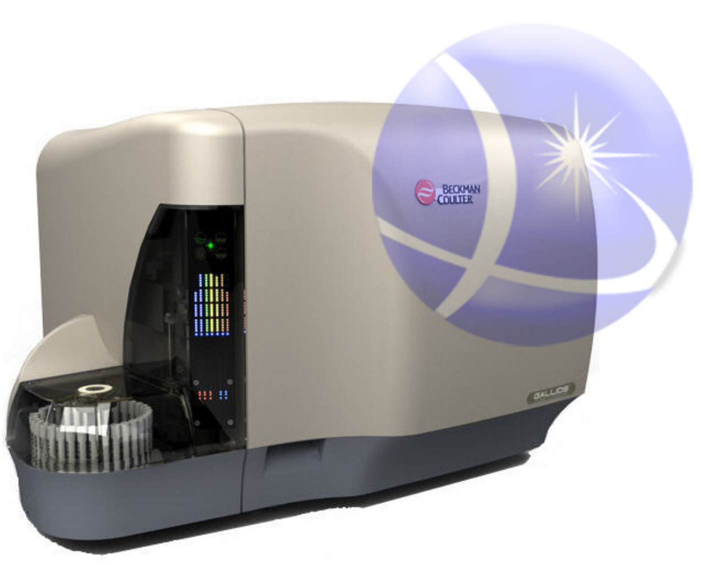
Instructions For Use

Gallios Flow Cytometer

with Kaluza for Gallios Software



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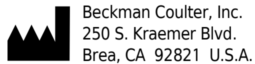
January 2014



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

Kaluza for Gallios Instructions for Use
B25062AA (January 2014)

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Revision History

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.

Initial Issue, 1/14
Kaluza for Gallios, version 1.0

Safety Notice

Overview

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate the 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.

Beckman Coulter, Inc. urges its customers and employees to comply with all national health and safety standards such as the use of barrier protection. This may include, but is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory instrumentation.

If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the equipment may be impaired.

Alerts for Danger, Warning, Caution, Important, and Note

All Warnings and Cautions in this document include an exclamation point, framed within a triangle.

The exclamation point symbol is an international symbol which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing are attempted.

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Instrument Safety Precautions

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.**
- **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.**
- **Use the proper tools when troubleshooting.**

System integrity could be compromised and operational failures could 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.**

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, contact your Beckman Coulter Representative.

Electrical Safety

To prevent electrical-related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a Beckman Coulter Representative for any servicing of equipment requiring the removal of covers or panels.

High Voltage

This symbol indicates the potential of an electrical shock hazard existing from a high-voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) outlets.

Laser Light

This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Laser Specifications

- **Laser Types:**

  — Class II Laser Product
  — Class 3B Laser Product
  — Class 3B Laser Product (EN60825-1)
  — Class 1 Laser Product
  — Class Nlb Laser Product (CDRH)

- **Maximum Output:** <500 mW (red and violet), <100mW (blue), 1.0 mW (barcode reader)
- **Wavelength:** 633-643nm (red), 400-410nm (violet), 488nm (blue), and 670nm (barcode reader)

NOTE See CHAPTER 15, *Troubleshooting* for additional laser-specific information.

Disposal of Electronic Equipment

It is important to 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 devices.

Chemical and Biological Safety

If a hazardous substance such as blood is spilled onto the instrument, clean up the spill by using a 10% bleach solution, or use your laboratory decontamination solution. Then follow your laboratory

procedure for disposal of hazardous materials. If the instrument needs to be decontaminated, contact your Beckman Coulter Representative.

Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

WARNING

Normal operation of the instrument might involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- **Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.**
- **Observe all cautionary information printed on the original solutions' containers prior to their use.**
- **Dispose of all waste solutions according to your facility's waste disposal procedures.**
- **Operate the instrument in accordance with the instructions outlined in this manual and take all the necessary precautions when using pathological, toxic, or radioactive materials.**
- **Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.**
- **Use an appropriately-contained environment when using hazardous materials.**
- **Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.**
- **Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.**

NOTE Observe all warnings and cautions listed for any external devices attached or used during operation of the instrument. Refer to applicable external device user's manuals for operating procedures of that device.

NOTE For Safety Data Sheets (SDS/MSDS) information, go to the Beckman Coulter website at **www.beckmancoulter.com**.

Moving Parts

Risk of personal injury. To avoid injury due to moving parts, observe the following:

- **Never attempt to exchange labware, reagents, or tools while the instrument is operating.**
- **Never attempt to physically restrict any of the moving components of the instrument.**
- **Keep the instrument work area clear to prevent obstruction of the movement.**

Cleaning

Observe the cleaning procedures outlined in this user's manual for the instrument. Prior to cleaning equipment that has been exposed to hazardous material, contact the appropriate Chemical and Biological Safety personnel.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

IMPORTANT It is your responsibility to decontaminate components of the instrument before requesting service by a Beckman Coulter Representative or returning parts to Beckman Coulter for repair. Beckman Coulter will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

RoHS Notice

These labels and materials declaration table (the Table of Hazardous Substances Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

China RoHS Caution Label

This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

China RoHS Environmental Label

This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center "e" indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

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Introduction

Overview

This manual contains the information you need to:

- Run the Gallios Flow Cytometer.
- Use Kaluza for Gallios Software.
- Review the instrument specifications.
- Understand operation principles and methods.
- Clean, adjust, and replace components.

Conventions Used in This Manual

The system help uses the conventions described in Table 1:

Table 1 Conventions Used in This Manual

Convention	Supplemental Information
Blue text	Clicking on the blue text allows you to access related information.
Bold font	Indicates a software option, such as **Acquire**.
Italicized 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 the two keys listed, such as (Ctrl) + (S), are linked for a specific function and must be pressed in this sequence: **1.** Press down on the first key listed and, while continuing to press it, press down on the second key listed. **2.** Release both keys at the same time.
( ) ( )	Indicates pressing and releasing the first key listed, and then press and release the next key listed. ***For example***: (Y) (Enter)

Table 1 Conventions Used in This Manual

Convention	Supplemental Information
Icons and Buttons	Indicates selecting functions on the software screen as shown within text. ***For example***: Select Clear worklist.
NOTE	Indicates information that is important to remember or helpful in performing a procedure.
“screen” and “window’	These two terms are used interchangeably.
“Gallios Flow Cytometry System”	This term is referred to as “system” or “instrument” throughout this manual.

Description of Reporting Units

Reporting units for absolute count when using a CAL Factor are cells/µL. A CAL Factor is entered in the Parameter Description field of the Worklist (see CHAPTER 8, *Worklist Pane*).

Graphics

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

CHAPTER 1

Use and Function

Introduction

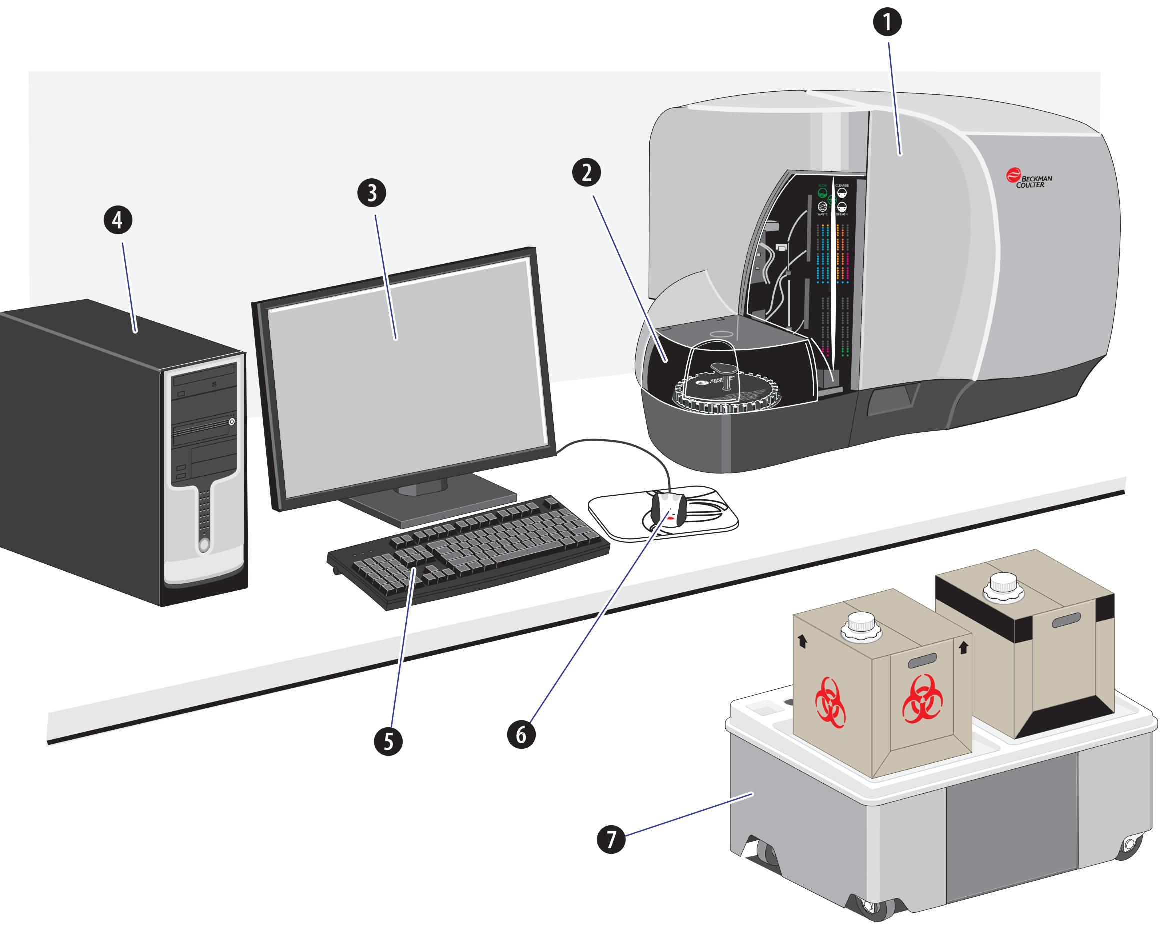
This chapter covers information on the following topics:

- *System Components*: An overview of the system components and their intended use.
- *Options*: A description of optional equipment that can be used with your Gallios Flow Cytometry System.
- *Reagents and Quality Control Materials*: An overview of the reagents and quality control materials recommended by Beckman Coulter.
- *Safety Data Sheets (SDS/MSDS)*: Instructions for obtaining an SDS/MSDS for Beckman Coulter reagents used on the Gallios Flow Cytometry System.

System Components

The system components are shown in Figure 1.1.

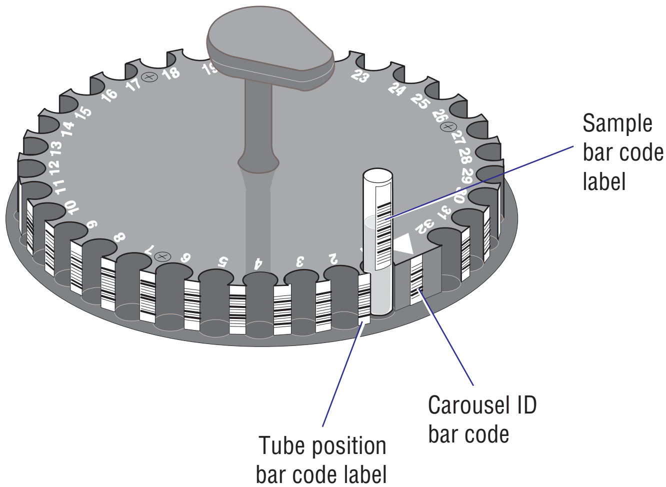
Figure 1.1 Gallios Flow Cytometer System



Item	Description
1. Cytometer	This unit analyzes samples. It contains internal sheath fluid and cleaning agent containers.
2. Multi-Tube Carousel Loader (MCL)	The multi-tube carousel loader (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 barcode types: • Codabar • Code 39 barcode • Code 128 • Interleaved 2-of-5. For additional information on the barcode specifications, see APPENDIX A, Barcode Specifications. The MCL mixes each sample before analysis. You can use the MCL to automatically analyze multiple samples or analyze single samples. During an acquisition, samples can be accessed through the tube access door, by using the **Access Sample** feature (see CHAPTER 12, *Accessing the Sample* for details). Figure 1.2 shows the location of the carousel number, tube position, and sample tube barcode labels on the MCL carousel.

Item	Description
3. Monitor	The monitor, computer, keyboard, and mouse are part of the Gallios workstation. The computer runs: • **Kaluza for Gallios Software**, which controls the instrument and displaying sample results in real time. • **Kaluza Analysis Software**, for data analysis.
4. Computer	
5. Keyboard	
6. Mouse	
7. Pneumatic Supply	This unit provides pressure and vacuum to the Cytometer. See the *Gallios Flow Cytometer Supply Cart Addendum* (P/N A85285) for additional information.

Figure 1.2 MCL Carousel Barcode Labels



Options

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 is available for your Gallios Flow Cytometry System.

Fourth Laser

An optional 561 nm laser is available for your 6-, 8- or 10-color Gallios Flow Cytometry System configurations.

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, 561-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 liquid-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:

Material	Used for...
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.
CYTO-COMP Cell Kit	CYTO-COMP Cells stained with a single color are used to adjust color 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 on a flow cytometer.
QuickCOMP 4 Kit	Four single-color antibody reagents (FITC, PE, ECD, and PC5) that can be used to adjust color on a flow cytometer.
VersaComp Antibody Capture Beads	Positive and negative microspheres that can be used to set multicolor flow cytometry experiments.

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

Safety Data Sheets (SDS/MSDS)

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

1. On the internet, go to http://www.beckmancoulter.com and select **Safety Data Sheets (SDS/MSDS)** from the **Support** 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.

CHAPTER 2

Installation

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.

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.

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	Measurements
Height	60.5 cm (23.8 in.)
Additional clearance above for servicing	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	3.8 cm (1.5 in.)
Total clearance needed	73.9 cm (29.1 in.)

Electrical Input

The Supply Cart/Pneumatic Supply requires one dedicated outlet with an isolated ground. 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

Temperature fluctuations within the ambient temperature range can affect performance. For optimal performance, Beckman Coulter recommends the ambient room temperature is maintained between 15.5°C and 32°C (60°F to 90°F) and does not fluctuate more than +/-2.8°C (+/-5°F) from the ambient temperature at alignment. Keep the humidity between 30% and 85%, 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.

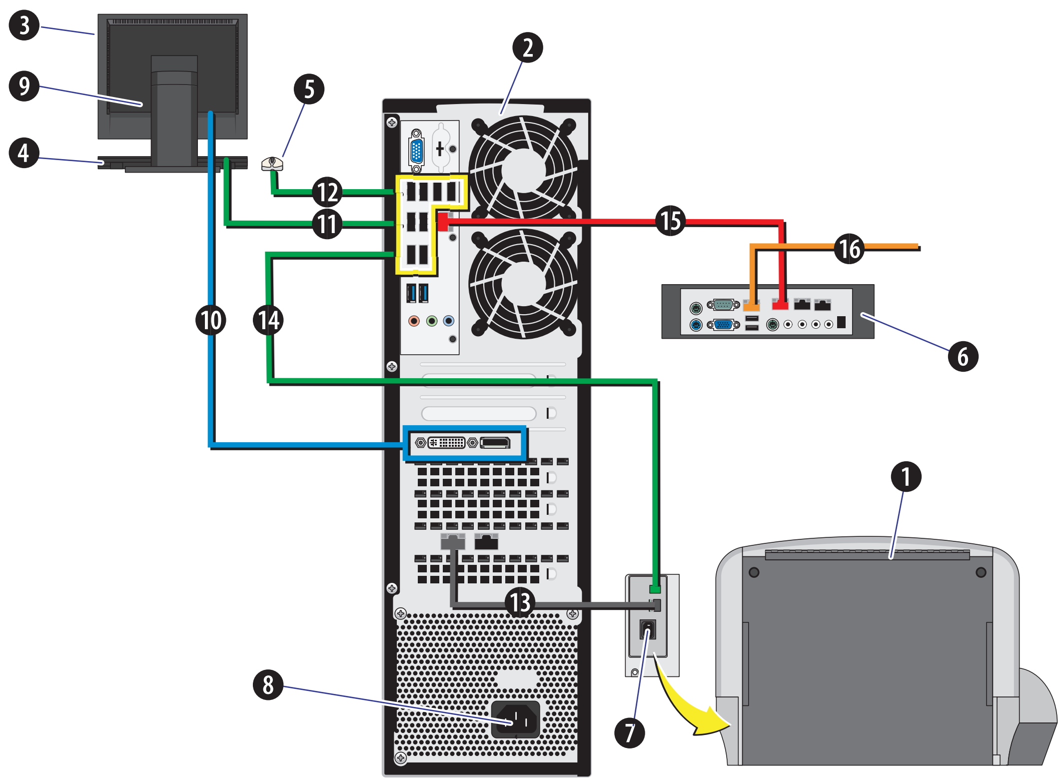
Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of 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.

System Connections

Interunit connections are shown in Figure 2.1.

Figure 2.1 Cable Connections



1. Instrument
2. Kaluza for Gallios Workstation
3. Monitor
4. Keyboard
5. Mouse
6. RAP Box
7. Instrument Power Supply
8. Kaluza for Gallios Workstation Power Supply
9. Monitor Power Supply
10. Monitor-to-Workstation Connection (DisplayPort Cable)
11. Keyboard-to-Workstation Connection (USB Cable)
12. Mouse-to-Workstation Connection (USB Cable)
13. Instrument-to-Workstation Connection (Network Cable)
14. Instrument-to-Workstation Connection (USB Cable)
15. RAP Box-to-Workstation Connection (Network Cable)
16. System-to Local Network Connection (Network Cable)

Reconnecting the Instrument to the Kaluza for Gallios Workstation

If you need to reconnect your instrument to the Kaluza for Gallios workstation, perform the following steps:

1. Connect the instrument power supply (7) to a power source, but do not power it on.

2. Connect the workstation power supply (8) to a power source.

3. Connect the workstation monitor power supply (9) to a power source.

4. Connect the cables to the Kaluza for Gallios workstation according to Figure 2.1. Specifically, ensure the following are connected:
   - Monitor to Kaluza for Gallios Workstation (10)
   - Keyboard to Kaluza for Gallios Workstation (11)
   - Mouse to Kaluza for Gallios Workstation (12)

   IMPORTANT The network cables mentioned below can be plugged into any of the ethernet ports. Following Step 11 in *Reinstalling Kaluza for Gallios* configures these ports.

   - Instrument to Kaluza for Gallios workstation, which includes both a network cable (13) and a USB cable (14) connecting the flow cytometer to the Kaluza for Gallios workstation. The instrument must be off during this process, but keep it connected to a power source.
   - ***Optional***: RAP Box to the workstation using a network cable (15)
   - ***Optional***: Network cable to the local network (16)

   NOTE If you need a VGA port for video, a DVI to VGA converter is included.

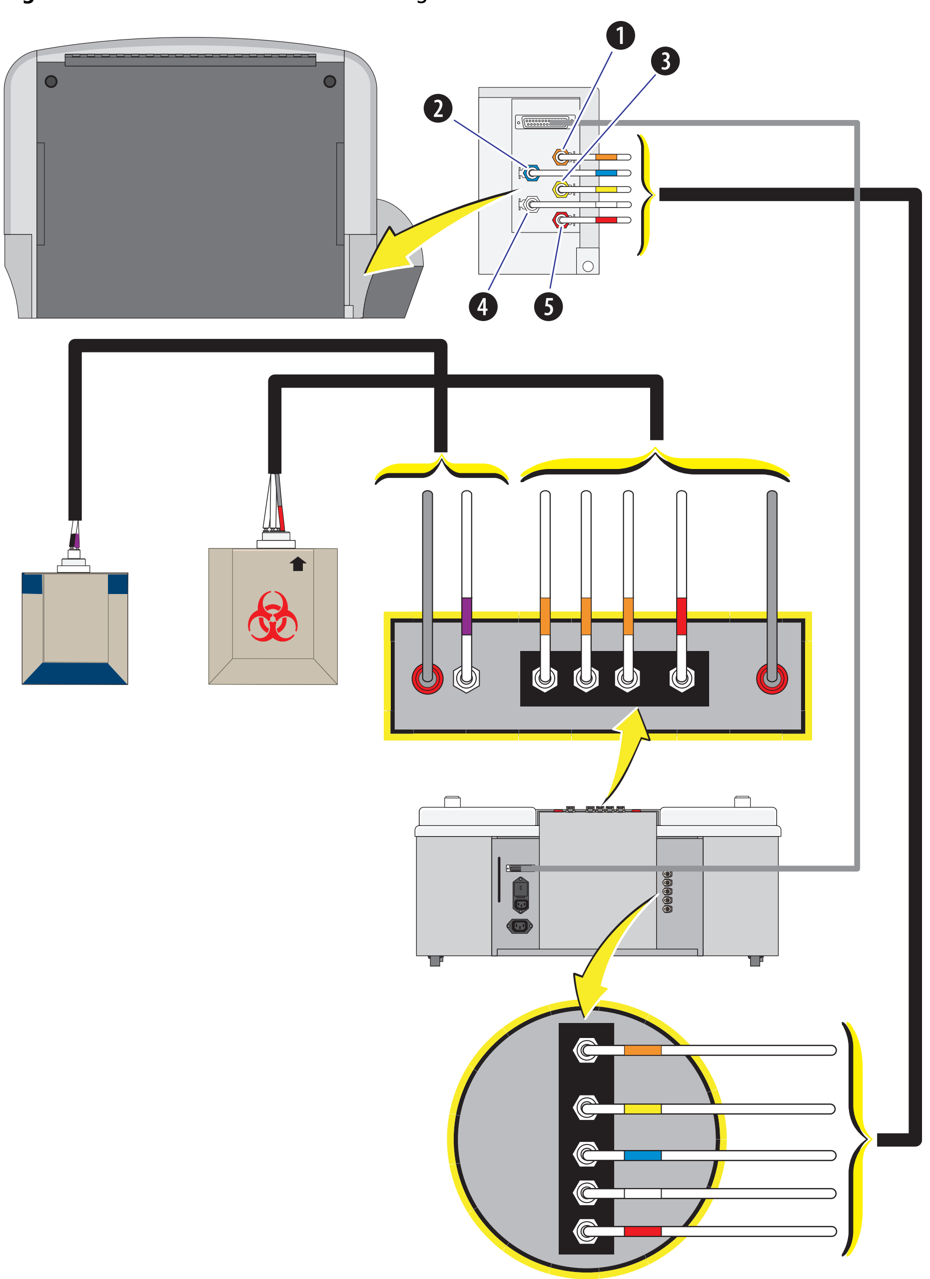
5. Power up the Kaluza for Gallios workstation and log in.

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



1. Vent (orange)
2. Air (blue)
3. Vac (yellow)
4. Sheath (white)
5. Waste (red)

Software Installation

Kaluza for Gallios and *Offline* Kaluza for Gallios have different system requirements and installation procedures. For complete instructions, refer to the appropriate section below for the type of software that you need to install:

- *Reinstalling Kaluza for Gallios*
- *Installing Offline Kaluza for Gallios*

Reinstalling Kaluza for Gallios

Table 2.1 contains the standards specified for the Kaluza for Gallios workstation.

Table 2.1 Kaluza for Gallios System Standards

Component	Standard
Operating System	Windows 7, 64-bit, with Service Pack 1
Processor	The processor must support SSE2 Instruction Set.
Monitor	The layout of the main workspace is optimized for high-resolution wide screen monitors; however, the software can function with a resolution as low as 1440 x 900.

To install Kaluza for Gallios:

NOTE Installing Kaluza for Gallios on your computer requires an Administrator account. Standard accounts can be used for normal operation of Kaluza for Gallios once the software is installed.

IMPORTANT Do not install *Offline* Kaluza for Gallios on the Kaluza for Gallios workstation.

1. After powering up and logging into the Kaluza for Gallios workstation (as Administrator), insert the Kaluza for Gallios installation disc into the DVD drive.

   NOTE If Kaluza for Gallios had already been installed on the workstation, the current version of Kaluza for Gallios will automatically be removed during this installation process. Uninstalling Kaluza for Gallios **does not** remove any of your data files, Protocols, or Worklists.

2. Double click the **Computer** icon on the desktop.

3. Double click the DVD drive containing the installation disc.

4. Locate **Kaluza for Gallios Setup.exe** and double-click to launch the installation process.

5. Acknowledge any warnings that appear, allowing changes to be made to the computer.

6 Once all warnings have been acknowledged, the **Online, Kaluza for Gallios Installer** window appears. As demonstrated in the installer window, the application is dependent upon successful installation of the other prerequisites, which are included on your Kaluza for Gallios installation disc. If any of the prerequisites have not already been installed on your computer, an appears next to the name of each prerequisite that needs to be installed. Install each pending prerequisite, **one at a time**, by selecting the **Install** link, and then following all prompts and accepting all default settings for each pending installation.

NOTE You might be prompted to install a device driver while installing CytoSystem. If this security prompt appears, install this device.

When a prerequisite has been successfully installed, a will display next to the product name. In the example in Figure 2.3, the first three prerequisites are installed, and the last two are still pending installation.

Figure 2.3 Kaluza for Gallios Installer Window

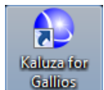
7 When all of the prerequisites have been installed, select the **Install Online, Kaluza for Gallios** button located in the lower right corner of the dialog box.

NOTE The **Install Online, Kaluza for Gallios** button will not be enabled until all prerequisites have been installed.

8 The **Kaluza for Gallios Setup** dialog box appears. Read the license agreement, and select the check box signifying that you accept the terms of the license agreement. Select **Install**.

9 Once the installation process is complete, select **Finish**. The **Kaluza for Gallios** icon (Figure 2.4) should now be located on your desktop. Double-click the icon to launch the software.

Figure 2.4 Kaluza for Gallios Desktop Icon



10 Eject the installation disc from the DVD drive.

11 If you are using multiple ethernet ports (Gallios to workstation ***and*** a network cable to the local network), you will need to reconfigure the network cards in case they were plugged into different ports so that the computer correctly identifies the purpose for each port. To reconfigure network cards:

- Contact your local IT department.

  OR

- Search for ***Configuring multiple gateways on a network*** in Windows 7 System Help and follow the instructions provided.

12 If you need to reconfigure an RMS connection, contact your Beckman Coulter Service Representative.

Installing Offline Kaluza for Gallios

Offline Kaluza for Gallios contains the functions necessary to allow you to set up your acquisition, including Protocols and Worklists, but it does not include the acquisition controls. Refer to Table 2.2 for the standards specified for *Offline* Kaluza for Gallios.

Table 2.2 Offline Kaluza for Gallios System Standards

Component	Standard
Operating System	*Offline* Kaluza for Gallios requires one of the following operating systems: • Windows 7, 64-bit, with Service Pack 1 • Windows XP, 32-bit, with Service Pack 3
Processor	The processor must support SSE2 Instruction Set.
Monitor	The layout of the main workspace is optimized for high-resolution wide screen monitors; however, the software can function with a resolution as low as 1440 x 900.

To install *Offline* Kaluza for Gallios:

NOTE Installing Offline Kaluza for Gallios on your workstation requires an Administrator account. Standard accounts can be used for normal operation of Offline Kaluza for Gallios once the software is installed.

---

1 Power up the computer and log in (as Administrator).

---

2 Insert the Kaluza for Gallios installation disc into the DVD drive.

NOTE If *Offline* Kaluza for Gallios had already been installed in your workstation, the current version will automatically be removed during this installation process. Uninstalling *Offline* Kaluza for Gallios **does not** remove any of your Protocols or Worklists.

---

3 Double click the **My Computer** icon on the desktop.

---

4 Double click the DVD drive containing the installation disc.

---

5 Locate **Kaluza Offline Authoring Setup.exe** and double-click to launch the installation process.

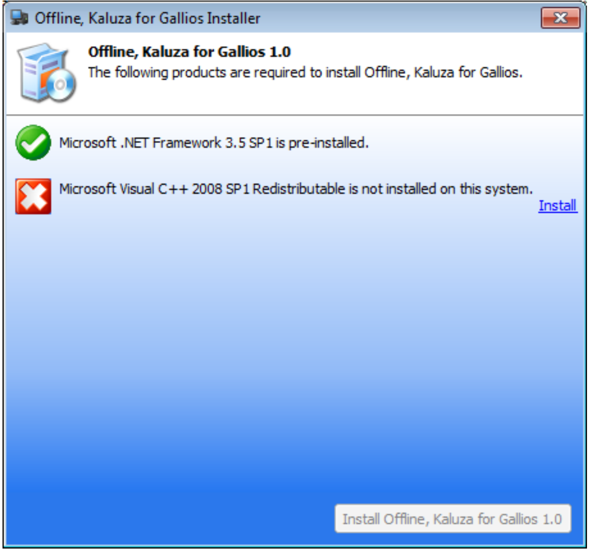
IMPORTANT Depending on the version of Windows, the operating system, the drive used for the installation, and the permissions that have been set up for your computer, you may receive security warnings, which must be acknowledged prior to installing *Offline* Kaluza for Gallios.

---

6 Once all warnings have been acknowledged, the Kaluza Installer dialog box appears. As demonstrated in the dialog box, the application is dependent upon successful installation of the other prerequisites, which are included on your Kaluza for Gallios installation disc. If any of the prerequisites have not already been installed on your computer, an will appear next to the name of each prerequisite that needs to be installed. Install each pending prerequisite, **one at a time**, by selecting the **Install** link, and then following all prompts and accepting all default settings for each pending installation.

When a prerequisite has been successfully installed, a will display next to the product name. In the example in Figure 2.5, the first prerequisite is installed, and the last is still pending installation.

Figure 2.5 Offline Kaluza for Gallios Installer Window



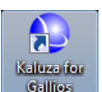
7 When all of the prerequisites have been installed, select the **Install Offline, Kaluza for Gallios** button located in the lower right corner of the window.

NOTE The **Install Offline, Kaluza for Gallios** button will not be enabled until all prerequisites have been installed.

8 The **Kaluza for Gallios Setup** dialog box appears. Read the license agreement, and select the check box signifying that you accept the terms of the license agreement. Select **Install.**

9 Once the installation process is complete, select **Finish.** The *Offline* **Kaluza for Gallios** icon (Figure 2.6) should now be located on your desktop. Double click the icon to launch the software.

Figure 2.6 Offline Kaluza for Gallios Desktop Icon



10 Eject the installation disc.

CHAPTER 3

Operation Principles

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.

Sample Flow

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 barcode labels that identify the carousel and the tube position number. Also, you can put barcode labels on the sample tubes. See APPENDIX A, Barcode Specifications.

The MCL has a barcode reader that reads the carousel number, the sample tube position, and the sample tube barcode 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.

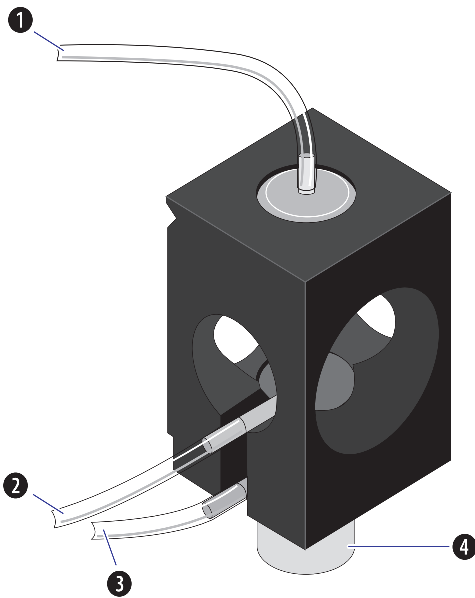
Hydrodynamic Focusing

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



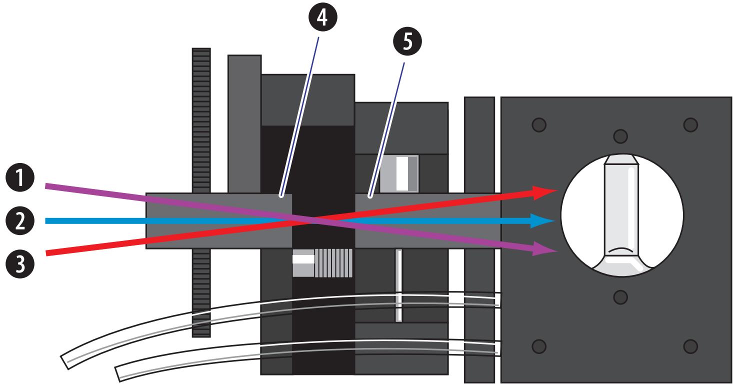
1. Waste out
2. Debubble port
3. Sheath stream enters here
4. Sample stream enters here

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

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.

Light Collection, Separation, and Measurement

Forward Scatter Collection

The FALS (Forward Angle Light Scatter) detector collects scattered light from a particle that intersects with a laser and delivers information roughly proportional to the size of the particle. 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 *Signal Processing*, the signals are processed to measure the characteristics of the cells that scattered the light.

The forward scatter detection in the Gallios flow cytometer is designed to optimize the detector for your desired particle-size sensitivity. The Gallios FS detector uses three software controlled settings chosen for optimal versatility of a wide range of particle size characterization. The three FS angle collection options are:

- **Small**—used for the majority of particles between 1 micron and 20 microns. This forward angle collection provides the best non-specific size performance blend. (1 to 19° FALS angle collection).
- **Large**—used for larger particle resolution <40 microns. (1 to 8° FALS angle collection).
- **Submicron**—used for sub-micron particle resolution.

Side Scatter and Fluorescent Light Collection

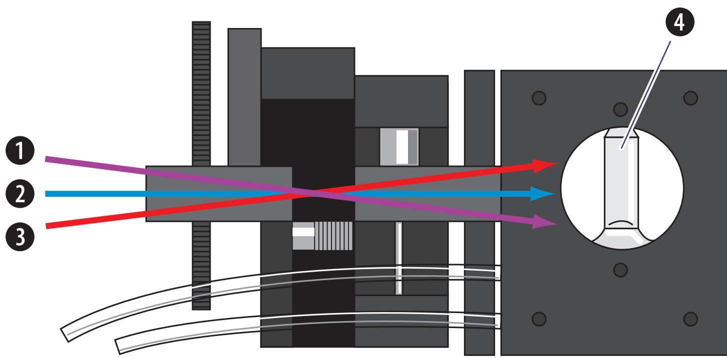
Both side scatter and fluorescence are measured 90 degrees from the laser excitation angle. Side scatter on this system is collected opposite the fluorescence collection.

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 fluorescence. SS is filtered with a 488 nm band-pass (488 BP) filter that is mounted inside the fiber optic cable (located on the right side of the flow cell).

Figure 3.3 Side Scatter Collection



1. Violet laser beam
2. Blue laser beam
3. Red laser beam
4. Flow cell

Fluorescent Light

Band pass and long pass filters are used to transmit color bands. The color bands are designed to measure fluorescent light from the fluorochromes such as FITC, PE, ECD, PC5 or PC5.5, APC, APC AlexaFluor700, APC AlexaFluor750, Pacific Blue and Krome 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 fluorescent 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

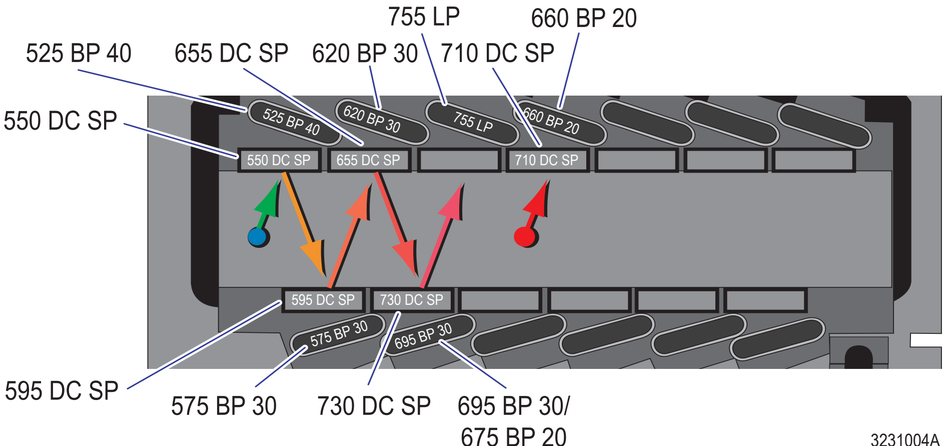


Figure 3.5 Two Laser, 8 Color Filter Block Configuration

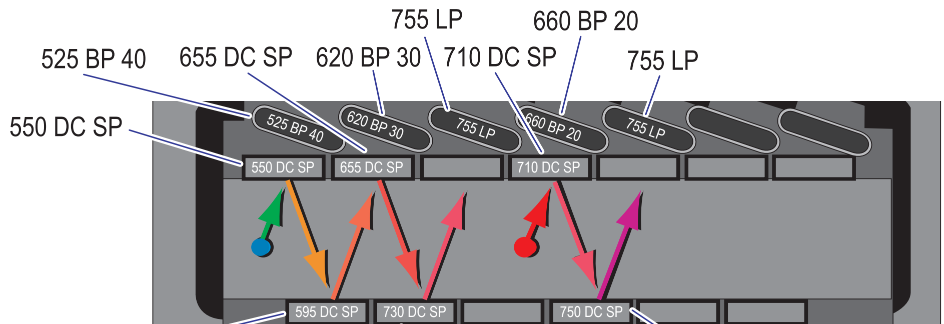
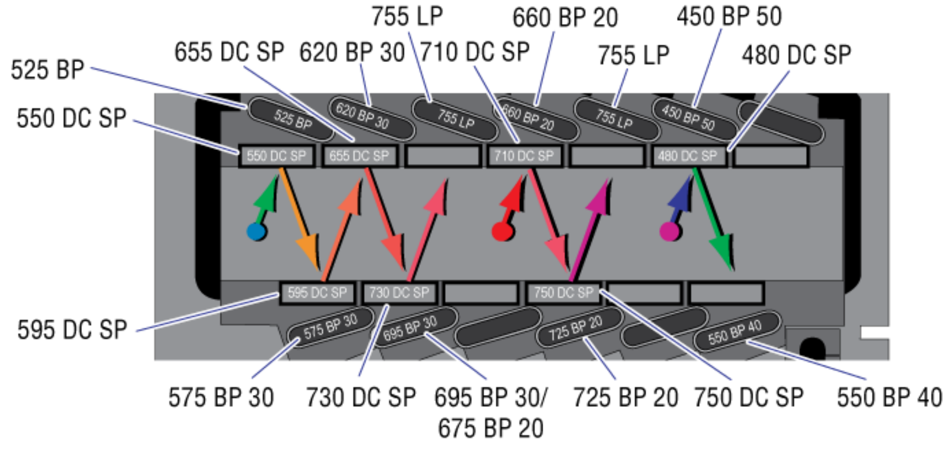


Figure 3.6 Three Laser, 10 Color Filter Block Configuration



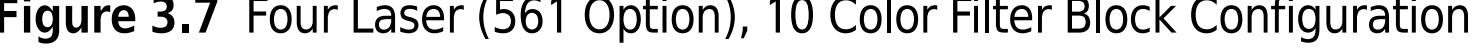
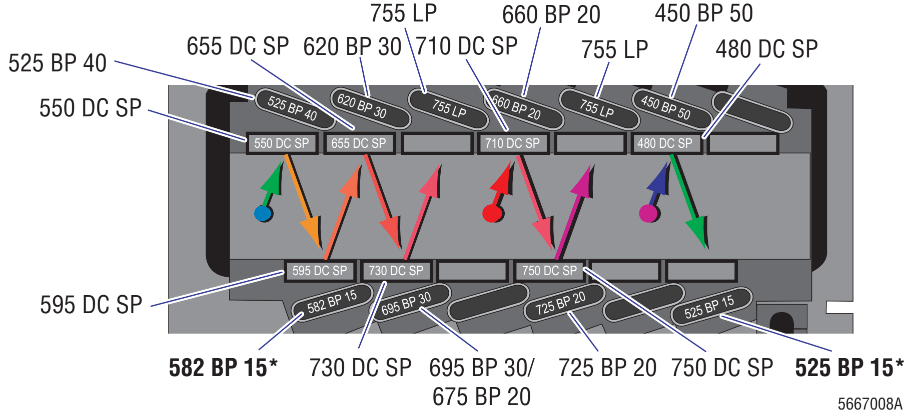


Figure 3.7 Four Laser (561 Option), 10 Color Filter Block Configuration



*These filters are for the optional 561 Laser.

Signal Processing

Voltage Pulse Signals

The Cytometer has up to twelve sensors (FS, SS, FL1-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 amplify, condition, integrate, and analyze these pulses.

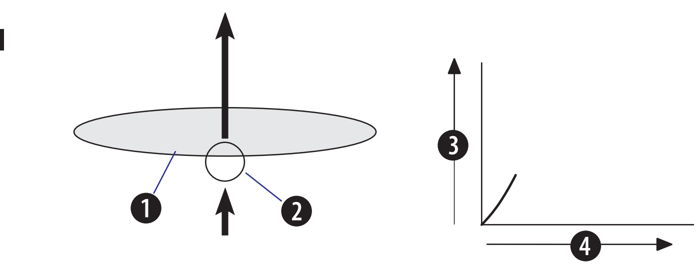
Height Signal

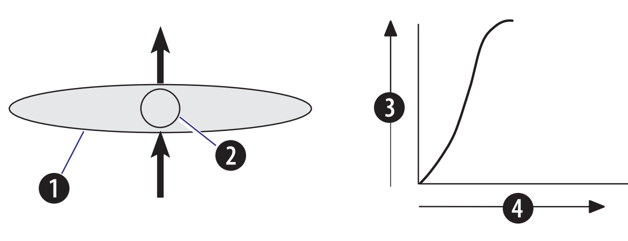
Figure 3.8 shows how a voltage pulse signal forms as a cell crosses the laser beam.

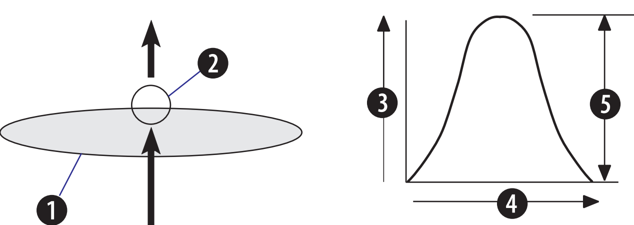
- Part I of Figure 3.8 shows when the cell enters the laser beam and some light is scattered.
- Part II of Figure 3.8 shows when the cell is in the center of the laser beam and the scattered light, and therefore, the pulse height, reaches a maximum.
- Part III of Figure 3.8 shows when the cell leaves the laser beam and the scattered light decreases.

The intensity of light scatter or fluorescence determines the pulse height (see Figure 3.8). 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 3.9 shows how three cells with the same amount of total fluorescence but with different fluorescence intensities, produce different height pulses.

Figure 3.8 Voltage Pulse Formation, Height Signal







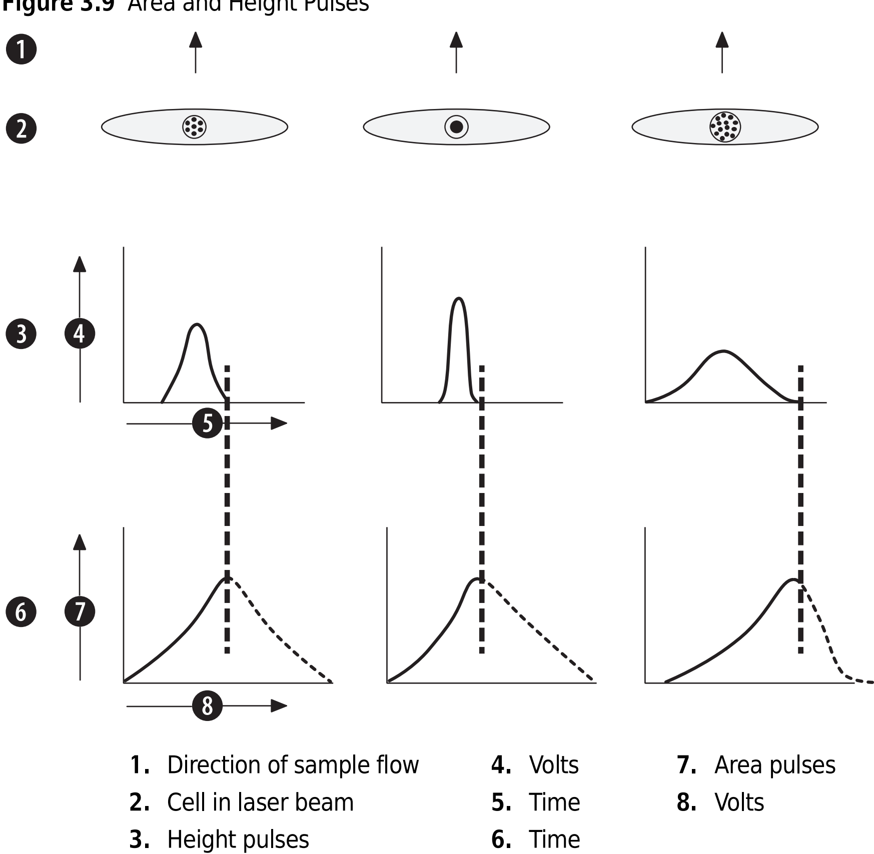
1. Laser beam
2. Cell/Particle
3. Volts
4. Time
5. Pulse height

Area 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 area signal (see Figure 3.9).

The area under the 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.

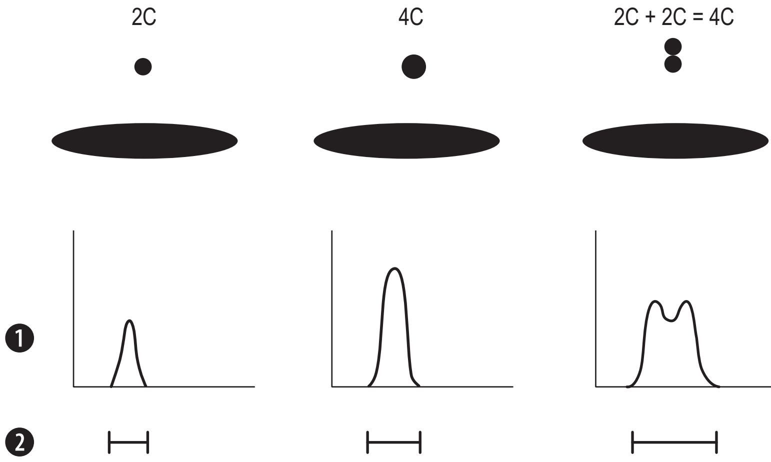
Figure 3.9 Area and Height Pulses



Width Signal

Width is the transit time of a cell or particle to traverse the laser beam. The width is a measurement of the actual pulse width. An application for width is doublet discrimination.

Figure 3.10 Width 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 area, height, and width signals.
- Logarithmically transform the linear area, height, and width signals.

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

Signals Generated

Depending on whether you have a 6, 8, or 10 color configuration, some or all of the following signals are available:

- FS Area, FS Height, FS Width
- SS Area, SS Height, SS Width
- FL1 Area, FL1 Height, FL1 Width
- FL2 Area, FL2 Height, FL2 Width
- FL3 Area, FL3 Height, FL3 Width
- FL4 Area, FL4 Height, FL4 Width
- FL5 Area, FL5 Height, FL5 Width
- FL6 Area, FL6 Height, FL6 Width
- FL7 Area, FL7 Height, FL7 Width
- FL8 Area, FL8 Height, FL8 Width
- FL9 Area, FL9 Height, FL9 Width
- FL10 Area, FL10 Height, FL10 Width

CHAPTER 4

Specifications

Sample Requirements

CHAPTER 12, *Sample Requirements*, provides sample requirement details.

Instrument Specifications and Characteristics

Dimensions

Component	Height	Width	Depth	Weight
Computer	42.7 cm (1.8 in.)	13.0 cm (5.1 in.)	44.4 cm (17.5 in.)	9.07 kg (20 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)
Supply Cart	29.8 cm (11.75 in.)	72.4 cm (28.5 in.)	49.5 cm (19.5 in.)	30.39 kg (67 lb)

Installation Category

Category II (per IEC 61010-1 standard).

Acoustic Noise Level

Measure Level: $\leq$ 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 µL/min
- Medium approximately 30 µL/min
- High approximately 60 µL/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.

Optional Fourth Laser

- A solid-state, 21.5 mW 561 nm laser co-linear with the 488 nm blue laser. The 561 and 488 nm lasers share the same optical path and detector array.

Laser Power Monitoring

Laser power is monitored by each laser individually. If the laser power deviates more than ±10% a Laser Power Error is displayed prominently in red on the Kaluza for Gallios workspace. The system will not run a sample until corrective measures rectify the fault. Follow the instructions in Table 15.1 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.

561 nm Laser Beam Spot Size

An elliptical spot 10.5 µm high by 88 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 dependent upon your system configuration. See Figure 3.4, Figure 3.5, Figure 3.6, and Figure 3.7.

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 1,000, 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.
- Up to 7-decade (4-decade default) digital logarithmic transformation of FS, SS and FL1-FL10.
- Logicle scale enabled. Events with negative compensation values are correctly displayed by means of using the logicle scale. This scale enables sliders on plots, which split the axis into two regions, where the linear region is on one side and the log is on the other side. This allows you to keep the log scale, but negative values are transformed into the linear scale, preserving the symmetrical appearances of correctly compensated data.
- Fluorescence color compensation can be viewed in 0.0001 increments and adjusted in .01 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

Data acquisition is 20-bit for **Area**, 18-bit for **Height**, 10-bit for **Width**, and 30-bit for **Time**.

Digital Sampling rate

40Mhz

Digital Accuracy

<5% error

Workstation

The descriptions below are minimum configurations.

Computer

Table 4.1 provides computer specifications for Kaluza for Gallios and *Offline* Kaluza for Gallios.

IMPORTANT Do not install *Offline* Kaluza for Gallios on the Kaluza for Gallios workstation.

Table 4.1 Computer Specifications

Software Type	Component	Specification
Kaluza for Gallios	**Operating System**	The operating system selected for Kaluza for Gallios is: Windows 7, 64-bit, with Service Pack 1.
	Computer	Intel® Xeon, 6 core microprocessor and 16 GB of RAM.
	Monitor	The layout of the main workspace is optimized for high-resolution wide screen monitors; however, the software can function with a resolution as low as 1440 x 900.
	Data Storage	• 160-GB (or larger) non-removable hard disk. • DVD±RW/CD-RW drive
	Networking	• 3 Ethernet adapters
	Input Devices	• Mouse • Keyboard.

Table 4.1 Computer Specifications

Software Type	Component	Specification
***Offline* Kaluza for Gallios**	**Operating System**	*Offline* Kaluza for Gallios requires one of the following operating systems: • Windows 7, 64-bit, with Service Pack 1 • Windows XP, 32-bit, with Service Pack 3
	Computer	Any computer with one of the operating systems specified above that is equipped with at least 2GB of RAM.
	Monitor	The layout of the main workspace is optimized for high-resolution wide screen monitors; however, the software can function with a resolution as low as 1440 x 900.
	Data Storage	• 160-GB (or larger) non-removable hard disk. • DVD±RW/CD-RW drive
	Input Devices	• Mouse • Keyboard.

Software Specifications

This section contains a high-level overview of Kaluza for Gallios specifications. For additional information, see CHAPTER 6, *Introduction to Kaluza for Gallios*.

Data Output

Data output uses Flow Cytometric 3.1 Standard (FCS) file format for listmode files. Kaluza Analysis Software provides a variety of features for formatting and editing data (refer to the *Kaluza Analysis Software Instructions for Use*, P/N A75667, for additional information).

Setup Mode

During Setup mode, the workspace is a rolling display of events being acquired. The maximum number of events can be set through the Setup Mode drop-down on the **Instrument Control** panel (see CHAPTER 12, *Setup Mode* for details). The incoming data is not saved.

Acquisition

During data acquisition, the plots are updated in real time. When one plot is displayed with statistics, the statistics are also updated in real time. Up to 200 plots are available for any given sample.

One-parameter plots have 1,024-channel resolution.
Two-parameter plots have adjustable resolution up to 2,048- x 2,048-channels.

Parameters

All signals, including Time, can be acquired simultaneously. Three different signals are available from each detector, including Area, Height, and Width.

Gates

Up to 318 gates are available for analysis per Protocol. Up to 31 of those gates can be assigned a color. The following types of gates are available for gating and analysis:

- Linear
- Quadrant
- Hinged Quadrant
- Polygon (up to 128 points)
- Freehand
- Rectangle
- Ellipse

Data Analysis

Data analysis is available through Kaluza Analysis Software. Refer to the K*aluza Analysis Software Instructions for Use* (P/N A75667) for details.

Default Files & File Extensions

Standard files and file extensions include:

File Extension	Type
*.analysis	Analysis file.
*.compensation	Compensation file.
*.fcs	Listmode data file.
*.lmd	Legacy Gallios Listmode data file.
*.pro	Legacy Gallios Protocol file.
*.protocol	Protocol file.
*.worklist	Worklist file.

Default Worklist and Protocols

The following generic Protocols and Worklist are included with Kaluza for Gallios:

NOTE The Protocols listed below will need to be optimized for your instrument. These protocols are available at the following location: **C:\Users\Public\Public Documents\Beckman Coulter\Flow Cytometry**

- Cleaning.worklist (CHAPTER 13, *Cleaning Procedures*)
- Flow-Check Pro (CHAPTER 11, *Quality Control*):
  - Flow-Check Pro - 6 color.protocol
  - Flow-Check Pro - 8 color.protocol
  - Flow-Check Pro - 10 color.protocol
- Flow-Set Pro (CHAPTER 11, *Quality Control*):
  - Flow-Set Pro - 6 color.protocol
  - Flow-Set Pro - 8 color.protocol
  - Flow-Set Pro - 10 color.protocol

Additionally, Protocols for service use are distributed with Kaluza for Gallios.

FCS Header - Keyword Reference

The listmode data generated by Gallios cytometers during the process of acquisition of a sample is stored in a file for further analysis. This file contains all the raw, unprocessed data generated by the instrument and the Beckman Coulter proprietarily formatted Protocol. Each data file contains only the results of a single acquisition run.

FCS 3.1 Section

The FCS 3.1 data set consists of three segments: HEADER, TEXT and DATA. This data set contains the uncompensated, raw data.

HEADER Segment

The HEADER segment contains the offsets to the beginning and end of the other segments as specified by the FCS 3.1 standard. The standard also calls for an optional ANALYSIS segment. The data files generated by the Gallios cytometers do not contain an ANALYSIS segment and its offsets are 0 in the HEADER segment. Each offset specified in the HEADER segment is referred to the beginning of the HEADER segment itself.

TEXT Segment

The TEXT segment contains a set of keyword - keyword value combinations delimited by the "/" character. The set contains all the required keywords as specified by the FCS 3.1 standard as well as some optional ones. In addition, the keyword set includes a number of custom keywords that provide additional information about the data being collected. Custom keywords are preceded by the "@" character.

In Table 4.2, Kaluza for Gallios FCS 3.1 Standard Keywords and Table 4.3, Kaluza for Gallios Custom Keywords are keywords included in the TEXT segment:

Table 4.2 Kaluza for Gallios FCS 3.1 Standard Keywords

FCS 3.1 Keyword	Key Value
$BEGINANALYSIS	Offset to the beginning of the ANALYSIS segment. This value is always 0.
$BEGINDATA	Offset to the beginning of the DATA segment. This value matches the offset specified in the header section.
$BEGINSTEXT	Offset to the beginning of the supplemental TEXT segment. This value is always 0 because no supplemental TEXT segment is used.
$BTIM	The time that acquisition started for this data set (hh:mm:ss.cc).
$BYTEORD	The byte order of the data in the data segment. The data for each parameter is right justified and is in little endian order, where the least significant byte goes first. This value is always "1,2,3,4".
$CYT	The name or type of the cytometer used to collect the data set.
$CYTSN	The serial number of the cytometer used to collect the data set.
$DATATYPE	Type of data in the DATA segment. This value is always "I".
$DATE	The date that acquisition started for this data set (dd-mmm-yyyy)
$ENDANALYSIS	Offset to the end of the ANALYSIS segment. This value is always 0.
$ENDDATA	Offset to the end of the DATA segment. This value matches the offset specified in the header section.
$ENDSTEXT	Offset to the end of a supplemental TEXT segment. This value is always 0 because no supplemental TEXT segment is used.
$ETIM	The time that acquisition ended for this data set (hh:mm:ss.cc).
$FIL	The name of the file to which the data set was originally saved.
$INST	The institution or laboratory where the data set was collected. Optional. Will not appear if this value is not set.
$MODE	Data mode. This value is always "L".
$NEXTDATA	Byte offset into binary data for next data item. This value is always 0.
$OP	The logon name of the current Windows user when the data set was acquired.
$ORIGINALITY	Describes whether the data set has been modified. For files generated by Kaluza for Gallios, this value is always "Original".
$PAR	Number of parameters in an event.
$PROJ	The name of the protocol used to acquire the data set.
$PnB	Number of bits reserved for parameter number "n". This value is always 32. Values are stored as 20 bits, plus 12 bits of padding.
$PnE	Amplification for parameter "n". This value is always "0,0", which represents a linear value.
$PnG	Amplifier gain used for acquisition of parameter "n".
$PnN	The short name for parameter "n".
$PnR	Range for parameter "n". An "area" parameter is 1048576, representing 20-bit data. A "height" parameter is 262144, representing 18-bit data. A "width" parameter is 1024, representing 10-bit data. The "time" parameter is 1073741824, representing 30-bit data.

Table 4.2 Kaluza for Gallios FCS 3.1 Standard Keywords

FCS 3.1 Keyword	Key Value
$PnS	The description for parameter "n".
$PnV	The voltage of the sensor associated with parameter "n".
$SPILLOVER	Specifies the information necessary to carry out fluorescence compensation using a spillover matrix. See the FCS 3.1 specification for details. Optional. Will not appear if the spillover matrix is empty.
$SYS	The type of computer and operating system under which the data set was collected.
$TIMESTEP	The time step of the TIME parameter. If the time step is 1/100 of a second, then 0.01 is the value for this keyword. When an event is captured s seconds after acquisition has started, the value s * 100 should be entered into the time parameter. Therefore, it follows that the maximum time range for the *.fcs file is range/100 seconds.
$TOT	Total number of events acquired.

Table 4.3 Kaluza for Gallios Custom Keywords

Custom Keyword	Key Value
@ABSCALFACTOR	Defines the calibration factor of the beads used with this sample if Flow-Count or similar absolute calibration particles are used. If the calibration factor is not provided, this keyword contains "NOT SET".
@ACQSOFTWARE	The name of the software that was used to acquire the data set.
@ACQTIME	The duration of the acquisition, excluding pause time.
@AUTOFLUORESCENCE	Specifies the information necessary to perform autofluorescence correction during compensation. The value is a one-dimensional vector of autofluorescence values: n,P1,P2,…,Pi,V1,V2,…,Vi, where n is the number of parameters in the autofluorescence vector, Pi is the name of the “i"-th parameter in the vector, and Vi is the double-precision value of the “i"-th parameter in the vector. Optional. Will not appear if the autofluorescence vector is empty.
@BARCODE	The barcode associated with the acquired data set, or "NoRead" if nothing is read.
@BEGINKALUZA	Offset to the beginning of the KALUZA segment.
@BLUELASERPOWER_END	The blue laser power in milliwatts at the end of the acquisition.
@BLUELASERPOWER_START	The blue laser power in milliwatts at the start of the acquisition.
@BLUETARGETPOWER	The requested blue laser power in milliwatts at the end of the acquisition.
@CAROUSEL	The carousel number from which the data set was acquired.
@DISCRIMINATOR	Discriminator detector and level. Example: "FL1,20"
@ELAPSEDTIME	The duration of the acquisition, including pause time.
@ENDKALUZA	Offset to the end of the KALUZA segment.
@REDLASERPOWER_END	The red laser power in milliwatts at the end of the acquisition.
@REDLASERPOWER_START	The red laser power in milliwatts at the start of the acquisition.

Table 4.3 Kaluza for Gallios Custom Keywords

Custom Keyword	Key Value
@REDTARGETPOWER	The requested red laser power in milliwatts at the end of the acquisition.
@VIOLETLASERPOWER_END	The violet laser power in milliwatts at the end of the acquisition.
@VIOLETLASERPOWER_START	The violet laser power in milliwatts at the start of the acquisition.
@VIOLETTARGETPOWER	The requested violet laser power in milliwatts at the end of the acquisition.
@SAMPLEID1	User defined string.
@SAMPLEID2	User defined string.
@STOPREASON	The reason that the acquisition was stopped. Possible values are: ERROR, PROTOCOL, MANUAL, or MAX EVENTS.
@TUBENO	The tube number (location on the carousel) from which the data set was acquired.

DATA Segment

The DATA segment contains the uncompensated raw data produced by the instrument. Each event contains data for the parameters specified in the TEXT segment. The number of parameters per event is specified by the value of the $PAR keyword. The order of the parameters within each event is specified by the parameter indices used in the TEXT segment. The parameter data bit range depends on the measurement type (20 bits for **Area**, 18 bit for **Height**, 10 bit for **Width**, and 30 bit for **Time**), and occupies 32 bits (4 bytes) in the file. The parameters are aligned to the byte boundary. The data for each parameter is right justified with 12 bits of padding in front in little endian order, meaning that the least significant byte goes first.

Following, there is the ordered list of parameters as reported by the cytometer. Parameter indices are assigned incrementally, starting from 1 following the order listed.

FS Height, FS Area, FS Width, SS Height, SS Area, SS Width, FL1 Height, FL1 Area, FL1 Width, FL2 Height, ..., FL9 Width, FL10 Height, FL10 Area, FL10 Width, TIME.

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 20µl of Acridine Orange, the system was cleaned according to the *Routine Cleaning Procedure* in CHAPTER 13, *Cleaning Procedures*. 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 instrument 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%.

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 voltage = 700, Gain at 200, Discriminator at 47 and setting the particle size to the **Small** 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 area signal intensity values using Flow-Check Pro fluorospheres is <2% from the blue laser.

Fluorescence

The HPCV of the area 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 three 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

Laser Characteristics

Day-To-Day

The mean channel value of the area 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 area signal channel number obtained over a period of 8 days.

Within Day

The mean channel value of the area 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 area signal channel number obtained within a period of 24 hours.

CHAPTER 5

System Overview

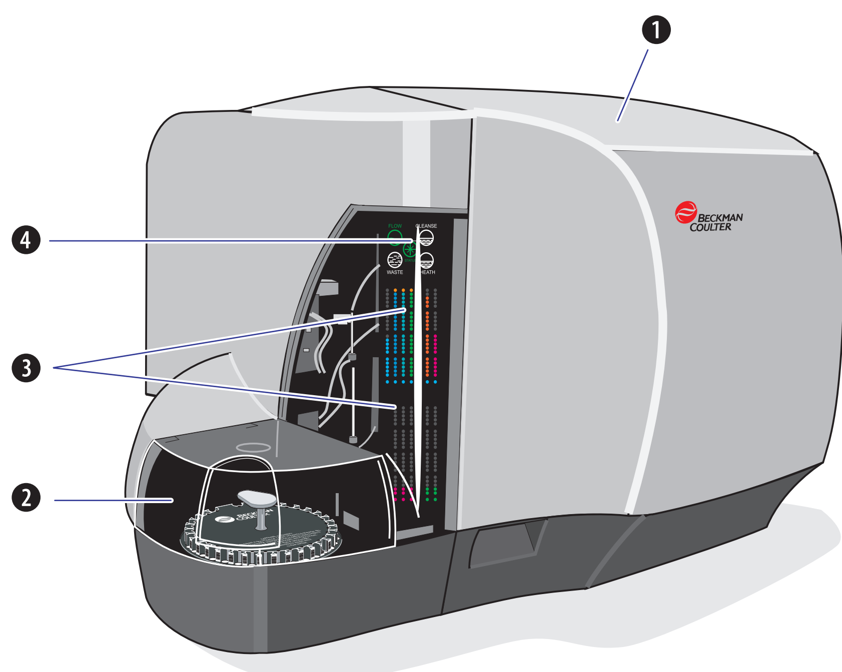
Product Description

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 multi-parametric analysis.

The instrument can simultaneously measure forward scatter, side scatter, and up to ten fluorescent dyes using up to four solid-state lasers at 488 nm, 638 nm, 405 nm and 561 nm. Therefore, the instrument can perform correlated multiparameter analyses of individual cells.

Cytometer Controls and Indicators

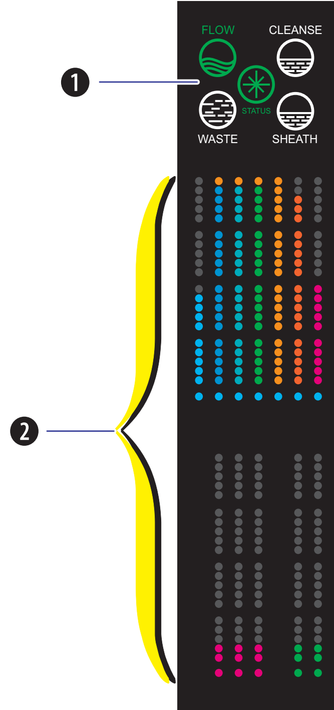
Gallios Flow Cytometer



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

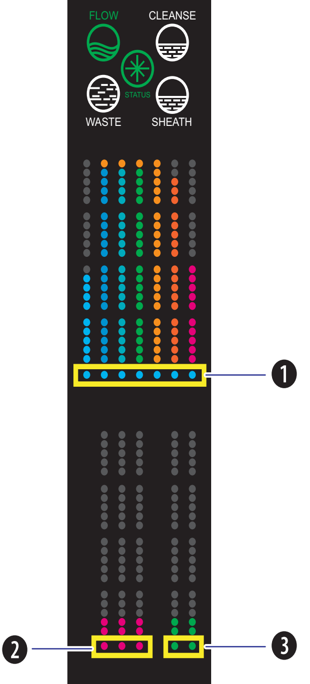
Cytometer Indicator Panel

1. Level Sense and Flow Indicators
2. Signal Amplitude Indicators



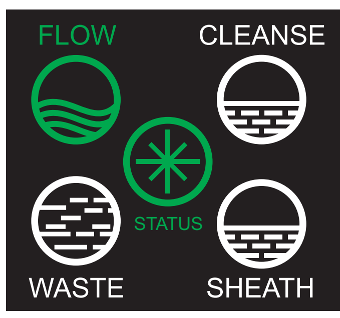
Signal Amplitude Indicators

NOTE When the log, linear, and logicle parameters are selected for the same sensor, the amplitude display defaults to the linear area signal.



1. Blue indicates the signals from the 488 and 561 lasers.
   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

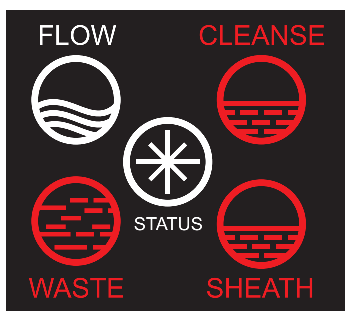
Cytometer Ready and Sheath Flow Indicators



FLOW (ready when green)

STATUS (ready when green)

Level Sense Indicators

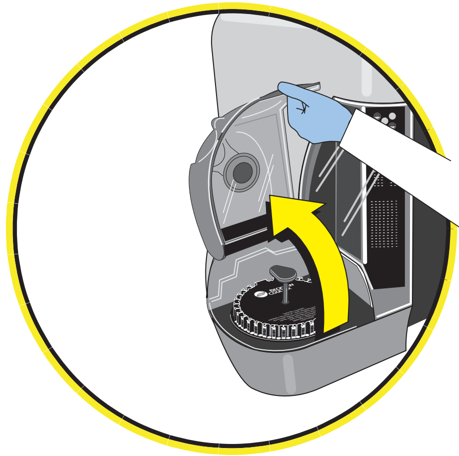


CLEANSE (red when low)

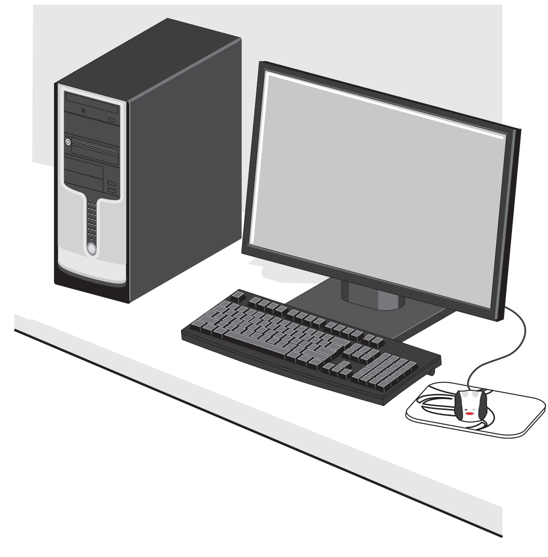
WASTE (red when full)

SHEATH (red when low)

MCL (Multi-tube Carousel Loader)



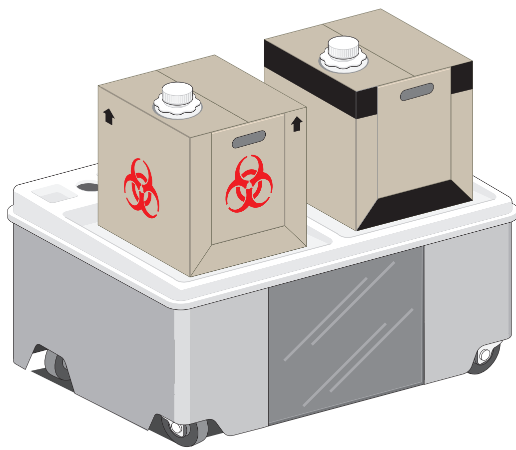
Workstation



Pneumatic Supply

If your instrument is equipped with a pneumatic supply, refer to the *Gallios Flow Cytometer Instructions for Use* (p/n 773231) for additional information.

Supply Cart



Printer (Optional)

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

Learning the Basic Operating Techniques

Practice the basic techniques until you feel comfortable using them. If, later on, you need to use a basic technique but cannot remember how, refer back to this manual for step-by-step instructions.

MCL Carousels

The Gallios Flow Cytometry System starter kit has:

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

Barcode Labels

You can put a barcode label on each sample tube. See APPENDIX A, *Barcode Specifications* for additional information.

NOTE Barcode labels are not required on sample tubes for system operation.

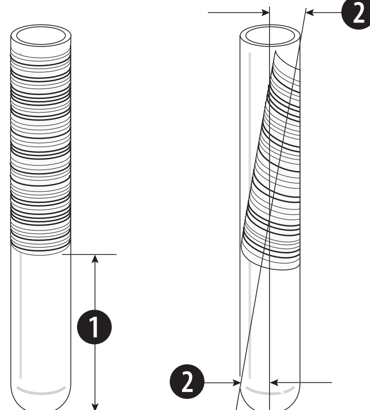
Sample misidentification can occur from the use of incorrect, poor quality, damaged, dirty or improperly placed barcode labels. Follow the Barcode Specifications to create your barcode labels to prevent incorrect sample identification. Barcode label incorrectly placed on sample tubes could cause misidentified tubes. To prevent misidentified samples, affix the barcode label as shown below so the MCL can read the label.

To use barcode labels:

---

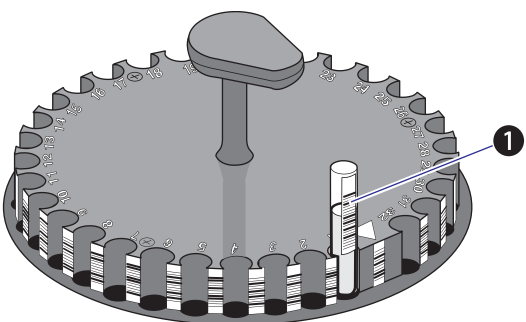
1 Carefully align the label with the tube. Put labels on the tubes so that the bars follow one another in a vertical sequence. The barcode reader scans the tube vertically. Do not tilt the label more than ±7.5 degrees from the axis of the tube.

1. 25.4 mm (1.0 in.) minimum
2. 7.5 degrees.



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

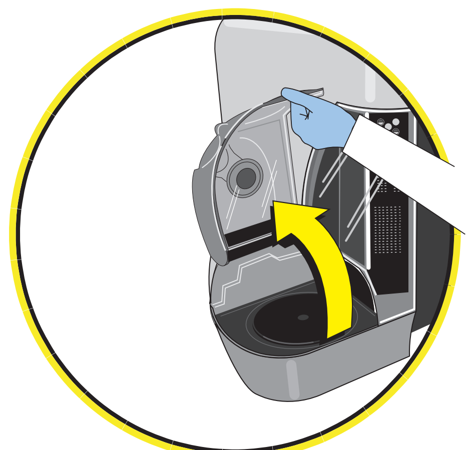
3 Place the sample in the MCL.



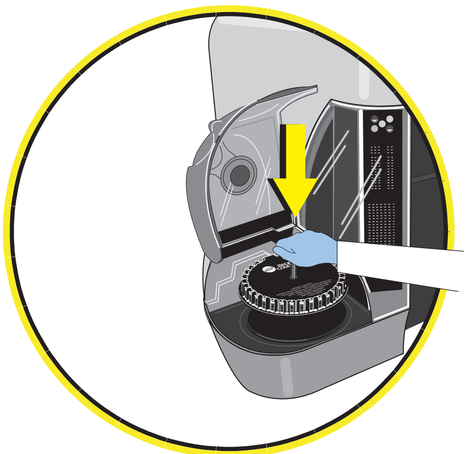
1. The orientation of a tube with a barcode label does not matter. The MCL rotates the tube to find the barcode label.

Putting a Carousel in the MCL

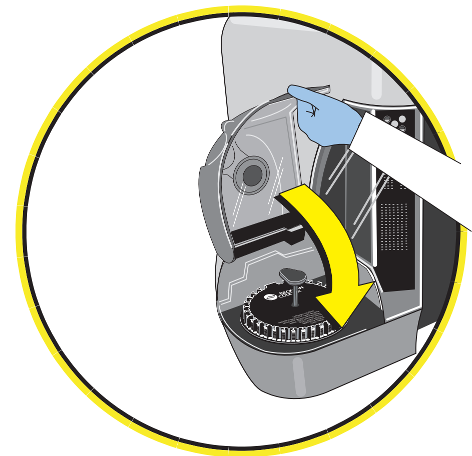
1 Open the MCL cover.



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.

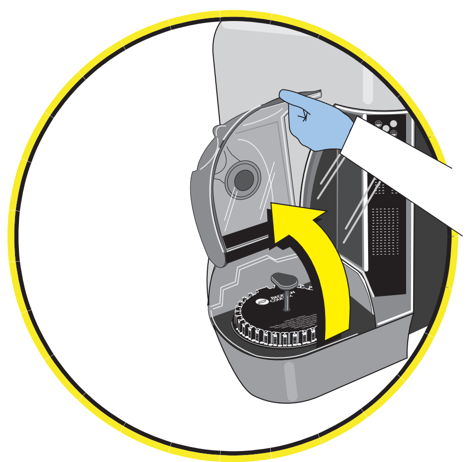


3 Close the MCL cover.

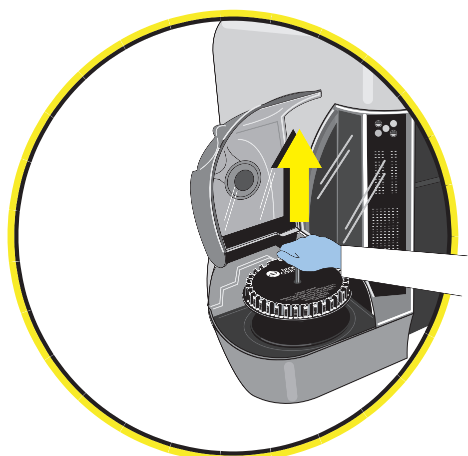


Removing a Carousel from the MCL

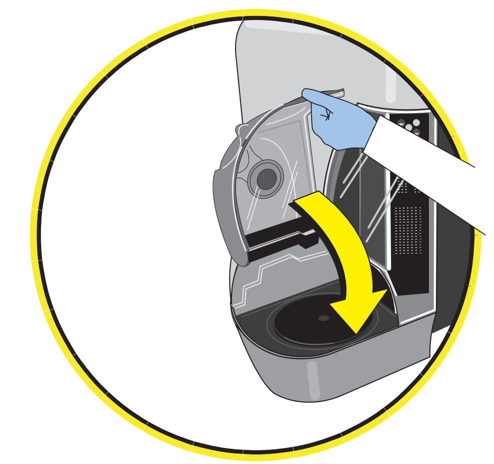
1 Open the MCL cover.



2 Remove the carousel.



3 Close the MCL cover.



CHAPTER 6

Introduction to Kaluza for Gallios

Overview

Kaluza for Gallios is designed to make acquiring samples easy, through its intuitive design and easy-to-navigate user interface. The straightforward nature of Kaluza for Gallios allows you to spend less time at the instrument, and more time performing research. Listmode data acquired using Kaluza for Gallios is loaded into Kaluza Analysis Software with the touch of a button, providing a seamless flow as you analyze your data.

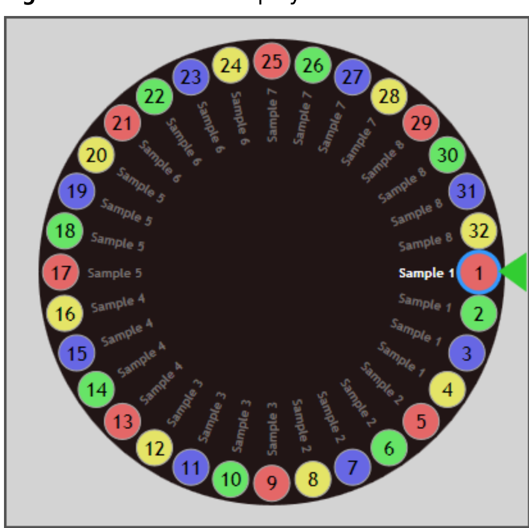
Kaluza for Gallios Features

The following innovative Kaluza for Gallios features enable you to create Protocols and Worklists, and acquire samples with ease.

Carousel Display

The Carousel Display (Figure 6.1) is a graphical representation of the samples in your carousel. Through the Carousel Display, you can choose Protocols for each sample, make edits to a sample's attributes, and copy data to the next available sample position. Edits can be made at almost any time and in any order, allowing you a great degree of control over your Worklists.

Figure 6.1 Carousel Display



Radial Menus

Radial menus (Figure 6.2) provide quick access to the tools necessary for making changes on the Plot Sheet. Radial menus appear by right-clicking directly on a plot, gate, or on the whitespace. As you hover over an icon, a menu appears for that icon, allowing you to make changes instantly.

Figure 6.2 Plot Radial Menu

Extensive Maximum Event Limit

When using an 8-parameter setup, Kaluza for Gallios offers a 25 million event maximum limit; this extended limit allows you to detect the very rare events. Table 7.1 provides maximum limits for the number of enabled measurements.

Offline Authoring of Protocols and Worklists

Kaluza for Gallios includes an offline version of the user interface where you can set up Protocols and Worklists for your acquisition beforehand, saving you time at the instrument.

Integration with Kaluza Analysis Software

With the touch of a button, you can load acquired data (*.fcs files) into Kaluza Analysis for sample analysis. The user interfaces for Kaluza Analysis and Kaluza for Gallios are very similar, which makes moving between the two very easy. In addition, acquiring samples and analyzing previously acquired samples can be done simultaneously.

Getting Started

This section contains instructions and important information for improving your experience with Kaluza for Gallios.

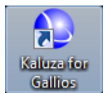
Launching Kaluza for Gallios

The shortcut for Kaluza for Gallios was created on your desktop during the installation process (as described in the software CD package).

To launch the software:

1. Double-click the Kaluza for Gallios shortcut (Figure 6.3).

Figure 6.3 Kaluza for Gallios Shortcut



OR

Click the **Windows Start Button** > **All Programs** > **Beckman Coulter** > **Kaluza for Gallios 1.0**.

Command-Line Switches

The following command-line switches are available for Kaluza for Gallios:

- **/RESET**: Instructs Kaluza for Gallios to reset the current user's window sizes and position, as well as other options, to the defaults.
- **/NoCleanOnClose**: Instructs Kaluza for Gallios to not initiate a clean cycle when the application is closed.

Using the License Key

A license key needs to be attached to the computer to enable Kaluza for Gallios to control the cytometer. A license key comes in the form of a USB device.

NOTE Offline Kaluza for Gallios does not require a license key.

To set up a license key:

1. Install Kaluza for Gallios on your computer using the instructions in CHAPTER 2, *Reinstalling Kaluza for Gallios*.

2. Plug the USB key into host computer USB port. This allows full access to Kaluza for Gallios.

NOTE The license key is a custom USB device and is not a flash drive. No information can be read or written to it using the file system. It is not a potential vector for malware.

NOTE For additional information regarding the license key, refer to the instructions on the website at **http://localhost:1947**.

User Preferences and Security

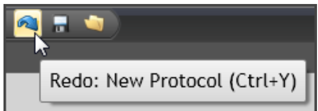
On a shared system, you may wish to secure your Kaluza for Gallios files and define options specifically for your needs. Kaluza for Gallios works in conjunction with your Windows operating system to provide you with the additional control you require. This is achieved through creating separate user accounts on your Windows operating system. For instructions on creating multiple Windows user accounts, consult **Windows Explorer > Help > View help**, and then enter "**User Accounts**" in the ***Search Help*** field and follow the instructions provided.

NOTE On a system with multiple active users, only one user can be logged into Kaluza for Gallios at a time.

Tooltips

Hover your mouse cursor over hotspot areas of the screen to display information related to your current location. This information, known as tooltips, provides clear instructions, saving you time and eliminating guesswork. Figure 6.4 is an example of a tooltip that appears when hovering over the redo icon.

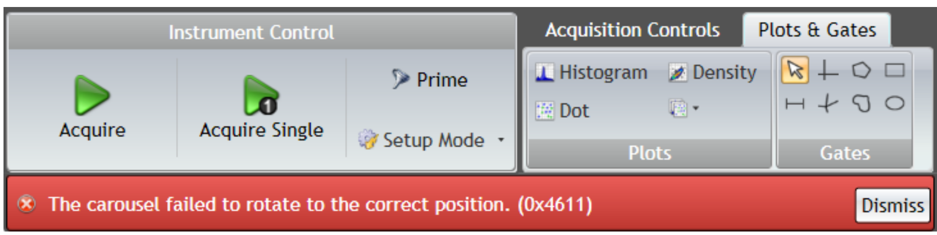
Figure 6.4 Tooltip Example



Cytometer Messages

Cytometer messages appear below the ribbon, as shown in red in Figure 6.5. If there are multiple messages, "*Click to show additional messages*" appears, allowing you to view multiple messages simultaneously. See CHAPTER 15, *Cytometer Messages*, for additional information on cytometer messages, and refer to Table 15.2, to view a complete list of the cytometer messages, along with the recommended action for correcting each issue.

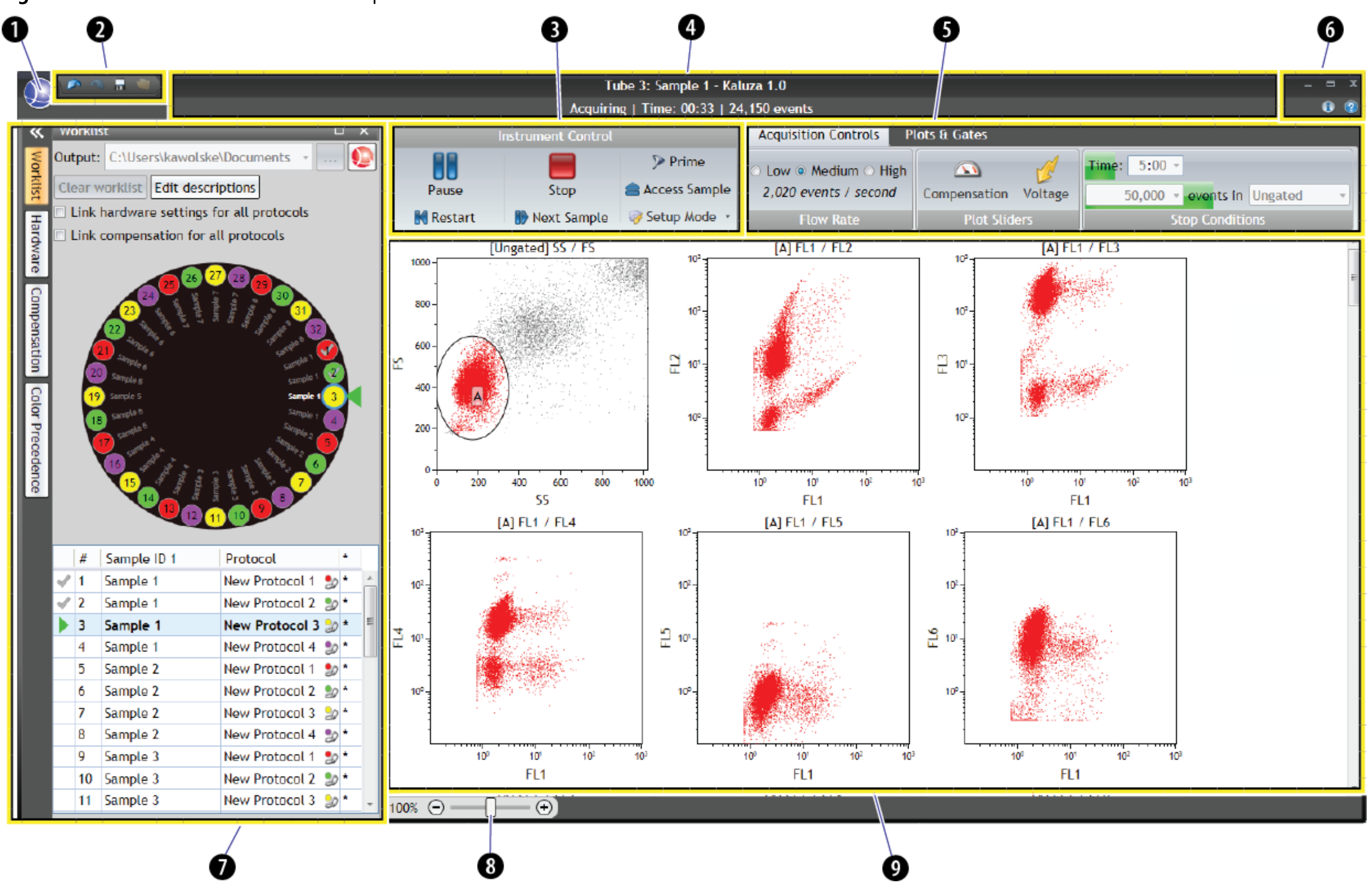
Figure 6.5 Error Message Example



Components of the Main Workspace

The components of the Kaluza for Gallios main workspace are shown in Figure 6.6, and descriptions of each component are found in Table 6.1.

Figure 6.6 Kaluza for Gallios Main Workspace



1. Application Button
2. Quick Access Toolbar
3. Instrument Control Panel
4. Title/Status Bar
5. Ribbon
6. Title Bar Options
7. Acquisition Attributes Panes
8. Zoom
9. Plot Sheet

Table 6.1 Components of the Main Workspace

Component	Function
1. Application Button	Opens the Application menu, providing access to creating, opening, or saving Protocols and Worklists, importing hardware and/or compensation settings, changing the state of the cytometer, cleaning the cytometer, cytometer logs, and powering lasers off and on. See *Application Menu Items* for details.
2. Quick Access toolbar	Provides quick access to functions, including undoing and redoing actions, opening a file, and saving a Protocol. See *Quick-Access Toolbar* for details.
3. Instrument Control Panel	Provides the instrument controls required for performing and preparing for acquisitions. See *Instrument Control Panel* for details. **NOTE** The Instrument Control Panel is not included in the Offline Kaluza for Gallios workspace.
4. Title/Status Bar	Provides the program name and version, the current status of the instrument, and, if applicable, the Sample ID/Protocol name and the time elapsed and the number of events captured for the current sample being acquired. See *Title/ Status Bar* for details.
5. Ribbon	Allows you to specify acquisition controls and select plots and gates to place on the Plot Sheet. See *Ribbon* for details.
6. Title Bar Options	Provides buttons to minimize, maximize, and close Kaluza for Gallios and open the help file and About screen. See *Title Bar Options* for details.
7. Acquisition Attributes Panes	Contains the Worklist, and the Hardware, Compensation, and/or Color Precedence panes. See *Acquisition Attributes Panes* for details.
8. Zoom	Allows you to modify the zoom value of the plot sheet. See *Zoom* for details.
9. Plot Sheet	Plots, gates, statistics, etc. are placed in this location. See *Using the Plot Sheet* for details.

Right-Click Options

NOTE Normally right-click options provide alternatives to standard procedures and are not included in the instructions in this manual, unless they are the only way to use a particular option.

When you click the right mouse button, menu options that apply to a particular region of the screen appear. Specifically, right-click menus are available in the Worklist pane (both the Carousel Display and the Parameter Descriptions Grid), and the Hardware pane.

A unique type of menu, the radial menu, is available with a right-click in the Plot Sheet.

Using Radial Menus

Radial menus are incredibly useful tools, as they enable convenient access to the menu items that are applicable to your current location on the plot sheet. Radial menus appear by right-clicking on one of three areas: plots, gates, and sheet whitespace.

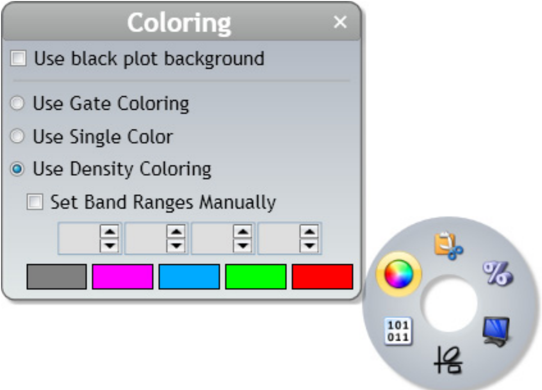
You can move a radial menu to any location of the screen. To move a radial menu, left-click on any blank part of the menu and drag it to the preferred location. See CHAPTER 7, *Plot Set-Up*, CHAPTER 7, *Setting Up Gates*, or CHAPTER 6, *Plot Sheet Radial Menus Options*, for details.

To use a Radial Menu:

1 Right-click on the location that you wish to update. A radial menu appears.

2 Move your mouse over the menu. As you hover over the icons located on the radial menu, the menu for that icon appears. For example, hovering over the Coloring icon brings the Coloring menu, as shown in Figure 6.7.

Figure 6.7 Coloring Menu



3 Make the necessary changes within the appropriate menu. When you are satisfied with your changes, close the menu by selecting [X] or by clicking on some other part of the software.

Drag and Drop

NOTE Kaluza for Gallios includes multiple methods for achieving a particular outcome. When the “drag and drop” method is available for a task, it is the option noted in the instructions.

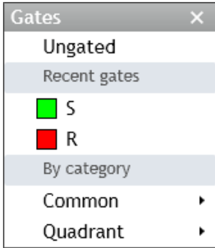
Many functions in Kaluza for Gallios use the drag and drop method. Examples include:

- Creating plots by dragging/dropping an icon from the Ribbon onto the sheet.
- Moving a sheet item to a different location.
- Associating a Protocol to a carousel position by dragging/dropping the Protocol file from Windows Explorer.
- Rearranging samples in the Worklist.

Pop-up Menus

Pop-up menus, which appear after selecting hyperlinks, may include headings and subheadings within the menu, both of which are not selectable; however, they do include information that is available for selecting under applicable headings. See Figure 6.8 to view an example of the Gates heading. Headings appear in white font/gray highlight. The subheadings (**Recent gates** and **By category** in Figure 6.8) use a dark gray font and are highlighted in light gray. An arrow located next to a menu item indicates that additional sub-menu options are available, as demonstrated by **Common** and **Quadrant**. Sub-menus pop-up when you hover your mouse cursor over a row that includes an arrow.

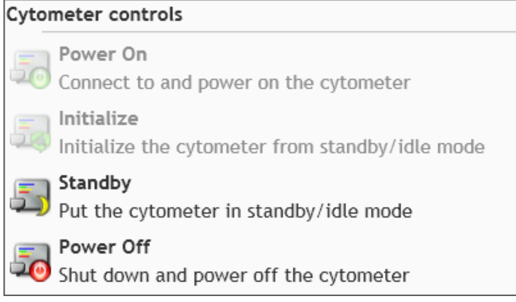
Figure 6.8 Pop-Up Menus



Indication of Option Availability

The availability of options depends on the current state of the instrument. When options are not available, they appear transparent compared to the options that are available. In Figure 6.9, **Power On** and **Initialize** are not available because the instrument has already been powered on and initialized.

Figure 6.9 Unavailable Options



System Performance

To optimize the performance of the application, do not conduct a full disk virus scan while running Kaluza for Gallios.

File Compatibility

The following file types can be imported into Kaluza for Gallios:

Table 6.2 Compatible File Types

Originating Software	File Extension	File Type
Gallios Software	*.lmd	Listmode Data
	*.pro[a]	Protocol
Kaluza Analysis Software	*.analysis[a]	Analysis
	*.compensation	Compensation
	*.protocol	Protocol
	*.txt	Text-Formatted Compensation
Kaluza for Gallios	*.compensation	Compensation
	*.fcs	Listmode Data
	*.protocol	Acquisition Protocol
	*.txt	Text-Formatted Compensation
	*.worklist	Worklist

a. Some features of this file type are not supported in Kaluza for Gallios.

Kaluza for Gallios File Type Summary

Table 6.3 lists the types of files that you can create using Kaluza for Gallios, as well as important details about the content included in each file type. Review the table to determine the file type you need to create, and then refer to the appropriate section for details on creating each file type:

Table 6.3 Kaluza for Gallios File Types

File Type	Extension	Saving Mechanism	What is Saved	What is NOT Saved	For More Information, Refer to:
Compensation	*.compensation[a] *.txt[a]	Save as	• Spillover Matrix • Autofluorescence Vector Values	• Protocols • Data Sets • Worklists	CHAPTER 7, *Compensation*
Data Set	*.fcs	Files are automatically saved once the acquisition has stopped.	• Raw data • Embedded runtime protocol	• Protocols • Worklists	CHAPTER 12, *Sample Acquisition*
Protocol	*.protocol	Save *or* Save as	• Plots (including all customizations) • Gates (including gate coloring definitions) • Parameters • Parameter Descriptions • Instrument Settings • Acquisition Controls • Compensation Spillover Matrix & Autofluorescence Vector Values[b]	• Data Sets • Metadata • Worklists	CHAPTER 7, *Creating a New Protocol*
Worklist	*.worklist[a]	Save as or Save selected as	• Protocols[b] • Sample IDs • Carousel positions • Parameter descriptions	• Data Sets	CHAPTER 8, *Worklists*

a. **Save as** must be selected ***each*** time you wish to save an entry as any file type other than a ***.protocol** file.

b. If you had opened a saved Protocol or Compensation file into the Worklist, the Protocol and/or Compensation file is saved with the Worklist and becomes independent of the original file. Any changes made to the Protocol or Compensation file after saving the Worklist must also be saved in a **"Save as"** manner, where the original file is replaced with the updated file.

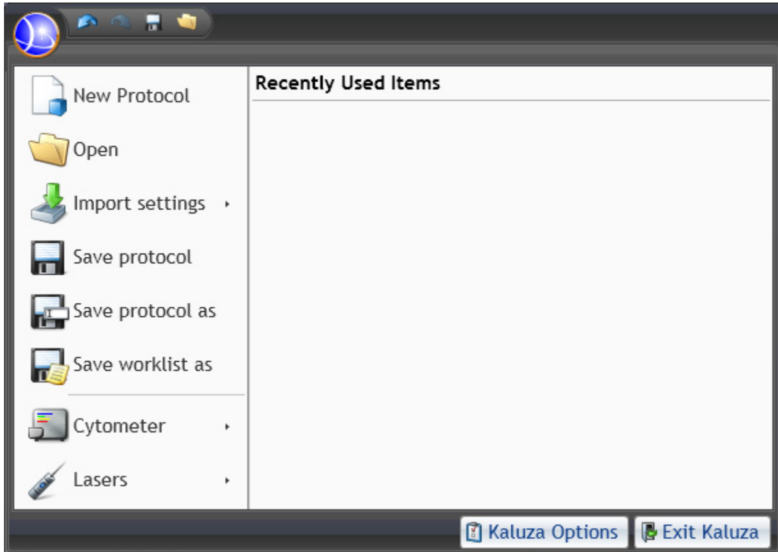
Main Workspace

See Figure 6.6, to view the location of each component in the Kaluza for Gallios workspace. The components that make up the Kaluza for Gallios workspace are described in detail in the following sections.

Application Menu

The is referred to as the Application button, and is located in the upper left-hand corner of the application workspace. Select this button to open the Application menu (Figure 6.10).

Figure 6.10 Application Menu



Application Menu Items

The Application menu provides the options described in Table 6.4.

Table 6.4 Application Menu

Menu Item	Description
Recently Used Items	Provides access to the most recently used files. The files are listed in chronological order, with the most recently used file at the top of the list. To open a file on the list, click on the file name.
New Protocol	Allows you to create a new Protocol. For additional information, see CHAPTER 7, *Creating a New Protocol*. **NOTE** Ctrl + N also creates a new Protocol.

Table 6.4 Application Menu

Menu Item	Description
Open	Opens a file into the Kaluza for Gallios application. **NOTE** Other options for opening files in Kaluza for Gallios include: • Selecting **Open** from the home screen after opening the application. • Selecting from the Quick Access Toolbar. • Using the shortcut feature by pressing Ctrl + O keys on your keyboard to display the Open Dialog window.
Import Settings	Allows the user to select a file from which to import compensation and/or voltages, gains, and discriminator settings. See CHAPTER 7, *Importing Hardware Settings* for additional information.
Save protocol	Saves the current Protocol as a *.protocol file.
Save protocol as	Enables you to save the current Protocol as a *.protocol file to a new location or with a new name.
Save worklist as	Enables you to save the current Worklist as a *.worklist file to a new location or with a new name.
Cytometer[a]	Initiates actions related to the cytometer, including the following: • **Power On**: Connects to and powers on the cytometer. • **Initialize**: Initializes the cytometer when the instrument is in Standby/Idle mode. • **Standby**: Puts the cytometer into Standby/Idle mode. • **Power Off**: Powers off the cytometer. **IMPORTANT** When **Power Off** is selected, the Clean cycle is **not** initiated. • **Clean**: Flushes the instrument sample lines with FlowCLEAN cleaning solution. The cytometer automatically transitions to the Idle mode after the cleanse cycle completes. See CHAPTER 13, *Cleaning Procedures* for details. • **Cytometer Log**: Provides a listing of cytometer messages, including the time stamp, the ID of the error code, and the message that displayed. See CHAPTER 15, *Cytometer Log File* for details
Lasers[a]	By selecting the **On** or **Off** button associated with the red and violet lasers, you can change the current state of each laser. See CHAPTER 7, *Creating a New Protocol* for details.
Kaluza Options	Allows you to adjust settings for the application. See *Kaluza Options Menu* for details.
Exit Kaluza	Closes the application. **IMPORTANT** When the instrument is in the Ready or the Standby state, an automatic Clean cycle occurs when you exit the program. As this process takes 2-3 minutes to complete, attempts to power off the cytometer using the desktop shortcut (CHAPTER 13, *Power OFF the Cytometer*) while the Clean cycle is in process will be unsuccessful.

a. This option is not included in Offline Kaluza for Gallios.

Kaluza Options Menu

The Kaluza Options menu allows you to adjust settings for using the Kaluza for Gallios application. Table 6.5 describes the settings that can be adjusted using this menu.

NOTE Kaluza Options are specific to individual Windows 7 User Accounts. For information on creating User Accounts, refer to *User Preferences and Security*.

Table 6.5 Kaluza Options Menu

<table>
<tr><th>Tab</th><th>Component</th><th>Description</th></tr>
<tr><td rowspan="2">Options
(Figure 6.11)</td><td>Statistics</td><td>• Include a thousands separator for both whole and fractional numbers.
• Display between 0 and 4 decimal places for both fractional numbers and percents.</td></tr>
<tr><td>Compensation</td><td>• Display between 0 and 4 decimal places in the Spillover Matrix, including the Autofluorescence Vector column.</td></tr>
<tr><td rowspan="4">Defaults
(Figure 6.12)</td><td colspan="2">NOTE When default settings are applied, changes affect only newly created plots or newly created data files.</td></tr>
<tr><td>Coloring</td><td>• Change the default plot background color from white to black.</td></tr>
<tr><td>File Name</td><td>• Change the default file name format for *.fcs files.
• Choose the default delimiter type (a space, underscore, or hyphen) to be used in file names.

NOTE If file naming conventions are changed during an acquisition, the new conventions apply to future acquisitions only. The current acquisition is named using the former convention.

NOTE The File Name section is not included in Offline Kaluza for Gallios.</td></tr>
<tr><td>Keywords</td><td>• Allows you to enter the name of your institution, which is included in the listmode data (*.fcs file). This information can be viewed through the Information table when the *.fcs file is opened in Kaluza Analysis Software.

NOTE The Keywords section is not included in Offline Kaluza for Gallios.</td></tr>
<tr><td>Available on both tabs</td><td>Restore initial settings</td><td>• Reinstates all settings listed in the Kaluza Options menu to the defaults. The Restore initial settings functionality applies to any settings made on both the Options and Defaults tabs.</td></tr>
</table>

Figure 6.11 Kaluza Options Menu > Options

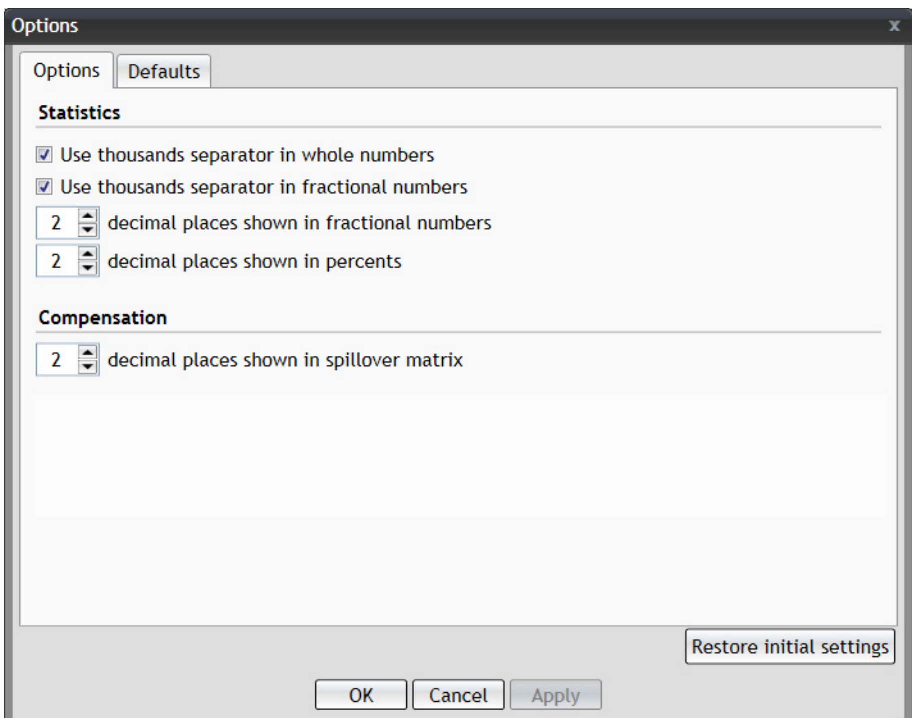
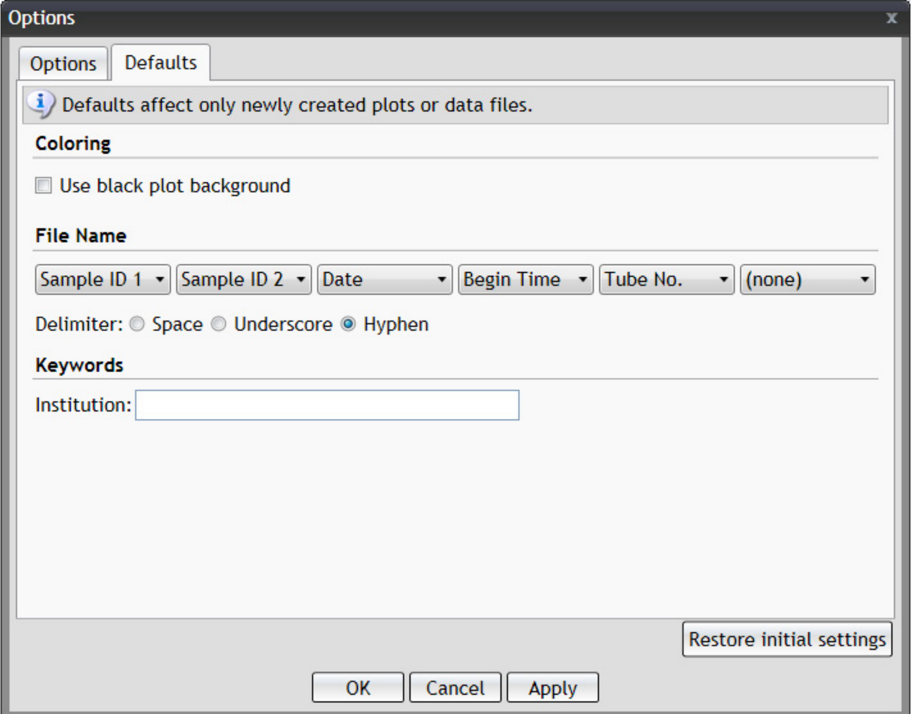


Figure 6.12 Kaluza Options Menu > Defaults



To make changes to the **Kaluza Options** menu:

1 Select > Kaluza Options. The **Kaluza Options** menu appears.

2 Make your changes using the radio buttons, check boxes, and up/down arrows, or use the Restore initial settings button to reset all values.

If you wish to view the changes you made prior to closing the **Kaluza Options** menu, select **Apply**.

3 Select **OK** to implement changes and close the menu.

Quick-Access Toolbar

The Quick-Access toolbar (Figure 6.13) provides convenient access to Kaluza for Gallios functions, including undo, redo, save, and open file.

When you use the Quick-Access toolbar, the save function is limited to saving the current Protocol. Additional options for saving are available through the Application menu (see *Application Menu*) and the Carousel context menu. The functions available on the Quick-Access toolbar are described in Table 6.6.

Figure 6.13 Quick-Access Toolbar

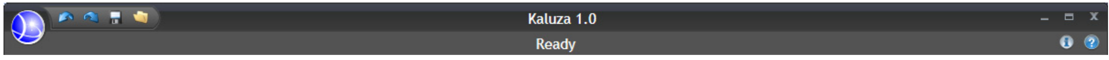
Table 6.6 Quick-Access Toolbar Functions

Icon	Description	Function
	Undo **Redo**	• **Undo**: Steps the software back one action per click of this icon. **NOTE** Ctrl + Z is an additional method for undoing previous actions. • **Redo**: Steps the software forward one action per click of this icon (only available after using the undo function). **NOTE** Ctrl + Y is an additional method for redoing actions. Actions completed prior to starting a sample acquisition are not eligible for undo/redo *once an acquisition is started*. Similarly, once Setup mode is exited, the actions taken while in Setup mode are no longer able to be undone/redone. **IMPORTANT** Undo/redo are not available on functions that do not impact program data. These functions include zoom, scrolling a window, etc.
	Save	Saves the current Protocol file to a location of your choice.
	Open File	Opens a file into the application.

Title/Status Bar

The Title/Status Bar, which is located at the top of the main workspace (see Figure 6.6), displays the software name and version, instrument status, and contains the application button, the quick access toolbar, and title bar options buttons.

Figure 6.14 Title/Status Bar



Title Bar Options

The buttons are available on the Title/Status bar are described in Table 6.7:

Table 6.7 Title/Status Bar Options

Icon	Description	Function
	Minimize	Minimizes the Kaluza for Gallios screen.
	Maximize	Maximizes the Kaluza for Gallios screen to fit the full dimensions of the monitor.
	Restore	Restores the Kaluza for Gallios screen to the previous size.
	Close	Closes the application. **IMPORTANT** When the instrument is in the ***Ready*** or the ***Standby*** state, an automatic Clean cycle occurs when you exit the program. As this process takes 2-3 minutes to complete, attempts to power off the cytometer using the desktop shortcut (CHAPTER 13, *Power OFF the Cytometer*) while the Clean cycle is in process will be unsuccessful.
	Information	Provides access to the Log Directory, a link to the Kaluza website, information about Kaluza for Gallios, including the version, serial number, license type, and copyright information.
	Help	Provides the complete *Kaluza for Gallios Instructions for Use* in a PDF file format.

Acquisition Attributes Panes

Worklist

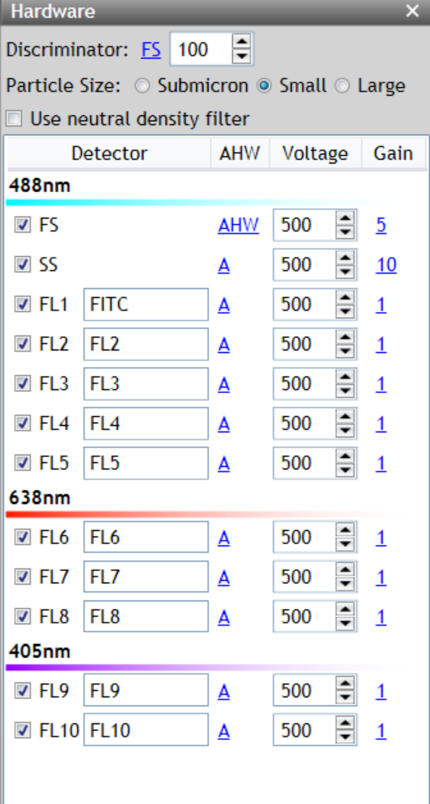
The Worklist (CHAPTER 6, *Worklist—Pane View*) is the hub for sample set-up activities. Through the Worklist, you provide the software with the details about the samples loaded onto the carousel. You can identify which carousel positions contain a sample, give the sample a name, assign a Protocol to each sample, link protocols, and make parameter specifications. The Worklist can be viewed in a pane on the left side of the workspace, or in the expanded view, which fills the screen, allowing you to view all of the data entry fields. For additional information on Worklists, see CHAPTER 8, *Worklists*.

Figure 6.15 Worklist—Pane View

Hardware Pane

The Hardware Pane (Figure 6.16) contains the items necessary for defining and updating parameters, parameter descriptions and settings, particle size, discriminator settings, and choosing to use the neutral density filter on side scatter. For additional information, see CHAPTER 7, *Hardware Configuration*.

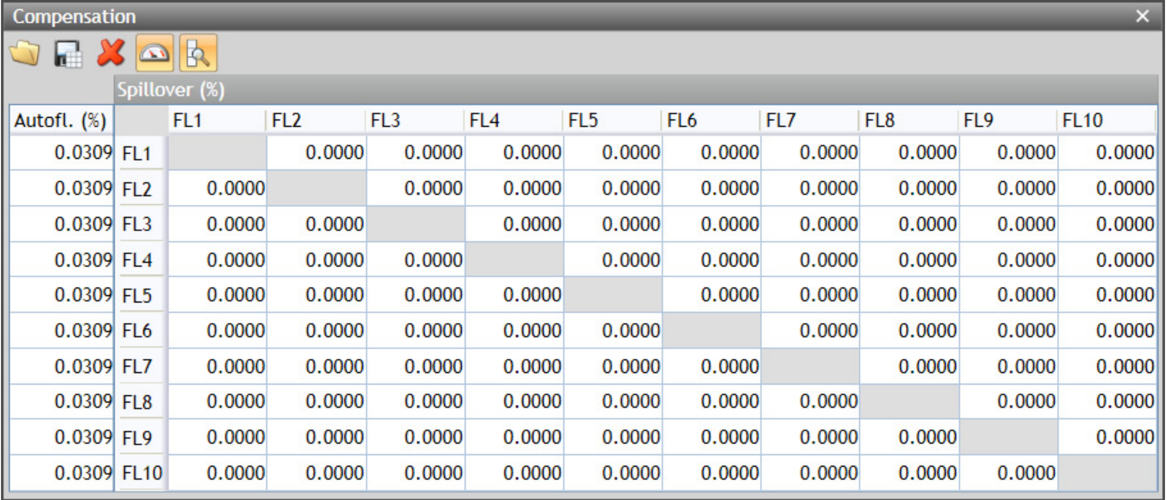
Figure 6.16 Hardware Pane



Compensation Pane

The Compensation pane (Figure 6.17) contains tools for adjusting the Spillover and Autofluorescence Vector values. See CHAPTER 7, *Adjusting Compensation*, for in-depth instructions on how to use the Compensation pane.

Figure 6.17 Compensation Pane

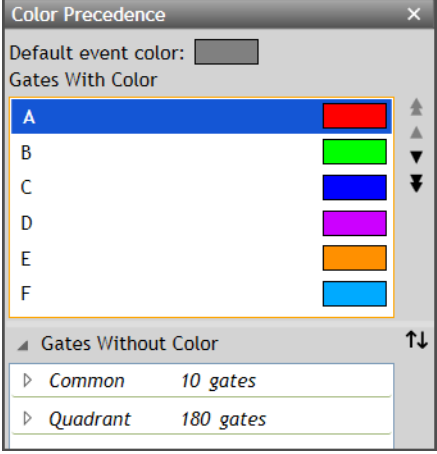


Autofl. (%)		FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8	FL9	FL10
0.0309	FL1		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0309	FL2	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0309	FL3	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0309	FL4	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0309	FL5	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000
0.0309	FL6	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000
0.0309	FL7	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000
0.0309	FL8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000
0.0309	FL9	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000
0.0309	FL10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

Color Precedence Pane

The Color Precedence pane displays event coloring and precedence of coloring for gates in the current Protocol. See CHAPTER 7, *Establishing Color Precedence of Gates*, for in-depth instructions on how to use the Color Precedence pane.

Figure 6.18 Color Precedence Pane



Display Options for the Acquisition Attributes Pane

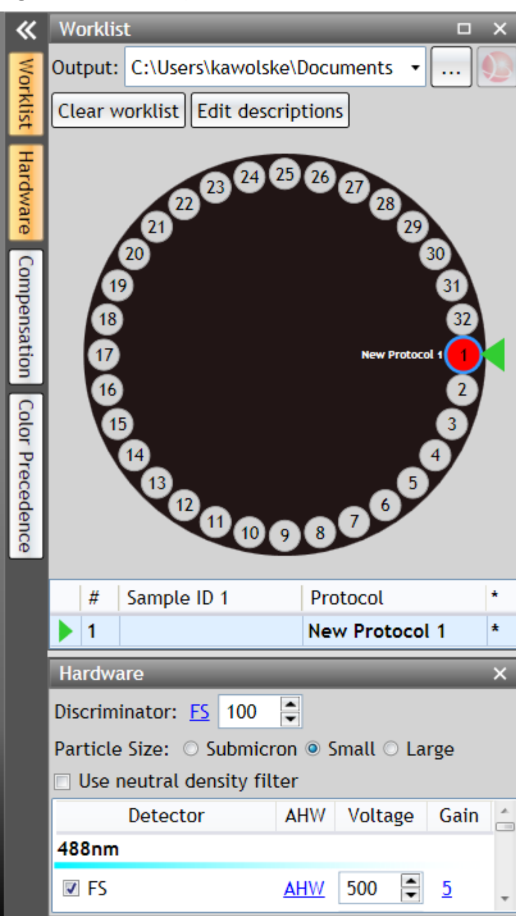
To optimize your workspace, you may wish to change the size of a pane or hide the entire Acquisition Attributes pane.

Hiding a Component Pane

To hide a component pane, select the button in the component pane you wish to close.

The four Acquisition Attributes component panes each have vertically-docked buttons, where the color indicates the status of the pane. The white button indicates the pane is closed, and a gold button indicates that the pane is open. For example, in Figure 6.19, the Compensation and the Color Precedence panes have been closed.

Figure 6.19 Component Pane

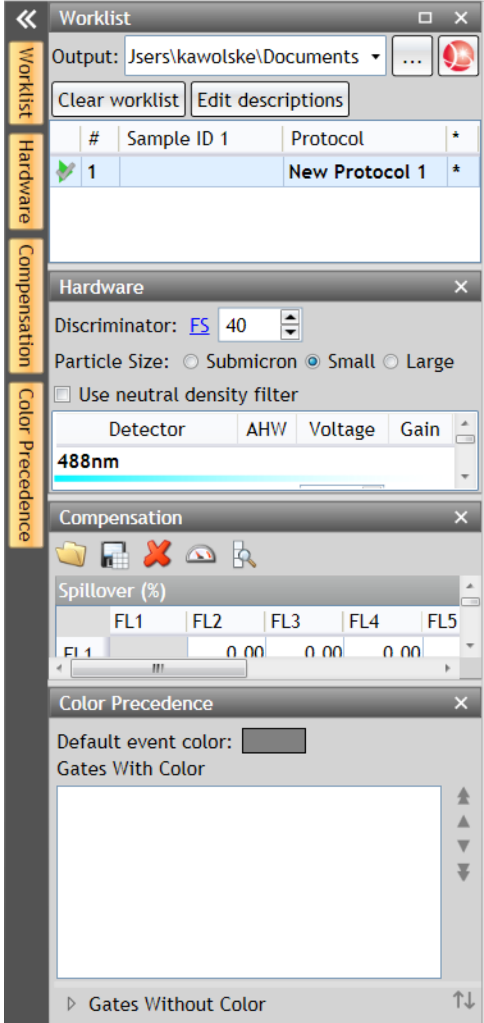


NOTE An additional way to close an Acquisition Attributes component pane is to select the gold button corresponding to the pane you wish to hide.

Displaying a Component Pane

To re-open a pane, select the white button corresponding to the pane you wish to open. See Figure 6.20, which shows all four components of the Acquisition Attributes pane open/active.

Figure 6.20 Acquisition Attributes Pane—Components



Hiding the Acquisition Attributes Pane

To hide the entire Acquisition Attributes pane, select , located at the top of the component pane buttons.

Displaying the Acquisition Attributes Pane

Restore the hidden Acquisition Attributes pane by selecting , which is located at the top of the component buttons.

NOTE When the Acquisition Attributes pane is restored, it is restored with the same active component panes as when it was hidden from view.

Resizing the Acquisition Attributes Panes

To resize the Acquisition Attributes pane:

1. Hover your mouse over the right edge of the pane until the cursor changes to a double-sided arrow (⇔).

2. Click and drag the edge of the pane to the right or left, depending on whether you need to make it smaller or larger.

3. When you are satisfied with the size, release the mouse button.

Resizing Component Panes

To resize (lengthen or shorten) the Worklist, Hardware, Compensation, or Color Precedence panes:

1. Hover your mouse over the bottom edge of the pane until the cursor changes to a double-sided arrow (⇳).

2. Click and drag the edge of the pane up or down, depending on whether you need to make it smaller or larger.

3. When you are satisfied with the size, release the mouse button.

Instrument Control Panel

The **Instrument Control** panel contains the controls for acquisition processes. The panel changes based on the current state of the instrument (Figure 6.21 and Figure 6.22 are examples). These controls are described in detail in CHAPTER 12, *Instrument Control Panel*.

Figure 6.21 Instrument Control Panel—Acquiring Menu

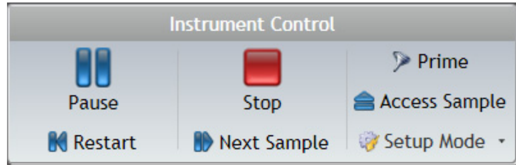
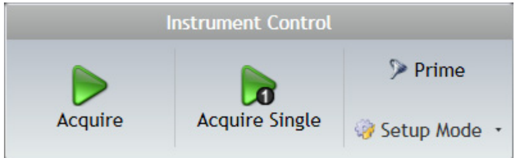


Figure 6.22 Instrument Control Panel—Ready Menu



Ribbon

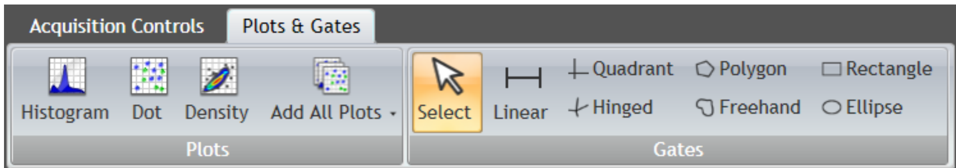
The Ribbon, which is located directly above the plot sheet, provides convenient access to the most-used items within the application. There are two tabs on the Ribbon, which can change slightly, given the current task you are completing. Refer to the following sections for details on each element of the ribbon:

- *Acquisition Controls Tab*
- *Plots & Gates Tab*

Switching Active Ribbon Tabs

To switch between active tabs, select the title of a different tab on the Ribbon Toolbar. In Figure 6.23, the Plots & Gates tab is selected.

Figure 6.23 Ribbon Tabs

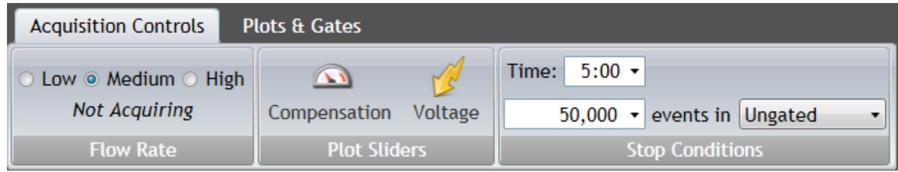


Acquisition Controls Tab

The Acquisition Controls tab (Figure 6.24) provides means for making specifications for acquiring samples and viewing data in the plots. This tab is divided into three sections, including Flow Rate, Plot Sliders, and Stop Conditions. For details, refer to the following sections:

- CHAPTER 7, *Flow Rate*
- CHAPTER 7, *Plot Sliders*
- CHAPTER 7, *Stop Conditions*

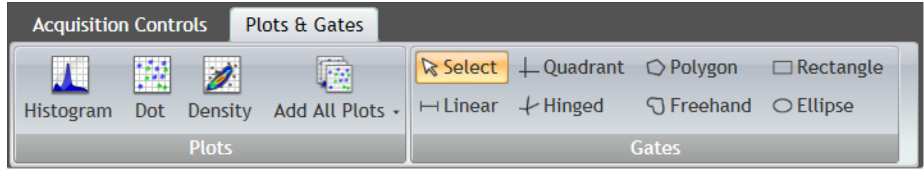
Figure 6.24 Acquisition Controls Tab



Plots & Gates Tab

The Plots & Gates tab (Figure 6.25) is divided into two sections, Plots and Gates.

Figure 6.25 Plots & Gates Tab



Using the Plots & Gates Tab

To make changes or add items to a sheet, use one or both methods described below:

- **Selecting the icon located on the tab**: Select the icon for the specific item you need; this either changes your cursor or adds the new item you selected below any items already on the sheet.
- **Dragging and dropping:** Select the item that you wish to add to the sheet, and then drag and drop it in the location of your choice.

Plots

The Plots section of the Plots & Gates tab displays all plots that are available. Refer to the following sections for details:

- CHAPTER 7, *Histograms*
- CHAPTER 7, *Dot and Density Plots*
- CHAPTER 7, *Add All Plots*

Gates

The Gates section of the Plots & Gates tab displays all options available for gating data. Refer to the following sections for details:

- CHAPTER 7, *Linear Gates*
- CHAPTER 7, *Quadrant Gates*
- CHAPTER 7, *Hinged Quadrant Gates*
- CHAPTER 7, *Polygon Gates*
- CHAPTER 7, *Freehand Gates*
- CHAPTER 7, *Rectangle Gates*
- CHAPTER 7, *Ellipse Gates*
- CHAPTER 7, *Selection Tool*

Zoom

Zoom (Figure 6.26) allows you to bring items on the Plot Sheet closer or move them farther away. Zoom ranges from 50% to 500%. You can either use the slider to change the zoom or the + or - buttons to zoom up or down incrementally by 10%.

Figure 6.26 Zoom

Basic Editing for Plots, Gates, and Plot Sheet Items

You can use the Edit radial menu (available through the icon), or keyboard shortcuts to perform basic editing functions, including cut, copy, paste, and delete. Table 6.8 provides details regarding the availability of these functions and any specific details regarding use.

Table 6.8 Editing Plots, Gates, and Sheet Items

Description	Icon/Shortcut		Use on...		
	Edit Radial Menu	Keyboard Shortcut	Plots	Gates	Sheet Whitespace
Cut is used to remove an item from the sheet or plot. The removed item is available for pasting to any valid location.		Ctrl + X	✓	✓	-
Copy is used to duplicate selected items. The selected item is available for pasting to any valid location.		Ctrl + C	✓	✓	-
Paste inserts data made available by **Cut** or **Copy** to the location of your choice.		Ctrl + V	✓	✓	✓
Delete eliminates a selected item. **NOTE** The only way to retrieve an item that has been deleted is by selecting the (undo) icon.		Delete	✓	✓	-

NOTE Plots or gates must be selected prior to performing editing tasks. These functions are available for multi-selection.

Using the Plot Sheet

Plot Sheet Radial Menus Options

The plot sheet radial menu (Figure 6.27) provides access for changing or adding items to your sheet. The following sections provide an overview of the options available when you right-click on the sheet white space to access the radial menu.

Figure 6.27 Sheet Radial Menu

Display Menu

Use the (Display menu) to add compensation or voltage sliders to applicable plots. See CHAPTER 7, *Plot Sliders*.

Gates & Tools Menu

Use the (Gates & Tools menu) to draw a gate on a plot. See CHAPTER 7, *Gates*.

Add Item Menu

Use the (Add Item menu) to add a plot to the Plot Sheet. See CHAPTER 7, *Plots*.

Edit Menu

Use the (Edit menu) to make edits to items on the Plot Sheet. See *Basic Editing for Plots, Gates, and Plot Sheet Items*.

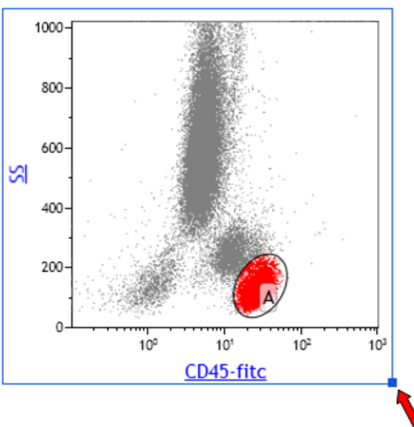
Adjusting the Size of a Plot

To adjust the size of a plot:

1 Select the item you wish to adjust.

2 Select the handle (indicated by the red arrow in Figure 6.28) and drag in the direction you wish to make your change.

Figure 6.28 Resizing a Plot



3 Release your mouse button when you are satisfied with the size.

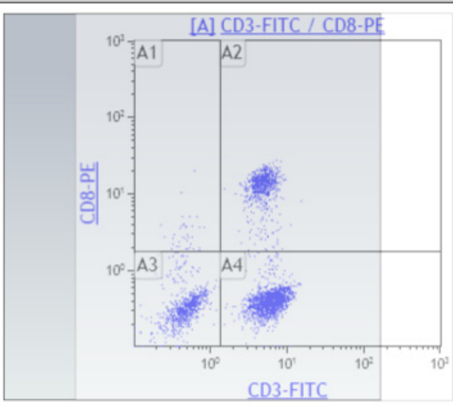
Moving Plots

To move a plot to a new location on your sheet:

1 Select the plot you wish to move.

2 Drag the plot to the new location. As you drag you plot, a gray box will appear (as shown in Figure 6.29) indicating the locations where you can drop the plot. When the gray box appears in the location of your preference, release your mouse button.

Figure 6.29 Moving a Plot



CHAPTER 7

Protocols

Introduction

Protocols, which are made up of parameters, plots, and gates, are key to accurate data collection and analysis. Through its plots, the Protocol allows you to view the results of the data collected by the flow cytometer, as the plots translate the raw data into a meaningful format. This allows you to compare the event populations of selected parameters. Furthermore, the use of gating allows you to zero in on specific event subpopulations, where you can separate the events that are positive or negative for specific phenotypes.

Protocols are essential for accurately collecting raw data during the sample acquisition process, as they provide the means for adjusting instrument settings, including discriminator, voltages, gains, and compensation; when adjusted correctly, this ensures that results are accurate as possible. Once Protocols are set up and saved, they can be used for multiple samples, allowing you to standardize results and simplify the process.

This chapter covers the following topics, which are all part of the process of creating Protocols:

- *Creating a New Protocol*
- *Parameters*
- *Plots*
- *Gates*
- *Hardware Configuration*
- *Acquisition Controls*
- *Compensation*

Creating a New Protocol

Each sample must have a Protocol associated with it prior to beginning the acquisition process. To create a new Protocol:

1 From the Kaluza for Gallios home screen, select **New protocol**.

OR

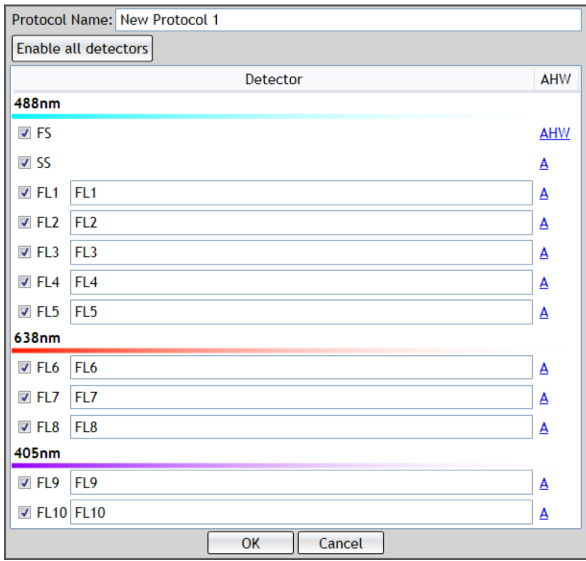
Select > **New Protocol**.

OR

Ctrl + N

A screen opens (Figure 7.1), allowing you to select and define parameters. See *Parameters* for additional information on the parameters to select for your acquisition.

Figure 7.1 Parameter Setup Screen



NOTE A new Protocol is assigned to the first empty sample location in the carousel.

2 If desired, in the **Protocol Name** field, enter a name for the Protocol.

3 Choose the parameters you wish to include in the acquisition by clicking in the check boxes located next to the applicable detector names.

OR

Select [Enable all detectors] if you are using all of the detectors.

IMPORTANT Risk of Protocol errors. Offline Kaluza for Gallios uses a 10-color configuration, but your instrument might be equipped with a 6- or 8-color configuration. Be sure to only use the parameters on your instrument when working offline.

NOTE Parameter settings can also be updated in the Hardware pane. See CHAPTER 7, *Hardware Configuration* for details.

4 Select the link in the **AHW** (Area = **A**, Height = **H**, Width = **W**) column corresponding to the parameter you wish to update. The software defaults to displaying results for Area only (**A**) on most detectors. Height (**H**) and Width (**W**) are also enabled by default on FS to allow for doublet discrimination. To change the measurement type, or to choose additional types, select the A link for the parameter. In the pop-up menu (Figure 7.2), choose the type(s) you wish to collect by clicking on each needed measurement type, or deselect measurement types by clicking on the row again.

NOTE Measurement selections can be updated in the Hardware pane; however, the selections cannot be changed during an acquisition. See *Hardware Configuration* for details.

Figure 7.2 Measurement Pop-Up Menu

5 If desired, in the **Description** field, enter a description for each parameter. The parameter descriptions display on plots and in the Worklist.

6 Select **OK** to continue setting up your Protocol.

7 Create the plots you wish to include in your Protocol. See *Plots* for details on the plots available in Kaluza for Gallios, adding plots to the plot sheet, and setting up plots.

8 If an analysis will be performed during acquisition, create the required gates. See *Gates* for complete details on creating and setting up gates.

9 Select > **Save Protocol.**

OR

From the Carousel Display on the Worklist, right click on the sample location and select **Save Protocol As...**.

10 In the **Save protocol as** window, navigate to the location where you wish to save the file, and select **Save**. The file is saved with the ***.protocol** extension and is now ready to apply to other samples. To apply this Protocol to other samples, see CHAPTER 8, *Creating a New Worklist*, step 9.

Parameters

The parameters you need to include in your acquisition depend on the number of fluorescent dyes in your sample or parameters available, depending on your instrument. Your instrument is either equipped with a 6-, 8-, or 10-color configuration. The Time parameter is automatically included in every acquisition. Table 7.2 describes each parameter type in detail.

Because Forward Scatter, Side Scatter, and each fluorescence parameter can be measured using up to three measurement types (see *Parameter Measurements* for details) and Time is automatically included in the acquisition, each sample could potentially contain an extensive amount of information, as ***each event*** can record up to the following number of parameters:

- **37** parameters for a **10-color** configuration
- **31** parameters for an **8-color** configuration
- **25** parameters for a **6-color** configuration

Kaluza for Gallios can record data for up to 25 million events for an 8-parameter configuration. Each additional enabled parameter lowers the event maximum, as more information (parameters/measurements) is being recorded for each event. Refer to Table 7.1 to view the maximum number of events for your configuration.

Each parameter is further specified by the scale selected for plot display. See *Choosing Scale Type* for details.

Table 7.1 Maximum Events Per Configuration

Parameters Enabled	Maximum Events	Parameters Enabled	Maximum Events
1	25,000,000	20	10,000,000
2	25,000,000	21	9,523,809
3	25,000,000	22	9,090,909
4	25,000,000	23	8,695,652
5	25,000,000	24	8,333,333
6	25,000,000	25	8,000,000
7	25,000,000	26	7,692,307
8	25,000,000	27	7,407,407
9	22,222,222	28	7,142,857
10	20,000,000	29	6,896,551
11	18,181,818	30	6,666,666
12	16,666,666	31	6,451,612
13	15,384,615	32	6,250,000
14	14,285,714	33	6,060,606
15	13,333,333	34	5,882,352
16	12,500,000	35	5,714,285
17	11,764,705	36	5,555,555
18	11,111,111	37	5,405,405
19	10,526,315	*N/A*	*N/A*

Table 7.2 Parameters

Parameter	Description
Forward Scatter	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 can be measured in area, height, and/or width; see *Parameter Measurements* for a description of each type.
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 can be measured in area, height, and/or width; see *Parameter Measurements* for a description of each type.
Fluorescence	Fluorescence is 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. Depending on the number of detectors enabled on your instrument, you could capture up to 30 fluorescence parameters, as each fluorescence detector can record up to three different types of measurements (see *Parameter Measurements* for details.)
Time	The Time parameter is the amount of time, in integral 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). The Time parameter is automatically included as part of the acquisition process. **NOTE** 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 in Figure 7.3. Unexpected fluctuations in the pattern of fluorescence may indicate compromised fluidics or optic conditions. **Figure 7.3** Time vs. Fluorescence Plot [Ungated] TIME / FITC FITC TIME

Parameter Measurements

Forward scatter, side scatter, and fluorescence parameters are measured in area, height, and/or width. See Table 7.3 for a brief description of each. Each parameter measurement is displayed on plots using either the log, linear, or logicle scale; see *Choosing Scale Type* for details.

IMPORTANT You cannot change the measurement selections while an acquisition is in process. If you need to change your selections, you must stop the acquisition, make changes, and then restart the acquisition.

Table 7.3 Parameter Measurements

Type	Description
Area	A voltage pulse with height and area proportional to the total amount of light generated by an event. For additional information, see CHAPTER 3, *Area Signal*.
Height	A voltage pulse that rises to its highest point as a particle reaches the center of the laser beam and indicates the maximum brightness of the event. For additional information, see CHAPTER 3, *Height Signal*.
Width	The transit time of a cell or particle to traverse the laser beam. For additional information, see CHAPTER 3, *Width Signal*.

NOTE If multiple measurements are selected for a parameter, the measurement type displays with the parameter name on the plot. For example, in Figure 7.4, FS was measured on Height, Area, and Width during acquisition; because the Area measurement was selected for FS, **FS Area** displays on the axis.

Figure 7.4 Parameters/Measurement Types

Choosing Scale Type

Choosing the appropriate scale for your data is very important. The ability to produce meaningful plots is dependent upon selecting the appropriate scale. Scale type selections may also be controlled through the **Data** radial menu.

Logarithmic (Log) Scale

The log scale is useful when the data includes a large range of values, as the logc scale changes the range by using ratios (for example, cell surface marker fluorescence parameters). Fluorescence parameters are best displayed in log scales because the scale is expanded to display weak signals and compressed to show strong signals. In Kaluza for Gallios, the number of decades can be adjusted to fit the data.

Linear Scale

The linear scale contains divisions that are uniformly spaced. The linear scale is good for showing forward scatter and side scatter parameters and width measurements.

Logicle Scale

When using the log scale, correctly compensated data may appear to be incorrectly over-compensated because events with negative values tend to pile along the axes; this distortion occurs because negative values do not exist on a log scale. Kaluza for Gallios includes the logicle scale, which provides a means to correctly display compensated data. Changing an axis from log to logicle scale splits the axis into two different regions, where the positive values remain in log scale and negative values are transformed into linear scale. The two different scales are divided by a slider, which provides the ability to interactively control the width of each region. When you use the logicle scale, negative values display correctly, preserving the desired symmetrical appearance of correctly compensated data. See *Using the Logicle Scale* for additional details.

Plots

The following sections describe creating and configuring the following plots:

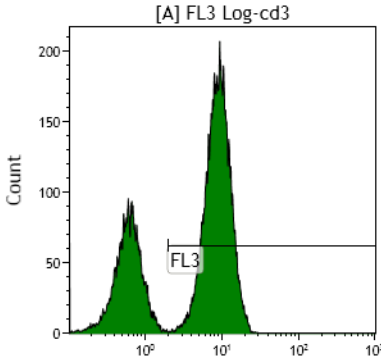
- *Histograms*
- *Dot Plots*
- *Density Plots*
- *Add All Plots*

Histograms

A histogram (Figure 7.5) represents a frequency distribution, where height depicts corresponding frequencies. The following parameter options are available for each axis:

X-Axis	Y-Axis
Any parameter within the Data Set in linear, log, or logicle scale, or in integral seconds.	• Count • % Gated **NOTE** Selecting a parameter other than **Count** or % **Gated** for the Y-Axis parameter changes the plot to a dot plot. Any gates created for the histogram plot are removed when the plot type is changed.

Figure 7.5 Histogram Plots



Setting Up Histograms

IMPORTANT The content within the Hardware pane directly affects how parameters are displayed on plots. See *Hardware Configuration* for complete instructions on updating parameter descriptions.

To set up a histogram:

1. From the Plots & Gates Ribbon tab, select the Histogram icon, and drag it to the preferred location on your sheet.

2. Hover your mouse over the parameter hyperlink at the bottom of the histogram plot.

3. Select the hyperlink. A list of parameters appears.

4. Select the new parameter.

If...	Then...
Using the default scale type for the parameter,	Select the parameter from the pop-up. You do not have to select the scale type. Default scale types are as follows: • **FS/SS**: Linear scale • **Fluorescence**: — **Area** or **Height,** Log scale — **Width**, Linear scale • **Time**: Seconds (integral)
Using a scale type other than the default,	**1.** Hover your mouse over the parameter. A pop-up appears, allowing you to choose the scale type for your parameter. **2.** Select the scale that best suits your data. For additional details on available scale types, see *Choosing Scale Type*.

5 Select the hyperlink located on the Y-axis of the plot if you need to change the measurement type.

6 Choose the appropriate measurement type from the pop-up list.

NOTE Events in histogram plots default to being scaled on **Count**.

7 Choose a gate for your plot, if needed, using the hyperlink located at the top of the plot.

8 Continue customizing your plot using the radial menus. Table 7.4 provides specific information, as well as links to general options, for setting up a histogram plot.

Table 7.4 Histogram Set-Up Options

Radial Menu	Plot Set-Up Details
Data	The **Histogram Options** section within the Data radial menu is for customizing your histogram plots for optimal data presentation. Specifically, these options are available: • Smoothing your data for a more pleasant appearance. • Clipping the first and last channels (for scaling purposes only). • Adjusting the Y-axis scale to better fit your data. See *Setting Up Plot Data*, for general information on making changes to plot data, including parameter axis data, input gates, and bivariate resolution.
Coloring	Use the Coloring radial menu to update the coloring associated with a plot. See *Using the Coloring Menu*.
Edit	Use the Edit radial menu to perform basic plot editing functions, including cut, copy, paste, and delete. See *Basic Editing for Plots, Gates, and Plot Sheet Items*.
Statistics	Use the Statistics radial menu to choose statistics to display at the bottom of the plot. See *Setting Up Statistics*.
Display	Use the Display radial menu to alter the size of a plot or to change the information that displays on a plot. See *Setting Up Plot Display*.
Gates & Tools	Use the Gates & Tools radial menu to add a gate to a plot. See *Using the Gates & Tools Plot Radial Menu*. See *Linear Gates* for details on gating a histogram plot.

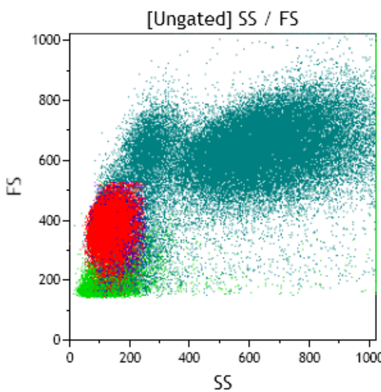
Dot and Density Plots

Dot Plots and *Density Plots* compare two parameters to determine their relationship. You can choose any parameter within the Protocol for an axis. A density plot is a specific type of dot plot with different starting options for the coloring algorithm. *Setting Up Dot and Density Plots* provides details on setting up dot and density plots.

Dot Plots

A **dot plot** (Figure 7.6) compares two parameters to determine their relationship. Each event that contains markers for the two sets of data being compared appears as a dot.

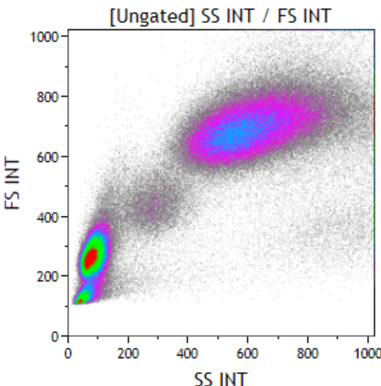
Figure 7.6 Dot Plot



Density Plots

A **density plot** (Figure 7.7) is a five-color representation of the number or percentage of events that occur in comparing X-axis and Y-axis parameters. Greater and lesser density of event occurrences are represented by different colors.

Figure 7.7 Density Plot



Setting Up Dot and Density Plots

To set up a dot or density plot:

1 From the Plots & Gates Ribbon tab, select the Dot or Density icon, and drag it to the preferred location on your sheet.

2 Select the **<Choose a parameter>** hyperlink. A pop-up appears with a list of parameters.

NOTE When you are updating a parameter, the currently selected parameter is displayed instead of **<Choose a parameter>**, the pop-up list contains a dot next to the parameter name/description (**e.g., FL1** in Figure 7.8), and the scale used for the parameter contains a check mark next to the scale type (**e.g., FL1-A-Log**).

Figure 7.8 Parameter Updates

3 Make your selection by clicking on the appropriate parameter from the pop-up list. The scale for the parameter you choose defaults to **log** for fluorescence parameters, **seconds** (integral) for Time, and **linear** for all others. If you wish to choose a scale other than the default, hover your mouse over the parameter you wish to display, and choose the scale type for the parameter from the pop-up list.

NOTE Selecting **Count** or **% Gated** as a Y-axis parameter changes a dot or density plot to a histogram.

4 Repeat this process for the other parameter, if necessary.

5 If needed, choose a gate for your plot using the hyperlink located at the top of the plot.

6 Continue customizing your plot using the radial menus. Table 7.5 provides specific information, as well as links to general options, for setting up a dot or density plot.

Table 7.5 Dot and Density Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Data	The **Bivariate Options** section of the data menu also provide the option to swap axes with the click of a button. This automatically reorients any gates on the plot. See CHAPTER 7, *Setting Up Plot Data*, for general information on making changes to plot data including parameter axis data, and input gates.
Edit	Use the Edit radial menu to perform basic plot editing functions, including cut, copy, paste, and delete. See CHAPTER 6, *Basic Editing for Plots, Gates, and Plot Sheet Items*.
Statistics	Use the Statistics radial menu to choose statistics to display at the bottom of the plot. See CHAPTER 7, *Setting Up Statistics*.
Display	Use the Display radial menu to: • Alter the size of a plot. • Change information that displays on a plot. • Change the bivariate resolution; options include: — 128 X 128 — 256 X 256 — 512 X 512 — 1024 X 1024 — 2048 x 2048 See CHAPTER 7, *Setting Up Plot Display*.

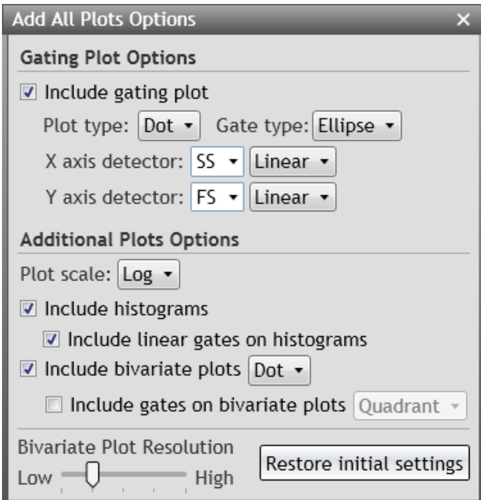
Table 7.5 Dot and Density Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Gates & Tools	Use the Gates & Tools radial menu to add a gate to a plot. See *Using the Gates & Tools Plot Radial Menu*. See *Gates* for details on gating the plot.
Coloring	Use the Coloring radial menu to: • Change the background to black. • **Use Gate Coloring**, where only events located in gates are colored, as specified in the Color Precedence pane. • **Use Single Color**, where all events located in the plot are changed to the default event color, as specified in the Color Precedence pane. • **Use Density Coloring**, where event coloring is based on the density of events. — **Set Band Ranges Manually** allows you to specify the banded color ranges. ▪ Select the check box to enable manually setting band ranges. ▪ Select the up/down arrows or select the entry and type the new value into the field. See *Using the Coloring Menu*, for additional details and options associated with using the Coloring radial menu.

Add All Plots

Add All Plots is a customizable option that automatically adds multiple plots/gates to the plot sheet. The plot types and gate types that are added depend on the configurations you select. To define the options you would like to include when **Add All Plots** is selected, select the drop-down arrow on the Add All Plots icon; this opens the **Add All Plots Options** window (Figure 7.9), which is described below. Once configurations are made, select the icon to add all of your selected plots to the Plot Sheet.

Figure 7.9 Add All Plots



Gating Plot Options

- **Include Gate Plot**: When this check box is selected, a gating plot is included.
  - **Plot Type**: Dot (default) or Density.
  - **Gate Type**: Ellipse (default) or Rectangular.
  - **X and Y Axis Detector**: Use to assign parameters and scale to the X and Y axis of this gating plot.

Additional Plot Options

- **Plot Scale**: Linear, Log (default), or Logicle.
- **Include Histograms**: Adds histograms of each fluorescence parameter are added.
  - **Include linear gates on histograms**: Adds a linear gate is added to each histogram.
- **Include bivariate plots**: Includes bivariate plots comparing all fluorescence parameters to each other are added. Dot (default) or Density.
  - **Include gates on bivariate plots**: Adds the selected gate type to the bivariate plots.
- **Gate Type Options**: Quadrant (default), Hinged, Ellipse, Rectangle.
- **Bivariate Plot Resolution**: Use the slider to control the plot resolution.
- **Restore initial settings**: Use this button to restore the default selections.

Adding Plots to the Plot Sheet

To add a plot to the plot sheet:

1 Select the Plots & Gates Ribbon tab.

2 To add the new plot:

- **To the bottom of the plots already included on the sheet**: Select the icon corresponding to the type of plot you wish to include in your analysis.
- **To a specific location on the plot sheet**: Click the specific plot icon and drag it to the location you prefer, and then release the mouse button.

NOTE This option is not available for Add All Plots.

Plot Set-Up

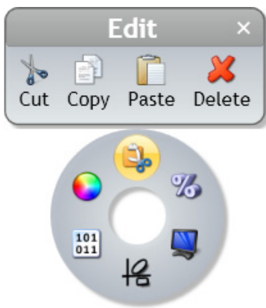
Kaluza for Gallios offers multiple options for customizing your plots. The following sections describe these options and provide instructions for setting up the plots to meet your needs. Because radial menus are the main source for making changes to your plots, the sections to follow focus primarily on using the radial menus for making your changes.

NOTE Radial menu options will vary, depending on the plot type and if multiple plots are selected.

Editing Plots

To perform basic plot editing functions, including cut, copy, paste, and delete, use the Edit radial menu (see Figure 7.10). See CHAPTER 6, *Basic Editing for Plots, Gates, and Plot Sheet Items*, for a description of the functions available on the Edit radial menu.

Figure 7.10 Edit Radial Menu



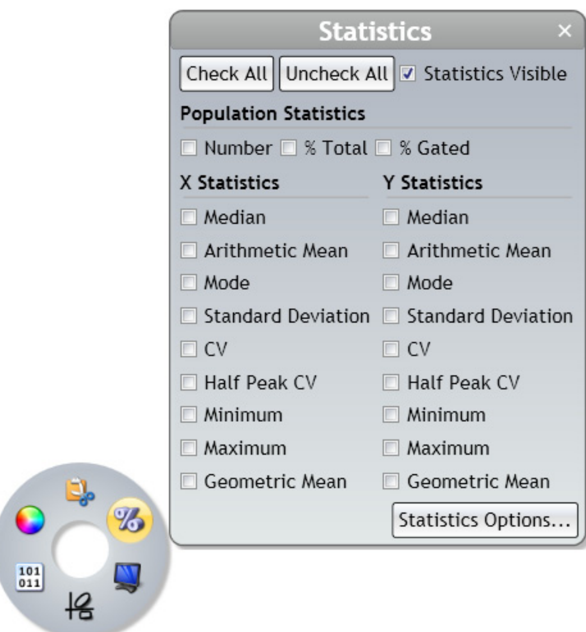
Setting Up Statistics

To choose statistics to display at the bottom of the plot, use the Statistics radial menu (see Figure 7.11), which provides access for selecting statistics to display on the plot.

- See *Using the Statistics Radial Menu*, for general instructions on using the Statistics radial menu.
- Figure 7.12 is an example of statistics displayed on a plot.
- Refer to APPENDIX B, *Statistics* for information on how statistics are derived.

NOTE Available statistics are different, depending on the plot type.

Figure 7.11 Statistics Radial Menu

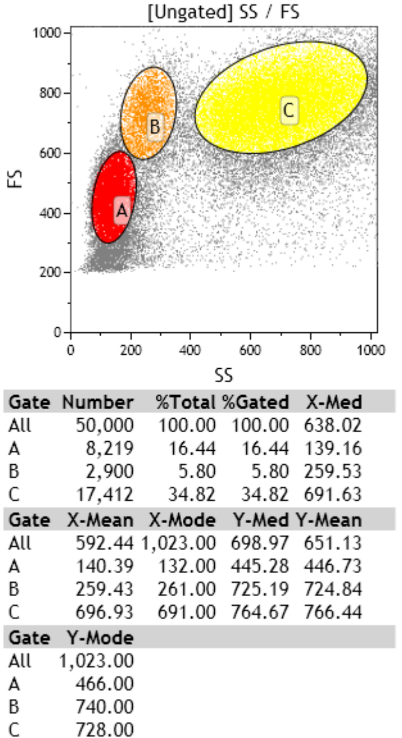


The Statistics menu contains the options listed in Table 7.6:

Table 7.6 Statistics Menu Options

Menu Option	Description
Check All	Selects all statistics available for a plot.
Uncheck All	Removes all selections previously set for a plot.
Statistics Visible	Displays chosen statistics on a plot. When deselected, any statistics you previously chose will be retained, but will not display on your plot. By default, **Statistics Visible** is selected.
Population Statistics	Allows you to choose statistics related to all events within a plot, including **Number**, % **Total**, and % **Gated**.
X Statistics/ Y Statistics	Allows you to choose statistics related to the X and Y axes, including **Median**, **Arithmetic Mean**, **Mode**, **Standard Deviation, CV**, **Half Peak CV**, **Minimum**, **Maximum**, and **Geometric Mean**. **NOTE** Histogram plots only display X statistics.
Statistics Options...	Allows you to make systemic changes to the appearance of statistics, including using thousands separators in whole numbers and/or fractions and choosing between 0 and 4 decimal places in fractional numbers and/or percents. See CHAPTER 6, *Kaluza Options Menu*.

Figure 7.12 Plot Statistics



Gate	Number	%Total	%Gated	X-Med
All	50,000	100.00	100.00	638.02
A	8,219	16.44	16.44	139.16
B	2,900	5.80	5.80	259.53
C	17,412	34.82	34.82	691.63

Gate	X-Mean	X-Mode	Y-Med	Y-Mean
All	592.44	1,023.00	698.97	651.13
A	140.39	132.00	445.28	446.73
B	259.43	261.00	725.19	724.84
C	696.93	691.00	764.67	766.44

Gate	Y-Mode
All	1,023.00
A	466.00
B	740.00
C	728.00

Using the Statistics Radial Menu

To use the Statistics menu:

1. Select the plot(s) for which you wish to display statistics.
2. Access the Statistic radial menu by right-clicking on the selected plot.
3. Hover your mouse over the icon to access the Statistics menu.
4. Make your selection(s) using the buttons and/or check boxes.
5. Select ✕ to complete the process.

Setting Up Plot Display

Use the Data radial menu to change data associated with a plot, including plot resolution, title, and axes (See Figure 7.13).

See *Using the Display Radial Menu*, for general instructions on using the Display radial menu.

NOTE The Display menu contains different options, depending on the type of plot.

Figure 7.13 Display Radial Menu

The Display menu contains the options listed in Table 7.7.

Table 7.7 Display Menu Options

Menu Option	Description
Maximize	Increase the size of the plot to fit within the sheet portion of the application. **NOTE** Double-clicking on a plot also maximizes a plot, and double-clicking on a maximized plot returns the plot to the previous size. After you have maximized a plot, three buttons appear below the plot: • **Previous**: Shows the previous plot located on the sheet in the maximized view. • **Restore**: Returns the plot to its previous size. • **Next**: Shows the next plot located on the sheet in maximized view.
Reset Size	Return a plot that has been resized back to the default size and shape.
Plot Resolution	Adjust the resolution of bivariate plots using the slider. Options include: • 128 x 128 • 256 x 256 • 512 x 512 • 1024 x 1024 • 2048 x 2048 Higher resolutions should be used on data sets with large numbers of events to assist in viewing populations.
Title	Allows you to customize the title that appears at the top of the bivariate plots. You may select **Use Custom Title**, which prompts a **Title** field, allowing you to create your own title. Additional options include showing the following information in the plot title: • Input Gate • Data Set Identifier • Name • Description • Scale (linear, log, or logicle)
Axes	Allows you to customize the axes included in your plot. Choose **Use Custom Axes** to prompt a field to display for each axis in the plot, which allows you to create your own title. If **Use Custom Axes** is not selected, you may include: • Axis Tick Marks • Axis Grid Lines • Name • Description • Scale **NOTE** The parameter measurement is automatically included in the axis labels when it is needed to differentiate between Area, Height, or Width measurements.

Using the Display Radial Menu

To use the Display menu:

1. Select the plot for which you wish to change the display.
2. Access the Display radial menu by right-clicking on the selected plot.
3. Hover your mouse over the icon to access the Display menu.
4. Make your updates in the Display menu.
5. Select to complete the process.

Using the Gates & Tools Plot Radial Menu

To add a gate, the Gates & Tools menu (see Figure 7.14) provides immediate access for choosing these options.

NOTE The Gates & Tools menu includes the same options no matter where you are currently located on the sheet. If a gate selected from the menu is not appropriate for the current sheet location, the cursor appears as a symbol. When you are in an appropriate location for the gate type, the cursor changes to the symbol for the gate type you selected, indicating that you may begin drawing.

See *Gates*, for an in-depth description of gating.

Figure 7.14 Gates & Tools Radial Menu

Gates

Gates that are accessible through the Gates & Tools menu include the following:

- Linear
- Quadrant
- Hinged
- Polygon
- Freehand
- Rectangle
- Ellipse

To enable Gate-Drawing mode:

1. Access the Gates & Tools radial menu by right-clicking on a plot.

2. Hover your mouse over the icon to access the Gates & Tools menu.

3. Select the gate type you need from the menu.

   NOTE As you hover your mouse over each icon, the tooltip shows the name of the gate that corresponds with the icon.

4. Your cursor changes to resemble the type of gate you wish to draw. See *Gates*, for details on drawing specific gate types.

Setting Up Plot Data

To change data associated with a plot, including the parameter axis data, input gates, and scale, use the Data radial menu (see Figure 7.15).

For general instructions on using the Data radial menu, see *Using the Data Radial Menu*.

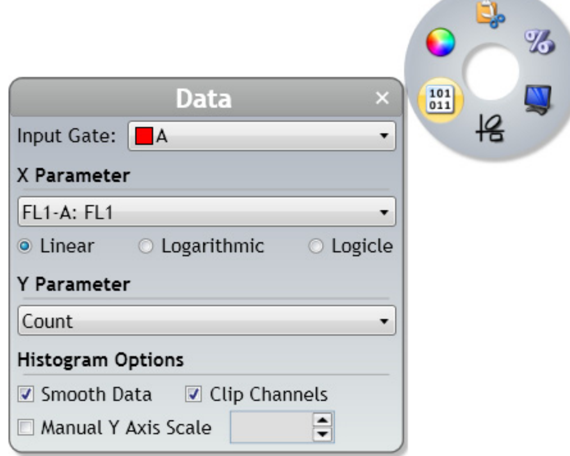
Data Menu Options—All Plots

The Data menu for all plots contains the options listed in Table 7.8.

Table 7.8 Data Menu Options for All Plots

Menu Option	Description
Input Gate	Allows you to change the input gate from which a plot is gated.
X Parameter/ Y Parameter	Allows you to make axis-related data changes, including the following: • Change the X or Y parameter. • Select a different scale; options include linear, log, or logicle. • Select the number of decades displayed (log or logicle). • Change the negative percentage (logicle only). • Swap axes (bivariate plots only). **NOTE** Other options are available, depending on the plot type.

Figure 7.15 Data Radial Menu



Using the Data Radial Menu

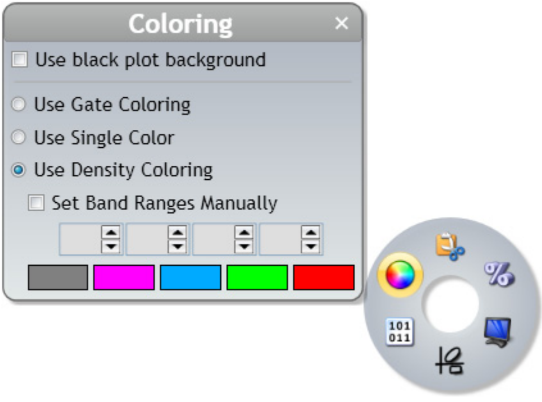
To use the Data menu:

1. Select the plot for which you wish to change the data.
2. Access the Data radial menu by right-clicking on the selected plot.
3. Hover your mouse over the icon to access the Data menu. The Data menu for the specific plot type appears.
4. Enter your updates.
5. Select to complete the process.

Using the Coloring Menu

To update the coloring associated with a plot, use the Coloring radial menu (Figure 7.16 is an example of the Coloring menu when on a density plot), which provides access to making multiple types of coloring updates to plots.

Figure 7.16 Coloring Radial Menu



Updating Colors

Color blocks are designed for changing the color of bands in density plots.

NOTE Other than the ability to turn on/off gate coloring, changes to gate coloring are not made through the Plot radial menu. Use the Color Precedence pane or the Gating radial menu to change the color associated with events in a gate (see *Color Events*, for details).

To make changes to density coloring:

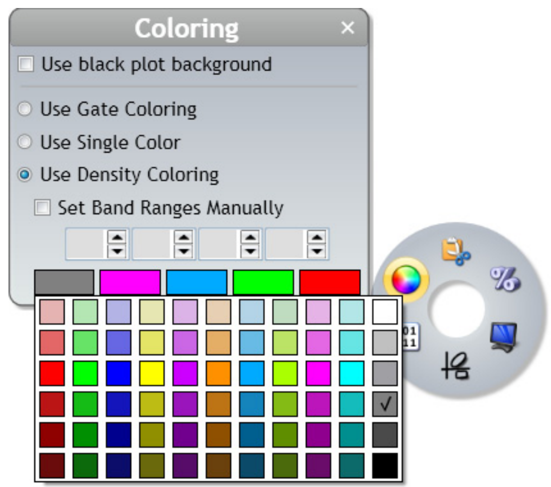
1 Access the Colors radial menu by right-clicking on a plot.

2 Hover your mouse over the icon to access the Coloring menu.

3 Select **Use Density Coloring**.

4 Select the color block you wish to change. The color palette appears.

5 Select any color from the palette that does not contain a check mark (the check mark indicates the current color). Refer to the details on the following color swatch.



6 Select ✕ to finish the process.

Gate Coloring

You may choose between displaying the gate coloring set up in your Protocol or using single color for the plot. These options are described in Table 7.9.

Table 7.9 Gate Coloring Menu

Menu Option	Description
Use Single Color	Changes all events on the plot to the default event color (set up in the Color Precedence pane).
Use Gate Coloring	Displays gate coloring used in the Protocol. Use the Color Precedence pane to change gate coloring.

NOTE The **Use Density Coloring** is a third option for Dot and Density plots, as described in Table 7.5.

To turn on/off gate coloring on a plot:

1 Right click on the plot and hover your mouse over the icon to access the Coloring menu.

2 Choose the radio button for the gate-coloring option you prefer.

3 Select ✕ to finish the process.

Gates

Kaluza for Gallios offers seven different gate types, allowing you to precisely define your data. These gate types are described in detail in Table 7.10:

Table 7.10 Kaluza for Gallios Gates

Icon	Gate Type
	Linear Gates
	Quadrant Gates
	Hinged Quadrant Gates
	Polygon Gates
	Freehand Gates
	Rectangle Gates
	Ellipse Gates

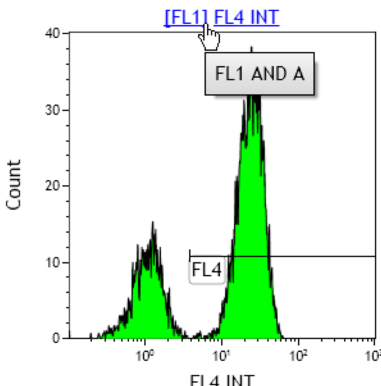
IMPORTANT If you wish to make multiple gates of the same type, hold down the (Shift) key to remain in drawing mode. Release the (Shift) key to escape.

Each Data Set being analyzed may contain a maximum of 318 gates. Up to 31 gates can be assigned a color for each data set.

Viewing Gate Logic

Hovering over the gate assignment at the top of a plot (Figure 7.17), as well as hovering over the gate on the Color Precedence List, allows viewing the gate logic for any gate.

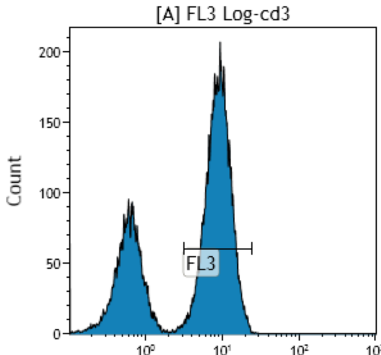
Figure 7.17 Gate Logic



Linear Gates

Linear gates are used for histogram plots. A linear gate encompasses events that fall within the linear range that you define and includes all events within the vertical/horizontal frame.

Figure 7.18 Linear Gate



To create a linear gate:

---

1 From the Plots & Gates Ribbon tab, select the Linear icon.

---

2 On the histogram plot, click and drag your mouse over the area that you wish to be included in the gate.

---

3 Release your mouse button at the end of the line.

---

4 Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

---

5 Customize your gate by following instructions in *Setting Up Gates*.

Quadrant Gates

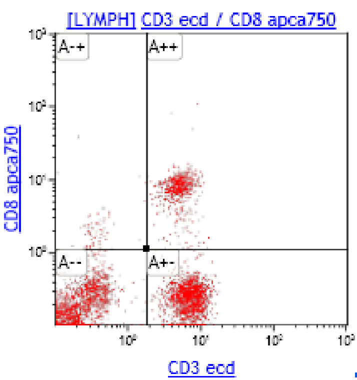
The quadrant gate is available for use on dot and density plots. When you choose this option, each plot is divided into four gated sections by perpendicular lines. Quadrant gates can be moved at your discretion. Each quadrant of the gate is assigned positive/negative values, depending on the position (see Table 7.11).

Table 7.11 Quadrant Values

Values	Location
-+ (Negative, Positive)	Upper Left Quadrant
++ (Positive, Positive)	Upper Right Quadrant
-- (Negative, Negative)	Lower Left Quadrant
+- (Positive, Negative)	Lower Right Quadrant

NOTE Multiple quadrants can be placed on a single plot.

Figure 7.19 Quadrant Gate



To create a quadrant gate:

1. From the Plots & Gates Ribbon tab, select the

icon.

2. Click your mouse anywhere within the dot or density plot to create the new gate; this adds a quadrant gate to the plot, with the center-point in the location where you clicked your mouse.

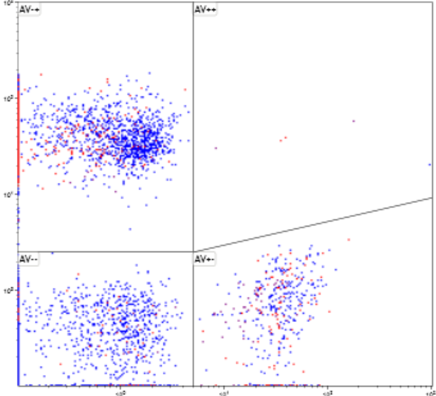
3. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

4. Customize your gate by following instructions in *Setting Up Gates*.

Hinged Quadrant Gates

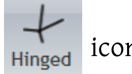
Similar to the quadrant gate, the hinged quadrant gate divides plots into four sections, each containing positive/negative values depending on the location of the quadrant. However, unlike the quadrant gate, the hinged quadrant allows you the flexibility to move each quadrant borderline to an angle of your choosing. The movement of each quadrant borderline is limited to its current plot axis.

Figure 7.20 Hinged Quadrant Gate



To create a hinged quadrant gate:

---

1 From the Plots & Gates Ribbon tab, select the  icon.

---

2 Click your mouse where you would like the center-point of the hinged quadrant in the dot, contour, or density plot.

---

3 Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

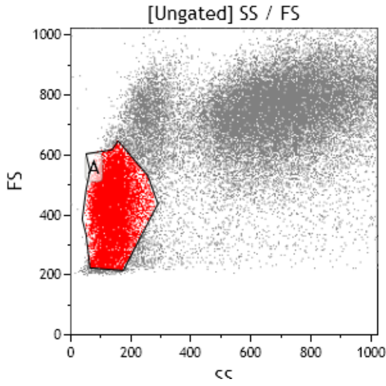
---

4 Customize your gate by following instructions in *Setting Up Gates*.

Polygon Gates

The polygon gate allows you to create a gate with up to 128 points; this allows you set up a very specific zone of events to include in your gate. The polygon gate is available on dot and density plots.

Figure 7.21 Polygon Gate



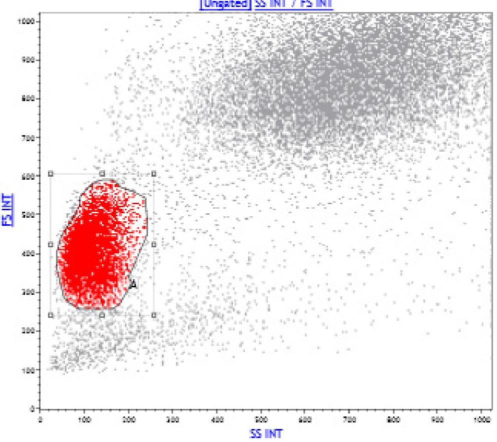
To create a polygon gate:

1. From the Plots & Gates Ribbon tab, select the Polygon icon.
2. Click your mouse where you wish to begin creating your gate.
3. Determine the path you need for your gate and continue clicking your mouse at the location of each direction change. As you draw the gate, a new line will be added each time you click your mouse, and the default gate color will display, working as a guide to show your progress.
4. Double-click or select the initial point when the gate is completed.
5. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.
6. Customize your gate by following instructions in *Setting Up Gates*.

Freehand Gates

The freehand gate is a very flexible option that gives you complete control over the size and shape of your gate. The freehand gate is available on dot and density plots.

Figure 7.22 Freehand Gates



To create a freehand gate:

---

1 From the Plots & Gates Ribbon tab, select the icon.

---

2 Determine the path you need for your gate.

---

3 Click your mouse where you wish to begin creating your gate, and, without releasing your mouse button, draw your gate to the size and shape you need. As you draw the gate, the default gate color will display, working as a guide to show your progress.

---

4 Release your mouse when you are finished.

---

5 Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

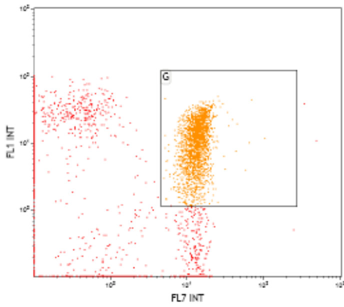
---

6 Customize your gate by following instructions in *Setting Up Gates*.

Rectangle Gates

The rectangle gate is available on dot and density plots.

Figure 7.23 Rectangle Gates



To create a rectangular gate:

1. From the Plots & Gates Ribbon tab, select the Rectangle icon.

2. Determine the size you need for your rectangular gate.

3. Click your mouse where you would like to begin your rectangle and drag to the size you need. Release your mouse when finished.

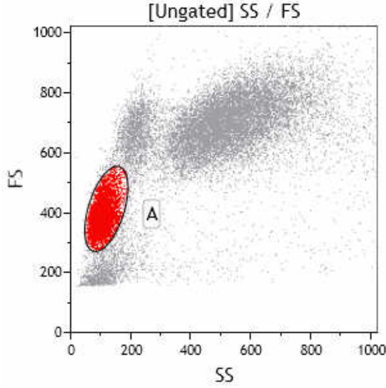
4. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

5. Customize your gate by following instructions in *Setting Up Gates*.

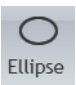
Ellipse Gates

The Ellipse gate consists of curved lines. These gates can be sized and shaped using the eight default handles and rotated using the handle. The ellipse gate is available on the dot and density plots.

Figure 7.24 Ellipse Gates



To create an ellipse gate:

1. From the Plots & Gates Ribbon tab, select the  icon.

2. Determine the path you need for your gate.

3. Click your mouse where you would like to begin your ellipse and drag to the size you need. Release your mouse when finished.

4. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

5. Customize your gate by following instructions in *Setting Up Gates*.

Setting Up Gates

Kaluza for Gallios offers many options for customizing your gates. The following sections highlight these options and give instructions on how to employ the techniques for using these options.

Editing Gates

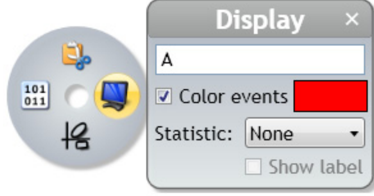
Use the Edit radial menu (see Figure 7.25) to perform basic editing functions for a selected gate, including cut, copy, paste, and delete. See CHAPTER 6, *Basic Editing for Plots, Gates, and Plot Sheet Items*, for a description of the functions.

Figure 7.25 Edit Radial Menu

Setting Up Gate Display

Use the Display radial menu (see Figure 7.26) to alter the name of a gate, change the color of events that fall within the gate, and choose a statistic to appear next to the gate.

Figure 7.26 Display Radial Menu



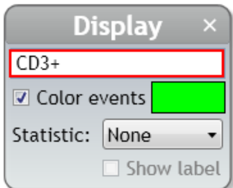
Gate Name

The gate name field allows you to enter a custom name for your gate. To update a gate name:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Display menu. The Display menu appears.

3 Delete the current gate name and enter the revised name into the field (field location is outlined in red in the figure below).



4 Select ☒ to complete the process.

Color Events

Use the **Color events** section of the Display menu to define a new color for your gate or remove gate coloring. The two procedures below describe the process for completing these tasks.

To update the color of the events that fall within a gate:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Display menu. The Display menu appears.

3 Select the color block. The color palette appears.

4 Choose the new color for the gated events.

5 Select ☒ to complete the process.

To add/remove gate coloring:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Display menu. The Display menu appears.

3 Select/deselect the **Color events** check box to change your event coloring preference.

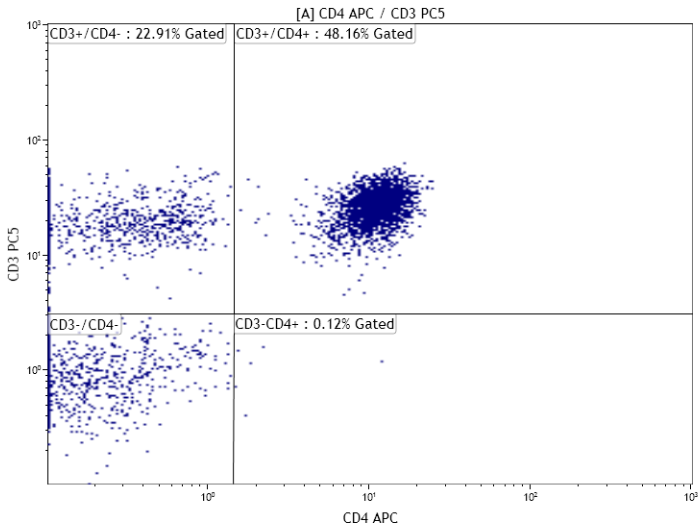
4 Select ☒ to complete the process.

Gate Statistics

The **Statistic:** drop-down is where you can choose to display a statistic directly on the plot. Statistic options include **Number**, **% Total**, and **% Gated**. You can also choose to include a statistic label.

Figure 7.27 shows an example of a gate displaying the **% Gated** statistic, including the statistic label.

Figure 7.27 % Gated Statistic and Statistic Label



To display a statistic directly on the plot:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Display menu. The Display menu appears.

3 Select the **Statistic:** drop-down list and choose the preferred statistic.

4 If you wish to show a label, select the check box located next to **Show Label.**

5 Select ☒ to complete the process.

Adding a New Gate

Use the Gates & Tools radial menu (see Figure 7.28) to add a gate to a plot.

NOTE The Gates & Tools menu includes the same options no matter where you are currently located on the sheet. If a gate selected from the menu is not appropriate for the current sheet location, the cursor will display as a ⃠ symbol. When you are in an appropriate location for the gate type, the cursor changes to the symbol for the gate type you selected, indicating that you may begin drawing.

See *Using the Gates & Tools Plot Radial Menu*, for details on using this menu.

See *Gates*, for an in-depth description of gating.

Figure 7.28 Gates & Tools Radial Menu

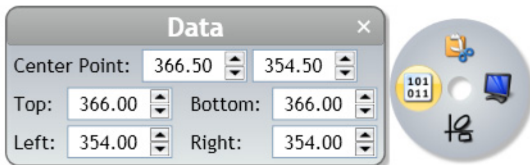
Data Menu

Use the Data radial menu (see Figure 7.29) for one or more of the following activities:

- Viewing coordinates
- Changing location
- Changing the size or angle
- Linking to other gates

For additional methods for changing data, see *Resizing, Reshaping, and Moving Gates*.

Figure 7.29 Data Radial Menu (Hinged Quadrant Gate)

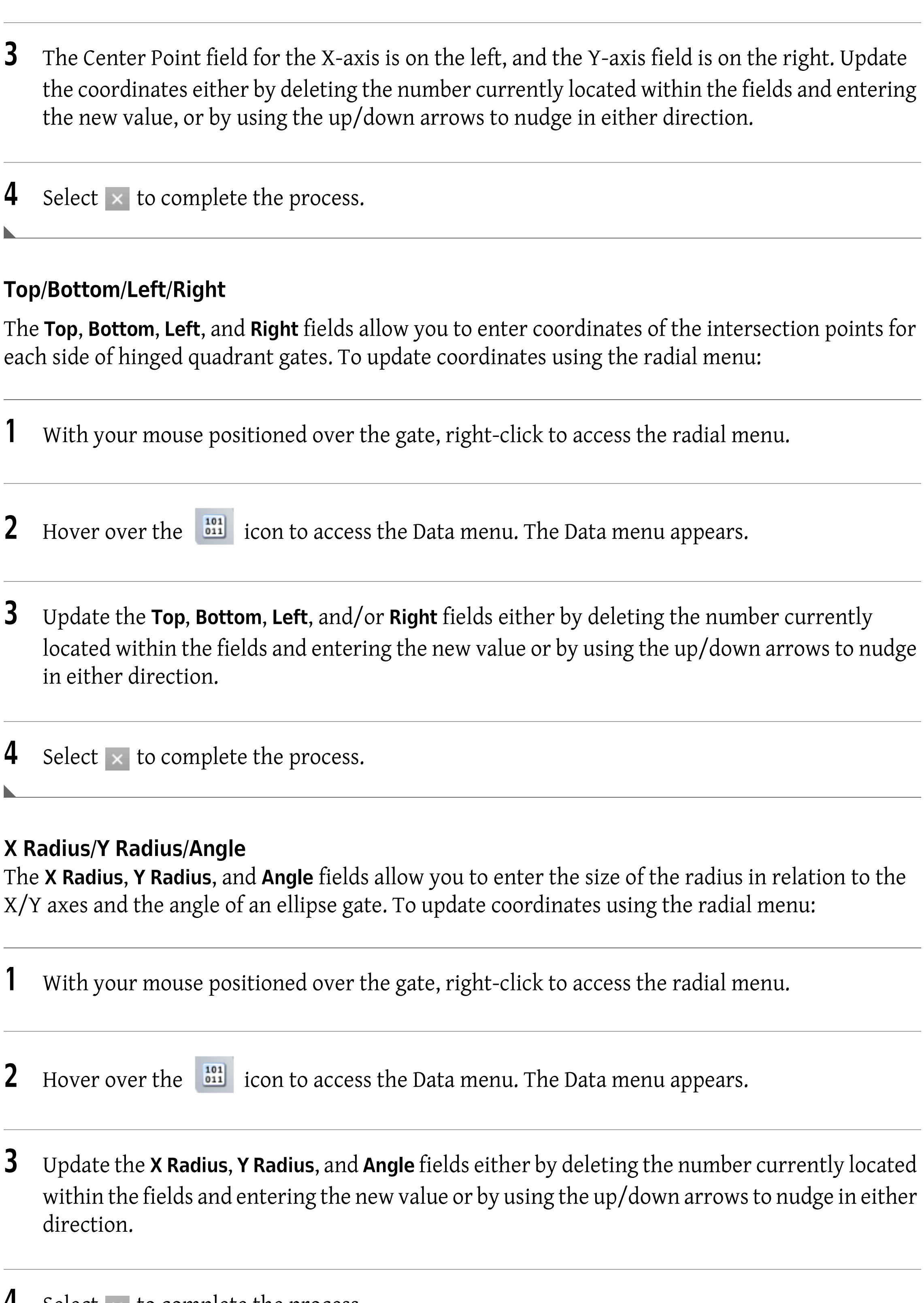


Center Point

Center Point allows you to change the location of the center point of the gate. (Applies to ellipse, quadrant, and hinged quadrant gates.) To change the center point location using the radial menu:

1 With your mouse positioned over the gate, right click to access the Data radial menu.

2 Hover over the [101/011 icon] icon to access the Data menu. The Data menu appears.



3 The Center Point field for the X-axis is on the left, and the Y-axis field is on the right. Update the coordinates either by deleting the number currently located within the fields and entering the new value, or by using the up/down arrows to nudge in either direction.

4 Select to complete the process.

Top/Bottom/Left/Right

The **Top**, **Bottom**, **Left**, and **Right** fields allow you to enter coordinates of the intersection points for each side of hinged quadrant gates. To update coordinates using the radial menu:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Data menu. The Data menu appears.

3 Update the **Top**, **Bottom**, **Left**, and/or **Right** fields either by deleting the number currently located within the fields and entering the new value or by using the up/down arrows to nudge in either direction.

4 Select to complete the process.

X Radius/Y Radius/Angle

The **X Radius**, **Y Radius**, and **Angle** fields allow you to enter the size of the radius in relation to the X/Y axes and the angle of an ellipse gate. To update coordinates using the radial menu:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Data menu. The Data menu appears.

3 Update the **X Radius**, **Y Radius**, and **Angle** fields either by deleting the number currently located within the fields and entering the new value or by using the up/down arrows to nudge in either direction.

4 Select to complete the process.

X0/X1/Y0/Y1

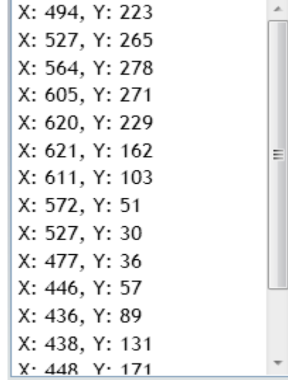
The **X0**, **X1**, **Y0**, and **Y1** fields allow you to change the size of a rectangle gate by changing the coordinates of each side of the rectangle. To update coordinates using the radial menu:

1. With your mouse positioned over the gate, right-click to access the radial menu.
2. Hover over the icon to access the Data menu. The Data menu appears.
3. Update the **X0**, **X1**, **Y0**, and **Y1** fields either by deleting the number currently located within the fields and entering the new value or by using the up/down arrows to nudge in either direction.
4. Select to complete the process.

X/Y Coordinates

In polygon and freehand gates, the Data menu displays the X and Y coordinates of each handle (change of direction), as well as the angle and length located on the gate. Figure 7.30 shows the X/Y coordinates list for a freehand gate.

Figure 7.30 X/Y Coordinates List



NOTE To make changes to the shape of polygon or freehand gates, follow the methods described in *Resizing, Reshaping, and Moving Gates*.

Link to Gates

Link to Gates... allows you to change other freehand, polygon, linear, rectangle, and ellipse gates within your Protocol to the same size, shape and X/Y coordinate locations as the current gate. Gates cannot be linked to gates of other types, except for polygon and freehand, which can be linked together. When gates are linked, changes made to one gate automatically apply to all gates that are linked.

NOTE Changes to the axes of a plot on which one linked gate is defined will not affect the other linked gates.

To link gates:

1 With your mouse positioned over the gate, right-click to access the radial menu.

2 Hover over the icon to access the Data menu. The Data menu appears.

3 Select the **Link to Gates...** hyperlink. A pop-up window appears with a list of gates applicable for linking.

4 Select the gate(s) that you wish to link to the current gate.

5 Select to complete the process.

X0/X1/Height

The **X0**, **X1**, and **Height** fields allow you to change the length and height of a linear gate by changing the coordinates of each side of the line or the height coordinate on the Y-axis.

To update coordinates using the radial menu:

1 With your mouse positioned over the linear gate, right-click to access the radial menu.

2 Hover over the icon to access the Data menu. The Data menu appears.

3 Update the **X0**, **X1**, and **Height** fields either by deleting the number currently located within the fields and entering a new value or by using the up/down arrows to nudge in either direction.

4 Select to complete the process.

Resizing, Reshaping, and Moving Gates

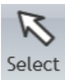
See Table 7.12 for complete instructions on updating the physical characteristics of a gate.

Table 7.12 Resizing, Reshaping, and Moving Gates

Item	Details
	Resize and Reshape Gates: Handles allow for resizing and/or reshaping a portion of, or an entire, gate.
	Move Gates: When your mouse enters the confines of a gate, the cursor changes to a , indicating that the movement of an entire gate is enabled. Move a gate by selecting and dragging when you see this cursor. • To move the gate a fixed amount, select the gate, and then press the appropriate arrow key(s) on your keyboard until you are satisfied with the position. • Smaller adjustments can be made by pressing the Ctrl key while using the arrow keys.
	Lengthen or Shorten, Reposition, Change Angle, and Stretch or Compress Gates: The cursor changes to a double-sided arrow when you move your mouse near a handle. The type of arrow indicates the direction of movement. Select and drag a handle to: • Lengthen or shorten **linear** gates. • Reposition quadrants in the **quadrant** and **hinged** gates. • Change angles of the quadrants in the **hinged** gates. • Horizontally, vertically, or diagonally stretch or compress entire gates, including **polygon**, **freehand**, **rectangular**, and **elliptical** gates.
	Reshape Polygon Gates: The cursor changes to a when you hover your mouse over a polygon handle. This cursor indicates that you can reshape the polygon. Select and drag a handle to reshape.
	Rotate Elliptical Gates: By moving your mouse over the circular handle, the indicates it is ready for you to rotate an elliptical gate on the center of axis.
	Move a Gate Name: When you move your mouse over a gate name, the indicates that a gate name is ready for movement. Select and drag the name to move to a new location.

Selection Tool

The Selection Tool, which allows you to make changes to gates, as well as individual plots, is available in the Gates section of the Plots & Gates tab.

Choose 

on the Plots & Gates tab to change the cursor to Selection mode.

Methods for Applying Gates to Plots

In addition to using the Data menu (see CHAPTER 7, *Setting Up Plot Data*), there are three other methods for assigning a gate to a plot.

Gating Plots Using the Plot Hyperlink

To gate a plot using the hyperlink:

1 Select the hyperlink located at the top of a plot. A pop-up appears, containing a list of gates, including recently created gates and gates by category.

2 Select the gate for your plot from the pop-up lists. The events within your plot are now filtered, based on the events in the gate you selected.

Gating Plots Using the Color Precedence Pane

To gate a plot using the Color Precedence pane:

1 In the Color Precedence pane, select the gate that you wish to apply to the plot, and with the gate still selected, drag the gate onto the plot and release your mouse button to complete the process.

Gating Plots by Dragging/Dropping

To gate a plot using the drag/drop method:

1 Press the Alt key, and, while pressed, select the gate that you wish to use as the input gate for a plot.

2 Drag the gate onto the plot and release your mouse button/ Alt key to complete the process.

Establishing Color Precedence of Gates

The Color Precedence pane displays the event coloring and precedence of coloring for gates in the current Protocol. The pane contains three main sections, including Default Event Color, Gates With Color, and Gates Without Color. These sections, along with the procedures for making changes within the Color Precedence pane, are described in the following section:

NOTE If the Color Precedence pane does not appear on the left side of the screen, it has been docked. See CHAPTER 6, *Display Options for the Acquisition Attributes Pane* for additional information on docking and undocking panes.

IMPORTANT If a .PRO file is opened and the Protocol contains an orphan gate, the orphaned gate will be present in color precedence, but there is no geometric entity (no gate viewed in a plot) for the user to manipulate. A plot can be created for the gate using Show Gate option, or the gate can be deleted from the Color Precedence pane.

NOTE An orphan gate is a gate that is included in a Protocol, but not present on any plot.

Table 7.13 Color Precedence Options

<table>
<tr><th>Item</th><th>Description</th></tr>
<tr><td>Default Event Color</td><td>Displays the color of events that have not been assigned to a gate. This color is also the default color of events on a plot when Use Single Color is selected from the Coloring radial menu (see CHAPTER 7, Using the Coloring Menu for details).

Figure 7.31 Default Event Color
Default event color:</td></tr>
<tr><td>Gates With Color</td><td>Displays gates in order of precedence. The gate located at the top of the list has the highest precedence, and the gate at the bottom has the lowest precedence (Figure 7.32). When an event belongs to more than one gate, it appears on the plot sheet in the color with the highest applicable precedence. All enabled gates in the current Protocol are displayed in the Gates With Color section of the Color Data Set Precedence pane. You can assign color to up to 31 gates per Data Set. If the same color is selected for more than one gate, an Information icon displays (Figure 7.33) indicating “This color is used multiple times.”

Figure 7.32 Gates with Color
Gates With Color
FL1
FL2
FL3
FL5
FL4
A

Figure 7.33 Duplicate Color Icon
Color Precedence
Default event color:
Data Set: 2C CD3-CD19 004
Gates With Color
C
B
A
This color is used multiple times.</td></tr>
<tr><td>Gates Without Color</td><td>Displays a list of gates that are not color enabled (Figure 7.34). These gates are separated into Common, and Quadrant gates, but only applicable categories are visible. The Gates Without Color section also displays gates that had been previously assigned a color, but are now disabled.

As a default, the Color Precedence pane does not display the list of gates without color. To enable this listing, see CHAPTER 7, Displaying the Gates Without Color Listing.

Figure 7.34 Gates Without Color
Gates Without Color
Quadrant 40 gates
B1
B2
B3
B4</td></tr>
</table>

NOTE Multi-selection is available for the following procedures.

Updating Color Precedence

To change the precedence of a gate:

1 From the Color Precedence pane, select the gate you need to move.

2 At this point, there are two ways to change the gate precedence hierarchy:

a. **Drag and Drop Method**: With the row still selected, drag it to the new location and release the mouse button.

OR

b. **Using Buttons (located on the right-hand side of the pane)**:

- **To move the gate up or down one space**: select the ▲ or ▼ buttons until the gate is in the appropriate location.
- **To move the gate to the top or bottom of the list:** select the [top] or [bottom] button.

Displaying the Gates Without Color Listing

To display/hide the Gates Without Color section of the Color Precedence pane:

1 Click the arrow (outlined in red in the figure below) located at the bottom of the Color Precedence pane, to display gates without color. The Gates Without Color section uses categories (Common and Quadrant).

To hide this portion of the Color Precedence screen, click on the ◢ icon located next to Gates Without Color.

2 The software defaults to a collapsed view of the Common/Quadrant/Boolean gate categories.

To display the contents a category, click on the ▷ icon located next to the section you wish to expand.

To collapse the list, click on the ◢ icon located next to the section you wish to collapse.

Assigning Color to a Gate Without Color

To assign color to a gate without color:

1 Select anywhere within the row of the gate you need to color.

2 There are three ways to enable gate coloring:

- **Click and Drag:** With the row still selected, drag the row to the Gates With Color section of the pane and release the mouse button.
- **Up/Down Icon:** Select the ↑↓ button to move the gate to the Gates With Color section.

  NOTE If gate coloring had been disabled, the gate will retain the former level of precedence when re-enabled.

- **Right-Click:** Right-click on the selected gate(s) and select **Enable coloring**.

Changing the Name of a Gate

Gate names are editable and can be changed by:

NOTE Changing the gate name using the Color Precedence pane changes the gate name in all locations within the application.

1 Position your mouse over the gate name that you wish to change and click twice. An editable field is indicated by the text appearing highlighted.

NOTE Copy, cut, and paste is available by right-clicking on the text field or using keyboard shortcuts once the field is editable (highlighted).

2 Enter the new name in the field.

IMPORTANT When changing gate names, keep the following in mind:

- Gate names cannot be left blank.
- The maximum length is 255 characters.
- Gate names cannot be repeated.

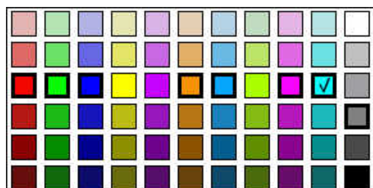
3 To save your changes, press (Enter) on your keyboard or click on another gate.

Changing the Color of a Gate

New gates are automatically assigned a unique color. Gates that have been moved from the Gates Without Color section, however, might not be assigned a unique color. When this occurs, you will see an Information icon. To correct the duplicated color assignment, update one of the colors by following the procedure below.

To change the color of a gate:

1 Click on the color swatch of the gate color you wish to change. The color palette appears. The colors that have a bold, black outline are the colors that are already in use. The color that contains a check mark is the current color assigned to that gate. Refer to the figure below for an example.



2 Choose a new color from the palette. This action changes the color of all events shown on the plot sheet that fall within that gate and have the higher precedence.

Deleting a Gate From the Color Precedence Pane

To delete a gate:

1 From the Color Precedence pane, select within the row of the gate you wish to delete.

2 There are two ways to delete a gate:

a. **Right-Click:** Right-click on the selected gate and select **Delete** from the menu.

b. **Keyboard**: Press (Delete) on your keyboard.

NOTE You may also delete a gate directly from the plot by selecting the gate and pressing (Delete) on your keyboard.

Disabling Color From the Color Precedence Pane

To disable a gate:

1 From the Color Precedence pane, select within the row of the gate you wish to disable coloring on.

2 Right-click and select **Disable Coloring**. This places the gate in the Gates Without Color section of the pane.

Show Gate From the Color Precedence Pane

To locate a gate:

1. From the Color Precedence pane, select within the row of the gate you wish to locate.

2. Right-click and select **Show Gate**. This places into view, on the screen, the plot where a specific gate is located, or creates a plot if one does not exist.

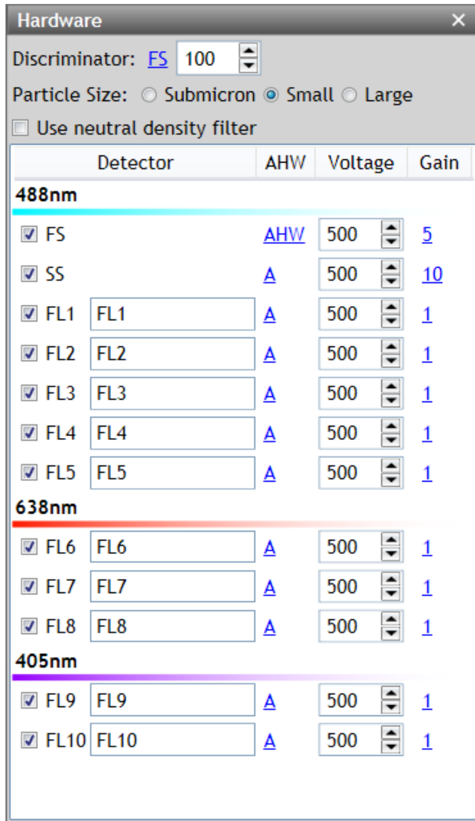
   NOTE This option is not available for multi-selection.

Hardware Configuration

The Hardware Pane (Figure 7.35) allows you to define instrument settings and make changes to parameters. The following sections describe the settings available in the Hardware Pane:

- *Hardware Settings*
- *Particle Size*
- *Neutral Density Filter*
- *Detector*
- *Measurements*

Figure 7.35 Hardware Pane



NOTE If the Hardware pane does not appear on the left side of the screen, it has been docked. See CHAPTER 6, *Display Options for the Acquisition Attributes Pane* for additional information on docking and undocking panes.

Hardware Settings

Discriminator, voltage, and gain values can be imported, or you can manually update the values in the Hardware Pane.

- ***To import settings that have already been defined***, follow the instructions in *Importing Hardware Settings*.
- ***To create new hardware settings***, follow the instructions in the following sections:
  - *Discriminator*
  - *Voltage*
  - *Gains*

Importing Hardware Settings

Hardware settings, including discriminator, voltage, and gain values, can be imported into Protocols from *.fcs, *.lmd, *.pro, *.analysis, or *.protocol files. These settings typically originate from files created while performing Quality Control procedures at the beginning of each day (see CHAPTER 11, *Quality Control*). Because changing hardware settings alters data results, changing these settings during a sample acquisition automatically switches the instrument to Setup Mode, and, as a result, any data collected is discarded once these values are changed. Therefore, you need to define hardware settings prior to sample acquisition. Once these settings are optimized, they can be imported into sample Protocols containing the same parameters.

To import hardware settings:

1. Select > **Import Settings**.
2. Select **Hardware settings** to import hardware settings only. If you also wish to import compensation values, select **Hardware settings & compensation** instead. For additional information on compensation, see *Compensation*.
3. In **Import Hardware Settings**, navigate to the file, select it, and then select **Open**.

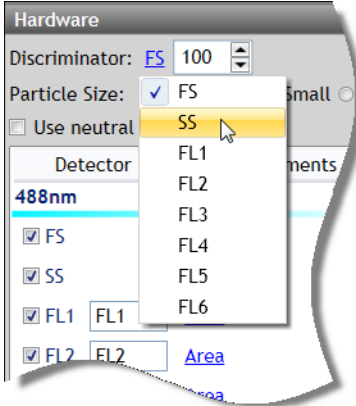
Discriminator

The discriminator eliminates signals caused by debris by excluding signals below the threshold that you define. Any of the signals, including forward scatter, side scatter, or fluorescence, can be set as the discriminator, but only one discriminator can be specified for a sample acquisition.

To set the discriminator:

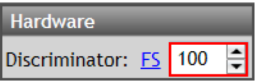
1 Select the **Discriminator** link and choose the desired signal from the drop-down list (Figure 7.36).

Figure 7.36 Discriminator Selection



2 Enter a discriminator channel value into the field (Figure 7.37) or click on the up or down arrows to change the current value by and increment of 1. The maximum value for this field is **1000**.

Figure 7.37 Discriminator Field



Voltage

Voltage amplification can be changed for individual parameters to increase the visibility of events.

To change a the voltage of a parameter:

1 Click your mouse within the **Voltage** entry field, and type in the new value.

OR

Click on the up or down arrows to adjust the current value up or down by an increment of 1.

NOTE Voltage can also be changed using sliders. See *Plot Sliders* for details.

2 Click your mouse within another entry field or on the Plot Sheet, or press (Enter) on your keyboard after you type in a change to ensure the change is accepted by the software.

Gains

Gain changes the amplification factor of voltages. A change of 1 to 750 represents a 1-to-4 change in gain. Linear amplification (gain) is 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) is by 1.0 or 2.0 for FL1-FL10.

To change the gain value:

1 Select the Gain value that you wish to change.

2 From the pop-up list, select the favored value.

Particle Size

Defining the particle size for your acquisition helps to eliminate noise, as this adjusts the detector sensitivity based on the type of particle in your sample. Table 7.14 describes the particle sizes and how the detector sensitivity differs for each size option.

Table 7.14 Particle Size

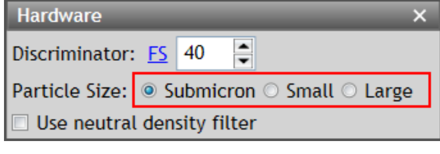
Particle Size	Description
Large (21 - 40µm)	Large particles require less detector sensitivity, and therefore, the **Large** particle setting narrows the area that receives signal to the center portion of the detector, eliminating the detection of debris and small particles that would only be viewed with a wider detection area
Small (1 - 20µm)	Small particles require moderate detector sensitivity. The **Small** setting takes readings from the entire width of the detector; however, the outer portion of the detector is filtered to remove submicron-sized particles.
Submicron	Submicron particles require increased detector sensitivity. The **Submicron** setting uses the entire width of the detector, and the sensitivity of readings registered on the outer portion of the detector is amplified so that the very small particles are identified as events.

To select the particle size:

1. Determine the appropriate particle size for your sample based on the information in Table 7.14.

2. Choose the radio button next to the size of the particles in your sample (Figure 7.38).

Figure 7.38 Particle Size Selection



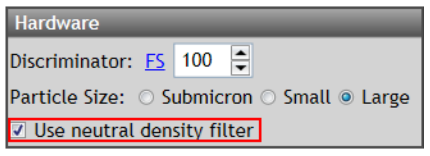
Neutral Density Filter

The Side Scatter Neutral Density Filter is an electronic attenuation designed to behave similar to a physical filter (labeled by its power of 10 reduction of light) that will only allow a percentage of any light to pass. This is not a wavelength-specific physical filter.

To enable the Neutral Density Filter:

1. Select the **Use neutral density filter** check box (Figure 7.39)

Figure 7.39 Use Neutral Density Filter Check Box



Detector

Detectors can be enabled or disabled before you begin an acquisition. For additional information on detectors, see CHAPTER 3, *Light Collection, Separation, and Measurement*.

To update the detectors for your Protocol:

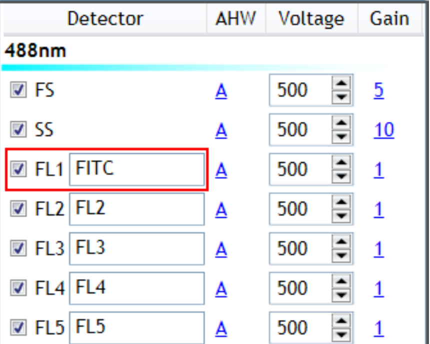
1. Click your mouse in the check box next to the detector name to enable or disable a detector; check boxes that currently contain a check mark indicate that a detector is enabled, and no check mark indicates the detector is currently disabled.

   IMPORTANT Risk of Protocol errors. Offline Kaluza for Gallios uses a 10-color configuration, but your instrument might be equipped with a 6- or 8-color configuration. Be sure to only use the detectors on your instrument when working offline.

2. The enabled fluorescence detectors contain an entry field, allowing you to change the detector label, if desired. To change the detector label, click once in the entry field, and then enter the new name using your keyboard. In Figure 7.40, the FL1 detector name was changed to **FITC**.

   NOTE If the Protocol is used in multiple locations in the Worklist, the label will only affect the currently selected location. To copy it to other locations, refer to CHAPTER 8, *Editing Metadata in Kaluza for Gallios*. To use your updated Protocol in other locations, refer to **Duplicate** in Table 8.1.

Figure 7.40 Detector Entry Field



3 Press the (Enter) key on your keyboard, or click anywhere outside of the entry field to complete the process.

Measurements

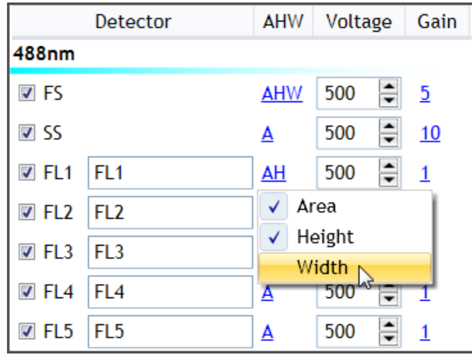
You are able to choose up to three measurement types for each parameter, including **Area**, **Height**, and/or **Width.** To determine which type of signal(s) to collect for each parameter, see CHAPTER 3, *Voltage Pulse Signals*.

To update the measurements:

1 Select the link in the **AHW** (Area = **A**, Height = **H**, Width = **W**) column corresponding to the parameter you wish to update.

2 In the pop-up menu, select the measurement type(s) you wish to collect by clicking on each needed type. In Figure 7.41, **Area** and **Height** have been selected for FL1, as indicated by the check marks in the pop-up menu and in the "**AH**" in the **AHW** column. Selected measurements can be deselected by clicking again on the selected measurement. If all of the measurements are deselected, the parameter will become deselected as well.

Figure 7.41 Measurement Selection



Acquisition Controls

Acquisition controls are available through the ribbon (CHAPTER 6, *Acquisition Controls Tab*). Through these controls, you can change the flow rate, set the stop conditions, and update the compensation or voltages on the plots by choosing to use plot sliders. Refer to the sections below for a description of each part of the Acquisition Controls tab.

Flow Rate

The flow rate can be changed to meet the needs of your acquisition. A low flow rate provides lower coefficients of variation (CVs) for data populations than a medium or high flow rate.

To choose a flow rate:

1. Determine the appropriate flow rate for your acquisition. The approximate flow rate for each speed is specified as:
   - **Low:** 10 µL/min
   - **Medium:** 30 µL/min
   - **High:** 60 µL/min

2. Select the radio button next to the desired rate.

Stop Conditions

You can choose to set the acquisition to stop either by a set amount of time **or** when the acquisition has reached a specific number of events in a gate or in total (ungated). The acquisition is stopped once either stop condition reaches the set capacity.

NOTE The values set in Stop Conditions cannot be changed during an acquisition. The instrument must be paused or in Setup Mode to change the stop conditions.

To create stop conditions:

---

1 Select the **Time** drop-down arrow to select either **1**, **2**, **3**, **5**, **10**, **15**, or 20 minutes.

OR

Manually enter a value into the **Time** field for the minute and/or second mark, up to 20 minutes.

---

2 Select the number of events to stop at by selecting the drop-down arrow (Figure 7.42) and selecting a value from the drop-down list.

OR

Manually enter a value by clicking in the field and entering a specific number of events, and then click outside of the field to accept the change.

NOTE Based on the parameters/measurements selected, Kaluza for Gallios automatically calculates the maximum number of events able to be captured in the acquisition (see *Parameters* for additional information on maximum allowable events per configuration). This number is the last value in the **events** drop-down; e.g., **13,333,333** in Figure 7.42.

IMPORTANT If you change your configuration by including additional parameters/measurements, an error similar to Figure 7.43 will display if the new configuration exceeds the previously-specified maximum event count.

Figure 7.42 Number of Events Field

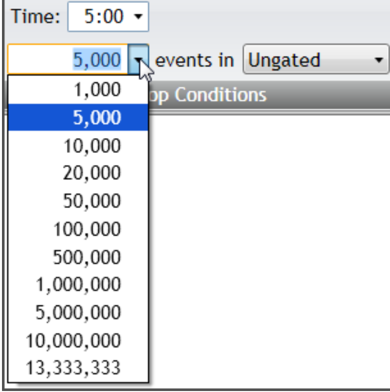


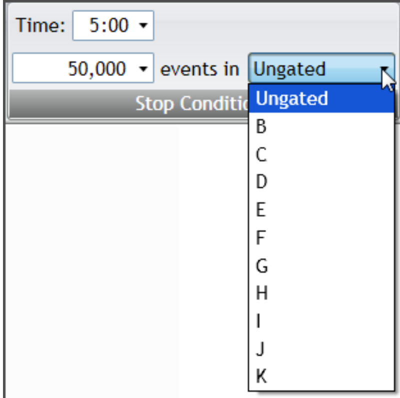
Figure 7.43 Event Count Error

The event count stop condition for location(s) 1 is not reachable. Select fewer parameters or decrease the count.

3 If you wish to specify that the number of events defined in Step 2 is in a gated population, select the drop-down arrow (Figure 7.44) and choose the gate.

IMPORTANT If you have a gated stop condition and delete the gate during acquisition, the stop condition will default to **Ungated**, and the acquisition might stop earlier than expected if the number of ungated events already exceeds the gated stop condition. Changes to the gating hierarchy or gate positions during acquisition could also affect when the acquisition stops.

Figure 7.44 Gate Selection Field



Plot Sliders

Compensation and/or voltage can be changed through sliders on plots. By enabling sliders, you can make adjustments based on the appearance of data on the plot, rather than providing a specific numerical value. Changes made using the sliders alter the data results in real time.

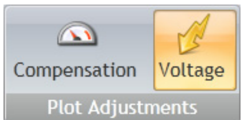
Voltage adjustments restart data collection, and the system automatically transitions into Setup mode. When running daily quality control, standardization and compensation protocols are used to optimize the settings for your application (see CHAPTER 11, *Quality Control*). These settings can be imported into your sample protocols (see *Importing Hardware Settings* for additional information).

Compensation can be changed at any time. Changing the compensation during an acquisition does not result in a restart to the acquisition, and compensation can also be adjusted in Kaluza Analysis Software after the acquisition is completed. Compensation settings can be imported; see *Importing Compensation* for additional information.

To use the compensation and/or voltage sliders:

1 Select the icon on the ribbon that corresponds to the type of slider to add to your plots. The icon is highlighted when the slider is enabled. In Figure 7.45, the Voltage sliders are enabled.

Figure 7.45 Voltage Sliders Enabled



For additional information, see:

- *Compensation*
- *Voltage*

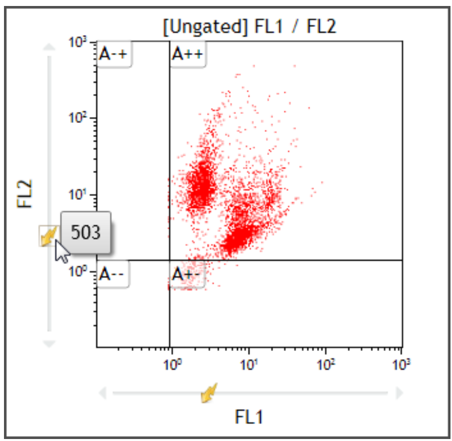
---

2 To use the sliders:

- **To freehand the change**: Select the slider handle on the plot and drag in the direction you wish to make the change. The numerical value is displayed next to the slider, as shown in Figure 7.46.
- **To make fine adjustments (0.1%** for **Compensation** or **1 volt** for **Voltage**): Select the appropriate arrow located on either end of the slider.
- **To make course adjustments (1%** for **Compensation** or **10 volts** for **Voltage**): Click on the slider bar on either side of the handle.

IMPORTANT When the voltage is changed during an acquisition, the instrument automatically switches to Setup Mode, and the data is discarded.

Figure 7.46 Slider Value Display



Compensation

Introduction to Compensation

To understand the need for fluorescence compensation, you must start with basic flow cytometry concepts. These concepts are described in this section.

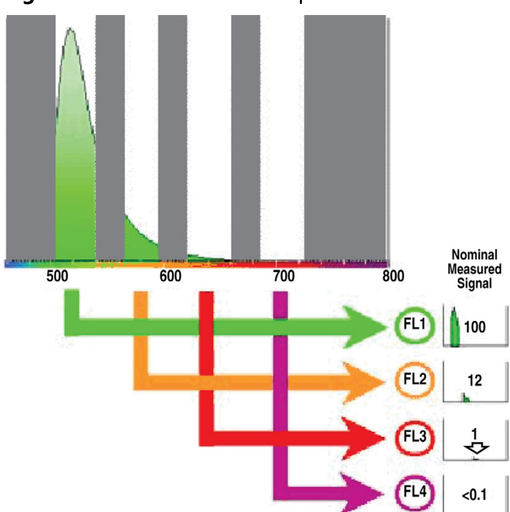
When particles are processed through a flow cytometer, they (or the attached fluorochromes) are excited by a laser. As these laser-excited events return to their former, unexcited state, they release energy. The intensity of the released energy depends on two factors, including:

- The type of fluorochrome(s) attached to, or intrinsic to (autofluorescence) the event.
- The amount of fluorescence molecules that are attached to each event.

The energy released from each event is detected by photomultiplier tubes (PMTs), via a series of dichroic mirrors and optical band-pass filters that allow only a specific region of the spectrum to reach each PMT. Each PMT located within a flow cytometer detects a different color range; however, because the emission spectra for different fluorescence stains overlap and signals cross over to PMTs other than the one specified for a particular fluorochrome, it is necessary to correct spillover.

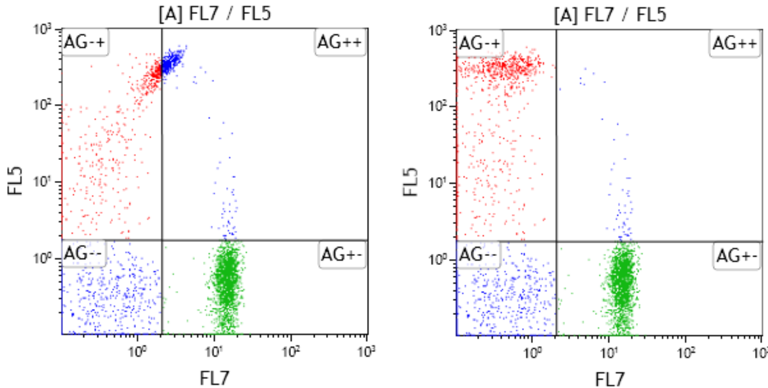
Figure 7.47 illustrates fluorescence spillover (FL1) from a particle labeled with FITC, a green dye fluorescing into PMT2 (FL2), PMT3 (FL3), and (PMT4) FL4. Some of the FITC-emitted light reaches every one of the four fluorescence detectors shown in this example. Because PMTs are very high-gain devices, even a very small amount of light can be measured, and it is quite possible for three, or even all four, detectors (as in this example), to generate a measurable signal. This phenomenon, where part of the signal from a fluorochrome spills over into a detector other than its primary or “intended” detector, is called “crosstalk” or "spillover." This may occur for all fluorochromes in use and must be dealt with. Color compensation electronically removes the crosstalk or spillover. The Spillover Matrix shows the combined spillover effects for all fluorochromes present.

Figure 7.47 Fluorescence Spillover—FITC



In Figure 7.49, the plot on the left is not compensated, and the plot on the right shows correctly compensated fluorescence parameters. When fluorescence parameters are correctly compensated, they are aligned both horizontally and vertically.

Figure 7.48 Compensating Event Populations - Before and After Correctly Compensating



The following sections describe the methods for making manual adjustments to compensation using Spillover Sliders (see *Using Spillover Sliders*) or the Spillover Matrix (see *Adjusting Spillover Values in the Compensation Pane*). Compensation can also be calculated automatically using Kaluza Analysis and then imported back into Kaluza for Gallios (refer to *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature* in the *Kaluza Analysis Software Instructions for Use* (P/N A75667) for details.

Compensation Worklists

Complete instructions for creating a Compensation Worklist, preparing and acquiring compensation samples, and performing compensation procedures, see CHAPTER 9, *Compensation Worklists*.

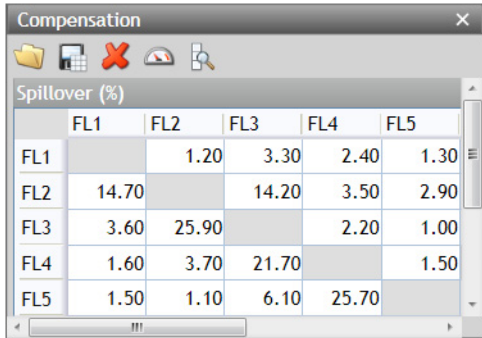
Compensation Pane

The Compensation pane (see Figure 7.49) contains tools for adjusting Spillover and Autofluorescence Vector values related to a particular Protocol. The main component of the Compensation pane is the Spillover Matrix, which includes all fluorescence parameters associated with a Protocol and, if applicable, a column that displays the autofluorescence vector. Other tools, which are described in the following sections, are also available in the Compensation pane:

- *Saving a Compensation File*
- *Importing Compensation*
- *Resetting Spillover and Autofluorescence Vector Values*
- *Using Spillover Sliders*
- *Displaying the Autofluorescence Vector*

NOTE If the Compensation pane does not appear on the left side of the screen, it has been docked. See CHAPTER 6, *Display Options for the Acquisition Attributes Pane* for additional information on docking and undocking panes.

Figure 7.49 Compensation Pane



Compensation

Spillover (%)

	FL1	FL2	FL3	FL4	FL5
FL1		1.20	3.30	2.40	1.30
FL2	14.70		14.20	3.50	2.90
FL3	3.60	25.90		2.20	1.00
FL4	1.60	3.70	21.70		1.50
FL5	1.50	1.10	6.10	25.70	

Kaluza for Gallios Compensation matrix uses the following convention: FL# (vertical axis) - %FL# (horizontal axis). For example, in Figure 7.49, **FL2-%FL1 = 14.7**.

Adjusting Compensation

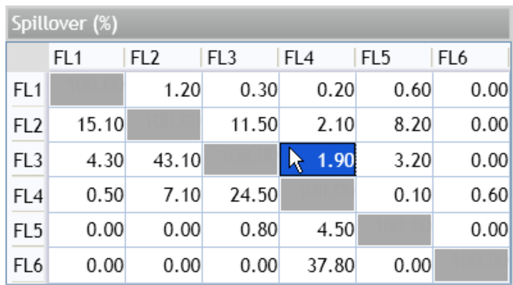
The following sections describe the methods for adjusting compensation.

Adjusting Spillover Values in the Compensation Pane

The Spillover Matrix allows you to manually enter Spillover percentages into the matrix and/or to view Spillover percentages that have already been adjusted using the Spillover Sliders on the plot sheet.

To manually update a value within a cell of the Spillover Matrix:

1. Click your mouse button within the cell you wish to update. As shown in the figure below, the cell is highlighted in blue when it is ready to update.



Spillover (%)

	FL1	FL2	FL3	FL4	FL5	FL6
FL1		1.20	0.30	0.20	0.60	0.00
FL2	15.10		11.50	2.10	8.20	0.00
FL3	4.30	43.10		1.90	3.20	0.00
FL4	0.50	7.10	24.50		0.10	0.60
FL5	0.00	0.00	0.80	4.50		0.00
FL6	0.00	0.00	0.00	37.80	0.00	

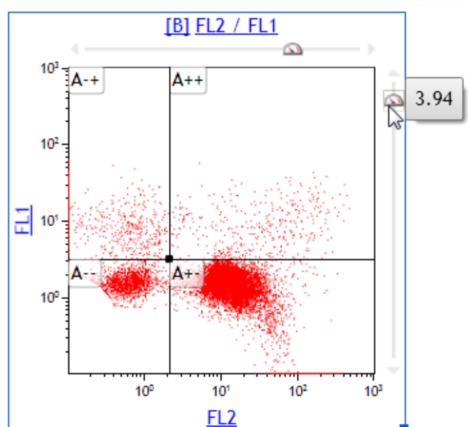
2. Type the new Spillover percentage into the cell and press Enter on your keyboard or click your mouse on another location of your screen.

Using Spillover Sliders

Spillover Sliders allow you to compensate for fluorescence Spillover by using real-time visual cues on plots. The sliders can be generated on all applicable plots.

- **To enable the Spillover Sliders on the plot sheet**: Select the icon from the Compensation pane or the Acquisition Controls Ribbon tab.
- **To remove the Spillover Sliders from the plot sheet**: Select the icon from the Compensation pane or the Acquisition Controls Ribbon tab.
- **To update Spillover using the Spillover Sliders on the plot sheet**: Select the slider and drag in the direction you wish to change. The Spillover value is displayed next to the slider, as shown in Figure 7.50.
- **To make incremented adjustments on the plot sheet**:
  - **To move in increments of .1%**: Select the appropriate arrow located on either end of the slider. Each time an arrow is selected, the slider handle moves 0.1%. This change can also be viewed in the Compensation pane.
  - **To move in increments of 1%**: Click on the slider bar on either side of the slider handle. Each time the slider bar is clicked, the slider moves 1%.

Figure 7.50 Spillover Sliders



Saving a Compensation File

The Spillover Matrix and Autofluorescence Vector can be saved into a ***.compensation** or ***.txt** file for use with other Protocols. To save a ***.compensation** or ***.txt** file:

1. From the Compensation pane, select the icon, which is located above the Spillover Matrix. The Save Compensation dialog box appears.

2. Select the destination for the file by navigating to the location using icons in the dialog box or the drop-down list in the **Save in** field.

3 Enter a file name into the **File name** field.

4 Select **Save**.

Importing Compensation

The Spillover Matrix and Autofluorescence Vector from a previously saved file can be applied to a Data Set. Applicable types files for importing compensation include the following:

- *.analysis
- *.compensation
- *.fcs
- *.lmd
- *.protocol
- *.txt

To import compensation:

1 Select the icon above the Spillover Matrix.

OR

Select **> Import Settings > Compensation.**

OR

From the Worklist, right click on the sample number in the carousel or grid **> Import Settings > Compensation.**

The Import Compensation dialog box appears.

2 Navigate to the file using the icons in the dialog box or the drop-down list in the **Look in** field. Once you find the file, select it, and verify that the file name appears in the **File Name** field.

3 Select **Open.** The new Spillover percentages appear in the Spillover Matrix.

Resetting Spillover and Autofluorescence Vector Values

The icon located above the Spillover Matrix resets Spillover and Autofluorescence Vector values associated with the Data Set to **0.00**.

Accounting for Autofluorescence

To increase the accuracy of fluorescence compensation, Autofluorescence Vector values need to be factored in prior to determining Spillover percentages. An Autofluorescence Vector is the value that is subtracted from the data prior to Spillover compensation and then added back afterwards.

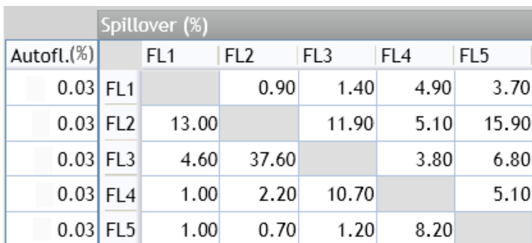
Accounting for autofluorescence allows a greater degree of accuracy when interpreting data due to the increased ability to decipher which fluorochromes have attached to the surface of a cell. The true level of absorption of fluorochromes, or fluorescence intensity, is also more evident when factoring in autofluorescence.

When autofluorescence is considered, it enables a more "true" compensation because it changes the coordinates of each event to a state as if they contained no autofluorescence, compensates the events based on this assumption, and then adds the value back in to re-account for the intensity level.

Displaying the Autofluorescence Vector

To show the Autofluorescence Vector in the Spillover Matrix, select the icon; this adds a column to the left-hand side of the Spillover Matrix (highlighted in red in Figure 7.51).

Figure 7.51 Autofluorescence Vector Column (2 decimals)



	Spillover (%)					
Autofl.(%)		FL1	FL2	FL3	FL4	FL5
0.03	FL1		0.90	1.40	4.90	3.70
0.03	FL2	13.00		11.90	5.10	15.90
0.03	FL3	4.60	37.60		3.80	6.80
0.03	FL4	1.00	2.20	10.70		5.10
0.03	FL5	1.00	0.70	1.20	8.20	

NOTE The Autofluorescence Vector value might be rounded, depending on the number of decimal places you elected to display in the Kaluza Options dialog box.

To hide the Autofluorescence Vector, select the icon.

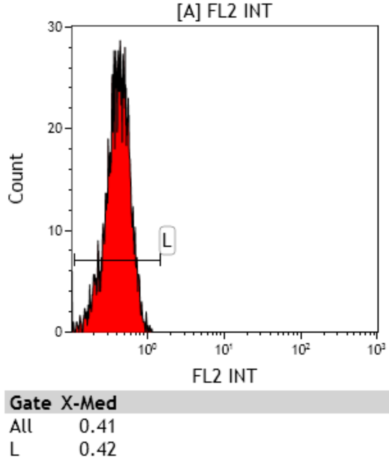
Determining Autofluorescence Vector Values

To manually determine an Autofluorescence Vector value for a fluorescence parameter:

IMPORTANT Optimally, you should start by using an unstained sample. If this is not available, negative populations allow for Autofluorescence Vector calculations.

1 Create a histogram plot for the fluorescence parameter by setting the Y-axis to **Count** and the X-axis to the specific fluorescence parameter.

2 Create a linear gate on the histogram that includes events from the negative population (the events located in the first decade, as indicated by the red outline in the figure below).



3 Using the Statistics radial menu, choose to display the X-Median value.

4 Find the value corresponding to negative population for the gate that you created in step 2.

For example, in the figure in step 2, the "L" gate includes the negative population for FL2. Take the X-Med value shown, divide by 1024 and multiply by 100 to get a percentage.

5 Enter the result from step 4 for the negative population into the Autofluorescence Vector column in the Compensation pane.

Updating the Autofluorescence Vector

To update the Autofluorescence Vector:

1 Click within the cell you wish to update. The cell is highlighted in blue when it is ready to update.

2 Type the new autofluorescence value into the cell and press (Enter) .

NOTE Valid Autofluorescence Vector values range from **0** to **100, since it is a percentage value**.

Using the Logicle Scale

To display the logicle scale (see CHAPTER 7, *Logicle Scale*, for an overview) and use the sliders, follow the steps below.

1 Hover your mouse over the parameter you wish to change to logicle. The parameter changes to a hyperlink.

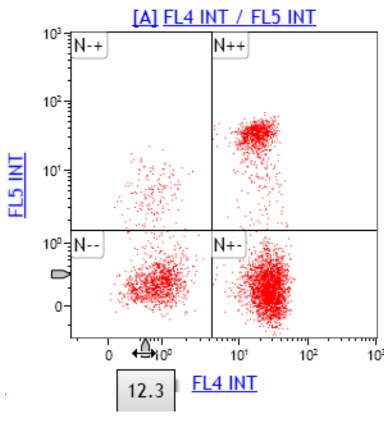
2 Select the hyperlink. The list of parameters appears.

NOTE Currently selected parameters contain a dot next to the parameter name/description, and the current scale used for the parameter (if applicable) contains a check mark next to the scale type.

3 Hover your mouse over the parameter that you wish to display using the logicle scale, and from the pop-up menu, choose the **logicle** scale type for that parameter.

Repeat this step for the other plot parameter, if necessary.

Changing a parameter to the logicle scale adds a slider to the axis, as shown in the figure below.



4 Select and drag the slider to adjust the scale to display negative values. As demonstrated on the X-axis in the figure above, the numerical value appears and changes as you move the slider.

5 Release your mouse button once you are satisfied with the display of the compensated events.

CHAPTER 8

Worklists

Introduction

The Worklist is the location where sample information is entered in preparation for acquisition activities. During an acquisition, the information entered into the Worklist is communicated to the instrument, and the instrument uses this information to acquire samples from the appropriate carousel locations. Protocols are linked to specific sample locations in the carousel through the Worklist. In addition, the Worklist uses a Parameter Descriptions grid, allowing you to enter Sample ID names, edit Parameter descriptions, and provide a Calibration Factor.

This chapter covers the following topics:

- *Worklist Pane*
- *Expanded View*
- *Creating a New Worklist*
- *Saving a Worklist*
- *Opening a Saved Worklist*
- *Customizing the Worklist*
- *Editing a Worklist*
- *Multi-Selecting Samples*

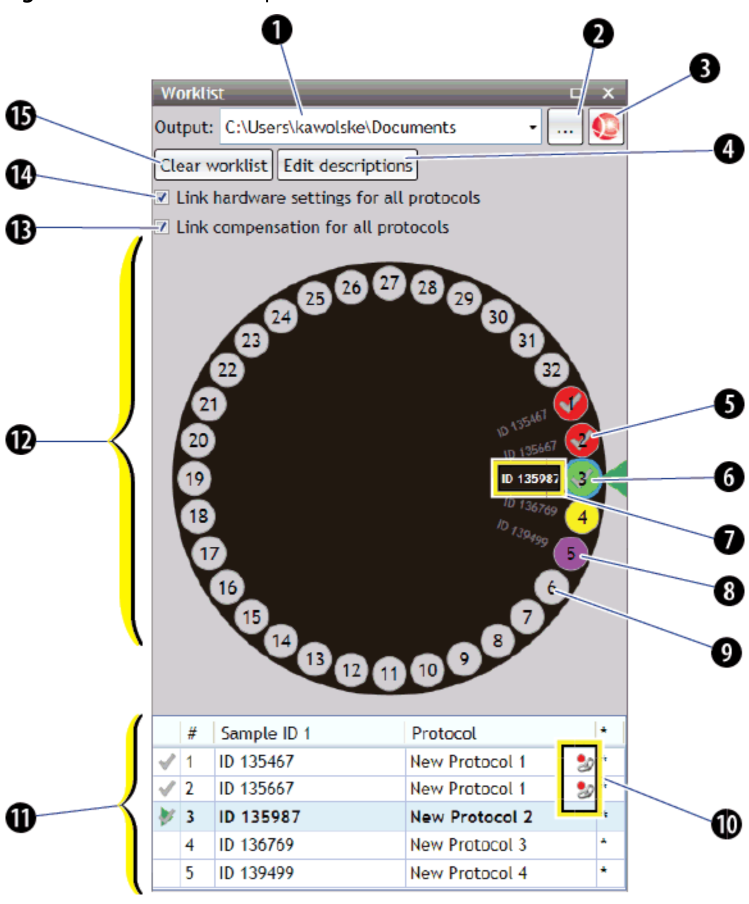
NOTE Instructions on creating Compensation Worklists can be found in CHAPTER 9, *Compensation Worklists*.

Worklist Pane

The Worklist Pane (Figure 8.1), which is located on the left side of the Kaluza for Gallios workspace, is a condensed view of the Worklist, containing only the key elements. The carousel image is visible if space permits. The expanded view (see *Expanded View*) of the Worklist provides complete Worklist details, including the Parameter Descriptions Grid and the carousel image.

NOTE If the Worklist pane does not appear on the left side of the screen, it has been docked. See CHAPTER 6, *Display Options for the Acquisition Attributes Pane* for additional information on docking and undocking panes.

Figure 8.1 Worklist Components



Item	Description
1. Output[1]	By selecting the drop-down, this field allows you to choose from the most recently used directories for the location of acquired data files.
2. Select an output directory[1]	Allows you to browse to a location where acquired *.fcs files are saved.
3. Load acquired data in Kaluza[1]	When selected, this button opens any unloaded data files from the current Worklist into Kaluza Analysis Software. The button animates when new files are available to load.
4. Edit descriptions	Opens the expanded Worklist view, allowing you to enter all relevant parameter descriptions. Double clicking on the carousel also opens the expanded view. See *Expanded View* for details.
5. Acquired Sample	A check mark indicates a sample that has been acquired.
6. Current Sample	The green arrow indicates either the currently selected sample, or the sample that is currently being acquired. If an acquisition is in progress, the green arrow defaults to the sample currently being acquired.
7. Sample ID or Protocol Name	The Protocol name, or, if entered, Sample ID 1.

Item	Description
8. Occupied Carousel Position	Occupied positions are signified by appearing in color. A carousel position is considered occupied once a Protocol is associated with the position. Each unique Protocol is represented by a unique color. All copies of the same Protocol are represented by the same color.
9. Vacant Carousel Position	Vacant carousel positions are indicated by light grey shading.
10. Linked Protocols Symbol	When protocols are linked, the positions become the same color in the carousel, and this symbol is displayed in the Protocol column in the Parameter Descriptions Grid. The color swatch on this symbol corresponds to the color of these samples in the carousel. Additional information on linking can be found in *Editing a Worklist* and *Linking Functionality in Kaluza for Gallios*.
11. Parameter Descriptions Grid	Data specifications are entered in the Parameter Descriptions grid. Additional data entry columns, including an additional Sample ID column, fluorescence detectors, and calibration factor, are available in the expanded view. See *Expanded View* for details.
12. Carousel	Graphical representation of the Gallios carousel. Each number corresponds to the carousel position marked on the instrument.
13. Link Compensation for all protocols	Allows you to link the compensation values between all Protocols when multiple Protocols are used in a Worklist. Once the compensation is linked, changes made to the compensation in one Protocol affect the compensation in all other Protocols. *This option is only available when all Protocols contain the same parameters.* CHAPTER 7, *Introduction to Compensation* provides information on compensation, and Table 8.2 provides details on linking compensation. **NOTE** This option does not affect previously acquired files. However, if a sample is reacquired, the compensation will be updated.
14. Link hardware settings for all protocols	Allows you to link the voltage, gains, and discriminator settings between all Protocol when multiple Protocols are used in a Worklist. Once these settings are linked, changes made to the settings for one Protocol affect the settings for all other Protocols. *This option is only available when all Protocols contain the same parameters.* CHAPTER 7, *Hardware Settings* provides information on hardware settings, and Table 8.2 provides details on linking hardware settings. **NOTE** This option does not affect previously acquired files. However, if a sample is reacquired, the volts, gains, and discriminator settings will be updated.
15. Clear worklist	Clears all input currently entered into the Worklist and returns to application to the home screen. Clearing the Worklist does not remove any saved files associated with the Worklist. **IMPORTANT** Selecting **Clear worklist** does not prompt you to save the Worklist. If necessary, be sure to save the Worklist prior to selecting **Clear Worklist**.

1. This option is not available in Offline Kaluza for Gallios.

Expanded View

The expanded view contains all data entry fields relevant to your acquisitions. In addition to the Worklist items introduced in *Worklist Pane*, the data fields available through the expanded view, which are described in Figure 8.2, allow you to further define your data.

To use the expanded view:

1. To enter the expanded view, double click on the carousel image in the Worklist Pane.

   OR

   Select the Edit parameter descriptions button.

   OR

   Select the button located at the top of the Worklist pane.

2. To exit the expanded view, double click on the carousel image.

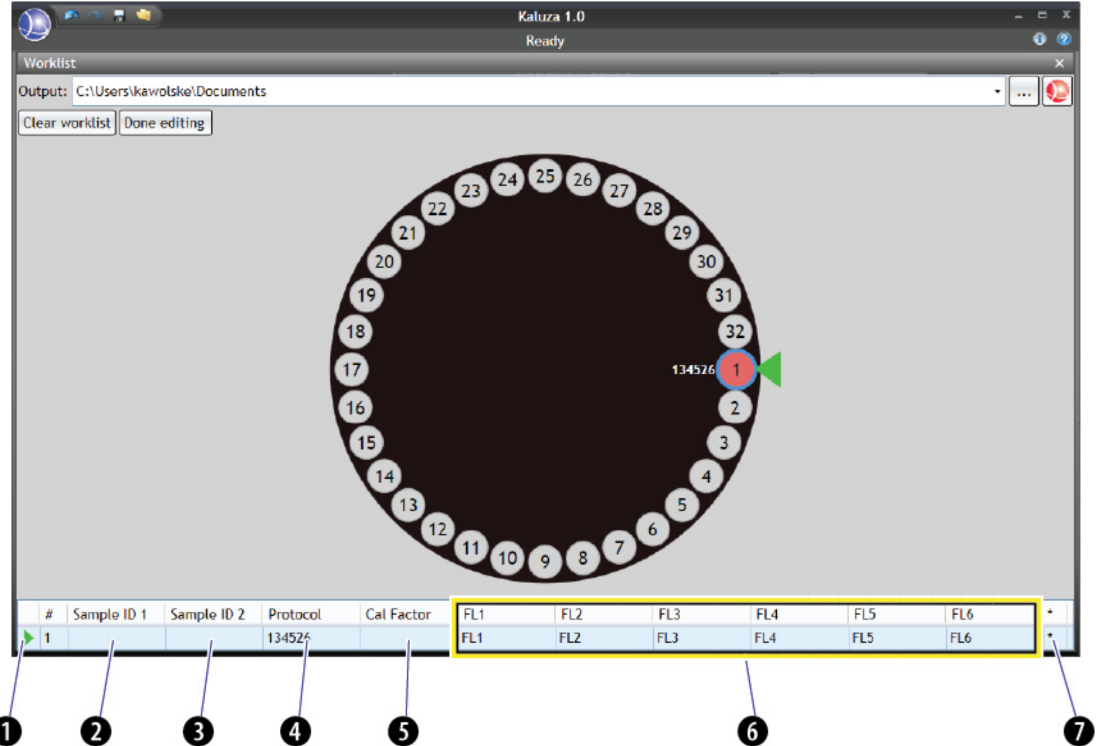
   OR

   Select the Done editing button.

   OR

   Select the button located at the top of the expanded view.

Figure 8.2 Expanded Worklist



Column	Description
1. Indicator	A green arrow in this column indicates the currently selected carousel position, or if an acquisition is in process, the green arrow defaults to the sample currently being acquired. Once a sample has been acquired, a check mark appears in this column.
#	The carousel sample position. The number of this position corresponds to the carousel position on the instrument. **NOTE** If you wish to change the order of samples or choose a specific location for a sample in the carousel, double click the number, enter a new number, and then select the (Enter) key. If a sample has already been assigned to the desired position, you must first change the location of sample residing in that position to an open carousel location. OR You can also move samples by selecting the carousel position(s) that you wish to move, dragging to the new location, and releasing your mouse button. If a sample was already in the location, that sample, and all of the following samples are pushed clockwise around the carousel.
2. Sample ID 1	Enter an ID corresponding to the sample into this column, if desired.
3. Sample ID 2	If applicable, enter second sample ID into this field, allowing you to further identify the sample. This ID does not display on the carousel graphic.
4. Protocol	The name of the Protocol associated with the sample. This field cannot be edited. If you wish to change the name of the Protocol, you must perform a **Save As**, changing the name to the desired name.
5. Cal Factor	Enter the calibration factor, as provided in the FlowCount assay sheets.

Column	Description
6. Fluorescence Detectors (FL1 – FL10)	These fields allow you to enter a description for each fluorescence detector. Any detectors that are not part of the Protocol are signified by shaded cells or do not appear on the Worklist at all.
7. *	When an asterisk appears in this column, it signifies that there are unsaved changes in the Protocol.

Creating a New Worklist

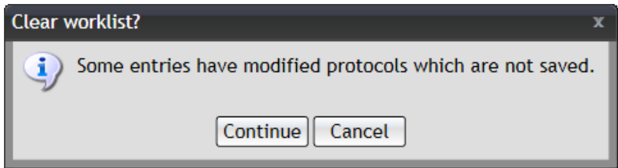
To prepare for the acquisition process, each sample is associated with a Protocol and all related metadata, which is entered into the Worklist. To create a new Worklist:

NOTE Additional methods for creating and/or editing a Worklist are provided in Table 8.1

1 If a Worklist is currently open in Kaluza for Gallios, select Clear worklist from the Worklist pane.

If there are Protocols in the Worklist that have not been saved, you will get a message (Figure 8.3) prompting you to continue clearing the Worklist without saving the Protocols. If you wish to continue, select Continue; however, if you wish to save the Protocols, select Cancel and save the Protocols as necessary.

Figure 8.3 Clear Worklist Prompt



2 From the Kaluza for Gallios home screen, select **Open** to open a saved Protocol. This starts a new Worklist, where selected Protocol is automatically assigned to **Sample 1** in the carousel.

NOTE If you have not yet created the Protocol(s) for the samples that will be part of this Worklist, create and save the Protocol(s) by completing instructions in CHAPTER 7, *Creating a New Protocol*.

NOTE When using a Protocol originated from Kaluza Analysis Software, be aware that items not available in Kaluza for Gallios will not be present, and will not result in an error. These items include the following:

- Contour, Radar, Overlay, Tree, and Comparison Plots
- Gate Statistics and Information Tables
- Boolean Gates
- Sheet Items (Image or Text Boxes)
- Report Sheets
- Multiple Sheets

When saving this Protocol in Kaluza for Gallios and reopening in Kaluza Analysis software, these items will not return as part of the Protocol.

3 From the Worklist pane, select Edit descriptions to enter the expanded Worklist view, which provides additional data entry fields and more space.

4 New Protocols are assigned to the first available open position on the carousel. If you wish to change the order of samples or choose a specific location for a sample in the carousel:

- Select the carousel position(s) that you wish to move, drag to the new location, and release your mouse button. If a sample was already in the location, that sample, and all of the following samples are pushed clockwise around the carousel.

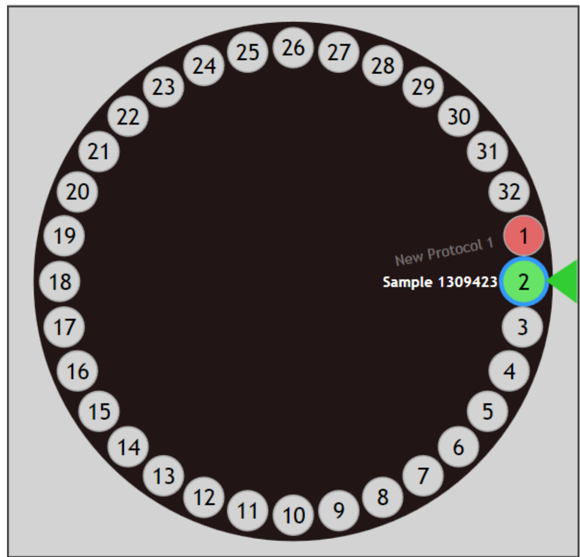
  OR

- Double click the number (in the # column), enter a new number, and then select the Enter key. If a sample has already been assigned to the desired position, you must first change the location of sample residing in that position to an open carousel location.

5 In the grid, enter a sample ID in the **Sample ID 1** column, if desired.

NOTE The text entered into the **Sample ID 1** column becomes the sample label, overriding the Protocol name. In Figure 8.4, **Sample 1309423** was entered into the **Sample ID 1** field for **Sample 2**. The **Sample ID 1** field for **Sample 1** was left blank, defaulting to the Protocol name as the sample label.

Figure 8.4 Sample IDs Visible on Carousel



6 Enter an optional second sample ID in the **Sample ID 2** column.

7 If desired, enter the calibration factor provided on the calibrator assay sheet into the **Cal Factor** field.

8 Enter descriptions for each of the fluorescence detectors, if desired.

NOTE The software defaults to using the abbreviated form for fluorescence detectors if no text is entered. For example, **FL1** will be used for the first fluorescence detector.

9 For the next sample in the Worklist, associate a Protocol to the sample by performing the appropriate option below.

IMPORTANT When a Protocol is used for more than one sample in a Worklist, the Protocols are not linked by default, and, therefore, any changes made to the Protocol only apply to that sample. If you wish to link a specific Protocol between sample locations, select the location to be linked, right-click on the carousel position, and select **Link**.

- **To associate a currently saved Protocol**, hover your mouse over an open sample location When the sample number changes to a icon, select the icon. Navigate to the folder containing the file, select it, and then select **Open**.
- **To use the same Protocol as another sample**, right click on the sample location containing the Protocol you wish to use, and select **Duplicate**. This positions the new sample in the first available carousel location.
- **To create a new Protocol for the next sample**, right click on the sample, and select **New Protocol**. Create and save a Protocol by completing instructions in CHAPTER 7, *Protocols*.

NOTE You can also open saved Worklists into the current Worklist. See *Customizing the Worklist* for details.

10 Update the Parameter Descriptions Grid by following instructions in steps 5 - 8.

11 Continue adding samples to the Worklist by completing steps 9 and 10 until the Worklist is completed.

12 If desired, select the **Link volts and gains for all protocols** and/or the **Link compensation for all protocols** check box (see Figure 8.1 for details).

NOTE This option is only available when all Protocols in the Worklist contain the same parameters.

Saving a Worklist

A Worklist can be saved as a complete unit, or you can choose specific sample locations to be saved as a separate Worklist:

- See *Saving All Items as a Worklist* to save the current Worklist as is.
- See *Saving Selected Carousel Positions as a Worklist* to save specific sample locations as a separate Worklist.

Saving All Items as a Worklist

To save the current Worklist, including associated Protocols, compensation values, sample IDs, carousel positions, and parameter descriptions:

1. Select > **Save worklist as**.

CAUTION

Risk of incongruent data. Saving a Worklist saves all Protocols, compensation files, sample IDs, carousel positions, and parameter descriptions as a unit. Any changes made within the Worklist after it was last saved, including changes to Protocols or Compensation files (even if the *.protocol file or *.compensation file was saved separately) requires you to re-save the Worklist by choosing "Save Worklist As" so that all changes are captured.

2. Enter a name for the Worklist, choose the destination, and select **Save** to complete the process.

Saving Selected Carousel Positions as a Worklist

To save selected carousel positions on the current Worklist, including the associated Protocols, compensation values, sample IDs, carousel positions, and parameter descriptions associated with the selected positions:

1. Select the samples you wish to include as a separate Worklist. If you wish to select:
   - **Multiple consecutive samples**, see *Multi-Selecting a Consecutive Group of Samples*.
   - **Random samples**, see *Multi-Selecting Random Samples in the Worklist*.

2. Right click on the selected samples and choose **Save as worklist**.

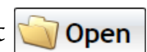
CAUTION

Risk of incongruent data. Saving a Worklist saves all Protocols, compensation files, sample IDs, carousel positions, and parameter descriptions as a unit. Any changes made within the Worklist after it was last saved, including changes to Protocols or Compensation files (even if the *.protocol file or *.compensation file was saved separately) requires you to re-save the Worklist by choosing "Save Worklist As" so that all changes are captured.

3. Enter a name for the Worklist, choose the destination, and select **Save** to complete the process.

Opening a Saved Worklist

To open a saved Worklist:

1 From the Kaluza for Gallios home screen, select  .

2 Navigate to the location of the *.worklist file, and select **Open**. This opens the saved Worklist into Kaluza for Gallios. You may now wish to open additional saved Worklists or Protocols into the current Worklist. See *Customizing the Worklist* for instructions.

Customizing the Worklist

The Kaluza for Gallios Worklist can be customized to meet your needs. You can open multiple *.worklist and *.protocol files to make a new Worklist for your specific needs.

To open multiple saved files to create a custom Worklist:

1 Open the first file into the Worklist:

   a. From the Kaluza for Gallios home screen, select Open .

   b. Navigate to the location of the file, select it, and then select **Open**. This opens the saved Protocol or Worklist into Kaluza for Gallios.

   NOTE If opening a *.worklist file as the first file, the original positions are retained.

2 For the next sample(s):

   a. Select the position where you wish to place the next Protocol or Worklist.

   b. From the Open dialog, navigate to the location of the file, select it, and then select **Open**.

   IMPORTANT If the next file you open is a *.worklist file, the sample positions originally saved in the Worklist are **not** retained; instead, they are placed in the carousel in sequential order at the location where you opened the file. You can edit the location of any samples; refer to *Updating Sample Locations* for additional information.

3 Continue adding to the carousel by repeating Step 2.

4 Once you are satisfied, save the Worklist by following the instructions in *Saving All Items as a Worklist*.

Editing a Worklist

The Worklist can be edited using the options described in Table 8.1. These options are available by right clicking on a sample in the Worklist, or by selecting multiple samples (see *Multi-Selecting Samples* for additional information).

Table 8.1 Worklist Editing Options

Function	Description	Availability		
		Vacant Carousel Position	Occupied Carousel Position	Multiple Samples
Open	Opens a file into the selected location.	✓	–	–
New Protocol	Creates a new Protocol for the selected location. This Protocol can be applied to other locations using the **Duplicate** option.	✓	–	–
Duplicate	Duplicates the Protocol and all data associated with the selected position in the next available carousel position. When a Protocol is duplicated, the positions become linked, meaning changes made to the protocol are linked to all positions using the same Protocol (see *Linking Functionality in Kaluza for Gallios* for details on linked protocols). Protocols that are linked become the same color in the carousel and are signified with a symbol in the Protocol column of the Parameter Descriptions Grid; the color swatch on this symbol corresponds to the color of these samples in the carousel.	–	✓	✓
Delete	Deletes the Protocol(s) and all data associated with the sample from the Worklist. **IMPORTANT** Deleting a sample from the Worklist does **not** delete files associated with that carousel position.	–	✓	✓
Link	Links the protocol for when multiple carousel positions are selected. When positions containing different original configurations are linked, all positions are switched to the Protocol of the lowest numbered carousel position in the multi-selected group; therefore any prior configurations made are discarded. After linking multiple positions, changes made to a Protocol, no matter which position is currently selected, make the same changes to all other positions assigned to that Protocol. Protocols that are linked are the same color in the carousel, and are also signified with a symbol in the Protocol column of the Parameter Descriptions Grid. For additional details on linking Protocols, see *Linking Functionality in Kaluza for Gallios*.	–	–	✓

Table 8.1 Worklist Editing Options

Function	Description	Availability		
		Vacant Carousel Position	Occupied Carousel Position	Multiple Samples
Unlink	Removes the selected carousel position from the linked Protocol. **IMPORTANT** Unlinking the Protocol **does not** prompt you to change the file name of the Protocol. Once you make changes, be sure to save the Protocol as a different name so that you do not save over the original protocol.	–	✓	✓
Import settings	Allows you to select a file from which to import compensation and/or voltages, gains, and discriminator settings.	–	✓	✓
Save protocol	Saves the Protocol associated with a carousel position.	–	✓	–
Save protocol as...	Saves the Protocol for the selected position, and allows you to specify a different name. This updates the Protocol name and color on the carousel graphic. Any other samples using the original Protocol remain using that Protocol.	–	✓	–
Save selected as worklist	Saves the selected Protocols together as a *.worklist file.	–	–	✓
Load in Kaluza...	Loads the *.fcs file from the selected sample into Kaluza Analysis Software.	–	✓[1]	✓[1]

1. This option is available only after a sample has been acquired.

Updating Sample Locations

You can edit the location of samples in the carousel through one of the procedures below:

From the Carousel:

1 Select the carousel position(s) that you wish to move

2 Drag to the new location

3 Release your mouse button

If a sample was already in the location, that sample, and all of the following samples, are pushed back on the queue, behind the moved samples.

From the Parameter Descriptions Grid:

1. Double click the sample number (in the # column),
2. Enter a new number, and then press the Enter key.

If a sample has already been assigned to the desired position, you must first change the location of sample residing in that position to an open carousel location.

Linking Functionality in Kaluza for Gallios

Table 8.2 provides details of linking functionality in Kaluza for Gallios.

NOTE Importing hardware settings and compensation differ from linking. Importing is a single action that makes the Protocol match what is in a the imported file. Importing does not create or maintain a relationship so that changes to one entry propagate to additional entries.

Table 8.2 Linking Functionality

Feature	Requirement(s)	What Becomes Linked	What is NOT Linked	How to Link	Visual Indicator After Linking
Protocols	No requirements	• Plots • Gate Positions • Hardware Settings • Compensation • Color Precedence • Stop Conditions • Flow Rates	• Parameter Descriptions	**1.** Load multiple Protocols into the carousel. **2.** Multi-select the carousel positions you wish to link. **3.** Right click on them and select **Link**. OR **1.** Duplicate a Protocol in the Worklist.	Carousel positions are the same color. Protocol 1 (1) Protocol 1 (2) Protocol 1 (3) Link icon displayed with the protocol name in the carousel grid. Protocol **Protocol 1** Protocol 1 Protocol 1
Hardware Settings	Same detectors in all protocols	• Voltages • Gains • Discriminators	• Plots • Gates • Color Precedence • Compensation • Stop Conditions • Flow Rates	Check the **Link hardware settings for all protocols** check box in the Worklist pane.	Check box in the worklist pane. ☑ Link hardware settings for all protocols
Compensation Settings	Same compensated detectors in all protocols	• Compensation	• Hardware Settings • Plots • Gates • Color Precedence • Stop Conditions • Flow Rates	Check the **Link compensation for all protocols** check box in the Worklist pane.	Check box in the worklist pane. ☑ Link compensation for all protocols

Editing Metadata in Kaluza for Gallios

Entries in the Parameter Descriptions Grid can be copied and pasted into other cells or deleted. Table 8.3 describes the actions to take complete these tasks.

Table 8.3 Editing the Parameter Descriptions in Kaluza for Gallios

Task	Action
Copy a single cell.	Select the cell and press Ctrl + C .
Copy a rectangular group of cells.	Select the first cell in the group; then Shift +click the last cell in the group. Press Ctrl + C on your keyboard.
Paste a single copied cell.	Select the cell you wish to paste into, and then press Ctrl + V on your keyboard.
Paste a single copied cell into multiple cells.	Select the first cell in the group; then Shift +click the last cell in the group. Press Ctrl + V on your keyboard.
Paste a group of copied cells.	Select the first cell in the group; then Shift +click the last cell in the group. Press Ctrl + V on your keyboard.
Delete a single cell.	Select the cell, and then press the Delete key on your keyboard.
Delete a group of cells.	Select the first cell in the group; then Shift +click the last cell in the group that you wish to delete. Press the Delete key on your keyboard.

Editing Metadata in Excel

Kaluza for Gallios provides the ability to edit Worklist metadata in Microsoft Excel, allowing you to take advantage of the added flexibility that Microsoft Excel offers. This is especially beneficial when changing or inputting a significant amount of metadata.

NOTE The procedure below can be done in the Worklist pane (*Worklist Pane*) and the expanded view (*Expanded View*). If attempting to paste content that was originally copied while in the expanded view back into the Worklist pane (after editing in Excel), only the content from the # and **Sample ID 1** columns will be updated. You must be in the expanded view to paste all other content, such as parameter labels.

To edit metadata in Excel:

1 In the Parameter Descriptions Grid, select the cells you wish to edit:

- **To select a rectangular group of cells**, click on the first cell you wish to edit; then, Shift + click the last cell in the group. For example, in Figure 8.5, the dark blue cells were selected as a group by first selecting the cell outlined in red and then Shift + clicking the cell outlined in yellow.

Figure 8.5 Rectangular Group Selected

#	Sample ID 1	Sample ID 2	Protocol	Cal Factor	FL1	FL2	FL3	FL4
1			New Protoc...		FL1	FL2	FL3	FL4
2			New Protoco...		FL1	FL2	FL3	FL4
3			New Protoco...		FL1	FL2	FL3	FL4

OR

- **To select all of the cells in the grid**, press Ctrl + A on your keyboard.

2 Press Ctrl + C on your keyboard.

3 Open Microsoft Excel, and on your keyboard, press Ctrl + V .

4 Make necessary edits, and when completed, select the data and press Ctrl + C on your keyboard.

IMPORTANT The first, last, and **Protocol** columns cannot be updated by changing the text. Therefore, any updates made to these columns in Excel will not make changes to Parameter Descriptions Grid.

5 Paste the data back into Kaluza for Gallios:

- **To paste the data in a specific location**, click at the starting cell where you wish to place your data, and press Ctrl + V on your keyboard. Verify that the metadata pasted as desired.

OR

- **To replace all of the metadata when there is a 1:1 relationship with the copied content,** click within the Worklist and press Ctrl + A to select all entries; then enter Ctrl + V .

OR

- **To replace all of the metadata when there is NOT a 1:1 relationship with the copied content:** *For example, your Worklist contains 10 samples, but you wish to use the edited metadata from Sample 1 for samples 3, 5, 7, and 9 and the edited metadata from Sample 2 for samples 4, 6, 8, and 10.* After editing and then copying rows 1 and 2 in Excel, click in the Worklist, press Ctrl + A to select all entries; then press Ctrl + V to paste them.

6 Save the Excel file for later use, if desired.

Multi-Selecting Samples

By selecting multiple samples (or rows) in the Worklist, a different set of options become available for the samples (see Table 8.1 for details). The following sections describe the methods for multi-selecting samples in the Worklist.

Multi-Selecting a Consecutive Group of Samples

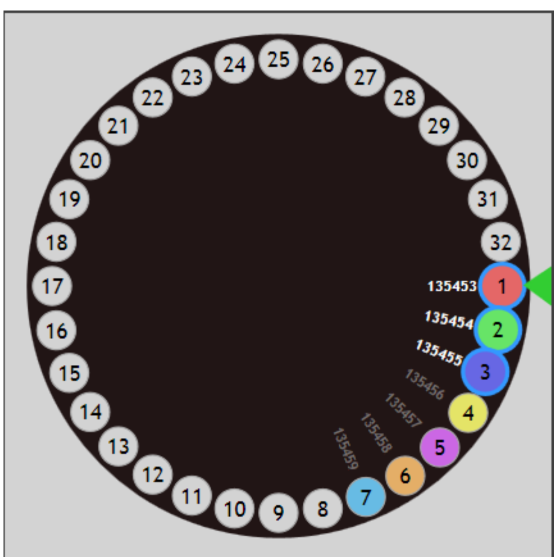
To multi-select a consecutive group of samples:

1 Select the sample with the lower number first.

2 Press and hold the Shift key and select the sample with the higher number.

3 When you are finished, release the Shift key. The entries are now ready to act as a group. On the carousel graphic, selected samples appear with a bold blue outline and Protocol names or sample IDs are in bold white font as shown on samples 1, 2, and 3 in Figure 8.6. In addition, grid rows for the selected samples are highlighted in blue.

Figure 8.6 Multiple Selected Samples



Multi-Selecting Random Samples in the Worklist

To multi-select random samples:

1. Press and hold the (Ctrl) key while selecting the samples you wish to include in your selection.

2. Release the (Ctrl) key when you have finished making your selections. The entries are now ready to act as a group. On the carousel graphic, selected samples appear with a bold blue outline and Protocol names or sample IDs are in bold white font as shown on samples 1, 2, and 3 in Figure 8.6. In addition, grid rows for the selected samples are highlighted in blue.

CHAPTER 9

Compensation Worklists

Introduction

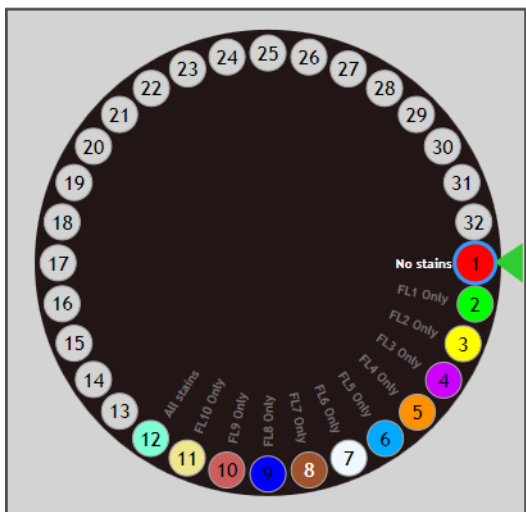
Compensation Worklists are different from standard Worklists in that the software automatically creates a Protocol for each sample. These Protocols are designed to include the items necessary for accurately adjusting compensation. The color compensation values derived while running the Compensation Worklist can be imported into future assays using the same stains.

NOTE For a basic overview on compensation, and to understand the need for accurately compensating events, see CHAPTER 7, *Introduction to Compensation*.

Compensation Worklist Components

Compensation Worklists are typically comprised of three components, the Setup (**No stains**) sample, the Control (single stain fluorescence) samples, and the Verification (**All stains**) sample. Figure 9.1 shows the carousel view of a 10-color Worklist. Each of these types of samples are described in the sections below.

Figure 9.1 Ten-Color Compensation Worklist—Carousel View



Setup Sample

The Setup sample (**No stains** in Figure 9.1) is the first sample in the Worklist. The Setup Protocol is designed for determining autofluorescence levels, as well as setting the voltages, which increases the accuracy of results when applied to the stained samples. The Setup Protocol contains the following:

- One FS/SS dot plot containing an ellipse gate marked, **Cells**.
- A histogram for each fluorescence parameter in the Compensation Worklist. Each histogram is gated on **Cells** and displays the **median** of events that naturally fluoresce on the available fluorescence detectors.

For additional information on autofluorescence, see CHAPTER 7, *Accounting for Autofluorescence*.

Control Samples

Following the **No stains** sample, are the Control samples containing single stains (samples **2** through **11** in Figure 9.1). The number of stains depends on the number of detectors available for your cytometer and the number of stains required for your assay. Running single stains allows you narrow the emission spectrum of each stain so that each dye is properly represented. The Control Protocols contain the following:

- One FS/SS dot plot containing an ellipse gate marked **Cells.**
- One dot plot for each fluorescence parameter in the Worklist compared to the current stain. The current stain is located on the X-axes, and the other stains are located on the Y-axes. These plots are gated on the **Cell** gate and contain two gates, one for events emitting dim signals, and the other for events emitting bright signals. Statics are displayed at the bottom of the plot, which help you achieve proper compensation.

Verification Sample

The Verification sample is the last sample in your Worklist (**All stains** in Figure 9.1), which directly follows the last sample containing a Control. This sample is made using the same cell type that you are using for your assay, and is stained with all of the desired antibody cocktail.

NOTE The Verification Protocol contains the plots and gates defined in **Add All Plots Options**. Make sure that the **Add All Plots Options** are set the way you want *prior to* generating the Verification Protocol. See CHAPTER 7, *Add All Plots* for details.

Sample Preparation

To prepare the samples for adjusting color compensation:

NOTE In Kaluza for Gallios, Compensation Worklists are always set up using the same workflow, which is specified below and shown in Figure 9.1. The order of samples in your carousel must reflect this pattern. If you do not wish to not include a setup (**No stains)** sample or a verification (**All Stains**) sample, make sure to set up your carousel as instructed below; the software automatically adds these samples, but they can be deleted from the Worklist.

1 Prepare the Setup sample, and place in carousel location no. **1**.

2 Prepare a control sample for each detector used in the application:

a. Stain each sample with the appropriate fluorochrome that corresponds to each detector used in the application. Make sure to include negatives or unstained cells in your samples in addition to the positively-stained cells.

b. Place each single color sample in the carousel in the order of detectors used, starting with carousel position **2** for the FL1 Control stain. The table below specifies the single-stain locations.

IMPORTANT All of the ten detectors specified below might not be available, depending on the setup of your instrument or the number of fluorochromes required for your assay.

Carousel Position	Detector
2	FL1
3	FL2
4	FL3
5	FL4
6	FL5
7	FL6
8	FL7
9	FL8
10	FL9
11	FL10

3 Prepare the Verification sample using the desired (fluorochrome-related) cocktail, and place it in the open carousel position directly following the last single-stained sample.

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

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 Close the MCL cover.

6 The samples are now ready to acquire for compensation adjustment procedures. You must now set up the Compensation Worklist in Kaluza for Gallios; refer to *Creating a Compensation Worklist* for details.

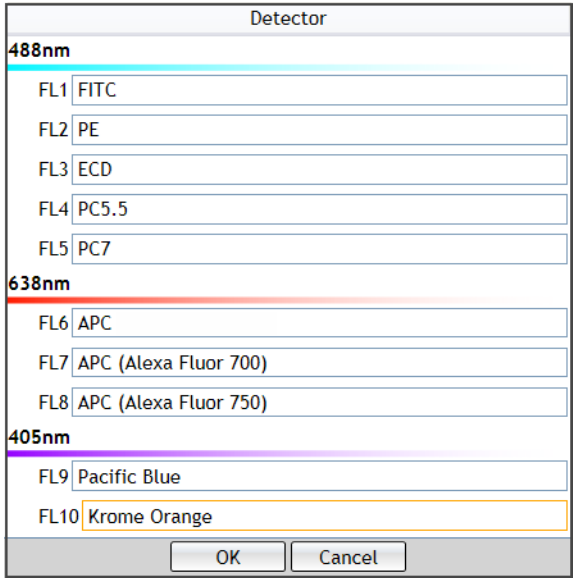
Creating a Compensation Worklist

To create a Compensation Worklist:

1 From the Kaluza for Gallios home screen, select **Create compensation worklist**. The screen changes, allowing you to prepare your Compensation Worklist.

2 Enter a description for each single-color control in your Compensation Worklist. Figure 9.2 is an example of the fluorochromes used in a 10-color application.

Figure 9.2 Ten-Color Compensation Worklist Descriptions



3 Select **OK**. This creates a separate Worklist entry for each control stain, preceded by the **No stains** sample and followed by the **All Stains** sample (Figure 9.3). In addition, a Protocol specifically designed for performing compensation is automatically created for every sample in the Worklist.

NOTE For a description of each sample type and the contents of automatic Protocols, see *Compensation Worklist Components*.

NOTE Compensation and Voltage sliders are enabled once the Compensation Worklist is created. For additional information on Compensation and Voltage sliders, see CHAPTER 7, *Plot Sliders*.

Figure 9.3 Compensation Worklist—Pane View

Worklist
Output: C:\Users\kawolske\Documents
Clear worklist | Edit descriptions
☑ Link hardware settings for all protocols
☑ Link compensation for all protocols

#	Sample ID 1	Protocol	*
1	**No stains**	**Setup**	*
2	FITC	FL1 control	*
3	PE	FL2 control	*
4	ECD	FL3 control	*
5	PC5.5	FL4 control	*
6	PC7	FL5 control	*
7	APC	FL6 control	*
8	APC (Alexa Fluor...	FL7 control	*
9	APC (Alexa Fluor...	FL8 control	*
10	Pacific Blue	FL9 control	*
11	Krome Orange	FL10 control	*
12	All stains	Verification	*

4 As a default, **Link hardware settings for all protocols** and **Link compensation for all protocols** are selected; verify that they are still selected.

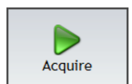
5 If desired, make updates to the Parameter Descriptions Grid by double clicking on the carousel to open the expanded view. Select Done editing when completed.

You are now ready to perform the compensation adjustment procedures. See *Acquiring Compensation Samples and Adjusting Compensation* for details.

Acquiring Compensation Samples and Adjusting Compensation

To acquire compensation samples and adjust compensation:

1 From the Worklist Pane, ensure that the **No stains** sample is selected, and then select



.

2 In the FS/SS plot, resize and/or move the gate marked **Cells** to ensure the event population of interest is captured.

3 On each of the histograms, make all of the necessary hardware adjustments, including voltages, gains, etc., ensuring that the populations are clearly visible on the scale.

NOTE Setup Mode is activated once the Hardware settings are modified.

See CHAPTER 7, *Hardware Configuration* for additional information.

4 Once all of the hardware adjustments are made, remove the software from Setup mode by selecting Setup Mode.

IMPORTANT Throughout the compensation adjustment procedure, it might become apparent that the hardware settings were initially not adjusted properly. If voltages settings change during the compensation adjustment procedure you need to re-acquire the entire Compensation Worklist.

Once the **No stains** sample has been acquired, the instrument automatically moves to the single-stain samples, starting with the single-color stained sample corresponding to the FL1 detector.

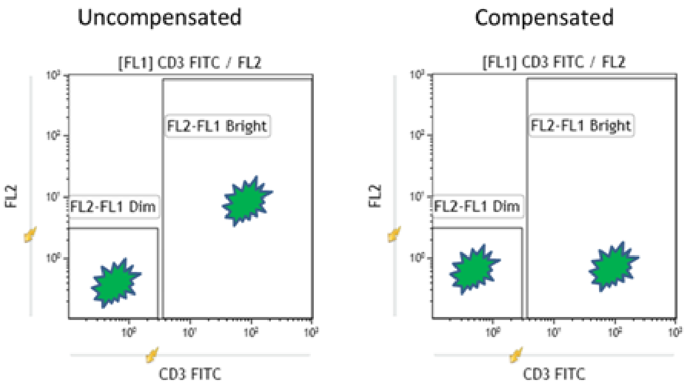
IMPORTANT Steps 5 through 8 are optional, and apply to those who wish to manually adjust compensation during acquisition. If you wish to have the acquisition auto-compensated, skip to Step 9.

5 In the FS/SS plot, resize and/or move the gate to ensure the event population of interest is captured.

6 Using the Compensation sliders (CHAPTER 7, *Plot Sliders*), adjust the compensation on each of the plots so that event populations in the Bright and Dim gates are aligned horizontally and are symmetrical in appearance, as demonstrated in Figure 9.4. If necessary, adjust the location of the gates to ensure the events are centered in the gate. Compensation has been achieved when the **Y-Med** values for the Bright and Dim gates are essentially the same value.

IMPORTANT Compensation adjustment is active during Pause Mode, so you may select **Pause** (on the Instrument Control panel) during the compensation process to ensure all adjustments are made before acquisition for this sample is completed.

Figure 9.4 Uncompensated vs. Compensated Bright and Dim Gates



7 Continue compensating all of the single-stain samples by repeating the procedures in steps 5 and 6.

Once all of the single-stain samples have been acquired, the instrument moves to the **All Stains** sample. This sample serves as a verification, allowing you to check and fine-tune the compensation settings derived from the single-stain samples against a sample using the same cell type and fluorochromes that you are using for your assay.

8 View the **All Stains** plots, verify results are as expected, and make any final adjustments, if necessary. You will typically use the compensation values from the **All Stains** *.fcs file to import into your assays containing the same stains, as this file contains the resultant values from the previous adjustments in the Worklist. For instructions on importing these values into you assays, see CHAPTER 7, *Importing Compensation*.

9 Once the acquisition is completed, you can fine-tune your compensation values using Kaluza Analysis Software by selecting the button; refer to *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature* in the *Kaluza Analysis Software Instructions for Use* (P/N A75667) for details. Compensation values that have been set in Kaluza Analysis can be imported back into Kaluza for Gallios; see CHAPTER 7, *Importing Compensation* for details.

10 If desired, save the Worklist for future use:

a. Select > **Save worklist as.**

b. In the **Save worklist as** dialog box, enter a name in the **File name** field.

c. Navigate to the location where you wish to save the file, and then select **Save**.

CHAPTER 10

Daily Routine

Before You Begin

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

1 Read CHAPTER 3, *Operation Principles*. Using your system is easier if you have a general understanding of how it works.

2 Read CHAPTER 5, *System Overview*. 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.

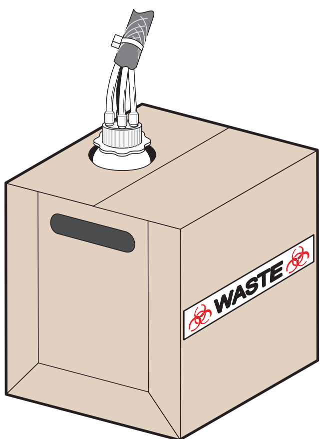
Daily Startup

Perform the following steps to start up the system. If you have set up Cytometer Auto Power On/ Power Off, and the Cytometer is running, skip ahead to Additional Start Up Tasks.

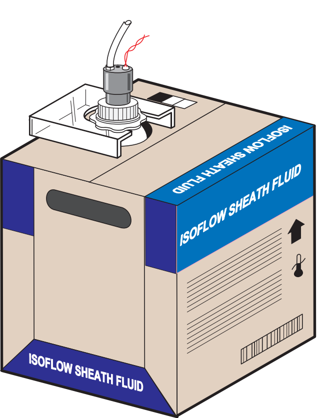
1. Check Waste and Reagent Levels
2. Power the Computer and Cytometer ON
3. Check the Power Supply
4. Additional Start Up Tasks

Check Waste and Reagent Levels

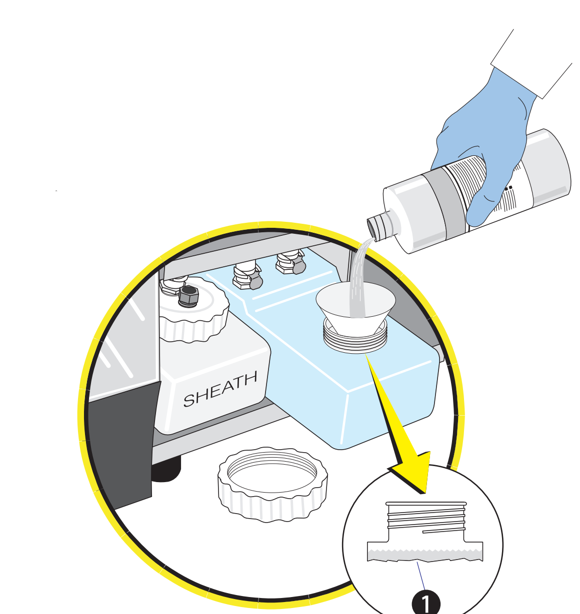
1 Empty the waste container and verify tubing is connected to the cap.



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



3 Check the cleaning agent fill level **(1)** and fill the cleaning agent container if necessary.



Power the Computer and Cytometer ON

To power the computer and cytometer on:

1 Turn on the computer.

2 When the Windows Log on screen appears:

a. Enter your Windows **User Name.**

b. Enter your **Password** and press the (Enter) key.

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

3 Double-click the Kaluza for Gallios shortcut (Figure 10.1) located on your desktop.

Figure 10.1 Kaluza for Gallios Shortcut

OR

Click the **Windows Start Button** > **All Programs** > **Beckman Coulter** > **Kaluza for Gallios 1.0**.

NOTE Do not start a full disk virus scan while running Kaluza for Gallios.

4 Launching Kaluza for Gallios simultaneously powers on the instrument. After the program launches, you will notice the instrument status progress through the following statuses:

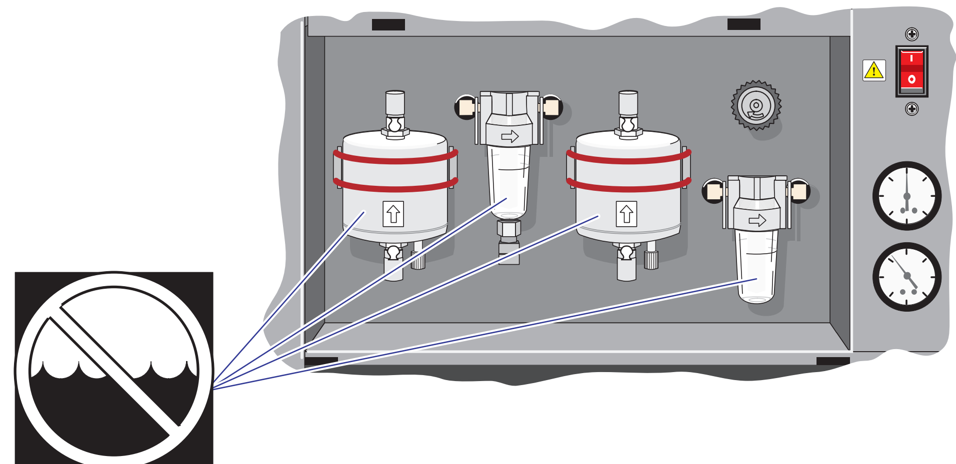
- *Off*
- *Initializing*
- *Ready*

IMPORTANT Warm up requirements depend on how the instrument has been powered off:

- **If the instrument had been completely shut down, including the Supply Cart power switch turned off,** allow about 40 minutes to warm up the system before performing QC or running samples
- **If the instrument has been shut down, but the Supply Cart pneumatics switch was left on**, allow about 10 minutes to warm up the system.

Check the Power Supply

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

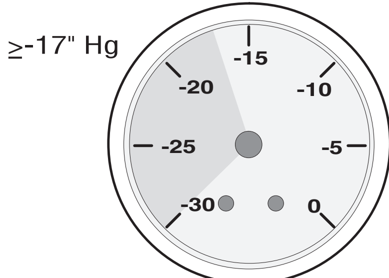


Call your Beckman Coulter Representative if:

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

---

2 Check the SYStem VACuum gauge to verify it reads between 17 and 30 in. Hg (dark grey shaded area). If it reads less than 17 in. Hg, call your Beckman Coulter Representative.



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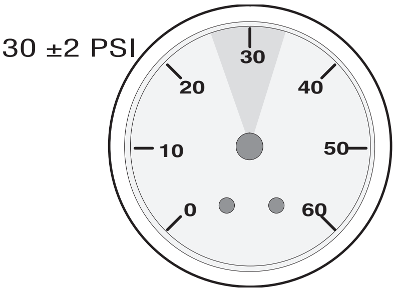
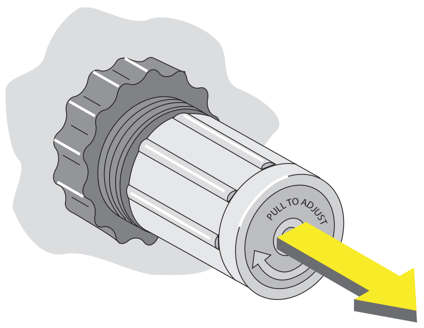
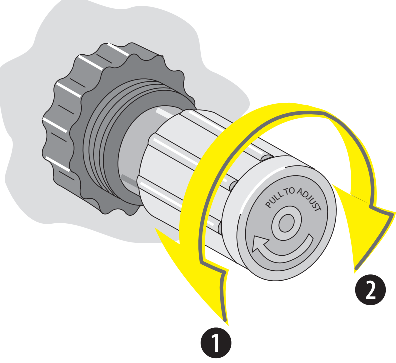
3 Check the SYStem PRESSure gauge. If it does not read between 28 and 32 psi, do the following:



a. Pull the PRESSure ADJuster knob out toward you.

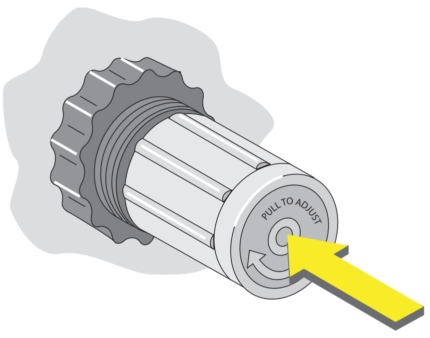


b. Adjust the pressure to 30 ±2 psi.



1. To decrease, turn to the left.
2. To increase, turn to the right.

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



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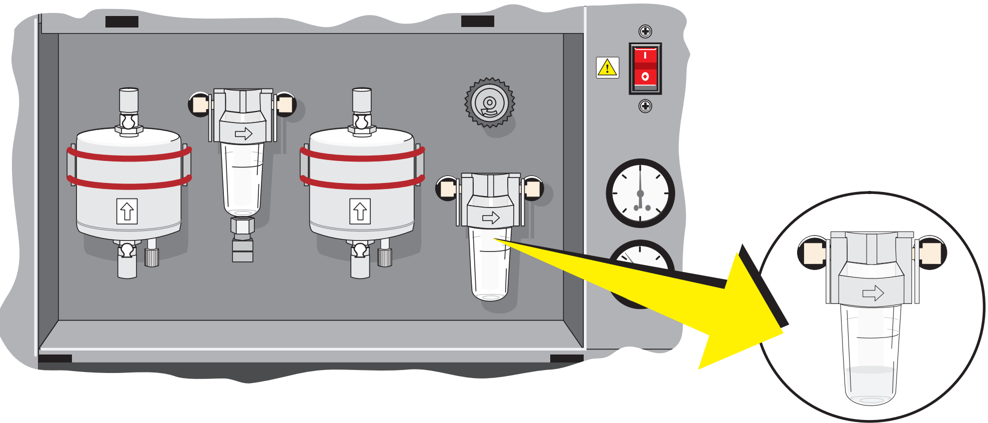
4 Check the VACuum TRAP.

If it is >1/4 full of fluid Clean the Vacuum Trap.



5 Close the Supply Cart door.

Additional Start Up Tasks

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

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.

Before Performing Shut Down

Perform the *Routine Cleaning Procedure* in CHAPTER 13, *Cleaning Procedures*.

Power the Computer and Cytometer OFF

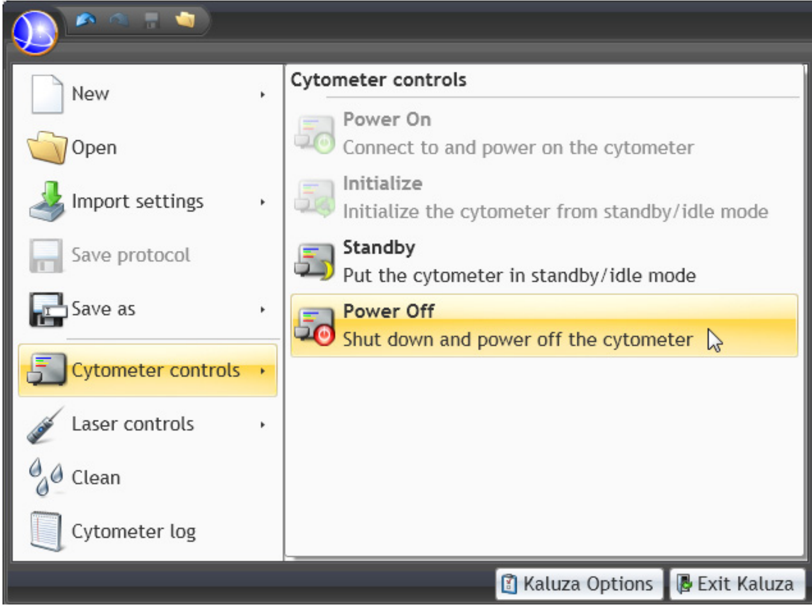
To power the computer and cytometer OFF:

1 Make sure the instrument is not actively acquiring data. If so, wait for the acquisition to finish before moving to step 2.

2 If applicable, save any unsaved changes to your Protocols or Worklist files.

3 Select > **Cytometer controls** > **Power Off** to power down the cytometer (Figure 10.2).

Figure 10.2 Powering Off the Cytometer



4 Select > Exit Kaluza to close the application.

5 If applicable, close any other open applications.

6 Select the Windows **Start** button, and then select the **Shut down** button to turn off the computer.

7 Turn off the monitor.

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.

Extended Shutdown

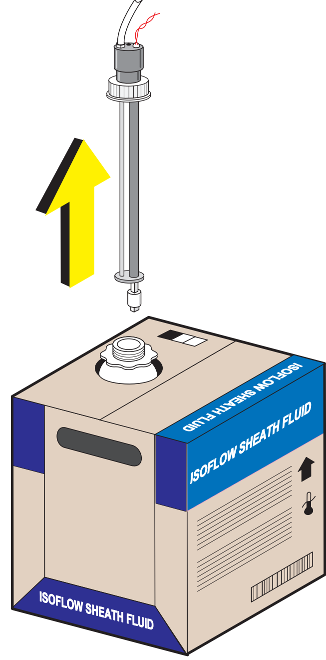
If you intend to leave the instrument in the shutdown state for an extended amount of time:

1 Make sure the instrument is not actively acquiring data. If so, wait for the acquisition to finish before moving to step 2.

CAUTION

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.

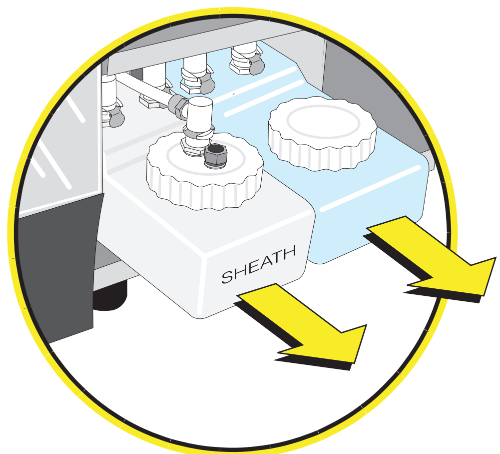
2 Disconnect the support collar from the external sheath fluid container.



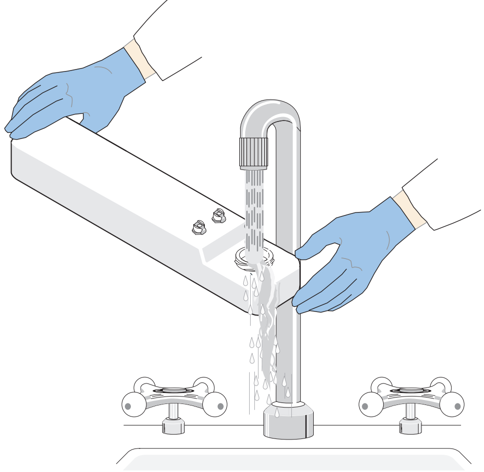
3 Lift the pickup tube assembly straight up and out.

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

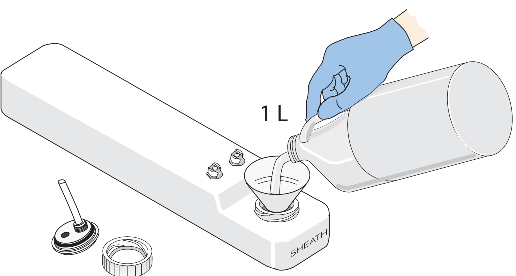
5 Remove the internal sheath fluid and cleaning agent containers.



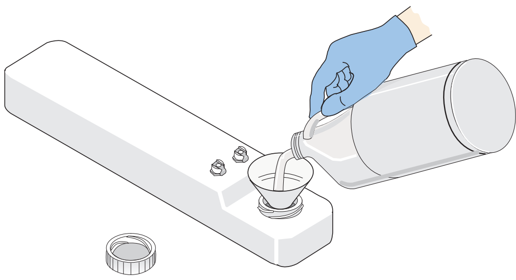
6 Rinse the inside of both containers with water.



7 Pour 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.



8 Fill the cleaning agent container with distilled water.

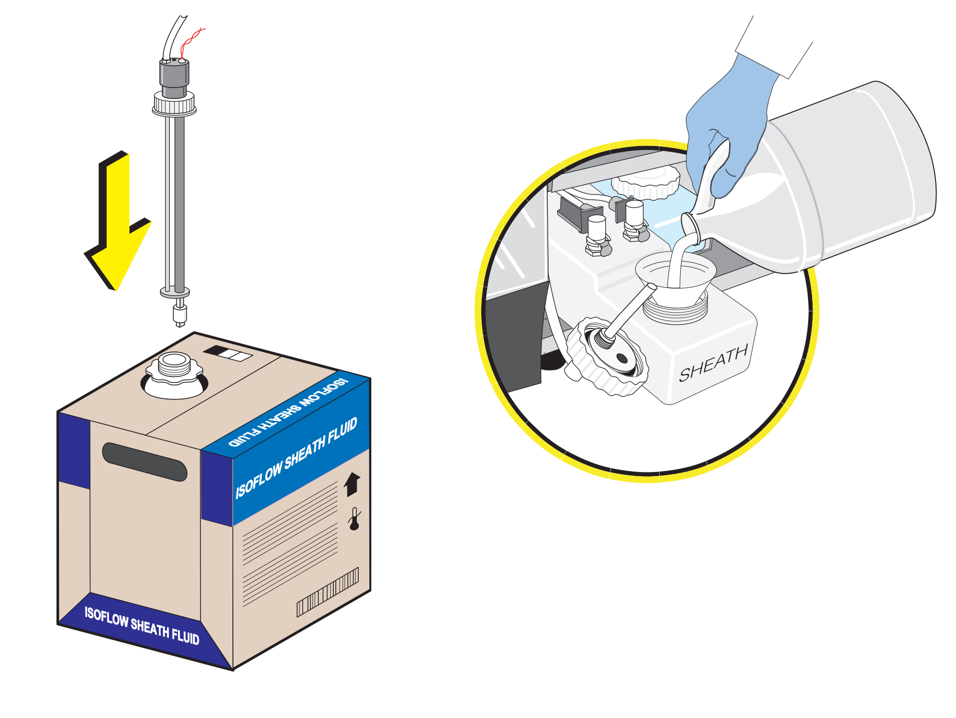


9 Cap and replace the internal sheath fluid and cleaning agent containers.

10 Perform the *Routine Cleaning Procedure* in CHAPTER 13, *Cleaning Procedures*, except use tap water in all four tubes. Do not use any bleach or IsoFlow sheath fluid.

11 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



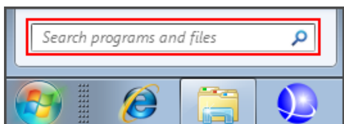
Cytometer Auto Power On/Power Off

You can set up the system to automatically power on or power off the Cytometer. The computer must be ON with Windows running to allow auto startup to run.

To set up auto startup or shutdown:

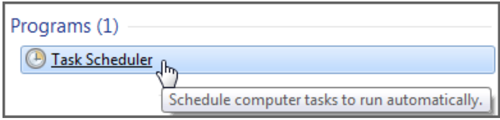
1 Select the Windows **Start** button and enter the text "**Task Scheduler**" into the *Search Programs and Files* field (Figure 10.3).

Figure 10.3 Search Programs and Files Field



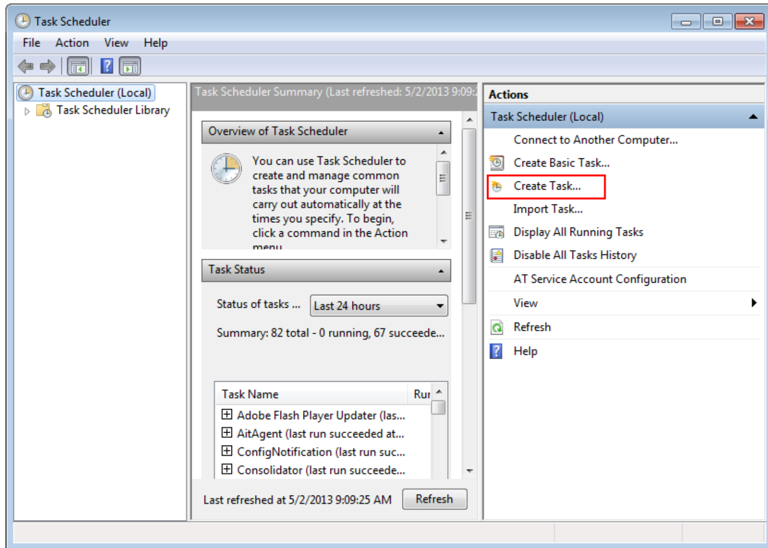
2 Select **Task Scheduler** (Figure 10.4) to open the **Task Scheduler** window.

Figure 10.4 Task Scheduler—Windows Start Menu



3 In the Actions section, select **Create Task**.

Figure 10.5 Actions—Create Task



4 On the **General** tab, enter “**Turn Cytometer On**” or “**Turn Cytometer Off**” in the **Name** field.

5 Select the **Actions** tab, and select **New...**.

6 Select the **Browse...** button.

- To power the cytometer **ON**, browse to: **C:\Program Files (x86)\Beckman Coulter\Flow Cytometry\CytoSystem\bin\BCI.Cytomics.CRS.CXPSupport.InstrumentOn.exe**

  OR

- To power the cytometer **OFF**, browse to: **C:\Program Files (x86)\Beckman Coulter\Flow Cytometry\CytoSystem\bin\BCI.Cytomics.CRS.CXPSupport.InstrumentOff.exe**

7 Select **OK**.

8 Select the **Triggers** tab, and then select **New...**.

9 In **Settings**, select **Daily**, and then configure the time to start in the **Start** field. Select **OK** when completed.

10 Select **OK** in the **Create Task** window, and then close the **Task Schedule** window to complete the auto setup procedure.

CHAPTER 11

Quality Control

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 Kaluza for Gallios, but need optimized for your experiment/instrument. Specifically, these protocols are listed below and are available at the following location:
C:\Users\Public\Public Documents\Beckman Coulter\Flow Cytometry

- **Flow-Check Pro**:
  - Flow-Check Pro - 6 color
  - Flow-Check Pro - 8 color
  - Flow-Check Pro - 10 color
- **Flow-Set Pro**:
  - Flow-Set Pro - 6 color
  - Flow-Set Pro - 8 color
  - Flow-Set Pro - 10 color

In addition to doing the daily quality control procedures in this chapter, you should make a quality control check for the specific application/experiment you are running.

QC Materials

The QC materials needed for each QC process are listed in Table 11.1.

Table 11.1 QC Materials

QC Process	QC Material Used
Verify fluidics and laser alignment	Flow-Check Pro Fluorospheres. Verify HPCV versus expected value.
Adjust high voltage and gain for a given application	Flow-Set Pro Fluorospheres. Ascertain target mode position based upon application/experiment and adjust high voltage and gain daily to that target.
Perform absolute counts	Flow-Count Fluorospheres.

Table 11.1 QC Materials

QC Process	QC Material Used
Adjust color compensation for a given application	VersaComp Antibody Capture Beads, Cyto-Comp Cells, or whole blood stained with single-color antibodies. Use single color stained samples with each fluorochrome used in your application/experiment.
Verify correct settings with an application Control	Update the experiment 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.

Daily QC

Daily QC consists of:

Action	Description
1. Check Fluid & Optics	• Run Flow-Check Pro to verify fluidics and optics. • Verify HPCVs per instrument specifications.
2. Set Voltages & Gains	• Run Flow Set Pro[a] Fluorospheres. • Adjust voltages to place populations in target positions.
3. Color Compensation & Verification	• Open the compensation worklist. • Import the Flow-Set Pro hardware settings.[a] • Run the unstained, single color, and verification sample. • Generate / save compensation matrix in Kaluza Analysis. • Ensure the Verification sample achieves expected values.
4. Run Samples	• Open the experiment protocol. • Import saved hardware settings and compensation. • Run experiment samples.

a. Flow-Set Pro requires FS Discriminator value equal to approximately **20**. Biological samples require a higher FS Discriminator value.

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 Quality Control Samples

Before running quality control samples, perform the following:

1 Check that the Daily Startup procedure was performed. See CHAPTER 10, *Daily Startup* for instructions.

2 Warm up the system; warm up requirements depend on how the instrument has been powered off:

- **If the instrument had been completely shut down, including the Supply Cart power switch turned off,** allow about 40 minutes to warm up the system before performing QC or running samples
- **If the instrument has been shut down, but the Supply Cart pneumatics switch was left on,** allow about 10 minutes to warm up the system.

3 Verify that the instrument status is ***Ready***, and then acknowledge and resolve current error messages, as described in Table 15.2.

4 Follow the directions in the Flow-Check Pro fluorospheres Instructions for Use (IFU) for details on storage and handling.

5 Follow the directions in the Flow-Set Pro fluorospheres IFU for details on storage and handling and how to establish Flow-Set Pro fluorospheres target channels for your application/ experiment.

6 Optimize the settings for your instrument using the protocols provided for Flow-Check Pro and Flow-Set Pro. Select the files corresponding to your instrument configuration from the following location: **C:\Users\Public\Public Documents\Beckman Coulter\Flow Cytometry**

7 Follow the IFU for any additional reagents used in your application/experiment.

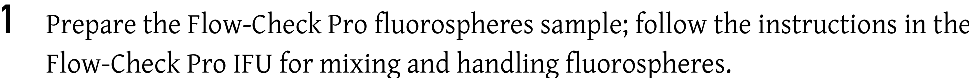
8 Ensure the file **Output** directory is in the location of your preference. See CHAPTER 12, *Selecting an Output Directory* for details.

Running Daily Quality Control Procedures

Checking Fluidics and Optics

To check fluidics and optics:

1 Prepare the Flow-Check Pro fluorospheres sample; follow the instructions in the Flow-Check Pro IFU for mixing and handling fluorospheres.

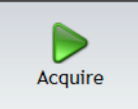


2 Open the Flow-Check Pro Protocol in Kaluza for Gallios to adjust settings for your instrument.

NOTE Default Flow-Check Pro Protocols are accessed from the following location:
C:\Users\Public\Documents\Beckman Coulter\Flow Cytometry

3 Ensure the file **Output** directory is in the location of your preference. See CHAPTER 12, *Selecting an Output Directory* for details.

4 Place the Flow-Check Pro fluorospheres sample tube into the carousel position identified in the Worklist.

5 From the **Instrument Control** panel, select 

to initiate the acquisition.

6 Once data acquisition starts, view all histograms to ensure peaks fall within regions.

7 Adjust voltages to place peaks within regions (Setup Mode will automatically invoke when you begin to adjust voltages).

8 From the **Instrument Control** panel, select [Setup Mode] to exit Setup Mode once populations are within the regions (refer to the Flow-Check Pro IFU as needed).

9 Verify the HPCVs are within the instrument specifications before running any application/ experiment samples.

a. If the HPCVs are not within the upper limits identified in the protocol's region name, you may need to perform one or more of the following troubleshooting steps:
   - Ensure the Flow Rate is LOW and repeat the run.
   - Prime the system (see CHAPTER 12, *Priming the Sample Pathway*) and repeat the run.
   - Run a Clean Cycle by selecting > **Cytometer** > **Clean**.
   - Run a Clean Worklist (see CHAPTER 13, *Routine Cleaning Procedure*).

b. Repeat running the Flow-Check Pro fluorospheres to verify HPCVs.

Standardizing—Setting Voltages and Gains

To set voltages and gains:

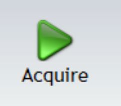
1 Prepare the Flow-Set Pro fluorospheres sample. Follow the Flow-Set Pro IFU for mixing and handling the fluorospheres.

2 Open the Flow-Set Pro protocol into Kaluza for Gallios to adjust settings for your instrument and specific application/experiment.

NOTE Default Flow-Check Pro Protocols are accessed from the following location:
C:\Users\Public\Documents\Beckman Coulter\Flow Cytometry

3 Ensure the file **Output** directory is in the location of your preference. See CHAPTER 12, *Selecting an Output Directory* for details.

4 Place the Flow-Set Pro fluorospheres sample tube into the carousel position specified in the Worklist.

5 From the **Instrument Control** panel, select 

to initiate the acquisition.

6 Once data acquisition starts, view all histograms to ensure peaks fall within target regions established for your application/experiment. Refer to the Flow-Set Pro IFU for instructions on determining application/experiment target channels for Flow-Set Pro.

7 Adjust voltages to place peaks within regions (Setup Mode will automatically invoke when you begin to adjust voltages).

8 Once populations are within the regions (refer to the Flow-Set Pro IFU as needed), select Setup Mode from the **Instrument Control** panel to exit Setup Mode.

Color Compensation

To set color compensation values:

1 Prepare the following samples, following the applicable Instructions for Use (IFU) for all reagents in use:

- **Unstained cells sample**
- **Color compensation samples**: VersaComp Antibody Capture Beads or cells stained with single color antibodies for each fluorochrome used in your application/experiment antibody cocktail.
- **Verification sample**: Cells stained with your application/experiment antibody cocktail, i.e., 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.

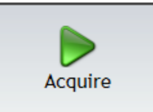
2 Open (or create) the Compensation Worklist in Kaluza for Gallios. See CHAPTER 9, *Compensation Worklists* for details on creating a Compensation Worklist.

3 Ensure the voltage and gain hardware settings from the Flow-Set Pro are imported into the Worklist. See CHAPTER 7, *Importing Hardware Settings* for details.

NOTE Flow Set Pro requires a different FS discriminator (typically the FS discriminator is **20**) than what is used in protocols running biological cells. Verify the appropriate FS discriminator is set for the Compensation Worklist samples. See CHAPTER 7, *Discriminator* for details on setting the discriminator.

4 Ensure the file **Output** directory is in the location of your preference. See CHAPTER 12, *Selecting an Output Directory* for details.

5 Place the compensation sample tubes into the carousel positions identified in the Worklist.

6 From the **Instrument Control** panel, select  to initiate the acquisition.

7 Once data acquisition completes for all samples, select the button (**Load acquired data in Kaluza**) to generate a compensation matrix in the Kaluza Analysis. Refer to the *Kaluza Analysis Software Instructions for Use* (P/N A75667) for instructions on generating the compensation matrix.

Verification

Verification of the application/experiment settings is achieved by reviewing the Verification sample data results with the compensation applied. The Verification tube is the last sample tube acquired in the Compensation Worklist (see *Color Compensation*) and may be included, but is not required, when generating the compensation matrix in Kaluza Analysis (*Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature* in the *Kaluza Analysis Software Instructions for Use* (P/N A75667).

IMPORTANT When verifying the application/experiment settings:

- Be sure the compensation matrix is imported into the Verification data set prior to reviewing the verification data results.
- Verify results achieved are within the assayed values.

CHAPTER 12

Sample Acquisition

Introduction

This chapter describes how to prepare samples and perform an acquisition, and provides information on the other functions integral to the acquisition process. These functions are in the following sections:

- *Sample Requirements*
- *Preparing Samples*
- *Instrument Control Panel*
- *File Output*
- *Sample Acquisition*

Sample Requirements

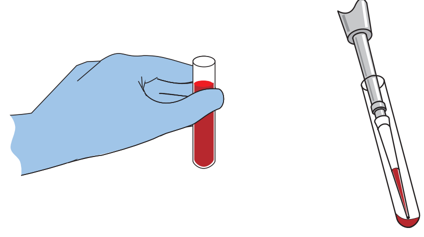
At least 170 µL of prepared sample is needed. It must be in a 12 x 75-mm test tube. Samples analyzed on the instrument must be in a single-cell suspension. Typically, cells are prepared before they are analyzed. The method used to prepare a specimen depends on the sample type and the assay desired. For 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.

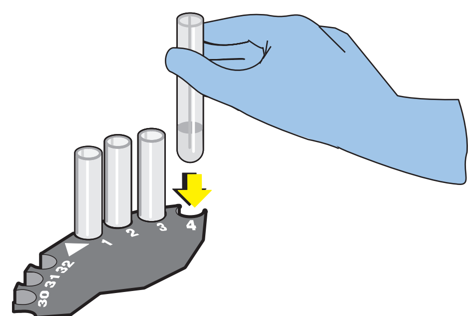
Preparing Samples

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.



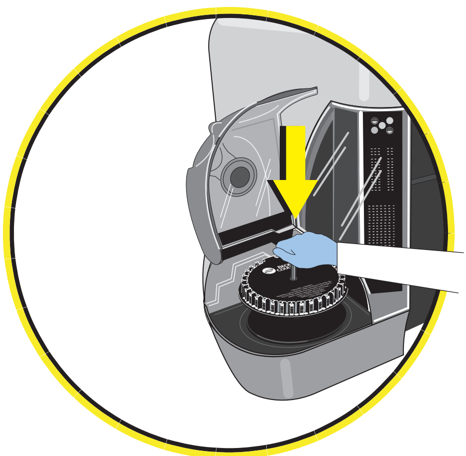
2 Place the sample tubes in a carousel.



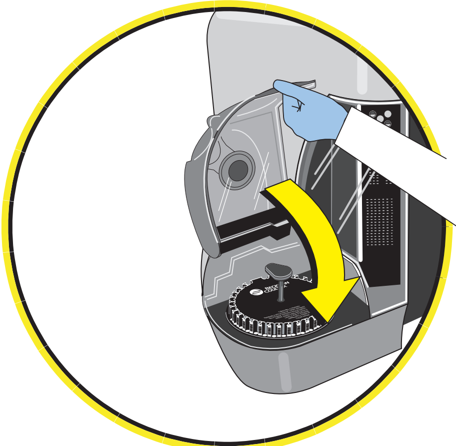
⚠ WARNING

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. The samples are now ready to be acquired. For complete instructions on acquiring samples, refer to *Sample Acquisition*.

Instrument Control Panel

The Instrument Control panel contains the controls for acquisition processes. The panel changes based on the current state of the instrument (Figure 12.1 and Figure 12.2 are two examples).

Figure 12.1 Instrument Control Panel—Acquiring Menu

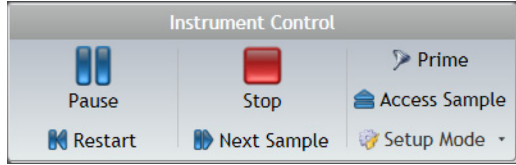
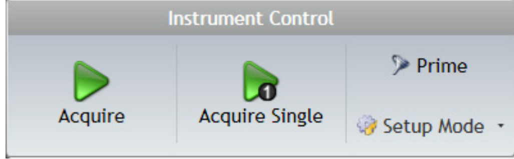


Figure 12.2 Instrument Control Panel—Ready Menu



To familiarize yourself with the controls used during sample acquisition, refer to Table 12.1.

Table 12.1 Instrument Control Panel Options

Icon	Description
Power On	Connects to and powers ON the instrument.
Initialize	Prepares the instrument to begin acquiring samples.
	This area of the Instrument Control panel animates when the instrument is initializing or in cleansing mode.
Acquire	Acquires samples, beginning with the selected sample and continuing to the end of the Worklist. Once the acquisition process is completed, the *.fcs files are saved into the output directory defined in the Worklist pane.
Acquire Single	Only acquires the sample currently selected in the Worklist.

Table 12.1 Instrument Control Panel Options

Icon	Description
Prime	Declogs or removes bubbles or blockages from the sample line using sheath solution. Priming should be done during an acquisition if you notice a slow-down of the flow rate. **IMPORTANT** Performing a Prime during an acquisition discards any collected data and restarts the acquisition once priming is completed.
Setup Mode	Setup Mode allows you to adjust the hardware settings; i.e., voltages, gains, discriminator values while the instrument continuously cycles acquired data. This enables uninterrupted changes to hardware settings based on the appearance of events on plots. Data acquired during Setup Mode is not saved, and all data acquired before entering into Setup Mode is discarded. Setup Mode is most commonly used during Quality Control procedures (CHAPTER 11, *Quality Control*), as you will want hardware settings optimized prior to acquiring samples. The drop-down feature allows you to choose or enter the number of events that display on the plot while in Setup Mode.
Pause	Temporarily stops the acquisition on the sample currently being run.
Continue	Continues the data acquisition process. This option is available after the instrument is paused.
Stop	Stops the current acquisition and writes the acquired data to a *.fcs file.
Restart	Begins reacquiring the current sample. Any data previously collected is discarded, and the process is restarted. **NOTE** Pressing the (F5) key also restarts an acquisition.
Next Sample	Stops the current acquisition and begins acquiring the next sample. Data from the current sample is written to a *.fcs file before proceeding to the next sample.
Access Sample	Pauses the acquisition and rotates the sample to the tube access door.
Power Off	Powers the instrument OFF.

File Output

FCS files are automatically saved once the acquisition is completed for each sample. Because the process is automatic, you must define the output directory and set up file naming conventions prior to beginning the acquisition, unless you are using defaults or have already made your specifications.

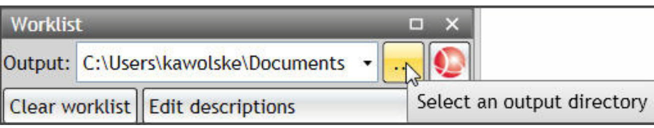
NOTE For systems with multiple users, file security is achieved by setting up individual User Accounts on your Windows 7 operating system. For additional information, see CHAPTER 6, *User Preferences and Security*.

Selecting an Output Directory

The default output directory for acquisition files is your user Documents folder. To choose a different output directory:

1. From the Worklist Pane, select the ... icon (Figure 12.3) located in the **Output** field.

Figure 12.3 FCS Output Directory Field

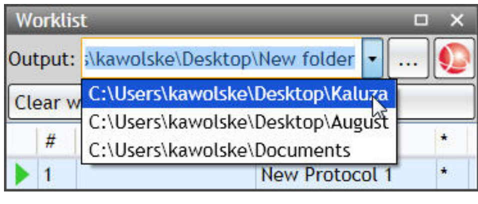


2. In the **Browse For Folder** window, navigate to the desired folder and select it.

3. Select **OK** to complete the process. The selected output directory becomes the default location until a new location is selected.

   Previous directories are available for quick selection by clicking the down arrow in the **Output** field (Figure 12.4) and selecting the desired directory.

Figure 12.4 Output Directory Drop-Down



FCS File Naming

FCS files are named based on the defaults set up in the Kaluza Options dialog (see CHAPTER 6, *Kaluza Options Menu*), which is available through the Application Menu.

To set the default file name:

1. Select [icon] > Kaluza Options.

2. Choose the **Defaults** tab.

3. In the File Name section, select the identifier(s) from the drop-down lists that you would like to use as your file naming convention. You can specify up to six identifiers to use as the file name.

4. Select a delimiter type to use to separate the selected identifiers.

5. Select **OK** to complete the process.

   NOTE Defaults affect only newly created files.

   NOTE Samples are named using the conventions defined at the start of the acquisition for each sample. If an acquisition is in process while the naming convention is being changed, the in-process sample uses the former naming convention, unless the acquisition for that sample is restarted; if multiple samples are being processed as start of the acquisition, all remaining samples will use the newly defined naming convention.

Sample Acquisition

For best results, all of the actions in *Before Running Samples* should take place prior to sample acquisition. Once these activities are completed, you can begin acquiring samples; see *Performing an Acquisition* for instructions.

Before Running Samples

Before running samples, the following steps should be taken to achieve the best results:

1. Perform daily start-up activities. CHAPTER 10, *Daily Routine*, provides instructions for how to turn on and prepare the instrument and the computer.

2 Perform quality control procedures. Following the instructions in CHAPTER 11, *Quality Control*, ensures that your system is working accurately and precisely.

3 Prepare the samples and place in the instrument carousel (see *Preparing Samples*).

4 Set up a Worklist (CHAPTER 8, *Worklists*) and choose and/or create Protocols (CHAPTER 7, *Protocols*) to go with each sample. During an acquisition, the information entered into the Worklist is communicated to the instrument, and the instrument uses this information to acquire samples from the appropriate carousel locations.

Performing an Acquisition

To perform an acquisition:

NOTE In addition to the Instrument Control options described in the procedure below, refer to *Available Options While Acquiring* for other options available during an acquisition.

1 If the Worklist or Protocol is not already open:

a. Select > **Open**.

b. Navigate to the location of the Worklist or Protocol using the fields and/or icons and select the file.

c. Select **Open** to open the file into Kaluza for Gallios. *If the Worklist does not appear on the user interface, the Worklist pane has likely been docked. Refer to* CHAPTER 6, *Displaying a Component Pane to view the Worklist.*

2 If the lasers required for your acquisition have been powered off:

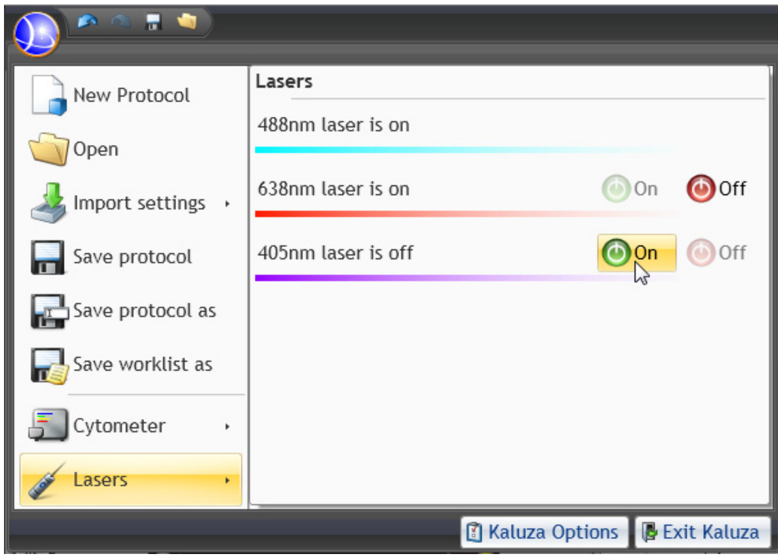
a. Select and move your mouse over **Lasers**.

b. In **Lasers**, select the **On** button corresponding to the laser(s) required for the acquisition. When the **On** or **Off** button is transparent, that is the current state of the laser, and is therefore not selectable. For example, in Figure 12.5, the red, 638nm laser is already powered on, and the violet, 405 nm laser is currently powered off, but is ready to be selected, as indicated by the highlighted **On** button when mousing over the button. Follow the warm-up requirements specified in Step 4 of CHAPTER 10, *Power the Computer and Cytometer ON*.

NOTE If the Protocol associated with the sample requires a laser that is currently powered off, an error message(s) appears above the plot sheet, prompting you turn the laser on.

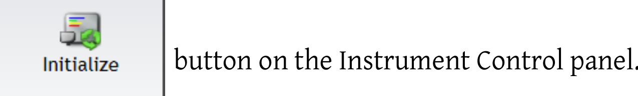
IMPORTANT If your instrument is equipped with a **561 Laser**, refer to the *561 mn Laser Option Addendum* (p/n B00207) for instructions on using this optional accessory with your Gallios Flow Cytometer.

Figure 12.5 Application Menu—Laser Power



3 Verify that the instrument carousel matches the order and location of samples in the Worklist.

4 If the instrument is currently in ***Standby*** mode, initialize the instrument by selecting the



button on the Instrument Control panel.

5 If one or more of the three factors listed in the caution below applies to the current state of your instrument, the sample pathway will need to be flushed by performing a prime. To prime the instrument, select Prime on the Instrument Control panel.

⚠ CAUTION

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.**

---

6 Verify your Protocol(s) to ensure that all the configurations that cannot be changed during the acquisition process are correct. These configurations include the following:

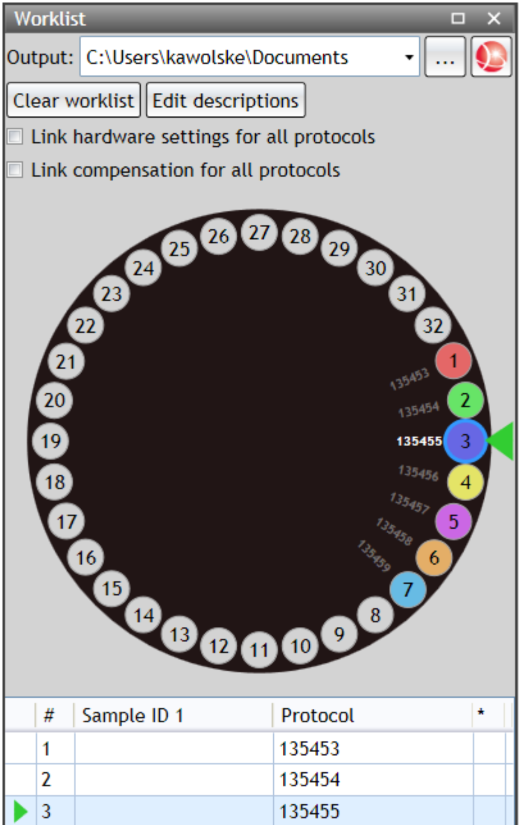
- The detectors used and measurements selected for each parameter. See CHAPTER 7, *Hardware Configuration*.
- The *.fcs file output directory. See *Selecting an Output Directory*.
- The *.fcs file output name format. See *FCS File Naming*.

NOTE The stop conditions specified on the Acquisition Controls tab **can** be changed during the acquisition if the acquisition is paused. See CHAPTER 7, *Stop Conditions* for details.

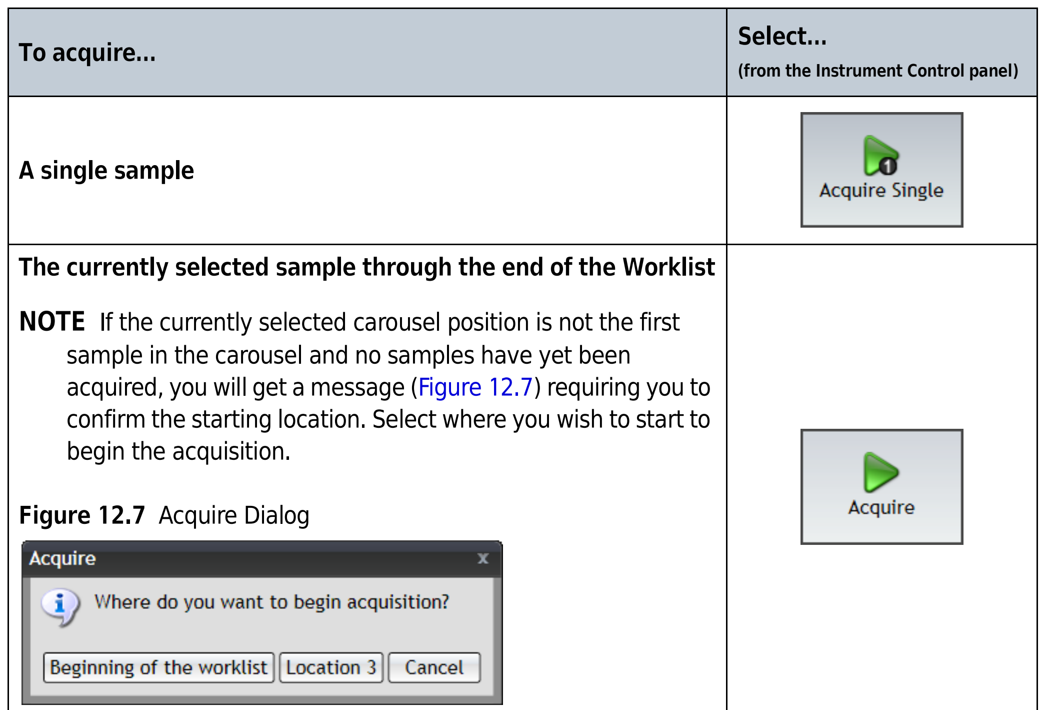
---

7 Select the sample in the Worklist that is the starting point for the acquisition. The sample can be selected by either clicking on the sample number in the carousel or the sample row in the Parameter Descriptions grid. In Figure 12.6, sample **3** is selected, as indicated by the green arrow on both the carousel graphic and the Parameters Descriptions grid.

Figure 12.6 Sample 3 Selected



8 The instrument and software are now ready to begin acquiring samples:



To acquire...	Select... (from the Instrument Control panel)
A single sample	Acquire Single
The currently selected sample through the end of the Worklist **NOTE** If the currently selected carousel position is not the first sample in the carousel and no samples have yet been acquired, you will get a message (Figure 12.7) requiring you to confirm the starting location. Select where you wish to start to begin the acquisition. **Figure 12.7** Acquire Dialog Acquire Where do you want to begin acquisition? Beginning of the worklist · Location 3 · Cancel	Acquire

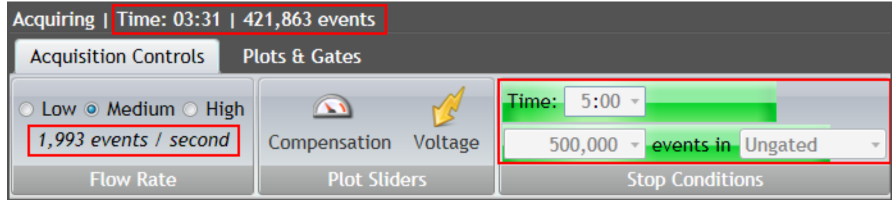
Once the acquisition begins, the Title/Status bar and the Acquisition Controls tab display details of the acquisition process for the current sample. These details include the following, which are shown in Figure 12.8:

- **Title/Status Bar**: The cytometer state (e.g., ***Loading carousel***, ***Aspirating sample***, ***Acquiring***, ***Finishing acquisition***, ***Unloading carousel***), the amount of time the acquisition has been running, and the number of events captured so far.
- **Acquisition Controls** tab:
  - **Flow Rate**: The number of events recorded per second.

  IMPORTANT If you notice a slow-down of the flow rate during an acquisition, prime the instrument to flush the sample pathway. Be aware that performing a Prime during an acquisition discards any collected data and restarts the acquisition once priming is completed.

  - **Stop Conditions**: Two progress bars show the progression of time and number of events captured, based on the limits specified prior to the acquisition. When one of the green progress bars reaches the end, the acquisition is completed.

Figure 12.8 Acquisition Details



IMPORTANT While an acquisition is in progress, keep in mind the following:

- The state of the cytometer cannot be placed into ***Standby*** mode or powered off while the cytometer is acquiring samples.
- If the acquisition files are actively being written to a network drive, and the network loses connectivity, the acquisition is stopped. Similarly, if the acquisition files are actively being written to a USB flash drive, and the flash drive is removed, the acquisition is stopped.

---

9 Check your plots to ensure data follows expected patterns, and make changes, if required.

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.

Data review should include the following:

- Review the light scatter patterns.
  - Verify any population of interest is above the discriminator to ensure no cell loss. Refer to the following sections to make changes:
    - CHAPTER 7, *Discriminator*
    - CHAPTER 7, *Particle Size*

- CHAPTER 7, *Neutral Density Filter*

— Verify any population of interest is separated as much as possible from other populations that are present (within biological constraints). Refer to the following section to make changes:

- CHAPTER 7, *Compensation*

- Review the antibody staining patterns.

— Verify overall expected staining patterns are observed; in other words, verify the fluorescence staining correlates to the antibody used in sample preparation.

— Verify abnormally dim or negative sample staining patterns are consistent within the context of the research experiment. Refer to the following sections to make changes:

- CHAPTER 7, *Voltage*
- CHAPTER 7, *Gains*

IMPORTANT Making changes to the particle size, neutral density filter usage, and/or voltages, gains, and discriminator values puts the acquisition into Setup Mode. Be aware that Setup Mode discards any previously collected data when invoked. Data collection restarts once Setup Mode is turned off. If possible, these settings should be defined as part of the daily Quality Control procedures; see CHAPTER 11, *Quality Control* for details.

- CHAPTER 7, *Compensation*

- Review gate boundaries.

— Verify gates encompass any populations of interest while at the same time exclude undesired events. To make changes, refer to the following section:

- CHAPTER 7, *Editing Gates*

IMPORTANT If you are acquiring multiple samples, you cannot make changes to Protocols belonging to other samples. However, if a Protocol is used in multiple locations, changes made during the acquisition are reflected on all other samples linked to that Protocol.

---

10 If you need to make changes during the acquisition, refer to the following sections, depending on your need(s):

- *Pausing an Acquisition*
- *Restarting an Acquisition*
- *Stopping an Acquisition*
- *Moving to the Next Sample*
- *Priming the Sample Pathway*
- *Accessing the Sample*
- *Setup Mode*

IMPORTANT Kaluza Analysis must be installed for this feature to work.

11 The *.fcs file for each sample is saved to the location you selected (see *Selecting an Output Directory*) as soon as the instrument is done acquiring. Once a sample is acquired, the button (**Load acquired data in Kaluza**) animates, indicating that the *.fcs file is ready for analysis.

To load *.fcs files into Kaluza Analysis Software, select the button. This immediately opens any unloaded *.fcs files from the current Worklist into Kaluza Analysis Software.

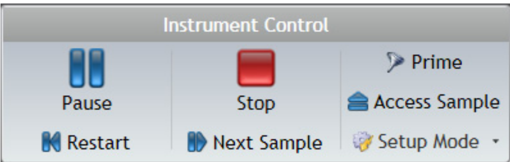
NOTE In addition to the Standard FCS 3.1 information, the runtime Protocol, including all of the plots and gates, is also saved with Kaluza for Gallios *.fcs files, and appears intact in Kaluza Analysis.

Available Options While Acquiring

While an acquisition is in progress, you might need to make changes to the software or the samples. These options are available on the Instrument Control panel (Figure 12.9). Listed below are the options available during an acquisition:

- *Pausing an Acquisition*
- *Restarting an Acquisition*
- *Stopping an Acquisition*
- *Moving to the Next Sample*
- *Priming the Sample Pathway*
- *Accessing the Sample*
- *Setup Mode*

Figure 12.9 Instrument Control Panel—Acquiring



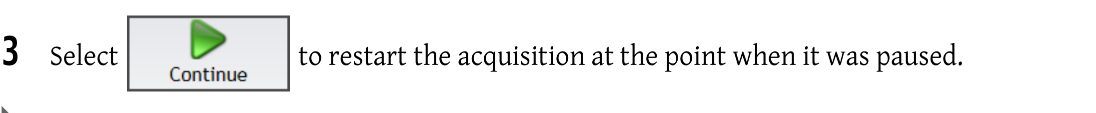
Pausing an Acquisition

Pausing an acquisition, rather than stopping and losing data, is the preferred method for making prolonged changes to the Protocol.

To pause the acquisition:

1 Select [Pause] on the Instrument Control panel.

2 Make the necessary changes.



3 Select Continue to restart the acquisition at the point when it was paused.

Restarting an Acquisition

Restarting an acquisition discards any data that has been collected for the current sample and begins the acquisition again.

To restart an acquisition:

1 Select Restart from the Instrument Control panel.

Stopping an Acquisition

Choosing **Stop** ends data acquisition for the current sample and saves all data collected to an *.fcs file. **Stop** also stops acquiring the remaining samples in the Worklist.

NOTE If you stop an acquisition while it is in Setup Mode, no *.fcs file is written.

To stop an acquisition:

1 Select Stop from the Instrument Control panel.

Moving to the Next Sample

You can stop acquiring data for the current sample and move to the next sample. This option immediately stops acquiring the current sample, saves all of the data collected for that sample, and begins processing the next sample in the Worklist.

NOTE This option is unavailable if the last sample in the Worklist is being processed.

To stop acquiring the current sample, and start acquiring the next sample in the Worklist:

1 From the Instrument Control panel, select Next Sample.

Priming the Sample Pathway

If you notice the acquisition data rate dramatically slows down, the sample pathway likely needs to be flushed. Selecting **Prime** corrects this issue by declogging or removing bubbles or blockages from the sample line with sheath solution.

To prime the sample pathway:

1. Select **Prime** from the Instrument Control panel.
   After priming is completed, the acquisition process is restarted for the current sample. Any data collected prior to priming is discarded.

Accessing the Sample

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 **Access Sample** option allows you to pause processing of a single sample and rotate the MCL to position the sample at the MCL Tube Access door so that additional reagent, for example, can be added to the sample.

To pause the acquisition and allow access to the current sample:

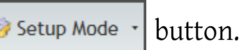
1. From the Instrument Control panel, select **Access Sample**. This pauses the acquisition and rotates the sample to the MCL tube access door.
2. Remove the sample from the carousel and make the necessary changes.
3. Place the tube back into the carousel in the same position from which it was removed.
4. Select **Continue** to start the acquisition at the point when it was paused.

Setup Mode

Setup Mode allows you to adjust the hardware settings; i.e., voltages, gains, and discriminator values while continuously cycling acquired data. This enables uninterrupted changes to hardware settings based on the appearance of events on plots. Data acquired during Setup Mode is not saved, and any data acquired from the current sample before entering into Setup Mode is discarded. Setup Mode is most commonly used during Quality Control procedures (CHAPTER 11, *Quality Control*), as you'll want hardware settings perfected prior to acquiring samples.

Changing the Number of Events to Display on Plots

To verify the number of events displayed on plots during Setup Mode:

1 From the **Instrument Control** Panel, select the drop-down arrow on the 

button.

2 Verify the number of events to display. If you wish to change the number:

- Select the **events** drop-down and choose one of the amounts listed.

  OR

- Click once within the field and enter any amount from **10** to **100,000** events.

3 Click anywhere within the software to complete the process.

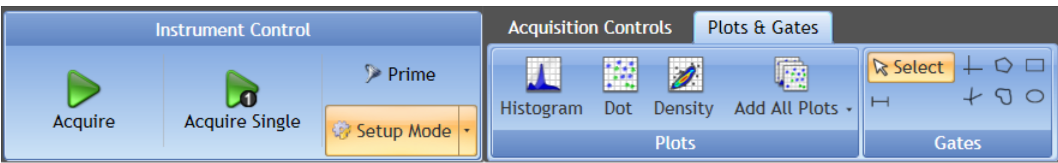
Using Setup Mode

To use Setup Mode:

1 Using the procedure in *Changing the Number of Events to Display on Plots*, verify the amount of events to display on plots during Setup Mode is correct.

2 From the Instrument Control panel, select Setup Mode. The ribbon turns blue, indicating the instrument is in Setup Mode (see Figure 12.10).

Figure 12.10 Setup Mode Enabled



3 Make the necessary changes to the Hardware Settings. For additional information, see:

- CHAPTER 7, *Discriminator*

- CHAPTER 7, *Voltage*
- CHAPTER 7, *Gains*
- CHAPTER 7, *Plot Sliders*
- CHAPTER 7, *Particle Size*
- CHAPTER 7, *Neutral Density Filter*

---

4 Select Setup Mode to leave Setup Mode and start acquiring data.

OR

Select Stop, and then select > **Save Protocol** to save the hardware settings defined during Setup Mode.

CHAPTER 13

Cleaning Procedures

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 into Standby/Idle Mode*
- *Remove the Reagent Containers*
- *Replace the Reagent Containers*
- *Power the Cytometer Only On/Off*

Cleaning Schedule

Table 13.1 contains the cleaning schedule.

Table 13.1 Cleaning Schedule

Frequency	Item
Daily Shutdown	• Sampling System — Sample Head — Sample Probe
Every 60 Days	• Internal Sheath Fluid Container • Cleaning Agent Container
As Needed	• Air Filters • Vacuum Trap

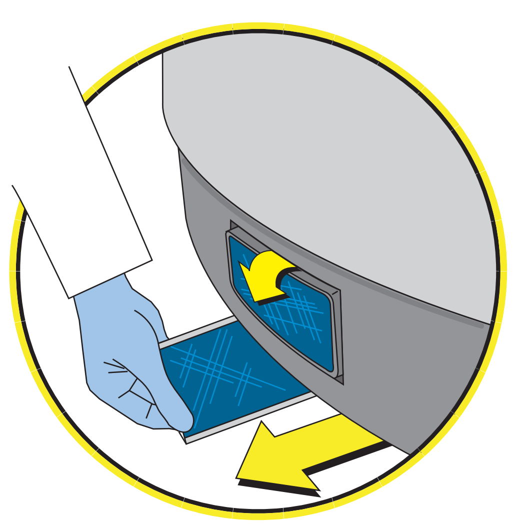
Clean the Air Filters

Clean the air filters on an as-needed basis. It is easiest to clean the air filters after performing the shutdown procedure.

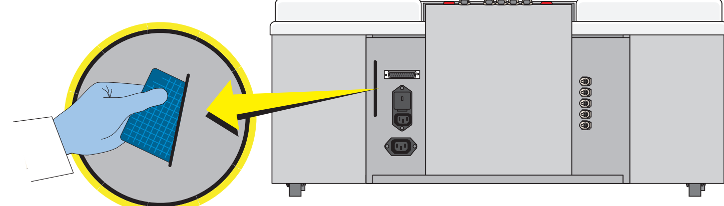
Location of Air Filters

The instrument has four air filters located on the:

- Cytometer Left Side (2)



- Supply Cart Rear Panel (2)

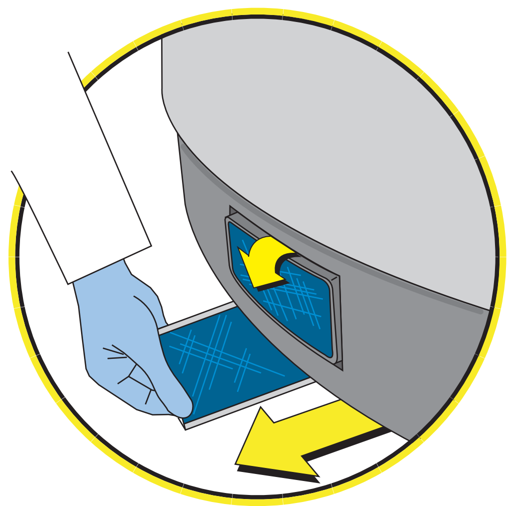


Prepare to Clean the Air Filters

1 Power OFF the Cytometer.

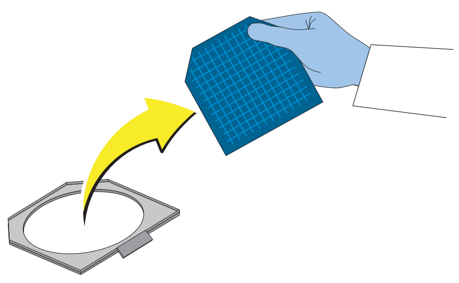
2 Turn the Supply Cart switch off and unplug the Supply Cart power cord from the wall outlet.

3 Remove the Cytometer filters from the left side of the Cytometer.

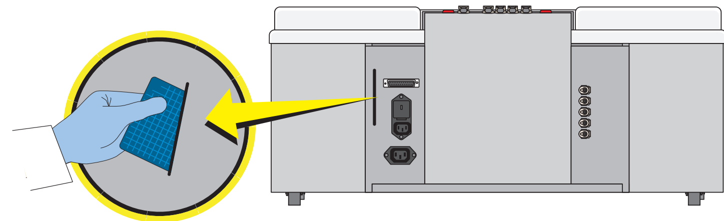


4 Remove the Cytometer filters from their frames.

NOTE Replace any torn filters.

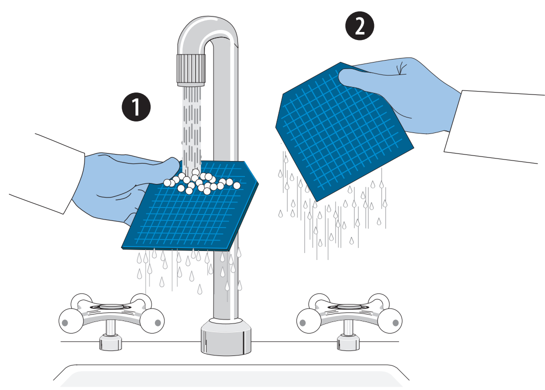


5 Remove the filter from the rear of the Supply Cart. Replace any torn filters.



Rinse and Return the Air Filters

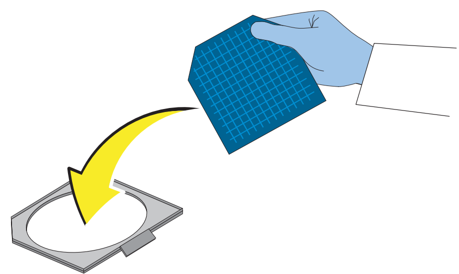
1 Rinse the filters in water (1), and then shake them out (2).



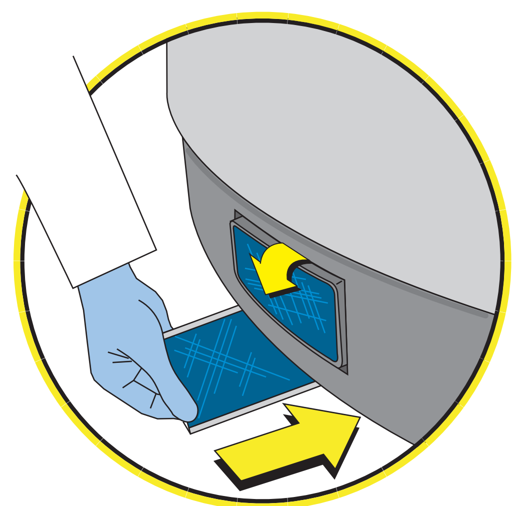
2 Set the filters aside and let them dry out for about 30 minutes.

Use paper towels to check that the filters are completely dry.

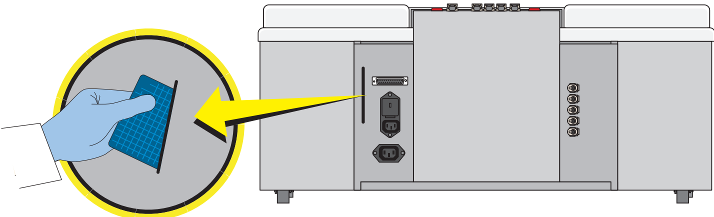
3 Return the Cytometer filters to their frames.



4 Slide the Cytometer filters back into their respective locations on the left side of the Cytometer.



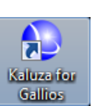
5 Return the Supply Cart filter to its location on the rear of the Supply Cart.



6 Plug the Supply Cart power cord into the wall outlet and turn the switch on.

7 Power ON the Cytometer Only

OR

Power the Cytometer and Kaluza for Gallios ON by double clicking on the  shortcut.

8 Record that the air filters were cleaned in the instrument log book.

9 Perform the Daily Startup procedure before running samples.

Put the Cytometer into Standby/Idle Mode

To clean, replace, or fill the reagent containers you need to put the cytometer into Standby mode.

To put the cytometer into Standby mode:

1 Select > **Cytometer** > **Standby**.

2 Wait about 10 seconds for the Cytometer to depressurize. Standby appears on the Application Title/Status bar, and the **Instrument Control** panel displays ***Initialize*** and ***Standby*** (Figure 13.1) when the Cytometer is depressurized.

Figure 13.1 Instrument Control Panel—Standby Mode

Remove the Reagent Containers

Remove a reagent container to perform these procedures:

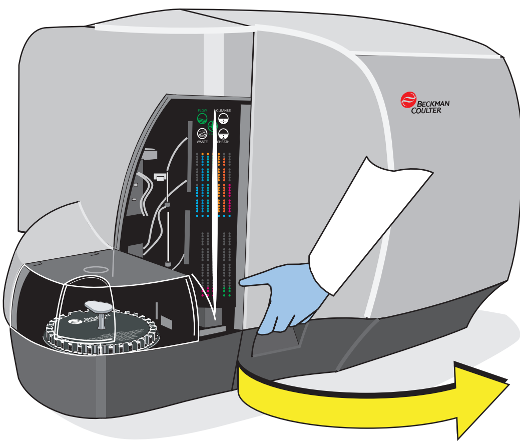
- Clean the sheath fluid container
- Clean the cleaning agent container
- Replace a reagent container. Clean any new reagent container before using it.

Procedure

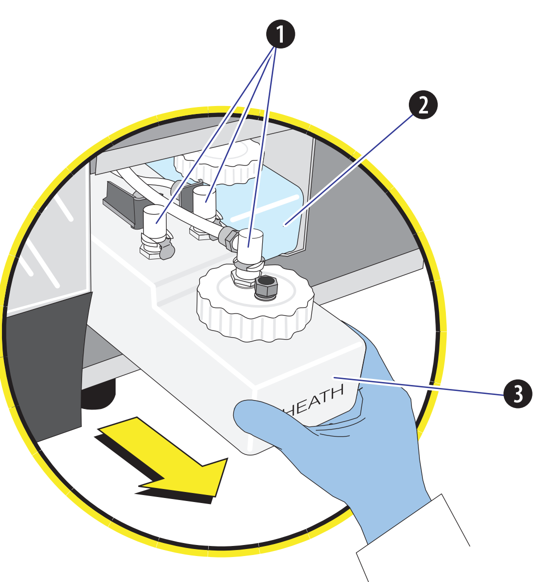
1 Check if the instrument is currently displaying the ***Standby*** mode:

- If yes (***Standby*** appears in the Instrument Control panel), go to step 2.
- If no, Put the Cytometer into Standby/Idle Mode.

2 Open the Front Cover.

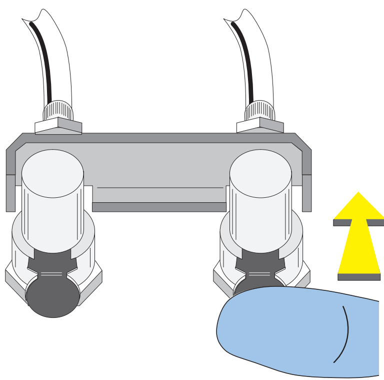


3 Pull out a reagent container just far enough to access the connectors.

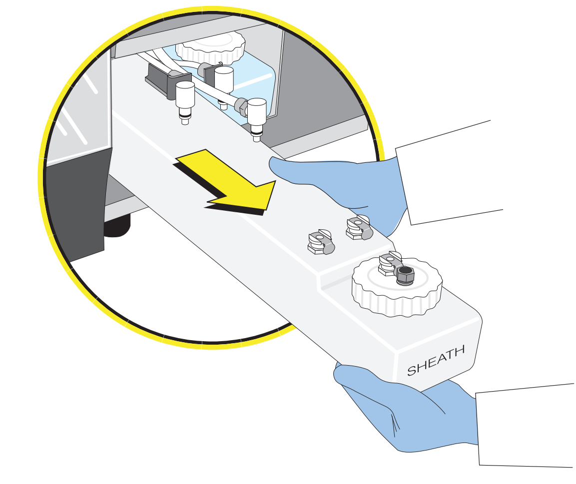


1. Reagent container connectors.
2. Cleaning agent container.
3. Sheath fluid container.

4 Disconnect the tubing on the top of each reagent container by pushing in on the metal clips on the connectors.



5 Slide the container out of the instrument.



Clean the Internal Sheath Fluid Container

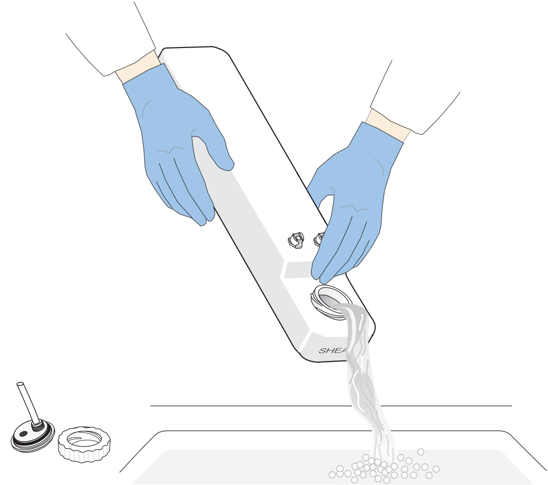
CAUTION

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 every 60 days.
- Clean a new sheath fluid container before placing it into the reagent drawer.

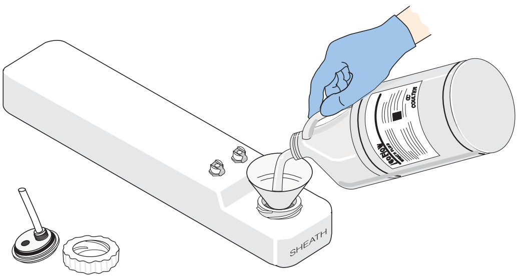
1 See Remove the Reagent Containers to remove the sheath fluid container.

2 Empty the container as completely as possible.

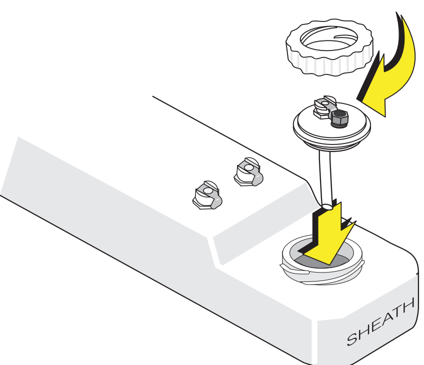


3 Position a funnel into the sheath fluid container.

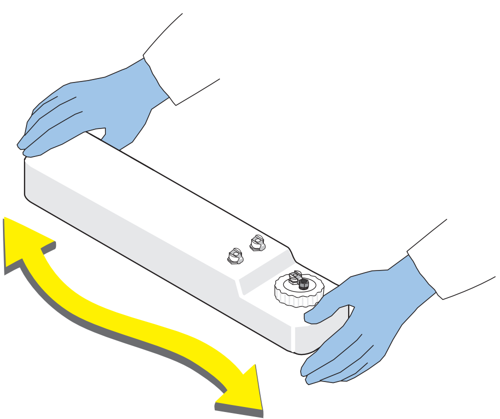
Pour about 50 to 100 mL of fresh IsoFlow sheath fluid or equivalent into the sheath fluid container.



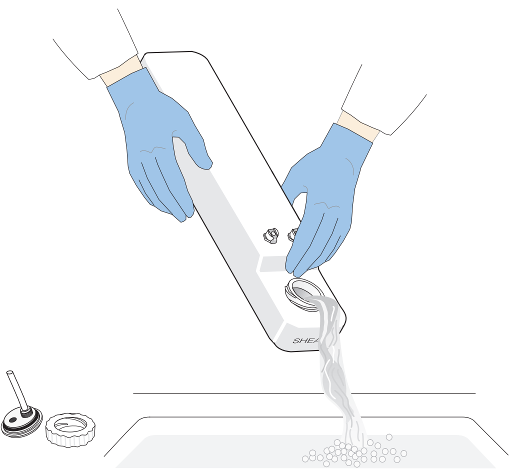
4 Screw the cap back on the sheath fluid container.



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 in the instrument log book.

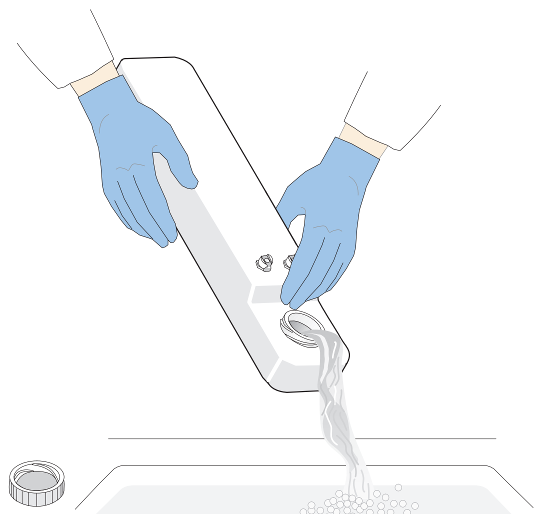
9 See *Replace the Reagent Containers* to replace the sheath fluid container.

Clean the Cleaning Agent Container

- Remove and clean the cleaning agent container every 60 days.
- Clean a new cleaning agent container before placing it into the reagent drawer.

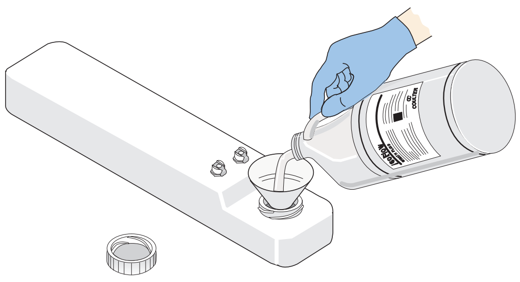
1 See Remove the Reagent Containers to remove the cleaning agent container.

2 Empty the container as completely as possible.



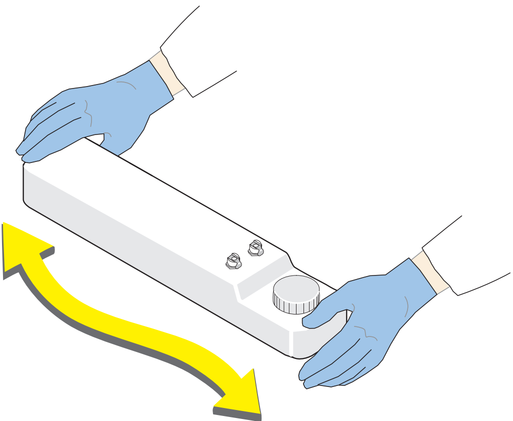
3 Position a funnel into the cleaning agent container.

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.

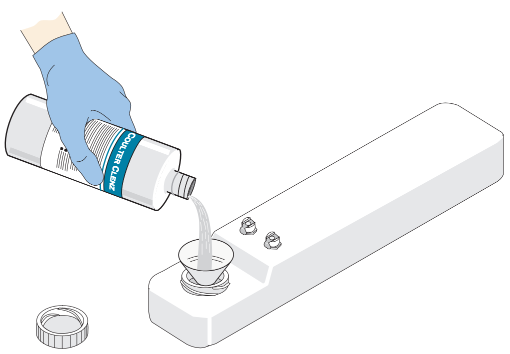
5 Swirl the sheath fluid in the sheath fluid container, rinsing all surfaces.



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.

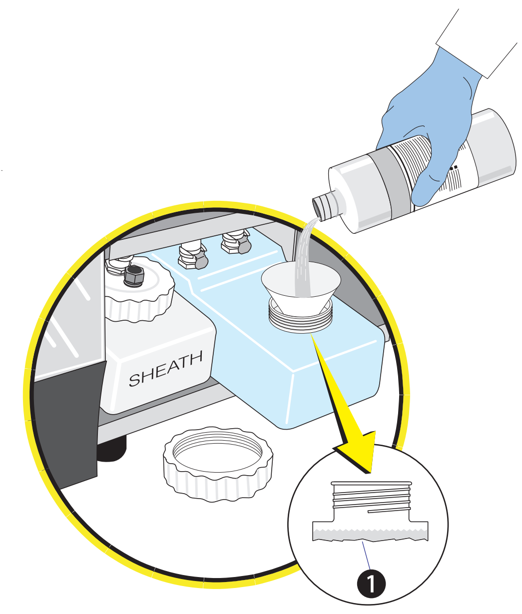


8 Empty the container as completely as possible.

⚠ 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 Carefully pour cleaning agent into the cleaning agent container (approx. 1L), filling it just to the bottom of its neck (1).



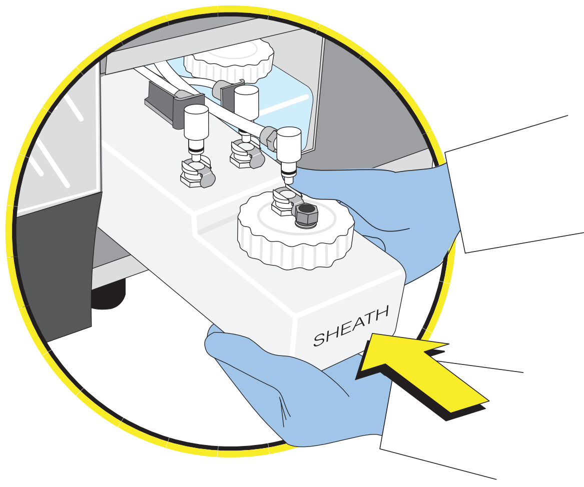
10 Record that the cleanse container was cleaned in the instrument log book.

11 See Replace the Reagent Containers to replace the cleaning agent container.

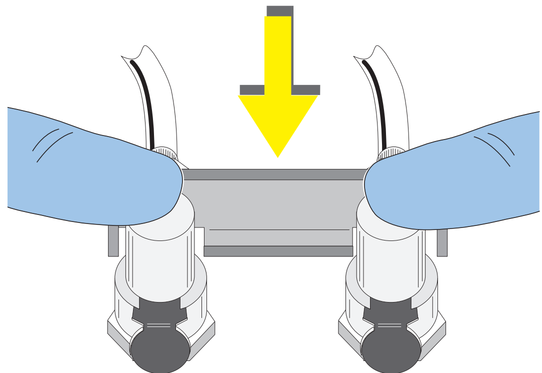
Replace the Reagent Containers

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.

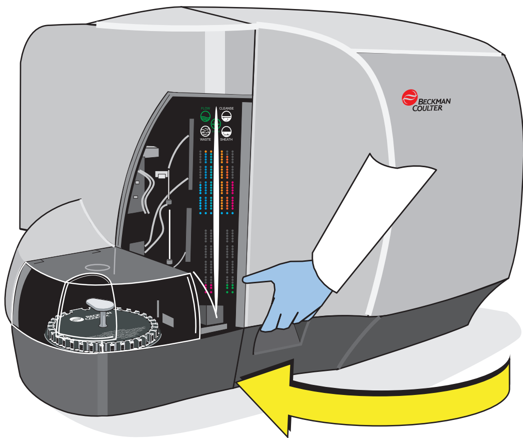


3 Fill each reagent container as instructed in these procedures:

- Fill the Internal Sheath Fluid Container, or
- Fill the Cleaning Agent Container.

4 Slide the reagent container back into place.

5 Close the Front Cover



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 the routine cleaning procedure, daily head/probe cleaning, and the daily 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.

To remove any crystal or debris buildup, perform the Clean the MCL Sample Head and the Sample Probe procedure as needed.

Routine Cleaning Procedure

Perform this procedure as often as described in the heading When to Clean the Sampling System.

⚠ WARNING

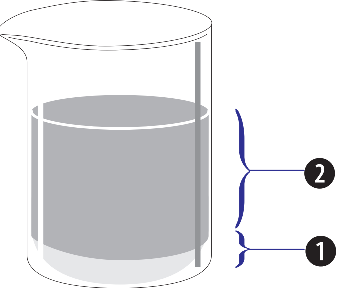
The cleaning solution is hazardous and can cause personal injury or damage clothing. Beckman Coulter urges its customers to comply with all national health and safety standards such as the use of barrier protection. This may include, but it is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

⚠ CAUTION

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 **(1)**(5% or 6% solution of sodium hypochlorite - available chlorine) and 9 parts distilled water or IsoFlow sheath fluid **(2)**.



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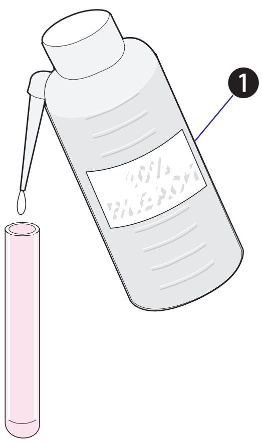
2 Put 2 mL of the bleach solution **(1)** in a test tube.



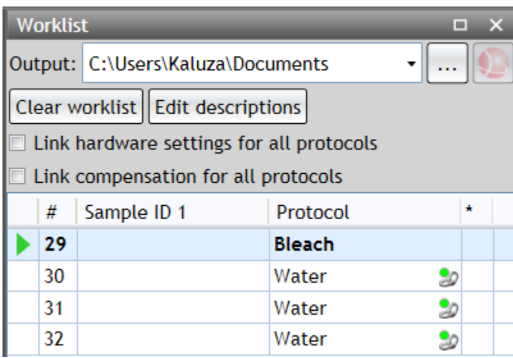
3 Load the carousel:

a. Put the test tube of bleach solution into carousel position 29.

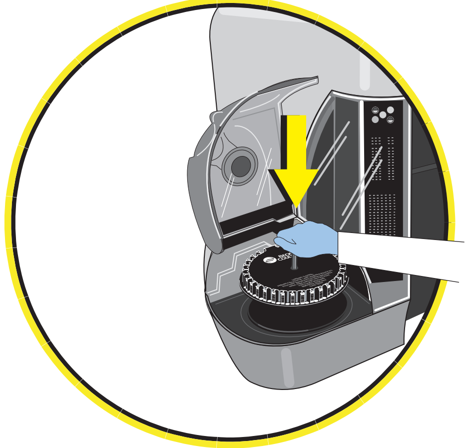
b. Put three freshly prepared tubes, each containing about 2 mL of distilled water or IsoFlow sheath fluid, into positions 30, 31, and 32 of the carousel.

4 Open the Cleaning Worklist into Kaluza for Gallios (Figure 13.2). The Cleaning Worklist (Cleaning.worklist or Cleaning) can be found at the following location:
C:\Users\Public\Public Documents\Beckman Coulter\Flow Cytometry

Figure 13.2 Cleaning Worklist

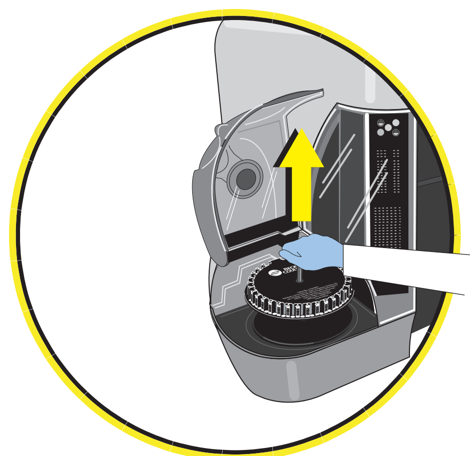


5 Put the carousel into the MCL sample loader and close the MCL cover.



6 Select Acquire.

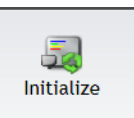
7 When the Cleaning Worklist is done, remove the carousel.



8 Close the MCL cover and select > **Cytometer** > **Clean**.

When the Clean cycle completes, the Supply Cart pneumatics will automatically turn off and ***Standby*** appears in the Application Title/Status bar when the Cytometer is depressurized.

9 Record that the routine cleaning procedure was performed in the instrument log book.

10 Before running samples, select 

on the **Instrument Control** panel 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 cells, i.e., 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.

MCL Sample Head and Sample Probe Cleaning Procedure

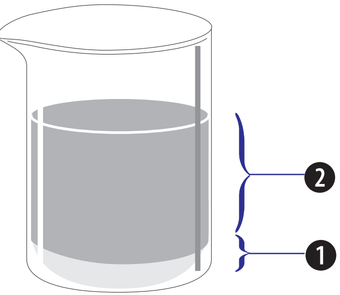
Perform this procedure as often as described in the heading When to Clean the Sampling System.

⚠ WARNING

Potential for chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details concerning exposure before using bleach.

---

1 Prepare a cleaning solution of 1 part of high-quality, fragrance-free bleach (1) (5% or 6% solution of sodium hypochlorite - available chlorine) and 9 parts distilled water or IsoFlow sheath fluid (2).



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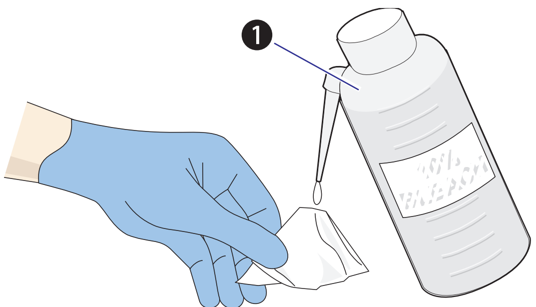
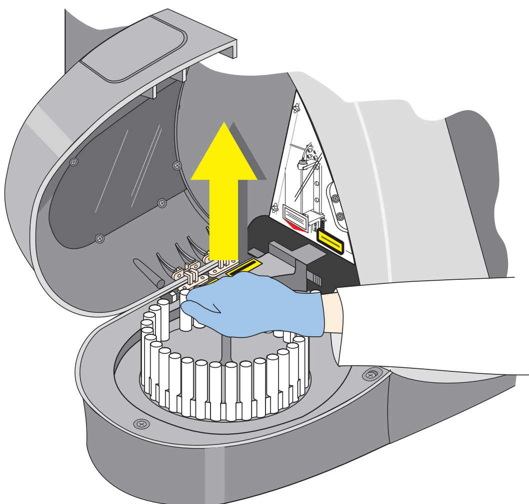
2 While wearing suitable laboratory protective gloves, apply the 10% bleach solution (1) to a gauze pad.

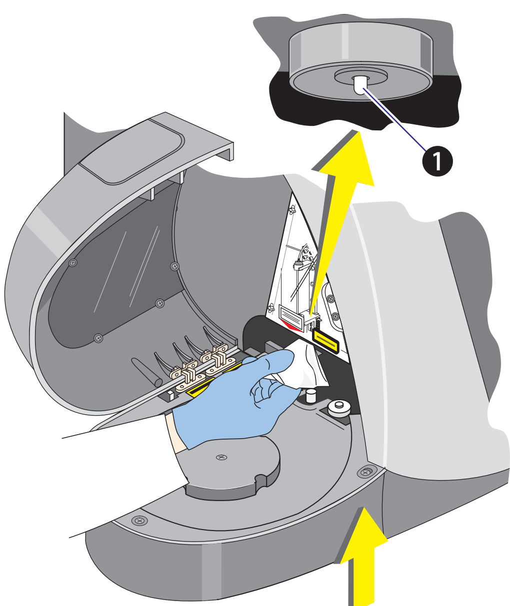


3 Open the MCL cover.

NOTE If a carousel is present, remove it.



4 Carefully push the moistened gauze pad up against the inside of the MCL sample head (1) and scrub away any debris inside and around the sample probe.



5 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.

6 Rinse the MCL sample head and probe with gauze moistened with water.

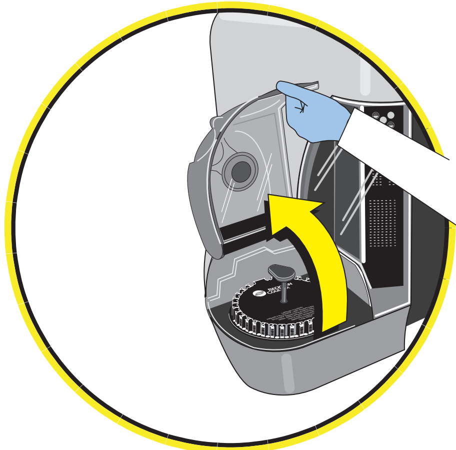
7 Record that the daily sample head cleaning procedure was performed in the instrument log book.

Clean the MCL Sample Head and the Sample Probe

To remove any crystal or debris buildup, perform this procedure as needed.

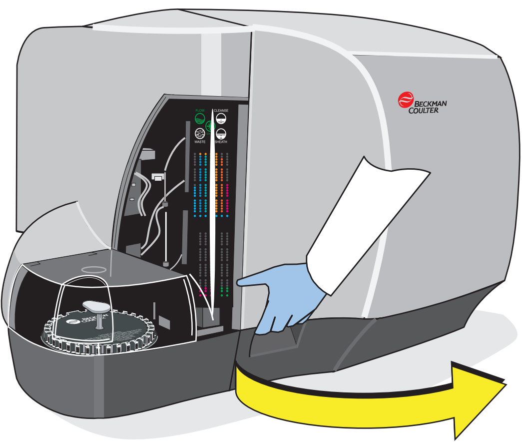
1 Power OFF the Cytometer.

2 Lift up the MCL cover.



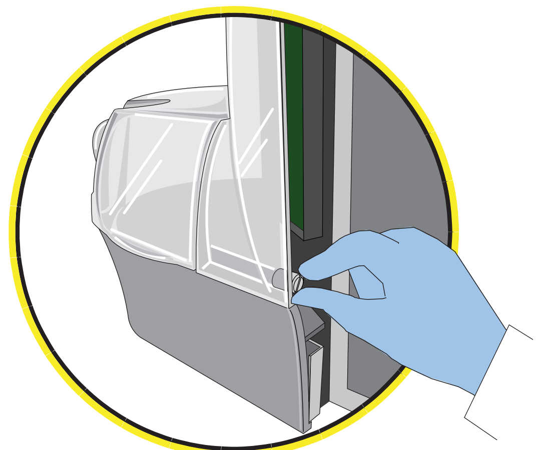
NOTE If a carousel is present, remove it.

3 Open the Front Cover.

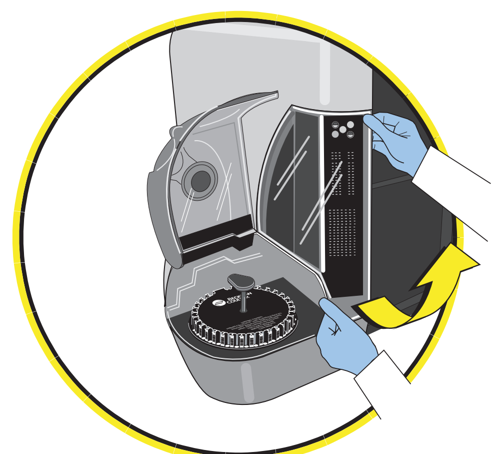


4 Remove the Front Left Side Panel.

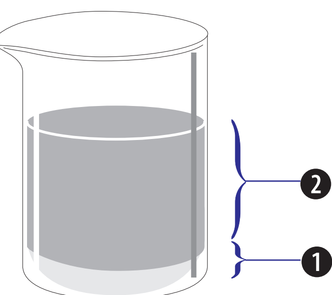
a. Unscrew the thumbscrew that attaches the left side panel to the front frame.



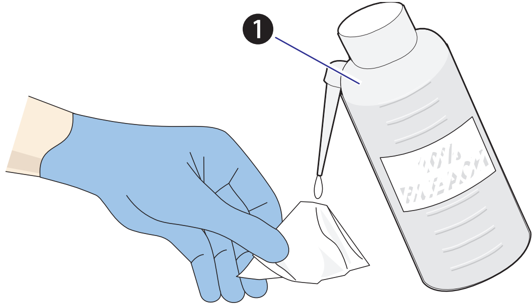
b. Remove the left side panel by pulling it towards you and down.



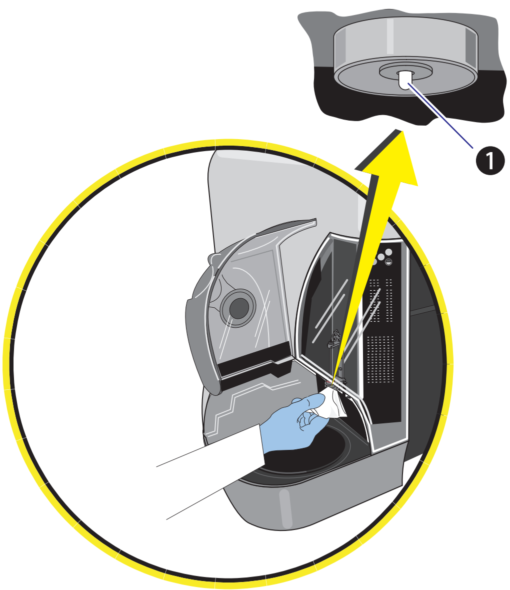
5 Prepare a cleaning solution of 1 part of high-quality, fragrance-free bleach **(1)** (5% or 6% solution of sodium hypochlorite - available chlorine) and 9 parts distilled water or IsoFlow sheath fluid **(2)**.



6 While wearing suitable laboratory protective gloves, apply the 10% bleach solution **(1)** to a gauze pad.



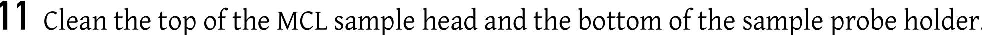
7 Carefully push the moistened gauze pad up against the inside of the MCL sample head **(1)** and scrub away any debris inside and around the sample probe.



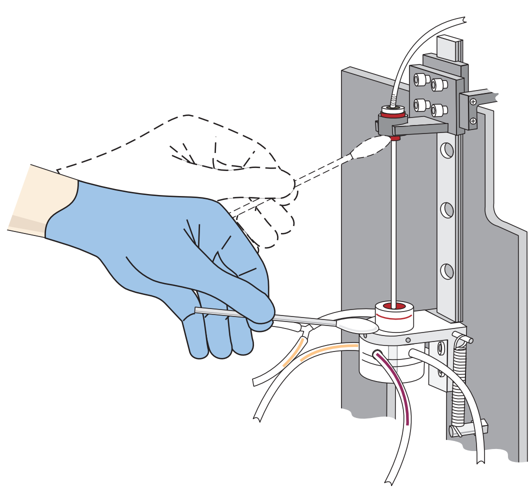
8 Continue scrubbing the sample head and probe by pushing the head up and down 10 times during a 60-second period. Replace moistened gauze as needed.

9 Rinse the MCL sample head and probe with gauze moistened with water.

10 Moisten a Q-tip with distilled water.

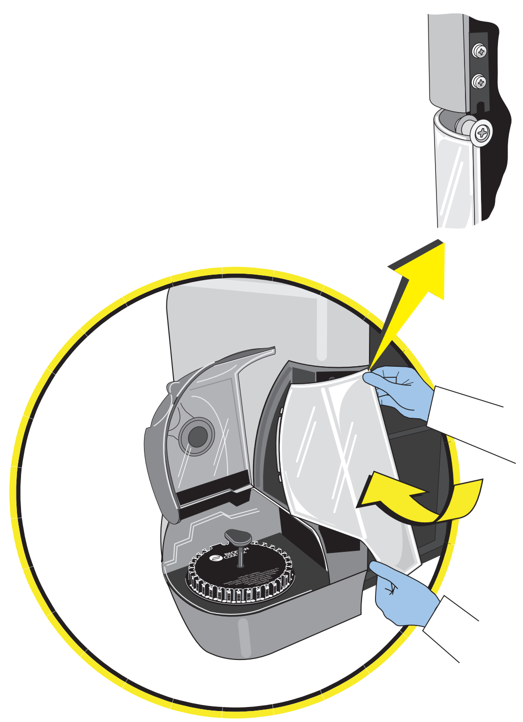


11 Clean the top of the MCL sample head and the bottom of the sample probe holder.

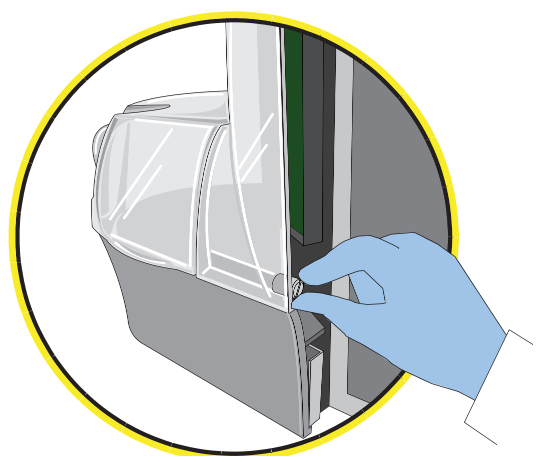


12 Replace the Front Left Side Panel.

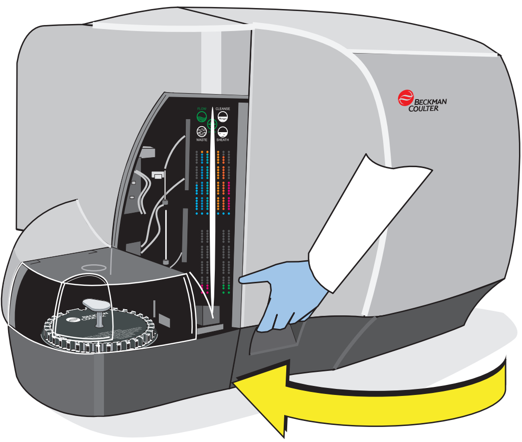
a. Slide in the left side panel, aligning the post on the top of the panel with the cut out on the frame.



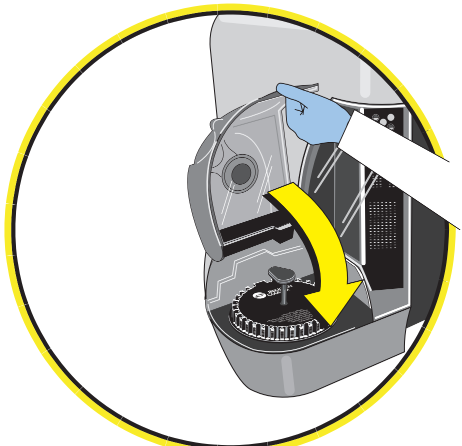
b. Screw in the thumbscrew to attach the left side panel to the front frame.



13 Close the Front Cover.



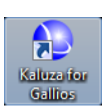
14 Close the MCL cover.



15 Record that the sample head cleaning procedure was performed in the instrument log book.

16 Power ON the Cytometer Only

OR

Power the Cytometer and Kaluza Software ON by double clicking on the  shortcut.

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.

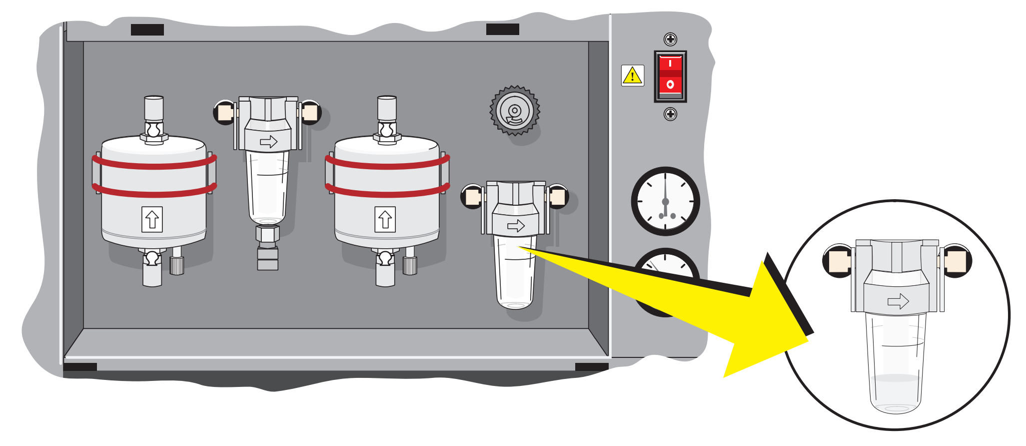
To clean the Vacuum Trap, perform these procedures:

- 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

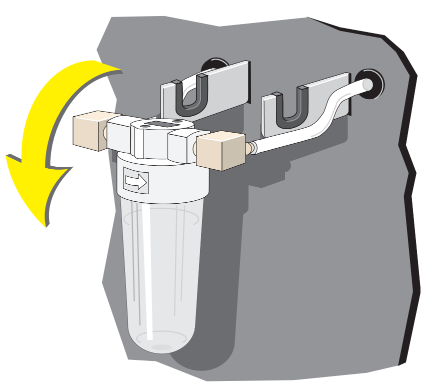
1 Power OFF the Cytometer, turn the Supply Cart switch off, and unplug both Supply Cart power cords from the wall outlet.

2 Open the Supply Cart front door and locate the vacuum trap.



Find and Pull Out the Vacuum Trap

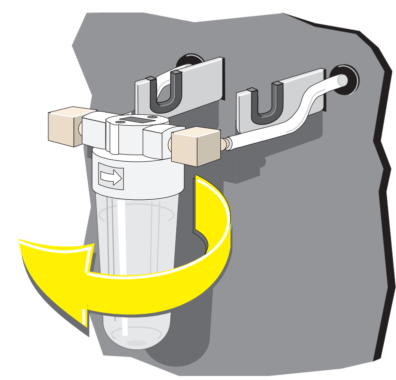
1 The vacuum trap is the trap on the left. Lift the vacuum trap assembly out of its bracket so that you can grasp the top of the assembly.



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.

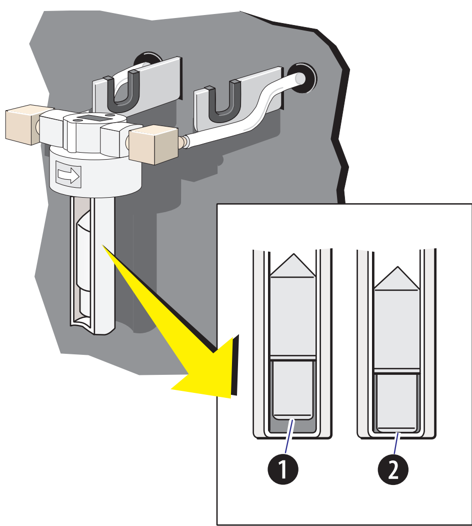


Rinse and Return the Vacuum Trap to Its Bracket

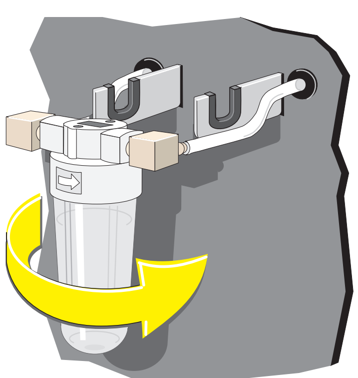
1 Rinse the vacuum trap with water, and then shake out the excess water.

2 Insert the white center post, pointed end up, into the vacuum trap assembly.

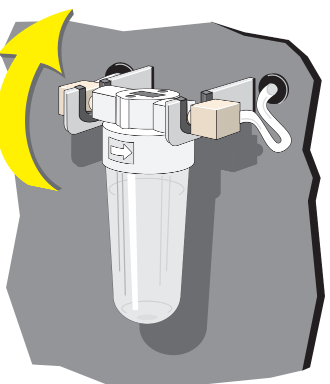
If the white center post in the vacuum trap assembly is stuck in the up position **(1)**, pull it into the down position **(2)**.



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.

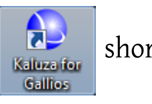


5 Wipe up any spills.

6 Plug the Supply Cart power cord into the wall outlet and turn the Supply Cart switch on.

7 Power ON the Cytometer Only

or

Power the Cytometer and Kaluza for Gallios ON by double clicking on the  shortcut.

8 Check that no error messages are displayed.

NOTE If an error message appears, see Table 15.2 for possible causes and operator actions.

9 Ready appears in the Application Title/Status bar when system initialization is done.

10 Record that the vacuum trap was cleaned in the instrument log book.

11 Perform the Daily Startup procedure before running samples.

Power the Cytometer Only On/Off

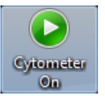
Use the procedures below if the instrument has not been fully shut down.

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 ON the Cytometer Only

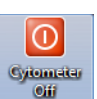
Use this procedure if the computer is already on and you do not need to start Kaluza for Gallios:

- On the Windows desktop, double click the 

icon to power up ONLY the Cytometer.

Power OFF the Cytometer

Use this procedure to turn off the Cytometer. You can still work with the Windows software after the Cytometer shuts off.

- On the Windows desktop, double click the 

icon to turn off ONLY the Cytometer.

NOTE If the Supply Cart power switch remains on, the instrument maintains the temperature in the Flow-Cell area. If the Supply Cart power switch is turned off, the Flow-Cell area thermal control is turned off and impacts warm up time requirements for startup.

CHAPTER 14

Replace/Adjust Procedures

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 20L 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*

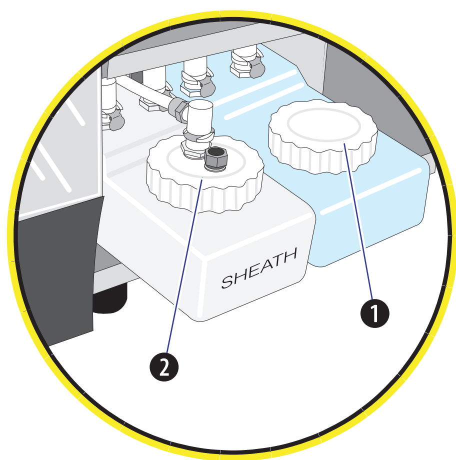
Replacement/Adjustment Schedule

All replacement and adjustment procedures should be done on an as needed basis.

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

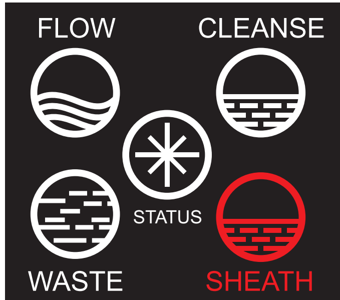
The cleaning agent container has a working capacity of about 500 mL. This is the amount of reagent needed when you are filling the cleaning agent container after *Cleanse Level Warning* (0x3008) or *Cleanse Level Error* (0x4009) appears. When you fill a completely empty cleaning agent container (after 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.

Replace the 10 L External Sheath Fluid Container

Perform this procedure whenever:

- The **Sheath Low** indicator is red.



- The *Sheath Cube Level Error* (0x3002) appears.

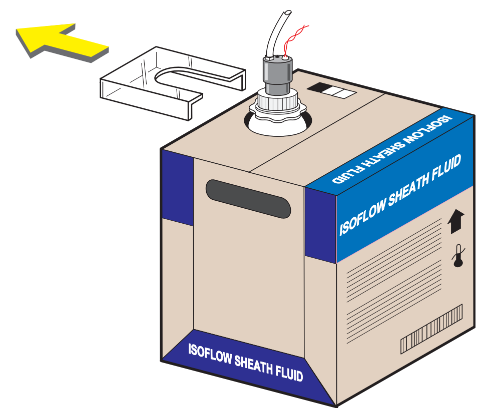
---

1 Check if the instrument is currently in ***Standby*** mode:

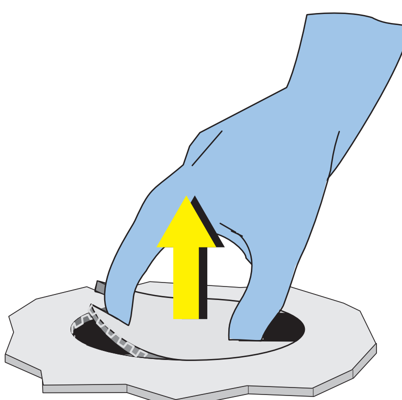
- If yes (***Standby*** appears in the **Instrument Control** panel), go to step 2.
- If no, Put the Cytometer into Standby/Idle Mode.

---

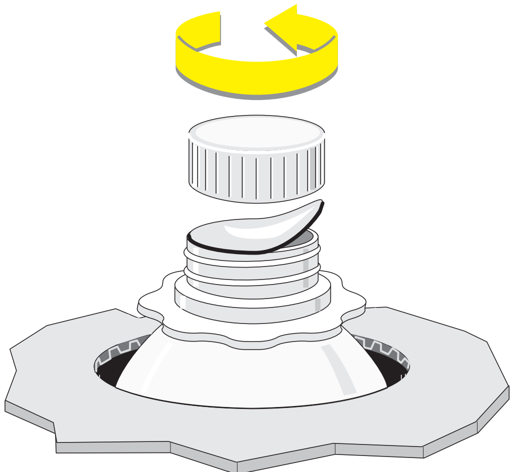
2 Remove the support collar from the empty sheath fluid container.



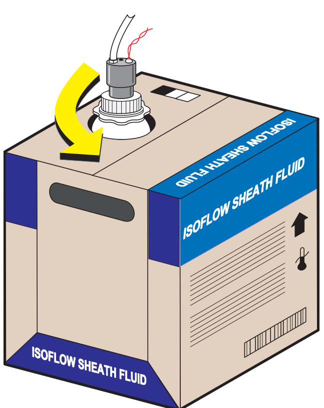
3 Remove any cardboard cutouts from the new sheath fluid container.



Remove the cap and seal from the new sheath fluid container. Be sure to completely remove the foil seal.



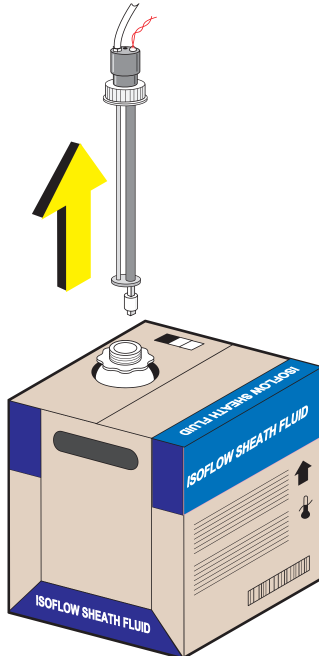
4 Unscrew the plastic cap that secures the pickup tube assembly into the old sheath fluid container.



⚠ CAUTION

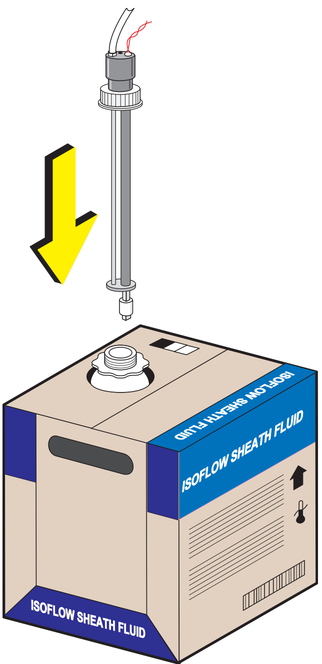
Misleading results could occur if you contaminate the sheath fluid. Be careful not to contaminate the sheath fluid. Do not let your fingers, paper towels, or other objects touch the pickup tube assembly.

5 Lift the pickup tube assembly straight up and out.

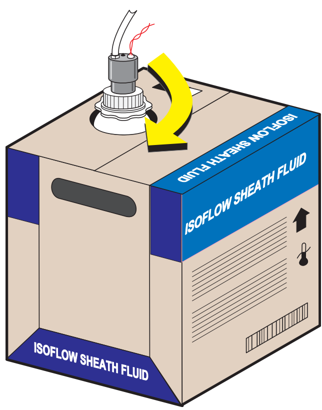


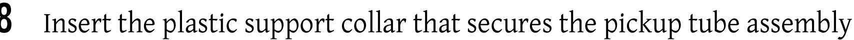
6 Inspect the pickup tube assembly and replace it if necessary.

7 Carefully insert the pickup tube assembly straight into the new sheath fluid container.

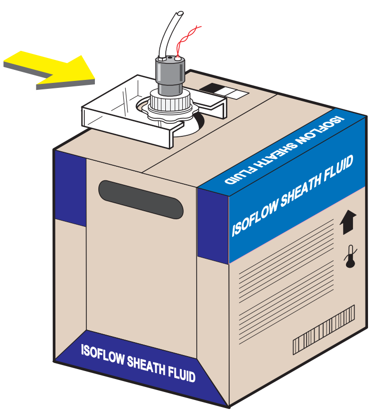


Tighten the cap.



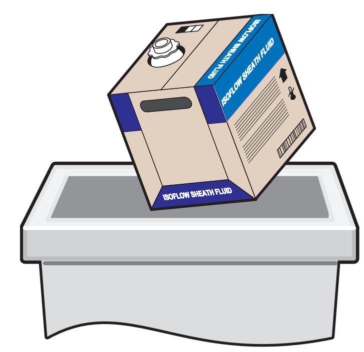


8 Insert the plastic support collar that secures the pickup tube assembly.



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.



Fill the Internal Sheath Fluid Container

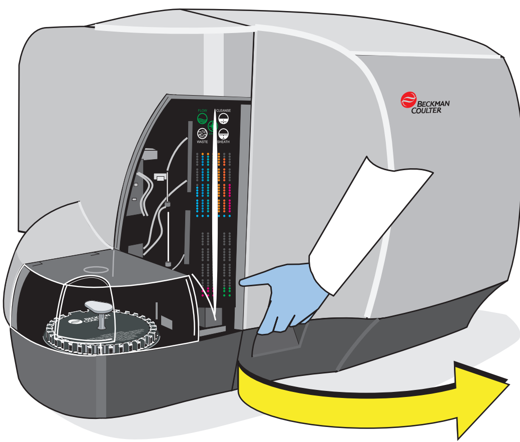
Perform this procedure whenever:

- You clean or replace the sheath fluid container.
- The error message *Internal Sheath Tank Level Warning* (0x3130) appears.

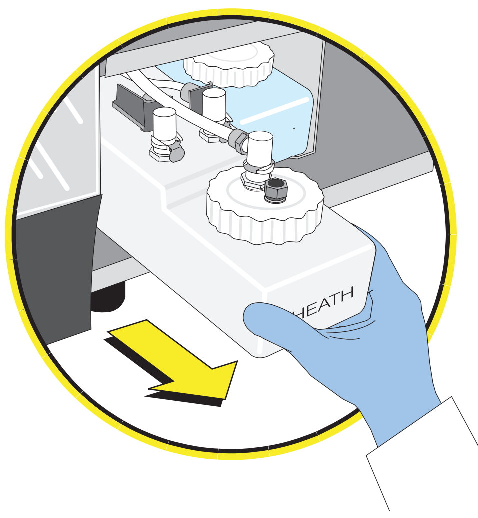
1 Check if the instrument is currently in ***Standby*** mode:

- If yes (***Standby*** appears in the **Instrument Control** panel), go to step 2.
- If no, Put the Cytometer into Standby/Idle Mode.

2 Open the Front Cover.



3 Pull out the sheath fluid container far enough to access the cap.

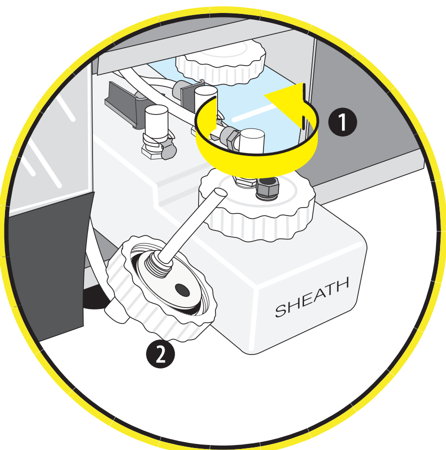


⚠ CAUTION

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:

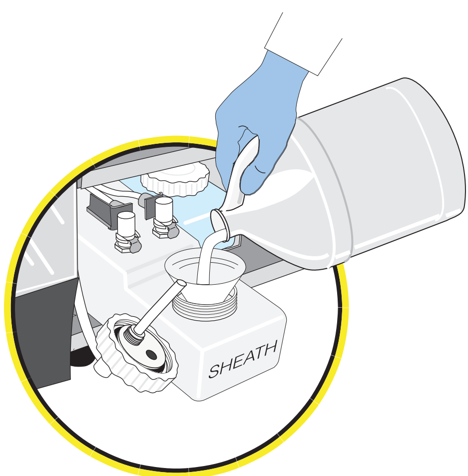
1. Unscrew the cap on the sheath fluid container.
2. To avoid contaminating the sheath fluid, lay the cap upside down on the counter.



⚠ CAUTION

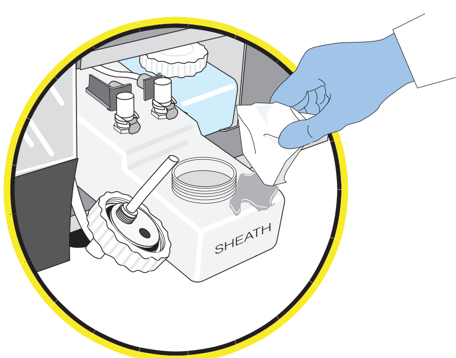
To prevent damage to the instrument, do not overfill the sheath fluid container. Avoid spills. Do not tilt the container or remove it from the drawer to fill it.

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.

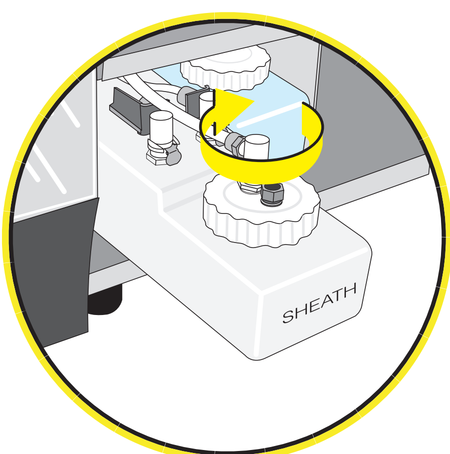
7 Carefully wipe up any spills.



⚠ CAUTION

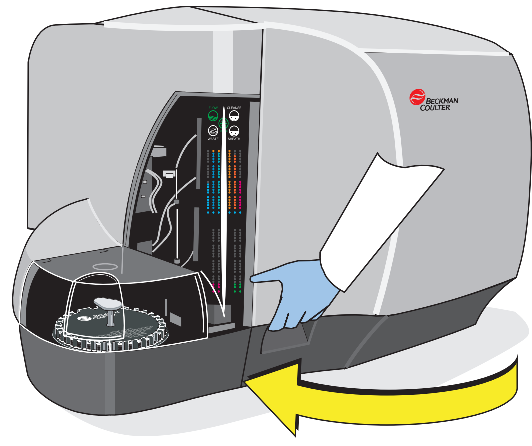
Misleading results could occur if you analyze samples without the cap on the sheath container. Be sure to put the cap back on the sheath fluid container after you fill it.

8 Screw the cap back on.



9 Slide the sheath fluid container back into place.

10 Close the Front Cover.



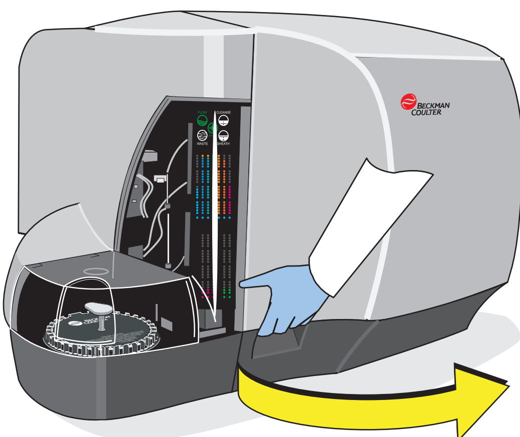
Fill the Cleaning Agent Container

Perform this procedure whenever *Cleanse Level Warning* (0x3008) or *Cleanse Level Error* (0x4009) appears.

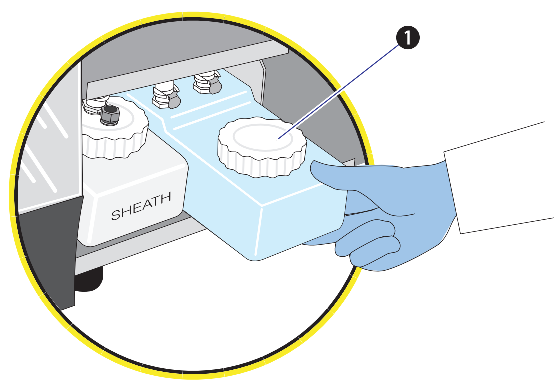
1 Check if the instrument is currently displaying ***Standby*** mode:

- If yes (***Standby*** appears in the **Instrument Control** panel), go to step 2.
- If no, Put the Cytometer into Standby/Idle Mode.

2 Open the Front Cover.



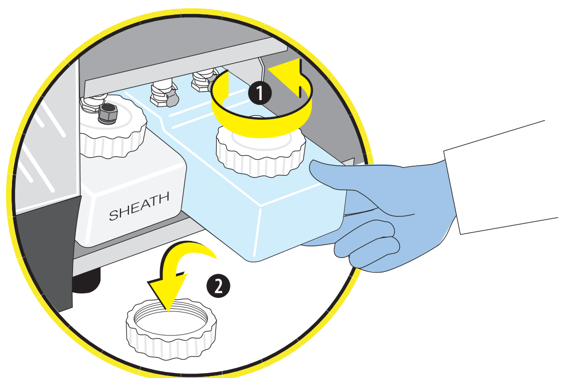
3 Pull out the cleaning agent **(1)** container far enough to access the cap.



⚠ CAUTION

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.

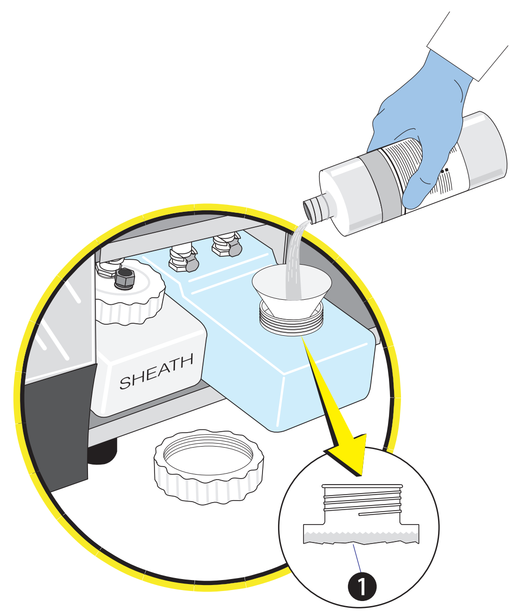
4 Unscrew the cap on the cleaning agent container **(1)**. To avoid contaminating the cleaning agent, lay the cap upside down on the counter **(2).**



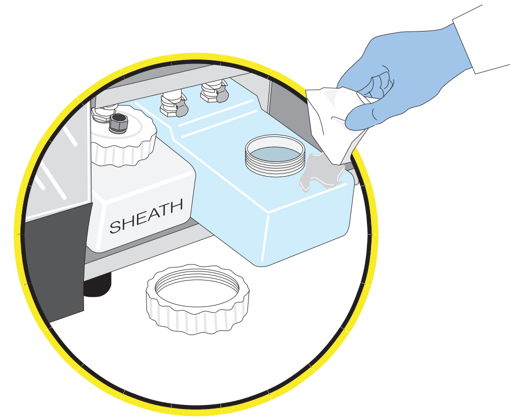
⚠ CAUTION

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 Carefully pour cleaning agent into the cleaning agent container, filling it just to the bottom of its neck **(1)**.



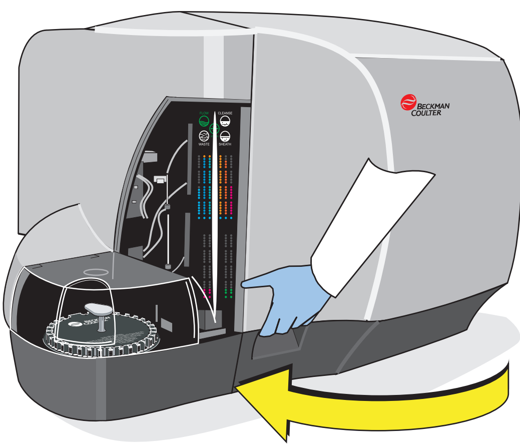
6 Carefully wipe up any spills.

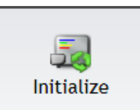


7 Screw the cap back on.

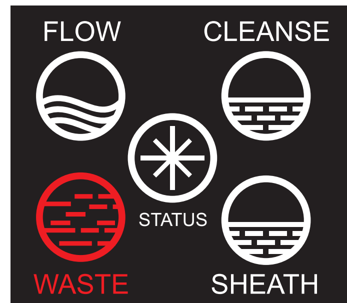
8 Slide the cleaning agent container back into place.

9 Close the Front Cover.



10 Before running samples, select  to initialize the system.

Empty the 20L Waste Container



- Empty the 20 L waste container when:
  - You perform your daily startup.
  - The **Waste Full** indicator is red.
  - *Waste Cube Full* (0x3005) or *Waste Level Error* (0x4006) 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 ***Standby*** mode:

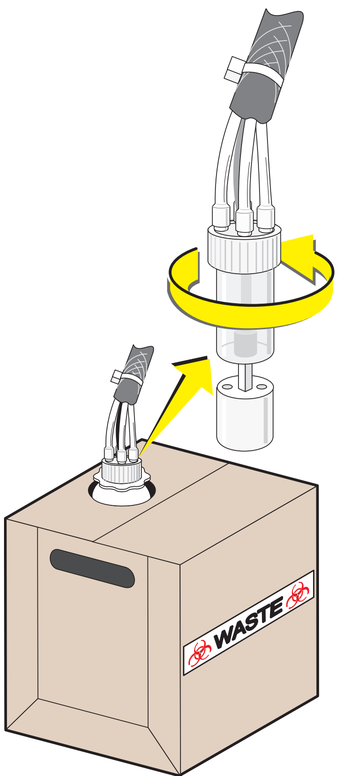
- If yes (***Standby*** appears in the **Instrument Control** panel), go to step 2.
- If no, Put the Cytometer into Standby/Idle Mode.

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.

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.

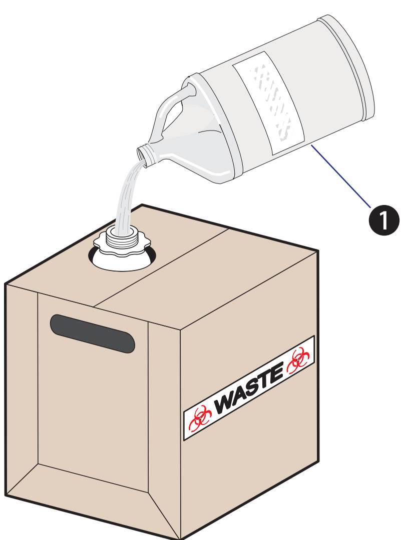
3 Unscrew the cap and lay it on a leakproof disposable container, such as a glove or beaker.



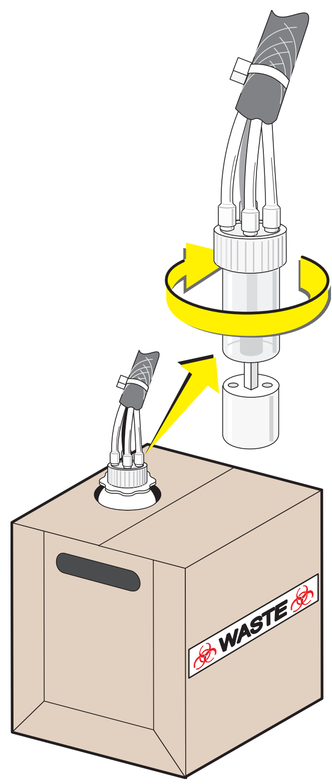
4 Empty the waste container according to your laboratory's procedures.

NOTE Take proper precautions to avoid spills if you are emptying the waste container into a sink, drain, or larger container. When moving the waste container to dispose of its contents, be sure the cap is secure to avoid spills.

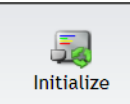
5 Put about 2 L of high-quality, fragrance-free, gel-free bleach **(1)** (5 to 6% solution of sodium hypochlorite - available chlorine) into the waste container to cover the bottom of the container.



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 From the **Instrument Control** panel, select  to initialize the instrument.

Replace the Sheath Fluid Filter

Replace the 0.2-µm sheath fluid filter:

- Once a year.

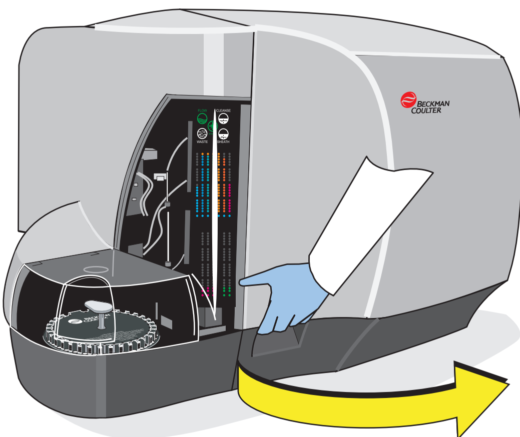
  or

- Whenever the sample flow rate is too high (repeated *Data Rate Warning* (0x3011) or *System Pressure Error* (0x4109) messages appear).

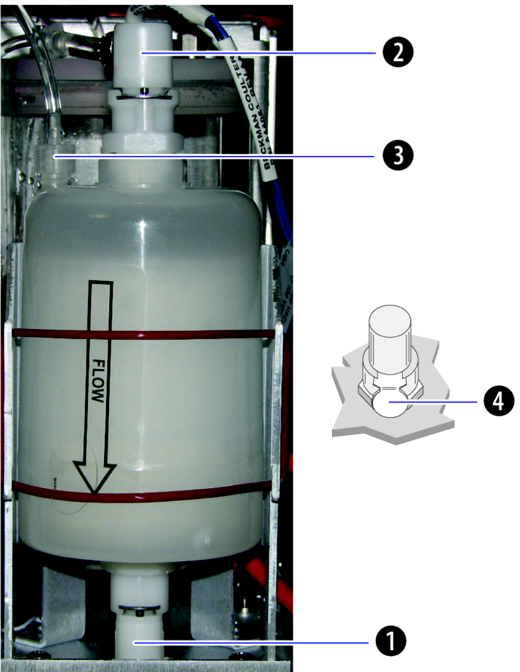
Procedure

1. Check if the instrument is currently displaying **Standby** mode:
   - If yes (Standby appears in the **Instrument Control** panel), go to step 2.
   - If no, Put the Cytometer into Standby/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.

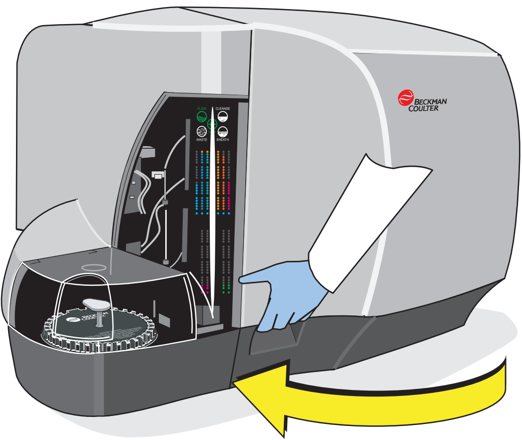
8 Wipe up any spills, and then put the filter in the bracket.

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.



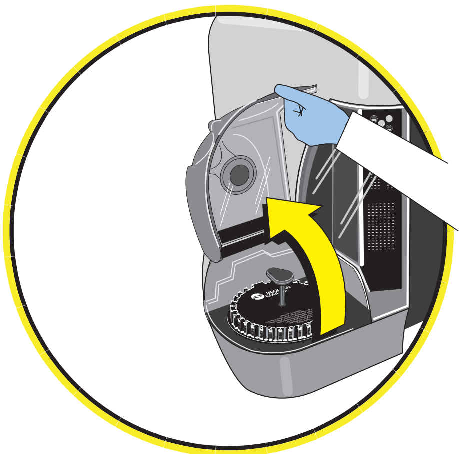
Replace the Sample Probe and Sample Pickup Tubing

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.

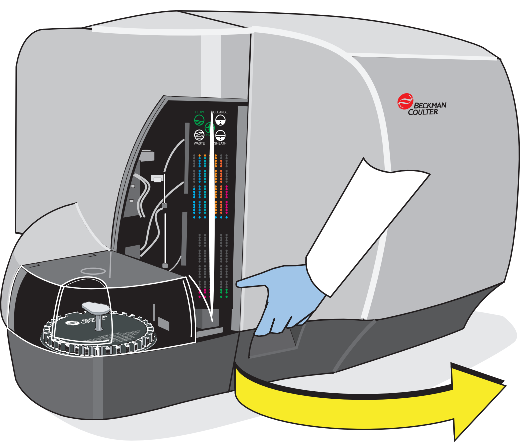
1 Power OFF the Cytometer.

2 Open the MCL cover.



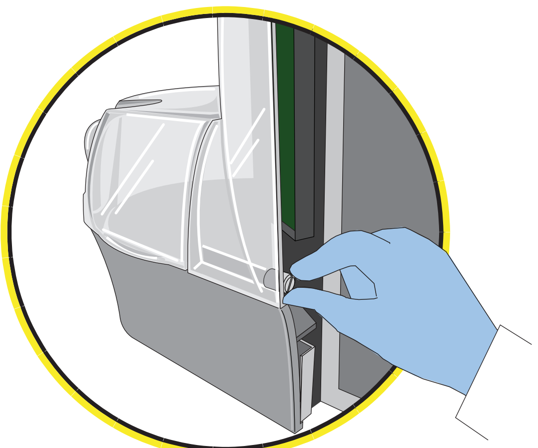
NOTE If a carousel is present, remove it.

3 Open the Front Cover.

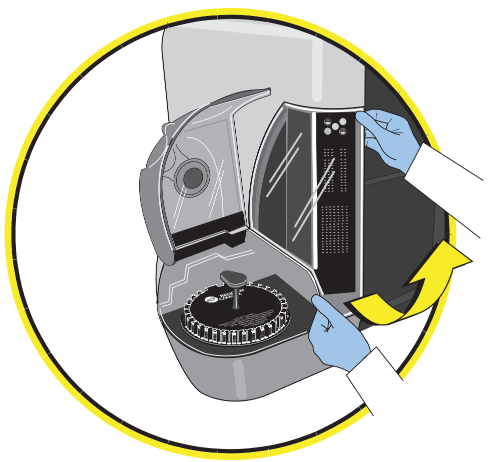


4 Remove the Front Left Side Panel.

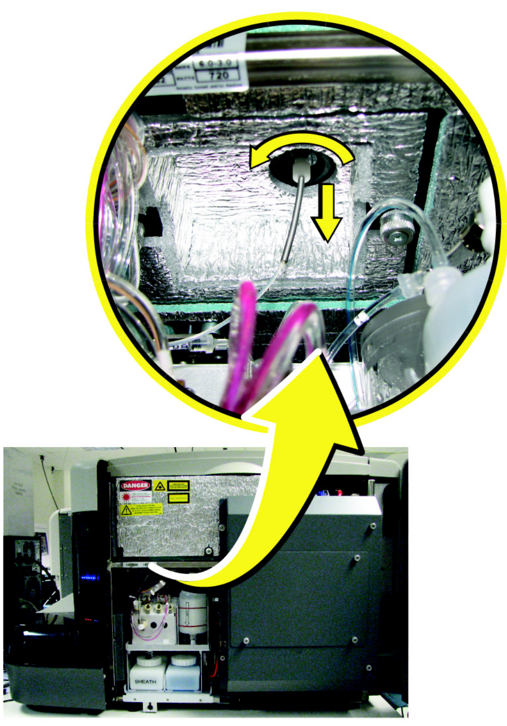
a. Unscrew the thumbscrew that attaches the left side panel to the front frame.



b. Remove the left side panel by pulling it towards you and swivel it up and out of the instrument.

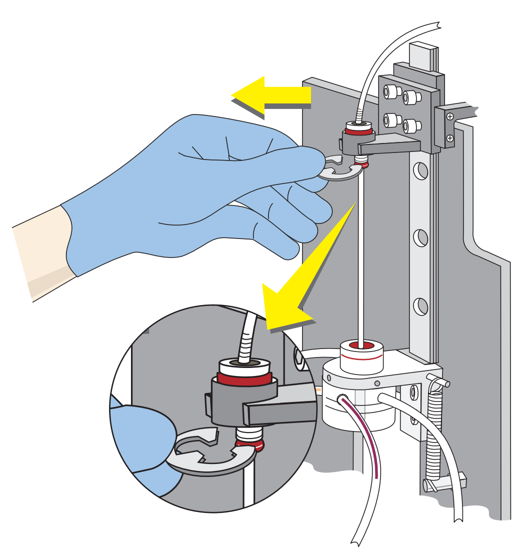


5 Unscrew the sample pickup tubing connector from the bottom of the flow cell compartment.

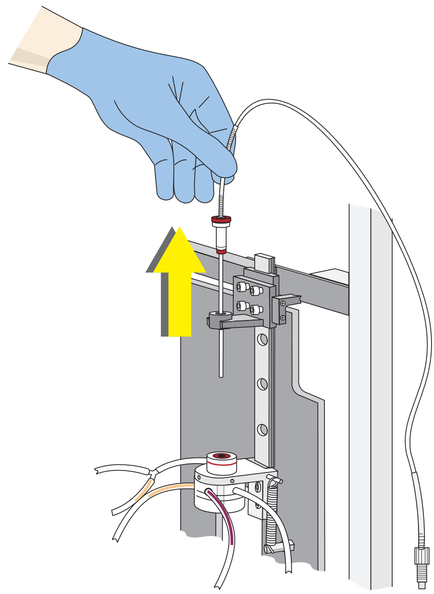


6 Pull the sample pickup tubing out through the left (MCL) side of the instrument.

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.

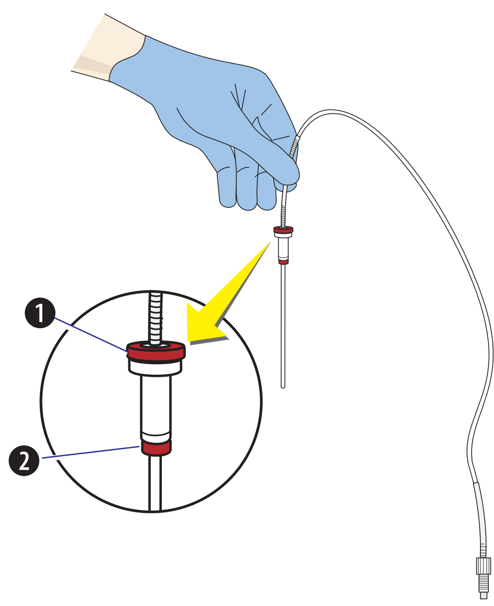


⚠ WARNING

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.

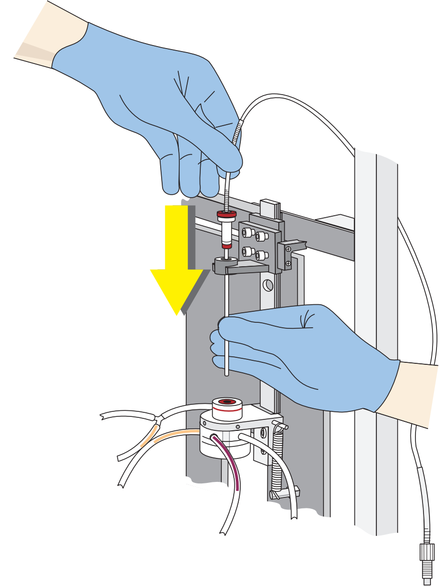
9 Discard the old sample pickup tubing and probe assembly in accordance with your local regulations and acceptable laboratory procedures.

10 Ensure that the rubber washer **(1)** and O-ring **(2)** are positioned correctly on the new sample probe.



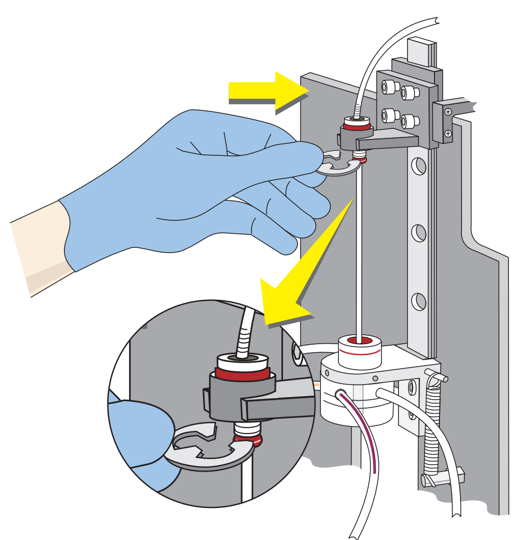
11 Thread the sample pickup tubing through the instrument.

12 Insert the new sample probe into the sample probe holder.



13 Guide the sample probe tip into the MCL sample head.

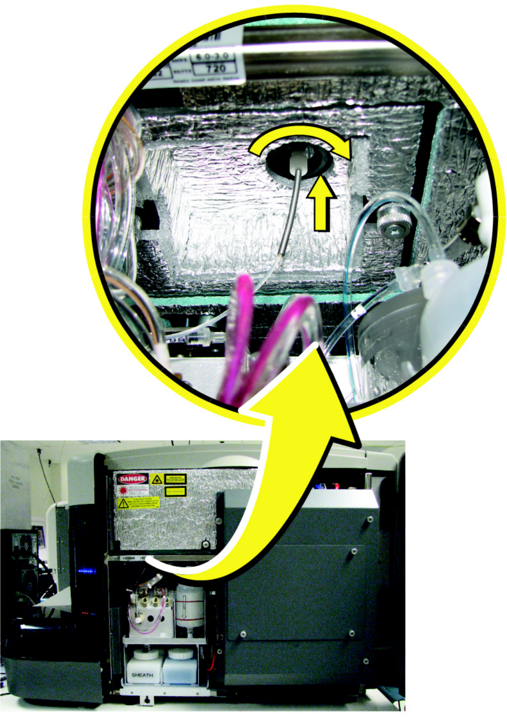
14 Insert the clip removed in step 7 into the groove on the sample probe.



⚠ CAUTION

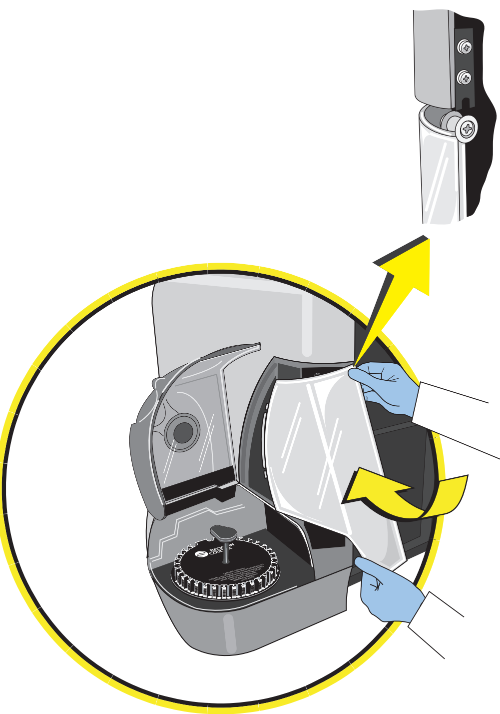
Risk of erroneous results if the flow cell is misaligned. Overtightening the connector from the sample pickup tubing to the flow cell can cause misalignment of the flow cell. Only screw on the sample pickup tubing connector "finger tight."

15 Screw on the connector from the sample pickup tubing to the bottom of the flow cell compartment until it is "finger tight."

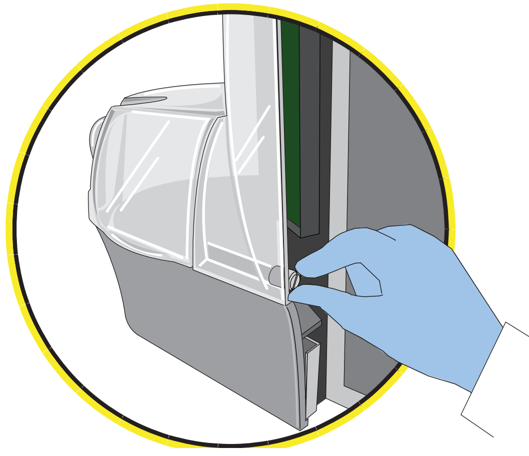


16 Replace the Front Left Side Panel.

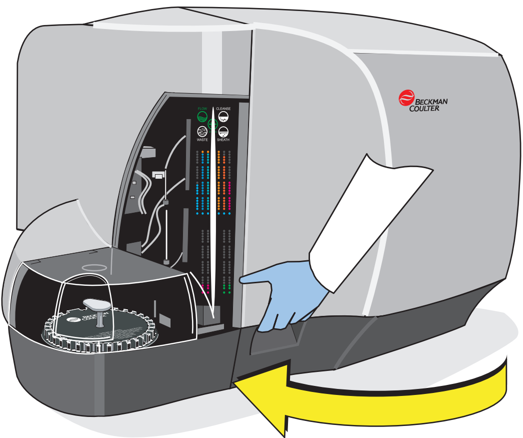
a. Swivel the left side panel into the instrument, aligning the post on the top of the panel with the cut out on the frame and then push the panel back into place.



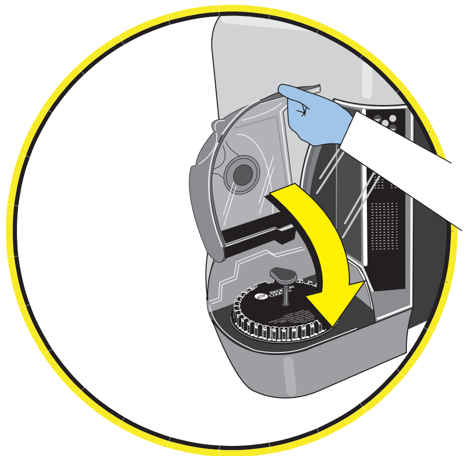
b. Screw in the thumbscrew to attach the left side panel to the front frame.



17 Close the front cover.



18 Close the MCL cover.



19 Power ON the Cytometer Only

or

Power the Cytometer and Kaluza for Gallios ON by double clicking on the

icon on your desktop.

20 On the **Instrument Control** panel, select Prime.

21 After the prime cycle is done, select [Prime] again.

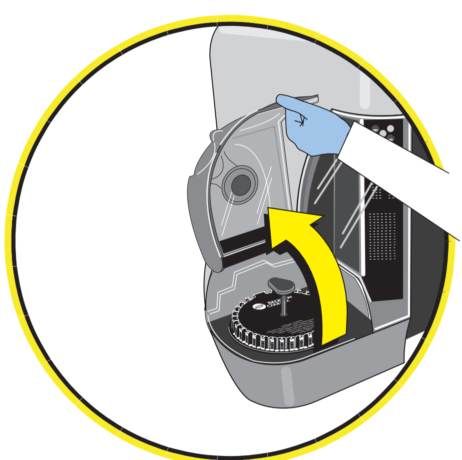
Replace the MCL Sample Head

Use this procedure when:

- Cleaning the sample head does not fix your excessive carryover problem.
- Numerous *Sample Pressure Error* (0x4112) or *Tube Up Down Error* (0x4605) messages occur.

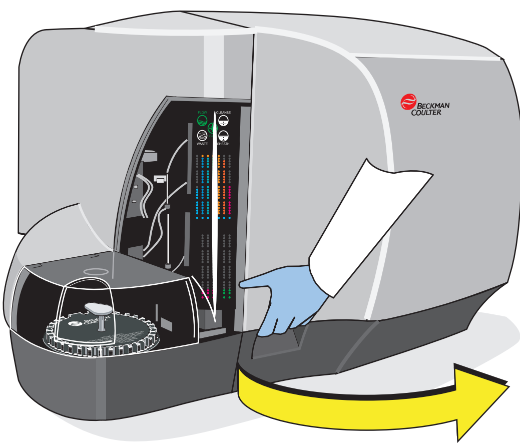
1 Power OFF the Cytometer.

2 Open the MCL cover.



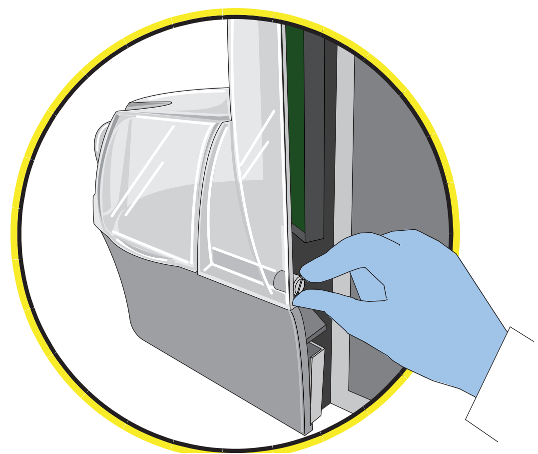
NOTE If a carousel is present, remove it.

3 Open the Front Cover.

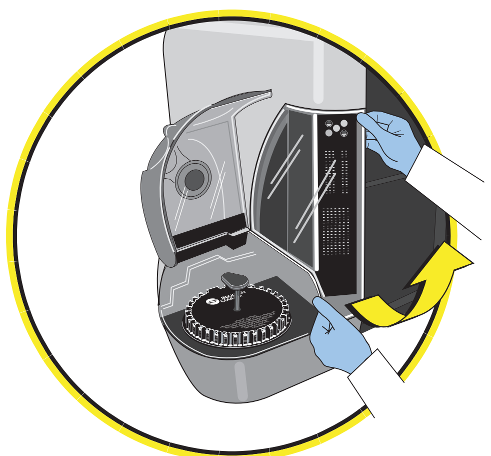


4 Remove the Front Left Side Panel.

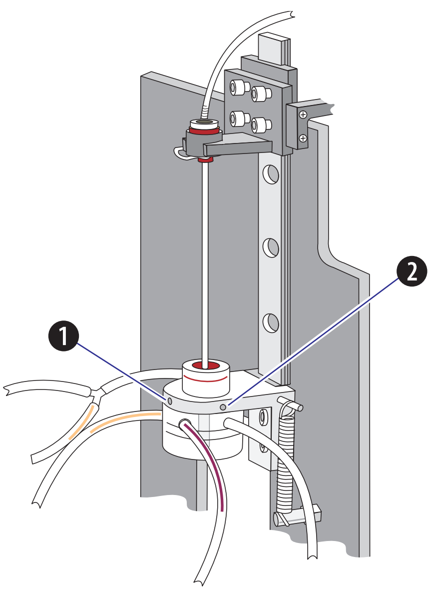
a. Unscrew the thumbscrew that attaches the left side panel to the front frame.



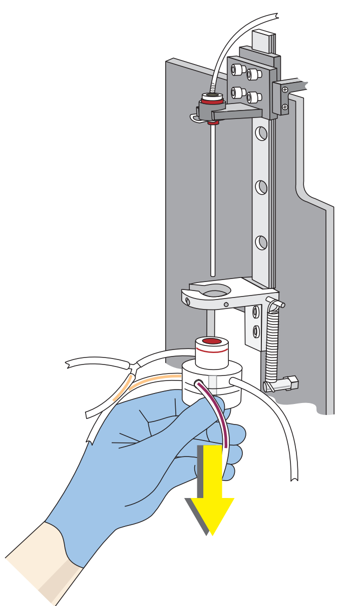
b. Remove the left side panel by pulling it towards you and swivel it up and out of the instrument.



5 Use a 0.050 in. Allen wrench to loosen the side **(1)** and front **(2)** setscrews on the sample head.

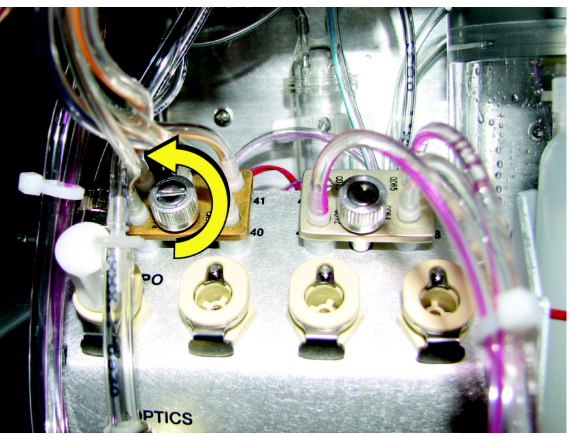


6 Pull off the sample head.



7 Pull the sample head and tubing through the instrument behind the frame.

8 Loosen the thumbscrew holding the left tubing manifold.



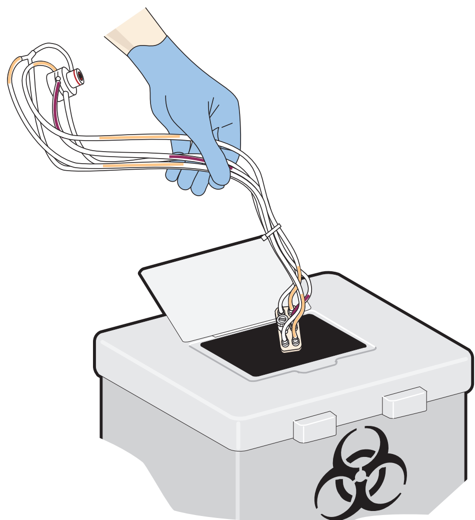
NOTE You might find it easier to unscrew the thumbscrew with a screwdriver.

9 Pull off the tubing manifold.

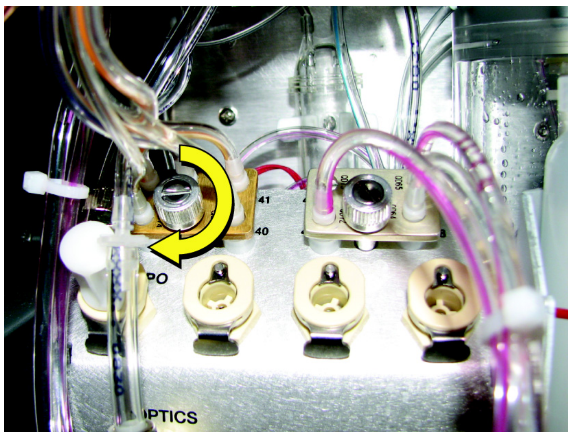
⚠ WARNING

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.



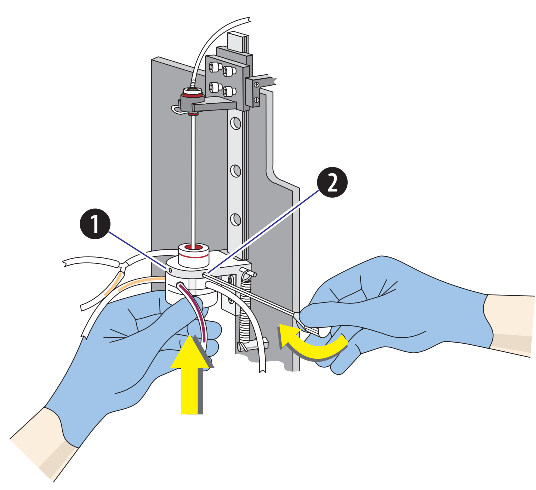
11 Place the new tubing manifold into the bracket in the pneumatic drawer and tighten the thumbscrew.



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.

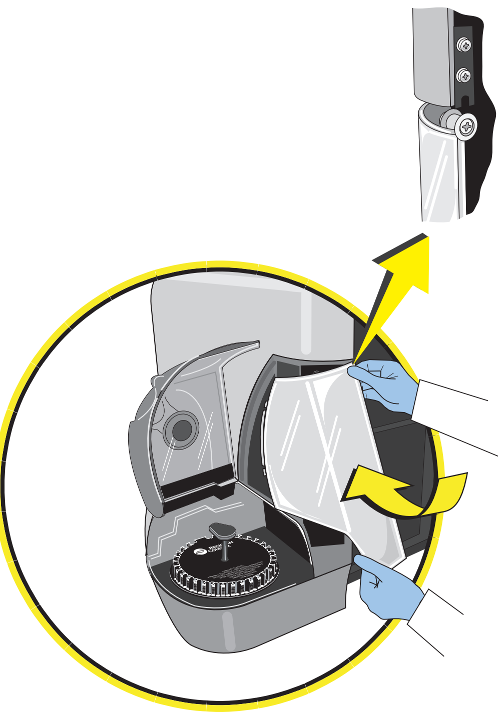


14 Tighten the side **(1)** setscrew first.

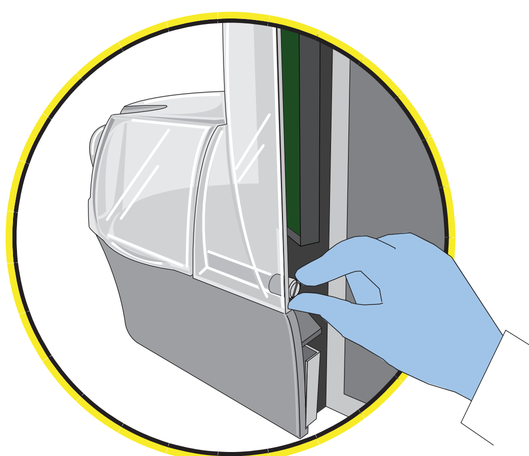
Then tighten the front **(2)** setscrew.

15 Replace the Front Left Side Panel.

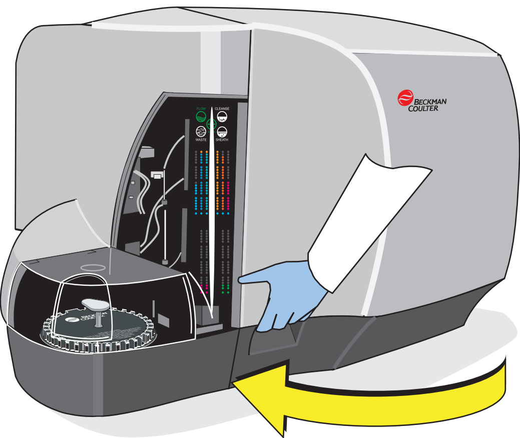
a. Swivel the left side panel into the instrument, aligning the post on the top of the panel with the cut out on the frame and then push the panel back into place.



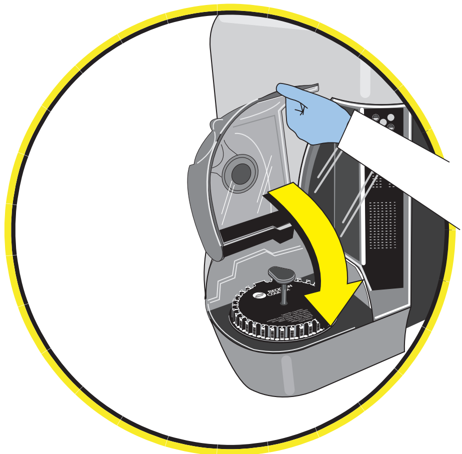
b. Screw in the thumbscrew to attach the left side panel to the front frame.



16 Close the front cover.

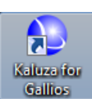


17 Close the MCL cover.



18 Power ON the Cytometer Only

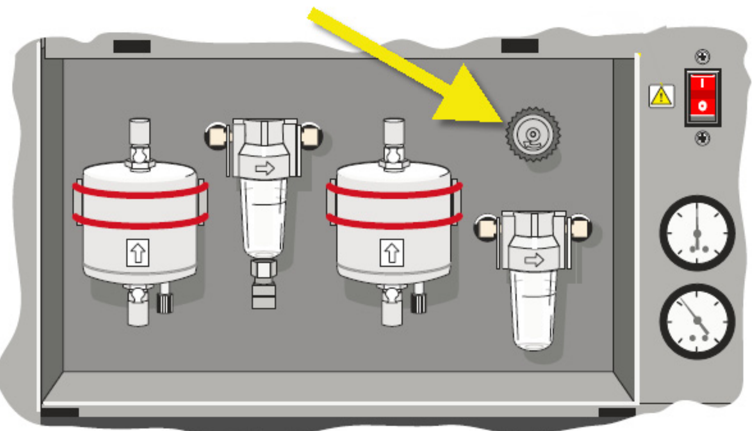
or

Power the Cytometer and Kaluza for Gallios ON by double clicking on the  shortcut on your desktop.

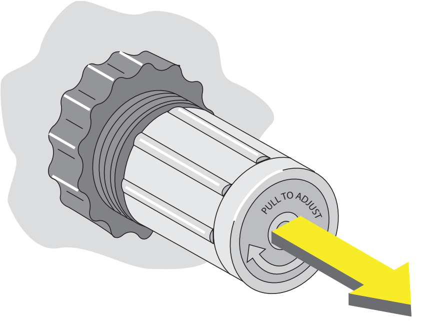
Adjust the System Pressure

- 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.

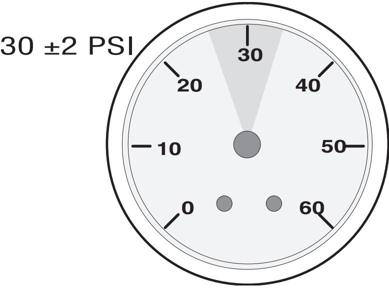
1 Open the Pneumatic Supply front door and locate the Pressure Adjustment knob.



2 Pull the collar around the Pressure Adjust knob out toward you.

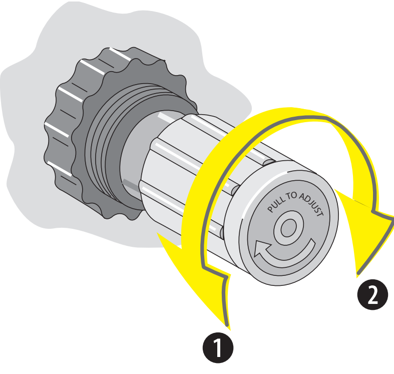


3 Adjust the pressure to 30 ±2 psi.

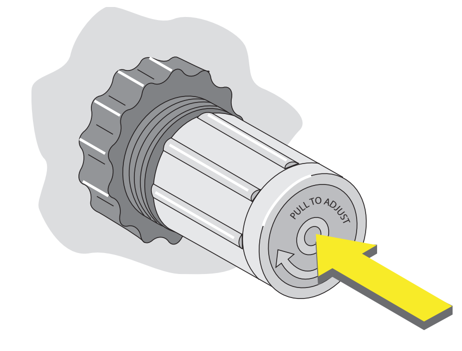


(1) To decrease, turn to the left.

(2) To increase, turn to the right.



4 Push in on the collar to lock it into place.



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

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.

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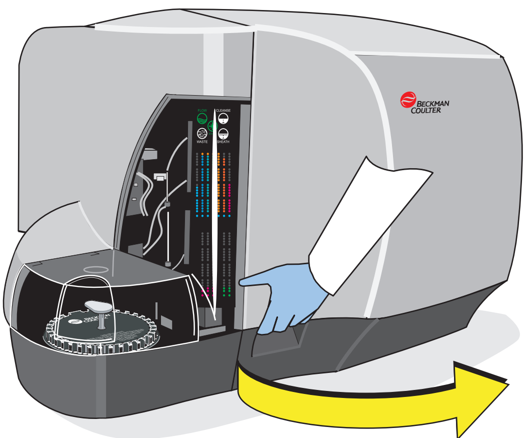
1 Wear powder-free gloves to perform this procedure.

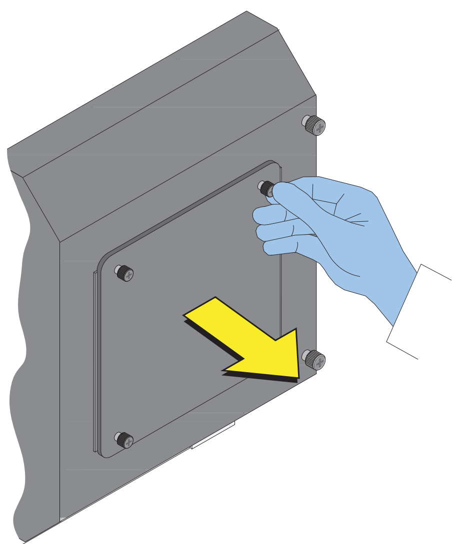
---

2 Open the Front Cover.

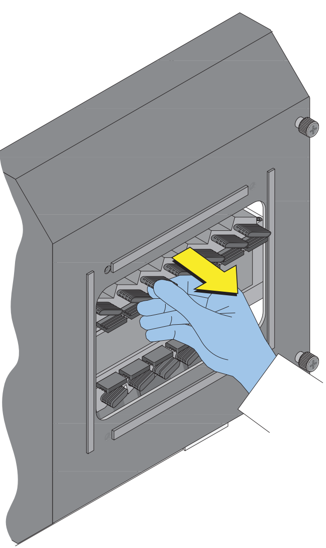


3 Loosen the four thumbscrews on the filter array cover and remove it.

NOTE You need to unscrew the upper left thumbscrew with a screwdriver.



4 Remove the filter holder containing the filter you want to replace.



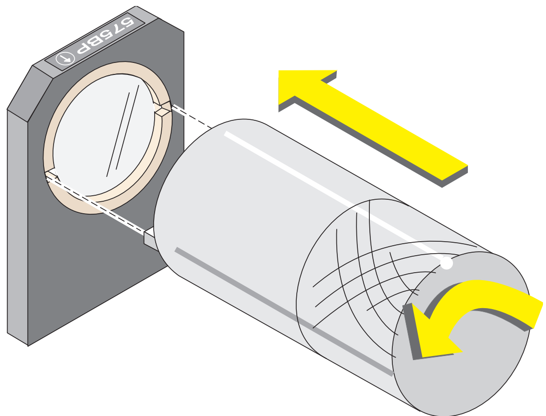
NOTE There are two types of filter holders. See,

- 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

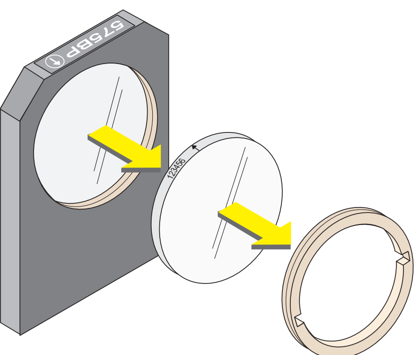
1 Use the special tool provided to loosen the metal ring on the filter holder.



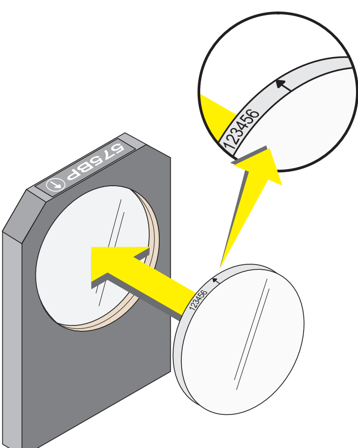
NOTE You might find it easier to finish loosening the metal ring by turning it with your gloved fingers.

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.

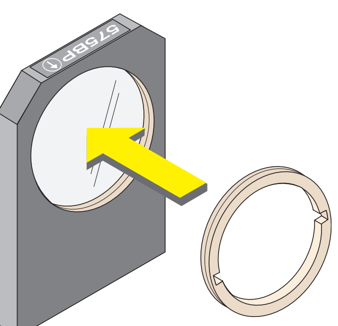


- For BCI filters:

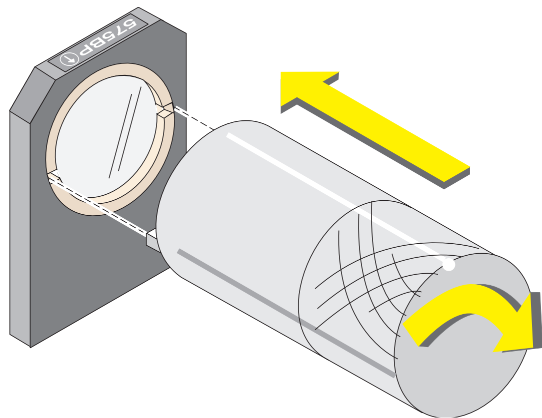
  Position the filter into the filter holder so the arrow points to the metal ring.
- For non-BCI filters:

  See Identify Coated Side Of Dichroic Filter to determine correct orientation.

5 Place the metal ring over the filter in the filter holder.



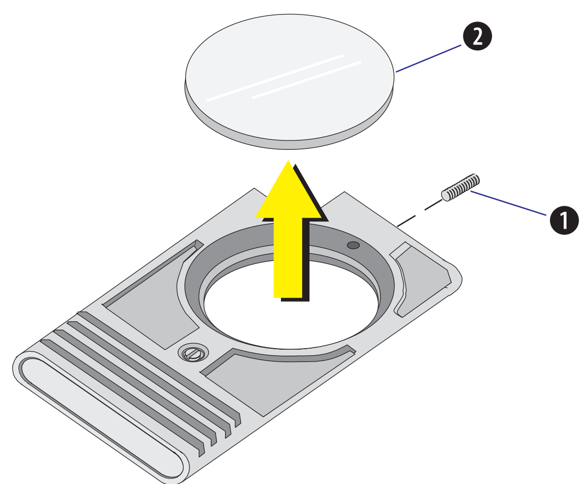
6 For the metal ring fastener, insert the special tool into the metal ring's two slots and turn to the right to tighten.



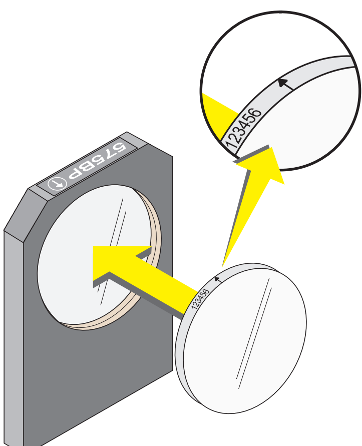
NOTE You might find it easier to begin tightening the metal ring by turning it with your gloved fingers.

Replace Bandpass Filter

1 Remove the set screw (1) and remove the filter (2).

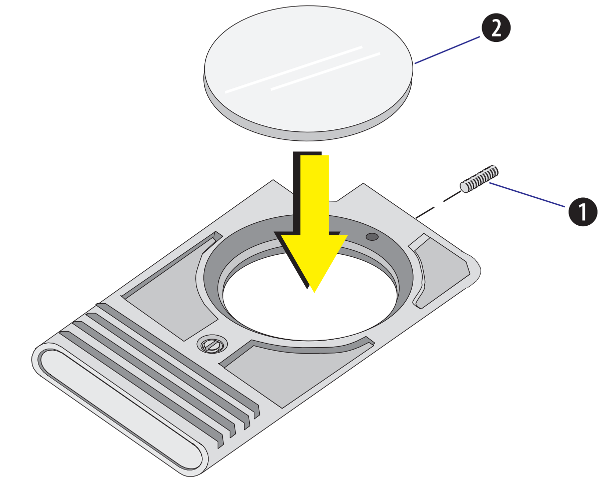


2 Orient the new filter correctly and insert the filter into the filter holder.



- For BCI filters:
  Position the filter into the filter holder so the arrow points away from the counterbore.
- 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

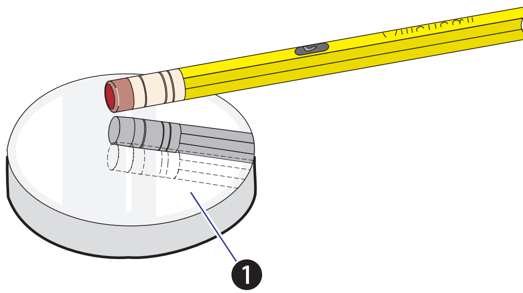
1 Determine which is the coated side **(1)** of a non-BCI filter:

a. Take the eraser end of a pencil and hold it close to the filter, near its edge.

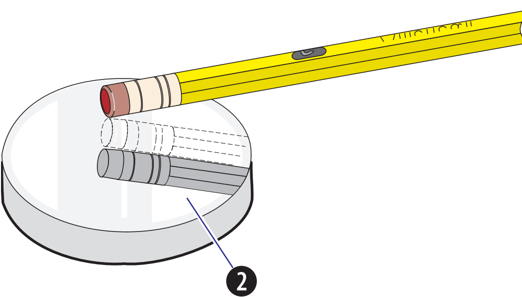
b. Look at the two reflections, dark- and light-colored, of the pencil.

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 **(1)**.



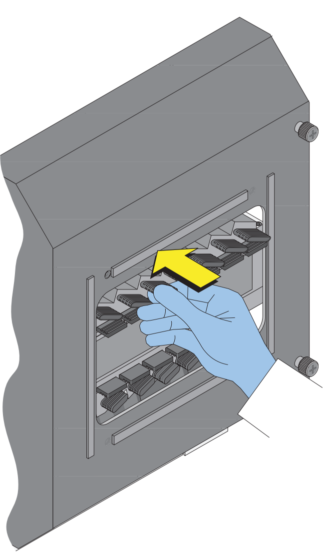
The uncoated side **(2)** shows the pencil touching the light-colored reflection.



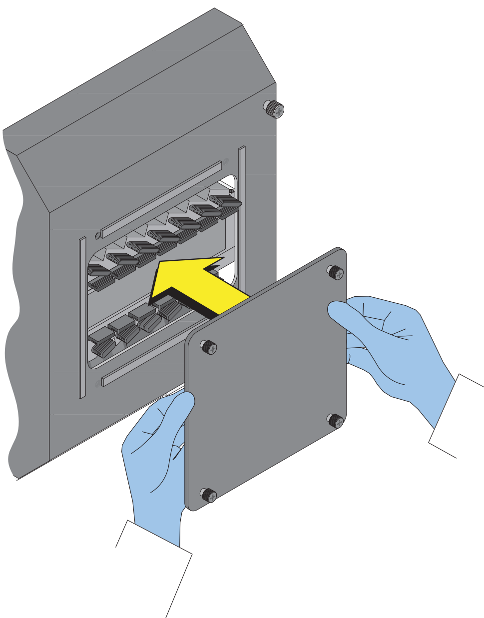
e. The coated side **(1)** should face the metal ring when you insert it.

Replace Filter Holder

1 Place the filter holder containing the filter you replaced back in the filter array.

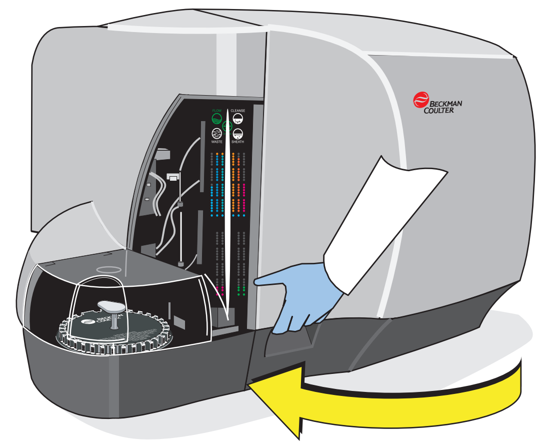


2 Replace the filter array cover.



NOTE You need to use a screwdriver to tighten the upper left thumbscrew.

3 Close the Front Cover



CHAPTER 15

Troubleshooting

Precautions/Hazards

Laser/Radiation Precautions

The Cytometer contains two or three lasers (488, 638, and 405 lasers to support the 6-color/2-laser, 8-color/2-laser, or the 10-color/3-laser Gallios Flow Cytometer configurations), but can also include an optional fourth laser (561). The MCL barcode reader contains one 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 15.1 for the Sensing Compartment cover warning label.

See Figure 15.2 for the Sensing Compartment interior (cover removed) warning labels.

See Figure 15.3 for the Lasers in the Sensing Compartment warning labels.

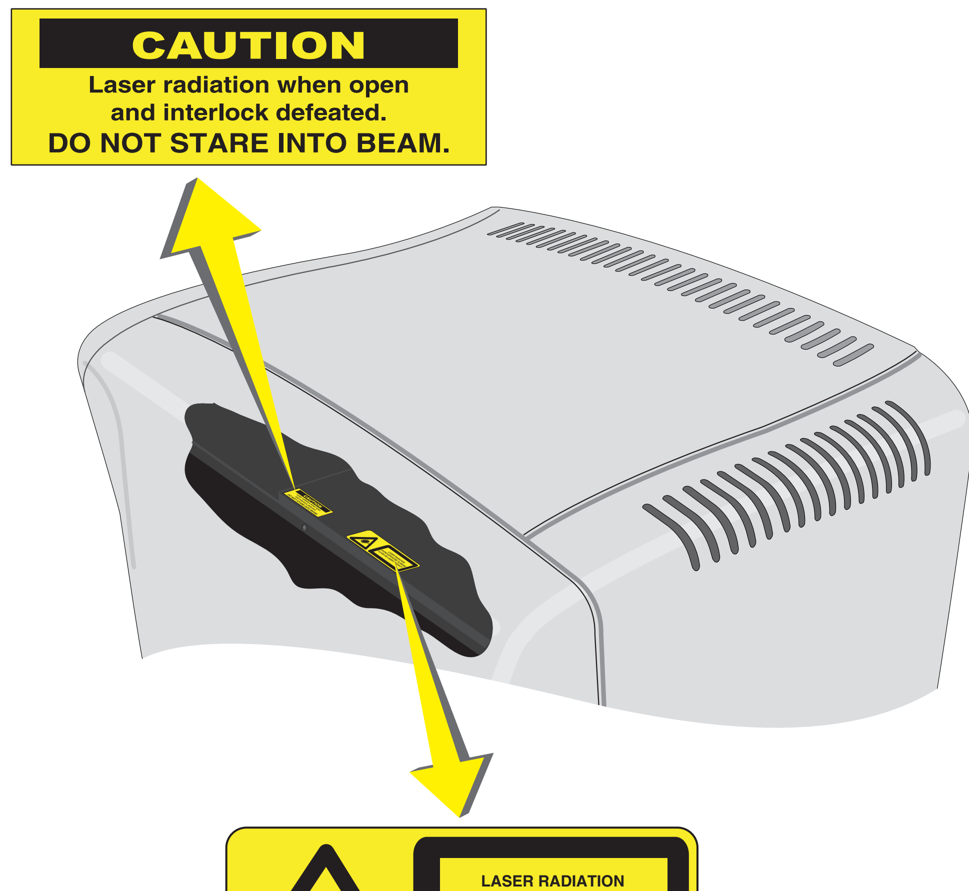
See Figure 15.4 for the Filter Array (cover removed) warning labels.

See Figure 15.5 for the Laser Label on the Cytometer Back Panel.

See Figure 15.6 for the MCL barcode reader warning labels.

Figure 15.1 Laser Labels on the Sensing Compartment Cover

Figure 15.2 Laser Labels in the Sensing Compartment, Cover Removed



Shown left to right below are, the red laser, the blue laser and the violet laser.

Figure 15.3 Labels on the Lasers in the Sensing Compartment, Cover Removed

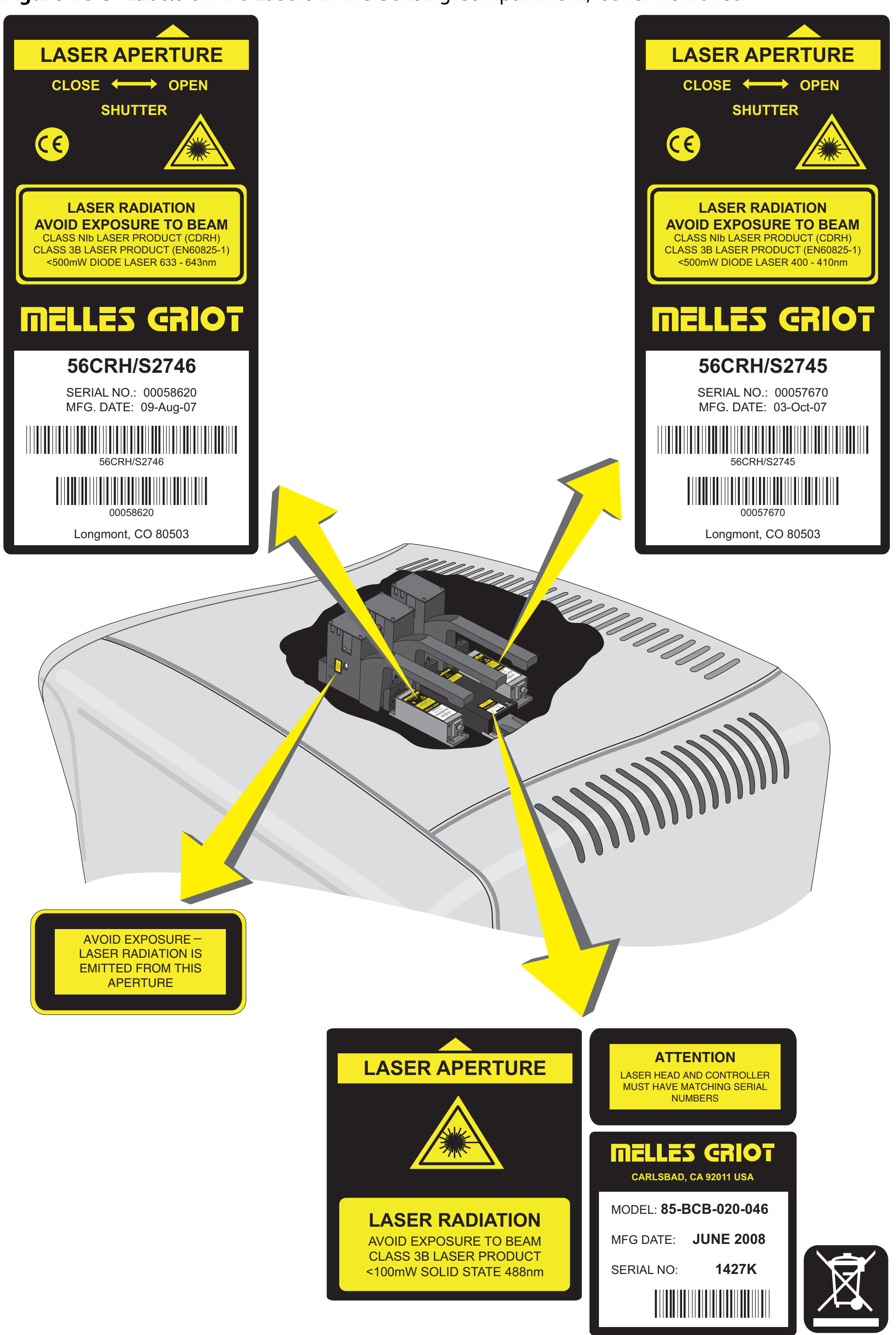


Figure 15.4 Laser Labels on the Filter Array, Cover Removed

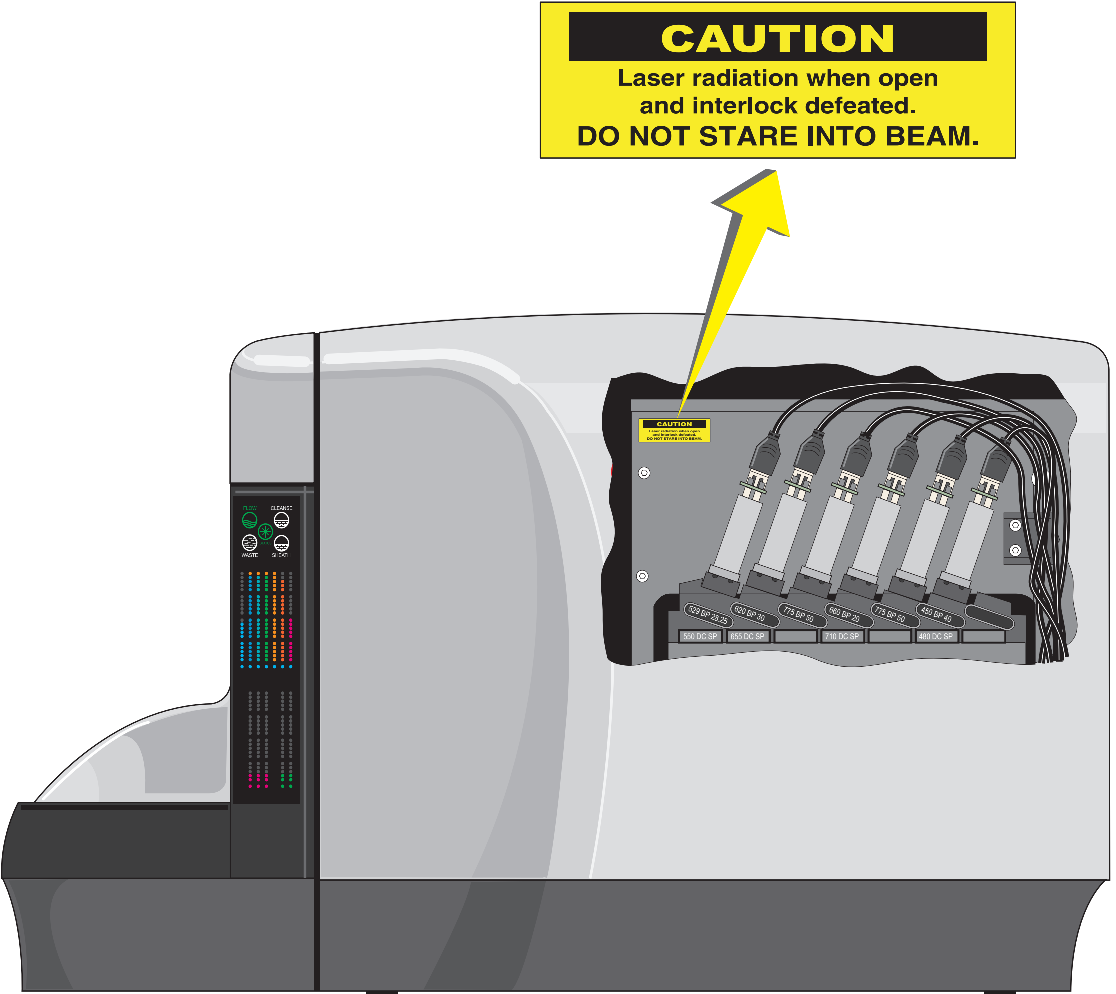


Figure 15.5 Laser Label on the Cytometer Back Panel

THIS LASER PRODUCT CONFORMS
TO THE PROVISIONS OF 21 CFR,
SUBCHAPTER J, SECTIONS
1040.10 AND 1040.11

Manufactured:
DATE: SEPTEMBER 2009
BY: BECKMAN COULTER, INC.
250 S. Kraemer Blvd.
Brea, CA 92821 USA

2427796

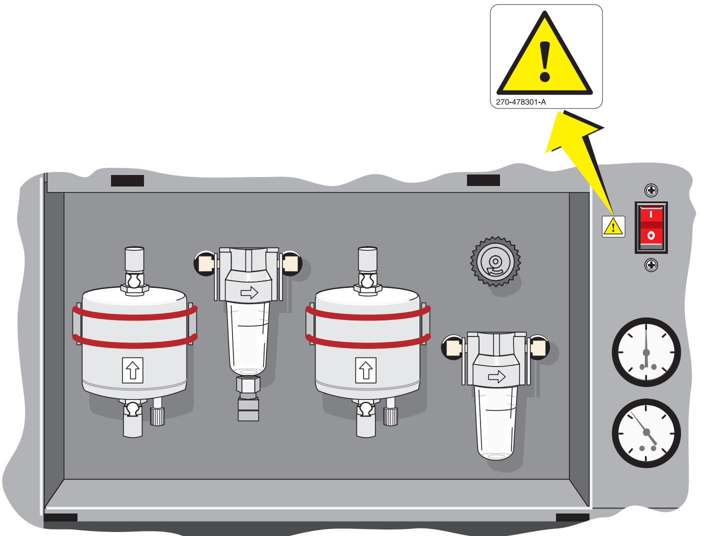
Figure 15.6 Laser Labels on the MCL Barcode Reader

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 15.7 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

These labels and materials declaration table (the Table of Hazardous Substances Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

RoHS Environmental Label

This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center "e" indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

Disposal Precaution

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.

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.

Kaluza for Gallios Log Files

Log files document exceptions within Kaluza for Gallios and provide clues to understanding issues and viewing user set-up configurations. In *Program Failure Log Files* are instructions for providing Kaluza for Gallios log files to a Beckman Coulter Representative for troubleshooting. *User Options File* provides the location of user configuration log files.

Program Failure Log Files

When contacting Beckman Coulter for troubleshooting information, you will need to provide the log files.

To access the log files and upload for troubleshooting:

1 Locate the Kaluza for Gallios log directory:

a. Select the (**About**) icon in the upper right corner of the Kaluza for Gallios screen.

b. In the **About Kaluza for Gallios** screen, select the Open Log Directory... button, which takes you directly to the location of the files.

OR

Locate the files manually by navigating to the appropriate location listed below:

Operating System	Storage Location
Microsoft Windows 7 or **Microsoft Windows Vista**	C:\ProgramData\Beckman Coulter\Kaluza for Gallios\<version>
Microsoft Windows XP	C:\Documents and Settings\All Users\Application Data\Beckman Coulter\Kaluza for Gallios\<version>

2 Close Kaluza for Gallios.

3. Zip the entire contents of the log directory, and place the zip file in the location of your choice.

4. Access the Kaluza troubleshooting page by entering *www.kaluzasoftware.com* into your internet browser, and then selecting the **FAQ** tab.

5. Select the **Troubleshooting File Upload** button.

6. Fill out the short form, describing the problem, and then select **Submit your information** to access the upload page.

7. Upload the zip file to complete the process. Once the file is uploaded, it will be listed on the page. You can upload additional files or close the browser when you are finished.

System NFO Files

When contacting Beckman Coulter for troubleshooting information, you might be requested to provide a *.nfo file. To access the *.nfo file and upload for troubleshooting:

1. From the Windows **Start** button, select **All Programs** > **Accessories** > **System Tools**.

2. Select **System Information**.

3. In the System Information window, select **File** > **Save**.

4. In the **File name** field, enter your name, navigate to the location where you wish to save the file, and then select **Save**. The default file type is ***.nfo**.

   NOTE It could take between 2 and 4 minutes for the *.nfo file to be created.

5. Access the Kaluza troubleshooting page by entering *www.kaluzasoftware.com* into your internet browser, and then selecting the **FAQ** tab.

6. Select the **Troubleshooting File Upload** button.

7. Fill out the short form, describing the problem, and then select **Submit your information** to access the upload page.

8 Upload the *.nfo file to complete the process. Once the file is uploaded, it will be listed on the page. You can upload additional files or close the browser when you are finished.

User Options File

Kaluza for Gallios stores user options in a user configuration file that can be found in the following locations:

- Windows 7 and Vista:
  C:\Users\<user-name>\AppData\Local\Beckman_Coulter,_Inc\Kaluza.Gallios.exe_Url_<random>\<version>\user.config
- On Microsoft Windows XP:
  C:\Documents and Settings\<user-name>\Local Settings\Application Data\Beckman_Coulter,_Inc\Kaluza.Gallios.exe_Url_<random>\<version>\user.config

Troubleshooting Kaluza for Gallios

The table below lists tips to guide you if you encounter any problem during installation, as well as the respective recommended action. If you are unable to solve the problem, contact your Beckman Coulter Representative.

NOTE Some troubleshooting issues are due to the Sentinel HASP subsystem. Sentinel HASP is the technology used to license-protect Kaluza for Gallios. The names "Sentinel" and "HASP" are used interchangeably in error text.

Issue: .NET Framework installation errors.	
Comment:	**Recommended Action:**
• Error messages display during .NET installation. • Kaluza for Gallios crashes during launch.	• Contact your IT Department. • See *Kaluza for Gallios Log Files*.

Issue: Kaluza for Gallios installation prerequisites dialog box persists that the .NET is not installed on a Windows 7 PC.	
Comment:	**Recommended Action:**
The Kaluza for Gallios Setup.exe properties are set to Compatibility Mode. (This can be viewed in Windows Explorer by right-clicking the Kaluza for Gallios Setup.exe file and selecting **Properties**.)	If installing on Windows 7 and the install is being run by Windows XP in Compatibility Mode, the .NET installation will always show a warning on the Prerequisite screen (even after successful installation). If this is the case, deactivate Compatibility Mode via the Install File and repeat the installation. If this does not work, try copyig the Kaluza for Gallios Setup.exe file from the installation disc to a hard drive aand repeating the steps.

Issue: • **Unable to start Aksfridge Service, error occurs at the end of the installation.** • **Unable to start Aksfridge Service with parameters 1080 1058 1. Error code: 48 5 550 1058.**	
Comment:	**Recommended Action:**
This error occurs because the Sentinel HASP service cannot get started correctly.	Reboot the computer and attempt to run the application one more time.

Issue: Sentinel HASP error during installation.	
Comment:	**Recommended Action:**
This error occurs because the Sentinel HASP service is not installed correctly. Three possible causes are: • Other “Sentinel” services are running and are interfering with or have modified the Kaluza for Gallios licensing service. • A firewall is blocking port 1947 for the Sentinel HASP. • Another program is holding on to port 1947 for Sentinel HASP.	• Reboot and repeat install. • If error persists, temporarily disable virus checker and repeat installation. • If error persists, uninstall any instances of Kaluza for Gallios and then remove any Sentinel HASP Local License Manager license keys installed on the PC, reboot, and then re-install Kaluza for Gallios. • If error still persists, browse to http://localhost:1947 in your web browser. Click the Sentinel HASP keys on the left side of the screen, take a screen shot and save this image file. Click on **Features** on the left side of the screen and take another screenshot, and save the file. Files (*.jpg, *.gif, *.pdf, *.txt) can be uploaded for troubleshooting. To access the troubleshooting page, go to *www.kaluzasoftware.com*, and select the **FAQ** tab. Fill out the short form, describing the problem. Select **Submit** to access the upload page.

Issue: Unable to access HASP SRM Run-Time Environment (H0033) displays on the HASP Protection System for the Kaluza for Gallios window.	
Comment:	**Recommended Action:**
This occurs because the Sentinel HASP run-time has not been started.	**1.** Find Kaluza for Gallios in **Add**/**Remove** programs. **2.** Select the **Repair** option to ensure all necessary files are in place. **3.** Reboot the computer. **4.** Attempt to launch Kaluza for Gallios. If the error message still persists: **1.** Click **START** > **My Computer** > right-click **Manage**. **2.** Select **Services** from the left side of the screen. **3.** Locate **HASP License Manager for Kaluza.** **4.** Right-click on the **HASP service**. **5.** Select **Properties**. **6.** Ensure that the **Startup Type** is set to **Automatic**. **7.** Ensure that the message **Service is started** is displayed. **8.** Attempt to launch Kaluza for Gallios. **9.** If the problem persists, contact your Beckman Coulter Representative.

Issue: Unable to start hardlock service displays on the HASP SRM Run-time installation window.	
Comment:	**Recommended Action:**
This is known to occur occasionally at the end of the installation. It occurs because the HASP service could not get started correctly.	Reboot the computer and attempt to run the application one more time.

Issue: Unable to start hasplms service displays on the HASP SRM Run-time installation window.	
Comment:	**Recommended Action:**
This is likely a bad interaction with McAfee antivirus software.	**1.** Reboot the computer. **2.** Rerun the Kaluza for Gallios installer. If the error message still persists: **1.** Click **START** > **My Computer** > right-click **Manage**. **2.** Select **Services** from the left side of the screen. **3.** Locate any McAfee services. **4.** Attempt to right-click and halt the McAfee services. **5.** If problem persists, contact your local IT department to verify McAfee services are halted. **NOTE** HASP contains a known interaction McAfee Host Intrusion Protection Service.

Issue: Using the Repair option from Add/Remove programs on a 64-bit version of Windows 7 leads to an uninstallable Sentinel HASP License Manager service.	
Comment:	**Recommended Action:**
If the user repairs the Kaluza for Gallios installation on a 64-bit version of Windows 7 and later uninstalls Kaluza for Gallios, Sentinal HASP License Manager service remains installed.	If software repair is needed, uninstall/install Kaluza for Gallios by doing the following: **1.** Find Kaluza for Gallios in the **Add**/**Remove** programs. **2.** Uninstall Kaluza for Gallios. **3.** Reboot the computer. **4.** Reinstall Kaluza for Gallios using the installation disk.

<table>
<tr><th colspan="2">Issue: Upon launching the software, Kaluza for Gallios displays an H0007 error message.</th></tr>
<tr><th>Comment:</th><th>Recommended Action:</th></tr>
<tr><td>Error displays: “Kaluza requires a hardware key. Please insert the USB hardware key. (H0007)”
This occurs because Kaluza for Gallios cannot locate or a physical hardware key to connect to.</td><td>Ensure that the computer was rebooted after Kaluza for Gallios was installed. This will ensure that HASP services have been started. Instructions for starting HASP services can be found in steps 1-7 in “Issue: Unable to access HASP SRM Run-Time Environment (H0033) displays on the HASP Protection System for the Kaluza for Gallios window.” above.
If the error message still persists:
1. Find Kaluza for Gallios in the Add/Remove programs.
2. Select the Repair option to ensure all necessary files are in place.
3. Reboot the computer.
4. Attempt to launch Kaluza for Gallios.
If the error message still persists:
1. Check the file path C:\Program Files\Common Files\Aladdin Shared\Hasp or C:\Program Files (x86)\Common Files\Aladdin Shared\HASP to ensure it does not contain any non-Latin characters. (This sometimes happens on non-English Windows installations.)
2. If problem persists, contact your Beckman Coulter Technical Support Desk.
Users with the Danish version of Windows XP installation may experience this difficulty. HASP is aware of this difficulty, and currently there is no workaround the situation. Users must purchase the license with a hardware key for the software to work fine. This issue does not occur on Danish Vista or Danish Windows 7 operating systems.</td></tr>
</table>

<table>
<tr><th colspan="2">Issue: AVG flags HASP as a virus. Using the AVG antivirus software version 9.0.818 or prior version.</th></tr>
<tr><th>Comment:</th><th>Recommended Action:</th></tr>
<tr><td>This is a known interaction between AVG and HASP. Currently .AVG flags the file haspvlib_87749.dll as a virus. This .dll file is specific the Kaluza for Gallios. AVG has been contacted about this issue, and they have now updated their product so this file will no longer be flagged as a virus.</td><td>Upgrade the AVG software to version 9.0.819 or later.</td></tr>
</table>

<table>
<tr><th colspan="2">Issue: You currently have a HASP license key but are unable to access Kaluza for Gallios due to a license expiration error. Error code 11009.</th></tr>
<tr><th>Comment:</th><th>Recommended Action:</th></tr>
<tr><td>Your computer is having trouble recognizing the hardware (USB) license key.</td><td>Unplug and then plug the hardware key back in. Ensure that the red light on the hardware key is turned on.</td></tr>
</table>

Issue: Error on startup. The Kaluza for Gallios application used to operate properly, but now will not load.	
Comment:	**Recommended Action:**
This issue could be due to the license manager service no longer running.	**1.** Verify that the license manager service that Kaluza for Gallios uses is properly running. **a.** Navigate to http://localhost:1947. **b.** If the website displays with content, then the service is running. If the error still persists: **1.** Select **Run** from the Windows **Start** menu. **2.** Enter **services.msc** in the **Open** field. **3.** Locate **Sentinel HASP License Manager Service**. and right-click. **4.** Start or restart **Sentinel HASP**.

Issue: "An error occurred while trying to load persisted settings for the Kaluza application. Kaluza settings, including any user configured options, will be restored to initial values." Error code: 99991.	
Comment:	**Recommended Action:**
The user's configuration is saved in a file titled **user.config** when Kaluza for Gallios closes. In this case, something was saved in the **user.config** file that Kaluza for Gallios is unable to load.	Delete the **user.config** file from the location specified in *User Options File*. **1.** Close and re-launch the Kaluza for Gallios. **2.** If the error persists after re-launching, zip the entire log directory as specified in *Kaluza for Gallios Log Files*. **3.** Report the issue to your local Beckman Coulter representative. Files can be uploaded for troubleshooting. To access the troubleshooting page, go to *www.kaluzasoftware.com*, and select the **FAQ** tab. Fill out the short form, describing the problem. Select **Submit** to access the upload page. **NOTE** The **user.config** file stores the pane positions, collapsed pane states and the recently used list. Kaluza for Gallios automatically recreates this file when the software is re-launched.

Cytometer Messages

See Table 15.2 for a list of Cytometer messages.

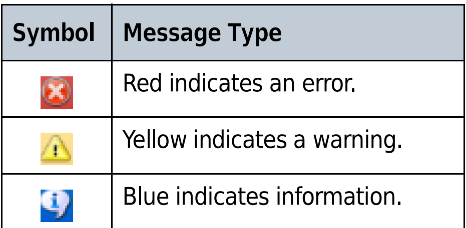
Display Locations

Cytometer messages appear below the Kaluza for Gallios ribbon and in the Cytometer log file.

Kaluza for Gallios Screen

Cytometer messages appear below the ribbon, as shown in red in Figure 15.8. If there are multiple messages, "*Click to show additional messages*" appears, allowing you to view multiple messages simultaneously. Messages appear in three different colors (Table 15.1); however, when multiple messages need to be addressed, all messages appear in the color of the message of the greatest severity, and the symbol indicates the message type (Figure 15.9). Refer to Table 15.2, to view a complete list of the cytometer messages, along with the recommended action for correcting the issue. Most messages can be cleared once they acknowledged by selecting the Dismiss button, but some require you to take the action specified in the message before it is cleared.

Table 15.1 Messages



Symbol	Message Type
(red error icon)	Red indicates an error.
(yellow warning icon)	Yellow indicates a warning.
(blue information icon)	Blue indicates information.

Figure 15.8 Error Message Example

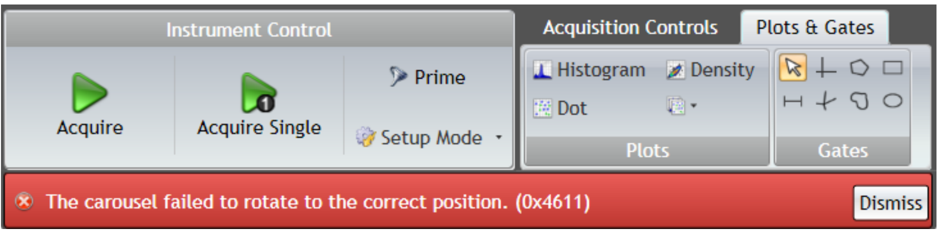
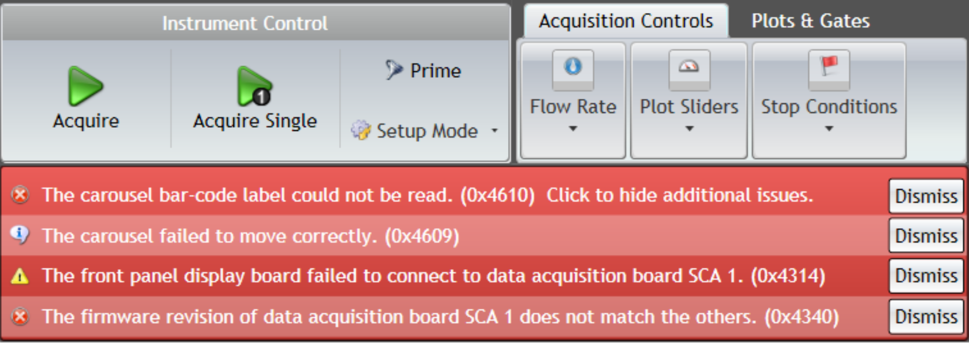


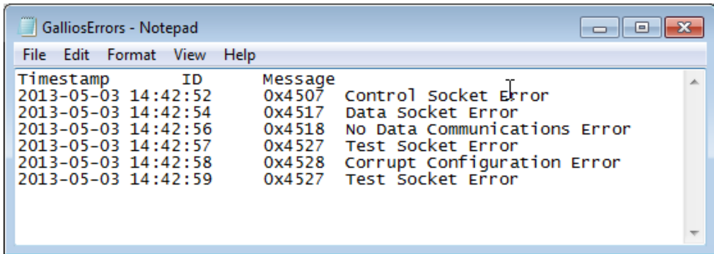
Figure 15.9 Multiple Messages



Cytometer Log

All error messages appear in the Cytometer Log. Refer to *Cytometer Log File* for details. See Figure 15.10.

Figure 15.10 Example of Error Messages in Cytometer Log



Timestamp	ID	Message
2013-05-03 14:42:52	0x4507	Control Socket Error
2013-05-03 14:42:54	0x4517	Data Socket Error
2013-05-03 14:42:56	0x4518	No Data Communications Error
2013-05-03 14:42:57	0x4527	Test Socket Error
2013-05-03 14:42:58	0x4528	Corrupt Configuration Error
2013-05-03 14:42:59	0x4527	Test Socket Error

Cytometer Log File

The Cytometer messages are located in the Cytometer Log file.

NOTE The log contains all errors and is never cleared, unless the Cytometer Log file is deleted.

How to Access the Cytometer Log File

From Kaluza for Gallios

- Select > **Cytometer log**. The **GalliosErrors** text file appears (Figure 15.10).

IMPORTANT If you are experiencing problems when opening the Cytometer Log file, it might have become very large, as this log is never cleared. Close Kaluza for Gallios, and move or delete the file so that a new, and much smaller, file is created. The location of this file is provided in *From Windows Desktop*.

From Windows Desktop

IMPORTANT Hidden folders must be visible prior to completing this procedure. To make hidden folders visible, from the Windows **Start** menu, select **Control Panel** > **Folder Options**. Then, select the **View** tab. Under **Advanced settings**, click **Show hidden files, folders, and drives**, and then click **OK**.

1. Right click on the Windows **Start** button > **Open Windows Explorer** > **Local Disk (C:)** > **ProgramData** > **Beckman Coulter** > **Kaluza for Gallios**.

2. Double click on folder corresponding to the currently installed version.

3. Double click on **GalliosErrors** to open. See Figure 15.10.

Cytometer Log Entry Descriptions

All cytometer log message entries are posted in chronological order. Each message entry consists of three columns (see Figure 15.10):

- **Timestamp**—Time and date the message occurred.
- **ID**—Four-digit number, which is displayed with the error (see Table 15.2 for a complete list). In addition, this number assists your Beckman Coulter Representative in troubleshooting efforts.
- **Message**—Text describing the error. See Table 15.2 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. In Microsoft Notepad, select **Edit > Find.**
2. Type in the word or phrase you want to find (Example: ***Waste***).
3. Select the direction of the search: Up or Down.
4. Select **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: Select **File > Print**.

Cytometer Messages Table

Table 15.2 lists the messages by ID codes, 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 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x3002	The 10 L sheath cube is empty.	Replace the external sheath fluid container. See CHAPTER 14, *Replace the 10 L External Sheath Fluid Container*.
0x3005	The waste container is full.	Empty the waste container. See CHAPTER 14, *Empty the 20L Waste Container*.
		Contact your local Beckman Coulter Representative.
0x3008	The instrument is low on cleaning agent.	Fill cleaning agent container. Refer to CHAPTER 14, *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.
0x3011	Data is being lost because the discriminated data rate is too high.	**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, CHAPTER 14, *Replace the Sheath Fluid Filter*.
0x3017	The MCL cover is open.	Close the MCL cover.
0x3019	The sample tube pressure is low.	If warning persists, see Code 0x4015.
0x3102	The waste chamber is full.	**1.** Empty the 20 L waste container. See CHAPTER 14, *Empty the 20L 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.
0x3105	The system pressure is out of range.	If warning persists, see 0x4106.
0x3108	The sheath fluid pressure is out of range.	If warning persists, see 0x4109.
0x3111	The sample pressure is out of range.	If warning persists, see 0x4015.
0x3122	The system vacuum is out of range.	If error persists, see 0x4123.
0x3125	The ambient temperature is out of range.	Reduce or wait until ambient temperature is within specification.
0x3130	The internal sheath tank is low.	If error persists, contact your local Beckman Coulter Representative.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x3134	The drip chamber is almost full.	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 until 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 0x4135.
0x3142	The ambient temperature sensor may have failed.	If warning persists, contact your local Beckman Coulter Representative.
0x3144	The pressures are not calibrated.	Pressures cannot be calibrated because pressure control board cannot be detected or calibration coefficients are corrupted. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x3202	The 488nm laser is initializing.	Wait until the laser initializes.
0x3205	The 488nm laser power is out of range.	If laser does not stabilize within a reasonable time or frequently goes unstable, restart the entire system. If problem persists, contact your local Beckman Coulter Representative.
0x3208	The 488nm laser has a fault.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative
0x3212	The 638nm laser is initializing.	Wait until the laser initializes.
0x3215	The 638nm laser power is out of range.	If laser does not stabilize within a reasonable time or frequently goes unstable, restart the entire system. If the problem persists, contact your local Beckman Coulter Representative.
0x3218	The 638nm laser has a fault.	Check optics covers are properly installed and tightened. See 0x4219.
0x321a	The 638nm laser failed to communicate.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
0x3222	The 405nm laser is initializing.	Wait until the laser initializes.
0x3225	The 405nm laser power is out of range.	If laser does not stabilize within a reasonable time or frequently goes unstable, restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x3228	The 405nm laser has a fault.	Check optics covers are properly installed and tightened. See 0x4229.
0x322a	The 405nm laser failed to communicate.	Restart the entire system. If warning persists, contact your local Beckman Coulter Representative.
0x3231	The optics temperature differs considerably from the alignment temperature.	Wait until the temperature of the optics is closer to that when the instrument was aligned or realign the optics for different ambient temperature range. If the problem persists, contact your local Beckman Coulter Representative.
0x3233	The optics temperature is stabilizing.	If the optics temperature does not stabilize within a reasonable time, restart the entire system. If the problem persists then contact your local Beckman Coulter Representative.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x3421	The instrument is not calibrated to trigger the 405nm laser.	Calibrate the 405nm laser.
0x3422	The instrument is not calibrated to trigger the 488nm laser.	Calibrate the 488nm laser.
0x3423	The instrument is not calibrated to trigger the 638nm laser.	Calibrate the 638nm laser.
0x3612	The carousel failed to move correctly.	If warning persists, see 0x4609.
0x3613	The probe failed to move correctly.	None. The instrument will automatically retry to raise or lower the probe. If warning persists, contact your local Beckman Coulter Representative.
0x3616	The loader failed to move the tube correctly.	If warning persists, see 0x4605.
0x4003	The 10 L sheath cube is empty.	Replace the external sheath fluid container. See CHAPTER 14, *Replace the 10 L External Sheath Fluid Container*.
0x4006	The waste container is full.	Empty the waste container. See CHAPTER 14, *Empty the 20L Waste Container*.
0x4009	The instrument is out of cleaning agent.	The requested cleanse procedure could not be performed due to the low level of cleaning agent. Fill cleaning agent container. Refer to CHAPTER 14, *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.
0x4014	No sample tube was found in location X.	Verify the intended sample is placed in the location identified in the carousel and select **Continue**.
0x4015	The sample tube pressure is low.	Inspect sample tube and sample head for damage. Change as required. CHAPTER 14, *Replace the MCL Sample Head*.
0x4103	The waste chamber is full.	**1.** Empty the 20 L waste container. See CHAPTER 14, *Empty the 20L 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.
0x4106	The system pressure went out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative
0x4109	The sheath fluid pressure went out of range.	**1.** Check sheath fluid container cap for tightness. **2.** If problem continues, CHAPTER 14, *Replace the Sheath Fluid Filter*. **3.** If problems continues, contact your local Beckman Coulter Representative.
0x4112	The sample pressure went out of range.	Inspect sample tube and sample head for damage. Change as required. CHAPTER 14, *Replace the MCL Sample Head*.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x4115	The waste back pressure went out of range. Check the waste vent filter and waste container.	**1.** Empty the 20 L waste container. See CHAPTER 14, *Empty the 20L 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.
0x4123	The system vacuum went out of range. Check for liquid in the trap.	Liquid in the vacuum trap. Check that the vacuum trap (on the front of the Supply Cart) is tight and is less than 1/4 full of fluid. If it is more full, empty it (see CHAPTER 13, *Clean the Vacuum Trap*).
		Vacuum line is not connected between the Supply Cart and the Cytometer. Connect the vacuum line at the back of the instrument.
		Hardware problem. Contact your local Beckman Coulter Representative.
0x4131	The internal sheath tank is empty.	If error persists, contact your local Beckman Coulter Representative.
0x4135	The drip chamber filled.	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.
0x4138	The drip or waste chamber overflowed.	Contact your local Beckman Coulter Representative.
0x4140	The system pressure went out of range. Check the pressure line from the supply cart to the cytometer.	The pressurized air supply could be 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.
		There could be a short circuit. Check fuses.
		The pressure line might not be connected between the Supply Cart and the Cytometer. Connect the pressure line.
0x4143	The ambient temperature sensor failed.	If error persists, contact your local Beckman Coulter Representative.
0x4200	The 488nm laser type does not match the configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4203	The 488nm laser failed to initialize.	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.
0x4206	The 488nm laser power went out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4209	The 488nm laser had a fault.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x420a	The 488nm laser failed to communicate.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4210	The 638nm laser type does not match the configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4213	The 638nm laser failed to initialize.	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.
0x4216	The 638nm laser power went out of range.	If the error persists, contact your local Beckman Coulter Representative.
0x4219	A fault occurred on the 638nm laser during acquisition and the acquisition was stopped.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
0x4220	The 405nm laser type does not match the configuration.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
0x4223	The 405nm laser failed to initialize.	If the laser does not initialize within a reasonable time, restart the entire system. If the problem persists, contact your local Beckman Coulter Representative.
0x4226	The 405nm laser power went out of range.	If error persists, contact your local Beckman Coulter Representative.
0x4229	The 405nm laser had a fault.	Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
0x4301	The CAN master board was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4302	The fluidics I/O board was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4303	The optics I/O board was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4304	The sampler I/O board was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4306	The voltages on the fluidics I/O board went out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4307	The voltages on the optics I/O board went out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4308	The voltages on the sampler I/O board went out of range.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4310	The data acquisition board SCA 1 was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4311	The data acquisition board SCA 2 was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4312	The data acquisition board SCA 3 was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x4313	The pico motor control board was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4314	The front panel display board failed to connect to data acquisition board SCA 1.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4315	The front panel display board failed to connect to data acquisition board SCA 2.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4316	The front panel display board failed to connect to data acquisition board SCA 3.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4317	The optics temperature control board was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4321 (Detector 1) 0x4322 (Detector 2) 0x4323 (Detector 3) 0x4324 (Detector 4) 0x4325 (Detector 5) 0x4326 (Detector 6) 0x4327 (Detector 7) 0x4328 (Detector 8) 0x4329 (Detector 9) 0x432a (Detector 10) 0x432b (Detector 11) 0x432c (Detector 12)	Detector X was not found.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x4331 (Detector 1) 0x4332 (Detector 2) 0x4333 (Detector 3) 0x4334 (Detector 4) 0x4335 (Detector 5) 0x4336 (Detector 6) 0x4337 (Detector 7) 0x4338 (Detector 8) 0x4339 (Detector 9) 0x433a (Detector 10) 0x433b (Detector 11) 0x433c (Detector 12)	The type of detector X does not match the configuration.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4340	The firmware revision of data acquisition board SCA 1 does not match the others.	Contact your local Beckman Coulter Representative.
0x4341	The firmware revision of data acquisition board SCA 2 does not match the others.	Contact your local Beckman Coulter Representative.
0x4342	The firmware revision of data acquisition board SCA 3 does not match the others.	Contact your local Beckman Coulter Representative.
0x4343	Each acquisition board has a different firmware revision.	Contact your local Beckman Coulter Representative.
0x4344	The acquisition windows do not match.	Contact your local Beckman Coulter Representative.
0x4507	A control socket error occurred.	Check the Ethernet cable between the workstation and the cytometer. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4517	A data socket error occurred.	Check the Ethernet cable between the workstation and the instrument. Restart the entire system. If the error persists, contact your local Beckman Coulter Representative.
0x4518	A data communication error occurred.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4527	A test socket error occurred.	Check the Ethernet cable between the workstation and the cytometer. Restart the entire system. If error persists, contact your local Beckman Coulter Representative.

Table 15.2 Cytometer Messages

ID Code	Message	Recommended Action
0x4528	The instrument configuration is corrupt.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4604	The probe failed to move correctly.	Restart the entire system. If error persists, contact your local Beckman Coulter Representative.
0x4605	The MCL tube lifter failed to move the tube correctly.	**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
0x4609	The carousel failed to move correctly.	**1.** Check that there is no obvious obstruction (sample tube) in the MCL area. **2.** Check that the 30 psi gauge on the Supply Cart is okay. **3.** If no obstruction is found and pressure okay, contact your local Beckman Coulter Representative.
0x4610	The carousel barcode label could not be read.	**1.** Check that the barcode label is not torn or written on. **2.** Try using another carousel. **3.** If problem continues, contact your local Beckman Coulter Representative.
0x4611	The carousel failed to rotate to the correct position.	**1.** Check that a carousel is in place in the MCL. **2.** Check that there is no obvious obstruction (sample tube) in the MCL area. **3.** Check that the 30 psi gauge on the Pneumatic Supply is okay. **4.** Try using another carousel. **5.** If no obstruction is found and pressure okay, contact your local Beckman Coulter Representative.
0x6305	The instrument failed to turn off.	CHAPTER 13, *Power the Cytometer Only On/Off*. If error persists, contact your local Beckman Coulter Representative.

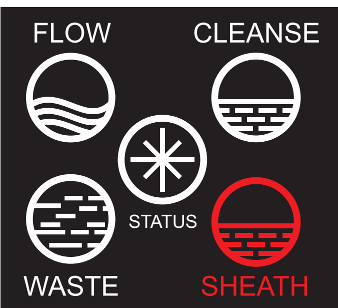
Level Sense Indicators

Sheath Low

When the **Sheath Low** indicator (see Figure 15.11) 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 15.11 Sheath Low Indicators

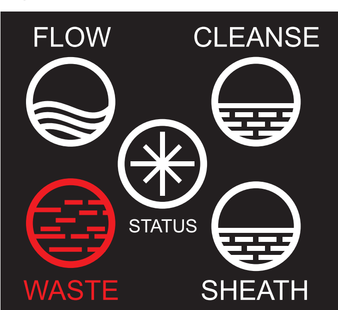


Waste Full

When the **Waste Full** indicator (see Figure 15.12) 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 20L Waste Container.

Figure 15.12 Waste Full Indicators



APPENDIX A

Barcode Specifications

Barcode Sample Identification

Barcode symbols are a highly accurate and efficient procedure for identifying and processing laboratory samples. Beckman Coulter instruments use four barcode symbologies (types) to identify specimens:

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

The barcode reader senses the difference between enabled barcode symbologies in a run.

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 barcode specifications is important to achieve highly accurate reading. Follow the barcode specifications to avoid inaccurate reading of the barcode label.

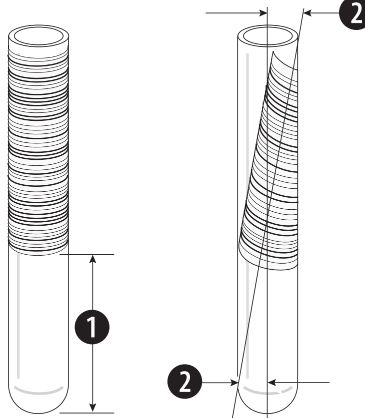
Figure A.1 Barcode Label

1. Quiet zone
2. Barcode symbol
3. Sample ID

Correct Placement of the Barcode Label

The barcode 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 Barcode 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 barcode 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 barcode 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 barcode label can be read.

Barcode Label Specifications

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 barcode specifications is important to achieve highly accurate reading. Follow the barcode label specifications to keep the rate of misread labels to a minimum.

The quality of the barcode 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 barcode information if the barcode symbol cannot be read.

When a barcode is read on a tube it is put in the @BARCODE keyword of the FCS file. There is no barcode field in Kaluza for Gallios to pre-enter barcodes for automatic positive identification; however, you can scan the tube barcode into the **Sample ID 1** or **Sample ID 2** field in

Kaluza for Gallios and manually verify the @BARCODE FCS keyword matches the Sample ID field used for the manually-scanned barcode.

Label Size and Thickness

The length of the label must be less than 44.45 mm (1.75 in.). The label includes the barcode 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 Barcode Label Specifications

1. Quiet zones 3.5 mm (0.14 in.) minimum
2. Barcode symbol height 19.05 mm (0.75 in.) minimum
3. Barcode label length 44.45 mm (1.75 in.) maximum

The width of the barcode 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 barcode symbol must be 19.05 mm (0.75 in.) minimum.

See Table A.2.

Label and Print Quality

All barcode symbols must agree with the American Identification Manufacturer's (AIM) Uniform Symbology Specification.[11]

All barcode symbols must be printed at print quality class "B" or better as defined by the American National Standards Institute (ANSI).[22] Several factors affect print quality:

- Labels must be clean, not yellowed, and used before the expiration date.
- Print the barcode 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 barcode symbol must be well-defined. Edges must be constant (not irregular), so the bars and spaces have the correct widths for the barcode symbology used.

Barcode Error Rate

A misread label can cause one sample ID to be read as another sample ID. Whenever possible use a barcode symbology and configuration choices that provide the most accurate barcode reading.

The quality of the barcode 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 barcode 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 barcode 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.[33]
- 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.**

Barcode Symbologies

Beckman Coulter instruments use four barcode symbologies for specimen identification, see Table A.1.Within the given specifications, the MCL reader and the optional handheld barcode reader automatically distinguish the following barcodes:

Table A.1 Barcode Symbologies

Barcode 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 barcode • 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 / Minimum 3 alphanumeric characters • Code 128C - Maximum 16 numeric characters / Minimum 3 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 barcode • Maximum 10 characters (9 data characters + 1 checksum character).

Barcode Labels

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

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

Sample misidentification can occur from the use of incorrect, poor quality, damaged, dirty or improperly placed barcode labels. Follow the specifications in this section to create your barcode labels to prevent incorrect sample identification. See also CHAPTER 5, *Barcode Labels*.

The instrument supports preprinted labels.

Barcode 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

MCL Barcode 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.

Barcode 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.

To prevent incorrect identification of sample tubes, do not use FNC1, FNC4, and FS (hexadecimal 1C) characters in your barcode information.

Checksum Algorithm

Beckman Coulter strongly recommends the use of barcode checksums to provide automatic checks for read accuracy.

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.

APPENDIX B

Statistics

Overview

This chapter contains information about the statistics that Kaluza for Gallios supports:

Component	Definition
1. Number	The number of events in the Input Gate.
2. % Total	Ratio of the number of events in the input gate to the total events in the protocol. $\%Total = 100 * \frac{Count}{Total\ number\ of\ events\ in\ protocol}$
3. % Gated	Ratio of the number of events in the input gate to the number of events in the parent gate. $\%Gated = 100 * \frac{Count}{Parent\ Gate\ Count}$

Component	Definition
4. Median	Median of the values of the events in the input gate. Kaluza for Gallios computes the frequency histogram of the events to generate this statistic. The frequency histogram has 1024 bins. Using the frequency histogram avoids the time consuming sort operation that is typically used to find the median. $$b = i \left\| \sum_{i=1}^{j} C_i > \frac{n}{2} \right.$$ $[j \leq n, C_i = Count\ in\ bin\ i, n = Total\ number\ of\ events\ in\ input\ gate]$ The median *m* can then be computed using the following steps. 1. $A = \frac{\sum_{i=1}^{b-1} x_i}{n} * 100$ 2. $B = 50 - A$ 3. $D = \frac{k_b}{n} * 100$ 4. $E = \frac{B}{D}$ 5. $m = b + E$ In step 3, k_b is the count in bin *b*. **NOTE** If the parameter that the statistics are computed on are displayed using the log or logicle transformation, the frequency histogram computed for the mode, HPCV and median statistics are computed in that transformation space. The following computations need to be performed for the result to be reported in linear transformation space. If the display parameter is log: $mode_{linear} = LinearTransform(LogarithmicTransform^{-1}(mode))$ $HPCV_{linear} = LinearTransform(LogarithmicTransform^{-1}(HPCV))$ $m_{linear} = LinearTransform(LogarithmicTransform^{-1}(m))$ If the display parameter is logicle: $mode_{linear} = LinearTransform(LogicleTransform^{-1}(mode))$ $HPCV_{linear} = LinearTransform(LogicleTransform^{-1}(HPCV))$ $m_{linear} = LinearTransform(LogicleTransform^{-1}(m))$

Component	Definition
	The various transformations used by Kaluza for Gallios are based on the following definitions of the transformations: $LinearTransform(x) = a * x + b$ $LogarithmicTransform(x) = \log_{10}\left(\max(a * x, 1)\right) * b$ $LogicleTransform^{-1}(x) = BiExponentialTransform(x) = ae^{bx} - ce^{-dx} + f$ The coefficients ***a, b, c, d*** and ***f*** in these transformations are computed dynamically based on factors like the range of the data, number of decades, width of the negative zone (in case of the logicle) etc. Some of these coefficients change as the user interacts with Kaluza for Gallios.
5. Arithmetic Mean	The Arithmetic Mean of the values of the events in the input gate. The arithmetic mean of a sequence of numbers {a1, a2, ...ai... an} is defined by: $\mu = \frac{1}{n}\sum_{i=1}^{n} a_i$
6. Mode	The channel number with largest population among the events in the input gate. For multimodal data, the mode with the smallest value is returned. Kaluza for Gallios computes the frequency histogram of the events to generate this statistic. The frequency histogram has 1024 bins. If every value occurs only once, the Mode is set to NaN. E.g.: If there were only one (1) event or if there were two (2) events each in different channels.
7. Standard Deviation	The standard deviation of the values of the events in the input gate. The standard deviation of the sample with a sequence of numbers {a1, a2, ...ai... an} and arithmetic mean µ, is the square root of the sample variance and is defined by: $S = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(a_i - \mu)^2}$
8. CV (Coefficient of Variation)	The coefficient of variation of the values of the events in the input gate. $CV = \frac{S}{\mu}$
9. HPCV (Half Peak of Coefficient of Variation)	Kaluza for Gallios computes the frequency histogram of the events to generate this statistic. The frequency histogram has 1024 bins. Where FWHM = Full Width Half Max value of a Normal or Gaussian peak. $HPCV = \frac{1}{2.36} * \frac{FWHM}{Mode} * 100$ Refer to *Practical Flow Cytometry*, by Howard M. Shapiro, Fourth Edition 2003, Wiley-Liss, Inc., page 235 for the definition.

Component	Definition
10. Minimum	The minimum value among all events in the input gate. $x_{min} = \min_{1 \le i \le n} \{x_i\}$ $[n = Total\ number\ of\ events\ in\ input\ gate]$
11. Maximum	The maximum value among all events in the input gate. $x_{max} = \max_{1 \le i \le n} \{x_i\}$ $[n = Total\ number\ of\ events\ in\ input\ gate]$
12. Geometric Mean	The mean of the values of the events in the input gate. The geometric mean of a sequence of numbers {a1, a2, ...ai... an} is defined by: $G = \left(\prod_{i=1}^{n} a_i\right)^{1/n}$

NOTE Under certain conditions, **N/A** will display in a given statistics field. For example, **0%** will display if there are events on your plot, but none are encompassed in the particular gate. However, if there are no events in the input gate to the plot, then any gates on that plot will display **N/A**. This is because in the first case the % gated is 0/N, which equals **0**; whereas in the second case it is 0/0, which is a divide by zero error and will display **N/A**.

References

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2. American National Standards Institute (ANSI) *Barcode Print Quality Guidelines*. X3. 182-1990 (R2000). http://www.ansi.org
3. Clinical and Laboratory Standards Institute (CLSI), *Laboratory Automation: Barcodes for* Specimen Container Identification; Approved Standard. AUTO2-A. http://www.clsi.org

Glossary

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.

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

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.

Autofluorescence Vector — The value that is subtracted from the data prior to spillover compensation and then added back afterwards. This increases the accuracy of fluorescence compensation.

Autofluorescence* — Fluorescence associated with a cell, usually caused by components and chemicals within the cell structure itself.

Barcode symbol — A group of parallel bars and spaces with encoded characters. A barcode symbol generally contains a leading quiet zone, a start character, data characters, a check character, a stop character, and a trailing quiet zone.

Bar — A strip (or element) that is usually black and has minimum reflectance.

Bivariate Plot — A plot containing two variables, one is displayed on the X-axis and the other on the Y-axis.

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

Button — The Workstation screens have pictures/icons 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 gate counts obtained.

Channel — In an analog-to-digital converter, the number of equally spaced divisions of the amplified input signal voltage.

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 barcode 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 Precedence — The hierarchy of colors associated with gated events or when an event falls within several different gates, then the color precedence determines the display color.

Color — 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.

Compensation — The mathematical process by which multi-parameter flow cytometric data is corrected for spectral overlap.

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.

CV — Coefficient of Variation

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

Data Set — Raw data derived from events within a sample, as captured by the flow cytometer.

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.

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.

Discrete code — Each character in the barcode symbol starts with a black bar and ends with a black bar. A white space gap (intercharacter gap) is between each character in the barcode symbol.

Continuous code — Each character in the barcode symbol starts with a black bar and ends with a white space. Characters follow after each other to form a continuous flow of code.

Element — A bar or space in a barcode symbol. There are narrow elements and wide elements.

Event — A particle passing through the laser beam.

Event — An individual particle, detected by a flow cytometer, from which raw data is derived.

FCS* — Flow Cytometry Data File Standard. A set of standards developed for reading and writing flow cytometry data files in a standardized format.

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.

FCS (Flow Cytometry Standard)* **—** (FCS 2.0 or 3.0) File format – format used to save flow cytometry data. Includes identifying information about the sample in the “header” and measurement information for each cell or event analyzed. The header is in text format while the measurement information is in binary format.

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.

Fluorescence* **—** Excitation light energy is absorbed by fluorescent molecule, the molecule transitions to an excited state and as it returns to unexcited ground-state, a specific wavelength of light is emitted.

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.

Fluorochrome* **—** Fluorescent substance used in biological staining to produce fluorescence in a specimen.

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

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.

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 log amplification.

Gate — A subset of events, defined by a boundary, that allows for further examination.

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.

Intercharacter gap — The space between two characters in a barcode symbol. Refer to discrete code. Not in all barcode types.

Laser — Abbreviation for light amplification by stimulated emission of radiation. Three standard lasers are in the instrument: one in the MCL for reading barcodes and two in the flow cell for analyzing cells.

Linear amplification — See gain.

Linear Scale — A scale that contains divisions that are uniformly spaced. The linear scale is good for showing forward scatter and side scatter parameters.

Listmode data — A list of measurements from each cell.

Log 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.

Log Scale — A scale that contains divisions based on exponential values. Log scales are useful when the data includes a large range of values. Fluorescence parameters are best displayed in the log scale because both weak and strong signals are appropriately accounted for.

Logicle Scale — A scale that allows for correctly displaying compensated data. When using the logicle scale, negative space can be displayed on one or both axes. This optimizes your ability to compensate fluorescence parameters, giving events a Gaussian appearance.

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.

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.

Offline Computer Workstation — A computer workstation (with specified minimum hardware specifications) that is not connected to a cytometer and which can run the software for offline analysis.

Opacity — The degree to which light can/cannot penetrate an object. The greater the opacity, the more defined the object appears.

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.

Parameter — Types of data collected from the flow cytometer detectors, including such measurements as light scatter and fluorescence, area, height, and width.

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.

Plot — Used as a data analysis tool, a plot is a graphical representation of the raw data collected from the flow cytometric sample. Plots are customized based on the parameters chosen to represent the data.

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

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.

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.

Protocol — The plots, parameters, and gates used for data analysis.

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

Quiet zone — An area at each end of the barcode symbol, which must be clear of marks, including readable text.

RAP Box — Connection point for the RMS connector.

RAP — Remote Access Point. Allows for Technical Support to reach the system.

RD1 — Abbreviation for phycoerythrin dye.

Reagent — A system consumable such as a diluent or cleaner, and applications-related consumables such as lysing reagents or antibodies.

Ribbon — The section above the sheet area of the application screen for which you may perform a number of tasks from within the Acquisition Controls and Plots & Gates tabs.

RMS — Remote Diagnostic Maintenance Software. After granting permission, this software allows Technical Support to remote into the desktop.

ROHS — European Union Directive to implement restrictions on the use of certain hazardous substances.

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

Self-checking — A barcode that uses a checking algorithm to make sure the barcode 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 (SS) sensor — Collects the side scatter and generates voltage pulse signals.

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.

Space — A strip (or element) of a barcode that is usually white and has maximum reflectance.

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

Spillover — The amount of light emitted from a fluorochrome that is detected by unintended detectors.

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.

Standardization — The use of a control material to establish the appropriate cytometer hardware settings to consistently run an application eliminating hardware variability; can be beads or unstained control sample.

Symbology — A set of rules for encoding and decoding information contained in a barcode symbol. Examples of symbologies include Code 39, Code 128, Interleaved 2-of-5, and Codabar.

Start and Stop characters — The characters that start and end the barcode symbol and show the scan direction.

Verification — The act of verifying that the statistics obtained after optimizing application cytometer settings match known values.

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.

* Flow Cytometry Glossary http:/www.flocyte.com. October 14, 2013
http://www.flocyte.com/glossary

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Related Documents

Your Kaluza documentation can be found on our website at www.beckmancoulter.com.

Kaluza Analysis Software Instructions for Use
P/N A75667

- Chapter 1, *Introduction to Kaluza Analysis Software*
- Chapter 2, *Data Analysis*
- Chapter 3, *Sheet Set-Up*
- Appendix A, *Statistics*
- Appendix B, *Quick Reference Sheet*
- Appendix C, *Frequently Asked Questions*
- Appendix D, *Troubleshooting*

561 nm Laser Option Addendum
P/N B00207

Gallios Flow Cytometer Supply Cart Addendum
P/N A85285

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