Instructions for Use

MoFlo XDP

High-speed Cell Sorter

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

C10712AA
June 2017

Beckman Coulter, Inc.
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Brea, CA 92821 U.S.A.

BECKMAN COULTER

Instructions for Use
PN C10712AA (June 2017)

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Revision History

Initial Issue, June 2017
Software Version 5.2

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released.

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released.

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 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.**
- **SmartSampler presents a pinch hazard. When a sample run is initiated, the sample chamber door closes. Do not place your hand in the sample chamber after you have initiated a sample run and the door begins to close.**
- **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

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.

⚠ WARNING

California Proposition 65: This product can expose you to chemicals including phthalates, which are known to the State of California to cause cancer and birth defects or other reproductive harm. For more information go to www.P65warnings.ca.gov.

Instrument Safety Precautions

IMPORTANT For safety precautions specific to this instrument, see below.

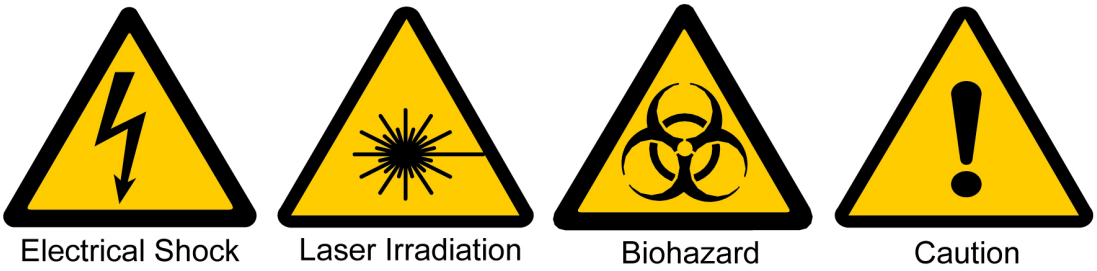
The MoFlo product line has been engineered with safety as one of its primary features. Safety of the operator, field service personnel, bystanders, and of valuable samples, is paramount to Beckman Coulter's commitment to high performance design and engineering.

This section explains some general safety and hazard symbols and necessary precautions operators of the MoFlo XDP 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

Electrical Safety

The MoFlo product line conforms to international regulations encompassing the accessibility of high voltages by the user (IEC 61010-1). Please familiarize yourself with the following features of MoFlo XDP and their corresponding potential hazards:

Safety Interlocks

The MoFlo XDP is equipped with two safety interlocks designed to protect the operator from inadvertent exposure to high voltage and laser irradiation.

- When the Sort Chamber door opens, the safety interlock disables the voltage to the deflection plates and halts any CyCLONE assembly movement.
- When the Illumination Chamber door slides open, the safety interlock closes the laser shutters and disables the electrical charge to the sort deflection plates. Note: The 635 nm diode laser does not include a shutter. Therefore, when the Illumination Chamber safety interlock is opened the red diode laser is automatically shut off.

DO NOT attempt to defeat these interlocks except when this document specifically instructs you to do so.

Safety Interlock Keys

Illumination (Interrogation) Chamber Safety Interlock Key

Sort Chamber Door Safety Interlock 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.
- Do not touch the steel nut connecting the sample line to the CytoNozzle 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 CytoNozzle. The frequency is set by operator.

Sort Deflection Plates

The range of voltage applied to these plates is 0-4000 VDC. This high voltage is only present when the plate voltage is turned on and the interlock is closed. High voltage is only accessible if the user inserts an object between these charged plates if the interlock is defeated. Once enabled by the operator, this high voltage is constant unless changed by the operator. Do not touch the charged plates when power is applied.

Sort Deflection Plate Arcing

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

1. Use the Stream Configuration screen on the aXcess Control Panel to 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 Illumination Table.
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 on the aXcess Control Panel Stream Configuration screen to prevent fanning of the side streams and wetting the plates.

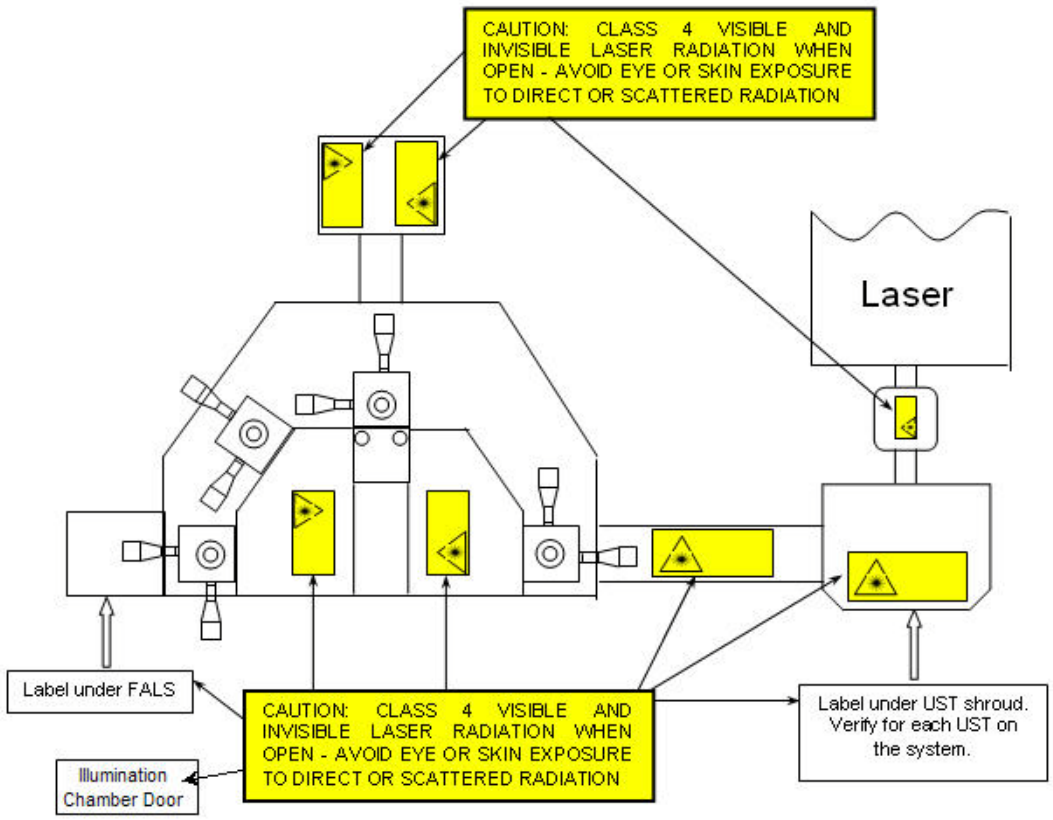
Optical/Laser Safety

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 XDP 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, interlocks, and shields as identified in this manual.

Location of Laser Safety Warning Labels



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

Universal precautions should be followed when handling samples and waste fluid containing samples:

- Gloves, a laboratory coat, and eye protection should be worn whenever handling samples including insertion and removal of sample tubes from the sample station.
- Waste fluid may contain hazardous levels of biological contamination. Gloves, a laboratory coat and eye protection should be worn whenever exposure to waste fluid exists.
- 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.

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*.

General Safety

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

- The MoFlo XDP is intended for Professional Use Only. All operators should be trained on the proper use and limitations of the instrument prior to its operation.
- 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.
- Wear appropriately sized gloves providing good manual dexterity to reduce the likelihood of skin pinches and abrasions.
- 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, be aware that they can cause a tripping hazard.
- Always check with the site safety officer for correct disposal of waste products.
- Use proper lifting techniques, or seek assistance when handling full tanks. To reduce the likelihood of back injury, empty waste at least once per day.
- The MoFlo XDP is capable of pressures up to 100 psi (689 kPa). Check sample and sheath pressures when changing nozzle size.
- Avoid incidental contact with the metal corners and edges of the optical table, cable tie downs and waste tube edges which can cause abrasions.

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.

RoHS Notice

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

RoHS Caution Label

This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

RoHS Environmental Label

This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center 'e' indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

Contents

Illustrations

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Introduction

How to Use Your Manual

Scope

The MoFlo XDP manual contains basic information regarding the use and operation of the MoFlo XDP 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.

Product Description

IMPORTANT Use of this Product in the field of Sperm Sorting is subject to Third Party Patent Rights including, but not limited to U. S. Patent No. 9,134,220. Beckman Coulter does not grant the user any express or implied license to use this device in the Sperm Sorting Field.

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, reviewing QC data, 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 recommendation.

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

Chapter 3, aXcess 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, Alignment and Shutdown
Provides information on starting, aligning, and shutting down the instrument.

Chapter 6, Performance Validation and Calibration
Contains an example validation and calibration procedure.

Chapter 7, Sorting and IntelliSort Setup
Provides information on how to set up for sorting including optimizing droplet formation, starting IntelliSort, determining drop delay, configuring CyCLONE, acquiring data, setting sort decisions, specifying sorting to plates, slides, or tubes, and saving acquired data.

Chapter 8, Cleaning and Maintenance
Describes the cleaning procedures for daily cleaning as well as the biannual and yearly decontamination process.

Chapter 9, Troubleshooting and Replacement Procedures
Provides a basic troubleshooting matrix and procedures for replacing customer-replaceable parts.

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

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

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

Appendix D, Additional Sorting Information
Provides information regarding pressure differential and processing speed limitations, sorting parameters, sort stream precedence, sort mode examples, abort terminology, and doublet discrimination.

Appendix E, 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 F, Setting Laser Delay
Provides instructions on how to reset laser delay after changing a nozzle or making adjustments to operating pressure.

Appendix G, Symbols
Defines the symbols used on MoFlo XDP labels.

Conventions

This manual uses the following conventions:

- Bold font indicates instrument manual titles.
- Bold indicates a screen icon.

- Italics font indicates screen text displayed by the instrument.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

CHAPTER 1

Installation

General Laboratory Information

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

MoFlo XDP Specifications

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

Table 1.1 General System Specification and Environment Requirements

Specification	Requirements
Electrical Requirements (XDP Electronics)	100–230 Vac, 6-3 A, 50–60 Hz Main power is not to exceed ±10% of nominal input voltage.
XDP Electronics Weight	33 kg (72 lbs)
Instrument Bench	122 cm x 91 cm (4 ft x 2 ft) Table or 152 cm x 122 cm (5 ft x 4 ft) Table
Service Access	46 cm (18 in.) on all sides of the instrument bench
Installation Category	II
Pollution Degree	2
Laser Product Classification	Class I Laser Product (IEC/EN60825-1/A2:2001)
Dimensions (not including Auxiliary Components)	4 ft x 3 ft Table 74 cm (29 in.) x 122 cm (48 in.) x 91 cm (36 in.) 4 ft x 5 ft Table 81 cm (32 in.) x 122 cm (48 in.) x 152 cm (60 in.)
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)
Humidity and temperature range for instrument storage and operation	15–30°C (59–86°F), not facing direct sunlight 20–80% RH (non-condensing humidity) Maximum 80% RH up to 30°C decreasing linearly to 50% RH at 40°C ±2°C
Maximum Altitude	Do not operate at an altitude greater than 2000 m (6561 ft)

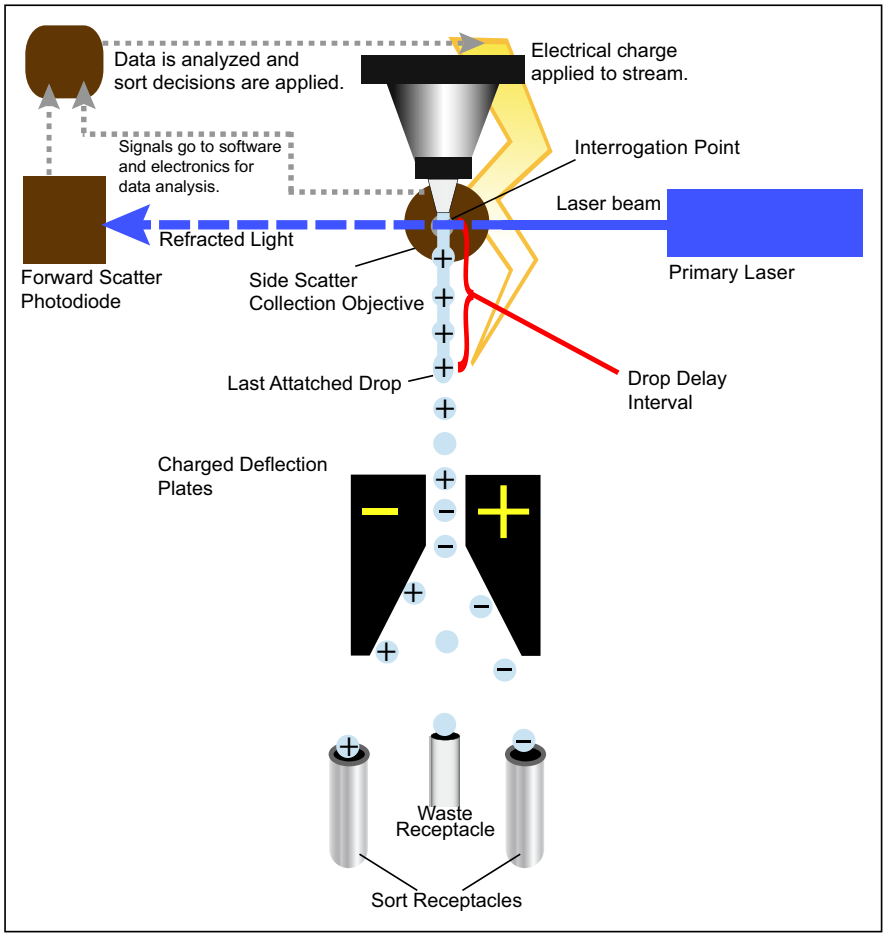
CHAPTER 2

System Overview

Overview of the MoFlo XDP System

This section provides an overview of the MoFlo XDP system architecture. After the general principles of operation are explained, the instrument subsystems are also described.

Figure 2.1 Sort Overview Diagram



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

MoFlo XDP examines individual particles that are propelled in a buffered saline solution through one or more 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. When used in a multi-laser configuration, MoFlo XDP is capable of acquiring and analyzing up to 12 fluorescence and two light-scatter parameters for each particle. Additional computed parameters can be created based on collected data.

To sort, MoFlo XDP acquires data and consults sort decisions that are defined by the operator. The CytoNozzle applies a positive or negative charge to the sheath stream based on the information that was collected after the particle was interrogated by the laser, together with the specified sort decisions. During this time, a piezoelectric crystal in the CytoNozzle 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.

MoFlo XDP Features

Open Architecture

MoFlo XDP provides modular flexibility that allows you to choose from one to three laser paths, and from three to twelve color parameter detectors.

Compact Footprint

The compact footprint of MoFlo XDP conserves laboratory space.

Ease of Use

MoFlo XDP includes an easy-to-use touch screen, called the aXcess Control Panel that facilitates instrument alignment and sort setup. The aXcess Control Panel not only makes instrument alignment simple, it allows for uncomplicated sort stream and CyCLONE configuration. The aXcess Control Panel also displays large-format sort statistics for each sort stream so that you do not have to be near the instrument in order to monitor a sort.

Summit Software

Summit software provides control of MoFlo XDP sorting functions, as well as user- and application-specific data acquisition, analysis, storage, reduction, and retrieval. Summit software provides a MS Windows-based environment using protocol-based menus. Summit software also provides high-content data acquisition, database capabilities, and open architecture for data exchange. Additional features include off-line analysis and easy page layout.

MoFlo XDP Subsystems

In order to describe the MoFlo XDP in greater detail, the system will be divided into six subsystems that comprise the following:

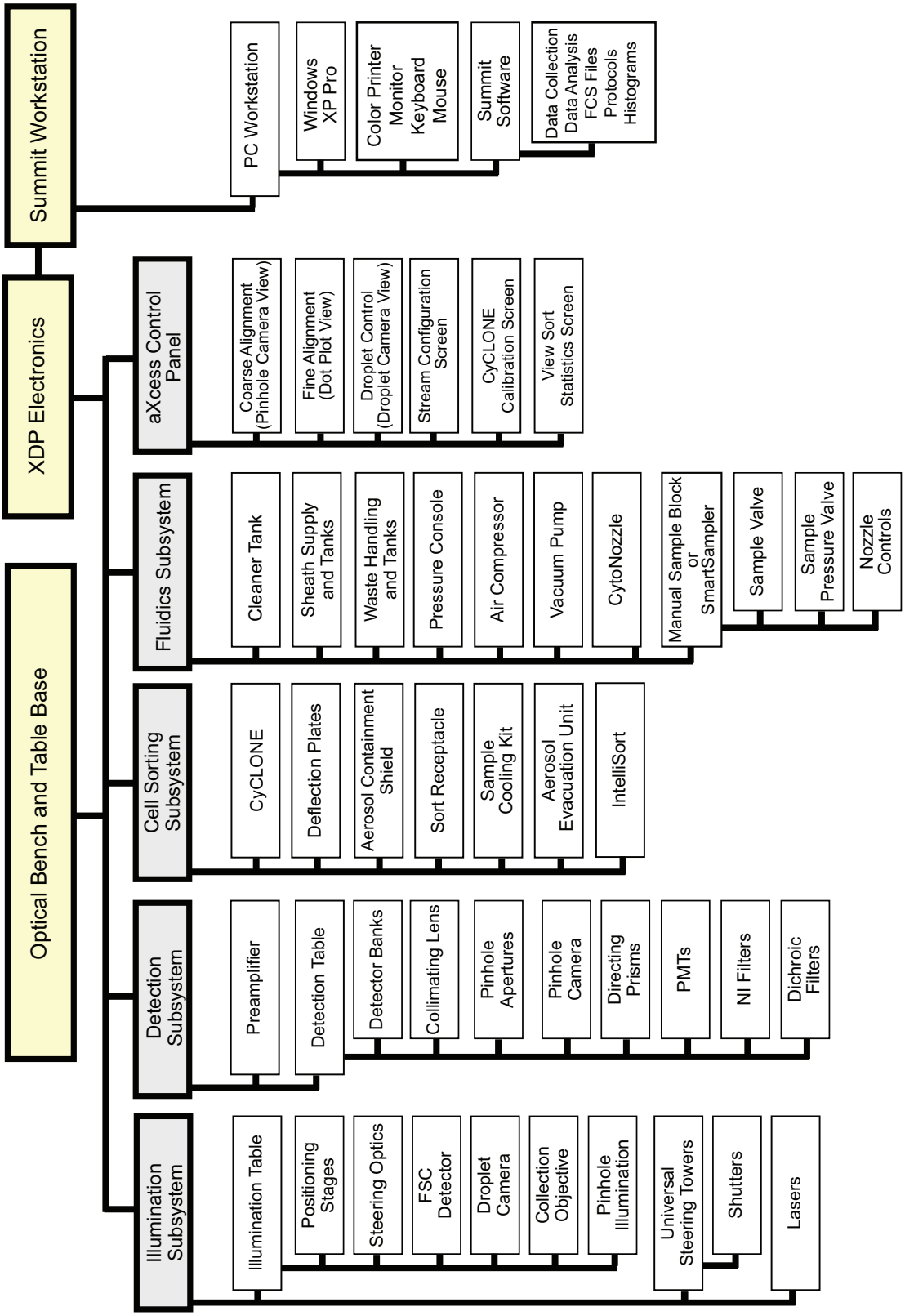
- Illumination
- Detection

- Cell sorting
- Fluidics
- aXcess Control Panel
- Summit Software Workstation

MoFlo XDP Flow Cytometer System Architecture

See Figure 2.2, MoFlo XDP System Architecture.

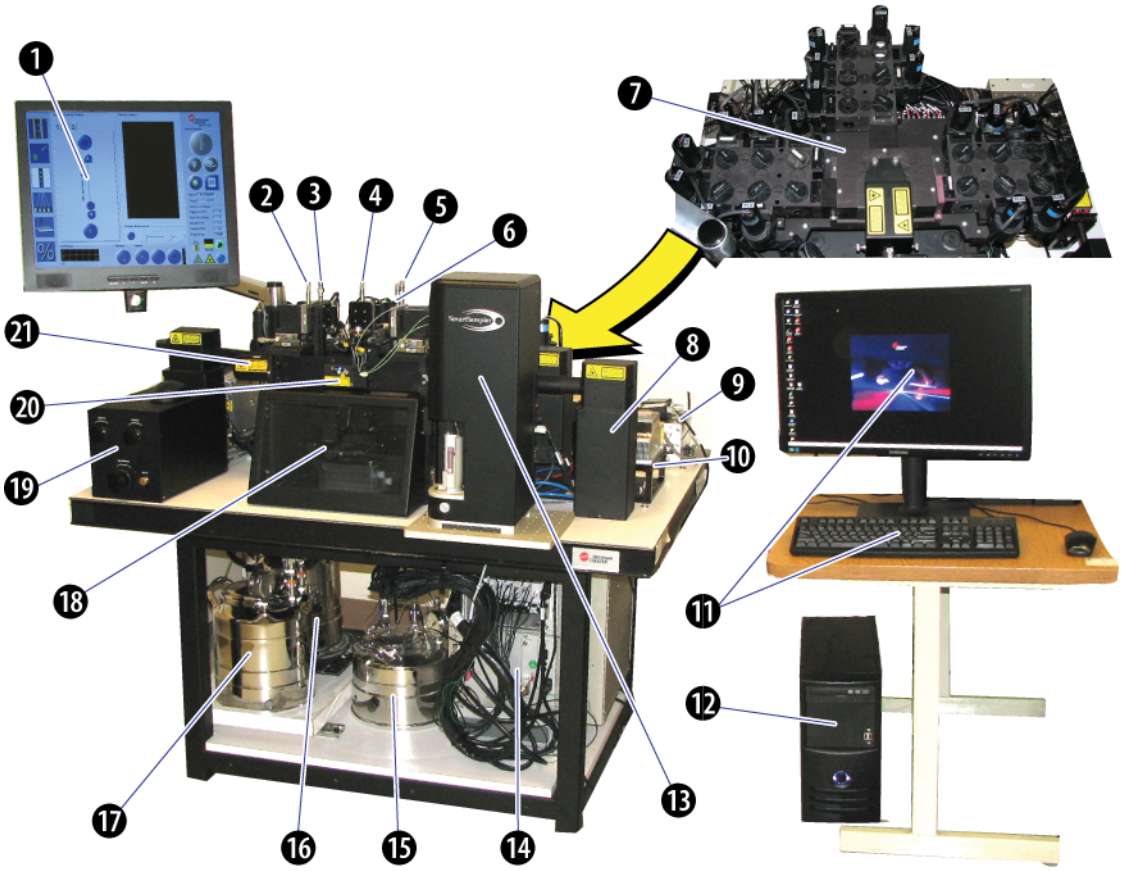
Figure 2.2 MoFlo XDP System Architecture



Optical Bench and Table Base

The optical bench is the structural platform for the MoFlo XDP. All instrument subsystems except the Summit Software Workstation are located on the optical bench or on the table base. These subsystems include the Illumination Table, the Detection Subsystem, the Cell Sorting Subsystem, the Fluidics Subsystem, the XDP Electronics, and the aXcess Control Panel.

Figure 2.3 MoFlo XDP Platform



1. aXcess Control Panel
2. Forward Scatter Positioning Stage
3. Laser Path 3 Positioning Stage
4. CytoNozzle Positioning Stage
5. Laser Path 2 Positioning Stage
6. Laser Path 1 Positioning Stage
7. Illumination Table (IT)
8. Steering Tower (one per laser)
9. Laser Power Supply (will vary)
10. Laser 1, Laser 2 & 3 (not visible)
11. Summit Software Monitor and Keyboard
12. Summit Software Workstation
13. SmartSampler
14. XDP Electronics Chassis and Controller
15. Cleaner Tank
16. Sheath Tank
17. Waste Tank
18. Sort Chamber
19. Pressure Console
20. Illumination (Interrogation) Chamber
21. Forward Scatter Photodiode

Illumination Subsystem

The Illumination Subsystem provides laser illumination, operator safety, and control of laser alignment.

Illumination Table

The Illumination Table (IT) is located at the center front of the Optical Bench and provides a platform for the instrument alignment controls. The IT supports the micrometer positioning stages and gimbals for laser and stream alignment. Also located on the IT are the Droplet Camera, the Forward Scatter Photodiode, the Pinhole Aperture Illumination, the Side Scatter Collection Objective, beam shaping optics and the 635 nm diode laser. Below the IT are the components that make up the Cell Sorting Subsystem described on page 2-13. Two safety interlocks described on page viii protect the operator from inadvertent exposure to laser radiation and high voltage.

Illumination Chamber

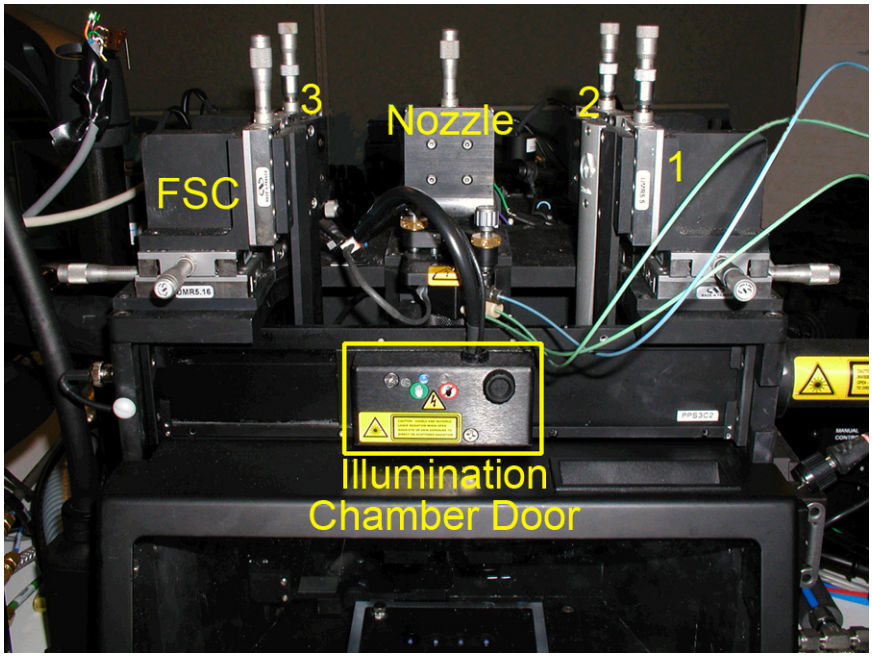
The Illumination (or Interrogation) Chamber is the area of the instrument where the sample and sheath stream intersect with laser light. This point of intersection is known as the interrogation point. In addition, the XDP electronics monitor the door location to create the HV/Optical Interlock to protect the user if the Illumination Chamber Door is opened. Display LEDs are also provided on this module to display the High Voltage Warning as generated by the High Voltage Plates Shelf. The CytoNozzle is located in the Illumination Chamber and is described on page 2-18.

Positioning Stage Assemblies

The Positioning Stages provide fine-movement control of the CytoNozzle, Forward Scatter Collection optic and Beam Shaping Optics for the individual lasers.

If you are standing in front of the instrument, laser path one is located on the far right side of the IT. Moving counterclockwise, laser path two is next, then the nozzle stage, laser path three, and finally the Forward Scatter stage is located on the far left.

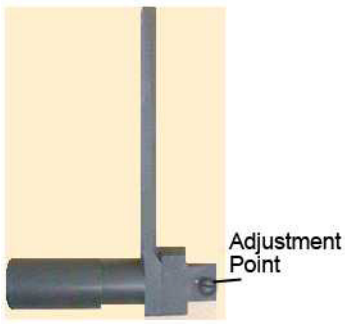
Figure 2.4 Positioning Stages and Illumination Chamber



The **CytoNozzle Gimbaled Positioning Stage** has five axes of movement and is located on the top of the Illumination Table at its center. It is typically used to ensure that the stream is perpendicular to the plane of the table and is positioned correctly in the Pinhole Camera view on the aXcess Control Panel.

The **Forward Scatter Collection Positioning Stage** and optics are located on the top front left of the Illumination Table directly in line with the first laser path. It consists of a forward-scatter collection lens and obscuration bar. This positioning stage and collection objective focus forward-scattered light from the first laser path, typically a 488 nm line. The Obscuration Bar is adjusted with a flathead screwdriver to differentially block direct laser illumination of the Forward Scatter photodiode so that the intensity of direct laser light does not overwhelm the detector. The angle of the obscuration bar is dependent on the size of the sample particles and the sensitivity of the instrument.

Figure 2.5 Forward Scatter Collection Optic



The **Laser Positioning Stages** have three axes of movement and are each located on a laser path in line with the individual light containment tubes. These stages allow the operator to focus each laser at the precise focal point of the collection optic and at the core of the sample stream.

Forward Scatter Photodiode

The Forward Scatter Photodiode is located on the left side of the Illumination Table, covered by a light-containment tube. See 9-12 for directions on how to change the filters in the Forward Scatter Detector. Some applications require the placement of a Forward Scatter Collection Objective for resolution of bacteria, other similarly small particles, or asymmetric cells.

Side Scatter Collection Objective

The Side Scatter Collection Objective is a fixed component of the Illumination Table. Placed at a right angle to the primary laser paths, the Side Scatter Collection Objective focuses the light from each interrogation point onto a pinhole aperture. Passage of light through the spatially separated pinholes, and through the spatially separated pinhole apertures, results in individual laser illumination of each separate detector bank and minimizes crossbeam interference.

Universal Steering Towers

Some optical pathways are associated with a universal shutter tower that contains a laser shutter that is opened and closed through the aXcess Control Panel. The shutter tower comprises a series of silica mirrors coated to optimally reflect the wavelength of the laser on the same pathway. These mirrors should not be touched or cleaned by the user. Note: the 635 nm red diode laser does not include a shutter and therefore can only be turned on and off through the aXcess Control Panel.

Lasers

The MoFlo XDP can be configured with up to three free-standing lasers. A selection of solid state, air-cooled, and/or water cooled lasers can be accommodated on the optical bench.

- When selecting a laser, the following should be considered:
- Wavelength needed for the desired protocol or application.
- In-house facilities to accommodate water or air cooling.
- Laser power output for the desired application.
- Cost of the different laser types.

Detection Subsystem

The Detection Subsystem encompasses the Detection Table and the Preamplifier assemblies. This subsystem includes the components that collect, direct, filter, and convert emitted light to electrical potential.

Detection Table

The Detection Table serves as a platform for either the Detector Banks, or the Precision Optical Detection (PODs), as well as the Collimating Lens, Pinhole Apertures, Pinhole Camera, Beam Directing Prisms, Photomultiplier Tubes (PMTs), Band-pass Filters, and Dichroic Mirrors.

NOTE Figure 2.6 depicts the detection paths for a typical three-laser configuration with a Z-configuration on detection path one. Figure 2.7 illustrates a detection table configured with PODs.

Figure 2.6 Detection Table with Detector Banks (prior to 2009)

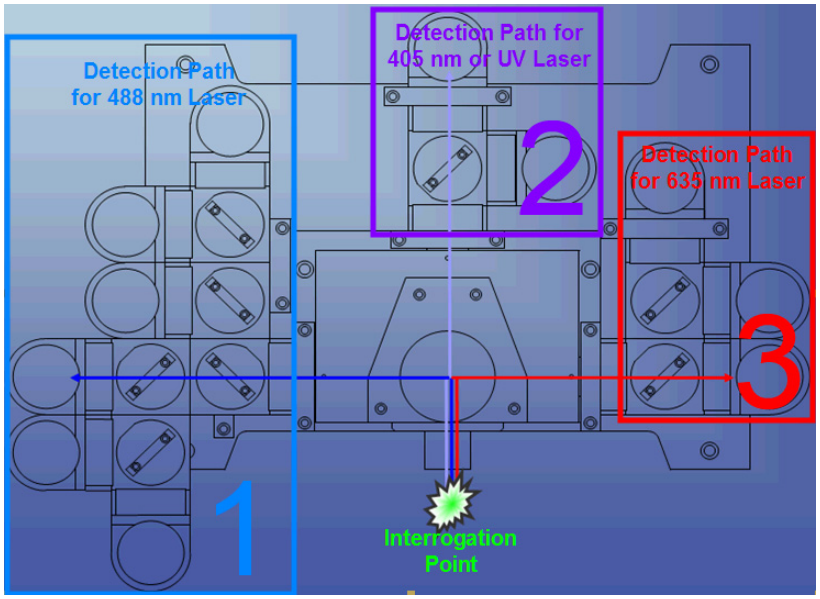
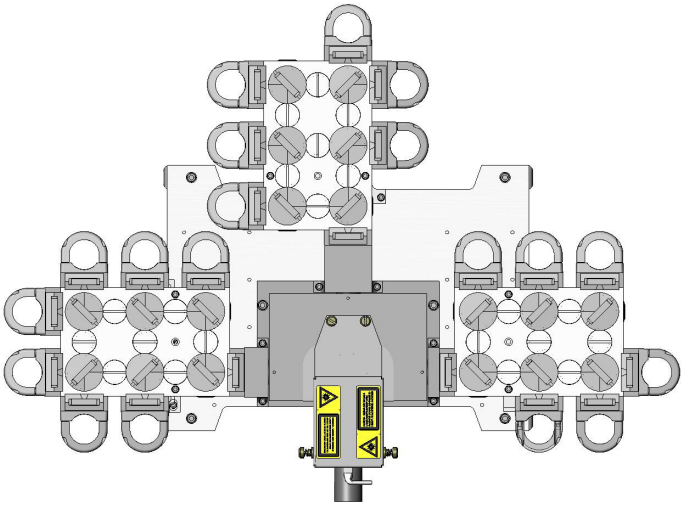


Figure 2.7 Detection Table with Detection PODs (2009 forward)



Detector Banks or Detection PODs

MoFlo XDP can be configured with up to three detector banks, or after 2009, up to three Precision Optical Detectors (PODs). A **Detector Bank** is associated with one laser, a single pinhole, and a beam directing prism (if required). A **POD** can be associated with a free-standing laser. As with the Detector Banks, one POD is associated with one pinhole. Each detector bank or POD may accommodate multiple PMTs, various dichroic mirrors, optical filters, and a collimating lens.

Detector Bank One, or POD One is located on the left side of the Detection Table and is associated with the first laser path, which typically hosts the 488 nm laser. **Detector Bank One, or POD One** includes the 90 degree Side Scatter Detector, which differs from the fluorescence detectors only because it is used with a 488 ±10 nm band-pass filter.

Detector Bank Two, or POD Two is located in the center of the Detection Table. **Detector Bank Three, or POD Three** is located the right of the table.

Photomultiplier Tube (PMT)

Photomultiplier Tubes accept emitted light, focus and multiply the signal, and convert the light into electrical current that is then output to the Preamplifier.

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.

- 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.

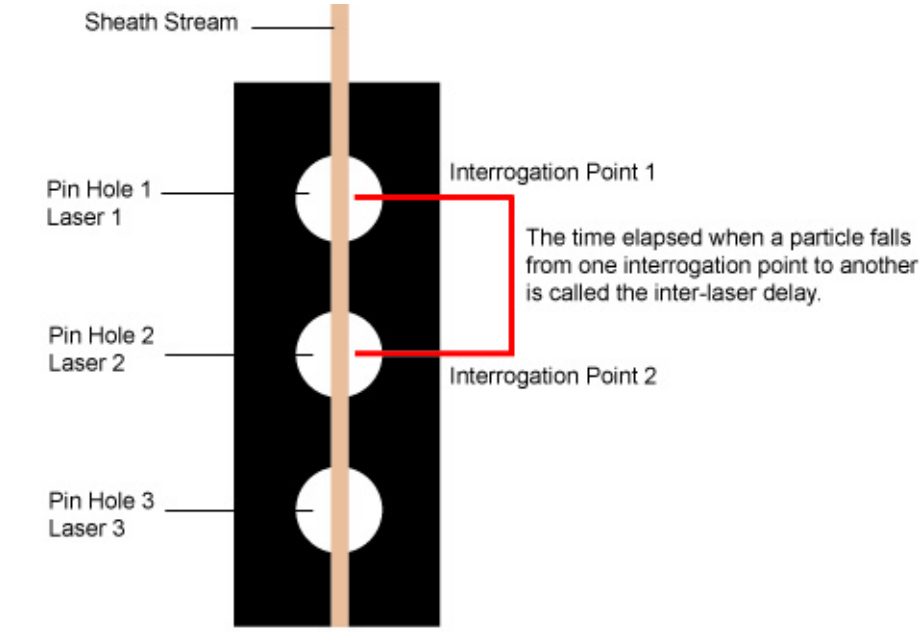
Standard 25 mm diameter short-pass and long-pass dichroic mirrors and band-pass optical filters are positioned at various points in each detector bank. These filters are selected to pass only the emission spectra that the PMT is intended to receive. Below are some examples of Detection Table configurations.

NOTE For instructions on how to align the Z-configuration filters see CHAPTER 5, *Z-configuration Filter Alignment Order*. For instructions on how to align POD filter configurations refer to the package insert that accompanied the filter set and read CHAPTER 5, *Detection POD Filter Alignment Overview*.

Pinhole Aperture Strip

The Pinhole Aperture strip is attached to the Detection Table directly behind the Collection Objective. Each pinhole is associated with one of the three light-detection pathways. The pinholes are designed as a mask to make possible a view of a single interrogation point and spatially separate emission spectra.

Figure 2.8 Pinhole Aperture Strip



Pinhole Camera

The Pinhole Camera makes it possible to view the Pinhole Apertures during instrument alignment. The image of the Pinhole Apertures can be viewed on the Coarse Alignment screen on the aXcess Control Panel.

Directing Prisms

Prisms are associated with the first and third Detection Banks. The prisms reflect emitted light 90 degrees to either the left or right Detection Bank. No prism is associated with the second Detection Bank pathway. Therefore, emitted light is permitted to pass directly into the second Detection Pathway.

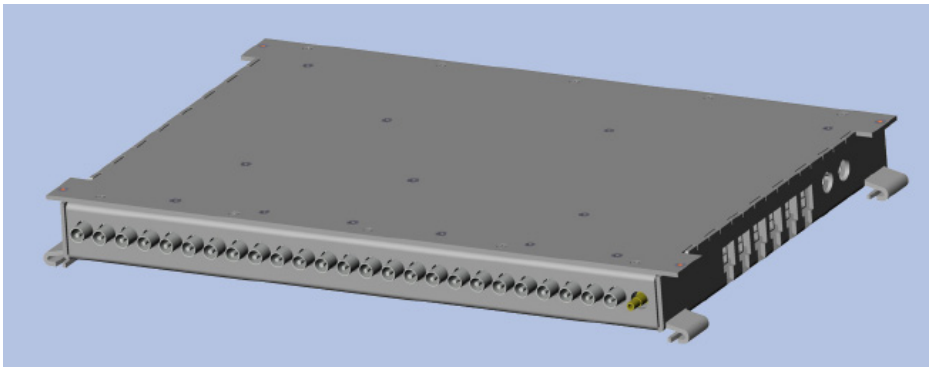
Collimating Lens

Emitted light is passed through a Collimating Lens immediately following the Directing Prisms. The collimated light signal permits signals of approximately equal intensity to reach each PMT along the detection path.

Preamplifier

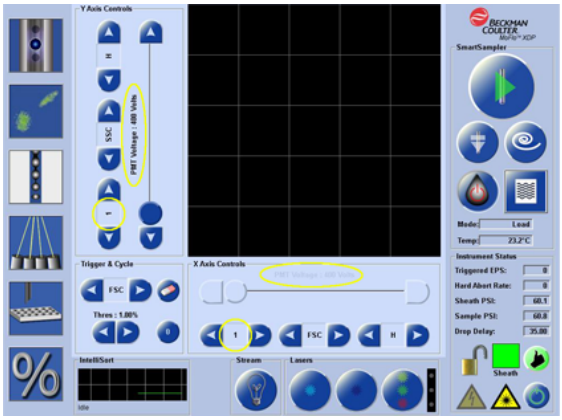
The Preamplifier module controls the PMTs to adjust detector gain, and converts the current output into a voltage for analysis by the ADC (Analog to Digital Converter) Cards. A special channel for interfacing to a photodiode is also provided for compatibility with normal Forward Scatter configurations. The preamplifier allows each channel to select the PMT input through software control. Each preamplifier can control and interface to 12 PMTs and one photodiode. Two preamplifiers are necessary to increase the number of detectors.

Figure 2.9 Preamplifier



The operator may use the aXcess Control Panel Fine Alignment screen or the Acquisition tab in Summit software to adjust each PMT voltage and gain.

Figure 2.10 Fine Alignment Screen and Summit Software Acquisition Parameters



Threshold (%) 2 Trigger FS

Name	Signal	Voltage	Gain	Path
FS	H/A	N/A	4	1
SS	H/A	400	1	1
FL1	H/L/W	400	1	1
FL2	L/A/W	540	1	1
FL3	L/LA/W	628	1	1
FL4	L/W	609	1	1
FL5	Disabled	650	1	1
FL6	Disabled	584	1	2
FL7	Disabled	449	1	2
FL8	Disabled	755	1	3
FL9	Disabled	678	1	3

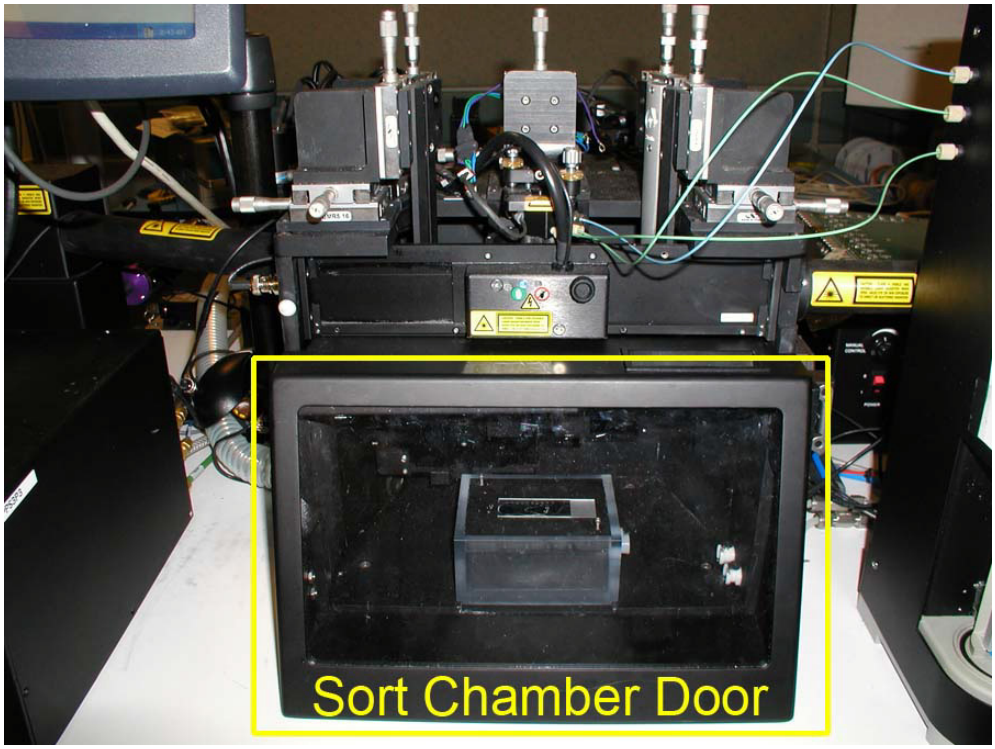
Cell Sorting Subsystem

The Cell Sorting Subsystem resides within the Illumination Table and consists of the Illumination Chamber, CytoNozzle, CyCLONE, Deflection Plates, Aerosol Containment Shield, Sort Receptacle, SortRescue, IntelliSort, and the optional Aerosol Evacuation Unit and Sample Cooling Kit. These components allow the user to separate particles of interest from the sample solution at high speed and great purity.

Aerosol Containment Shield

The Aerosol Containment Shield, also known as the Sort Chamber Door, is part of a passive aerosol containment assembly that acts to isolate the lower portion of the Illumination Table from the optical bench and from the laboratory. When closed, the door prevents gross movements of air into the sort chamber and provides a chamber that is easily decontaminated. When the Sort Chamber door opens, the safety interlock disables the voltage to the deflection plates.

Figure 2.11 Sort Chamber



IntelliSort and Droplet Camera

IntelliSort monitors and stabilizes the sorting process via the Droplet Camera that captures an image of the droplet stream for subsequent image analysis by software. When IntelliSort is enabled it monitors the precise location of the droplet break-off point and controls it during a sort. Several factors can alter the droplet break-off point including room temperature, fluid temperature, and pressure changes. If IntelliSort detects droplet instability, it will modify 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 the CyCLONE arm retracts to protect the sorted samples (with SortRescue).

Deflection Plates

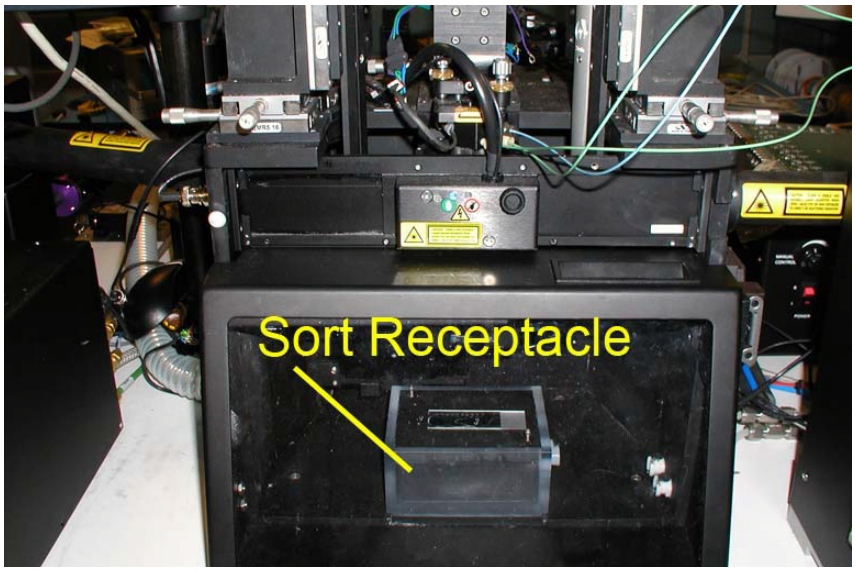
The Deflection Plates provide the electric field that deflects the individually charged droplets into the appropriate receptacles. These plates can be polarized with up to 4000 Vdc. Caution should be exercised when the plate voltage is enabled. The Sort Chamber door and the safety interlocks prevent access to the plates when they are energized.

Figure 2.12 Deflection Plates

Sort Receptacle

The Sort Receptacle is used during the sort setup procedure to optimize the deflection of sort streams. A number of lids are provided with the Sort Receptacle. When used without the lid during instrument alignment, the Sort Receptacle is used to catch any sheath that misses the waste-aspiration tube.

Figure 2.13 Sort Receptacle



CyCLONE

The CyCLONE is a computer driven robotic arm that permits the accurate, rapid deposition of sorted events into user-defined matrices. This unit accommodates several sizes of micro-well plates, including 96-, 384-, and 1536-well plates, as well as slides and tubes or custom plates and slides. Deposition of drops onto slides is easily defined for numerous combinations of rows and columns. The CyCLONE unit is also used for setting up Drop Delay.

Figure 2.14 CyCLONE with Slide

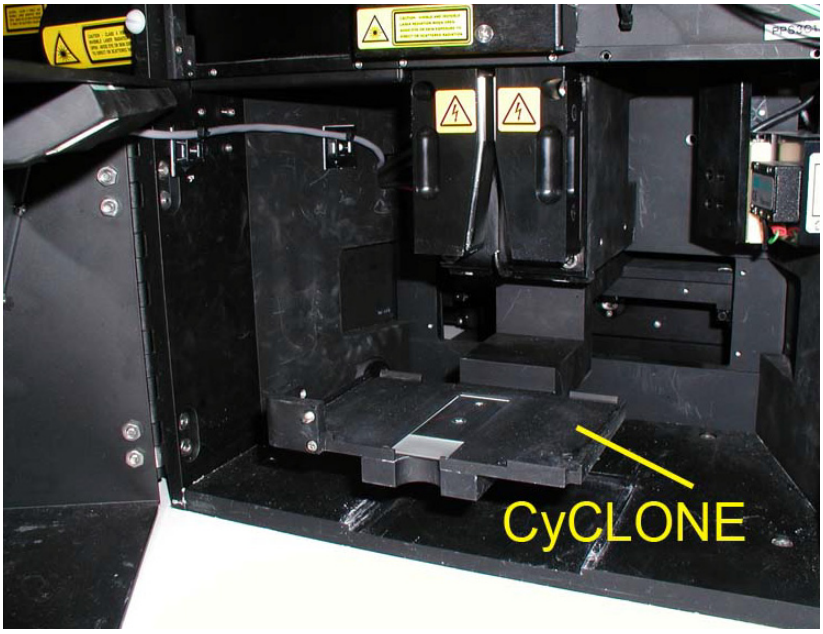
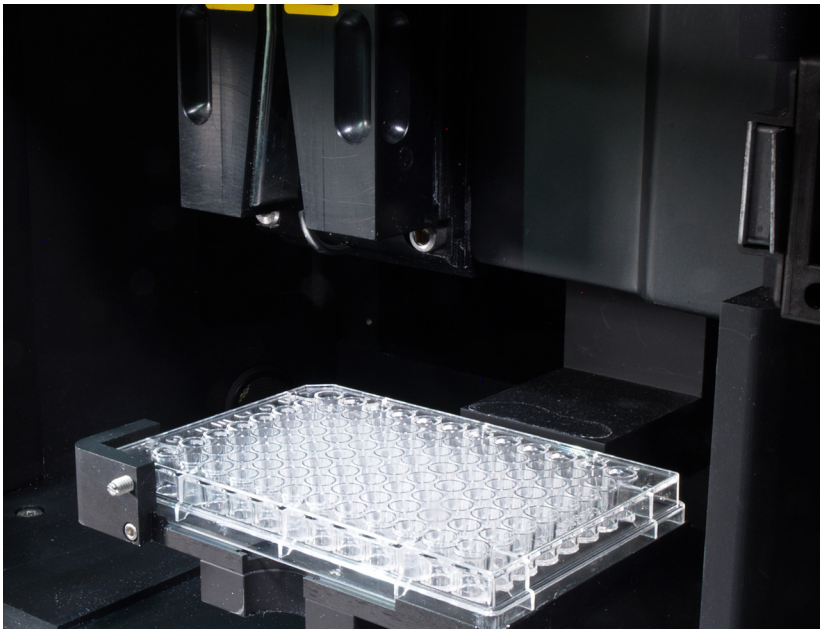


Figure 2.15 CyCLONE with Plate



SortRescue

The SortRescue is a four-way sort receptacle that mounts to the CyCLONE arm. SortRescue also has cooling capability to work with the optional sample cooling kit (Water Bath). In the event that IntelliSort detects a sort failure, the CyCLONE arm will retract in order to keep the SortRescue samples from being contaminated.

Figure 2.16 SortRescue

Water Bath and Sample Cooling Kit

The Water Bath makes it possible to maintain samples at a constant, regulated temperature that is defined by the operator. The Water Bath is a stand-alone unit that is placed on the floor next to the MoFlo XDP, or on the bench underneath the instrument. Refrigerated components may cause water condensation around the unit so avoid any slipping hazards. This component is optional.

Figure 2.17 Sample Cooling Kit (1)

Figure 2.18 Sample Cooling Kit (2)

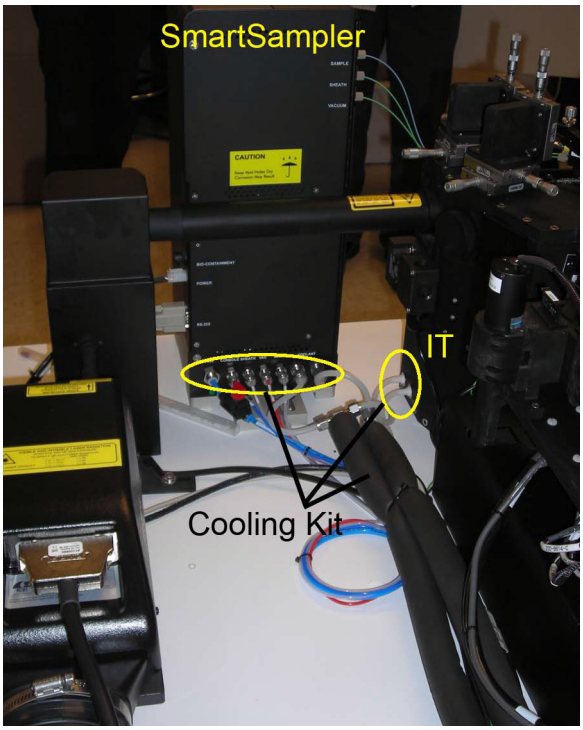
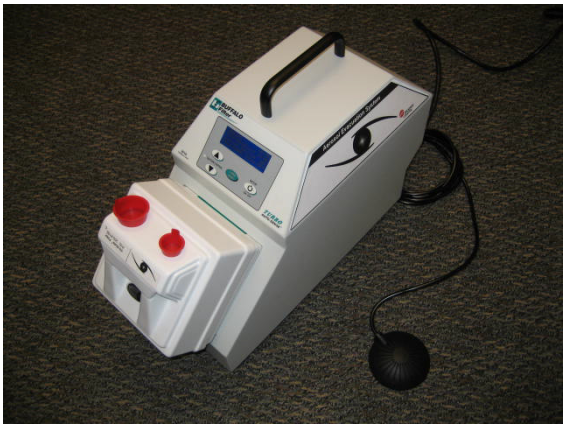


Figure 2.19 Water Bath Console

Aerosol Evacuation System

The optional Aerosol Evacuation System removes aerosols and micro droplets, which may be generated during the course of normal operation or sort failure mode conditions, from the sort chamber without disturbing sorting. The system features an ultra-quiet motor (40 dB Min and 69.5 dB Max) and a high-suction, high flow rate centrifugal action pump. Micro droplets and particulates greater than 0.12 µm are removed under vacuum and trapped in an Ultra Low Penetration Air (ULPA) filter. The air flow rate of the Aerosol Evacuation System is fully user adjustable, providing clearance of the sort chamber at rates of 10 to 40 complete air exchanges per minute. A single disposable filter is used on the system to simplify installation and removal of filters. The filter is completely enclosed to protect the operator from potential contamination when changing filters. This feature is optional.

Figure 2.20 Aerosol Evacuation System



Fluidics Subsystem

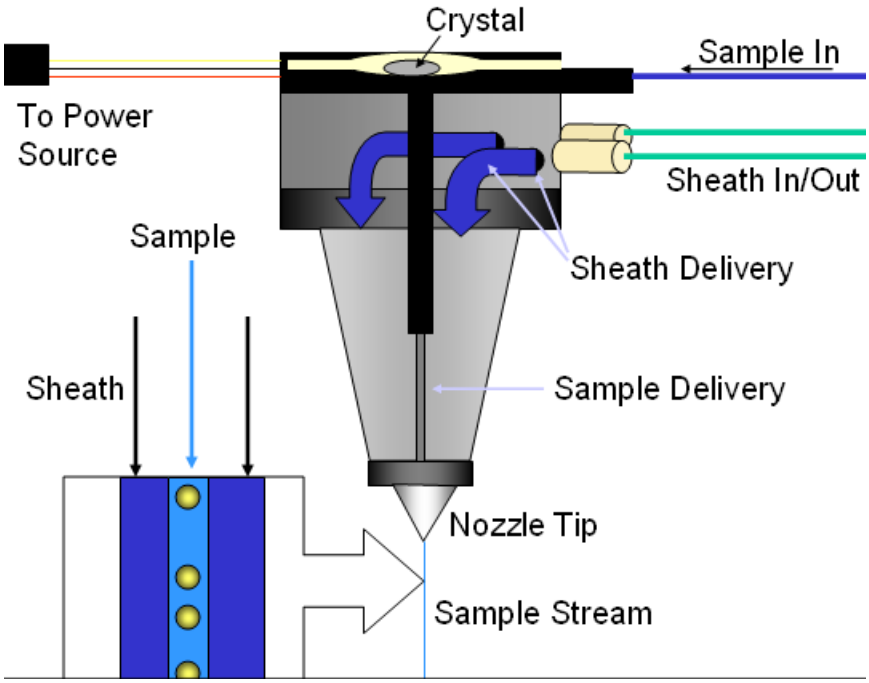
The Fluidics Subsystem consists of the sheath and waste subassemblies. Air pressure and vacuum to these subassemblies are controlled by the Pressure Console. The tubing, valves, connectors, tanks, gauges and regulators used in these subsystems are rated to withstand pressures of up to 100 psi. Exceeding this pressure may result in instrument failure and possible operator injury. Follow proper fluidics startup and shutdown procedures to assure the waste stream flows into the waste tank and monitor this during normal operation. The Manual Sample Station, the SmartSampler, and the CytoNozzle are also described in this section.

CytoNozzle

The CytoNozzle delivers sheath and sample to the laser interrogation point, charges the sheath and sample stream, and breaks the stream into droplets by use of a vibrating piezoelectric crystal. The

CytoNozzle can be fitted with a variety of nozzle tip sizes including 50, 70, 80, 90, 100, 120, 150 and 200 µm. Select a nozzle tip size three to five times larger than the particles in the sample.

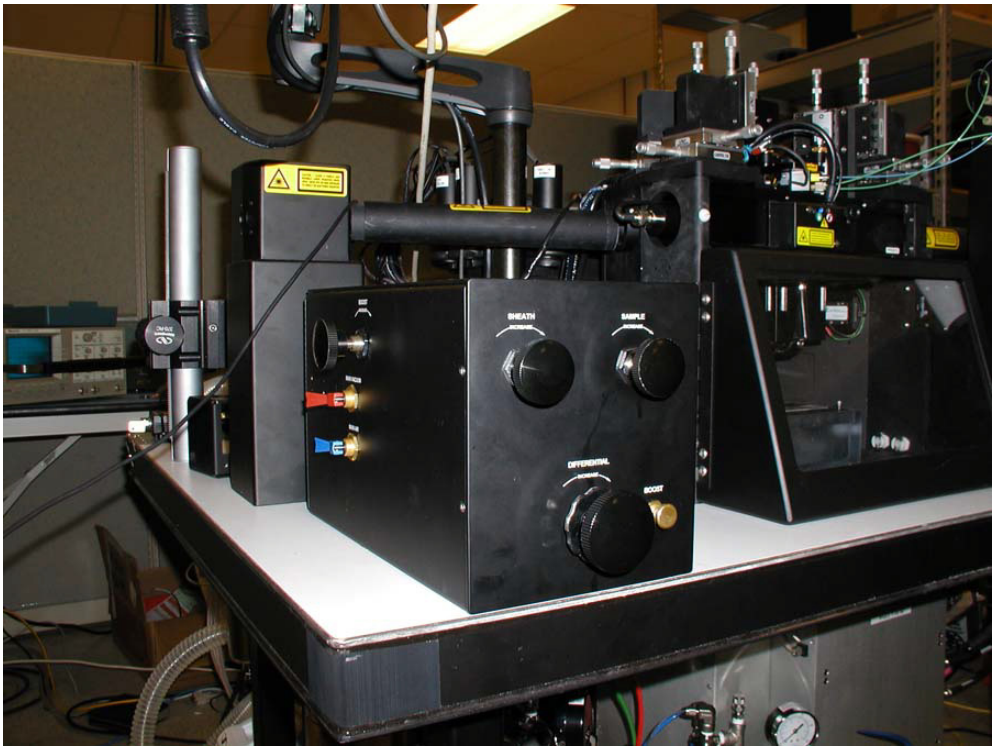
Figure 2.21 CytoNozzle Diagram



Pressure Console

The Pressure Console measures instrument air pressure and vacuum. It also regulates sheath pressure and sample pressure. Improved sensors and measurement devices increase resolution and accuracy, and allow software calibration of the pressure sensors and load cells. On the front of the pressure console are the boost button, and the adjustment controls for sample pressure, sheath pressure, and the fine adjustment for pressure differential. On the left side of the Pressure Console are the main vacuum and air switches and the adjustment knob for Boost pressure. Opening the red vacuum switch applies vacuum to the waste tank and the rest of the instrument. Opening the blue air pressure switch applies pressure to the sheath tank which drives the delivery of sheath fluid to the sample station. The Pressure Console delivers the sample to the instrument by applying a slightly greater pressure to the sample 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. This modest pressure differential ensures laminar fluid flow while minimizing the sample aspiration rate. Avoid using a pressure differential greater than 0.5 psi. This can disrupt laminar flow and reduce sort purity, recovery, and degrade %CV values.

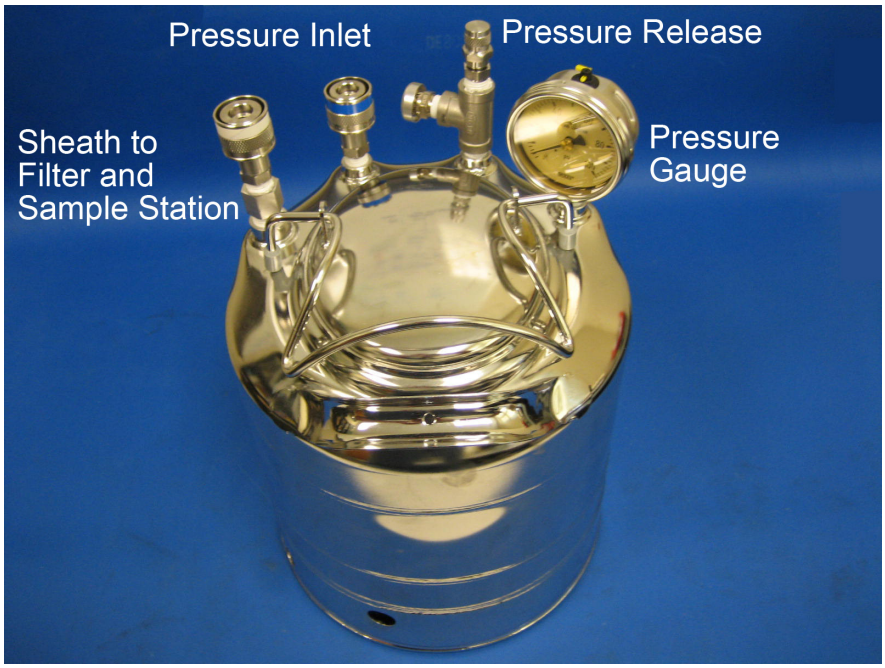
Figure 2.22 Pressure Console



Sheath Tank

Sheath fluid is stored in an autoclaveable two-gallon, electroplated, stainless-steel sheath tank located under the optical bench. 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. All fittings are provided with color-coded quick connects to enable reliable and fast connection. Sheath fluid is transported to the Manual Sample Station or SmartSampler through the clear sheath tubing. The sheath valves on the right side of the manual sample station or the aXcess Control Panel menu for the SmartSampler control the sheath flow. Between the sheath tank and either the Manual Sample block or the SmartSampler is an in-line sheath filter that filters particles larger than 0.2 µm.

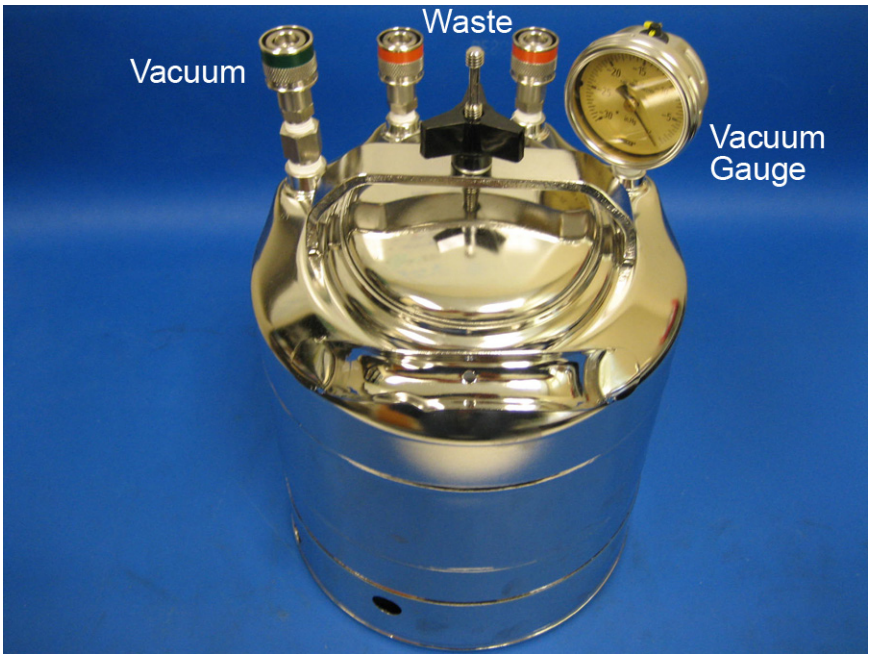
Figure 2.23 Sheath Tank



Waste Tank

The autoclaveable two-gallon waste tank is located under the optical bench next to the sheath tank. It is fitted with a vacuum gauge, two quick-connect fittings for waste fluid, and one for vacuum. Vacuum is regulated by the main vacuum switch on the pressure console. Opening the main vacuum switch applies vacuum to the waste tank and hence the rest of the instrument. Waste fluids are collected from the waste aspiration tube, from the left and right sheath/vacuum ports on the CytoNozzle upper body assembly, and from the waste ports on any of the connected sample handling options. All waste tubing on the system is red. Either orange quick connect can connect to either orange fitting on the tank. The vacuum tube is green.

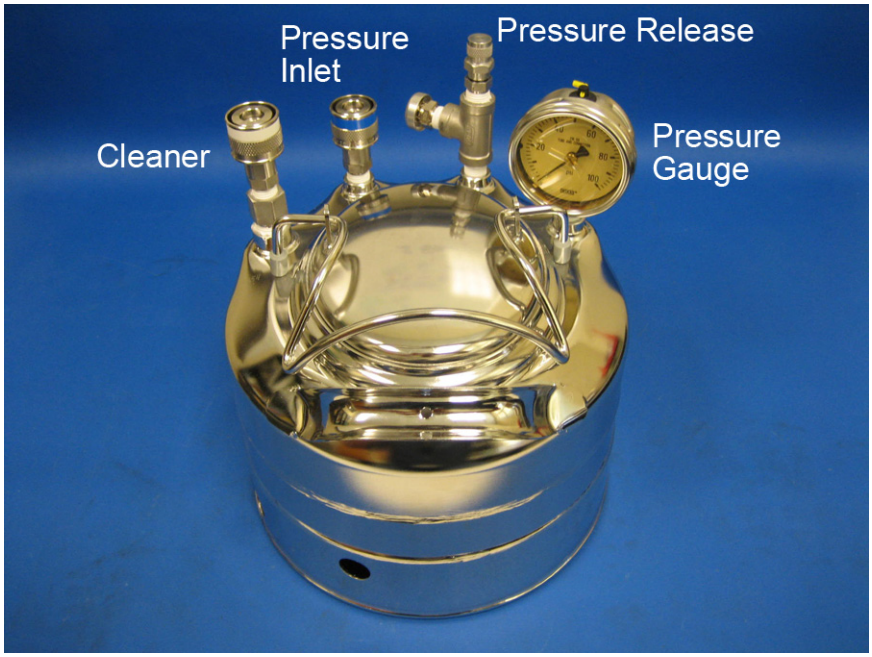
Figure 2.24 Waste Tank



Cleaner Tank

The one-gallon cleaner tank is an autoclaveable electroplated, stainless-steel tank located below the optical bench. The pressure gauge and relief valve, as well as fittings for cleaning fluid supply and pressure lines, are mounted on the cleaner tank. All fittings are provided with color-coded quick connects to enable reliable and fast connection. Cleaner fluid is transported to the SmartSampler through the clear tubing. The cleaner tank is only required when the instrument is paired with a SmartSampler.

Figure 2.25 Cleaner Tank



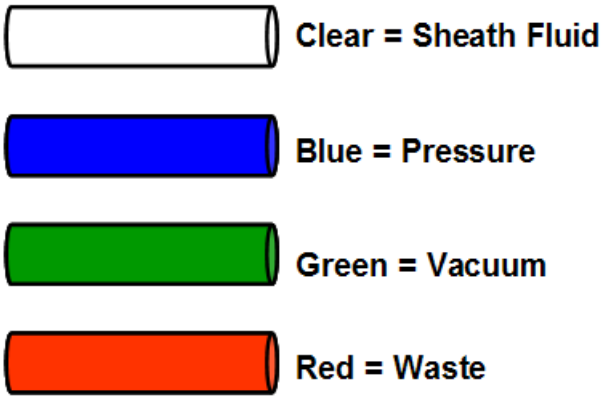
Tubing

Throughout the MoFlo XDP system is 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 sheath fluid; raw, filtered and unfiltered. It is also used for Rinse Agents with the SmartSampler.
- Blue - The blue tubing designates a pressure line that carries the air pressure specified on the aXcess Control Panel.
- Green - The green tubing carries 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 Sample Station and waste aspiration tube below the CytoNozzle.

Exceptions: The sheath tubing between the Sample Station and the CytoNozzle is green tubing. The sample tubing running from the Sample Station to the CytoNozzle is blue tubing. The green tubing and blue tubing are different in inner diameter (i.d.) and should not be interchanged.

Figure 2.26 Tubing



Manual Sample Station

The manual sample block is mounted to the MoFlo XDP optical bench, typically to the right of the Illumination Table. It accepts standard 5 mL 12 x 75 mm polypropylene tubes.

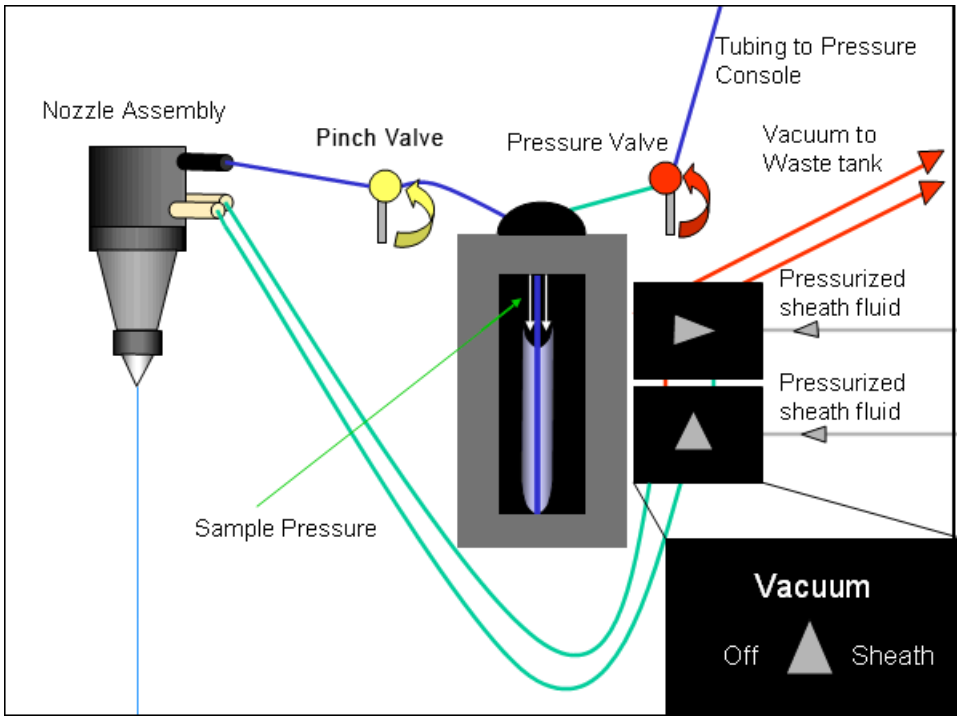
NOTE Do not use glass or polystyrene sample tubes due to the high pressure levels of the system.

Sample tubes are loaded into the tube block. The PEEK tubing has a 0.25 mm (0.010 in) internal diameter and less than 2.5 micro-liters dead volume.

Raising the tube block lever to its upright, locked position seals the sample tube. This seal, located in the sample cap, is maintained by an o-ring that contacts the outer neck of the tube and prevents sample contamination.

Pressure is applied to the sample by opening the red valve on the top of the manual sample block. This must be done prior to opening the pinch valve. Sheath valves are also located on the sample block. Sheath fluid is supplied to the CytoNozzle through dual sheath lines, each of which is controlled by a sheath valve. Each sheath valve may be set to one of three positions: off, sheath or vacuum. Located at the top of the sample cap is the kinetics port. This port facilitates the addition of sample or reagents to the sample tube without releasing the tube block lever. Located at the bottom of the tube block is a waste port that supplies vacuum to remove liquid waste. When one or both sheath valves are in the sheath position and the sample valve is in the open position, sheath fluid will backflush through the sample tubing into the tube block and down the waste port. If a sample is in place and the sample pressure valve is in the closed position, sheath fluid will dilute the sample. A diagram of the Manual Sample Station fluidics is shown below. The sheath, sample, and pressure valves allow you to analyze a sample, backflush, debubble, or unclog the nozzle tip.

Figure 2.27 Manual Sample Station Diagram



SmartSampler

The SmartSampler, which is operated via the aXcess Control Panel, provides support for operators performing long term, temperature controlled sorts. The SmartSampler can accommodate a large variety of sample tubes allowing for a more flexible sample handling option for the customer compared to the Manual Sample Station. The SmartSampler can be programmed to provide sample agitation to keep the sample homogeneously suspended. The Smart Sampler can be programmed to automatically boost the sample pressure when the SmartSampler is instructed to run a sample.

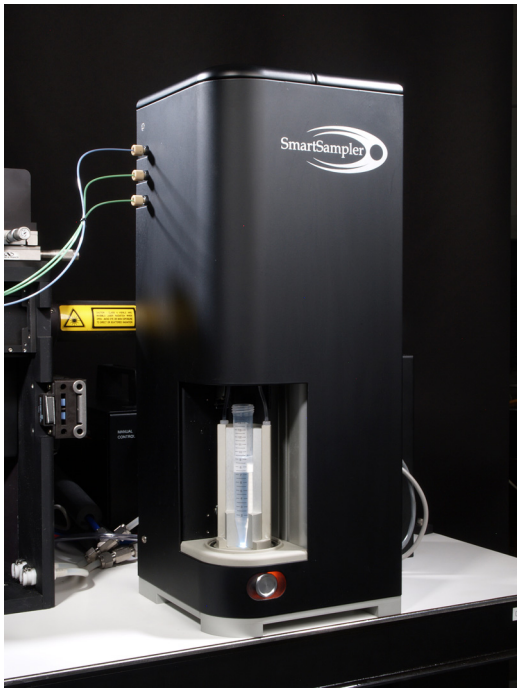
The SmartSampler has been designed to streamline sample handling operations that are normally performed manually by the instrument operator. Tube sizes from 0.5 to 50 mL can be easily accommodated by the SmartSampler. Other ease-of-use features include agitation and temperature control (through the use of a Water Bath), and single pushbutton operation for starting or stopping a run.

SmartSampler Features

SmartSampler features include:

- Accepts most commonly used tube formats
- Adjustable automatic agitation settings
- Automatic boost and backflush
- Single pushbutton start/stop operation
- Automated cleaning and shutdown
- Sample line air detection
- Debubble, unclog, clean, rinse, and drain
- Changeable probe
- Settings are available in Summit software and on the aXcess Control Panel

Figure 2.28 SmartSampler Photo



XDP Electronics

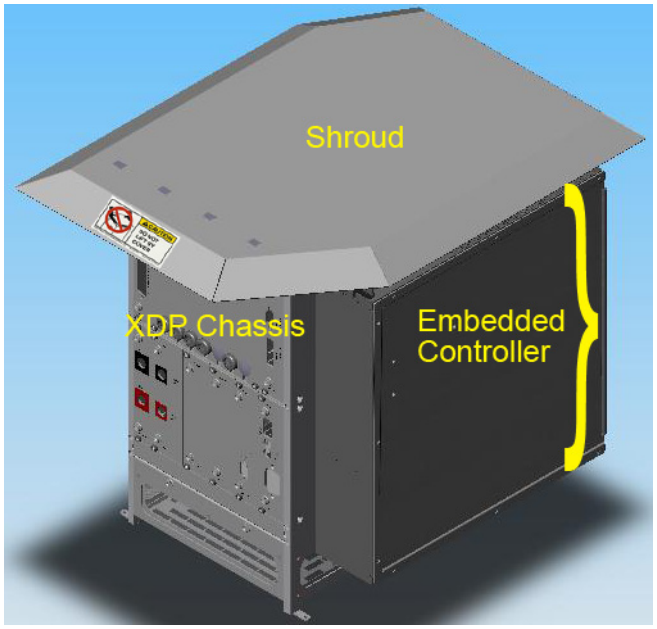
XDP Chassis

The XDP Chassis provides a backplane to connect all XDP Shelves and Cards. Power and control is distributed to tie system operation together. A microcontroller monitors system status and manages power sequencing of the various system components. This microcontroller is powered from the trickle supply provided by the AC Entry Shelf to monitor the system power switch and to provide a controlled system startup. A control cable links the XDP Chassis to the XDP Embedded Controller for status and control. A high-flow, high-reliability fan provides cooling for the entire chassis.

XDP Embedded Controller

The XDP Embedded Controller provides all instrument control and status of all system components, and contains a dedicated sort engine for high speed computation of sort decisions. Redundant, controllable fans cool the system internal components for reliable operation. The Embedded Controller also drives the aXcess Control Panel and controls interactions with the Summit Software Workstation.

Figure 2.29 XDP Electronics Drawing



CHAPTER 3

Cytometer Control Panel

aXcess Control Panel

The Cytometer Control Panel is the graphical user interface that allows you to interact with the XDP Electronics. The aXcess Control Panel is used for aligning and fine-tuning the instrument, as well as configuring sort streams and CyCLONE. During a sort, the aXcess Control Panel also displays sort statistics.

IMPORTANT The power button to the Embedded Controller is in the center on the front of the monitor. In general the system should remain turned on. If the system is not on, press the button to perform a Startup.

Although the system power should usually remain on, you can perform a controlled Shutdown by pressing the Shutdown button in the software. The hardware button on the monitor can be used to perform a Shutdown if the software is not available.

Figure 3.1 aXcess Control Panel Startup and Shutdown Buttons

Common Screen Elements

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

The buttons and display icons around the perimeter of the aXcess Control Panel are always visible. The elements on the left side of the screen include the selection buttons for the Coarse Alignment, Fine Alignment, Droplet control, Stream Configuration, CyCLONE Configuration, and Sort Statistics screens. Along the bottom of the aXcess Control Panel are the IntelliSort Status Display, Laser Control Panel selection button, and the Laser Shutter Controls. On the right side of the aXcess Control Panel are the SmartSampler buttons (if a SmartSampler is present), 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 3.2 aXcess Control Panel

1. Coarse Alignment screen (pinhole view)
2. Fine Alignment screen (dot plot)
3. Droplet Control screen
4. Stream Configuration screen
5. CyCLONE Configuration screen
6. Sort Statistics screen
7. IntelliSort view
8. Stream Illumination
9. Laser Shutter on/off for the laser directed to pinhole one.
10. Laser Shutter on/off or the laser directed to pinhole two
11. Laser Shutter on/off or the laser directed to pinhole three.
12. Shutter open/closed indicator
13. Controlled Shutdown button
14. Instrument Status Indicators
15. SmartSampler controls (Displayed only when a SmartSampler is installed.)
16. Area is gray because the elements within it are not common to other screens

Laser Control Tabs

The aXcess Control Panel displays a Laser Control Tab for each pinhole that is associated with a laser. Touch the appropriate tab to reveal the laser controls.

Figure 3.3 Laser Control Tab for a Free-standing 488 nm Laser Through Pinhole One

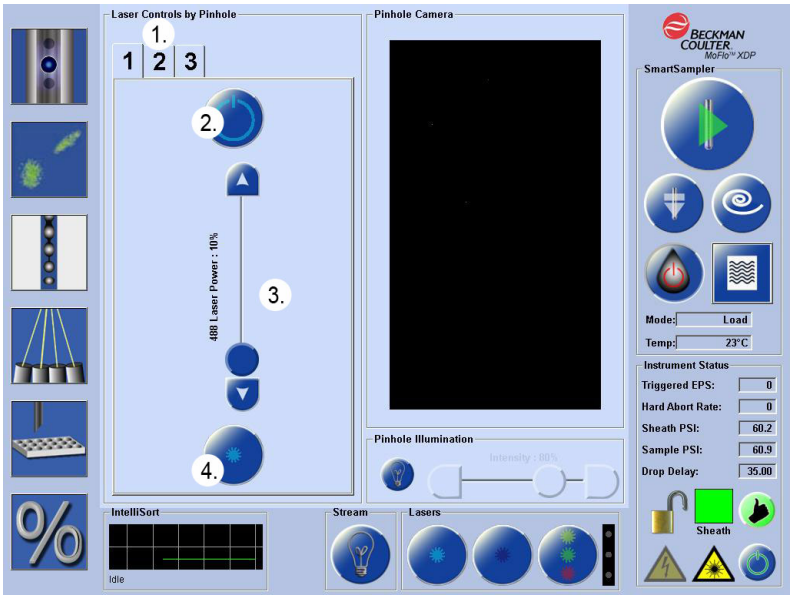


Table 3.1 Status Indicators - Screen Elements and Functions

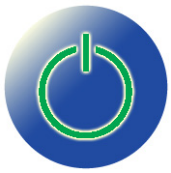
Screen Element	Function
	This symbol indicates that the instrument is ready for operation. • The safety interlock is closed. • The sheath tank contains an acceptable level of fluid. • No errors are detected in the hardware, software, or communications between the two. • No bubbles are detected in the sample line (With a SmartSampler installed).
	**IMPORTANT** Follow the standard Shutdown procedure before pressing this button. This button shuts down the entire MoFlo XDP instrument. Beckman Coulter recommends that the instrument remain powered on unless it will not be used for a significant period of time.
	This symbol indicates that the instrument is not ready. • Safety interlock is open. • The sheath does not contain enough fluid. • Errors are detected. (Press this button to view a screen that lists the error.)

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

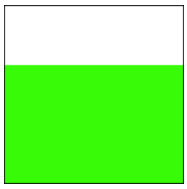
Screen Element	Function
	This symbol indicates that at least one safety interlock is open.
	This symbol indicates that the safety interlocks are closed.
	When this symbol is bright, high voltage is applied to the droplet stream and/or the charge plates. When this symbol is dim, high voltage is not applied.
	When this symbol is bright, a laser is powered and the corresponding shutter is open, or the red diode laser is on. 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 Red = Empty
Mode	This field contains the status of the SmartSampler. Off, Standby, Analyze, Load, Backflush, Debubble, Unclog, Rinse, Change Probe, Drain, Clean
Temp	Sample temperature
Triggered EPS	Number of triggered events that are detected per second
Hard Abort Rate	Rate of Hard Aborts
Sheath PSI	Sheath pressure at the nozzle
Sample PSI	Pressure applied to the sample
Drop Delay	Drop Delay value from Summit software

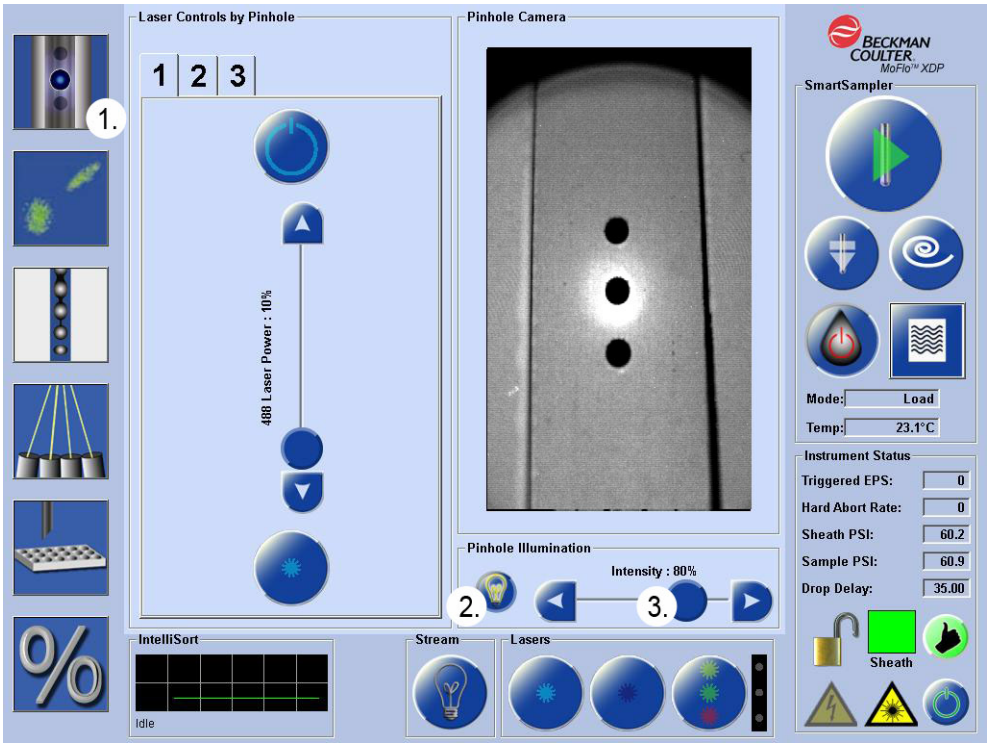
Table 3.1 Status Indicators - Screen Elements and Functions *(Continued)*

Screen Element	Function
IntelliSort	The IntelliSort Monitor in the lower-left portion of the screen allows you to observe the status of IntelliSort as well as a graph of the changes IntelliSort makes to amplitude to maintain droplet stability. **Status Messages** The following messages indicate the IntelliSort status: • Idle - The instrument is started, but IntelliSort is not enabled. • Preparing - After you press the IntelliSort button, IntelliSort prepares for approximately three minutes. Do not make instrument adjustments during this time. • Maintain - IntelliSort is maintaining drop delay. • Monitor - IntelliSort detected a minor instability in the system and is suspending control for up to one minute. • Monitor timeout - While monitoring, IntelliSort did not return to a stable state within the specified time and cannot continue to maintain. • Init Failed - IntelliSort could not start up the service correctly (communications or hardware failure). • System Settings - IntelliSort detected a change in the system settings (i.e. plate voltage turned off); cannot continue to maintain. • Drop Drive Voltage - IntelliSort reached the upper or lower bound of the adjustable drop drive amplitude range; cannot continue to maintain. • Image Error - Failed to capture image from camera. • No drops / streams - IntelliSort cannot detect drops or a valid stream image; cannot continue to maintain. • Top Drop Position - Top drop is not in the middle third of the camera image when IntelliSort was enabled; cannot prepare and maintain. • No objects found - IntelliSort cannot detect droplet objects in the image; cannot continue to maintain. • Motor error - IntelliSort could not initialize the camera motor. • Delay changed - The position of the top drop changed suddenly by more than one wavelength; drop delay may no longer be valid.

Coarse Alignment Screen

The Coarse Alignment Screen is used for initial alignment of the instrument. Press the Coarse Alignment button and then press the Pinhole Illumination button to view the image of the Pinhole Apertures while you are aligning the sheath stream and while you are positioning the bead flash in the appropriate pinhole.

Figure 3.4 Coarse Alignment Screen (Pinhole View)



1. Coarse Alignment screen
2. Pinhole Illumination on/off
3. Intensity Adjustment

Table 3.2 Coarse Alignment - Screen Elements and Functions

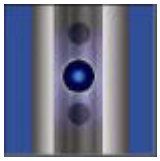
Screen Element	Function
Coarse Alignment Screen 	Displays the Coarse Alignment Screen.

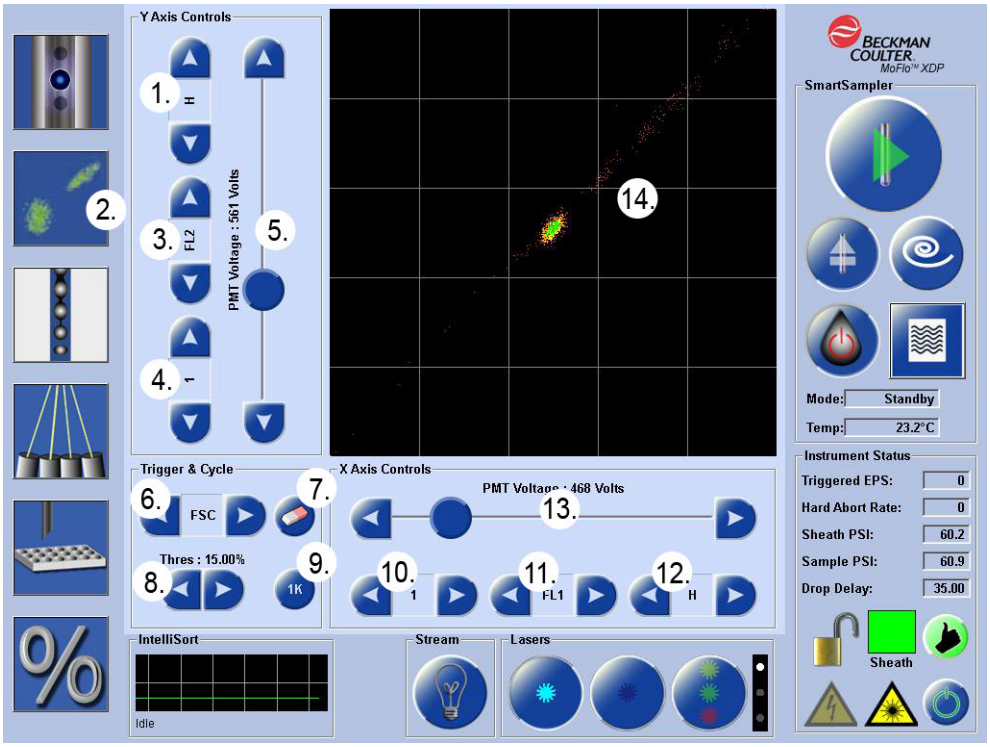
Table 3.2 Coarse Alignment - Screen Elements and Functions

Screen Element	Function
Pinhole Illumination 	Turns on and off the light that illuminates the pinhole apertures.
Intensity Control (slider control)	Dims and brightens pinhole illumination.

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 icon 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 3.5 Fine Alignment Screen (Dot Plot View)



1. Y-axis Data Type
2. Fine Alignment Screen
3. Y-axis Parameter
4. Y-axis Gain
5. PMT Voltage Control (Y-axis parameter)
6. Trigger Parameter
7. Clear Displayed Events
8. Threshold Setting
9. Data Cycle Rate
10. X-axis Gain
11. X-axis Parameter
12. X-axis Data Type
13. PMT Voltage Control (X-axis parameter)
14. Dot Plot Data Display

Table 3.3 Fine Alignment - Screen Elements and Functions

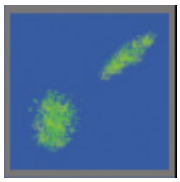
Screen Element	Function
Fine Alignment Screen 	Displays the Fine Alignment screen.
Adjust PMT Voltage (slider control)	Adjusts voltage for the PMT that is associated with the selected parameter.
Select a Parameter 	Selects the parameter for the corresponding axis. FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6, FL7, FL8, FL9, FL10
Select a Data Type 	This is the data type displayed on the aXcess Control Panel but it does not reflect data type set for acquiring data in Summit software. The aXcess 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 page 4-9. H = linear height L = log height A = linear area LA = log area W = pulse width
Adjust Gain 	Adjusts the gain on the PMT in increments of 1 with a range of 1 - 100.
Select Trigger 	Selects the trigger parameter, which is the height signal. It is common for Forward Scatter to be the trigger parameter, but 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 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.

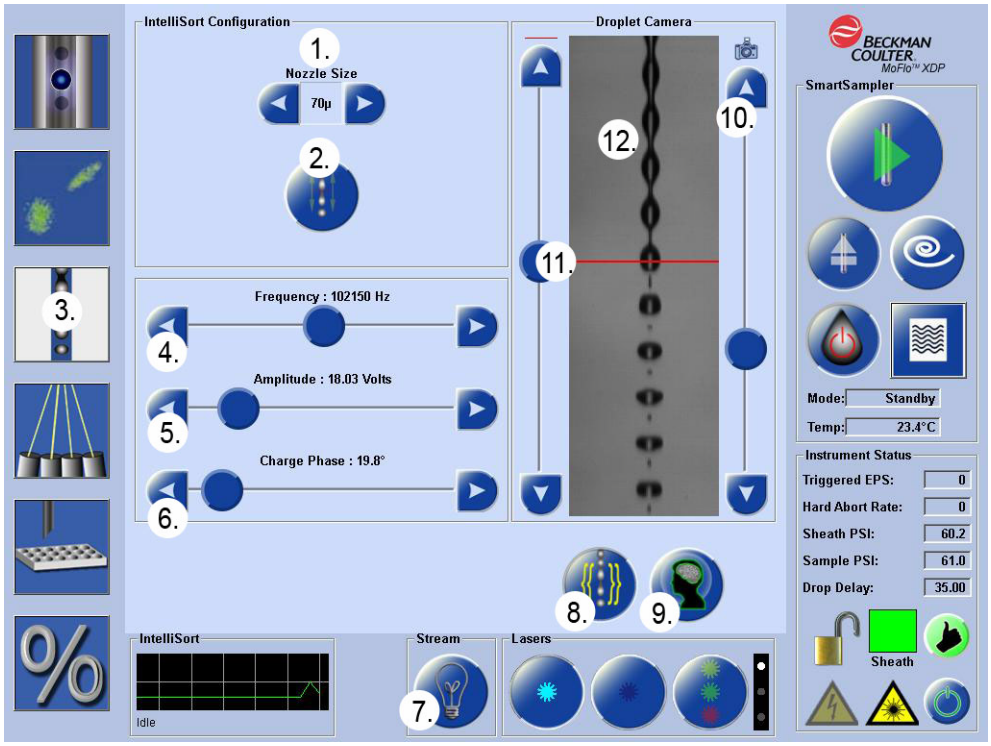
Table 3.3 Fine Alignment - Screen Elements and Functions *(Continued)*

Screen Element	Function
Cycle Rate 	Sets the cycle mode to 100, 1000 (1K), or 5000 (5K) events.
Data Clear 	Clears data and refreshes the aXcess Control Panel.

Droplet Control Screen

The Droplet Control screen is used for creating and viewing droplets. When the Drop Drive (DD) button is pressed, DD Amplitude and DD Frequency are applied to the piezoelectric crystal in the nozzle body. The Drop Drive button also causes infrared LEDs to strobe, enabling the Droplet Camera to produce an image of the droplet stream. The Droplet Camera can be raised or lowered in order to find the last attached drop in the stream.

Figure 3.6 Droplet Control Screen



1. Enter Nozzle Size
2. IntelliSort Camera Reset
3. Droplet Control screen
4. Frequency
5. Amplitude
6. Charge Phase
7. Sort Chamber Illumination on/off
8. Drop Drive on/off
9. IntelliSort on/off
10. Move Droplet Camera
11. Last Attached Drop Marker
12. Droplet Image

Table 3.4 Droplet Control - Screen Elements and Functions

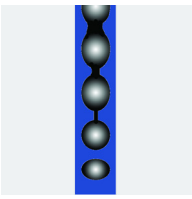
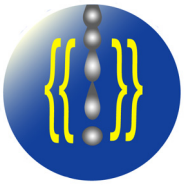
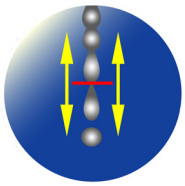
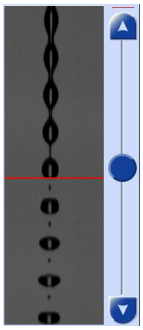
Screen Element	Function
Droplet Control Screen 	Displays the Droplet Control screen.
Nozzle Size 	Set a new nozzle size only if the nozzle has been changed.
IntelliSort on/off 	Enables or disables IntelliSort.
Drop Drive on/off 	Turns on and off the piezoelectric crystal in the nozzle that vibrates to form droplets.
IntelliSort Camera Reset 	Resets IntelliSort camera if necessary.
Frequency (slider control)	Controls the Drop Drive frequency. (The rate with which the crystal vibrates.)
Amplitude (slider control)	Adjusts the Drop Drive amplitude. (The force with which the crystal vibrates.)
Charge Phase	The test pattern must be enabled before you adjust Charge Phase. Adjust the value to achieve the tightest side streams.

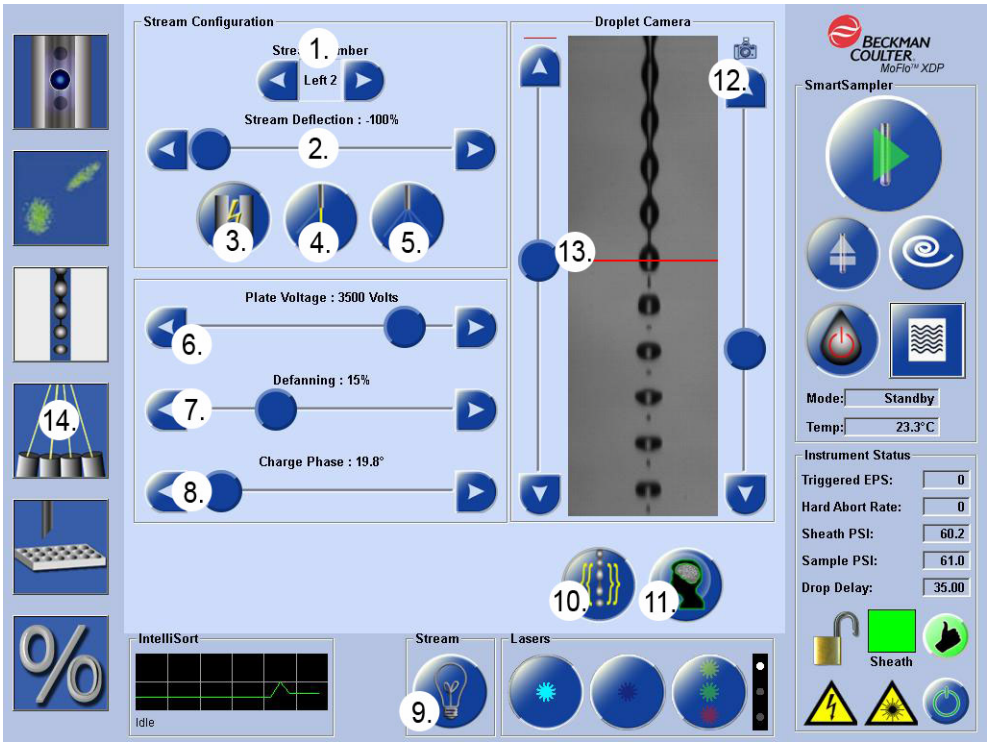
Table 3.4 Droplet Control - Screen Elements and Functions *(Continued)*

Screen Element	Function
Camera controls 	Moves the droplet camera.
Set Red Line 	It is optional to move the red marker to the Last Attached Drop to create a reference for viewing stream stability.

Stream Configuration Screen

The Stream Configuration screen allows you to individually select and adjust sort streams prior to beginning a sort. This screen is also used to control Deflection Plate voltage, Charge Phase, Defanning, Stream Test Pattern, and Drop Drive.

Figure 3.7 Stream Configuration Screen



1. Select Stream
2. Stream Deflection Percentage
3. Power Charge Plates on/off
4. Stream on/off
5. Test Pattern on/off
6. Plate Voltage Adjustment
7. Defanning Adjustment
8. Charge Phase Adjustment
9. Sort Chamber Illumination on/off
10. Drop Drive on/off
11. IntelliSort on/off
12. Move Droplet Camera
13. Last Attached Drop Marker
14. Stream Configuration screen

Table 3.5 Stream Configuration - Screen Elements and Functions

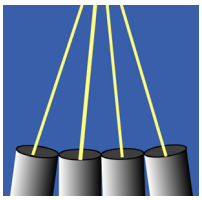
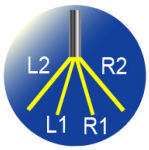
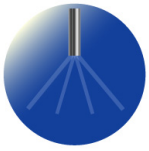
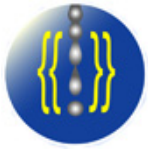
Screen Element	Function
Access Stream Configuration Screen 	Displays the Streams Configuration screen.
Stream Number 	Selects the stream number for which you intend to adjust deflection. Left 2 = stream furthest left of the waste receptacle Left 1 = stream directly to the left of the waste receptacle Center = stream directly into the waste receptacle Right 1 = stream directly to the right of the waste receptacle Right 2 = stream furthest right of the waste receptacle  **NOTE** The graphic above is only for clarification. It does not appear on screen.
Stream Deflection (slider control)	Adjusts stream deflection percentage.
Stream Deflection on/off 	Enables deflection for the selected stream.
Plate Voltage (slider control)	Selects the voltage that is applied to the Deflection Plates.
Defanning (slider control)	Adjusts the value to achieve the tightest waste stream. The test pattern should be enabled before you adjust Defanning.
Charge Phase (slider control)	Adjusts the value to achieve the tightest side streams. The test pattern should be enabled before you adjust Charge Phase.

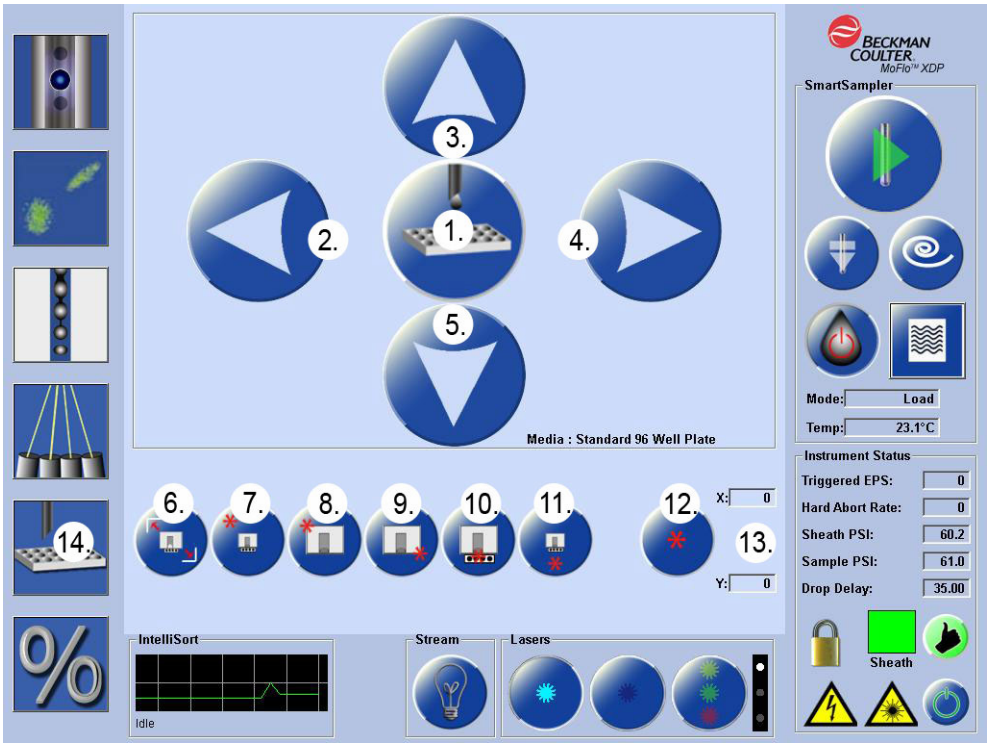
Table 3.5 Stream Configuration - Screen Elements and Functions *(Continued)*

Screen Element	Function
Charge Plates on/off 	Turns on and off the voltage to the Deflection Plates.
Test Pattern on/off 	Turns on and off the charge applied to the streams. Enables the stream(s) previously set up with the stream configuration controls.  
Drop Drive on/off 	Turns on and off the piezoelectric crystal in the nozzle that vibrates to form droplets.

CyCLONE Configuration Screen

The CyCLONE Configuration screen is used to specify the size and location of a plate, slide, or tube. When the Find Limits button is pushed, the CyCLONE determines the limits of the receptacle medium and then the remaining buttons are enabled. This screen allows you to set, change, and test positions such as home, end, tubes, load, and park.

Figure 3.8 CyCLONE Configuration Screen



1. Squirt Fluid
2. Moves CyCLONE left
3. Moves CyCLONE back
4. Moves CyCLONE right
5. Moves CyCLONE forward
6. Find Extents
7. Park
8. Home
9. End
10. Tubes
11. Load
12. Set
13. Numerical Position
14. CyCLONE Configuration Screen

Table 3.6 CyCLONE Configuration - Screen Elements and Functions

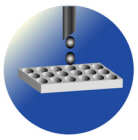
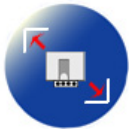
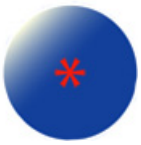
Screen Element	Function
Directional 	Moves the nozzle to a specific X or Y coordinate. When the CyCLONE reaches its mechanical limit in a particular direction the button will be inactive and grayed out.
Squirt 	Squirts fluid when testing the accuracy of a CyCLONE position.
Find Limits 	Reinitializes CyCLONE if it loses its calibration.
Park 	Sends the CyCLONE to the stored Park position. This moves the mechanical arm of the CyCLONE back and out of the way. If you want to set a new park position, press the directional buttons until the CyCLONE moves to the desired position and then press the set button.
Home 	Sends the CyCLONE to the stored Home position. If you want to set a new home position, press the directional buttons until the CyCLONE moves to the desired position and then press the set button.
End 	Sends the CyCLONE to the stored End position. If you want to set a new end position, press the directional buttons until the CyCLONE moves to the desired position and then press the set button.
Tubes 	Sends the CyCLONE to the stored Tubes position. If you want to set a new tubes position, press the directional buttons until the CyCLONE moves to the desired position and then press the set button.

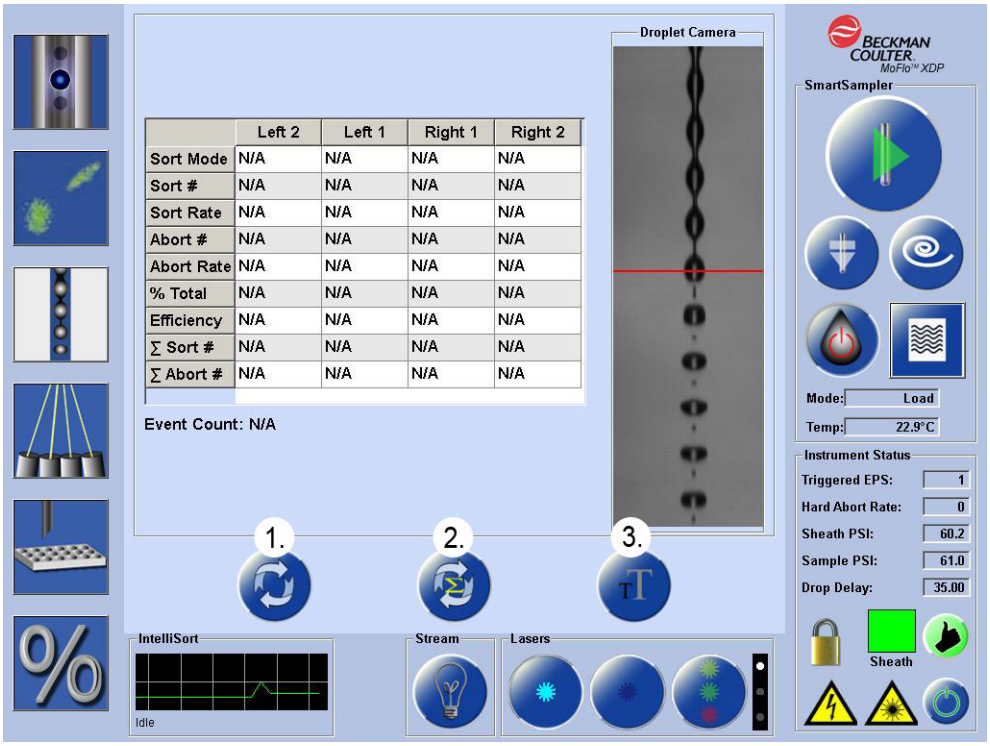
Table 3.6 CyCLONE Configuration - Screen Elements and Functions *(Continued)*

Screen Element	Function
Load 	Sends the CyCLONE to the stored Load position. This moves the mechanical arm of the CyCLONE forward so that you may load test tubes or plates. If you want to set a new load position, press the directional buttons until the CyCLONE moves to the desired position and then press the set button.
Set 	Stores the new XY 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 fields	These fields display the numerical X and Y coordinates of the current position of the CyCLONE.

Sort Statistics Screen

The Sort Statistics screen allows you to view in large or small format the sort statistics for each stream. The screen below displays the large format. The small format display includes a droplet camera image.

Figure 3.9 Sort Statistics Screen



1. Refresh Screen
2. Refresh Total Sort and Abort Counts
3. Change Font Size

Table 3.7 Sort Statistics - Screen Elements and Functions

Screen Element	Function
Left 2	The outermost stream to the left of the waste receptacle.
Left 1	The stream directly to the left of the waste receptacle.
Right 1	The stream directly to the right of the waste receptacle.
Right 2	The outermost stream 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 #	Total positive events that have been sorted for the stream.

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

Screen Element	Function
Sort Rate	Total sorted events per second for the stream.
Abort #	Total positive events that have been aborted for the stream.
Abort Rate	Total aborted events per second for the stream.
% Total	The percent of positive sorted events relative to the Total Events for the acquisition.
Efficiency	The number of positive events sorted, divided by the total events that could have been sorted for the stream. sorted/(sorted+aborted)
∑ Sort Number	Total Sorted Events
∑ Abort Number	Total Aborted Events
Refresh	Press this button to clear the sort statistics that display in the grid.
Font Size	Press this button to change the font in the grid to large format. In large format mode the droplet image is not displayed. Press the button again to change back to small format.

SmartSampler Screen

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

Figure 3.10 SmartSampler Abbreviated Menu

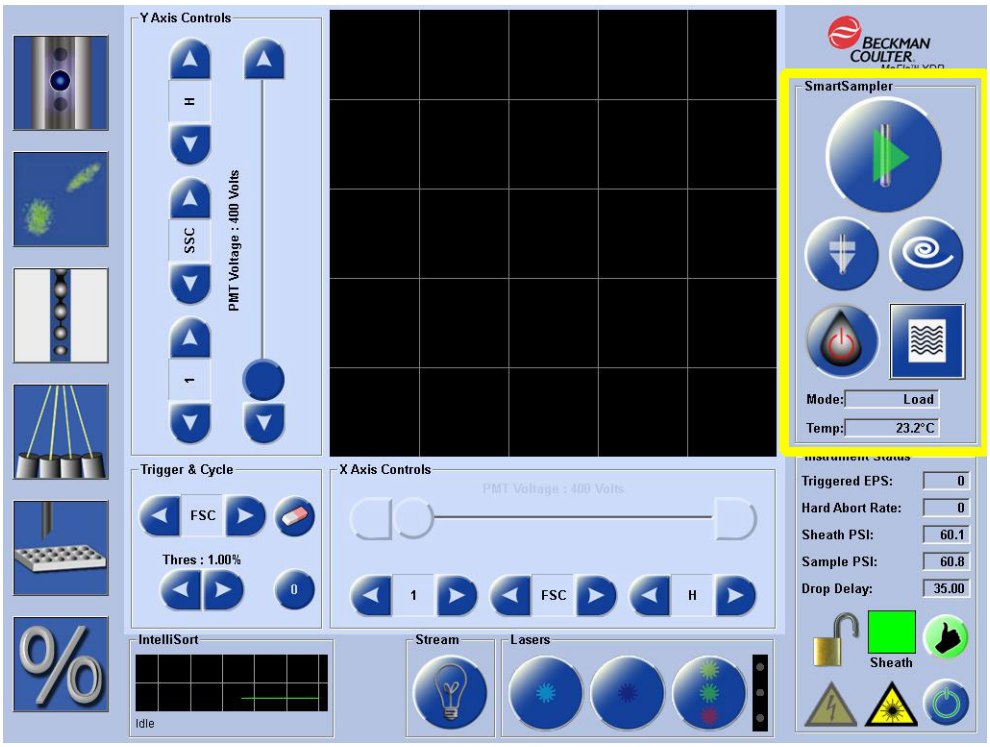


Figure 3.11 SmartSampler Full Menu

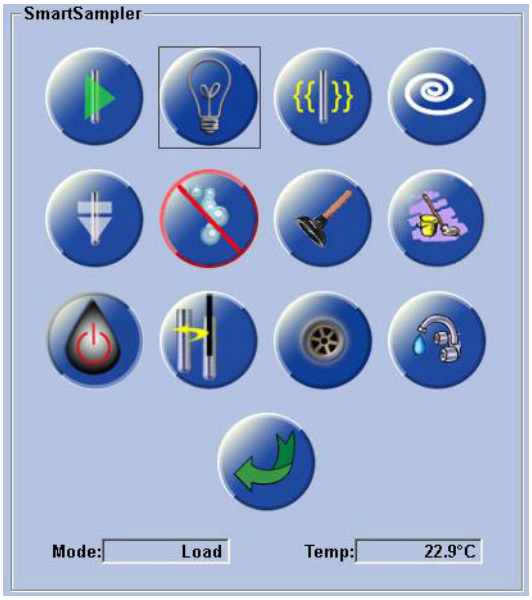


Table 3.8 SmartSampler - Screen Elements and Functions

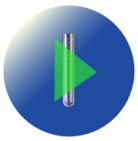
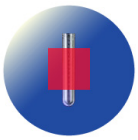
Screen Element	Function
	**SmartSampler Menu button** Displays a dialog box that contains the following buttons: • Start/Stop Sample • Sample Illumination on/off • Agitate Sample • Backflush • Chamber up/down • Debubble • Unclog • Clean and Rinse • Rinse • Start/stop Fluidics • Change Probe • Drain • 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, Clean.
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 3.8 SmartSampler - Screen Elements and Functions

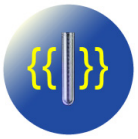
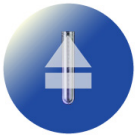
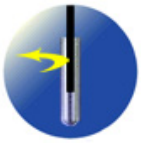
Screen Element	Function
Agitate Sample 	The chamber must be closed for this button to be active. Press this button to a agitate the sample until the button is pressed again to stop agitation. If the chamber opens, agitate will automatically stop.
Backflush 	When the chamber is closed and Backflush is pressed, sample stops flowing. You must open the chamber to backflush. Press Backflush to: • Draw fluid back through the sample line and vacuum it to the waste tank. • Backflush stops after a user-defined time.
Open Chamber 	Press this button to: • Open the chamber. • Depressurize the sample. • Close the pinch valve. • Automatic backflush if set in Summit software.
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: • Debubble until button is pressed again. • Close the pinch valve if it is open. • Alternate vacuum and sheath between the two sheath lines that attach to the nozzle.
Unclog 	Press this button to: • Apply vacuum to both sheath lines at the same time. Some fluid should be held under the nozzle tip. • Close the pinch valve if it is open.

Table 3.8 SmartSampler - Screen Elements and Functions

Screen Element	Function
Clean and Rinse 	• The pinch valve stays closed and vacuum is on. • Press this button to: • Clean the outside of the probe. The probe chamber moves up and down as it cycles Cleaner through the wiper body. • Rinse the outside of the probe. The probe chamber moves up and down as it cycles sheath through the wiper body.
Start/Stop Fluidics 	Press this button to automatically perform the following tasks when you first start up the instrument: • Start sheath flow. • Debubble. • Backflush. To Shutdown, push this button again. Follow the on-screen prompts to clean, rinse and shutdown the fluidics.
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 9-14.
Drain 	Press this button to drain fluid from the sample chamber to waste if necessary.
Rinse 	Press this button to rinse the sample probe.
Return 	Press this button to return to the previous screen.

CHAPTER 4

Summit Software

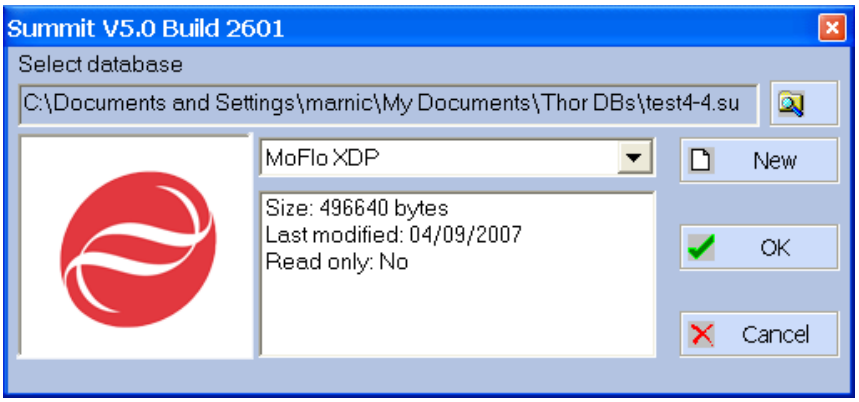
Overview

Summit software is a Windows based application that has a series of menus, hot keys, and buttons which allow 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, and auto-compensate data.

How to Open Summit Software

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

Figure 4.1 Select Database Dialog Box



2 Select **MoFlo XDP** from the dropdown 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.

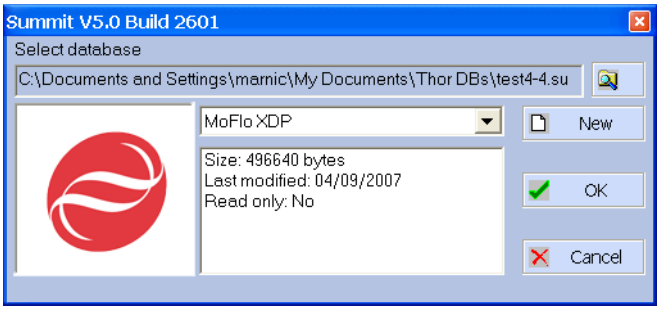
Summit Software Database

A Summit software database is a collection of protocols, samples, and data. 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

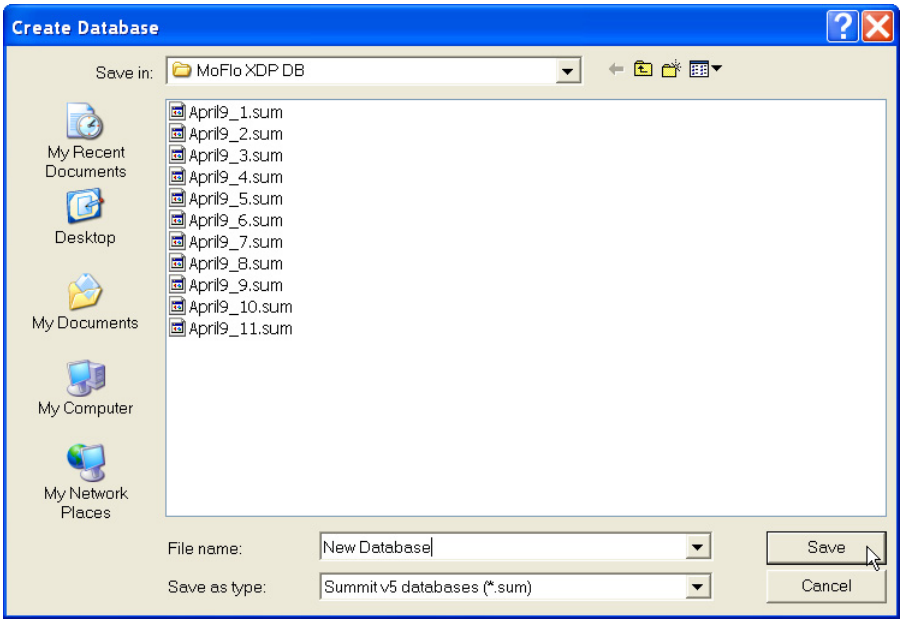
1 Open Summit Software and click **New.**

Figure 4.2 Select Database Dialog Box



The **Create Database** dialog box appears.

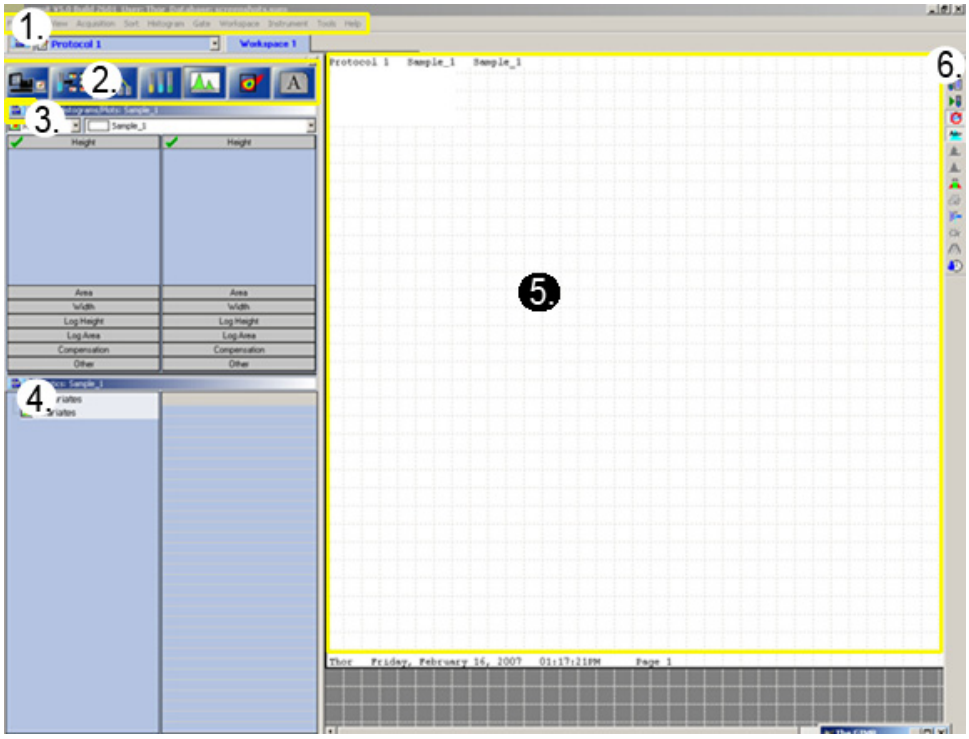
Figure 4.3 Create Database Dialog Box



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



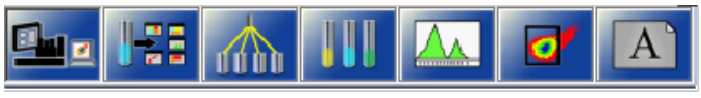
1. Summit software Main Menu
2. Summit Control Panel
3. Additional Menu
4. Additional Menu
5. Workspace
6. Toolbar Icons

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)



When a SmartSampler is Connected

Instrument Tab

The Instrument tab is active when a SmartSampler is connected to the MoFlo XDP.

SmartSampler Settings

Figure 4.6 Instrument Tab

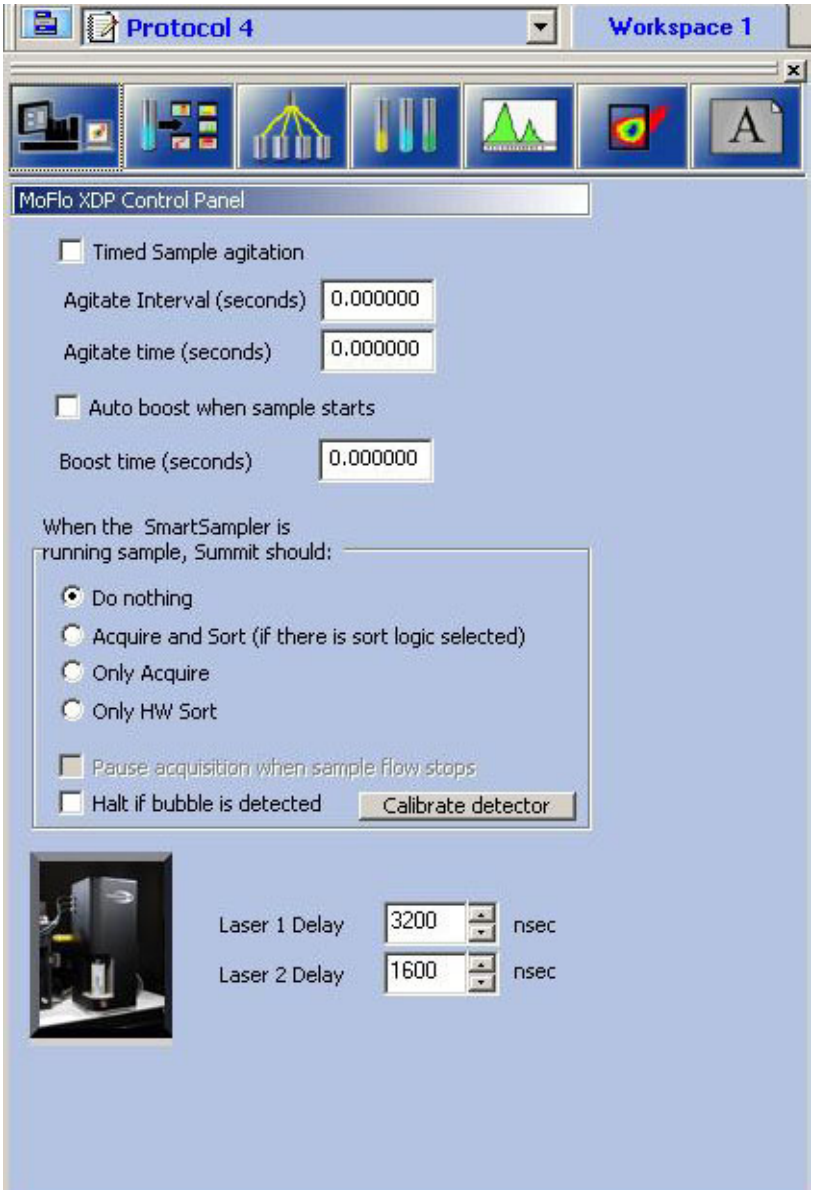


Table 4.1 Instrument Tab - Screen Elements and Functions

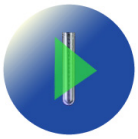
Screen Element	Function
Timed Sample agitation Agitate Interval Agitate Time	Select the checkbox when you want to specify the agitate time and the interval between agitations. **NOTE** When the checkbox is not selected, the SmartSampler Agitate button must be turned on and off manually through the aXcess 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 (See Figure 3.10), Summit software should: 	
1. Do nothing (See Figure 4.6)	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 4.6)	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 4.6)	When the Start Sample button is pressed, the sample flows, and data is acquired in Summit software.
4. Only HW Sort (See Figure 4.6)	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.

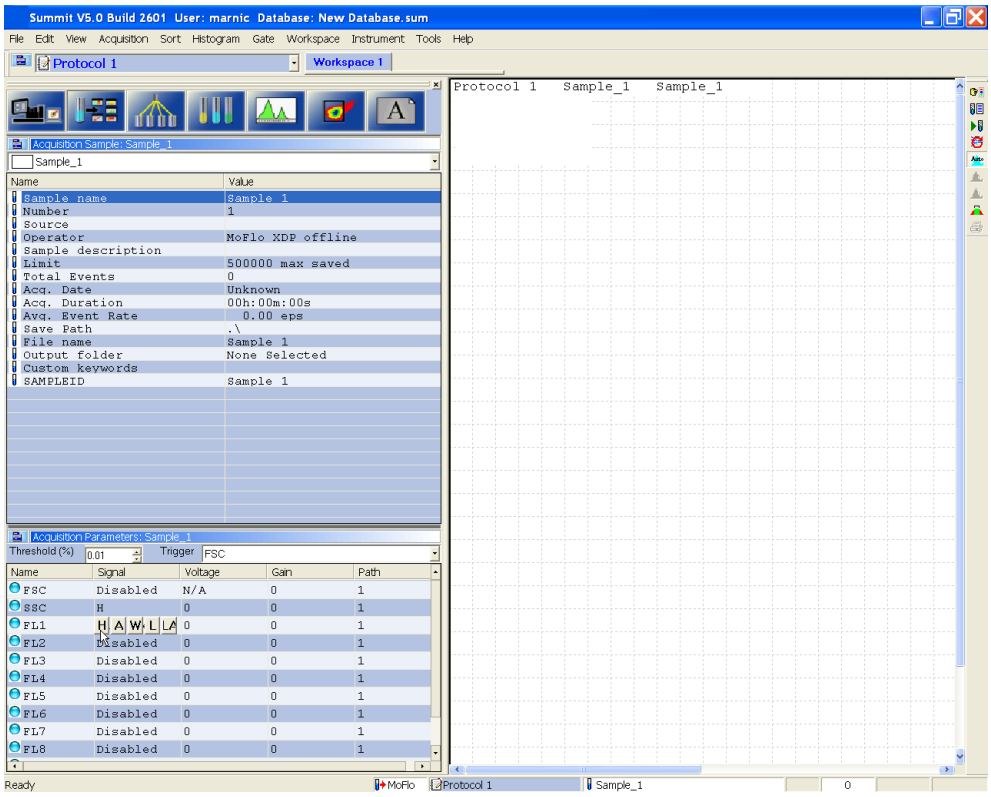
Table 4.1 Instrument Tab - Screen Elements and Functions *(Continued)*

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.
SmartSampler button (See Figure 4.6) 	Click the SmartSampler button to display the SmartSampler control panel and instrument status indicators in Summit software. 
Laser 1 Delay	Displays the nanoseconds that elapse when an event passes from pinhole one to three. See APPENDIX F, *Setting Laser Delay* for recommended values.
Laser 2 Delay	Displays the nanoseconds that elapse when an event passes from pinhole two to three. See APPENDIX F, *Setting Laser Delay* for recommended values.

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.

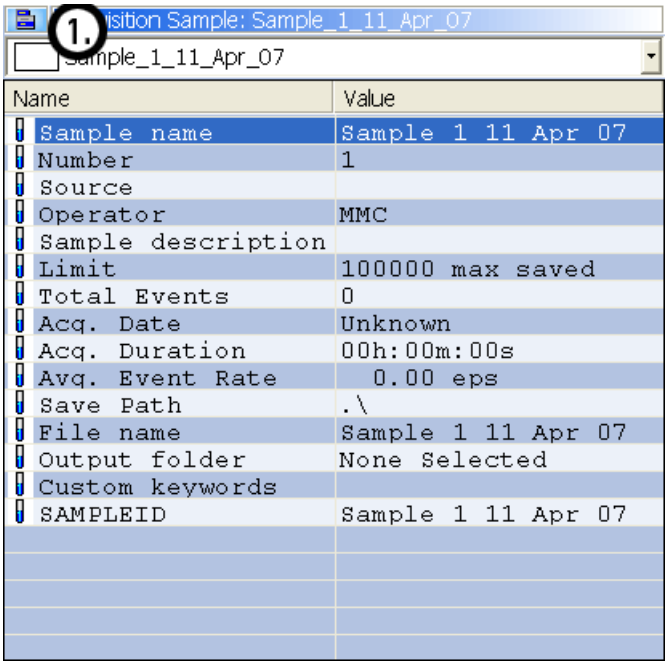
Figure 4.7 Acquisition Tab



Acquisition Sample Panel

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

Figure 4.8 Acquisition Sample Panel



How to Edit Information Specific to a Sample Run

1 To view editable fields, click the blue additional menu icon (See #1. on Figure 4.8) and select **Edit View**. The fields that are editable will activate.

Figure 4.9 Acquisition Sample - Accessing Edit View

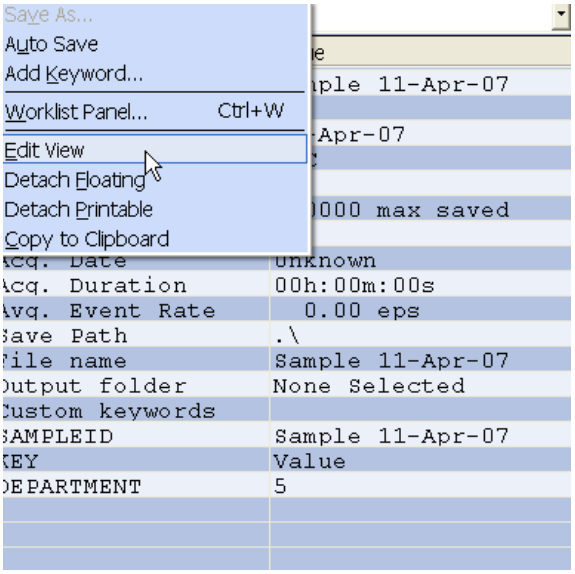
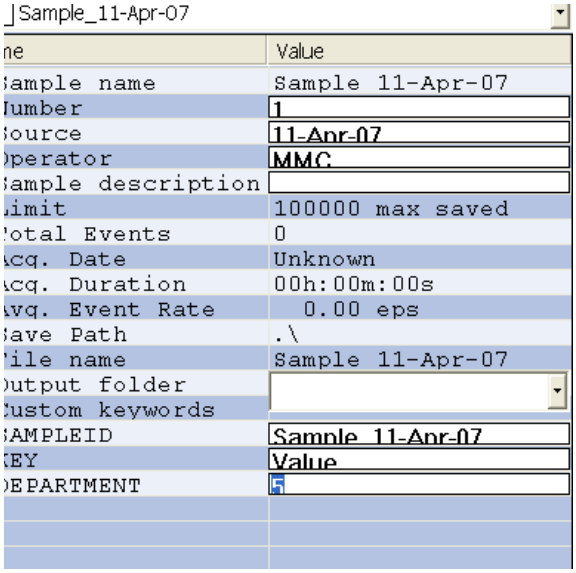


Figure 4.10 Acquisition Sample - Editing Example



NOTE To return to the previous view click **Edit View** again.

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**.

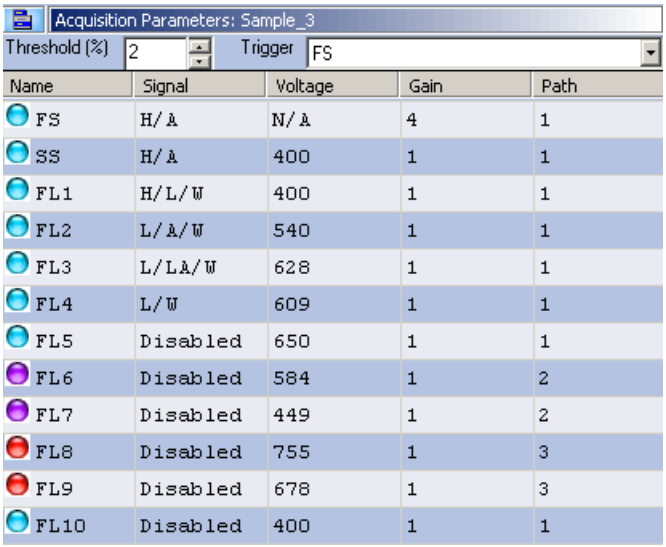
Enable Parameters

Before you can set up histograms or dot plots you must enable the parameters that you intend to use for your experiment.

How to Enable Parameters

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

Figure 4.11 Acquisition Parameters Panel



2 To enable parameters, double-click the word Disabled and select the letters that represent the parameters you would like to collect.

NOTE This can also be done by entering edit mode. To enter edit mode, click on the menu icon in the acquisition parameters tab and select edit mode.

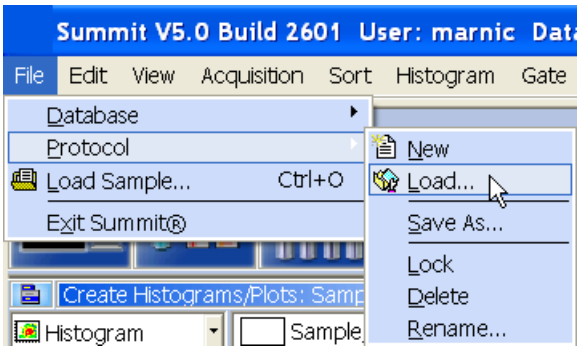
- H = linear height
- L = log height
- A = linear area
- LA = log area
- W = pulse width

Loading an Existing Protocol

How to Load an Existing Protocol

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

Figure 4.12 Loading an Existing Protocol 1



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

Figure 4.13 Loading an Existing Protocol 2

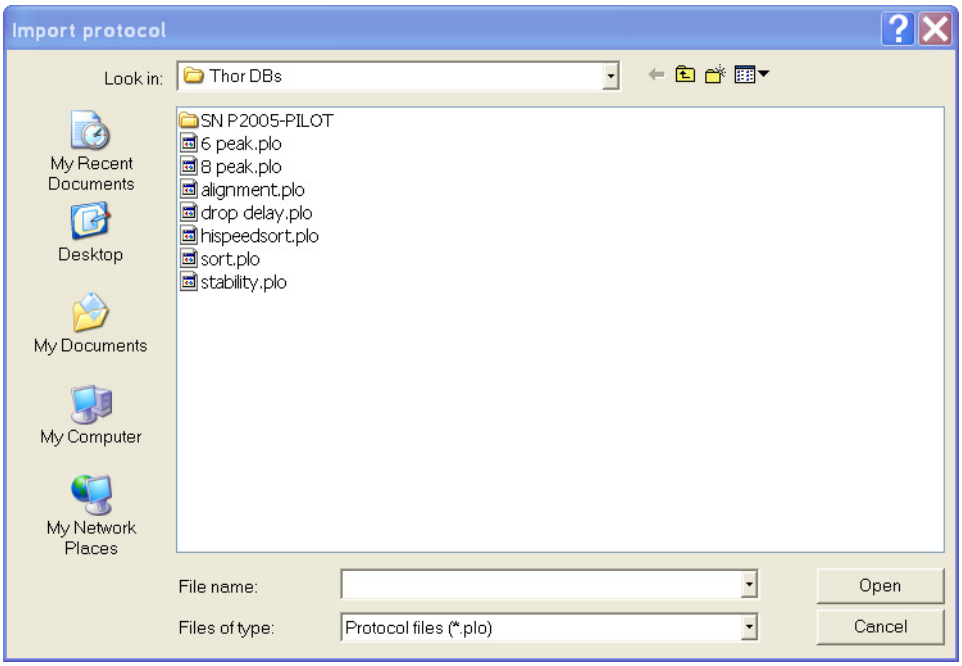
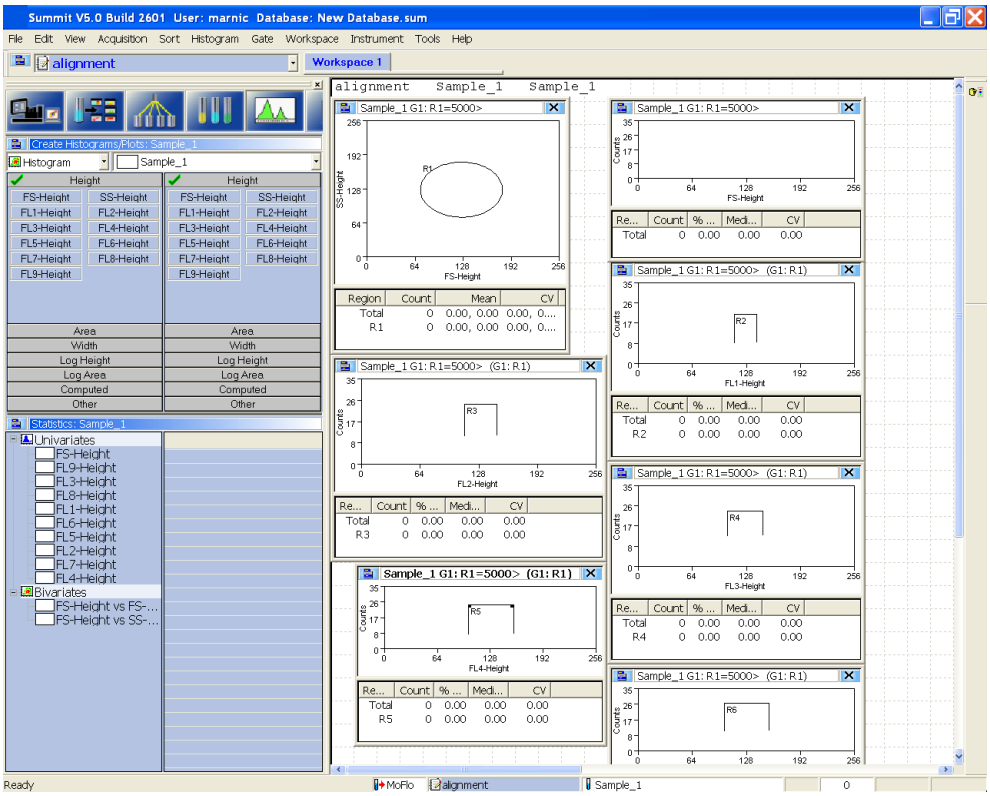


Figure 4.14 is an example of a typical alignment protocol that contains empty histograms in which to acquire data or display and analyze previously acquired data.

Figure 4.14 Loading an Existing Protocol 3



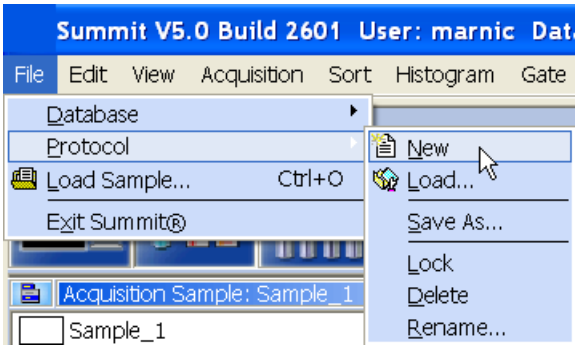
Creating Protocols

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 additional 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 4.15 Create a New Protocol



2 Ensure that you have enabled the desired parameters. See page 4-9.

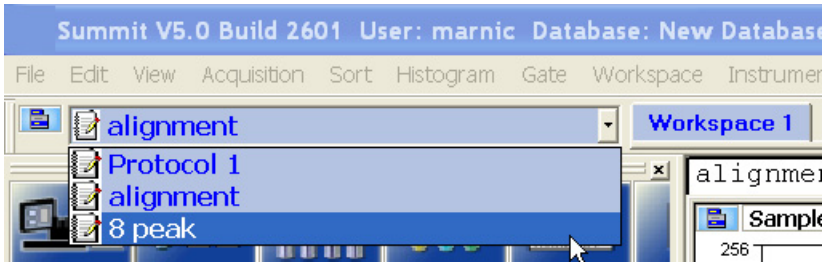
3 Create dot plots and histograms. See page 4-27.

Switching Protocols

To change protocols in Summit software, go to the Protocols toolbar and select a new protocol from the drop 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 4.16 Switching Protocols



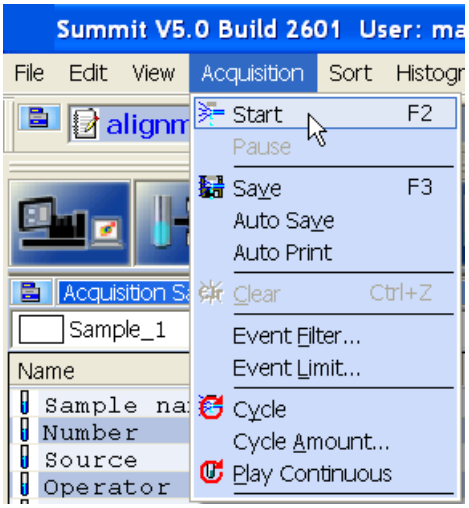
Acquiring Data in Summit Software

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

How to Start or Stop Data Acquisition

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

Figure 4.17 Acquiring Data



2 To stop data acquisition, click Stop (or press F2). Note: When you are running MoFlo XDP with a SmartSampler you will need to consider the settings on the Summit Software Instrument tab. See page 4-4.

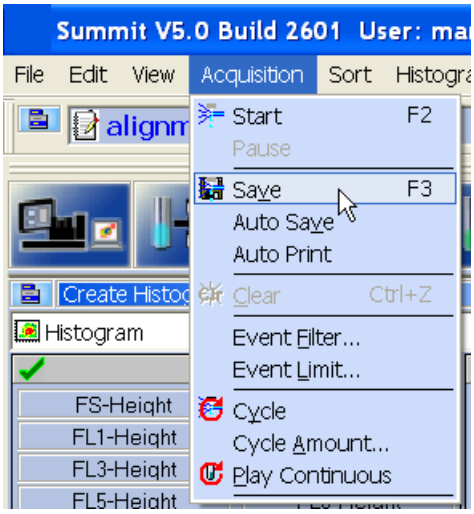
Saving Acquired Data

After you acquire data in Summit software you can save the information in FCS format. For more details see page 7-33.

How to Save Acquired Data

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

Figure 4.18 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

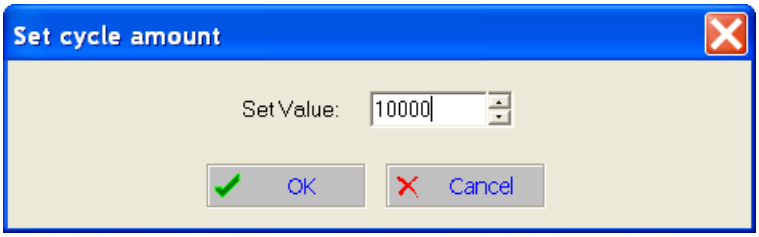
The Cycle Mode in Summit software cycles the events through a buffer to display only the most recent data events. This is useful during alignment activities. The number of data events displayed at any one time is adjustable.

How to Display the Most Recent Data During Alignment Activities

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

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

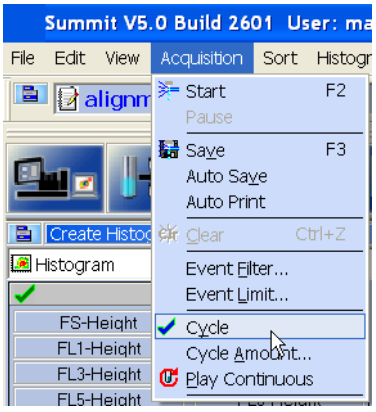
Figure 4.19 Setting the Cycle Amount for Cycle Mode



3 Click **OK**.

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

Figure 4.20 Enabling Cycle Mode

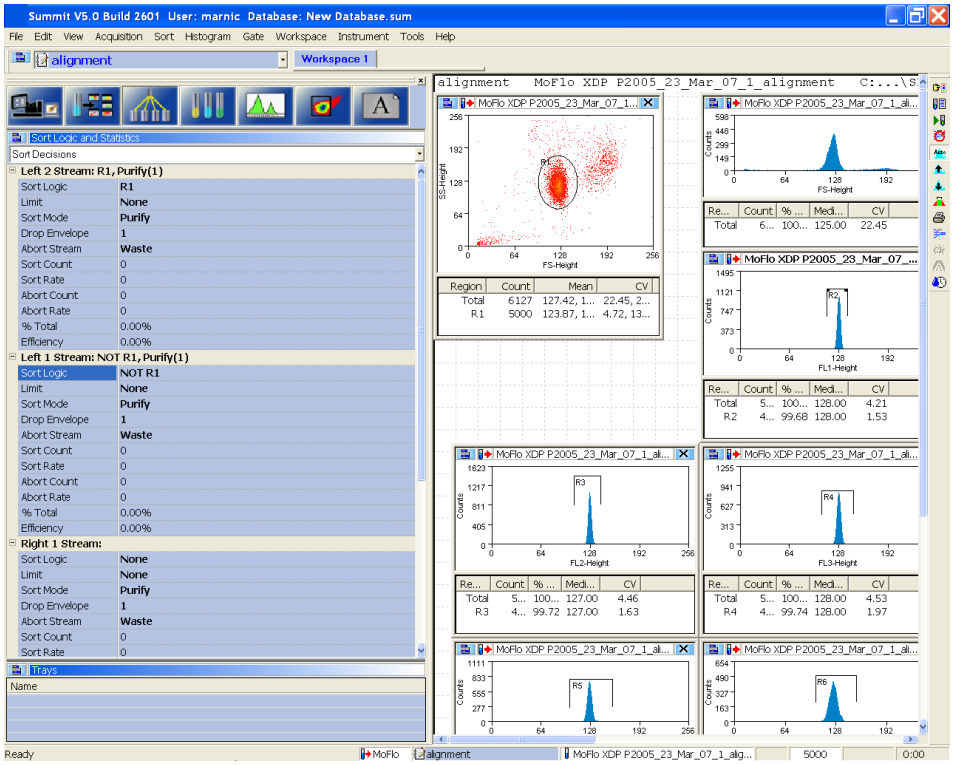


5 After you set the Cycle Mode restart acquisition for Cycle Mode to become effective.

Sort Tab

The Sort tab allows you to specify sort logic based on previously set regions and gates. For more information see page 7-1.

Figure 4.21 Sort Tab



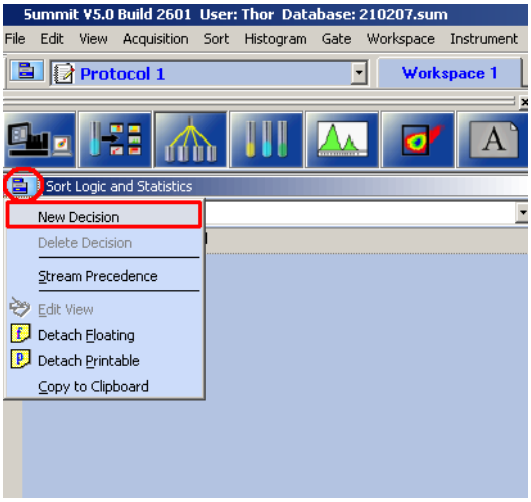
Set Sort Decisions

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 4.22 Set Sort Decisions



2 Double-click on the sort logic topic for a stream in the sort window.

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.

NOTE The user may select a particular region from the inside or outside list, but not both.

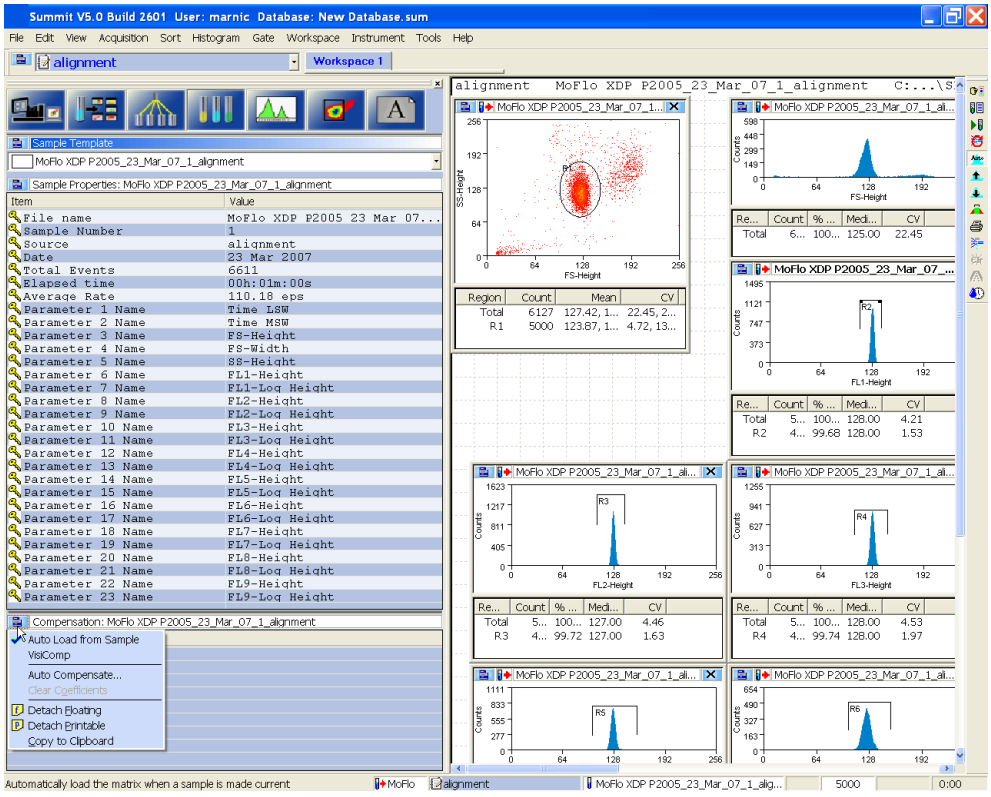
IMPORTANT The information in this section is only an overview. For more details on sorting see page 7-1.

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 Population**. A submenu appears listing each available stream. When a stream is selected, the sort logic editor appears. 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. **Example:** Histogram 1 contains region R4 and is gated by G1: R1 AND R2 AND R3. Right clicking on R4 and selecting the left stream from the Sort Population menu causes the sort logic editor to appear with R1, R2, R3, and R4 selected in the inside gates list. This happens regardless of the current setting for left stream sort logic.

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.

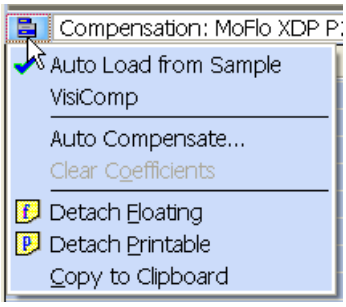
Figure 4.23 Sample Tab



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 4.24 Auto Load from Sample



Auto Compensation Wizard for Single Positive Controls

Summit software provides an automatic method to obtain a full compensation matrix for multicolor analysis. The compensation matrix is calculated from single stained controls by the auto-compensate function.

The following procedure describes how to use the Summit software Auto Compensation feature within single stained controls. Although this feature provides an automatic method to compensate data, compensation can still be adjusted, fine-tuned, or performed manually or via the compensation matrix.

NOTE Additional information regarding compensation can be found in Appendix A.

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.

3. Load all control sample files into an experiment folder.

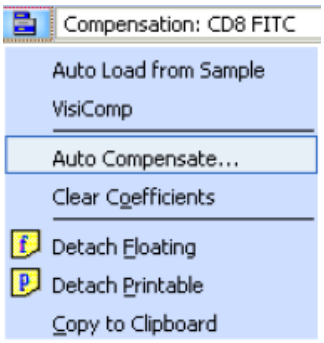
4. Click the Sample tab.

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 Click the upper-left icon to access the main dot plot menu and then click **Auto Compensate**.

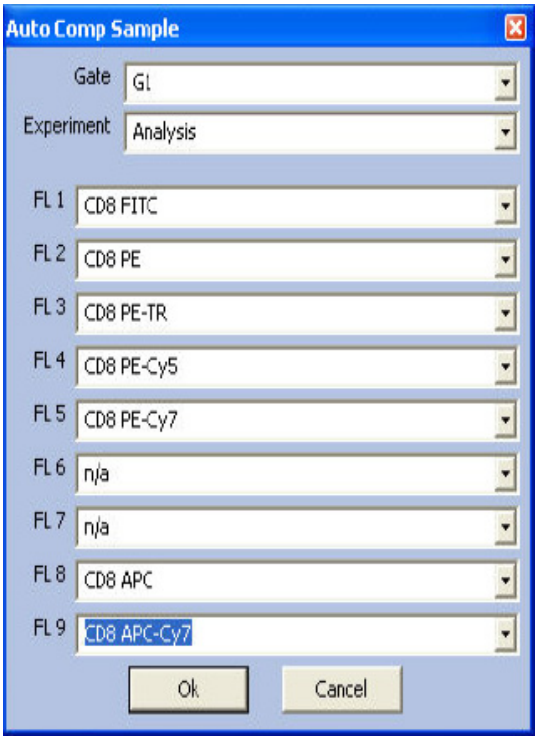
7 In the **Sample Compensation** panel, click the small, blue icon in the upper-left corner and select **Auto Compensate** from the list.

Figure 4.25 Select Auto Compensate



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

Figure 4.26 Auto Comp Sample Dialog



9 Select a gate from the Gate list, if applicable.

10 From the **Experiment** list, select the experiment folder that contains your control samples

11 Select all of the single control samples included in the experiment.

12 Click **OK**.

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

13 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 4.27 Auto Compensate Wizard

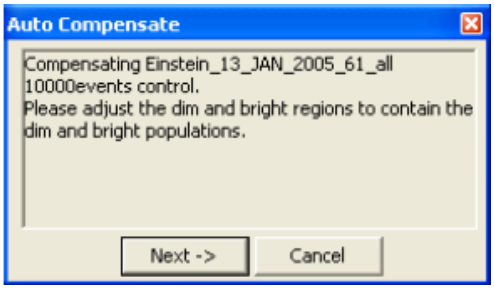
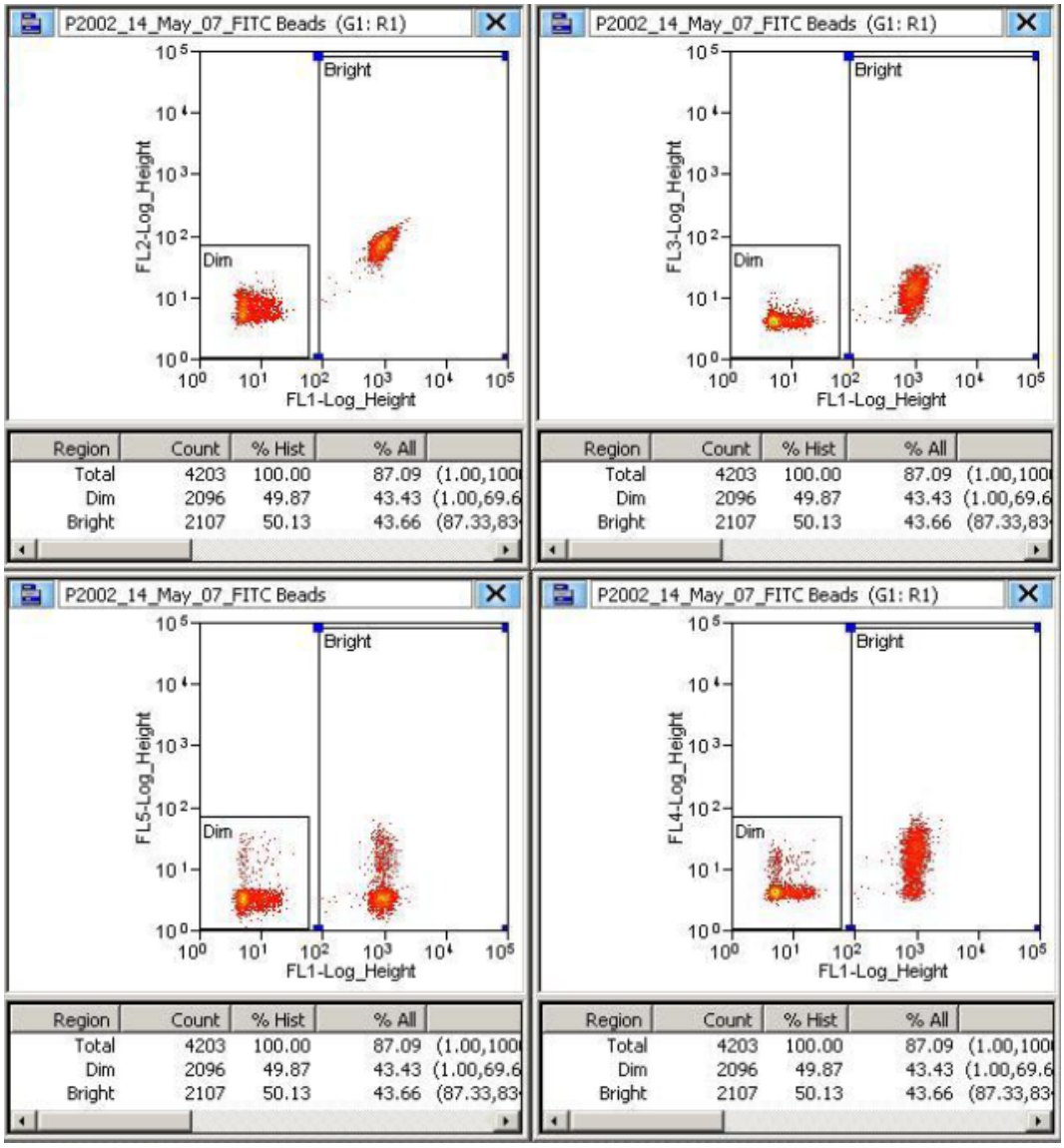


Figure 4.28 Single Control Sample Dot Plots



14 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.

15 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.

16 Repeat step 14 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.

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.

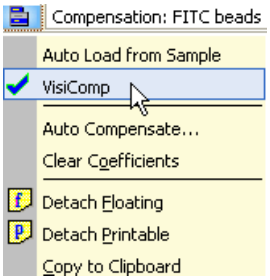
How to Use VisiComp to Visualize Compensation Results

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

2 Create all plots, regions, and gates.

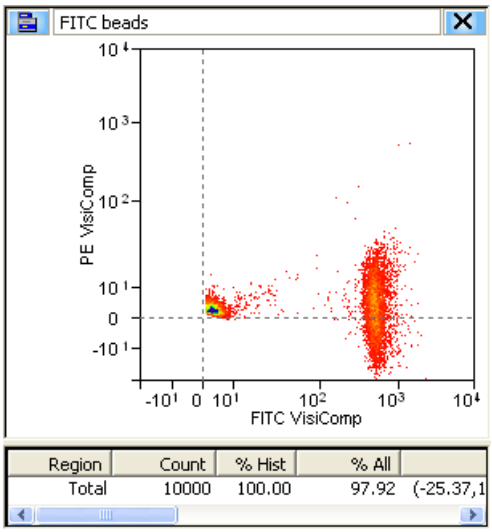
3 On the Sample tab, click the **Compensation** panel icon and select **VisiComp**.

Figure 4.29 Apply VisiComp



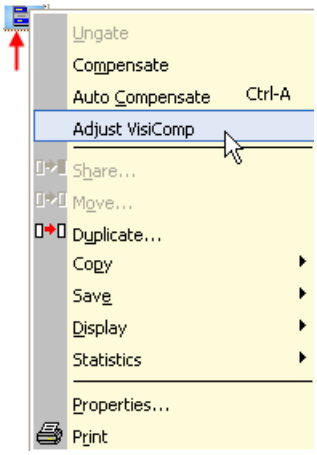
4 Use either the auto compensation wizard to set up plots, or manually set up all of the plots that you want to use for compensation analysis.

Figure 4.30 VisiComp Example



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 4.31 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.

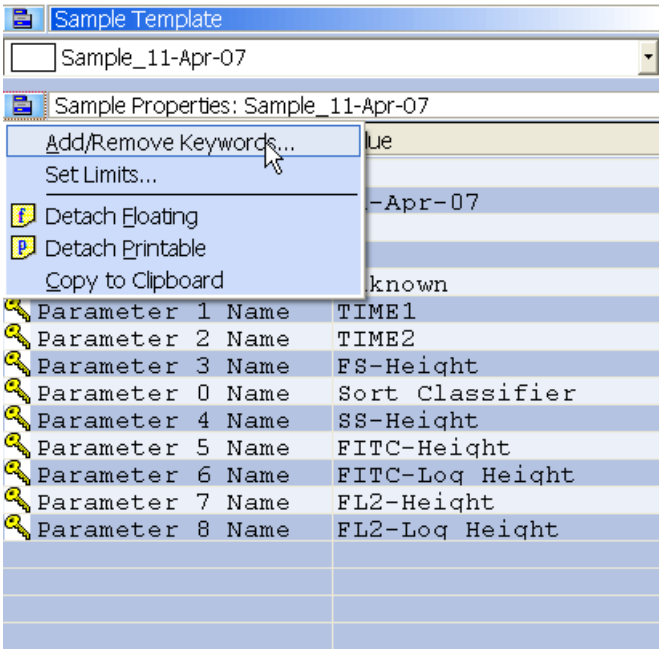
FCS Keywords

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

How to Add or Remove Keywords

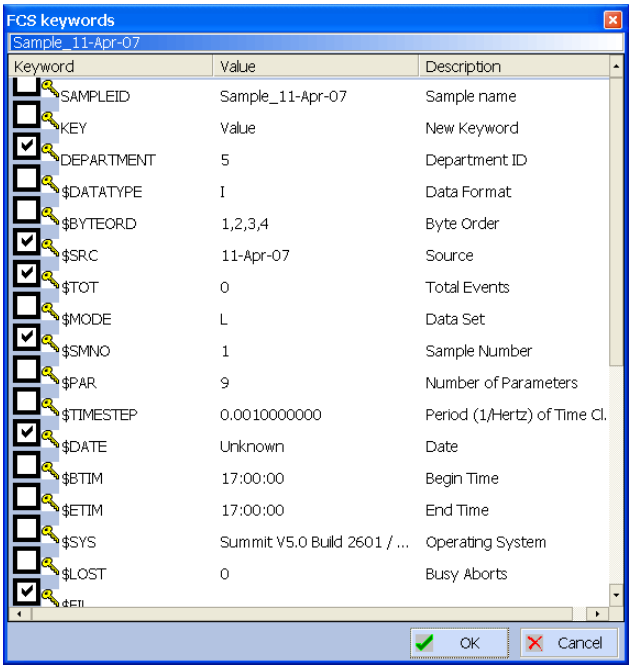
1 Click the blue menu icon and select **Add/Remove Keywords**.

Figure 4.32 Add/Remove Keywords 1



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

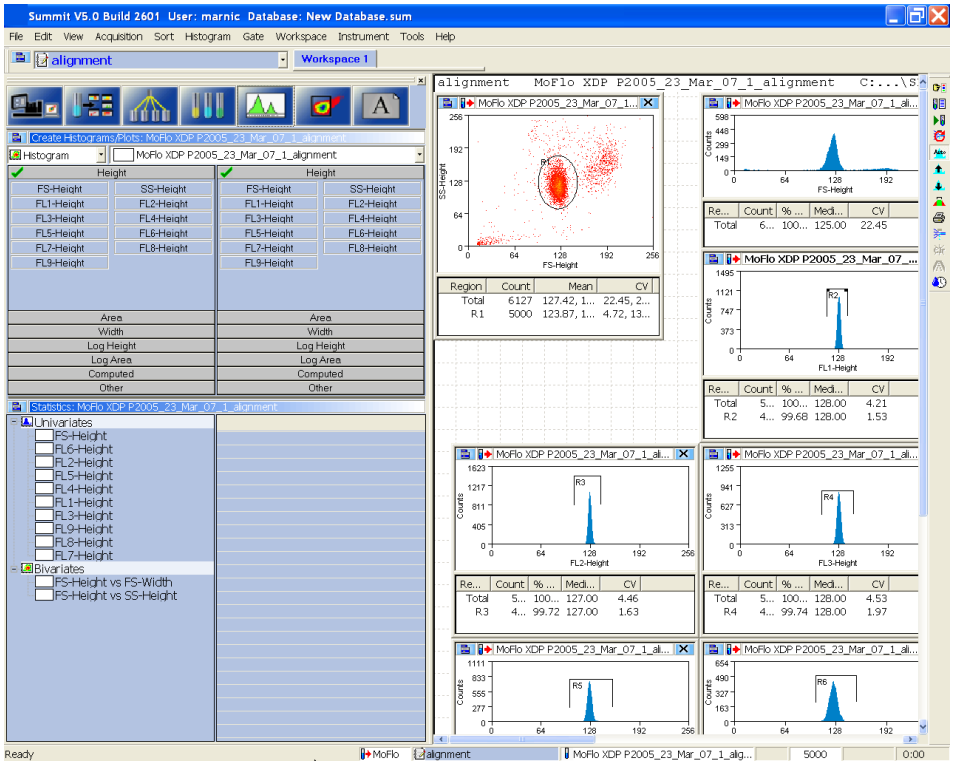
Figure 4.33 Add/Remove Keywords 2



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 4.34 Histogram Tab



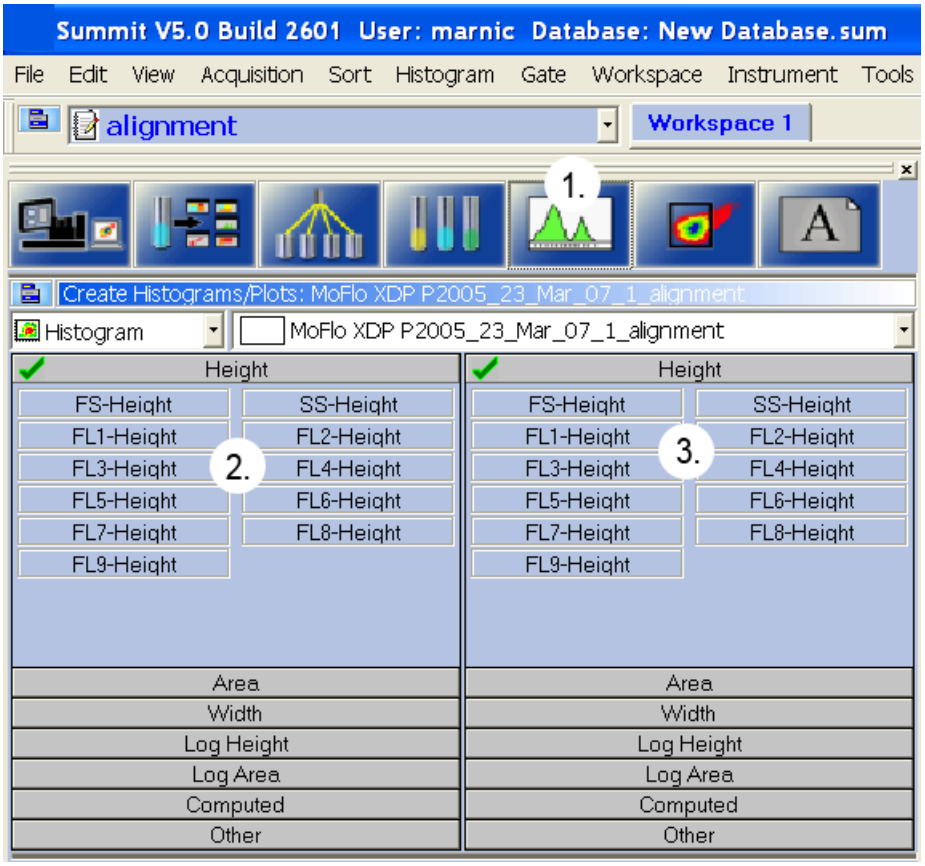
Creating Histograms and Dot Plots

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 4-9.

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 4.35). The histograms and dot plots that you create will build a Protocol that you can elect to save.

Figure 4.35 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.

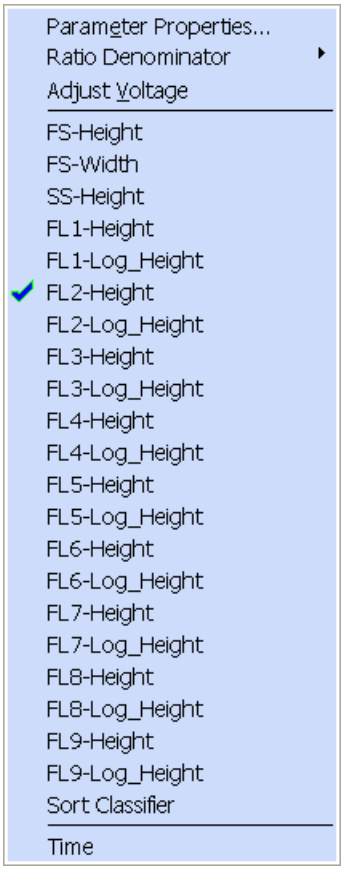
Maximize Dot Plots and Histograms

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. The options in the list include the parameters that were collected with the current data file.

Figure 4.36 Change Axis Parameters



Change Axis Labels

To change the label that is displayed on the X or Y axis of a histogram or dot plot, right-click on the axis you want to change and select **Parameter Properties** from the menu. You can enter additional fluorochrome, marker, or antibody information in the field labeled **Long Name**. The information entered in this field will be the label displayed along the axis.

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. Go to the main histogram menu and select **Statistics > Edit Display**. The **Edit Columns** dialog box appears. The checkboxes that are selected will appear in the statistics window below the histogram or dot plot. You can also drag and drop to change the order of statistics. Choose to apply these settings to all histograms by selecting the check box at the bottom of the dialog.

Manually Scaling Data

To manually rescale date within a dot plot or histogram, click the scale up or down buttons on the right side of the screen.

Contouring Data

To Enable Contouring, go to the main dot plot menu and select **Display > Contour**. Select the **Enable contours** checkbox. The dropdown 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, go to the main histogram menu 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 drop 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.

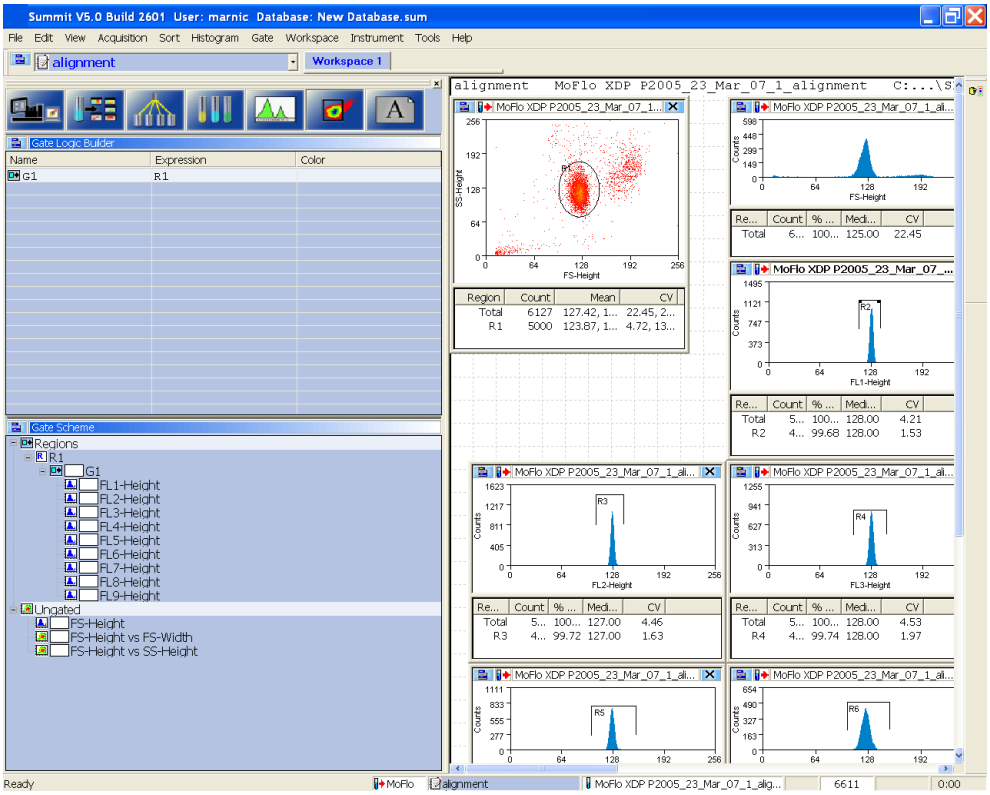
5. To include additional sample data, go to the Summit Software Main Menu and select **New > Samples**.

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 4.37 Gate Logic Tab



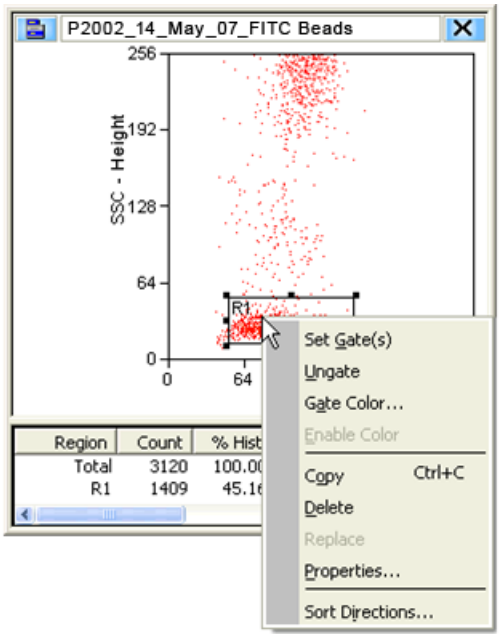
Setting a Gate from a Single Region

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

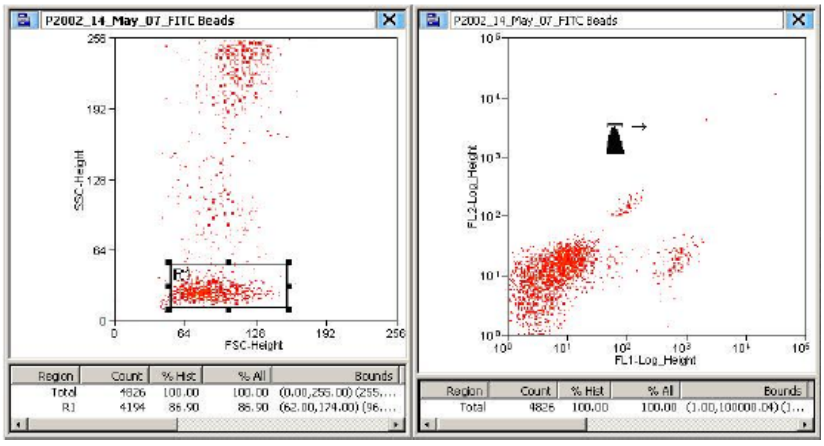
1 To gate one histogram or dot plot, right-click in the region from which you would like to gate, and select **Set Gates** from the menu. The appearance of the curser will change.

Figure 4.38 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 4.39 Set Gate 2



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**.

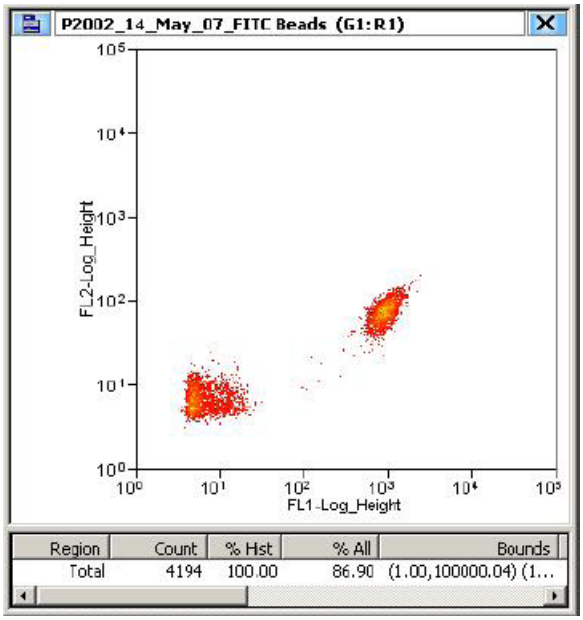
Setting a Serial Gate

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 an 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 dot plot gated on region (R1).

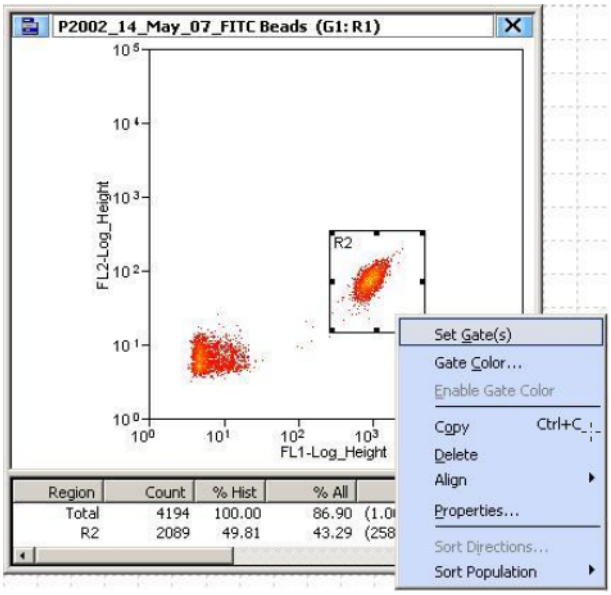
Figure 4.40 Set Serial Gate 1



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

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

Figure 4.41 Set Serial Gate 2



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

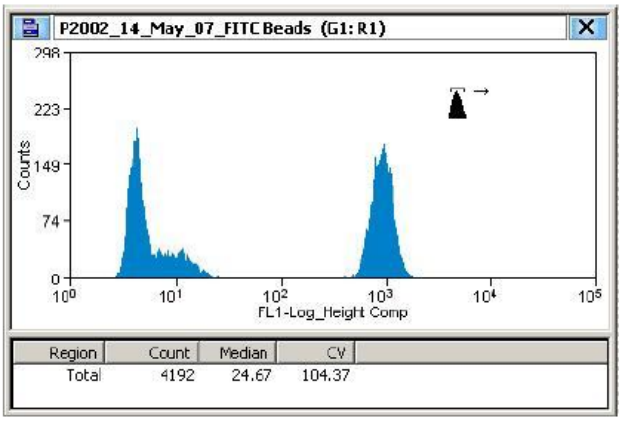
4 Click **Yes** to activate serial gating. Click **No** to gate using only the specified region.

Figure 4.42 Set Serial Gate 3

5 Check the **Make my choice the default and don't ask me again** checkbox to disable this dialog box in the future. If checked, this option is set as the default in Summit software. The default can be changed in the **Preferences** dialog box.

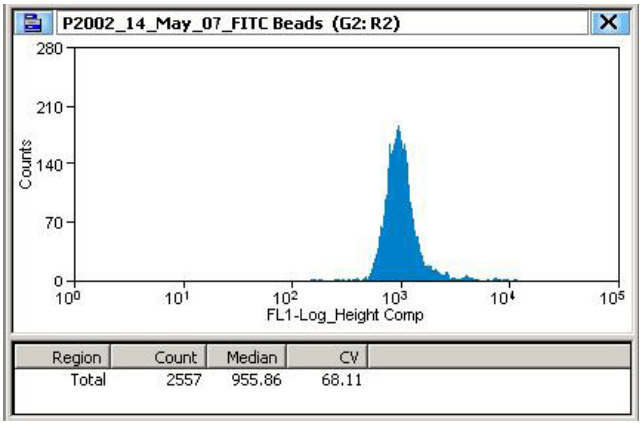
6 Single or double click in a histogram(s) to apply the gate.

Figure 4.43 Set Serial Gate 4



7 If you selected Yes in step 4, the serial (or sequential) gate logic is applied to the histogram or dot plot. The graphic below shows a gated histogram involving regions 1 and 2.

Figure 4.44 Set Serial Gate 5



Gate Logic Builder

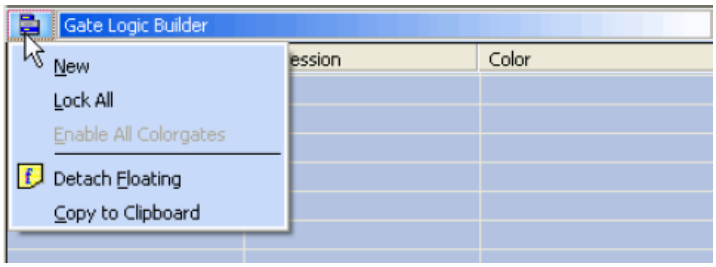
The **Gate Logic Builder**, located on the upper-left portion of the Gating tab, allows you to define gate logic and view it graphically.

How to Define (Edit) Gate Logic

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

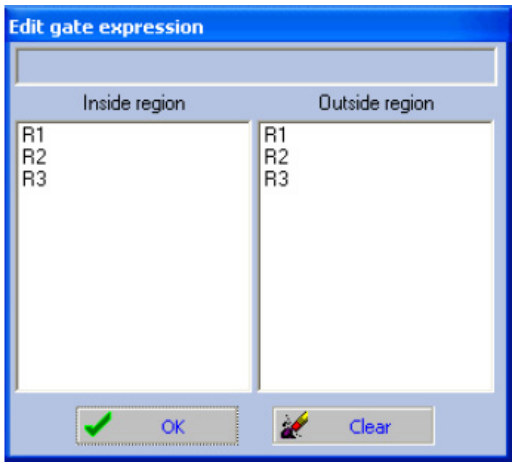
2 Click the upper-left icon in the **Gate Logic Builder** panel and then click **New** to create a new gate.

Figure 4.45 Gate Logic Builder



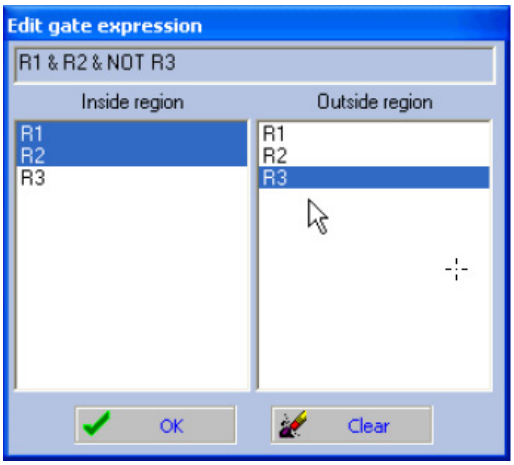
3 Double-click a text field in the **Expression** column. The **Edit gate expression** dialog box appears.

Figure 4.46 Edit Gate Expression 1



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**.

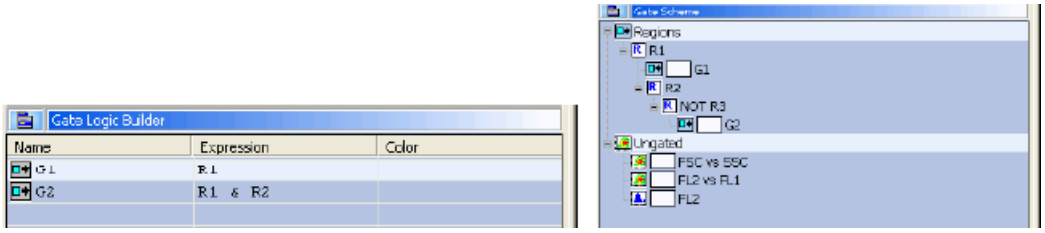
Figure 4.47 Edit Gate Expression 2



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.

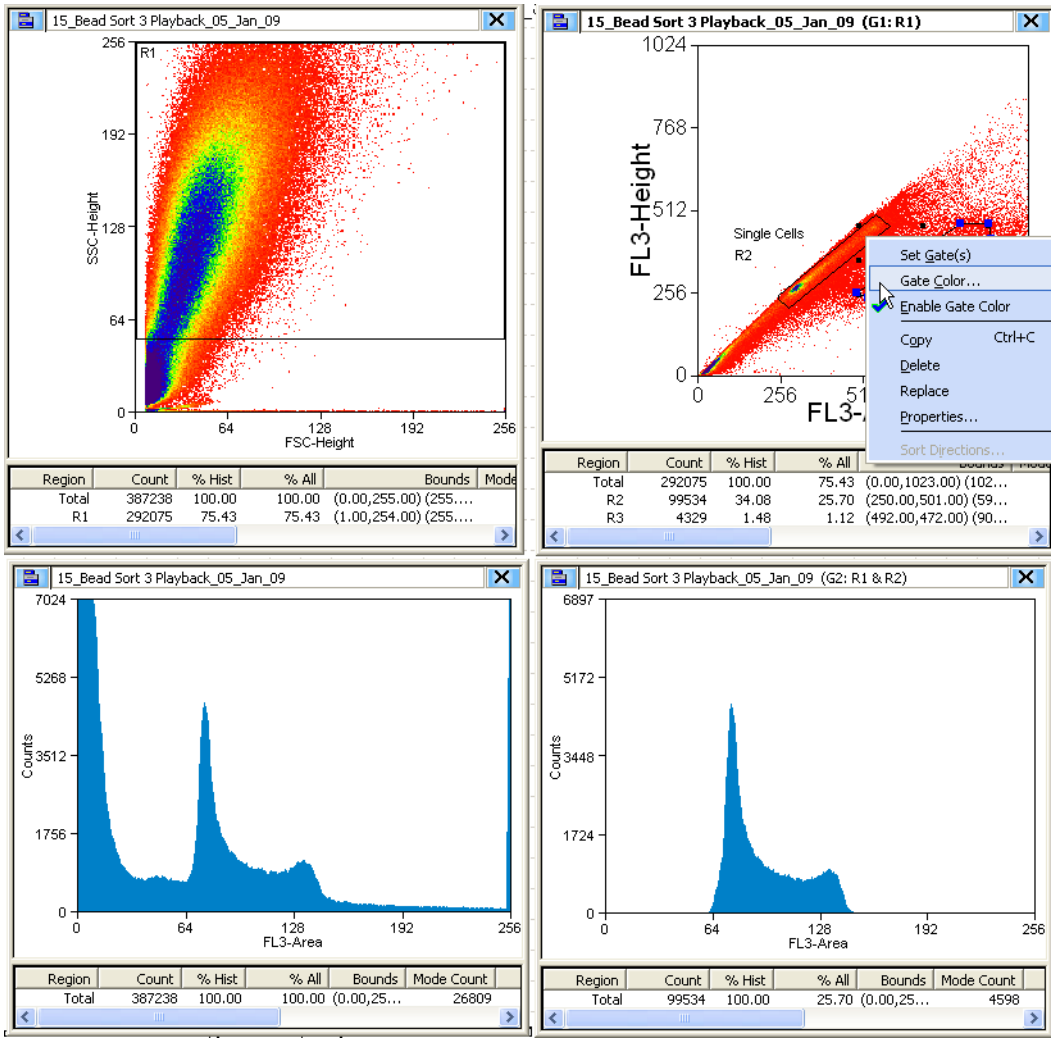
Figure 4.48 Gate Logic Builder and Gate Scheme Panels



Color Gating

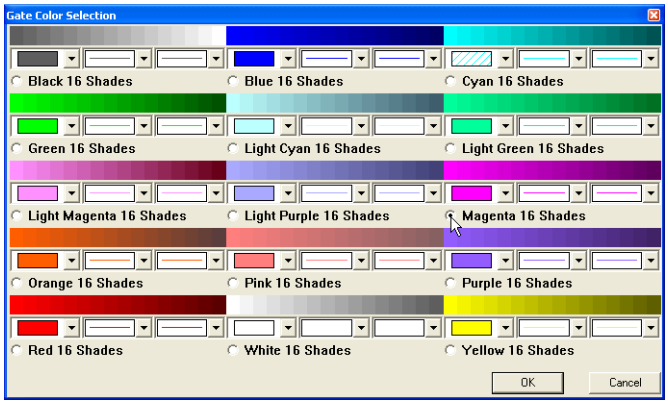
1 To view the single cells and doublet cells populations with color gating, right click in the region around the single cell population Figure 4.49 and select **Gate Color**.

Figure 4.49 Color Gating Diagram



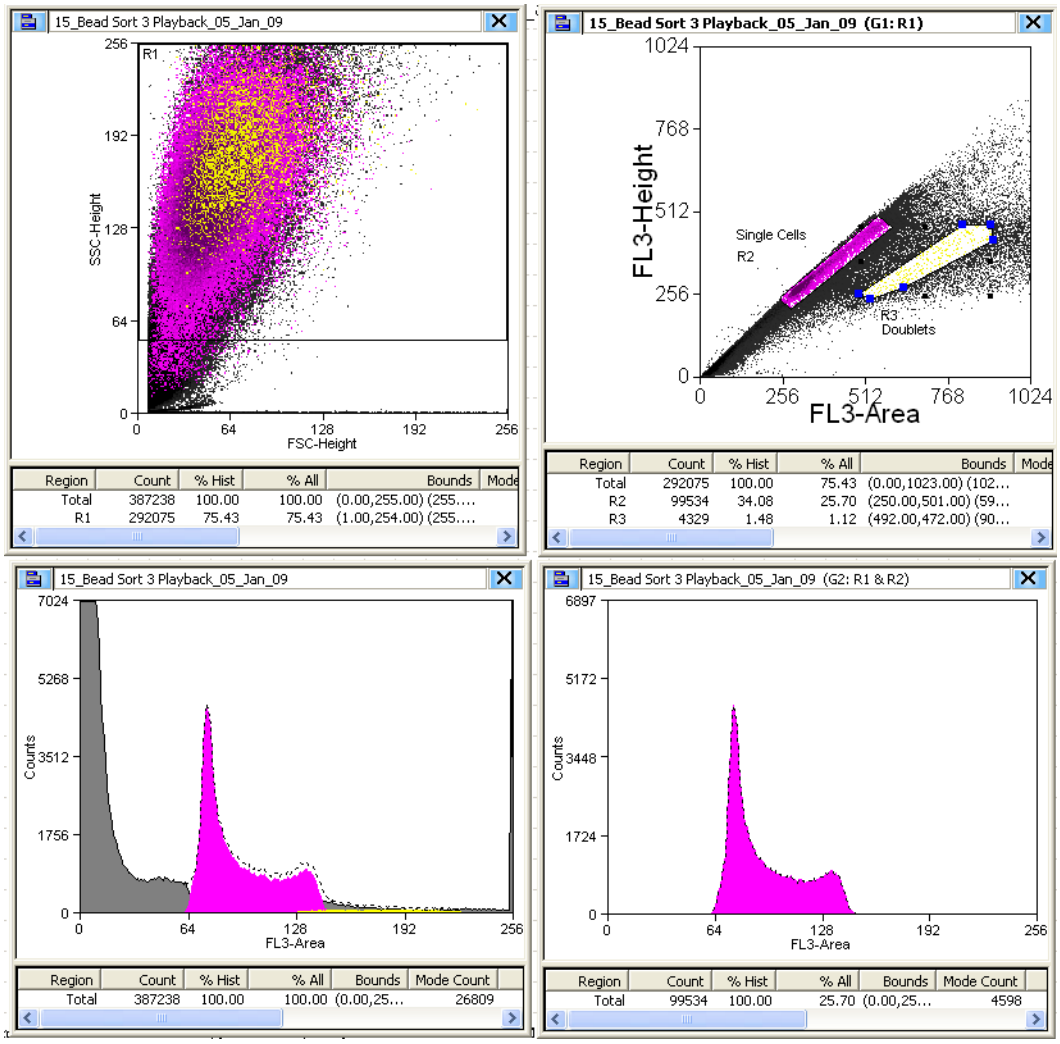
2 Select a color from the color selection pallet and click **OK**.

Figure 4.50 Color Gating Color Pallet



3 The single cell population from Figure 4.49 appears in Figure 4.51 in magenta. The doublet population is displayed in yellow.

Figure 4.51 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 4.52.

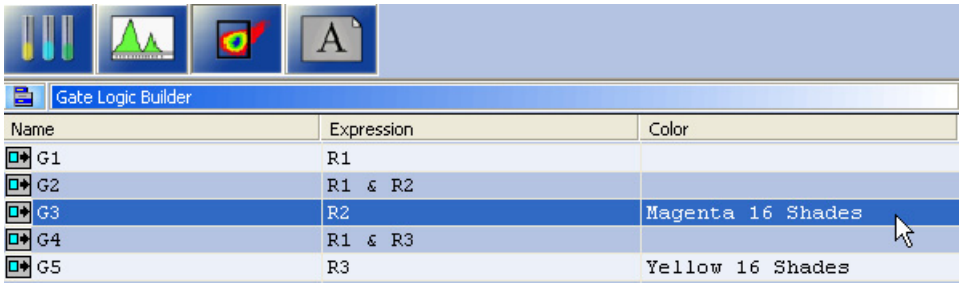
Figure 4.52 Toggle Color Gating

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 4.53 Edit Color Gates

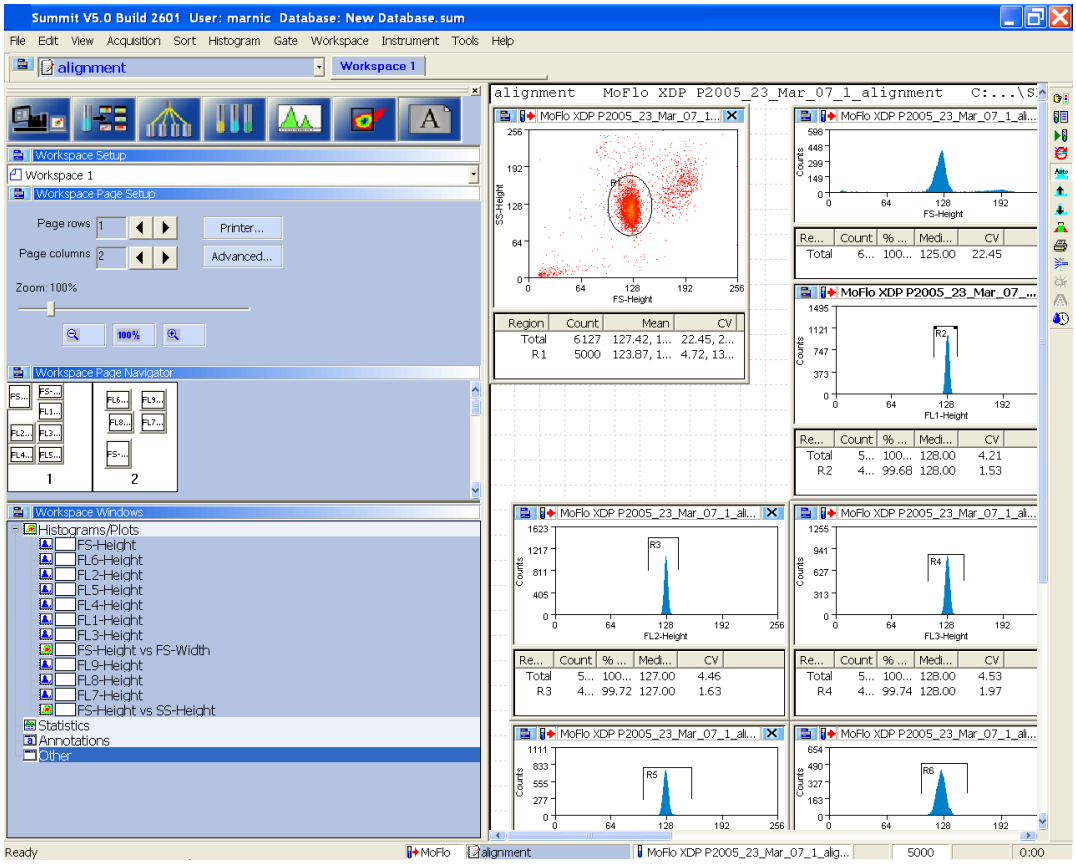


NOTE The order of the color dot plots and histograms correlates to the order of the gates listed in the **Gate Logic Builder**. As an example, if you have a color gate applied to a rare population that is being hidden in the background, you can reorder color to bring it to the front and more easily identify 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 4.54 Layout Tab



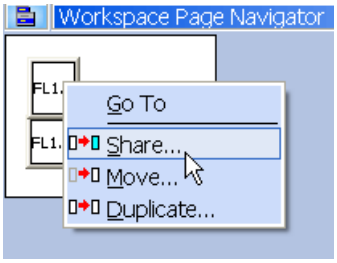
Workspace Page Setup

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 4.55 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.
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**.
Left arrow key	Sets the displayed sample in the current workspace or protocol as the first file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region to the left when selected.

Table 4.2 Shortcut Keys and Functions *(Continued)*

Shortcut Key	Function
Right arrow key	Sets the displayed sample in the current workspace or protocol as the last file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region to the right when selected.
Down arrow key	Sets the displayed sample in the current workspace or protocol as the next file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region downward when selected.
Up arrow key	Sets the displayed sample in the current workspace or protocol as the previous file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region upward when selected.
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, Alignment and Shutdown Procedures

Reagents

Required Reagents for Startup and Shutdown

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

Startup

IMPORTANT Please refer to the MoFlo XDP Quick Start Guide if you prefer a high-level version of the Startup procedures.

Startup Procedures

Turn On the aXcess Control Panel

1 In general, power to the MoFlo XDP system and aXcess Control Panel should remain on.

2 If the system is powered on, proceed to the steps to *Ignite Laser(s)*.

3 If the system is off, power up the Embedded Controller by pressing the power button located in the lower center of the aXcess Control Panel. In general the system should remain turned on.

Ignite Laser(s)

1 Start the lab cooling water supply and return, or laser fan, if applicable.

2 Turn on the laser power supplies per manufacturer instructions (if lasers cannot be controlled through the aXcess Control Panel). Turn the key to ignite the laser(s).

3 On the Laser Control panel set lasers to appropriate power, if applicable.

4 Wait for lasers to warm up and stabilize (minimum of 30 minutes).

Start Fluidics

1 Ensure that the Waste Tank is empty and the Sheath Tank is full.

2 Turn on main air and vacuum supplies, if necessary. Apply pressure and vacuum to the MoFlo XDP by opening blue and red valves on the left side of the Pressure Console.

3 After pressure and vacuum stabilize, debubble the Sheath Filter by lifting the vent lever for three seconds and then close the lever.

4 To continue:

- If your instrument has a Manual Sample Station, go to the heading Manual Sample Station Fluidics.
- If your instrument has a SmartSampler, go to the heading SmartSampler Fluidics

Manual Sample Station Fluidics

1 Set both sheath valves to **Sheath**.

2 Open the pinch valve to backflush the sample line into the waste receptacle. Back flushing for 10 - 15 seconds will clear the sample tubing.

3 To remove air from the CytoNozzle you must debubble. Turn the sample valve off. Rotate the top sheath valve to vacuum while leaving the other sheath valve in the **Sheath** position. Run for 10-15 seconds. Rotate the top sheath valve back to **Sheath** and move the lower valve to vacuum. Run for 10-15 seconds. Set both sheath valves back to the **Sheath** position.

4 Check for trapped air and repeat the debubble procedure if necessary. See page 9-7 for instructions on checking for trapped air.

5 To continue, go to the heading Start Summit Software.

SmartSampler Fluidics

1 On the aXcess Control Panel, press the Start Fluidics button. This causes the SmartSampler to start sheath flow, backflush, and debubble.

NOTE Power to the SmartSampler should always stay on to ensure connectivity with the XDP electronics.

2 Check for air in the lines. Repeat the Debubble procedure, if necessary.

Start Summit Software

1 Log into the Windows operating system. The Summit Software Workstation should generally remain on.

2 Double-click the Summit software icon to open Summit software.

3 Select the appropriate database, or create a new database.

4 From the **Acquisition** tab in Summit software, enable the parameters you would like to use. Verify the laser path selection for each parameter. See page 4-9.

5 Load the alignment protocol if necessary, or create histograms.

Alignment

IMPORTANT Please refer to the MoFlo XDP Quick Start Guide if you prefer a high-level version of the Alignment procedures.

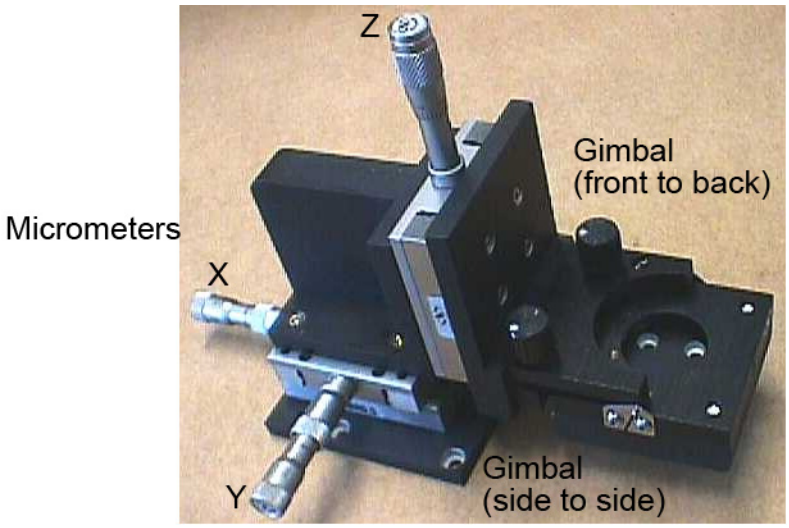
Coarse Instrument Alignment

When the instrument is extremely out of alignment and there is no trigger rate or data displayed, you must rely on sight rather than data to perform alignment. After the instrument is coarsely aligned, you must subsequently perform the Fine Alignment procedure before using the instrument.

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 Illumination Table and controls the position of the CytoNozzle, 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 Aspiration Tube.

Figure 5.1 Stream Positioning Adjustment Stage



Stream Alignment Procedure

1 On the aXcess Control Panel coarse alignment screen turn on the Illumination Chamber Light.

2 With the stream visible, lower the CytoNozzle tip to the top of the camera view. Adjust the X- and Y-axis micrometers to focus the stream and center it over the pinholes, and adjust the gimbals so the stream flows directly into the waste receptacle.

3 To check stream verticality, rotate the Z-axis micrometer counterclockwise to raise the stream. If the stream moves out of focus or migrates right or left, the stream is not in alignment.

4 If the stream is aligned, move the nozzle tip until it is just in view of the pinhole image.

Primary Laser Alignment

Aligning the primary laser is the first step to multiple laser alignment. Once the stream, forward scatter detector, and the primary laser are aligned, you will align the secondary and tertiary lasers.

Primary Laser Coarse Alignment Procedure

1 If necessary, align the primary laser. This step may not be necessary every time you use the instrument.

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

2 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.

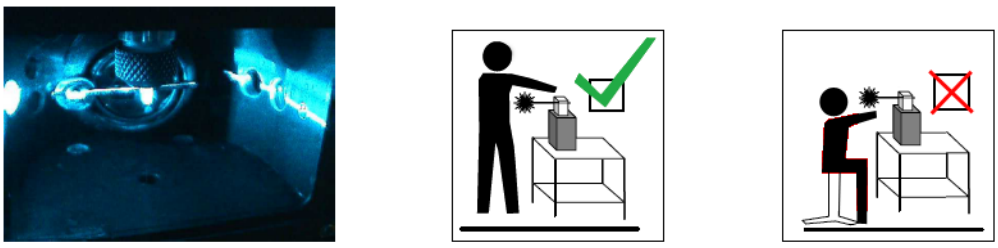
3 Open the shutter for the primary laser, which is typically the 488 nm laser.

4 Adjust the laser to hit just below the tip of the CytoNozzle.

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

5 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. Adjust the micrometer until the ring of diffraction is maximized in intensity.

Figure 5.2 Ring of Diffraction and Laser Safety



6 Close the Illumination Chamber door and remove the safety interlock key.

Bead Flash

NOTE It may not be necessary to align the bead flash every day.

Collinear Laser Alignment Procedure (using Bead Flash)

1 Load a sample tube of Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (concentration 10^6) or other brightly fluorescing particles.

2 Do one of the following:

- If your instrument has a Manual Sample Station, start the sample flow then go to step 3 to continue.
- If your instrument has a SmartSampler, press the SmartSampler Start Sample button then go to step 3 to continue.

3 Decrease the illumination intensity of the Illumination Chamber so you can see the silhouette of the pinholes on the Coarse Adjustment screen.

4 Press and hold the boost button on the Pressure Console in order to see the bead flash.

5 If you are aligning the primary laser, use the micrometers to guide the bead-flash directly in the middle of the top pinhole. The bead-flash from the second laser should align with the center pinhole. And the bead-flash from the third laser should align in the lowest pinhole.

Fine Instrument Alignment

If you have maximized the ring of diffraction and the bead flash is over the top pinhole, you are ready to fine tune the alignment. The rest of the alignment should be done by viewing the bead population data on either the aXcess Control Panel Fine Alignment screen or histograms and dot plots in Summit software.

For general alignment it is common to plot two fluorescence parameters (FL1 vs. FL2) on the fine alignment panel.

Primary Laser Fine Alignment Procedure

1 On the aXcess Control Panel Fine Alignment screen select *H* (or linear height) for the parameters displayed.

2 Using Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (concentration 10^6), acquire data (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 4-16 for Cycle Mode details.

3 Use the aXcess Control Panel Fine Alignment screen to plot FSC vs SSC. Adjust gains and PMT voltage as necessary to bring the bead population on scale.

4 On the aXcess Control Panel Fine Alignment screen change the parameters to FL1 height vs FL2 height and adjust gains and voltages to bring the population on scale.

5 Adjust the pressure to achieve an event rate of 100-120 EPS. For detailed information on setting the pressure differential see D-1.

6 Use the aXcess Control Panel Fine Alignment screen and Summit software to optimize fluorescence intensity. Intensity is maximized when the dot plot population is as far right and as high on the plot as possible Figure 5.3. Similarly, single parameter histograms are optimized when the population is as far right on the graph and compact as possible Figure 5.4. Use the micrometers to adjust the primary laser, also change the gains and PMT voltages as necessary to center the population until the intensity is maximized.

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 *Filter Alignment for Z-configuration Detection Path* to view the appropriate diagrams. To align filters for Detection PODs refer to *Detection POD Filter Alignment Overview* and the filter alignment diagram that was shipped with the filter set.

Figure 5.3 Summit Software and aXcess Control Panel Fine Alignment

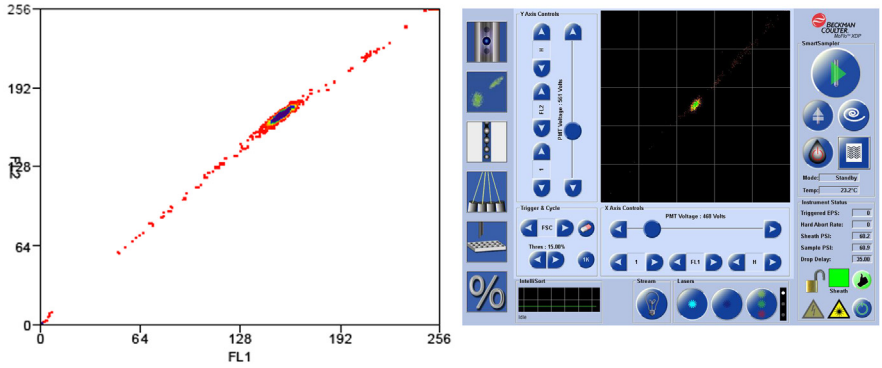
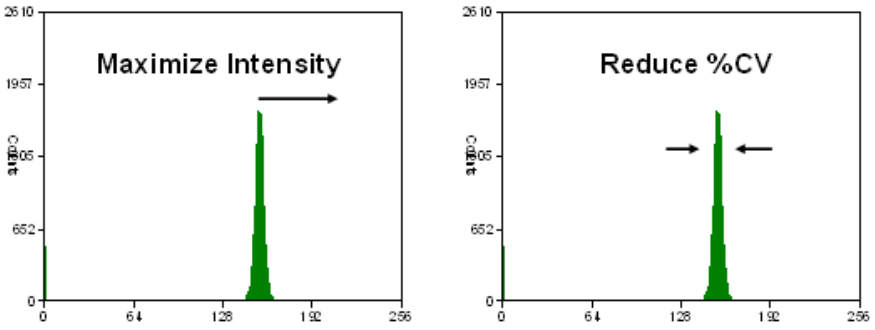


Figure 5.4 Histograms Maximize Intensity and Reduce %CV

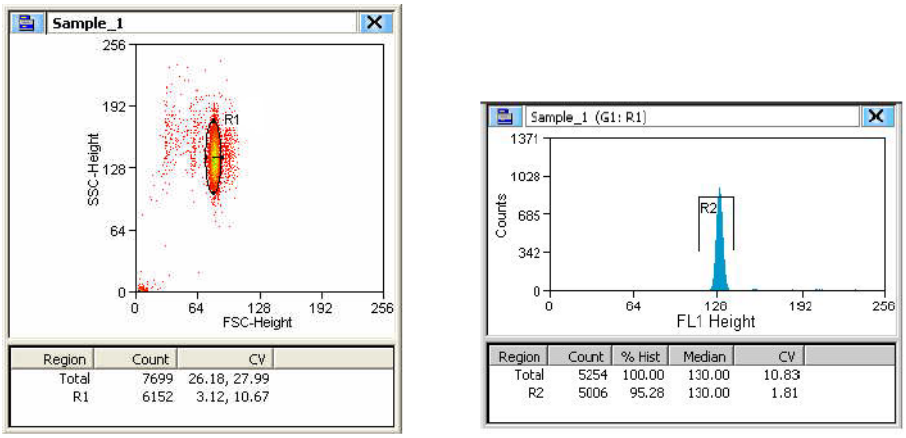


NOTE In Summit software, to view %CV statistics in a histogram you must first create a region over the population.

7 In Summit software create a FSC vs. SSC dot plot, and single-parameter (linear height) histograms for the available parameters. See CHAPTER 4, How to Create a Histogram or Dot Plot.

8 Right-click in the FSC vs. SSC dot plot and select the ellipse to create a region. Click and drag the shape so that a region is set around the most densely populated area of the FSC vs. SSC plot. Right-click and choose **Set Gates** the appearance of the curser will change. Use the newly changed curser to click in each single-parameter histogram in your protocol. Double-click on the last histogram to set the gate for all of them. Once the gate is applied, there will be an annotation in the histogram title bars to indicate a gate is applied Figure 5.5.

Figure 5.5 Gate Set on R1 and Histogram Representing R1



9 In the single parameter histograms right-click and create a bar region. The region will cause the histogram to display %CV statistics for the population. If the %CV statistics do not appear at the bottom of the histogram, see page 4-30.

10 Adjust PMT voltages to set all parameters to median channel 128 ±5 on each linear height histogram.

11 On the aXcess Control Panel Droplet screen, toggle the Drop Drive button on and off while adjusting the amplitude voltage to verify the absence of optical noise when the Drop Drive is on.

Secondary Laser Coarse Alignment

1 Adjust only the appropriate laser focusing optics for the secondary laser. Do not move the nozzle micrometers or the primary laser micrometers.

NOTE If the primary laser, stream, or forward scatter positions are accidentally changed when you are aligning the secondary laser, realign them before proceeding.

2 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.

3 Open the shutter for the laser you intend to align.

4 Adjust the secondary laser to hit just below the tip of the CytoNozzle.

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

5 Shutter the primary laser and check the secondary laser beam for the ring of diffraction. Adjust the micrometer until the ring of diffraction is maximized in intensity.

6 Close the Illumination Chamber door and remove the safety interlock key.

7 If the secondary laser has not been aligned over the second pinhole, follow the directions in the *Bead Flash* section above.

Secondary Laser Fine Alignment

1 Open the shutter for the primary laser.

NOTE The shutter for the secondary laser should already be open.

2 Use the aXcess Control Panel or Summit to plot a secondary laser parameter verses a primary laser parameter (or another secondary parameter.)

3 Run the appropriate particles and optimize fluorescence intensity, then fluorescence %CV.

Tertiary Laser Coarse Alignment

1 Adjust only the appropriate laser focusing optics for the tertiary laser. Do not move the nozzle micrometers, the primary laser or the secondary laser micrometers.

NOTE If the stream, forward scatter detector, primary laser, or secondary laser positions are accidentally changed when you are aligning the tertiary laser, realign them before proceeding.

2 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.

3 Open the shutter for the laser you intend to align.

4 Adjust the tertiary laser to hit just below the tip of the CytoNozzle.

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

5 Shutter the primary and secondary lasers and check the tertiary laser beam for the ring of diffraction. Adjust the micrometer until the ring of diffraction is maximized in intensity.

6 Close the Illumination Chamber door and remove the safety interlock key.

7 If the tertiary laser has not been aligned over the third pinhole, follow the directions in the *Bead Flash* section above.

Tertiary Laser Fine Alignment

1 Open the shutters for the primary and secondary lasers.

NOTE The shutter for the tertiary laser should already be open.

2 Use the aXcess Control Panel or Summit to plot a tertiary laser parameter verses a primary laser parameter (or another tertiary parameter.)

3 Run the appropriate particles and optimize fluorescence intensity, then fluorescence %CV.

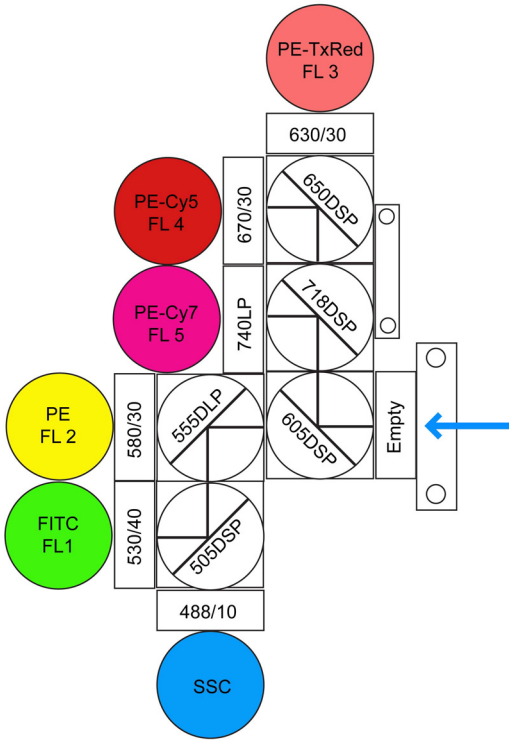
Filter Alignment for Z-configuration Detection Path

A filter layout in a Z-configuration allows five colors and Side Scatter to be detected from one laser path with a 488 nm laser.

Follow the Filter Alignment diagram Figure 5.6 to place filters in the appropriate positions in the Z-configuration detection path. See Figure 5.7 for an alignment order diagram. For additional filter layout diagrams view Figure 5.8, Figure 5.9, and Figure 5.10.

NOTE Filter alignment differs for instruments that are configured with Detection PODs. Refer to *Detection POD Filter Alignment Overview* for details.

Figure 5.6 Filter and PMT Layout for the Z-configuration Detection Path



Z-configuration Filter Alignment Order

1. When aligning filters in the Z-configuration, align the most direct detector paths first. Then introduce dichroic filters one at a time to align the closest dichroic back to the laser source.

2. Remove all filters from the Z-configuration and ensure that PMTs are placed in the correct brackets. Inspect the filters to ensure that they are clean and secure in their holders.

3. Create a dot plot using parameter one (which is usually FL2) and FSC. Adjust the PMT to ensure that the signal is optimized for parameter one. Place the appropriate filter in position two Figure 5.7 and create a dot plot for parameters FL2 and FL3. Gently adjust the filter until the signal is optimized.

4. Place the appropriate filter in position four and create a dot plot for parameters FL2 and SSC Figure 5.7. Gently adjust the filter until the signal is optimized.

5. Continue in this manner until the detection block is fully populated and aligned.

NOTE For detection PODs filter alignment, follow the diagrams packaged and shipped with the filter sets and refer to *Detection POD Filter Alignment Overview*.

Figure 5.7 Filter Alignment Order for the Z-configuration Detection Path

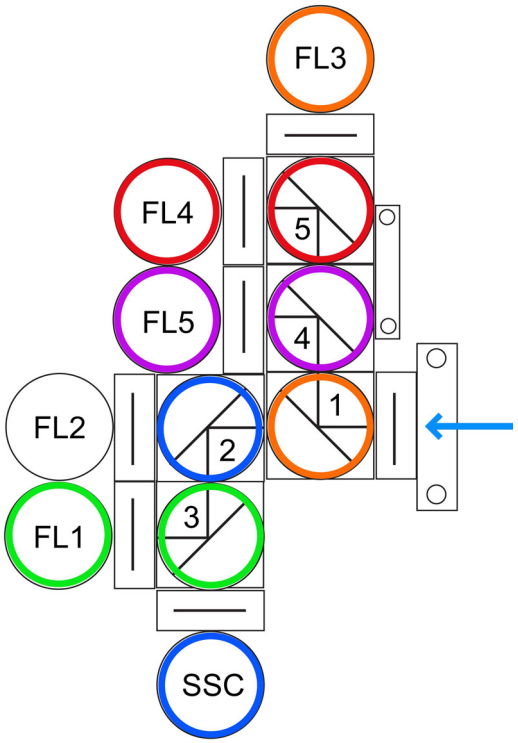


Figure 5.8 Detection Table and Filters Schematic (Configuration with 405 nm Laser)

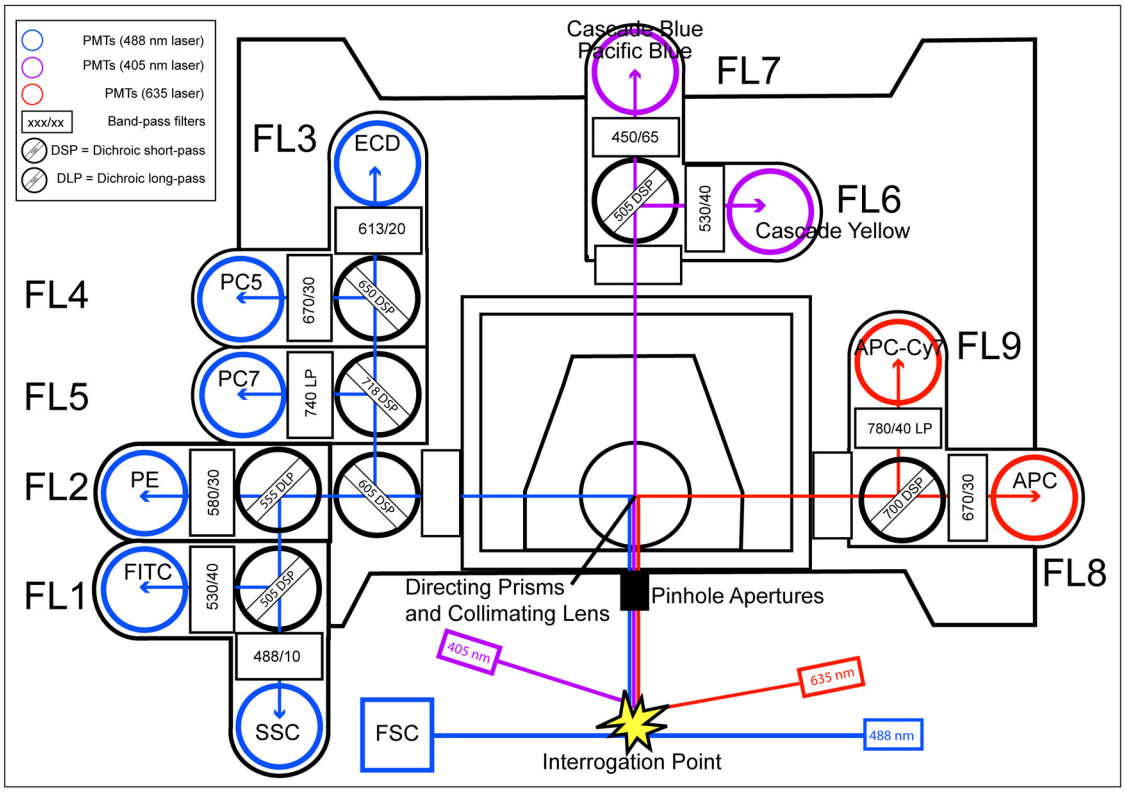
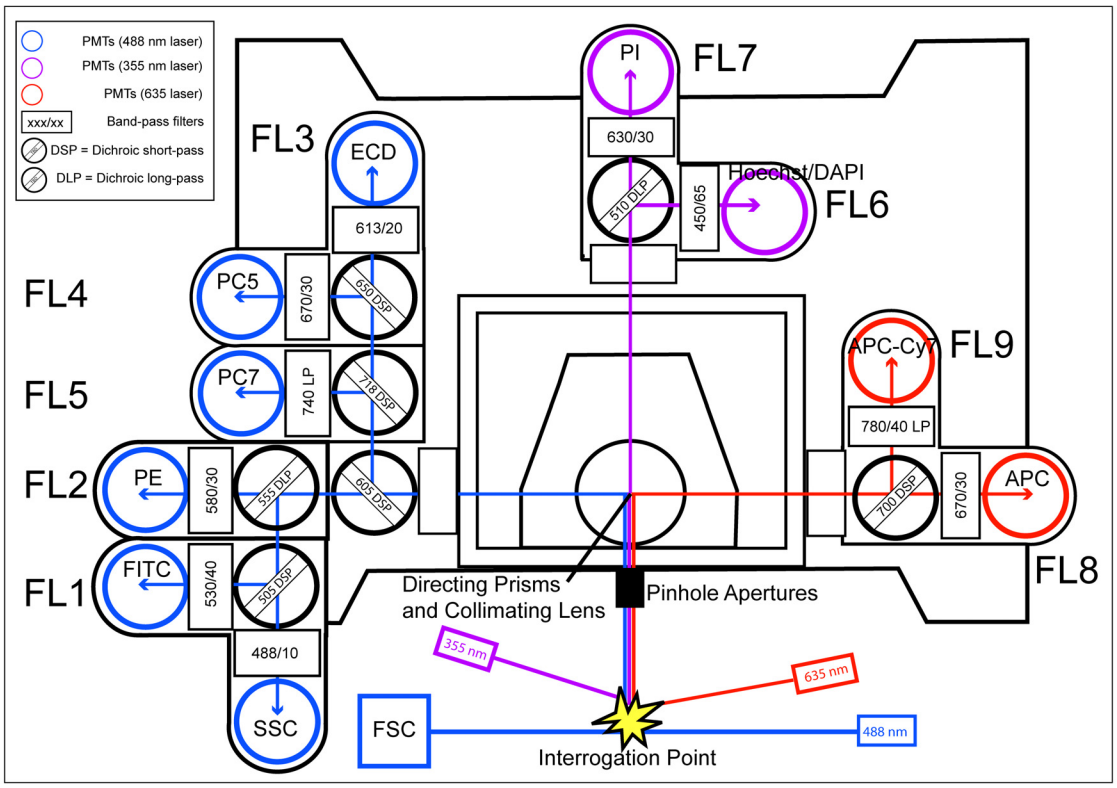
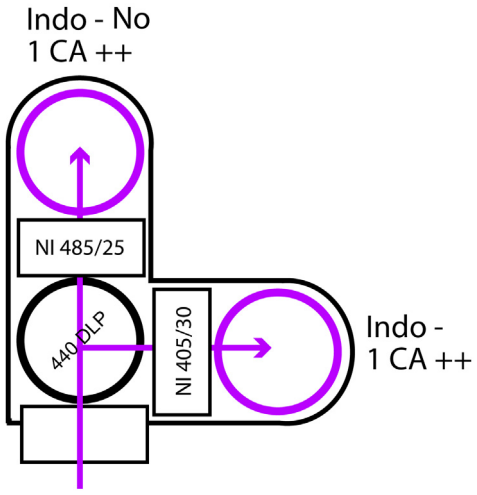


Figure 5.9 Detection Table and Filters Schematic (Configuration with 355 nm Laser and Hoechst/DAPI)



When the 355 nm laser is used with INDO dyes, the filters are configured as shown in Figure 5.10.

Figure 5.10 Detection Table and Filters Schematic (Configuration with 355nm Laser and INDO)



Detection POD Filter Alignment Overview

Filter Alignment Theory

Consider the following guidelines when aligning and optimizing filter sets within the PODs.

- Detection POD filter layout diagrams are shipped with the filter sets. The diagrams vary depending on the configuration of the instrument.
- 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.
- Filter alignment diagrams are shipped with the filter sets. Refer to the diagrams for specific alignment instructions.
- 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 filters and mirrors from the POD.

2 Ensure that PMTs are placed in the POD to collect the parameters you are optimizing.

3 Viewing the applicable filter layout diagram place the first mirror or dichroic on the far end of the straight-through path.

4 Create a dot plot on the aXcess 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.

Shutdown

IMPORTANT Please refer to the MoFlo XDP Quick Start Guide if you prefer a high-level version of the Shutdown procedures.

Shutdown Procedures

Laser

1 Turn off the lasers per manufacturer instructions, or through the aXcess Control Panel as applicable.

2 Turn off power supplies if necessary.

3 After an appropriate cooling time, close the water valve(s) as applicable.

Stream Configuration Screen

1 On the Stream Configuration Screen, turn off the Drop Drive, Deflection Plates, and Stream Illumination.

2 On the Coarse Alignment Screen turn off Pinhole Illumination.

3 To continue:

- If your instrument has a Manual Sample Station, go to the heading Fluidics System - Manual Sample Station.
- If your instrument has a SmartSampler, go to the heading Fluidics System - SmartSampler.

Fluidics System - Manual Sample Station

1 Set both sheath valves to Sheath.

2 Open the pinch valve to backflush the sample line into the waste receptacle. Backflushing for 10-15 seconds will clear the sample tubing of sample.

3 Run one tube of mild disinfectant by turning one sheath valve to Vacuum and the other to Sheath then press the Boost button on the Pressure Console.

4 Once the tube of disinfectant is half empty, reverse the position of valves.

5 Remove the tube and run a tube of DI water.

6 Press the Boost button, while turning both sheath valves to the off position and run 90% of the tube.

7 Close the Sample Pressure Valve and the Sample Valve.

8 Remove pressure and vacuum to the MoFlo XDP by closing the red and blue valves on the Pressure Console.

IMPORTANT Do not loosen this valve more than one full turn because you will liberate the compressed spring and ball bearing.

9 Vent the pressure from the sheath tank and the cleaner tank by turning the vent valve one full turn.

NOTE Waste and sheath tanks can be maintained now or the following day.

10 Disconnect the color-coded quick connect fittings at the Waste Tank and Sheath Tank by pulling the fitting collars up until the fittings are released.

11 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 (If adding bleach, it should not be allowed to sit in the tank overnight). See the Fluidics Decontamination procedure on page 8-3. Reattach the Waste Tank to the system. For list of approved cleaners, see A-1.

12 Open the Sheath Tank by pulling up on the handle and removing the lid. Fill the Sheath Tank to the upper weld line with sheath fluid, close the vent valve, and reattach the tank to the system.

NOTE If it is necessary to clean the Sheath Tank, see the Fluidics Decontamination procedure on page 8-3.

13 To continue, go to aXcess Control Panel Monitor.

Fluidics System - SmartSampler

1 Run a tube of mild disinfectant.

2 Run a tube of DI water to remove the disinfectant.

3 Place another tube of DI water in the chamber.

4 Press the Stop Fluidics button.

5 Remove pressure and vacuum to the MoFlo XDP by closing the red and blue valves on the Pressure Console.

IMPORTANT Do not loosen this valve more than one full turn because you will liberate the compressed spring and ball bearing.

6 Vent the pressure from the sheath tank and the cleaner tank by turning the vent valve one full turn.

NOTE Waste and sheath tanks can be maintained now or the following day.

7 Disconnect the color-coded quick connect fittings at the Waste Tank and Sheath Tank by pulling the fitting 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 (If adding bleach, it should not be allowed to sit in the tank overnight). See the Fluidics Decontamination procedure on page 8-3. Reattach the Waste Tank to the system. For list of approved cleaners, see A-1.

9 Open the Sheath Tank by pulling up on the handle and removing the lid. Fill the Sheath Tank to the upper weld line with sheath fluid, close the vent valve, and reattach the tank to the system.

NOTE If it is necessary to clean the Sheath Tank, see the Fluidics Decontamination procedure on page 8-3.

aXcess Control Panel Monitor

1 Do not turn off the aXcess Control Panel unless the instrument will be unused for a significant period of time.

2 If it is necessary to turn off the whole system, press the on-screen power button on the aXcess Control Panel and follow the on-screen prompts to perform the shut down procedure.

Summit Software Workstation

1 Perform data backup as required and close the Summit Software Workstation.

2 Log off the Windows operating system.

3 It is recommended that the Summit Software Workstation remain on.

CHAPTER 6

Performance Validation

Performance Validation

Daily performance validation provides the assurance that the MoFlo XDP 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.

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.

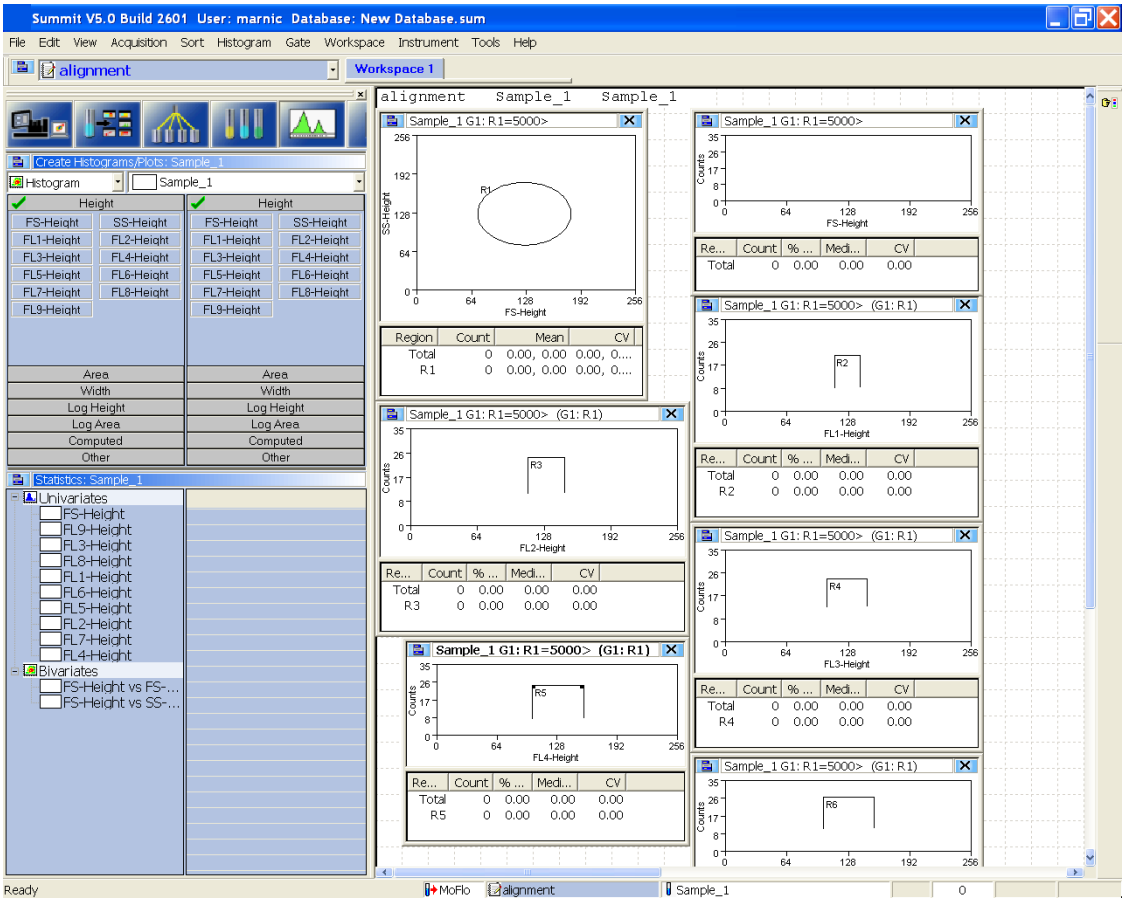
Performance Validation Procedure

1 Perform the instrument Startup Procedures on page 5-1.

2 Perform Coarse Instrument Alignment on page 5-4.

3 Align the instrument to a set of established parameters using Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (concentration 10^6). See Fine Instrument Alignment on page 5-6. Create an alignment protocol to reflect the configuration of your instrument. Figure 6.1 can be used as a reference. For each available parameter, collect data for the median channel, %CV, PMT voltage, and laser power.

Figure 6.1 Performance Validation Protocol Example



4 Acquire 5,000 events at 100-120 EPS and save the results for future reference.

NOTE If results vary dramatically from previous measurements, or fall outside the target %CV values in Table 6.1, refer to CHAPTER 9, Troubleshooting.

Calibration Parameters

%CV

The percent Coefficient of Variation is a common term used to judge the resolution or width of a peak. The better the resolution, the more likely that neighboring populations can be counted and sorted distinctly. The %CV is determined automatically in Summit software by determining the standard deviation of the population in terms of channel number and then dividing by the mean channel number and multiplying by 100%.

Table 6.1 Alignment Target %CV Values

Laser	Parameter	%CV	PMT Voltage
355 nm 100 mW	UV 1	<3.0	<650 V
	UV 2	<3.5	<800 V
405 nm 25 mW	Violet 1	<4.0	<800 V
	Violet 2	<4.0	<600 V
488 nm 100 mW	FITC	<2.5	<700 V
	PE	<2.5	<700 V
	ECD (PE-Texas Red)	<3.5	<800 V
	PC5 (PE-Cy5)	<4.0	<800 V
	PC7 (PE-Cy7)	<5.0	<800 V
635 nm 25 mW	APC	<6.5	<900 V
	APC Cy-7	<5.0	<800 V
	Alexa 700	<5.0	<800 V

The results in Table 6.1 were obtained by gating on FSC vs. SSC and drawing a region over the peak on median 128 as seen in Figure 5.5.

IMPORTANT The following %CV values are intended to be used as guidelines. It is important to track the performance of your instrument to determine trends over time.

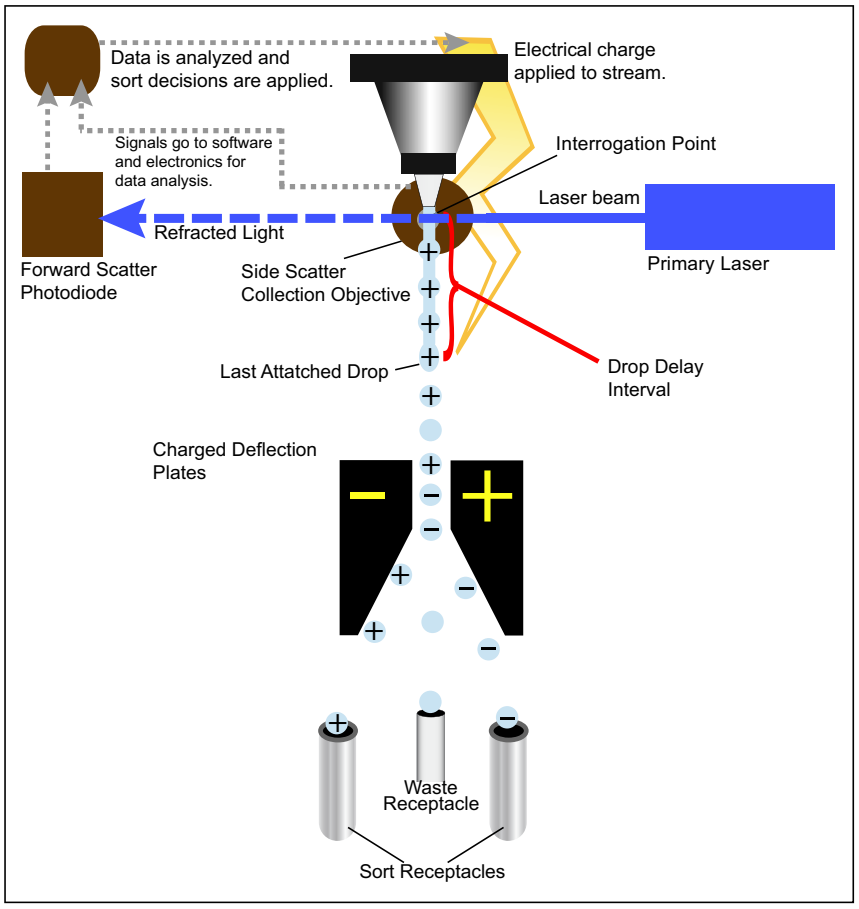
CHAPTER 7

Sorting

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.

Figure 7.1 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 XDP 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%.

Sort Setup

Prior to sorting a sample, the instrument must be started and aligned with the sheath stream flowing. Below is an outline of the steps required to set up a sort run. Detailed instructions are included later in this section.

1. Optimize droplet formation.
2. Optimize droplet deposition.
3. Optimize droplet deflection.
4. Enable IntelliSort if desired.
5. Determine Drop Delay.
6. Acquire data in Summit software and set regions and gates.
7. Set Sort Decisions in Summit software.
8. Configure CyCLONE.
9. Configure Slides or Plates in Summit software (if necessary).

NOTE You should not attempt to sort with air in the CytoNozzle 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 page 9-7 to determine how to check for trapped air.

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 split second when the cell will be contained in 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 XDP 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.

Setting Up a Sort Run

Optimize Droplet Formation

IMPORTANT Before you begin, ensure that the CytoNozzle is clean and the system is air free. See page 9-7.

In order for the instrument to make reliable sort decisions, the time it takes a particle to travel between the laser interrogation point and the last attached droplet must be precisely defined.

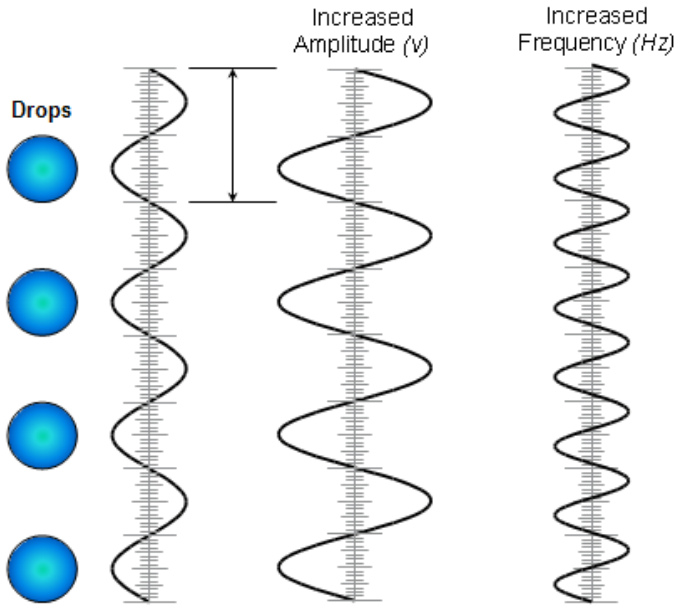
1 In order to create droplets from the sheath and sample stream you must press the Drop Drive button on the aXcess Control Panel Droplet Control screen. When the Drop Drive Amplitude is on, you can view the droplet image.

2 The Droplet Camera can be raised or lowered to find the Last Attached Drop in the stream. The control arrows for the camera position are located on the upper-right portion of the aXcess Control Panel Droplet Control screen.

3 Set the Drop Drive Amplitude according to the recommended voltage given in the CytoCalc table on page E-1. Typically DD Amplitude for a 70 µm nozzle tip is between 8 and 18 volts.

NOTE 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.

Figure 7.2 Drop Drive Amplitude and Frequency Diagram



4 Find the shortest droplet break off point by adjusting the drop drive frequency. You may need to adjust the camera position during this step.

5 Fine tune the DD Amplitude adjustment to achieve a clean break-off of the first satellite drop. This will help to create a good charge phase and reduce fanning on the side streams. See Figure 7.3, Figure 7.4, and Figure 7.5 for examples of acceptable and unacceptable droplet images.

Figure 7.3 Last Attached Drop Diagram

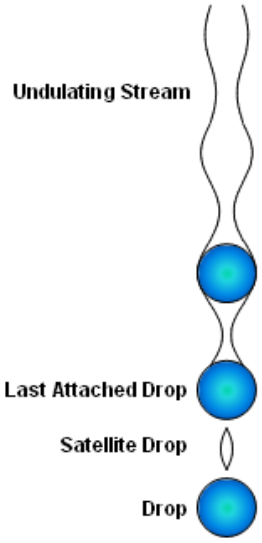


Figure 7.4 Droplet Stream Images for Comparison 1

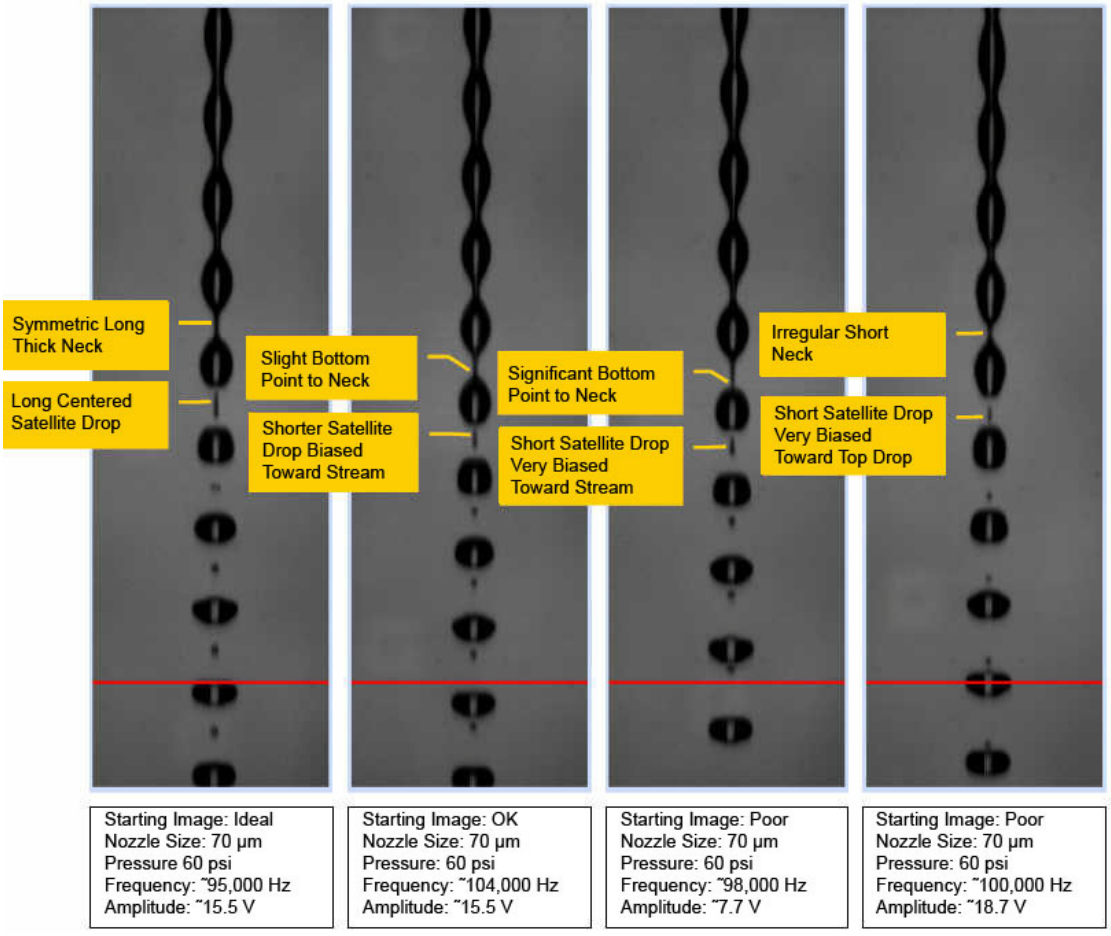
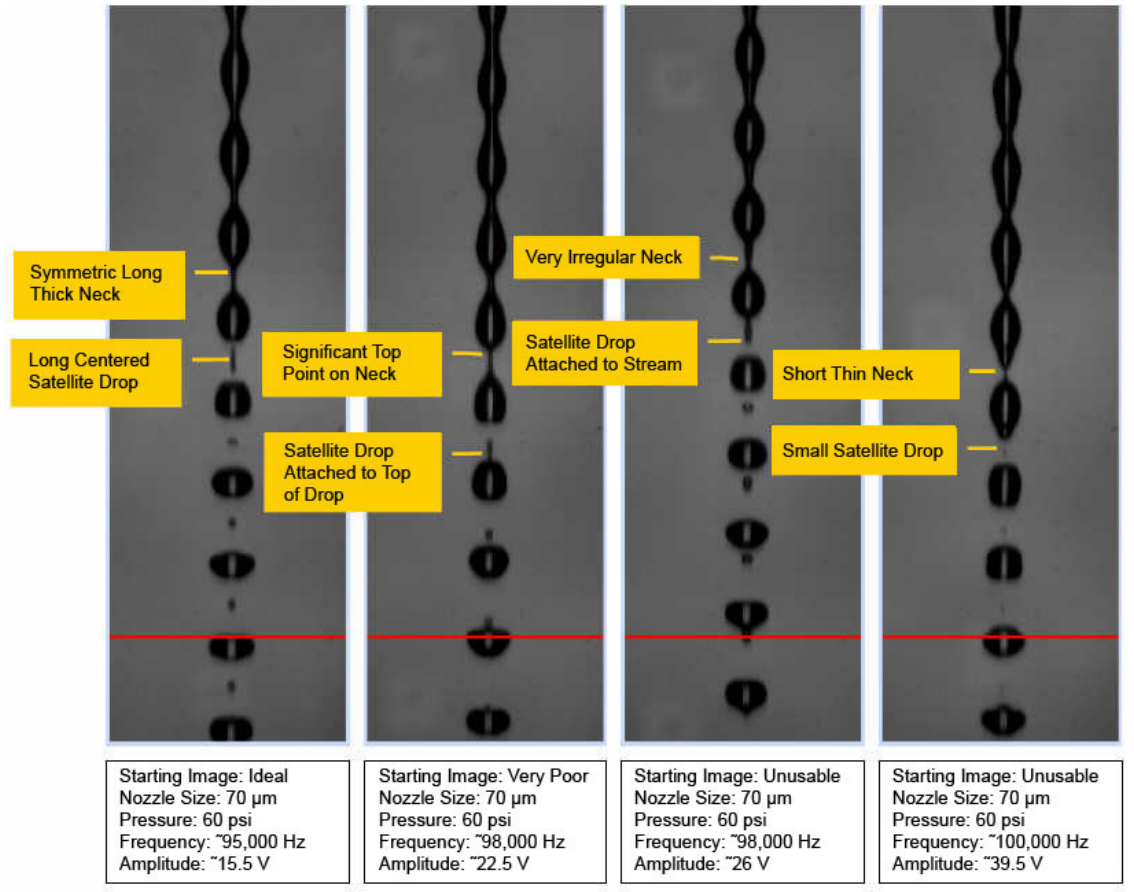


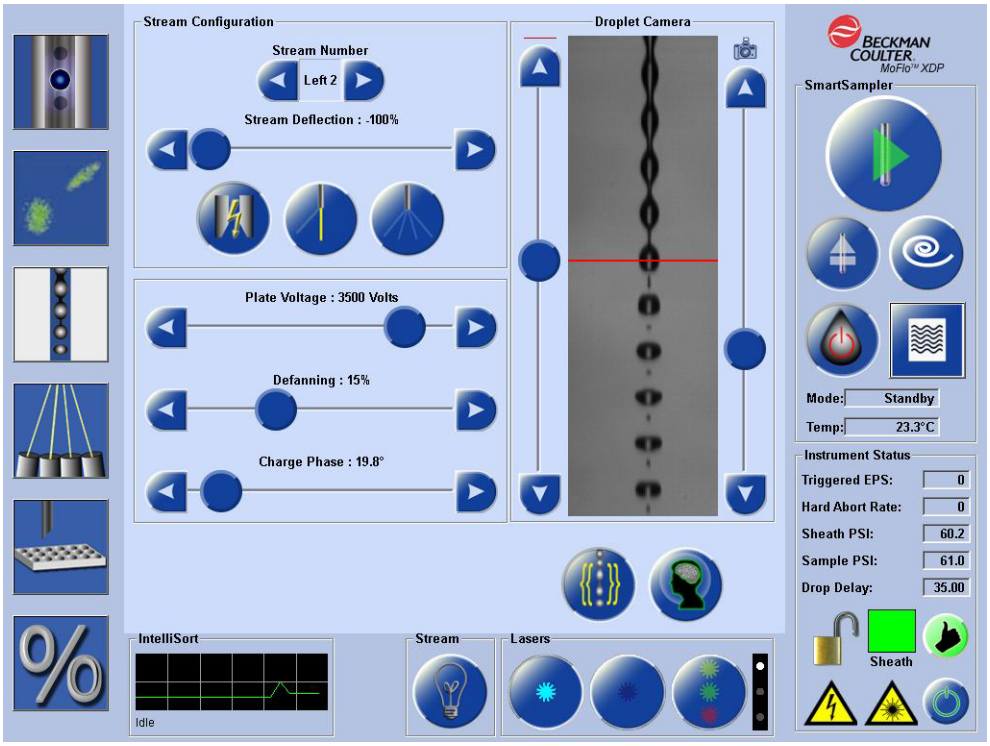
Figure 7.5 Droplet Stream Images for Comparison 2



Optimize Droplet Break-off and Start IntelliSort

After droplet break-off is optimized, the aXcess Control Panel Stream Configuration screen is used for adjusting sort parameters and enabling IntelliSort.

Figure 7.6 Optimizing Droplet Deposition and Starting IntelliSort



1 Turn on the Sort Chamber light.

2 On the aXcess Control Panel Stream Configuration screen, press the Charge Plates button to turn them on.

3 Adjust the plate voltage value. If you are performing a four-way sort, set the voltage to 4000 V.

4 Set a Stream Number you intend to use for the sort, and then enable the deflection for the stream by pressing Enable Stream button.

- Left 2 = the stream furthest left of the waste receptacle.
- Left 1 = the stream to the immediate left of the waste receptacle.
- Right 1 = the stream to the immediate right of the waste receptacle.
- Right 2 = the stream furthest right of the waste receptacle.

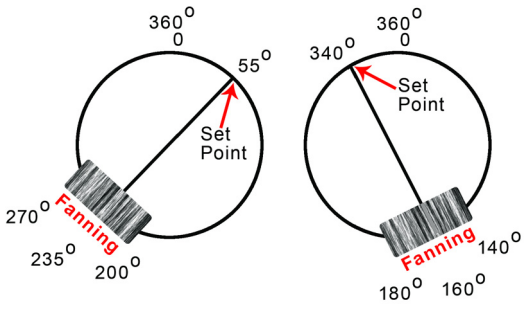
5 Repeat step 4 until you have enabled all of the streams you intend to use.

6 Press the Test Pattern Button.

7 On the Stream Configuration screen, adjust Charge Phase to determine the bounding values that cause the streams to fan the most, and take note of the values.

8 Adjust the Charge Phase in between the two extreme values. See Figure 7.7 below.

Figure 7.7 Adjust the Charge Phase Between Two Extremes



9 Adjust Defanning to tighten the waste stream.

10 Adjust Stream Deflection to steer the streams into the tubes.

NOTE The values for L1 and R1 cannot be set higher than the values for L2 and R2 respectively.

11 Press the Test Pattern button to turn it off.

IMPORTANT Allow IntelliSort to complete the Preparing cycle. Do not bump the instrument, or make adjustments to the system during this cycle. Adjust the droplet camera so that the last attached drop is in the center of the droplet window and move the red marker line to a set point, such as just below the last attached drop.

12 If you would like to use IntelliSort, press the IntelliSort (brain) button.

NOTE The controls for adjusting Frequency, Amplitude, and Charge Phase are disabled when IntelliSort is on.

IMPORTANT The Last Attached Drop Marker can be used to visually indicate the starting position of the Last Attached Drop. However, changes in sheath fluid temperature and pressure will cause the Last Attached Drop to drift from the starting position. This is normal. IntelliSort is controlling other parameters that maintain the correct number of attached drops.

13 It is optional to position the Last Attached Drop Marker just below the Last Attached Drop.

IMPORTANT If IntelliSort exits **Maintain** mode, consider the following:

- The Manual Sample Station may be clogged or require debubbling.
- The fluidics system was not set up correctly. (Bouncy images and drifting top drop under steady state conditions are indications of fluidics instability.)
- The image of the stream/drops was lost. (Some causes could be that the Illumination chamber door was opened, the instrument ran out of fluid, or the instrument lost pressure.)
- Normal operation caused too much variation in stream conditions for IntelliSort to manage. (This is usually indicative of fluidic instability.)
- DO NOT back flush, debubble, or jostle the sample tubing when IntelliSort is in Maintain mode.

14 When the prompt under the IntelliSort Monitor displays Maintain, perform the Drop Delay procedure.

NOTE IntelliSort can maintain Drop Delay within 10% for a temperature change of ±3 degrees Celsius and for a sheath pressure change of ± 1 psi.

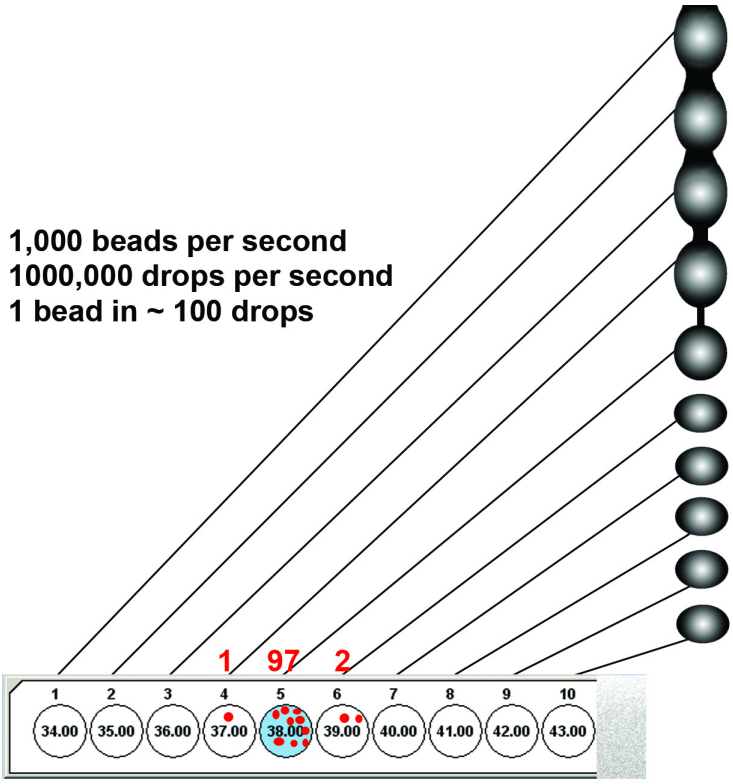
IntelliSort and SortRescue

It is optional to attach the SortRescue receptacle to the CyCLONE before you sort to tubes. In the event that IntelliSort detects a sort failure, the Smart Sampler will stop the flow of sample, the CyCLONE arm will retract in order to keep the SortRescue samples from being contaminated, and the sort run will be terminated.

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.

Figure 7.8 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 software.

- 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.

IMPORTANT IntelliSort must be enabled before you determine Drop Delay. See page 7-6.

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

NOTE Beckman Coulter recommends that you use IntelliSort to maintain Drop Delay.

1 Run Drop Delay Beads at 100 EPS.

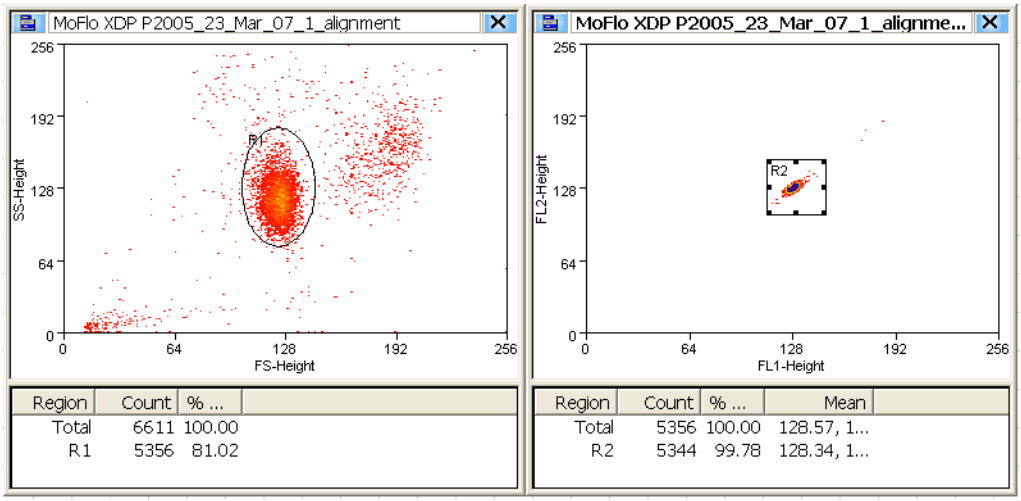
2 Open an established Drop Delay Protocol, or create a FSC vs. SSC dot plot and a FL1 vs. FL2 dot plot.

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 FL1 vs. FL2 population.

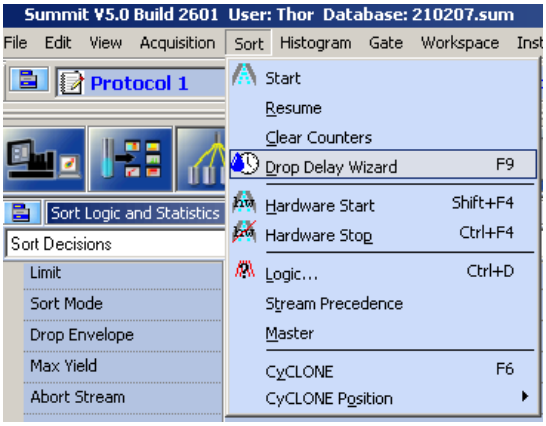
Figure 7.9 Drop Delay Dot Plots



6 Remove the Sort Receptacle from the Sort Chamber.

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

Figure 7.10 Drop Delay Wizard 1

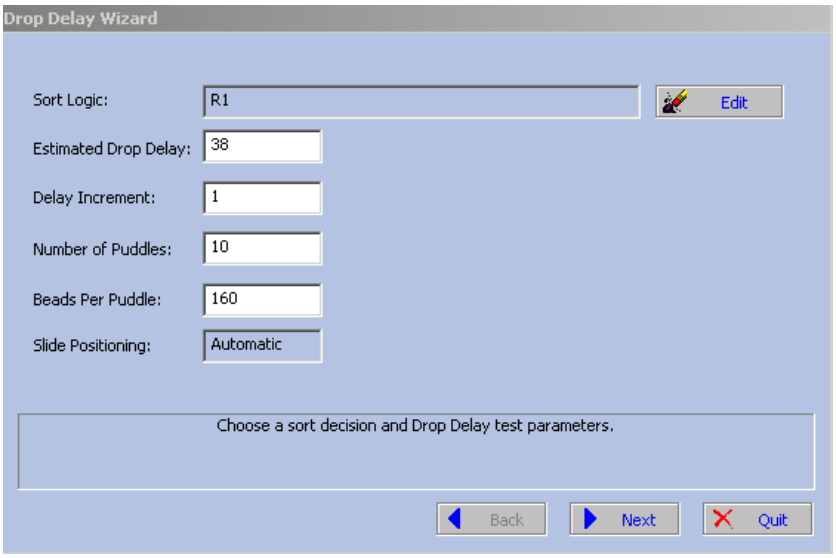


8 The **Drop Delay Wizard** appears. Move all objects out of the path of CyCLONE and place a clean slide on the CyCLONE.

NOTE If the CyCLONE does not move, verify the chamber door is closed.

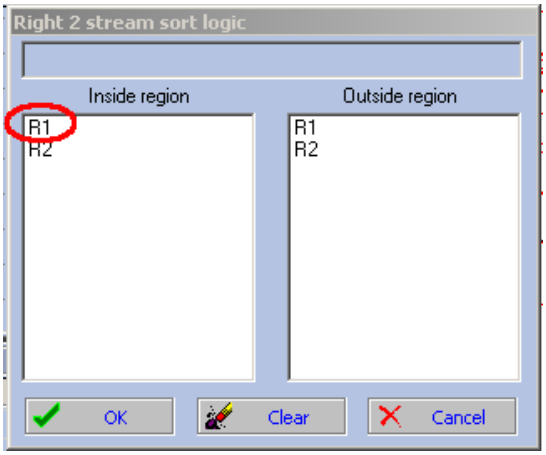
NOTE The Estimated Drop Delay value will be the same as the last time you used the instrument. If you think this value is not an appropriate starting place, you may consult the CytoCalc table to determine an Estimated Drop Delay value, and enter the value in the Estimated Drop Delay field. See page E-1 to view CytoCalc.

Figure 7.11 Drop Delay Wizard 2



9 Click the **Edit** Button. The **Right 2 Stream Sort Logic** dialog box appears.

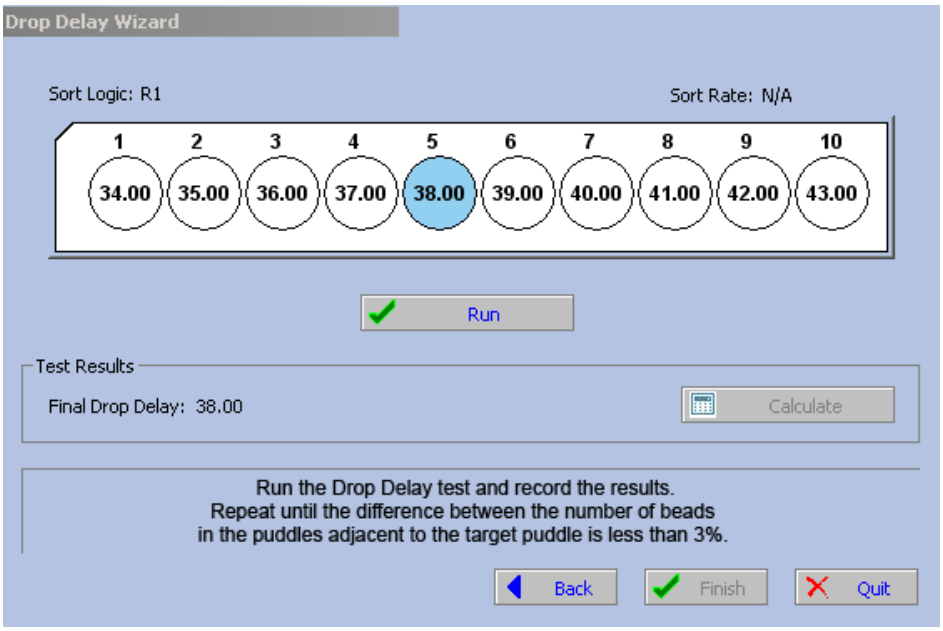
Figure 7.12 Drop Delay Wizard 3



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

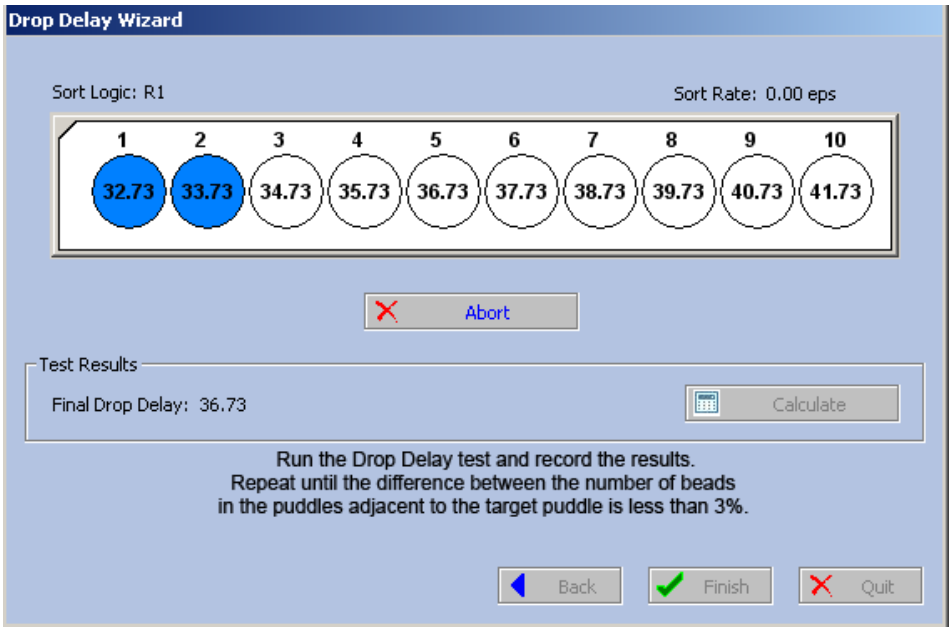
11 Click **Next**. The circles represent the puddles that will be deposited on the slide.

Figure 7.13 Drop Delay Wizard 4



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

Figure 7.14 Drop Delay Wizard 5



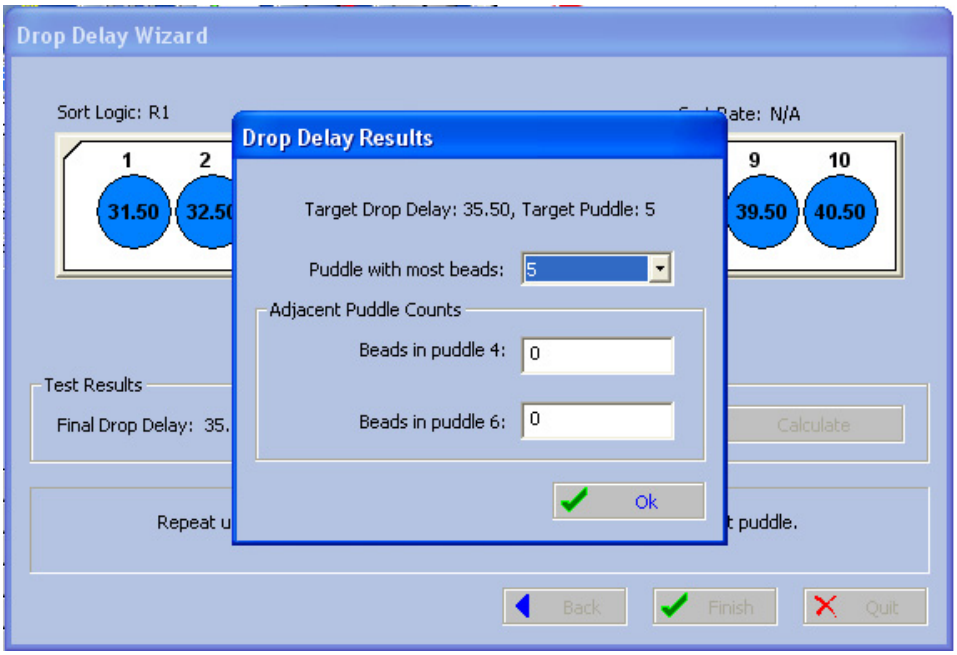
13 When all puddles have been created, remove the slide and inspect the puddles under a fluorescent microscope.

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

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

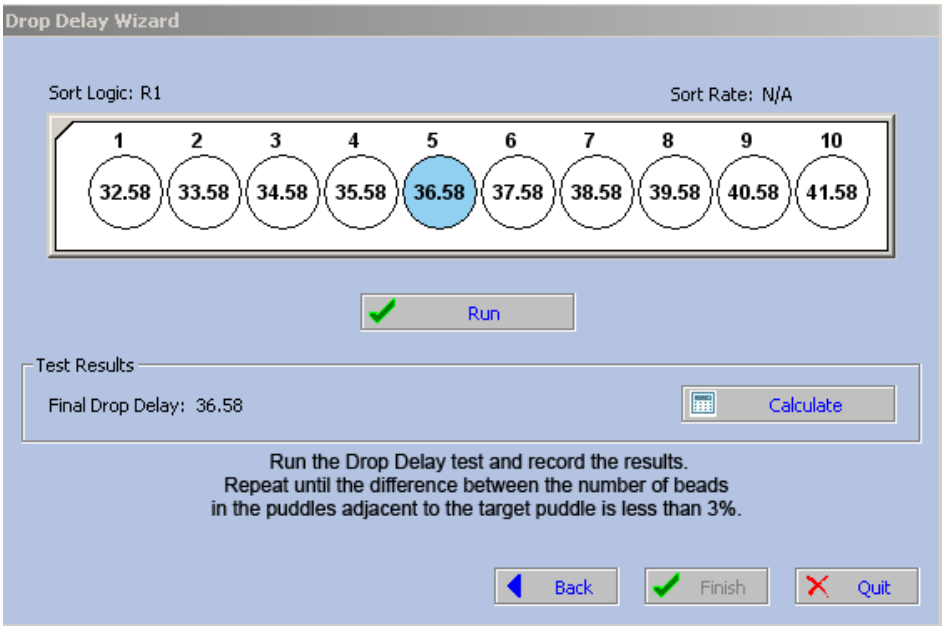
16 Enter the values in the **Drop Delay Wizard**.

Figure 7.15 Drop Delay Wizard 6



17 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 7.16 Drop Delay Wizard 7



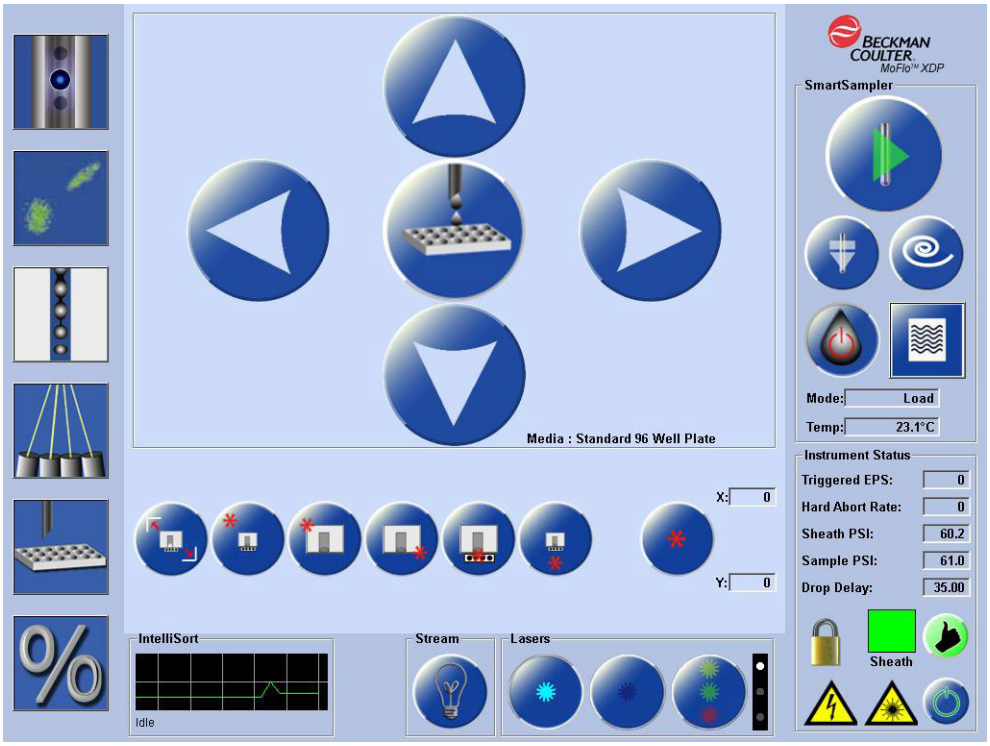
18 Click **Finish**.

Configure CyCLONE

It is necessary to configure CyCLONE to recognize the sort receptacle medium you intend to use for your sort.

NOTE If you intend to sort to a plate or a slide, proceed to the Acquire Data section on page 7-17. You will return to configure CyCLONE later.

Figure 7.17 Configure CyCLONE Screen



1 In Summit software, select the media you intend to use for the sort using the Sort CyClone menu.

2 Go to the aXcess Control Panel Configure CyCLONE screen.

3 If all buttons are disabled, ensure the Sort Chamber door is closed and press the Find Extents button.

4 Next you will test, and if necessary reset, the Home, End, and Tubes positions (if you are sorting to tubes.)

5 Press the Home button. The CyCLONE will move to the last set position. If you want to change the home position, use the arrow buttons to move the CyCLONE to the desired location. Press the Set button to store the new position.

6 To test the new position, press the Squirt button and observe if the fluid is deposited in the desired location.

7 If the fluid is deposited in the desired location, press the **Set** button (number 12 on Figure 3.8.)

IMPORTANT If you do not press the **Set** button the position will be lost.

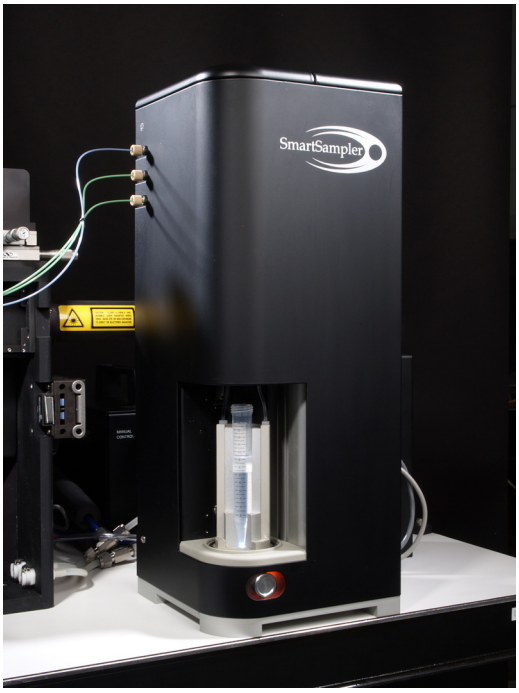
8 Follow this process until all of the CyCLONE positions are configured.

Acquire Data, Set Regions and Gates

Now that the instrument is aligned, droplets and streams are optimized, and drop delay is set, you are ready to acquire data from your sample then set Regions and Gates from which to set Sort Decisions.

1 Load the sample tube on the SmartSampler or the Manual Sample Station.

Figure 7.18 SmartSampler



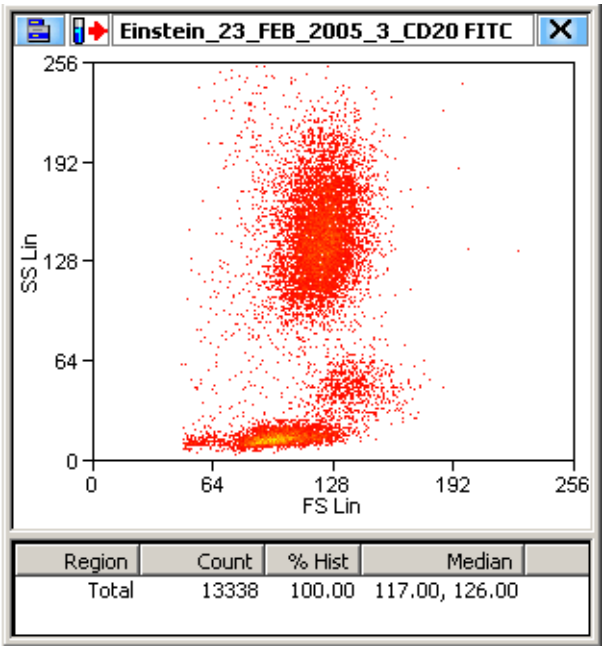
2 In Summit software load a pre-defined Protocol or create the appropriate dot plots and histograms.

NOTE For directions on how to make dot plots and histograms see page 4-27.

3 Acquire data in Summit software by clicking the Acquire menu and selecting Start, (or by pressing the F2 key).

NOTE If your system includes a SmartSampler, check the settings on the **Instrument** tab in Summit software. See page 4-4.

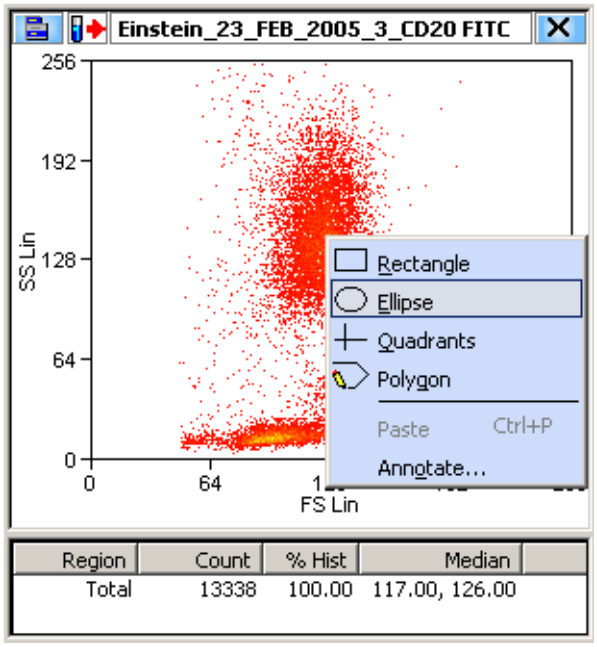
Figure 7.19 Acquire Data



4 Press the F2 key to stop acquisition.

5 Right-click to create Regions from which to make sort decisions.

Figure 7.20 Set Regions



Set Sort Decisions

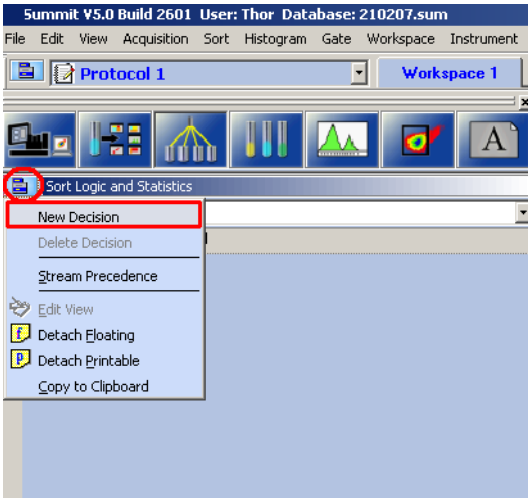
After you have created regions around the various constituents you would like to sort from the sample population it is time to define sort decisions. There are many sort modes and droplet envelopes available to customize your sort. Each Sort Mode is specifically designed to provide high recovery, high purity, single cell deposition, or both high purity and recovery. The Droplet Envelope gives the sort logic the freedom to charge more than one drop in a single sort decision to maximize recovery.

Sort Decisions are set in the **Sort Logic Editor** in Summit software. You must select sort decisions for each stream you intend to sort.

1 Click the **Sort** tab in Summit software.

2 Click the menu icon in the **Sort Logic and Statistics** panel and select **New Decision**.

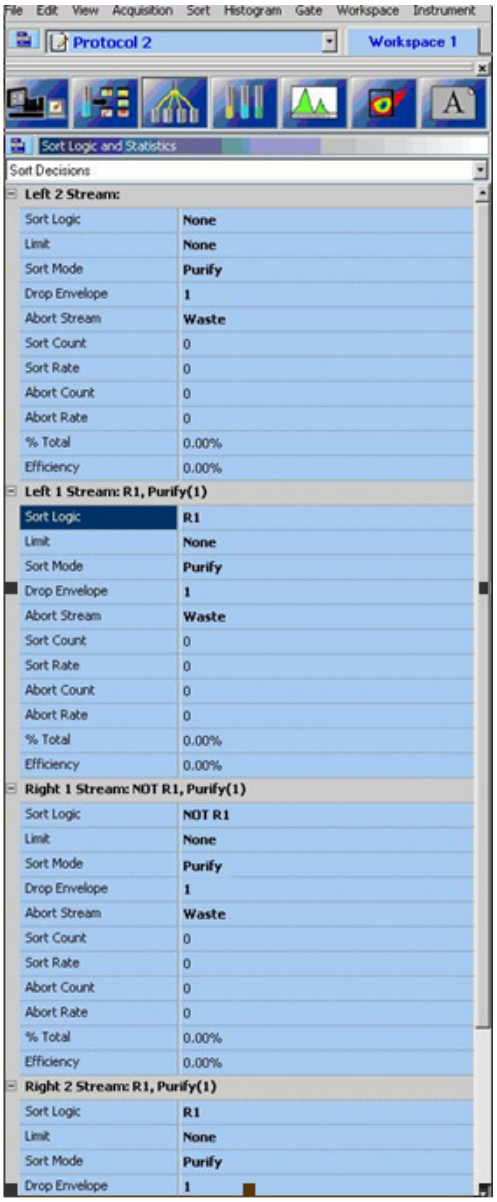
Figure 7.21 Set Sort Decisions 1



3 The **Sort Logic and Statistics** editor appears.

NOTE You may need to click and drag the column to the right to display the options you see below.

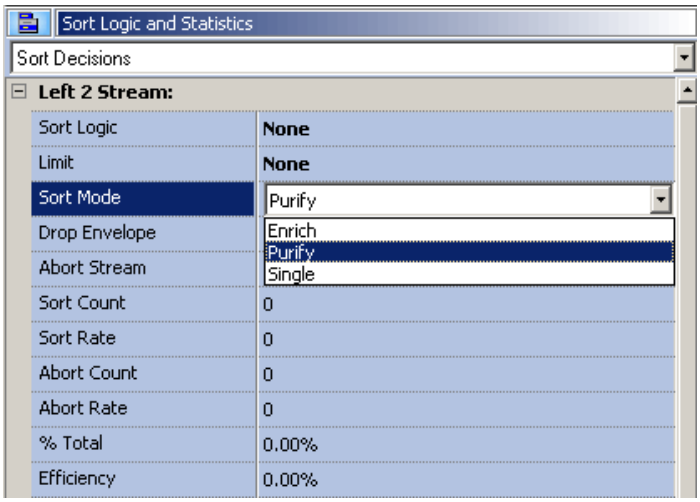
Figure 7.22 Set Sort Decisions 2



Select a Sort Mode

1 On the Sort tab, with the **Sort Logic and Statistics** panel open, double-click the field to the right of **Sort Mode** to display the dropdown menu.

Figure 7.23 Select Sort Mode



2 Select the appropriate option explained below. See Figure D.2 for more information.

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.

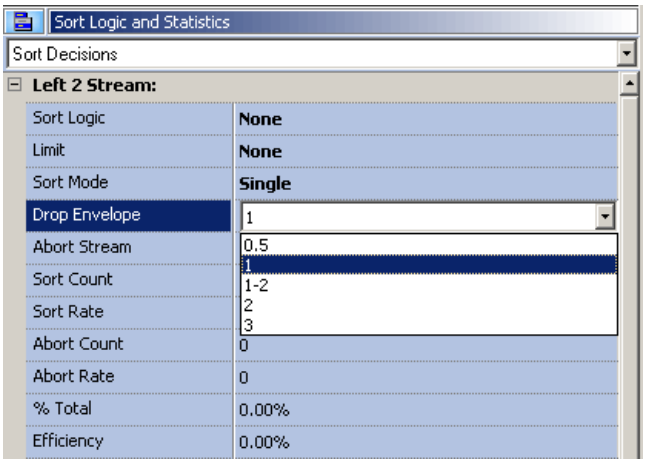
Single Mode Single Mode can be thought of as Single Cell Mode. In other words, only one positive event must be contained within the Droplet Envelope to pass the sort logic. If two positives or a negative are within the Droplet Envelope, the sort logic will fail and the positive(s) will be aborted. If 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.

Select a Droplet Envelope

The Droplet Envelope defines the number of droplets to which sort mode will be applied.

1 Double-click the field to the right of **Drop Envelope** to display the dropdown menu.

Figure 7.24 Select Drop Envelope



2 Select the appropriate option 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. It should not be used with the Single Sort Mode because events calculated to be on the edge of a drop cannot be guaranteed to actually sort with that drop. 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.

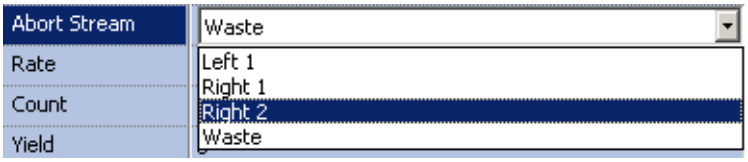
- 3 Drop - Three drops are always sorted. The drop containing the positive events plus both adjacent drops are sorted. Use this envelope only when the drop delay stability cannot be maintained within ¼ drop. At high event rates with either the Single or Purify Sort Mode, this will provide very poor or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

Selecting an Abort Stream

There are three means by which an event will fail to be sorted. One is a Hard Coincidence. The other two are considered software-related or Soft Aborts. A Soft Abort is a result of an event failing the criteria that was defined in the Summit software by the operator. When determining sort decisions, you must appoint a destination stream for aborted events.

1 Double-click the field to the right of Abort Stream to display the dropdown menu.

Figure 7.25 Select Abort Stream



2 Select the stream to which you would like the aborted events be directed.

- If you intend to use CyCLONE to sort to a plate or a slide, proceed to the Plate or Slide Configuration section. See page 7-25.
- If you intend to sort into tubes, or any sort receptacle other than a plate or a slide, proceed to the Begin Sorting section. See page 7-30.

Plate or Slide Configuration in Summit Software

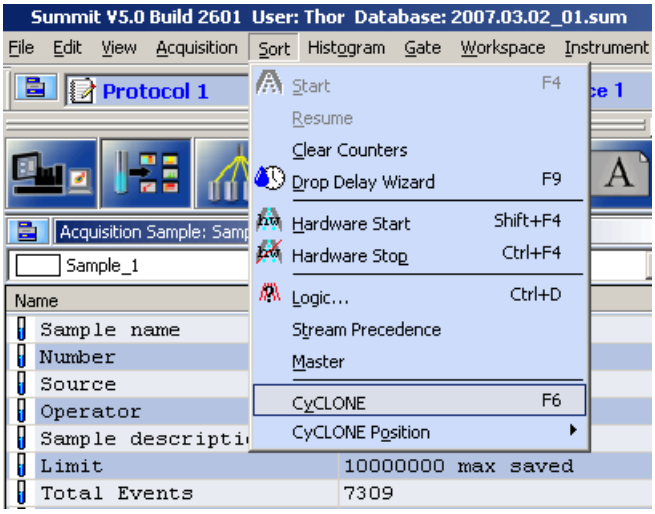
IMPORTANT If you are using a sort receptacle other than a plate or a slide, do not follow this procedure.

When you intend to use CyCLONE 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 before sorting.

---

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

Figure 7.26 Select CyCLONE



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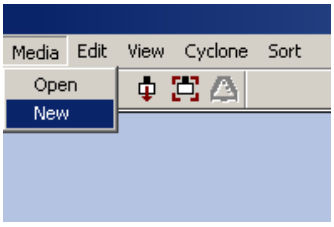
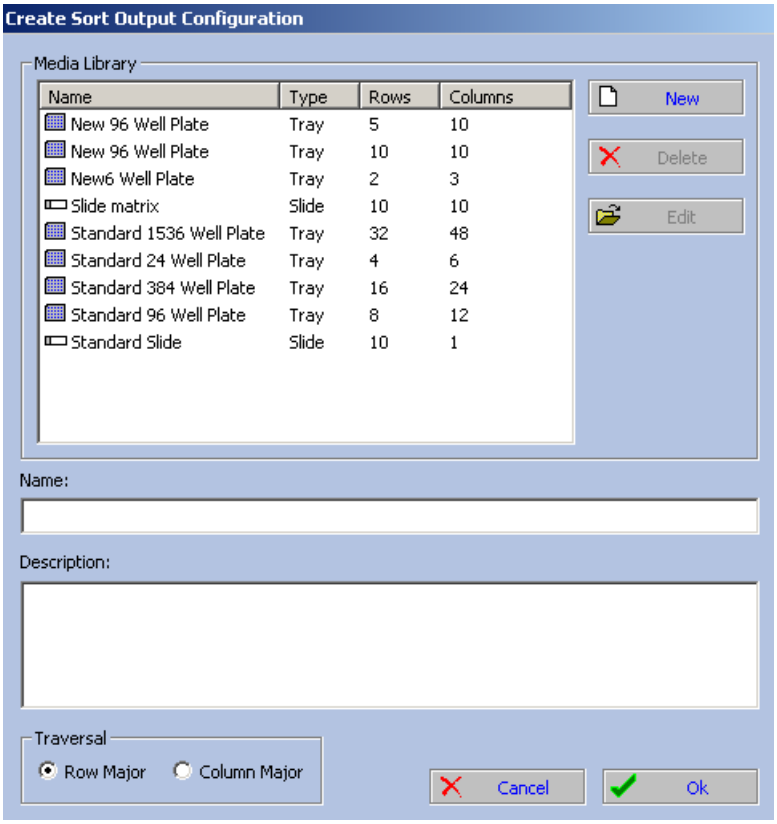
2 Click **Media** and select **New.**

Figure 7.27 CyCLONE Media New



3 A dialog box appears. Select the appropriate medium from the list.

Figure 7.28 CyCLONE Select Slide or Plate Type



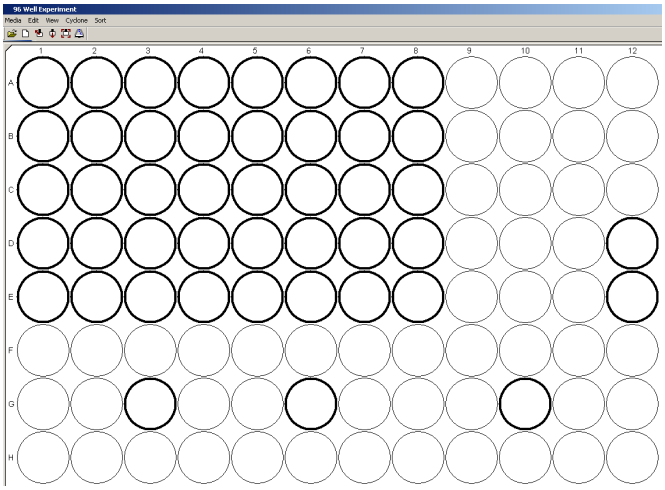
4 Enter a file name in the **Name** field, and click **OK**.

5 Proceed to the Configure CyCLONE section.

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

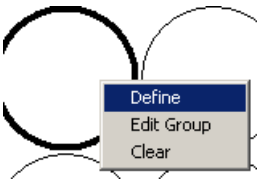
NOTE To select individual circles, press the CTRL key while you click the circle. To select a group of circles, click one circle, press the SHIFT key while you click another circle. To clear a selected circle, click it again.

Figure 7.29 CyCLONE Layout



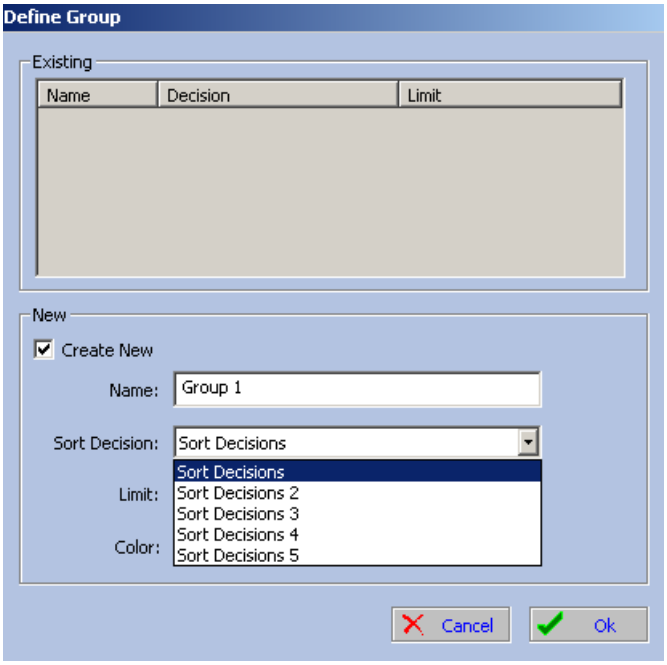
7 Right-click in a selected circle and choose **Define**.

Figure 7.30 CyCLONE Define Layout



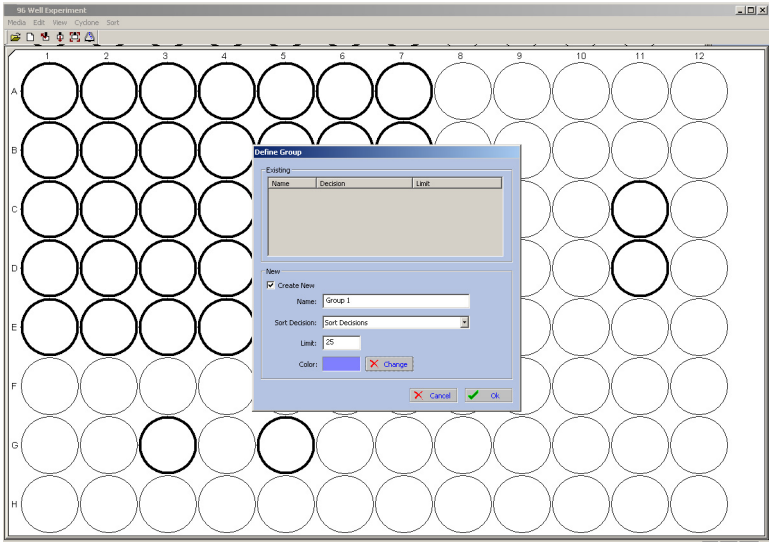
8 Specify sort decisions if not already set. You must set the decisions for stream Right 2 (R2) only.

Figure 7.31 CyCLONE Define Layout Sort Decisions



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

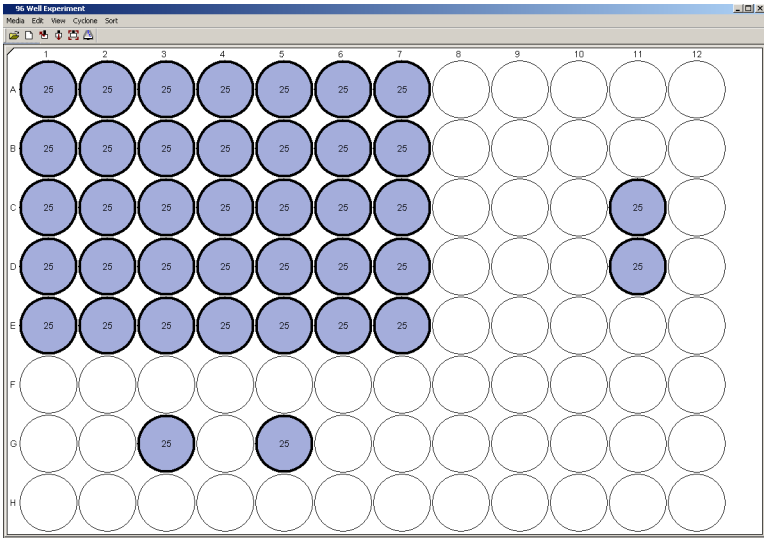
Figure 7.32 CyCLONE Define Layout Colors 1



10 Select a color.

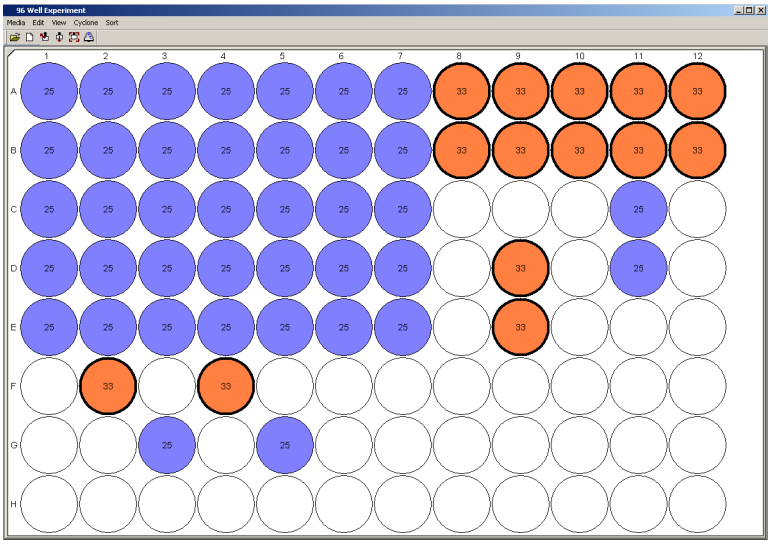
11 Click **OK**.

Figure 7.33 CyCLONE Define Layout Colors 2



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

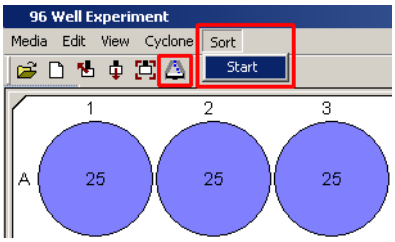
Figure 7.34 CyCLONE Define Layout Colors 3



Begin Sorting to a Plate or Slide

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

Figure 7.35 CyCLONE Start Sort



Begin Sorting to Tubes

To begin sorting to tubes, use the instructions that are appropriate for your instrument – Manual Sample Station or SmartSampler.

Manual Sample Station

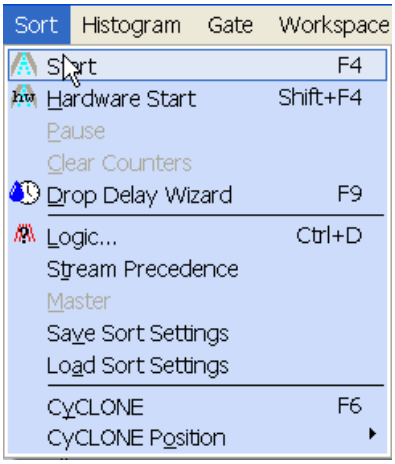
1 Ensure that a sort receptacle other than a slide or a plate is in place.

NOTE To sort to slides or plates see the Plate or Slide Configuration in Summit Software section.

2 From the Sort Menu in Summit software, select Start (or press F4).

NOTE Pressing F4 again will stop the sort.

Figure 7.36 Start Sort to Tubes



SmartSampler

1 Ensure that a sort receptacle is in place.

2 Ensure that the SmartSampler settings on the Summit Software Instrument tab are set to your preference. See page 4-4.

3 Press the Start Sample button on the aXcess Control Panel.

4 From the Sort Menu in Summit software, select Start (or press F4).

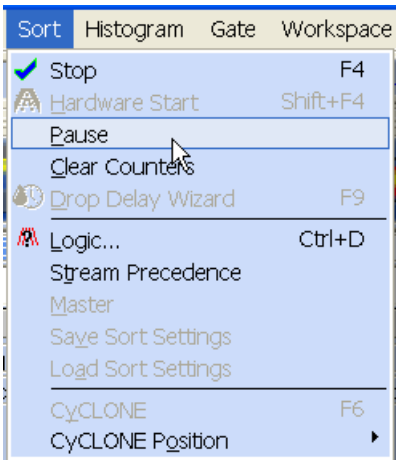
NOTE Pressing F4 again will stop the sort.

Pause Sorting and Data Acquisition

In certain situations during a sort run, you may want to pause data acquisition but continue to run the sample so you can continue to view events on the aXcess Control Panel, and then resume data acquisition later. If a sort receptacle has become full, but the sort is not complete, pause the sort while you change the receptacle.

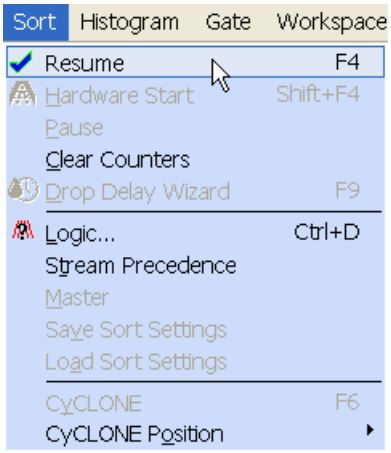
1 To pause a sort in this manner, go to the main **Sort** menu in Summit software and select **Pause**.

Figure 7.37 Pause Sort



2 To resume sorting and acquiring data in Summit software, go to the main **Sort** menu and select **Resume.**

Figure 7.38 Resume Sort



NOTE If you are using a plate or slide, MoFlo XDP will resume sorting at the same position CyCLONE was in when you paused the sort. If you are sorting to tubes, use the Configure CyCLONE panel to check the positioning of the tubes.

Saving Acquired Data

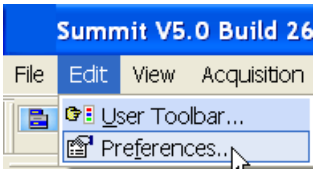
Summit software for MoFlo XDP can save up to one billion events in an FCS file (up to 200 GB) in fewer than five seconds provided that certain settings are enabled. Data can be saved in FCS format that can be viewed and analyzed in Summit software online or offline. FCS files can be saved to the local hard drive or to a network location.

Saving Large FCS Files to a Local Drive

Summit software for MoFlo XDP can save large FCS files in fewer than five seconds if they are saved to the local hard drive, and if the proper settings are enabled.

1 Prior to acquiring data, select **Edit > Preferences**.

Figure 7.39 Edit Preferences



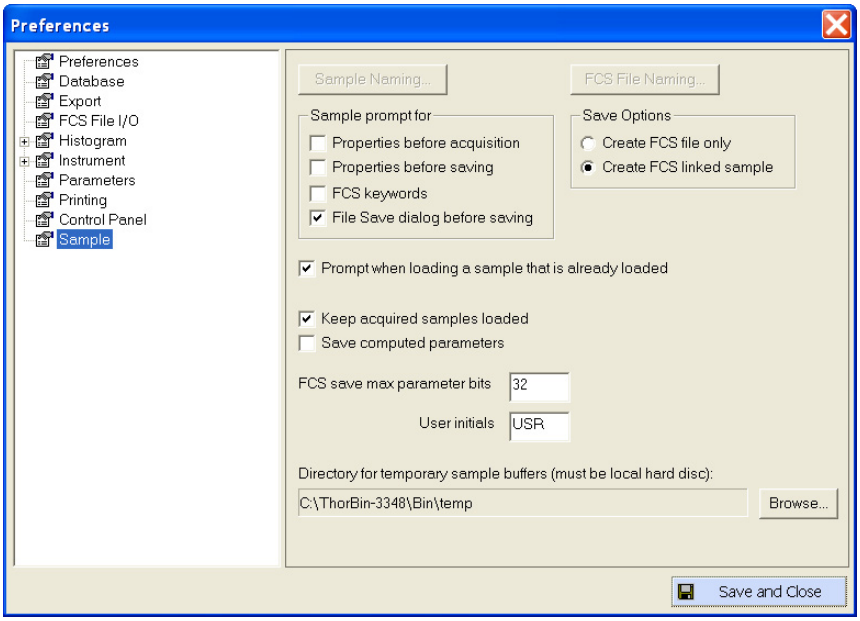
The **Preferences** dialog box appears.

2 Ensure that the value in the field next to **FCS save max parameter bits** is 32.

NOTE FloJo 8.43 cannot read FCS files over 16 bits. If you want to analyze data in FloJo, change the value to 16. FloJo cannot display any Summit software time parameters.

3 Ensure that the checkbox next to **Save computed parameters** is clear.

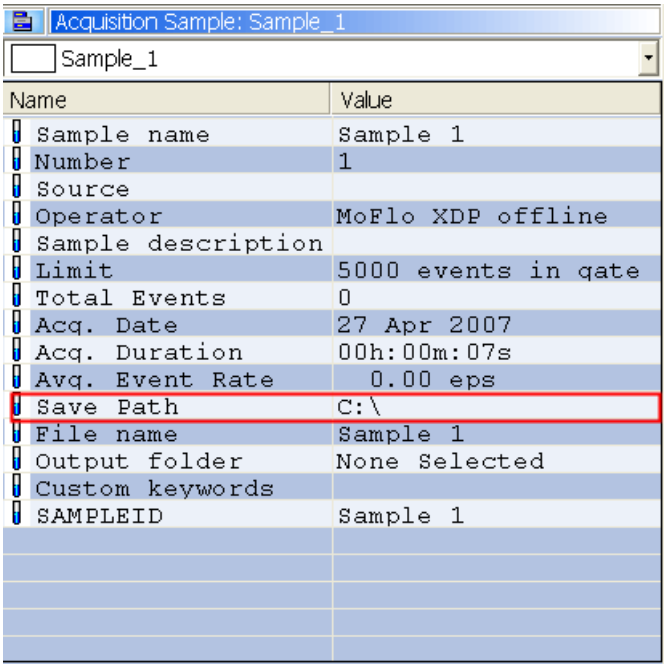
Figure 7.40 Edit Preferences Sample Settings



4 Click **Save and Close**.

5 In the Summit Software Acquisition tab ensure that the **Save Path** is designated to a location on the local drive.

Figure 7.41 Acquisition Tab Sample Save Path



6 Acquire data and stop acquisition.

7 Go to the main **Acquisition** menu and select **Save**, or press the F3 key.

8 The save destination must be the same as the location specified in the **Save Path** in step 5.

9 Enter a name for the file. In the file type dropdown menu select **FCS file (PC Byte Order)**.

IMPORTANT If you save an FCS file to the local network, but did not use the optimal settings, the file will save to the destination you selected, but it will take longer. The file will be saved as a background process and you may continue to work in Summit software during this time.

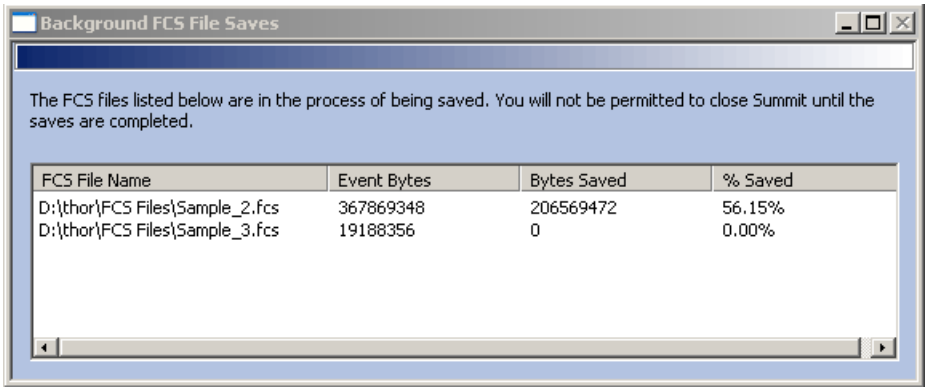
10 Click **Save**. The file will be saved in fewer than five seconds.

NOTE It is possible to move locally saved files to a network location later.

Saving Large FCS Files to a Network Location

You can save large FCS files to a Network Location and continue to work in Summit software while they are being saved. Data is saved as a background process, so you can continue to acquire and save data files. Additional files will be added to a queue until they can be processed.

Figure 7.42 Background Saves



CHAPTER 8

Cleaning and Maintenance

Introduction

Regular maintenance of the MoFlo XDP 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

Daily Decontamination

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 procedure that is appropriate for your instrument (Manual Sample Station Daily Decontamination Procedure or SmartSampler Daily Decontamination Procedure).

Manual Sample Station Daily Decontamination Procedure

1. Set both sheath valves to Sheath.
2. Open the pinch valve to backflush the sample line into the waste receptacle. Backflushing for 10-15 seconds will clear the sample tubing of sample.
3. Run one tube of mild disinfectant by turning one sheath valve to Vacuum and the other to Sheath then press the Boost button on the Pressure Console.
4. Once the tube of disinfectant is half empty, reverse the position of valves.
5. Remove the tube and run a tube of DI water.
6. Press the Boost button, while turning both sheath valves to the off position and run the whole tube.

7 Close the Sample Pressure Valve and the Sample Valve.

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

9 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 XDP tanks before autoclaving.

SmartSampler Daily Decontamination Procedure

1 On the aXcess Control Panel, press the Stop Fluidics button and follow the prompts to run a tube of cleaner and a tube of DI water.

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

3 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 XDP tanks before autoclaving.

NOTE See APPENDIX A, *Approved Cleaners and Disinfectants* for additional cleaning information.

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 XDP every year. To schedule annual maintenance service, contact your local Beckman Coulter Support Representative.

Sheath Filter

The in-line 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. See page 9-10.

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 XDP 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. (Spare part number 998003 - SPARE, FILTER, INLINE, 0.2 MICRON, W/VITON O-RING)
- A bleach solution containing 2000 ppm active chlorine is also acceptable (115 mL household bleach + 2885 mL water).
- Paper towels

3 Ensure system is fully depressurized.

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

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

6 Turn instrument on according to CHAPTER 5, *Startup Procedures* and ensure all fluidic lines are properly connected. 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.

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

8 After 20 minutes, perform a debubble operation at the sample station for one minute.

9 Remove the sample tube and backflush for one minute.

10 At the sheath filter canister, perform debubble operation for three minutes and then stop.

11 Shut down and depressurize system.

12 Remove the canister and rinse it with DI water. Reconnect the canister without a sheath filter installed.

13 Remove sheath tank and empty any residual decontamination solution into sink and flush with water. Rinse sheath tank with DI water and then fill tank halfway with fresh DI water.

14 Reconnect sheath tank to instrument. Prepare a new 5 mL tube with fresh DI water for placement on the sample station.

15 Start instrument and ensure all fluidic lines are properly hooked up. The rinse cycle of this procedure shall run at the same pressure as the decontamination cycle.

16 Once the sheath stream is observed coming out of the nozzle, engage the sample and run fluidics and sample for 90 minutes.

NOTE DI water in the sample station may need to be refilled during the rinse time. Refill as necessary.

17 After 90 minutes have elapsed, perform a debubble operation at the sample station for one minute.

18 Remove the sample tube and backflush for 30 seconds.

19 At the sheath filter, perform debubble operation for three minutes and then stop.

20 Shut down and depressurize system.

21 Remove and rinse the canister with DI water. Install a new sheath filter, and reconnect the canister.

22 Disconnect sheath tank and discard any unused DI water.

23 Fill sheath tank with desired fluid and reconnect to system.

24 The system should be run with the standard sheath fluid for thirty minutes prior to running any samples.

25 The sheath filter should be replaced at least once a year.

CHAPTER 9

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 XDP laser components. Laser maintenance should only be performed by specially trained, certified Beckman Coulter Field Service Representatives.

Problem/Solution Tables

The following tables are a guide for troubleshooting MoFlo XDP problems. If in doubt, call your Beckman Coulter Representative.

- Table 9.1, General Troubleshooting
- Table 9.2, SmartSampler Troubleshooting
- Table 9.3, IntelliSort Troubleshooting

Table 9.1 General Troubleshooting

Problem	Possible Cause	Possible Solution
Poor CVs on the florescence parameters.	Poor laser alignment.	Realign the lasers.
	Poor stream alignment.	Realign the stream.
	Poor laser beam quality. (Bad mode.)	Realign the laser or replace the laser.
	Poor laser beam focus. (Dirty, scratched or faulty BSO).	Clean, or change out the BSO components.
	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 CytoNozzle. Replace the CytoNozzle.
	Dirty objective lens.	Clean the lens.
	Dirty or faulty optical mirrors or dichroics.	Clean, align, or replace the mirrors.
	Dirty, misaligned, or faulty PMT.	Clean, align, or replace the PMT.
	Photo bleached or damaged beads	Prepare a new sample with fresh beads.
	Sample event rate too high.	Lower the sample pressure.
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 CytoNozzle, or replace the CytoNozzle.
	Sample event rate too high.	Lower the sample pressure.
	Misaligned or dirty FSC detector.	Clean and align the FSC detector.
	Misaligned obscuration bar.	Reposition the obscuration bar.
	Faulty nozzle assembly.	Replace the CytoNozzle.
Unstable droplet.	Air bubbles in the sheath lines or nozzle.	Remove trapped air. See page 9-7.
	Partially clogged nozzle.	Clean the CytoNozzle. See page 9-9.
	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 CytoNozzle.
	Air leaks inside the pressure console.	Tighten the fittings.

Table 9.1 General Troubleshooting *(Continued)*

Problem	Possible Cause	Possible Solution
Unstable sample event rate.	Air leaks inside the pressure console.	Tighten the fittings.
	Air leaks inside the sample handling station.	Tighten the fittings. Troubleshoot and replace if necessary.
	Air leaks inside the fittings and tubing between the sample handling station and the nozzle.	Tighten the fittings and replace the fittings and tubing if required.
	Partial nozzle clog.	Clean the CytoNozzle. See 9-9.
	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 on page ix in the Safety section.
No signal on a particular parameter.	Faulty PMT.	Isolate the PMT (change out if possible).
	PMT power 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.
Histograms show excessive noise.	Obscuration bar is not in the correct position.	Adjust the obscuration bar on the FSC collection optic.
	The threshold is not adjusted correctly.	Adjust the threshold setting on the aXcess Control Panel Fine Alignment screen.
	Microbial contamination.	Decontaminate the system. See page 8-3.
Event rate equals drop delay frequency.	Bad vertical alignment of FSC detector or nozzle.	Adjust the vertical alignment of either the nozzle stage or the FSC detector.
	Nozzle body is too high.	Adjust the vertical alignment of either the nozzle stage.

Table 9.1 General Troubleshooting *(Continued)*

Problem	Possible Cause	Possible Solution
Bead Flash is present but Count is not present. (Event Rate = 0).	The system electronics are not triggering.	Adjust the threshold setting on the aXcess Control Panel Fine Alignment screen.
	Threshold is too high.	Lower threshold to 2-5%.
	Faulty photodiode.	Trigger off a different parameter and/or replace photodiode.
	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 XDP server.	Restart Summit software.
EPS high with no sample flowing.	Dirty FSC objective.	Use cotton swab to clean FSC and SSC objectives with ethanol, rinse with DI water, dry.
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.
When using the Manual Sample Station, the Event Rate varies more than 20 percent of the Average Sample Rate.	Poorly sealed sample tube.	Verify that the O-ring is intact and is lubricated with vacuum grease.

Table 9.2 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 on IT front column.	Insufficient vacuum.	Turn up vacuum source (must be at least 440 mbar (13 in. Hg).
	Salt crystal has built up inside waste tank quick connect fitting or table manifold.	Disconnect the waste tank quick connect at the orange tubing line, hold connector upside down and tap on a solid surface until salt crystals are removed.

Table 9.2 SmartSampler Troubleshooting *(Continued)*

Problem	Possible Cause	Possible Solution
Probe strikes side of tube during agitation.	Bent probe.	Replace sample probe.
O-ring that seals chamber to the base is dislodged.	Overpressure 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.
	CytoNozzle 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.

IntelliSort Troubleshooting

Ensure that you have followed the procedures for correctly setting up a sort and enabling IntelliSort. (See CHAPTER 7, *Sorting*.)

IMPORTANT If IntelliSort exits **Maintain** mode consider the following:

- The Manual Sample Station may be clogged or require debubbling.
- The fluidics system was not set up correctly. (Bouncy images and drifting top drop under steady state conditions are indications of fluidics instability.)
- The image of the stream/drops was lost. (Some causes could be that the Illumination chamber door was opened, the instrument ran out of fluid, or the instrument lost pressure.)
- Normal operation caused too much variation in stream conditions for IntelliSort to manage. (This is usually indicative of fluidic instability.)
- DO NOT back flush, debubble, or jostle the sample tubing when IntelliSort is in Maintain mode.

Table 9.3 IntelliSort Troubleshooting

Problem	Possible Cause	Possible Solution
Status Message: Monitor Time-out	System was in Monitor Mode longer than the time-out period. Bubbles in the nozzle. Bubbles in the sheath line. Rapid and continuos pressure change.	Clear nozzle. Run sheath until system stabilizes. Check for leaks in the air pressure supply lines.
Status Message: Init Failed	IntelliSort failed to load.	Power down the instrument and restart.
Status Message: Drop Drive Voltage	Drop Drive voltage was set too low (below 0.1 V)	Change Drop Drive Voltage setting and restart IntelliSort.
Status Message: No Drops/Stream	Image did not contain drops or stream object because the sheath lines were bumped. Sheath flow was interrupted. A large bubble in nozzle. Stream Camera USB cable is loose or unplugged.	Check the sheath tank level. Remove trapped air. Check the Stream Camera USB connection.
Status Message: Top Drop Position	Top drop is not in the middle third of the droplet image.	Adjust the image using the Droplet Camera slider to the center of the top drop in the image.
Status Message: Motor Error	Droplet Camera vertical positioning stage encoder count does not match command position. Unexpected end of travel hard-stop reached. USB cable limiting range of motion. Foreign object limiting range of motion. Encoder mounting screws are loose.	Home Droplet Camera stage. Contact a Beckman Coulter representative.
Status Message: Delay Changed	Top drop position shifted more than threshold allows. Large bubble in nozzle. Pressure change greater than 1.5 psi.	Clear trapped air. Run sheath until system stabilizes. Check for leaks in the air pressure supply lines. Check sheath tank bleed valve.
Status Message: Undefined	An error with no defined status message.	Ignore because error does not interrupt IntelliSort control.

Table 9.3 IntelliSort Troubleshooting

Problem	Possible Cause	Possible Solution
Droplet Image Blurry	Objective not focused. Objective body loose.	Focus objective. Contact a Beckman Coulter representative.
Droplet Image Black Specs	Dust on CMOS sensor.	Contact a Beckman Coulter representative.
Droplet Image too dark	Dirty optical filter. Interrogation Chamber door is open.	Clean the filter glass in the Interrogation Chamber with lens paper and alcohol. Ensure that the Interrogation door is securely closed.

Indicator of Electronic Malfunctions

The general indicators of electrical component malfunctions are listed below.

- The smell of hot electronic components.
- Excessive heat.
- Electrical arcing.
- The LEDs on the XDP electronics main power supply fail to light.
- Abnormal mechanical noise from fans indicating obstruction or problems with bearings.

While it is unlikely that the MoFlo XDP electronics will exhibit excessive heat or the smell of hot components, arcing is likely when the sort deflection plates become wet. Arcing across the sort deflection plates should be addressed as soon as it is noticed.

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, you can perform either the Droplet Stability Test or the Stream Shutoff 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 aXcess Control Panel Droplet Control screen. The following procedure can be used:

1 Select the aXcess Control Panel Droplet Control screen. Make sure both valves are turned to Sheath and the Sample Valve is closed.

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 aXcess Control Panel 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 Proceed as follows:

- If you have a Manual Sample Station, go to step 5 to continue.
- If you have a SmartSampler go to step 6 to continue.

5 If you have a Manual Sample Station, debubble as follows:

a. Turn one Sheath Valve to Vacuum (this will disrupt the fluidics and cause the droplets to break into a stream). While observing the droplet image, turn the valve back to Sheath

b. If the fluidics system is free of air, the droplets will snap back to the same location as you marked on the stream when debubble is turned off. Air in the system will cause the droplets to relocate on the screen and possibly appear to undulate.

6 If you have a SmartSampler debubble as follows:

a. Press the Debubble button.

b. 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.

Stream Shutoff Test (Manual Sample Station Only)

It should take fewer than two seconds for the stream to shut off after you have closed the sheath valves. When the sheath valves are shut off, any air bubbles left in the fluidics system will slowly decompress causing the stream to continue flowing for several (more than two) seconds.

1 Select the aXcess Control Panel Droplet Control screen.

2 Verify that the Drop Drive Amplitude is turned off.

3 While viewing the stream image on the screen, turn one Sheath Valve to off then turn the second valve to off. After shutting the second valve off, the stream should stop flowing in fewer than two seconds.

NOTE When debubbling or operating the Manual Sample Station, avoid the condition where both Sheath Valves are in the Vacuum position. This will pull air up through the CytoNozzle tip and introduce air into the fluidics system. Turning to Vacuum on both Sheath Valves is appropriate for unclogging the nozzle tip.

4 From either of the tests above, if it appears that there is air in the CytoNozzle, 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 CytoNozzle Blockages

1 Close the laser shutters and turn off the 635 nm diode laser.

2 Cleaning methods are listed in order of complexity:

- **a.** Run a tube of bleach solution, 5% Triton-X, or 70% Ethanol, followed by DiH2O through the CytoNozzle with the sheath streams turned off (Manual Sample Station.)
- **b.** Hold a cap of bleach solution or 70% Ethanol to the nozzle tip and apply vacuum.
- **c.** Remove the tip from the CytoNozzle and clean it directly with cleaning solution. Use caution when handling a disassembled CytoNozzle because the exposed injection tube increases the possibility of a skin puncture.
- **d.** Sonicate the CytoNozzle tip for 10-20 minutes in cleaning solution.

NOTE Do not touch the CytoNozzle tip. It can become clogged with debris from your hands.

3 Reassemble the CytoNozzle if necessary.

4 Debubble.

5 To determine if a partial clog remains, select the aXcess Control Panel Droplet Control screen and turn on the Drop Drive Amplitude to view the droplet stream. 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.

6 Realign the sheath stream and lasers.

Sealing a Sample Tube on the Manual Sample Station

A poorly sealed sample tube may cause the event rate to vary widely from the average sample rate. This procedure should be performed monthly.

The O-ring is located above the sample tube, within the sample tube cap.

1 To lubricate, apply a light coat of vacuum grease to the outside lip of an empty sample tube.

2 Load the tube as normal into the sample tube cap.

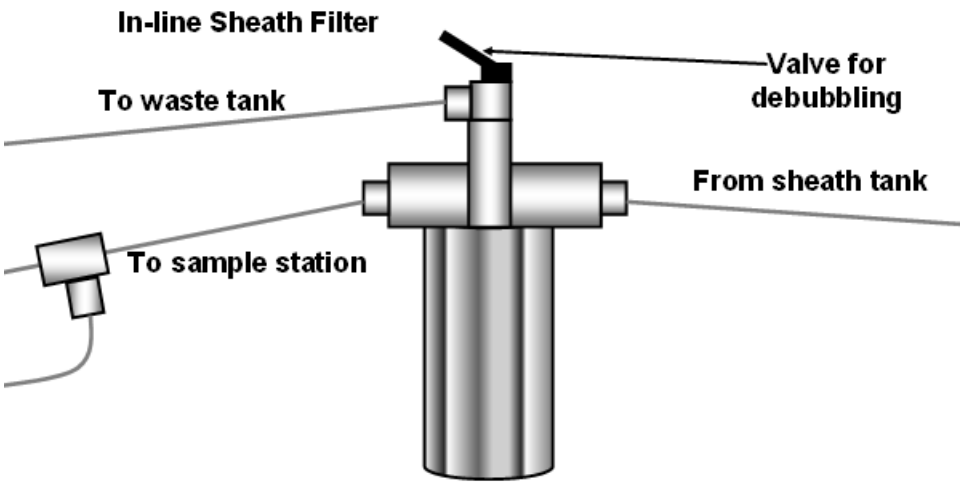
3 Remove the tube. Load and unload the tube several times to achieve even application.

Replacement Procedures

Replacing the Inline 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 9.1 Sheath Filter Diagram



How to Replace the Inline Sheath Filter

1 Ensure that sheath is turned off at the sample station.

2 On the Pressure Console switch off the blue main air switch.

IMPORTANT Do not loosen the valves more than one full turn because you will liberate the compressed spring and ball bearing.

3 Vent the pressure from the sheath tank and the cleaner tank by turning the vent valve on each tank one full turn.

4 After the pressure has equalized, tighten the vent valves on the tanks.

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 On the Pressure Console, turn the blue pressure switch on.

14 Open the black debubble valve.

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.

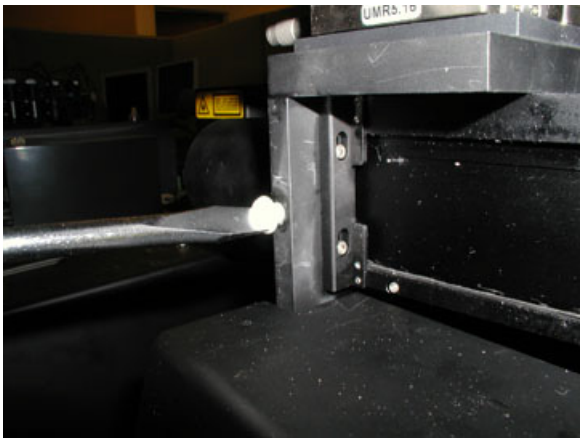
Replacing the Forward Scatter Photodiode Filters

The forward-scatter photodiode is located on the left side of the Illumination Table, covered by the forward-scatter photodiode light-containment shield. Note the warning declaration on the forward-scatter photodiode light-containment shield.

How to Replace the Forward Scatter Photodiode Filters

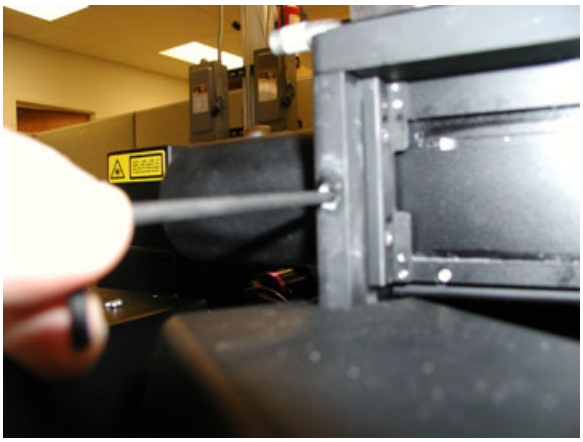
1 To access the photodiode use a flat bladed screwdriver and remove the white plastic screw.

Figure 9.2 Remove Plastic Screw



2 Loosen the 3.174 mm [1/8 in] hex cap head screw that anchors the light-containment shield in place.

Figure 9.3 Remove Set Screw



3 Once the light-containment shield is removed, the photodiode assembly is accessible. The photodiode assembly is housed in an internally threaded cylinder that fits snugly around the rear of the forward-scatter laser-focusing optics. An externally threaded retaining ring is used to secure the face of the photodiode and the laser cleanup filter. You may remove the retaining ring to insert other 25 mm [0.98 in.] filters into the 488 nm laser path. Direct laser irradiation of the detector will result in erroneous event rates and saturation of the detector.

Figure 9.4 Disassembled Photodiode

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 autoclavable. 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.

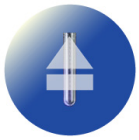
The sample tubing on the SmartSampler can also become damaged during use. Some cells can stick to the tubing and cause a partial clog. If the sample tubing is damaged, it cannot be repaired; it must be replaced.

If the sample probe becomes bent or the sample tubing becomes damaged, you should replace them immediately.

How to Replace the SmartSampler Probe

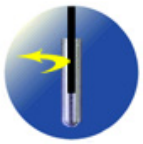
1 Press the Open Chamber button on the aXcess Control Panel.

Figure 9.5 Open Chamber Button



2 Press the change probe button on the aXcess Control Panel

Figure 9.6 Change Probe Button

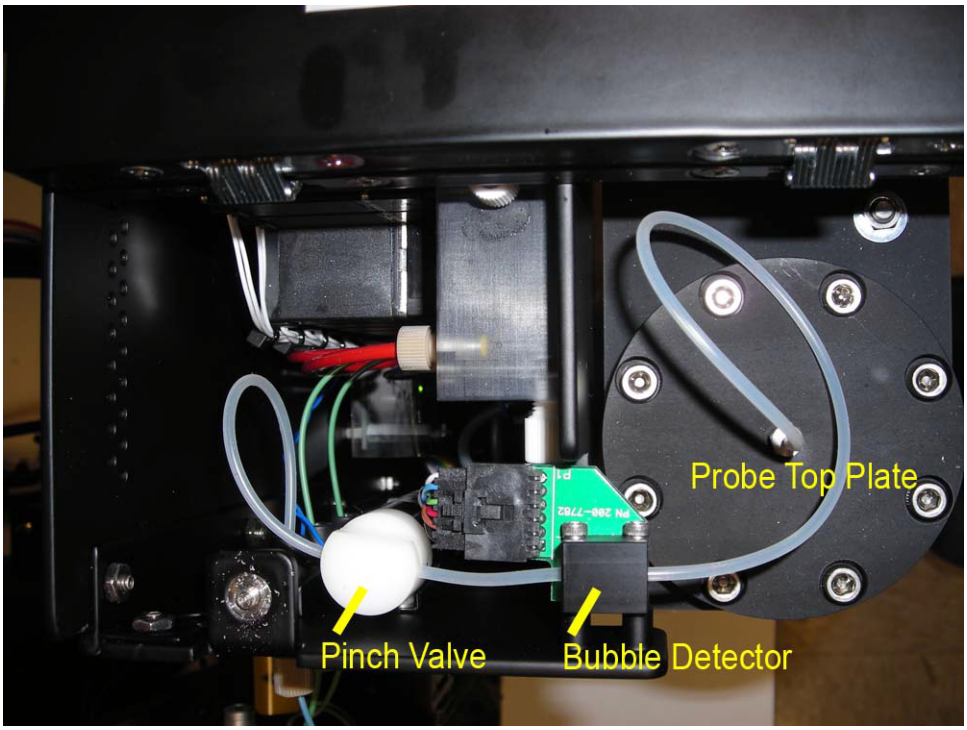


3 Unfasten the (4) screws on the SmartSampler cover and remove the cover.

Figure 9.7 Remove Cover

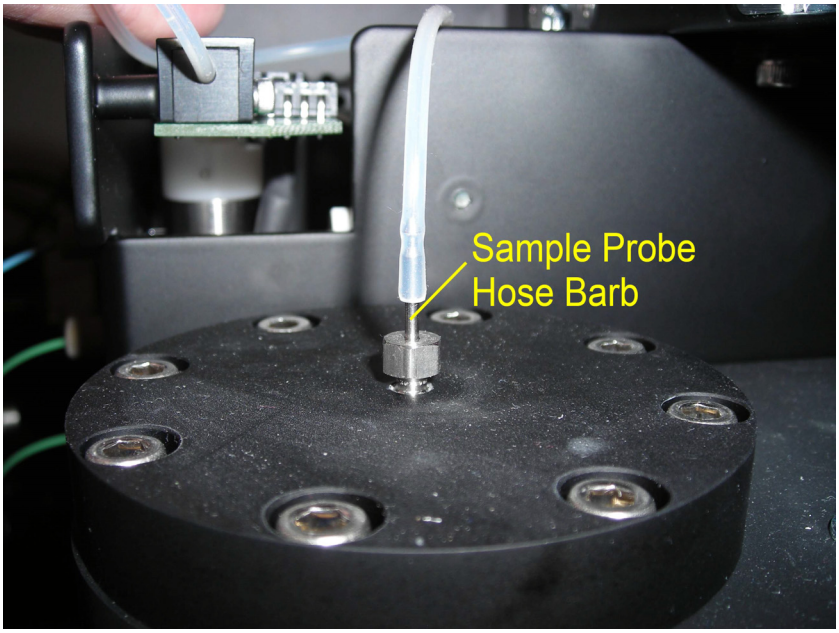
4 Open the top plate on the SmartSampler.

Figure 9.8 Top View of the SmartSampler with Lid Removed



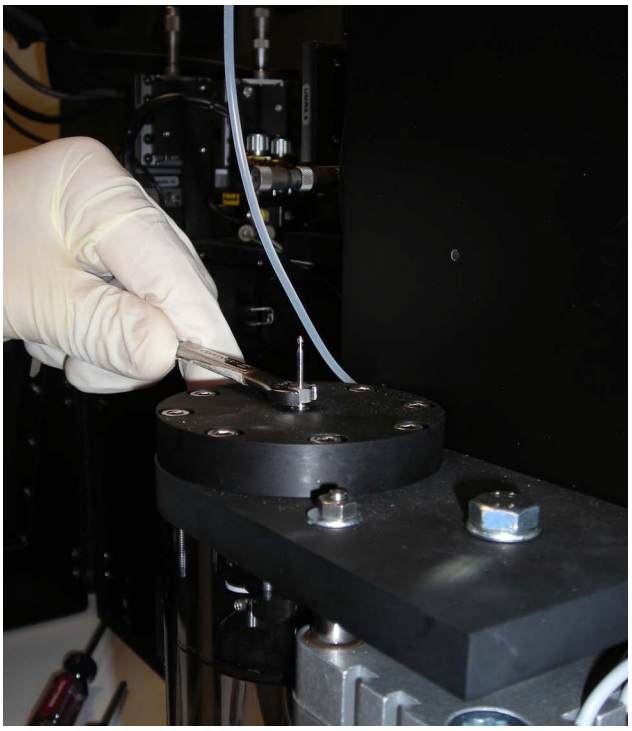
5 Gently pull the silastic tubing off of the sample probe hose barb.

Figure 9.9 Side View of the Probe Top Plate with Hose Barb



6 Using a ¼" open-end wrench, loosen the probe nut.

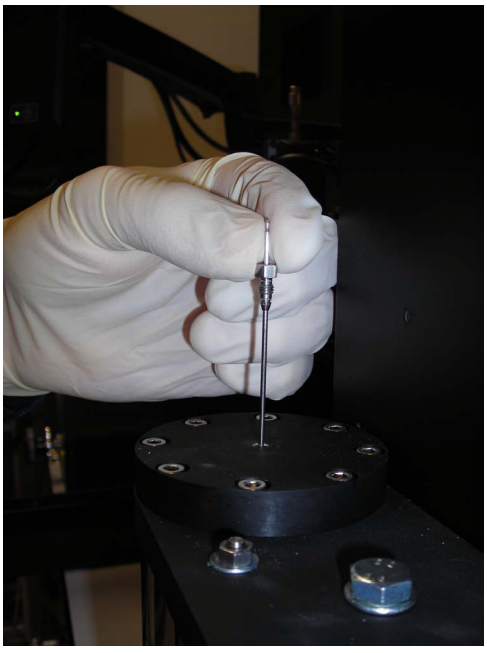
Figure 9.10 Loosen Probe Nut on Probe Top Plate



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 9.11 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 CytoNozzle before performing this procedure.

How to Replace SmartSampler Tubing

1 Press the Chamber Open button on the aXcess Control Panel. This will place the SmartSampler in the Open state and move the probe plate to the top.

Figure 9.12 Open Chamber Button

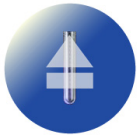
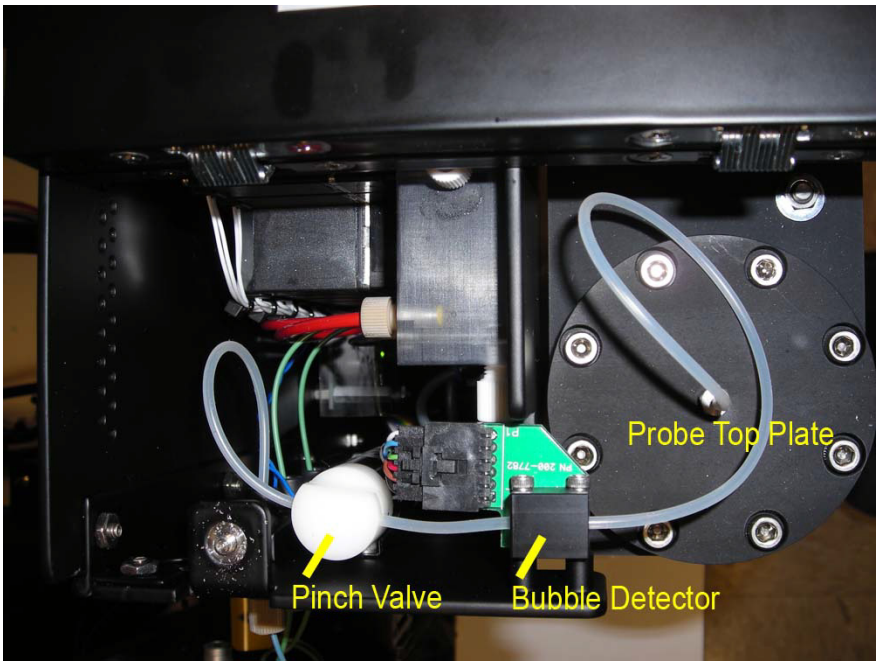


Figure 9.13 Top View of SmartSampler with Lid Removed



2 Carefully pull the silastic tubing off of the sample probe hose barb.

Figure 9.14 Side View of the Probe Top Plate with Hose Barb and Tubing

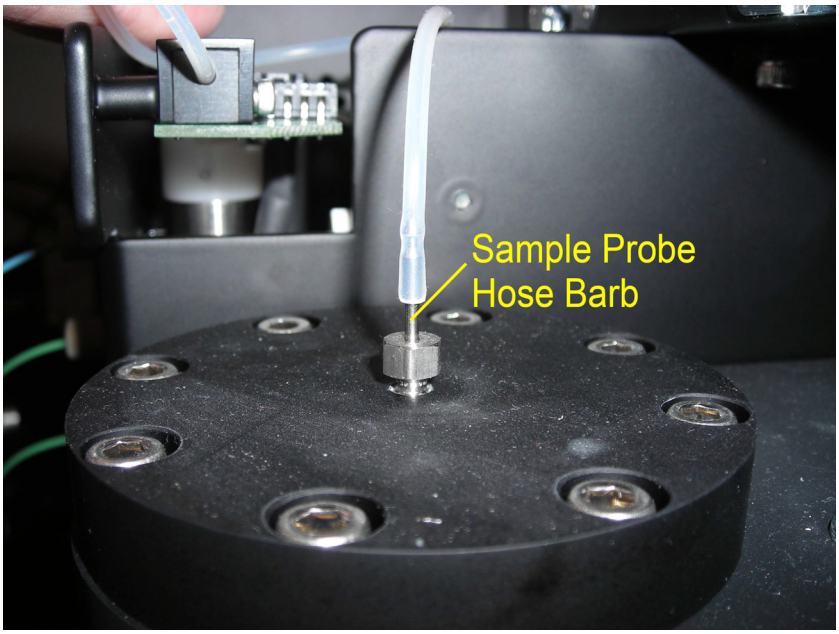
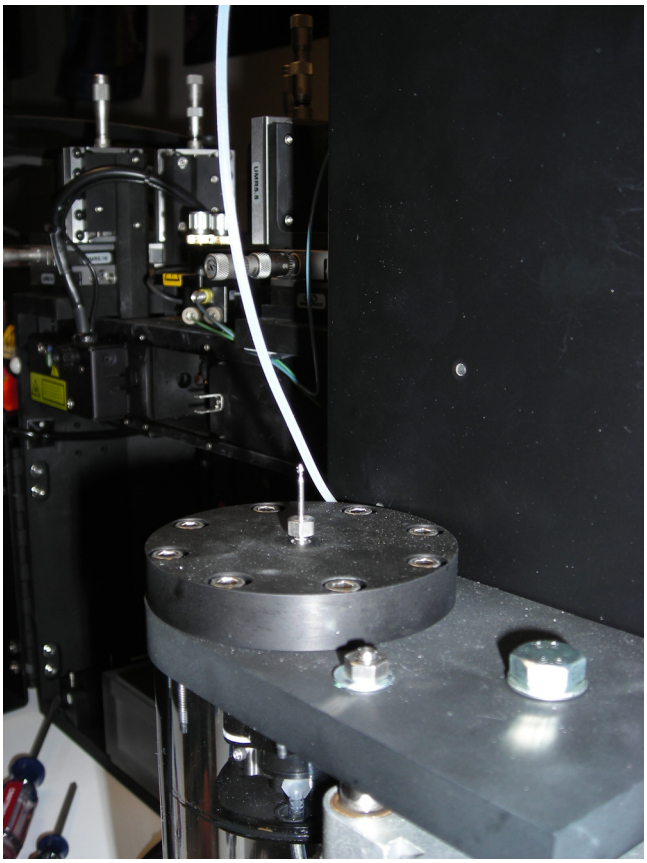
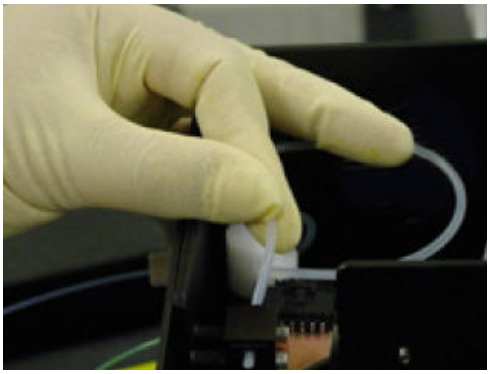


Figure 9.15 Side View of the Hose Barb with Tubing Removed



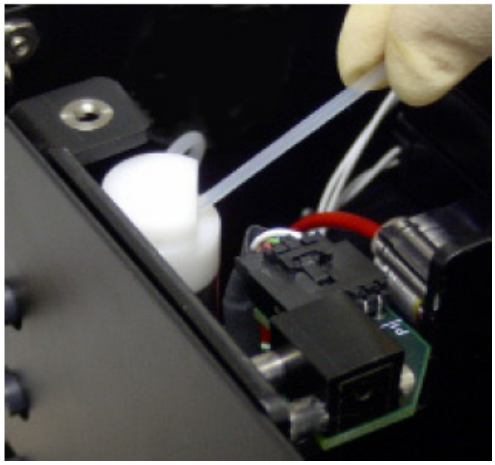
3 Pull the silastic tubing through the bubble detector, away from the sample chamber.

Figure 9.16 SmartSampler Bubble Detector



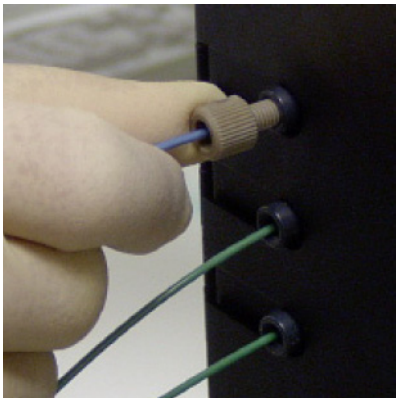
4 Remove the silastic tubing from the pinch valve slot.

Figure 9.17 SmartSampler Pinch Valve



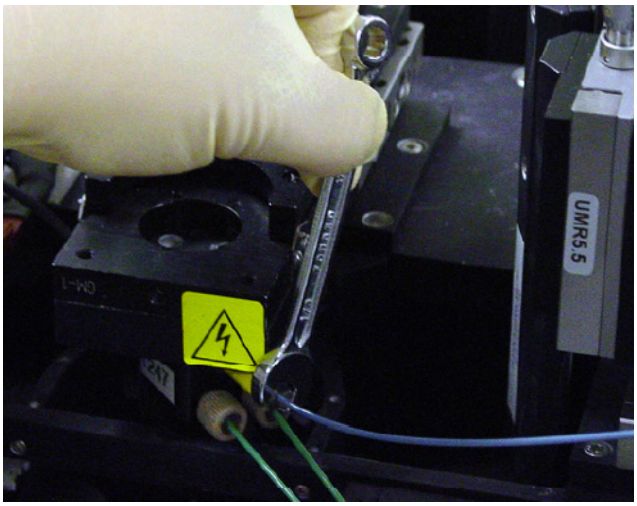
5 Loosen the finger-tight fitting that secures the tubing to the union by turning the fitting counterclockwise with your fingers.

Figure 9.18 SmartSampler Finger-Tight Fittings



6 Using a ¼" open wrench, loosen the nut that goes into the top port on the side of the CytoNozzle.

Figure 9.19 Loosen Top Port Nut

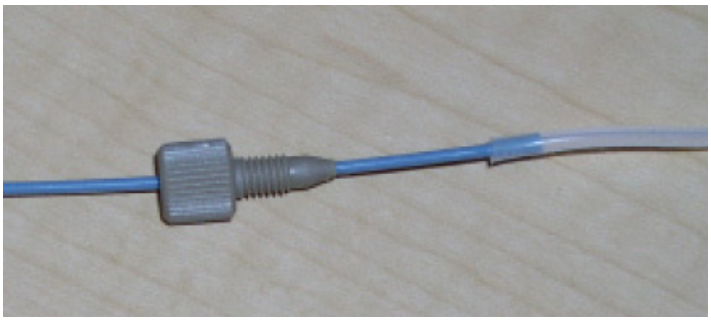


7 Pull the sample tubing assembly out of the SmartSampler unit.

8 Dispose of the entire sample tubing assembly according to your laboratory's approved procedures.

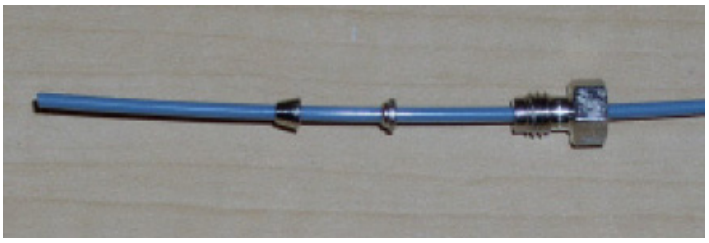
9 Using a new sample tubing assembly, slide the finger tight fitting on the blue PEEK tubing until it almost reaches the junction of the blue PEEK and silastic tubing. The threads on the fitting should point toward the silastic end of the tubing.

Figure 9.20 Fitting on Tubing



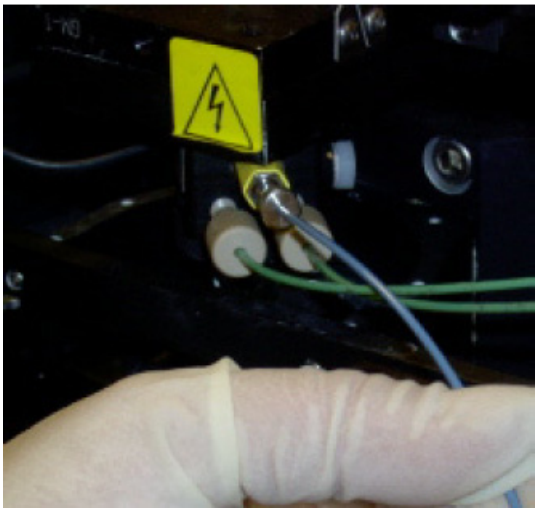
10 Using the sample tubing assembly, slide the stainless steel nut on the blue PEEK tubing, and then slide on the ferrules (smaller ferrule first). The smaller ferrule should be flat against the nut threads and all three parts should point toward the end of the blue PEEK tubing.

Figure 9.21 Add Ferrules



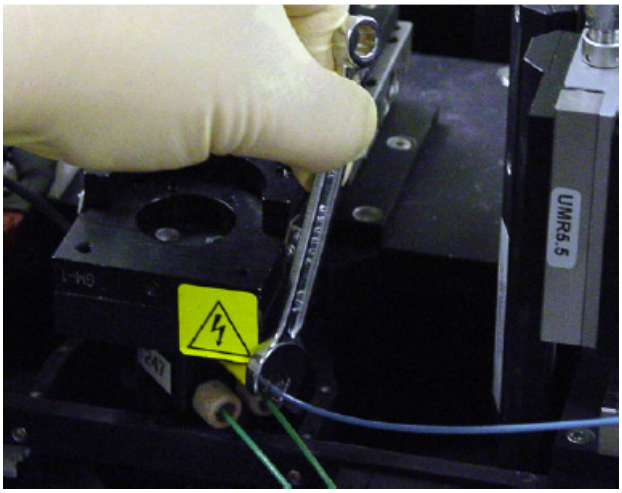
11 Install the blue PEEK tubing into the top port on the side of the CytoNozzle using the stainless steel nut and ferrules at the end of the tubing.

Figure 9.22 Attach Tubing to the Top Port



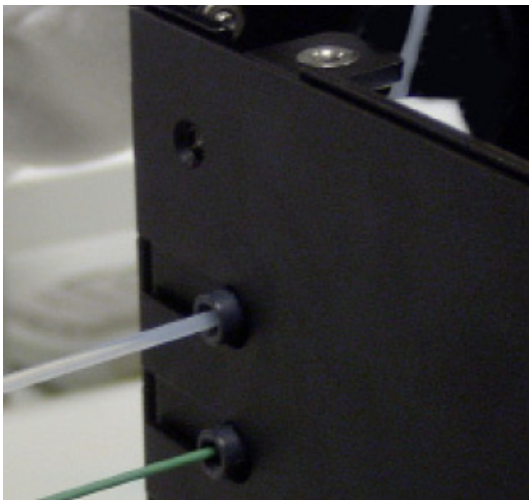
12 Tighten the nut on the sample tubing to the CytoNozzle using a ¼" open wrench (snug + ¼ turn).

Figure 9.23 Tighten Nut on Sample Tubing



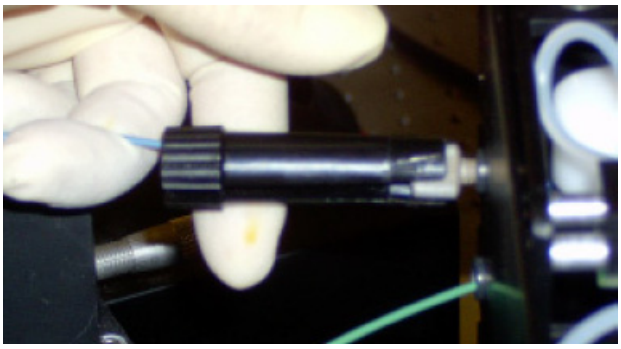
13 Pull the silastic end of the tubing through the top union on the side of the SmartSampler.

Figure 9.24 Pull Silastic End Through Top of SmartSampler



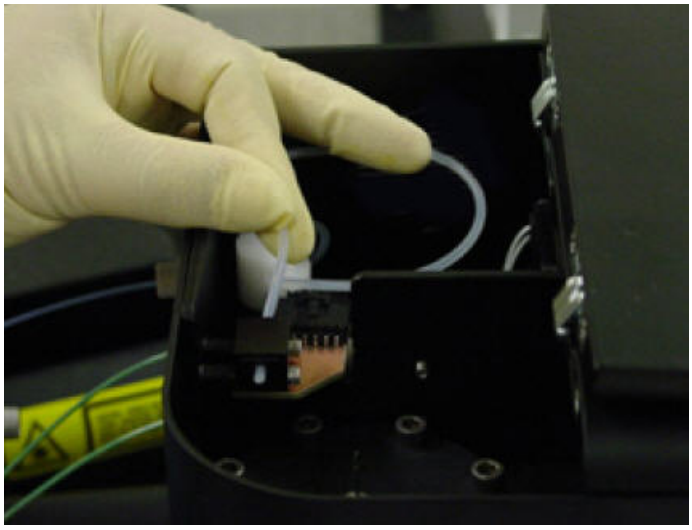
14 When the junction of the PEEK and silastic tubing enters the union, tighten the finger tight fitting into the union using the torque tool.

Figure 9.25 Torque Tool



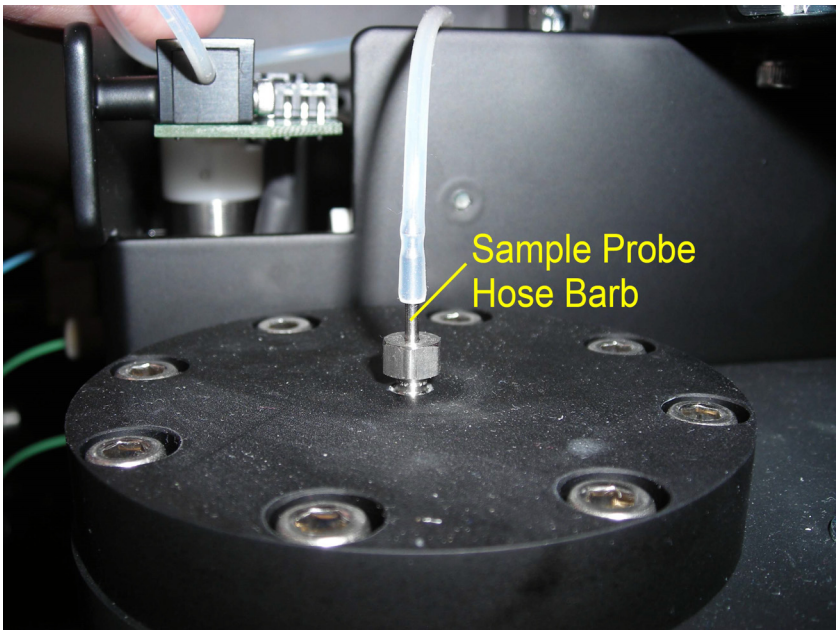
15 Feed the silastic tubing through the hole in the side of the bubble detector. Start through the hole closest to the union and pull through the hole closest to the sample probe.

Figure 9.26 Pull Silastic Tubing through Bubble Detector



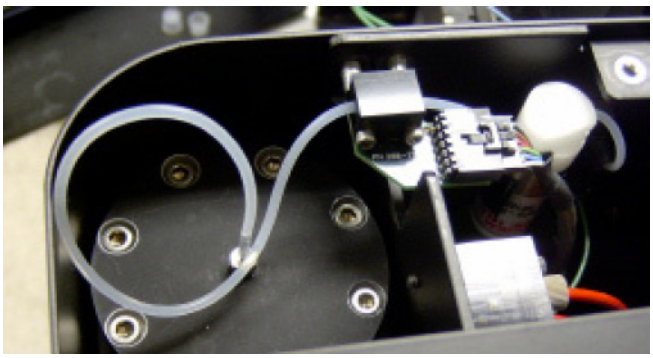
16 When the tubing is through both bubble detector holes, guide the tubing out through the chassis cutout and slide the tubing onto the hose barbed end of the probe. The tubing should cover the probe by approximately 3/8".

Figure 9.27 Attach Tubing to Hose Barb



17 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 9.28.

Figure 9.28 Small Loop of Tubing



18 Push the tubing into the pinch valve by holding the tubing in both hands and pushing it into the valve slot.

Figure 9.29 Tubing in Pinch Valve Slot

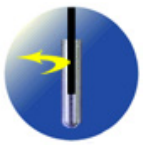
19 Ensure that the tubing is completely seated to the bottom of the slot so that the pinch valve will function properly.

20 Verify that you have left the proper amount of tubing on each side of the pinch valve.

21 Check that there are no sharp bends in the tubing.

22 Press the Change Probe button on the aXcess 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 9.30 Remove Probe Button



APPENDIX A

Approved Cleaners and Disinfectants

Overview

The following list of cleaners and disinfectants can be used on the MoFlo XDP. 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 XDP should be directed to Beckman Coulter Customer Service.

Cleaners

- Beckman Coulter LH Series Formaldehyde-Free Clenz
- 0.1% Triton-X100 in DI water
- 70% Ethanol in DI water

Disinfectants for Use in Sample Line*

- 70% Ethanol in DI water
- Bleach solution with a maximum concentration of 200 ppm active chlorine (11.5 mL household bleach 2875 mL water, 1:250 dilution) This is a recurring committee meeting for the first Friday of every month. Sometimes these meetings take only 15 minutes.

Disinfectants for Use in Sheath Line†

- Bleach solution containing 200 ppm active chlorine (11.5 mL household bleach 2875 mL water, 1:250 dilution) This is a recurring committee meeting for the first Friday of every month. Sometimes these meetings take only 15 minutes.
- For yearly decontamination, use a bleach solution containing 2000 ppm active chlorine (115 mL household bleach 2885 mL water, 1:25 dilution).

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).

* ANY disinfectant used on the sample probe must be rinsed with an equal amount of DI water.

† ANY disinfectant used in the sheath lines must be rinsed with DI water for a minimum of 90 minutes.

- 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.

Use COULTER CLENZ, or any of the approved Approved Cleaners and Disinfectants in the Cleaner tank.

APPENDIX B

Consumables

Consumables

Table B.1 Beads

Code	Product
URFP-30-2	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 exciting and emitting across 355 nm, 405 nm, 488 nm, and 635 nm.
RCP-30-5	FluoroSpheres – 6-peak, 3.2 µm, 1 x 10^7/mL, 40 tests
RCP-30-5A	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 E.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 XDP 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)

APPENDIX C

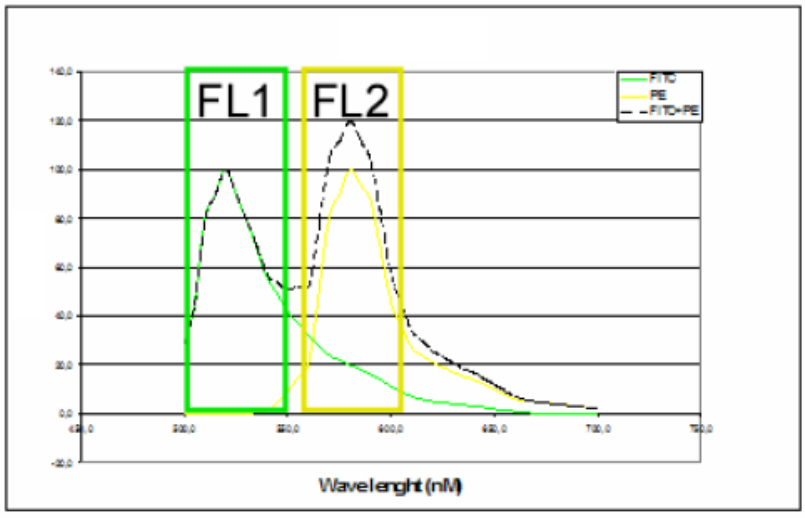
Compensation Background Information

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 4-20.

Figure C.1 FL1, FL2 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**.

APPENDIX D

Additional Sorting Information

Pressure Differential and Processing Speed Limitations

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 CytoNozzle.

The Pressure Console regulates the Sheath Pressure and Sample Pressure. It also includes a fine adjustment knob that allows you to make small changes to Sample Pressure. 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 in turn to the third. If you change the CytoNozzle, or the sheath pressure, you will need to adjust the inter-laser time delay. Follow the automatic prompts in the Wizard.

Changing the Operating Pressure

Nominal operating pressure is usually 60 psi as paired with a 70 µm CytoNozzle. 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 close the Sample Valve, before making any of the following pressure changes.

Changing the Inter-laser Time Delay

After a significant pressure change you will need to adjust the inter-laser time delay. See Appendix F-1 for additional information.

1. Create an area histogram for the primary laser and the secondary or tertiary lasers.
2. Run calibration beads at 100 EPS.
3. While acquiring, access the CHAPTER 4, *Instrument Tab* in Summit and use the arrows on the screen to adjust the values while viewing the medians on the histograms.
4. Maximize the median values and close the window.

Decreasing the Pressure

When decreasing the nominal operating pressure, lower the sheath pressure to the desired level using the Sheath Pressure Regulator. Next, lower the sample pressure to approximately 0.2 psi above the sheath pressure. Then, vent the pressure at the sheath tank.

Increasing the Pressure

When increasing the nominal operating pressure, raise the sheath pressure to the desired level using the Sheath Pressure Regulator. Next, raise the sample pressure to approximately 0.2 psi above that of the sheath with the Sample Pressure Regulator.

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.

Correcting the Fine Adjustment Knob on the Pressure Console

The fine adjustment knob for sample pressure is located on the front of the Pressure Console below the coarse adjustment knobs and has a limited range for adjustment. If the fine adjustment knob is rotated too far in or out, this can be corrected by the following steps:

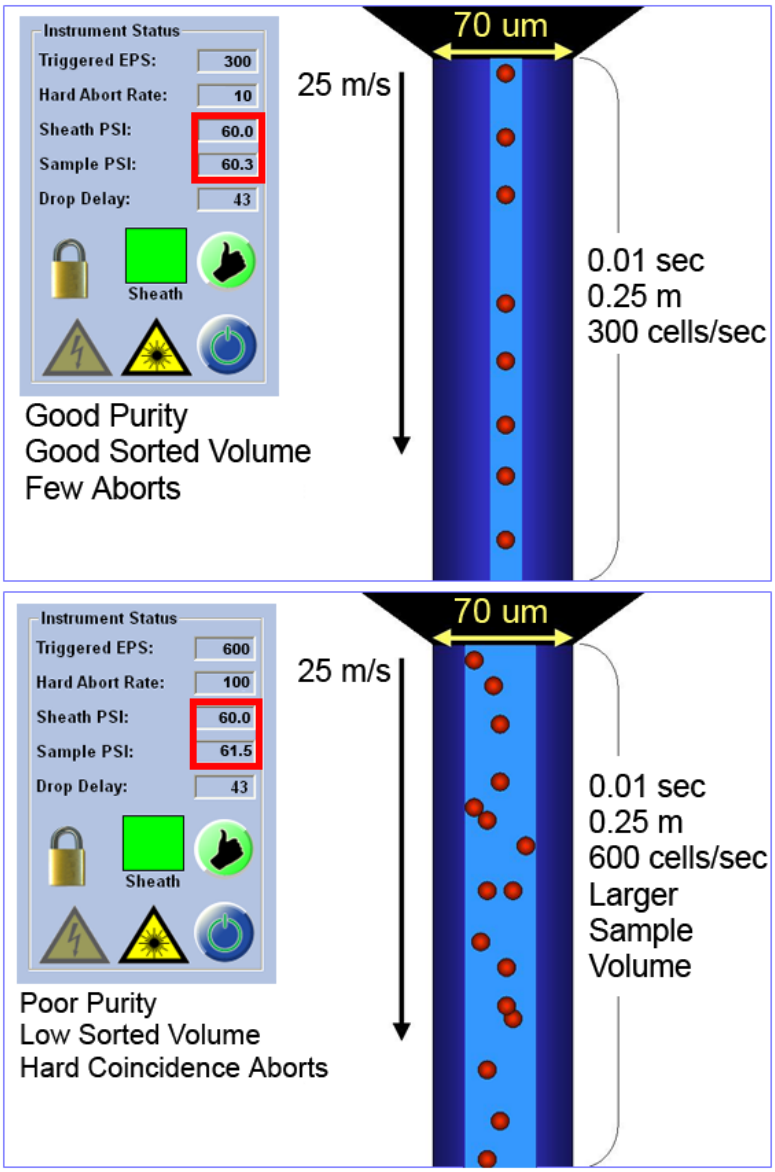
1. Rotate the fine adjustment knob to the middle of its travel.
2. Adjust the coarse sample control on the front of the Pressure Console to approximate your desired event rate.
3. Use the fine adjustment knob to fine-tune the event rate.

Processing Speed Limitations

The MoFlo XDP 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 XDP electronics prefer to process cells arriving 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 D.1 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.

XDP Electronics, the aXcess Control Panel, and Sorting Parameters

The aXcess Control Panel provides the user interface for the XDP Electronics that are responsible for controlling some of the parameters associated with sorting.

Table D.1 Details concerning Sort Parameters involving XDP Electronics

Sort Parameter	Screen	Range	Effect
Drop Drive Amplitude	Droplet Control screen	1–140 V	Force with which the nozzle is vibrating
Drop Drive Frequency	Droplet Control screen	0–200 KHz	Nozzle vibration cycles per second, represented in Hz
Charge Phase	Droplet Control screen	0–360 degrees	Side streams
Defanning	Stream Configuration screen	0–50 percent	Center waste stream
Stream Deflection	Stream Configuration screen	0–100 percent	Side stream deflection
Plate Voltage	Stream Configuration screen	0–4000 Vdc	Side stream deflection

Summit Software and Sorting Parameters

Summit software is responsible for the following parameters that are associated with sorting.

Table D.2 Details concerning Sort Parameters involving Summit Software

Sort Parameter	Range	Effect
Drop Delay	See the CytoCalc Table on page E-1.	Controls the time it takes for a particle of interest to travel from the laser interrogation point to the position of the Last Attached Drop.
Condition	L2, L1, R1, R2	Selects the stream for which the sort decision will be set.
Sort Mode	Enrich, Purify, or Single	Effects purity and recovery.
Drop Envelope	0.5, 1.0, 1-2. 2.0, 3.0	Defines the number of droplets deflected (sorted) for each positive event.
Abort Stream	Waste, Left 2, Left 1, Right 1, Right 2	Determines the stream to which the aborted events will go.
Sort Rate	Varies	View the number of sorted events per second (EPS) for the stream.
Sort Count	Varies	View the total events that have been sorted for the stream.

Sort Stream Precedence

Sometimes an analyzed cell meets the criteria to be sorted into more than one stream. When this happens, the following precedence is applied based on the Sort Mode that was set for that population.

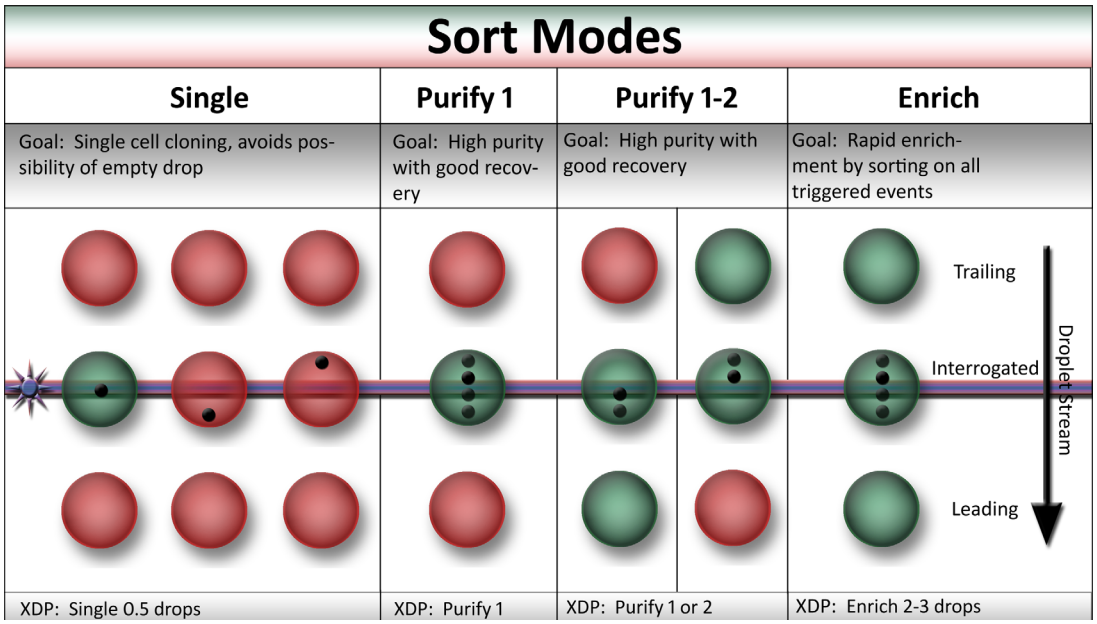
1. Single
2. Purify
3. Enrich

This is an ordering from most restrictive to most permissive, which is probably always the intent (because it provides the most flexibility in setting up protocols). However, within a sort mode, the stream precedence defaults from far left (L2) to far right (R2). The precedence between streams sharing the same mode can be changed in the Summit software under the sort menu.

Sort Mode Examples

The XDP divides sorting decisions into two parts, Sort Mode and Drop Envelope. The user first selects the precision of the sort output: Single, Purify, or Enrich. The user then selects the Drop Envelope: 0.5, 1, 1-2, 2, or 3 drops. For detailed Drop Envelope descriptions see CHAPTER 7, Select Drop Envelope.

Figure D.2 Sort Mode Example



Green indicates sorted drops and red indicates aborted. Particles shown in the green drops can be in any one location within the drop, but in only one. If there is more than one particle in a drop, the drop will be aborted.

Abort Terminology

Hard Aborts

A hard abort on MoFlo XDP occurs when a flow cytometer cannot process sort information in time to make the sort decision. However, due to the speed of MoFlo XDP electronics, MoFlo XDP produces very few hard aborts. For example, MoFlo XDP generally will not produce hard aborts during analysis until the processing speed has exceeded 200,000 EPS. MoFlo XDP can sort beads at 100,000 EPS and experience approximately 1,700 EPS hard aborts. 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 Regions in Summit, fails to pass the criteria defined by the Sort Mode Figure 7.23 and Droplet Envelope Figure 7.24, or Sort Stream Precedence. This failure causes an abort of the positive event and is tallied in the abort statistics and the Abort Rate data on the aXcess Control Panel Statistics screen.

When an event fails the Sort Logic, as defined by the Regions 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.

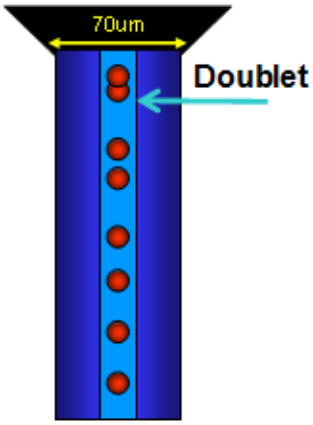
Sort Decisions and Doublets

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.

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 D.3 Doublet Diagram



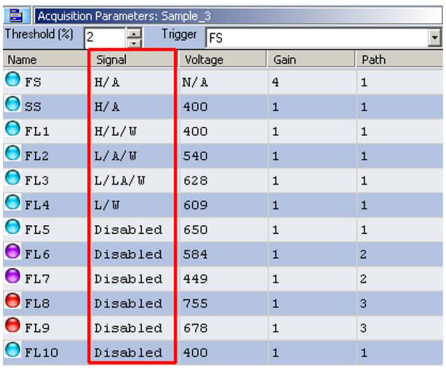
The linear area and log area signals for a doublet yield a greater value than of a singlet. However, linear height and log height signals do not provide any information that distinguishes a singlet from a doublet. To determine doublets you must collect area or pulse width data.

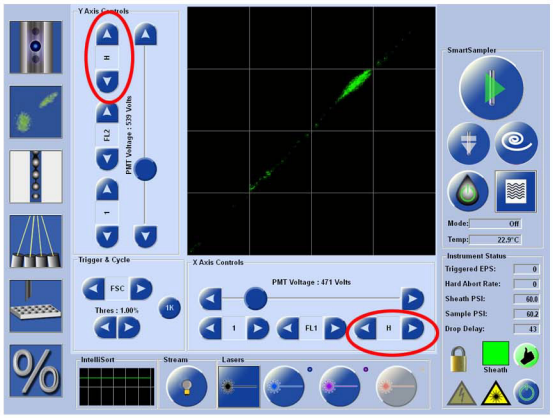
Data Type Parameters

Data Type parameters are set in Summit and on the aXcess Control Panel Fine Alignment screen and are defined as:

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

Figure D.4 Data Type Parameter Settings in Summit and on the Fine Alignment Screen





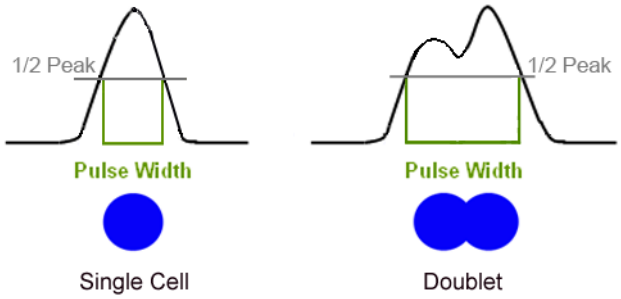
Threshold

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) in XDP electronics is determined at 1/2 the signal peak as measured from .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.

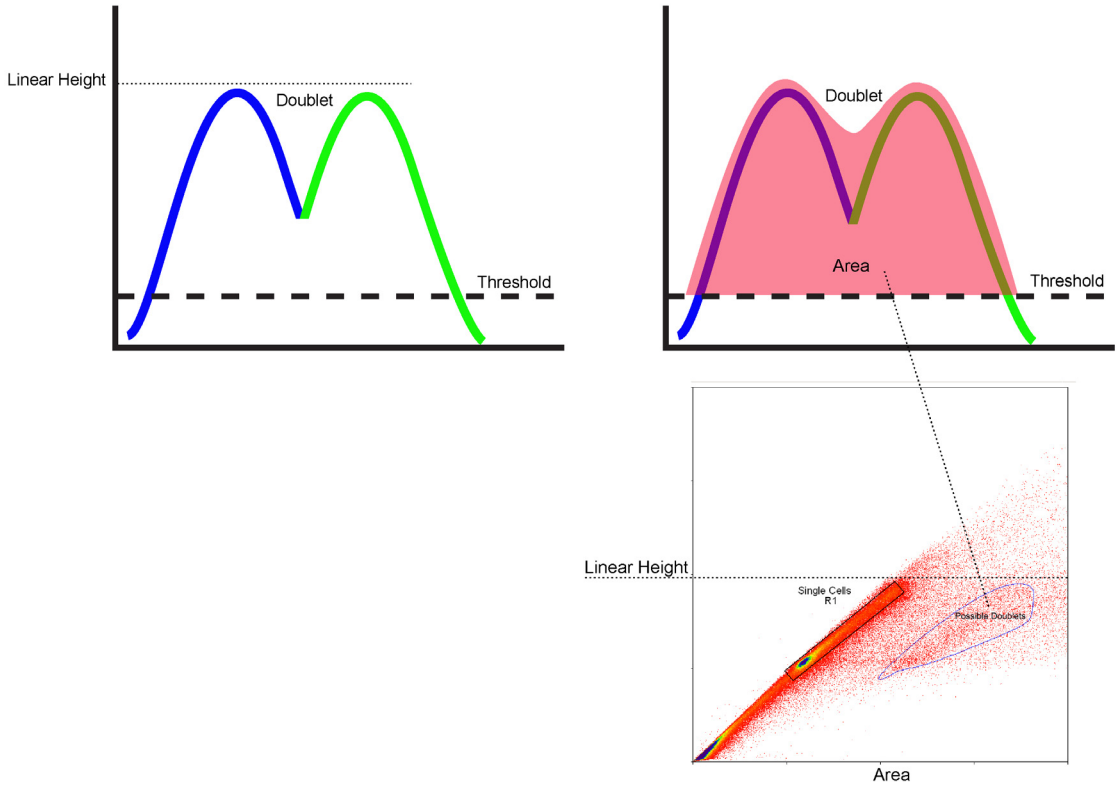
Figure D.5 Pulse Width Single and Doublet Peaks



Height vs. Area Signals for Doublets

As can be seen in Figure D.6 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 D.6 Height and Linear Area and Doublets



Doublet Discrimination

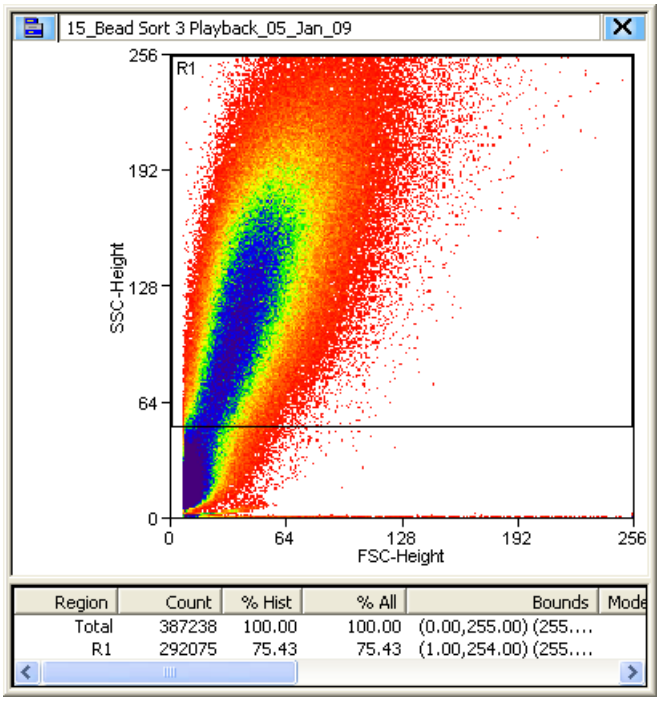
It is important to determine the doublet population in acquired data before setting sort decisions in order to obtain an accurate sort. The generic example shown below depicts cells that were treated with a DNA dye. Adjust your methods according to the needs of your specific experiments.

1 In Summit, set the data type parameters to Area, Height, and Pulse Width. See CHAPTER 4, *Enable Parameters*. 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*.

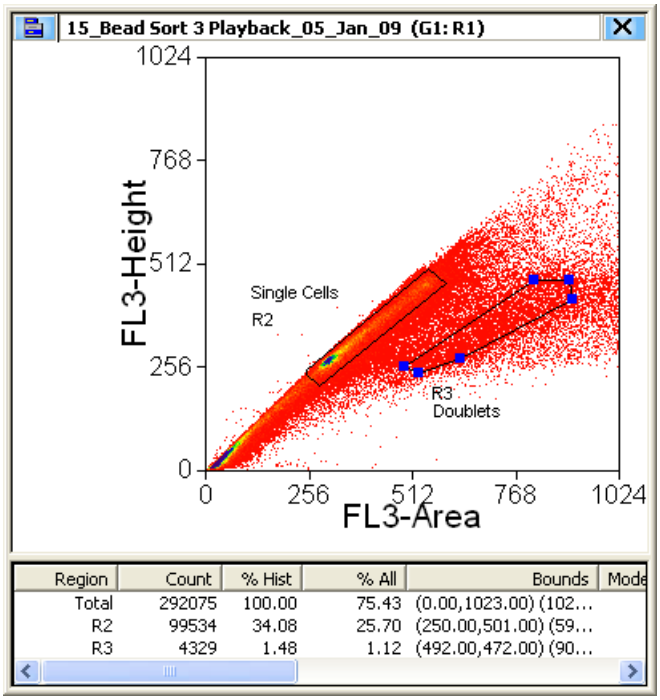
NOTE Either the Pulse Width or Area dot plot will display the singlet and doublet populations more distinctly depending on the characteristics of the sample.

Figure D.7 FSC vs. SSC dot plot



3 Set a region to include the suspected single cell population as shown in Figure D.8. 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 D.8 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*.

Applications and References

If you would like a copy of the *MoFlo and CyAn Selected References* booklet, please contact your sales or service representative.

APPENDIX E

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.

NOTE When setting the Drop Delay in Summit software, the value in the **Approximate Drop Delay** field is the same value that was used the last time you ran the instrument. Therefore, you may want to start with the Drop Delay value that is already in Summit software rather than a value listed below.

Table E.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 F

Laser Delay

Preliminary Actions

Change the nozzle tip, adjust sheath and sample pressure, check the sheath lines and nozzle assembly for trapped air. Allow the system to stabilize.

Laser Procedures

Laser Alignment

Alignment should be done using Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles (or comparable) beads at a concentration of 10^6/mL. Create a Summit Protocol displaying a FS vs. SS dot plot and linear area histograms for all laser parameters. Display median and CV statistics.

Laser Alignment

1. Set system to trigger off of FS. Align the 488 laser and optimize the signal CVs and median by adjusting the sheath stream and laser line micrometers. Ensure the threshold is low enough (0.5% to 1%) to establish 100-150 events per second. After optimizing, mark medians with bar regions and note CV values for each parameter.

2. Repeat step one for lasers in pinholes two and three changing the trigger source for the laser path being aligned. Again, after optimizing, mark medians and note CV values.

Setting Laser Delay

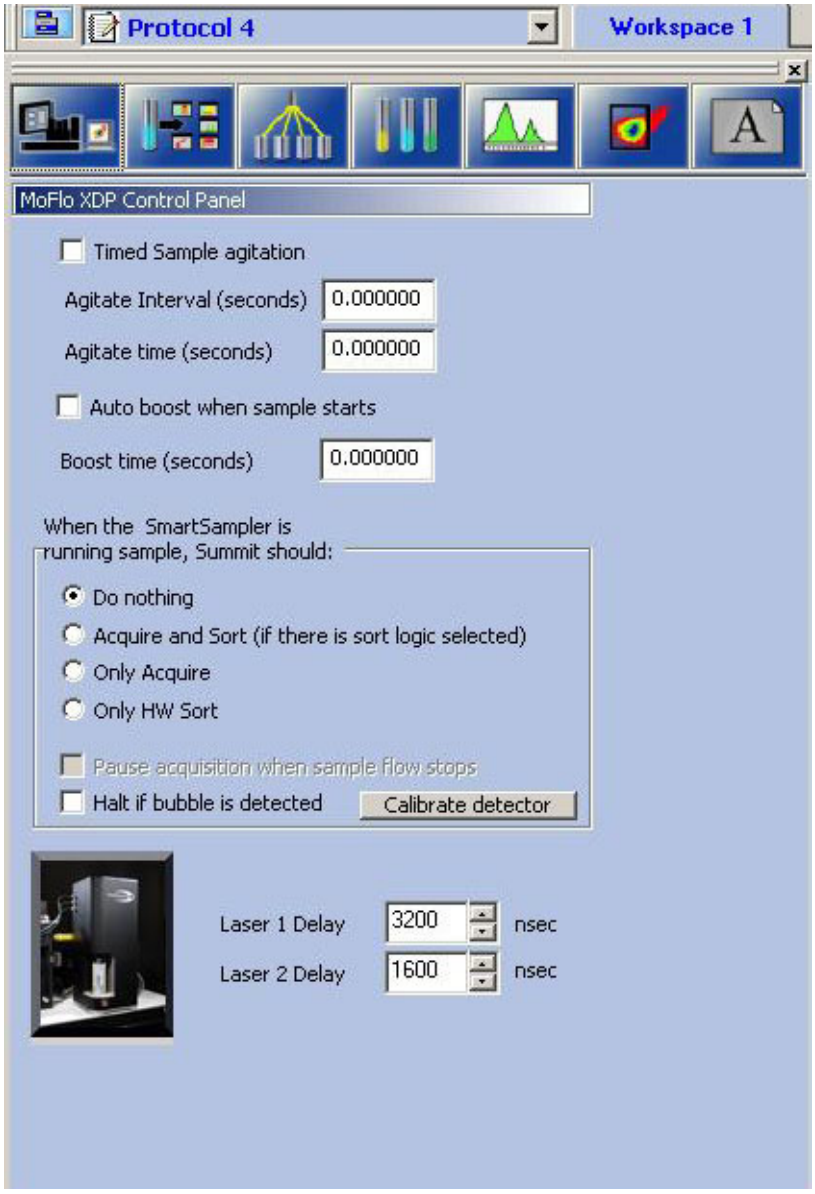
IMPORTANT Triggering populations may move or disappear from histograms depending on laser delay settings.

Setting Laser Delay

1. Click the Instrument tab in Summit software. The two fields in the lower portion of the screen allow you to adjust the laser delay of path two and path three.

   NOTE Laser delay one box displays values from pinholes 1 to 3 in nanoseconds (e.g., 3200 ns for 60 psi). Laser delay two box displays values from the pinholes 1 to 2 (e.g., 1600 ns for 60 psi). RECORD THE CURRENT VALUES - these can be reentered when returning to the starting sheath pressure.

Figure F.1 Setting Laser Delay



2 Gate the FS vs. SS population to parameter histograms.

3 Place Summit in cycle display mode (cycle count- 100 events) and set the event rate to ~100 events per second.

4 While triggering on FSC increase the threshold until pinhole one populations (FL1, FL2, FL3, etc.) begin to disappear. Lower the threshold so the population peaks return and can be easily viewed. As the threshold increases, the pulse width becomes smaller, and the median values decrease. If necessary, mark these new median values by creating new or moving bar regions.

5 Repeat step 4 for the middle and bottom pinholes by changing the trigger source and adjusting the threshold so the relevant populations are just visible. Increasing the threshold ensures that only the top of each pulse is being displayed. This will make determining the laser delay quicker and more precise in the following steps.

6 Return to triggering off of a pinhole one parameter (e.g., FS).

7 Adjust the laser delay values to return to the highest median and smallest CV values recorded for laser two and three parameters found when aligning the lasers by increasing (if sheath pressure was lowered) or decreasing (if sheath pressure was increased) the laser delay in nanoseconds. The most accurate method to do this is to adjust the relevant path's laser delay (using the laser delay input box arrows) until the median and CVs move as far as possible to the right (record laser delay value) and continue adjusting the laser delay in the same direction until both the median and CV begin to degrade (record this value). Set the laser delay to the average of these two values.

Typical values:

Tip	psi	Laser 1 (ns)	Laser 2 (ns)
70	60	3600	1800
80	40	4400	2200
100	30	5000	2500

8 Record values. Under most conditions these can be input when changing tip and sheath pressures without predetermining the laser delay. Close the laser delay adjustment window.

9 Lower trigger thresholds.

APPENDIX G

Symbols

Symbol Definitions

Table G.1 Symbol Definitions

Symbol	Definition
	Instrument Serial Number
	Beckman Coulter Model Number
	A “CE” mark indicates that a product has been assessed before being placed on the market, and has been found to meet European Union safety, health, and/or environmental protection requirements.
	Date of Manufacture
	Alternating Current Input
	Fuse
	Caution, Consult Accompanying Documents
	Identification of Manufacturer

Table G.1 Symbol Definitions *(Continued)*

Symbol	Definition
	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.
	The XDP Electronics Chassis must not be lifted by the cover. The cover is designed only to shield the electronics from possible fluid spills.

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

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

C — centigrade

CD-ROM — compact disk - read only memory

CDRH — National Center for Devices and Radiological Health

cm — centimeter

COM — serial communications port

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

DT1 — detection table one, located on the left side of the IT on the MoFlo and MoFlo XDP

DT2 — detection table two, located in the middle of the IT on the MoFlo and MoFlo XDP

DT3 — detection table three, located on the right side of the IT on the MoFlo and MoFlo XDP

EMI — electromagnetic interference

EPROM — erasable programmable read only memory

ESD — electrostatic discharge

F — Fahrenheit; fuse

FC — flow cell

FF — fitting

FRU — field replaceable 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

pot — potentiometer

psi — pounds per square inch

PWA — printed wiring assembly

PWB — printed wiring board

QA — quality assurance

QC — quality control

R — potentiometer; resistor

RAM — random access memory

RG — regulator

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

V — volts

Vac — volts alternating current

vac — vacuum

Vdc — volts direct current

VL — valve

W — watt; wide

WM — wire marker

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U

W

X

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MoFlo XDP Documentation

MoFlo XDP Instructions for Use
PN C10712

- Introduction
- Installation
- System Overview
- Cytometer Control Panel
- Summit Software
- Startup, Alignment and Shutdown Procedures
- Performance Validation
- Sorting
- Cleaning and Maintenance
- Troubleshooting and Replacement Procedures
- Appendices
- References

MoFlo XDP Quick Start Guide

Summit Software Online Help

www.beckmancoulter.com

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