Instruction For Use

MoFlo Astrios

High Speed Cell Sorter

PN A99481D
January 2012

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BECKMAN COULTER

Instructions for Use
PN A99481D (January 2012)

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Minor changes were made throughout the document when necessary.

Safety Notice Chapter: Updated pictures and Laser Safety Warning Labels; removed the statement: "This Class A digital apparatus complies with Canadian KES-001"; replace the statement part 15 of the FCC Rules with CISPR 11; and added China RoHS Caution Label.

Chapter 1: Added Table 1.3 Laser Specifications and detailed some laser references.

Chapter 2: Updated Figure 2.6.

Chapter 3: Changed "Stream Template" to "Stream Indicator".

Chapter 8: Revised some steps and figures in Manually Determined Drop Delay.

Chapter 9: Added Deflection Plates Block Assembly and Charge Deflection Plate Cleaning Procedure, and Optical Cleaning Procedure.

Chapter 10: Improved the Replacing SmartSampler Tubing procedure.

Appendix A: Added Cleaning materials for Deflection Plates Block Assembly and Charge Deflection Plate Cleaning Procedure and Optical Cleaning procedure.

Appendix B: Added Consumables.

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Other moderate changes were made throughout the document.

Safety Notice: Added China RoHS Environmental Label and Serial Nameplate.

Introduction: Conventions Used was added and other minor changes were made.

Chapter 3: Updated the Laser and Stream Intercept Configuration Screen and Quality Control Screen sections.

Chapter 6: Updated the Laser Spot Determination section. Added Laser Delay section.

Chapter 7: Updated this chapter with minor edits and graphic replacement.

Chapter 8: Under Set Sort Decisions section, a subsection was added called Sort Modes. Minor edits were made to the Sort Decisions section under Step 3. Under the Additional Sorting Information section, a sentence was added to the Changing Inter-laser Delay Values subsection.

Added APPENDIX F, Biosafety Cabinet Accessory.

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.

⚠ 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.

⚠ WARNING

Risk of operator injury if:

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

To avoid injury:

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

⚠ CAUTION

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

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

contamination.

⚠ CAUTION

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

⚠ CAUTION

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

Instrument Safety Precautions

⚠ WARNING

Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

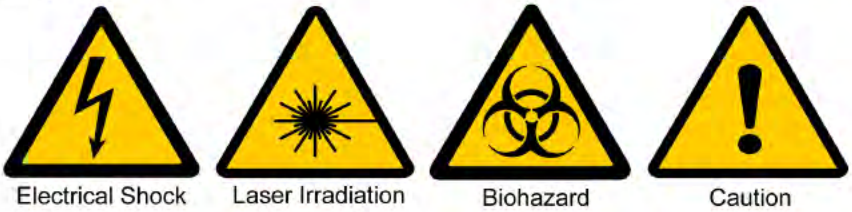
The MoFlo Astrios High-speed Sorter has been engineered with safety as one of its primary features. Safety of the operator, field service personnel, bystanders, and of valuable samples, is paramount to Beckman Coulter's commitment to high performance design and engineering.

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

Symbols

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

Safety Symbols



Electrical Shock — Risk of Electric Shock

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

Biohazard — Biological Hazard/Risk

Caution — Important; Attention; Refer to Accompanying Documentation

General Safety

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

- The MoFlo Astrios is intended for Professional Use Only. All operators should be trained on the proper use and limitations of the instrument prior to its operation.
- Be aware that the Sort Chamber, Illumination Chamber, and cabinet doors on the MoFlo Astrios present possible pinch points. While the doors are light-weight and do not have sharp edges, care should be taken when opening and closing doors.
- The sash on the Biosafety Cabinet can be moved manually up and down thereby creating a possible pinch point. Position hands appropriately when moving the sash on the Biosafety Cabinet.
- Be aware of the edges of the Biosafety Cabinet and instrument base.
- Familiarize yourself with the sample station. The SmartSampler has electronically controlled moving parts. When a sample run is initiated, do not insert your hand in the sample chamber.
- The sample probe on the SmartSampler poses a possible puncture hazard. Use caution when working around the sample probe.
- The input air pressure to the system should never exceed 125 psi. Over pressurization of the SmartSampler chamber can cause an O-ring to blow out causing a very loud, but short pop sound.
- Wear appropriately sized gloves providing good manual dexterity to reduce the likelihood of skin pinches and abrasions.
- Be aware that fluidic and electrical lines are secured with zip ties that can cause skin abrasion if they are contacted with force.
- The nozzle injection tube is exposed when the interior of the nozzle is cleaned. It may cause skin abrasion if it is contacted with force.
- Carefully replace tanks in the fluidics cabinet as to avoid pinching between a heavy tank and the metal supports and quick connect fittings inside the lower enclosure.

- Change the sheath and waste tanks daily and inspect the catch basin for fluid leakage. The catch basin is located below the tanks in the fluidics cabinet. If leakage occurs underneath the instrument covers, it should drain to this location.
- Use proper lifting techniques or seek assistance when handling the UPS, instrument covers, or full tanks. To reduce the likelihood of back injury, empty waste at least once per day.
- Condensation or leakage from the refrigerated water bath can drip on the floor and cause a slipping hazard. The Water Bath Auxiliary cart has the capacity to contain some amount of liquid per EN61010.
- Protect the skin and eyes whenever handling chemicals of any kind, regardless of how benign they may appear.
- Summit workstations include a keyboard interface. Evaluate the ergonomic suitability of the location of the keyboard and the user to avoid injury.
- Cords and cables may be located on the floor around the unit. Drawers and detector assemblies can be positioned by the user. Be aware that these things can cause a tripping hazard.
- Check with the site safety officer for correct disposal of waste products and for spill clean-up protocols.
- The MoFlo Astrios is capable of pressures up to 100 psi (689 kPa). Check sample and sheath pressures when changing nozzle size.
- The user should rest appropriately to avoid strain due to repetitive use, awkward movements, or sitting too long.

Electrical Safety

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

Safety Interlocks

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

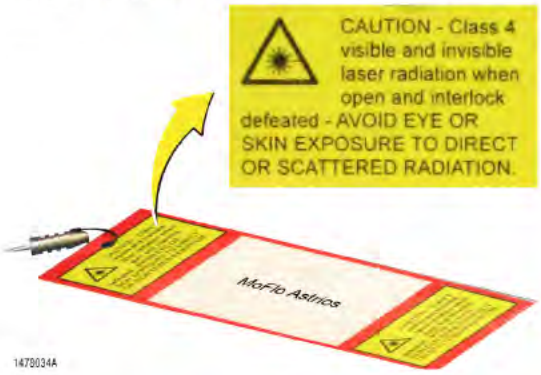
- When the Sort Chamber door opens, the safety interlock disables the voltage to the deflection plates, SortRescue moves into place, sample flow halts, and CyClone movement is stopped.
- When the Illumination Chamber door opens, the safety interlock closes the laser shutters.
- When the latch for the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

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

Safety Interlock Override Key

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

Illumination (Interrogation) Chamber Safety Interlock Override Key



Stream Charge

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

Drop Drive Voltage

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

Sort Deflection Plates

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

⚠ CAUTION

Do not touch the charged plates when power is applied.

Deflection Plate Arcing

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

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

Laser Power Supplies

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

Disposal of Electrical Instrumentation

It is very important that customers understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

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

- that the device was put on the European Market after August 13, 2005 and
- that the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

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

Optical/Laser Safety

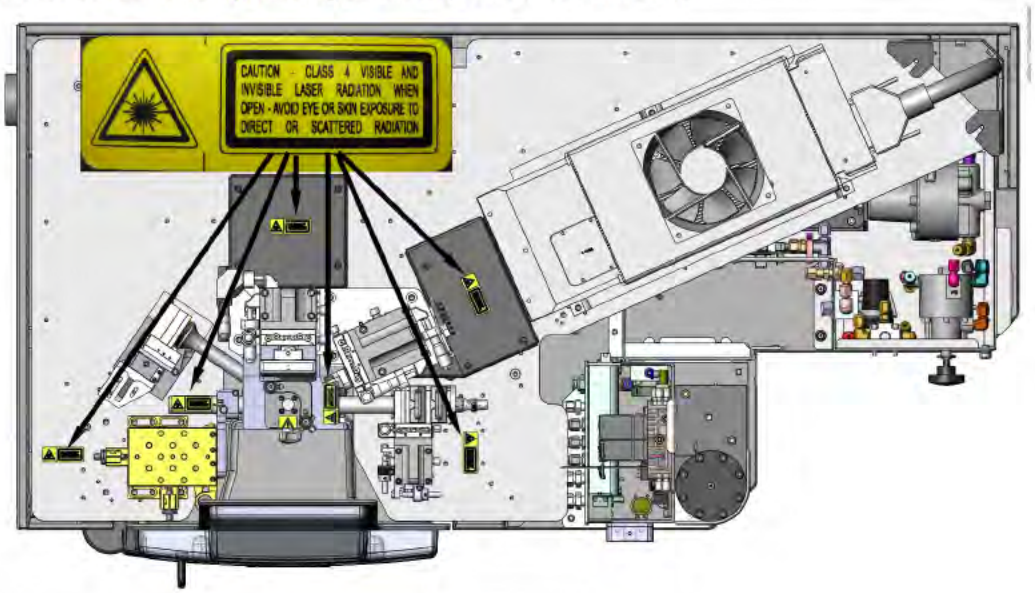
Laser Product Hazard Classification

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

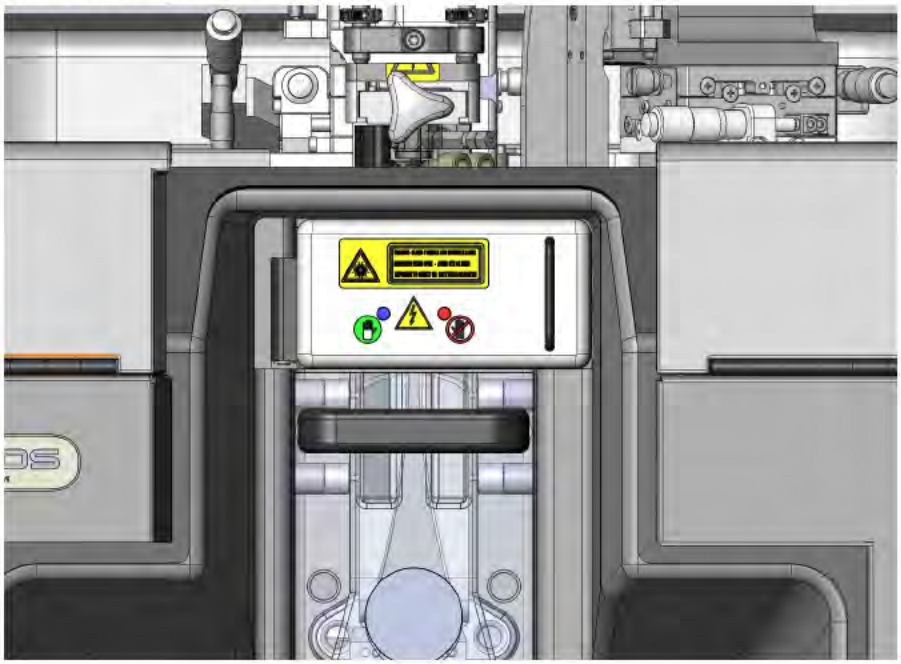
Remove all jewelry when working with an open beam and do not place shiny or reflective objects into the path of the laser beam as to prevent reflection of the beam in unprotected directions. Use all protective housings, Safety Interlocks, and shields as identified in this manual.

Class 1 Laser Product Label

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



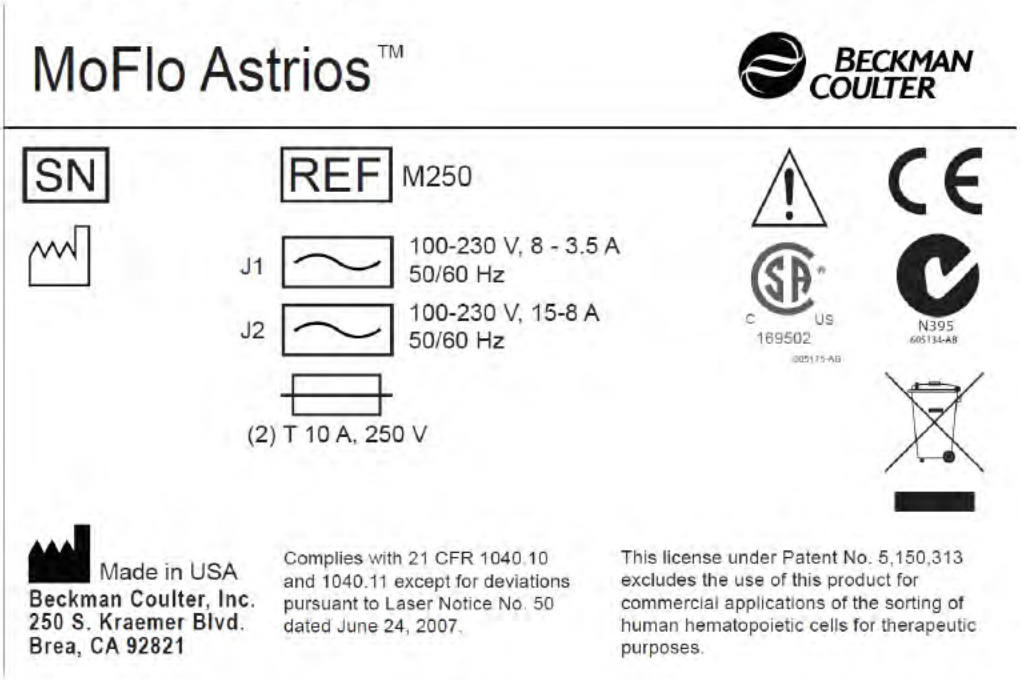
Front View Showing Radiation Exposure Hazard Labels



Serial Nameplate

The Astrios Serial Number Nameplate is illustrated below.

Astrios Serial Number Nameplate



Biological Effects of Laser Irradiation

Eye Injury

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

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

Skin Injury

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

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

Biohazard Safety

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

- Gloves, a laboratory coat, and eye protection should be worn whenever handling samples including insertion and removal of sample tubes from the sample station.
- If the system loses vacuum or the waste tube becomes clogged, waste fluid could spill into the sort chamber. Immediately turn off sheath and sample flow, wear proper personal protective equipment and attend to the spill.
- Waste fluid may contain hazardous levels of biological and chemical contamination. Gloves, a laboratory coat and eye protection should be worn whenever exposure to waste fluid exists. See APPENDIX A, *Disinfectants for Use in the Waste Tank*.
- To ensure inactivation of biological organisms in the waste tank, an appropriate type and quantity of an EPA registered disinfectant should be placed in the tank initially upon use, and every time the waste tank is emptied and reinstalled.
- The Aerosol Containment Shield, also known as the Sort Chamber door, is part of a passive aerosol containment assembly that isolates the contents of a sort from the rest of the instrument, the operator, and the laboratory. When closed, the door prevents movement of air into and out of the Sort Chamber. It is optional to purchase an Aerosol Evacuation system for additional protection from aerosols. See CHAPTER 2, *Aerosol Evacuation System*.
- A BSL-2, Level A2 Biosafety Cabinet is available for purchase as an optional system accessory. See APPENDIX F.

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

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

Electromagnetic Information

⚠ CAUTION

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to CISPR 11 Class A. In a domestic environment it could cause radio interference, in which case, you may need to take measures to mitigate the interference. 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.

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.

China RoHS Caution Label

This logo indicates that this electronic information product contains certain toxic or hazardous substances or elements, and can be used safely during its environmental protection use period. The number in the middle of the logo indicates the environmental protection use period for the product. The outer circle indicates that the product can be recycled. The logo also signifies that the product should be recycled immediately after its environmental protection use period has expired. The date on the label indicates the date of manufacture.

China RoHS Environmental Label

This logo indicates that the product does not contain any toxic or hazardous substances or elements. The "e" stands for electrical, electronic and environmental electronic information products. This logo indicates that this electronic information product does not contain any toxic or hazardous substances or elements, and is green and environmental. The outer circle indicates that the product can be recycled. The logo also signifies that the product can be recycled after being discarded, and should not be casually discarded.

Contents

Illustrations

Tables

How to Use Your Manual

Document Overview

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

This instrument is intended for research use only.

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

Conventions Used

This document uses the following conventions:

- Bold face font indicates buttons or selections that appear on the Summit Workstation screen.
- The term select is used to indicate either one or both of the following actions:
  - To tap or touch with your finger.
  - To click with a mouse.

  NOTE The verb "press" is reserved for mechanical buttons, such as keys on the keyboard.

- Sections that contain entirely new content are flagged with a New Section icon  at the end of the section title.
- The software path to a specific function or screen appears with the greater then (>) symbol between screen options.

- Links to information in another part of the document for additional information are in blue and are underlined. To access the linked information, select the blue, underlined text.

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 use, or maintenance of this equipment.

About this Manual

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

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

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

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

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

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

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

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

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

Chapter 9, Cleaning and Maintenance
Provides the daily decontamination procedure, deflection plates block assembly and charge deflection plate cleaning procedure, optical cleaning procedure, and yearly fluidics decontamination procedure. Information regarding changing the sheath filter and annual preventative maintenance by a Beckman Coulter Representative is also included.

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

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

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

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

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

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

Appendix F, Biosafety Hood Accessory
Defines the cautions and warning for the optional Biosafety Cabinet.

CHAPTER 1

Installation

General Laboratory Information

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

MoFlo Astrios Specifications

Heating and air conditioning vents or fans are not recommended directly above the MoFlo Astrios because of the resulting temperature fluctuation, vibration, and possible dust. Specifications for the optional Biosafety Cabinet are discussed in APPENDIX F.

Table 1.1 General System Specification and Environmental Requirements

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

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

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

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

Table 1.2 Regional Electrical Requirements

Country	Dedicated Lines with Isolated Grounds	Non-dedicated Lines
USA	Two dedicated lines at 115 Vac, 50/60 Hz at 15 A Main Chassis ON/OFF Power connection and main AC input from the UPS, J1 Laser Power connection plugs directly into a wall outlet, J2	Three non-dedicated lines at 115 Vac, 50/60 Hz at 15 A - one for the Summit computer, a second for the monitor, and a third for the printer.
Europe	Two dedicated lines at 220 Vac, 50/60 Hz at 10 A Main Chassis ON/OFF Power connection and main AC input from the UPS, J1 Laser Power connection plugs directly into a wall outlet, J2	Three non-dedicated lines at 220 Vac, 50/60 Hz at 10 A - one for the Summit computer, a second for the monitor, and a third for the printer.
Japan	Two dedicated lines at 100 Vac, 50/60 Hz at 15 A Main Chassis ON/OFF Power connection and main AC input from the UPS, J1 Laser Power connection plugs directly into a wall outlet, J2	Three non-dedicated lines at 100 Vac, 50/60 Hz at 15 A - one for the Summit computer, a second for the monitor, and a third for the printer.

Table 1.3 Laser Specifications

Typical[a] Laser Wavelength	Nominal[a] Laser Power	Spot Size at Stream	Laser Separation
355 nm	100 mW	Horizontal: Gaussian beam of 50 µm, full width 1/e2 intensity Vertical: Gaussian beam of 25 µm, full width 1/e2 intensity	127 (When properly aligned by the operator.)
405 nm	55 mW	Horizontal: Flat-top full width half max. intensity approx. 35-55 µm Vertical:Gaussian beam of 5-15 µm, full width 1/e2 intensity	127 ± 3 µm
488 nm	165 mW	Horizontal: Flat-top full width half max. intensity approx. 35-55 µm Vertical: Gaussian beam of 5-15 µm, full width 1/e2 intensity	127 ± 3 µm
532 nm	150 mW	Horizontal: Flat-top full width half max. intensity approx. 35-55 µm Vertical: Gaussian beam of 5-15 µm, full width 1/e2 intensity	127 ± 3 µm
560 nm[b]	200 mW	Horizontal: Flat-top full width half max. intensity approx. 35-55 µm Vertical: Gaussian beam of 5-15 µm, full width 1/e2 intensity	127 ± 3 µm
592 nm	200 mW	Horizontal: Flat-top full width half max. approx. 35-55 µm Vertical: Gaussian beam of 5-15 µm, full width 1/e2 intensity	127 ± 3 µm
645 nm[c]	105 mW	Horizontal: Flat-top full width half max intensity approx. 35-55 µm Vertical: Gaussian beam of 5-15 µm, full width 1/e2 intensity	127 ± 3 µm

a. The laser wavelength and power have a small amount of expected part to part variation, and may differ between and within laser manufacturer and the laser model.

b. Historically the 561 nm green lasers have been used in flow systems, therefore, the software still uses 561 to identify green lasers.

c. Historically the 640 nm red lasers have been used in flow systems, therefore, the software still uses 640 to identify red lasers.

Table 1.4 System Noise

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

System Connections

Figure 1.1 AC Entry Panel Connections

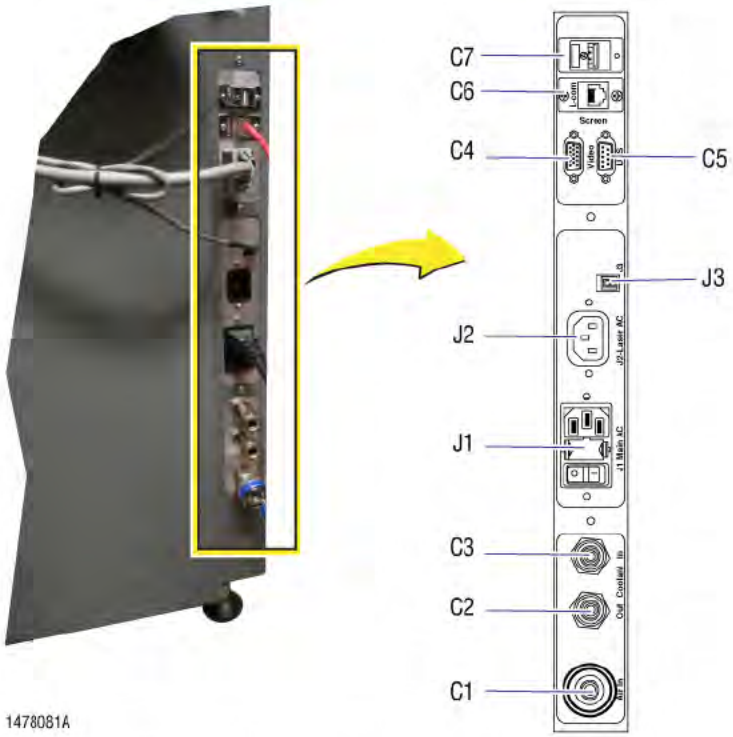


Table 1.5 AC Entry Panel Connections and Definitions

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

Figure 1.2 Astrios Electronics Chassis Connections Drawing

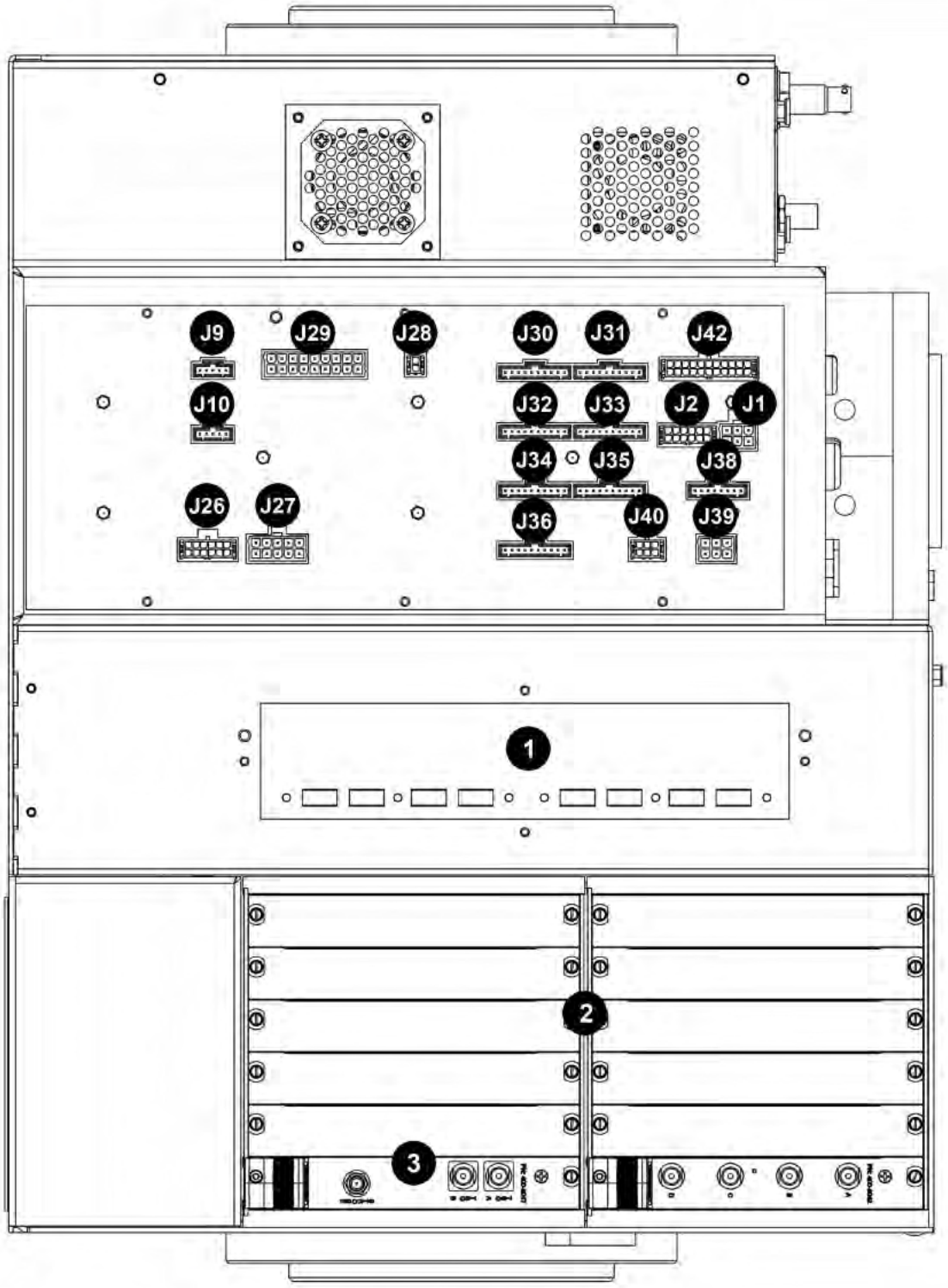


Table 1.6 Astrios Electronics Chassis Connections Labeled

J1	Not used
J2	Not used

Table 1.6 Astrios Electronics Chassis Connections Labeled *(Continued)*

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

Figure 1.3 Summit Workstation Connections Photo

Table 1.7 Summit Workstation Connections Labeled

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

Installing Summit Software

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

CHAPTER 2

System Overview

Overview of the MoFlo Astrios System

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

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

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

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

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

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

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

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

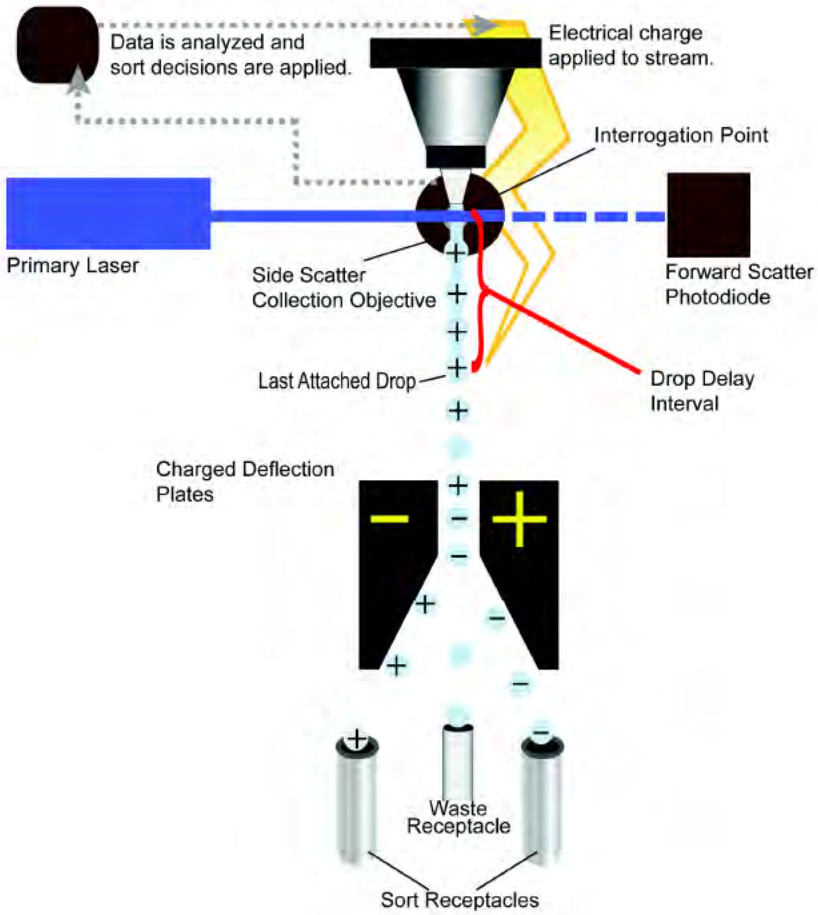
Indexed sorting allows the user to view a data file and observe the location of sorted particles within a plate that is represented graphically on screen.

General Principles of Operation

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

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

Figure 2.1 Principles of Operation Diagram



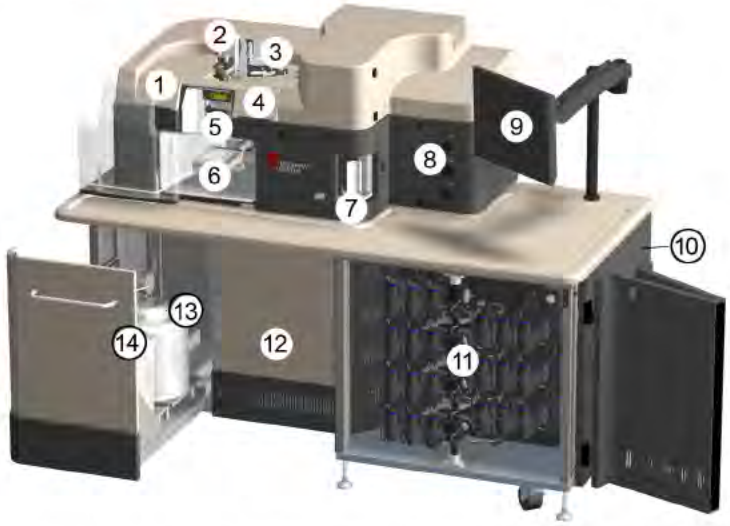
System Layout

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

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

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

Figure 2.2 MoFlo Astrios Annotated

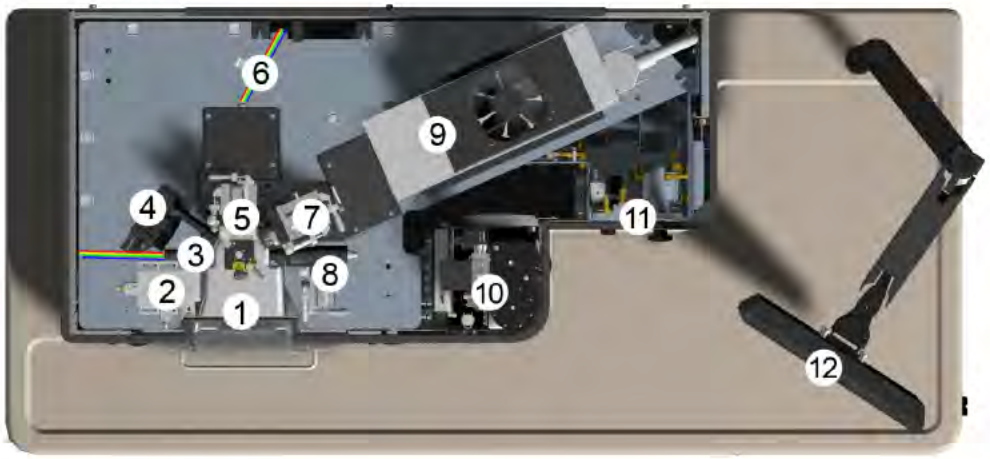


1.	Fiber-coupled Beam Shaping Optic (FBSO) Positioning Micrometers (behind panel)
2.	Nozzle Positioning Micrometers and Gimbals
3.	UV Laser BSO Positioning Micrometers
4.	Forward Scatter Sensor Positioning Micrometers
5.	High-Voltage Deflection Plates
6.	CyClone (with microplate attached)
7.	SmartSampler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

13.	Sheath Tank
14.	Waste Tank

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

Figure 2.3 Upper Enclosure Annotated



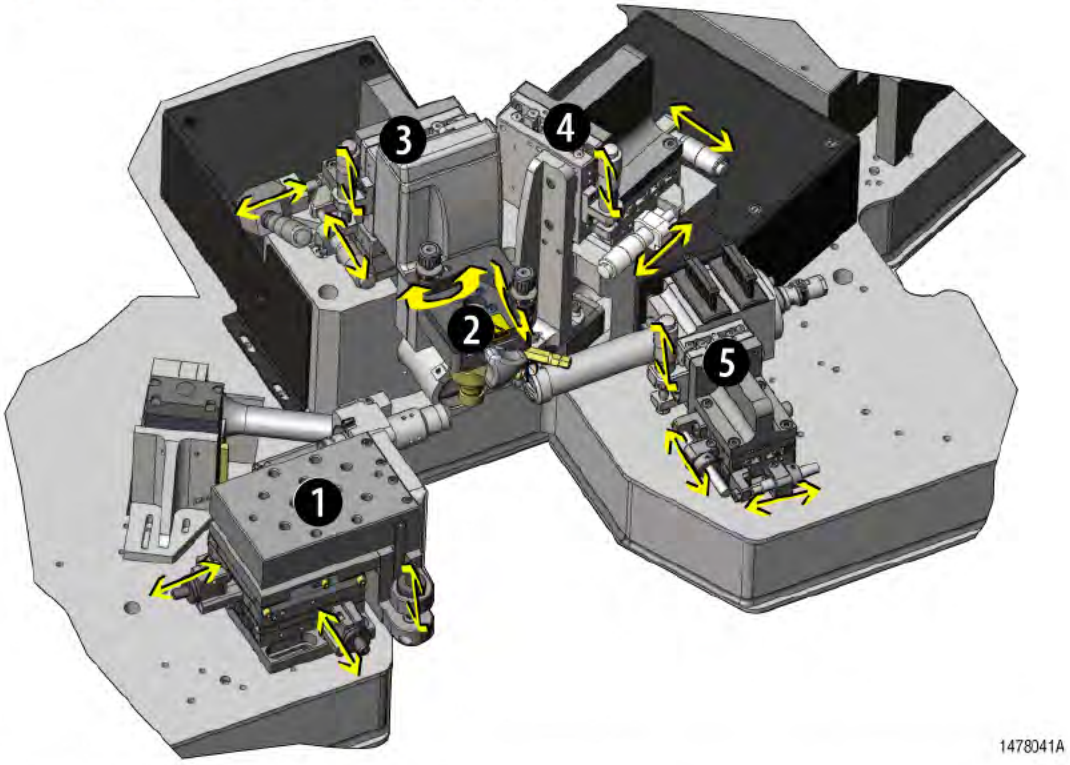
1.	Sort Chamber
2.	FBSO Positioning Micrometers
3.	FBSO with Fiber Optics
4.	IntelliSort Camera
5.	Nozzle Positioning Micrometers
6.	Fiber Optics delivering emitted and scattered light to the PODs.
7.	UV Laser BSO Positioning Micrometers
8.	Forward Scatter Positioning Micrometers
9.	UV Laser
10.	SmartSampler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

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

Micrometer Positioning Controls

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

Figure 2.4 Positioning Micrometers (Instrument Cover Removed)



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

Illumination

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

Fiber-Coupled Lasers

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

Laser Separation

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

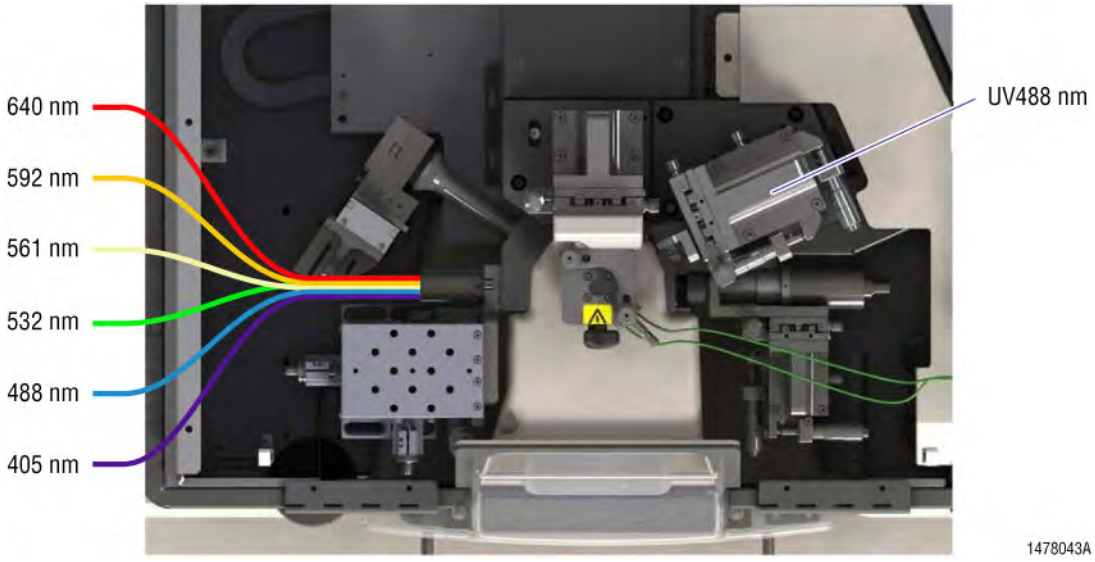
Laser Spot Size

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

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

Figure 2.5 Fiber-Coupled Lasers

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



Ultraviolet Laser

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

Laser Separation

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

Laser Spot Size

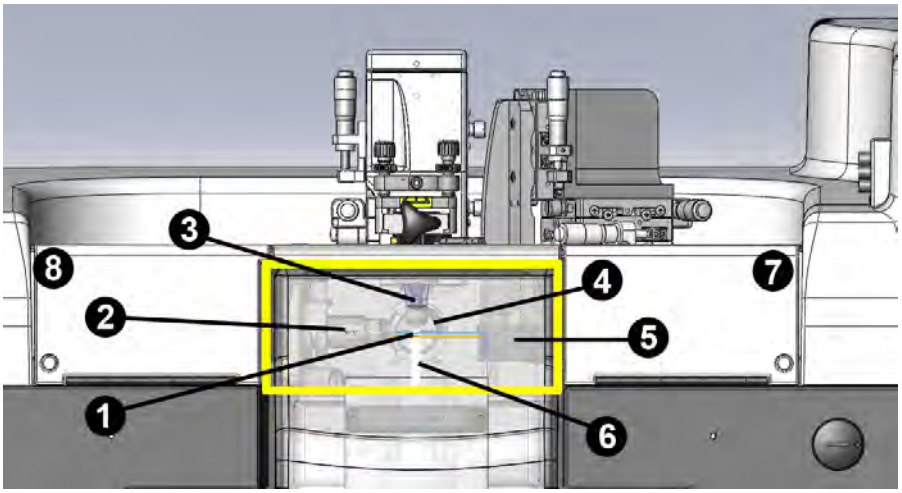
Horizontal: Gaussian beam of 50 µm, $1/e^2$

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

Illumination Chamber

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

Figure 2.7 Illumination Chamber Annotated



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

Forward Scatter Light Collection

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

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

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

Figure 2.8 Forward Scatter Collection Objective

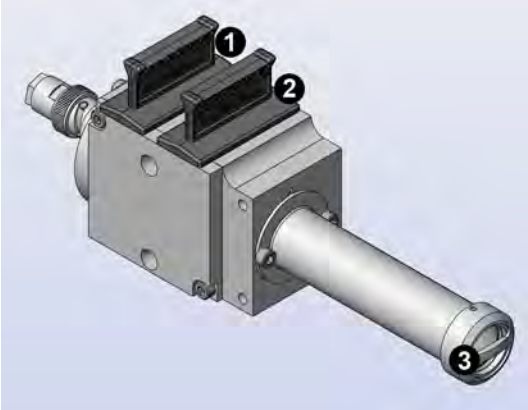


Table 2.1 Forward Scatter Collection Objective Filters and Obscuration Cap

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

Side Scatter Light Collection

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

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

Detection

Pinhole Camera and Seven Pinhole Aperture

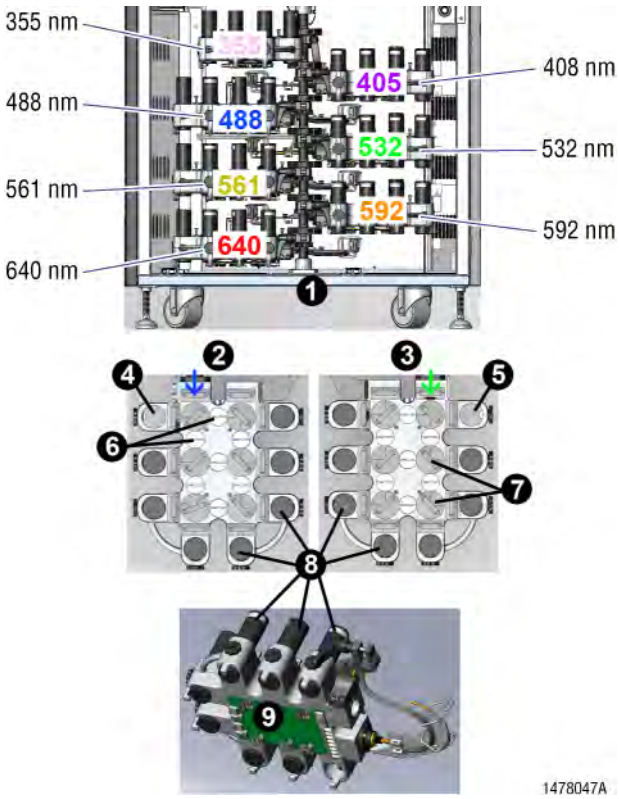
The pinhole camera makes it possible to view the seven pinhole apertures on the Coarse Alignment screen of the Touch Screen Control Panel. Upon installation, a Beckman Coulter Representative will align the laser beams coming from the fiber-coupled lasers and through the FBSO to the appropriate spatially-separated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

Figure 2.9 Pinhole Screen

Precision Optical Detector (POD)

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

Figure 2.10 PODs, PMTs and Preamplifiers



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

Collimating Lens

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

Dichroic Mirrors and Optical Filters

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

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

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

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

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

Photomultiplier Tubes (PMTs)

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

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

Preamplifiers

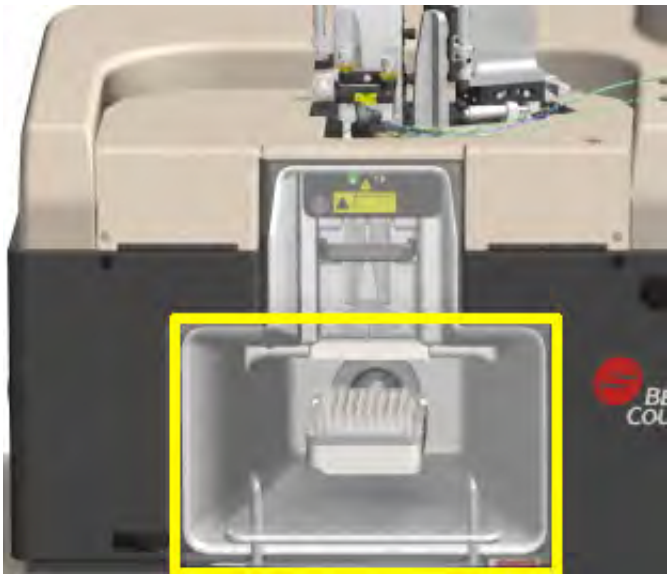
The underside of each POD is fitted with a dedicated preamplifier. See Figure 2.10. The preamplifiers control the PMTs to adjust detector gain, and convert current output into voltage output that can be analyzed by the Analog to Digital Converter Cards (ADCs.) Each preamplifier can control and interface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

Cell Sorting

Sort Chamber and Aerosol Containment Shield

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

Figure 2.11 Sort Chamber and Aerosol Containment Shield



CyClone

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

Table 2.2 CyClone Accessories for Sort Output

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

Table 2.2 CyClone Accessories for Sort Output

15 mL Tube Holder	Holds up to two tubes.
50 mL Tube Holder	Holds up to two tubes.
50 mL & 5 mL Holder	Holds one 50 mL and up to four 5 mL tubes.

Sample Cooling

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

Deflection Plates

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

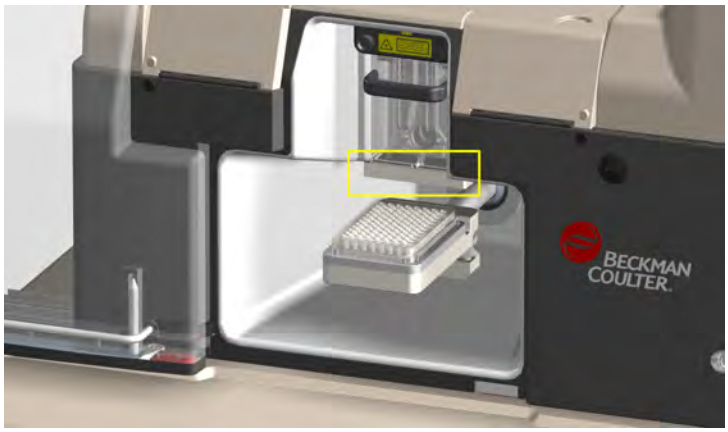
The Deflection Plates are designed to be easily removed and cleaned. The operator can use the handle on the Deflection Plate block to pull the block out of the Sort Chamber. The individual charge plates slide out for cleaning, see Chapter 9 Deflection Plates Block Assembly and Charge Deflection Plate Cleaning Procedure.

Figure 2.12 Deflection Plates Block Assembly

SortRescue

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

Figure 2.13 Sort Rescue Extended



IntelliSort

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

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

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

Streams Camera and Streams Screen

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

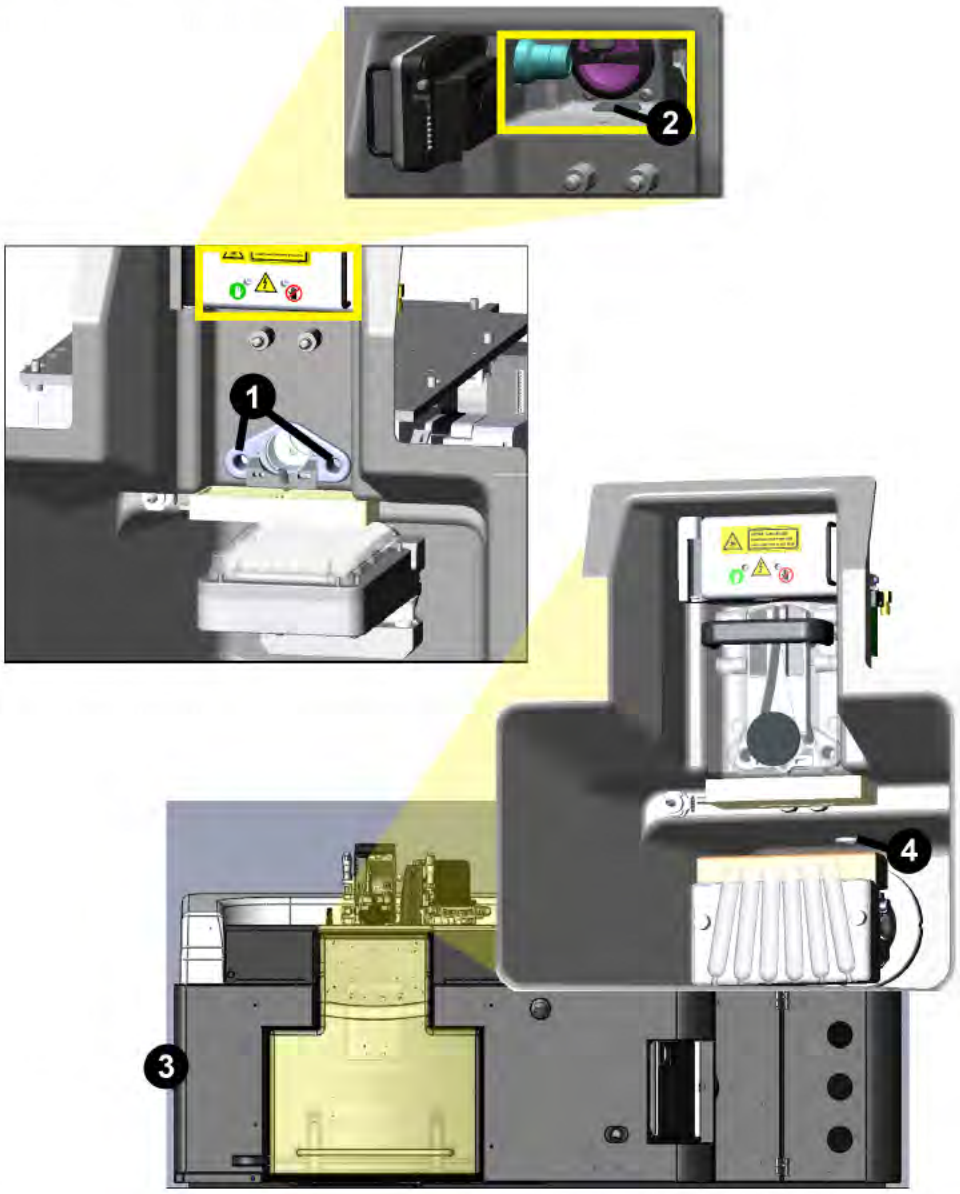
Aerosol Evacuation System

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

Figure 2.14 Aerosol Evacuation Console

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

Figure 2.15 Aerosol Evacuation Vents



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

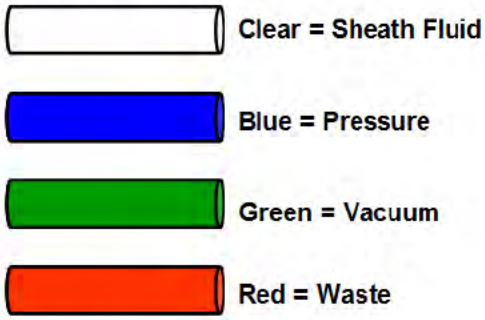
Fluidics

Tubing

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

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

Figure 2.16 Tubing Colors



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

Sheath Tank

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

Figure 2.17 Sheath Tank

Waste Tank

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

Figure 2.18 Waste Tank

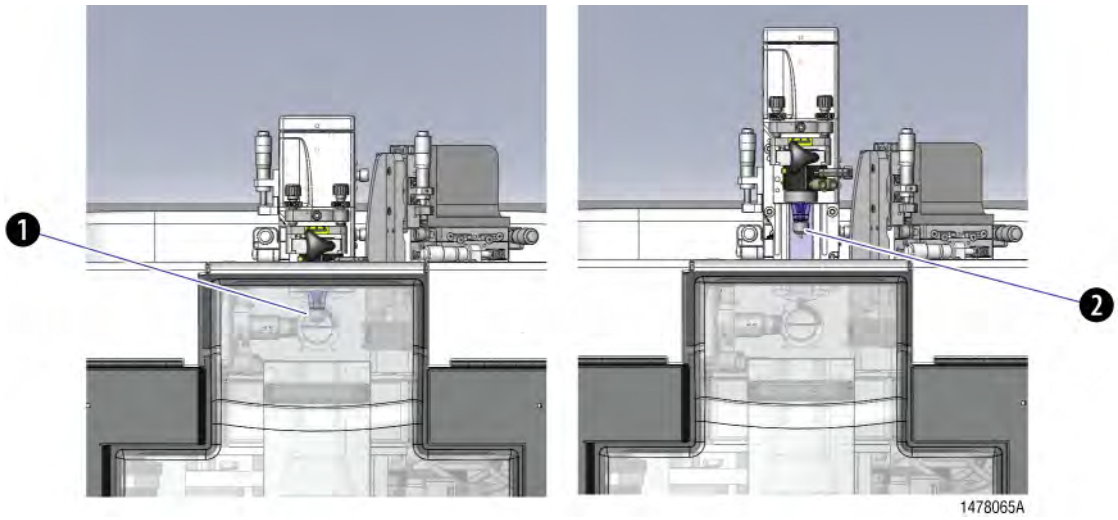
Nozzle

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

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

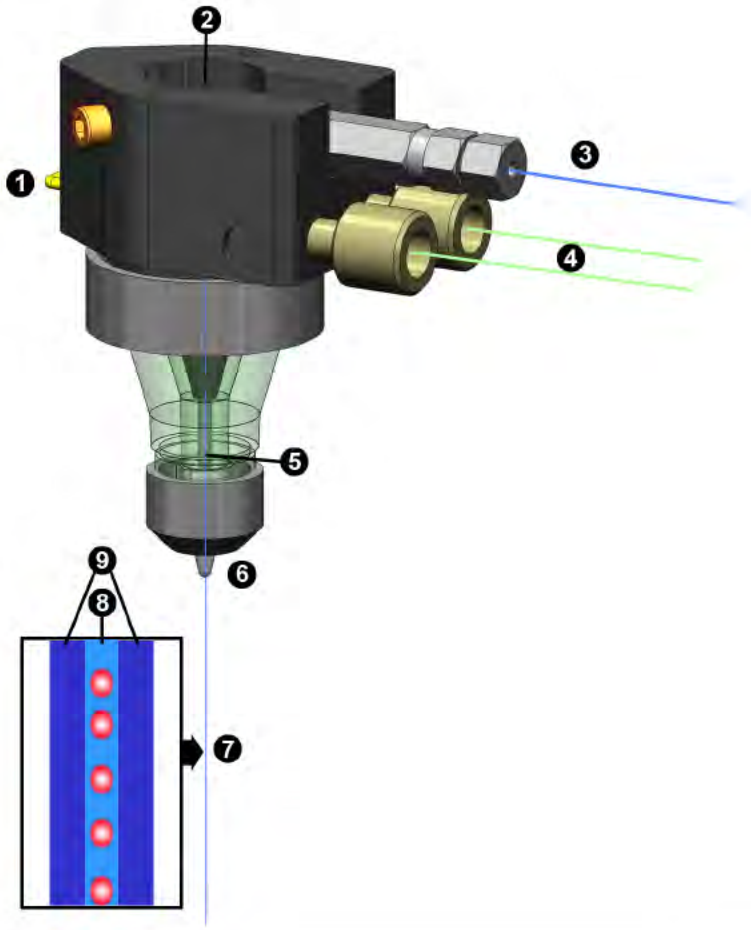
The nozzle positioning stage can be raised for access during nozzle cleaning or replacement. See Figure 2.19.

Figure 2.19 Instrument Nozzle



1.	Nozzle in operating position.
2.	Nozzle raised for cleaning or replacement.

Figure 2.20 Nozzle Interior



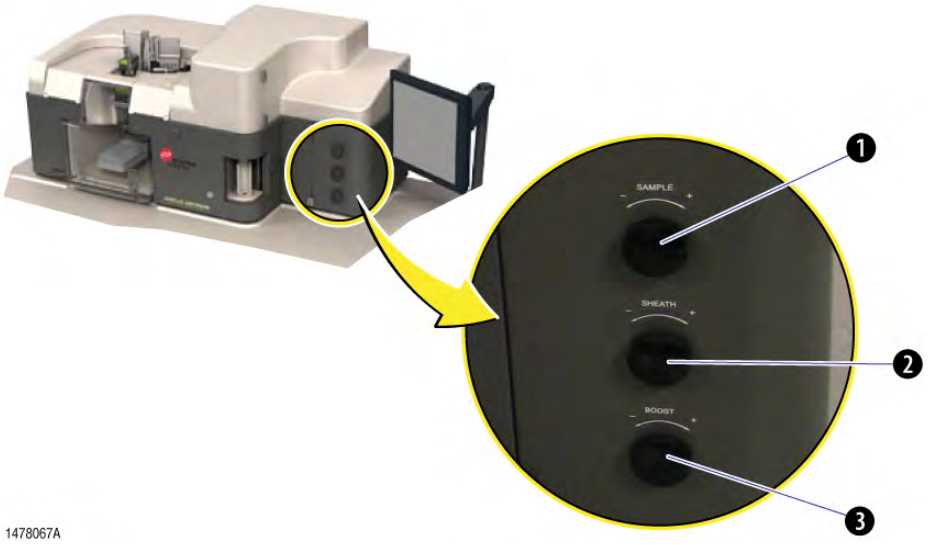
1.	To power source
2.	Piezoelectric Crystal
3.	Sample line in
4.	Sheath lines in/out
5.	Sample delivery
6.	Nozzle Tip
7.	Sample and Sheath Stream
8.	Sample
9.	Sheath

Pressure Console

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

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

Figure 2.21 Pressure Console



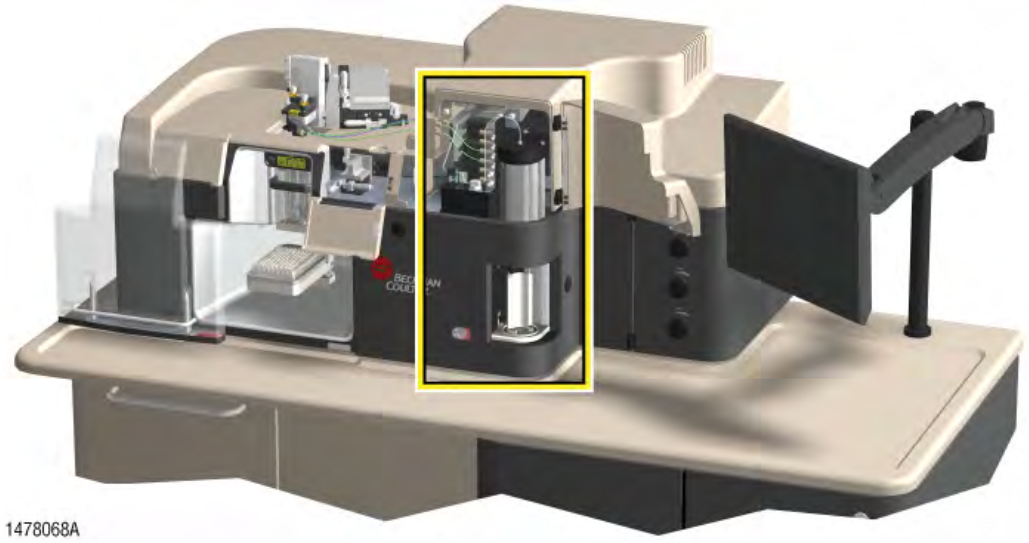
1.	Sample pressure coarse adjustment
2.	Sheath pressure coarse adjustment
3.	Sample Boost coarse adjustment. (Used in conjunction with the boost button on the Touch Screen Control Panel to adjust the amount of pressure that will be applied.)

SmartSampler

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

See CHAPTER 3, *SmartSampler Controls*.

Figure 2.22 SmartSampler



MoFlo Astrios Electronics

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

CHAPTER 3

Touch Screen Control Panel Overview

Touch Screen Control Panel

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

Common Screen Elements

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

The buttons and status icons around the perimeter of the Touch Screen Control Panel are common to the main screens and are visible when the main screens are active. The elements on the left side of the screen include the selection tabs for Coarse Alignment, Laser Intercept Configuration, Fine Alignment, Quality Control, Sort Setup, Sort Statistics, and POD Alignment. Along the bottom of the Touch Screen Control Panel are the Stream Illumination button and the Laser Shutter Controls as well as a representation of the seven-pinhole aperture strip. The right side of the Touch Screen Control Panel contains the SmartSampler buttons and instrument status indicators.

NOTE The image displayed on the button is the state in which the instrument is operating. For instance, a button that displays a bright light bulb indicates that the light is on. When you press the button the light will turn off and the button will display a dim bulb.

Figure 3.1 Touch Screen Control Panel Common Elements

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

Table 3.1 Status Indicators - Screen Elements and Functions

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

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

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

Coarse Alignment (Pinhole) Screen

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

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

Figure 3.2 Coarse Alignment Screen

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

Laser Control Tabs

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

Table 3.2 Coarse Alignment - Screen Elements and Functions

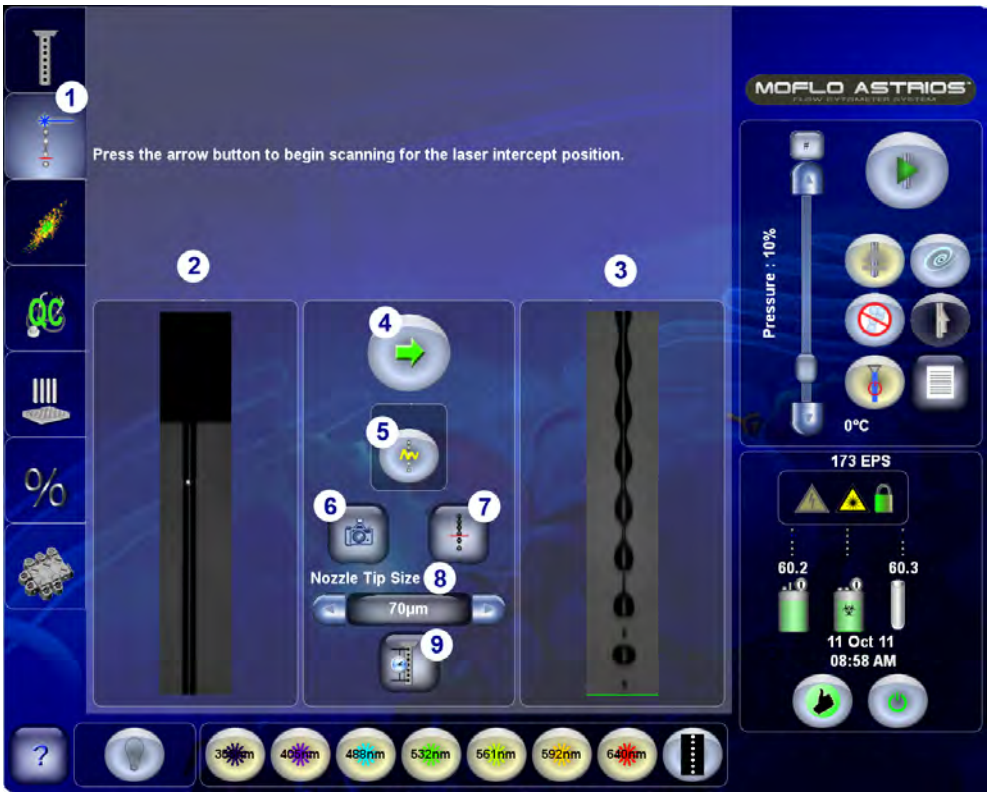
Screen Element	Function
Coarse Alignment tab 	Displays the Coarse Alignment screen.
Pinhole Illumination 	Turns ON and OFF the light that illuminates the pinhole apertures.
Intensity Control (slider control)	Dims and brightens pinhole illumination.
Laser Power ON/OFF 	Turns ON and OFF the power to the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

Laser and Stream Intercept Configuration Screen

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

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

Figure 3.3 Laser and Stream Intercept Configuration Screen



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

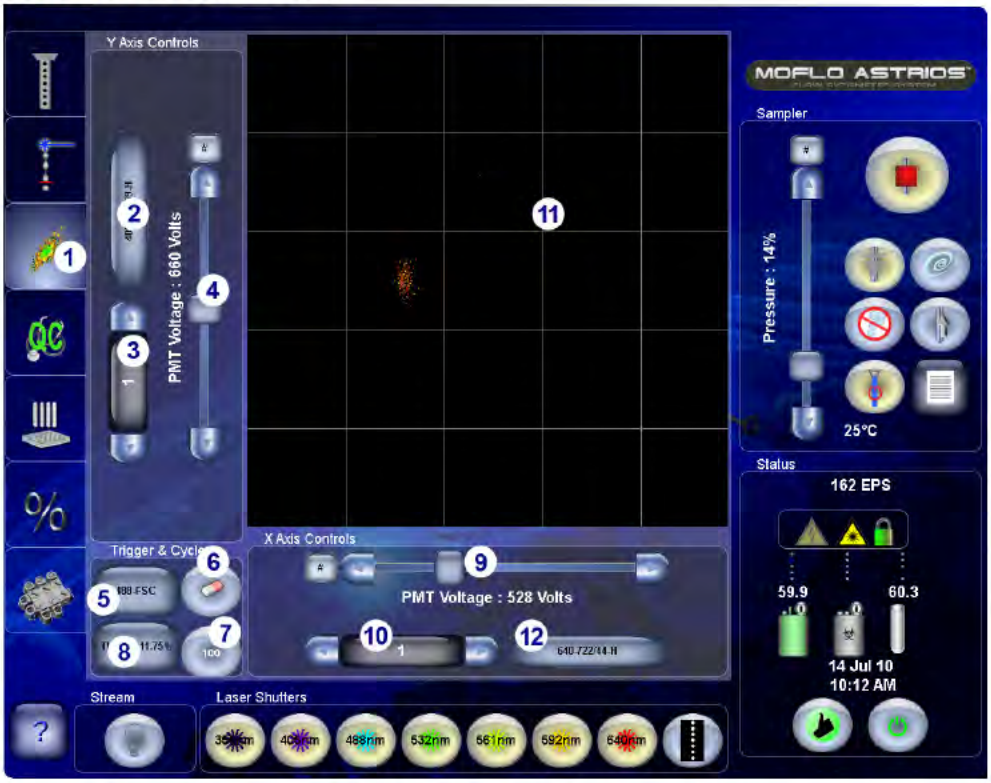
Table 3.3 Laser and Stream Intercept Screen

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

Fine Alignment Screen

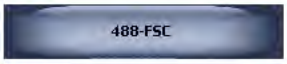
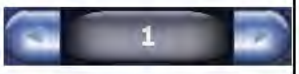
The Fine Alignment screen is used for fine adjustments to instrument alignment as well as setting parameters, data types, trigger, threshold, and the data cycle rate. Press the Dot Plot tab to view data in a dot plot format while you make fine adjustments with the appropriate micrometers, and while you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

Figure 3.4 Fine Alignment Screen



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

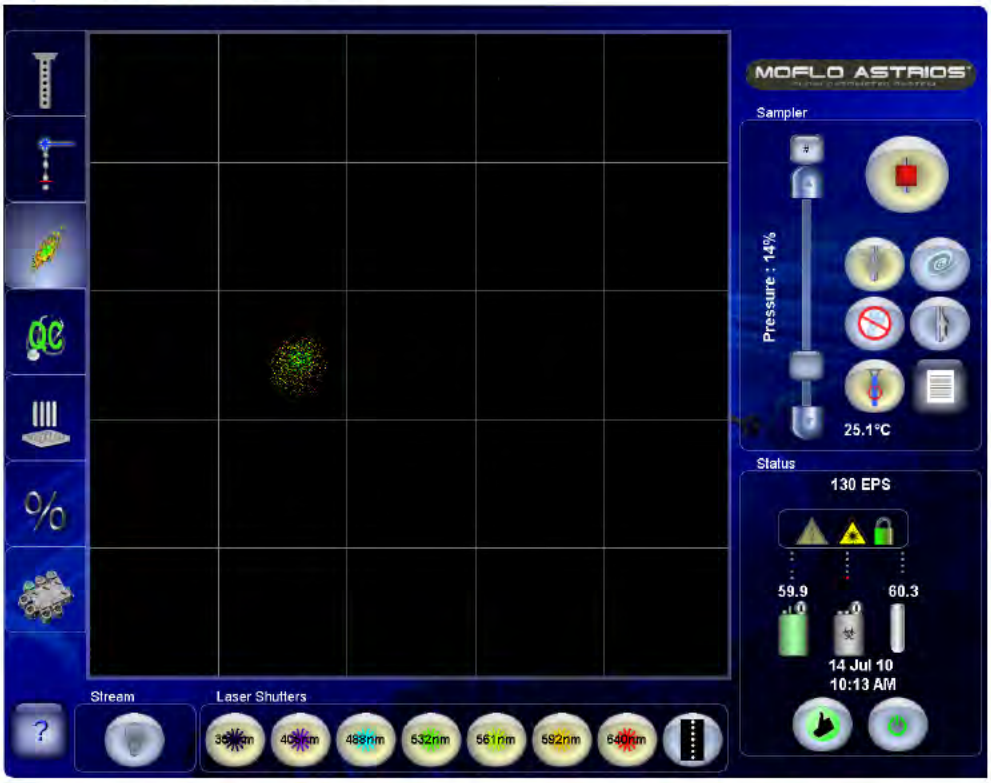
Table 3.4 Fine Alignment - Screen Elements and Functions

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

Enlarged Fine Alignment Data Display

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

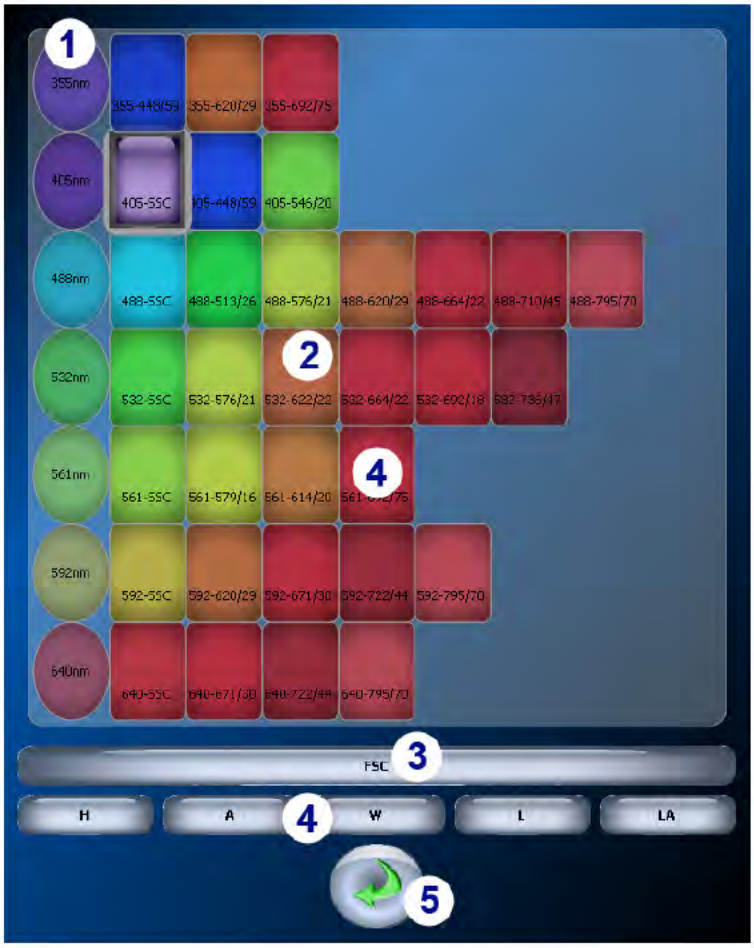
Figure 3.5 Data Display Maximized



Parameter Selection Tool

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

Figure 3.6 Parameter Selection Tool



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

Table 3.5 Parameter Selection Tool - Elements and Functions

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

Quality Control Screen

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

Figure 3.7 Quality Control Screen

1. QC tab	4. Start QC Procedure
2. Laser Line	5. QC Valid for QC Trend Analysis Report
3. PMTs and Filters	6. Status of QC Procedure

Table 3.6 QC Screen - Elements and Functions

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

Table 3.6 QC Screen - Elements and Functions *(Continued)*

Screen Element	Function
Cancel QC button 	Cancels the QC procedure.
Valid QC Button 	Marks the QC report as a valid report.

Sort Screen

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

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

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

Figure 3.8 Sort Screen

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

Figure 3.9 Sort Output Types

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

Table 3.7 Sort Screen - Elements and Functions

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

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

Screen Element	Function
Edit 	Access the Definition screen and edit a previously saved Custom Sort Output Type.
Delete 	Deletes custom Sort Output Type.
IntelliSort Initialize 	Sets drop drive frequency, and sets amplitude. This step must be done before the QC procedure is run.
IntelliSort Drop Delay Determination 	Performs automated drop delay determination and sets drop delay between 32 and 45, for a 70 µm tip, 60 psi sheath pressure setting. (Before pressing this button, view the streams image and adjust charge phase if necessary.)
IntelliSort Maintain 	Starts IntelliSort Maintain Mode, which can monitor a sort and maintain drop delay within 10% for a temperature change of ±3 degrees Celsius for a sheath pressure change of ±3 psi.

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

Screen Element	Function
Manual Droplet Setup 	Press this button to access the Manual Droplet Setup screen. **NOTE** This screen is necessary only when you intend to set up a sort manually. If IntelliSort is maintaining the drop delay some of the controls on this screen will be disabled.
Stream Setup 	Press this button to access the Stream Setup screen and: • Set Charge Phase. • Set up a sort manually. • Adjust deflection for sort output • Adjust plate voltage