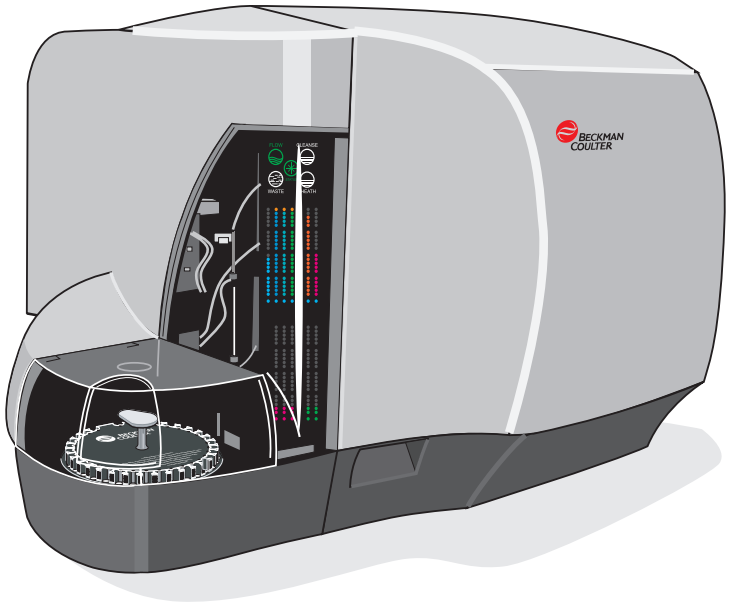
Gallios Flow Cytometer

INSTRUCTIONS FOR USE



773231AB (May 2010)

Beckman Coulter, Inc.
250 S. Kraemer Blvd.
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.

REVISION STATUS

Initial Issue, 2/2009
Gallios Software Version 1.0.
Gallios System Help version 1A.090621

Issue AB2, 5/2010
Software Version 1.0.
Gallios System Help version 1A.102701

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

OVERVIEW

Contents

The system help contains the information you need to:

- Perform the day-to-day running of the Gallios™ Flow Cytometer.
- Clean, adjust and replace components.
- Understand the operation principles and methods.
- Review the instrument specifications.

How to Find Information

To find the information you need in the system help:

- Select the **Contents** Tab.
  - Select a heading, table or illustration to view it.
- Select the **Index** Tab.
  - Type in a keyword.
  - Double click on any of the related index entries that appear to view them.
- Select the **Search** Tab.
  - Type in a word or phrase, for example: sample probe.
  - Double click on any of the related headings that appear to view them.
  - All instances of the word(s) are highlighted in the text (for this example: sample, sample probe, and probe).
- Select the **Favorites** Tab.
  - Select any of the favorites that you previously selected. See USING THE SYSTEM HELP for instructions to create your favorites.

USING THE SYSTEM HELP

See USING THE SYSTEM HELP for detailed instructions how the system help works.

CONVENTIONS

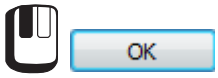
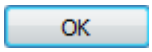
The system help uses the following conventions:

- Throughout the system help, your Gallios Flow Cytometry System is also referred to as the system or instrument.
- Blue and Underlined text indicates that you can click on the text to access related information.
- **Bold font** indicates a software option, such as **Cytometer**.
- *Italics font* indicates screen text displayed on the instrument, such as *Preparing Samples*.
- `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:

a. Press down on the first key listed and while continuing to press it, press down on the second key listed.

b. 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].
- Icons/buttons to select functions on the software screen are shown within text.

  Example:  .
-  indicates to use the mouse to select the screen button labeled  .
-  **File ▸▸ Save** indicates to use the mouse to select the **Save** item on the **File** menu.
- [F1] through [F12] are special function keys.
- A **Note** contains information that is important to remember or helpful in performing a procedure.
- The terms “screen” and “window’ are used interchangeably.

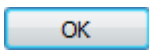
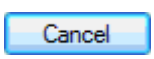
To Choose A Command With The Keyboard

After you press [Alt], each menu name has one letter underlined to indicate which letter to use to pull down the menu. For example, the letter F in the File menu is underlined, press [F] to pull down the File menu; the letter E in the Edit menu is underlined, press [E] to pull down the Edit menu.

Command	Function
[Enter]	Accepts your selection.
[Esc]	Stops the operation, discarding your choices.
[Tab]	Moves cursor over different choices if there are multiple options - see Windows® manuals for Windows operation via keyboard.
[Alt]+[Tab]	When you have more than one application Window open, use [Alt]+[Tab] to switch between tasks.

Dialog Box

Dialog boxes receive commands or information; for example, a file name dialog box receives information about a file name.

	Accepts the information you have selected or typed.
	Stops the operation, ignoring your choices.

Description of Reporting Units

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

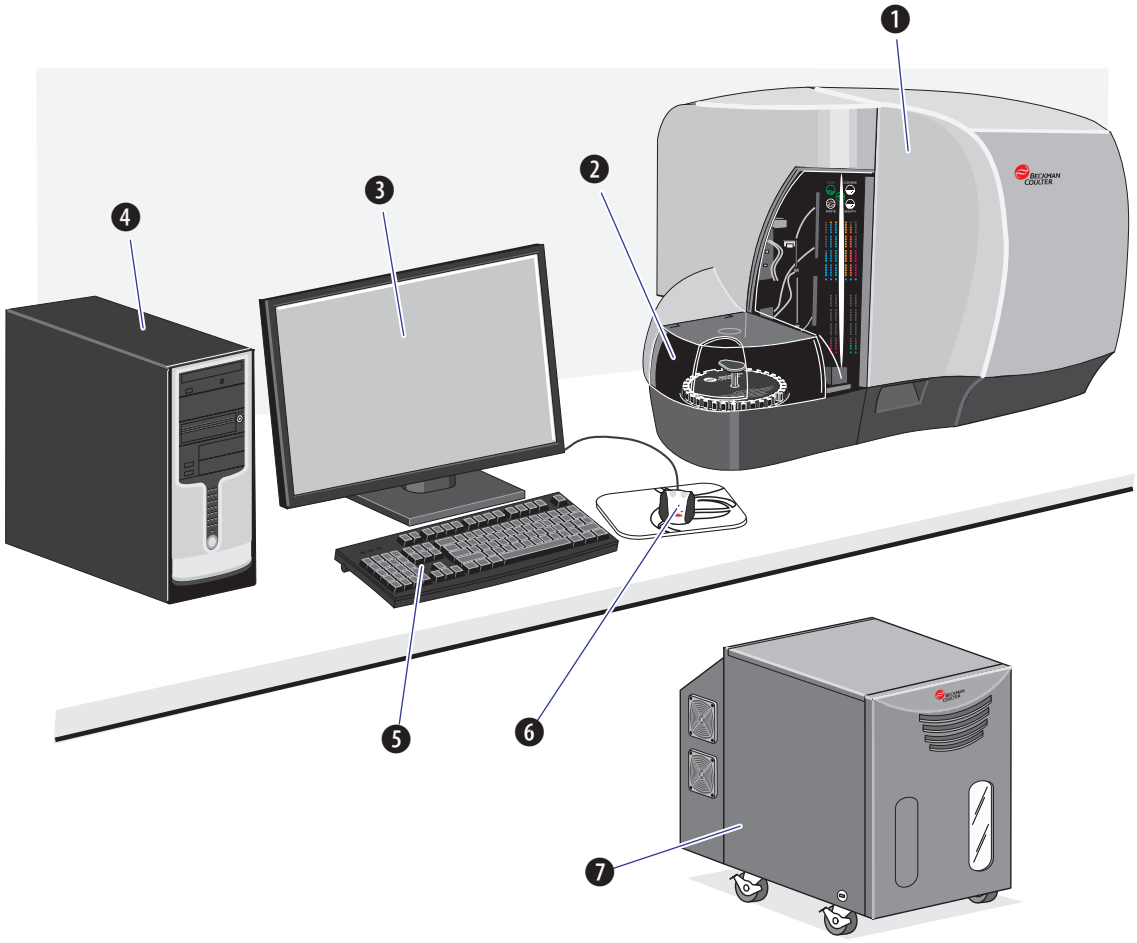
GRAPHICS

All graphics, including screens and printouts, are for illustration purposes only and must not be used for any other purpose.

1.1 SYSTEM COMPONENTS

The system components are shown in Figure 1.1.

Figure 1.1 Gallios Flow Cytometer System



1	Cytometer	5	Keyboard
2	MCL	6	Mouse
3	Monitor	7	Pneumatic Supply
4	Computer		

Cytometer

This unit analyzes the sample. It contains internal sheath fluid and cleaning agent containers.

Pneumatic Supply

This unit provides pressure and vacuum to the Cytometer.

Workstation

The Workstation runs the software that controls 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.

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:

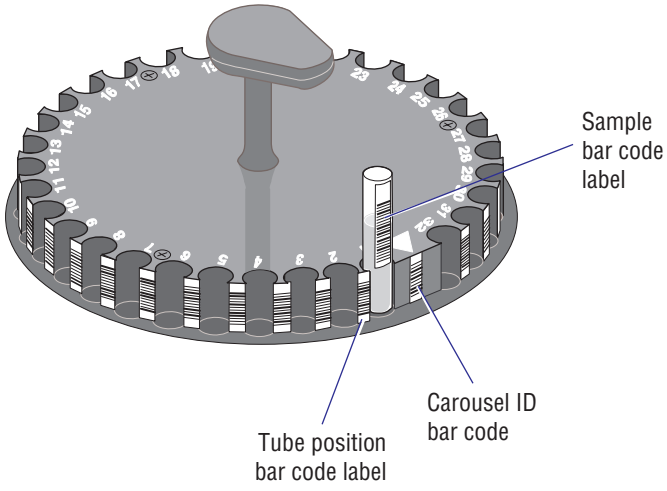
- Codabar
- Code 39® bar code
- Code 128
- Interleaved 2-of-5.

For additional information on the bar-code specifications, see Appendix A, BAR-CODE SPECIFICATIONS.

The MCL mixes each sample before analysis. You can use the MCL to automatically analyze multiple samples or analyze single tubes manually through the tube access door.

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



Gallios Flow Cytometer Software

The Gallios Flow Cytometry System contains the Acquisition software that is used when running the Cytometer.

1.2 OPTIONS

Hardware Options

Printer

Provides a printout of sample results and other information. The Printers available for the system are subject to change, so contact your Beckman Coulter Representative for the current model selection.

Additional PMTs

Additional PMTs and filters are available to configure your system for 8 colors (with the standard 2 lasers) or 10 colors (with the optional third laser).

Third Laser

A 405 nm violet solid-state laser providing a minimum of 40 mW light regulated laser power.

1.3 REAGENTS AND QUALITY CONTROL MATERIALS

Beckman Coulter recommends these reagents or their equivalents. All stated analytical characteristics and specifications in this manual are based on the use of the Gallios Flow Cytometry System with the following reagents.

Sheath Fluid

In the Cytometer, the 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, 405-nm and 638-nm laser light
- Low background
- Compatible with the characteristics of the sample being measured (such as pH, osmolality, conductivity).

The internal sheath container has a working capacity of about 1.8 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, FlowClean 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.

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™ Pro Fluorospheres	Fluorospheres used to check the stability of the optical and fluidic systems.
Flow-Set™ Pro 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 and 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.
IMMUNO-TROL Low 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.
Immuno-Brite™ Fluorospheres	Uniform size fluorospheres with varying fluorescence intensities that are used to monitor instrument linearity.
CYTO-COMP™ Cell Kit	CYTO-COMP Cells stained with a single color are used to adjust color compensation settings for multicolor analysis using monoclonal antibodies.
QuickCOMP 2 Kit	Two single-color antibody reagents (FITC and PE) that can be used to adjust color compensation on a flow cytometer.
QuickCOMP 4 Kit	Four single-color antibody reagents (FITC, PE, ECD, and PC5) that can be used to adjust color compensation on a flow cytometer.

Additional quality control reagents are available. Contact your Beckman Coulter representative or access http://www.beckmancoulter.com.

1.4 MATERIAL SAFETY DATA SHEETS (MSDS)

To obtain an MSDS for Beckman Coulter reagents used on the Gallios Flow Cytometry System:

1. On the internet, go to http://www.beckmancoulter.com and select **MSDS** from the **Customer Support** drop down menu.
2. If you do not have internet access:
   - 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
   - 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:

Specifications	
Height	60.5 cm (23.8 in.)
Additional clearance above for servicing and lifting the Data Acquisition card cage above the sensor	45.7 cm (18 in.) min.
Total clearance needed	106.2 cm (41.8 in.)
Width	95.3 cm (37.5 in.)
Additional clearance on right for servicing	15.2 cm (6 in.)
Additional clearance on left for servicing	15.2 cm (6 in.)
Total clearance needed	125.7 cm (49.5 in.)
Depth	70.1 cm (27.6 in.)
Additional clearance behind instrument for sufficient cooling and room for servicing	3.8 cm (1.5 in.)
Total clearance needed	73.9 cm (29.1 in.)

Electrical Input

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

The Pneumatic Supply requires one dedicated outlet with an isolated grounds The computer requires a separate outlet, but it does not have to be a dedicated line.

Country	Dedicated Lines with Isolated Grounds	Non-Dedicated Lines
USA	One dedicated line at 115 Vac, 50/60 Hz at 15 A	Two non-dedicated lines at 115 Vac, 50/60 Hz at 15 A - one for the tower computer and a second, for the monitor. A third non-dedicated line is required if the optional printer is being installed.
Europe and other applicable countries	One dedicated lines at 220 Vac, 50/60 Hz at 10 A or One dedicated lines at 240 Vac, 50/60 Hz at 10 A	Two non-dedicated lines at 220 Vac, 50/60 Hz at 10 A - one for the tower computer and a second, for the monitor. A third non-dedicated line is required if the optional printer is being installed.
Japan	One dedicated lines at 100 Vac, 50/60 Hz at 15 A	Three non-dedicated lines at 100 Vac, 50/60 Hz at 15 A - one for the tower computer and a second, for the monitor. A third non-dedicated line is required if the optional printer is being installed.

Power Consumption

<1500 Watts

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 30% and 80%, without condensation.

Heat Dissipation

Heat dissipation is 720W (2457 Btu/hour). Provide sufficient air conditioning (refer to Ambient Temperature and Humidity).

Acoustic Noise Level

≤60db

Drainage

The waste line from the Cytometer is connected to a 20-L waste container. Dispose of the waste in accordance with your local regulations and acceptable laboratory procedures.

WARNING Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of the waste container in accordance with your local regulations and acceptable laboratory procedures.

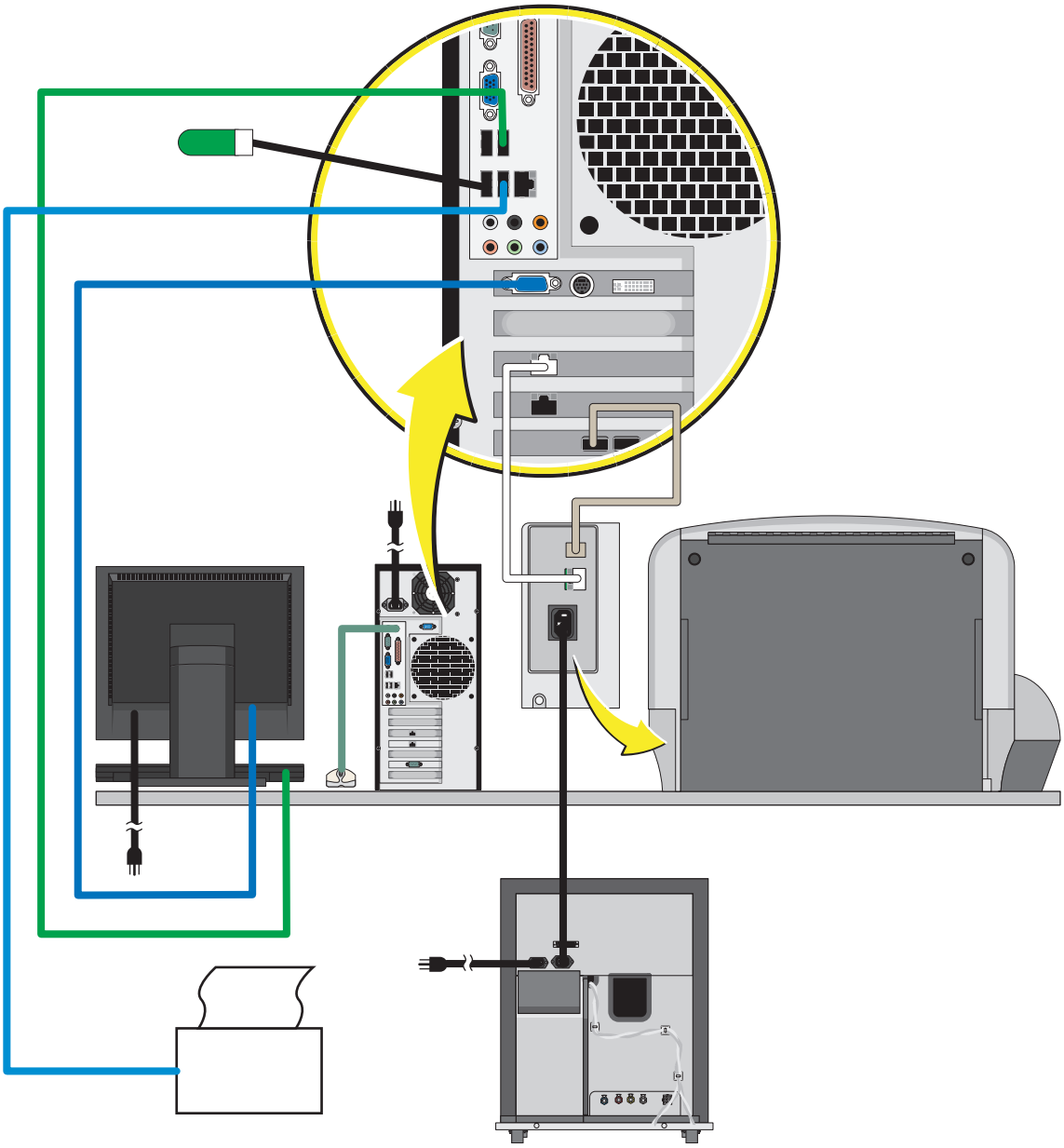
The waste line supplied with the instrument can be connected to an open drain. If you use an open drain, mechanically secure the waste tube into the drain so the tube cannot accidentally come out of the drain. This prevents spillage.

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

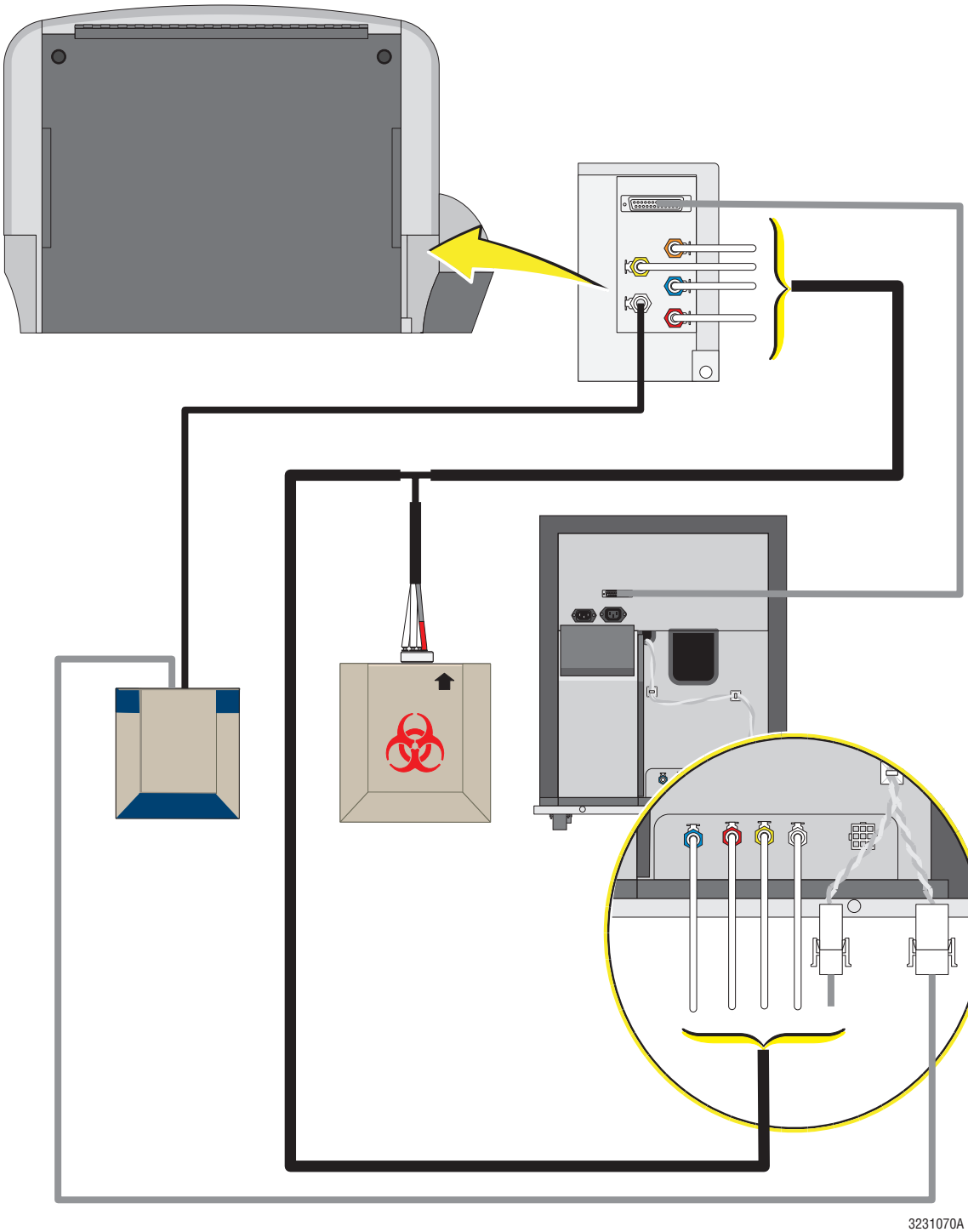


Waste and Pneumatic Tubing

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

Note: Ensure the waste tubing does not exceed the height of the MCL head.

Figure 2.2 Waste and Pneumatic Tubing Connections



3231070A

2.4 INSTALLING GALLIOS SOFTWARE

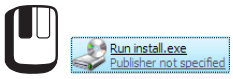
Before Installing Gallios Software

In order to install Gallios software you must have Administrator access rights in the file system, operating system, and SQL Server.

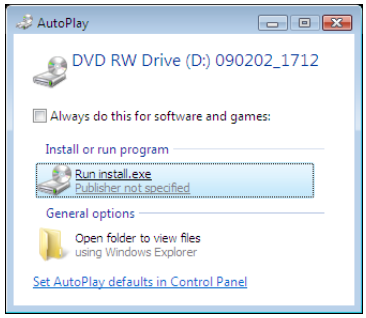
Install Gallios Software

If you are reinstalling Gallios Software, remove the existing Gallios software before proceeding with the installation. Uninstalling the Gallios software does not remove any of your data files, protocols, panels or worklists.

---

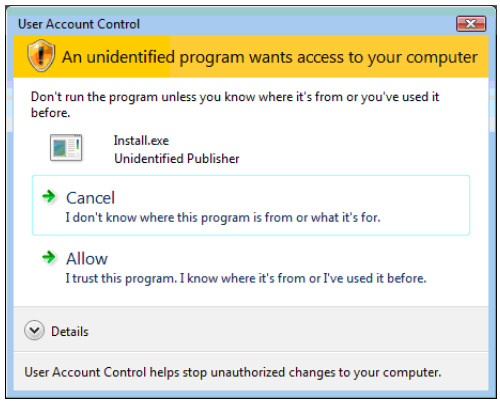
1 Insert the Gallios installation CD into the CD-Rom drive. The CD-Rom should Autorun. 

.

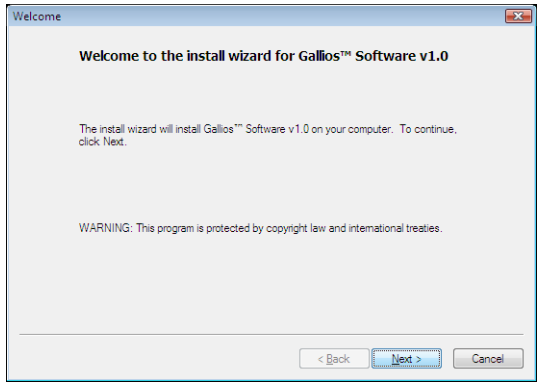


---

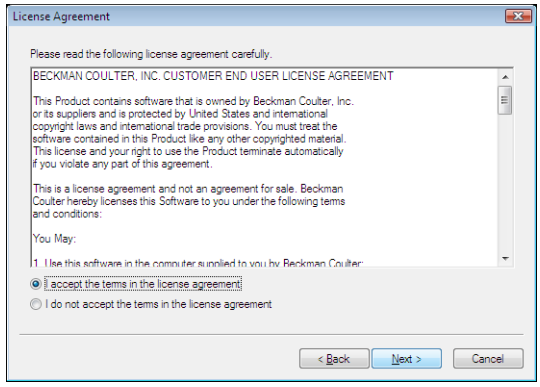
2  → Allow .



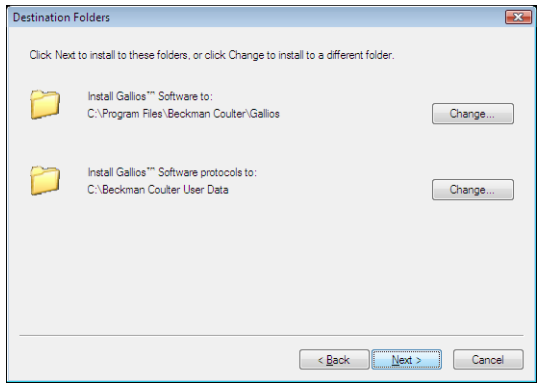
3 Next > to begin installing the software.



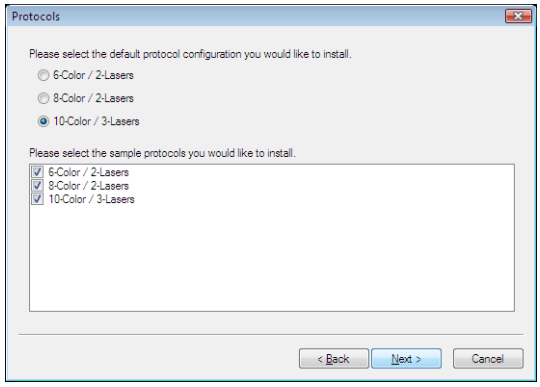
4 Read the license agreement and if acceptable, **I accept the terms in the license agreement** and Next >.



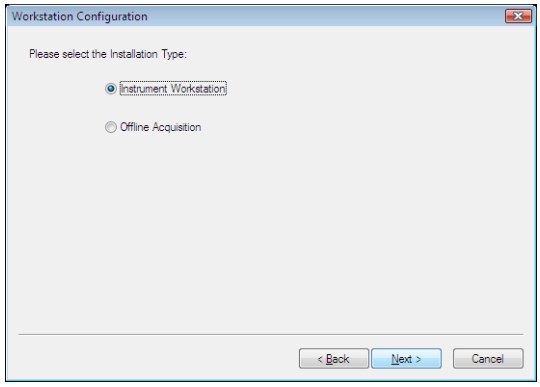
5 Next > to install files in the default folder or Change... to install in a different folder.



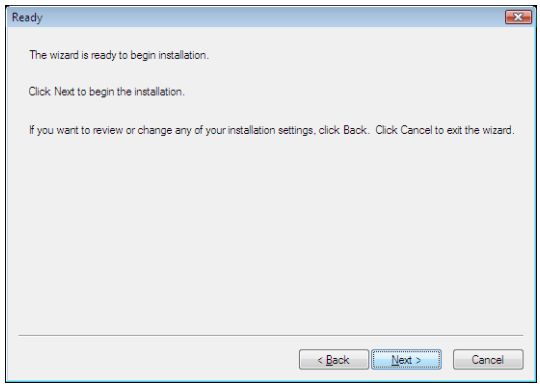
6 which protocols to install and Next >.



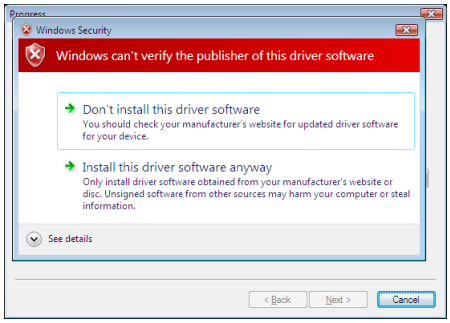
7 the Installation Type and Next >.



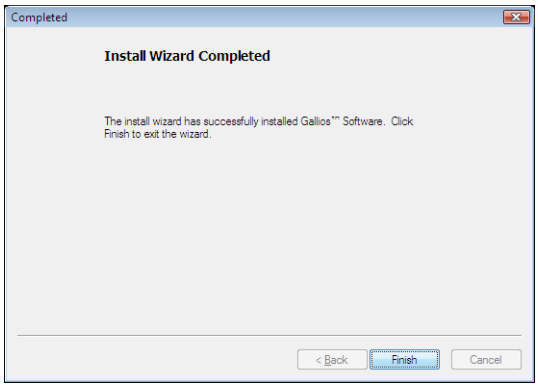
8 Next > to start the Gallios installation.



9 Install this driver software anyway.



10 Finish to exit the installation wizard.



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 x 75 mm test tubes are free of debris before you use them.

Sample Loading

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 Appendix A, 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 to mix the sample.
- It lowers its sample probe into the tube and the tube is pressurized. Sample flow begins.

The sample probe is cleaned automatically when sample flow ends.

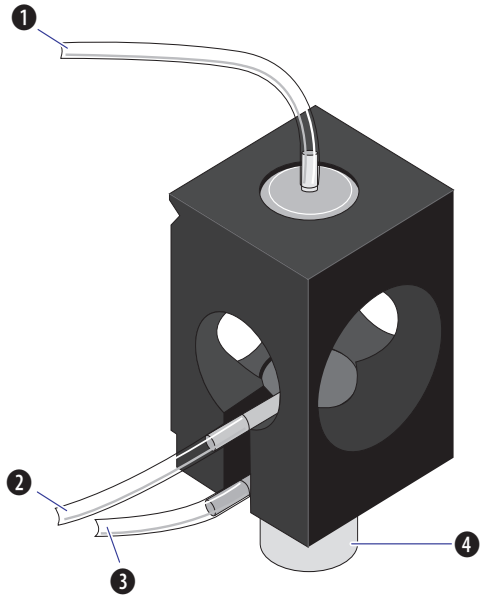
Hydrodynamic Focusing

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 through the flow cell.

The flow cell (Figure 3.1) contains a rectangular 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 3.1, 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. If the cells were to move through the laser beam in different ways during sample flow, sample analysis could be distorted.

Figure 3.1 Flow Cell



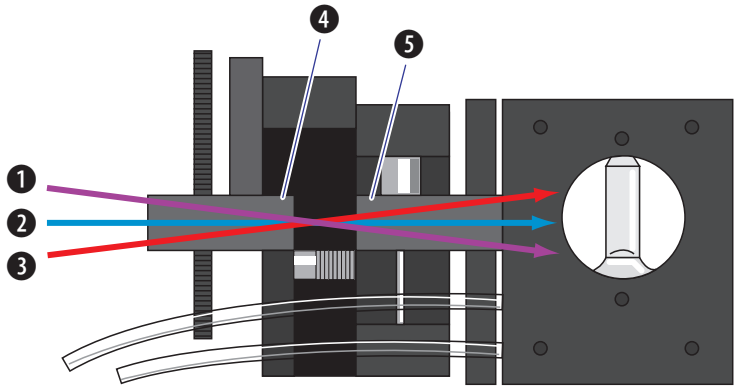
1 Waste out
2 Debubble port
3 Sheath stream enters here
4 Sample stream enters here

3.3 LASER BEAM SHAPING

Before the laser beams reach the sample stream, cross-cylindrical lenses focus the beams (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



1 Violet laser beam
2 Blue laser beam
3 Red laser beam
4 Horizontal beam shaping lens
5 Vertical beam shaping lens

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 collects the forward scatter—the laser light that is scattered at narrow angles to the axis of the laser beam. The forward angle light is filtered with a 488 nm band pass before it reaches the FS sensor which 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.

The FS sensor on this system allows you to collect different angles of Forward Scatter. The wide position collects FS angles of 1 to 19° and is ideal for smaller particles. The narrow position allows you to collect a lower angle of FS, 1 to 8°, which is ideal for larger particles. See Collection Angle**.** In addition, this system allows you to collect an enhanced wide angle (W2) to enable submicron particle resolution.

Side Scatter and Fluorescent Light Collection

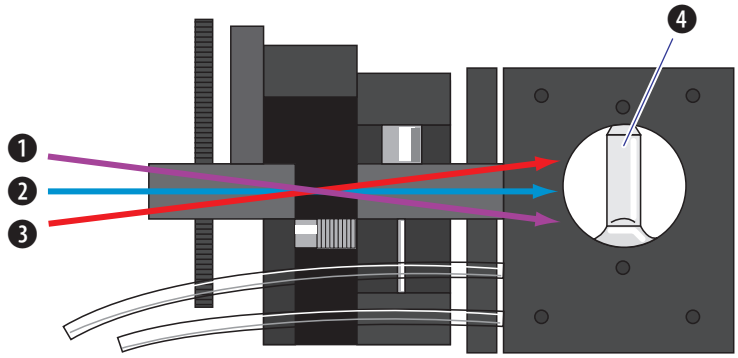
Both side scatter and fluorescence are measured 90 degrees from the laser excitation angle. Side scatter on this system is collected opposite the fluorescence collection.

The fluorescence pickup lens filter assembly is gel-coupled to the flow cell and collects FL from the flow cell, and focuses it.

Side Scatter

The wavelength of SS is 488 nm. It is much more intense than FL. SS is filtered with a 488 nm band-pass (488 BP) filter that is mounted inside the fiber optic cable.

Figure 3.3 Side Scatter Collection



1	Violet laser beam	3	Red laser beam
2	Blue laser beam	4	Side scatter collected through fiber optic cable mounted on the right side of flow cell

Fluorescent Light

Band pass and Long pass filters are used to transmit color bands. The color bands are designed to measure fluorescence light from the fluorochromes such as FITC, PE, ECD, PC5, or PC5.5, APC, APC AlexaFluor700, APC AlexaFluor750, Pacific Blue and Pacific Orange (with PMT and violet laser upgrades installed) that are excited by illumination from the lasers. Dichroic filters are used to reflect colors. Positions of the dichroic filters have been efficiently designed to reduce the number of optical surfaces fluorescence light must pass to reach the photo sensors. Their locations relative to the optical axis have also been optimized for light to pass symmetrically through each filter. You can individually interchange the optical filters. There is no need to realign the optical system when the filters are changed.

Figure 3.4 Two Laser, 6 Color Filter Block Configuration

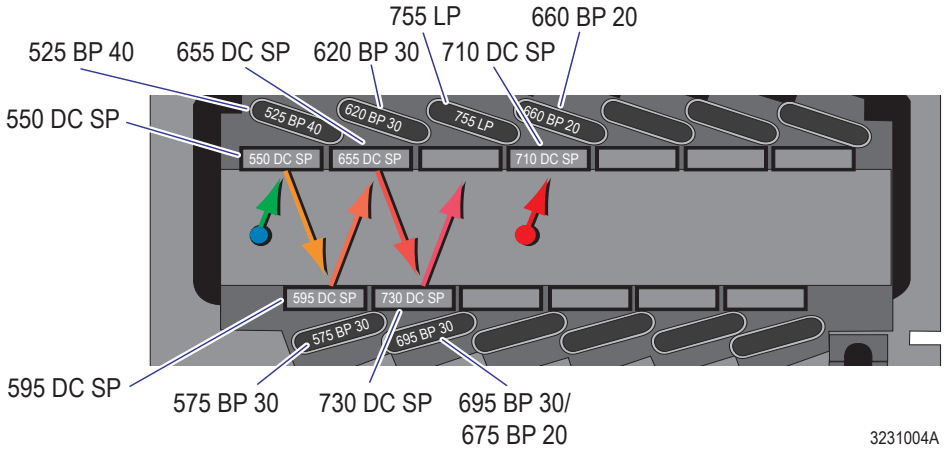


Figure 3.5 Two Laser 8 Color Filter Block Configuration

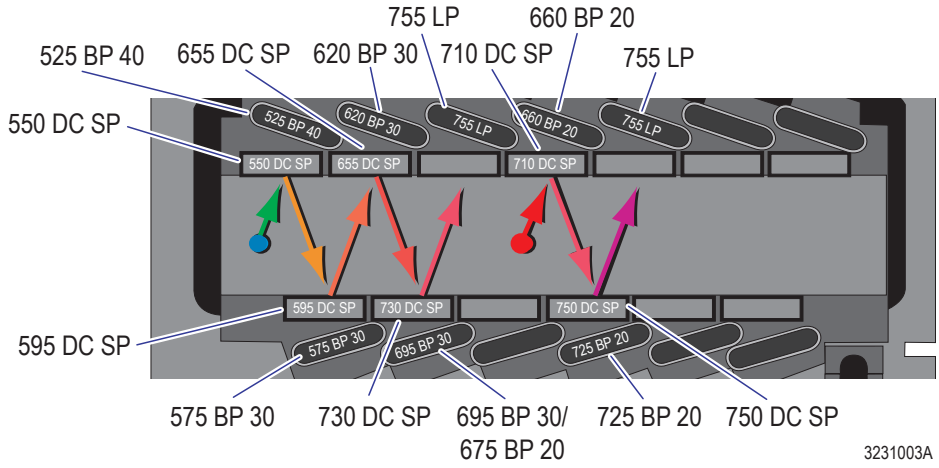
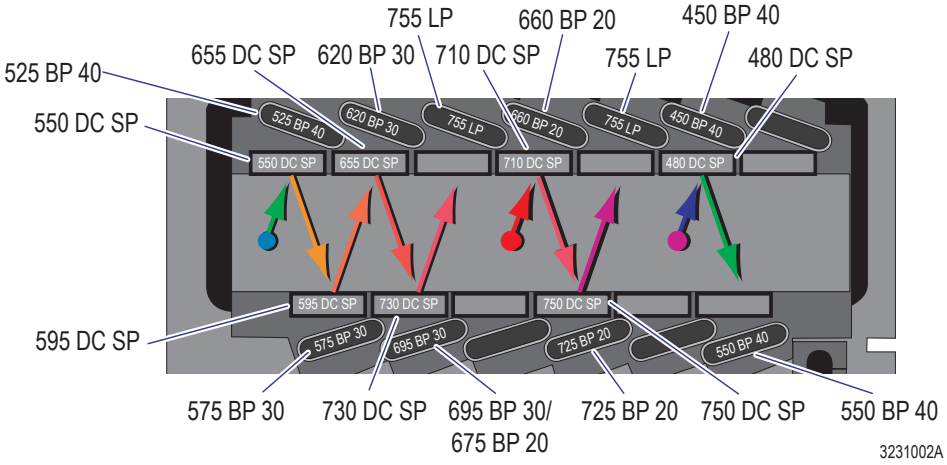


Figure 3.6 Three Laser 10 Color Filter Block Configuration



3.6 SIGNAL PROCESSING

Voltage Pulse Signals

The Cytometer has up to twelve sensors (FS, SS,FL-FL10), each generating a voltage pulse signal as each cell passes through 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.

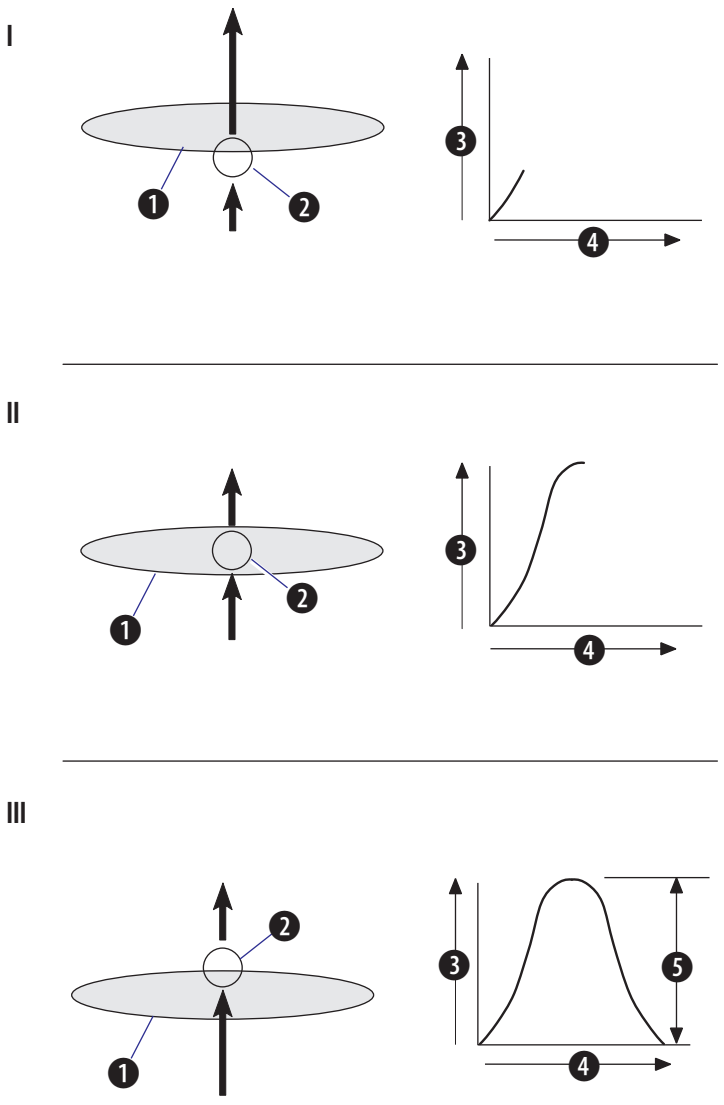
Peak Signal

Figure 3.7 shows how a peak voltage pulse signal forms as a cell crosses the laser beam.

- Part I of Figure 3.7 shows when the cell enters the laser beam and some light is scattered.
- Part II of Figure 3.7 shows when the cell is in the center of the laser beam and the scattered light, and therefore, the pulse height, reaches a maximum.
- Part III of Figure 3.7 shows when the cell leaves the laser beam and the scattered light decreases.

The intensity of light scatter or fluorescence determines the height of the peak pulse (see Figure 3.7). The time the particle is in the laser beam determines the width of the pulse. Therefore, the total fluorescence (intensity and time) determines the area under the pulse. Figure 3.8 shows how three cells with the same amount of total fluorescence but with different fluorescence intensities, produce different peak pulses.

Figure 3.7 Voltage Pulse Formation, Peak Signal



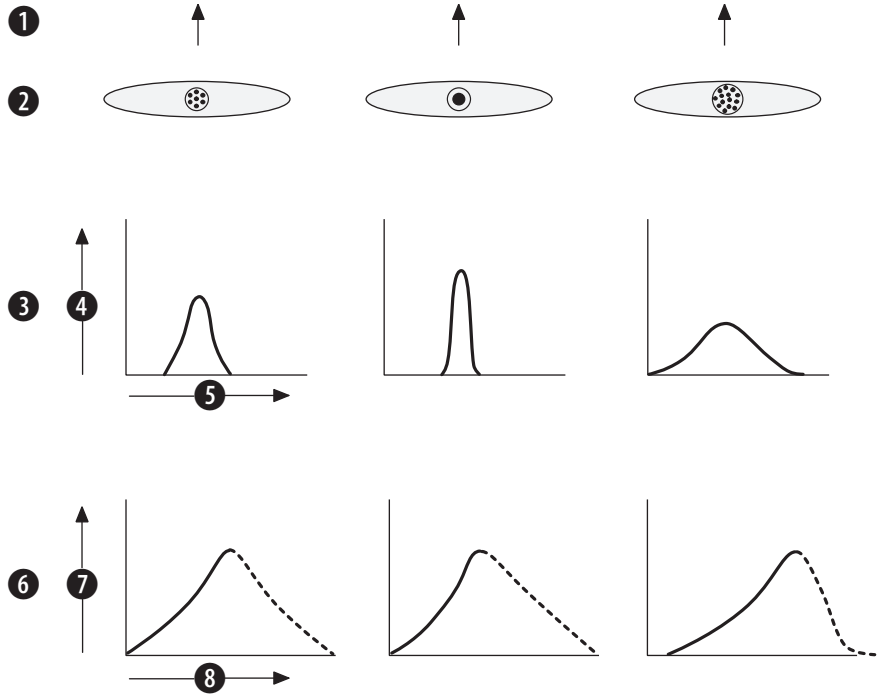
1	Laser beam	4	Time
2	Cell/Particle	5	Pulse height
3	Volts		

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 3.8).

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 3.8 Integral and Peak Pulses

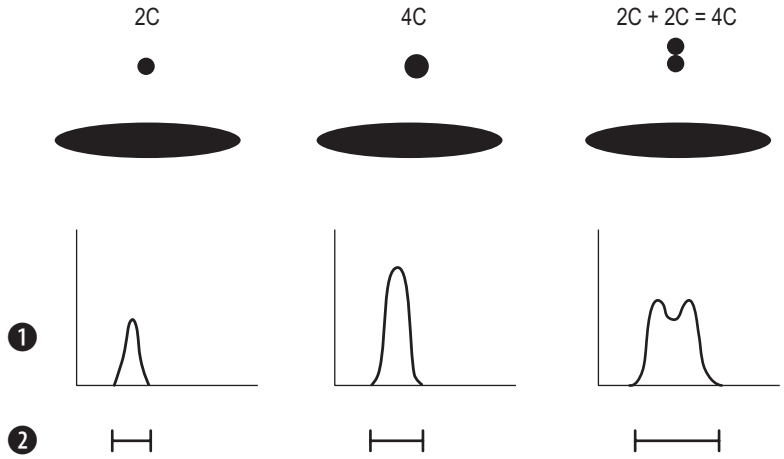


1	Direction of sample flow	4	Volts	7	Volts
2	Cell in laser beam	5	Time	8	Time
3	Peak pulses	6	Integral pulses		

Time-Of-Flight Signal

Time-of-Flight is the transit time of a cell or particle to traverse the laser beam. The pulse width of a peak pulse is measured and can be assigned to any peak signal. Two of the applications for Time-of-Flight are doublet discrimination and calculating cell size. There is a beam width normalization adjustment on the cytometer that allows you to subtract the effects of the laser beam width from the Time-of-Flight measurement.

Figure 3.9 Time-Of-Flight Pulses



Amplification

Some voltage pulses must be amplified so that the characteristics of the cells can be measured. The system lets you:

- Increase the gain to linearly amplify the integral, peak and Time-of-Flight signals.
- Logarithmically transform the linear intregral and peak signal data.

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

Signals Generated

The signals available are dependant upon whether you have a 6, 8, or 10 color configuration. INT stands for Integral and TOF stands for Time-Of-Flight.

- FS INT, FS PEAK, FS TOF
- SS INT, SS PEAK, SS TOF
- FL1 INT, FL1 PEAK, FL1 TOF
- FL2 INT, FL2 PEAK, FL2 TOF
- FL3 INT, FL3 PEAK, FL3 TOF
- FL4 INT, FL4 PEAK, FL4 TOF
- FL5 INT, FL5 PEAK, FL5 TOF
- FL6 INT, FL6 PEAK, FL6 TOF
- FL7 INT, FL7 PEAK, FL7 TOF
- FL8 INT, FL8 PEAK, FL8 TOF
- FL9 INT, FL9 PEAK, FL9 TOF
- FL10 INT, FL10 PEAK, FL10 TOF

The software allows you to select a maximum of 16 parameters, including derived parameters. Log and linear scaling of the Integral and Peak signals are also available but when Lin and Log parameters are selected for the same signal (Peak or Integral), this will be counted as one parameter. TOF is available in linear only.

3.7 PROTOCOLS

A Protocol is a collection of information about how the Cytometer and Gallios software is set up. Protocols can be created for acquiring samples or analyzing stored data. It contains the following information:

- Display configuration
- Parameter names and configuration
- Regions
- Gates
- Color definitions
- Statistics
- Instrument settings
- Reporting Templates (FlowPAGEs)

Special Protocols and Panels

QC Protocols (Export): Any Protocol name beginning with the characters “QC” (upper-case only) over-rides the current Export Data Format setting on the Workspace Preferences / Publish tab causing Published data to be formatted as a single row of values. In the case that the Publish Data to MS Excel option is selected, this progressively builds a table on a single spreadsheet.

Cleanse Protocols: Any Protocol name beginning with the word “Cleanse” overrides the Output Options setting for Save LMD on the Workspace Preferences - Acquisition Options tab. This allows you to create protocols that do not write a listmode file to disk on completion.

AS Protocols: There is a series of special protocols which include “AS” in their names. These are components of the AutoSetup applications which operate with the Auto Setup Wizard to set up Volts, Gains and Compensation.

Special Panels

The cleanse panel utilizes two cleanse protocols (‘cleanse bleach.PRO’ and ‘cleanse di water.PRO) that you should use at the end of the working day or shift on your Gallios flow cytometer.

3.8 AUTOMATED SOFTWARE FEATURES

Gallios software contains the following automated software features.

- AutoSetup Wizard
- QuickCOMP
- QuickSET
- Automatic Gate Creation
- Automatic Gate Maintenance
- Automatic Color Precedence
- Elliptical and Contour AutoGating

- AutoMATOR Analysis

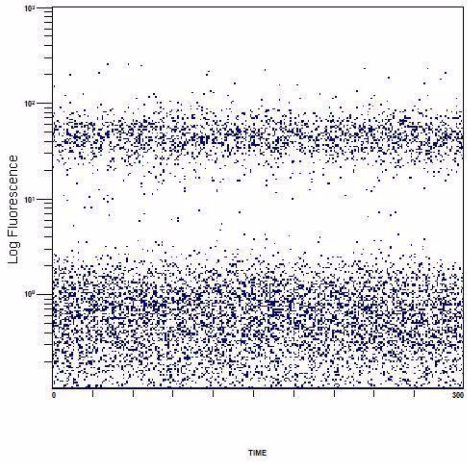
3.9 PARAMETERS

TIME Parameter

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

Note: Including TIME as a selected parameter in all protocols allows for an internal quality check on the data acquisition. A TIME versus fluorescence plot may be helpful to monitor system fluidic and optic conditions during acquisition of any given sample. Monitor consistent fluorescence over time as shown below. Unexpected fluctuations in the pattern of fluorescence may indicate compromised fluidics or optic conditions.

Figure 3.10 Time vs Fluorescence plot



The minimum stop time is 10 seconds, the maximum stop time (maximum duration) is 1,200 seconds and the default stop time is 300 seconds (5 minutes).

When you assign the TIME parameter to a plot 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 plot, divide by 64, 128, 256 or 512 depending upon the plot resolution you are using.

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 plot 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 plot, divide by 64, 128, 256 or 512 depending upon the plot resolution you are using.

3.10 PLOT DISPLAY

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

- Single-parameter histogram plot
- Dual-parameter dot plot
- Dual-parameter density plot
- Prism plot

Dual-parameter plots can be displayed in 64 x 64, 128 x 128, 256 x 256, or 512 x 512 resolution. Plots can be displayed on a black or white background.

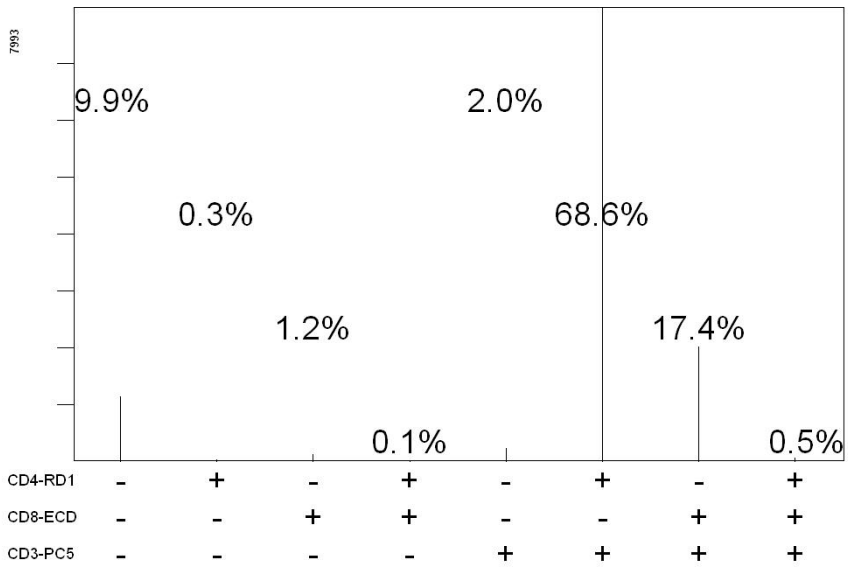
PRISM

Prism is used to analyze multicolor immunofluorescence samples. With multicolor immunofluorescence a cell is either positive or negative for each of up to ten cell surface markers. A particular combination is called a phenotype. Prism allows you to display percentages on all phenotypic populations in a single plot. It is software derived and can be acquired in either run time or listmode.

Prism is available on up to ten parameters. TIME, RATIO, and Prism itself cannot be used for Prism. All other signals can be used for Prism. Generally, the fluorescence parameters are used.

A Prism plot 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 plot. See Figure 3.11.

Figure 3.11 Prism Plot



See Prism Plot for instructions on using Prism.

Regions

To analyze data or gate plots, you must first create and assign regions to these tasks. You can create five different types of regions (see Regions Introduction). The region types are:

- Linear
- Rectangular
- Quadrant/FlexQuad
- Polygonal
- Elliptical
- Contour

Once a region is created it can be assigned to function in a specific way. The functions that are available include:

- Analysis
- Prime
- CAL (calibration)
- Gating
- Linked
- Listmode gating (LIVEGATE)
- Contour AutoGate
- Elliptical AutoGate
- Positives analysis
- Minimum Count.

These functions are not available for all region types.

Gating

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

Data Storage

Sample results can be printed out, saved to removable media, saved to a local hard drive or saved to a network drive. 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 plots or archived for analysis later. Histograms can also be saved to a file. The runtime protocol is always saved to the listmode file.

Histogram Statistics

Linear Region Statistics

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

$$\text{total} = \frac{\text{Number of events in region}}{\text{Total number of events in the gated display}} \times 100$$

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

Number = Number of cells in the region
Mode = Intensity containing the largest number of cells within the region
Total is expressed as a percent (%).

$$\text{Mean} = \sum_{ML}^{MH}\left(\frac{Cn * Cch}{N}\right)$$

$$\text{SD} = \sqrt{\sum_{ML}^{MH}\frac{(Cn - \overline{X})^2}{N}}$$

$$\text{CV} = \frac{\delta}{\overline{X}} \times 100$$

Where δ = Standard deviation
CV = Coefficient of Variation (expressed in % CV)
M_H = High region marker channel
M_L = Low region marker channel
C_n = Raw digital channel number
C_{ch} = Channel count
N = Integral of all counts between & including marker channels
$\overline{X}$ = Mean channel number (rounded to nearest whole channel)

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

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

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

$$\text{Half Peak CV} = \frac{\text{FWHM}}{2.354}$$

Where FWHM=the Full Width Half Max value of a Normal or Gaussian peak.

Log Region Statistics

The method of geometric calculations of Means depends on your Advanced Statistics Configuration.

Log-Log Mean Method

Weighting each channel according to its face value as given by the log scale performs this calculation of Mean.

Example
When using a four-decade uncalibrated logarithmic display reporting output as Channels, you might give channel zero (the lowest channel) a value $10^{-1} = 0.1$ and on a 1024 channel display, channel 1023 is given the value $10^{-3} = 1000$. All raw digital channel values are converted to an absolute value (Relative Channel Number) in the range 0.1 to 1000 before calculations are performed for the mean.

The mean can then be reported in Channel numbers in the range 0 to 1023 thus:

$$\overline{X}_{channels} = Cd * LOG10\left(\sum_{ML}^{Mh} \frac{10^{\left(\frac{Cn}{Cd}\right)*Cch}}{N}\right)$$

Where:
C_n = Raw digital channel number
C_{ch} = Channel count
C_d = Channels per decade
N = Integral of all counts between & including marker channels
$\overline{X}$ = Mean channel number (rounded to nearest whole channel)
For Calibrated values, the calculation is reported as the calibrated value in the range Log Offset to

$$\overline{X} = Log\_Offset * 10^{\left(\frac{\overline{X}Channels}{Cd}\right)}$$

The log offset cannot be zero since a log offset of zero is 10 (minus infinity), which is not possible to represent on paper or on screen. Gallios software does not allow log offset values

of less than 0.1. Numbers entered in the logarithmic offset are clamped at 0.1 if a number less than this is entered.

Lin-Log Mean Method

This method of Mean calculation is worked out by performing a simple arithmetic mean channel calculation on raw data according to its channel value in the range 0 to 1023 (for 1024 channel data). The Mean value can then be reported as channels (in the range 0 to 1023) or converted to the Relative Channel value.

Using the same data as in the previous example…

Lin-Log Mean method reported in Channel numbers in the range 0-1023 thus:

$$\overline{X}channels = \sum_{M_L}^{M_H}\left(\frac{Cn * Cch}{N}\right)$$

Where:
C_n = Raw digital channel number
C_{ch} = Channel count
C_d = Channels per decade
N = Integral of all counts between & including marker channels
$\overline{X}$ = Mean channel number (rounded to nearest whole channel)

For calibrated values, the Mean calculation is reported as a value in the range Log Offset to (Log offset* 10 $^{\text{Decades Full Scale}}$) thus:

$$\overline{X} = Log\_Offset * 10\left(\frac{\overline{X}Channels}{Cd}\right)$$

The log offset cannot be zero since a log offset of zero is 10 (minus infinity). Since the graphical scale is actually proportional to the exponent, it is not possible to represent this on paper or on screen. Because of this problem, Gallios software does not allow log offset values of less than 0.1. Numbers entered in the Logarithmic Offset edit window are clamped at 0.1 if a number less than 0.1 is entered.

4.1 SAMPLE REQUIREMENTS

See SAMPLE REQUIREMENTS in the RUNNING SAMPLES chapter for details.

4.2 INSTRUMENT SPECIFICATIONS AND CHARACTERISTICS

Dimensions

Component	Height	Width	Depth	Weight
Computer	43.2 cm (17 in.)	20.3 cm (8 in.)	45.7 cm (19 in.)	12.3 kg (27 lb)
Cytometer and MCL	60.5 cm (23.8 in.)	95.3cm (37.5 in.)	70.1 cm (27.6 in.)	104 kg (230 lb)
Monitor (typical)	53.34 cm (21 in.)	50.8 cm (20 in.)	25.4 cm (10 in.)	7.0 kg (15.5 lb)
Pneumatic Supply	58.4 cm (23 in.)	45.7 cm (18 in.)	71.1 cm (28 in.)	31.8 kg (70 lb)

Installation Category

Category II (per IEC 1010-1 standard).

Acoustic Noise Level

Measure Level: ≤60 dBa

Cytometer

Flow Cell

Sensing area: BioSense 150 µm x 460 rectangular channel with an integral lens, mounted with a vertical (upward) flow path. See Figure 3.1.

Flow Rate

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

- Low approximately 10 uL/min
- Medium approximately 30 uL/min
- High approximately 60 uL/min

Sheath Consumption

- 780mL / hour (acquisition)
- 0mL / hour (standby)

Lasers

- Solid-state, software controlled, 22 mW, blue laser operating at 488 nm and
- Solid-state, software controlled, 25 mW, diode laser operating at 638 nm.

Optional third laser:

- Solid-state, software controlled, 40 mW, violet laser operating at 405 nm.

Laser Power Monitoring

Laser power is monitored by each laser individually. If the laser power deviated more than ±10% a Laser Power Error is displayed on the Status Bar**.** The system will not run a sample until corrective measures rectify the fault. Follow the instructions in the Troubleshooting section for handling this error.

Beam-Shaping Optics

Cross cylindrical lenses 10 mm by 80 mm.

Blue Laser Beam Spot Size

An elliptical spot 10 µm high by 84 µm wide.

Red Laser Beam Spot Size

An elliptical spot 9.6 µm high by 72 µm wide.

Violet Laser Beam Spot Size

An elliptical spot 8.9 µm high by 70 µm wide.

Laser Beam Separation

The laser beams are 125 µm (±12.5 µm) apart.

Optical Filters

The filters used in the Gallios system are dependant upon your system configuration. See Figure 3.4, Figure 3.5 and Figure 3.6.

Sensors

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

Signal Processing

- High voltage amplification, minimum 250 up to 1,100, in increments of 1, for FL1-FL10.
- Vernier gain (fine amplification), up to 1,000 (labeled volts), in increments of 1, for FS and SS. A change of 1 to 750 represents a 1-to-4 change in gain:
- Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, 20, 50, 75, 100, 200, 500 or 750 for FS and SS.
- Linear amplification (gain) by 1.0 or 2.0 for FL1-FL10.
- Four-decade digital logarithmic transformation of FS, SS and FL1-FL10.

  Note: A scale of 0.1 to 1,000 is displayed on the plot axis for logarithmic parameters, but the default statistics are based on an actual scale of 0.1024 to 1024. The displayed scale can be changed to 1 to 10,000.

- Six decade with True View enabled. Data is transformed in a 'log' style at high values, but 'linearly' near the axis. Up to 2 negative decades of data may be displayed. Transform 'reflects' around origin and becomes Logarithmic at high negative values.
- Fluorescence color compensation is available in 0.1 increments, from 0 to 100%, for FL1-FL10.
- A discriminator (maximum value of 1,000) is available for any one of the signals. Only one discriminator can be specified for any one sample acquisition.

Dynamic range

20-bit data acquisition

Workstation resolution

1,048,576 channels

Digital Sampling rate

40Mhz

Digital Accuracy

<5% error

Workstation

The descriptions below are minimum configurations.

Computer

Intel® Pentium® Core™ 2 Duo microprocessor and 4 GB of RAM.

RAM (Random Access Memory)

The amount of available RAM in the computer determines the maximum size of a listmode file. This software is a Windows application and, as such, shares RAM with other processes. To help prevent error messages and system lock-ups from occurring, be aware of the factors that use available RAM. RAM is limited by other factors such as:

- other software applications running simultaneously
- total number of events acquired
- number of parameters
- data rate
- FlowPAGEs present
- autogating
- movement of regions with many events in cache memory
- linked regions

For example, if you have several parameters, FlowPAGEs, a high data rate, and you are moving regions, you may receive a RAM memory error message. If this occurs during acquisition, the listmode file will not be saved.

Note: A protocol's "Maximum Acquirable Events" limit is denoted on the Cytometer Control Acquisition Setup Tab and is determined by the number of parameters selected for use in the protocol.

Data Storage

- 160-GB (or larger) nonremovable hard disk.
- DVD±RW/CD-RW drive

Interfaces

- Microsoft® Windows®
- Bidirectional asynchronous serial interfaces for communication with mainframe and personal computers.

Input Devices

- PS/2 optical Intellimouse®
- Full size PS/2 keyboard.

Monitor Options

- Color LCD with a 22-in. flat screen.
- Optional second monitor

4.3 SOFTWARE SPECIFICATIONS

Data Output and Compatibility

Output and compatibility features include:

- Flow Cytometric Standard (FCS) file format for listmode and histogram files. Listmode files contain an FCS 2.0 dataset followed by an FCS 3.0 dataset.
- Copy and Paste, or Drag and Drop plots images to third party programs such as MS Word.
- Full-color printouts with the appropriate optional Printer.
- Printout of sample results and patient reports with FlowPAGE and desktop printing
- PDF format for Acquisition printouts or FlowPAGE reports
- Printout of Worklist for the MCL
- Compatibility with ALTRA™, Elite™, XL™, XL-MCL™ and FC 500 flow cytometer files (listmode files only)
- Export data to MS Excel 2003
- Save/export data as a text file.

Reporting Units

Reporting units for absolute count calibrators are cells/µL for US units or cells/L for SI units.

Setup Mode

During Setup mode the workspace is a rolling display that is updated up to three times per second. The maximum number of events can be set on the Cytometer Control Acquisition Setup Tab. The incoming data is not saved.

Acquisition

During data acquisition, the plots are updated in real time. When one plot is displayed with statistics underneath, the statistics are also updated in real time. Unlimited one or two parameter plots are available for any given sample.

One-parameter plots have 1,024-channel resolution.
Two-parameter plots have up to 512- x 512-channel resolution.

Parameters

16 different signals, including Time and Ratio (derived parameter) can be acquired simultaneously in logarithmic and linear modes, giving up to 32 acquisition parameters from a possible 82.

5 different signals available from each detector:

- Integral linear
- Integral logarithmic
- Peak linear
- Peak logarithmic
- Time of Flight linear.

Regions

Up to 32 gates and 256 analysis regions are available for gating, analysis, and autogating per protocol. Up to 8 of those regions can be used to create any one gate. The following types of regions are available for gating and analysis:

- Linear
- Rectangular
- Quadrant
- Polygonal
- Elliptical
- Contour.

Listmode Analysis

The instrument can store up to 16 parameters including 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. See RAM (Random Access Memory).

Locked Protocols

Protocols can be locked or unlocked by a System Administrator and password protected to prevent inadvertent modifications. The following software screens are disabled when using locked protocols. You cannot make any changes on these screens.

Dotplot Properties
Region Properties (QC product selection and export region statistics are not locked)
Create / Modify Gates
Color Precedence
Color Blend
Cytometer Control
Plots Toolbar
Plots Menu
Select Results
Edit FlowPAGES
Edit FCS Headers

Default File Extensions

The software has a variety of standard file extensions. Many of these file extensions are not necessary for Gallios itself but are required for compatibility with other programs.

Standard (default) files & file extensions

*.LMD	FCS listmode data file (includes ASCII header information and binary listmode data).
*.HST	FCS histogram file, single parameter only per file. (Includes ASCII text file descriptions and binary histogram channel information).
*.PRO	Protocol files.
*.PNL	Panel files.
*.WLS	Worklist files.
*.CMP	Compensation files.
*.PPP	Listmode compensation panel.
*.PDF	Files created when “Print to PDF” is selected.
*.WLQ	Queue of worklist files.
*.ADF	Application Definition file.
*.ALQ	Queue of listmode files saved in AutoMATOR.

The following characters cannot be included in a file name: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), comma (,), question mark (?), quotation mark ("), pipe symbol (|), colon (:), or apostrophe (‘). Leading, Trailing and consecutive spaces are not allowed.

Default AutoSetup Protocols and Panels

The following AutoSetup panels and protocols are included with Gallios software.

Note: Do not delete any protocol that is associated with an Application Name. All protocols must be present for an application to run correctly.

Application Name	Flow-Set™ Pro Protocol	Compensation Protocols	Verification Protocol	Settings Protocol	Base Protocol
AS 6C 2L	AS 6C 2L_STAND.Pro	AS 6C 2L_FL01.Pro AS 6C 2L_FL02.Pro AS 6C 2L_FL03.Pro AS 6C 2L_FL04.Pro AS 6C 2L_FL05.Pro AS 6C 2L_FL06.Pro	AS 6C 2L_VERIFY.Pro	AS 6C 2L Settings.Pro	AS 6C2L_APP.Pro

The initial protocol in each application, which you use to adjust or confirm correct PMT High Voltage and parameter gain settings, utilizes Flow-Set Pro fluorospheres.

If you wish to use a negative control sample use the "AS *_STAND.Pro" protocol in the above panels. You must modify the protocol for the number of colors you are analyzing.

Each AutoSetup II application requires a user defined base protocol with the desired parameter names to generate the application. The Application Definition Wizard creates a unique base protocol with the same name as the application.

The guidelines below show approximate region widths generated by the Application Definition Wizard. **Flow-Set Pro region widths generated by the Application Definition Wizard may be edited. If different regions widths are desired, open the appropriate *_Stand protocol onto the workspace and edit the regions.** Save the protocol. Subsequent scheduling of the application uses the edited regions.

- Forward Scatter (linear): ±20
- Side Scatter (linear): ±50
- Fluorescence Parameters (logarithmic);
  - 1st decade - beginning or low end: ±0.1
  - 1st decade - high end: ±0.3
  - 2nd decade - beginning or low end: ±1.0
  - 2nd decade - high end: ±3.0
  - 3rd decade - beginning or low end: ±10
  - 3rd decade - high end: ±30
  - 4th decade - beginning or low end: ±100
  - 4th decade - high end: ±300

Default Generic Panels and Protocols

The following generic protocols are included with Gallios software.

Panel Name	Protocol
Cleanse.PNL	Cleanse Bleach.PRO Cleanse Water.PRO

Application Name	Protocol	Base Protocol
Alignment Blue Red 6c	Alignment Blue Red 6c_ALIGN.pro	qc_flowcheck_br6c.pro
Alignment Blue Red 8c	Alignment Blue Red 8c_ALIGN.pro	qc_flowcheck_br8c.pro
Alignment Blue Red Violet	Alignment Blue Red Violet_ALIGN.pro	qc_flowcheck_brv.pro
Alignment Blue Violet	Alignment Blue Violet_ALIGN.pro	qc_flowcheck_bv.pr
Alignment Blue	Alignment Blue_ALIGN.pro	qc_flowcheck_b.pro

FCS Header - Keyword Reference

The Following FCS keyword information is included in Gallios listmode and histogram file header section.

Those prefixed by a "$" are FCS Standard defined keywords, those prefixed by an "@" or nothing are Gallios defined keywords.

Table 4.1 FCS Keyword Information

FCS Keyword	Key value
$DATATYPE	Single character defining base data type of binary data, I=16 bit integer, F=IEEE 32 bit floating point, D=double precision 64 bit floating point, A=ASCII
$PAR	Number of parameters
$MODE	Single character defining mode of data, L=listmode, C=dual parameter correlated, U=single parameter uncorrelated.
$PnB	Number of bits allocated to store data for parameter n, 8 means 256 channel data, 16 means up to 64 k channel data
$PnR	Channel resolution of parameter n, for example: 256,1024 and so on
$BYTEORD	INTEL platform '1,2', Motorola platform '2,1'
$NEXTDATA	Byte offset into binary data for next data item, FCS files can be saved as a pseudo-linked list, most often 0
$DATE	DD-MMM-YY
$EXP	Experiment name (from Workspace Preferences)
$PROJ	Project name (from Workspace Preferences)
$OP	Operator ID, that is, user ID.
$INST	Institute name (from Workspace Preferences)
$FIL	File name excluding path
$CYT	Type of flow cytometer, for example: Gallios, FC 500, XL, ALTRA, ELITE and so on
$SMNO	System defined run number, that is, a number which is incremented each time the flow cytometer is used to acquire data

Table 4.1 FCS Keyword Information

FCS Keyword	Key value
$SRC	Sample Source (from Workspace preferences)
$SYS	Gallios Intel Windows
$CELLS	Cells name (from Workspace Preferences)
$BTIM	Start time (HH:MM:SS 24 hour clock ':' delimiters)
$ETIM	End time (HH:MM:SS 24 hour clock ':' delimiters)
$TOT	Total number of events acquired
$PnV	Voltage of sensor n
@PnGAIN	Amplifier Gain setting of a sensor
$PnN	Name of parameter n, for example: Manufacturer assigned name, that is, FS or FL1 LOG
$PnS	User assigned name of parameter
$PnE	Lin/Log parameter calibration (0,0) means linear, (decades, offset [for example: 4.0, 0.1024]) means log
$DFCiToj	% of FLi to subtract from FLj
@Y2KDATE	Date in format YYYYMMDD
@SAMPLEID1	User defined string
@SAMPLEID2	User defined string
@SAMPLEID3	User defined string
@SAMPLEID4	User defined string
@PnZ	Calibration for parameter n is active when this key value is 'ON'
@PnX	Linear calibration values in xx, yy order, for example: xx= channel number, yy = channel value
@PnU	Units for this parameter n
@PnQ	Sensor name for parameter n, from the CCUI.
@PnC	Calculation method for parameter n, 'ARITHMETIC' means use arithmetic method for calculation - may be overridden by analysis engine
@PnADDRESS	Mutliplexor address of parameter n
$RUNNUMBER	(See $SMNO) System defined run number, that is, a number which is incremented each time the flow cytometer is used to acquire data
@BASELINEOFFSET	Default is 'OFF' any other means ON, that is, this sample was acquired using baseline offset
@FILEGUID	Unique 128 bit number identifying this file, changed every time the file is saved.
@CYTOMETERID	The unique ID given to the flow cytometer by the user, for example: the flow cytometer serial number
@LOCATION	The address of the institute who owns the flow cytometer
TESTNAME	PROTOCOL NAME
TESTFILE	PROTOCOL FILE NAME

Table 4.1 FCS Keyword Information

FCS Keyword	Key value
@BARCODE	Barcode read from MCL
@CAROUSEL	Carousel number read from MCL
@TUBENO	Tube number read from MCL
@Discriminator	Displays the discriminator parameter and level
@RESAVEDFILE	Displays Runtime Protocol if the current file is exactly as at the end of acquisition, if any changes are made post acquisition and the LMD file is resaved the new file displays New Protocol.
@RATIONUMERATORMUX	Internal Mux address of the parameters used for the Ratio Parameter Numerator.
@RATIODENOMINATORMUX	Internal Mux address of the parameters used for the Ratio Parameter Denominator.
@PANEL	Name of the panel file used for acquisition of the sample.
@ACQUISITIONPROTOCOLOFFSET	Defines where in the LMD file the Runtime Protocol is stored, the value displayed is number of bytes from start of file to the start of the protocol section.
@ABSCALFACTOR	Defines the calibration factor of the beads used with this sample if Flow-Count™ or similar absolute calibration particles are used.
$PnG	Amplifier gain used for acquisition of parameter n
$INSTADDRESS	The address of the facility
@SETTINGSFILE	Instrument settings file used for acquisition
@SETTINGSFILEDATETIME	The date and time of the instrument settings file used for acquisition
@ACQTIME	Acquisition time from start to end of acquisition (excluding pause time)
@ELAPSEDTIME	Total elapsed time from start to end of acquisition (including acquisition and pause time)
@BUILDNUMBER	Software build number
@REDLASERPOWER_START	Red laser power at start of acquisition
@REDLASERPOWER_END	Red laser power at end of acquisition
@REDTARGETPOWER	Red laser target power
@REDLASERSHUTTER	Red laser shutter status
@BLUELASERPOWER_START	Blue laser power at start of acquisition
@BLUELASERPOWER_END	Blue laser power at end of acquisition
@BLUETARGETPOWER	Blue laser target power
@BLUELASERSHUTTER	Blue laser shutter status
@VIOLETLASERPOWER_START	Violet laser power at start of acquisition
@VIOLETLASERPOWER_END	Violet laser power at end of acquisition
@VIOLETTARGETPOWER	Violet laser target power
@VIOLETLASERSHUTTER	Violet laser shutter status

Table 4.1 FCS Keyword Information

FCS Keyword	Key value
@STOPREASON	Shows acquisition was stopped due to reaching the maximum acquirable event count
@CRS20BITFORMAT	Data stored in Gallios 20bit format (written to FCS3.0 header section only)
$COMP	FCS3.0 format compensation keyword (written to FCS3.0 header section only)
$SPILLOVER	FCS3.1 format compensation keyword (written to FCS3.0 header section only)
@CYTOLINKMULTIPLECLIENT	Indicates if other cytolink client was connected to CytoLink during acquisition of the LMD file
@RATIO_NUMERATOR	Name of Parameter used as Ratio Numerator
@RATIO_DENOMINATOR	Name of Parameter used as Ratio Denominator

Database Structure

The information in this section specifies the read-only access to Gallios software for 3rd party LIS vendors. The information in this section includes,

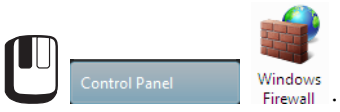
- Firewall Setup
- Database Login
- Report Generator Database Schema

Note: Beckman Coulter does not warranty data after 3rd party middleware has retrieved data from the Gallios report database.

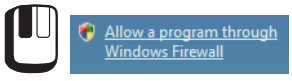
Firewall Setup

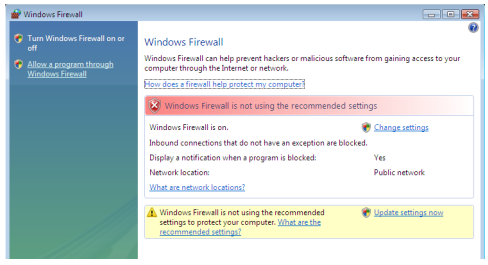
Use these instructions to setup the Windows Firewall to allow SQL Server application access.

---

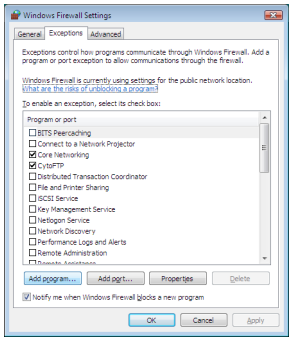
1 

---

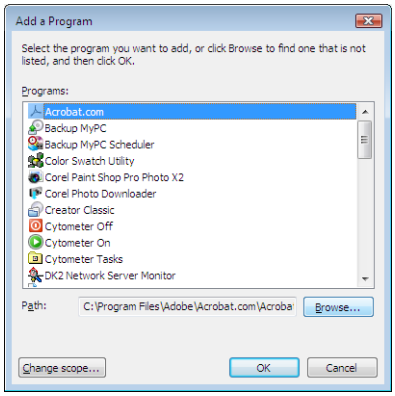
2 



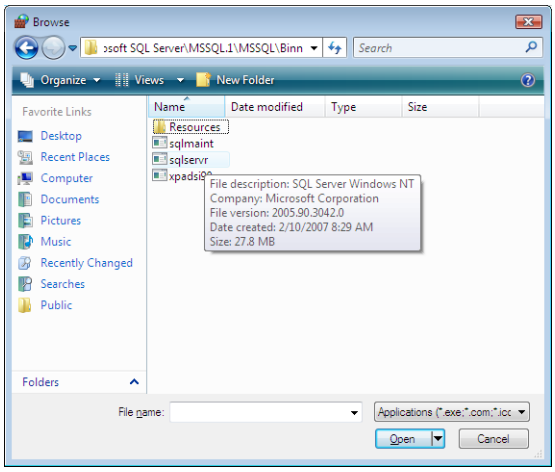
3 Add program...

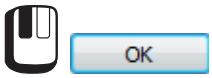


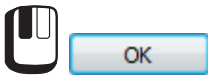
4 Browse...



5 Navigate to C:\Program Files\Microsoft SQL Server\MSSQL.1\MSSQL\BINN and double click on **sqlservr**.



6 

7 

and close the Control Panel windows.

Database Login

For read-only access to the database you will need the following,

- username **cr600crown**
- password **Feb33d1n7**

Report Generator Database Schema

This section lists the tables contained in the Report Generator database, and details the fields within each table.

Table 4.2 cytoimage Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	Instance	int	4	
Y	idx	tinyint	1	
	file_path	nvarchar	256	
	image_file	nvarchar	48	
	protocol	nvarchar	48	
	plot_id	int	4	
	plot_string	nvarchar	48	

Table 4.3 cytoinfo Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	instance	int	4	
Y	line_number	smallint	2	
	description	nvarchar	32	Y
	info_count	decimal	15, 3	Y
	lo_count	decimal	9, 3	
	hi_count	decimal	9, 3	
	info_percent	decimal	15, 4	Y

Table 4.3 cytoinfo Table

Key	Column Name	Data Type	Length	Allow Nulls
	lo_percent	decimal	7, 3	
	hi_percent	decimal	7, 3	
	region	nvarchar	30	Y
	optstat1_name	nvarchar	14	Y
	optstat1_val	float	8	Y
	optstat2_name	nvarchar	14	Y
	optstat2_val	float	8	Y
	gate	nvarchar	3	Y
	prt	char	1	
	transfer	bit	1	
	prot_eq	varchar	48	Y
	do_percent	bit	1	
	do_count	bit	1	
	do_opt1	bit	1	
	do_opt2	bit	1	
	do_result	bit	1	

Table 4.4 cytorun Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	instance	int	4	
	first_run	nvarchar	10	
	last_run	nvarchar	10	
	first_lmd	nvarchar	260	Y
	last_lmd	nvarchar	260	Y
	cyto_serial_run	nvarchar	10	
	panel_name	nvarchar	48	
	panel_path	nvarchar	199	
	panel_complete	nvarchar	1	
	panel_match	nvarchar	1	
	run_datetime	datetime	8	
	entry_datetime	datetime	8	
	run_source	nvarchar	1	
	ivd	nvarchar	1	
	algorithm_name	nvarchar	12	Y
	algorithm_version	nvarchar	8	Y

Table 4.4 cytorun Table

Key	Column Name	Data Type	Length	Allow Nulls
	user_id	nvarchar	24	
	si_units	nvarchar	1	
	count_method	nvarchar	1	
	comment	nvarchar	750	Y
	specimen_id	nvarchar	24	
	patient_id	nvarchar	31	Y
	lname	nvarchar	31	Y
	fname	nvarchar	20	Y
	mi	nvarchar	1	Y
	birth	smalldatetime	4	Y
	sex	nvarchar	1	Y
	ssn	nvarchar	12	Y
	specimen_type	tinyint	1	Y
	physician	nvarchar	31	Y
	hemo_instrument	nvarchar	12	Y
	hemo_datetime	smalldatetime	4	Y
	lymphs	decimal	4, 2	Y
	monos	decimal	4, 2	Y
	ne	decimal	4, 2	Y
	eo	decimal	4, 2	Y
	ba	decimal	4, 2	Y
	platelets	int	4	Y
	other_name	nvarchar	15	Y
	other_val	int	4	Y
	wbc	int	4	Y
	rbc	int	4	Y
	specimen_datetime	smalldatetime	4	Y
	print_ranges	nvarchar	1	
	first_tube_id	nvarchar	24	Y
	last_tube_id	nvarchar	24	Y
	dil_factor	decimal	6, 2	Y
	harvest_vol	decimal	6, 2	Y
	body_weight	decimal	5, 2	Y
	results_decimal_places	smallint	2	

Table 4.4 cytorun Table

Key	Column Name	Data Type	Length	Allow Nulls
	first_lmd_revision_number	int	4	Y
	last_lmd_revision_number	int	4	Y
	panel_revision_number	int	4	Y
	check_file_path	nvarchar	199	Y

Table 4.5 cytosamples Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	specimen_type	tinyint	1	
	sname	nvarchar	31	Y
	sortorder	int	4	Y

Table 4.6 cytospecimen Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	specimen_id	nvarchar	24	
	patient_id	nvarchar	31	Y
	lname	nvarchar	31	Y
	fname	nvarchar	20	Y
	mi	nvarchar	1	Y
	birth	smalldatetime	4	Y
	sex	nvarchar	1	Y
	ssn	nvarchar	12	Y
	specimen_type	tinyint	1	Y
	physician	nvarchar	31	Y
	hemo_instrument	nvarchar	12	Y
	hemo_datetime	smalldatetime	4	Y
	lymphs	decimal	4, 2	Y
	monos	decimal	4, 2	Y
	ne	decimal	4, 2	Y
	eo	decimal	4, 2	Y
	ba	decimal	4, 2	Y
	platelets	int	4	Y
	other_name	nvarchar	15	Y
	other_val	int	4	Y

Table 4.6 cytospecimen Table

Key	Column Name	Data Type	Length	Allow Nulls
	wbc	int	4	Y
	rbc	int	4	Y
	specimen_datetime	smalldatetime	4	Y
	entry_datetime	datetime	8	
	dil_factor	decimal	6, 2	Y
	harvest_vol	decimal	6, 2	Y
	body_weight	decimal	5, 2	Y
	first_tube_id	nvarchar	24	Y
	extMod	nvarchar	100	Y
	lock_user_id	nvarchar	24	Y
	lock_cyto_serial_num	nvarchar	10	Y
	lock_datetime	smalldatetime	4	Y

Table 4.7 cytotemplate Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	panel_name	nvarchar	48	
Y	cyto_serial_num	nvarchar	10	
Y	panel_path	nvarchar	199	
	si_units	nvarchar	1	
	count_method	nvarchar	1	
	auto_print	nvarchar	1	
	auto_pdf	nvarchar	1	
	auto_xls	nvarchar	1	
	ivd	nvarchar	1	
	algorithm_idx	smallint	2	
	comment	nvarchar	750	Y
	entry_datetime	datetime	8	
	user_id	nvarchar	24	
	print_ranges	nvarchar	1	
	results_decimal_places	smallint	2	

Table 4.8 cytotemplate_image Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	panel_name	nvarchar	48	
Y	cyto_serial_num	nvarchar	10	

Table 4.8 cytotemplate_image Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	panel_path	nvarchar	199	
Y	plot_num	smallint	2	
	protocol	nvarchar	48	
	panel_idx	smallint	2	
	plot_id	int	4	
	plot_string	nvarchar	48	

Table 4.9 cytotemplate_line Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	panel_name	nvarchar	48	
Y	cyto_serial_num	nvarchar	10	
Y	panel_path	nvarchar	199	
Y	line_num	smallint	2	
	description	nvarchar	32	
	prot_eq	nvarchar	48	
	panel_idx	smallint	2	
	region	nvarchar	30	
	gate_type	nvarchar	3	Y
	lo_percent	decimal	7, 3	
	hi_percent	decimal	7, 3	
	lo_count	decimal	11, 3	
	hi_count	decimal	11, 3	
	optstat1_name	int	4	Y
	optstat2_name	int	4	Y
	prt	nchar	1	
	do_percent	bit	1	
	do_count	bit	1	
	do_opt1	bit	1	
	do_opt2	bit	1	
	do_result	bit	1	

Table 4.10 in_use_flags Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	user_id	varchar	24	
Y	table_name	varchar	64	
	in_use	bit	1	

Table 4.10 in_use_flags Table

Key	Column Name	Data Type	Length	Allow Nulls
	cytometer	varchar	12	Y
	lockDateTime	smalldatetime	4	Y

Table 4.11 InstallationID Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	InstallationID	varchar	50	

Table 4.12 instrument_directories Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	cytometer	nvarchar	10	
Y	user_id	nvarchar	24	
Y	subject	nvarchar	16	
	directory	nvarchar	256	

Table 4.13 instrument_flags Table

Key	Column Name	Data Type	Length	Allow Nulls
	Table_Name	varchar	32	
	Updated	varchar	1	
	when_updated	datetime	8	Y

Table 4.14 instrument_installation Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	subject	varchar	32	
	content	varchar	64	
Y	cytometer	varchar	10	
Y	user_id	varchar	24	

Table 4.15 instrument_signals Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	signal_index	int	4	
	signal_name	varchar	10	

Table 4.16 instrument_statistic Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	statistic_index	int	4	
	statistic_name	varchar	10	

Table 4.17 lis_transfer Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	instance	int	4	

Table 4.18 qc_base Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	qc_instance	int	4	
	qc_date	datetime	8	
	qc_time	varchar	10	
	qc_cytometer	varchar	10	
	qc_settings_file	varchar	48	
	qc_seconds	int	4	
	qc_application	varchar	48	
	qc_protocol	varchar	48	
	FL1_FL2	float	8	
	FL1_FL3	float	8	
	FL1_FL4	float	8	
	FL1_FL5	float	8	
	FL1_FL6	float	8	
	FL1_FL7	float	8	
	FL1_FL8	float	8	
	FL1_FL9	float	8	
	FL1_FL10	float	8	
	FL2_FL1	float	8	
	FL2_FL3	float	8	
	FL2_FL4	float	8	
	FL2_FL5	float	8	
	FL2_FL6	float	8	
	FL2_FL7	float	8	
	FL2_FL8	float	8	
	FL2_FL9	float	8	
	FL2_FL10	float	8	
	FL3_FL1	float	8	
	FL3_FL2	float	8	
	FL3_FL4	float	8	
	FL3_FL5	float	8	
	FL3_FL6	float	8	

Table 4.18 qc_base Table

Key	Column Name	Data Type	Length	Allow Nulls
	FL3_FL7	float	8	
	FL3_FL8	float	8	
	FL3_FL9	float	8	
	FL3_FL10	float	8	
	FL4_FL1	float	8	
	FL4_FL2	float	8	
	FL4_FL3	float	8	
	FL4_FL5	float	8	
	FL4_FL6	float	8	
	FL4_FL7	float	8	
	FL4_FL8	float	8	
	FL4_FL9	float	8	
	FL4_FL10	float	8	
	FL5_FL1	float	8	
	FL5_FL2	float	8	
	FL5_FL3	float	8	
	FL5_FL4	float	8	
	FL5_FL6	float	8	
	FL5_FL7	float	8	
	FL5_FL8	float	8	
	FL5_FL9	float	8	
	FL5_FL10	float	8	
	FL6_FL1	float	8	
	FL6_FL2	float	8	
	FL6_FL3	float	8	
	FL6_FL4	float	8	
	FL6_FL5	float	8	
	FL6_FL7	float	8	
	FL6_FL8	float	8	
	FL6_FL9	float	8	
	FL6_FL10	float	8	
	FL7_FL1	float	8	
	FL7_FL2	float	8	
	FL7_FL3	float	8	
	FL7_FL4	float	8	

Table 4.18 qc_base Table

Key	Column Name	Data Type	Length	Allow Nulls
	FL7_FL5	float	8	
	FL7_FL6	float	8	
	FL7_FL8	float	8	
	FL7_FL9	float	8	
	FL7_FL10	float	8	
	FL8_FL1	float	8	
	FL8_FL2	float	8	
	FL8_FL3	float	8	
	FL8_FL4	float	8	
	FL8_FL5	float	8	
	FL8_FL6	float	8	
	FL8_FL7	float	8	
	FL8_FL9	float	8	
	FL8_FL10	float	8	
	FL9_FL1	float	8	
	FL9_FL2	float	8	
	FL9_FL3	float	8	
	FL9_FL4	float	8	
	FL9_FL5	float	8	
	FL9_FL6	float	8	
	FL9_FL7	float	8	
	FL9_FL8	float	8	
	FL9_FL10	float	8	
	FL10_FL1	float	8	
	FL10_FL2	float	8	
	FL10_FL3	float	8	
	FL10_FL4	float	8	
	FL10_FL5	float	8	
	FL10_FL6	float	8	
	FL10_FL7	float	8	
	FL10_FL8	float	8	
	FL10_FL9	float	8	

Table 4.19 qc_comment Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	qc_instance	int	4	
	qc_comment	varchar	32	

Table 4.20 qc_data Table

Key	Column Name	Data Type	Length	Allow Nulls
	qc_instance	int	4	
	qc_seconds	int	4	
	qc_region	varchar	31	
	qc_parameter	varchar	31	
	qc_hpcv	float	8	
	qc_cv	float	8	
	qc_mean	float	8	
	qc_median	float	8	
	qc_count	float	8	
	qc_abs_count	float	8	
	qc_volts	float	8	
	qc_gain	float	8	
	qc_percent_pos	float	8	
	qc_reference	char	1	
	qc_user_id	varchar	24	
	qc_inc	int	4	
	qc_code	int	4	
	qc_lot	varchar	9	
	qc_mode	float	8	
	qc_gate_pcnt	float	8	
	qc_total_pcnt	float	8	
	qc_xy	bit	1	

Table 4.21 qc_instance Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	qc_instance	int	4	
	qc_characteristic	char	1	

Table 4.22 qc_maintenance Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	mn_cytometer	nvarchar	10	
Y	mn_date	smalldatetime	4	
Y	mn_maint_type	nvarchar	32	
	mn_user	nvarchar	24	
	mn_comment	nvarchar	256	Y

Table 4.23 qc_mainttype Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	mn_cytometer	nvarchar	10	
Y	mn_color	nvarchar	32	
	mn_user	nvarchar	24	

Table 4.24 qc_products Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	qc_code	int	4	
	qc_product_deleted	bit	1	
	qc_type	char	1	
	qc_product	varchar	24	
	qc_part_no	varcahr	10	Y
	qc_lot_no	varchar	9	
	qc_assay	int	4	Y
	qc_expiration	smalldatetime	4	Y
	qc_received	smalldatetime	4	Y
	qc_enter_service	smalldatetime	4	Y

Table 4.25 qc_servicelog Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	sv_cytometer	char	10	
Y	sv_date	smalldatetime	4	
Y	sv_user	nvarchar	24	
Y	sv_condition	nvarchar	256	
	sv_action_date	smalldatetime	4	Y
	sv_action_user	nvarchar	24	Y
	sv_action_taken	nvarchar	256	Y

Table 4.26 qc_template Table

Key	Column Name	Data Type	Length	Allow Nulls
	qc_date	smalldatetime	4	
Y	qc_template_name	varchar	31	
Y	qc_plot_num	int	4	
	qc_plot_mean	float	8	
	qc_plot_sd	float	8	
	qc_plot_manual_limit	float	8	
	qc_plot_percent_mean	float	8	
	qc_plot_num_sd	float	8	
	qc_plot_mean_selection	int	4	
	qc_plot_limit_selection	int	4	
	qc_plot_protocol	varchar	48	
	qc_plot_product	varchar	31	
	qc_code	int	4	
	qc_plot_parameter	varchar	31	
	qc_plot_statistic	varchar	31	
	qc_plot_region	varchar	31	
Y	qc_user_id	varchar	31	
Y	qc_instrument	varchar	31	
	qc_application	varchar	48	Y
	qc_user_label	varchar	20	
	qc_xy	bit	1	

Table 4.27 Settings Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	Description	varchar	50	
	Value	varchar	50	

Table 4.28 Sex Table

Key	Column Name	Data Type	Length	Allow Nulls
Y	Sexcode	varchar	1	
	SexName	varchar	10	

4.4 ANALYTICAL CHARACTERISTICS AND SPECIFICATIONS

Carryover

Particle carryover is <0.1% from one specimen to another when the number of gated events is 10,000.

Fluorescence carryover on the Gallios system was assessed by analyzing an unstained control sample after the acquisition of a sample stained with a vital dye. Following the acquisition of three tubes of Cyto-trol Control Cells stained with 20ul of Acridine Orange, the system was cleaned according to the Routine Cleaning Procedure. Subsequently, 3 tubes of unstained Cyto-trol Control Cells were analyzed. The average mean channel fluorescence shift obtained in the unstained sample acquisitions was less than 1%.

Acquisition Rate

The Gallios was verified to analyze at least 200,000 events from FS, SS, FITC, PE, ECD, APC, PC5.5, PC7, and APC parameters with event rates from 1384 to 40822 events per second. Electronic pulses were counted from the beads passing through the aperture as measured by discriminator events. The count from the histogram display/printout was assessed against the number of pulses counted (electronic count). Yield percentage was taken from the Stored Events counter. At 25,000 events per second, the yield was measured to be 90%.

Data Acquisition Throughput

Throughput, including printing, of 80 tubes/hour of 10,000 normal lymphocytes with a leukocyte count of 10,000 was obtained at a high flow rate. The throughput of a concentrated sample was 88 tubes/hour was obtained at 10,000 events per second when the stop count was set to 100,000 gated events.

Acquisition throughput is dependant on many variables, including flow rate, data rate, number of parameters collected, gated events, data plots, FlowPAGE reports and printouts generated. Changing these variables, such as decreasing the number of events collected, increasing sample concentration or increasing the flow rate can increase your acquisition throughput; while increasing the number of events collected or running at a lower data rate can decrease your acquisition throughput.

Precision for Surface Markers

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

Scatter Resolution

Scatter resolution on the Gallios Flow Cytometer was measured using 0.404 µm particles from Thermo Scientific. The noise was set to the bottom of the scale, using FS HV = 700, Gain at 200, Discriminator at 47 and setting the Forward Scatter detector to the Wide2 setting. The Gallios was able to clearly show baseline resolution between the noise and the 0.404 µm diameter particles.

Forward Scatter

The HPCV of the integral signal intensity values using Flow-Check Pro fluorospheres is <2% from the blue laser.

Fluorescence

The HPCV of the integral signal intensity values using Flow-Check Pro fluorospheres is <2% for FL1-FL4 and <2.5% for FL5 from the blue laser, <3.0% for FL6-FL8 from the red laser and <4.0% for FL9-FL10 from the violet laser.

Sensitivity

Fluorescence

Three measurements of multi-level fluorescence sensitivity particles were taken on 3 Gallios Flow Cytometers. Acquisition was conducted on 10,000 bead stop count at the medium flow rate. For the FITC and PE measurements, 8 peak Spherotech™ RCP30-5A Rainbow particles were used. For the PeCY5, and APC, URCP-38-2K Ultra Rainbows were used. The following values represent the average of the measurements taken.

- <112 MESF for FITC
- <78 MESF for PE
- <15 MESF for PC5
- <75 MESF for APC

Stability

Day-To-Day

The mean channel value of the integral signal intensity of alignment verification fluorospheres from the standard PMT's off the blue, red and violet lasers does not vary more than ±5% from any integral signal channel number obtained over a period of 8 days.

Within Day

The mean channel value of the integral signal intensity of alignment verification fluorospheres from the standard PMT's off the blue red, and violet lasers does not vary more than ±5% from any integral signal channel number obtained within a period of 24 hours.

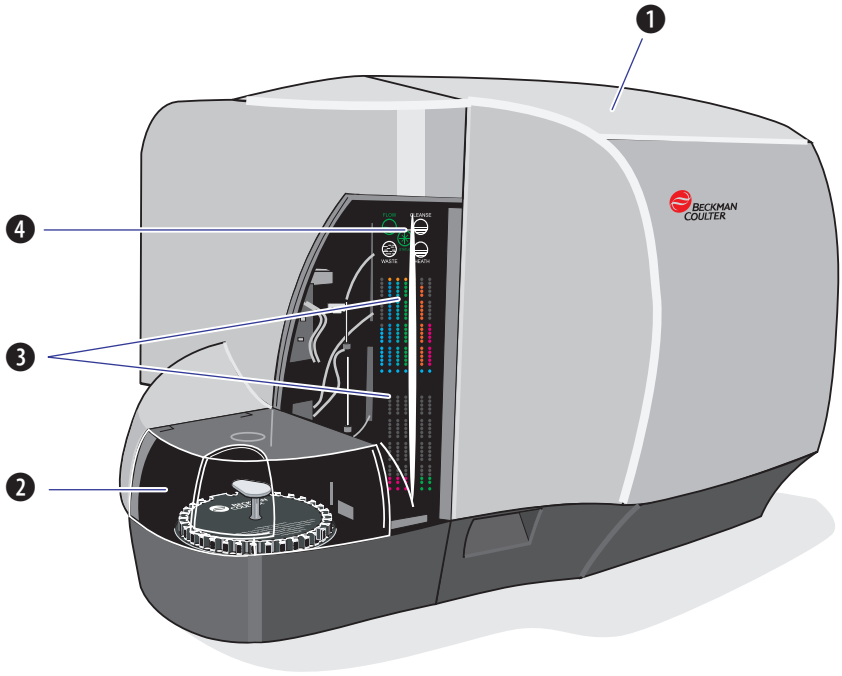
5.1 PRODUCT DESCRIPTION

The Gallios Flow Cytometer is a system designed for the qualitative and quantitative research of biological and physical properties of cells and other particles using multiparametric analysis.

The instrument can simultaneously measure forward scatter, side scatter, and up to ten fluorescent dyes using three solid-state lasers at 488 nm, 638 nm and 405 nm. Therefore, the instrument can perform correlated multiparameter analyses of individual cells.

5.2 CYTOMETER CONTROLS AND INDICATORS

Gallios Flow Cytometer

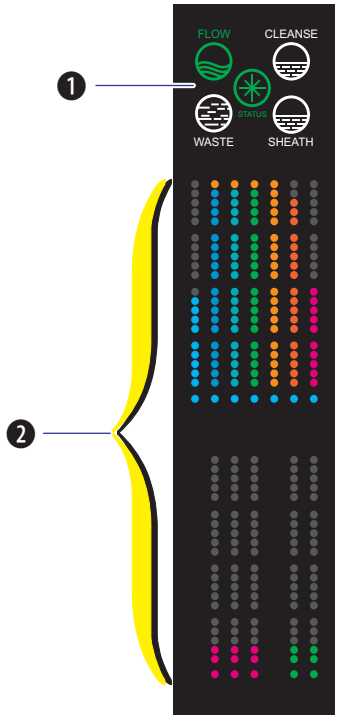


1	Cytometer	3	Signal Amplitude Indicators
2	MCL	4	Level Sense and Flow Indicators

Cytometer Indicator Panel

1 Level Sense and Flow Indicators

2 Signal Amplitude Indicators



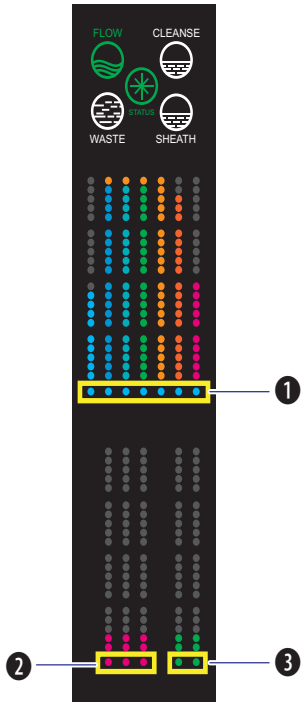
Signal Amplitude Indicators

Note: When both log and linear parameters are selected for the same sensor, the amplitude display defaults to the log signal.

1 Blue indicates the signals from the 488 laser.
FS, SS, FL1, FL2, FL3, FL4 and FL5

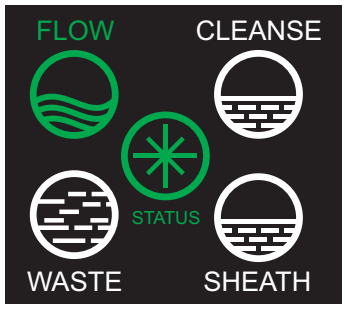
2 Red indicates the signals from the 638 laser.
FL6, FL7 and FL8

3 Green indicates the signals from the 405 laser.
FL9 and FL10



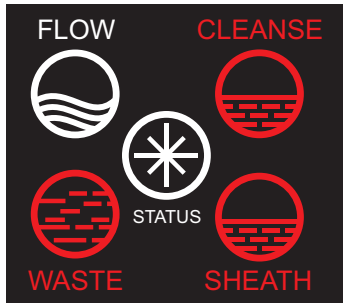
Cytometer Ready and Sheath Flow Indicators

FLOW (ready when green)
STATUS (ready when green)

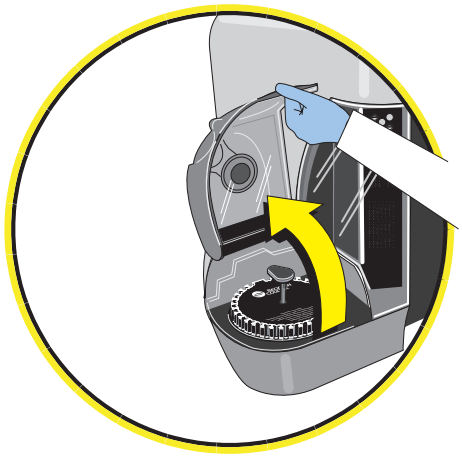


Level Sense Indicators

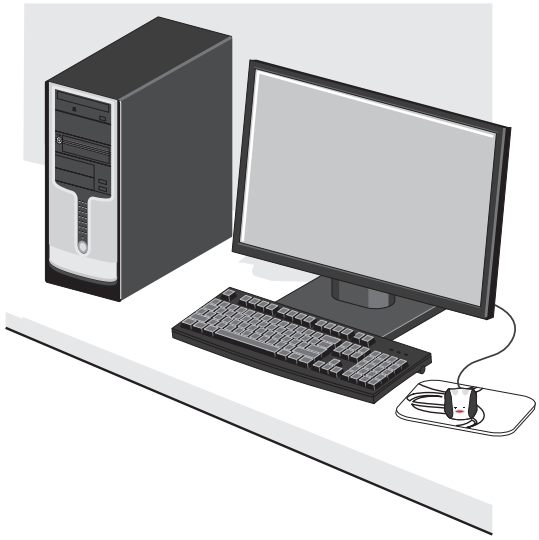
CLEANSE (red when low)
WASTE (red when full)
SHEATH (red when low)



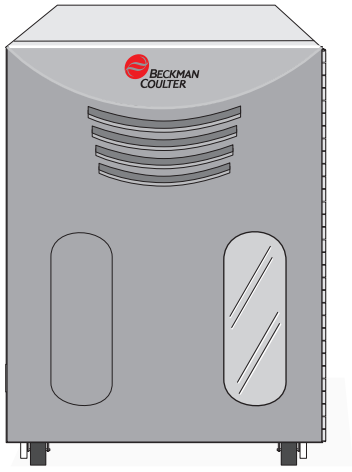
MCL (Multi-tube Carousel Loader)



Workstation



Pneumatic Supply



Printer (Optional)

Operating instructions from the Printer manufacturer are included with the Printer.

5.3 MICROSOFT® WINDOWS® DESKTOP

Windows Administrator Password

Be sure to maintain your Windows Administrator password in a secure location. If you lose or forget the Windows Administrator password, you must reimage your hard drive, causing the loss of all data.

Taskbar

At the bottom of the screen is the Taskbar. It contains the Start button on the left-hand side and a clock on the right. Other icons are displayed on the Taskbar depending on the configuration of the hardware and software of your particular computer.

When a program is opened, a button for that particular program appears within the Taskbar. You can switch between programs by clicking with the mouse on these buttons.

5.4 LEARNING THE BASIC OPERATING TECHNIQUES

Before reading the other chapters in this manual:

- Read about the Cytometer Control screens.
- Read this chapter to become experienced with using the MCL.

Practice the basic techniques until you feel comfortable using them. If, later on, you need to use a basic technique but cannot remember how, use the System Help Index or Search tab to look it up and get the step-by-step instructions.

MCL Carousels

The Gallios Flow Cytometry System starter kit has:

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

Bar-Code Labels

You can put a bar-code label on each sample tube. See the BAR-CODE SPECIFICATIONS appendix.

IMPORTANT Sample misidentification can occur from the use of incorrect, poor quality, damaged, dirty or improperly placed bar-code labels. Follow the BAR-CODE SPECIFICATIONS to create your bar-code labels to prevent incorrect sample identification. Risk of erroneous results if the bar-code label is placed incorrectly on sample tubes. To prevent misidentified samples, affix the bar-code label as shown below so the MCL can read the label.

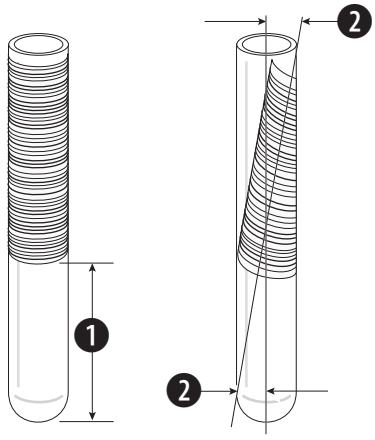
Putting a Bar-Code Label on a Sample Tube

1 Carefully align the label with the tube.

2 Press the label down securely, including edges and corners, without wrinkles or folds.

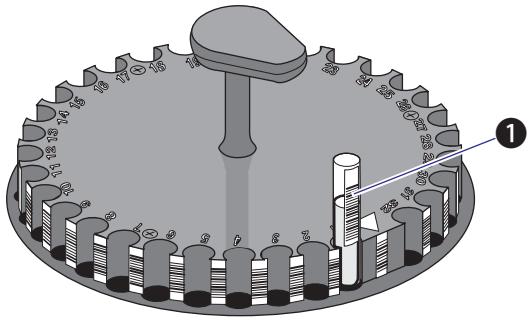
❶ 25.4 mm (1.0 in.) minimum

❷ 7.5 degrees.



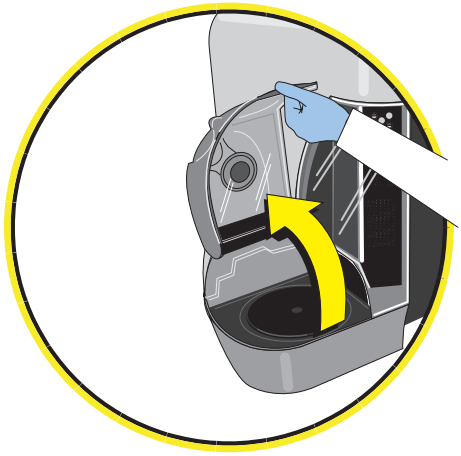
Putting Sample Tubes in a Carousel

- The orientation of a tube with a bar-code label ❶ does not matter. The MCL rotates the tube to find the bar-code label.
- Do not skip tube positions within a panel. The Gallios flow cytometer does not skip a protocol in a panel when a carousel tube position is empty. If you lose a sample, delete that protocol from the panel using the Acquisition Manager.
- You can skip a single-tube position to separate two panels based on your tube location setup in Acquisition Manager.

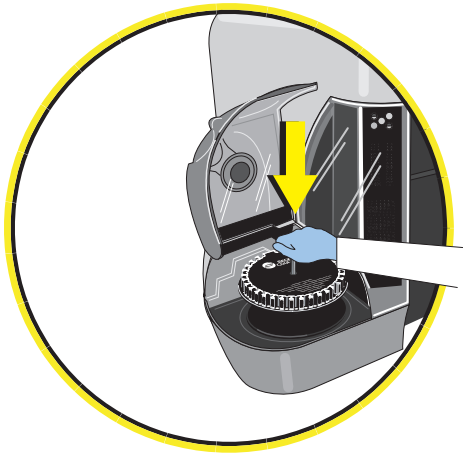


Putting a Carousel in the MCL

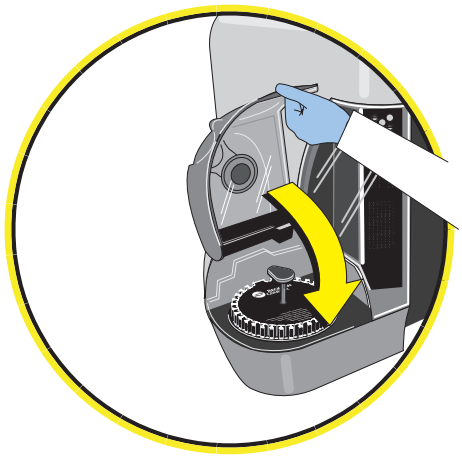
1 Open the MCL cover.



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

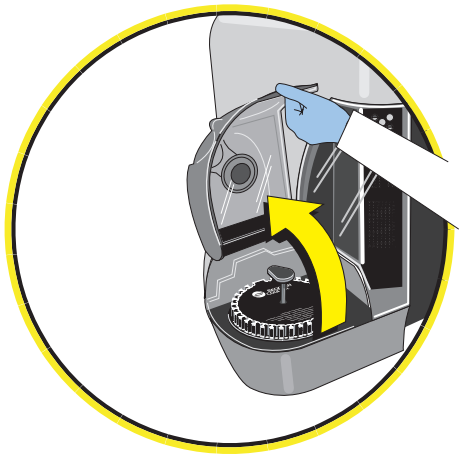


3 Close the MCL cover.

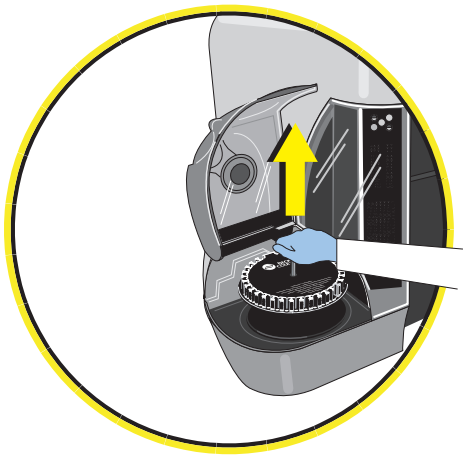


Removing a Carousel from the MCL

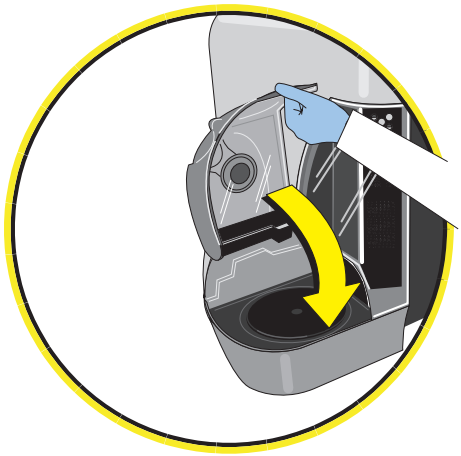
1 Open the MCL cover.



2 Remove the carousel.



3 Close the MCL cover.



5.5 GALLIOS SOFTWARE SHORTCUTS

Gallios software uses several standard Windows techniques for providing shortcuts to functions.

Keyboard Shortcuts
Drag And Drop
Toolbar Buttons

Keyboard Shortcuts

Where Ctrl+X means hold Ctrl down and press X.

Ctrl+C	Copy
Ctrl+V	Paste
Ctrl+X	Cut
Ctrl+Z	Undo
Ctrl+Y	Redo
Ctrl+W	Workspace Preferences
Ctrl+T	Tile Special
Ctrl+P	Print
Ctrl+N	New Protocol
Ctrl+S	Save Protocol
Ctrl+O	Open Listmode File
Ctrl+F	Format Plot
Ctrl+D	Duplicate Plot
Ctrl+Q	Listmode QuickCOMP
Ctrl+L	View Cytometer Log
Ctrl+1	Create new Dot Plot
Ctrl+2	Create new Histogram Plot
Ctrl+4	Create new Density Plot
Ctrl+8	Create new Prism Plot
Ctrl+9	Create new Legend Plot
Ctrl+0	Create new Info Plot
Alt+F4	Exit active window
F1	Context Sensitive Help
F5	Refresh Screen
F7	Publish to Excel (or text file)
F9	Start Acquisition
F10	Stop Acquisition
F11	Pause Acquisition
F12	Abort Acquisition

Drag And Drop

Dragging and dropping is a shortcut method of opening, moving and deleting files or other objects.

Within Gallios software the Drag and Drop technique can be used for several functions, with some operations Ctrl can be held down to modify the default behavior.

Table 5.1 Drag And Drop Table

Drag From…	Action
Resource Explorer	
Worklist	Drag a stored Worklist to Acquisition Manager.
Panels	Add a panel to the current Worklist by dragging a panel to the current Worklist.
Protocols	Drag a protocol to the Workspace or Acquisition Manager.
Listmode files	Drag a file to a series of plots, or with Ctrl pressed ONLY the current plot.
Protocol Explorer	
Gates	Drag a gate to gate ALL plots, or with Ctrl pressed ONLY the current plot.
AutoMATOR Setup	
Rearrange files	Reorder files for playback using the AutoMATOR application.
Modify Color Precedence	
Reorder Gates	Drag gates into the required precedence order.
Plots	
Regions	Drag copies of regions from one plot to another, with Ctrl pressed gate logic is also copied.
Plot Images	Drag the current plot image to a third party application (such as, MS Paint, Power Point®).
Statistics	Reorder the statistics under the plots.
Tile Special	
Plots	Reorder plots on the desktop.
Cytometer Control (Cytometer Only)	
Parameters	Reorder parameters in the “selected Parameter” list.
Acquisition Manager	
Worklist Columns	Reorder Worklist columns to any desired order.

Toolbar Buttons

Toolbars form an essential part of the Gallios software. The application entered and the mode you are operating at the time determines whether a particular Toolbar display is available.

Toolbars can be moved using the Drag and Drop method with the mouse around the Gallios software desktop or customized to suit the preferences of each user. See, Toolbars - Customize Toolbars.

The Toolbar elements are:

FILE OPTIONS TOOLBAR
PLOT OPTIONS TOOLBAR
REGIONS OPTIONS TOOLBAR
GATE, COLOR, STATS AND HELP TOOLBAR
FLOWPAGE TOOLBAR
ACQUISITION MANAGER TOOLBAR
AUTOMATOR TOOLBAR
CYTOMETER TOOLBAR.
REPORT GENERATOR TOOLBAR

5.6 CREATING PROTOCOLS

---

1 **File ▸▸ New ▸▸ New Protocol.**

---

2 and **Parameters** button on the Cytometer Control Acquisition Setup Tab.

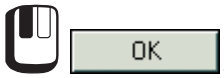
---

3 Choose the Parameters to be acquired and OK.

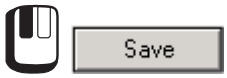
Note: Including TIME as a selected parameter in all protocols allows for an internal quality check on the data acquisition. A TIME versus fluorescence plot may be helpful to monitor system fluidic and optic conditions during acquisition of any given sample.

IMPORTANT Risk of erroneous results if the parameter order is changed or parameters are added or deleted. To prevent reporting erroneous results, verify the protocols plots, regions and gates before reporting results.

4 If the parameters have changed,

a. 

.

b. Enter the parameter name and 

.

c. 

on the Cytometer Control screen.

5 Create the Plots required for acquisition.

6 If analysis is to be performed during acquisition, create the required Regions and Gates, and also select the required statistics from the **Analysis ▸▸ Select Results** option.

7 **File ▸▸ Save Protocol As** to save the protocol.

8 Enter the sample information into the correct position in the Acquisition Manager Worklist. See Acquisition Manager to modify the Acquisition Manager display.

9 Set the listmode file name options from the Workspace Preferences - LMD File Name tab.

10 Set the acquisition options from the Workspace Preferences - Acquisition Options tab.

11 Set up the Worklist and run samples.

12  Setup Mode on the Cytometer Control Acquisition Setup Tab and adjust the instrument settings.

13 When satisfied with the data, deselect Setup Mode and continue acquisition.

14 Once sufficient data is collected,  **File ▸▸ Save Protocol** to update the protocol with the new instrument settings.

5.7 CREATING REGIONS

Create Polygonal Regions

See also: Interactive Polygonal Region Editing

1 Highlight a dual parameter plot (click on the title bar).

2  .

3 A Polygonal Region cursor ✛ is displayed on screen.

4 Place the cursor at the point where you want the Region to begin.

5 Click and release the mouse button, to fix the point (a start box is displayed on screen).

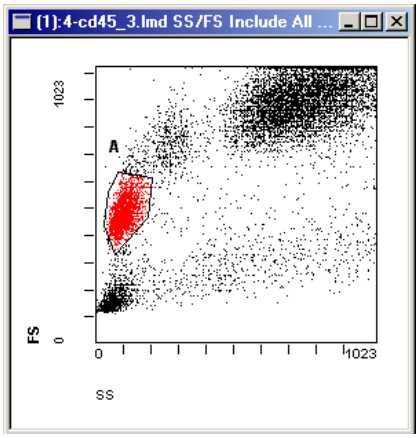
6 Continue in this manner until the desired number of points has been chosen.

When drawing a Region, if you decide that it is not the one you want, press Esc before the region is completed. The Region is deleted.

To change the Region type, stay in the current plot, choose the desired region type from the Region menu or click a Region shortcut button for whatever Dual Parameter Region type you wish.

7 Close the Region by returning the cursor to the starting box and clicking to conclude Region creation.

8 The Region Name appears on the plot.



9 See Dot Plot Data Source to display percentages with the Region name.

Create Rectangular Regions

See also: Interactive Rectangular Region Editing

1 Highlight a dual parameter plot.

2  .

3 A Rectangular Region cursor appears in the highlighted plot.

4 Position the cursor to the point where you want the Region to begin.

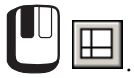
5 Click and release the mouse button. A Rectangular Region start box is displayed on screen. By moving the mouse you can increase and decrease the size of the Rectangular Region. When the desired Rectangular Region appears, click the mouse button to complete the Region.

6 The Region Name appears on the plot.

Create Quadrant Regions

The Quadrant Region is a FlexQuad Quadrant Region. You can adjust the angle of quadrant dividers to adequately analyze populations that are properly compensated with the negative data display. The dividers between FlexQuad quadrants 1 and 2 and between quadrants 2 and 4 are 'flexible' which allows you to better analyze broadly spread populations.

1 Highlight a dual parameter plot.

2  .

3 A crosshair cursor ⊞ is displayed in the current window.

4 Position the cursor on the plot where you want the intersection point of the region and  to show the quadrant lines (cross hair).

5 Move the regions to the required position, if needed, then  to set this position.

6 Gallios software shows the Quadrant's name in each of its four Regions.

When drawing a Region, if you decide that it is not the one you want, press Esc before the Region is completed. The Region is deleted.

Repositioning a Quadrant Region

1 Mouse over the Quadrant region lines to get the ↔, ↕ or ✥ cursor.

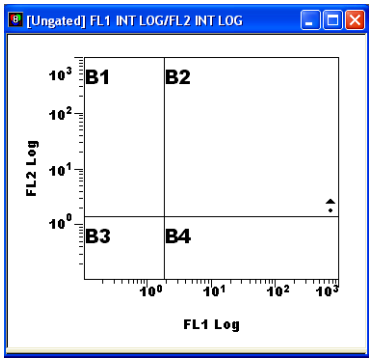
2 Reposition the region.

a. + hold and drag ↔ to move the vertical divider.

b. + hold and drag ↕ to move the horizontal divider.

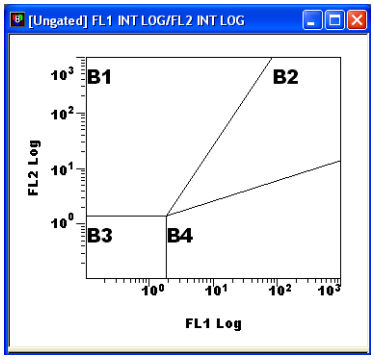
c. + hold and drag ✥ to move the Quadrant region.

Before repositioning FlexQuad dividers.



3 Release the mouse button.

After repositioning FlexQuad dividers.



See also: Interactive Quadrant Region Editing

Create Linear Regions

This option requires two points to be set giving an upper and lower channel boundary for every Region.

1 Highlight a single parameter histogram.

2  .

A Linear Region cursor appears in the current window.

3 Position the cursor on the chosen plot to the point where you want the Region to begin. Click, two vertical lines with a horizontal bar appears.

4 Move the cursor left or right, up or down until the desired end point of the Region is reached. to accept the settings and anchor the region.

When drawing a Region, if you decide that it is not the one you want, press Esc before the Region is completed. The Region is deleted.

After setting the Region position, the long vertical lines are replaced by short channel markers to prevent cluttering of the plot display.

The horizontal bar is positioned approximately at the horizontal cursor position when the region is set.

See also:
Interactive Linear Region Editing
Status Bar (Linear)
X Coordinate
Integral

Create Multiple Linear Regions

1 Highlight a single parameter histogram and  .

2 A Parallel cursor appears in the current window.

3 Position the cursor on the chosen plot to the point where you want the Region to begin. Click, two vertical lines with a horizontal bar is displayed. A stop box appears in the right hand top corner or the window.

4 Move the cursor left or right, up or down until the desired end point of the Region is reached.  to accept the settings and anchor the region.

5 Repeat this procedure until all Regions are set.

6 When you have drawn all of your Multi-Linear Regions, press Esc or move the cursor onto the stop box in the right hand top corner, click. This ends the Multi-Linear sequence.

See also: Interactive Multi-Linear Region Editing

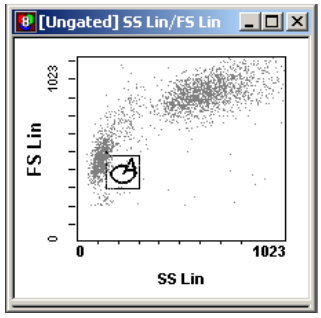
5.8 CREATING GATES

Create AutoGate

Note: If a stop count is used on a plot that contains an AutoGate region, the stop count is not exact.

---

1 in the plot you want to create an AutoGate on.

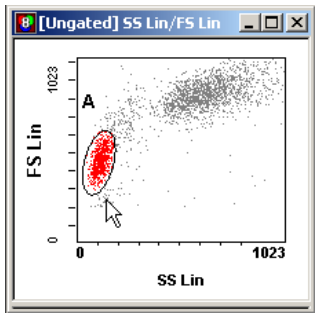


---

2 or .

---

3 on the population to AutoGate.



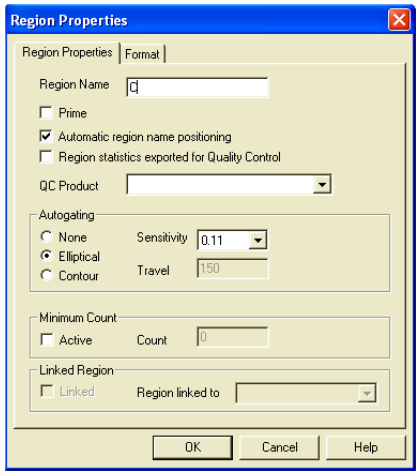
---

4 To specify the AutoGate Sensitivity and Travel,

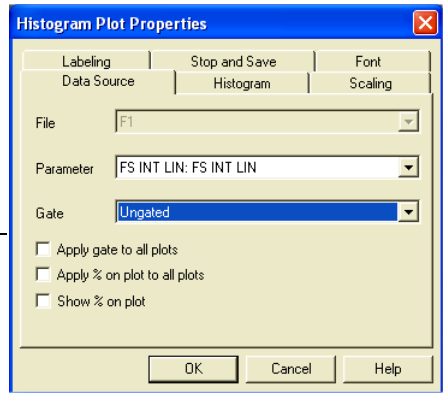
a. the region.

b. Click the right mouse button on the region and **Region Properties**.

c. Specify the AutoGating Sensitivity and Contour Travel, if necessary, and OK.



5 Right mouse click on the plot you want to assign the AutoGate to and select Format to display the Plot Properties dialog.



6 the gating drop down list and select the AutoGate to gate the plot on the AutoGate.

7 Repeat steps 5 and 6 to assign the AutoGate to other plots or **Apply gate to all plots**.

Note: If you select **Apply gate to all plots** you must go back to the plot that contains the AutoGate and unassign the AutoGate from the plot.

Convert a Polygonal Region to an AutoGate Region

1 region to make it active.

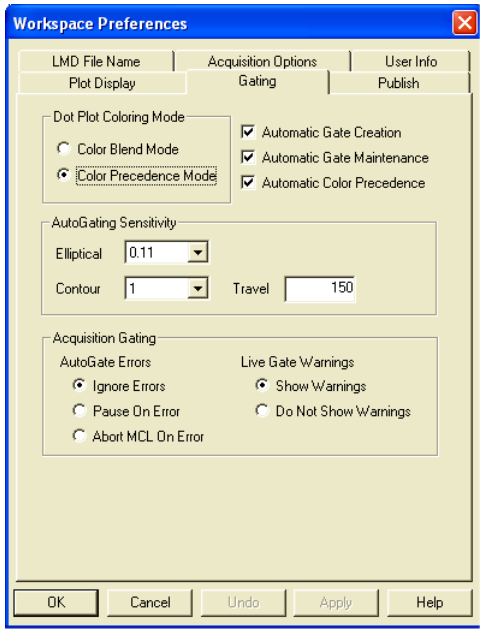
2 Click the right mouse button on the region and **Region Properties**.

3 either **Elliptical** or **Contour in** the Polygonal Region Properties screen.

Automatic Gate Creation

This option allows you to create a new gate automatically when a new Region is drawn. If this option is not selected you must use **Analysis ▸▸ Create Modify Gates** to create gating logic.

Regions copied using Ctrl+Drag and Drop into a plot assign the region as a gate.



5.9 CREATING FLOWPAGES

FlowPAGE Example

Below is a typical example of a FlowPAGE. This is a screen view only. A full printed page outputs at a much higher resolution. See also: FlowPAGE Menu in the Using Gallios Software chapter.

---

1  to create a blank FlowPAGE.

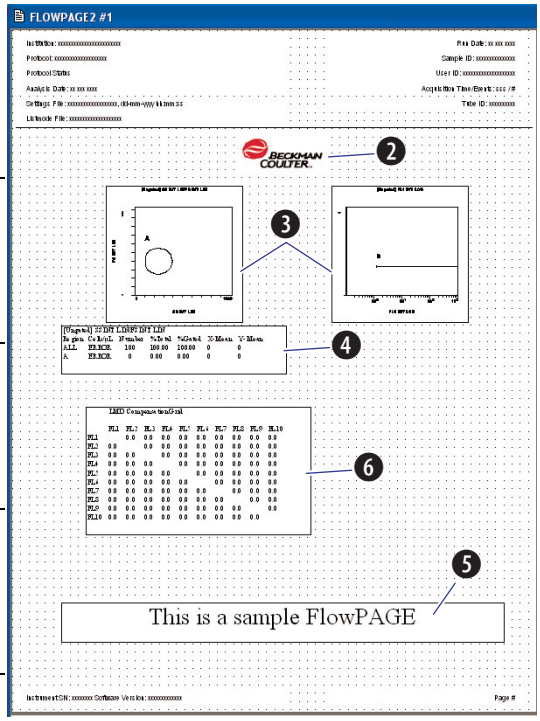
2  to insert a picture.

3 Insert Flowpage Plots.

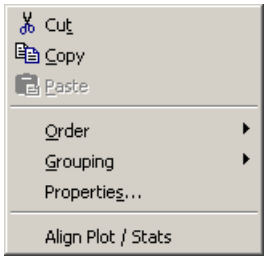
4 Insert a FlowPAGE Statistics Table.

5 Insert a FlowPAGE Textbox.

6 Insert a FlowPAGE LMD Compensation Grid.



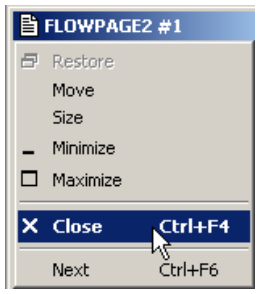
7 Click the right mouse button on any object on the FlowPAGE to select additional formatting options.



Delete FlowPAGES

1 in the upper left corner of the FlowPAGE window to be deleted and **Close**.

Note: FlowPAGES in a locked protocol can not be inserted, deleted or edited.



5.10 CREATING PANELS

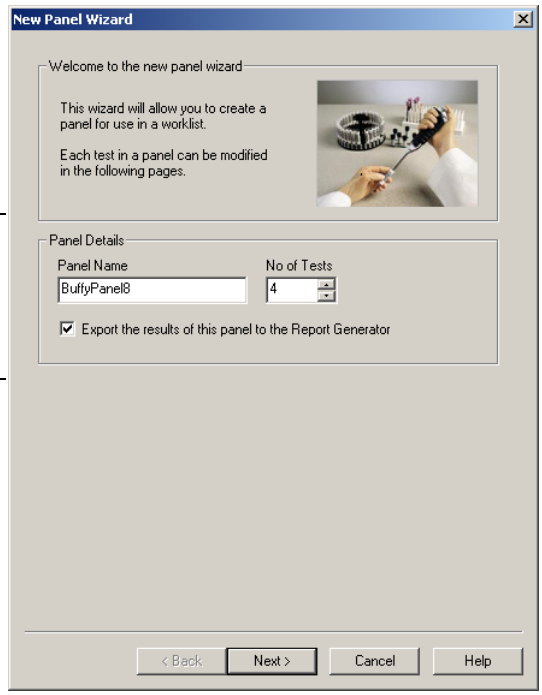
The Panel Wizard allows you to create a Panel for use in the Acquisition Manager.

IMPORTANT When editing and saving a panel, any worklist containing the edited panel must be recreated. If the worklist in not re-created, the panel in the worklist may not match the newer version of the panel.

1 **File ▸▸ New Panel** to start the Panel Wizard.

2 Enter the New Panel name.

3 Select the Number of Tests required. The default value is 1 and the maximum is 32.

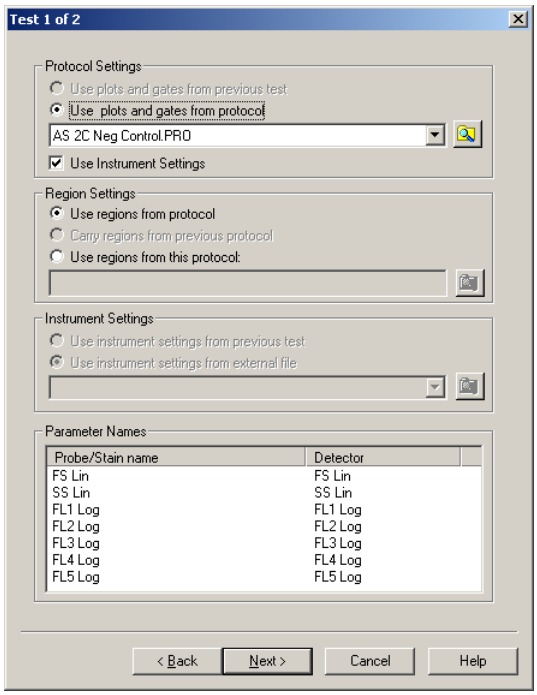


4 **Export the results of this panel to the Report Generator** if you want a Patient Panel Report generated when you run this panel. See Panel Report.

5 Next >.

6 Choose your Protocol Settings.

Use Plots and Gates from Previous Test
Use Plots and Gates from Protocol
Use Instrument Settings



7 Choose your Region Settings.

Use Regions from Protocol
Carry Regions from Previous Protocol
Use Regions from This Protocol:

Next >.

8 Choose your Instrument Settings.

Use Instrument Settings from Previous Test
Use Instrument Settings from External File

9 Double on parameter names under **Probe/Stain name** to assign the Parameter Names.

10 Repeat steps 6 through 9 for each test.

11 

Use Plots and Gates from Previous Test

Allows you to use Gates and Plots from a previous Test but is not active for the first Test within a Panel.

This option is not available if **Use plots and gates from protocol** is chosen.

Use Plots and Gates from Protocol

Allows you to select Plots and Gates from a specific Protocol, chosen from the drop down list box.

This option is not available if **Use plots and gates from previous test** is chosen.

Use Instrument Settings

Uses the instrument settings from the selected Protocol. If the checkbox is disabled, this allows the **Instrument Settings** below to be active.

Use Regions from Protocol

This option loads all regions stored within the current protocol.

Carry Regions from Previous Protocol

Selecting this option allows region positions to be carried from the previous test in a panel. Any regions of the same type and drawn on the same parameters are carried.

Use Regions from This Protocol:

This option loads the regions and region positions from the selected protocol.

Use Instrument Settings from Previous Test

Uses the Instrument settings from the previous Test but is not active for the first Test within a Panel.

This option is not available if **Use instrument settings from external file** is selected.

Use Instrument Settings from External File

Use Instrument Settings from an External File such as Listmode or Protocol [Settings] files that you choose from the drop down list box.

This option is not available if **Use instrument settings from previous test** is selected.

Parameter Names

Allows you to choose the Parameter Names for each tube within a Panel.

5.11 CREATING WORKLISTS

Before Creating Worklists

- If no Worklist is visible, **View ▸▸ Acquisition Manager** to display the Worklist pane.
- Unless the current Worklist has been saved using the **Save Worklist** button within the ACQUISITION MANAGER TOOLBAR, current worklist settings are not available.
- Do not save scheduled AutoSetup Applications as a Worklist. Applications are intended to be "scheduled". Do not add any protocols or panels to Acquisition Manager that has scheduled Applications or AutoSetup II protocols. Always select "New Worklist" after scheduled QC Applications or Autosetup protocols are run.
- Modifications to a multi-tube panel may impact any associated panel templates or worklists. Beckman Coulter recommends that all templates and worklists containing a panel that has been modified be validated before use.

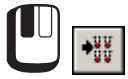
IMPORTANT When editing and saving or deleting a protocol or panel, any worklist containing the edited or deleted protocol or panel must be recreated. If the worklist is not re-created, the protocol or panel in the worklist may not match the newer version of the protocol or panel.

1 to create a new Worklist or to clear current Worklist from the Acquisition Manager screen.

2 Drag And Drop panels and protocols from the Resource Explorer to the Acquisition Manager. Information added in this way always appears at the end of the Worklist.

3 After all the information has been added, you can use Drag And Drop to change the order.

Worklist Panel

1  .

2 Select which panel you wish to open and 

.

3 After all the information has been added, you can use Drag And Drop to change the order.

You can use this option to add another Panel to an existing Worklist.

Worklist Test

 to insert a single Row at the end of the current Panel.

Note: A duplicate of the first protocol in the panel is added to the current panel. If a different protocol is required,  in the protocol field.

5.12 VERIFYING A WORKLIST

Always verify carousel and tube location fields before starting acquisition and ensure that the Acquisition Manager information accurately reflects the tubes in the carousel being run. The order of the tubes in the carousel must match the order of the tubes in the Worklist.

The default protocol (Ctrl N) must be saved prior to being used in the Worklist. If the default protocol is not saved with a specific name prior to using it, that tube may be run with unexpected protocol settings depending on the Save Protocol selection in Workspace Preferences. Also, the default protocol name will default to "setup.def" in any associated panel template and have no regions.

5.13 USING THE SYSTEM HELP

Your Gallios system provides System Help that allows you to search for information on specific system-related topics through the **Contents, Index, and Search** options.

The Help system is an electronic version of the Instructions For Use Manual. It includes a table of contents, an index for finding information quickly, and a glossary of definitions. See also, HELP MENU.

Access System Help

Use one of these options to access the System Help.

 .

 **Help** ▸▸ **Gallios Help**.

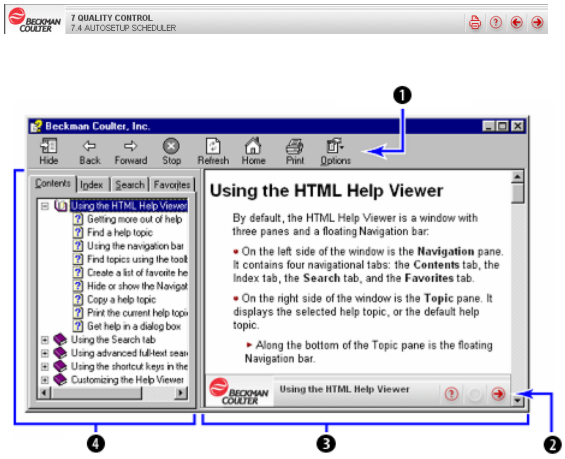
F1.

Note: If you cannot access the System Help, contact your Beckman Coulter Representative.

How to Use Help

 on the Help Navigation Bar.

Information about the Help window and instructions for using help are displayed in the navigation pane.



❶ Toolbar
❷ Navigation Bar
❸ Topic Pane
❹ Navigation Pane

6.1 BEFORE YOU BEGIN

This chapter explains the daily startup procedures. Before doing these procedures:

---

1 Read the OPERATION PRINCIPLES chapter. Using your system is easier if you have a general understanding of how it works.

---

2 Read the SYSTEM OVERVIEW chapter. It contains instructions for

- CYTOMETER CONTROLS AND INDICATORS
- LEARNING THE BASIC OPERATING TECHNIQUES.

---

3 Read each procedure entirely.

---

4 If conditions cause static charge to exist in your lab, be sure to properly ground yourself before touching the instrument.

---

5 Shutdown and restart the system computer once per day to allow the virus protection program to run. See Power the Computer and Cytometer OFF and Power the Computer and Cytometer ON. Do not start a full disk virus scan while running Gallios software.

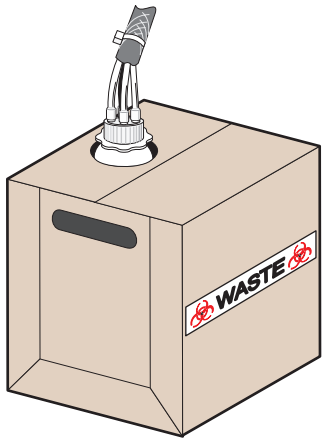
6.2 DAILY STARTUP

Perform the following steps to start up the system. If you have set up CYTOMETER AUTO STARTUP and the Cytometer is running, skip ahead to Additional Start Up Checks.

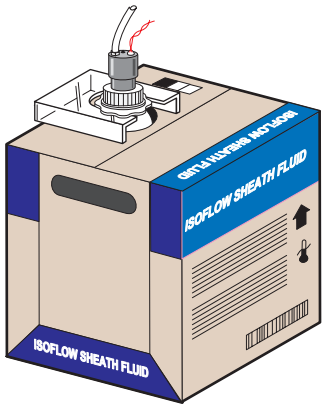
- Check Waste and Reagent Levels
- Power the Computer and Cytometer ON
- Check the Power Supply
- Additional Start Up Checks

Check Waste and Reagent Levels

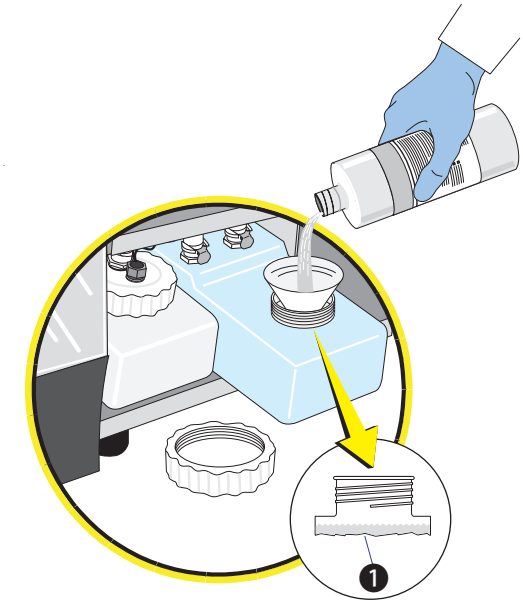
1 Empty the waste container and verify tubing is connected to the cap.



2 Check the sheath fluid level and replace the external sheath fluid container if necessary.



3 Check the cleaning agent fill level ❶ and fill the cleaning agent container if necessary.



Power the Computer and Cytometer ON

Turning On Power

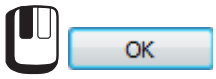
Turn on the system at the computer.

Logging Onto Windows Software

When the **Log on to Windows** screen appears,

a. Select your username icon.

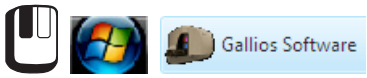
b. Enter your Password and



.

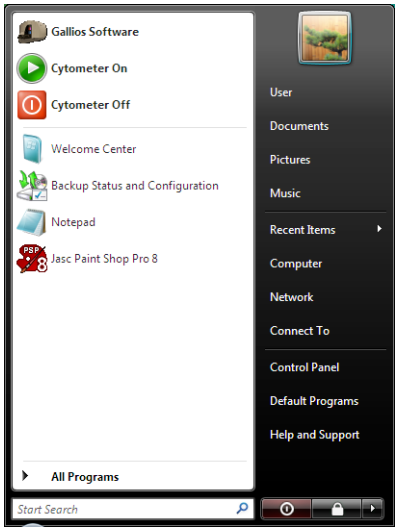
Note: If your computer is part of a network, you may need to enter the User name and Password assigned by your network manager.

Logging Onto Gallios Software

1 

to start the software and power up the Cytometer.

Allow about 40 minutes to warm up the system before performing QC or running samples. Do not start a full disk virus scan while running Gallios software.



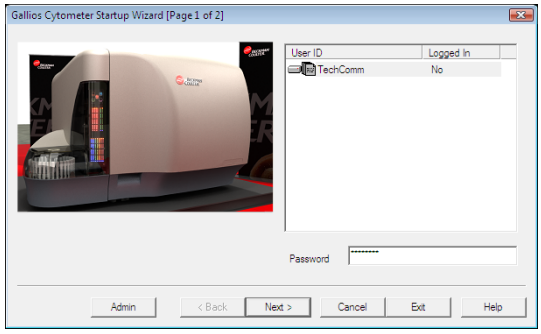
2 At the **Gallios Cytometer Startup Wizard [Page 1 of 2]** screen:

a. Highlight your User ID.

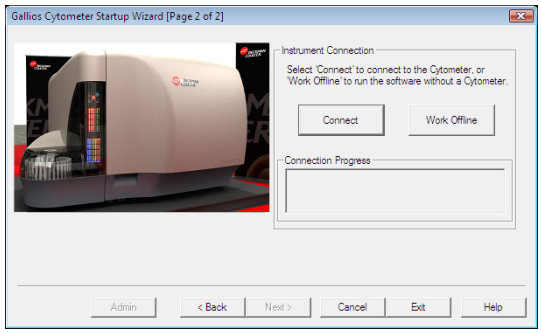
b. Enter your Password.

c. 

Note: For additional information about this screen, see MULTI-USER SIGN ON. If you need to set up User IDs, see User Administration.



3 



4 During system startup, the following series of Cytometer status messages are displayed. The startup cycle includes a prime cycle.

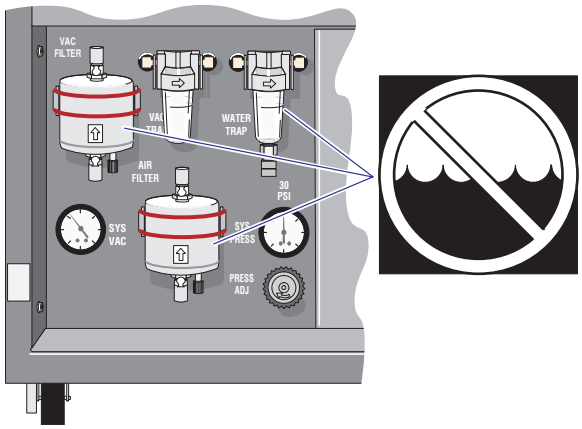
Initialization
Awaiting Sample

Check the Power Supply

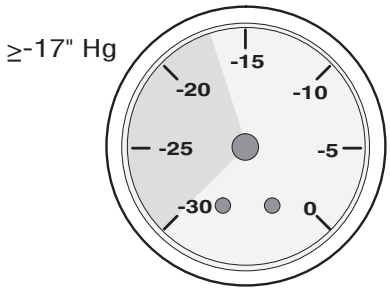
1 Open the Power Supply door and check the WATER TRAP, AIR FILTER, and VACuum FILTER.

Call your Beckman Coulter Representative if:

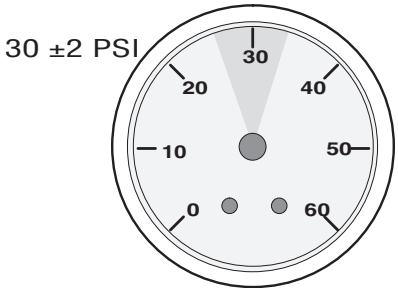
- The TRAP is >1/3 full.
- The FILTERS have any fluid.



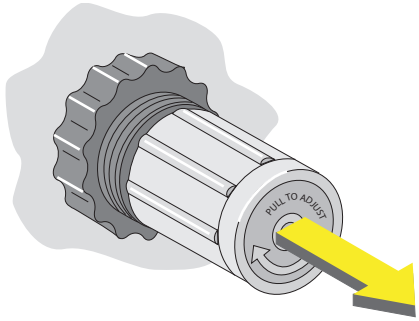
2 Check the SYStem VACuum gauge. If it reads ≥-17 in. Hg, call your Beckman Coulter Representative.



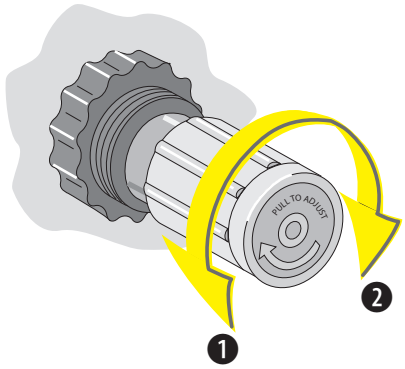
3 Check the SYStem PRESSure gauge. If it does not read between 28 and 32 psi, do the following:



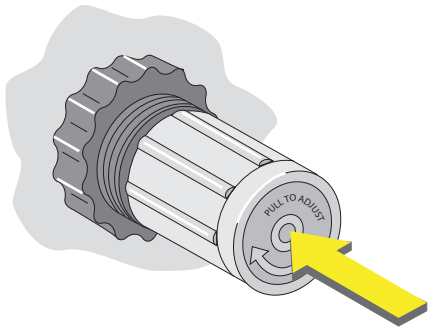
a. Pull the PRESSure ADJuster knob out toward you.



b. Adjust the pressure to 30 ±2 psi.
   - ❶ To decrease, turn to the left.
   - ❷ To increase, turn to the right.

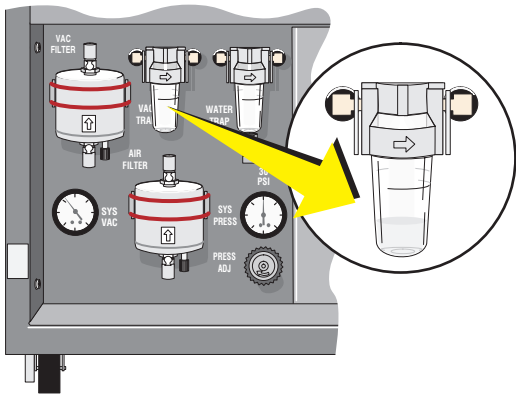


c. Push in on the knob to lock it into place.



4 Check the VACuum TRAP.

If it is >1/4 full of fluid CLEAN THE VACUUM TRAP.



5 Close the Power Supply door.

6 Record the startup checks on the Maintenance Log.

Additional Start Up Checks

1 Check that the MCL vortex function mixes samples by running a blank sample.

2 Refer to the manuals that came with your Printer to:

- Perform Printer diagnostics.
- Check that there is an adequate paper supply in the Printer.
- Check ink cartridges if you have a color Printer and replace if necessary.

6.3 DAILY SHUTDOWN

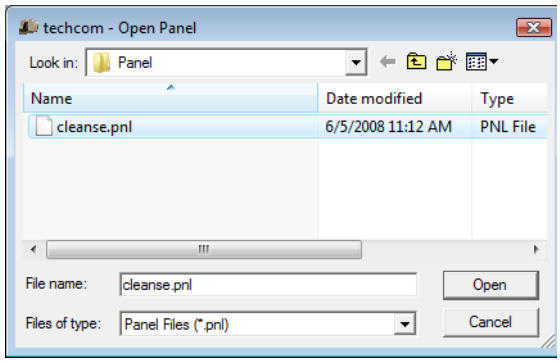
When to Shut Down the Cytometer

- Shut down the instrument at least once a day, even if it is intended for use 24 hours per day.
- Leave the instrument shut down for at least 30 minutes before restarting.

- Shutdown and restart the system computer once per day to allow the virus protection program to run. See Power the Computer and Cytometer OFF and Power the Computer and Cytometer ON. Do not start a full disk virus scan while running Gallios software.

Before Performing Shut Down

1 Perform the Routine Cleaning Procedure.

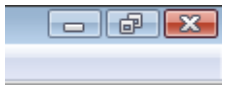


Power the Computer and Cytometer OFF

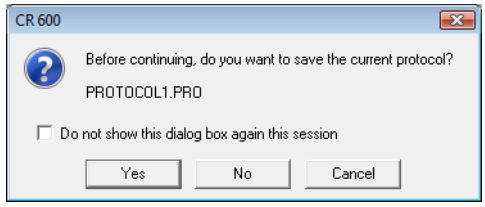
1 Check if the instrument is currently displaying the Idle mode:

- If yes, the message *Press Idle Mode button to initialize* appears at the bottom of the screen. Go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE

2  in all open windows.



3 Yes or No.



4 ▸▸ Cytometer Off to turn off the Cytometer.

5 ▸▸ to shutdown the Workstation.

6 Turn off the monitor and printer separately.

After Instrument Shut Down

1 Wipe down all exposed surfaces with 10% bleach solution and then 70% ethanol. Pay special attention to the Sampling area.

2 Keep the system shut down for 30 minutes. Before running samples, do the daily startup and quality control procedures.

Reminder:

Clean the air filters once a week.

Clean the sample probe and sample head once a week.

Clean the internal sheath fluid container once a month.

Clean the cleaning agent container every 60 days.

3 Record daily shutdown and cleaning on the electronic Maintenance Log.

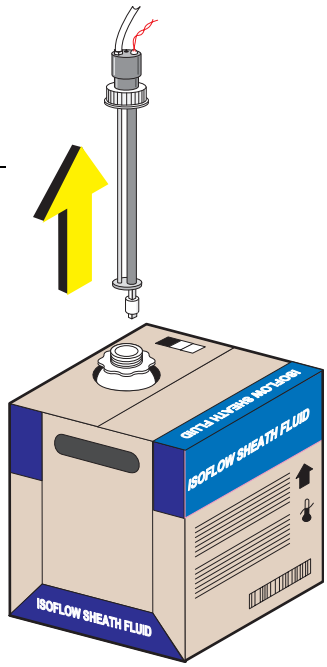
6.4 EXTENDED SHUTDOWN

If you intend to leave the instrument in the shutdown state for an extended amount of time:

1 Check if the instrument is currently displaying the Idle mode:

- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

2 Disconnect the support collar from the external sheath fluid container.

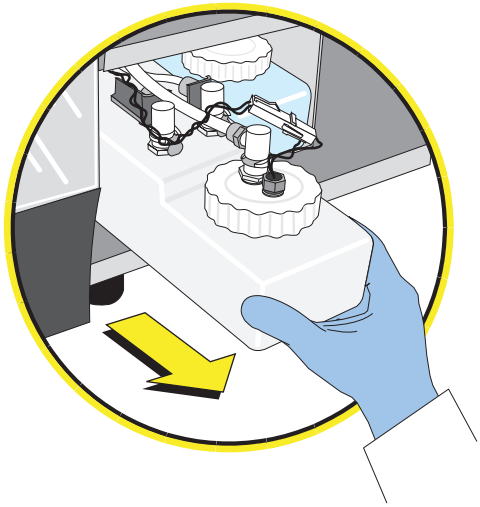


IMPORTANT Misleading results could occur if you contaminate the sheath fluid. Be careful not to contaminate the sheath fluid. Do not let your fingers, paper towels, or other objects touch the pickup tube assembly.

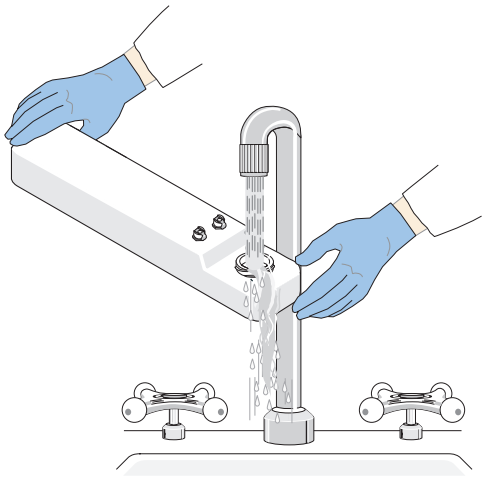
3 Lift the pickup tube assembly straight up and out.

4 Insert the pickup tube assembly into a container of distilled water.

5 Remove the internal sheath fluid and cleaning agent containers.

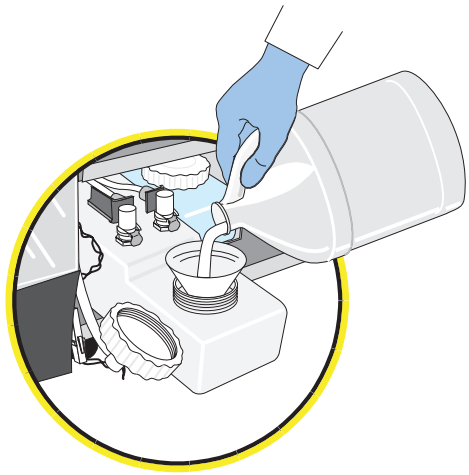


6 Rinse the inside of both containers with water.

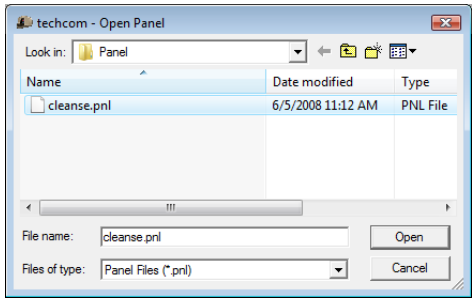


7 Replace the internal sheath fluid and cleaning agent containers. Fill both containers with distilled water instead of reagent.

- Only put 1 L of distilled water into the internal sheath fluid container. Do NOT fill this container. A partially filled container triggers water to be pumped from the external container of distilled water into the lines during this procedure.
- Fill the cleaning agent container with distilled water.

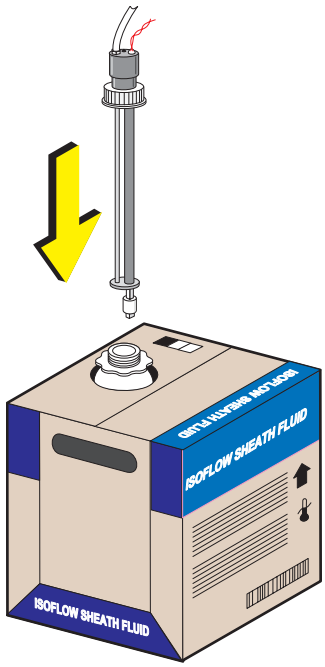


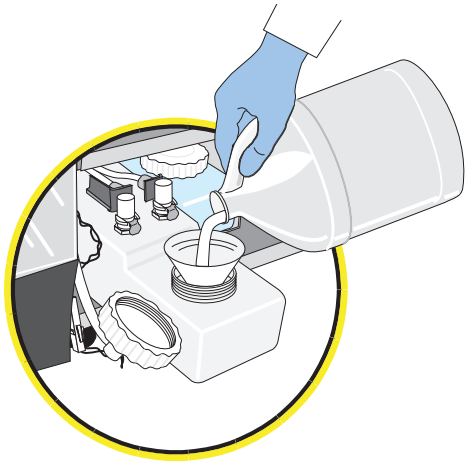
8 Perform the Routine Cleaning Procedure except use tap water in all four tubes. Do not use any bleach or IsoFlow sheath fluid.



9 When you start up the instrument for the first time after the extended shutdown,

- If disconnected, reconnect the pickup tube assembly and tubing to the instrument.
- REPLACE THE 10 L EXTERNAL SHEATH FLUID CONTAINER
- Clean the internal sheath fluid container
- Clean the cleaning agent container
- Fill the internal sheath fluid container with sheath fluid.
- Fill the cleaning agent container with cleaning agent.
- Perform the Routine Cleaning Procedure
- Perform DAILY QC before running samples.



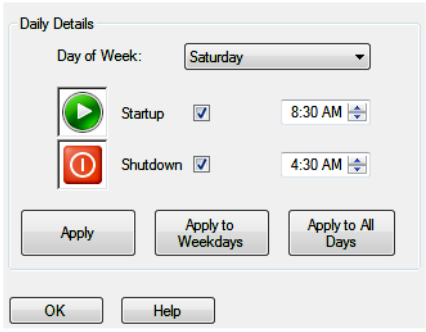


6.5 CYTOMETER AUTO STARTUP

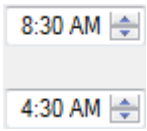
You can set up the system to automatically Startup or Shutdown the Cytometer. The computer must be on with Windows running to allow Auto Startup to run.

1 ▸▸ ▸▸ **All Programs ▸▸ Gallios ▸▸ Cytometer Tasks**

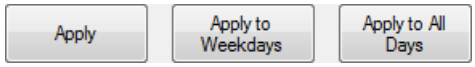
2 ▸▸ Startup ☑ and/or ▸▸ Shutdown ☑



3 Set the desired **Startup** and/or **Shutdown** time.



4 Apply the settings as desired.



5 ▸▸ OK

7.1 INTRODUCTION

Perform the following quality control checks to ensure that your system is working accurately and precisely. The protocols needed for these quality control (QC) procedures are included with your system Software.

In addition to doing the daily quality control procedure in this chapter, you should make a quality control check for the specific applications you are running.

7.2 QC PROCESSES

The chart below shows which QC materials are needed for each QC process.

QC Process	QC Material Used
Verify fluidics and laser alignment	Flow-Check™ Pro Fluorospheres. Verify HPCV versus expected value. Export results to the QC Database and review the QC data.
Adjust high voltage and gain for a given application	Flow-Set™ Pro Fluorospheres. Ascertain target mean position based upon application and adjust high voltage and gain daily to that target. Export results to the QC Database and review the QC data.
Perform absolute counts	Flow-Count™ Fluorospheres.
Adjust color compensation for a given application	For AutoSetup applications, Cyto-Comp™ Cells or whole blood stained with QuickCOMP™ 2 or QuickCOMP 4 kit. Use single color stained samples with each fluorochrome used in your application.
Verify correct settings with an application Control	Update the control protocol with the settings derived from above. Run a biological control equivalent to the application, such as Immuno-Trol™ Cells, Immuno-Trol Low Cells, Cyto-trol™ Control Cells, or a normal whole blood. Export results to the QC Database and review the QC data.

7.3 DAILY QC

Daily QC consists of:

- Preparing AutoSetup Samples
- Running the AutoSetup Scheduler
- Running the AutoSetup II Wizard

IMPORTANT Risk of erroneous results if the Cytometer has been idle for an extended period of time or you have just performed Daily Startup. To ensure correct results, perform a prime after:

- Daily Startup.
- The Cytometer has been idle for an extended period of time.
- You place a new carousel on the MCL and light scatter signals appear abnormal.

Preparing AutoSetup Samples

1 Prepare the Flow-Check Pro fluorospheres Follow the package insert instructions for mixing and handling fluorospheres.

2 Prepare the Flow-Set Pro fluorospheres. The Flow-Set Pro fluorospheres tube is used to set the detector gains and voltages to the required level. Follow the package insert instructions for mixing and handling the fluorospheres.

3 Prepare AutoSetup compensation tubes for each fluorochrome in the application you need to run.

Stain with appropriate single color reagents in each fluorochrome.

4 Prepare an AutoSetup verification tube.

a. Use the appropriate amount of Immuno-Trol Cells or Immuno-Trol Low Cells according to the instructions on the package insert or reconstitute the Cyto-trol Control Cells according to the instructions on the package insert.

b. Stain the cells with the monoclonal antibodies you use for the protocol or panel.

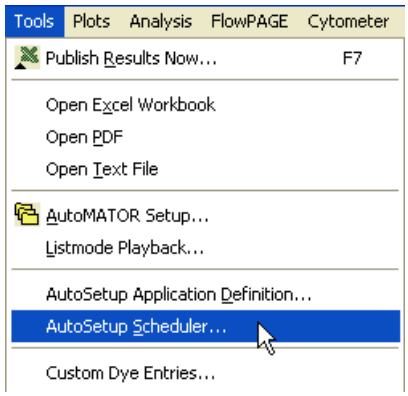
5 Run the AutoSetup Scheduler.

7.4 AUTOSETUP SCHEDULER

Use the AutoSetup Scheduler to select the applications that you need to run on the instrument in a given day or "shift". AutoSetup Scheduler groups the selected applications and provides the carousel load report to facilitate setting up and loading samples for daily QC.

---

1 **Tools ▸▸ AutoSetup Scheduler.**

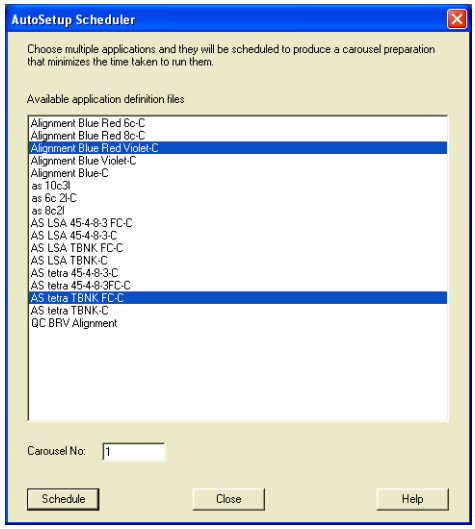


---

2 the applications you want to schedule for a given day or shift.

If you want to schedule an application that does not appear in the list, use the Application Definition Wizard to define the application.

Note: Using the descriptor QC in front of any protocol name appends the results of multiple Flow-Check Pro runs to a single spreadsheet. See Workspace Preferences - Acquisition Options to automatically export data to MS Excel for Quality Control monitoring or basic spreadsheet data entry.

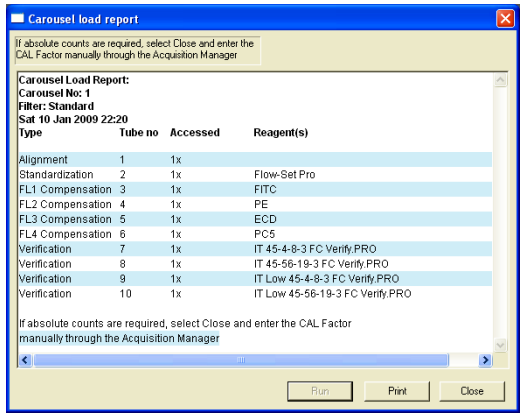


---

3 Enter the Carousel number and Schedule.

4  Print to print the Carousel Load Report.

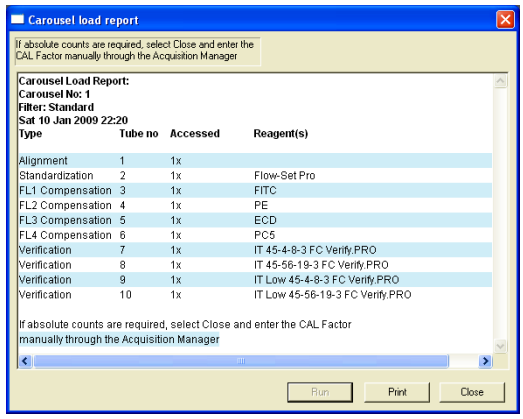
Note: If absolute counts are required,  Close and use the Acquisition Manager to enter the CAL Factor.



5 Place tubes in the carousel in the order shown on the Carousel Load Report.

6 If you entered a CAL Factor in step 4 above,  on the Cytometer Toolbar to run AutoSetup II.

7 If absolute counts are not required,  Run on the Carousel Load Report screen to run AutoSetup II.



7.5 AUTOSETUP II WIZARD

AutoSetup II simplifies and automates application setup and QC monitoring for multiple applications simultaneously. You can perform a QC setup for all of your multicolor applications that you run during a single day or shift at the same time using the Application Definition Wizard and the AutoSetup Scheduler.

IMPORTANT Risk of erroneous results if the Cytometer has been idle for an extended period of time or you have just performed Daily Startup. To ensure correct results, perform a prime after:

- Daily Startup.
- The Cytometer has been idle for an extended period of time.
- You place a new carousel on the MCL and light scatter signals appear abnormal.

Before Running the AutoSetup II Wizard

- Check that the DAILY STARTUP procedure was performed.

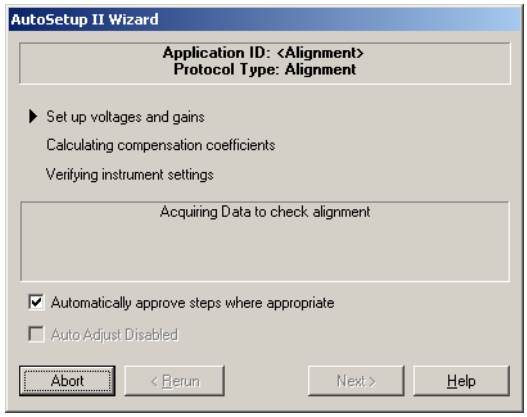
- Allow about 40 minutes to warm up the system.

8 Check the Cytometer status message. Start this procedure when the Cytometer status message *Awaiting Sample* appears. Record any error messages on the electronic Maintenance Log.

- Set up Workspace Preferences - Acquisition Options to print results.
- Set up Workspace Preferences - LMD File Name to specify listmode file names.
- Follow the directions in the Flow-Check Pro fluorospheres package for details on storage and handling.
- Establish Flow-Set Pro fluorospheres target channels for your laboratory and modify the default application definitions if necessary. Follow the directions in the Flow-Set Pro fluorospheres package insert.
- Set up the QC Products that you need to use with AutoSetup applications.
- Set up region statistics exporting and assign QC products to regions.
- If acquisition ends as a result of duration (not stop count) while running AutoSetup II, select ■ on the Cytometer Control toolbar to continue.
- Do not save scheduled AutoSetup Applications as a Worklist. Applications are intended to be "scheduled". Do not add any protocols or panels to Acquisition Manager that has scheduled Applications or AutoSetup II protocols. Always select "New Worklist" after scheduled QC Applications or Autosetup protocols are run.

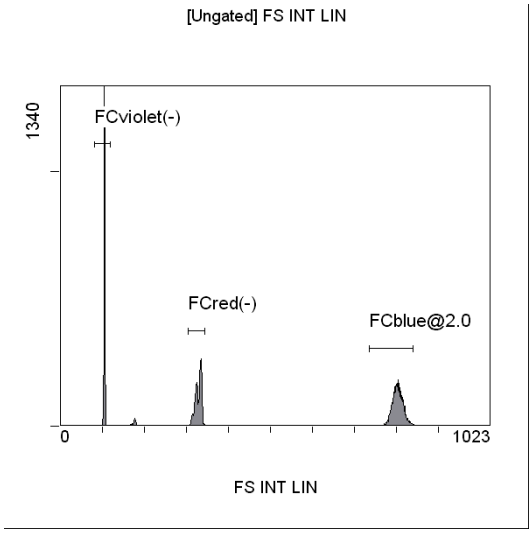
---

1 Run on the Carousel Load Report screen in the AutoSetup Scheduler.



ALIGNMENT APPLICATIONS

2 After data acquisition for the Alignment Application starts, view all histograms to ensure peaks fall within regions.



a. Setup Mode on the Cytometer Control Acquisition Setup Tab.

b. Adjust voltages to place peaks within regions

c. Uncheck Setup Mode.

d. When acquisition of the Alignment stops, check that the AutoSetup II Wizard dialog does not have QC Failed due to the HPCV exceeding the limit identified in each region name.

e. If the HPCVs pass, Finish on the Autosetup Wizard or Next > to continue with Additional scheduled QC applications.

f. If the HPCVs are not within the upper limits identified in the protocol's region name, you may need to < Rerun after performing one or more of the following troubleshooting steps:

   1) Ensure the Flow Rate is LOW

   2) Prime the system

g. If the HPCVs are still not within the upper limits, select Abort and perform the following troubleshooting steps and repeat scheduling and running the alignment application.

   1) Run a Cleanse Cycle

   2) CLEAN THE SAMPLING SYSTEM

STANDARIZATION APPLICATIONS

3 When the _STAND protocol's data acquisition starts, view all histograms to ensure peaks fall within regions.

Note: When running a negative control verify that the AutoGate is around the population of interest in the FS vs SS dotplot. If you need to, adjust populations.

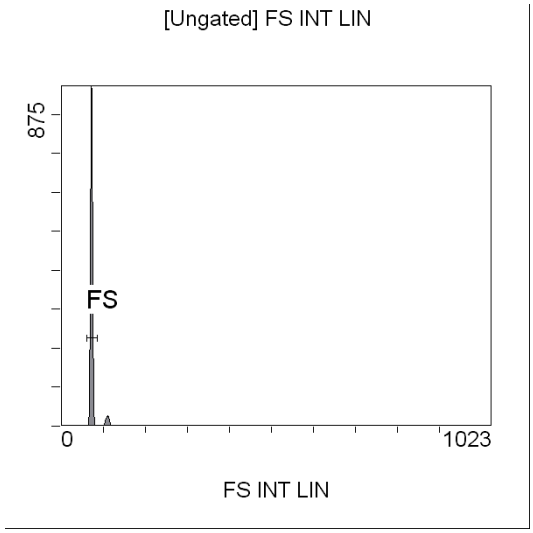
a. **Auto Adjust Disabled** as soon as the AutoSetup wizard displays.

b. adjust voltages to place the lymphocytes in a suitable position.

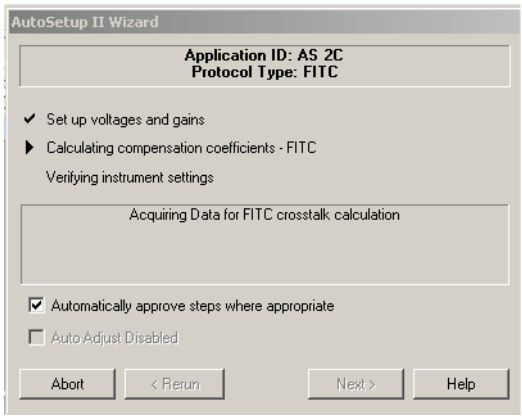
c. adjust voltages to place the fluorescence populations within the first log decade.

d. Acquire.

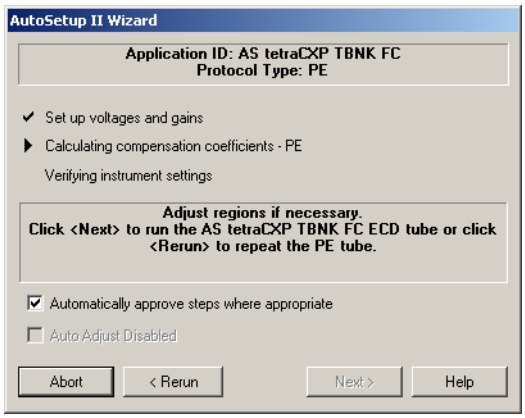
The Wizard adjusts Volts and Gains to place the Flow-Set Pro fluorospheres within the regions. The Wizard dialog box displays a list of the parameters remaining to be adjusted. If the Wizard cannot adjust peaks to the regions within 60 seconds, AutoSetup aborts and the parameters that failed are listed.



The compensation tubes are used to calculate the compensation coefficients required, all these tubes are run in the same way.

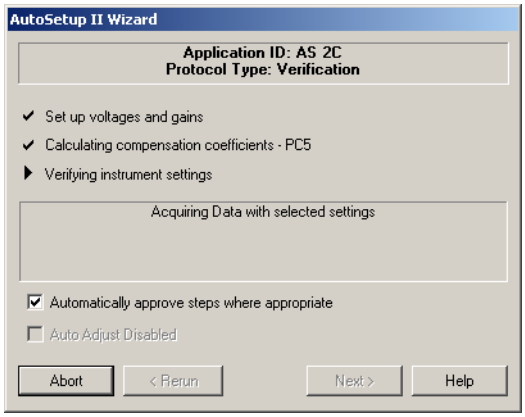


This process is repeated for each of the compensation tubes (for example: FL2, FL3, FL4, and FL5).

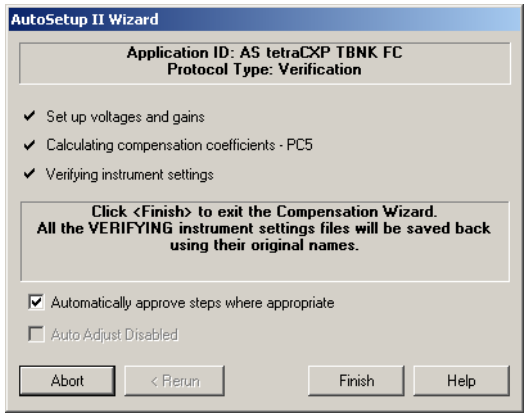


Verification samples are run to ensure the calculated coefficients are satisfactory for the samples and antibodies you are using in your tests.

Note: If multiple applications are scheduled together, samples common to the applications are processed in tandem without dropping the tube. The protocol is displayed during processing.

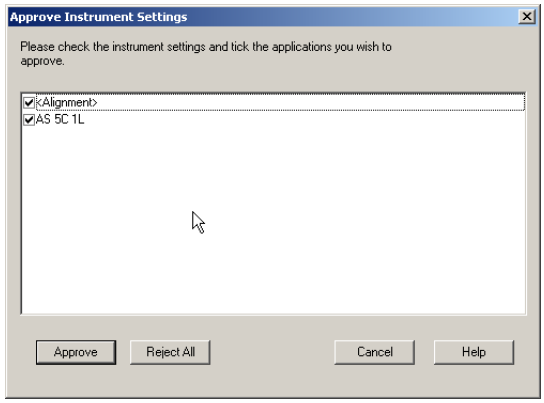


4 Finish to print verification tube results and advance to the approve screen.

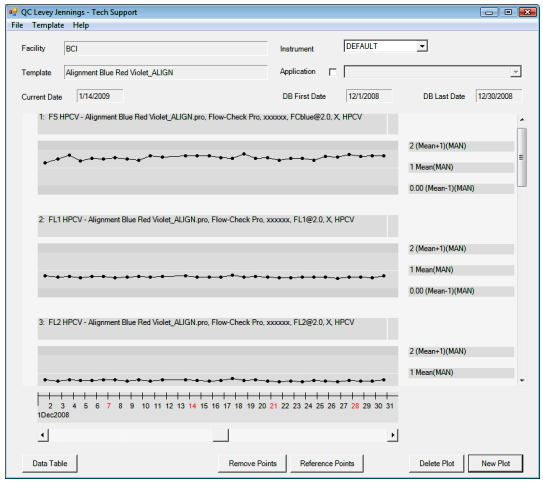


5 Check the printed verification tube results to ensure instrument settings are correct and Approve.

Note: Due to differences between individual instruments, settings files should not be transferred from one instrument to another. If you attempt to use settings files from another instrument, the software displays *Incorrect Cytometer Serial Number.*



6 Review QC Results in the QC Database.



7.6 APPLICATION DEFINITION WIZARD

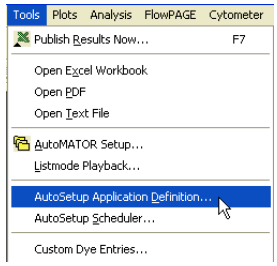
Use the Application Definition Wizard to define your applications and save the definitions for use by the AutoSetup Scheduler. The application definition captures the instrument setup, lasers, parameters, fluorochromes, target channels, verification and alignment requirements of a particular application.

Before Running the Application Definition Wizard

- Establish Flow-Set Pro fluorospheres target channels for your laboratory. Follow the directions in the Flow-Set Pro fluorospheres package insert.
- Ensure that the base protocol you use to define an application contains only the parameters that you use in the application. If it contains extra unused parameters, the compensation matrix will be incorrect.

---

1 **Tools ▸▸ AutoSetup Application Definition.**



---

2 one of the following options:

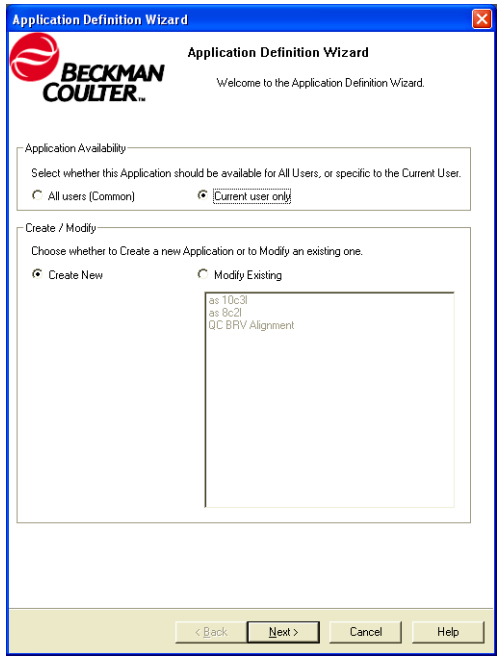
Application available only to current user
Application available to all users (common).

---

3 one of the following options:

Create a new application definition
Modify an existing application definition

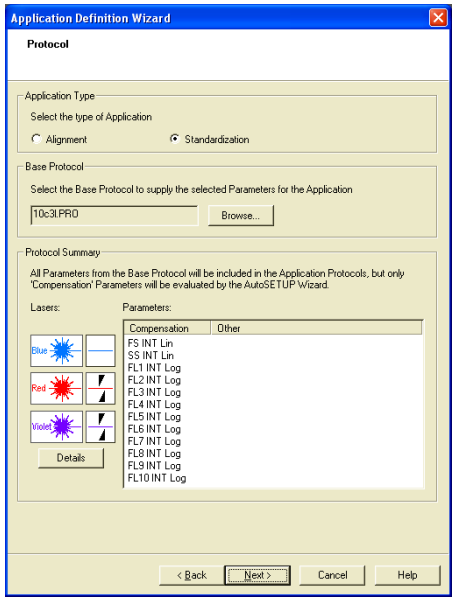
and then Next >.



4 Browse... and select the protocol to use as the base protocol for the application.

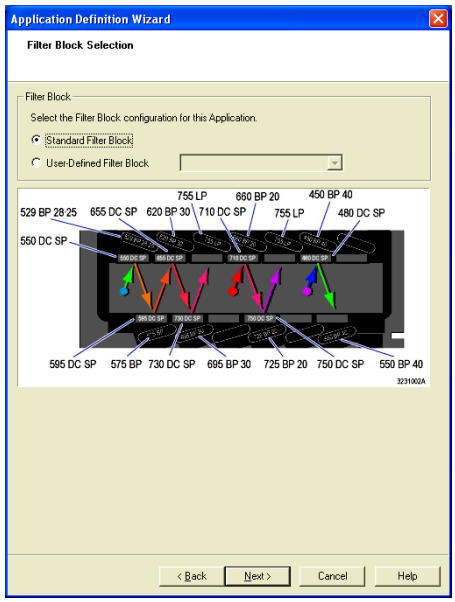
The Protocol Summary displays a description of the parameters and laser setup for the selected protocol.

Next >.

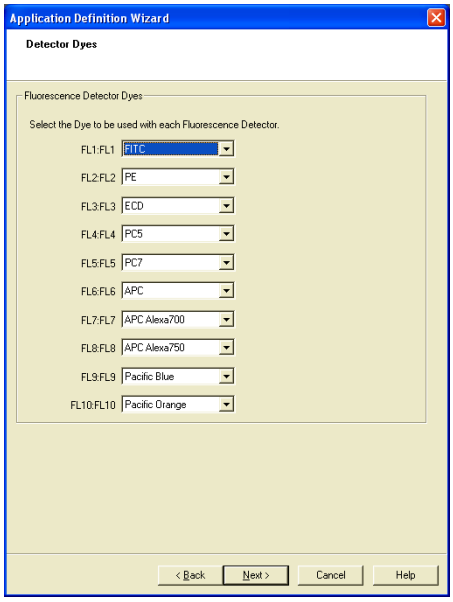


5 the filter block to use for the application and Next >.

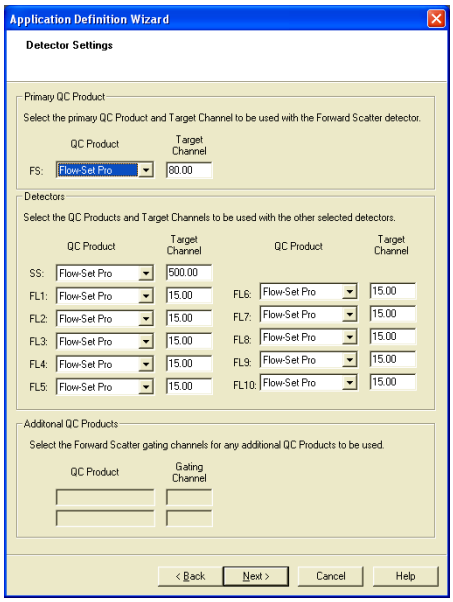
Note: An alignment application does not require a Filter Block selected. Alignment applications must have the appropriate laser selection made. See Cytometer Control Parameter Setup.



6 the dyes you use for each fluorescence detector and Next >.

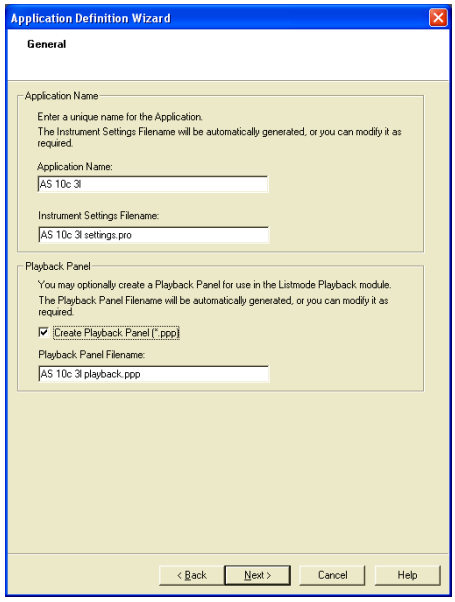


7 Select a QC product and enter a target channel for Forward Scatter, Side Scatter and each fluorescence detector.



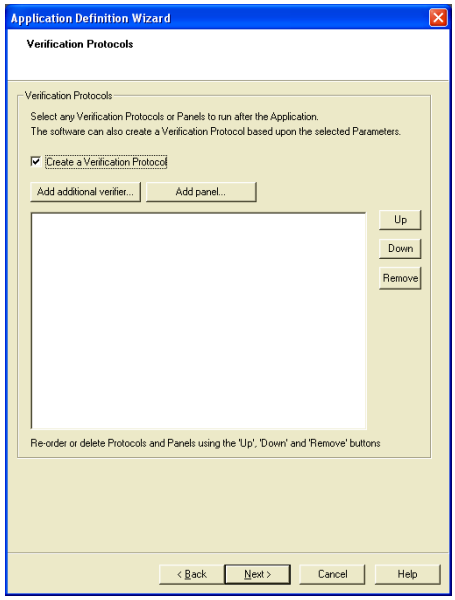
8 Enter names for the application and the instrument settings file and

- Using the nomenclature "AS" as a prefix for the application name will cause the Application Definition Wizard to create the applications' protocols with "AS" as a part of the protocol name. This will readily distinguish qc application protocols from other protocols.
- Selecting the Create Playback Panel (*.ppp) checkbox automatically creates an equivalent panel for the Listmode Playback Tool to create compensation files, if desired.



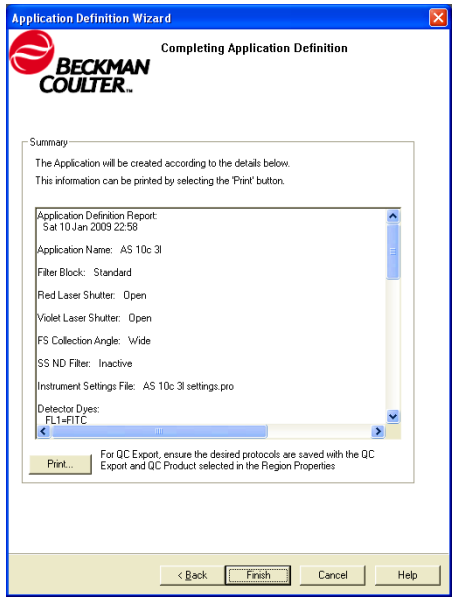
9 Choose to have the wizard create a verification protocol or add a saved verification protocol or panel. Use the buttons to add, remove and reorder multiple verification protocols.

Note: Any panels used for Quality Control must be constructed with unique individual protocols.



10 A summary report is displayed for the application you just defined. The application definition is available for use with the AutoSetup Scheduler.

Next > to print the summary report and Finish to exit the wizard.

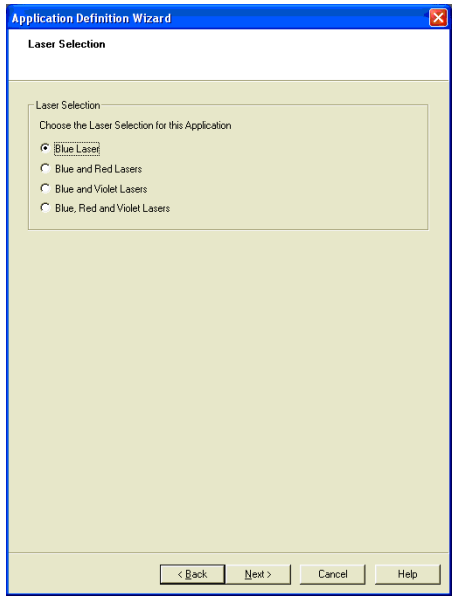


Laser Selection

The Laser Selection page is only displayed for Alignment Applications and replaces the Filter Block Selection page.

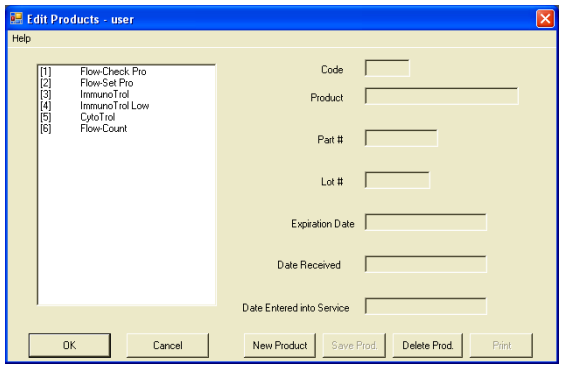
You can select which combination of lasers the Alignment Application is to process. Only Laser Selection combinations appropriate to the current instrument hardware configuration will be enabled.

The selected Lasers will be used by the software to determine the template Alignment Protocol to be used by the Application.



Setting Up QC Products

1 on the Report Generator toolbar to display the Edit Products screen.



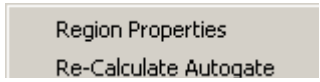
2 Refer to the package insert and enter information about the QC Product in the appropriate fields.

3 Save Prod. and OK .

Exporting Region Results To QC Database and Assigning QC Products

1 on a region to make it active.

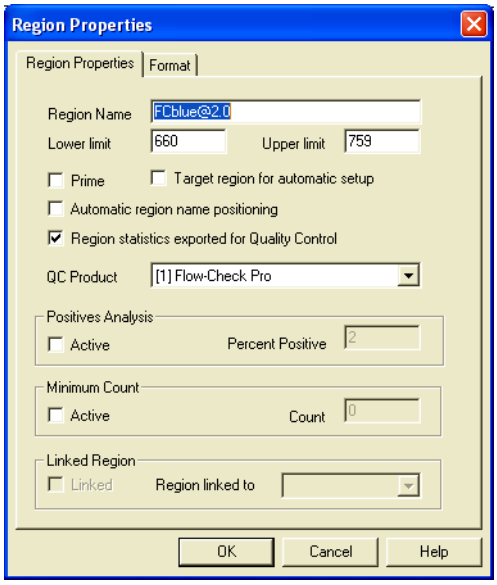
2 Right mouse click on the region and **Region Properties**.



3 Ensure that ☑ **Region Statistics exported for Quality Control**.

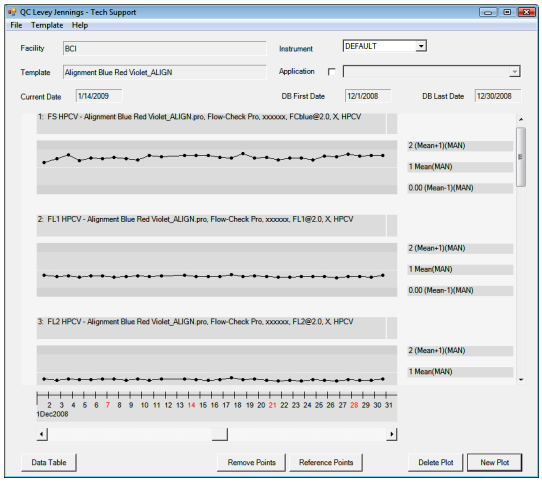
4 next to **QC Product** and select the QC Product to assign to the region.

5 OK and save the protocol.



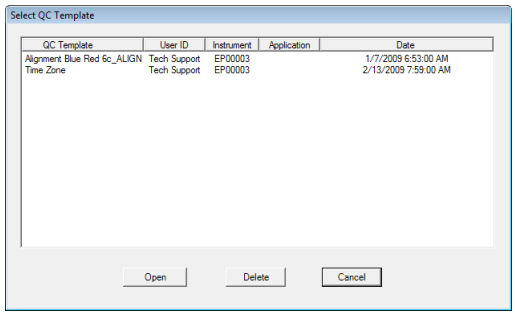
Review QC Results

1 on the Report Generator toolbar to display the QC Levey Jennings screen.



2 next to **Application** and to select an application.

3 **Template ▸▸ Open Template.**



4 the desired template and Open.

5 Repeat steps 2 - 4 to view other QC data plots.

See QC Levey Jennings Screen for information about creating and editing QC plots.

8.1 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, a TQ-Prep workstation combined with a PrepPlus or PrepPlus 2 lets 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.

8.2 BEFORE RUNNING SAMPLES

1 Check that the DAILY STARTUP procedures were done.

2 Check that the DAILY QC procedures were done.

3 Ensure Acquisition Options and LMD file name are set up in Workspace Preferences.

4 Ensure there is sufficient space on your hard drive for sample processing and data acquisition.

IMPORTANT Risk of erroneous results if the Cytometer has been idle for an extended period of time or you have just performed Daily Startup. To ensure correct results, perform a prime after:

- Daily Startup.
- The Cytometer has been idle for an extended period of time.
- You place a new carousel on the MCL and light scatter signals appear abnormal.

IMPORTANT Risk of sample misidentification if a power failure occurs during sample processing. In the event of a power failure, discard any in-process samples.

8.3 RUNNING SAMPLES - MCL AUTOMATIC MODE

Verifying A Worklist

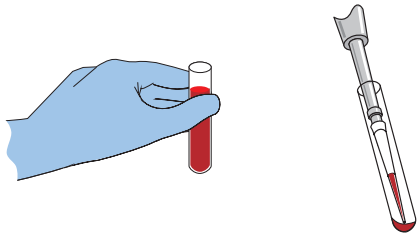
Always verify carousel and tube location fields before starting acquisition and ensure that the Acquisition Manager information accurately reflects the tubes in the carousel being run. The order of the tubes in the carousel must match the order of the tubes in the Worklist.

The default protocol (Ctrl N) must be saved prior to being used in the Worklist. If the default protocol is not saved with a specific name prior to using it, that tube may be run with unexpected protocol settings depending on the Save Protocol selection in Workspace Preferences. Also, the default protocol name will default to "setup.def" in any associated panel template and have no regions.

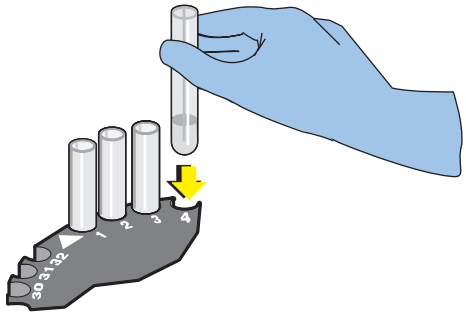
IMPORTANT When editing and saving a protocol or panel, any worklist containing the edited protocol or panel must be recreated. If the worklist is not re-created, the protocol or panel in the worklist may not match the newer version of the protocol or panel.

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

1 Prepare samples according to the reagent package insert.

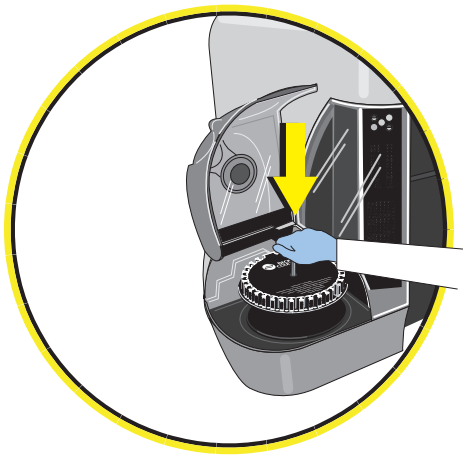


2 Place the sample tubes in a carousel.

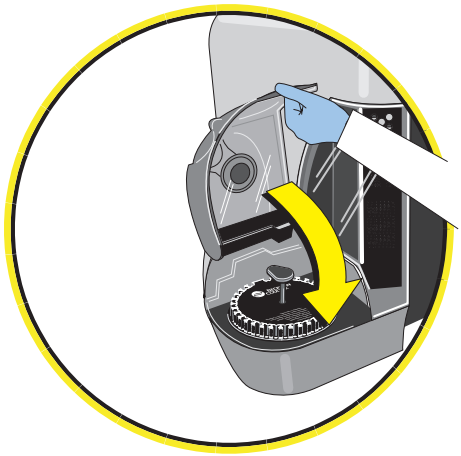


WARNING Risk of injury. Do not open the MCL cover while the MCL is moving. To avoid injury, wait until the MCL stops moving before opening the MCL cover.

3 Open the MCL cover and place the carousel on the MCL.



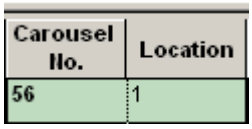
4 Close the MCL cover.



5 Use the Acquisition Manager Toolbar or Drag And Drop to select the Worklist, Panel or Protocol you want to use.

Note: If you load a Panel or Worklist that contains a deleted Protocol, the software prompts you to use the New Panel Wizard to correct the problem. Panel names for the corrected protocols are not displayed in the Worklist however they are displayed on the Panels tab.

6 Enter the carousel ID number and tube location in the Acquisition Manager.



7  to begin processing samples.

During the sample cycle the following series of Cytometer status messages appears:

Awaiting Sample

Preparing Sample

Acquiring

Stopping.

8 Observe the Events/Sec counter on the Status Bar to monitor data acquisition.

Note: While running a Worklist and using bar codes on your sample tubes, if the Tube ID in the Worklist does not match the bar code on the tube, a system message appears. After you acknowledge the message, the mismatched tube is aborted and the processing stops for the rest of the carousel.

9 Verify all sample IDs before reporting results Review all data plots and results, including exported results, prior to reporting results.

IMPORTANT Risk of reporting incorrect results. Data displays for light scatter patterns, antibody staining profiles, and all gates and boundaries used to arrive at the test result should be reviewed by a laboratory professional when interpreting the data. If results are suspect, follow your laboratory procedures to resolve.

10 Print Patient Panel Report, if needed. See Panel Report.

8.4 RUNNING SAMPLES - MCL MANUAL MODE

MCL Manual Mode operates the same as MCL Automatic Mode except the MCL stops after processing each tube to allow you to enter the location of the next tube to process.

The MCL Manual Mode works from a systematic processing of the Worklist in Acquisition Manager by starting with the sample identified in Row 1, then moving to the sample identified in Row 2, Row 3 etc. Although the tube location may be edited, the order in which sample processing takes place is always based on the sequential rows in Acquisition Manager. With a partially completed worklist, the next sample to be processed will always be the next row in the Worklist that has not been run (completed rows display in blue in Acquisition Manager).

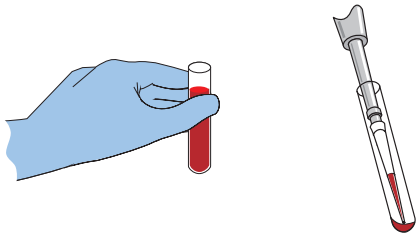
The sequence of processing samples using the MCL Manual Mode is as follows:

- The Location field in Acquisition Manager auto-fills when the Carousel Number is entered.
- When MCL Manual Mode is selected, the 'Enter Next Tube Location to process' dialog displays.
- The 'Enter Next Tube Location to process' dialog's “Tube Location” field allows a TUBE LOCATION to be chosen for the selected TEST identified in the row that the arrow is pointing to.
- The selected test (row identified by the arrow) cannot be altered at this time.

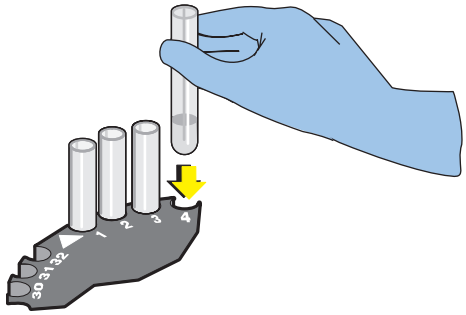
The rules of sample identifiers and listmode file names will always persist when processing samples regardless of tube location defined. Therefore, prior to manually changing the tube location, ensure the sample identifiers match the listmode description intended for each specimen.

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

1 Prepare samples according to the reagent package insert.

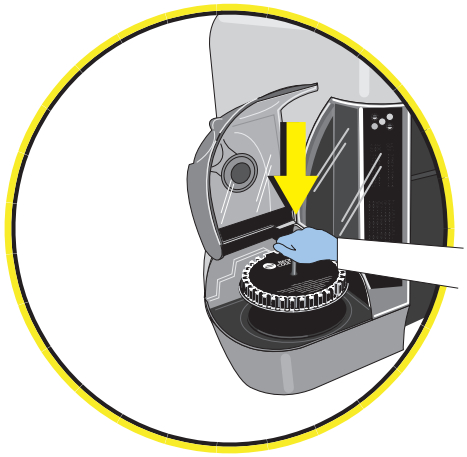


2 Place the sample tubes in a carousel.

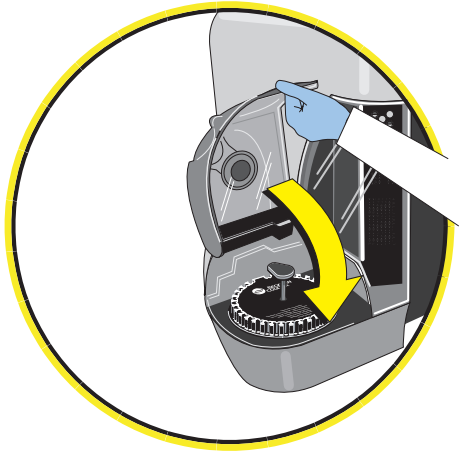


WARNING Risk of injury. Do not open the MCL cover while the MCL is moving. To avoid injury, wait until the MCL stops moving before opening the MCL cover.

3 Open the MCL cover and place the carousel on the MCL.



4 Close the MCL cover.



5 To stop after processing each tube and edit the Sample ID,

 **Edit Sample IDs** on Workspace Preferences - Acquisition Options.

6 Use the Acquisition Manager Toolbar or Drag And Drop to select the Worklist, Panel or Protocol you want to use.

Note: If you load a Panel or Worklist that contains a deleted Protocol, the software prompts you to use the New Panel Wizard to correct the problem. Panel names for the corrected protocols are not displayed in the Worklist however they are displayed on the Panels tab.

7 Enter the carousel ID number in the Acquisition Manager.

Carousel No.	Location
56	1

8  to place the Cytometer in MCL Manual Mode.

9  to begin processing samples.

10 Enter the tube location to process and

.

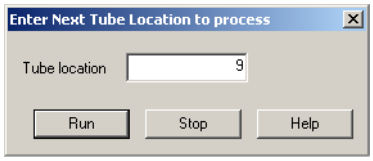
During the sample cycle the following series of Cytometer status messages appears:

Awaiting Sample

Preparing Sample

Acquiring

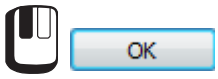
Stopping.



11 Observe the Events/Sec counter on the Status Bar to monitor data acquisition.

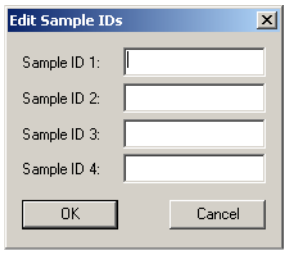
Note: While running a Worklist, if the Tube ID in the Worklist does not match the bar code on the tube, a system message appears. After you acknowledge the message, the mismatched tube is aborted and the processing stops for the rest of the carousel.

12 If you enabled **Edit Sample IDs** on Workspace Preferences - Acquisition Options, edit the sample ID after the tube finishes processing and



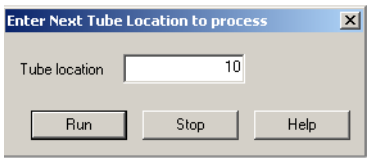
.

Note: After the first tube in a panel, Sample ID 1 cannot be changed.



13 Enter the location of the next tube to process and

.



14 Repeat steps 12 and 13 to process additional tubes.

15 Verify all sample IDs before reporting results. Review all data plots and results, including exported results prior to reporting results.

IMPORTANT Risk of reporting incorrect results. Data displays for light scatter patterns, antibody staining profiles, and all gates and boundaries used to arrive at the test result should be reviewed by a laboratory professional when interpreting the data. If results are suspect, follow your laboratory procedures to resolve.

16 Print Patient Panel Report, if needed. See Panel Report.

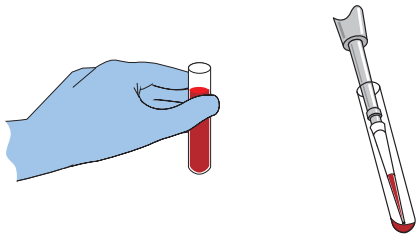
8.5 RUNNING SAMPLES - SINGLE TUBE MODE

The Single Tube Mode operates by introducing tubes individually into the MCL through the MCL Tube Access door.

Note: When operating in the Single Tube Mode, the Prime function is not available when the system is between samples, even though the toolbar is active.

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

1 Prepare samples according to the reagent package insert.



2 If you want the MCL to stop after processing each tube to allow you to edit the Sample ID,  **Edit Sample IDs** on Workspace Preferences - Acquisition Options.

3 Use the Acquisition Manager Toolbar or Drag And Drop to select the Worklist, Panel or Protocol you want to use.

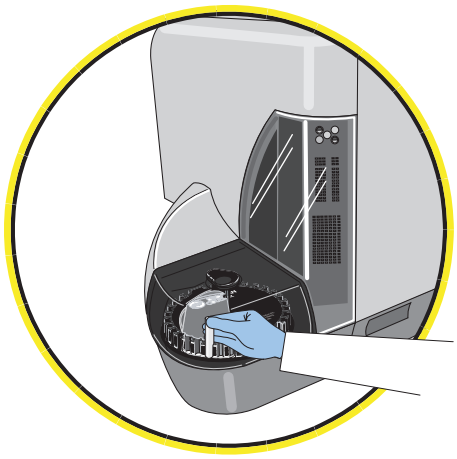
Note: If you load a Panel that contains a deleted Protocol, the software prompts you to use the New Panel Wizard to correct the problem.

Note: If you load a Panel or Worklist that contains a deleted Protocol, the software prompts you to use the New Panel Wizard to correct the problem. Panel names for the corrected protocols are not displayed in the Worklist however they are displayed on the Panels tab.

4  to place the Cytometer in Single Tube Mode. The MCL moves to put carousel position 21 under the Tube Access door.

WARNING Risk of injury. Do not open the MCL cover while the MCL is moving. To avoid injury, wait until the MCL stops moving before opening the MCL cover.

5 Open the MCL Tube Access door and place the sample tube in carousel position 21.



6 Close the MCL Tube Access door and  to begin processing samples.

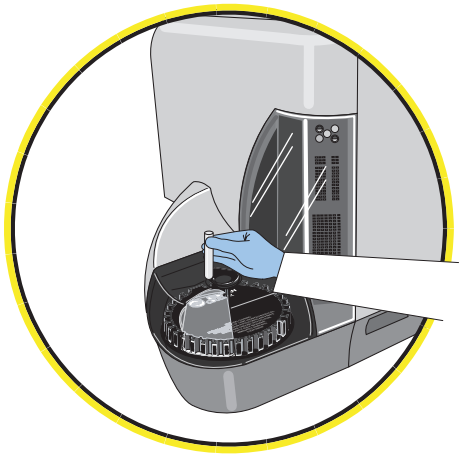
During the sample cycle the following series of Cytometer status messages appears:

Awaiting Sample

Preparing Sample

Acquiring

Stopping.



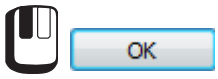
7 Observe the Events/Sec counter on the Status Bar to monitor data acquisition.

Note: While running a Worklist, if the Tube ID in the Worklist does not match the bar code on the tube, a system message appears. After you acknowledge the message, the mismatched tube is aborted and the processing stops for the rest of the carousel.

8 Verify all sample IDs before reporting results. Review all data plots and results, including exported results prior to reporting results.

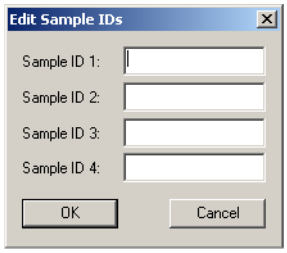
IMPORTANT Risk of reporting incorrect results. Data displays for light scatter patterns, antibody staining profiles, and all gates and boundaries used to arrive at the test result should be reviewed by a laboratory professional when interpreting the data. If results are suspect, follow your laboratory procedures to resolve.

9 If you enabled **Edit Sample IDs** on Workspace Preferences - Acquisition Options, edit the sample ID and



.

Note: After the first tube in a panel, Sample ID 1 cannot be changed.



10 Repeat steps 5 through 9 to process additional tubes.

to exit Single Tube Mode and return to Automatic Mode.

11 Print Patient Panel Report, if needed. See Panel Report.

9.1 SOFTWARE PROCEDURES

For system help, click on the links below to go to the detailed procedure.

These procedures are in the System Overview chapter.

- CREATING PROTOCOLS
- CREATING REGIONS
- CREATING GATES
- CREATING FLOWPAGES
- CREATING PANELS
- CREATING WORKLISTS

These procedures are in the Quality Control chapter.

- AutoSetup II Wizard
- Application Definition Wizard (define all your applications)
- AutoSetup Scheduler (multiple application AutoSetup)

These procedures are in the Using Gallios Software chapter.

- Batch AutoMATOR
- Advanced Color Precedence
- Listmode Compensation
- Setting a CAL Region

9.2 HARDWARE PROCEDURES

For system help, click on the links below to:

- Clean the MCL sample head and the sample probe - procedure.
- Replace the sample probe and sample pickup tubing - procedure.
- Replace the MCL sample head - procedure
- Replace an optical filter - procedure

10.1 WELCOME TO GALLIOS SOFTWARE

This exciting new software, designed in response to customer feedback, is simpler, faster and more reliable, allowing you to obtain the best from your Cytometer.

Gallios Software can analyze a wide variety of listmode files.

10.2 MULTI-USER SIGN ON

Overview

When you start Gallios software, the Cytometer Startup Page 1 is displayed. If a System Administrator has been configured on your system, skip to Cytometer Startup Page 1, if not, see Configure System Administrator.

Configure System Administrator

On the initial Startup after installation, there needs to be at least one System Administrator configured however it is recommended that more than one System Administrator is configured. The default Admin Password is password. Users must be set up by the Administrator.

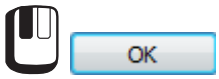
---

1 Log in as Admin.

a. 

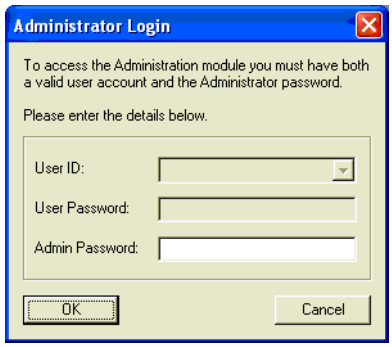
on Cytometer Startup Page 1.

b. Enter the Admin Password.

c. 

.

Note: It is vital that the default Admin Password is changed as soon as possible by using the User Profile – User ID option within the User Administration screen. The Admin password can be set to a previously used password.

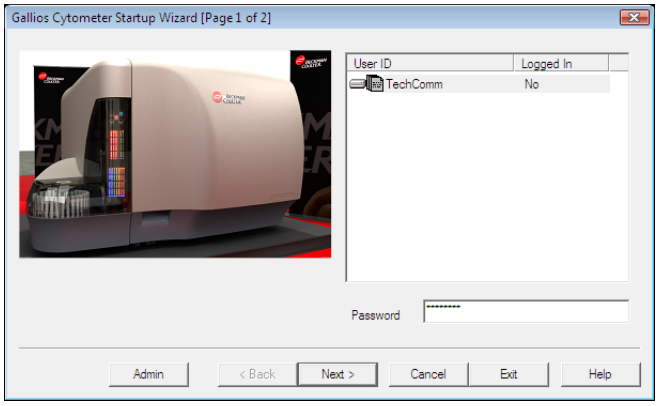


---

2 Add a User and assign them System Administrator Privileges using the Add User button and the instructions in User Profile – User ID.

Cytometer Startup Page 1

User ID
Logged In
Password
Admin Button
Next Button



Displayed are the User IDs of all those who have access rights to Gallios software and whether they are logged in to the system or not.

If you have been previously set up on the system, highlight your User ID and enter your Password. If you do not have a User ID and Password, see your System Administrator.

Note: If a red X appears next to your User ID (techcomm)

- Ensure the pathway for your user directory is,
  - not a network drive if the network connection is not met.
  - not a CD-ROM drive.

Ensure all of your user folders are intact. If any of the following folders are missing from your user directory, use Windows Explorer to create the missing folders.

- AcquisitionProtocol
- AnalysisProtocol
- HST
- HTML
- Images
- LMD
- Panel
- PDF
- Results
- Worklist

To access the administration tools, Admin and enter the Password. See User Administration. Admin is for administrative functions only. You cannot log in to Gallios software with the admin button.

User ID

This lists all the Users that are allowed access to the system. User IDs are a minimum of 3 characters and a maximum of 24 characters. If your name is not included, see your System Administrator.

Logged In

Indicates whether or not the Users are logged in.

Password

Enter your User Password. Passwords are a minimum of 6 characters and a maximum of 24 characters. Blanks are not allowed.

Admin Button

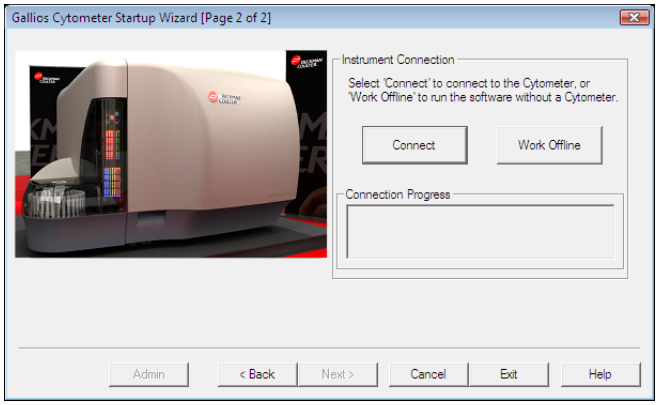
Allows access to the User Administration Tools, which allow the addition, removal and modification of User profiles and Workgroups plus the creation of Export Logs.

Next Button

Click this option to accept the current logged-in name and move to Page 2.

Page 2

Connect
Work Offline



Connect

Initializes connection to the Cytometer. If all steps in the connection process are successful, the Gallios software is launched.

Work Offline

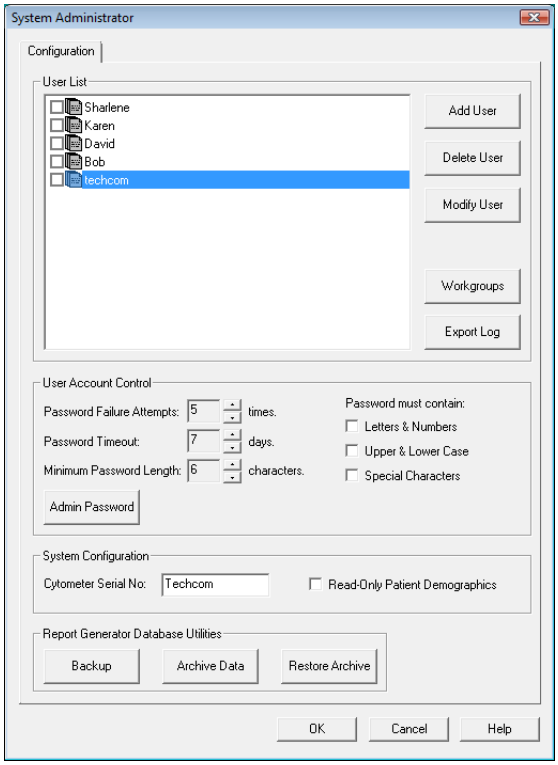
Launches the Gallios software without connecting to the Cytometer. The software is configured according to the last instrument configuration to which the workstation was connected.

10.3 SIGN-ON ADMINISTRATION

User Administration

This screen allows the Administrator to add, remove and modify User profiles, including passwords as well as the creation of user Workgroups and the creation of Export Logs.

System Administrator Screen



Add User

Use this button to add a new user. See User Profile – User ID.

Delete User

IMPORTANT Deleting a User ID can cause loss of data. If you delete a User ID all the directories and files including listmode files that are in your Windows desktop under that User ID are deleted. Before deleting a User ID be sure to move or copy all needed directories and files for that User ID to different directories.

Note: At least one folder must be selected for deletion in order for the User to be deleted. If the User is a part of a workgroup, remove the User from the Workgroup prior to deleting the User.

Highlight the User ID you wish to delete from the system and  Delete user. A confirmation dialog box is displayed. Follow the screen prompts to indicate which folders you want to delete or retain. Choose **Yes** to delete the user or **No** to revert to the User Access Control dialog box.

Modify User

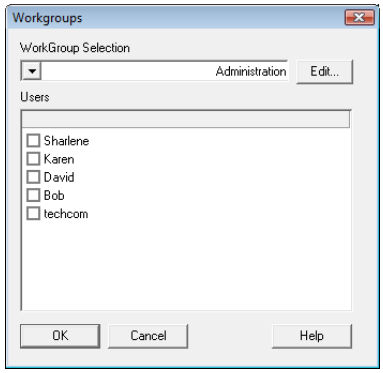
Use this button to modify an existing user. See User Profile – User ID.

Workgroups

Selecting the Workgroup option allows the Administrator to set up various Workgroups of your choice depending upon your requirements. Choose any names to suit your requirements and then enable the checkbox next to the names of users you wish to add to the Workgroup.

Workgroup Selection
Users
Edit Button

Within the display, ALL Users assigned to the system are shown BUT only those within the particular Workgroup shown in the Workgroup Selection box have the checkbox beside the User ID enabled.



See also:
User Administration

Workgroup Selection

Displays the current Workgroup. Select the drop down list box to display and select other Workgroups.

Users

Lists ALL the Users assigned to the system. Those with the checkbox activated by their User IDs are members of the particular Workgroup shown in the **Workgroup Selection** box. Users can be assigned to multiple Workgroups. To add a user to the Workgroup, click to enable the checkbox. To remove a user from the Workgroup, click to disable the checkbox.

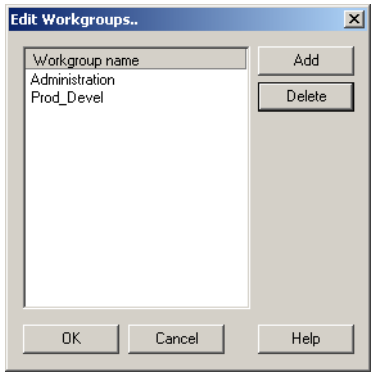
Edit Button

Opens the **Edit Workgroups** screen.

Edit Workgroups

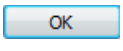
Use this screen to Add or Delete specific Workgroup names.

Add
Delete



Add

to add a new Workgroup to the system. Type the new Workgroup name in the Text Edit box and  

.

Delete

Highlight the appropriate Workgroup from the list displayed and  

to delete a particular Workgroup from the system. To edit the name of a particular Workgroup, first delete the Workgroup and then add it as a "new" Workgroup.

See also:
Workgroups
User Administration

Export Log

Select Export Log to create a text log file showing the date and time each User has spent logged on to Gallios software.

---

1 Log in as Admin.

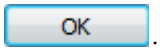
a.  

on Cytometer Startup Page 1.

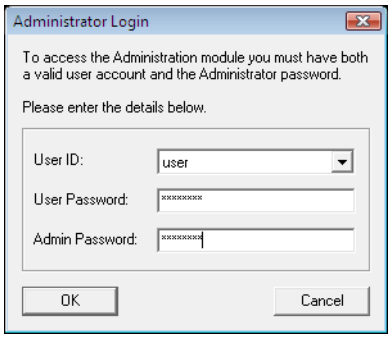
a. Select a User ID from the pull down list.

a. Enter the password for that user.

b. Enter the Admin Password.

c.  

.

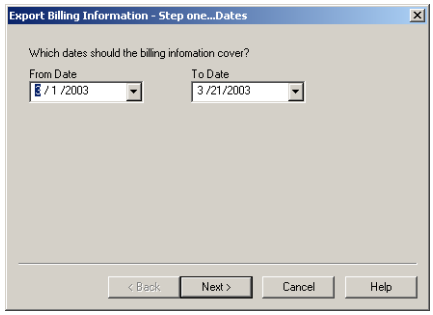


---

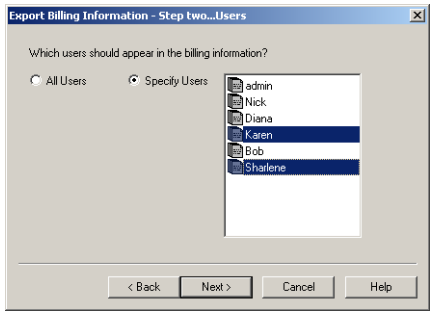
2  

.

3 Enter the date range for the log and 

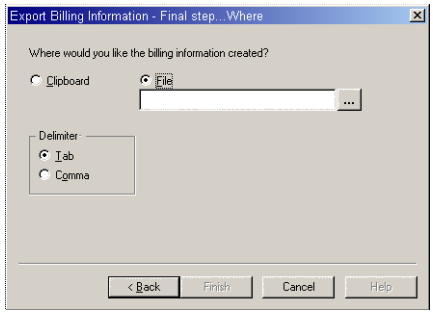


4 Select the users to include in the log and 



5 Choose to direct the information to the **Clipboard** or to a **File**. If **File** is selected, you can either enter a valid File Name in the box or use ... to select an appropriate file.

Select between **Tab** delimited (MS Excel default) or a **Comma** delimited text format file.



6 

to end.

See also:
User Administration

User Account Control

The System Administrator specifies the following user password parameters,

- Password Failure Attempts
- Password Timeout
- Minimum Password Length
- Password Must Contain
  - Letters & Numbers
  - Upper & Lower Case
  - Special Characters

If a network user is unable to log into Gallios software, use the DESkey Configuration (Start ▸▸ Control Panel) utility to check for duplicates of the user log in.

System Configuration

Enter the serial number of the Cytometer connected to the Workstation. You must be logged on as Administrator to enter the serial number. If the Read-Only Patient Demographics is checked then the Database Information screen will be set to read only.

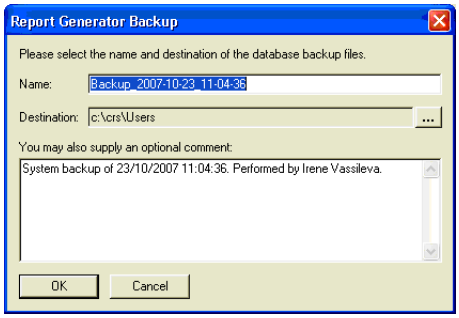
Report Generator Database Utilities

Use these controls to Backup the Report Generator Database, Archive Data or Restore an Archive. To Archive or Restore data see DATABASE MANAGEMENT.

Backup

Name
Destination
Comment

Once the backup has finished, a message is displayed showing the full path and filenames of the newly created Backup file.



Name

Specify a name for the Report Generator Backup. This name will be used to generate the filenames of the backup file and the txt file containing the comments created by the Report Generator Backup. The default name is based on the current date and time and is in the format Backup_YYYY-MM-DD_HH-MM-SS. The extension for Backup comment file is txt and for report generator data backup it is bak.

Destination

The default destination is your user directory in the Gallios installation folder. You can change it via the browse button. You can not type into the edit box.

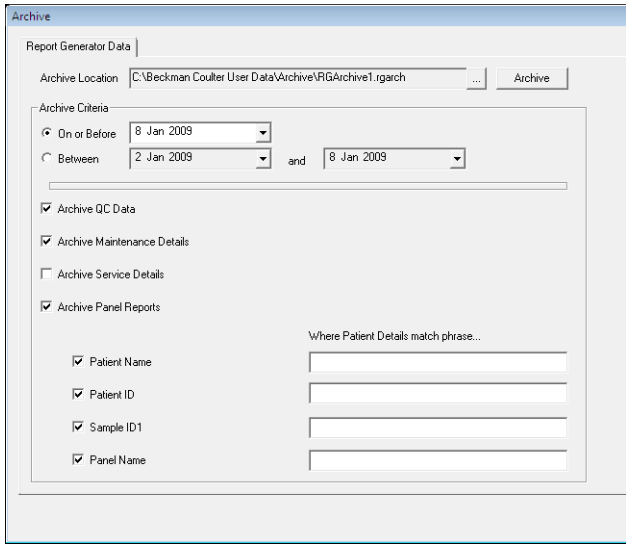
Comment

The comment is optional. The comment is saved in a text file associated with the database Backup files.

Archive Data Screen

Only information stored in the Report Generator / QC database is archived via the Archive screen.

Archive Location
Archive Criteria On or Before [Date]
Archive Criteria Between [Date] and [Date]
Archive QC Data
Archive Maintenance Details
Service Maintenance Details
Archive Panel Reports
Patient Name
Patient ID
Sample ID1
Panel Name



Archive Location

Specify the destination and filename for the new Archive file. This can be any local hard drive or network directory. The Archive Location field is automatically be populated with a default filename for the new Archive. The default directory is the Gallios installation directory 'Archive' subdirectory. The default filename is RGArchiveXX.rgarch where 'XX' is an automatically incrementing number.

Archive Criteria On or Before [Date]

Set the Archive Criteria to Archive data which was written on or before the selected Date. The clicking the Date field allows you to specify the Date using the Date Selection control.

Archive Criteria Between [Date] and [Date]

Set the Archive Criteria to Archive data which was written on or between the two selected Dates. The first Date must always be prior to, or the same as, the second Date. The default value for the 'To' field is 23:59:59 on the current date. Only the current date is shown in the control, however the time is used to ensure that all of today's activities are included. The default value for the 'From' field is 00:00:00 on the date six days before the current date. These values give a default range of seven whole days, which includes the current day. Clicking the Date fields allows you to specify the Dates using the Date Selection control.

Archive QC Data

Set the Archive Criteria to include QC Data. The QC Data is archived according to the selected Dates.

Archive Maintenance Details

Set the Archive Criteria to include Maintenance Details. The Maintenance Details are archived according to the selected Dates.

Service Maintenance Details

Set the Archive Criteria to include Service Details. The Service Details are archived according to the selected Dates.

Archive Panel Reports

Set the Archive Criteria to include Panel Reports. The Panel Reports are archived according to the selected Dates and may be further filtered by selecting one or more of the following Panel Report-specific criteria.

Patient Name

Only Panel Reports for patients whose last name contains the phrase entered into this field are included in the archive. All printable characters are allowed as input.

Patient ID

Only Panel Reports for patients who's ID contains the phrase entered into this field are included in the archive. All printable characters are allowed as input.

Sample ID1

Only Panel Reports for which the Sample ID1 contains the phrase entered into this field are included in the archive. All printable characters are allowed as input.

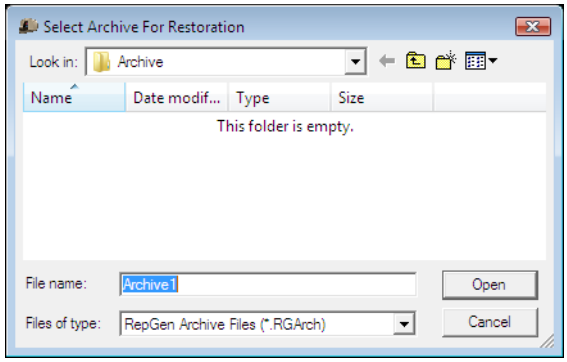
Panel Name

Only Panel Reports which were run for Export Panels whose name contains the phrase entered into this field are included in the archive. All printable characters are allowed as input.

Restore Archive

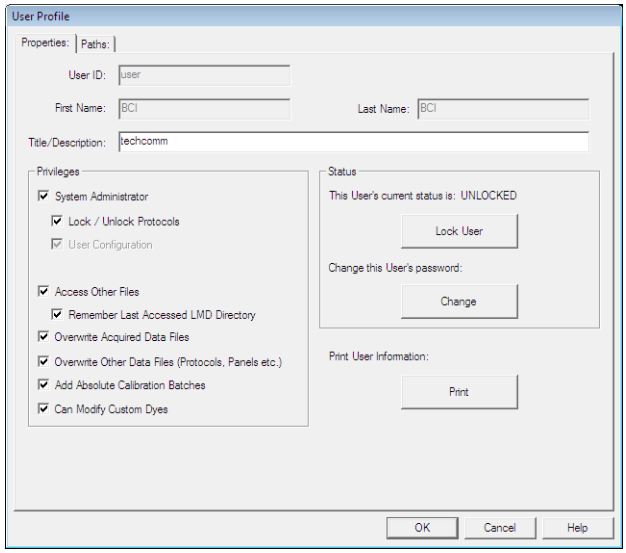
The Restore Archive dialog is a standard Windows File Open dialog which allows you to select an Archive file to restore.

While Restoration is in progress, a warning message is displayed to indicate that Restoration may take some time and that you should not exit the software until the Restoration is complete



User Profile – User ID

When a new user is added with the **Add user** button, or an existing one requires their details changed under **Modify user** button, the User Profile screen is displayed.



See also:
User Profile – Paths
User Administration

User ID

Enter the new User ID if a new user, or Modify the User ID if an existing one. The Admin name cannot be modified or deleted. User IDs are a minimum of 3 characters and a maximum of 24 characters.

First Name

Accepts a minimum of 1 and a maximum of 100 characters. All printable characters are allowed. Leading and trailing spaces are removed.

Last Name

Accepts a minimum of 1 and a maximum of 100 characters. All printable characters are allowed. Leading and trailing spaces are removed.

Title

Accepts a minimum of 1 and a maximum of 100 characters. All printable characters are allowed. Leading and trailing spaces are removed.

Privileges

The Administrator can assign various privileges, which allow or restrict a user's ability to access and overwrite files, and the ability to add Absolute Calibration Batches.

System Administrator

Allows access to the Administrator module.

Lock / Unlock Protocols

Grants permission to lock and unlock protocols.

User Configuration

Disabled and un-checked when System Administrator is unchecked. Disabled and checked when System Administrator is checked.

Access other files?

Check this checkbox to enable the User to access other files. Leaving the box Unchecked allows access only to the User's own Directories.

Note: If you open protocols from other users folders and you want to save the protocols to your folder, you must use the Save Protocol As option and navigate to your folder and save the protocol.

Remember last accessed LMD directory?

If this option is checked, it allows the last accessed LMD (listmode) directory to be selected by that particular User.

This option is available only if the **Access other files?** checkbox was enabled.

Automatically Overwrite acquired data files?

Enabling this checkbox, allows the overwriting of your acquired data files (listmode and histograms). If **Access other files** is enabled, enabling this checkbox allows overwriting any other User's acquired data files.

Overwrite other data files?

If the checkbox is enabled, the User is allowed to overwrite their other files (protocols, panels, Worklists, and so on). If **Access other files** is enabled, enabling this checkbox allows overwriting any other User's files.

Add Absolute Calibration Batches

Enabling this checkbox allows the selected user to enter and modify information available in the **Analysis** ▸▸ **Absolute Count Calibration** dialog box.

Can Modify Custom Dyes

Allow a user access the Custom Dyes Entry dialog to modify the dye entries.

User Status

Displays the current status of this User ID. Locked means this user cannot log into the software. A User is locked:

- Until the System Administrator unlocks the User after setting up the User ID information for the first time.

- Whenever the user reaches the defined number of unsuccessful password attempts allowed.
- Manually by the System Administrator

Unlocked means this user can log into the software with all of their defined privileges.

Change Password

Displays the Password Change dialog.

See also, User Administration

User Profile – Paths

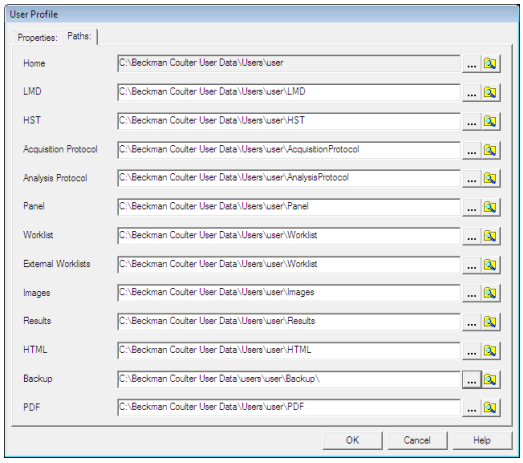
The Administrator uses this screen to assign User directory Paths for different file types, as well as a Backup Directory.

This Backup directory may be any valid disk or Network area where listmode files may be automatically archived.

If you use a network connection for the Backup directory you must validate the network connection. Refer to Windows help for information about the recommended Windows network configuration.

If a Backup directory is present, you are prompted with *Archive Now* on exiting the program, followed by an option to delete local files.

Browsing Buttons
Home
Path Details
Backup



See also:
User Profile – User ID
User Administration

Browsing Buttons

The Ellipses ... allow browsing for a target directory. The  button opens Windows Explorer allowing you to browse for a directory name.

Home

Shows the Home Directory Path that applies to that User.

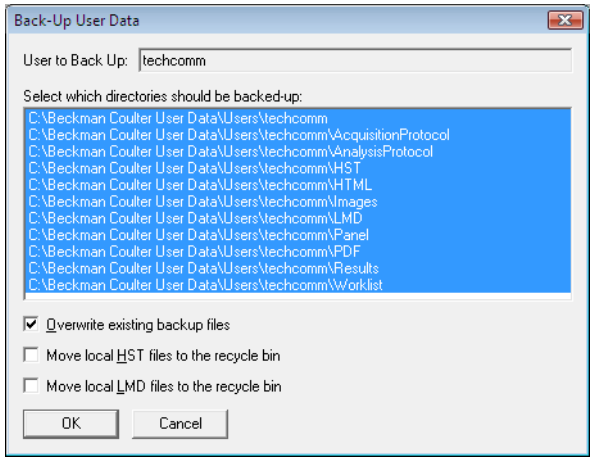
Path Details

Shows the paths to the destination directories for the different File types within Gallios software.

Backup

Shows the path to the Backup or directory for data (listmode) files. If a Backup path is specified for the current user in the User Profile dialog - Paths tab, the Back-up User Data is launched when the user logs out or exits the software.

User to Back Up
Directories list
Overwrite existing backup files
Move local HST files to the recycle bin
Move local LMD files to the recycle bin



User to Back Up

A read only field displaying the User ID of the user that will be backed up.

Directories list

The list displays all the directories of the current user. It is a multi-select list control. You can select the directories that you want backed up. Everything is selected by default.

Overwrite existing backup files

If checked, existing backup files are overwritten without prompt. If not checked and a standard Windows Confirm File Replace dialog is displayed and you can decide if you want files to be overwritten.

Move local HST files to the recycle bin

Disabled if Overwrite existing backup files is not checked. Disabled if the HST folder is not selected in the list control. If selected, the HST files of the user are moved to the recycle bin after they have been backed up.

Move local LMD files to the recycle bin

Disabled if Overwrite existing backup files is not checked. Disabled if the LMD folder is not selected in the list control. If selected, the LMD files of the user are moved to the recycle bin after they have been backed up.

10.4 FILE OPTIONS TOOLBAR

The following Icon Buttons are shown within the File Options Toolbar.

 **Open**	Open the **Open Listmode Data File** dialog box.
 **Save**	Save all the FCS listmode data files that are currently open.
 **Save Protocol**	Save the Protocol with the current name. If the Protocol has not yet been Saved, the **Save Protocol As** dialog box is displayed.
 **Print**	Open the Print Plot printing dialog box.
 **Cut**	Cut an item previously selected and place it on the Clipboard.
 **Copy**	Copy a previously selected item into Clipboard while retaining it in its original position.
 **Paste**	Paste a previously Cut or Copied item from Clipboard into the desired position.
 **Undo**	Undo the previous command. Use the down arrow symbol to see a list of previous actions that can be Undone.
 **Redo**	Redo the previous Undo command. Use the down arrow symbol to see a list of previous actions that can be Redone.

10.5 PLOT OPTIONS TOOLBAR

Note: The Plots toolbar is disabled when running a locked protocol.

 **Color Dot Plot**	Create a Color Dot Plot and specify the plot properties.
 **Histogram Plot**	Create a Histogram Plot and specify the plot properties.
 **Density Plot**	Create a Density Plot and specify the plot properties.
 **Prism Plot**	Create a Prism Plot and specify the plot properties.
 **Legend Plot**	Create a Legend Plot and specify the plot properties.
 **Info Plot**	Create a Info Plot and specify the FCS Keywords.

10.6 REGIONS OPTIONS TOOLBAR

 **Polygonal Region**	Insert a Polygonal Region into a dual parameter plot. The region is a free-form with up to 100 points. It can include horizontal acute angles but not vertical re-entrant angles.
 **Rectangular Plot**	Insert a Rectangular Region into a dual parameter plot.
 **Quadrant Region**	Insert a Quadrant Region into a dual parameter plot.
 **Elliptical AutoGate**	Create an Elliptical AutoGate around the selected population on a plot.
 **Contour AutoGate**	Create a Contour AutoGate around the selected population on a plot.
 **Edit Both Prism Dividers**	Create or Edit a Dual Parameter plot with a set of Dual Prism Dividers.
 **Single Linear Region**	Insert a Linear Region.
 **Multiple Linear Region**	Insert a Multiple Linear Region.
 **Edit Single Prism Divider**	Create or Edit a Single Parameter Prism Divider.

10.7 GATE, COLOR, STATS AND HELP TOOLBAR

 **Create / Modify Gate**	Access the Create / Modify Gates dialog box.
 **Modify Color Gate Blend Setup**	Access the Modify Color Gate Blend Setup dialog box.
 **Create / Modify Color Gate Precedence Setup**	Access the Create / Modify Color Gate Precedence Setup dialog box.
 **View Regions**	View/edit a Region's properties, Region points or to delete a Region that is no longer required.
 **Publish Results**	Publish Statistics to a Microsoft Excel spreadsheet or a Text file.
 **Select Results**	Choose Statistics for display and export.
 **Help**	Access the Help System.
 **Beckman Coulter**	Go to the Beckman Coulter, Inc. web site.

10.8 FLOWPAGE TOOLBAR

 **New FlowPAGE**	Open a New FlowPAGE – Also see Insert/Blank FlowPAGE.
 **Insert bitmap**	Insert a Picture into a FlowPAGE.
 **Annotation**	Add a Text Box to a FlowPAGE.
 **Line**	Draw a Line on a FlowPAGE to enhance the graphical layout of the page.
 **Rectangle**	Insert a Rectangle into a FlowPAGE that can be used as a border for Pictures or Text Boxes.
 **Compensation Grid**	Insert a Listmode Compensation Grid.
 **Align Top**	Align all the selected items to the top of the last selected item (grey handles).
 **Align Bottom**	Align all the selected items to the bottom of the last selected item (grey handles).
 **Align Left**	Align all the selected items to the left of the last selected item (grey handles).
 **Align Right**	Align all the selected items to the right of the last selected item (grey handles).
 **Space Evenly Across**	Space selected items within a FlowPAGE evenly across a page.
 **Space Evenly Down**	Space selected items within a FlowPAGE evenly down a page.
 **Grow to largest**	Resize the selected items to all be the same size as the reference item.
 **Shrink to smallest**	Resize the selected items to all be the same size as the reference item.

10.9 ACQUISITION MANAGER TOOLBAR

 **New Worklist**	Clear the Acquisition Manager panel to create a new Worklist.
 **File Open**	Open a selected stored Worklist file into Acquisition Manager
 **Save Worklist**	Save the current Worklist with a user definable name.
 **Save Panel**	Save the current Panel with a user definable name
 **Delete**	Delete the currently selected test(s).

Insert Test	Inserts a blank row at the end of the current panel, which allows for the insertion of extra tests into the selected panels.
Insert Panel	Insert a Panel from file into the current worklist.
Customize Worklist Columns	Display the Customize Worklist Columns dialog box, allowing hiding of unwanted columns and customization of column titles.
Print	Print the current Worklist.
AutoScheduler	Run the AutoSetup Scheduler

Note: The Listmode File Name column displays only a provisional file name prior to acquisition. If Time or Run Number is included as part of the name the actual value of these fields can only be determined once acquisition of each sample is complete. Therefore, if you wish to print a worklist including the final listmode file names, only do so once acquisition of all samples in the Worklist is complete.

Note: When attempting to save a panel directly from the worklist, the Save Worklist Panel icon becomes active only when the row number is selected to highlight one line of a worklist. Selecting the Save Worklist Panel icon then saves the panel of which the highlighted tube is a member.

Note: Scheduled Applications cannot be saved as Panels or Worklists.

10.10 AUTOMATOR TOOLBAR

The AutoMATOR Toolbar allows shortcuts to commonly used AutoMATOR functions.

AutoMATOR Setup	Display the AutoMATOR Setup dialog box.

Once all alterations have been made, 

. The toolbar becomes active once all files have been entered. A list of files for each panel is shown allowing you to choose the batch required to run first.

The rest of the files follow in order after the selected panel.

Stop	Cancel the batch run. This aborts the current panel. When the panel runs again, the test starts from the beginning.

Run Start the batch running. The AutoMATOR Status Bar appears showing the status of the tests, that is, if it is currently paused, still running, and so on.

Pause Pause has two states:

- Continuous Pause

  Pause the tests for modifications and remain on pause until you decide to continue. This function allows the test to stop without reverting to the beginning of the panel again. To resume the test, click the Pause  button again.

- Auto Pause

  The run of tests pauses and then carries on with the rest of the panel automatically after a 15-second wait. The countdown is displayed on the AutoMATOR Status Bar. When the tests are in the Auto Pause setting, you can hold down Shift and click the Pause button. Shift overrides the Auto Pause, placing the AutoMATOR into Continuous Pause. To restart, click the Pause button again and the test resumes from the point at which it was paused.

10.11 CYTOMETER TOOLBAR

Restart Acquisition Reset the current acquired events to zero and clear the current data in memory. Acquisition restarts at zero events and continues until a stop condition is reached.

Start/Continue Acquisition (F9) Start acquisition or continue an acquisition if previously paused.

Pause Acquisition (F11) Pause acquisition of the current sample.

Pause / Rotate Pause Acquisition and rotate sample to the tube access door.

Stop Acquisition (F10) Stop the Acquisition of the current sample and output results.

Abort Acquisition (F12) Stop the Acquisition without saving data or outputting reports.

Idle Mode Place the Cytometer in Idle mode in order to perform various cleaning and replacing procedures.

Prime Flush the sample line and flow cell with sheath fluid to declog or remove bubbles.

Cleanse Flush the sample line and flow cell with cleaning agent.

MCL Manual Mode Run a carousel of samples one tube at a time.

Single Tube Mode Run a single tube in fixed position # 21.

Cytometer Controls Display the Cytometer Control dialog box to adjust the Cytometer settings. Click the button again to hide the dialog box.

10.12 LISTMODE PLAYBACK TOOLBAR

The listmode playback tool utilizes two sets of buttons - one for saving and restoring worklists (panels populated with listmode files) and one for saving and restoring queues (lists of listmode files).


Create a new Worklist (clear Worklist)


Save a Panel


Add a Panel to the Worklist


Add a Tube to the Worklist


Delete a tube from the Worklist


Play


Pause


Stop


Sort the Worklist


Minimize the Listmode Playback window


Load Playback Worklist


Save Playback Worklist


Load Listmode Queue

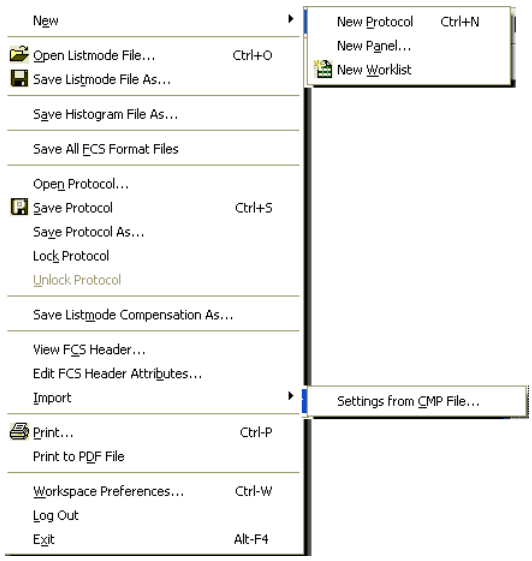

Save Listmode Queue

10.13 REPORT GENERATOR TOOLBAR

Quality Control Report	Display Levey-Jennings plots based on information selected in the QC Template and data located in the QC tables.
Panel Template	A Panel Template defines the content of the Panel Report that is produced when the export panel is run.
Panel Report	Display a list of Patient Panel Reports available for display or print.
Database Entry	Enter or view the specimen information in the database.
Product Editor	Add or edit QC products.
Maintenance Log	Electronic log of instrument routine maintenance, tracked by user name.
Service Log	Electronic log of instrument service performed, tracked by user name.

10.14 FILE MENU

New Protocol
New Panel
New Worklist
Open Listmode File
Save Listmode File As
Save Histogram File As
Save All FCS Format Files
Open Protocol
Save Protocol
Save Protocol As
Save Listmode Compensation As
View FCS Header
Edit FCS Header Attributes
Import Settings From CMP File
Print
Print to PDF
Workspace Preferences
Log Out
Exit



New Protocol

Use this option to create a New Protocol.

1 **File ▸▸ New ▸▸ New Protocol**. The **Save Existing Protocol?** dialog box is displayed.

2 If you **Yes**, the **Save Protocol File As…** dialog box is displayed. the folder in which you wish to Save the Protocol. Enter a valid File Name in File Name box and **Save**.

3 If the File Name already exists, a warning box is displayed.

- If you wish to replace the existing Protocol, **Yes**.
- Selecting the **No** option here allows you to enter a different Protocol File Name. Clicking **No** closes the existing Protocol file and clears the workspace.

See also:
Save Protocol As
Creating a Protocol for Analysis

Creating a New Acquisition Protocol

1 **File ▸▸ New ▸▸ New Protocol.**

2 **Parameters** button on the Cytometer Control Acquisition Setup Tab and choose the Parameters to be acquired.

3 Create the Plots required for acquisition.

4 If analysis is to be performed during acquisition, create the required Regions and Gates, and also select the required statistics from the **Analysis ▸▸ Select Results** option.

5  **File ▸▸ Save Protocol As** to save the protocol.

6 Set the required Listmode File name options from the Workspace Preferences – LMD File Name tab.

7 Set up the Worklist (sample information, CAL Factor, etc.) and run samples.

8 While running sample, adjust the instrument settings on the Cytometer Control Acquisition Setup Tab.

9  when data presentation is satisfactory and  **File ▸▸ Save Protocol Save…** to update the protocol with the new instrument settings.

Creating a Protocol for Analysis

1 **File ▸▸ New ▸▸ New Protocol** to clear the Workspace of all Regions, gates, statistical definitions, and plots and load the default protocol.

2 **File ▸▸ Open Listmode File to** open a representative listmode data file. You can also Drag and Drop a listmode file into the Workspace to load the runtime protocol.

3 To modify a plot:

Right mouse click on the plot and Format Plot.

4 To create a plot:

a. Choose one of the buttons on the Plots Toolbar or use the Plots Menu to select a plot type.

b. the correct parameters for the plot and OK.

c. Right mouse click on the plot and Format Plot.

5 Modify or create subsequent plots and choose the required File, Parameters, Regions and any gates to be applied.

6 


statistics output required


.

7  Save Protocol As and enter a protocol name.

New Panel

Use the Panel Wizard to create a Panel for use in the Acquisition Manager or in Manual Acquisition mode. See Creating Panels in the System Overview chapter.

New Worklist

Note: If no Worklist is visible,  **View ▸▸ Acquisition Manager** to display the Worklist pane. See Creating Worklists in the System Overview chapter.

Note: Unless the current Worklist has been saved using the **Save Worklist**  button within the ACQUISITION MANAGER TOOLBAR, current worklist settings are lost.

IMPORTANT When editing and saving a panel, any worklist containing the edited panel must be recreated. If the worklist in not re-created, the panel in the worklist may not match the newer version of the panel.

Open Listmode File

Use this option to open a new listmode file into all plots.

IMPORTANT Risk of erroneous results if the parameter order is changed or parameters are added or deleted. When parameters are de-selected from a protocol, the parameter order is changed which impacts remaining plots and associated regions and gates. To prevent reporting erroneous results, verify the protocols plots, regions and gates before reporting results.

Before any analysis can be done, a listmode file must be read into Gallios software. Most buttons on the Button Bar are disabled until this has been done.

There are two methods of opening listmode files, Using the File Menu and Using Drag and Drop.

Note: Listmode files with mismatched parameters cannot be opened on in a locked protocol, by either the **File ▸▸ Open Listmode File** menu item or by using Drag and Drop. The parameters in both the protocol and the listmode file must match in order to open a listmode file on in a locked protocol.

Using the File Menu

---

1 **File ▸▸ Open Listmode File**.

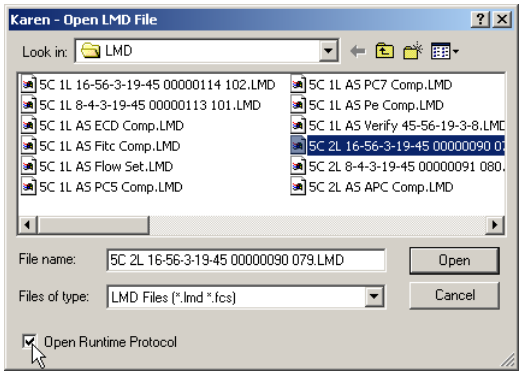
---

2 Select the desired listmode file.

a. a directory in the **Look in:** list box.

b. the type of file in the **Files of type**: list box.

c. the name of the listmode file you want to view.



---

3 **Open Runtime Protocol** to replay the LMD into the plots in the runtime protocol. If the open runtime protocol is not selected, the LMD replays into the plots currently displayed on the workspace.

Note: Listmode replay uses the runtime protocol Cal Factor unless you select a different Cal Factor on the Absolute Count Calibration dialog box.

Using Drag and Drop

A listmode file may be dragged and dropped from Resource Explorer to the workspace. If the listmode file is dragged and dropped into a grey or white space in the workspace (not within any plot currently displayed in the workspace), it will open in its embedded runtime protocol. If the listmode file is dragged and dropped on the workspace that contains protocol plots, the listmode file replays into the plots currently displayed on the workspace.

Note: With locked protocols, listmode files must be dragged and dropped outside of the plot frame and not directly in the plot.

When loading listmode files which have a parameter order different from the current protocol, you are warned with *Parameter Mismatch*.

- If you select **Continue**, the file is loaded and replaces the parameters with those of the listmode file.
- If you select **Abort**, the listmode file is not loaded.

Note: Listmode Compensation must be performed using the runtime protocol or a protocol with equivalent parameters as the runtime protocol.

When loading listmode files from a different instrument *This is not an Gallios file* appears. Select **Continue** to load the file.

Save Listmode File As

If, at any time, you decide to save your file under a different File Name:

---

1 **File ▸▸ Save Listmode File As**.

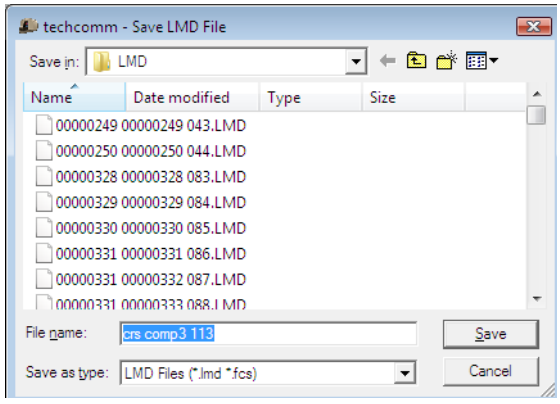
---

2 Specify the name of the file.

a. the desired directory in the **Save in:** list box.

b. the type of file in the **Save as type:** list box. In the **File name** text box, type the desired File Name.

Gallios software creates a file with the name you specified and stores it in the drive and directory you specified in FCS format.



File Name

A file name can have up to 200 alphanumeric characters, including the path. The following characters cannot be included: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), comma (,), question mark (?), quotation mark ("), pipe symbol (|), colon (:), or apostrophe (‘). Leading, Trailing and consecutive spaces are not allowed.

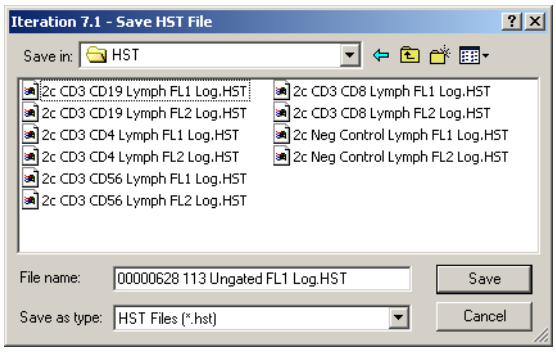
Save Histogram File As

The **Save Histogram File As** option allows a single histogram associated with the currently in-focus plot to be saved directly to an FCS histogram file.

Note: Gallios software only saves one Histogram Plot in a single FCS histogram file.

---

1 Highlight the Histogram Plot (Current window).



---

2 **File ▸▸ Save Histogram File As**.

---

3 Specify the desired drives and folders by selecting them in the list boxes or by typing the path in the File text box.

---

4 In the **File name** text box, type in the desired File Name.

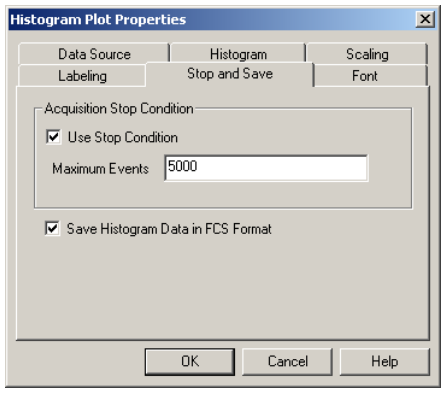
Gallios software creates a file with the name specified and stores it in the drive and folder you specified in FCS histogram format.

Save All FCS Format Files

This option saves the current listmode file to disk and saves any histograms marked for saving.

1 Highlight the required plot.

Note: The individual Histogram's Plot Properties must be set to save.



2 **Plots ▸▸ Acquisition Stop & Save** menu option.

3 **Save Histogram Data In FCS Format**.

Note: An (S) is added to the plot title if you select this option. Do not confuse this with an [S] that identifies a gate.

4 Repeat for each histogram plot you wish to be saved.

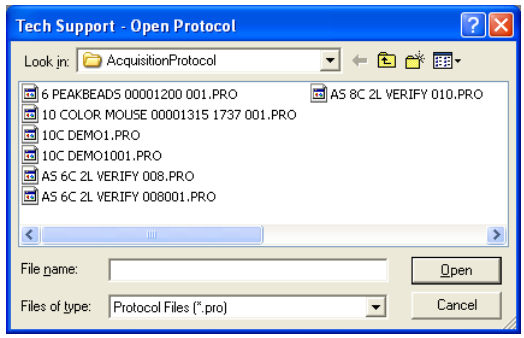
5 **File ▸▸ Save All FCS Format Files.**

Open Protocol

Use this option to open a protocol file from disk.

1 **File ▸▸ Open Protocol**.

The Open Protocol File dialog box appears.



2 the file you wish to open from the Folders list box.

3 Open.

Note: Double clicking with the mouse on the file name closes the dialog box and opens the file.

Save Protocol

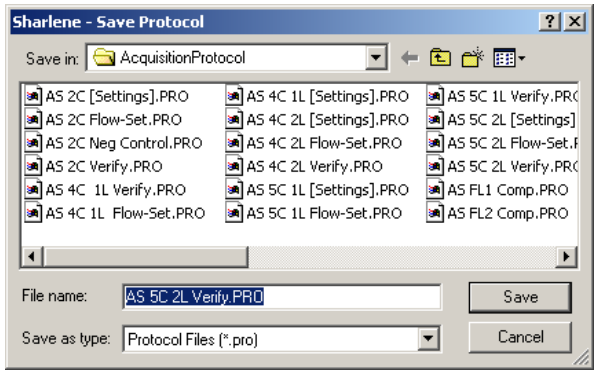
Selecting this option saves the Protocol in its current form. Ctrl+S is the shortcut option, as is the Save Protocol button.

Note: If you open protocols from other Users' folders and you want to save the protocols to your folder, you must use the Save Protocol As option and navigate to your folder and save the protocol.

Save Protocol As

Save a protocol file under a different name or to a different folder.

1 **File ▸▸ Save Protocol As**.



2 In the Save Protocol As dialog box,

the desired folder from the list or by typing the path in the **File name** text box.

3 In the **File name** text box, type the desired File Name.

Gallios software gives the default file extension *.PRO. The *.PRO extension is only a suggested extension, any standard DOS characters can be used for the full File Name. You are urged to keep the extension consistent for easy file searches.

Lock Protocol

Lock the current protocol. See Locked Protocols for details. Disabled if,

- the protocol is already locked.
- the current user does not have the Lock/Unlock Protocols Privilege.

Unlock Protocol

Unlock a previously locked protocol. See Locked Protocols for details. Disabled if,

- if the protocol is not locked.
- if the current user does not have the Lock/Unlock Protocols Privilege.

Save Listmode Compensation As

Opens the Save LMD compensation settings dialog.

Save in

By default set to the AcquisitionProtocol folder of the current user.

File name

Empty by default. You can type a file name or choose one from the list. A file name can have up to 200 alphanumeric characters, including the path. The following characters cannot be included: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*),

comma (,), question mark (?), quotation mark ("), pipe symbol (|), colon (:), or apostrophe (‘). Leading, Trailing and consecutive spaces are not allowed.

Save as type combo box

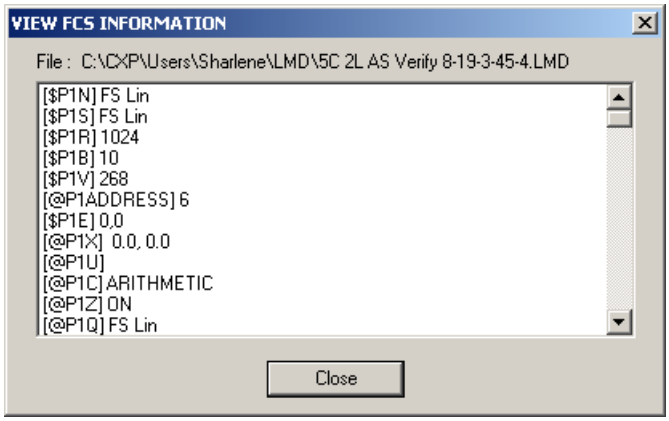
This combo box contains only one choice: LMD Compensation Files (*.cmp).

Save button

Validates the file name. If OK, close the Save LMD compensation settings dialog and saves the compensation settings in a file with the selected name.

View FCS Header

File ▸▸ View FCS Header Information to view the FCS header information on the current file.



FCS Keywords Used in Gallios Software

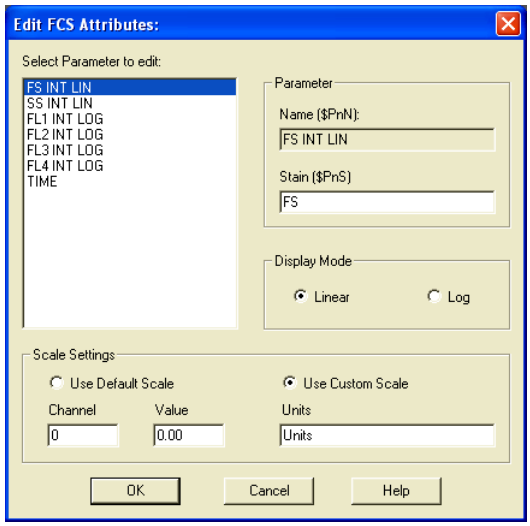
See FCS Header - Keyword Reference.

Edit FCS Header Attributes

Use this option to edit the FCS header information, including calibration information. You can change the displayed name of Parameters or change the scaling options. Axis calibration information can be entered here. This information is embedded in the listmode file.

1 **File ▸▸ Edit FCS Attributes** to view the Edit FCS Header Attributes dialog box.

Note: You cannot make any changes on this screen when running a locked protocol.



2 Make the desired changes.

3 OK or press Enter to accept.

4 Cancel or press Esc to exit the dialog box without saving the changes.

These changes may also be saved permanently within the open protocol by **File ▸▸ Save Protocol** or **File ▸▸ Save Protocol As**.

Note: Modifying the scale options should only be done during offline analysis. Replaying of the listmode files always uses the calibration set at acquisition.

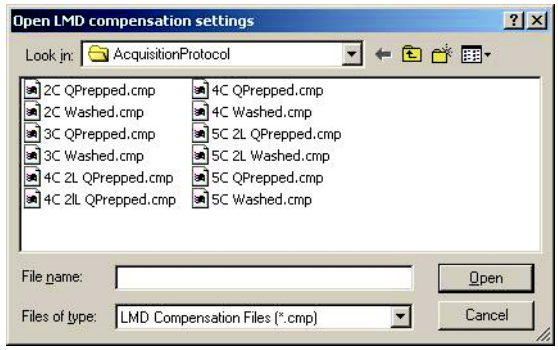
Import

Settings From CMP File

This option allows compensation settings obtained from the Listmode Playback option or Save Listmode Compensation As option to be imported into the current protocol.

---

1 **File ▸▸ Import ▸▸ Settings from CMP File.**



---

2 the appropriate file and Open.

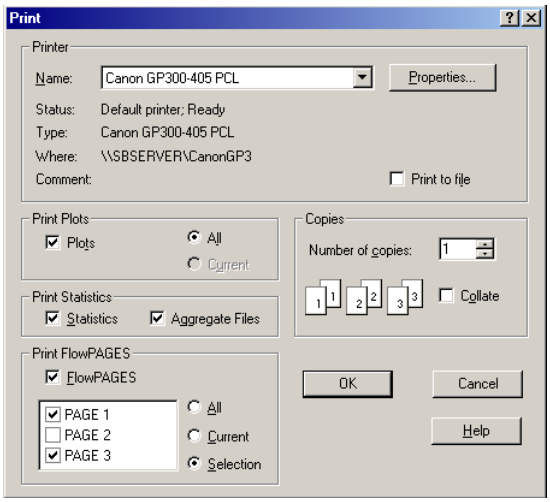
The compensation settings from the stored file are then read into the current Gallios software protocol.

Print

You can print plots and statistics in a variety of ways.

Note: To print to PDF use the **Print to PDF** option on the FILE MENU. Do not use **File ▸▸ Print** and select the PDF driver from the Print name on the Print dialog box.

1 **File ▸▸ Print**. The Print dialog box is displayed.



2 Make the desired changes.

3 OK or press Enter to commence printing.
See also:Print Plots
Print Statistics
Print FlowPAGEs
Print to PDF

4 Cancel or press Esc to exit the dialog box without saving the changes.

Print Plots

Select this option if you wish to Print Plots. Choose ALL or CURRENT Plots.

Print Statistics

- Statistics
  Select this option if you wish to Print Statistics.
- Aggregate Files
  This option groups together statistics from all the regions on a single file. Otherwise statistics are printed grouped by plot.

Note: If you want to include file information in the statistics printouts, an Info plot, formatted with desired FCS keywords, must be included in the protocol.

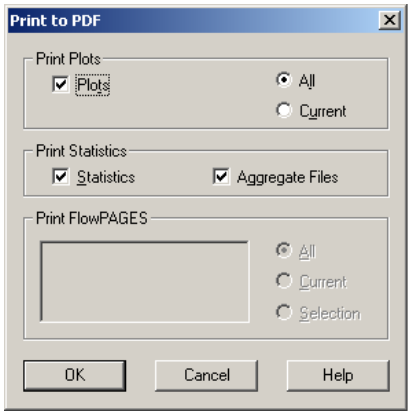
Print FlowPAGEs

Select this option to print FlowPAGEs. Choose ALL, CURRENT or a SELECTION.

To print individual pages, you can enable the page number checkbox.

Print to PDF

Print plots, statistics or FlowPAGES in PDF format.



Note: During installation a GalliosPDF printer driver is added to the Windows Printers folder. This driver is used for the "Print To PDF" options only. If you require additional functionality to create PDF reports, you need to use a full version of Adobe® Acrobat® software. This additional software is not included with Gallios Software.

If you install the full version of Adobe Acrobat, you should use the Adobe distiller to create PDF files rather than the GalliosPDF - PDF compatible print driver. If you continue to use the "Print to PDF" options within Gallios Software when Adobe Acrobat full version is installed, some Flowpages are cropped. This issue does not occur when Acrobat Reader® only is installed.

Workspace Preferences

Use Workspace Preferences option to set the defaults for the workspace and plot options according to your own preference. Some of the options are global, so they immediately apply the specific changes required to all plots. Other options only apply the changes to the plots created after the option has been selected. Use **Plots ▸▸ Format Plot** to modify the Default Plot options.

Workspace Preferences - Gating
Workspace Preferences - Publish
Workspace Preferences - Plot Display
Workspace Preferences - LMD File Name
Workspace Preferences - User Info
Workspace Preferences - Acquisition Options

Workspace Preferences - LMD File Name

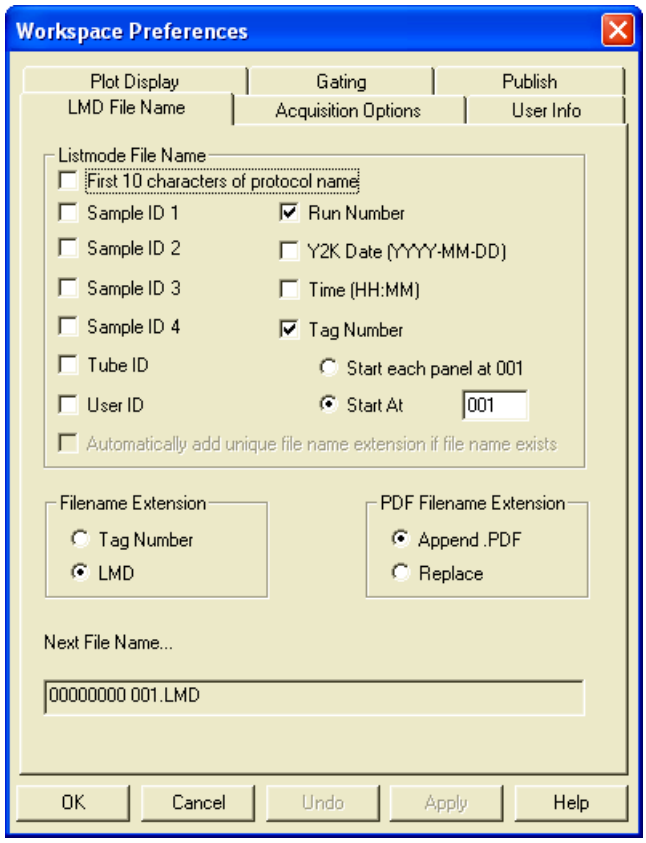
This tab allows you to specify a compound listmode file name, including components such as the date and the time that the sample was run as well as sample ID fields.

IMPORTANT Risk of erroneous results if you overwrite listmode file names. Either Tag number or Run Number must always be selected to ensure that unique filenames are created.

The File Name displayed is only a provisional file name. Some available options (for example: Run Number and Time) can only be defined at the time of acquisition. If a printout of the Worklist is required with the final assigned file name it is only correct once acquisition of samples is complete.

A file name can have up to 200 alphanumeric characters, including the path. However, the following characters cannot be included: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), comma (,), question mark (?), quotation mark ("), pipe symbol (|), colon (:), or apostrophe (‘). Leading, Trailing and consecutive spaces are not allowed.

First 10 characters....
Sample ID 1, 2, 3, 4
User ID
Tube ID
Automatically add unique....
Run Number
Y2K Date
Time
Tag Number
File Name Extension
PDF File Name Extension



Note: If more than one Listmode File Name option is selected, the priority of the options used for the file name are,
1. Characters from protocol name
2. Sample ID 1
3. Sample ID 2
4. Sample ID 3
5. Sample ID 4
6. Tube ID
7. User ID
8. Run Number
9. Y2K Date
10. Time
11. Tag Number

See also: Workspace Preferences
Workspace Preferences - Acquisition Options
Workspace Preferences - User Info
Workspace Preferences - Plot Display
Workspace Preferences - Gating
Workspace Preferences - Publish

Note: If the Worklist Column names are changed using the Customize Worklist Columns option within Acquisition Manager, these changes are reflected in the notation labels on the above dialog.

First 10 characters....

The first ten characters of the protocol filename is used to generate the listmode filename for acquisition. If the protocol name is longer, then the name is truncated to 10 characters.

Sample ID 1, 2, 3, 4

The Sample ID columns allow you to enter information about the particular sample being run. When any of the checkboxes are selected in Workspace Preferences, tthe information in the corresponding columns is is included as part of the listmode file name. If Sample ID 2-4 are not displayed, use Customize Worklist Columns to add them to the Acquisition Manager. Sample ID 1 is the same for all tubes within a panel.

Note: Be sure to verify any manually entered Sample IDs.

User ID

When checked, this includes the User ID as part of the listmode file name.

Tube ID

When checked, this includes the Tube ID as part of the listmode file name.

Automatically add unique....

If a file already exists with the same name and extension, it automatically uses the next available extension number. This option is only available when a user is not given data file overwrite capability in the user access rights section of the Admin setup. Depending on User overwrite privledges, if Tag # is selected and this option is not selected, LMD filenames may be overwritten.

Run Number

The system automatically generates an incrementing run number every time that a sample is run. When this option is selected, the run number is included as part of the listmode file name – the easiest way to ensure unique file names are always generated. If not selected, the run number is still generated, saved and can be accessed in the FCS Header section of the file. The Run Number cannot be de-selected unless one of the Sample ID's or Tube ID are selected. De-selecting all the Sample IDs and Tube ID check boxes results in Run Number being automatically selected.

Y2K Date

When selected, this option includes the date as part of the listmode file name. The format used is the standard Year 2000 format (YYYY-MM-DD), showing when the sample was analyzed.

Time

When this option is selected, the time (HH-MM-SS) that the sample was analyzed is included as part of the listmode file name.

Note: The Listmode File Name column displays only a provisional file name prior to acquisition. If Time or Run Number is included as part of the name the actual value of these fields can only be determined once acquisition of each sample is complete. Therefore, if you wish to print a worklist including the final listmode file names, only do so once acquisition of all samples in the Worklist is complete.

Tag Number

This allows you to choose whether the Tag number should be included as part of the listmode file name. If this option is selected, it activates the following items:

- Start each Panel at 001
  This starts the Tag Numbering option from 001 for each new panel.
- Start At
  This starts the Tag Numbering option from any number that you require, and continues incrementing until manually reset.

File Name Extension

This sets the file extension to be either Tag Number or LMD:

- Tag Number
  Selecting this option allows you to have the Tag Number as the file name extension. This is dependent on a Tag Number being previously selected.
- LMD
  This option sets the file name extension to be LMD.

PDF File Name Extension

Change the LMD File Name extension to one of the options listed below.

Append .PDF File Name

Append PDF to the listmode file name extension.

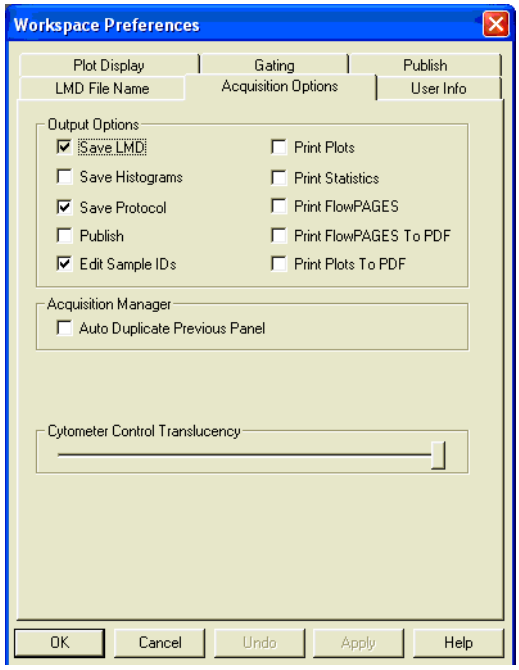
Replace

Replace the current listmode file name extension with .PDF. This option should only be used when LMD is being used as the listmode file name extension.

Workspace Preferences - Acquisition Options

Sets the default Acquisition Options for Gallios software during Acquisition, allowing automatic Saving, Printing and Exporting of Data.

Output Options
Acquisition Manager
Cytometer Control Translucency



See also: Workspace Preferences
Workspace Preferences - LMD File Name
Workspace Preferences - User Info
Workspace Preferences - Plot Display
Workspace Preferences - Gating
Workspace Preferences - Publish

Output Options

The Output actions occur automatically at the completion of the acquisition of each sample tube. Check the appropriate options for the actions you wish to perform when acquiring data using the Acquisition Manager. See also, Workspace Preferences - Publish.

Note: Save Protocol applies to any changes made to a protocol, whether acquiring or not. You cannot alter the Save LMD default, as this is a mandatory setting. If a protocol name begins with Cleanse, the protocol does not create a listmode file when the protocol is run.

Acquisition Manager

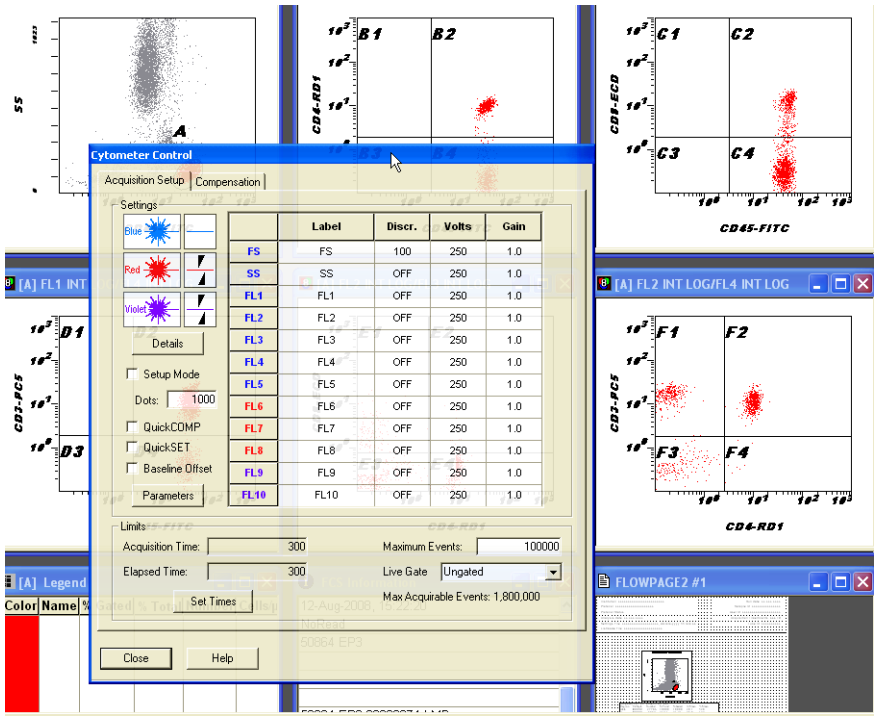
Auto Duplicate Previous Panel adds a copy of the current panel into the Acquisition Manager at the completion of the current panel, and is useful if you wish to run samples in Panel Mode but do not wish to define a complete worklist in advance.

- In Automatic mode, the software will duplicate the last panel each time it reaches the end of the worklist and automatically begins acquisition on the first tube in that Panel.
- Acquisition will stop when either the maximum 32 tubes have been acquired or when the instrument attempts to acquire from a location which does not contain a tube.
- Regardless of the manually entered numbering of the first Panel, all AutoDuplicated Panels will be numbered consecutively, continuing from the last run test.
- Sample ID1s will always be populated with the Run Number.

- Sample ID2-4 will not be entered and consequently cannot be selected as part of the LMD filename.
- If the last acquired tube location was 32, the Panel will not be AutoDuplicated. The worklist will NOT wrap around to tube location 1.
- If there are insufficient available locations to insert a complete Panel without exceeding tube location 32, the Panel will not be AutoDuplicated.

Cytometer Control Translucency

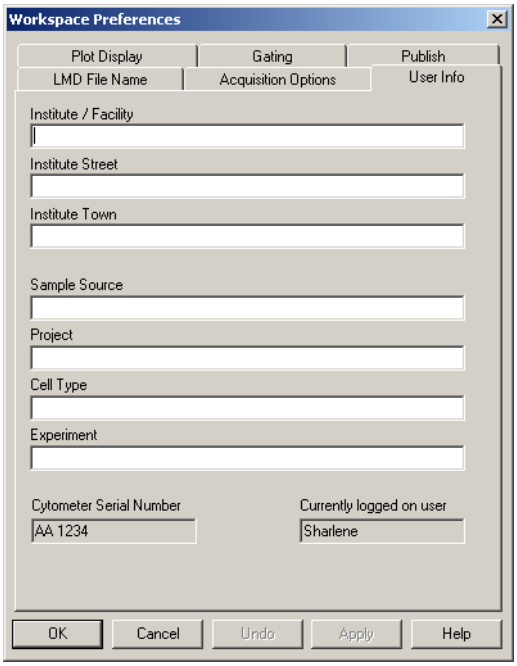
Use this slider to fade the Cytometer Controls dialog so it appears translucent.



Workspace Preferences - User Info

This option allows defined information and sample/project information to be stored as part of the FCS header in listmode files. This can be printed out as required via the Legend plot or with the exported data. All of these are optional and can be filled in according to your preference.

Institute
Institute Street
Institute Town
Sample Source
Project
Cell Type
Experiment
Cytometer Serial Number
Currently logged on user



See also: Workspace Preferences
Workspace Preferences - LMD File Name
Workspace Preferences - Acquisition Options
Workspace Preferences - Plot Display
Workspace Preferences - Gating
Workspace Preferences - Publish

Institute

Enter the Institute name with a minumum of 3 characters . It can be printed out as part of the statistics output.

Institute Street

Enter the Institute street address. It can be printed out as part of the statistics output.

Institute Town

Enter the Institute town. It can be printed out as part of the statistics output.

Sample Source

This option allows you to enter information regarding where the sample came from.

Project

The name for the project is entered here.

Cell Type

This labels the sample with the specific cell type being analyzed in that sample.

Experiment

The name of the experiment being conducted is entered into this section.

Cytometer Serial Number

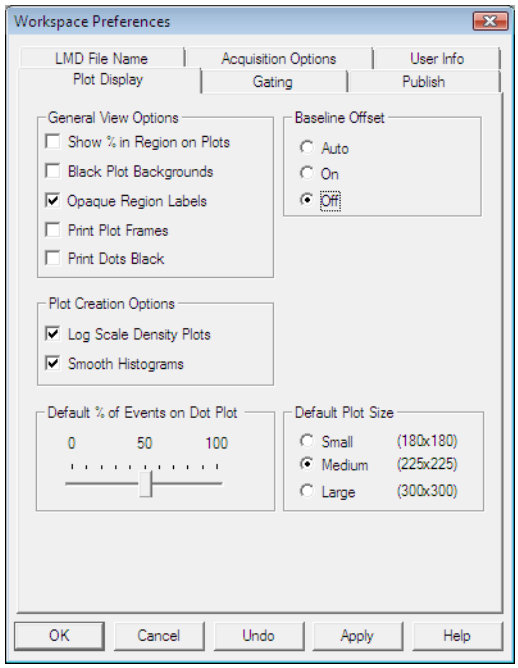
The serial number from the specific Cytometer being used is displayed here and recorded in the listmode file, as more than one Cytometer may be utilized in the same experiment. You must be logged on as a System Administrator to enter the serial number.

Currently logged on user

The User ID of the currently logged on user is displayed here and recorded in the listmode file.

Workspace Preferences - Plot Display

Show % in Region on Plots
Black Plot Backgrounds
Opaque Region Labels
Print Plot Frames
Print Dots Black
Log Scale Density Plots
Smooth Histograms
Default % of Events on Dot Plot
Baseline Offset
Default Plot Size



See also: Workspace Preferences
Workspace Preferences - LMD File Name
Workspace Preferences - Acquisition Options
Workspace Preferences - User Info
Workspace Preferences - Gating
Workspace Preferences - Publish

Show % in Region on Plots

When the show % in region option is selected, the percentage of events in a particular region is automatically displayed on new plots. For new plots all regions created will display the % gated value of that region. When this option is selected, the % is also displayed on all existing plots in the protocol.

Black Plot Backgrounds

This displays the plots with a black background instead of white. This can be useful as it makes the viewing of smaller populations of cells much easier. When you print, drag or copy a plot to other software programs, the background remains white.

Opaque Region Labels

This option displays an area around the annotation within black backgrounds, making it visible.

Print Plot Frames

This option can be used to show or hide frames on plots printed directly from the File Print menu and exported plot images.

If this is checked, all frames are displayed around plots. If it is clear, frames are not printed or exported.

Print Dots Black

Select this option to print all dots in a color dot plot in black irrespective of their colors. If it is clear, dots are printed in various shades of gray or, if using a color Printer, the best matched colors available. This option does not affect the printing of FlowPAGEs.

Log Scale Density Plots

Selecting this option displays a density plot with log scaling of the density levels. If unchecked, linear scaling of the density levels is used. These changes are only applied to plots created after this option has been selected, and not to the ones previously created.

Smooth Histograms

This automatically smooths single histograms by default allowing for easier viewing. When selected, this option displays smoothed data on all new histograms as they are created. It does not apply any of the changes to previously created plots.

Default % of Events on Dot Plot

This allows you to set the default number of dots to be displayed when acquiring data in a dot plot. These changes are only applied to plots created after this option has been selected, and not to the ones previously created. Reducing the default percentage increases the speed of software functions when reading large listmode files.

Baseline Offset

IMPORTANT Risk of erroneous results. The Baseline Offset function should only be used after first viewing data with the baseline offset function turned off (unchecked). You must be satisfied that the overall results of any assay are not significantly affected by turning baseline offset on. If the lower marker of a statistics region is higher than the first decade (of log displayed data), then there should be no effect on statistics from that region by using baseline offset. However, you should not use baseline offset when determining appropriate cytometer settings as with AutoSetup. Baseline offset On should only be used for visual purposes after analysis.

This function allows log data in very low level signals to be moved up from low numbered channels by adding a randomized Gaussian positive offset. This allows easier comparison of data from other Cytometers. Baseline Offset includes the following three options:

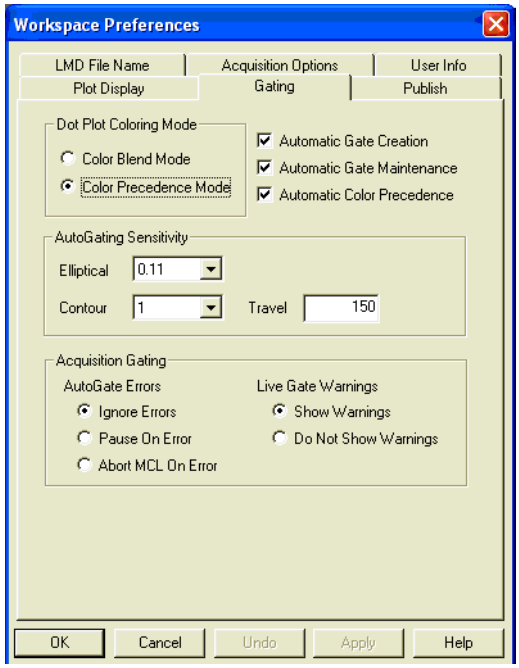
- Auto
  This option displays the listmode data according to a keyword in the FCS file header. If the Baseline Offset keyword value is ON, then data is automatically displayed with Baseline Offset switched on. If it is OFF, then data is displayed with no Baseline Offset.
- On
  This reads a file and modifies the displayed data only by adding a randomized Gaussian offset to the low-level signals. Data on disk is not modified and is left as it was when acquired from the Cytometer.
- Off
  Reads a file exactly as it is with no Baseline Offset. This setting ignores the runtime setting of Baseline Offset and always displays data without the Baseline Offset effect.

Default Plot Size

Allows you to select the size of the plot display. for newly created plots.

Note: To change the size of plots already present on the workspace, **Window ▸▸ Tile Special** and select the desired plot size to update the size of the existing plots displayed on the workspace.

Workspace Preferences - Gating



See also: Workspace Preferences
Workspace Preferences - LMD File Name
Workspace Preferences - Acquisition Options
Workspace Preferences - User Info
Workspace Preferences - Plot Display
Workspace Preferences - Publish

Automatic Gate Creation

This option allows you to create a new gate automatically when a new Region is created. If this option is not selected you must use **Analysis ▸▸ Create Modify Gates** to create gating logic. Regions copied using Ctrl+Drag and Drop into a plot assign the region as a gate.

Automatic Gate Maintenance

Enabling this option ensures that when the gating of a plot changes, the associated gate of any region drawn onto this plot also changes to reflect the new gating state.

Automatic Color Precedence

As a new region is drawn, the color for that gate is placed at the top of the list in the color precedence dialog box. If this option is disabled, a color is not associated with a gate.

Dot Plot Coloring Mode

This alternates Color Dot plot mode between Color Blend and Color Precedence modes:

- Color Blend Mode
- Color Precedence Mode (default)

AutoGating Sensitivity

Select the sensitivity of the AutoGate region drawn.

Elliptical Sensitivity

Settings are 0.11, 0.33 and 1.1 (% of processed peak). Selecting 0.11 creates a larger region, whereas selecting 1.1 creates a smaller radius region around the target population.

Contour Sensitivity

Settings are 1, 2, 3, 4, and 5. Level 1 = 0.11% processed peak at a resolution of 64x64, levels 2, 3 and 4 are 0.11, 0.33 and 1.1% at a resolution of 128x128. Level 5 is 1.1% processed peak at a resolution of 256x256.

Contour Travel

Input a maximum value the contour AutoGate region travels from the mean. The Minimum value is 25; Maximum is 250 channels. Use this option to slightly adjust the AutoGate from tube to tube if slight changes in lysis cause the target population to move slightly. This option is not available for elliptical AutoGates.

AutoGating Failure

You can select an error option when the AutoGate algorithm fails during acquisition in the MCL automatic mode.

Pause on Error

The system pauses the current acquisition when an AutoGating error occurs and awaits further input before proceeding.

Abort On Error

The system automatically aborts the Worklist when an AutoGating error occurs.

Ignore Errors

The system continues processing the Worklist when an AutoGating error occurs.

Live Gate Warnings Options

Notifies that the protocol uses a Live Gate. Only one of the following radio buttons can be selected at any one time.

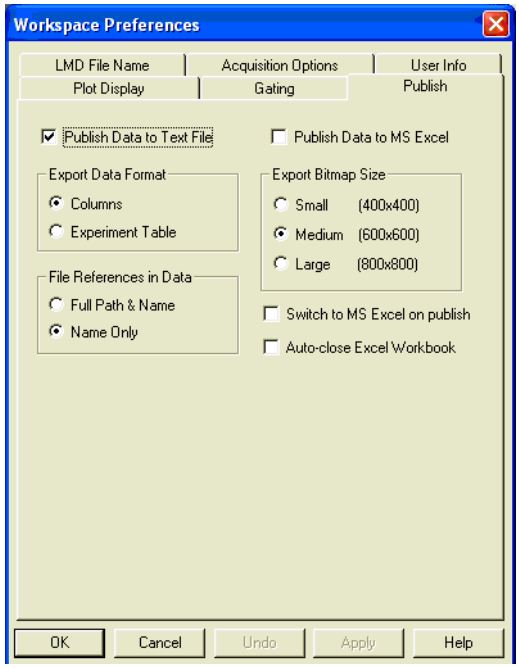
Show Warnings radio button

Display Live Gate warning messages.

Do Not Show Warnings

Do not display Live Gate warning messages.

Workspace Preferences - Publish



See also: Workspace Preferences
Workspace Preferences - LMD File Name
Workspace Preferences - Acquisition Options
Workspace Preferences - User Info
Workspace Preferences - Plot Display
Workspace Preferences - Gating

Publish Data to Text File

Enable this checkbox to publish data to an ASCI text file with a file extension of .TXT. The text file starts with the name of the application: Gallios Cytometer and the date in the format Day, Month date, year time (Day, Month DD, YYYY HH:MM:SS).

Note: Legend Plot data, Prism Plot data and FCS Info Plot information cannot be exported to text files.

Publish Data to MS Excel

This function enables you to publish data to a Microsoft Excel 2003 Workbook, used in conjunction with the **Tools ▸▸ Publish Results Now** menu item. Ensure that only one session of MS Excel is open prior to exporting.

Note: Microsoft Excel is not included with your Gallios software and must be purchased separately.

Export Data Format

Select the Export Data Format.

- Columns to export the data from all files to a single worksheet

- Experiment Table to export the data in experiment table format for inclusion in a pivot table.

File References in Data

Choose to reference the file name by Full Path and Name or by Name Only.

Export Bitmap Size

Allows you to select the size of the Bitmap used in when exporting.

Switch to MS Excel on Publish

MS Excel starts and displays the published data. When enabled, Excel moves to the front of Windows desktop as the active application with each export. If left unchecked, Excel runs in the background with each subsequent export.

Auto-close Excel Workbook

When enabled, the Excel workbook automatically closes with the Excel session still active.

When automatic publishing (**Publish Data to MS Excel**) is selected and data is appended to a given worksheet, the worksheet closes and immediately reopens with the added data if **Auto-close Excel Workbook** is selected.

Log Out

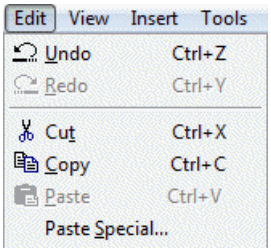
Allows a particular user to Log Out of Gallios software so that another person can Sign-On. This menu item is disabled during acquisition, including between tubes processing.

Exit

This option closes down the Gallios software and returns you to your Windows Desktop.

10.15 EDIT MENU

Undo
Redo
Cut
Copy
Paste
Paste Special



Undo

Edit ▸▸ Undo reverses software operations such as Region drawing, Region label editing and FlowPAGE editing. Each New operation is appended to an Undo list. The valid Undo operations are then performed in reverse order with each Undo request.

Note: Undo does not undo such operations as Save and Acquisition of data.

The **Edit ▸▸ Undo** list is cleared after each change of plot focus.

The **Edit ▸▸ Undo** function is applicable to the Window Menu, Regions, FlowPAGE and fonts.

Redo

Edit ▸▸ Redo reverses the last Undo operation.

If a new operation is performed after a series of **Edit ▸▸ Redo** operations, the **Edit ▸▸ Redo** list is cleared and all previous Undo actions are lost.

The **Edit ▸▸ Redo** list is cleared after each change of plot focus.

Cut

Edit ▸▸ Cut deletes the highlighted items from the display, placing them within the Clipboard. These items can be recalled either by using the **Copy**, **Paste** or **Paste Special** options within the **Edit** menu.

Histogram plot displays can be Cut from one Overlay plot to another by using the **Paste** or **Paste Special** options.

If you **Edit ▸▸ Cut** another item before the first item is Pasted, this first item is overwritten.

See also:
Copy
Paste
Paste Special

Copy

To Copy Statistics, Regions or Plot Images onto the Windows Clipboard,  **Edit ▸▸ Copy**.

This allows for Pasting these items into other areas of Gallios software or to other Windows Applications such as MS PowerPoint®, Word, Excel, and so on.

The exact nature of what can be pasted depends upon the target application and can be controlled or modified using the target applications **Edit ▸▸ Paste Special** command.

See also:
Cut
Paste
Paste Special

Paste

After copying into memory, Gallios software allows the pasting of a number of different items.

The default paste result depends upon what you are trying to paste.

- If the target is a FlowPAGE, the Plot Image and Statistics are Pasted.

---

1 Select the Plot of interest to be copied.

2 the Plot and hold down the left mouse button. (The No Drop Cursor is displayed.)

3 Drag the cursor onto the FlowPAGE. (The Drop Cursor is now shown.)

4 Release the mouse button to paste the Regions onto the Plot or the Plot Image plus the Statistics onto a FlowPAGE.

Note: If you use Ctrl+ to Drag and Drop, you get the Plot Image but not the Statistics.

- If the Paste target is a Plot, then the previously copied regions are Pasted.

1 Select the Plot with the region of interest to be copied.

2 Drag the cursor to the destination Plot.

3 Release the mouse button to paste the Regions onto the Plot.

Note: If the copied region is required as a gate, use Ctrl+ to Drag and Drop the region.

See also:
Cut
Paste
Paste Special

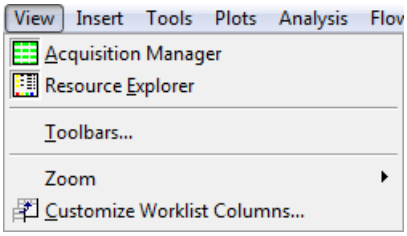
Paste Special

Allows which form of a copied object is pasted. Bitmaps, Text or Region may be pasted depending on the copy source and the paste target.

See also:
Cut
Copy

10.16 VIEW MENU

Acquisition Manager
Resource Explorer
Toolbars - Customize Toolbars
Zoom
Customize Worklist Columns

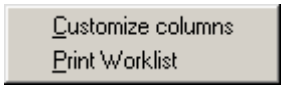


Acquisition Manager

The Acquisition Manager is used to create a Worklist for the Cytometer. It may also be used to create or modify existing panels. Sample and Test information may be entered here, this data is stored as part of the FCS listmode file. Use **View ▸▸ Acquisition Manager** to show or hide the Acquisition Manager.

Panels and Protocols can be added to the Acquisition Manager Worklist by using the Resource Explorer to Drag and Drop or by using the ACQUISITION MANAGER TOOLBAR. Items may be reordered within groups or entire groups may be reordered using Drag and Drop. Thick black lines define groups, and items within the groups are separated by thin dotted lines.

By clicking the right mouse button in the column headings the following menu choice is available.



Select the appropriate option to Customize Worklist Columns, allowing you to add, remove or rename the columns, or print the Worklist when a Print dialog box is displayed.

See also:
ACQUISITION MANAGER TOOLBAR
Worklist Columns Available

Acquisition Manager Docking Options

The Acquisition manager may be docked to the application window or floated in the main application window. Right clicking in the grey area of the manager (not on the worklist columns) displays the following context menu.

Allow Docking

When checked, allows the manager to be docked. If Allow Docking is un-checked while the manager is docked, it is floated in the desktop. If allow docking is selected and the Acquisition Manager is floating, double-clicking on the Acquisition Manager title bar docks the window. The Acquisition Manager can also be docked by dragging it to any side of the application.

Hide

Closes the Acquisition Manager. To re-open it use View->Acquisition Manger menu option.

Float in Main Window

Checking this item floats the manager in the Main application window only; un-checking it allows it to float anywhere on the desktop. If Float in Main Window is checked, double clicking the Acquisition Manager title bar maximizes it.

Acquisition Manager – Modifying Panels and Worklists

The Acquisition Manager is used to create a Worklist for the Cytometer. It may also be used to create or modify existing Panels or Worklists.

IMPORTANT Risk of erroneous quality control statistics. Panels containing QC regions must be constructed from individual unique protocols. Do not use a panel constructed from one protocol (with different parameter names) for QC database purposes.

Panels

1 Open the Panel to be modified.

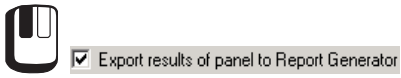
2 To add tests to a Panel, use the Insert Test  button on the Acquisition Manager Toolbar. Repeat this process as necessary. You may also insert multiple tests by using the Insert Panel button. Alternate protocols may be selected once the tests have been added using the File  button inside the Worklist.

IMPORTANT Risk of reporting incorrect results. If you edit panels that are a part of different worklists, the panels are not automatically updated in those worklists. When opening a worklist that contain panels that have been modified, the worklist still contains the original version of the panel. You need to re-construct the worklist in order for it to have the latest versions of panels.

3 To remove tests, delete the entire row from the Worklist.

4 To change any other panel information, such as how instrument settings are loaded, use the appropriate columns. When the changes are complete,  to save the panel.

5 If you intend to generate Panel Reports from this panel,



.

Worklists

1 Open the Worklist to be modified.

2 To add samples to the Worklist, use Drag and Drop from the Resource Explorer. This starts a new panel or protocol that is automatically added to the end of the Worklist.

3 To remove tests, delete the entire row from the Worklist.

4 Save the Worklist using the Save Worklist button on the Acquisition Manager Toolbar.

See also:
Acquisition Manager
Customize Worklist Columns

Worklist Columns Available

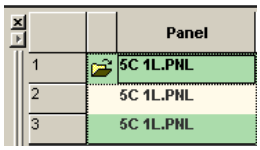
The following columns are available for display within the Acquisition Manager:

Panel
Protocol
Plots
Region Source
Cytosettings
Parameter Names
Tube ID
Carousel Number and Location
Sample ID 1 – 4
CAL Factor
LMD Filename
Stop Condition and Value
P1 - P32

Acquisition Manager - Panel

IMPORTANT Risk of erroneous quality control statistics. Panels containing QC regions must be constructed from individual unique protocols. Do not use a panel constructed from one protocol (with different parameter names) for QC database purposes.

Panel



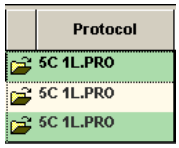
Displays the name of the currently selected panel. An alternative panel may be selected using the File button or a New Panel created using the Panel Wizard.

See also:
Acquisition Manager
Worklist Columns Available
Acquisition Manager – Modifying Panels and Worklists
Customize Worklist Columns
Deleting a Worklist Row
Adding a Panel
Adding a single Test to the Worklist

Acquisition Manager - Protocol

Protocol

Displays the name of the currently selected protocol.



An alternative protocol may be selected  .

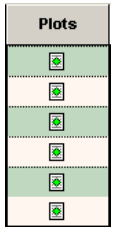
Acquisition Manager – Plots

Plots – A two-state toggle button displayed as icons. This column is de-selected by default. The two available states are:

Load the plots, and gates from the protocol, this is the default option.

Pass the plots, and gates through from the previous acquisition.

The "Pass Plots" option is not available for auto-setup applications.



Note: Passing Plots is effectively passing the protocol to the next line in Acquisition Manager. If the panel has different protocols in place and Pass Plots is selected, the entire protocol will be passed to the next tube in the panel. When the Pass Plots option is selected then this takes precedence over the Load Regions from Current Protocol option.

See also:
Worklist Columns Available
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - Region Sources

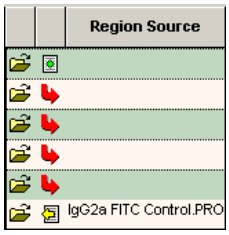
Region positions can be passed between different protocols within a panel – regions can, for example, be set on a control sample then passed through all the tests in a panel.

Note: In order for a region position to be carried into a new protocol the source and target region must satisfy all the following criteria:

- Regions must have the same name
- Regions must be of the same type
- Regions must be drawn on the same parameters.

All regions satisfying these criteria are passed from one protocol to another.

There are three options for the region source:



This option loads the region positions from the current protocol

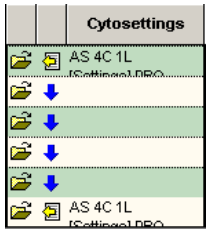
This option passes the region positions from those of the previous tube in a panel.

This option loads region positions from any suitable protocol, use the Browse button to select the required protocol. The name of the selected protocol then appears.

See also:
Worklist Columns Available
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - Cytosettings

Cytosettings – three-state toggle displayed as icons or bitmaps. These states being:



Loads the instrument **cytosettings** from the protocol,

Passes the instrument **cytosettings** through from the previous acquisition.

Note: When using the Instrument Settings column of the Aquisition Manager to pass settings between protocols within a panel, you should ONLY pass settings between protocols where the parameter number and order are identical.

Retrieve the instrument **cytosettings** from an external file. For example, from an AutoSetup protocol, another protocol or listmode file.

Note: When using the Instrument Settings column of the Aquisition Manager to retrieve settings from an external file within a panel, you should ONLY retrieve settings from external files where the parameter number and order are identical.

When the **Retrieve instrument settings from external file** option is selected, the external file name is displayed in this field. A Browse button is provided so that a setting file can be opened.

See also:
P1 - P32
Worklist Columns Available
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - Parameter Names

Parameter Names – An editable box displaying the default parameter names from the protocol or panel. This allows free text entry of parameter names to be added as required.

Changes made here are updated in the protocol once acquisition begins. Protocol Save can then be used to save these parameter names in the panel. See Edit FCS Header Attributes for an alternative way of saving parameter names in a protocol prior to acquisition.

In this example, the columns are numbered P1 to P6. The actual number present is determined by the number of parameters selected in the protocol; the maximum number you can have is 32.

P1	P2	P3	P4	P5	P6
FS PEAK	FS	SS	CONTROL FITC	CONTROL PE	CD45 ECD
FS PEAK	FS	SS	CD3 FITC	CD19 PE	CD45 ECD
FS PEAK	FS	SS	CD3 FITC	CD4 PE	CD45 ECD
FS PEAK	FS	SS	CD3 FITC	CD8 PE	CD45 ECD
FS PEAK	FS	SS	HLA-DR FITC	CD19 PE	CD45 ECD
FS PEAK	FS	SS	CD10-FITC	CD19 PE	CD45 ECD

See also:
Worklist Columns Available
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - Tube ID

Tube ID	Carousel No.	Location	Sample ID 1	Sample ID 2	Sample ID 3	Sample ID 4
89268232953	1	1	Sample 1	Whole Blood	Normal	
89268232954	1	2	Sample 1	Whole Blood	Normal	
89268232955	1	3	Sample 1	Whole Blood	Normal	
89268232956	1	4	Sample 1	Whole Blood	Normal	
89268232957	1	5	Sample 1	Whole Blood	Normal	

Tube ID – Displays the bar-code number from the tube. Allows positive sample identification and an error is generated if the Tube ID does not match the bar-code label on the sample tube. If no Tube ID is entered, but a bar-code label is present on the sample tube and run on the Gallios, the bar code is read and the Tube ID field is updated with this information.

Acquisition Manager - Carousel No. and Location

Tube ID	Carousel No.	Location	Sample ID 1	Sample ID 2	Sample ID 3	Sample ID 4
89268232953	1	1	Sample 1	Whole Blood	Normal	
89268232954	1	2	Sample 1	Whole Blood	Normal	
89268232955	1	3	Sample 1	Whole Blood	Normal	
89268232956	1	4	Sample 1	Whole Blood	Normal	
89268232957	1	5	Sample 1	Whole Blood	Normal	

Carousel No. and Location – Allows you to to specify the Carousel number and the location of a tube in the carousel. You can run a tube at multiple points in a worklist (for example: a set-up or cleaning tube) and random access to sample tubes.

Acquisition Manager - Sample ID

Tube ID	Carousel No.	Location	Sample ID 1	Sample ID 2	Sample ID 3	Sample ID 4
89268232953	1	1	Sample 1	Whole Blood	Normal	
89268232954	1	2	Sample 1	Whole Blood	Normal	
89268232955	1	3	Sample 1	Whole Blood	Normal	
89268232956	1	4	Sample 1	Whole Blood	Normal	
89268232957	1	5	Sample 1	Whole Blood	Normal	

Sample IDs – Up to four Sample ID fields may be displayed. Any required information can be entered into these fields. Sample ID1 is the same for all tubes of a panel. Sample ID's 2-4 can be edited for any tube of the panel. If you do not enter Sample ID1, then the first run number of the panel will auto fill as Sample ID1 for all tubes in a panel.

See also:
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - CAL Factor

CAL Factor – Edit box containing absolute count CAL factor, designed to allow the use of multiple Flow-Count, Leuko-Count or Stem-Count fluorospheres batches. The CAL Factor  button allows the display of the **Absolute Count Calibration** dialog box. See Absolute Count Calibration for more details.

Note: If the same CAL Factor is used for multiple panels in a worklist, use Shift +  to autofill the entire worklist with the CAL Factor.

See also:
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - LMD File Name

LMD File Name – The File Name displayed in this column is only a provisional file name. Some available options (for example: Run Number and Time) can only be defined at the time of acquisition. If these elements are included in the file name and a printout of the Worklist with the final assigned file name is required. They only print correctly once acquisition of samples is complete. A Browse  button is available, allowing file name definition through the Workspace Preferences - LMD File Name option.

See also:
Worklist Columns Available
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - Stop Condition and Stop Value

Stop Condition – By clicking the mouse within this box, you can select the Stop Condition to apply. This option allows you to override the stop conditions present within a protocol.

The options are:

Protocol	to use the preset stop condition.
Time	to Stop at a particular time.
Maximum events	when the require number of event have been collected.

Stop Value – Detected from protocol and can be edited, allowing you to override currently defined stop value.

Note: If a stop count is used on a plot that contains an AutoGate region, the stop count is not exact. The Stop Condition and Stop Value selections are disregarded when running locked protocols.

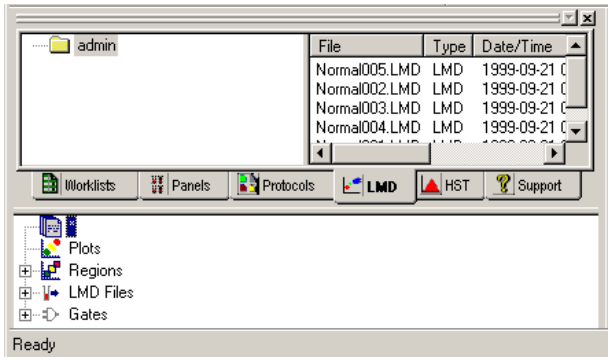
See also:
Acquisition Manager
Customize Worklist Columns

Acquisition Manager - P1-P32

P1-P32 refers to the number of parameters in the protocol or listmode file. Selecting or deselecting P1 results in also selecting or deselecting P2 through P32 from being viewed as an Acquisition Manager column.

Resource Explorer

The Resource Explorer occupies a definable area within the Workspace. It allows you to browse files, and can be used to Drag and Drop items into Gallios software. Protocols, Panels and Worklists can be dragged from the Resource Explorer and dropped into an Acquisition Manager Worklist. Information added in this way always appears at the end of the Worklist. The order may be changed using Drag and Drop after the information has been added.



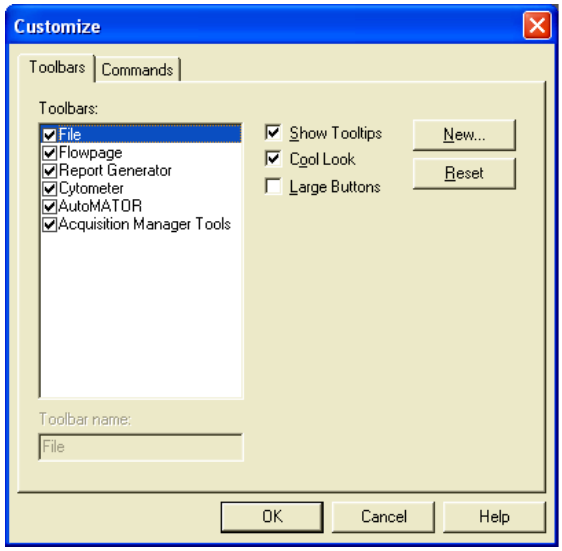
The view that you see depends upon your particular access rights. User levels show only a particular user Listmode, Histogram and Protocol directory, depending on the tab selected.

If you are a member of a particular Workgroup, all the user folders within that Workgroup are visible. The Support tab provides a link to the Beckman Coulter web site.

Toolbars - Customize Toolbars

This allows you to select the style and which Toolbars are displayed.

Toolbars
Toolbar Name
Show ToolTips
Cool Look
Large Buttons
New...
Reset



If a customized Toolbar has been created and this is highlighted, Delete is available to allow the deletion of that Toolbar.

See also, Toolbars - Customize Commands Tab

Toolbars

Select which Toolbars you wish to be displayed. Any customized Toolbars are listed.

Toolbar Name

Allows you to modify the name of any customized Toolbar. It is grayed out for the standard Toolbars.

Show ToolTips

Show Fly-over help when the mouse is pointed to the Icon.

Cool Look

This changes the style to a "cool" look.

Large Buttons

Changes the Icon buttons to a Large size.

New...

Displays a dialog box to enter your customized name of your new Toolbar.

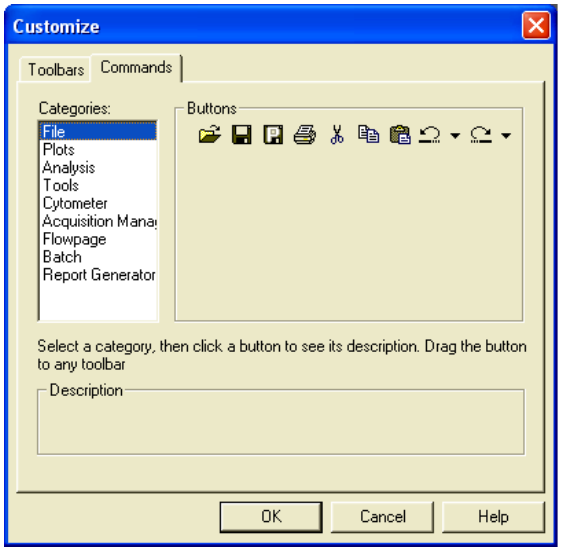
Reset

Resets the Toolbar to the default values.

Toolbars - Customize Commands Tab

This allows you to customize the Toolbars by dragging the button to the desired position within any Toolbar.

Categories
Buttons
Description



See also, Toolbars - Customize Toolbars

Categories

Displays the Toolbars that are currently available.

Buttons

Shows the buttons that may be selected for any Toolbar.

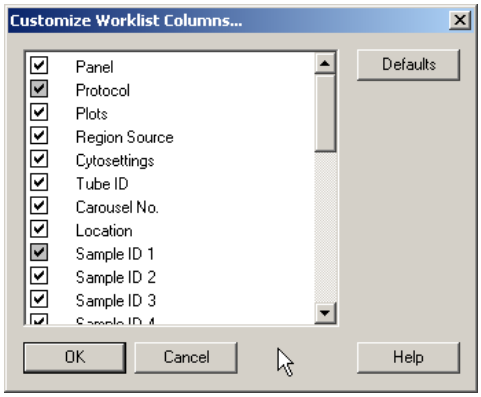
Description

Displays a description of the Icon button selected.

Zoom

The following option allows you to zoom in on a FlowPAGE plot to 50%, 75% 100%, 200%, and To Fit. Select the desired view. Selecting 100% returns to the default size.

Customize Worklist Columns



Use the Customize Worklist Columns dialog box to add, remove, or edit Worklist Columns displayed. Columns can also be renamed or reordered.

A column is included or removed by enabling or disabling the checkbox. If the checkbox is grayed out it cannot be altered.

Note: Checking P1 will effectively select all parameters that are present in the protocol to be viewed as columns in Acquisition Manager

The Column Name can be edited by double clicking on the name and renaming as appropriate. If the name has a lock icon, no changes are allowed.

The Default button resets the Worklist Columns to the standard default and consequently resets the Workspace Preferences – LMD File Name.

Note: If a particular Worklist Column is not displayed, the standard default heading still shows on the Workspace Preferences – LMD File Name tab dialog box.

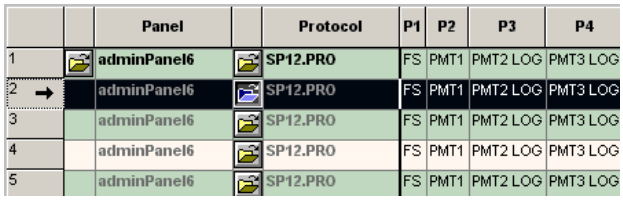
If the Names are changed, this reflects in the name labels within Workspace Preferences – LMD File Name.

Reordering Columns

The columns may be reordered by selecting a column or a group and using Drag and Drop to move it to the desired location. To move a column within a group, use Drag and Drop. To move a group of columns use Ctrl+Drag and Drop. Groups are defined by thick black lines, and items within the groups are separated by thin dotted lines.

Deleting a Worklist Row

To delete a Row within a Worklist, highlight the appropriate Row or Rows by clicking the mouse in the Row Number column shown by the ➜.



		Panel		Protocol	P1	P2	P3	P4
1		adminPanel6		SP12.PRO	FS	PMT1	PMT2 LOG	PMT3 LOG
2 ➜		adminPanel6		SP12.PRO	FS	PMT1	PMT2 LOG	PMT3 LOG
3		adminPanel6		SP12.PRO	FS	PMT1	PMT2 LOG	PMT3 LOG
4		adminPanel6		SP12.PRO	FS	PMT1	PMT2 LOG	PMT3 LOG
5		adminPanel6		SP12.PRO	FS	PMT1	PMT2 LOG	PMT3 LOG

Use  or Delete within the Acquisition Manager Toolbar to delete the appropriate rows.

If you inadvertently mark a row or rows for deletion, click the mouse within the body of the Worklist outside of those rows highlighted.

Carousel Overflow

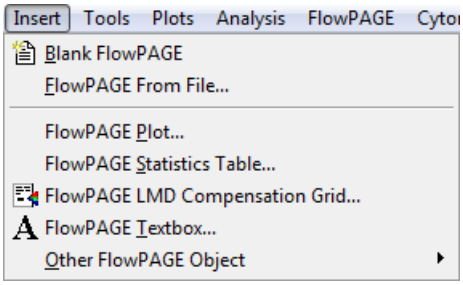
A Gallios Flow Cytometer carousel can hold a maximum of 32 tubes. Creating a worklist with more than 32 tubes in one carousel generates a carousel overflow message.

 OK to jump to the point in the worklist where a new carousel number must be added. This is the initial tube of the next complete panel. Panels can not be split across carousels.

 Cancel to clear all the carousel information (when running in manual mode, for example).

10.17 INSERT MENU

Blank FlowPAGE
FlowPAGE from File
FlowPAGE Plot
FlowPAGE Statistics Table
FlowPAGE LMD Compensation Grid
FlowPAGE Textbox
Other FlowPAGE Object



Note: In certain circumstances, when you insert additional plots or statistics into a FlowPAGE, they become locked outside the boundary. To remedy this, print the FlowPAGE. After printing, the added plots or statistics are unlocked and can be moved into the boundary of the FlowPAGE.

Blank FlowPAGE

To open a new blank FlowPAGE,  .

Note:

- The page displayed in the FlowPAGE is the same aspect ratio and paper orientation as used in the default Printer.
- The page scaling can be changed at any time after a FlowPAGE is created.
- A maximum of 24 FlowPAGEs can be created per protocol.
- FlowPAGES in a locked protocol can not be inserted, deleted or edited.

All FlowPAGEs contain:

- Institution name
- Acquisition or playback protocol
- Acquisition date and time
- User ID
- Settings file name, date and time
- Listmode file name
- Total acquisition time and stop condition
- Sample ID 1
- Protocol Status
- Analysis date
- Instrument serial number
- Software version

Protocol Status

There are three options:

- Runtime Results - displayed when the FlowPAGE is printed as part of the acquisition output options.
- Listmode Replay: Runtime Protocol - displayed when the data is replayed through the same protocol it was acquired under. The protocol content is used to determine whether this is the runtime protocol, not the protocol filename. If an AutoGate is included in protocol, it may display New Protocol if the autogating algorithm repositions the region when the data is replayed.
- Listmode Replay: New Protocol - Displayed when the data is replayed through a different protocol to the one it was acquired under.

Note: In the case of a locked protocol the options for Protocol Status are as described above, but, Locked Protocol is added to the status; e.g. Listmode Replay: New Protocol, Locked Protocol.

FlowPAGE from File

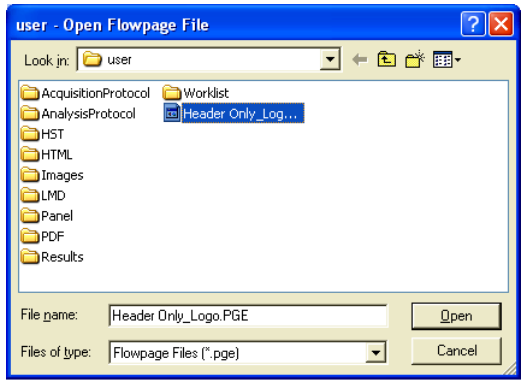
When a FlowPAGE has been created, (including all the departmental logos, addresses, plots, statistics and comment boxes), the whole FlowPAGE can be saved and retrieved.

Note: If the retrieved FlowPAGE contains plots or objects not present in the current protocols, they will display as blanks or red crosses.

Use the open FlowPAGE option to open a FlowPAGE template from disk. FlowPAGE uses the default file extension *.PGE. The *.PGE extension is only a suggested extension, any standard DOS characters can be used for the full file name.

---

1 **Insert ▸▸ Open FlowPAGE From File** to view the **Open FlowPAGE** dialog box.



---

2 the desired drive in the Drive List box.

---

3 the desired directory in the Folders List box.

---

4 the type of file in the Files List box and click.

---

5 the name of the file to view. OK .

Or

Double click on the file name to close the dialog boxes and display the file.

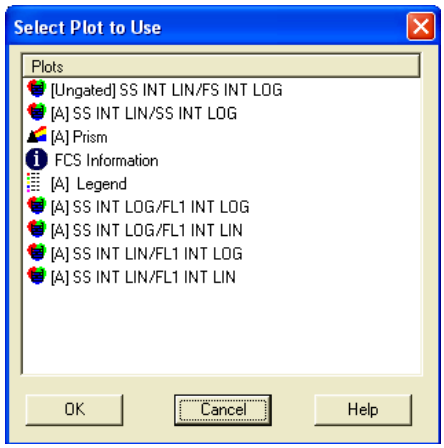
FlowPAGE Plot

Insert Plot

To insert plots currently displayed on the desktop into FlowPAGE.

---

1 **Insert** ▸▸ **FlowPAGE Plot** and a plot in the list.



---

2 OK. The plot is placed in the upper left corner of the FlowPAGE.

Inserting Drag & Drop Plot

Insert currently displayed plots on the desktop into FlowPAGE.

---

1 inside the plot to be inserted into FlowPAGE and hold down the left mouse button. The No Drop cursor is displayed.

---

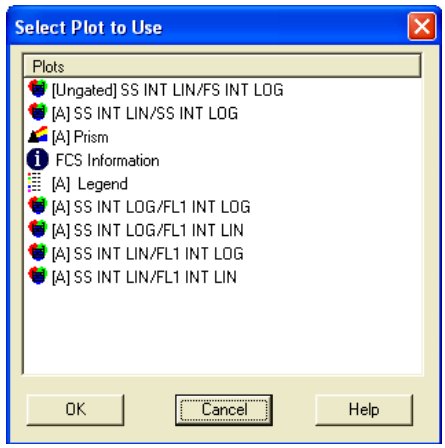
2 Drag the cursor onto the FlowPAGE. The Drop cursor is now shown. Release the mouse button. The plot is now inserted into FlowPAGE.

Note: If you Drag and Drop the plot, the plot and the statistics are inserted. If you Ctrl+Drag and Drop the plot, the statistics are not inserted with the plot.

FlowPAGE Statistics Table

This option is used to insert statistics into FlowPAGE.

1 **Insert ▸▸ FlowPAGE Statistics Table**.



2 From the list of plots currently open in Gallios software, the one containing the statistics you wish to insert.

Cancel or press Esc to exit the dialog box without accepting the changes.

3 The statistics for the selected plot are inserted into the FlowPAGE and can then be moved to the desired position.
Only one plot is permitted per statistics table.

FlowPAGE LMD Compensation Grid

The LMD Compensation Grid shows the compensation settings used to generate the data seen on screen and in plots on the FlowPAGE.

FlowPAGE Textbox

This option is used to insert text into FlowPAGE.

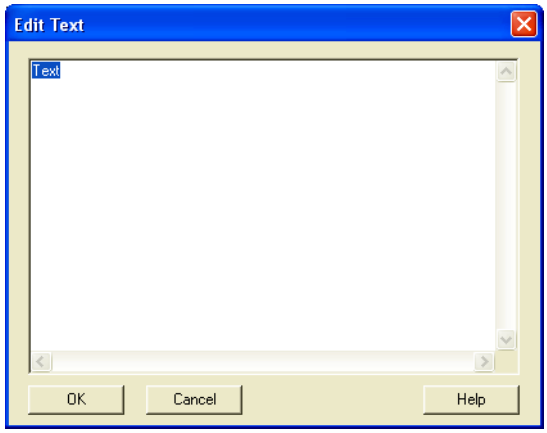
1 **Insert ▸▸ FlowPAGE Textbox**. An insert text cursor A is displayed.

2 Move the cursor to the place for the textbox and .

3 Type the required text and 

.

Note: To preserve the integrity of the header and footer information, any text boxes placed with in the FlowPAGE header or footer space will not persist.



4 outside the text box to accept the changes.

5 Click the right mouse button on the text box to edit properties, enter text, edit the font or change the alignment.

Other FlowPAGE Object

Inserting a Rectangle

1 **Insert ▸▸ Other FlowPAGE Object** and the Rectangle option.
A cross-hair cursor + is displayed.

2 and hold down the left mouse button while you drag to size the rectangle.

---

3  outside the rectangle to anchor it.

See also:
Inserting a Line
Inserting a Picture
Inserting a Text File

Inserting a Line

Draw straight lines using **FlowPAGE ▸▸ Insert ▸▸ Line**.

---

1  **Insert ▸▸ Other FlowPAGE Object** and  the Line option.
A cross-hair cursor + is displayed.

---

2 Drag the cursor to desired line size and release the mouse button. The line is drawn and the cursor reverts back to the Chooser cursor  .

See also:
Inserting a Picture
Inserting a Text File

Inserting a Picture

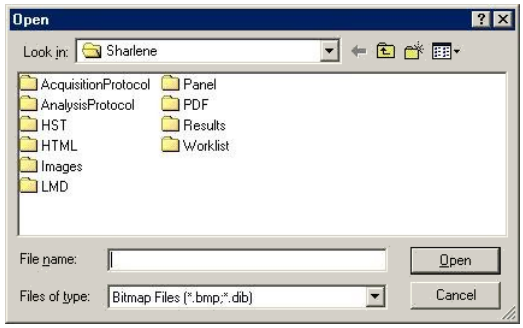
FlowPAGE allows the import of a Windows Bitmap file (extension *.bmp).

---

1 Use MS Paint or other graphics editor to create a bitmap file to be placed on a FlowPAGE and save it as a file.

 **Insert ▸▸ Other FlowPAGE Object** and  the **Picture** option.

2 Select the desired bitmap file.



a. the desired directory in the **Look in:** list box.

b. the type of file in the **Files of type**: list box.

c. the name of the bitmap file you want to insert.
Note: Double click on the file name to close the dialog box and display the file.

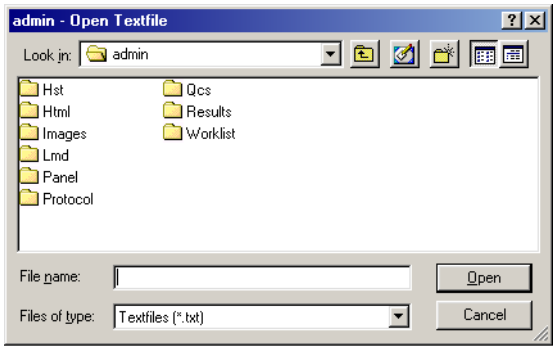
3 the FlowPage location where you want to place the picture.

See also:
Inserting a Line
Inserting a Rectangle
Inserting a Text File

Inserting a Text File

1 **Insert ▸▸ Other FlowPAGE Object** and the Text File option.
Note: Use MS Notepad or other text editor to create text (*.txt) files.

2 Select the desired text file.



a. the desired directory in the **Look in:** list box.

b. the type of file in the **Files of type:** list box.

c. the name of the text file you want to insert.
Note: Double click on the file name to close the dialog box and display the file.

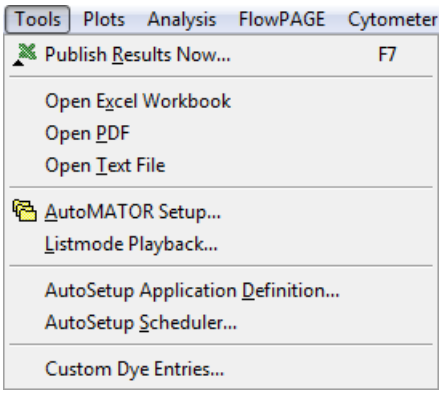
3 The text box is placed in the upper left corner of the FlowPage.

a. Use to move the text box to the desired location.

b. ouside the text box to anchor it.

See also:
Inserting a Line
Inserting a Picture
Inserting a Rectangle

10.18 TOOLS MENU



Publish Results Now

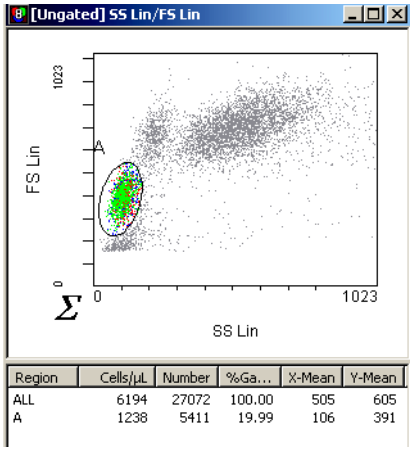
Statistics are displayed under plots in resizable columns and can be exported to Microsoft Excel or ASCII text (.txt) file format.

The Workspace Preferences – Publish tab option **Publish Data to Text File** and/or **Publish Data to MS Excel** must be enabled. See Workspace Preferences - Publish.

To open the Statistics Pane, move the mouse over the Plot so that the Σ (Sigma Cursor) is displayed and click the left mouse button. The Statistic Pane drops down showing ALL the Plot statistics. To hide the Statistics Pane, click the mouse on the Sigma cursor again.

If other Regions are added or deleted while the Statistics Pane is open, it is automatically resized to accommodate the change.

Plots, including the Statistics Pane, can be resized at any time by dragging any part of the Window Frame although the height of the Statistics Pane remains unchanged.



The Statistics Pane can only be resized by using the Splitter Bar while holding down Ctrl. Use the mouse to drag the revealed Statistics Pane to the required position. If resizing is achieved using the splitter bar, the automatic sizing using the Σ (Sigma Cursor) is overridden.

Auto sizing is the default mode selected when the Plot is first created whereas user-defined sizing is selected as soon as resizing occurs using the Splitter Bar. To reinstate the Auto sizing, hold down Ctrl while clicking the Σ (Sigma Cursor) with the mouse.

Open Excel Workbook

Displays the Open XLS dialog which allows your to select an Excel workbook from the Windows file system. The dialog will default to your User Results directory.

Note: An error may display if a workbook is opened manually by a user and subsequently Gallios attempts to open the same workbook.

Open PDF

Displays the Open PDF File dialog which allows you to select a PDF file from the Windows file system. The dialog will default to your User Results directory. Once a file has been selected, Adobe Reader is launched to display the selected file.

Open Text File

Displays the Open TXT dialog which allows you to select a Text file from the Windows file system. The dialog will default to your User Results directory. Once a file has been selected, WordPad is launched to display the selected file.

AutoMATOR

Welcome to AutoMATOR

Use the AutoMATOR option to sequentially read, analyze and report a set of listmode files. AutoMATOR performs batch analysis of stored data using a multi-file protocol.

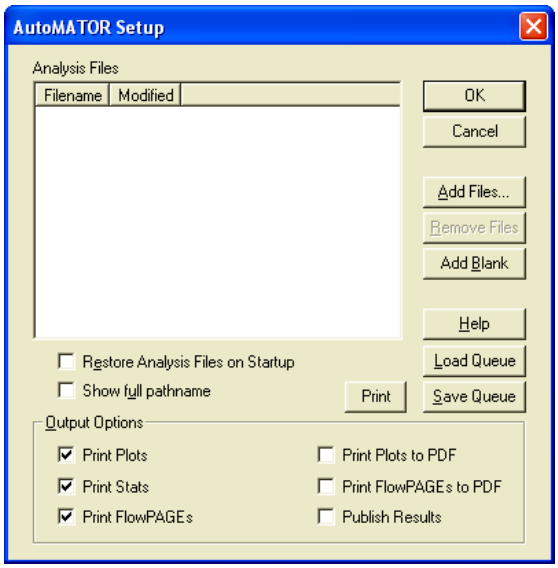
AutoMATOR allows listmode files to be read and analyzed in a sequential or a predetermined batch order.

AutoMATOR Setup

---

1  to display the set of files last used. If these files are not required, highlight them and  Remove Files.

Reordering Files
Add Files
Remove Files
Add Blank
Load Queue
Save Queue
Print
Restore Analysis Files on Startup
Show full pathname
Output Options



2 Add Files... or Load Queue to select the required files.

3 Navigate to the correct directory and locate the relevant files.

4 Select the relevant files by highlighting with the mouse. To select more than one file, hold down Ctrl and click the relevant files with the mouse button (to deselect, click again while Ctrl is held down).

5 **Open**.
The files are listed in the AutoMATOR Setup dialog box.

6 OK to accept changes. Cancel does not apply any of the changes made.

Note: To select all the files, press Ctrl+A.

Status Icon:

Pause	The Auto Pause function pauses the run of tests pauses and then carries on with the rest of the panel automatically after a 15-second wait. The countdown shows on the AutoMATOR Status Bar. When the tests are in the Auto Pause setting, you can hold down Shift and click the Pause button. Shift overrides the Auto Pause, placing the AutoMATOR into Continuous Pause. To restart, click the Run button and the test resumes from the point at which it was paused.

	Continuous Pause	Pauses the tests for modifications and remains on pause until you decide that processing continue. This function allows the test to stop without reverting to the beginning of the panel again. To resume the test, click the Run button.
	Continue	Plays back the indicated file without pausing.

These icons allow you to insert a pause or a stop into the panel, or let the panel run straight through from start to finish. Clicking repeatedly on the icon until it displays the desired status modifies the status in the highlighted file.

...\File001.LMD
...\File002.LMD
...\File003.LMD
...\File004.LMD

In this example, the four files would run and then pause before printing. This allows you to make any necessary alterations to the plots and regions before outputting results.

Note: When replaying multiple batches of listmode files or running a batch of large listmode files, it is recommended to use the AutoPause or Continuous Pause selections

Reordering Files

Once all files are listed, they can be arranged in File Name or Date and Time order by clicking on the relevant column title. Rearrange files by Dragging and Dropping them.

---

1 on the relevant file so that it is highlighted.

---

2 Hold the mouse button down and drag the file to the appropriate position so that it is between two file names.

---

3 Release the mouse button.

If there are a number of files in the AutoMATOR Setup dialog box, you can either use the scroll bar, or utilize the Drag and Drop option.

Select a file using the mouse, keep the mouse button held down and drag to either the top or the bottom of the window. Moving the cursor outside the window scrolls the list. The further the cursor is pushed to the top/bottom, the faster it scrolls.

Add Files

Select this option to display the Open Listmode File dialog box. You can then peruse other Files in various drive and directories and add them to the list.

Select the name of the listmode file(s) you want to add.

Remove Files

When selected, this option removes any highlighted files within the AutoMATOR Setup dialog box. The Delete button performs the same function.

Add Blank

A Blank is used to fill a space when there is an incomplete panel of listmode files, so that the results from the tests are kept in the correct order and a complete panel is maintained.

When selected, this option puts a blank at the end of the file list. This can then be Dragged and Dropped into the required position.

Load Queue

Displays a dialog box to load a queue file.

Save Queue

Displays a save dialog box to save the queue to a file. The files are saved in the LMD subdirectory under the file extension ALQ.

Print

Print a listing of the files in the queue.

Restore Analysis Files on Startup

If the checkbox is enabled, all the files displayed in the Analysis File list appear in the list the next time that you log in.

If the checkbox is not ticked, the list is empty the next time you log in.

Show full pathname

Shows the paths to the destination directories for the selected LMD files.

Output Options

These options consist of several checkboxes found at the bottom of the AutoMATOR Setup dialog box. Check the required output options to print or publish data at the completion of each sample. If none of the output options are selected the Report icon is crossed out  .

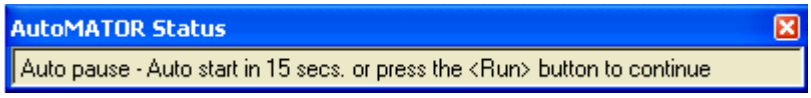
The options are:

- Print plots as displayed.

- Print statistics only.
- Prints all FlowPAGEs.
  If these are not selected, the Page icon appears crossed out, and a report is not printed from the test.
- Publish Plots or FlowPAGES in PDF format.
- Publish Results
  Allows the automatic publishing of results to a text file or Microsoft Excel spreadsheet at the completion of each sample.

AutoMATOR Status Bar

This shows the current file being run and the current state of that file.



The AutoMATOR Status Bar, which displays the function that the test is performing, can be removed from the screen.

Click on the Close  button featured on the Status Bar while the AutoMATOR is running. This hides the bar from view. It automatically reappears again the next time the AutoMATOR is run. The Status Bar cannot be brought back onto the screen once closed down without reopening AutoMATOR.

- Processing
  This is when the file is being analyzed by the software.
- Auto Pause
  When this command is active, it informs you as to the length of time left in pause before the analysis resumes. If this length of time is not required, selecting the Run  button again can restart the analysis.
- Continuous Pause
  This informs you that the processing is paused and can only continue if you restart the analysis manually by selecting the Run  button again.

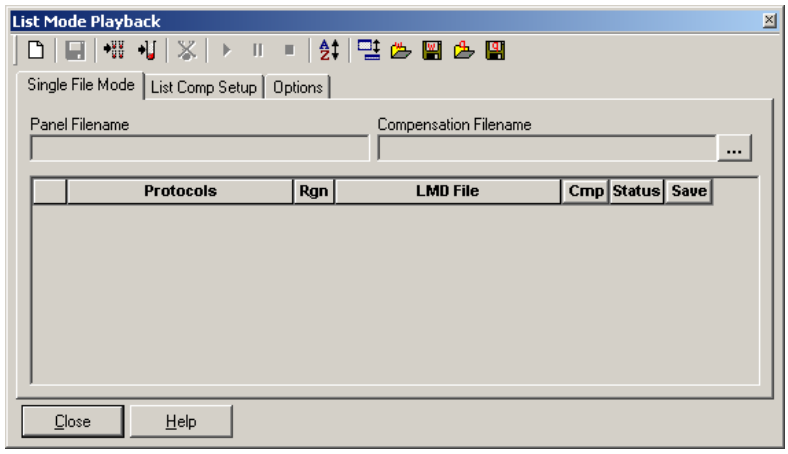
Listmode Playback

The Listmode Playback option allows for listmode playback with runtime protocol/panel or with a new protocol/panel. This option also allows listmode compensation of 20-bit data files in a fully automated manner, replicating the procedure used during analysis. Compensation settings may be verified and manually adjusted if required. The Options tab allows setting of default options for outputs during listmode playback.

Note: Panel Reports generated from listmode replay must be run using the Listmode Playback tool, replaying the listmode files through the desired export panel.

Note: A protocol from a listmode file cannot be used for further acquisitions until it has been saved with a new name.

Single File Mode



This mode allows for single file or panel based listmode file playback, similar to acquisition of samples.

A single panel may be selected; multiple copies of this panel may be run at a time. Tubes may be added or removed from individual panels.

Load Queue loads a listmode queue file (a list of listmode files).

Regions may be Loaded or carried within a panel, AutoGating and positives analysis can be active on any protocol within a panel

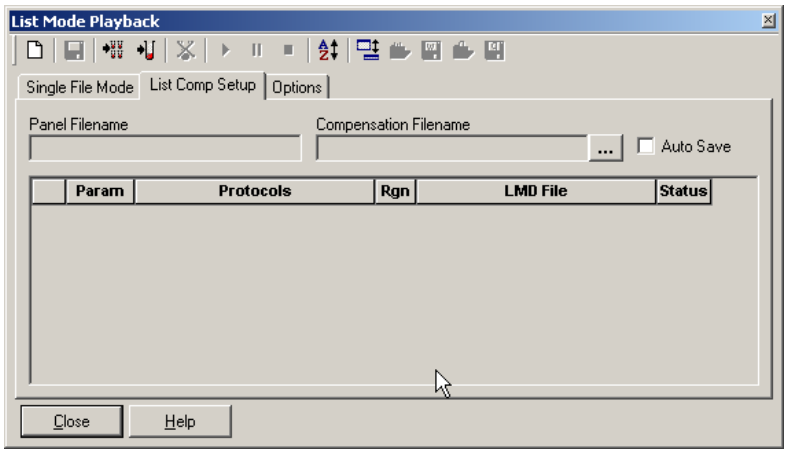
Where the source listmode file is a 20-bit data file, comp settings can be from the runtime protocol (stored within the LMD file), from the Compensation settings file created in ListComp Setup or any *.CMP file may be used.

During playback the title bar displays the current file being analyzed.

Optionally a new file may be saved containing newly compensated 10-bit data. The original LMD file cannot be overwritten automatically.

Saved files have the suffix entered in the Options tab appended to each saved file.

List Comp Setup



This dialog provides an Acquisition Manager style worklist for the selection of a compensation panel and associated protocols. *.ppp files created from the protocols used in the AutoSetup II applications do not need the *_STAND protocol. These *.ppp panels allow you to replay their associated LMD files to create *.cmp files.

Note: only one AutoSetup application *.ppp file may be selected at any given time.

The dialog is resizable by dragging the corners to the required size.

The settings file name defaults to the name of the panel but with a new *.cmp extension.

The Auto Save checkbox allows settings to be automatically written to the settings file that has the name of the panel selected.

Panels can be created within this dialog, but cannot be used for acquisition. These listmode panels are saved with a *.ppp extension.

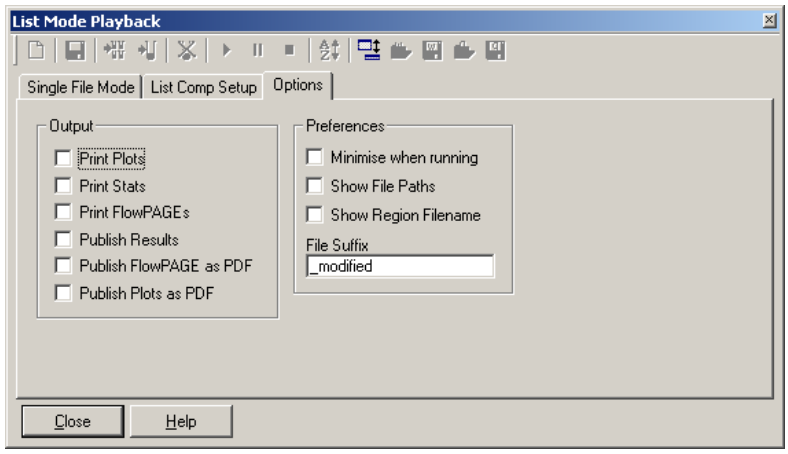
Note: The Save button becomes active when one of the rows in the panel is highlighted.

The Parameter field indicates the Compensation parameter within the protocol.

The Status field allows a pause or stop between each file playback.

Note: Listmode Compensation must be performed using the runtime protocol or a protocol with equivalent parameters as the runtime protocol.

Options Tab



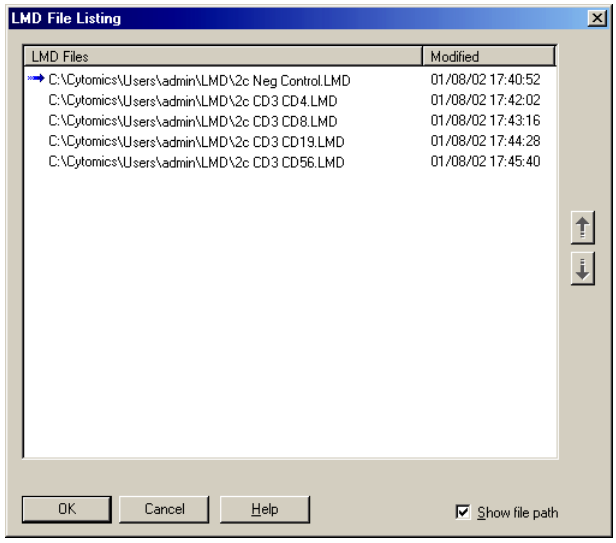
This tab allows setting of default options for the Listmode Playback. Print options, publish functions, file save naming options are set in this dialog box.

Note: If you playback a listmode file through 2 different protocols and have Print to PDF selected, the PDF file is saved after each playback with the same name. If you have the capability to overwrite files, the PDF file will only have the information from the last playback.

File Sorting - In the Listcomp setup or Single file mode options, listmode files may be sorted using the LMD File Listing option. This allows files to be sorted alphabetically, or by Time / Date modified. Files may be Dragged and Dropped. Individual files may be selected and moved up or down the list using the arrow buttons.

The start of each panel is indicated by an arrow.

A **Show File Path** checkbox allows the full file path for each file.



AutoSetup Application Definition

IMPORTANT Risk of erroneous quality control statistics. Panels containing QC regions must be constructed from individual unique protocols. Do not use a panel constructed from one protocol (with different parameter names) for QC database purposes.

Use the Application Definition Wizard to define your applications and save these definitions to be used in the Autosetup Scheduler. The application definition captures the instrument setup, lasers, parameters, fluorochromes, target channels, verification and alignment requirements of a particular application. See APPLICATION DEFINITION WIZARD .

AutoSetup Scheduler

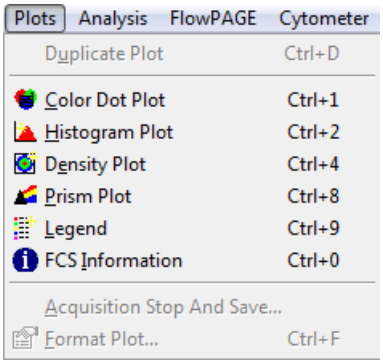
Use the AutoSetup Scheduler to select the applications to be run on the instrument in a given day or "shift". AutoSetup Scheduler groups the selected applications for efficient set up of multiple applications simultaneously and provides the carousel load report to facilitate the setup and loading of samples for daily QC. See AUTOSETUP SCHEDULER to run the scheduler.

Custom Dye Entries

Displays the Custom Dye Entries dialog. This dialog allows you to customize the list of detector dyes for the Application Definition Wizard. The customized list will appear in the dropdown combo boxes in the Detector Dyes page of the Application Definition Wizard.

10.19 PLOTS MENU

Duplicate Plot
Color Dot Plot
Histogram Plot
Density Plot
Prism Plot
Legend
FCS Information
Acquisition Stop and Save
Format Plot



Plots - Introduction

Gallios software can display six Plot types including the FCS Information plot and the Legend for Color Gated Plots. When a Plot is displayed, click the right mouse button to display further menu options.

Note: You cannot add or duplicate plots when running a locked protocol.

Color Dot Plot
Histogram Plot
Density Plot
Prism Plot

Legend
FCS Information

Duplicate Plot

This option creates an exact duplicate of the currently selected Plot.

Ctrl+D may be used as a keyboard shortcut for the same function.

Color Dot Plot

The Color Dot Plot option provides two-parameter displays of data and allows any combination of the parameters to be selected. Each dot in a Color Dot Plot represents one event (cell or particle). The dot location gives the parameter values for each event. Each parameter can have a channel number from 0 to 255 for 256 channel data or 0 to 1023 for 1024 channel data. The colors of the dots reflect the Gate or combination of Gates used to create the plot.

To Create a Color Dot Plot:

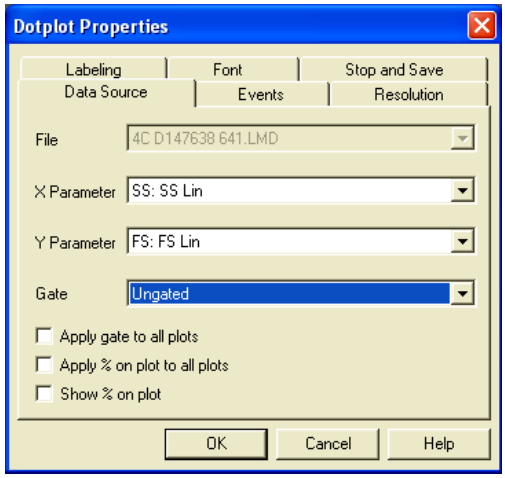
---

1  .

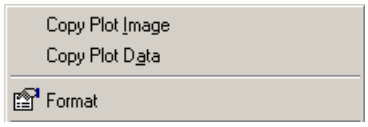
---

2 From the dialog box displayed, select the required File, then select desired Gates, Parameters and Regions for display.
Dot Plot Data Source
Dot Plot Events
Dot Plot Resolution
Dot Plot Labeling
Font
Stop and Save (Acquisition only)

Note: You cannot make any changes on this screen when running a locked protocol.



3 When a Plot is displayed, click the right mouse button within the plot to display further menu options.
Copy Plot Image
Copy Plot Data
Format Plot



Copy Plot Image

Copies the current plot image into memory for pasting into other applications. See Copy for more details.

Copy Plot Data

Copies the raw plot data into memory for pasting into other applications, such as Microsoft Excel.

Format Plot

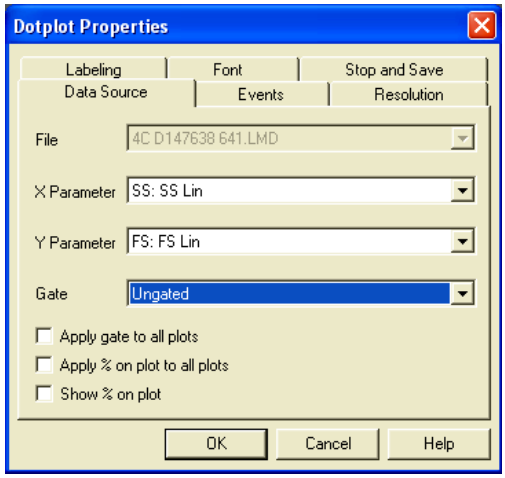
Displays the Plot Properties dialog box for the current plot. See Format Plot.

Dot Plot Data Source

Choose the X and Y parameters for the current plot. Gating equation and regions to be displayed can be selected.

File
Gate
X and Y Parameter
Apply Gate to All Plots
Apply % on plot to all plots
Show % on plot

Note: You cannot make any changes on this screen when running a locked protocol.



File

Read-only field of file currently populating the plot.

Gate

Available Gates are listed. Gates required for Advance Precedence should be selected at this point. See Modify Color Precedence.

X and Y Parameter

Select the required parameter for display from those available in the list.

Apply Gate to All Plots

To select this option, enable the checkbox and apply the gate selected to all plots.

Apply % on plot to all plots

Displays the % of events in the regions on all plots.

Show % on plot

Displays the % of events in the regions on this plot only.

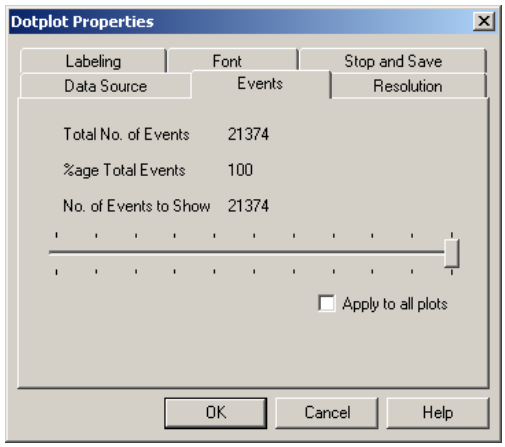
Dot Plot Events

Use this to specify the percentage of events to view. This option significantly increases the speed at which dot plots refresh if large data files are being analyzed. For an event interval less than 100%, data is displayed uniformly from within the whole of the data file, that is, if 10% of events are selected then event numbers 1, 11, 21, 31, and so on, are displayed if they meet the Gating criteria for the plot.

The default value for the number of events to display can be set in the **File ▸▸ Workspace Preferences** and selecting the Plot Display tab.

No. of Events to Show
Apply to All Plots

Note: You cannot make any changes on this screen when running a locked protocol.



See also:
Workspace Preferences - Plot Display
Dot Plot Data Source
Font

No. of Events to Show

Place the cursor on the Slide bar and move to change the **No. of Events to Show**.

Apply to All Plots

Enable this checkbox to apply the **No. of Events to Show** to all Plots.

Dot Plot Resolution

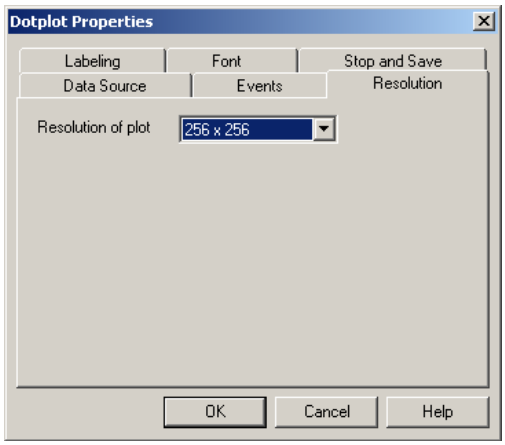
Set the number of channels for the X and Y Axis of the Plot.

Note: You cannot make any changes on this screen when running a locked protocol.

The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

Note: If a higher resolution is selected, a sufficient number of events is required to produce a visually acceptable graph.

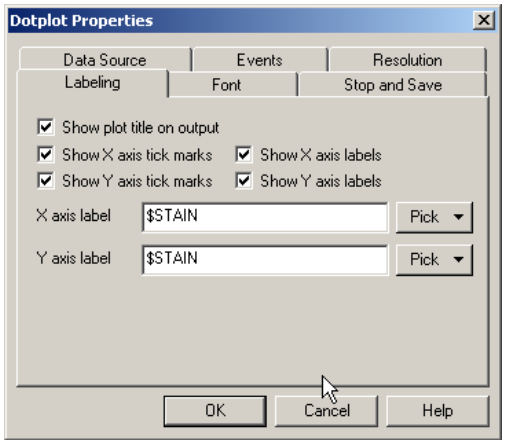


Dot Plot Labeling

The options here allow you to override the default plot display options for the current plot. This is useful when capturing images for publication.

Show Plot Title on Output
Show Axis Tick Marks
Show Axis Labels
X and Y Axis Labels

Note: You cannot make any changes on this screen when running a locked protocol.



Show Plot Title on Output

Unchecking this option prevents the plot title from appearing when the plot is printed or exported.

Show Axis Tick Marks

Unchecking these options hides the axis scale tick marks and labels.

Show Axis Labels

Unchecking these options allows the axis parameter name to be hidden.

X and Y Axis Labels

Allows custom naming of the parameters on the current plot, any FCS keyword or text may be entered. Alternatively select **Pick** to select from File Name, Stain name ($PnS) or Parameter name ($PnN).

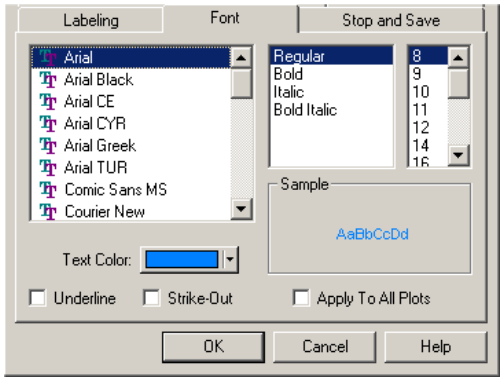
Font

Note: You cannot make any changes on this screen when running a locked protocol.

Select the required Font Type, Font Style and Point Size.

Other font attributes available are **Underline**, **Strike-Out** and **Text Color**. The default color is black.

The Apply To All Plots checkbox allows the selected font to be applied to all the currently displayed plots. You should choose True Type fonts so that X and Y axis displays are available.



Histogram Plot

Single parameter histograms show the frequency distribution of the chosen parameter. All histograms can be customized, that is, Colors, Gate for histogram, Scaling, and so on.

To create a Histogram Plot:

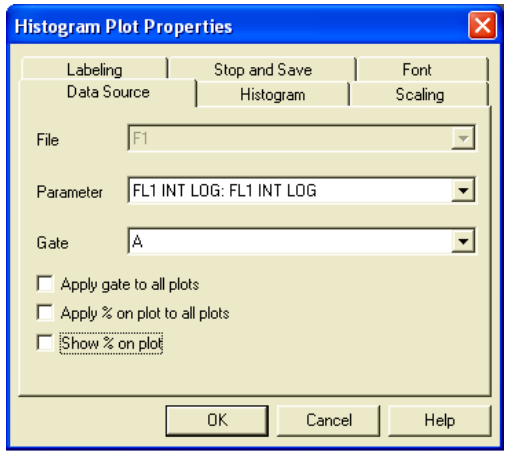
---

1  .

2 From the Data Source dialog box displayed, select the required File, then select desired Gates, Parameter and Regions for display.

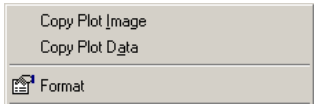
Histogram Plot Data Source
Histogram
Histogram Scaling
Histogram Labeling
Stop and Save
Font

Note: You cannot make any changes on this screen when running a locked protocol.



3 When a Plot is displayed, click the right mouse button to display further menu options.

Copy Plot Image
Copy Plot Data
Format Plot

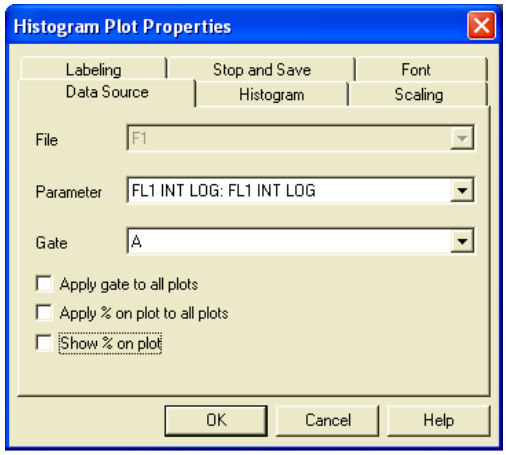


Histogram Plot Data Source

Choose the parameter and gate for the current plot. Gating equation and regions to be displayed can be selected.

File
Gate Selection
Parameter
Apply % on plot to all plots
Show % on plot

Note: You cannot make any changes on this screen when running a locked protocol.



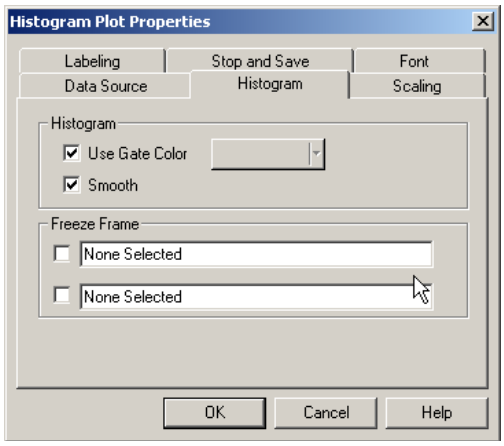
Parameter

Select the required parameter for display from those available in the list.

Histogram Plot Configuration

Use Gate Color
Smooth
Freeze Frame

Note: You cannot make any changes on this screen when running a locked protocol.



Use Gate Color

If the **Use Gate Color** option is checked, the Histogram fill color is the same as the default color for the Gate that the plot is gated upon, and the Color Select button is unavailable. To change the color of the Histogram in the selected plot, clear the **Use Gate Color** checkbox and click the Color Select button. The colors returned are only those which the display is capable of rendering.

Smooth

If the **Smooth** box is checked, the visual representation of the Histogram is smoothed.

Freeze Frame

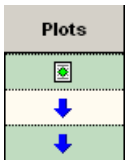
Check a **Freeze Frame** box to assign the current Histogram. The top button creates a dotted overlay and the bottom button creates a dashed Histogram.

The current Histogram is assigned to whichever Overlay button is selected and its name appears in the edit box adjacent to the button. This legend may be modified if desired.

During acquisition, the freeze frame checkbox should be enabled at the end of each acquisition. If the checkbox is enabled during acquisition of a tube, only the outline of the histogram at the time of enabling, is captured (Data acquired afterwards will not be included in the freezeframe).

Using Manual Mode, Single Tube Fixed Position mode gives the ability to 'Stop' and enable the Freeze Frame checkboxes

In order for Freeze Frame to work in Acquisition, ensure that “Plots” is enabled as a column in the Acquisition Manager. Selecting "Pass Plots" is effectively passing the protocol in a panel in Acquisition Manager. If the panel consists of different protocols, using Pass Plots will cause the same protocol to be passed to the next tube in the panel.



Histogram Freeze Frame

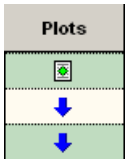
Gallios software allows the display of up to three Histograms on each single parameter Histogram. The base Histogram is the one that relates to the current listmode file and the other two can be overlays from any other compatible file. Statistics are calculated from the base Histogram only.

The other two overlays are for comparison only and no statistical information can be obtained from them.

See also:
Create a Histogram Freeze Frame
Delete a Histogram Freeze Frame Overlay

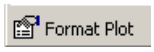
Create a Histogram Freeze Frame

In order for Freeze Frame to work in Acquisition, ensure that pass protocol is set up in the Acquisition Manager.



1 Read in a file or acquire data to be assigned as an overlay (for example: a negative control).

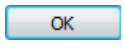
2 Highlight the chosen single parameter Histogram to be used as an overlay plot (click on the title bar).

3 Click the right mouse button on the plot and  

, then  the Histogram tab. See also Histogram Formatting.

4 Check a **Freeze Frame** box to assign the current Histogram. The top button creates a dotted overlay and the bottom button creates a dashed Histogram.

5 The current Histogram is assigned to whichever overlay button is selected and its name appears in the edit box adjacent to the button, this legend may be modified if desired.

6  

or press Enter to accept any changes.

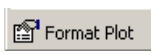
7  

or press Esc to exit the dialog box without saving the changes.

Now you can open another file or acquire a positive sample. The new data is displayed as the base histogram and the previous sample is seen as an overlay.

Delete a Histogram Freeze Frame Overlay

1 Highlight the plot containing the unwanted overlay (click on the title bar).

2 Click the right mouse button on the plot and  

, then  the Histogram tab.

3 Clear the appropriate Overlay Histogram checkbox to delete an overlay.

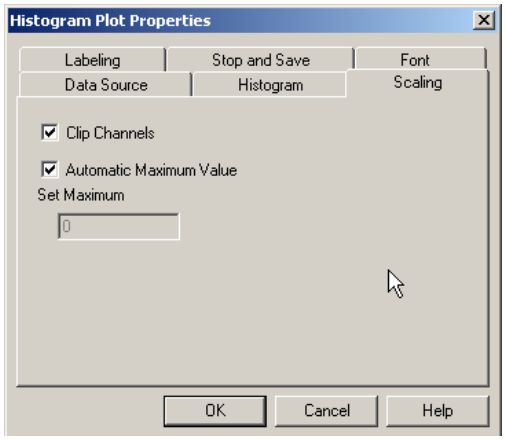
4 OK or press Enter to accept any changes.

5 Cancel or press Esc to exit the dialog box without saving the changes. Gallios software then updates the display.

Histogram Scaling

Clip Channels
Automatic Maximum Value
Set Maximum

Note: You cannot make any changes on this screen when running a locked protocol.



Clip Channels

If Automatic Maximum Value calculation is used then toggling the Clip Channels on ensures that the bottom and top channels are excluded from the calculation of the plots full-scale value for the purpose of scaling.

Automatic Maximum Value

This option searches the histogram for the maximum value and then sets the value found to be the full scale for the histogram.

Set Maximum

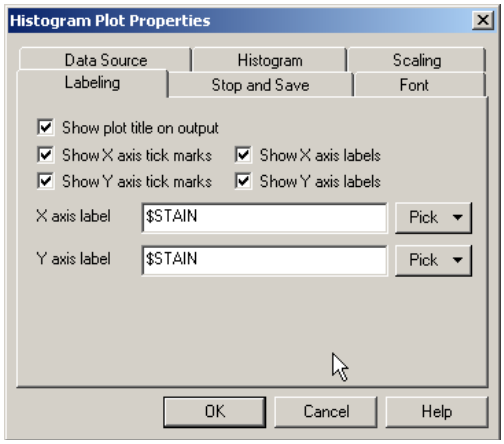
If the Automatic Maximum Value button is cleared, then any value entered here is the full scale for the histogram.

Histogram Labeling

Use the options here to override the default plot display options for the current plot. This is useful when capturing images for publication.

Show Plot Title on Output
Show Axis Tick Marks
Show Axis Labels
X and Y Axis Labels

Note: You cannot make any changes on this screen when running a locked protocol.

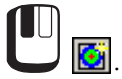


Density Plot

The Density Plot option provides two-parameter data display, any combination of parameters can be selected. Each colored area in a Density Plot represents an ISO-count of the number of events (cells or particles). The color shows the number of events at any X and Y parameter value.

To create a Density Plot:

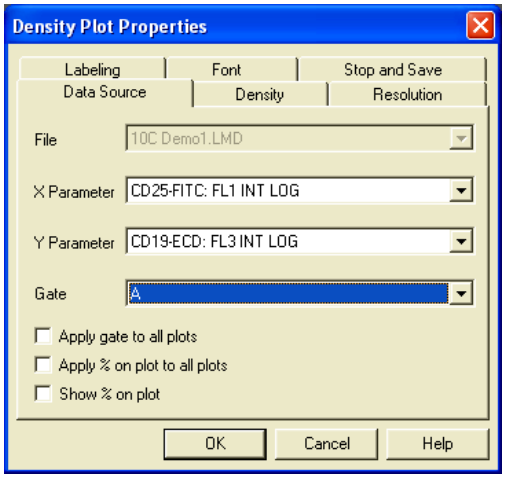
---

1  .

2 From the Data Source dialog box displayed, select the required File, then select desired Gates, Parameters and Regions for display.

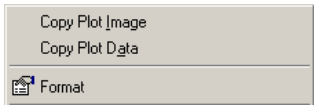
Density Plot Data Source
Density Level Configuration
Density Plot Resolution
Density Plot Labeling
Stop and Save (acquisition only)

Note: You cannot make any changes on this screen when running a locked protocol.



3 When a Plot is displayed, click the right mouse button to display further menu options.

Copy Plot Image
Copy Plot Data
Format Plot

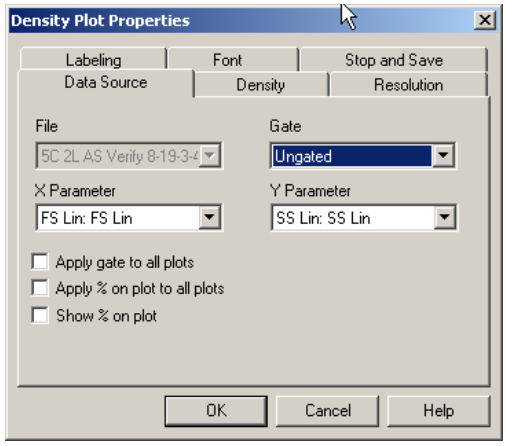


Density Plot Data Source

Choose the X and Y parameters for the current plot. Gating equation and regions to be displayed can be selected.

File
Gate Selection
X and Y Parameter
Apply Gate to All Plots
Apply % on plot to all plots
Show % on plot

Note: You cannot make any changes on this screen when running a locked protocol.

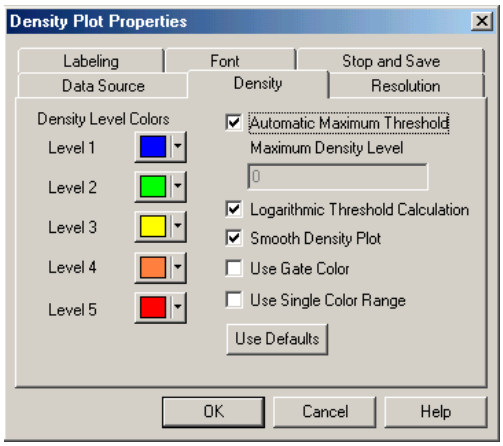


Density Level Configuration

Use this option to specify the Density levels and other configuration preferences.

Density Level Colors
Automatic Maximum Threshold
Maximum Density Level
Logarithmic Threshold Calculation
Smooth Density Plot
Use Gate Color
Use Single Color Range

Note: You cannot make any changes on this screen when running a locked protocol.



Density Level Colors

This Plot may have up to 5 different ISO-count levels, each of which is represented by a color. Choosing the appropriate button may change the default color for each level.

---

1 the Level 1 button to view the Color dialog box.

---

2 Select the desired color that is to be allocated to Level 1.

---

3 Continue as above until all desired changes have been made. OK to accept all changes.

Cancel or press Esc to exit the dialog box without saving the changes.

Automatic Maximum Threshold

This option allows automatic configuration of the maximum value that is used to calculate the Density Color Levels. If this option is not selected you may manually enter the value which is used to calculate the Density Color Levels.

Maximum Density Level

Configure the Density Plot to manually enter the value that is used to calculate the Density Color Levels. This option is only active when the **Automatic Maximum Threshold** button is cleared.

Logarithmic Threshold Calculation

Density color thresholds may be calculated in one of two ways:

- Linear threshold calculation - option toggled off
  The maximum value is used to determine the threshold value for each color. The actual threshold value is calculated as follows: -

  Value = (Level No) x (Maximum In Plot /5) - 1
- Logarithmic threshold calculation - option toggled on
  The maximum value is used to determine the threshold value for each color. The actual threshold value is calculated as follows: -

  Value = 10(Level No) x (log10 (Maximum In Plot /5)) - 1

Smooth Density Plot

Choose this option to smooth the Density plot. Clear it to display raw unsmoothed data.

Use Gate Color

Creates a single color density range based on the currently assigned gate color.

Use Single Color Range

Creates a range of density level colors based on the selected Level 1 and Level 5 colors.

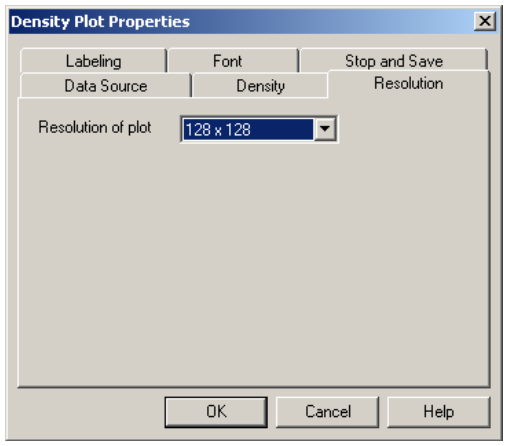
Density Plot Resolution

Set the number of channels for the X and Y Axis of the Plot.

The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

Note: You cannot make any changes on this screen when running a locked protocol.

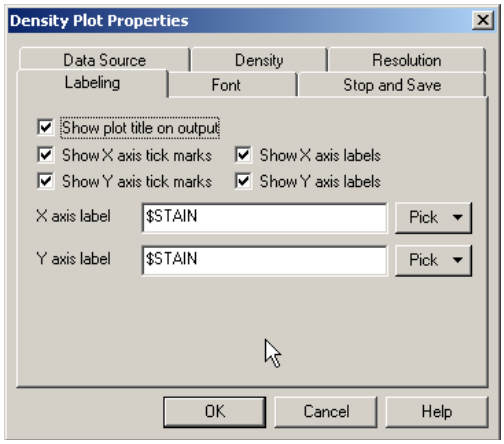


Density Plot Labeling

Use the options here to override the default plot display options for the current plot. This is useful when capturing images for publication.

Show Plot Title on Output
Show Axis Tick Marks
Show Axis Labels
X and Y Axis Labels

Note: You cannot make any changes on this screen when running a locked protocol.



Prism Plot

A Prism Plot is a way of displaying all the subpopulations or phenotype percentages of a multi-color immunocytometry analysis protocol.

The Plot represents the relative percentages of each subpopulation (phenotype) and the number of phenotypes depends on the number of color parameters being simultaneously analyzed. Prism Plots can be customized, for example: Gate for Prism, Scaling, and so on.

To create a Prism plot:

---

1 

---

2 An empty Prism plot window is displayed with the Data Source dialog box overlaying it.

---

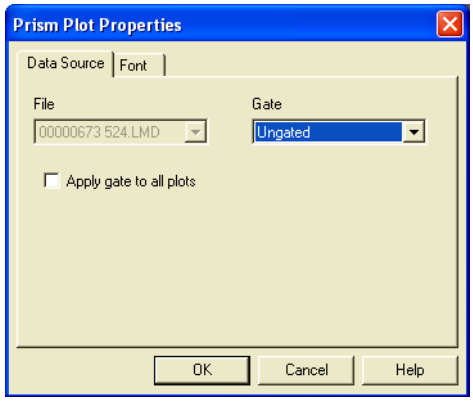
3 Make the desired changes.
Data Source (Prism)
Format Plot

The plot type with the current Gate is displayed in the plot window. Ungated is the default.

Data Source (Prism)

Choose the **File** and **Gate** to be used for the Prism plot.

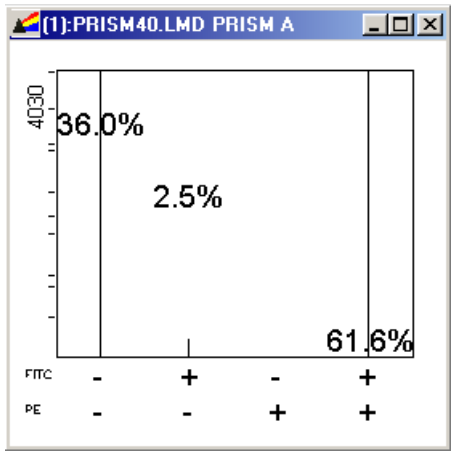
File
Gate Selection
Apply Gate to All Plots



What is Prism?

The Prism parameter is a method of summarizing multi-parameter surface marker data as a one-dimensional Prism plot representing all possible subpopulations or phenotypes.

The Prism plot is a simple bar graph. The height of each bar represents the count of each phenotype. The count as a percentage of all events or gated events is written adjacent or above each bar. The prism plot can be gated, in which case the % values represent the percentage of gated prism events and the gate is indicated in the title bar.

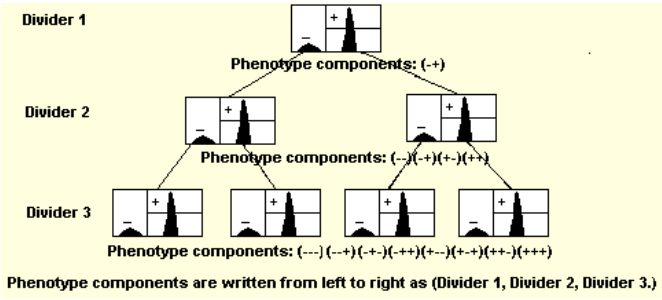


How is Prism Created?

In single parameter analysis of surface marker data, one or two regions are normally set to measure both Negative and Positive cells. We can then look at a second parameter and calculate the 2 phenotypes from parameter-1 positive populations and the two phenotypes from parameter-1 negative populations (four phenotypes in total).

We can repeat this with 3, 4 or more parameters.

The Prism Divider can be used instead to mark the dividing line between the Negative and Positive cells.



Prism Dividers

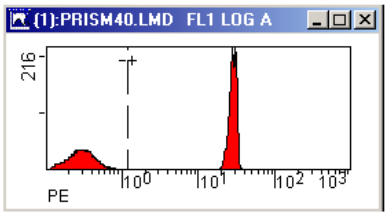
A Prism is created by setting one or more prism dividers. There are three ways of setting prism dividers, two of them involve graphical positioning (single and dual prism dividers). The third uses **Analysis ▸▸ View/Modify Prism** that can set the divider numerically on any divider. Any changes to the divider on one plot is replicated on any other plot using that parameter.

Each new parameter that has a prism divider set on it multiplies the total number of phenotypes by 2.

Only one divider can be set per parameter.

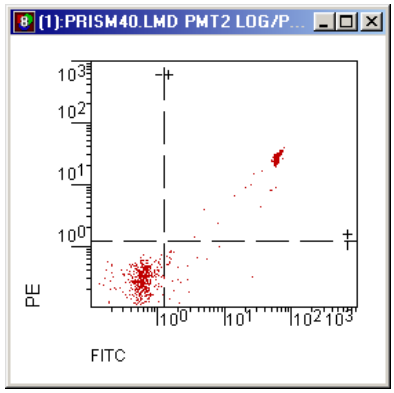
Setting a Single-Parameter Prism Divider

Single parameter prism dividers are set by placing a Single-parameter histogram in focus and selecting . The divider can then be set by clicking on the selected plot, positioning the divider, and then clicking once more to fix the divider position.



Dual Parameter Prism

Dual parameter prism dividers are set by placing a Dual-parameter plot in focus and selecting . This simultaneously creates two prism dividers.



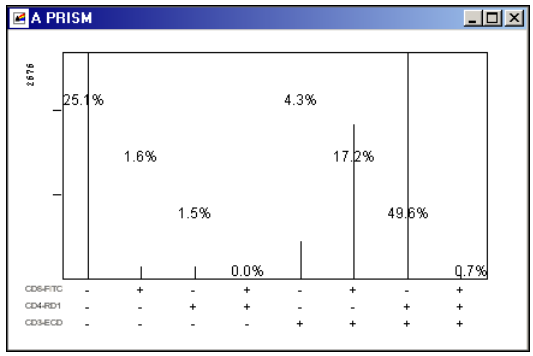
The combinations of dividers are combined to produce 2^p regions
Where p is the number of prism dividers set.

$$2^p \text{ regions}$$

This table shows the number of phenotypes created for up to six Prism dividers set.

Number of Prism Dividers set	Number of phenotypes created
1	2
2	4
3	8
4	16
5	32
6	64

This Plot shows a 3 color Prism Plot (3 dividers set, 8 phenotypes created).
The percentages printed on the prism plot from left to right match one-to-one with the phenotype nomenclature indicated on the axes.
Example:



Triple Positive phenotype (+ + +) is 0.7%.
Triple Negative phenotype (- - -) is 25.1%

The phenotype percentages are staggered to prevent excessive obscuration of the numerical values when a high density of prism information is presented.

Editing Prism Dividers

1 Mouse over the divider to get the ↔ cursor.

2  and drag the divider to the new position.

Removing Prism Dividers

1  the Prism divider you want to delete.

2 ***Press*** Delete**.**

Gallios Software - Prism Compatibility

The Gallios software Prism plot can interpret the Prism parameter derived from:

- RXP/CXP data acquisition
- EXPO™ data acquisition
- EXPO 32 data acquisition
- Elite or ALTRA data acquisition or
- COULTER Elite version 4.x DOS software acquisition.

Gallios software cannot display Prism using listmode files saved from COULTER XL SYSTEM II™ software however, Gallios software can generate Prism plots from XL SYSTEM II files.

Note: Reanalysis of listmode files overrides the analysis protocol's Prism dividers. You have to adjust the dividers when this occurs.

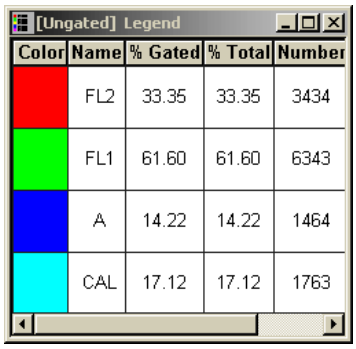
Third party software may not display Prism data correctly since a special Prism data display algorithm is involved to display Prism in Gallios software.

Legend

Use this option to view the population colors and population names,

1 **Plots ▸▸ Legend Plot** to create a legend plot.
When a Plot is displayed, click the right mouse button within the plot to display further menu options.

2 Every time the population names or colors are edited Gallios software updates the Legend Window.
If Gallios software is in Dot Plot Mode-Color Blend mode, Legend displays the names, colors and associated statistics of the color Gate combinations.



3 If Gallios software is in Dot Plot Mode-Color Precedence mode, Legend displays the names, colors and associated statistics of the Gates with the highest precedence at the top.

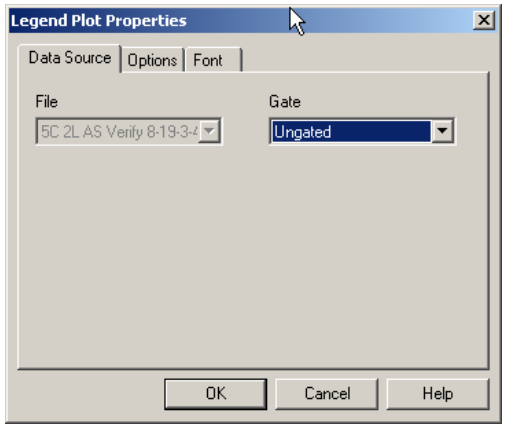
See also:
Data Source (Legend)
Legend - Options Tab
Color Blend Mode - Introduction
Modify Color Precedence
Advanced Precedence

Data Source (Legend)

Choose the **File** and **Gate** to be used for the Legend plot.

File
Gate

Note: You cannot make any changes on this screen when running a locked protocol.



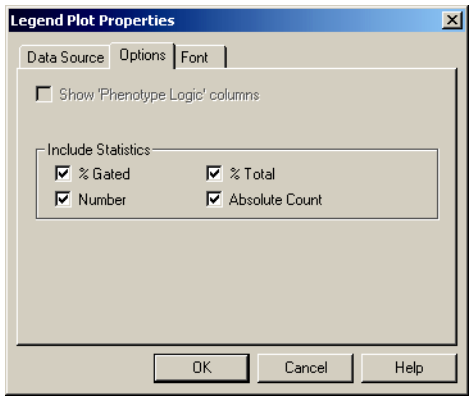
See also:
Legend
Data Source (Legend)
Legend - Options Tab
Format Plot

Legend - Options Tab

The Options tab displays various checkboxes that may be enabled as appropriate.

Show "Phenotype Logic" Columns
% Gated
% Total
Number
Absolute Count

Note: You cannot make any changes on this screen when running a locked protocol.



See also:
Legend
Data Source (Legend)
Format Plot

Show "Phenotype Logic" Columns

Displays the logical combination of Color Gates for each population. Available in Color Blend Mode only.

% Gated

Total number of events in region, as a percentage of events in a gated display.

% Total

Total number of events in region as a percentage of total events in the file.

Number

Total number of events in a Region.

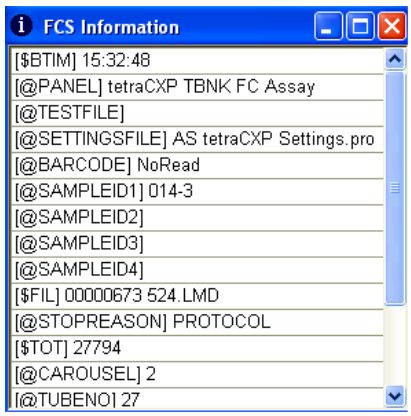
Absolute Count

This displays the absolute count (cells/ul) of a population based on the calibration factor (CAL Factor) and the number of particles within a defined CAL region.

FCS Information

Set up the Info plot to include instrument serial number and User ID information on statistical printouts. Use the Info Plot FCS tab to select the FCS Keywords for the information you want on your statistical printouts.

Plots ▸▸ FCS Information to create an Info plot.



Info Plot Data Source

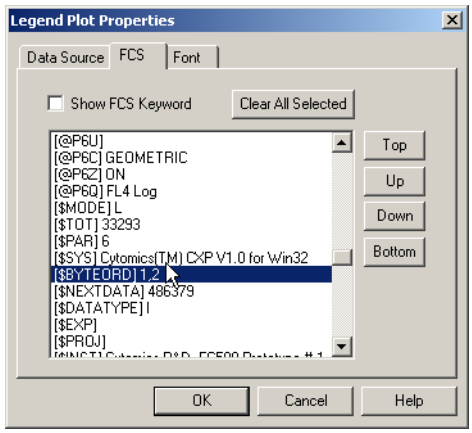
Displays the name of the file that is the data source for the Info Plot.

Info Plot - FCS Tab

Show FCS Keyword – Enable this checkbox to display the FCS Keyword as well as the associated keyword value.

Multiple Keywords can be selected from the scroll box.

Select **Clear All Selected** to deselect the items.



Acquisition Stop and Save

Use Acquisition Stop and Save to terminate the acquisition of a sample at a preset stop value. If there is more than one stop value set, then the first stop counter reached terminates the current acquisition.

Note: The values set in the Cytometer Control Acquisition Limits (Acquisition Time, Elapsed Time and Maximum Events) overrides the stop values set here in the event of an acquisition limit being reached before the Acquisition Stop value has been achieved.

Single Parameter Histograms can be selected for automatic saving in FCS Format.

Note: An acquisition from lysed whole blood is to be stopped on 5,000 lymphocytes to allow the collection and storage of all event data.

In normal blood, where lymphocytes account for about 30% of the white cell count, we have no problem with the above setup. We can predict with a normal sample that the total count from a 5,000 cell lymphocyte count is around 5,000 x 100/30 or 16,666 events total.

If the sample is from a lymphopenic patient, this could be a problem. A 5,000 lymphocyte count may not be achieved before the sample tube is empty or RAM memory is full.

It is normal under these circumstances to set two counters, one for the stop count and one for the maximum permissible count, that is, the maximum total event count. If a stop count is used, it is recommended that the stop count be set to a large number, for example, 100,000 events. Thus, with a lymphopenic patient, if the lymphocytes are less than 5% of the white cell count, an acquisition would terminate on the total event count of 100,000 before a 5,000 cell lymphocyte count could be achieved.

Note: If a Stop condition is reached while you are adjusting voltages and gains, acquisition is not restarted and the data plots are not refreshed. You can be avoid this situation by making all cytosetting adjustments while in Setup Mode.

Setting a Stop Counter

Note: If a stop count is used on a plot that contains an AutoGate region, the stop count is not exact.

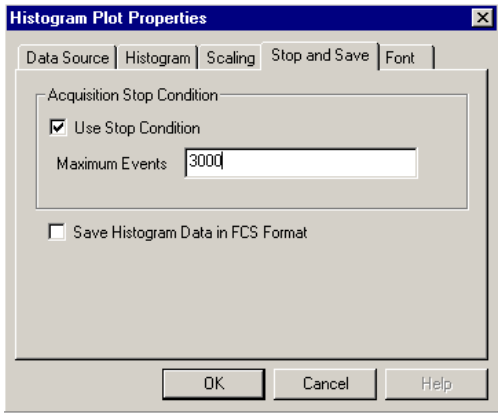
---

1 Highlight the plot on which you wish to set a stop counter.

---

2 **Plots ▸▸ Acquisition Save and Stops** command to view the Acquisition Plot Set-up dialog box.
Use Stop Condition
Maximum Events
Save Histogram Data in FCS Format

Note: You cannot make any changes on this screen when running a locked protocol.



---

3 **Use Stop Condition** and enter **Maximum Events**.

---

4 To enable an FCS Histogram to be saved from this plot, check the **Save Histogram Data in FCS Format** checkbox.
Note: The actual histogram save action is automatically performed if the Save Histogram checkbox is checked in Workspace Preferences - Acquisition Options.

See also:
Color Dot Plot
Histogram Plot
Density Plot
Format Plot
Acquisition Stop and Save

Use Stop Condition

Check this checkbox if the stop counter is to be activated.

Maximum Events

Set the maximum number of events you wish to be recorded.

Save Histogram Data in FCS Format

Check this checkbox to save the Histogram Data in FCS Format.

Format Plot

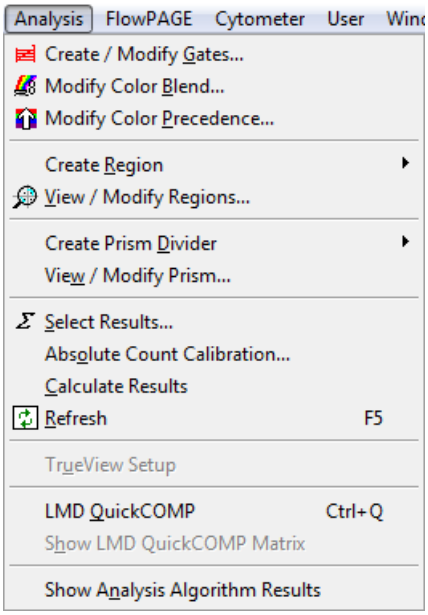
To display the Plot Properties dialog box, click the right mouse button on the plot and

.

Format options differ depending on the Plot type. Select a formatting option from the table below to view detailed information about the format options available for the different types of plots.

Plot Type	Plot Name	Available Formatting Options					
Single Parameter	**Histogram Plot**	Data Source	Histogram	Scaling	Labeling	Font	Stop & Save
Dual Parameter	**Dot Plot**	Data Source	Events	Resolution	Labeling	Font	Stop & Save
	Density Plot	Data Source	Density	Resolution	Labeling	Font	Stop & Save
Special Types	**Legend Plot**	Data Source	Options	Font			
	Prism Plot	Data Source	Font				
	Info Plot	Data Source	FCS	Font			

10.20 ANALYSIS MENU



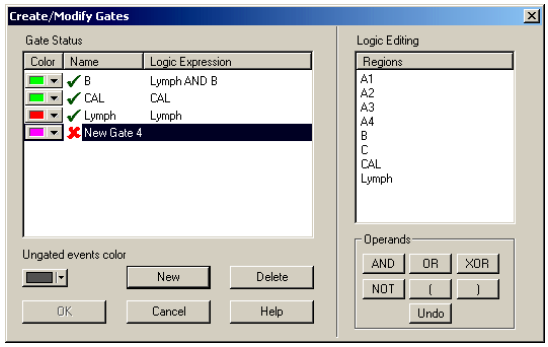
Create / Modify Gates

Use the Gating function to define populations of interest either for plot display or for sorting.

---

1 .

Note: You cannot make any changes on this screen when running a locked protocol.



---

2 New.

Gate and Logical Expression
Gate Color
New
Delete
Operands

---

3 **New Gate 4** is highlighted so you can modify the name if desired.

---

4 Create new logical expressions.

See also:
Gate Selection
Gate Color
Boolean Gating

Gate and Logical Expression

Shows the Gate name and the logical combination of Gates.

New

Allows you to create a new Gate with a new combination of Regions.

Delete

The **Delete** button erases any selected gates highlighted in the Create/Modify Gates dialog box.

Operands

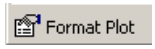
The Operands function allows you to insert a combination of regions into the gating equation using the Operands buttons. Once a Region has been entered next to the new file name, the Operands have to be utilized to enable a new combination of cells to be viewed. The Operands work according to Boolean Gating.

Gate Selection

The Gate option can be used once Gates have been created.

To apply a Gate to a plot:

---

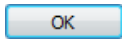
1 Click the right mouse button on the plot and  

.

---

2  the Gate you wish to apply from the **Gate** drop down list box.

---

3  

or press Enter to accept the changes.

or press Esc to exit the dialog box without saving the changes.

Gate Color

When a region is drawn on a plot, the first region is automatically shown as red, meaning events inside this gate are colored red when viewed on a color dot plot. The second is in green, and the third in blue.

These colors can be altered through the Color function if more or different colors are required.

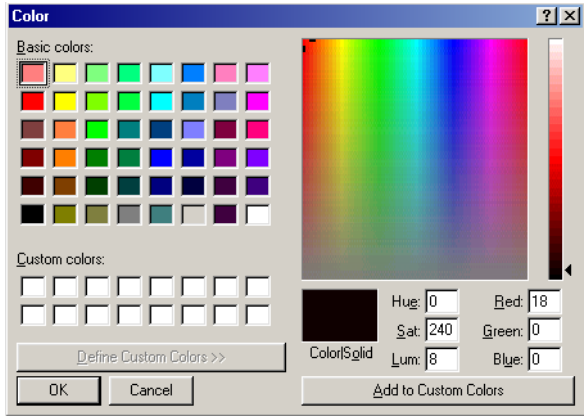
---

1  the down arrow next to the Color Well for the selected Gate or select the Ungated events color down arrow to change the color for Ungated events.

2 the required color.
This automatically changes the events within the gate to this color.

3 If further colors are required,

Other to reveal a larger color palette. a defined color or drag the cursor around on the color board to select the color of choice.



4 When the appropriate color has been found, you can click the **Add to Custom Colors** button for easy use at a later date or you can OK to add to a plot.
The same principles can also be applied to the Ungated Events Color option.

Boolean Gating

Gallios software implements Boolean gating. Up to eight regions can be combined to form a gate. A gate is used to select a specific subpopulation of data.

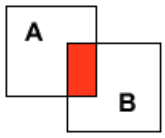
Gallios software uses a combination of regions and operators to achieve the Boolean gating process. The Operands are utilized in order to create a combination of regions.

Gate Logic

The shaded areas show the following logical combinations of regions.

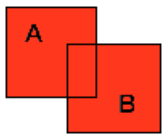
AND

Events which are in both regions A AND region B. This is written as A AND B.



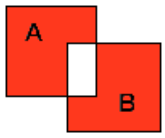
OR

Events that are in region A OR in region B. This is written as A OR B.



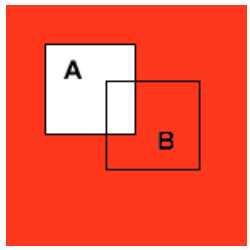
XOR

Events, that are EXCLUSIVELY in region A OR in region B, but NOT in both. This is written as A XOR B.



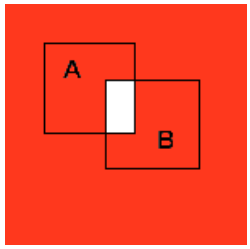
NOT

Events that are NOT in region A. This is written as NOT (A).



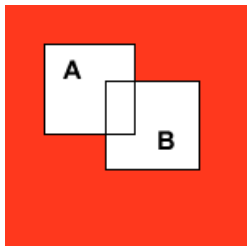
NOT

Events which are NOT in region A AND in region B. This is written as NOT (A AND B).



NOT

Events, which are NOT in region A OR in region B. This is written as NOT (A OR B).



PARENTHESES

These are used to define a precise logical statement. An open parentheses must be placed after a NOT operator even if the operand is only a single value.

Example: Lymphs AND NOT (CD8).

Color Blend Mode - Introduction

Gallios software allows the use of combinations of Gates to determine the color of dots in a dot plot. The key concept behind this process is similar to color printing and depends upon the use of colored combinations of Gates to define a unique subpopulation. Each Gate (up to three) is given a color and the logical gate combinations give rise to populations with blended colors.

Consider three gates: Gate A, set on FL1 positive cells; Gate B, set on FL2 positive cells; Gate C, set on FL3 positive cells. Any event may be in either none, one, two or all three of these Gates and therefore, combinations of the gates may be used to define eight different subpopulations (phenotypes) of events as follows:

Each of these subpopulations may be represented by a different color. Thus up to eight differently colored subpopulations may be visualized on a Color Gated Dot Plot, and each group can be given a user defined name.

Default Name	Primary A (FL1)	Secondary B (FL2)	Tertiary C (FL3)
Group 0	-	-	-
Group 1	+	-	-
Group 2	-	+	-
Group 3	+	+	-
Group 4	-	-	+
Group 5	+	-	+
Group 6	-	+	+
Group 7	+	+	+

Where: +ve data is inside a Gate, and -ve data is outside a Gate

When in color blend mode ONLY color statistics from the blended colors are displayed in the Legend plot. Use  to view Region statistics.

See also:
Modify Color Blend

Modify Color Blend

To use **Modify Color Blend**:

---

1 **Analysis ▸▸ Modify Color Blend** to view the **Modify Color Blend** dialog box.

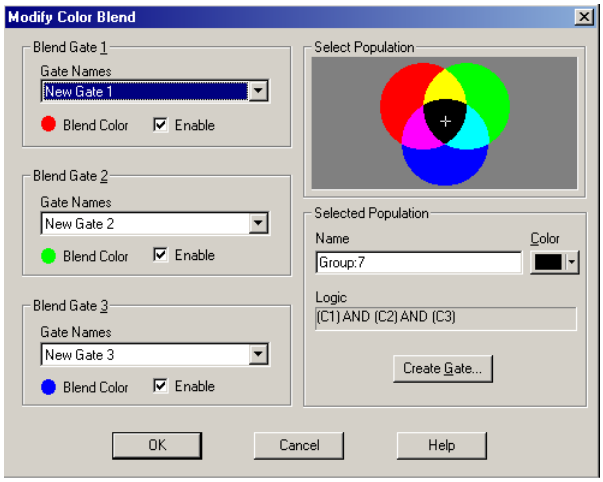
---

2 the Gate you wish to modify.

---

3 Make the desired changes.
Gate Names
Blend Color
Enable
Name
Logic
Color
Create Gate

Note: You cannot make any changes on this screen when running a locked protocol.



4  or press Enter to accept the changes.

 or press Esc to exit the dialog box without saving the changes.

See also:
Create / Modify Gates
Workspace Preferences - Gating

Gate Names

Allows the selection of a Gate that defines a population of interest.

Enable

Enables the selection of one, two or all three blend Gates.

Blend Color

Displays the color to be associated with the selected Gate logic.

Name

Allows a user-defined name for the selected combination.

Color

Allows you to modify the predefined colors.

Logic

Displays the full coloring Gate logic for the selected population.

Create Gate

Creates a logical Gate based on the coloring logic. A Create Gate screen is displayed showing the Gate Logic to be applied. Press **Yes** to Create the Gate or **No** to cancel.

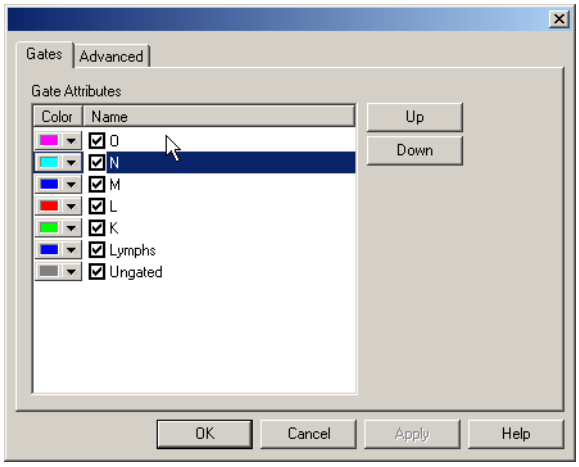
Modify Color Precedence

When a subpopulation is very small and a larger blended color would swamp a smaller population, Color Precedence can be used instead of Color Blending. In Color Precedence Mode, the subpopulation (or color) given the highest precedence overrules any color with lower precedence when the same event satisfies more than one color gate, or, when two or more events are to be displayed at the same location.

1 **Analysis ▸▸ Modify Color Precedence** to view the Color Precedence – Gates tab dialog box.

2 the required gate so as to highlight the gate name ONLY.

Note: You cannot make any changes on this screen when running a locked protocol.



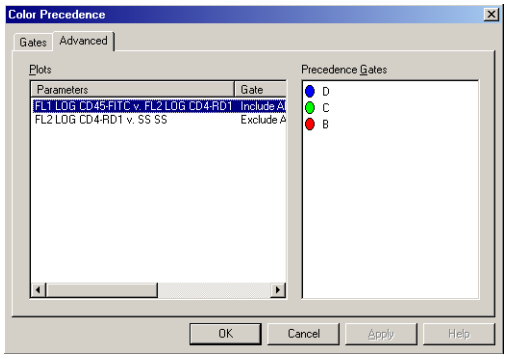
3 Use Up and Down to move Gates into the required order of precedence. Those with the highest precedence are at the top of the list.

See also:
Workspace Preferences - Gating
Advanced Precedence

Advanced Precedence

Note: You cannot make any changes on this screen when running a locked protocol.

Advanced Precedence allows you to assign particular Gate colors to specific plots.



This is of particular interest if different plots are gated on completely different criteria and the color from one gate, therefore, has no relevance to another plot or file.

To configure what colored events will display on a given plot,

1. Highlight the plot in the left pane of this dialog,
2. Select the Precedence Gate(s) desired to be viewed (as colored events) on that plot.
3. Select Apply to view changes and OK to accept the change.

Regions Introduction

A Region identifies a specific population of cells.

Up to 256 regions (total) can be set on any combination of Parameters, 32 of these are usable as Gates.

Regions are drawn on a Single Parameter Histogram by setting the position of an upper and lower boundary (Region markers).

Regions are drawn on a 2-Dimensional Dot Plot by enclosing a subpopulation of events with rectangles, polygons (nonrectangular Regions) or using quadrant markers (four rectangular Regions, set with only one point).

To speed up the analysis of data, when a Single Parameter Histogram Region or a Rectangular or Polygonal Region is created, it is also assigned to a Gate. This assignment can be accepted or edited later. The automatic assignment of Regions to Gate can be switched on or off if desired, using **Gates** ▸▸ Automatic Gate Creation.

Region Properties

 on a region to make it active and then click the right mouse button on it to display the Region Properties dialog box. 

.

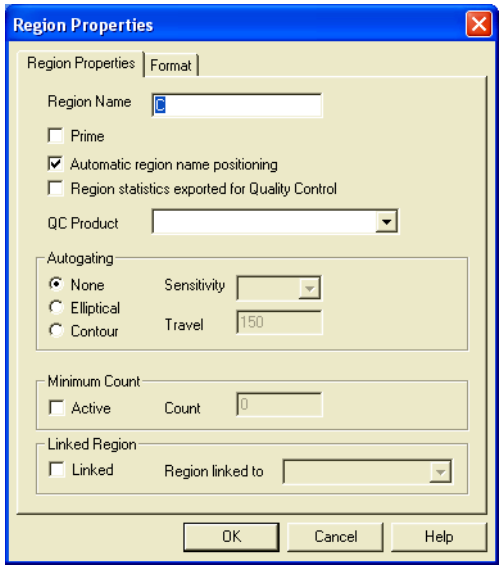
The **Re-Calculate AutoGate** option allows a return to AutoGating after manual manipulation of an Auto region. All the AutoGates are re-calculated, including positives analysis in a protocol.

Polygonal Region

The Polygonal Region allows you to draw a free-form Region with up to 100 points and can include horizontal acute angles on a dual parameter plot. To draw a Polygonal Region in a dual parameter display see, Create Polygonal Regions.

Polygonal Region Properties

Region Name
Prime
Automatic Region Name Positioning
Region statistics exported for QC
QC Product
None
Elliptical
Contour
Sensitivity
Travel
Minimum Count
Linked Region



Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.

Region Name

Displays the name of the region. If the name is not grayed, you can rename it.

Prime

When checked, this option allows the system to determine if the peak of the events on the plot is within this region. If the peak is outside this region (due to an air bubble or partial clog), the system performs a Prime cycle. A maximum of three sequential Prime cycles are allowed; if the peak of the plot is still not within this region, the MCL aborts.

Automatic Region Name Positioning

Region labels default to an automatic position. You may move a region label to any desired position within a plot, this user modification automatically unchecks this option. To revert to the automatic position recheck this option.

Region Statistics exported for Quality Control

The region's results are exported to the Quality Control feature of the Report Generator.

Note: Exporting region statistics and selecting the QC product for FlexQuad regions operates differently depending on whether the Region Properties dialog is opened via,

- right click on the selected region in the plot and using the context menu or
- right click on the region in Protocol explorer or double click the region name in the View / Modify regions dialog.

If the Region Properties dialog is opened via right click on the selected region in the plot and using the context menu, when a QC product is selected or changed, it is applied to all quadrants.

If the Region Properties dialog for any of the quadrants is opened via right click in Protocol explorer or double clicking the name in the View / Modify regions dialog, then changing a QC product for a quadrant is only applied to this quadrant.

QC Product

Select the QC product from the drop down list. See also, Setting Up QC Products

AutoGating

None

This is the default when the AutoGate option is not selected.

Elliptical

Toggle the AutoGate to an elliptical region.

Contour

Toggle the AutoGate region to a contour region.

Sensitivity

Eliptical AutoGate settings are 0.11, 0.33 and 1.1 (% of processed peak). Contour AutoGate settings are 1, 2, 3, 4, and 5.

- Level 1 = 0.11% processed peak at a resolution of 64x64.
- Levels 2, 3 and 4 = 0.11, 0.33 and 1.1% at a resolution of 128x128.
- Level 5 = 1.1 % processed peak at a resolution of 256x256.

Selecting 0.11 creates a larger region, whereas selecting 1.1 creates a smaller radius region around the target population.

Travel

Input a maximum value the contour AutoGate region travels from the mean. The Minimum value is 25; Maximum is 200 channels. Use this option to slightly adjust the AutoGate from tube to tube if slight changes in lysis cause the target population to move slightly. This option is not available for elliptical AutoGates.

Minimum Count

Check this option and enter a number in the **Count** field to enable the minimum count function. This overrides an existing stop count to ensure a minimum number of events within a particular region are collected. The actual stop count in this case is approximate (but is always in excess of the minimum count value). This is limited by the Duration and Total Events.

Linked Region

Select a region to be linked to another region and the region tracks the selected region. A region may only link to a region of the same type and parameters. For dual parameter regions, the parameters may be transposed. A polygonal region may link to a quadrant region/FlexQuad region, but a quadrant region/FlexQuad region may not link to a polygonal region.

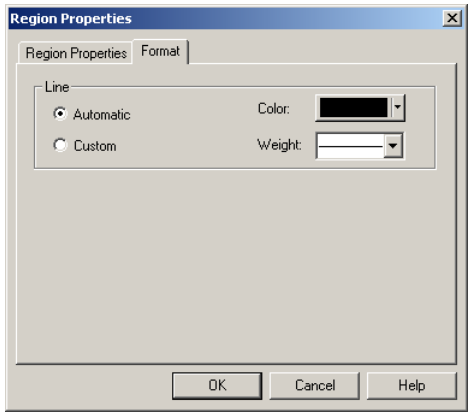
When a region is linked to another, changes to either region's coordinates (by dragging the region) are propagated to both regions. The exception is when the region is an auto analysis region (positives analysis, Elliptical and Contour AutoGate). Then the regions track the auto analysis region and the link is unidirectional.

If a region is linked to another, positives analysis and autogating are disabled for that region.

Polygonal Region Format Tab

Specify the **Color** and line **Weight** for the Polygonal Region boundary.

Note: You cannot make any changes on this screen when running a locked protocol.



Interactive Polygonal Region Editing

When a Region's position is changed, any Gates and statistics that use the Region are automatically updated and each plot is redrawn to reflect the changes in that Region. When the Region is deleted, any Gate or statistics that used the Region are also automatically deleted. When a Gate has been deleted, any plot that used the deleted gate is reset to Ungated. Gallios software allows you to resize an already created Region.

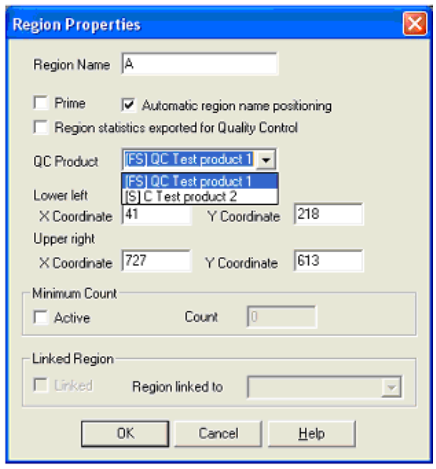
Rectangular Region

This option draws a Rectangular Region on a dual parameter plot. See also: Create Rectangular Regions.

Rectangular Region Properties

Region Name
Prime
Automatic Region Name Positioning
Region statistics exported for QC
QC Product
X and Y Coordinate
Minimum Count
Linked Region

Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.



X and Y Coordinate

Set the region to defined position by entering X and lower Y coordinates for the region.

Interactive Rectangular Region Editing

When a Region's position is changed, any Gates and statistics that use the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When the Region is deleted, any Gate or statistics that used the Region are also automatically deleted. When a Gate has been deleted, any plot, which used the deleted Gate, is reset to Ungated. Gallios software allows resizing of previously created Regions.

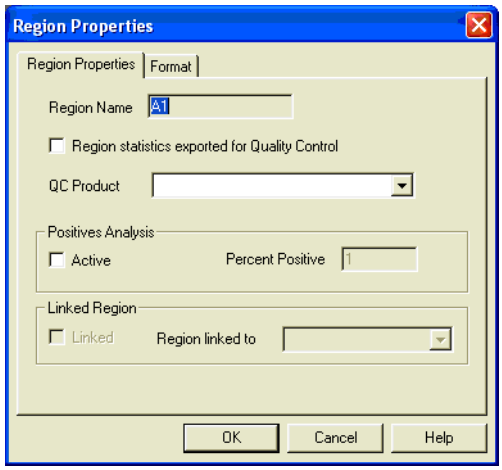
Quadrant Region

Insert a Quadrant Region into a dual parameter plot. All quadrants comprise four sets of Rectangular shaped polygonal Regions on a dual parameter plot. The Quadrants are automatically assigned an alphabetical region name and a number for each quadrant. Quadrant regions have flexquad capabilities allowing adjustment of the dividers between FlexQuad quadrants 1 and 2 and between quadrants 2 and 4. See also: Create Quadrant Regions and Repositioning a Quadrant Region.

Quadrant Region Properties

Region Name
X and Y Coordinate
Region statistics exported for QC
QC Product
Positives Analysis
Linked Region

Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.



Interactive Quadrant Region Editing

When a Region's position is changed any Gates and statistics derived from the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When a Region is deleted any Gate or statistics that used the Region are also automatically deleted. When a Gate has been deleted, any plot, which used the deleted Gate, is reset to Ungated. Gallios software allows you to reposition an already created Region.

Elliptical AutoGate

Create an Elliptical AutoGate around the selected population on a plot.

See also: Create AutoGate.

Contour AutoGate

Create a Contour AutoGate around the selected population on a plot.

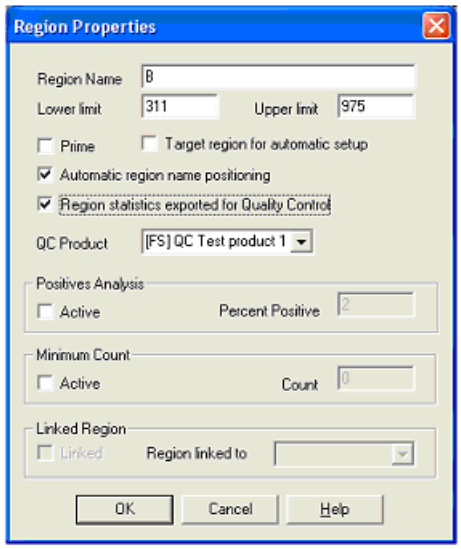
See also: Create AutoGate.

Linear Region

Insert a Linear Region. See also: Create Linear Regions.

Linear Region Properties

Region Name
Lower Limit and Upper Limit
Prime
Target Region for Automatic Setup
Automatic Region Name Positioning
Region statistics exported for QC
QC Product
Positives Analysis
Minimum Count
Linked Region



Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.

Lower Limit and Upper Limit

Set the region to defined limits by entering upper and lower limits for the region. These limits can be viewed in the statistics box for each plot by selecting **Analysis ▸▸** Select Results **and select Min/Max.**

Target Region for Automatic Setup

Required only when a multiple standardization bead preparation is used in the _STAND protocol containing multiple regions in the Forward Scatter plot. This feature is not used when a single bead product is used to standardize the system.

Positives Analysis

This option moves the region to the selected percent result. Specify a positive percentage value from 0.1 to 99.9. If the positive analysis option is not selected, the percentile is unavailable. Use this option to automatically set the region position for a negative control sample.

Interactive Linear Region Editing

When a Region's position is changed, any Gates that use the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When the Region is deleted any Gate which used the Region is also automatically deleted. When a Gate has been deleted, any plot that used the deleted Gate is reset to Ungated. Gallios software allows you to resize an already created Region.

Status Bar (Linear)

The status bar normally gives a brief description of selected menu items.

When drawing or modifying Regions the status bar divides into three areas of information. These update as a region is modified:

- The status bar shows Channel for X parameter
- The total integral count of the region
- The percentage of events currently within the region.

X Coordinate

The X Axis channel value is the coordinate of the cursor position given by a scaling factor. This scaling factor depends on whether the Histogram parameter is using linear or logarithmic amplification and whether you have calibrated that parameter or not.

If the parameter is linear and uncalibrated, the X channel varies between 0 - 255 or 0 - 1024 depending on the data source. If the parameter is logarithmic and uncalibrated, the value varies between 0.1024 - 1024 and span four decades. For most other manufacturers, the data varies between 1 - 10,000, spanning four decades also.

Integral

When you create a Region the status bar reflects the integral values given along the scale for that parameter at the cursor position. That is, before the first marker is set.

If the cursor is on channel 10, the integral shows the count value of the populations at channel 10.

After first marker is set and as you move the mouse to either the left or right of this point, the integral value continuously updates to reflect the summation of each channel between the Region starting position and the current mouse position.

Multiple Linear Regions

This option sets multiple consecutive linear Regions on one display. When linear regions are created using the Multiple Linear Regions option, gates are not created even though Automatic Gate Creation is selected in Workspace Preferences. See: Create Multiple Linear Regions.

Interactive Multi-Linear Region Editing

When a Region's position is changed, any Gates that use the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When the Region is deleted any Gate which used the Region is also automatically deleted. When a Gate has been deleted, any plot that used the deleted Gate is reset to Ungated. Allows you to resize an already created Region.

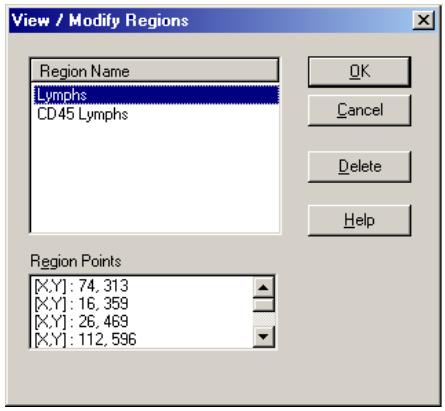
View / Modify Regions

Use this option to view/edit a Region's properties, Region points or to delete a Region that is no longer required.

1  .

2 Make the desired changes.

See also:
Region Name
Region Points



3  or press Enter to accept the changes.

 or press Esc to exit the dialog box without saving the changes.

See also:
Deleting Regions
Editing and Moving a Region Name
Moving a Region
Resizing a Region

Region Name

Select the Region to view, edit, or delete from the Regions list box. Highlight the **Region Name** and  to delete the region(s). Double click the **Region Name** to rename the region.

Region Points

This list box gives details of the Region bounds. This is Upper/Lower channel for single parameter histograms. For rectangles the lower left and upper right vertices are specified and for polygons the X/Y coordinates of all the vertices. To display a Region's points (or bounds), click on the desired Region in the Regions list box.

Resizing a Region

If you want to enlarge or shrink a Region:

---

1  the Region.

The Region is now active and handles appear. Vertices also appear for Contour AutoGate and Polygon Regions

---

2 Hold the left mouse button down while moving the handle (or moving a vertex as in Contour AutoGate and Polygon Regions).

Moving a Region

To move a Region to another position:

---

1 Mouse over the Region to get the  cursor.

---

2 Hold the left mouse button down while moving the Region anywhere in the current window. If the Region has been moved, the statistics automatically recalculate. The Region name moves to the same relative position alongside the Region's new position.

See also:
View / Modify Regions

Editing and Moving a Region Name

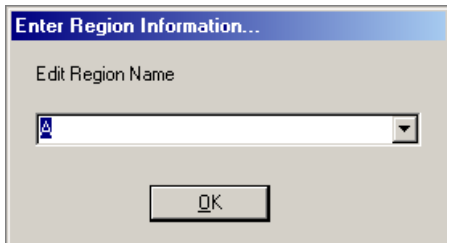
To edit or move the Region name:

Note: Quadrant/FlexQuad region names can also be edited through the View/Modify Regions dialog.

1 Double click on or near the first letter of the Region name to activate the Region Name dialog box.

Note: CAL and Quadrant/FlexQuad regions can not be renamed, therefore double clicking CAL or FlexQuad region name does not launch the Enter Region Information dialog.

2 To edit the Region name, type in the desired changes, if any, click OK.



3 To move the Region Name, mouse over the region to get the cursor. If the region name has multiple characters, mouse over the first character to get the cursor.

4 + hold and drag to move the region name.

5 to fix the location of the region name.

Deleting Regions

There are two methods used to delete degions, From Within a Plot and Using the Delete Button in the View / Modify Regions Dialog Box.

Note: If a plot is deleted, its regions are still retained.

From Within a Plot

1  the Region.
The Region is now active and handles appear.

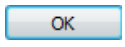
2 Press Delete. The Region is now deleted. Any Gates and statistics that were associated with this Region are also deleted.

Using the Delete Button in the View / Modify Regions Dialog Box

1  .

2  the Region to delete and   .

Use Ctrl+  to select multiple regions to delete.
Use Ctrl+A to select all of the regions.

3   or press Enter to delete the Regions.

  or press Esc to exit the dialog box without deleting any selected Regions.

When you exit the **View / Modify Regions** dialog box, Gallios software updates all the Windows. If a deleted Region was also specified in any statistics or Gates, then Gallios software updates to reflect these changes.

Re-Calculate All AutoGates

Use this function to re-calculate multiple autogates in a protocol, rather than re-calculating them individually.

Create Prism Divider

Dual Prism Divider

The **Analysis ▸▸ Create Prism Divider ▸▸ Dual Prism Divider** menu option allows you simultaneously set two Prism dividers in a “quadrant fashion" on a dual parameter plot. Any displayed Prism Plot is automatically updated with the new Prism statistics. If the number of Prism dividers is changed during the divider creation, the corresponding Prism plot also reflects this by changing its format according to the number of active Prism dividers.

Note: Gallios Cytometer software only allows one Prism divider per parameter and the dividers are GLOBAL across the application. As a consequence, modifying the Prism divider of any parameter on any plot automatically updates the divider of that parameter if it is displayed on any other plot. This includes both dual and single parameter plots.

See also:
Setting a New Dual Prism Divider
Editing a Dual Prism Divider

Setting a New Dual Prism Divider

1 Highlight the chosen dual parameter plot (current window).

2 **Analysis ▸▸ Create Prism Divider ▸▸ Dual Prism Divider**.

3 A Dual Prism cursor is displayed in the current window.

4 Position the cursor anywhere on the window, click to show the prism divider lines. If Esc is pressed at this point the operation is abandoned.

5 Move cursor to position crosshair then click to set position.

Editing a Dual Prism Divider

Editing an existing Dual Parameter plot with a set of dual Prism dividers displayed essentially consists of creating a new set of dual Prism dividers. When the second set of dual Prism dividers is defined the first set is deleted.

1 Highlight the required dual parameter plot.

2 Mouse over the Prism divider to get the ↔, ↕ or ✥ cursor.

3 + hold to set the new Dual Prism crosshair. When the new dividers are set they replace the previously set dividers. Any associated Prism plot is updated with the new Prism statistics.

Any associated Prism plot changes its format and has its statistics updated to reflect a smaller number of Prism dividers and a smaller number of displayed phenotypes.

4 To delete Prism divider, on the divider to make it active and press Delete . The Prism divider for the associated parameter is deleted altogether from this plot and from any other plot that has this divider set. Any associated Prism plot changes its format and has its statistics updated to reflect a smaller number of Prism divider(s).

Single Prism Divider

The **Analysis ▸▸ Create Prism Divider ▸▸** Single Prism Divider menu option allows a single Prism divider to be set on a single parameter histogram plot. Any displayed Prism plots is automatically updated with the current Prism statistics. If the number of Prism dividers is changed during the divider creation, the corresponding Prism plot also reflects this by changing its format according to the number of active Prism dividers. For further explanation on Prism parameter see What is Prism?

Note: Gallios software only allows one Prism divider per parameter. As a consequence modifying the Prism divider of any parameter on any plot automatically updates the divider of that parameter if it is displayed on any other plot. This includes both histogram and dual parameter plots.

See also:
Setting a New Single Prism Divider
Editing a Single Prism Divider

Setting a New Single Prism Divider

1 Highlight the required histogram plot.

2 **Analysis ▸▸ Create Prism Divider ▸▸ Single Prism Divider**.

3 A Single Prism cursor is displayed in the current window.

4 Position the cursor anywhere on the histogram, click to show the prism divider lines. If Esc is pressed at this point the operation is abandoned.

5 Move cursor to position crosshair then click to set position.

Editing a Single Prism Divider

Editing an existing histogram plot with a set of single Prism dividers displayed essentially consists of creating a new single Prism dividers. When the second single Prism dividers are defined, the original is deleted.

1 Highlight the chosen histogram plot (click on the title bar).

2 Mouse over the Prism divider to get the ↔, ↕ or ✥ cursor.

3  + hold to set the new divider. The old divider is deleted and the display is once again updated to show only one single Prism divider. Any associated Prism plot is updated with the new Prism statistics.

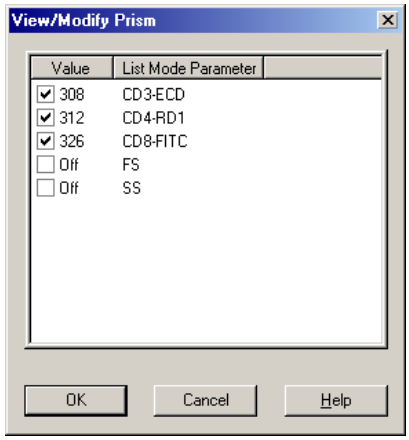
4 To delete Prism divider,  on the divider to make it active and press Delete. The Prism divider for the associated parameter is deleted altogether from this plot and from any other plot that has this divider set. Any associated Prism plot changes its format and its statistics are updated to reflect a smaller number of Prism dividers.

View/Modify Prism

Under most circumstances, Prism is modified graphically by using Dual or Single Prism Divider cursor operations. Occasionally, it is necessary to modify the Prism dividers numerically to reproduce exactly the setup of another experiment. View/Modify Prism allows you to do this.

1  **Analysis ▸▸ View/Modify Prism**. The Prism Setup dialog box is displayed.

2 the parameter whose divider you wish to edit.



3 Place a check in the box next to the Listmode Parameter value you wish to enable.

Note: If no check is in the checkbox, the particular value is automatically set to OFF. This switches off the prism divider and deletes it from any displays that were using it. If a prism divider is switched off, any active prism plots and plots with prism divider displays also update them to reflect the modified number of prism parameters in use.

4 the value that you wish to change and enter the required value within the range 0 to 1023.

5 OK to accept all changes.

Cancel to abandon all changes.

Select Results

Gallios software gives the flexibility of generating many different statistics and these are produced using combinations of Regions and Gates.

A Gate is used to select events that may potentially be counted while Regions mark subpopulations and determine whether an event is actually counted.

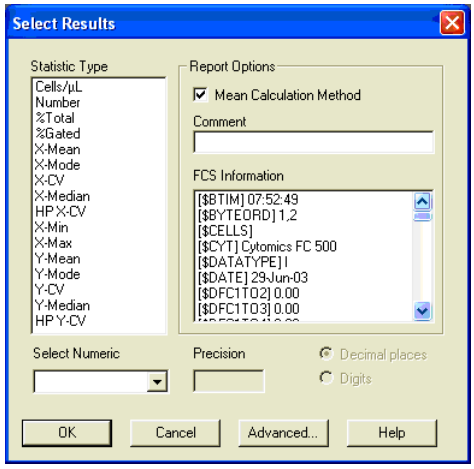
All Regions and Gates can be named according to your preference, allowing easy recognition of your results.

The statistics calculated and displayed depend upon those selected in the current protocol. If no statistics have been selected then the default statistics are displayed. FCS options are either generated automatically by Gallios software or obtained from sample information entered by the User. Desired items can be selected and exported during the publishing of an Excel Spreadsheet. The selected FCS information is only published and does not print with the plots within Gallios software.

When in Color Blend Mode, only the color blend color statistics from the blended colors are displayed in the Legend plot. Use  to view Region statistics.

Statistic Type
Number
% Total
% Gated
Mean
CV
Median
HP-CV
Mode
Cells / µL
Min and Max

Mean Calculation Method
FCS Information
Select Numeric Precision
Enter Numeric Value
Advanced Statistics Configuration



For more information concerning the Publication of Results, see Publish Results Now.

Statistic Type

There are the different types of statistics that can be utilized. Any number of these can be selected and the relevant statistic appears in the box beneath the plot.

Mean Calculation Method

This exports the method of calculating the mean as part of a Published Excel spreadsheet.

FCS Information

These options are either generated automatically by Gallios software or obtained from sample information entered by you. Desired items can be selected and exported during the publishing of an Excel Spreadsheet. The selected FCS information is only published and does not print with the plots within Gallios software.

Number

Total number of events in a Region.

% Total

Total number of events in region as a percentage of total events in the file.

% Gated

Total number of events in region, as a percentage of events in a gated display.

Mean

The Mean channel is the arithmetic mean channel defined as the sum of the region channel numbers, times counts, divided by the integral of the region.

The Mean (or the average) is found when all selected events are added together and then divide by the total number of events.

The Advanced button allows for the calculation of a Geometric mean.

CV

The CV is Standard Deviation divided by the mean of the data between the markers.

Note: Standard Deviation calculations are only valid between markers that have a Normal or Gaussian distribution. CV is not strictly valid for any other shape distribution. CV is reported as ### on a log scaled histogram. The derivation of CV can be found in any standard statistic text.

Median

Median is used to indicate the point on the scale of measures where the population is centered. The median of a population is the point that divides the distribution of scores in half. Numerically, half of the scores in a population have values that are equal to or larger than the median, and half have values that are equal to or smaller than the median.

HP-CV

(HP X-CV and HP Y-CV) The Half Peak Coefficient of Variation (HP-CV) is derived by the fixed mathematical relationship between the Standard Deviation (SD) and the Full Width Half Max (FWHM) value of a Normal or Gaussian peak. The relationship between them is defined as SD=FWHM/2.354.

This is an approximation to the real value of CV. The advantage of calculation HP-CV is that the value remains fairly independent of the position of a pair of region markers, provided that the markers are set either side of a peak and the marker channel counts are less than half the value of the peak channel count. In general, the HP-CV approximation is usually smaller than the CV calculation. See standard statistics texts for further explanation of HP-CV and CV differences. HPCV is reported as # # # on a log scaled histogram.

Mode

The mode channel is the channel with the largest number of counts between region markers.

Gate colors can be customized using the drop down color box and selecting the required color.

Cells / µL

This displays the absolute count of a population based on the calibration factor (CAL Factor) and the number of particles within a user defined CAL region.

Min and Max

Displays linear region boundaries.

Select Numeric Precision

Choose the precision you want to specify for, Percent, Mean or CV.

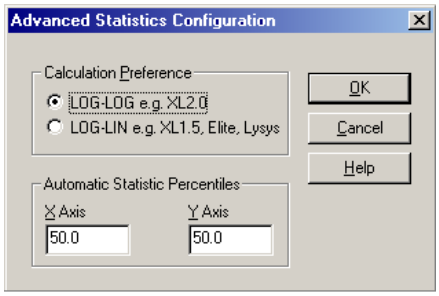
Enter Numeric Value

Maximum 3 decimal places for CV, 4 decimal places for Percent and maximum 5 digits for Mean.

Advanced Statistics Configuration

Selecting the Advanced button offers further options to select the way that the mean is calculated.

Log-Log
Log-Lin
Automatic Statistic Percentiles



Differences Between Log-Log and Log-Lin Statistical Calculations

For most practical applications users may never notice the difference between the two. In general, Log-Log mean appears to have its value skewed further to the right than a Log-Lin mean calculation on the same data. This skew is more pronounced when the distribution of data extends greater than ±half a decade from the mean value, however calculated.

Log-Log

Selecting Log-Log calculates the mean values of Log data using geometric conversion of each individual channel before a mean value is calculated.

Log-Lin

Selecting the Log-Lin mode calculates the mean values by determining the arithmetic mean of the raw ADC channels and then converting this to its relative linear channel equivalent.

Automatic Statistic Percentiles

This is the channel, which divides the events in a region by the percentile value. The default percentile is 50 (the median) but can be set to request any desired value. Different values may be set for both the X and Y axis.

Absolute Count Calibration

Use absolute count calibration particles for the calculation of absolute numbers of cells present in a sample.

As a sample is analyzed, the numbers of a particular cell type in a region, and the numbers of absolute count calibration particles present in a CAL region are compared, and the absolute concentration of the cell type is then calculated automatically.

A listmode files replays with its runtime Cal Factor. Use this screen to enter a different Cal Factor to calculate individual listmode absolute counts.

Note:

- A CAL region MUST be set for the system to be able to calculate an absolute count. See Setting a CAL Region.
- Listmode replay uses the runtime protocol Cal Factor unless you select a different Cal Factor before replaying the file.

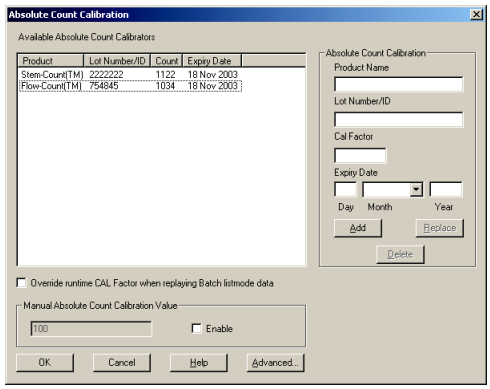
To display the absolute count, **Cells/µL** from the Analysis/Select Results menu option.

Setting a CAL Factor for Absolute Counts

From the list of **Available Absolute Count Calibrators**, select the Lot Number currently in use.

Enter a **Manual Absolute Count Calibration Value** if the required Lot is not present in the list. A manual calibration value is only used for the current session, it cannot be saved. A listmode file replays with its runtime Cal Factor.

Note: The number of events in the CAL region must be greater than 1000, otherwise absolute counts are not determined.



Override runtime CAL Factor...

Check this option if you want to replay a batch of listmode files using a different Cal Factor.

Advanced

If your Administrator has assigned you access rights, the **Advanced** button is active and you can enter and modify batch and count information. Enter the **Product name**, **Expiry Date**, **Lot Number/ID** and **CAL Factor.**See Add Absolute Calibration Batches in the User Administration section.

ADD

Use the ADD button to enter new lot information (only available on the Advanced screen).

Replace

Use the Replace button to replace the selected information with updated values (only available on the Advanced screen).

Delete

Use the Delete button to remove a selected lot from the list (only available on the Advanced screen).

Setting a CAL Region

Create a Region around the absolute count calibration particles, the region MUST be named CAL. Set or select the calibration factor for the batch of beads in use from the **Analysis ▸▸** Absolute Count Calibration menu item.

Note: If the Cal Factor is set to 0 or there is no CAL Region present in the protocol, “Error” appears in the Cells/uL field. If the number of events in the CAL region equal to or less than 1000, "Invalid" appears in the Cells/uL field.

Calculate Results

This option calculates the statistics for all files selected on the screen.

Refresh

Selecting **View ▸▸ Refresh**, or pressing F5, causes Gallios software to refresh the screen, all plots and FlowPAGEs. This option is useful when Windows fails to refresh the screen correctly leaving some graphical errors visible.

TrueView Setup

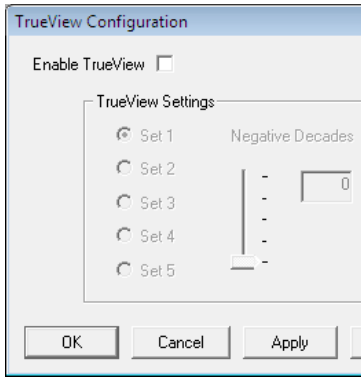
TrueView is a patented tool which provides the ability to optimally view compensated data. Using TrueView, you can display all 1,048,576 channels of data acquisition as well as graphically display negative values in order to facilitate compensation.

Data is transformed via a mathematical function that you can configure. The compensated data is transformed seamlessly in a manner resembling a logarthim but permitting the display of values less than 0. The full range of data is displayed with a default scaling of 6-decades. You can select one of five TrueView Transform settings and the number of Negative Decades to be displayed on on TrueView plots.

Note: The following conditions apply when using TrueView,

- When toggling between the TrueView settings, stats and region counts will differ slighly as each of the TrueView settings represents a different mathematical transformation.
- TrueView should not be enabled while running the AutoSetup Wizard.
- Quadstat Regions are lost on axis if TrueView is turned off and region was set in negative decades area.
- With TrueView, when a region is drawn below the minimum permissible logarithm value, disabling Trueview and subsequently re-enabling will result in the drawn region defaulting all coordinates to the minimum log value.

Enable TrueView checkbox
Set 1 - 5 radio buttons
Negative Decades slider

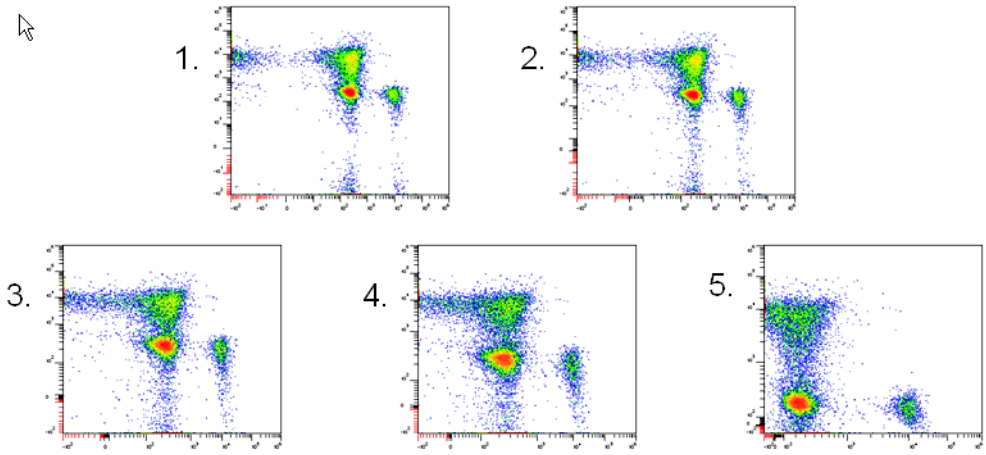


Enable TrueView checkbox

The Enable TrueView checkbox toggles the TrueView Display mode on and off.

Set 1 - 5 radio buttons

This group of radio buttons selects the required TrueView Transform. Only one Set can be selected at any one time. Set 1 - 5 affects the location of the Lin/Log threshold and the compression of the linear data range. An example of each set is as follows:

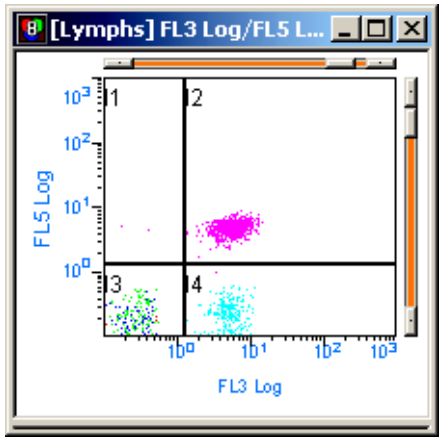


Negative Decades slider

This slider allows you to select the number of Negative Decades to be displayed on compensated parameter plots. The slider allows between 0 and 2 decades to be selected, in 0.5 decade increments

LMD QuickCOMP

Enabling LMD QuickCOMP displays orange slider bars on all dual fluorescence plots.



These sliders are used to intuitively adjust the compensation coefficients and update the compensation values applied to the listmode file.

Clicking the arrows adjusts the settings by ±0.1%, clicking either side of the slider adjusts by ±1.0% or drag the slider to place the cells in the required position. You can view the new settings in the LMD QuickCOMP Matrix

Show LMD QuickCOMP Matrix

When you use the LMD QuickCOMP slider bars to change compensation settings, the new settings are displayed in the LMD QuickCOMP Matrix dialog box. You cannot use the LMD QuickCOMP Matrix to change a protocol's compensation settings .

Note: To view compensation adjustments of Listmode data, always view the LMD QuickCOMP Matrix. The compensation matrix in the Cytometer Control dialog represents the compensation settings last used in that protocol and may not necessarily be the compensation settings used for any given Listmode file.

LMD QuickCOMP Settings

	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8	FL9	FL10
FL1		0.4	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
FL2	18.1		7.4	0.4	1.0	0.0	0.0	0.0	0.0	0.0
FL3	12.7	71.4		0.2	0.7	0.0	0.0	3.0	0.0	0.0
FL4	3.3	24.3	77.3		0.4	1.3	0.4	0.0	0.0	0.0
FL5	3.1	1.8	9.8	13.0		0.1	0.7	1.4	0.0	0.0
FL6	4.5	0.0	0.2	51.0	0.0		10.1	6.9	0.0	0.0
FL7	3.5	0.0	1.3	18.7	0.2	36.7		11.8	0.0	0.0
FL8	0.0	0.0	1.1	3.2	4.2	6.3	19.7		0.0	0.0
FL9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
FL10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

Close

Show Analysis Algorithm Results

Not available in Gallios software.

10.21 FLOWPAGE MENU

FlowPAGE - Introduction

FlowPAGE is the reporting area for Data, Plots, Statistics, and user created text. FlowPAGE enables custom formatting of multiple pages of flow cytometer information without the need to swap between other third party applications.

Once a FlowPAGE is created, the contents of its plots and statistics windows are automatically updated to reflect new listmode data or configurations made in the Gallios software analysis.

All FlowPAGEs are saved with a Protocol, this retains the link between the Plots you display and how you want to format the output to the Printer.

FlowPAGEs contain fixed header and footer information generated from FCS Keywords. The automatic header information as displayed on the FlowPage reports always displays xxxxxx on the Gallios Workspace. Upon printing, all automatic header fields are populated with the listmode file Keyword information on the printout ONLY.

FlowPAGEs are updated during Data Acquisition in live time if Do Not Show Plot Events has not been selected.

Note: Occasionally, when using a FlowPAGE, one or more items cannot be deleted from a page. If this occurs, the items can be cleared from the FlowPAGE by selecting **Files ▸▸ Save As...** to save the protocol with a new name. If you reload this new protocol, the items are now cleared from the FlowPAGE.

Editing FlowPAGEs

You cannot make any changes to a FlowPAGE when running a locked protocol. See Creating FlowPAGES in the System Overview chapter.

Select a Single Item Within FlowPAGE

1 Mouse over the item until you see the cursor.

2 the item and handles appear around the item.

Move a Selected Item Within FlowPAGE

1 Mouse over the item until you see the cursor and +hold and drag the selected item to its new position.

Select a Group of Items Within FlowPAGE

1 Click and hold the mouse button, selecting just outside the group of items required.

2 Drag the cursor until the marquee (dotted outline) completely encompasses the items you want to select.

3 Release the mouse button. Each item has handles around it.

4 Mouse over the selected items until you see the cursor and +hold and drag the selected items to move them.

5 Click the right mouse button on the selected items to edit them.

Deleting Objects in FlowPAGE

1 the object that is to be deleted. Handles appear around the object.

2 Press Delete.

Resizing Objects in FlowPAGE

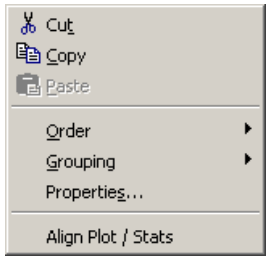
1 the object that is to be resized. Handles appear around the object.

2 Drag a handle to the size required and release the mouse button.

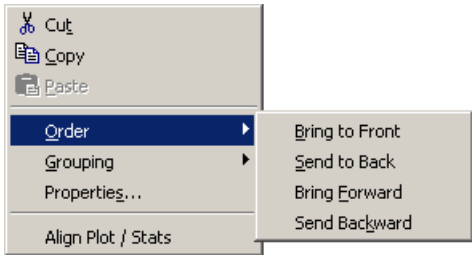
Additional FlowPAGE Formatting Options

Click the right mouse button on any object on the FlowPAGE to select additional formatting options.

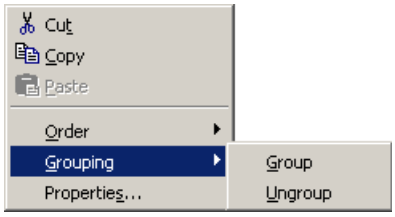
You can **Cut**, **Copy** and **Paste** items that are selected.



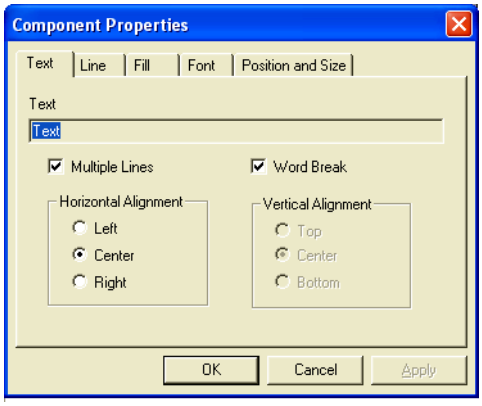
You can change the order of items that are selected.



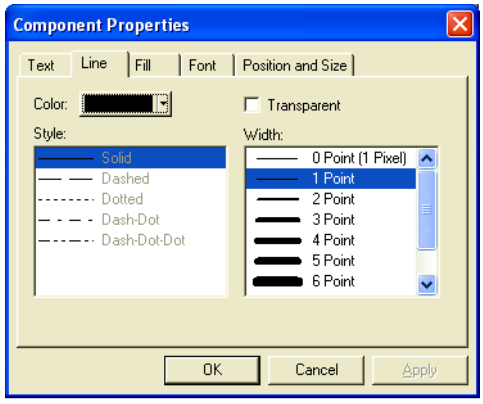
You can group and ungroup items that are selected.



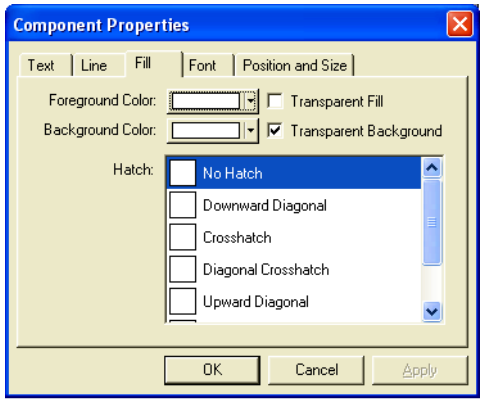
You can edit and change the alignment of text.



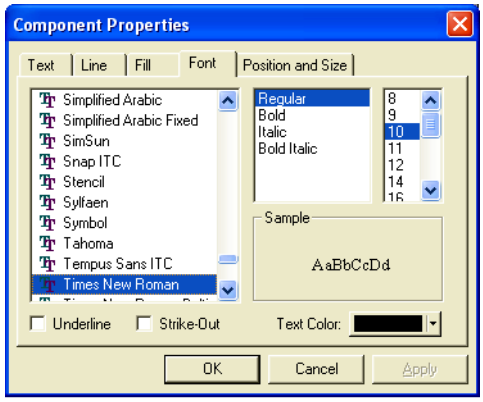
You can change how lines are displayed.



You can change the fill properties of items that are selected.



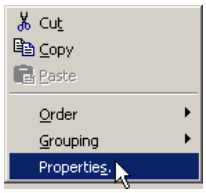
You can change the font properties of items that are selected.



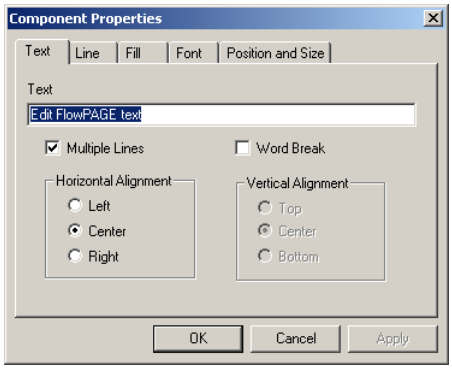
Edit Text

1 Textbox in the FlowPAGE that you wish to edit. The edit handles appear.

2 Right mouse click on the textbox and **Properties.**



3 Type in the new text.



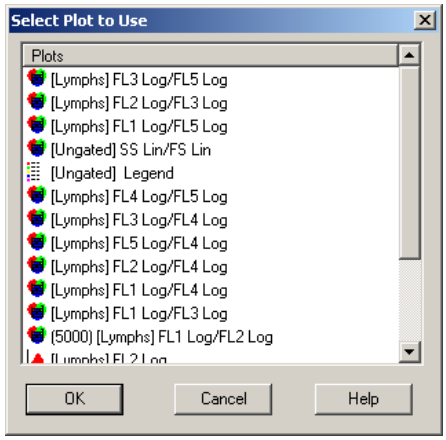
4 OK.

Change Plot

Use Change Plot to change which Plot is displayed from those available on the Gallios desktop.

1 On the FlowPAGE, the Plot you want to replace. The edit handles appear.

2 **FlowPAGE ▸▸ Change Plot**.



3 Select the plot you want to use.

4 OK .

Grow to Largest

1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.

2 . All items in the group grows to the size of the item which has the largest area.

Shrink to Smallest

1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.

2 the button. All items in the group shrink to the size of the item which has the smallest area.

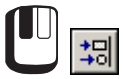
Align Left

1 Select the items to be aligned. See Select a Group of Items Within FlowPAGE.

2  . All items are now aligned to the left of the last selected (grey handles) item.

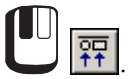
Align Right

1 Select the items you wish to align. See Select a Group of Items Within FlowPAGE.

2  . All items are now aligned to the right of the last selected (grey handles) item.

Align Top

1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.

2  . All items are now aligned to the top of the last selected (grey handles) item.

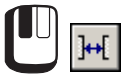
Align Bottom

1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.

2  . All items are now aligned to the bottom of the last selected (grey handles) item.

Space Evenly Across

1 Select the items to be changed. See ***Select a Group of Items Within FlowPAGE.***

2  . All items are now horizontally spaced equally.

Space Evenly Down

1 Select the items you wish to change. See Select a Group of Items Within FlowPAGE.

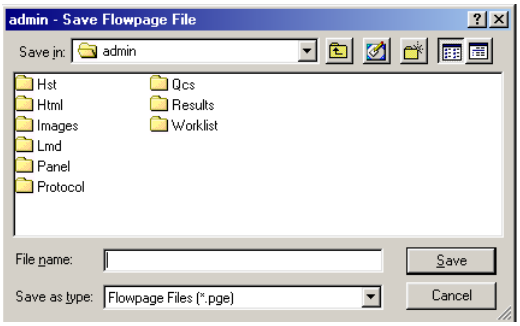
2  . All items are now vertically spaced equally.

Save FlowPAGE As

To save an existing FlowPAGE as a FlowPAGE template:

1 **FlowPAGE ▸▸ Save FlowPAGE As** to view the Save FlowPAGE dialog box.

2 Specify the desired drive and folder by selecting them in the list boxes or by typing the path in the File text box.



3 In the **File name** text box, type the desired File Name.

4 FlowPAGE gives the default file extension *.PGE. The *.PGE extension is only a suggestion, any standard DOS characters can be used for the full file name.

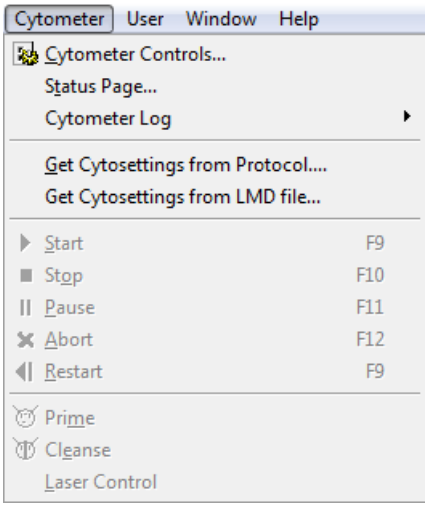
Note: Keep the extension consistent for easy file searches.

Do Not Show Plot Events

Toggles the FlowPAGE 'Hide Events' mode. When in 'Hide Events' mode, all dot, density, histogram and prism plots displayed on FlowPAGEs will not display event information. Other plot information (labels, axes, regions and prism dividers) will continue to display and will be updated appropriately when changes are made to the protocol. Other FlowPAGE components will not be affected by the enable/disable state of 'Hide Events' mode.

10.22 CYTOMETER MENU

Cytometer Control
Status Page
Cytometer Log
Get Cytosettings from Protocol
Get Cytosettings from LMD file
Start
Stop
Pause
Abort
Restart
Prime
Cleanse
Laser Control



The Cytometer menu is concerned with online connection and control of the flow cytometer. The Cytometer Control menu option allows the Cytometer Control screens to be activated. Until this is done the other Cytometer menu items are grayed out.

The Status Page displays current instrument settings, which can be printed out for future reference.

The Cytometer Log displays a log of errors generated by the system.

The Get Cytosettings from Protocol option allows Cytometer Instrument Settings to be obtained from a Protocol other than the current Protocol in memory.

The Get Cytosettings from LMD file option allows Cytometer Instrument Settings to be obtained from a listmode file.

The Controls for Acquisition are also found within the Cytometer menu.

See also:
Flow Rate
Status Bar

Cytometer Control

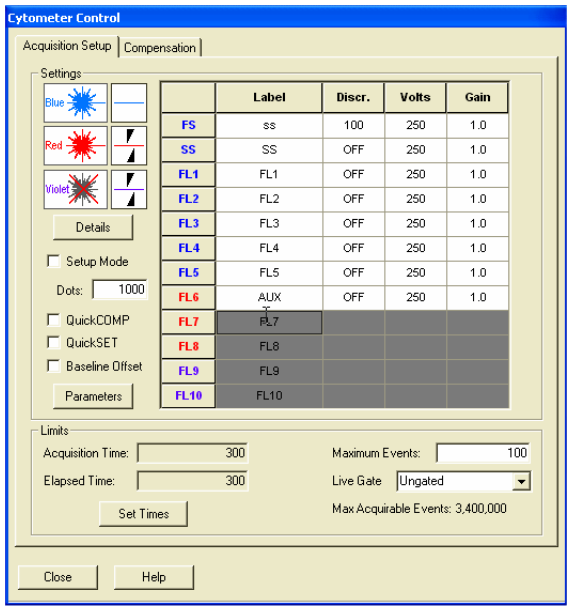
The Cytometer Control button  opens the Cytometer Control screen. This screen is a show/hide dialog box. The type of Cytometer you have installed determines the screens displayed and the functions that are available.

See also:
Cytometer Control Acquisition Setup Tab
Cytometer Control Compensation Tab
Flow Rate

Cytometer Control Acquisition Setup Tab

This screen is used to set the Acquisition Mode and Acquisition Limits. Additionally, the Discriminator values for the selected Parameters can be set.

Label
Discriminators
Volts
Details
Setup Mode
Dots
QuickCOMP Mode
QuickSET Mode
Baseline Offset
Parameter Button
Acquisition Time
Elapsed Time
Maximum Events
Live Gate
Max Acquirable Events



Note: You cannot make any changes on this screen when running a locked protocol.

See also:
Cytometer Control
Cytometer Control Compensation Tab
Cytometer Control Parameter Setup
Flow Rate
Status Page

Settings

The Settings grid allows you to specify Label, Discriminator, Voltage and Gain values for each of the Scatter and Fluorescence detectors installed on the instrument. Detector row headings will be colored according to the Laser to which the Detector relates. If the Laser or PMT to which a Detector relates is not installed on the instrument, then all fields on that row will be disabled.

Label

These input fields allow the editing the Names assigned to the default manufacturer's detectors. Changes are ONLY reflected in the names shown within the Discriminator box in the Acquisition Setup tab and the Compensation tab. This allows the various fluorochromes to be easily recognized when adjusting instrument settings.

Discriminators

Lists the currently selected parameter and allows the setting and modification of discriminator values. To change a value, select the discriminator you wish to alter and use the Slide Control on the right hand part of the display to change the value as appropriate.

Volts

Select the appropriate Voltage for the particular Detector. This is done by using the Slide Bar on the right side of the screen and adjusting it to the required value. For fine adjustment, the Up and Down arrows at the ends of the Slide Bar can be used.

Gain

Adjusts the Gain amplifier. Set the Gain to the appropriate setting you require for that Detector. This is chosen from the drop down box, which appears when you click in the box.

Details

Display the Laser Control dialog.

Note: Do not use an AutoGate as a Live Gate. A Live Gate should be exclusionary.

Setup Mode

Enable this checkbox to allow the continuous acquisition of data from the Cytometer. In this mode, Stops are disabled, Printing is disabled and listmode files are not stored.

Setup Mode allows continuous acquisition of data from the Cytometer giving continuous real-time feedback of instrument performance on the displayed plots. Any Stop & Save values set are ignored in this mode.

Display of data continues until **Abort** is selected, to end data acquisition, or the **Setup Mode** box is unchecked, to begin normal data acquisition.

In Setup Mode the plots are updated real-time with only the most recently acquired events displayed. The incoming data is not saved. During Setup Mode, older events are cleared from the screen and from memory allowing the Cytometer settings to be adjusted in real-time without reaching a set stop condition.

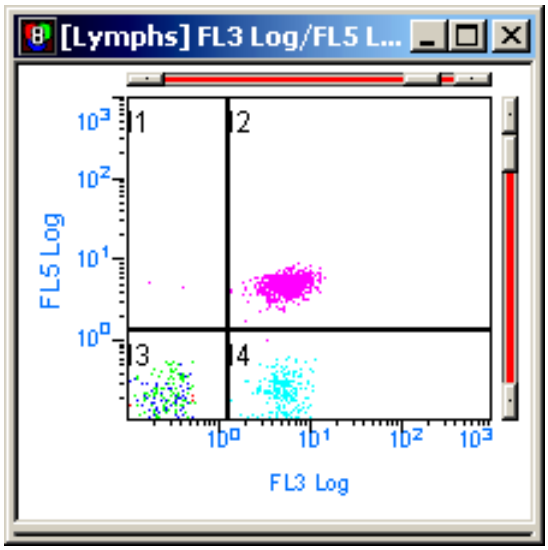
Data is displayed on a “first in; first out” basis. The number of events to be displayed can be changed by entering the required value into the Dots input box.

Once you have finished using Setup Mode click **Abort**, or uncheck the **Setup Mode** checkbox and then perform the acquisition.

Dots

This sets the maximum events to be displayed during **Setup Mode**. The default size is 1,000 events. The **Setup Mode** rolling displays are updated up to three times per second. To change the size, edit the Dots field to the number of Dots required. If a zero is entered, an error message displays showing range of 10 - 9999. The Dots value is stored in the Protocol

QuickCOMP Mode



Enabling **QuickCOMP** on the Cytometer Control Acquisition Setup Tab dialog box displays red slider bars on all dual fluorescence plots.

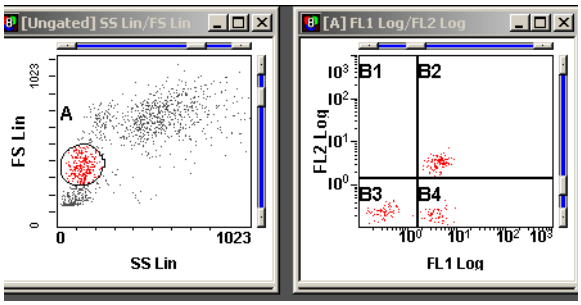
These sliders are used to intuitively adjust the compensation coefficients and update the compensation values in the Cytometer Control Compensation Tab.

Clicking the arrows adjusts the settings by ±0.1%, clicking either side of the slider adjusts by ±1.0% or drag the slider to place the cells in the required position.

When **QuickCOMP** is enabled **QuickSET** mode is not available.

Note: Listmode Compensation must be performed using the runtime protocol or a protocol with equivalent parameters as the runtime protocol.

QuickSET Mode



Enabling **QuickSET** on the Cytometer Control Acquisition Setup Tab dialog box displays slider bars on all plots.

These sliders are used to adjust voltages at the data plots and update the high voltages in the Cytometer Control Acquisition Setup Tab.

Clicking the arrows adjusts the voltage by ±0.1 volt, clicking either side of the slider adjusts by ±1.0 volts or drag the slider to place the cells in the required position.

When **QuickSET** is enabled **QuickCOMP** mode is not available.

Baseline Offset

Enable this checkbox to switch on Baseline Offset. Enabling this checkbox will override the Baseline Offset selections on the Workspace Preferences - Plot Display tab. Further information concerning the setting of this option can be found under Workspace Preferences - Plot Display.

Parameter Button

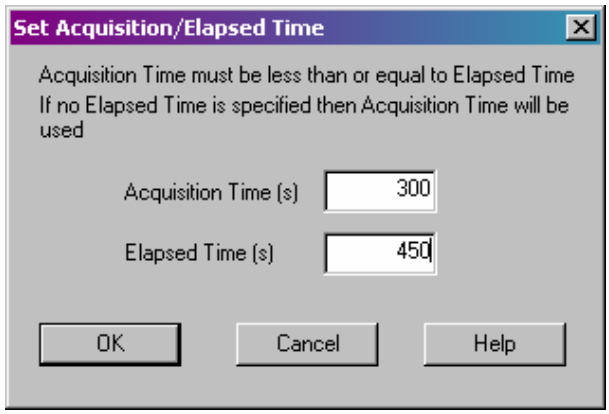
Selecting this displays the Setup Parameter Screen.

Limits

The Limits grid allows you to specifyAcquisition Limits for Acquisition Time, Elapsed Time, Maximum Events and Live Gate.

Set Acquisition/Elapsed Time

The 'Set Acquisition/Elapsed Time' dialog will allows you to set the required Acquisition and Elapsed times for the current protocol.



The Acquisition Time must be less than or equal to the Elapsed Time. If no Elapsed Time is specified then the Acquisition Time will be used. Both Acquisition and Elapsed Times must be between 3 and 99,999 seconds.

Acquisition Time

This field will allows you to set Acquisition Time for the current Protocol.

Elapsed Time

This field will allows you to set the Elapsed Stop Time, which accounts for time in Pause mode, for the current Protocol.

OK

Pressing OK will validate the Acquisition Time and Elapsed Time values. If one or more values is invalid then you will be notified and the dialog will remain open. If both values are valid then the dialog will close and the specified values will be indicated in the Cytometer Control dialog.

Cancel

Pressing Cancel will close the dialog without updating the Acquisition or Elapsed Time settings in the Cytometer Control dialog.

Maximum Events

This is the maximum number of events, which may be acquired on the next Acquisition. To change this, enter the new number required in the input field. However, if the Duration is reached before the maximum number of events acquisition ceases at this point.

Live Gate

This displays all Gates that are available for the Live Gating of data during acquisition. The primary use of a Live Gate is for the intentional removal of unwanted events, such as debris. The data outside of the Live Gate is NOT STORED to the final listmode file by gating out all unnecessary events, thus reducing the data set size.

The software was also designed to enable you to adjust the Live Gate for cell population drift during long acquisition times; this does not automatically refresh the events. The listmode file will include events that are a combination of the original and modified gate(s) used throughout the Live Gate acquisition.

IMPORTANT Improper use of the Live Gate region may generate erroneous but credible results if the Live Gate region is modified during acquisition. If a Live Gate region is modified during acquisition the data set will be a combination of the original and modified gate(s). If this is not desired, then a restart must be invoked manually.

- Ensure that the Live Gate is correctly positioned on the population(s) of interest prior to the acquisition of data. Do not make changes to the Live Gate region or assignment during acquisition.
- Make changes to Live Gate regions only after activating the SETUP option in the Cytometer Control screen resulting in a refresh of the data. If not in setup mode when changes are made to a Live Gate region, a manual restart is required.
- Review all data plots and results prior to reporting results.

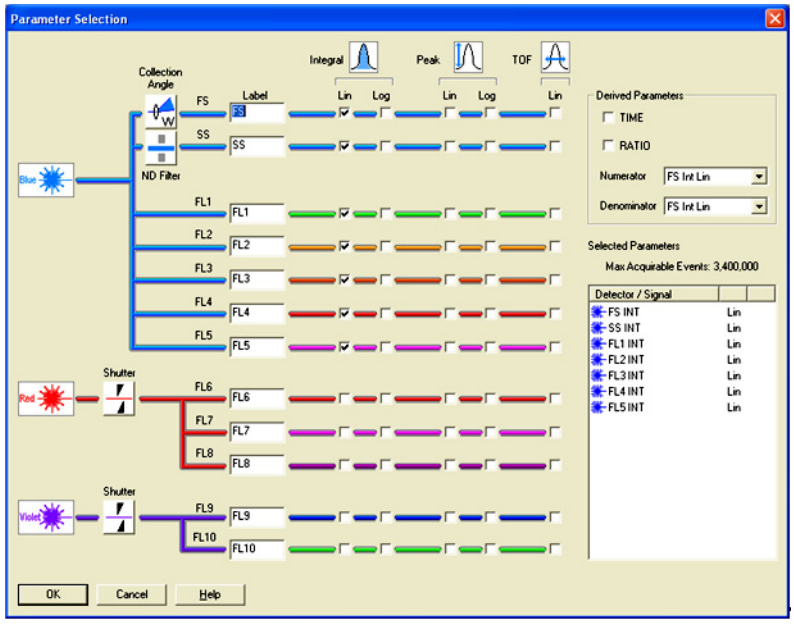
Max Acquirable Events

A system preset limit of the Maximum Acquirable Events based on the number of parameters selected in the protocol.

Cytometer Control Parameter Setup

This screen is used to select the Signals required in the protocol. The signals that are available are selectable by checking the options you require. **Note:** If you add a parameter to an existing protocol, the Detector Names revert to the default names shown below. You cannot make any changes on this screen when running a locked protocol.

IMPORTANT Risk of erroneous results if you assign the same parameter name to more than one detector. Use a different parameter name for each detector.



You can arrange the order within the **Selected Signals** list box by highlighting the appropriate item(s) and Drag and Drop into your chosen order within the **Selected Signals** list box.

IMPORTANT Risk of erroneous results if you modify a protocol by removing or changing the order of selected parameters. Verify that the protocol's plots, regions and gate assignments are as desired before reporting results.

To remove a previously selected item from the **Selected Signals** box, uncheck the parameter no longer required.

Ratio – Choose from the drop down box the appropriate option for the Numerator and Denominator if required.

A detector name may be entered, this is displayed in all **Cytometer Control** screens but does not appear on plots.

Collection Angle

Select the required Forward Scatter Collection Angle for the Protocol. The FS Angle icon will toggle between 'Wide' degrees 'W2' (enhanced wide angle) and 'Narrow' degrees.

ND Filter

Activate 

or deactivate 

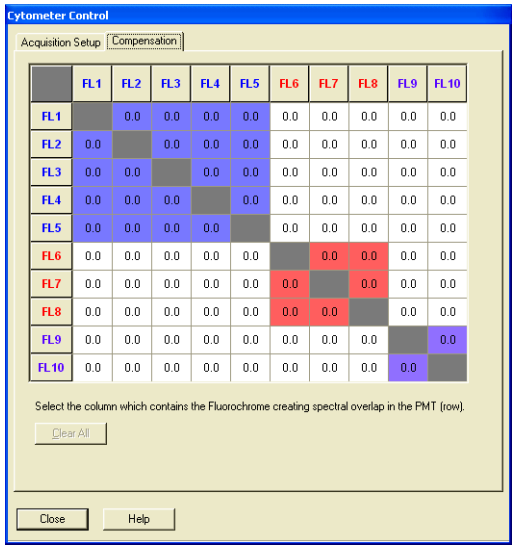
Neutral Density Filters on the Side Scatter Detector.

Cytometer Control Compensation Tab

Compensation allows for the subtraction of a percentage of the signal of one fluorescence detector from the signal of another fluorescence detector to correct for the overlap of one dye's emission into another dye's detector.

Compensation Matrix
Clear All Button

You cannot make any changes on this screen when running a locked protocol.



See also:
Cytometer Control
Cytometer Control Acquisition Setup Tab

Compensation Matrix

Click in the box and type a value or use the Slide Bar to set the desired value. Use the Up and Down arrows to make any fine adjustments to the value.

Clear All Button

Selecting the **Clear All** button resets ALL the values within the **Compensation Matrix** to zero.

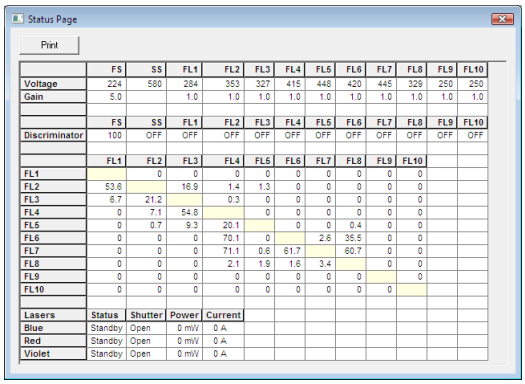
Status Page

To view the cytosettings of an application, display or print the Cytometer Status screen for the verification tube of the application.

The **Status Page** displays the Cytometer settings currently in use and allows you to print these if required.

Selecting the **Print** button displays the standard Windows Print dialog box.

Printed status pages also contain the protocol filename, date, time and cytometer serial number.



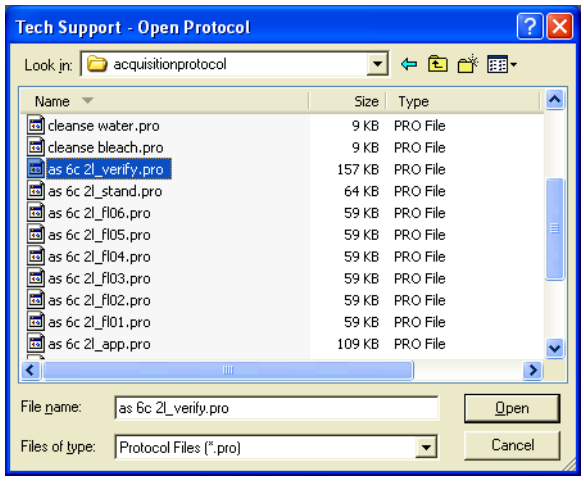
Cytometer Log

Use this menu item to view the log of errors generated by the system. It also allows you to clear this log as needed.

Get Cytosettings from Protocol

The **Get Cytosettings From Protocol** option allows Cytometer Instrument Settings to be obtained from a Protocol other than the current Protocol in memory.

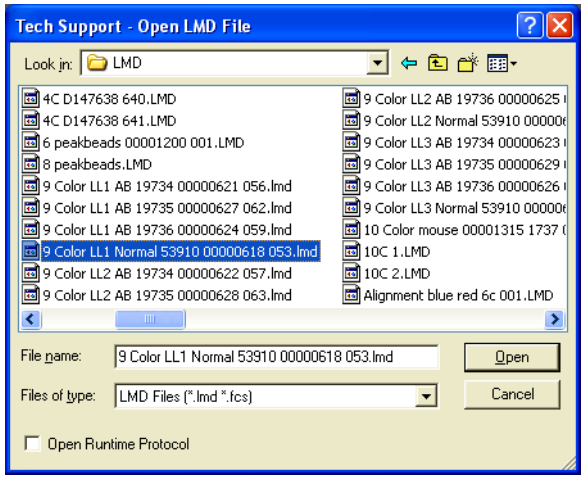
Choose and Open the required Protocol.



Get Cytosettings from LMD file

The **Get Cytosettings From LMD File** option allows Cytometer Instrument Settings to be obtained from a listmode file.

Choose and Open the required listmode file.



Start

The Start button  initiates acquisition of the current sample.

Stop

The Stop button  ends data acquisition. Any Output options such as saving data, calculating statistics, printing reports, and so on, are then performed.

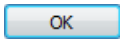
Pause

The Pause button  pauses data acquisition as does clicking. To continue Acquisition, select  . To restart acquisition from zero events select  .

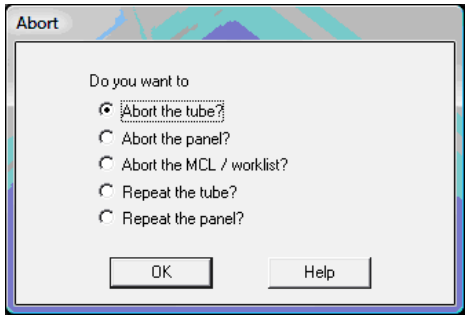
Abort

Selecting the Abort button  displays the Abort screen. This function is only available if Acquisition is running.

Note: No Files are saved if a tube is Aborted.

Select the option required and   .

Abort the Tube?
Abort the Panel?
Abort the MCL / worklist?
Repeat the Tube?
Repeat the Panel?



Abort the Tube?

Stops acquisition, marks the current tube as aborted and the instrument proceeds to the next tube.

Abort the Panel?

Stops acquisition, marks the remainder of the current panel as aborted and the instrument proceeds to the first tube of the next panel.

Abort the MCL / worklist?

Ejects the current carousel and leaves the remainder of the Worklist unread allowing resumption from the current tube.

Repeat the Tube?

Allows the current tube to be resampled.

Repeat the Panel?

Allows the current panel to be resampled. The MCL returns to the first tube of the current panel.

Restart

If a sample is already being acquired, the Restart button  restarts the Acquisition from zero events.

Pause and Rotate

IMPORTANT Risk of sample misidentification. Sample misidentification can occur if you pause the carousel and remove the sample tube and replace it with a different sample tube. To prevent sample misidentification, do not swap sample tubes when the carousel is paused.

The Pause and Rotate button  allows you to pause processing of a single sample and rotate the MCL to a fixed position on the carousel so that additional reagent, for example, can be added to the sample.

Note: To prevent excessive air from entering the flowcell, do not leave the Cytometer paused for extended periods of time.

Note: When you use the Pause and Rotate button function, and remain in the Pause and Rotate state, the Elapsed Time setting limit is ignored.

Idle Mode

The Idle Mode button  places the Cytometer in Idle mode so that various cleaning and replacing procedures can be performed.

Prime

The Prime button  allows you to declog or remove bubbles or blockages from the sample line with sheath solution.

Cleanse

The Cleanse  button allows you to initiate a cycle to flush the instrument sample lines with cleaning solution. The Cytometer will automatically transition to the idle mode  after the 60 second cleanse cycle completes.

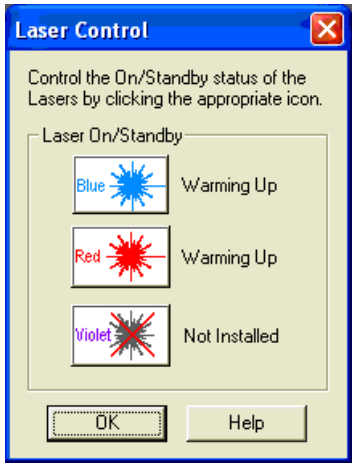
Laser Control

The **Laser Control** Dialog allows you to place the Red and Violet lasers into a Standby state to extend their lifetimes. The Blue laser cannot be placed into Standby mode by the software.

Note: If the Laser Control dialog is open when a laser changes state(e.g. from Warming Up to On), the current state of the laser may not display correctly. Close the Laser Control dialog and re-open it to display the current status of the lasers.

You control the On/Standby state of the Red and Violet lasers by clicking on the appropriate icon. Additionally, if the Blue laser is in the Standby state, switch it on by clicking on the Blue laser icon.

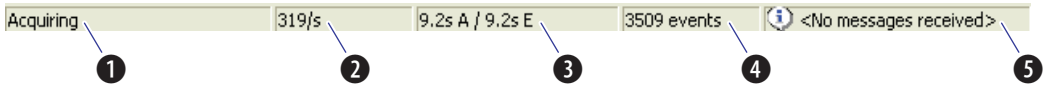
On - Laser is in full power mode
Standby - Laser is in standby power mode
Warming Up - Laser is returning to full power from Standby
Not Installed - Laser is not installed



Status Bar

The Status Bar which is shown at the bottom of the screen displays the following information when in Acquisition.

Note: When you select a menu item or open a dialog during acquisition, the Status Bar does not refresh. Close any open dialogs during acquisition to allow the Status Bar to refresh.



1	Cytometer Status	4	Number of Events
2	Event Rate	5	Cytometer Error/Warning Messages
3	Acquisition Time/Elapsed Time		

Cytometer Status

Displays the current status of the Cytometer (e.g. Initializing, Awaiting Sample, Loading Carousel, Acquiring, Cleansing, Priming, Draining).

Event Rate

Displays the number of events being acquired per second. When not acquiring, it displays 'Not acquiring'. This refers to the Processed Data Rate, that is, the number of events which have exceeded the discriminator, subsequently processed and saved in memory.

Acquisition Time/Elapsed Time

Displays the acquisition time and elapsed time in seconds.

When in Pause mode, the Acquisition time on the status bar does not continue to increment although the Elapsed time does continue to increment. The total Acquisition/Elapsed time can also be viewed from the FCS Keywords associated with the beginning time and end time of acquisition.

Number of Events

The current total number of events acquired is displayed here.

When acquiring, this value increments to the maximum events set or until one of the Stop conditions has been met. See Acquisition Stop and Save.

When acquiring data in Setup Mode, this item cycles in the range from 0 to 10 times the number of Events shown in the Dots Displayed. The value appears to wraparound, that is, if you have requested 5,000 dots to be displayed, this item increments to 4,999. On the 5,000th event the total events counter resets to 0.

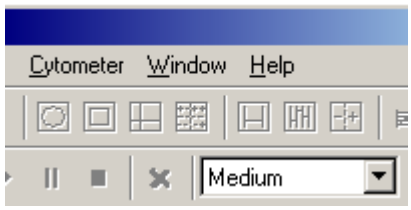
This ensures that you can continue to display data without reaching a set stop condition.

Cytometer Error/Warning Messages

Error/Warning messages generated by the Cytometer. The software translates any received error codes and displays the appropriate text in the status bar. When there are no messages coming from the instrument, this field defaults to "No messages Received". Double clicking this field brings up the Cytometer Status Messages dialog. See CYTOMETER MESSAGES in the Troubleshooting chapter.

Flow Rate

This item shows the relative flow rate of the Cytometer during acquisition. Click on the drop down box and choose the required rate.



The Flow Rate can only be displayed as Low, Medium or High. The Flow Rates are approximately: 10, 30 and 60 µL/min respectively.

Note: _STAND protocols are hard-coded to a Flow Rate of Medium. This flow rate is not passed to remaining tubes in the AutoSetup Application.

Windows Status Bar

The Windows Status Bar normally gives a brief description of selected menu items.

When drawing or editing Regions, the Status Bar shows Channel for X and Y parameters respectively as they are drawn.

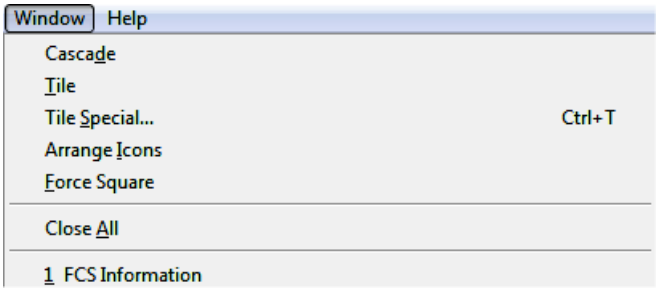
X and Y Coordinates

The X and Y channel values are the coordinates of the cursor position given by a scaling factor. This scaling factor depends upon whether the respective parameters are linear or logarithmic amplification and whether you have calibrated the parameters or not.

- If the parameters are linear and uncalibrated, the X and Y channels vary between 0 - 255 or 0 - 1024 depending on the data source.

If the parameters are logarithmic and uncalibrated, the values vary between 0.1024 - 1024, and span four decades. For most other manufacturers, the data varies between 1 - 10,000, spanning four decades.

10.23 WINDOW MENU



Cascade

Use **Cascade** to display the window so that the plots overlay.

Window ▸▸ Cascade.

Tile

Arranges each plot so every window display is visible. The pattern depends on the number of open Windows.

Window ▸▸ Tile.

Tile Special

Tile Special overrides the normal Windows **Tile** command, this allows plots within the workspace to be arranged in a logical manner.

The plot size in **Tile Special** resizes plots currently displayed in the workspace.

New plots are sized equally according to the default size setting in **File ▸▸ Workspace Preferences ▸▸ Plot Display**.

Plots may be ordered by File, Gate, or Parameter or any combination of these.

At the start of each row, the Plot Type Icon is displayed.

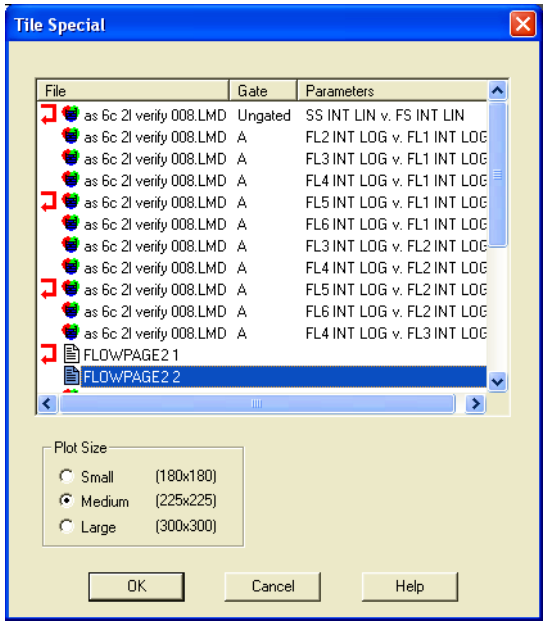
---

1 **Window ▸▸ Tile Special** to display the **Tile Special** dialog box.

2 By clicking the mouse on the **File**, **Gate** or **Parameter** column heading, the plots are displayed across the screen in alphabetical order. Clicking the appropriate column heading again displays the plots in reverse order.

3 The order is shown in the **Tile Special** dialog box. OK to apply the change.

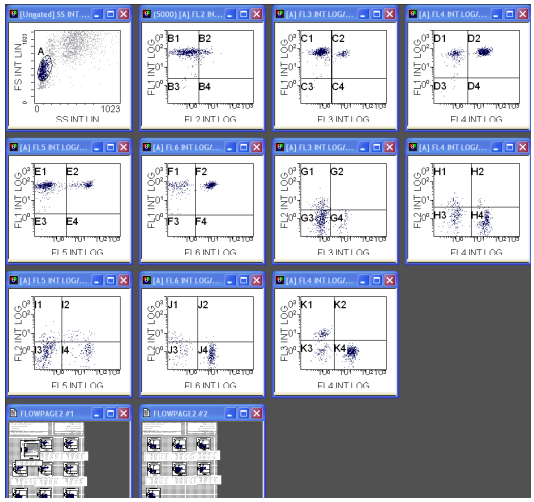
You can also Drag and Drop the plots into any order.



The icon is activated by clicking to the left side of a plot listed in the File column. This forces that plot to be displayed on the following row. If an existing is clicked, the icon marker is removed and New Row is not forced.

In addition, if Ctrl is depressed while the **File**, **Gate** or **Parameters** selection is made, plots can be sorted by Gates within File, and Parameters within Gates/Files.

When OK is selected in the example **Tile Special** screen shown above, the resulting plot display is shown to the right.



Arrange Icons

This option is used to arrange minimized plot windows. Plot windows can be minimized and placed anywhere on screen. **Window ▸▸ Arrange Icons** to place all icons along the bottom of the screen.

Note: This option is very useful when minimized plots have been "lost" on the desktop. Choosing **Window ▸▸ Arrange Icons** places all the icons along the bottom of the Gallios software Window.

Force Square

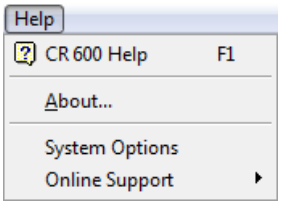
Each window is squared so that horizontal and vertical sides are the same length. That is, aspect ration is forced to 1:1. This is used mainly for dual parameter plots.

Close All

Choosing this option closes all Gallios software windows.

10.24 HELP MENU

Gallios Help
About...
System Options
Online Support



When you access the Help menu, there are four options available: **Gallios Help**, **About…**, **System Options,** and **Online Support**. See also, USING THE SYSTEM HELP.

Gallios Help

Opens the Gallios system help.

About…

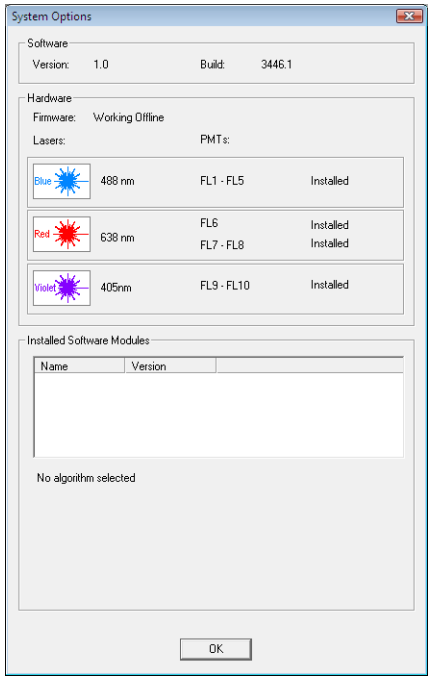
Displays:

- Gallios software copyright
- Gallios software version information
- Whether the currently displayed protocol is a,
  - Runtime Protocol or
  - New Protocol (when listmode file is replayed) or
  - Locked Protocols.

System Options

The System Options screen will display details of the current instrument hardware, together with any installed software modules.

If you are asking for technical support by telephone, fax, or email, please quote the Gallios software build number, which is accessible from menu **Help ▸▸ System Options**, and ensure you know the Romlock number which is written on the body of the Romlock.



Software

The information in the Software group describes the software version and build number.

Hardware group

The information in the Hardware group describes the current instrument configuration. If no instrument is currently connected to the workstation, then the last known settings are displayed, except where otherwise specified.

Hardware group - Firmware

This field displays the version number reported by the instrument firmware. If no instrument is currently connected, the field will display "Working Offline".

Hardware group - Lasers

The emission wavelengths for all installed Lasers is displayed. If a Laser is not present the Laser information reads "Not Installed"

Hardware group - PMTs

The installed PMTs are detailed, broken down by the appropriate Laser. Each PMT group is labelled "Installed" or "Not Installed" according to the current instrument configuration.

Installed Software Modules group

Shows the optional Software Modules that are installed. Not applicable for Gallios software.

List control

The list control displays the names and versions of the installed algorithms Only one item can be selected at a time.

Description field

A read only field that describes the selected algorithm. The description is provided by the algorithm itself.

Online Support

- **Beckman Coulter Web Site**

  If you have an Internet connection and you click the mouse on http://www.beckmancoulter.com you can access the Beckman Coulter Web Site through your Web Browser program.

You can then access the latest information concerning the up-to-date software releases and other items of interest.

10.25 QUALITY CONTROL REPORT

The Quality Control Report consists of Levey Jennings plots based on information selected in the QC Template and data stored in the QC tables in the Report Generator database. The information selected in the QC Template may be results (that is, %, cells/µL, mean, mode) or cytosettings (that is, voltages, gains). The data is plotted against time in the Levey Jennings Plots and can be saved to spreadsheet format.

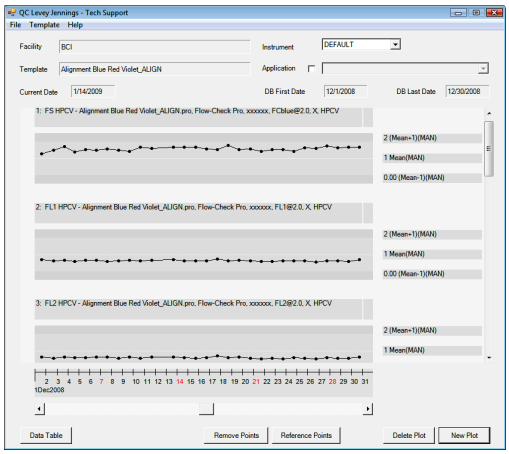
Note: A QC protocol must be run prior to viewing data associated with your instrument (serial number).

QC Levey Jennings Screen

---

1  on the Report Generator toolbar to display the QC Levey Jennings screen.

Instrument
Application
Data Table
Remove Points
Reference Points
Delete Plot
New Plot



Instrument

Choose the instrument that you want to view QC data from.

Application

and choose an AutoSetup II application that is associated with a template.

Note: To view QC results that were run without being associated with an application (for example, Flow-Check Pro QC results not scheduled when run), leave this option unchecked.

Data Table

Displays the information from the Levey Jennings plots in tabular format.
See also, QC Data Table.

Remove Points

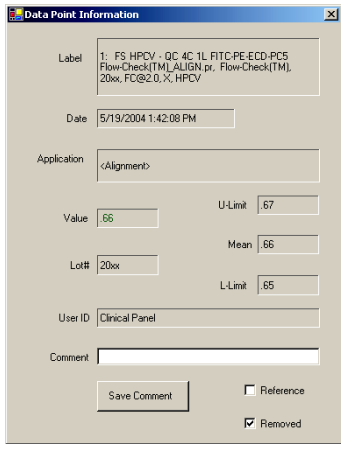
To remove a data point from a plot,

on a data point and **Remove**.

To restore the data point,

Remove to clear the checkbox.

Removed data points are displayed green and are excluded when calculating the mean and SD on the Levey Jennings plot.



To remove a range of data points,

1 Remove Points and on the first data point to remove.

---

2 Right mouse click on the last data point to remove and Remove Points.

Reference Points

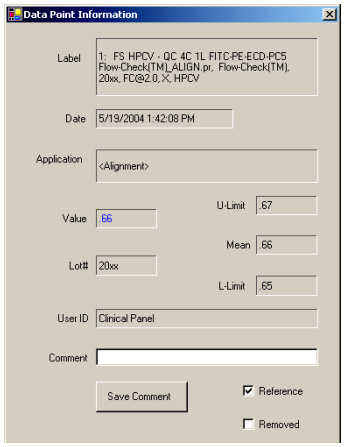
To assign a data point as a Reference,

on a data point and **Reference**.

To restore the data point,

Reference to clear the checkbox.

Reference points are displayed orange and when assigned, the Mean and SD of the Levey Jennings plot can be calculated from the Reference points.



To assign a range of data points as reference,

1 Reference Points and on the first data point.

2 Right mouse click on the last data point and Reference Points.

Delete Plot

1  (to the right of the plot title). Multiple data plots can be selected.

2  and  .

New Plot

Add a new plot.

File Menu

Print

Print the displayed QC data.

Print Preview

Preview the QC data printout.

Page Setup

Set page orientation and margins.

Close

Close the QC Levey Jennings screen.

Template Menu

The dataplot definitions of the Levey Jennings screen can be saved as a QC template.

Clear Template

Clear the current template

Select Template

Select a saved template.

Save Template

Save the current template

Save Template As

Save a template as a different name.

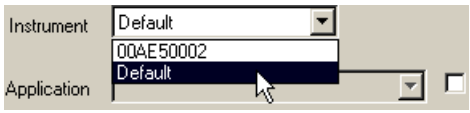
Associate Default Template Files To Your Instrument

Default QC templates are installed with the software. You need to save the default template files with your instrument serial number for the database to populate them correctly.

---

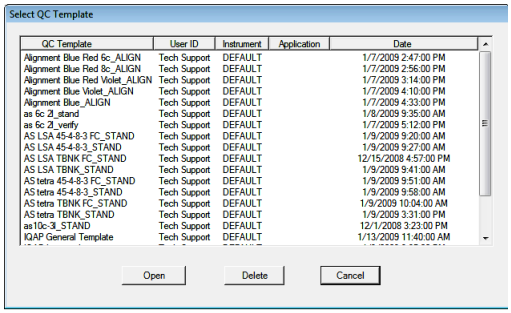
1 On the QC Levey Jennings screen

Default in the **Instrument** field.



---

2 **Template ▸▸ Select Template.**



---

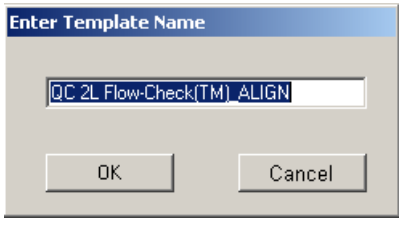
3 Highlight the desired template and

Open.

---

4 **Template ▸▸ Save Template As**, enter the template name and

OK.



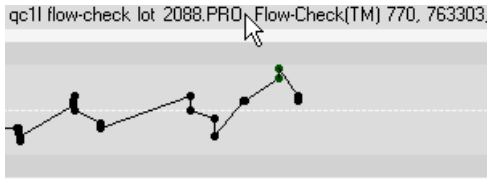
Modifying Template Files

You can modify a default template to collect QC data from different protocols and parameters.

Note: Do not modify or edit IQAP templates. Use the IQAP templates to submit control data to the IQAP Program. Refer to the IQAP manual, PN 4206384, or www.beckmancoulter.com for additional information.

Select Parameter

1 on the title bar of a plot.



2 Use ▼ to choose the appropriate,

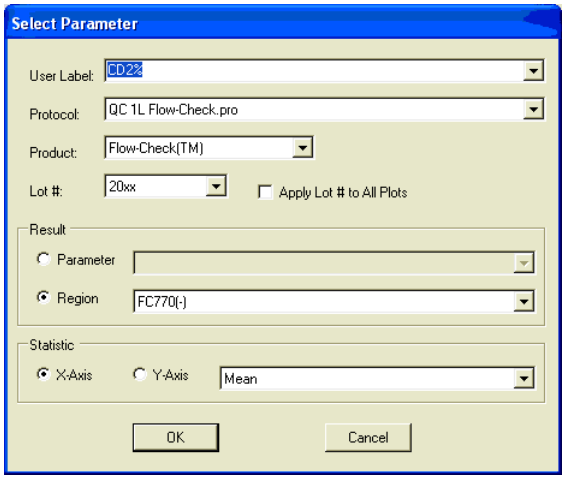
a. **User Label**

b. **Protocol**

c. **Product**

d. Lot #

e. Apply Lot # to all plots.



3 Click one of the choices,

a. **Parameter** ◉ and choose the parameter or compensation setting or,

b. **Region** ◉ and choose,

- the **Region**
- **X** or **Y**
- the **Statistic.**

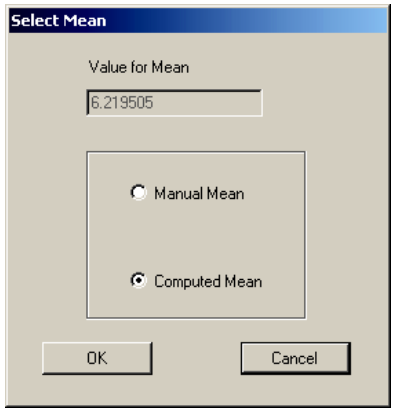
4 Click OK.

Select Mean

1 Click **(Mean)**.

2 one of the choices,

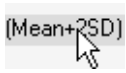
- **Computed Mean.**
- **Manual Mean** and enter value.



3 OK.

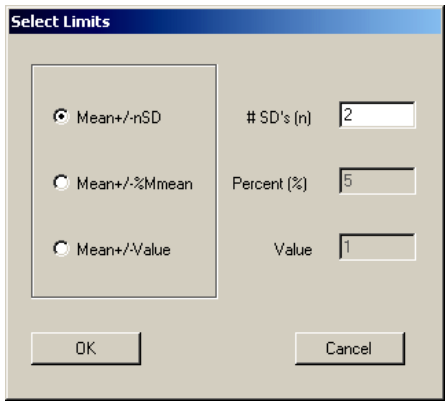
Select Limits

1 **(Mean+)** or **(Mean-)**.



2 one of the choices,

- **Mean+/-nSD** and enter a number.
- **Mean+/-%Mmean** and enter percent.
- **Mean+/-Value** and enter value.



3 OK.

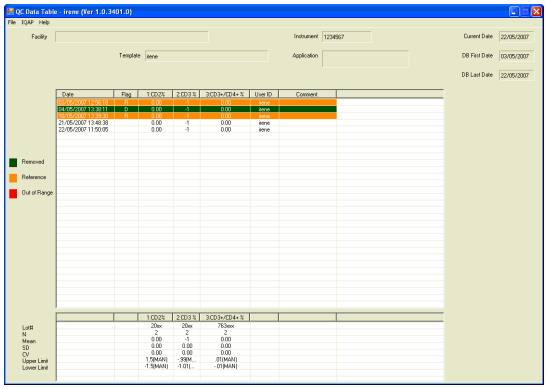
QC Data Table

The QC Data Table displays the information from the Levey Jennings plots in tabular format. Each column of the table displays the data from one Levey Jennings plot. The statistics for each column are displayed at the bottom of the table.

Data Table to display the Data Table.

and or in the scroll box to scroll both data tables together.

and drag a scroll bar to scroll a data table individually.



File Menu

Create Spreadsheet File

Creates a text file (*.txt) that contains the data table information. You can import the file into a spreadsheet program for analysis.

Print

Print the QC data table. When you use an IQAP template for control results, print the data table and fax or mail the results to the IQAP Program.

Print Preview

Preview the QC data table printout.

Page Setup

Set page orientation and margins.

Close

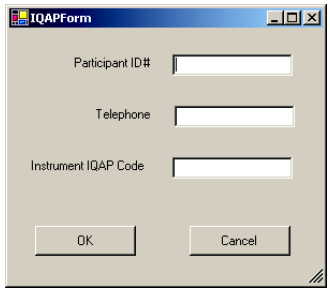
Close the QC Data Table screen.

IQAP Menu

The IQAP information entered here is saved to the printout and spreadsheet file.

Edit IQAP

1 **IQAP ▸▸ Edit IQAP** to display the IQAP information.



2 Enter your IQAP information and OK.

10.26 PANEL REPORT TEMPLATE

IMPORTANT Risk of panel report not generating if you have not saved the template before running the assay for the first time. Edit the limits and save the template before first-time use.

Risk of misleading results if you do not specify the correct cell population. Specify the correct cell population even if you do not want to print the line since other lines may refer to this line.

Overview

Results are exported to the database when they are generated from panels that are "export" panels. This means that when you run a sample in an export panel, or replay a listmode in an export panel, the results are automatically exported to the database. There is a one-to-one match between export panels and their corresponding templates.

Any panel can be saved as an export panel. See Creating a New Panel Report Template or Creating Panels. When you open the desired panel report template, it is important to understand how the template affects the printed report.

Each line in the template represents information to be used in the final printed report. Certain lines of information, such as those used for calculating other results, are not printed on the report. Each template has three tabs: Global, Local and Data Plots.

Note: Panel Reports may not contain all of the FCS information from the protocols in a panel. If a Panel Report is constructed from from multiple protocols, the FCS keywords from one protocol may not match the FCS keywords in another protocol in the Panel, causing some FCS information to be omitted from the Panel Report.

10.27 CREATING A NEW PANEL REPORT TEMPLATE

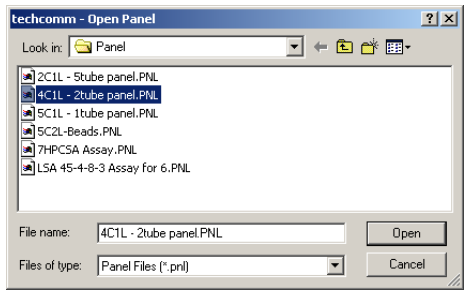
In order to print patient panel reports, you need to create a panel report template that you use to define what to print on the report.

Create A CPF File

A CPF file contains the settings for a panel report template.

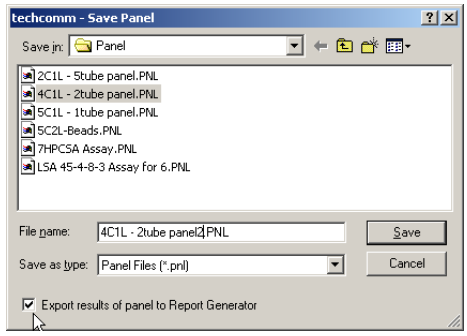
---

1 , select the panel you want to use to create a panel report template and Open.



---

2 Right click on the panel in Acquisistion Manager and Save as Panel / Panel Wizard.

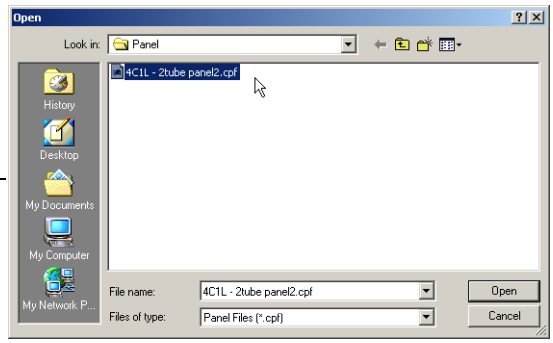


---

3 Type a name for the panel, **Export Results of panel to Report Generator** and Save.

Create the Panel Report Template

4  .



5 **File ▸▸ Open,** select the CPF file you want to use for patient reports and  .

6 Use the Global, Local and Data Plots tabs to enter the information you want on the patient report.

7 **File ▸▸ Save** to save the template. See Verifying A Panel Report Template

Note: When you save an export panel using the "Save As Panel" option, you must clear the worklist and re-select the panel before running the export panel.

Verifying A Panel Report Template

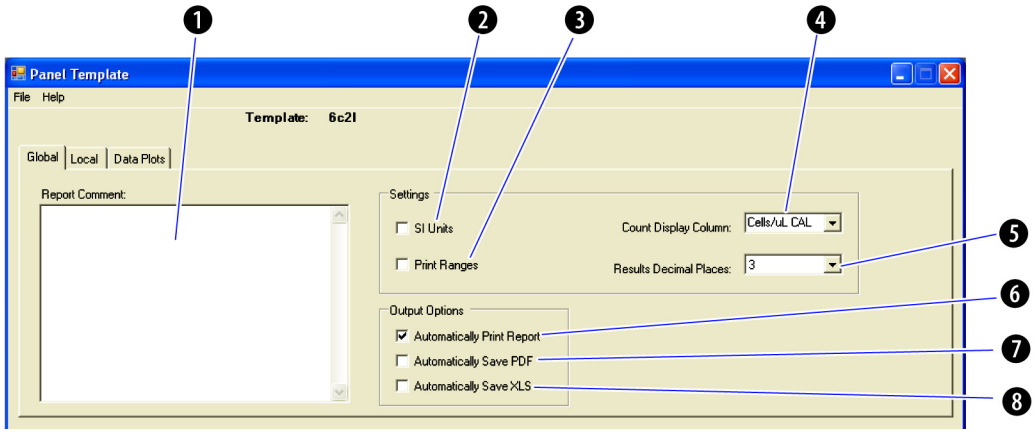
Before using the report template, verify that it is working as expected. Check that the result lines are organized in the desired sequence. **Note**: The row that an equation is created on must be constructed using information from rows above the equation row. **Note**: You must save a protocol **prior** to using it as part of a panel.

To verify a panel report template after creating or editing it:

1. Print a copy of the panel report template.
2. Verify that the report includes all desired lines in the proper sequence.
3. Verify that the equations are referencing the appropriate rows and that these rows are **above** the equation row in the template.

Run a test panel on a normal sample and verify each result printed on the panel report. If there is an inconsistency, verify the report template again for proper formulas and references; then verify again.

Global Tab Settings



1 Allows you to define a global report comment that prints on **every** panel report generated from this template
Note: You can edit the comment on individual reports if necessary. See Editing Report Comments.

2 Allows you to select the reporting unit format: SI Units or US format (which is the default if SI Units is not selected).

3 Allows you to choose whether or not to print ranges on the report.

4 Allows you to choose Cells/µl CAL, Cells/µl Hem or Number. Cells/µl CAL adjusts absolute counts based on the CAL factor. Cells/µl Hem calculates absolute counts based on the Cell Population selected from the hematology patient information and the number of events in the result region. Number displays the unadjusted count in the region.

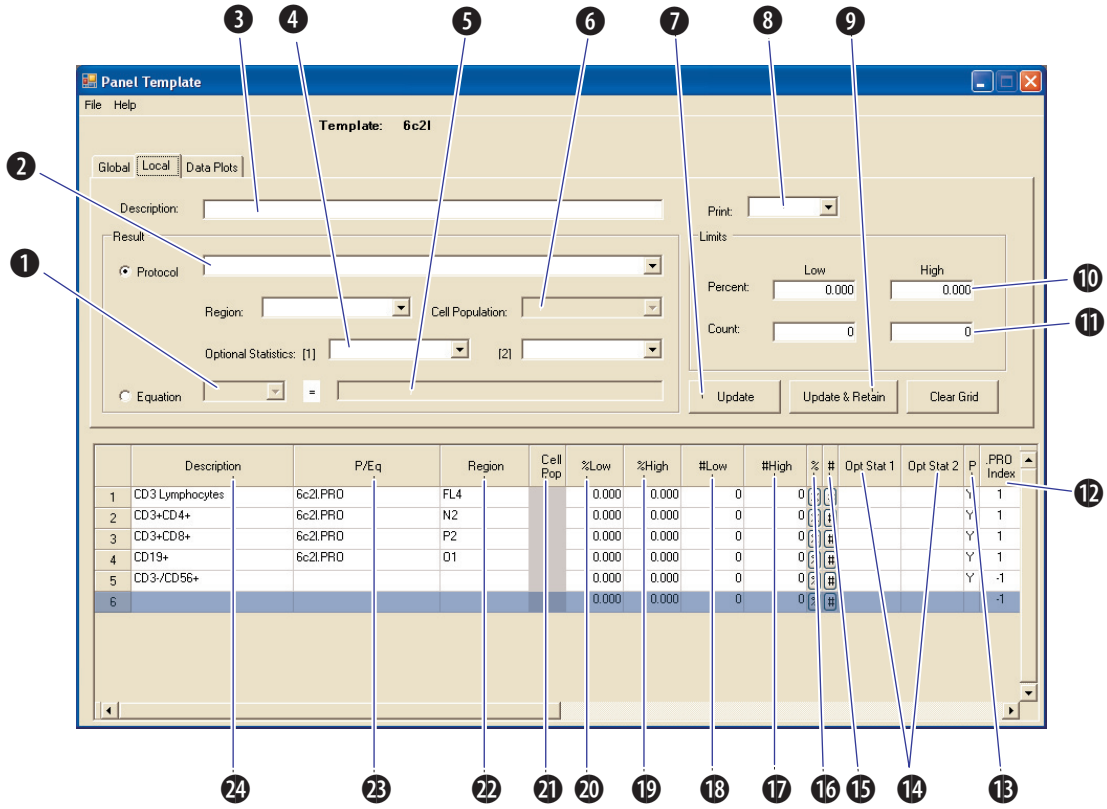
5 Allows you to select the number of decimal places used. Decimal places are just for results and considered in flagging. Select only combo box. The options are **0**, **1**, **2**, **3**, & **4**.

6 Allows you to enable auto-printing so that the panel reports automatically print at the end of acquisition.

7 Allows you to enable auto-save to .PDF.

8 Allows you to enable auto-save to an XLS file.

Local Tab Settings



Note: Special information below about some of the fields on the report.

- The Description, Protocol Region and Print Status fields must include entries in order for the template row input to be fully implemented.
- If you exceed 26 characters for the Description field, the Description may appear truncated on the printout or in the PDF and Excel files.

1 The equation result is selected from a drop-down list that includes:

%&# (Both) = Percent result is calculated from percents and is placed in the Result column and Count result is calculated from counts and is placed in the Count column.

Percent = Result of the equation is placed in the Result column with a % sign.

Count = Result of the equation is placed in the Count column.

Result = Result of the equation is placed in the Result column, no % sign.

See explanation for ❺ for additional equation options.

IMPORTANT Risk of misleading results if you do not specify the correct cell population. Specify the correct cell population even if you do not want to print the line since other lines may refer to this line.

2 When selected, lists all the protocols in the panel. From the drop down list select the protocol to extract results from.

3 Text description of the current template line

4 Displays the optional statistics for the result that was selected in the Region field in the Protocol area of the Local tab. Select up to two of the following for each Results Row: X-Mean, Y-Mean, X-Mode, Y-Mode, X-Median, Y-Median, X-CV, Y-CV, X-HPCV, Y-HPCV. Only statistics that were selected in the protocol during acquisition are available as options. Optional statistics cannot be selected when Equation is selected.

5 Use one of the following methods to define a custom equation.

- Type an equation using the (), *, /, +, -, @, & operators. Result Rows are designated in the equation as R001 to R999.
- The Row number can be preceded by,

  "**%** = Use the value in the Result column of the row

  "**#** = Use the value in the Count column of the row

  "**@** = Use the value in the Opt Stat 1 column of the row

  "**&** = Use the value in the Opt Stat 2 column of the row
- Define an equation by clicking on a cell in the Results Section to add it to the equation. For example, clicking on the Count column of Row 5 adds #R005 to the equation.

Right clicking on this field displays the following options,

+ Add

- Subtract

***** Multiply

/ Divide

DF = Dilution Factor

HV = Harvest Volume

BW = Body Weight

6 Select a cell population (LY, MO, NE, EO, BA, Other, WBC, RBC, PLT, or blank) based on the gating that was used. The available selections correspond to the hematology information in the database. A selection must be made when the calculation method is **Cells/µL HEM**; otherwise, Cell Pop is grayed but retains its value. Cell Pop is blank when Equation is selected.

7 Adds a row using the information entered and blanks all of the fields.

8 Indicates if item prints on the report:

N = not printed; used for calculations; do not enter ranges for these items

Y = printed

B = blank

9 Adds a row using the information entered and retains the information in all of the fields.

10 Fields where you enter the low and high percentage range limits that appear in ⓲ and ⓳, respectively. Low range is -1000 to 1000 with 3 decimal places. High range is -1000 to 1000 with 3 decimal places.

11 Fields where you enter the low and high absolute count range limits that appear in ⓰ and ⓱, respectively. Ranges are is -99999999 to 99999999 for both low and high

The index of the protocol. The index is 0 if equation was used and -1 for a blank line.

13 Indicates if item prints on the report:

N = not printed; used for calculations; do not enter ranges for these items

Y = printed

B = blank

14 Displays the optional statistics for the result that was selected in the Region field in the Protocol area of the Local tab.

15 Indicates that result of the equation is calculated from counts and placed in the Count column.

16 Indicates that result of the equation is calculated from percents and placed in the Result column.

17 Displays the high count range limit entered in ⓫.

18 Displays the low count range limit entered in ⓫.

19 Displays the high percentage range limit entered in ⓾.

20 Displays the low percentage range limit entered in ⓾.

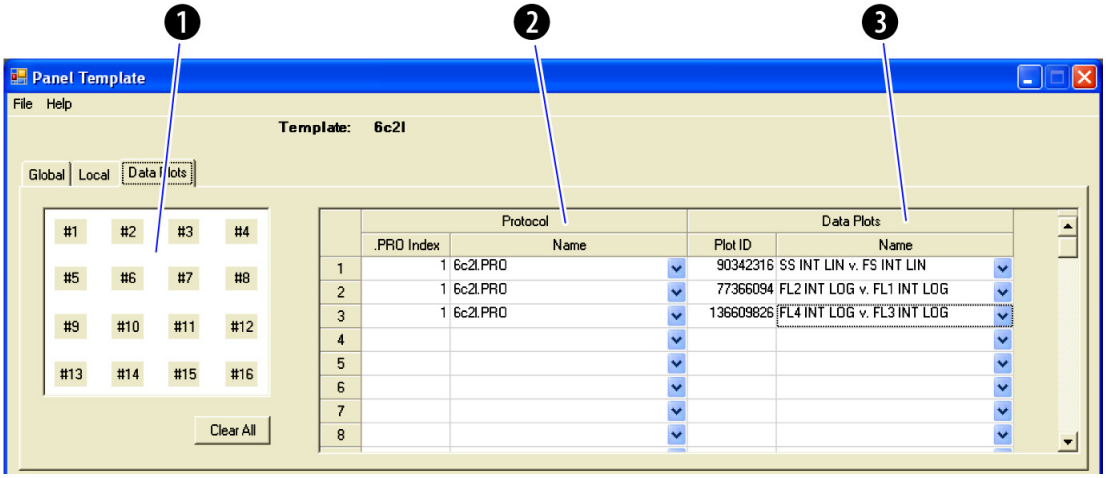
21 Represents the cell population used for gating absolute counts from a hematology instrument on non-Flow-Count panels.

22 Displays the region from where data was extracted.

23 Displays the protocol name (**P**) or an equation (**Eq**) used for arriving at the results.

24 Text description of the current template line.

Data Plots Tab Settings



1 Allows you to select up to 16 data plots to include in the report.

2 Displays the protocol name and index number. The index number represents the protocol order in the panel.

3 Displays the data plot name and index number. The index number is a unique number for plot identification.

10.28 EDITING PANEL REPORT TEMPLATES

Before Running an Export Panel for the First Time

Before you run an export panel for the first time, update the template and save the limits. If you do not save the updated template, the following message appears:

Procedure for Editing/Saving Panel Report Templates

Do this procedure to edit and save a any export panel report template to:

- select/deselect SI units
- enable/disable the auto-print feature (when enabled, the report prints automatically at the end of acquisition) **Note:** The panel report auto-print feature is in addition to FlowPAGEs, plots and/or statistics that auto-print per the Workspace Preferences setup (see Workspace Preferences, Acq Options tab)
- add a global report comment (which is printed on every report generated from the affected template)
- edit flagging limit ranges
- add or delete plots

Note: When you edit an export panel and then save it using the "Save As Panel" option, you must clear the worklist and re-select the panel before running the export panel.

---

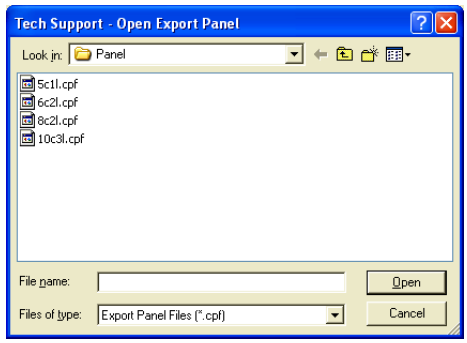
1 .

---

2 View the templates (with .cpf extension):

a. **File ▸▸ Open**.

b. Navigate to the desired panel folder.

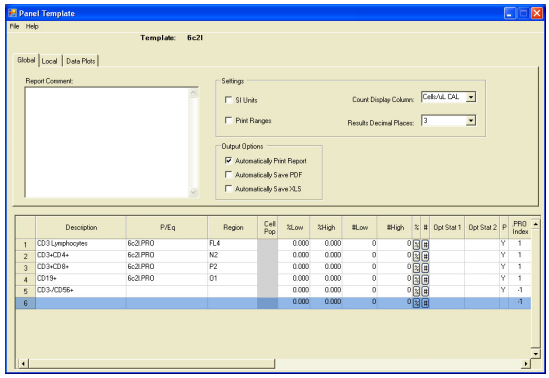


3 To view the contents of a template, select the desired .cpf and **Open**.

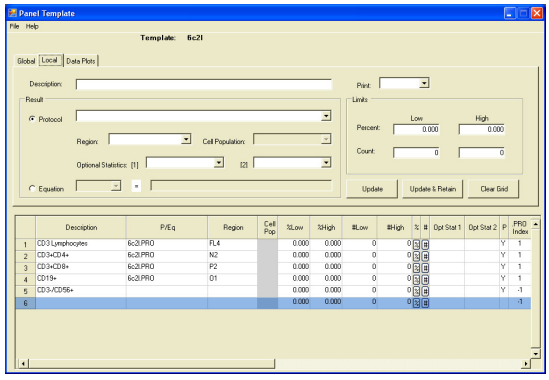
Note: Any export panel created by a user must have the corresponding Panel Report Template created by that user.

4 To select/deselect SI units, enable/disable auto-print, or to add/enter a report comment, **Global**.

Note: If editing a global report comment, only future reports will be printed with the updated text; reports generated prior to the change will not contain the updated comment.



5 To edit flagging limits, **Local**.

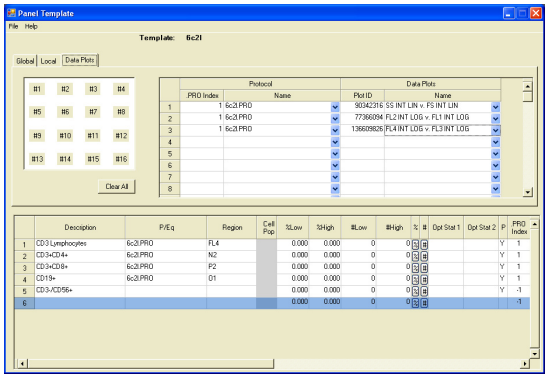


6 Enter/edit the information as needed.

Note: The Description, Protocol Region and Print Status fields must include entries in order for the template row input to be fully implemented.

7 To select data plots for the report,

Data Plots.



8 **File** ▸▸ **Save** to save the template with your changes.
See Verifying A Panel Report Template.

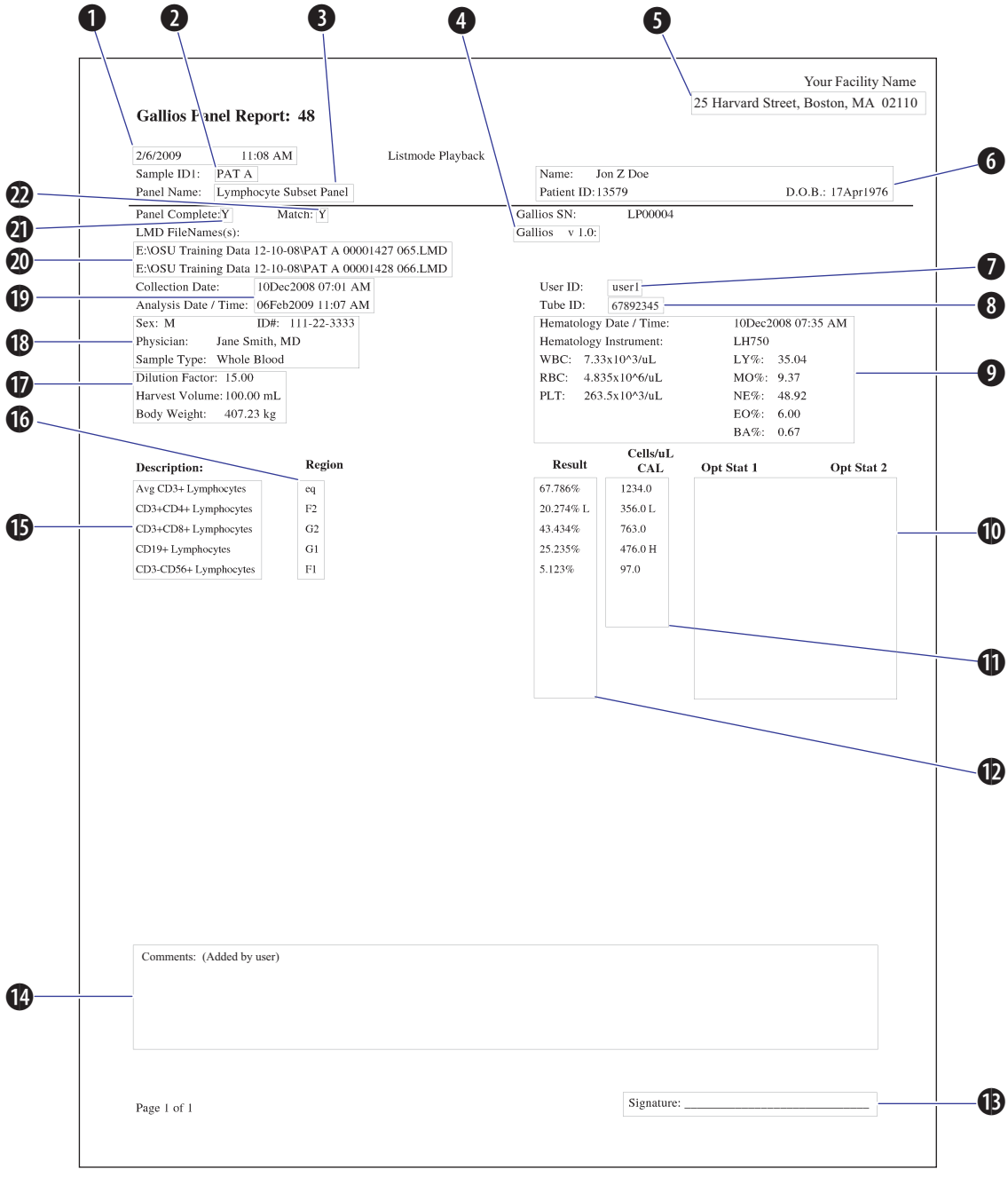
9 **File** ▸▸ **Print** to print a copy of the template for your records.

10 **File** ▸▸ **Close** to close the template screen.

10.29 PANEL REPORT

ATTENTION: The panel must be selected for export so results go to the database.

Below is an example of a patient panel report with data only. Each area is defined for the purpose of helping you better understand the origin of the report information.



1 Date and time report was printed

2 Sample identification (obtained from Gallios listmode file)

3 Indicates the name of the panel used (obtained from Gallios listmode file)

4 Gallios software version and application plug in software version, if installed (obtained from system registry)

5 Institution's name and address where report was produced

6 Patient information (entered on Database Information screen; see Entering/Editing Database Information)

7 User who logged on to Gallios and generated the report

8 Tube ID (barcode)

9 Hematology information (entered on Database Information screen; see Entering/Editing Database Information)

10 Optional statistics: X or Y mean, X or Y mode, X or Y median, X or Y CV, X or Y HPCV

11 Cell count results (may come from Gallios listmode file or can be calculated by the Report Generator depending on the template selected) and expected range of cell count (for expected ranges entered at Local Tab in report template; see EDITING PANEL REPORT TEMPLATES). If a non-Flow-Count™ fluorospheres template is used and hematology information is added, Cells/µL are reflected as **Cells/µL HEM**.

12 Percent positive results (obtained from Gallios listmode file) and expected range of percent (entered at Local Tab in report template; see EDITING PANEL REPORT TEMPLATES)

13 Signature blank for your lab's use

14 Comments (entered by user at the template or at individual report)

15 Reported results (measured or calculated)

16 Indicates that an equation was used and that results were generated by Report Generator

17 Specimen information (entered on Database Information screen; see Entering/Editing Database Information)

18 Patient information (entered on Database Information screen; see Entering/Editing Database Information)

19 Date and time sample was collected and date and time of sample analysis (obtained from Gallios listmode file)

20 Listmode filenames (obtained from Gallios listmode file)

21 Indicates if the complete panel (for example, all tubes) was processed (obtained from Gallios listmode file)

Y = yes

N = no (Rerun entire panel if **N** appears.) *Incomplete Report* prints on the report.

22 Indicates if protocols or regions in the file match the template definition (obtained from Gallios listmode file)

Y = yes

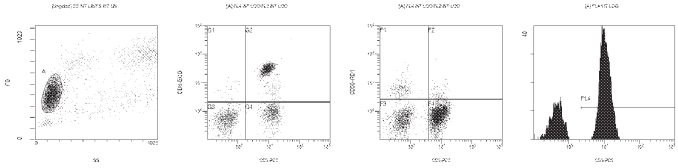
N = no (Indicates that the protocol used different regions than the template. Rerun data/sample through original protocol.)

Below is an example of a panel report with data and plots.

Gallios Panel Report: 49

2/6/2009 11:10 AM Listmode Playback
Sample ID1: PAT A Name: Jon Z Doe
Panel Name: Lymphocyte Subset Panel Patient ID: 13579 D.O.B.: 17Apr1976

Panel Complete: Y Match: Y Gallios SN: LP00004
LMD FileNames(s): Gallios v 1.0:
E:\OSU Training Data 12-10-08\PAT A 00001427 065.LMD
E:\OSU Training Data 12-10-08\PAT A 00001428 066.LMD
Collection Date: 10Dec2008 07:01 AM User ID: user1
Analysis Date / Time: 06Feb2009 11:10 AM Tube ID: NoRead
Sex: M ID#: 111-22-3333 Hematology Date / Time: 10Dec2008 07:35 AM
Physician: Jane Smith, MD Hematology Instrument: LH750
Sample Type: Whole Blood WBC: 7.33x10^3/uL LY%: 35.04
Dilution Factor: 15.00 RBC: 4.835x10^6/uL MO%: 9.37
Harvest Volume: 100.00 mL PLT: 263.5x10^3/uL NE%: 48.92
Body Weight: 407.23 kg EO%: 6.00
BA%: 0.67



Description:	Region	Result	Cells/uL CAL	Opt Stat 1	Opt Stat 2
Avg CD3+ Lymphocytes	eq	67.786%	1234.0		
CD3+CD4+ Lymphocytes	F2	20.274% L	356.0 L		
CD3+CD8+ Lymphocytes	G2	43.434%	763.0		
CD19+ Lymphocytes	G1	25.235%	476.0 H		
CD3-CD56+ Lymphocytes	F1	5.123%	97.0		

Page 1 of 1

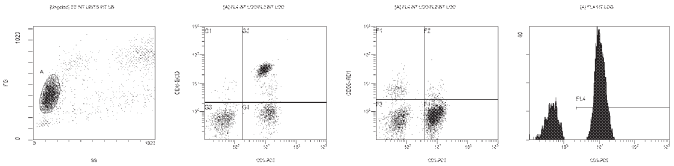
Signature: _____

Below is an example of a panel report with plots only.

Gallios Panel Report: 50

2/6/2009 11:12 AM Listmode Playback
Sample ID1: PAT A Name: Jon Z Doe
Panel Name: Lymphocyte Subset Panel Patient ID: 13579 D.O.B.: 17Apr1976

Panel Complete: Y Match: Y Gallios SN: LP00004
LMD FileNames(s): Gallios v 1.0:
E:\OSU Training Data 12-10-08\PAT A 00001427 065.LMD
E:\OSU Training Data 12-10-08\PAT A 00001428 066.LMD
Collection Date: 10Dec2008 07:01 AM User ID: user1
Analysis Date / Time: 06Feb2009 11:12 AM Tube ID: NoRead
Sex: M ID#: 111-22-3333 Hematology Date / Time: 10Dec2008 07:35 AM
Physician: Jane Smith, MD Hematology Instrument: LH750
Sample Type: Whole Blood WBC: 7.33x10^3/uL LY%: 35.04
Dilution Factor: 15.00 RBC: 4.835x10^6/uL MO%: 9.37
Harvest Volume: 100.00 mL PLT: 263.5x10^3/uL NE%: 48.92
Body Weight: 407.23 kg EO%: 6.00
BA%: 0.67



Page 1 of 1

Signature: _____

Note: When a tube in an multi-tube export panel is aborted, no listmode file is created, however, the panel report is printed as INCOMPLETE REPORT and includes results for the aborted tube. Upon completion of the export panel, the panel report prints as INCOMPLETE REPORT and ERR for the aborted tube.

Printing Panel Reports

Auto-Printing

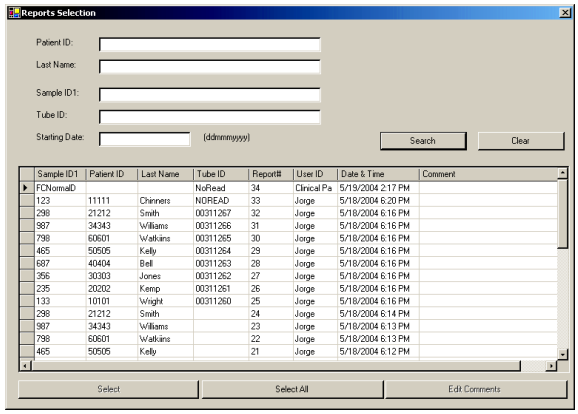
If auto-print was selected in the template, the panel report prints automatically after acquisition ends.

Manually Printing Panel Reports

Do this procedure to manually print a panel report (if auto-print is disabled).

---

1  .



---

2 Search for the desired report(s) using the following search criteria: patient ID, last name, sample ID1, or analysis date.

a. Enter the search criteria information.

b. **Search**.

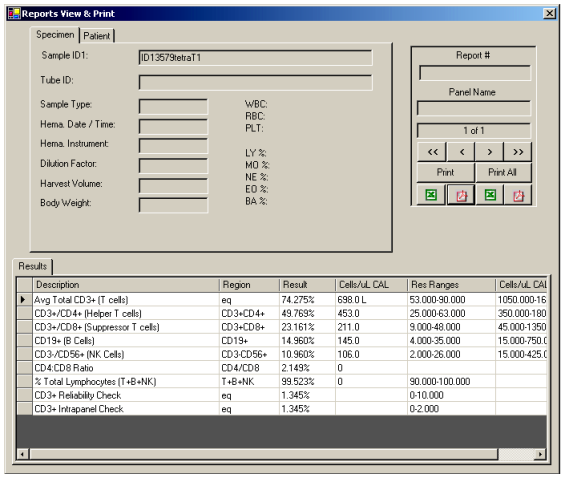
The last sample run appears at the top of the list by default. You can sort the list by Sample ID, Patient ID, Last Name, etc. by simply clicking the desired column title.

Note: To do a global search and locate every sample report in the database, leave all search criteria fields empty and **Search**.

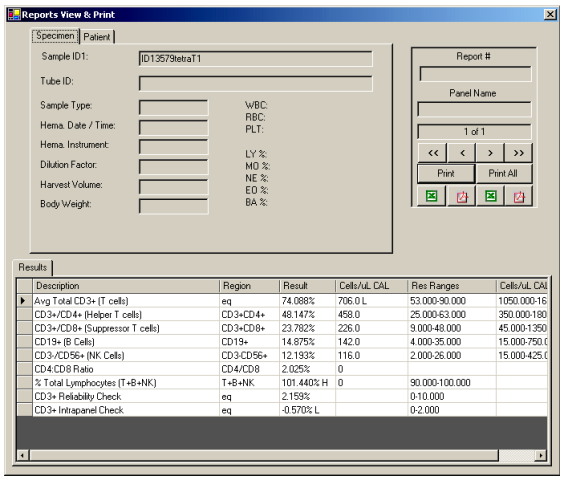
---

3 Select the desired report(s). For details on selecting, see Tips for Selecting Reports.

4 Once you have selected the desired report(s), the Reports View & Print screen appears.



5 **Patient** tab.



6 Using << < > >>, review the reports as needed.

<< displays first report

< displays previous report

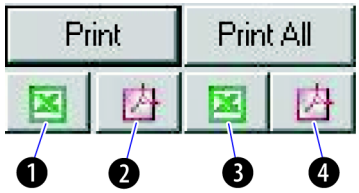
> displays next report

>> displays last report

7 Print the desired reports:

- To print all reports as a batch, Print All.
- To print the report currently displayed, Print.

8 Print (save) the desired reports as a PDF or an XLS file:



❶ saves the selected report to XLS file.

❷ saves the selected report as a PDF file.

❸ saves all reports in this set as XLS files.

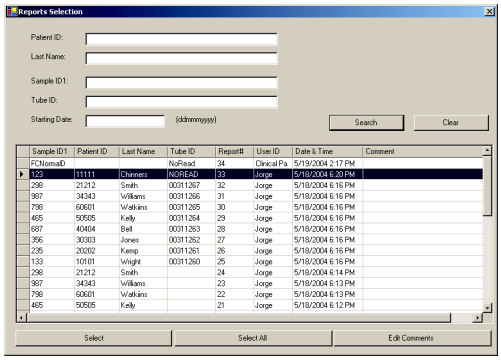
❹ saves all reports in this set as PDF files.

For a better understanding of the printed report, see PANEL REPORT

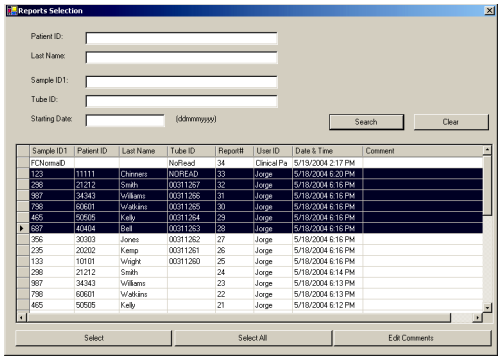
9 **Exit** when finished.

Tips for Selecting Reports

- To select only one report, highlight the desired row and **Select**.



- To select all reports, **Select All.**
- To select multiple reports in sequence:
  a. Highlight the first report you want to select.
  b. Press Shift while you the far left column of the last file to be selected.
  c. Release Shift and the mouse button.
  d. The selected rows are highlighted.
  e. **Select**.



- To select multiple reports out of sequence:
  a. Press and hold Ctrl while you the far left column of each file.
  b. The selected rows are highlighted.
  c. Release Ctrl after all desired files are highlighted.
  d. **Select**.

- To deselect a report, press and hold Ctrl while you the far left column of the highlighted file. The deselected file is no longer highlighted.

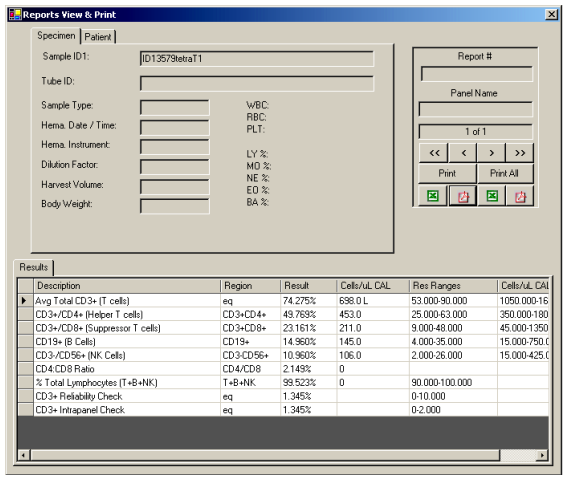
Editing Report Comments

If a comment was added to a template, all reports generated from that template will print that comment. However, you can add or delete comments from individual sample reports via the Edit Comments feature.

Do this procedure to edit comments for a specific report.

---

1 .



---

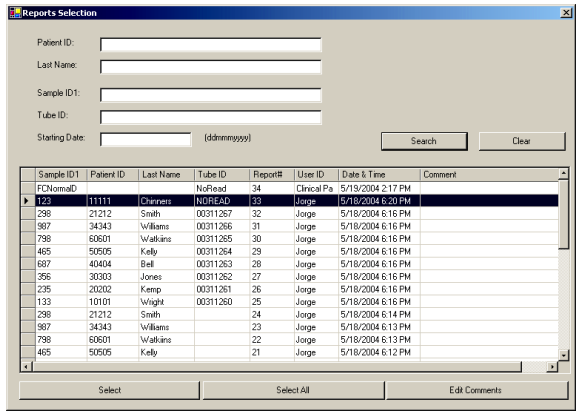
2 Search for the desired report using the following search criteria: patient ID, last name, sample ID1, or analysis date:

a. Enter the search criteria information.

b. **Search**.

Note: To do a global search and locate every sample report in the database, leave all search criteria fields empty and **Search**. The last sample run appears at the top of the list.

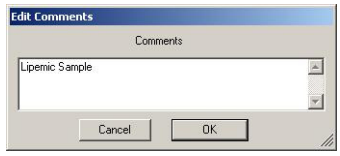
3 To select a report, highlight the desired row.



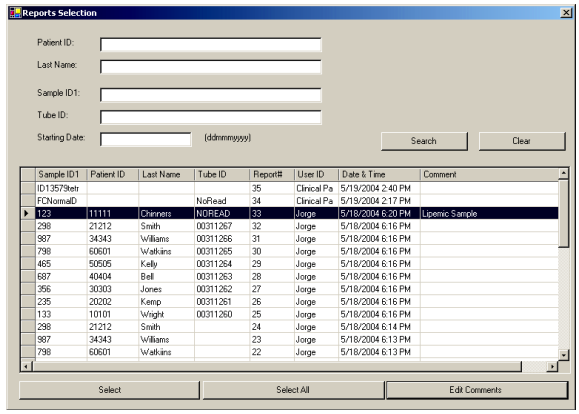
4 Enter/edit the comment information (750 characters maximum).

a. **Edit Comments** and enter/edit the text.

b. **OK** when finished.



5 The comments appear on the Reports Selection screen.



10.30 PATIENT DATABASE EDITOR

The Database Information screen is where you enter/edit information for:

- Patient Demographics,
- Specimen Information, and
- Hematology Information.

The database information prints on the patient panel report.

ATTENTION: When entering a date of birth into patient demographics, you must enter all four digits of the year (for example, 2005 not 05).

Entering/Editing Database Information

Keep in mind that you should enter/edit information prior to acquisition or listmode replay. The database links the information to Gallios by Sample ID1.

---

1  .

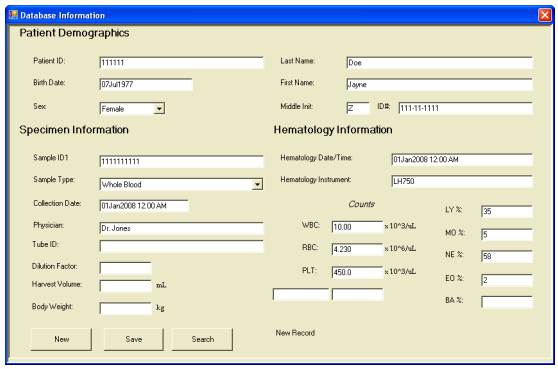
ATTENTION: Regarding hematology information, if Flow-Count was not used for a single-platform absolute count, the WBC and differential parameters may be entered prior to saving or replacing the listmode data files through a non-Flow-Count panel. Absolute counts will then be determined as a dual-platform with the manually entered hematology information and printed on the panel report by replaying the listmode files through a non-Flow-Count export panel.

---

2 To **edit** information or to add specimen information for an existing patient:

a. Enter the search criteria information (such as patient #).

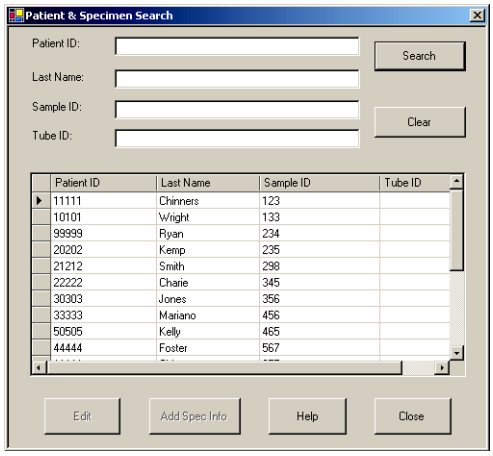
b. **Search**.



3 Highlight the desired information to edit.

4 Verify that the correct information appears.

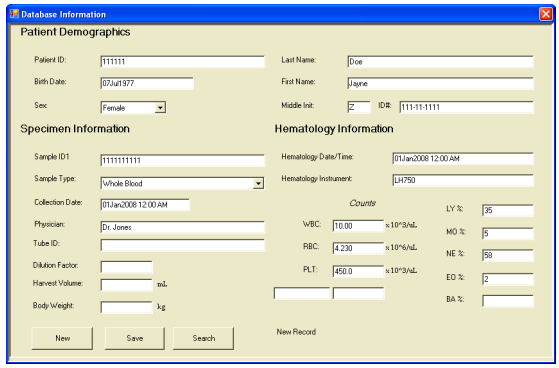
5 Edit or Add Spec Info.



Note: Edit allows you to edit all information except Sample ID. Add Spec Info allows you to enter new specimen information for this patient, as well as edit existing information, including Sample ID; all demographic information is retained.

6 Edit the information as needed and

Save to save the changes.



To enter information for a new patient:

a. **Clear**.

b. Enter the information.

c. **Save** to save the input.

10.31 DATABASE MANAGEMENT

You must be assigned System Administrator rights in order to Archive Data or Restore an Archive.

Archive Data

Data from the Report Generator and QC databases can be archived out of the live database for storage offline.

1 Log in as Admin.

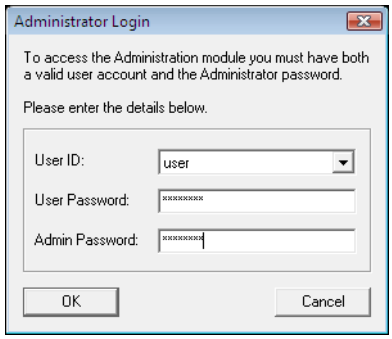
a. Admin on Cytometer Startup Page 1.

a. Select your User ID from the pull down list.

a. Enter your password.

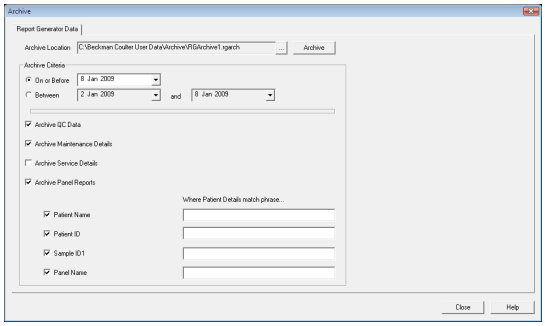
b. Enter the Admin Password.

c. OK.

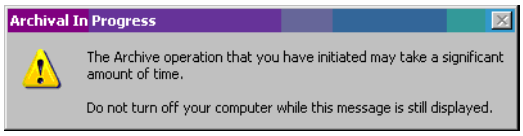


2 Archive Data.

3 Choose the archive location, archive criteria and what data you want to archive. Refer to the detailed description of the Archive Data Screen.



4 Archive.



Restore Archive

The Restore Archive dialog is a standard Windows File Open dialog which allows you to select an Archive file to restore.

1 Log in as Admin.

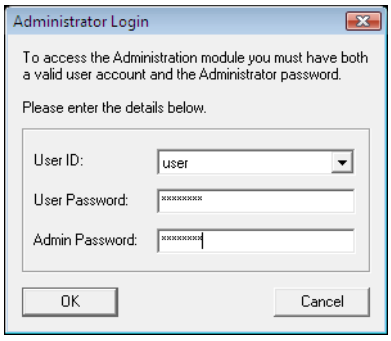
a. Admin on Cytometer Startup Page 1.

a. Select your User ID from the pull down list.

a. Enter your password.

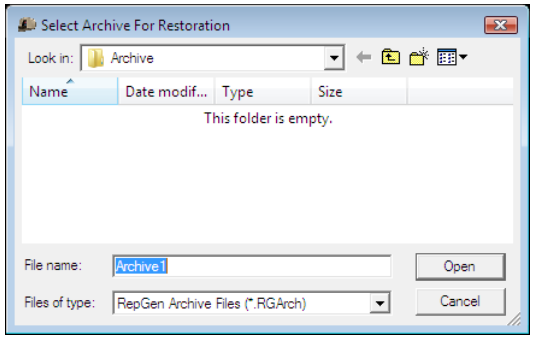
b. Enter the Admin Password.

c. OK.

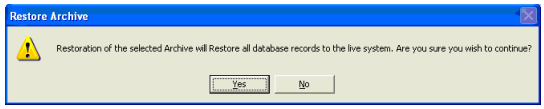


2 Restore Archive.

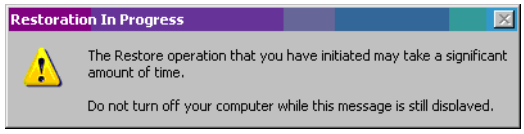
3 Select the Archive file (*.rgarch)



The system validates the archive file. If it is valid, a confimation message is displayed



While Restoration is in progress, a warning message is displayed to indicate that Restoration may take some time and that you should not exit the software until the Restoration is complete

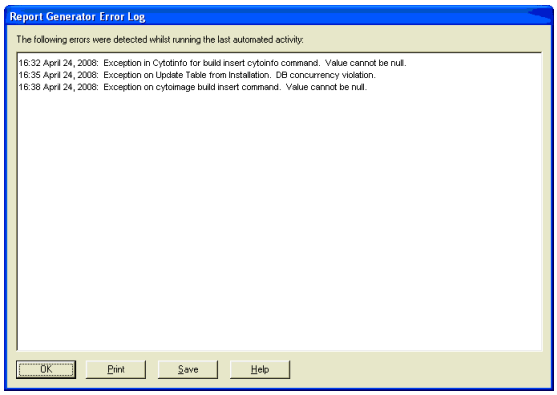


10.32 REPORT GENERATOR ERROR LOG

The Report Generator writes error messages that occur during an automated activity to the Report Generator Error Log. The name of the log file is RepGenError.rgl and it is created in the current user folder. The software will display the Report Generator Error Log at the completion of any automated activity during which errors were written to it. If the error log file is present, the Report Generator Error Log will also be displayed when the software is launched.

Error Log Screen

Error messages list
OK
Print
Save



Error messages list

The dialog displays a list of the errors (the contents of RepGenError.rgl file). All error messages display the time and date at which they were created, together with information to allow you identify the cause of the issue.

OK

If the log file has been saved, selecting the OK button closes the dialog and deletes the log file. If it has not been saved, then the software displays a message prompting you to save the log file. Selecting Yes closes the message and displays a save dialog, leaving the Report Generator log dialog open. Selecting No closes the message and the Report Generator error log dialog and deletes the log file RepGenError.rgl.

Print

Print the Error Log details. The page format defaults to Landscape to ensure that the error message text can be displayed in full.

Save

Select a file into which to save the Error Log. The file will be saved in simple Text format. Selecting Save closes the Report Generator Error Log dialog the log file RepGenError.rgl is deleted.

11.1 WHAT THIS CHAPTER EXPLAINS

This chapter contains the following cleaning procedures:

- CLEAN THE SAMPLING SYSTEM
- CLEAN THE MCL SAMPLE HEAD AND THE SAMPLE PROBE
- CLEAN THE AIR FILTERS
- CLEAN THE INTERNAL SHEATH FLUID CONTAINER
- CLEAN THE CLEANING AGENT CONTAINER
- CLEAN THE VACUUM TRAP.

Other general procedures in this chapter are:

- PUT THE CYTOMETER IN THE IDLE MODE
- REMOVE THE REAGENT CONTAINERS
- REPLACE THE REAGENT CONTAINERS
- POWER THE CYTOMETER ONLY ON/OFF.

11.2 CLEANING SCHEDULE

See Table 13.1, Cleaning Schedule in the TROUBLESHOOTING chapter.

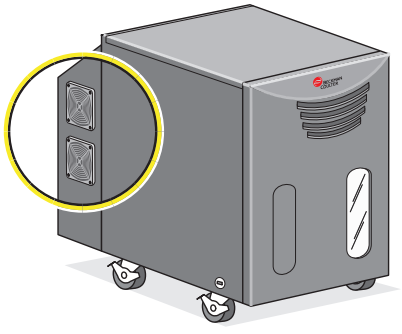
11.3 CLEAN THE AIR FILTERS

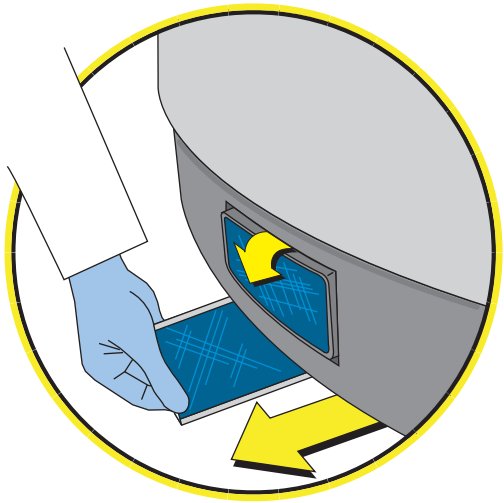
Clean the air filters per Table 13.1, Cleaning Schedule. It is easiest to clean the air filters after performing the shutdown procedure.

Location of Air Filters

The instrument has four air filters located on the:

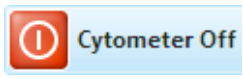
- Cytometer Left Side (2)
- Pneumatic Supply, Left Side Panel (2).





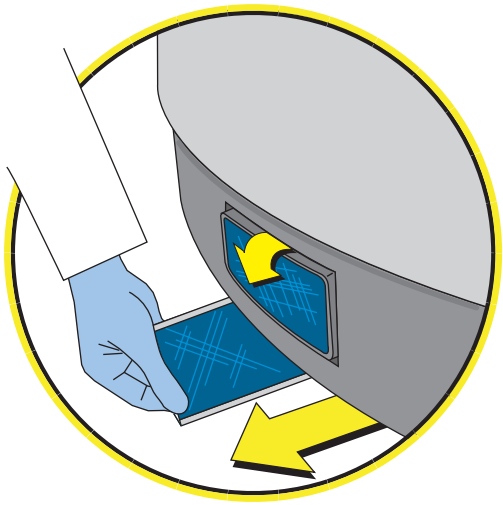
Prepare to Clean the Air Filters

1 Power the Cytometer OFF.



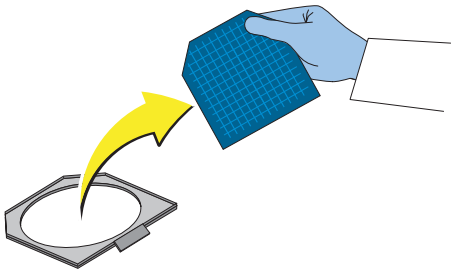
2 Unplug the Pneumatic Supply power cord from the wall outlet.

3 Remove the Cytometer filters from the left side of the Cytometer.



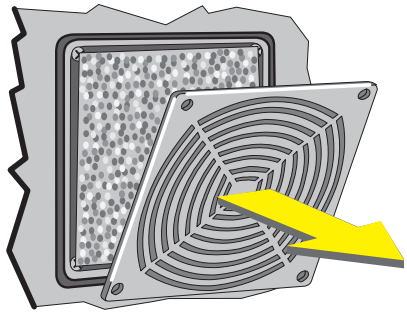
4 Remove the Cytometer filters from their frames.

Note: Replace any torn filters.



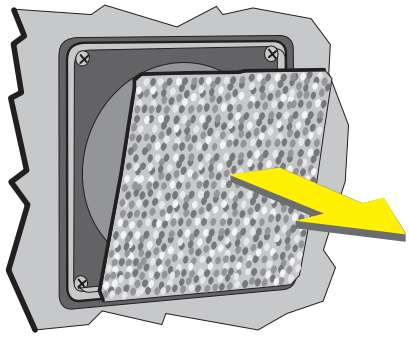
5 Pull off the Pneumatic Supply filter covers. Even though the covers look like they are screwed in, they are not.

The filter covers are made of flexible plastic; they snap out when you pull them. Grab a segment of the grille between your thumb and index finger and then pull.



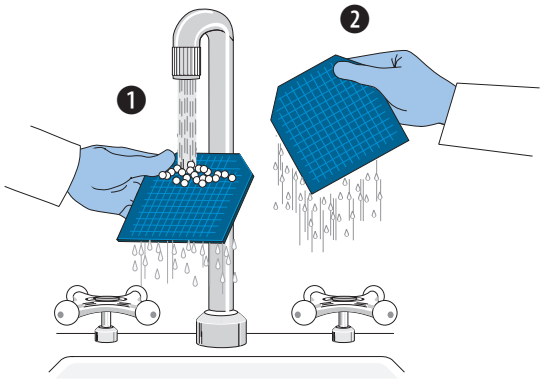
6 Gently pinch and pull out each filter.

Handle them gently to avoid damaging them.



Rinse and Return the Air Filters

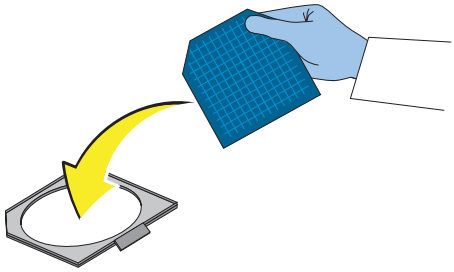
1 Rinse the filters in water (1), and then shake them out (2).



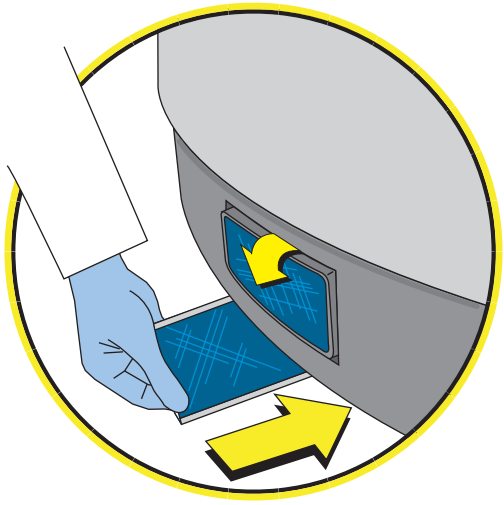
2 Set the filters aside and let them dry out for about 30 minutes.

Use paper towels to check that the filters are completely dry.

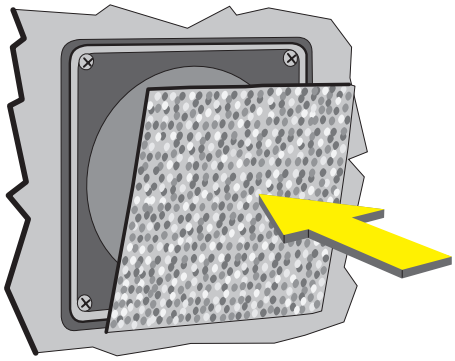
3 Return the Cytometer filters to their frames.



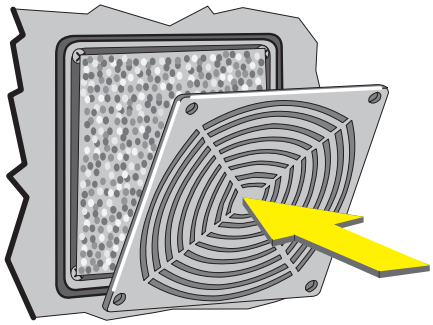
4 Slide the Cytometer filters back into their respective locations on the left side of the Cytometer.



5 Return each Pneumatic Supply filter into its holder. Replace any torn filters.



6 Put each filter cover back on.

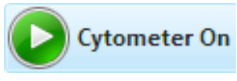


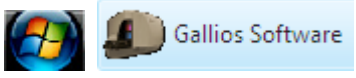
7 Plug the Pneumatic Supply power cord into the wall outlet.

8 Power the Cytometer Only ON

or

Power the Cytometer and Gallios Software ON.





9 Record that the air filters were cleaned on the electronic Maintenance Log.

10 Perform the Daily Startup procedure before running samples.

11.4 PUT THE CYTOMETER IN THE IDLE MODE

To clean, replace, or fill the reagent containers you need to put the Cytometer in the Idle mode.

1 To put the Cytometer in the Idle mode:

2 Wait about 10 seconds for the Cytometer to depressurize. The message *Press Idle Mode button to initialize* appears at the bottom of the screen when the Cytometer is depressurized.

11.5 REMOVE THE REAGENT CONTAINERS

Remove a reagent container to perform these procedures:

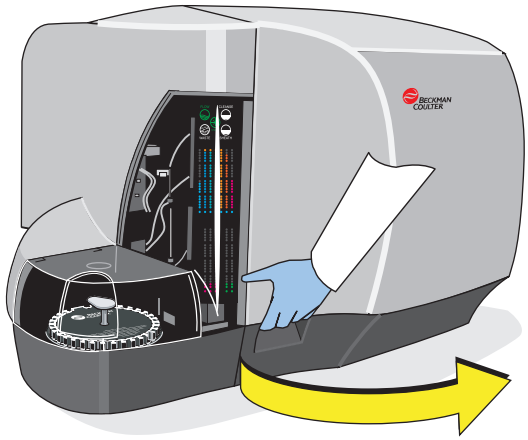
- Clean the sheath fluid container
- Clean the cleaning agent container
- Replace a reagent container. Clean any new reagent container before using it.

Procedure

1 Check if the instrument is currently displaying the Idle mode:

- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

2 Open the Front Cover.



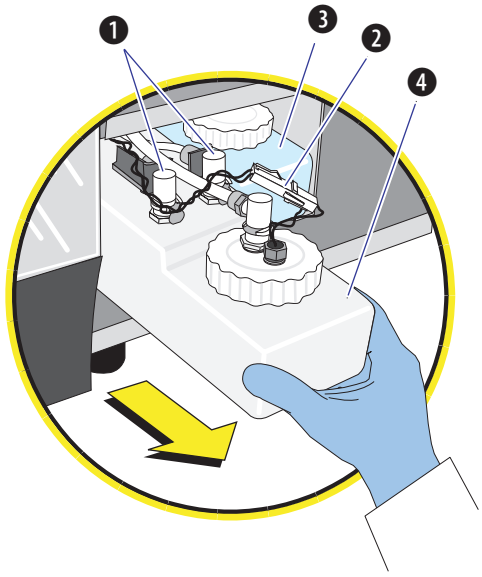
3 Pull out a reagent container.

(1) Reagent container connectors.

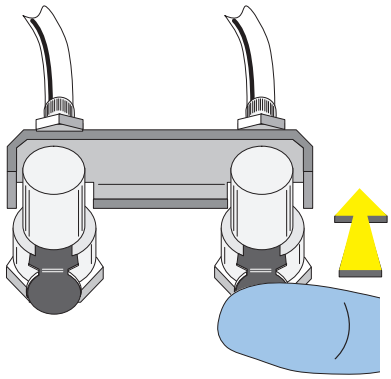
(2) Sheath level sense connector

(3) Cleaning agent container.

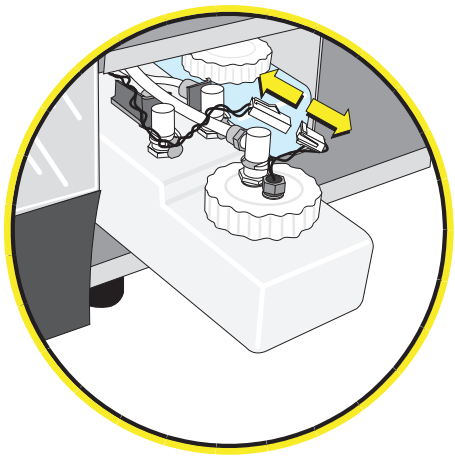
(4) Sheath fluid container.



4 Disconnect the tubing on the top of each reagent container by pushing in on the metal clips on the connectors.



5 Disconnect the Sheath Level Sense connector.



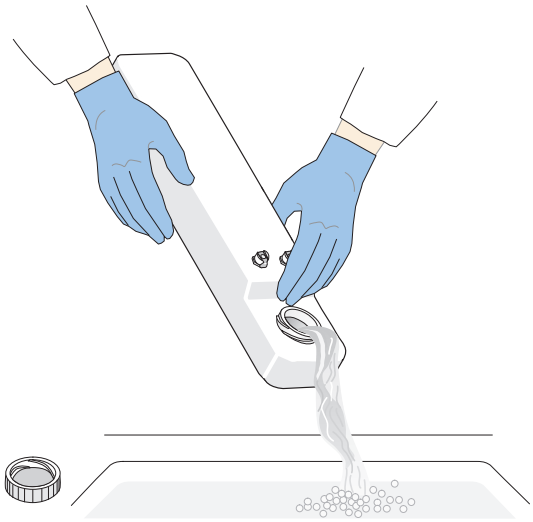
11.6 CLEAN THE INTERNAL SHEATH FLUID CONTAINER

IMPORTANT Misleading results could occur if you contaminate the sheath fluid container. Be careful not to contaminate the sheath fluid container. Do not let your fingers, paper towels, or other objects touch the inside of the container or the inside of its cap.

- Remove and clean the internal sheath fluid container according to Table 13.1, Cleaning Schedule.
- Clean a new sheath fluid container before placing it into the reagent drawer.

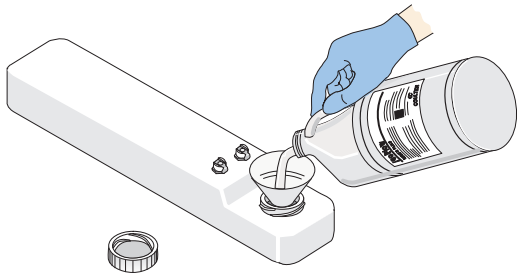
1 See REMOVE THE REAGENT CONTAINERS to remove the sheath fluid container.

2 Empty the container as completely as possible.

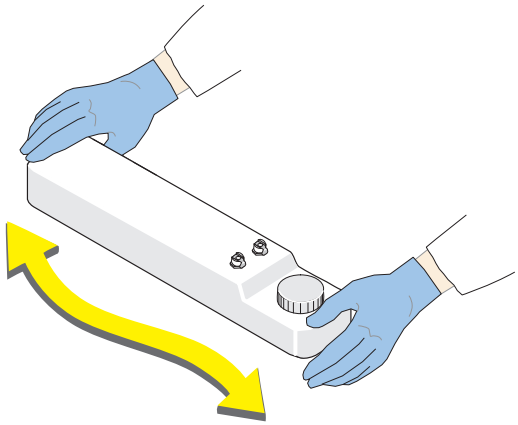


3 Position a funnel into the sheath fluid container.

Pour about 50 to 100 mL of fresh IsoFlow™ sheath fluid or equivalent into the sheath fluid container.

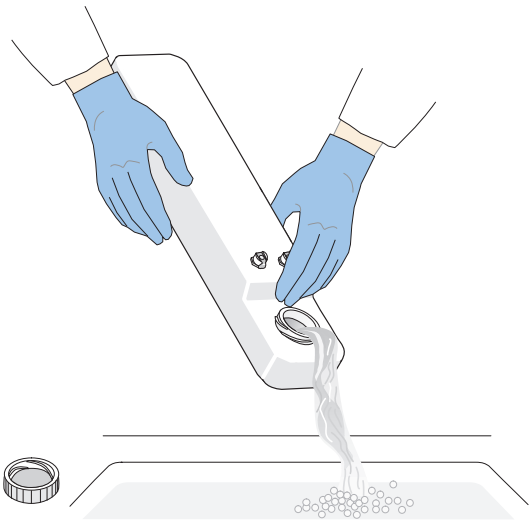


4 Screw the cap back on the sheath fluid container.



5 Swirl the sheath fluid in the sheath fluid container, rinsing all surfaces.

6 Empty the container as completely as possible.



7 FILL THE INTERNAL SHEATH FLUID CONTAINER

8 Record that the sheath container was cleaned on the electronic Maintenance Log.

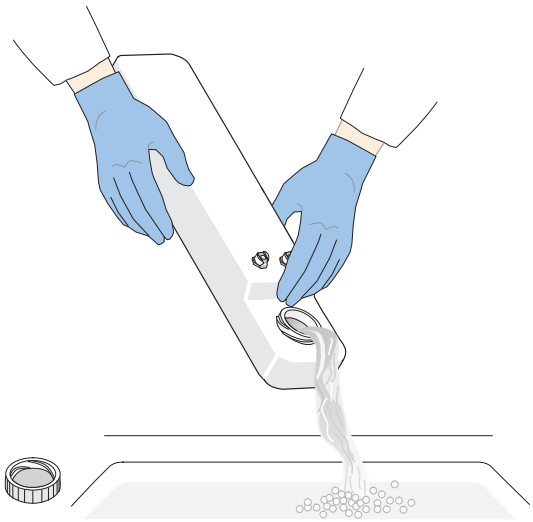
9 See REPLACE THE REAGENT CONTAINERS to replace the sheath fluid container.

11.7 CLEAN THE CLEANING AGENT CONTAINER

- Remove and clean the cleaning agent container every 60 days. See Table 13.1, Cleaning Schedule.
- Clean a new cleaning agent container before placing it into the reagent drawer.

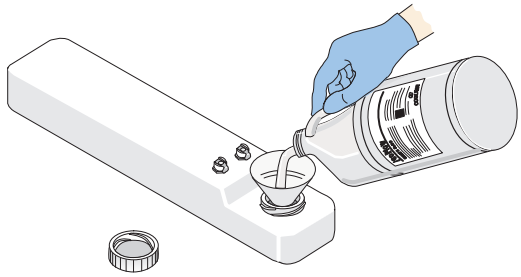
1 See REMOVE THE REAGENT CONTAINERS to remove the cleaning agent container.

2 Empty the container as completely as possible.

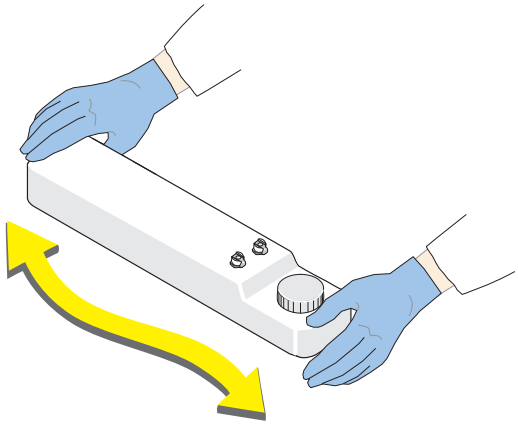


3 Position a funnel into the cleaning agent container.

Pour about 50 to 100 mL of fresh IsoFlow sheath fluid or equivalent into the cleaning agent container.



4 Screw the cap back on the cleaning agent container.

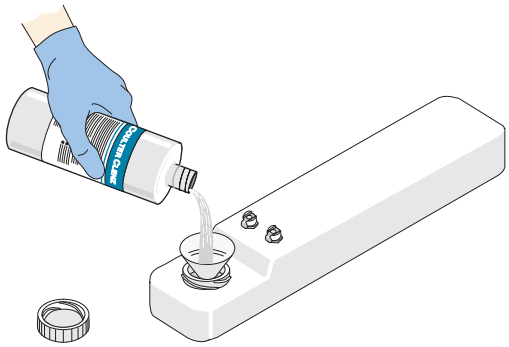


5 Swirl the sheath fluid in the sheath fluid container, rinsing all surfaces.

6 Empty the container as completely as possible.

7 Position a funnel into the cleaning agent container.

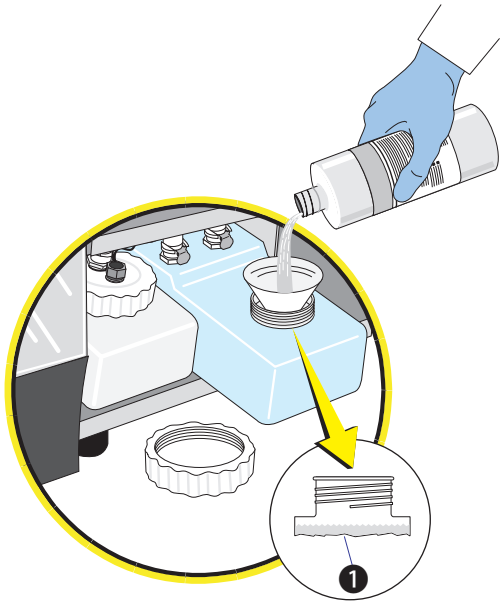
Pour about 50 to 100 mL of fresh FlowClean cleaning agent or equivalent into the cleaning agent container.



8 Empty the container as completely as possible.

CAUTION Risk of damage to the instrument if you overfill the cleaning agent container. Overfilling the cleaning agent container causes the cleaning agent to enter the pressurized line. Avoid spills. Do not tilt the container or remove it from the drawer to fill it.

9 Position a funnel into the cleaning agent container.



10 Carefully pour cleaning agent into the cleaning agent container (approx 1L), filling it just to the bottom of its neck (1).

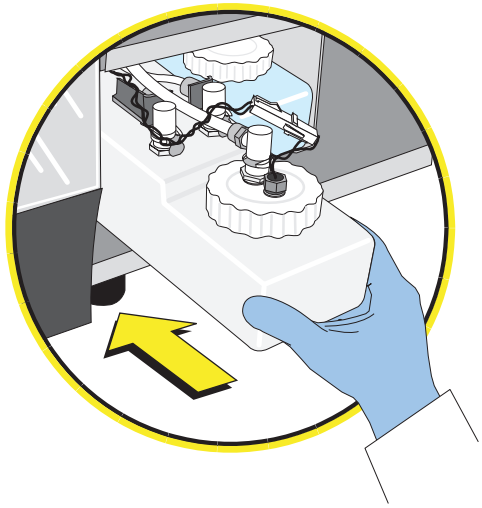
11 Record that the cleanse container was cleaned on the electronic Maintenance Log.

12 See REPLACE THE REAGENT CONTAINERS to replace the cleaning agent container.

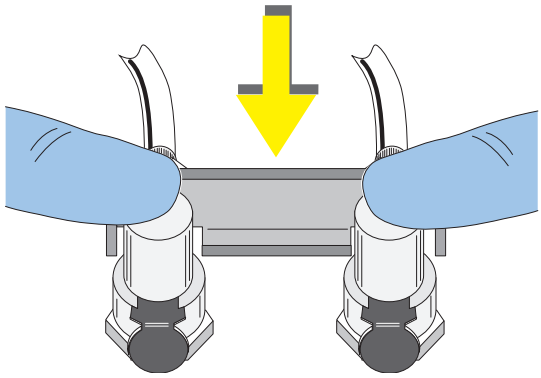
11.8 REPLACE THE REAGENT CONTAINERS

Use this procedure to return a cleaned reagent container into the reagent drawer.

1 Slide the reagent container back in part way. Keep the neck of the reagent container out.



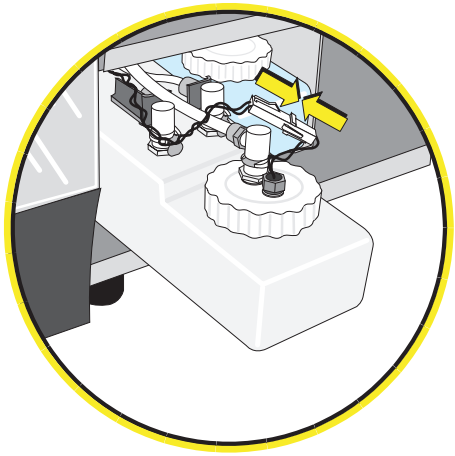
2 Reconnect the tubing assembly by pushing down on the tubing inserts so that the tubing snaps into the connector.



3 Fill each reagent container as instructed in these procedures:

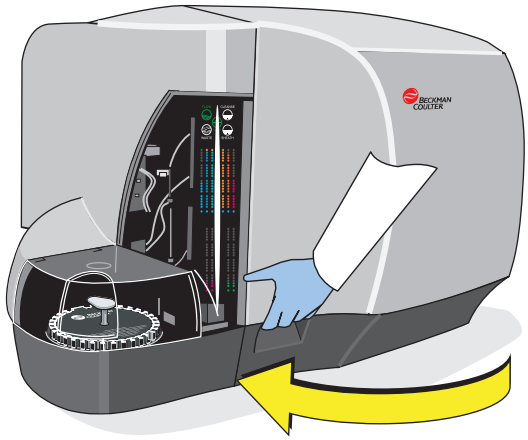
- FILL THE INTERNAL SHEATH FLUID CONTAINER, or
- FILL THE CLEANING AGENT CONTAINER.

4 Reconnect the Sheath Level Sense connector.



5 Slide the reagent container back into place.

6 Close the Front Cover



11.9 CLEAN THE SAMPLING SYSTEM

Routine daily cleaning helps to minimize instrument downtime.

When to Clean the Sampling System

Routine and Sample Head Cleaning Procedures

Perform both the routine and the sample head/probe cleaning procedures before you perform Daily Shutdown and:

- When you change laboratory application procedures, especially if you are using vital fluorescent stains. If vital stains such as propidium iodide, ethidium bromide, acridine orange, thiazole orange, Coriphosphine-O, Fura 3, or fluorescein diacetate, are used, perform these cleaning procedures immediately after using the dyes.
- Immediately prior to running any immunophenotyping application if vital stains are being used on the same instrument.
- When you observe a significant increase in debris or background counts.

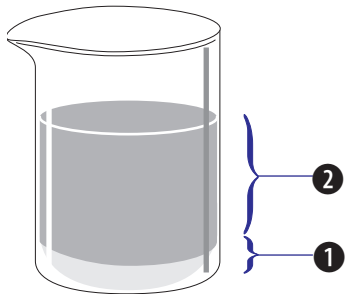
Routine Cleaning Procedure

Perform this procedure as often as described in the heading When to Clean the Sampling System.

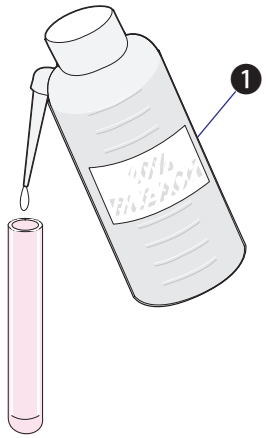
WARNING The cleaning solution is hazardous and can cause personal injury or damage clothing. Beckman Coulter urges its customers to comply with all national health and safety standards such as the use of barrier protection. This may include, but it is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

IMPORTANT A cleaning solution that is not fresh can leave residual stain in the system and misleading results could occur when you change laboratory applications. Be sure to prepare a fresh cleaning solution before performing the cleaning procedure and use it within the same day.

1 Prepare a cleaning solution of 1 part high-quality, fragrance-free bleach ❶ (5% or 6% solution of sodium hypochlorite - available chlorine) and 9 parts distilled water or IsoFlow sheath fluid ❷.



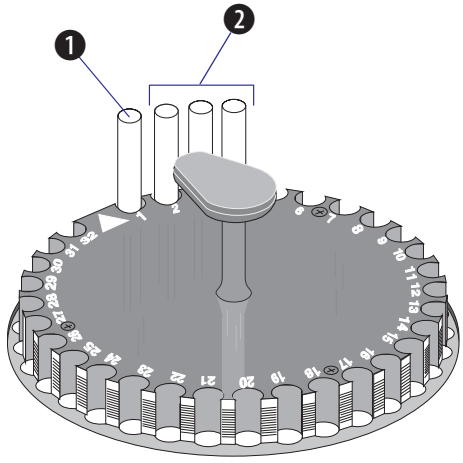
2 Put 2 mL of the bleach solution ❶ in a test tube.



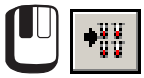
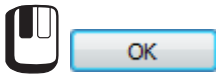
3 Load the carousel:

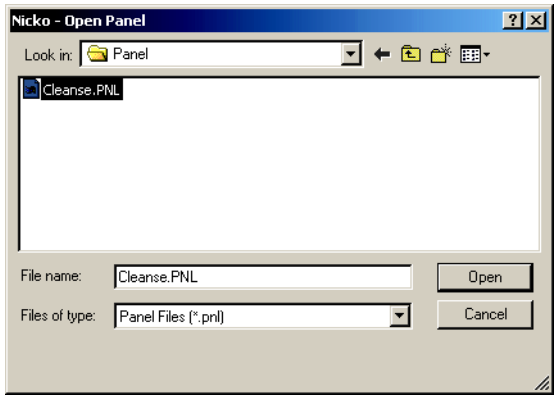
❶ Put the test tube of bleach solution into carousel position 1.

❷ Put three freshly prepared tubes, each containing about 2 mL of distilled water or IsoFlow sheath fluid, into positions 2, 3, and 4 of the carousel.

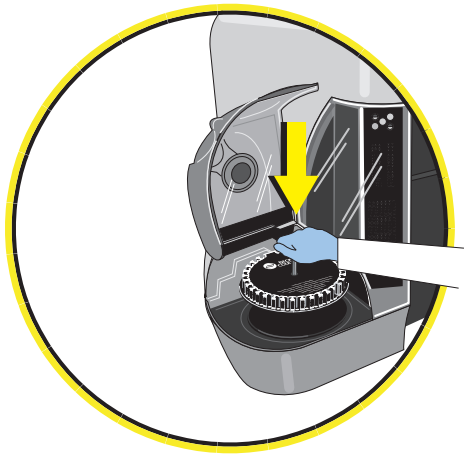


4 Select the cleaning panel if it is not currently selected:

-  .
- Select **Cleanse.PNL** from the list of panels.
-  .



5 Put the carousel into the MCL sample loader and close the MCL cover.

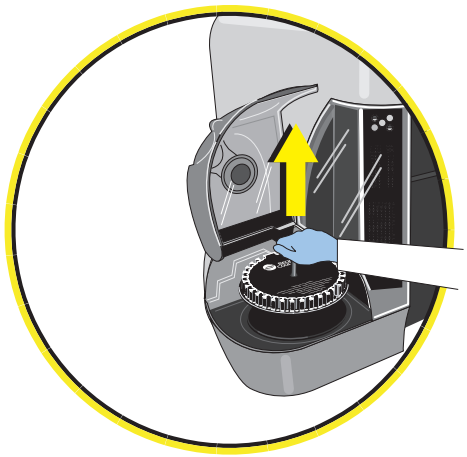


6 Enter the **Carousel No.** in the Worklist. The tube **Location** numbers automatically appear.

Carousel No.	Location
22	1
22	2
22	3
22	4

7  .

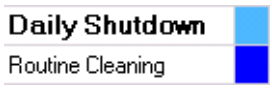
8 When the cleaning panel is done, remove the carousel.



9 Close the MCL cover and  .

When the cleanse cycle completes, the Pneumatic Supply will automatically turn off and *Press Idle Mode button to initialize* appears at the bottom of the screen when the Cytometer is depressurized.

10 Record that the routine cleaning procedure was performed on the electronic Maintenance Log.



11 Before running samples  to initialize the system.

Testing for Residual Stain

If you use vital stains such as propidium iodide, ethidium bromide, acridine orange, thiazole orange, Coriphosphine-O, Fura 3, or fluorescein diacetate, you may want to test for residual stain after performing the routine cleaning procedure and before proceeding to your next application.

To test for residual stain, run unstained Immuno-Trol™ cells or CYTO-TROL™ control cells for your application to ensure that the autofluorescent population is where you normally expect it. If it is not, repeat the routine cleaning procedure.

11.10 CLEAN THE MCL SAMPLE HEAD AND THE SAMPLE PROBE

To remove any crystal or debris buildup, perform this procedure as often as described in the heading When to Clean the Sampling System.

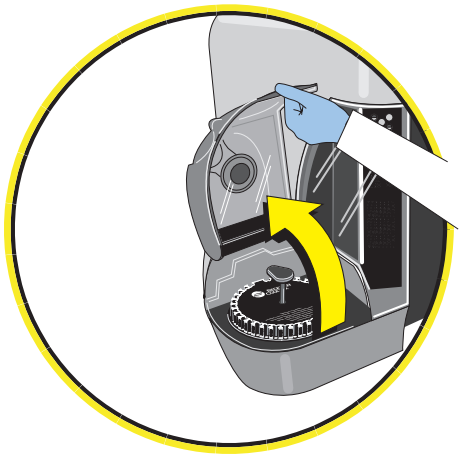
---

1 Power the Cytometer OFF.

---

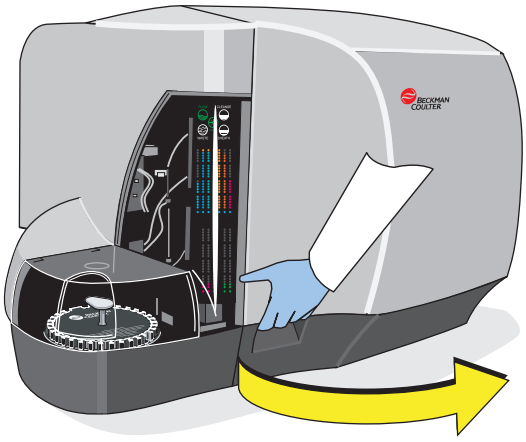
2 Lift up the MCL cover.

Note: If a carousel is present, remove it.



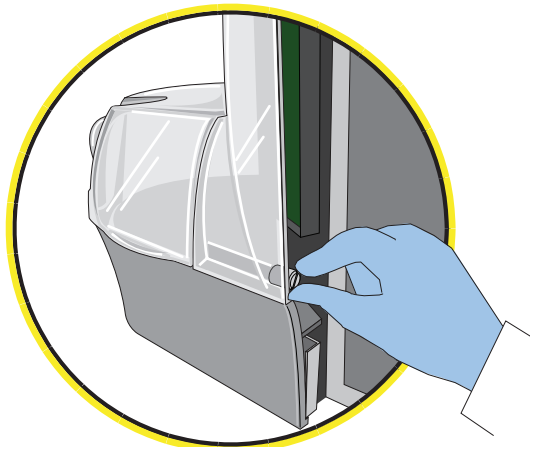
---

3 Open the Front Cover.

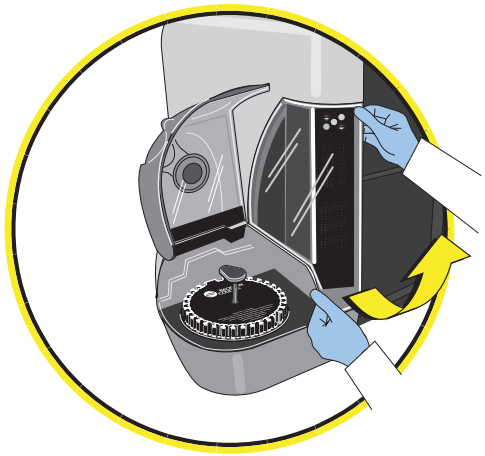


4 Remove the Front Left Side Panel.

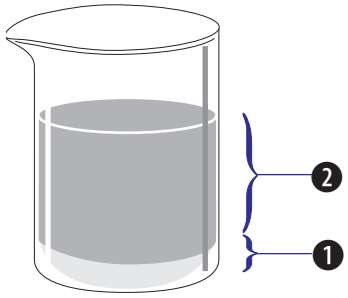
a. Unscrew the thumbscrew that attaches the left side panel to the front frame.



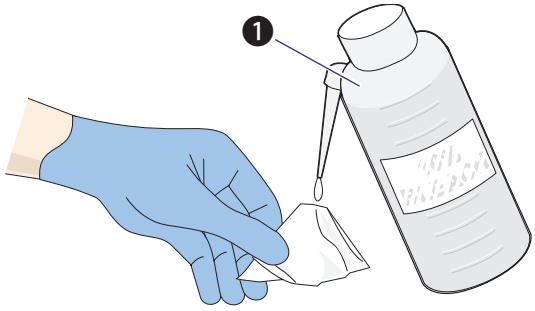
b. Remove the left side panel by pulling it towards you and down.



5 Prepare a cleaning solution of 1 part of high-quality, fragrance-free bleach ❶ (5% or 6% solution of sodium hypochlorite - available chlorine) and 9 parts distilled water or IsoFlow sheath fluid ❷.

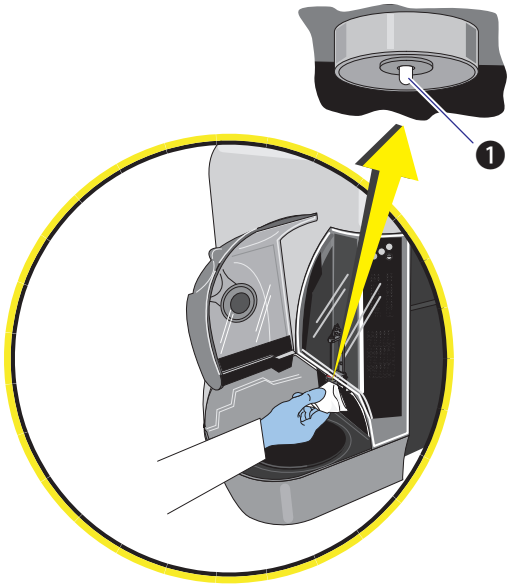


6 While wearing suitable laboratory protective gloves, apply the 10% bleach solution ❶ to a gauze pad.



7 Carefully push the moistened gauze pad up against the inside of the MCL sample head ❶ and scrub away any debris inside and around the sample probe.

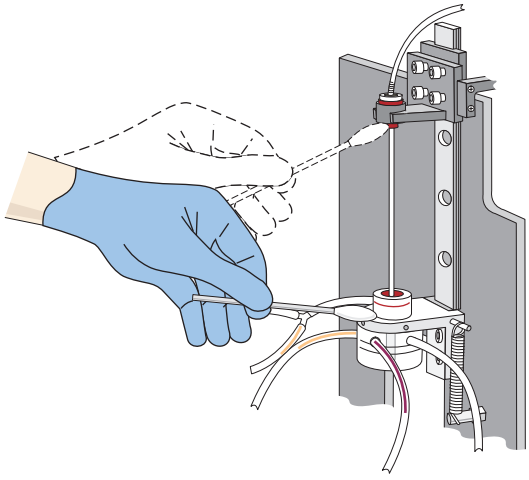
8 Continue scrubbing the sample head and probe by pushing the head up and down 10 times during a 60-second period. Replace moistened gauze as needed.



9 Rinse the MCL sample head and probe with gauze moistened with water.

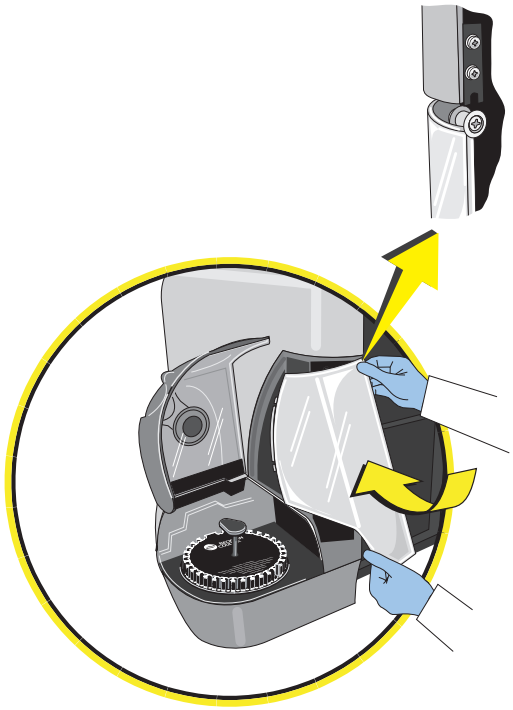
10 Moisten a Q-tip with distilled water.

11 Clean the top of the MCL sample head and the bottom of the sample probe holder.

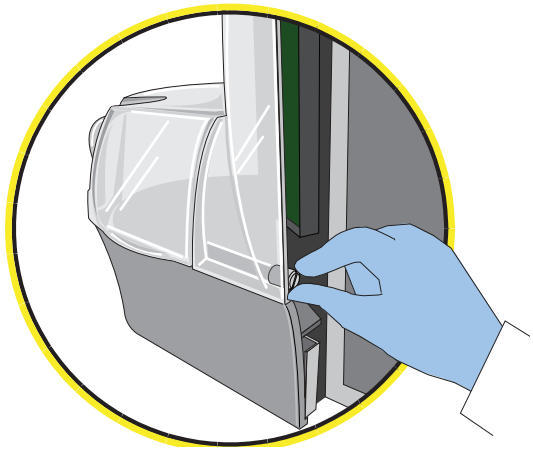


12 Replace the Front Left Side Panel.

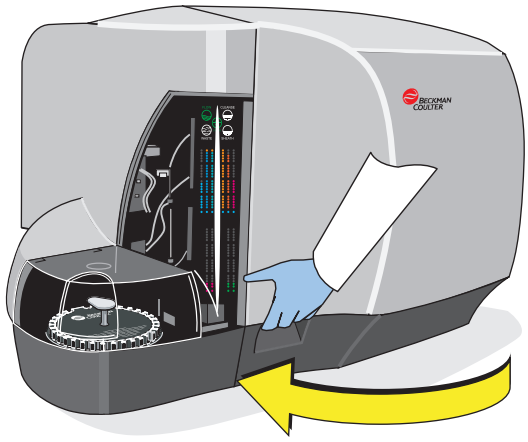
a. Slide in the left side panel, aligning the post on the top of the panel with the cut out on the frame.



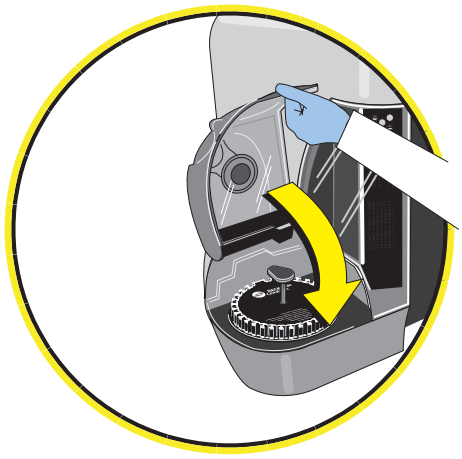
b. Screw in the thumbscrew to attach the left side panel to the front frame.



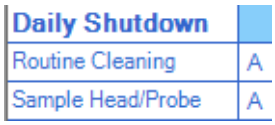
13 Close the Front Cover.



14 Close the MCL cover.



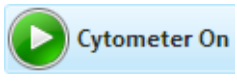
15 Record that the sample head cleaning procedure was performed on the electronic Maintenance Log.



16 Power the Cytometer Only ON

or

Power the Cytometer and Gallios Software ON.



11.11 CLEAN THE VACUUM TRAP

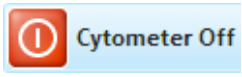
- Clean the vacuum trap as needed.
- If the vacuum trap is more than one-quarter full of fluid, empty it and rinse with tap water.

To clean the Vacuum Trap, perform these procedures:

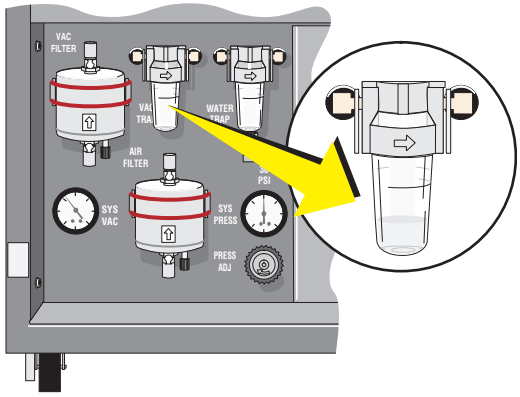
- Prepare to Clean the Vacuum Trap
- Find and Pull Out the Vacuum Trap
- Rinse and Return the Vacuum Trap to Its Bracket

Prepare to Clean the Vacuum Trap

1 Power the Cytometer OFFand unplug both Pneumatic Supply power cords from the wall outlet.

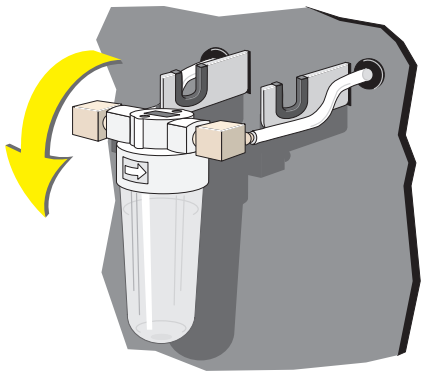


2 Open the Pneumatic Supply front door and locate the vacuum trap (1).

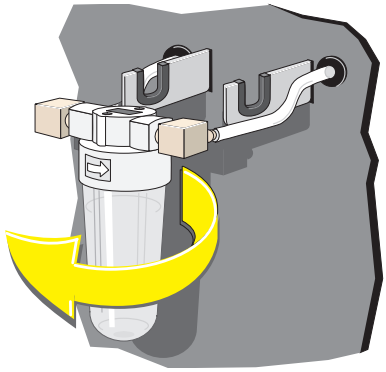


Find and Pull Out the Vacuum Trap

1 The vacuum trap is the trap on the left. Lift the vacuum trap assembly out of its bracket so that you can grasp the top of the assembly.



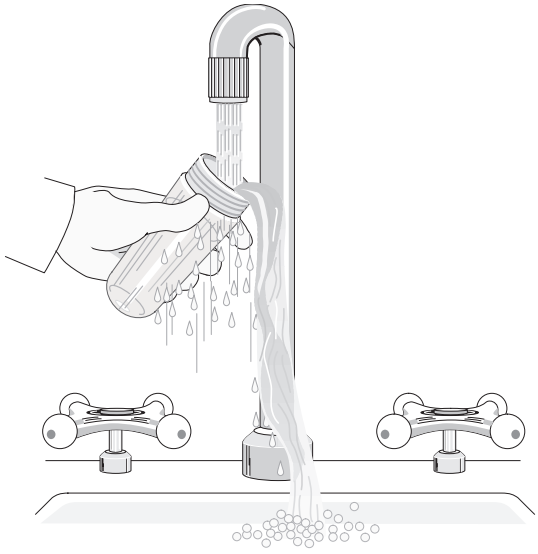
WARNING To prevent injury, avoid skin contact with the vacuum trap and its associated tubing. The vacuum trap and its associated tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of the vacuum trap in accordance with your local environmental regulations and acceptable laboratory procedures.



2 While using one hand to hold the top of the vacuum trap assembly, use the other hand to unscrew the vacuum trap. Then, empty the vacuum trap according to your local environmental regulations and your laboratory's procedures.

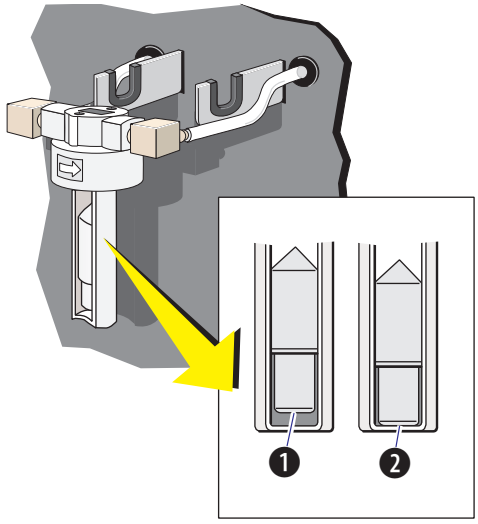
Rinse and Return the Vacuum Trap to Its Bracket

1 Rinse the vacuum trap with water, and then shake out the excess water.

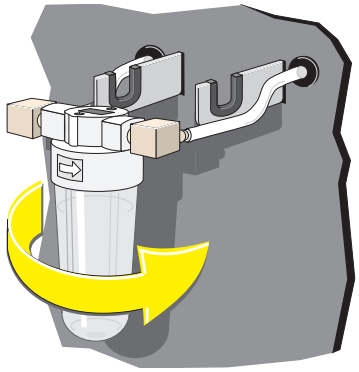


2 Insert the white center post, pointed end up, into the vacuum trap assembly.

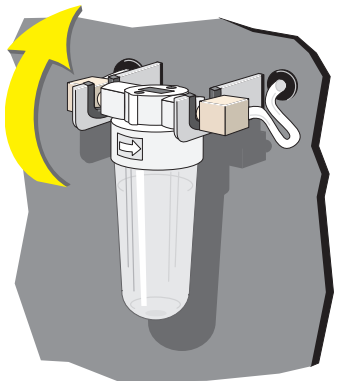
If the white center post in the vacuum trap assembly is stuck in the up position ❶, pull it into the down position ❷.



3 Carefully align the threads on the vacuum trap jar with the threads on the vacuum trap assembly and screw the vacuum trap back into place.



4 Return the vacuum trap assembly to its bracket.



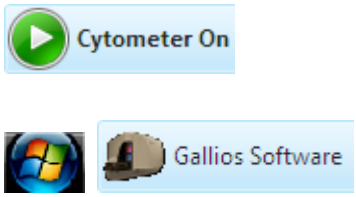
5 Wipe up any spills.

6 Plug both Pneumatic Supply power cords into the wall outlet.

7 Power the Cytometer Only ON

or

Power the Cytometer and Gallios Software ON.

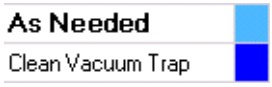


8 Check that no error messages are displayed.

Note: If an error message appears, see Table 13.2, Cytometer Messages for possible causes and operator actions.

9 *Awaiting Sample* appears at the bottom of the screen when system initialization is done.

10 Record that the vacuum trap was cleaned on the electronic Maintenance Log.



11 Perform the Daily Startup procedure before running samples.

11.12 POWER THE CYTOMETER ONLY ON/OFF

Use the procedures below if the instrument has not been fully shut down.

Otherwise use these more detailed procedures:

- Use the Power the Computer and Cytometer ON procedure if you need to start up the instrument and computer from a fully shut down condition.
- Use the Power the Computer and Cytometer OFF procedure if you need to fully shut down the instrument and the computer.

Power the Cytometer Only ON

Use this procedure if the computer is already on and you do not need to start the Gallios software.

---

1 On the Windows desktop:

to power up ONLY the Cytometer.

Power the Cytometer OFF

Use this procedure to turn off the Cytometer. You can still work with the Windows® software after the Cytometer shuts off.

---

1 On the Windows desktop:

to turn off ONLY the Cytometer.

12.1 WHAT THIS CHAPTER EXPLAINS

List of Replacement and Adjustment Procedures

This chapter has these replacement and adjustment procedures:

- REPLACE REAGENTS
- REPLACE THE 10 L EXTERNAL SHEATH FLUID CONTAINER
- FILL THE INTERNAL SHEATH FLUID CONTAINER
- FILL THE CLEANING AGENT CONTAINER
- EMPTY THE 20 L WASTE CONTAINER
- REPLACE THE SHEATH FLUID FILTER
- REPLACE THE SAMPLE PROBE AND SAMPLE PICKUP TUBING
- REPLACE THE MCL SAMPLE HEAD
- ADJUST THE SYSTEM PRESSURE
- REPLACE AN OPTICAL FILTER

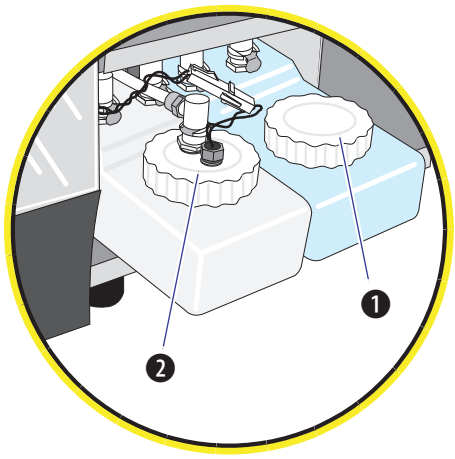
12.2 REPLACEMENT/ADJUSTMENT SCHEDULE

See the Replacement Schedule in the TROUBLESHOOTING Chapter.

12.3 REPLACE REAGENTS

About the Reagent Containers

- The Cytometer has an external 10 L sheath fluid container and internal containers for cleaning agent (1) and sheath fluid (2).
- For best use of reagents, refill the internal reagent containers only when the instrument indicates that they are low.
- If you replace a reagent container, clean it before you put it into the instrument and fill it. See CLEAN THE INTERNAL SHEATH FLUID CONTAINER or CLEAN THE CLEANING AGENT CONTAINER.



Reagent Container Capacity

The internal sheath fluid container has a working capacity of about 500 mL. It is automatically replenished from the external 10 L sheath fluid container. When you fill a completely empty sheath fluid container (after cleaning or replacement), you need about 1 L of sheath fluid due to pressurization and level sensing requirements. **Note:** A bottle of IsoFlow sheath fluid holds 1.8 L.

Cleaning Agent Container

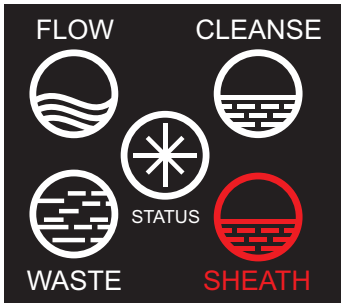
The cleaning agent container has a working capacity of about 500 mL. This is the amount of reagent needed when you are filling the cleaning agent container after *Cleanse Level Warning or Cleanse Level Error* appears. When you fill a completely empty cleaning agent container (after cleaning or replacement), you need about 1 L of cleaning agent due to pressurization and level sensing requirements.

Note: A bottle of FlowClean cleaning agent holds 500 mL.

12.4 REPLACE THE 10 L EXTERNAL SHEATH FLUID CONTAINER

Perform this procedure whenever:

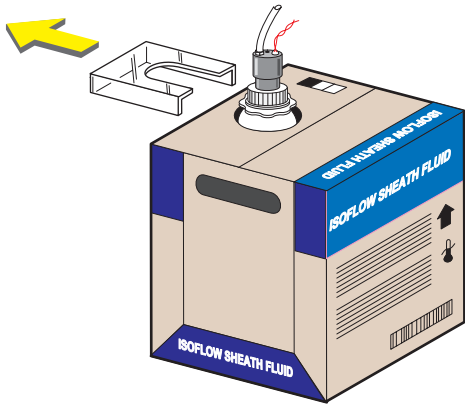
- The **Sheath Low** indicator is red.
- The *Sheath Cube Level Error* appears.



1 Check if the instrument is currently in the Idle mode:

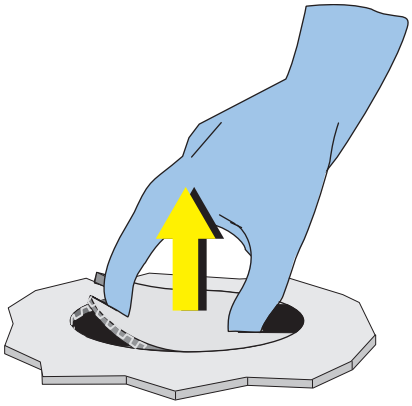
- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

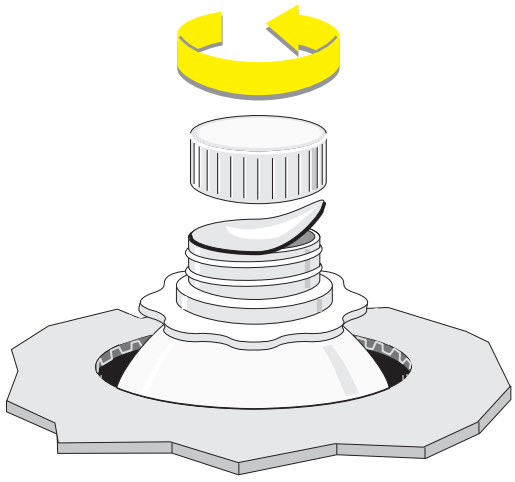
2 Remove the support collar from the empty sheath fluid container.



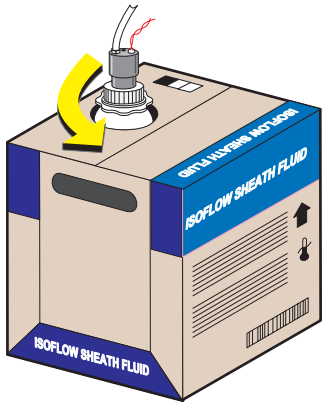
3 Remove any cardboard cutouts from the new sheath fluid container.

Remove the cap and seal from the new sheath fluid container. Be sure to completely remove the foil seal.



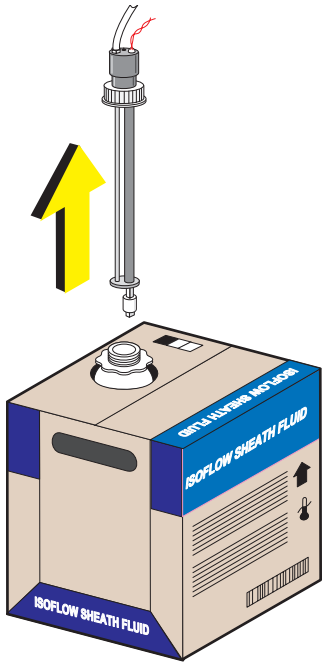


4 Unscrew the plastic cap that secures the pickup tube assembly into the old sheath fluid container.



IMPORTANT Misleading results could occur if you contaminate the sheath fluid. Be careful not to contaminate the sheath fluid. Do not let your fingers, paper towels, or other objects touch the pickup tube assembly.

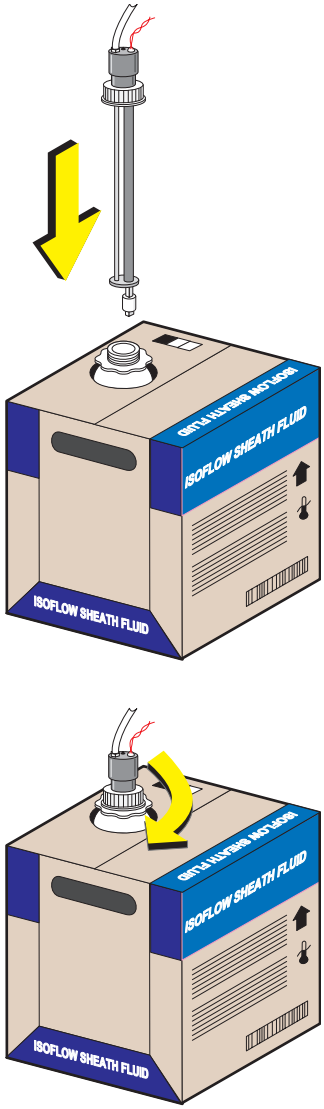
5 Lift the pickup tube assembly straight up and out.



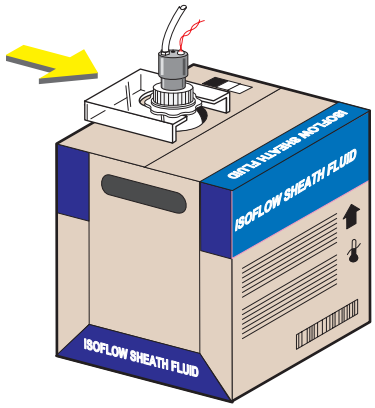
6 Inspect the pickup tube assembly and replace it if necessary.

7 Carefully insert the pickup tube assembly straight into the new sheath fluid container.

Tighten the cap.

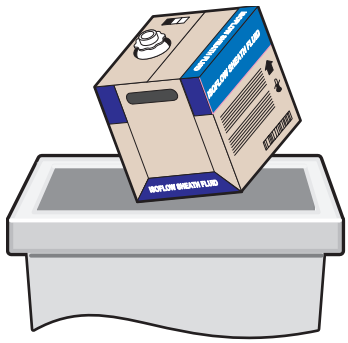


8 Insert the plastic support collar that secures the pickup tube assembly.



9 Place the 10 L external sheath fluid container in a location that is lower than the internal sheath fluid container. This prevents siphoning of the sheath fluid.

10 Put the cap from the new container onto the old container and dispose of the container properly.



11 Record the new sheath fluid container information on the electronic Maintenance Log.

12.5 FILL THE INTERNAL SHEATH FLUID CONTAINER

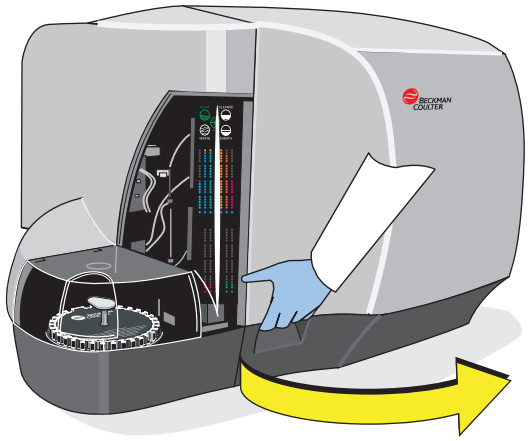
Perform this procedure whenever:

- You clean or replace the sheath fluid container.
- The error message *Internal Sheath Tank Level Warning* appears.

1 Check if the instrument is currently in the Idle mode:

- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

2 Open the Front Cover.



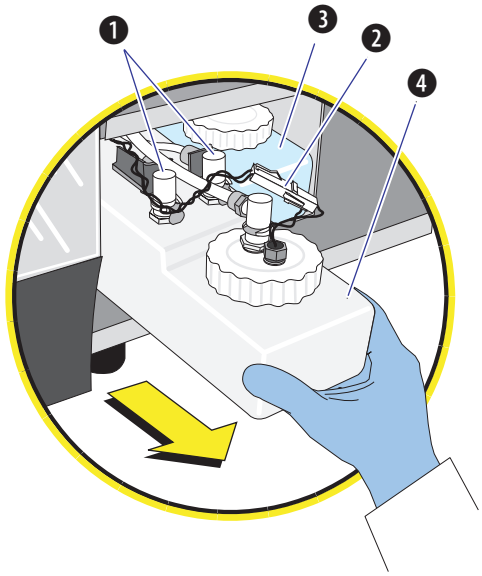
3 Pull out the sheath fluid container.

(1) Reagent container connectors.

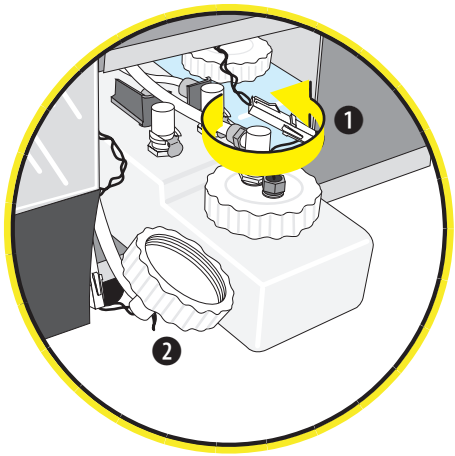
(2) Sheath level sense connector

(3) Cleaning agent container.

(4) Sheath fluid container.



IMPORTANT Misleading results could occur if you contaminate the sheath fluid. Be careful not to contaminate the sheath fluid. Do not let your fingers, paper towels, or other objects touch the inside of the container or the inside of its cap.

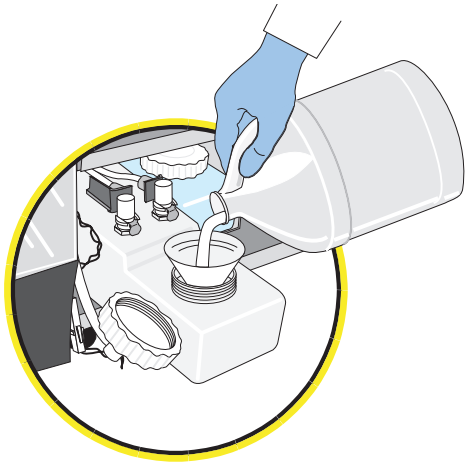


4 Remove the cap:

(1) Unscrew the cap on the sheath fluid container.

(2) To avoid contaminating the sheath fluid, lay the cap upside down on the counter.

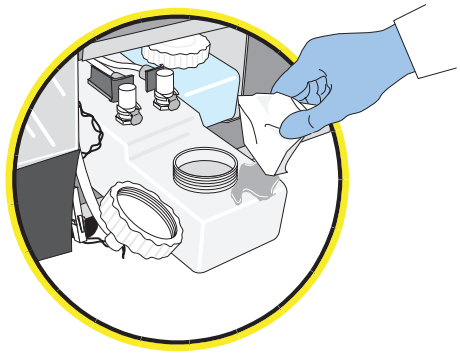
CAUTION To prevent damage to the instrument, do not overfill the sheath fluid container. Avoid spills. Do not tilt the container or remove it from the drawer to fill it.



5 Position a funnel into the sheath fluid container.

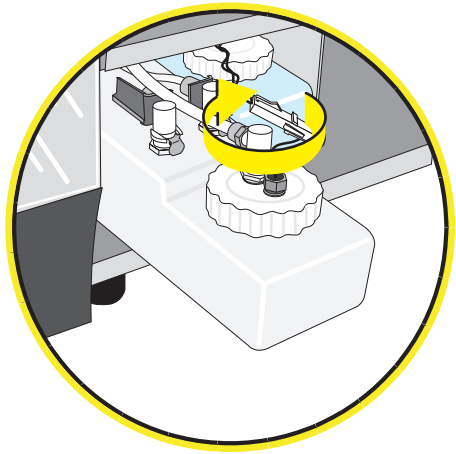
6 Carefully pour sheath fluid into the sheath fluid container, filling it just to the bottom of its neck.

7 Carefully wipe up any spills.

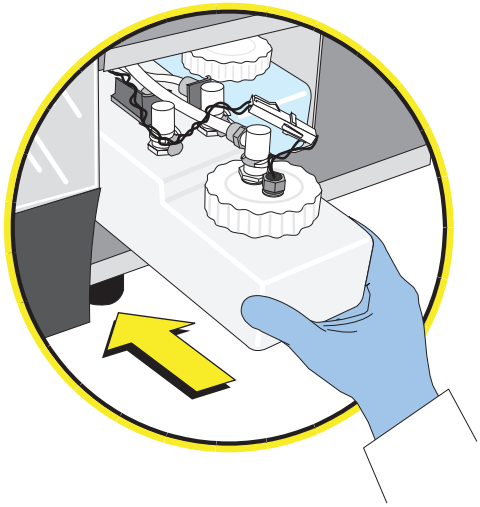


IMPORTANT Misleading results could occur if you analyze samples without the cap on the sheath container. Be sure to put the cap back on the sheath fluid container after you fill it.

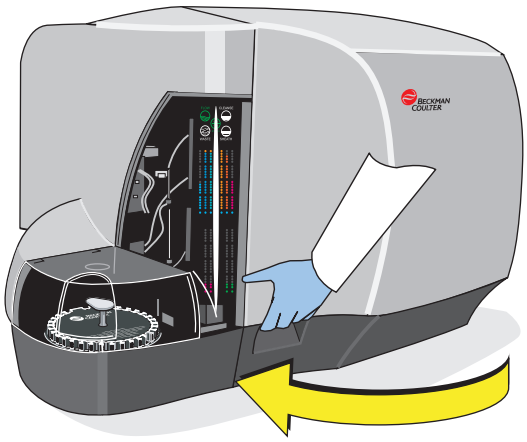
8 Screw the cap back on.



9 Slide the sheath fluid container back into place.



10 Close the Front Cover.



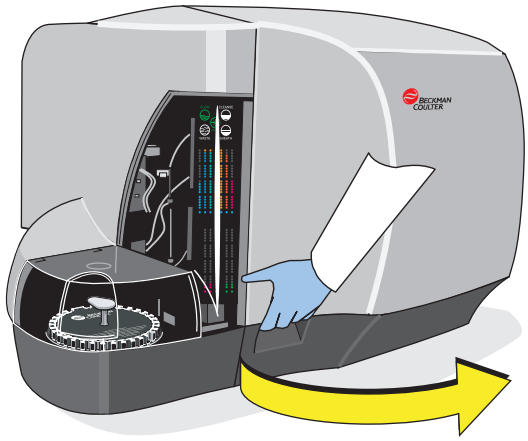
12.6 FILL THE CLEANING AGENT CONTAINER

Perform this procedure whenever *Cleanse Level Warning* or *Cleanse Level Error* appears.

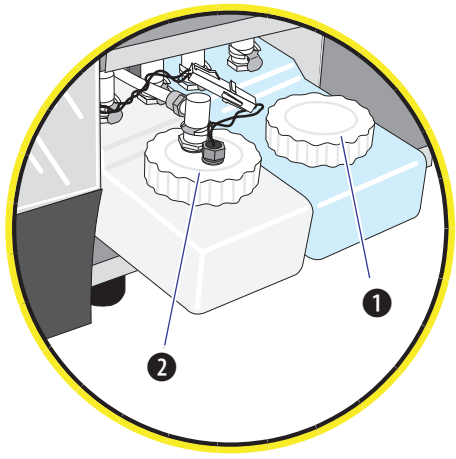
1 Check if the instrument is currently displaying the Idle mode:

- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

2 Open the Front Cover.

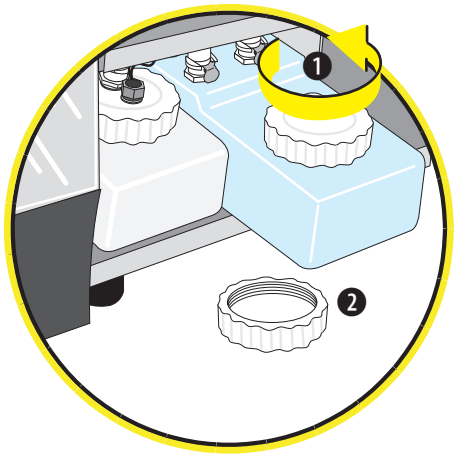


3 Pull out the cleaning agent (1) container.



IMPORTANT Misleading results could occur if you contaminate the cleaning agent. Be careful not to contaminate the cleaning agent. Do not let your fingers, paper towels, or other objects touch the inside of the container or the inside of its cap.

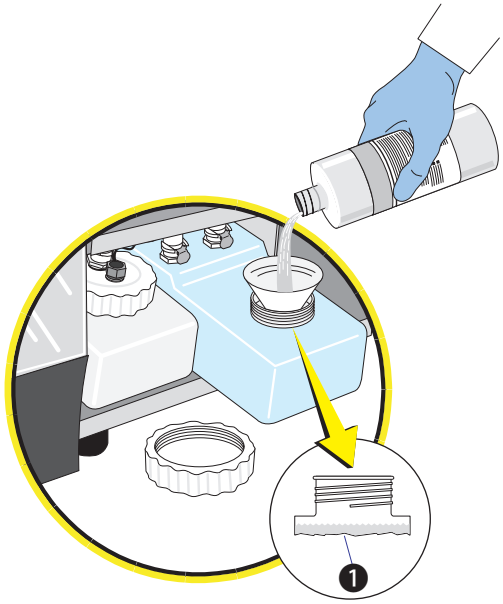
4 Unscrew the cap on the cleaning agent container (1). To avoid contaminating the cleaning agent, lay the cap upside down on the counter (2).



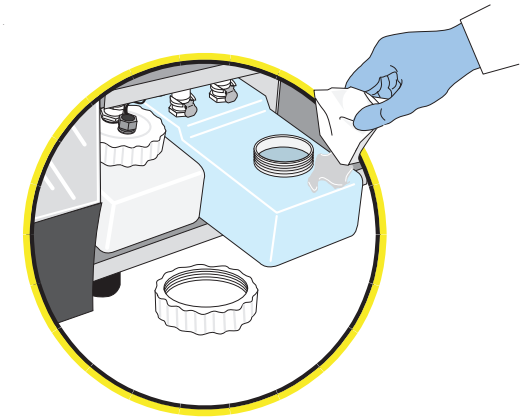
CAUTION Risk of damage to the instrument if you overfill the cleaning agent container. Overfilling the cleaning agent container causes the cleaning agent to enter the pressurized line. Avoid spills. Do not tilt the container or remove it from the drawer to fill it.

5 Position a funnel into the cleaning agent container.

6 Carefully pour cleaning agent into the cleaning agent container, filling it just to the bottom of its neck (1).



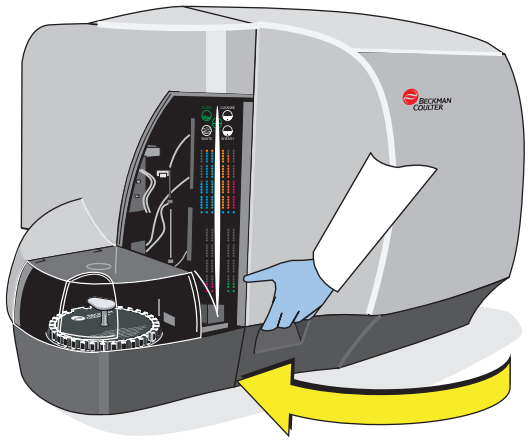
7 Carefully wipe up any spills.



8 Screw the cap back on.

9 Slide the cleaning agent container back into place.

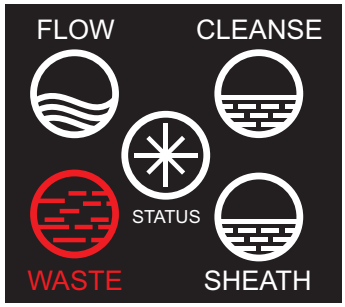
10 Close the Front Cover.



11 Before running samples,  to initialize the system.

12.7 EMPTY THE 20 L WASTE CONTAINER

- Empty the 20 L waste container when:
  - You perform your daily startup.
  - The **Waste Full** indicator is red.
  - *Waste Level Warning* or *Waste Level Error* appears.
  - An audible alarm on the 20 L waste container sounds.
- The 20 L waste container is positioned on the floor near the instrument.



Procedure

---

1 Check if the instrument is currently displaying the Idle mode:

- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

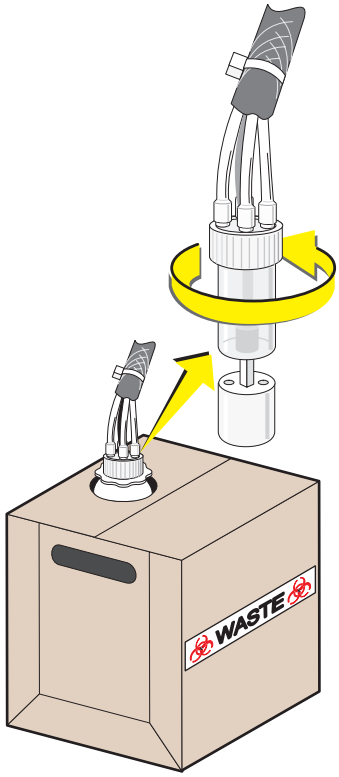
Note: Wait until any instrument function is done before emptying the waste container.

---

2 Lift the waste container and swirl it before removing the cap.

WARNING Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of the waste container in accordance with your local regulations and acceptable laboratory procedures.

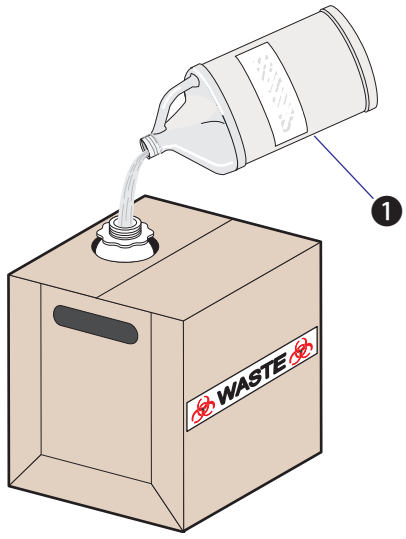
3 Unscrew the cap and lay it on a leakproof disposable container, such as a glove or beaker.



4 Empty the waste container according to your laboratory's procedures.

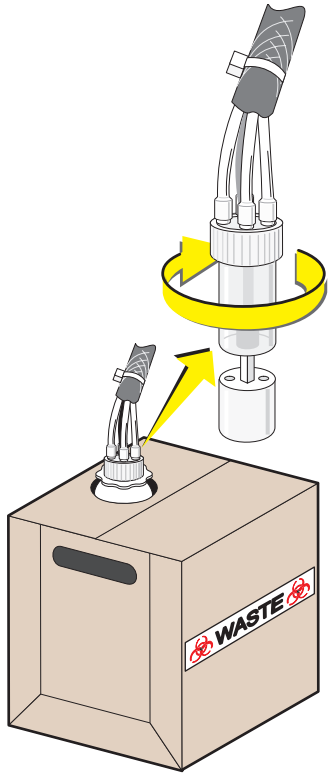
Note: Take proper precautions to avoid spills if you are emptying the waste container into a sink, drain, or larger container.

5 Put about 2 L of high-quality, fragrance-free, gel-free bleach (1) (5 to 6% solution of sodium hypochlorite - available chlorine) into the waste container to cover the bottom of the container.



6 Replace the cap on the new waste container and securely tighten.

Note: Properly dispose of the leakproof disposable container used in step 3 after you screw the cap back on the waste container.



7 The system automatically performs an initialization cycle if you emptied the waste container after the **Waste Full** indicator appeared.

Note: If you emptied the waste container before the **Waste Full** indicator appeared:

to initialize the system.

12.8 REPLACE THE SHEATH FLUID FILTER

Replace the 0.2-µm sheath fluid filter:

- Every 6 months.

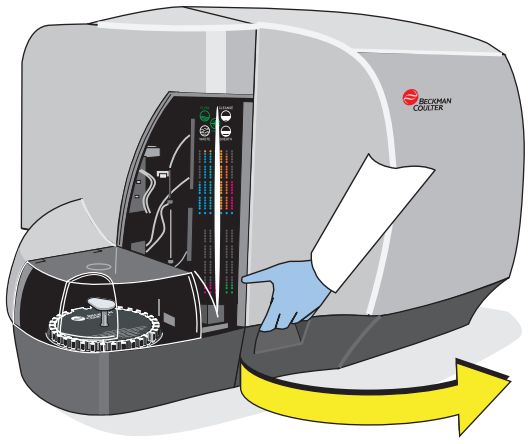
  or
- Whenever the sample flow rate is too high (repeated *Data Rate Warning* or *System Pressure Error* messages appear).

Procedure

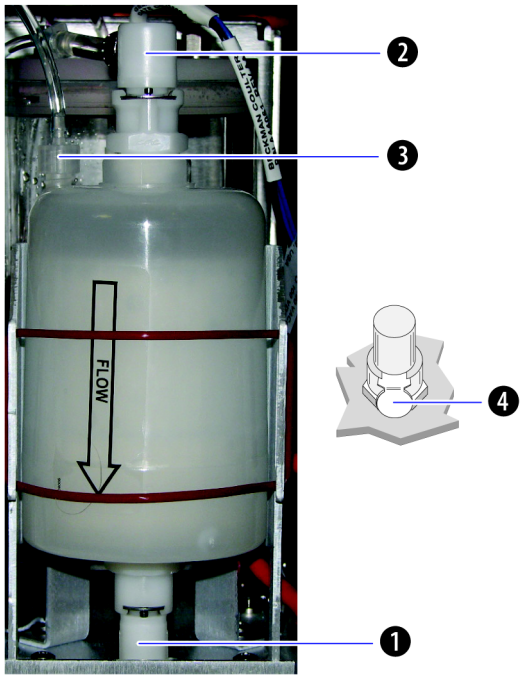
1 Check if the instrument is currently displaying the Idle mode:

- If yes (*Press Idle Mode button to initialize* appears), go to step 2.
- If no, PUT THE CYTOMETER IN THE IDLE MODE.

2 Open the Front Cover.



3 Undo the flexible strap holding the sheath fluid filter.



CAUTION Risk of damage to the instrument if you do not install the sheath fluid filter correctly. It allows fluid to flow in one direction only. Make sure you install the new sheath fluid filter correctly.

4 Pick up the old sheath fluid filter, and notice how the three tubes are connected (1), (2) and (3) and notice the direction of the arrow on it.

5 Get the new filter and hold it with the arrow going in the same direction as the arrow on the old filter.

Note: In the next step, immediately install the new filter to avoid spills.

6 Disconnect and reconnect each tube to the new filter, one at a time, in this order: (1), (2) and (3).

Tubes (1), (2) are disconnected by pushing in on the metal clip on the connector (4).

When reconnected, the connectors snap into place.

7 Discard the old sheath fluid filter.

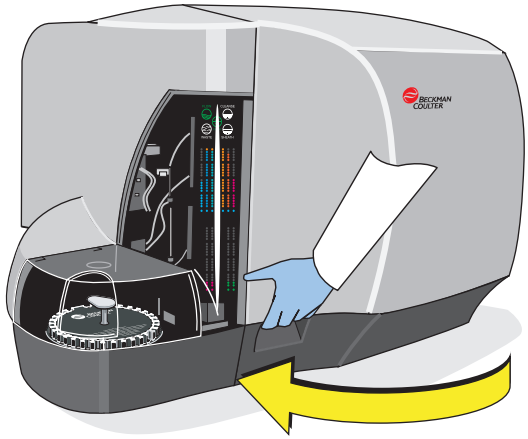
8 Wipe up any spills, and then put the filter in the bracket.

a. Ensure that the arrow is pointing down.

b. Reattach the flexible strap that holds the sheath fluid filter.

c. Check that the tubing is not kinked or twisted.

9 Close the Front Cover.



10 Record that the sheath fluid filter was replaced on the electronic Maintenance Log.

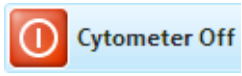
12.9 REPLACE THE SAMPLE PROBE AND SAMPLE PICKUP TUBING

Replace the sample probe and sample pickup tubing when:

- The sample probe is bent.
- The sample probe leaks.
- There is erratic sample flow or no sample flow from the sample probe.

---

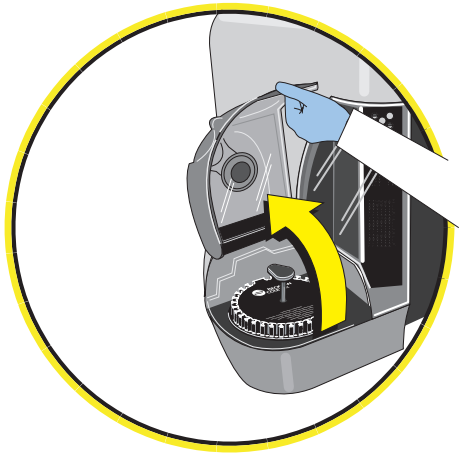
1 Power the Cytometer OFF.



---

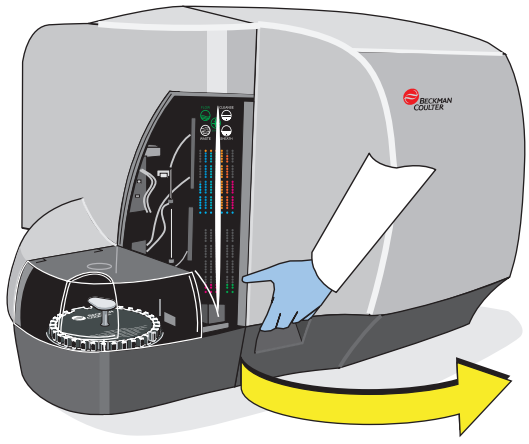
2 Open the MCL cover.

Note: If a carousel is present, remove it.



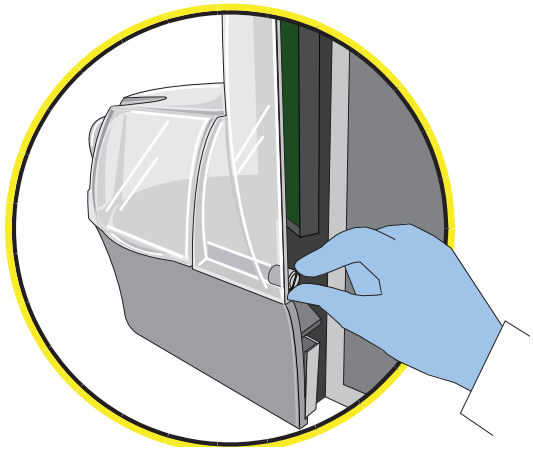
---

3 Open the Front Cover.

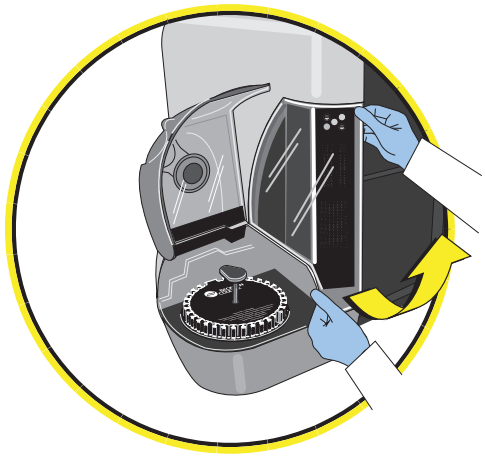


4 Remove the Front Left Side Panel.

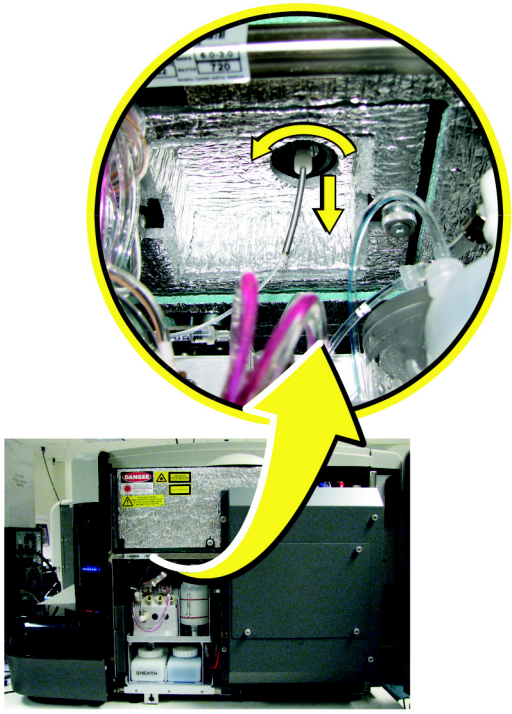
a. Unscrew the thumbscrew that attaches the left side panel to the front frame.



b. Remove the left side panel by pulling it towards you and swivel it up and out of the instrument.

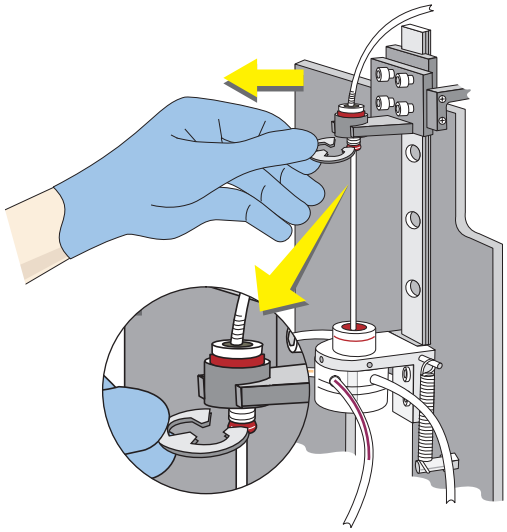


5 Unscrew the sample pickup tubing connector from the bottom of the flow cell compartment.

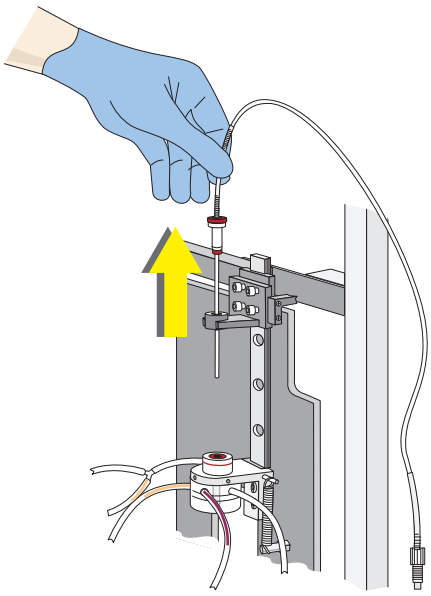


6 Pull the sample pickup tubing out through the left (MCL) side of the instrument.

7 Remove the e-ring from the sample probe using needle nose pliers or a hemostat. Retain the clip.



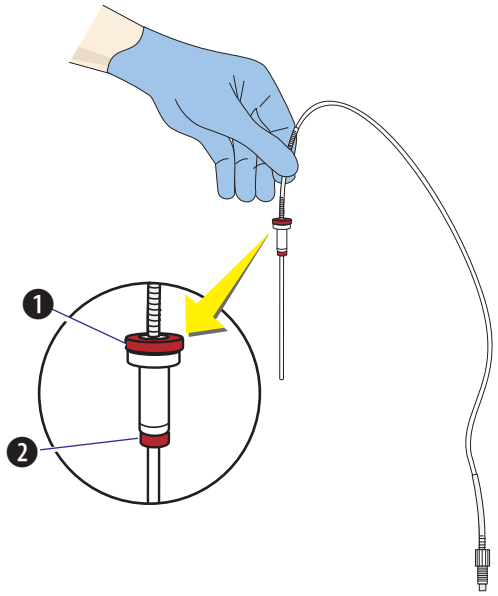
8 Lift the sample probe up and out of its holder.



WARNING Risk of biohazardous contamination if you have skin contact with the sample pickup tubing. The sample pickup tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample pickup tubing in accordance with your local regulations and acceptable laboratory procedures.

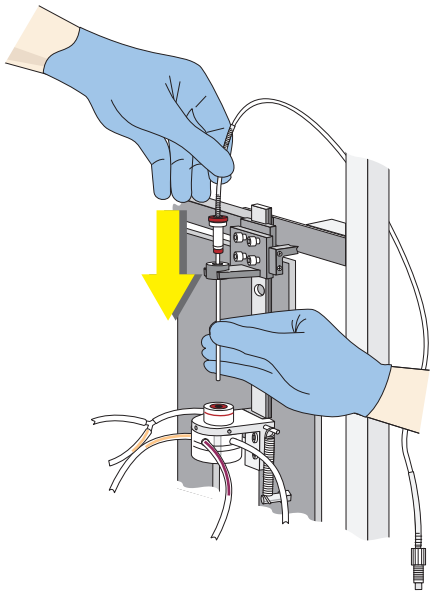
9 Discard the old sample pickup tubing and probe assembly in accordance with your local regulations and acceptable laboratory procedures.

10 Ensure that the rubber washer ❶ and O-ring ❷ are positioned correctly on the new sample probe.



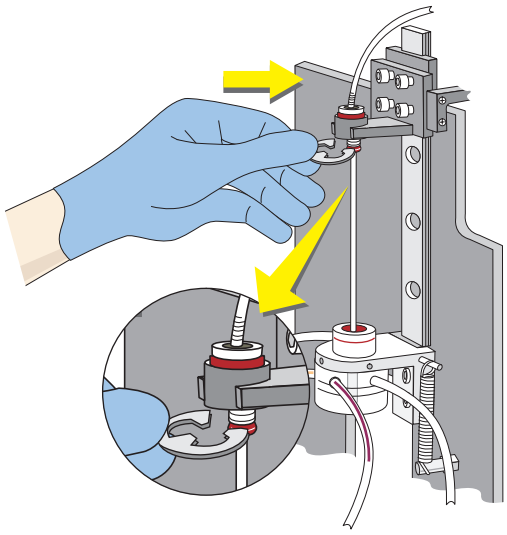
11 Thread the sample pickup tubing through the instrument.

12 Insert the new sample probe into the sample probe holder.



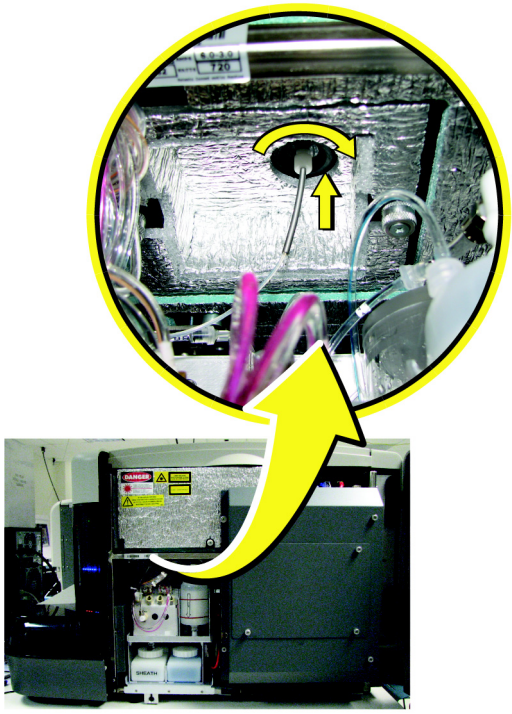
13 Guide the sample probe tip into the MCL sample head.

14 Insert the clip removed in step 7 into the groove on the sample probe.



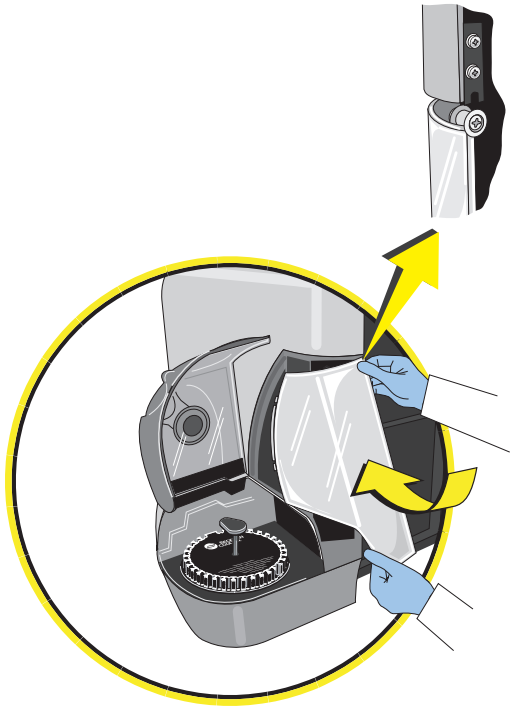
IMPORTANT Risk of erroneous results if the flow cell is misaligned. Overtightening the connector from the sample pickup tubing to the flow cell can cause misalignment of the flow cell. Only screw on the sample pickup tubing connector "finger tight."

15 Screw on the connector from the sample pickup tubing to the bottom of the flow cell compartment until it is "finger tight."

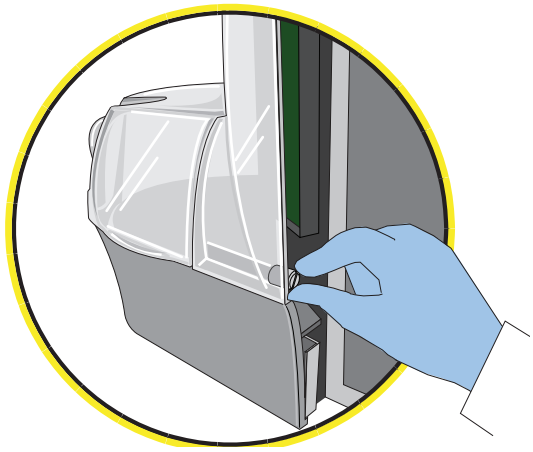


16 Replace the Front Left Side Panel.

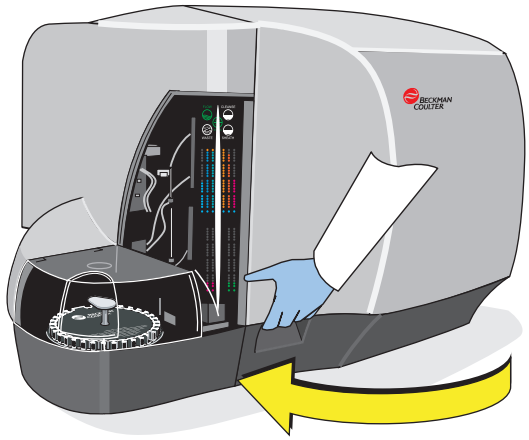
a. Swivel the left side panel into the instrument, aligning the post on the top of the panel with the cut out on the frame and then push the panel back into place.



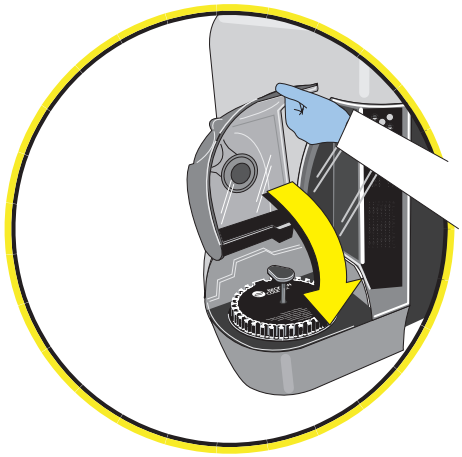
b. Screw in the thumbscrew to attach the left side panel to the front frame.



17 Close the front cover.



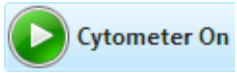
18 Close the MCL cover.

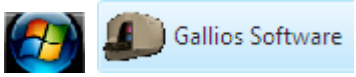


19 Power the Cytometer Only ON

or

Power the Cytometer and Gallios Software ON.





20  .

21 After the prime cycle is done,  again.

12.10 REPLACE THE MCL SAMPLE HEAD

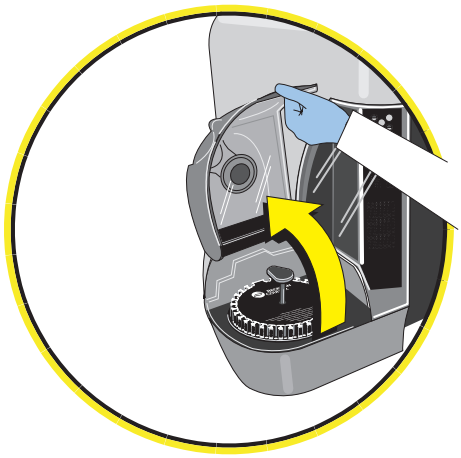
Use this procedure when:

- Liquid from the cleaning adaptors does not draw up properly.
- Cleaning the sample head does not fix your excessive carryover problem.
- Numerous *Sample Pressure Error* or *MCL Tube Up/Down Error* messages occur.

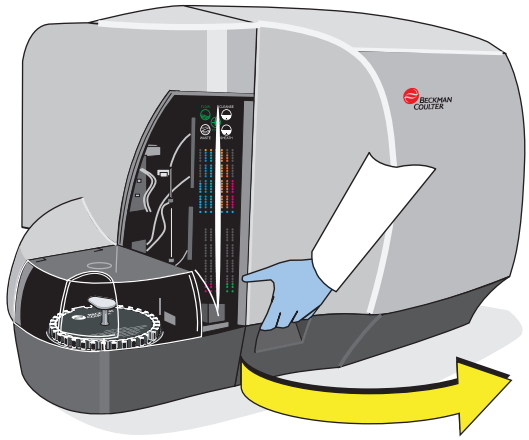
1 Power the Cytometer OFF.

2 Open the MCL cover.

Note: If a carousel is present, remove it.

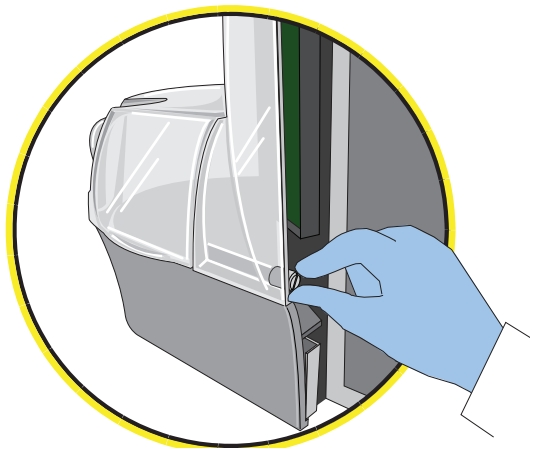


3 Open the Front Cover.

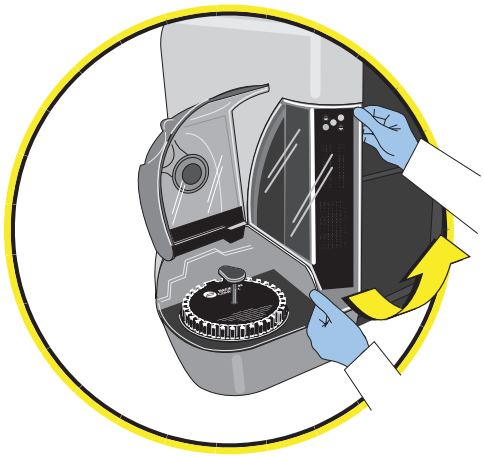


4 Remove the Front Left Side Panel.

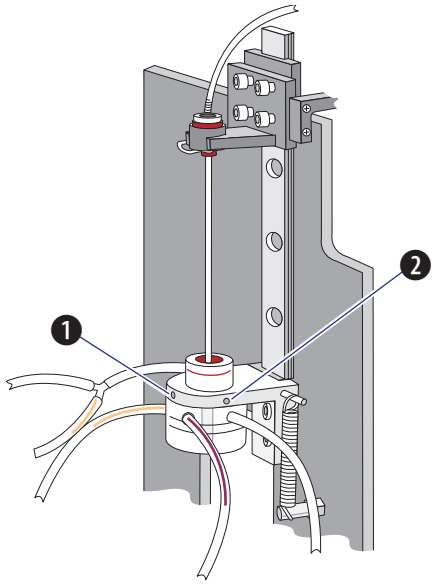
a. Unscrew the thumbscrew that attaches the left side panel to the front frame.



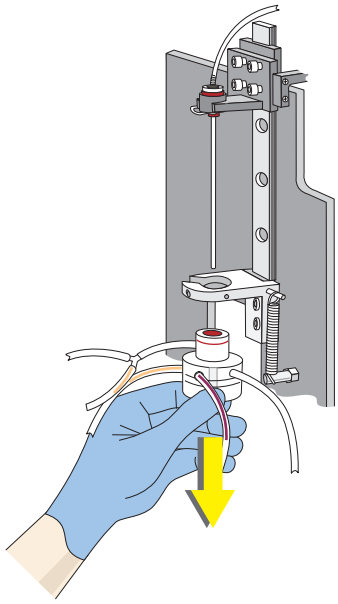
b. Remove the left side panel by pulling it towards you and swivel it up and out of the instrument.



5 Use a 0.050 in. Allen wrench to loosen the side ❶ and front ❷ setscrews on the sample head.



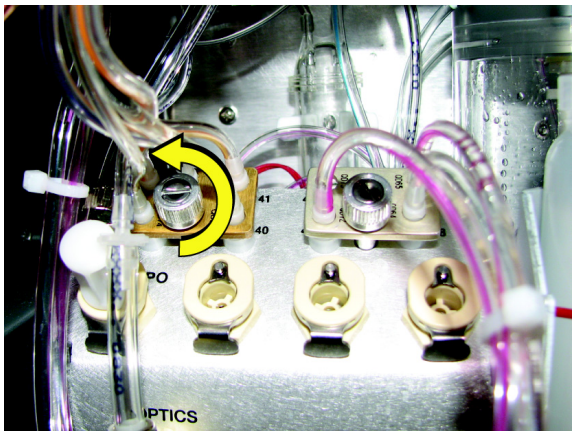
6 Pull off the sample head.



7 Pull the sample head and tubing through the instrument behind the frame.

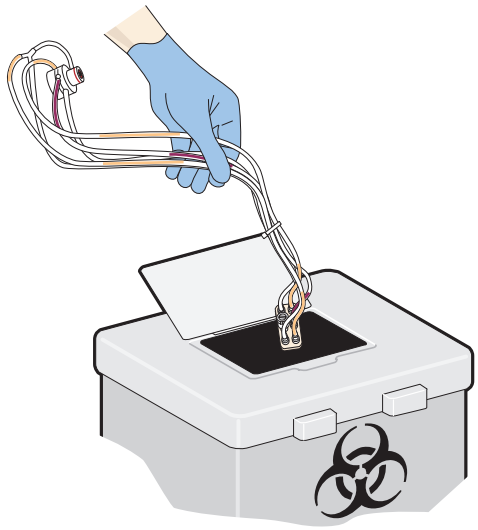
8 Loosen the thumbscrew holding the left tubing manifold.

Note: You might find it easier to unscrew the thumbscrew with a screwdriver.



9 Pull off the tubing manifold.

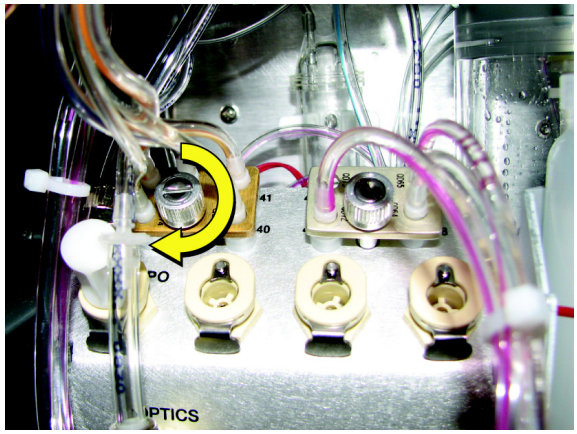
WARNING Risk of biohazardous contamination if you have skin contact with the sample head and its tubing. The sample head tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample head and tubing in accordance with your local regulations and acceptable laboratory procedures.



10 Discard the old sample head and tubing assembly in accordance with your local regulations and acceptable laboratory procedures.

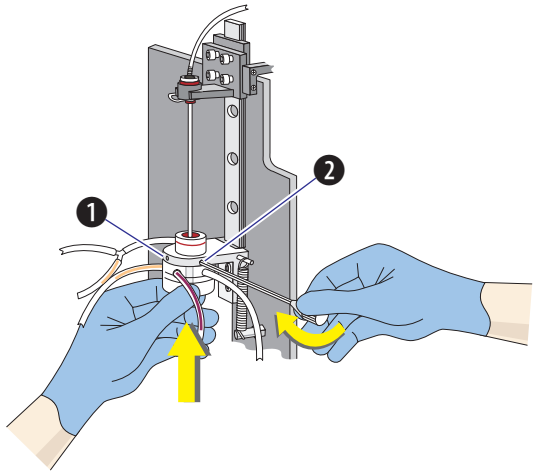
11 Place the new tubing manifold into the bracket in the pneumatic drawer and tighten the thumbscrew.

Note: You might find it easier to screw in the thumbscrew with a screwdriver.



12 Route the sample head and tubing through the instrument.

13 Position and hold the sample head up against its bracket.

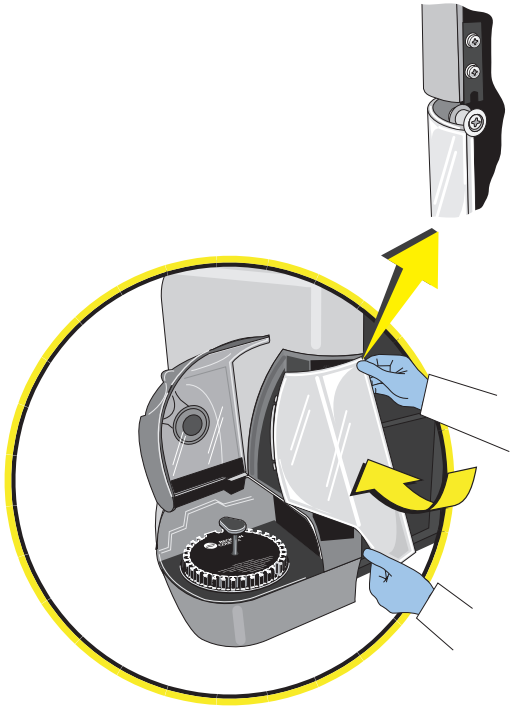


14 Tighten the side ❶ setscrew first.

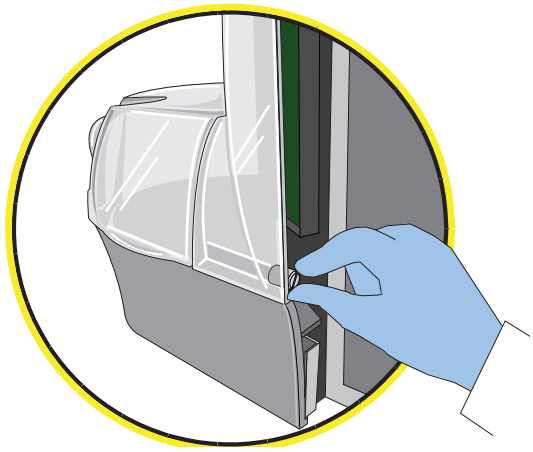
Then tighten the front ❷ setscrew.

15 Replace the Front Left Side Panel.

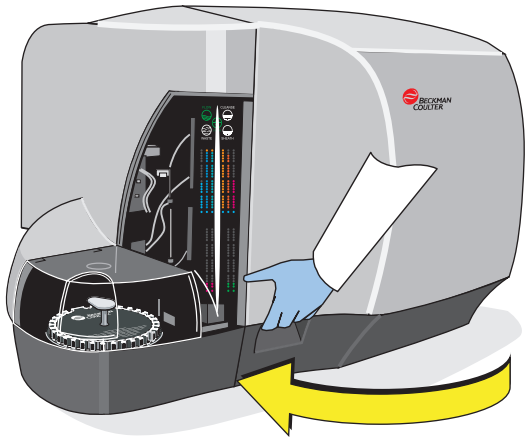
a. Swivel the left side panel into the instrument, aligning the post on the top of the panel with the cut out on the frame and then push the panel back into place.



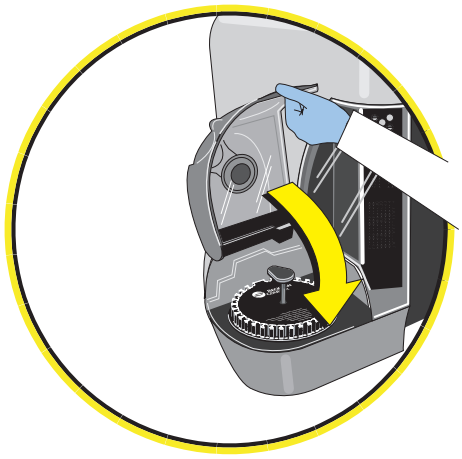
b. Screw in the thumbscrew to attach the left side panel to the front frame.



16 Close the front cover.



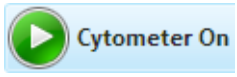
17 Close the MCL cover.

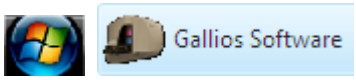


18 Power the Cytometer Only ON

or

Power the Cytometer and Gallios Software ON.



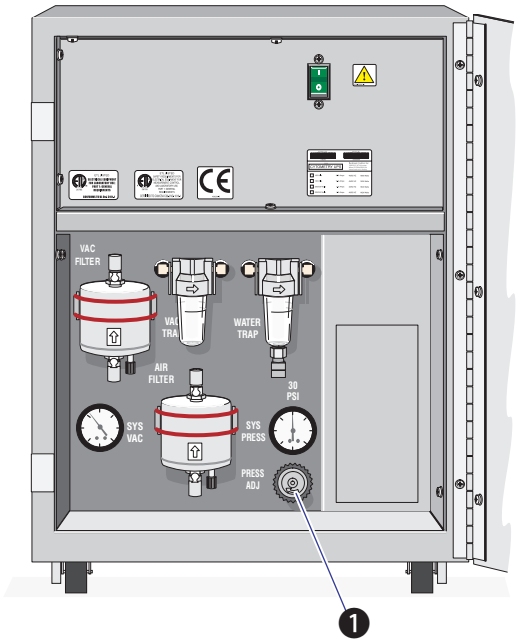


12.11 ADJUST THE SYSTEM PRESSURE

- Adjust the system pressure if the System Pressure gauge is not reading 30 ±2 psi.
- Daily Startup describes how to check the System Pressure gauge reading on the Pneumatic Supply.

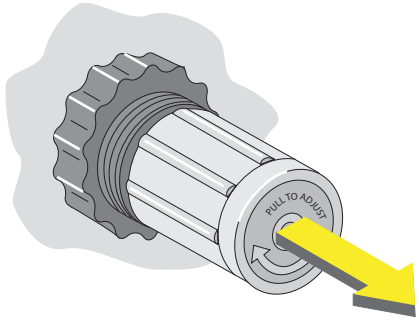
---

1 Open the Pneumatic Supply front door and locate the Pressure Adjustment knob (1).



---

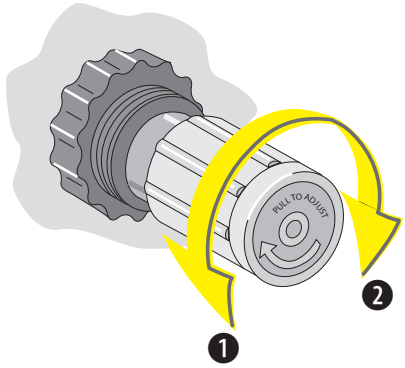
2 Pull the collar around the Pressure Adjust knob out toward you.

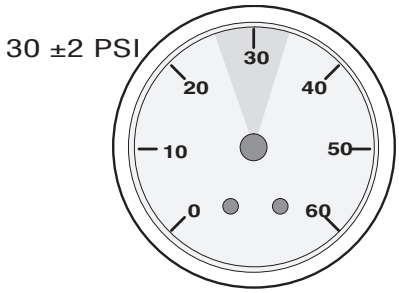


3 Adjust the pressure to 30 ±2 psi.

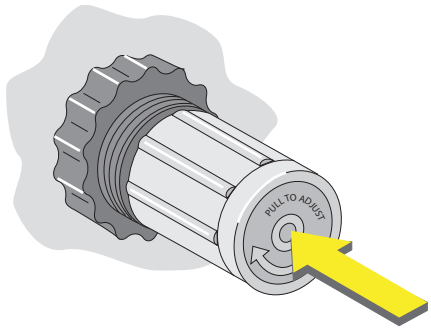
(1) To decrease, turn to the left.

(2) To increase, turn to the right.





4 Push in on the collar to lock it into place.



12.12 REPLACE AN OPTICAL FILTER

Perform this procedure when:

- When there is a loss of signal power - replace the old filter with a new filter of the same type.
- When you are running a different application and need a different filter in that filter holder.

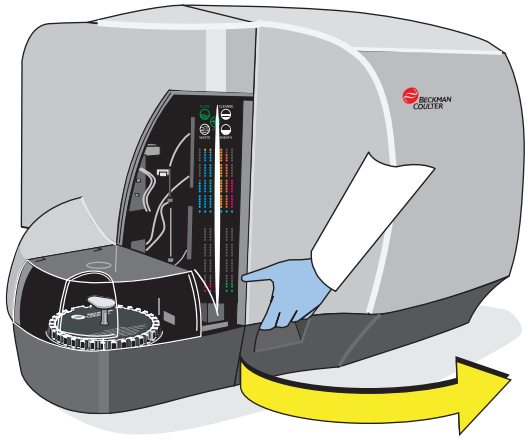
Note: If you replaced a damaged filter with the same type of filter, check that you retrieve similar autostandardization mean intensity values with the new filter

Remove Filter Holder

IMPORTANT Risk of incorrect readings from a contaminated filter if you wear gloves with powder to perform this procedure. Powder from the gloves can contaminate the filter and cause incorrect readings. Wear powder-free gloves whenever you are working with any optical filter components.

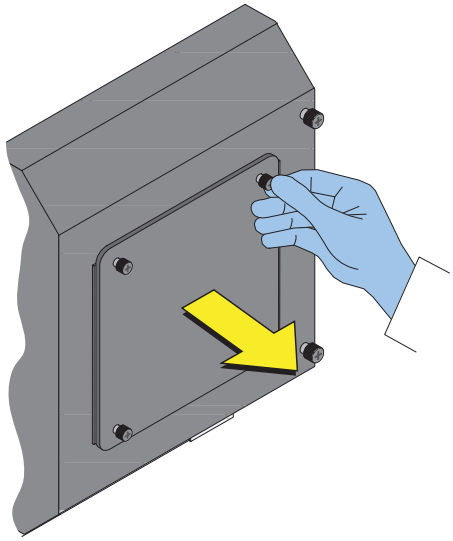
1 Wear powder-free gloves to perform this procedure.

2 Open the Front Cover.

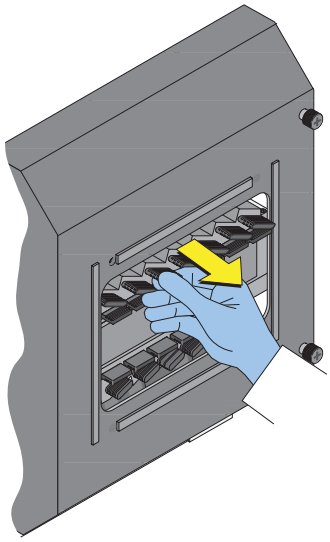


3 Loosen the four thumbscrews on the filter array cover and remove it.

Note: You need to unscrew the upper left thumbscrew with a screwdriver.



4 Remove the filter holder containing the filter you want to replace.



Note: There are two types of filter holders. See,

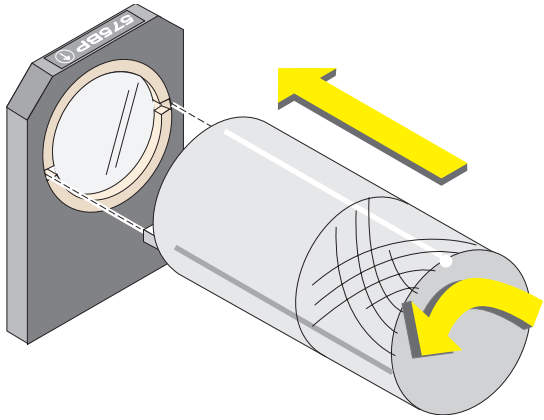
- Replace Dichroic Filter or
- Replace Bandpass Filter.

Use the appropriate instructions for the type of filter holder used on the filter you are going to replace.

Replace Dichroic Filter

1 Use the special tool provided to loosen the metal ring on the filter holder.

Note: You might find it easier to finish loosening the metal ring by turning it with your gloved fingers.



2 Insert the tool into the metal ring's two slots and turn to the left.

3 Remove the metal ring and the filter.

4 Orient the new filter correctly and insert the filter into the filter holder.

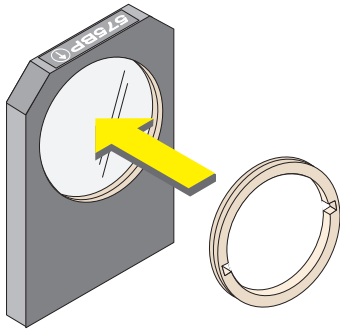
- For BCI filters:

  Position the filter into the filter holder so the arrow points to the metal ring.

- For non-BCI filters:

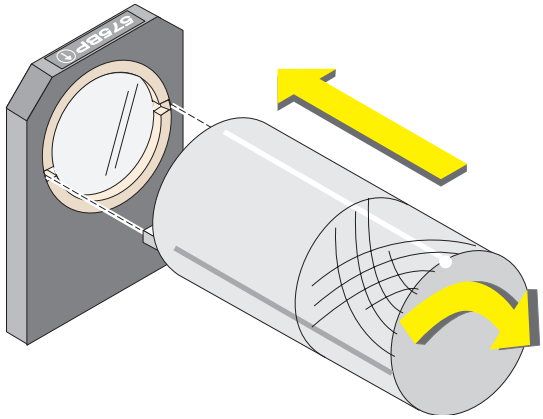
  See Identify Coated Side Of Dichroic Filter to determine correct orientation.

5 Place the metal ring over the filter in the filter holder.



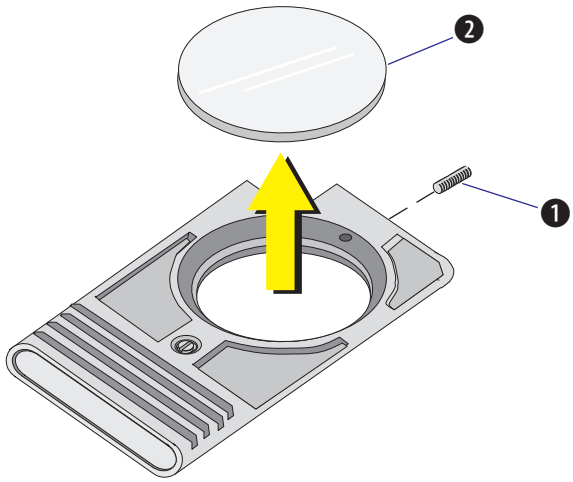
6 For the metal ring fastener, insert the special tool into the metal ring's two slots and turn to the right to tighten.

Note: You might find it easier to begin tightening the metal ring by turning it with your gloved fingers.



Replace Bandpass Filter

1 Remove the set screw (1) and remove the filter (2).



2 Orient the new filter correctly and insert the filter into the filter holder.

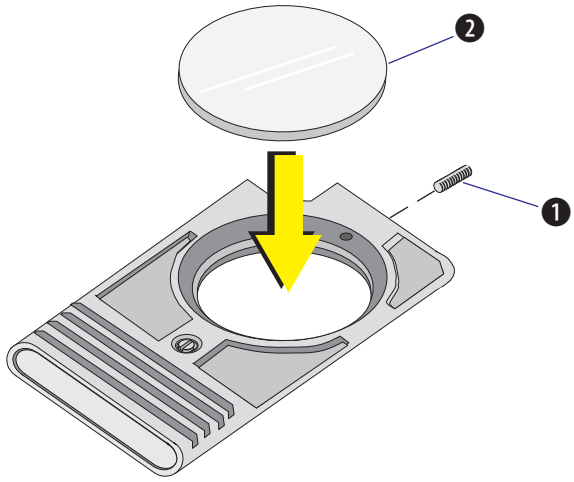
- For BCI filters:

  Position the filter into the filter holder so the arrow points to the metal ring.

- For non-BCI filters:

  See Identify Coated Side Of Dichroic Filter to determine correct orientation.

3 Place the filter (2) in the filter holder and tighten the set screw (1).



Identify Coated Side Of Dichroic Filter

1 Determine which is the coated side ❶ of a non-BCI filter:

a. Take the eraser end of a pencil and hold it close to the filter, near its edge.

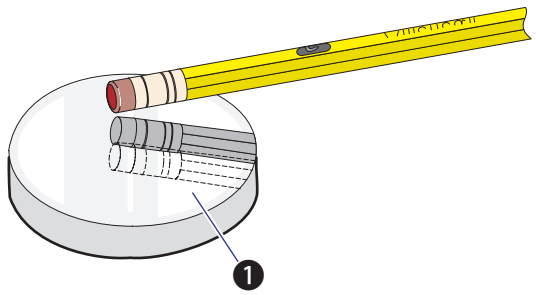
b. Look at the two reflections, dark- and light-colored, of the pencil.

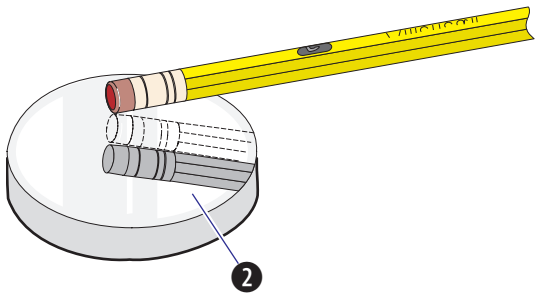
c. Turn the filter over and repeat steps a and b.

d. The side where the pencil touches the dark-colored reflection is the coated side ❶.

The uncoated side ❷ shows the pencil touching the light-colored reflection.

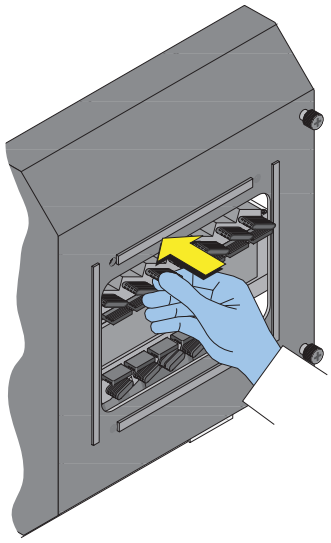
e. The coated side ❶ should face the metal ring when you insert it.





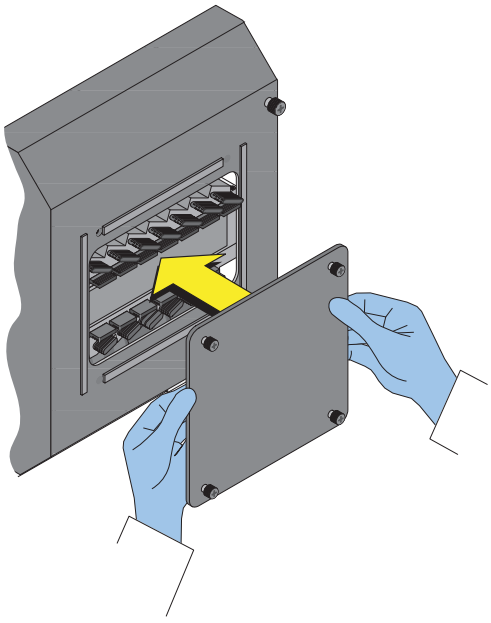
Replace Filter Holder

1 Place the filter holder containing the filter you replaced back in the filter array.

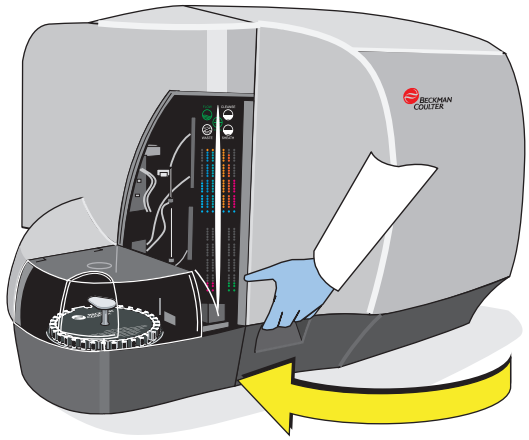


2 Replace the filter array cover.

Note: You need to use a screwdriver to tighten the upper left thumbscrew.



3 Close the Front Cover



13.1 PRECAUTIONS/HAZARDS

Laser/Radiation Precautions

The Cytometer contains two lasers (standard configuration) and the MCL bar-code reader contains one laser. The Cytometer can also include an optional third 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.

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 lasers are 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 contact your local Beckman Coulter Representative.

Laser 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.

CDRH-required warning labels are located:

See Figure 13.1 for the Sensing Compartment cover warning label.

See Figure 13.2 for the Sensing Compartment interior (cover removed) warning labels.

See Figure 13.3 for the Lasers in the Sensing Compartment warning labels.

See Figure 13.4 for the Filter Array (cover removed) warning labels.

See Figure 13.5 for the Laser Label on the Cytometer Back Panel

See Figure 13.6 for the MCL bar-code reader warning labels.

Figure 13.1 Laser Labels on the Sensing Compartment Cover

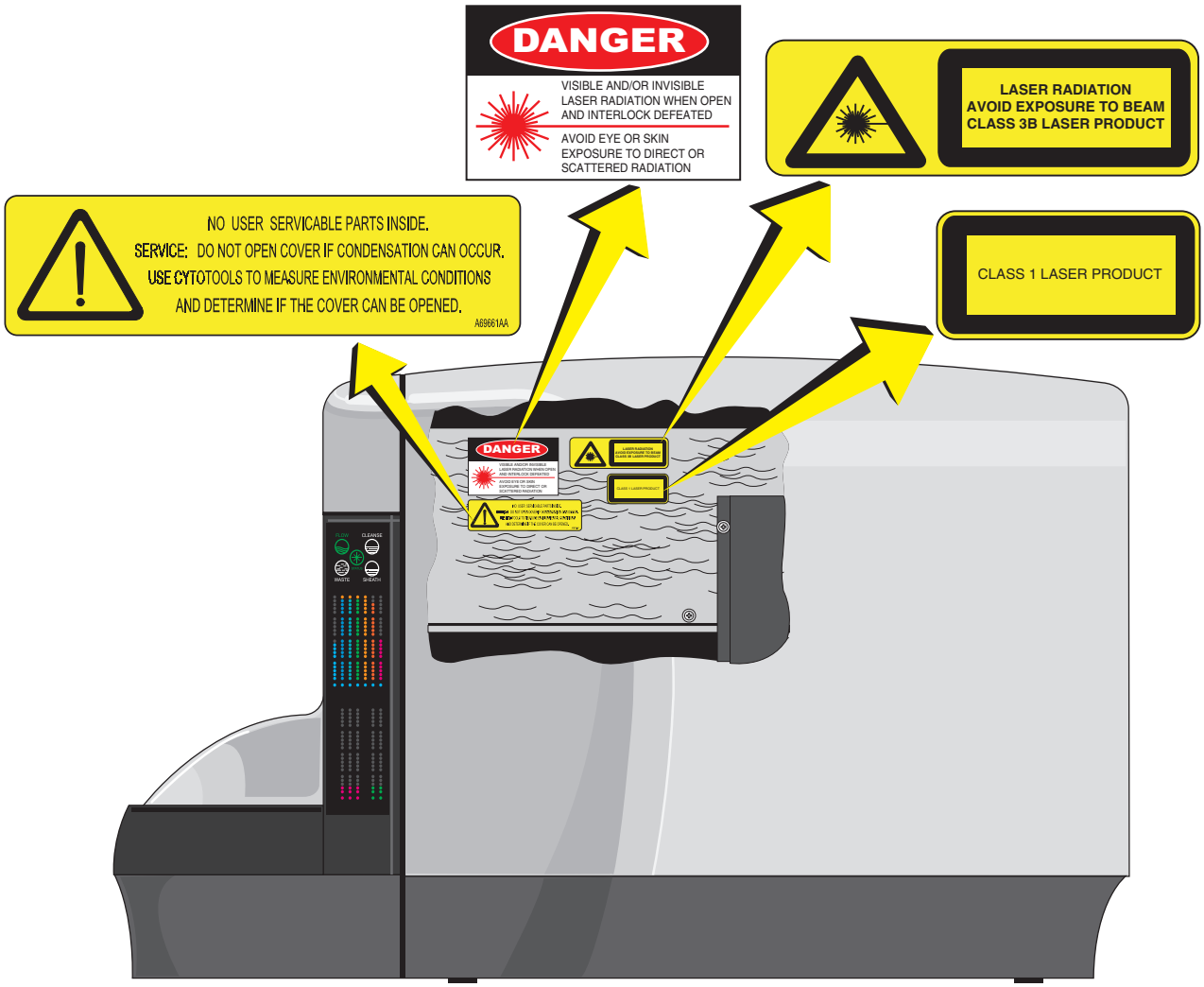


Figure 13.2 Laser Labels in the Sensing Compartment, Cover Removed

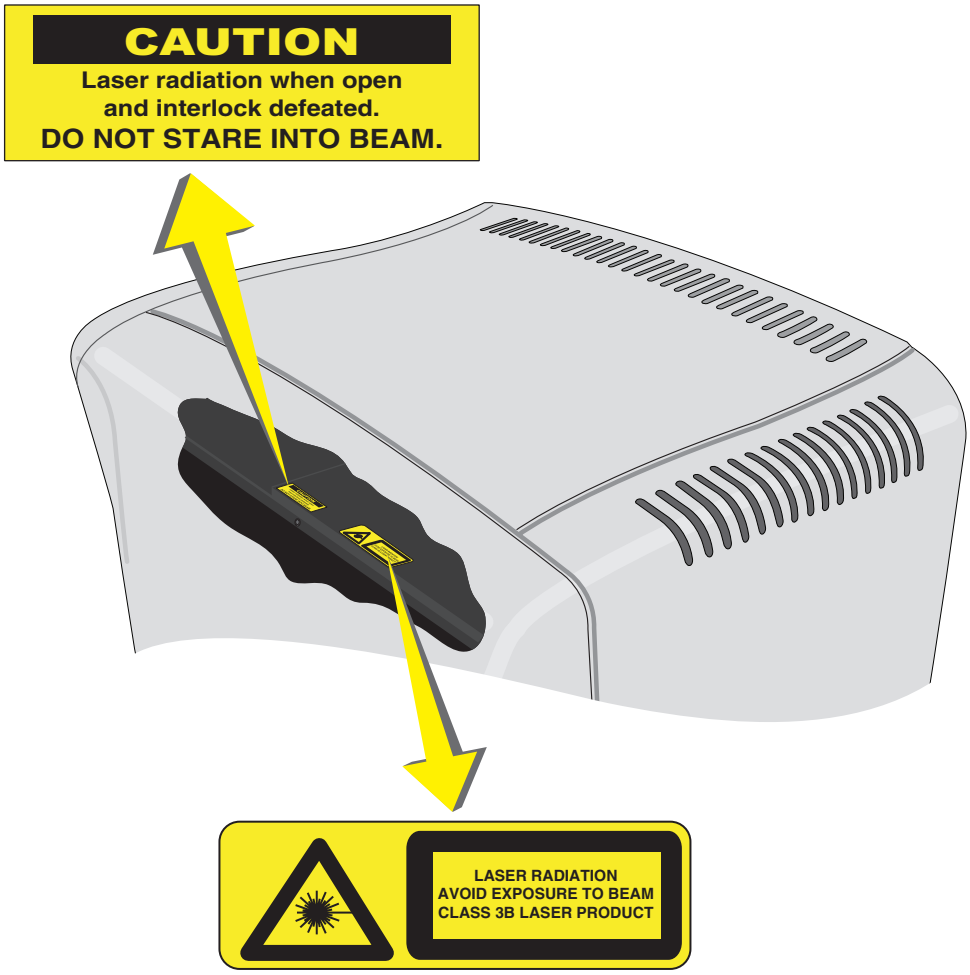


Figure 13.3 Labels on the Lasers in the Sensing Compartment, Cover Removed

Shown left to right below are, the red laser, the blue laser and the optional violet laser.

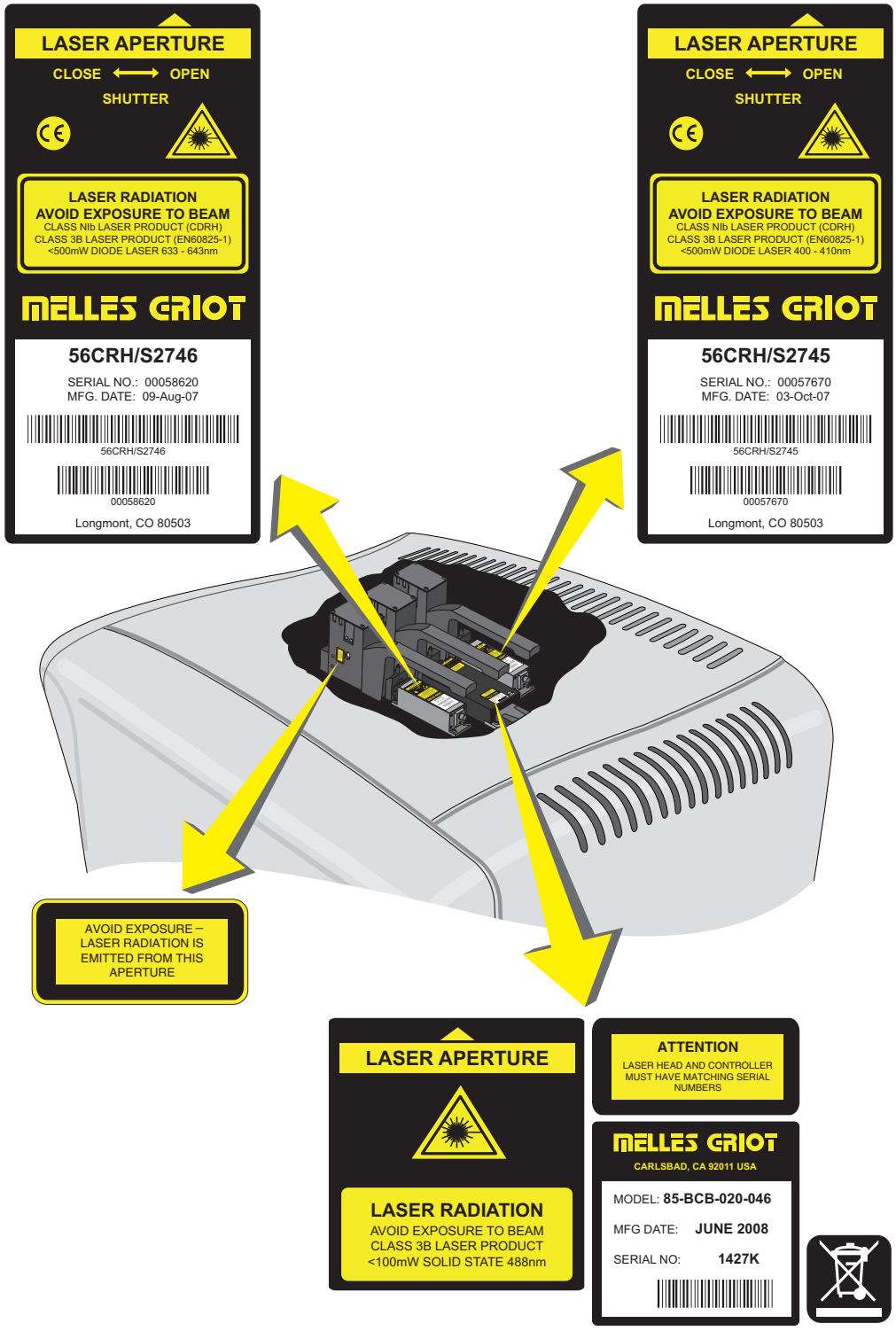


Figure 13.4 Laser Labels on the Filter Array, Cover Removed

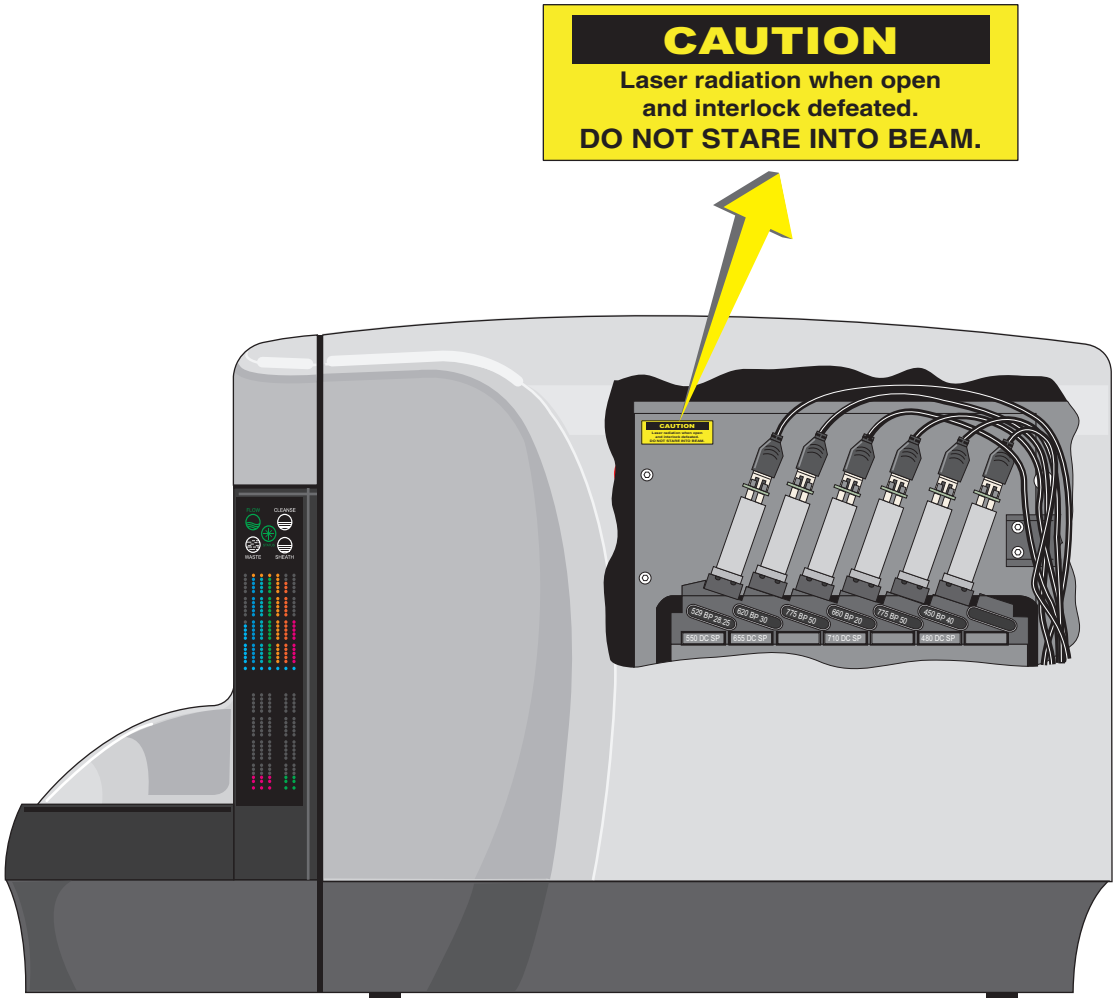


Figure 13.5 Laser Label on the Cytometer Back Panel

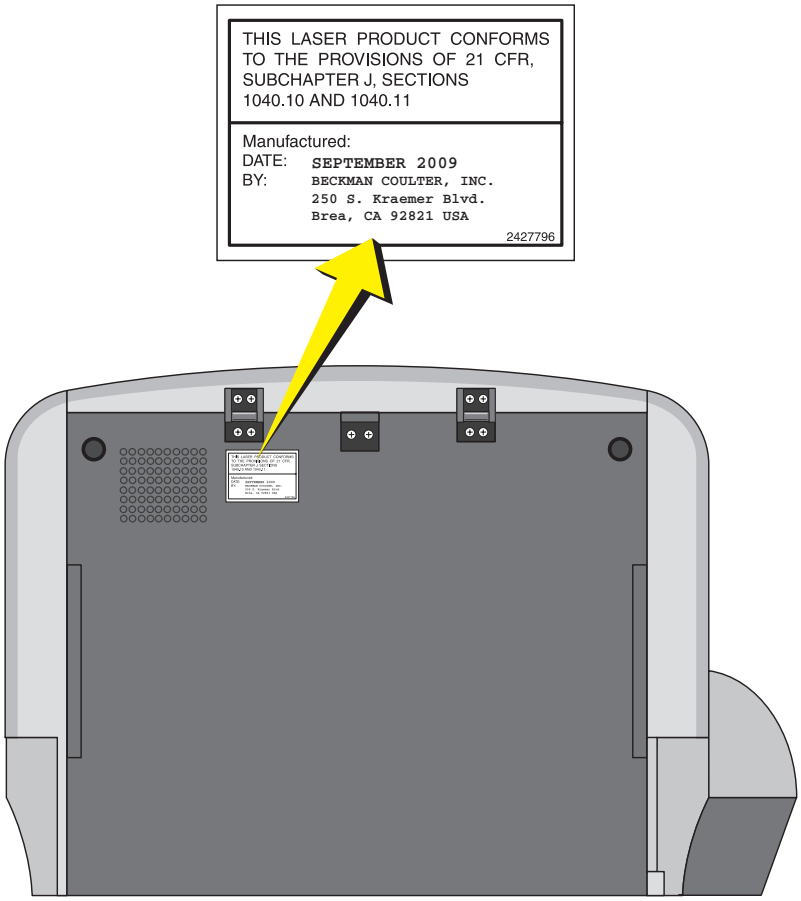
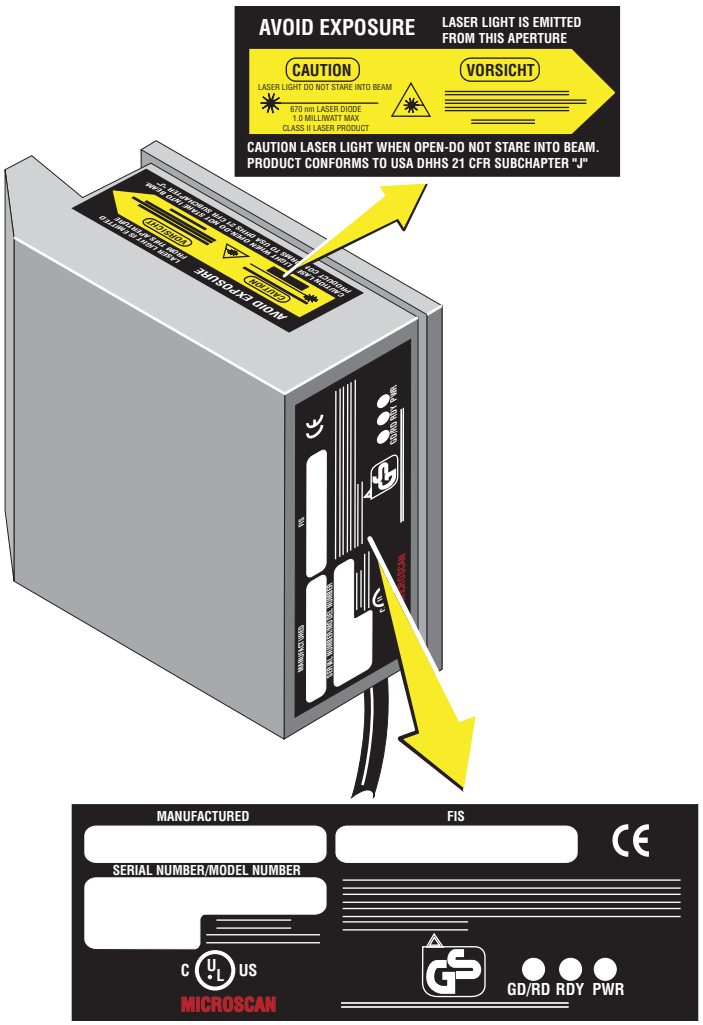


Figure 13.6 Laser Labels on the MCL Bar-Code Reader

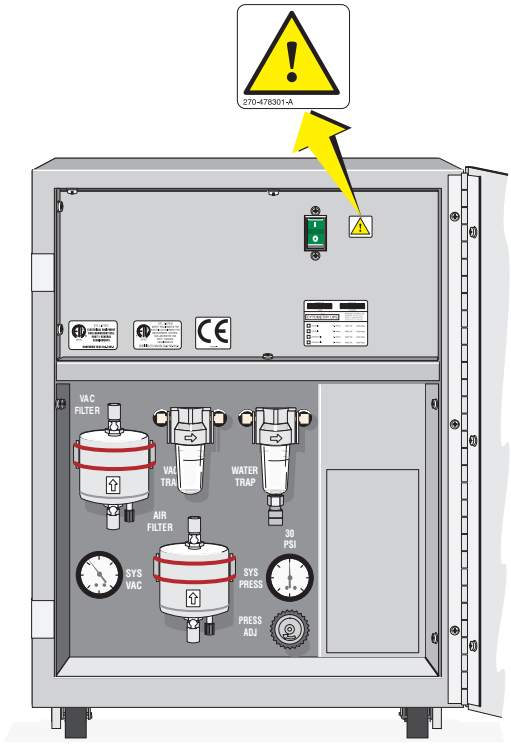


7272001A

Warning Labels on UPS

The  label located next to the power switch on the Pneumatic Supply instructs you to refer to product documentation before powering up the instrument.

Figure 13.7 International Warning Symbol Locations



Disposal Of Electrical Instrumentation

It is very important that customers understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates:

- that the device was put on the European Market after August 13, 2005 and
- that the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of device.

RoHS Caution Label

This logo indicates that this electronic information product contains certain toxic or hazardous substances or elements, and can be used safely during its environmental protection use period. The number in the middle of the logo indicates the environmental protection use period for the product. The outer circle indicates that the product can be recycled. The logo also signifies that the product should be recycled immediately after its environmental protection use period has expired. The date on the label indicates the date of manufacture.

RoHS Environmental Label

This logo indicates that the product does not contain any toxic or hazardous substances or elements. The "e" stands for electrical, electronic and environmental electronic information products. This logo indicates that this electronic information product does not contain any toxic or hazardous substances or elements, and is green and environmental. The outer circle indicates that the product can be recycled. The logo also signifies that the product can be recycled after being discarded, and should not be casually discarded.

Disposal Precaution

WARNING Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of the waste container in accordance with your local regulations and acceptable laboratory procedures

EMC Information

This equipment complies with the emission and immunity requirements described in IEC 61326-2-6.

IMPORTANT This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it could cause radio interference, in which case, you may need to take measures to mitigate the interference. It is advised that prior to operation of the device, the electromagnetic environment should be evaluated. Do not use this device in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources), as these could interfere with the proper operation.

13.2 MAINTENANCE SCHEDULES

Cleaning Schedule

See Table 13.1 for the cleaning schedule.

Table 13.1 Cleaning Schedule

Component	Daily	Weekly	Monthly	Every 60 Days	As Needed
CLEAN THE AIR FILTERS (Cytometer)	-	✓	-	-	-
CLEAN THE AIR FILTERS (Pneumatic Supply)	-	-	✓	-	-
CLEAN THE CLEANING AGENT CONTAINER	-	-	-	✓	-
CLEAN THE MCL SAMPLE HEAD AND THE SAMPLE PROBE	-	✓	-	-	-

Table 13.1 Cleaning Schedule

Component	Daily	Weekly	Monthly	Every 60 Days	As Needed
CLEAN THE SAMPLING SYSTEM	✓	-	-	-	-
CLEAN THE INTERNAL SHEATH FLUID CONTAINER	-	-	✓	-	-
CLEAN THE VACUUM TRAP	-	-	-	-	✓

- = Not Applicable

Replacement Schedule

The sheath fluid filter needs to be replaced every 6 months. All other replacement and adjustment procedures should be done on an as needed basis.

13.3 CYTOMETER MESSAGES

See Table 13.2 for a list of Cytometer messages.

Display Locations

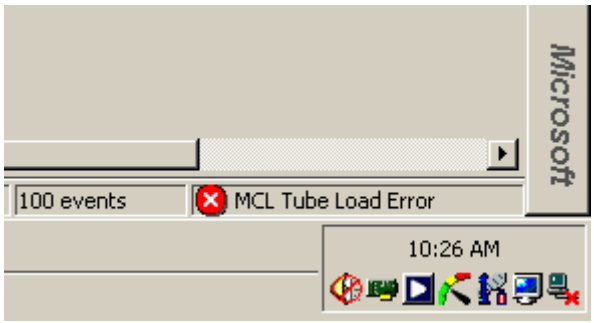
Cytometer messages can appear at the bottom of the Gallios software screen, in the Cytometer Status Messages screen, and in the cytometer.log file.

Gallios Software Screen

At the bottom of the Gallios software screen, the last message appears. See Figure 13.8.

> **Note:** If multiple messages are posted at the same time, only the last one posted appears here. All of the messages posted appear in the Cytometer Status Messages screen and the cytometer.log file.

Figure 13.8 Error Message on Gallios Software Screen, Example

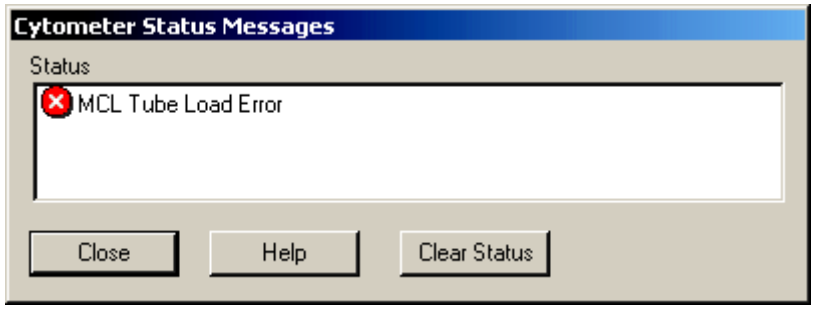


Cytometer Status Messages Screen

In the Cytometer Status Messages screen, the last message and all uncleared messages appear. See Figure 13.9.

- To access the Cytometer Status Messages screen, double click the message at the bottom of the Gallios software screen.
- **Clear Status** when you want to delete all messages in the Cytometer Status Messages screen.

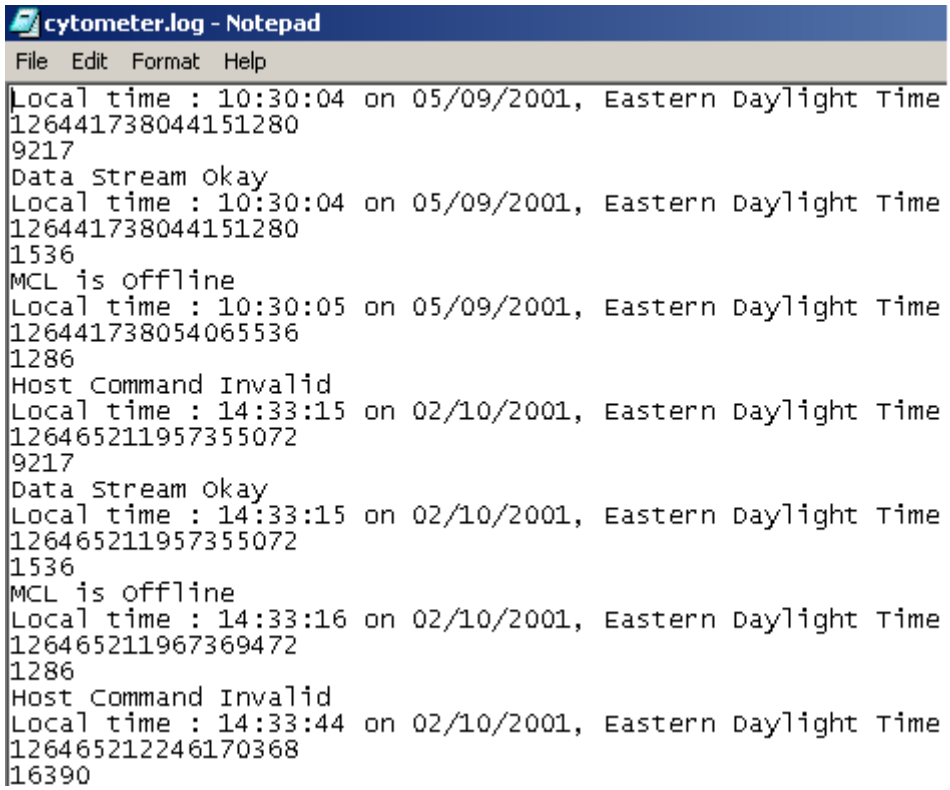
Figure 13.9 Cytometer Status Messages Window, Example



Cytometer.log File

In the cytometer.log file, all messages appear from the last 30 days unless they were manually cleared. Refer to CYTOMETER.LOG FILE for details. See Figure 13.10.

Figure 13.10 Example of Error Messages in Cytometer.log File



13.4 CYTOMETER.LOG FILE

The Cytometer messages are located in the cytometer.log file.

Note: Messages over 30 days old are automatically removed from the cytometer.log file and placed into the cytometerarchive.log file.

How to Access the Cytometer.log File

From Gallios Software

- Press Ctrl+L. See Figure 13.10.

  OR,

- **Cytometer ▸▸ Cytometer Log ▸▸ View Log**. See Figure 13.10.

From Windows Desktop

1. **Start ▸▸ Programs ▸▸ Accessories ▸▸ Windows Explorer ▸▸ My Computer ▸▸ (C:) drive ▸▸ Gallios**.
2. Highlight **cytometer.log**.
3. Double click on **cytometer.log** to open. See Figure 13.10.

Cytometer.Log Entry Description

All cytometer.log message entries are posted in chronological order. Each message entry consists of four lines (see Figure 13.10):

- Date and time when the message occurred
- 18-digit number - for Service use only
- Four-digit number - for Service use only
- Message text. See Table 13.2 for a list of messages and operator actions.

How to Search the Cytometer.log File

To search for a specific word or phrase listed anywhere in the cytometer.log file:

1. **Edit ▸▸ Find...**
2. Type in the word or phrase you want to find (Example: `Waste`).
3. The direction of the search: Up or Down.
4. **Find Next** and the next occurrence of the word in the error log is highlighted.
5. Repeat step 4 as needed or until *Cannot find "XXXXX"* appears.

Other Functions Available

Here are some of the more often used functions available from the cytometer.log file pull down menus: **File**, **Edit**, **Format**, and **Help**:

Print

To print the cytometer.log file: **File ▸▸ Print**.

Change Font

To change the font: **Format ▸▸ Font**, and select the new font.

Find Help Topics

To find Help on other topics: **Help ▸▸ Help Topics**.

Cytometerarchive.log File

The cytometerarchive.log file contains messages that were automatically moved from the cytometer.log file after 30 days. This log is for Service use.

13.5 CYTOMETER MESSAGES TABLE

Table 13.2 lists the messages in alphabetical order, with their cause and what to do about them. These are the messages produced by the Cytometer.

contact your local Beckman Coulter Representative if:

- The recommended action does not solve the problem.
- You need help.

Table 13.2 Cytometer Messages

Message	Probable Cause	Recommended Action
Acquisition Yield Warning	The threshold events exceeds the processed and displayed events.	1. Dilute the sample . 2. Change the discriminator setting. 3. Change the sample flow rate to medium or low. 4. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Ambient Sensor Error	The ambient temperature sensor failed during acquisition and the acquisition was stopped.	If error persists, contact your local Beckman Coulter Representative.
Ambient Sensor Warning	There has been no change in the measured ambient temperature for some time and the sensor may have failed.	If warning persists, contact your local Beckman Coulter Representative.
Ambient Temperature Warning	Ambient Temperature is outside the system's specifications.	Reduce or wait till ambient temperature is within specification.
Blue Laser Comms Error	Unable to communicate with blue laser.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Blue Laser Fault	A fault occurred on the blue laser during acquisition and the acquisition was stopped.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Blue Laser Fault Warning	There is a fault condition on the blue laser.	Check optics covers are properly installed and tightened. See Blue Laser Fault.
Blue Laser Initialization Error	The blue laser failed to initialize.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Blue Laser Initializing	The blue laser is initializing	If the laser does not initialize within a reasonable time then restart the entire system. If the problem persists then contact your local Beckman Coulter Representative.
Blue Laser Not Calibrated	The instrument is not calibrated to trigger off the blue laser.	Calibrate the blue laser.
Blue Laser Power Error	During acquisition the blue laser output power went out of range and the acquisition was stopped.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Blue Laser Power Warning	The blue laser power is unstable.	If laser does not stabilize within a reasonable time or frequently goes unstable then restart the entire system. If problem persists then contact your local Beckman Coulter Representative.
CAN Master Board Not Found	The CAN master board could not be detected	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Carousel In/Out Error	The carousel failed to move in or out correctly after several attempts.	1. Check that there is no obvious obstruction (sample tube) in the MCL area. 2. Check that the 30 psi gage on the Pneumatic Supply is okay. 3. If no obstruction is found and pressure okay, contact your local Beckman Coulter Representative.
Carousel In/Out Warning	The first attempt to move the carousel in or out failed.	If warning persists, see Carousel In/Out Error.
Carousel Label Read Error	The carousel bar-code label could not be read after several attempts.	1. Check that the bar-code label is not torn or written on. 2. Try using another carousel. 3. If problem continues, contact your local Beckman Coulter Representative.
Carousel Label Read Warning	The first attempt to read the carousel bar-code label failed.	If warning persists, see Carousel Label read Error.
Carousel Rotate Error	The carousel failed to rotate to the correct position after several attempts.	1. Check that there is no obvious obstruction (sample tube) in the MCL area. 2. Check that the 30 psi gage on the Pneumatic Supply is okay. 3. Try using another carousel. 4. If no obstruction is found and pressure okay, contact your local Beckman Coulter Representative.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Cleanse Level Error	The requested cleanse procedure could not be performed due to the low level of cleaning agent.	Fill cleaning agent container. Refer to FILL THE CLEANING AGENT CONTAINER.
	Cleanse sensor failed if the cleaning agent container is full but this error message displayed.	contact your local Beckman Coulter Representative.
Cleanse Level Warning	Low cleaning agent.	Fill cleaning agent container. Refer to FILL THE CLEANING AGENT CONTAINER.
	Cleanse sensor failed if the cleaning agent container is full but this error message displayed.	contact your local Beckman Coulter Representative.
Control Socket Error	A control communication error has occurred between the workstation and the instrument.	Check the Ethernet cable between the workstation and the cytometer. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Corrupt Configuration Error	The instruments configuration information is corrupted.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Data Rate Warning	The discriminated data rate is higher than can be handled by the instrument and data is being lost.	1. Dilute the sample or change the discriminator setting. 2. Check that the sheath fluid container cap is tightened. 3. Change the sample flow rate to medium or low. 4. If problem continues, REPLACE THE SHEATH FLUID FILTER.
Data Socket Error	A data communication error has occurred between the workstation and the instrument.	Check the Ethernet cable between the workstation and the instrument. Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Detector X Incorrect Type	The type of detector X does not agree with the type in the instrument configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Detector X Missing	The instrument is configured for detector X but no detector could be found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Drip Chamber Level Error	The drip chamber is full and the acquisition has been stopped.	The drip chamber will be automatically drained at the end of the acquisition. To avoid the error in the future change the sample concentration or protocol to reduce the acquisition time. If the error persists, contact your local Beckman Coulter Representative.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Drip Chamber Level Warning	The first time this message is displayed during acquisition there is enough spare volume for at least another five minutes of sample analysis. The second time or if acquisition is not in progress it indicates that the drip chamber is full.	Stop the acquisition in a controlled way or allow it to continue till the chamber is full and the acquisition automatically stopped. To avoid the message in the future change the sample concentration or protocol to reduce the acquisition time. If warning persists, see *Drip Chamber Level Error*.
Drip Or Waste Overfill Error	The drip or waste chamber has overflowed and the acquisition has been stopped.	contact your local Beckman Coulter Representative.
Event Checksum Failures	The checksum of an event passed from the instrument to the workstation is incorrect.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Failed to Power Off	The instrument failed to turn off.	POWER THE CYTOMETER ONLY ON/OFF. If error persists,contact your local Beckman Coulter Representative.
Fluidics Board Not Found	The fluidics I/O board could not be detected.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Fluidics I/O Board Voltage Error	The voltages on the fluidics I/O board are out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Front Panel Connection Fault – SCA1	A fault was detected in the connection between the data acquisition board SCA 1 and the front panel display board.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Front Panel Connection Fault – SCA2	A fault was detected in the connection between the data acquisition board SCA 2 and the front panel display board.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Front Panel Connection Fault – SCA3	A fault was detected in the connection between the data acquisition board SCA 3 and the front panel display board.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Hardware Made Safe	An error has occurred or a request has been made from CytoTools and the harware has been put into a safe state.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Host Command Invalid	The Cytometer software could not process the requested command from the Workstation.	1. Reboot the computer and restart the Cytometer. 2. If problem continues, contact your local Beckman Coulter Representative.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Incorrect Laser Type - Blue	The type of the blue laser does not agree with the type in the instrument configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Incorrect Laser Type - Red	The type of the red laser does not agree with the type in the instrument configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Incorrect Laser Type - Violet	The type of the violet laser does not agree with the type in the instrument configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Internal Sheath Tank Level Error	There is no more sheath fluid in the internal sheath tank and the acquisition has been stopped.	If error persists, contact your local Beckman Coulter Representative.
Internal Sheath Tank Level Warning	The internal sheath tank is empty.	If error persists, contact your local Beckman Coulter Representative.
MCL Door Open Error	MCL cover is open while the MCL is in use.	Close the MCL cover.
MCL Door Open Warning	MCL cover is open while the MCL is in use.	Close the MCL cover.
Optics Board Not Found	The optics I/O board could not be detected.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Optics I/O Board Voltage Error	The voltages on the optics I/O board are out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Optics Temperature Stabilizing	The temperature of the optics is stabilizing.	If the optics temperature does not stabilize within a reasonable time then restart the entire system. If the problem persists then contact your local Beckman Coulter Representative.
Optics Temperature Warning	The temperature of the optics differs considerably from the temperature when the optics were aligned such that the instrument may not meet its specification.	Wait till the temperature of the optics is closer to that when the instrument was aligned or realign the optics for different ambient temperature range.
Pico Motor Board Not Found	The pico motor control board could not be detected.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Place tube in position 21	Single Tube Fixed Mode selected	Place tube in position 21 and select Run to continue in this mode. To discontinue operation in this mode, depress the Single Tube Fixed Mode button on the Cytometer Control toolbar.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Pressures Not Calibrated	Pressures can not be calibrated because pressure control board can not be detected or calibration coefficients corrupted.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Probe Up/Down Error	The probe failed to move up or down correctly after several attempts.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Probe Up/Down Warning	The first attempt to raise or lower the probe failed.	None. The instrument will automatically retry to raise or lower the probe. If warning persists, contact your local Beckman Coulter Representative.
Red Laser Comms Warning	Unable to communicate with red laser.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Red Laser Fault	A fault occurred on the red laser during acquisition and the acquisition was stopped.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Red Laser Fault Warning	There is a fault condition on the red laser.	Check optics covers are properly installed and tightened. See Red Laser Fault.
Red Laser Initialization Error	The red laser failed to initialize.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Red Laser Initializing	The red laser is initializing.	If the laser does not initialize within a reasonable time then restart the entire system. If the problem persists then contact your local Beckman Coulter Representative.
Red Laser Not Calibrated	The instrument is not calibrated to trigger off the red laser.	Calibrate the red laser.
Red Laser Power Error	During acquisition the red laser output power went out of range and the acquisition was stopped.	If the error persists, contact your local Beckman Coulter Representative.
Red Laser Power Warning	The red laser power is unstable.	If laser does not stabilize within a reasonable time or frequently goes unstable then restart the entire system. If the problem persists then contact your local Beckman Coulter Representative.
Sample Pressure Error	During acquisition the sample pressure went out of range and the acquisition was stopped.	If the error persists, contact your local Beckman Coulter Representative.
Sample Pressure Warning	The sample pressure is outside the operating range.	If warning persists, see *Sample Pressure Error.*
Sample Tube Not Found	No tube is present in the carousel location identified in Acquisition Manager.	Verify the intended sample is placed in the location identified in Acquisition Manager and select Run.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Sample Tube Pressure Error	After several attempts the sample tube could not be pressurized. There may be a leak caused by a bad sample tube or a bad sample head.	Inspect sample tube and sample head for damage. Change as required. See REPLACE THE MCL SAMPLE HEAD.
Sample Tube Pressure Warning	The first attempt to pressurize the tube failed. There may be a leak caused by a bad sample tube or a bad sample head	If warning persists, see *Sample Tube Pressure Error.*
Sampler Board Not Found	The sampler I/O board could not be detected.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Sampler I/O Board Voltage Error	The voltages on the sampler I/O board are out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
SCA Board 1 Missing	The data acquisition board SCA 1 could not be detected	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
SCA Board 2 Missing	The data acquisition board SCA 2 could not be detected	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
SCA Board 3 Missing	The data acquisition board SCA 3 could not be detected	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Set Manual Mode	Status confirming samples will be run in the MCL Manual Mode.	Select Run to continue in this mode. To discontinue operation in this mode depress the MCL Manual Mode button on the Cytometer Control toolbar to return to the MCL Automatic Mode.
Set Single Tube Mode	Status confirming samples will be run in the Single Tube Fixed Mode.	Select Run to continue in this mode. To discontinue operation in this mode, depress the Single Tube Fixed Mode button on the Cytometer Control toolbar to return to the MCL Automatic Mode.
Sheath Cube Level Error	The 10 L sheath cube is empty and the instrument has entered the idle state.	Replace the external sheath fluid container. See REPLACE THE 10 L EXTERNAL SHEATH FLUID CONTAINER.
Sheath Cube Level Warning	The 10 L sheath cube is empty.	Replace the external sheath fluid container. See REPLACE THE 10 L EXTERNAL SHEATH FLUID CONTAINER.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Sheath Pressure Error	During acquisition, the sheath pressure went out of range and acquisition was stopped.	1. Check sheath fluid container cap for tightness. 2. If problem continues, REPLACE THE SHEATH FLUID FILTER. 3. If problems continues, contact your local Beckman Coulter Representative.
Sheath Pressure Warning	The sheath fluid pressure is outside the system's operating range.	If warning persists, see *Sheath Pressure Error.*
Start of Data Decode Error	The header at the beginning of the data that is passed from the instrument to the workstation could not be decoded.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
System Pressure Error	During acquisition the system pressure was lost and the acquisition was stopped.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative
System Pressure Warning	The system pressure is outside the operating range.	If warning persists, see *System Pressure Error.*
System Pressurization Error	Pressure line is not connected between the power module and the Cytometer.	Connect the pressure line.
	The pressurized air supply is outside the system's operating range.	1. Go to the Ready State, then check that the system pressure is 30 psi. 2. Run a sample and monitor the system pressure. If the system pressure drops below the range specified in the instrument manual, then contact your local Beckman Coulter Representative.
	Short circuit.	Check fuses.
System Vacuum Error	During acquisition, the system vacuum went outside the operating range and acquisition was stopped.	If error persists, see *System Vacuum Warning.*
System Vacuum Warning	Liquid in the vacuum trap.	Check that the vacuum trap (on the front of the Pneumatic Supply) is tight and is less than 1/4 full of fluid. If it is more full, empty it (see CLEAN THE VACUUM TRAP).
	Vacuum line is not connected between the power module and the Cytometer.	Connect the vacuum line at the back of the instrument.
	Hardware problem.	contact your local Beckman Coulter Representative.
TEC Board Not Found	Optics temperature control board could not be detected.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Tube Up/Down Error	Unable to load or unload the sample tube from the MCL sampling position.	1. Check that the labels on the sample tubes are secure and are not adhering to the walls of the carousel. 2. Check that there is no crack in the sample tube. 3. If problem continues, contact your local Beckman Coulter Representative
Tube Up/Down Warning	The first attempt to load or unload the sample tube from the MCL sampling position failed.	If warning persists, see Tube Up/Down Error.
Violet Laser Comms Warning	Unable to communicate with the violet laser.	Restart the entire system. If warning persists, contact your local Beckman Coulter Representative.
Violet Laser Fault	A fault occurred on the violet laser during acquisition and the acquisition was stopped.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Violet Laser Fault Warning	There is a fault condition on the violet laser.	Check optics covers are properly installed and tightened. See Violet Laser Fault.
Violet Laser Initialization Error	The violet laser failed to initialize.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
Violet Laser Initializing	The violet laser is initializing	If the laser does not initialize within a reasonable time then restart the entire system. If the problem persists then contact your local Beckman Coulter Representative.
Violet Laser Not Calibrated	The instrument is not calibrated to trigger off the violet laser.	Calibrate the violet laser.
Violet Laser Power Error	During acquisition, the violet laser output power went out of range and acquisition was stopped.	If error persists, contact your local Beckman Coulter Representative.
Violet Laser Power Warning	The violet laser power is unstable.	If laser does not stabilize within a reasonable time or frequently goes unstable then restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Waste Backpressure Error	The filter on the waste container vent line is probably wet, clogged, or disconnected. There is not enough empty volume in the 20 L waste container for further sample analysis.	1. Empty the 20 L waste container. See EMPTY THE 20 L WASTE CONTAINER. 2. Check the waste vent filter for the presence of liquid and a proper connection. 3. If problem continues, contact your local Beckman Coulter Representative.

Table 13.2 Cytometer Messages *(Continued)*

Message	Probable Cause	Recommended Action
Waste Chamber Level Error	The liquid level in the internal waste chamber is too high and acquisition has been stopped.	1. Empty the 20 L waste container. See EMPTY THE 20 L WASTE CONTAINER. 2. Check the waste vent filter for the presence of liquid and a proper connection. 3. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Waste Chamber Level Warning	The liquid level in the internal waste chamber is too high.	1. Empty the 20 L waste container. See EMPTY THE 20 L WASTE CONTAINER. 2. Check the waste vent filter for the presence of liquid and a proper connection. 3. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
Waste Cube Level Error	The is not enough empty volume in the waste container for further sample analysis and the idle state has been entered.	Empty the waste container. See EMPTY THE 20 L WASTE CONTAINER.
Waste Cube Level Warning	The waste container is full.	Empty the waste container. See EMPTY THE 20 L WASTE CONTAINER.
	If the waste container is not full, the waste sensor failed.	contact your local Beckman Coulter Representative.

13.6 SOFTWARE MESSAGES

Gallios Software Messages

Table 13.3 lists the Gallios software messages, with their cause and what to do about them. These are messages produced by the Software.

contact your local Beckman Coulter Representative if:

- The recommended action does not solve the problem.
- You need help.

Table 13.3 Software Messages

Message	Probable Cause	Recommneded Action
Unspecified error	An unknown error occurred.	Contact your local Beckman Coulter representative.
Nothing to modify!	Modify button pressed with no dies listed.	Select Dye

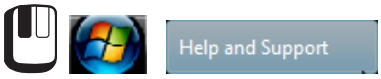
Table 13.3 Software Messages

Message	Probable Cause	Recommneded Action
Unable to read Error Code Strings	Failed to read error code string	Reboot the computer, if problem persists contact your local Beckman Coulter representative.
The file contains errors. The data checksum does not match the data.	Listmode data checksum stored does not match checksum re-calculated.	The listmode data is corrupt and the you should recover the original listmode file from backup or archive.
Baseline Offset - not available	On starting software the 3mv binary file is not found.	Reboot the computer, if problem persists contact your local Beckman Coulter representative.
Multiplexor Address of new labels does not match existing parameters Continue?	Parameter label does not match, asks you to continue.	Reboot the computer, if problem persists contact your local Beckman Coulter representative.
Missing NoOfTest field in file <<file-name>>	On inserting a panel into the listmode playback it is discovered that the number of tests information is missing from the file.	Recreate the panel file.
Missing NoOfPanels field in file <<file-name>>	Number of panels information missing from the worklist.	Recreate the worklist.
Missing NoOfTest field in section Panel <<panel-number>> Info in file <<file-name>>	Number of tests information missing from the panel specified in the worklist.	Recreate the panel file.
The BCAP service does not appear to be running.\nSoftware cannot start without BCAP.	Starting software without BCAP causes this error message.	Reboot the computer, if problem persists contact your local Beckman Coulter representative.
The program will not be able to work properly because it failed to access the administrator user settings. Please close the application immediately.	The software failed to find the administrator account.	Reboot the computer, if problem persists contact your local Beckman Coulter representative.
The program will not be able to work properly because it failed to access the administrator user settings. Please close the application immediately.	The software failed to find the administrator account.	Reboot the computer, if problem persists contact your local Beckman Coulter representative.
Unable to open '<session default protocol name>' in home directory. \nPlease select a protocol file to copy.	The specified default protocol is not accessible in the expected location.	Select a protocol file to use as the default protocol file.
This protocol contains an obsolete QuadStat region: <region> This region will not be loaded.	Protocol loaded contains an obsolete QuadStat	This protocol contains a QuadStat region from a previous software version and cannot be converted. Recreate the QuadStat region
The QC Product file could not be opened.	The file path is wrong, the name is wrong or the file is corrupt.	Contact your local Beckman Coulter representative.

Table 13.3 Software Messages

Message	Probable Cause	Recommneded Action
The changes to regions have resulted in the following regions having their status as linked regions removed: <<comma separated list of region names>>	User has made changes that have broken the links between regions.	Re-establish the desired links..
The QC Product file could not be opened.	The QC product file could not be opened due to the file not being present in the specified location or the file is invalid.	Contact your local Beckman Coulter representative.
Error writing file.	An error occurred writing the file.	Check hard drive space, access rights and write protection on media.
COleException. SCODE: <<error-code>>.	An error occurred when opening the link to Excel.	Close Excel if it is open and re-try the export.
Error out of memory	System could not allocate memory.	Close down unnecessary applications and reboot the computer. If problem persists contact your local Beckman Coulter representative.
Critical error reading file.	A critical error occurred when reading the file.	Check for hard drive space and access rights.
The Instrument Settings file has been altered. A complete Panel needs to be run before processing samples.	ASI Panel is aborted.	You must rerun the AutoSetup panel before acquiring samples.
The file name association is incomplete or invalid.	The log file is not supported by the viewing application.	Endure *.log files are associated with the Notepad application in order to view the log file.
The log cannot be cleared.	The cyto log file could not be overwritten.	Close down any applications that may be accessing the log file.
The selected file is not a valid file type and cannot be inserted.	User selected an image file of an unsupported format for FlowPage.	Image type must be *.bmp or *.dib.
Error opening a connection to the database.	Database information – connection to database failed	Restart software; if problem persists contact a representative of Beckman Coulter
Could not save this record. specimen_id'<sample ID>'	Database information – failed to save the record when save was clicked	Try again; Restart software; if problem persists contact a representative of Beckman Coulter
This archive was not created with this version of report generator	Trying to restore an archive that was not created with this version of report generator	Select a different archive to restore. See DATABASE MANAGEMENT.

Operating System Error Messages

The Windows Operating system may generate and display error messages. To access help for Windows Operating system error messages, 

.

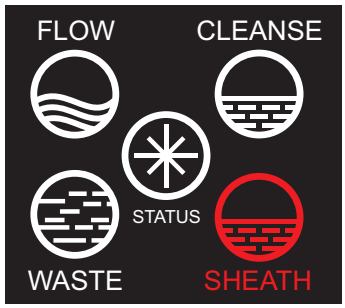
13.7 LEVEL SENSE INDICATORS

Sheath Low

When the **Sheath Low** indicator (see Figure 13.11) appears:

- During sample analysis, you have 5 minutes to finish analyzing the current sample.
- You cannot analyze samples or use the instrument until the sheath fluid container is filled.
- FILL THE INTERNAL SHEATH FLUID CONTAINER.

Figure 13.11 Sheath Low Indicators

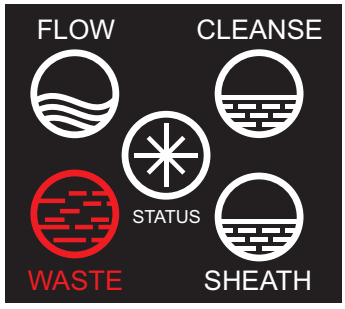


Waste Full

When the **Waste Full** indicator (see Figure 13.12) appears:

- During sample analysis, you have 5 minutes to finish analyzing the current sample.
- You cannot analyze samples or use the instrument until the waste container is emptied.
- EMPTY THE 20 L WASTE CONTAINER.

Figure 13.12 Waste Full Indicators



13.8 CYTOMETER CONTROL WINDOW CANNOT BE VIEWED

If the Cytometer Control window cannot be viewed (it is hidden behind the Windows Taskbar at the bottom of the screen), the Auto hide feature is currently selected. Change the Windows settings to deselect the Auto hide feature and keep the Cytometer Control window in view.

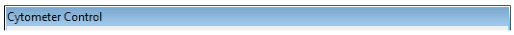
To deselect the Auto hide feature:

1 **Start ▸▸ Control Panel ▸▸ Taskbar & Start Menu**.

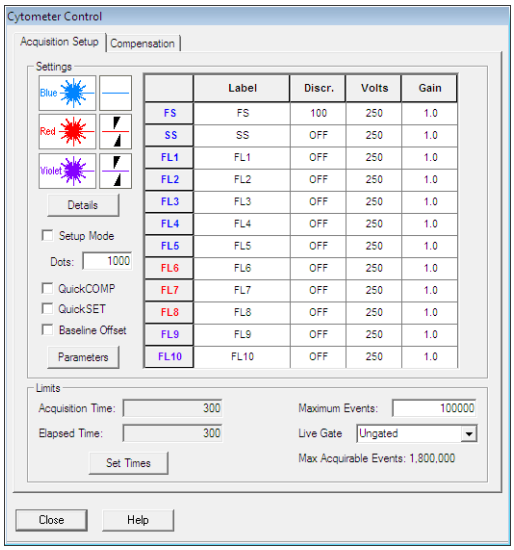
2 On the **Taskbar** tab, **Auto hide** and deselect **Keep the taskbar on top of other windows**.

OK.

3 The Cytometer Control window should now be partially in view.



4 Drag the Cytometer Control window into full view.



A.1 BAR-CODE SAMPLE IDENTIFICATION

Bar-code symbols are a highly accurate and efficient procedure for identifying and processing laboratory samples. Beckman Coulter instruments use four bar-code symbologies (types) to identify specimens:

- Code 128
- Code 39®
- Codabar
- Interleaved 2-of-5.

The bar-code reader senses the difference between enabled bar-code symbologies in a run.

IMPORTANT A misread label can cause one sample ID to be read as another sample ID. The laboratory's process for printing, placing, and meeting all bar-code specifications is important to achieve highly accurate reading. Follow the bar-code specifications to avoid inaccurate reading of the bar-code label.

Figure A.1 Bar-Code Label

1 Quiet zone

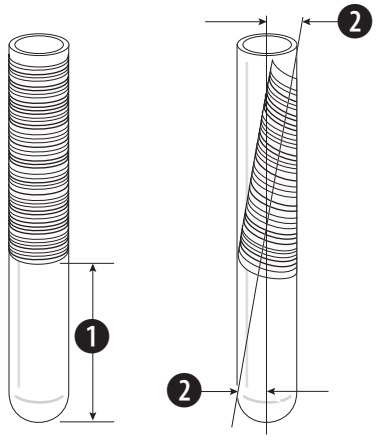
2 Bar-code symbol

3 Sample ID

A.2 CORRECT PLACEMENT OF THE BAR-CODE LABEL

The bar-code label must be placed a minimum of 25.4 mm (1.0 in.) from the bottom of the tube. Refer to Figure A.2.

Figure A.2 Bar-Code Label Placement



1	25.4 mm (1.0 in.) minimum
2	7.5 degrees

Put labels on the tubes so that the bars follow one another in a vertical sequence. Refer to Figure A.2. The bar-code reader scans the tube vertically. Do not tilt the label more than ±7.5 degrees from the axis of the tube.

Put the tubes in the carousel so that the bar-code symbols are visible through the slots in the front of the carousel. When viewed at eye level, the full symbol, including the quiet zones, must be visible through the slot and above the bottom of the carousel.

Note: The Gallios Flow Cytometry System rotates the tube as needed so the bar-code label can be read.

A.3 BAR-CODE LABEL SPECIFICATIONS

IMPORTANT A misread label can cause one sample ID to be read as another sample ID. The laboratory's process for printing, placing, and meeting all bar-code specifications is important to achieve highly accurate reading. Follow the bar-code label specifications to keep the rate of misread labels to a minimum.

The quality of the bar-code symbol and the label is important for accurate reading. For high accuracy, use labels that meet all of the specifications.

When possible, print the sample ID on the label in alphanumeric characters so the operator can manually enter the bar-code information if the bar-code symbol cannot be read.

Label Size and Thickness

The length of the label must be less than 44.45 mm (1.75 in.). The label includes the bar-code symbol and a minimum quiet zone of 3.5 mm (0.14 in.) at each end of the symbol. Refer to Figure A.3.

Figure A.3 Bar-Code Label Specifications

1	Quiet zones 3.5 mm (0.14 in.) minimum
2c	Bar-code symbol height 19.05 mm (0.75 in.) minimum
3	Bar-code label length 44.45 mm (1.75 in.) maximum

The width of the bar-code label must be 5 mm (0.2 in.) less than the circumference of the sample tube.

Label thickness, including adhesive, must be 0.09 mm (0.0036 in.) maximum. Total thickness for all labels and adhesives put together must be 0.36 mm (0.0144 in.) maximum.

Symbol Dimensions

The height of the bar-code symbol must be 19.05 mm (0.75 in.) minimum.

See Table A.2.

Label and Print Quality

All bar-code symbols must agree with the American Identification Manufacturer's (AIM) Uniform Symbology Specification.[1]

All bar-code symbols must be printed at print quality class "B" or better as defined by the American National Standards Institute (ANSI).[2] Several factors affect print quality:

- Labels must be clean, not yellowed, and used before the expiration date.
- Print the bar-code symbol on material that is reflective and has a matte finish. Use a background diffuse reflectance of 80% or more for maximum contrast.
- The labels must not have defects such as spots, lines, missing sections, cuts, folds, or density problems.
- The bars in the bar-code symbol must be well-defined. Edges must be constant (not irregular), so the bars and spaces have the correct widths for the bar-code symbology used.

A.4 BAR-CODE ERROR RATE

IMPORTANT A misread label can cause one sample ID to be read as another sample ID. Whenever possible use a bar-code symbology and configuration choices that provide the most accurate bar-code reading.

The quality of the bar-code symbol and the label is important for accurate reading. To get the highest possible accuracy only use labels that meet all the specifications described for labels and symbols. Deviations from these specifications make the bar code more difficult to read and allow for a possible increase in the error rate.

The symbology and the configurable parameters that the laboratory selects have an effect on the error rate. Certain features of the symbologies and the selections made by the laboratory have an important effect on the accuracy of the bar-code reading system. In general:

- Code 128 and Code 39 are more accurate and have lower error rates than Codabar or Interleaved 2-of-5.
- NCCLS recommends Code 128 because of its accuracy, compact form, and self-checking capabilities.[3]
- A checksum greatly increases accuracy. Use a checksum with Interleaved 2-of-5 and Codabar because they are less accurate symbologies.
- Select the fixed length option, if available, because it is more accurate than the variable length option.
- To keep label and printing flaws to a minimum, use a narrow element of more than 0.25 mm (0.010 in.).

Beckman Coulter recommends the use of:

- **Code 128**
- **Checksum for all other symbologies**
- **Fixed length code symbols**
- **Narrow bar sizes of 0.25 mm (0.010 in.) minimum.**

A.5 BAR-CODE SYMBOLOGIES

Beckman Coulter instruments use four bar-code symbologies for specimen identification, see Table A.1.Within the given specifications, the MCL reader and the optional handheld bar-code reader automatically distinguish the following bar codes:

Table A.1 Bar-Code Symbologies

Bar-Code Type	Description
Code 128 (also known as USD-6)	• Variable length • Alphanumerics; 107 character set • Self-checking • Continuous code; intercharacter space is part of code structure for higher density of code per square inch; compact bar code • Code 128 is recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for its accuracy, compact form, and self-checking capabilities[3] • Code 128B - Maximum 8 alphanumeric characters • Code 128C - Maximum 16 numeric characters (The use of 15 numeric characters is invalid)
Code 39 (also known as 3-of-9 and USD-3)	• Variable length • Includes 43 data characters; 26 letters (uppercase A-Z), 10 digits (0-9), six symbols (. $ / + % -) and a space • Strong self-checking properties • Checksum • Discrete code; white spaces are not part of this code • Maximum 7 characters (6 data characters + 1 checksum character).
Interleaved 2-of-5 (also known as I2 of 5, USD-1, and USD-1.25)	• Numerics only • Checksum • Lower density of code per square inch; longer label • Requires an even number of digits to be encoded, a leading “0” must be added if the number count is odd • Fixed 14 characters (13 data characters + 1 checksum character).
Codabar (also known as USD-4 and NW7)	• Variable length • Includes 16 data characters; 10 digits (0-9), and six symbols (. $ / + % -) • Has specific start and stop characters which lead to improvement in readability • Checksum • Lower density of code per square inch; longer bar code • Maximum 10 characters (9 data characters + 1 checksum character).

A.6 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, poor quality, damaged, dirty or improperly placed bar-code labels. Follow the specifications in this section to create your bar-code labels to prevent incorrect sample identification. See also Putting a Bar-Code Label on a Sample Tube.

The instrument supports preprinted labels.

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 A.2 Code-Related Specifications

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

* See AIM Uniform Symbology Specification, Rev. 1995 for detailed specification.
** Includes checksum character

A.7 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.

A.8 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 identification of sample tubes, do not use FNC1, FNC4, and FS (hexadecimal 1C) characters in your bar-code information.

A.9 CHECKSUM ALGORITHM

Beckman Coulter strongly recommends the use of bar code checksums 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 checksum digits, maximize accuracy in reading Codabar, Code 39 and Interleaved 2-of-5 labels. In one study, the use of checksum 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 checksums, Beckman Coulter recommends that you verify each bar-code reading to assure correct patient identification.

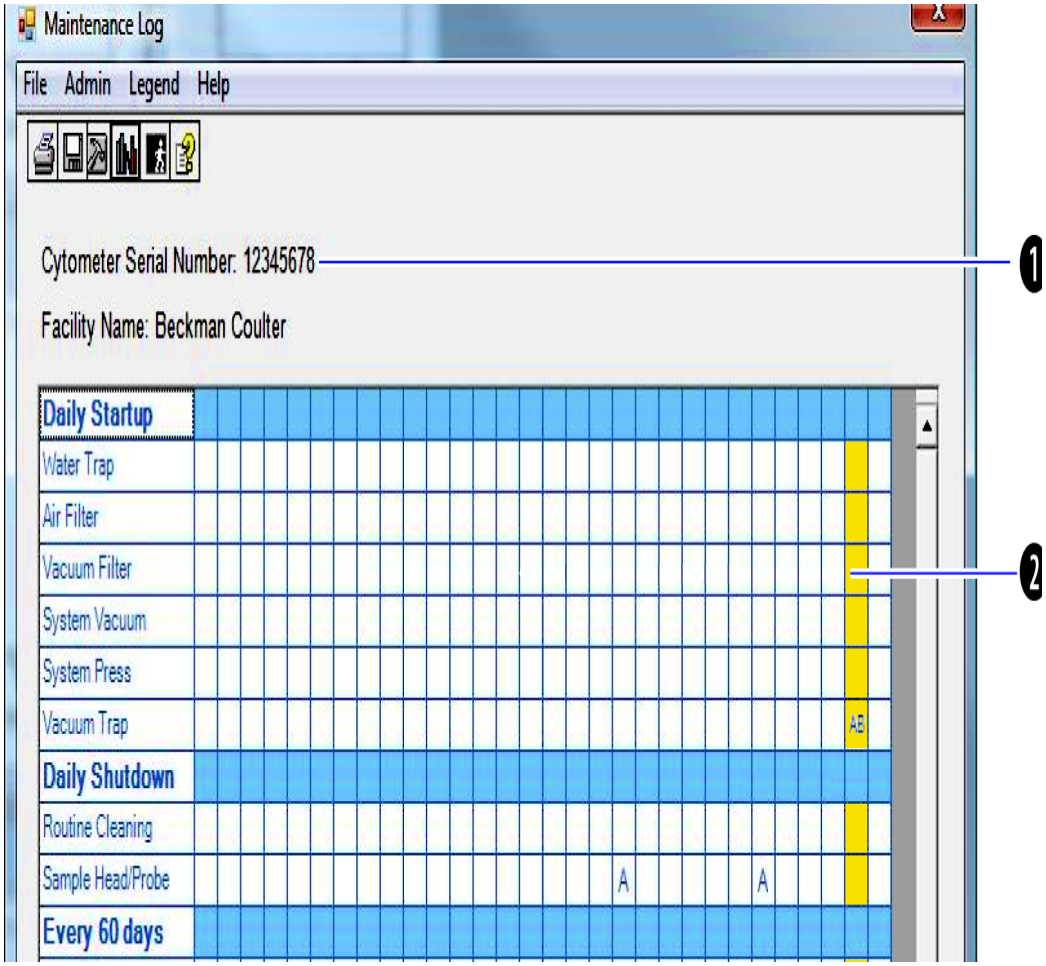
B.1 INTRODUCTION

The Report Generator contains two log screens you can use to record maintenance and service activities. Use the MAINTENANCE LOG to record daily and periodic maintenance. Use the SERVICE LOG to record service conditions noted and actions taken.

B.2 MAINTENANCE LOG

The Maintenance Log lists actions that need to be performed and how often they are needed.

If you switch to Larger scale (120 DPI) font settings in **Control Panel ▸▸ Personalization**, the columns on the Maintenance Log screen become mis-aligned and truncated.



1 The instrument serial number and facility name are displayed at the top.

2 The boxes corresponding to the current day's date are highlighted. These are the only ones that may be selected by a user.

3 The dates are shown across the bottom. The most recent month is shown by default.

4 Arrow buttons on the bottom line can be used to show other months.

Menu Options

The following menu items are available.

File Menu

- **Print -** Prints the Maintenance Log of the current month.  performs the same function.
- **Save -** Saves a new entry on the Maintenance Log.  performs the same function.
- **Exit -** Exits the Maintenance Log.  performs the same function.

Admin

Prompts for the Gallios Administrator password.  performs the same function.

Legend

Displays a legend that shows the list of user names and the corresponding color used for the visual display.  performs the same function.

Help

Displays the Maintenance Log help.  performs the same function.

Using The Maintenance Log

The system automatically assigns letter "A" to the Administrator and assigns a unique color and letter to all other users. The color assignment is used for visual display and the letter assignment is used for printing. The letter legend is printed with the log. The pre-assigned letter "A" for Administrator is used for both the visual display and the printout.

User Entry

Double-click on a box for the current date to indicate that you have performed the specified action.

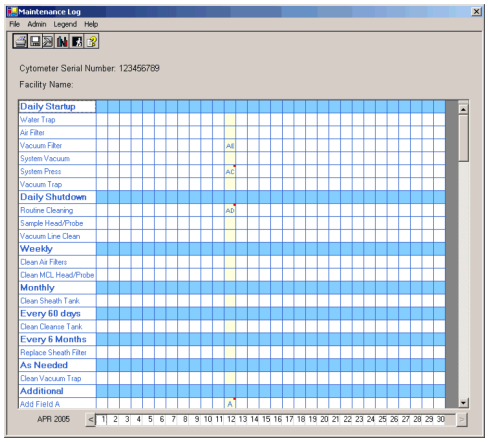
You can erase your own entry for the current date by clicking on the box again. You cannot change entries for other users or previous dates.

To enter a comment on any of the boxes, right-click on the box and select **Insert Comment**.

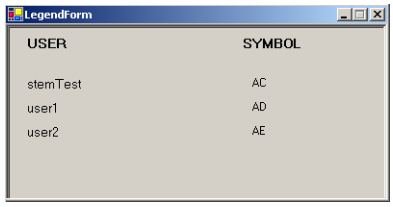
The presence of a comment in a box is indicated by a small red square in the upper right-hand corner of the box and is visible on the printout. The Gallios User ID precedes the comment.

■ on the Maintenance Log indicates there is a comment associated with this entry.

* on a printout indicates there is a comment associated with this entry. Comments print on separate pages along with a legend (shown below), which identifies the users by an automatically assigned two-character symbol rather than color.



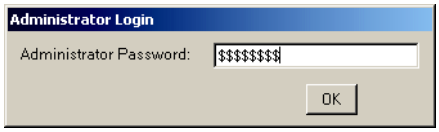
You can view the legend on the screen and print if desired.



Administrator Entry

To log in, and enter the Gallios Admin password.

Admin will log out when you exit the screen.



on any empty box and the letter A is displayed in the box.

Right click on any box and **Delete** to erase it and a hyphen '-' is displayed in the box.

To enter a comment on any of the boxes, right-click on the box and select **Insert Comment**. If the box already contains a comment and the Administrator adds a comment, the previous comment is replaced and the letter A precedes the comment however, the original user color designation remains in the box.

The Administrator can add a task to the bottom of the fixed list by clicking on one of the empty rows. The task is added to the section starting with the current month. A total number of 5 additional task rows are available.

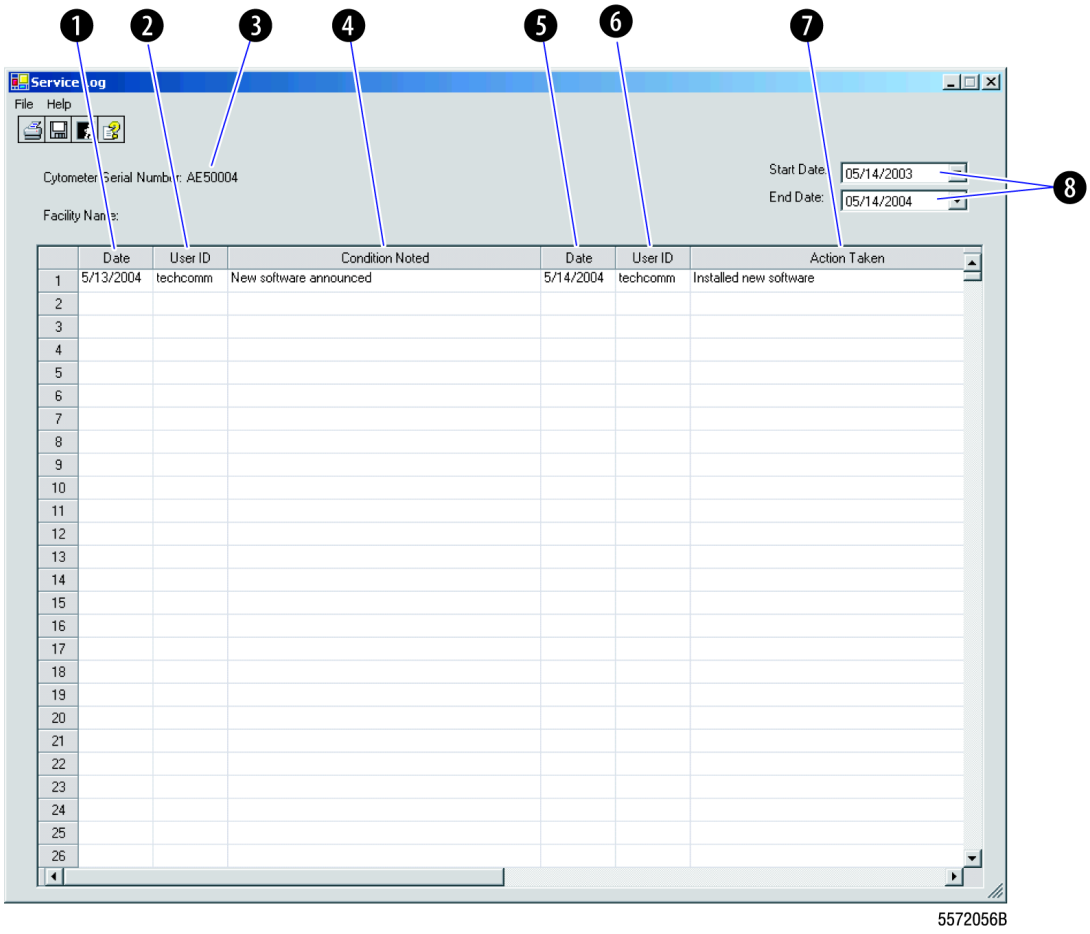
The Administrator can edit or delete any task that was added. Default (system-defined) tasks may not be edited or deleted.

To edit an added task, on the task and make the desired changes.

To delete an added task,  on the task and press Delete.

The task is deleted from the current month going forward. A task may not be deleted if there are entries for it in the current month.

B.3 SERVICE LOG



1 Date that the Condition Noted entry was made.

2 User who made the entry. This field is non-editable and it is filled in automatically by the system when the Date is entered.

3 The instrument serial number and facility name are displayed at the top.

4 Enter text (up to 255 characters) to describe the condition noted.

5 The second Date applies to the Action Taken entry and tracks the date of the action.

6 Applies to the User who made the Action Taken entry. This field is non-editable and it is filled in automatically by the system when the Date is entered.

7 Enter text (up to 255 characters) to describe the action taken.

8 The Start and End Dates are used for visual display - the log will automatically scroll to the time period selected.

Menu Options

The following menu items are available.

File Menu

- **Print -** Prints the Service Log of the current month.  performs the same function.

  Only the selected range of the service log prints when you choose **File** ▸▸ **Print** or  .

  Note: Select a date range that has < 500 entries when printing.

- **Save -** Saves a new entry on the Service Log.  performs the same function.

- **Exit** - Exits the Service Log.  performs the same function.

Help

Display the Service Log help.  performs the same function.

Using The Service Log

The Service Log is a scrollable grid with entries in chronological order.

Enter Condition Noted

1. Double-click the Date column and select the date on the calendar corresponding to the condition noted.

2. Double-click the Condition Noted field and type the text describing the condition noted.

3.  to save the Service Log.

Enter Action taken

1 Double-click the Date column and select the date on the calendar corresponding to the action taken.

2 Double-click the Action Taken field and type the text describing the action taken.

3  to save the Service Log.

Start Date: and End Date:

Use these fields to display the entries between Start Date and the End Date. The log displays the time period selected. The dates are based on Condition Noted dates only.

REFERENCES

1. American Identification Manufacturer's group (AIM), U*niform Symbology Specifications Code 39, Interleaved 2 of 5, Codabar,* and *International Symbology Specifications Code 128.* ANSI/AIM BC1, BC2, BC3, BC4, 1995. http://www.aimusa.org
2. American National Standards Institute (ANSI) *Bar Code Print Quality Guidelines.* X3. 182-1990 (R2000). http://www.ansi.org
3. Clinical and Laboratory Standards Institute (CLSI), *Laboratory Automation: Bar Codes for Specimen Container Identification; Approved Standard.* AUTO2-A. http://www.clsi.org

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

APC - Abbreviation for allophycocyanin dye.

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.

AutoGate - A gate that automatically sizes to the population contained within the boundaries of the region. There are two types: Elliptical and Contour.

AutoSetup Wizard - A software feature that guides you through the processing of quality control materials to automatically establish your application settings.

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

Bar - A strip (or element) that is usually black and has minimum reflectance.

Bar-code symbol - A group of parallel bars and spaces with encoded characters. A bar-code symbol generally contains a leading quiet zone, a start character, data characters, a check character, a stop character, and a trailing quiet zone.

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

Button - The Workstation screens have pictures/icons (for example, ) that you select with the mouse to tell the software what to do. They are arranged on Toolbars for related functions.

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

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

Character - The smallest group of elements that makes a number, letter, or punctuation mark.

Check character (digit, check digit, checksum) - A character used to mathematically check that the bar-code symbol was read correctly.

Cleaning agent - A detergent used to flush sample from tubing and minimize 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.

Continuous code - Each character in the bar-code symbol starts with a black bar and ends with a white space. Characters follow after each other to form a continuous flow of code.

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.

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.

Cytosettings - Cytometer hardware settings. Consists of acquisition duration, (Elapsed/Acquisition Time) acquisition maximum events, gating settings, disciminators, voltages, gains, baseline offset and compensation settings. Same as Instrument Settings in Acquisition Manager.

db - Abbreviation for decibels.

dc - Abbreviation for direct current.

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

Digit - See checksum.

DiOC5(3) - Abbreviation for oxacarbocyanine dye.

Discrete code - Each character in the bar-code symbol starts with a black bar and ends with a black bar. A white space gap (intercharacter gap) is between each character in the bar-code symbol.

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

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

Element - A bar or space in a bar-code symbol. There are narrow elements and wide elements.

Event - A particle passing through the laser beam.

Export file (*.XLS or *.TXT) - File containing selectable statistics and other sample information from each sample run.

Export Panel - A panel that exports results to the Report Generator to create a Panel Repor.t

FDA - Abbreviation for fluorescein diacetate dye.

FITC - Abbreviation for fluorescein isothiocyanate dye.

Fixed code length - A specific length of sample ID code, (usually enabled when all sample IDs are the same length) to make sure that only one length sample ID is accepted.

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.

Flow Cytometry Standard (FCS) - The flow cytometry data file standard that provides the specifications needed to completely describe flow cytometry data sets within the confines of the file containing the data.

FlowPAGE - A template for creating a report. It can include: data plots, statistics, and user attached text and/or graphics.

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, FL5, FL6, FL7, FL8, FL9 and FL10) sensors (PMTs) - 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.

HPCV - Half peak coefficient of variation

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.

Indicators - See Controls and Indicators.

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

Intercharacter gap - The space between two characters in a bar-code symbol. Refer to discrete code. Not in all bar-code types.

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. Three standard lasers are in the instrument: one in the MCL for reading bar codes and two in the flow cell for analyzing cells.

Linear amplification - See gain.

Listmode data - A list of measurements from each cell.

Listmode playback tool - A tool used: 1) to replay 20-bit linear listmode data though new compensation settings, 2) for panel playback of listmode files, and 3) to replay AutoSetup listmode files to generate a new compensation file.

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

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 automated sample loader for the instrument.

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.

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.

Panel - In general, a group of protocols for analyzing a series of samples corresponding to one specimen. The Cytometer settings and regions can be passed on through the panel with identification of the primary samples.

Panel Wizard - A software feature that guides you through creating or editing a panel.

Panel Report - Patient data reports that are generated by the Report Generator database.

PC7 - **Abbreviation for phycoerythrin-cyanine tandem dye.**

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.

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.

Pneumatic 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 plot 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 replay listmode data.

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

QuickCOMP - Direct histogram manipulation to adjust Cytometer color compensation.

QuickSET - Direct histogram manipulation to adjust Cytometer voltages and gain.

Quiet zone - An area at each end of the bar-code symbol, which must be clear of marks, including readable text.

RD1 - Abbreviation for phycoerythrin dye..

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

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 Resource Explorer 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.

Self-checking - A bar code that uses a checking algorithm to make sure the bar-code symbol was read correctly.

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.

Slider bars - They appear on dual fluorescence plots when you enable QuickCOMP mode on the Cytometer Control dialog box. These sliders are used to intuitively adjust the compensation coefficients and update the compensation values in the Cytometer Control Compensation Tab.

Space - A strip (or element) that is usually white and has maximum reflectance.

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.

Standard Panel - A panel that does not export results to the Report Generator.

Start and Stop characters - The characters that start and end the bar-code symbol and show the scan direction.

Symbology - A set of rules for encoding and decoding information contained in a bar-code symbol. Examples of symbologies include Code 39, Code 128, Interleaved 2-of-5, and Codabar.

TrueView - TrueView is a patented tool which provides the ability to optimally view compensated data. Using TrueView, you can display all 1,048,576 channels of data acquisition as well as graphically display negative values in order to facilitate compensation. See also TrueView Setup.

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.

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

Symbols

Numerics

A

B

C

D

E

F

G

H

K

L

M

N

O

P

Q

R

S

T

U

V

W

Z

BECKMAN COULTER, INC. CUSTOMER END USER LICENSE AGREEMENT

This Product contains software that is owned by Beckman Coulter, Inc. or its suppliers and is protected by United States and international copyright laws and international trade provisions. You must treat the software contained in this Product like any other copyrighted material. This license and your right to use the Product terminate automatically if you violate any part of this agreement.

This is a license agreement and not an agreement for sale. Beckman Coulter hereby licenses this Software to you under the following terms and conditions:

You May:

1. Use this software in the computer supplied to you by Beckman Coulter;
2. Maintain one copy of this software for backup purposes (the backup copy shall be supplied by Beckman Coulter);
3. After written notification to Beckman Coulter, transfer the entire Product to another person or entity, provided you retain no copies of the Product software and the transferee agrees to the terms of this license agreement.

You May Not:

1. Use, copy or transfer copies of this Software except as provided in this license agreement;
2. Alter, merge, modify or adapt this Software in any way including disassembling or decompiling;
3. Loan, rent, lease, or sublicense this Software or any copy.

Limited Warranty

Beckman Coulter warrants that the software will substantially conform to the published specifications for the Product in which it is contained, provided that it is used on the computer hardware and in the operating system environment for which it was designed. Should the media on which your software arrives prove defective, Beckman Coulter will replace said media free of charge within 90 days of delivery of the Product. This is your sole remedy for any breech of warranty for this software.

Except as specifically noted above, Beckman Coulter makes no warranty or representation, either expressed or implied, with respect to this software or its documentation including quality, performance, merchantability, or fitness for a particular purpose.

No Liability for Consequential Damages

In no event shall Beckman Coulter or its suppliers be liable for any damages whatsoever (including, without limitation, damages for loss of profits, business interruption, loss of information, or other pecuniary loss) arising out of the use of or inability to use the Beckman Coulter Product software. Because some states do not allow the exclusion or limitation of liability for consequential damages, the above limitation might not apply to you.

General

This agreement constitutes the entire agreement between you and Beckman Coulter and supersedes any prior agreement concerning this Product software. It shall not be modified except by written agreement dated subsequent to the date of this agreement signed by an authorized Beckman Coulter representative. Beckman Coulter is not bound by any provision of any purchase order, receipt, acceptance, confirmation, correspondence, or otherwise, unless Beckman Coulter specifically agrees to the provision in writing. This agreement is governed by the laws of the State of California.

BECKMAN COULTER

TRADEMARKS

ALTRA, BECKMAN COULTER logo, COULTER, CYTO-COMP, CYTO-TROL, Elite, EPICS, FlowCentre, Flow-Check Pro, Flow-Count, Flow-Set Pro, Gallios, Immuno-Brite, ImmunoPrep, Immuno-Trol, IsoFlow, Multi-Q-Prep, Q-Prep, TQ-Prep, XL, and XL-MCL are trademarks of Beckman Coulter, Inc.

All other trademarks, service marks, products, or services are trademarks or registered trademarks of their respective holders.

Gallios Flow Cytometer System Documentation

- Instructions For Use
  PN 773231

Introduction • Use and Function • Installation • Operation Principles • Specifications • System Overview • Daily Routine • Quality Control • Running Samples • How to... • Using Gallios Software • Cleaning Procedures • Replace Adjust Procedures • Troubleshooting • Bar-Code Specifications • Maintenance Log • References • Glossary • Index

Come visit us at www.beckmancoulter.com

EC REP
Beckman Coulter Ireland, Inc.
Mervue Business Park,
Mervue Galway, Ireland 353 91 774068

Printed on Recycled Paper

BECKMAN COULTER