and initial setup of the MoFlo Astrios. Contact your Beckman Coulter Representative before relocating your MoFlo Astrios.

MoFlo Astrios Specifications

Heating and air conditioning vents or fans are not recommended directly above the MoFlo Astrios because of the resulting temperature fluctuation, vibration, and possible dust.

Table 1.1 General System Specification and Environmental Requirements

Specification	Requirements
Service Access	46 cm (18 in.) on left side, 72 cm (36 in.) on right side, no access to the back of the instrument is needed.
Installation Category	II
Pollution Degree	2
Laser Product Classification	Class I Laser Product (IEC/EN60825 -1:Ed.2: 2007)
Instrument Dimensions (not including Auxiliary Components)	Height – 129.5 cm (51 in.) Width – 165 cm (65 in.) Depth – 77 cm (30.5 in.) Weight – 567 kg (1250 lbs)
Electronics Chassis	Height – 49.5 cm (19.5 in.) Width – 35.9 cm (14.1 in.) Depth – 23 cm (9 in.) Weight – 18 kg (40 lbs)
Dimensions Summit Software Workstation	Height – 42.9 cm (16.9 in.) Width – 19.1 cm (7.5 in.) Depth – 45.7 cm (18.0 in.) Weight – 10.5 kg (23 lbs)

Table 1.1 General System Specification and Environmental Requirements *(Continued)*

Specification	Requirements
Humidity and temperature range for instrument storage and operation	15–26°C (59–79°F), not facing direct sunlight 20–80% RH (non-condensing humidity) Maximum 80% RH up to 26°C
Maximum Altitude	Do not operate at an altitude greater than 2000 m (6561 ft)
AC Entry Panel	Height – 43 cm (17 in.) Width – 4.4 cm (1.75 in.) Depth – 17 cm (6.75 in.) Weight – 0.9 kg (2 lbs) Input J1 – 100–230 Vac, 8–3.5 A, 50–60 Hz Input J2 – 100–230 Vac, 15–8 A, 50–60 Hz **Main power is not to exceed ±10% of nominal input voltage.** Output J5 – (UV Laser) 100–230 Vac, 8–3.5 A, 50–60 Hz Output J6 – (Laser Engine) 100–230 Vac, 8–3.5 A, 50–60 Hz Output J7 – (Electronics Chassis) 100–230 Vac, 8–3.5 A, 50-60 Hz

The pneumatic supply, water bath, and aerosol evacuation unit each require a dedicated outlet with an isolated ground. The Summit workstation computer requires a separate outlet, but it does not have to be a dedicated line.

Table 1.2 Regional Electrical Requirements

Country	Dedicated Lines with Isolated Grounds	Non-dedicated Lines
USA	Two dedicated lines at 115 Vac, 50/60 Hz at 15 A UPS output connects to J1 Lasers connect to J2	Three non-dedicated lines at 115 Vac, 50/60 Hz at 15 A - one for the Summit computer, a second for the monitor, and a third for the printer.
Europe	Two dedicated lines at 220 Vac, 50/60 Hz at 10 A UPS output connects to J1 Lasers connect to J2	Two non-dedicated lines at 220 Vac, 50/60 Hz at 10 A - one for the Summit computer, a second for the monitor, and a third for the printer.
Japan	Two dedicated lines at 100 Vac, 50/60 Hz at 15 A UPS output connects to J1 Lasers connect to J2	Two non-dedicated lines at 100 Vac, 50/60 Hz at 15 A - one for the Summit computer, a second for the monitor, and a third for the printer.

Table 1.3 System Noise

Component	Noise Level
MoFlo Astrios System	<70 dB
Aerosol Evacuation System	Fan running at maximum power 62 ±2 dB

System Connections

Figure 1.1 AC Entry Panel Connections

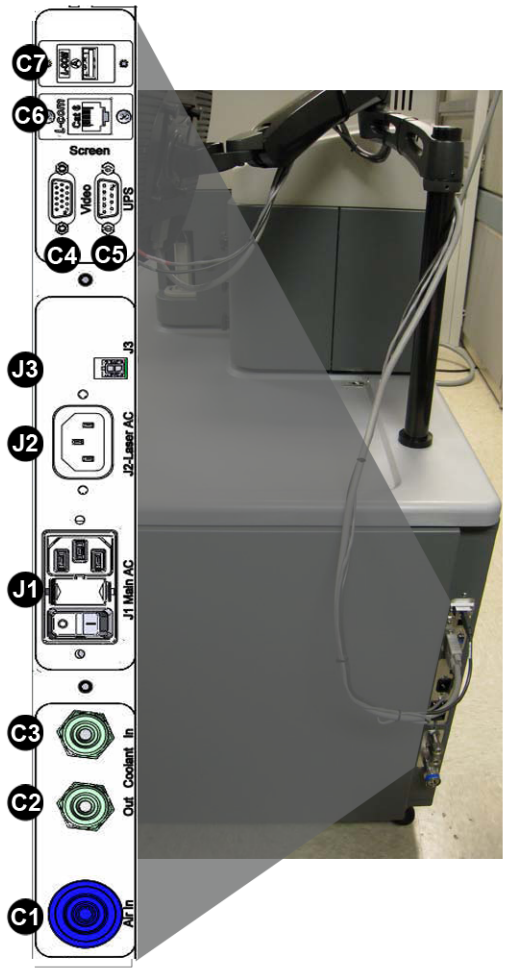


Table 1.4 AC Entry Panel Connections and Definitions

C1	Main Air IN (either Jun Air or house air) Do not set air pressure above 125 psi.
C2	Cooling Water OUT
C3	Cooling Water IN
C4	Touch Screen Monitor connection
C5	Not used
C6	Network Crossover Cable to the Summit Workstation Computer
C7	USB Touch Screen connection
J1	Main Chassis ON/OFF Power connection and main AC input from the UPS
J2	Laser Power connection plugs directly into a wall outlet
J3	Touch Screen Power connection

Figure 1.2 Astrios Electronics Chassis Connections Drawing

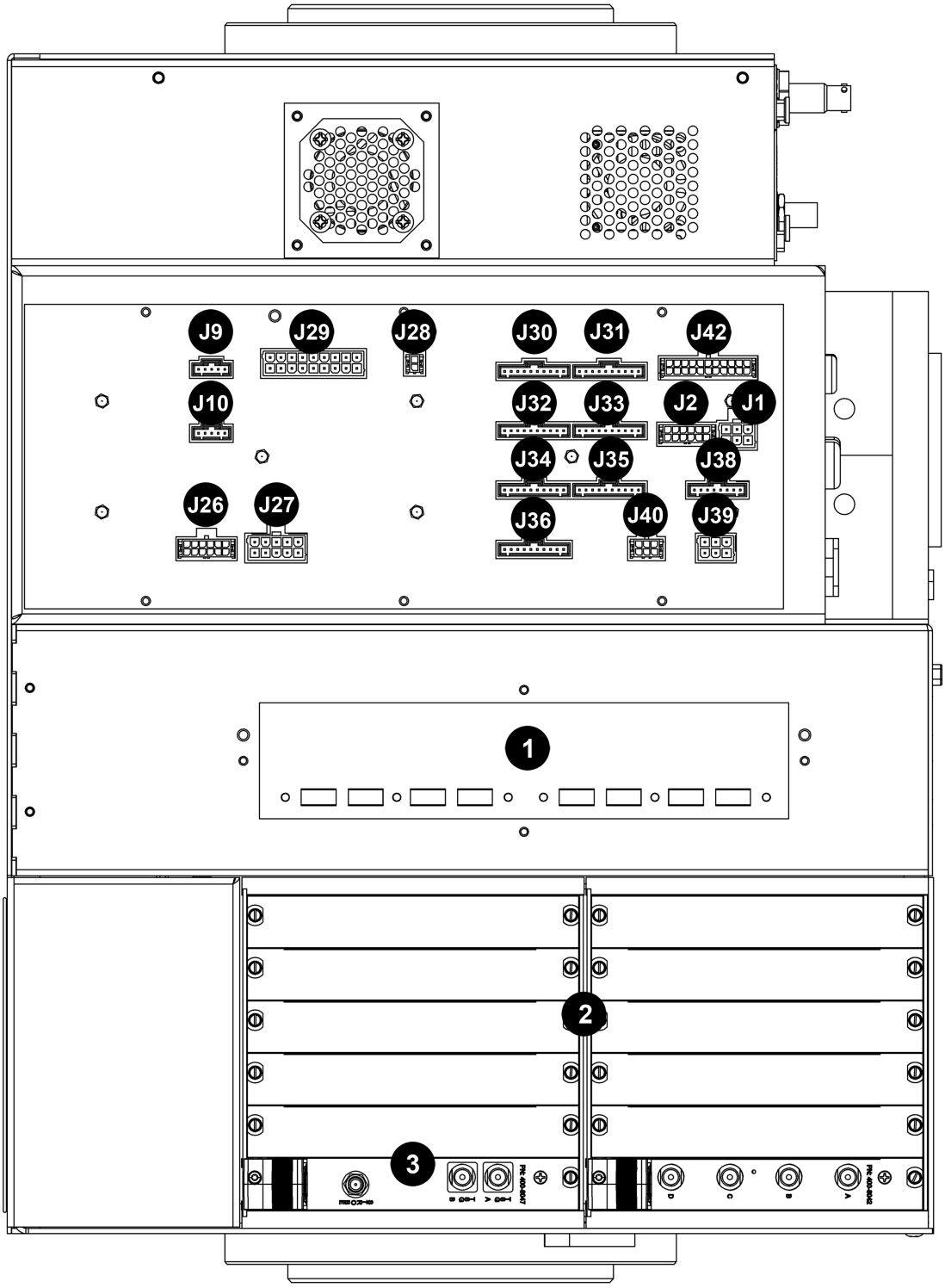


Table 1.5 Astrios Electronics Chassis Connections Labeled

J1	Not used
J2	Not used

Table 1.5 Astrios Electronics Chassis Connections Labeled *(Continued)*

J9	UV Laser Control
J10	Wired but not used.
J26	Fluidics Load Cell (waste and sheath) connector
J27	Pneumatics connector
J28	Bio-safety Hood Interface connector
J29	Upper Distribution Panel Power connector
J30	POD 1 Preamplifier Control connector
J31	POD 2 Preamplifier Control connector
J32	POD 3 Preamplifier Control connector
J33	POD 4 Preamplifier Control connector
J34	POD 5 Preamplifier Control connector
J35	POD 6 Preamplifier Control connector
J36	POD 7 Preamplifier Control connector
J38	Laser Engine Control connector
J39	AC Entry and Touch Panel Control connector
J40	System Power Switch and LED Illumination connector
J42	Power Supply Assembly Control connector
1	USB connections used for: Upper Distribution Board, Laser Engine, AC entry P (Touch Panel)
2	Analog to Digital Cards (ADCs)
3	Sort card

Figure 1.3 Summit Workstation Connections Photo

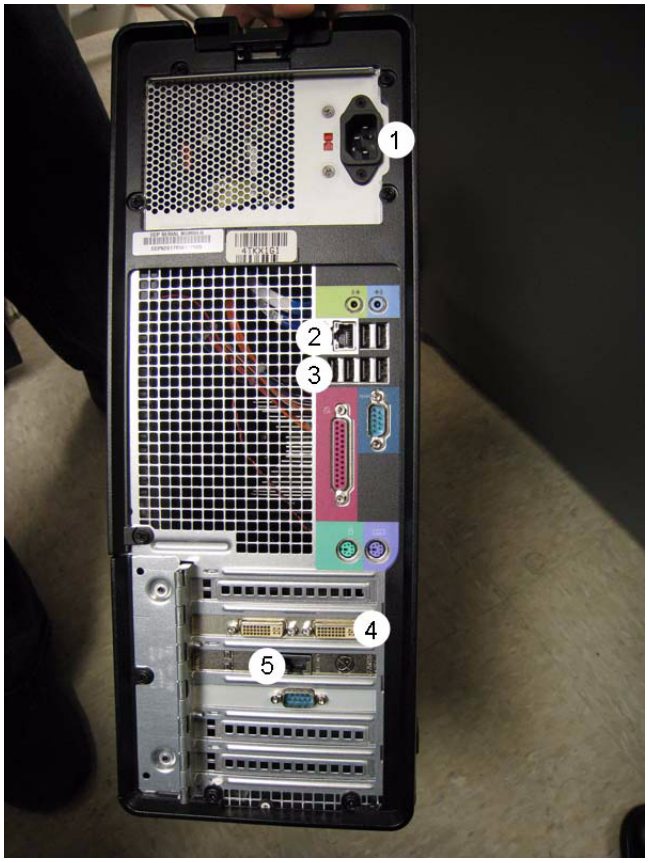


Table 1.6 Summit Workstation Connections Labeled

1	AC Power cable connects to the UPS.
2	Crossover cable connects to the bulkhead located on the rear right corner of the instrument table.
3	USB cables connect the mouse and keyboard.
4	Monitor cable connects to the monitor.
5	Optional network cable connects to the laboratory's network.

Installing Summit Software

Summit software will be installed by Beckman Coulter personnel upon instrument installation. To install Summit software on additional computers, insert the CD into the CD-ROM and follow the on-screen prompts.

CHAPTER 2

System Overview

Overview of the MoFlo Astrios System

MoFlo Astrios is a research instrument that analyzes and sorts single-cell suspensions of cells and other similarly sized particles.

The instrument achieves an acquisition rate of 100,000 particles per second and a sort rate of 70,000 sort decisions per second. Electronics and 32-bit software can acquire more than one billion events and store the information in a single data file.

The system can be configured with up to six fiber-coupled lasers and a free-standing ultraviolet (UV) laser, each directed to its own spatially-separated collection path. A flat-top beam shaping optic simplifies alignment of the fiber-coupled lasers and delivers focused laser power to the sample stream. Each laser can be configured with up to six detectors. However, when multiple lasers are used, a maximum of 32 simultaneous color parameters can be analyzed for each sample run. Computed parameters based on collected data can be created to provide a 20 x 20 compensation matrix.

The Automatic Quality Control (QC) feature allows the operator to monitor daily system performance for all available parameters, view on-screen results, create QC reports, and track instrument performance over time.

Summit software is used for acquiring, sorting, and analyzing flow cytometry data.

IntelliSort provides fully-automated sort setup including droplet optimization, bead-free drop delay determination, and sort monitoring.

Sample can be sorted into one, or up to six, temperature-controlled tubes. Alternatively, sample can be sorted into one of five standard-size, temperature-controlled, microplates, as well as standard microscope slides. It is also possible to customize sort output using CyClone.

SortRescue is a custom tray that protects samples before, during, and after sorting, and captures spray in fault conditions.

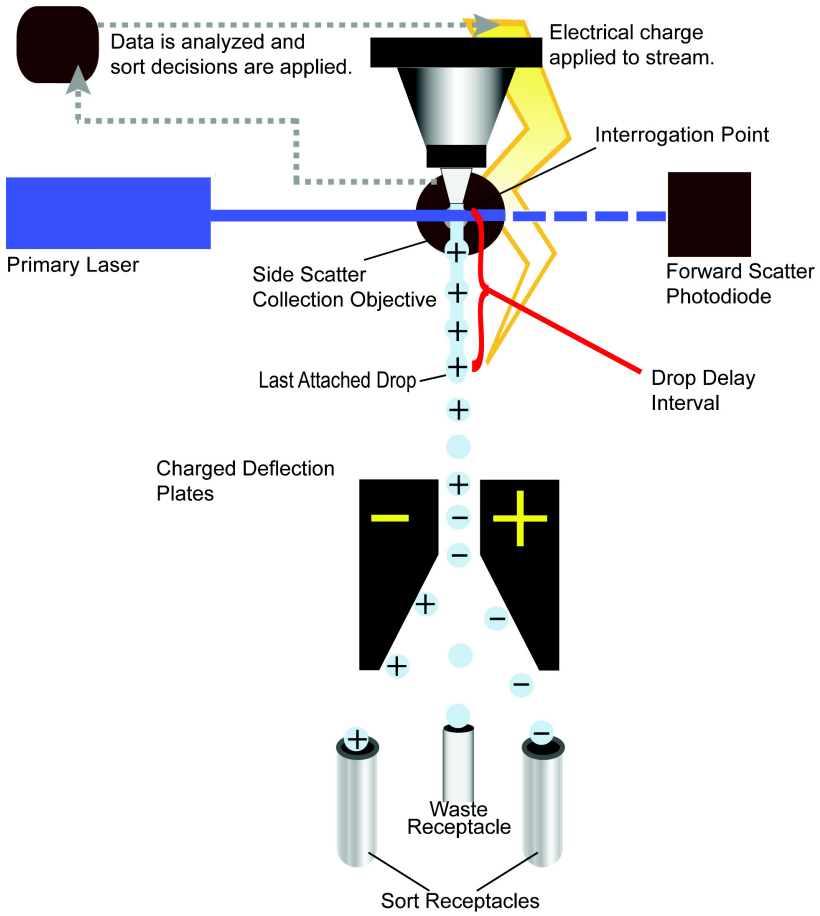
Indexed sorting allows the user to view a data file and observe the location of sorted particles within a plate that is represented graphically on screen.

General Principles of Operation

MoFlo Astrios examines individual particles that are propelled in a buffered saline solution through one to seven spatially separated laser beams of differing wavelengths. If the properties of the particle or fluorescent dye added to the particle are excited by the wavelength of laser light, the particle emits broadband fluorescence and scattered light. The emitted light is collected, focused, reflected, and filtered so that discrete wavelengths of light are detectable by photomultiplier tubes (PMTs). The PMTs convert the light signals to electronic signals that are sent to the instrument electronics. Data is then acquired by Summit software according to the parameters set by the operator.

To sort, MoFlo Astrios acquires data and consults sort decisions as defined by the operator. The nozzle applies a positive or negative charge to the sheath stream based on an event and the sort decisions. During this time, a piezoelectric crystal in the nozzle continually vibrates to break the charged stream into droplets. Charge plates positioned on either side of the droplet stream attract or deflect the charged droplets into the appropriate receptacles.

Figure 2.1 Principles of Operation Diagram



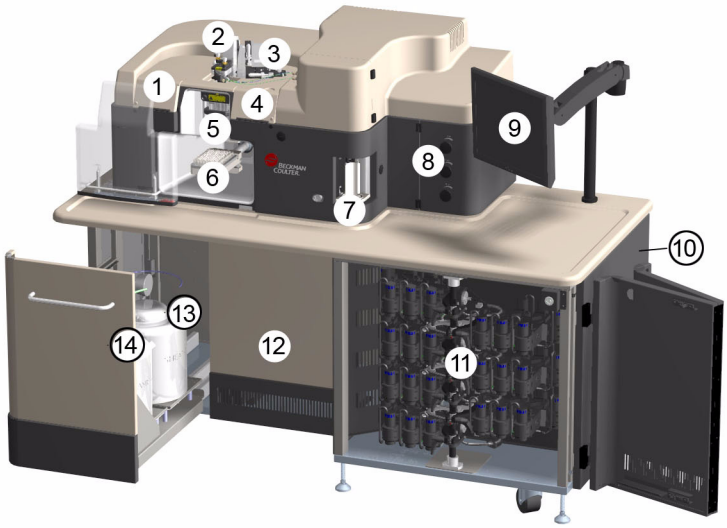
System Layout

MoFlo Astrios is designed with workflow, operator safety, sample isolation, and ergonomics in mind. All components that require operator interaction are accessible from the front of the instrument.

The upper portion of the instrument includes the flat-top Fiber-coupled Beam Shaping Optic (FBSO) attached to the fiber optics that extend from the laser engines, forward scatter sensors, the UV laser and BSO, nozzle, sample input, pressure console, alignment micrometers, high-voltage deflection plates, Sort Chamber, CyClone, and the Touch Screen Control Panel.

Sheath and waste tanks are stored in the fluidics drawer on the left side of the lower enclosure. Beneath each tank is a load cell that allows the instrument to monitor sheath and waste volume. The lower-right enclosure houses the Precision Optical Detectors (PODs) that contain the Photomultiplier Tubes (PMTs) and filter sets for the lasers. The PODs rotate forward and out of the enclosure when access to the detectors is necessary.

Figure 2.2 MoFlo Astrios Annotated

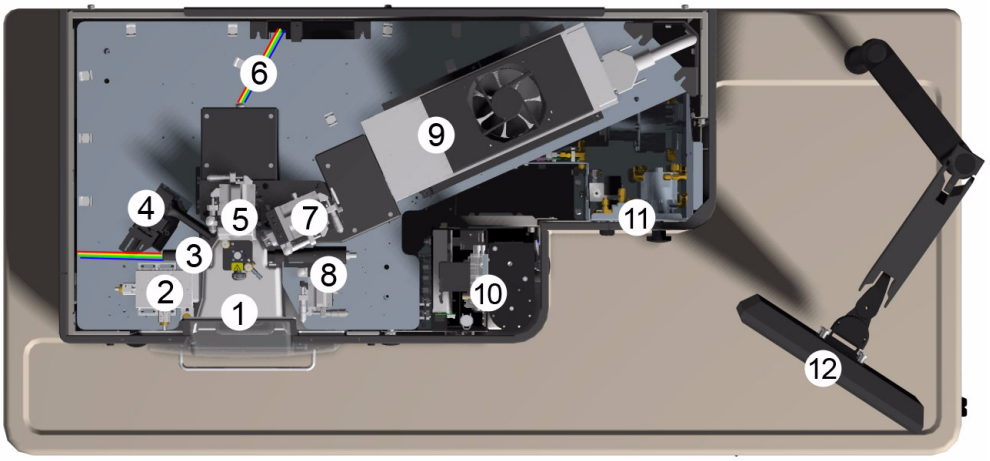


1.	Fiber-coupled Beam Shaping Optic (FBSO) Positioning Micrometers (behind panel)
2.	Nozzle Positioning Micrometers and Gimbals
3.	UV Laser BSO Positioning Micrometers
4.	Forward Scatter Sensor Positioning Micrometers
5.	High-Voltage Deflection Plates
6.	CyClone (with microplate attached)
7.	SmartSampler
8.	Pressure Console
9.	Touch Screen Control Panel
10.	Fiber-Coupled Lasers (behind panel)
11.	Detection PODs and PMTs
12.	Electronics (behind panel)

13.	Sheath Tank
14.	Waste Tank

Light from the fiber-coupled lasers is focused and delivered to the stream via the FBSO. The FBSO adjustment micrometers as well as the micrometers for the Forward Scatter Detector are enclosed behind front-access doors because daily alignment is not necessary The UV laser BSO and the nozzle have dedicated alignment stages that are exposed to the operator.

Figure 2.3 Upper Enclosure Annotated



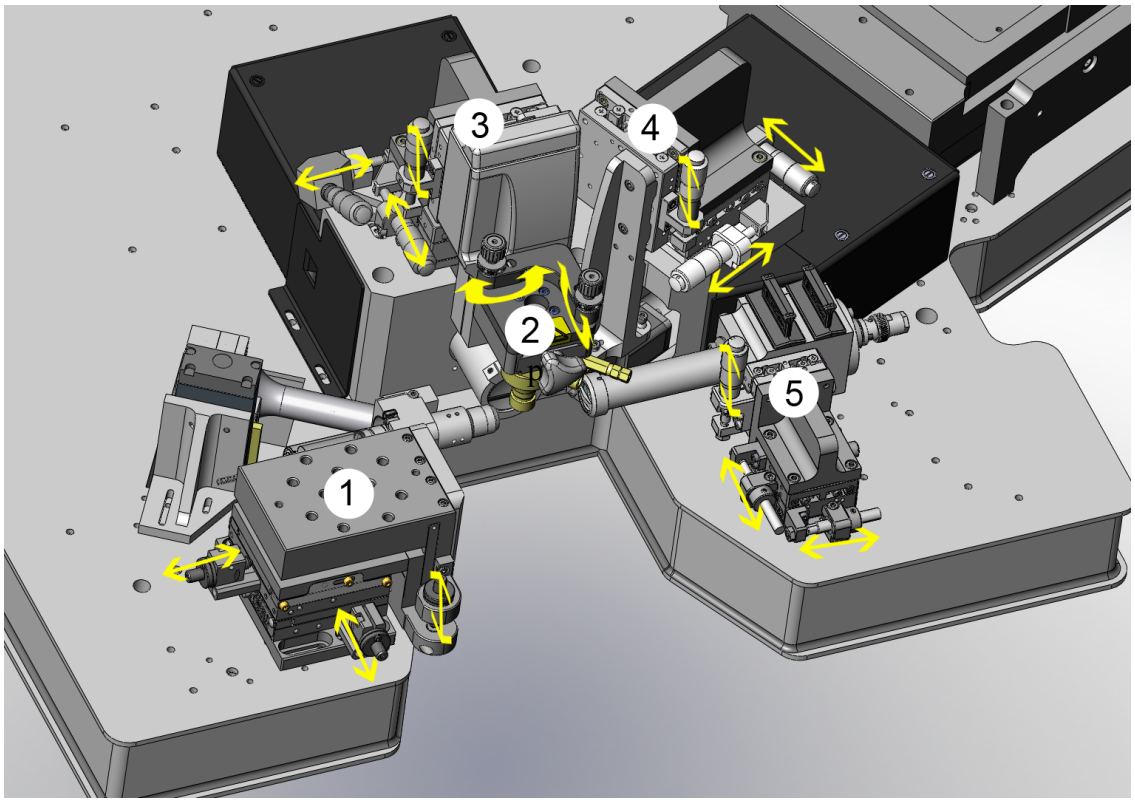
1.	Sort Chamber
2.	FBSO Positioning Micrometers
3.	FBSO with Fiber Optics
4.	IntelliSort Camera
5.	Nozzle Positioning Micrometers
6.	Fiber Optics delivering emitted and scattered light to the PODs.
7.	UV Laser BSO Positioning Micrometers
8.	Forward Scatter Positioning Micrometers
9.	UV Laser
10.	SmartSampler
11.	Pressure Console
12.	Touch Screen Control Panel

The electronics and the fiber-coupled lasers are located in the lower enclosure and do not require operator interaction.

Micrometer Positioning Controls

The positioning controls provide fine-movement control of the beam shaping optics for the fiber-coupled lasers (FBSO), the nozzle, the beam shaping optics for the free-standing UV laser, and the Forward Scatter Collection sensor.

Figure 2.4 Positioning Micrometers (Instrument Cover Removed)



1.	Positioning stage for the Fiber-coupled beam shaping optics (FBSO). These micrometers rarely require adjustment.
2.	Positioning gimbals for the nozzle rock the stream from left to right and from front to back.
3.	Positioning stage for the nozzle.
4.	Positioning stage for the free-standing UV laser BSO.
5.	Positioning stage for the Forward Scatter Collection sensor.

Illumination

As cells in the sample and sheath stream intersect with the laser beam, they illuminate. The cells scatter laser light and emit fluorescent light if they have been treated with reagents that fluoresce.

Fiber-Coupled Lasers

The fiber-coupled lasers are housed in two laser engines in the lower enclosure. Fiber optics that extend from the laser engines deliver laser light to the FBSO, which focuses the laser beam onto the sample and sheath stream.

Laser Separation

The separation between lasers when they intersect with the sheath and sample stream is 127 ± 3 µm.

Laser Spot Size

Horizontal: Flat top with half width of approximately 35-55 µm.

Vertical: Gaussian beam of 5-15 µm, $1/e^2$.

Figure 2.5 Fiber-Coupled Lasers

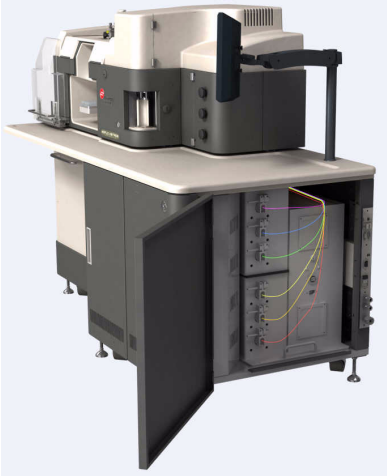
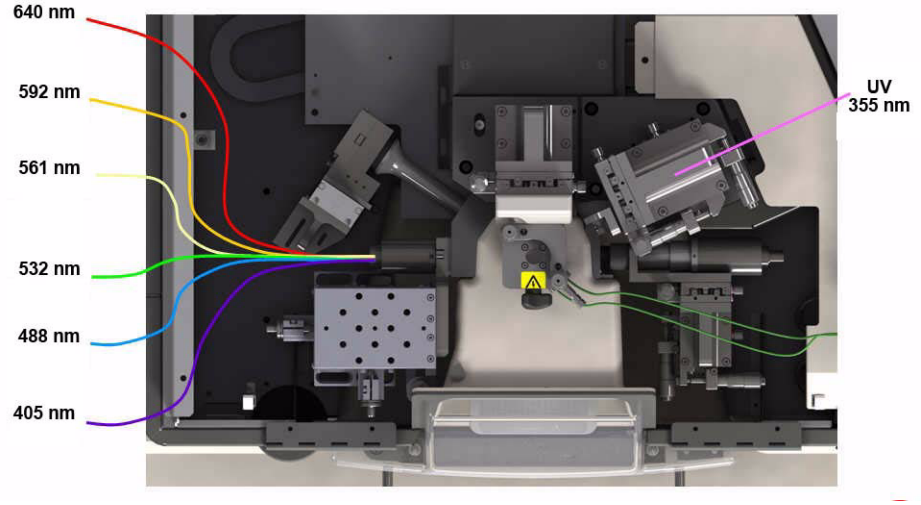


Figure 2.6 Laser Fibers Entering the Upper Encloser, Free-standing UV Laser



Ultraviolet Laser

The ultraviolet (UV) laser is a 355 nm, solid-state, software controlled laser operating at 100 mW. It is located on the right side of the upper enclosure. See Figure 2.3. The UV laser is the only laser that the operator should align daily, and therefore, the positioning micrometers for the UV BSO are exposed. While fluorescent parameters may be collected using the UV laser, side scatter parameters cannot be collected.

NOTE When the UV laser is run inside a biosafety cabinet, Beckman Coulter will honor the warranty only if the instrument decontamination procedure is run four, or fewer, times per year.

Laser Separation

When properly aligned by the operator, the separation between UV laser and the closest fiber-coupled laser is 127 ± 3 µm at the intersection with the sheath and sample stream.

Laser Spot Size

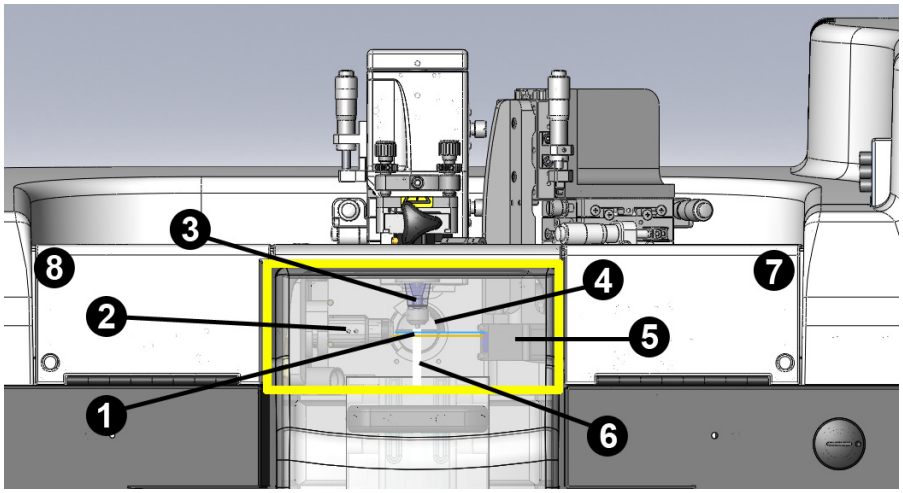
Horizontal: Gaussian beam of 50 µm, $1/e^2$

Vertical: Gaussian beam of 25 µm, $1/e^2$

Illumination Chamber

The Illumination (or Interrogation) Chamber is the area of the instrument where the sample and the sheath stream intersect with laser light. This point of intersection is known as the interrogation point. Light is collected by the Forward Scatter Collection and the Side Scatter Collection sensors.

Figure 2.7 Illumination Chamber Annotated



1.	Interrogation Point - the point at which the stream and laser light intersect.
2.	Fiber-coupled Beam Shaping Optic (FBSO) attached to the fiber-coupled lasers.
3.	Nozzle - delivers sheath and sample stream, charges the stream, vibrates to create droplets.
4.	Side Scatter Collection objective - collects light scattered at a 90 degree angle as well as emitted fluorescent light.
5.	Forward Scatter Collection objective - collects light scattered at narrow angles to the axis of the laser beam.
6.	Sheath and Sample Stream
7.	Door that covers the Forward Scatter Collection Objective Micrometers and Filters
8.	Door that covers the FBSO Micrometers

Forward Scatter Light Collection

The Forward Scatter objective collects laser light that is scattered at narrow angles to the axis of the laser beam. It is located on the right side of the instrument directly across from the FBSO. See Figure 2.7 and Figure 2.8. The signal generated by the forward scattered light is proportional to the size of the cell that was illuminated by the laser.

The Forward Scatter objective includes inter-changeable scatter bar caps and two filter slots intended for a wavelength specific filter and a neutral density filter. It is possible to acquire forward scatter information using any one of the fiber-coupled lasers and its corresponding wavelength filter. Forward Scatter bar caps are available in sizes 3.0 mm to 7.5 mm.

NOTE In general, a 70 µm tip, with the instrument running at 60 psi, can be fitted with the 4.5 mm SSC bar and the 7.0 mm FSC bar. Ideal combinations will vary according to the nature of the application.

Figure 2.8 Forward Scatter Collection Objective

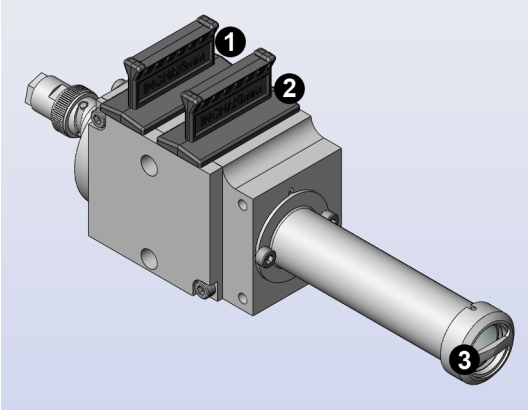


Table 2.1 Forward Scatter Collection Objective Filters and Obscuration Cap

1.	Forward Scatter Wavelength Filter
2.	Neutral Density Filter
3.	Forward Scatter Bar Cap

Side Scatter Light Collection

The Side Scatter Collection objective is placed at a right angle to the fiber-coupled laser beam and the stream intersection. See Figure 2.7. Side scattered light and fluorescence are collected by the Side Scatter Collection objective. The amount of side scattered light is proportional to the granularity of the cell that was interrogated by the laser. In addition to side-scattered light, cells emit fluorescent light at all angles to the axis of the laser beam. Fluorescent emission enables the instrument to measure characteristics of the cells, such as cell-surface antigens. The Side Scatter objective includes inter-changeable scatter bar caps that are bow tie shaped with the narrowest dimension measured in sizes 3.0 mm to 7.5 mm.

NOTE In general, a 70 µm tip, with the instrument running at 60 psi, can be fitted with the 4.5 mm SSC bar and the 7.0 mm FSC bar. Ideal combinations will vary according to the nature of the application.

Detection

Pinhole Camera and Seven Pinhole Aperture

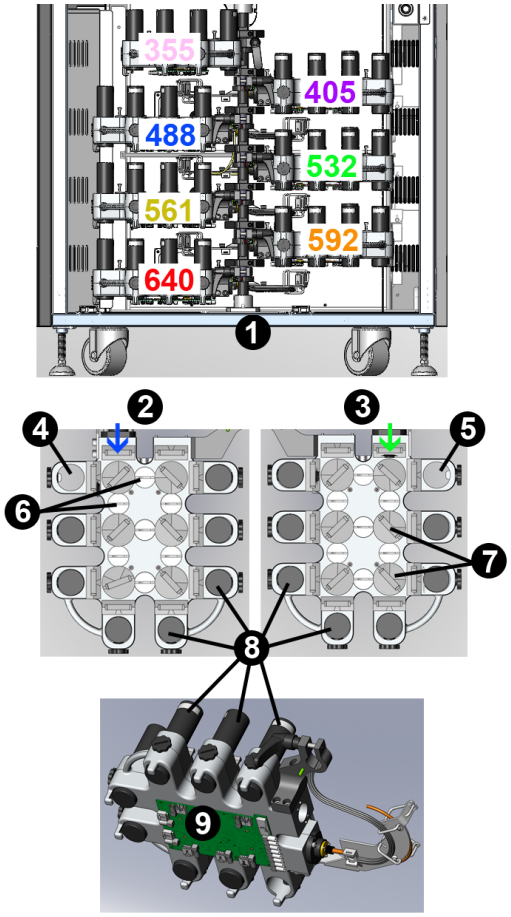
The pinhole camera makes it possible to view the seven pinhole apertures on the Coarse Alignment screen of the Touch Screen Control Panel. Upon installation, a Beckman Coulter representative will align the laser beams coming from the fiber-coupled lasers and through the FBSO to the appropriate spatially-separated pinholes. The alignment of the fiber optics should not need further adjustment by the operator. The UV laser will be aligned through the seventh pinhole. The UV laser may need to be realigned periodically by the operator.

Figure 2.9 Pinhole Screen

Precision Optical Detector (POD)

Seven Precision Optical Detectors (PODs) can be included in the MoFlo Astrios system. A standard MoFlo Astrios configuration dedicates each laser wavelength to a dedicated POD. See Figure 2.10. One preamplifier is attached to the base of each POD. A POD is capable of housing seven PMTs as well as the required dichroic filters and mirrors.

Figure 2.10 PODs, PMTs and Preamplifiers



1.	PODs and the laser wavelengths that are assigned to them.
2.	Left side POD
3.	Right side POD
4.	Left side empty PMT holder (not used)
5.	Right side empty PMT holder (not used)
6.	Light containment gates
7.	Dichroic filters and mirrors
8.	PMTs
9.	Preamplifier board

Collimating Lens

Emitted light passes through a Collimating Lens immediately before entering a POD. The collimated light permits signals of approximately equal intensity to reach each PMT along the detection path in the POD.

Dichroic Mirrors and Optical Filters

Dichroic mirrors and optical filters are designed to block, pass, or reflect light of certain bandwidths and in the case of the dichroic filter, reflect and pass light of different wavelengths at the same time. Filters are either made from dyed glass, which will absorb certain wavelengths of light, or metallic coatings that have been vapor deposited on a glass substrate. The coated filters function by internal reflection and interference between the metal deposition layers. The list below describes the features of some commonly used filters in flow cytometry.

IMPORTANT The Astrios filter sets are designed to optimize emitted light while reducing compensation for each laser path. We recommend any changes to the standard filter configuration or addition of custom filters be evaluated by the operator prior to use.

The Astrios filter sets and instrument are designed for the standard laser wavelengths offered. Any future additions of wavelengths may require filter changes to optimize performance.

- Band Pass Filters transmit light within a defined spectral band ranging from less than one to many nanometers wide.
- Long Pass and Short Pass filters transmit above or below a certain cut-on or cut-off wavelength and continue to transmit a wide energy band.
- Dichroic Beam splitters are used at a non-normal angle (usually 45 degrees). The long pass and short pass dichroic filters are designed for optimal reflection of one specified region of the spectrum and high transmission of another.
- Neutral Density Filters will uniformly attenuate the intensity of light over a broad spectral range.
- Rejection Band filters are designed to block a narrow spectral band, such as a monochromatic light from a laser while transmitting other wavelengths efficiently.

Standard 25 mm diameter short-pass and long-pass dichroic mirrors and band-pass optical filters are positioned at various points in each POD. These filters are selected to pass only the emission spectra that the PMT is intended to receive. See CHAPTER 10, *Filter Alignment Diagrams*.

Photomultiplier Tubes (PMTs)

Photomultiplier Tubes accept emitted light, focus and multiply the signal, and convert the light into electrical current that is then output to a preamplifier that is located under each POD. The PMTs have a 185 nm to 900 nm spectral range.

The operator adjusts PMT voltages and gains through the Touch Screen Control Panel or the Acquisition tab in Summit software.

Preamplifiers

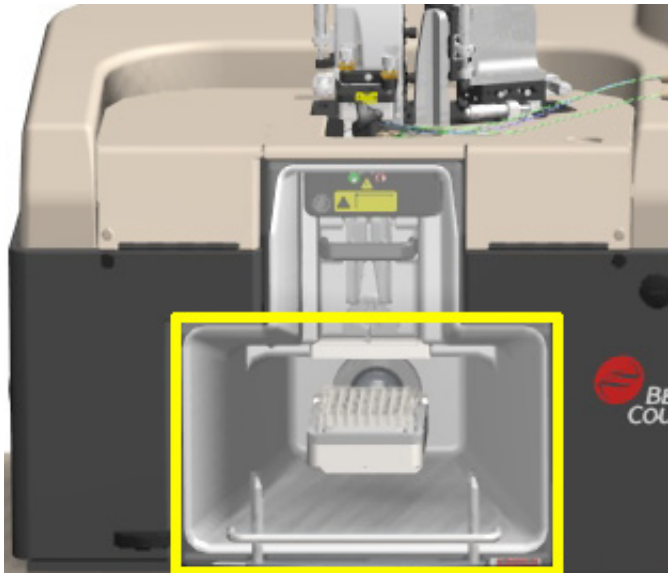
The underside of each POD is fitted with a dedicated preamplifier. See Figure 2.10. The preamplifiers control the PMTs to adjust detector gain, and convert current output into voltage output that can be analyzed by the Analog to Digital Converter Cards (ADCs.) Each preamplifier can control and interface with seven PMTs.

Cell Sorting

Sort Chamber and Aerosol Containment Shield

The Sort Chamber is located in the upper enclosure. It is well lit, and designed for easy access and cleaning. The Aerosol Containment Shield, also known as the Sort Chamber door, is part of a passive aerosol containment assembly that isolates the contents of a sort from the rest of the instrument, the operator, and the laboratory. When closed, the door prevents movement of air into and out of the Sort Chamber. When the door is opened, the safety interlock disables the voltage to the deflection plates and halts CyClone movement.

Figure 2.11 Sort Chamber and Aerosol Containment Shield



CyClone

The CyClone is located in the Sort Chamber. See Figure 2.11. CyClone includes four accessories that accommodate microscope slides, and a variety of disposable tubes and microplates. Pre-configured sort output definitions determine plate voltage and defanning to automatically direct sort streams to the appropriate receptacles.

Table 2.2 CyClone Accessories for Sort Output

Plate and Slide Holder	6-well flat bottom microplates
	24-well flat bottom microplates
	96-well flat bottom microplates
	384-well flat bottom microplates
	1536-well flat bottom microplates
	NOTE All microplates were verified using Corning Costar™ flat bottom microplates. The operator should empirically confirm compatibility when using microplates from other manufacturers.
5 mL Tube Holder	Holds up to six tubes.
15 mL Tube Holder	Holds up to two tubes.
50 mL Tube Holder	Holds up to two tubes.
50 mL & 5 mL Holder	Holds one 50 mL and up to four 5 mL tubes.

Sample Cooling

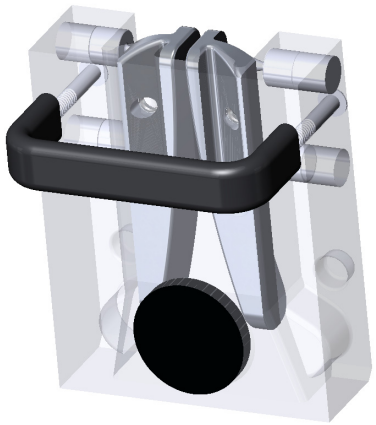
The CyClone and accessories are designed with built-in sample cooling capability that can be used if the optional Haake Water Bath console is purchased. The Water Bath console is a stand-alone unit placed next to the instrument. Temperature controlled water flows from the console through the CyClone arm then through the body of the tube or plate holder. The operator selects a constant, regulated temperature at which to maintain samples.

Deflection Plates

The Deflection Plates, located in the Sort Chamber, provide the electric field that deflects individually charge droplets into the appropriate receptacles. These plates can be polarized with up to 5000 Vdc. Caution should be exercised when the plate voltage is enabled. The Sort Chamber door and the safety interlocks prevent access to the plates when they are energized.

The Deflection Plates are designed to be easily removed and cleaned. The operator can use the handle on the Deflection Plate block to pull the block out of the Sort Chamber. The individual charge plates slide out for cleaning.

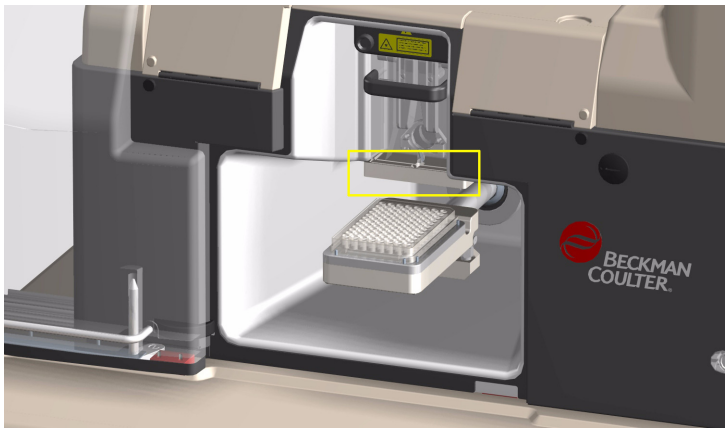
Figure 2.12 Deflection Plates Block Assembly



SortRescue

The SortRescue tray is located between the Deflection Plates and the sort output. During normal operation, the SortRescue tray is retracted so that sorted sample can be deposited in the appropriate tube or plate well. In the event that IntelliSort detects a sort failure, SortRescue extends to protect the sample that has already been sorted. See Figure 2.13. SortRescue can be removed for cleaning.

Figure 2.13 Sort Rescue Extended



IntelliSort

During a sort setup, IntelliSort makes use of the IntelliSort camera and software to automatically optimize droplets, and determine drop delay without the use of calibration particles.

When a sort is in process, IntelliSort monitors the droplet stream for instability. Several factors can alter droplet stream stability including ambient temperature, fluid temperature, and pressure changes. If IntelliSort detects instability, it modifies control parameters to ensure that the sort continues uninterrupted and without operator intervention.

In the event that IntelliSort detects a dramatic sort failure, sample flow is stopped, and SortRescue Figure 2.13 moves into place to protect the sorted sample.

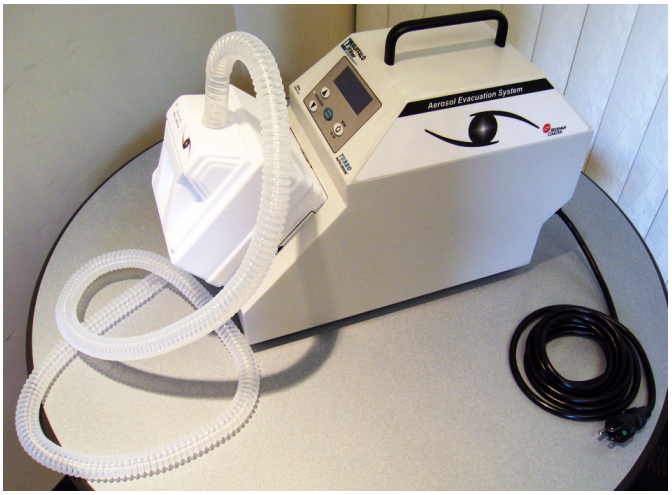
Streams Camera and Streams Screen

The Streams Camera and the Streams screen, on the Touch Screen Control Panel, make it possible to view the sort streams in order to direct them to their targeted sort output device and to send waste to the waste aspiration tube. See CHAPTER 3, *Deflection Tab*.

Aerosol Evacuation System

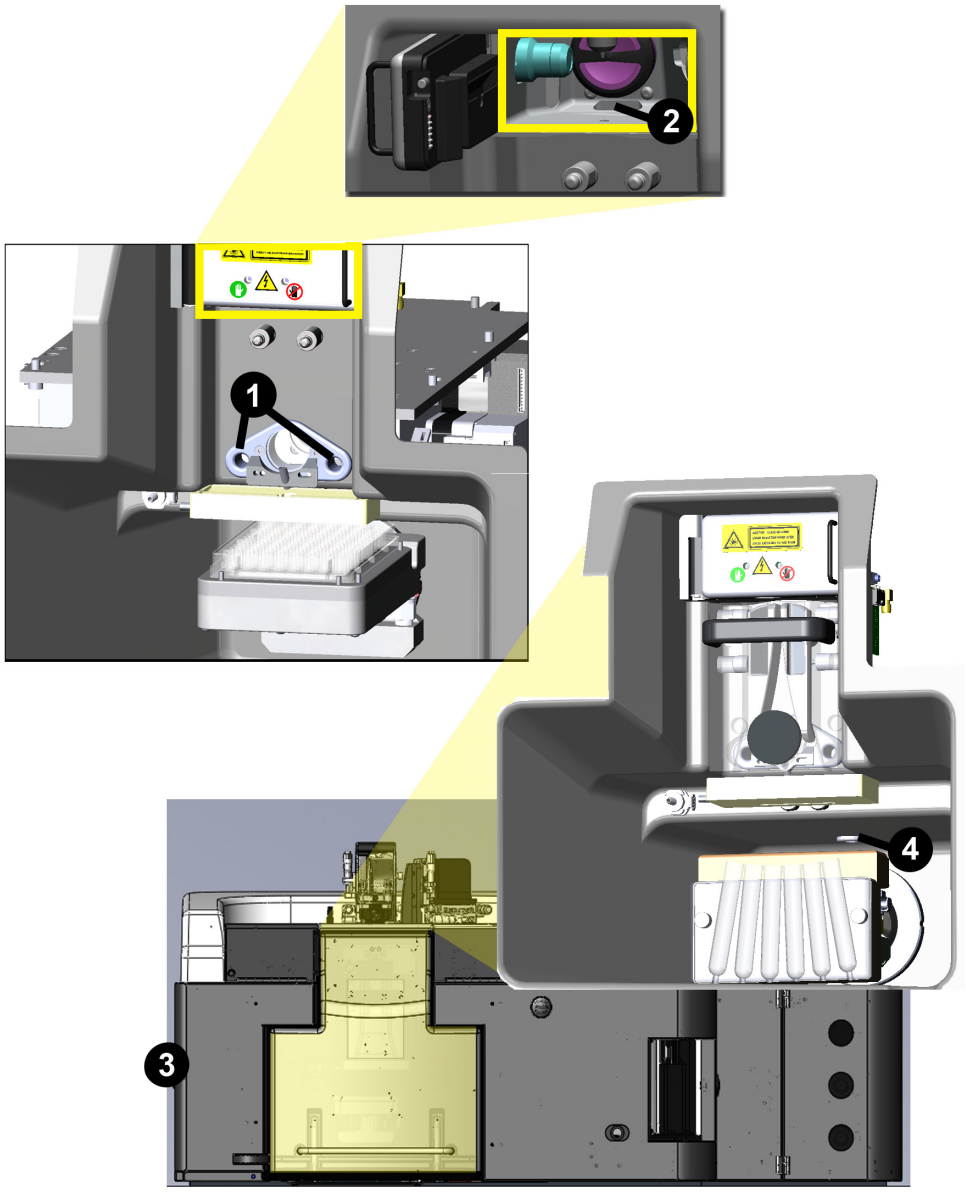
The optional Aerosol Evacuation system removes aerosols and micro droplets, generated during the course of normal operation or a sort failure, from the sort chamber. The system makes use of a high-suction, high-flow-rate centrifugal action pump to remove particles greater than 0.12 µm and trap them in an Ultra Low Penetration Air (ULPA) filter. The flow rate of the Aerosol Evacuation System is user adjustable, providing clearance of the sort chamber at rates of 5 to 15 complete air exchanges per minute. The filter is completely enclosed to protect the operator from potential contamination when filters are changed.

Figure 2.14 Aerosol EVacuation Console



The Aerosol Evacuation system vacuums aerosols from ports in the Interrogation Chamber and the Sort Chamber Figure 2.15 and vents them out the left side of the instrument trapping them in the filter on the front of the unit.

Figure 2.15 Aerosol Evacuation Vents



1.	Two vents located in the Sort Chamber behind the Deflection Plates.
2.	One vent located in the bottom of the Interrogation Chamber.
3.	A vacuum hose vents aerosols from the instrument to the filter on the Aerosol Evacuation unit.
4.	One vent located in the far back of the Sort Chamber.

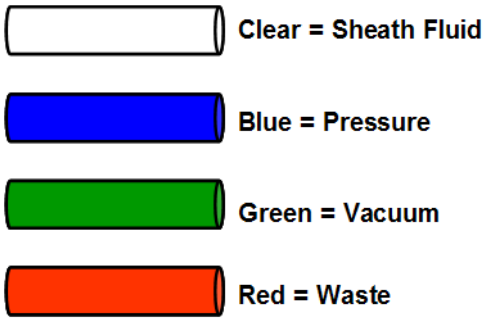
Fluidics

Tubing

The MoFlo Astrios system contains tubing of four different colors. The color of a tube determines the function of the tubing. This can be useful in tracking the origin or destination of a particular tube.

- Clear - The clear tubing carries filtered and unfiltered sheath fluid. It is also used for the rinse function in the SmartSampler.
- Blue - The blue tubing indicates a pressure line.
- Green - The green tubing is used for Vacuum that runs from the pressure console to the waste tank.
- Red - The red tubing carries all waste back to the waste tank from the SmartSampler and the waste tube in the Sort Chamber.

Figure 2.16 Tubing Colors

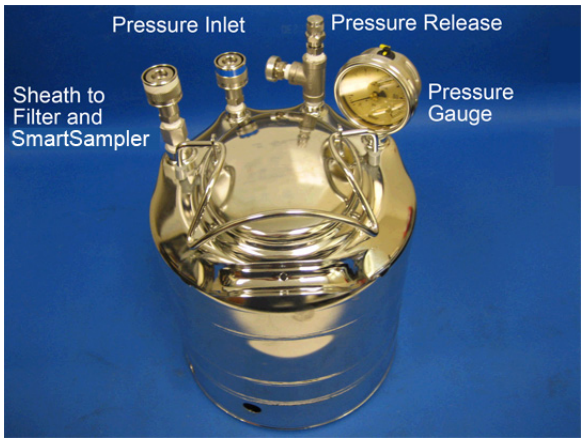


NOTE The PEEK tubing from the SmartSampler does not follow this color-coded convention. Sheath tubing on the SmartSampler is green and the sample tubing is blue.

Sheath Tank

Sheath fluid is stored in an autoclaveable, two-gallon, electroplated, stainless-steel tank located on the left side of the lower enclosure. The sheath pressure gauge and relief valve, as well as fittings for sheath fluid supply and sheath pressure lines, are mounted on the sheath tank. See Figure 2.17. All fittings are provided with color-coded quick connects to enable reliable and fast connection. Sheath fluid is transported to the SmartSampler through clear sheath tubing. An in-line sheath filter is located between the tank and the SmartSampler to filter particles larger than 0.2 µm. Sheath flow is controlled through the Touch Screen Control Panel and the status of sheath tank volume is shown there as well.

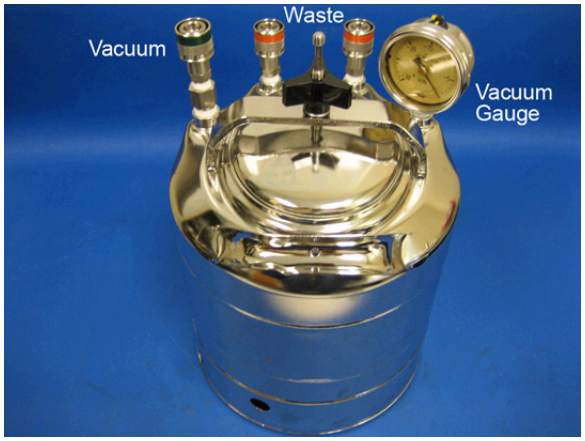
Figure 2.17 Sheath Tank



Waste Tank

The autoclaveable two-gallon, electroplated, stainless-steel waste tank is located on the left side in the front of the lower enclosure. It is fitted with a Vacuum gauge, two quick-connect fittings for waste fluid, and one for Vacuum. See Figure 2.18. Vacuum is regulated by the Touch Screen Control Panel. Waste fluids are collected from the waste aspiration tube, the SmartSampler during debubbling, and the purge valve on the in-line Sheath filter. All waste tubing on the system is red. The orange quick connect fittings can connect to either orange fitting on the tank. The green quick connect fittings and tubing are used for Vacuum.

Figure 2.18 Waste Tank



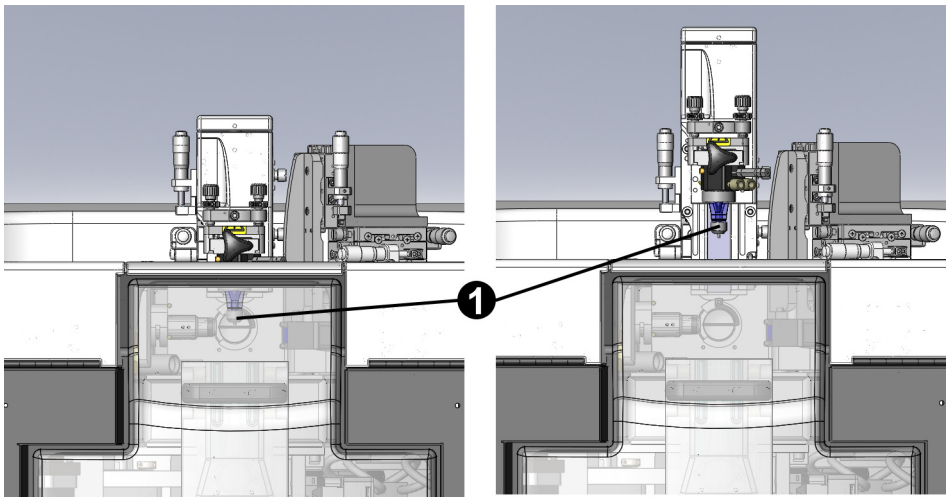
Nozzle

The MoFlo Astrios nozzle delivers sheath and sample to the laser interrogation point via hydrodynamic focusing. Hydrodynamic focusing causes cells to move through the stream and intersect with the laser beams one at a time. Information from user-defined sort decisions and analysis is used to direct the nozzle body to positively or negatively charge the sheath and sample stream. When drop drive is applied, the nozzle body constantly vibrates to break the stream into droplets that can be sorted. The nozzle body can be fitted with a 70 or 100 µm tip.

NOTE Astrios nozzle tips are specific to MoFlo Astrios and are not interchangeable with older MoFlo and MoFlo nozzle tips.

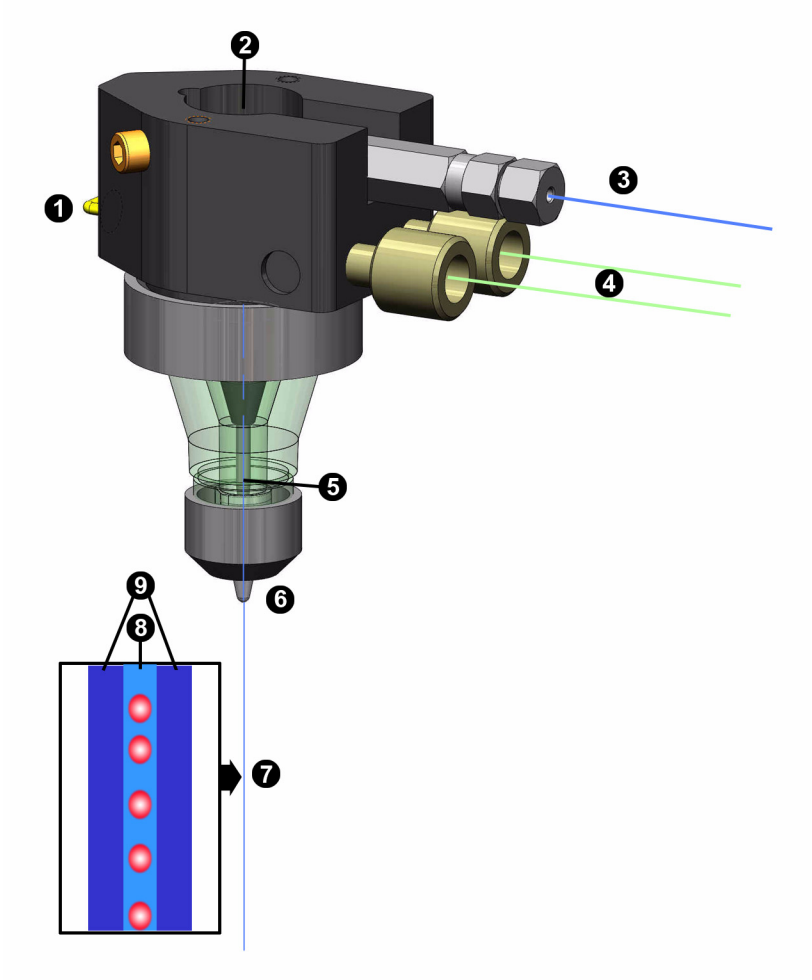
The nozzle positioning stage can be raised for access during nozzle cleaning or replacement. See Figure 2.19.

Figure 2.19 Instrument Nozzle



1.	Nozzle in operating position (left). Nozzle raised for cleaning or replacement (right).

Figure 2.20 Nozzle Interior



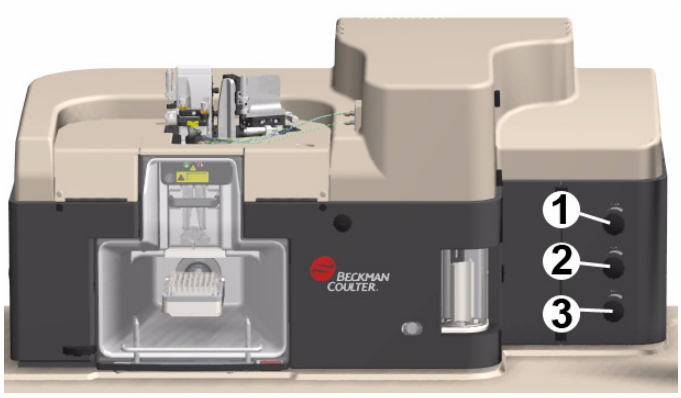
1.	To power source
2.	Piezoelectric Crystal
3.	Sample line in
4.	Sheath lines in/out
5.	Sample delivery
6.	Nozzle Tip
7.	Sample and Sheath Stream
8.	Sample
9.	Sheath

Pressure Console

The Pressure Console allows the operator to coarsely control sheath and sample pressure using the knobs on the front of the upper enclosure. See Figure 2.21. Fine adjustment to sample pressure is made on the Touch Screen Control Panel. The Pressure Console provides the ability to temporarily boost sample pressure through the Touch Screen Control Panel. The Pressure Console also senses and reports sheath pressure, sample pressure, air supply pressure, and waste vacuum.

Sample is delivered to the instrument at a slightly higher pressure than is applied to the sheath fluid. Generally the sample pressure should be between 0.1-0.3 psi greater than the sheath pressure at a nominal sheath pressure of 60 psi for a 70 µm nozzle tip. This modest pressure differential ensures laminar fluid flow while minimizing the sample aspiration rate.

Figure 2.21 Pressure Console



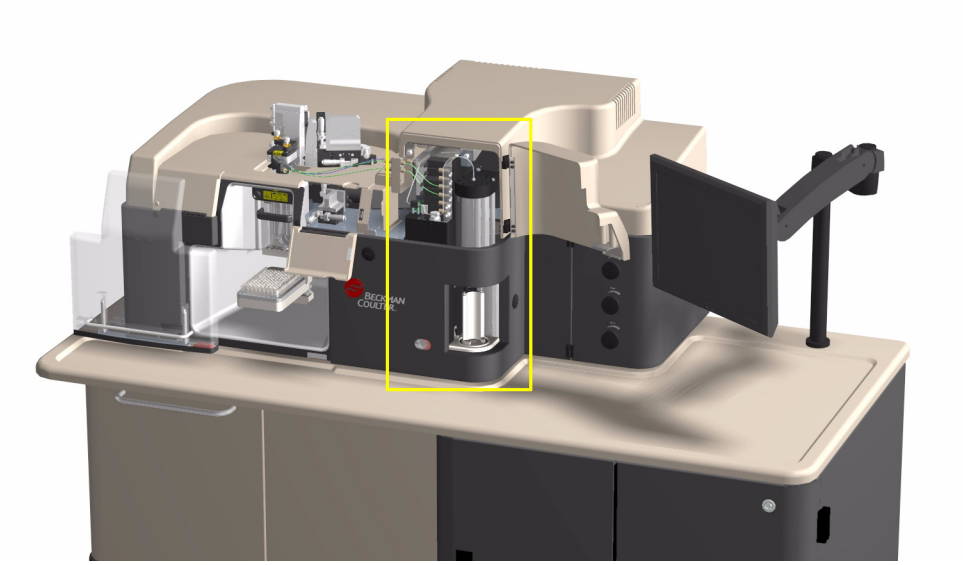
1.	Sample pressure coarse adjustment
2.	Sheath pressure coarse adjustment
3.	Sample Boost coarse adjustment. (Used in conjunction with the boost button on the Touch Screen Control Panel to adjust the amount of pressure that will be applied.)

SmartSampler

The SmartSampler, which is operated via the Touch Screen Control Panel, provides support for operators performing long, temperature controlled sorts. It is located in the upper enclosure on the MoFlo Astrios. Tube sizes from 0.5 to 50 mL can be accommodated, and samples can be temperature controlled if a water bath option is selected. The SmartSampler can be set up to provide sample agitation, and the probe and tubing are user-replaceable.

See CHAPTER 3, *SmartSampler Controls*.

Figure 2.22 SmartSampler



MoFlo Astrios Electronics

The instrument achieves an acquisition rate of 100,000 particles per second and a sort rate of 70,000 sort decisions per second. Electronics and 32-bit software can acquire more than one billion events and store the information in a single data file. MoFlo Astrios electronics are not user-accessible.

CHAPTER 3

Touch Screen Control Panel Overview

Touch Screen Control Panel

The Touch Screen Control Panel is the user interface that allows you to interact with the instrument. The panel is used for aligning and fine-tuning the instrument, configuring IntelliSort, performing the quality control protocol, optimizing photomultiplier tube (PMT) performance, as well as setting up and maintaining a sort. During a sort, the Touch Screen Control Panel also displays sort statistics.

Common Screen Elements

IMPORTANT The SmartSampler buttons display the state to which the instrument will go when the button is pressed.

The buttons and status icons around the perimeter of the Touch Screen Control Panel are common to the main screens and are visible when the main screens are active. The elements on the left side of the screen include the selection tabs for Coarse Alignment, Laser Intercept Configuration, Fine Alignment, Quality Control, Sort Setup, Sort Statistics, and POD Alignment. Along the bottom of the Touch Screen Control Panel are the Stream Illumination button and the Laser Shutter Controls as well as a representation of the seven-pinhole aperture strip. The right side of the Touch Screen Control Panel contains the SmartSampler buttons and instrument status indicators. Note: The image displayed on the button is the state in which the instrument is operating. For instance, a button that displays a bright light bulb indicates that the light is on. When you press the button the light will turn off and the button will display a dim bulb.

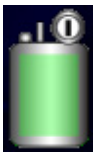
Figure 3.1 Touch Screen Control Panel Common Elements

1. Area is gray because the elements within are not common to other screens.
2. Coarse Alignment tab (pinhole view)
3. Laser Intercept Configuration screen
4. Fine Alignment screen (dot plot)
5. Quality Control screen
6. Sort Setup screen
7. Sort Statistics screen
8. POD Alignment screen
9. Screen Element Names
10. Stream Illumination
11. Laser Shutters
12. Master Shutter on/off (a lit pinhole indicates that light is passing through that pinhole.)
13. Controlled Shutdown button
14. Instrument Status Indicators (See Table 3.1 for definitions.)
15. SmartSampler controls

Table 3.1 Status Indicators - Screen Elements and Functions

Screen Element	Function
	This symbol indicates that the instrument is ready for operation. • The safety interlock is closed. • The sheath tank contains an acceptable level of fluid, and the waste tank is sufficiently empty. • No errors are detected in the hardware, software, or communications between the two. • No bubbles are detected in the sample line with bubble detector enabled.
	This button initiates the controlled shutdown dialog and should be used at the end of each day. It is also the controlled method by which the electronics can be shut down.
	This symbol indicates that errors were detected. (Press this button to view a screen that lists the errors.) When the error is resolved and the button is pressed, the button changes to the green “thumbs up” icon.

Table 3.1 Status Indicators - Screen Elements and Functions *(Continued)*

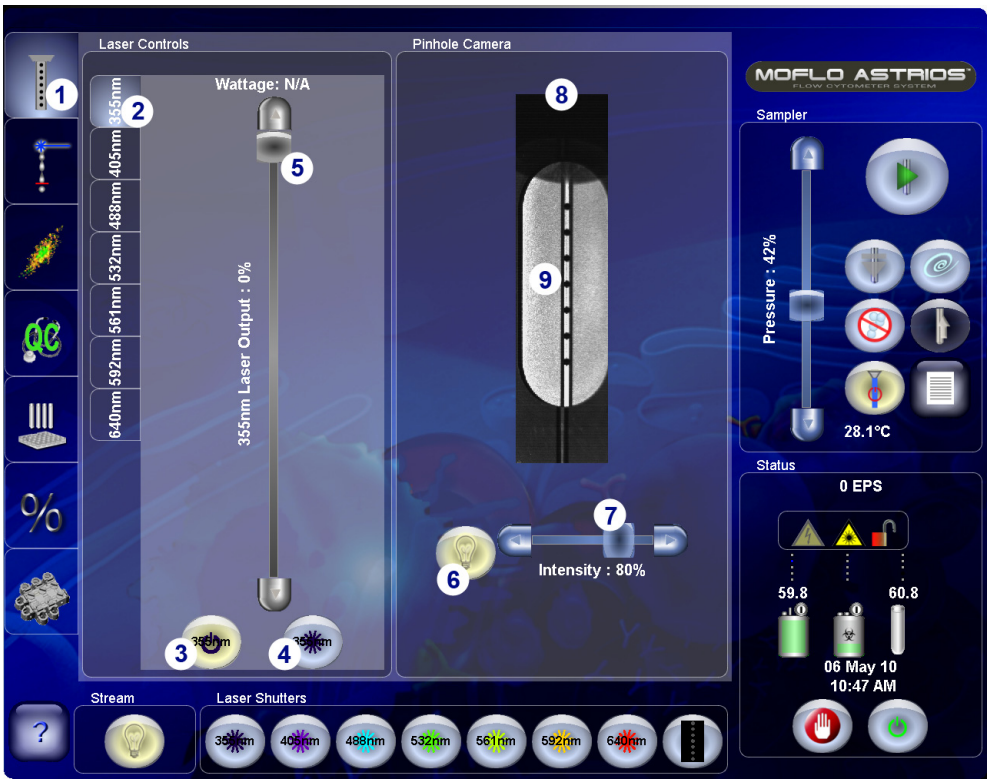
Screen Element	Function
	This symbol indicates that at least one safety interlock is open.
	This symbol indicates that the safety interlocks are closed.
	When this symbol is bright, high voltage is applied to the droplet stream and/or the charge plates. When this symbol is dim, high voltage is not applied.
	When this symbol is bright, a laser is powered and the corresponding shutter is open. When this symbol is dim, no laser light in the Illumination Chamber.
	This symbol indicates the status of the sheath tank. Green = Full Yellow = Approaching empty (Tank first displays yellow when it reaches 10% full.) Red = Extremely low, add sheath fluid (The system will shut down the fluidics when the tank reaches this status.) The value above the symbol indicates the sheath pressure. **NOTE** Typically the sheath tank is filled during the Startup or Shutdown process. If the sheath tank needs filled during your work shift, go to the Change Tanks procedure in CHAPTER 5, *Startup and Shutdown Procedures*.
	This symbol indicates the status of the waste tank. Green = Empty or low Yellow = Approaching full (Tank first displays red when it reaches 90% full.) Red = Extremely full, empty waste (The system will shut down the fluidics when the tank reaches this status.) **NOTE** Typically the waste tank is emptied during the Startup or Shutdown process. If the waste tank needs emptied during your work shift, go to the Change Tanks procedure in CHAPTER 5, *Startup and Shutdown Procedures*.
	The value above the icon indicates the sample pressure.
Temp	Sample temperature at the SmartSampler
EPS	Number of triggered events that are detected per second

Coarse Alignment (Pinhole) Screen

The Coarse Alignment Screen is used for initial alignment of the instrument and to access laser control. Press the Coarse Alignment tab and then press the Pinhole Illumination button to view the image of the Pinhole Apertures while you are aligning the sheath stream.

Upon installation, a Beckman Coulter representative will align the laser beams coming from the fiber-coupled lasers and through the FBSO to the appropriate spacially-separated pinholes. The alignment of the fiber optics should not need further adjustment by the operator. The UV laser will be aligned through the seventh pinhole. The UV laser should be checked daily by the operator, and may periodically need to be realigned.

Figure 3.2 Coarse Alignment Screen



1. Coarse Alignment tab
2. Laser Control tabs
3. Laser Power ON/OFF
4. Laser Shutter open/close (Provides same function as shutter buttons below.)
5. Laser Intensity Adjustment (NA for UV laser)
6. Pinhole Illumination ON/OFF
7. Illumination Intensity
8. Nozzle tip
9. Pinholes and Stream

Laser Control Tabs

The Touch Screen Control Panel displays a Laser Control Tab for each laser on the system. See number 2 on Figure 3.2. Each laser control tab enables the operator to power the laser ON and OFF and to open and close the laser shutter. Laser power intensity can be adjusted for most of the fiber-coupled lasers. If the slider bar is available then power can be adjusted using the Touch Screen. The UV laser power must be adjusted manually.

Table 3.2 Coarse Alignment - Screen Elements and Functions

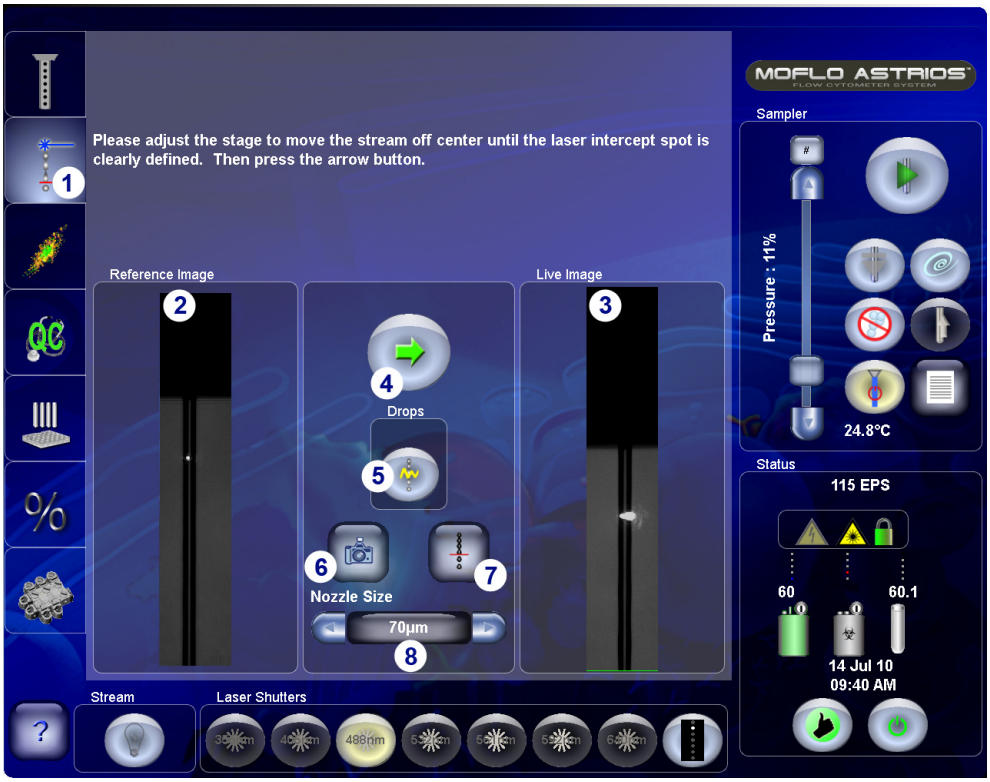
Screen Element	Function
Coarse Alignment tab 	Displays the Coarse Alignment screen.
Pinhole Illumination 	Turns ON and OFF the light that illuminates the pinhole apertures.
Intensity Control (slider control)	Dims and brightens pinhole illumination.
Laser Power ON/OFF 	Turns ON and OFF the power to the laser.
Laser Shutter Open/Close 	Opens and closes the laser shutter.

Laser and Stream Intercept Configuration Screen

The Laser and Stream Intercept Configuration Screen sets the system up so that IntelliSort can function properly.

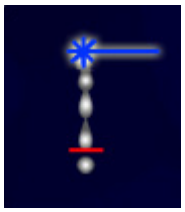
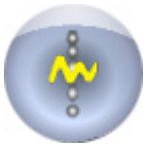
The screen provides a reference image and a live image of the laser and stream interception point. It also allows the user to perform the background subtraction procedure when necessary. For more information see CHAPTER 10, *Background Image Subtraction*.

Figure 3.3 Laser and Stream Intercept Configuration Screen



1. Laser and Stream Intercept tab	**5.** Initialize IntelliSort
2. Reference Image	**6.** Background Subtraction
3. Live Image	**7.** Manual Droplet Setup
4. Next Arrow	**8.** Nozzle Size Selector

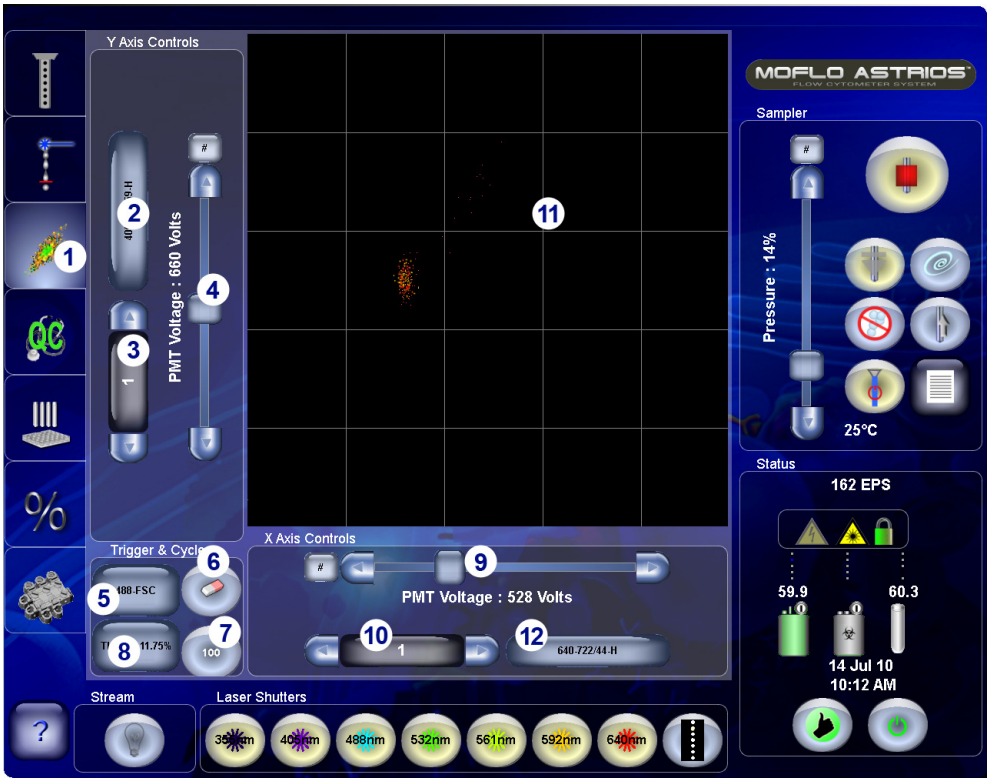
Table 3.3 Laser and Stream Intercept Screen

Screen Element	Function
Laser and Stream Intercept tab 	Displays the Laser and Stream Intercept screen.
Reference Image	Displays the image that was captured from the droplet camera before the laser intercept procedure was started.
Live Image	Displays the live image from the droplet camera.
Next Arrow 	Allows the Find Laser procedure to move to the next step.
Initialize IntelliSort 	Sets frequency and amplitude. This step must be completed before the QC procedure is performed.
Background Image Subtraction 	Takes an image of the area around the droplet stream and then subtracts the image, so that Drop Delay Determination can work correctly. This does not need to be done every day.
Manual Droplet Setup 	Displays the controls for manual droplet setup.

Fine Alignment Screen

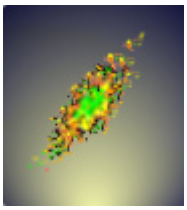
The Fine Alignment screen is used for fine adjustments to instrument alignment as well as setting parameters, data types, trigger, threshold, and the data cycle rate. Press the Dot Plot tab to view data in a dot plot format while you make fine adjustments with the appropriate micrometers, and while you adjust voltage and gain for the PMTs.

Figure 3.4 Fine Alignment Screen



1. Fine Alignment tab
2. Y-axis Parameter
3. Y-axis PMT Gain
4. Y-axis PMT Voltage Control
5. Trigger Parameter
6. Clear Displayed Events
7. Data Cycle Rate
8. Threshold Setting
9. X-axis PMT Voltage Control
10. X-axis PMT Gain
11. Data Display Area
12. X-axis Parameter

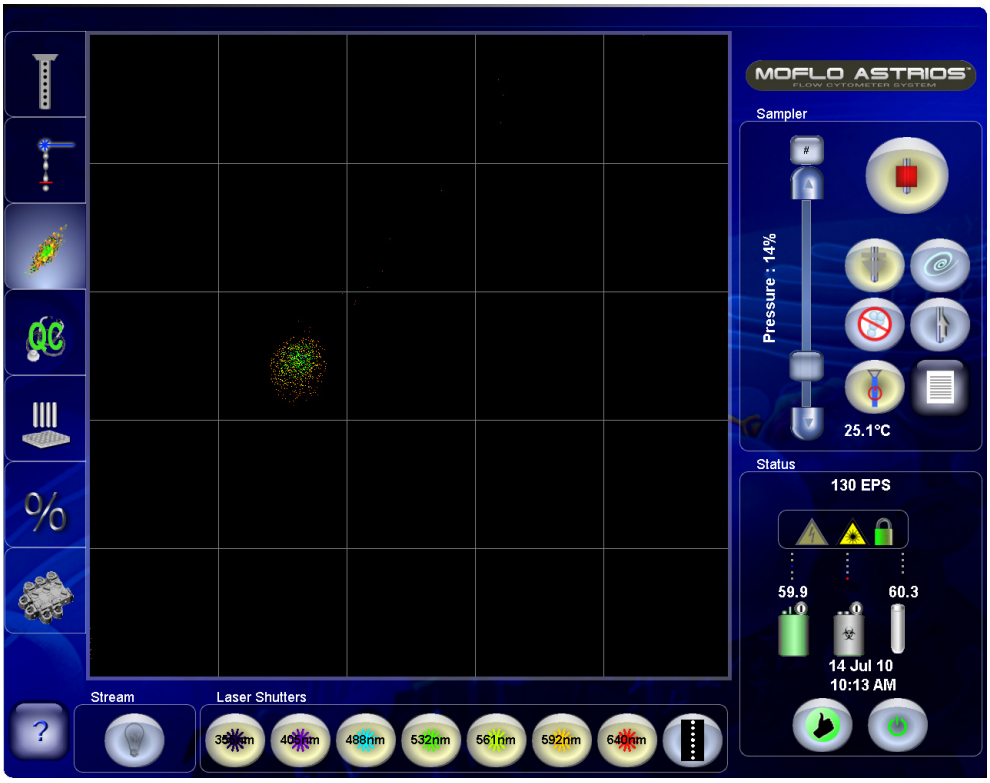
Table 3.4 Fine Alignment - Screen Elements and Functions

Screen Element	Function
Fine Alignment tab 	Displays the Fine Alignment screen.
Adjust PMT Voltage (slider control)	Adjusts voltage for the PMT that is associated with the selected parameter.
Select Parameter 	Launches the Parameter Selection Tool for the corresponding axis. See Figure 3.6.
Adjust Gain 	Adjusts the gain on the PMT in increments of 1 with a range of 1 - 100.
Select Trigger 	Selects the trigger parameter, any parameter can be set as the trigger.
Set Threshold 	The purpose of the threshold is to desensitize the electronics to low-level noise caused by very small particles or auto fluorescence from the data. The threshold-level selector allows the user to determine the minimum voltage at which signal processing is initiated. This range is selectable from 0.01 percent to 100 percent, with a full-scale selection equivalent to 10 V.
Cycle Rate 	Sets the cycle mode to 0,100, 1000 (1K), or 5000 (5K) events.
Data Clear 	Clears data and refreshes the Touch Screen Control Panel.

Enlarged Fine Alignment Data Display

It is possible to maximize and minimize the data display of the Fine Alignment screen by touching the grid in the data display area.

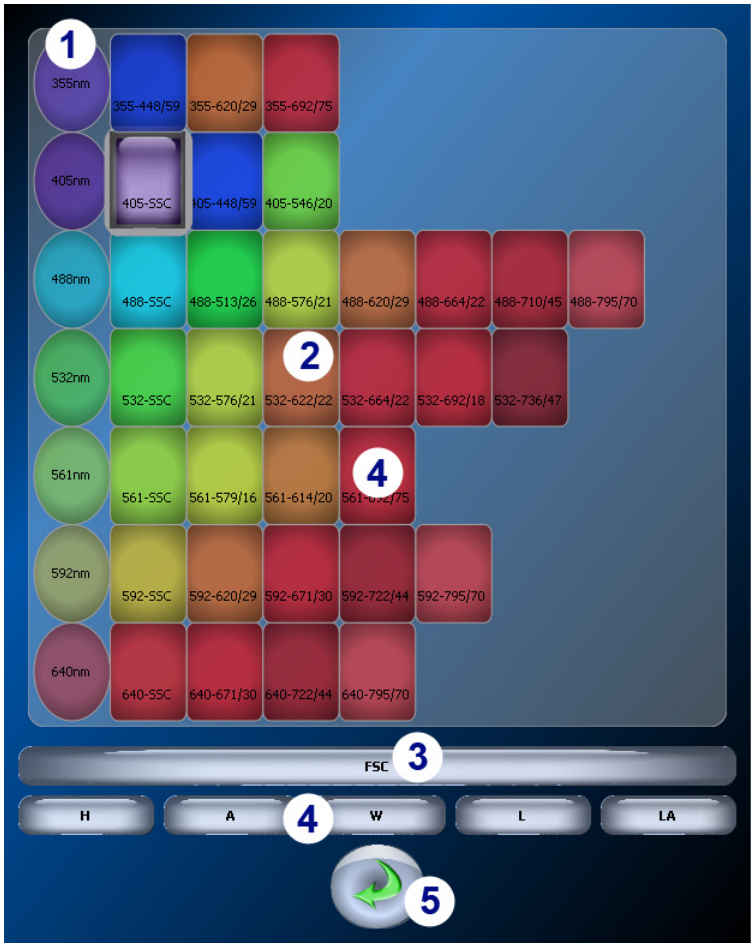
Figure 3.5 Data Display Maximized



Parameter Selection Tool

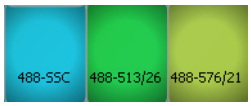
The Parameter Selection Tool allows you to select laser line, PMT, parameter, and the data type of the parameter.

Figure 3.6 Parameter Selection Tool



1. Laser Wavelength **2.** Filters per POD **3.** FSC Selection	**4.** Data Type Selection • H = height • A = Area • W = Pulse Width • L = Log • LA = Log Area **5.** Return to Fine Alignment Screen

Table 3.5 Parameter Selection Tool - Elements and Functions

Screen Element	Function
Laser Line 	The circles represent the lasers included on the system.
PMTs 	The squares represent the PMTs and corresponding filters per laser line.
Parameter 	Selects the Forward Scatter (FSC) parameter.
Data Type 	The data type displayed on the Control Panel does not reflect data type set for acquiring data in Summit Software. The Control Panel can display data from any parameter at all times. Summit software displays and collects only the enabled parameters on the Acquisition panel. See CHAPTER 4, *Enable Parameters*. H = linear height A = linear area L = log height LA = log area W = pulse width
Return 	Returns to the Fine Alignment screen.

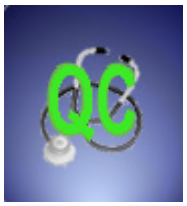
Quality Control Screen

The QC screen is a representation of lasers and detectors on the instrument. Circles represent laser lines. Squares represent PMT positions. The user presses a button to start the wizard for QC and is guided through the QC procedure. A progress dialog informs the operator of the current activity. After the QC procedure is run, detectors that meet specification show a green checkmark. Failing detectors show a red X. Parameters that the system was not able to analyze show a question mark.

Figure 3.7 Quality Control Screen

1. QC tab	**4.** Start QC Procedure
2. Laser Line	**5.** Cancel QC
3. PMTs and Filters	**6.** Status of QC Procedure

Table 3.6 QC Screen - Elements and Functions

Screen Element	Function
QC tab 	Displays the QC screen.
Laser Line 	The circles represent the lasers included with the system.
PMTs 	The boxes represent the PMTs per laser line.
Start QC button 	• Turns on Drop Drive if IntelliSort Initialization has been completed. • Initializes voltages, gains, and thresholds for all parameters. • Automatically starts acquisition and adjusts event rate to 300 EPS (approximately 30 seconds). • Sets trigger FSC to the 488 nm laser. Sets gain and threshold to the values the user selected for forward scatter. • Sets laser delay for all powered lasers. • Sets all gains on PMT voltage to 1 except on the trigger parameter. • Adjusts SSC voltage for the trigger laser. Sets a gate from FSC vs. SSC from the trigger laser to all other parameters. • Adjusts the voltages on all remaining parameters simultaneously to center the population on each histogram in median 128. • Sets EPS to 100-120. • Collects 5000 events. • Checks each detector against QC pass/fail criteria. • Reports the CV and PMT voltage with a green check (passing) or red X (failing) • Exports to a CSV file that can be viewed and edited using a spreadsheet program such as Excel. (These files can be accessed through Summit Software.)
Cancel QC button 	Cancels the QC procedure.

Sort Screen

The Sort screen is used for setting up IntelliSort and selecting a standard Sort Output Type (Table 3.7) to prepare for starting a sort. The MoFlo Astrios includes pre-configured sort output definitions. See Figure 3.9. When you select a standard sort output definition, the instrument automatically sets the position of the CyClone arm beneath the charge plates.

Custom Sort Output types can be created and edited but standard Sort Output types cannot be changed. The controls for IntelliSort, manual droplet setup, and manual stream setup are also accessible from this screen.

NOTE Some controls on this screen will be disabled when IntelliSort is in Maintain Mode.

Figure 3.8 Sort Screen

1. Sort tab	**7.** IntelliSort Initialize
2. Select Sort Output Type	**8.** IntelliSort Automatic Drop Delay Determination
3. Create a new Sort Output Type	**9.** IntelliSort Maintain Mode
4. Copy an existing Sort Output Type	**10.** Manual Droplet Setup
5. Edit a custom Sort Output Type	**11.** Streams Camera and Deflection Controls
6. Delete a custom Sort Output Type	

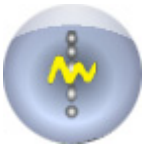
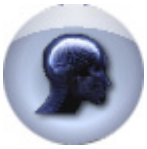
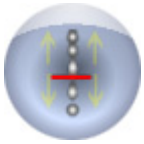
Figure 3.9 Sort Output Types

NOTE All microplates were verified using Corning Costar™ flat bottom microplates. The operator should empirically confirm compatibility when using microplates from other manufacturers.

Table 3.7 Sort Screen - Elements and Functions

Screen Element	Function
Sort tab 	Press to access the Sort screen.
Sort Output Type	Use the drop-down list to select a Sort Output Type. Standard Sort Output Types: 6-well, 24-well, 96-well, 384-well, and 1536-well microplates 5mL, 15 mL, 50 mL, and 50 mL with 5 mL tube holders Slide Custom Sort Output Types will also appear in the list.
Create New 	Access the Definition screen and create a new Sort Output Type.
Copy 	Access the Definition screen and create a copy of a standard Sort Output Type that can be edited.

Table 3.7 Sort Screen - Elements and Functions *(Continued)*

Screen Element	Function
Edit 	Access the Definition screen and edit a previously saved Custom Sort Output Type.
Delete 	Deletes custom Sort Output Type.
IntelliSort Initialize 	Sets drop drive frequency, and sets amplitude. This step must be done before the QC procedure is run.
IntelliSort Drop Delay Determination 	Performs automated drop delay determination and sets drop delay between 32 and 45. (Before pressing this button, view the streams image and adjust charge phase if necessary.)
IntelliSort Maintain 	Starts IntelliSort Maintain Mode, which can monitor a sort and maintain drop delay within 10% for a temperature change of ±3 degrees Celsius for a sheath pressure change of ±3 psi.
Manual Droplet Setup 	Press this button to access the Manual Droplet Setup screen. **NOTE** This screen is necessary only when you intend to set up a sort manually. If IntelliSort is maintaining the drop delay some of the controls on this screen will be disabled.
Stream Setup 	Press this button to access the Stream Setup screen and: • Set Charge Phase during IntelliSort setup. • Set up a sort manually. • Adjust deflection for sort output • Adjust plate voltage

Definition, Deflection, and CyClone Tabs

The Definition, Deflection, and CyClone tabs can be accessed through the Sort screen. Typically these tabs are needed only if you intend to create or edit a custom Sort Output Type.

Definition Tab

The Definition tab is used to create or edit a custom Sort Output Type.

Figure 3.10 Definition Tab

1. Sort Output Type	**4.** Cancel
2. Keyboard	**5.** Return to Sort Output Screen
3. Save Definition	

Table 3.8 Definition Screen - Elements and Functions

Screen Element	Function
Type	Sort Output type such as: 6-well, 24-well, 96-well, 384-well, and 1536-well microplates 5mL, 15 mL, 50 mL, and 50 mL with 5 mL tube holders (or custom) Slide
Name	Text field to allow naming of the sort output type.
Rows	Number of rows in custom sort output definition. (Not applicable with Tubes output type.)
Columns	Number of columns in custom sort output definition.
Keyboard	Touch screen keyboard
Set 	Saves changes to Sort Output definition.
Cancel 	Cancels changes made on the Definition screen and returns to the Sort screen.
Return 	Returns to the Sort screen.

Deflection Tab

The Deflection tab can be accessed by setting up a new Sort Output Type and selecting the Deflection tab.

This screen is used to individually select and adjust sort streams prior to beginning a sort. It is also used to edit the deflection settings for a Sort Output Type. From this screen you can turn ON and OFF the charge plates, adjust deflection plate voltage, stream targets, and stream positions.

Figure 3.11 Deflection Tab

1. Sort Output Type Name	**7.** Test Pattern ON/OFF
2. Stream Deflection Percentage	**8.** Retract SortRescue
3. Stream Selection	**9.** Set Stream Position
4. Stream Template	**10.** Charge Plates ON/OFF
5. Stream Target Adjustment	**11.** Plate Voltage Adjustment
6. Stream Positioning Adjustment	**12.** Return

Table 3.9 Deflection Tab - Screen Elements and Functions

Screen Element	Function
Sort Output Type	Label such as: 6-well, 24-well, 96-well, 384-well, and 1536-well microplates (or custom) 5mL, 15 mL, 50 mL, and 50 mL with 5 mL tube holders (or custom) Slide
Stream Deflection (slider control)	Adjusts stream deflection percentage for the selected stream.
Stream Selection 	Selects the stream for which you intend to adjust deflection and displays the deflection percentage. Left 3 = stream furthest left of the waste receptacle Left 2 = second stream left of the waste receptacle Left 1 = stream directly to the left of the waste receptacle Center = stream that flows straight down and is used for the waste stream. Right 1 = stream directly to the right of the waste receptacle Right 2 = second stream right of the waste receptacle Right 3 = stream furthest right of the waste receptacle
Stream Template	Provides a dotted line to use as reference while adjusting the stream position. Press to turn ON/OFF the test stream.
Stream Position Target 	Press to select the Stream Positioning target that represents the location of the sort receptacle.
Stream Positioning (slider control)	Adjusts the position of the Stream Positioning Target when a Stream Position Target is selected.
Charge Plates ON/OFF 	Turns ON/OFF the voltage to the Deflection Plates
Test Pattern ON/OFF 	Turns ON/OFF the charge applied to the test streams. Enables the stream(s) that have been previously selected on the stream template.
Plate Voltage (slider control)	Selects the voltage that is applied to the Deflection Plates.
Sort Rescue Retraction 	Press to move Sort Rescue out of the way from the streams. Sort Rescue remains retracted as long as the button is depressed.

Table 3.9 Deflection Tab - Screen Elements and Functions *(Continued)*

Screen Element	Function
Set 	Saves the changes made to stream positioning.
Return 	Returns to Sort screen.

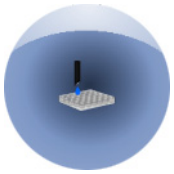
CyClone Configuration Tab

MoFlo Astrios comes with pre-configured sort output definitions. However, the CyClone Configuration screen is available if the operator chooses to set up a custom sort output receptacle. The CyClone Screen is used to specify the size and location of a custom plate, slide, or tube. When the Find Limits button is pushed, the CyClone determines the limits of the sort output receptacle and then the remaining buttons are enabled. This screen allows you to set, change, and test positions. Typically, this screen will be used only to set up custom tubes or plates. Standard sort output definitions use pre-configured CyClone positions.

Figure 3.12 CyClone Tab

1. Squirt Fluid	**6.** Return
2. Find Limits	**7.** Moves CyClone back
3. Home	**8.** Moves CyClone right
4. End	**9.** Moves CyClone forward
5. Set	**10.** Moves CyClone left

Table 3.10 CyClone Configuration Screen - Elements and Functions

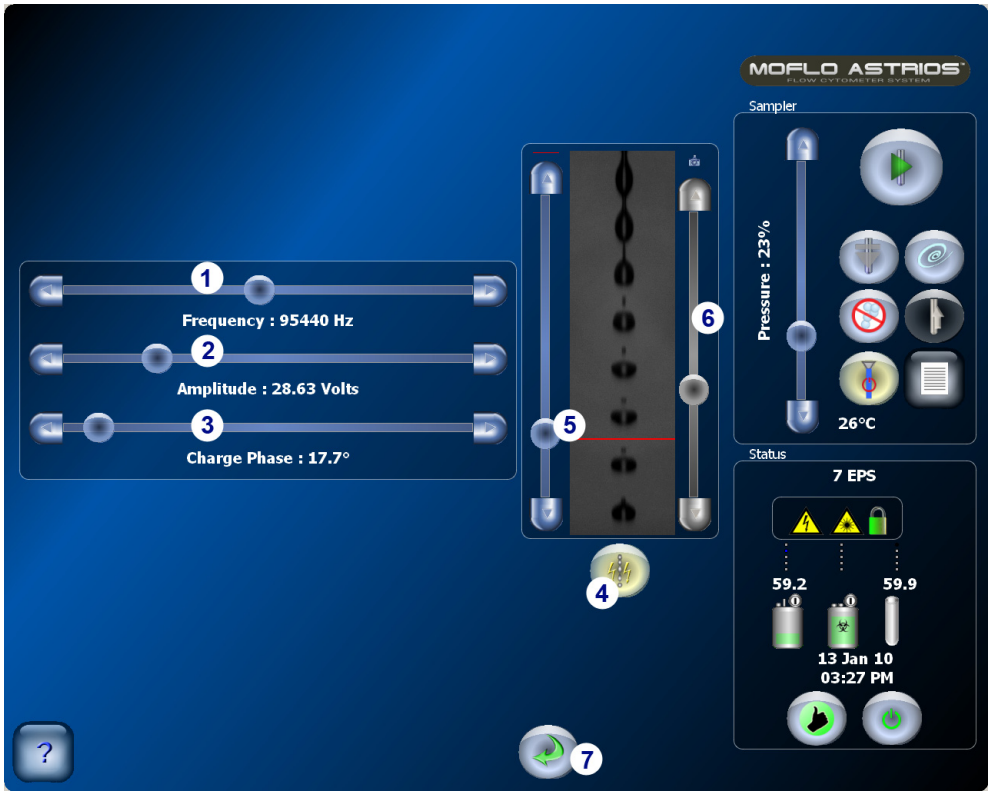
Screen Element	Function
Squirt 	Press to squirt fluid when testing the accuracy of a CyClone position. SortRescue retracts momentarily so that fluid can be deposited and then it moves back into place.
Directional 	Moves the nozzle to a specific coordinate. When the CyClone reaches its mechanical limit in a particular direction the button will be inactive and grayed out.
Find Limits 	Re-initializes CyClone if it loses its calibration. Press to initialize CyClone and active the remaining screen elements. Remove tubes from tube holder before pressing this button.
Home 	Moves the CyClone to the stored Home position. If you want to set a new Home position, press the directional arrows until the CyClone moves to the desired position and then press the Set button.
End 	Moves the CyClone to the stored End position. If you want to set a new End position, press the directional arrows until the CyClone moves to the desired position and then press the Set button.
Set 	Press to store the new coordinate. This button will not be active until a directional button is pressed and the CyClone moves to a new XY coordinate.
X and Y	Displays the numerical X and Y coordinates of the current position of the CyClone.
Return 	Returns to the Sort screen.

Manual Droplet Setup Screen

The Droplet screen is used for manually setting up droplets. Some elements on this screen will be disabled if IntelliSort is running.

NOTE The IntelliSort Initialize button automatically performs all of the functions on this screen. However, the user may adjust the Last Attached Drop Marker and Charge Phase if necessary.

Figure 3.13 Manual Droplet Setup Screen



1. Frequency	**5.** Last Attached Drop Marker
2. Amplitude	**6.** Move Droplet Camera
3. Charge Phase	**7.** Return to Sort Screen
4. Drop Drive ON/OFF	

Table 3.11 Manual Droplet Setup Screen - Elements and Functions

Screen Element	Functions
Frequency (slider bar)	Controls the Drop Drive frequency. (The rate at which the crystal in the nozzle vibrates.)
Amplitude (slider bar)	Adjusts the Drop Drive amplitude. (The force with which the crystal in the nozzle vibrates.)
Charge Phase (slider bar)	Adjusts the value to achieve the tightest side streams. The test pattern should be enabled before you adjust the Charge Phase.

Table 3.11 Manual Droplet Setup Screen - Elements and Functions *(Continued)*

Screen Element	Functions
Drop Drive ON/OFF 	Turns ON/OFF the piezoelectric crystal in the nozzle that vibrates to form droplets.
Camera Controls	Moves the droplet camera.
Last Attached Drop Marker	It is optional to move the red marker to the last attached drop to create a reference for viewing stream stability.

Sort Statistics Screen

The Sort Statistics screen allows you to view in large or small format the sort statistics for each stream, and observe the droplet image if selected.

Figure 3.14 Sort Statistics Screen

1. Sort Statistics tab	**2.** Expand Statistics Display

Table 3.12 Sort Statistics - Screen Elements and Functions

Screen Element	Function
Left 3	Statistics for the stream furthest left of the waste receptacle
Left 2	Statistics for the stream second to the left of the waste receptacle
Left 1	Statistics for the stream directly to the left of the waste receptacle
Right 1	Statistics for the stream directly to the right of the waste receptacle.
Right 2	Statistics for the stream second to the right of the waste receptacle.
Right 3	Statistics for the stream furthest to the right of the waste receptacle.
Sort Mode	Displays the Sort Mode that was selected in Summit software for the stream. Enrich Mode - All positive events are sorted except Hard Aborts. Purify Mode - All negative events are aborted. Single Mode - All negative events are aborted and the droplet must contain only one positive event.
Sort #[a]	Total positive events that have been sorted for the stream.
Sort Rate[a]	Sorted events per second for the stream.
Abort #[a]	Total positive events that have been aborted for the stream.
Abort Rate[a]	Aborted events per second for the stream.
% Total[a]	The percent of positive sorted events relative to the Total Events for the acquisition.
Efficiency[a]	The number of positive events sorted, divided by the total events that could have been sorted for the stream. sorted/(sorted+aborted)
∑ Sort #	Sum Total Sorted Events
∑ Abort #	Sum Total Aborted Events
Expand Display 	Expands the statistics display. In large format mode the droplet image is not displayed. Press the button again to change back to small format.

a. Use the Summit software Sort tab to clear this statistic. All sort statistics, except ∑ Sort # and ∑ Abort #, are cleared automatically between sorts.

NOTE To clear sort statistics identified in Table 3.12, see Clearing Sort Statistics under the Sort Tab heading in CHAPTER 4, *Summit Software*.)

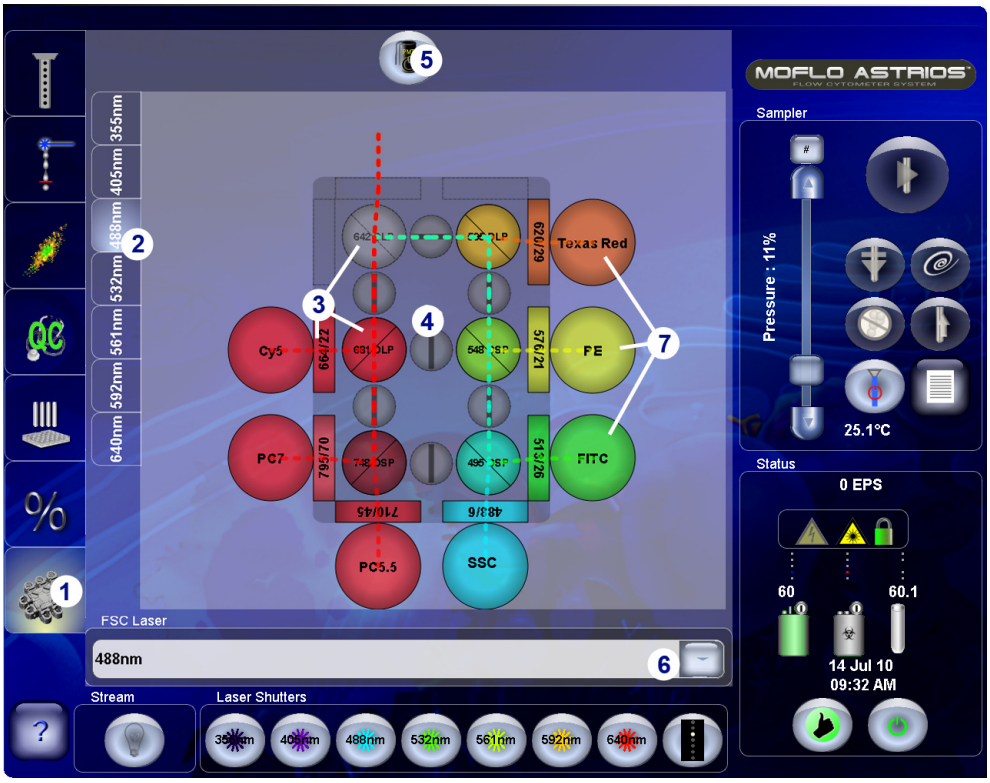
PMT and Filter Update Screen

The PMT and Filter Update screen allows you to update filter, PMT, and Forward Scatter laser information and then store it so the system can recognize the new configuration.

IMPORTANT Never physically change the PMT configuration of a POD without first disabling power to the PMTs by pressing the PMT Power ON/OFF button. (See number 5 in Figure 3.15.) After power to the PMTs is disabled it is possible to physically move PMTs and to edit filters on screen. Always update the Touch Screen to reflect the physical condition of the POD and then turn power to the PMTs ON.

See CHAPTER 10, *Edit Mode - Changing PMTs and Filters* and *Edit Mode - Designating a Forward Scatter Laser and Filter* for details.

Figure 3.15 PMT Update Screen



1. PMT and Filter Alignment tab	**5.** PMT Power ON/OFF
2. Laser tabs	**6.** Forward Scatter Laser Selection
3. Filters and/or Mirrors	**7.** PMTs
4. Light Containment Gate	

Table 3.13 PMT and Filter Alignment Screen - Elements and Functions

Screen Element	Function
PMT and Filter Alignment tab 	Press to access the PMT and Filter Update screen.
Laser tab	Displays the POD layout for the selected laser.
PMTs	Press to assign fluorochrome names. Text field only.
Dichroic Filters and Mirrors	Press to change the filter information.
Light Containment Gates	Manually position Light Containment Gates to block or allow light to travel through the POD. Edit the on-screen positions to represent the physical configuration.
PMT Power ON/OFF 	Turns power ON/OFF to all PMTs and PODs. Power must be turned OFF before moving a PMT and before editing filter information. Power ON scans to detect new PMT locations and loads filter information into memory. This enables the system to recognize the new PMT and filter configuration.
FSC Laser	Selects the laser that will be used to trigger events.

SmartSampler Controls

IMPORTANT SmartSampler buttons display the state the instrument goes to when the button is pressed.

Figure 3.16 SmartSampler Abbreviated Menu

1. Pressure Differential (between Sheath and Sample)	**5.** Debubble
2. Start Sample (Stop Sample)	**6.** Boost Sample
3. Chamber Down (Chamber Up)	**7.** Start Sheath Flow (Stop Sheath Flow)
4. Backflush	**8.** SmartSampler Full Menu

Figure 3.17 SmartSampler Full Menu

1. Start Sample (Stop Sample)	**8.** Sample Boost
2. Sample Illumination	**9.** Rinse Probe
3. Agitate Sample	**10.** Change Probe
4. Backflush	**11.** Drain
5. Close Chamber (Open Chamber)	**12.** Start Sheath Flow (Stop Sheath Flow)
6. Debubble	**13.** Change Tanks (ON/OFF Pressure to Tanks)
7. Unclog	**14.** Return

Table 3.14 SmartSampler - Screen Elements and Functions

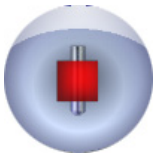
Screen Element	Function
	**SmartSampler Menu button** Displays a dialog box that contains the following buttons: • Start/Stop Sample • Sample Illumination on/off • Agitate Sample • Backflush • Chamber up/down • Debubble • Unclog • Boost • Rinse • Change Probe • Drain • Start/stop Sheath Stream • Change Tanks (ON/OFF Pressure to Tanks) • Return to Previous Screen
Mode	State of the SmartSampler as reported by the firmware. The possible modes are: Off, Standby, Analyze, Load, Backflush, Debubble, Unclog, Rinse, Change Probe, Drain, Boost.
Temp:	Temperature of SmartSampler Sample Holder
Start Sample 	Press this button to: • Close the chamber (if open). • Open the pinch valve and boost the sample (if Auto-boost is selected in Summit software). • Activate F2 in Summit software (begin acquiring data or sort if Summit software is set to respond to the SmartSampler). **NOTE** When this button is pressed the Stop icon replaces it.
Stop Sample 	Press this button to: • Close the pinch valve. • Pause or stop acquiring data depending on the user-defined settings in Summit software.
Sample Illumination 	Press this button to turn on/off the Sample Illumination.

Table 3.14 SmartSampler - Screen Elements and Functions *(Continued)*

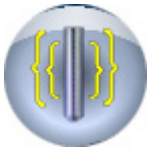
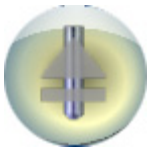
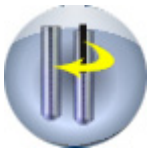
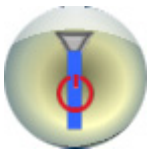
Screen Element	Function
Agitate Sample 	The chamber must be closed in order to agitate the sample. Press this button to agitate the sample. When the button is pressed again agitation stops. If the chamber opens, agitate will automatically stop but the button will not reset until it is pressed again.
Backflush 	When the chamber is closed and Backflush is pressed, sample stops flowing. The chamber opens. Backflush draws fluid back through the sample line and it is vacuumed to the waste tank.
Open Chamber 	Press this button to: • Close the pinch valve. • Depressurize the sample. • Open the chamber.
Close Chamber 	Press this button to: • Close the chamber. • Pressurize the sample. • The pinch valve remains closed and the fluidics are ready to run sample.
Debubble 	Press this button to: • Open the chamber if it is closed. • Close the pinch valve if it is open. • Debubble until button is pressed again. • Alternate vacuum and sheath between the two sheath lines that attach to the nozzle.
Unclog 	Press this button to: • Open the chamber if it is closed. • Close the pinch valve if it is open. • Apply vacuum to both sheath lines at the same time. Some fluid should be held under the nozzle tip.
Sample Boost 	Press this button to temporarily boost the sample pressure. The pressure will be boosted as long as the button is pressed. **NOTE** To change the sample pressure differential press and hold the Sample Boost button while adjusting the physical knob labeled Boost Figure 2.21.

Table 3.14 SmartSampler - Screen Elements and Functions *(Continued)*

Screen Element	Function
Rinse Probe 	Press this button to rinse the sample probe with sheath.
Change Probe 	Press this button to close the chamber and prepare to change the sample probe. The chamber will not be pressurized. For instructions see page 10-48.
Drain 	Press and hold this button to drain fluid from the sample chamber to waste.
Start/Stop Sheath Flow 	Press this button to: • Start sheath stream • Backflush **NOTE** If the fluidics system is not pressurized when you press this button, the system will turn ON pressure and vacuum to the tanks and then turn on the sheath stream. To stop sheath stream, push this button again. **NOTE** If the Start Sheath Flow function is deactivated and you are unable to shut off the fluidics, press the Change Tanks button.
Change Tanks 	This button is used when tanks need to be changed during the work day when the system has already been powered up and running. (Place a tube of deionized water in the sample station prior to pressing this button.) This button leaves lasers powered ON. Press this button to: • Close the sample chamber. • Run water through the sample line. • Reopen the chamber. • Power OFF pressure and vacuum. (change tanks) and press again to power ON.
Return 	Press this button to return to the previous screen.

CHAPTER 4

Summit Software

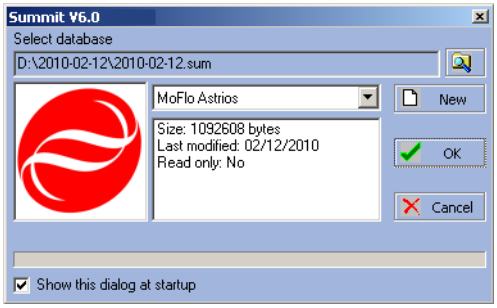
Summit Software Overview

Summit Software allows you to acquire, sort, and analyze flow cytometry data then save the data in FCS format. With Summit Software you can monitor and control the instrument, define protocols, configure compensation settings and workspaces, define batch protocol panels, reagents, and tubes, auto-compensate data, and view indexed sorting.

How to Open Summit Software

1 To open Summit Software double-click the **Shortcut** icon on the computer desktop. The **Select database** dialog box appears.

Figure 4.1 Select Database Dialog Box



2 Select **MoFlo Astrios** from the pull-down menu. This will allow you to interact with the instrument in real time. It is also possible to work with Summit Software offline to analyze previously saved FCS data files.

Now you will either create a new database or open a previously saved database. A Summit Software database is a collection of protocols, samples, and data.

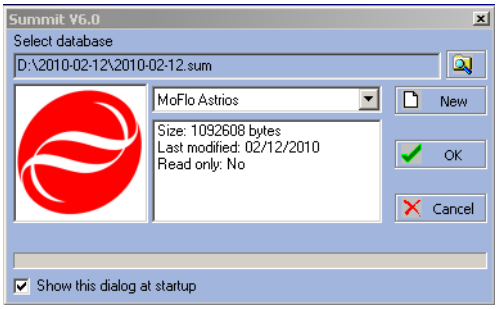
Summit Software Database

A Summit Software database is a collection of protocols, samples, and links to data collected or viewed during a particular session. After you open a new database a workspace appears in which to create histograms and dot plots. It is also possible to open existing protocol files that may already contain histogram and dot plot forms.

How to Create a New Database

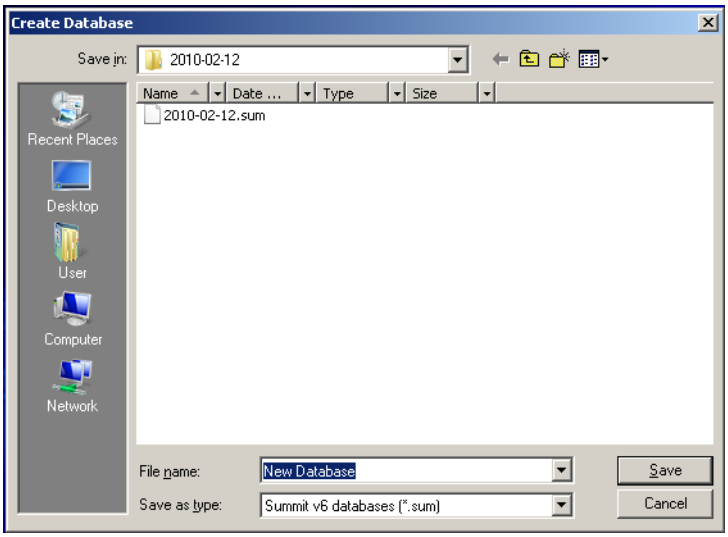
1 Open Summit Software and click **New**.

Figure 4.2 Select Database Dialog Box



The **Create Database** dialog box appears.

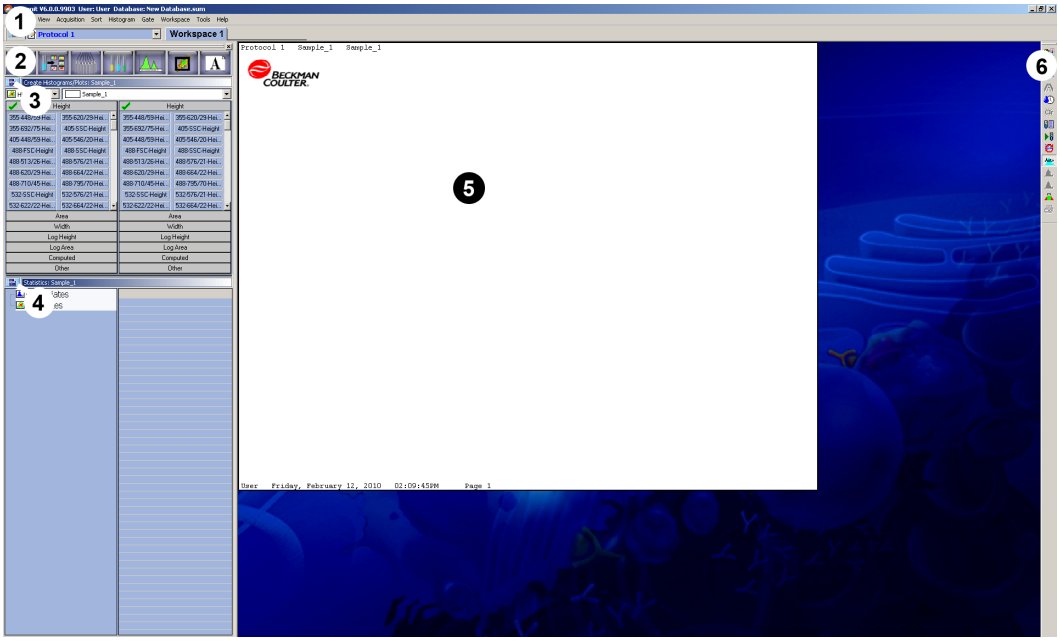
Figure 4.3 Create Database Dialog Box



2 Specify the folder in which you will save the database. Specify a name for the database and click **Save**. The main Summit Software screen will appear.

Summit Software Screen Overview

Figure 4.4 Summit Software Screen Overview



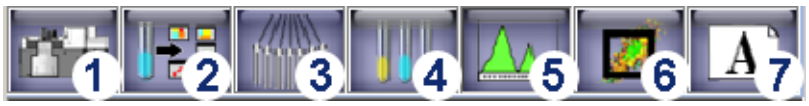
1. Summit Software Main Menu
2. Summit Software Control Panel
3. Additional Menu
4. Additional Menu
5. Workspace
6. Toolbar Icons

Summit Software Control Panel

Most of the operations in Summit software can be accessed through the Summit Software Control Panel. The panel is located on the left side of the screen and has a series of buttons across the top. You can select each of these buttons to get information related to a particular topic. Each tab contains submenus that have options specific to that menu.

Any of these windows can be detached by clicking the Summit Software Control Panel additional menu icon (see number 3. on Figure 4.4) and selecting **Detach Floating**.

Figure 4.5 Summit Software Control Panel (see number 2. on Figure 4.4)



1. Instrument tab	5. Histogram tab
2. Acquisition tab	6. Gate Logic tab
3. Sort tab	7. Layout
4. Sample tab	

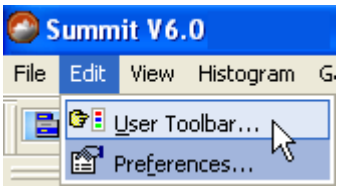
User Toolbar Buttons

Toolbar buttons can be customized to appear on the right side of the screen. These buttons give you immediate access to the features in Summit that you use most often, such as Toggle cycle mode, Toggle color gating, and Replay sample.

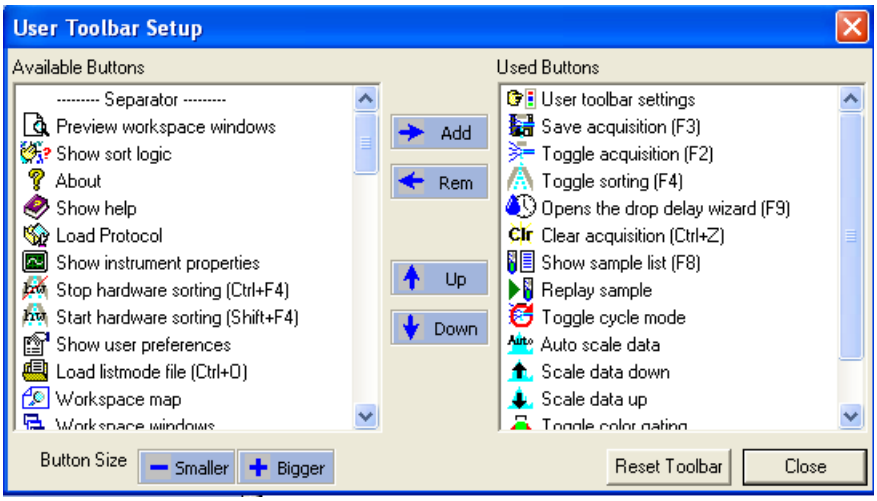
Figure 4.6 User Toolbar Icons

Customize the User Toolbar

1 Click the **Edit** menu in Summit Software and select User Toolbar.



2 The User Toolbar Setup dialog box appears.



3 Select from the list of **Available Buttons** and click **Add**. The icon is added to the toolbar.

4 To remove icons from the toolbar select from the Used Buttons list and click **Rem**.

5 Click **Smaller** or **Bigger** to change the size of the toolbar buttons. Click **Up** or **Down** to change the location of the button on the toolbar. The **Reset Toolbar** button restores the User Toolbar to default settings.

Instrument Tab

Instrument Tab

The Instrument tab is active only when Summit Software is connected to an instrument. From here you can specify SmartSampler settings. See Table 4.1 for settings definitions.

Figure 4.7 Instrument Tab

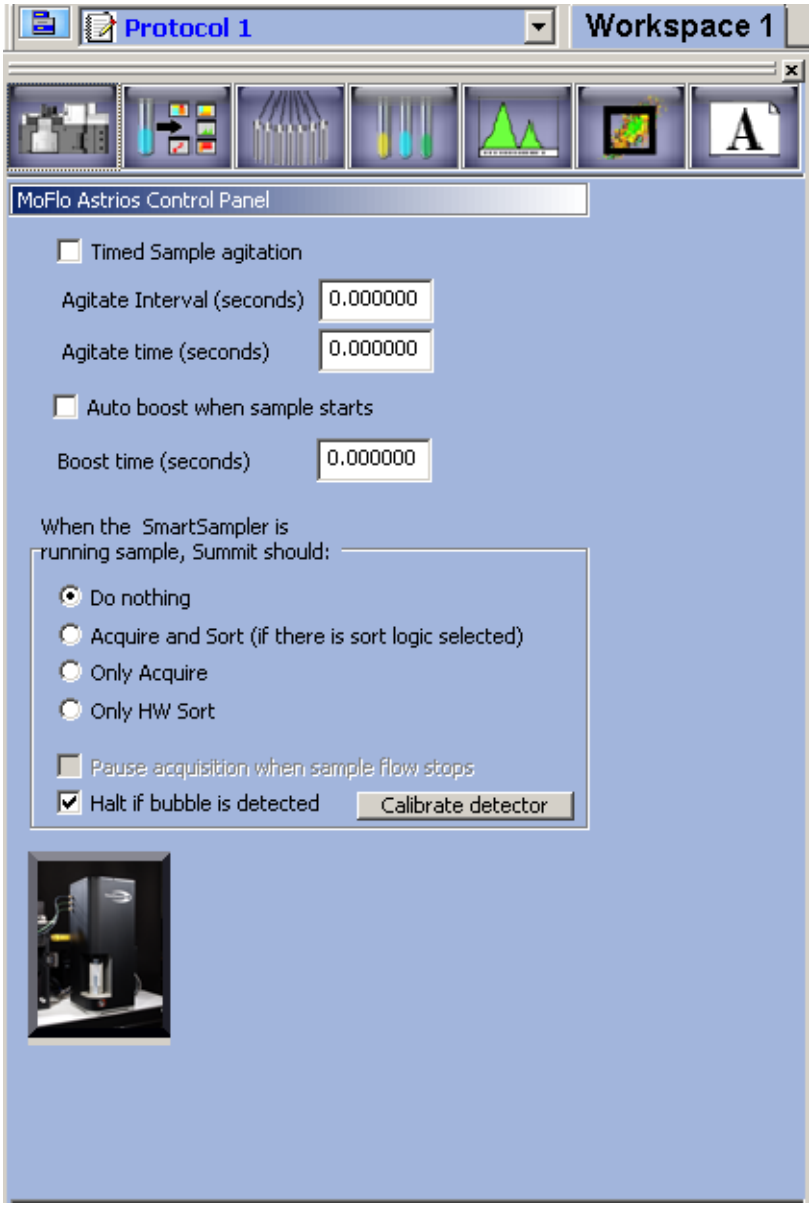


Table 4.1 Instrument Tab - Screen Elements and Functions

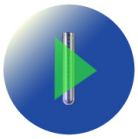
Screen Element	Function
Timed Sample agitation Agitate Interval Agitate Time	Select the checkbox when you want to specify the agitate time and the interval between agitations. **NOTE** When the checkbox is not selected, the SmartSampler Agitate button must be turned on and off manually through the Touch Screen Control Panel.
Auto boost when sample starts Boost time	Select the checkbox to set the SmartSampler to automatically boost when sample flow starts and to specify how long auto boost will continue.
When the SmartSampler is running sample, Summit software should: 	
1. Do nothing (See Figure 3.16)	Summit software automatically does nothing when the Start Sample button is pressed. Acquisition (F2) and Sorting (F4) can be started and stopped manually.
2. Acquire and Sort (See Figure 3.16)	When the Start Sample button is pressed, the sample flows, sorting starts and data is acquired in Summit software. Note: Sort logic must be set up in Summit software in order for the sort feature to run. If sort logic is not set up, Summit Software will still acquire data.
3. Only Acquire (See Figure 3.16)	When the Start Sample button is pressed, the sample flows, and data is acquired in Summit Software.
4. Only HW Sort (See Figure 3.16)	When the Start Sample button is pressed, the sample flows, sorting starts but data is not automatically acquired in Summit Software. It is possible to manually acquire data in Summit Software while in this mode. **1.** Press the Start Sample button to automatically start the HW sort. **2.** Set cycle mode and start data acquisition (F2). **3.** Pause data acquisition but continue to sort (F2). **4.** All intervals of acquired data will be saved to the same FCS file at the end of the sort.
Pause acquisition when sample flow stops	Select the checkbox to set the Start Sample button to start sample and acquire data. When pressed again, it will pause data acquisition.

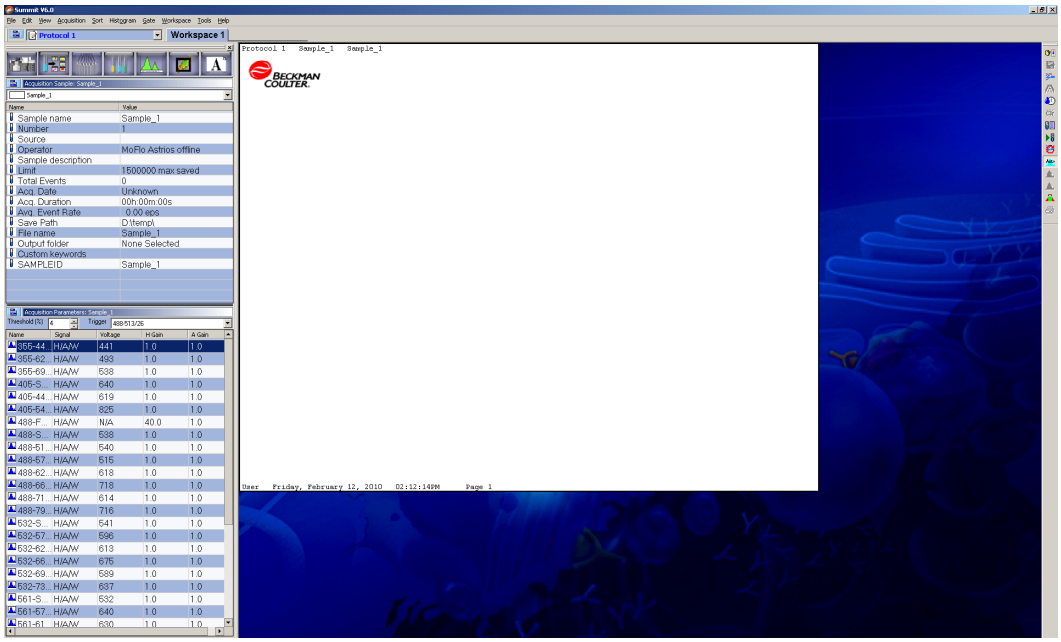
Table 4.1 Instrument Tab - Screen Elements and Functions *(Continued)*

Screen Element	Function
Halt if bubble is detected	**IMPORTANT** This feature works correctly only if you calibrate the air detector as needed. If you do not intend to calibrate the air detector leave the checkbox blank. Select to stop sample flow if the SmartSampler air detector detects a bubble.
Calibrate detector	Click this button to backflush the sample line and calibrate the SmartSampler air detector. Note: Fluidics must already be turned ON.
SmartSampler button (See Figure 3.16) 	Click the SmartSampler button to display the SmartSampler control panel and instrument status indicators in Summit software. 

Acquisition Tab

The Acquisition tab allows you to specify the data types that will be acquired in Summit Software. From this location you can also set up specific sample run information and view sample run statistics.

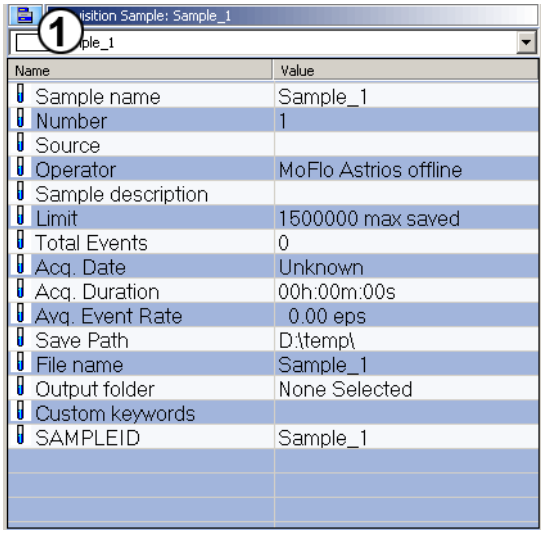
Figure 4.8 Acquisition Tab



Acquisition Sample Panel

The Acquisition Sample Panel can be customized to display, and later save, information specific to a sample run.

Figure 4.9 Acquisition Sample Panel



How to Edit Information Specific to a Sample Run

1 Double-click a **Value** field you intend to edit.

2 Change the information in the **Value** fields as desired.

NOTE To individually change a field, double-click in that field, enter the change, and click away from the field.

3 To add a new **Name** and **Value** to the panel select **Add Keyword**. The **Edit Keyword** dialog box appears.

4 Enter the new information, and click **OK**.

Enable Parameters

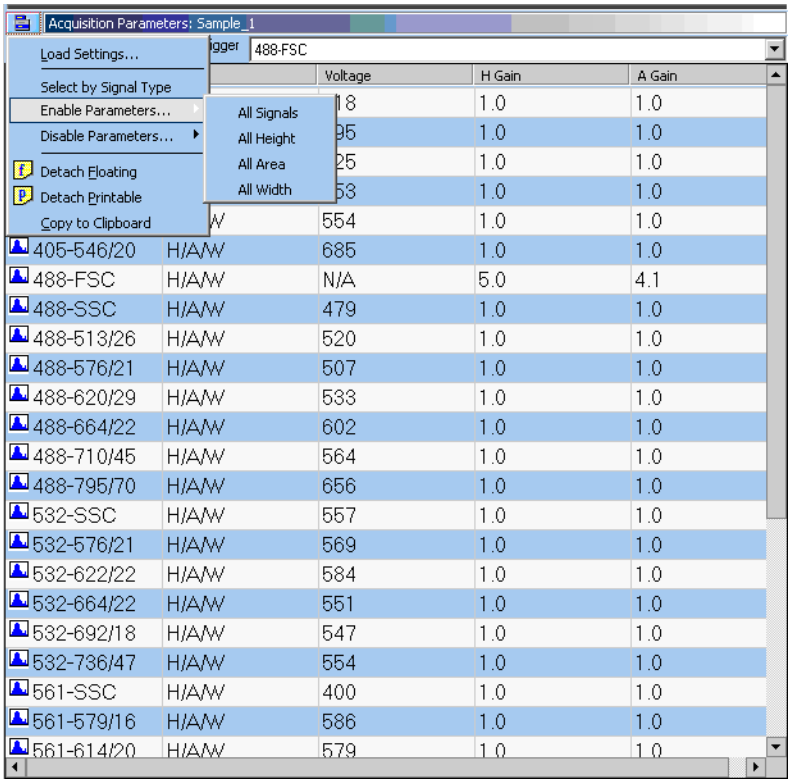
Before you can set up histograms or dot plots you must enable the parameters that you intend to use for your experiment. When a parameter is enabled, the instrument collects linear height, area, and width information. All other parameters, such as log values, are computed using the linear data. Unlike this feature in older versions of Summit, parameters in Summit 6.0 may be either all enabled or all disabled. (For instructions, see How to Enable Specific Parameters for All Data.) You may also enable height, area, width, log height, and log area individually. (For instructions, see How to Enable Individual Parameters.)

How to Enable Specific Parameters for All Data

1 Click the **Acquisition** screen tab and locate the **Acquisition Parameters** panel.

2 Click the Menu icon and then select **Enable Parameters...** to access the submenu shown in Figure 4.10.

Figure 4.10 Enabling Specific Parameters for All Data



- H/A/W = Enabled Height/Area/Width

NOTE The **Disable Parameters...** option below the **Enable Parameters...** option allows you to disable height, area, or width for all data.

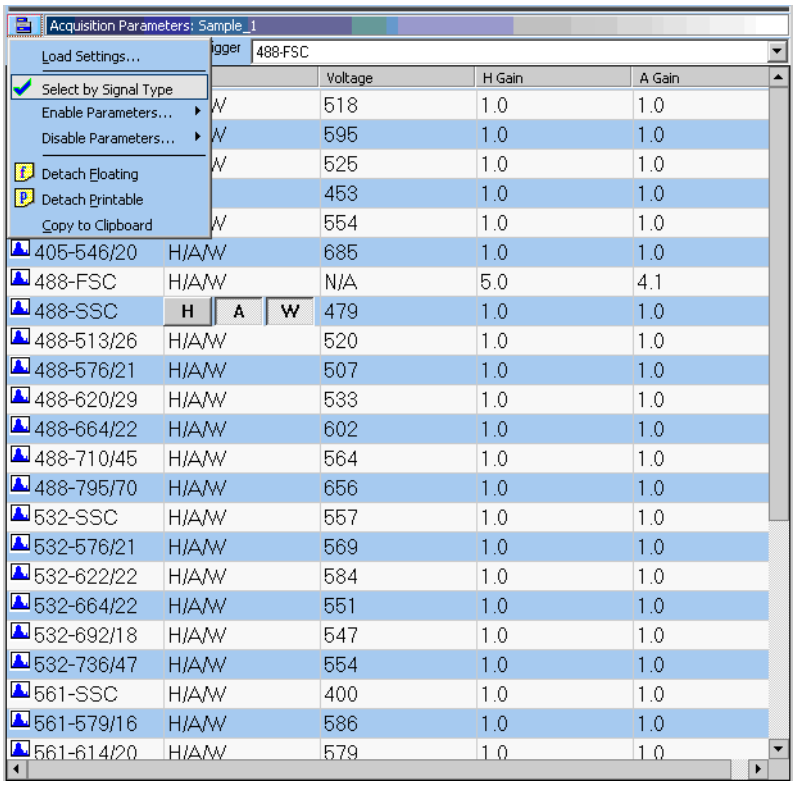
3 To enable parameters, select the appropriate option(s).

How to Enable Individual Parameters

1 Click the **Acquisition** screen tab and locate the **Acquisition Parameters** panel.

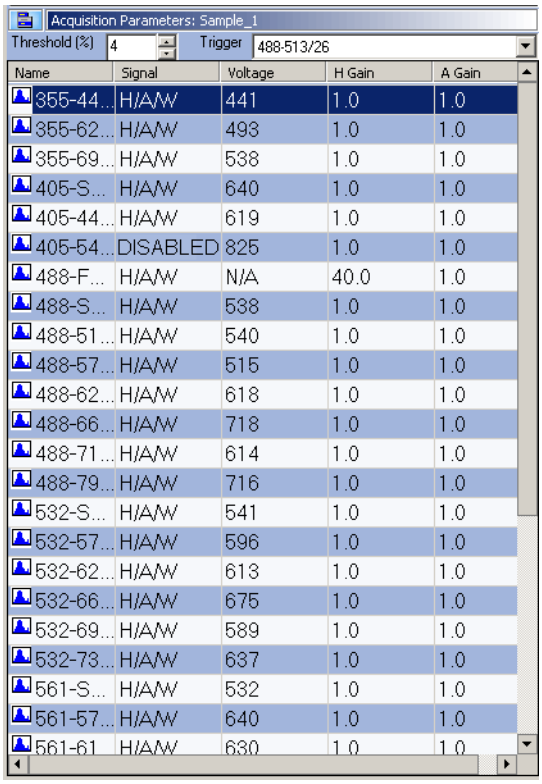
2 Click the Menu icon **> Select by Signal Type** (see Figure 4.11). Double click on the signal column for the parameter to be enabled.

Figure 4.11 Enabling Individual Parameters



3 To enable parameters, select a line in the grid that contains the parameter you intend to enable. Double-click the word **Disabled**, then click away from that line in the grid to see H/A/W, which means all parameters for that detector are enabled.

Figure 4.12 Acquisition Parameters Panel



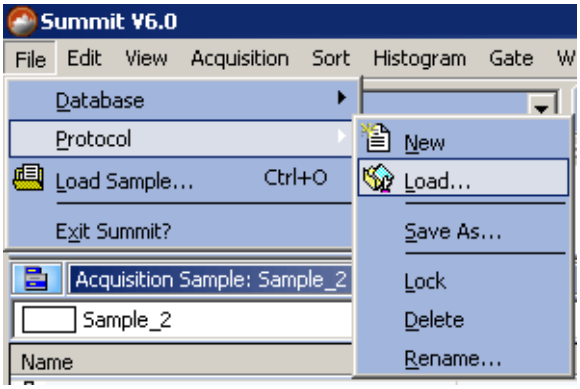
- H/A/W = Enabled Height/Area/Width
- DISABLED = All data types disabled for the parameter.

Loading an Existing Protocol

How to Load an Existing Protocol

1 To load a previously saved protocol select **File > Protocol > Load**.

Figure 4.13 Loading an Existing Protocol 1



2 A list of previously saved PLO files appears. Select the desired file and click **Open**.

Figure 4.14 Loading an Existing Protocol 2

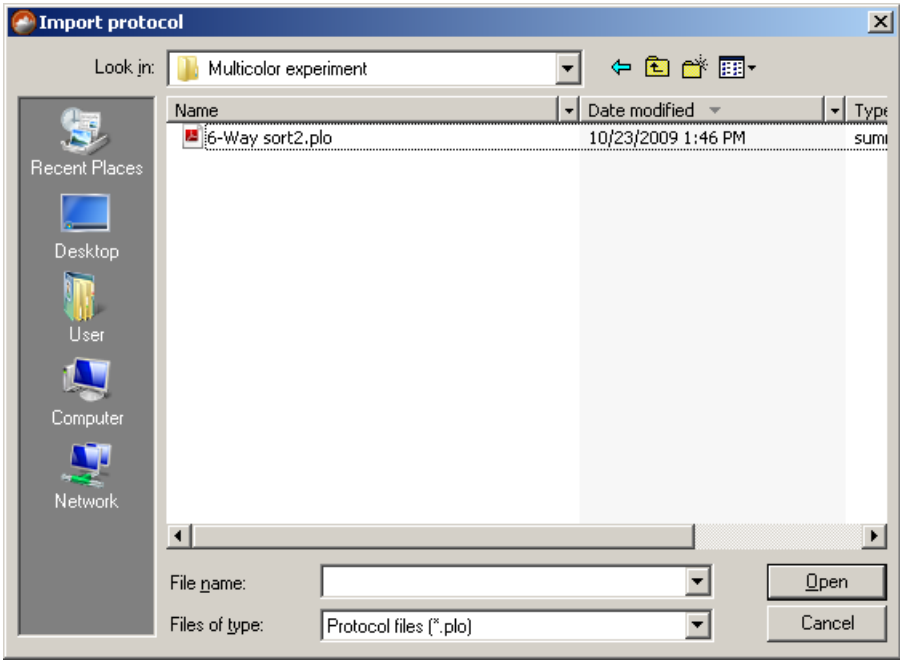
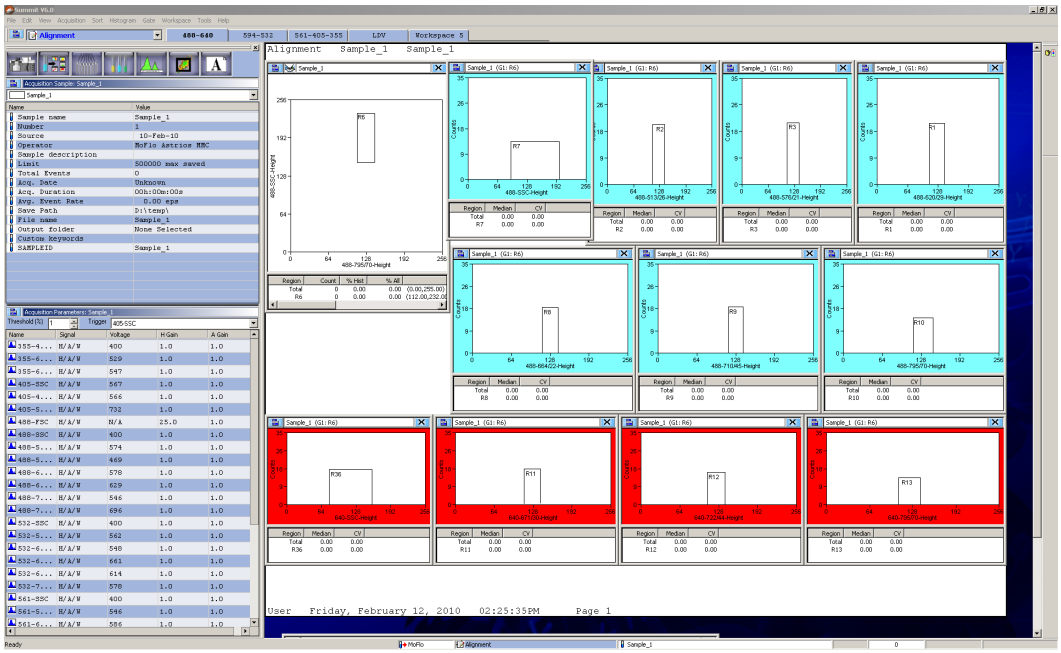


Figure 4.15 is an example of a typical alignment protocol that contains empty histograms in which to acquire data or display and analyze previously acquired data.

Figure 4.15 Loading an Existing Protocol 3



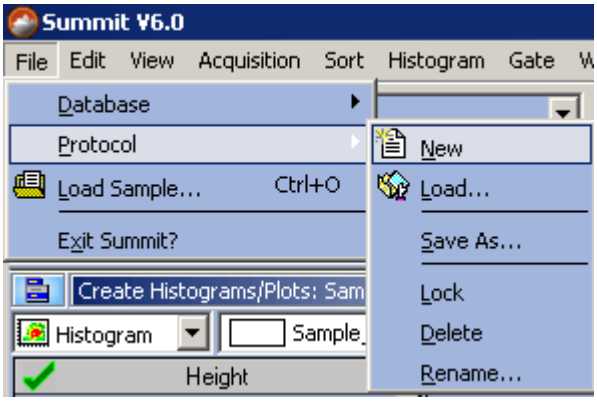
Creating Protocols

When you open a new database there is a workspace in which to create histograms and dot plots. The histograms and dot plots that you create become Protocol 1. It is possible to create additional new protocols for this database, or load pre-existing protocols.

How to Create a New Protocol

1 To create a new protocol, go to the main menu and select **File > Protocol > New.** A new workspace appears in which to create dot plots and histograms for the new protocol.

Figure 4.16 Create a New Protocol



2 Ensure that you have enabled the desired parameters. See page 4-11.

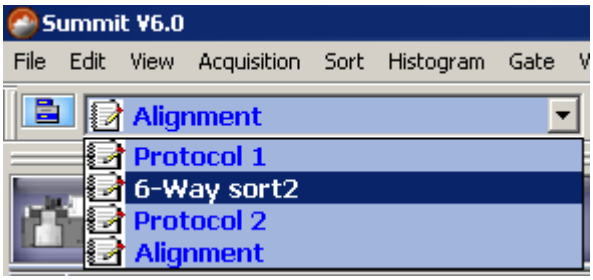
3 Create dot plots and histograms. See page 4-39.

Switching Protocols

To change protocols in Summit Software, go to the Protocols toolbar and select a new protocol from the pull-down menu.

NOTE Only the protocols that you have loaded into the current database or that you have recently created will appear in this list.

Figure 4.17 Switching Protocols



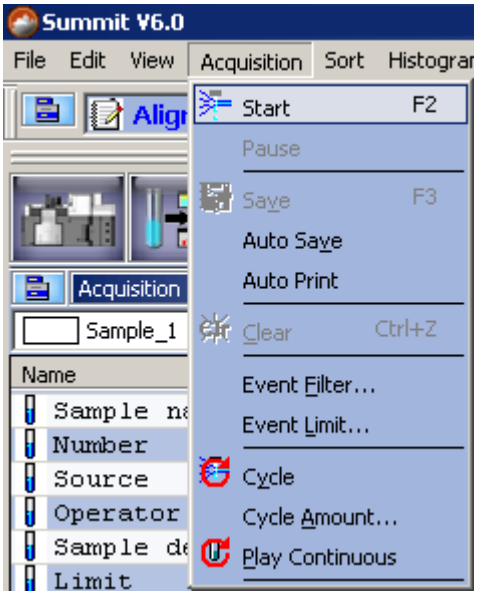
Acquiring Data in Summit Software

Sample must be running before you can acquire data in Summit Software.

How to Start or Stop Data Acquisition

1 Click the Acquisition pull-down menu and select Start (or press F2).

Figure 4.18 Acquiring Data



2 To stop data acquisition, click Stop (or press F2).

NOTE The SmartSampler settings can be changed on the Summit Software Instrument tab.

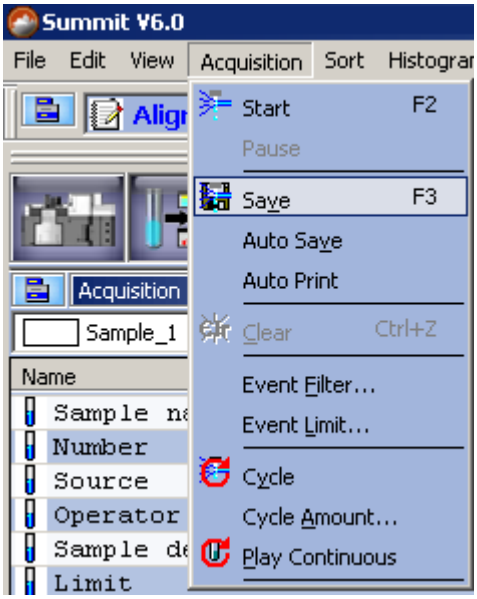
Saving Acquired Data

After you acquire data in Summit Software you can save the information in FCS format. Summit 6.0 stores the protocol used to acquire data, including the gating scheme, in the resulting FCS file.

How to Save Acquired Data

1 Click the Acquisition pull-down menu and select Save (or press F3).

Figure 4.19 Saving Acquired Data



2 Select a folder in which to save the data. Enter a file name and select an FCS file type.

3 Click **Save**.

Cycle Mode

The Cycle Mode in Summit Software cycles the events through a buffer to display only the most recent data events. This is useful during alignment activities. The number of data events displayed at any one time is adjustable.

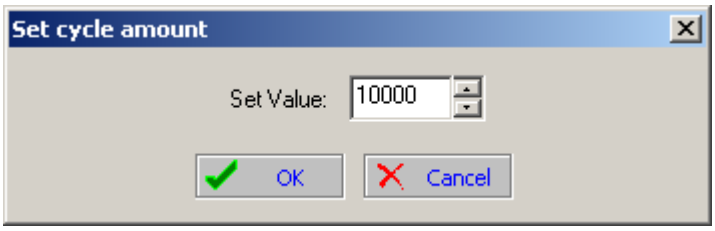
How to Display the Most Recent Data During Alignment Activities

1 Ensure that you are not currently acquiring data.

2 Click on the **Acquisition** pull-down menu.

3 Select **Cycle Amount**. Set the number of events that should be reached before the data cycles.

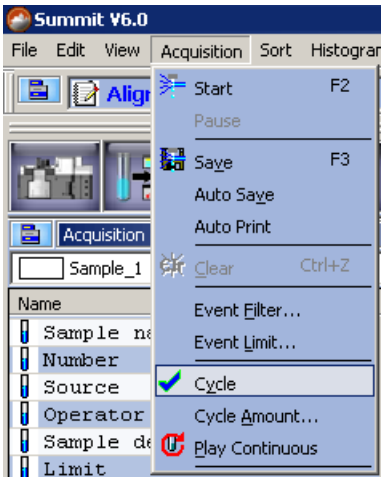
Figure 4.20 Setting the Cycle Amount for Cycle Mode



4 Click **OK**.

5 From the Acquisition pull-down menu, select **Cycle** or click the Cycle Mode icon on the right side of the screen.

Figure 4.21 Enabling Cycle Mode

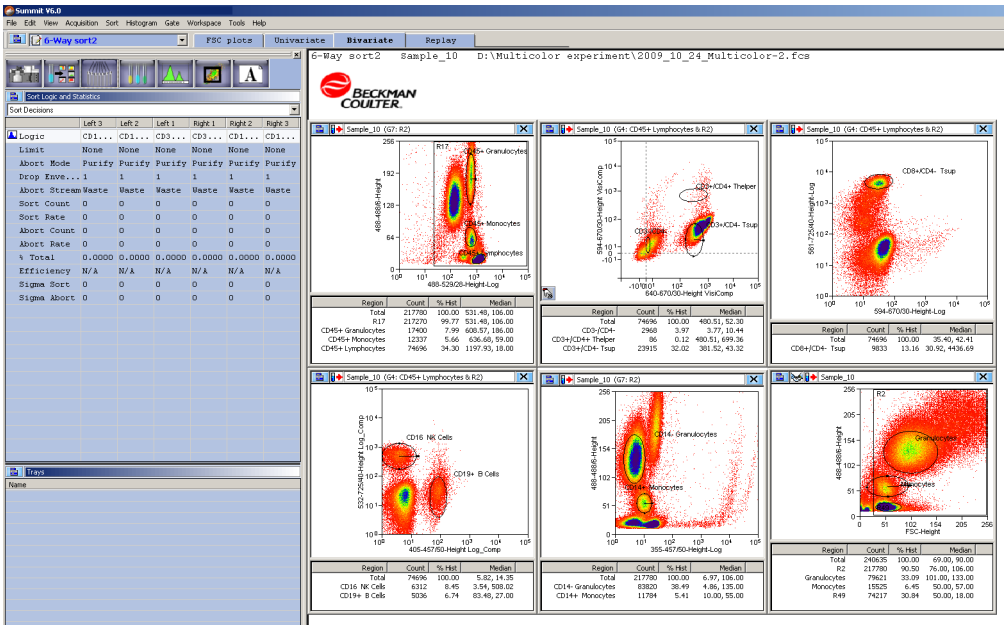


6 After you set the Cycle Mode start acquisition for Cycle Mode to become effective.

Sort Tab

The Sort tab allows you to specify sort logic based on previously set regions and gates. You can view sort statistics as the sort is running.

Figure 4.22 Sort Tab



Clearing Sort Statistics

To clear sort statistics, click the Menu icon for the Sort Logic and Statistics Panel and select **Clear Accumulated Sort Stats**.

On the Sort Statistics screen,

- **Sort #**, **Sort Rate**, **Abort #**, **Abort Rate**, **% Total**, and **Efficiency** clear (update to zero) immediately if the system is sorting. If the system is not sorting, these values automatically reset to zero at the beginning of the next sort.
- ∑ **Sort #** and ∑ **Abort #** do not clear (update to zero) automatically between sorts. These counts continue to accumulate until manually zeroed. This feature allows counts to continue in the event a sort tube is filled and the sort needs to be stopped to replace the full tube with an empty one. Zeroing these values during a sort will display immediately. Zeroing these values after a sort is completed will display the zeroed values at the beginning of the next sort.

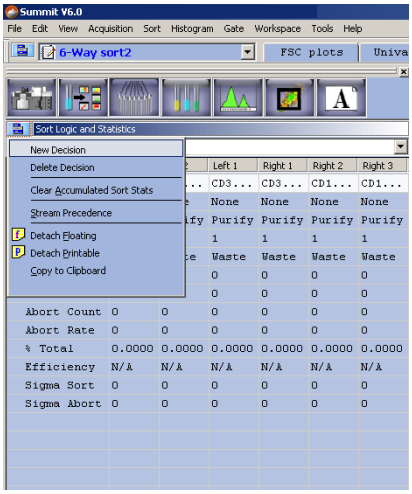
Set Sort Decisions

Before you can set sort decisions you must acquire data from the sample that you intend to sort. You must also set one or more regions in the data so that you can define the population that will be sorted.

How to Create or Edit Sort Decisions

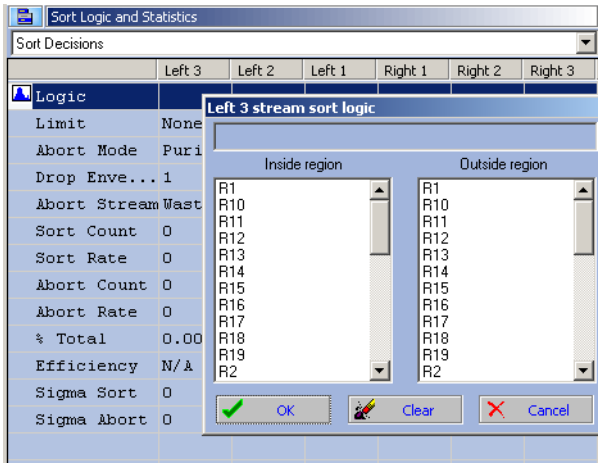
1 Create and edit sort decisions in the Sort Logic and Statistics Panel. Launch the sort logic editor by clicking the Menu icon and selecting **New Decision**.

Figure 4.23 Set Sort Decisions



2 Double-click on a field in the blank **Logic** field (below the column header) that corresponds to the stream you want to set up.

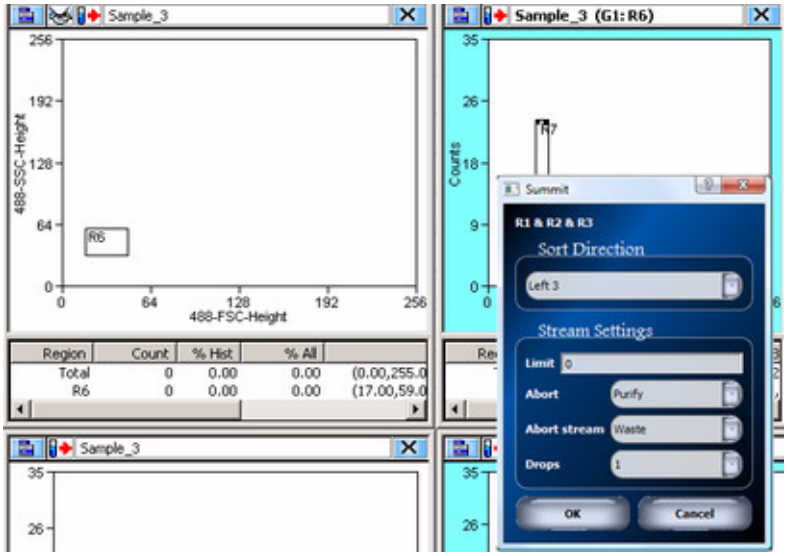
Figure 4.24 Select a Stream



3 You may select one or more regions from the inside or outside region list. All of the regions selected are used to create the sort logic. The resulting expression is displayed in a static text box at the top of the window.

4 You may also set a sort decision by right-clicking on a region and selecting the sort stream from a submenu. Right-click and select **Sort Directions**. A submenu appears listing each available stream. The regions selected in the editor do not reflect the current sort logic for that stream, but rather the region that was right clicked as well as any regions in the logical gate applied to the histogram.

Figure 4.25 Right-click a Region Set Sort Decisions



View Sorts and Aborts Per Stream in Histograms

There are three ways to view sorts and aborts per stream. They can be viewed on Touch Screen Sort Statistics Screen, on the Acquisition tab in Summit, and in Histograms while you are sorting. To view sorts and aborts in histograms, you must first go to the Histograms tab and create a **Sorts and Aborts** histogram.

Double-click Sorts And Aborts per Stream Figure 4.26 to create a histogram like Figure 4.27.

Figure 4.26 Create a Sorts and Aborts Histogram

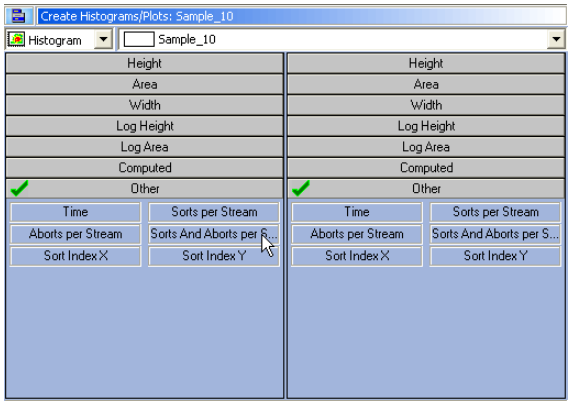
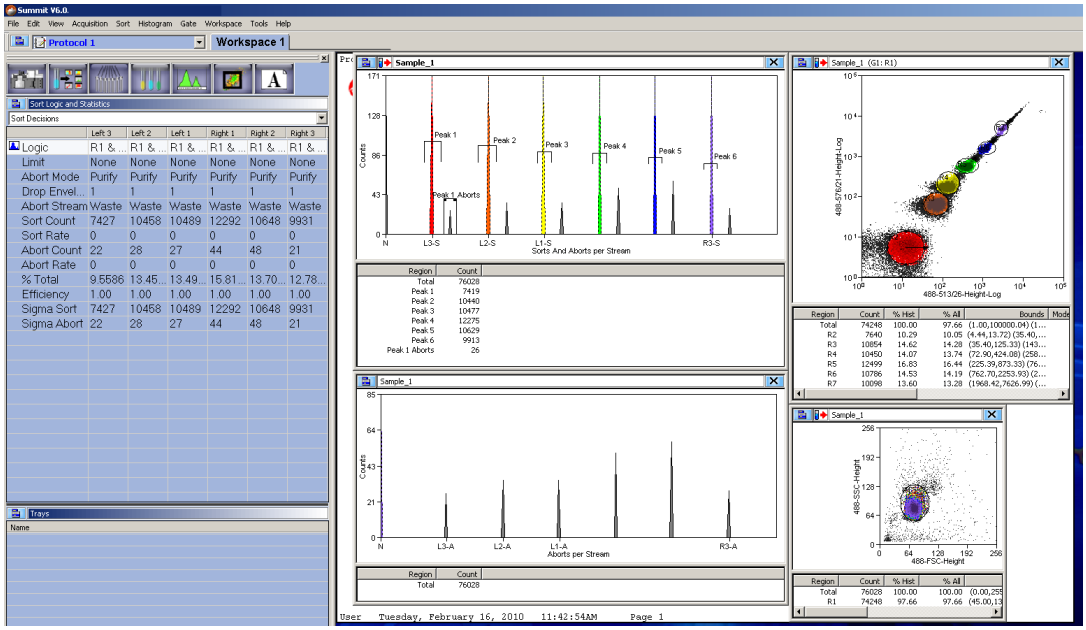


Figure 4.27 View Sorts and Aborts Per Stream



Index Sorting

Index Sorting provides an on-screen representation of a microplate that helps you determine the contents of each well after a sort. Color gating can be used to view the location on the dot plot that is associated with the well.

Figure 4.28 Create an Index Histogram

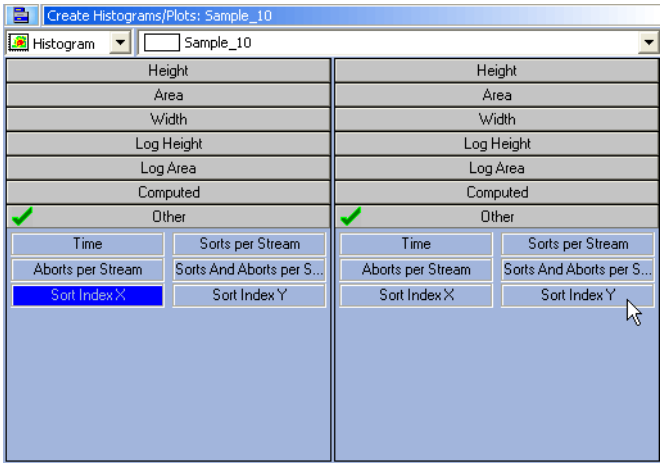
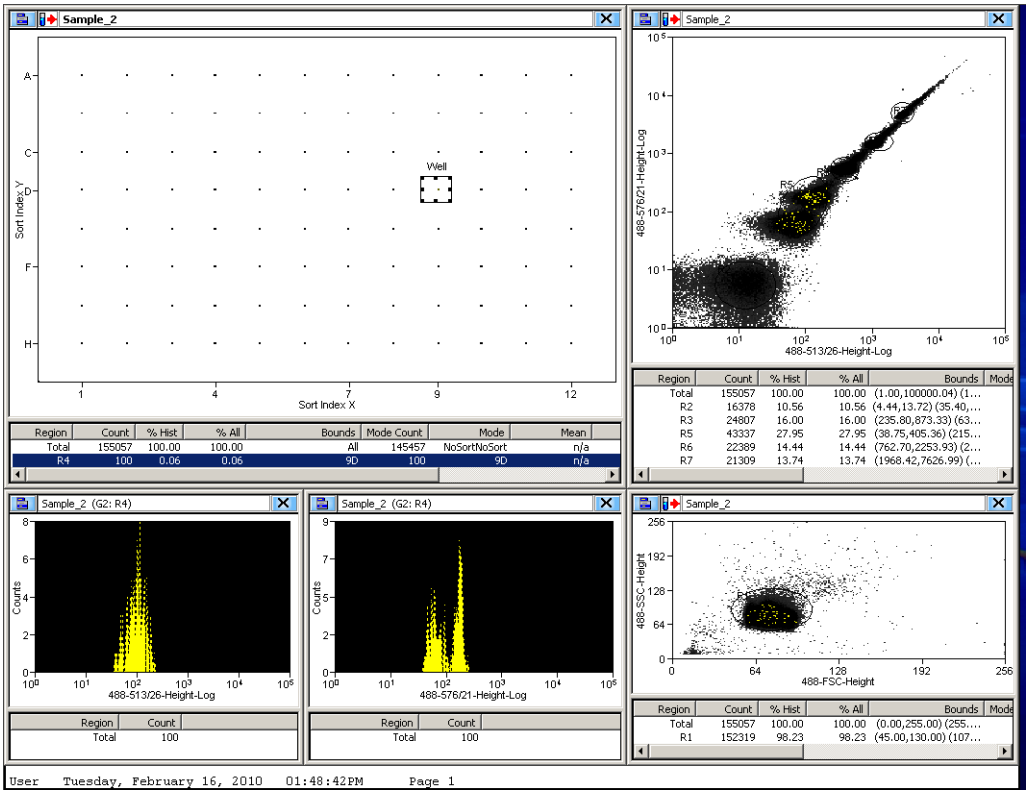


Figure 4.29 Indexed Sorting Display With Color Gating



Sort Report

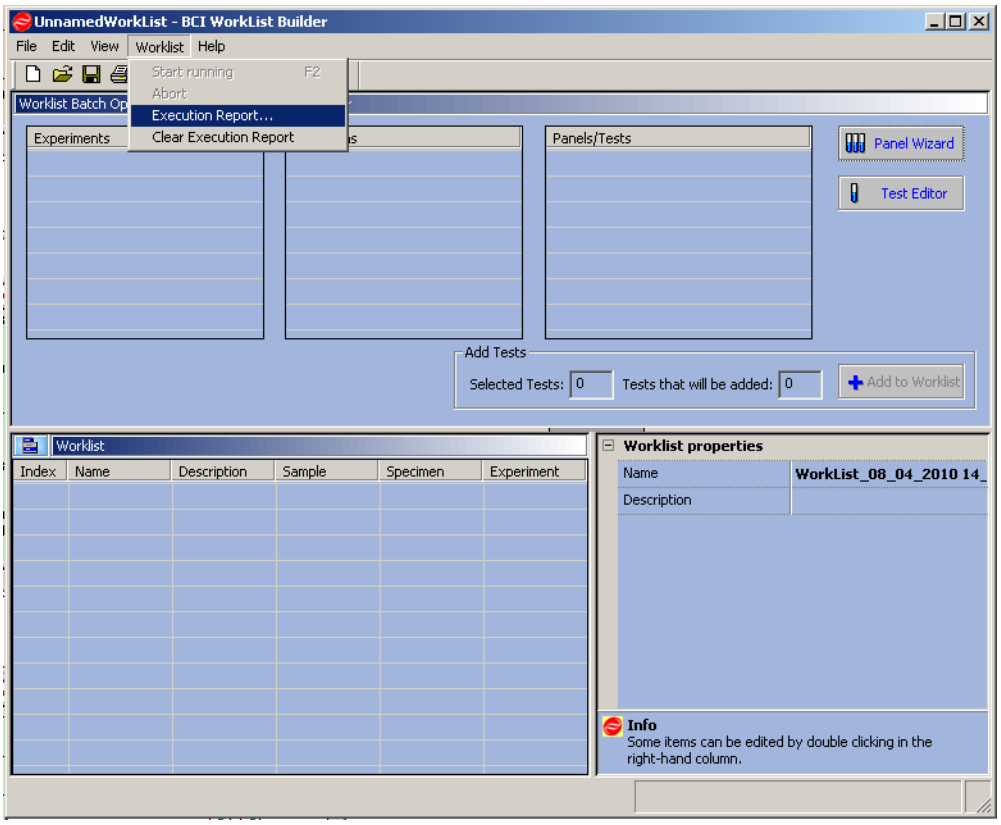
The Sort Report displays the system parameters that were used to create the sort as well as the sort statistics that were produced. The report can be printed if desired.

1 Begin sorting in Summit Software. (F4)

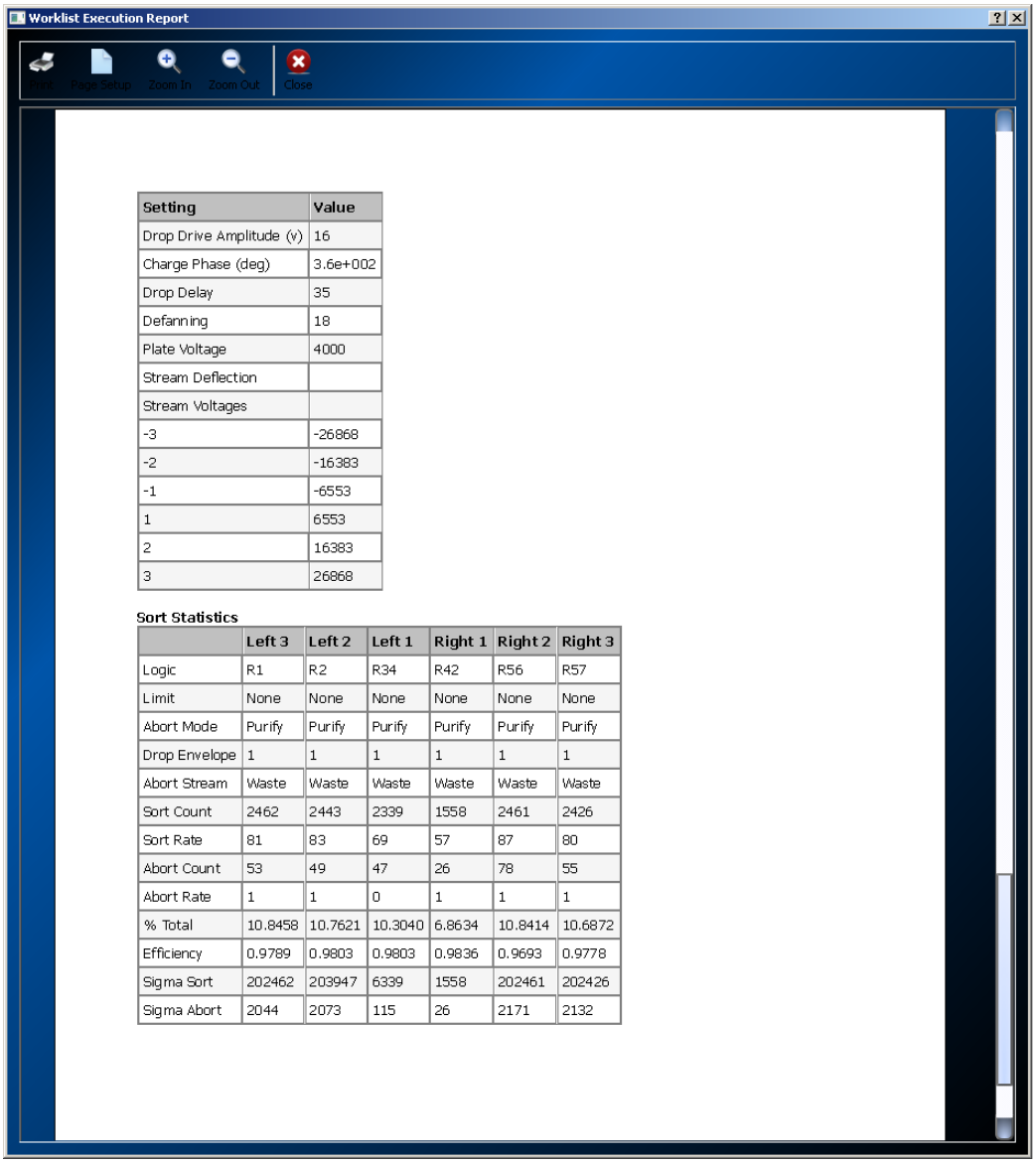
2 Double-click the Worklist Builder icon, or from the main Summit menu, select **View > Worklist Panel**.

3 When you are finished sorting, stop the sort. (F4)

4 Select **Worklist > Execution Report**.



5 The Sort Report appears on screen. You can print the report if desired.



Setting	Value
Drop Drive Amplitude (v)	16
Charge Phase (deg)	3.6e+002
Drop Delay	35
Defanning	18
Plate Voltage	4000
Stream Deflection	
Stream Voltages	
-3	-26868
-2	-16383
-1	-6553
1	6553
2	16383
3	26868

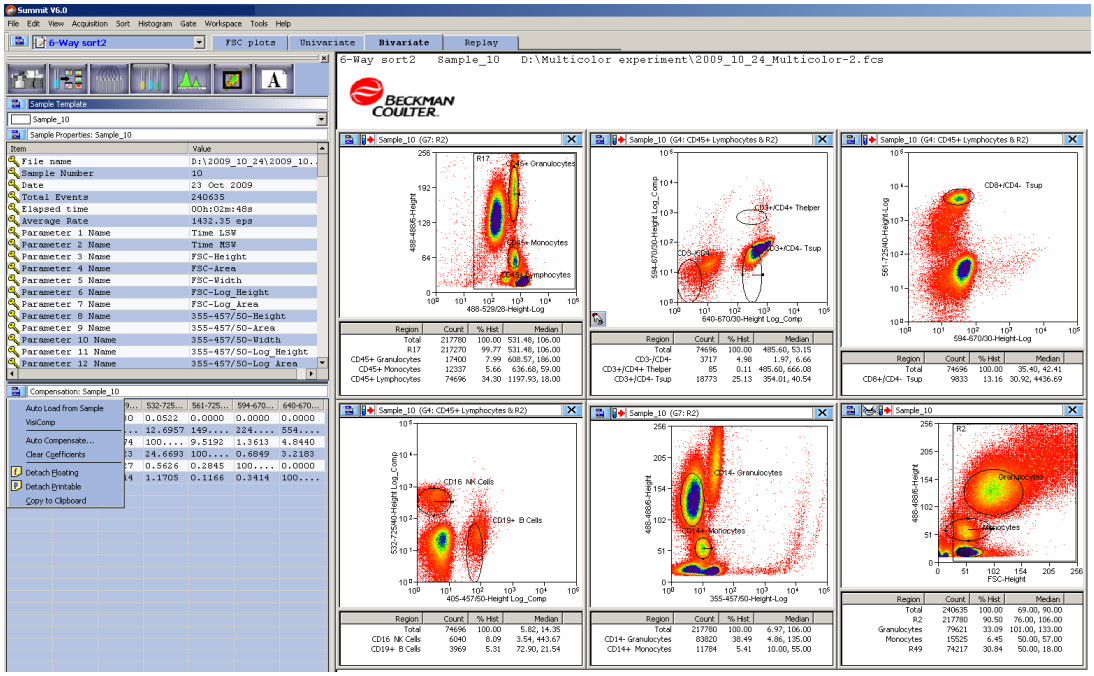
Sort Statistics

	Left 3	Left 2	Left 1	Right 1	Right 2	Right 3
Logic	R1	R2	R34	R42	R56	R57
Limit	None	None	None	None	None	None
Abort Mode	Purify	Purify	Purify	Purify	Purify	Purify
Drop Envelope	1	1	1	1	1	1
Abort Stream	Waste	Waste	Waste	Waste	Waste	Waste
Sort Count	2462	2443	2339	1558	2461	2426
Sort Rate	81	83	69	57	87	80
Abort Count	53	49	47	26	78	55
Abort Rate	1	1	0	1	1	1
% Total	10.8458	10.7621	10.3040	6.8634	10.8414	10.6872
Efficiency	0.9789	0.9803	0.9803	0.9836	0.9693	0.9778
Sigma Sort	202462	203947	6339	1558	202461	202426
Sigma Abort	2044	2073	115	26	2171	2132

Sample Tab

The Sample tab displays the parameters of the selected sample file, and allows you to change the list of parameters visible on screen. From this tab you can also compensate data.

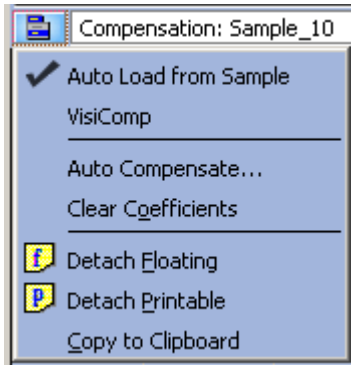
Figure 4.30 Sample Tab



Auto Load from Sample

When you compensate data, the **Compensation Matrix** is established and can be saved with the FCS file. Selecting the **Auto Load from Sample** option located on the **Sample** tab, causes the **Compensation Matrix** to automatically display when you load a data file for analysis.

Figure 4.31 Auto Load from Sample



Auto Compensation Wizard

Summit Software provides an automatic method to obtain a full compensation matrix for multi-color analysis. The compensation matrix is calculated from single stained controls by the Auto Compensation function.

The issue with Auto Compensation on an Astrios with the stock filter configuration is that for a given fluorochrome, multiple channels can detect the signal. The following table shows some examples of these duplicated channels (by no means is it exhaustive):

Fluorochrome	Detector Channels with Signal		
PE	488-576/21	532-576/21	561-579/16
PE-Texas Red	488-620/29	532-622/22	561-614/20
PE-Cy5	488-664/22	532-664/22	592-671/30
PE-Cy5.5	488-710/45	532-692/18	
APC	592-671/30	640-671/30	
APC-Alexa 750	592-795/70	640-795/70	

It is essential that each fluorochrome to be compensated appears only once in the spillover matrix. Since the spillover values are determined by the data from detector channels and more than one channel can detect a single fluorochrome, there is a risk that a fluorochrome could appear multiple times in the matrix. This would cause the following problems:

- Parameters that must have spillover corrected from a signal that is detected in multiple channels effectively have the spillover subtracted multiple times. This results in significant overcompensation.
- The positive signal in the duplicated parameters is significantly reduced or even eliminated when auto-compensated against a parameter that essentially has the same signal. This effect is similar to compensating a parameter against itself.

To prevent these problems, use the best channel from each of the duplicated channel sets and only allow that channel in the compensation matrix.

How to use the Auto Compensation Wizard for a Single Stained Control

1 Acquire the first single-control sample required for your experiment. The first control sample should include an unstained or isotype control for which you will set PMT voltages. From the resulting dot plot, you can determine gating if required. Any gates that you want to use must be set before you apply Auto Compensation.

NOTE During the Auto Compensation operation, adjustments to only the size and placement of regions are allowed.

2 Run the remaining single control samples and save the data files.

NOTE It is helpful to use the cell type, epitope, and conjugated fluorochrome in the file name for future reference. For instance: CHO_CD45_FITC.fcs

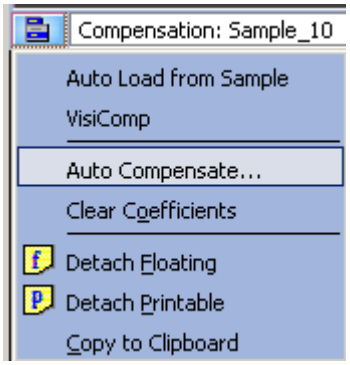
3 Load all control sample files into an experiment folder.

4 Click the Sample tab.

5 Identify or create a dot plot that will be used to adjust compensation. Ensure that the parameter for which you are compensating is on the x-axis.

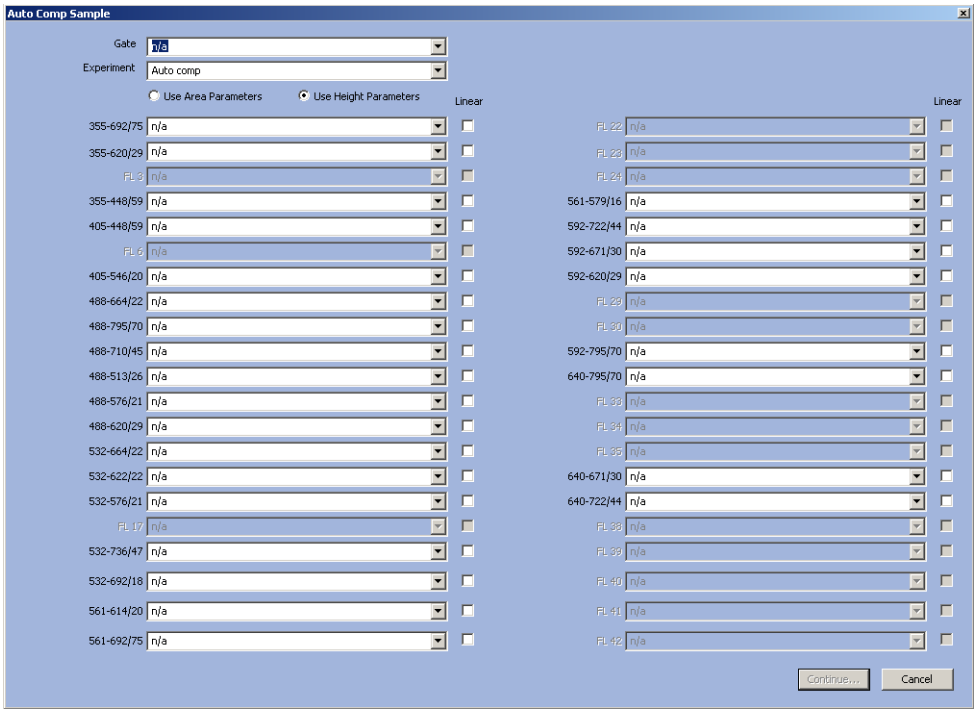
6 In the **Sample Compensation** panel, click the extended menu icon  and select **Auto Compensate** from the list.

Figure 4.32 Select Auto Compensate



7 The **Auto Comp Sample** dialog box appears.

Figure 4.33 Auto Comp Sample Dialog Blank

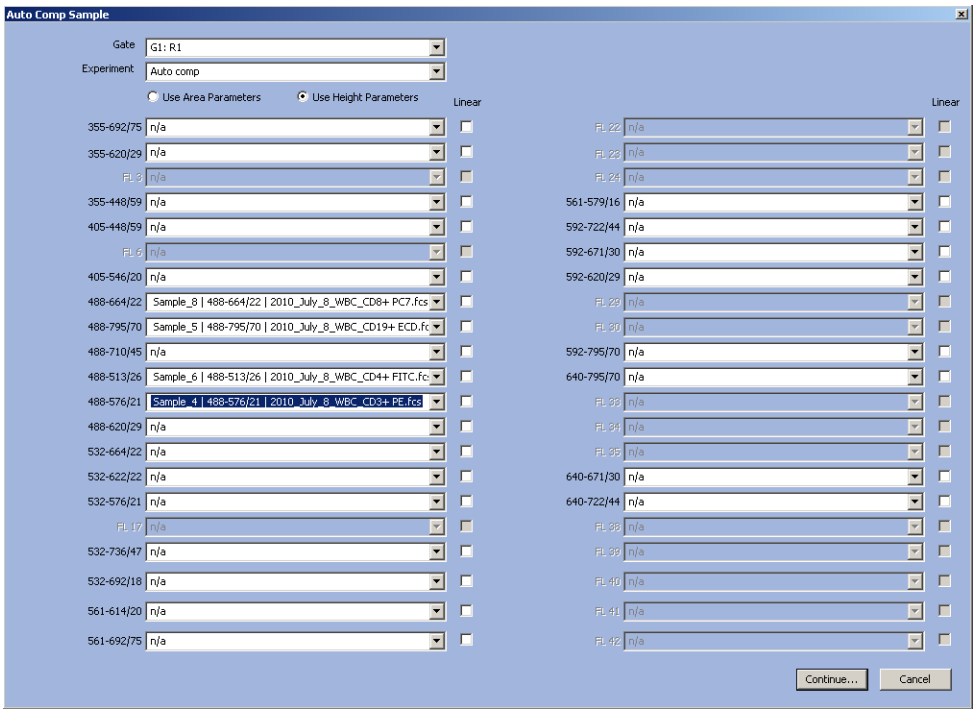


8 Select a gate from the Gate pull-down menu, if applicable.

9 From the **Experiment** pull-down menu, select the experiment folder that contains your control samples.

10 One-at-a-time select all of the single control samples included in the experiment. A parameter fluorochrome can only be selected once, irrespective of the number of detectors sensing the signal.

Figure 4.34 Auto Comp Sample Dialog Box Populated



11 Click **Continue**.

IMPORTANT If you click **Cancel** at any point in the auto compensation process, you will clear the compensation matrix and the **AutoComp Workspace.**

12 A new Workspace labeled **AutoComp** is created and the first set of dot plots is displayed. Each dot plot places the control parameter on the x-axis and a parameter to compensate against on the y-axis. Default auto compensation **Dim** and **Bright** regions are displayed and, if a gate was selected, it is applied to each dot plot. The **Auto Compensate** wizard appears.

Figure 4.35 Auto Compensate Wizard

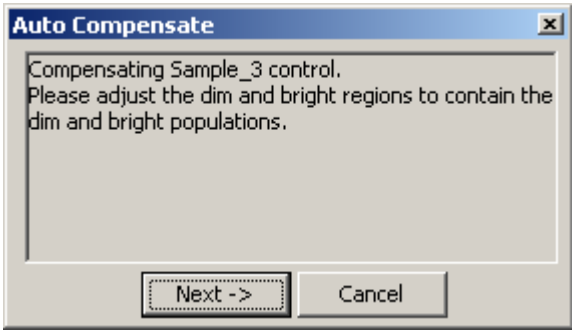
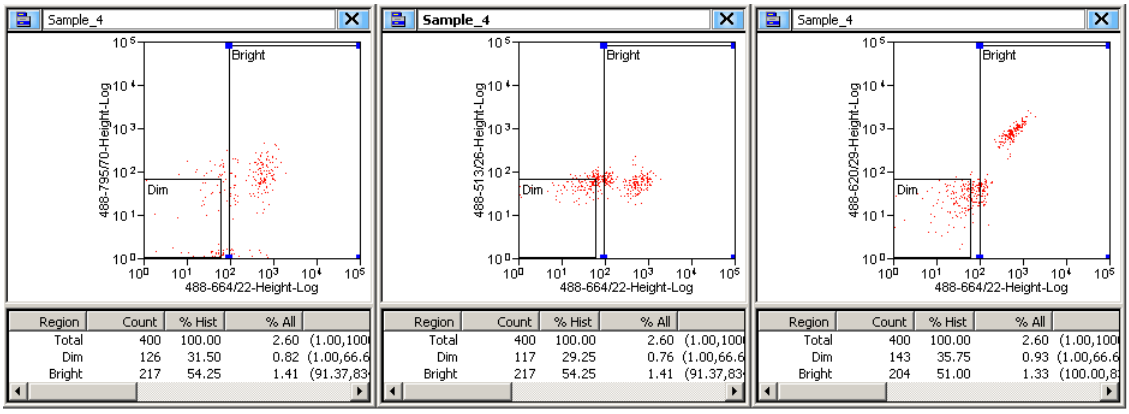
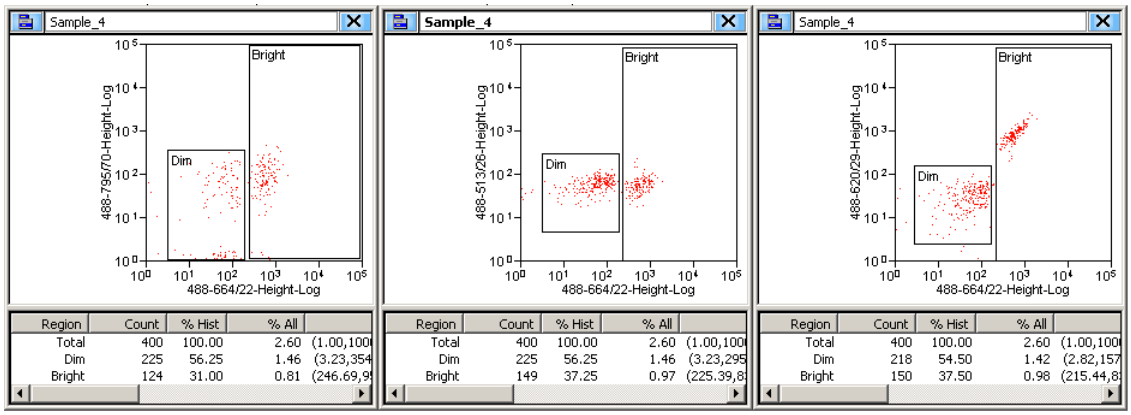


Figure 4.36 Single Control Sample Dot Plots



13 Examine the *% Hist* statistics for each histogram. If either the **Dim** or **Bright** region contains less than 5% of the data for the dot plot, click-and-drag the region until greater than 5% of the data appears in both the **Dim** and **Bright** regions.

Figure 4.37 Auto Comp Adjusted Regions



14 When all regions on all plots contain greater than 5% of the data, Click **Next** on the **Auto Compensate** dialog box. The next set of dot plots will appear.

15 Repeat step 13 until all single-control samples have been compensated. When auto compensation is complete, the compensation matrix contains the appropriate values and the **AutoComp** workspace is removed.

Figure 4.38 Compensation Matrix

Compensation: Sample_5

Parameter	488-513/26-Height	488-620/29-Height	488-664/22-Height	488-795/70-Height
488-513/26-...	100.0000	0.0000	0.0000	0.0000
488-620/29-...	8.0706	100.0000	160.8759	0.3554
488-664/22-...	5.1605	101.1257	100.0000	0.2111
488-795/70-...	0.0000	14.3896	16.4902	100.0000

Applying VisiComp

To help you better visualize the results of compensation, Summit Software includes a scaling algorithm called VisiComp that displays 0 and negative values. VisiComp provides a good way to verify the results of the Summit Software Auto Compensation feature, and allows you to fine tune and make adjustments to compensation.

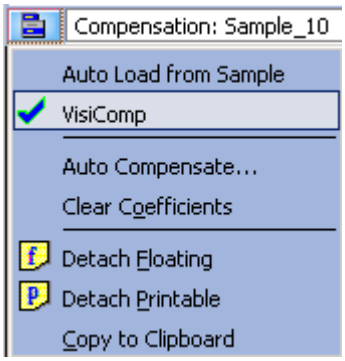
How to Use VisiComp to Visualize Compensation Results

1 Pre-load all necessary samples (Listmode FCS files) that are required to perform compensation.

2 Create all plots, regions, and gates.

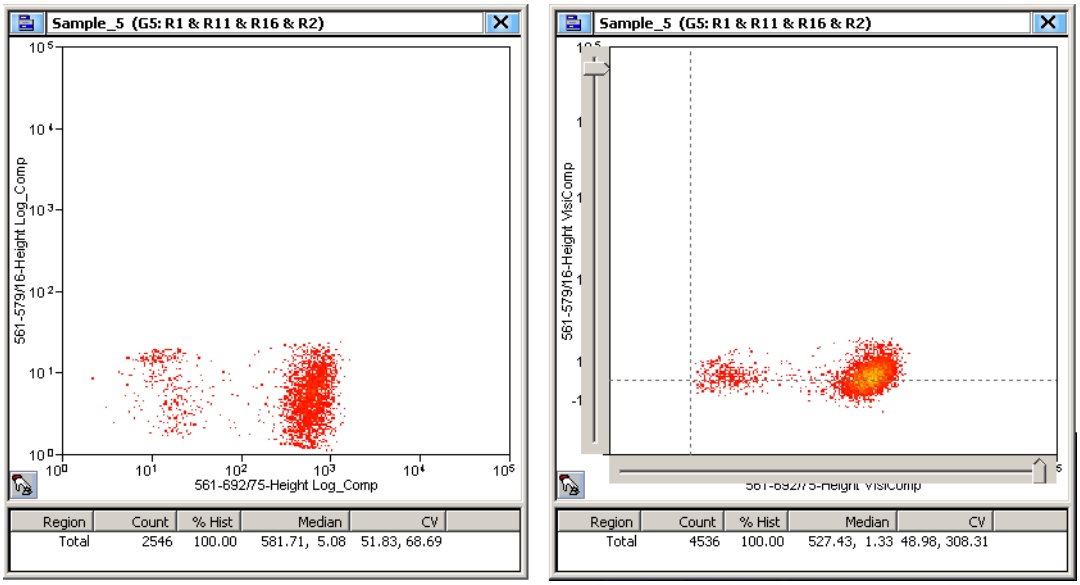
3 On the Sample tab, click the **Compensation** panel icon and select **VisiComp**.

Figure 4.39 Apply VisiComp



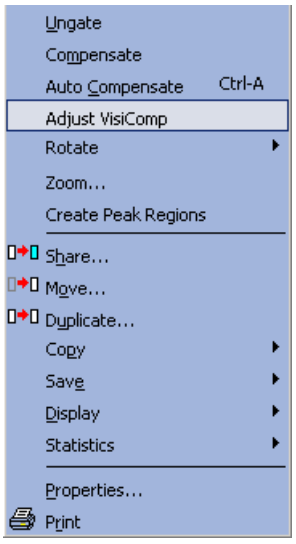
4 Use either the auto compensation wizard to set up plots, or manually set up all of the plots that you want to use for compensation analysis.

Figure 4.40 Data Displayed Before and After Applying VisiComp



5 To adjust the width of the VisiComp linear region click the Sample icon in the upper left portion of the window and select **Adjust VisiComp**. Use the slider tool, or enter a specific value to complete the adjustment.

Figure 4.41 Adjust VisiComp



NOTE The adjusted width of the VisiComp linear region applies to all plots and histograms that display compensated parameters in any one sample template. Because of this, it is important to display all data before you adjust the width. What is ideal for one parameter pair may not be perfect for another. Therefore, adjust the width to display the best compromise across all plots.

IMPORTANT If you turn off VisiComp, any regions that extend into the negative area of the VisiComp scale will be moved where they can be displayed on the log scale. Any regions that were entirely in the negative area will have a 0 width and 0 height.

6 Create regions and gates to complete your analysis.

NOTE If you created regions and gates before you applied VisiComp, you will need to verify the location of the regions.

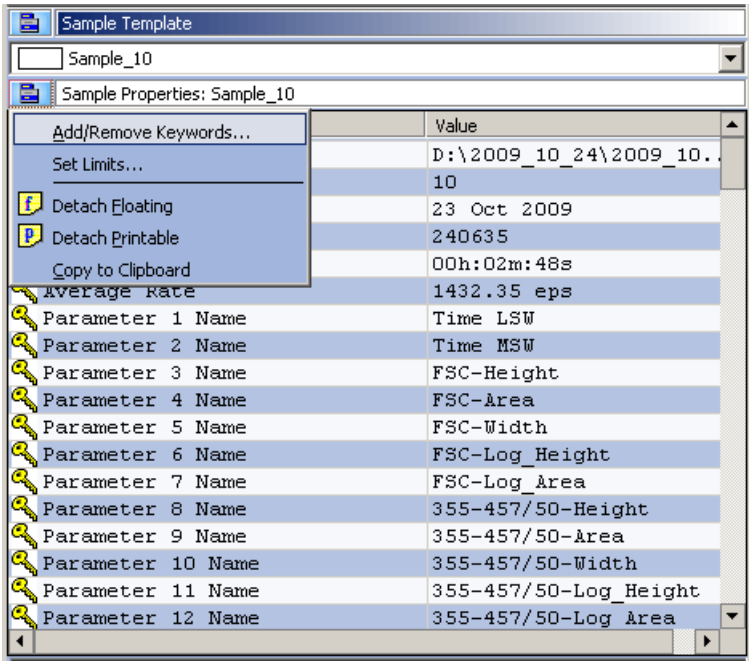
FCS Keywords

To customize your view of sample data you can add and remove Keywords.

How to Add or Remove Keywords

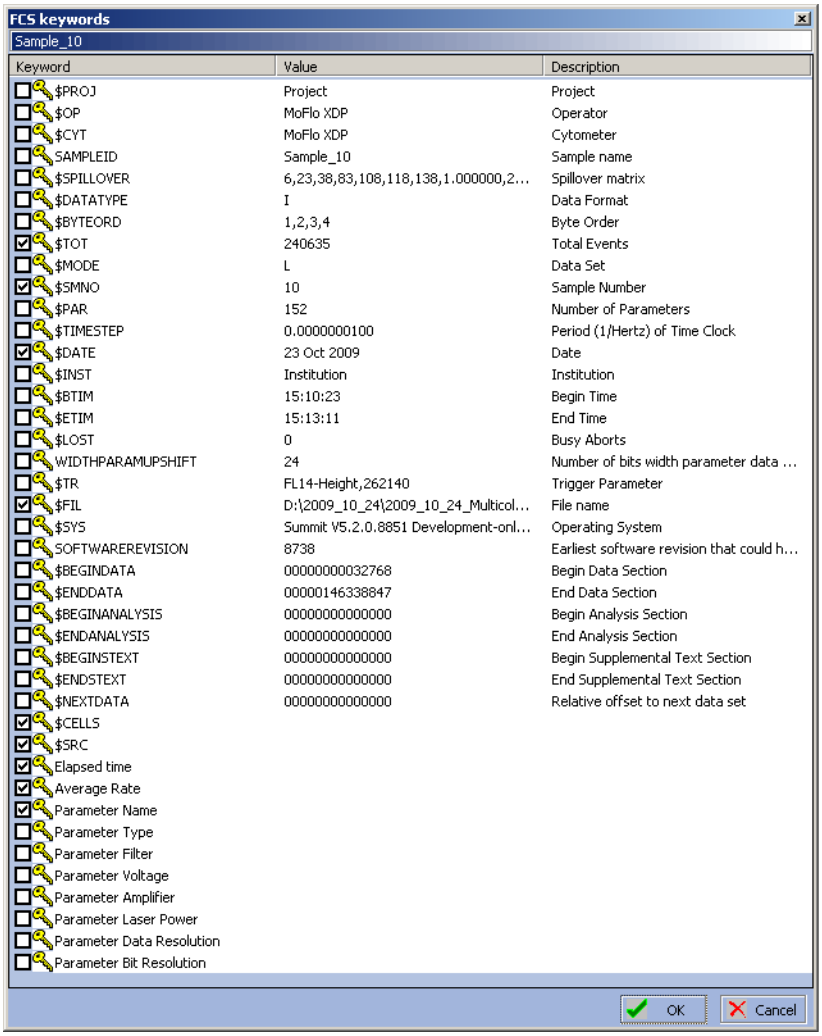
1 Click the blue menu icon and select **Add/Remove Keywords**.

Figure 4.42 Add/Remove Keywords 1



2 Select the checkboxes next to the Keywords you would like to display, and click **OK**.

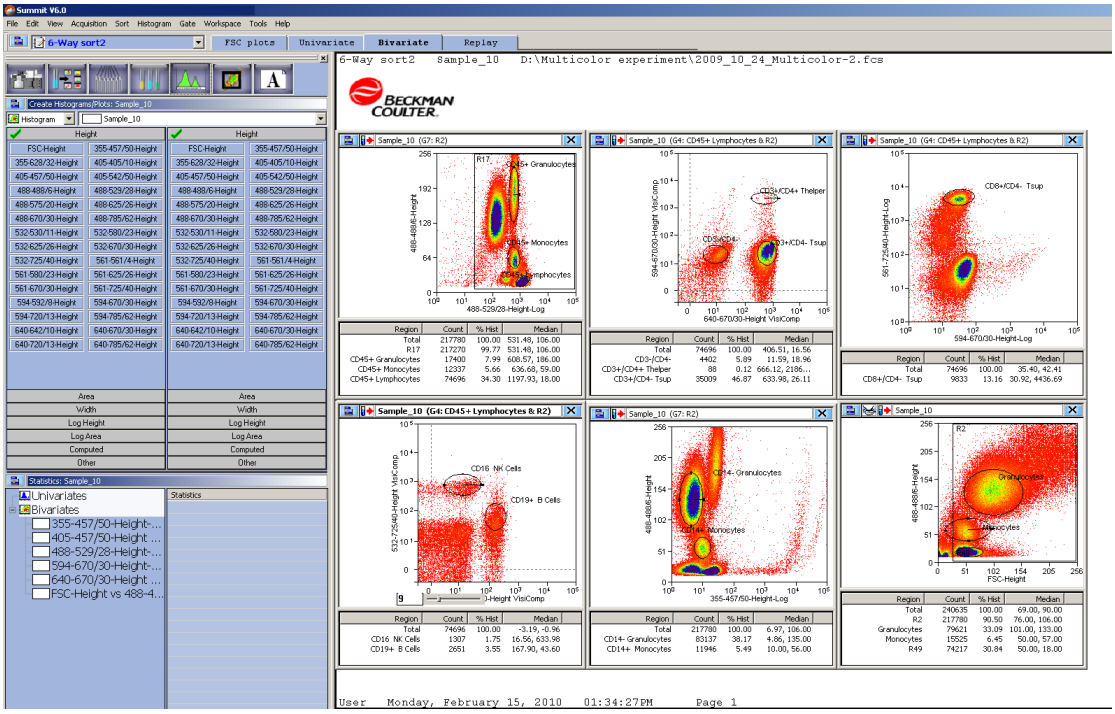
Figure 4.43 Add/Remove Keywords 2



Histogram Tab

Histograms and dot plots (bivariate histograms) are created in the **Histogram** tab. The **Create Histograms** panel displays all of the parameters that are enabled in the **Acquisition** tab.

Figure 4.44 Histogram Tab



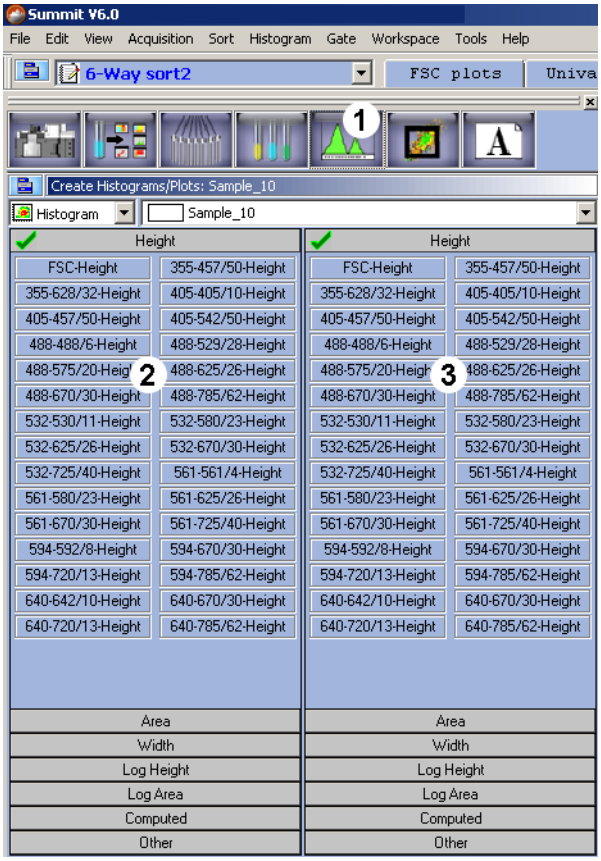
Creating Histograms and Dot Plots

You must create histograms and dot plots in order to display the data you acquire. Prior to creating dot plots and histograms you must enable the parameters you would like to collect. See page 4-11.

How to Create a Histogram or Dot Plot

1 Create dot plots and histograms by selecting the **Histogram** tab in the Summit **Software Control** panel (see #1. in Figure 4.45). The histograms and dot plots that you create will build a Protocol that you can elect to save.

Figure 4.45 Creating Histograms and Dot Plots



1. Histogram Tab
2. X-axis Parameters
3. Y-axis Parameters

2 Select one of the following:

- To create a single parameter histogram, double-click on the X-axis parameter for the histogram you would like to create. The frame for the histogram will appear in the Workspace on the right of the screen.
- To create a dual parameter dot plot, click once on the X parameter and twice on the Y parameter. The newly created frame for the dot plot will appear in the Workspace.

3 It is also possible to create a histogram or plot by right-clicking in a Workspace and selecting **New Histogram** or **New Plot**. It is then necessary to right-click the axes and select the desired parameters.

Maximize Dot Plots and Histograms

To maximize dot plots and histograms double-click on the title bar. This option is useful to better see the data, create regions, or set gates. Double-click the title bar again to restore the image.

Change Axis Parameters

To change the displayed parameter in a dot plot or histogram, right-click on the axis you want to change and select a new parameter from the menu. After you have selected a parameter, click the data type you would like to view. The histogram will change to reflect your selection.

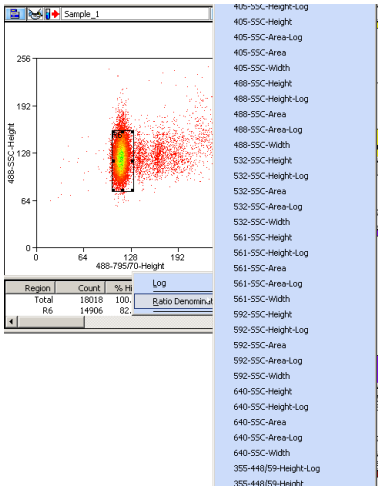
- H = linear height
- A = linear area
- W = pulse width
- L = log height
- LA = log area

Figure 4.46 Change Axis Parameters

Display Ratio

To display the data as a ratio, right-click axis of a histogram to display the Parameter Selector and then click the **More Options** button.

Figure 4.47 Display Data as a Ratio



Create Regions in Histograms

To create bar regions in single parameter histograms right-click in the histogram and select **Bar** from the menu. In a dual parameter histogram, right click to create a rectangle, ellipse, polygon, or quadrants. Once created, you can click and drag to resize and reposition the region. Once you have created regions, the statistics for those regions will appear in the status window below the histogram. As you move regions the statistics will update in real time. To delete a region right-click and select **Delete**.

Renaming Regions

Regions can be renamed to reflect the population inside the region. To rename a region right-click in the region you want to rename and select **Properties**. A dialog box appears. Enter a new name for the region in the upper-left text field and click **OK**.

Copy and Paste Regions

You can copy all regions from histograms and dot plots and paste into another histogram or dot plot. Right-click inside the region and select **Copy**, go to the next histogram or dot plot, right-click and select **Paste**.

Customizing Statistics Display

You can customize the display of statistics in both histograms and dot plots. Click on a plot to make it active. Right-click inside the plot and select **Edit Statistics Display**.

Manually Scaling Data

To manually rescale date within a dot plot or histogram, click the scale up or down buttons on the User Toolbar. If the buttons are not present on the toolbar see Customize the User Toolbar.

Contouring Data

To Enable Contouring, click the extended menu icon within the dot plot  and select **Display > Contour**. Select the **Enable contours** checkbox. The pull-down menu directly below the checkbox lists the available contouring algorithms. The dialog box contains additional options for maximizing data and smoothing the contouring.

Exporting Histograms to Word

To export a dot plot or histogram to Word, click the extended menu icon within the dot plot  and select **Copy > Window as Bitmap**. Open Word and paste the histogram image into the document. The **Copy as Graphic** option does not include the histogram frame or statistics.

Multi File Display

- It is possible to display more than one data file or sample. Select the Sample tab in the Summit Software Control panel. Click the menu icon and select **Duplicate**. This will copy the existing dot plots and histograms in the protocol.

  NOTE All copied versions will be indicated with a different color.

- You can manually arrange the dot plots and histograms, or you can right-click on the white sheet, select **Arrange Windows** and select the desired option.
- To load additional samples, go to the Summit Software Main Menu and select **New > Samples**. Click on a sample name and drag and drop to load additional samples into the templates.

Create Overlays

Overlays are special histograms where you can display data from more than one sample within a single parameter or within a single histogram for one parameter.

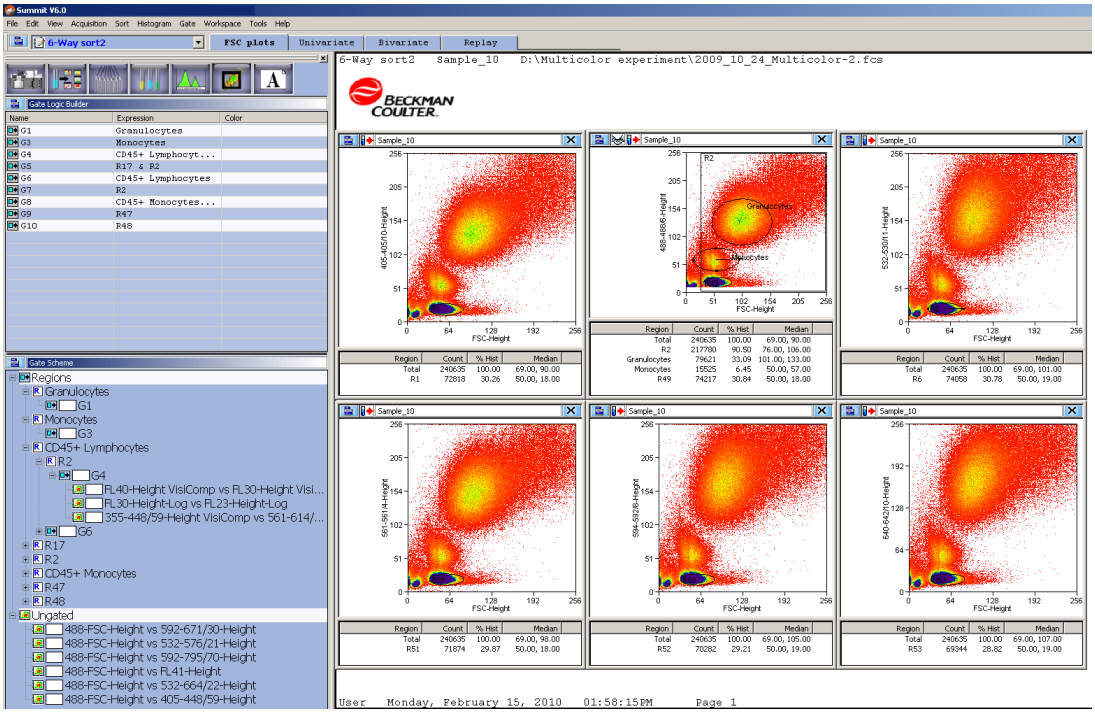
How to Overlay Multiple Histograms

1. To create an overlay, select the Histogram tab from the Summit Software Control panel.
2. Click the pull-down menu on left side and select **Overlay**. Double-click on the parameter you would like to use on the overlay.
3. To add data, go to the Main Overlay Menu and select **Add Data**. The curser will change.
4. Click on the histogram of the data you would like to add to the overlay.
5. To include additional sample data, go to the Summit Software Main Menu and select **New > Samples**.
6. Click on the sample of interest and drag and drop it on the overlay.

Gate Logic Tab

The Gate Logic tab allows you to view and adjust gate logic as well as apply color gating to histograms.

Figure 4.48 Gate Logic Tab



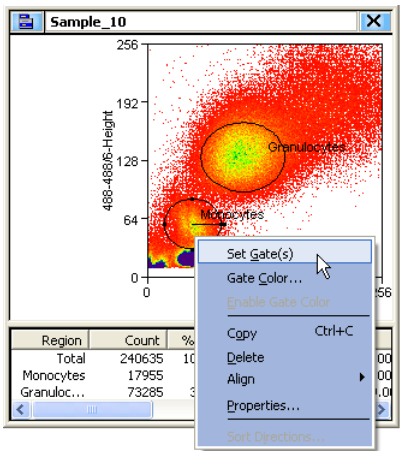
Setting a Gate from a Single Region

Gating on the main population in the FSC vs. SSC histogram will clean up the data and %CV values in other histograms. This eliminates bead or cell fragments and doublets from being considered in the alignment of fluorescent parameters. Gating can be done directly in dot plots and histograms in which regions have been created.

How to Gate One or More Histograms or Dot Plots

1 To gate one histogram or dot plot, right-click in the region from which you would like to gate, and select **Set Gates** from the menu. The appearance of the curser will change.

Figure 4.49 Set Gate 1



2 Use the newly changed curser to double-click in a histogram or dot plot in your protocol. Once the gate is applied there will be an annotation in the title bar to indicate a gate is applied.

Figure 4.50 Set Gate 2

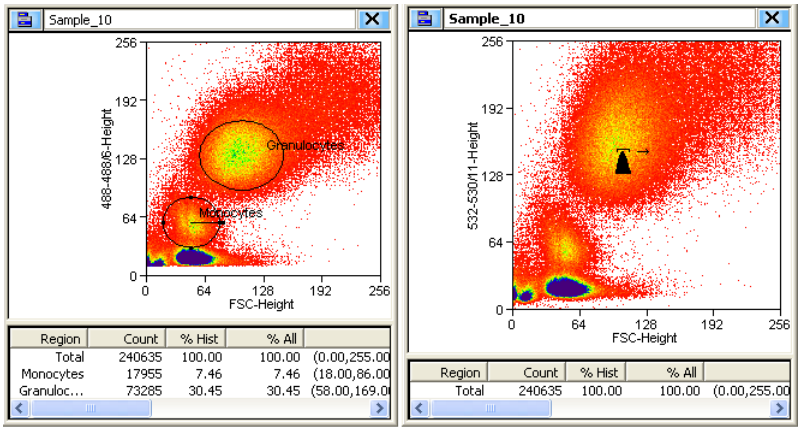
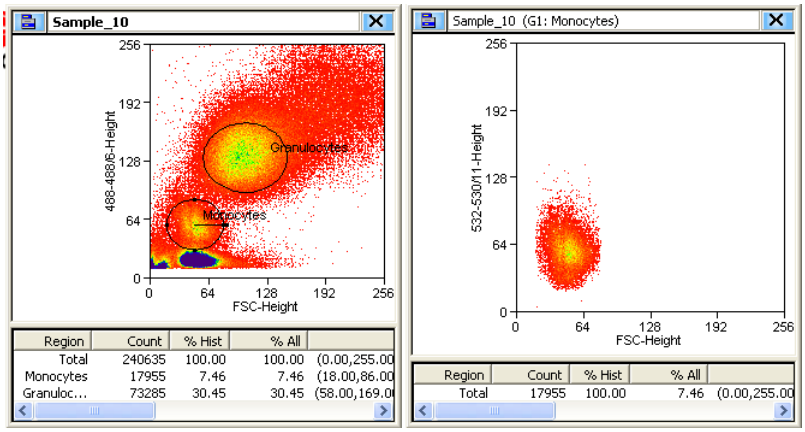


Figure 4.51 Set Gate Result



3 To gate more than one histogram or dot plot, right-click in the region from which you would like to gate, and select **Set Gates** from the menu. The appearance of the curser will change.

4 Use the newly changed curser to single-click in all of the histograms or dot plots in your protocol that you would like gated. When you come to the last histogram or dot plot, double-click to apply the gate.

5 To remove a gate, click the main histogram or dot plot menu icon and select **Ungate**, or click right-click outside of the gated region and select **Ungate**.

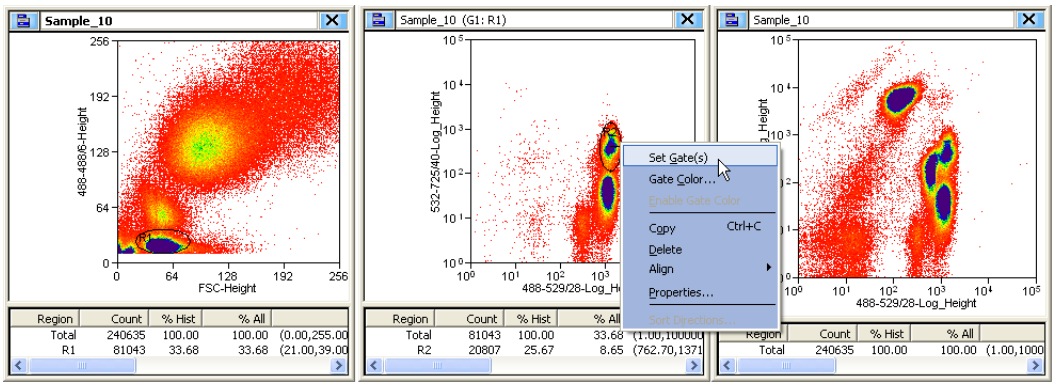
Setting a Serial Gate

When a region in a dot plot or histogram is used for gating, the serial gate option works by automatically appending any gate regions applied to that plot to the new gate. For example, a dot plot is gated based on two regions (R1 & R2). If a R4 region is created in that plot and is used as a gate on another histogram or dot plot, the serial gate option defines the newly applied gate as (R1 & R2 & R4). Electing not to serial gate would apply a gate involving region (R4) only to the target histogram or dot plot.

How to Serial Gate Histograms or Dot Plots

1 Open a protocol, create a region (R1) in the first dot plot. Right-click and set a gate from R1 to the second dot plot.

Figure 4.52 Set Serial Gate 1



2 Create a region (R2) in the second dot plot.

3 Right-click in region (R2) and then click **Set Gates**.

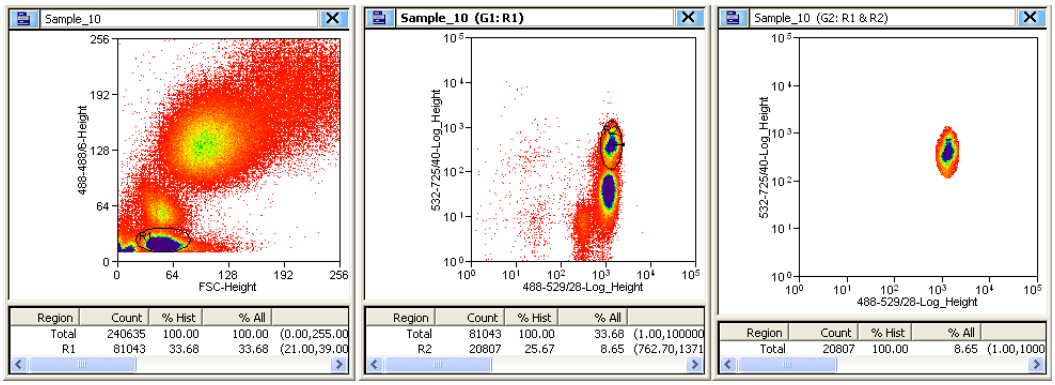
The **Combine region and gate?** Dialog box appears.

4 Click **Yes** to activate serial gating. Click **No** to gate using only the specified region.

5 Check the **Make my choice the default and don't ask me again** checkbox to disable this dialog box in the future.

6 Double-click in the final dot plot to apply the gate.

Figure 4.53 Set Serial Gate Result



Gate Logic Builder

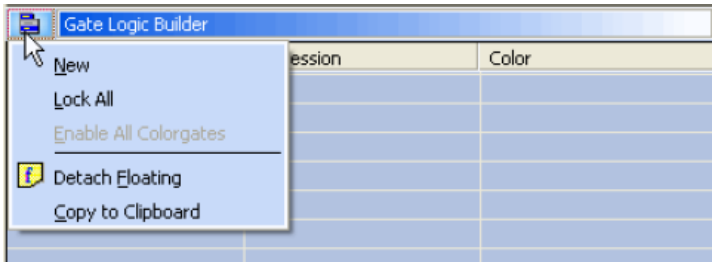
The **Gate Logic Builder**, located on the upper-left portion of the Gating tab, allows you to define gate logic and view it graphically.

How to Define (Edit) Gate Logic

1 Select the Gating tab in the **Summit Software Control Panel**.

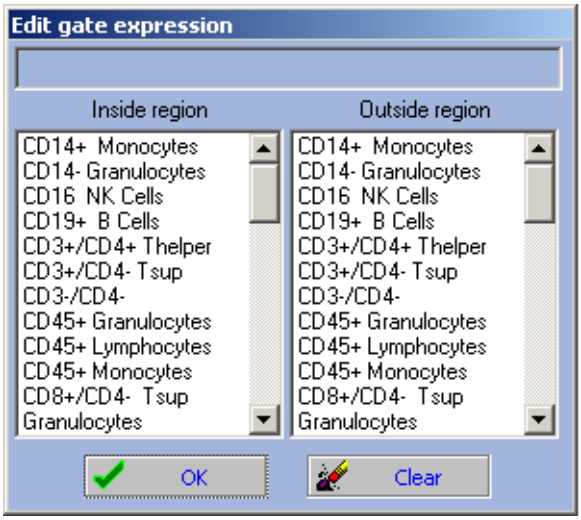
2 Click the upper-left icon in the **Gate Logic Builder** panel and then click **New** to create a new gate.

Figure 4.54 Gate Logic Builder



3 Double-click a text field in the **Expression** column. The **Edit gate expression** dialog box appears.

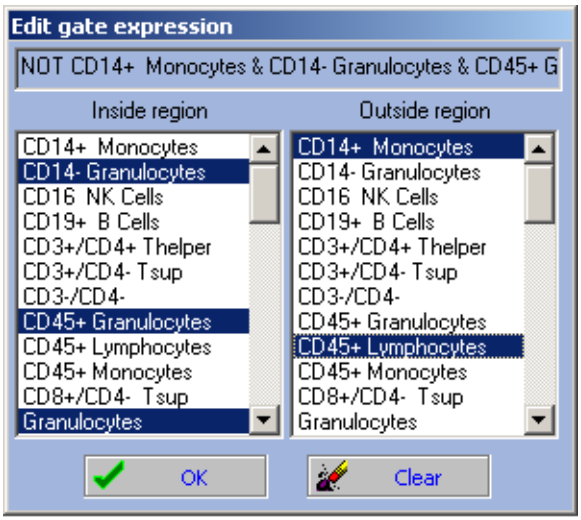
Figure 4.55 Edit Gate Expression Options



NOTE The number of regions available correlates to the number of regions created in the current workspace. If you did not name your regions, they will appear as R1, R2, R3, and so on.

4 Select one or more regions to be included in the gate, and click **OK**. Gates can be defined to include those events that fall inside or outside specific regions. To clear all selected regions, click **Clear**.

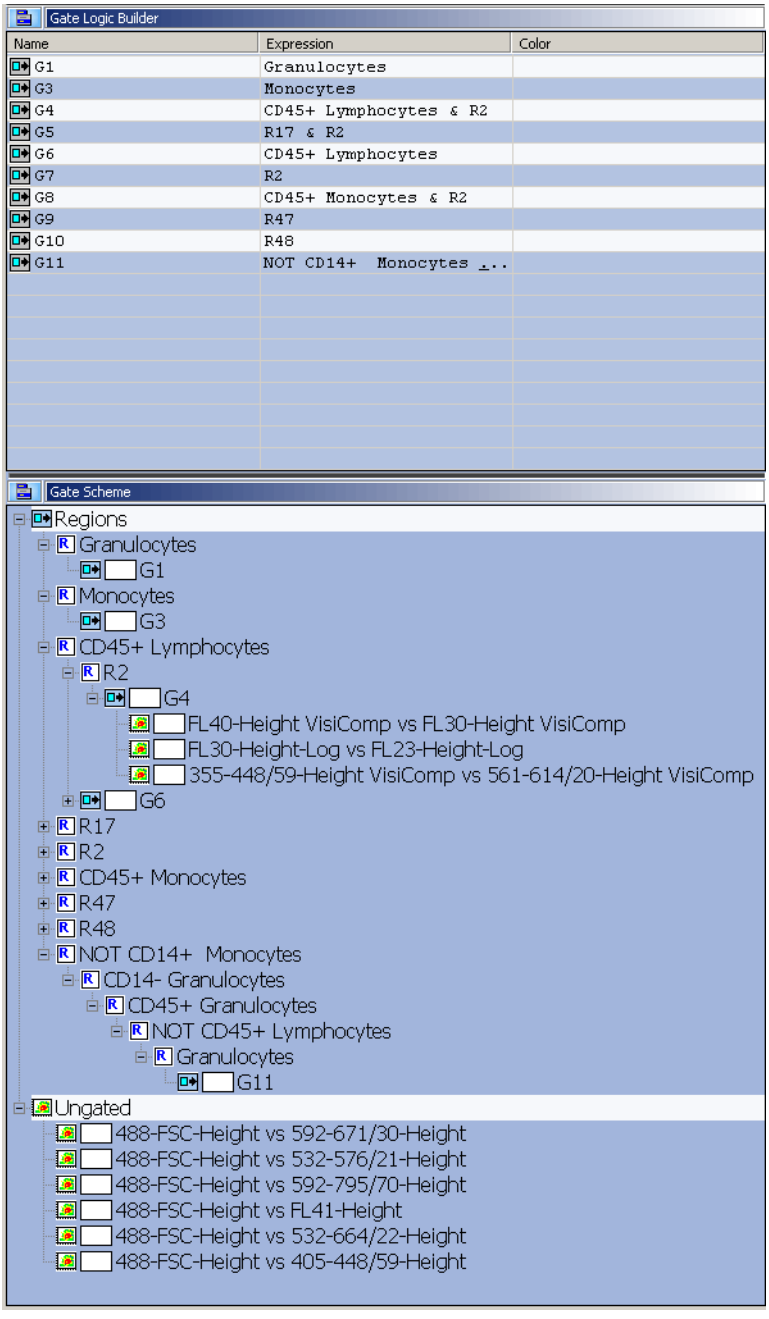
Figure 4.56 Select Gate Expressions



NOTE The number of regions available correlates to the number of regions created in the current workspace.

The newly defined gate expression is displayed in the **Expression** column of the **Gate Logic Builder** column and the current gate scheme is displayed in the **Gate Scheme** panel.

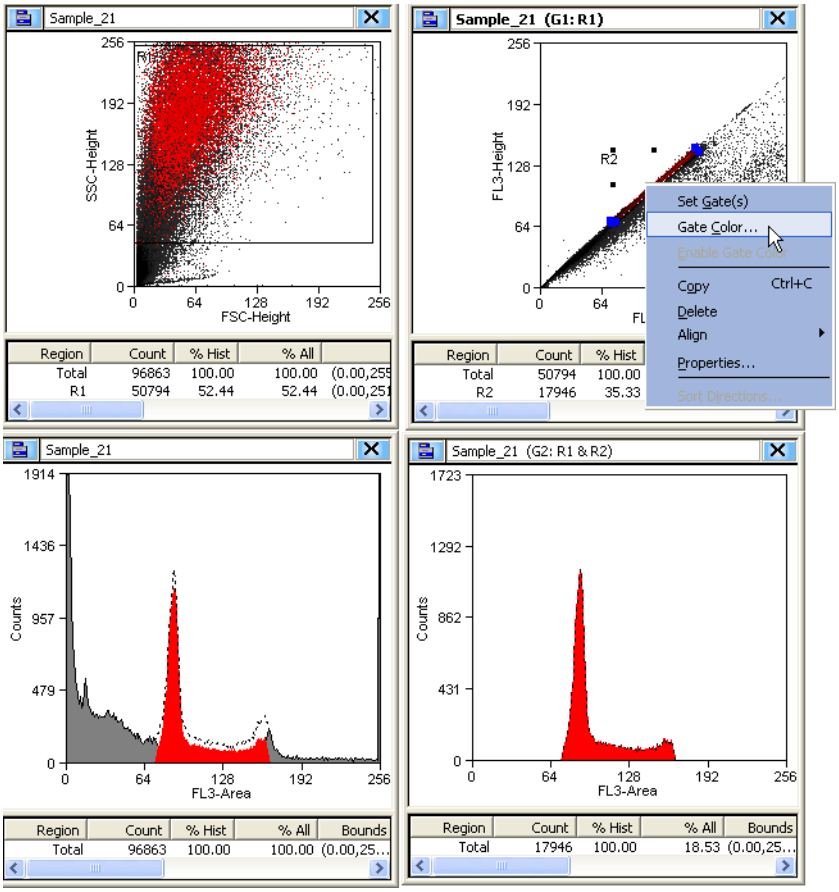
Figure 4.57 Gate Logic Builder and Gate Scheme Panels



Color Gating

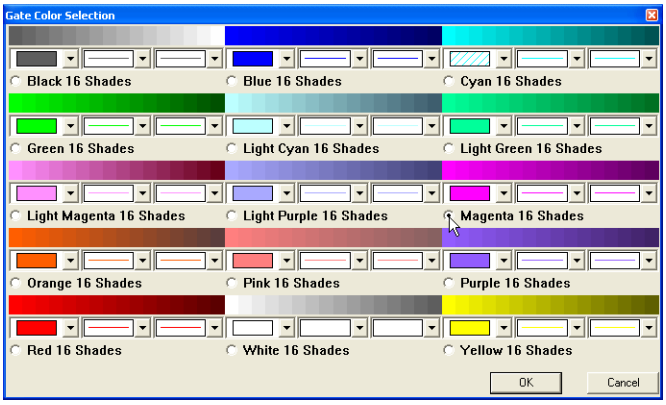
1 To view the single cells and doublet cells populations with color gating, right click in the region around the single cell population Figure 4.58 and select **Gate Color**. For information on doublet discrimination, see CHAPTER 8, *Sort Decisions and Doublets*.

Figure 4.58 Color Gating Diagram



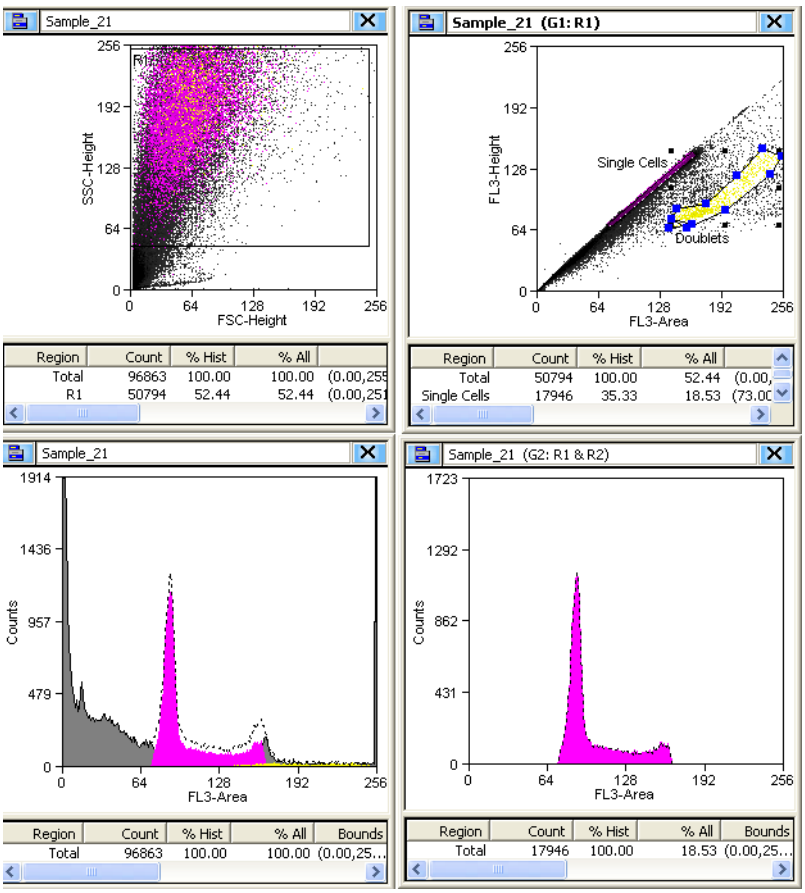
2 Select a color from the color selection pallet and click **OK**.

Figure 4.59 Color Gating Color Pallet



3 The single cell population from Figure 4.58 appears in Figure 4.60 in magenta. The doublet population is displayed in yellow.

Figure 4.60 Color Gated Single Cell Population and Doublet Population



NOTE You can toggle back and forth from color gating by clicking the icon in the upper right portion of the screen Figure 4.61.

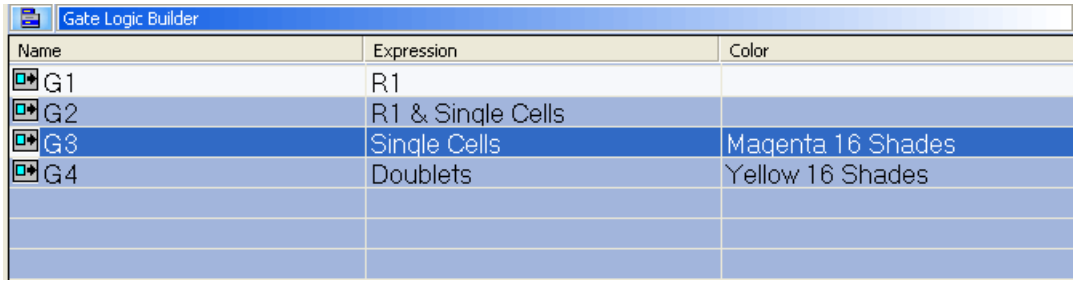
Figure 4.61 Toggle Color Gating

4 Create other regions if necessary to identify doublets. Click and drag the regions around the dot plot until you are confident you have located the doublet population.

5 Set sort decisions such that doublets are eliminated from the sort.

Another method of applying colors to gates is to double-click in the **Color** column for a particular gate and select a color.

Figure 4.62 Edit Color Gates

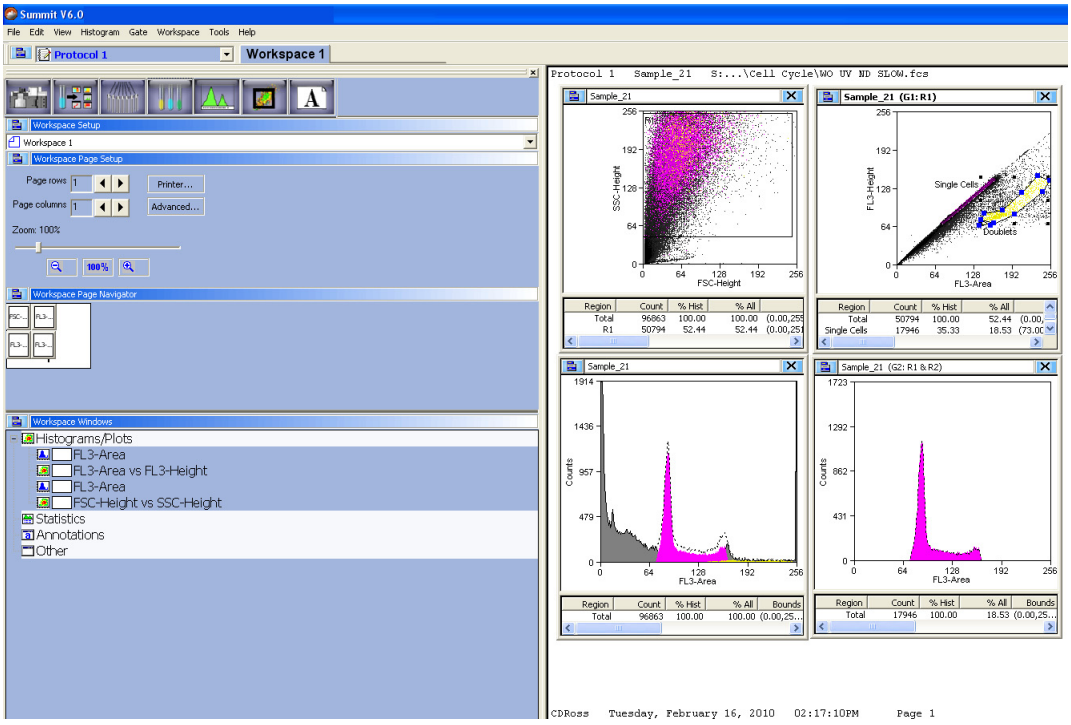


NOTE The order of the color dot plots and histograms correlates to the order of the gates listed in the **Gate Logic Builder**. As an example, if you have a color gate applied to a rare population that is being hidden in the background, you can reorder color to bring it to the front and more easily identify those rare events.

Layout Tab

The Layout tab assists you in manipulating the appearance of your Workspace as well as duplicate and share histograms, and print some or all of your Workspace.

Figure 4.63 Layout Tab



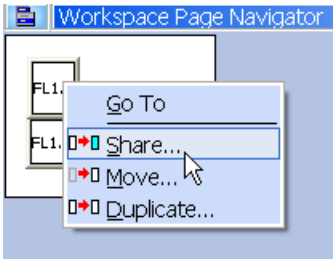
Workspace Page Setup

The Workspace Page Setup panel allows you to add and subtract pages from your Workspace, reduce or enlarge the layout, and print the layout.

Workspace Page Navigator

The **Workspace Page Navigator** allows you to click and drag thumbnails of your histograms to reposition them on your layout. From **Workspace Page Navigator** you can also **Share**, **Move**, and **Duplicate** histograms between Workspaces.

Figure 4.64 Workspace Page Navigator



Go To

The Go To option activates the selected histogram.

Share

The **Share** option duplicates the histogram allows you to specify the Workspace where it will appear, and keeps the data in both histograms the same if changes are made.

Move

The **Move** option allows you to move a histogram from one Workspace to another.

Duplicate

The **Duplicate** option copies the histogram but does not link the data between the old and the new copy.

Shortcut Keys

The following list describes the keyboard shortcuts in Summit software.

Table 4.2 Shortcut Keys and Functions

Shortcut Key	Function
F1	Opens the Summit software online Help system.
F2	Starts/Stops acquiring events.
F3	Saves acquisition data to a drive (**C:**, **D:**, etc.), the network, disk, or CD.
F4	Starts/Stops sorting.
F5	Pause
F6	Opens the CyClone menu.
F7	Opens the **Create New Histograms** dialog box for the current workspace.
F8	Opens a window that displays all loaded sample files in the current database.
F9	Opens the **Drop Delay Wizard**.
SHIFT + ARROW	Expands a region in the arrow direction (left, right, up, down) when selected.
CTRL + ARROW	Contracts a region in the arrow direction (left, right, up, down) when selected.
CTRL + O	Opens a dialog box to open one or more FCS listmode files.
CTRL + S	Saves modified items on the Summit software desktop that have changed (or auto-save is invoked at periodic intervals and when Summit software is closed).
CTRL + P	Prints the current view of the Summit software desktop.
CTRL + C	Copies the selected region, which can be pasted into a histogram.
CTRL + V	Pastes the copied region into a histogram.
CTRL + D	Opens the **Sort Logic and Statistics** menu.
CTRL + G	Opens the **Gate Logic** menu.
CTRL + Z	Clears the event buffer of all acquired events.
CTRL + W	Opens the Worklist Panel.
SHIFT + F4	Starts a Hardware Sort.
ALT + F4	Exits Summit software.
+	Expands one node when viewing a folder list in a window (use + on the numeric keypad).
-	Collapses one node when viewing a folder list in a window (use - on the numeric keypad).
*	Expands an entire folder tree contained in a window (use * on the numeric keypad).

CHAPTER 5

Startup and Shutdown Procedures

Reagents

Refer to APPENDIX A, *Approved Cleaners and Disinfectants*.

Manual Startup

Startup Procedure - Main System Power and Summit Computer OFF

1. In general, the main power to the MoFlo Astrios, Touch Screen Control Panel, and Summit Workstation should remain ON. However, if power to the system is completely OFF follow the steps below.
2. Turn on the Jun-Air compressor or the house air pressure.
3. Ensure that the main chassis power switch on the rear-right side of the lower enclosure is switched ON and wait 10 seconds. See Figure 1.1.
4. Power ON the MoFlo Astrios by pressing the power button on the front-right side of the lower enclosure.
5. Power ON the Summit Workstation computer. Start Summit Software.

Startup Procedure - Main System Power Already ON

1. Power ON the Touch Screen Control Panel monitor using the power button on the side bezel.
2. Select a laser tab on the Laser Control Panel (Figure 5.1) and press the Laser Power button to turn laser power ON.

Figure 5.1 Laser Control Tab

3 Use the Laser Power slider to set laser power to the desired value.

NOTE The UV laser power can be changed manually but not via the Touch Screen.

4 Repeat steps 2 and 3 until you have powered ON all of the lasers you intend to use.

NOTE Lasers should warm up and stabilize for a minimum of 30 minutes. Fluidics can be started during this time. The shutter for the UV laser will not open unless the laser has been on for 30 minutes.

Start Summit Software

1 Log into the Windows operating system. The Summit Workstation should generally remain powered ON.

2 Double-click the Summit icon 

to open Summit Software.

3 Select the appropriate database or create a new database. See CHAPTER 4, *Summit Software Database*.

4 Load the alignment protocol if necessary, or create histograms for each parameter on the system.

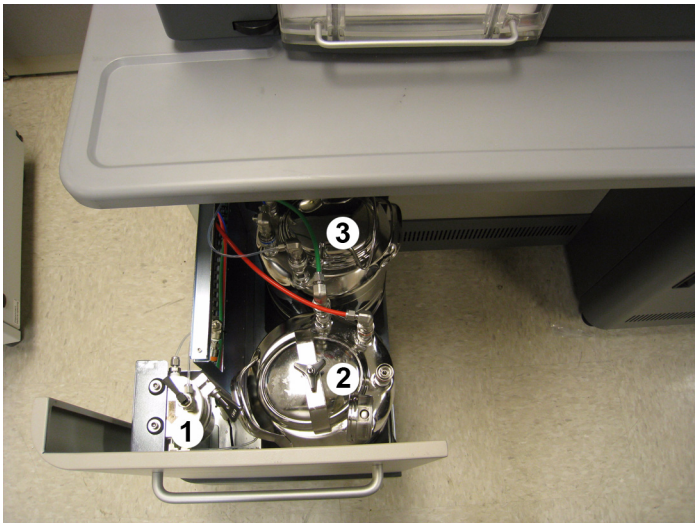
NOTE When creating a new protocol it is necessary to enable the parameters you intend to use and verify the laser path selection for each parameter. See CHAPTER 4, *Enable Parameters*.

5 After the instrument is started and has warmed-up for 30 minutes, continue to CHAPTER 6, *Instrument Alignment*.

Start Fluidics Manually

1 Ensure that the Waste Tank is empty and the Sheath Tank is full. (See Table 3.1 for status indicators.)

Figure 5.2 Fluidics Tanks and Sheath Filter Vent



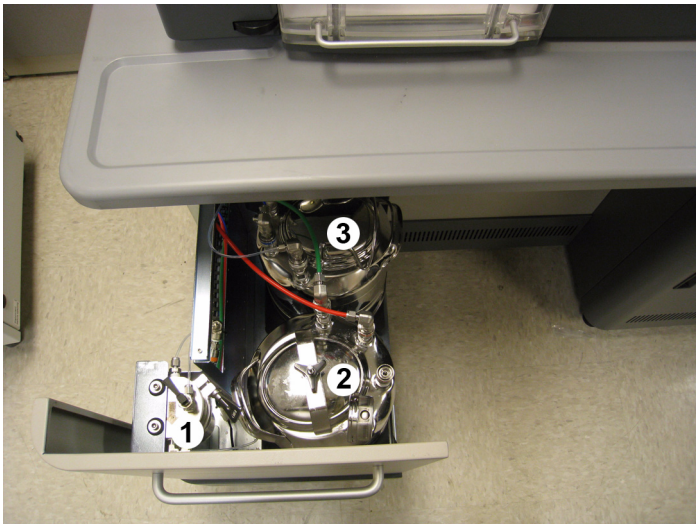
1. Sheath Filter Vent Lever
2. Waste Tank
3. Sheath Tank

2 Press the SmartSampler extended menu button.

3 Press the Change Tanks button to pressurize the fluidics.

4 After pressure and vacuum stabilize, open the fluidics drawer on the left side of the instrument. Debubble the Sheath Filter by lifting the vent lever for three seconds then closing the lever. Close the fluidics drawer.

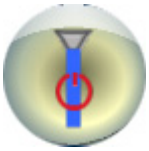
Figure 5.3 Fluidics Tanks and Sheath Filter Vent



1. Sheath Filter Vent Lever
2. Waste Tank
3. Sheath Tank

NOTE For best results, let the fluidics warm up and stabilize for a minimum of 30 minutes.

5 Press the Start Sheath Flow button to pressurize the tanks and start the sheath stream flowing.



Automatic Startup

During the Shutdown procedure, you can set the instrument to start up automatically at a future date and time. Thereby making it possible for the instrument to be warmed up and stabilized when you are ready to begin work.

A wizard guides you to select the lasers that will be powered ON, and whether or not fluidics will be pressurized automatically when the system starts up. Once the Automatic Startup selections are made, a timer counts down to the start time. Automatic Startup can be cancelled at any time. See *Shutdown Procedure For Setting Automatic Startup*.

Shutdown

Shutdown Procedure Selecting Manual Startup

IMPORTANT Once you begin, make sure you complete the entire shutdown procedure **before** leaving the instrument.

1 Press the Power button on the lower-right portion of the Touch Screen Control Panel to initiate the shutdown procedure.

- Charge Plates are disabled.
- Drop Drive is disabled.
- Lasers are turned off.
- Sample Chamber is opened.
- You are prompted to place a tube of cleaner in the Sample Chamber.

2 Place a tube of cleaner in the SmartSampler. Press the green arrow to continue.

- Cleaner is run through the probe for 30 seconds.
- You are prompted to place a tube of deionized water in the Sample Chamber.

3 Place a tube of deionized water in the SmartSampler. Press the green arrow to continue.

- Deionized water is run through the probe for 60 seconds.
- A message appears asking if you want to set the instrument to start automatically.

4 Select **No**. A message asks if you want to shut off electronics. In general, the electronics should be left powered ON. You may want to turn off the electronics when the instrument will not be used for several days in a row.

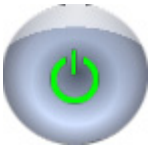
5 Turn OFF power to the Touch Screen monitor using the physical power button on the right side of the monitor bezel.

6 Close Summit. See *Exit Summit Workstation*.

Shutdown Procedure For Setting Automatic Startup

IMPORTANT Once you begin, make sure you complete the entire shutdown procedure **before** leaving the instrument.

1 Press the Power button on the lower-right portion of the Touch Screen Control Panel to initiate the shutdown procedure.



- Charge Plates are disabled.
- Drop Drive is disabled.
- Lasers are turned off.
- Sample Chamber is opened.
- You are prompted to place a tube of cleaner in the Sample Chamber.

2 Place a tube of cleaner in the SmartSampler. Press the green arrow to continue.

- Cleaner is run through the probe for 60 seconds.
- You are prompted to place a tube of deionized water in the Sample Chamber.

3 Place a tube of deionized water in the Smart Sampler. Press the green arrow to continue.

- Deionized water is run through the probe for 60 seconds.
- A message appears asking if you want to set the instrument to start automatically.

4 Select **Yes**. The Automatic Startup screen appears.

Figure 5.4 Automatic Startup Screen

5 Select the lasers you would like to turn ON during Automatic Startup.

6 Press the Start Fluidics button if you want pressure to the sheath tank and vacuum to the waste tank to be turned on automatically.

7 If you select to automatically start fluidics, ensure that the sheath tank is full and the waste tank is empty. Open the fluidics drawer on the lower-left side of the instrument. Disconnect the color-coded quick-connect fittings at the waste tank and sheath tank by pulling the metal collars up until the fittings are released.

8 Open the waste tank. Unscrew the threaded knob on the lid of the tank. When the knob is sufficiently loose, the lid can be removed. Empty the waste tank, rinse and add an appropriate disinfectant to the tank. See CHAPTER 9, *Daily Decontamination Procedure During Shutdown* for more information.

NOTE For a list of approved cleaners see APPENDIX A, *Approved Cleaners and Disinfectants*.

IMPORTANT Bleach should not be left in the waste tank over night. Use an alternate approved disinfectant if you intend to automatically start fluidics.

9 Open the sheath tank by pulling up on the handle and removing the lid. See CHAPTER 9, *Daily Decontamination Procedure During Shutdown*. Fill the sheath tank to the upper weld line with sheath fluid and ensure that the vent valve is closed.

10 Reattach the tanks to the system.

11 Touch the Date and Time area of the Touch Screen and use the arrow buttons to select the time and date for Autostartup to begin.

12 Press the green “thumbs up” button to confirm the Autostartup settings and cause the instrument begin the count down.

13 Turn OFF power to the Touch Screen monitor using the physical power button on the right side of the monitor bezel.

Exit Summit Workstation

1 Perform data backup as required and close Summit Software.

2 Log off the Windows operating system.

3 It is recommended that power to the Summit Workstation remain ON.

NOTE Automatic Startup can be cancelled at any time by pressing the red X button. When the instrument has been started with the Autostartup procedure, it will turn itself off if there is no user interaction within four hours.

Change Tanks

Use the following procedure only if you need to either fill the sheath tank or empty the waste tank during your work shift (not at the beginning or end of the work day).

Status Indicators

The Touch Screen Control Panel displays the sheath tank and waste tank status indicators:

This symbol indicates the status of the sheath tank.

Green = Full

Yellow = Approaching empty (Tank first displays yellow when it reaches 10% full.)

Red = Extremely low, add sheath fluid (The system will shut down the fluidics when the tank reaches this status.)

The value above the symbol indicates the sheath pressure.

This symbol indicates the status of the waste tank.

Green = Empty or low

Yellow = Approaching full (Tank first displays red when it reaches 90% full.)

Red = Extremely full, empty waste (The system will shut down the fluidics when the tank reaches this status.)

Fill Sheath Tank and Empty the Waste Tank

NOTE If the sheath tank status indicator is red, the system has already shut down the fluidics. Go to step 3 to proceed.

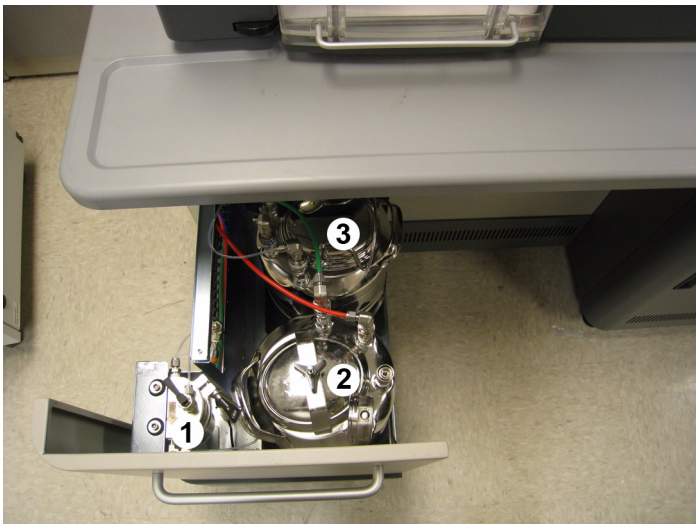
How to Fill the Sheath Tank During Your Work Shift

1 Press the SmartSampler extended menu button.

2 Press the Change Tanks button to turn off the fluidics which depressurizes the tanks.

3 Slide out the fluidics drawer on the left side of the instrument and locate the sheath tank.

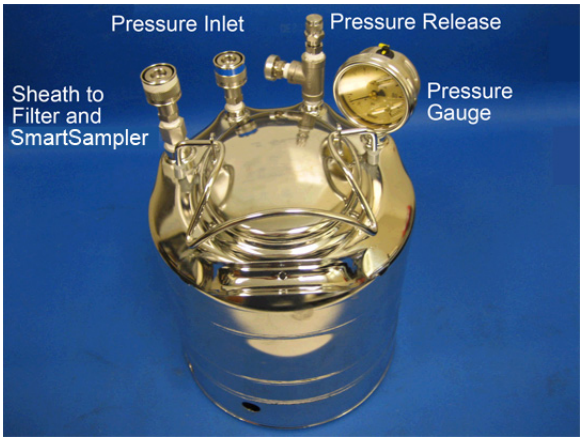
Figure 5.5 Components Inside the Fluidics Drawer



1. Sheath Filter Vent Lever
2. Waste Tank
3. Sheath Tank

4 When the pressure gauge registers zero, disconnect all the sheath and pressure tubings (sheath to filter and SmartSampler, and pressure inlet). Do not disconnect the pressure gauge.

Figure 5.6 Sheath Tank



NOTE If the pressure gauge is not registering zero, twist the pressure release valve until you hear the pressure escaping (a hissing sound). Do not remove the pressure release valve from the sheath tank. Once the pressure has escaped, retighten the pressure release valve. (Figure 5.6).

5 Remove the lid from the sheath tank (rotate counterclockwise). To avoid contamination, do not touch the inside of the lid and set the lid aside on a clean surface such as a paper towel.

6 Fill the sheath tank to a little more than 2/3 full. Use IsoFlow sheath fluid or similar fluid.

7 Place the lid back on the sheath tank and tighten (turn clockwise).

⚠ WARNING

Risk of personal injury. The full sheath tank is heavy. When placing the sheath tank back in the fluidics drawer, use proper lifting techniques or seek assistance to reduce the likelihood of back injury. When positioning the sheath tank inside the fluidics drawer, avoid pinching your fingers between the sheath tank and the metal supports.

8 Carefully place the sheath tank back inside the fluidics drawer. Avoid pinching your fingers between the sheath tank and metal supports.

9 Reconnect all sheath and pressure tubings (sheath to filter and SmartSampler, and pressure inlet). Each connector is a different size to facilitate proper reconnection. See Figure 5.6 as needed.

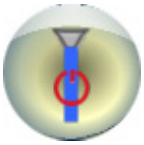
10 Press the SmartSampler extended menu button.

11 Press the Change Tanks button to turn on the fluidics and pressurize the tanks.

12 After pressure and vacuum stabilize, debubble the sheath filter by lifting the vent lever for three seconds then closing the lever.

13 Close the fluidics drawer. For best results, let the fluidics warm up and stabilize for a minimum of 30 minutes.

14 Press the Start Sheath Flow button to start the sheath stream flowing.



NOTE If the waste tank status indicator is red, the system has already shut down the fluidics. Go to step 3 to proceed.

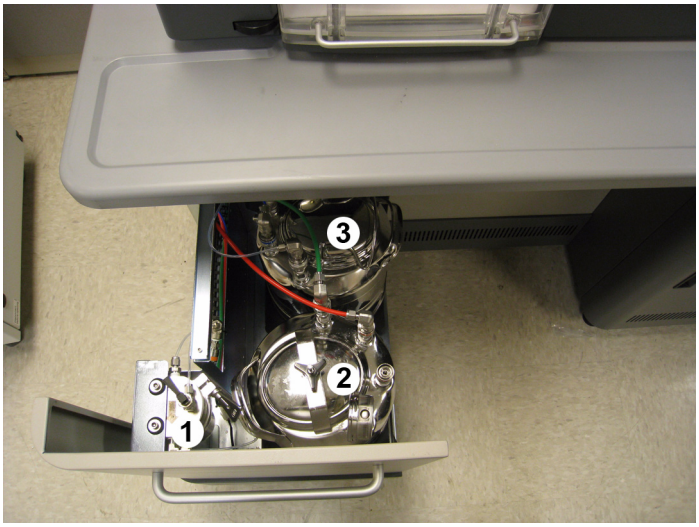
How to Empty the Waste Tank During Your Work Shift

1 Press the SmartSampler extended menu button.

2 Press the Change Tanks button to turn off the fluidics which depressurizes the tanks.

3 Slide out the fluidics drawer on the left side of the instrument and locate the waste tank.

Figure 5.7 Components Inside the Fluidics Drawer



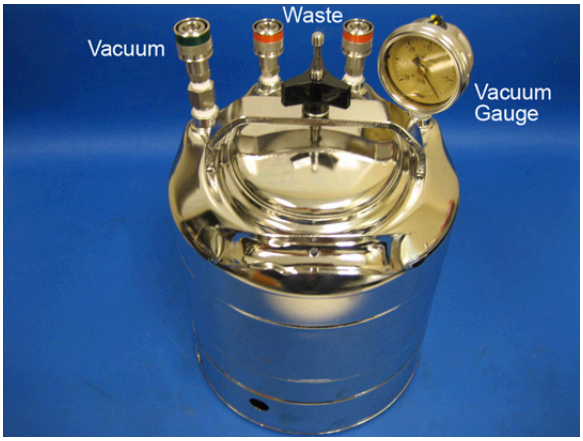
1. Sheath Filter Vent Lever
2. Waste Tank
3. Sheath Tank

⚠ WARNING

Biohazard contamination could occur from contact with biohazardous waste fluid. Avoid skin contact.

4 When the vacuum gauge registers zero, disconnect the vacuum and two waste tubings. Do not disconnect the vacuum gauge.

Figure 5.8 Waste Tank



⚠ WARNING

Risk of personal injury. A full waste tank is heavy. When removing the waste tank from the fluidics drawer, use proper lifting techniques or seek assistance to reduce the likelihood of back injury.

5 Carefully lift the waste tank out of the fluidics drawer.

6 Remove the lid from the waste tank (rotate counterclockwise).

⚠ WARNING

Biohazard contamination could occur from contact with biohazardous waste fluid. Avoid skin contact. Dispose of biohazardous waste in accordance with your local regulations and acceptable laboratory practices.

7 Dispose of the waste in accordance with your local regulations and acceptable laboratory practices.

8 Add approximately 20 mL of an approved disinfectant to the waste tank to reduce the risk of contamination. For the approved list of disinfectants, see APPENDIX A, *Approved Cleaners and Disinfectants*.

9 Place the lid back on the waste tank and tighten (turn clockwise).

10 Position the waste tank back inside the drawer.

⚠ WARNING

Biohazard contamination could occur from contact with biohazardous waste fluid. Avoid skin contact.

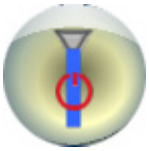
11 Reconnect the vacuum and waste tubings. The two waste tubings are interchangeable.

12 Close the fluidics drawer.

13 Press the SmartSampler extended menu button.

14 Press the Change Tanks button to turn on the fluidics and pressurize the tanks.

15 After pressure and vacuum stabilize, press the Start Sheath Flow button to start the sheath stream flowing and continue operation.



CHAPTER 6

Instrument Alignment

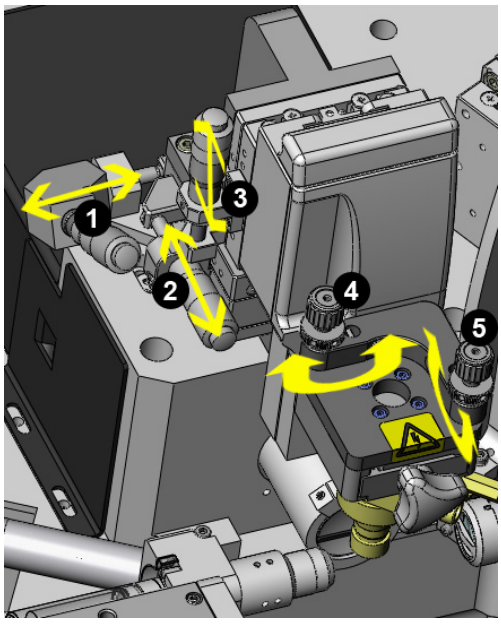
Every Day Instrument Alignment

Instrument alignment for MoFlo Astrios should be stable from day to day, provided that the nozzle has not been removed and the pressure settings have not been changed since the last time the instrument was aligned. See CHAPTER 10, *Troubleshooting and Replacement Procedures* for information on coarse laser alignment, PMT and Filter optimization, and Forward Scatter Diode alignment.

Stream Alignment

The stream positioning stage has five axes of movement, involving three micrometers and two gimbals. This stage is located at the center top of the instrument and controls the position of the nozzle, and therefore, the stream. These adjustments allow the precise positioning of the stream at the focal point of the Collection Objective and the center of the waste collection tube.

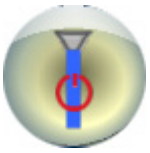
Figure 6.1 Stream Positioning Adjustment Stage



1. Micrometer moves stream left and right.	**4.** Gimbal rocks stream left and right
2. Micrometer moves stream front and back (focus).	**5.** Gimbal rocks stream front and back
3. Micrometer moves stream up and down.	

Stream Alignment Procedure

1 Turn on the sheath stream.



Verify that:

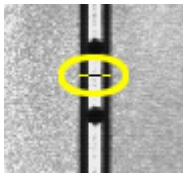
- the sheath tank gauge displays 60 psi.
- the waste tank gauge displays 10 -15 Hg.
- the sheath filter has been debubbled.
- the SmartSampler has been debubbled for one minute.

2 On the Touch Screen Control Panel Coarse Alignment screen turn on the Illumination Chamber Light so you can view the stream over the pinholes.

Consider the following to determine if the stream requires alignment.

- Verify that the sheath stream is centered over the pinholes.

- Verify that the dark stream edges and the bright stream center appear proportioned in thirds.



- Turn micrometer (3) Figure 6.1 clockwise to raise the nozzle and verify that the stream remains centered over the pinholes.

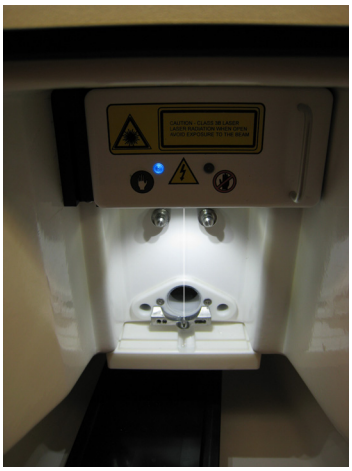
If the stream needs alignment, continue to step 3. If the stream is aligned, proceed to Laser Spot Determination.

---

3 Open the Sort Chamber door and remove the charge plates in order to view the waste receptacle.

---

4 Open the Interrogation Chamber, remove the charge plates, and use the micrometers and gimbals to direct the stream into the center of the waste tube.



---

5 Adjust micrometer (1) Figure 6.1 to center the stream over the pinholes. Adjust micrometer (2) Figure 6.1 to focus the stream until the dark sides of the stream and the bright center appear proportioned in thirds.

---

6 To check stream verticality, micrometer (3) Figure 6.1 clockwise to raise the stream. If the stream moves out of focus or migrates right or left, the stream is not in alignment, and the gimbals must be adjusted.

7 Move the nozzle to the raised position and adjust gimbal (4) Figure 6.1. It is necessary to move the gimbal so that it appears you are significantly over correcting the position. Lower and raise the nozzle to see if the stream moves to the right or left. If it does, repeat the process until the gimbal positioning is correct. Fine tune micrometers (1 and 2) if necessary.

8 Move the nozzle to the raised position and adjust gimbal (5) Figure 6.1. It is necessary to move the gimbal so that it appears that you are significantly over correcting the position. Lower and raise the nozzle to see if the stream moves out of focus. If it does, repeat the process until the gimbal positioning is correct. Fine tune micrometers (1 and 2) if necessary.

9 When the stream is aligned, move the nozzle tip until it is positioned within view of the camera Figure 6.2.

Figure 6.2 Stream Aligned and Nozzle Tip Within View

IMPORTANT Align the stream until the image displays three equal thirds.

Laser Spot Determination

After stream alignment is complete, and coarse laser alignment has been verified, the electronics must discern the laser and stream interception point.

Figure 6.3 Laser Spot Determination Screen

IMPORTANT Familiarize yourself with CHAPTER 10, *Background Image Subtraction*.

Determine the Laser Spot Procedure

1 Press the green arrow button. An on-screen prompt appears either stating the laser spot was found or stating that the laser spot was too large.

2 If you see the message that the laser spot was too large or poorly defined, view the Reference Image of the small, focused laser spot intersecting with the sheath stream. Adjust the stream focus (micrometer 2, Figure 6.1) until the Live image is similar to the Reference image. Do not adjust micrometer 3 or the gimbals.

3 Press the green arrow button. An on-screen prompt appears stating that the laser spot was found. The Reference Image changes.

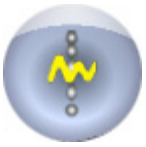
4 Adjust the stream focus (micrometer 2) to make the Live Image look like the newly changed Reference Image.

5 Press the green arrow button again to confirm laser spot determination. A green reference line appears.

6 Adjust nozzle micrometer (3) Figure 6.1 so that the nozzle is even with the green line.

7 Press the arrow button again to complete the process. The green line will move out of view.

8 Press the IntelliSort Initialize button. This sets drop drive frequency and amplitude, which causes the stream to form droplets. Now you are ready to fine align the instrument and subsequently run QC.



Laser Alignment

The fiber-coupled lasers are aligned as a unit by a Beckman Coulter representative during instrument installation. The alignment of the fiber-coupled lasers should remain stable from day-to-day. The UV laser is a free-standing laser that may require more frequent alignment by the user.

To verify and adjust coarse laser alignment see CHAPTER 10, *Laser Alignment*.

Fine Laser Alignment

After the stream is aligned, the lasers are coarsely aligned, the laser spot is found, and IntelliSort has been initialized, the rest of the alignment should be done by viewing the bead population data on the Fine Alignment screen and histograms and dot plots in Summit Software.

Fine Alignment Procedure (Fiber-coupled Lasers)

1 Access the Fine Alignment screen.

2 On the Fine Alignment screen press the X-axis parameter selector and choose 640-722/44 (H), or another parameter if your system does not have a 640 nm laser.

NOTE The parameters you select will depend on the laser configuration of your system. For best results select lasers that have the greatest spacial separation on the pinhole strip (not including the 355 nm laser).

Below is the pinhole order for a seven-laser system. When a system has fewer than seven lasers, the pinhole order remains the same.

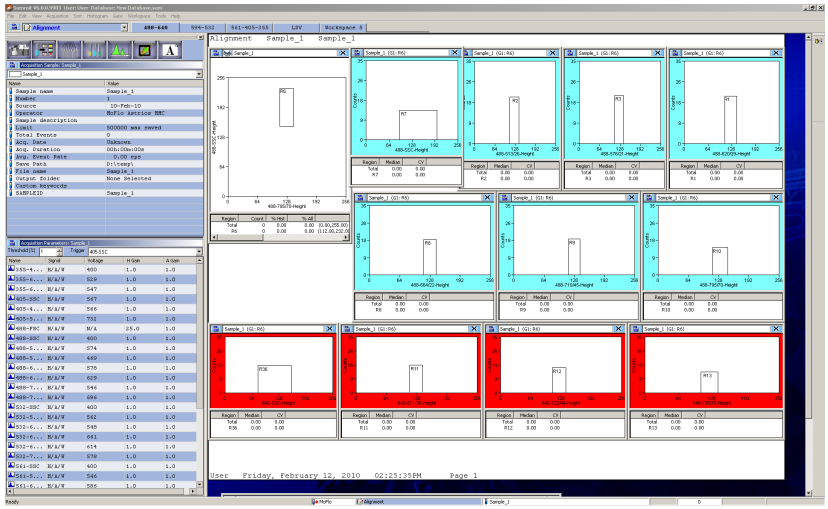
- 640 nm
- 488 nm
- 592 nm
- 561 nm
- 532 nm
- 405 nm
- 355 nm (The UV laser is free-standing and is directed through the seventh pinhole.)

3 Press the Y-axis parameter selector and select 405-448/59 (H), or another parameter if your system does not have a 405 nm laser.

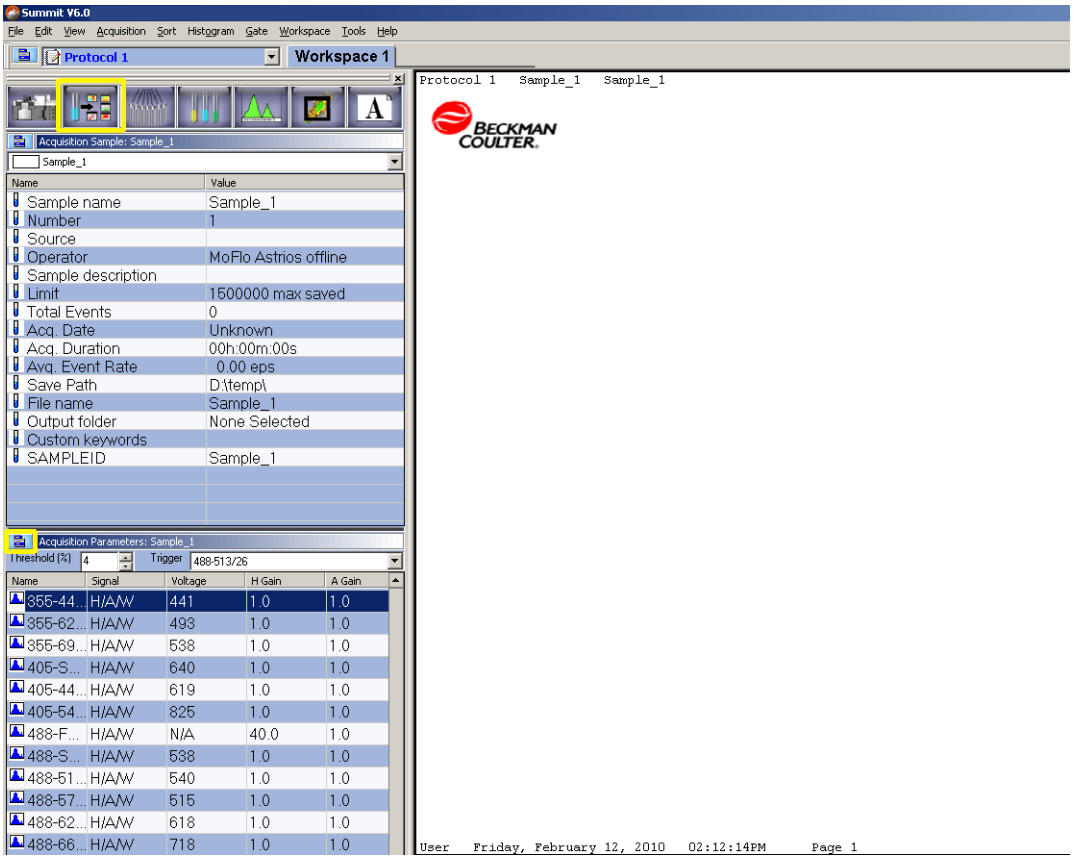
4 Press the Trigger parameter selector Figure 3.4 and choose FSC as the alignment trigger.

5 Open all laser shutters.

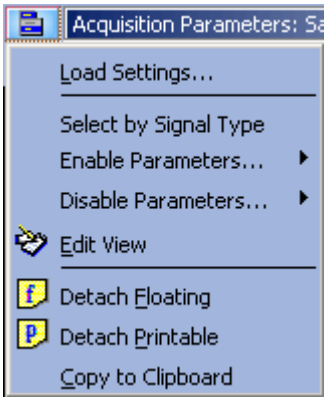
6 In Summit Software, select File/Protocol/Load Protocol and open the Alignment Protocol.



7 Go to the Acquisition tab in Summit and click the extended menu icon  .



8 Select **Load Settings** and open the settings file from a recent session. This will provide preliminary parameter voltages that can be used as a starting place for fine alignment.



NOTE If you do not have an instrument settings file, skip this step. You can manually adjust voltages for each parameter.

9 Load a tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (diluted 1:10 with deionized water), acquire data in Summit (F2).

NOTE It is helpful to set Summit Software in Cycle Mode while you perform fine alignment. Click the Cycle Mode button on the left side of the screen to turn Cycle Mode off and on. See page 4-19 for Cycle Mode details.

10 Ensure that sample pressure is 0.3 psi above sheath pressure and press the Start Sample button.

11 Press the Boost button until data is present on the screen.

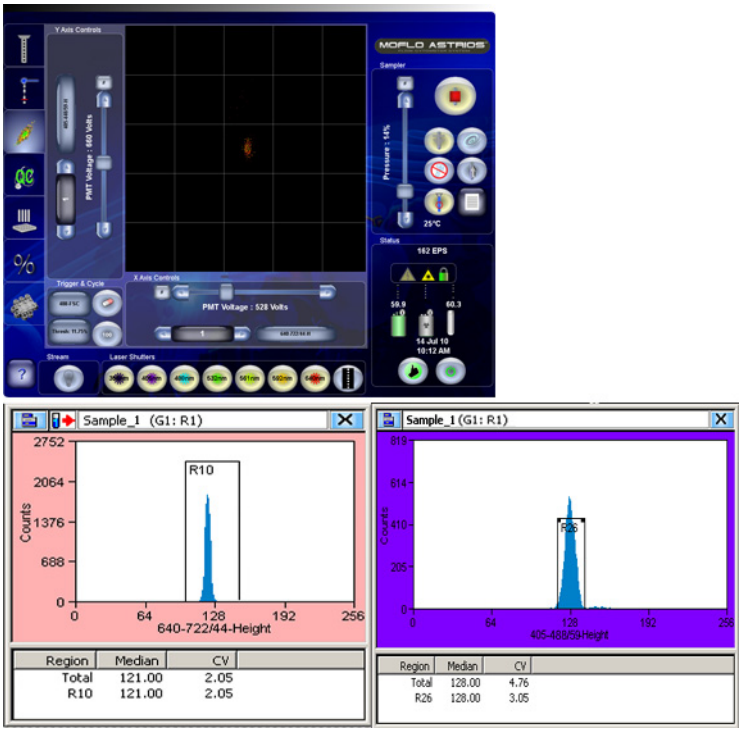
12 Adjust the sample differential pressure to achieve an event rate of 100-120 EPS.

13 Adjust gains and PMT voltage as necessary to bring the bead population on scale.

14 Use the Fine Alignment screen and Summit Software to optimize fluorescence intensity relative to each parameter. The goal is to achieve a relative optimal signal where one parameter may not be as intense as is possible, but both parameters will be optimally intense relative to each other. See Figure 6.4. To do this, adjust micrometers (1) and (2) but do not touch the vertical positioning micrometer (3) Figure 6.1.

NOTE When you are aligning the instrument and attempting to maximize fluorescence signal intensity, it is important that the dichroic filters are arranged and aligned correctly. If the filters on your system require alignment see CHAPTER 10, *PMT and Filter Alignment* to view the appropriate diagrams.

Figure 6.4 Summit Software and Touch Screen Control Panel Fine Alignment



NOTE In Summit Software, to view %CV statistics in a histogram you must first create a region over the population.

15 Proceed to aligning the free-space UV laser if it is included in your system. After the instrument is aligned, perform the *Quality Control Procedure* in Chapter 7.

Free-space UV Laser Fine Alignment

The BSO for the free-space UV laser requires coarse alignment more often than the FBSO for the fiber-coupled lasers. See CHAPTER 10, *UV BSO Alignment - Ring of Diffraction* and *UV Laser Co-linear Alignment Bead Flash Procedure* for more information on how to perform coarse alignment.

UV Laser Fine Alignment

1 Open the shutter for the trigger laser and for the UV laser.

2 Use the Touch Screen Control Panel or Summit to plot 355-692/75 (H) vs. 355-448/59 (H).

3 Run the alignment particles. Optimize fluorescence intensity, then fluorescence %CV by adjusting the UV BSO micrometers.

IMPORTANT Do not adjust the position of the nozzle.

CHAPTER 7

Quality Control

Quality Control and Performance Validation

Daily performance validation provides the assurance that the MoFlo Astrios is producing quality data from day-to-day. Documentation of performance results in a good quality control program will support the integrity of the data, provide a good track record of performance, and flag the operator when the instrument may need repair. After completing the startup and alignment procedures, validate the performance of the instrument.

The QC screen is a representation of lasers and detectors on the instrument. Circles represent laser lines. Squares represent PMT positions. After the QC procedure is complete, detectors that meet specification display a green checkmark. Failing detectors display a red X. Detectors with undefined QC values will display a question mark.

IMPORTANT Performance Validation should be repeated if you make changes to the instrument, such as changing a nozzle tip, fluidic pressure, dichroic mirrors, laser power output, etc. The automated QC process will qualify only those parameters where a laser is powered ON.

Quality Control Procedure

1. Perform the instrument Startup procedure. See CHAPTER 5, *Startup and Shutdown Procedures*.

2. Perform Instrument Alignment including Laser Spot Determination. See CHAPTER 6, *Every Day Instrument Alignment*.

3. Ensure that sample is not running and that you are not acquiring data in Summit before you begin. If you have performed instrument alignment, the Alignment protocol will be open in Summit and you will already have a tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles diluted 1:10 with deionized water.

4 Access the QC screen on the Touch Screen.

Figure 7.1 QC Screen Before Procedure is Run

5 Ensure that data is not being acquired in Summit. Then with the alignment particles in the SmartSampler and the sample flowing at about 100 EPS, press the Start QC button.

Auto QC performs the following actions:

- Initializes voltages, gains, and thresholds for all parameters.
- Automatically starts acquisition and adjusts event rate to 300 EPS.
- Uses the selected FSC laser, gain, and threshold.
- Sets laser delay for all powered lasers.
- Sets all gains on PMT voltage to 1 except on the trigger parameter.
- Adjusts SSC voltage for the trigger laser. Sets a gate from FSC vs. SSC from the trigger laser to all other parameters.
- Adjusts the voltages on all remaining parameters simultaneously to center the population on each histogram in median 128. Side scatter parameters are set to median 64.

- Sets EPS to 100.
- Checks each detector against QC pass/fail criteria.
- Calculates CV values at half-height (half of the peak height).

  NOTE Summit values generally run on full-height.

- Exports to a CSV file that can be viewed and edited using a spreadsheet program such as Excel. (These files can be accessed through Summit Software.)

---

6 When the QC procedure is complete, a QC report is generated that can be accessed through Summit. See CHAPTER 7, *QC Report*. The Touch Screen displays detectors that meet specification with a green checkmark, failed detectors with a red X, and detectors with undefined QC values with a question mark.

Figure 7.2 QC Procedure Complete

QC Report Per Detector

488-513/26	Laser and Filter Wavelength
CV	Percent Coefficient of Variation Achieved
V	Optimized PMT Voltage
Median	Peak Median Channel

IMPORTANT Infrequent errors pertaining to event rate control can generally be corrected by running Auto QC again.

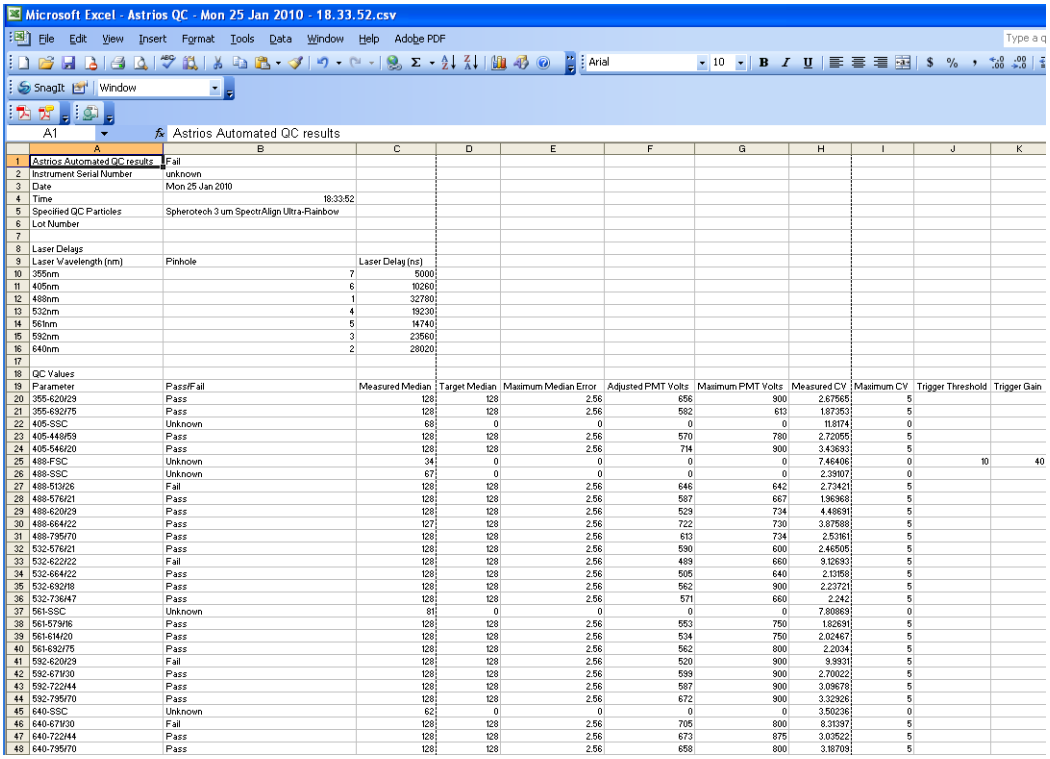
7 Failed parameters indicated by red check marks are generally correctable. Troubleshoot the parameters and rerun QC.

- Ensure that the instrument and lasers were allowed a minimum of 30 minutes to warm up. The shutter on the UV laser cannot open until the laser is powered ON for 30 minutes.
- If a single parameter in a POD has failed, check the alignment of the dichroic filter and PMT in that position. See CHAPTER 10, *PMT Alignment Procedure*.
- If all of the parameters in a POD fail, verify that the laser is powered ON and that the laser warmed up for 30 minutes prior to starting QC. If a laser control slider exists, ensure that the laser power percentage is set to 100 percent.
- If all the parameters for the fiber-coupled lasers fail, check the FBSO alignment. If parameters in the 355 nm POD fail, check the BSO alignment for the 355 nm laser.
- Check CHAPTER 10, *QC Setup Tool* and compare settings to the QC results.

QC Report

After the QC procedure is complete, a report is available through Summit Software. Select **Tools Copy QC Reports**, select a folder in which to save the data and click **Save**. Browse to the folder in which you saved the reports and double-click the desired report.

Figure 7.3 QC Report



Microsoft Excel - Astrios QC - Mon 25 Jan 2010 - 18.33.52.csv

	A	B	C	D	E	F	G	H	I	J	K
1	Astrios Automated QC results	Fail									
2	Instrument Serial Number	unknown									
3	Date	Mon 25 Jan 2010									
4	Time	18:33:52									
5	Specified QC Particles	Spherotech 3 um SpectrAlign Ultra-Rainbow									
6	Lot Number										
7											
8	Laser Delays										
9	Laser Wavelength (nm)	Pinhole	Laser Delay (ns)								
10	355nm	7	5000								
11	405nm	6	10260								
12	488nm	1	32780								
13	532nm	4	19230								
14	561nm	5	14740								
15	592nm	3	23560								
16	640nm	2	28020								
17											
18	QC Values										
19	Parameter	Pass/Fail	Measured Median	Target Median	Maximum Median Error	Adjusted PMT Volts	Maximum PMT Volts	Measured CV	Maximum CV	Trigger Threshold	Trigger Gain
20	355-620/29	Pass	128	128	2.56	656	900	2.67565	5		
21	355-692/75	Pass	128	128	2.56	582	613	1.87353	5		
22	405-SSC	Unknown	68	0	0	0	0	11.8174	0		
23	405-448/59	Pass	128	128	2.56	570	780	2.72055	5		
24	405-546/20	Pass	128	128	2.56	714	900	3.43633	5		
25	488-FSC	Unknown	34	0	0	0	0	7.46406	0	10	40
26	488-SSC	Unknown	67	0	0	0	0	2.39107	0		
27	488-513/26	Fail	128	128	2.56	646	642	2.73421	5		
28	488-576/21	Pass	128	128	2.56	587	667	1.96968	5		
29	488-620/29	Pass	128	128	2.56	529	734	4.48691	5		
30	488-664/22	Pass	127	128	2.56	722	730	3.87588	5		
31	488-795/70	Pass	128	128	2.56	613	734	2.53161	5		
32	532-576/21	Pass	128	128	2.56	590	600	2.46505	5		
33	532-622/22	Fail	128	128	2.56	489	660	9.12693	5		
34	532-664/22	Pass	128	128	2.56	505	640	2.13158	5		
35	532-692/18	Pass	128	128	2.56	562	900	2.23721	5		
36	532-736/47	Pass	128	128	2.56	571	660	2.242	5		
37	561-SSC	Unknown	81	0	0	0	0	7.80869	0		
38	561-579/16	Pass	128	128	2.56	553	750	1.82691	5		
39	561-614/20	Pass	128	128	2.56	534	750	2.02467	5		
40	561-692/75	Pass	128	128	2.56	562	800	2.2034	5		
41	592-620/29	Fail	128	128	2.56	520	900	9.9931	5		
42	592-671/30	Pass	128	128	2.56	599	900	2.70022	5		
43	592-722/44	Pass	128	128	2.56	587	900	3.09678	5		
44	592-795/70	Pass	128	128	2.56	672	900	3.32926	5		
45	640-SSC	Unknown	62	0	0	0	0	3.50236	0		
46	640-671/30	Fail	128	128	2.56	705	800	8.31397	5		
47	640-722/44	Pass	128	128	2.56	673	875	3.03522	5		
48	640-795/70	Pass	128	128	2.56	658	800	3.18709	5		

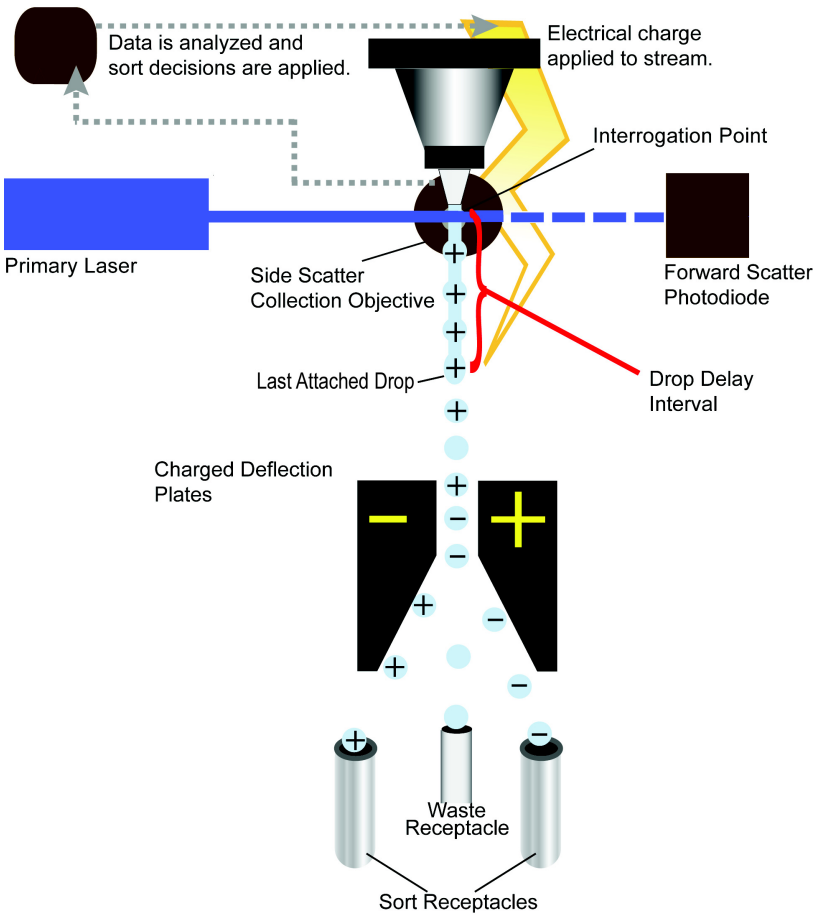
CHAPTER 8

Sorting and IntelliSort

Sort Overview

Sorting takes place following analysis of the particle in the Illumination Chamber. When a cell of interest is detected by the lasers and optics, a pulse of charge is sent through the stream when the cell of interest reaches the last attached drop. The droplet then breaks off with a charge that can be either positive or negative, depending on the desired sort direction. The droplets fall through the electric field created by the charge plates, and get deflected accordingly. The cells are then collected in the sample tubes or plate wells.

Figure 8.1 Sort Overview Diagram



The sorting function involves many facets of the instrument that require precise timing and calibration. The stability of the fluidics and droplet formation are critically important. MoFlo Astrios has been engineered to provide an extremely stable droplet break-off and charge on the droplets of interest. This translates directly to sort purity.

An accurate drop delay assessment is also critical to high sort purity. The drop delay defines the time duration for a particle to travel from the interrogation point at the laser to the last attached drop. With stable fluidics and an accurate drop delay, cell sorting can reach very high purities of greater than 99%.

During a Sort

During a sort the following events occur:

1. A decision is made at the interrogation point whether to sort or abort a cell.
2. If it is determined that a cell is to be sorted, the system waits until the cell reaches the Last Attached Drop.
3. The Last Attached Drop breaks off carrying a charge that can be positive, negative, or neutral depending on the direction it will be sorted.
4. The electronics send a charge through the sheath and sample stream.
5. As the charged droplet falls through the electric field created by the Sort Deflection Plates, it is deflected into the proper sorting receptacle.

Sort Setup Using IntelliSort with Automatic Drop Delay Determination

Below is an outline of the steps required to set up a sort run using IntelliSort. **Detailed instructions are included later in this section.**

NOTE You should not attempt to sort with air in the nozzle or fluidics system. An air bubble in the nozzle or sheath filter will cause the drop delay to be unstable and could lead to poor sort purity. Refer to CHAPTER 10, *Inspecting for Trapped Air* to determine how to check for trapped air.

IMPORTANT When a sort is started, make sure the sort plates are on. It takes a few seconds for the plates to achieve a full charge.

1. Determine laser spot location and perform background subtraction.
2. Perform Amplitude and Frequency scan and fine tune the alignment.
3. Perform the QC procedure.
4. Define a Sort Output Type (tubes, slide, or plate).
5. Set up Deflection.
6. Verify CyClone positions.
7. Verify the Charge Phase.
8. Perform automatic Drop Delay Determination, and enable IntelliSort Monitoring.

9. Acquire sorting data in Summit Software and set regions and gates.
10. Set Sort Decisions in Summit Software.
11. Configure Slides or Plates in Summit software (if necessary).

Select and Edit Sort Output Type

The Sort Output Type is the receptacle into which your sample will be sorted.

Figure 8.2 Select a Sort Output Type

Press the Copy button to create an editable copy of a the standard Sort Output Type you selected. A screen with three tabs appears. These tables will allow you to create a custom Sort Output Type that will contain your specialized settings for naming, deflection, and CyClone positions.

Define the Specifics of the Sort Output Type

This tab allows you to customize the Sort Output Type and give it a unique name.

Figure 8.3 Define the Sort Output Type

Touch on a text field and then type the information you would like to save. When you are satisfied with the information, select the checkmark button, and go to the Deflection tab.

When setting up tubes, the rows value will always be (1) and the columns value will vary with the number of tubes in the tube holder.

When setting up a microplate, consider the following diagram:

Set Stream Deflection for the Sort Output Type

This screen is used to individually select and adjust sort streams prior to beginning a sort. It is also used to edit the deflection settings for a Sort Output Type. From this screen you can turn ON and OFF the charge plates, adjust deflection plate voltage, stream targets, and stream positions.

Figure 8.4 Set up Deflection

Adjust Stream Deflection

1 Turn Plate Voltage ON.

2 Turn ON the test pattern.

3 Touch the on-screen dashed-line stream to select it Figure 8.4.

NOTE When you intend to sort to a microplate, select L1, the stream directly to the left of the waste stream. If you are sorting to 1536-well plates, move the waste tube and direct the sort stream straight down.

4 Adjust the stream deflection slider bar if necessary to ensure that it is lined up with its target and is not hitting the waste tube.

5 When you are satisfied with deflection setup, select the checkmark button, and go to the CyClone tab.

Set CyClone Positions

Default positions are set for each sort type. For best results, check the positions and adjust them if necessary.

Figure 8.5 Set CyClone Positions

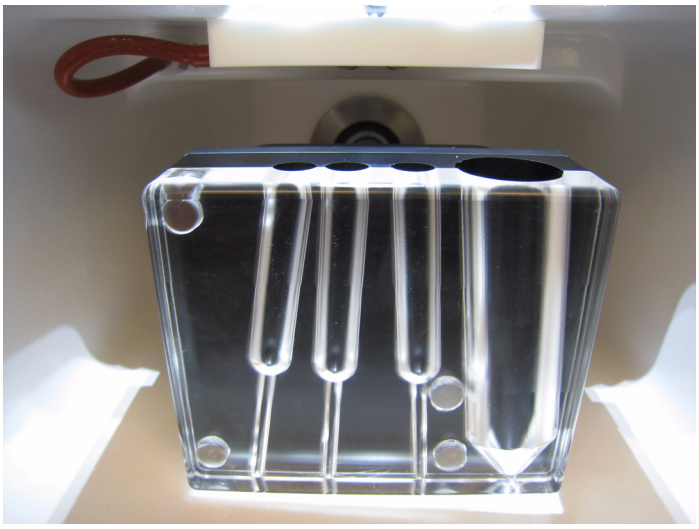
NOTE Ensure that the correct Sort Output is displayed on the screen. If not, access the Deflection tab and select the appropriate Sort Output type before continuing.

IMPORTANT While setting up CyClone, check the red waste tubing that extends from Sort Rescue. If the tubing extends low enough to rub against the microplate, rotate the tubing up and out of the way.

Set up Cyclone Positions for Tubes

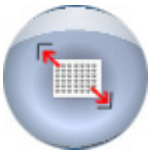
1 Place the appropriate sort output holder on the CyClone arm.

NOTE To remove a sort output holder, reach behind the holder and press the two quick-connect fittings while pulling up.



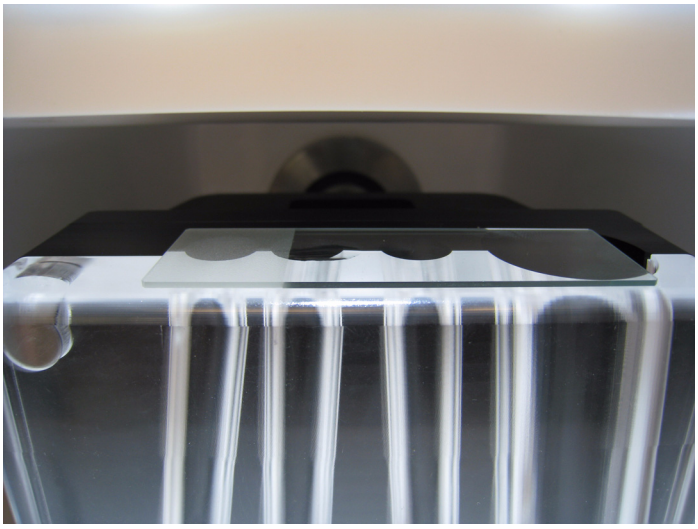
2 Press the Find Extents button.

IMPORTANT Ensure that there are no tubes in the tube holder before you press the Find Extents button.



3 Press the Home button. The CyClone will move to the stored Home position. If necessary use the arrow buttons to position the sort output holder under the charge plates.

4 Place a clean slide over the top of the tube slots.



5 Close the Sort Chamber door.

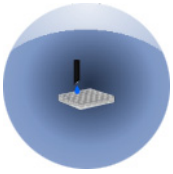
6 Ensuring that the streams test pattern is ON, press the SortRescue button.

7 View the drops that were deposited on the slide. If the drops are near the center of the tubes, *Adjust Stream Deflection* until the drops are correctly positioned.

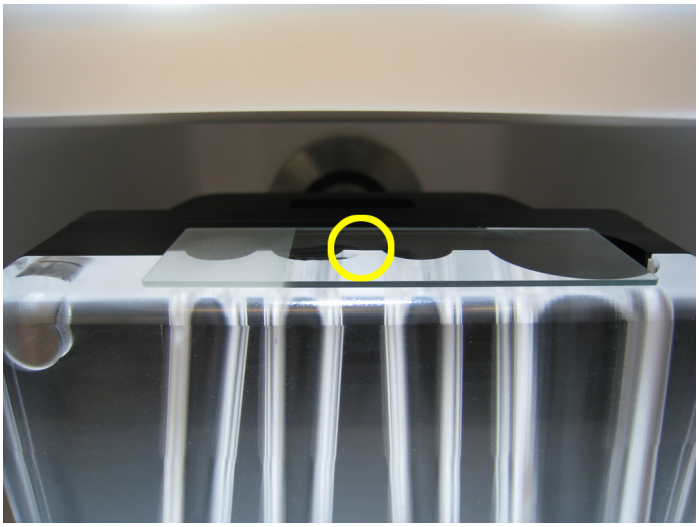
8 If droplet deposition was significantly misaligned continue to the following steps.

9 Wipe the slide and close the Sort Chamber door.

10 Ensuring that the streams test pattern is OFF. Press and hold the Squirt button to create a puddle on the slide.



11 Use the arrow buttons to move the CyClone so the puddle will be deposited half way between the clear and the dark materials on the sort output holder.



12 When the drop is positioned correctly for the Home position, select the Checkmark button to set the new Home position.

13 Select the End button and ensure that you will be able to remove the tubes from this position. If not, use the arrow buttons such that the tubes can be removed and select the Checkmark button to set the new End position.

14 Ensuring that the streams test pattern is ON, press the SortRescue button.

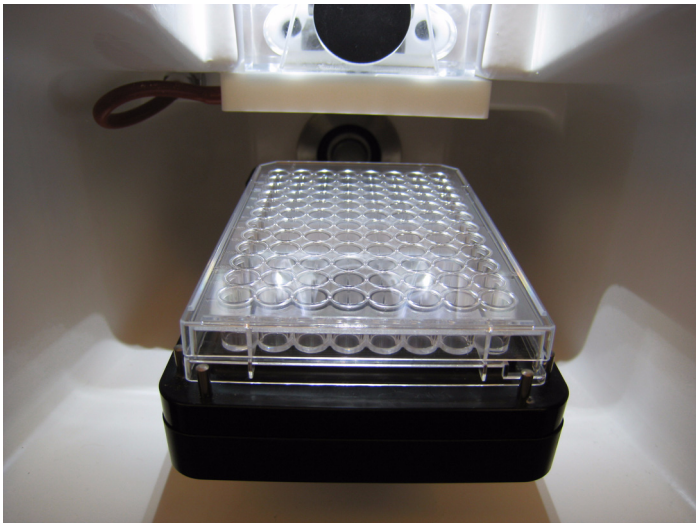
15 View the drops that were deposited on the slide. If the drops are near the center of the tubes, you can *Adjust Stream Deflection* until the drops are correctly positioned.

16 When stream deflection is correct, adjust the lower stream positioning targets to line up with the dashed line streams and select the Checkmark button one-at-a-time.

Set up CyClone Positions for Plates

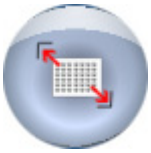
1 Place the plate sort output holder on the CyClone arm. Place a microplate (with the lid on) on the sort output holder.

NOTE To remove a sort output holder, reach behind the holder and press the two quick-connect fittings while pulling up.



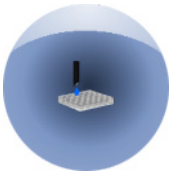
2 Close the Sort Chamber door.

3 Press the Find Extents button.

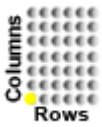


4 Press the Home button. The CyClone will move to the stored Home position.

5 Press and hold the Squirt button to create a puddle on the plate lid.



6 If the puddle is in the center of the Home well (yellow dot), the Home position is correct.

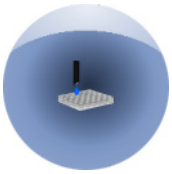


If the puddle is not in the center of the Home well, wipe the microplate lid, use the arrow buttons to move the CyClone arm and deposit another puddle.

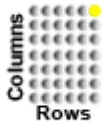
7 When the drop is positioned correctly for the Home position, select the Checkmark button to set the new Home position.

8 Select the End button. The CyClone will move to the stored End position

9 Press and hold the Squirt button to create a puddle on the plate lid.



10 If the puddle is in the center of the End well (yellow dot), the End position is correct.

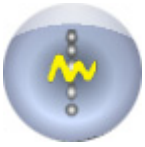


If the puddle is not in the center of the End well, wipe the microplate lid, use the arrow buttons to move the CyClone arm and deposit another puddle.

11 When the drop is positioned correctly for the End position, select the Checkmark button to set the new End position.

Start IntelliSort

The first step to automatically setting up IntelliSort is to Initialize IntelliSort, which sets frequency, amplitude, and charge phase. This step is typically performed before alignment and QC are run. If IntelliSort has not already been initialized, perform initialization.



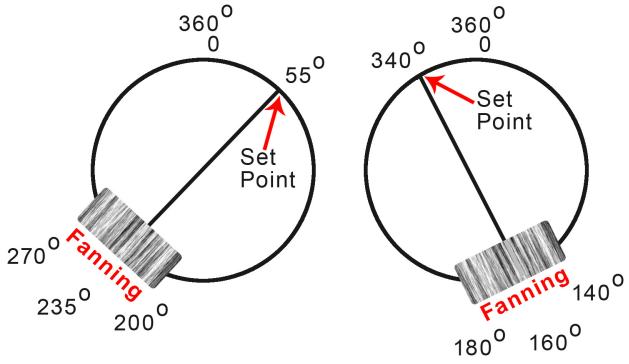
Now you will verify charge phase, determine drop delay, and enable IntelliSort Maintain Mode.

1 From the Sort Screen select the Stream Setup button.

2 Check the Charge Phase to ensure that IntelliSort picked the optimal value. Adjust Charge Phase to determine the point that causes the streams to fan the most. Set the phase 180° from that value.

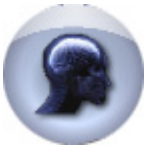
3 Adjust the Charge Phase in between the two extreme values. See Figure 8.6 below.

Figure 8.6 Adjust the Charge Phase Between Two Extremes



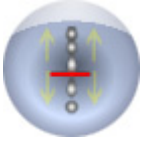
NOTE Over time you may find that the value that IntelliSort selects is sufficient making this step unnecessary.

4 Press the Drop Delay button to start the automatic Drop Delay Determination procedure.



NOTE Once Automatic Drop Delay Determination is complete, It is optional to manually verify the Drop Delay value, and if desired, change the value.

5 Press the Maintain button, which monitors and maintains drop delay within 10% for a temperature change of ±2 degrees Celsius for a sheath pressure change of ±1 psi.



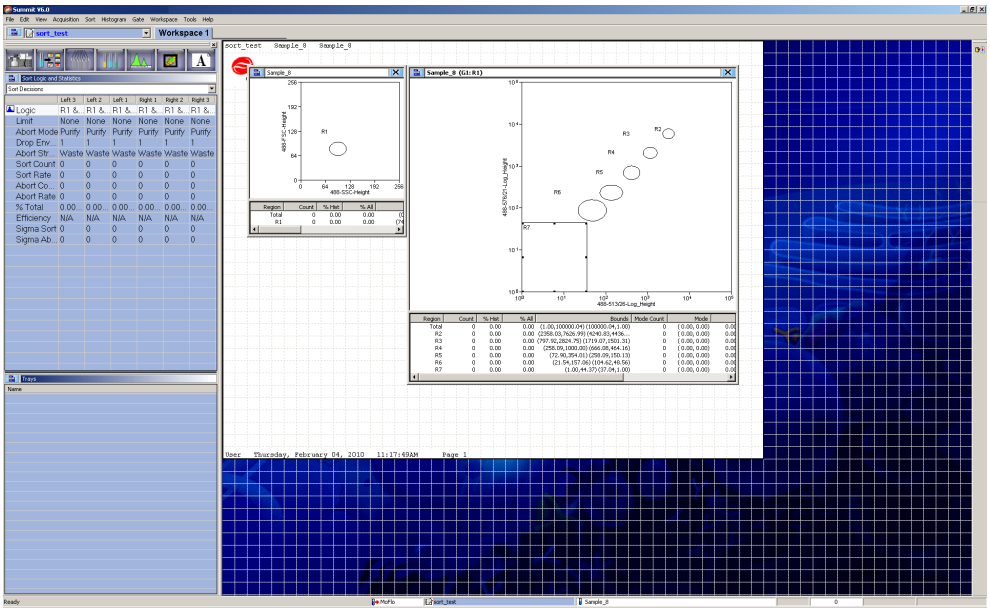
NOTE When IntelliSort is in Maintain mode you cannot adjust Drop Delay.

Set Sort Decisions

In order to set sort decisions you need to acquire sample data and from that data you must specify sort criteria.

---

1 Create a sort protocol in Summit Software. Set regions and gates.



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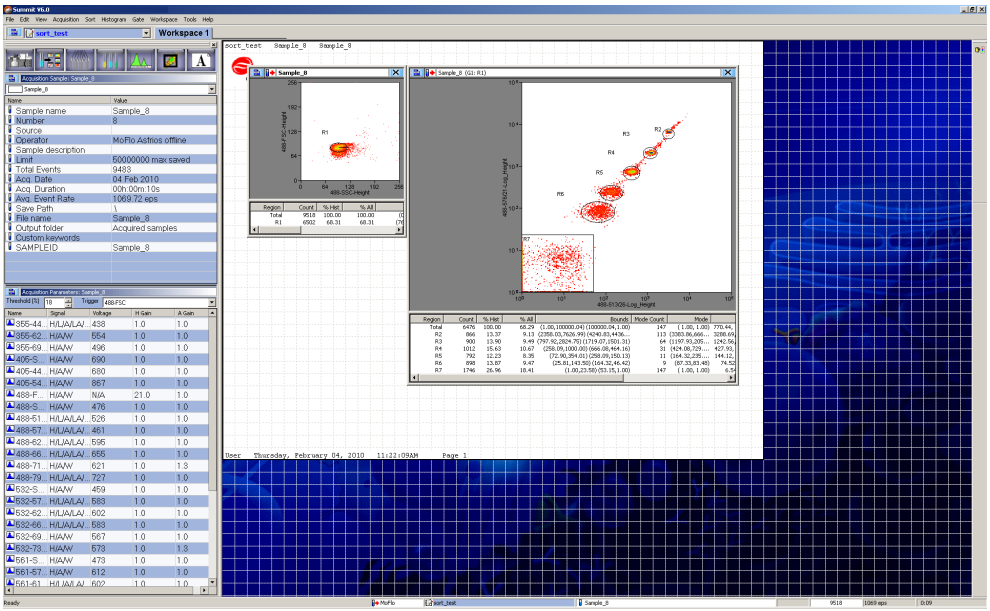
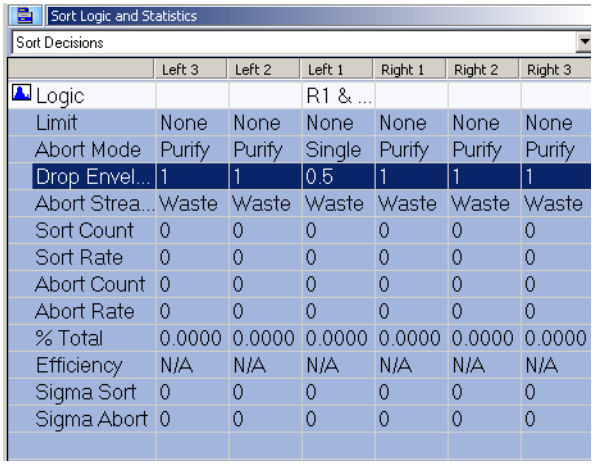
2 Place the sample on the SmartSampler, select the Start Sample button, and press the F2 button in Summit to acquire data on screen.



3 Click the Sort tab in Summit and set sort decisions. Double-click on a field you want to edit and then make a new selection. You can also right-click a region in a histogram to set sort decisions. See Figure 4.25.

NOTE If you are sorting to plates and using IntelliSort, it is important to set Abort Mode to Single and Drop Envelope to 0.5. Plate sorts must always use stream Left 1.

- If you are sorting to 1536-well plates, move the waste tube and direct the sort stream straight down.
- Six-way sorting requires the Drop Envelope to be set to 1. Any Abort Mode can be used.



	Left 3	Left 2	Left 1	Right 1	Right 2	Right 3
Logic			R1 & ...			
Limit	None	None	None	None	None	None
Abort Mode	Purify	Purify	Single	Purify	Purify	Purify
Drop Envel...	1	1	0.5	1	1	1
Abort Strea...	Waste	Waste	Waste	Waste	Waste	Waste
Sort Count	0	0	0	0	0	0
Sort Rate	0	0	0	0	0	0
Abort Count	0	0	0	0	0	0
Abort Rate	0	0	0	0	0	0
% Total	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Efficiency	N/A	N/A	N/A	N/A	N/A	N/A
Sigma Sort	0	0	0	0	0	0
Sigma Abort	0	0	0	0	0	0

4 Select an Abort Mode described below.

Enrich Mode The Enrich Mode is used when recovery is the most important aspect of the sort. With Enrich, all positive events are sorted with the exception of Hard Coincidence events.

Purify Mode Purify Mode is used when purity of the sort is most important. This Sort Mode will sort all positive events only when there are no negatives within the window of the Droplet Envelope. If a negative cell is on the edge of a droplet adjacent to the drop envelope, the sort logic will fail and the drop containing the positive event will be aborted. The aborts can be sent to waste or to a separate tube that will then be resorted for greater recovery.

Single Mode Single Mode can be thought of as Single Cell Mode. In other words, only one positive event must be contained within the Droplet Envelope to pass the sort logic. If two positives or a negative are within the Droplet Envelope, the sort logic will fail and the positive(s) will be aborted. If a negative cell is on the edge of a droplet adjacent to the drop envelope, the sort logic will fail and the drop containing the positive event will be aborted. Single Mode is most useful for sorting single positive events into wells. Also, Single (1 Drop) Mode is used for the Drop Delay calculation in Summit software.

NOTE Sometimes an analyzed cell meets the criteria to be sorted into more than one stream. When this happens, the following sort precedence is applied based on the Abort Mode that was set for that population. This order is from the most restrictive to the most permissive.

1. Single
2. Purify
3. Enrich

Within an Abort Mode, stream precedence defaults to the far left stream (L3) taking precedence, then L2, L1, R1, R2, and finally the far right stream (R3). The precedence of streams that share the same Abort Mode can be changed in Summit under the Sort menu.

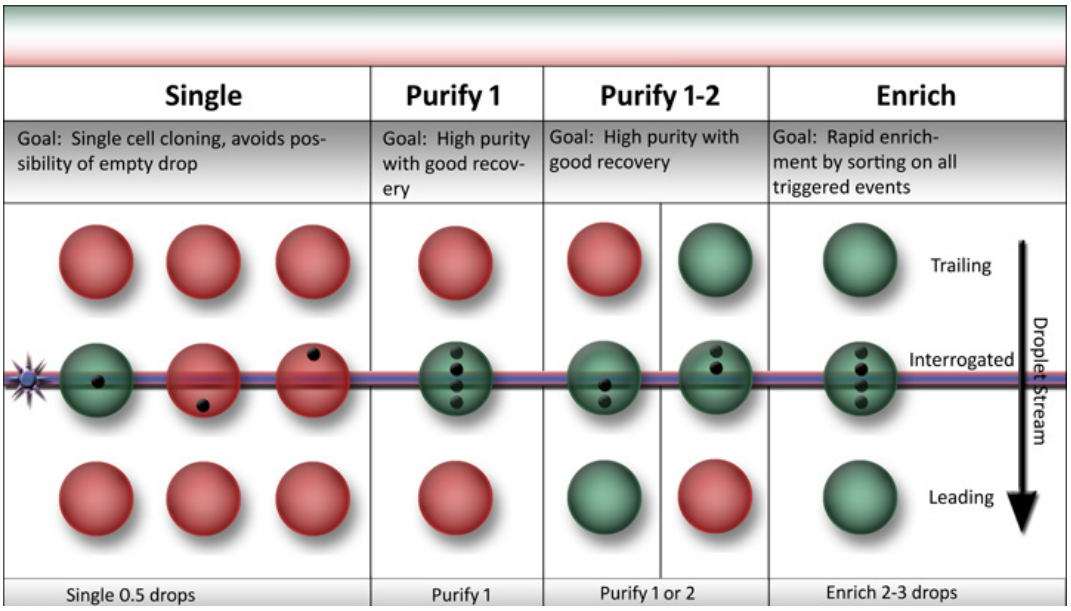
---

5 Select a Drop Envelope described below.

- 0.5 Drop - One drop is sorted if all positive events are in the center half of the droplet. This envelope typically provides the poorest yield (most soft aborts). This is generally only used for single cell deposition (Single Sort Mode). It should never be used with the Enrich Sort Mode because positive events will be aborted.
- 1 Drop - One drop is sorted if the positive events fall anywhere in the drop. This envelope provides the best yield (fewest soft aborts) at high event rates when using the Purify Sort Mode. This envelope is used when determining drop delay.
- 1-2 Drop - One drop is sorted if all positive events are in the center of the droplet. If a positive event is outside the center, then the drop adjacent to the edge containing that event is also sorted. If there is a positive event in both edges, then both adjacent drops are sorted. This envelope helps ensure that the positive events are always sorted and is typically the best envelope to use with the Enrich Sort Mode. When used with either the Single or Purify Sort Modes at high event rates, this will give similar results as the 0.5 Drop Envelope since the adjacent drops will frequently contain a negative event.

- 2 Drop - At least two drops are always sorted. The drop containing the positive events plus the nearest adjacent drop are sorted. If there are positive events in both halves of the drop of interest, then both adjacent drops are also sorted. Use this envelope only when the drop delay stability cannot be maintained within ¼ drop. At high event rates with either the Single or Purify Sort Mode, this will provide very poor yield (high soft aborts) because the adjacent drops will frequently contain a negative event.
- 3 Drop - Three drops are always sorted. The drop containing the positive events plus both adjacent drops are sorted. Use this envelope only when the drop delay stability cannot be maintained within ¼ drop. At high event rates with either the Single or Purify Sort Mode, this will provide very poor or no yield (high soft aborts) because the adjacent drops will frequently contain a negative event.

Figure 8.7 Abort Mode and Drop Envelope Illustration



Begin Sorting to Tubes

1 Ensure that you have pressed the CyClone Find Extents button, and that you have set up stream deflection.

2 Place tubes on the CyClone.

3 Ensure that the SmartSampler settings on the Summit Software *Instrument Tab* are set to your preference.

4 Press the Start Sample button on the Touch Screen.

IMPORTANT Before starting the sort, make sure the sort plates are on. It takes a few seconds for the plates to achieve a full charge. During this time, any sorts that occur will be incorrectly deflected and contamination can occur.

5 From the Sort Menu in Summit select Start (or press F4).

NOTE Pressing F4 again will stop the sort.

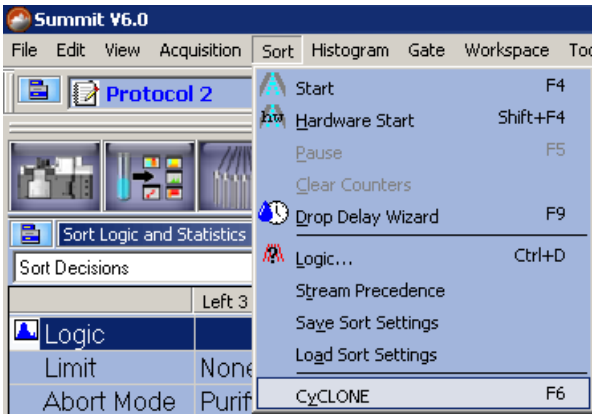
Plate or Slide Configuration in Summit Software

IMPORTANT If you are sorting to tubes do not follow this procedure.

The first time you intend to sort to a slide or a plate, you must first acquire data from the sample, set regions and gates, set sort decisions, configure the slide or plate in Summit Software, and configure CyClone. From the Touch Screen you can create custom sort output type definitions that save CyClone configurations so you can use them again.

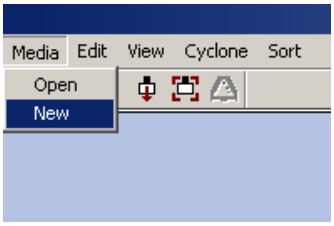
1 From the main **Sort** menu in Summit software select **CyClone**.

Figure 8.8 Select CyClone



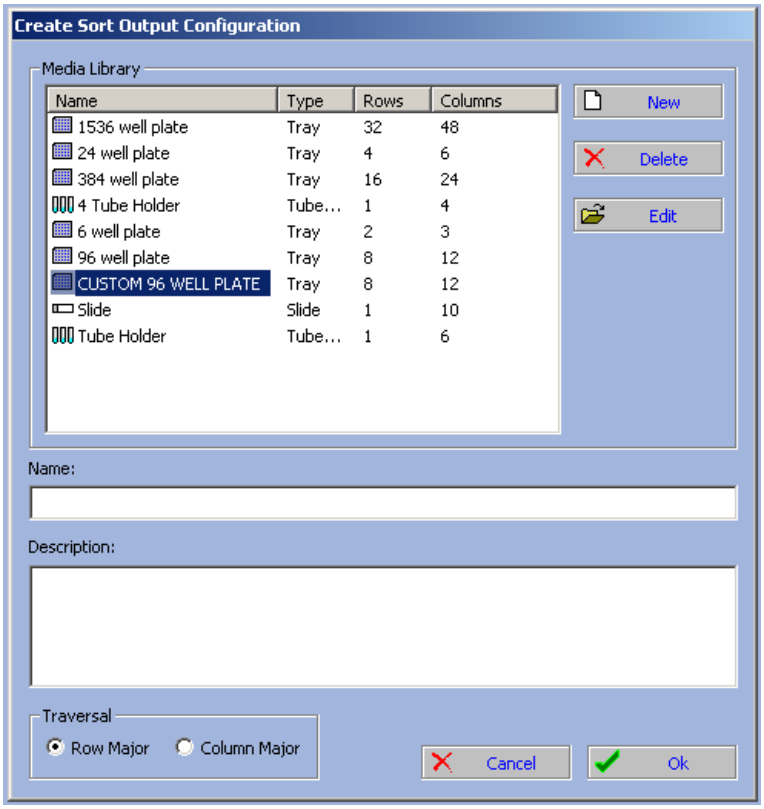
2 Click **Media** and select **New**.

Figure 8.9 CyClone Media New



3 A dialog box appears. This list includes the default sort output types, and customer sort output types from the Definition tab on the Touch Screen.

Figure 8.10 CyClone Select Slide or Plate Type



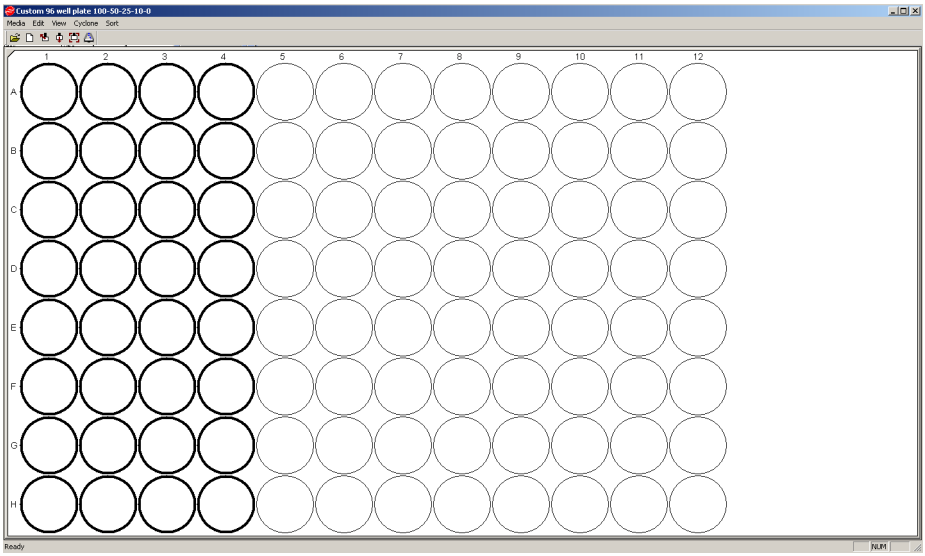
IMPORTANT The sort output types shown in Figure 8.10 are the default settings (and one custom definition). It is recommended that you set up custom sort output types including the CyClone Home and End positions. Once you have created a custom sort output type, you can skip setup on the Touch Screen Definition, Deflection, and CyClone tabs and instead select the custom sort output type from this list.

4 Enter a file name in the **Name** field, and click **OK**.

5 A layout depicting the chosen medium appears. Click on all of the circles that will receive one of your sort decisions.

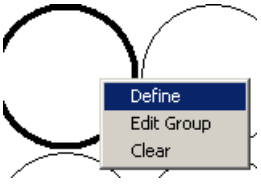
NOTE To select individual circles, press the CTRL key while you click the circle. To select a group of circles, click one circle, press the SHIFT key while you click another circle. To clear a selected circle, click it again.

Figure 8.11 CyClone Layout



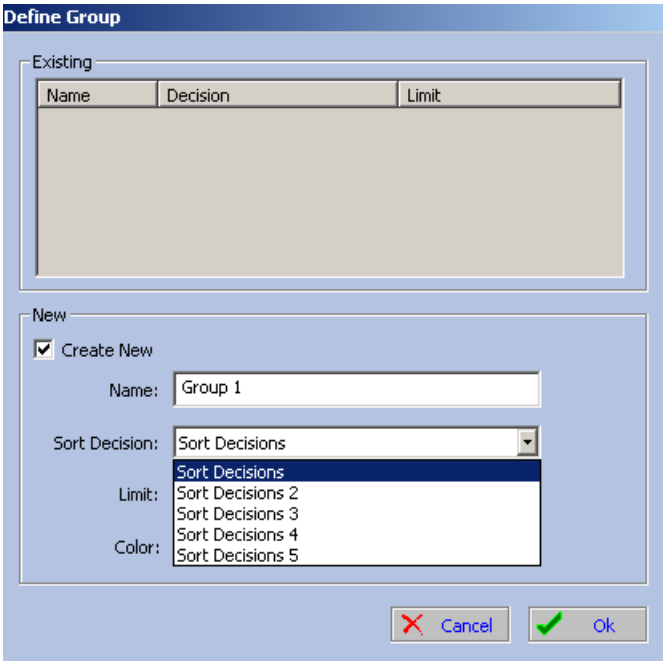
6 Right-click in a selected circle and choose **Define**.

Figure 8.12 CyClone Define Layout



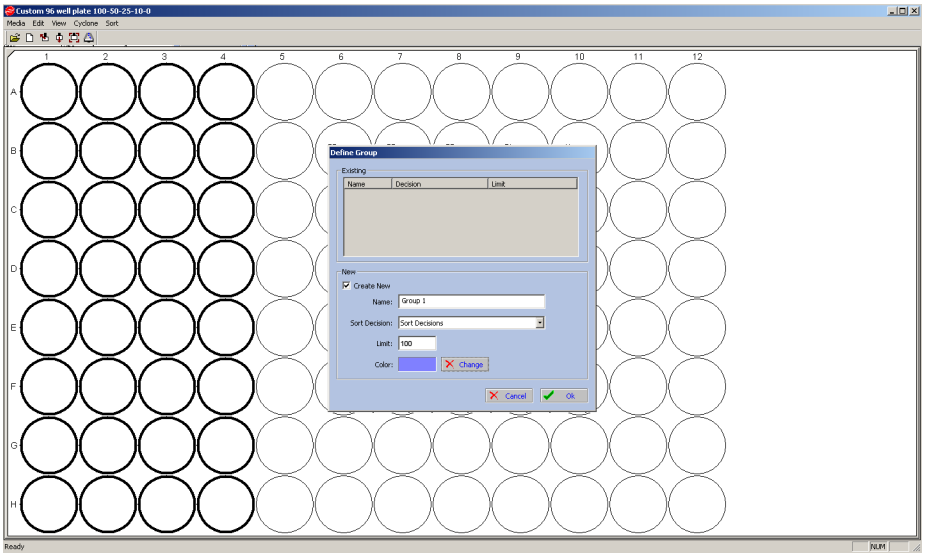
7 Specify sort decisions if not already set. You must set the decisions for stream Left 1 (L1) only.

Figure 8.13 CyClone Define Layout Sort Decisions



8 Set a Limit for the maximum number of cells that will be sorted in one location.

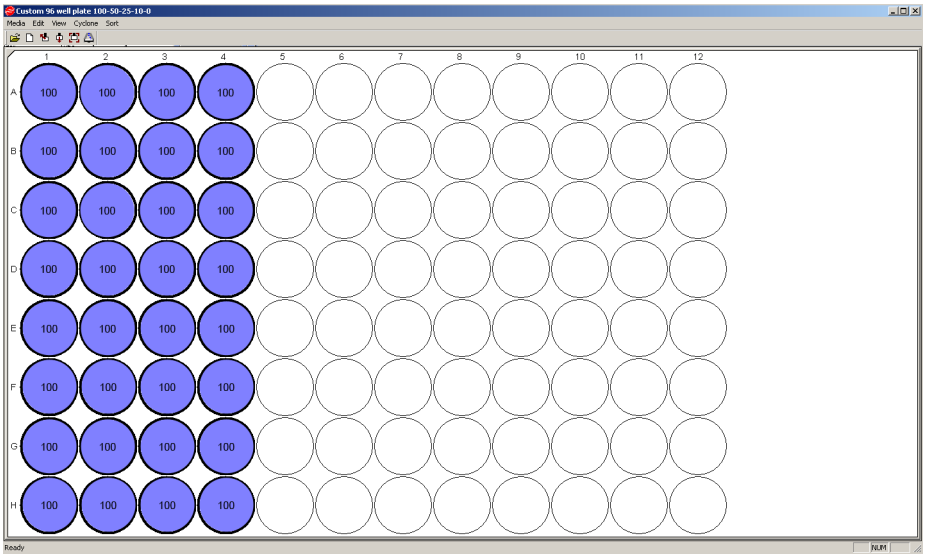
Figure 8.14 CyClone Define Layout Colors 1



9 Select a color.

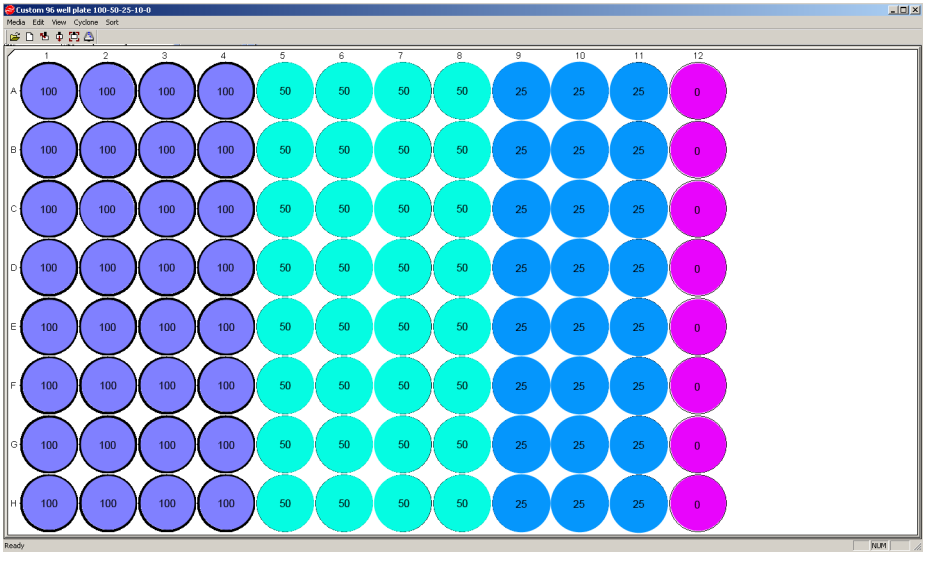
10 Click **OK**.

Figure 8.15 CyClone Define Layout Colors 2



11 If you intend to sort more than one set of sort decisions, repeat steps 1-10. The graphic below depicts a configuration of four sort decisions.

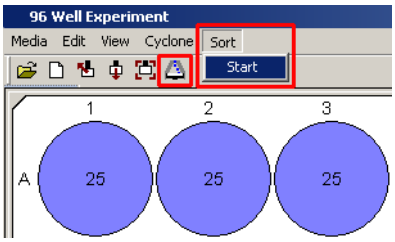
Figure 8.16 CyClone Define Layout Colors 3



Begin Sorting to a Plate or Slide

1 To begin sorting, click the sort icon or click **Start**.

Figure 8.17 CyClone Start Sort



Additional Sorting Information

During a sample run, the sample pressure should be approximately 0.1-0.5 psi greater than the sheath pressure. This is known as the pressure differential. Nominal operating sheath pressure is usually 60 psi (approximately 61.5 psi at the sheath tank) as paired with a 70 µm nozzle.

The pressure console regulates the sheath pressure and sample pressure. The sample differential pressure is controlled through the Touch Screen Control Panel. The goal of the operator is to adjust the pressure differential based on the desired events-per-second (EPS) rate when processing a particular sample.

NOTE Sheath Pressure and nozzle size are the only variables that will affect the stream velocity, and therefore, will warrant a change in the inter-laser delay value. Inter-laser delay is the time it takes a particle to travel from the first laser pinhole, to the second laser pinhole, and so on until it passes pinhole seven. If you change the nozzle, or the sheath pressure, you must rerun the QC procedure in order to automatically reset inter-laser delay.

Changing the Operating Pressure

Nominal operating pressure is usually 60 psi as paired with a 70 µm nozzle. However, it is possible to adjust the pressure up to 100 psi using the coarse knobs on the front of the pressure console. Always remove the sample from the instrument, and ensure the SmartSampler chamber is open, before making any of the following pressure changes.

Changing Inter-laser Delay Values

Inter-laser delay is the time it takes a particle to travel from the first laser pinhole, to the second laser pinhole, and so on until the particle travels all the way past pinhole seven. After a significant pressure change, it is necessary to rerun the QC procedure, which automatically resets the inter-laser delay values. See CHAPTER 7, *Quality Control*.

Decreasing the Pressure

When decreasing the nominal operating pressure, lower the sheath pressure to the desired level using the sheath regulator knob on the pressure console Figure 2.21. Next, lower the sample pressure to approximately 0.2 psi above the sheath pressure. Then, vent the pressure at the sheath tank.

NOTE After making adjustments at the pressure console, check the pressure at the sheath tank. Do not run samples until the sheath tank pressure gauge is correct.

Increasing the Pressure

When increasing the nominal operating pressure, raise the sheath pressure to the desired level using the sheath regulation knob on the pressure console Figure 2.21. Next, raise the sample pressure to approximately 0.2 psi above that of the sheath with the sample regulation knob on the pressure console.

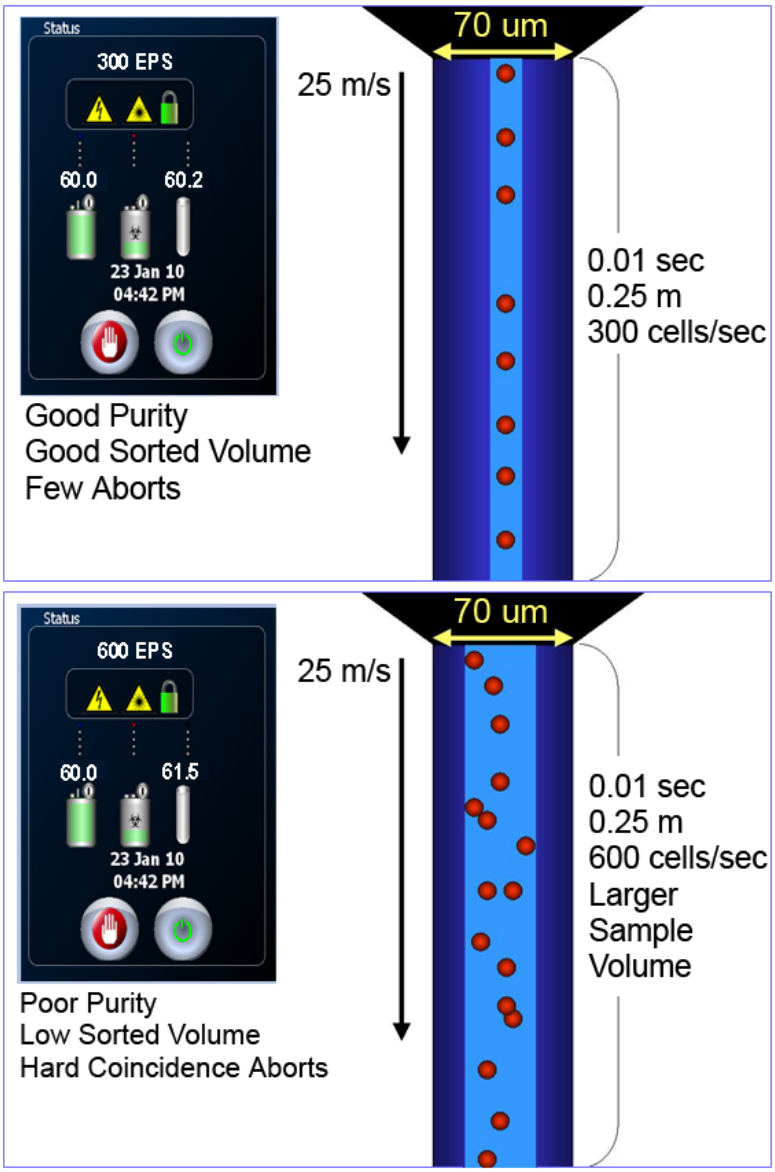
NOTE After making adjustments at the pressure console, check the pressure at the sheath tank. Do not run samples until the sheath tank pressure gauge is correct.

Processing Speed Limitations

The MoFlo Astrios is designed to sort events very quickly. In fact, good purity and recovery can be achieved at speeds of >70,000 events per second. However, you must make sure that your cell concentration is such that you can achieve high event rates at an acceptable pressure differential.

The MoFlo Astrios electronics most efficiently process cells that arrive at the interrogation point in single file. If you increase the pressure differential by more than 0.8 psi (60 psi; 70 µm tip), this will cause the cells to arrive at the interrogation point simultaneously and will therefore increase coincidence and sort logic aborts. Therefore, if you wish to process cells at a high event rate, make sure your sample is adequately concentrated.

Figure 8.18 Sort Purity vs. Hard Coincidence Aborts



General Rule for Cell Concentration — For every 1,000 events per second that you wish to obtain, you should have one million cells per milliliter. For example, if you wish to run at 40,000 events per second, you should have at least 40 million cells per milliliter. This will ensure that you can obtain a high event rate at an acceptable pressure differential.

Hard Aborts

A hard abort on MoFlo Astrios occurs when the instrument cannot process sort information in time to make the sort decision. However, due to the speed of MoFlo Astrios electronics, very few hard aborts are produced. For example, MoFlo Astrios generally will not produce hard aborts during analysis until the processing speed has exceeded 200,000 EPS. Processing speeds and abort rates will vary depending on the nature of the sample.

Soft Aborts

A Soft Abort occurs when a positive event, as defined by the Sort Decisions set in Summit, fails to pass the criteria defined by the Abort Mode and Droplet Envelope Figure 8.7, or sort precedence. This failure causes an abort of the positive event and is tallied in the abort statistics and the Abort Rate data on the Touch Screen Control Panel Statistics screen.

When an event fails the Sort Logic, as defined by the Sort Decisions set in Summit, it is identified as a negative event, which is aborted. These negative events are not counted in the abort statistics, but are included in the total events and event rate.

Sort Decisions and Doublets

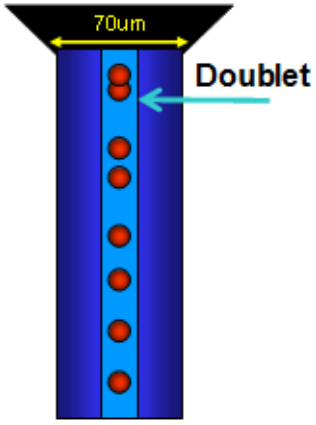
When particles flow at very high event rates, it is possible that two particles overlap and appear as one larger particle. This is called a doublet.

For many sorting applications it is critical that only single cells be analyzed and sorted.

Doublets

A doublet occurs when two particles cross the interrogation point at or near the same time. The probability of doublets increases when analyzing cells that tend to stick together, or when cells are flowing at a high event rate or a high pressure differential. Proper sample preparation and pre-filtering the sample can minimize doublets or clumps of cells. Sample can be agitated to minimize doublets.

Figure 8.19 Doublet Diagram



The linear area and log area signals for a doublet yield a greater value than of a singlet. However, linear height and log height signals do not provide any information that distinguishes a singlet from a doublet. To determine doublets you must collect area or pulse width data.

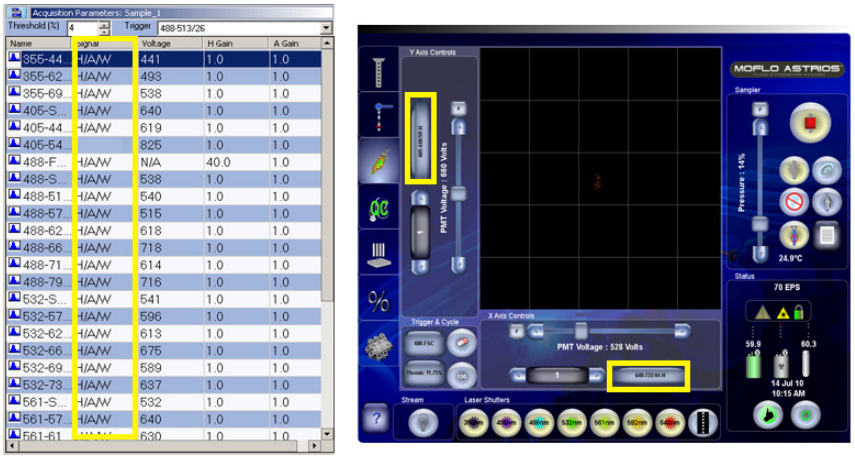
Data Type Parameters

Data Type parameters are enabled or disabled in Summit and on the Touch Screen Control Panel Fine Alignment screen and are defined as:

- H = linear height
- A = linear area
- W = pulse width
- L = log height
- LA = lot area

Before you can set up histograms or dot plots you must enable the parameters that you intend to use for your experiment. When a parameter is enabled, the instrument collects linear height, area, and width information. All other parameters, such as log values, are computed using the linear data. Unlike this feature in older versions of Summit, parameters in Summit 6.0 are either all enabled or all disabled. You do not have to enable height, area, width, log height, and log area individually.

Figure 8.20 Data Type Parameter Settings in Summit and on the Fine Alignment Screen



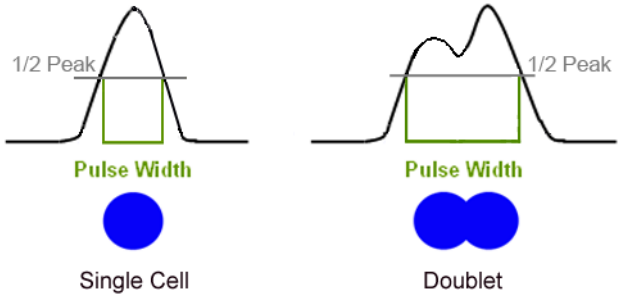
Threshold

The purpose of the threshold is to desensitize the electronics to low-level noise caused by very small particles or auto fluorescence. The threshold-level selector allows the user to empirically determine the minimum voltage at which signal processing is initiated. This range is selectable from 0.01 percent to 100 percent, with a full-scale selection equivalent to 10 V.

Pulse Width

The Pulse Width (W = pulse width) is determined at 1/2 the signal peak as measured from 0.01 above baseline. When particles are too close together to be measured as distinct events they are called a doublet. The pulse width for doublets is wider and is measured at 1/2 of the highest peak. See Figure 8.21.

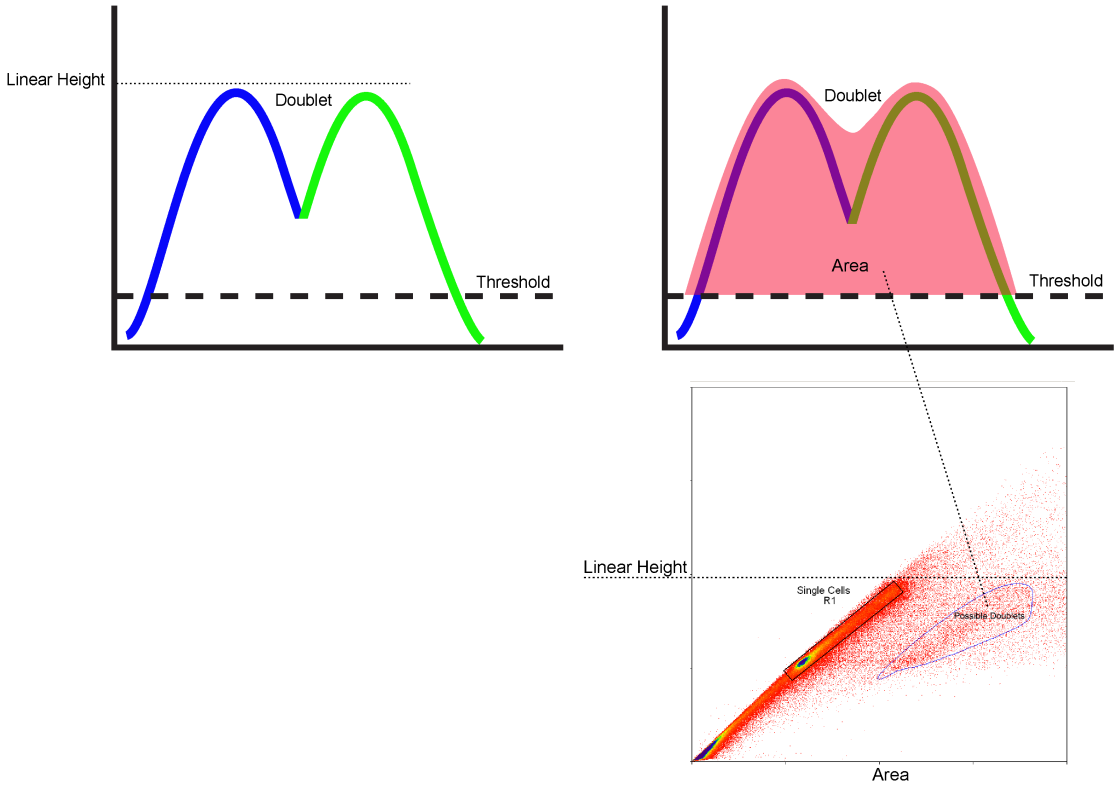
Figure 8.21 Pulse Width Single and Doublet Peaks



Height vs. Area Signals for Doublets

As can be seen in Figure 8.22 linear height (H = linear height) and log height (L = log height) signals do not provide any information that distinguishes a singlet from a doublet. Area (A = linear area) includes the area between the threshold and the peak.

Figure 8.22 Height and Linear Area and Doublets



Doublet Discrimination

It is important to determine the doublet population in acquired data before setting sort decisions in order to obtain an accurate sort. The generic example shown below depicts cells that were treated with a DNA dye. Adjust your methods according to the needs of your specific experiments.

---

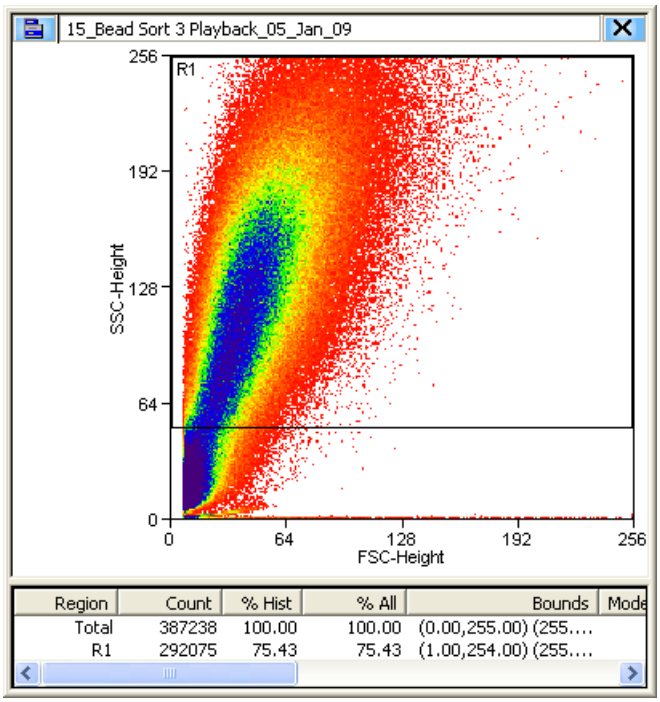
1 In Summit, create a FSC vs. SSC dot plot, an Area vs. Height dot plot, and a Height vs. Pulse Width dot plot. Acquire data.

---

2 Create a region that will exclude suspected debris. Set a gate using the region, and gate into the Area vs. Height and the Height vs. Pulse Width dot plots. See CHAPTER 4, *Setting a Gate from a Single Region*.

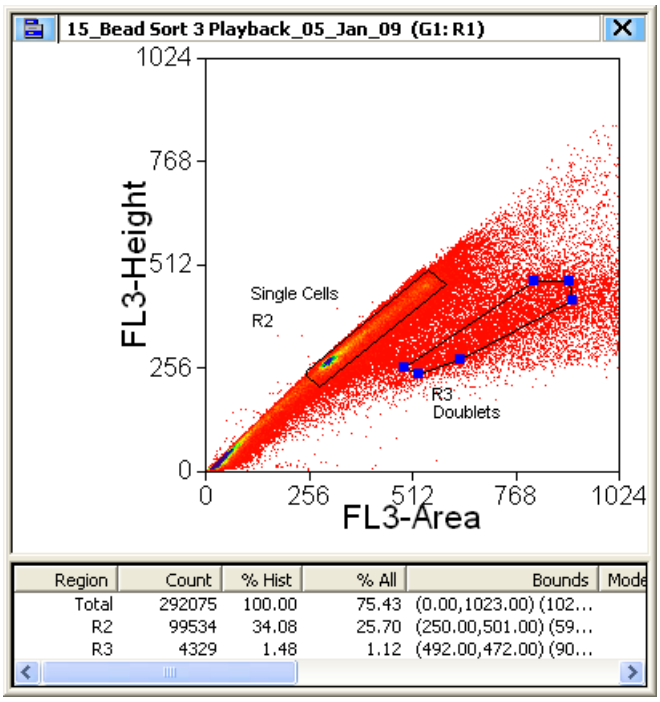
NOTE Either the Pulse Width or Area dot plot will display the singlet and doublet populations more distinctly depending on the characteristics of the sample.

Figure 8.23 FSC vs. SSC dot plot



3 Set a region to include the suspected single cell population as shown in Figure 8.24. You can also create a region around the suspected doublet population. When you set sort decisions the doublet population can be excluded from the sort.

Figure 8.24 Area vs. Height dot plot Gated on R1 of the FSC vs. SSC plot



NOTE Color gating is a method to help view the doublet population in various histograms after the doublet population has been determined. See CHAPTER 4, *Color Gating*.

Manually Determine Drop Delay

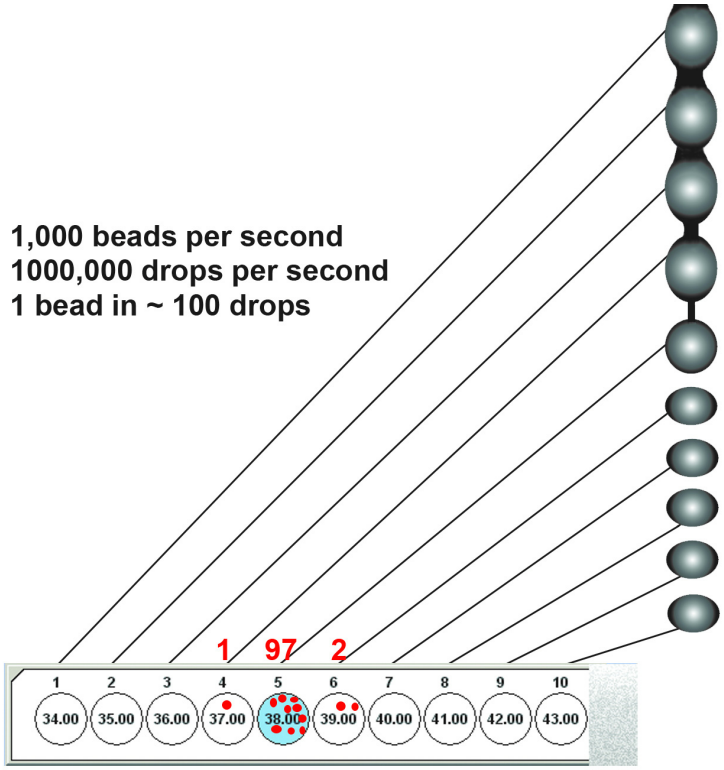
Drop Delay is defined as the amount of time it takes for a particle to travel from the interrogation point of the primary laser to the Last Attached Drop in the stream. The accuracy and stability of the Drop Delay is crucial to effective sorting. During normal operation of the instrument, with 70 µm and 100 µm nozzle tips, Drop Delay is determined automatically with IntelliSort.

Drop Delay must be determined manually for nozzle tips other than 70 µm and 100 µm. Nozzle selection is made in the *Laser and Stream Intercept Configuration Screen*.

The information in this section can be used to manually determine Drop Delay or to verify the results of automatic Drop Delay Determination.

NOTE If you use automatic Drop Delay, and you want to manually verify Drop Delay results, do not enter IntelliSort Maintain Mode, you can make adjustments, and then enter IntelliSort Maintain Mode.

Figure 8.25 Drop Delay Diagram



The Drop Delay calibration is a statistical experiment, which operates in the following manner:

- Ten puddles are deposited on a microscope slide.
- Each of the ten puddles uses a different drop delay setting, which differs by one whole number as shown in the example above.
- Precisely 100 droplets are deposited into each puddle at these 10 different drop delay settings.

  NOTE The droplet value can be changed in Summit.

- Within these 10 drop locations will be 100 beads. By statistical probability, the beads will be deposited onto the slide with the majority of the beads (>50) appearing in one puddle, and the remainder (<50) in the adjacent puddles.
- The number of beads in the adjacent puddles is an indication of the amount the drop delay needs to be changed in order to be accurate to the nearest 1/100th of a droplet.
- A change is made to the drop delay to accommodate this.

Prior to manually determining Drop Delay, you must first follow the steps to start and align the instrument, optimize the droplet stream, and optimize droplet deposition.

---

1 Run Flow-Check FluoroSpheres™ (approximately 0.5 mL undiluted) at 100 EPS.

---

2 Open an established Drop Delay Protocol, or create a FSC vs. SSC dot plot using parameters from the same laser.

---

3 Acquire data in Summit Software (F2).

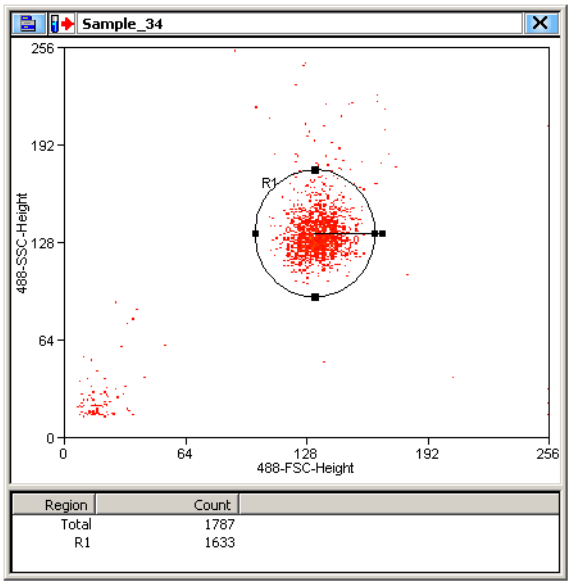
---

4 Right-click and select a region around the main FSC vs. SSC population.

---

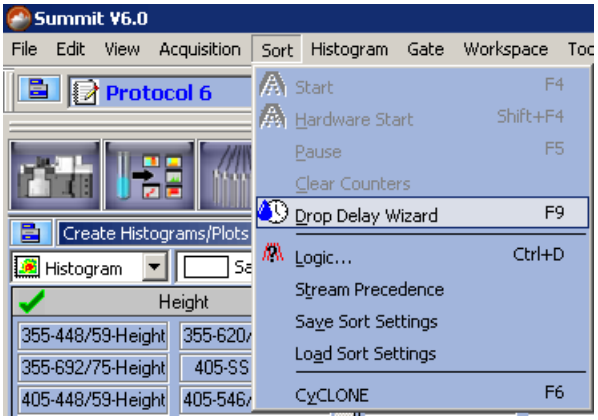
5 Right-click and select a region around the main fluorescent population.

Figure 8.26 Drop Delay FSC vs. SSC Dot Plot



6 Go to the main **Sort** menu in Summit and select **Drop Delay Wizard**.

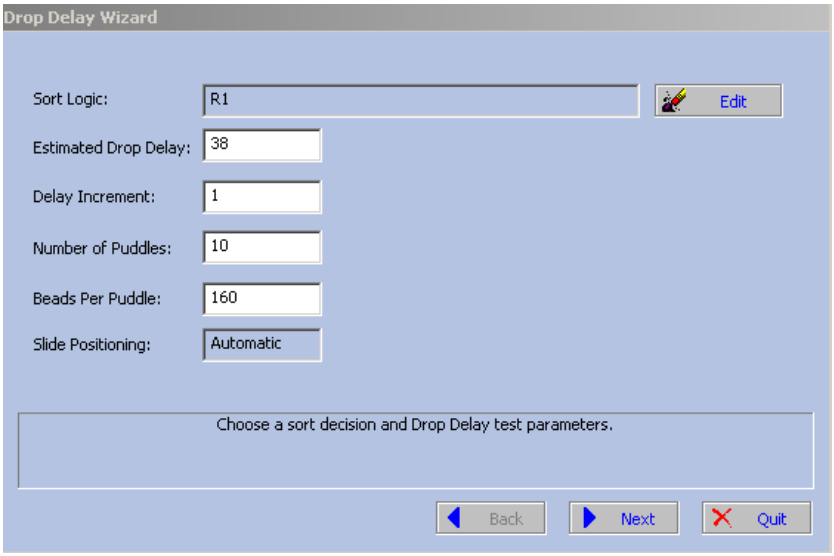
Figure 8.27 Drop Delay Wizard 1



7 The **Drop Delay Wizard** appears. Move all objects out of the path of CyClone and place a clean slide on the CyClone.

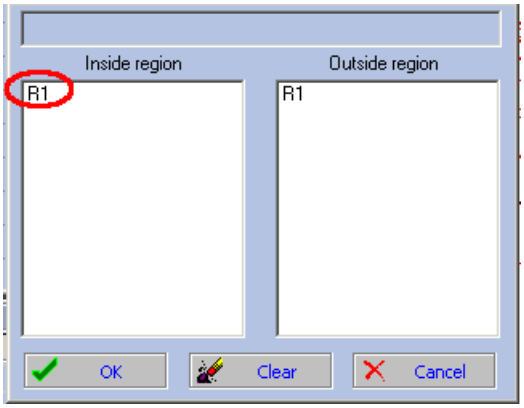
- If you ran automatic Drop Delay using IntelliSort, and you are now performing manual Drop Delay to check the results, the Estimated Drop Delay value displayed in Figure 8.28 will be the value that was determined by IntelliSort.
- If you did not run automatic Drop Delay, but intend to run Drop Delay manually, the Estimated Drop Delay value in Figure 8.28 will be the same as the last time you used the instrument.

Figure 8.28 Drop Delay Wizard 2



8 Click the **Edit** Button. The **Left 1 Stream Sort Logic** dialog box appears.

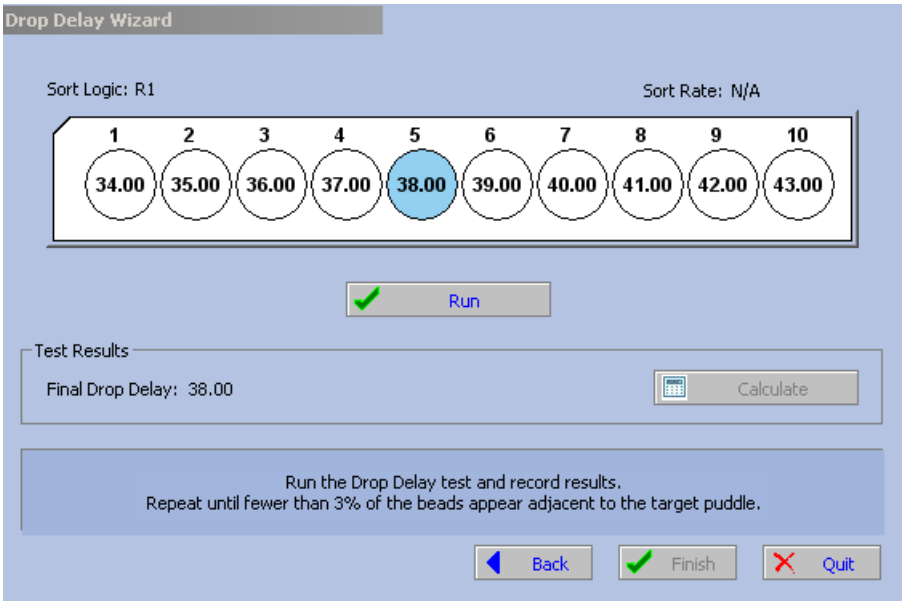
Figure 8.29 Drop Delay Wizard 3



9 Select **R1**, and click **OK**.

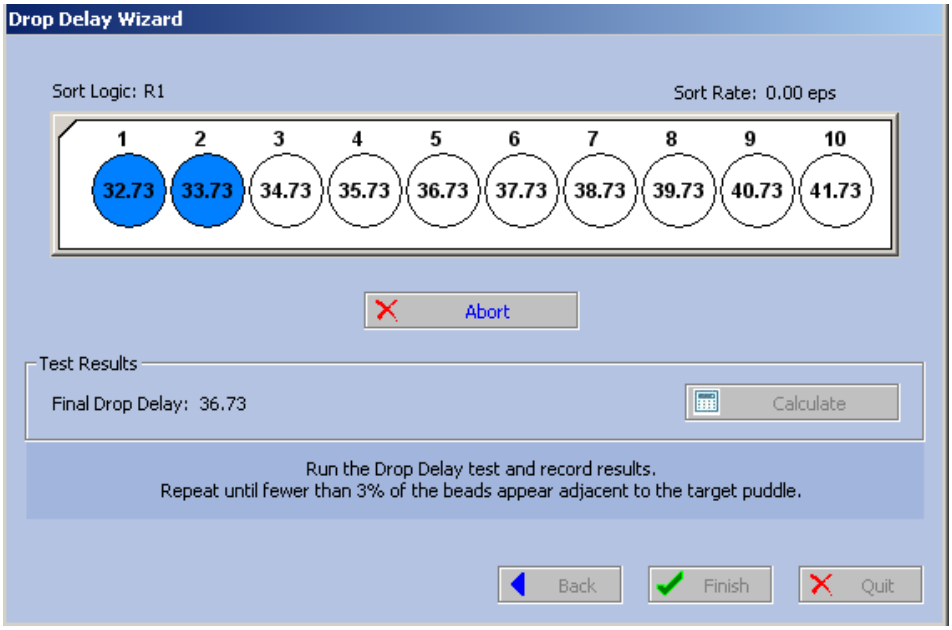
10 Click **Next**. The circles represent the puddles that will be deposited on the slide.

Figure 8.30 Drop Delay Wizard 4



11 Click **Run**. You will see the circles turn blue as the puddles are created.

Figure 8.31 Drop Delay Wizard 5



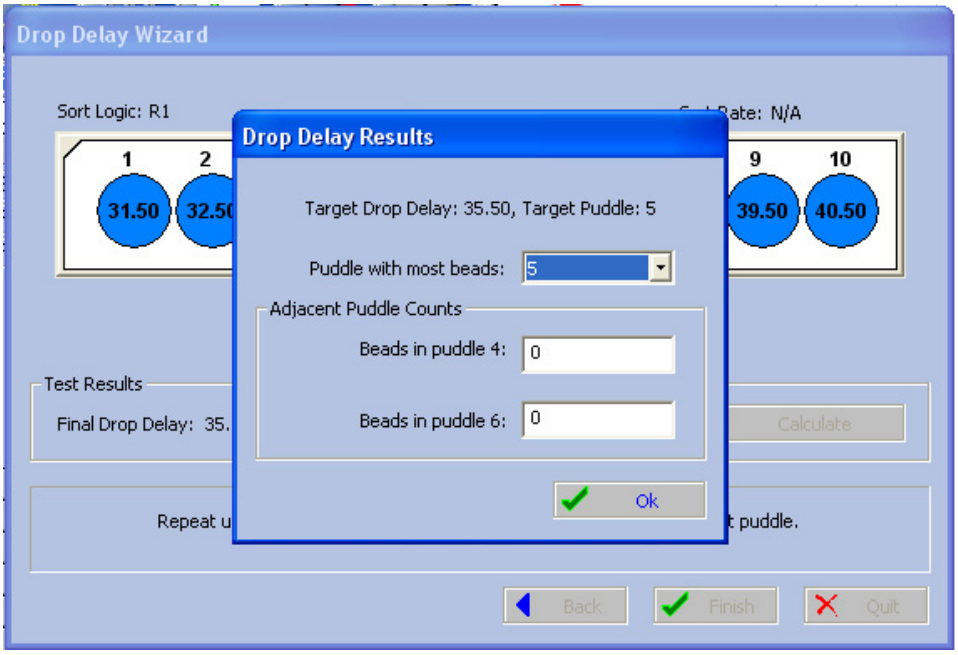
12 When all puddles have been created, remove the slide and inspect the puddles under a fluorescent microscope.

13 Determine the puddle that contains the most beads. (Puddle number one is located on the edge of the slide closest to the user when the test was run.)

14 Count the beads in the puddles adjacent to the puddle that contains the most beads.

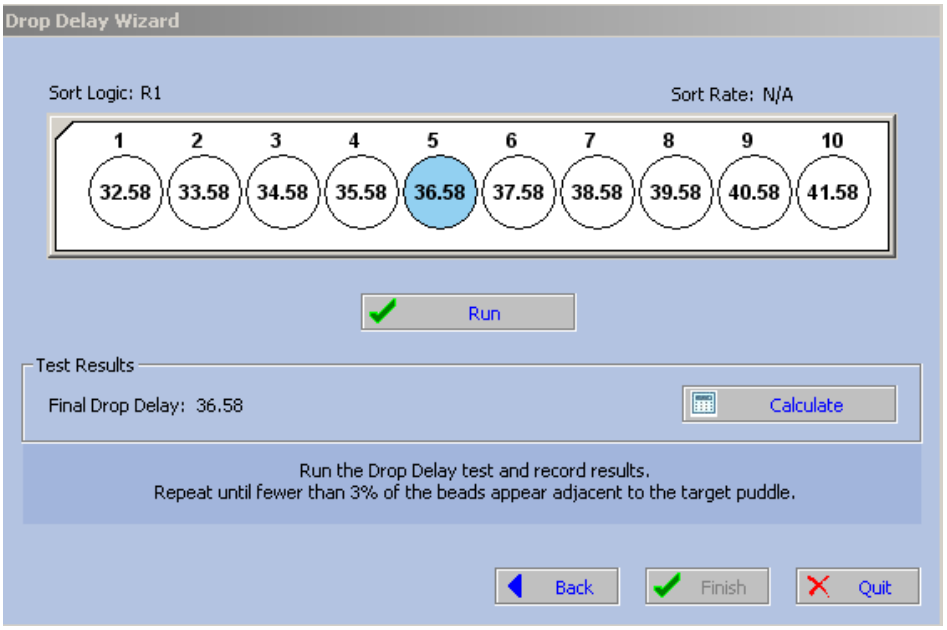
15 Enter the values in the **Drop Delay Wizard**.

Figure 8.32 Drop Delay Wizard 6



16 Click **OK**. Rerun the test and record the results. Repeat the process until the difference between the number of beads in the puddles adjacent to the target puddle is less than three percent.

Figure 8.33 Drop Delay Wizard 7



17 Click **Finish**.

18 Enable the IntelliSort Maintain button.

Sort Report

It is possible to view, save, and print the sort statistics that were recorded during a sort. In order to record the statistics you must open Worklist Builder before you begin sorting. See CHAPTER 4, *Sort Report*.

CHAPTER 9

Cleaning and Maintenance

Introduction

Regular maintenance of the MoFlo Astrios is recommended as described in this section. System integrity can be effectively maintained by assuring cleanliness of the fluidics. In addition to performing preventive maintenance procedures, Beckman Coulter also recommends that you establish and perform other laboratory procedures for routine operations such as backing up your data and experimental protocols.

Cleaning

The decontamination procedure will vary according to your laboratory requirements, but the following information can be used as a guide.

At the end of the work day or before you shut down the instrument, use the following cleaning procedure.

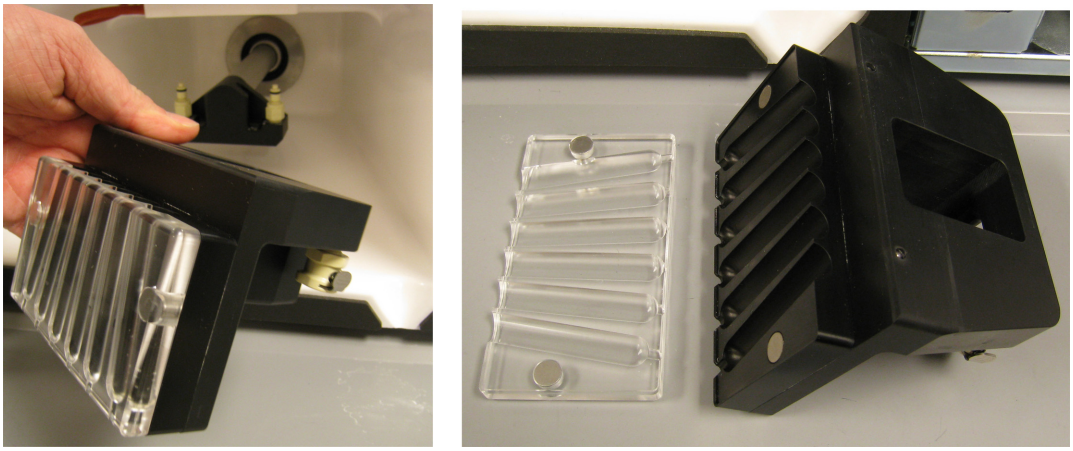
Daily Decontamination Procedure During Shutdown

IMPORTANT Once you begin, make sure you complete the entire shutdown procedure **before** leaving the instrument.

1 On the Touch Screen Control Panel, press the Power button and follow the prompts to run a tube of cleaner and a tube of deionized water during the shutdown procedure.

2 Press the release buttons on the sides of the sort receptacle fittings and lift the receptacle off of the CyClone arm.

Figure 9.1 Remove the Sort Receptacle



3 Pull the SortRescue tray forward off of the mounting posts and disconnect the waste evacuation tubing.

Figure 9.2 Disconnect the SortRescue Tray and Tubing

4 Spray the surfaces of the components in the sort chamber with 70 percent ethanol and wipe clean.

5 Open the fluidics drawer on the lower left side of the instrument. Disconnect the color-coded quick connect fittings at the waste tank and sheath tank by pulling the collars up until the fittings are released.

6 Open the waste tank. Unscrew the threaded knob on the lid of the tank. When the knob is sufficiently loose, the lid can be removed. Empty the waste tank and clean it with an approved cleaner. See APPENDIX A, *Approved Cleaners and Disinfectants*.

7 Open the sheath tank by pulling up on the handle and removing the lid. Empty the sheath tank and clean it with an approved cleaner. Rinse the tank with deionized water before refilling with sheath fluid.

NOTE Cleaning the sheath tank can be done daily or weekly as deemed appropriate by your laboratory manager.

8 After you remove the tanks from the system and empty them, it is optional to autoclave the tanks. There is no need to remove the pressure gauges or fittings from the MoFlo Astrios tanks before autoclaving.

NOTE See APPENDIX A, *Approved Cleaners and Disinfectants* for more information. See also CHAPTER 9, *Yearly Fluidics System Decontamination*. Laboratory cleaning schedules will vary according to individual needs.

Maintenance

IMPORTANT Weekly and monthly preventative maintenance will be determined by the requirements of your laboratory. In most cases, the Fluidics Decontamination procedure should be run on a yearly basis; however, individual laboratory needs may vary.

Yearly Maintenance

A Beckman Coulter Field Service Representative should perform a maintenance check on the MoFlo Astrios every year. To schedule an annual maintenance service, contact your local Beckman Coulter Support Representative.

Sheath Filter

The Inline Sheath Filter should be changed periodically to ensure free flow of the sheath across the filter membrane. Beckman Coulter recommends the filter change every six months to one year, depending on the usage and nature of the sheath fluid.

Yearly Fluidics System Decontamination

IMPORTANT In most cases, the Fluidics Decontamination procedure should be run on a yearly basis; however, individual laboratory needs may vary.

This procedure is for the decontamination of the MoFlo Astrios fluidics system. The purpose of the decontamination is to eliminate any bacterial growth that may be proliferating in the sheath tank, sheath lines, sample lines, or other fluidic components. It is good practice to perform routine maintenance of the fluidic system to keep it clean and functioning properly. This procedure may be necessary for yearly maintenance only, or more often depending on the needs of your laboratory.

Fluidics System Decontamination Procedure

1 Obtain and put on appropriate personal protective equipment. A lab coat, gloves and safety glasses are required.

2 Obtain the following supplies:

- One new in-line sheath filter.
- A bleach solution containing 2000 ppm active chlorine is also acceptable (115 mL household bleach + 2885 mL water).
- Paper towels
- Large bucket

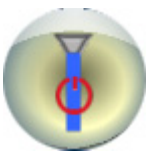
NOTE This procedure is to be performed at a system pressure of 60 psi. Sample pressure should be slightly higher (0.1-0.3 psi greater) than sheath pressure.

3 Press the Change Tanks button to depressurize the system.

4 Remove and discard the in-line sheath filter. Reconnect the canister without a sheath filter installed.

5 Place 3 L of decontamination solution into an empty sheath tank and connect to instrument. Prepare a 5 mL tube of decontamination solution for placement on the SmartSampler.

6 Press the Start Fluidics button.



7 Once the sheath stream is observed coming out of the nozzle, engage the sample and run fluidics and sample for 20 minutes. Stop the sheath flow.

8 Turn the knob on the nozzle stage and raise the nozzle.

9 Loosen the knob on the front of the nozzle body and then pull the nozzle body out from the stage.

⚠ WARNING

Risk of personal injury. When removing the nozzle tip, the exposed injection tube can puncture your skin. Do not touch the exposed injection tube.

10 Turn the nozzle body upside-down, unscrew the black retaining ring, and remove the nozzle tip.

11 Locate a large bucket in which to drain the nozzle.

12 Start fluidics and run the 3 L of decontamination solution into the bucket.

13 When the sheath tank is empty of the decontamination solution, press the Change Tanks button.

IMPORTANT The tanks and canister and must be thoroughly rinsed.

14 Remove the sheath and waste tanks as well as the sheath filter canister. Rinse the tanks and the canister with deionized water.

15 Fill the sheath tank with deionized water.

16 Return the tanks and canister to the instrument.

17 Start fluidics and let the tank drain into the bucket.

18 Repeat steps 13 through 17 for another tank of deionized water.

19 Using sterile technique, install a sterile sheath filter in the canister.

20 Fill the sheath tank with deionized water and run fluidics to let the tank drain into the bucket.

NOTE Repeat this step two times so that two tanks of deiionized water have run through the new sheath filter.

21 Fill the sheath tank with sheath.

⚠ WARNING

Risk of personal injury. When reinstalling the nozzle tip, the exposed injection tube can puncture your skin. Be careful not to touch the exposed injection tube.

22 Reattach the nozzle tip.

23 Reinstall the nozzle body in the stage.

⚠ WARNING

Risk of personal injury. When lowering the nozzle stage, you can pinch your fingers between the bottom of the stage and the instrument frame. Lower the nozzle stage using the upper portion of the stage to avoid pinching points.

24 Lower the nozzle stage back into the instrument and lock it in place.

25 De-bubble for one hour.

26 Check the nozzle and fluidics lines for visible bubbles and de-bubble again if necessary.

CHAPTER 10

Troubleshooting and Replacement Procedures

Troubleshooting

Please contact your local Beckman Coulter Field Service Representative immediately for assistance with any instrument malfunction or service need.

⚠ WARNING

Do not attempt any maintenance on the MoFlo Astrios laser components. Laser maintenance should only be performed by specially trained, certified Beckman Coulter Field Service Representatives.

⚠ CAUTION

You should not perform laser alignment until you have received proper laser safety training.

- **Follow ANSI Standard 136.1 for laser safety.**
- **Do not wear jewelry with reflective surfaces during laser alignment.**
- **Never position your eyes within the same plane as the laser beam.**
- **Wear appropriate personal protective equipment.**

Laser Alignment

When the instrument is extremely out of alignment and there is no trigger rate or data displayed for a laser, you must align the FBSO to regain the use of the instrument.

FBSO Alignment - Ring of Diffraction for Fiber-coupled Lasers

The fiber-coupled lasers are adjusted as a unit, therefore all fiber-coupled lasers should be aligned when a middle laser is aligned.

Fiber-coupled Lasers Ring of Diffraction Procedure

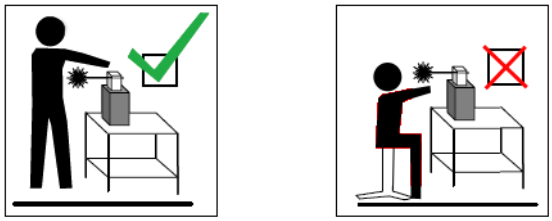
1 Ensure that the stream is visually aligned. See CHAPTER 6, *Stream Alignment*.

2 Once the stream is aligned, adjust only the appropriate laser focusing optics for the FBSO.

IMPORTANT Refer to *Optical/Laser Safety* for specific laser safety information.

3 With the sheath stream running, use the High Voltage/Optical safety interlock key to override the safety interlock on the outside of the Illumination Chamber, and slide the Illumination Chamber door open.

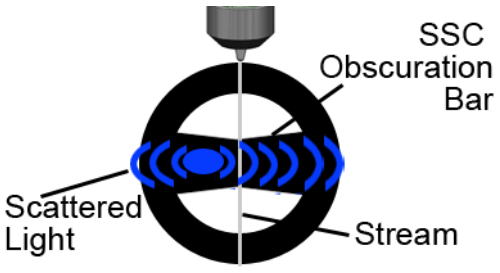
Figure 10.1 Laser Safety (Eyes should never be in the same horizontal plane as laser light.)



4 Open the shutter for the 488 nm laser.

5 From a top-down angle, view the Forward Scatter obscuration bar directly across from the laser beam. Verify that the laser light is evenly distributed on the FSC bar.

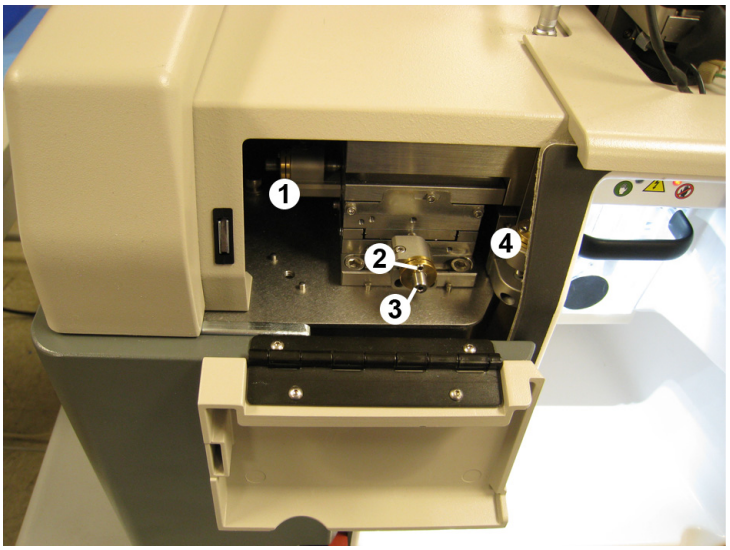
6 From a top-down angle, view the Side Scatter obscuration bar directly behind the stream. Verify that the laser light is of even intensity on the SSC bar. You should observe that the light forms a bull's-eye shape. The center of the bull's-eye for the 488 nm laser will not be in the center of the bar.



7 If the laser light is not evenly distributed on the SSC and FSC obscuration bars, the FBSO requires alignment.

8 Open the door on the left side of the instrument to access the FBSO adjustment micrometers. Use 2 mm ball driver to unlock the micrometer, and then use the same tool to adjust it. Each micrometer has a lock fitting and an adjustment fitting.

Figure 10.2 Access to the FBSO Micrometers



1. Left to Right Adjustment (Beam focus on stream.)
2. Set Screw Lock (One per micrometer.)
3. Front to Back Adjustment (Beam alignment with stream.)
4. Up and Down Adjustment (Beam alignment with pinholes.)

9 Adjust the 488 nm laser beam to intersect with the stream just below the tip of the nozzle.

10 Check the laser beam for a 360-degree emission of light scatter that completely circles the interrogation point at the stream. This is called a ring of diffraction.

IMPORTANT When performing laser alignment, ensure that your eyes are not in the horizontal plane of the laser.

11 Adjust the micrometers until the laser light is evenly distributed on the FSC bar.

12 Adjust the micrometers until the laser light is evenly distributed on the SSC bar. Look for a bull's-eye shape, and note that the center of the bull's eye will not be exactly in the center of the bar for the 488 nm laser.

Co-linear (FBSO) Laser Alignment Bead Flash

1 With the sheath stream running, load a sample tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (diluted 1:10 with deionized water).

2 Press the SmartSampler Start Sample button.

3 Decrease the illumination intensity of the Illumination Chamber so you can see the silhouette of the pinholes on the Coarse Alignment (Pinhole) screen.

4 Press and hold the boost button on the Touch Screen in order to see the bead flash.

5 The bead-flash from the fiber-coupled lasers must align with the first through sixth pinholes. Adjust the vertical micrometer until the bead flash for 488 nm laser is centered in the second pinhole.

NOTE Below is the pinhole order for a seven-laser system. When a system has fewer than seven lasers, the pinhole order remains the same and pinholes for empty laser spots should not display bead flash.

- 640 nm
- 488 nm
- 592 nm
- 561 nm (center pinhole)
- 532 nm
- 405 nm
- 355 nm (The UV laser is free-standing and is directed through the seventh pinhole.)

6 The fiber-coupled lasers are adjusted as a unit, therefore all fiber-coupled lasers should be aligned when a middle laser is aligned. You can verify the bead flash for each fiber-coupled laser by opening and closing the corresponding shutters one-at-a-time.

Co-linear Laser Alignment Pulse Width

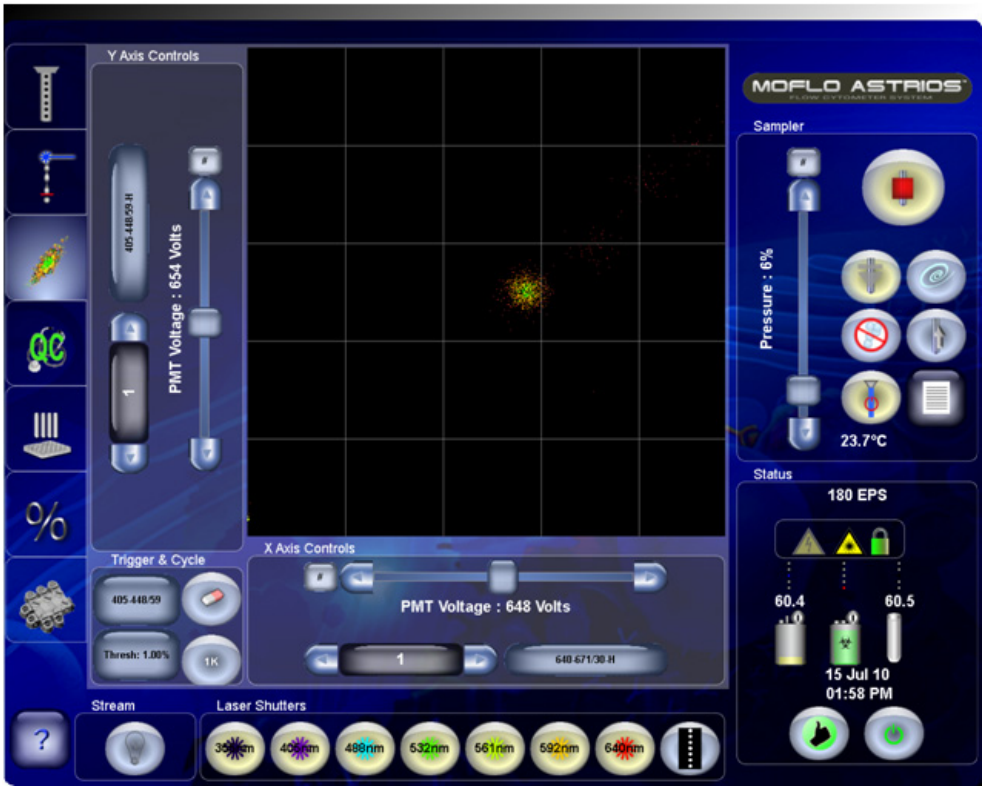
1 Set the trigger parameter on a fluorescent parameter from the 561 nm laser (or another laser near the center pinhole).

2 Set the touch screen 561-579/16 (H) vs. (W). (If the system does not have a 561 nm laser, set a parameter from the center-most laser.) Adjust the stage focus to minimize the Pulse-width parameter and maximize Height parameter.

Figure 10.3 561-579/16 (H) vs. (W) Bivariate

3 Set the touch screen parameters to 640-722/44 (H) and 405-448/59 (H) Figure 6.4, or two fluorescent parameters on the lasers from the furthest extents of the spectrum for your configuration.

Figure 10.4 640-722/44 (H) vs. 405-448/59 (H) Bivariate.



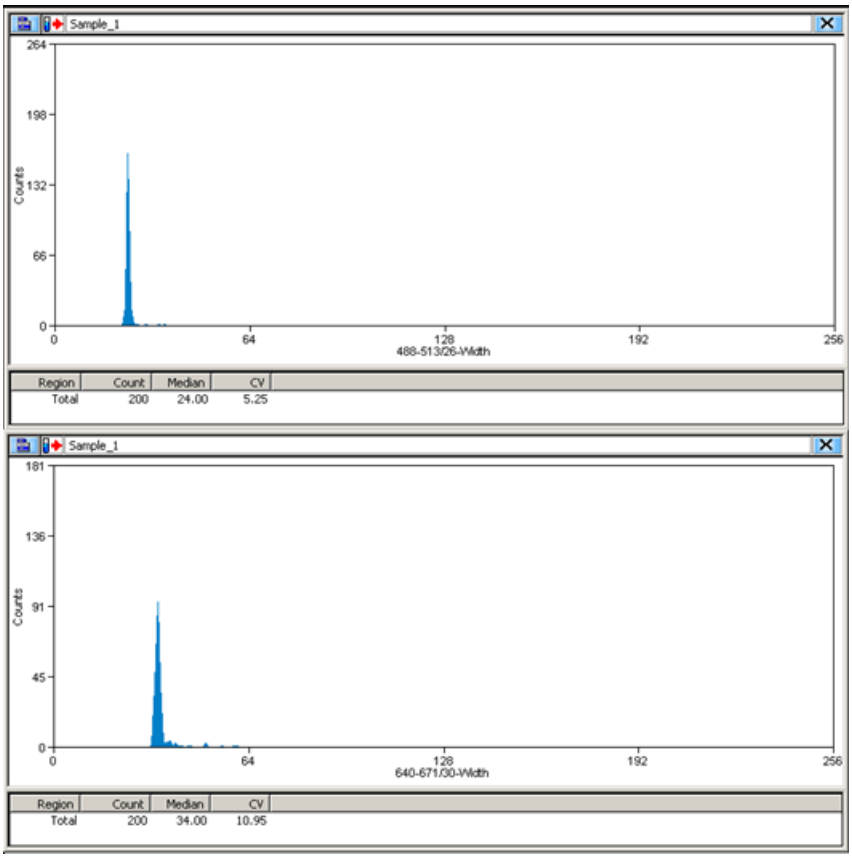
4 Adjust FBSO micrometer (3) Figure 10.2 together with the nozzle focus micrometer (1) Figure 6.1 to maximize the height signal in both parameters.

NOTE An iteration with the FBSO vertical micrometer (4) may be needed.

5 Adjust the nozzle micrometer (2) to further maximize the signal.

6 Create single-parameter Pulse Width histograms in Summit for 488-513/26 and 640-671/30. Acquire data in Summit (F2). Adjust stage focus to optimize Pulse-width. The 488 nm median channel should be 20-25, while the 640 nm median channel should be 30-35 (These values are based on a 70 µm tip with sheath pressure at about 60 psi.)

Figure 10.5 488-513/26 and 640-671/30 Pulse Width Histograms



7 Check the bivariate parameters Figure 10.4 to verify that the BSO stage is in the best position before tightening the stage locks.

8 Lock the FBSO stage locks.

UV BSO Alignment - Ring of Diffraction

When the instrument is extremely out of alignment and there is no trigger rate or data displayed for a laser, you must rely on sight rather than data to perform alignment.

UV Laser Ring of Diffraction Procedure

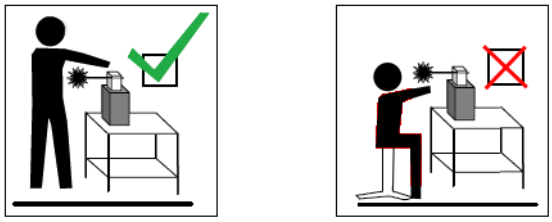
1 Adjust only the appropriate laser focusing optics for the BSO associated with the UV laser. Do not move the nozzle micrometers.

NOTE If the stream position is accidentally changed when you are aligning the UV laser, realign the stream to the FBSO before proceeding.

IMPORTANT Refer to *Optical/Laser Safety* for specific laser safety information.

2 With the sheath stream running, use the High Voltage/Optical safety interlock key to override the safety interlock on the outside of the Illumination Chamber, and slide the Illumination Chamber door open.

Figure 10.6 Laser Safety (Eyes should never be in the same horizontal plane as laser light.)



3 Open the shutter for the UV laser. Shutter all remaining lasers.

4 Adjust the laser beam to intersect with the stream just below the tip of the nozzle.

IMPORTANT When performing laser alignment, ensure that your eyes are not in the horizontal plane of the laser.

5 From a top-down angle, view the Side Scatter obscuration bar directly behind the stream. Adjust the micrometers so the laser light is of even intensity on the SSC bar.

6 Remove the interlock defeat key and close the chamber door.

7 Proceed to the UV BSO Co-linear Alignment procedure, to direct the UV laser into the seventh pinhole.

UV Laser Co-linear Alignment Bead Flash Procedure

1 Ensure that the shutter for the UV laser is open.

NOTE This shutter will not open unless the laser has been powered on for 30 minutes.

2 On the Fine Alignment Screen, set the trigger laser to a UV parameter.

3 With the sheath stream running, load a sample tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (diluted 1:10 with deionized water), or other brightly fluorescing particles.

4 Press the SmartSampler Start Sample button.

5 Decrease the illumination intensity of the Illumination Chamber so you can see the silhouette of the pinholes on the Coarse Adjustment screen.

6 Press and hold the boost button on the Touch Screen in order to see the bead flash through the pinhole.

7 Adjust the vertical micrometer on the UV BSO until the bead flash is centered in the seventh pinhole.

NOTE Below is the pinhole order for a seven-laser system. When a system has fewer than seven lasers, the pinhole order remains the same and pinholes for empty laser spots should not display bead flash.

- 640 nm
- 488 nm
- 592 nm
- 561 nm
- 532 nm
- 405 nm
- 355 nm (The UV laser is free-standing and is directed through the seventh pinhole.)

8 Proceed to CHAPTER 6, *UV Laser Fine Alignment*.

Forward Scatter Optical Alignment

The forward scatter parameter (FSC) will be aligned when the MoFlo Astrios is installed and should not require alignment on a daily basis. If the instrument is unable to detect any signal, or if it is displaying a high event rate when no sample is flowing, the FSC detector may require cleaning or alignment.

1. Press the Trigger parameter selector and choose 488-710/45.

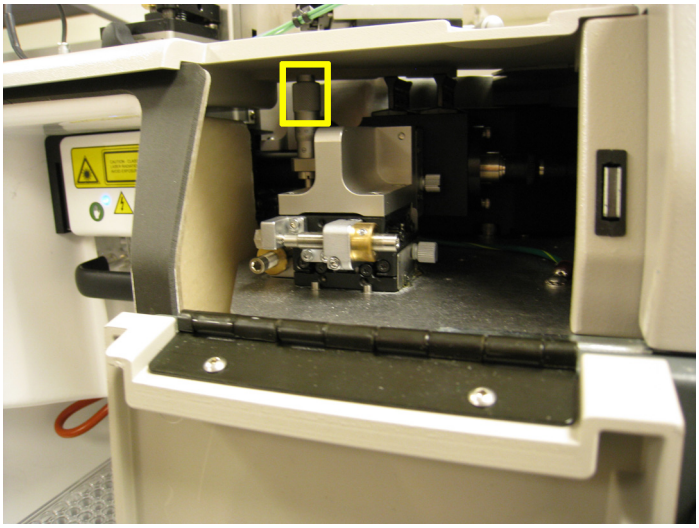
2. Set the FSC gain to 10.

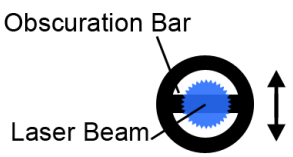
3. Set the Touch Screen parameters to 488-FSC (H) and another optimized parameter such as 355-448/59 (H). Create an identical dot plot in Summit.

4. Ensure that Drop Drive is turned on.

5. Load a tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles diluted 1:10 with deiionized water. Run the sample at 500-1000 EPS.

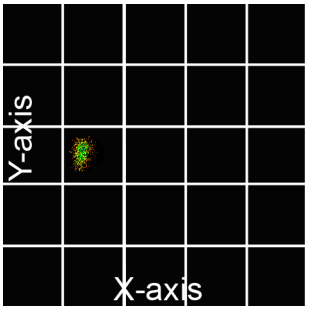
6. The FSC micrometers are located under a door on the right side of the Illumination Chamber. Adjust only vertical positioning. This changes the position of the FSC obscuration bar relative to the laser light.

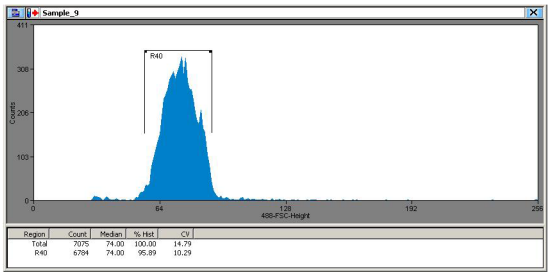




7 Adjust the FSC vertical positioning micrometer to achieve the minimum signal, indicated by local minima.

8 Adjust the FSC vertical positioning micrometer to achieve optimum signal. This is usually slightly off of the minimum signal when the population appears as compact as possible. Optimize the %CV in Summit. Set the median to 64 and adjust the Gain (in the range of 10-20) to move the population to the median.





9 View the current EPS and take note of the value.

10 Set the Trigger to 488-FSC, set the Threshold to 10 percent and verify that the EPS rate remains the same. This step is to verify that the instrument is detecting little or no noise at the current threshold setting.

11 To further optimize the threshold setting, turn OFF the sample flow and take note of EPS. Lower the threshold and view the EPS. Continue to lower the threshold until the EPS value increases. When the EPS value increases, the threshold setting is too low. Finally, set the threshold at the lowest level that prevents the instrument from detecting noise.

NOTE When forward scatter is aligned and sample is not running, the EPS value should be less than 10.

PMT and Filter Alignment

PMTs, filters, and mirrors are located in the PODs, which are in the lower-right side of the cabinet. See CHAPTER 2, *Precision Optical Detector (POD)*. The signal obtained from each PMT may need to be optimized periodically. The filter sets must be aligned and optimized if filters are moved or PMTs are added.

NOTE This procedure should be performed as needed rather than daily.

⚠ CAUTION

It is necessary to bend in order to reach the lower PODs while operating the Touch Screen Control Panel at the same time. Use careful body positioning to avoid strain, and be aware of the table top edge when you return to standing.

PMT Alignment Procedure

1. Identify the correct POD for the laser line Figure 2.10, unlock the POD, and swing the POD forward out of the cabinet.
2. Looking down at the POD identify the PMT and filters to be adjusted.
3. On the Fine Alignment screen plot the parameter to be adjusted vs. another parameter on the same POD.
4. Load a tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles diluted 1:10 with deionized water. Press the Start Sample button.
5. Gently loosen the knob on the PMT holder just until the PMT can rotate inside the holder.
6. Slowly rotate and change the height of the PMT while watching the bead population on the dot plot and maximize the signal strength.
7. Tighten the knob to secure the PMT in place.
8. Rerun Automatic QC if desired.

Filter Alignment Theory

Consider the following guidelines when aligning and optimizing filter sets within the PODs.

IMPORTANT If you change filter, mirror, or PMT positions the instrument will not record the information until you change the information on the Touch Screen PMT and Filter Update Screen. See CHAPTER 3, *PMT and Filter Update Screen*.

- Remove all dichroics and filters, except for the band pass filters and the mirror or filter you are aligning. Additional mirrors and filters will be added and adjusted as you go.
- Dichroic Filters are optimized based on the relationship between transmitted verses reflected light in a two-parameter plot.
- Mirrors are optimized based on the forward scatter parameter (or any optimized parameter) and the parameter to which the light is reflected.

General Filter Alignment Procedure

1. Remove all dichroic filters and mirrors from the POD. Alternately, if you are troubleshooting one parameter that failed QC, remove only the relevant filter.

2. Ensure that PMTs are placed in the POD to collect the parameters you are optimizing.

3. Viewing the applicable Filter Alignment Diagrams place the first mirror or dichroic on the near end of the straight-through path closest to the laser beam entry.

4. Create a dot plot on the Touch Screen Control panel (or in Summit) to view the parameters you are aligning.

5. View the dot plot as you adjust the filter or mirror. When the population on the graph is maximized for intensity and the %CV is reduced as much as possible, the filter is optimized.

   NOTE Touch up may be necessary when filter alignment is complete. This varies by configuration.

6. Viewing the filter layout diagram, place the next filter in the POD, ensure that two PMTs are available to collect the signal, create a dot plot, and optimize the signal. Continue in this fashion until the filters and mirrors are optimized.

   NOTE Mirrors can be optimized relative to the FSC parameter or to another optimized parameter. Dichroics should be optimized relative to the two parameters they affect.

Filter Changes and Future Laser Wavelengths

Filter Changes — The MoFlo Astrios filter set is designed to optimize emitted light while reducing compensation for each laser path. We recommend any changes to the standard filter configuration or addition of custom filters be carefully evaluated by the operator prior to use.

Future Laser Wavelengths — The Astrios filter sets and instrument are designed for the standard laser wavelengths offered. Any future additions of wavelengths may require filter changes to optimize performance.

Filter Alignment Diagrams

Standard filter sets are provided with each laser on the system. The following diagrams illustrate how the filters are organized in the PODs. See CHAPTER 2, *Precision Optical Detector (POD)* for a visual description of which POD is associated with which laser.

Figure 10.7 355 nm Laser, Filter Diagram

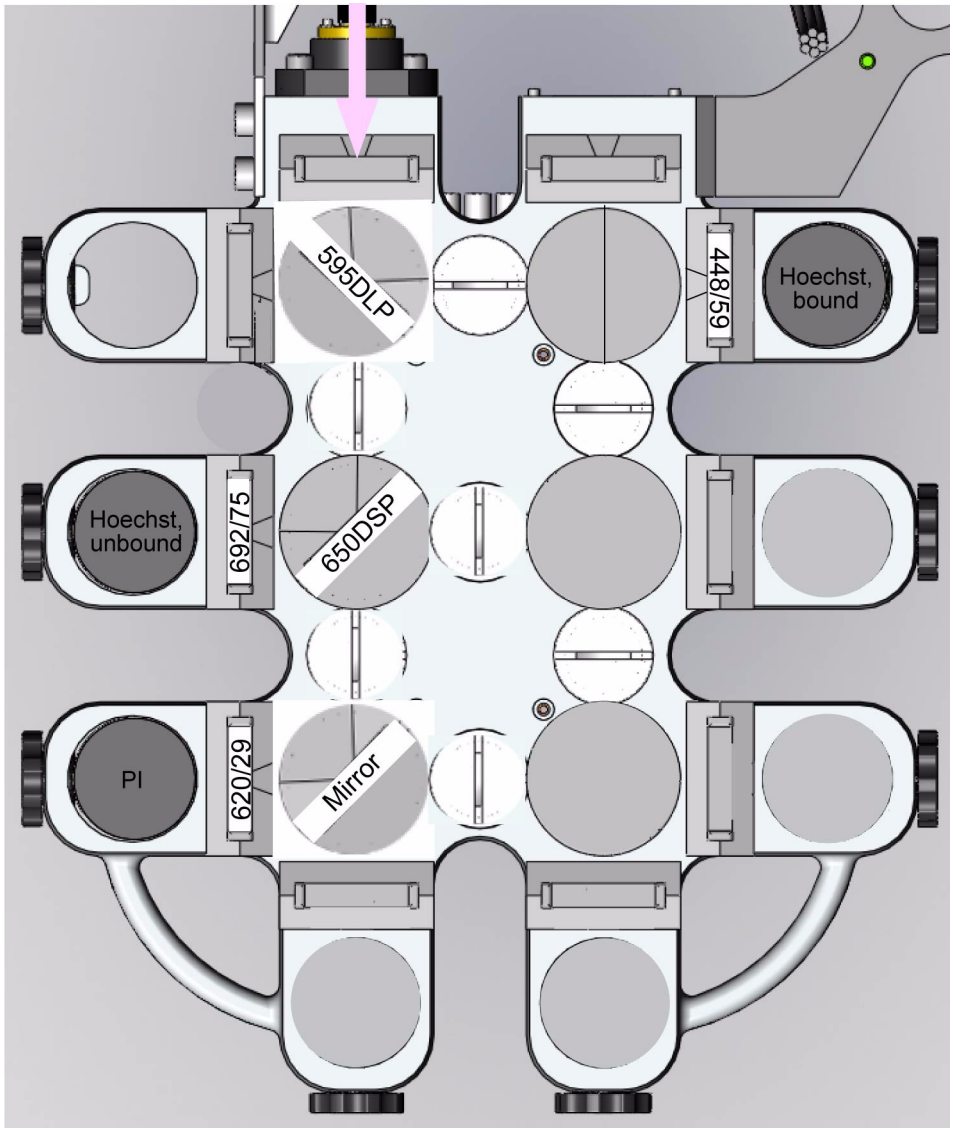


Figure 10.8 405 nm Laser, Filter Diagram

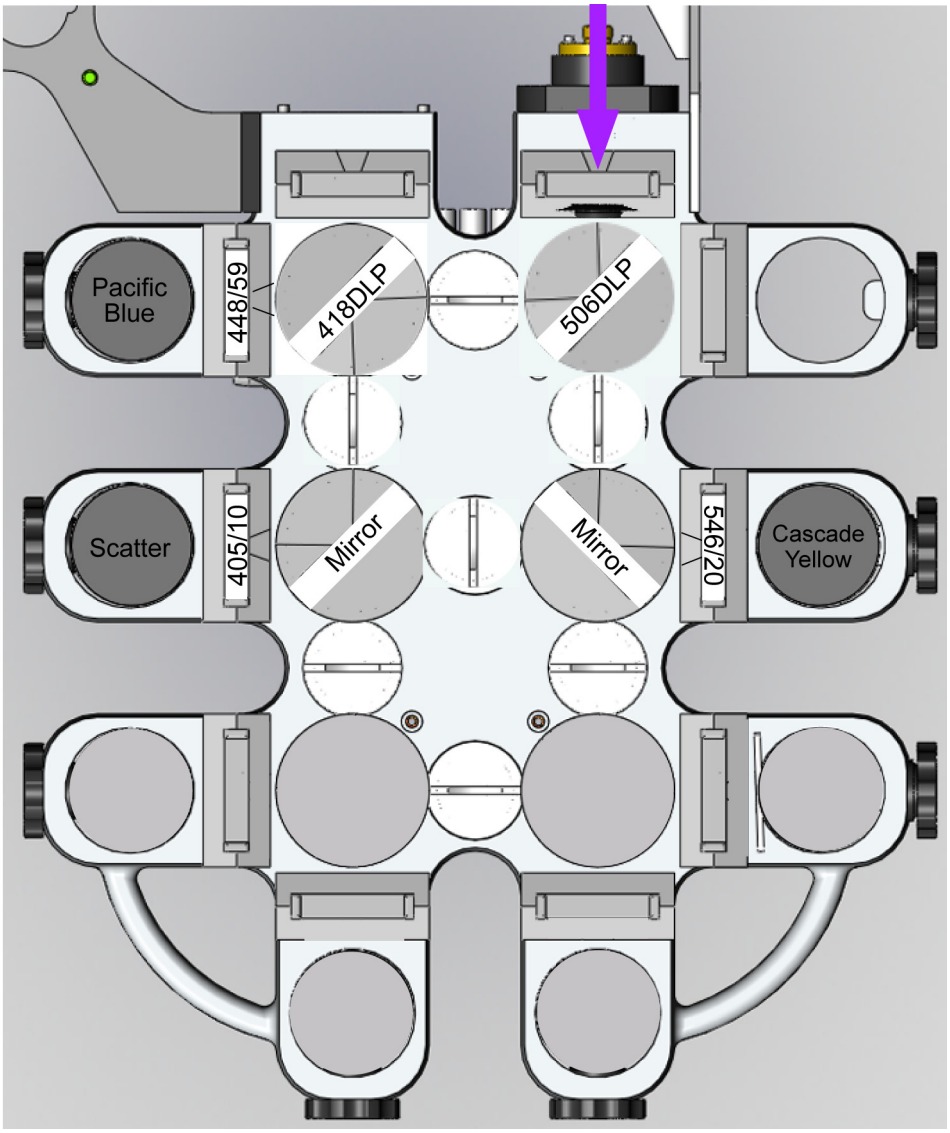


Figure 10.9 488 nm Laser, Filter Diagram

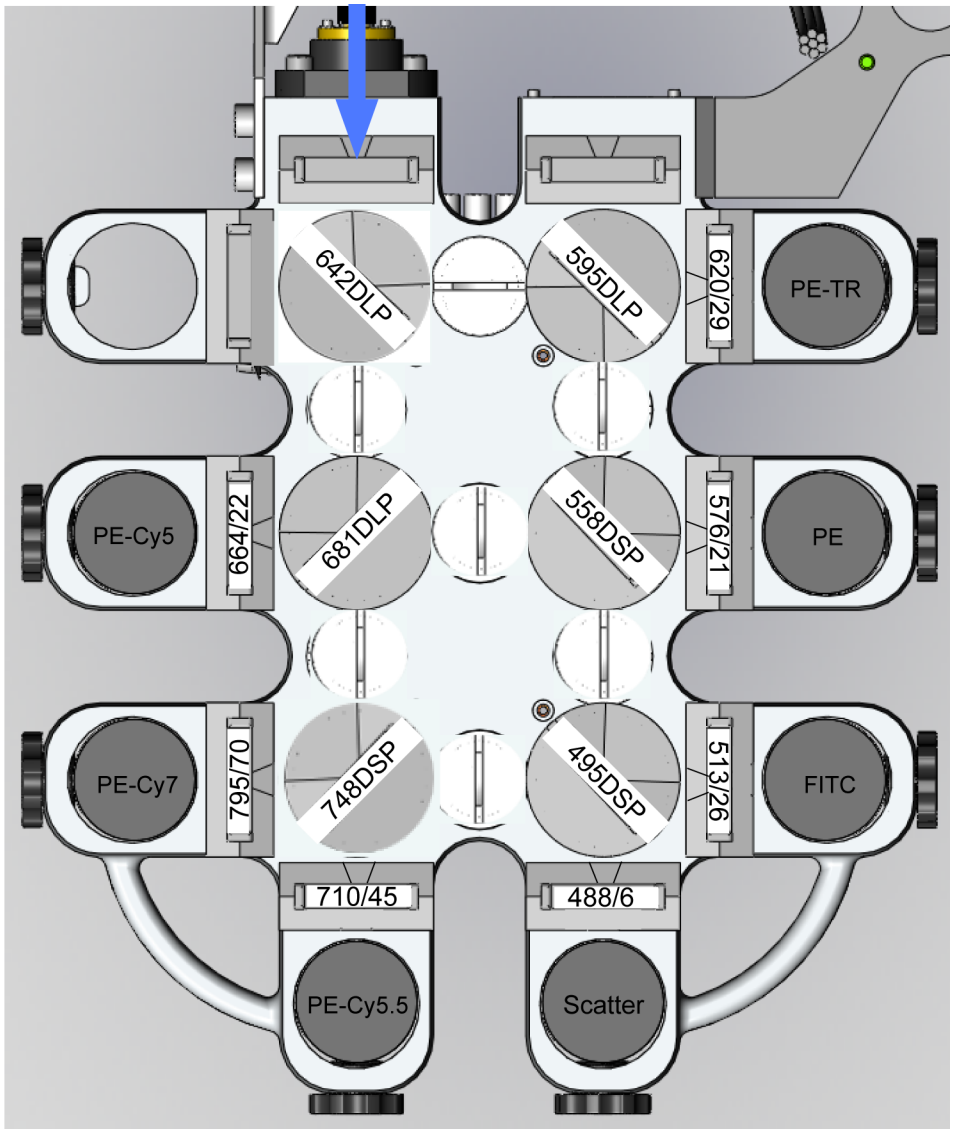


Figure 10.10 532 nm Laser, Filter Diagram

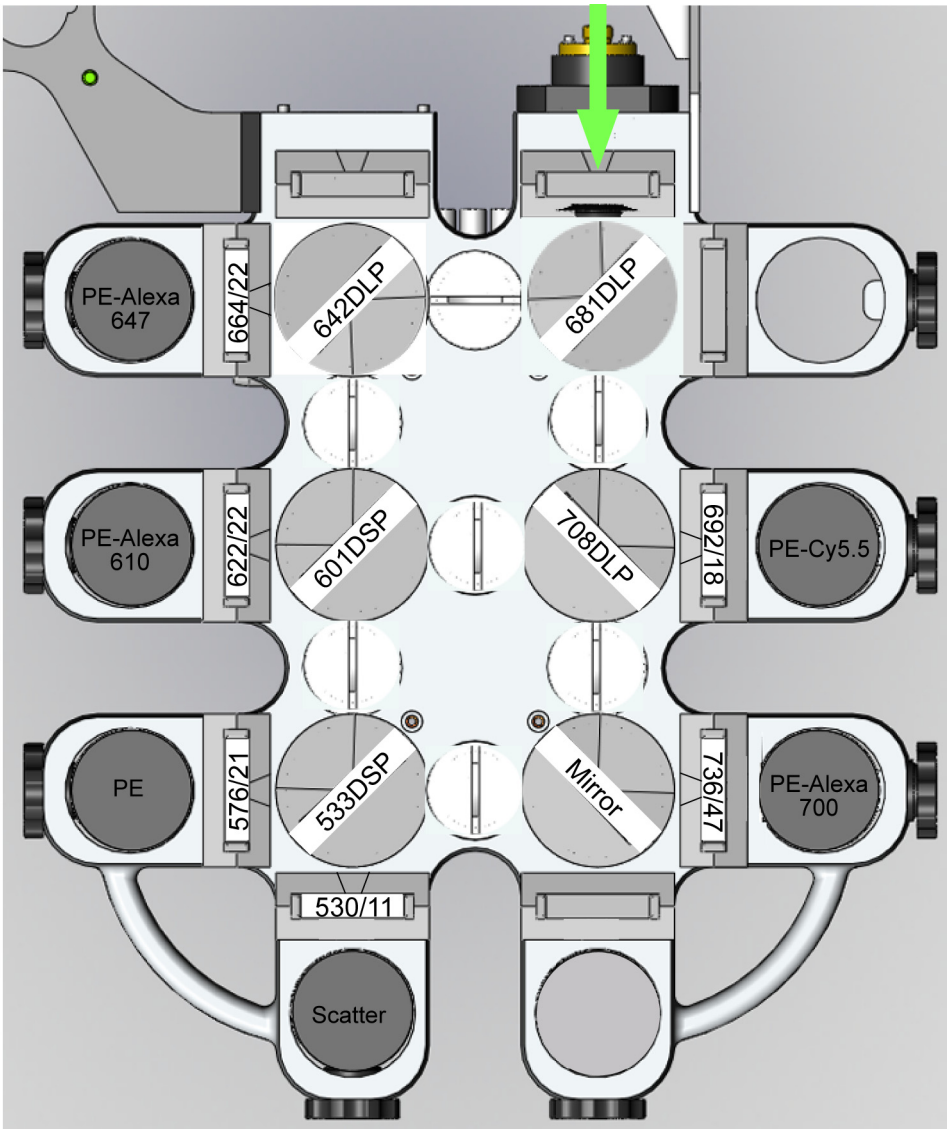


Figure 10.11 561 nm Laser, Filter Diagram

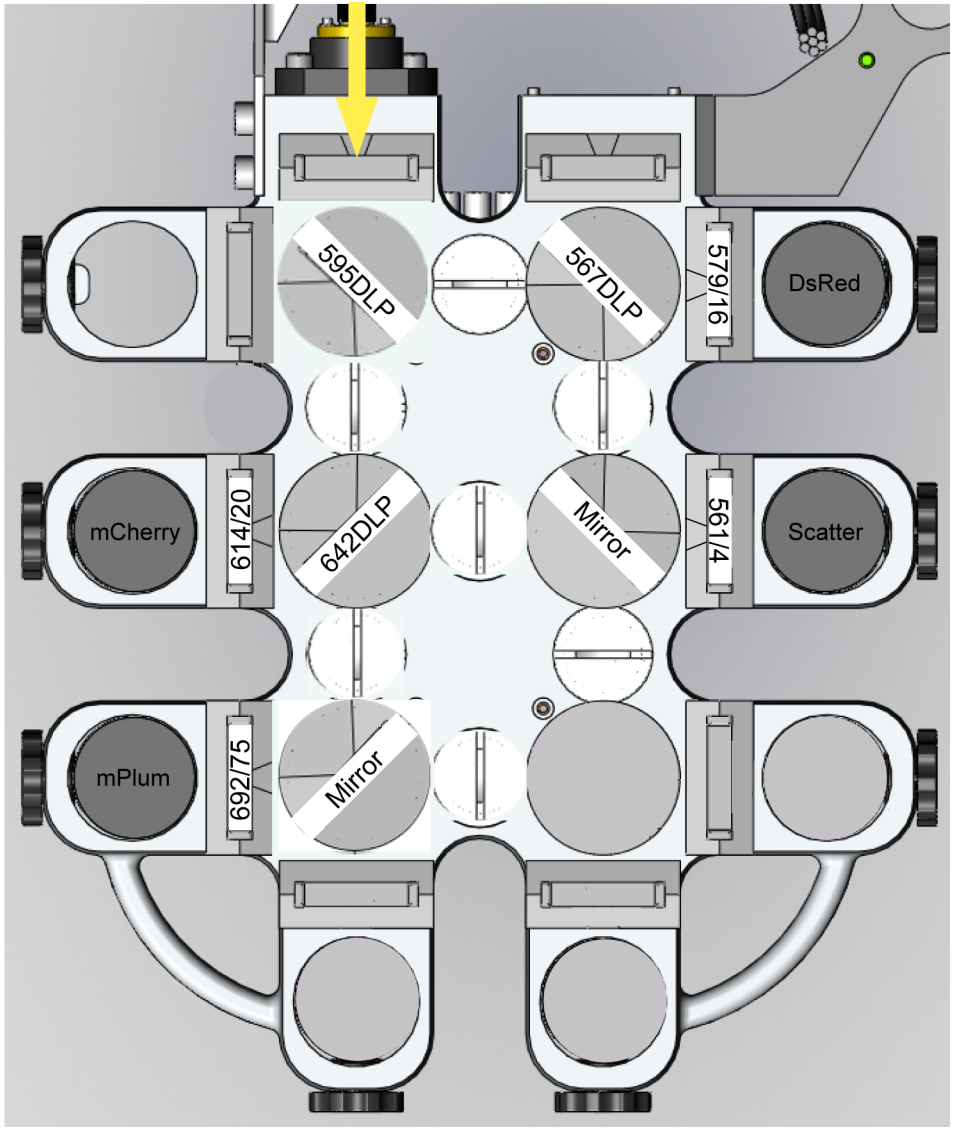


Figure 10.12 592 nm Laser, Filter Diagram

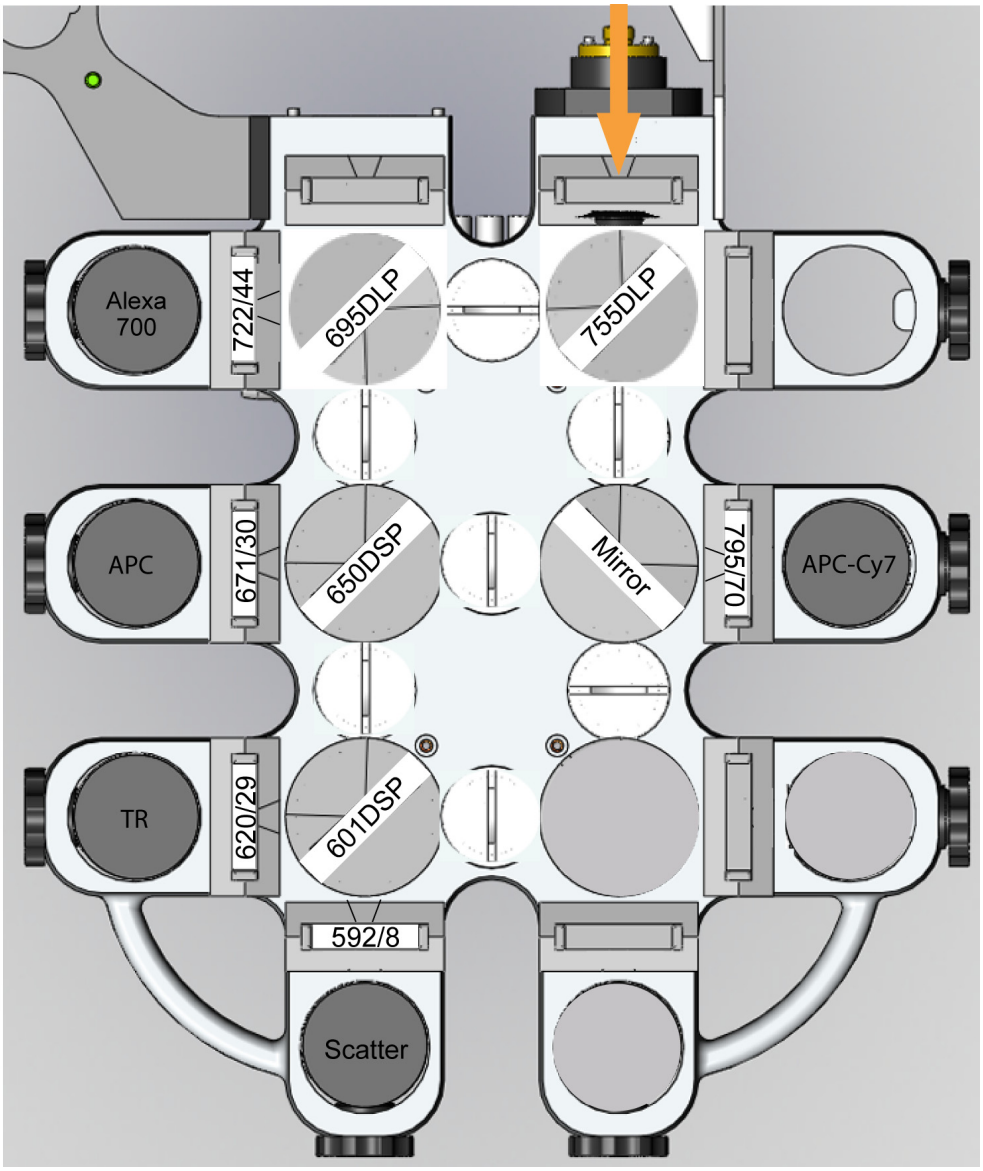
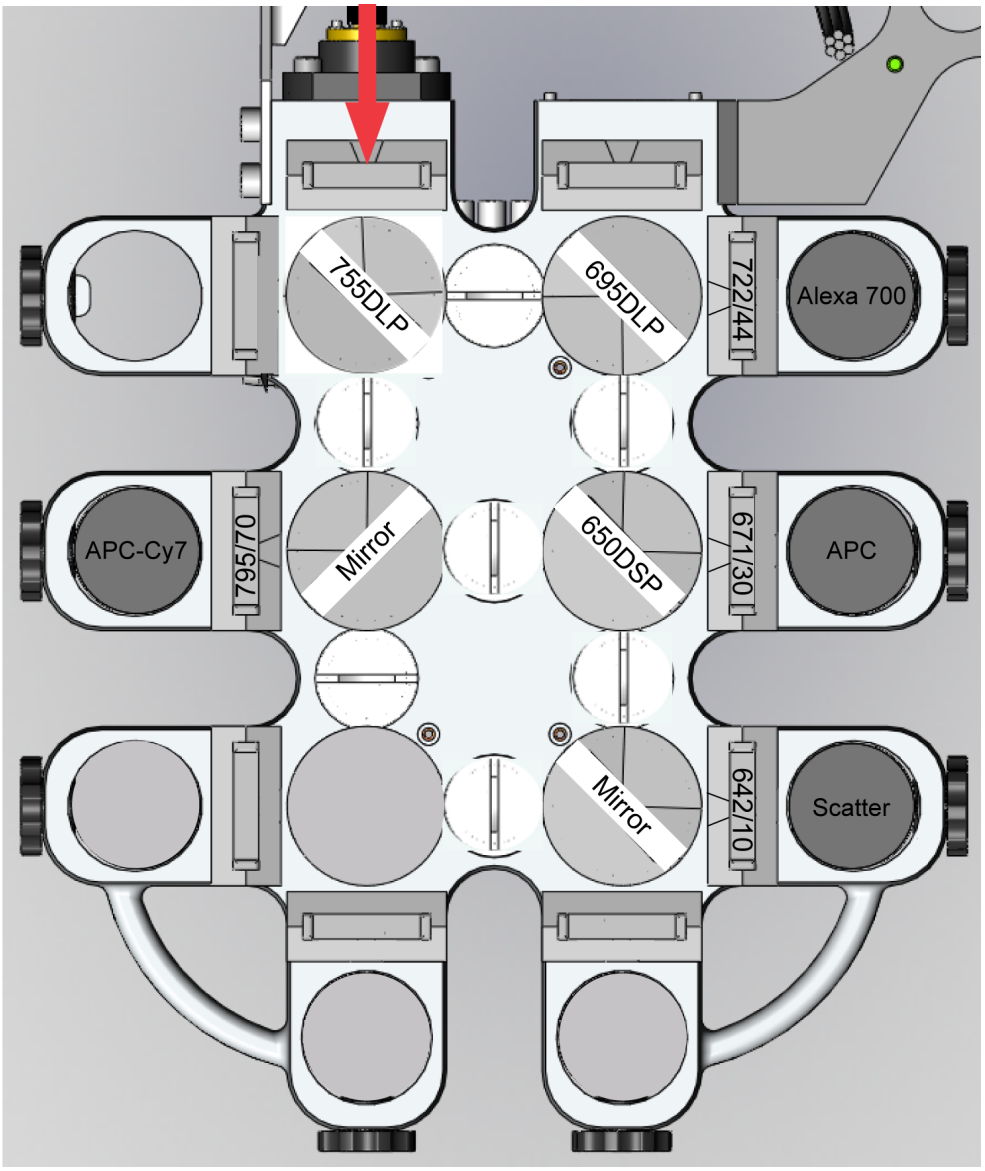


Figure 10.13 640 nm Laser, Filter Diagram



Edit Mode - Changing PMTs and Filters

The PMT and Filter Update screen allows you to change filter and PMT information and then store the information so the system can recognize the new configuration.

IMPORTANT Never physically change the PMT configuration of a POD without first disabling power to the PMTs by pressing the PMT Power ON/OFF button. (See number 5 in Figure 3.15.)

1 On the Touch Screen PMT and Filter Update screen, press the PMT Power ON/OFF button to disable power to all the PMTs in all the PODs and enter Edit Mode.

2 Once power has been disabled, move PMTs and change filters as desired.

3 On the Touch Screen, press the corresponding PMT or filter and change the information to reflect the new instrument configuration.

Make changes on the Touch Screen for each physical change you made to the PODs.

4 Press the Power ON/OFF button again. The system scans to detect new PMT locations and loads filter information into memory. This enables the system to recognize the new PMT and filter configuration.

Edit Mode - Designating a Forward Scatter Laser and Filter

The PMT and Filter Update screen allows you to change the laser designated to detect Forward Scatter and then store the information so the system can recognize the new configuration.

IMPORTANT You cannot enter Edit Mode without first disabling power to the PMTs by pressing the PMT Power ON/OFF button. (See number 5 in Figure 3.15.)

1 On the Touch Screen PMT and Filter Update screen, press the PMT Power ON/OFF button to disable power to all the PMTs in all the PODs. and enter Edit Mode.

2 Press the arrow on the **FSC Laser** list box to access the laser choices.

3 Select the desired laser.

4 Open the door that covers the Forward Scatter Collection Objective Figure 2.7.

5 Replace the Forward Scatter Filter Figure 2.8 with a filter that corresponds to the laser you selected in step 3.

6 Press the Power ON/OFF button again. The system scans to detect new information and loads it into memory.

Background Image Subtraction

It is necessary for the Background Image Subtraction procedure to be performed before the Laser Spot is determined, and before IntelliSort is set up. This procedure allows IntelliSort to discern relevant stream image information and remove background optical artifacts detected by the camera. A Beckman Coulter Representative will perform Background Image Subtraction during instrument installation.

NOTE This procedure does not need to be performed every time IntelliSort is used, but may need to be repeated periodically depending on system performance.

NOTE The two error notices below indicate that IntelliSort could not complete initialization, and you must perform Background Image Subtraction.

- Error - Could not calibrate microns per pixel.
- Error - Width data is too wide.

Background Image Subtraction Procedure

1 Start the MoFlo Astrios.

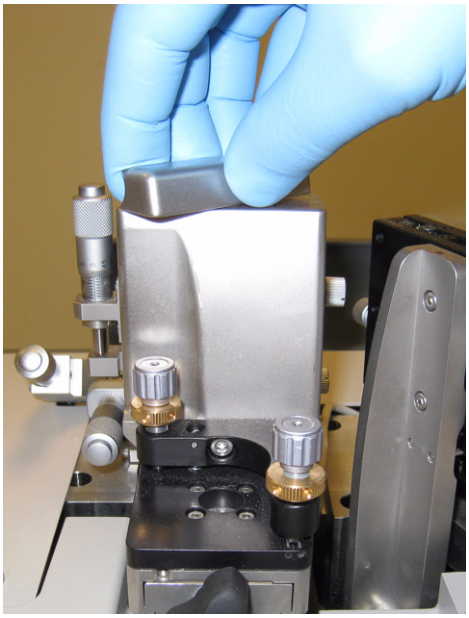
2 Ensure that the nozzle size is set to 70 µm or 100 µm depending on the nozzle that is installed.

3 Press the camera button. After an approximate 30-second pause, a message states that the stream is about to be shutdown.

4 Press OK to turn the stream off.

5 Wait for the stream to completely stop.

6 Turn the knob on the nozzle stage and raise the nozzle.



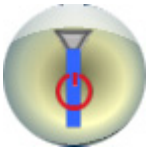
7 After the stream and nozzle are no longer in view, press the button on the dialog box to begin background image subtraction.

⚠ WARNING

Risk of personal injury. When lowering the nozzle stage, you can pinch your fingers between the bottom of the stage and the instrument frame. Lower the nozzle stage using the upper portion of the stage to avoid pinching points.

8 Lower the nozzle stage back into the instrument and lock it in place.

9 Turn the sheath stream back on.



10 Proceed to CHAPTER 6, *Laser Spot Determination*.

QC Setup Tool

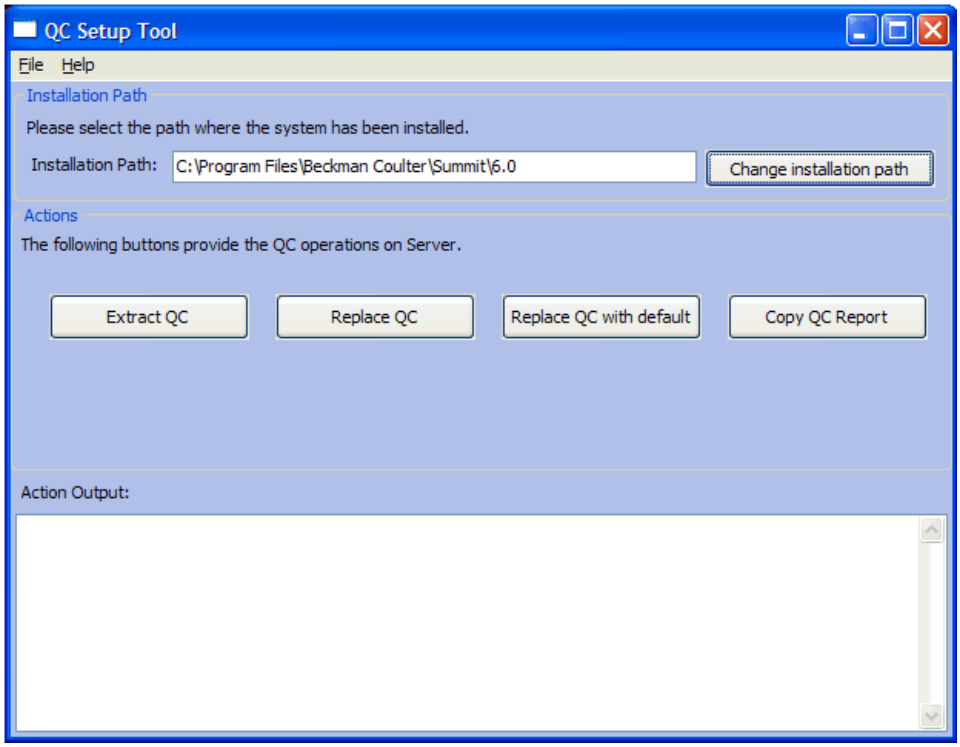
The QC Setup Tool contains the information used to evaluate the instrument during the Quality Control procedure and is used to determine what constitutes a passing or failing value for each detector.

IMPORTANT This file should be edited when the system receives new lasers or filters, or when the bead lot changes.

Accessing the QC Setup Tool

1 On the Summit workstation click **Start > Programs > Beckman Coulter > Tools > QC Tools.**

Figure 10.14 QC Setup Tool



Extract QC button – Pulls the current QC criteria from the instrument and allows you to save it in a spreadsheet.

Replace QC – Applies the changes you have made and saved in the spreadsheet to the instrument.

Replace QC with default – Restores the QC criteria on the instrument with the original factory values.

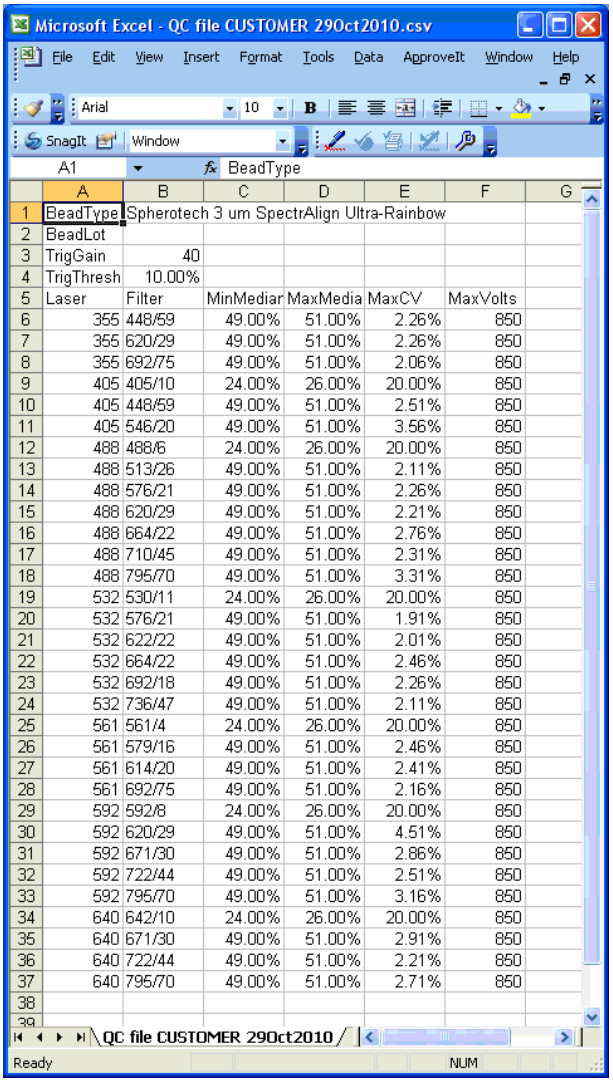
Copy QC Report – Displays QC procedure results.

2 Click the Extract QC button. You are prompted to save the information as a CSV file.

3 After the CSV file is saved, you can open and edit the file.

NOTE Read the rules listed below Figure 10.15 before attempting to edit the spreadsheet you saved.

Figure 10.15 Example of a QC Criteria File Spreadsheet



	A	B	C	D	E	F	G
1	BeadType	Spherotech 3 um SpectrAlign Ultra-Rainbow					
2	BeadLot						
3	TrigGain	40					
4	TrigThresh	10.00%					
5	Laser	Filter	MinMediar	MaxMedia	MaxCV	MaxVolts	
6	355	448/59	49.00%	51.00%	2.26%	850	
7	355	620/29	49.00%	51.00%	2.26%	850	
8	355	692/75	49.00%	51.00%	2.06%	850	
9	405	405/10	24.00%	26.00%	20.00%	850	
10	405	448/59	49.00%	51.00%	2.51%	850	
11	405	546/20	49.00%	51.00%	3.56%	850	
12	488	488/6	24.00%	26.00%	20.00%	850	
13	488	513/26	49.00%	51.00%	2.11%	850	
14	488	576/21	49.00%	51.00%	2.26%	850	
15	488	620/29	49.00%	51.00%	2.21%	850	
16	488	664/22	49.00%	51.00%	2.76%	850	
17	488	710/45	49.00%	51.00%	2.31%	850	
18	488	795/70	49.00%	51.00%	3.31%	850	
19	532	530/11	24.00%	26.00%	20.00%	850	
20	532	576/21	49.00%	51.00%	1.91%	850	
21	532	622/22	49.00%	51.00%	2.01%	850	
22	532	664/22	49.00%	51.00%	2.46%	850	
23	532	692/18	49.00%	51.00%	2.26%	850	
24	532	736/47	49.00%	51.00%	2.11%	850	
25	561	561/4	24.00%	26.00%	20.00%	850	
26	561	579/16	49.00%	51.00%	2.46%	850	
27	561	614/20	49.00%	51.00%	2.41%	850	
28	561	692/75	49.00%	51.00%	2.16%	850	
29	592	592/8	24.00%	26.00%	20.00%	850	
30	592	620/29	49.00%	51.00%	4.51%	850	
31	592	671/30	49.00%	51.00%	2.86%	850	
32	592	722/44	49.00%	51.00%	2.51%	850	
33	592	795/70	49.00%	51.00%	3.16%	850	
34	640	642/10	24.00%	26.00%	20.00%	850	
35	640	671/30	49.00%	51.00%	2.91%	850	
36	640	722/44	49.00%	51.00%	2.21%	850	
37	640	795/70	49.00%	51.00%	2.71%	850	
38							

NOTE Data populating this spreadsheet consist of current QC criteria that includes the changes your laboratory made and saved to the instrument. It does not reflect instrument specifications.

- Bead Type is a simple string describing the type of beads used for the QC process.
- Bead Lot is a simple string with the lot information about the beads used for the QC process.
- Trigger Gain - not used at this time.
- Trigger Threshold - not used at this time.
- The first four rows of column A should not be changed by the user.
- Row five should not be changed by the user.
- No blank rows are permitted from the first row to the end.
- All columns after column F must be blank.

- Each row from row six through the last row corresponds to a detector that may or may not be on the system. Each of these rows must have a non-blank value present in all fields. For the columns holding percentages, the value must be expressed either with a trailing % character or as a decimal fraction such as 0.20.
- The MinMedian and MaxMedian columns contain a percentage of the range of values (channels 0 through 256) between which the median of the population should lie.
- The MaxCV is the maximum coefficient of variance percentage that is allowed for the detector when the population median is within its range.
- The MaxVoltage is the maximum allowed voltage to which the detector may be set when the population median is within its range.

4 Save the changes to the spreadsheet.

5 To apply the new QC criteria to the instrument, click the **Replace QC** button.

Problem/Solution Tables

The following tables are a guide for troubleshooting MoFlo Astrios problems. If in doubt, call your Beckman Coulter Representative.

Table 10.1 General Troubleshooting

Problem	Possible Cause	Possible Solution
Poor CVs on the fluorescence parameters.	Sample event rate too high.	Lower the sample pressure.
	Photo damaged beads.	Prepare a new sample.
	Dirty or faulty optical mirrors or dichroics.	Clean, align, or replace the mirrors.
	Dirty, misaligned, or faulty PMT.	Clean, align, or replace the PMT.
	Poor sample injection: • Clogged or partially clogged nozzle tip. • Inadequate stream verticality. • Dirty or clogged sample injection needle on the nozzle.	Perform the procedure to unclog the nozzle. Replace the nozzle.
	Poor stream alignment.	Realign the stream.
	Dirty objective lens, or FBSO.	Clean the lens, or FBSO window.
	Poor laser beam focus. (Dirty, scratched, or faulty FBSO).	Clean or change the FBSO components.
	Poor laser alignment.	Realign the lasers.
	Poor laser beam quality.	Realign the laser or replace the laser.
Poor CVs on the FSC parameter.	Poor sample injection: • Dirty or clogged sample injection needle on the nozzle. • Clogged or partially clogged nozzle tip. • Inadequate stream verticality.	Perform the procedure to unclog the nozzle, or replace the nozzle.
	Sample event rate too high.	Lower the sample pressure.
	Misaligned or dirty FSC detector.	Clean and align the FSC detector.
	Faulty nozzle assembly.	Replace the nozzle.

Table 10.1 General Troubleshooting *(Continued)*

Problem	Possible Cause	Possible Solution
Unstable droplet.	Air bubbles in the sheath lines or nozzle.	Remove trapped air. See page 10-33.
	Partially clogged nozzle.	Clean the nozzle. See page 10-34.
	Fluidic leaks including sheath tank, sheath filter, sample handling station, and the fittings up to the nozzle.	Check for leaks and tighten fittings.
	Faulty nozzle.	Replace the nozzle.
	Air leaks inside the pressure console.	Call Technical Support.
Unstable sample event rate.	Air leaks inside the pressure console.	Call Technical Support.
	Air leaks inside the sample handling station.	Call Technical Support.
	Air leaks inside the fittings and tubing between the sample handling station and the nozzle.	Call Technical Support.
	Partial nozzle clog.	Clean the nozzle. See page 10-34.
	Sample is settling in tube.	Agitate the sample and reacquire.
Unstable side streams.	Result of unstable droplets.	See the *Unstable Droplets* category above.
	Wet high-voltage plates.	Turn of the plate voltage and dry the plates. See the complete procedure in *Deflection Plate Arcing* in the Safety section.
No signal on a particular parameter.	Faulty PMT.	Isolate the PMT (change out if possible).
	PMT power or signal cable is unplugged.	Plug in PMT.
	PMT is misaligned.	Gently adjust the PMT within its bracket while viewing a histogram for that parameter.
	Dichroic mirror in front of the PMT is misaligned.	Realign Dichroic filter.

Table 10.1 General Troubleshooting *(Continued)*

Problem	Possible Cause	Possible Solution
Histograms show excessive noise.	Obscuration bar is not the optimum size.	Change the obscuration bars on the FSC and/or SSC collection optics to better suit your application.
	The threshold is not adjusted correctly.	Adjust the threshold setting on the Touch Screen Control Panel Fine Alignment screen.
	Microbial contamination.	Decontaminate the system. See page 9-4.
	Drop Drive amplitude is set too high.	Reduce Drop Drive amplitude.
Event rate equals drop delay frequency.	Bad vertical alignment of FSC detector or nozzle.	Adjust the vertical alignment of the nozzle stage.
	Nozzle body is too high.	Adjust the vertical alignment of either the nozzle stage.
Bead Flash is present but Count is not present. (Event Rate = 0).	The system electronics are not triggering.	Adjust the threshold setting on the Touch Screen Control Panel Fine Alignment screen.
	Threshold is too high.	Lower threshold to 2-5%.
	Faulty photodiode or PMT.	Trigger off a different parameter and/or replace photodiode or PMT.
	Trigger set to parameter of a laser that is not turned on.	Trigger off of a different parameter.
Bead Flash present, the Counts on ACP are fine, but the Summit Software histogram shows no events (Summit Software Event Rate = 0).	Incorrect gating through Summit software.	Delete all gates on all histograms.
	Poor communication between Summit software and Astrios server.	Restart Summit Software.
Summit Software does not display sorting options.	The offline version of Summit Software is installed.	Verify that the version of Summit Software is an online version.
	An offline database was selected when Summit was started.	Close Summit and open an online database.
No vacuum on the waste tank.	Vacuum source is turned off.	Verify the vacuum source is turned on.
	Waste tank lid is not tight enough.	Tighten the waste tank lid. Verify that the O-ring is intact and is lubricated with vacuum grease.
	Vacuum switch on side of pressure console turned off.	Enable vacuum to the system using the pressure console switch.

Table 10.2 SmartSampler Troubleshooting

Problem	Possible Cause	Possible Solution
Sheath is backflushed into sample tube while in **Run** mode.	Sample pressure is lower than sheath pressure.	Increase sample differential setting on pressure console.
Sheath fluid spilling out of waste tube.	Insufficient vacuum.	Check waste tank gauge (must be at least 440 mbar (13 in. Hg).
	Salt crystals have built up inside waste tank quick connect fitting.	Disconnect the waste tank quick connect at the red tubing line, hold connector upside down and tap on a solid surface until salt crystals are removed.
Probe strikes side of tube during agitation.	Bent probe.	Replace sample probe.
O-ring that seals chamber to the SmartSampler base is dislodged.	System pressure not at least 20 psi above sample pressure.	Increase air pressure to the instrument to 517 or 138 KPa (85 or 20 psi) overpressure, whichever is greater.
Chamber opens only part way.	Wiper catch pan O-ring not properly lubricated.	Call Technical Support.
	Insufficient air flow at the low-pressure regulator.	Call Technical Support.
Discontinuous sample flow - sudden fluctuation in Event Rate or Trigger Rate not observed during Boost.	Inadequate sample volume.	Increase sample volume.
	Sample is clumping.	Agitate sample.
	Nozzle is clogged.	Debubble.
		Unclog.
	Sample tubing is clogged.	Replace sample tubing.
Agitation not working properly; stops for a long period of time or binds up.	Weight on motor not properly attached or sliding off of motor shaft.	Call Technical Support.

Inspecting for Trapped Air

It is very important that the fluidics system is air-free before proceeding with alignment and sorting. To check for presence of air in the system perform the Droplet Stability test that follows.

Droplet Stability Test

The condition of the fluidics system can be determined by observing the location of the last attached drop on the Touch Screen Control Panel Droplet Control screen. The following procedure can be used:

1. Select the Touch Screen Control Panel Droplet Control screen. Make sure the sheath stream is flowing but the sample stream is not.

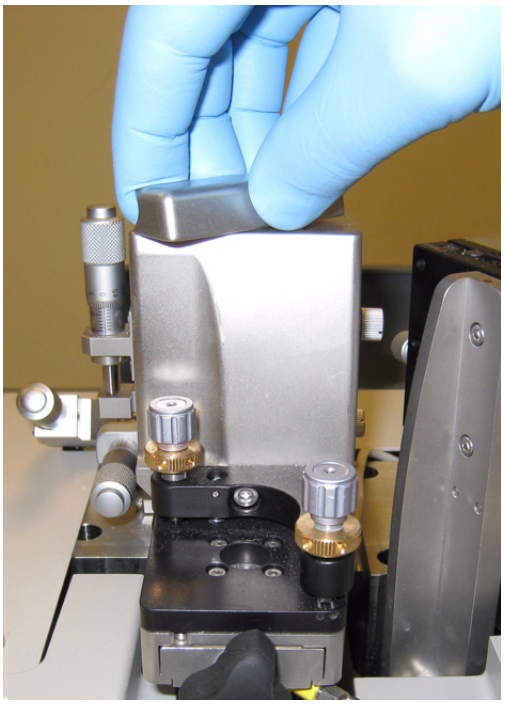
2. Make sure the Drop Drive Amplitude is on so that you are making droplets and can see them on the screen.

3. Using arrows on the Droplet Control screen move the red line to denote the exact position of a droplet (use the last attached droplet if it is visible).

4. Press the SmartSampler Debubble button.
   - If the fluidics system is free of air, the droplets will snap back to the same location as you marked on the stream. Air in the system will cause the droplets to relocate on the screen and possibly appear to undulate.
   - If it appears that there is air in the nozzle, return to the Debubbling procedure and then test for air again. It is very important that the fluidics system is air-free before proceeding with alignment.

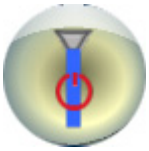
Clearing Nozzle Blockages

1 Turn the knob on the nozzle stage and raise the nozzle.



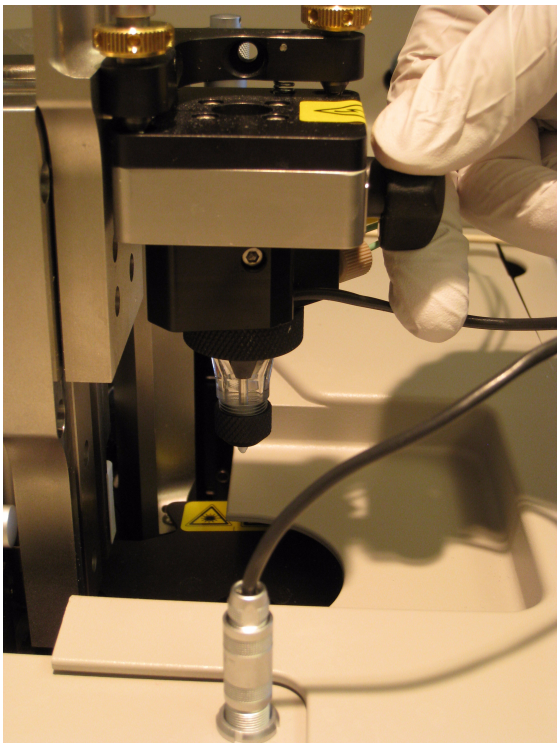
2 Hold a cap of 2000 ppm active chlorine bleach solution or 70% ethanol to the nozzle tip and press the Unclog button on the Touch Screen. If the tip is not clear, proceed to the next step.

3 Press the Start Sheath Flow button to stop the sheath stream.



4 Unplug the nozzle stage by separating the Lemo® connector to the left of the nozzle stage.

5 Loosen the knob on the front of the nozzle body and then pull the nozzle body out from the stage.



⚠ WARNING

Risk of personal injury. When removing the nozzle tip, the exposed injection tube can puncture your skin. Do not touch the exposed injection tube.

6 Turn the nozzle upside down and unscrew the nozzle tip retaining ring. Remove the tip from the nozzle and clean it directly with cleaning solution.

7 If the nozzle is not clear, sonicate the nozzle tip for 30 seconds with deionized water.

NOTE Do not touch the nozzle tip. It can become clogged with debris from your hands.

8 Clean the nozzle tip with 10 mL of ethanol through a 10 mL syringe followed by deionized water.

9 Visually inspect the nozzle under magnification for salt crystals or cellular debris. Repeat the previous step if there is any debris present.

10 Using your gloved hands, place a small amount of silicon O-ring grease on the black O-ring of the nozzle tip.

11 Apply a thin coating of O-ring grease to the plastic threads on the nozzle body.

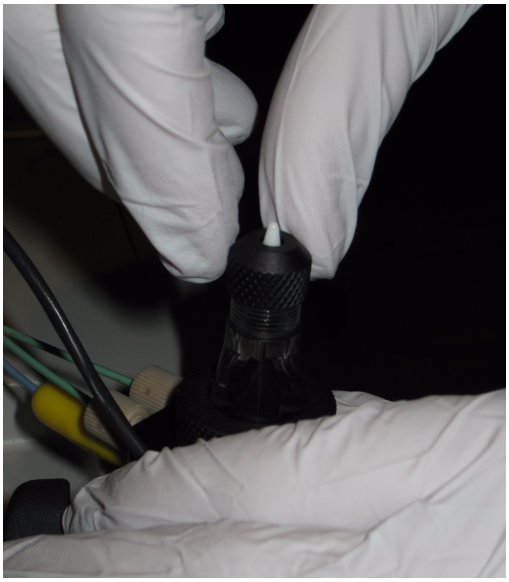
12 Wipe off the excess silicone O-ring grease using a lint-free tissue.

13 Slide the O-ring onto the nozzle tip, placing the O-ring 1/3 of the way up the nozzle tip. Make sure the O-ring is properly seated — not tilted or twisted.

⚠ WARNING

Risk of personal injury. When reinstalling the nozzle tip, the exposed injection tube can puncture your skin. Do not touch the exposed injection tube.

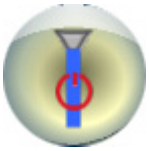
14 Turn the nozzle upside down. Set the nozzle tip into the clear plastic nozzle body and attach the small retaining ring.



15 Finger tighten all parts with smooth, even pressure. Make sure the retaining ring screws smoothly onto the nozzle body.

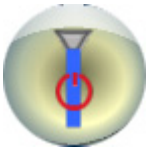
16 Cover the nozzle body with a paper towel.

17 Press the Start Sheath Flow button to start the sheath flowing.

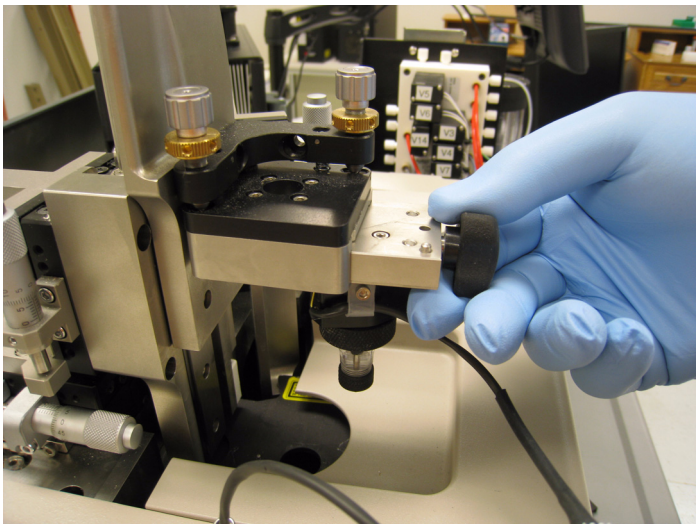


18 If necessary, tap the side of the clear plastic nozzle body gently to remove bubbles.

19 Press the Start Sheath Flow button to stop the sheath stream.



20 Reinstall the nozzle body in the stage.



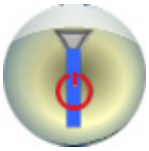
⚠ WARNING

Risk of personal injury. When lowering the nozzle stage, you can pinch your fingers between the bottom of the stage and the instrument frame. Lower the nozzle stage using the upper portion of the stage to avoid pinching points.

21 Lower the nozzle stage back into the instrument and lock it in place.

22 Reconnect the power to the nozzle body (via the Lemo connector).

23 Press the Start Sheath Flow button to start the sheath flowing.



24 Debubble.

25 To determine if a partial clog remains, select the Touch Screen Control Panel Droplet Control screen and turn on the Drop Drive Amplitude to view the droplet stream.

NOTE If the drops are vertically symmetric and regular when the Droplet Camera is panned downward from the position of the last attached drop, the clog has been completely purged. Occasionally, a clog will only be partially removed. A partial clog can be identified by asymmetric and irregular droplets when viewed near the last attached drop. In addition to this asymmetry, a partial clog will generally shorten the position of the last attached drop.

26 Realign the sheath stream.

Replacement Procedures

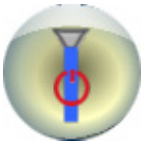
Exchanging the Nozzle Tip

When exchanging one nozzle tip for another, it is essential that all parts are clean and that surface tension is reduced so that bubbles can be quickly eliminated.

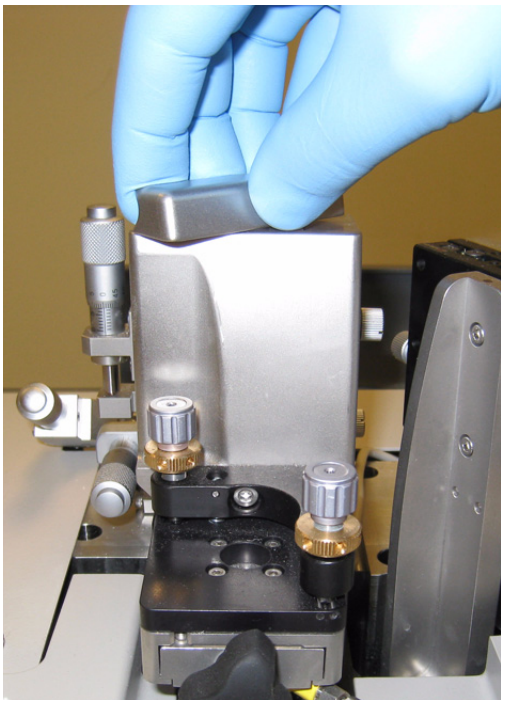
IMPORTANT For best results, wear nitrile lab gloves.

How to Exchange a Nozzle Tip

1 Shut off the sheath flow by pressing the Start Sheath Flow button.

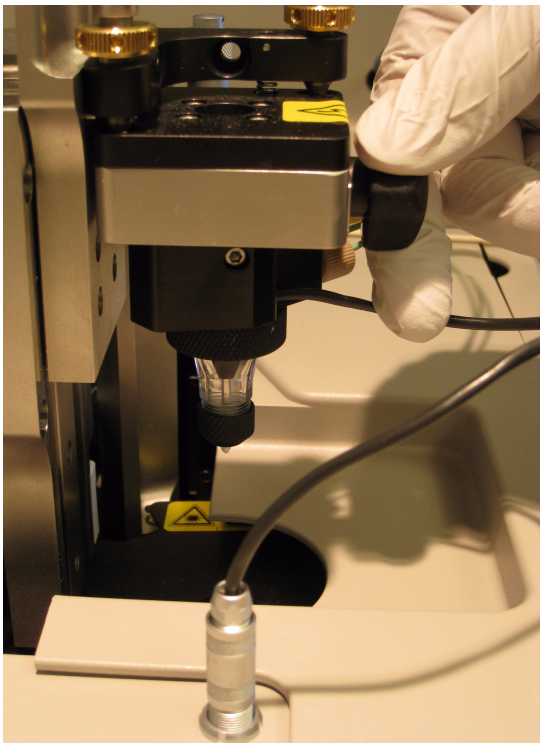


2 Turn the knob on the nozzle stage and raise the nozzle.



3 Unplug the nozzle assembly by separating the Lemo connector to the left of the nozzle stage.

4 Loosen the knob on the front of the nozzle body and then pull the nozzle body out from the stage.



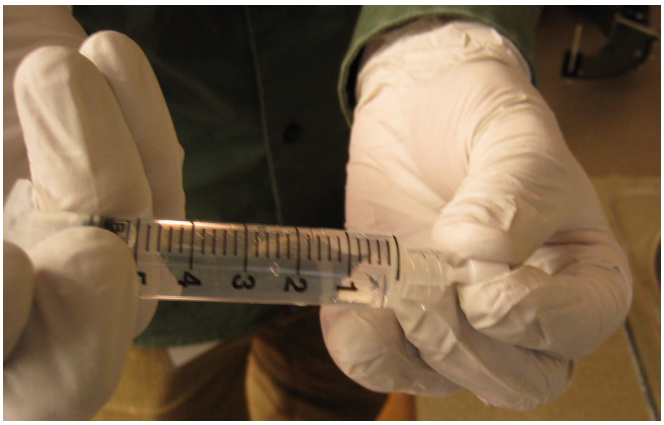
⚠ WARNING

Risk of personal injury. When removing the nozzle tip, the exposed injection tube can puncture your skin. Do not touch the exposed injection tube.

5 Unscrew the nozzle tip from the nozzle body and then store it where it will not be damaged.

6 Get the new nozzle tip and if you have not already done so, put on a pair of nitrile gloves.

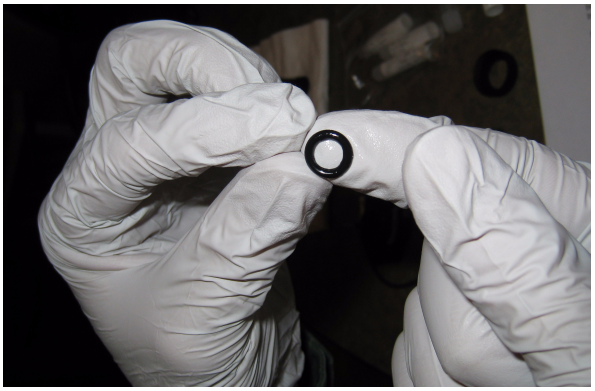
7 Clean the nozzle tip with 10 mL of ethanol through a 10 mL syringe followed by deionized water.



8 Visually inspect the nozzle under magnification for salt crystals or cellular debris. Repeat the previous step if there is any debris present.

9 Remove the black O-ring from the nozzle tip retaining ring.

10 Using your gloved hand, place a small amount of silicon O-ring grease on the black O-ring of the nozzle tip.



11 Apply a thin coating of O-ring grease to the plastic threads on the nozzle body.

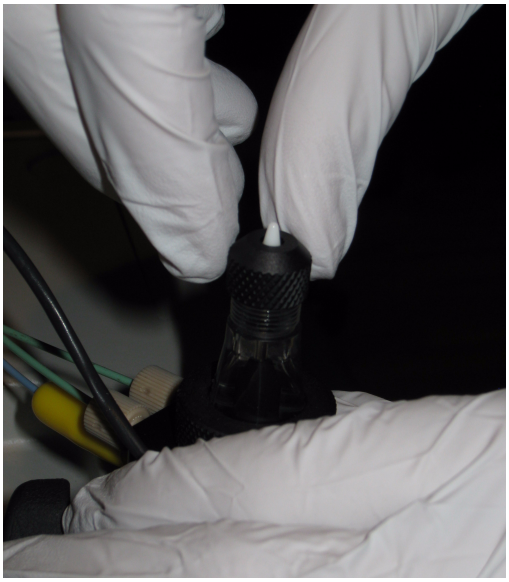
12 Wipe off the excess silicone O-ring grease using a lint-free tissue.

13 Slide the O-ring onto the nozzle tip, placing the O-ring 1/3 of the way up the nozzle tip. Make sure the O-ring is properly seated — not tilted or twisted.

⚠ WARNING

Risk of personal injury. When reinstalling the nozzle tip, the exposed injection tube can puncture your skin. Do not touch the exposed injection tube.

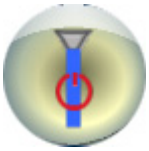
14 Turn the nozzle upside down. Set the nozzle tip into the clear plastic nozzle body and attach the small retaining ring.



15 Finger tighten all parts with smooth, even pressure. Make sure the retaining ring screws smoothly onto the nozzle body.

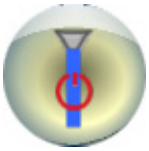
16 Cover the nozzle body with a paper towel.

17 Press the Start Sheath Flow button to start the sheath flowing.

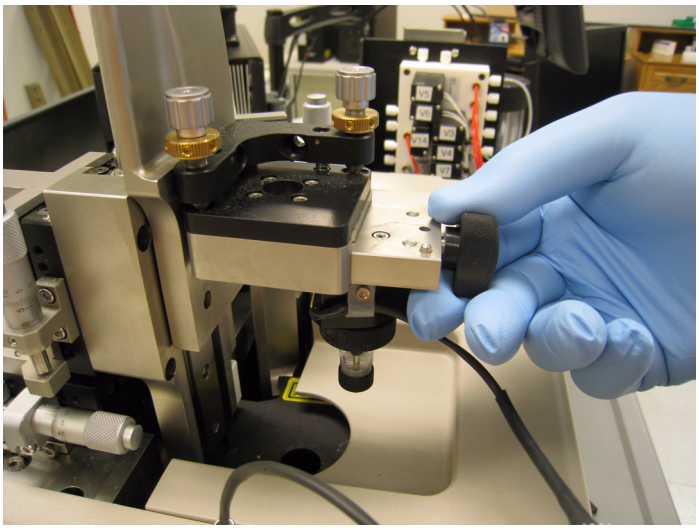


18 If necessary, tap the side of the clear plastic nozzle body gently to remove bubbles.

19 Press the Start Sheath Flow button to stop the sheath stream.



20 Reinstall the nozzle body in the stage.



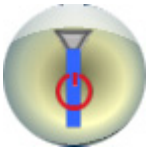
⚠ WARNING

Risk of personal injury. When lowering the nozzle stage, you can pinch your fingers between the bottom of the stage and the instrument frame. Lower the nozzle stage using the upper portion of the stage to avoid pinching points.

21 Lower the nozzle stage back into the instrument and lock it in place.

22 Reconnect the power to the nozzle body (via the Lemo connector).

23 Press the Start Sheath Flow button to start the sheath flowing.



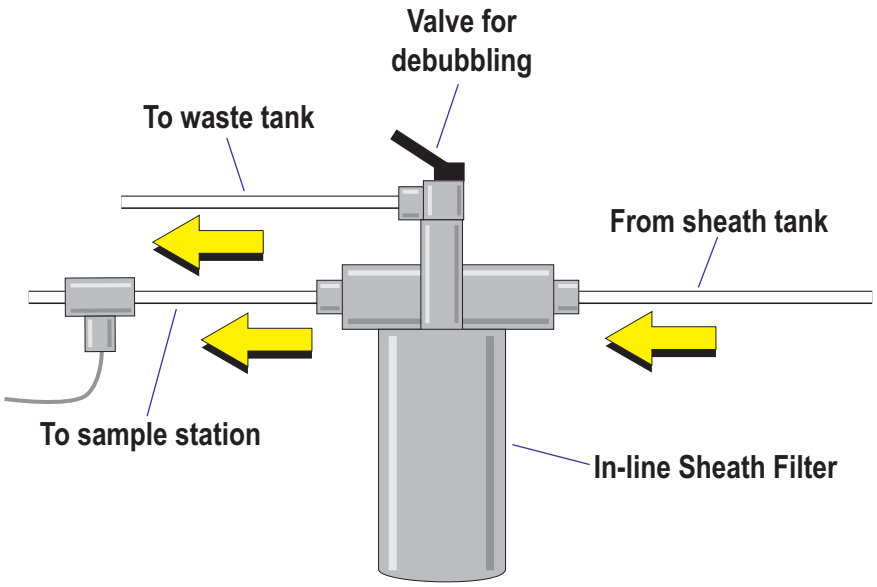
24 Debubble.

25 Complete the Stream Alignment instructions under CHAPTER 6, *Instrument Alignment*.

Replacing the In-line Sheath Filter

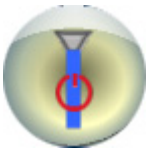
Prior to removing the sheath filter you must turn off the sheath stream and depressurize the sheath tank. The canister and filter will contain sheath, so it is a good idea to position a bucket under the canister before you begin.

Figure 10.16 Sheath Filter Diagram



How to Replace the In-line Sheath Filter

1 Turn OFF the sheath stream.



2 Turn OFF pressure to the tanks.

3 Vent the pressure from the sheath tank by turning the vent valve one full turn.

4 After the pressure has equalized, tighten the vent valve on the tank.

5 Loosen the wing nut on the canister and rotate the bolt out.

6 Pull the bracket open and remove the canister.

7 Rotate and pull out the sheath filter. Dispose of the filter. Notice approximately how much sheath fluid remains in the canister. This will be useful later when you reattach the canister.

8 Inspect the O-ring in the rim of the canister and replace it if it shows signs of wear.

9 Rotate and push the new sheath filter onto the mounting post.

10 Fill the canister with fresh sheath fluid to the level you observed earlier.

11 Place the new filter in canister.

12 Replace the canister and bracket. Rotate the wing nut back into position and tighten the bracket.

13 Turn pressure to the tanks ON.

14 Open the black debubble valve on the sheath canister Figure 10.16.

15 Observe the waste tubing until the air bubbles are gone and fluid fills the line.

16 Close the debubble valve.

17 Perform a debubble procedure on the instrument.

Replacing the SmartSampler Probe

Maintenance of the sample probe and sample tubing is critical to the proper operation of SmartSampler. The sample probe is constructed of stainless steel and is autoclaveable. The sample tubing is disposable; its assembly consists of silastic tubing connected to blue PEEK tubing, a finger-tight fitting, a stainless steel nut, and ferrules.

It is very important to keep the SmartSampler sample probe and sample tubing in proper working order. If the sample probe strikes the side of the sample tube during agitation, the sample probe is probably bent. A bent probe can cause many problems, including damage to the SmartSampler unit, damage to sample tubes and adaptors, or damage to samples.

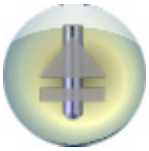
The sample tubing on the SmartSampler can also become damaged during use. Some cells can stick to the tubing and cause a partial clog. If the sample tubing is damaged, it cannot be repaired; it must be replaced.

If the sample probe becomes bent or the sample tubing becomes damaged, you should replace them immediately.

How to Replace the SmartSampler Probe

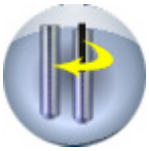
1 Press the Open Chamber button on the Touch Screen Control Panel.

Figure 10.17 Open Chamber Button



2 Press the change probe button on the Touch Screen Control Panel

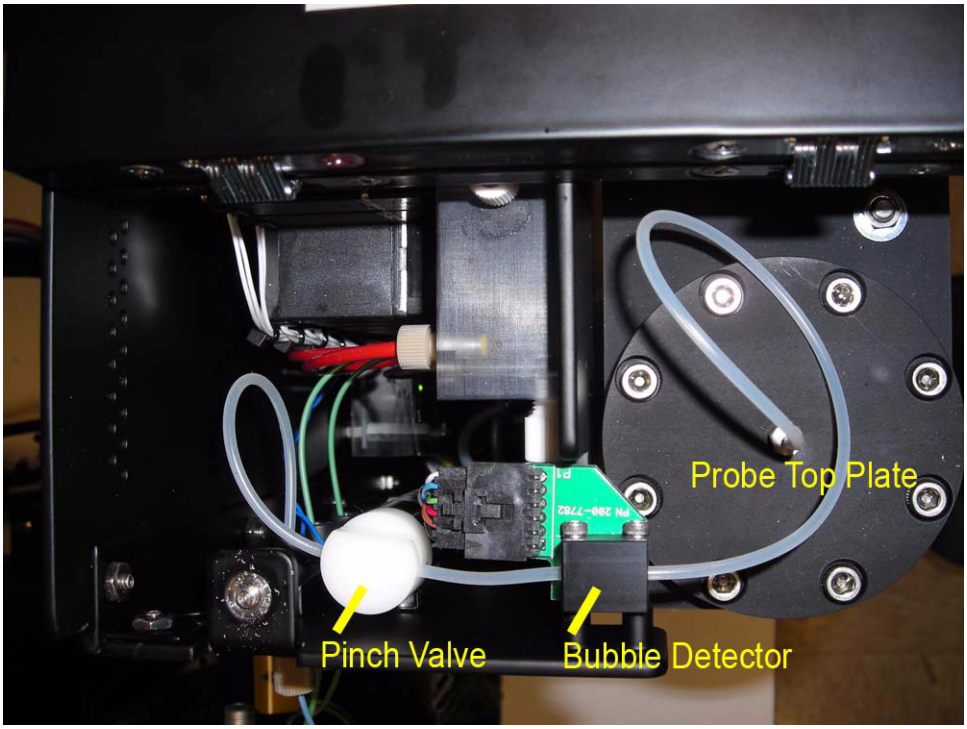
Figure 10.18 Change Probe Button



3 Remove cover.

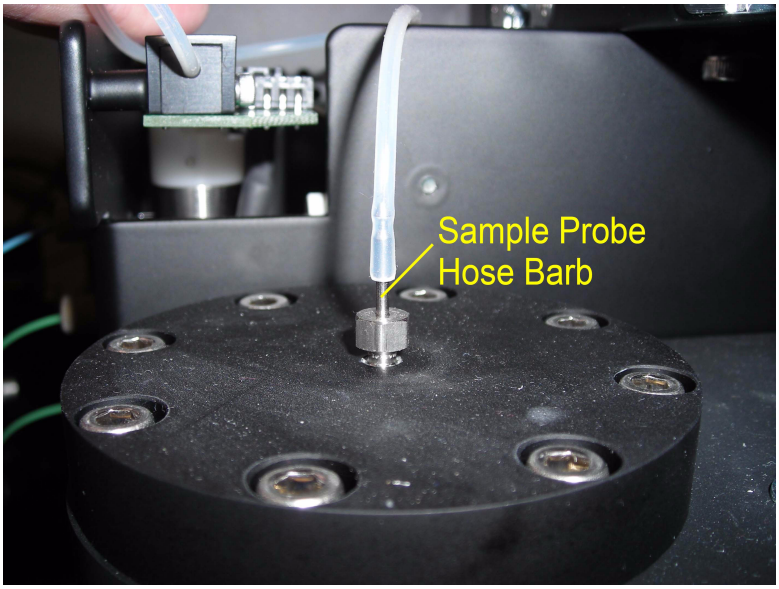
4 Open the top plate on the SmartSampler.

Figure 10.19 Top View of the SmartSampler with Lid Removed



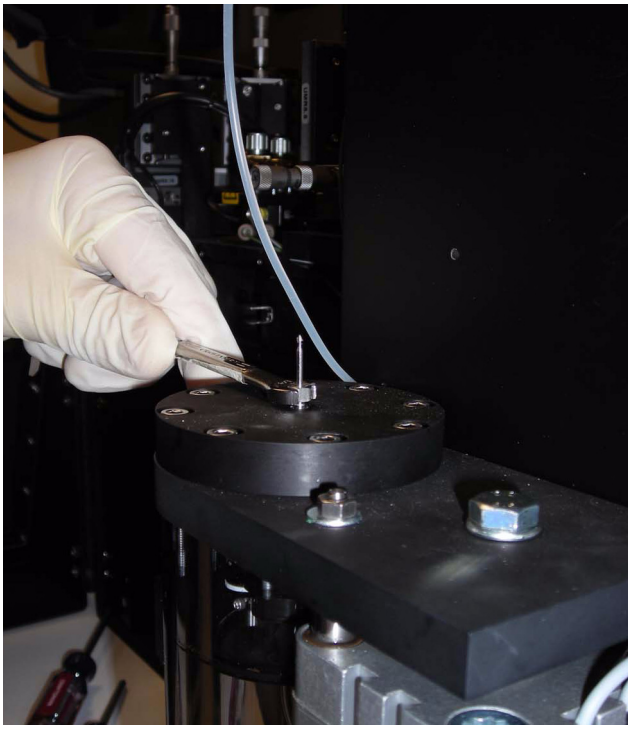
5 Gently pull the silastic tubing off of the sample probe hose barb.

Figure 10.20 Side View of the Probe Top Plate with Hose Barb



6 Using a ¼" open-end wrench, loosen the probe nut.

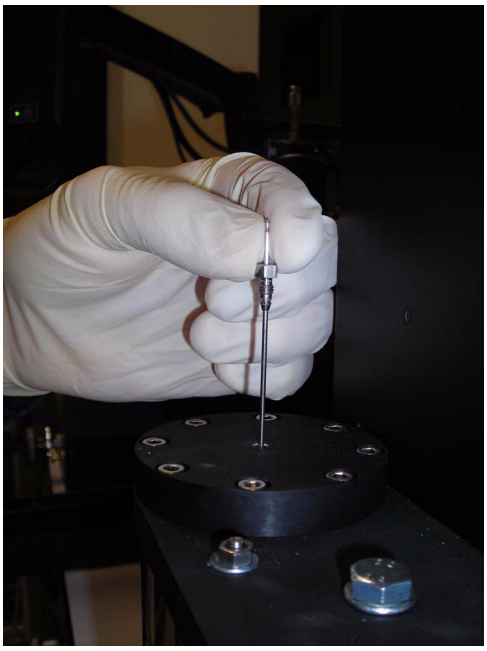
Figure 10.21 Loosen Probe Nut on Probe Top Plate



7 Slowly pull the sample probe upward until it is completely removed from the probe plate.

NOTE : If the sample probe is not damaged, it can be autoclaved for sterilization and reinstalled. If the probe is damaged, dispose of it according to your company's approved procedures.

Figure 10.22 Sample Probe Pulled from Probe Top Plate



IMPORTANT Do not use force when moving the probe through the two holes in order to avoid bending the probe.

8 Feed the probe through the probe top plate and wiper assembly.

9 Using your fingers, slightly tighten the nut attached to the probe.

10 Using a ¼" open-end wrench, tighten (snug plus a ¼ turn) the nut that is attached to the probe.

Replacing SmartSampler Tubing

Disconnect all power to the nozzle before performing this procedure.

How to Replace SmartSampler Tubing

1 Press the Chamber Open button on the Touch Screen Control Panel. This will place the SmartSampler in the Open state and move the probe plate to the top.

Figure 10.23 Open Chamber Button

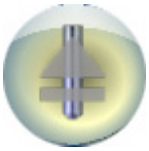
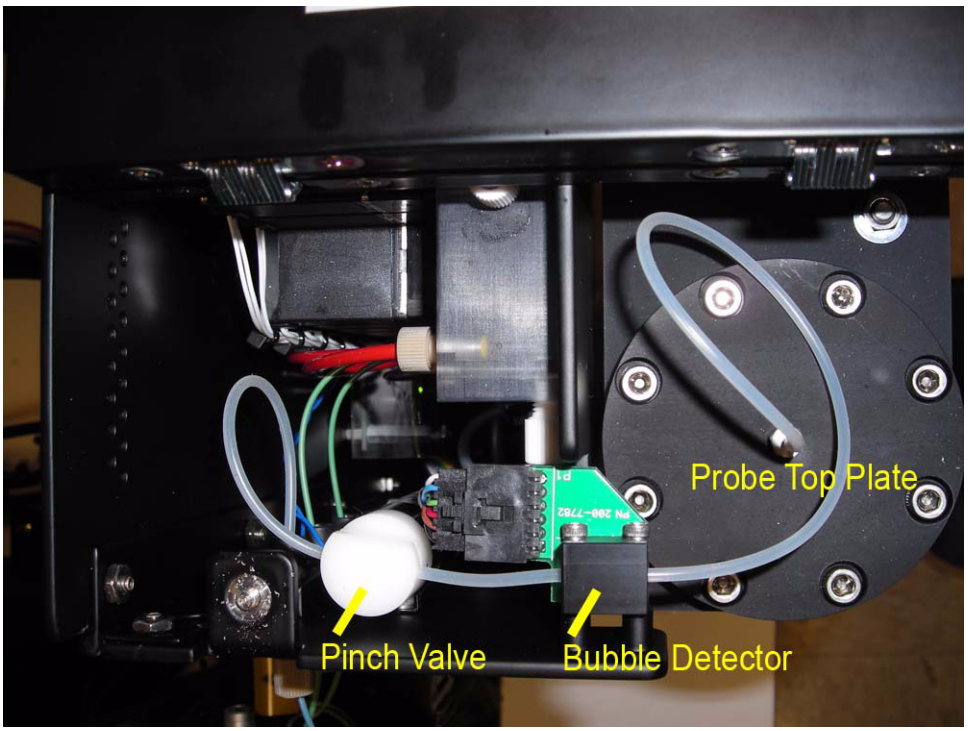


Figure 10.24 Top View of SmartSampler with Lid Removed



2 Carefully pull the silastic tubing off of the sample probe hose barb.

Figure 10.25 Side View of the Probe Top Plate with Hose Barb and Tubing

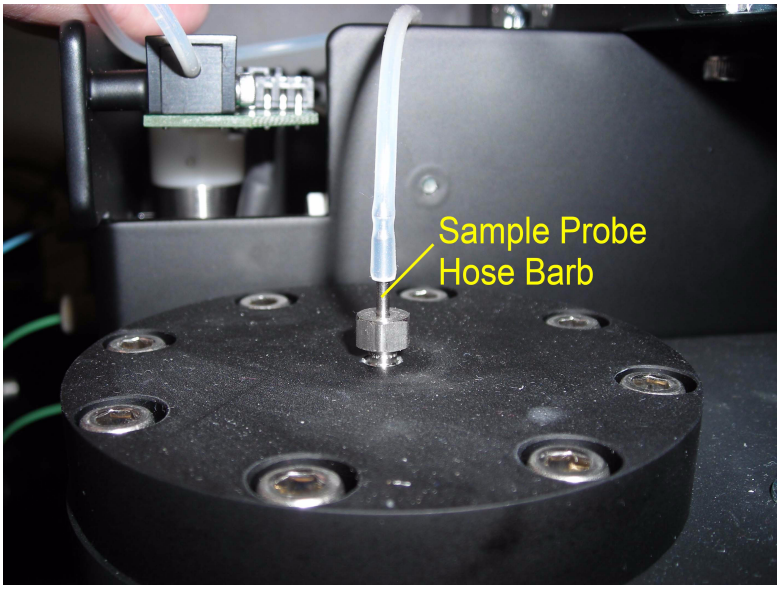
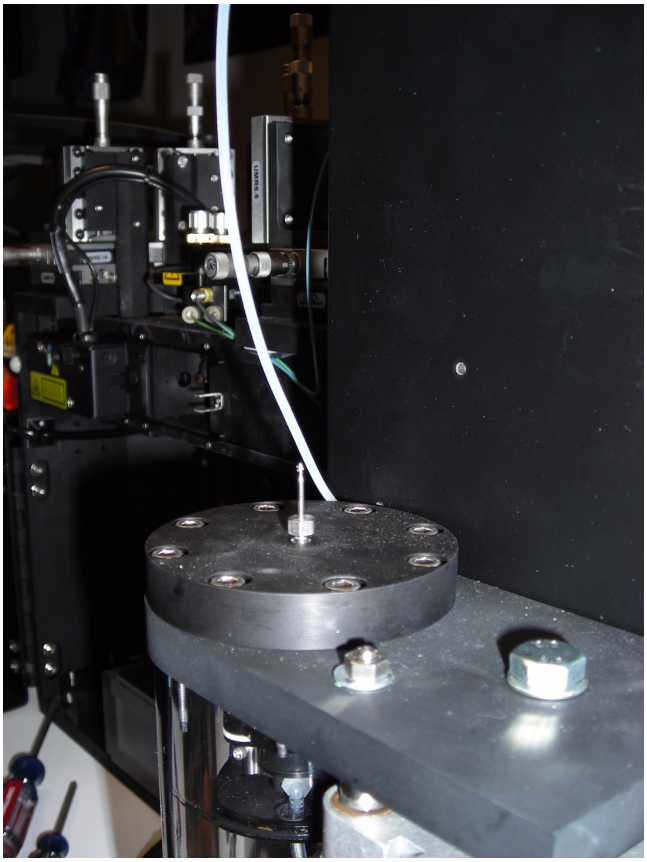
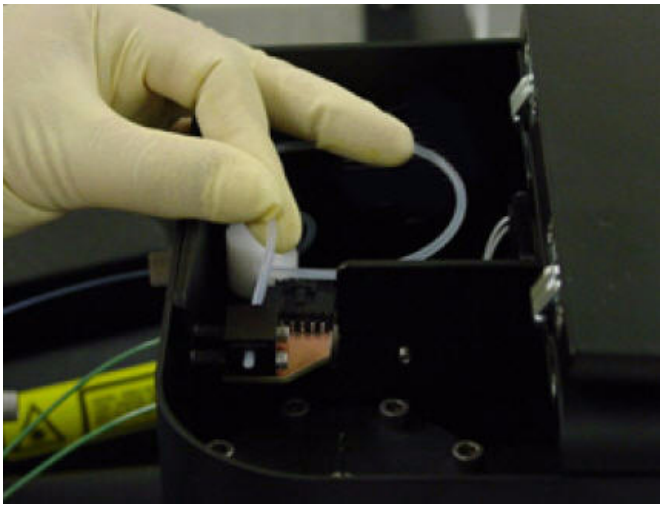


Figure 10.26 Side View of the Hose Barb with Tubing Removed



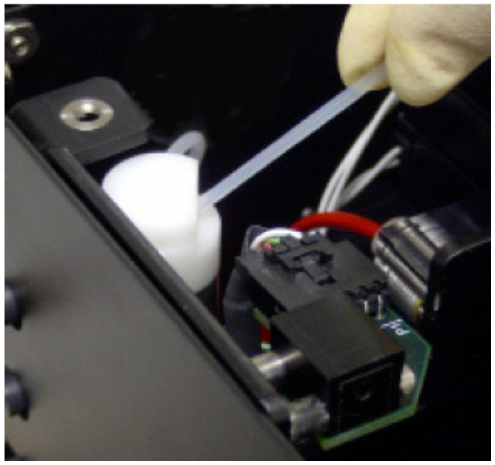
3 Pull the silastic tubing through the bubble detector, away from the sample chamber.

Figure 10.27 SmartSampler Bubble Detector



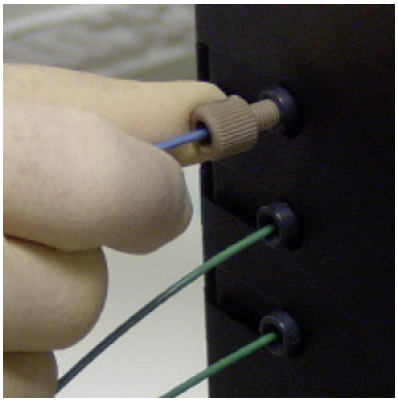
4 Remove the silastic tubing from the pinch valve slot.

Figure 10.28 SmartSampler Pinch Valve

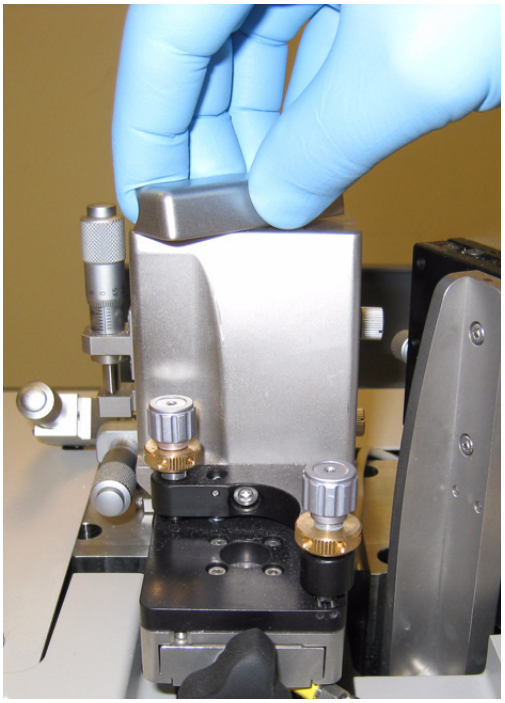


5 Loosen the finger-tight fitting that secures the tubing to the union by turning the fitting counterclockwise with your fingers.

Figure 10.29 SmartSampler Finger-Tight Fittings

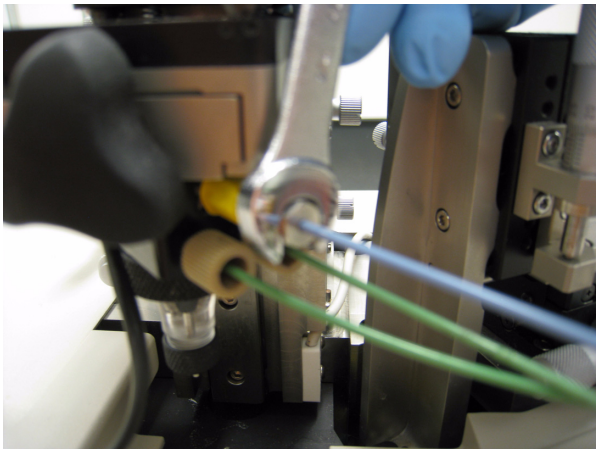


6 Turn the knob on the nozzle stage and raise the nozzle.



7 Using a ¼" open wrench, loosen the nut that goes into the top port on the side of the nozzle.

Figure 10.30 Loosen Top Port Nut

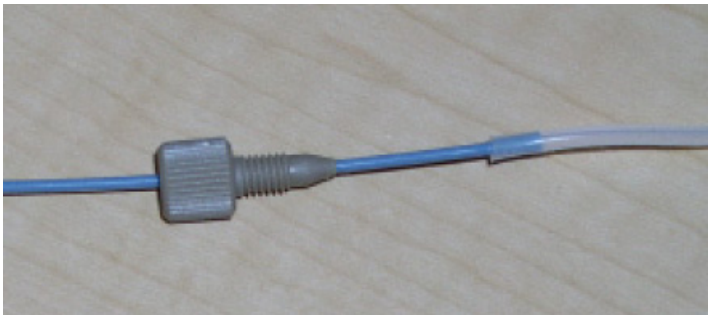


8 Pull the sample tubing assembly out of the SmartSampler unit.

9 Dispose of the entire sample tubing assembly according to your laboratory's approved procedures.

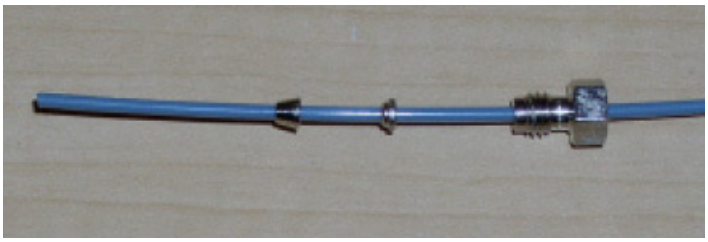
10 Using a new sample tubing assembly, slide the finger tight fitting on the blue PEEK tubing until it almost reaches the junction of the blue PEEK and silastic tubing. The threads on the fitting should point toward the silastic end of the tubing.

Figure 10.31 Fitting on Tubing



11 Using the sample tubing assembly, slide the stainless steel nut on the blue PEEK tubing, and then slide on the ferrules (smaller ferrule first). The smaller ferrule should be flat against the nut threads and all three parts should point toward the end of the blue PEEK tubing.

Figure 10.32 Add Ferrules

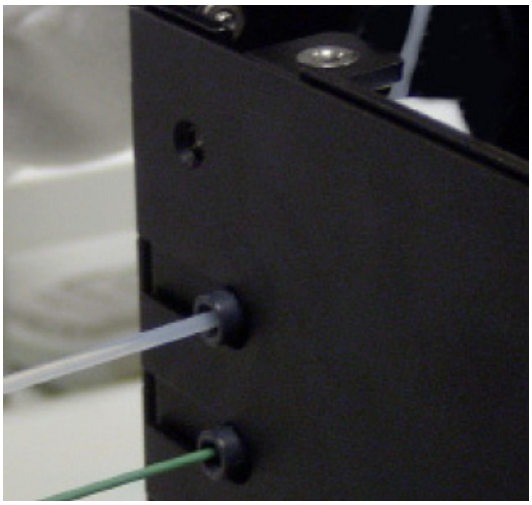


12 Install the blue PEEK tubing into the top port on the side of the nozzle using the stainless steel nut and ferrules at the end of the tubing.

13 While using a wrench to support the nozzle, tighten the nut on the sample tubing using a ¼" open wrench (snug + ¼ turn).

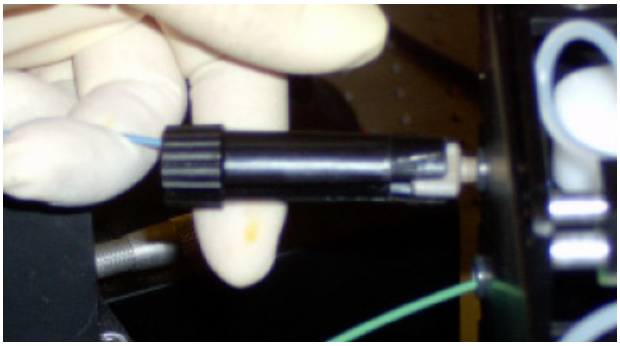
14 Pull the silastic end of the tubing through the top union on the side of the SmartSampler.

Figure 10.33 Pull Silastic End Through Top of SmartSampler



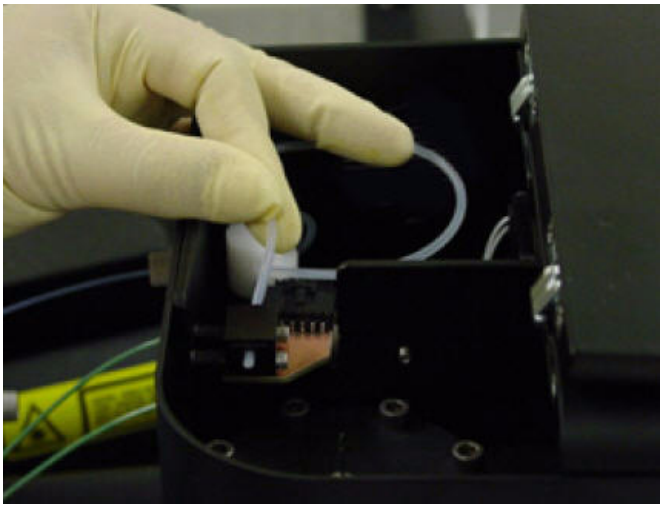
15 When the junction of the PEEK and silastic tubing enters the union, tighten the finger tight fitting into the union using the torque tool.

Figure 10.34 Torque Tool



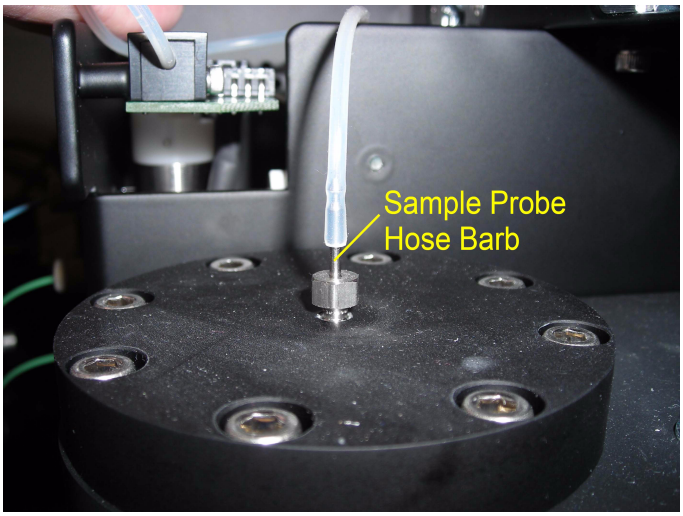
16 Feed the silastic tubing through the hole in the side of the bubble detector. Start through the hole closest to the union and pull through the hole closest to the sample probe.

Figure 10.35 Pull Silastic Tubing through Bubble Detector



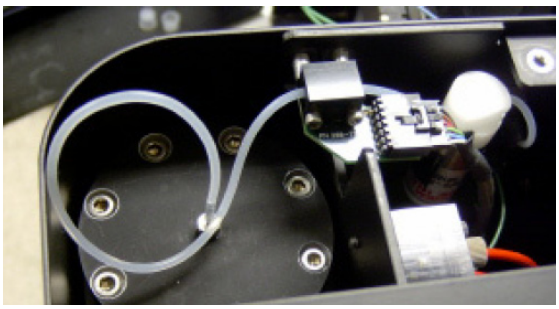
17 When the tubing is through both bubble detector holes, guide the tubing out through the chassis cutout and slide the tubing onto the hose barbed end of the probe. The tubing should cover the probe by approximately 3/8".

Figure 10.36 Attach Tubing to Hose Barb



18 To prevent a sharp bend in the tubing when it is pushed into the pinch valve, make sure you pull enough tubing through the air detector on the probe side to form a small loop as shown in Figure 10.37.

Figure 10.37 Small Loop of Tubing



19 Push the tubing into the pinch valve by holding the tubing in both hands and pushing it into the valve slot.

Figure 10.38 Tubing in Pinch Valve Slot

20 Ensure that the tubing is completely seated to the bottom of the slot so that the pinch valve will function properly.

21 Verify that you have left the proper amount of tubing on each side of the pinch valve.

⚠ WARNING

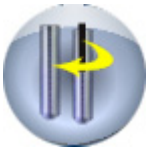
Risk of personal injury. When lowering the nozzle stage, you can pinch your fingers between the bottom of the stage and the instrument frame. Lower the nozzle stage using the upper portion of the stage to avoid pinching points.

22 Lower the nozzle stage back into the instrument and lock it in place.

23 Check that there are no sharp bends in the tubing.

24 Press the Change Probe button on the Touch Screen Control Panel. When the chamber closes, verify that the tubing is not stressed between the bubble detector and the probe. There should be a small amount of slack in the tubing.

Figure 10.39 Change Probe Button



APPENDIX A

Approved Cleaners and Disinfectants

Overview

The following list of cleaners and disinfectants can be used on the MoFlo Astrios. If products not specified on this list are used, it may cause damage to the system and void the warranty. Any questions or concerns regarding chemical usage on the MoFlo Astrios should be directed to Beckman Coulter Customer Service.

Cleaners

- Beckman Coulter LH Series Formaldehyde-Free Clenz
- 0.1% Triton-X100 in deionized water
- 70% ethanol in deionized water

Disinfectants for Use in the Sample Line*

- 70% ethanol in deionized water
- Bleach solution with a maximum concentration of 200 ppm active chlorine (11.5 mL household bleach 2875 mL water, 1:250 dilution). Use only high-quality, fragrance-free, gel-free bleach (5 to 6% solution of sodium hypochlorite - available chlorine). Ten minutes of contact time is needed for disinfection.

Disinfectants for Use in the Sheath Line†

- Bleach solution containing 200 ppm active chlorine (11.5 mL household bleach 2875 mL water, 1:250 dilution). Use only high-quality, fragrance-free, gel-free bleach (5 to 6% solution of sodium hypochlorite - available chlorine). Ten minutes of contact time is needed for disinfection.
- For yearly decontamination use a bleach solution containing 2000 ppm active chlorine (115 mL household bleach 2885 mL water, 1:25 dilution).

---

* Any disinfectant used on the sample probe must be rinsed with an equal amount of deionized water.

† Any disinfectant used in the sheath lines must be rinsed with deionized water for a minimum of 90 minutes.

Disinfectants for Use in the Waste Tank

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

- 10% Sodium hypochlorite or bleach. Use only high-quality, fragrance-free, gel-free bleach (5 to 6% solution of sodium hypochlorite - available chlorine).
- Quaternary ammoniums
- Thymols
- Phenols

Tanks

Keep in mind that over time bleach will cause materials to corrode. If you clean a tank with bleach solution, remove the bleach and rinse the tank.

APPENDIX B

Consumables

Consumables

Table B.1 Beads

Code	Product
URFP-30-2 (Spherotech PN)	Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles - 3.0 µm, 1 x 10^7/mL, 2 mL/vial (for installation only; RUO) Single population of beads excited from 355 nm through 647 nm.
RCP-30-5 (Spherotech PN)	FluoroSpheres – 6-peak, 3.2 µm, 1 x 10^7/mL, 40 tests
RCP-30-5A (Spherotech PN)	FluoroSpheres – 8-peak, 3 µm, 1 x 10^7/mL, 100 tests
6605359	Beckman Coulter Flow-Check Beads

NOTE The first three products listed in Table B.1 must be purchased directly from Spherotech.

Table B.2 Sheath[a]

Code	Product
8546859	IsoFlow Epics Sheath Fluid 10 L
8546719	ISOTON II, 20 L
CY30230	Puraflow 8X Sheath Fluid, 24 L (6 L x 4 bags)

a. Review the product insert information for any sheath fluid used with the MoFlo Astrios to evaluate preservatives or incompatibilities with sample buffers.

Table B.3 Maintenance

Code	Product
721542	Beckman Coulter Formaldehyde Free Clenz
	Bleach solution containing 2000 ppm active chlorine (115 mL household bleach 2885 mL water)
ML03630	0.2 µm Inline Sheath Filter.

APPENDIX C

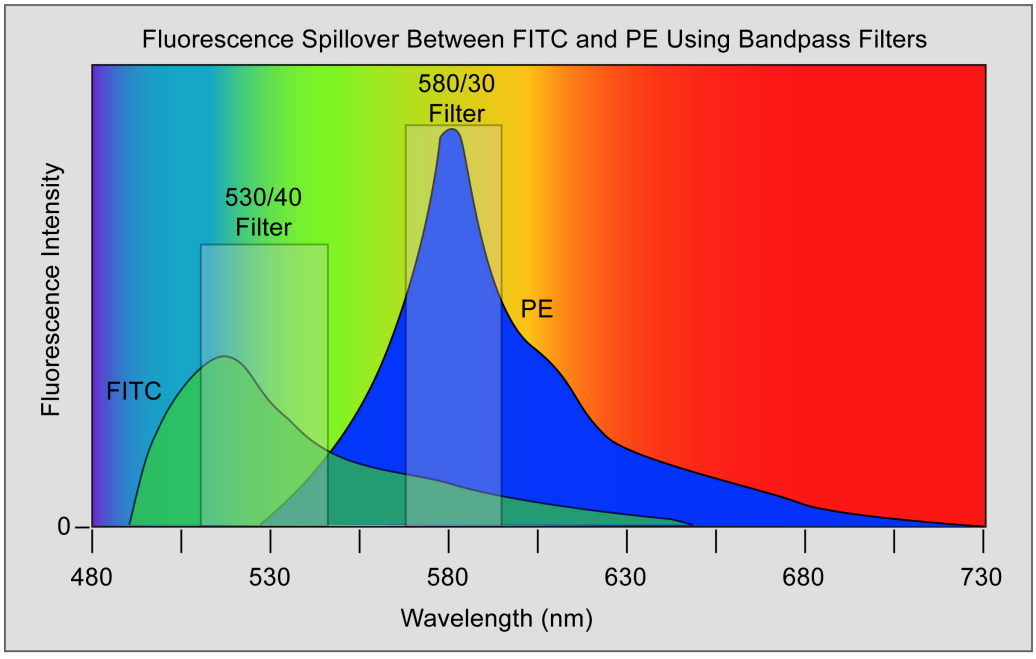
Compensation Background Information

Compensation

Compensation is the process of resolving the actual intensities from each antibody conjugate in a multicolored sample. Using single color staining or single color fluorochromes as a compensation control makes it possible to define the relative amount of light that ends up in another detector. For example, compensation can be used to determine how much light from FITC ends up in the FL2 detector and, conversely, how much light from PE ends up in the FL1 detector (see figure below). This percentage is defined as the spillover coefficient of FITC into FL2 and PE into FL1. By having these spillover coefficients defined for all colors and channels in an experiment, it is possible to calculate the compensation of data in a multicolor sample using linear algebra. This is the way that compensation is performed mathematically in Summit software and other offline flow analysis software.

NOTE For instructions on how to use the Auto Compensate wizard see page CHAPTER 4, *Auto Compensation Wizard*.

Figure C.1 FITC and PE Spectra Diagram



The amount of light that is detected in each fluorescence parameter (FL1, FL2) is the area under the curve of each spectra. If two fluorochromes are bound to the same cell, the sum of the light from each fluorochrome is detected. Knowing the spillover in both directions makes it possible to mathematically determine the light from each fluorochrome.

For two fluorochromes, the following math is used:

FL1(FITC) = FL1(total) - a_{12}FL2(total compensated)

FL2(PE) = FL2(total) - a_{21}FL1(total compensated)

Where a_{12} is the spillover of FITC into FL2 and a_{21} is the spillover of PE into FL1. The (total) is the signal measured on the dual stained cell in each channel. In a multi-color experiment each total signal includes spillover from more than one color. Therefore, to prevent “overcompensation” by subtracting too much, the **compensated total** is subtracted rather than the **total**.

See CHAPTER 4, *Auto Compensation Wizard*.

APPENDIX D

CytoCalc Table

CytoCalc Table

The CytoCalc Table provides suggested starting values that can be used when you are adjusting settings. These values are approximate. You will empirically find the optimal values.

Table D.1 CytoCalc Table

Nozzle Size (µm)	Recommended Pressure (psi)	Approximate Frequency (Hz)	Approximate DD Amplitude (Volts)	Approximate Drop Delay
50	80–100	120000	25	25
70	60	100000	15	40
80	60	80000	30	45
90	40	60000	40	40
100	25	40000	30	40
120	20	30000	50	35
150	15	20000	50	30
200	5	7000	50	15

APPENDIX E

Symbols

Symbol Definitions

Table E.1 Symbol Definitions

Symbol	Definition
	Instrument Serial Number
	Beckman Coulter Model Number
	Date of Manufacture
	Alternating Current Input
	Fuse
	Caution, Consult Accompanying Documents

Table E.1 Symbol Definitions *(Continued)*

Symbol	Definition
	Identification of Manufacturer
	Correct Disposal of this Product (According to Directive 2002/96/EC on Waste Electrical and Electronic Equipment [WEEE] applicable in the European Union and other European countries with separate collection systems.) Contact a Beckman Coulter Representative for disposal of the equipment at the end of its working life. This product should not be mixed with other commercial waste for disposal.

Abbreviations

The following list is a composite of the symbols, abbreviations, acronyms, and reference designators either used in this manual or related to the information in it. When the same abbreviation (or reference designator) is used for more than one word (or type of component), all meanings relevant to this manual are included, separated by semicolons.

> — greater than

< — less than

≥ — greater than or equal to

≤ — less than or equal to

% — percent

+ — plus

- — minus

± — plus or minus

°C — degrees Celsius

°F — degrees Fahrenheit

® — registered trademark

™ — trademark

µ — micron

µL — microliter

µs — microsecond

A — ampere

ac — alternating current

ADC — analog-to-digital conversion

Amp — amplifier

ANSI — American National Standards Institute

baud — bits per second

BCI — Beckman Coulter Incorporated

BIOS — basic input/output system

BSO — beam shaping optics associated with the UV laser

FBSO — fiber-coupled lasers' BSO

C — centigrade

CD-ROM — compact disk - read only memory

CDRH — National Center for Devices and Radiological Health

cm — centimeter

CPU — Central Processing Unit

CV — check valve; coefficient of variation

CV% — coefficient of variation

DAC — digital-to-analog converter

dc — direct current

DCN — document control number

DIP switch — dual in-line package switch

DVM — digital volt meter

EMI — electromagnetic interference

EPROM — erasable programmable read only memory

ESD — electrostatic discharge

F — Fahrenheit; fuse

FBSO — beam shaping optics associated with the fiber-coupled lasers

FC — flow cell

FF — fitting

FRU — field replacable unit

ft — foot; feet

g — grams

GA — gauge

gal. — gallon

GND — ground

Hz — Hertz

i.d. — internal diameter

in. — inches

I/O — input/output

IPL — illustrated parts list

IT — illumination table

IVD — in vitro diagnostics

J — receptacle connector

K — constant

kg — kilogram

L — liter; long

lb — pound

LCD — liquid crystal display

LED — light emitting diode

M — mega; motor

m — meter

max — maximum

MB — megabyte

MF — manifold

MHz — megahertz

min — minimum

mL — milliliter

mm — millimeter

ms — millisecond

MTBF — mean time between failures

MTTR — mean time to repair

mV — millivolt

N/A — not applicable

NA — Numerical Aperture

NDFW — Neutral Density Filter Wheel

nm — nanometer

o.d. — outer diameter

OS — operating system

P — plug connector

PC — personal computer

PCB — printed circuit board

PLCU — power and laser control unit

PM — pump

PMI — preventative maintenance inspection

PMT — photomultiplier tube

PN — part number

POD — precision optical detection

psi — pounds per square inch

QA — quality assurance

QC — quality control

R — potentiometer; resistor

RAM — random access memory

RG — regulator

RH — relative humidity

ROM — read only memory

S — switch; sensor

SD — standard deviation

sec — second

SN — sensor

SRK — Service Resource Kit

SVP — system verification procedure

SW — software; switch

TBD — to be determined

thd — thread

thk — thick

TP — test point

UL — Underwriter's Laboratory

USB — universal serial bus

UV — ultraviolet

V — volts

Vac — volts alternating current

vac — vacuum

Vdc — volts direct current

Index

T

U

V

W

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