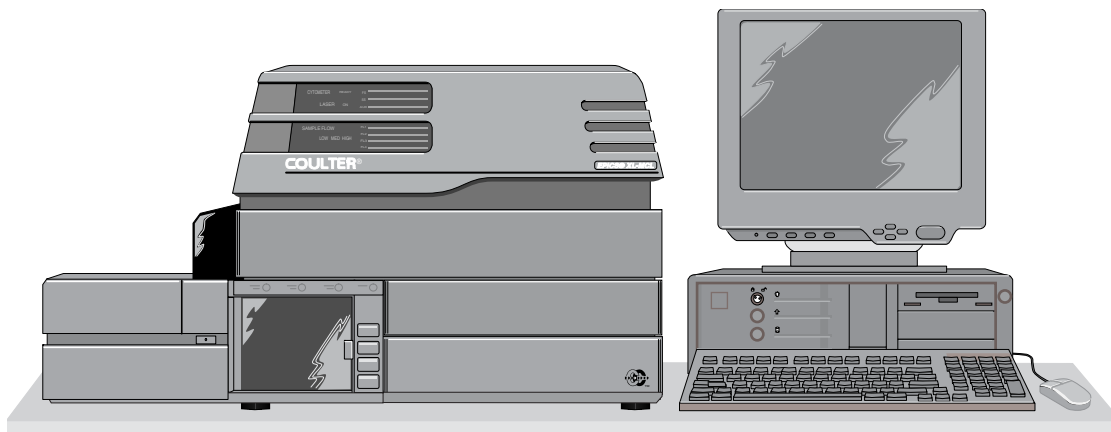


COULTER® EPICS® XL™ Flow Cytometer
COULTER EPICS XL-MCL™ Flow Cytometer
SYSTEM II™ Software

Getting Started



PN 4237238BA (October 2010)



Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821



WARNINGS AND PRECAUTIONS

READ ALL PRODUCT MANUALS AND CONSULT WITH BECKMAN COULTER-TRAINED PERSONNEL BEFORE ATTEMPTING TO OPERATE INSTRUMENT. DO NOT ATTEMPT TO PERFORM ANY PROCEDURE BEFORE CAREFULLY READING ALL INSTRUCTIONS. ALWAYS FOLLOW PRODUCT LABELING AND MANUFACTURER'S RECOMMENDATIONS. IF IN DOUBT AS TO HOW TO PROCEED IN ANY SITUATION, CONTACT YOUR BECKMAN COULTER REPRESENTATIVE.

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Can cause injury.

CAUTION - Can cause damage to the instrument.

IMPORTANT - Can cause misleading results.

BECKMAN COULTER, INC. URGES ITS CUSTOMERS TO COMPLY WITH ALL NATIONAL HEALTH AND SAFETY STANDARDS SUCH AS THE USE OF BARRIER PROTECTION. THIS MAY INCLUDE, BUT IT IS NOT LIMITED TO, PROTECTIVE EYEWEAR, GLOVES, AND SUITABLE LABORATORY ATTIRE WHEN OPERATING OR MAINTAINING THIS OR ANY OTHER AUTOMATED LABORATORY ANALYZER.

WARNING Risk of operator injury if:

- All doors, covers and panels are not closed and secured in place prior to and during instrument operation.
- The integrity of safety interlocks and sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.
- You contact moving parts.
- You mishandle broken parts.
- Doors, covers and panels are not opened, closed, removed and/or replaced with care.
- Improper tools are used for troubleshooting.

To avoid injury:

- Keep doors, covers and panels closed and secured in place while the instrument is in use.
- Take full advantage of the safety features of the instrument. Do not defeat safety interlocks and sensors.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter Representative.
- Open/remove and close/replace doors, covers and panels with care.
- Use the proper tools when troubleshooting.

CAUTION System integrity might be compromised and operational failures might occur if:

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

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

Issue A Initial Issue, 1/96

SYSTEM II Software, Version 1.0. Initial issue for customer distribution.

Issue B, 12/98

Complete revision. SYSTEM II Software, Version 3.0. Includes new quality control features, patient report export feature, and user-defined run time reports.

Issue BA, 10/10

SYSTEM II Software, Version 3.0.

Updates were made to the company corporate address.

Note: Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released to the Beckman Coulter website. For labeling updates, go to www.beckmancoulter.com and download the most recent manual or system help for your instrument.

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	TRADEMARKS

This introductory section contains the following topics:

- Using your COULTER EPICS XL and XL-MCL Flow Cytometer Manuals
- About this Manual
- Conventions
- Icons.

USING YOUR XL AND XL-MCL FLOW CYTOMETER MANUALS

Use the **Reference** manual for in-depth information on the principles of flow cytometry, information about what your system does, the methods it uses, its specifications, and information on installation, safety, and system options.

Use the **Getting Started** manual to become familiar with the controls and indicators for your system and to learn about protocols, regions, panels, and the basic skills you need to operate the system. This manual also has an overview of the software.

Use the **Operator's Guide** for the day-to-day running of your instrument. Go through the detailed step-by-step procedures of startup, quality control (QC), running samples, analyzing data, printing reports, reviewing QC data, and shutdown.

Use the **Data Management** manual for instructions on how to export, save, copy, move, archive, and delete files. It also has information about the types of files your system creates and uses, instructions for working with QC features, and instructions for setting up the report template that you use to create your patient reports.

Use the **Special Procedures and Troubleshooting** manual to clean, replace, or adjust a component of the instrument. The Troubleshooting tables and error messages are at the back of the manual.

Use the **Operating Summary** as a quick reference for basic procedures.

Use the **User's Comment Card** in the Reference manual to give us your comments about the manual and ways to improve it.

ABOUT THIS MANUAL

Your EPICS XL and XL-MCL Flow Cytometer Getting Started manual has information on the skills and concepts you need to learn before operating the instrument. It also contains an overview of the system software.

This information is organized as follows:

- Chapter 1, Controls and Indicators
Describes the parts of the instrument used during normal operation and how you interact with them
- Chapter 2, Learning the Basics
Contains instructions for basic operating techniques, loading samples, using the MCL, and using the mouse.
- Chapter 3, Software Overview
Contains illustrations, descriptions for each software screen and instructions for using the screen buttons and keyboard.
- Chapter 4, Creating Protocols
Contains instructions on creating, editing and saving protocols, defining histograms, creating regions, assigning gates and adjusting cytometer settings.
- Chapter 5, Using Regions
Contains information on region types and instructions for creating, assigning and editing regions.
- Chapter 6, Creating Panels
Contains information on what panels and worklists are and how to use them to process batches of samples.
- Chapter 7, The Operating System
Contains instructions on starting the XL and XL-MCL software from the MS-DOS® operating system and warnings about Microsoft® Windows™ and XL and XL-MCL software.
- Index
Use the Index to easily locate specific information in this manual.

CONVENTIONS

This manual uses the following conventions:

Throughout this manual your EPICS XL or XL-MCL flow cytometer is referred to as the system.

Italics indicate screen messages.

Bold indicates a menu item.

Courier font indicates text you have to type using the keyboard.

 indicates a key (such as .

+ indicates that the two keys listed (such as +) are linked for a specific function and must be pressed in this sequence:

1. Press down on the first key listed and while continuing to press it, press down on the second key listed.
2. Release both keys at the same time.

  indicates to press and release the first key listed then press and release the next key listed. For example,  .



File >> Save

Select the **Save** item on the **File** menu.



[OKAY]

Use the mouse to click on the screen button labeled [OKAY].



F1 through **F12**

Special function keys.

Description of Reporting Units

Unless otherwise stated, all parameter units are shown in the US unit format (cells/ μ L) throughout the manuals.

ICONS



Press and release the left mouse button.



Press and release the right mouse button.



Move the mouse without pressing either button.



Press and hold the left mouse button while moving the mouse.



Press and hold the right mouse button while moving the mouse.



Operational check.



Turn off the system at the computer.



Turn on the system at the computer.



Power Supply.



No fluid.



Close the Power Supply door.

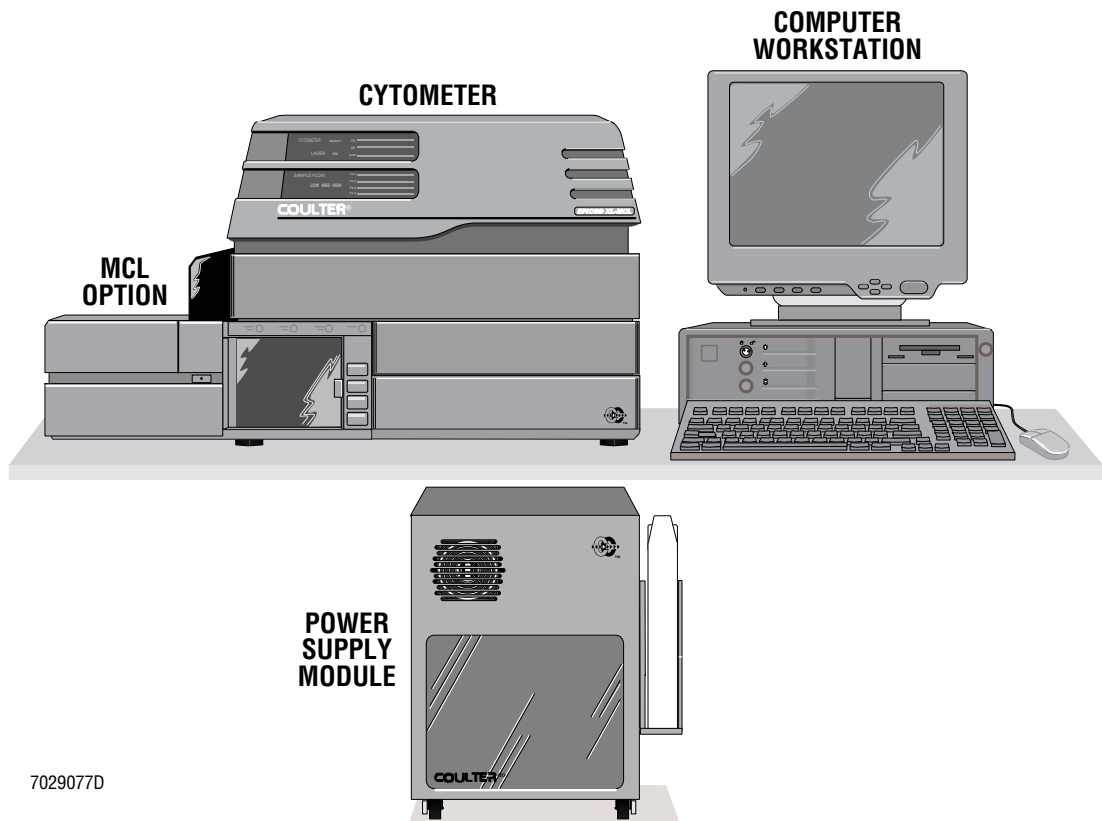


Open the Power Supply door.



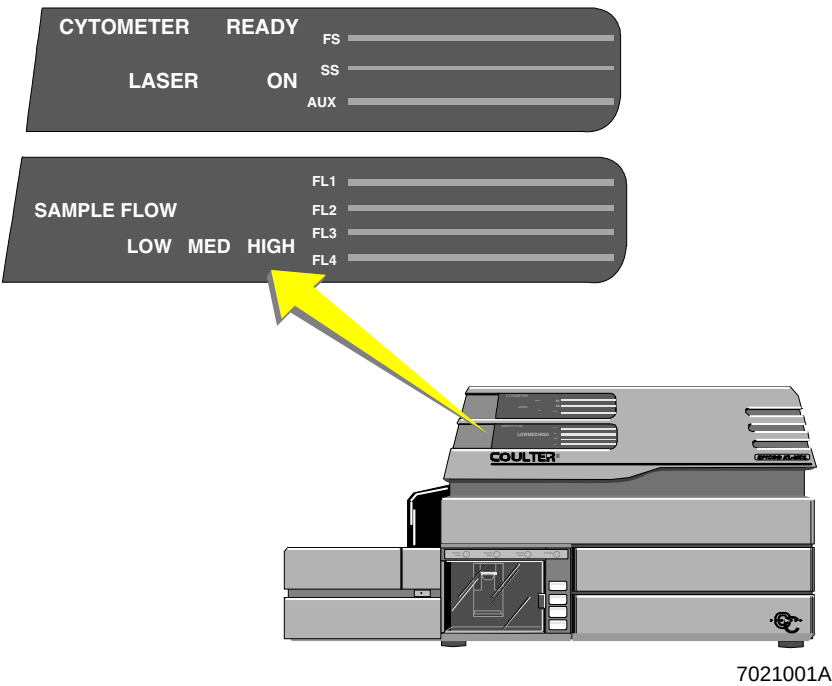
Call your Coulter Representative.

1.1 XL AND XL-MCL FLOW CYTOMETRY SYSTEM

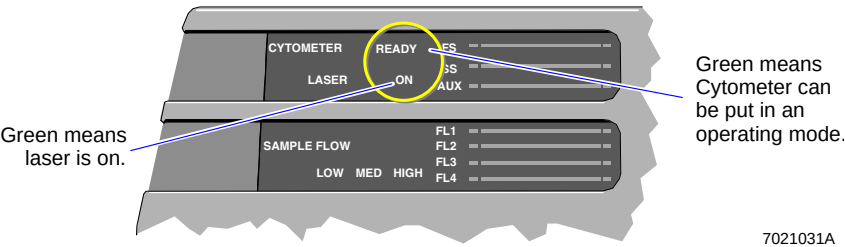


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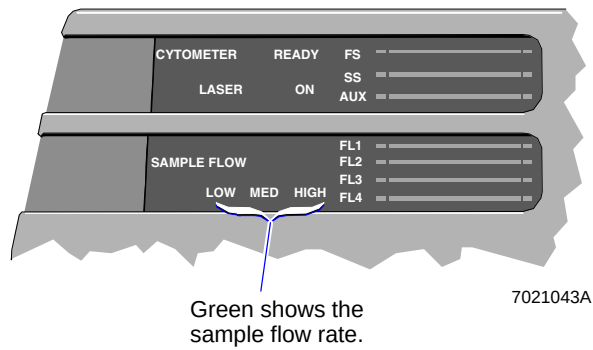
1.2 CYTOMETER INDICATOR PANELS



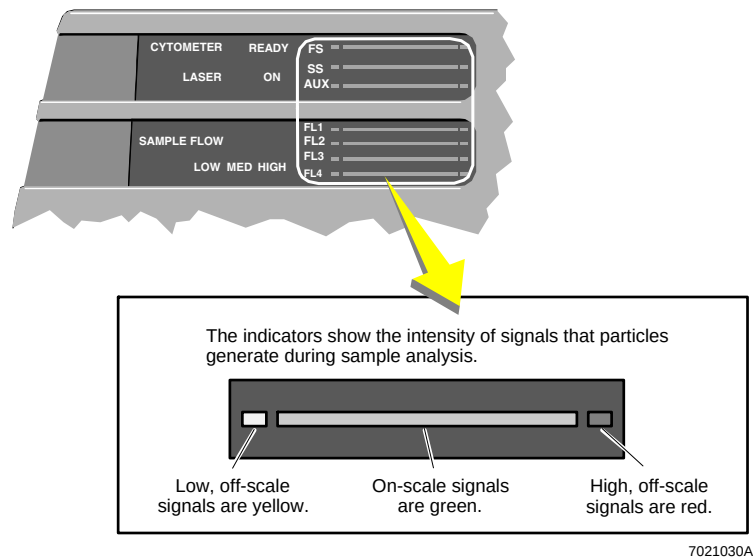
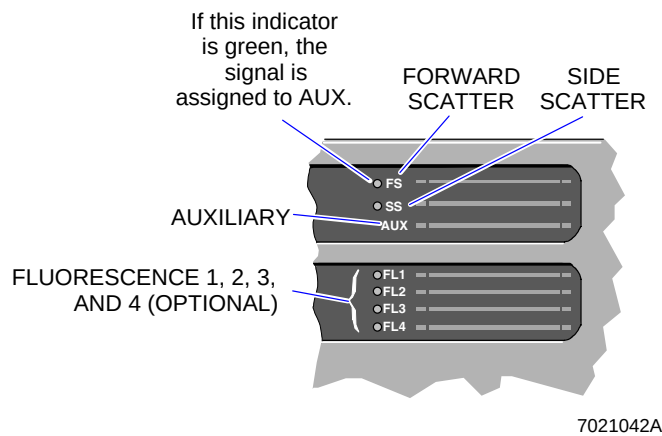
Cytometer Ready and Laser On Indicators



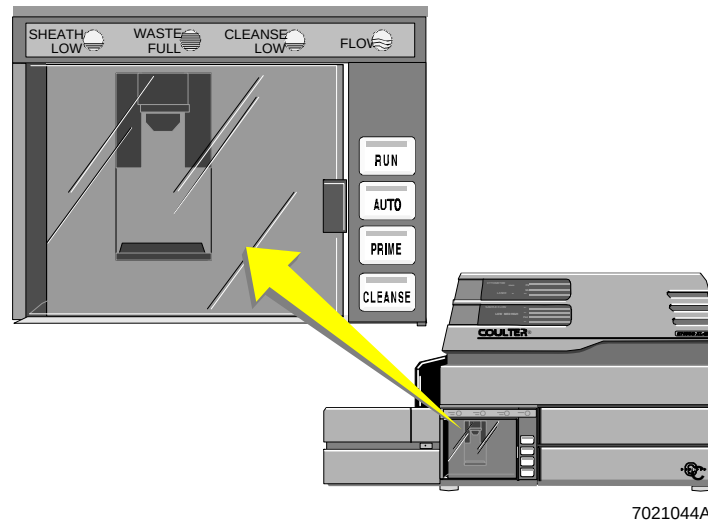
Sample Flow Rate Indicators



Signal Amplitude Indicators



1.3 CYTOMETER CONTROL PANEL AND SAMPLE STAGE AREA (XL-MCL FLOW CYTOMETER)



Operating Mode Buttons

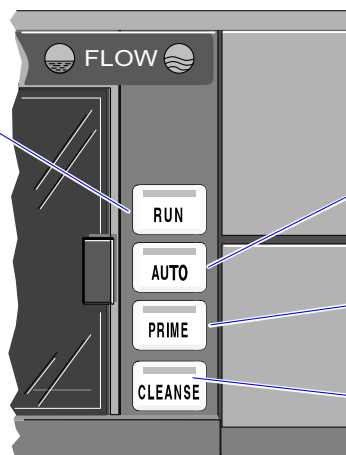
The XL-MCL flow cytometer is shown in this figure. The information also applies to the XL flow cytometer except there is no AUTO button.

You must have the Acquisition Run screen displayed to use these buttons.

Press to use the Run mode.
Press again for Idle mode.
The indicator color shows the Cytometer operating mode:

Glowing (solid) green	Run mode. Cytometer is waiting for you to insert a sample tube.
Flashing green	Idle mode.
Glowing (solid) orange	Run mode during sample cycle.

7238136B

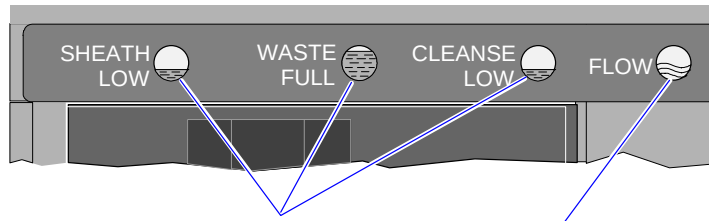


XL-MCL flow cytometer only (Shown).
Press to use the optional Automatic mode.
Press again to use the Manual mode.
Glowing (solid) green indicator means the Cytometer is in the Auto mode and a carousel is in the MCL.

Press to flush the flow cell with sheath fluid. Data acquisition pauses until the prime cycle is done. During the 10-second cycle, indicator is green.

Press to flush the flow cell with cleaning agent. During the 60-second cycle, indicator is green. There must not be a tube on the sample stage.

Level Sense and Flow Indicators: XL-MCL Flow Cytometer



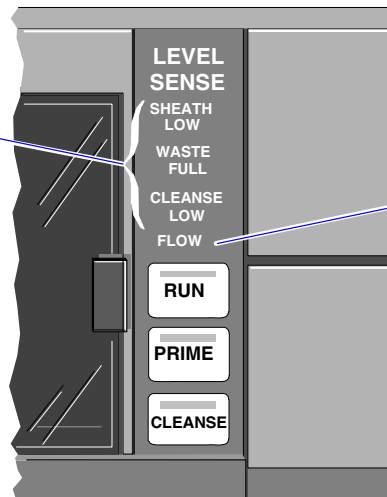
Red symbol means a reagent container needs refilling or the waste container needs emptying.

Green symbol means sample is going through the flow cell.

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Level Sense and Flow Indicators: XL Flow Cytometer

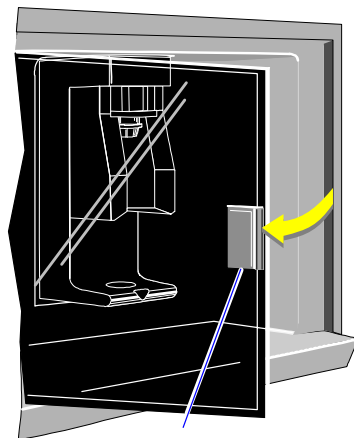
Red means a reagent container needs refilling or the waste container needs emptying.



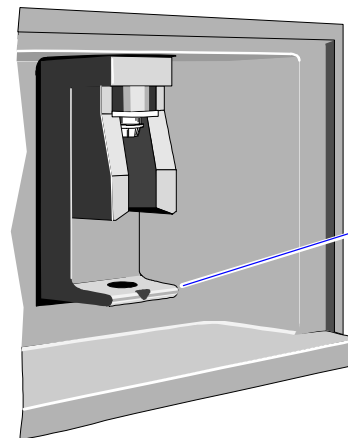
Green means sample is going through the flow cell.

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Sample Stage Area



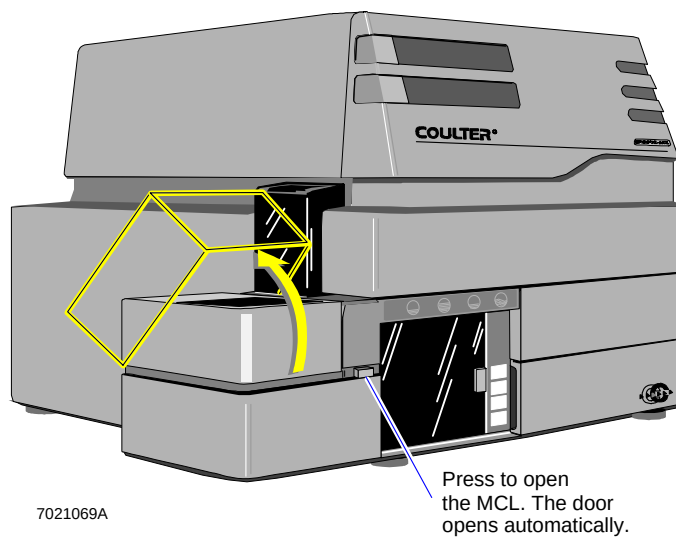
DOOR HANDLE



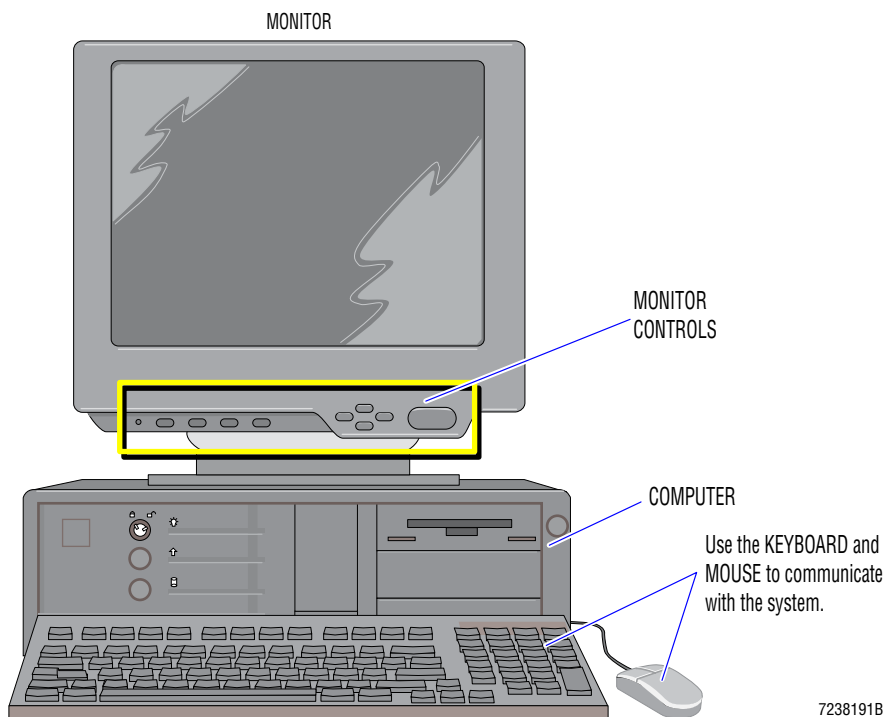
Put a tube on the SAMPLE STAGE to start the sample cycle.

7021037A

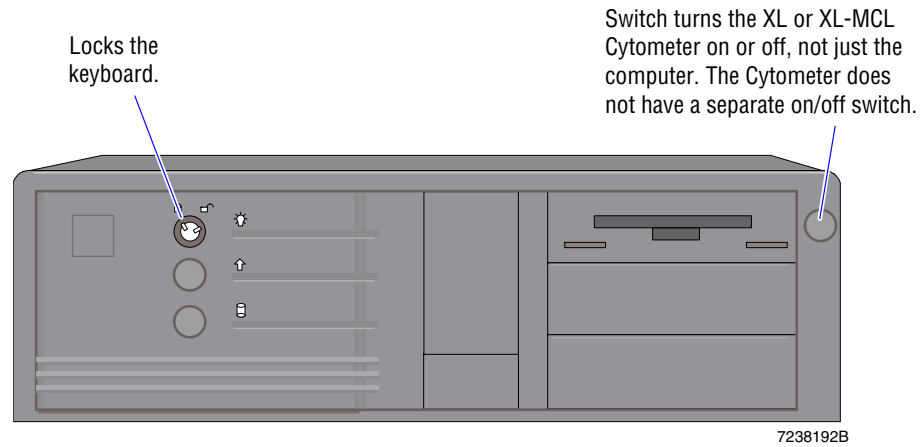
1.4 MCL (MULTI-TUBE CAROUSEL LOADER)



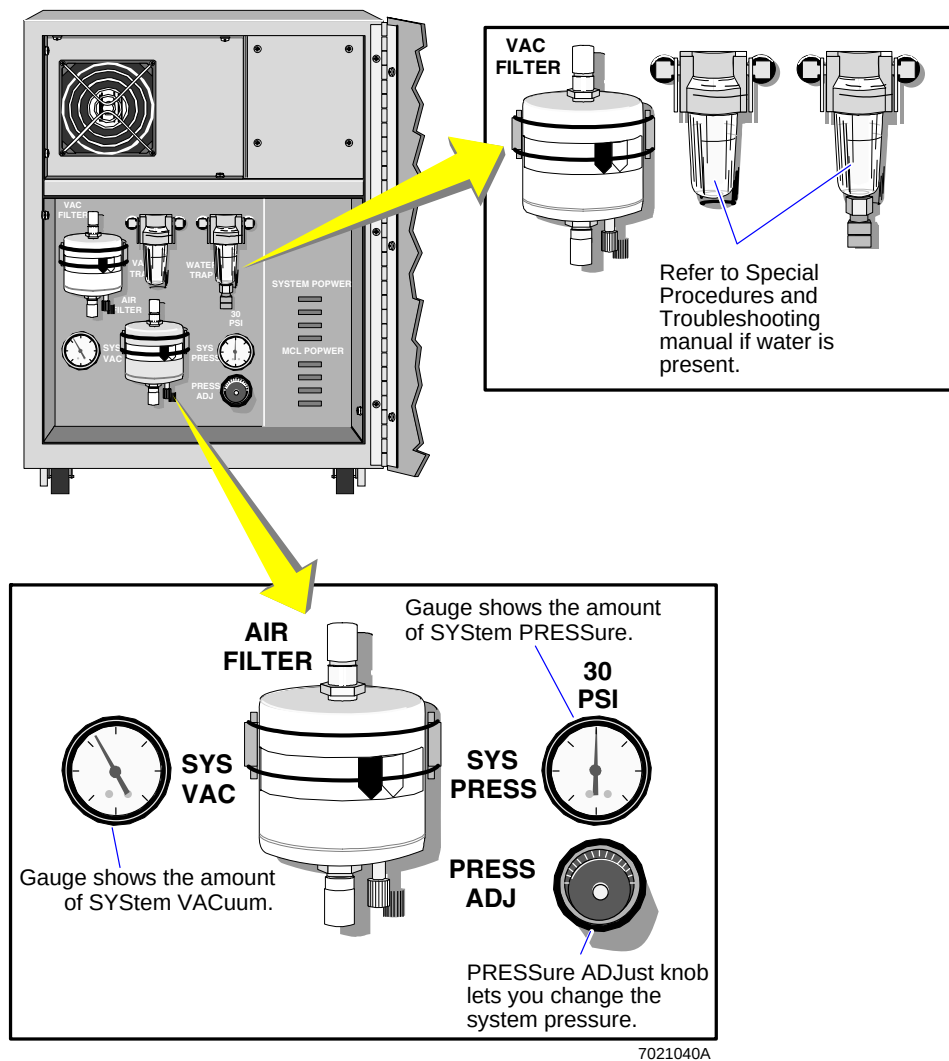
1.5 WORKSTATION



Computer



1.6 POWER SUPPLY



1.7 PRINTER (OPTIONAL)

This guide does not explain how to use the optional Printer. Operating instructions from the Printer manufacturer are included with the Printer.

2.1 LEARNING THE BASIC OPERATING TECHNIQUES

Before reading the other chapters in this manual:

- Read about the Cytometer operating mode buttons in Heading 1.3, Cytometer Control Panel and Sample Stage Area.
- Read this chapter to become experienced with:
 - Using the Sample Stage.
 - Using the MCL (if equipped).
 - Using the Mouse to navigate through the different software screens.

Practice the basic techniques until you feel comfortable using them. If, later on, you need to use a basic technique but cannot remember how, look it up in the Index (at the back of this manual or in the Master Index booklet) and get the page number for step-by-step instructions.

Note: Your screens might look slightly different from the ones in this manual, depending on the selections you make. The step-by-step instructions in this chapter are generic; they are not specific to any particular immunological application.

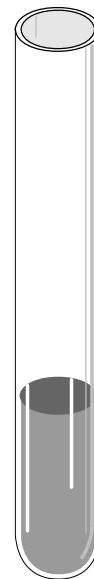
2.2 USING THE SAMPLE STAGE

Test Tube and Sample Requirements

You need at least 0.5 mL of sample, but the tube must not be more than half full.

If you use stop-on-volume (aspirate a pre-set volume of sample), you need at least 12.5 times more prepared sample than the volume you specify to properly fill the volume chamber.

For example, if you specify a stop-on-volume of 40 μ L, you need at least 500 μ L of sample.



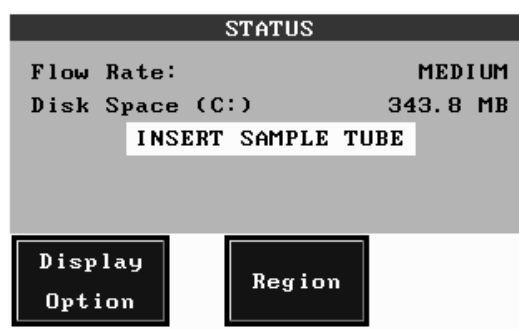
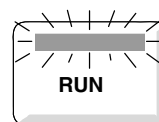
12 x 75 mm TEST TUBE

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Putting a Tube on the Sample Stage

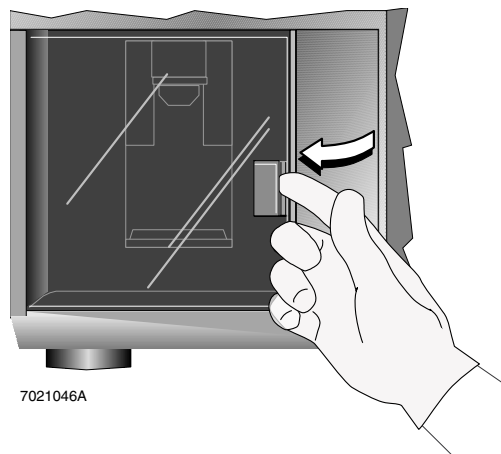
1. Ensure that the Cytometer is in Run mode (the RUN button light is steady green).

The Acquisition Run screen must be displayed and must show the message *Insert sample tube*.



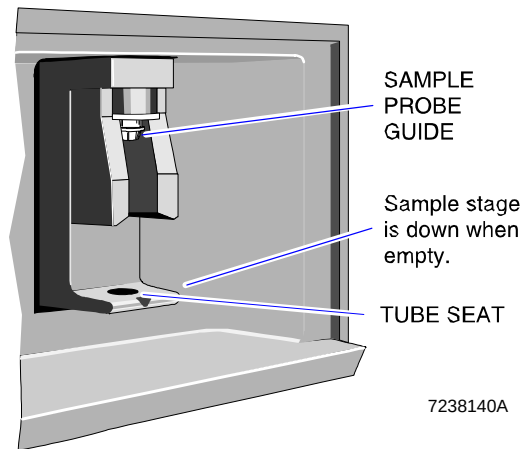
7239063A

2. Open the door.



7021046A

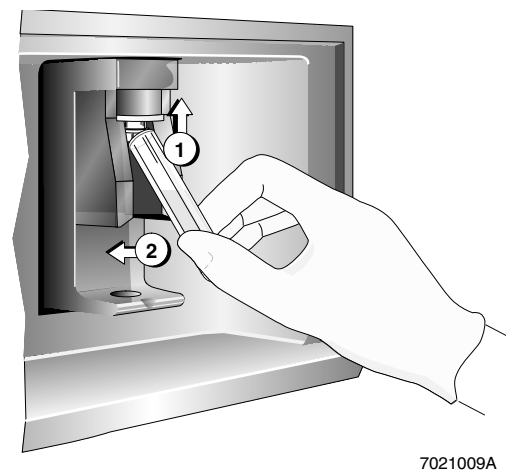
WARNING Risk of injury from broken sample tube. If the tube is not seated correctly, it might break. When you put a tube on the stage, be sure that the sample probe guide covers the tube opening, and be sure to position the tube's bottom in its seat on the Sample Stage.



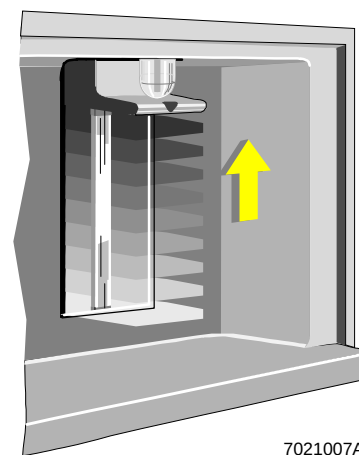
3. Insert the tube:

- a. Top-first
- b. Bottom-second.

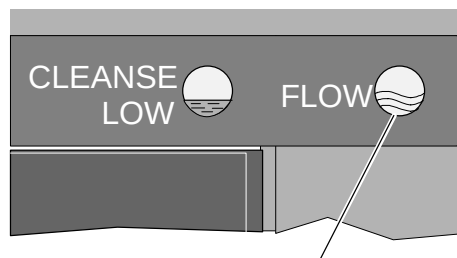
The Cytometer beeps quickly as a reminder to remove your hand from the Sample Stage before it rises.



4. The stage rises, positioning the tip of the sample probe near the bottom of the tube.



The FLOW indicator turns green when the tube is pressurized.



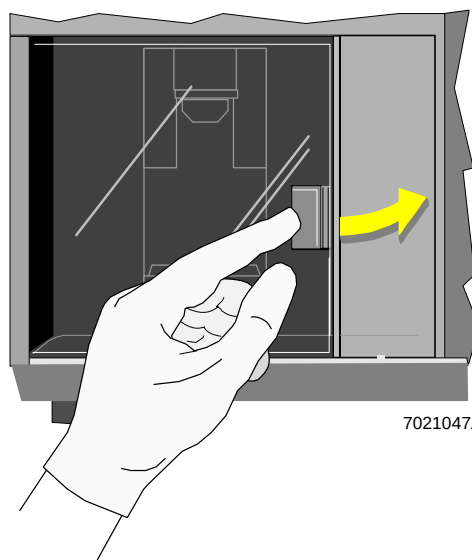
7238145A

Green symbol means
sample is going through
the flow cell.

5. Close the door.

When data acquisition ends, the tube is depressurized, the FLOW indicator turns off and the Sample Stage lowers.

Remove the tube (see instructions for removing a tube on the next page).

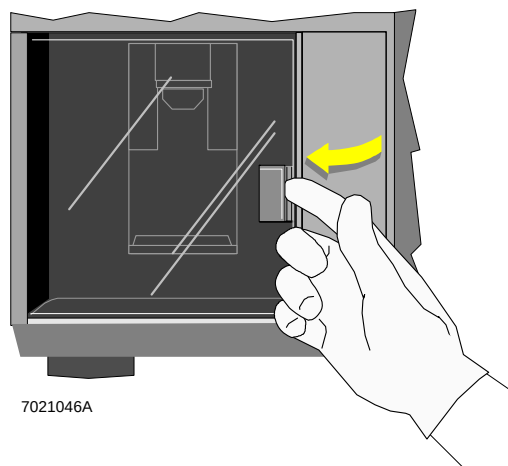


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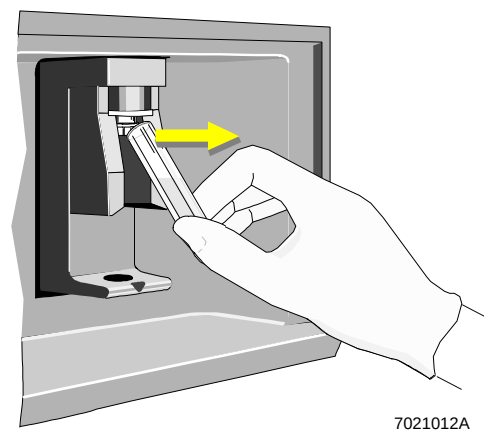
CAUTION Air bubbles can form in the system if you do not remove the tube immediately after the Sample Stage lowers, following data acquisition.

Removing a Tube from the Sample Stage

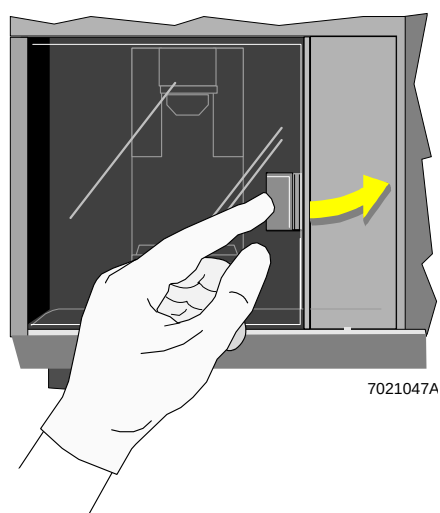
1. Open the door.



2. Pull from the bottom of the tube and remove it from the Sample Stage.



3. Close the door.



2.3 USING THE MCL (XL-MCL FLOW CYTOMETER ONLY)

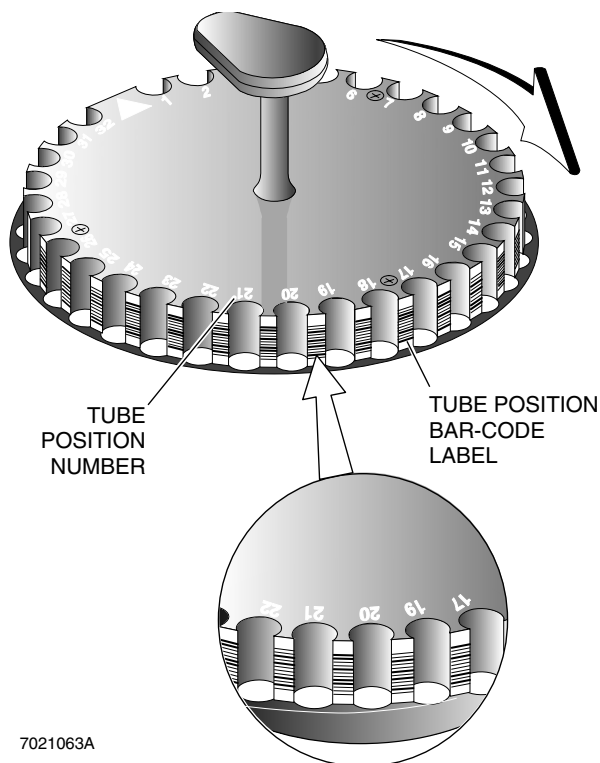
Carousels

The XL-MCL flow cytometer starter kit has:

- Two carousels, each with 32 tube positions.
- A sheet of bar-code labels, numbered 01 to 99, for you to use to identify the carousels.

Putting Sample Tubes in a Carousel

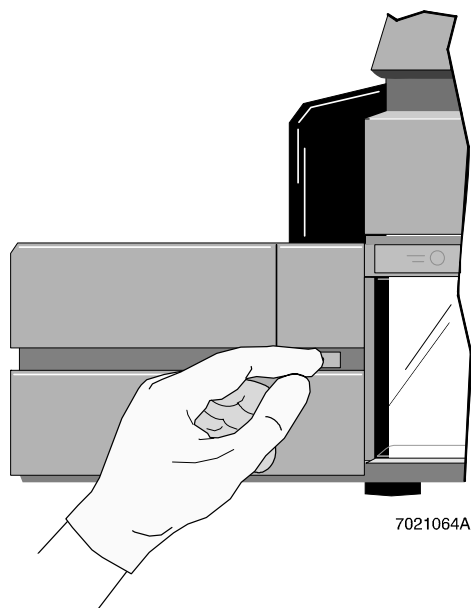
- The orientation of a tube with a bar-code label does not matter. The MCL rotates the tube to find the bar-code label.
- Do not skip tube positions within a panel. The XL-MCL flow cytometer does not skip a protocol in a panel when a carousel tube position is empty. If you lose a sample, replace it with a blank tube containing sheath fluid.
- You can skip a single-tube position to separate two panels. The system stops analyzing carousel tubes when it finds two consecutive empty tube positions or reaches the last position on the carousel.



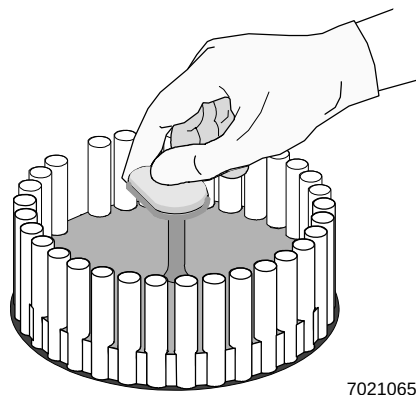
7021063A

Putting a Carousel in the MCL

1. Press the MCL door release.



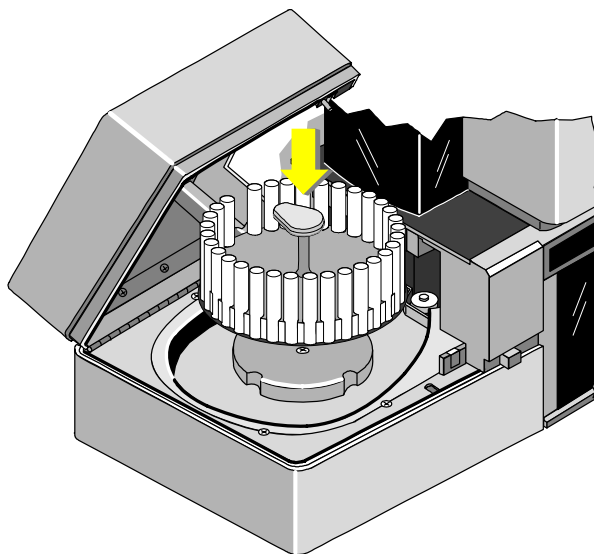
2. Pick up the carousel.



LEARNING THE BASICS

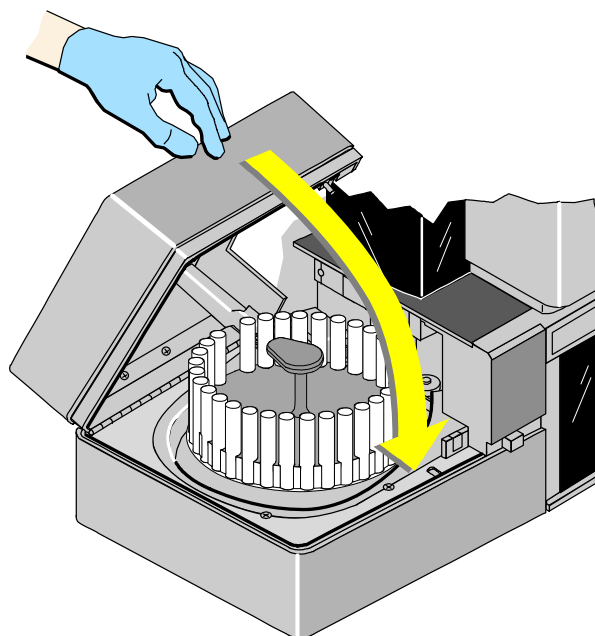
USING THE MCL (XL-MCL FLOW CYTOMETER ONLY)

3. Line up the carousel with its turntable, and then push down. The carousel is in home position when the handle points toward the back.



7021066A

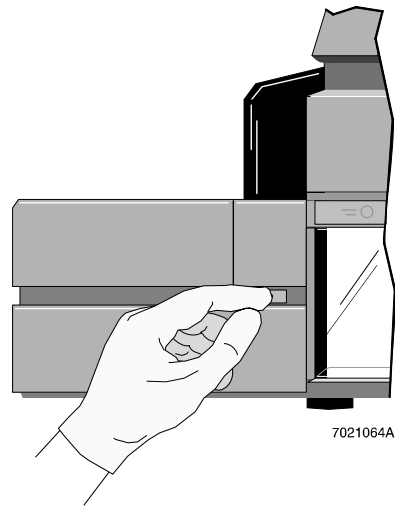
4. Close the MCL.



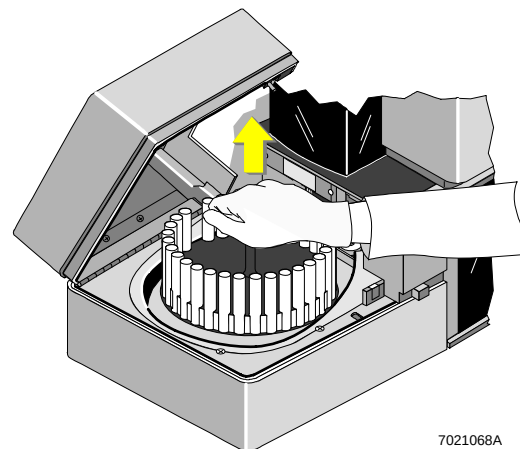
7021-067

Removing a Carousel from the MCL

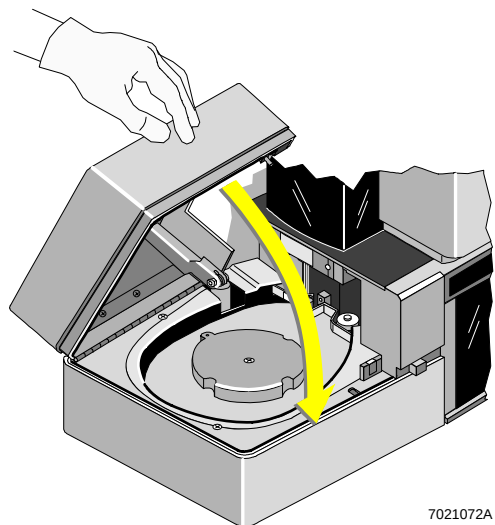
1. Press the MCL door release.



2. Remove the carousel.



3. Close the MCL.



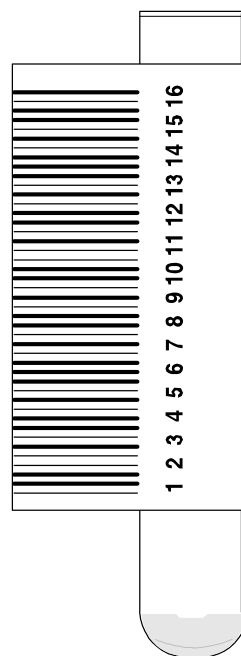
Bar-Code Labels

You can put a bar-code label on each sample tube. Bar-code label specifications are in the Specifications Chapter in the Reference manual.

IMPORTANT To prevent misidentified samples, affix the bar-code label as shown below so the MCL can read the label correctly.

Putting a Bar-Code Label on a Sample Tube

1. Carefully align the label with the tube.
2. Press the label down securely, including edges and corners, without wrinkles or folds.



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2.4 USING THE MOUSE

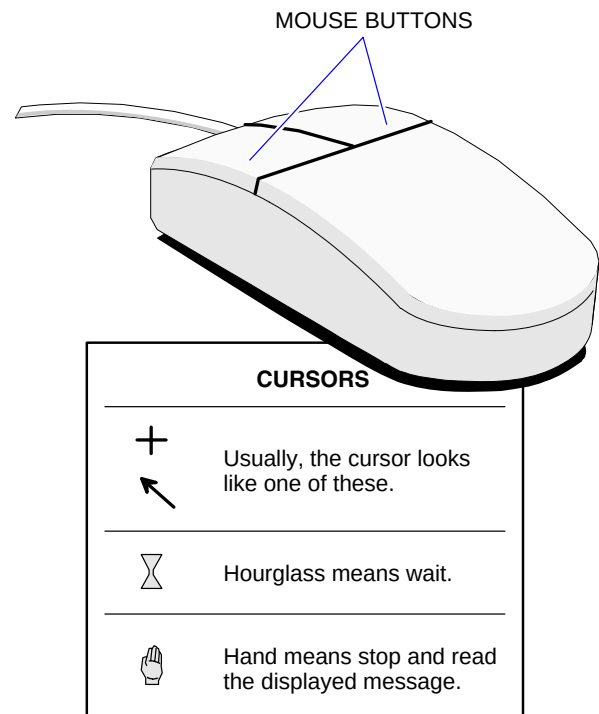
Selecting Screen Items

The mouse is a pointing device you use to select items on the screen. When you move the mouse, a graphic image called a cursor moves on the screen. The cursor changes depending on what is happening in the software.

Use the cursor to point to an item on the screen and then press or click one of the mouse buttons to select the item.

You also use the mouse to drag screen items. You enlarge a region or move a population on a histogram by dragging. To drag something:

- Point to the object with the cursor.
- Press and hold the left mouse button.
- Move the mouse while holding down the left button.
- Release the button when the object is sized or positioned the way you want it.
- Press the right mouse button to anchor the object.



7021013A

Symbols for Selecting Menu Items

1. This manual uses the convention shown in the right-hand column to tell you to select a specific menu or menu item.

Menu names or menu buttons appear in **bold**.

Menu item selections appear on the same line after the ►► symbol.

2. Position the cursor on **Setup Screen**. It is highlighted.

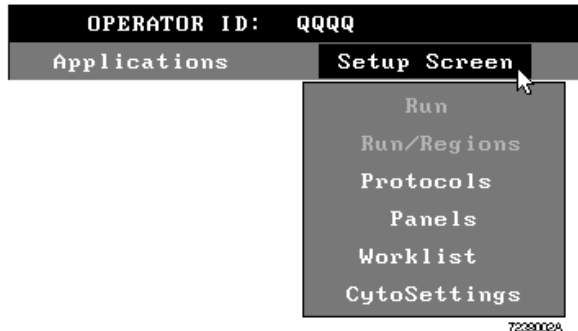


Setup Screen ►► Protocol

OPERATOR ID:	XXX
Applications	Setup Screen

7238001A

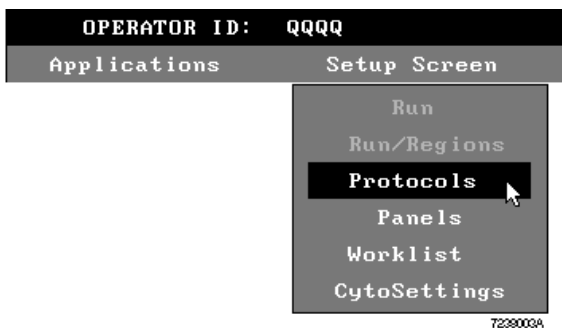
3. Press and release either mouse button once. The drop-down menu appears.



4. Move the cursor down the menu. As the cursor passes items, some of them highlight with a colored background. Items that do not highlight cannot be selected now.

When **Protocols** is highlighted, press either mouse button once.

The Protocol Setup screen appears.



Symbols for Selecting Screen Buttons

Many of the software screens or windows have "buttons." This manual uses the convention shown in the right-hand column to tell you to select a specific "button" on a screen.



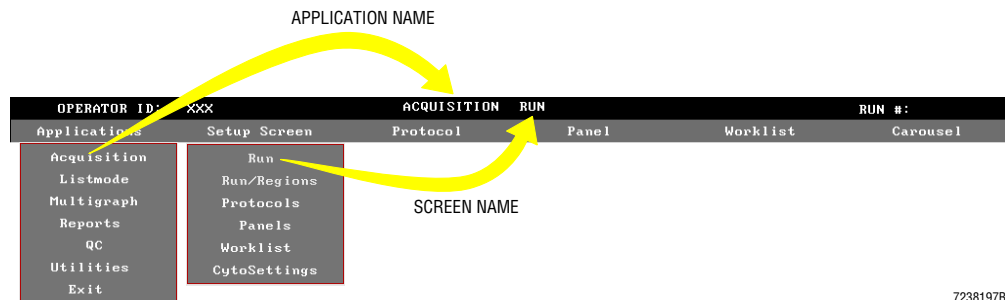
Screen buttons are indicated by the button label written in Small Caps in square brackets.

Usually, it does not matter which mouse button you press. Specific exceptions are noted in the text, and the mouse icon is modified.



2.5 MOVING BETWEEN SCREENS

At the second menu on every screen you can choose among the screens that are part of the current application. This menu is called either Setup Screen (in the Acquisition application), Setup (in the Listmode application), or Screen (in the other applications). First you select the application you want from the Application menu, then you select which screen to use in that application from the Setup Screen or Screen menu. Each screen title combines the current Application name and the current screen name so that you know where you are in the software.



7238197B

2.6 USING YOUR NETWORK WORKSTATION AND SERVER

The Network Workstation and Server are optional computer systems. The Network Workstation can be used to analyze data and create Worklists off-line. The Server can be used to archive data and as a network database server.

The Server can only be used as a network database server and should not be used as a SYSTEM II software Workstation. Create Worklists and perform off-line data analysis on a separate Workstation. SYSTEM II software cannot be used on the Server while it is in use as a network database server.

Your Network Workstation uses LANTastic® software to connect to one or more XL or XL-MCL systems.

You can use the Network Workstation or server to bridge to your own network. You must validate your own network connection. Coulter has validated the connection between the XL or XL-MCL flow cytometer and the Workstation or server using LANTastic software.

The Network is used to download to the Workstation data files [Listmode (LMD), Histogram (HST), Patient Report, exported data files (EPT), and Cytometer setting files (CYT) and database records (DBF)] for archival, off-line analysis, or report generation.

The Network is used to upload worklists, protocols, panels, and database records to the XL or XL-MCL flow cytometer.

Your XL or XL-MCL flow cytometer can send files to and receive files from a Workstation or Server; however, the Workstation or Server cannot pull files from an XL or XL-MCL flow cytometer or transfer files to an XL or XL-MCL flow cytometer.

There can be more than one Workstation or Server on the network. The XL or XL-MCL flow cytometer cannot be a Server.

The Network is configured in the config.sys and autoexec.bat files at installation. The XL or XL-MCL flow cytometer software does not treat a network drive any differently from other drives.



LEARNING THE BASICS

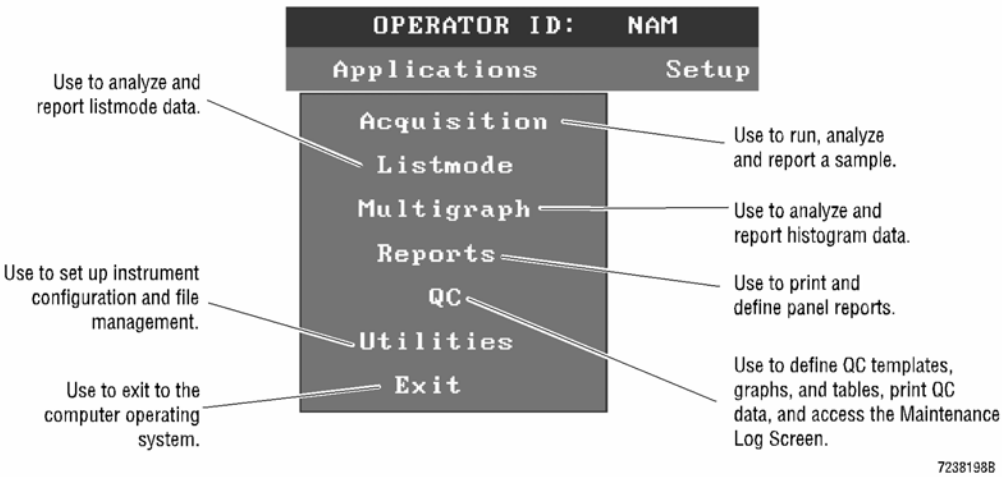
USING YOUR NETWORK WORKSTATION AND SERVER

3.1 APPLICATIONS MENU

You control most of the functions of the XL and XL-MCL flow cytometer from a series of different software screens.

The screens are grouped into applications and give you access to a group of logically related actions. A set of related screens are grouped into an "Application."

Use the Applications menu to change among applications according to your current needs.



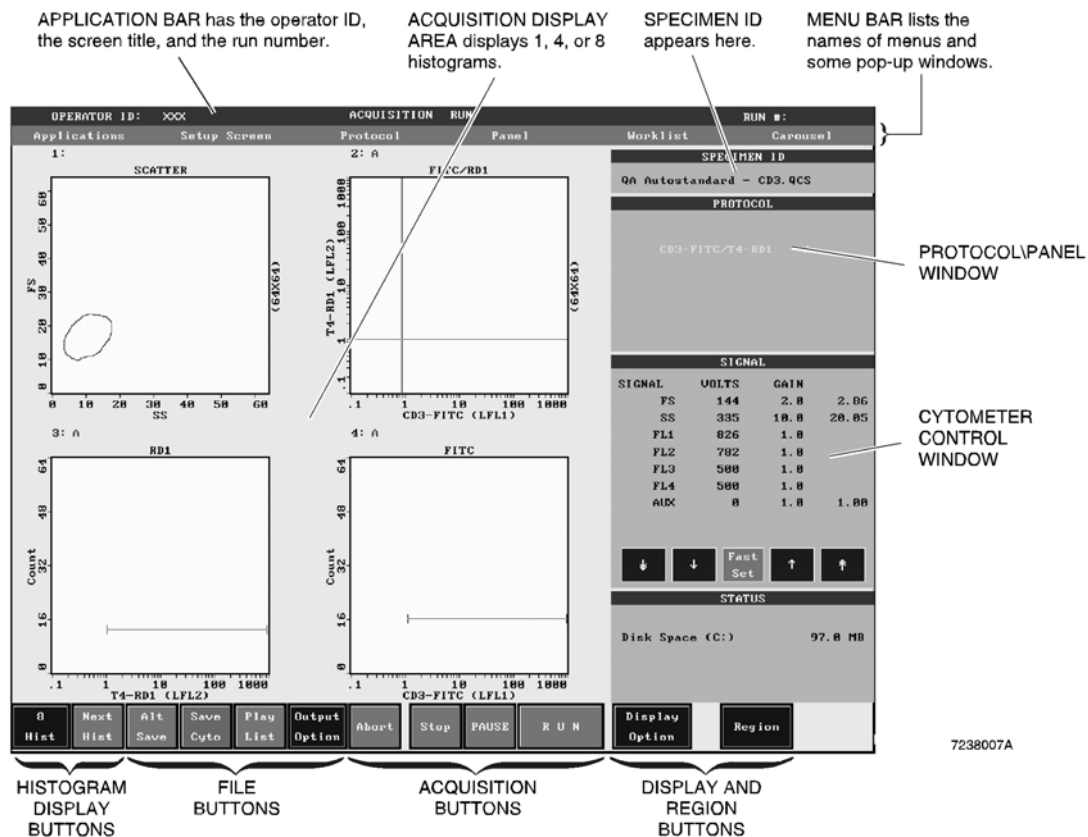
Description of the Applications

The following pages illustrate the main menu choices for each application in turn. The Acquisition Run screen is described first.

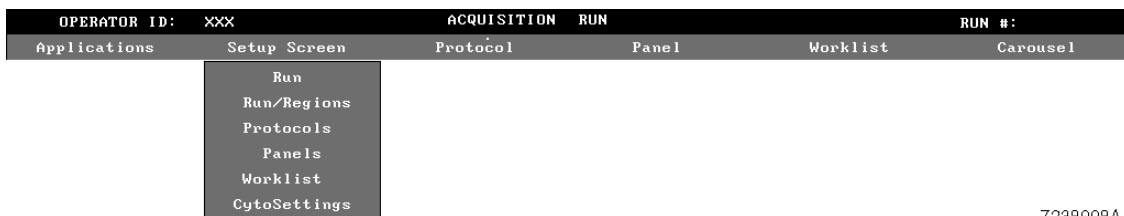
Screen Features

A set of features common to all the other screens follows the descriptions of the applications.

3.2 ACQUISITION APPLICATION



Acquisition Setup Screen Menu



Run

Goes to Run screen to acquire data from a sample using a worklist, panel, or protocols.

Run/Regions

Goes to Run screen and activates Region button to create, edit, erase, move, copy, or redraw regions.

Protocols

Goes to Protocol screen to select, define, setup, and save protocols.

Panels

Goes to Panel screen to select, define, setup, and save panels.

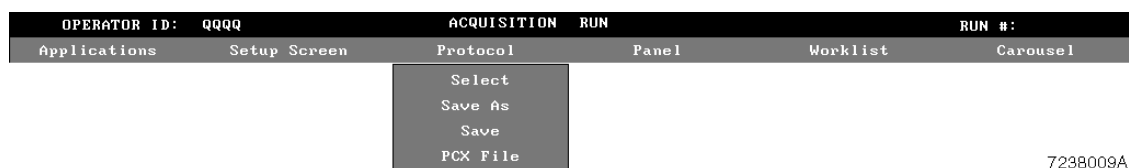
Worklist

Goes to the Worklist screen to define sample loading and specimen information for up to 32 tubes.

CytoSettings

Goes to CytoSettings screen to adjust Cytometer settings.

Acquisition Protocol Menu



Select

Opens a protocol file.

Save

Saves a protocol file with the current name.

Save As

Saves a protocol file with a new name.

PCX File

Saves the screen (or a portion of the screen) as a PCX graphics file.

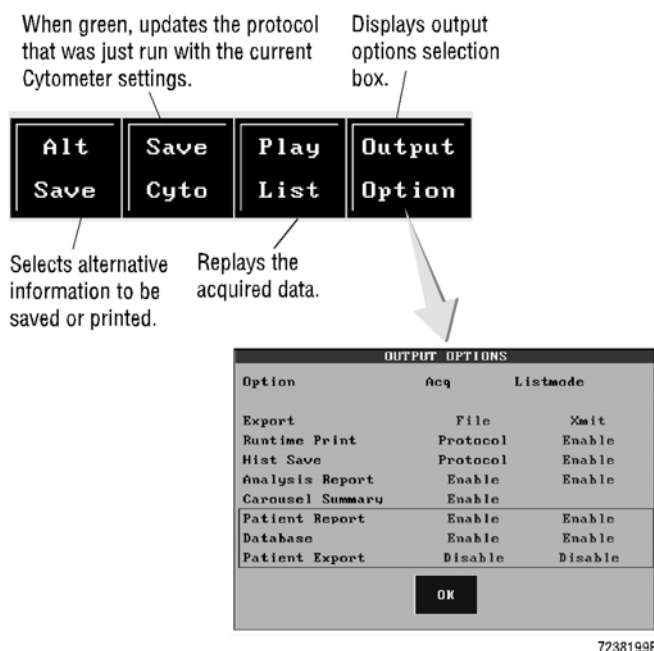
Acquisition Screen Buttons

Histogram Display Buttons

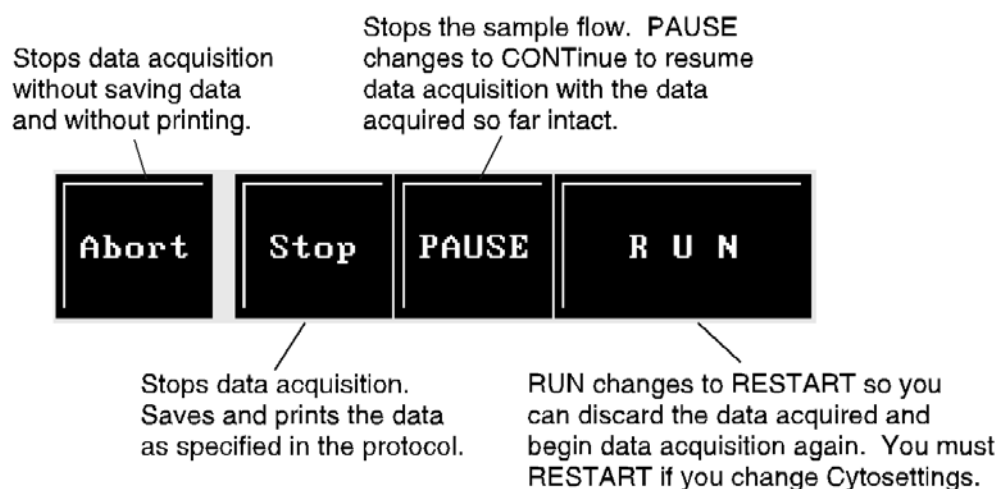
Note: Click inside a histogram to display it at full size.



File Buttons



Acquisition Buttons



7238012A

Display and Region Buttons



7238194B

Output Options Window

Select File to write the run reports output (EPT/CYT files) to a file.
Select Xmit to write the run reports output (EPT/CYT files) to a file and transmit the file out of the serial port.

Select Enabled in Listmode to print a replay data report. Protocol is the only selection for Acquisition, because the protocol setup determines if the runtime report is printed.

Select Enabled in Listmode to save the histograms. Protocol is the only selection for Acquisition, because the protocol setup determines if the histograms are saved.

Select Enabled to print the Analysis Report for an Optional Special Analysis. (Such as tetraONE™ and reticONE™ reagents).

Select Enabled to print a carousel summary immediately after running an MCL carousel.

Select Enabled to print patient reports.

Select Enabled to save results to the database. You must enable Patient Report before you can enable Database.

Selections only apply to Acquisition application. Select Disabled for any option you do not want.

Selections only apply to Listmode application. Select Disabled for any option you do not want.

Option	Acq	Listmode
Export	File	Xmit
Runtime Print	Protocol	Enable
Hist Save	Protocol	Enable
Analysis Report	Enable	Enable
Carousel Summary	Enable	Enable
Patient Report	Enable	Enable
Database	Enable	Enable
Patient Export	Disable	Disable

OK

Select Enabled to generate a patient report export file (PXP). Patient Report must be enabled to enable Patient Export.

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Cytometer Control Windows

Counters and Stops Window

COUNTERS		
28 NOV 1994		15:10:37
Total Events		0
Elap Secs		0
Events/Sec		0

STOPS		
Acq Secs	0	0
HIST 2	5000	0

Displays the Acquisition seconds (may be preset).

Displays preset number of events to stop on.

7238017B

Signal Window

The Signal Window displays a table of signal parameters and control buttons. Annotations explain the functionality of various elements:

- Total gain:** Points to the '25.00' value in the 'GAIN' column.
- Click on the value you want to change:** Points to the '10.0' value in the 'GAIN' column for the 'FL1' signal.
- Click these to increase or decrease value by small amount:** Points to the small up and down arrow buttons at the bottom.
- Click these to increase or decrease value by large amount:** Points to the large up and down arrow buttons at the bottom.
- Click here to drag population on histogram. Settings will update automatically:** Points to the 'Fast Set' button.

SIGNAL	VOLTS	GAIN
FS	500	10.0
SS	500	10.0
FL1	500	10.0
FL2	500	10.0
FL3	500	10.0
FL4	500	10.0
AUX	500	10.0

7238014B

Discriminators Window

The Discriminators Window shows settings for the sensor and gating. Annotations explain the controls:

- Click here to select the signal to use:** Points to the 'FS' button in the 'Sensor' section.
- Click here to change the value:** Points to the 'Off' button in the 'Value' section.
- Click these to increase or decrease value:** Points to the small up and down arrow buttons in the 'Value' section.
- Click here to enable gated listmode data acquisition:** Points to the 'OFF' button in the 'GATING' section.

DISCRIMINATORS

Sensor: FS

Value: Off

GATING

Listmode Gating: OFF

7238016A

Color Compensation Window

The Color Compensation Window displays a matrix of compensation values and control buttons. Annotations explain the controls:

- Click on the value you want to change:** Points to the '0.0' value in the 'FL1' row, 'FL2' column.
- Click here to drag population on histogram. Settings will update automatically:** Points to the 'Fast Comp' button.
- Click these to increase or decrease the value:** Points to the small up and down arrow buttons at the bottom.

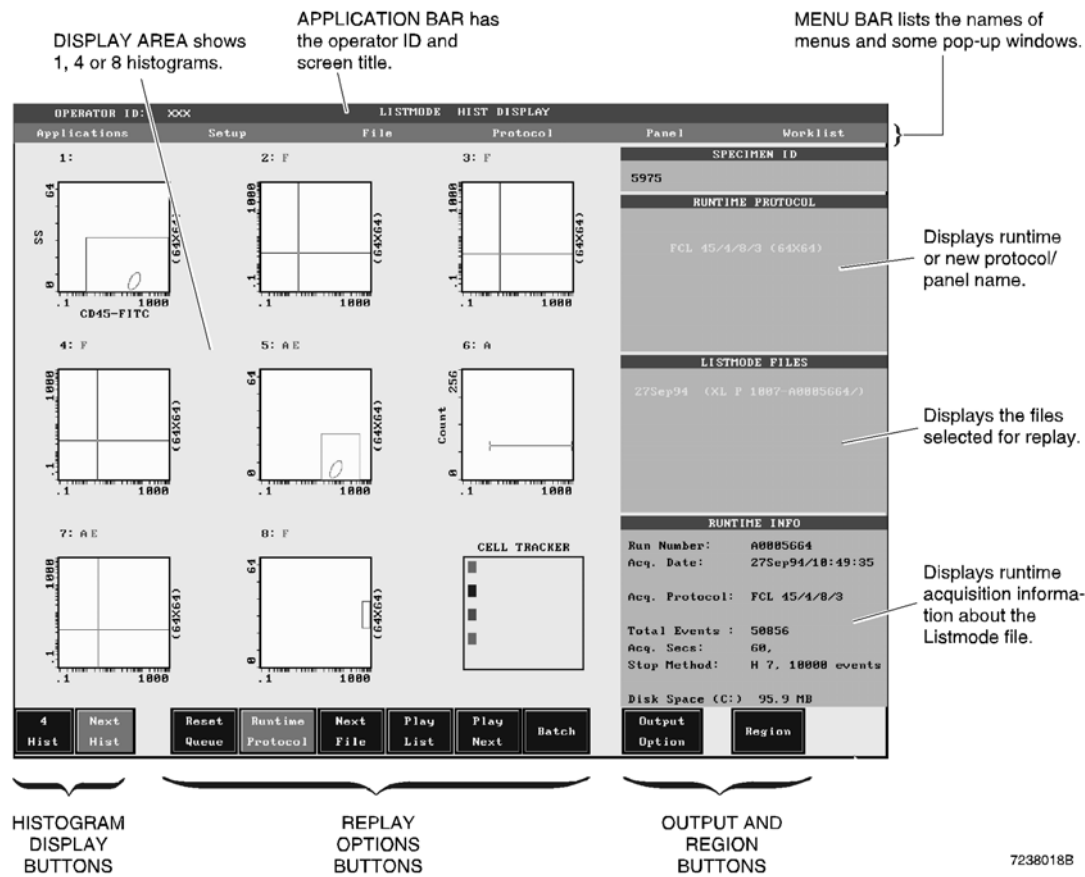
COMPENSATION

Signal out = $B - \%A$

B A→	FL1	FL2	FL3	FL4
FL1	0.0	0.0	0.0	0.0
FL2	0.0	0.0	0.0	0.0
FL3	0.0	0.0	0.0	0.0
FL4	0.0	0.0	0.0	0.0

7238015B

3.3 LISTMODE APPLICATION



Listmode Setup Menu



- Analysis**
Goes to Analysis screen to display histograms.
- Regions**
Goes to Analysis screen and activates Region button to create, edit, erase, move, copy, or redraw regions.
- Protocol**
Goes to Protocol screen to select, define, setup, and save protocols.

- Panels**
Goes to Panel screen to select, define, setup, and save panels.
- List Queue**
Goes to List Queue screen to put listmode files in a queue for batch analysis.
- File Info**
Goes to listmode file selection pop-up window to select file for replay.

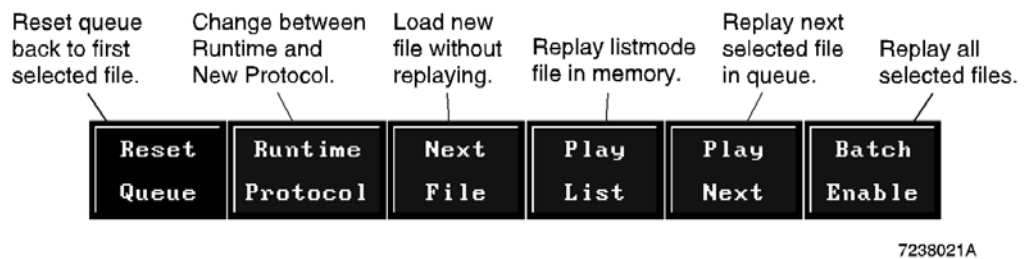
Listmode Screen Buttons

Histogram Display Buttons

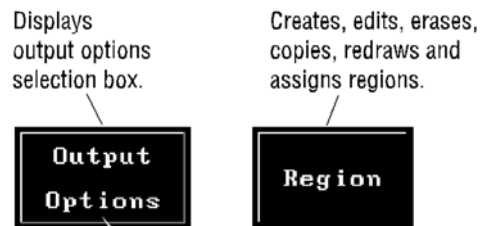
Note: Click inside a histogram to display it at full size.



Replay Options Buttons



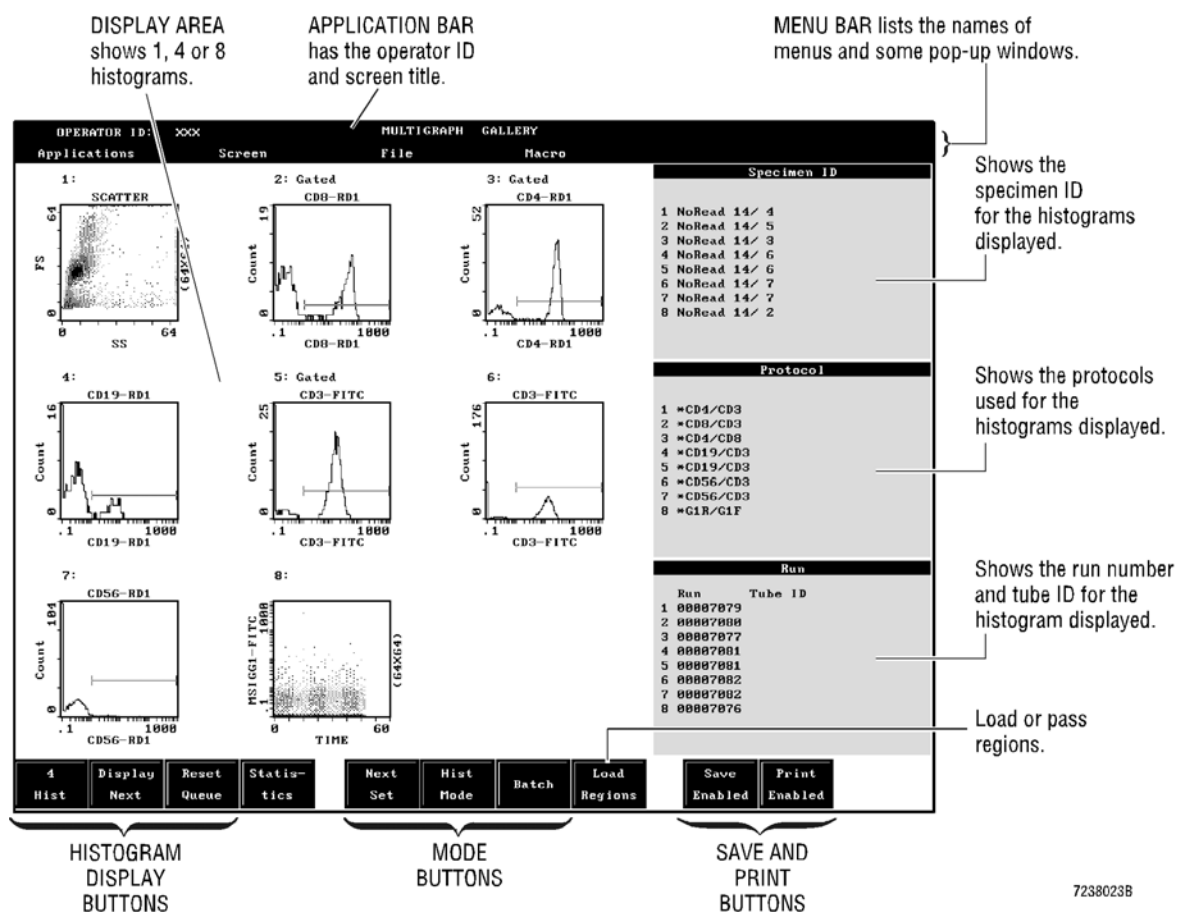
Output and Region Buttons



OUTPUT OPTIONS		
Option	Acq	Listmode
Export	File	Xmit
Runtime Print	Protocol	Enable
Hist Save	Protocol	Enable
Analysis Report	Enable	Enable
Carousel Summary	Enable	
Patient Report	Enable	Enable
Database	Enable	Enable
Patient Export	Disable	Disable
OK		

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3.4 MULTIGRAPH APPLICATION



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Multigraph Screen Menu

OPERATOR ID: QQQQ	MULTIGRAPH GALLERY
Applications	Screen
	File
	Gallery
	Single Overlay
	Dual Overlay
	Hist Queue
	Hist Data

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Gallery

Goes to Gallery screen to display and analyze up to eight histograms at one time.

Single Overlay

Goes to Overlay screen to superimpose up to eight single-parameter histograms.

Dual Overlay

Goes to Overlay screen to superimpose up to eight dual-parameter histograms.

Hist Queue

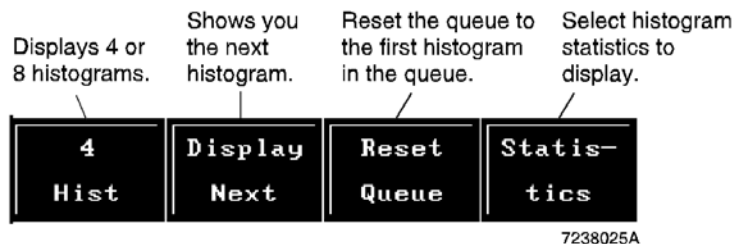
Goes to Hist Queue screen to select groups of histograms for analysis and display.

Hist Data

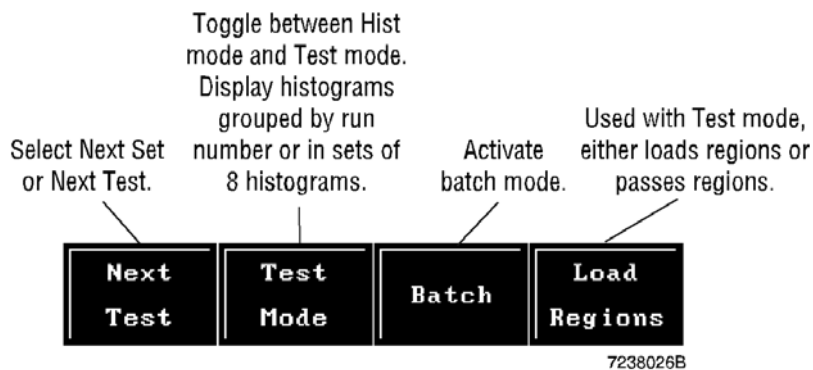
Goes to Hist Data screen to display information about the histogram.

Multigraph Screen Buttons

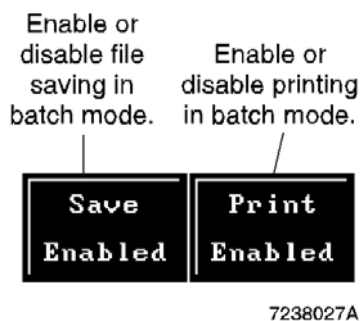
Histogram Display Buttons



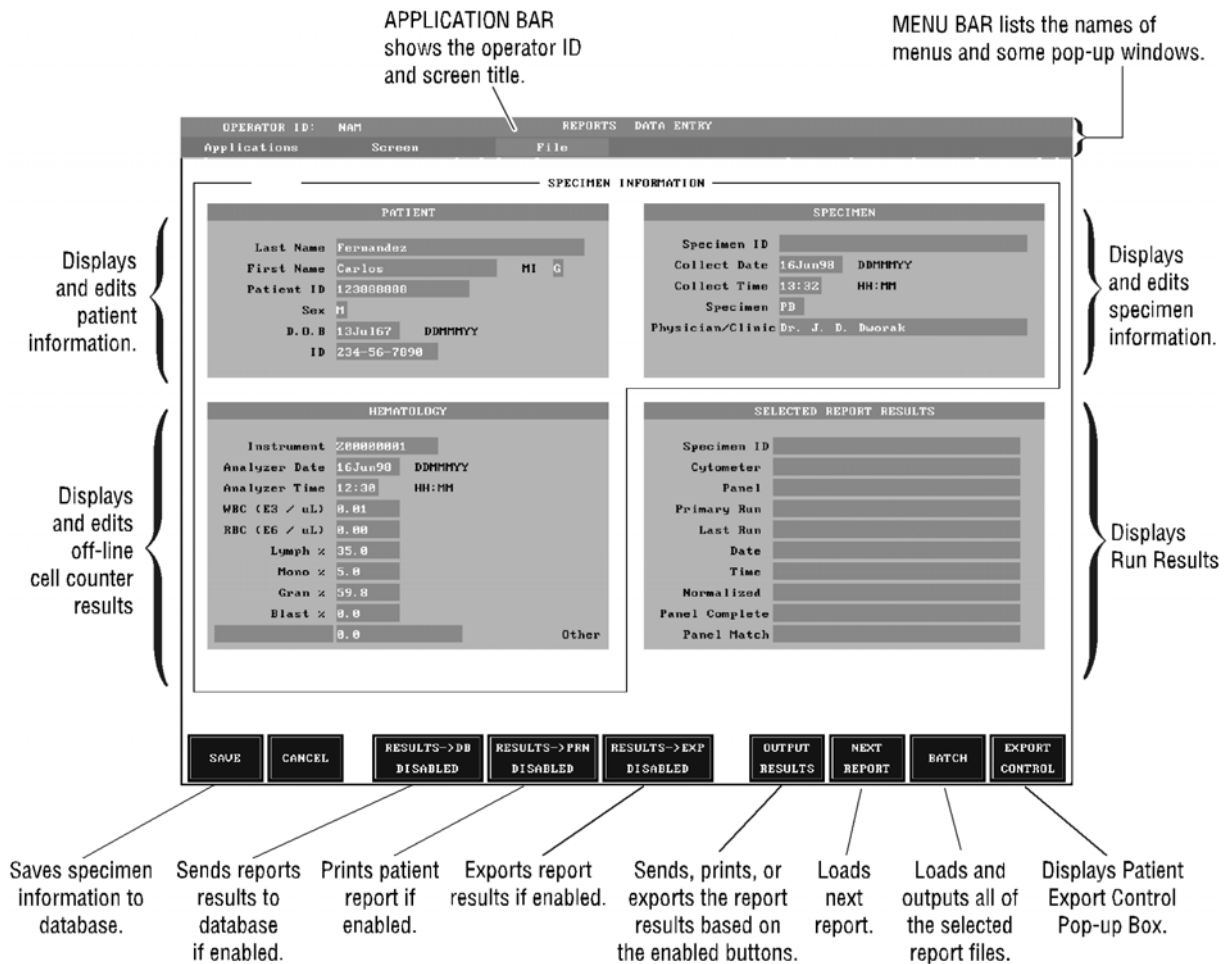
Mode Buttons



Save and Print Buttons



3.5 REPORTS APPLICATION



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Reports Screen Menu

OPERATOR ID: QQQQ		REPORTS DATA ENTRY	
Applications	Screen	File	Purge Database
	Data Entry		
	Template		

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Data Entry

Goes to Reports Data Entry screen to enter patient and specimen information and print reports.

Template

Goes to Reports Template Setup screen to define a template for your report layout.

3.6 QC APPLICATION

Displays the QC template associated with the displayed protocol file.

Displays all regions in the displayed protocol.

APPLICATION BAR has the operator ID and screen title.

Displays the protocol file that you select.

MENU BAR lists the names of menus and some pop-up windows.

Displays up to 40 QC parameters that you select.

Displays all available gains, total gains, and voltages.

Displays all available compensation values.

Scrolls the QC parameters on the template table.

Restores the screen to its last saved condition.

Deletes a row from the template table. When on (green), deletes the next row you select.

Sorts template table. When on (green), sorts by the field (Region or Name) that you select.

Clears all entries from the template table.

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QC Screen Menu

OPERATOR ID: C3P0		QC TEMPLATE	
Applications	Screen	File	
	QC-Template		
	Levey-Jennings		
	Data Table		
	Compare		
	Maintenance		

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QC Template

Defines a QC template for a protocol.

Levey-Jennings

Displays QC data in Levey-Jennings graphs according to the layout designed in the QC Template.

Data Table

Displays QC data in table format according to the layout designed in the QC template.

Compare

Goes to QC Compare screen to compare two QC files.

Maintenance

Goes to the QC Maintenance screen to record and print system maintenance log.

3.7 UTILITIES APPLICATION

Displays configuration file in use.

APPLICATION BAR shows the operator ID and screen title.

MENU BAR lists the names of menus.

Sets up facility name and report header

Sets up network node, serial communication, SQL database errors, and reporting units.

Sets up printers and bar-code characters

Displays cytometer configuration

Sets up system startup options

Sets up maximum histogram resolution and display memory allocation.

7238203B

Utilities Screen Menu

OPERATOR ID: QQQQ

UTILITIES CONFIGURATION

ApplicationsScreenFile

ConfigurationData Management

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Configuration

Changes the system configuration and startup options.

Data Management

Sets up file paths, and automatic archiving and deleting.

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3-13

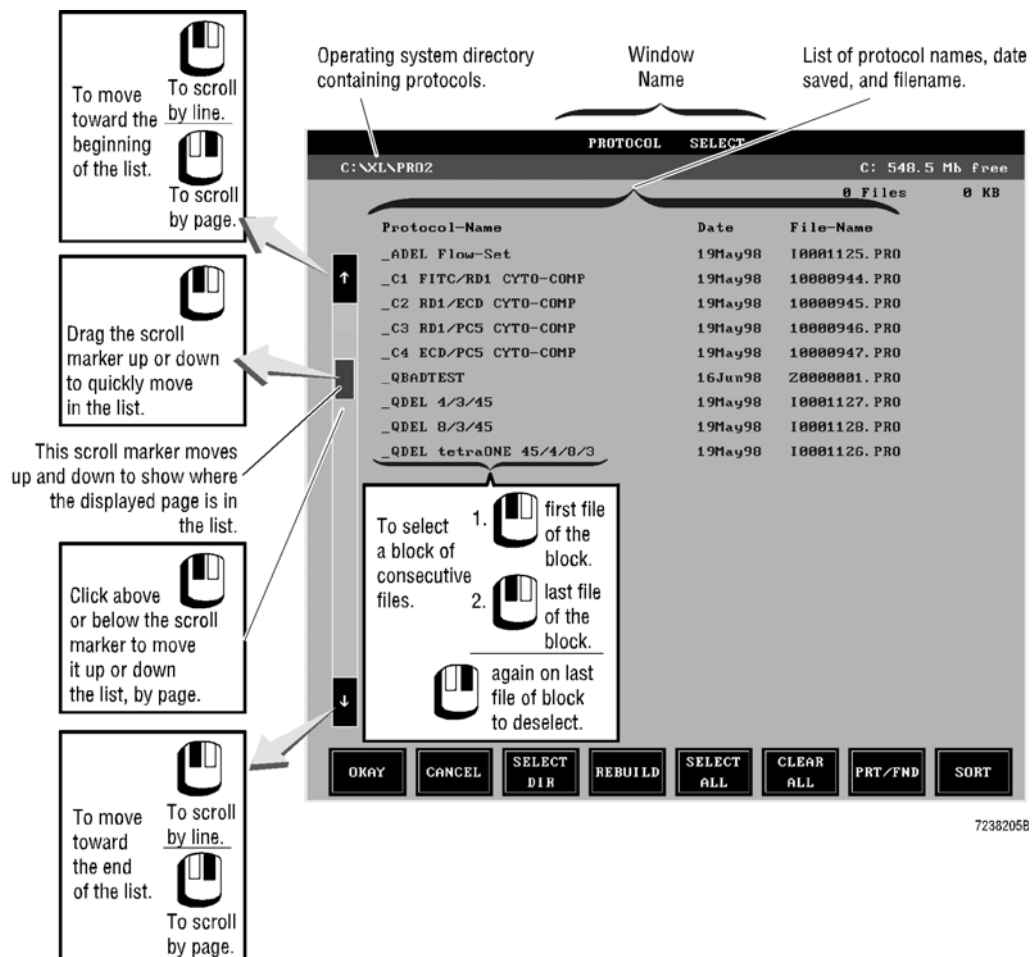
3.8 POP-UP WINDOWS

Many screens have buttons on the menu bar, which access pop-up windows. A pop-up window is a screen that is displayed on top of the current screen. The window does not completely cover the current screen, but you must close the window before you can use the current screen again.



To display a pop-up window:

1. **Applications ► Acquisition.**
2. **Setup Screen ► Protocols.**
3. **File ► Select.**



Click  on line to select it.

Click  on line again to deselect it.

Displays the previous page. 

Displays the next page. 

Displays the beginning of the list. 

Displays the end of the list. 

Accepts selections and closes the window.

Ignore changes to selections and closes the window.

Changes to a different drive or directory.

Rebuilds the directory.

Selects all the protocols.

Deselects all the protocols.

Click  to sort the files in ascending order using the column heading you select as the sort criteria.

Click  to sort them in descending order.

Click  to print a list of all the user selected protocols.

Click  to search for a string of characters you enter at the system prompt.

Note: Always sort listmode files for a listmode queue in ascending order to preserve panel functionality.

Protocol-Name	Date	File-Name
_ADEL Flow-Set	19May98	10001125. PRO
_C1 FITC/RD1 CYTO-COMP	19May98	10000944. PRO
_C2 RD1/ECD CYTO-COMP	19May98	10000945. PRO
_C3 RD1/PC5 CYTO-COMP	19May98	10000946. PRO
_C4 ECD/PC5 CYTO-COMP	19May98	10000947. PRO
_QBADTEST	16Jun98	Z0000001. PRO
_QDEL 4/3/45	19May98	10001127. PRO
_QDEL 8/3/45	19May98	10001128. PRO
_QDEL tetraONE 45/4/8/3	19May98	10001126. PRO

OKAY

CANCEL

SELECT DIR

REBUILD

SELECT ALL

CLEAR ALL

PRT/FND

SORT

Click here to select a disk or network drive.

Click here to change path by typing it in.

Click here to select a directory.

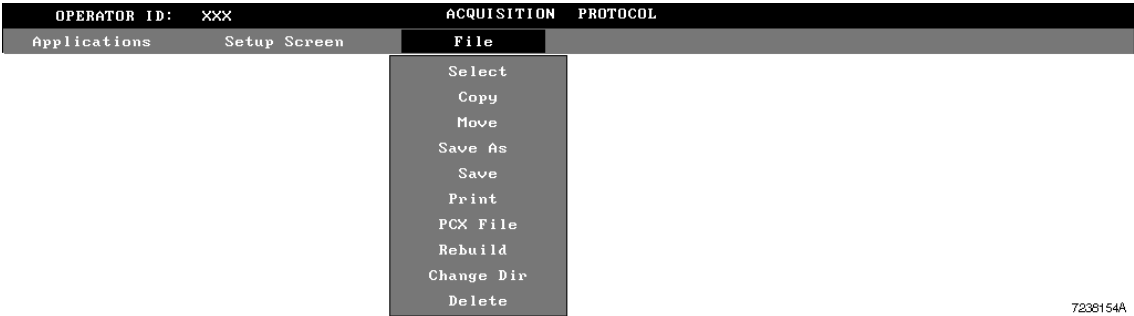
Enter

Selects the name under the cursor; it is highlighted. Press again to deselect.

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3.9 FILE MENU

The File menu is a generic menu that appears on most screens. From certain screens, specific items from the File menu are temporarily disabled. These disabled options appear gray and cannot be selected.



Select

Opens a pop-up window to select files.

Copy

Opens a pop-up window to copy files to a different directory.

Move

Opens a pop-up window to move files to a new directory. (Deletes the files from the old directory).

Save As

Saves a file with a new name.

Save

Saves a file with the same name.

Print

Prints a formatted version of the screen (not a screen dump).

PCX File

Saves the screen as a PCX graphics file.

Rebuild

Rebuilds the directory of the screen you are on.

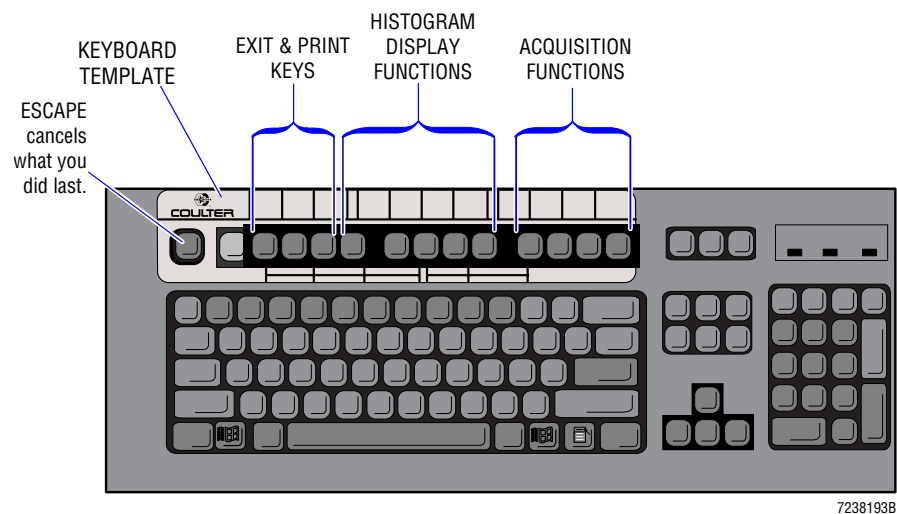
Change Dir

Changes to a different directory to search for files.

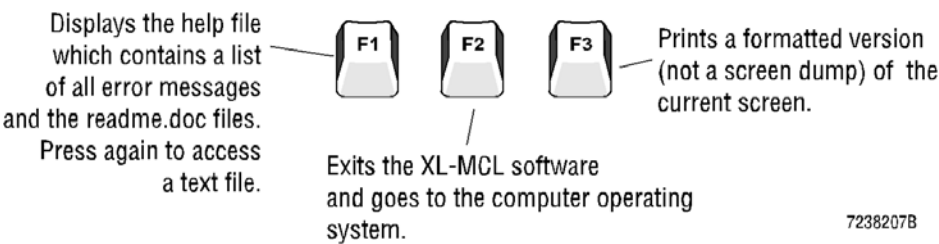
Delete

Opens a pop-up window to delete files.

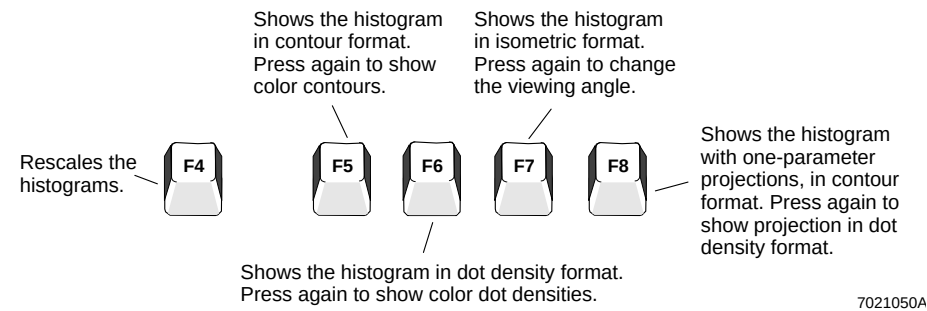
3.10 USING THE KEYBOARD



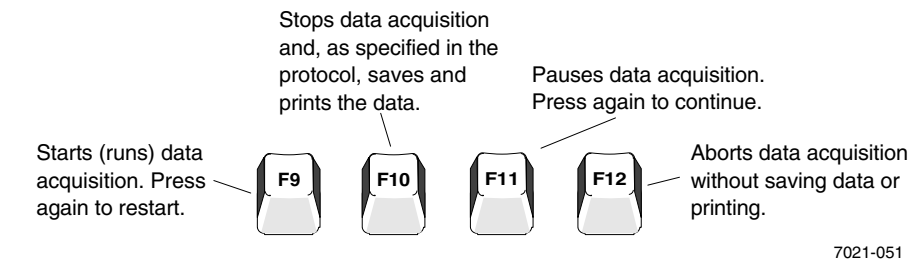
Utility Keys



Histogram Display Keys



Acquisition Keys



Selecting Menu Items with the Cursor Keys

1. Press **Ctrl**+**←** to pull down the Applications menu or press and hold down **←** or **→** until the menu title you want is highlighted.
2. Press **Enter**.
3. Press **↑** or **↓** until the item you want is highlighted.
4. Press **Enter**.

Keyboard Shortcuts/Hot Keys

In the Acquisition and Listmode applications, you can change screens without using the menus by pressing and holding down **Alt** while pressing the appropriate letter key. Keyboard shortcuts are also called hot keys.

Key	Acquisition Screen	Listmode Screen
Alt C	Cyto settings	N/A
Alt G	Run Regions	Analysis Regions
Alt H	N/A	Analysis
Alt I	N/A	File Info
Alt N	Panel	Panel
Alt P	Protocol	Protocol
Alt Q	N/A	List Queue
Alt R	Archive Status (pop-up box)	Archive Status (pop-up box)
Alt T	Protocol	Protocol
Alt U	Run	Analysis
Alt V	Software Version Number, Cytometer Version Number, Operating System Version Number, and the database file path and whether it is connected.	Software Version Number, Cytometer Version Number, Operating System Version Number, and the database file path and whether it is connected.
Alt W	Worklist	N/A
Alt Z	Cytometer Error Log	N/A

3.11 RESPONDING TO PROMPTS AND MESSAGES

Prompts

When a prompt appears on the screen, the software needs information from you.

The software uses the information displayed (called the default) unless you change it.

Sample Name: **Current sample name**

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Clearing the Prompt from the Screen

Press **Esc**. You are returned to the screen that was displayed before the prompt appeared. Any changes to the default information are ignored.

Using the Default Information

To accept the default information,

press **Enter** or  or .

Entering Information

1. Start typing. The default information disappears and the text you enter appears.
2. When finished, press **Enter** or  or .

Editing the Default or the Information You Type

- To move the cursor one character at a time, press **←** or **→**.
- To move the cursor to the end of the line, press **End**.
- To move the cursor to the beginning of the line, press **Home**.
- To insert the information, press **Insert**. To type over information, press **Insert** again.
- To erase the character under the cursor, press **Delete**.
- To erase the character to the left of the cursor, press **Backspace**.
- When finished, press **Enter** or  or .

Enter new PANEL name: **Current panel name**

7238120A

Messages

When a message appears on the screen in a green, amber, or red box something unexpected has happened. Green is for the least severe message; red, the most severe.

The sample pressure failed.

PLEASE ACKNOWLEDGE

7021-110

- The Special Procedures and Troubleshooting manual lists the error messages in the Troubleshooting chapter.
- **F1** displays a list of all possible error messages on the screen.
- **Alt+Z** displays up to 200 of the last error messages posted for your instrument.

Acknowledging a Message

To acknowledge an error message:

Press **Enter** or  or .

3.12 ADJUSTING CYTOMETER SETTINGS

Depending on the types of applications you are running, you might want to change the default values for amplification, the discriminator, and other Cytometer settings.

To adjust the Cytometer settings using the Acquisition Cytosettings screen before data acquisition,

1.  **Applications ► Acquisitions.**
2.  **Setup Screen ► Cytosettings.**

Note: Cytosettings are displayed and can be adjusted for FL4 even if you do not have the optional fourth PMT installed on your system. The Utilities Configuration screen will show your system's current configuration.

Acquisition Cytosettings Screen

When you click here, you are prompted to enter a vernier gain (fine amplification). The range is 0 to 1000. A change of 1 to 1000 represents a 1 to 4 change in gain.

Click here to change the sample flow rate (low, medium or high).

When you click here, you are prompted to enter a value (maximum 1023).

Click here to toggle to signal to use for discriminator.

PROTOCOL CYTO-COMP				FLOW RATE MEDIUM				LASER POWER 15 mW Regulated			
SIGNAL				COMPENSATION Signal out = B - %A				DISCRIMINATORS			
SIGNAL	VOLTS	GAIN	TOTAL GAIN	B	A →	FL1	FL2	FL3	FL4	Sensor	FS
FS	53	5.0	5.00							Value	100
SS	190	20.0	31.40							Listmode Gating	Disable
FL1	687	1.0		FL1		0.5	0.0	0.0			
FL2	793	1.0		FL2		14.0		16.0	4.5		
FL3	650	1.0		FL3		0.0	37.0		0.5		
FL4	687	1.0		FL4		0.0	0.0	41.0			
AUX	500	10.0	25.00								

When you click here, you are prompted to enter a value for voltage (0 to 2000).

Click in here to toggle the linear amplification (integral gain) value.

When you click here you are prompted to enter a value (0.1 increments, from 0 to 100%).

Click here to enable gated listmode acquisition.
7238077B

4.1 WHAT IS A PROTOCOL?

A protocol tells the Cytometer what data to acquire. It specifies:

- The parameters to be used
- The Cytometer settings
- The statistics to report
- The histogram definitions
- The type of analysis to be run
- The regions to use
- The histograms and regions to print
- Other information.

4.2 CREATING AND EDITING PROTOCOLS

Edit an existing protocol to create a new protocol.

1.  **Setup Screen >> Protocols.**

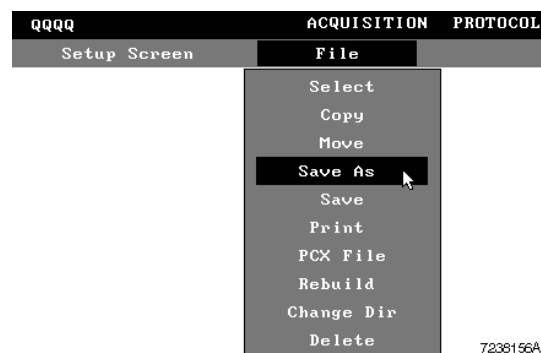


2.  **File >> Save As**, type a name for the protocol and press **Enter**.
3. Edit the protocol (select signals, define histograms, and specify functions).

When you edit a protocol, save the protocol to save the changes.

After editing and saving a protocol, reselect the protocol to use it to acquire data.

Note: The software defaults to a single space whenever you enter double spaces in a protocol name.



4.  **File >> Save.**

4.3 SELECTING SIGNALS

You can select up to eight of the signals the system generates, including AUX, RATIO, and TIME and you can assign descriptive names to some signals.

1.  **Setup Screen ► Protocols.**
2.  in the Signal Sources box, the signals you want, including any you need for RATIO or AUX.
The selected signals are highlighted in the Signal Sources box

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM:??	DEN:??
AUX	X	SIG:??	

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and appear in the Parameters box.

PARAMETERS	
Signal	User Name
FS	FS
SS	SS
FL1	FL1
FL1 LOG	FL1 LOG
FL2	FL2
FL2 LOG	FL2 LOG
FL3	FL3
FL3 LOG	FL3 LOG

7238157A

Naming Signals

1.  the signal name you want to change in the User Name column in the Parameters box.

PARAMETERS	
Signal	User Name
FS	FS
SS	SS
FL1	FL1
FL1 LOG	FL1 LOG
FL2	FL2
FL2 LOG	FL2 LOG
FL3	FL3
FL3 LOG	FL3 LOG

7238158A

- You are prompted for the new parameter name. Type a new name for the signal and press **Enter**.

Enter parameter title :

7238113A

The new name appears in the User Name column in the Parameters box.

PARAMETERS	
Signal	User Name
FS	FS
SS	SS
FL1	FL1
FL1 LOG	FITC
FL2	FL2
FL2 LOG	FL2 LOG
FL3	FL3
FL3 LOG	FL3 LOG

Erase Hist/Param

7238159A

Erasing Signals

If you decide not to use a selected signal:



the highlighted signal you do not want in the Signal Sources box.

It is removed from the Parameters box and is no longer highlighted in the Signal Sources box.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM:??	
		DEN:??	
AUX	X	SIG:??	

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Selecting and Assigning AUX

The AUX signal can only use Lin or Peak signals as its source. AUX cannot use a Log signal.

1.  **Setup Screen ► Protocols.**

2.  **??** next to **SIG:**

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: ??	DEN: ??
AUX	X	SIG: ??	

7238160A

3.  the X next to the signal you want to use for AUX, from the Signal Sources box.

The signal name appears next to SIG:

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: ??	DEN: ??
AUX	X	SIG: FL3	PEAK

7238161A

4.  the X next to AUX in the Signal Sources box.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: ??	DEN: ??
AUX	X	SIG: FL3	PEAK

7238162A

- The AUX parameter and its signal name are now displayed in the Parameters box.

PARAMETERS	
Signal	User Name
FS	FS
SS	SS
AUX	AUX(FL3 PEAK)
Erase Hist/Param	

7238163A

Editing AUX Parameter

If you want to change the AUX parameter assignment:

-  the signal name next to SIG:. It highlights.
-  the X next to the new signal you want to assign to AUX.
The new signal name appears next to SIG:.

Selecting and Assigning Ratio Parameter

-  Setup Screen ► Protocols.
-  ?? next to NUM:
You can use any of the parameters already selected, including AUX, for NUM (numerator) and DEN (denominator).

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: ??	DEN: ??
AUX	X	SIG: ??	

7238164A

3.  the X next to the signal to use as the numerator.

The signal name displays next to NUM:.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: FL1 LOG	
		DEN: ??	
AUX	X	SIG: ??	

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4.  ?? next to DEN:

You can use any of the parameters already selected, including AUX, for NUM and DEN.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: FL1 LOG	
		DEN: ??	
AUX	X	SIG: ??	

7238166A

5.  the X next to the signal to use as the denominator.

The signal name appears next to DEN:.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: FL1 LOG	
		DEN: FL2 LOG	
AUX	X	SIG: ??	

7238167A

6.  the X next to Ratio.
The X is highlighted and Ratio is displayed in the Parameters box.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: FL1 LOG	DEN: FL2 LOG
AUX	X	SIG: ??	

7238168A

Selecting and Assigning TIME Parameter

The stop time, in seconds, is entered in the Functions box on the Protocol screen.

1.  Setup Screen ►► Protocols.
2.  the X next to Time in the Signal Sources box.
The X is highlighted and Time is displayed in the parameters box.
Note: Time is scaled to a value of 1,000 by the system. Even though single-parameter histograms are scaled to 1024, time data only appears up to the 1,000 mark.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS	X	X	X
SS	X	X	X
FL1	X	X	X
FL2	X	X	X
FL3	X	X	X
FL4	X	X	X
TIME	X		
PRISM	X		
RATIO	X	NUM: ??	DEN: ??
AUX	X	SIG: ??	

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Activating PRISM in Acquisition

1.  **Protocol » Select.**
2.  the protocol you want to use.
3.  [Okay].
4.  **Setup Screen » Protocols.**
5.  the X next to Prism.
6.  **File » Save** or **File » Save As** to save the protocol.
7.  **Setup Screen » Run.**

Assign the prism equations as explained in Heading 5.6, Prism Equation Regions.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS		X	X
SS		X	X
FL1	X		X
FL2	X		X
FL3	X	X	X
FL4	X	X	X
TIME			
PRISM			
RATIO		NUM: FL1 LOG	
		DEN: FL2 LOG	
AUX	X	SIG: ??	

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Activating PRISM in Listmode

To activate PRISM in Listmode when it was not activated when the Listmode file was acquired,

1.  [Runtime Protocol] to change it to [New Pnl/ Pro].
2.  **Protocol » Select.**
3.  the protocol you want to use.
4.  [Okay].
5.  **Setup » Protocol.**
6.  the X next to Prism.
7.  **File » Save** or **File » Save As** to save the protocol.
8.  **Setup » Analysis.**

Assign the prism equation as explained in Heading 5.6, Prism Equation Regions.

SIGNAL SOURCES			
Sensor	Lin	Log	Peak
FS		X	X
SS		X	X
FL1	X		X
FL2	X		X
FL3	X	X	X
FL4	X	X	X
TIME			
PRISM			
RATIO		NUM: FL1 LOG	
		DEN: FL2 LOG	
AUX	X	SIG: ??	

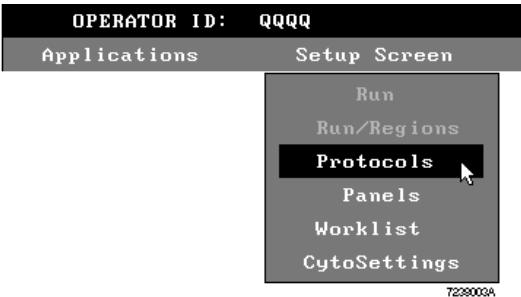
7238170A

4.4 DEFINING HISTOGRAMS

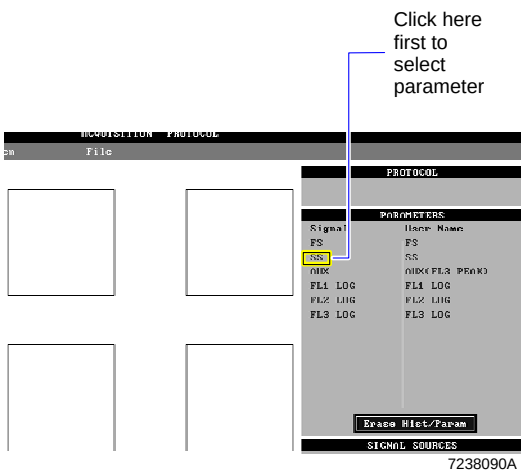
In any protocol, you can have up to eight histograms. For each of those histograms, you can specify different regions, gates, stop counts, and other settings.

Assigning Parameters and Titles to Histograms

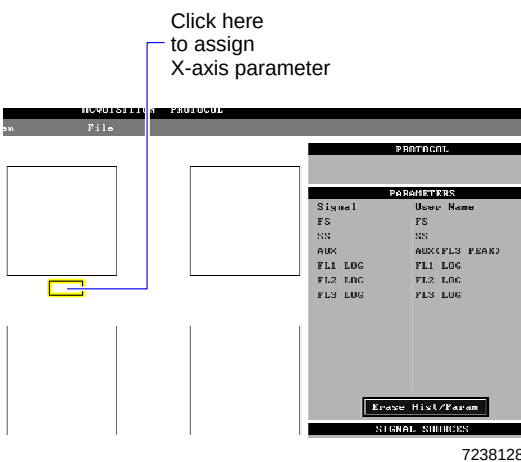
1.  Applications ► Acquisition.
 Setup Screen ► Protocols.



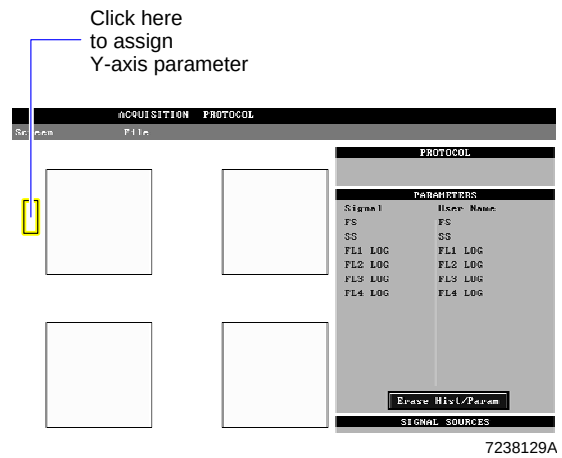
2.  the desired X-axis parameter in the Parameters box Signal column.



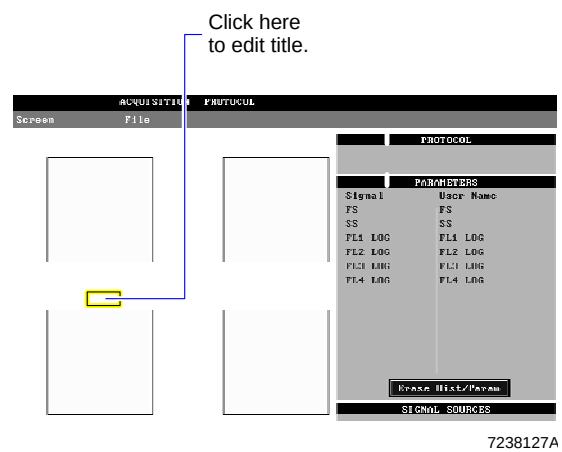
3.  the X-axis of the histogram you want to assign the parameter to.



4.  the desired Y-axis parameter, if any, in the Parameters box Signal column.
5.  the Y-axis of the histogram you want to assign the parameter to.



6.  on the top outside border of the histogram to enter a Title, if desired. Type the title and press **Enter**.



7. Repeat steps 2 through 6 to:
 Assign parameters to and name the other histograms.
 Change the assignments and names.

Erasing Parameters and Histograms

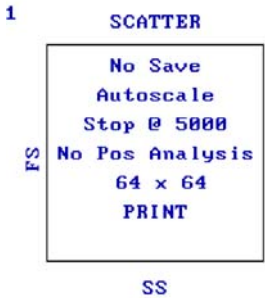
- 1.  Applications >> Acquisition.
-  Setup Screen >> Protocols.
-  Erase Hist/Param in the Parameters box.

PARAMETERS	
Signal	User Name
FS	FS
SS	SS
AUX	AUX(FL3 PEAK)
FL1 LOG	FL1 LOG
FL2 LOG	FL2 LOG
FL3 LOG	FL3 LOG

Erase Hist/Param

7238171A

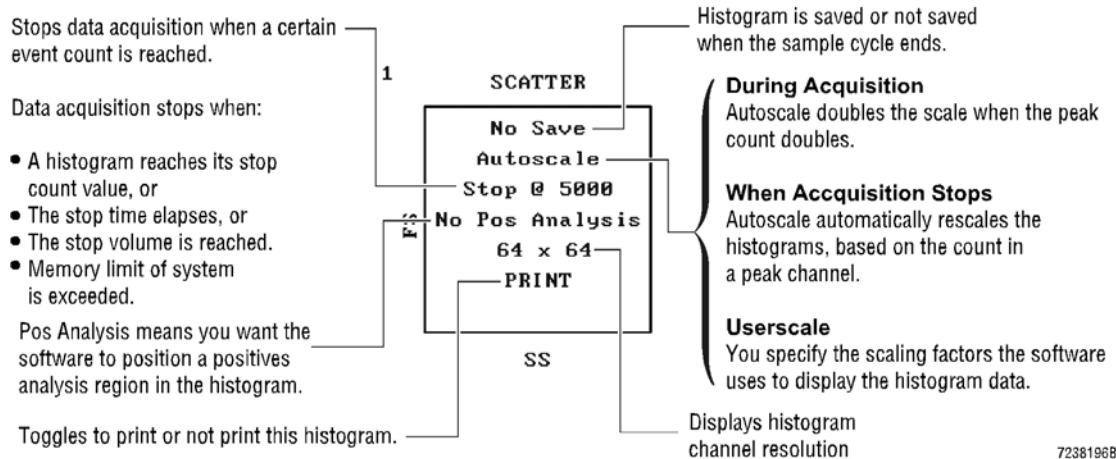
- 2.  The parameter names on the histograms you want to erase.
Note: When you click inside the histogram or erase all the parameter names on a histogram, the histogram is erased.
- 3.  Erase Hist/Param to deselect it.



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Specifying Histogram Options

When you assign a parameter to an undefined histogram, the histogram options appear inside the histogram. Select an option to change it to the desired setting. The following illustration shows the defaults. You are prompted to enter values.



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Histogram Resolution

Two-parameter histograms can be displayed in 64 x 64, 128 x 128, or 256 x 256 resolution. Histogram resolution affects region statistics because region boundaries are different at different histogram resolutions. The affected statistics can be normalized by toggling [Normalize]on (green) in the Statistics pop-up window from the Protocol screen.

Positives Analysis

When you select the positives analysis (PosAnly) option, the software positions a positives analysis region (linear or quad-stat) in the histogram at the end of the sample cycle.

If the histogram does not already have a region, the software creates one and names it Positive.

If any regions already exist, but they are not positives analysis regions, the first region in a histogram becomes a positives analysis region.

Positives analysis is typically used with the control histogram.

One-Parameter Histograms

For a one-parameter histogram (linear region), the software sets the:

- Upper boundary of the region to the highest channel of the histogram.
- Lower boundary of the region to the lowest channel needed to make the percentage of events in the region approximate the percentage defined in the Functions box on the protocol screen.
- Autoscale to a multiple of four.

Two-Parameter Histograms

For a two-parameter histogram (quad-stat region), the software sets the region boundaries to the lowest channels needed to make the sum of the percentage of events in:

- Quadrants 1 + 2, not more than n%.
- Quadrants 2 + 4, not more than n%.

You specify the value of n% in the Functions box on the Acquisition Protocol screen. In a printout generated at the end of a sample cycle, the region's name is the only indication that the region used positives analysis.

4.5 SPECIFYING FUNCTION OPTIONS

The Functions box is in the lower right of the Acquisition Protocol screen. Select an option to change it to the desired setting; the defaults are shown here.

Note: Use the statistics selection pop-up box to select which statistics to display.

RETIC-STAT PERFORMS A RUN-TIME ANALYSIS USING THE RETIC-STAT REAGENT AND CALCULATES RETICULOCYTE PERCENTAGE AND DISTRIBUTION INDICES

AVAILABLE ANALYSIS DLLs

Standard	
Cell-Stat	1.0
Retic-Stat	1.92
cellSTAT 3D	1.02T

Automatically prints histogram hardcopy at end of acquisition.

Saves listmode files.

Selects stop on time value. (Time parameter histogram scales to this setting even if you manually stop a run.)

Selects stop on volume value (overrides stop time).

Sets the minimum number of events that must be acquired in a region prior to stopping on any other stop counter (excluding time or volume).

Autoprint OFF

List Save OFF

Time Stop 300

Vol. Stop OFF

Min. Counter OFF

Listmode Gate OFF

Analysis Standard

Version

Pos Analysis : 0.0

Statistics Select

Activates listmode gating regions.

Selects between standard or optional analysis such as Cell-Stat™ or Retic-Stat™ software in pop-up window.

Shows the current software version for your instrument.

Specifies a percent for positive analysis region.

Selects type of statistical report.

Click on the statistics you want to display or not display.

SINGLE STATS	DUAL STAT	REGION PRINT
Identifier (ID)	Identifier (ID)	A GADJ AMOR
Percentage (%)	Percentage (%)	B LABJ AMOR
Peak Position X (PkPosX)	Peak Position X (PkPosX)	C1 ADJ QUAD
Peak Count (PkCnt)	Peak Position Y (PkPosY)	C2 ADJ QUAD
Count (Count)	Peak Count (PkCnt)	C3 ADJ QUAD
Mean X (Mn X)	Count (Count)	C4 ADJ QUAD
Mean Intensity X (MnI X)	Mean X (Mn X)	D CD4-IC2 RECT
Median X (Md X)	Mean Y (Mn Y)	E1 ADJ QUAD
Standard Deviation X (SD X)	Mean Intensity X (MnI X)	E2 ADJ QUAD
Full Peak CV X (FPCUX)	Mean Intensity Y (MnI Y)	E3 ADJ QUAD
Half Peak CV (HPCU)	Median X (Md X)	E4 ADJ QUAD
Minimum (Min)	Median Y (Md Y)	F CD8-IE21 RECT
Maximum (Max)	Standard Deviation X (SD X)	G ADJLIN LIN
	Standard Deviation Y (SD Y)	H1 ADJ CD4/CD8 QUAD
	Full Peak CV X (FPCUX)	H2 ADJ CD4/CD8 QUAD
	Full Peak CV Y (FPCUY)	H3 ADJ CD4/CD8 QUAD

Click on the regions you want to print. They change from blue to yellow. Regions appear grayed out and cannot be selected if the related histogram is not selected for printing.

Enters a calibrator value, for example, from Flow-Count™ Fluorospheres package insert.

Shows a scale of 0-1023 for displays and statistics of linear signals, regardless of resolution.

Selects the default statistics.

Deselects all the statistics.

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4.6 SAVING OR UPDATING A PROTOCOL

At any point in the creation of a protocol, you can save what you have done so far. As a safeguard, you should:

- Save a protocol whenever you make any changes to it.
- Archive your protocols on a weekly basis.

Saving a Protocol

1. Display the Acquisition Protocol screen.

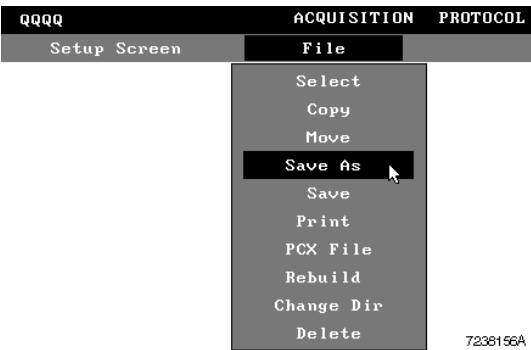


Setup Screen ► Protocols.

2.  **File ► Save** or,



File ► Save As.



Note: The software defaults to a single space whenever you enter double spaces in a protocol name.

Save saves the file with the current file name, overwriting the existing file.

Save As lets you save the file under a new file name in the same directory, prompting you for the new name.

Use **Copy** or **Move** after saving if you want to put the protocol in a different directory.

Use **File ► Select** to select a previously saved protocol.

5.1 REGION TYPES

Create regions by selecting the **Region** button at the bottom of the Acquisition Run and Listmode Analysis screens. Use the Region Definition box to specify their type.

Acquisition Regions

REGION DEFINITION box has all the histogram regions in the protocol.

Region button

Region List

Identifier that appears on the histogram when you finish drawing a region.

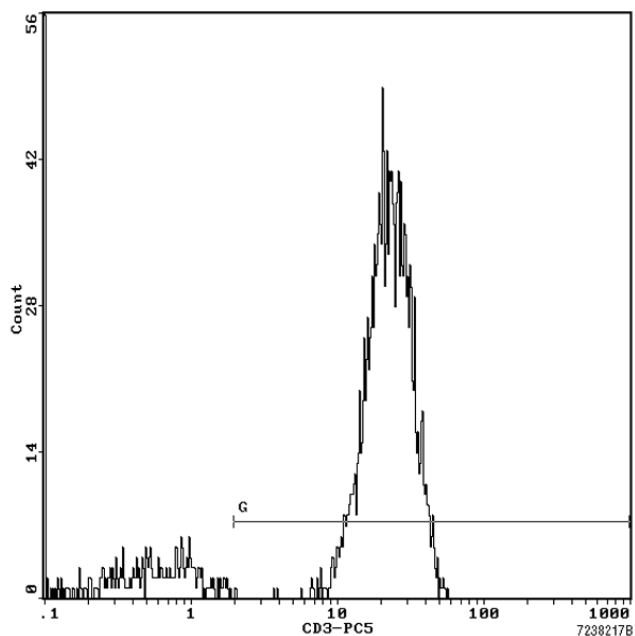
SCROLL BAR

Region types are:
 Numeric
 Linear
 Rectilinear
 Quadrant statistic (quad-stat)
 Amorphous

Up to 14-character name you enter to replace the default name.

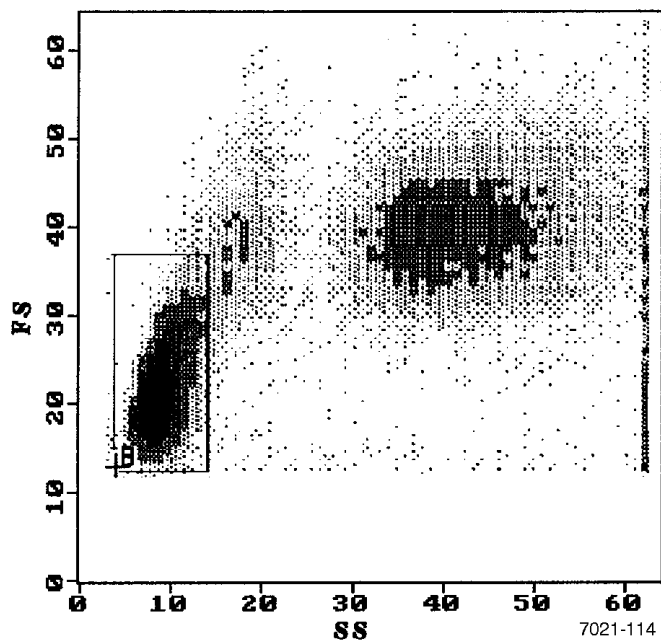
One-Parameter Histogram Regions

Numeric and Linear Regions

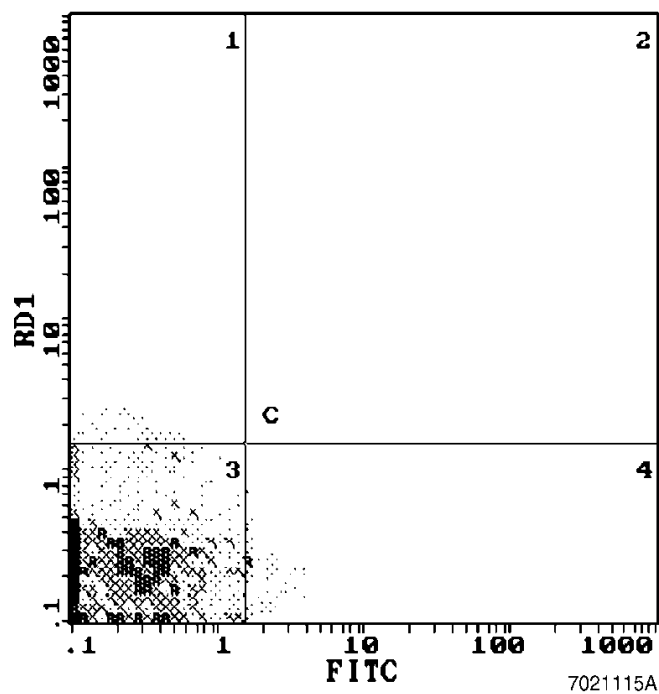


Two-Parameter Histogram Regions

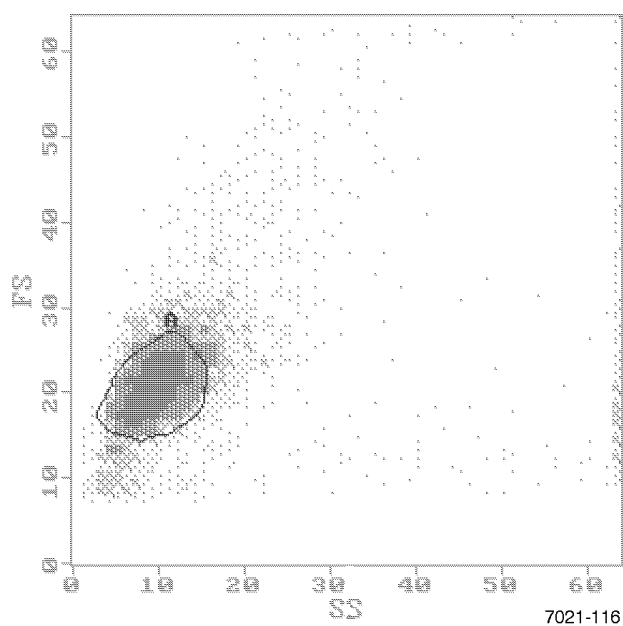
Numeric and Rectilinear Regions



Quad-stat Region



Amorphous Region



5.2 CHANGING THE CURSOR MODE

When you are working with regions, the cursor is in one of five modes: **Edit**, **Create**, **Redraw**, **Copy**, and **Erase**. The cursor mode affects what happens to screen items you select.

When you select **Region** the default cursor mode is **Edit**. Unless told otherwise, use that mode.

Acq. Date:	27Sep94/10:49:35
Acq. Protocol:	FCL 45/4/8/3 (64
Total Events :	50856
Acq. Secs:	60,
Stop Method:	H 7, 10000 events
Disk Space (C:)	97.2 MB
Output Option	Region Edit

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To change the cursor mode with the mouse:



Click left mouse button to select among:
Edit, **Create**, **Redraw**, **Copy** and **Erase**.

To change the cursor mode with the keyboard:

Press **Tab** to select among: **Edit**, **Create**,
Redraw, **Copy** and **Erase**.

5.3 ASSIGNING REGIONS

You can assign regions to be:

- Cell-Seeker™ (autogating)
- Cell-Tracker™
- Listgate
- Prime
- QC
- Calibration
- Gating
- Mapping
- Prism equation
- Lot number.

Region Type	Description
Cell-Seeker (Autogating)	Must be amorphous regions.
Cell-Tracker	Must be linear, rectilinear, or amorphous regions.
Listgate	Must be linear or rectilinear regions. Must contain "LISTGATE" in the region name. Negative listmode gates must contain "LISTGATENOT" or "LISTGATE-" in the region name.
Lot number	Must be rectilinear or amorphous regions. Must start region name with "LOT#=". Can only be in _A, _C, or _Q protocols.
Mapping regions	Must be rectilinear or amorphous regions. Must contain in the region name: • [Region ID], where Region ID is the ID letter of the region to map, or • [Lm+n], where L is a quad-stat Region ID and m and n are adjacent quad-stat quadrants' numbers.
Positives analysis	Must be linear or quad-stat regions.
Prime	Must be rectilinear regions. Must have PRIME in the region name.
Prism equation	Must be linear or quad-stat regions.
QC	Must be rectilinear, linear, or amorphous regions. Must contain "QC" at the beginning of the region name. Must only be assigned in _Q protocols.

Assigning Gates to Histograms

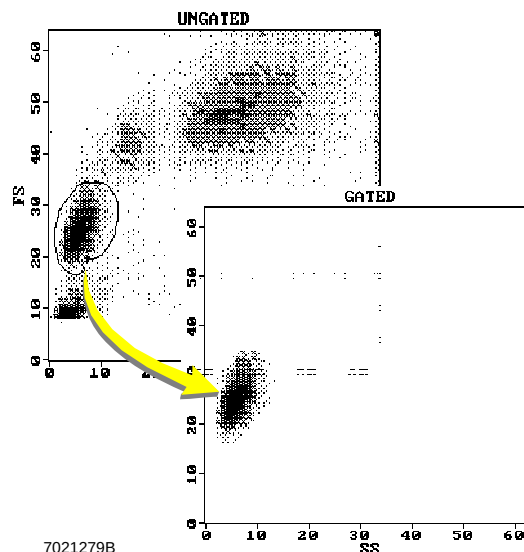
The "gating" process consists of:

Creating regions appropriate for the types of analyses you are doing.

Assigning the regions as gates so that other histograms include data from only those regions.

The software lets you create up to 24 regions in a protocol. You can use:

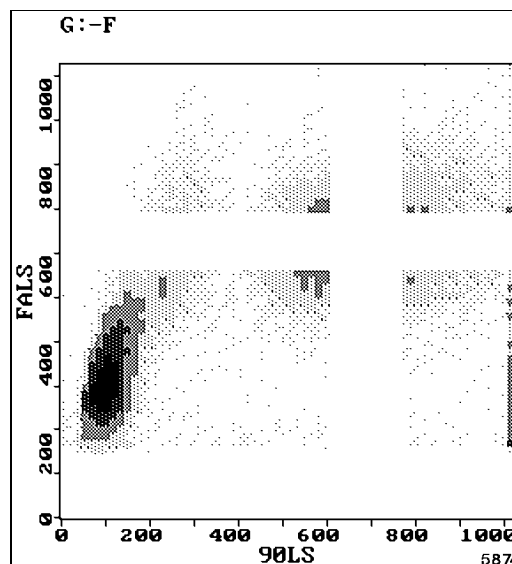
- Up to 16 of those 24 regions to gate other histograms. Each rectilinear region counts as two regions.
- The not (—) sign to exclude those events in a region.



IMPORTANT If you use rectilinear gates for — (not) gating you can exclude desired populations and report incorrect results. Use only amorphous or linear regions for — (not) gating.

To the right is an example of — (not) gating with a rectilinear region which shows why you should not use this type of gating.

Note: "OR" gating is not supported.



Assigning a Region as a Gate

1.  **Setup Screen** >> **Run/Regions**.
2.  [4 Hists] or [8 Hists] so that you are displaying more than one histogram at a time.
3.  [Region]

Acq. Date: 27Sep94/10:49:35

Acq. Protocol: FCL 45/4/8/3 (64

Total Events : 50856

Acq. Secs: 60,

Stop Method: H 7, 10000 events

Disk Space (C:) 97.2 MB

Output Option Region Edit

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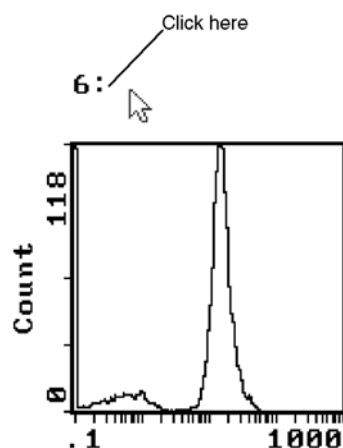
4.  **Edit** until it changes to **Create**.
5.  the region in the region list you want to use as a gate.

Note: Quad-stat regions cannot be assigned as gates.

REGIONS DEFINITION		
ID	Name	Type
A	AUTO	Amo
B	B	Rec
C	C	Qua
D	D	Lin
E		Rec
F		Rec
G		Rec
H		Rec
I		Rec
J		Rec
K		Rec
L		Rec

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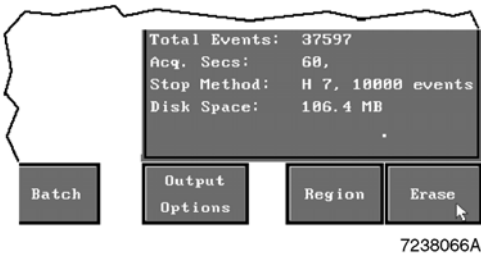
6.  the number of the histogram you are assigning the gate to. The region ID appears.
7. Repeat step 5 to gate the histogram on additional regions.



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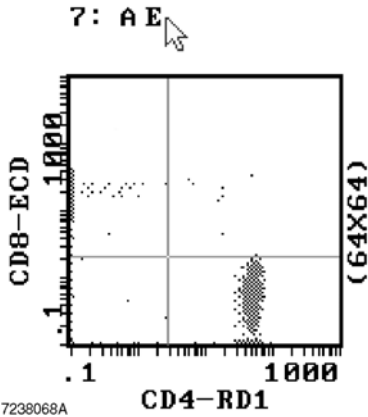
To remove an assigned gate:

1.  the cursor mode to change it to **Erase**.
2.  on the region in the Regions Definition box you do not want to use as a gate.
3.  the histogram number that has the gate you want to remove.



REGIONS DEFINITION		
ID	Name	Typ
A	AUTO	Amo
B	B	Rec
C	C	Qua
D	D	Lin
E		Lin
F		Lin
G		Lin
H		Lin
I		Lin
J		Lin
K		Lin
L		Lin

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Gating Equations

To gate a histogram using the — sign, or to edit an existing histogram equation:

1.  [Region].
2.  The histogram number above the histogram you want to gate.
Type the gate equation and press **Enter**.



Sequential Gating

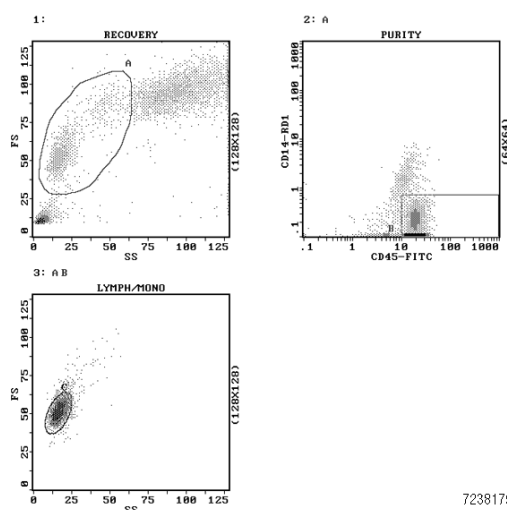
IMPORTANT For a sequentially gated histogram, gating may not work as intended resulting in possible incorrect analysis results, if you do not include all the gate regions in the gate equation.

To the right is an example of sequential gating.

Histogram 2 is gated on region A from histogram 1.

Since histogram 3 is gated on region B, it must also have region A in its gate equation because region B and histogram 2 are gated on region A.

If histogram 3 had only region B in its gate equation, the analysis results for histogram 3 would be include events outside of region A.



5.4 CELL-SEEKER (AUTOGATING) REGIONS

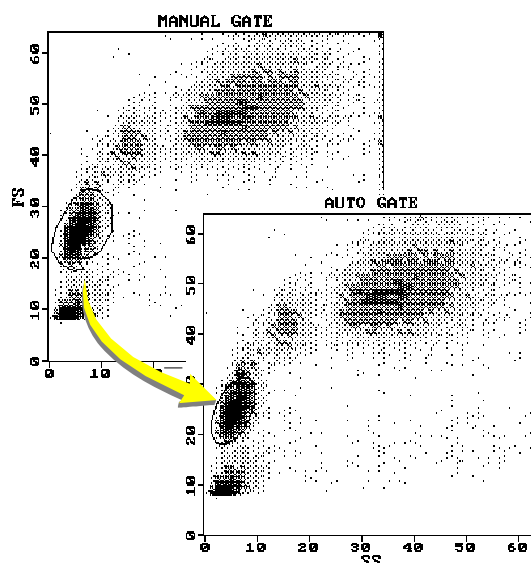
Any amorphous region with a name that begins with AUTO is a Cell-Seeker region. After 10 seconds of acquisition or 10,000 events have been acquired (whichever comes first):

- The original region is replaced by an elliptical region that encloses the cell population.
- The data acquired is replayed in the new region.

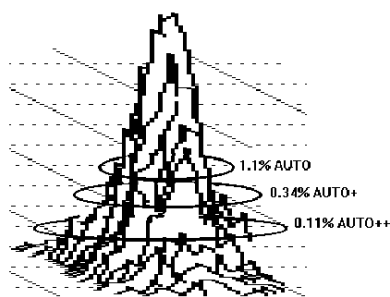
A message appears on the screen if the software cannot autogate (that is, there are insufficient events). If you are using the MCL and autogating, the message is in the carousel summary report.

You can select from three sizes of autogating regions, by changing the region name:

- AUTO draws an elliptical region around the population with the border set at 1.1% of the peak height of the processed population.
- AUTO+ draws an elliptical region around the population with the border set at 0.34% of the peak height of the processed population.
- AUTO++ draws an elliptical region around the population with the border set at 0.11% of the peak height of the processed population.



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Autogating works differently under certain circumstances:

Panel	The primary and secondary protocols are used for Autogating; the autogated region is passed on. You can edit that region in the control protocol; the edited region is passed on.
Stop-on-volume	Autogating occurs after data acquisition is complete.
Autogated histogram	You cannot autogate on a histogram if its gate equation has an autogated region in it.
[PAUSE]	Autogating does not occur whether in manual or automatic (MCL) mode.

Assigning Autogating Regions

1. Run the sample you want to assign an autogating region to.
2. Draw an amorphous region around the population you want to autogate.
3. Include AUTO, AUTO+, or AUTO++ in the region name.
4. Save the Protocol to save the region.

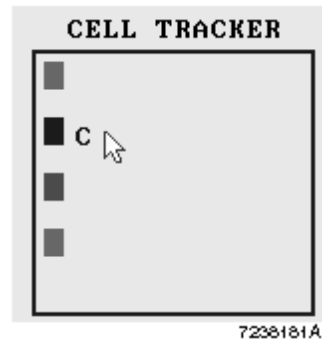


5.5 CELL-TRACKER REGIONS

Cell-Tracker software allows you to assign the events in a region to one of four colors. You can use Cell-Tracker color analysis to identify populations without using gating. The color assignments are stored with the protocol. Cell-Tracker software can be used for acquisition or listmode replay.

Assigning Cell-Tracker Regions

1.  [Region].
2.  [Edit] until it changes to [Create].
3.  [4 Hists] to change to [8 Hists], if necessary.
4.  on the region in the region box you want to assign to a color.
5.  one of the four available colors in the Cell-Tracker box that you want to use.
6.  [Region].

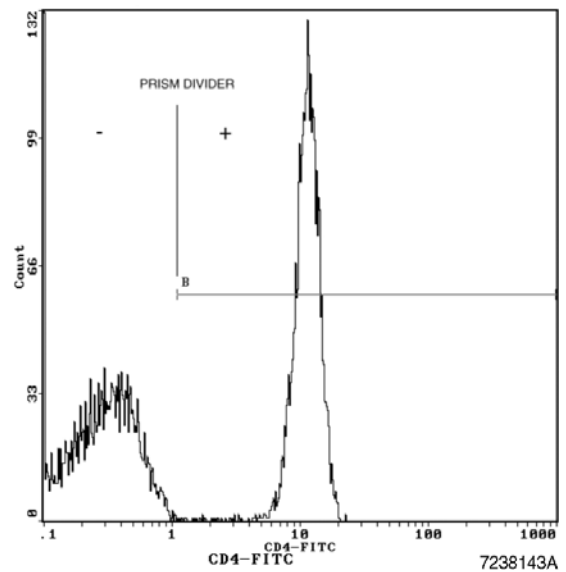


5.6 PRISM EQUATION REGIONS

Each Prism parameter requires a divider value to separate the negative from the positive population. You set the prism dividers by assigning linear or quad-stat regions to a prism equation. The prism equation can have up to four regions.

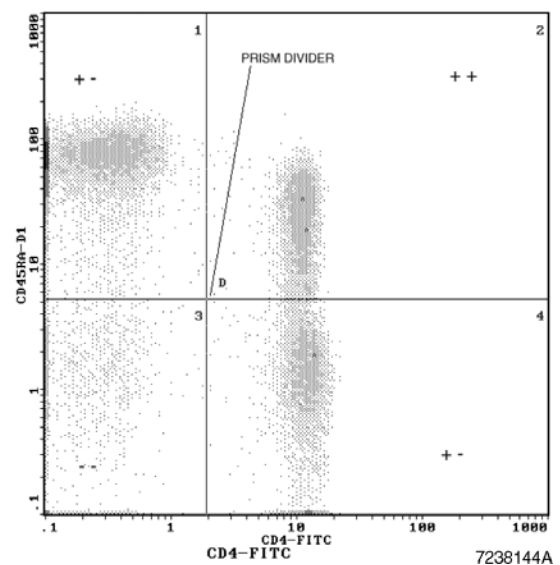
On single-parameter histograms the minimum position of the linear region assigned to the Prism Equation is the divider.

Events below the divider are negative for the surface marker and events above the divider are positive for the surface marker.



On dual-parameter histograms the intersection of the Quad-stat region is the divider.

Events below and to the left of the dividers are negative for the surface markers and events above and to the right of the dividers are positive for the surface markers.

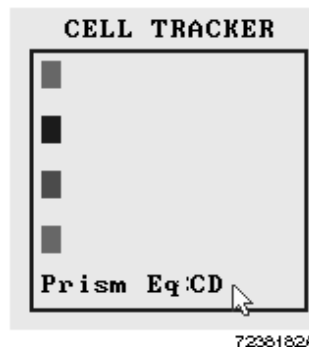


Assigning Prism Equations

Assign regions to the prism equation to display the Prism parameter as a histogram.

- Up to four parameters can be used in the prism equation.
- Only one region per histogram can be used.
- If a parameter occurs more than once in the prism equation, only the lower alphabet region ID letter is used.

1.  [Region].
2.  [Edit] until it changes to [Create].
3.  on linear or quad-stat regions in the Regions box to assign the Prism equation.
4.  Prism Eq: in the Cell-Tracker box.
5.  [OK].
6.  [Region].
7. Save the protocol using **File ► Save** to overwrite the protocol or **File ► Save As** to save the protocol under a new name.



5.7 LISTMODE GATING

Listmode gating allows you to filter data from the Cytometer to exclude events that do not satisfy the listmode gate, before they are sent to the computer for histogram building. Listmode gating can be used to reduce the size of listmode files. Only a linear or rectilinear region can be assigned as a listmode gate. Listmode gating can only be used during acquisition. Only one listgate region can be used in a protocol.

Assigning Listmode Gates

1. Run some of the sample.
2. Draw a rectilinear or linear region around the population you want to include in the listmode file (all events outside the region are excluded).
3. Name the region. The name LISTGATE (all caps) must be included in the name.

REGIONS DEFINITION		
ID	Name	Type
A	AUTO	Amo
B	Positive	Qua
C	Positive	Lin
D	LymphsLISTGATE	Rec
E		Rec

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Activating Listmode Gating

1.  Setup Screen ► Protocols.
2.  Listmode Gate in the Functions box to change it to ON.
OR
1.  [DISPLAY OPTION] until the Discriminators box is displayed.
2.  [LISTMODE GATING] to change it to ON.

Autoprint	ON
List Save	ON
Time Stop	1200
Vol. Stop	OFF
Min. Counter	OFF
Listmode Gate	ON
Analysis	Standard
Pos. Anal %	0.0
Statistics	Select

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Assigning Negative Listmode Gates

Negative listmode gating allows you to retain all the data outside of the gated region and to exclude the data inside the region. Only linear or rectilinear regions can be assigned as a negative listmode gate.

1. Run some of the sample.
2. Draw a linear or rectilinear region around the population you want to exclude in the listmode file (all events outside the region are included).
3. Name the region. LISTGATENOT (all caps) or LISTGATE- must be included in the region name.

5.8 PRIME REGIONS

Prime regions are used to initiate an autoprime if the peak of a histogram does not accumulate in the region within 10 seconds after beginning acquisition, or every second thereafter, or 10,000 events are acquired (whichever comes first). Only linear or rectilinear regions can be assigned as Prime regions. If three autoprimes are issued without the peak of a population accumulating in the Prime region, the run aborts.

Prime regions are disabled if you set a stop on volume in the Functions box on the protocol screen.

Assigning Prime Regions

Include the name PRIME (all caps) in the name of a linear or rectilinear region to assign it as an autoprime region.

REGIONS DEFINITION		
ID	Name	Type
A	AUTO	Amo
B	POSITIVES	Qua
C	POSITIVES	Lin
D	LYMPHPRIME	Rec
E		Rec

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5.9 MAPPING REGIONS

Any rectilinear or amorphous region can be assigned to map:

- An amorphous region
- A rectilinear region
- A single quad-stat region quadrant
- Two adjacent quadrants of a quad-stat region.

The rectilinear or amorphous mapping region assumes the coordinates of the mapped region or quadrant. If the mapped region is an amorphous region, then the rectilinear mapping region is resized to exactly fit around the limits of the amorphous region.

Use mapping regions:

- To gate on one or two quadrants of a quadstat region.
- To automatically adjust a rectilinear region around a population (map a rectilinear to an autogate).

Assigning Mapping Regions

Create a rectilinear or amorphous region on the histogram where you want a mapping region.

To assign a mapping region to a rectilinear or an amorphous region, use [] (square brackets) around the ID letter of the rectilinear or amorphous region for the name of the mapping region.

To assign a mapping region to a quadrant of a quad-stat region, use [] (square brackets) around the quadrant ID for the name of the mapping region. For example, mapping region name [B4] tracks quadrant 4 of quad-stat region B.

To assign a mapping region to two adjacent quadrants of a quad-stat region, use [] (square brackets) around the two quadrant IDs joined by a plus sign for the name of the mapping region. For example, mapping region name [B1+3] tracks quadrants 1 and 3 of quad-stat region B.

Note: Due to statistical rounding, the percent from a region that maps two quadrants can differ slightly from the sum of the percents of the two quadrants.

The mapping region must be created on either the same type of histogram as the region it maps to or on a histogram with the same parameters on the same axes as the region it maps to.

REGIONS DEFINITION		
ID	Name	Type
A	AUTO	Amo
B	Positive	Qua
C	Positive	Lin
D	[A] tracksA	Rec
E	[B4] tracksB4	Rec
F		Rec

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REGIONS DEFINITION		
ID	Name	Type
A	A	Rec
B	B	Qua
C	[B1+3]	Rec

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5.10 CALIBRATION REGIONS

If a region in a histogram contains a population of a known concentration, name the region CAL to use it as a calibration region. There can be only one CAL region in each protocol or panel. Do not enter the calibration factor in the region name.

To enter the calibration factor, for example, from the Flow-Count Fluorospheres package insert:

1.  **Setup Screen ►► Protocol.**
2.  Statistics in the Functions box.
3.  CAL FACTOR in the Statistics box.
4. Type the calibration factor found in the Flow-Count Fluorospheres package insert and press .

The system multiplies the count and peak height statistics for all the regions by the calibration factor, divided by the number of events in the calibrator region. The screen and the printout display the adjustment factor. If the number of events in the calibrator region is less than 1,000, the statistics are not adjusted.

The CAL Factor is a system function, not a protocol setting, and is not saved with the protocol but applies to all protocols that have a Calibrator region.

Statistics adjusted by the CAL Factor are saved to the database record and can be printed on patient reports.

CAL Factor adjustments to the statistics take precedence over hematology adjustments to the statistics on patient reports.

The adjusted statistics can also be saved to the histogram file, to the export file, or to a listmode file.

5.11 QC REGIONS

A QC region in a _Q protocol contributes its full statistics to the QC data (*.QCC) file for viewing in the Levey-Jennings graphs or QC data table. You define which statistics appear when you define the QC template for the _Q protocol.

QC regions:

- Must contain QC in their file names.
- Can only be rectilinear, linear, or amorphous.
- Can only be made in _Q protocols.

REGIONS DEFINITION		
ID	Name	Type
A	A	Rec
B	B	Qua
C	QC QUAD1	Rec

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Assigning a QC Region

1.  **Protocol >> Select.**
2.  a _QxyzABCD protocol.
3.  [Okay].
4. Create any rectilinear, linear, or amorphous QC regions you want to generate statistics like PkPosX for this protocol. Be sure to include QC in the region name.

5.12 LOT NUMBER REGIONS

Lot number regions are made as a means of identifying the lot number of the control materials and reagents used for Autostandardization panel. The lot number (from the region name) is written to the QC data file (*.QCS or *.QCC) for the related protocol and can be viewed in the QC template, the Levey-Jennings graphs, and the QC data table.

Lot number regions:

- Must be named LOT#= followed by up to eight letters and/or numbers.
- Can only be rectilinear or amorphous.
- Can only be assigned once per protocol.
- When assigned to _Q protocols, writes its lot number into the associated QC data (*.QCC) file - in the file header.
- When assigned to _A protocols, writes its lot number into the associated QC Cytometer setting baseline (*.QCS) file - in the file header.
- When assigned to _C protocols, writes its lot number into the associated QC Cytometer setting baseline (*.QCS) file - with its file entry.
 - When there are multiple _C protocols in an Autostandardization panel, the lot number of each _C protocol replaces the lot number of the previous _C protocol, so that the recorded lot number is from the last _C protocol. It is recommended to use the lot number from the CYTO-COMP™ kit as the lot number in the _C protocols.

REGIONS DEFINITION		
ID	Name	Type
A	LOT#=1234ABCD	Rec
B		Num
C		Rec

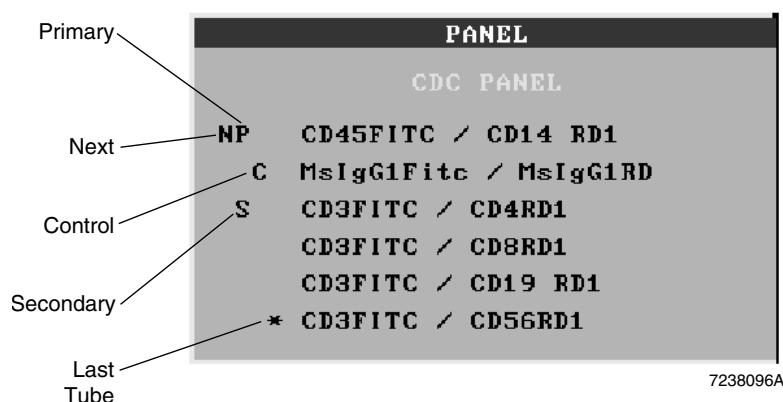
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6.1 WHAT IS A PANEL?

A panel is a group of linked protocols that you can save under a name you choose.

When you create a panel:

- You list the protocols in a certain order. Each protocol corresponds to a sample tube.
- You specify assignments of protocols as Secondary (S), Control (C), or Next (N).
- The software automatically assigns Primary (P) and Last Tube (*) markers.



A panel contains only protocol names (up to 32), not the protocols themselves. The panel directs the software to the appropriate protocol for the content of the protocol. The passing of Cytometer settings and regions from the primary, secondary, and control protocols apply only in the panel. Outside of the panel, the protocols remain as they are.

The protocol assignments apply only to the panel in which they are made. They do not affect other panels, or the protocol itself. For example, a protocol that is assigned as a primary in one panel can be assigned as a control in another.

The specimen ID is the same for all of the tubes in a panel.

6.2 PANEL PROTOCOL ASSIGNMENTS

The chart below shows how protocols function within a panel when they are assigned as either a primary, secondary, or control protocol.

Function	Primary	Secondary	Control	Test
First tube in a panel	✓			
Gets Cytometer settings and regions from the protocol or from the Cytometer settings baseline file (*.QCS) for protocols with the same prefix as the Autostandardization panels.	✓	✓		
In Autostandardization panels: A _Axyz protocol gets the Cytometer settings from the xyz.QCS data file that is referenced by the "xyz" prefix in the protocol name. It also adjusts the cytosettings to standardize fluorescence intensities per the application.	✓			
In Autostandardization panels: A _C protocol gets the Cytometer settings that were updated by the _A protocol run before the _C protocol in the panel. It also adjusts the compensation values per the application and updates the same xyz.QCS file.		✓		
In Autostandardization panels: A _QxyzABCD protocol in the panel gets the Cytometer settings from the xyz.QCS data file that is referenced by the xyz prefix in the protocol name. It also creates or updates the xyzABCD.QCC file with quality control statistics from the QC regions defined in the protocol.		✓		
Gets regions and region names from previous primary, secondary, or control protocol.			✓	✓
Passes regions to next tube.	✓	✓	✓	
Passes Cytometer settings to next tube.	✓	✓		
Autogating performed.	✓	✓		
Positives analysis performed.			✓	
Initiates patient report.	✓			
Initiates export file.	✓			
Initiates database record.	✓			
Can be converted to a secondary.	✓		✓	✓
Can also be a control.	✓	✓	✓	
Ends panel.	✓	✓	✓	✓

Primary Assignment

The software automatically assigns Primary as the first tube in a panel. When you create a panel, the Primary tube must also be Next because Next denotes the first tube to be run.

Cytosettings, regions, and region names are passed from the primary protocol to the protocols that follow it.

Control Assignment

If a protocol in a panel is assigned as the control:

- You can create and edit regions in that protocol while it is the current protocol in the panel or utilize positive analysis feature.
- Those regions are passed on to the other protocols in that panel.

Secondary Assignment

A protocol assigned as a Secondary is similar to one assigned as a Primary except it does not start a new panel. Cytosettings and regions are passed from the secondary protocol to the protocols that follow it. This is useful when adding panels together to analyze the same specimen. Protocols that were assigned as primary in the individual panels can be assigned as Secondary in the larger panel. You can assign more than one secondary protocol in a panel.

Next Assignment

When you display the Acquisition Panel screen to create a panel, the protocols in the panel are displayed in a numbered list. By default, protocol 1 is assigned as Next, but you can reassign the next protocol to reorder the panel. Next cannot be used to skip or repeat a tube.

Passing Cytometer Settings in a Panel

The software passes the Cytometer settings in the primary or secondary protocol to subsequent protocols until the next secondary protocol is reached.

This means that the settings for:

- Flow rate
- Volts
- Gains
- Compensation
- Discriminators

which were used for the first tube (the primary protocol), are also used for subsequent tubes up to the next secondary protocol.

If you want to change the Cytometer settings and have those changes passed on throughout the panel, you must make those changes to the primary or secondary protocols during Acquisition. The only exception is when you use protocols that have the same prefix as the Autostandardization protocols. In that case, the settings are automatically updated.

Changes made to the Cytometer settings in the other protocols in the panel are used and saved only to those specific protocols (with [Save Cyto] turned on); they are not passed on.

The software can also pass the Cytometer settings from the QC data (*.QCS) file. For protocols that have the same prefix used in the Auto Setup panels, for example 3CL 45-FITC/4-RD1/3-PC5, the Cytometer settings are accessed from the *.QCS data file (3CL.QCS in this example) stored on the hard drive.

Passing Regions in a Panel

IMPORTANT Incorrect analysis results can be reported if regions are not passed in a panel. If histogram X-axis and Y-axis parameter assignments do not match, regions are not passed. If you want to pass the regions in a panel, make sure that the parameters on the X- and Y-axes of a particular histogram number match throughout a panel.

For example, the parameters on the X- and Y-axes of histogram 3 in the primary protocol must match the parameters on the X- and Y-axes of histogram 3 in the other protocols in the panel if the regions in histogram 3 are to pass from the primary protocol. If the parameters on the axes do not match, the regions that existed in the new protocol are used; no passing into that histogram occurs.

Passing Region Names in a Panel

Region names are passed from the primary protocol to the protocols that follow it.

Gating in a Panel

Histogram gate equations, which are used for gating, are not passed in a panel. If you want the same gating used throughout a panel, give all the protocols in the panel identical gates and gate equations, using the same region ID letters.

Cell-Seeker (Autogating) Regions in a Panel

In a panel, autogating works only in the primary and secondary protocols. The autogated regions can be edited in the primary, secondary, and control protocols and passed on from there.

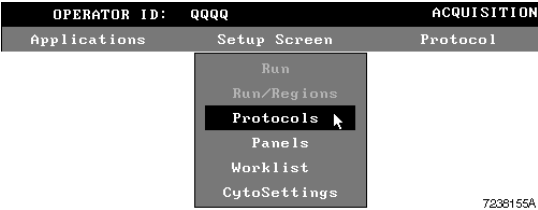
Passing Other Regions in a Panel

Cell-Tracker (color assignment) equations, Calibration (CAL) regions, and Prism equations are not passed in a panel.

6.3 CREATING A PANEL

Selecting Protocols

1. Display the Acquisition Protocol screen.
-  Applications ► Acquisition.
-  Setup Screen ► Protocols.
2. Clear the Protocol Select window:
-  File ► Select.
-  [Clear All].



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3.  The protocols you want in the panel.
-  [Okay].



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Making Assignments and Ordering Protocols

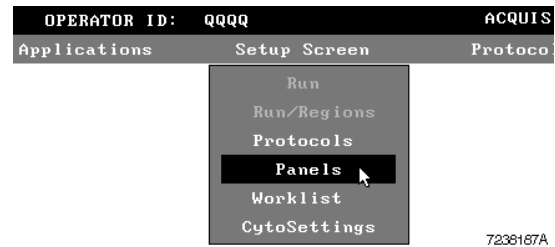
1. After selecting protocols, display the Acquisition Panel screen.



Setup Screen ► Panels.

The screen lists the protocols, in alphabetic order, with all of them highlighted.

The first protocol is flagged as N (Next) and P (Primary).



2. Reorder the protocols, if necessary.



[Next].



the protocol you want to run first.



[Order].



the remaining protocols in the order you want.



[Order]. The protocols are listed in numeric order. The first protocol is now flagged as Primary and Next.



3. Make the control protocol assignments.



[Control].



the protocol or protocols you want to flag as C (controls). The letter C appears next to each protocol selected.



[Control].

- Make the secondary protocol assignments.

 [Secondary].

 the protocol or protocols you want to flag as Secondary (S). The letter S appears next to each protocol selected.

 [Secondary].

- Check that the protocol assignments and order are what you want.

To delete a protocol from a panel, change the cursor mode to Erase and then select the protocol to be deleted. To cancel the deletion, select the deleted protocol again.

The date displayed for the protocol is the date the protocol was last modified at the time the panel was created.

Panel Name: CDC PANEL

1	NF	CD45FITC / CD14 ED1	20Dec94	G0024002.PRO
2	C	Mo1gC1Fito / Mo1gC1RD1	20Dec94	G0024003.PRO
3	S	CD3FITC / CD4RB1	20Dec94	G0024004.PRO
4		CD3FITC / CD8RB1	20Dec94	G0024005.PRO
5		CD3FITC / CD19 RB1	16Jan95	G0024006.PRO
6		CD3FITC / CD56RB1	20Dec94	G0024001.PRO

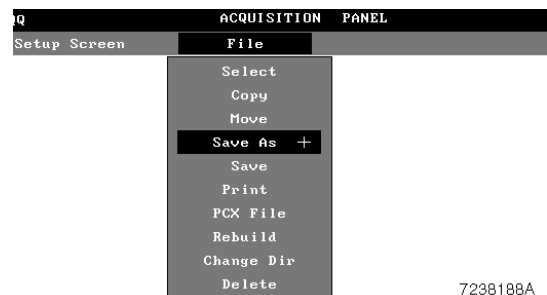
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Saving a Panel

After making assignments and ordering the protocols, save a panel.

 **File ► Save As.**

You are prompted for a panel name.



Adding Panels

To add panels together to make a larger panel to analyze one specimen:

1.  [Panel].
2.  the first panel you want to use.
3.  [Okay].
4.  **Setup Screen ► Panels.**
5.  [ADD Panel].
6.  the panel you want to add.
7.  [Okay].
8.  [Secondary].
9.  the primary protocol from the added panel, to change it to a secondary.
10.  [Secondary].
11. Repeat steps 5 through 10 to add other panels.
12.  **File ► Save As.**
Type a name for the new panel and press .



Adding Protocols

To add a protocol to an existing panel:

1.  [Panel].
2.  the first panel you want to use.
3.  [Okay].
4.  **Setup Screen ► Panels.**
5.  [ADD Protocol].
6.  the protocol you want to add.
7.  [Okay].
8. Repeat steps 5 through 7 to add other protocols.
9. Change the order of the protocols if necessary.
10. Assign control protocol if necessary.
11. Assign secondary protocol if necessary.
12.  **File ► Save As.**
Type a name for the new panel and press .



7.1 THE XL OR XL-MCL FLOW CYTOMETER AND THE MS-DOS® OPERATING SYSTEM

CAUTION System integrity might be compromised and operational failures might occur if:

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

Your XL or XL-MCL flow cytometer software runs under the MS-DOS operating system. When you select the Exit item from the Applications menu, you turn off the XL or XL-MCL flow cytometer software and enter the operating system. (See the MS-DOS manuals for more information.) The Cytometer remains on.

The computer drive designations are:

C:\ hard disk
A:\ 3.5-in. diskette drive

Note: For instruments with a 5.25-in diskette drive:

C:\ hard disk
A:\ 5.25-in. diskette drive
B:\ 3.5-in. diskette drive

Restarting the Software from the MS-DOS Operating System

To return to the XL or XL-MCL flow cytometer software from the MS-DOS operating system, type XL2 at the C:\XL> prompt and press **[Enter]**. If the Cytometer is off, restarting the software turns it back on.

The XL command has the following syntax:

```
C:\XL> XL [+h] [+l] [+m] [-a] [-l] [-m] [-u]
```

where the items in square brackets are optional switches.

The [+letter] switches force rebuilding of the Histogram, Listmode, and Macros directories, respectively.

The [-letter] switches force the software to start in a particular application. Respectively, these are Acquisition, Listmode, Multigraph, and Utilities.

To turn off the Cytometer from the operating system, enter this command at the C:\XL> prompt:

```
C:\XL> xloff
```

Note: Do not use MS-DOS keyboard commands (such as **[Ctrl]+[S]**) while using the XL or XL-MCL flow cytometer.

7.2 THE XL OR XL-MCL FLOW CYTOMETER AND MICROSOFT® WINDOWS™ SOFTWARE

The XL and XL-MCL flow cytometer SYSTEM II software is not compatible with Microsoft Windows software but can coexist with Windows 95 on a FlowCentre Multimedia Workstation.

If you have a FlowCentre™ Multimedia Workstation, you can request your Coulter Representative to set up the XL/XL-MCL flow cytometer icons on the Windows 95 desktop. You can then launch the XL or XL-MCL flow cytometer software from this icon within the Windows 95 software on your FlowCentre Multimedia Workstation.

Do not install smartdrv.exe. This device driver is not compatible with XL and XL-MCL flow cytometer software.

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