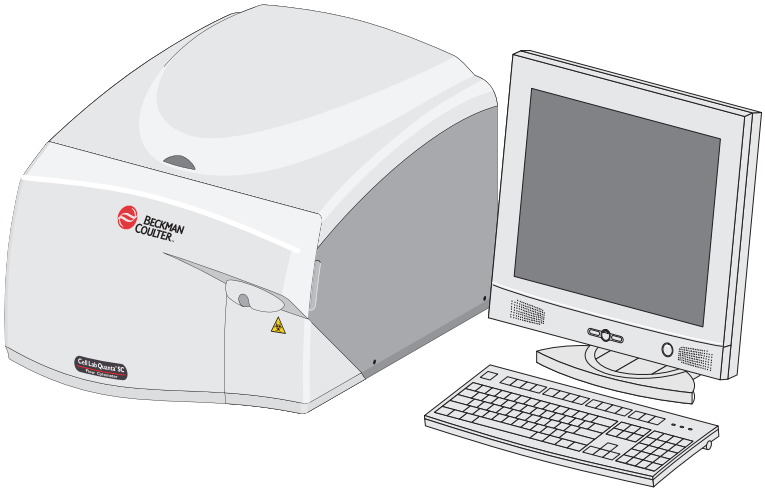
Cell Lab Quanta SC

Instructions for Use



PN 721742AD (October 2011)

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

BECKMAN COULTER

WARNINGS AND PRECAUTIONS

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

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Can cause injury.
CAUTION - Can cause damage to the instrument.
IMPORTANT - Can cause misleading results.

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

WARNING Risk of operator injury if:

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

To avoid injury:

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

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

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

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

Initial Issue A, 10/05
Software Version 1.0

Issue AA, 11/08
Software Version 1.0
Revision AA contains updates or new information in the following chapters:

- Chapter 1:
  - Updates to the laser warning labels instructions, and graphic depicting location of all of these labels. See Heading 1.6, WARNING LABELS AND PRECAUTIONS section.
  - Updates to Table 1.1, List of Approved Reagents, under Heading 1.8, REAGENTS.
  - Indicates the BCI website to access reagent information and search by Reagent Name or Part Number, under Heading 1.8, REAGENTS.
  - Indicates the BCI website to access Cell Lab Quanta System Application Notes, under Heading 1.8, REAGENTS.
  - Added instructions for three new warning labels:
    - Heading 1.6, WARNING LABELS AND PRECAUTIONS
- Chapter 4:
  - Updates to Table 4.7, Cups Approved for Use , under Heading 4.2, CUPS

Issue AB, 07/09
Software Version 1.0
Revision AB contains an update to the new corporate address.

Issue AC, 03/10
Software Version 1.0
Revision AC contains updates or new information in the following chapters:

- Cover page:
  - Replaced the manufacturer's symbol to show the Brea, California address.
- Chapter 1:
  - Replaced the label on the rear of the instrument to show the Brea, CA address.
- Chapter 4:
  - Updated Table 4.1, Absolute Count Performance Specifications under Heading 4.1, PERFORMANCE SPECIFICATIONS.
- Chapter 7:
  - Added an important notice under the Playing Back Files section.
  - Updated the following sections: Create/Delete Protocol Groups, Assign a User to a Protocol Group and Remove a User Assigned to a Protocol Group.

Issue AD, 10/11
Software Version 1.0.

Changes were made to pages 2-3.

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

This document applies to the latest software listed and higher versions. When a subsequent software version affects 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 latest version of the manual or system help for your instrument

REVISION STATUS

CONTENTS

Illustrations

Tables

OVERVIEW

This introductory section contains the following topics:

- ABOUT THIS MANUAL
- CONVENTIONS, and
- GRAPHICS.

ABOUT THIS MANUAL

The manual covers the description, installation, operation and maintenance of the Cell Lab Quanta™ SC system.

The information in your Quanta SC Instructions For Use manual is organized as follows:

- Chapter 1, USE AND FUNCTION
  Contains a short description of the major instrument components and options, and the reagents and quality control materials used.
- Chapter 2, INSTALLATION
  Contains instrument requirements, and diagrams of the interunit cable connections.
- Chapter 3, OPERATION PRINCIPLES
  Contains a brief description of how the system uses light scatter analysis to perform cellular enumeration.
- Chapter 4, SPECIFICATIONS
  Details the instrument and performance specifications.
- Chapter 5, GETTING STARTED
  Provides information needed to get started, including Powering Up and Powering Down.
- Chapter 6, DAILY ROUTINE
  Provides instructions for procedures that need to be done daily, including Start Up and Shut Down.
- Chapter 7, SOFTWARE
  Provides details on how to use the software.
- Chapter 8, QUALITY CONTROL
  Provides information on how to run quality control material to verify instrument setup.
- Chapter 9, SAMPLE ANALYSIS
  Provides information on how to run patient samples.
- Chapter 10, CLEANING/REPLACEMENT
  Provides information on cleaning and replacement procedures.
- Chapter 11, TROUBLESHOOTING
  Provides information on error messaging and instrument troubleshooting guide.
- INDEX
  Provides page numbers for indexed information.

CONVENTIONS

Text Conventions

- **Bold font** indicates a software option, such as **Startup**.
- *Italics font* indicates screen text displayed on the instrument, such as *Run Shutdown*.
- A **Note** contains supplemental information.
- An ATTENTION contains information that is important to remember or helpful when performing a procedure.
- The terms “screen” and “window’ are used interchangeably.
- Quanta is used interchangeably with Quanta SC System and instrument.
- ***Bold, italics font*** indicates a procedure heading.

Graphic Conventions

-  indicates “select” with or “click” the left mouse button.
-  indicates double-click with the left mouse button.
-  indicates “click” the right mouse button.

SYMBOLS

Safety Symbols

Safety symbols alert you to potentially dangerous conditions. These symbols, together with text, apply to specific procedures and appear as needed throughout this manual.

Symbol	Warning Condition	Action
	**Biohazard**. Consider all materials (specimens, reagents, controls, and calibrators, and so forth) and areas these materials come into contact with as being potentially infectious.	Wear standard laboratory attire and follow safe laboratory procedures when handling any material in the laboratory.
	**Electrical shock hazard**. Possibility of electrical shock when instrument is plugged in to the power source.	Before continuing, unplug the instrument from the electrical outlet.
	**Hot Surface hazard**. Possibility of injury from a hot surface.	Before continuing, use caution when touching a surface that may be hot.
	**Light hazard**. Consider all light sources and light emissions as being potentially hazardous to your eyes.	Before continuing, verify that you are wearing the proper protective eye wear to avoid damage to your eyes from beams of light. Never look directly into a beam of light.
	**Laser hazard**. Consider all laser sources as being potentially hazardous to your eyes.	Before continuing, verify that you are wearing the proper protective eye wear to avoid damage to your eyes from beams of light. Never look directly into a beam of light.
	**International warning**. Whenever this symbol is present, refer to the product labeling for detailed description of the warning.	Before continuing, verify that you have read and understood the warning described in the product labeling.
	**WEEE International warning**. Whenever this symbol is present, refer to local disposal requirements in the event that the labeled parts need replacement or disposal.	Before continuing, please contact your dealer or local Beckman Coulter office for proper decontamination information and take back program to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of device.

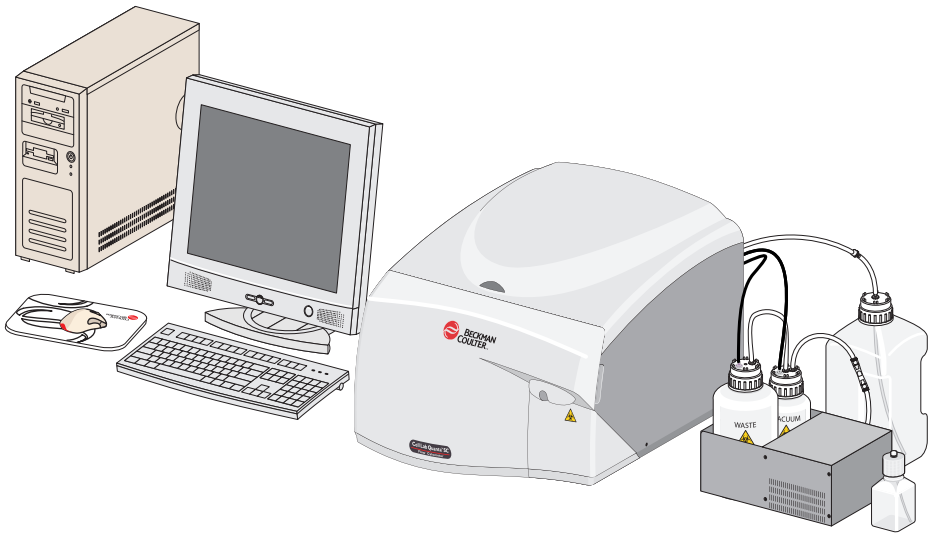
GRAPHICS

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

1.1 SYSTEM OVERVIEW

Figure 1.1 shows the Cell Lab Quanta SC system. The Quanta SC system is a three-color flow cytometer, which provides the additional sizing parameter of Electronic Volume (EV) and the granularity differentiating parameter of Side Scatter. Two light sources are provided; the mercury arc lamp and the 488 nm solid state laser.

Figure 1.1 Quanta SC System



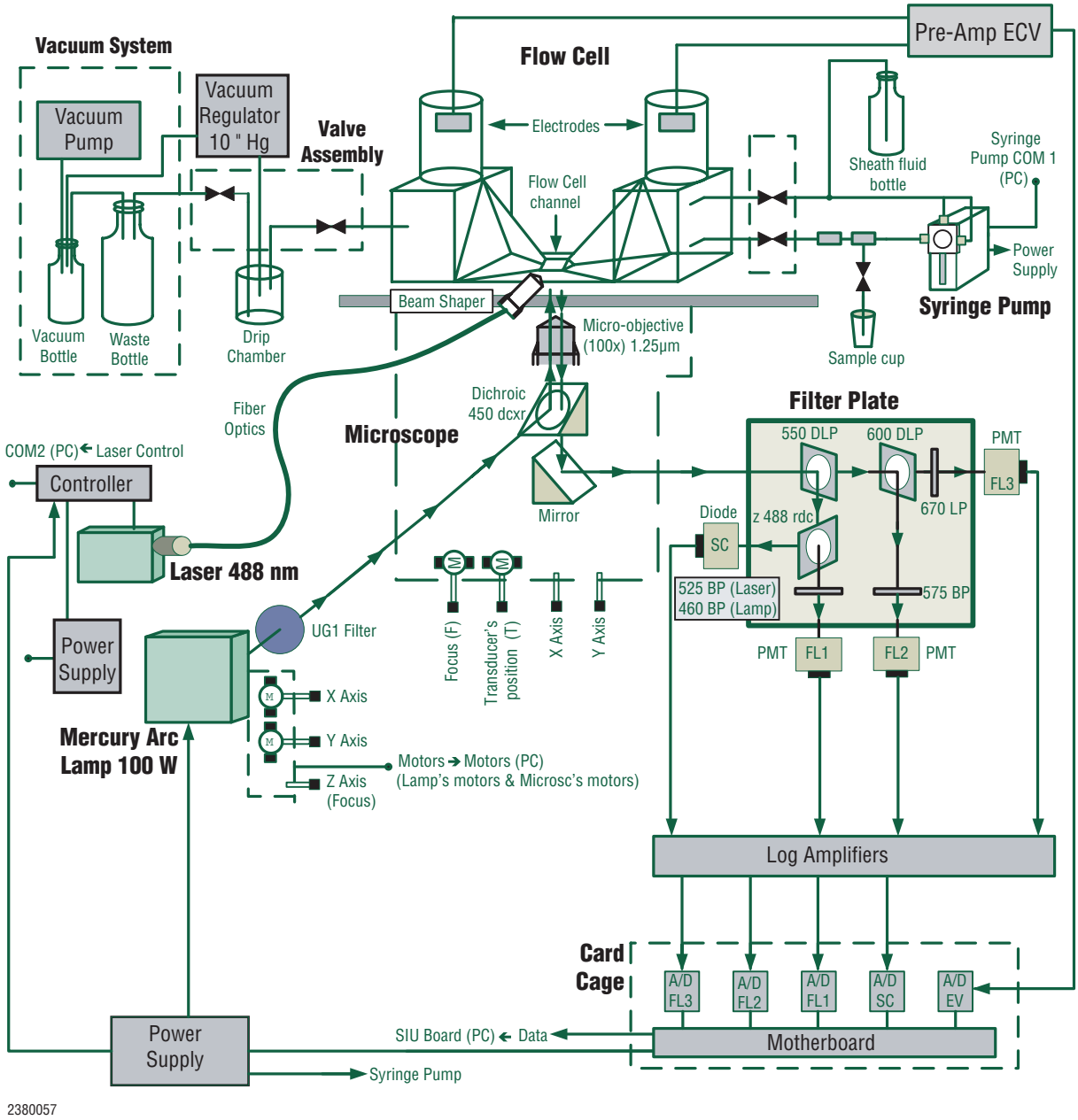
1.2 INTENDED USE

The Cell Lab Quanta SC instrument is intended for General Laboratory Use. The instrument simultaneously measures the fluorescence and electronic volume (EV) based on the Coulter Principle.[1]

1.3 QUANTA SC SYSTEM OVERVIEW

Figure 1.2 shows the major components of the Quanta instrument.

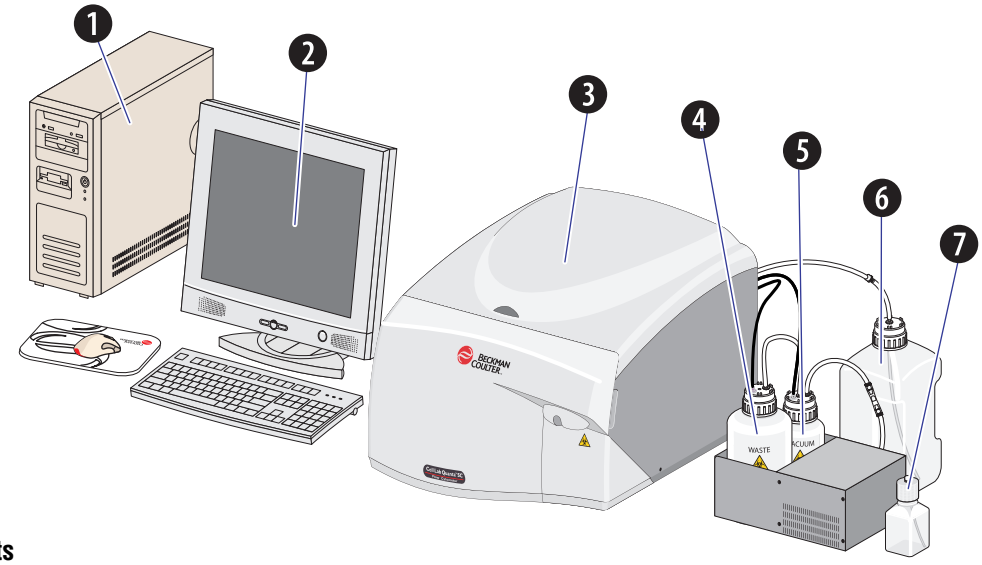
Figure 1.2 Schematic Overview of Quanta Instrument



1.4 SYSTEM COMPONENTS

See Figure 1.3

Figure 1.3 System



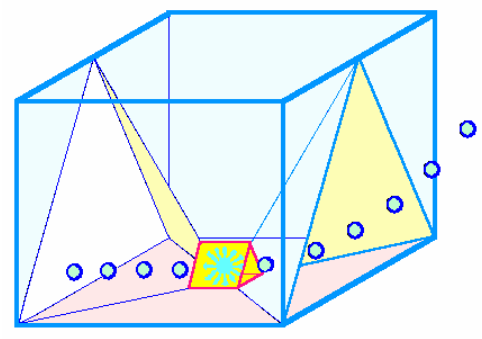
Components

1. Workstation Computer.
   To control the cytometer and analyze data from the cytometer.
2. Workstation Monitor.
   Displays data from the Workstation computer.
3. Flow Cytometer.
   Contains fluidics, optical flow chamber, electronics, laser and arc lamp.
4. Waste Collection.
   Contains Waste bottle and Vacuum bottle for waste collection.
5. Vacuum Module.
   Provides system vacuum.
6. Sheath Container.
   Contains sheath fluid.
7. Shutdown Solution.
   Contains shutdown solution.

1.5 PRINCIPLES OF OPERATION

Electronic volume (EV) and optical measurements are made simultaneously in the same spatial location consisting of a flow cell with an equilateral triangular cross section. See Figure 1.4.

Figure 1.4 Triangular Flow Cell: Overview



This triangular flow geometry produces large hydrodynamic forces that focus the sample stream to the center of the triangular aperture and allows the simultaneous collection of optical and electronic volume measurements.

The optical system consists of a 100X oil immersion micro-objective with a numerical aperture of 1.25 used to collect the fluorescence emission. A 100 W stabilized arc lamp with wavelengths of 365, 404, and 435; and a 488 nm laser are used as the light sources for the florescence measurements. The fluorescence signals are collected with photomultiplier tubes.

The fluidics system is controlled with a metering pump, which also consists of 17 computer-controlled valves that automatically perform all the sample handling and flushing operations. Fluid transfer is accomplished by vacuum.

1.6 WARNING LABELS AND PRECAUTIONS

The Cell Lab Quanta SC system contains a 100-Watt Arc Lamp (Figure 1.5) emitting high intensity heat and a 488 nm diode laser (Figure 1.6). The instrument, therefore, may pose certain hazards associated with this lamp and laser if misused.

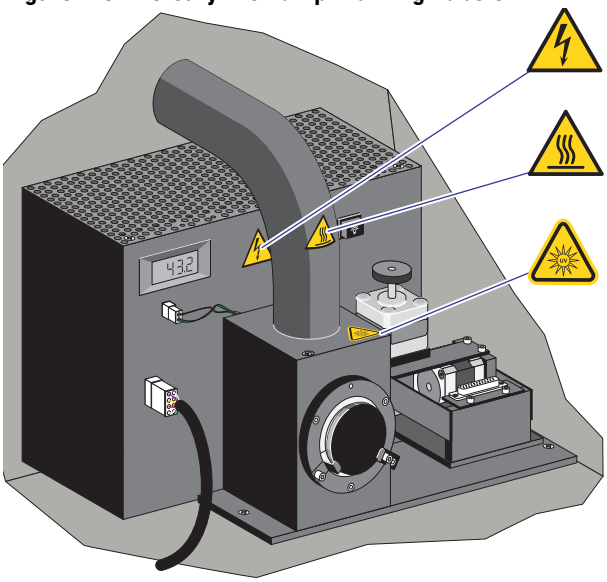
Mercury Arc Lamp

WARNING The Arc Lamp has power to 100 W and is accessible when the instrument cover is opened. Avoid contact with the lamp.

When operating the instrument with the cover opened, coming into contact with the Arc Lamp may cause severe burns. Avoid coming into contact with the lamp.

To avoid injury: Limit access to the inside of the instrument while the lamp is turned on to only those occasions when there is an absolute need to open the instrument cover (Start Up or Calibration). Never look directly into the lamp.

Figure 1.5 Mercury Arc Lamp Warning Labels



Laser

The Cell Lab Quanta SC is a Class I laser product.

WARNING Avoid direct exposure to the beam.

Reflected laser light can be as damaging as the original beam. Remove rings, watchbands, metal pens, pendants and any other reflective accessories from hands and clothing.

Eye and skin exposure to direct and reflected laser light is hazardous and may be extremely harmful.

Ensure that all mirrors and optics are securely positioned and fixed. Prevent stray reflections from other surfaces.

Do not place reflective objects in the laser beam.

Limit access to the laser to personnel who are familiar with the equipment. The laser must not be installed, operated or repaired by inexperience or untrained personnel.

Do not open the Controller or Laser Head enclosure for any reason. Always return the units to the manufacturer for repair.

Provide bright light around the laser equipment to reduce the operator's pupil size.

Always wear eye protection appropriate to the beam wavelength and intensity when in the vicinity of the laser equipment. **Note**: Glasses may make the beam invisible, increasing the risk of skin burns.

The laser equipment must be turned off when not in use.

Never operate the unit in the presence of flammable gases or fumes.

Laser radiation may be emitted from the end of the fiber optic cable. Never look directly into the fiber while the laser is ON.

Figure 1.6 Laser Warning Labels

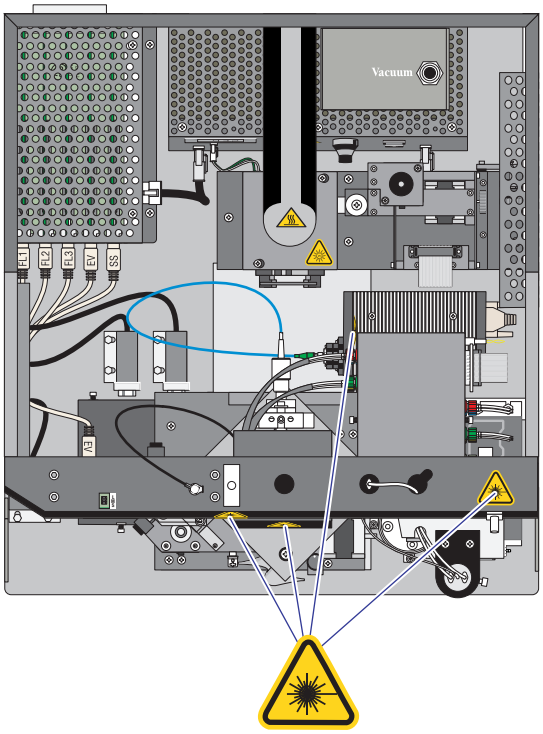
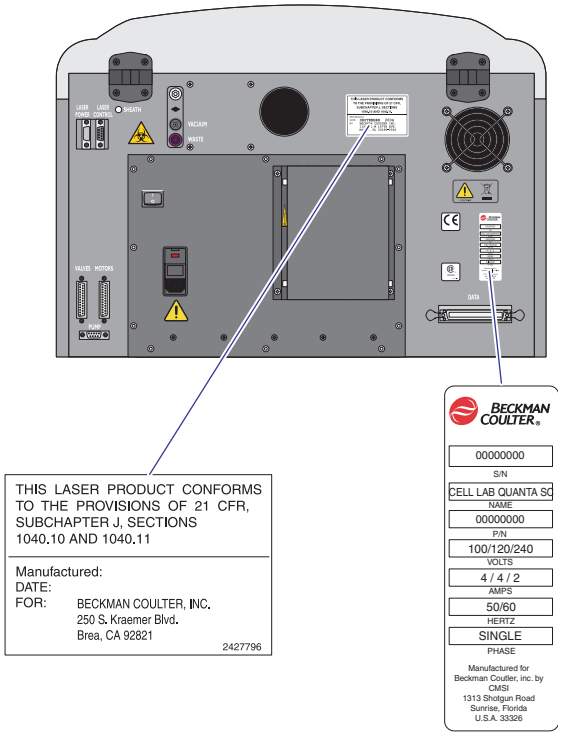


Figure 1.7 Laser Warning Labels

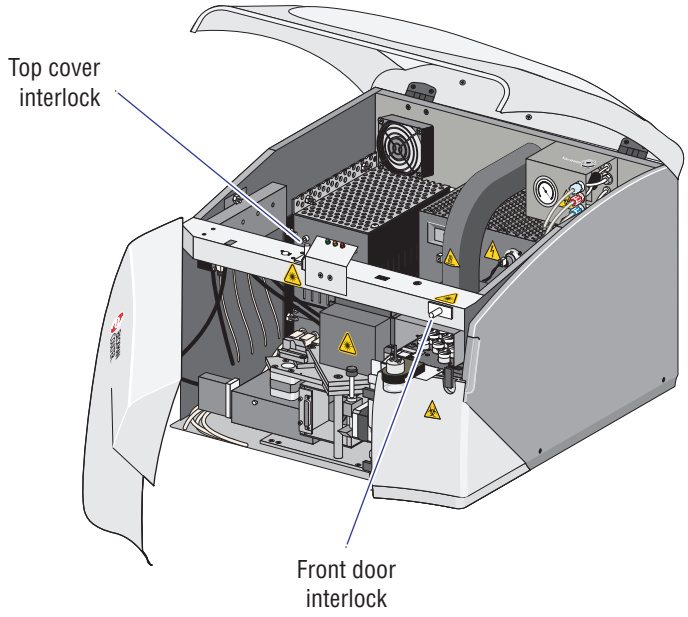


Laser Interlocks

WARNING Risk of personal injury if the laser safety interlock is bypassed. Do not tamper with the laser interlock unless otherwise instructed in this manual.

Figure 1.8 shows the laser interlocks.

Figure 1.8 Laser Interlocks



Handling Precautions

Proper handling procedures for samples and reagents used in flow cytometry analysis should be adhered to at all times. Consult appropriate Material Safety Data Sheets for all diluents and reagents used.

1.7 OPTIONS

Hardware Options

Figure 1.9 Optical Filter Configuration -- Laser 488 nm

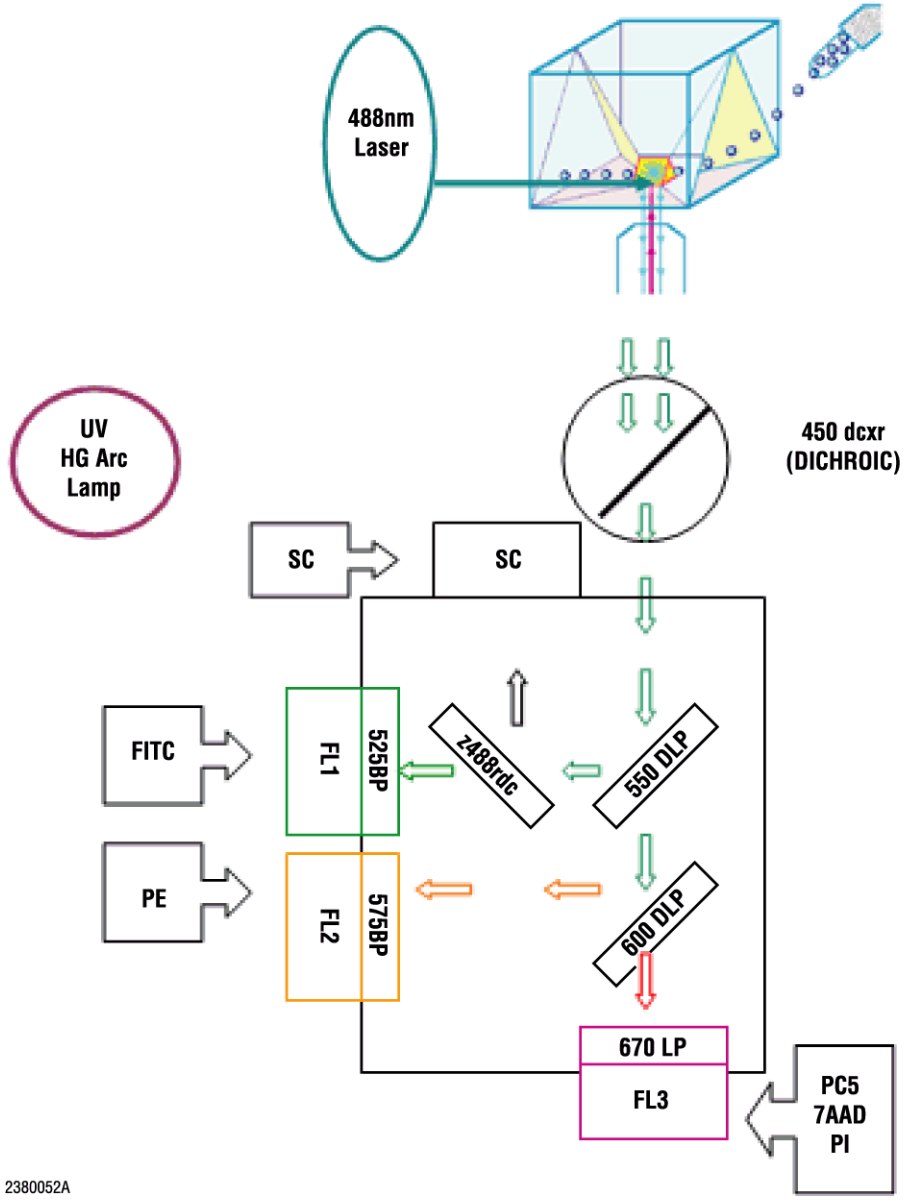
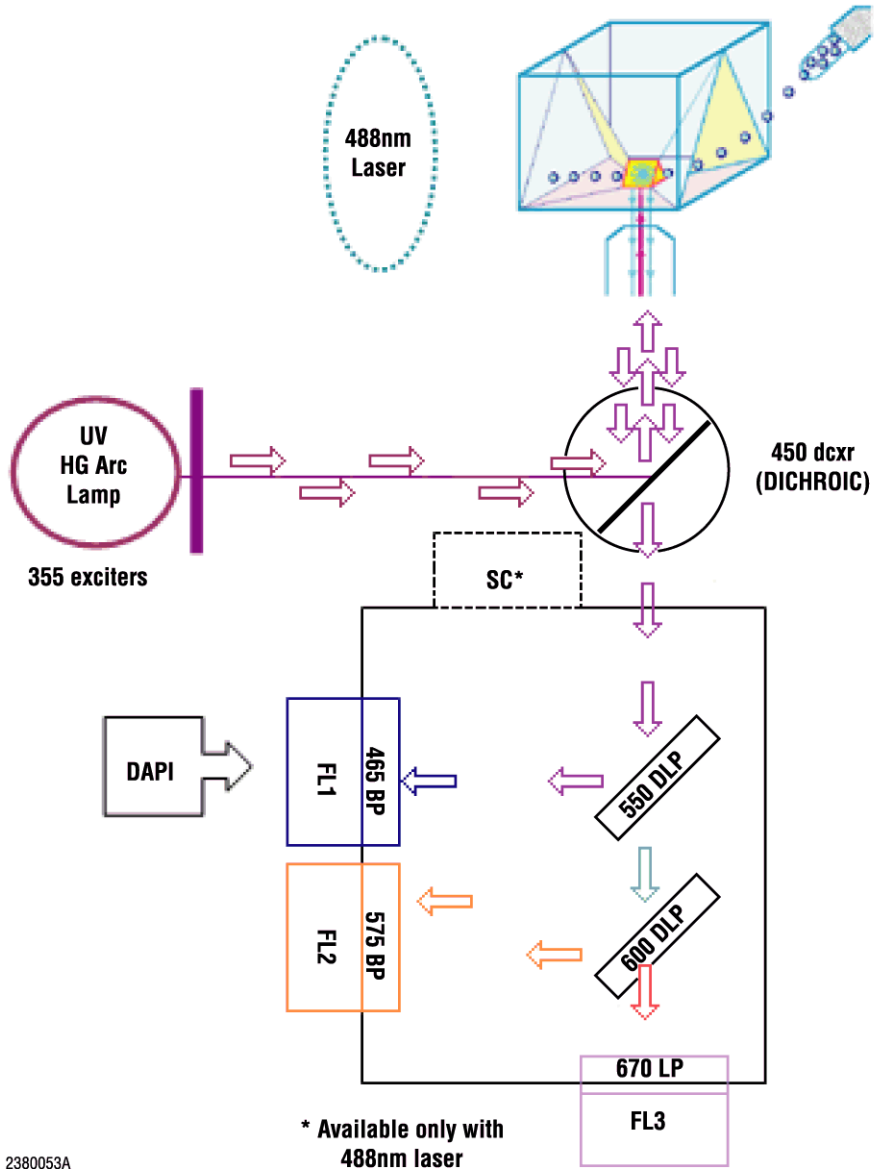


Figure 1.10 Optical Filter Configuration -- Lamp 365 nm



Flow Cell Configurations

Description	Configuration
Standard	125 x 125 µm

Jumper Positions for Different Size Particles µm

Flow Cell Aperture µm	Particle Size limits µm	Recommended EV Jumper Position for different Size Particles µm*			Suggested particles to be analyzed
		L	M	S	
125	3-40	15-40	10-15	3-10	Cells, large beads

* Sample mean size is depicted in the above table for Electronic Volume (EV)

Light Source Configuration

Quanta SC Systems configuration contains both light sources, Mercury Arc Lamp and the 488 nm Laser with side scatter and three fluorescence parameters.

IMPORTANT Erroneous results can occur if both light systems (Mercury Arc Lamp and 488 nm Laser) are active at the same time. Ensure that the appropriate light source is selected during the startup procedure, refer to Heading 6.1, PERFORM STARTUP for detailed instructions.

Printer

A printer is not supplied with the instrument but is available as an option.

1.8 REAGENTS

Do not use any reagents that are not compatible with the specific wetted surfaces of the sample instrument, such as non-aqueous solvents.

For a list of approved reagents for use on this system, see Table 1.1.

Table 1.1 List of Approved Reagents

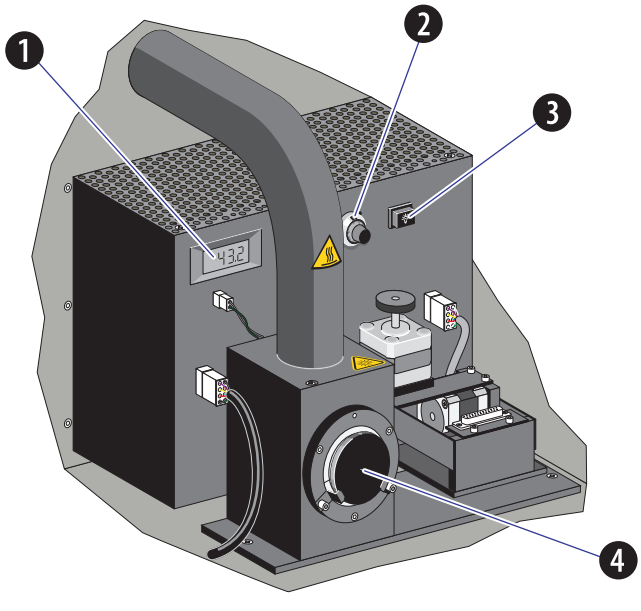
Reagent Name	Part Number
IsoFlow Sheath Fluid 1x10L	8546859
IsoFlow Sheath Fluid 4x1.8L	8547008
Marine Iso-Diluent 10 L	731088
Shutdown Solution 5 L	629968
Cleaning Solution Kit	629969
COULTER CLENZ 500 mL	8546929
COULTER CLENZ 5 L	8546930
COULTER CLENZ 10 L	8546931

1.9 CONTROLS AND INDICATORS

For details, see the following illustrations:

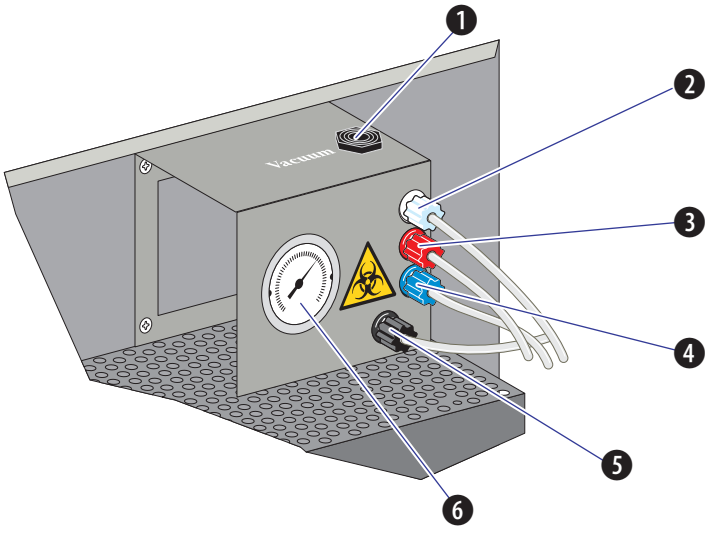
- Figure 1.11, Inside the Instrument: Controls and Indicators
- Figure 1.12, Vacuum Regulator: Controls and Indicators
- Figure 1.13, Jumper Positions: Controls and Indicators.

Figure 1.11 Inside the Instrument: Controls and Indicators



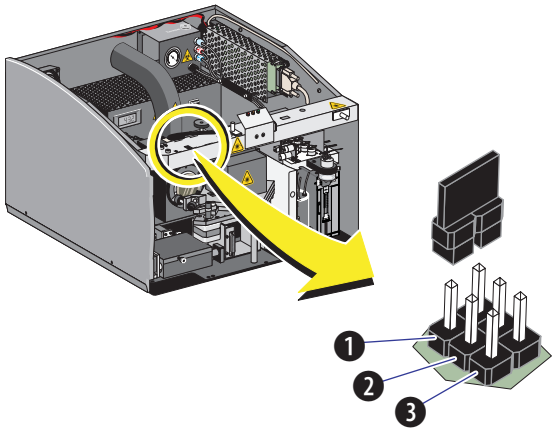
1. Mercury Arc Lamp voltage display
2. Adjustment dial for Mercury Arc Lamp power
3. Mercury Arc Lamp power starter
4. Arc Lamp Excitation Filter

Figure 1.12 Vacuum Regulator: Controls and Indicators



1. Vacuum regulator adjustment
2. Sheath
3. Air
4. Waste
5. Vacuum
6. Vacuum gauge

Figure 1.13 Jumper Positions: Controls and Indicators



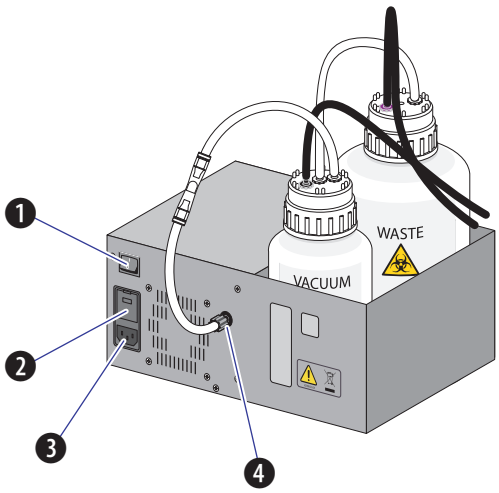
1. Jumper Position S
2. Jumper Position M
3. Jumper Position L

Waste Bottle and Vacuum Bottle

For details see the following illustrations:

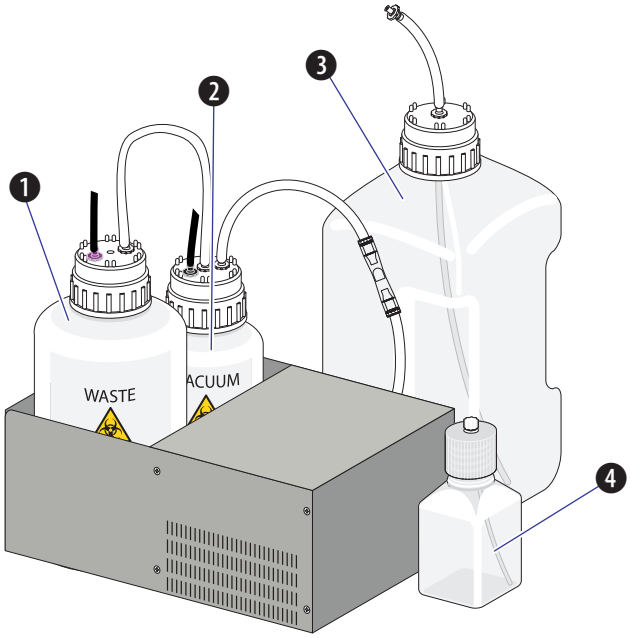
- Figure 1.14, Reservoirs: Controls and Indicators
- Figure 1.15, Waste System and Reagents

Figure 1.14 Reservoirs: Controls and Indicators



1. Power ON/OFF for pump
2. Fuse
3. Power cord, connects to surge protector
4. Connects to instrument vacuum bottle

Figure 1.15 Waste System and Reagents

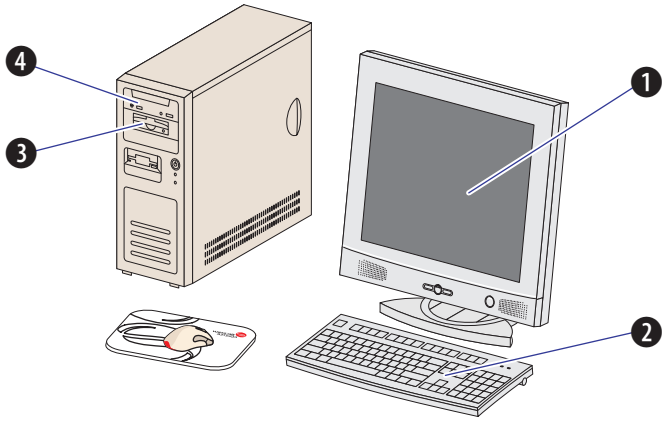


1. Waste Bottle
2. Vacuum Bottle
3. Sheath Fluid Bottle
4. Shutdown Solution Bottle

PC

See Figure 1.16.

Figure 1.16 Workstation PC: Controls and Indicators



1. Monitor
2. Keyboard
3. 1.44 Floppy Drive
4. CDRW Drive
   CD-ROM Read/Write Drive

1.10 MATERIAL SAFETY DATA SHEETS (MSDS)

To obtain an MSDS for reagents used on this system:

1. On the internet, go to http://www.beckmancoulter.com:
   a. Select **MSDS** from the **Customer Support** drop-down menu.
   b. Follow the instructions on the screen
   c. Contact your Beckman Coulter Representative if you have difficulty locating the information.
2. If you do not have internet access:
   - In the USA, either call Beckman Coulter Customer Operations (800-526-7694) or write to:

     Beckman Coulter, Inc.
     Attention: MSDS Requests
     P.O. BOX 169015
     Miami, FL 33116-9015
   - Outside the USA, contact your Beckman Coulter Representative.

2.1 DELIVERY INSPECTION

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

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

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

2.2 SPECIAL REQUIREMENTS

Before your system is installed, determine where you want the system placed. Consider the following:

- Space and Accessibility
- Installation Category
- Electrical Input
- Ambient Temperature and Humidity
- Heat Dissipation
- Drainage

Space and Accessibility

Allow room to interconnect the system components. Consider:

- A comfortable working height
- Adequate space for ventilation, and access for maintenance and service
- Placement of the system so that:
  - the vents are not blocked and proper airflow is allowed
  - the system is at least 6 inches from any wall or object, such as a filing cabinet or other instrument.
- Proper electrical requirements in the location chosen for system placement. To prevent electrical shock, be sure to plug equipment into properly grounded electrical outlets.
- Once the computer and the system have been setup, store the shipping boxes in a dry area.

Table 2.1 System Dimensions and Accessibility

Specifications	Instrument	Workstation Monitor	Workstation Computer*
Height	44.5 cm (17.5 in.)	43.3 cm (17.1 in.)	43.18 cm (17 in.)
Clearance above for servicing	48.3 cm (19 in.) min.	N/A	
Total clearance needed	92.3 cm (36.5 in.)	43.3 cm (17.1 in.)	
Width	55.9 cm (22 in.)	42.2 cm (16.6 in.)	20.32 cm (8 in.)
Clearance on right for servicing	15.2 cm (6 in.)	N/A	
Clearance on left for servicing	15.2 cm (6 in.) min.	N/A	
Total clearance needed	86.3 cm (34 in.)	42.2 cm (16.6 in.)	
Depth	70.5 cm (27.8 in.)	17.6 cm (6.9 in.)	43.18 cm (17 in.)
Clearance behind instrument for sufficient cooling and room for servicing	15.2 cm (6 in.)	N/A	
Total clearance needed	85.7 cm (33.8 in.)	17.6 cm (6.9 in.)	
Weight	34.9 kg (77 lbs)	5.6 kg (12.4 lbs)	5.0 kg (11 lbs)
Sound Pressure Level	<85 dBA	<85 dBA	<85 dBA

*Workstation Computer must be placed on the floor.

Note: Keep all shipping containers in case your system needs to be shipped to Beckman Coulter, Inc. for repair.

Installation Category

Category II (per IEC 1010-1 standard). Pollution degree 2.

Electrical Input

CAUTION Possible instrument damage could occur if you plug the Quanta SC System on the same electrical circuit as another instrument or use an extension cord or a power strip to connect the Quanta SC System. Use a dedicated outlet with isolated ground for the Quanta SC System plug.

AC Input Specifications Instrument	Voltage Rating: 100/120 VAC	Voltage Rating: 220/240 VAC
	Current Rating: 6 A	Current Rating: 3 A
	Frequency Rating: 50/60 Hz	Frequency Rating: 50/60 Hz
	Connection Type: IEC 320/C14	Connection Type: IEC 320/C14
AC Input Specifications Pump Station	Voltage Rating: 100/120 VAC	Voltage Rating: 220/240 VAC
	Current Rating: 6 A	Current Rating: 3 A
	Frequency Rating: 50/60 Hz	Frequency Rating: 50/60 Hz
	Connection Type: IEC 320/C14	Connection Type: IEC 320/C14
AC Input Specifications Computer	Voltage Rating: 100/120 VAC	Voltage Rating: 220/240 VAC
	Current Rating: 6 A	Current Rating: 3 A
	Frequency Rating: 50/60 Hz	Frequency Rating: 50/60 Hz
	Connection Type: IEC 320/C14	Connection Type: IEC 320/C14

Ambient Temperature and Humidity

This device is intended for indoor use only.

Keep the room temperature between 16°C and 29°C (60°F and 84°F), and do not let it change more than 3°C (5°F) since the last alignment verification. Keep the humidity between 30% and 80%, without condensation. Maximum Altitude 2000m.

Heat Dissipation

Heat dissipation is 500 W (1,706 Btu/hour) for the total system. Provide sufficient air conditioning (refer to Ambient Temperature and Humidity).

Drainage

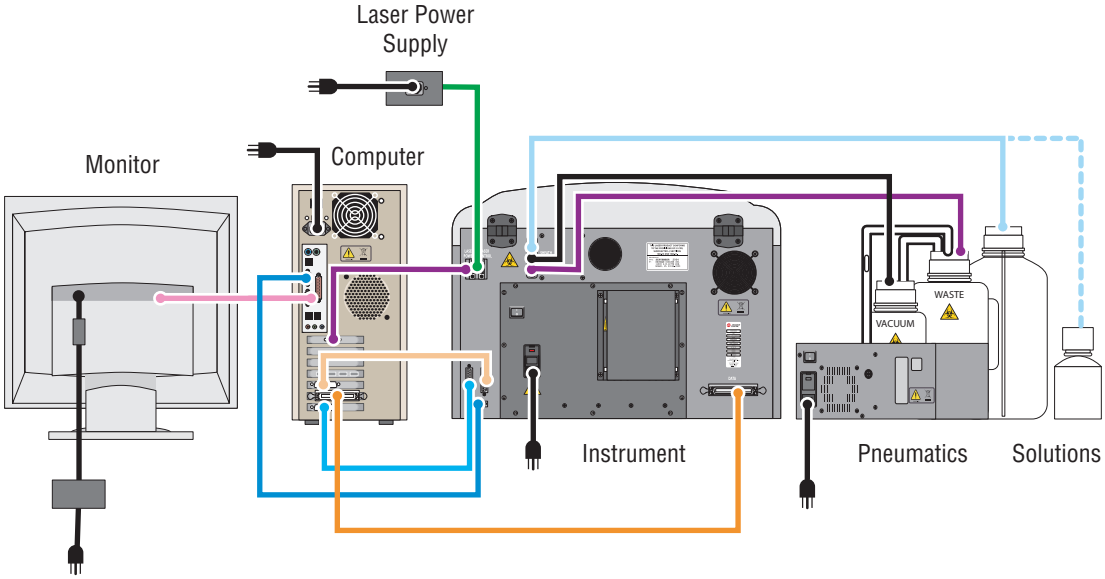
WARNING Risk of biohazardous contamination if you have skin contact with the waste container or vacuum bottle, its contents, and its associated tubing. The waste container and its associated tubing might contain residual biological material and must be handled with care. Clean up spills immediately.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

The waste line from the Cytometer is connected to a waste bottle, which sits next to the vacuum module. Dispose of the waste in accordance with your local environmental regulations and acceptable laboratory procedures.

2.3 INSTALLATION PROCEDURES

Your system will be installed by a Beckman Coulter Representative.

Interunit Connections



Lifting and Carrying

WARNING Possible operator injury. One person only should not lift the instrument. Lifting handles are not provided and the instrument weighs over 40 lbs. Lifting of the instrument should only be done by a minimum of two persons following the requisite safety precautions.

3.1 OVERVIEW

The Quanta SC System is a three-color flow cytometer, which provides the additional sizing parameter of Electronic Volume (EV), and the granularity differentiating parameter of side scatter.

The Quanta SC System uses a triangular flow cell (Figure 1.4) filled with conductive sheath liquid. Cells suspended in a weak electrolyte solution are drawn through a small aperture that separates two electrodes between which a slight current flows. As each cell passes through the electrical sensing zone of the aperture, the cell displaces its own volume of conducting liquid, momentarily increasing the impedance. This change in resistance produces a voltage pulse large enough to accurately measure; the Coulter Principle states that the amplitude of this pulse is directly proportional to the volume of the cell. The Coulter Principle, which is not affected by shape, color, or refractive index, is the accepted reference method for cell and particle counting and sizing.

A lamp or laser light source is used to excite fluorescent dyes attached to the particles. This light is directed toward the aperture in the flow cell.

As particles pass through the light source, the dye emission is reflected to a mirror and then to filters that direct the fluorescent light in turn to three PMTs (FL1, FL2 and FL3). The PMTs generate voltage pulses proportional to the amount of fluorescence.

If the laser is ON, then laser light bounces off the particles as they pass through the laser light. The light which bounces off at a right angle is passed to the side scatter detector. This detector also generates a voltage pulse proportional to the amount of light which bounced off the particle.

The Electronic Volume (EV) pulse, fluorescent pulse(s) and the side scatter pulse are amplified, digitized and then analyzed for pulse height. The pulse height of each measurement is then used to create the plots seen on the main screen.

A suspension of cells or particles, of precise volume, is drawn through the orifice of an aperture. A slight current is maintained, by two electrodes, across the orifice. Single cell suspensions enter the “sensing zone” resulting in an increase in resistance ultimately producing a voltage pulse proportional to the volume of the cell or particle. This measurement is not affected by color, shape, or refractive index of the sample. The Coulter Principle is the reference method for automated cell counting and sizing.

3.2 SAMPLE FLOW

When a sample is placed on the Cell Lab Quanta SC instrument and the **Start** button is selected, the metering pump aspirates the sample from the sample cup into the sample loop (holding station for the sample). Once the sample is in the sample loop, the sample is then boosted from the sample loop to the entrance of the flow cell.

When the sample is at the entrance of the flow cell, the metering pump slows the sample down to allow the triangulating forces of the sheath flow to line the particles in the sample up as they pass through the flow cell. Once a stable flow has been achieved, the software begins data collection.

Data collection continues until the user manually stops it, a user defined stop criteria is met, or the volume of sample that was aspirated into the sample loop has been dispensed through the flow cell.

After a stop criteria is met or the sample volume has been dispensed, several automated post sample processes may occur. These can include:

- Saving the file to disk
- Printing a file report
- Recovering the remaining sample into sample cup
- Rinsing the fluidics to prepare for the next sample.

4.1 PERFORMANCE SPECIFICATIONS

Absolute Count

Table 4.1 Absolute Count Performance Specifications

Description	Specification
Accuracy	± 5%
Reproducibility (CV)	< 5%
Concentration	3 x 10^4 to 2 x 10^6 particles per mL. **ATTENTION:** For greatest accuracy, samples should have a concentration in the range of 2.5 x 10^5 to 2 x 10^6 particles per mL.

Fluorescence

Table 4.2 Fluorescence Performance Specifications

Description	Specification
Resolution	≤ 2.5% HPCV using TRBC DNA Reference Calibrator in linear scale with NIM-DAPI and the mercury arc lamp
	≤ 3% HPCV using COULTER Flow-Check Fluorospheres in linear scale with the 488 laser

Sizing

Table 4.3 Sizing Performance

Description	Specification
Sizing	Electronic impedance with a measurement range of 3.0 to 40 microns diameter with the standard 125 µm flow cell.

Optics

Table 4.4 Optics Performance

Description	Specification (in nms)
Excitation*	365, 404 or 435 arc excitation and a 488 nm laser *May only operate one light source at a time.
Fluorescence Detectors	3 Ultra Sensitive Photomultipliers tube
Optical Alignment	Automated Computer Controlled
Optical Coupling	1.25 NA oil immersion objective
Scatter	Solid State Photo Diode

Parameters

- Fluorescence 1 Linear/Log display
- Fluorescence 2 Linear/Log display
- Fluorescence 3 Linear/Log display
- Side Scatter Linear/Log display
- EV Linear display
- Time display
- Counts per mL display
- Calculated Fluorescence Surface Density (FSD)/ Fluorescence Concentration (FC) display

Calculated Parameters

The following parameters take advantage of the Coulter Volume used in Flow Cytometry:

- FC – Fluorescent Concentration
- FSD – Fluorescent Surface Density

FC and FSD are parameters calculated for every data point, and the calculation is treated as a parameter for display, as well as statistics.

FC and FSD Relevance

FC and FSD are relevant whenever the quantity of stain is proportional to the size of the particle (that is, when a bigger particle will allow for more staining than a smaller particle). If this is the case, then FC (used for internal markers) or FSD (used for external markers) can normalize the fluorescence to stain density, instead of total quantity of stain.

When the quantity of stain is proportional to the size of the particle the stain density (FC and FSD) can help to separate populations that blend together using the total fluorescent measurement.

Mathematical Definitions

FC = Fluorescent Channel/Volume Channel
FSD = Fluorescent Channel/(Volume Channel)^(2/3)

To get Surface Area of a Sphere from the Volume:

Volume = 4/3Pi R^3
Surface Area = 4Pi R^2
Surface Area = Volume^(2/3) * 4Pi*(3/(4*Pi)) ^ (2/3)

The surface area is equal to 2/3 root of the volume times a constant. For arbitrary channel assignment, constants can be ignored as they are equivalent to gain.

Numeric Range of FSD and FC

The range of FC and FSD parameters is the addition of the range of the Fluorescent and Volume parameters which are the basis of the calculation.

FC range = 1/EV max channel to FL Max Channel
FSD range = 1/(EV max channel)^2/3 to FL Max Channel

If EV is 1000 linear channels, and FL is 4 decades of Log then:
FC range = .001 to 10000 (7 decades)
FSD range = .01 to 10000 (6 decades)

FSD and FC Calibration

The FSD parameter can be calibrated to Antigen Density assuming that the stain density is directly proportional to the Antigen Density.

The FC parameter can be calibrated for Absolute Concentration (the concentration of the item that the internal marker binds to). This also assumes that the stain concentration is proportional to the concentration of the item being calibrated.

The FC parameter may also be calibrated for NPE (Nuclear Packing Efficiency). This is the inverse of the Absolute Concentration, and is specific for Cell Nuclei.

Possible FSD and FC Calculation Errors

The FSD parameter assumes the particle is spherical when calculating the surface area. If the particle is not spherical, then the ratio of the volume to the surface area may not match the calculated parameter which uses the 2/3 root of the volume to calculate the surface area.

Software

- Microsoft® Windows® XP Professional
- Microsoft Office
- FCS 2.0 Generated Files

Fluidics

Table 4.5 Fluidics Specifications

Description	Specification
Flow Cell	Patented 125 µm triangular flow cell (standard)
Sample Rate	4.17 µL to 100 µL per minute for 125 µm
Valves	Computer-controlled fluidics system
Delivery System	Vacuum pump/motorized metering pump
Minimum Sample Size	150 µL
Maximum Sample Size	2 mL
Carryover	< 1% from one specimen to another when # of gated events is between 100 and 10,000.

Installation Requirements

Table 4.6 Installation Requirements

Description	Specification
Power	100 Vac, 120 Vac, or 220 Vac, 50/60 cycles
Wattage Consumed	500 watts (total system)
Operating Temperature	between 16°C and 29°C (60°F and 84°F)
Physical Dimensions	22 W x 28 D x 18 H (in.) 56 W x 71 D x 45 H (cm)
Instrument Weight*	34.9 kg (77 lbs)

** Workstation weight is separate and varies with the workstation components manufacturers.*

4.2 CUPS

The Quanta SC System analyzes samples in cups.

Beckman Coulter does not recommend the use of one cup in preference to another nor guarantees the acceptability of the sample cup to produce quality results. If you need information on a sample cup not listed here, contact your Beckman Coulter Representative.

Table 4.7 Cups Approved for Use

Cup Size	Manufacturer	Part Number
4 mL Note: Maximum Sample Volume, 2 mL	Beckman Coulter, Inc.	383721

4.3 FLOW CELL

The standard flow cell is 125 µm.

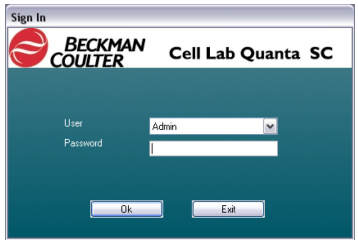
5.1 POWERING UP THE SYSTEM

Turn On the Instrument and the Computer

1 Power ON the Workstation computer.

2 Launch the Cell Lab Quanta SC software.

3 If the Sign-In window appears, select the User ID from the drop-down list and enter password. For additional information regarding User IDs and passwords, refer to Heading 7.12, WORKING WITH THE SECURITY MENU.



4 Run the startup item from the Instrument Menu, and follow the steps as prompted. Refer to Heading 6.1, PERFORM STARTUP for detailed instructions regarding instrument startup.

5.2 INTRODUCTION TO THE QUANTA SC SOFTWARE

Overview

Cell Lab Quanta SC software is compatible with Microsoft Windows XP based systems. The Analysis software functions include:

- Data Collection
- Instrument Control
- Data Analysis
- Acquisition
- Reporting
- Gating

- Statistical Analysis
- Alignment
- Listmode Playback
- Calculate EV
- Printing
- Compensation

Microsoft Windows Basics

Desktop

After your Workstation is powered up and runs through its routines, the Windows desktop (Figure 5.1) appears. Because desktops can be customized, yours may look different. Beckman Coulter, Inc. recommends that the Windows scheme set up on your Workstation at installation be maintained and not altered.

Figure 5.1 Desktop

Windows Password

The Cell Lab Quanta SC software is password protected and log on specific which protects against unauthorized changes to your data or system. This protection is provided at the application level only and does not apply to the Microsoft Windows XP Operating System (OS).

Beckman Coulter recommends that you utilize the Microsoft Windows password and screen saver password if you are concerned about protecting unauthorized changes to data files as well as OS settings. Refer to your Microsoft Windows documentation or contact your

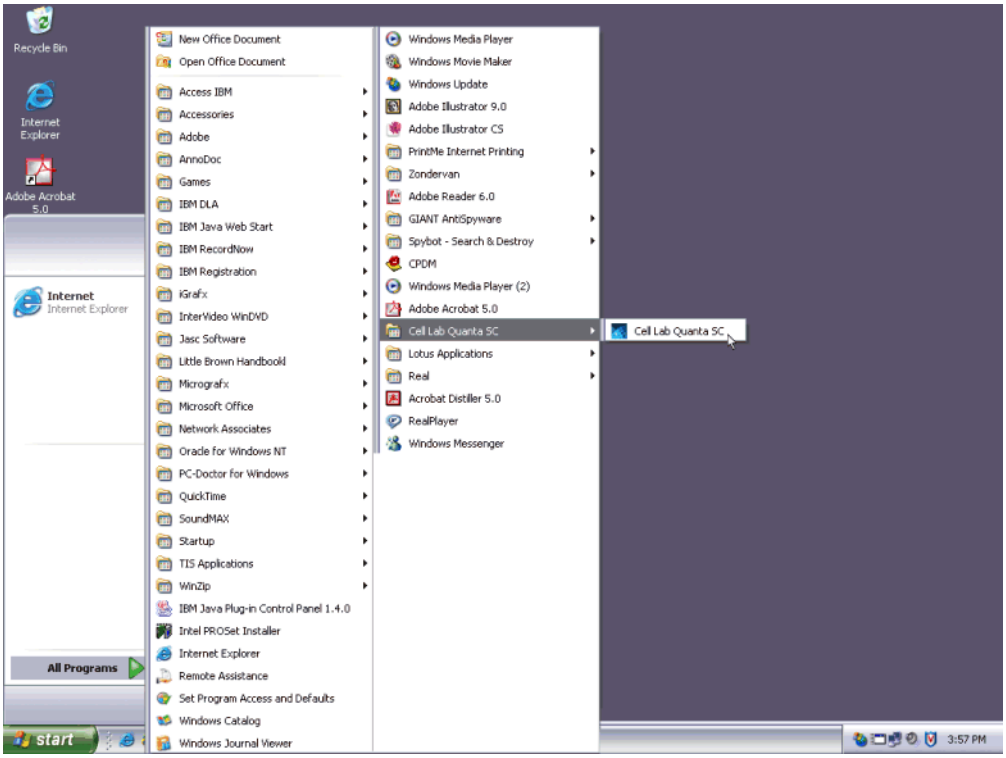
Network Administrator should you have difficulties setting the appropriate permissions for a user ID.

Launching the Software

You can either launch the software from the Start menu or from your Windows desktop.

To Launch from the Start Menu

1. Start .
2. **All Programs ▸▸ Cell Lab Quanta SC ▸▸ Cell Lab Quanta SC**. The Main screen (Figure 5.2) appears.



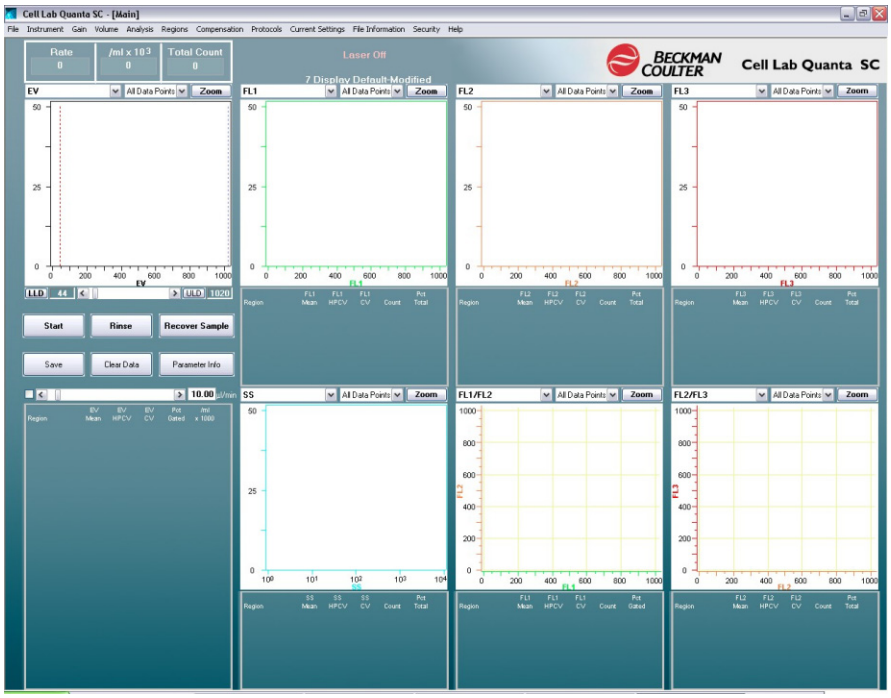
To Launch from the Desktop

From the Windows desktop, Cell Lab Quanta SC . The Main screen (Figure 5.2) appears.

Main Screen

When you launch the software, the Main screen (Figure 5.2) appears. The Main menu (Figure 5.3) appears at the top of the screen. For details about the Main screen, see Heading 7.1, UNDERSTANDING THE MAIN SCREEN.

Figure 5.2 Main Screen



Main Menu

See Figure 5.3.

Figure 5.3 Main Menu

Menu Options

The Main menu options include:

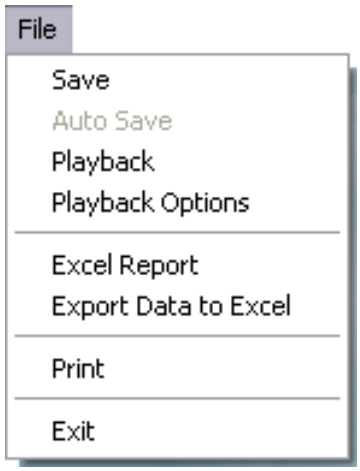
- File Menu
- Instrument Menu
- Gain Menu
- Volume Menu
- Analysis Menu
- Regions Screen
- Compensation Screen
- Protocols Screen
- Current Settings Screen

- File Information Screen
- Security Menu
- Help Menu

File Menu

When you **File** from the Main menu (Figure 5.4), appears. For details about this menu, see Heading 7.2, WORKING WITH THE FILE MENU.

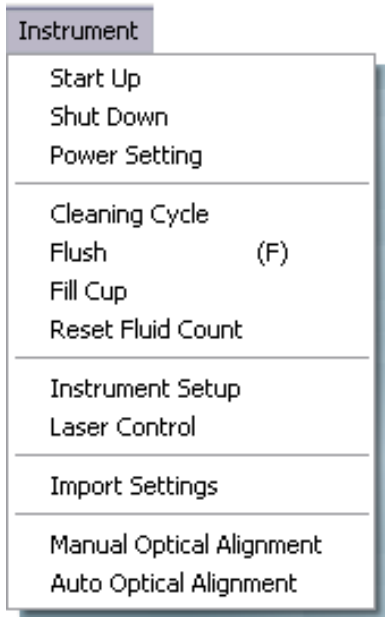
Figure 5.4 File Menu



Instrument Menu

When you **Instrument** from the Main menu, Figure 5.5 appears. For details about this menu, see Heading 7.3, WORKING WITH THE INSTRUMENT MENU.

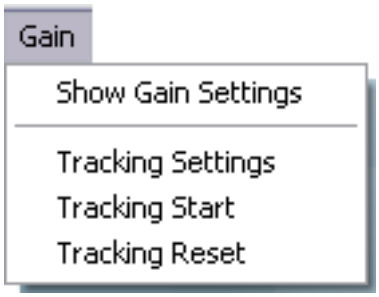
Figure 5.5 Instrument Menu



Gain Menu

When you **Gain** from the Main menu, Figure 5.6 appears. For details about this menu, see Heading 7.4, WORKING WITH THE GAIN MENU.

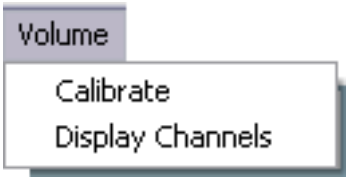
Figure 5.6 Gain Menu



Volume Menu

When you **Volume** from the Main menu, Figure 5.7 appears. For details about this menu, see Heading 7.5, WORKING WITH THE VOLUME MENU.

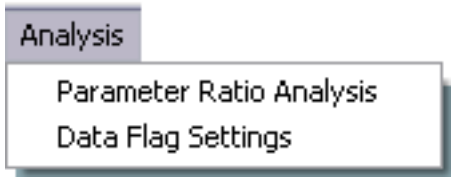
Figure 5.7 Volume Menu



Analysis Menu

When you **Analysis** from the Main menu, Figure 5.8 appears. For details about this menu, see Heading 7.6, WORKING WITH THE ANALYSIS MENU.

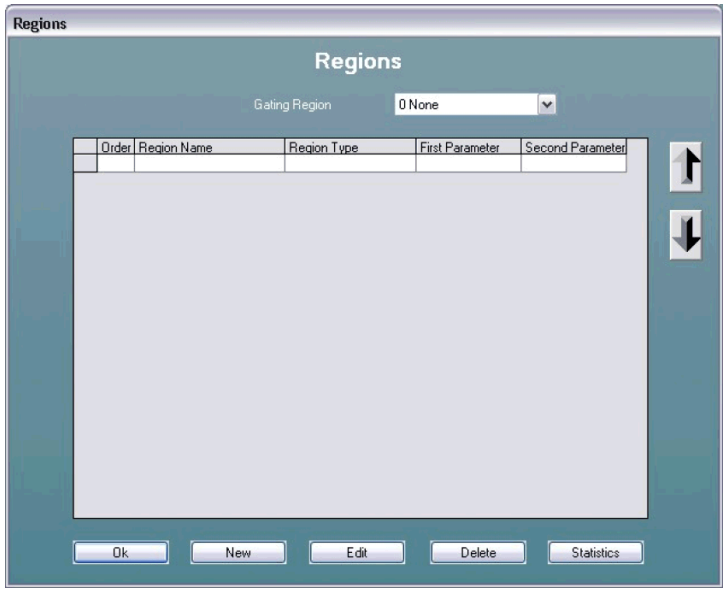
Figure 5.8 Analysis Menu



Regions Screen

When you **Regions** from the Main menu, Figure 5.9 appears. For details about this menu, see Heading 7.7, WORKING WITH THE REGIONS MENU.

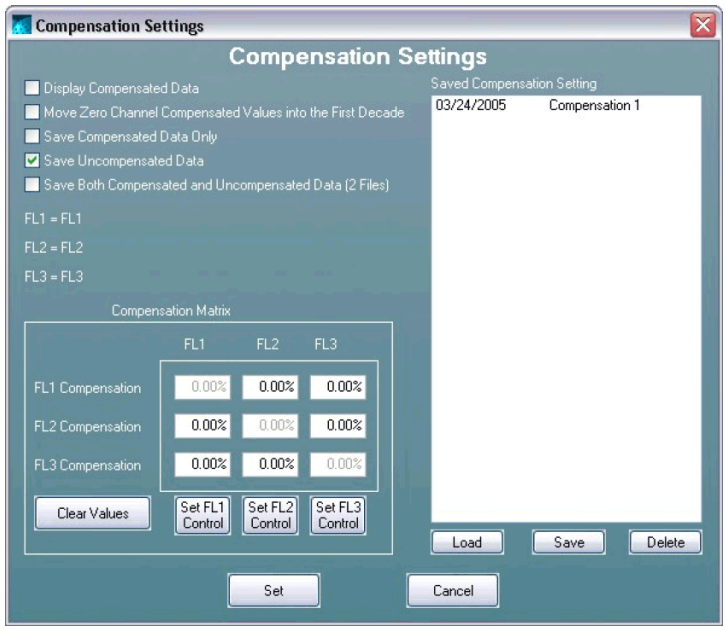
Figure 5.9 Regions Screen



Compensation Screen

When you **Compensation** from the Main menu, Figure 5.11 appears. For details about this menu, see Heading 7.9, WORKING WITH THE MANAGE PROTOCOLS SCREEN.

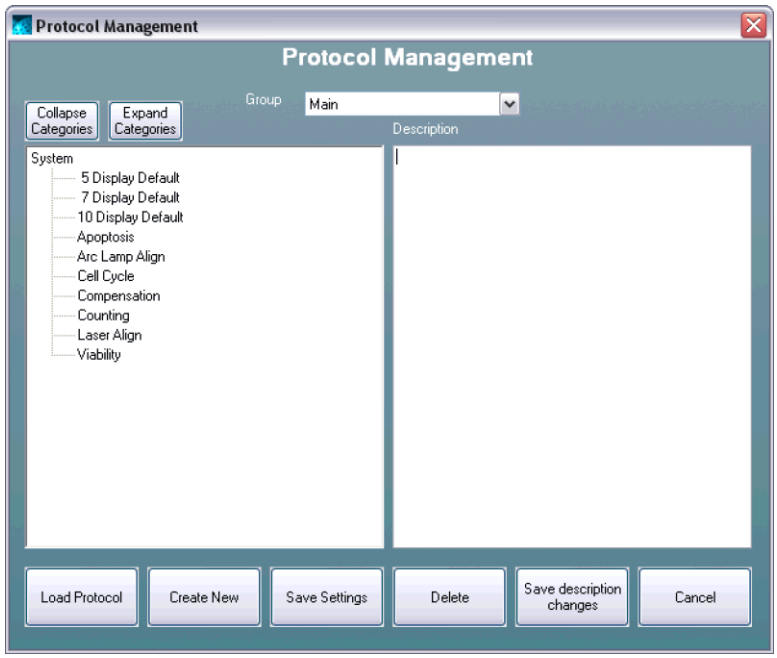
Figure 5.10 Compensation Screen



Protocols Screen

When you **Protocols** from the Main menu, Figure 5.11 appears. For details about this menu, see Heading 7.9, WORKING WITH THE MANAGE PROTOCOLS SCREEN.

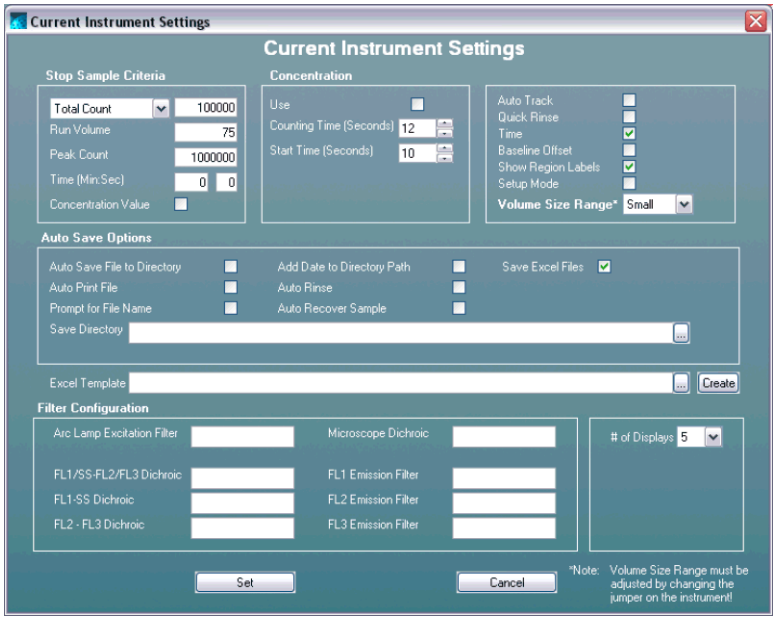
Figure 5.11 Protocols Screen



Current Settings Screen

When you **Current Instrument Settings** from the Main menu, Figure 5.12 appears. For details about this menu, see Heading 7.10, WORKING WITH THE CURRENT SETTINGS SCREEN.

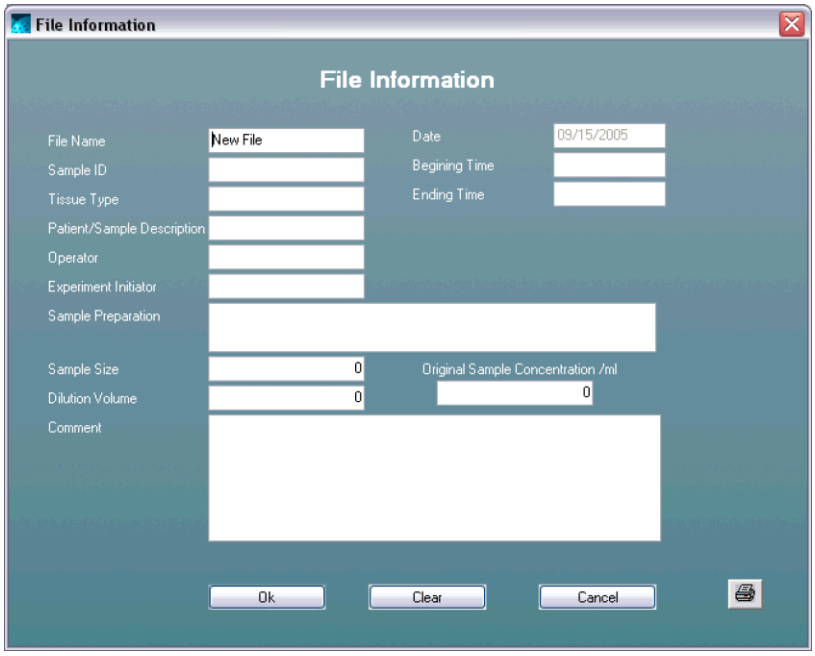
Figure 5.12 Current Instrument Settings Window



File Information Screen

When you **File Information** from the Main menu, Figure 5.13 appears. For details about this menu, see Heading 7.11, WORKING WITH THE FILE INFORMATION SCREEN.

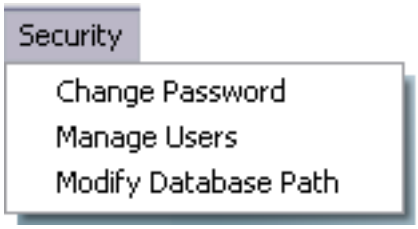
Figure 5.13 File Information Screen



Security Menu

When you **Security** from the Main menu, Figure 5.15 appears. For details about this menu, see Heading 7.12, WORKING WITH THE SECURITY MENU.

Figure 5.14 Security Menu



Help Menu

When you **Help** from the Main menu, Figure 5.15 appears. For details about this menu, see Heading 7.13, WORKING WITH THE HELP MENU.

Figure 5.15 Help Menu

Closing a Window or Closing the Software

Throughout this manual, you may be instructed to close a software window or to close (shutdown) the Quanta software. For details, see:

- Closing a Window
- Closing the Software

Closing a Window

To close an open software window and leave the software open, on the window to close. The previous window appears.

Closing the Software

To close the Quanta software entirely and return to the Windows desktop, from the Main screen. Your Windows desktop appears.

5.3 POWERING DOWN THE SYSTEM

Turn Instrument and Workstation OFF

1 Prior to powering down the system, perform shutdown procedure, see Heading 6.3, PERFORM SHUTDOWN.

2 Close the Quanta software (see Closing the Software.)

3 At the Workstation,  ▸▸ **Shutdown** ▸▸ **Shutdown or TURN OFF**.

4 Allow the system time to shut down.

5 Turn off the PC and monitor.

5.4 PLACING A CUP ON THE INSTRUMENT

Perform this procedure to place a sample cup securely on the instrument at the sample stage (Figure).

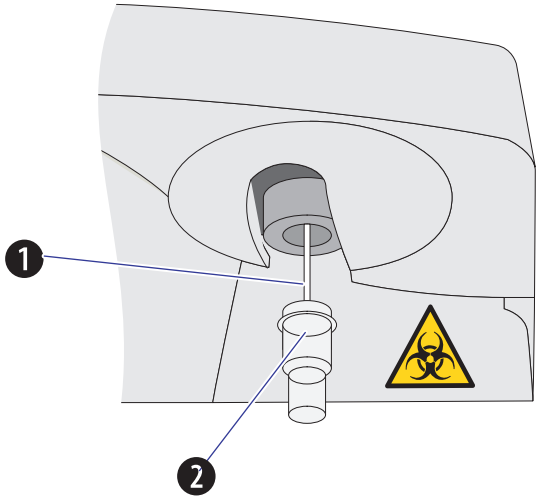
Sample Stage

WARNING Risk of contact with biohazardous material if you use a cup not approved for use on this instrument. Only use approved cups listed in Table 4.7.

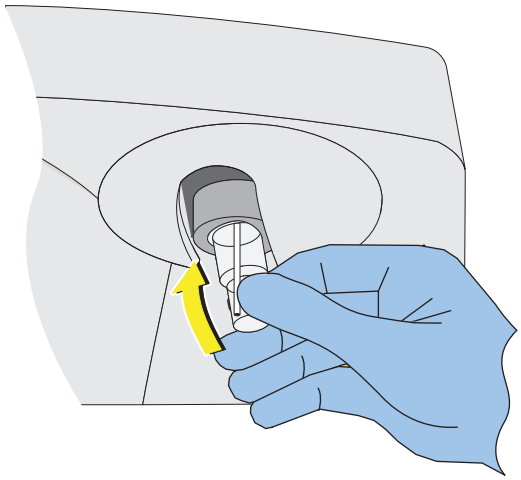
1 Place the cup on the instrument:

a. Introduce cup so that the probe (❶) is inserted in the cup.

b. Tilt the cup and push up so that the cup (❷) is secure in the holder.



c. Push up until the cup is completely secure.



2 When you are certain the cup is secure, release it so that it is supported by the holder.

6.1 PERFORM STARTUP

Perform this procedure at the beginning of each day and after switching between light sources or filters.

---

1 Verify correct filters are installed for the selected application and light source.

---

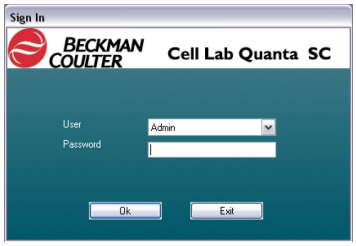
2 Power ON the Workstation computer.

---

3 Launch the Cell Lab Quanta SC software.

---

4 Once the Quanta software has launched, the following screen appears if a username and password are required. For additional information regarding User IDs and passwords, refer to Heading 7.12, WORKING WITH THE SECURITY MENU.

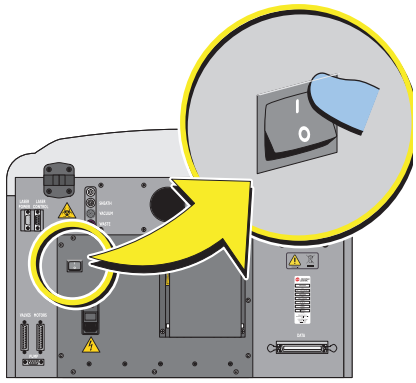


---

5 **Instrument ▸▸ Start Up**. The following screen appears.

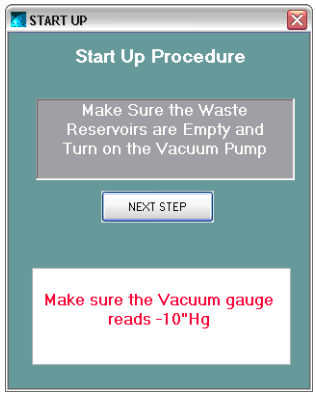
---

6 Turn ON the Quanta instrument.



7 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Start Up Procedure to startup the instrument.

For additional information regarding this Start Up step, refer to Heading 10.4, EMPTYING THE WASTE BOTTLE and Heading 11.2, MONITOR VACUUM READINGS.



8 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Start Up Procedure to startup the instrument.

Refer to Figure 1.15, Waste System and Reagents to locate the appropriate containers.

9 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Start Up Procedure to startup the instrument.

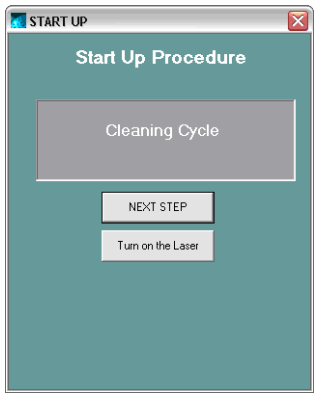
CAUTION Possible damage to the instrument if Mercury Arc Lamp power starter is continuously depressed after visible light is evident. Do not continue to depress the Mercury Arc Lamp power starter after observing visible light.

CAUTION Possible damage or reduced bulb life expectancy if Mercury Arc Lamp is ignited when lamp is HOT. If the Mercury Arc Lamp is turned OFF for any reason, including brief power failures, the lamp must be allowed to cool before reignition. Wait at least 15-20 minutes before attempting to reignite Mercury Arc Lamp. Very high or unstable CVs or HPCVs may occur as the result of a damaged Mercury Arc Lamp.

IMPORTANT Possible erroneous results if laser is operated with a laser base temperature >7°C (45°C) above ambient. Operating the laser at a temperature >7°C (45°C) above ambient may result in high HPCVs and CVs and/or the gain and voltage may have to be adjusted significantly from original settings.

IMPORTANT Erroneous results can occur if both light systems are active at the same time. Ensure that the appropriate light source is selected during the startup procedure.

10 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Start Up Procedure to startup the instrument.



6.2 RUN QC (QUALITY CONTROL) SAMPLES

Beckman Coulter recommends the use of QC materials to gauge instrument performance of reportable parameters. See Chapter 8, QUALITY CONTROL for details.

6.3 PERFORM SHUTDOWN

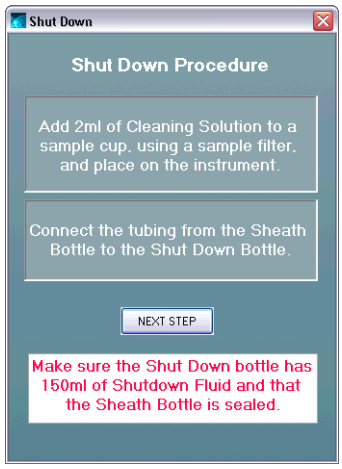
Perform this procedure at the end of each day.

Note: COULTER CLENZ can be used as the shutdown fluid if the instrument will be shutdown for a time period not to exceed one (1) day.

CAUTION Risk of damage to the instrument if you exit the Quanta application or turn off the instrument and computer without performing the Shutdown procedure. Perform the Shutdown procedure before you exit the Quanta application or turn off the instrument and computer to prevent damage to the instrument.

1 **Instrument ▸▸ ShutDown.** The following screen displays. Follow the directions on the screen to continue with the Shut Down Procedure to shutdown the instrument.

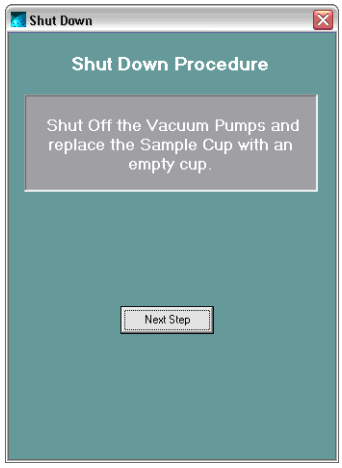
Refer to Figure 1.14, Reservoirs: Controls and Indicators to locate the appropriate containers.



CAUTION Instrument damage can occur if instrument is shut down for more than one (1) day using COULTER Clenz as the shutdown solution. Use Shutdown Solution, referenced in Table 1.1, List of Approved Reagents.

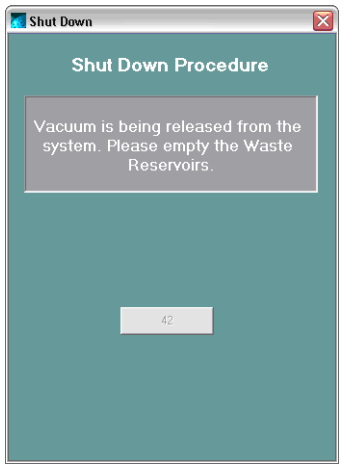
2 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Shut Down Procedure to shutdown the instrument.

Refer to Figure 1.14, Reservoirs: Controls and Indicators to locate the appropriate containers.

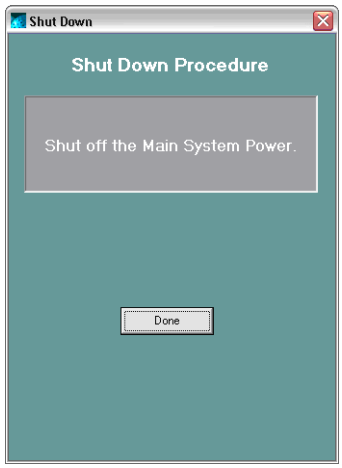


3 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Shut Down Procedure to shutdown the instrument.

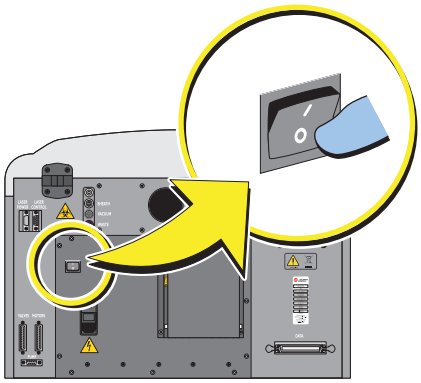
Refer to Figure 1.14, Reservoirs: Controls and Indicators to locate the appropriate containers and Heading 10.4, EMPTYING THE WASTE BOTTLE for proper waste removal.



4 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Shut Down Procedure to shutdown the instrument.



5 Turn OFF the Quanta instrument.

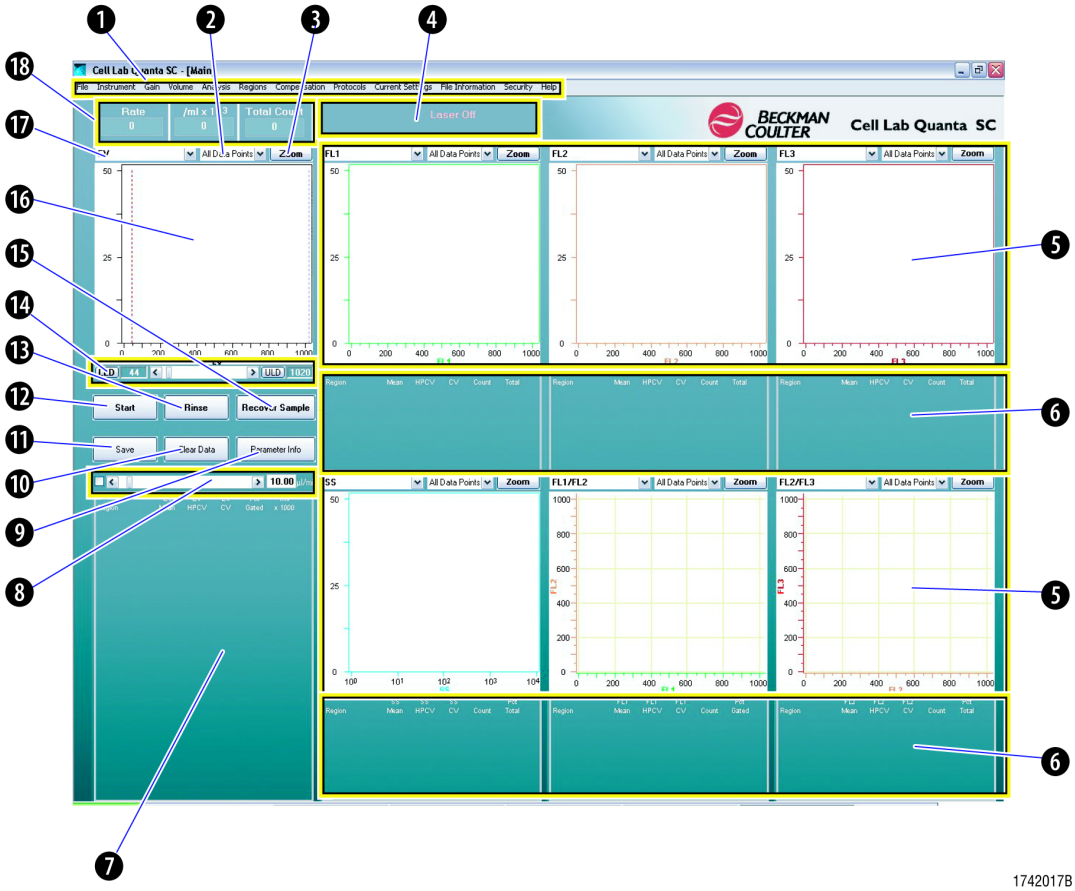


7.1 UNDERSTANDING THE MAIN SCREEN

The Main screen (Figure 7.1) can display up to ten graphs which may be either single or dual parameter. The seven graph display includes statistics under each graph for its corresponding regions. The X-axis of the first display is always the primary (trigger) parameter. The following parameters are available for collection:

- FL1 (Fluorescent Channel 1)
- FL2 (Fluorescent Channel 2)
- FL3 (Fluorescent Channel 3)
- SS (Side Scatter)
- EV (Electronic Volume)
- Time (must be selected on the Current Setting screen; see Heading 7.10, WORKING WITH THE CURRENT SETTINGS SCREEN); only displayed in the dual-parameter scatterplots.

Figure 7.1 Main Screen: Defined



❶ Main menu (see Figure 5.3)

❷ Select a region to be the gate for the display or select All Data Points. If a region is selected from the drop down list, then only data points within that region are shown on the display.

❸ Zoom button: Show pop-up screens that enlarge the display of the histograms and the scatterplots. Use the slide bar on the left side to adjust the Y-axis scale for single-parameter histograms.

❹ Message area that displays the Laser state, Compensation state, Data Flags and Protocol Name, as appropriate.

Note: If the settings are modified after a protocol is loaded, (modified) appears after the protocol name.

❺ Display areas for the parameter selected from the parameter drop down list.

The Main screen can display up to 10 graphs, each of which may be single or dual parameters, see Setting Up the Instrument for detailed instructions.

❻ Region statistics for the display directly located above area.

❼ Region statistics, gain and tracking windows are displayed in this area.

❽ Flow Rate Control bar: Controls the flow rate of the sample through the instrument. If the box is checked beside the bar, the instrument automatically adjusts the flow rate to keep the count rate constant during the sample run.

❾ Parameter Information button displays the Parameter Information dialog which allows users to customize certain parameter properties (Name, Color, etc.). For additional information about this dialog, see Parameter Information.

❿ Clear Data button resets the total count and erases all data points collected up to that point. This should only be done if the data collected is no longer desired. The rinse automatically does this between samples.

⓫ Save button saves the listmode data file in FCS 2.0 format to the operating system.

⓬ Aspirates sample into the instrument and begins data collection.

⓭ Rinse button cleans the fluidics and prepares the instrument for the next sample.

⓮ Lower Level Discriminator (LLD)/Upper Level Discriminator (ULD) bar: activates the scroll bar allowing you to adjust the selected discriminator value. Setting these values is important for every experiment.

Coordinate discriminator values with gain adjustments on the front of the instrument.

In most cases, the LLD should be set to a level where the noise signal is not visible or just slightly visible on the graph.

IMPORTANT Risk of invalid data collection or lost data if the discriminator values are not properly set.

⓯ Recover Sample button allows most of the sample that was aspirated into the instrument to be recovered from the sample loop. You can recover sample even if the run volume has been completely dispensed.

IMPORTANT Misleading results can occur due to the potential of dilution. Use care when selecting Auto Recover Sample.

⓰ X-axis is the primary parameter display; triggers data collection and determines which events are collected. There are five settings for this button: EV, FL1, FL2, FL3, SS.

Note:

- If this graph is set to FL1, FL2, or FL3 and fluorescence is not detected during data acquisitions, no data is collected.
- If the setting is for EV and no volume data is detected, no data is collected.
- If the setting is for SS and no side scatter is detected, no data is collected.

⓱ Drop down list of parameters available for collection, includes: FL1, FL2, FL3, EV, SS, Time, FLn-FSD, FLn-FC.

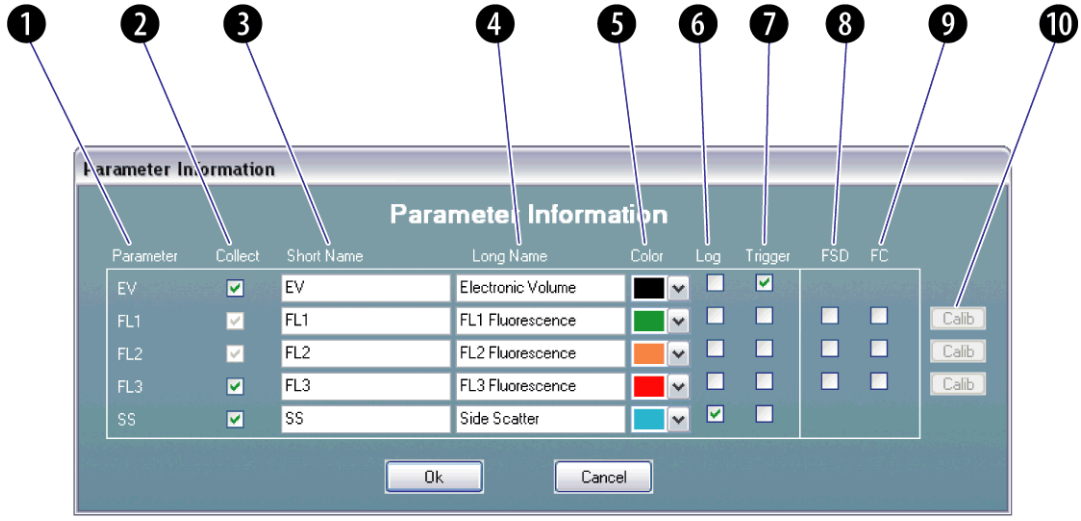
Note: FLn represents either FL1, FL2 or FL3. FSD (Fluorescence Surface Density); FC (Fluorescence Concentration).

⓲ Counting Display: Displays the count rate, concentration/mL x 10^3, and total count of what is being collected while the sample is running.

Parameter Information

The Parameter Information button displays a dialog box with the list of available parameters allowing the user to customize certain fields.

Figure 7.2 Main Screen: Parameter Information



❶ Parameter lists the available parameters for collection.

❷ Collect checkbox allows the user to select which parameters are to be collected.

❸ Short Name allows the user to customize the parameter name for buttons and labels; name length is limited to 9 characters.

❹ Long Name is saved to the FCS listmode file as $PnS keyword.

❺ Color drop down list allows the user to select a default color assigned to the histogram data.

IMPORTANT Misleading results can occur if the same color is assigned to parameters and regions. Do not assign the same color to a parameter if region has been assigned duplicate color.

❻ Log checkbox switches the parameter between linear and logarithmic data collection.

❼ Trigger checkbox determines the primary parameter.

❽ FSD checkbox determines the fluorescence signal of the parameter to be used for FSD data collection.

Note: FSD or FC, not both, can be checked.

❾ FC checkbox determines the fluorescence signal of the parameter to be used for FC data collection.

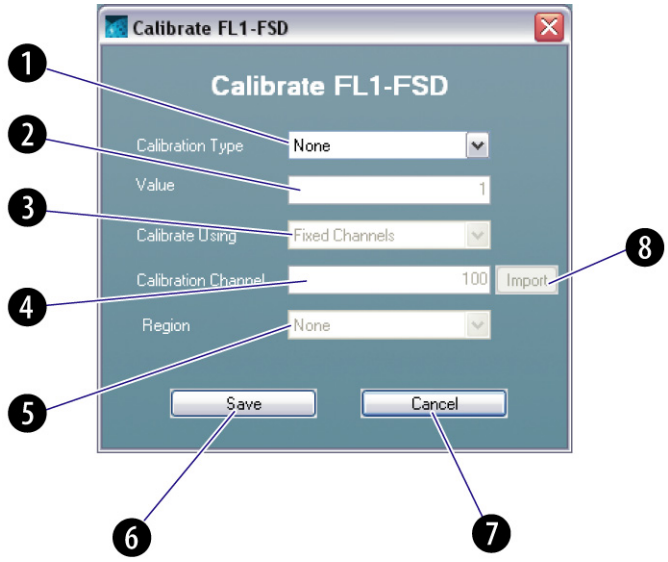
Note: FSD or FC, not both, can be checked.

❿ Calib (Calibration) button when pressed allows calibration of FSD for antigen density or FC for NPE and absolute concentration fluorescence ratios, see Figure 7.3.

Calibrate FSD or FC

For detailed information regarding the FSD or FC calculated parameter, refer to Calculated Parameters found in Chapter 4.

Figure 7.3 Main Screen: Calibrate



❶ Calibration Type drop down list displays the type of antigen density, absolute concentration, NPE analysis.

❷ Value field allows the user to input the assay value for calibration.

❸ Calibrate Using drop down list allows the user to selection Region or Fixed Channel for calibration.

❹ Calibration Channel field allows the user to input the channel manually for calibration.

❺ Region drop down list allows the user to select a region around the standard to be used for calibration.

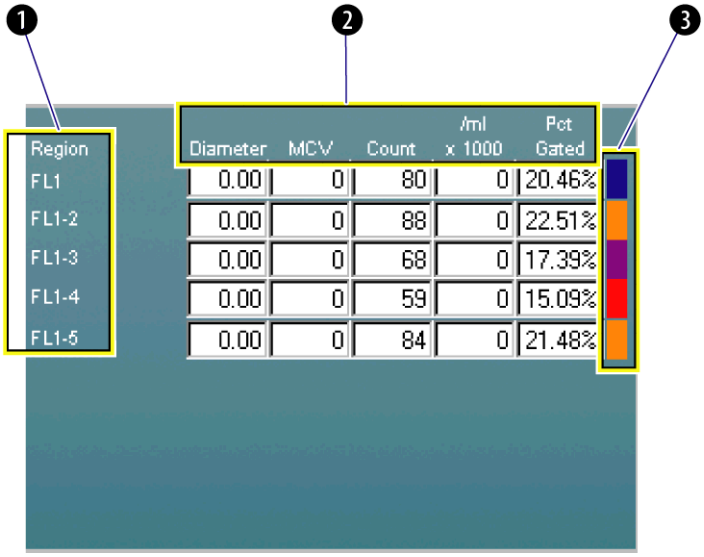
❻ Save button saves your settings and returns to the Parameter Information dialog.

❼ Cancel button returns you to the Parameter Information dialog with updating the settings.

❽ Import button allows the user to import the current value of the selected region to be used for calibration

Region Statistics

Figure 7.4 Main Screen: Region Statistics



❶ Region name can be double-clicked to edit the region, see Heading 7.7, WORKING WITH THE REGIONS MENU.

❷ Column header names can be double-clicked to edit the heading label.

❸ Double-clicking the desired parameter color displays the Region Statistics screen, see Heading 7.7, WORKING WITH THE REGIONS MENU.

7.2 WORKING WITH THE FILE MENU

The File menu (Figure 5.4) allows you to save files, playback files, generate reports, export data, and print files.

For details, see:

- Saving Listmode (.LMD) Files
- Auto Save Option
- Playing Back Files
- Defining Playback Options
- Generating a Microsoft Excel Report
- Exporting Data to Excel

- Printing
- Exiting (Closing) the Application

Saving Listmode (.LMD) Files

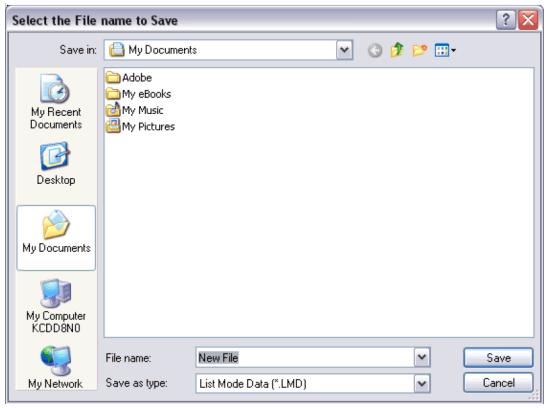
Perform this procedure to save the data to the disk as a listmode FCS 2.0 file.

---

1 **File** ▸▸ **Save**.

---

2 Select the desired folder where you want the file to be saved.



---

3 Type the file name.

---

4 **Save**.

Auto Save Option

Perform this procedure to automatically save or print results as defined in Heading 7.10, WORKING WITH THE CURRENT SETTINGS SCREEN. When enabled, this option allows for the LMD file, reports, Excel Files to be saved or printed and to perform flagging if Data Flags were defined.

1 **File** ▸▸ **Auto Save**.

Note: When Auto Save is unavailable, it will appear gray in the File Menu.

2 Browse to indicate the location where the files are to be stored.

Playing Back Files

Perform this procedure to simulate running files that have previously been run. You can also modify the file and re-save it with the new information.

IMPORTANT Erroneous results can occur if using FCS 2.0 files that have been generated on other systems, other than the Quanta instruments.

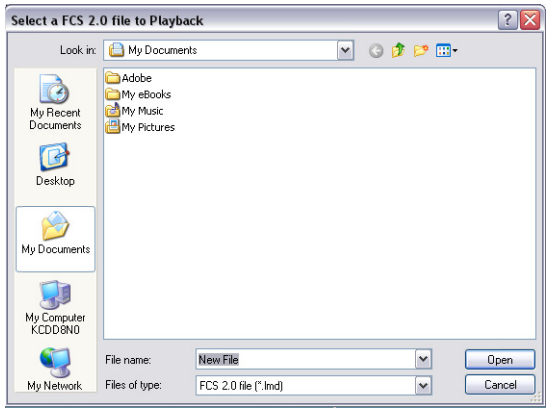
1 **File** ▸▸ **Playback**.

2 Select the desired file to be played back:

a. Locate the file in the appropriate folder.

b. Select the file.

c. **Open**.



3 During file playback, a slider appears on the left of the first histogram that allows you to control the speed of the file playback.

Note: Return to normal operation of acquiring data from the instrument by **File ▸▸ Normal Mode**.

Defining Playback Options

Perform this procedure to define playback options, which allow you to customize the playback information. The options include:

Quick Playback allows you to view the results of a past run without having to wait through the length of collection time.

Playback Regions, **Playback Volume**, and **Playback Compensation Value** (when selected) play back the chosen file using the file's original settings. If these options are not selected, the files play back using the instrument's current settings.

1 **File ▸▸ Playback Options**.

2 Select the desired playback options:

a. ☐ next to the desired option.

b. ☑ appears when the option is selected.

Note: To deselect an option, ☑ until ☐ appears.

3 **OK** to save the options.

Generating a Microsoft Excel Report

Perform this procedure to generate an Excel report based on the currently selected Template file, see Heading 7.10, WORKING WITH THE CURRENT SETTINGS SCREEN. If no Template is selected in the protocol, then one of the Standard Template files will be used based on the number of parameters configured, or you can create a customer Template file.

---

1 **File ▸▸ Excel Report**. to open the currently selected Excel template populated with the active File Information.

Exporting Data to Excel

Perform this procedure to send raw listmode data to an Excel file.

---

1 **File ▸▸ Export Data to Excel**.

Printing

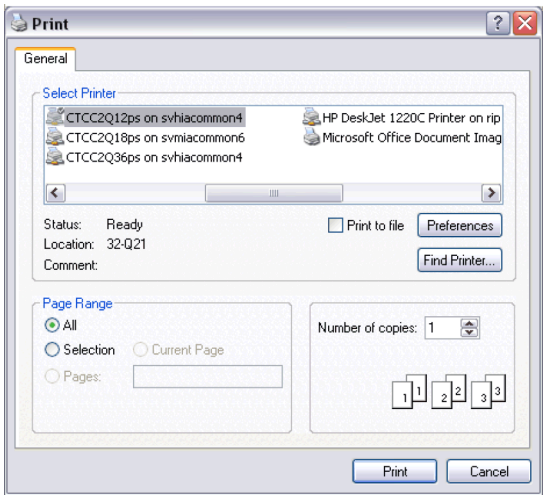
Perform this procedure to print. If you want file information printed along with the data.

---

1 **File ▸▸ Print**.

---

2 Select the desired printer and print settings.



3 **Print**.

Exiting (Closing) the Application

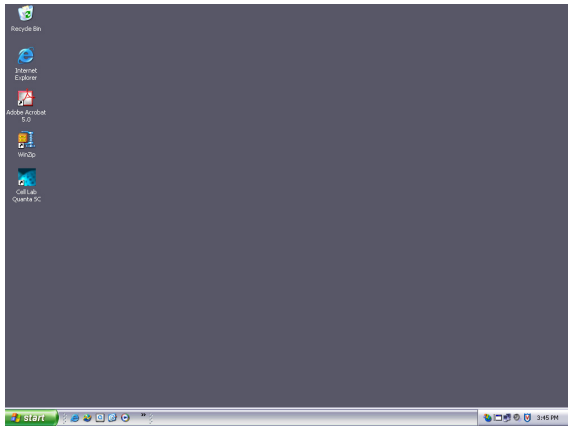
Perform this procedure to close the application, which automatically sends a Valves Off command to the instrument.

---

1 **File** ▸▸ **Exit**.

---

2 Your Windows desktop appears after the Quanta software is closed.



7.3 WORKING WITH THE INSTRUMENT MENU

The Instrument menu (Figure 5.5) allows you to do the following procedures. This menu is disabled after playing back a listmode file; select **File** ▸▸ **Normal Mode** to end Playback Mode.

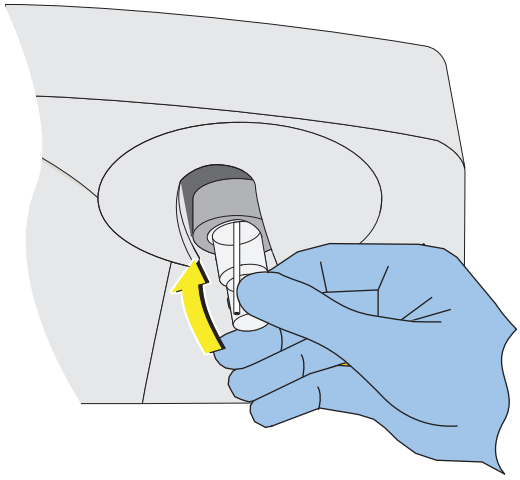
- Start Up
- Shut Down
- Defining the Power Setting
- Running the Cleaning Cycle
- Flushing
- Fill Cup
- Resetting Fluid Count
- Setting Up the Instrument
- Importing Settings
- Automatically Aligning the Optics

Start Up

Perform this procedure to do a Start Up routine on the instrument.

---

1 Place an empty sample cup on the instrument.

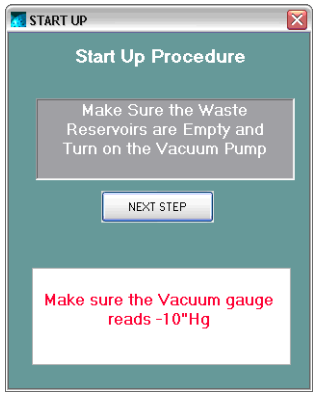


---

2 **Instrument** ▸▸ **Start Up**.

---

3 Follow the instructions on the screen. Refer to Heading 6.1, PERFORM STARTUP for detailed instructions.



---

4 **Next Step**. The screen will prompt you through the proper procedure to startup the instrument.

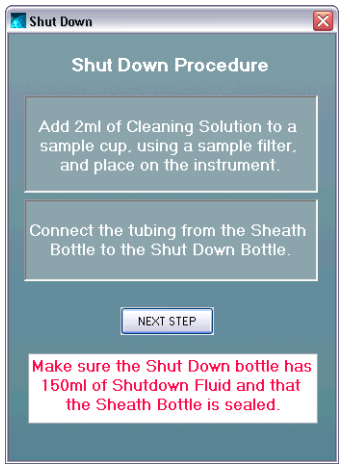
IMPORTANT Erroneous results can occur if both light systems are active at the same time. Ensure that the appropriate light source is selected during the startup procedure, refer to Heading 6.1, PERFORM STARTUP for detailed instructions.

Shut Down

Perform this procedure run a Shut Down routine on the instrument.

1 **Instrument** ▸▸ **Shut Down**.

2 Follow the instructions on the screen. Refer to Heading 6.3, PERFORM SHUTDOWN for detailed instructions.



3 **Next Step**. The screen will prompt you through the proper procedure to shutdown the instrument, see Heading 6.3, PERFORM SHUTDOWN.

Defining the Power Setting

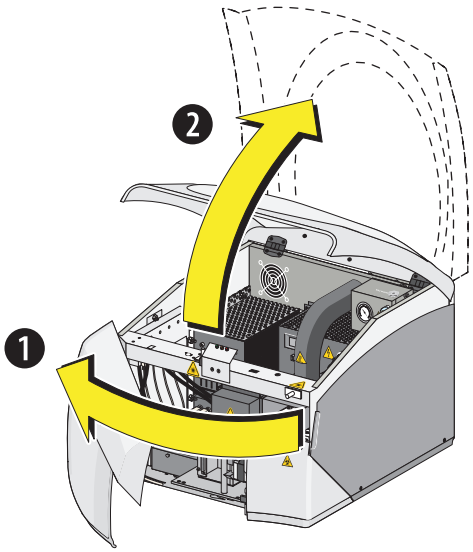
After the lamp has completed its initial warm up cycle of about 20 minutes, perform this procedure once weekly to define the correct setting for the arc lamp potentiometer located on the power supply next to the voltage display.

---

1 **Instrument** ▸▸ **Power Setting**.

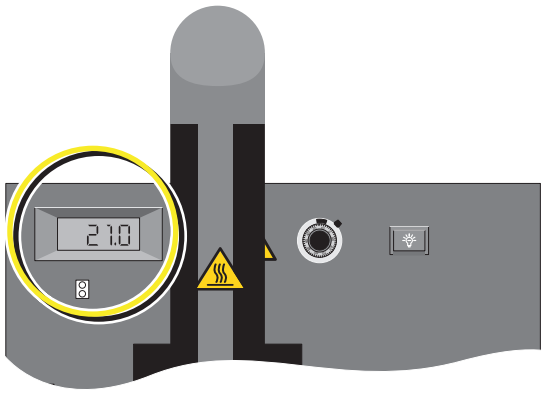
---

2 Open the cover.



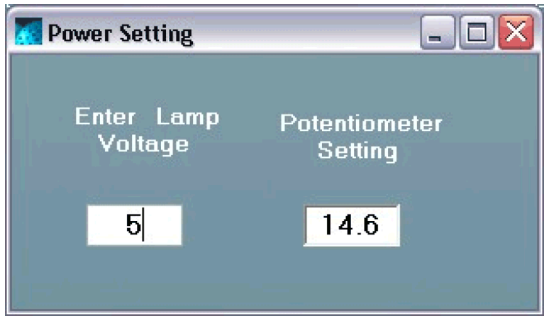
---

3 Obtain the current voltage from the power supply inside the instrument. Voltage reading should be between 19-28 volts.

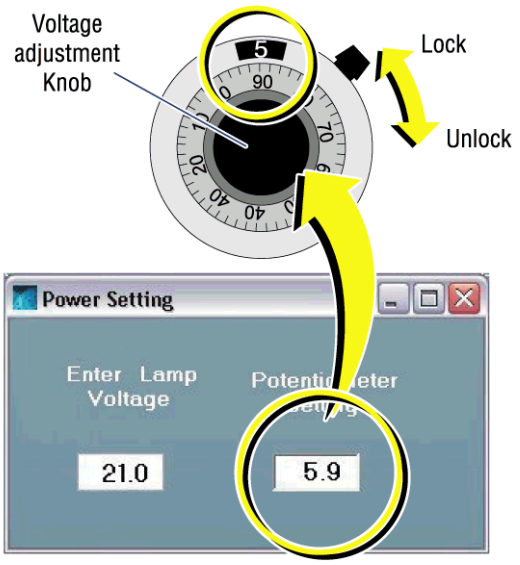


---

4 Type the lamp voltage; notice that the Potentiometer Setting automatically changes.



5 Set the Potentiometer to the new Potentiometer setting value.



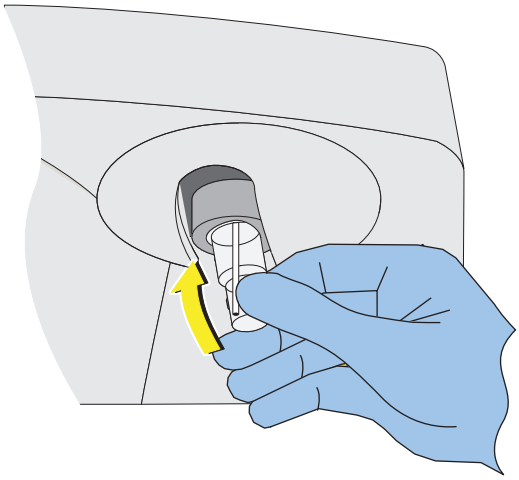
6 Record the lamp potentiometer setting in the instrument logbook per your laboratory requirements.

Running the Cleaning Cycle

Perform this procedure to remove flow cell clogs. The system runs the cleaning solution through the aperture at a high speed.

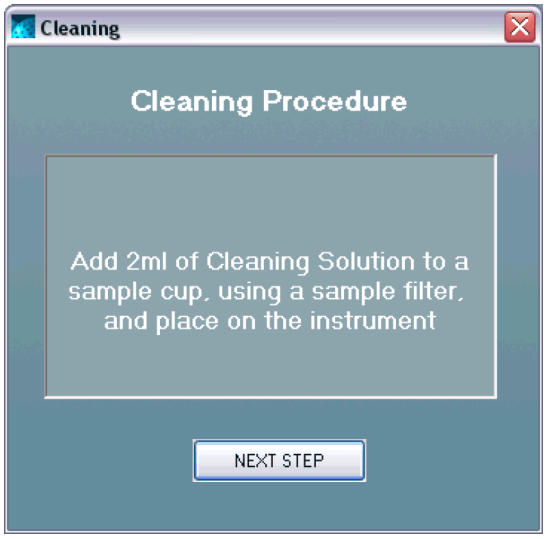
---

1 Place an empty sample cup on the instrument.



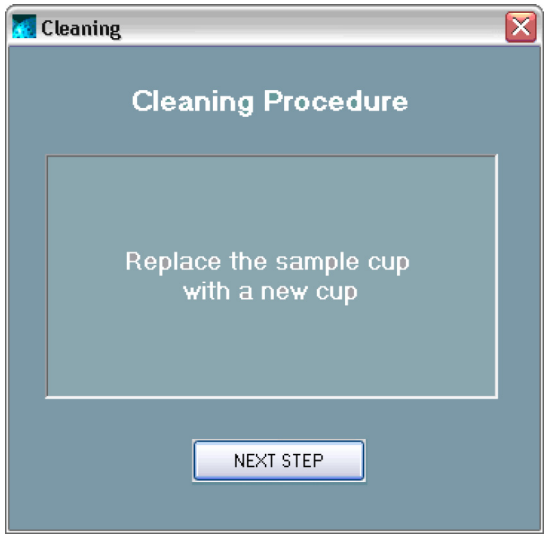
---

2 **Instrument** ▸▸ **Cleaning Cycle**. The following screen displays. Follow the directions on the screen to continue with the Cleaning Procedure.



---

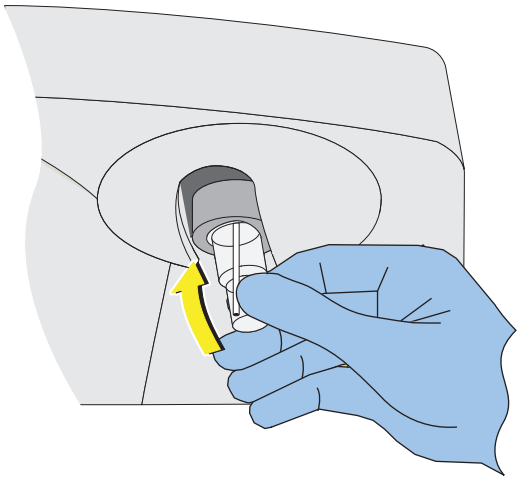
3 **Next Step**. The following screen displays. Follow the directions on the screen to continue with the Cleaning Procedure.



Flushing

Perform this procedure to empty fluid from the electrodes and certain areas of the flow cell and to refill the locations. The system does this by running sheath fluid through the flow cell at a high speed.

1 Place an empty sample cup on the instrument.

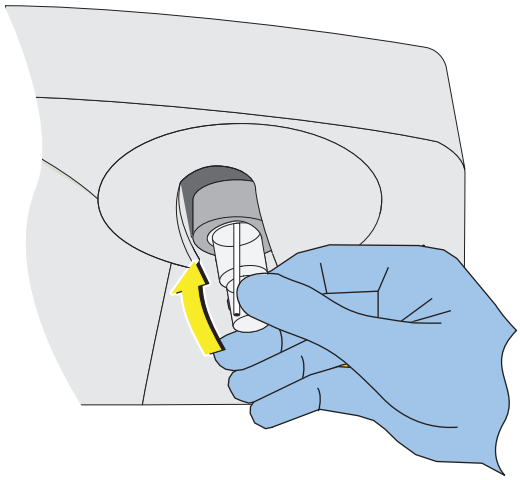


2 **Instrument ▸▸ Flush (F)**.

Fill Cup

Perform this procedure to fill the sample cup with diluent.

1 Place an empty sample cup on the instrument.



2 **Instrument ▸▸ Fill Cup**.

Resetting Fluid Count

Perform this procedure to reset the fluid count after the sheath bottle has been emptied and re-filled or after the waste container has been emptied to ensure proper level sensor monitoring.

1 **Instrument ▸▸ Reset Fluid Count**.

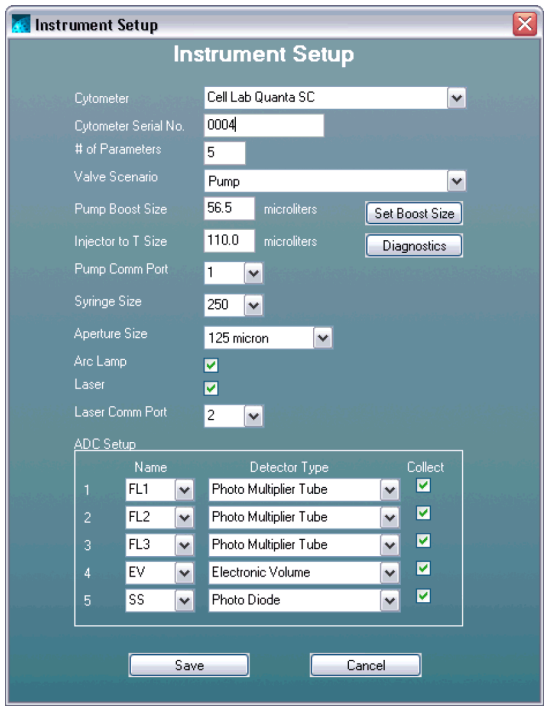
2 **OK**.

Setting Up the Instrument

Only your Beckman Coulter Representative can perform this procedure to define the instrument settings. For additional information on the Instrument Setup screen, see Understanding the Instrument Setup Screen.

1 **Instrument** ▸▸ **Instrument Setup**.

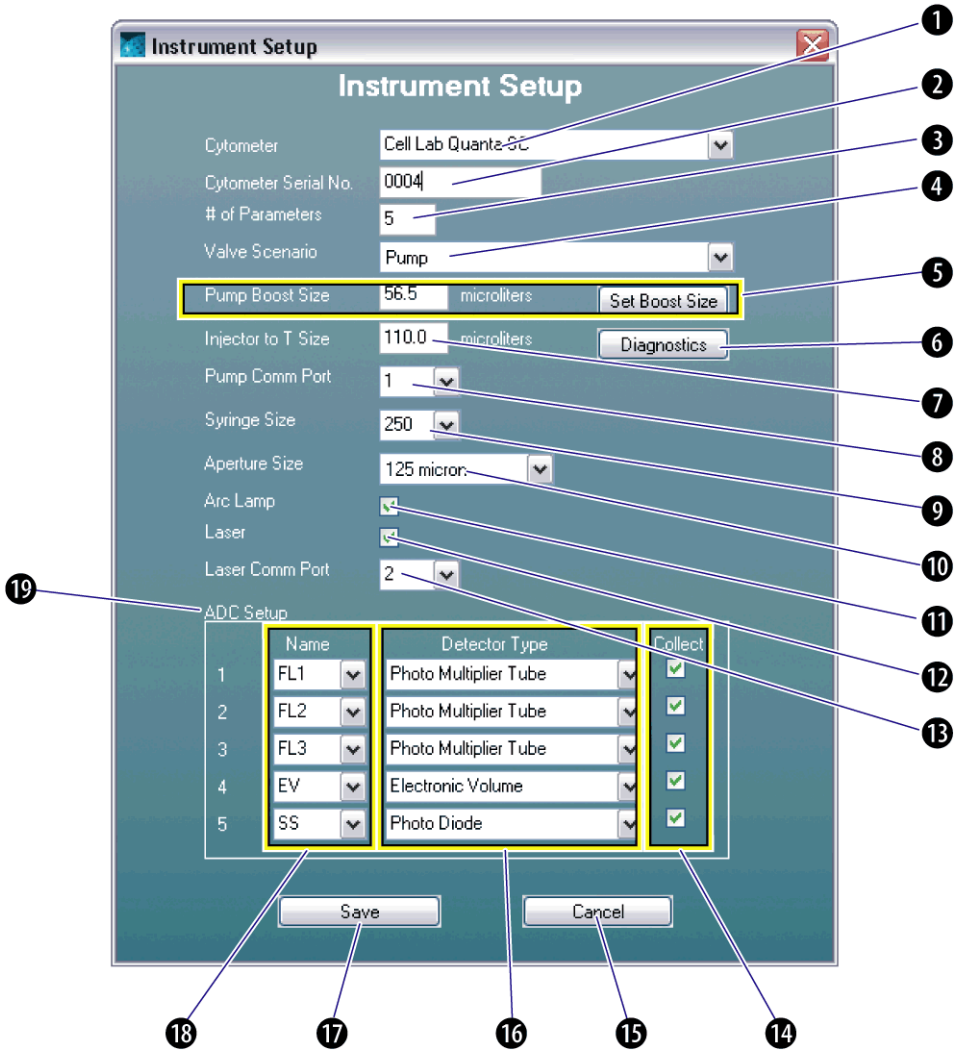
2 This screen is displayed for reference information only. The user cannot modify any settings which appear on this screen display.



Understanding the Instrument Setup Screen

See Figure 7.5.

Figure 7.5 Instrument Setup Screen: Defined



❶	Identifies the instrument model	❷	Identifies the serial number on back of the instrument
❸	Defines the maximum number of parameters that the instrument can collect	❹	Identifies the valve scenario (e.g. Pump) used by the instrument
❺	Defines the sample volume (about 54.5 µL) that must be boosted from the sample loop to the flow cell. Set Boost Size button allows you to automatically configure the boost size.	❻	Diagnostics button displays the Diagnostics screen.
❼	Defines the sample volume (about 57.0 µL) from the aspirator tip to the first T, where valve 7 is located	❽	Defines the comm port on the PC where the metering pump is connected

❾ Defines the syringe size

❿ Defines the aperture size

⓫ Indicates (when checked) that an arc lamp is present on the instrument

⓬ Indicates (when checked) that a laser is present on the instrument

⓭ Defines the com port on the PC where the laser is connected

⓮ Collect checkbox turns ADC on or off. These options are available to Service only.

⓯ Cancels unsaved settings.

⓰ Detector Type drop down list provides the detection options for each parameter to be collected. These options are available to Service only.

⓱ Saves settings.

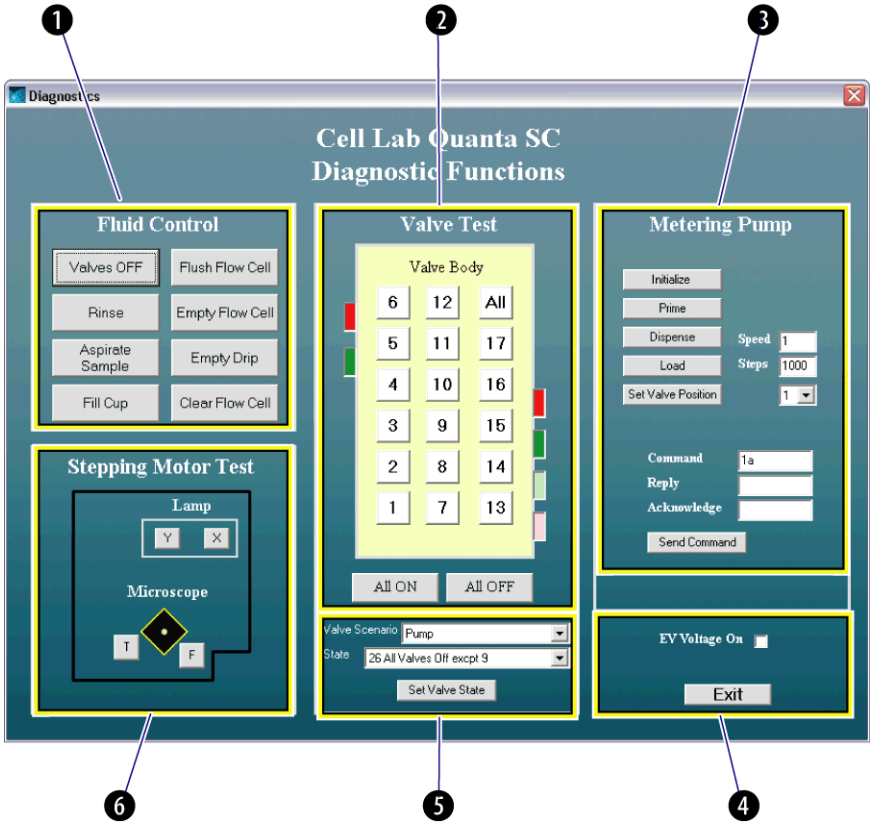
⓲ Parameter Name drop down list allows the selection of the parameter to be used for collection. These options are available to Service only.

⓳ ADC Setup box contains configurable parameter name, detector type and on/off checkbox. These options are available to Service only.

Understanding the Diagnostic Functions Screen

See Figure 7.6.

Figure 7.6 Diagnostic Functions Screen: Defined



CAUTION Instrument damage can occur if diagnostics functions are attempted by the user without supervision of a Beckman Coulter Representative. Do not attempt to perform these Diagnostic Functions without direction from a Beckman Coulter Representative.

❶ This section is used to activate different fluidics functionality.

❷ This section is used to individually verify functionality of the valves or different programmed valve scenarios.

❸ This section is used to activate, control and manage the syringe pump performance.

❹ This section is used to activate, control and manage the syringe pump performance.

❺ This section is used to individually verify functionality of the valves or different programmed valve scenarios.

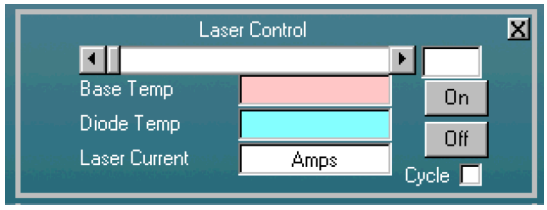
❻ This section is used to activate the four stepping motors for Auto Alignment and Lamp functions.

Laser Control

Perform this procedure to adjust the laser power.

---

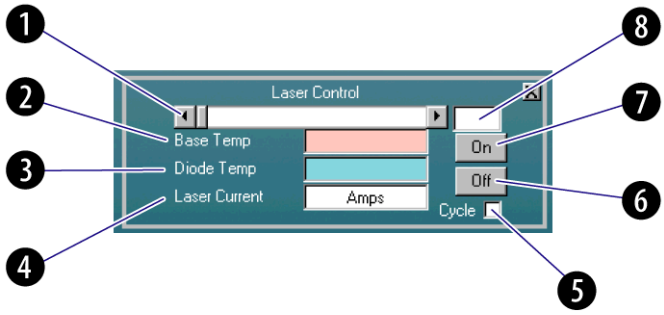
1 **Instrument ▸▸ Laser Control**.



Understanding the Laser Control Options

See Figure 7.7.

Figure 7.7 Laser Control Options: Defined



❶ Scroll bar adjusts the laser power to a lower value to optimize CVs when the sample is extremely bright and is flooding the detectors.

Note: Laser Power is normally set to 22 mW.

❷ Displays the container temperature.

❸ Displays the temperature of the diode.

❹ Displays the laser current.

❺ Cycle checkbox when enabled turns the laser ON at the start of acquisition and OFF when acquisition stops.

If unchecked, laser remains ON at all times.

❻ Off button turns the laser OFF.

Note: If Cycle checkbox not enabled, turn OFF the laser when not using the instrument.

❼ On button turns the laser ON.

❽ Laser Power displayed in mW.

Importing Settings

Perform this procedure to import instrument settings and protocols from other software versions transferred to your instrument. For additional information, see Understanding the Import Settings Screen.

---

1 **Instrument** ▸▸ **Import Settings**.

---

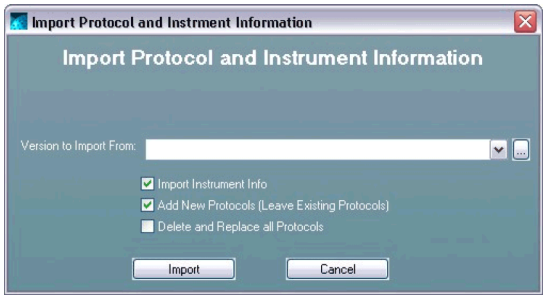
2 Select the desired options:

a. ... to browse your computer for the desired database file (Quanta.mdb).

b. ☐ next to the desired option.

c. ☑ appears when the option is selected.

Note: To deselect an option, ☑ until ☐ appears.



---

3 **Import**.

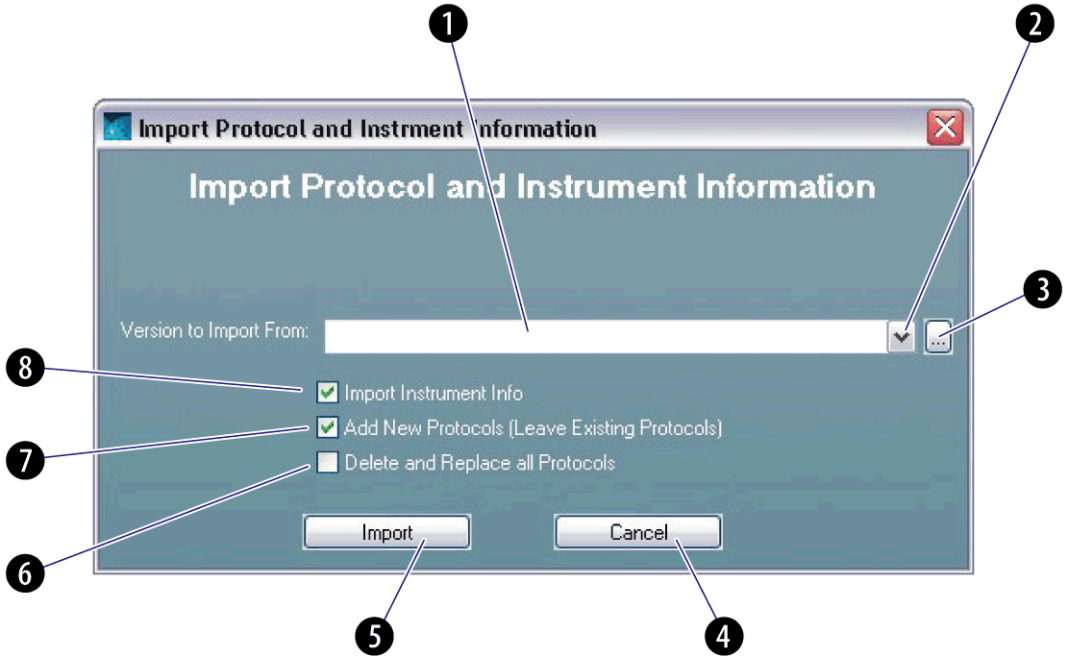
a. When the import is complete, an *Import Complete* message appears.

b. **OK** to return to the main screen.

Understanding the Import Settings Screen

See Figure 7.8.

Figure 7.8 Import Setting Screen: Defined



1	Version/File from which to import	2	Installed versions from which to select
3	Browse to pick a file rather than type in a name	4	Cancels unsaved settings.
5	Button to perform the import	6	Replace existing protocols with the same name
7	Import protocols yes/no	8	Import instrument settings yes/no

Automatically Aligning the Optics

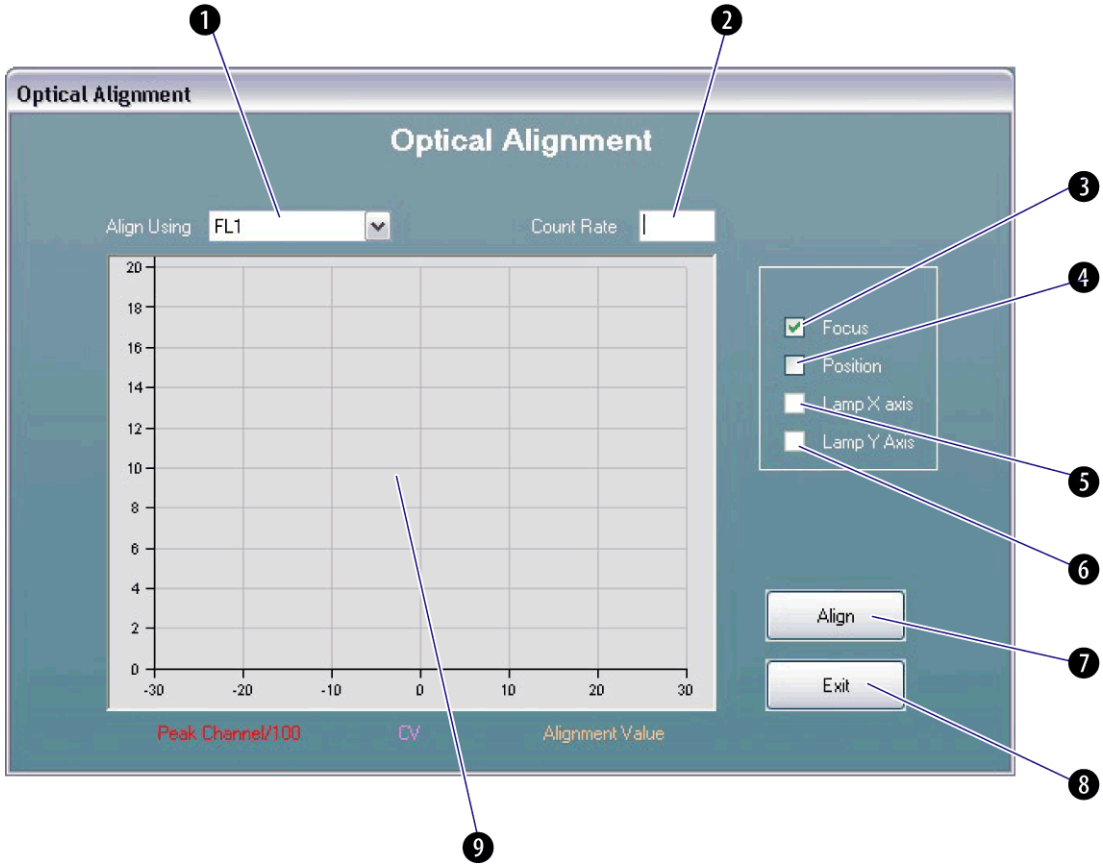
Note: This menu option is only available when running a sample.

Perform this procedure if your HPCVs are still not within specifications. For detailed instructions, refer to Heading 8.5, AUTOMATICALLY ALIGNING THE OPTICS.

Understanding the Auto Optical Alignment Screen

See Figure 7.9.

Figure 7.9 Auto Optical Alignment Screen: Defined



❶	Align Using drop down list selects the fluorescence parameter that corresponds to the beads being run.	❷	Count Rate displays the count rate.
❸	Focus checkbox when enabled adjusts the microscope focus when Align button is selected.	❹	Position checkbox when enabled adjusts the microscope position when Align button is selected.
❺	Lamp X axis checkbox when enabled adjusts the arc lamp along the X-axis when the Align button is selected.	❻	Lamp Y axis checkbox when enabled adjusts the arc lamp along the Y-axis when the Align button is selected.
❼	Align button begins the automatic alignment.	❽	Exit button closes the Optical Alignment window.
❾	Graph displays the Peak Channel, CV and Alignment value at each motor position during alignment.		

7.4 WORKING WITH THE GAIN MENU

For additional information, see Understanding the Tracking Settings Screen.

Tracking is the auto gain adjustment done by the computer to keep a known sample in the same channel number. As the Mercury Arc lamp ages, its intensity increases. The effects of the arc lamp's aging are usually nominal, but tracking may be necessary on runs where maximum resolution is needed to separate close populations.

The Gain menu (Figure 5.6) allows you to do the following procedures:

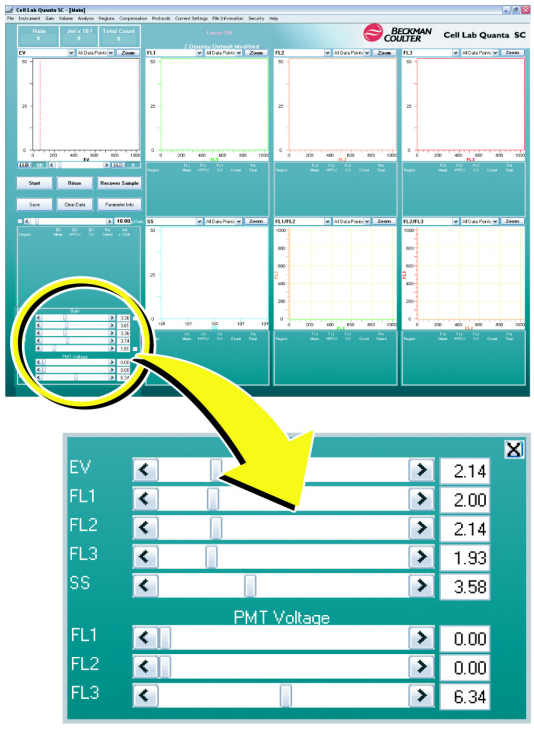
- Show Gain Settings
- Defining Tracking Settings
- Tracking Start
- Reset Tracking

Show Gain Settings

Perform this procedure to display the Gain Settings on the Main Screen of each fluorescent parameter and PMT voltage of the fluorescence parameters.

---

1 **Gain ▸▸ Show Gain Settings.**



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Defining Tracking Settings

Perform this procedure to define the tracking settings.

---

1 **Gain** ▸▸ **Tracking Settings**.

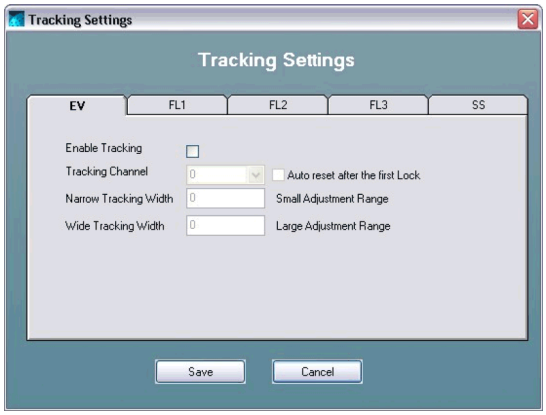
---

2 Select the specific parameter tab to modify the tracking settings.

a. the Enable Tracking box.

b. Enter the Narrow Tracking Width and Wide Tracking Width.

c. **Save** to close dialog box and save your new settings or **Cancel** to discard the updated settings.



---

3 To begin Tracking, **Gain** ▸▸ **Tracking Start**.

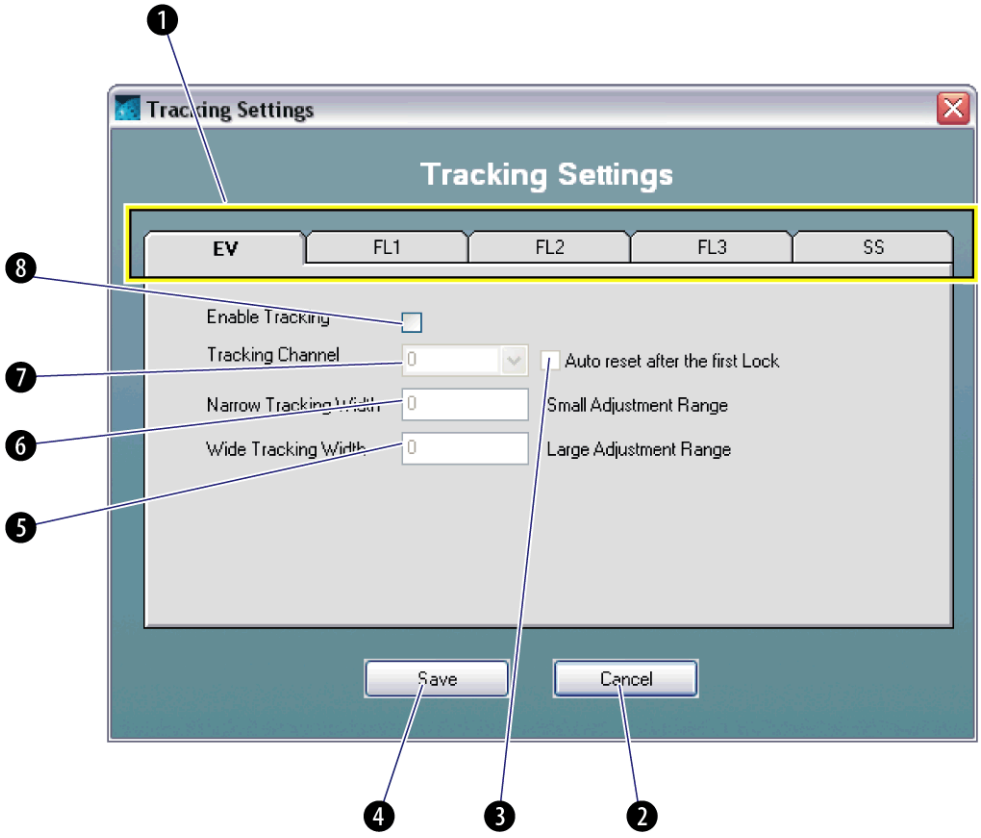
---

4 To stop Tracking, **Gain** ▸▸ **Tracking Stop**.

Understanding the Tracking Settings Screen

See Figure 7.10.

Figure 7.10 Tracking Settings Screen: Defined



❶	Parameter tabs appear in order defined by the user depending upon the trigger parameter and order set.	❷	Select to cancel your setting changes.
❸	Enable checkbox to clear the data after the standard is first locked to the correct channel	❹	Select to save your setting changes
❺	Set to the width of the standard peak	❻	Set to a value approximately equal to one-half of the standard peak width
❼	Channel in which the standard should reside	❽	Enable checkbox to track the channel on this parameter tab

Tracking Start

Tracking is the auto gain adjustment performed by the computer to keep a known sample within the same channel number. This adjustment may become necessary as an arc lamp ages and its intensity increases. The effects of arc lamp aging is not usually large; however, tracking may be required for maximum resolution on long sample runs.

Perform this procedure to start tracking. The button toggles between Start and Stop. It controls if tracking is ON or OFF.

---

1 **Gain ▸▸ Tracking Start**.

---

2 The Tracking Display on the Main screen overlays the Region Display. By default, when tracking is enabled, the Tracking Display is set to visible.

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Reset Tracking

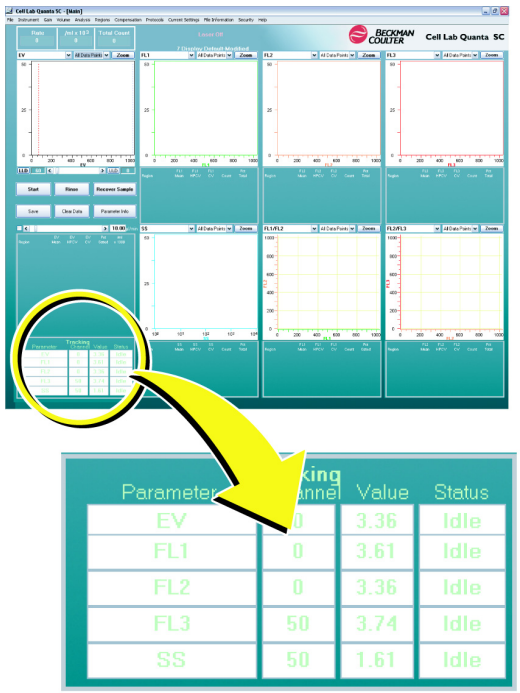
Perform this procedure to restore the gain settings to their original values before tracking started.

---

1 **Gain** ▸▸ **Tracking Reset**.

---

2 The Tracking Display on the Main screen is reset when this selection is pressed.



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7.5 WORKING WITH THE VOLUME MENU

The Volume menu (Volume Menu) allows you to calibrate the volume and display channels. For details, see:

- Calibrating the Volume
- Displaying Channels

Calibrating the Volume

Perform this procedure to calibrate the EV axis of the data graphs and the mean cell volume, diameter and surface area statistics to absolute units. To ensure proper calibration, use the correct size of Coulter Calibration beads. For additional information, see Understanding the Volume Calibration Screen.

Calibration Beads

Dilute the Coulter Calibration beads as follows:

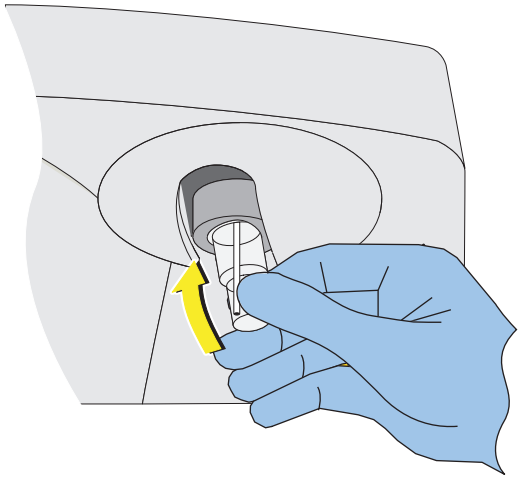
Bead µm	Part Number	Dilution Ratio
1 µm	6602790	1:2000
2 µm	6602792	1:5
3 µm	6602793	1:5
5 µm	6602794	1:3
10 µm	6602796	1:2
20 µm	6602798	1:1

---

1 Place 1 mL of beads in a sample cup.

a. Mix gently and place onto sample holder.

b. This sample will be used to set up the single parameter gate region.



---

2 **Start**.

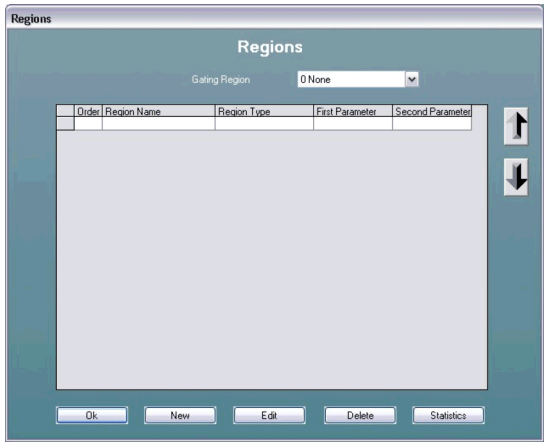
---

3 Adjust the EV gain to place the beads in Channel 200 on the EV graph. Refer to Heading 7.4, WORKING WITH THE GAIN MENU for detailed instructions.

---

4 Adjust the lower discriminator to eliminate the noise. Refer to Heading 7.1, UNDERSTANDING THE MAIN SCREEN for detailed instructions.

5 Go to the Regions Menu and set up a Single Parameter Region. Refer to Managing Regions for detailed instructions on setting up this region.



6 Collect at least 5000 data points.

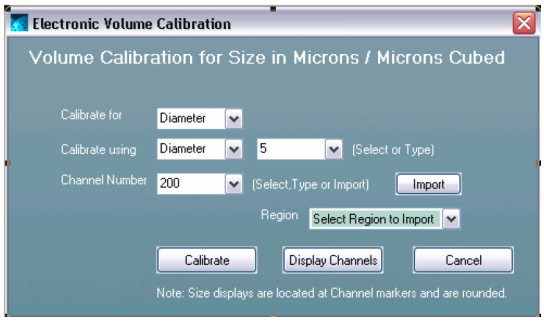
a. **Stop** to stop the sample.

7 **Volume** ▸▸ **Calibrate**.

a. Select **Diameter** from the **Calibrate For** and **Calibrate Using** drop down lists.

b. Insert the diameter assayed value of the beads in the drop down list to the right of **Calibrate using** list. Refer to the COULTER CC Size Standard reagent package insert for the assay values.

c. **Import** and select the region around the beads. The Mean Channel of the region should appear in the box labeled **Channel Number.**



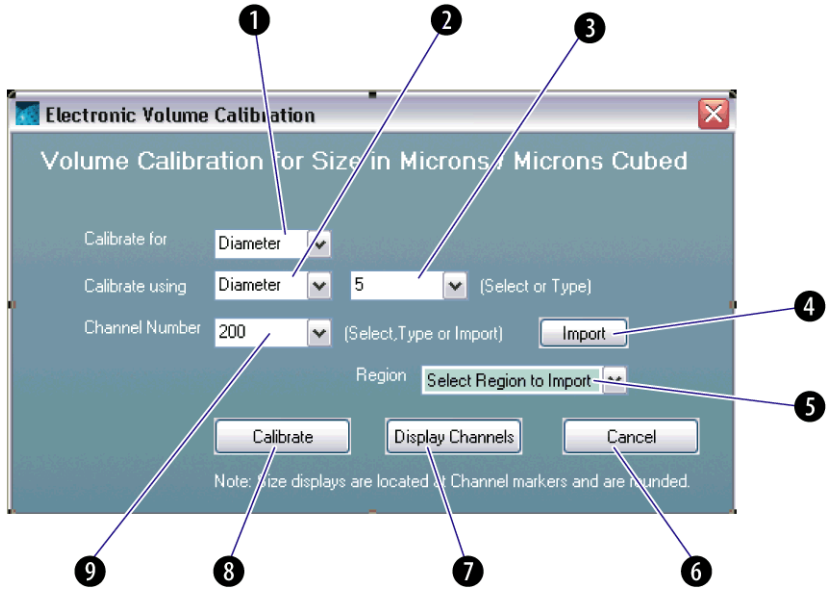
8 **Calibrate.**

Note: Volume automatically recalibrates if the EV gain is adjusted after calibration.

Understanding the Volume Calibration Screen

See Figure 7.11.

Figure 7.11 Volume Calibration Screen: Defined



❶	Volume/Diameter selection for display	❷	Volume/Diameter selection on standard
❸	Bead size	❹	Import button allows the calibration to be based on a region, the mean of which becomes the Channel Number
❺	Drop down list from which to select region to import	❻	Cancels unsaved settings
❼	Clear the calibration and display channel numbers	❽	Apply the calibration
❾	Channel Number where the calibration beads appear		

Displaying Channels

Perform this procedure if you want to change the X-axis from microns to channel numbers.

For additional information, see Understanding the Volume Calibration Screen.

---

1 **Volume ▸▸ Display Channels**.

---

2 The instrument resumes normal flow channel display.

7.6 WORKING WITH THE ANALYSIS MENU

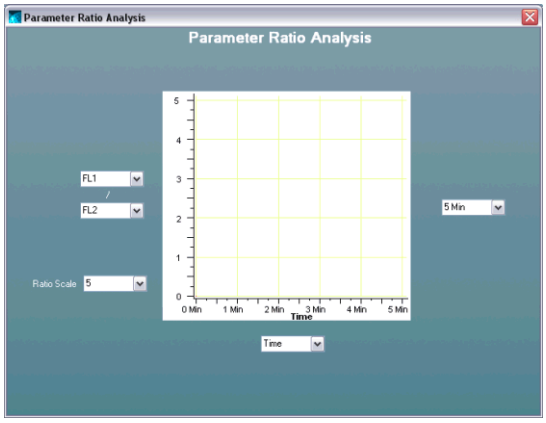
The Analysis menu (Figure 5.9) allows you to analyze the Parameter Ratio and Data Flag Settings of each parameter. See:

- Parameter Ratio Analysis
- Data Flag Settings

Parameter Ratio Analysis

Perform this procedure to determine the ratio between any of the parameters from the drop down lists.

1 **Analysis ▸▸ Parameter Ratio Analysis.**

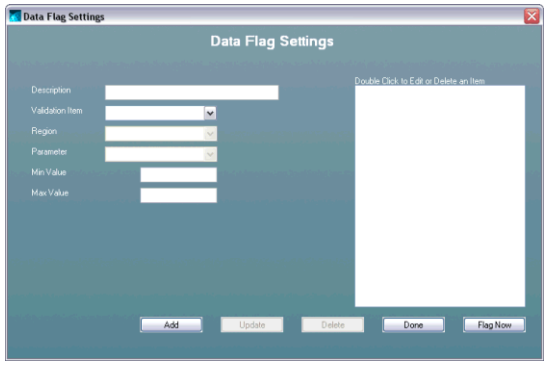


Data Flag Settings

Perform this procedure to specify the region, parameter, and statistic of each data flag. You can assign a lower and upper limit to any statistic that is displayed. If the statistic value falls outside of the limits, it will be highlighted in red on the Main screen and the Excel Report.

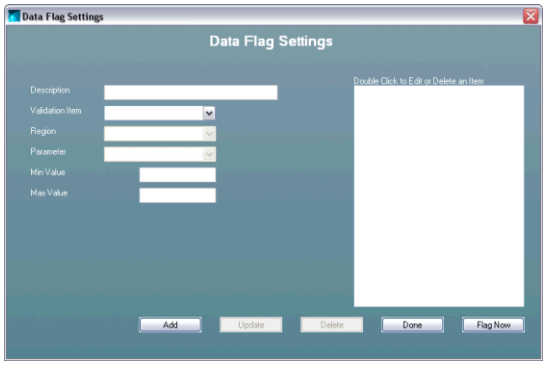
---

1 **Analysis** ▸▸ **Data Flag Settings**. The following window displays.



---

2 **Validation Item**, **Region** and **Parameter** from the drop down lists for each flag to be defined.



a. Enter a Minimum and Maximum value.

b. **Add** to include the flagging item in the Data Flag Setting list on the right of the window.

Note: To set up a flag for Mean Diameter, Mean Surface Area or Mean Cell Volume, you must first calibrate Volume, see Calibrating the Volume for detailed instructions.

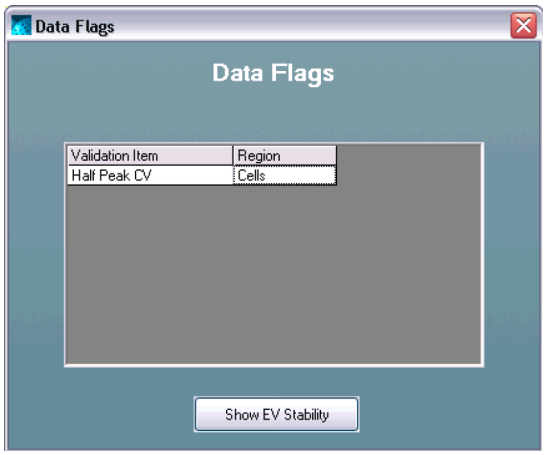
c. **Update** to save changes made to an existing data flag.

d. **Delete** to delete a highlighted flag from the list on the right of the window.

e. **Done** when finished setting up data flags to exit the window.

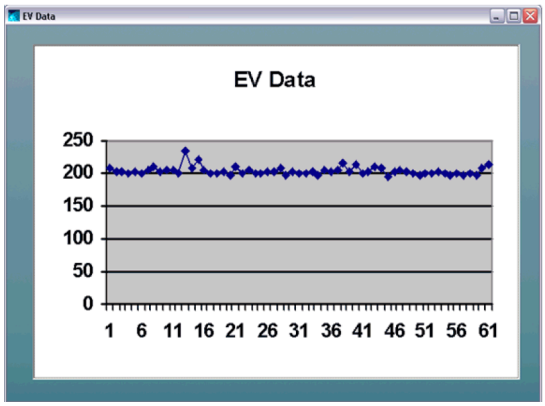
3 **Flag Now** to activate data flagging at any time.

Note: If any of the statistics are flagged, the message Data Flag is displayed on the Main screen. the message to display a list of the statistics outside of the established limits.



a. **Show EV Stability** to view a graph of the EV Mean Channel versus Time for the most recent run.

Note: Fluctuations in the graph can occur if there was a clog or a bubble in the system.



7.7 WORKING WITH THE REGIONS MENU

The Regions menu (Figure 5.9) allows you to manage regions and to show region statistics. See:

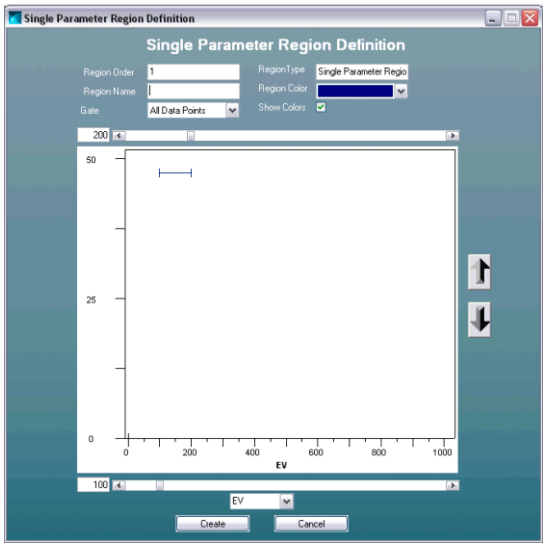
- Managing Regions
- Showing Region Statistics

Managing Regions

Perform this procedure to create, edit, or delete region. For additional information, see Understanding the Manage Regions Screen.

1 **Regions**.

2 **New**, see Figure 7.13, Single Parameter Region Definition Screen: Defined.



a. Choose the type of region you want to create:

- **Ellipse**: An elliptical area of the graph.
- **Polygon**: A multi-sided, closed figure.
- **Quadrant**: A vertical and a horizontal line, that divides the graph into four (4) areas.
- **Single Parameter**: A continguous area of a single-parameter graph.
- **Parameter Divider**: A vertical line that separates a single-parameter graph into two (2) areas.
- **Logical Regions**: These region types allow gating logic to be applied to a histogram using the overlap of two (2) or more existing regions.

b. **Create**.

3 Fill in the Region Order, Region Name, Region Type, Region Color, Gate and select the data source.

a. To create a Single Parameter Gate Region, move the gates using the slider bars located above and below the graph.

b. The graph can be enlarged or reduced by using the up and down arrow buttons on the right hand side of the screen. When desired region has been created, proceed to step 8.

IMPORTANT Misleading results can occur if the same color is assigned to parameters and regions. Do not assign the same color to a parameter if region has been assigned duplicate color.

4 Select the desired parameters from the drop down lists.

a. To create a Quadrant region, the red quadrant line and drag to the desired location. When desired region has been created, proceed to step 8.

5 **Create** to draw the region.

6 the dot plot to set the end points. to finish the region. Once a region has been set, the region and drag to the desired location.

7 **Edit** to redraw the region.

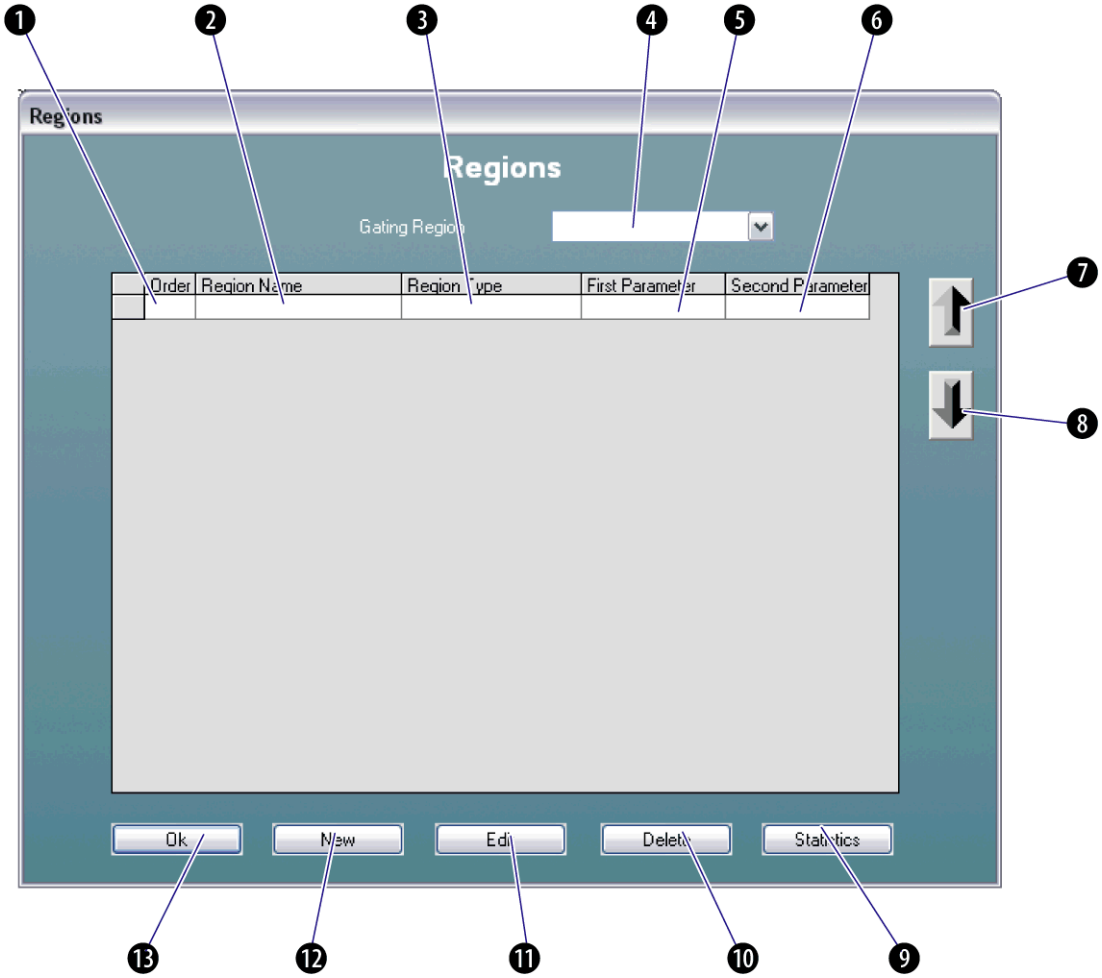
8 **Create** to set the region once it is drawn to the desired size.

9 **Exit** to set the Region and return to the Main screen.

Understanding the Manage Regions Screen

See Figure 7.12.

Figure 7.12 Manage Regions Screen: Defined



❶	The display order important for color assignment, data source selection	❷	Region name
❸	Type of region	❹	Allows you to select certain data points to be collected and saved. Achieved by creating a region around the desired points then selecting the region as the live gate (appears in the pull-down menu after it is created).

❺ Parameter for which the region is defined

❻ Second parameter for which the region is defined

❼ Allows the user to change the region order. The higher order regions will overwrite the lower order regions for dot color.

❽ Allows the user to change the region order. The higher order regions will overwrite the lower order regions for dot color.

❾ Displays region statistics

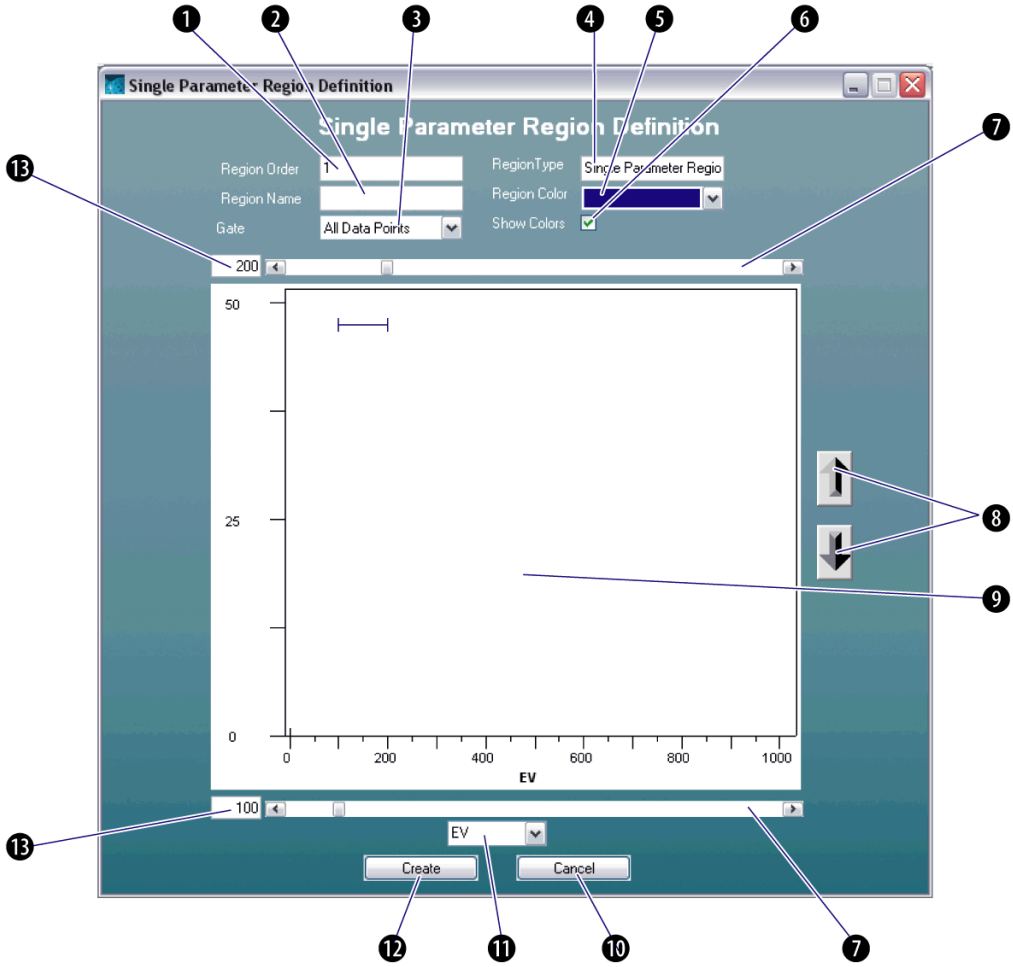
❿ Deletes a selected region

⓫ Allows you to edit the selected region

⓬ Allows you to create new regions

⓭ Sets the current configuration to the instrument and refreshes the display

Figure 7.13 Single Parameter Region Definition Screen: Defined



❶ Region Order is a sequential number assigned by the Quanta SC.

❷ Region Name allows the user to enter a name for the region.

❸ Gate drop down list allows the user to select All Data Points or an existing region as the source.

❹ Region Type is not editable and is populated once the Create button has been selected.

❺ Region Color allows the user to select a color for those data points falling within the specified region.

❻ Show Colors checkbox applies the Region Color selected from the drop down list.

❼ Scroll Bars allow the user to adjust the region's boundaries.

❽ Arrow buttons scale the graph by clicking either the up or down button.

❾ Data plot display

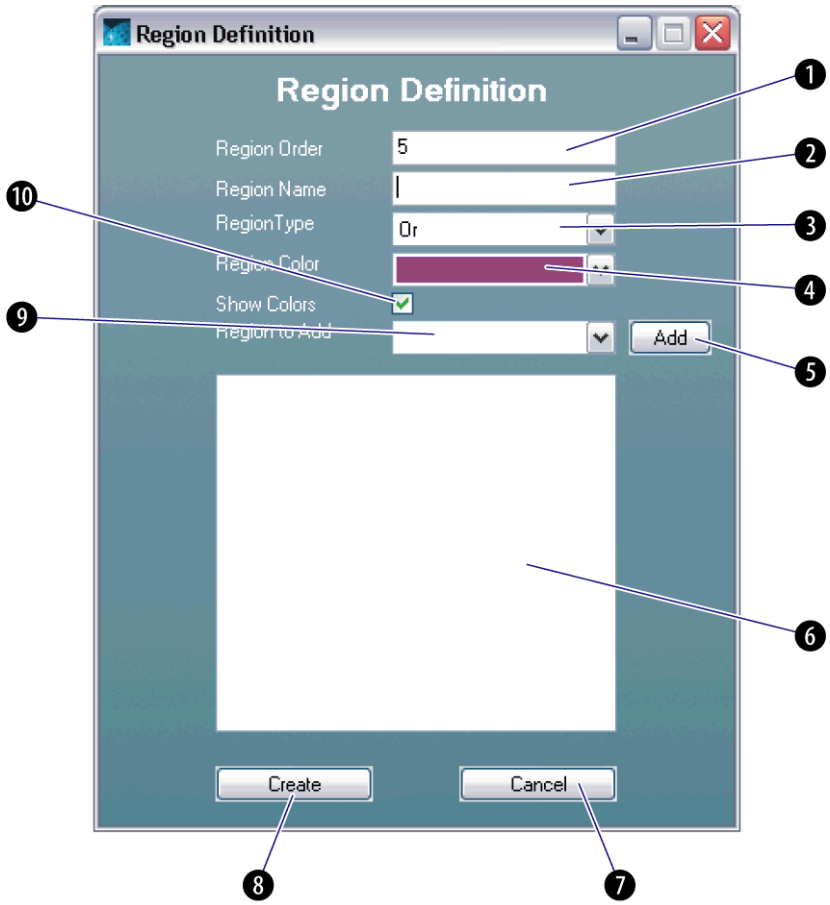
❿ Cancels unsaved settings.

⓫ Parameter drop down list allows the user to select the parameter for each axis.

⓬ Creates a Single Parameter Region.

⓭ Percent of data contained in each side of the divider (Divider Regions only).

Figure 7.14 Region Definition Screen for And/Or Regions: Defined



❶ Region Order is a sequential number assigned by the Quanta SC.

❷ Region Name allows the user to enter a name for the region.

❸ Region Type is not editable and is populated once the Create button has been selected.

❹ Region Color allows the user to select a color for those data points falling within the specified region.

❺ Adds Region Definition to list. Double-click a Region Definition to remove from the list.

❻ Displays all Region Definitions created.

❼ Cancels unsaved settings.

❽ Creates a Region Definition.

❾ Region to add drop down list allows you to select a region to add to the list.

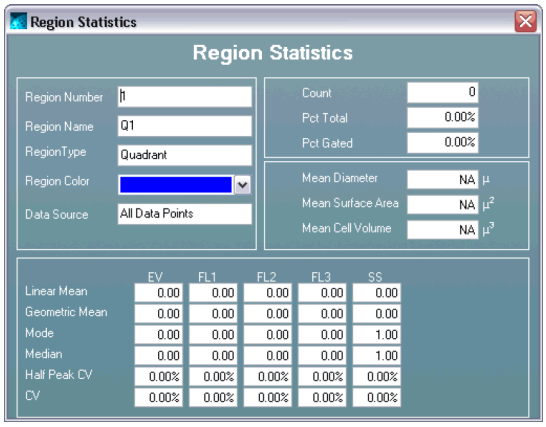
❿ Show Colors checkbox applies the Region Color selected from the drop down list.

Showing Region Statistics

Perform this procedure to display region statistics for the selected region.

---

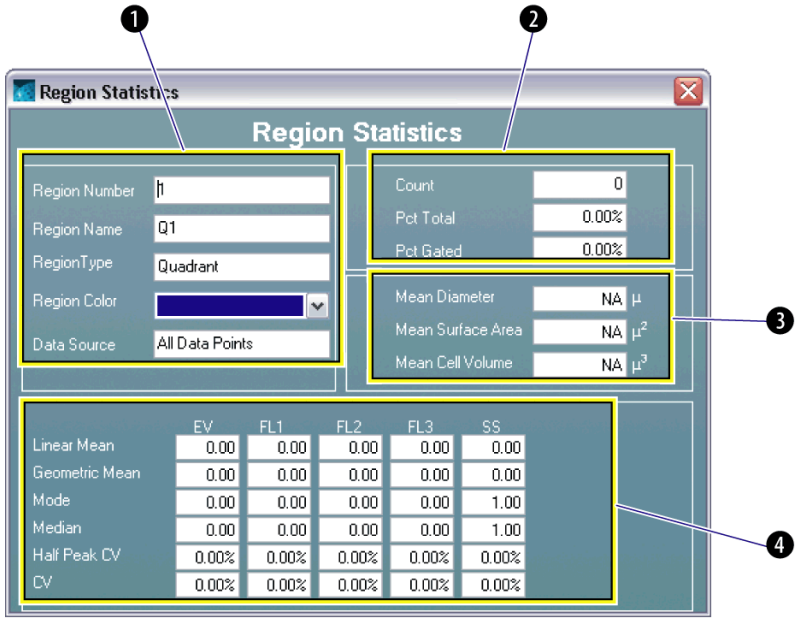
1 **Statistics** from the Regions menu to display the Region Statistics window.



Understanding the Region Statistics Screen

See Figure 7.15.

Figure 7.15 Region Statistics Screen: Defined



❶
- Region Number
  Sequential number assigned by the system.
- Region Name, Type and Color
  User-specific information entered by the operator.
- Data Source
  Region's gate.

❷
- Count
  Number of data points contained in the region.
- Pct Total
  Percent of total counts that are in the region.
- Pct Gated
  Percent of counts in the histogram gate that are contained in the region.

❸
- Mean Diameter
  Calculated as spherical equivalent of the mean cell volume.
- Mean Surface Area
  Calculated as spherical equivalent of the mean cell volume.
- Mean Cell Volume
  Linear mean channel of EV adjusted by the volume calibration factor.

❹ For each parameter that was collected (EV, FL1, FL2, FL3, and SS):
- Linear Mean Channel
  Arithmetic mean of the data points in the region.
- Geometric Mean Channel
  Geometric mean of the data points in the region.
- Mode Channel
  Peak (highest) channel.
- Median Channel
  50th percentile of the data.
- Half Peak CV
  CV using the half peak formula.
- CV
  Coefficient of variation.

7.8 WORKING WITH THE COMPENSATION MENU

The Compensation menu (Figure 5.9) allows you to set up compensation and control the display and saving of compensated data.

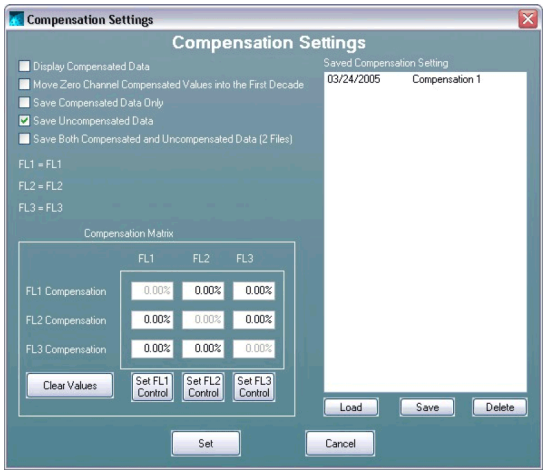
Defining Compensation Settings

Perform this procedure to display the compensated data of the sample runs on the screen and to define the compensation settings.

For additional information, see Understanding the Compensation Settings Screen.

---

1 **Compensation.** The following screen displays.



a. to uncheck the Display Compensated Data box.

b. **Clear Values**, then **Set**.

---

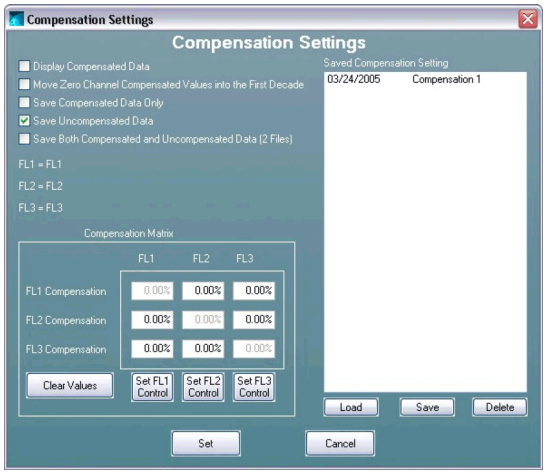
2 Set up the appropriate protocol or current settings for your application. (An example system protocol named Compensation is provided.)

---

3 Run an unstained isotype control. Set the gain so that the signals appear in the first decade of both X and Y directions for the three (3) fluorescence plots. Refer to Heading 7.4, WORKING WITH THE GAIN MENU for detailed instructions.

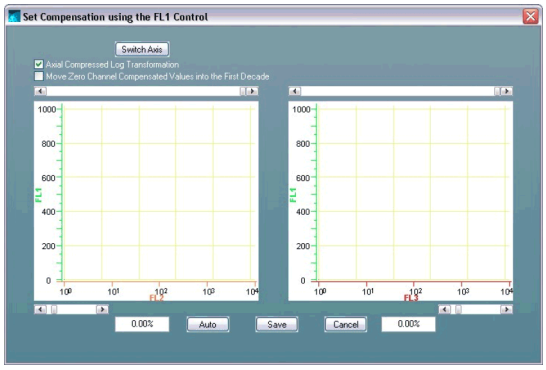
---

4 Run a control stained with the FL1 fluorochrome. **Compensation** and **Set FL1 Control.**



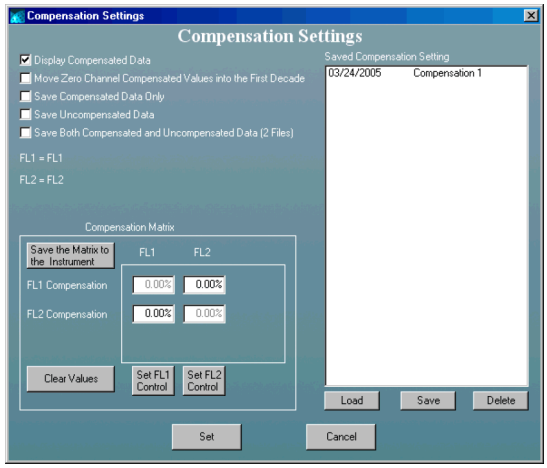
5 **Auto** from the Set Compensation dialog displayed to set the correct amount of compensation.

a. **Save**.



6 Repeat steps 4 and 5 for FL2 and FL3.

7 **Display Compensated Data** checkbox to show the compensated data on the Main screen.

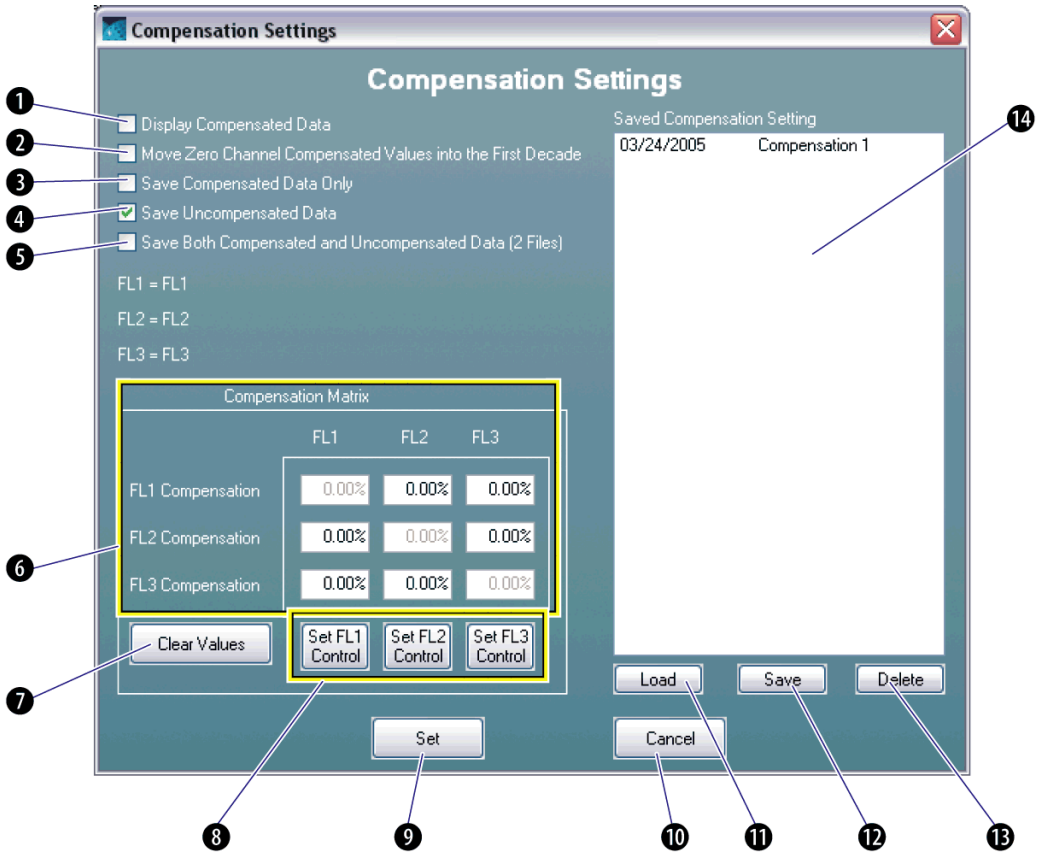


8 **Set.**

Understanding the Compensation Settings Screen

See Figure 7.16.

Figure 7.16 Compensation Settings Screen: Defined



❶	Enable checkbox to display all data compensated versus uncompensated.	❷	Enable checkbox to randomly assign a value in the range of 1 to 7 for the data assigned to the zero channel after compensation
❸	Enable checkbox to save only the compensated data	❹	Enable checkbox to save the uncompensated data as well as the compensation matrix
❺	Enable checkbox to save both compensated and uncompensated data, including differentiating the file names.	❻	Compensation Matrix shows the percent of subtraction for each combination of fluorescence signals. Compensation values may be entered here.
❼	Clear Values button clears the values for parameters in the Compensation Matrix table.	❽	Use FL1 Control to set compensation for FL2, FL3; whichever button you set controls the other two parameters.
❾	Set button saves the Compensation Settings.	❿	Cancels unsaved settings.

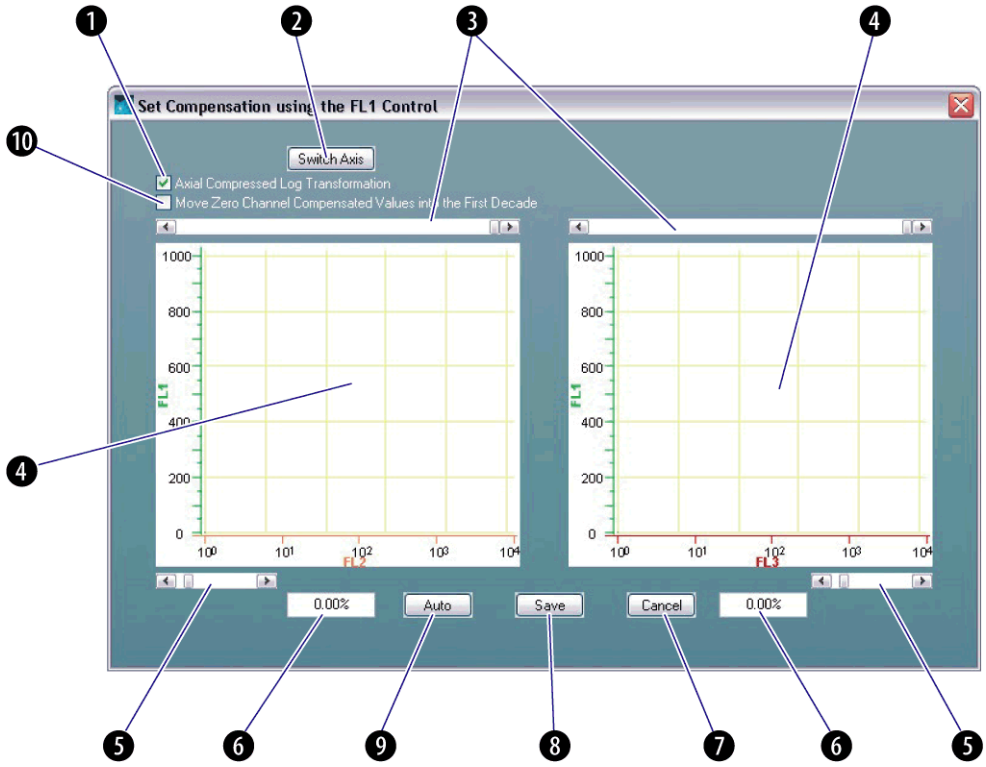
⓫ Loads previously saved settings of a compensation setting from the list.

⓬ Saves current compensation settings to a specified name.

⓭ Deletes a selected entry from the list.

⓮ List of Saved Compensation Settings can be loaded, save as new, or deleted.

Figure 7.17 Set Compensation using the FLn Control Screen: Defined



❶ Axial Compressed Log Transformation (ACLT) checkbox enables or disables the ACLT display. This display provides the ability to view negatively as well as positively compensated data, and is necessary when trying to determine the correct amount of compensation.

Note: Disabling the ACLT will revert to a normal log or linear display.

❷ Switch Axis button reverses the X- and Y-axis parameters on the display.

❸ Scroll bars allow compensation to be manually adjusted for the displayed parameter.

❹ Data plot displays.

❺ Manually adjusts percentage of compensation.

❻ Compensation percent displays the percent of subtraction under each graph.

❼ Cancels button exits the screen without saving.

❽ Save button applies the updated compensation percentages to the Compensation Settings screen.

❾ Auto Button automatically sets the correct amount of compensation as calculated by fitting the data of a single positive control.

❿ Move Zero Channel Compensated Values into the First Decade checkbox will only be available if the ACLT has been disabled.

7.9 WORKING WITH THE MANAGE PROTOCOLS SCREEN

By selecting **Manage Protocols** from the main menu, the Manage Protocols screen (Figure 5.11) appears. The Manage Protocols screen allows you to use permanent, pre-programmed protocols and to create and save new protocols.

Beckman Coulter, Inc. recommends that regular backup copies of your data files and protocols are created on a consistent basis.

At this screen, you can perform the following procedures:

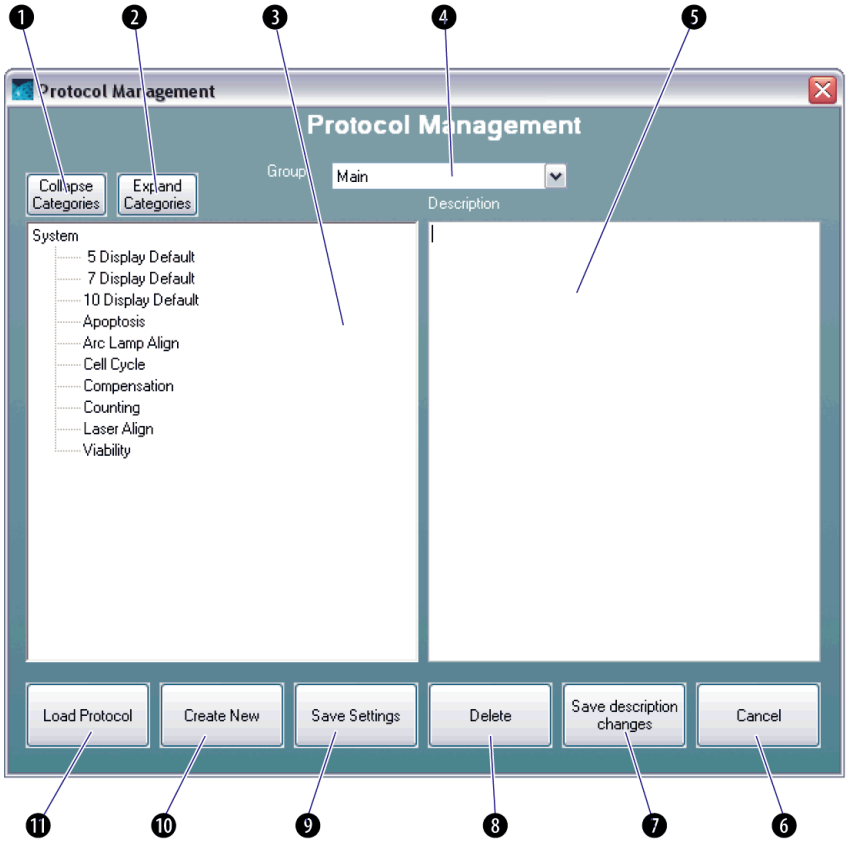
- Loading Protocols
- Creating New Protocols
- Saving Current Settings to a Protocol
- Deleting Protocols
- Save Description Changes (Editing a Protocol)
- Create/Delete Protocol Groups
- Assign a User to a Protocol Group
- Remove a User Assigned to a Protocol Group

For additional information, see Understanding the Protocol Management Screen.

Understanding the Protocol Management Screen

See Figure 7.18.

Figure 7.18 Protocol Management Screen: Defined



❶	Collapse Categories.	❷	Expand Categories.
❸	Lists the available protocols.	❹	Protocol Group drop down list.
❺	Lists any descriptive text saved with the protocol.	❻	Cancels any changes made to the selected protocol.
❼	Saves changes made to the description of a selected protocol.	❽	Deletes any highlighted protocol.
❾	Saves the current settings to a previously created protocol.	❿	Creates a new protocol using current user settings.
⓫	Loads any highlighted protocol		

Loading Protocols

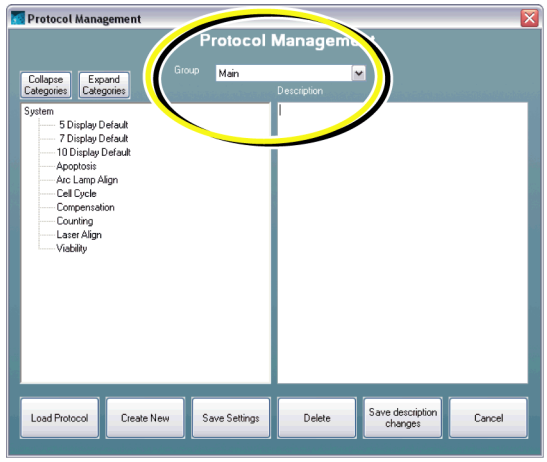
Perform this procedure to load an existing protocol.

---

1 **Protocols**.

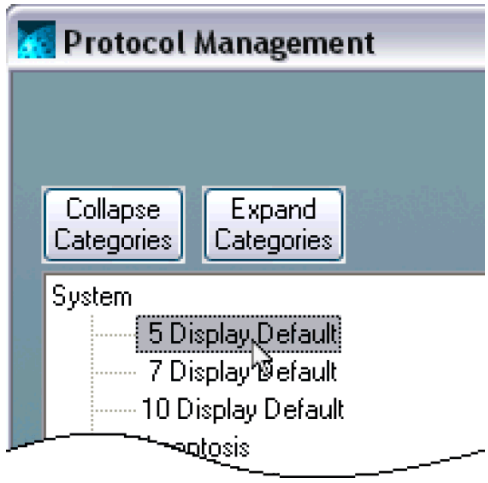
---

2 **Protocol Group** from the drop down list.



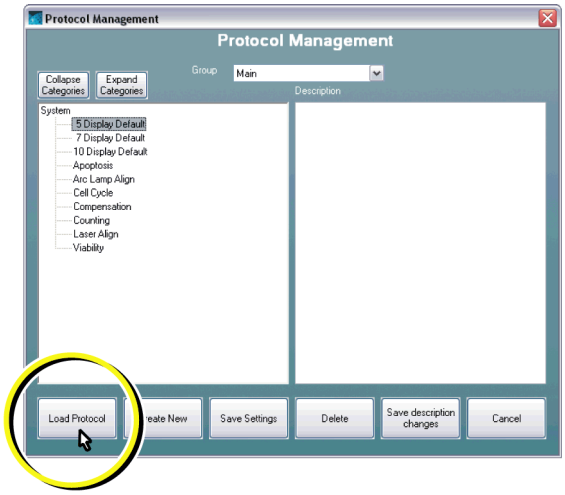
---

3 Highlight the desired protocol.



---

4 Load Protocol.

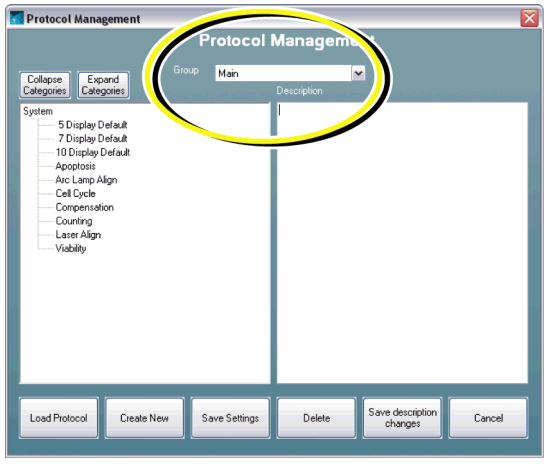


Creating New Protocols

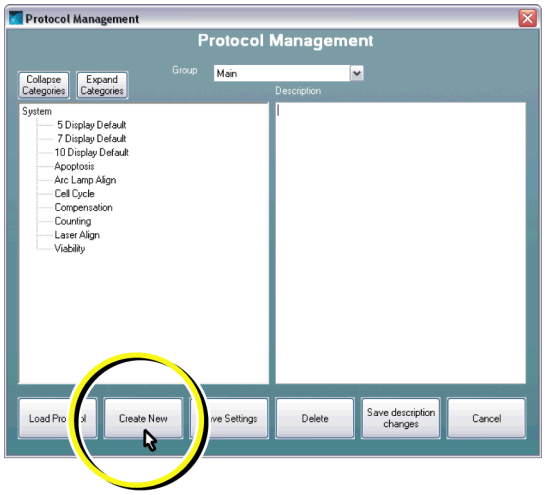
Perform this procedure to create a new protocol with the current settings you are using.

1 **Protocols**.

2 **Protocol Group** from the drop down list.



3 Create New.

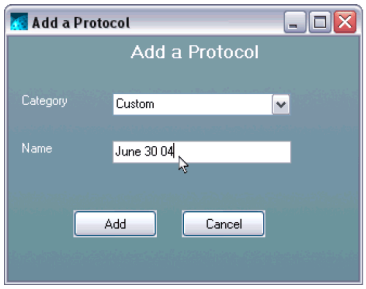


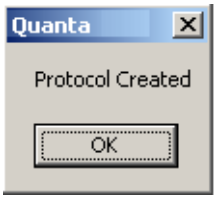
4 Name the new protocol:

a. Type the protocol name (e.g. `June 30 04`).

b. Choose the Category from the drop down list or type new category name.

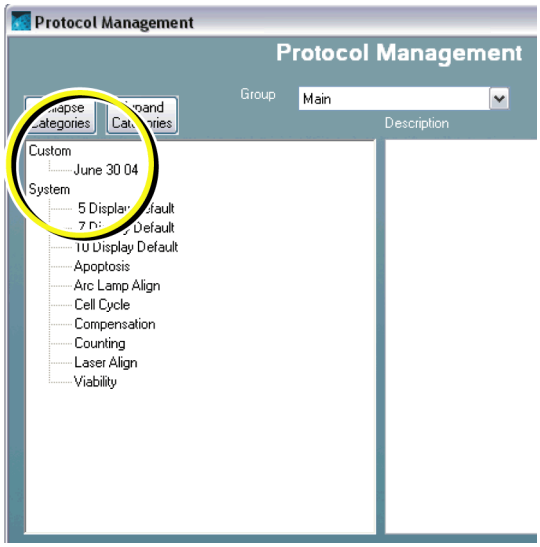
c. **Add**.





d. appears.

e. **OK**. The protocol name appears in the protocol list.



Saving Current Settings to a Protocol

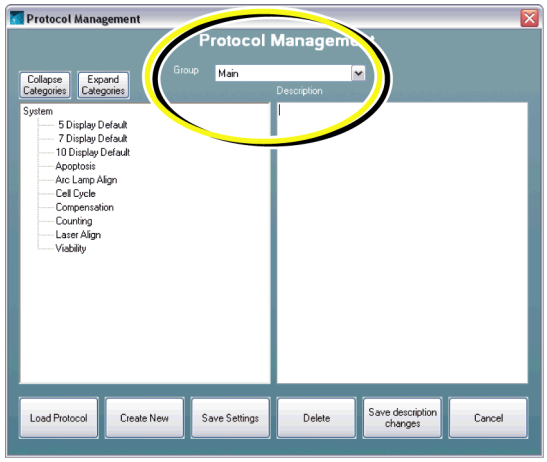
Perform this procedure to save the instrument's current settings to a previously created protocol that you or another user created. Default protocols provided with the system cannot be changed.

---

1 **Protocols**.

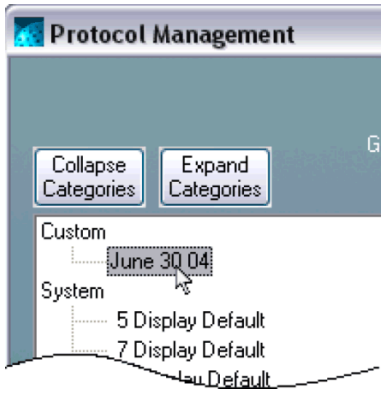
---

2 **Protocol Group** from the drop down list.



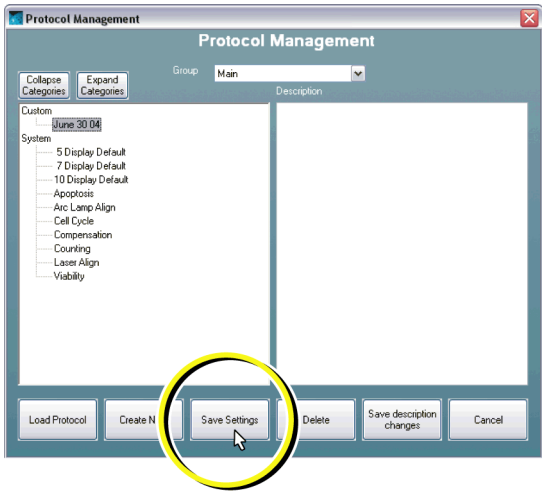
---

3 Highlight the desired protocol to be saved.



---

4 Save Settings.

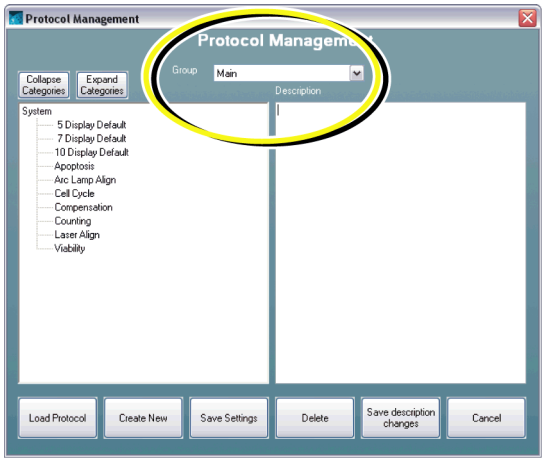


Deleting Protocols

Perform this procedure to delete a protocol.

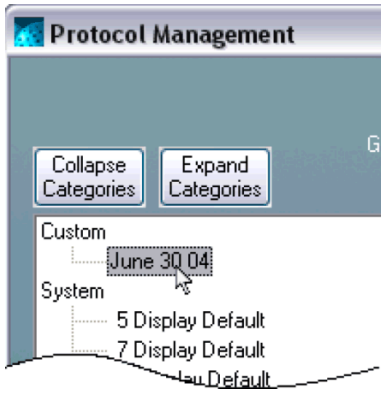
1 **Protocols**.

2 **Protocol Group** from the drop down list.



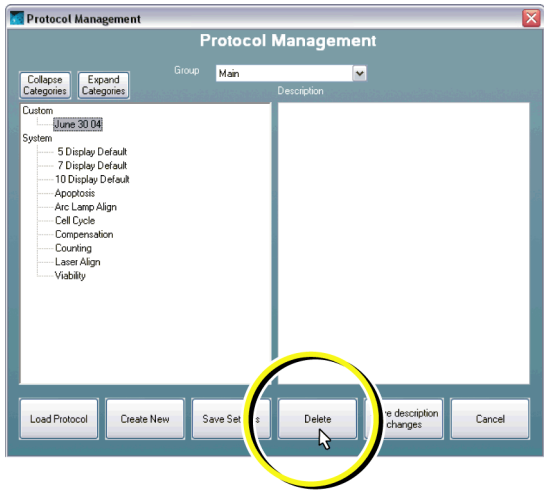
3 Highlight the desired protocol to be deleted.

Note: You cannot delete system protocols; you can only delete protocols that you or another user created.



4 

.



Save Description Changes (Editing a Protocol)

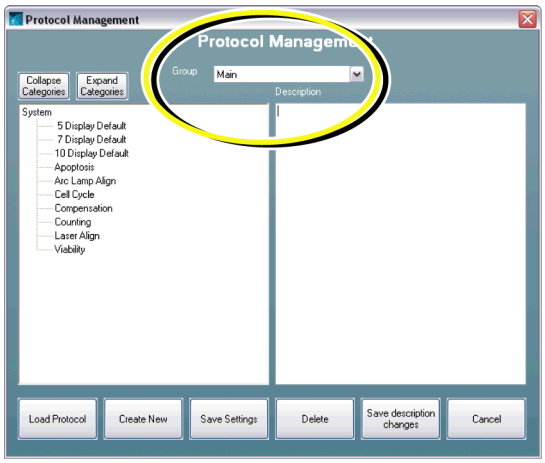
Once you have selected a protocol, you can Perform this procedure to create a new protocol or edit the protocol description, except for default protocols which are LOCKED and cannot be changed.

---

1 **Protocols**.

---

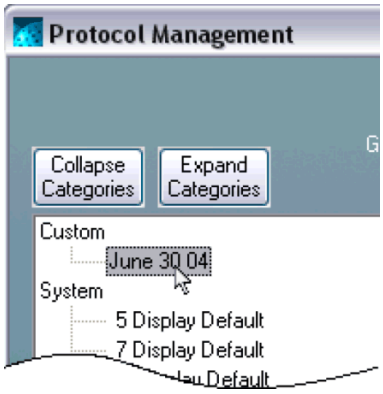
2 **Protocol Group** from the drop down list.



---

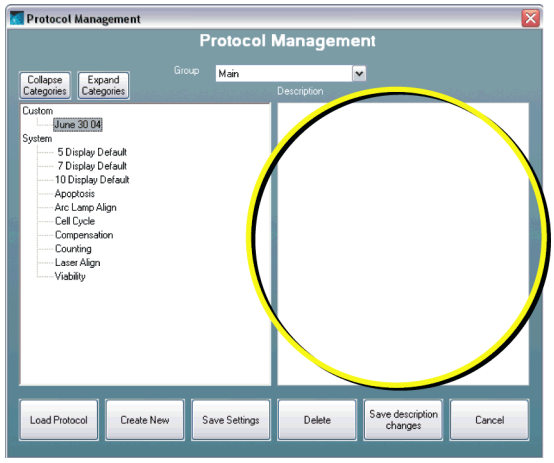
3 Highlight the desired protocol to be edited.

Note: You cannot edit system protocols; you can only edit protocols that you or another user created.

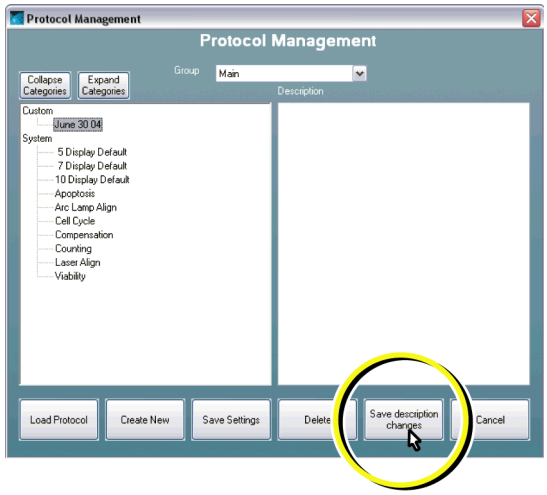


---

4 Type in new description text.



5 Save description changes.



Create/Delete Protocol Groups

Perform this procedure to create or delete a Protocol Group.

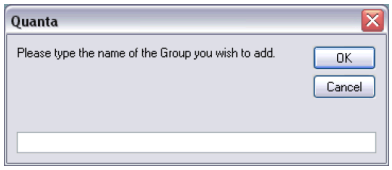
When working with protocols, a user can only load or save protocols from the Protocol Group they are assigned.

1 **Security ▸▸ Manage Users.**

2 **New** and enter a name for the new Protocol Group.

-OR-

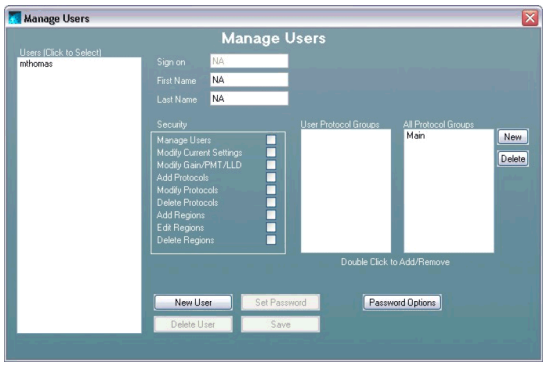
Highlight the Protocol Group to delete and **Delete.**



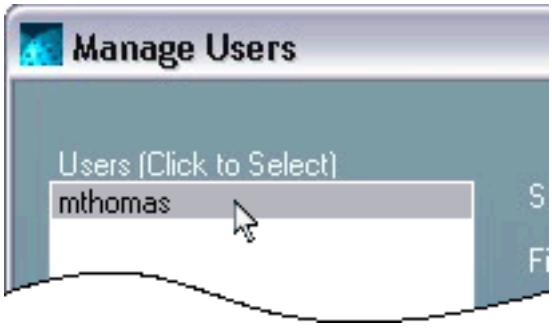
Assign a User to a Protocol Group

Perform this procedure to assign a user to a Protocol Group. One or more users can be assigned to a Protocol Group. When working with protocols, a user can only load or save protocols from the Protocol Groups that they are assigned to.

1 **Security ▸▸ Manage Users.**



2 to select the specific user from the list on the left side of the window and assign it to the specific Protocol Group.

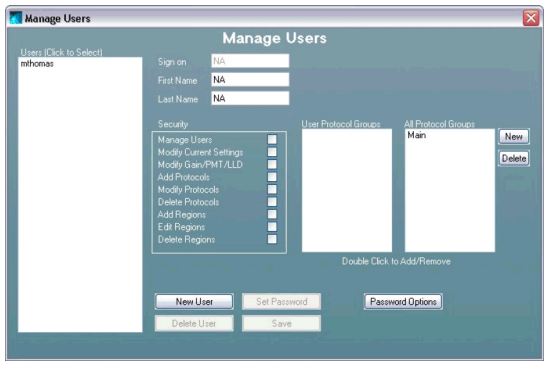


3 on the Protocol Group in the **All Protocol Groups** window to complete assigning the current user to this protocol group.

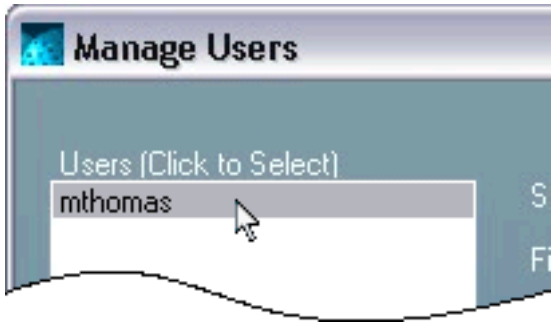
Remove a User Assigned to a Protocol Group

1 **Security ▸▸ Manage Users.**

Note: The Security list displays only if the 21 CFR Option is installed; otherwise this area is blank.



2 to select the specific user from the list on the left side of the window and delete it from a specific Protocol Group.



3 on the Protocol Group in the All Protocol Groups window to remove the user's access.

7.10 WORKING WITH THE CURRENT SETTINGS SCREEN

When you select **Current Settings** from the Main menu, the Current Setting screen (Figure 5.12) appears. This screen allows you to define many settings as defined in the following procedures.

- Defining Stop Sample Criteria
- Defining the Concentration (Use, # of Seconds, and Start Time)
- Enabling/Disabling Checkboxes
- Defining Auto Save Options
- Customizing an Excel Report
- Defining Filter Configurations

For additional information, see Understanding the Current Instrument Settings Screen.

Defining Stop Sample Criteria

Perform this procedure to define how much sample will be run through the instrument and to define the end of the run. When any of the defined criteria is met, sample analysis stops and the system automatically performs any selected Auto Save Options.

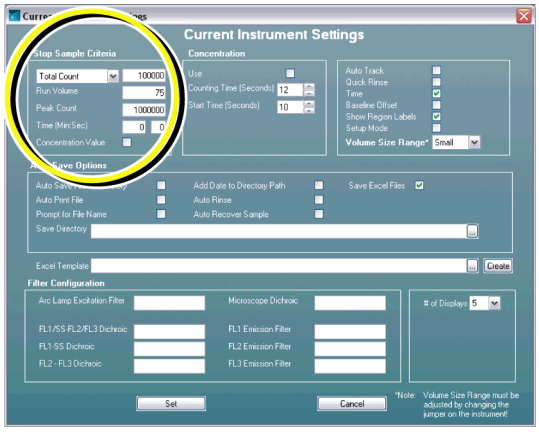
---

1 **Current Settings**.

---

2 Define the Stop Sample Criteria.

For details about the options for defining the end of the sample, see Understanding the Current Instrument Settings Screen.



---

3 **Set**.

Defining the Concentration (Use, # of Seconds, and Start Time)

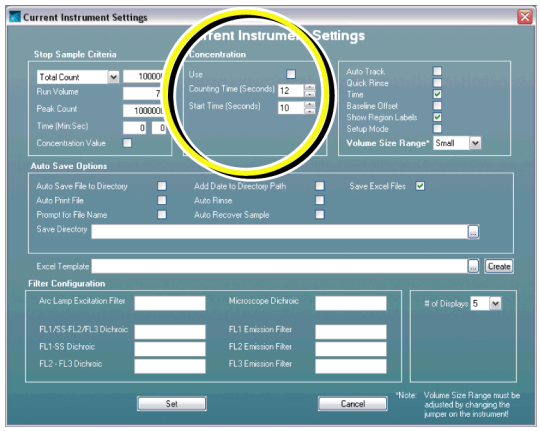
Perform this procedure to set the concentration to be collected, to define the # of seconds for sample collection and to define when the concentration is to begin collection.

---

1 **Current Settings**.

---

2 Define the Concentration settings



---

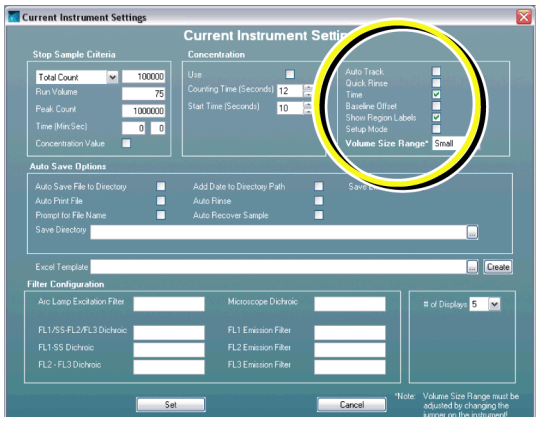
3 **Set**.

Enabling/Disabling Checkboxes

Perform this procedure to enable/disable checkboxes for the following options:

- Auto Track
- Quick Rinse
- Time Parameter
- Baseline Offset
- Show Region Labels
- Setup Mode
- Volume Size Range

1 **Current Settings**.



2 Enable/disable checkboxes:

- To enable, ☐ until ☑ appears.
- To disable, ☑ until ☐ appears.

3 **Set**.

Defining Auto Save Options

Perform this procedure to define the following Auto Save options:

Auto Save File to Directory automatically saves the results of each run to a pre-selected directory. An auto-incrementing number is added to the filename. This feature sequentially adds a number after the filename.

Auto Print File automatically prints the Excel Report after the run is completed.

Prompt for File Name allows you to enter a name for the file instead of using the automatic incrementing feature.

Add Date to Directory Path automatically assigns the current date to the chosen directory path.

Auto Rinse automatically runs a Rinse cycle after each sample run.

ATTENTION: If you select **Auto Rinse**, you must remove the sample immediately after data collection is complete or the sample will be destroyed.

Auto Recover Sample automatically recovers the remaining portion of the aspirated sample for use during another run.

IMPORTANT Misleading results can occur due to the potential of dilution. Use care when selecting Auto Recover Sample.

Save Excel Files allows you to save a report in the form of an Excel spreadsheet file.

Save Directory allows you to choose where the data will be saved.

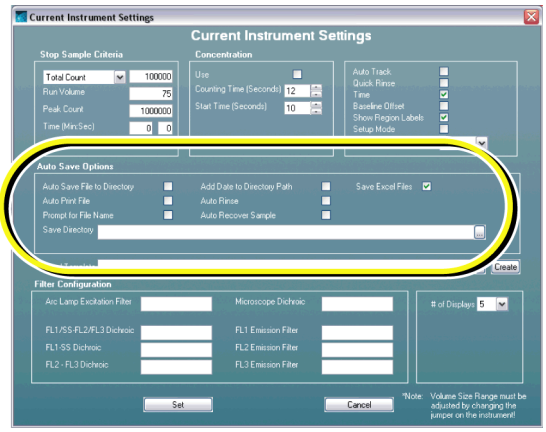
Excel Template allows you to create a new Excel template or use a pre-existing template.

---

1 **Current Settings**.

---

2 Define the Auto Save options.



---

3 **Set**.

Customizing an Excel Report

Pre-defined Excel Templates

If you do not define an Excel template, the system will automatically select a pre-defined template.

Template Name	# of Graphs
1DTemplate.xls	one (1)
3DTemplate.xls	three (3)
5DTemplate.xls	five (5)
7DTemplate.xls	seven (7)
10DTemplate.xls	ten (10)

IMPORTANT Misleading results can occur if a pre-defined Excel template is modified. All Excel templates, including pre-defined templates, should be validated prior to their use.

In order for graphs and statistics to appear in the Excel Report, they must be displayed on the Main screen.

Creating/Modifying Text in Excel Report

Perform this procedure to create or modify the Excel report text.

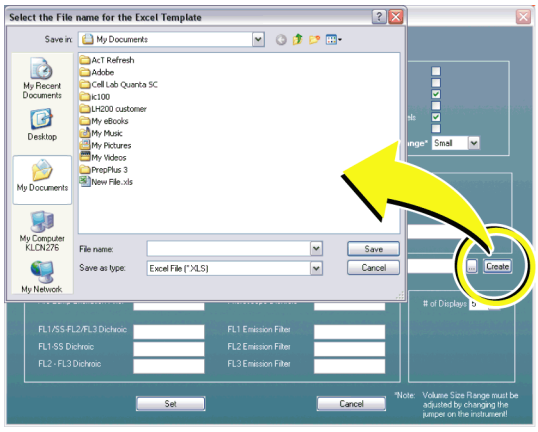
---

1 **Current Settings** ▸▸ **Create** to create a new template and type in a filename, or

Current Settings ▸▸ **…** to display a list of available Excel templates.

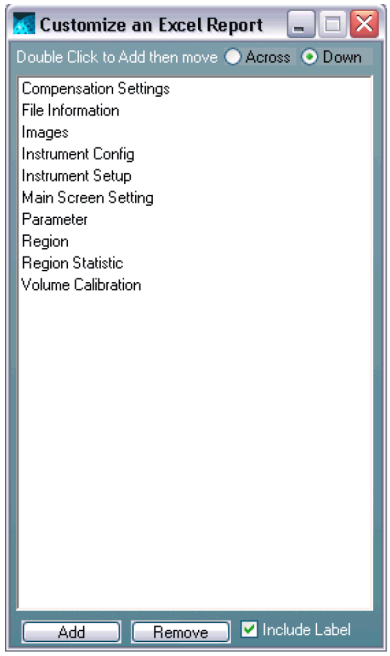
a. the desired template, or type in the new filename.

b. When the template name appears on the Current Settings screen, the template name to open the file.



---

c. The selected Excel template is opened and a list of report items is displayed.



IMPORTANT Misleading results can occur. When creating an Excel Report template, include unique identifiers, such as filename, sample ID, instrument serial number, and date and time of analysis. Be sure to validate every Excel template prior to use.

2 Add a text field to the report by:

a. Enable the Include Label checkbox to place the label to the left of the text field.

b. Click on the spreadsheet cell where you want the label to appear.

Note: If you enabled the Include Label checkbox, the label appears to the left of the text. If you select Column A for the text, the label will not display.

c. From the Customize an Excel Report dialog, you can double-click a category to display the text fields.

d. Select the desired text field, and **Add**.

3 Remove a text field by highlighting the desired text and **Remove**.

4 Edit a graph by selecting the graphic in the spreadsheet and either move, resize, or delete the graph. The graph numbers correspond to the graphs displayed on the Main Screen.

5 The new report template is automatically saved when the dialog box is closed.

Defining Filter Configurations

Perform this procedure to enter the following information regarding the specific filters used on your instrument:

Arc Lamp Excitation Filter allows you to enter the filter name/description.

Microscope Dichroic allows you to enter the filter name/description.

FL1 Emission Filter allows you to enter the filter name/description.

FL2 Emission Filter allows you to enter the filter name/description.

FL3 Emission Filter allows you to enter the filter name description.

FL2-FL3 Dichroic allows you to enter the filter name/description.

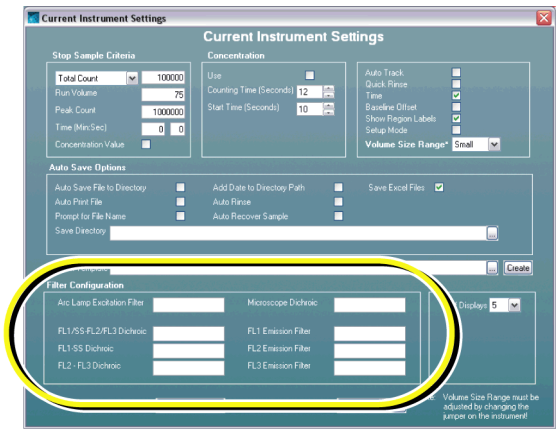
FL1/SS-FL2/FL3 Dichroic allows you to enter the filter name/description.

FL1-SS Dichroic allows you to enter the filter name/description.

IMPORTANT Misleading results can occur if the filter names do not match the names used in the system software. Ensure filter names reflect the instrument setup currently in use. For additional information, refer to Step 9 of Heading 10.5, CHANGING A FILTER for a diagram of the filters.

1 **Current Settings**.

2 Define the filter configurations as desired.

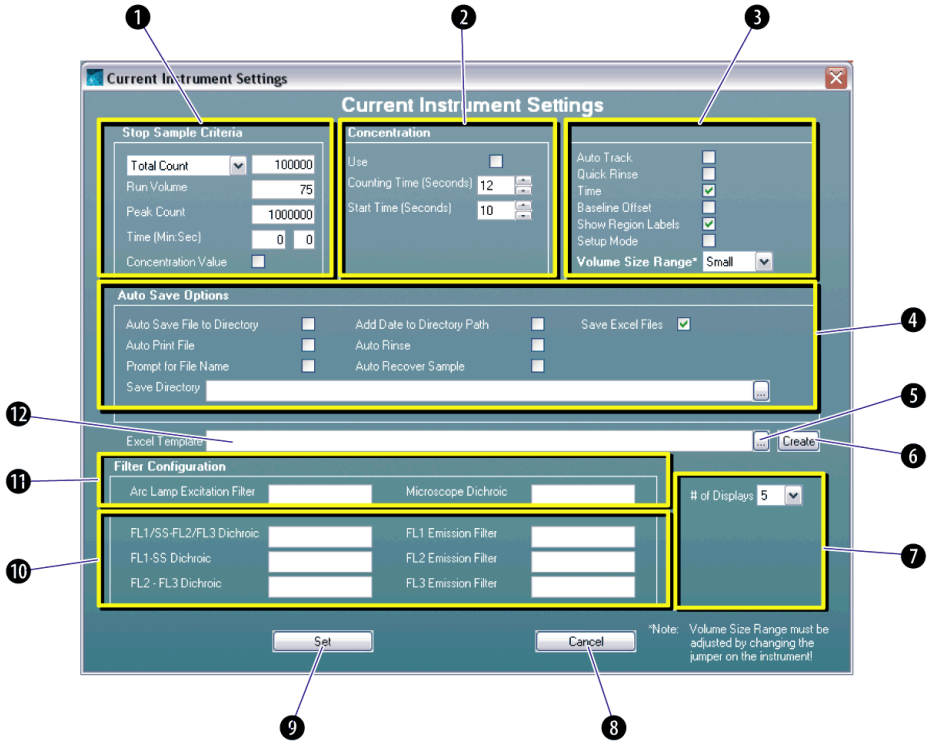


3 **Set**.

Understanding the Current Instrument Settings Screen

See Figure 7.19.

Figure 7.19 Current Instrument Settings Screen: Defined



1 Stop Sample Criteria.

- **Total Count** stops the sample run when the number of particles collected either in total or for the selected region matches the number in the field.
- **Run Volume** defines the sample amount through the flow cell. Once volume is met, sample is stopped.
- **Peak Count** stops the sample when the mode of the Primary single-parameter histogram reaches the value in the field.

2 Concentration.

- **Use** enabled checkbox indicates concentration is to be collected.
- **Counting Time (Seconds)** indicates how many cycles will be collected per sample.
- **Start Time (Seconds)** indicates when the concentration is to begin collecting.

- **Time (Min:Sec)** stops the sample when the given time is counted from the end of Stabilization has elapsed. If 0:0 is set as the value, then the system ignores the Stop time.
- **Concentration Value** stops the sample run when the concentration value is met.

❸
- **Auto Track** enabled checkbox allows tracking to automatically start once samples has started running.
- **Quick Rinse** enabled checkbox allows 16 second rinse.
- **Time** enabled checkbox allows time parameter to be collected.
- **Show Region Labels** enabled checkbox allows display of region name next to region on graphs.
- **Setup Mode** enabled checkbox allows only the most recent 200 data points to be displayed.
- **Volume Size Range** allows you to enter the EV jumper position (S,M,L). Refer to Figure 1.13, Jumper Positions: Controls and Indicators.
- **Baseline Offset** assigns a random gaussian value to redistribute zero channel data across the first decade.

IMPORTANT Risk of erroneous results. The Baseline Offset function should only be used after first viewing data with the baseline offset function turned off (unchecked). You must be satisfied that the overall results of any assay are not significantly affected by turning baseline offset on. However, you should not use baseline offset when determining appropriate cytometer settings. Baseline offset on should only be used for visual purposes after analysis.

❹ Auto Save Options.
- **Auto Save File to Directory** enabled checkbox allows the results of each run to be automatically saved to a pre-selected directory.
- **Auto Print File** enabled checkbox, allows user to auto print Excel reports.
- **Prompt for File Name** enabled checkbox allows the user to enter the filename rather than use the automatic incrementing feature.
- **Save Directory** allows user to specify the location for saved data.
- **Add Date to Directory Path** enabled checkbox automatically saves files to a sub-directory with current date as directory name.
- **Auto Rinse** enabled checkbox automatically rinses after each sample run.
- **Auto Recover Sample** enabled checkbox provides that the remaining portion of the aspirated sample will automatically be recovered for another run.

Save Excel Files enabled checkbox allows Excel reports to be automatically saved at the same time as the FCS 2.0 List Mode Data files.

❺ Browse button to use an existing Excel template.

❻ Creates a new Excel template.

❼ Drop down list to select number of data plot displays on the Main menu.

❽ Cancels unsaved settings.

❾ Sets the selected instrument settings.

❿ Filter Configuration.

- Allows user to input the specific filters being used in the instrument.
  - ▸ FL1/SS-FL2/FL3 Dichroic
  - ▸ FL1-SS Dichroic
  - ▸ FL2-FL3 Dichroic
  - ▸ FL1 Emission Filter
  - ▸ FL2 Emission Filter
  - ▸ FL3 Emission Filter

⓫ Filter Configuration.

- Allows user to input the specific filters being used in the instrument.
  - ▸ Arc Lamp Excitation Filter
  - ▸ Microscope Dichroic

⓬ Name of Excel template, new or existing.

7.11 WORKING WITH THE FILE INFORMATION SCREEN

When you select **File Information** from the Main menu, the File Information screen (Figure 5.13) appears. This screen allows you to enter the following information about the sample and file that will be saved with the FCS standard output file and included in the Excel report:

File Name allows you to name the file. (FCS Keyword $FIL)

Sample ID allows you to assign a specific Sample ID. (FCS Keyword $SMNO)

Tissue Type allows you to define the type of tissue for the sample. (FCS Keyword $CELLS)

Patient/Sample Description allows you to enter a description for the patient/sample. (FCS Keyword $SRC)

Operator allows you to enter the name or ID of the operator who is running this sample. (FCS Keyword $OP)

Experiment Initiator allows you to enter the name of the initiator. (FCS Keyword $EXP)

Sample Preparation allows you to define the sample preparation method.

Sample Size allows you to specify the sample size in before dilution.

Dilution Volume displays the total volume after dilution (including the sample).

Date automatically displays the current system date. (FCS Keyword $DATE)

Beginning/Ending Time allows you to define the start and end of the analysis data collection time. (FCS Keyword $BTIM / $ETIM)

Original Sample Concentration/ML allows you specify the original sample concentration.

Comments allows you to enter specific comments regarding this sample. (FCS Keyword $COM)

For additional information, see Understanding the File Information Screen.

Entering Sample Information

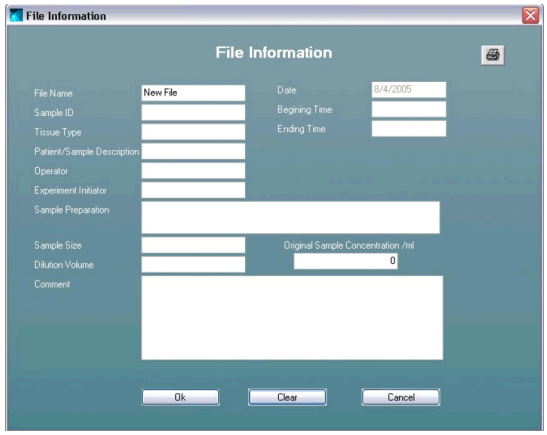
Perform this procedure to enter information for a specific sample.

---

1 **File Information**.

---

2 Enter the file information for your sample.



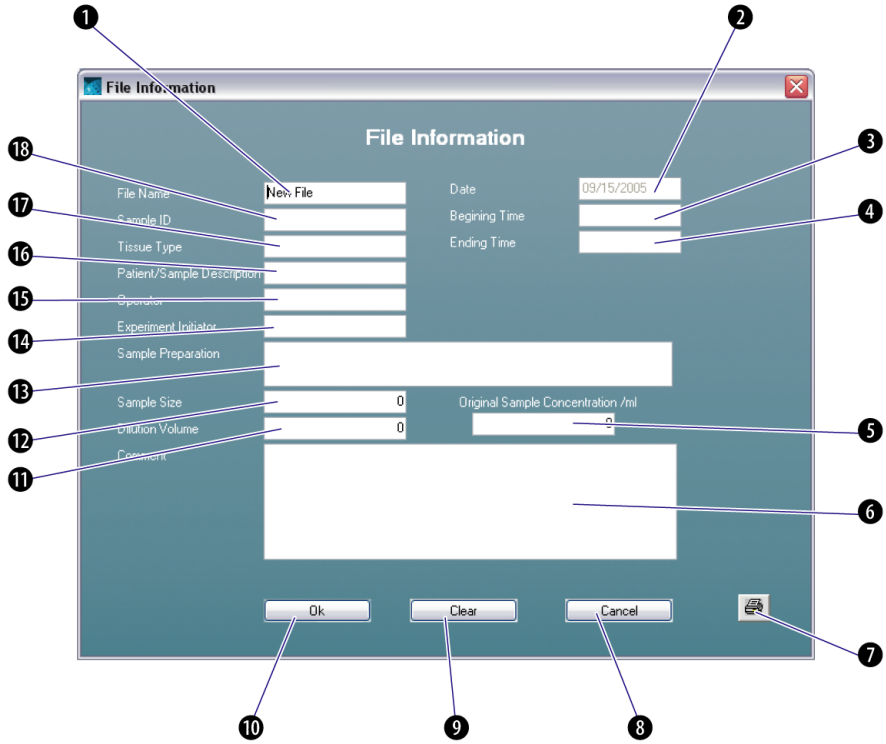
---

3 **OK**.

Understanding the File Information Screen

See Figure 7.20.

Figure 7.20 File Information Screen: Defined



❶	File Name allows you to name the file	❷	Date automatically displays the current system date; this date is populated by the Quanta SC system.
❸	Beginning Time displays the time at the start of data collection; this time is populated by the Quanta SC system.	❹	Ending Time displays the time at the end of data collection; this time is populated by the Quanta SC system.
❺	Original Sample Concentration/MLis calculated from the Concentration on the Main screen, the sample size, and dilution volume.	❻	Comments allows you to enter specific comments regarding this sample
❼	Print button.	❽	Cancel changes and close screen
❾	Clear data from the screen	❿	Save changes and close screen
⓫	Dilution Volume displays the total volume after dilution (including the sample).	⓬	Sample Size allows you to specify the sample size before dilution.
⓭	Sample Preparation describes method used to prepare the sample for analysis	⓮	Experiment Initiator allows you to enter the name of the initiator

⓯	Operator allows you to enter the name or ID of the operator who is running this sample	⓰	Patient/Sample Description allows you to enter a description for the patient/sample
⓱	Tissue Type allows you to define the type of tissue for the sample	⓲	Sample ID allows you to assign a specific Sample ID

7.12 WORKING WITH THE SECURITY MENU

The Security menu (Figure 5.9) allows you to change your password, manage other users, and modify the database path of your data collection. See:

- Change Password
- Manage Users
- Password Options
- Modify Database Path

Change Password

Perform this procedure to change the password of the current user.

---

1 **Security ▸▸ Change Password.**

---

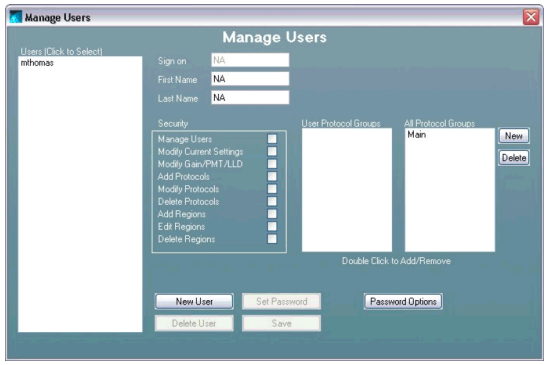
2 Enter your old password first, then type in the new password. Retype new password to confirm.

Manage Users

Perform this procedure to manage users and their security rights.

---

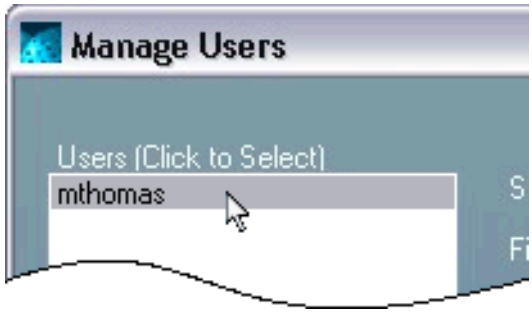
1 **Security ▸▸ Manage Users.**



---

2 Select a user from the list to change the security settings for that username. Security Settings include:

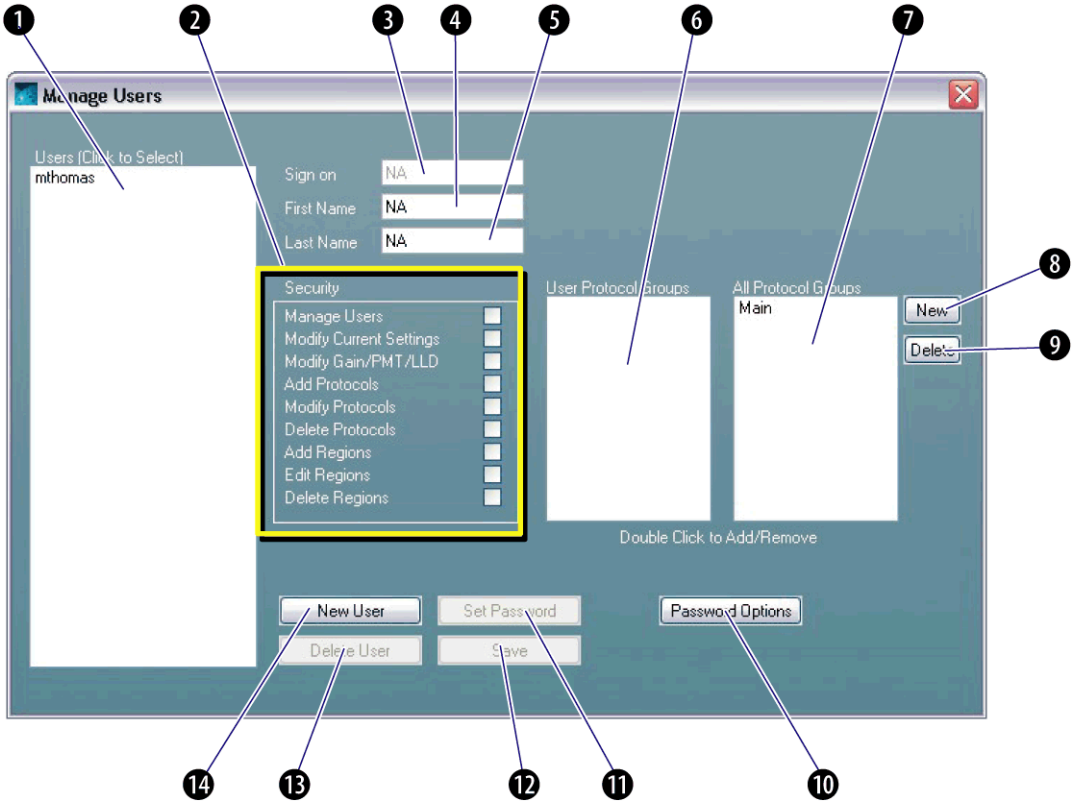
- Security Rights
- User Protocol Groups
- All Protocol Groups, and
- Password Options.



Understanding the Security Menu Screen

See Figure 7.21.

Figure 7.21 Security Menu: Manage Users



1. List of users established for the Quanta SC system.
2. Security Rights checkboxes allows the system administrator to choose which rights are to be associated with a given username.
3. Sign on field displays username.
4. First Name field displays the selected user's first name or user can enter text.
5. Last Name field displays the selected user's last name or user can enter text.
6. User Protocol Groups lists the protocol groups the username can use.
7. All Protocol Groups lists all protocol groups available on the Quanta system.
8. New button allows for a new protocol group to be added.
9. Delete button deletes a protocol group from the list.
10. Password Options displays a dialog, see Password Options.
11. Set Password saves the new password for the specified user.
12. Save button exits and saves all changes made during the edit session.
13. Delete user deletes the selected user.
14. New User button adds a new user.

Password Options

Perform this procedure to set the password security options.

1 **Security ▸▸ Password Options**.

2 Set the password security options according to your laboratory's requirements. Options include:

- Auto Log on as Admin bypasses the log in screen and access protections
- Remember last user requires username to be typed or selected from drop down list
- Require alpha/numeric password
- Minimum password length
- Specified time limit of password, and
- Save / Cancel changes.

Modify Database Path

Perform this procedure to allow a Windows restricted user the ability to access the software. This procedure is only available if user is logged on as Admin.

Protocols, user information, current settings, regions and instrument settings are all stored in a database file. By default, this database is in the installation directory. Windows security allows the creation of windows users that do not have the ability to modify the installation

directory on some computers. Please contact your Windows Administrator to provide the proper read/write permissions for users.

---

1 **Security ▸▸ Modify Database Path.**

7.13 WORKING WITH THE HELP MENU

The Help menu (Figure 5.15) has two options:

- **Help**, which launches the online Help file (see Launching Help)
- **About**, which provides information about the Quanta software (see Viewing Software Information)

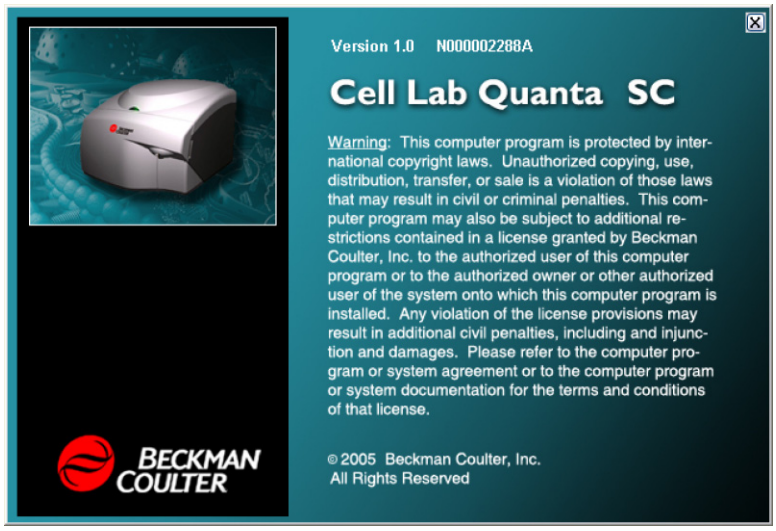
Launching Help

To launch the online Help, **Help ▸▸ Help**.

When you are finished viewing the Help, to close.

Viewing Software Information

To view the software details, such as manufacturer and version number, **Help ▸▸ About**.



8.1 OVERVIEW

Quality control is extremely important to the daily operation of the Quanta SC System. The cost and time associated with the quality control procedures outlined in this manual are minimal compared to the loss of quality in results if quality control procedures are not followed.

8.2 QC MATERIALS

The following Quality Control materials are used to address all of Quanta's parameters. Quanta operation supports the following materials:

- Flow-Check Fluorospheres (PN 6605359)
- Coulter CC Size Standard L5 (PN 6602794)
- Sphero Rainbow Alignment Particles (Arc Alignment Beads; PN RAP-38-5 and RAP-38-10)

If commercial controls are not available, whole blood specimens may be used if there are corresponding values available from other instruments for reference purposes.

8.3 DAILY QC

Beckman Coulter recommends the use of QC materials to gauge instrument performance of reportable parameters. Daily QC depends on the light source and application that will be run that day.

For 488 laser applications, daily QC consists minimally of running Flow-Check Fluorospheres.

For Mercury Arc lamp applications, daily QC consists of minimally running the Arc Alignment Beads (refer to Heading 8.2, QC MATERIALS).

Additional QC may be required depending on the applications to be used.

For applications using volume, ensure you follow the calibration procedure, Heading 7.5, WORKING WITH THE VOLUME MENU, using the COULTER CC Size Standard L5.

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

- Daily Startup.
- The Cytometer has been idle for an extended period of time.
- A syringe malfunction related to aspiration, mixing, or dispensing.

Before Running Flow-Check Fluorospheres

- Check that the DAILY STARTUP procedure was performed, refer to Heading 6.1, PERFORM STARTUP.

Running Flow-Check Fluorospheres

1 Prepare the Flow-Check Fluorospheres according to package insert instructions, refer to Heading 8.4, QC METHOD for mixing and handling of fluorospheres.

2 Load the Laser Alignment protocol.

3 Ensure sample is properly mixed prior to placing a sample cup on the instrument.

4 **Start** from the Main screen. The process goes through *Aspirating*, *Boosting*, *and Stabilizing*, then *Stop* appears. Data acquisition begins.

5 Ensure the flow rate is 150-200 events per second.

6 Adjust fluorescense peaks FL1 (525 BP), FL2 (575 BP) and FL3 (670 LP) to channel 200.

7 Adjust the regions and check the HPCVs.

8 Check that the HPCV values for all parameters are within your laboratory's acceptance limits and record them in a logbook.

Note: Follow the package insert instructions for the Flow-Check Fluorospheres to establish expected range values for your laboratory.

9 If the HPCVs are greater than 3%, perform the AUTOMATICALLY ALIGNING THE OPTICS found in Heading 8.5.

Running the Arc Lamp Alignment Beads

1 Prepare the beads as required for your application, refer to Heading 8.4, QC METHOD.

2 Load the Lamp Alignment protocol.

3 Ensure sample is properly mixed prior to placing a sample cup on the instrument.

4 **Start** from the Main screen. The process goes through *Aspirating*, *Boosting*, *and Stabilizing*, then *Stop* appears. Data acquisition begins.

5 Set flow rate (slider bar) to 4.17 mL per minute.

6 If necessary, adjust the FL1 (465/30 BP) to channel 200.

7 Adjust the regions and check the HPCV.

8 Check that the HPCV value for FL1 is within your laboratory's acceptance limits and record them in a logbook.

9 If the HPCVs are greater than 3%, perform the AUTOMATICALLY ALIGNING THE OPTICS found in Heading 8.5.

8.4 QC METHOD

Prepare QC Material

Flow-Check Fluorospheres

1 Prepare Flow-Check Fluorospheres according to the package insert instructions.

2 Follow the package insert instructions for mixing and handling fluorospheres.

3 Check that the values for Mean, and the half-peak coefficient of variation (HPCV) for FL1, FL2 and FL3 are within your laboratory's acceptance limits and record them in a logbook.

Note: Follow the directions in the Flow-Check Fluorospheres package insert to establish expected range values for your laboratory.

8.5 AUTOMATICALLY ALIGNING THE OPTICS

The Automatic Optical Alignment procedure will automatically focus the light onto the flow cell to obtain maximum fluorescence and greatest possible signal strength while maintaining acceptable HPCV values. It is recommended that this procedure be performed **only** if HPCVs are greater than 3%.

Arc Lamp Alignment Beads

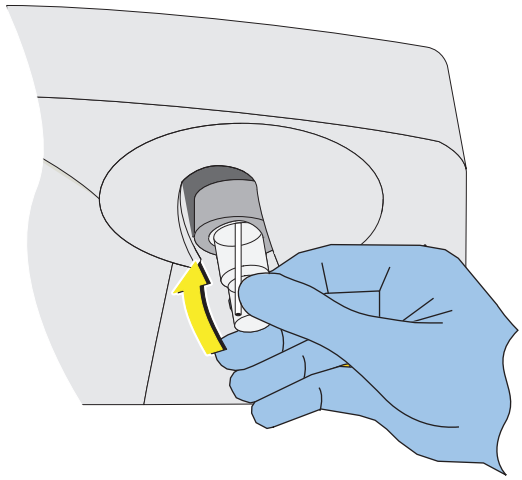
Use Sphero Rainbow Alignment Particles:

- PN RAP-38-5
  3.8 µm size, 5 mL bottle
- PN RAP-38-10
  3.8 µm size, 10 mL bottle

1 Prepare the required QC materials, refer to Heading 8.3, DAILY QC for detailed instructions

2 Load the Optical Alignment protocol, refer to Loading Protocols in Chapter 7 of the Quanta SC manual.

3 Place an empty sample cup on the instrument.



4 **Rinse** from the Main screen.

5 After rinse is completed, place sample cup containing QC material on the instrument and **Start.**

Note: Ensure count rate is greater than 100.

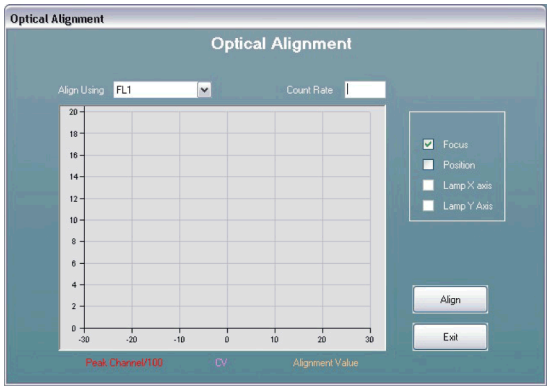
- If using Laser Alignment Protocol, run a sample of Flow-Check beads.
- If using Arc Lamp Alignment Protocol, run a sample of alignment beads.

6 **Auto Optical Alignment** from the Instrument Menu.

7 Select the fluorescence parameter (FL1, FL2, or FL3) that corresponds to the application being run.

8 Select one of the following options:

- Focus: Adjusts Microscope Focus
- Position: Adjusts Microscope Position
- Lamp X: Adjusts Lamp X-axis (arc lamp only)
- Lamp Y: Adjusts Lamp Y-axis (arc lamp only).



Note:

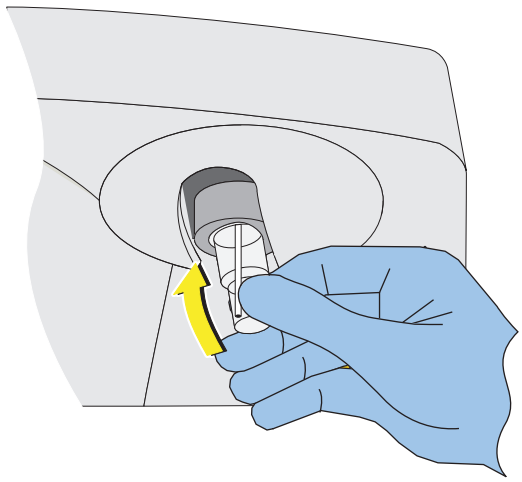
- Most alignments can be achieved by selecting Focus.
- The Lamp X and Lamp Y alignment options are only available if the laser is OFF.

9 **Align**.

10 If the HPCVs are still greater than 3%, repeat steps 7 to 9 until the best value is obtained.

11 When HPCVs are less than 3%, **Exit.**

12 Place an empty sample cup on the instrument and perform a **Rinse**.



13 **Main** to return to the Main Screen.

9.1 BEFORE RUNNING SAMPLES

1 Verify correct filters are installed and correct protocols are loaded for the selected application and light source.

2 If the instrument has been idle for more than one hour with an empty sample cup, press **RINSE** from the Main Screen. The instrument performs a rinse cycle and primes for presentation of the next sample.

Note: If the instrument is idle for more than two hours, perform a shutdown procedure as instructed in Heading 6.3, PERFORM SHUTDOWN.

3 You can set the gain, PMT Voltage and discriminator values as instructed in Heading 9.5, SETTING INITIAL GAIN, VOLTAGE AND DISCRIMINATOR VALUES.

4 Ensure that QC has been done. See Chapter 8, QUALITY CONTROL.

5 Enter sample information. See Heading 7.11, WORKING WITH THE FILE INFORMATION SCREEN.

Note: If identification by Sample ID is desired, you must enter a Sample ID with the sample information.

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

9.2 PREPARING SAMPLES

Available applications are provided as application notes and are separate from this manual.

Prepare the sample and according to the instructions for the specific protocol that you are following. Protocol documents are separate from this manual.

9.3 RUNNING SAMPLES

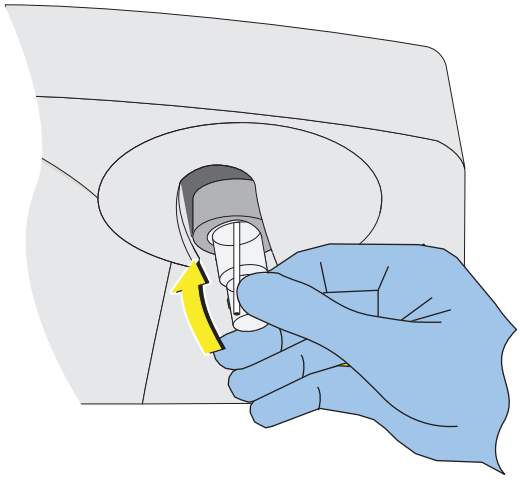
IMPORTANT Risk of sample misidentification if a power failure occurs during sample processing. In the event of a power failure, discard any in-process samples.

Available applications are provided as application notes and are separate from this manual.

Analyze the sample according to the instructions for the specific protocol that you are following.

---

1 Place the sample cup on instrument.
See Heading 5.4, PLACING A CUP ON THE INSTRUMENT.



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2 Press **Start** button on Main screen.

- Sample is aspirated and data collection begins.
- Start button changes to “Aspirating”.
- Aspirating button then changes to “Boosting” as the sample moves from the sample loop toward the flow cell.
- Boosting button changes to “Stabilizing” when the sample is tested for stable flow through the flow cell.
- After stabilization is completed, Stabilizing button changes to “Stop”.

---

3 To stop the sample flow and data collection, press the **Stop** button. Stop button changes to “Resume” button.

---

4 To resume sample flow and data collection, press the **Resume** button.

IMPORTANT Possible erroneous results can occur if the sample has stopped and you press **Resume** or **Continue** during sample processing. Sample settling can occur in the tubing; the amount of settling will depend upon the type of sample and the length of time the sample has been stopped.

5 When the stop criteria (defined in the Current Settings screen) is met, the button reflects "Continue" if there is sample remaining.

For details about defining the stop criteria, see Heading 7.10, WORKING WITH THE CURRENT SETTINGS SCREEN.

6 To allow the sample to continue running, press **Continue**.

7 If the sample stops due to the run volume (defined in the Current Settings screen) being met, the button reflects "Reload". You can load more sample into the sample loop and resume data collection by pressing the **Reload** button.

Note: There is a 1-minute time-out if a sample is not presented and "Reload" is pressed.

For details about defining the run volume, see Heading 7.10, WORKING WITH THE CURRENT SETTINGS SCREEN.

9.4 AFTER RUNNING SAMPLES

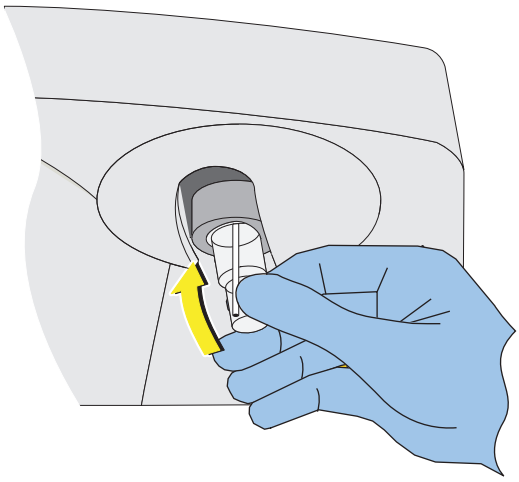
1 After the sample has been run, you can recover sample from the sample loop.

a. Press **Recover Sample** on the Main screen.

b. Recovery occurs even if the run volume selected has been completely dispensed through the flow cell because extra volume is aspirated during the sample "Aspirating" process.

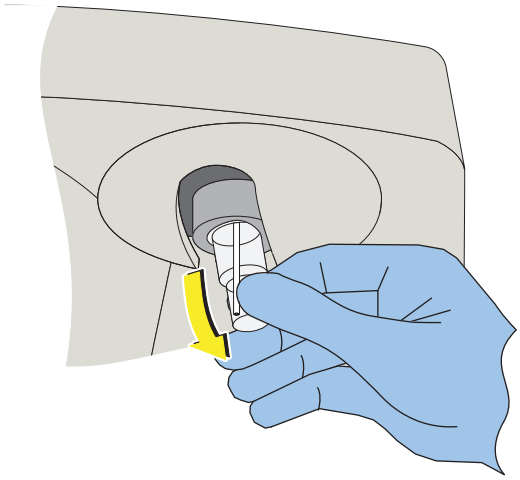
IMPORTANT Misleading results can occur due to the potential of dilution. Use care when selecting Auto Recover Sample.

2 Place an empty sample cup on instrument. See Heading 5.4, PLACING A CUP ON THE INSTRUMENT.



3 Press **Rinse** to clean the fluidics and to prepare the instrument for the next sample.

4 Slightly tilt the sample cup to release the vacuum seal, then gently pull the cup down



5 Check the Waste Bottle to determine if it needs to be emptied. If so, do Heading 10.4, EMPTYING THE WASTE BOTTLE.

6 Review the data according to your laboratory procedures.

9.5 SETTING INITIAL GAIN, VOLTAGE AND DISCRIMINATOR VALUES

It is important to set the Gain, PMT Voltage, and Discriminator values properly to differentiate sample from background noise. Gain and PMT Voltage values are adjusted from the Gain Settings box, refer to Heading 7.4, WORKING WITH THE GAIN MENU and Heading 7.1, UNDERSTANDING THE MAIN SCREEN for detailed instructions regarding ULD and LLD.

Note: When using the Laser, you may also want to adjust the Laser power in addition to Gain and PMT Voltage values.

9.6 WORKING WITH REGIONS

If you create a region, it is displayed on the Main screen (Figure 5.2). For details about the Manage Regions screen, see Heading 7.7, WORKING WITH THE REGIONS MENU.

10.1 INSTRUMENT CLEANING AND HANDLING REQUIREMENTS

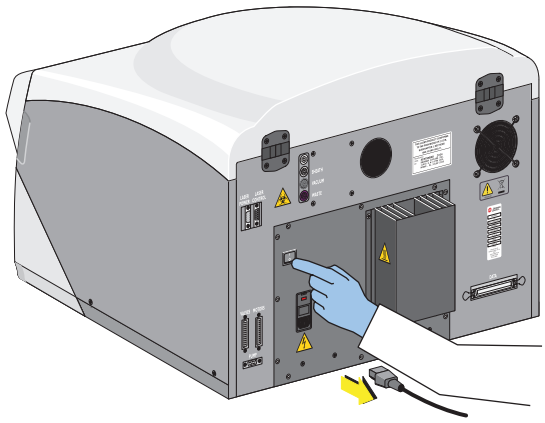
CAUTION Risk of damage to the instrument if any fluid comes into contact with the electronic components. To prevent damage to the instrument, do not let any fluid come into contact with the internal instrument components.

- Use soap or a mild detergent and water to clean the outer surfaces of the Quanta SC System. Do not allow any fluids to permeate the inside of the instrument.
- Observe Good Laboratory Practices when handling samples. Protective laboratory gloves are strongly recommended.
- If sample is spilled, thoroughly clean and disinfect the area with a 1:10 dilution of high-quality, fragrance-free, gel-free bleach (5 to 6% solution of sodium hypochlorite - available chlorine).
- In case of breakage or spillage of liquid onto the Quanta Instrument, perform a shutdown prior to cleanup.

10.2 INSTRUMENT QUICK DISCONNECT

Prior to performing maintenance, replacement or troubleshooting procedures, ensure that the instrument power has been disconnected from the electrical unit by unplugging the power cord from the back of the instrument.

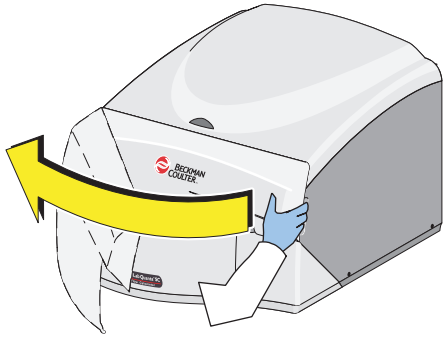
Figure 10.1 Disconnect Analyzer Power



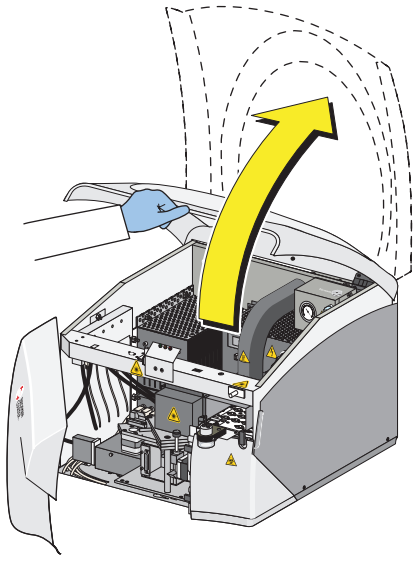
10.3 OPENING/CLOSING THE COVER

Certain maintenance, replacement or troubleshooting procedures may require the instrument cover to be opened.

1 Pull front cover open from the right side of instrument.

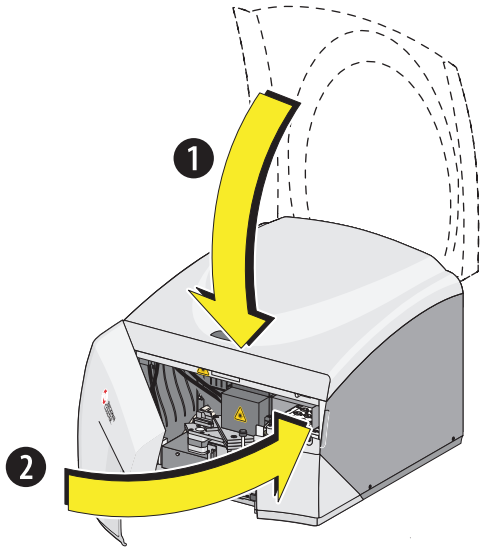


2 Pull top of cover up until it locks in the upright position.



3 To close the cover properly, reverse the above steps pulling the top cover down first and then closing the front cover.

CAUTION Closing the front cover before the top cover can damage the covers and interrupt the laser interlock. Pull the top cover down first, then close the front cover.

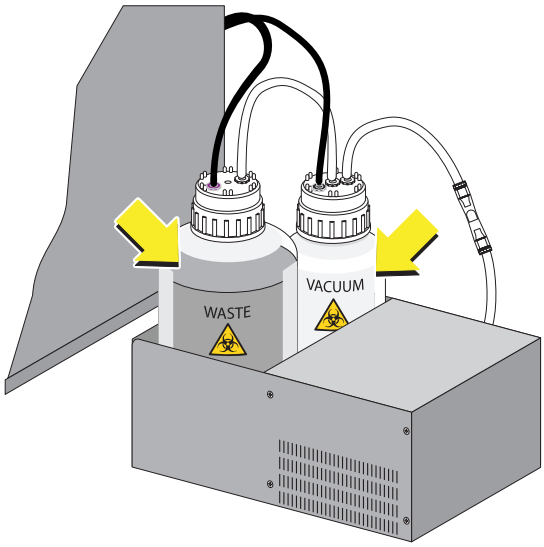


10.4 EMPTYING THE WASTE BOTTLE

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

1 Empty the Waste Bottle when full.

Note: If liquid is evident, the Vacuum Bottle should also be emptied.



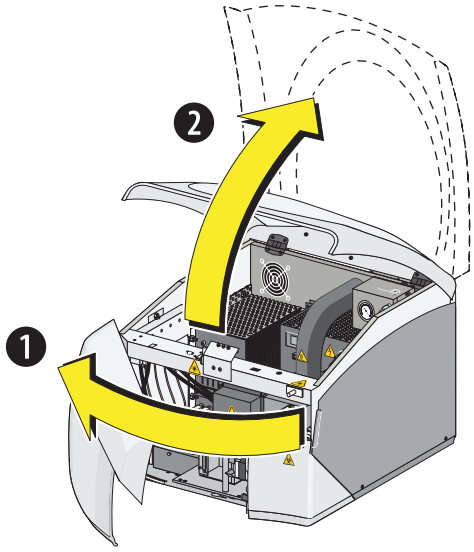
2 Dispose of the contents of the waste and vacuum bottles in accordance with your local regulations and acceptable laboratory procedures.

Note: Take proper precautions to avoid spills if you are emptying the waste container into a sink, drain or larger container.

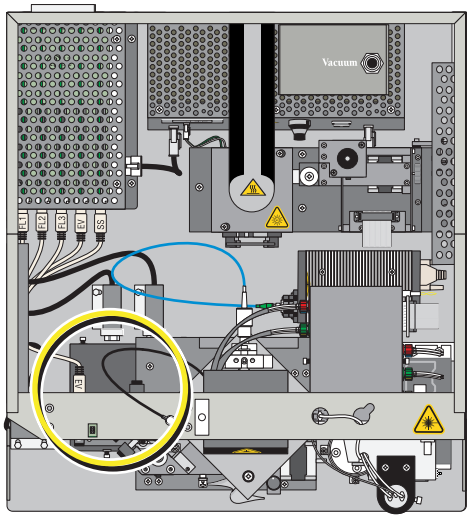
10.5 CHANGING A FILTER

1 Perform Shutdown, refer to Heading 6.3, PERFORM SHUTDOWN for detailed instructions.

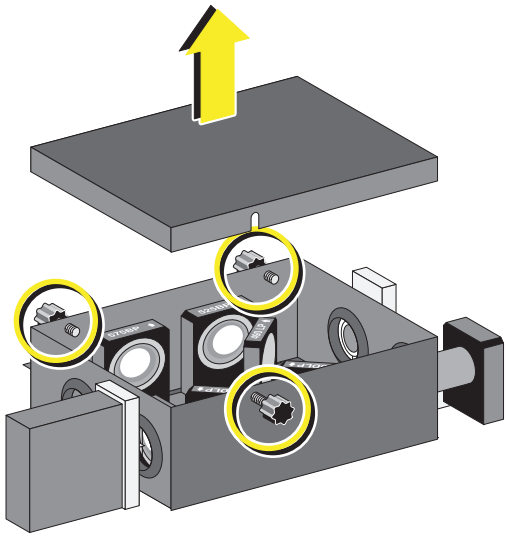
2 Open the cover, refer to Heading 10.3, OPENING/CLOSING THE COVER for detailed instructions.



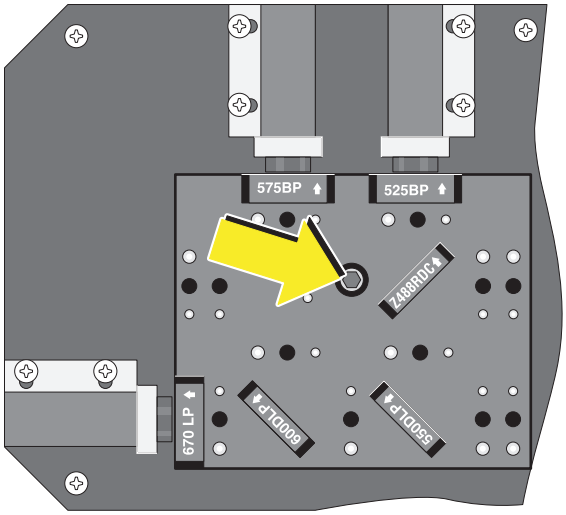
3 Locate filter area.



4 Loosen thumbscrews and lift off filter cover.

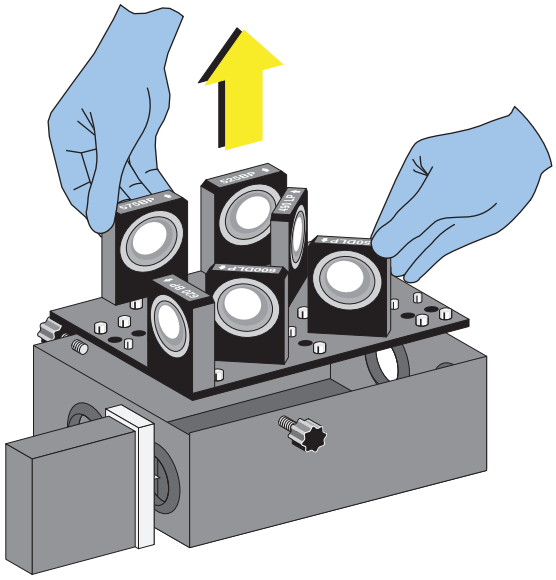


5 Use a 3/16 in. Allen wrench to unscrew the bolt in the middle of the optical filter plate.

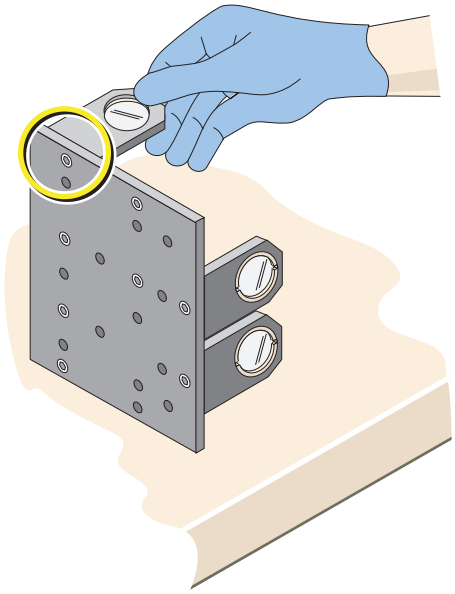


6 Lift the optical filter plate up and off. Set this optical filter aside.

Note: Do not touch lens. Inspect lens to ensure free from smudges or debris. If smudges are present, clean with lens cloth.



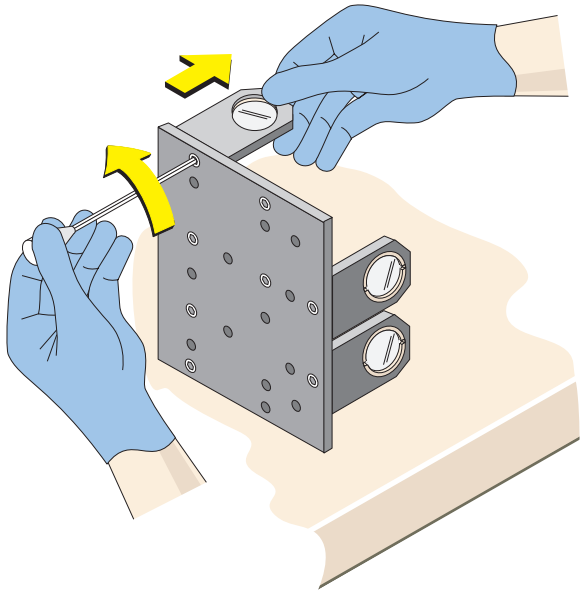
7 Turn the optical filter on its side and locate the screw that holds the filter holder to be removed.



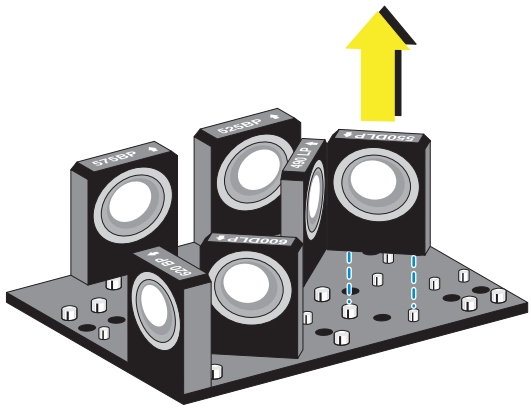
8 Use a 9/64 in. Allen wrench to remove the screw that holds the filter to be replaced from the optical filter plate.

Note: Do not touch lens. Inspect lens to ensure free from smudges or debris. If smudges are present, clean with lens cloth.

CAUTION Risk of damage to instrument if filter block is dropped. Use care when handling filter block and changing a filter.



9 Remove the filter holder to be replaced by pulling it up and out of the optical base. See below for typical locations of filters:



❶ FL1 Filter and holder (525 BP)
For Mercury Arc Lamp (465 BP)

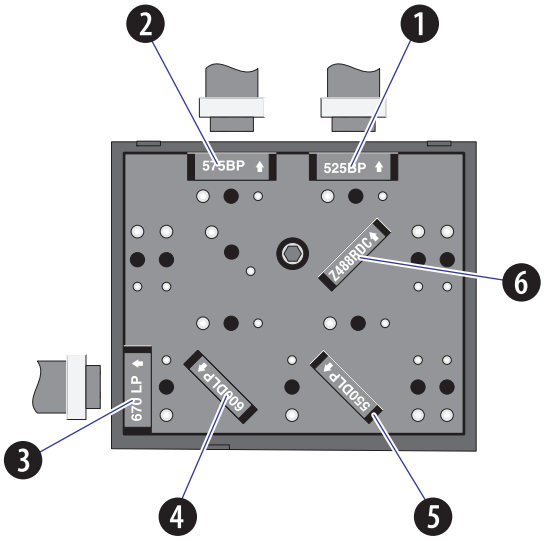
❷ FL2 Filter and holder (575 BP)

❸ FL3 Filter and holder (670 LP)

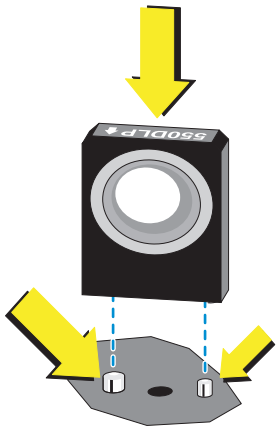
❹ Dichroic/Splitter and holder (600 DLP)

❺ Dichroic/Splitter and holder (550 DLP)

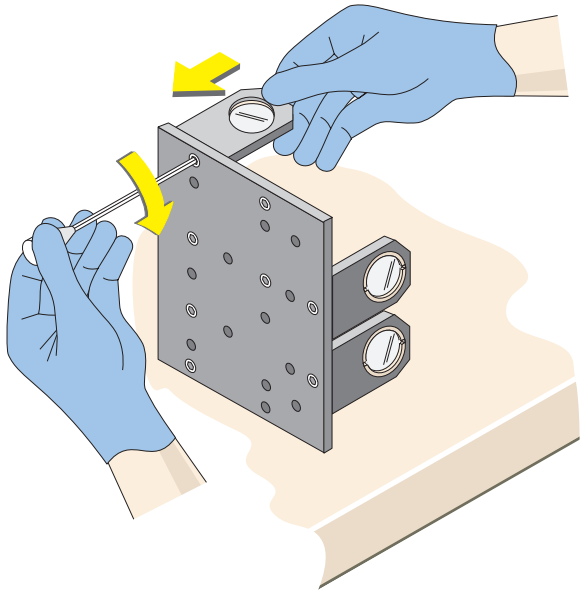
❻ Dichroic/Splitter and holder (Z488RDC)
For Mercury Arc Lamp, this filter is not used.



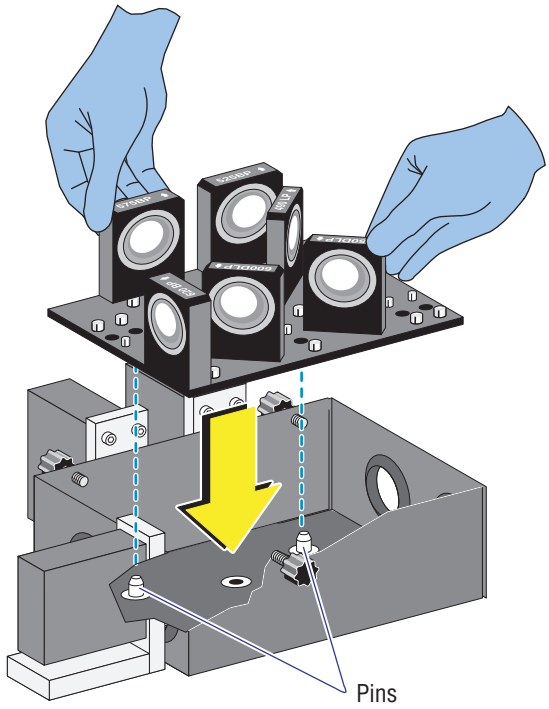
10 Orient the filter holder and filter correctly. Match up the filter holder pins so that the large pin and small pin fit securely inside filter holder.



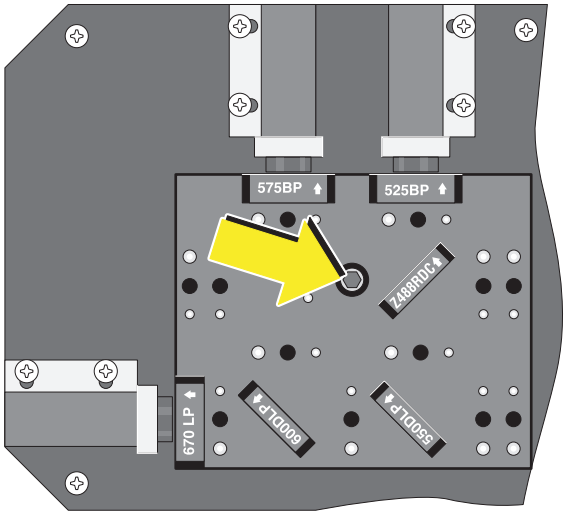
11 Insert the filter holder into the optical filter plate and screw in the holding screw.



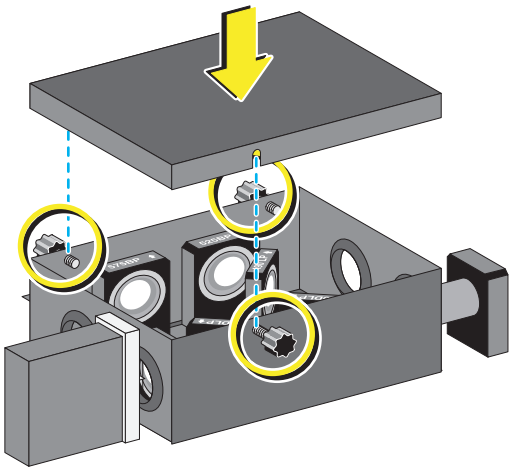
12 Replace the optical filter plate into the instrument. Check that it is firmly seated.



13 Use a 3/16 in. Allen wrench to screw in the bolt in the middle of the optical filter plate.

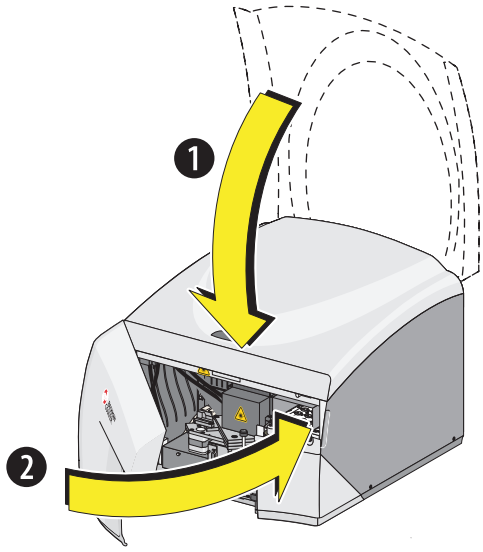


14 Replace filter cover and tighten thumbscrews.



15 Close the cover.

CAUTION Closing the front cover before the top cover can damage the covers and interrupt the laser interlock. Pull the top cover down first, then close the front cover.



16 Perform Startup, refer to Heading 6.1, PERFORM STARTUP for detailed instructions.

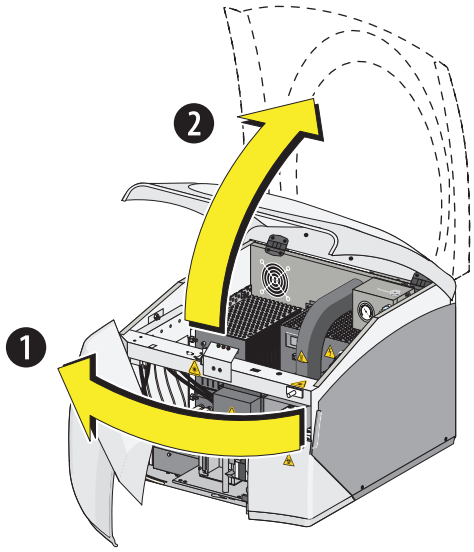
17 Perform Daily QC, refer to Heading 8.3, DAILY QC for detailed instructions.

10.6 CHANGING AN ARC LAMP EXCITATION FILTER

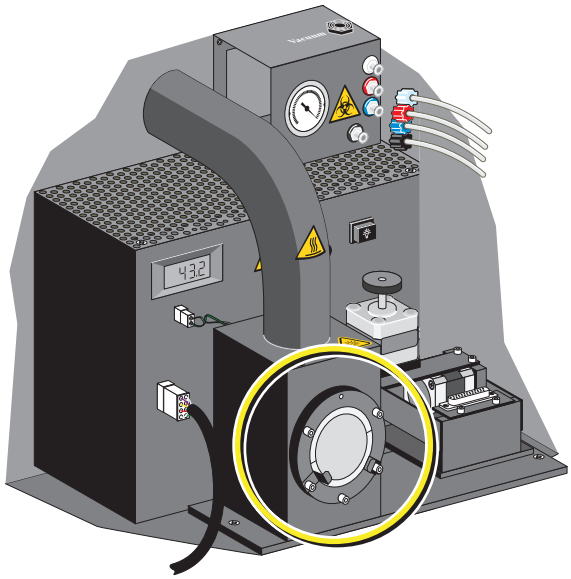
For use with 365, 405, 435 nm Arc Lamp.

1 Perform Shutdown, refer to Heading 6.3, PERFORM SHUTDOWN for detailed instructions.

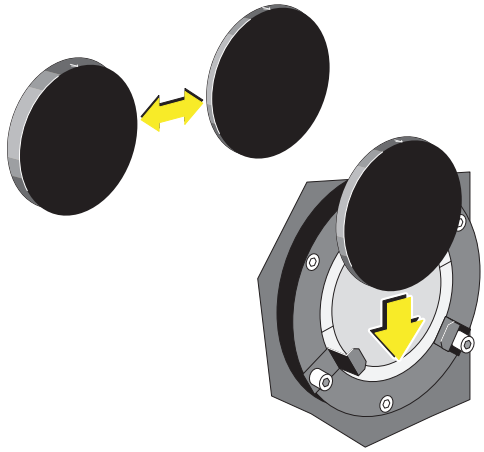
2 Open the cover, refer to Heading 10.3, OPENING/CLOSING THE COVER for detailed instructions.



3 Locate filter area.

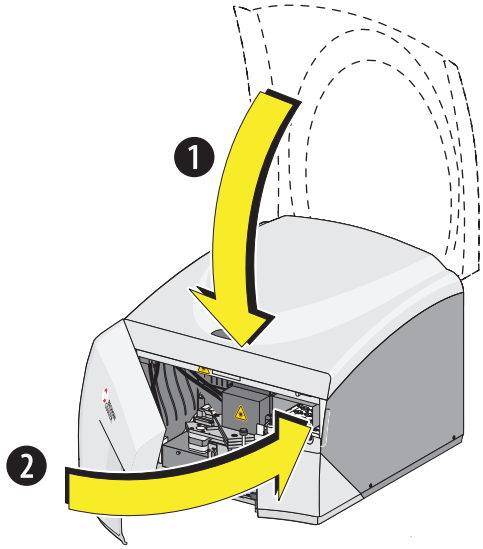


4 Place selected Arc Lamp Excitation Filter in front of the Mercury Arc Lamp.



5 Close the cover.

CAUTION Closing the front cover before the top cover can damage the covers and interrupt the laser interlock. Pull the top cover down first, then close the front cover.



6 Perform Startup, refer to Heading 6.1, PERFORM STARTUP for detailed instructions.

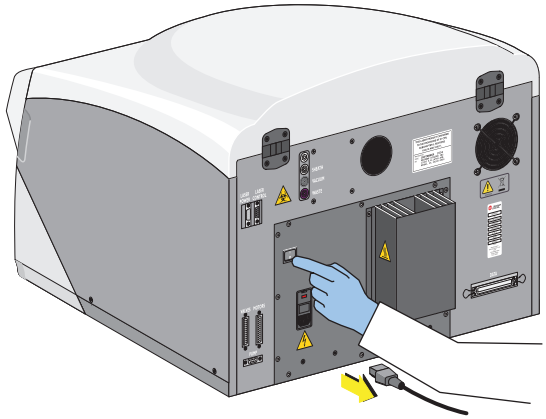
7 Perform Daily QC, refer to Heading 8.3, DAILY QC for detailed instructions.

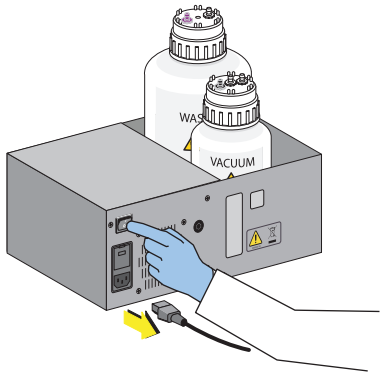
10.7 REPLACING THE FUSE

Perform this procedure to replace a blown fuse. Prior to beginning this procedure, ensure that the power cord has been disconnected from the instrument, for details refer to Heading 10.2, INSTRUMENT QUICK DISCONNECT.

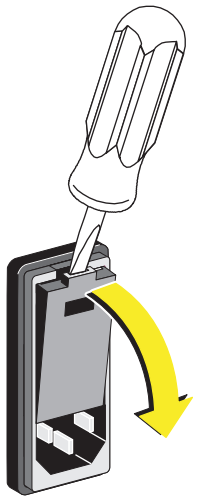
Fuse	Voltage Rating	Current Rating
1.25” MDL Time Delay Glass Fuse	100/120	6A
1.25” 3AG Slo-Blo Glass Fuse	220/240	3A
1.25” Slo-Blo Glass Fuse (for pump cabinet)	100/120	1A
1.25” Slo-Blo Glass Fuse (for pump cabinet)	220/240	.5A

1 Locate the power cord connection and remove power cord from instrument or pump cabinet.

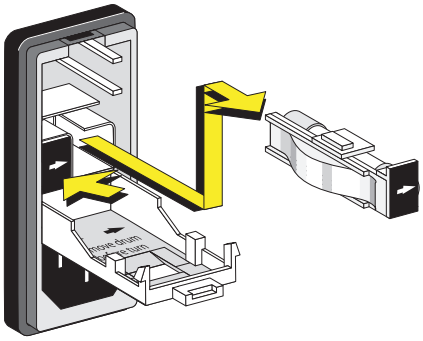




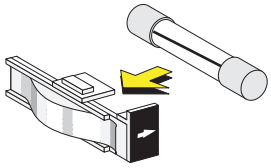
2 Using a flat head screwdriver, open the fuse holder door by inserting in notch at the top of the holder.



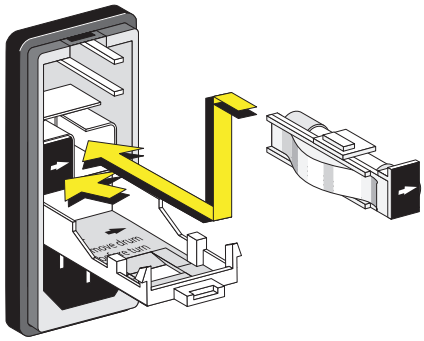
3 Remove fuse to be replaced from the fuse holder.



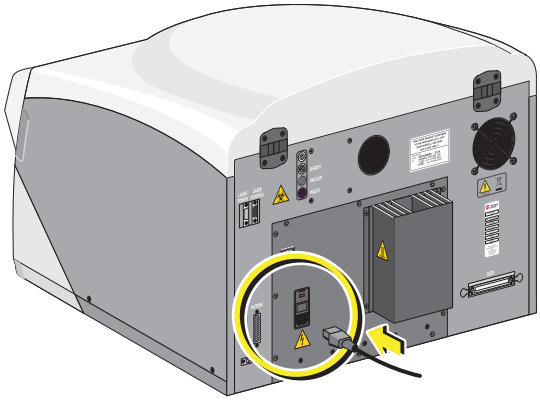
4 Insert new fuse into holder.

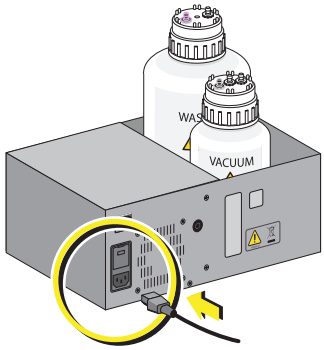


5 Replace fuse holder into the fuse module.



6 Replace fuse holder door and reconnect power to the instrument.

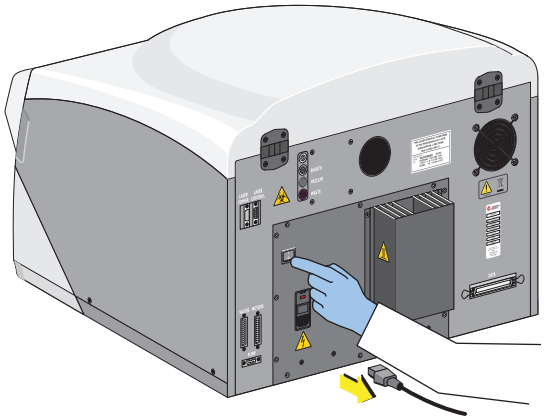


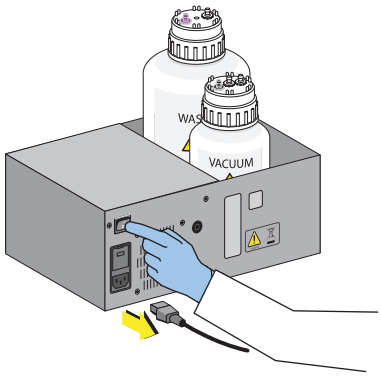


10.8 ADJUSTING FUSE VOLTAGE

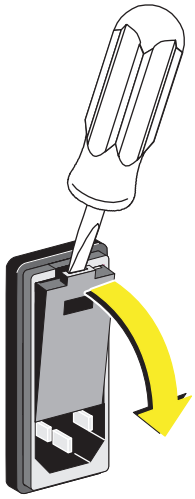
Perform this procedure to adjust or change the fuse voltage for those countries operating outside of the 120 V AC. Prior to beginning this procedure, ensure that the power cord has been disconnected from the instrument, for details refer to Heading 10.2, INSTRUMENT QUICK DISCONNECT.

1 Locate the power cord connection and remove power cord from instrument.

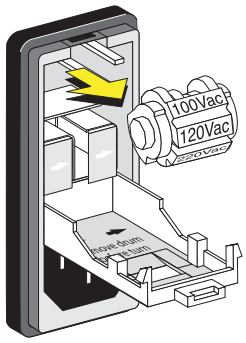




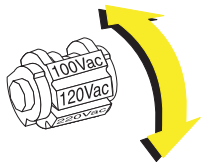
2 Using a flat head screwdriver, open the fuse holder door by inserting in notch at the top of the holder.



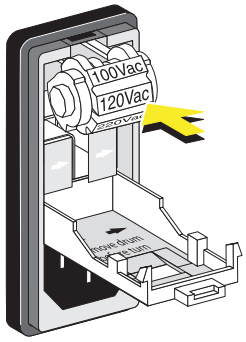
3 Pull the voltage indicator out of the fuse holder.



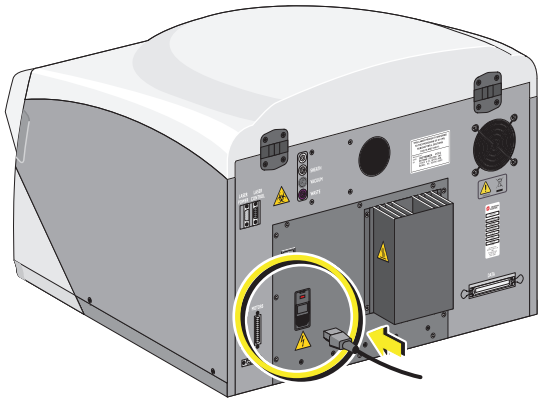
4 Turn the voltage indicator to the appropriate voltage rate.

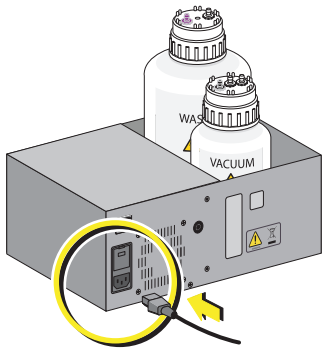


5 Reinsert the voltage indicator in the fuse holder ensuring the correct voltage is displayed through the fuse holder window.



6 Replace fuse holder door and reconnect power to the instrument.





11.1 SYSTEM CONNECTIONS

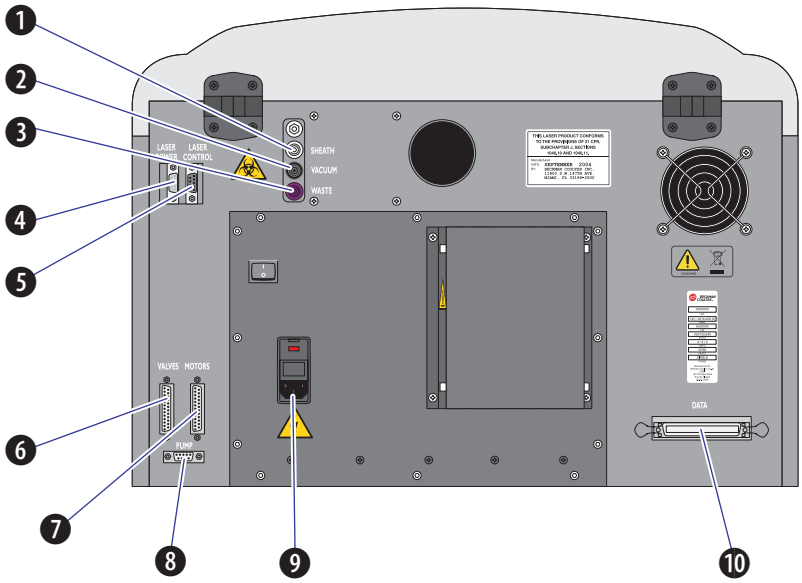
There system is connected:

- from the PC to the instrument, monitor, and printer
- from the Analyzer to the Waste and Vacuum Bottles

For details regarding these connections, see:

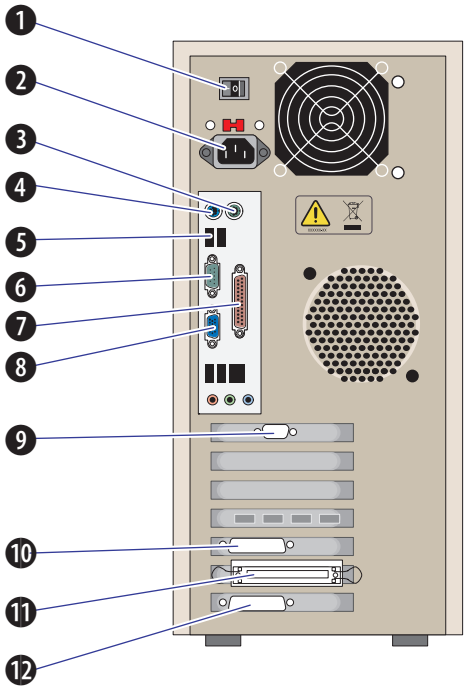
- Figure 11.1, Cable Connections: Back of Instrument
- Figure 11.2, Cable Connections: Back of PC
- Figure 11.3, Cable Connections: Monitor
- Figure 11.4, Tubing Connections: Waste Bottle and Vacuum Bottle

Figure 11.1 Cable Connections: Back of Instrument



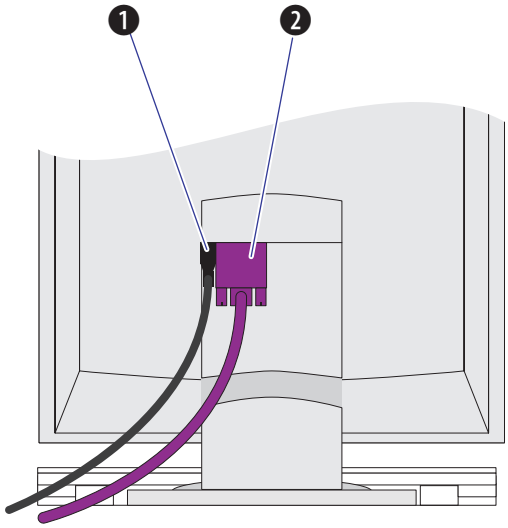
1	Sheath	2	Vacuum
3	Waste	4	Laser Power Supply
5	Laser to computer com port	6	Valves, connects to PC but not to printer parallel port.
7	Stepping Motors, connects to PC	8	Sample pump to com port
9	Power cord, UPS, fuse holder, plugs into surge protector	10	ADC Boards, connects to PC

Figure 11.2 Cable Connections: Back of PC



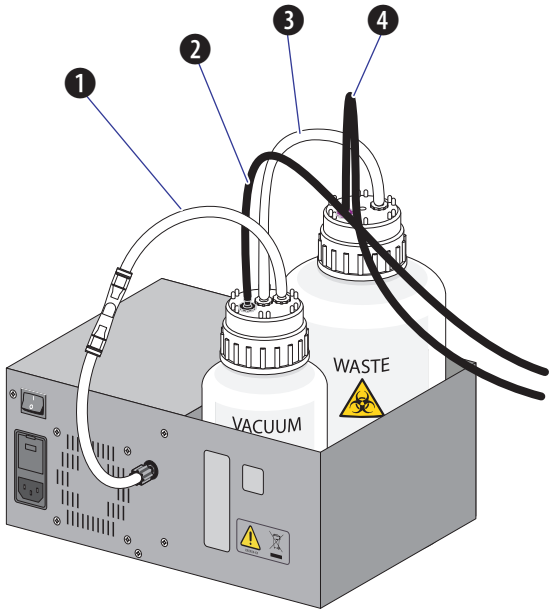
1. Power ON/OFF switch
2. Power cord, plugs into the surge protector
3. Keyboard connection
4. Mouse connection
5. Printer/USB
6. Pump
7. Printer Port (only)
8. Monitor
9. Laser
10. Motors
11. ADC
12. Valves

Figure 11.3 Cable Connections: Monitor



1. Power cord, plugs into the surge protector
2. Cable, connects to the PC

Figure 11.4 Tubing Connections: Waste Bottle and Vacuum Bottle

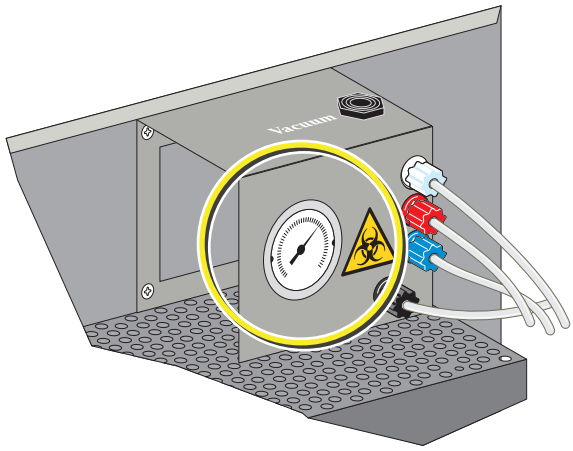


1. Tubing connection to pump
2. Tubing connection to vacuum to instrument
3. Tubing connection connects waste to vacuum bottles
4. Tubing connection to waste from instrument

11.2 MONITOR VACUUM READINGS

The vacuum monitoring occurs as part of the Start Up procedure. For detailed startup instructions refer to Heading 6.1, PERFORM STARTUP.

Ensure the vacuum gauge reads -10”Hg. Refer to Figure 1.12, Vacuum Regulator: Controls and Indicators for location of vacuum gauge.



11.3 ERROR MESSAGES

See Table 11.1.

Table 11.1 Error Messages

Message	Probable Cause	Recommended Corrective Action
Metering Pump communications error	Cable is not plugged in, or not working properly. The power is off.	Check the cable, power.
Metering Pump error	Hardware Failure with the pump.	Initialize the pump.
Laser communication error	Cable is not plugged in, or not working properly. The power is off.	Check the cable, power.
Laser error	Hardware Failure with the Laser	Power down, power up.
ADC power on error	The system power is off	Turn on power
Software error	System lock-up.	Exit software, restart.
Yellow Warning Box	Waste Reservoirs full and/or Sheath Bottle empty.	Empty the Waste Reservoirs and/or Fill the Sheath Bottle.

11.4 TROUBLESHOOTING GUIDE

Table 11.2 provides a troubleshooting guide.

Table 11.2 Troubleshooting Guide

Problem Area	Situation	Suggested Action
Analysis	If the instrument stays at "Stabilizing" for more than 10 seconds: • discriminator value may be improperly set • gain value may be improperly set • insufficient sample volume • improper setting of the collection parameter has occurred.	Press Stabilizing button to end the stabilization process and to begin normal data collection.
Mercury Arc Lamp	Lamp did not ignite due to: • lamp bulb is hot and must cool before reigniting • lamp bulb is defective.	Press the lamp button until the lamp ignites. If ignition does not occur within 10 seconds, release lamp button. Wait two to five seconds, press lamp button again and hold until the lamp ignites. If ignition does not occur within ten seconds, release the lamp button and repeate the above.

IMPORTANT Possible erroneous results if laser is operated with a laser base temperature >7°C (45°C) above ambient. Operating the laser at a temperature >7°C (45°C) above ambient may result in high HPCVs and CVs and/or the gain and voltage may have to be adjusted significantly from original settings.

CAUTION Possible damage or reduced bulb life expectancy if Mercury Arc Lamp is ignited when lamp is HOT. If the Mercury Arc Lamp is turned OFF for any reason, including brief power failures, the lamp must be allowed to cool before reignition. Wait at least 15-20 minutes before attempting to reignite Mercury Arc Lamp. Very high or unstable CVs or HPCVs may occur as the result of a damaged Mercury Arc Lamp.

Table 11.2 Troubleshooting Guide *(Continued)*

Problem Area	Situation	Suggested Action
Performance	If performance problems occur due to: • Bubbles in tubings and erratic flow rate • HPCV out of limits • Unstable volume • After running biologicals, high HPCV and unstable EV • EV signal has lots of particles • If signal appears on FL2 when running Arc Lamp.	For all performance problems, perform **Flush** twice and then a Cleaning Cycle. If performance problems still persist: • HPCV out of limits, perform an Optical Auto Alignment. • High HPCV and unstable EV, soak flow cell in COULTER CLENZ for a minimum of two hours or overnight. • EV signal has debris, try new sample cup. • FL2 signal appears with Arc Lamp, ensure laser is OFF.
Printer	Printer will not print	Refer to the printer manual provided by the printer manufacturer.
System	System is not working due to: • System locks up, not responding • Cannot switch to Log or go back to Linear • Vacuum pump will not turn on • Loss of vacuum • Power supply turns OFF.	For all system problems, check all connections in Heading 11.1, SYSTEM CONNECTIONS. If system problems still persist: • System lock ups and unable to switch to Log or Linear, exit software and restart • Vacuum pump inactive, check vacuum pump fuses and replace if faulty • Loss of vacuum, ensure waste container cap is tight. • Power supply OFF, check instrument fuses and verify AC line is at the correct current setting.

REFERENCES

1. Coulter WH. High speed automatic blood cell counter and cell size analyzer. Paper presented at National Electronics Conference, Chicago, IL, 1956; October 3.
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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.

Arc lamp alignment beads - Mercury Arc Lamp alignment is obtained by using these beads to insure proper alignment, optimal resolution and sensitivity with the systems optics.

Arc lamp excitation filter - Optical filter used to select the desired wavelength available from the Mercury Arc Lamp.

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.

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

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

Boost - Moving the sample from the sample loop to the flow cell.

Character - The smallest group of elements that makes a number, letter, or punctuation mark.

Cleaning solution - 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.

Color compensation - The subtraction of:

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

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

Control - A substance used to routinely monitor the performance of an analytical process that has 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.

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

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.

DiOC5(3) - Abbreviation for oxacarbocyanine dye.

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.

Electronic Volume (EV) - The signal produced as a particle passes through the flow cell resulting in an increased impedance to the current flow that is proportional to the volume of the particle.

Event - A particle passing through the laser beam.

Export file (*.XLS) - File containing selectable statistics and other sample information from each sample run.

FC - Fluorescent Concentration is the amount of fluorescent light (FL1, FL2, FL3) divided by the Electronic Volume (EV).

FDA - Abbreviation for fluorescein diacetate dye.

FITC - Abbreviation for fluorescein isothiocyanate dye.

FSD - Fluorescent Surface Density is the amount of fluorescent light (FL1, FL2, FL3) divided by two-thirds root of the Electronic Volume (EV).

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

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

Flow Cytometry Standard (FCS) - The flow cytometry data file standard that provides the specifications needed to completely describe flow cytometry data sets within the confines of the file containing the data.

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

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

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

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

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

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

GB - The abbreviation for gigabyte.

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

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

HPCV - Half peak coefficient of variation

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

Indicators - See Controls and Indicators.

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

Laser - Abbreviation for light amplification by stimulated emission of radiation.

Linear amplification - See gain.

Listmode data - A list of measurements from each cell.

Listmode playback tool - A tool used: 1) to replay 20-bit linear listmode data though new compensation settings, 2) for panel playback of listmode files, and 3) to replay AutoSetup listmode files to generate a new compensation file.

LiveGate - Listmode Gate used to exclude events from the raw (listmode) data.

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

MB - Abbreviation for megabyte.

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

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

Mercury arc lamp - High pressure lamp which emits ultraviolet light. The Mercury Arc Lamp also emits light at several wavelengths (365, 405, 434, 548 and 578 nm).

Microscope focus - Accurately adjusts the beam of light directly at the cells/particles in the flow stream which will then provide optimal resolution.

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

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.

NIM-DAPI - Nuclear Isolation Media - 4'6' Diamidino Z Phenyl Indol.

Optical alignment - focuses the light onto the flow cell to achieve maximum signal strength and optional HPCV values.

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 ratio analysis - Displays a calculated parameter based on the ratio of two measured parameters.

PC7 - Abbreviation for phycoerythrin-cyanine tandem dye.

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

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.

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

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

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

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

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

RD1 - Abbreviation for phycoerythrin dye.

Sample cup - Recptacle that holds the sample to be analyzed.

Scroll bar - The area on the left of a pop-up window. The bar's arrows let you move (scroll) the window's content up or down so that you can see other parts of it.

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

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

Sheath fluid - A balanced electrolyte solution.

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

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

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

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.

Numerics

A

B

C

D

E

F

G

H

I

S

T

U

V

W

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