Definition, Deflection, and CyClone Tabs

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

Definition Tab

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

Figure 3.10 Definition Tab

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

Table 3.8 Definition Screen - Elements and Functions

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

Deflection Tab

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

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

Figure 3.11 Deflection Tab

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

Table 3.9 Deflection Tab - Screen Elements and Functions

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

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

Screen Element	Function
Sort Rescue Retraction 	Press to move Sort Rescue out of the way from the streams. Sort Rescue remains retracted as long as the button is depressed.
Set 	Saves the changes made to stream positioning.
Return 	Returns to Sort screen.

CyClone Configuration Tab

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

Figure 3.12 CyClone Tab

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

Table 3.10 CyClone Configuration Screen - Elements and Functions

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

Table 3.10 CyClone Configuration Screen - Elements and Functions *(Continued)*

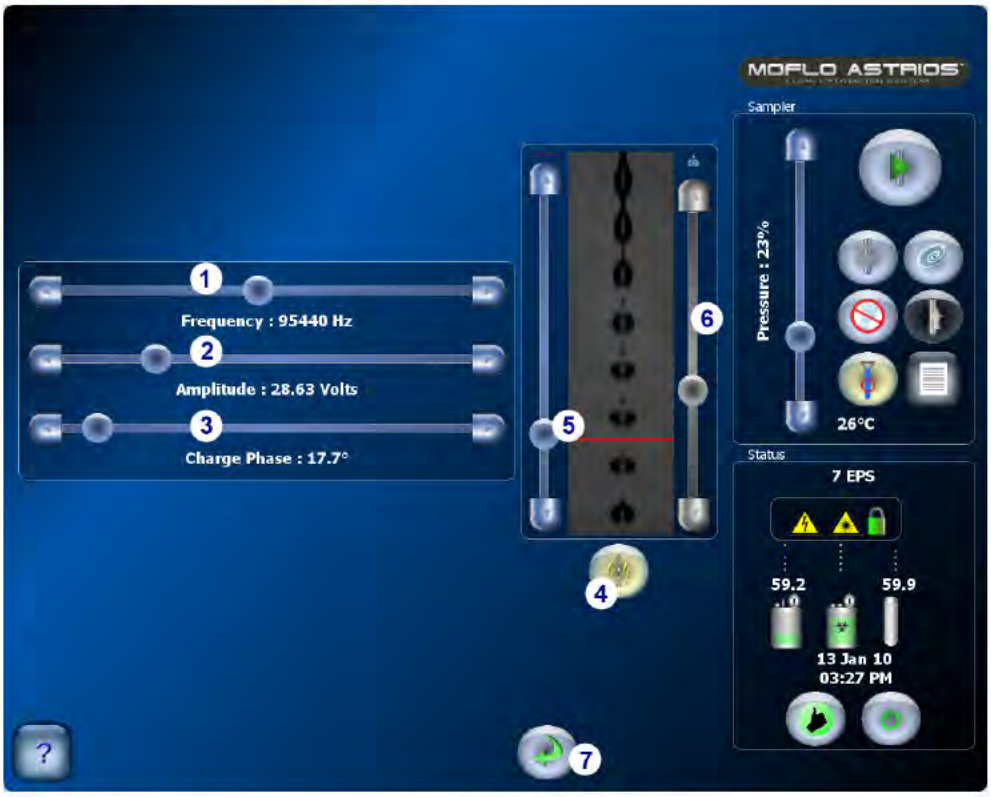
Screen Element	Function
X and Y	Displays the numerical X and Y coordinates of the current position of the CyClone.
Return 	Returns to the Sort screen.

Manual Droplet Setup Screen

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

NOTE The IntelliSort Initialize button automatically determines the optimum frequency for the sheath pressure and nozzle tip combination and sets a default amplitude value that may be adjusted. Users may also adjust the Last Attached Drop Marker and Charge Phase, if necessary.

Figure 3.13 Manual Droplet Setup Screen



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

Table 3.11 Manual Droplet Setup Screen - Elements and Functions

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

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

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

Sort Statistics Screen

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

Figure 3.14 Sort Statistics Screen

1. Sort Statistics tab	2. Expand Statistics Display

Table 3.12 Sort Statistics - Screen Elements and Functions

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

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

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

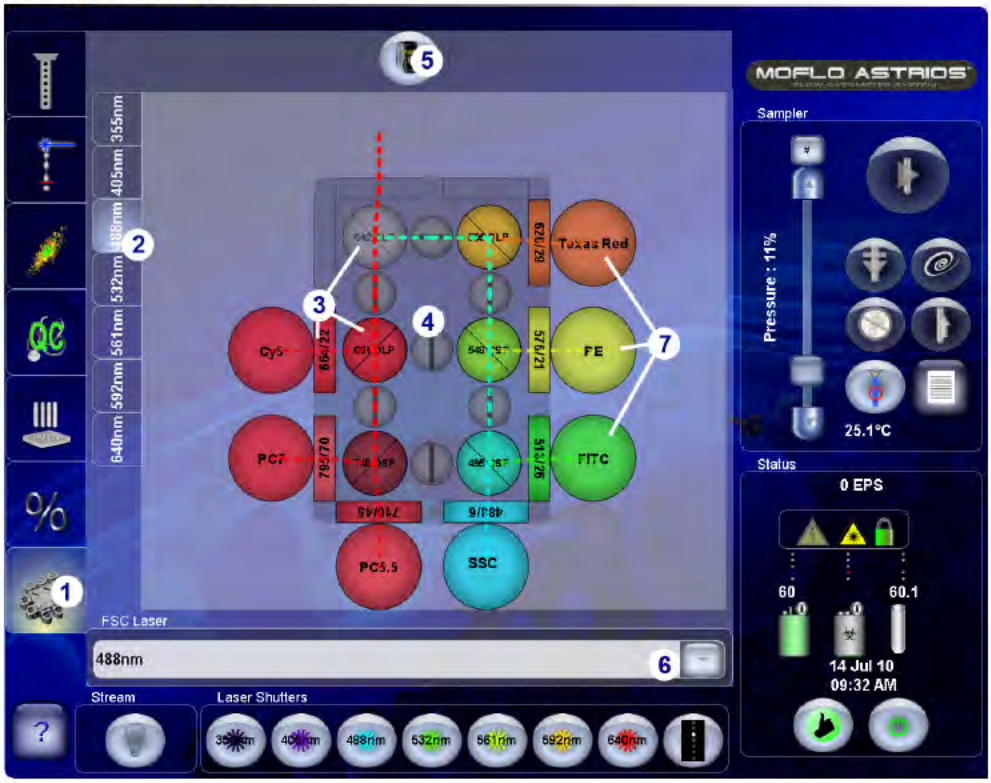
PMT and Filter Update Screen

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

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

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

Figure 3.15 PMT Update Screen



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

Table 3.13 PMT and Filter Alignment Screen - Elements and Functions

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

SmartSampler Controls

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

Figure 3.16 SmartSampler Abbreviated Menu

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

Figure 3.17 SmartSampler Full Menu

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

Table 3.14 SmartSampler - Screen Elements and Functions

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

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

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

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

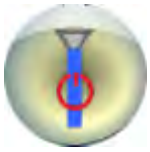
Screen Element	Function
Sample Boost 	Press this button to temporarily boost the sample pressure. The pressure will be boosted as long as the button is pressed. **NOTE** To change the sample pressure differential press and hold the Sample Boost button while adjusting the physical knob labeled Boost Figure 2.21.
Rinse Probe 	Press this button to rinse the sample probe with sheath.
Change Probe 	Press this button to close the chamber and prepare to change the sample probe. The chamber will not be pressurized. For instructions see page 10-49.
Drain 	Press and hold this button to drain fluid from the sample chamber to waste.
Start/Stop Sheath Flow 	Press this button to: • Start sheath stream • Backflush **NOTE** If the fluidics system is not pressurized when you press this button, the system will turn ON pressure and vacuum to the tanks and then turn on the sheath stream. To stop sheath stream, push this button again. **NOTE** If the Start Sheath Flow function is deactivated and you are unable to shut off the fluidics, press the Change Tanks button.

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

Screen Element	Function
Change Tanks 	This button is used when tanks need to be changed during the work day when the system has already been powered up and running. (Place a tube of deionized water in the sample station prior to pressing this button.) This button leaves lasers powered ON. Press this button to: • Close the sample chamber. • Run water through the sample line. • Reopen the chamber. • Power OFF pressure and vacuum. (change tanks) and press again to power ON.
Return 	Press this button to return to the previous screen.

CHAPTER 4

Summit Software

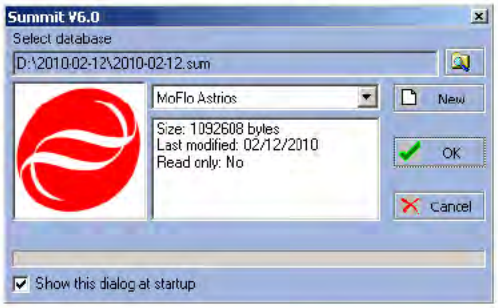
Summit Software Overview

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

How to Open Summit Software

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

Figure 4.1 Select Database Dialog Box



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

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

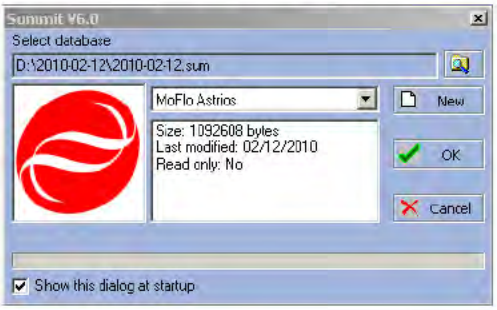
Summit Software Database

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

How to Create a New Database

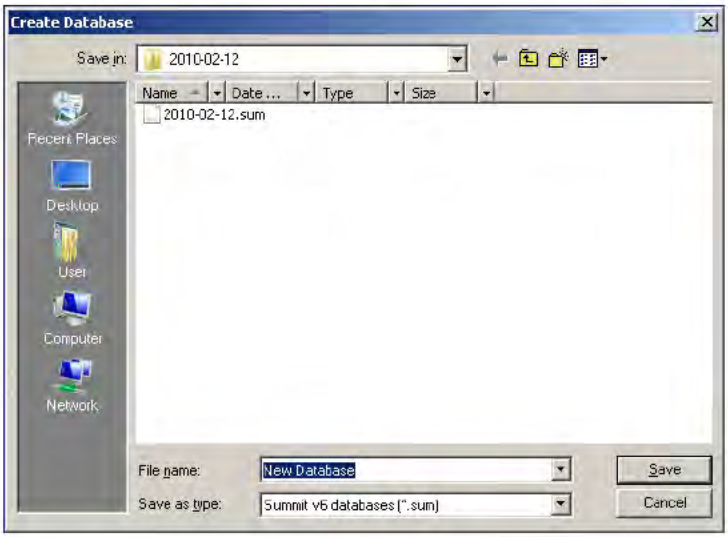
1 Open Summit Software and click **New.**

Figure 4.2 Select Database Dialog Box



The Create Database dialog box appears.

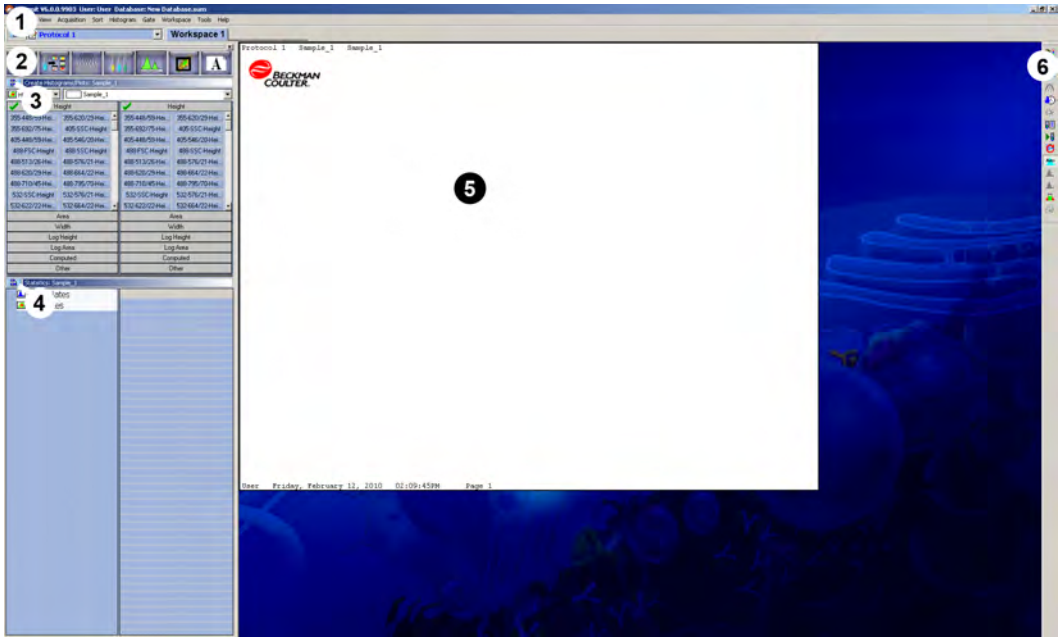
Figure 4.3 Create Database Dialog Box



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

Summit Software Screen Overview

Figure 4.4 Summit Software Screen Overview



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

Summit Software Control Panel

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

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

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

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

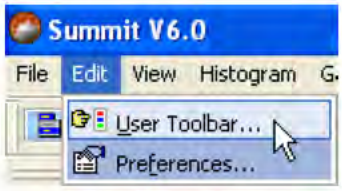
User Toolbar Buttons

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

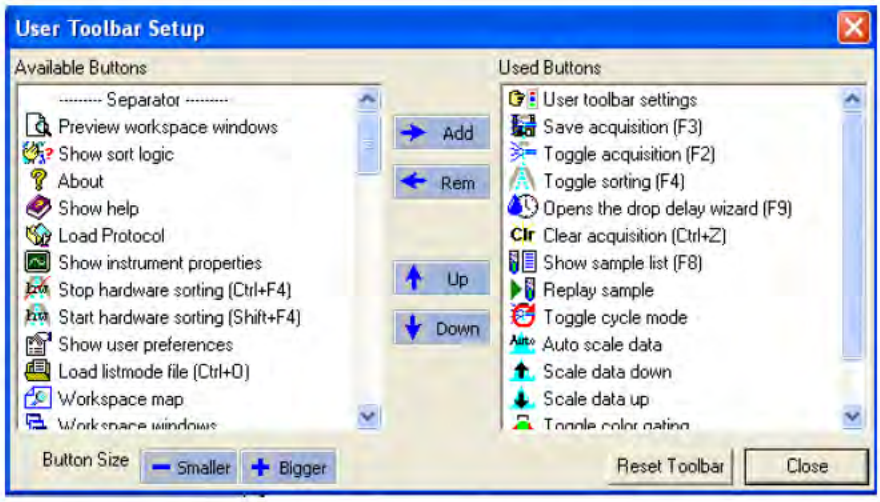
Figure 4.6 User Toolbar Icons

Customize the User Toolbar

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



2 The User Toolbar Setup dialog box appears.



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

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

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

Instrument Tab

Instrument Tab

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

Figure 4.7 Instrument Tab

Table 4.1 Instrument Tab - Screen Elements and Functions

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

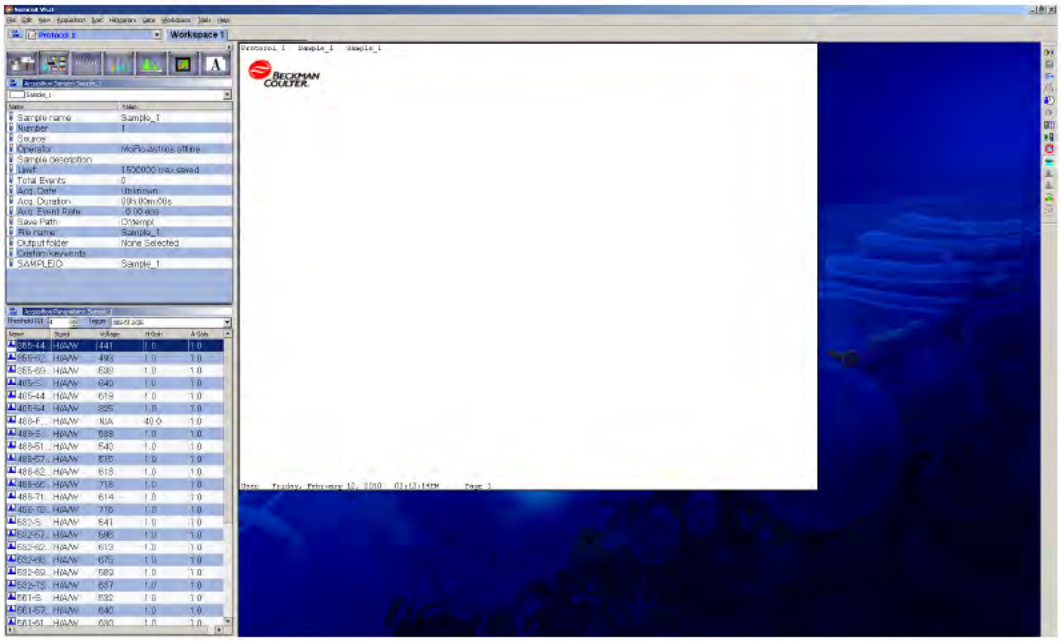
Table 4.1 Instrument Tab - Screen Elements and Functions *(Continued)*

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

Acquisition Tab

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

Figure 4.8 Acquisition Tab



Acquisition Sample Panel

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

Figure 4.9 Acquisition Sample Panel

How to Edit Information Specific to a Sample Run

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

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

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

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

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

Enable Parameters

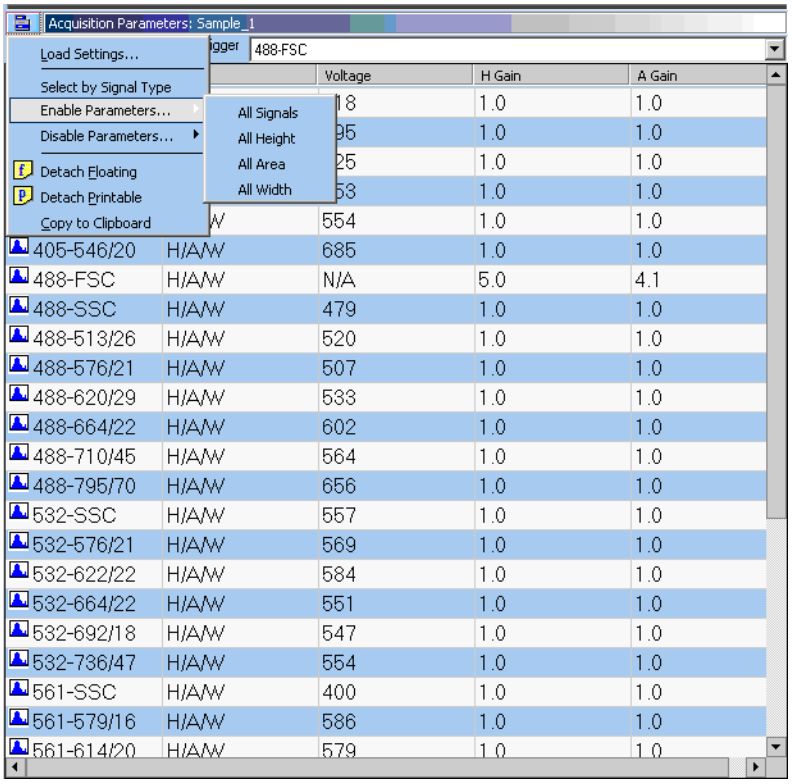
Before you can set up histograms or dot plots you must enable the parameters that you intend to use for your experiment. When a parameter is enabled, the instrument collects linear height, area, and width information. All other parameters, such as log values, are computed using the linear data. Unlike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

How to Enable Specific Parameters for All Data

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

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

Figure 4.10 Enabling Specific Parameters for All Data



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

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

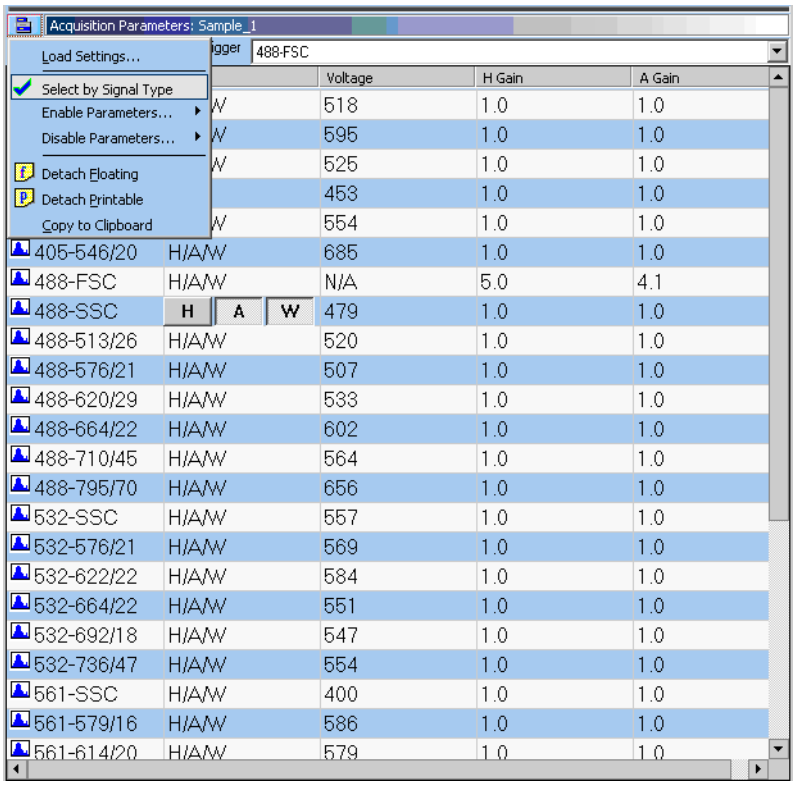
3 To enable parameters, select the appropriate option(s).

How to Enable Individual Parameters

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

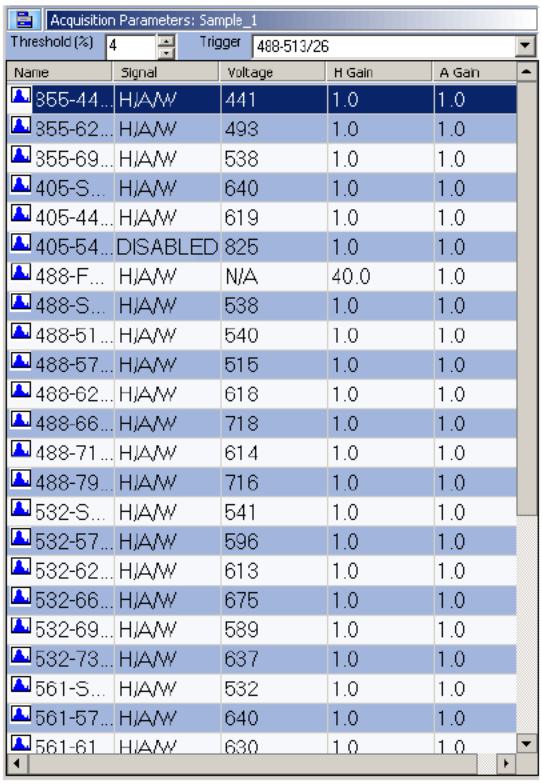
2 Click the Menu icon **> Select by Signal Type** (see Figure 4.11). Double click on the signal column for the parameter to be enabled.

Figure 4.11 Enabling Individual Parameters



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

Figure 4.12 Acquisition Parameters Panel



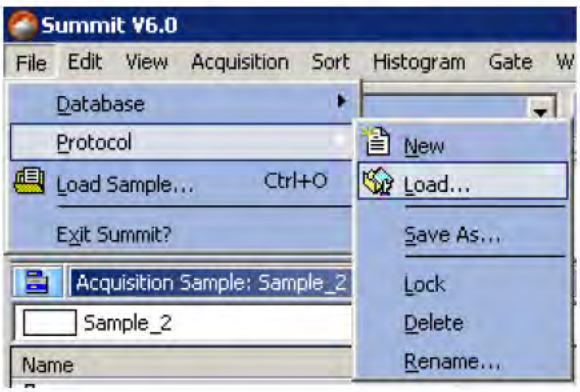
- H/A/W = Enabled Height/Area/Width
- DISABLED = All data types disabled for the parameter.

Loading an Existing Protocol

How to Load an Existing Protocol

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

Figure 4.13 Loading an Existing Protocol 1



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

Figure 4.14 Loading an Existing Protocol 2

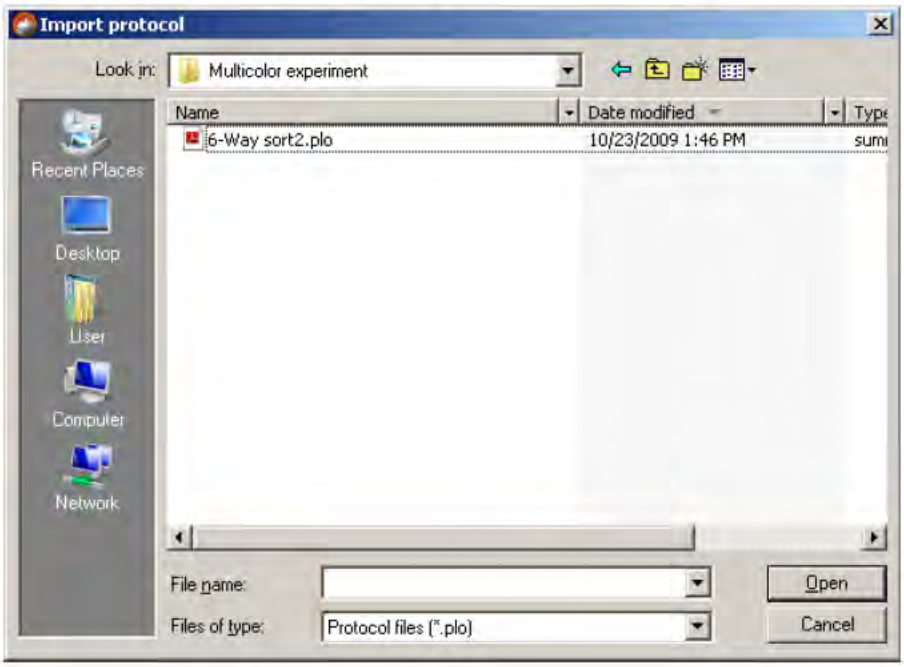
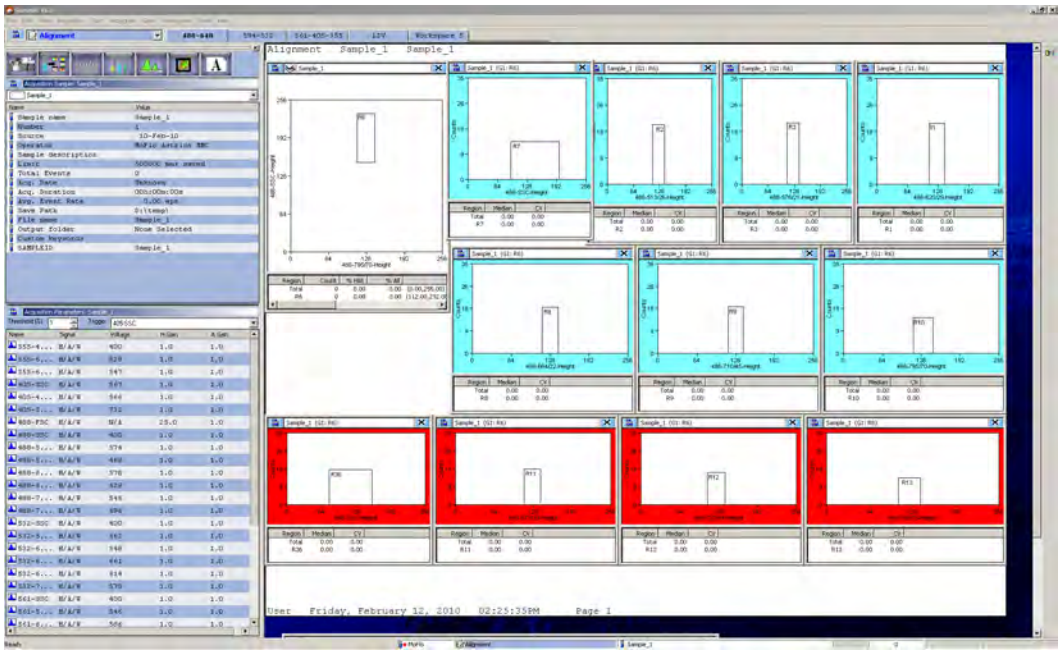


Figure 4.15 is an example of a typical alignment protocol that contains empty histograms in which to acquire data or display and analyze previously acquired data.

Figure 4.15 Loading an Existing Protocol 3



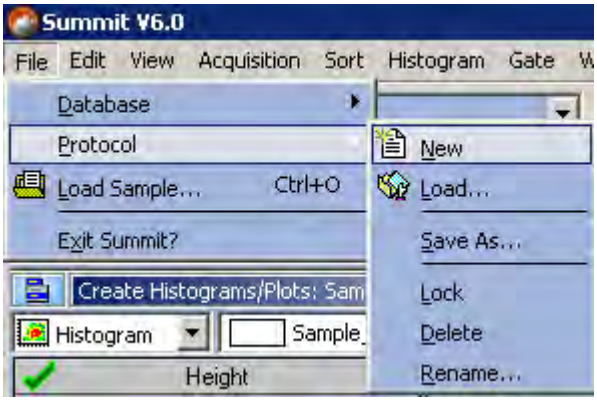
Creating Protocols

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

How to Create a New Protocol

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

Figure 4.16 Create a New Protocol



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

3 Create dot plots and histograms. See page 4-37.

Switching Protocols

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

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

Figure 4.17 Switching Protocols

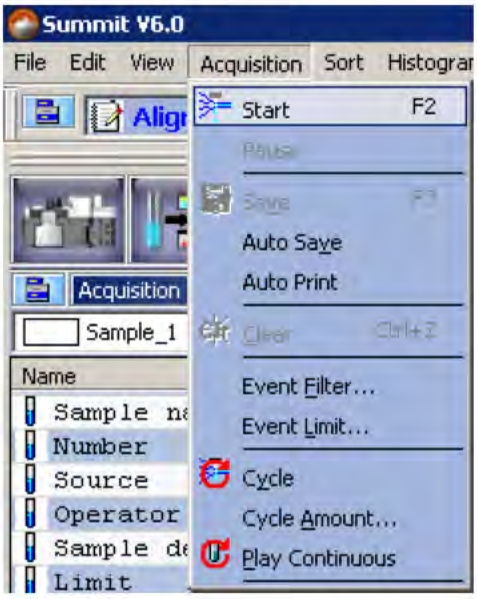
Acquiring Data in Summit Software

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

How to Start or Stop Data Acquisition

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

Figure 4.18 Acquiring Data



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

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

Saving Acquired Data

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

How to Save Acquired Data

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

Figure 4.19 Saving Acquired Data

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

3 Click **Save.**

Cycle Mode

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

How to Display the Most Recent Data During Alignment Activities

1 Ensure that you are not currently acquiring data.

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

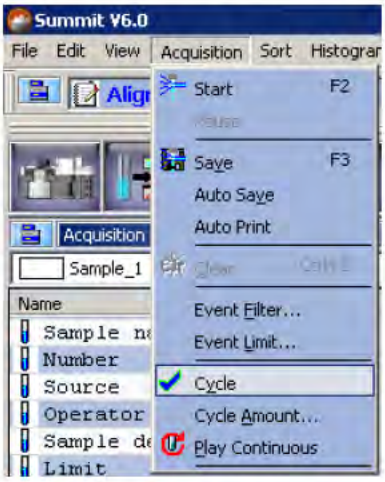
3 Select **Cycle Amount**. Set the number of events that should be reached before the data cycles.

Figure 4.20 Setting the Cycle Amount for Cycle Mode

4 Click **OK**.

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

Figure 4.21 Enabling Cycle Mode

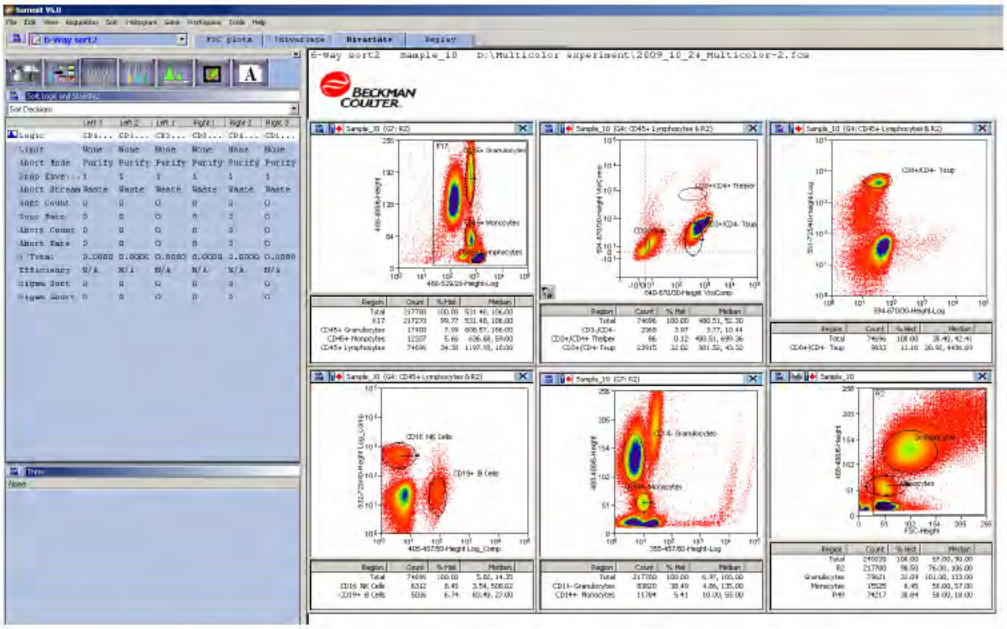


6 After you set the Cycle Mode start acquisition for Cycle Mode to become effective.

Sort Tab

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

Figure 4.22 Sort Tab



Clearing Sort Statistics

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

On the Sort Statistics screen,

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

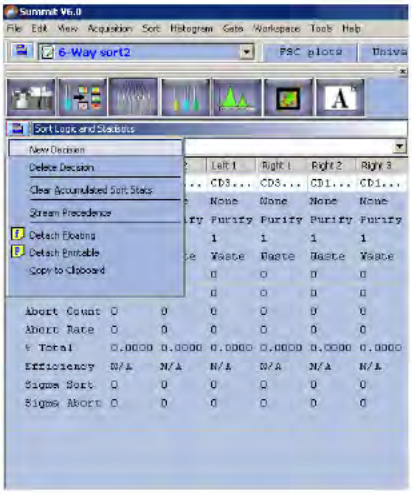
Set Sort Decisions

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

How to Create or Edit Sort Decisions

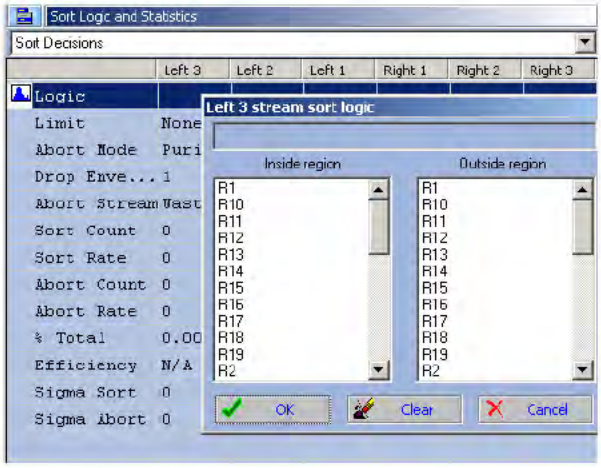
1 Create and edit sort decisions in the Sort Logic and Statistics Panel. Launch the sort logic editor by clicking the Menu icon and selecting **New Decision.**

Figure 4.23 Set Sort Decisions



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

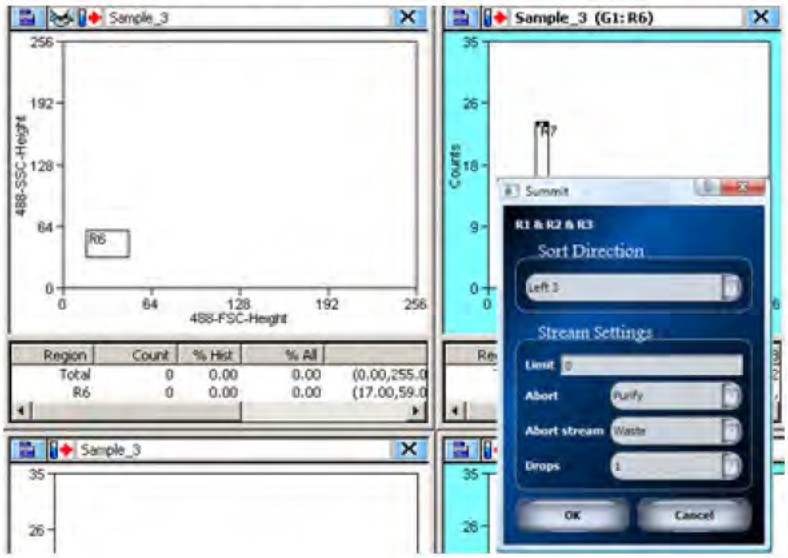
Figure 4.24 Select a Stream



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

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

Figure 4.25 Right-click a Region Set Sort Decisions



View Sorts and Aborts Per Stream in Histograms

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

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

Figure 4.26 Create a Sorts and Aborts Histogram

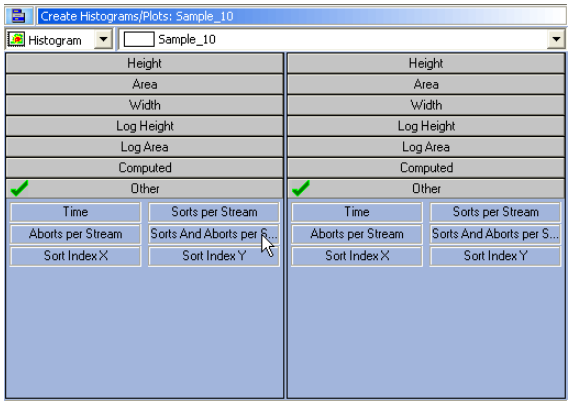


Figure 4.27 View Sorts and Aborts Per Stream

Index Sorting

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

Figure 4.28 Create an Index Histogram

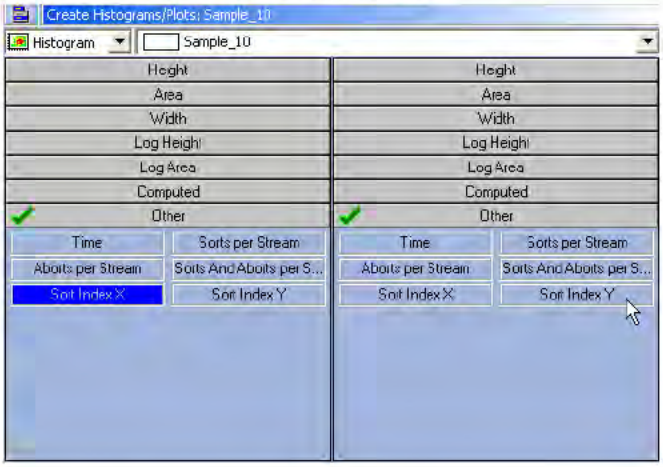
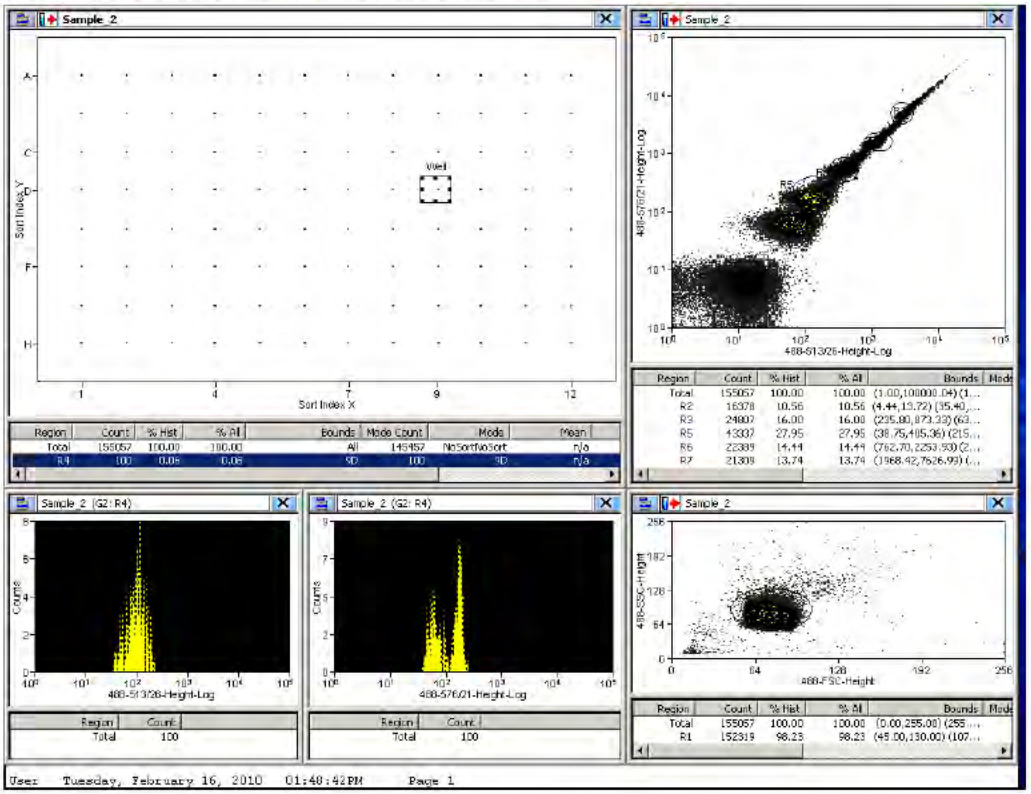


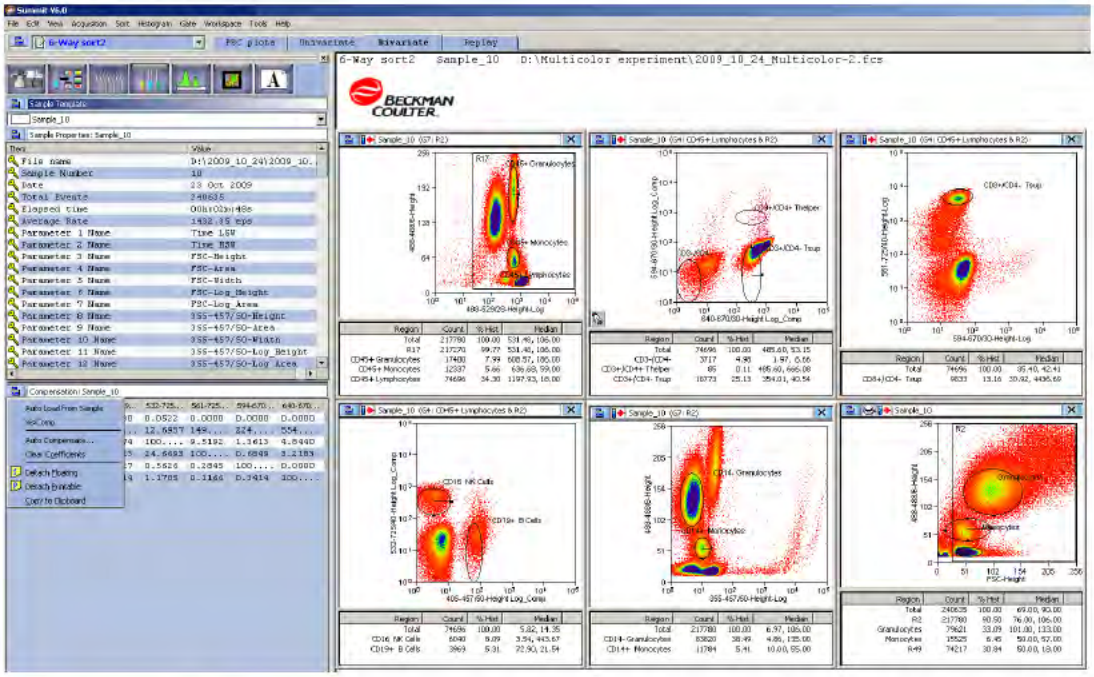
Figure 4.29 Indexed Sorting Display With Color Gating



Sample Tab

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

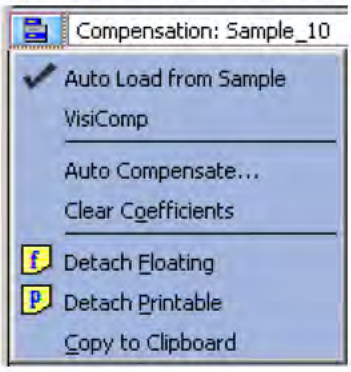
Figure 4.30 Sample Tab



Auto Load from Sample

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

Figure 4.31 Auto Load from Sample



Auto Compensation Wizard

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

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

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

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

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

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

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

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

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

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

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

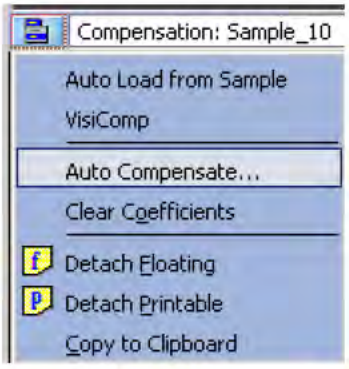
3 Load all control sample files into an experiment folder.

4 Click the Sample tab.

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

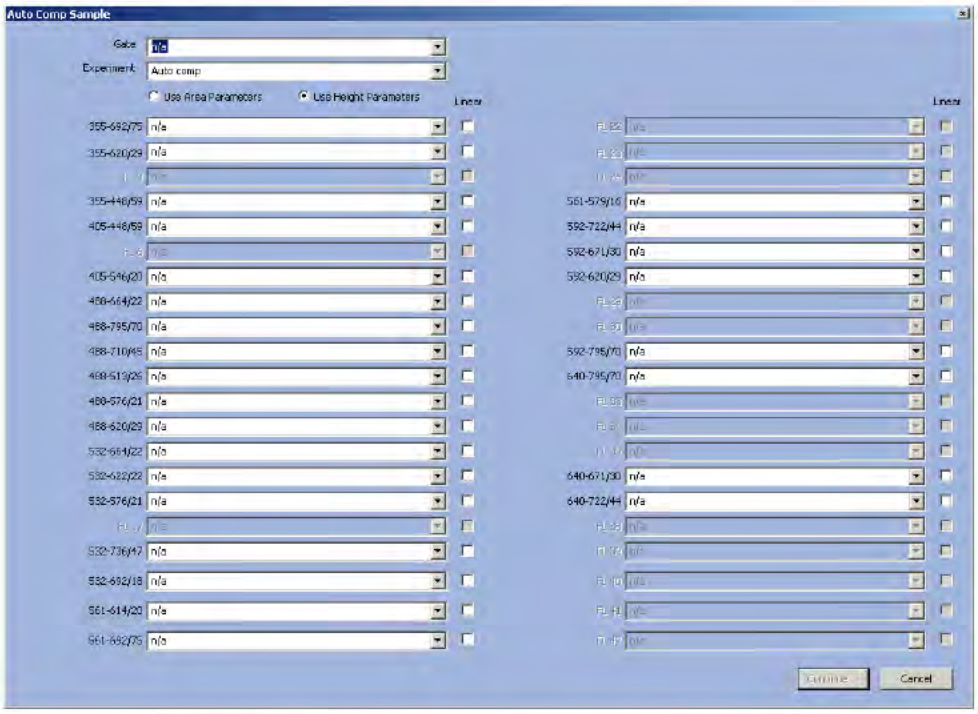
6 In the Sample Compensation panel, click the extended menu icon and select **Auto Compensate** from the list.

Figure 4.32 Select Auto Compensate



7 The Auto Comp Sample dialog box appears.

Figure 4.33 Auto Comp Sample Dialog Blank

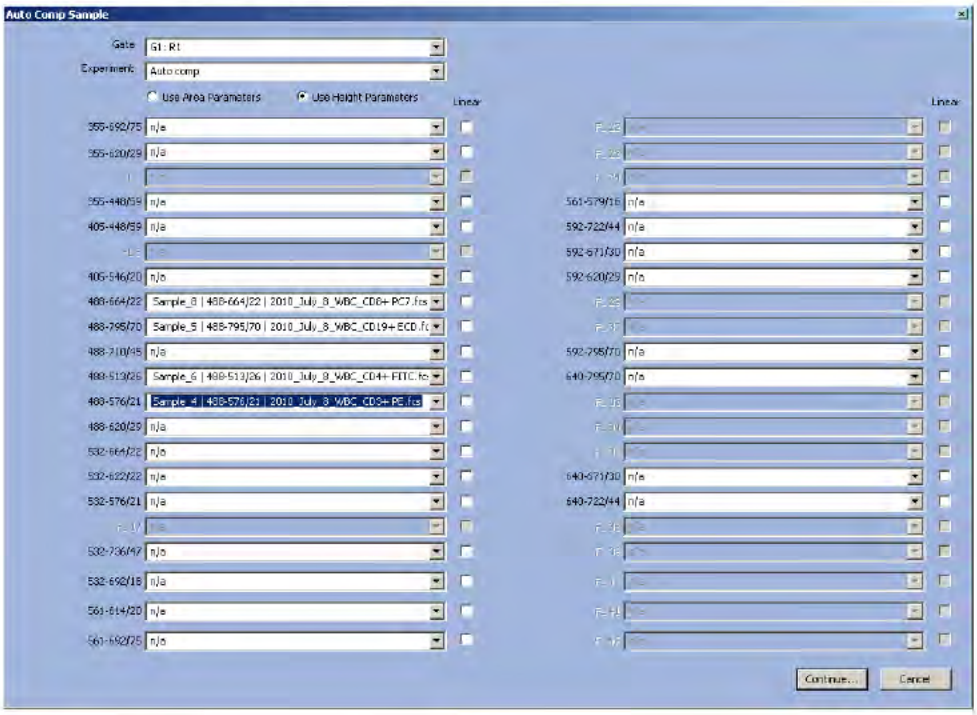


8 Select a gate from the Gate pull-down menu, if applicable.

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

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

Figure 4.34 Auto Comp Sample Dialog Box Populated



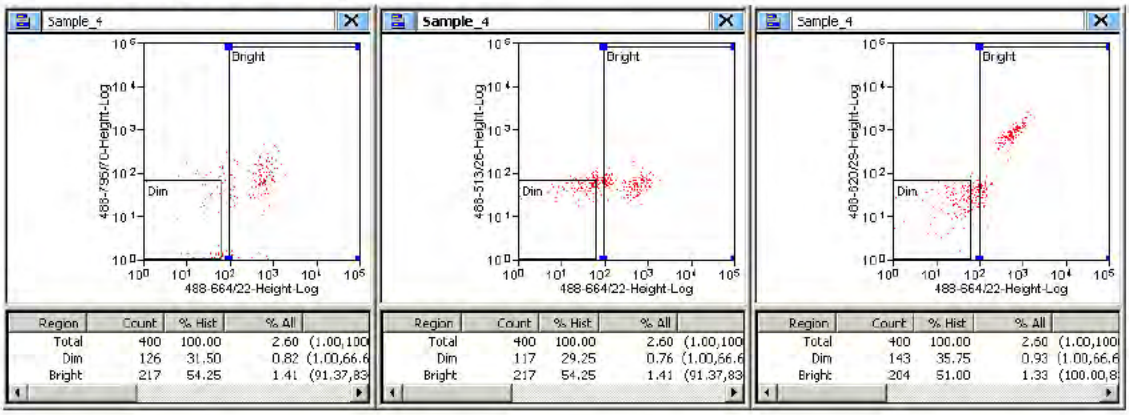
11 Click **Continue.**

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

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

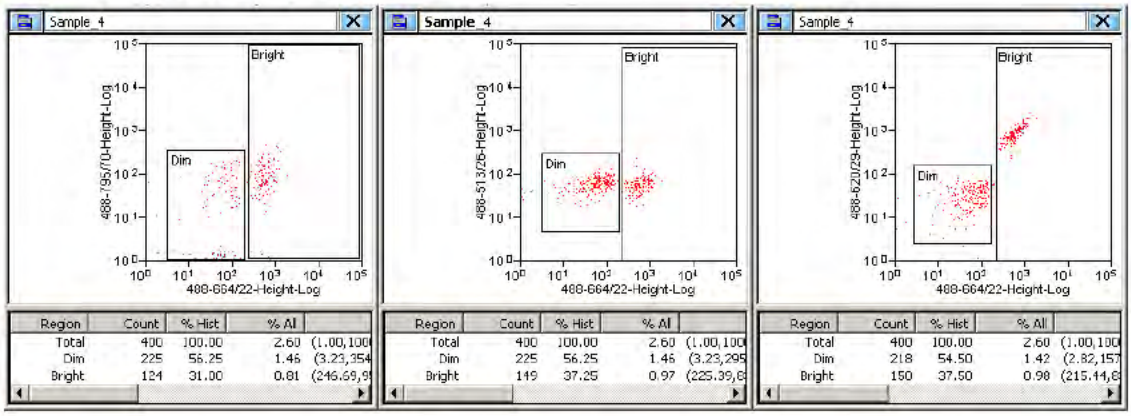
Figure 4.35 Auto Compensate Wizard

Figure 4.36 Single Control Sample Dot Plots



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

Figure 4.37 Auto Comp Adjusted Regions



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

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

Figure 4.38 Compensation Matrix

Compensation: Sample_5

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

Applying VisiComp

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

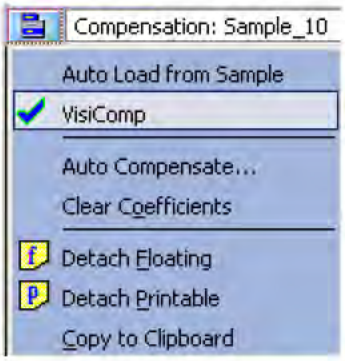
How to Use VisiComp to Visualize Compensation Results

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

2 Create all plots, regions, and gates.

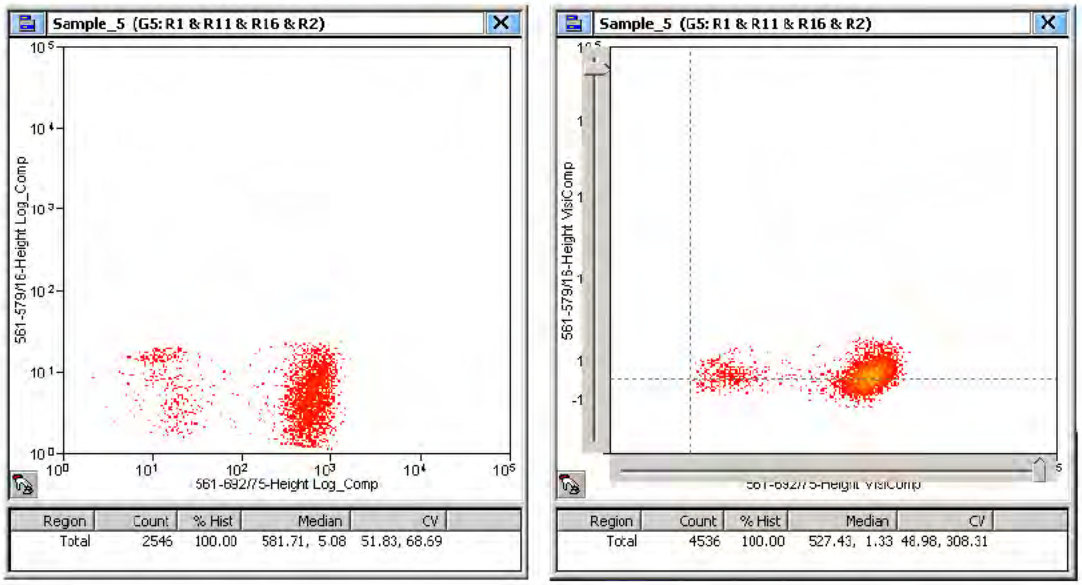
3 On the Sample tab, click the Compensation panel icon and select **VisiComp**.

Figure 4.39 Apply VisiComp



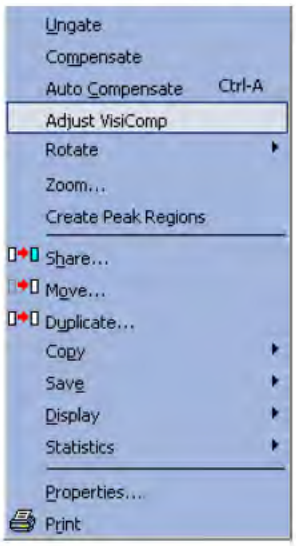
4 Use either the auto compensation wizard to set up plots, or manually set up all of the plots that you want to use for compensation analysis.

Figure 4.40 Data Displayed Before and After Applying VisiComp



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

Figure 4.41 Adjust VisiComp



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

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

6 Create regions and gates to complete your analysis.

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

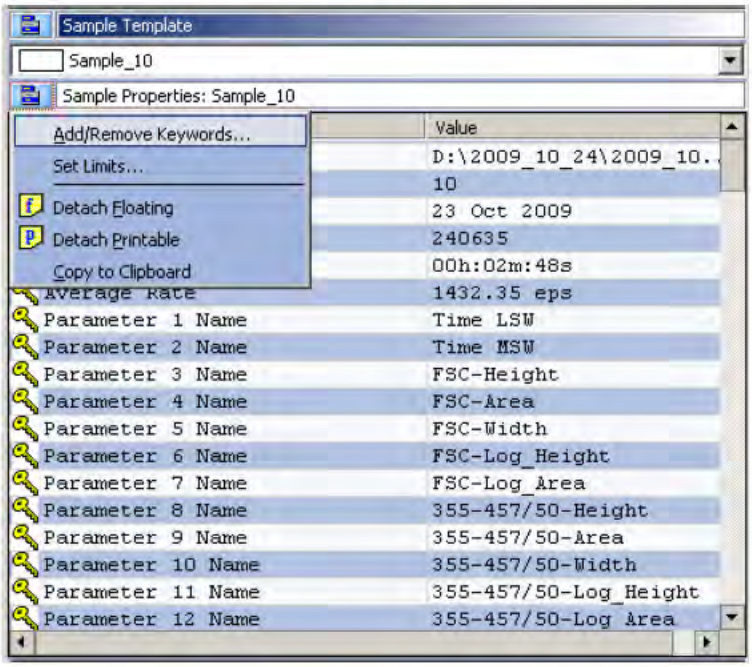
FCS Keywords

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

How to Add or Remove Keywords

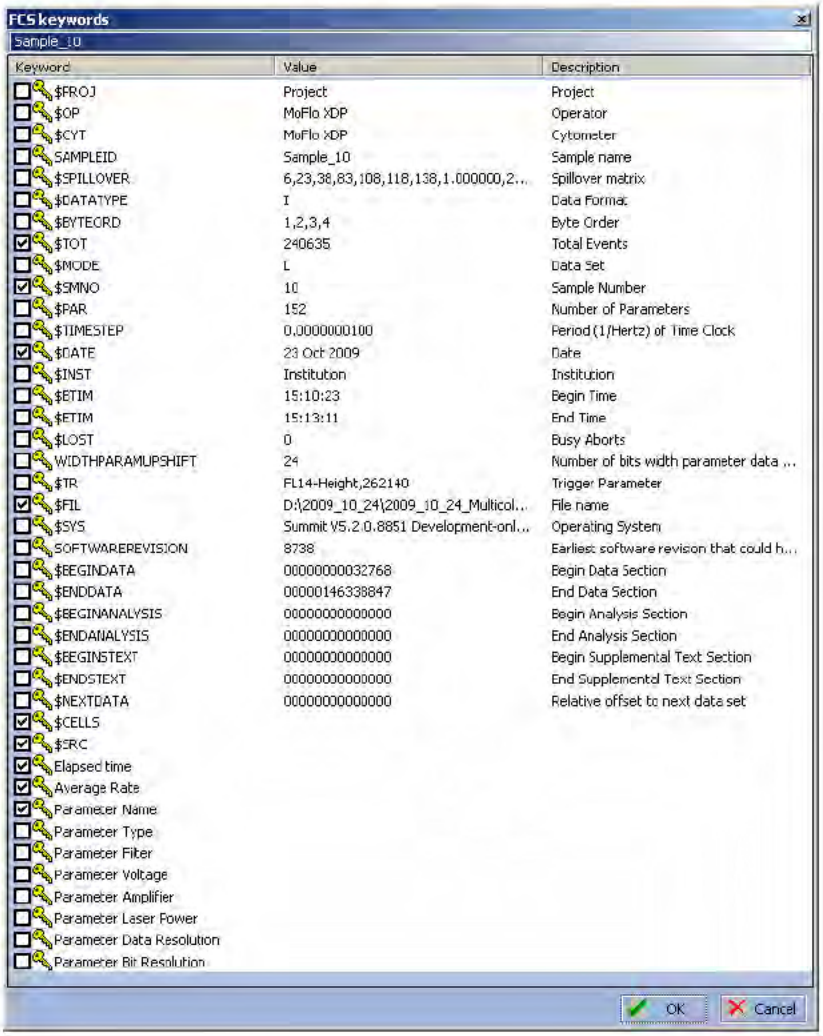
1 Click the blue menu icon and select **Add/Remove Keywords.**

Figure 4.42 Add/Remove Keywords 1



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

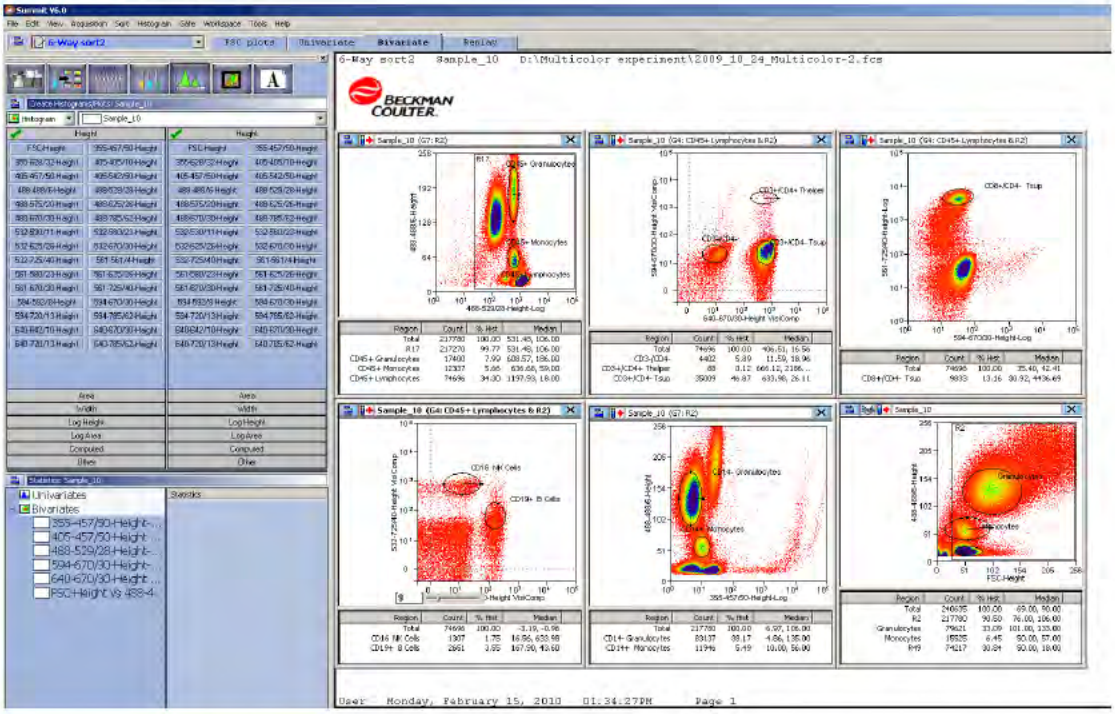
Figure 4.43 Add/Remove Keywords 2



Histogram Tab

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

Figure 4.44 Histogram Tab



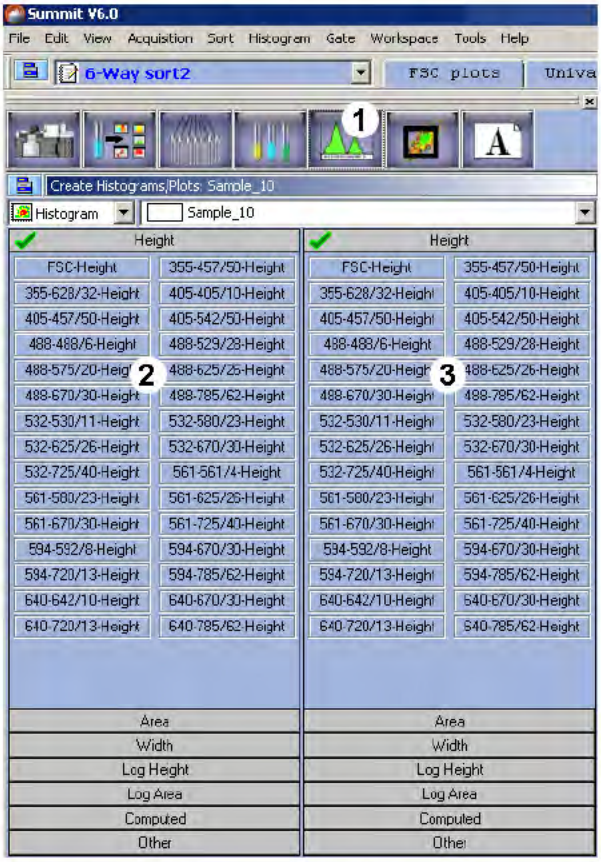
Creating Histograms and Dot Plots

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

How to Create a Histogram or Dot Plot

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

Figure 4.45 Creating Histograms and Dot Plots



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

2 Select one of the following:

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

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

Maximize Dot Plots and Histograms

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

Change Axis Parameters

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

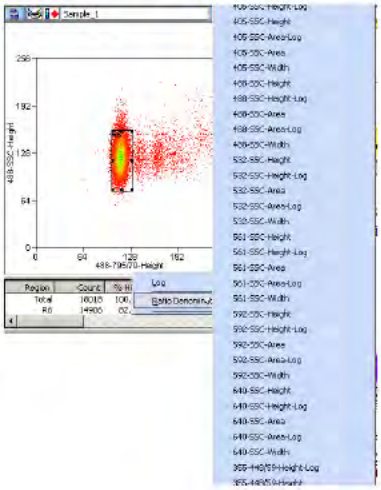
- H = linear height
- A = linear area
- W = pulse width
- L = log height
- LA = log area

Figure 4.46 Change Axis Parameters

Display Ratio

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

Figure 4.47 Display Data as a Ratio



Create Regions in Histograms

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

Renaming Regions

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

Copy and Paste Regions

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

Customizing Statistics Display

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

Manually Scaling Data

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

Contouring Data

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

Exporting Histograms to Word

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

Multi File Display

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

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

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

Create Overlays

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

How to Overlay Multiple Histograms

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