Instructions for Use



High-speed Analyzer

Summit 4.3

0000050G June 2008



Beckman Coulter, Inc. 4300 N. Harbor Blvd. Fullerton, CA 92835



Summit Software Overview

Overview

Summit software is a Windows[®] based application that has a series of menus, hot keys, and buttons which allow you to acquire, sort, and analyze flow cytometry data then save the data in FCS format. With Summit software you can monitor and control the instrument, define protocols, configure compensation settings and workspaces, define batch protocol panels, reagents, and tubes, and auto-compensate data.

How to Open Summit Software

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



Summit ¥4.3		X
Select database		
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	CYAN	
ТМ	·\\	🗙 Cancel
Show this dialog a	t startup	

2 Select **CYAN** from the dropdown menu. This will allow you to interact with the instrument in real time. It is also possible to work with Summit software offline to analyze previously saved FCS data files.

Now you will either create a new database or open a previously saved database.

Summit Software Database

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

How to Create a New Database

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

Figure 3.2 Create a New Database

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1		
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The Create Database dialog box appears.

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.

How to Open an Existing Database

1 Open Summit Software.

Figure 3.3 Browse to an Existing Database

Summit V4.3		×
Select database		<u></u>
C:\Documents and S	ettings\marnic\MyDocuments\Thor	
	OFFLINE analysis	New
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2 Browse to the folder that contains the database you would like to open, select the file and click **Open**.

Figure 3.4 Select an Existing Database

Open Datab	ase				? ×
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Summit Software Screen Overview

Figure 3.5 Summit Software Screen Overview

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Name Sample name	Malachowski 16 Mav		
Number	70	FITC Lin PE Lin PE Texas Red Lin	
Source	Spectrà	Region Median CV Region Median CV Region Median CV	
Sample descriptio	n	Total 131.00 9.43 Total 129.00 9.59 Total 131.00 9.39	
Limit	1h:00m:5s		
Acg. Date	Unknown	B B Malachowski 16 May 08 X 7. B Malachowski 16 May 08 X B B Malachowski 16 May 08 X	
Acq. Duration	00h:00m:00s	72213 60033 38208	
Avg. Event Rate	0.00 eps	54159 - g2656 - R3	
File name	Malachowski 16 May	3 30/05 - 3 30/05 - 3 19/04 - 0 9/05	
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FITC Peak	569 1.0		
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PE-Te Peak	665 1.0	R9 133.00 3.85 R2 125.00 2.11 R10 127.00 2.20	
PE-Cy5 Peak	715 1.0		
PE-Cy7 Peak	716 1.0		
Violet 1 Peak	604 1.0	Alignment Malachowski 16 May 08 SpectrA	
Violet 2 Peak	748 1.0		
APC Peak	718 1.0		
APC-Cy7 Peak	746 1.0		
ady		🕞 CyAn 🛃 Alignment 📴 Malachowski_16_May_08_SpectrA 0	0:01

- 1. Summit software Main Menu
- 2. Protocol List
- 3. Summit Control Panel (to gain access to 7. Workspace (with blank histograms) additional screens)
- 4. Additional Menu

- 5. Sample List
- 6. Additional Menu
- 8. 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.

Figure 3.6 Summit Software Control Panel (see number 3. on Figure 3.5)



Acquisition Tab

The Acquisition tab allows you to set the threshold at which an event will be detected by the instrument, set the event triggering parameter, designate parameter names, specify the data types that will be collected, and set the voltage and gain to be applied to each parameter. From Acquisition tab you can also set up specific sample run information and view sample run statistics.



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PE-Te Peak	665 1.0					
PE-Cy5 Peak	715 1.0					
PE-Cy7 Peak	716 1.0					
Niolet 1 Peak	604 1.0					
🔿 Violet 2 Peak	748 1.0					
APC Peak	718 1.0					
	746					

Acquisition Sample Panel

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



	Acquisition Sample: Malachowski_16_May_08_SpectrA 1hour No.						
	Malachowski_16_May_08_SpectrA						
Na	ame	Value					
0	Sample name	Malachowski_16_May					
	Number	70					
	Source	SpectrA					
	Operator						
	Sample description						
	Limit	1h:00m:5s					
	Total Events	0					
	Acq. Date	Unknown					
	Acq. Duration	00h:00m:00s					
	Avg. Event Rate	0.00 eps					
	Save Path	D:\Consumables\					
	File name	Malachowski_16_May					
	Output folder	Acquired samples					
	Custom keywords						
	SAMPLEID	Malachowski_16_May					

How to Edit Information Specific to a Sample Run

1 To view editable fields, click the blue additional menu icon and select **Edit View**. The fields that are editable will activate.

Figure 3.9 Acquisition Sample - Accessing Edit View



Figure 3.10 Acquisition Sample - Editing Example

Acquisition	Parame	ters: Mala	achow	ski_16_№	1ay_0	8_SpectrA	
Threshold (%)).5	Trigge	r [-s			•
Name	Peak/A	Area/Log		Voltage	е	Gain	
FS	Р 河	A 🗤	Z L	N/A		22.2	•
SS 🔁	Р 河	M A 💀	ΖL	480	÷Ŀ.	1.0	-
FITC	₽	м A 💀	ΖL	569	·1	1.0	-
A PE	Р 🗹	M A 💀	ΖL	564	÷1	1.0	•
🖲 exas Red	Р 🗹	м A 💀	ΖL	665	<u>1</u>	1.0	•
PE-Cy5	Р 🗹	M A 💀	Z L	715	÷1	1.0	•
PE-Cy7	Р 🗹	.▼ A 15	ΖL	716	<u>1</u>	1.0	•
AViolet 1	Р 🦻	A 💀	Z L	604	÷1	1.0	•
AViolet 2	Р 河	м A 💀	ΖL	748	- <u>)</u>	1.0	•
APC	Р 🤄	🗷 A 💀	Z L	718	÷1	1.0	•
APC-Cy7	P		Z L	746	-)	1.0	-

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

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

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

- **3** To add a new Name and Value to the panel select **Add Keyword**. The **Edit Keyword** dialog box appears. For more information on keywords see page 3-19.
- **4** Enter the new information, and click **OK**.

How to Change Acquisition Settings

IMPORTANT Before data can be acquired, data type parameters must be enabled. See Figure 3.10

How to Change Acquisition Settings

Figure 3.11 Set Event Threshold Percentage

1 To set the Threshold percentage at which an event will be detected by the instrument, click the up or down arrows in the **Threshold** % field Figure 3.11. To change the event Trigger parameter, choose an item from the **Trigger** dropdown list Figure 3.12.

Sample Parameters				E
Sample Parameters				
Threshold (%)	Trigger FS			-
Name	Peak/Area/Log	Voltage	Gain	
🐲 FS	Peak/Area/Log	N/A	8.0	
# 55	Peak/Area/Log	400	1.0	
488 FITC	Peak/Area/Log	400	1.0	
488 PE	Peak/Area/Log	400	1.0	
488 PE-Texas Red	Peak/Area/Log	400	1.0	
488 PE-Cy5	Peak/Area/Log	400	1.0	
<mark>488</mark> PE-Cy7	Peak/Area/Log	400	1.0	
🛄 Violet 1	Peak/Area/Log	400	1.0	
🛄 Violet 2	Peak/Area/Log	400	1.0	
APC	Peak/Area/Log	400	1.0	
MR APC-Cy7	Peak/Area/Log	400	1.0	

Figure 3.12 Set Event Trigger Parameter

🚊 Sample Paramete	rs			
Threshold (%) 0.14	Trigger F	6		
Name	Peak/Area			
🐲 FS	Peak/AF	TC .		
49 SS	Peak/Ap	- E-Texas Red		
488 FITC	Peak/A	-Cy5	N	
488 PE	Peak/A	olet 1	43	
488 PE-Texas Red	Peak/A	PC		
488 PE-Cy5	Peak/Are	a/Log 400	1.0	
488 PE-Cy7	Peak/Are	a/Log 400	1.0	
Wiolet 1	Peak/Are	a/Log 400	1.0	
WViolet 2	Peak/Are	a/Log 400	1.0	
APC	Peak/Are	a/Log 400	1.0	
MAPC-Cy7	Peak/Are	a/Log 400	1.0	

2 To change the name of a parameter, double-click the name of the parameter in the Name column, type a new name Figure 3.13, and then press ENTER.

Figure 3.13	Change Parameter Name
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Sample Parameters							
Sample Parameters							
Threshold (%) 5 📑 Trigger PE-Cy7							
Name	Peak/Area/Log	Voltage	Gain				
🀲 FS	Peak/Area/Log	N/A	8.0				
🀲 SS	Peak/Area/Log	400	1.0				
488 FITC	Peak/Area/Log	400	1.0				
488 PE	Peak/Area/Log	400	1.0				
488 PE-Texas Red	Peak/Area/Log	400	1.0				
488 PE-Cy5	Peak/Area/Log	400	1.0				
438 PE-Cy7 🛛 🔓	Peak/Area/Log	400	1.0				
UM Violet 1	Peak/Area/Log	400	1.0				
UM Violet 2	Peak/Area/Log	400	1.0				
APC	Peak/Area/Log	400	1.0				
🕅 APC-Cy7	Peak/Area/Log	400	1.0				

How to Enable Data Type Parameters

- 1 Click the Acquisition tab and locate the Acquisition Parameters panel. Double click in the Peak/ Area/Log field. Select the check box that pertains to the data type you want to acquire. See Figure 3.10
 - **NOTE** This can also be done by entering edit mode. To enter edit mode, click on the menu icon in the acquisition parameters tab and select edit mode.

How to Change Voltage and Gain for a Parameter

1 Click the Acquisition screen tab and locate the Acquisition Parameters panel. Double click in the Voltage field. Type a new value or click and drag the slider until the desired value is reached.

Sample Parameters			
Threshold (%) 5	Trigger PE-Cy7		
Name	Peak/Area/Log	Voltage	Gain
🐲 FS	Peak/Area/Log	N/A	8.0
🏶 SS	Peak/Area/Log	600	1.0
488 FITC	Peak/Area/Log	400 h	\$1.0
488 PE	Peak/Area/Log	400	1.0
488 PE-Texas Red	Peak/Area/Log	400	1.0
488 PE-Cy5	Peak/Area/Log	400	1.0
488 PE-Cy7	Peak/Area/Log	400	1.0
🛄 Violet 1	Peak/Area/Log	400	1.0
🔰 Violet 2	Peak/Area/Log	400	1.0
APC	Peak/Area/Log	400	1.0
MR APC-Cy7	Peak/Area/Log	400	1.0

Figure 3.14 Set Voltage for a Parameter

Figure 3.15 Set Gain for the Voltage

Threshold (%) 5	Trigger PE-Cy7			
Name	Peak/Area/Log	Voltage	Gain	
🐲 FS	Peak/Area/Log	N/A	8.0	
8 55	Peak/Area/Log	400	1.5	T.
488 FITC	Peak/Area/Log	400	1.0	.0
488 PE	Peak/Area/Log	400	1.0	
8 PE-Texas Red	Peak/Area/Log	400	1.0	
488 PE-Cy5	Peak/Area/Log	400	1.0	
488 PE-Cy7	Peak/Area/Log	400	1.0	
Wiolet 1	Peak/Area/Log	400	1.0	
WViolet 2	Peak/Area/Log	400	1.0	
APC	Peak/Area/Log	400	1.0	
APC-Cy7	Peak/Area/Log	400	1.0	

Loading an Existing Protocol

How to Load an Existing Protocol

- **1** To load a previously saved protocol select **File > Protocol > Load**.
- **2** A list of previously saved protocol (.plo) files appears. Select the desired file and click **Open**.

Creating Protocols

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

How to Create a New Protocol

- 1 To create a new protocol, go to the main menu and select **File > Protocol > New**. A new workspace appears in which to create dot plots and histograms for the new protocol.
- **2** Ensure that you have enabled the desired parameters. See figure Figure 3.10.

3 Create dot plots and histograms. See page 3-21.

Switching Protocols

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

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

Acquiring Data in Summit Software

When you have enabled parameters and created or loaded a protocol you may acquire data.

How to Start or Stop Data Acquisition

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





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

Saving Acquired Data

After you acquire data in Summit software you can save the information in FCS format.

How to Save Acquired Data

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

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

How to Display the Most Recent Data During Alignment Activities

- **1** Click on the **Acquisition** pull-down menu.
- **2** Select **Cycle Amount**. Set the number of events that should be reached before the data cycles.

Figure 3.17 Setting the Cycle Amount for Cycle Mode

Set cycle amount	
Set Value:	10000
🖌 ок	× Cancel

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

Figure 3.18 Enabling Cycle Mode



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

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.

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em		Value									
File not	me	Malacho	waki 16								
Sample I	Number	69	·····								
Source		SpectrA									
Date		16 May	2008								
Total E	vents	372504									
Elapsed	time	01h:00m	:05s								
Average	Rate	103.33	eps								
Parametr	er 1 Name	Time LS	W								
Paramete	er 2 Name	Time MS	ស								
Paramete	er 3 Name	Pulse W	idth								
Paramete	er 4 Name	FS Lin									
Paramete	er 5 Name	FS Area									
Paramete	er 6 Name	FS Log									
Paramete	er 7 Name	SS Lin									
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Paramete Paramete Paramete Compensa rameter FITC PE Lo APC L	er 9 Name er 10 Name er 11 Name ation: Malachowski FITC Log . 100.0000 . 28.9383 . 0.0345	S3 Log FITC L1 FITC Ar PELog 0.7820 100.0000 0.0000	n ea hrA APC Log 0.0000 0.0000 100.0000								
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Parameti Parameti Parameti Compensa rameter FITC PE Lo APC L	r 9 Name er 10 Name er 11 Name ation: Malachowski FITC Log . 100.0000 . 28.9383 . 0.0345	SS Log FITC L1 FITC Ar PITC Ar PELog 0.7820 100.0000 0.0000	n ea APC Log 0.0000 100.0000								
Parameti Parameti Parameti Compensa arameter FITC APC L	r 9 Name er 10 Name er 11 Name tion: Malachowski FITC Log . 100.0000 . 28.9383 . 0.0345	SS Log FITC L1 FITC L2 FITC Ar PELog 0.7820 100.0000 0.0000	n ea APC Log 0.0000 0.0000 100.0000								
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Figure 3.19 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 3.20 Auto Load Compensation Matrix from FCS File



Auto Compensation Wizard for Single Positive Controls

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-compensate function.

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

NOTE Additional information regarding compensation can be found in APPENDIX C, *Compensation Background Information*.

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

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

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

- **2** Run the remaining single control samples and save the data files.
- **3** Load all control sample files into an experiment folder.
- 4 Click the **Sample** tab.
- **5** Identify or create a dot plot that will be used to adjust compensation. Ensure that the parameter for which you are compensating is on the x-axis.
- 6 In the Sample Compensation panel, click the small, blue icon in the upper-left corner and select Auto Compensate from the list.

Figure 3.21 Select Auto Compensate



7 The Auto Comp Sample dialog box appears.

Figure 3.22 Auto Comp Sample Dialog

	Gate	G1		•
r	iment	Analysis		•
	CD8 F	TITC		•
,				
ľ	CD8	PE		<u> </u>
}	CD8	PF-TR		•
		2		
ł	CD8 F	PE-Cy5		-
5		DE 0.47		
		-E-0y/		<u> </u>
5				-
	-			
ſ				-
2				
Í				
)				•
			1	
		OK	Cancel	

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

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

- **10** Select all of the single control samples included in the experiment.
- **11** Click **ок**.

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 3.23 Auto Compensate Wizard



Figure 3.24 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.
- 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.

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 3.25 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 3.26 VisiComp Example



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





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 3.28 Add/Remove Keywords 1

🖹 Sample Template	
Einstein_19_May_08_SpectrA 1h	nour NewAssy 🗾
Sample Properties: Einstein_19_M	1ay_08_SpectrA 1hour NewAssy
Add/Remove Keywords	Value 🔺
Set Limits	26
	SpectrA 1hour N
Detach Floating	Unknown
Detach Printable	0
Copy to Clipboard	Time LSW
Yarameter 2 Name	Time MSW
🔍 Parameter 3 Name	Pulse Width
🔍 Parameter 4 Name	FS Lin
🔍 Parameter 5 Name	FS Area
🔍 Parameter 6 Name	FS Log
🔍 Parameter 7 Name	SS Lin
🔍 Parameter 8 Name	SS Area
🔍 Parameter 9 Name	SS Log
🔍 Parameter 10 Name	FITC Lin
🔍 Parameter 11 Name	FITC Area
🔍 Parameter 12 Name	FITC Log
🔍 Parameter 13 Name	RPE Lin
🔍 Parameter 14 Name	RPE Area 👻
•	

 $\label{eq:select} 2 \quad \text{Select the check boxes next to the Keywords you would like to display, and click ok.}$

Figure 3.29 Add/Remove Keywords 2

FCS keywords		×
Einstein_19_May_08_Spec	trA 1hour NewAssy	
Keyword	Value	Description 🔺
	26.37	Electronics temperatur.
	3.76	Optics plate temperat
🗖 🔍 Laser1Delay	27.6	Laser delay - path 1
🗖 🔍 Laser2Delay	51.6	Laser delay - path 2
SAMPLEID	Einstein_19_May_08	Sample name
STATATYPE \$	Ι	Data Format
SNEXTDATA	0	Relative offset to nex
□ ^C \$BYTEORD	1,2,3,4	Byte Order
⊠ ≪ssrc	SpectrA 1hour NewAssy	Source
⊡ ≪stot	47492	Total Events
□ ^C _\$MODE	L	Data Set
⊠ ≪ssmno	26	Sample Number
□ ^{cs} spar	37	Number of Parameters
	0.000060	Period (1/Hertz) of Ti
Sector State Stat	19 May 2008	Date
	14:33:03	Begin Time
	14:33:03	End Time
SA2 Televise	Summit V4.3.01 Build	Operating System
□ ^{CC} _\$LOST	0	Busy Aborts
⊻ °sfil		
SCELLS €		
Elapsed time		
Average Rate		
Parameter Name		
Parameter Type		
Parameter Filter		•
•		
		OK X Cancel

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.

Summit V4 3	-					
Ele Edit View Acquicition Mic	togram Gata Workspace Instrum	ant Tools Help				
File cuic view Acquisicion Fils	togram Gate workspace instrum	ient toos nep				
📓 🛛 Alignment	Yorksp	pace 1				
		1				🗖 🙆 👩
						F III
Instrument Acquisition Sample	Histogram Gating Workspace					
Create Histograms/Plots: Ma	lachowski_16_May_08_SpectrA_1h_					6
🛃 Histogram 📩 🛄 Malach	nowski_16_May_08_SpectrA 1h 💌					
X-parameter •	Y-parameter •					
Y Pulse Width	Y Pulse Width					
Y FS Lin	Y FS LIN					
Y FS Log	Y FS Log					
YSS Lin	YSS Lin					M
🍟 SS Area	Y SS Area					
₩ SS Log	Y SS Log	1				
Y FITC Lin	Y FITC Lin					1
Y FITC Log	Y FITC Log					
Y PE Lin	Y PE Lin					
Y PE Area	Y PE Area					
Y PE Log	Y PE Log					
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Violet 2 Lin						
Violet 1 Area						
APC-Cy7 Lin						
PE-Cy5 Lin						
APC Area						
PE-Cy/Lin						
APC LIN						
Time vs EITC L in						
Pulse Width vs FS						
Violet 1 Lin vs Vio						
FITC Lin vs PE-Cy						
APC Lin vs APC-C						
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Figure 3.30 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 3-7.

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



Figure 3.31 Creating Histograms and Dot Plots

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

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

Maximize Dot Plots and Histograms

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

Change Axis Parameters

To change the displayed parameter in a dot plot or histogram, right-click on the axis you want to change and select a new parameter from the menu. The options in the list include the parameters that were collected with the current data file.

Figure 3.32 Change Axis Parameters

	Parameter Properties Data Display Ratio Denominator
✓	FSC Lin
	SSCLin
	FL 1 Log
	FL 2 Log
	FL 3 Log
	FL 4 Log
	FL 5 Log
	FL 6 Log
	FL 7 Log
	FL 8 Log
	FL 9 Log
	Time

Change Axis Labels

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

View Statistics in Histograms

To view statistics in histograms, click the additional menu icon within the histogram and select **Show**.



Figure 3.33 Show Statistics in Histograms

Edit Statistics Displayed in Histograms

To select the statistics that will be displayed in histograms, click the additional menu icon within the histogram and select **Edit Display**.

The check boxes that are selected will appear in the statistics window below the histogram or dot plot. You can also drag and drop to change the order of statistics. Click **OK** to apply these settings to all histograms by selecting the check box at the bottom of the dialog.





Figure 3.35 Edit Statistics Displayed in Histograms

Statistics Display	×
Histogram/Plot Statistics Count Gount Gount Gount Mode Mode Mode Median Std Dev. CV Corr	Dynamic Region Statistics
Attach to Region Apply to All Note: If <apply all="" to=""> is checked, then only the cu workspace will be affected</apply>	not rrent d

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**.

Manually Scaling Data

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

Figure 3.37 Manually Scaling Data



Contouring Data

To Enable Contouring, go to the main dot plot menu and select **Display > Contour**. Select the **Enable contours** check box. The dropdown menu directly below the check box lists the available contouring algorithms. The dialog box contains additional options for maximizing data and smoothing the contouring.

Exporting Histograms to Word

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

Multi File Display

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

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

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

Create Overlays

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

How to Overlay Multiple Histograms

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

Gate Logic Tab

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



Figure 3.38 Gate Logic Tab Displaying an Alignment Protocol and Gating Scheme

Setting a Gate from a Single Region

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

How to Gate One or More Histograms or Dot Plots

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

Figure 3.39 Set Gate 1



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



Figure 3.40 Set Gate 2

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

5 To remove a gate, click the main histogram or dot plot menu icon and select **Ungate**.

Setting a Serial Gate

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

How to Serial Gate Histograms or Dot Plots

1 Open a dot plot gated on region (R1).

Figure 3.41 Set Serial Gate 1



- **2** Create a region (R2) in the dot plot.
- **3** Right-click in region (R2) and then click **Set Gates**.

Figure 3.42 Set Serial Gate 2



The Combine region and gate? Dialog box appears.

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





- 5 Check the Make my choice the default and don't ask me again check box to disable this dialog box in the future. If checked, this option is set as the default in Summit software. The default can be changed in the Preferences dialog box.
- **6** Single or double click in a histogram(s) to apply the gate.

Figure 3.44 Set Serial Gate 4



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

Figure 3.45 Set Serial Gate 5



Gate Logic Builder

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

How to Define (Edit) Gate Logic

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

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

Figure 3.46 Gate Logic Builder

Gate	Logic Builder		X
	Gate Logic Builder		8
143	New	olor	Limit
	Look All		0
			0
	Default <u>T</u> ype		
	Copy to Clipboard		

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

Figure 3.47 Edit Gate Expression 1

Edit ga	te expression			
R1 & R	2			
	Inside region	 	Outside region	
R1 R2 R3		R1 R2 R3		
	OK		Clear	

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 3.48 Edit Gate Expression 2

Edit gate expression	
R1 & R2 & NOT R3	
Inside region	Outside region
R1 R2	R1 R2
R3	R3
	15
ОК	Clear

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 3.49 Gate Logic Builder and Gate Scheme Panels

Gate Logic	Builder		X	Gate Scheme
📑 Gate	Logic Builder		8	💼 Gate Scheme
Name	Expression	Color	Limit	📑 🕩 Regions
📑 G1	R1		0	R1
📑 G2	R1 & R2 & R3		0	
				R NOT R2
				E NOT R1
				(G4)
				(G6)
				Ungated

Color Gating

You can apply colors to gates to more easily distinguish gated populations. Double-click in the **Color** column for a particular gate and select a color.

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 3.50 Layout Tab Displaying an Alignment Protocol

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.

Figure 3.51 Workspace Setup Panel

🖹 Workspace Setup	
Workspace 1	•
Workspace Page Setup	
Page rows 2	Printer
Page columns 1	Advanced
Zoom: 90%	
Q 100% Q	

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 3.52 Workspace Page Navigator

📄 Work	space Page Navigator
Roc FL 1	FL 2
F R	<u>G</u> o To
0+0	<u>S</u> hare
L [•]	<u>M</u> ove
0+0	Duplicate

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.

Shortcut Key	Function
F1	Opens the Summit software online Help system.
F2	Starts/Stops acquiring events.
F3	Saves acquisition data to a drive (C: , D: , etc.), the network, disk, or CD.
F6	Opens the CyCLONE menu.
F7	Opens the Create New Histograms dialog box for the current workspace.
F8	Opens a window that displays all loaded sample files in the current database.
F9	Opens the Drop Delay Wizard.
Left arrow key	Sets the displayed sample in the current workspace or protocol as the first file in the Database samples window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region to the left when selected.

Table 3.1	Shortcut Keys and Functions
	Shortcut Keys and Functions

Shortcut Key	Function
Right arrow key	Sets the displayed sample in the current workspace or protocol as the last file in the Database samples window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region to the right when selected.
Down arrow key	Sets the displayed sample in the current workspace or protocol as the next file in the Database samples window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region downward when selected.
Up arrow key	Sets the displayed sample in the current workspace or protocol as the previous file in the Database samples window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region upward when selected.
SHIFT + ARROW	Expands a region in the arrow direction (left, right, up, down) when selected.
CTRL + ARROW	Contracts a region in the arrow direction (left, right, up, down) when selected.
CTRL + O	Opens a dialog box to open one or more FCS Listmode files.
CTRL + S	Saves modified items on the Summit software desktop that have changed (or auto- save is invoked at periodic intervals and when Summit software is closed).
CTRL + P	Prints the current view of the Summit software desktop.
CTRL + C	Copies the selected region, which can be pasted into a histogram.
CTRL + V	Pastes the copied region into a histogram.
CTRL + D	Opens the Sort Logic and Statistics menu.
CTRL + G	Opens the Gate Logic menu.
CTRL + Z	Clears the event buffer of all acquired events.
CTRL + W	Opens the Worklist Panel.
SHIFT + F4	Starts a Hardware Sort.
ALT + F4	Exits Summit software.
+	Expands one node when viewing a folder list in a window (use + on the numeric keypad).
-	Collapses one node when viewing a folder list in a window (use - on the numeric keypad).
*	Expands an entire folder tree contained in a window (use * on the numeric keypad).

 Table 3.1
 Shortcut Keys and Functions (Continued)