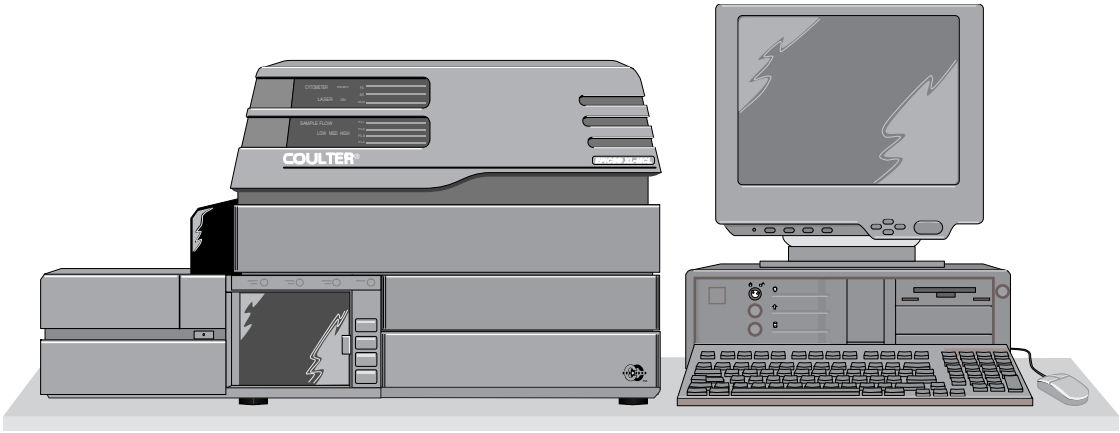
COULTER® EPICS® XL™ Flow Cytometer
COULTER EPICS XL-MCL™ Flow Cytometer
SYSTEM II™ Software

Reference



PN 4237298CA (September 2010)

Beckman Coulter, Inc.
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Brea, CA 92821

BECKMAN COULTER

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.

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 C, 01/08
Changes were made to the Intended Use statement on page 1-1 and to the laser description on page 4-2.

Issue CA, 09/10

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.

REVISION STATUS

CONTENTS

ILLUSTRATIONS

TABLES

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 instrument 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 need 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 appear at the back of the manual.

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

Use the **Master Index** to easily locate a topic in any of your manuals.

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 Reference manual provides in-depth information about what the instrument does, the methods it uses, its specifications, and information on installation, safety and software features.

This information is organized as follows:

- Chapter 1, Use and Function
  Contains the intended use of the instrument, the reagents used, and a short description of the major components and options.
- Chapter 2, Installation
  Contains instrument requirements, and diagrams of the interunit cable connections.
- Chapter 3, Operation Principles
  Contains a description of flow cytometry, the normal sample flow through the

instrument, how light collection and signal processing are accomplished and how the parameters are derived.

- Chapter 4, Specifications/Characteristics
  Details the instrument and performance specifications, the performance characteristics and interfering substances.
- Chapter 5, Precautions and Hazards
  Describes laser safety precautions and the location of the laser-related labels.
- Appendices
  The appendices provide reference material on the following topics:
  - Log Sheets
  - Protocols
- Glossary
  The glossary has definitions for many of the words and terms used in flow cytometry.
- 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 Enter).

▢+▢ indicates that the two keys listed (such as Alt+F2) 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, Y Enter.

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/µL) throughout the manuals.

1.1 INTENDED USE

The XL and XL-MCL flow cytometers are systems intended for use as in vitro diagnostic devices for the qualitative and quantitative measurement of biological and physical properties of cells and other particles. These properties are measured when the cells pass through a laser beam in single-file.

The instrument can simultaneously measure forward scatter, side scatter, and three or, optionally, four fluorescent dyes using a single laser at 488 nm. Therefore, the instrument can do correlated multiparameter analyses of individual cells.

Table 1.1 lists the major applications for the instrument. In addition to human cells, other cell types can be analyzed, such as:

- Plant cells
- Marine plankton
- Animal cells
- Bacteria

Table 1.1 Applications for the Instrument

Applications	Cell Types	Sample Preparation (refer to the reagent's package insert)	Measurements	Parameters
Cell surface antigens	Whole blood Buffy coats Mononuclear cells Dissociated tissue Platelets Bone Marrow	ImmunoPrep™ reagents with the Q-PREP™ Workstation, the Multi-Q-Prep™ Workstation or the TQ-Prep™ Workstation Whole blood lysing reagent kit Ficol Fluorescent-labeled antibodies IOTest 3 lysing OptiLyse C lysing VersaLyse	Cell size and granularity FITC, RD1, ECD, and PC5	Log/Linear Forward and side scatter Log/Linear fluorescence one, two, three, and four (optional) Prism Ratio Time AUX
Nucleic acids	Whole blood Dissociated tissue Frozen sections Paraffin sections Body fluids	Various staining methods, including: Ethidium bromide Propidium iodide DNA Prep reagents	Nucleic acid content Green, red, and orange fluorescence	Forward and side scatter Log/Linear Fluorescence one, two, and three (four is optional). Ratio, AUX

Table 1.1 Applications for the Instrument *(Continued)*

Kinetics	Whole blood Buffy coats Mononuclear cells Tissue culture cells	Various methods, including: Fluo 3 fluorescence	Cell size and granularity Green fluorescence Time	Forward and side scatter Fluorescence one Ratio Time
Cell function	Whole blood Buffy coats Mononuclear cells Tissue culture cells	Various methods and dyes, including: DCFH-DA $DiOC_5(3)$ FDA	Cell size and granularity Green fluorescence	Forward and side scatter Fluorescence one
Reticulocytes	Whole blood	Thiazole orange coriphosphine O (CPO)	Cell size and granularity Green fluorescence Red fluorescence	Log/Linear Forward and Side Scatter Log Fluorescence one Log Fluorescence four

1.2 SYSTEM COMPONENTS

The system components are shown in Figure 1.1.

Cytometer

This unit analyzes the sample. It contains the sheath fluid and cleaning agent bottles. A fourth fluorescence sensor and MCL (Multi-tube Carousel Loader) are optional.

Power Supply

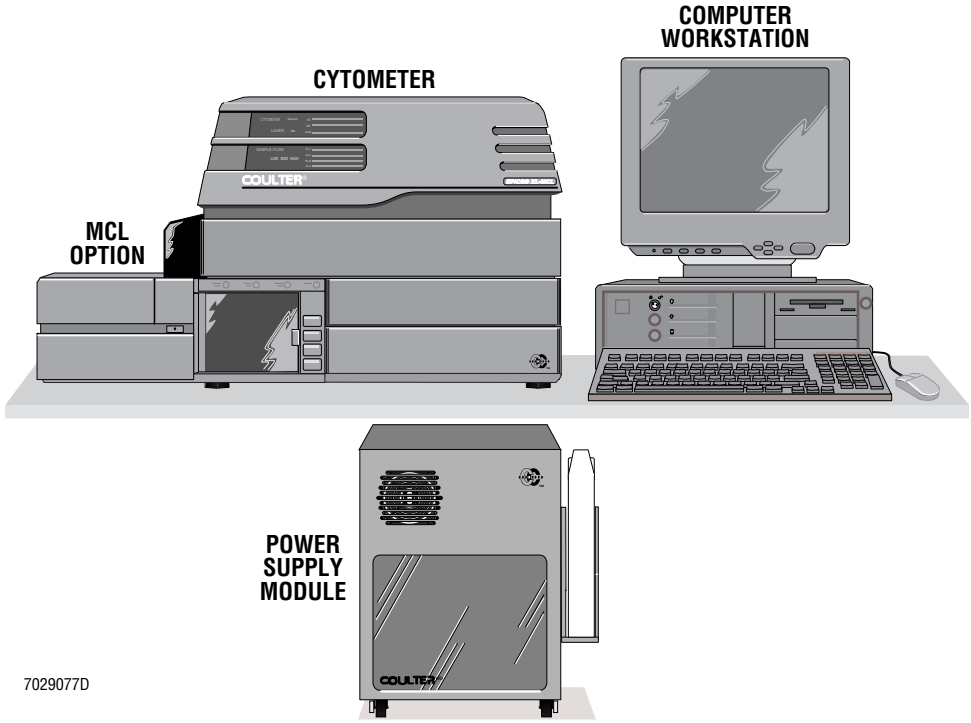
This unit provides dc power, pressure, and vacuum to the Cytometer. A 4-L waste bottle sits in a bracket on the right side of the Power Supply.

Workstation

The Workstation runs the software that enables you to control the instrument. It displays sample results and other information. It consists of:

- A monitor
- A computer with data storage devices
- A keyboard and a mouse.

Figure 1.1 EPICS XL-MCL Flow Cytometry System



1.3 OPTIONS

Hardware Options

Multi-tube Carousel Loader (MCL)

The MCL is an automated sample loader for the instrument. It uses a carousel that holds thirty-two 12- x 75-mm test tubes. The MCL reads the following bar-code types:

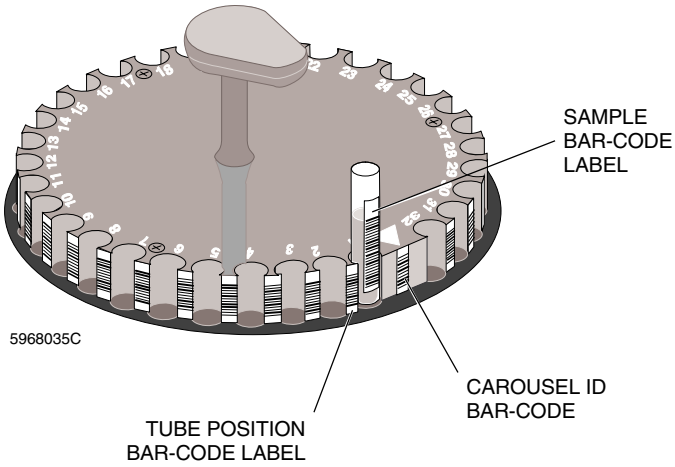
- Codabar
- Code 39® bar code
- Code 128
- Interleave 2-of-5.

For additional information on the bar-code specifications, see Heading ?, Bar-Code Specifications.

The MCL mixes each sample before analysis. You can interrupt the MCL to manually run Stat samples, and then have the MCL return to where it left off. A Worklist and MCL carousel report can be displayed or printed.

Figure 1.2 shows the location of the carousel number, tube position, and sample tube bar-code labels on the MCL carousel.

Figure 1.2 MCL Carousel Bar-Code Labels



Printer

Provides a printout of sample results and other information. The Printers listed here are subject to change, so contact your Beckman Coulter Representative for the correct model selection:

- HP LaserJet® V
- HP 1200C and 1600C color printers
- HP 560C color printer.

Bar-Code Printer

Used to print bar-code labels from the Worklist for identification of sample tubes in the MCL carousel.

Bar-Code Scanner

A hand-held scanner/reader used to read specimen collection bar-code labels. A fast, accurate method for inputting specimen and sample tube information and tube ID into the software database.

Data Storage

- 2.6-GB optical drive
- 650-GB external, recordable CD-ROM drive
- Iomega® Zip™ external 100-MB drive (parallel)

Network Kits

Single and multi-node network kits used to connect an XL or XL-MCL system to an existing workstation.

Workstation/Network Server

A COULTER FlowCentre™ Multimedia Workstation can be used as an off-line analysis workstation and network server for data and worklist transfer. The standard workstation that runs the flow cytometer can be upgraded to a FlowCentre Multimedia Workstation.

Fourth FL Sensor

This optional kit contains a photo-multiplier tube with a 200- to 800-nm spectral range and a 675-BP dichroic filter that are used to generate the following signals (parameters):

- FL4 (can be assigned to the AUX parameter)
- FL4 LOG
- FL4 PEAK (must be assigned to the AUX parameter).

RAM Upgrade Kits

Kits to upgrade 8 MB RAM systems to 16 MB RAM.

Software Options

tetraONE™ System

Software and reagent kits for performing automated four-color immunofluorescence analysis on the instrument.

reticONE™ System

Software and reagent kits for performing automated reticulocyte enumeration on the instrument.

MULTICYCLE® Software

DNA analysis software.

Artisoft® LANtastic® Software

Network operating system software.

Sybase® SQL Anywhere™ Software

Allows access to the System II database for extended queries.

1.4 REAGENTS AND QUALITY CONTROL MATERIALS

Beckman Coulter recommends these reagents or their equivalents. All stated performance characteristics in this manual are based on the use of the XL and XL-MCL system with the following reagents.

Sheath Fluid

In the Cytometer, sample is guided into a stream of sheath fluid to make the sample cells flow single file through the laser beam. IsoFlow™ sheath fluid, a nonfluorescent, balanced electrolyte solution, is made for this purpose.

IsoFlow sheath fluid has the following characteristics:

- Filtered to 0.2 µm
- Transparent and nonfluorescent to 488-nm laser light
- Low background
- Compatible with the characteristics of the sample being measured (such as pH, osmolality, conductivity).

The sheath container has a working capacity of about 2 L. The amount of sheath fluid the container holds beyond the working capacity is for pressurization and level sensing.

Cleaning Agent

When the Cytometer is in the Cleanse mode, COULTER CLENZ® cleaning agent flushes sample tubing and helps to reduce protein buildup and particles in the instrument. Each cleanse cycle uses about 15 mL of cleaning agent.

The cleaning agent container has a working capacity of about 500 mL. That is enough cleaning agent to use the Cleanse mode once a day for one month. The amount of cleaning agent the container holds beyond the working capacity is for pressurization and level sensing.

Read the container's label for more information on the cleaning agent.

Quality Control Materials

The quality control materials available from Beckman Coulter are:

Flow-Check™ Fluorospheres	Fluorospheres used to check the stability of the optical and fluidic systems.
Flow-Set™ Fluorospheres	Fluorospheres used to standardize light scatter and fluorescence intensity.
CYTO-TROL™ Control Cells	Lyophilized lymphocytes with assay values for specific surface antigens. Used to: • Assess monoclonal antibody function. • Verify proper flow cytometer setup.
Immuno-Trol™ Cells	Stabilized erythrocytes and leukocytes with a known quantity of surface antigens. Used to verify monoclonal antibody performance as well as verify the process of sample staining, lysing, and analysis.
CYTO-COMP™ Cell Kit	Lyophilized lymphocytes used with the CYTO-COMP Reagent Kit to adjust color compensation settings for multicolor analysis.
CYTO-COMP Reagent Kit	Four two-color fluorescent reagents used with the CYTO-COMP Cell Kit to adjust color compensation settings for multicolor analysis.

1.5 MATERIAL SAFETY DATA SHEETS (MSDS)

To obtain an MSDS for Beckman Coulter reagents used on the XL or XL-MCL flow cytometer:

1. In the USA, either call Beckman Coulter Customer Operations (800-526-7694) or write to:

   Beckman Coulter, Inc.
   Attention: MSDS Requests
   P.O. BOX 169015
   Miami, FL 33116-9015

2. Outside the USA, contact your Beckman Coulter Representative.

2.1 DELIVERY INSPECTION

The instrument is tested before shipping. International symbols and special handling instructions are printed on the shipping cartons to inform the carrier of the precautions and care applicable to electronic instruments.

CAUTION Possible instrument damage could occur if you uncrate the instrument, install it, or set it up. Keep the instrument in its packaging until your Beckman Coulter Representative uncrates it for installation and setup.

When you receive your instrument, carefully inspect all cartons. If you see signs of mishandling or damage, file a claim with the carrier immediately. If separately insured, file the claim with the insurance company.

2.2 SPECIAL REQUIREMENTS

Before your Beckman Coulter Representative arrives to install the instrument, you must determine where you want the system placed and the overall layout. Consider the factors described in the following paragraphs.

Space and Accessibility

Allow room to interconnect the system components. Also, arrange for:

- Comfortable working height
- Space for ventilation, and access for maintenance and service:

Cytometer	30.5 cm (12 in.) from the back 45.7 cm (18 in.) from the top 30.5 cm (12 in.) on both sides
Power Supply	Power Supply 12.7 cm (5 in.) from the back

Electrical Input

CAUTION Possible instrument damage could occur if you put the Power Supply plugs on the same electrical circuit or use an extension cord or a power strip to connect the Power Supply. Use two dedicated 115-V, 20-A outlets with isolated grounds for the Power Supply plugs.

The Power Supply requires two dedicated 115-V, 20-A outlets with isolated grounds. The computer requires a separate 115-V, 20-A outlet, but it does not have to be a dedicated line.

Ambient Temperature and Humidity

Keep the room temperature between 16°C and 32°C (60°F and 90°F), and do not let it change more than 5°F per hour. Keep the humidity between 5% and 80%, without condensation.

Heat Dissipation

Heat dissipation is 2,300 W (7,850 Btu/hour). Provide sufficient air conditioning (refer to Ambient Temperature and Humidity).

Drainage

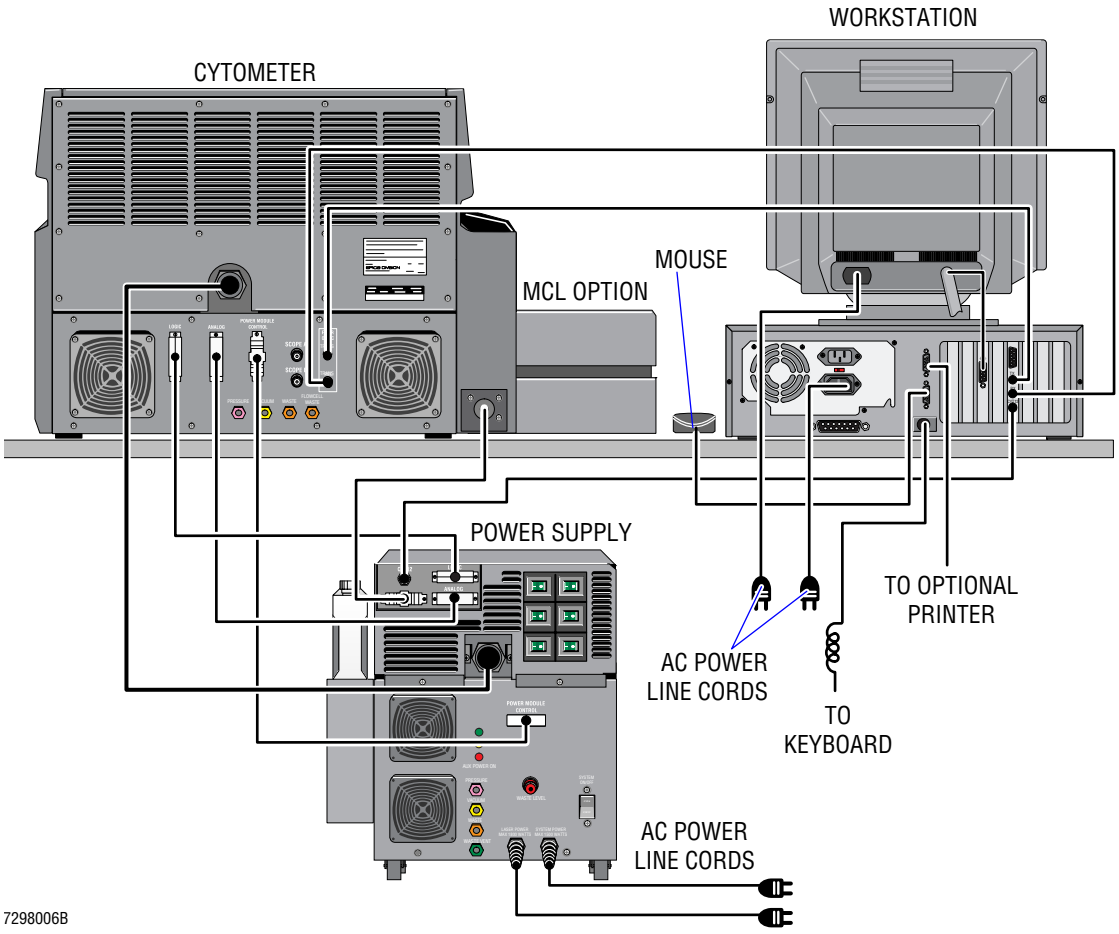
The waste line from the Cytometer is connected to a 4-L waste bottle, which sits in a bracket on the right side of the Power Supply. Dispose of the waste in accordance with your local environmental regulations and acceptable laboratory procedures.

2.3 SYSTEM CONNECTIONS

Power and Signal Cables

Figure 2.1 shows the interunit connections of the power and signal cables.

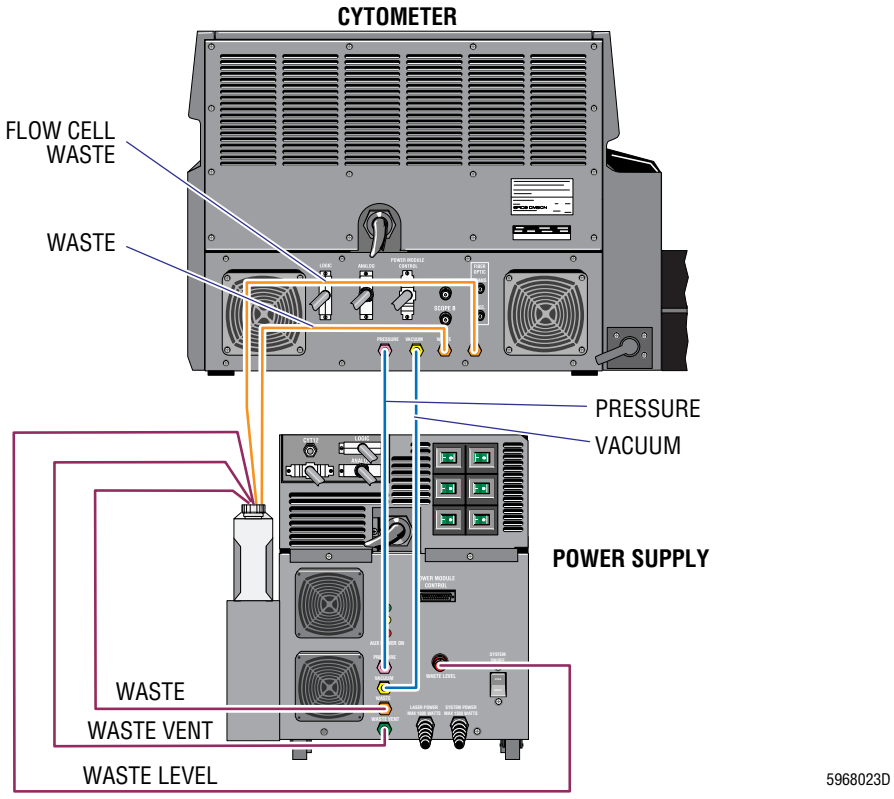
Figure 2.1 Power and Signal Cable Connections



Waste and Pneumatic Tubing

Figure 2.2 shows the interunit connections for waste and pneumatic tubing.

Figure 2.2 Waste and Pneumatic Tubing Connections



2.4 INSTALLING SOFTWARE VERSION 3.0

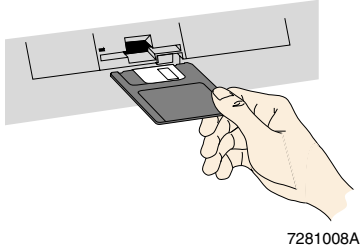
Use this procedure if you are installing software version 3.0 in the MS-DOS® operating environment.

If you have a FlowCentre™ Multimedia Workstation and want to start the XL/XL-MCL flow cytometer software from an icon on the Windows™ 95 desktop, call your local Beckman Coulter Representative.

To install software version 3.0 in the MS-DOS operating environment:

1. Insert the diskette labeled 1 of 2 into the diskette drive. See Figure 2.3.

Figure 2.3 Installing the Software Diskette



2. At the DOS prompt (C:\>) type:

   `a:` (or `b:`) and press Enter.
3. Type: `install` and press Enter.
4. Follow the instructions on the screen.
5. Be sure to register your software after installation is completed.
6. If you need to install the Utilities diskette in an off-line workstation, or install the Protocol diskette in a Cytometer workstation:
   - Insert the diskette and follow the instructions in steps 2 to 4 above.

3.1 WHAT THIS CHAPTER EXPLAINS

This chapter explains how the Cytometer measures scattered light and fluorescence as cells pass through the laser beam.

The illustrations in this chapter are not exact representations of the inside of the Cytometer. They are for explanatory purposes only.

3.2 SAMPLE FLOW

CAUTION Possible flow cell damage. To avoid clogging the sample probe, sample tubing or flow cell, ensure that 12 mm x 75 mm test tubes are free of debris before you use them.

Sample Loading

Manual

When you place a tube containing a prepared sample on the Cytometer sample stage, the stage rises to position the sample probe near the bottom of the tube. The tube is pressurized and sample flow begins.

Automated

The sample carousel has bar-code labels that identify the carousel and the tube position number. Also, you can put bar-code labels on the sample tubes. See Heading 4.6, Bar-Code Specifications.

The MCL has a bar-code reader that reads the carousel number, the sample tube position, and the sample tube bar-code labels as the carousel rotates. The MCL handles a sample tube as follows:

- It lifts the tube out of the carousel into a centering cup.
- It moves the bottom of the tube in a circular orbit for 3 seconds to mix the sample.
- It lowers its sample probe into the tube and the tube is pressurized. Sample flow begins.

In both manual and automated sample loading, the appropriate sample probe is cleaned automatically when sample flow ends.

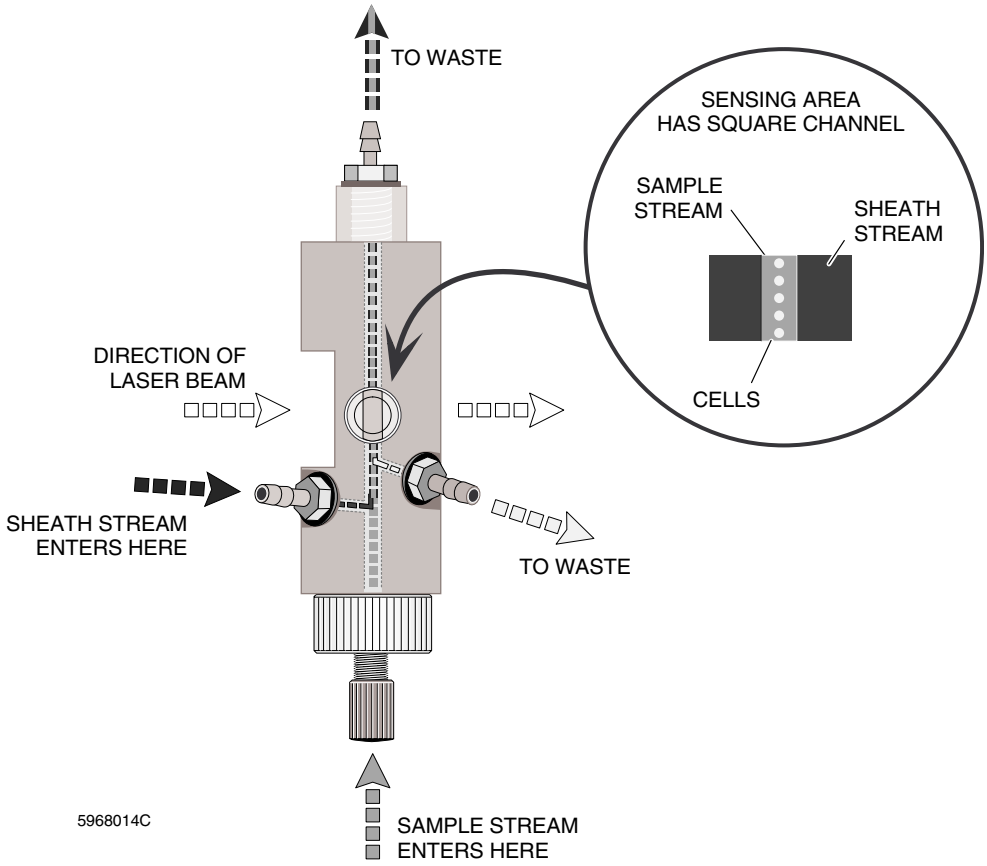
Hydrodynamic Focusing

If the cells were to move through the laser beam in different ways during sample flow, sample analysis could be distorted. The instrument uses a process called hydrodynamic focusing to ensure that the cells move through the laser beam one at a time, along the same path. Hydrodynamic focusing occurs in the flow cell.

The flow cell (Figure 3.1) contains a square channel. A pressurized stream of sheath fluid enters the channel at the lower end and flows upward. The sensing area of the flow cell is at the center of the channel.

While the sheath stream is flowing through the channel, a stream of sample is injected into the middle of the sheath stream. As shown in Figure 6, the sheath stream surrounds, but does not mix with, the sample stream. The pressure of the sheath stream focuses the sample stream so that the cells flow through the laser beam single file—that is, one at a time.

Figure 3.1 Flow Cell (Hydrodynamic Focusing)

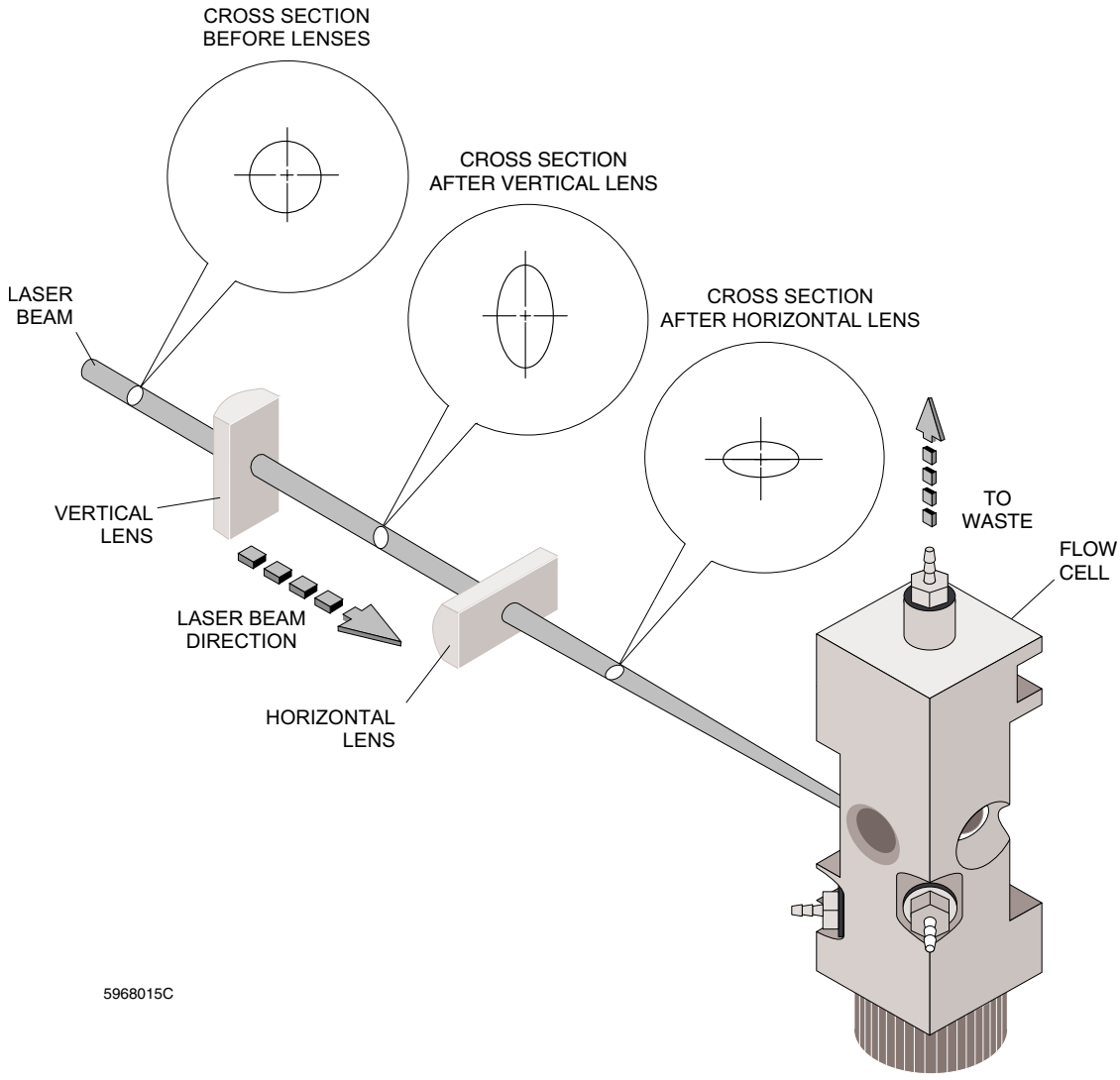


3.3 LASER BEAM SHAPING

Before the laser beam reaches the sample stream, cross-cylindrical lenses focus the beam (see Figure 3.2). Focusing keeps the beam perpendicular to the sample stream flow while making the beam small enough to illuminate only one cell at a time.

The first lens controls the width of the beam; the second, the height. The resulting elliptical beam is focused on the sensing area of the flow cell.

Figure 3.2 Laser Beam Shaping



3.4 CELL ILLUMINATION

As cells in the sample stream go through the sensing area of the flow cell, the elliptical laser beam illuminates them. The cells scatter the laser light and emit fluorescent light from fluorescent dyes attached to them.

Forward Scatter

The amount of laser light scattered at narrow angles to the axis of the laser beam is called forward scatter (FS). The amount of FS is proportional to the size of the cell that scattered the laser light.

Side Scatter and Fluorescent Light

The amount of laser light scattered at about a 90° angle to the axis of the laser beam is called side scatter (SS). The amount of SS is proportional to the granularity of the cell that scattered the laser light. For example, SS is used to differentiate between lymphocytes, monocytes, and granulocytes.

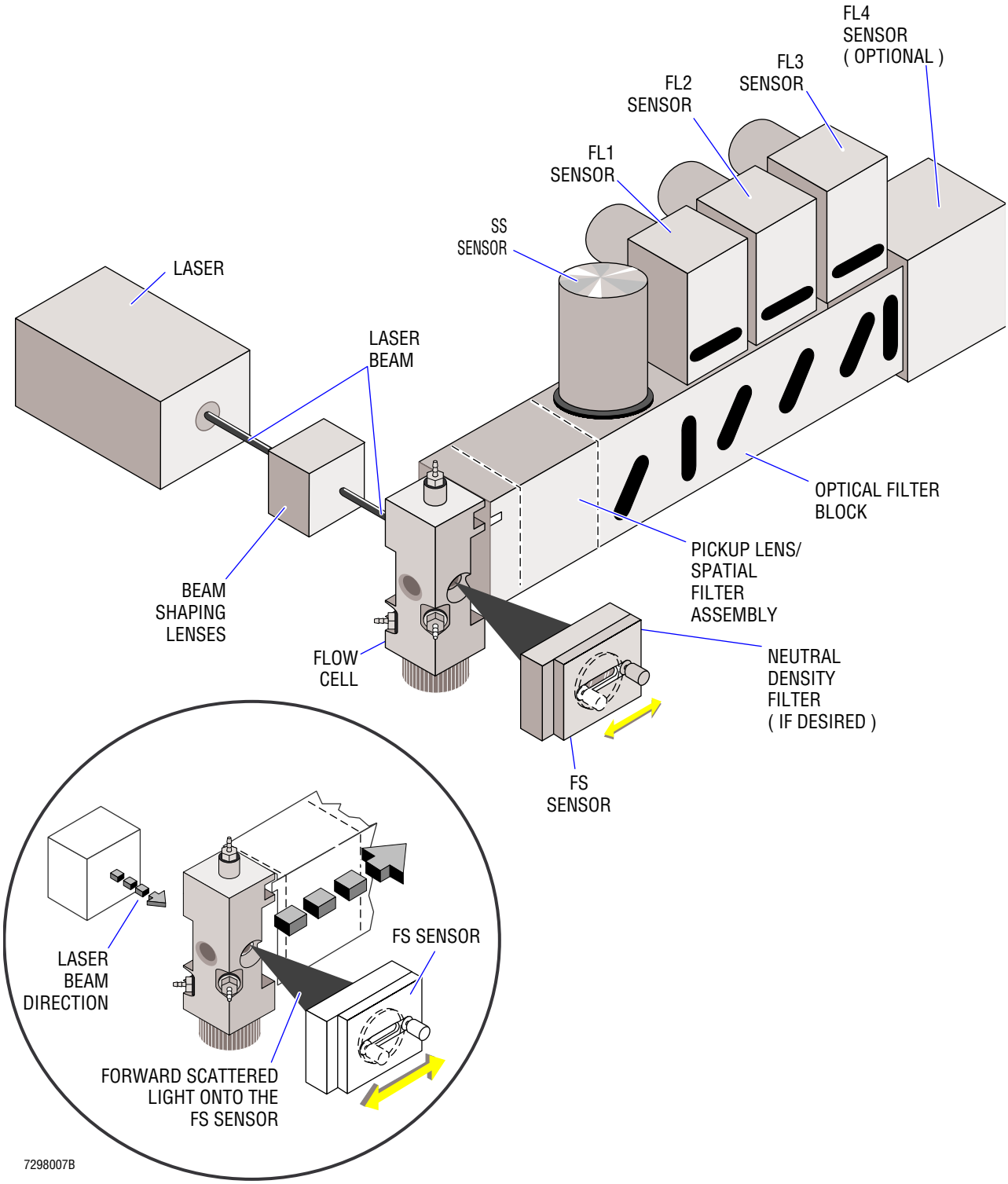
In addition to the SS, the cells emit fluorescent light (FL) at all angles to the axis of the laser beam. The amount of FL enables the instrument to measure characteristics of the cells emitting that light, depending on the reagents used. For example, FL is used to identify molecules, such as cell surface antigens.

3.5 LIGHT COLLECTION, SEPARATION AND MEASUREMENT

Forward Scatter Collection

The FS sensor (see Figure 3.3) collects the forward scatter—the laser light that is scattered at narrow angles to the axis of the laser beam. When light reaches the FS sensor, the sensor generates voltage pulse signals. These signals are proportional to the amount of light the sensor receives. As explained in Heading 3.6, Signal Processing, the signals are processed to measure the characteristics of the cells that scattered the light.

Figure 3.3 Optical System



Neutral Density Filter

When you analyze large cells (cells greater than 20 µm in diameter, such as plant cells), the large amount of FS can saturate the FS sensor. The instrument has a neutral density optical (ND1) filter that solves this problem by reducing the FS signal by a factor of about 10. The ND1 filter is not needed for cells less than 20 µm.

The ND1 filter is built-in and can be positioned for use as needed. The procedure to do so is in the Special Procedures and Troubleshooting manual, in the Replace/Adjust Procedures chapter.

Side Scatter and Fluorescent Light Collection

In order for the sensors to measure SS and FL, the light must be collected and the SS and fluorescent light must be separated.

The pickup lens/spatial filter assembly collects SS and FL from only the sensing area of the flow cell, and collimates it. This light then goes toward the SS sensor.

Side Scatter

The wavelength of SS is 488 nm. It is much more intense than FL. SS is the first light separated from the output of the pickup lens/spatial filter assembly.

SS is separated using a 488 nm dichroic long-pass (488 DL) filter at a 45-degree angle to the light path (see Figure 3.4 and Figure 3.5). The 488 DL filter reflects the SS to the SS sensor but transmits fluorescent light of longer wavelengths.

Fluorescent Light

The instrument has two fluorescence collection configurations. Three FL sensors are standard; a fourth FL sensor is optional.

Three FL Sensors. Figure 3.4 shows the filter configuration for three FL sensors. The light the 488 DL filter transmits goes to a 488 nm laser-blocking (488 BK) filter. The 488 BK filter blocks any remaining laser light, transmitting only fluorescent light. The remaining optical filters separate the light for the three FL sensors.

A 550 DL filter is at a 45-degree angle to the light path. It reflects light less than 550 nm to a 525 nm band-pass (525 BP) filter that transmits light between 505 nm and 545 nm to the FL1 sensor. The light the 550 DL filter transmits is between 555 nm and 725 nm.

The next dichroic long-pass filter, also positioned at a 45-degree angle to the light path, is a 600 DL. It reflects light between 555 nm and 600 nm to a 575 BP filter in front of the FL2 sensor. The 575 BP transmits light between 560 nm and 590 nm to the FL2 sensor.

The 600 DL filter transmits light between 605 nm and 725 nm to a 620 BP filter in front of the FL3 sensor. The 620 BP filter transmits light between 605 nm and 635 nm to the FL3 sensor.

Figure 3.4 Filter Configuration, Three Fluorescence Sensors

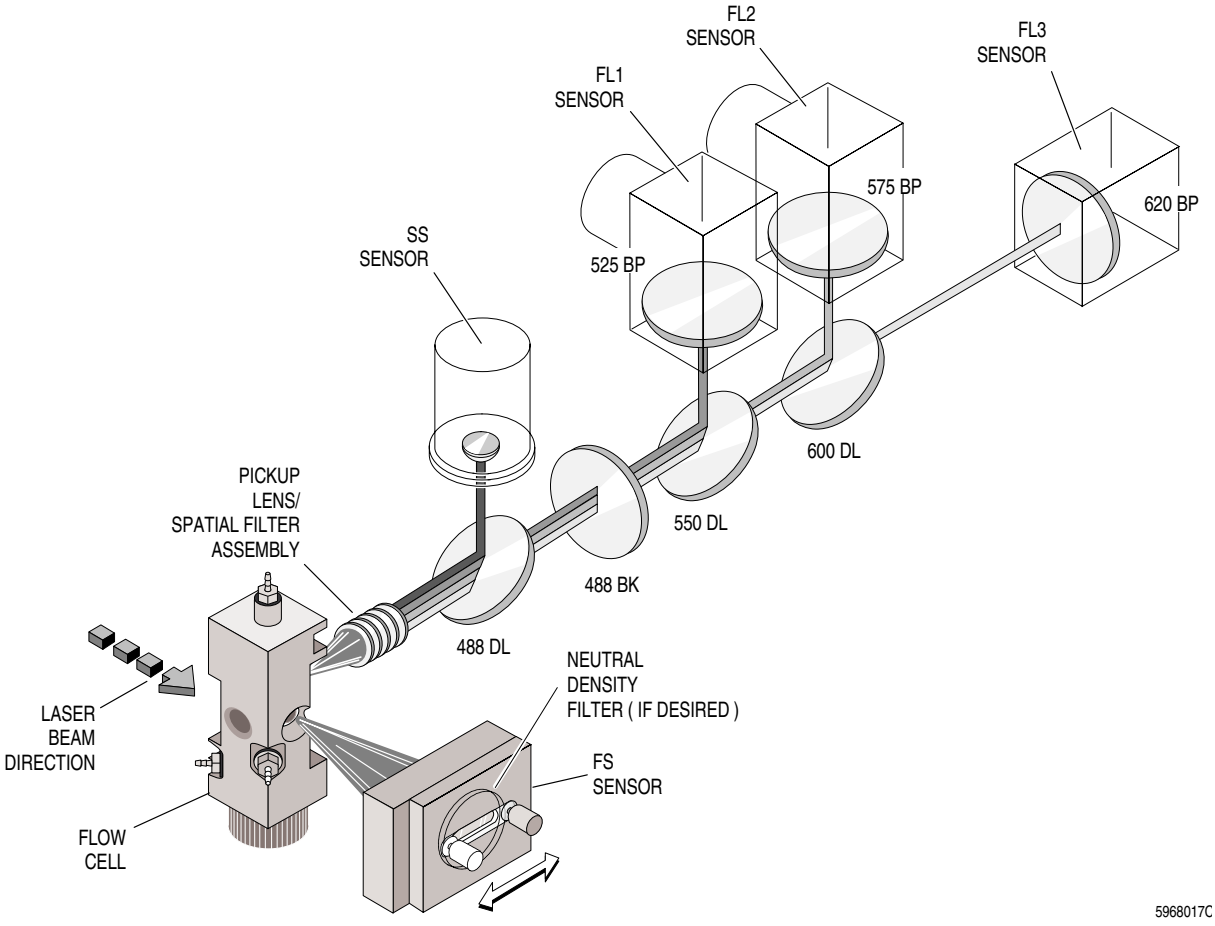
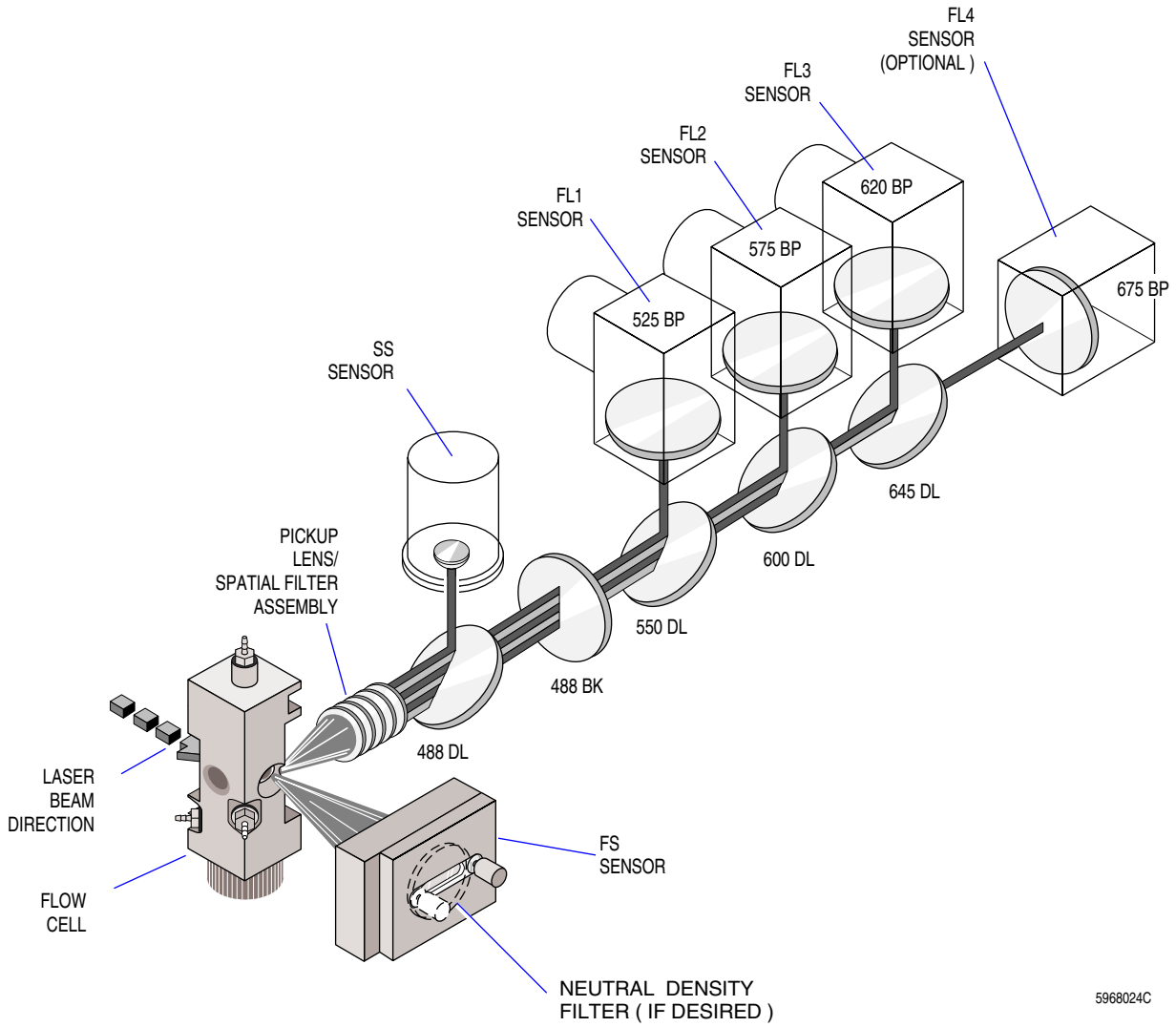


Figure 3.5 Filter Configuration, Four Fluorescence Sensors



Four FL Sensors. Figure 3.5 shows the filter configuration for four FL sensors. It uses the same filters as the three FL sensor configuration, up to and including the 600 DL filter.

A different dichroic long-pass filter, a 645 DL, reflects the light between 605 nm and 645 nm to a 620 BP filter in front of the FL3 sensor. The 620 BP filter transmits light between 605 nm and 635 nm to the FL3 sensor.

The 645 DL filter transmits light between 650 nm and 725 nm to a 675 BP filter in front of the FL4 sensor. The 675 BP filter transmits light between 660 nm and 700 nm to the FL4 sensor.

3.6 SIGNAL PROCESSING

Voltage Pulse Signals

The Cytometer has up to six sensors, each generating a voltage pulse signal as each cell crosses the laser beam. A voltage pulse signal is proportional to the intensity of light the sensor received. The Cytometer electronics amplifies, conditions, integrates, and analyzes these pulses.

Peak Signal

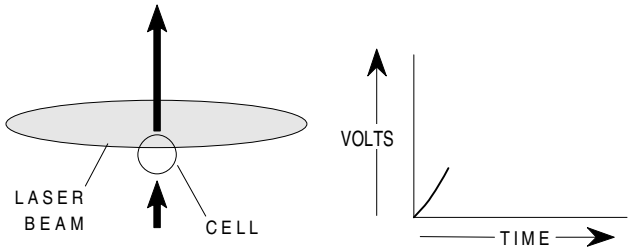
Figure 3.6 shows how a peak voltage pulse signal forms as a cell crosses the laser beam. The intensity of light scatter or fluorescence determines the height of the peak pulse (see Figure 3.6). The distribution of the fluorescence determines the width of the pulse. Therefore, the total fluorescence (intensity and distribution) determines the area under the pulse. Figure 3.6 shows how three cells with the same amount of total fluorescence but with different fluorescence intensities, produce different peak pulses.

Integral Signal

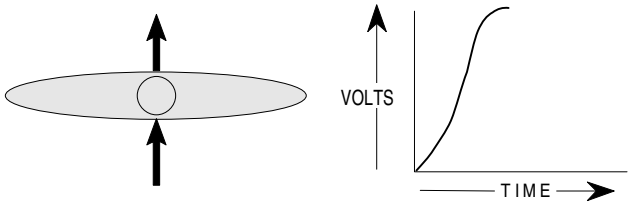
Because the total fluorescence in all three cells is the same, but the distribution is different, the pulse can be integrated to produce an integral signal (see Figure 3.7).

The height of the integral pulse is proportional to the total fluorescence and is obtained when the cell exits the laser beam. The pulse height, however, represents the most intense amount of fluorescence produced. The area under the pulse is proportional to the total fluorescence.

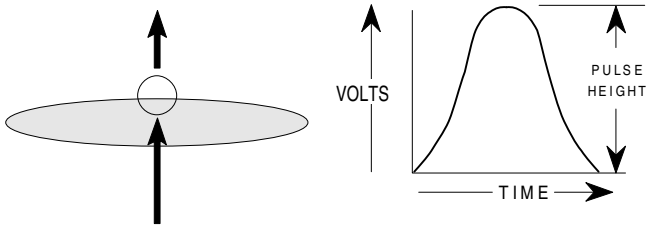
Figure 3.6 Voltage Pulse Formation, Peak Signal



Cell enters laser beam; some light is scattered.



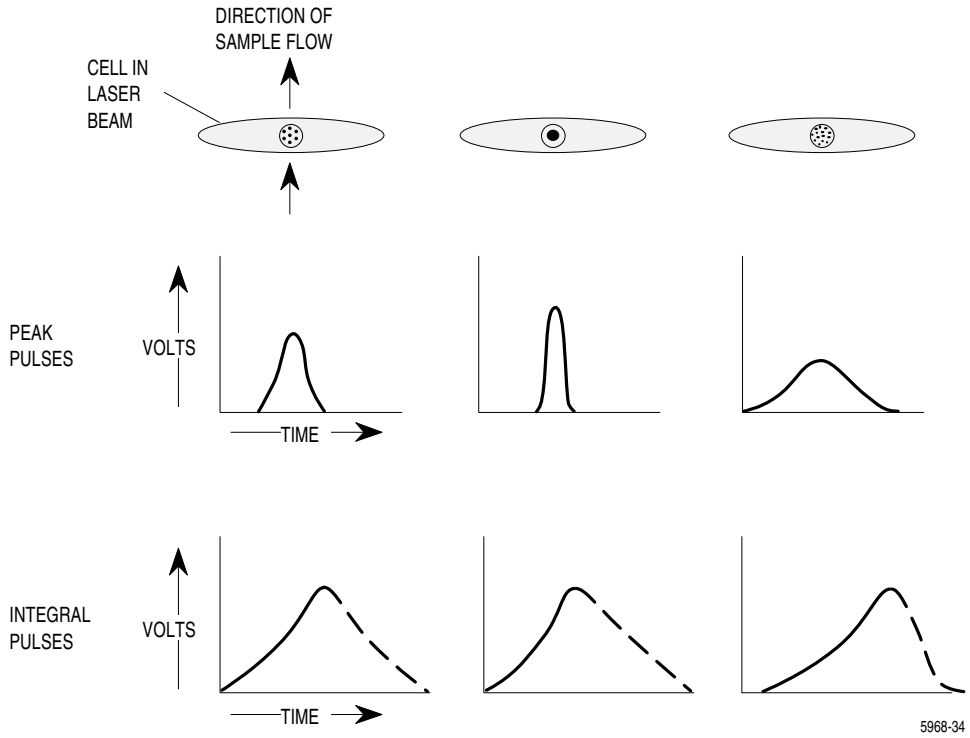
Cell is in center of beam. Scattered light, and therefore pulse height, reaches a maximum.



Cell leaves beam; scattered light decreases.

5968-18

Figure 3.7 Integral and Peak Pulses



Amplification

Some voltage pulses are so weak that they must be amplified so that the characteristics of the cells that scattered the light can be measured.

The instrument software lets you:

- Increase the gain to linearly amplify the integral and peak signals.
- Logarithmically transform the linear data.

A logarithmic transformation accentuates the differences between the smaller pulses and reduces the differences between the larger pulses.

Signals Generated

The instrument sensors—FS, SS, FL1, FL2, FL3, and FL4 (optional)—can generate these signals (integral unless stated otherwise; LOG stands for logarithmic):

- FS, FS LOG, FS PEAK
- SS, SS LOG, SS PEAK
- FL1, FL1 LOG, FL1 PEAK
- FL2, FL2 LOG, FL2 PEAK
- FL3, FL3 LOG, FL3 PEAK
- FL4, FL4 LOG, FL4 PEAK (optional).

For any given sample, the instrument can generate up to eight of these signals, plus the AUXiliary (AUX), Prism, TIME, and RATIO parameters.

3.7 PARAMETERS

AUXiliary Parameter

The only way to acquire a peak signal is to assign it to the AUX parameter. For any given sample, only one of the following integral or peak signals can be assigned to AUX:

- FS, FS PEAK
- SS, SS PEAK
- FL1, FL1 PEAK
- FL2, FL2 PEAK
- FL3, FL3 PEAK
- FL4 (optional), FL4 PEAK (optional).

When to Use the AUX Parameter

When you specify both linear and logarithmic amplification of the same signal, the gain for the linear amplification is also applied to the logarithmic amplification. However, when you specify only logarithmic amplification of a signal, the instrument automatically sets the gain for that signal to 1.0, and you cannot change it.

Use the AUX parameter when you want to:

- Amplify a signal at two different gains.
- Observe a peak signal or integral signal at the same time as the log signal.
- Observe a peak signal at the same time as the integral signal.

Also, the AUX parameter may be used for doublet discrimination. Assign a peak fluorescence signal to AUX so you can measure peak vs. integral fluorescence.

TIME Parameter

The TIME parameter is the amount of time, in seconds, the instrument acquires data. It is displayed on the histogram axis in 1-second resolution. The axis labels vary, depending on histogram resolution and stop time.

The minimum stop time is 10 seconds, the maximum stop time is 1,800 seconds (30 minutes) and the default stop time is 300 seconds (5 minutes).

When you assign the TIME parameter to a histogram axis, the divisions on the axis change accordingly.

To find the time (in seconds) per channel in a one-parameter histogram, divide the stop time (in seconds) by 1,024 (0.001 second = 1.0 ms).

For a two-parameter histogram, divide by 64, 128, or 256 depending upon the histogram resolution you are using.

When stop volume is used, the stop time is as shown in the right-hand column and cannot be changed because the stop volume is reached long before the stop time elapses.

Stop Volume	Stop Time
20 µL	60 seconds
40 µL	120 seconds
60 µL	180 seconds
80 µL	240 seconds
100 µL	300 seconds

RATIO Parameter

The RATIO parameter is calculated, not acquired directly. When you select a parameter, you specify which signal is the numerator and which is the denominator.

$$\text{RATIO} = \frac{\text{Numerator}}{\text{Denominator}} \times 1024$$

A ratio of _1 is at channel 1,023. If you assign RATIO to a histogram axis, RATIO events appear at a lower channel if the intensity of the numerator signal is less than the denominator signal.

To calculate the actual ratio at a particular intensity for a one-parameter histogram, divide the intensity by 1,024. For a two-parameter histogram, divide by 64, 128, or 256 depending upon the histogram resolution you are using.

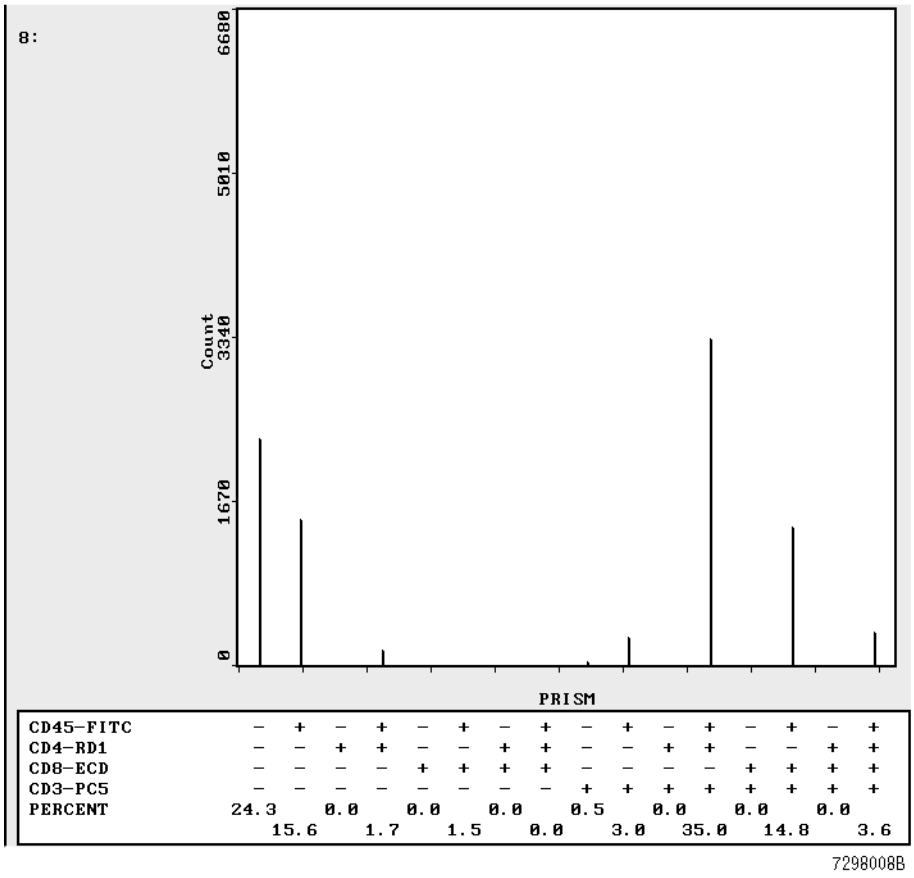
PRISM Parameter

Prism is used to analyze multicolor immunofluorescence samples. With multicolor immunofluorescence a cell is either positive or negative for each of two, three, or four cell surface markers. A particular combination is called a phenotype. The Prism parameter allows you to display percentages on all phenotypic populations in a single histogram. It is a software parameter that can be acquired in either run time or listmode.

Prism is available on up to four parameters. TIME, RATIO, and Prism itself cannot be used for the Prism parameter. All other signals can be used for the Prism parameter. Generally, FL1, FL2, FL3, and FL4 are used.

A Prism histogram shows a spike or population for each antibody combination with a percent of the total that represents the percent of the total events in the Prism histogram. See Figure 3.8.

Figure 3.8 Prism Histogram



To use Prism, you must first define regions that separate the negative and positive populations and assign these regions to the Prism Equation. See the heading, Prism Equation Regions, in the Getting Started manual for instructions on assigning regions to the Prism Equation.

3.8 AUTOSTANDARDIZATION PROTOCOLS

These protocols are run in an Autostandardization panel to automatically adjust the high voltage, gain, and compensation settings and to verify that the instrument is running correctly. The Quality Control chapter in the Operator's Guide has instructions for creating and running an Autostandardization panel.

_A Protocols

The _A protocols automatically adjust high voltage and gain settings. A three-letter name ("xyz" prefix) must follow the _A in the protocol name. The instrument settings are saved to the xyz.QCS file. Use the same three letter prefix at the beginning of any other protocol that you want updated with the standardized settings from the Autostandardization panel.

For example:

_A3QP Flow-Set	the autostandardization protocol for three-color immunofluorescence. It creates the 3QP.QCS baseline file.
3QP FITC/RD1/PC5	the primary and control protocol for three-color immunofluorescence
_Q3QP FITC/RD1/PC5	the QC protocol for three-color immunofluorescence. It creates the 3QPFITC.QCC quality control statistics file.

_A protocols use two regions to locate and move the peak of a Flow-Set Fluorospheres population to a specific channel, standardizing the Cytometer's light scatter and fluorescence intensity.

One region (the larger one) captures the population at its current position and a second region (the smaller one) identifies the target channel that the peak of the population will be moved to.

_A protocols must be assigned as Primary protocols in the Autostandardization panel.

_C Protocols

The _C protocols are used to standardize fluorescent (color) compensation. Compensation reagents and quad-stat regions are used to automatically adjust color compensation.

_C protocols must be assigned as Secondary protocols in the panel. Up to four can be used in a panel.

_Q Protocols

The _Q protocols are used to generate quality control statistics and verify the Cytometer setup. Regions with 'QC' in their names located in _Q protocols produce the quality control statistics which are stored in the xyzABCD.QCC file related to the _QxyzABCD protocol. Use the same first three letters in the _Q protocol name ("xyz" prefix) as in the _Axyz protocol.

When assigning _Q protocols to a panel:

- _Q protocols can be assigned as Secondary or Control protocols in a panel.
- Multiple _Q protocols can be assigned per panel. Use a different 'ABCD' descriptor after the '_Qxyz' (where 'xyz' represents the first three letters and designates which baseline

file to use) to distinguish each protocol. Spaces and hyphens (-) are not counted by the system as part of the xyzABCD that follows the _Q in the name.

Example of three _Q protocols:
_Q3QP 45/4/3, _Q3QP 45/8/3, _Q3QP 8/4/3.

- Use a _Q protocol to terminate the Autostandardization panel.

Examples of Autostandardization Protocols and Panels

First Panel:

- _Q3CL Flow-Check

Second Panel:

- _A3CL Flow-Set
- _C1 FITC/RD1 CYTO-COMP
- _C3 RD1/PC5 CYTO-COMP
- _Q3CL 45/4/3
- _Q3CL 45/8/3

3.9 QUALITY CONTROL (QC) FEATURES

QC Templates

Use the QC template to define what QC data appears in the Levey-Jennings graphs and data table. You can select from voltages, gains, compensations, and QC region statistics.

QC templates are only created for _A, _C, or _Q protocols, one QC template per protocol. The QC template needs to be loaded in the QC Template screen in order to view the QC data.

Levey-Jennings Graphs

Use the Levey-Jennings graph feature to automatically graph the data from the Cytometer settings baseline files (*.QCS) and QC statistical data files (*.QCC) over a period of time. You define the data you want to appear in the graph through the QC template. In the Levey-Jennings graph, you can visually review the QC data for trends, shifts, outliers, and violations of your laboratory's QC guidelines. In addition, you can request the system to check the data against Westgard rules.

Note: See the Data Management manual for a detailed explanation of the *.QCS and *.QCC files.

The Levey-Jennings graph shows a Mean value, and the inner and outer limits. The outer limits are 1.5 times the inner limits. Data points are plotted in relation to the Mean and these limits.

You can manually enter the Mean value or have the system calculate it. You determine whether to calculate and display the data points on the graphs in relation to the Mean ±nSD, Mean ±number, or Mean ±% of Mean.

Each graph shows the data for one QC region statistic or one Cytometer setting signal depending upon the QC template definition.

Data Table

Use the Data Table to view the data from the Cytometer settings baseline files (*.QCS) and QC statistical data files (*.QCC) in table format and to view the calculated statistics for the file. You define the data you want to appear in the Data Table in the QC template. Runs can be deleted from the calculated statistics, or you can select specific reference runs and calculate the statistics based on them.

Westgard Rules

Statistical QC data from the QC regions and instrument settings can be checked against Westgard rules. You can view or print the Westgard report at the QC Levey-Jennings screen and the QC Data Table screen. When you request the Westgard rules check at these two screens, any failure of the rules appears on the Westgard Report screen.

Here are the six Westgard rules used to check QC data by the XL software:

- Rule 1. The point is above the Mean +2 SD or below the Mean -2 SD.
- Rule 2. The point is above the Mean +3 SD or below the Mean -3 SD.
- Rule 3. The next two points are above the Mean +2 SD or below the Mean -2 SD.
- Rule 4. The point is above the Mean +2 SD, and the next point is below the Mean -2 SD, or vice versa.
- Rule 5. The next four points are above the Mean +2 SD or below the Mean -2 SD.
- Rule 6. The next ten points are above the Mean or below the Mean.

Note: Westgard rules used in this system only apply to ranges established with the calculated mean and standard deviation (SD). These rules do not apply to manually entered or system calculated means with Mean ±number or Mean ±%.

For more detailed information on the Westgard rules, visit their website, www.westgard.com.

Compare Screen

Use this screen to compare two different QC files (*.QCS or *.QCC). For example, you can compare the current *.QCC data file for a _Q protocol to a previous *.QCC data file for the same _Q protocol.

Note: New *.QCS and *.QCC files are created for a protocol when you change the lot number in the protocol's lot number region. So in the above example you would be comparing similar data for the same protocol for two different lot numbers of control material.

Maintenance Screen

Use the Maintenance screen to record daily startup and shutdown, and all maintenance performed on the XL/XL-MCL flow cytometer.

3.10 HISTOGRAM DISPLAY

The results of sample analysis appear on the Workstation screen as graphs called histograms. You assign the parameters to the histogram axes. Histograms can be displayed in black and white or color as:

- Single-parameter line
- Dual-parameter dot plot
- Dual-parameter contour
- Dual-parameter projection
- Dual-parameter isometric
- Single-or dual-parameter Prism.

Dual-parameter histograms can be displayed in 64 x 64, 128 x 128, or 256 x 256 resolution.

Regions

To analyze data or gate histograms, you must first create and assign regions to these tasks. You can create five different types of regions (see Creating Regions in the Getting Started manual). The region types are:

- Linear
- Rectilinear
- Numeric
- Quad-stat
- Amorphous.

Once a region is created it can be assigned to function in a specific way (see Assigning Regions in the Getting Started manual). The functions that you can assign a region to are:

- Analysis
- PRIME
- CAL (calibration)
- Cell-Seeker™ (autogating)
- Cell-Tracker™ (event coloring)
- Gating
- Listmode gating (LISTGATE)
- Prism
- Mapping
- Positives analysis
- QC (quality control statistics)
- Lot number.

Gating

The software lets you use gating to specify that only certain cells are to be analyzed. A gate can be defined as the cells that are inside or out of one or more regions.

Data Storage

Sample results can be printed out, saved to a diskette or other removeable media, saved to a network drive, or exported to another computer through a serial port. Specimen information and patient reports can be saved to a database. You can store sample results in the form of a list of the measurements from each cell, called listmode data. Listmode data can be replayed into histograms or archived for analysis later. Histograms can also be saved to a file.

Histogram Statistics

Note: Whenever more than 65,535 events accumulate in any one channel, ****** display instead of the statistics to indicate a channel overflow.

Linear Region Statistics

For linear signals, statistics for histogram regions are calculated as follows:

$$\text{Percent} = \frac{\text{Number of cells in region}}{\text{Total number of cells in the region}} \times 100$$

Count or Area = Number of cells in the region

Peak position = Intensity containing the largest number of cells within the region.

Peak count = Number of cells in the peak position within the region

$$\text{Mean} = \frac{\Sigma(\text{intensity number} \times \text{count in the position})}{\text{area}}$$

$$\text{SD} = \sqrt{\frac{\Sigma[(\text{intensity number} - \text{mean})^2 \times \text{count in the intensity}]}{\text{area}}}$$

For Mean and SD, the summations are performed over all the channels that lie within the region.

$$\text{Median} = \text{median intensity} + 0.5 - \frac{\left[\text{sum(median intensity)} - \frac{\text{area}}{2}\right]}{\text{count in median intensity}}$$

Median intensity = the smallest intensity such that sum (intensity) $\geq \frac{\text{area}}{2}$

Sum (intensity) = sum of events from the lower edge of the region to the intensity

$$\text{CV} = \frac{\text{SD} \times 100}{\text{Mean}}$$

$$\text{FPCV} = \text{CV}$$

$$\text{Half Peak CV} = \frac{42.46 \times \text{width of peak at half the peak height}}{\text{peak position}}$$

Log Region Statistics

For log signals, the formulas are:

$$\text{Mean intensity} = \frac{\Sigma\text{log to lin (channel number)} \times \text{count in the channel}}{\text{area}}$$

$$\text{Log to Lin (N)} = 1.024 \times 10^{\left[L + D \times \left(\frac{N}{1024}\right)\right]}$$

where L = -1 for four-decade logarithmic amplification
D = number of decades

$$\text{Log SD} = \text{Log to Lin}\left(\text{Mean} + \frac{SD}{2}\right) - \text{Log to Lin}\left(\text{Mean} - \frac{SD}{2}\right)$$

$$\text{Log CV} = \frac{\text{Log SD} \times 100}{\text{Log Mean}}$$

Log Mean = Log to Lin (Mean)

Log Peak Position = Log to Lin (Peak Position)

Log Median = Log to Lin (Median)

4.1 SAMPLE REQUIREMENTS

At least 0.5 mL of prepared sample is needed. It must be in a 12- x 75-mm test tube. Samples analyzed on the instrument must be in a single-cell suspension. Typically, cells are prepared before they are analyzed. The method used to prepare a specimen depends on the sample type and the assay desired. For example, the Q-Prep, Multi-Q Prep, or TQ-Prep workstations let you prepare antibody-labeled cells from an anticoagulated whole-blood specimen for surface marker analysis.

In general, the optimum concentration for analysis is 5 x 10 6 cells/mL. When this concentration is not possible, refer to the package insert for the preparation method you are using.

The instrument can measure cells that are between 0.5 µm and 40 µm in diameter. For fluorescent light measurements only, particles can be macromolecular or up to 40 µm in diameter.

4.2 INSTRUMENT SPECIFICATIONS

Dimensions

Component	Height	Width	Depth	Weight
Computer	15.75 cm (6.2 in.)	53.34 cm (21 in.)	42.55 cm (16.75 in.)	18.14 kg (40 lb)
Cytometer	50.8 cm (20 in.)	61 cm (24 in.)	57.2 cm (22.5 in.)	63.5 kg (140 lb)
with MCL	same as above	86.6 cm (34.1 in.)	same as above	84.8 kg (187 lb)
Monitor (typical)	42.7 cm (16.81 in.)	40.5 cm (15.94 in.)	43.8 cm (17.25 in.)	22 kg (48.4 lb)
Power Supply	48.3 cm (19 in.)	40.64 cm (16 in.)	50.8 cm (20 in.)	54.4 kg (120 lb)

Installation Category

Category II (per IEC 1010-1 standard).

Cytometer

Flow Cell

Sensing area: BioSense 250-µm square channel with an integral lens, mounted with a vertical (upward) flow path.

Flow Rate

Continuous pressure is applied to the sample tube. The amount of pressure depends on the flow rate you specify:

- Low
- Medium
- High

Laser

Air-cooled, software controlled, 15 mW, argon ion laser operating at 488 nm.

Argon Laser Power

Laser power is monitored 5 times per second within the system software and the reading is displayed on the STATUS bar of the cytometer software.

If the laser power deviates more than ±1%, a Laser Power Error is displayed on the STATUS bar and the system will not run a sample until the error message is no longer displayed. Follow the instructions in the Troubleshooting section for handling this error.

In order to extend the life of the argon laser, when the flow cytometer is in idle mode, the laser power will be less than 8mW. Once the instrument is taken out of idle mode, the laser power will return to 20mW.

Thus, while operational and out of idle mode, the laser power specifications are 20±1%mW.

Beam-Shaping Optics

Cross cylindrical lenses 10 mm by 80 mm.

Laser Beam Spot Size

An elliptical spot 10-µm high by 80-µm wide.

Optical Filters

- Neutral density (ND1) filter, if desired.
- 488-nm, 550-nm and 600-nm dichroic, long-pass (DL) filters.
- 488-nm laser-blocking (BK) filter.
- 525-nm, 575-nm, and 620-nm band-pass (BP) filters.
- 675-nm band-pass (optional).

Sensors

- The FS sensor and the SS sensor are photodiodes.
- The three FL sensors are photo-multiplier tubes that have a 200- to 800-nm spectral range (fourth FL sensor is optional).

Signal Processing

- High voltage amplification, up to 2,000, in increments of 1, for:

  FL1
  FL2
  FL3
  FL4 (optional).
- Vernier gain (fine amplification), up to 1,000 (labeled volts), in increments of 1, for the following. A change of 1 to 1,000 represents a 1-to-4 change in gain:

  FS
  SS
  AUX

- True (total) gain is the result of vernier gain (HV) and linear amplification (gain) for FS, SS and AUX. When HV is 100 and gain is 2.0, true gain is 2.6.

  True Gain = Gain x [1+ (0.003 x HV)]
- Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, 20, 50, 75, 100, 200, or 500 for:

  FS

  SS.
- Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, or 20 for:

  FL1

  FL2

  FL3

  FL4 (optional).
- Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, 20 or 50 for:

  AUX.
- Four-decade digital logarithmic transformation of:

  FS

  SS

  FL1

  FL2

  FL3

  FL4 (optional).

  Note: A scale of 0.1 to 1,000 is displayed on the histogram axes for logarithmic parameters, but the statistics are based on an actual scale of 0.1024 to 1024.
- Fluorescence color compensation is available in 0.1 increments, from 0 to 100%, for:

  FL1

  FL2

  FL3

  FL4 (optional).
- A discriminator (maximum value of 1,023) is available for one of the following signals. Only one discriminator can be specified for any one sample acquisition.

  FS

  SS

  FL1

  FL2

  FL3

  FL4 (optional)

  AUX.

Workstation

Computer

Intel® Pentium® microprocessor and 16 MB of RAM.

Data Storage

- 3.5-in., 1.44-MB diskette drive.
- 2-GB nonremovable hard disk.

Interfaces

Bidirectional asynchronous serial interfaces for communication with mainframe and personal computers.

Input Devices

- Two-button mouse.
- 101-key IBM® PC AT-compatible keyboard.

Monitor

Color (SVGA) with a 17-in. screen.

4.3 SOFTWARE SPECIFICATIONS

Data Output and Compatibility

You can set up the instrument for multiple users in the Utilities application. The Data Management screen lets you specify local and off-line directories for saving and retrieving files. Other output and compatibility features include:

- FCS 2.0 file format for listmode and histogram files.
- PCX format for image files.
- ASCII file format for converted Export (*.EPT) files.
- The ability to copy, move, and delete files.
- Full-color printouts with the appropriate optional Printer.
- SQL database for specimen information and patient reports.
- Automatic data archival.
- Printout of sample results and patient reports.
- Printout of Worklist and carousel report for the MCL.
- Compatibility with ALTRA™, Profile™, and Elite™ flow cytometer (without Prism, Gated Amp, or Time of Flight parameters) files.
- Compatibility with files from previous software versions of XL and XL-MCL flow cytometers (1.0, 1.5, SYSTEM II 1.0, SYSTEM II 2.0, and SYSTEM II 2.1).
- The ability to export specific line items from a patient report through a serial port or to an ASCII Patient report export (*.PXP) file.

Reporting Units

Reporting units (absolute units) are selected at the Utilities Configuration screen. They can be either standard US (cells/uL) or SI units (cells/L).

Two-Digit Date Format

For Non Date-of-Birth Dates

For two-digit date entries (other than date-of-birth entries) if the year entered is:

- 80 to 99, the system assumes the year is in the range of 1980 to 1999.
- 00 to 79, the system assumes the year is in the range of 2000 to 2079.

For Date-of-Birth Dates

For **D. O. B.** (date of birth) entries, if the year entered is:

- Equal to or less than the current year, the system assumes the year is in the current century.
  (Example: If it is currently 1998 and you enter a DOB of 21JUN91, the system assumes the D.O.B year is 1991.)
- Greater than the current year, the system assumes the previous century.
  (Example: If it is currently 2001 and you enter a DOB of 13JUL02, the system assumes the D.O.B year is 1902.)

Acquisition

During data acquisition, the histograms are updated in real time. When one histogram is displayed with statistics underneath, the statistics are also updated in real time.

Up to eight histograms are available for any given sample, and each histogram can have up to two parameters.

One-parameter histograms have 1,024-channel resolution.

Two-parameter histograms have up to 256- x 256-channel resolution.

Regions

Twenty-four regions are available for gating, analysis, and autogating. Up to 16 (eight amorphous) of those regions can be used at a time in histogram equations as gating regions.

The following types of regions are available for gating and analysis:

- Linear
- Rectilinear
- Numeric
- Amorphous
- Quad-stat (quadrant statistic) [analysis only]

You can select which statistics are printed for each region in a histogram.

Listmode Analysis

The instrument can store up to eight parameters including AUX, TIME, and RATIO as listmode data. The amount of available random access memory (RAM) in the computer determines the maximum size of a listmode file.

Multigraph Analysis

Multigraph analysis uses overlay and gallery displays to analyze or display up to eight histograms at a time.

4.4 PERFORMANCE SPECIFICATIONS

Carryover

Scatter and fluorescence carryover is less than 1% from one specimen to another when the number of gated events is between 100 and 10,000.

Data Acquisition Throughput

For two-color surface markers, processes 60 tubes/hour of normal whole-blood samples (2,500 lymphocytes/µL, 10,000 leukocytes/µL) when acquiring 10,000 lymphocytes at a rate of 1,000 leukocytes/second. The tubes were prepared on a Q-Prep workstation or a Multi-Q-Prep workstation. This assumes the use of a black and white laser printer.

Doublet Discrimination

Detects >90% of cellular doublets in cells _7 µm, using peak vs. integral discrimination, depending upon sample preparation method.

Precision for Surface Markers

See reagent package insert for precision specifications of other surface markers.

Resolution

Forward Scatter

Within ±25% of the half-peak coefficient of variation (HPCV) assay value for Flow-Check fluorospheres, without the ND1 filter. Generally a HPCV of <3% is acceptable for surface marker applications and <2% is acceptable for DNA applications.

Fluorescence

Within ±25% of the HPCV assay value for Flow-Check fluorospheres, with optical filters. Generally a HPCV of <3% is acceptable for surface marker applications and <2% is acceptable for DNA applications.

Sensitivity

Scatter

Detects 0.5-µm diameter particles.

Fluorescence

Less than 1,000 molecules of equivalent fluorochrome when measured with FCSC™ microbeads and IsoFlow sheath fluid.

Stability

Day-To-Day

Peak intensity channel values of Flow-Check fluorospheres for FS, FL1, FL2, FL3, and FL4 (optional) do not vary more than ±5% from the peak intensity obtained over a period of 7 days, when the temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check fluorospheres package insert for details.

Within Day

Peak intensity channel values of Flow-Check fluorospheres for FS, FL1, FL2, FL3, and FL4 (optional) do not vary more than ±5% from the peak intensity channel obtained within a period of 24 hours, when the temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check fluorospheres package insert for details.

4.5 PERFORMANCE CHARACTERISTICS

Refer to the package insert for the performance characteristics for the preparation method you are using.

4.6 BAR-CODE SPECIFICATIONS

Bar-Code Labels

A bar code consists of black lines (bars) and white lines (spaces), which are called elements.

There are narrow elements (NE) and wide elements (WE). The bar-code symbology determines their arrangement.

IMPORTANT Sample misidentification can occur from the use of incorrect bar-code labels. Follow the specifications in this section to create your bar-code labels to prevent incorrect sample identification.

The instrument supports preprinted labels.

Acceptable Bar Codes

Within the given specifications, the MCL reader and the optional hand-held bar-code scanner automatically distinguish the following bar codes:

Interleaved 2-of-5	fixed 14 characters (13 data characters + 1 check character, must be an even number of characters, leading zeros can be added)
Code 39 bar code	maximum 7 characters (6 data characters + 1 check character)
Codabar	maximum 10 characters (9 data characters + 1 check character
Code 128B	maximum 8 alphanumeric characters
Code 128C	maximum 16 numeric characters (The use of 15 numeric characters is invalid)

Bar-Code Label Optical Characteristics at 670 nm ±10%

- Print Contrast Signal (PCS): 80% minimum.

- Reflectivity of Media (RW): 80% minimum.
- Reflectivity of Ink (RB): 16% maximum.
- No spots or voids; no ink smearing.
- Edge roughness is included in the bar and space tolerances.

$$PCS = \frac{RW + RB}{RW} \times 100\%$$

Table 4.1 Code-Related Specifications

Code	Interleaved 2-of-5*	Codabar*	Code 39*	Code 128 *
Narrow element (NE) width	0.010" ±0.001"	0.010" ±0.001"	0.010" ±0.001"	0.010" ±0.001"
Wide element/narrow element ratio (WE/NE)	3:1	N/A	3:1	N/A
Intercharacter gap	No	0.010" Minimum.	_NE	No
Data digits	14**	1 to 10**	1 to 7**	2 to 16

* See AIM uniform Symbology specification, Rev. 1993 for detailed specification.
** Includes check sum character

NE Width

0.01 in.

WE/NE Ratio

3:1

Printing Methods

Optional bar-code printer. See Appendix A in the Special Procedures and Troubleshooting manual.

MCL Bar-Code Reader

The MCL uses a visible-laser type reader containing a Class II laser, operating at 670 nm, with a maximum power output of 1 mW.

Hand-Held Bar-Code Reader

The hand-held bar-code reader uses a visible-laser type reader containing a Class II laser, operating at 670 nm, with a maximum power output of 1 mW.

Bar-Code Decoder

The MCL sends a “GS” ASCII character (hexadecimal 1D) to the decoder to start operation.

The decoder:

- Turns the reader on.
- Decodes information that comes from the reader.
- Keeps the reader on for up to 4 seconds.
- Turns the reader off.
- Sends the decoded information (or no-read message) to the MCL.

IMPORTANT To prevent incorrect indentification of sample tubes, do not use FNC1, FNC4, and FS (hexadecimal 1C) characters in your bar-code information.

Check Sum Algorithm

Beckman Coulter strongly recommends the use of bar code check sums to provide automatic checks for read accuracy.

IMPORTANT Use of bar codes is an extremely accurate and effective method of positive patient identification. Certain features, such as check sum digits, maximize accuracy in reading Codabar, Code 39 and Interleaved 2-of-5 labels. In one study, the use of check sum digits detected 97% of misread errors.

Use checksums to provide protection against occasional misread errors caused by problems such as damaged or misapplied labels. If you must use bar codes without check sums, Beckman Coulter recommends that you verify each bar-code reading to assure correct patient identification.

5.1 LASER SAFETY

The Cytometer and the optional MCL each contain a laser. Beckman Coulter's design and manufacture of the instrument complies with the requirements governing the use and application of a laser as specified in regulatory documents issued by the:

- U.S. Department of Health and Human Services and
- Center for Devices and Radiological Health (CDRH).

In compliance with these regulatory documents, every measure has been taken to ensure the health and safety of users and laboratory personnel from the possible dangers of laser use.

Use the instrument according to the information in the manuals.

5.2 WARNINGS

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

To ensure your safety, the Cytometer laser is covered with protective shields. Do not remove these shields.

No user-serviceable assemblies are accessible. Do not attempt to remove the laser or open it.

The instrument has components that are dangerous to the operator. If any attempt has been made to defeat a safety feature, or if the instrument fails to perform as described in its manuals, disconnect the power and call your Beckman Coulter Representative.

5.3 WARNING LABELS

CDRH-required warning labels are placed near or on covers that, if removed, might expose laser radiation. They are also placed near openings that, if looked into, might expose you to laser radiation.

See Figure 5.1 for the Sensing Compartment cover warning label.

See Figures 5.2 and 5.3 for the Optical area warning labels.

See Figure 5.4 for the Laser head warning labels.

See Figure 5.5 for the Cytometer back panel laser label.

See Figure 5.6 for the MCL probe housing warning labels.

See Figure 5.7 for the MCL bar-code reader warning labels.

Figure 5.1 Laser Labels on the Sensing Compartment Cover

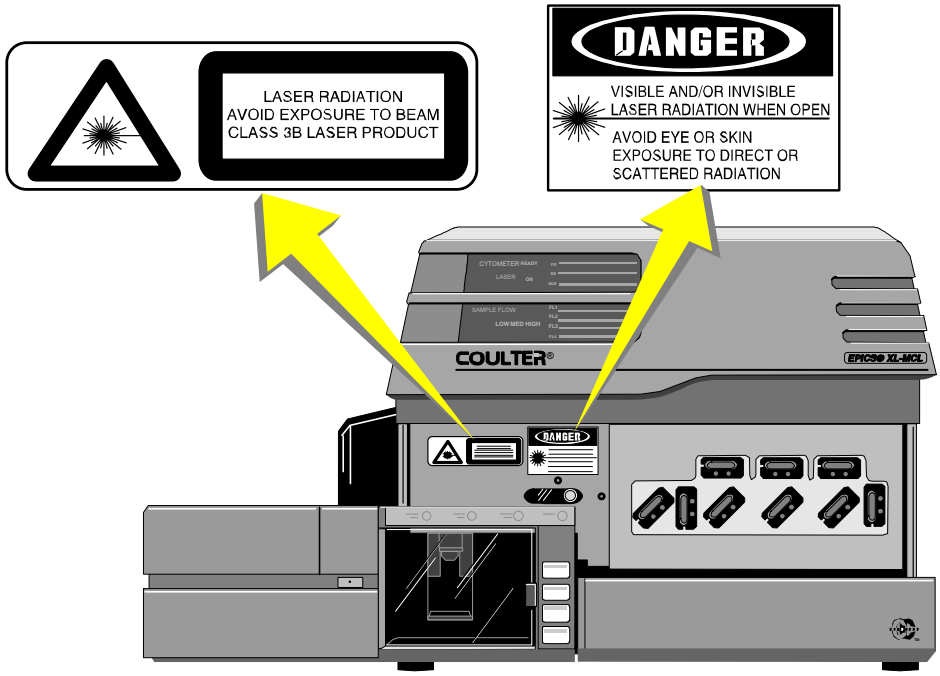


Figure 5.2 Laser Labels in the Optical Area, Side View

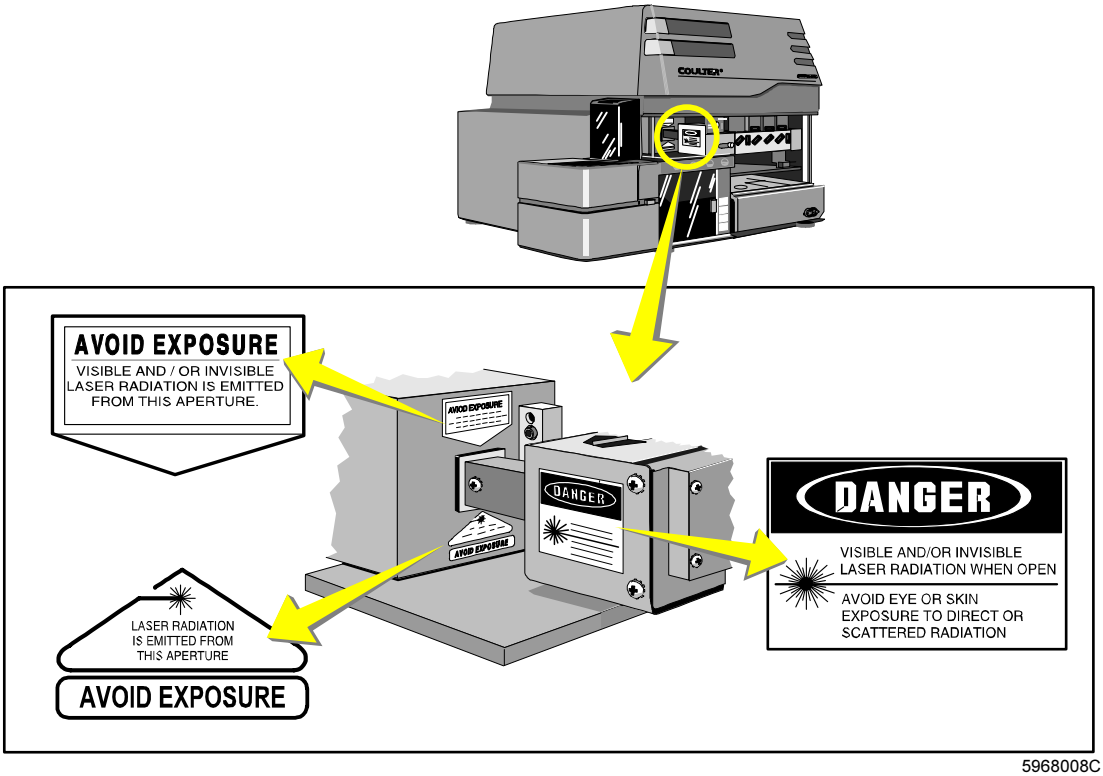


Figure 5.3 Laser Labels in the Optical Area, Front View

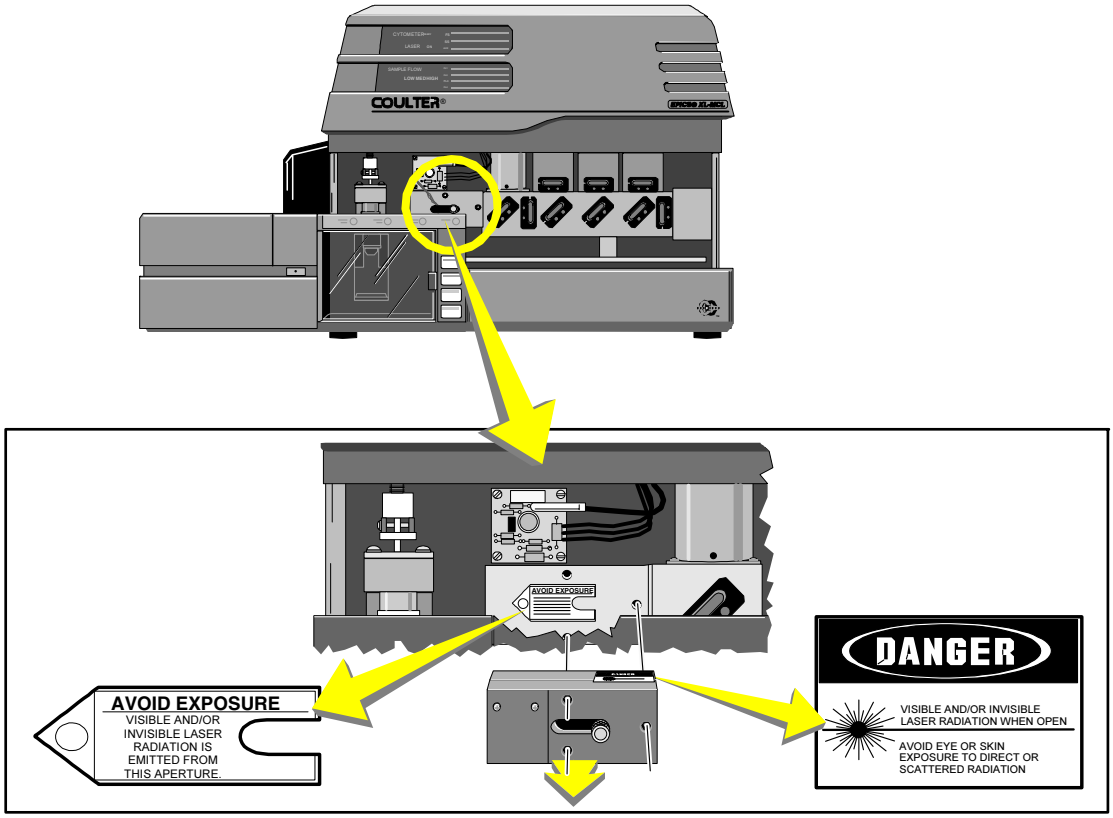


Figure 5.4 Laser Labels on the Laser Head

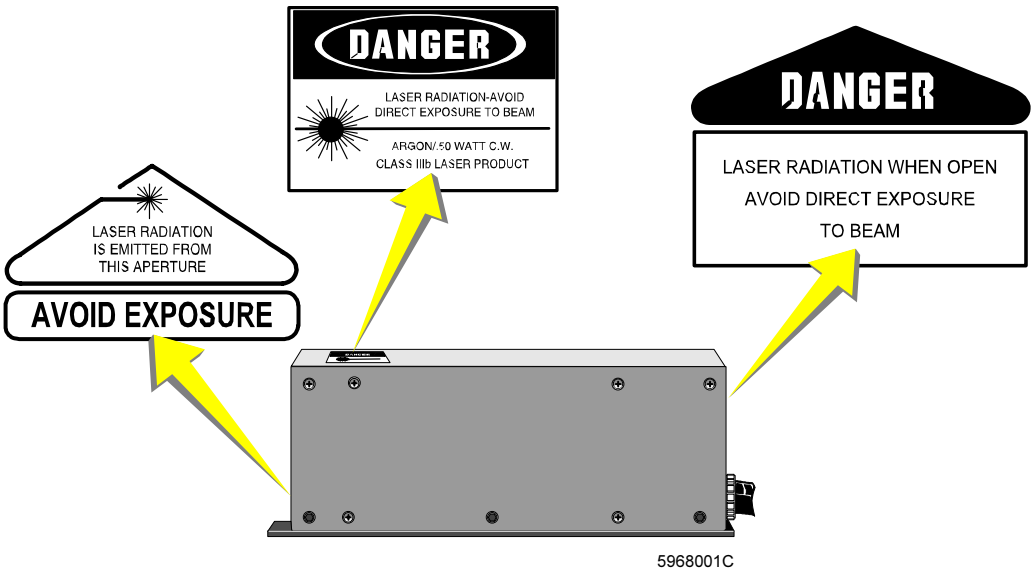


Figure 5.5 Laser Label on the Cytometer Back Panel

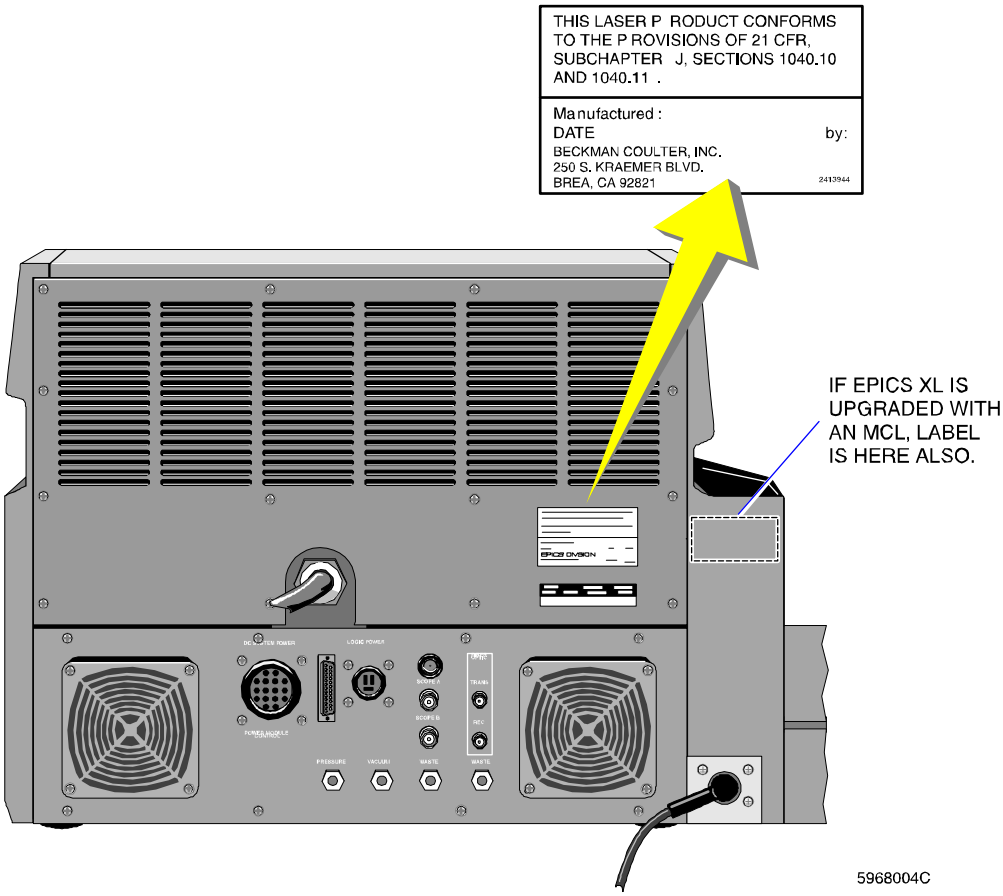


Figure 5.6 Laser Labels on the MCL Probe Housing Cover

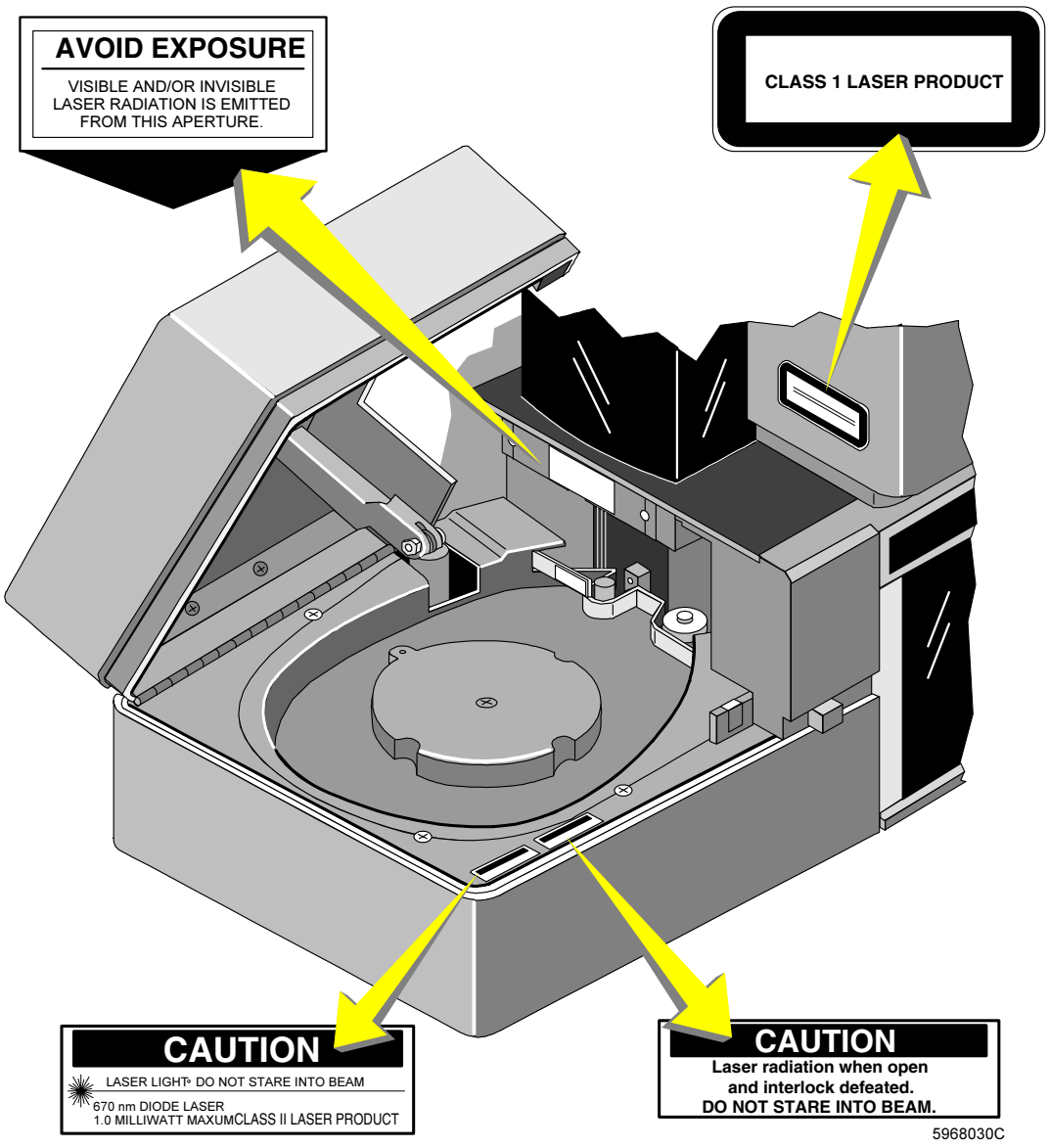
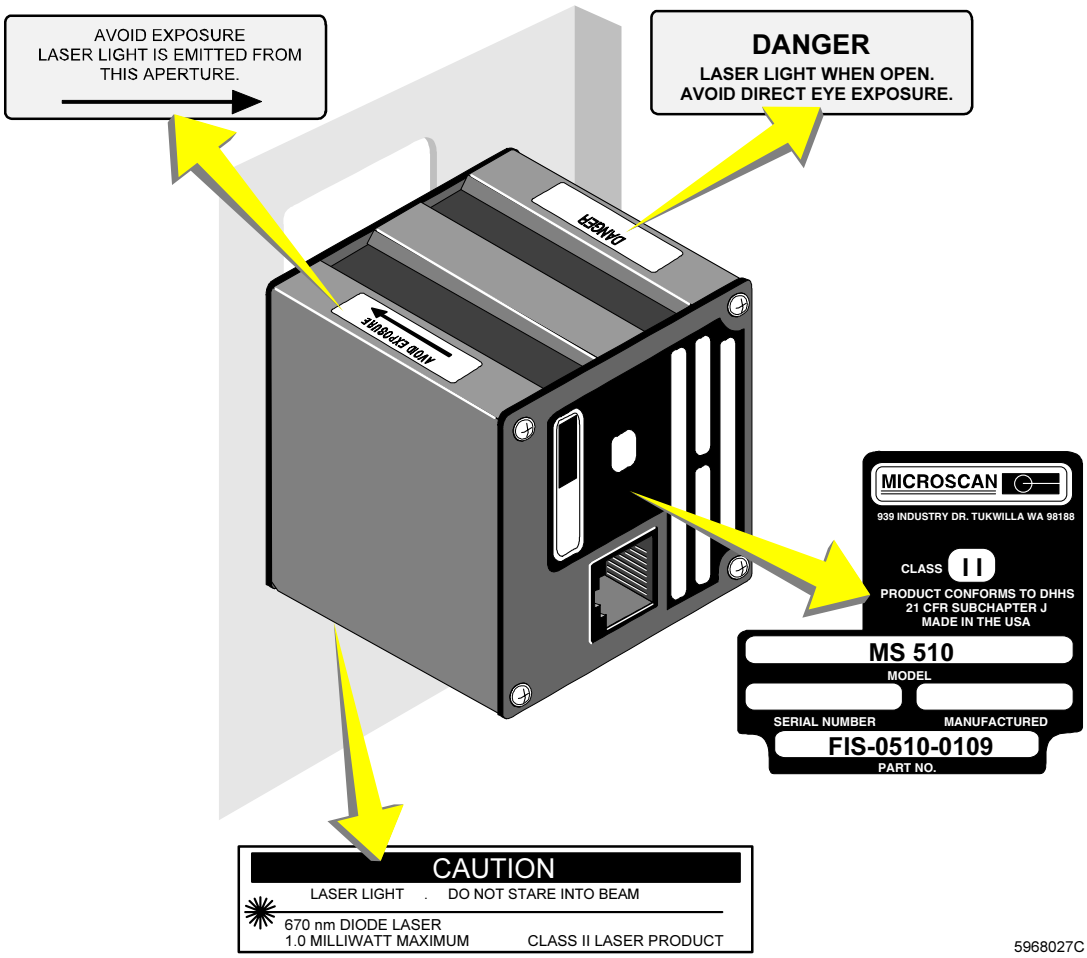


Figure 5.7 Figure 20 Laser Labels on the MCL Bar-Code Reader



LABEL ON BOTTOM OF BAR CODE READER

This appendix contains the following log sheets:

- Reagent Log
- Action Log
- Flow-Set Fluorospheres Charts
  - Daily Log for Instrument Standardization
  - Establishing HV/Total Gain Ranges
  - Establishing Fluorescence and/or Light Scatter Target Ranges
- Flow-Check Fluorospheres Charts
  - Daily Log for Instrument Verification of Alignment and Fluidics
  - Establishing Peak Position and HPCV Target Ranges

Make photocopies as needed.

REAGENT LOG

Reagent Name	Date Opened	Lot Number	Expiration Date	Tech

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298010C

ACTION LOG

Date	Condition Noted	Tech	Date	Action Taken	Tech

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298009C

DAILY LOG FOR INSTRUMENT STANDARDIZATION

Flow-Set™ Fluorospheres Lot Number _____

Application _____ Expiration Date _____

Instrument HV/Total Gain Target Ranges:

FS_____ LOG FL1_____ LOG FL3_____

SS/LOG SS_____ LOG FL2_____ LOG FL4_____

	FS			SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4		
Run	Peak	HV	Gain/ Total Gain*	Peak	HV	Gain/ Total Gain*	Peak	HV	Peak	HV	Peak	HV	Peak	HV	Tech/ Date
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
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19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
31															

*Record total gain if using autostandardization.

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298012C

ESTABLISHING HV/TOTAL GAIN RANGES

Flow-Set™ Fluorospheres Lot Number _____

Application _____ Expiration Date _____

Peak Intensity Target Ranges:

FS_____ LOG FL1_____ LOG FL3_____

SS/LOG SS_____ LOG FL2_____ LOG FL4_____

	FS			SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4		
Run	Peak	HV	Gain/ Total Gain*	Peak	HV	Gain/ Total Gain*	Peak	HV	Peak	HV	Peak	HV	Peak	HV	Tech/ Date
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
Average HV/Gain															
Average +2SD or +1%															
Average -2SD or -1%															

*Record total gain if using autostandardization.

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298013C

ESTABLISHING FLUORESCENCE AND/OR LIGHT SCATTER TARGET RANGES

Flow-Set™ Fluorospheres

Lot Number _____

Application _____

Expiration Date _____

	FS			SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4		
Run	Peak	HV	Gain/ Total Gain*	Peak	HV	Gain/ Total Gain*	Peak	HV	Peak	HV	Peak	HV	Peak	HV	Tech/ Date
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
n															
Average Channel															

*Record total gain if using autostandardization.

See package insert for directions on how to determine the target range for each parameter.

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298014C

DAILY LOG FOR INSTRUMENT VERIFICATION OF ALIGNMENT AND FLUIDICS

Flow-Check™ Fluorospheres

Lot Number _____

Expiration Date _____

Target Range	FS		FL1		FL2		FL3		FL4		
Run	Peak	HPCV	Peak	HPCV	Peak	HPCV	Peak	HPCV	Peak	HPCV	Tech/Date
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
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31											

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298016C

ESTABLISHING PEAK POSITION AND HPCV TARGET RANGES

Flow-Check™ Fluorospheres

Lot Number _____

Expiration Date _____

Run	FS		FL1		FL2		FL3		FL4		Tech/Date
	Peak	HPCV	Peak	HPCV	Peak	HPCV	Peak	HPCV	Peak	HPCV	
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
Mean											
SD											
+2SD											
-2SD											

Serial No. _____

Lab. _____

COULTER® EPICS® XL™ Flow Cytometer
COULTER® EPICS® XL-MCL™ Flow Cytometer

BECKMAN COULTER™

7298015C

This Appendix contains Tables 2.1 through 2.6 which describe the panels, protocols, and QC templates included with version 3.0 of the SYSTEM II software. These are generic templates that you can use "as is", or modify for your specific application needs. The protocols listed in theses panels can also be run separately or combined to form other panels.

Table 2.1 General Use Panels and Protocols

Panel Name	Protocol Name (and QC Template Name if applicable)	Description
Cleaning	BLEACH Rinse Rinse Rinse	Use this panel as the cleaning procedure at the end of a shift or application as per your laboratory procedure.
N/A	CLENZ	Use this protocol as a single protocol cleaning procedure.
N/A	_Axyz Flow-Check*	Use this protocol to set up the target Cytometer settings for the Flow-Check Fluorospheres.
N/A	_Qxyz Flow-Check*	Use this protocol after the target Cytometer settings have been set for a specific lot of Flow-Check Fluorospheres. The *n*xyz.qcs baseline file updates the Cytometer settings when running this protocol.

* Each _A and _Q protocol listed in this table has a corresponding QC template predefined in the software. The QC template has the same name as the protocol and resides in the same directory.

Table 2.2 Two-Color Application Panels and Protocols

Panel Name	Protocol Name (and QC Template Name if applicable)	Description
2 Color Auto Setup	_A2CL Flow-Set* _C1 FITC/RD1 CYTO-COMP* _Q2CL CD45-FITC/CD14-RD1* _Q2CL MsIgG1-RD1/MsIgG1-FITC* _Q2CL 1 CD3-FITC/CD4-RD1* _Q2CL 2 CD3-FITC/CD8-RD1* _Q2CL 3 CD3-FITC/CD19-RD1* _Q2CL 4 CD3-FITC/CD56-RD1*	_ Adjusts and monitors the Cytometer settings. _ Adjusts the compensation percentages. _ The _Q templates in this panel monitor the % positive, mean channel, and absolute counts (if using Flow-Count fluorospheres) for the pertinent regions in each protocol. Use the protocols to analyze control cells. Modify the panel and protocols as needed for your application.
N/A	_Q2CL FITC/RD1*	Use this protocol to create additional 2 Color Auto Setup protocols for different antibody combinations for the control cells. Subsequently, additional _Q protocols can be added to the original 2 Color Auto Setup Panel, as your applications require.

Table 2.2 Two-Color Application Panels and Protocols *(Continued)*

N/A	_Q2CL Kappa-FITC/CD19-RD1*	Add this protocol to the 2 Color Auto Setup Panel if your application includes Kappa analysis.
N/A	_Q2CL Lambda-FITC/CD19-RD1*	Add this protocol to the 2 Color Auto Setup Panel if your application includes Lambda analysis.
2 Color Analysis	2CL MsIgG1-RD1/MsIgG1-FITC 2CL CD45-FITC/CD14-RD1 2CL CD3-FITC/CD4-RD1 2CL CD3-FITC/CD8-RD1 2CL CD3-FITC/CD19-RD1 2CL CD3-FITC/CD56-RD1	Use the 2 Color Analysis panel to analyze your two-color applications. Modify the panel and protocols as needed for your application. The 2CL.qcs baseline file updates the Cytometer settings when running this panel.
N/A	2CL FITC/RD1	Use this protocol to create additional 2 Color Analysis protocols for different antibody combinations. Subsequently, these additional protocols can be added to the original 2 Color Analysis Panel as your applications require. These protocols may be run alone or combined to create panels specific to your applications. The 2CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	2CL Kappa-FITC/CD19-RD1	Add this protocol to the 2 Color Analysis panel if your application includes Kappa analysis.The 2CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	2CL Lambda-FITC/CD19-RD1	Add this protocol to the 2 Color Analysis panel if your application includes Lambda analysis. The 2CL.qcs baseline file updates the Cytometer settings when running this protocol.

* Each _A, _C, and _Q protocol listed in this table has a corresponding QC template predefined in the software. The QC template has the same name as the protocol and resides in the same directory.

Table 2.3 Three-Color Application [4 PMT System] Panels and Protocols

Panel Name	Protocol Name (and QC Template Name if applicable)	Description
3 Color Auto Setup [4 PMT System]	_A3CL Flow-Set [4 PMT]* _C1 FITC/RD1 CYTO-COMP* _C3 RD1/PC5 CYTO-COMP [3CL-4PMT]* _Q3CL 1 45-FITC/G1RD1/G1PC5 [4]* _Q3CL 3 45-FITC/4-RD1/3-PC5 [4]* _Q3CL 5 45-FITC/8-RD1/3-PC5 [4]* _Q3CL 7 45-FITC/19-RD1/3PC5 [4]* _Q3CL 9 45-FITC/56-RD1/3PC5 [4]*	_ Adjusts and monitors the Cytometer settings. _ Adjusts the compensation percentages. _ The _Q templates in this panel monitor the % positive, mean channel, and absolute counts (if using Flow-Count fluorospheres) for the pertinent regions in each protocol. Use the protocols to analyze control cells. Modify the panel and protocols as needed. The 3CL.qcs baseline file updates the Cytometer settings when running this panel.
N/A	_A3ECD Flow-Set	Adjusts and monitors Cytometer settings for three-color immunophenotyping using FITC, RD1, and ECD on either a 3 PMT or 4 PMT system.
N/A	_Q3ECD 2 FITC/RD1/ECD [4]*	Use this protocol to create additional 3 Color Auto Setup protocols for different antibody combinations of FITC, RD1, and ECD for control cells. Subsequently, additional _Q protocols can be added to the original 3 Color Auto Setup Panel, as your applications require. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	_Q3CL A4 FITC/RD1/PC5 [4]*	Use this protocol to create additional 3 Color Auto Setup protocols for different antibody combinations of FITC, RD1, and PC5 for control cells. Subsequently, additional _Q protocols can be added to the original 3 Color Auto Setup Panel, as your applications require. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	_Q3CL B2 G1-FITC/G1-RD1/G1-PC5 [4]*	Add this protocol to the 3 Color Auto Setup Panel if your application includes this antibody. Modify the protocol as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	_Q3CL B4 8-FITC/4-RD1/3-PC5 [4]*	Add this protocol to the 3 Color Auto Setup Panel if your application includes this antibody. Modify the protocol as required for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.

Table 2.3 Three-Color Application [4 PMT System] Panels and Protocols *(Continued)*

3 Color Analysis [4 PMT System]	3CL 45-FITC/G1-RD1/G1-PC5 [4] 3CL 45-FITC/4-RD1/3-PC5 [4] 3CL 45-FITC/8-RD1/3-PC5 [4] 3CL 45-FITC/19-RD1/3-PC5 [4] 3CL 45-FITC/56-RD1/3-PC5 [4]	Use the 3 Color Analysis panel to analyze your three-color applications. Modify the panel and protocols as needed for your application.The 3CL.qcs baseline file updates the Cytometer settings when running this panel.
N/A	3ECD FITC/RD1/ECD [4]	Use this protocol to create additional 3 Color Analysis protocols for different antibody combinations of FITC, RD1, and ECD. Subsequently, these additional protocols can be added to the original 3 Color Analysis Panel as your applications require. These protocols can be run alone or combined to create panels specific to your applications. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	3CL FITC/RD1/PC5 [4]	Use this protocol to create additional 3 Color Analysis protocols for different antibody combinations of FITC, RD1, and PC5. Subsequently, these additional protocols can be added to the original 3 Color Analysis Panel as your applications require. These protocols can be run alone or combined to create panels specific to your applications. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	3CL 8-FITC/4-RD1/3-PC5 [4]	Add this protocol to the 3 Color Analysis panel or use as a single protocol if your application includes analysis of this antibody combination. Modify the protocol as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	3CL G1-FITC/G1-RD1/G1-PC5 [4]	Add this protocol to the 3 Color Analysis panel or use as a single protocol if your application includes analysis of this antibody combination. Modify the protocol as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.

* Each _A, _C, and _Q protocol listed in this table has a corresponding QC template predefined in the software. The QC template has the same name as the protocol and resides in the same directory.

Table 2.4 Three-Color Application [3PMT System] Panels and Protocols

Panel Name	Protocol Name (and QC Template Name if applicable)	Description
3 Color Auto Setup [3 PMT System]	_A3CL Flow-Set [3 PMT]* _C1 FITC/RD1 CYTO-COMP* _C3 RD1/PC5 CYTO-COMP [3CL-3PMT]* _Q3CL 0 45-FITC/G1RD1/G1PC5 [3]* _Q3CL 2 45-FITC/4-RD1/3-PC5 [3]* _Q3CL 4 45-FITC/8-RD1/3-PC5 [3]* _Q3CL 6 45-FITC/19-RD1/3PC5 [3]* _Q3CL 8 45-FITC/56-RD1/3PC5 [3]*	_ Adjusts and monitors the Cytometer settings. _ Adjusts the compensation percentages. _ The _Q Templates in this panel monitor the % positive, mean channel, and absolute counts (if using Flow-Count fluorospheres) for the pertinent regions in each protocol. Use these protocols to analyze control cells. Modify the panel and protocols as required for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this panel.
N/A	_Q3ECD 1 FITC/RD1/ECD [3] 620 BP*	Use this protocol to create additional 3 Color Auto Setup protocols for different antibody combinations of FITC, RD1, and ECD. Subsequently, additional _Q protocols can be added to the original 3 Color Auto Setup Panel, as your applications require. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	_Q3CL A3 FITC/RD1/PC5 [3] 675 BP*	Use this protocol to create additional 3 Color Auto Setup protocols for different antibody combinations of FITC, RD1, and PC5. Subsequently, additional _Q protocols can be added to the original 3 Color Auto Setup Panel as your applications require. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	_Q3CL B1 G1-FITC/G1-RD1/G1-PC5 [3]*	Add this protocol to the 3 Color Auto Setup Panel if your application includes this antibody. Modify the protocol as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	_Q3CL B3 8-FITC/CD4-RD1/CD3-PC5[3]*	Add this protocol to the 3 Color Auto Setup Panel if your application includes this antibody. Modify the protocol as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
3 Color Analysis [3 PMT System]	3CL 45-FITC/G1-RD1/G1-PC5 [3] 3CL 45-FITC/4-RD1/3-PC5 [3] 3CL 45-FITC/8-RD1/3-PC5 [3] 3CL 45-FITC/19-RD1/3-PC5 [3] 3CL 45-FITC/56-RD1/3-PC5 [3]	Use the 3 Color Analysis panel to analyze your three-color applications. Modify the panel and protocols as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this panel.

Table 2.4 Three-Color Application [3PMT System] Panels and Protocols *(Continued)*

N/A	3ECD FITC/RD1/ECD [3] 620 BP	Use this protocol to create additional 3 Color Analysis protocols for different antibody combinations of FITC, RD1, and ECD. Subsequently, these additional protocols can be added to the original 3 Color Analysis Panel, as your applications require. These protocols can be run alone or combined to create panels specific to your applications. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	3CL FITC/RD1/PC5 [3] 675 BP	Use this protocol to create additional 3 Color Analysis protocols for different antibody combinations of FITC, RD1, and PC5. Subsequently, these additional protocols can be added to the original 3 Color Analysis Panel, as your applications require. These protocols can be run alone or combined to create panels specific to your applications. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	3CL 8-FITC/4-RD1/3-PC5 [3]	Add this protocol to the 3 Color Analysis panel or use as a single protocol if your application includes analysis of this antibody combination. Modify the protocol as required for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	3CL G1-FITC/G1-RD1/G1-PC5 [3]	Add this protocol to the 3 Color Analysis panel or use as a single protocol if your application includes analysis of this antibody combination. Modify the protocol as needed for your application. The 3CL.qcs baseline file updates the Cytometer settings when running this protocol.

* Each _A, _C, and _Q protocol listed in this table has a corresponding QC template predefined in the software. The QC template has the same name as the protocol and resides in the same directory.

Table 2.5 Four-Color Application Panels and Protocols

Panel Name	Protocol Name (and QC Template Name if applicable)	Description
4 Color Auto Setup	_A4CL Flow-Set* _C1 FITC/RD1 CYTO-COMP* _C2 RD1/ECD CYTO-COMP* _C3 RD1/PC5 CYTO-COMP [4CL]* _C4 ECD/PC5 CYTO-COMP* _Q4CL FITC/RD1/ECD/PC5*	_ Adjusts and monitors the Cytometer settings. _ Adjusts the compensation percentages. _ The _Q Templates in this panel monitor the % positive, mean channel, and absolute counts (if using Flow-Count fluorospheres) for the pertinent regions in each protocol. Modify the panel and protocols as needed for your application. Use these protocols to analyze control cells. The 4CL.qcs baseline file updates the Cytometer settings when running this panel.
4 Color Analysis	4CL FITC/RD1/ECD/PC5	Use the 4 Color Analysis panel to analyze your four-color applications. Modify the panel as required for your application. The 4CL.qcs baseline file updates the Cytometer settings when running this panel.

* Each _A, _C, and _Q protocol listed in this table has a corresponding QC template predefined in the software. The QC template has the same name as the protocol (but a different extension) and resides in the same directory.

Table 2.6 DNA Application Panels and Protocols

Panel Name	Protocol Name (and QC Template Name if applicable)	Description
N/A	_ADNA INDEX Control*	Use this Autostandardization protocol to adjust the Cytometer settings for DNA analysis. Use the QC template to monitor the Cytometer settings.
N/A	_QDNA INDEX Control*	Use this protocol to collect and monitor DNA INDEX Control percentages and mean channels. The dna.qcs baseline file updates the Cytometer settings when running this protocol.
N/A	DNA Analysis	Use this protocol to analyze your DNA specimens. Modify as needed for your application. The dna.qcs file updates the Cytometer settings when running this protocol.

* Each _A and _Q protocol listed in this table has a corresponding QC template predefined in the software. The QC template has the same name as the protocol and resides in the same directory.

_A protocols - These protocols are used to automatically adjust high voltage and gain settings. One is always assigned as a primary protocol in an Autostandardization panel so that the settings are passed to other protocols in the panel.

Accuracy - The ability of an instrument to agree with a predetermined reference value at any point within the operating range. Contrast with precision.

ASCII - Abbreviation for American Standard Code for Information Interchange. An ASCII file is a type of text file.

Assay values - Values for a control established by extensive repeat testing of that control.

AUX Signal - Auxillary acquisition pathway that allows either control of simultaneous Lin and Log signals or acquisition of a Peak signal.

Background count - Measure of the amount of electrical or particle interference.

BK filter - A laser-blocking optical filter that passes the fluorescence wavelengths but does not pass the laser wavelength.

BP filter - A band-pass optical filter that passes a band of wavelengths and blocks others.

Button - The Workstation screens have named areas (for example, a rectangle labeled Abort) that you select with the mouse to tell the instrument what to do. The Cytometer has buttons that you press to specify the operating mode (for example, the RUN button).

_C protocols - These protocols are used to standardize fluorescent compensation. They are always assigned as secondary protocols in an autostandardization panel, following the _Axyz protocol.

CAL Factor - A number used in conjunction with a known number of particles identified by a CAL region, that adjusts the region counts obtained.

Cell Seeker - 3 Levels of AutoGating.

Cell Stat - Algorithm that performs automatic gating and analysis used in tetraONE System.

Cell Tracker - Color event analysis with green, blue, red, and magenta colors assigned to regions and gates.

Channel - In an analog-to-digital converter, the number of equally spaced divisions of the amplified input signal voltage. All XL and XL-MCL flow cytometer signals are resolved into 1024 channels. For dual-parameter histograms, the number of channels is reduced to 64, 128, or 256.

Cleaning agent - A detergent used to flush sample from tubing and eliminate protein buildup.

Click - To press and release a mouse button.

Coefficient of variation (CV%) - A measure of the variability in signal intensity that is generated as particles pass repeatedly through the laser beam. This variability is expressed as a percentage of the average signal intensity.

Collimate - To make parallel (for example, collimate rays of light).

Color compensation - The subtraction of:

- a percentage of the signal from one fluorescence light sensor from
- the signal from another fluorescence light sensor

to correct for the overlap of one dye's emission into another dye's emission measurement.

Control - A substance used to routinely monitor the performance of an analytical process that does not have the characteristic being measured (for example, Immuno-Trol cells or CYTO-TROL control cells).

Controls and indicators - Instrument controls are the mechanisms you use to communicate with the instrument. Indicators are the mechanisms the instrument uses to communicate with you. Controls and Indicators is the first chapter in the Getting Started manual.

Cross-cylindrical lenses - Used in the Cytometer to focus the laser beam and form an elliptical beam spot.

Cytometer - The system component that analyzes the sample and contains the sheath fluid and cleaning agent bottles.

Database file - SQL format file that contains records of the patient report.

Database record - Individual patient panel report residing in the database file.

dc - Abbreviation for direct current.

Defaults - Original settings for the instrument. You can change them to customize the settings for your laboratory.

Discriminator - A channel setting for a parameter that lets you ignore events below the setting. This lets you eliminate signals caused by debris.

Display Options - Acquisition button that changes cytometer adjustment options in the Cytometer Control Window.

DL **filter** - A dichroic, long-pass optical filter that directs light in different spectral regions to different detectors.

DiOC5(3) - Abbreviation for oxacarbocyanine dye.

EPT2ASC2 - Program that converts Export (*. EPT) files to ASCII (*. EPA) files and (*. CYT) files to ASCII (*. CYA) files.

Export file (*.EPT) - Binary file generated by running a panel that contains the region statistics.

Event - A particle passing through the laser beam.

Fast Set - Direct histogram manipulation to adjust cytometer voltages and gain.

Fast Comp - Direct histogram manipulation to adjust cytometer color compensation.

FDA - Abbreviation for fluorescein diacetate dye.

File Trimming - Process that deletes data from the database file and from the QC history file by a date older than the time period you enter when to trim Auto Setup Records in the Data Management screen. Used to minimize the memory requirements of these files.

FITC - Abbreviation for fluorescein isothiocyanate dye.

Flow cell - A device through which particles pass, in a stream of fluid, one at a time, through a laser beam.

Flow cytometry - A process for measuring the characteristics of cells or other biological particles as they pass through a measuring apparatus in a fluid stream.

Fluorescent light - The emission of electromagnetic radiation that occurs when the emitting body absorbs radiation from some other source. For example, when a fluorescent dye is excited (absorbs radiation), it emits fluorescent light at a wavelength that is different from the wavelength of the light that excited it.

Fluorescent light (FL1, FL2, FL3, FL4 optional) sensors - Collect the fluorescent light and generate voltage pulse signals. The "1" refers to the first fluorescence sensor; "2" the second; and so forth.

Forward scatter (FS) - The laser light scattered at narrow angles to the axis of the laser beam. The amount of forward scatter is proportional to the size of the cell that scattered the laser light.

Forward scatter (FS) sensor - Collects the forward scatter and generates voltage pulse signals.

Gain - The amount of amplification applied to a signal. In linear amplification, all of a sensor's signals are increased by the same amount. Contrast with logarithmic amplification.

Gating - The use of criteria that must be met before an event is included in a histogram.

GB - The abbreviation for gigabyte.

High voltage - Can be adjusted to change the sensitivity of a fluorescent light sensor.

Histogram - A graph showing the relative number and distribution of events.

Hist Mode - Multigraph analysis of individual histograms from different run numbers.

Hot keys - A keyboard shortcut for changing screens. Instead of using the menu bar to change screens, you can press and hold down Alt while pressing a certain letter key. For example, pressing Alt and C simultaneously displays the Cytosettings screen.

Hydrodynamic focusing - A process that focuses the sample stream through the flow cell. It ensures that cells move through the laser beam one at a time, along the same path.

Instance Number - A Specimen can have multiple sets of results generated from acquisition and listmode. Each set of results saved to the Database is given an incremental instance number that internally links the results table to the cyto run table and the specimen table.

Integral signal - A voltage pulse with height and area proportional to the total amount of fluorescent material in a cell.

IQAP - Abbreviation for Beckman Coulter's Interlaboratory Quality Assurance Program. A service for all worldwide users of Immuno-Trol cells and CYTO-TROL control cells, the IQAP statistically compares your control data with that of other laboratories.

Laser - Abbreviation for light amplification by stimulated emission of radiation. Two lasers are in the instrument: one in the MCL for reading bar codes and one in the flow cell for analyzing cells.

Levey-Jennings - Graphs that display the control data for easy review for trends, shifts, outliers, and violations of your laboratory's guidelines. The graph consists of a middle line that shows the mean, and a set of inner limit lines and outer limit lines that appear above and below the mean. The control data appears as connected dots within the graph.

Linear amplification - See gain.

Listmode data - A list of measurements from each cell.

LISTGATE - Listmode Gate used as a live gate in acquisition and for listmode archival.

Loadlist - A group of panels.

Logarithmic amplification - A method of increasing the gain and dynamic range of a signal. A larger gain is applied to a sensor's smaller signals than to the sensor's larger signals. See also gain.

MB - Abbreviation for megabyte.

Mean - Arithmetic average of a group of data. See also standard deviation and coefficient of variation.

Menu - On a Workstation screen, a list of items from which you can choose.

Minimum Event Counter - The count that must minimally be achieved in order to stop acquisition. Used to ensure collection of rare events.

Mouse - A pointing device. The cursor on the Workstation screen moves as you slide the mouse on your desk or other flat surface.

Multi-tube Carousel Loader (MCL) - An optional automated sample loader for the instrument.

New Pnl/Pro - Used when listmode replay is required to be performed with a Panel or with a different protocol from the runtime protocol.

Next File - Listmode button that increments through the queue of listmode files without replaying the data.

Next Set - Button in Multigraph Application that is active in Histogram mode. Increments the display to the next eight histograms in the Hist queue.

Next Test - Button in Multigraph Application that is active in Test mode. Increments the display to the next run number in the Hist queue.

Neutral density (ND1) filter - An optical filter that can be used with the forward scatter sensor to reduce the intensity of the forward scatter, thus enabling the instrument to analyze large particles without saturating the sensor.

Normalization - Applied to linear statistics from different histogram resolutions to ensure results are comparable. Scales to 1,024.

Optical filters - Mediums, such as glass, that separate fluorescent light by wavelength, which is measured in nanometers (nm). See also BK, BP, and DL filters.

Output Options - Acquisition and Listmode global options for printing hardcopy and carousel summary. Also controls saving export files, database records, and patient report files.

Panel - A group of protocols for analyzing a series of tubes corresponding to one patient specimen. The Cytometer settings are passed on through the panel with identification of the primary tubes.

Pass/Load Regions - Button in Multigraph Application that is active in Test mode. Load regions display regions from the histogram file. Pass regions allow the currently displayed regions to pass on to the next test.

Patient Report - Report generated at the end of a Panel that contains results manipulated by the Report Template.

Patient Report Export File (*.PXP) - The data that is transmitted through a serial port or stored in a file according to the Patient Report template.

Patient Report Template - Template for a patient panel report that allows calculations to be performed on region statistics.

PCX File - A graphics file created using the mouse to identify the capture area.

Peak signal - A voltage pulse with height proportional to the amount of light the cell scatters or fluoresces.

Photo-multiplier tube (PMT) - A light-sensitive sensor that converts light energy into electrical current and generates a voltage pulse signal.

Pickup lens/spatial filter assembly - Collects side scatter and fluorescent light from only the sensing area of the flow cell, and collimates it.

Play List - Listmode button that replays the current listmode file in memory.

Play Next - Listmode button that loads and replays the next listmode file in the queue.

Pop-up window - A rectangular area that appears on top of the current screen displayed on the Workstation. You must close the window before you can use the current screen again.

Positives analysis - Analysis performed on the negative control to set regions automatically to exclude the negative population from the positives statistics.

Power Supply - The system component that provides direct current power, pressure, and vacuum to the Cytometer, and collects waste from the Cytometer.

Precision - Ability of an instrument to reproduce similar results when a sample is run repeatedly. Precision shows the closeness of test results when repeated analyses of the same material are performed. Also known as reproducibility. Contrast with accuracy.

PRIME region - When a histogram Peak is not within a PRIME Region the system performs an AutoPrime.

Printer - An optional system component that provides a printout of sample results and other information.

Prism - Phenotype parameter for multicolor analysis.

Prism histogram - Histogram that displays the phenotype of an identified population.

Protocol - A set of instructions that tells the Cytometer what and how to acquire data and relay listmode data.

_Q protocols - These protocols are used to generate quality control statistics from their QC regions. One or more can be assigned as secondary or control protocols at the end of an autostandardization panel.

QC Template - A customized design format instructing the system to display operator specified data from a specific test protocol.

QCC file - A QC data file generated by a _Q protocol that contains the statistics from all the defined QC regions in that protocol. The data can be viewed in Levey-Jennings and data table format.

QCS file - A QC Cytometer setting baseline file generated by a _A protocol that contains the voltage and gain settings from the _A protocol and the compensation values from the last _C protocol in the Autostandardization panel. The data can be viewed in Levey-Jennings and data table format.

Quality control (QC) - A comprehensive set of procedures a laboratory sets up to ensure that an instrument is working accurately and precisely.

RCVEXP3 - Program that resides in a Workstation that receives the serial transmission of an Export file from an XL or XL-MCL flow cytometer.

RD1 - Red dye 1.

Rebuild - Button used to update a directory listing whenever files are copied, moved, or deleted.

Region Mapping - Regions whose coordinates map to an identified region.

Results DB - That portion of the Database record that contains information pertaining to the results from the patient report.

Retic Stat - Algorithm that performs automatic gating and analysis used in reticONE System.

Runtime Protocol - The Protocol stored with the Listmode file at acquisition. Listmode replays identical to the acquisition protocol.

Runtime SQL - The database engine accessed from within SYSTEM II software.

Scroll bar - The area on the left of a pop-up window. The bar's arrows let you move (scroll) the window's content up or down so that you can see other parts of it. For example, the scroll bar in the Protocol Select window lets you scroll through the entire list of protocol names.

Select - To position the mouse cursor on an item, and then press and release a mouse button to choose that item.

Select Dir - A button in the file selection window that allows you to change to a different directory or drive to select files.

Sensitivity - The ability of the instrument to distinguish very low levels of light scatter and fluorescence from background light or electronic noise.

Sheath fluid - A balanced electrolyte solution.

Side scatter - The amount of laser light scattered at about a 90° angle to the axis of the laser beam. The amount of side scatter is proportional to the granularity of the cell that scattered the laser light.

Side scatter (SS) sensor - Collects the side scatter and generates voltage pulse signals.

Specimen DB - That portion of the Database record that contains information pertaining to the specimen and patient demographics.

Specimen ID - ID assigned to a Specimen draw as opposed to a tube.

SQL - Standard query language.

Standard deviation (SD) - A measure of difference from the mean. A measure of precision.

Test Mode - Multigraph analysis of histograms from the same run number either tube by tube or batched.

Tube ID - The Bar code ID on individual reaction sample tubes.

Voltage pulse signals - The signals that the forward scatter, side scatter, and fluorescence sensors generate. They are proportional to the intensity of light the sensor received.

Volume Stop - Stops acquisition based on a predefined volume of 20, 40, 60, 80, or 100 µL.

Westgard rules - A set of rules in the software that checks the quality control data against standard deviation related criteria.

Window - See pop-up window.

Workstation - The system component that runs the software that lets you control the instrument. It displays sample results and other information.

Symbols

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COULTER EPICS XL and XL-MCL Flow Cytometer Documentation

▪ Reference PN 4235298 (Black binding)	Use and Function • Installation • Operation Principles • Specifications/Characteristics • Precautions and Hazards • Log Sheets • Predefined Panels, Protocols, and QC Templates • Glossary • Index
▪ Special Procedures and Troubleshooting PN 4237296 (Blue binding)	Cleaning Procedures • Replace/Adjust Procedures • Troubleshooting • Bar-Code Options • QC Maintenance Screen • Index
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