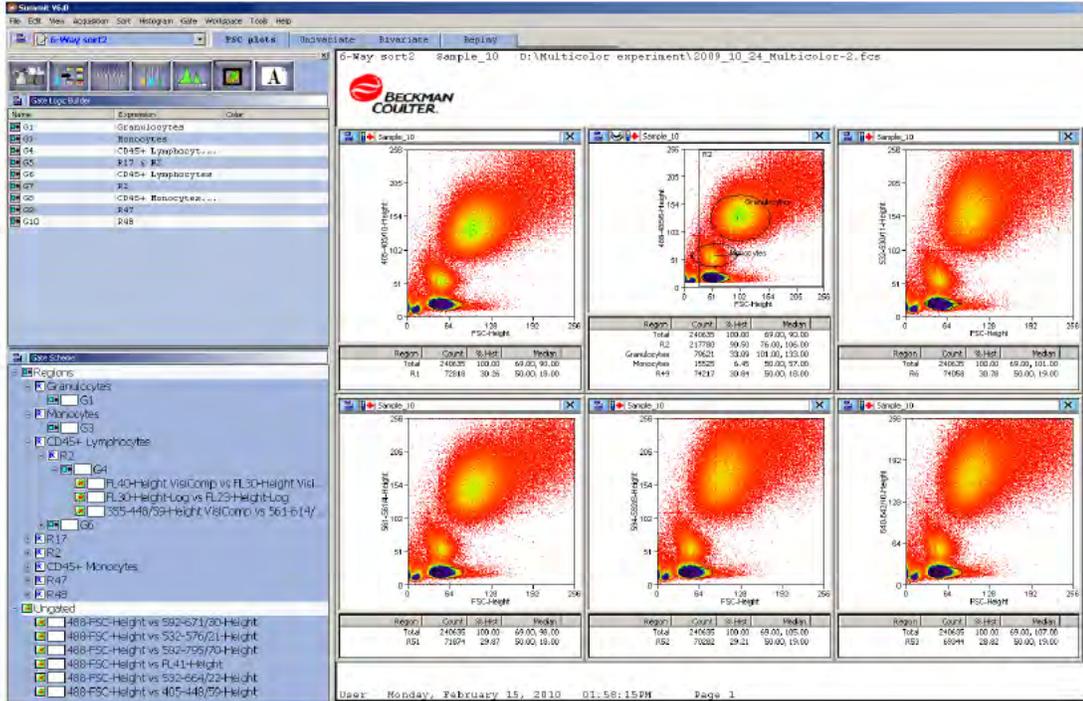


Gate Logic Tab

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

Figure 4.48 Gate Logic Tab



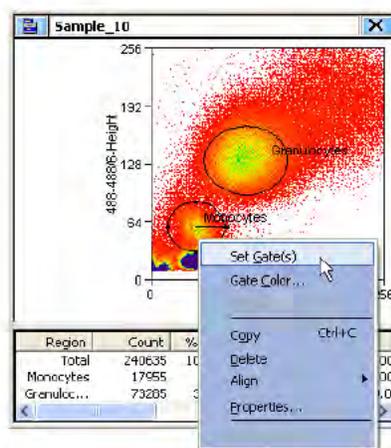
Setting a Gate from a Single Region

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

How to Gate One or More Histograms or Dot Plots

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

Figure 4.49 Set Gate 1



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

Figure 4.50 Set Gate 2

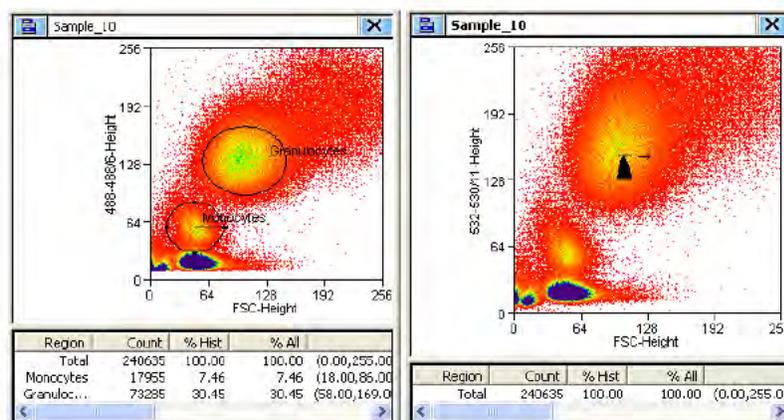
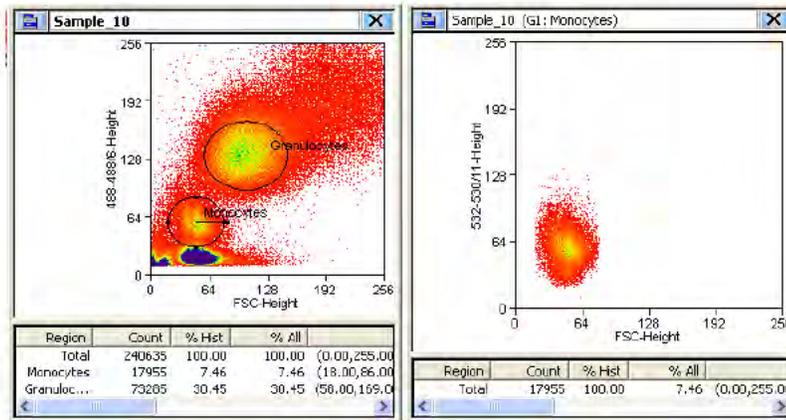


Figure 4.51 Set Gate Result



- 3 To gate more than one histogram or dot plot, right-click in the region from which you would like to gate, and select **Set Gates** from the menu. The appearance of the cursor will change.
- 4 Use the newly changed cursor to single-click in all of the histograms or dot plots in your protocol that you would like gated. When you come to the last histogram or dot plot, double-click to apply the gate.
- 5 To remove a gate, click the main histogram or dot plot menu icon and select **Ungate**, or click right-click outside of the gated region and select **Ungate**.

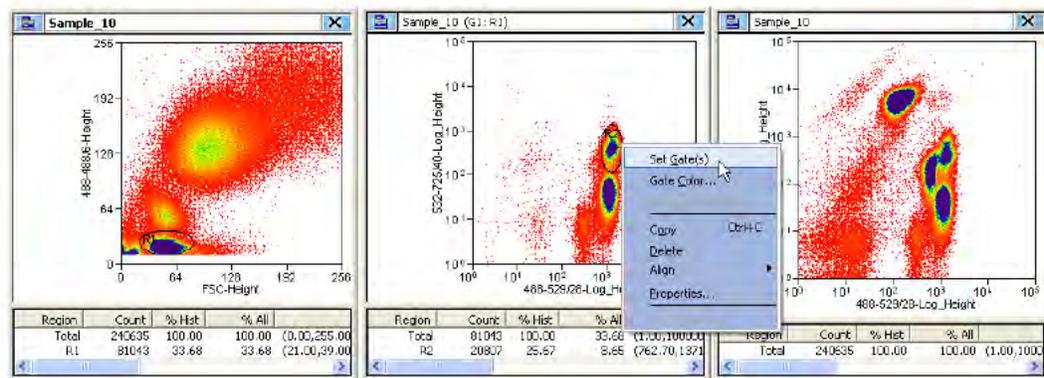
Setting a Serial Gate

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

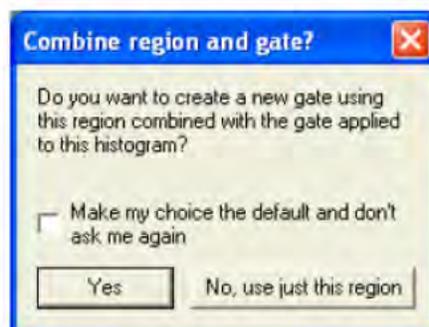
How to Serial Gate Histograms or Dot Plots

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

Figure 4.52 Set Serial Gate 1

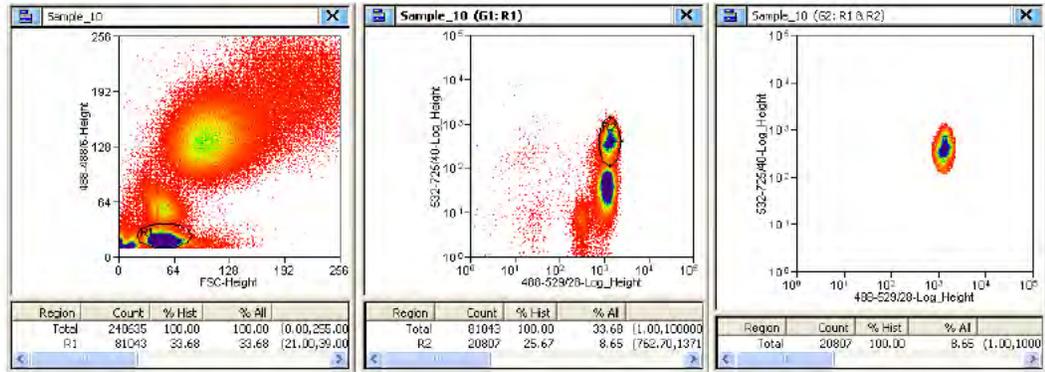


- 2 Create a region (R2) in the second dot plot.
- 3 Right-click in region (R2) and then click **Set Gates**.
The Combine region and gate? Dialog box appears.
- 4 Click **Yes** to activate serial gating. Click **No** to gate using only the specified region.



- 5 Check the **Make my choice the default and don't ask me again** checkbox to disable this dialog box in the future.
- 6 Double-click in the final dot plot to apply the gate.

Figure 4.53 Set Serial Gate Result



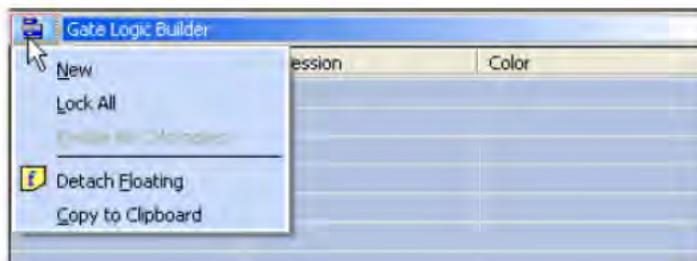
Gate Logic Builder

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

How to Define (Edit) Gate Logic

- 1 Select the Gating tab in the Summit Software Control Panel.
- 2 Click the upper-left icon in the Gate Logic Builder panel and then click **New** to create a new gate.

Figure 4.54 Gate Logic Builder



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

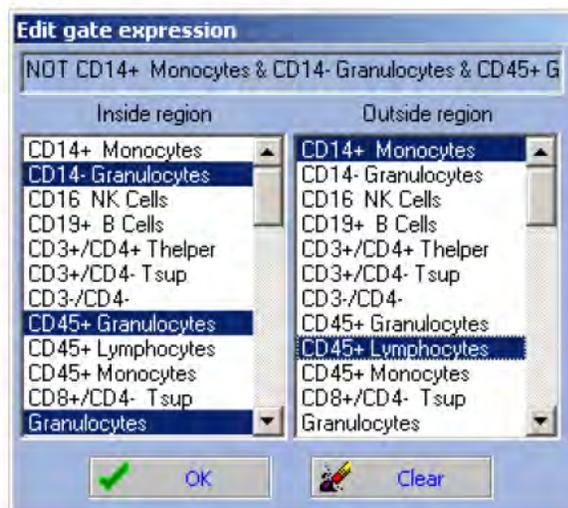
Figure 4.55 Edit Gate Expression Options



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

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

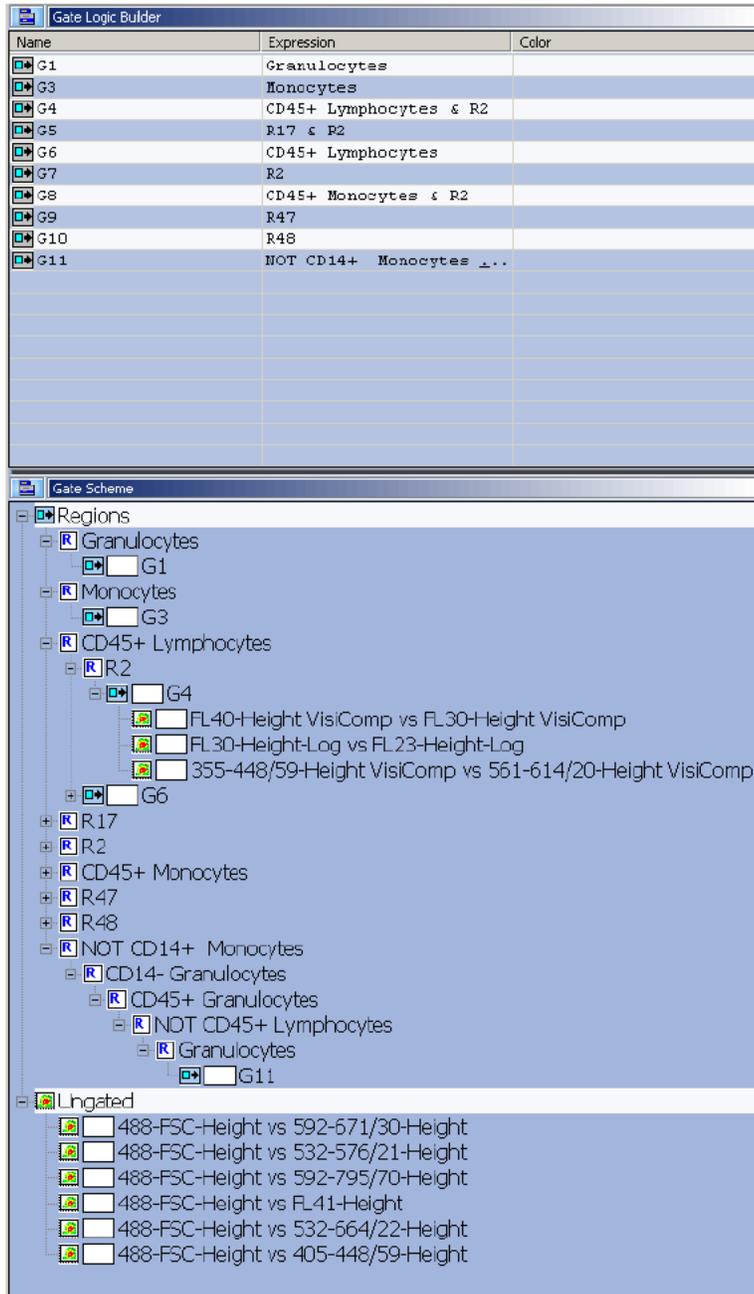
Figure 4.56 Select Gate Expressions



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

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

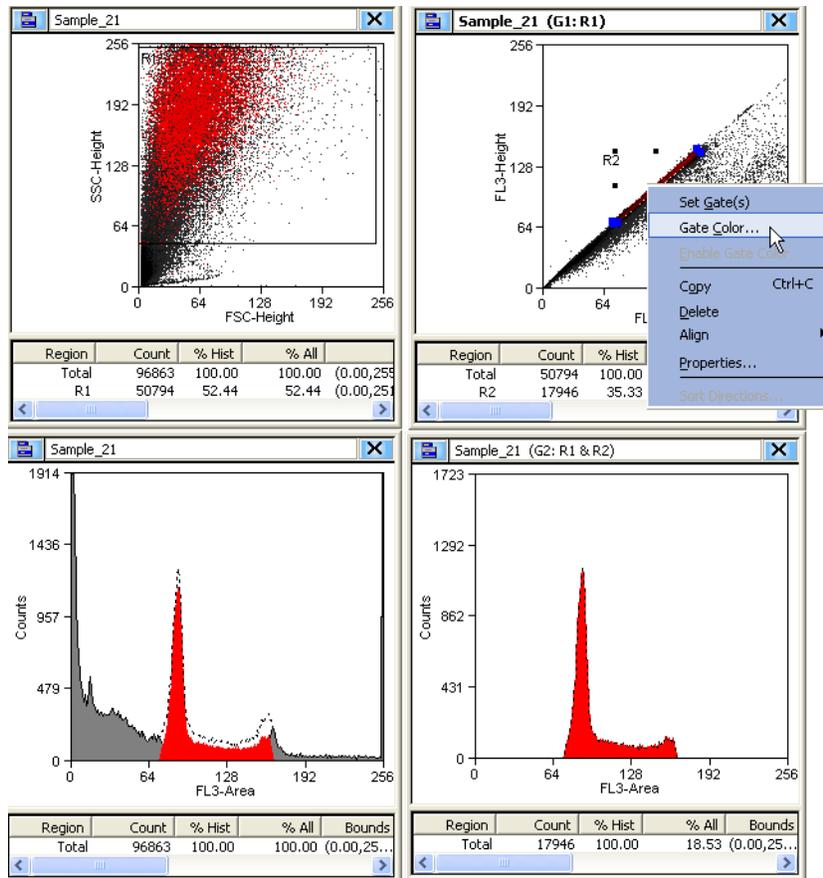
Figure 4.57 Gate Logic Builder and Gate Scheme Panels



Color Gating

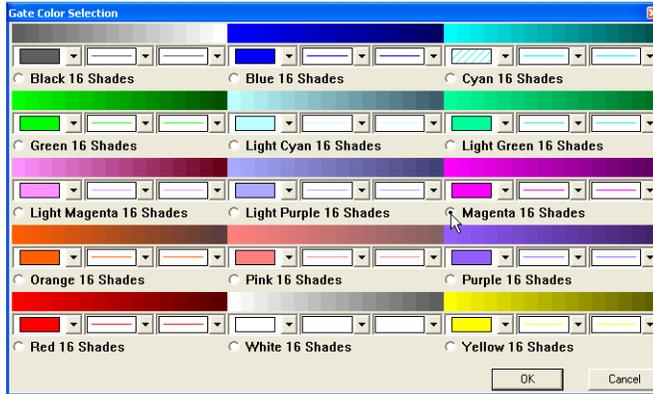
- To view the single cells and doublet cells populations with color gating, right click in the region around the single cell population [Figure 4.58](#) and select **Gate Color**. For information on doublet discrimination, see [CHAPTER 8, Sort Decisions and Doublets](#).

Figure 4.58 Color Gating Diagram



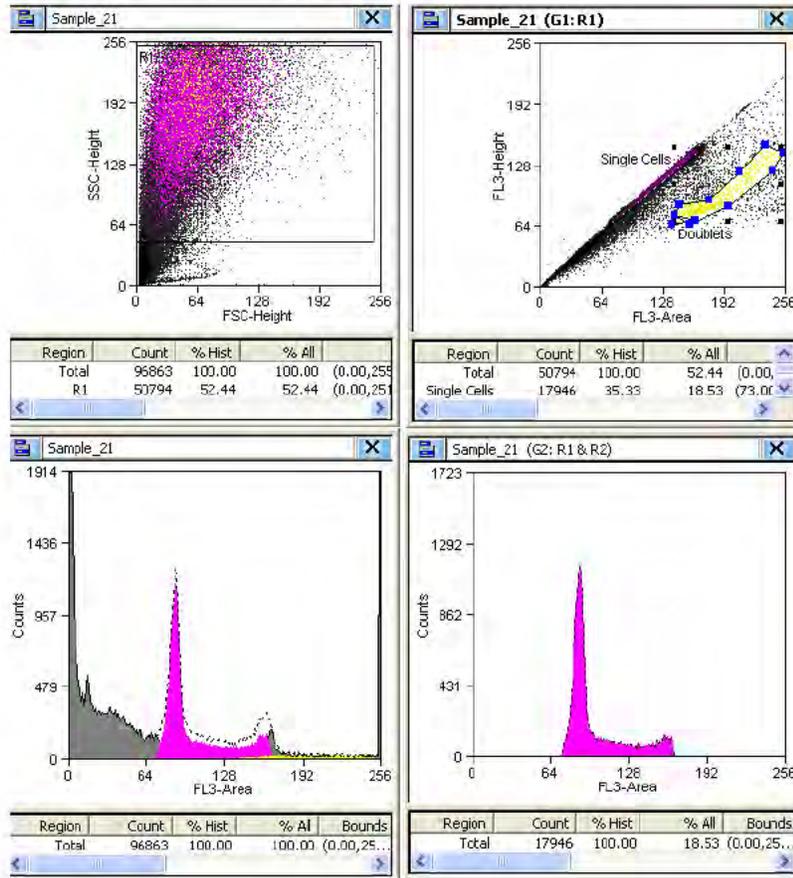
- 2 Select a color from the color selection palette and click **OK**.

Figure 4.59 Color Gating Color Palette



- 3 The single cell population from [Figure 4.58](#) appears in [Figure 4.60](#) in magenta. The doublet population is displayed in yellow.

Figure 4.60 Color Gated Single Cell Population and Doublet Population



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

Figure 4.61 Toggle Color Gating

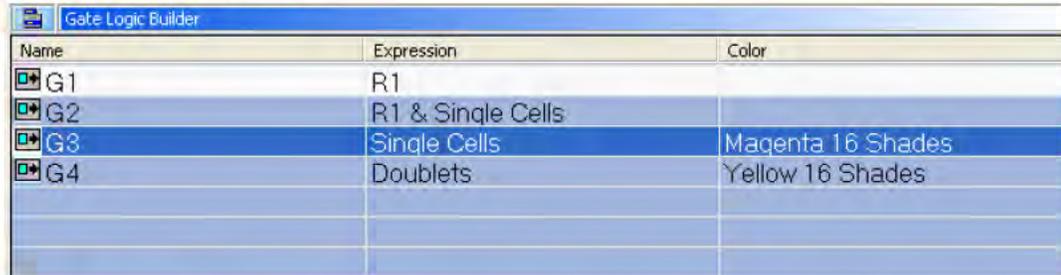


- 4 Create other regions if necessary to identify doublets. Click and drag the regions around the dot plot until you are confident you have located the doublet population.

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

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

Figure 4.62 Edit Color Gates



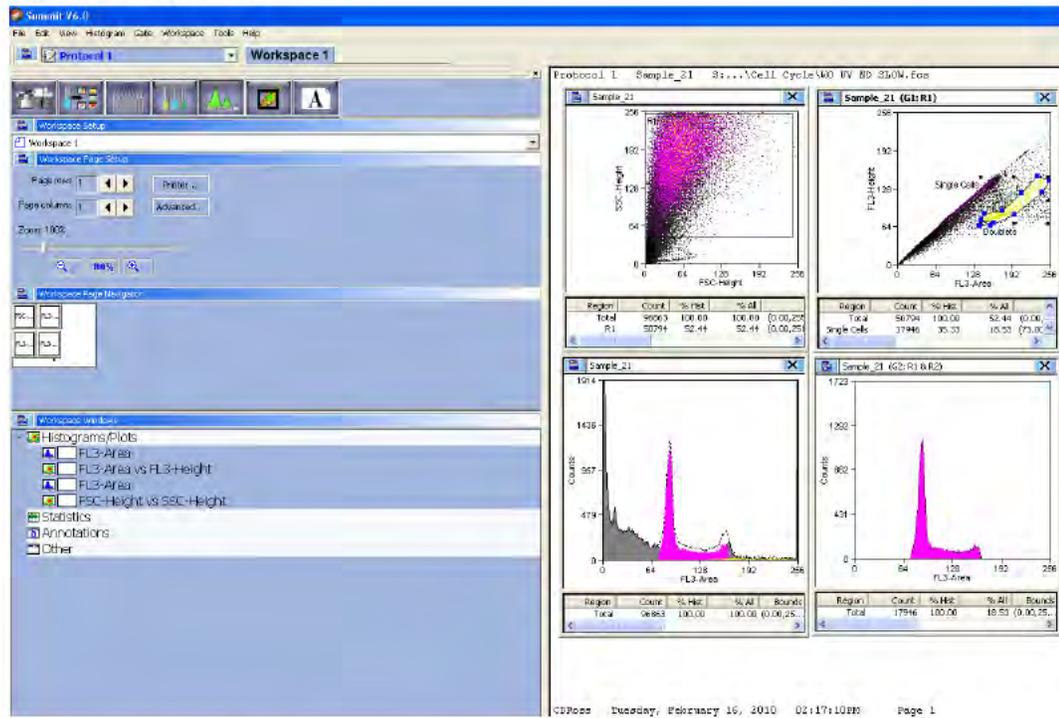
Name	Expression	Color
G1	R1	
G2	R1 & Single Cells	
G3	Single Cells	Magenta 16 Shades
G4	Doublets	Yellow 16 Shades

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

Layout Tab

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

Figure 4.63 Layout Tab



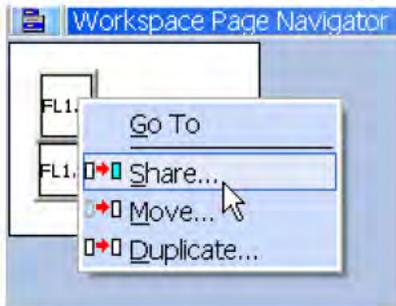
Workspace Page Setup

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

Workspace Page Navigator

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

Figure 4.64 Workspace Page Navigator



Go To

The Go To option activates the selected histogram.

Share

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

Move

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

Duplicate

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

Shortcut Keys

The following list describes the keyboard shortcuts in Summit software.

Table 4.2 Shortcut Keys and Functions

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

Startup and Shutdown Procedures

Reagents

Refer to [APPENDIX A, Approved Cleaners and Disinfectants](#).

Manual Startup

Startup Procedure - Main System Power and Summit Computer OFF

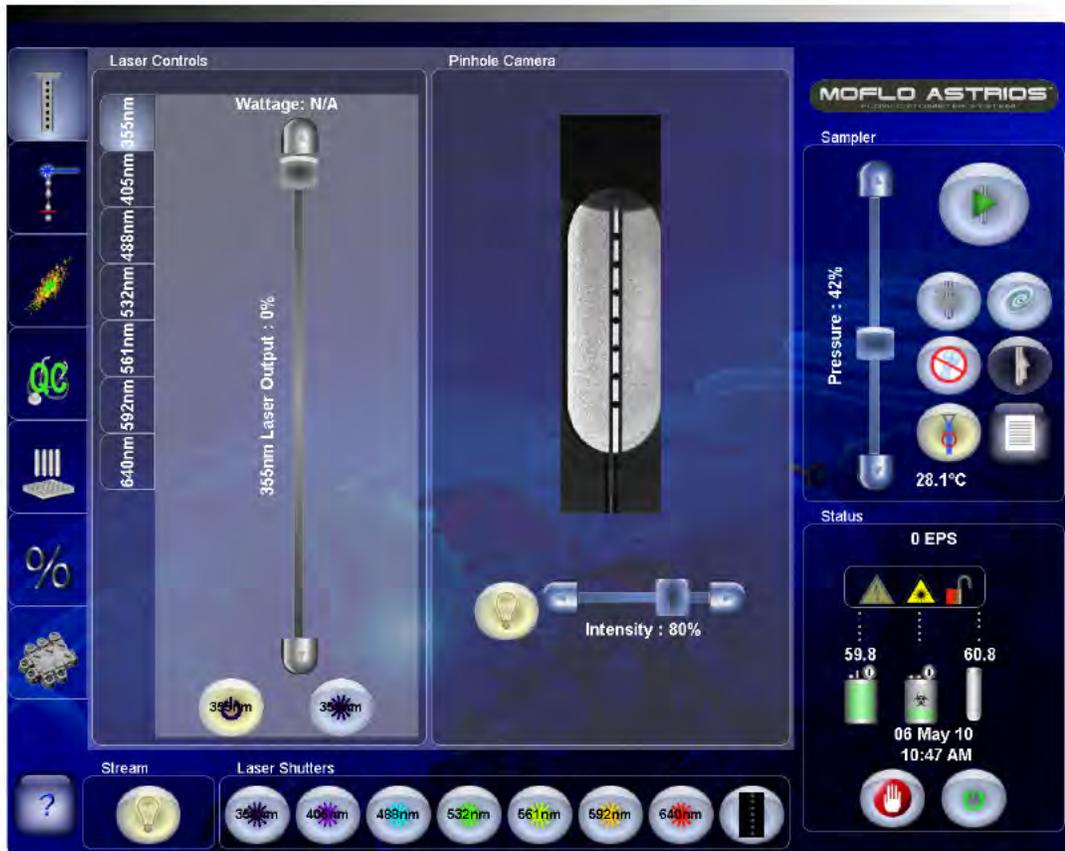
- 1 In general, the main power to the MoFlo Astrios, Biosafety Cabinet (if part of the system), Touch Screen Control Panel, and the Summit Workstation should remain ON. However, if power to the system is completely OFF follow the steps below.
- 2 Turn on the optional Biosafety Cabinet (if part of the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

Startup Procedure - Main System Power Already ON

- 1 Power ON the Touch Screen Control Panel monitor using the power button on the side bezel.

- 2 Select a laser tab on the Laser Control Panel (Figure 5.1) and press the Laser Power button to turn laser power ON.

Figure 5.1 Laser Control Tab



- 3 Use the Laser Power slider to set laser power to the desired value for lasers greater than or equal to 150 mW output in the laser engine.

NOTE The 355 nm, 405 nm, and 640 nm lasers can not be adjusted.

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

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

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

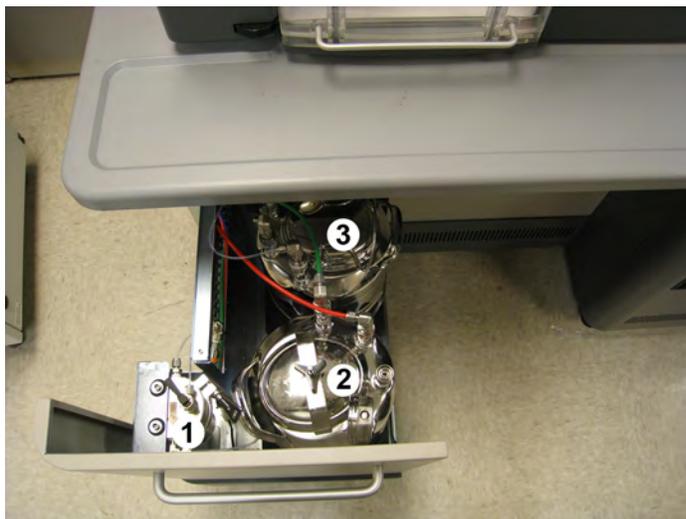
Start Summit Software

- 1 Log into the Windows operating system. The Summit Workstation should generally remain powered ON.
 - 2 Double-click the Summit icon  to open Summit Software.
 - 3 Select the appropriate database or create a new database. See [CHAPTER 4, Summit Software Database](#).
 - 4 Load the alignment protocol if necessary, or create histograms for each parameter on the system.
NOTE When creating a new protocol it is necessary to enable the parameters you intend to use and verify the laser path selection for each parameter. See [CHAPTER 4, Enable Parameters](#).
 - 5 After the instrument is started and has warmed-up for 30 minutes, continue to [CHAPTER 6, Instrument Alignment](#).
-

Start Fluidics Manually

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

Figure 5.2 Fluidics Tanks and Sheath Filter Vent



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

- 2 Press the SmartSampler extended menu button.



- 3 Press the Change Tanks button to pressurize the fluidics.



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

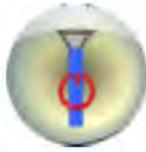
Figure 5.3 Fluidics Tanks and Sheath Filter Vent



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

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

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



Automatic Startup

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

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

NOTE For users who have the optional Biosafety Cabinet; the Biosafety Cabinet must have been turned on earlier (manually) or left on from the previous day for the instrument to be ready to run right away. Otherwise, allow at least 30 minutes for the system to reach thermal equilibrium.

Shutdown

Shutdown Procedure Selecting Manual Startup

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

- 1 Press the Power button on the lower-right portion of the Touch Screen Control Panel to initiate the shutdown procedure.
 - Charge Plates are disabled.
 - Drop Drive is disabled.
 - Lasers are turned off.
 - Sample Chamber is opened.
 - You are prompted to place a tube of cleaner in the Sample Chamber.
- 2 Place a tube of cleaner in the SmartSampler. Press the green arrow to continue.
 - Cleaner is run through the probe for 30 seconds.

- You are prompted to place a tube of deionized water in the Sample Chamber.
-
- 3 Place a tube of deionized water in the SmartSampler. Press the green arrow to continue.
 - Deionized water is run through the probe for 60 seconds.
 - A message appears asking if you want to set the instrument to start automatically.
-
- 4 Select No. A message asks if you want to shut off electronics. In general, the electronics should be left powered ON. You may want to turn off the electronics when the instrument will not be used for several days in a row.
-
- 5 Turn OFF power to the Touch Screen monitor using the physical power button on the right side of the monitor bezel.
-
- 6 Close Summit. See [Exit Summit Workstation](#).
-
- 7 Turn off Biosafety Cabinet (optional).

Shutdown Procedure For Setting Automatic Startup

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

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



- Charge Plates are disabled.
 - Drop Drive is disabled.
 - Lasers are turned off.
 - Sample Chamber is opened.
 - You are prompted to place a tube of cleaner in the Sample Chamber.
-
- 2 Place a tube of cleaner in the SmartSampler. Press the green arrow to continue.
 - Cleaner is run through the probe for 60 seconds.
 - You are prompted to place a tube of deionized water in the Sample Chamber.

- 3 Place a tube of deionized water in the Smart Sampler. Press the green arrow to continue.
 - Deionized water is run through the probe for 60 seconds.
 - A message appears asking if you want to set the instrument to start automatically.
- 4 Select Yes. The Automatic Startup screen appears.

Figure 5.4 Automatic Startup Screen



- 5 Select the lasers you would like to turn ON during Automatic Startup.
- 6 Press the Start Fluidics button if you want pressure to the sheath tank and vacuum to the waste tank to be turned on automatically.



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

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

NOTE For a list of approved cleaners see [APPENDIX A, Approved Cleaners and Disinfectants](#).

 **WARNING**

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

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

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

10 Reattach the tanks to the system.

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

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

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

14 Turn off the Biosafety Cabinet (optional).

Exit Summit Workstation

- 1 Perform data backup as required and close Summit Software.
- 2 Log off the Windows operating system.
- 3 It is recommended that power to the Summit Workstation remain ON.

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

Change Tanks

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

Status Indicators

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



This symbol indicates the status of the sheath tank.

Green = Full

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

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

The value above the symbol indicates the sheath pressure.



This symbol indicates the status of the waste tank.

Green = Empty or low

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

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

Fill Sheath Tank and Empty the Waste Tank

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

How to Fill the Sheath Tank During Your Work Shift

- 1 Press the SmartSampler extended menu button.



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



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

Figure 5.5 Components Inside the Fluidics Drawer



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

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

Figure 5.6 Sheath Tank

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

- 5 Remove the lid from the sheath tank (rotate counterclockwise). To avoid contamination, do not touch the inside of the lid and set the lid aside on a clean surface such as a paper towel.
- 6 Fill the sheath tank to a little more than 2/3 full. Use IsoFlow sheath fluid or similar fluid.
- 7 Place the lid back on the sheath tank and tighten (turn clockwise).

⚠ WARNING

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

- 8 Carefully place the sheath tank back inside the fluidics drawer. Avoid pinching your fingers between the sheath tank and metal supports.
- 9 Reconnect all sheath and pressure tubings (sheath to filter and SmartSampler, and pressure inlet). Each connector is a different size to facilitate proper reconnection. See Figure 5.6 as needed.

-
- 10** Press the SmartSampler extended menu button.



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



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

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

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



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

How to Empty the Waste Tank During Your Work Shift

- 1** Press the SmartSampler extended menu button.



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



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

Figure 5.7 Components Inside the Fluidics Drawer



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

⚠ WARNING

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

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

Figure 5.8 Waste Tank



⚠ WARNING

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

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

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

⚠ WARNING

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

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

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

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

10 Position the waste tank back inside the drawer.

⚠ WARNING

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

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

12 Close the fluidics drawer.

13 Press the SmartSampler extended menu button.



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



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



Instrument Alignment

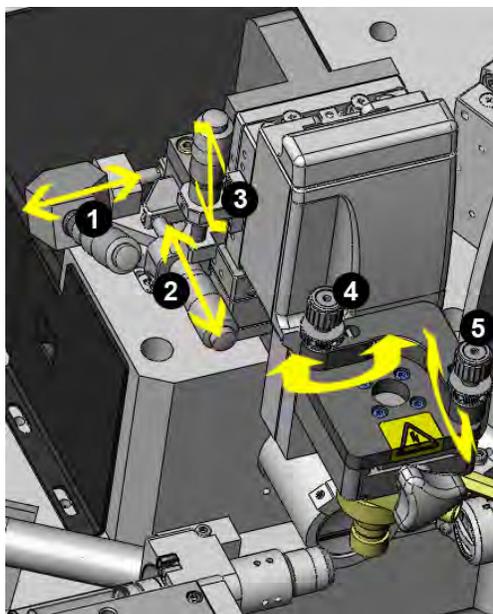
Every Day Instrument Alignment

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

Stream Alignment

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

Figure 6.1 Stream Positioning Adjustment Stage



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

Stream Alignment Procedure

- 1 Turn on the sheath stream.



Verify that:

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

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



Consider the following to determine if the stream requires alignment.

- Verify that the sheath stream is centered over the pinholes.
- Verify that the dark stream edges and the bright stream center appear proportioned in thirds.



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

If the stream needs alignment, continue to step 3. If the stream is aligned, proceed to [Laser Spot Determination](#).

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

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



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

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

- 7 Move the nozzle to the raised position and adjust gimbal (4) Figure 6.1. It is necessary to move the gimbal so that it appears you are significantly over correcting the position. Lower and raise the nozzle to see if the stream moves to the right or left. If it does, repeat the process until the gimbal positioning is correct. Fine tune micrometers (1 and 2) if necessary.
- 8 Move the nozzle to the raised position and adjust gimbal (5) Figure 6.1. It is necessary to move the gimbal so that it appears that you are significantly over correcting the position. Lower and raise the nozzle to see if the stream moves out of focus. If it does, repeat the process until the gimbal positioning is correct. Fine tune micrometers (1 and 2) if necessary.
- 9 When the stream is aligned, move the nozzle tip until it is positioned within view of the camera Figure 6.2.

Figure 6.2 Stream Aligned and Nozzle Tip Within View



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

Laser Spot Determination

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

Figure 6.3 Laser Spot Determination Screen



IMPORTANT Familiarize yourself with [CHAPTER 10, Background Image Subtraction](#).

Determine the Laser Spot Procedure

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

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



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



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



- 5 Press the green arrow button again to confirm laser spot determination. A green reference line appears. Adjust nozzle micrometer (3) Figure 6.1 so that the nozzle is even with the green line.



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



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



Laser Delay

Laser delay may be determined outside of the Quality Control (QC) tab if needed. When changing sheath pressures, the Laser Delay Determination is executed prior to fine alignment. The Laser Delay function precedes the Laser Spot Determination.

Finding Laser Delay

- 1 Obtain Spherotech 3 μm Ultra-Rainbow SpectrAlign beads at a concentration of 1×10^6 beads/ml.
- 2 Place the tube into the Smart Sampler.
- 3 Select the Laser Delay menu button.



- 4 Press the green arrow button to start the Laser Delay. Laser Delay will execute.



- 5 At the top of the menu screen, a dialog will appear that will describe steps completed during Laser Delay Determination.

Optimizing trigger channel delay

Laser Delay Instructions

1. Turn required lasers on
2. Verify nozzle tip size
3. Perform coarse alignment with less than 30 eps noise on trigger channel
4. Place alignment beads in SmartSampler
5. Press the green arrow button to begin

Automatic Laser Delay Results

State: Running

Date: Mon 10 Oct 2011 12:36:00

Nozzle: 70

Sheath PSI: 60.1

Trigger: 488-FSC

355nm
LD: 5000
(estimated)

405nm

488nm

532nm

561nm

592nm

640nm

- 6 At any time, you may press the red X to cancel Laser Delay.



- 7 When the Laser Delay Determination has completed, the results will be visible in “Automatic Laser Delay Results.” On the left-hand side of the window, the Laser Delay values will be visible for each laser. On the bottom window, the state, date, nozzle tip, sheath pressure and trigger will be reported at each Laser Delay Determination.

Laser delay optimization complete

Laser Wavelength	Laser Delay (LD)
355nm	5000 (estimated)
405nm	9320
488nm	27070
532nm	13820
561nm	18280
592nm	22680
640nm	31560

Laser Delay Instructions

1. Turn required lasers on
2. Verify nozzle tip size
3. Perform coarse alignment with less than 30 eps noise on trigger channel
4. Place alignment beads in SmartSampler
5. Press the green arrow button to begin

Automatic Laser Delay Results

State: Success

Date: Mon 10 Oct 2011 12:36:42

Nozzle: 70

Sheath PSI: 60.1

Trigger: 488-FSC

- 8 To return to the main screen, select the green return button.



Laser Alignment

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

To verify and adjust coarse laser alignment see [CHAPTER 10, Laser Alignment](#).

Fine Laser Alignment

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

Fine Alignment Procedure (Fiber-coupled Lasers)

- 1 Access the Fine Alignment screen.



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

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

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

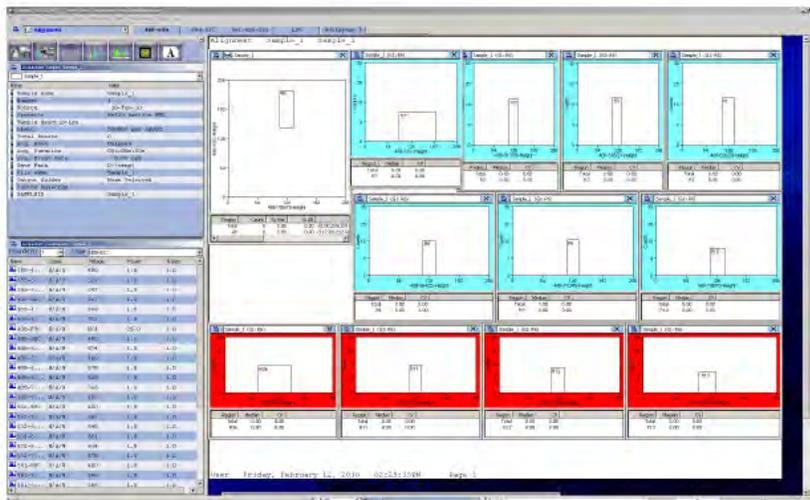
- 640 nm
- 488 nm
- 592 nm
- 561 nm
- 532 nm
- 405 nm
- 355 nm (The UV laser is free-standing and is directed through the seventh pinhole.)

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

- 4 Press the Trigger Parameter Selector [Figure 3.4](#) and choose FSC as the alignment trigger.

- 5 Open all laser shutters.

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



- 7 Go to the Acquisition tab in Summit and click the Extended Menu icon .



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



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

-
- 9** Load a tube of [Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles](#) 3.0 μm -3.4 μm 1 x 10⁷/mL. (diluted 1:10 with DI water), acquire data in Summit (F2).

NOTE It is helpful to set Summit Software in Cycle Mode while you perform fine alignment. Click the

Cycle Mode button  on the left side of the screen to turn Cycle Mode off and on. See page 4-19 for Cycle Mode details.

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



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



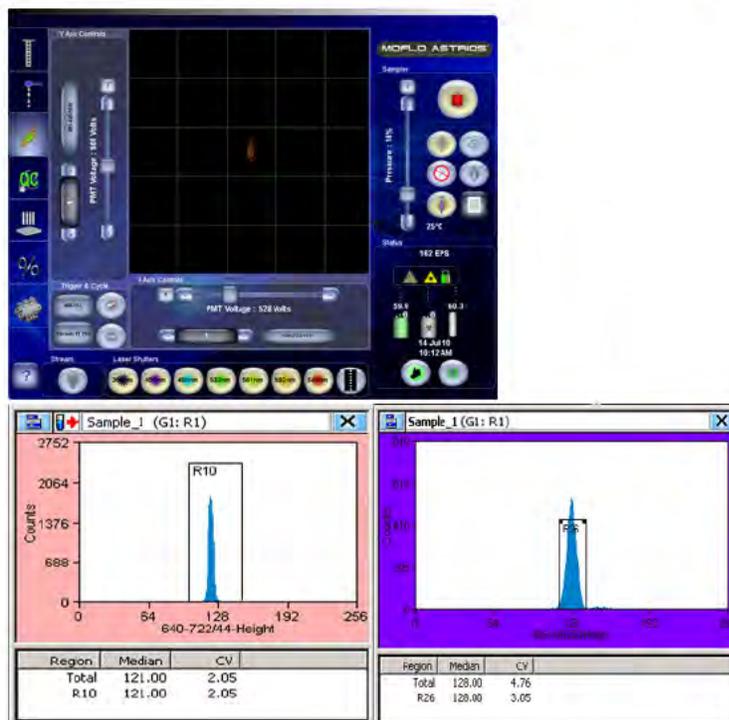
-
- 12** Adjust the sample differential pressure to achieve an event rate of 100-120 EPS.

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

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

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

Figure 6.4 Summit Software and Touch Screen Control Panel Fine Alignment



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

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

Free-space UV Laser Fine Alignment

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

UV Laser Fine Alignment

- 1 Open the shutter for the trigger laser and for the UV laser.
- 2 Use the Touch Screen Control Panel or Summit to plot 355-692/75 (H) vs. 355-448/59 (H).



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

IMPORTANT Do not adjust the position of the nozzle.

Quality Control

Quality Control and Performance Validation

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

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

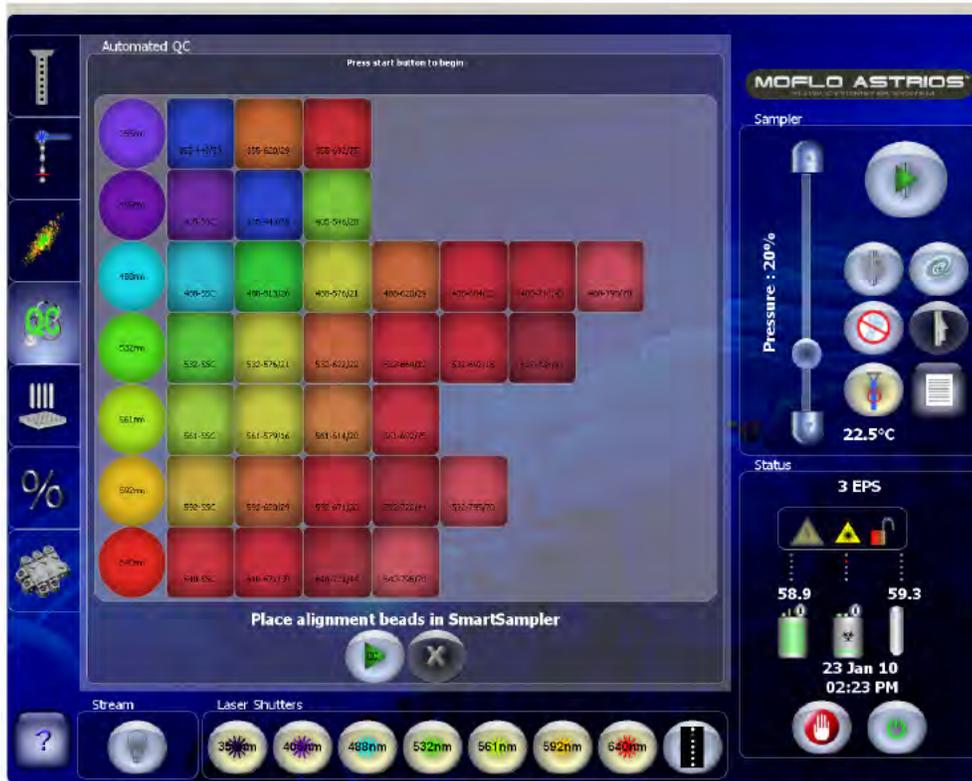
IMPORTANT Performance Validation should be repeated if you make changes to the instrument, such as changing a nozzle tip, fluidic pressure, dichroic mirrors, laser power output, etc. The automated QC process will qualify only those parameters where a laser is powered ON.

Quality Control Procedure

- 1 Perform the instrument Startup procedure. See [CHAPTER 5, Startup and Shutdown Procedures](#).
- 2 Perform Instrument Alignment including Laser Spot Determination. See [CHAPTER 6, Every Day Instrument Alignment](#).
- 3 Ensure that sample is not running and that you are not acquiring data in Summit before you begin. If you have performed instrument alignment, the Alignment protocol will be open in Summit and you will already have a tube of [Spherotech, Inc. SPHERO Ultra Rainbow Fluorescent Particles](#) diluted 1:10 with deionized water.

4 Access the QC screen on the Touch Screen.

Figure 7.1 QC Screen Before Procedure is Run



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



Auto QC performs the following actions:

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

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

NOTE Summit values generally run on full-height.

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

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

Figure 7.2 QC Procedure Complete



488-513/26
CV : 1.68657
V : 490
Median : 128.0

QC Report Per Detector

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

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

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

QC Report

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

Figure 7.3 QC Report

QC Values	Parameter	Measured Median	Target Median	Maximum Median Error	Adjusted PMT Yield	Maximum PMT Yield	Measured CY	Maximum CY	Trigger Threshold	Trigger Gain
1	QC Values									
2	Instrument Serial Number	Unknown								
3	Date	Mon 25 Jan 2010								
4	Time	18:33:52								
5	Specified QC Particles	Scheuchl 2.0um Spherulign Ultra-Fantox								
6	Lot Number									
7										
8	Laser Delay									
9	Laser Wavelength (nm)	635nm	Laser Delay (ns)							
10	355nm	7	5000							
11	420nm	5	10360							
12	480nm	1	32760							
13	520nm	4	18200							
14	560nm	5	14740							
15	580nm	3	23560							
16	640nm	2	28020							
17										
18										
19										
20	325-620-28	Pass	128	128	2.58	698	900	2.67909	5	
21	325-620-75	Pass	129	128	2.58	585	900	3.07350	5	
22	425-520	Unknown	182	0	0	0	0	1.82000	0	
23	425-448-89	Pass	128	128	2.58	670	780	2.22076	5	
24	425-546-20	Pass	129	128	2.58	711	900	3.83820	5	
25	480-FSC	Unknown	212	0	0	0	0	7.88408	0	10
26	480-SSC	Unknown	87	0	0	0	0	2.08877	0	10
27	480-SIZE	Fail	120	128	2.58	645	642	2.75400	5	
28	488-576-21	Pass	128	128	2.58	687	687	3.88992	5	
29	490-620-23	Pass	128	128	2.58	523	734	4.48308	5	
30	490-664-25	Pass	127	128	2.58	722	730	3.97938	5	
31	490-756-70	Pass	126	128	2.58	805	754	3.23386	5	
32	520-526-21	Pass	128	128	2.58	690	600	3.66505	5	
33	520-622-22	Fail	128	128	2.58	499	680	9.25932	5	
34	520-664-22	Pass	128	128	2.58	605	640	3.19616	5	
35	520-620-19	Pass	128	128	2.58	662	900	2.60721	5	
36	520-738-17	Pass	128	128	2.58	671	650	2.2642	5	
37	520-520	Unknown	91	0	0	0	0	7.88909	0	
38	520-570-6	Pass	128	128	2.58	662	780	3.83308	5	
39	520-640-23	Pass	128	128	2.58	634	750	2.62487	5	
40	520-647-5	Pass	129	128	2.58	582	830	2.21044	5	
41	520-620-29	Fail	128	128	2.58	520	900	6.8920	5	
42	520-675-9	Pass	128	128	2.58	598	900	2.70022	5	
43	520-720-14	Pass	128	128	2.58	687	900	3.09078	5	
44	520-780-70	Pass	128	128	2.58	673	900	3.62824	5	
45	640-520	Unknown	62	0	0	0	0	3.29000	0	
46	640-675-9	Fail	128	128	2.58	705	800	8.31312	5	
47	640-720-14	Pass	128	128	2.58	673	875	3.05522	5	
48	640-730-10	Pass	128	128	2.58	680	800	3.87038	5	

Quality Control

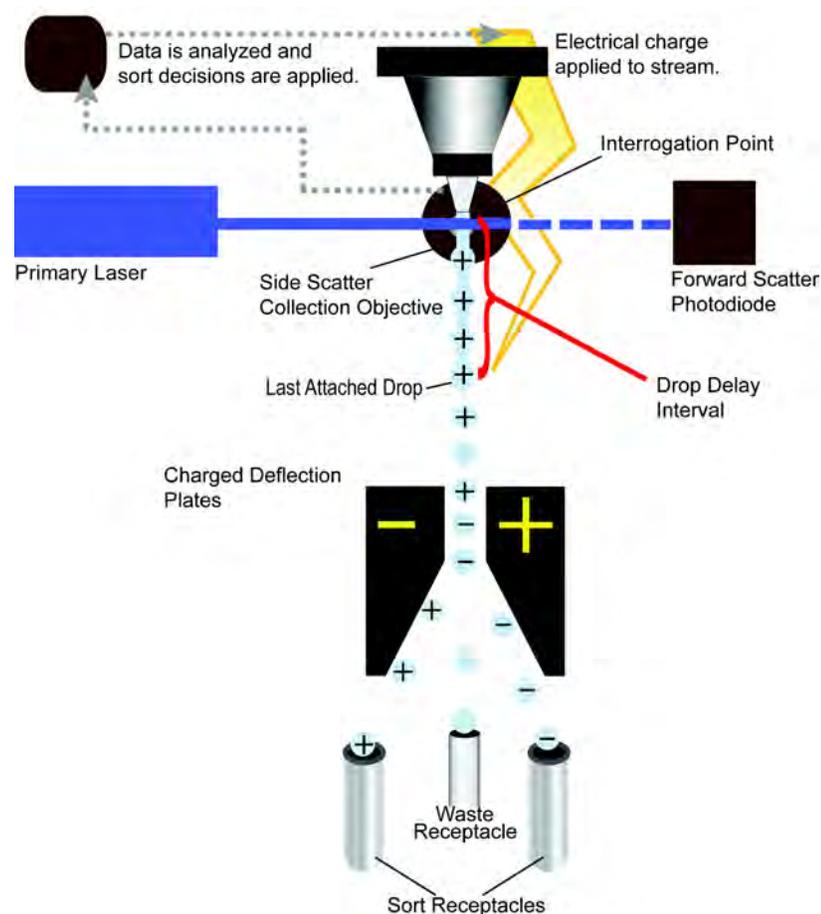
Quality Control and Performance Validation

Sorting and IntelliSort

Sort Overview

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

Figure 8.1 Sort Overview Diagram



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

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

To ensure accurate deflection of charged droplets schedule the cleaning of Deflection Plates, see [CHAPTER 9, Deflection Plates Block Assembly and Charge Deflection Plate Cleaning Procedure](#).

During a Sort

During a sort the following events occur:

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

Sort Setup Using IntelliSort with Automatic Drop Delay Determination

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

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

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

1. Determine laser spot location and nozzle height on the Find Laser Spot Tab.
2. Perform the Intellisort Initialization step (frequency and amplitude determination).
3. Perform the QC procedure.
4. Define a Sort Output Type (tubes, slide, or plate).
5. Set up Deflection.
6. Verify CyClone positions.

7. Verify the Charge Phase.
8. Perform automatic Drop Delay Determination, and enable IntelliSort Monitoring.
9. Acquire sorting data in Summit Software and set regions and gates.
10. Set Sort Decisions in Summit Software.
11. Configure Slides or Plates in Summit software (if necessary).

Select and Edit Sort Output Type

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

Figure 8.2 Select a Sort Output Type



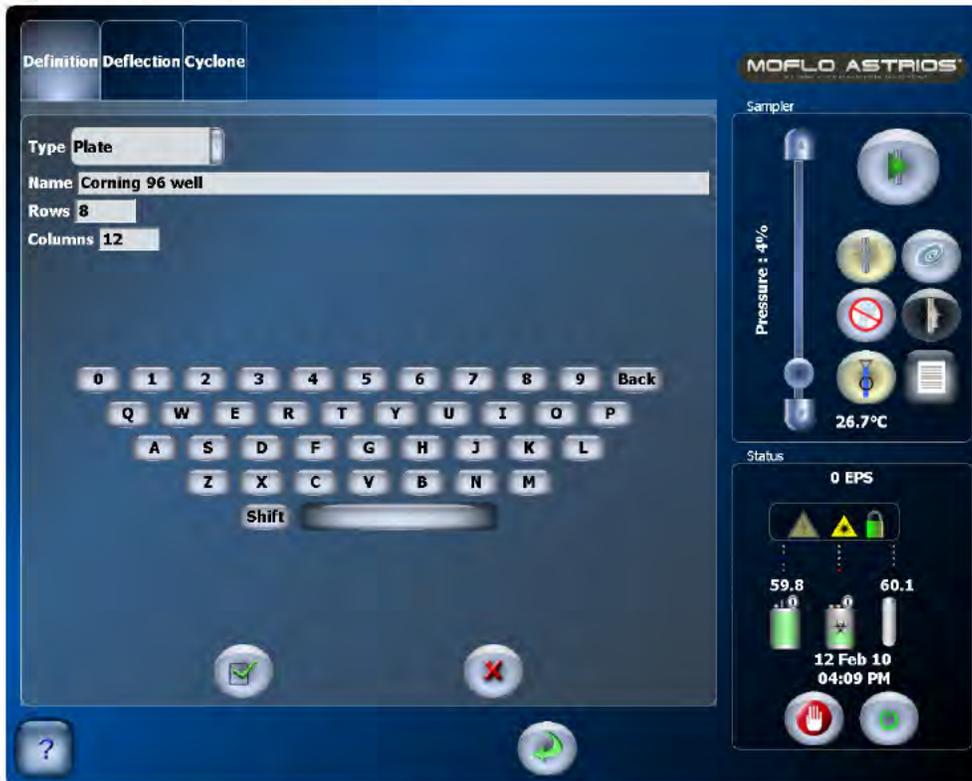
Press the Copy button to create an editable copy of a the standard Sort Output Type you selected. A screen with three tabs appears. These tables will allow you to create a custom Sort Output Type that will contain your specialized settings for naming, deflection, and CyClone positions.



Define the Specifics of the Sort Output Type

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

Figure 8.3 Define the Sort Output Type



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

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

When setting up a microplate, consider the following diagram:

