Cytomics FC 500 With CXP Software

Reference



WARNINGS AND PRECAUTIONS

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

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Can cause injury.

CAUTION - Can cause damage to the instrument.

IMPORTANT - Can cause misleading results.

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

WARNING Risk of operator injury if:

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

To avoid injury:

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

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

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

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

REVISION STATUS

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CXP Software Version 1.0. Initial issue for customer distribution.

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This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released.

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This introductory section contains the following topics:

- USING YOUR Cytomics FC 500 MANUALS
- ABOUT THIS MANUAL
- CONVENTIONS, and
- GRAPHICS.

USING YOUR Cytomics FC 500 MANUALS

The manuals listed below are available as PDF files in the FC 500 CXP software and the Operator's Manuals CD-ROM. Printed versions of these manuals are also available by order.

Use the **Reference** manual for instrument specifications and information on installation and system options.

Use the **Instructions** For Use manual for the day-to-day running of your instrument. Go through the detailed step-by-step procedures of startup, quality control (QC), running samples, analyzing data, printing reports, reviewing QC data and shutdown. It contains safety and troubleshooting information, error messages, as well as in-depth information on the principles of flow cytometry, information about what your instrument does, and the methods it uses.

Use the Special Procedures manual to clean, replace, or adjust a component of the instrument.

Use the **Getting Started** manual as a brief introduction to the system.

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

ABOUT THIS MANUAL

Your Cytomics FC 500 Reference manual provides instrument specifications and information on installation, safety and software features.

This information 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, SPECIFICATIONS
 Details the instrument and performance specifications.
- Appendices

The appendices provide reference material on the following topics:

- LOG SHEETS
- USING CXP SOFTWARE
- GLOSSARY

The glossary has definitions for many of the words and terms used in flow cytometry.

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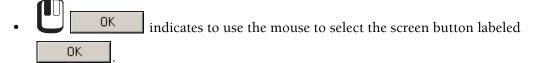
INDEX
 Use the Index to easily locate specific information in this manual.

CONVENTIONS

This manual uses the following conventions:

- Throughout this manual your FC 500 is also referred to as the system or instrument.
- **Bold font** indicates a software option, such as **Cytometer**.
- *Italics font* indicates screen text displayed on the instrument, such as *Preparing Samples*.
- Courier font indicates text you have to type using the keyboard.
- Indicates a key (such as Enter).
- - a. Press down on the first key listed and while continuing to press it, press down on the second key listed.
 - b. Release both keys at the same time.
- Indicates to press and release the first key listed then press and release the next key listed. For example: Y Enter.
- Icons/buttons to select functions on the software screen are shown within text.





- File >> Save indicates to use the mouse to select the Save item on the File menu.
- [F1] through [F12] are special function keys.
- A **Note** contains information that is important to remember or helpful in performing a procedure.
- The terms "screen" and "window" are used interchangeably.
- **SHOW ME** means there is a video available for the procedure in the online help.

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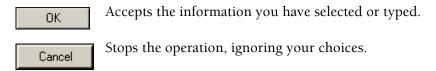
To Choose A Command With The Keyboard

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

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

Dialog Box

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



Description of Reporting Units

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

GRAPHICS

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

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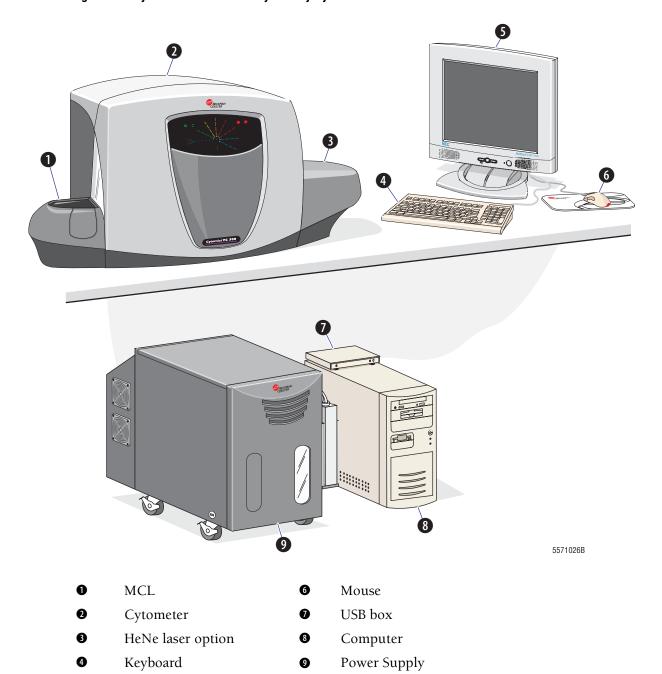
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1.1 SYSTEM COMPONENTS

The system components are shown in Figure 1.1.

Figure 1.1 Cytomics FC 500 Flow Cytometry System



Cytometer

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Monitor

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

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Power Supply

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

Workstation

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

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

Multi-tube Carousel Loader (MCL)

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

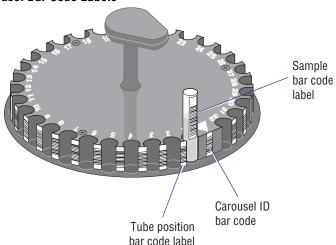
- Codabar
- Code 39® bar code
- Code 128
- Interleaved 2-of-5.

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

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

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

Figure 1.2 MCL Carousel Bar-Code Labels



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CXP Software

The CXP software consists of two separate applications:

- CXP Cytometer. This contains the Acquisition and Analysis software and is used when running the Cytometer.
- CXP Analysis. This contains the Analysis software for offline analysis. You can perform MultiFile Analysis with this software.

1.2 OPTIONS

Hardware Options

Printer

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

Bar-Code Scanner

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

- Refer to the bar-code reader manufacturer's manual for specific instructions about using it.
- This illustration shows how to connect the bar-code reader to your system. One bar-code connector plugs into the keyboard. The other connector • plugs into the keyboard connection on the back of the computer.



Workstation/Network Server

A Beckman Coulter FlowCentre[™] Multimedia Workstation can be used as an offline analysis workstation and network server for data and worklist transfer.

Second Laser

- A 635-638 nm red solid-state laser providing a minimum of 25 mW light regulated laser power, or
- A 633-635 nm air-cooled red Helium Neon (HeNe) laser providing a minimum of 20 mW light regulated laser power. Additional covers are added to the instrument to accommodate the HeNe laser.

The second laser has a shutter that allows you to block the laser output.

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Filter Kit

The optional filter kit includes 5 extra filter holders, an ND 1.0 filter, a full mirror (in a holder), a 50% mirror in a holder and an additional filter block base.

Single Laser Filter Block

An assembled filter block configured as shown in Figure 5.5 in the Instructions For Use manual.

Dual Laser Filter Block

An assembled filter block configured as shown in Figure 5.6 in the Instructions For Use manual.

Single Laser Filter Upgrade

The single laser filter upgrade kit includes two filters: a 620 BP filter and a 645 DSP filter.

Software Options

- Acquisition/Analysis
- Analysis only version
- Multi user Analysis packs
- Network user Analysis packs

1.3 REAGENTS AND QUALITY CONTROL MATERIALS

Beckman Coulter recommends these reagents or their equivalents. All stated performance characteristics in this manual are based on the use of the Cytomics FC 500 system with the following reagents.

Sheath Fluid

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

IsoFlow sheath fluid has the following characteristics:

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

The sheath container has a working capacity of about 1.8 L. The amount of sheath fluid the container holds beyond the working capacity is for pressurization and level sensing.

Cleaning Agent

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

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The cleaning agent container has a working capacity of about 500 mL. That is enough cleaning agent to use the Cleanse mode once a day for 1 month. The amount of cleaning agent the container holds beyond the working capacity is for pressurization and level sensing.

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

Quality Control Materials

Flow-Check™

The quality control materials available from Beckman Coulter are:

Fluorospheres used to check the stability of the optical and fluidic systems.

Fluorospheres	Traditosphoros assa to oncok the stability of the optical and halde systems.
Flow-Set [™] Fluorospheres	Fluorospheres used to standardize light scatter and fluorescence intensity for human leukocyte applications.
Flow-Count™ Fluorospheres	Fluorospheres with an assayed concentration that are used to determine absolute counts.
Stem-Count [™] Fluorospheres	Fluorospheres with an assayed concentration that are used to determine absolute counts.
Leuko-Count™ Fluorospheres	Fluorospheres with an assayed concentration that are used to determine absolute counts.
CYTO-TROL™ Control Cells	Lyophilized lymphocytes with assay values for specific surface antigens. Used to assess monoclonal antibody function and verify proper flow cytometer setup.
IMMUNO-TROL™ Cells	Stabilized erythrocytes and leukocytes with a known quantity of surface antigens. Used to verify monoclonal antibody performance as well as verify the process of sample staining, lysing, and analysis.
IMMUNO-TROL Low Cells	Stabilized erythrocytes and leukocytes with a known quantity of surface antigens. Used to verify monoclonal antibody performance as well as verify the process of sample staining, lysing, and analysis.
Immuno-Brite™ Fluorospheres	Uniform size fluorospheres with varying fluorescence intensities that are used to monitor instrument linearity.
CYTO-COMP™ Cell Kit	CYTO-COMP Cells stained with a single color, such as CD45, are used to adjust color compensation settings for multicolor analysis using monoclonal antibodies.
Stem-Trol™ Control Cells	Lyophilized KG-1a cells that have been modified and stabilized to approximate human hematopoietic progenitor cells (HPC).
QuickCOMP 2 Kit	Two single-color antibody reagents (FITC and PE) that can be used to adjust color compensation on a flow cytometer.
QuickCOMP 4 Kit	Four single-color antibody reagents (FITC, PE, ECD, and PC5) that can be used to adjust color compensation on a flow cytometer.
F F F F L F CC I C I L I F CC C C	Fluorospheres Flow-Set TM Fluorospheres Flow-Count TM Fluorospheres Stem-Count TM Fluorospheres Leuko-Count TM Fluorospheres CYTO-TROL TM Control Cells IMMUNO-TROL Low Cells IMMUNO-Brite TM Fluorospheres CYTO-COMP TM Cell Kit Stem-Trol TM Control Cells QuickCOMP 2 Kit

The reagents listed below are for "Research Use Only, not for use in diagnostic procedures".

PC7 (770/488) Setup Kit	Argon (488 nm laser) excitable PC7 channel alignment fluorospheres and argon excitable PC7 intensity standard.
APC (675/633) Setup Kit	Red laser excitable alignment fluorospheres and red laser excitable intensity standard.

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1.4 MATERIAL SAFETY DATA SHEETS (MSDS)

To obtain an MSDS for Beckman Coulter reagents used on the Cytomics FC 500 flow cytometer:

- 1. On the internet, go to http://www.beckmancoulter.com and select MSDS from the Customer Support drop down menu.
- 2. If you do not have internet access:
 - In the USA, either call Beckman Coulter Customer Operations (800-526-7694) or write to:

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

• Outside the USA, contact your Beckman Coulter Representative.

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2.1 DELIVERY INSPECTION

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

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

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

2.2 SPECIAL REQUIREMENTS

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

Space and Accessibility

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

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

Specifications	FC 500 with HeNe Laser	FC 500 w/o HeNe Laser or with Red Solid-State Laser.
Height	61.0 cm (24 in.)	61.0 cm (24 in.)
Additional clearance above for servicing and lifting the Data Acquisition card cage above the sensor	45.7 cm (18 in.) min.	45.7 cm (18 in.) min.
Total clearance needed	106.7 cm (42 in.)	106.7 cm (42 in.)
Width	111.8 cm (44 in.)	90.0 cm (35.5 in.)
Additional clearance on right for servicing	30.5 cm (12 in.)	30.5 cm (12 in.)
Additional clearance on left for servicing	30.5 cm (12 in.)	30.5 cm (12 in.)
Total clearance needed	172.7 cm (68 in.)	151.0 cm (59.5 in.)
Depth	88.9 cm (35 in.)	88.9 cm (35 in.)
Additional clearance behind instrument for sufficient cooling and room for servicing	30.5 cm (12 in.)	30.5 cm (12 in.)
Total clearance needed	119.4 cm (47 in.)	119.4 cm (47 in.)

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Electrical Input

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

The Power Supply requires two dedicated outlets with isolated grounds.

Country	Dedicated Lines with Isolated Grounds	Non-Dedicated Lines
USA	Two dedicated lines at 115 Vac, 50/60 Hz at 20 A (16 A continuous)	Three non-dedicated lines at 115 Vac, 50/60 Hz at 20 A
Europe and other applicable countries	Two dedicated lines at 220 Vac, 50/60 Hz at 15 A or Two dedicated lines at 240 Vac, 50/60 Hz at 15 A	Three non-dedicated lines at 220 Vac, 50/60 Hz at 10 A
Japan	Two dedicated lines at 100 Vac, 50/60 Hz at 25 A	Three non-dedicated lines at 100 Vac, 50/60 Hz at 20 A

The computer requires a separate outlet, but it does not have to be a dedicated line

Ambient Temperature and Humidity

Keep the room temperature between 16°C and 32°C (60°F and 90°F), and do not let it change more than 5°F per hour. Keep the humidity between 30% and 80%, without condensation.

Heat Dissipation

Heat dissipation is 2,300 W (7,850 Btu/hour) for a single laser system. Heat dissipation is 2,330 W (7,955 Btu/hour) for a two-laser system. Provide sufficient air conditioning (refer to Ambient Temperature and Humidity).

Drainage

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

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

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

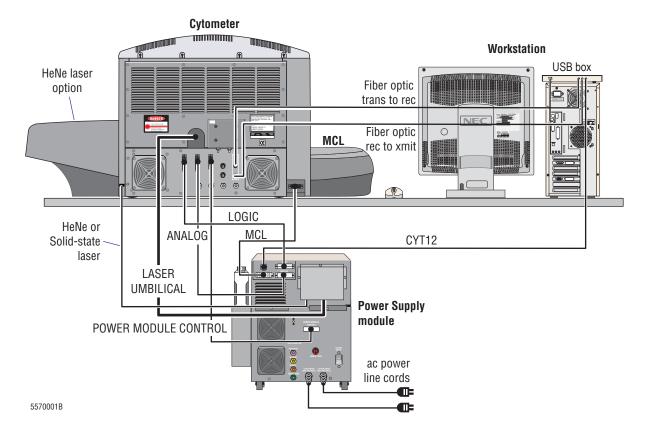
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2.3 SYSTEM CONNECTIONS

Power and Signal Cables

Figure 2.1 or Figure 2.2 shows the interunit connections of the power and signal cables, depending upon your Power Supply configuration. Computer configuration may vary based on current configuration availability.

Figure 2.1 Power and Signal Cable Connections, with Voltage-specific Power Supply



PN 175570B 2-3

1

Cytometer back cover Cytometer Workstation USB box Ve laser Fiber optic ption trans to rec Fiber optic MCL rec to xmit leNe or MCL d-state CYT12 laser Logic Power Analog module control ac power Power line cords Laser Supply umbilical module 5570005B

Figure 2.2 Power and Signal Cable Connections, with Universal Power Supply

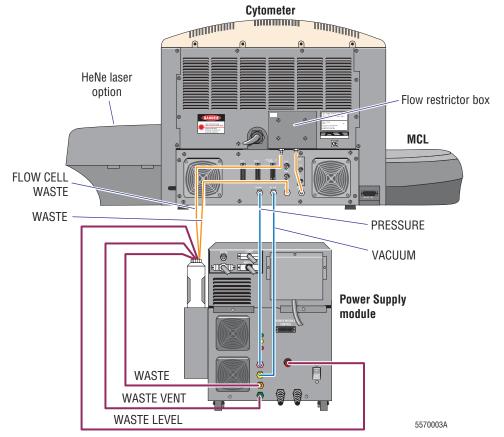
2-4 PN 175570B

Waste and Pneumatic Tubing

Figure 2.3 or Figure 2.4 shows the interunit connections for waste and pneumatic tubing, depending upon your Power Supply configuration.

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

Figure 2.3 Waste and Pneumatic Tubing Connections, with Voltage-specific Power Supply



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Cytometer back cover Cytometer HeNe laser option Flow restrictor box MCL FLOW CELL WASTE WASTE **PRESSURE** VACUUM WASTE **Power Supply** LEVEL module WASTE WASTE VENT 5570004A Power supply back cover

Figure 2.4 Waste and Pneumatic Tubing Connections, with Universal Power Supply

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2.4 INSTALLING CXP SOFTWARE

Your CXP Software is a 2 disk set. The contents of the set is,

- Disk 1 Microsoft[®] .NET Framework and MSDE2000
- Disk 2 CXP Software

Note: You must have Windows Administrator access rights to install the software.

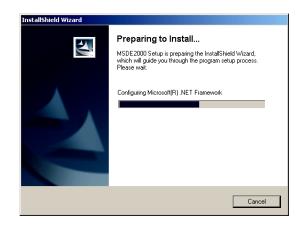
Install Disk 1

If you are reinstalling Disk 1, remove the existing Microsoft .NET Framework and MSDE2000 before proceeding with the installation.

1 Insert Disk 1 into the CD-Rom drive. The CD-Rom should Autorun the Microsoft .NET Framework and MSDE2000 installation.

If the CD-Rom does not Autorun click here for additional instructions.

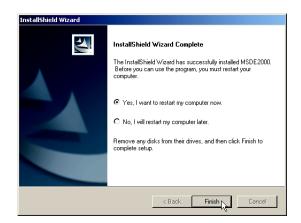
2 Follow the instructions on the installation screens to install the software.



3 Leave the CD-Rom in the drive and

Finish to restart your computer and finish installing the software.

Note: If you are reinstalling Disk 1, restore your database after restarting the computer.



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Install Disk 2

If you are reinstalling Disk 2, remove the existing CXP software before proceeding with the installation. Uninstalling the CXP software does not remove any of your data files, protocols, panels or worklists.

1 Insert Disk 2 into the CD-Rom drive.
The CD-Rom should Autorun the CXP
Software installation and display the
Welcome screen.

If the CD-Rom does not Autorun click here for additional instructions.

2 Follow the instructions on the installation screens to install the software.



Trivish to install the Romlock Drivers. These drivers allow CXP Software to communicate with the Romlock. The Romlock ensures that the person using the personal computer (PC) is a registered user of the CXP software. CXP Software does not function fully without the Romlock installed.

The installation process is now complete and CXP Software is ready to run.



4 If the Romlock drivers are installed, the system prompts you to restart the computer.

Note: The correct display of Tomogram Plots requires graphic card drivers to support Microsoft® Direct X® technology. Some graphic card drivers may show additional lines within the Tomogram cube. To remove the additional lines try updating your graphic card driver using the latest available drivers from the manufacturer's web site.

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If the problem persists, a registry file (**DisableTomogramCube.reg**) is provided in the CXP utilities folder of the Installation disk, which removes all lines from the Tomogram plot.

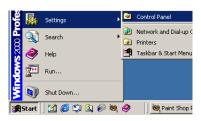
- To remove all Tomogram lines, double click the **DisableTomogramCube.reg** file.
- To re-enable all Tomogram lines, double click the **EnableTomogramCube.reg** file.

2.5 UNINSTALLING CXP SOFTWARE

Remove .NET Framework and MSDE 2000

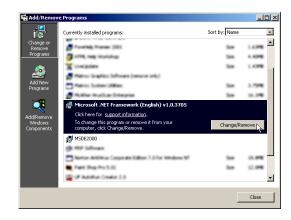
IMPORTANT Loss of all QC and Panel Report Data occurs when you remove Microsoft .NET Framework and MSDE2000. To preserve you QC and Panel Report Data, archive your database before removing Microsoft .NET Framework and MSDE2000.











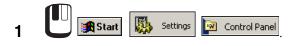


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5 Repeat steps 1 and 2 for MSDE2000.

Remove CXP Software

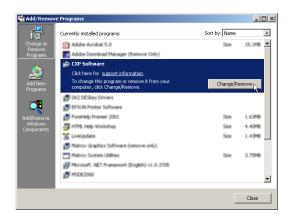
Note: If you remove CXP Software only and do not remove MSDE2000, your QC and Panel Report database is not removed.







3 CXP Software.





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- **5** Check that the shortcut icons have been deleted from the Start menu and the Windows Desktop. If any remain,
 - a. Right mouse click on the icon.
 - b. Select Delete.



If the CD-Rom Does Not Autorun

If the CD-Rom does not Autorun,



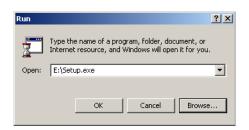


Use the Look In drop down window to locate the CXP Software CD-Rom. This is usually drive D on most systems but it may be another drive (E, F, and so on).





4 Return to step 2 in Installing CXP Software.



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INSTALLATION UNINSTALLING CXP SOFTWARE

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3.1 SAMPLE REQUIREMENTS

See SAMPLE REQUIREMENTS in the RUNNING SAMPLES chapter in the Instructions For Use manual for details.

3.2 INSTRUMENT SPECIFICATIONS

Dimensions

Component	Height	Width	Depth	Weight
Computer	15.75 cm	53.34 cm	42.55 cm	18.14 kg
	(6.2 in.)	(21 in.)	(16.75 in.)	(40 lb)
Cytometer and MCL	61.0 cm	111.8 cm*	105.4 cm	93.9 kg
(only for units with Universal	(24 in.)	(44 in.)	(41.5 in.)	(207 lb)
Power Supply)				
Cytometer and MCL	61.0 cm	111.8 cm*	88.9 cm	84.8 kg
	(24 in.)	(44 in.)	(35 in.)	(187 lb)
Monitor	42.7 cm	40.5 cm	43.8 cm	22 kg
(typical)	(16.81 in.)	(15.94 in.)	(17.25 in.)	(48.4 lb)
Power Supply	58.4 cm	45.7 cm	71.1 cm	59.0 kg
(Universal)	(23 in.)	(18 in.)	(28 in.)	(130 lb)
Power Supply	50.8 cm	45.7 cm	66.0 cm	54.4 kg
(Voltage-specific)	(20 in.)	(18 in.)	(26 in.)	(120 lb)

^{*} This is the width with the optional HeNe laser. The width of the FC 500 with a single argon laser or with the optional red solid-state laser is 90.0 cm (35.5 in.)

Installation Category

Category II (per IEC 1010-1 standard).

Cytometer

Flow Cell

Sensing area: BioSense 150 μ m x 450 rectangular channel with an integral lens, mounted with a vertical (upward) flow path.

Flow Rate

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

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

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Laser

Air-cooled, software controlled, 20 mW, Argon ion laser operating at 488 nm.

Optional second laser:

- Solid-state, software controlled, 25 mW, diode laser operating at 635 nm, or
- Air-cooled, software controlled, 20 mW, HeNe ion laser operating at 633 nm.

The second laser has a shutter that allows you to block the laser output to the flow cell.

Laser power is monitored 5 times per second. If the laser power deviated more than $\pm 1\%$ a Laser Power Error is displayed on the Status Bar. The system will not run a sample until corrective measures rectify the fault.

Beam-Shaping Optics

Cross cylindrical lenses 10 mm by 80 mm.

Argon Laser Beam Spot Size

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

Solid-State Laser Beam Spot Size

It approximates the beam size for the argon laser.

HeNe Laser Beam Spot Size

An elliptical spot 9.5-µm high by 85-µm wide.

Optical Filters

For filters used in the single filter block and the dual laser filter block configurations, see Figure 5.6 and Figure 5.5 in the Instructions For Use manual.

- 500-nm long pass (LP) filter
- 530-nm short pass (SP) filter
- 488-nm, 550-nm, 600-nm, and 710-nm dichroic, long-pass (DLP) filters
- 645-nm dichroic, short-pass (DSP) filter
- 525-nm, 575-nm, 620-nm, and 675-nm band-pass (BP) filters
- 755-nm long-pass (LP) filter

Sensors

- The FS sensor and the SS sensor are photodiodes.
- The five FL sensors are photo-multiplier tubes (PMTs) that have a 185-nm to 900-nm spectral range.

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Signal Processing

•	High voltage amplification	on, minimum	250 up	to 1,100,	in increments	of 1, for:

FL1 FL2

FL3

FL4 FL5

Vernier gain (fine amplification), up to 1,000 (labeled volts), in increments of 1, for the following. A change of 1 to 750 represents a 1-to-4 change in gain:

FS

SS

AUX

Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, 20, 50, 75, 100, 200, 500 or 750 for:

SS

Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, 20, 50 or 75 for:

FL1

FL2

FL3

FL4

FL5

Linear amplification (gain) by 1.0, 2.0, 5.0, 7.5, 10, 20 50 or 75 for:

AUX

Four-decade digital logarithmic transformation of:

FS

SS

FL1

FL2

FL3

FL4

FL5

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

Fluorescence color compensation is available in 0.1 increments, from 0 to 100%, for:

FL1

FL2

FL3

FL4

FL5

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SPECIFICATIONS SOFTWARE SPECIFICATIONS

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

FS SS

FL1

FL2

FL3

FL4

FL5

AUX

Workstation

The descriptions below are minimum configurations.

Computer

Intel® Pentium® 4 microprocessor and 256 MB of RAM.

Data Storage

- 3.5-in., 1.44-MB diskette drive.
- 20-GB nonremovable hard disk.
- 700-MB CD-RW drive
- 250-MB internal ZIP® drive

Interfaces

- Windows® 2000
- Bidirectional asynchronous serial interfaces for communication with mainframe and personal computers.

Input Devices

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

Monitor Options

- Color LCD with a 17-in. flat screen.
- Color CRT with a 22-in. screen.

3.3 SOFTWARE SPECIFICATIONS

Data Output and Compatibility

Output and compatibility features include:

- Flow Cytometric Standard (FCS) file format for listmode and histogram files. Listmode files contain an FCS 2.0 dataset followed by an FCS 3.0 dataset.
- Copy and Paste, or Drag and Drop plots images to third party programs such as MS Word.

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- Full-color printouts with the appropriate optional Printer.
- Printout of sample results and patient reports with FlowPAGE and desktop printing
- PDF format for Acquisition printouts or FlowPAGE reports
- Printout of Worklist for the MCL
- Compatibility with ALTRA[™], Elite[™], XL[™] and XL-MCL[™] flow cytometer files (listmode and histogram files only)
- Export data to MS Excel or similar spreadsheet program
- Save/export data as a text file.

Reporting Units

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

Setup Mode

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

Acquisition

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

Unlimited one or two parameter plots are available for any given sample.

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

Two-parameter plots have up to 512- x 512-channel resolution.

Regions

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

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

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

Listmode Analysis

The instrument can store up to 16 parameters including AUX, TIME, and RATIO as listmode data. The amount of available random access memory (RAM) in the computer determines the maximum size of a listmode file.

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Locked Protocols

Locked protocols are used with the optional tetraCXP application. The following software screens are disabled when using locked protocols. You cannot make any changes on these screens.

Dotplot Properties

Region Properties (QC product selection and export region statistics are not locked)

Create / Modify Gates

Color Precedence

Color Blend

Cytometer Control

Plots Toolbar

Plots Menu

Select Results

Edit FlowPAGES

Edit FCS Headers

Overlay

Overlay plot allows gallery, overlay and angled views of any number of single parameter histograms.

CXP Default File Extensions

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

Panel, Protocol and Worklist files are written as pure ASCII text files and can be read and edited by any ASCII text editor such as Windows Notepad or the MS-DOS Edit command.

The advantage of using ASCII text to describe the set-up information means that protocol save and restore queries or errors can easily be identified and perhaps even explained or corrected by fax or telephone support.

Standard (default) files & file extensions

*.LMD	FCS listmode data file (includes ASCII header information and binary listmode data).
*.HST	FCS histogram file, single parameter only per file. (Includes ASCII text file descriptions and binary histogram channel information).
*.PRO	Protocol files.
*.PNL	Panel files.
*.WLS	Worklist files.
*.CMP	Compensation files.
*.PPP	Listmode compensation panel.
*.PDF	Files created when "Print to PDF" is selected.
*.EWL	External Worklist files.

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*.WLQ Queue of worklist files.

*.ADF Application Definition file.

*.ALQ Queue of listmode files saved in AutoMATOR.

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

Note: When a panel name is read from an external worklist file (*.EWL), the system searches for the panel in the current user's panel folder and panel subfolders, and then searches the common panel folder and panel subfolders.

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Default AutoSetup Protocols and Panels

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

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

Application Name	Flow-Set™ Protocol	Compensation Protocols	Verification Protocol	Settings Protocol	Base Protocol
AS 2C1L	AS 2C1L_STAND.PR0	AS 2C1L_FL1.Pro	AS 2C1L_VERIFY.Pro	AS 2C1L Settings.Pro	AS 2C.Pro
		AS 2C1L_ FL2.Pro			
AS 3C1L	AS 3C1L_STAND.PRO	AS 3C1L_FL1.Pro	AS 3C1L_VERIFY.Pro	AS 3C1L Settings.Pro	AS 3C1L.Pro
		AS 3C1L_FL2.Pro			
		AS 3C1L_FL5.Pro			
AS 4C1L	AS 4C1L_STAND.Pro	AS 4C1L_FL1 .Pro	AS 4C1L_VERIFY.Pro	AS 4C1L Settings.Pro	AS 4C1L.Pro
		AS 4C1L_FL2.Pro			
		AS 4C1L_FL4.Pro			
		AS 4C1L_FL5.Pro			
AS 4C2L	AS 4C2L_STAND.Pro	AS 4C2L_FL1 .Pro	AS 4C2L_VERIFY.Pro	AS 4C2L Settings.Pro	AS 4C2L.Pro
		AS 4C2L_FL2 .Pro			
		AS 4C2L_FL4 .Pro			
		AS 4C2L_FL5.Pro			
AS 5C1L	AS 5C1L_STAND.Pro	AS 5C1L_FL1 .Pro	AS 5C1L_VERIFY.Pro	AS 5C1L Settings.Pro	AS 5C1L.Pro
		AS 5C1L_FL2 .Pro			
		AS 5C1L_FL3 .Pro			
		AS 5C1L_FL4 .Pro			
		AS 5C1L_FL5 .Pro			
AS 5C2L	AS 5C2L_STAND.Pro	AS 5C2L_FL1 .Pro	AS 5C2L_VERIFY.Pro	AS 5C2L Settings.Pro	AS 5C2L.Pro
		AS 5C2L_FL2 .Pro			
		AS 5C2L_FL3 .Pro			
		AS 5C2L_FL4 .Pro			
		AS 5C2L_FL5 .Pro			

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

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

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

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The guidelines below show approximate region widths generated by the Application Definition Wizard. Flow-Set, Flow-Set 675 and Flow-Set 770 region widths generated by the Application Definition Wizard may be edited. If different regions widths are desired, open the appropriate *_Stand protocol onto the workspace and edit the regions. Save the protocol. Subsequent scheduling of the application uses the edited regions.

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

Default Generic Panels and Protocols

The following generic protocols are included with CXP software.

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

Application Name	Protocol	Base Protocol
QC 1L Flow-Check(TM)	QC 1L Flow-Check(TM)_ALIGN.PRO	QC 1L Flow-Check.PRO
QC 2L Flow-Check(TM)	QC 2L Flow-Check(TM)_ALIGN.PRO	QC 2L Flow-Check.PRO
QC 4C 1L FITC-PE-ECD-PC5 Flow-Check(TM)	QC 4C 1L FITC-PE-ECD-PC5 Flow-Check(TM)_ALIGN.PRO	QC 4C 1L FITC-PE-ECD-PC5 Flow-Check.PRO
	2c 5test.PR0 (Analysis)	N/A
	4c 1l 2test.PRO (Analysis)	N/A
	HeNe Alignment.PRO	N/A

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FCS Header - Keyword Reference

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

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

FCS Keyword	Key value
\$DATATYPE	Single character defining base data type of binary data, I=16 bit integer, F=IEEE 32 bit floating point, D=double precision 64 bit floating point, A=ASCII
\$PAR	Number of parameters
\$MODE	Single character defining mode of data, L=listmode, C=dual parameter correlated, U=single parameter uncorrelated.
\$PnB	Number of bits allocated to store data for parameter n, 8 means 256 channel data, 16 means up to 64 k channel data
\$PnR	Channel resolution of parameter n, for example: 256,1024 and so on
\$BYTEORD	INTEL platform '1,2', Motorola platform '2,1'
\$NEXTDATA	Byte offset into binary data for next data item, FCS files can be saved as a pseudo-linked list, most often 0
\$DATE	DD-MM-YY
\$EXP	Experiment name (from Workspace Preferences)
\$PROJ	Project name (from Workspace Preferences)
\$0P	Operator ID, that is, user ID.
\$INST	Institute name (from Workspace Preferences)
\$FIL	File name excluding path
\$CYT	Type of flow cytometer, for example: FC 500, XL, ALTRA, ELITE and so on
\$SMNO	System defined run number, that is, a number which is incremented each time the flow cytometer is used to acquire data
\$SRC	SAMPLEID1
\$SYS	CXP Intel Windows
\$CELLS	Cells name (from Workspace Preferences)
\$BTIM	Start time (HH:MM:SS 24 hour clock ':' delimiters)
\$ETIM	End time (HH:MM:SS 24 hour clock ':' delimiters)
\$TOT	Total number of events acquired
\$PnV	Voltage of sensor n
@PnGAIN	Amplifier Gain setting of a sensor
\$PnN	Name of parameter n, for example: Manufacturer assigned name, that is, FS or FL1 LOG
\$PnS	User assigned name of parameter
\$PnE	Lin/Log parameter calibration (0,0) means linear, (decades, offset [for example: 4.0, 0.1024]) means log

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FCS Keyword	Key value
\$DFCiToj	% of FLi to subtract from FLj
@Y2KDATE	Date in format YYYYMMDD
@SAMPLEID1	User defined string
@SAMPLEID2	User defined string
@SAMPLEID3	User defined string
@SAMPLEID4	User defined string
@PnZ	Calibration for parameter n is active when this key value is 'ON'
@PnX	Linear calibration values in xx, yy order, for example: xx= channel number, yy = channel value
@PnU	Units for this parameter n
@PnQ	Sensor name for parameter n, from the CCUI.
@PnC	Calculation method for parameter n, 'ARITHMETIC' means use arithmetic method for calculation - may be overridden by analysis engine
@PnADDRESS	Mutliplexor address of parameter n
\$RUNNUMBER	(See \$SMNO) System defined run number, that is, a number which is incremented each time the flow cytometer is used to acquire data
@BASELINEOFFSET	Default is 'OFF' any other means ON, that is, this sample was acquired using baseline offset
@FILEGUID	Unique 128 bit number identifying this file, changed every time the file is saved.
@CYTOMETERID	The unique ID given to the flow cytometer by the user, for example: the flow cytometer serial number
@LOCATION	The address of the institute who owns the flow cytometer
TESTNAME	PROTOCOL NAME
TESTFILE	PROTOCOL FILE NAME
@BARCODE	Barcode read from MCL
@CAROUSEL	Carousel number read from MCL
@TUBENO	Tube number read from MCL
@PnDIVIDER	Prism channel divider (0xFFFF means OFF)
@COMPENSATIONMODE	Displays the method of compensation implemented Advanced or Classic
@Discriminator	Displays the discriminator parameter and level
@HENELASER	Displays the status of the Red Laser during analysis of the sample.
@TARGETLASERPOWER	Displays the Argon Laser power set during acquisition
@RESAVEDFILE	Displays Runtime Protocol if the current file is exactly as at the end of acquisition, if any changes are made post acquisition and the LMD file is resaved the new file displays New Protocol.
@RATIONUMERATORMUX	Internal Mux address of the parameters used for the Ratio Parameter Numerator.

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FCS Keyword	Key value
@RATIODENOMINATORMUX	Internal Mux address of the parameters used for the Ratio Parameter Denominator.
@PANEL	Name of the panel file used for acquisition of the sample.
@ACQUISITIONPROTOCOLOFFSET	Defines where in the LMD file the Runtime Protocol is stored, the value displayed is number of bytes from start of file to the start of the protocol section.
@ABSCALFACTOR	Defines the calibration factor of the beads used with this sample if Flow-Count or similar absolute calibration particles are used.

3.4 PERFORMANCE SPECIFICATIONS

Carryover

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

Data Acquisition Throughput

Acquisition throughput is dependant on many variables, including flow rate, data rate, number of parameters collected, gated events, data plots, FlowPAGE reports and printouts generated. As an example, a throughput of approximately 45 tubes per hour is expected when acquiring four parameters, running at approximately 1,000 gated lymphocytes/second, gated on 10,000 normal lymphocytes (~2,500 lymphocytes/ μ L), printing one FlowPAGE. Changing these variables, such as decreasing the number of events collected, increasing sample concentration or increasing the flow rate can increase your acquisition throughput; while increasing the number of events collected or running at a lower data rate can decrease your acquisition throughput.

Precision for Surface Markers

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

Resolution

Forward Scatter

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

Fluorescence

The HPCV of the integral signal intensity values using Flow-Check fluorospheres is <2% for FL1-FL4 from the Argon laser. The HPCV of the integral signal intensity values using the Flow-Check 675 fluorospheres is \leq 2.5% for FL4 from the red laser. The HPCV of the integral signal intensity values using Flow-Check 770 fluorospheres is \leq 4% for FL5.

Sensitivity

Scatter

Detects 0.5 µm - 40 µm diameter particles.

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Fluorescence

600 (FITC), 300 (PE) and 600 (APC) molecules of equivalent fluorochrome when measured with Spherotech™ Rainbow Calibration particles (FITC & PE), Molecular Probes LinearFlow™ Intensity Calibration particles (APC) and IsoFlow sheath fluid.

Stability

Day-To-Day

The mean channel value of the integral signal intensity of Flow-Check fluorospheres from the 488 nm laser for FS, FL1, FL2, FL3, and FL4 does not vary more than ±5% over a period of 7 days, when the temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check fluorospheres package insert for details.

Stability for FL5 (PC7) mean channel value of the integral signal intensity from the 488 nm laser using Flow-Check 770 beads does not vary more than ±5% from the average of the integral signal intensity mean channel values obtained over a 7 day period, when room temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check 770 fluorospheres package insert for details.

Stability for FL4 (APC) mean channel value of the integral signal intensity from the red laser using Flow-Check 675 beads does not vary more than ±5% from the average of the integral signal intensity mean channel values obtained over a 7 day period, when room temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check 675 fluorospheres package insert for details.

Within Day

The mean channel value of the integral signal intensity of Flow-Check fluorospheres from the 488 nm laser for FS, FL1, FL2, FL3, FL4 and FL5 does not vary more than ±5% from the mean integral signal channel number obtained within a period of 24 hours, when the temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check fluorospheres package insert for details.

Stability for FL5 (PC7) mean channel value of the integral signal intensity from the 488 nm laser using Flow-Check 770 beads does not vary more than $\pm 5\%$ from the average of the integral signal intensity mean channel values obtained within a period of 24 hours, when room temperature does not vary more than $\pm 5\%$ from temperature at alignment. Refer to the Flow-Check 770 fluorospheres package insert for details.

Stability for FL4 (APC) mean channel value of the integral signal intensity from the red laser using Flow-Check 675 beads does not vary more than ±5% from the average of the integral signal intensity mean channel values obtained within a period of 24 hours, when room temperature does not vary more than ±5°F from temperature at alignment. Refer to the Flow-Check 675 fluorospheres package insert for details.

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SPECIFICATIONSPERFORMANCE SPECIFICATIONS

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This appendix contains the following log sheets:

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

Make photocopies as needed.

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REAGENT LOG

Reagent Name	Concentration Units= (If applicable)	Date Received	Date Opened	Lot Number	Expiration Date	Tech

Cytometer Serial No:	
-,	

Laboratory: _

Cytomics FC 500



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ACTION LOG

Date	Condition Noted	Tech	Date	Action Taken	Tech

Cytometer Serial No:	
Laboratory:	
-	



Cytomics FC 500

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DAILY LOG FOR INSTRUMENT STANDARDIZATION

Flow-Set™	⁴ Fluoros	spheres		Lo	t Numb	er						. Е	xpiratio	n Date			
Flow-Set™ 675 Fluorospheres Lot Number												. E	xpiratio	n Date			
Flow-Set™ 770 Fluorospheres Lot Number													xpiratio				
												Apriatio	Dato				
Instrumen	nt HV/Tot	al Gain	Ta rget	Ranges	: :												
FS LOG FL1									LOG FL	3							
SS/LOG S	s			LOG F	L2				LOG FL	4			L	.0G FL5			
		FS		SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4 Red Laser		LOG FL5		
Run	Mode	HV*	Gain	Mode	HV	Gain	Mode	HV	Mode	HV	Mode	HV	488 Mode		Mode	HV	Tech/ Date
1			-														
2																	
4																	
<u>5</u>									-				1				
7																	
8																	
9 10									-								
11																	
12																	
13																	
15																	
16																	
17																	
<u>18</u> 19									-								
20																	
21																	
22									-								
24																	
25																	
26													-				
27 28																	
29 30				1		1	1										ı — —

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Cytomics FC 500

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ESTABLISHING HV/TOTAL GAIN RANGES

Flow-SatT	4 Eluoros	enhara	c	١o	t Num	hor						ı	- - - - - - -	n Nata				
·							Expiration Date Expiration Date											
Flow-Set™ 675 Fluorospheres Lot Number												_ [Expiratio	n Date				
Flow-Set ^{TN}	r-Set [™] 770 Fluorospheres Lot Number										_ [Expiratio	n Date					
Mode Tarç	get Rang	jes:																
FS LOG FL1									LOG FI	_3								
SS/LOG S	SS/LOG SSLOG FL2								LOG FL	_4				LOG FL	ō			
		F0		1 00	1.00	00	100	F1.4	1.00	FI O	100	EL O	100	F1.4	100			
		F\$		SS or LOG SS		LOG FL1		LOG FL2		LOG FL3		LOG FL4 Red Laser 488		LOG FL5		T		
Run	Mode	HV	Gain	Mode	HV	Gain	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Tech/ Date	
1																		
3																		
4																		
5																		
<u>6</u> 7																		
8																		
9																		
10							-											
<u>11</u> 12																		
13																		
14																		
15													1					
<u>16</u> 17									-				<u> </u>					
18									1				1					
19																		
20																		
Average HV/Gain																		
Average																		
+2SD or +1%																		
Average -2SD or																		
-1%																		
Cytomet															BE		<u>1</u> AN	

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Cytomics FC 500

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DAILY LOG FOR INSTRUMENT VERIFICATION OF ALIGNMENT AND FLUIDICS

Lot Number _ Lot Number _ FL1 Mode HPCV	FL2			[Expiration			Tech/Date
FL1	FL2	F	L3	F Red Lass 488	L4 er 🗌	Fl	.5	
				Red Last 488	er 🗌			Tech/Date
Mode HPCV	Mode H	HPCV Mode	HPCV	488		Mode	HPCV	Tech/Date
Mode HPCV	Mode H	HPCV Mode	HPCV	Mode	HPCV	Mode	HPCV	Tech/Date
			-					
			-					

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Cytomics FC 500

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7272015B

ESTABLISHING PEAK POSITION AND HPCV TARGET RANGES

Flow-Check™	Lot N	umber .	Expiration Date													
Flow-Check™ 675 Fluorospheres Flow-Check™ 770 Fluorospheres					Expiration Date											
	FS		FL1		FL2		FL3		FL4 Red Laser 488		FL5					
Run	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Tech/Dat			
1																
2																
3																
4																
5																
6																
7	_															
9																
10																
11																
12																
13																
14																
15																
16																
17																
18																
<u>19</u> 20	_															
Mean	+															
SD																
+2SD																
-2SD																
Cytometer S Laboratory:											BI	ECKI JLTE	1AN R			

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Cytomics FC 500

A-14 PN 175570B

B.1 WELCOME TO CXP SOFTWARE

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

CXP Analysis Software can analyze a wide variety of listmode files including those from the following Beckman Coulter Flow Cytometers:

- COULTER® EPICS® XL
- COULTER EPICS ALTRA
- COULTER EPICS Elite

B.2 MULTI-USER SIGN ON

Page 1

When you start CXP software, the following Startup screen is displayed allowing Password entry and access to the system.

On the initial Startup after installation, only the Admin User ID is available and the Admin Password is password. Users must be set up by the Administrator.

Note: It is vital that the default Admin Password is changed as soon as possible by using the User Profile – User ID option within the User Administration screen.

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

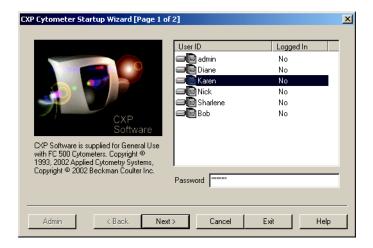
If you have been previously set up on the system, highlight your User ID and enter your Password. If you do not have a User ID and Password, see your system Administrator.

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

- Ensure the pathway for your user directory is,
 - not a network drive if the network connection is not met.
 - not a Zip drive.
 - not a CD-ROM drive.
- Ensure all of your user folders are intact. If any of the following folders are missing from your user directory, use Windows Explorer to create the missing folders.
 - AcquisitionProtocol
 - AnalysisProtocol
 - ► HST
 - ► HTML
 - Images
 - LMD
 - Panel
 - PDF

- Results
- ▶ Worklist

User ID Logged In Password Admin Button Next Button



To sign on and access the administration tools, highlight the admin User ID and enter the Password. The Admin button is then available. Select this button to access User Administration for adding, removing, and modifying User profiles, Workgroups, Export Logs, and User Passwords.

The User ID "admin" and Admin are for administrative functions only. You cannot log in to CXP software with the admin User ID.

User ID

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

Logged In

Indicates whether or not the Users are logged in to either Acquisition or Analysis software.

Password

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

Admin Button

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

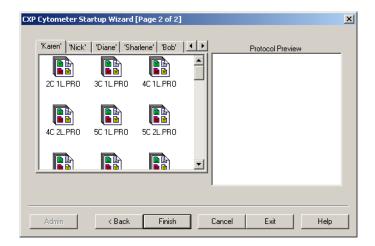
Next Button

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

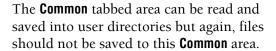
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Page 2

Tabbed Protocol Pane Protocol Preview Pane Finish Button



Other Users within your workgroup are displayed as tabbed User IDs across the top of the window. You may read other user protocols and save them to your directory. Each user may save files only to their own directory unless admin has assigned them rights to Overwrite other data files?. Files may be read from any of the other Users within a given Workgroup. To view any hidden tabs, use the $\$ > to move between multiple tabs.





Tabbed Protocol Pane

Allows you to select a Protocol from your Directory. Also allows Read Only access to the Protocols belonging to other members of your Workgroup if you have not been assigned rights to Overwrite other data files?.

Protocol Preview Pane

Displays the details of the currently highlighted protocol.

Finish Button

Starts up CXP software with the selected protocol or with a default protocol if no specific protocol has been selected.

B.3 SIGN-ON ADMINISTRATION

User Administration

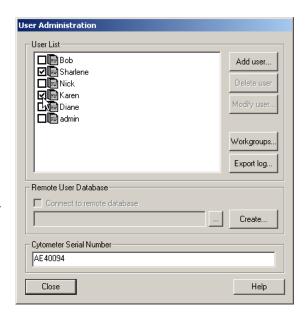
This screen allows the Administrator to add, remove and modify User profiles, including passwords as well as the creation of user Workgroups plus the creation of Export Logs.

Note: In the event of a system lockup, before users log back on to CXP software, the

Administrator can log all users off by on the User Administration screen next to any User ID displaying logged on for Export Log purposes.

Add User Modify User Delete User Workgroups Export Billing Information Remote User Database Cytometer Serial Number

Note: The Administrator must set up Cytometer Serial Number and Add Users in the CXP Cytometer software before adding users in CXP Analysis software.



Add User

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

Delete User

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

Highlight the User ID you wish to delete from the system and



A confirmation dialog box is displayed. Choose **Yes** to delete the user or **No** to revert to the User Access Control dialog box.

Modify User

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

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Workgroups

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

Workgroup Selection Users Edit Button

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



See also:

User Administration

Workgroup Selection

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

Users

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

Edit Button

Allows access to the **Edit Workgroups** screen.

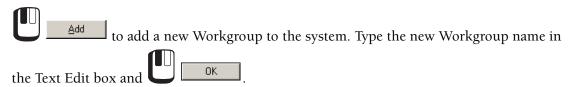
Edit Workgroups

This screen is used to Add or Delete specific Workgroup names.

Add Delete



Add



Delete

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

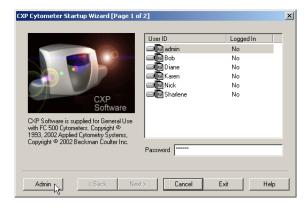
See also: Workgroups User Administration

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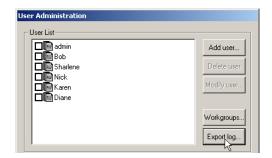
Export Billing Information

Selecting the Export option allows a text file log to be created showing the date and time each User has spent logged on to CXP software. The information shown records Analysis time and Cytometer time separately.

- **1** Log in as Admin.
 - a. Highlight Admin.
 - b. Enter the Admin Password.
 - c. Admin

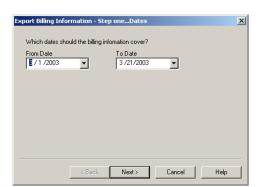


2 Export log...



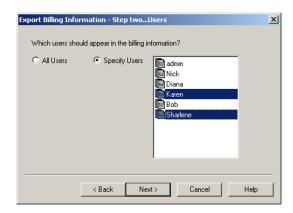
3 Enter the date range for the log and





4 Select the users to include in the log

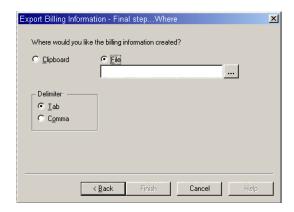




Choose to direct the information to the Clipboard or to a File. If File is selected, you can either enter a valid File Name in the box or use the Browse Button to select an appropriate file.

Select between Tab delimited (MS Excel

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



6 Finish to end.

See also:

User Administration

Remote User Database

Using a remote user database allows multiple users to share a central computer to store and retrieve data files. This remote computer acts as a server and MUST be able to be read from and written to by all users. The remote computer contains a database of all users, their passwords and folder locations on the server and allows users to save and restore files directly from the server rather than saving files locally.

Note: If you use a network connection for the Remote User Database you must validate the network connection. Refer to Windows 2000 help for information about the recommended Windows 2000 network configuration.

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Create Remote User Database

1 From any computer with CXP software installed, sign in to CXP software as the Administrator and select Admin.



2 From the User Administration dialog



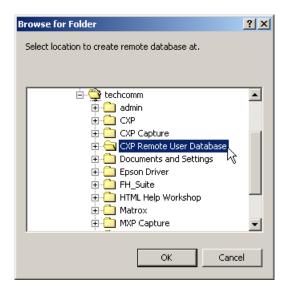


3 Browse the network for the computer and home directory to be used as the location for the Remote User Database



All intended users must have read/write access rights to the chosen location, as all User files are stored here.

Refer to Windows 2000 help for instructions about read/write access.



Connect To Remote User Database

1 From each user computer you wish to connect to this database, sign in to CXP software as Administrator and



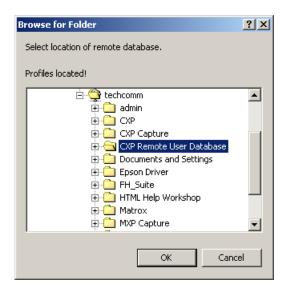


2 From the User Administration dialog

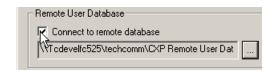




3 Locate the folder created in step 2 – The dialog box displays *Profiles located!*.



4 Check the **Connect to Remote Database** checkbox to enable the new location.



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- **5** Close the User Administration dialog box. On the main sign-on screen the hard disk icon changes to a networked drive icon .
- **6** Repeat steps 1 5 for each computer you wish to use the remote database.

See also, User Administration

Cytometer Serial Number

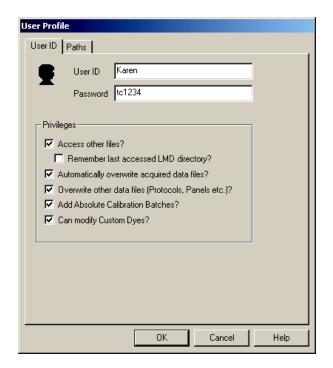
Enter the serial number of the Cytometer connected to the Workstation. You must be logged on as Administrator to enter the serial number.

User Profile – User ID

User ID

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

Password
User Privileges
Access other files?
Remember last accessed LMD
directory?
Automatically Overwrite acquired data
files?
Overwrite other data files?
Add Absolute Calibration Batches
Can Modify Custom Dyes



See also:

User Profile – Paths User Administration

User ID

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

Password

If you are assigning a Password for the first time, or changing an existing one for a particular User, type in the appropriate Password. Passwords must be a minimum 6 characters. Note that the screen is displayed so you can check it for accuracy before selecting the **Apply** or **OK** buttons. If you are changing the Admin Password, the Admin User ID is grayed out.

User Privileges

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

Access other files?

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

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

Remember last accessed LMD directory?

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

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

Automatically Overwrite acquired data files?

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

Overwrite other data files?

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

Add Absolute Calibration Batches

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

Can Modify Custom Dyes

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

See also, User Administration

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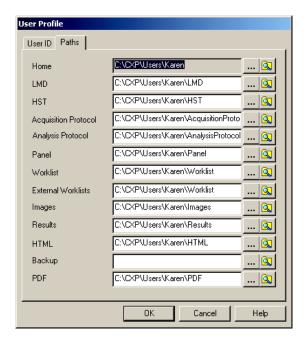
User Profile - Paths

This screen allows the Administrator to assign User directory Paths for different file types, as well as a Backup Directory.

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

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

Browsing Buttons Home Path Details Backup



See also:

User Profile – User ID User Administration

Browsing Buttons

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

Home

Shows the Home Directory Path that applies to that User.

Path Details

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

Backup

Shows the path to the Backup or directory for data (listmode) files.

B.4 FILE OPTIONS TOOLBAR

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

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

B.5 PLOT OPTIONS TOOLBAR

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

Color Dot Plot	Create a Color Dot Plot and specify the plot properties.
Histogram Plot	Create a Histogram Plot and specify the plot properties.
Contour Plot	Create a Contour Plot and specify the plot properties.
Density Plot	Create a Density Plot and specify the plot properties.
Overlay Plot	Create an Overlay Plot to compare single parameter histograms (CXP Analysis Software only).
Tomogram Plot	Create a Tomogram Plot and specify the plot properties (CXP Analysis Software only).
Surface Plot	Create a Surface Plot and specify the plot properties (CXP Analysis Software only).
Y Prism Plot	Create a Prism Plot and specify the plot properties.
Legend Plot	Create a Legend Plot and specify the plot properties.

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B.6 REGIONS OPTIONS TOOLBAR

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

B.7 GATE, COLOR, STATS AND HELP TOOLBAR

Ħ	Create / Modify Gate	Access the Create / Modify Gates dialog box.
4 8	Modify Color Gate Blend Setup	Access the Modify Color Gate Blend Setup dialog box.
	Create / Modify Color Gate Precedence Setup	Access the Create / Modify Color Gate Precedence Setup dialog box.
$^{\textcircled{p}}$	View Current Regions	View/edit a Region's properties, Region points or to delete a Region that is no longer required.
×	Publish Results	Publish Statistics to a Microsoft Excel spreadsheet or a Text file.
Σ	Select Results	Choose Statistics for display and export.
?	Help	Access the Help System.
	Beckman Coulter	Go to the Beckman Coulter, Inc. web site.
	ACS	Go to the Applied Cytometry Systems web site.

B.8 FLOWPAGE TOOLBAR

	New FlowPAGE	Open a New FlowPAGE – Also see Insert/Blank FlowPAGE.
	Insert bitmap	Insert a Picture into a FlowPAGE.
$ \mathbf{A} $	Annotation	Add a Text Box to a FlowPAGE.
	Line	Draw a Line on a FlowPAGE to enhance the graphical layout of the page.
	Rectangle	Insert a Rectangle into a FlowPAGE that can be used as a border for Pictures or Text Boxes.
<u>□</u>	Align Top	Align all the selected items to the top of the selection area.
<u>\$</u>	Align Bottom	Align all the selected items to the bottom of the selection area.
□ +	Align Left	Align all the selected items to the left of the selection area.
* □	Align Right	Align all the selected items to the right of the selection area.
] +• [Space Evenly Across	Space selected items within a FlowPAGE evenly across a page.
王	Space Evenly Down	Space selected items within a FlowPAGE evenly down a page.
	Grow to largest	Resize the selected items to all be the same size as the reference item.
K	Shrink to smallest	Resize the selected items to all be the same size as the reference item.

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B.9 ACQUISITION MANAGER TOOLBAR

New Worklist	Clear the Acquisition Manager panel to create a new Worklist.
File Open	Open a selected stored Worklist file into Acquisition Manager
Save Worklist	Save the current Worklist with a user definable name.
Save Panel	Save the current Panel with a user definable name
W Delete	Delete the currently selected test(s).
Insert Test	Inserts a blank row at the end of the current panel, which allows for the insertion of extra tests into the selected panels.
Insert Panel	Insert a Panel from file into the current worklist.
Customize Worklist Columns	Display the Customize Worklist Columns dialog box, allowing hiding of unwanted columns and customization of column titles.
Print	Print the current Worklist.
AutoScheduler	Run the AutoSetup Scheduler
External Worklist	Import an External Worklist. The acquisition manager is populated with the worklist.

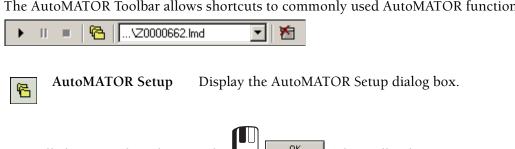
Note: The Listmode File Name column displays only a provisional file name prior to acquisition. If Time or Run Number is included as part of the name the actual value of these fields can only be determined once acquisition of each sample is complete. Therefore, if you wish to print a worklist including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

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

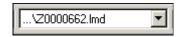
Note: Scheduled Applications cannot be saved as Panels or Worklists.

B.10 AUTOMATOR TOOLBAR

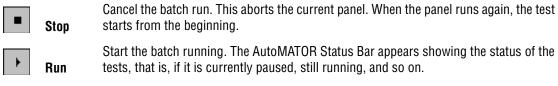
The AutoMATOR Toolbar allows shortcuts to commonly used AutoMATOR functions.



0K . The toolbar becomes active once all Once all alterations have been made, files have been entered. A list of files for each panel is shown allowing you to choose the batch required to run first.



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



Pause has two states:

Continuous Pause

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

again. To resume the test, click the Pause

· Auto Pause

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

Pause

Ш

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B.11 OVERLAY TOOLBAR

Note: The Overlay Toolbar is only available in CXP Analysis software.

	Gallery View	Arrange the histograms in a Gallery Style. Selecting one of the other Style buttons can alter this format.
	Overlay View	Arrange the histograms so that they are all overlaid on one set of axis.
*	3-D Overlay	Display all the plots in a 3-D format.
<	Rotate Clockwise	Rotate the plot clockwise so that it is easier to view. Can only be used when the plots are in the 3-D Overlay Mode.
\$	Rotate Counterclockwise	Rotate the 3-D plot counterclockwise.
!	Legend	Displays a key to the histograms on the Workspace. The Legend can be utilized in any of the layouts and is removed by clicking again on the Legend button.
Σ	Overlay Statistics	Generate a statistics summary within the overlay plot in any of the modes. This can be removed by clicking on the Overlay Statistics button again.
	Add Plot	Add a histogram plot created from a stored listmode file to the Gallery display.
1	Open Histogram	Open a stored histogram file.
A	Annotation	Place a free text annotation or FCS Keyword text into a plot.
8 2	Linked Marker	Set a region on all histograms simultaneously. This shows the percentage of events inside that region compared to the total number of events on the plot as a whole.
▶ *	Non-Linked Marker	Add an independent region to a plot. Any number of these can be added to all/some/one of the plots.

B.12 CYTOMETER TOOLBAR

4	Restart Acquisition	Reset the current acquired events to zero and clear the current data in memory. Acquisition restarts at zero events and continues until a stop condition is reached.
	Start/Continue Acquisition (F9)	Start acquisition or continue an acquisition if previously paused.
II	Pause Acquisition (F11)	Pause acquisition of the current sample.
$(\widehat{\mathbf{II}})$	Pause / Rotate	Pause Acquisition and rotate sample to the tube access door.
	Stop Acquisition (F10)	Stop the Acquisition of the current sample and output results.
×	Abort Acquisition (F12)	Stop the Acquisition without saving data or outputting reports
zzz	Idle Mode	Place the Cytometer in Idle mode in order to perform various cleaning and replacing procedures.
Ø	Prime	Flush the sample line and flow cell with sheath fluid to declog or remove bubbles.
A	Cleanse	Flush the sample line and flow cell with cleaning agent.
Î	AutoCleanse	Perform the Vacuum Line Cleaning procedure.
41114	MCL Manual Mode	Run a carousel of samples one tube at a time.
1	Single Tube Mode	Run a single tube in fixed position # 10.
	Cytometer Controls	Display the Cytometer Control dialog box to adjust the Cytometer settings. Click the button again to hide the dialog box.

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B.13 LISTMODE PLAYBACK TOOLBAR

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



B.14 REPORT GENERATOR TOOLBAR

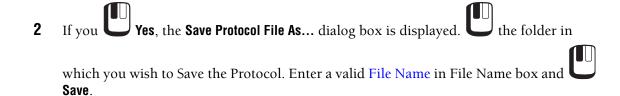
Quality Control Report	Display Levey-Jennings plots based on information selected in the QC Template and data located in the QC tables.
Panel Template	A Panel Template defines the content of the Panel Report that is produced when the export panel is run.
Panel Report	Display a list of Patient Panel Reports available for display or print.
Database Entry	Enter or view the specimen information in the database.
Data Management	Backup QC or patient records to an archive database and restore QC or patient records from an archive database.
Product Editor	Add or edit QC products.
Maintenance Log	Electronic log of instrument routine maintenance, tracked by user name.
Mullionalioo Eog	Electronic log of matrament routine maintenance, tracked by user maine.

B.15 FILE MENU

New Protocol

Use this option to create a New Protocol.





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

See also:

Save Protocol As Creating a Protocol for Analysis Creating a Multi-File Protocol

Creating a New Acquisition Protocol



- 2 Parameters button on the Cytometer Control Acquisition Setup Tab and choose the Parameters to be acquired.
- **3** Create the Plots required for acquisition.
- 4 If analysis is to be performed during acquisition, create the required Regions and Gates, and also select the required statistics from the **Analysis** → **Select Results** option.
- 5 File → Save Protocol As to save the protocol.

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

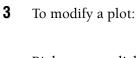
Note: Due to the flexibility of listmode file naming options, listmode filenames can become very long. When such listmode files are open in the Analysis Only software, the software assigns a file designator (for example, F1, F2, F3...) and displays that designator, with the filename in all statistic boxes on a FlowPage. Due to the long file name, this may result in overlapping statistic boxes, requiring reformatting of the FlowPage to view all results.

- **7** Set up the Worklist (sample information, CAL Factor, etc.) and run samples.
- **8** While running sample, adjust the instrument settings on the Cytometer Control Detector Tab.

Creating a Protocol for Analysis

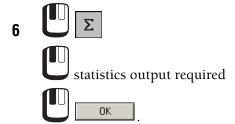
- 1 File >> New >> New Protocol to clear the Workspace of all Regions, gates, statistical definitions, and plots and load the default protocol. You can also Drag and Drop a listmode file into the Workspace to load the runtime protocol.
- 2 File > Open Listmode File to open a representative listmode data file.

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Right mouse click on the plot and Format Plot

- **4** To create a plot:
 - a. Choose one of the buttons on the Plots Toolbar or use the Plots Menu to select a plot type.
 - b. the correct parameters for the first plot and
 - c. Right mouse click on the plot and Format Plot.
- Modify or create subsequent plots and choose the required File, Parameters, Regions and any gates to be applied.



7 Save Protocol As and enter a protocol name.

Creating a Multi-File Protocol

When creating multi-file protocols, ensure that listmode files to be read into the protocol all contain the same parameters in the same order whenever possible. (CXP Analysis software only).

There are many possible ways to create a multi-file protocol. For example:

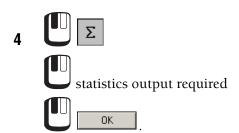
1 File >> New >> New Protocol to clear the Workspace of all regions, gates, statistical definitions, and plots and load the default protocol.

2 File → Open Listmode File to open a representative listmode file and its runtime protocol.

3 Create the desired plots for the additional listmode files.

Individual files can now be replaced by clicking on one of the windows and using Ctrl+Drag and Drop to add files from the Resource Explorer or by using the Data Source tab in the Dot Plot Properties dialog box. See Plots - Introduction for more details on Creating Plots.

The different listmode files are designated as F1, F2, F3, and so on in the plot titles.



5 Save Protocol As and enter a File Name for the protocol.

Note: The AutoMATOR has the ability to easily update the different files in a multi-file protocol in a controlled manner removing the tedium of having to repeatedly select individual files.

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New Panel

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

New Worklist

Note: If no Worklist is visible, View >> Acquisition Manager to display the Worklist pane. See Creating Worklists in the System Overview chapter in the Instructions For Use manual.

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

Open Listmode File

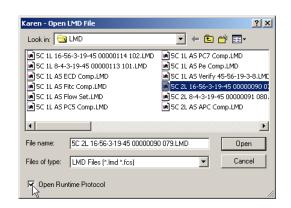
Analysis mode operates with either one FCS file or if using CXP ANALYSIS, several files simultaneously. Use this option to open a new listmode file into all plots. In CXP Analysis, this option updates all Plots relevant to one particular tube.

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

To open a listmode file for the first time:



- **2** Select the desired listmode file.
 - a. Look in: list
 - b. the type of file in the Files of type: list box.
 - c. the name of the listmode file you want to view.



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

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

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

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

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

When loading listmode files from a different instrument *This is not an FC 500 file* appears. Select **Continue** to load the file.

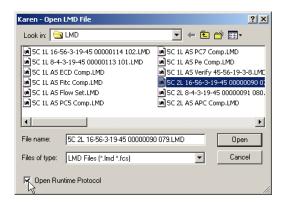
To replace all the plot windows using the same file name with another file:

1 Highlight one of the Plot windows containing the file name you wish to replace.



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- **3** Select the desired listmode file.
 - a. Look in: list box.
 - b. the type of file in the Files of type: list box.
 - c. the name of the listmode file you want to view.



All plot windows with the same file name are now replaced with the current file. The current file name is displayed in the title bar. To add files to individual plots, highlight the plot and use Ctrl +Drag and Drop from the Resource Explorer.

Save Listmode File As

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

1 File → Save Listmode File As.

When performing MultiFile Analysis, you must first highlight the plot with the desired listmode file name.

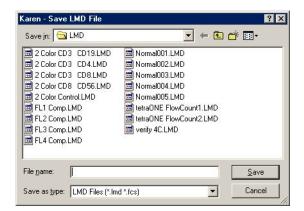
Note: Due to the flexibility of listmode file naming options, listmode filenames can become very long. When such listmode files are open in the Analysis Only software, the software assigns a file designator (for example, F1, F2, F3...) and displays that designator, with the filename in all statistic boxes on a FlowPage. Due to the long file name, this may result in overlapping statistic boxes, requiring reformatting of the FlowPage to view all results.

2 Specify the name of the file.

a. Save in: list box.

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

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



File Name

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

Open Histogram

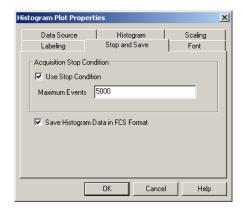
Use this menu option to add a stored single parameter histogram to a currently displayed overlay plot.

See also, Adding a Histogram to the Overlay Plot.

Save All FCS Format Files

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

Highlight the required plot.Note: The individual Histogram's Plot Properties must be set to save.



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

4 Repeat for each plot you wish to be saved.



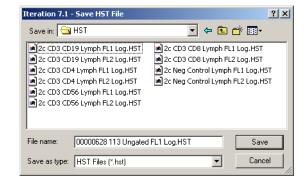
Save Histogram File As

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

Note: CXP software only saves one Histogram Plot in a single FCS histogram file. CXP software can only display FCS histogram files as part of an Overlay Plot.

1 Highlight the Histogram Plot (Current window).





- **3** Specify the desired drives and folders by selecting them in the list boxes or by typing the path in the File text box.
- In the **File name** text box, type in the desired **File Name**.

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

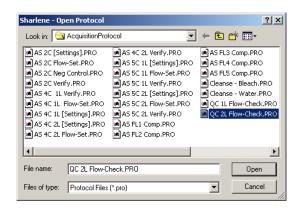
Open Protocol

Use this option to open a protocol file from disk.



The Open Protocol File dialog box appears.

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



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3



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

Save Protocol

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

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

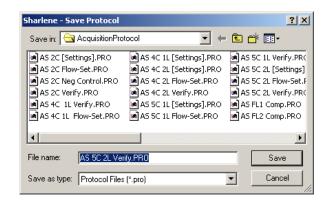
Save Protocol As

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



2 In the Save Protocol As dialog box,

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



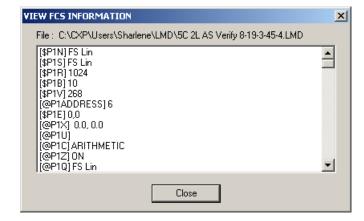
3 In the File name text box, type the desired File Name.

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

Note: If you are using the CXP Analysis software, protocols are saved in the Analysis Protocol folder. If you are using the CXP Acquisition software, protocols are saved in the Acquisition Protocol folder.

View FCS Header Information

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



FCS Keywords Used in CXP Software

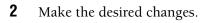
See FCS Header - Keyword Reference.

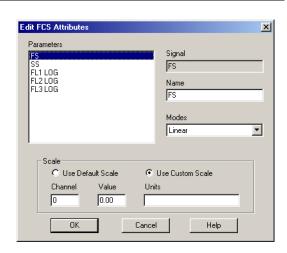
Edit FCS Header Attributes

Use this option to edit the FCS header information, including calibration information. You can change the displayed name of Parameters or change the scaling options. Axis calibration information can be entered here. This information is embedded in the listmode file. See Default Log Scaling for setting the default log tick marks.

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

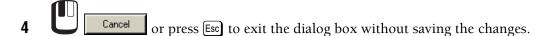
Note: You cannot make any changes on this screen when running a locked protocol.





3 OK or press Enter to accept.

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These changes may also be saved permanently within the open protocol by



Save Protocol or File → Save Protocol As.

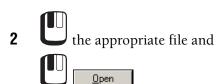
Note: Modifying the scale options should only be done during offline analysis. Replaying of the listmode files always uses the calibration set at acquisition.

Import

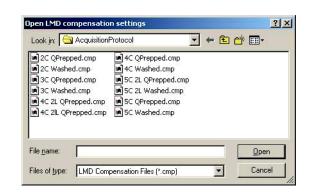
Settings From CMP File

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





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

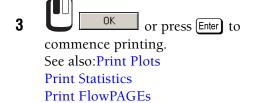


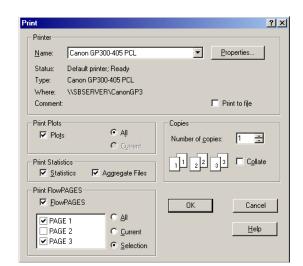
Print

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

1 File → Print. The Print dialog box is displayed.

2 Make the desired changes.





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

Print Plots

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

Print Statistics

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

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

Print FlowPAGEs

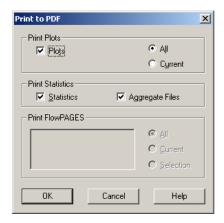
Select this option to print FlowPAGEs. Choose ALL, CURRENT or a SELECTION.

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

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Print to PDF

Print plots, statistics or FlowPAGES in PDF format. When printing a multifile analysis to a PDF file, the PDF filename is derived from the Listmode filename of the last file in each group.



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

If you install Adobe Acrobat 4.0 or 5.0 you should use the Adobe distiller to create PDF files rather than the ACSPDF - PDF compatible print driver. If you continue to use the "Print to PDF" options within CXP Software when Adobe Acrobat 4.0 or 5.0 is installed, some Flowpages are cropped. This issue does not occur when Acrobat Reader® only is installed.

Workspace Preferences - Introduction

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

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

Workspace Preferences - LMD File Name

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

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

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

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

Note: Due to the flexibility of listmode file naming options, listmode filenames can become very long. When such listmode files are open in the Analysis Only software, the software assigns a file designator (that is, F1) and displays that designator, with the filename in all statistics boxes on a FlowPage. Due to the long file name, this may result in overlapping statistics boxes, requiring reformatting of the FlowPage to view all results.

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First 10 characters.... Sample ID 1, 2, 3, 4

User ID

Automatically add unique....

Run Number

Y2K Date

Time

Tag Number

File Name Extension

PDF File Name Extension

Note: If more than one Listmode File Name option is selected, the priority of the options used for the file name are.

- 1. Sample ID 1
- 2. Protocol ID
- 3. Sample ID 2
- 4. Sample ID 3
- 5. Sample ID 4
- 6. Run Number
- 7. Tag Number
- 8. Tube ID
- 9. User ID
- 10. Date
- 10. Time



Workspace Preferences - User Info

Workspace Preferences - Plot Display

Workspace Preferences - Gating

Workspace Preferences - Publish

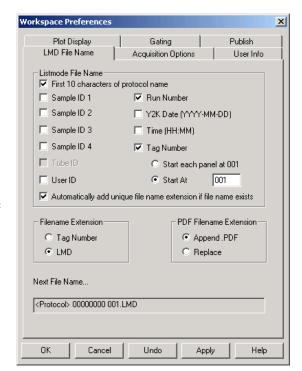
Note: If the Worklist Column names are changed using the Customize Worklist Columns option within Acquisition Manager, these changes are reflected in the notation labels on the above dialog.

First 10 characters....

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

Sample ID 1, 2, 3, 4

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



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

User ID

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

Automatically add unique....

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

Run Number

The system automatically generates an incrementing run number every time that a sample is run. When this option is selected, the run number is included as part of the listmode file name – the easiest way to ensure unique file names are always generated. If not selected, the run number is still generated, saved and can be accessed in the FCS Header section of the file.

Y2K Date

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

Time

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

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

Tag Number

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

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

File Name Extension

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

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

PDF File Name Extension

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

Append .PDF File Name

Append PDF to the listmode file name extension.

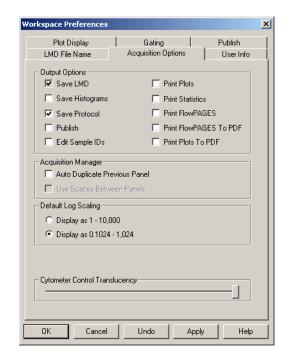
Replace

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

Workspace Preferences - Acquisition Options

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

Output Options Acquisition Manager Default Log Scaling Cytometer Control Translucency



See also: Workspace Preferences - Introduction

Workspace Preferences - LMD File Name

Workspace Preferences - User Info Workspace Preferences - Plot Display Workspace Preferences - Gating Workspace Preferences - Publish

Output Options

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

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

Acquisition Manager

Auto Duplicate Previous Panel - Adds a copy of the current panel into the Acquisition
Manager at the completion of the current panel, and is useful if you wish to run samples
in Panel Mode but do not wish to define a complete worklist in advance. This setting is
only available in Single Tube Mode.

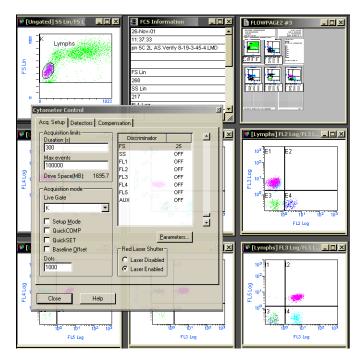
Default Log Scaling

Sets the default logarithmic scaling (tick marks) to be 0.1024 –1,024 or 1 - 10,000.

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Cytometer Control Translucency

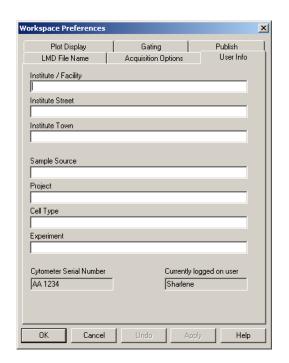
Use this slider to fade the Cytometer Controls dialog so it appears translucent.



Workspace Preferences - User Info

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

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



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

Institute

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

Institute Street

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

Institute Town

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

Sample Source

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

Project

The name for the project is entered here.

Cell Type

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

Experiment

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

Cytometer Serial Number

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

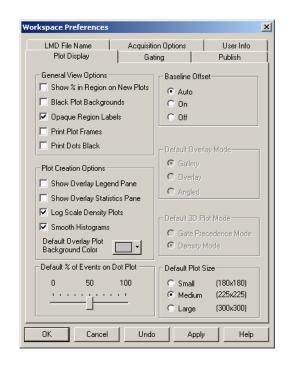
Currently logged on user

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

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Workspace Preferences - Plot Display

Show % in Region on New Plots Black Plot Backgrounds **Opaque Region Labels Print Plot Frames** Print Dots Black Show Overlay Legend Pane Show Overlay Statistics Pane Log Scale Density Plots **Smooth Histograms** Default Overlay Plot Background Color Default % of Events on Dot Plot Baseline Offset Default Overlay Mode Default 3D Plot Mode Default Plot Size



See also: Workspace Preferences - Introduction

Workspace Preferences - LMD File Name

Workspace Preferences - Acquisition Options

Workspace Preferences - User Info Workspace Preferences - Gating Workspace Preferences - Publish

Show % in Region on New Plots

When selected, this automatically displays the percentage of events in a region on newly created plots. All regions that have been created display this percentage.

Black Plot Backgrounds

This displays the plots with a black background instead of white. This can be useful as it makes the viewing of smaller populations of cells much easier. When you print, drag or copy a plot to other software programs, the background remains white.

Opaque Region Labels

This option displays an area around the annotation within black backgrounds, making it visible.

Print Plot Frames

This option can be used to show or hide frames on plots printed directly from the File Print menu and exported plot images.

If this is checked, all frames are displayed around plots. If it is clear, frames are not printed or exported.

Print Dots Black

Select this option to print all dots in black irrespective of their colors. If it is clear, dots are printed in various shades of gray or, if using a color Printer, the best matched colors available. This option does not affect the printing of FlowPAGEs.

Show Overlay Legend Pane

When this option is set as the default, any Overlay plot that is created is displayed with the Legend Pane active. When selected, this option displays the Legend pane on all new overlay plots as they are created. It does not apply these changes to any previously created plots.

Show Overlay Statistics Pane

When this option is enabled, Overlay plots that are created are displayed with the statistics pane present. This option is applied to new overlay plots as they are created. It does not apply these changes to plots created previously.

Log Scale Density Plots

Selecting this option displays any plot with a density mode (that is, density plot, surface plot and tomogram plot) with log scaling of the density levels. If unchecked, linear scaling of the density levels is used. These changes are only applied to plots created after this option has been selected, and not to the ones previously created.

Smooth Histograms

This automatically smooths single histograms by default allowing for easier viewing. When selected, this option displays smoothed data on all new histograms as they are created. It does not apply any of the changes to previously created plots.

Default Overlay Plot Background Color

Choose the default background color you require by clicking on the drop down arrow box and selecting from the color chart displayed. The default color set shows in the box to the left of the drop down arrow.

Default % of Events on Dot Plot

This allows you to set the default number of dots to be displayed when creating a dot plot. These changes are only applied to plots created after this option has been selected, and not to the ones previously created. Reducing the default percentage increases the speed of software functions when reading large listmode files.

Baseline Offset

IMPORTANT Risk of erroneous results. The Baseline Offset function should only be used after first viewing data with the baseline offset function turned Off (unchecked). You must be satisfied that the overall results of an assay are not significantly affected by turning baseline offset on. If the lower marker of a statistics region is higher than the first decade (of log displayed data) then there should be no effect at all by using Baseline Offset on Statistics data from that region.

This function allows log data in very low level signals to be moved up from low numbered channels by adding a randomized Gaussian positive offset. This allows easier comparison of data from other Cytometers. Baseline Offset includes the following three options:

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• Auto

This option displays the listmode data according to a keyword in the FCS file header. If the Baseline Offset keyword value is ON, then data is automatically displayed with Baseline Offset switched on. If it is OFF, then data is displayed with no Baseline Offset.

On

This reads a file and modifies the displayed data only by adding a randomized Gaussian offset to the low-level signals. Data on disk is not modified and is left as it was when acquired from the Cytometer.

Off
 Reads a file exactly as it is without modification during display.

Default Overlay Mode

This allows you to select the default mode for the overlay plots. Gallery, Overlay or Angled view can each be selected as the default. This is only applied to any plots created after this option has been checked and does not alter any of the previously created plots.

See also, Overlay Plot Mode.

Default 3D Plot Mode

This option sets the default mode for Surface and Tomogram plots. For further details see Gate Precedence Mode and Density Mode.

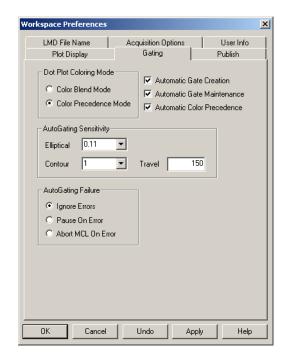
This option is only applied to plots created after this has been selected and does not alter any of the previously created plots.

Default Plot Size

Allows you to select the size of the plot display.

Workspace Preferences - Gating

Automatic Gate Creation
Automatic Gate Maintenance
Automatic Color Precedence
Dot Plot Coloring Mode
AutoGating Sensitivity
Elliptical Sensitivity
Contour Sensitivity
Contour Travel
AutoGating Failure
Pause on Error
Abort On Error
Ignore Errors



See also: Workspace Preferences - Introduction

Workspace Preferences - LMD File Name

Workspace Preferences - Acquisition Options

Workspace Preferences - User Info

Workspace Preferences - Plot Display

Workspace Preferences - Publish

Automatic Gate Creation

This option allows you to create a new gate automatically when a new Region is created. If this option is not selected you must use **Analysis Create Modify Gates** to create gating logic. Regions copied using Ctrl+Drag and Drop into a plot assign the region as a gate.

Automatic Gate Maintenance

Enabling this option ensures that when the gating of a plot changes, the associated gate of any region drawn onto this plot also changes to reflect the new gating state.

Regions with an associated gate CANNOT appear on more than one plot when this mode is enabled (CXP Analysis software only).

Automatic Color Precedence

As a new region is drawn, the color for that gate is placed at the top of the list in the color precedence dialog box. If this option is disabled, a color is not associated with a gate.

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Dot Plot Coloring Mode

This alternates Color Dot plot mode between Color Blend and Color Precedence modes:

- Color Blend Mode
- Color Precedence Mode (default)

AutoGating Sensitivity

Select the sensitivity of the AutoGate region drawn.

Elliptical Sensitivity

Settings are 0.11, 0.33 and 1.1 (% of processed peak). Selecting 0.11 creates a larger region, whereas selecting 1.1 creates a smaller radius region around the target population.

Contour Sensitivity

Settings are 1, 2, 3, 4, and 5. Level 1 = 0.11% processed peak at a resolution of 64x64, levels 2, 3 and 4 are 0.11, 0.33 and 1.1% at a resolution of 128x128. Level 5 is 1.1% processed peak at a resolution of 256x256.

Contour Travel

Input a maximum value the contour AutoGate region travels from the mean. The Minimum value is 25; Maximum is 250 channels. Use this option to slightly adjust the AutoGate from tube to tube if slight changes in lysis cause the target population to move slightly. This option is not available for elliptical AutoGates.

AutoGating Failure

You can select an error option when the AutoGate algorithm fails during acquisition in the MCL automatic mode.

Pause on Error

The system pauses the current acquisition when an AutoGating error occurs and awaits further input before proceeding.

Abort On Error

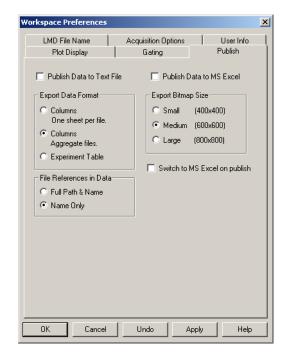
The system automatically aborts the Worklist when an AutoGating error occurs.

Ignore Errors

The system continues processing the Worklist when an AutoGating error occurs.

Workspace Preferences - Publish

Publish Data to Text File Export Data Format File References in Data Publish Data to MS Excel Export Bitmap Size



See also: Workspace Preferences - Introduction

Workspace Preferences - LMD File Name

Workspace Preferences - Acquisition Options

Workspace Preferences - User Info

Workspace Preferences - Plot Display

Workspace Preferences - Gating

Publish Data to Text File

Enable this checkbox to publish data to an ASCI text file with a file extension of .TXT.

Publish Data to MS Excel

This function enables you to publish data to a Microsoft Excel Workbook, used in conjunction with the **Tools → Publish Results Now** menu item.

Export Data Format

Select the Export Data Format.

- Columns New sheet per file to export the data with each file on a separate worksheet,
- Columns to export the data from all files to a single worksheet
- Experiment Table to export the data in experiment table format for inclusion in a pivot table.

File References in Data

Choose to reference the file name by Full Path and Name or by Name Only.

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Switch to MS Excel on Publish

MS Excel starts and displays the published data. When enabled, Excel moves to the front of Windows desktop as the active application with each export. If left unchecked, Excel runs in the background with each subsequent export.

Export Bitmap Size

Allows you to select the size of the Bitmap used in when exporting.

Log Out

Allows a particular user to Log Out of CXP software so that another person can Sign-On.

Exit

This option closes down the CXP software and returns you to your Windows Desktop.

B.16 EDIT MENU

Undo

Edit > Undo reverses software operations such as Region drawing, Region label editing and FlowPAGE editing. Each New operation is appended to an Undo list. The valid Undo operations are then performed in reverse order with each Undo request.

Note: Undo does not undo such operations as Save and Acquisition of data.

The **Edit > Undo** list is cleared after each change of plot focus.

The **Edit → Undo** function is applicable to the Window Menu, Regions, FlowPAGE and fonts.

Redo

Edit >> **Redo** reverses the last Undo operation.

If a new operation is performed after a series of **Edit** >> **Redo** operations, the **Edit** >> **Redo** list is cleared and all previous Undo actions are lost.

The **Edit PRedo** list is cleared after each change of plot focus.

Cut

Edit > Cut deletes the highlighted items from the display, placing them within the Clipboard. These items can be recalled either by using the Copy, Paste or Paste Special options within the Edit menu.

Histogram plot displays can be Cut from one Overlay plot to another by using the **Paste** or **Paste Special** options.

If you **Edit** > **Cut** another item before the first item is Pasted, this first item is overwritten.

See also:

Copy

Paste

Paste Special

Copy

To Copy Statistics, Regions or Plot Images onto the Windows Clipboard, Edit > Copy

This allows for Pasting these items into other areas of CXP software or to other Windows Applications such as MS PowerPoint®, Word, Excel, and so on.

The exact nature of what can be pasted depends upon the target application and can be controlled or modified using the target applications **Edit** >> **Paste Special** command.

See also:

Cut

Paste

Paste Special

Paste

After copying into memory, CXP software allows the pasting of a number of different items.

The default paste result depends upon what you are trying to paste.

- If the target is a FlowPAGE, the Plot Image and Statistics are Pasted.
- **1** Select the Plot of interest to be copied.
- 2 the Plot and hold down the left mouse button. (The No Drop Cursor sis displayed.)
- 3 Drag the cursor onto the FlowPAGE. (The Drop Cursor is now shown.)
- **4** Release the mouse button to paste the Regions onto the Plot or the Plot Image plus the Statistics onto a FlowPAGE.

Note: If you use Ctrl+ to Drag and Drop, you get the Plot Image but not the Statistics.

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•	If the Paste target is a Plot, then the previously copied regions are Pasted.
1	Select the Plot with the region of interest to be copied.
2	Drag the cursor to the destination Plot.
3	Release the mouse button to paste the Regions onto the Plot. Note: If the copied region is required as a gate, use to Drag and Drop the region.

See also:

Cut

Paste

Paste Special

Paste Special

Allows which form of a copied object is pasted. Bitmaps, Text or Region may be pasted depending on the copy source and the paste target.

See also:

Cut

Copy

Delete

When **Delete** is used, any selected items are deleted. In certain circumstances, it may be possible to reverse the **Delete** action by selecting the **Undo** option.

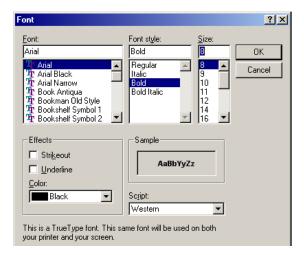
See also:

Undo

Edit Font

You can modify the Font type, size and color of selected text. The fonts available depend upon the fonts installed in Windows. If in doubt, try to use only True Type fonts.

The fonts selection dialog box is the standard Windows common dialog box for font selection and is fully explained in the Microsoft Windows documentation.



For True Type fonts, you can generate both horizontal and vertical fonts for the Plots.

For non-True Type fonts that do not have point sizes for both horizontal and vertical (portrait and landscape), Windows attempts to use a horizontal font for the vertical direction. Under these circumstances choose another font or reload the font of your choice making sure the font sizes required are available in both portrait and landscape types.

Please see the Microsoft Windows documentation on loading new fonts.

See also:

Edit Font (FlowPAGE)

Font

Font Annotations

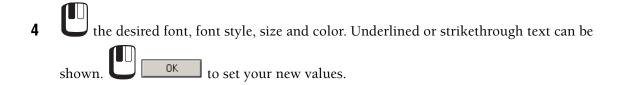
Edit Font (FlowPAGE)

To change the fonts in FlowPAGE:

- **1** Double click on a plot or text box to display the grap handles.
- **2** Click the right mouse button on the plot or text box to display the properties dialog box.

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5 once outside the plot or text box. The cursor reverts back to the Chooser cursor and the handles disappear.

See also:

Font

Font Annotations

B.17 VIEW MENU

Acquisition Manager

The Acquisition Manager is used to create a Worklist for the Cytometer. It may also be used to create or modify existing panels. Sample and Test information may be entered here, this data is stored as part of the FCS listmode file.

Panels and Protocols can be added to the Acquisition Manager Worklist by using the Resource Explorer to Drag and Drop or by using the ACQUISITION MANAGER TOOLBAR. Items may be reordered within groups or entire groups may be reordered using Drag and Drop. Thick black lines define groups, and items within the groups are separated by thin dotted lines.

By clicking the right mouse button in the column headings the following menu choice is available.

<u>C</u>ustomize columns <u>P</u>rint Worklist

Select the appropriate option to Customize the Worklist Columns allowing the addition, removal or renaming the columns, see Customize Worklist Columns or print the Worklist when a Print dialog box is displayed.

See also:

ACQUISITION MANAGER TOOLBAR Worklist Columns Available Acquisition Manager - Panel

Acquisition Manager – Modifying Panels and Worklists

The Acquisition Manager is used to create a Worklist for the Cytometer. It may also be used to create or modify existing Panels or Worklists.

IMPORTANT Risk of erroneous quality control statistics. Panels containing QC regions must be constructed from individual unique protocols. Do not use a panel constructed from one protocol (with different parameter names) for QC database purposes.

Panels

1	Open	the	Panel	to	be	modified.
---	------	-----	-------	----	----	-----------

- To add tests to a Panel, use the Insert Test button on the Acquisition Manager Toolbar. Repeat this process as necessary. You may also insert multiple tests by using the Insert Panel button. Alternate protocols may be selected once the tests have been added using the File button inside the Worklist.
- **3** To remove tests, delete the entire row from the Worklist.
- To change any other panel information, such as how instrument settings are loaded, use the appropriate columns. When the changes are complete, click the right mouse button inside the panel and Save As Panel.
- **5** If you intend to generate Panel Reports from this panel,

Export results of panel to Report Generator

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Worklists

1 Open the Worklist to be modified.

2 To add samples to the Worklist, use Drag and Drop from the Resource Explorer. This starts a new panel or protocol that is automatically added to the end of the Worklist.

3 To remove tests, delete the entire row from the Worklist.

4 Save the Worklist using the Save Worklist button on the Acquisition Manager Toolbar.

See also:

Acquisition Manager

Customize Worklist Columns

Worklist Columns Available

The following columns are available for display within the Acquisition Manager:

Panel

Protocol

Plots

Region Source

Cytosettings

Tube ID

Carousel Number and Location

Sample ID 1 – 4

CAL Factor

LMD Filename

Stop Condition and Value

Parameter Names

Acquisition Manager

Customize Worklist Columns

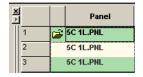
Acquisition Manager - Panel

IMPORTANT Risk of erroneous quality control statistics. Panels containing QC regions must be constructed from individual unique protocols. Do not use a panel constructed from one protocol (with different parameter names) for QC database purposes.

Panel

Displays the name of the currently selected panel. An

alternative panel may be selected using the File button or a New Panel created using the Panel Wizard.



See also:

Acquisition Manager
Worklist Columns Available
Acquisition Manager – Modifying Panels and Worklists
Customize Worklist Columns
Deleting a Worklist Row
Adding a Panel
Adding a single Test to the Worklist

Acquisition Manager - Protocol

Protocol

Displays the name of the currently selected protocol.

An alternative protocol may be selected



Acquisition Manager – Plots

Plots – A two-state toggle button displayed as icons. These states are:

Load the plots, and gates from the protocol, this is the default option.

Carry the plots, and gates through from the previous acquisition.



Note: This column is de-selected by default.

See also:

Worklist Columns Available Acquisition Manager Customize Worklist Columns

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Acquisition Manager - Region Sources

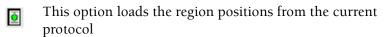
Region positions can be carried between different protocols within a panel – regions can, for example, be set on a control sample then carried through all the tests in a panel.

Note: In order for a region position to be carried into a new protocol the source and target region must satisfy all the following criteria:

- · Regions must have the same name
- Regions must be of the same type
- Regions must be drawn on the same parameters.

All regions satisfying these criteria are carried from one protocol to another.

There are three options for the region source:





This option carries the region positions from those of the previous tube in a panel.



This option loads region positions from any suitable protocol,

use the Browse button to select the required protocol. The name of the selected protocol then appears.



See also:

Worklist Columns Available Acquisition Manager Customize Worklist Columns

Acquisition Manager - Cytosettings

Cytosettings – three-state toggle displayed as icons or bitmaps. These states being:

Loads the instrument cytosettings from the protocol,

Carries the instrument cytosettings through from the previous acquisition.

Note: When using the Instrument Settings column of the Aquisition Manager to carry settings between protocols within a panel, you should ONLY carry settings between protocols where the parameter number and order are identical.

Retrieve the instrument cytosettings from an external file. For example, from an AutoSetup protocol, another protocol or listmode file.

When the Retrieve instrument settings from external file option is selected, the external file name is displayed in this field. A

Browse button is provided so that a setting file can be opened.

See also:

Worklist Columns Available Acquisition Manager Customize Worklist Columns

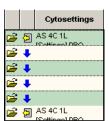
Acquisition Manager - Tube ID

Tube ID	Carousel No.	Location	Sample ID 1	Sample ID 2	Sample ID 3	Sample ID 4
89268232953	1	1	Sample 1	Whole Blood	Normal	
89268232954	1	2	Sample 1	Whole Blood	Normal	
89268232955	1	3	Sample 1	Whole Blood	Normal	
89268232956	1	4	Sample 1	Whole Blood	Normal	
89268232957	1	5	Sample 1	Whole Blood	Normal	

Tube ID – Displays the bar-code number from the tube. Allows positive sample identification and an error is generated if the Tube ID does not match the bar-code label on the sample tube. If no Tube ID is entered, but a bar-code label is present on the sample tube and run on the FC 500, the bar code is read and the Tube ID field is updated with this information.

Acquisition Manager - Carousel No. and Location

Tube ID	Carousel No.	Location	Sample ID 1	Sample ID 2	Sample ID 3	Sample ID 4
89268232953	1	1	Sample 1	Whole Blood	Normal	
89268232954	1	2	Sample 1	Whole Blood	Normal	
89268232955	1	3	Sample 1	Whole Blood	Normal	
89268232956	1	4	Sample 1	Whole Blood	Normal	
89268232957	1	5	Sample 1	Whole Blood	Normal	



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Carousel No. and Location – Allows you to to specify the Carousel number and the location of a tube in the carousel. You can run a tube at multiple points in a worklist (for example: a set-up or cleaning tube) and random access to sample tubes.

Acquisition Manager - Sample ID

Tube ID	Carousel No.	Location	Sample ID 1	Sample ID 2	Sample ID 3	Sample ID 4
89268232953	1	1	Sample 1	Whole Blood	Normal	
89268232954	1	2	Sample 1	Whole Blood	Normal	
89268232955	1	3	Sample 1	Whole Blood	Normal	
89268232956	1	4	Sample 1	Whole Blood	Normal	
89268232957	1	5	Sample 1	Whole Blood	Normal	

Sample IDs – Up to four Sample ID fields may be displayed. Any required information can be entered into these fields.

See also:

Acquisition Manager

Customize Worklist Columns

Acquisition Manager - CAL Factor

CAL Factor – Edit box containing absolute count CAL factor, designed to allow the use of multiple Flow-Count, Leuko-Count or Stem-Count fluorospheres batches. The CAL Factor

button allows the display of the **Absolute Count Calibration** dialog box. See Absolute Count Calibration for more details.

Note: If the same CAL Factor is used for multiple panels in a worklist, use to autofill the entire worklist with the CAL Factor.

See also:

Acquisition Manager Customize Worklist Columns

Acquisition Manager - LMD File Name

LMD File Name – The File Name displayed in this column is only a provisional file name. Some available options (for example: Run Number and Time) can only be defined at the time of acquisition. If these elements are included in the file name and a printout of the Worklist with the final assigned file name is required. They only print correctly once acquisition of

samples is complete. A Browse button is available to allowing file name definition through the Workspace Preferences - LMD File Name option.

See also:

Worklist Columns Available Acquisition Manager Customize Worklist Columns

Acquisition Manager - Stop Condition and Stop Value

Stop Condition – By clicking the mouse within this box, you can select the Stop Condition to apply. This option allows you to override the stop conditions present within a protocol.

The options are:

Protocol to use the preset stop condition.

Time to Stop at a particular time.

Maximum events when the require number of event have been collected.

Stop Value – Detected from protocol and can be edited, again allowing user to override currently defined stop value.

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

See also:

Acquisition Manager Customize Worklist Columns

Acquisition Manager - Parameter Names

Parameter Names – An editable box displaying the default parameter names from the protocol or panel. This allows free text entry of parameter names to be added as required. Changes made here are updated in the protocol once acquisition begins. Protocol Save can then be used to save these parameter names in the panel. See Edit FCS Header Attributes for an alternative way of saving parameter names in a protocol prior to acquisition.

In this example, the columns are numbered P1 to P6. The actual number present is determined by the number of parameters selected in the protocol; the maximum number you can have is 16.

P1	P2	Р3	P4	P5	P6
FS PEAK	FS	SS	CONTROL FITC	CONTROL PE	CD45 ECD
FS PEAK	FS	SS	CD3 FITC	CD19 PE	CD45 ECD
FS PEAK	FS	SS	CD3 FITC	CD4 PE	CD45 ECD
FS PEAK	FS	SS	CD3 FITC	CD8 PE	CD45 ECD
FS PEAK	FS	SS	HLA-DR FITC	CD19 PE	CD45 ECD
FS PEAK	FS	SS	CD10-FITC	CD19 PE	CD45 ECD

See also:

Worklist Columns Available Acquisition Manager Customize Worklist Columns

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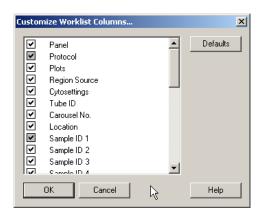
Customize Worklist Columns

Use the Customize Worklist Columns dialog box to add, remove, or edit Worklist Columns displayed. Columns can also be renamed or reordered.

A column is included or removed by enabling or disabling the checkbox. If the checkbox is grayed out it cannot be altered.

The Column Name can be edited by double clicking on the name and renaming as appropriate. If the name has a lock icon, no changes are allowed.

The Default button resets the Worklist Columns to the standard default and consequently resets the Workspace Preferences – LMD File Name.



Note: If a particular Worklist Column is not displayed, the standard default heading still shows on the Workspace Preferences – LMD File Name tab dialog box.

If the Names are changed, this reflects in the name labels within Workspace Preferences – LMD File Name.

Reordering Columns

The columns may be reordered by selecting a column or a group and using Drag and Drop to move it to the desired location. To move a column within a group, use Drag and Drop. To move a group of columns use The Drag and Drop. Groups are defined by thick black lines, and items within the groups are separated by thin dotted lines.

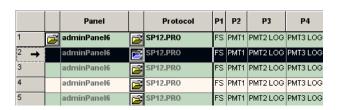
Deleting a Worklist Row

To delete a Row within a Worklist, highlight the appropriate Row or Rows by clicking the mouse in the Row Number column shown by the



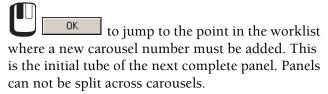
Use or Delete within the Acquisition Manager Toolbar to delete the appropriate rows.

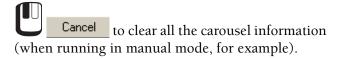
If you inadvertently mark a row or rows for deletion, click the mouse within the body of the Worklist outside of those rows highlighted.



Carousel Overflow

An FC 500 carousel can hold a maximum of 32 tubes. Creating a worklist with more than 32 tubes in one carousel generates a carousel overflow message.

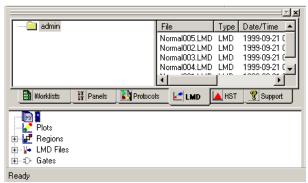






Resource Explorer

The Resource Explorer occupies a definable area within the Workspace. It allows you to browse files, and can be used to Drag and Drop items into CXP software. Tabs for Listmode files, Protocols and Histograms allow files to be dropped into the Workspace or Overlay Plots. Panels and Worklist can be dragged from the Resource Explorer and dropped into an Acquisition Manager Worklist. Information added in this way always appears at the end of the Worklist. The order may be changed using Drag and Drop after the information has been added.



The view that you see depends upon your particular access rights. User levels show only a particular user Listmode, Histogram and Protocol directory, depending on the tab selected.

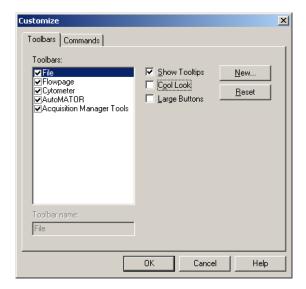
If you are a member of a particular Workgroup, all the user folders within that Workgroup are visible. The Support tab provides a link to the Beckman Coulter and ACS web sites as well as links to any Tutorials and ancillary Help files.

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Toolbars - Customize Toolbars

This allows you to select the style and which Toolbars are displayed.

Toolbars
Toolbar Name
Show ToolTips
Cool Look
Large Buttons
New...
Reset



If a customized Toolbar has been created and this is highlighted, ______ is available to allow the deletion of that Toolbar.

See also, Toolbars - Customize Commands Tab

Toolbars

Select which Toolbars you wish to be displayed. Any customized Toolbars are listed.

Toolbar Name

Allows you to modify the name of any customized Toolbar. It is grayed out for the standard Toolbars.

Show ToolTips

Show Fly-over help when the mouse is pointed to the Icon.

Cool Look

This changes the style to a "cool" look.

Large Buttons

Changes the Icon buttons to a Large size.

New...

Displays a dialog box to enter your customized name of your new Toolbar.

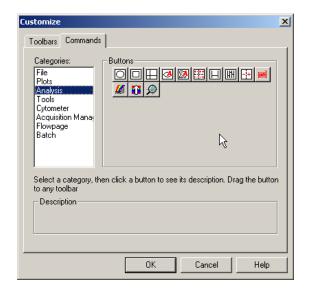
Reset

Resets the Toolbar to the default values.

Toolbars - Customize Commands Tab

This allows you to customize the Toolbars by dragging the button to the desired position within any Toolbar.

Categories ButtonsDescription



See also, Toolbars - Customize Toolbars

Categories

Displays the Toolbars that are currently available.

Buttons

Shows the buttons that may be selected for any Toolbar.

Description

Displays a description of the Icon button selected.

Zoom

The following option allows you to zoom in on a FlowPAGE, or Overlay Plot to 100%, 200% or 400%.

Select the desired view. Selecting 100% returns to the default size.

Refresh

Selecting **View** > **Refresh**, or pressing F5, causes CXP software to refresh the screen, all plots and FlowPAGEs. This option is useful when Windows fails to refresh the screen correctly leaving some graphical errors visible.

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B.18 INSERT MENU

Blank FlowPAGE

To open a new blank FlowPAGE,



Note:

- The page displayed in the FlowPAGE is the same aspect ratio and paper orientation as used in the default Printer.
- The page scaling can be changed at any time after a FlowPAGE is created.
- Several FlowPAGEs can be created.
- FlowPAGES in a locked protocol can not be inserted, deleted or edited.

All FlowPAGEs contain:

- Institution name
- Acquisition or playback protocol
- Acquisition date and time
- User ID
- Settings file name, date and time
- Listmode file name
- Total acquisition time and stop condition
- Sample ID 1
- Protocol status
- Analysis date
- Instrument serial number
- Software version

FlowPAGE from File

When a FlowPAGE has been created, (including all the departmental logos, addresses, plots, statistics and comment boxes), the whole FlowPAGE can be saved and retrieved.

Use the open FlowPAGE option to open a FlowPAGE template from disk. FlowPAGE uses the default file extension *.PGE. The *.PGE extension is only a suggested extension, any standard DOS characters can be used for the full file name.

1 Insert ➤ Open FlowPAGE From File to view the Open FlowPAGE dialog box.



2 the desired drive in the Drive List

- the desired directory in the Folders List box.
- 4 the type of file in the Files List box and click.
- 5 the name of the file to view.

Double click on the file name to close the dialog boxes and display the file.

FlowPAGE Statistics Table

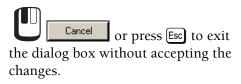
This option is used to insert statistics into FlowPAGE.

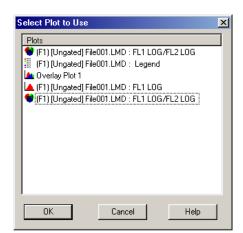
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2 From the list of plots currently open in CXP software, the one containing the statistics you wish to insert.

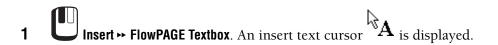




The statistics for the selected plot are inserted into the FlowPAGE and can then be moved to the desired position. Only one plot is permitted per statistics table.

FlowPAGE Textbox

This option is used to insert text into FlowPAGE.

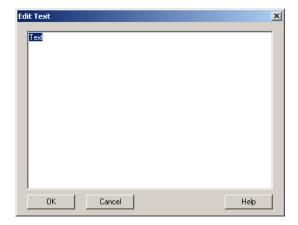


2 Move the cursor to the place for the textbox and

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3 Type the required text and





- 4 outside the text box to accept the changes.
- **5** Click the right mouse button on the text box to edit properties, enter text, edit the font or change the alignment.

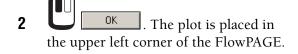
FlowPAGE Plot

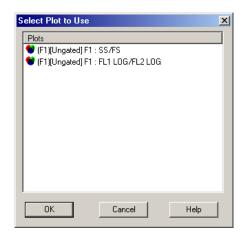
Insert Plot

To insert plots currently displayed on the desktop into FlowPAGE.

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Inserting Drag & Drop Plot

Insert currently displayed plots on the desktop into FlowPAGE.

inside the plot to be inserted into FlowPAGE and hold down the left mouse button.

The No Drop cursor is displayed.

2 Drag the cursor onto the FlowPAGE. The Drop cursor is now shown. Release the mouse button. The plot is now inserted into FlowPAGE.

Note: If you Drag and Drop the plot, the plot and the statistics are inserted. If you Ctrl+Drag and Drop the plot, the statistics are not inserted with the plot.

Other FlowPAGE Object

Inserting a Rectangle

1 Insert >> Other FlowPAGE Object and the Rectangle option.
A cross-hair cursor + is displayed.

2 and hold down the left mouse button while you drag to size the rectangle.

3 outside the rectangle to anchor it.

See also:

Inserting a Line Inserting a Picture Inserting a Text File

Inserting a Line

Draw straight lines using FlowPAGE → Insert → Line.

1 Insert >> Other FlowPAGE Object and the Line option.
A cross-hair cursor + is displayed.

2 Drag the cursor to desired line size and release the mouse button. The line is drawn and the cursor reverts back to the Chooser cursor .

See also:

Inserting a Picture Inserting a Text File

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Inserting a Picture

FlowPAGE allows the import of a Windows Bitmap file (extension *.bmp).

1 Use MS Paint or other graphics editor to create a bitmap file to be placed on a FlowPAGE and save it as a file.



- **2** Select the desired bitmap file.
 - a. Look in: list box.
 - b. the type of file in the Files of type: list box.
 - c. the name of the bitmap file you want to insert.

 Note: Double click on the file name to close the dialog box and display the file.



the FlowPage location where you want to place the picture.

See also:

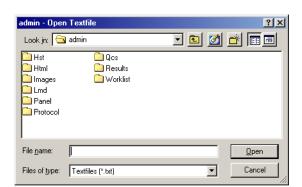
Inserting a Line Inserting a Rectangle Inserting a Text File

Inserting a Text File

Note: Use MS Notepad or other text editor to create text (*.txt) files.

- **2** Select the desired text file.
 - the desired directory in the Look in: list box.
 - b. the type of file in the Files of type: list box.
 - c. the name of the text file you want to insert.

Note: Double click on the file name to close the dialog box and display the file.



- **3** The text box is placed in the upper left corner of the FlowPage.
 - a. Use \bigoplus to move the text box to the desired location.
 - b. U ouside the text box to anchor it.

See also:

Inserting a Line Inserting a Picture Inserting a Rectangle

Other Insert Menu Items

Annotation

Inserts text into an Overlay Plot

Overlay Annotations - Introduction

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Auto Text

AutoText inserts the FCS Header Keyword Annotation into Overlay Plots.

The options are:

- Stain
- Source
- Parameter
- File Name
- Date

To insert other keywords, see FCS Keyword Annotation Mode.

Overlay Histogram

Inserts a stored histogram into an Overlay Plot.

See also, Adding a Histogram to the Overlay Plot.

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Publish Histogram Data Now

Opens Microsoft Excel and exports data from the Overlay histogram plot, the raw channel information is exported and additional statistics calculated. MS Excel must be present for this tool to function.

Publish Results Now

Statistics are displayed under plots in resizable columns and can be exported to Microsoft Excel or ASCII text (.txt) file format.

The Workspace Preferences – Publish tab option Publish Data to Text File and/or Publish Data to

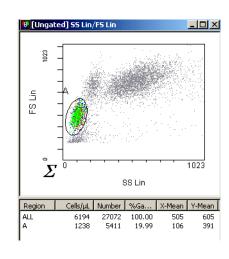


must be enabled. See Workspace Preferences - Publish.

To open the Statistics Pane, move the mouse over the Plot so that the Σ (Sigma Cursor) is displayed and click the left mouse button. The Statistic Pane drops down showing ALL the Plot statistics. To hide the Statistics Pane, click the mouse on the Sigma cursor again.

If other Regions are added or deleted while the Statistics Pane is open, it is automatically resized to accommodate the change.

Plots, including the Statistics Pane, can be resized at any time by dragging any part of the Window Frame although the height of the Statistics Pane remains unchanged.



The Statistics Pane can only be resized by using the $\frac{\mathbb{T}}{\Psi}$ Splitter Bar while holding down Use the mouse to drag the revealed Statistics Pane to the required position. If resizing is achieved using the splitter bar, the automatic sizing using the Σ (Sigma Cursor) is overridden.

Auto sizing is the default mode selected when the Plot is first created whereas user-defined sizing is selected as soon as resizing occurs using the Splitter Bar. To reinstate the Auto sizing, hold down [CH] while clicking the Σ (Sigma Cursor) with the mouse.

AutoMATOR

Welcome to AutoMATOR

Use the AutoMATOR option to sequentially read, analyze and report a set of listmode files. AutoMATOR performs batch analysis of stored data using a multi-file protocol.

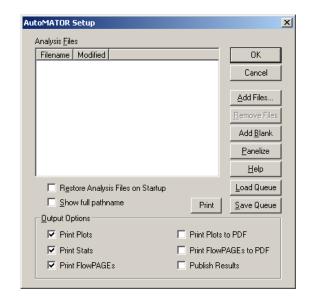
AutoMATOR allows listmode files to be read and analyzed in a sequential or a predetermined batch order.

AutoMATOR Setup

to display the set of files last used. If these files are not required, highlight them and Remove Files.

Remove Remove Reordering Files
Add Files
Remove Files
Add Blank
Panelize
Load Queue
Save Queue
Print
Restore Analysis Files on Startup

Show full pathname Output Options



2 Add Files... or Load Queue to select the required files.

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3	Navigate to the corre	ct directory and locate the relevant files.
4		es by highlighting with the mouse. To select more than one file, lick the relevant files with the mouse button (to deselect, click again vn).
5	Open. The files are listed in	the AutoMATOR Setup dialog box.
6	Note: To select all the	ccept changes. Cancel does not apply any of the changes made.
Sta	itus Icon:	
☒	Pause	The Auto Pause function pauses the run of tests pauses and then carries on with the rest of the panel automatically after a 15-second wait. The countdown shows on the AutoMATOR Status Bar. When the tests are in the Auto Pause setting, you can hold down shift and click the Pause button. Shift overrides the Auto Pause, placing the AutoMATOR into Continuous Pause.
		To restart, click the Run button and the test resumes from the point at which it was paused.
0	Continuous Pause	Pauses the tests for modifications and remains on pause until you decide that processing continue. This function allows the test to stop without reverting to the beginning of the panel again. To
		resume the test, click the Run button.
1	Continue	Plays back the indicated file without pausing.

USING CXP SOFTWARE

These icons allow you to insert a pause or a stop into the panel, or let the panel run straight through from start to finish. Clicking repeatedly on the icon until it displays the desired status modifies the status in the highlighted file.

In this example, the four files would run and then pause before printing. This allows you to make any necessary alterations to the plots and regions before outputting results.

Report Icon:

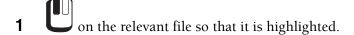


Once the files have been panelized, the report icon only appears next to the last file, as a report print out would only be produced after all files in a group have been run. (This differs from Acquisition Manager where there is a printout after each test that has run). This is providing that the correct Output Options have been selected.

Once the files have been panelized, you can select all or a block of the files so that they are highlighted. If the icon on one of the highlighted files is changed, all of the other selected files change to the same icon simultaneously. To change the status settings for one file only, highlight the file and then click on the icon until it changes to the desired status.

Reordering Files

Once all files are listed, they can be arranged in File Name or Date and Time order by clicking on the relevant column title. Rearrange files by Dragging and Dropping them.



- **2** Hold the mouse button down and drag the file to the appropriate position so that it is between two file names.
- **3** Release the mouse button.

If there are a number of files in the AutoMATOR Setup dialog box, you can either use the scroll bar, or utilize the Drag and Drop option.

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Select a file using the mouse, keep the mouse button held down and drag to either the top or the bottom of the window. Moving the cursor outside the window scrolls the list. The further the cursor is pushed to the top/bottom, the faster it scrolls.

Add Files

Select this option to display the Open Listmode File dialog box. You can then peruse other Files in various drive and directories and add them to the list.

Select the name of the listmode file(s) you want to add.

Remove Files

When selected, this option removes any highlighted files within the AutoMATOR Setup dialog box. The Delete button performs the same function.

Add Blank

A Blank is used to fill a space when there is an incomplete panel of listmode files, so that the results from the tests are kept in the correct order and a complete panel is maintained.

When selected, this option puts a blank at the end of the file list. This can then be Dragged and Dropped into the required position.

Panelize

This sets all the highlighted files into groups to easily select the Run, Pause or Stop replay options.

Load Queue

Displays a dialog box to load a queue file.

Save Queue

Displays a save dialog box to save the queue to a file. The files are saved in the LMD subdirectory under the file extension ALQ.

Print

Print a listing of the files in the queue.

Restore Analysis Files on Startup

If the checkbox is enabled, all the files displayed in the Analysis File list appear in the list the next time that you log in.

If the checkbox is not ticked, the list is empty the next time you log in.

Show full pathname

Shows the paths to the destination directories for the selected LMD files.

Output Options

These options consist of several checkboxes found at the bottom of the AutoMATOR Setup dialog box. Check the required output options to print or publish data at the completion of each sample.

The options are:

- Print plots as displayed.
- Print statistics only.
- Prints all FlowPAGEs.
 If these are not selected, the Page icon appears crossed out, and a report is not printed from the test.
- Publish Plots or FlowPAGES in PDF format.
- Publish Results
 Allows the automatic publishing of results to a text file or Microsoft Excel spreadsheet at the completion of each sample.

AutoMATOR Status Bar

This shows the current file being run and the current state of that file.



The AutoMATOR Status Bar, which displays the function that the test is performing, can be removed from the screen.

Click on the Close button featured on the Status Bar while the AutoMATOR is running. This hides the bar from view. It automatically reappears again the next time the AutoMATOR is run. The Status Bar cannot be brought back onto the screen once closed down without reopening AutoMATOR.

- Processing
 This is when the file is being analyzed by the software.
- Auto Pause
 When this command is active, it informs you as to the length of time left in pause before
 the analysis resumes. If this length of time is not required, selecting the Run
 again can restart the analysis.
- Continuous Pause
 This informs you that the processing is paused and can only continue if you restart the analysis manually by selecting the Run button again.

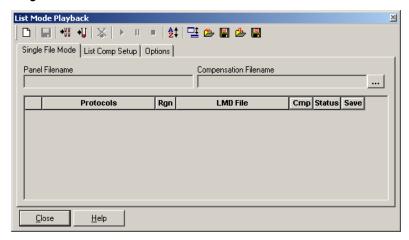
Listmode Playback

The Listmode Playback option allows for listmode playback with runtime protocol/panel or with a new protocol/panel. This option also allows listmode compensation of 20-bit data files in a fully automated manner, replicating the procedure used during analysis. Compensation settings may be verified and manually adjusted if required. The Options tab allows setting of default options during listmode playback.

Note: Panel Reports generated from listmode replay must be run using the Listmode Playback tool, replaying the listmode files through the desired export panel.

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Single File Mode



This mode allows for single file or panel based listmode file playback, similar to acquisition of samples.

A single panel may be selected; multiple copies of this panel may be run at a time. Tubes may be added or removed from individual panels.

Load Queue loads a listmode queue file (a list of listmode files).

Regions may be Loaded or carried within a panel, AutoGating and positives analysis can be active on any protocol within a panel

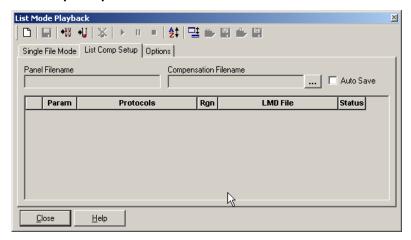
Where the source listmode file is a 20-bit data file, comp settings can be from the run time protocol (stored within the LMD file), from the Compensation settings file created in ListComp Setup or any *.CMP file may be used.

During playback the title bar displays the current file being analyzed.

Optionally a new file may be saved containing newly compensated 10-bit data. The original LMD file cannot be overwritten automatically.

Saved files have the suffix entered in the Options tab appended to each saved file.

List Comp Setup



This dialog provides an Acquisition Manager style worklist for the selection of compensation panels and associated protocols. *ppp files created from the protocols used in the AutoSetup II applications do not need the *_STAND protocol. These *.ppp panels allow you to replay their associated LMD files to create *.cmp files. For RXP AutoSetup I, if runtime panels are selected, as these contain Flow-Set fluorospheres protocols which have no use in this dialog, this protocol is removed from the displayed panel and a warning is displayed.

The dialog is resizable by dragging the corners to the required size.

The settings file name defaults to the name of the panel but with a new *cmp extension.

The Auto Save checkbox allows settings to be automatically written to the settings file that has the name of the panel selected.

Panels can be created within this dialog, but cannot be used for acquisition. These listmode panels are saved with a *.ppp extension.

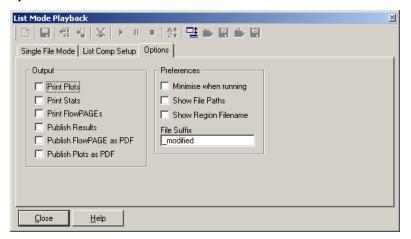
The Parameter field indicates the Compensation parameter within the protocol.

The Status field allows a pause or stop between each file playback.

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

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Options Tab

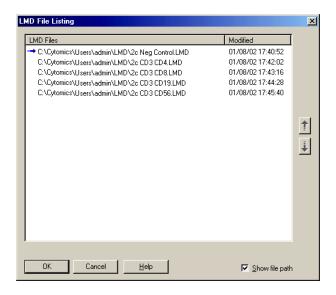


This tab allows setting of default options for the Listmode Playback. Print options, publish functions, file save naming options are set in this dialog box.

File Sorting - In the Listcomp setup or Single file mode options, listmode files may be sorted using the LMD File Listing option. This allows files to be sorted alphabetically, or by Time / Date modified. Files may be Dragged and Dropped. Individual files may be selected and moved up or down the list using the arrow buttons.

The start of each panel is indicated by an arrow.

A **Show File Path** checkbox allows the full file path for each file.



AutoSetup Application Defintion

IMPORTANT Risk of erroneous quality control statistics. Panels containing QC regions must be constructed from individual unique protocols. Do not use a panel constructed from one protocol (with different parameter names) for QC database purposes.

Use the Application Definition Wizard to define your applications and save these definitions to be used in the Autosetup Scheduler. The application definition captures the instrument setup, lasers, parameters, fluorochromes, target channels, verification and alignment requirements of a particular application. See APPLICATION DEFINITION WIZARD in the Instructions For Use manual to run the wizard.

AutoSetup Scheduler

Use the AutoSetup Scheduler to select the applications to be run on the instrument in a given day or "shift". AutoSetup Scheduler groups the selected applications for efficient set up of multiple applications simultaneously and provides the carousel load report to facilitate the setup and loading of samples for daily QC. See AUTOSETUP SCHEDULER in the Instructions For Use manual to run the scheduler.

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B.20 PLOTS MENU

Plots - Introduction

CXP software can display eight Plot types and the Legend for Color Gated Plots. When a Plot is displayed, click the right mouse button to display further menu options.

Note: You cannot add or duplicate plots when running a locked protocol.

Color Dot Plot
Histogram Plot
Contour Plot
Density Plot
Overlay Histogram Plots (CXP Analysis software only)
Tomogram Plot (CXP Analysis software only)
Surface Plot (CXP Analysis software only)
Prism Plot
Legend
Info Plot

Duplicate Plot

This option creates an exact duplicate of the currently selected Plot.

[Ctr]+[D] may be used as a keyboard shortcut for the same function.

Color Dot Plot

The Color Dot Plot option provides two-parameter displays of data and allows any combination of the parameters to be selected. Each dot in a Color Dot Plot represents one event (cell or particle). The dot location gives the parameter values for each event. Each parameter can have a channel number from 0 to 255 for 256 channel data or 0 to 1023 for 1024 channel data. The colors of the dots reflect the Gate or combination of Gates used to create the plot.

To Create a Color Dot Plot:



2 From the dialog box displayed, select the required File, then select desired Gates, Parameters and Regions for display.

Dot Plot Data Source

Dot Plot Events

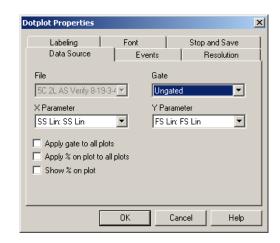
Dot Plot Resolution

Dot Plot Labeling

Font

Stop and Save (Acquisition only)

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



When a Plot is displayed, click the right mouse button within the plot to display further menu options.

Copy Plot Image Copy Plot Data Format Plot



Copy Plot Image

Copies the current plot image into memory for pasting into other applications. See Copy for more details.

Copy Plot Data

Copies the raw plot data into memory for pasting into other applications, such as Microsoft Excel.

Format Plot

Displays the Plot Properties dialog box for the current plot. See Format Plot.

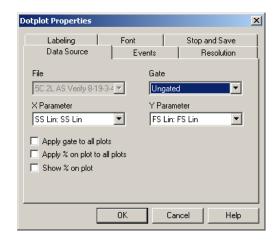
Dot Plot Data Source

Choose the X and Y parameters for the current plot. Gating equation and regions to be displayed can be selected.

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File
Gate
X and Y Parameter
Apply Gate to All Plots
Apply % on plot to all plots
Show % on plot

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



File

Select the required file from the files already in memory, displayed in the drop down box, or click the browse button to explore your directories for the required file (Analysis only).

Browse Button

Allows you to peruse other directory options and select files as appropriate.

X and Y Parameter

Select the required parameter for display from those available in the list.

Gate

Available Gates are listed. Gates required for Advance Precedence should be selected at this point. See Modify Color Precedence.

Apply Gate to All Plots

To select this option, enable the checkbox and apply the gate selected to all plots.

Apply % on plot to all plots

Displays the % of events in the regions on all plots.

Show % on plot

Displays the % of events in the regions on this plot only.

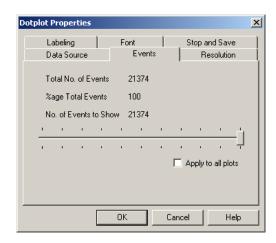
Dot Plot Events

Use this to specify the percentage of events to view. This option significantly increases the speed at which dot plots refresh if large data files are being analyzed. For an event interval less than 100%, data is displayed uniformly from within the whole of the data file, that is, if 10% of events are selected then event numbers 1, 11, 21, 31, and so on, are displayed if they meet the Gating criteria for the plot.

The default value for the number of events to display can be set in the File >> Workspace Preferences and selecting the Plot Display tab.

No. of Events to Show Apply to All Plots

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



See also:

Workspace Preferences - Plot Display Dot Plot Data Source Font

No. of Events to Show

Place the cursor on the Slide bar and move to change the No. of Events to Show.

Apply to All Plots

Enable this checkbox to apply the **No. of Events to Show** to all Plots.

Dot Plot Resolution

Set the number of channels for the X and Y Axis of the Plot.

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

The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

Note: If a higher resolution is selected, a sufficient number of events is required to produce a visually acceptable graph.



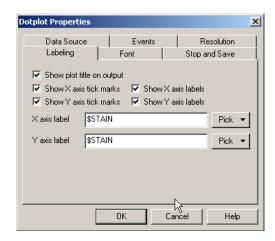
Dot Plot Labeling

The options here allow you to override the default plot display options for the current plot. This is useful when capturing images for publication.

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Show Plot Title on Output Show Axis Tick Marks Show Axis Labels X and Y Axis Labels

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



Show Plot Title on Output

Unchecking this option prevents the plot title from appearing when the plot is printed or exported.

Show Axis Tick Marks

Unchecking these options hides the axis scale tick marks and labels.

Show Axis Labels

Unchecking these options allows the axis parameter name to be hidden.

X and Y Axis Labels

Allows custom naming of the parameters on the current plot, any FCS keyword or text may be entered. Alternatively select **Pick** to select from File Name, Stain name (\$PnS) or Parameter name (\$PnN).

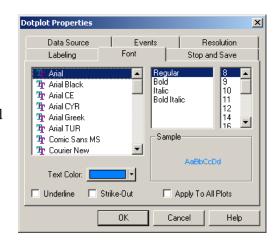
Font

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

Select the required Font Type, Font Style and Point Size.

Other font attributes available are **Underline**, **Strike-Out** and **Text Color**. The default color is black.

The Apply To All Plots checkbox allows the selected font to be applied to all the currently displayed plots. You should choose True Type fonts so that X and Y axis displays are available.



Histogram Plot

Single parameter histograms show the frequency distribution of the chosen parameter. All histograms can be customized, that is, Colors, Gate for histogram, Scaling, and so on.

To create a Histogram Plot:

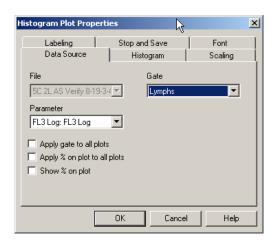
1



2 From the Data Source dialog box displayed, select the required File, then select desired Gates, Parameter and Regions for display.

Histogram Plot Data Source Histogram Formatting Histogram Scaling Histogram Labeling Font Annotations Stop and Save

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



3 When a Plot is displayed, click the right mouse button to display further menu options.

Copy Plot Image Copy Plot Data Format Plot

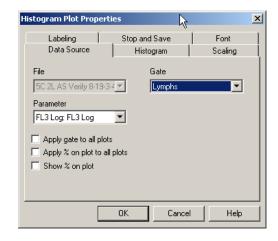


Histogram Plot Data Source

Choose the parameter and gate for the current plot. Gating equation and regions to be displayed can be selected. **Note:** You cannot make any changes on this screen when running a locked protocol.

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File
Gate Selection
Parameter
Apply % on plot to all plots
Show % on plot



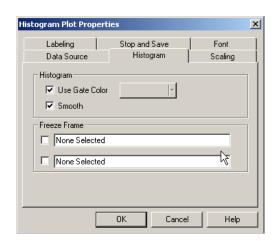
Parameter

Select the required parameter for display from those available in the list.

Histogram Plot Configuration

Use Gate Color Smooth Freeze Frame

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



Use Gate Color

If the **Use Gate Color** option is checked, the Histogram fill color is the same as the default color for the Gate that the plot is gated upon, and the Color Select button is unavailable. To change the color of the Histogram in the selected plot, clear the **Use Gate Color** checkbox and click the Color Select button. The colors returned are only those which the display is capable of rendering.

Smooth

If the **Smooth** box is checked, the visual representation of the Histogram is smoothed.

Freeze Frame

Check a **Freeze Frame** box to assign the current Histogram. The top button creates a dotted overlay and the bottom button creates a dashed Histogram.

The current Histogram is assigned to whichever Overlay button is selected and its name appears in the edit box adjacent to the button. This legend may be modified if desired.

In order for Freeze Frame to work in Acquisition, ensure that pass plots is set up in the Acquisition Manager.



Histogram Freeze Frame

CXP software allows the display of up to three Histograms on each single parameter Histogram. The base Histogram is the one that relates to the current listmode file and the other two can be overlays from any other compatible file. Statistics are calculated from the base Histogram only.

The other two overlays are for comparison only and no statistical information can be obtained from them.

See also:

Create a Histogram Freeze Frame Delete a Histogram Freeze Frame Overlay

CXP software Multi File Analyses allows any number of overlays to be displayed in a 2D or 3D Gallery Plot using the Overlay Histogram Plot.

Create a Histogram Freeze Frame

In order for Freeze Frame to work in Acquisition, ensure that pass plots is set up in the Acquisition Manager.



1 Read in a file or acquire data to be assigned as an overlay (for example: a negative control).

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- **2** Highlight the chosen single parameter Histogram to be used as an overlay plot (click on the title bar).
- 3 Click the right mouse button on the plot and Histogram tab. See also Histogram Formatting.
- 4 Check a **Freeze Frame** box to assign the current Histogram. The top button creates a dotted overlay and the bottom button creates a dashed Histogram.
- **5** The current Histogram is assigned to whichever overlay button is selected and its name appears in the edit box adjacent to the button, this legend may be modified if desired.
- 6 OK or press Enter to accept any changes.
- 7 Cancel or press (Esc) to exit the dialog box without saving the changes.

 Now you can open another file or acquire a positive sample. The new data is displayed as the base histogram and the previous sample is seen as an overlay.

Delete a Histogram Freeze Frame Overlay

1 Highlight the plot containing the unwanted overlay (click on the title bar).

2 Click the right mouse button on the plot and Histogram tab.

3 Clear the appropriate Overlay Histogram checkbox to delete an overlay.



5 CXP software then updates the display.

Histogram Scaling

Clip Channels Automatic Maximum Value Set Maximum

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



Clip Channels

If Automatic Maximum Value calculation is used then toggling the Clip Channels on ensures that the bottom and top channels are excluded from the calculation of the plots full-scale value.

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Automatic Maximum Value

This option searches the histogram for the maximum value and then sets the value found to be the full scale for the histogram.

Set Maximum

If the Automatic Maximum Value button is cleared, then any value entered here is the full scale for the histogram.

Histogram Labeling

Use the options here to override the default plot display options for the current plot. This is useful when capturing images for publication.

Show Plot Title on Output Show Axis Tick Marks Show Axis Labels X and Y Axis Labels

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



Contour Plot

The Contour Plot option (CXP Analysis Software only) provides two-parameter displays of data. Each event has a position on the plot according to its channel values for both parameters. Contour lines on the plot provide a third dimension by indicating the channel numbers containing the same event count or frequency count. (ISO-count contours).

To Create a Contour Plot:



2 From the Data Source tab box, select the required File, then select desired Gate, Parameters and Regions for display.

When a Plot is displayed, click the right mouse button to display further menu options.



Copy Plot Image Copy Plot Data Format Plot

See also:

Contour Plot Data Source Contour Plot Configuration Contour Plot Resolution Contour Plot Labeling Font Annotations Format Plot

Contour Plot Data Source

Choose the X and Y parameters for the current plot. Gating equation and regions to be displayed can be selected.

File
Browse Button
Gate Selection
X and Y Parameter
Apply Gate to All Plots
Apply % on plot to all plots
Show % on plot

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



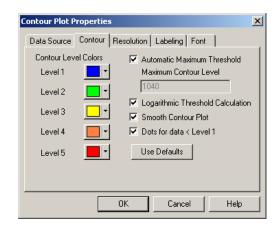
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Contour Plot Configuration

Use this option to specify the contour levels and other configuration preferences.

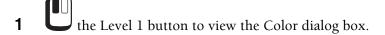
Contour Level Color Contour Plot Automatic Maximum Threshold Enter the Maximum Contour Level Contour Plot Logarithmic Threshold Calculation Smooth Contour Plot Dots for Data < Level 1

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



Contour Level Color

A Contour Plot may have up to 5 different ISO-count levels, each of which is represented by a color. Choosing the appropriate button may change the default color for each level.



- **2** Select the desired color that is to be allocated to Level 1.
- **3** Continue as above until all desired changes have been made. to accept all changes.

Contour Plot Automatic Maximum Threshold

This option allows automatic configuration of the maximum value, which is used to calculate the contour color levels. If this option is cleared you may manually enter the value.

If the Automatic Maximum Threshold is checked, next time the Contour Plot Attributes dialog box is entered the Automatic Maximum value is displayed.

Enter the Maximum Contour Level

This option allows the configuration of the Contour Plot manually. Enter the value, which is used to calculate the Contour Color Levels. The manual value can only be active when the **Automatic Maximum Threshold** checkbox is cleared.

Contour Plot Logarithmic Threshold Calculation

Contour color thresholds may be calculated in one of two ways:

Linear threshold calculation - option cleared
 The maximum value is used to determine the threshold value for each color. The actual threshold value is calculated as follows:

Value = (Level No) x (Maximum In Plot /5) - 1

Logarithmic threshold calculation - option checked
 The maximum value is used to determine the threshold value for each color. The actual threshold value is calculated as follows:

Value = 10(Level No) x (log10 (Maximum In Plot /5)) - 1

Smooth Contour Plot

Check this option to smooth the Contour Plot. Clear it to display raw unsmoothed data.

Dots for Data < Level 1

Check this option to see dots placed where data is present as 1 or more events but is at a level less than defined in level 1.

Note: This option may not appear to function unless smoothing is switched off first.

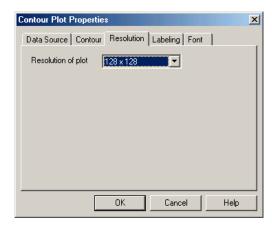
Contour Plot Resolution

Set the number of channels for the X and Y Axis of the Plot.

The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

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



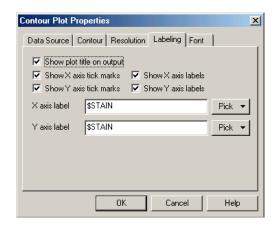
Contour Plot Labeling

Use the options here to over ride the default plot display options for the current plot. This is useful when capturing images for publication.

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Show Plot Title on Output Show Axis Tick Marks Show Axis Labels X and Y Axis Labels

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



Density Plot

The Density Plot option provides two-parameter data display, any combination of parameters can be selected. Each colored area in a Density Plot represents an ISO-count of the number of events (cells or particles). The color shows the number of events at any X and Y parameter value.

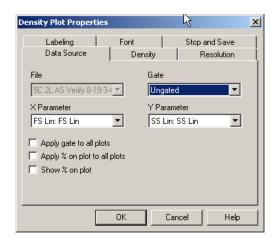
To create a Density Plot:



From the Data Source dialog box displayed, select the required File, then select desired Gates, Parameters and Regions for display.

Density Plot Data Source
Density Level Configuration
Density Plot Resolution
Density Plot Labeling
Font Annotations
Stop and Save (acquisition only)

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



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When a Plot is displayed, click the right mouse button to display further menu options.

Copy Plot Image
Copy Plot Data



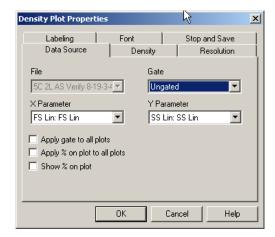
Density Plot Data Source

Format Plot

Choose the X and Y parameters for the current plot. Gating equation and regions to be displayed can be selected.

File
Gate Selection
X and Y Parameter
Apply Gate to All Plots
Apply % on plot to all plots
Show % on plot

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

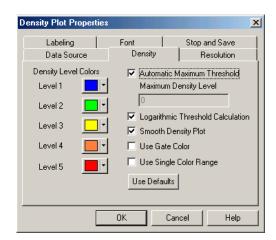


Density Level Configuration

Use this option to specify the Density levels and other configuration preferences.

Density Level Colors Automatic Maximum Threshold Maximum Density Level Logarithmic Threshold Calculation Smooth Density Plot Use Gate Color Use Single Color Range

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



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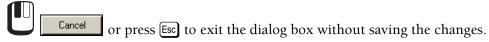
Density Level Colors

This Plot may have up to 5 different ISO-count levels, each of which is represented by a color. Choosing the appropriate button may change the default color for each level.

the Level 1 button to view the Color dialog box.

2 Select the desired color that is to be allocated to Level 1.

3 Continue as above until all desired changes have been made. to accept all changes.



Automatic Maximum Threshold

This option allows automatic configuration of the maximum value that is used to calculate the Density Color Levels. If this option is not selected you may manually enter the value which is used to calculate the Density Color Levels.

Maximum Density Level

Configure the Density Plot to manually enter the value that is used to calculate the Density Color Levels. This option is only active when the **Automatic Maximum Threshold** button is cleared.

Logarithmic Threshold Calculation

Density color thresholds may be calculated in one of two ways:

• Linear threshold calculation - option toggled off
The maximum value is used to determine the threshold value for each color. The actual
threshold value is calculated as follows: -

Value = (Level No) x (Maximum In Plot /5) - 1

Logarithmic threshold calculation - option toggled on
 The maximum value is used to determine the threshold value for each color. The actual threshold value is calculated as follows:

Value = 10(Level No) x (log10 (Maximum In Plot /5)) - 1

Smooth Density Plot

Choose this option to smooth the Density plot. Clear it to display raw unsmoothed data.

Use Gate Color

Creates a single color density range based on the currently assigned gate color.

Use Single Color Range

Creates a range of density level colors based on the selected Level 1 and Level 5 colors.

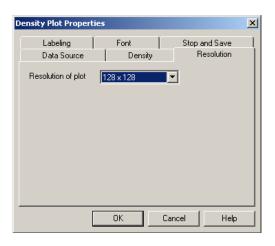
Density Plot Resolution

Set the number of channels for the X and Y Axis of the Plot.

The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

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

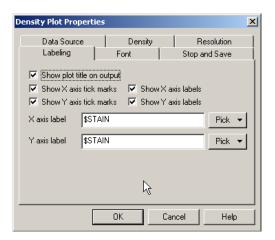


Density Plot Labeling

Use the options here to override the default plot display options for the current plot. This is useful when capturing images for publication.

Show Plot Title on Output Show Axis Tick Marks Show Axis Labels X and Y Axis Labels

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

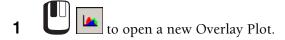


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Overlay Histogram Plots

Open Overlay Plot

The Overlay Plot (CXP Analysis only) displays and compares multiple histograms. The Overlay Toolbar becomes active once an Overlay Plot is created or selected.



Data can be retrieved from Listmode Files, from stored Histogram Files or dragged in using the Drag and Drop feature from histograms open in the Workspace. To include data from a Listmode File, from the overlay toolbar. To include data from a



See also, Adding a Histogram to the Overlay Plot

Overlay Plot Mode

The Overlay Plot has three possible views by selecting the appropriate icon from the Overlay Toolbar as follows:







The plots are automatically shown in Gallery Style. This is the default, which may be changed using Workspace Preferences - Plot Display and Default Overlay Mode.

The Overlay plot - Gallery Mode, allows the overlay of a control sample on all plots and subsequently allows histogram subtraction and the calculation of Kolmogorov Smirnov Statistics and Overton Statistics.

Adding a Histogram to the Overlay Plot

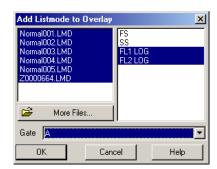
A histogram may be added to the overlay plot by either opening a stored histogram file, or inserting a histogram derived from a listmode file or by dragging a histogram plot from the workspace to the overlay plot.

To Add plots from a stored listmode file:

1 Click the Insert Histogram button, or



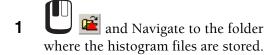
Files already in use are displayed on the left hand side, additional files can be added to the list using the **More Files** browse button.



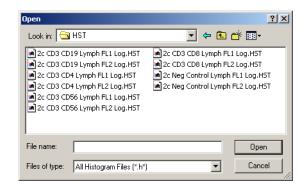
- **2** Highlight the required files parameters common to all selected files are displayed in a list on the right hand side of the dialog box.
- **3** Highlight the required parameters for display.
- 4 the required gate from the list of gates available.
- 5 OK to add these histograms to the Overlay plot. Cancel aborts the operation.

To open a stored histogram file:

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Highlight the file names to use. To select all, highlight the first file and press Shift + End.



3 Open . CXP software now displays all chosen files.

The plots are automatically shown in Gallery Style. This is the default, which may be changed using Workspace Preferences - Plot Display and Default Overlay Mode.

The Plots can be Annotated with relevant notes/comments, see Annotation.

See also, Overlay Plot Mode

Rearranging Overlay Histograms - Plot Sorter

Overlay Histograms can be rearranged within the Gallery View.

Moving an Individual Histogram

To rearrange the order of the histograms:

1 Select Gallery Style using the Gallery button

2 Select the desired histogram by clicking the left mouse button, holding it down and dragging the histogram to its new position.

3	A gray line appears to the left-hand side of the histogram as the plot is dragged.		
4	Using the gray line as a marker, Drag and Drop the highlighted histogram to its new location.		
5	Release the mouse button to complete the move.		
	ving Several Histograms move more than one histogram at a time:		
1	Select Gallery Style using the Gallery button.		
2	Select the desired histograms by holding down and, using the left mouse button, on each histogram until all relevant histograms are highlighted. Note: Ctf +A is a quick way of selecting all the plots.		
3	Still holding down Ctr), hold down the left mouse button and drag all the histograms to their new positions. A gray line appears to the left-hand side of the first chosen histogram.		
4	Using the gray line as a marker, drag the highlighted histograms to the new location.		
5	Release Ctrl and the mouse button to complete the move.		

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See also, Arrange

Deleting Overlay Histograms

1 Highlight the unwanted histogram with the left mouse button.

If more than one histogram is required, hold down (cm) and the required histograms with the left mouse button.

Note: [Ctr]+[A] is a quick way of selecting all the plots.

2 Press Delete or click the right mouse button and select **Cut**.

Selecting a Single Overlay Histogram Plot

- 1 Within the Overlay plot, on a histogram to select it.
- 2 Once a single Histogram Plot within the Overlay Plot Window has been selected, click the right mouse button and a menu containing the following options appears:

Cut

Copy

Paste

Set As Control

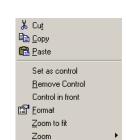
Remove Control

Control In Front

Format

Zoom to Fit

Zoom



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Cut

The Cut option takes a copy of the selected histogram and removes the original. This too can be pasted back into CXP software or into a spreadsheet program. This moves histograms to another position or to the Windows clipboard. It remains on the clipboard until other text, data or pictures are copied.

Copy

Copy places the text, data or picture onto the clipboard. The original remains in the application.

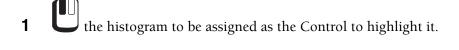
Paste

The Paste option is context sensitive. Pasting inside the overlay plot pastes histograms from the Windows clipboard at the end of the current histogram set.

You can paste text, data or a picture that were previously cut or copied to the clipboard using shortcut buttons, the menu, or the keyboard.

Set As Control

This option allows the comparison of a control sample with a test.



- 2 Click the right mouse button and Set as Control
- **3** This places the Control histogram onto all the other histograms featured on the overlay plot, allowing for comparison. The Control histogram is outlined in blue.
- **4** Only one histogram can be selected as the Control. If a you select a different histogram as the control, the previous Control histogram is deselected as the Control.

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Remove Control

This option removes a previously-set control.

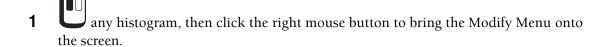
1 Select any histogram by clicking the left mouse button. Click the right mouse button and



Remove Control and the blue outline is removed.

Control In Front

If the histogram is filled with color, rather than a single line, the histogram can be set out so that the Control plot is in front of the other test, enabling easier comparison.





3 The action can be reversed by clicking on the menu and deselecting the **Control in Front** option again.

Format

To customize the appearance of histograms within the Overlay, use the Format, Scaling or General options within the Format Histogram dialog box.

Zoom to Fit

The **Zoom to Fit** option maximizes the selected histogram within the plot window allowing for easier viewing.

1 the required histogram, then click the right mouse button to get the Modify Menu.



3 To restore the histogram to its previous size, click the right mouse button and from the menu.



4 the 100% option, which brings the histogram back to its original size.

Zoom

The **View → Zoom** option allows you to zoom in on a FlowPAGE, or Overlay Plot to 100%, 200% or 400%.

Select the desired view. Selecting 100% returns to the default size.

Selecting Multiple Overlay Histogram Plots

1 Press and those histograms required. Click outside to histograms area to deselect.

Note: You can select all the Plots by pressing Ctrl+A.

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When multiple histograms are selected, within the Overlay Plot Window, click the right mouse button and a menu containing the following options appears.



Cut

Copy

Paste

Format

Zoom to Fit

Zoom

Where NO Histogram Plots within the Overlay Plot Window have been selected, and the Overlay Plot Window is in focus, click the right mouse button to view a menu containing the following options:

Arrange Plots Histogram Mode Overton Mode Kolmogorov Smirnov Mode Zoom Remove Control



Arrange Plots

Allows plots to be automatically arranged within the overlay plot. See Arrange for more details.

Histogram Mode

This is the default mode and displays the histograms in the order in which they were read.

This is the area in which most of the modifications to histogram formatting is undertaken, for example, adding statistics and annotations.

Overton Mode

This option subtracts a control histogram from the test histogram according to the algorithm defined by Roy Overton.

In order to activate the Overton Mode, you must first set a control by selecting the control histogram on the Overlay plot, click the right mouse button and select the **Set as Control** option. To activate Overton mode, verify that no histograms are selected, then click the right mouse button in the Overlay plot and select **Overton** mode.

Results are displayed in the statistics pane also the Overton Percentage can be selected using the FCS Keyword Annotation Mode or Shift + . This gives the percentage of cells that are calculated to be positive.

The Overton subtraction value can be displayed by selecting the Overlay Statistics button.



For additional information, see Modified Histogram Subtraction Technique for Analysis of Flow Cytometry Data; W Roy Overton; *Cytometry* 9:619-626 (1988).

Kolmogorov Smirnov Mode

This allows the statistical comparison of two tests to determine the probability of the two plots being different.

In order to activate the **Kolmogorov Smirnov** mode, you must first set a control by selecting the control histogram on the Overlay plot, click the right mouse button and select the **Set as Control** option. To activate **Kolmogorov Smirnov** mode, verify that no histograms are selected, then click the right mouse button in the Overlay plot and select **Kolmogorov Smirnov** mode.

The Kolmogorov Smirnov test D Value and probability value can be displayed by selecting the

Overlay Statistics button.

If an annotation is placed on the plot while in **Kolmogorov Smirnov** mode, and the FCS Keyword Annotation Mode is entered Shift/\$, the **Chi Squared** option can be selected giving the Chi Squared value for the plot compared to the control.

See also:

Overlay Plot Mode

Rearranging Overlay Histograms - Plot Sorter

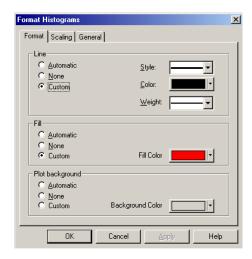
Overlay Histogram Plot (Format Tab)

Use the Format option to customize the line style, color, weight, background color and the fill color of the histograms.

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Line Fill

Plot Background



Line

Automatic Automatically assigns a color to the line.

None Makes the line around the histogram data points transparent.

Custom Selects the line style, color and weight.

Style Changes the line from a solid to a broken line.

Color Selects a line color or define a new line color.

Weight Changes the width of the line.

Fill

Automatic Automatically assigns a color to fill the histogram.

None Makes the area under the histogram line transparent.

Custom Selects a fill color or define a new fill color.

Plot Background

Automatic Automatically assigns a color to the background.

None Makes the background transparent.

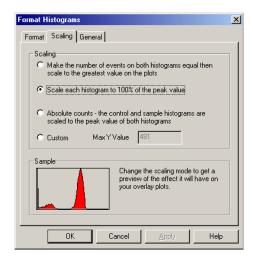
Custom Allows a user selectable background color for each histogram.

Overlay Histogram Plot (Scaling Tab)

When a control is overlaid on a test, select one of the options to change the scaling.

Make the Number of Events.... Scale Each Histogram.... Absolute Counts Custom

The Sample graph gives a preview of the effect a particular scale mode has on the plots.



Make the Number of Events....

This option normalizes the total number of events on both histograms and then scales both to the greatest value on the plots. Use this option for control and test files that have different numbers of events.

Note: If the control histogram has more events than the test histogram the control histogram is not normalized.

Scale Each Histogram....

This option scales each histogram to 100% of its peak value.

Absolute Counts

This option scales both test and overlaid control histograms to the greatest value found on either histogram.

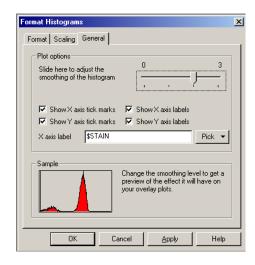
Custom

Use this option to enter the Max Y Value required. This scales both the test and the overlaid control to the same value.

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Overlay Histogram Plot (General Tab)

Plot Options Axis And Tick Mark Labels X Axis Labels



Plot Options

The slider bar allows adjustment of the smoothing level. Selecting <0> shows the data at full resolution. Levels of smoothing can be applied by sliding the bar to the right up to a maximum of <3>.

Axis And Tick Mark Labels

Checkboxes enable you to hide or show X/Y axis tick marks and X/Y axis labels. Select the desired options for display.

X Axis Labels

FCS Keywords can be selected using either the **Pick** drop down list option, which gives **Parameter**, **Stain** and **File Name**, or a user definable keyword may be used. Alternatively, free text may be entered.

To apply the changes made on the Format, Scaling, and General tabs, click the **Apply** button featured on the bottom of each tab.

Clicking OK applies the changes, and removes the Format Histograms box.

Cancel removes the box without applying the changes onto the required histograms.

All changes made in this option can be undone by selecting the **Edit >> Undo** menu.

Arrange

This allows the Overlay Histograms to be arranged in a particular order and displays four alternative ways of arranging histograms.

By file no By deviation By stain By parameter

Call up the Context Menu by clicking the right mouse button anywhere on the plot No histogram selected.

Select the Arrange option, which gives the choice of the following:

By File Number Arranges the histograms in order of file number.

By Deviation This orders histograms according to the difference between the test and the

control on a mean channel basis, one histogram must first be "set as a Control"

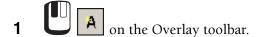
for this option to have an effect.

By Stain Arranges the histograms according to the user-defined stain parameter name.

By Parameter This arranges the histograms according to the original parameter name.

Annotations - Introduction

This section describes how to place an annotation onto an overlay plot.



- **2** Click the part of the histogram where the annotation is to be placed so that it is highlighted.
- **3** Type in the text.
- **4** Click outside the annotation area to fix the annotation into position.

Once an annotation is in place, it can be modified as required.

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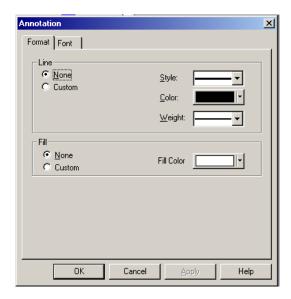
Format Annotations

See also the Font Annotations Tab.

- first on the annotation to select it, click the right mouse button once on the selected annotation to display the following menu.
- 2 Use **Cut**, **Copy** and **Paste** to move/remove/copy annotations.

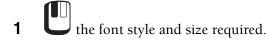


3 Use Format Annotation to select a **Fill Color** and the **Style**, **Color** and **Weight** of the line around the the annotation.

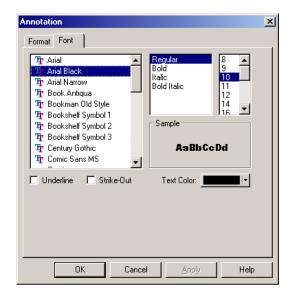


Font Annotations

The Font dialog box allows you to alter the style, size and color of the font.



- A preview of how the new font looks is shown in the **Sample** box contained within the Font dialog box.
- There is a checkbox to enable you to underline the text. This can be undone by unchecking the box. This also applies to the **Strike-Out** box. The text color can also be changed by clicking on the **Text Color** box, which reveals a drop down box. Select the color required.



- 4 Once modifications are complete, and the changes are transferred onto the histogram.
- **5** These changes can be undone through **Edit → Undo** on the toolbar, and the changes are undone in reverse order.

FCS Keyword Annotation Mode

The FCS Keyword Annotation Mode allows FCS Keywords contained in the file header to be placed within a histogram.

1 one histogram within the Overlay Plot.

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3 the histogram in the required area.

4 Press Shift + \$\\$ to display the FCS Keywords.



5 Double click on the desired field.

6 the plot to set the annotation on to the histogram.

Copying an Annotation onto the Other Plots

1 an annotation that is already on one of the histograms.

2 Press Ctrl+C to copy.

3	Press Ctrl+A to select all or Ctrl+ click each required plot.
4	Press Ctr)+V to paste onto all the other histograms (or use the right mouse button and the Paste option.)
5	Click outside the annotation to fix the annotations into position. Note: When copying the annotations onto all plots, it is best to Cut from the original annotation rather than Copy it, so that another annotation does not display over the original one.
Dra	e annotations can be moved within the histogram by clicking on them once and then agging and Dropping to a new position. Sting Data from Overlay Plots into a Spreadsheet
	ecting Paste in a spreadsheet program pastes the plot data into a column within the rksheet. The parameter name is pasted in the first cell.
То	transfer data from the CXP software to a spreadsheet:
1	Select the required plot.
2	Click the right mouse button and the Cut or Copy option.
3	Go into the spreadsheet program and Edit → Paste from the toolbar.

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4 This transfers the data from the plot.

5 Multiple plots can be copied in that manner by selecting the required plots (hold down



An alternative option is to **Tools** >> **Publish Histogram Data Now** when the Overlay plot is in focus. This directly exports all plot data to an Excel spreadsheet and performs statistical analysis of the selected files.

See also:

Publish Histogram Data Now

Tomogram Plot

The Tomogram Plot option (CXP Analysis Software only) provides three-parameter data display and any combination of parameters can be selected. Each colored area in a Tomogram Plot represents an ISO-count of the number of events (cells or particles). The color shows the number of events at any X and Y parameter value and the third parameter being Z.

Tomogram Plots use colors to show the relative frequency count of events on the display at any position by using the **Density Style View**.

Alternatively, a **Gate Precedence View** can be chosen. Both these views are set, by clicking the right mouse button and selecting either the **Density Style View** or the **Gate Precedence View** from the menu box displayed.

See the Note at the end of the INSTALLING CXP SOFTWARE procedure about graphic card drivers for the correct display of Tomogram Plots.

To create a Tomogram Plot:

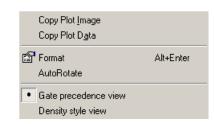


2 From the Data Source dialog box

displayed, the required File, then select desired Gates, and Parameters for display.

AutoRotate

Gate Precedence View/Density Style View



3 When a Plot is displayed, click the right mouse button to display further menu options for which context sensitive Help topics are available.

AutoRotate

Allows the Plot image to automatically rotate and is only available for Tomogram and Surface Plots.

Gate Precedence View/Density Style View

This option is used to set the **Density Style View** or the **Gate Precedence View**, which are mutually exclusive. See Modify Color Precedence for further information.

See also:

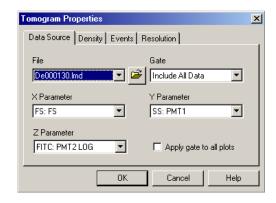
Tomogram Plot Data Source Tomogram Plot Density Tomogram Events Tomogram Resolution Format Plot

Tomogram Plot Data Source

Choose the X, Y and Z parameters for the current plot. Gating equation and regions to be displayed can be selected.

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File
Browse Button
Gate Selection
Select X Parameter
Select Y Parameter
Select Z Parameters
Apply Gate to All Plots



File

Select the required file from the files already in memory, displayed in the drop down box or click the Browse button to explore your directories for the required file.

Browse Button

Allows you to peruse other directory options and select files as appropriate.

Select X Parameter

Select the **X Parameter** from the choice displayed in the drop down box and click the mouse on the Parameter required.

Select Y Parameter

Select the **Y Parameter** from the choice displayed in the drop down box and click the mouse on the Parameter required.

Select Z Parameters

Select the **Z Parameter** from the choice displayed in the drop down box and click the mouse on the Parameter required.

Gate

Available Gates are listed in Precedence order, highest precedence being at the top. To rearrange the precedence order – click to highlight the Gate name ONLY then drag the Gate to the required position.

Gates required for Advance Precedence should be selected at this point.

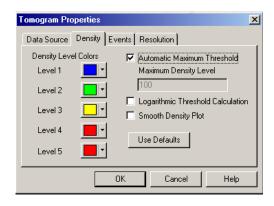
Apply Gate to All Plots

To select this option, enable the checkbox and apply the gate selected to all plots.

Tomogram Plot Density

Use this option to specify the Density levels and other configuration preferences.

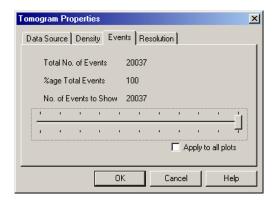
Density Level Colors Automatic Maximum Threshold Maximum Density Level Logarithmic Threshold Calculation Smooth Density Plot



Tomogram Events

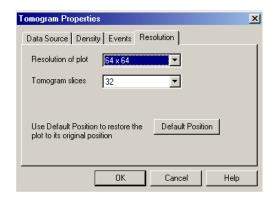
Use this to specify the percentage of events to view. This option significantly increases the speed at which plots refresh if large data files are being analyzed. For an event interval less than 100%, data is displayed uniformly from within the whole of the data file, that is, if 10% of events are selected then event numbers 1, 11, 21, 31, and so on, are displayed if they meet the Gating criteria for the plot.

No. of Events to Show Apply to All Plots



Tomogram Resolution

Resolution of Plot Tomogram Slices



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Resolution of Plot

Set the number of channels for the X and Y Axis of the Plot. The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

Click on the drop down box and select the required resolution.

Tomogram Slices

The Z Axis resolution is the number of **Tomogram slices** that are chosen for the third parameter.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.

Click on the drop down box and select the resolution factor required.

Surface Plot

The Surface Plot option (CXP Analysis only) provides two-parameter isometric display and any combination of parameters can be selected. Each colored area in a Surface Plot represents an ISO-count of the number of events (cells or particles). The color shows the number of events at any X and Y parameter value.

Alternatively, a Gate Precedence View can be chosen. The view is selected using the click the right mouse button and selecting either the Density Style View or the Gate Precedence View from the menu displayed.

To create a Surface Plot:



2 From the Data Source dialog box displayed, select the required File, then select desired Gates and Parameters for display.

3 When a Plot is displayed, click the right mouse button to display further menu options.



See also:

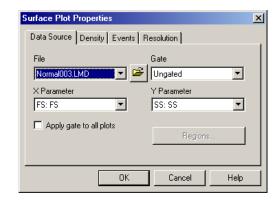
Surface Plot Data Source Surface Plot Density Surface Plot Events Surface Plot Resolution Format Plot

Surface Plot Data Source

Choose the X and Y parameters and the gating equation for the current plot.

File
Browse Button
Gate Selection
X and Y Parameter
Apply Gate to All Plots

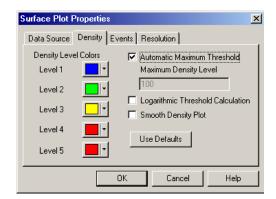
Note: The Region button is grayed out for the Surface Plot.



Surface Plot Density

Use this option to specify the Density levels and other configuration preferences.

Density Level Colors Automatic Maximum Threshold Maximum Density Level Logarithmic Threshold Calculation Smooth Density Plot

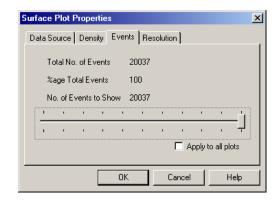


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Surface Plot Events

Use this to specify the percentage of events to view. This option significantly increases the speed at which surface plots refresh if large data files are being analyzed. For an event interval less than 100%, data is displayed uniformly from within the whole of the data file, that is, if 10% of events are selected then event numbers 1, 11, 21, 31, and so on, are displayed if they meet the Gating criteria for the plot.

No. of Events to Show Apply to All Plots

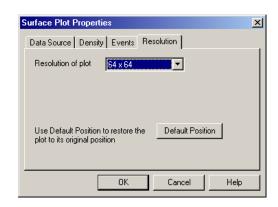


Surface Plot Resolution

Set the number of channels for the X and Y Axis of the Plot.

The available options allow for 64, 128, 256 and 512 channels to be displayed on the axis.

A higher resolution gives an improved graphic image whereas a lower resolution allows faster redrawing of that image.



Prism Plot

A Prism Plot is a way of displaying all the subpopulations or phenotype percentages of a multi-color immunocytometry analysis protocol.

The Plot represents the relative percentages of each subpopulation (phenotype) and the number of phenotypes depends on the number of color parameters being simultaneously analyzed. Prism Plots can be customized, for example: Gate for Prism, Scaling, and so on.

To create a Prism plot:



2 An empty Prism plot window is displayed with the Data Source dialog box overlaying it.

3 Make the desired changes.

Data Source (Prism) Format Plot

The plot type with the current Gate is displayed in the plot window. Ungated is the default.

Data Source (Prism)

Choose the File and Gate to be used for the Prism plot.

File
Browse Button
Gate Selection
Apply Gate to All Plots

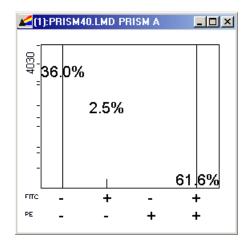


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What is Prism?

The Prism parameter is a method of summarizing multi-parameter surface marker data as a one-dimensional Prism plot representing all possible subpopulations or phenotypes.

The Prism plot is a simple bar graph. The height of each bar represents the count of each phenotype. The count as a percentage of all events or gated events is written adjacent or above each bar. The prism plot can be gated, in which case the % values represent the percentage of gated prism events and the gate is indicated in the title bar.

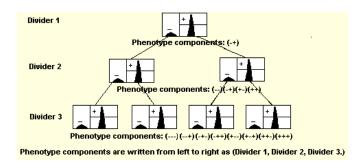


How is Prism Created?

In single parameter analysis of surface marker data, one or two regions are normally set to measure both Negative and Positive cells. We can then look at a second parameter and calculate the 2 phenotypes from parameter-1 positive populations and the two phenotypes from parameter-1 negative populations (four phenotypes in total).

We can repeat this with 3, 4 or more parameters.

The Prism Divider can be used instead to mark the dividing line between the Negative and Positive cells.



Prism Dividers

A Prism is created by setting one or more prism dividers. There are three ways of setting prism dividers, two of them involve graphical positioning (single and dual prism dividers). The third uses **Analysis** >> **View/Modify Prism** that can set the divider numerically on any divider. Any changes to the divider on one plot is replicated on any other plot using that parameter.

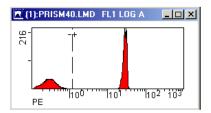
Each new parameter that has a prism divider set on it multiplies the total number of phenotypes by 2.

Only one divider can be set per parameter.

Setting a Single-Parameter Prism Divider

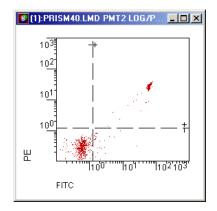
Single parameter prism dividers are set by placing a Single-parameter histogram in focus and selecting

• The divider can then be set by clicking on the selected plot, positioning the divider, and then clicking once more to fix the divider position.



Dual Parameter Prism

Dual parameter prism dividers are set by placing a Dual-parameter plot in focus and selecting . This simultaneously creates two prism dividers.



The combinations of dividers are combined to produce 2^p regions
Where p is the number of prism dividers set.

This table shows the number of phenotypes created for up to six Prism dividers set.

Number of Prism Dividers set	Number of phenotypes created		
1	2		
2	4		
3	8		
4	16		
5	32		
6	64		

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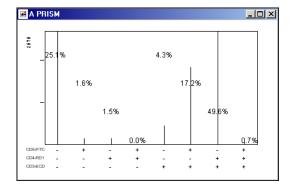
This Plot shows a 3 color Prism Plot (3 dividers set, 8 phenotypes created). The percentages printed on the prism plot from left to right match one-to-one with the phenotype nomenclature indicated on the axes.

Example:

Triple Positive phenotype (+ + +) is 0.7%.

Triple Negative phenotype (- - -) is 25.1%

The phenotype percentages are staggered to prevent excessive obscuration of the numerical values when a high density of prism information is presented.



Editing Prism Dividers

- **1** Mouse over the divider to get the \leftrightarrow cursor.
- 2 and drag the divider to the new position.

Removing Prism Dividers

- 1 the Prism divider you want to delete.
- 2 Press Delete .

CXP Software - Prism Compatibility

The CXP software Prism plot can interpret the Prism parameter derived from:

- RXP/CXP data acquisition
- EXPO[™] data acquisition
- EXPO 32 data acquisition
- Elite or ALTRA data acquisition or
- COULTER Elite version 4.x DOS software acquisition.

CXP software cannot display Prism using listmode files saved from COULTER XL SYSTEM II^{TM} software however, CXP software can generate Prism plots from XL SYSTEM II files.

Note: Reanalysis of listmode files overrides the analysis protocol's Prism dividers. You have to adjust the dividers when this occurs.

Third party software may not display Prism data correctly since a special Prism data display algorithm is involved to display Prism in CXP software.

Legend

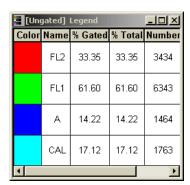
Use this option to view the population colors and population names,

1 Plots >> Legend Plot to create a legend plot.

When a Plot is displayed, click the right mouse button within the plot to display further menu options.

2 Every time the population names or colors are edited CXP software updates the Legend Window.

If CXP software is in Dot Plot Mode-Color Blend mode, Legend displays the names, colors and associated statistics of the color Gate combinations.



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3 If CXP software is in Dot Plot Mode-Color Precedence mode, Legend displays the names, colors and associated statistics of the Gates with the highest precedence at the top. Note: When analyzing multiple files using Color Precedence, the Legend plot shows all gates from all plots, regardless of precedence and file. To change this so that the Legend shows only those gates and plots associated with a given file, use the Advanced Precedence tab on the Color Precedence edit dialog.

See also:

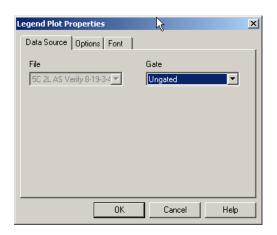
Data Source (Legend)
Legend - Options Tab
Color Blend Mode - Introduction
Modify Color Precedence
Advanced Precedence

Data Source (Legend)

Choose the File and Gate to be used for the Legend plot.

File Browse Button Gate

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



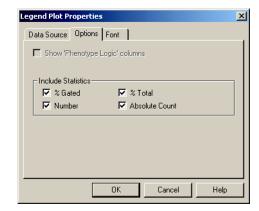
See also: Legend Data Source (Legend) Legend - Options Tab Format Plot

Legend - Options Tab

The Options tab displays various checkboxes that may be enabled as appropriate.

Show "Phenotype Logic" Columns % Gated % Total Number Absolute Count

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



See also: Legend Data Source (Legend) Format Plot

Show "Phenotype Logic" Columns

Displays the logical combination of Color Gates for each population. Available in Color Blend Mode only.

% Gated

Total number of events in region, as a percentage of events in a gated display.

% Total

Total number of events in region as a percentage of total events in the file.

Number

Total number of events in a Region.

Absolute Count

This displays the absolute count of a population based on the calibration factor (CAL Factor) and the number of particles within a defined CAL region.

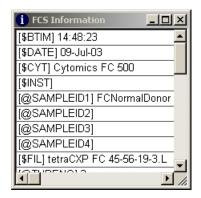
Info Plot

Set up the Info plot to include instrument serial number and User ID information on statistical printouts. Use the Info Plot FCS tab to select the FCS Keywords for the information you want on your statistical printouts.

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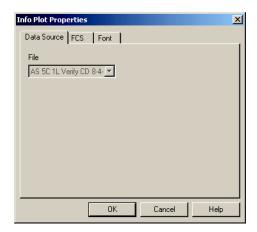


Plots → **FCS Information** to create an Info plot.



Info Plot Data Source

Displays the name of the file that is the data source for the Info Plot.

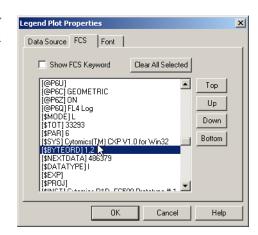


Info Plot - FCS Tab

Show FCS Keyword – Enable this checkbox to display the FCS Keyword as well as the associated keyword value.

Multiple Keywords can be selected from the scroll box.

Select Clear All Selected to deselect the items.



Acquisition Stop and Save

Use Acquisition Stop and Save to terminate the acquisition of a sample at a preset stop value. If there is more than one stop value set, then the first stop counter reached terminates the current acquisition.

Note: The values set in the Cytometer Control Acquisition Limits (duration and Max events) overrides the stop values set here in the event of an acquisition limit being reached before the Acquisition Stop value has been achieved.

Single Parameter Histograms can be selected for automatic saving in FCS Format.

Note: An acquisition from lysed whole blood is to be stopped on 5,000 lymphocytes to allow the collection and storage of all event data.

In normal blood, where lymphocytes account for about 30% of the white cell count, we have no problem with the above setup. We can predict with a normal sample that the total count from a 5,000 cell lymphocyte count is around $5,000 \times 100/30$ or 16,666 events total.

If the sample is from a lymphopenic patient, this could be a problem. A 5,000 lymphocyte count may not be achieved before the sample tube is empty or RAM memory is full.

It is normal under these circumstances to set two counters, one for the stop count and one for the maximum permissible count, that is, the maximum total event count. If a stop count is used, it is recommended that the stop count be set to a large number, for example, 100,000 events. Thus, with a lymphopenic patient, if the lymphocytes are less than 5% of the white cell count, an acquisition would terminate on the total event count of 100,000 before a 5,000 cell lymphocyte count could be achieved.

Setting a Stop Counter

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

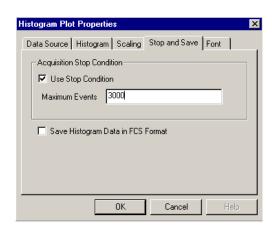
1 Highlight the plot on which you wish to set a stop counter.

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Plots → Acquisition Save and Stops command to view the Acquisition Plot Set-up dialog box.

Use Stop Condition
Maximum Events
Save Histogram Data in FCS Format

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



To enable an FCS Histogram to be saved from this plot, check the **Save Histogram Data in FCS Format** checkbox.

Note: The actual histogram save action is only performed if the Save Histogram checkbox is checked in Workspace Preferences - Acquisition Options.

See also:

Color Dot Plot Histogram Plot Density Plot Format Plot Acquisition Stop and Save

Use Stop Condition

Check this checkbox if the stop counter is to be activated.

Maximum Events

Set the maximum number of events you wish to be recorded.

Save Histogram Data in FCS Format

Check this checkbox to save the Histogram Data in FCS Format.

USING CXP SOFTWARE *PLOTS MENU*

Format Plot

To display the Plot Properties dialog box, click the right mouse button on the plot and



Format options differ depending on the Plot type. Select a formatting option from the table below to view detailed information about the format options available for the different types of plots.

Plot Type	Plot Name	Available Formatting Options					
Single Parameter	Histogram Plot	Data Source	Histogram	Scaling	Labeling	Font	Stop & Save*
Dual Parameter	Dot Plot	Data Source	Events	Resolution	Labeling	Font	Stop & Save*
	Contour Plot**	Data Source	Contour	Resolution	Labeling	Font	
	Density Plot	Data Source	Density	Resolution	Labeling	Font	Stop & Save*
Three Parameter	Tomogram Plot**	Data Source	Density	Events	Resolution	Font	
	Surface Plot**	Data Source	Density	Events	Resolution	Font	
Special Types	Overlay Plot**	Format	Scaling	General			
	Legend Plot	Data Source	Options	Font			
	Prism Plot	Data Source	Font				
	Info Plot	Data Source	FCS	Font			

^{*=}CXP Cytometer only

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^{**=}CXP Analysis only

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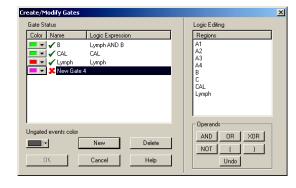
Create / Modify Gates

Use the Gating function to define populations of interest either for plot display or for sorting.

1



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



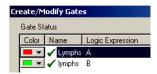
2



Gate and Logical Expression Gate Color New Delete Operands

- **3** New Gate 4 is highlighted so you can modify the name if desired.
- **4** Create new logical expressions.

Note: Gate names are case sensitive so it is possible to have 2 gates with the same name. When editing gate names, ensure that you do not use duplicate names.



See also:

Gate Selection Gate Color Boolean Gating

Gate and Logical Expression

Shows the Gate name and the logical combination of Gates.

New

Allows you to create a new Gate with a new combination of Regions.

Delete

The **Delete** button erases any selected gates highlighted in the Create/Modify Gates dialog box.

Operands

The Operands function allows you to insert a combination of regions into the gating equation using the Operands buttons. Once a Region has been entered next to the new file name, the Operands have to be utilized to enable a new combination of cells to be viewed. The Operands work according to Boolean Gating.

Gate Selection

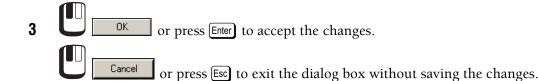
The Gate option can be used once Gates have been created.

To apply a Gate to a plot:

1 Click the right mouse button on the plot and



2 the Gate you wish to apply from the **Gate** drop down list box.



Gate Color

When a region is drawn on a plot, the first region is automatically shown as red, meaning events inside this gate are colored red when viewed on a color dot plot. The second is in green, and the third in blue.

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These colors can be altered through the Color function if more or different colors are required.

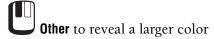
the down arrow next to the Color Well for the selected Gate or select the Ungated events color down arrow to change the color for Ungated events.

the required color.

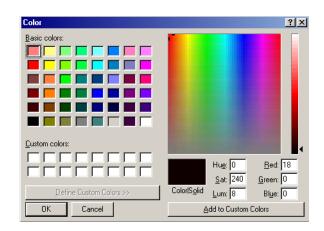
This automatically changes the events within the gate to this color.



3 If further colors are required,



palette. a defined color or drag the cursor around on the color board to select the color of choice.



4 When the appropriate color has been found, you can click the Add to Custom Colors buton

for easy use at a later date or you can to add to a plot.

The same principles can also be applied to the Ungated Events Color option.

Boolean Gating

CXP software implements Boolean gating. Up to eight regions can be combined to form a gate. A gate is used to select a specific subpopulation of data.

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CXP software uses a combination of regions and operators to achieve the Boolean gating process. The Operands are utilized in order to create a combination of regions.

Gate Logic

The shaded areas show the following logical combinations of regions.

AND

Events which are in both regions A AND region B. This is written as A AND B.



OR

Events that are in region A OR in region B. This is written as A OR B.



XOR

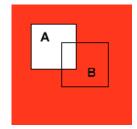
Events, that are EXCLUSIVELY in region A OR in region B, but NOT in both. This is written as A XOR B.



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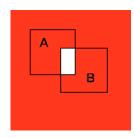
NOT

Events that are NOT in region A. This is written as NOT (A).



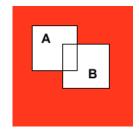
NOT

Events which are NOT in region A AND in region B. This is written as NOT (A AND B).



NOT

Events, which are NOT in region A OR in region B. This is written as NOT (A OR B).



PARENTHESES

These are used to define a precise logical statement. An open parentheses must be placed after a NOT operator even if the operand is only a single value.

Example: Lymphs AND NOT (CD8).

Color Blend Mode - Introduction

CXP software allows the use of combinations of Gates to determine the color of dots in a dot plot. The key concept behind this process is similar to color printing and depends upon the

use of colored combinations of Gates to define a unique subpopulation. Each Gate (up to three) is given a color and the logical gate combinations give rise to populations with blended colors.

Consider three gates: Gate A, set on FL1 positive cells; Gate B, set on FL2 positive cells; Gate C, set on FL3 positive cells. Any event may be in either none, one, two or all three of these Gates and therefore, combinations of the gates may be used to define eight different subpopulations (phenotypes) of events as follows:

Each of these subpopulations may be represented by a different color. Thus up to eight differently colored subpopulations may be visualized on a Color Gated Dot Plot, and each group can be given a user defined name.

Default Name	Primary A (FL1)	Secondary B (FL2)	Tertiary C (FL3)
Group 0	-	-	-
Group 1	+	-	-
Group 2	-	+	-
Group 3	+	+	-
Group 4	-	-	+
Group 5	+	-	+
Group 6	-	+	+
Group 7	+	+	+

Where: +ve data is inside a Gate, and -ve data is outside a Gate

When in color blend mode ONLY color statistics from the blended colors are displayed in the Legend plot. Use to view Region statistics.

See also:

Modify Color Blend

Modify Color Blend

To use Modify Color Blend:

1 Analysis -- Modify Color Blend to view the Modify Color Blend dialog box.

the Gate you wish to modify.

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3 Make the desired changes.

Gate Names

Blend Color

Enable

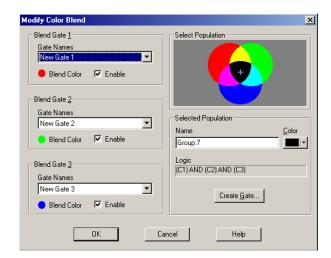
Name

Logic

Color

Create Gate

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



4 OK or press Enter to accept the changes.

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

See also:

Create / Modify Gates Workspace Preferences - Gating

Gate Names

Allows the selection of a Gate that defines a population of interest.

Enable

Enables the selection of one, two or all three blend Gates.

Blend Color

Displays the color to be associated with the selected Gate logic.

Name

Allows a user-defined name for the selected combination.

Color

Allows you to modify the predefined colors.

Logic

Displays the full coloring Gate logic for the selected population.

Create Gate

Creates a logical Gate based on the coloring logic. A Create Gate screen is displayed showing the Gate Logic to be applied. Press **Yes** to Create the Gate or **No** to cancel.

Modify Color Precedence

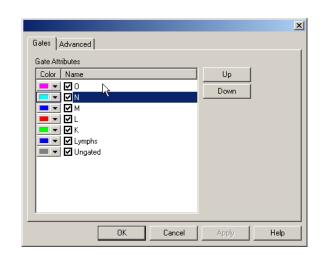
When a subpopulation is very small and a larger blended color would swamp a smaller population, Color Precedence can be used instead of Color Blending. In Color Precedence Mode, the subpopulation (or color) given the highest precedence overrules any color with lower precedence when the same event satisfies more than one color gate, or, when two or more events are to be displayed at the same location.

1 Analysis >> Modify Color Precedence to view the Color Precedence – Gates tab dialog box.

the required gate so as to highlight the gate name ONLY.

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

3 Use Up and Down to move Gates into the required order of precedence. Those with the highest precedence are at the top of the list.



See also:

Workspace Preferences - Gating Advanced Precedence

Advanced Precedence

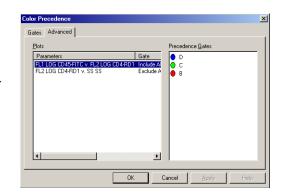
Note: You cannot make any changes on this screen when running a locked protocol.

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Advanced Precedence allows you to assign particular Gate colors to specific plots.

This is of particular interest if different plots are gated on completely different criteria and the color from one gate, therefore, has no relevance to another plot or file.

Advanced Precedence is useful when performing multifile analysis and you do not want a gate from one file to be represented as a color in another file.



Regions Introduction

A Region identifies a specific population of cells.

Up to 256 regions (total) can be set on any combination of Parameters, 32 of these are usable as Gates.

Regions are drawn on a Single Parameter Histogram by setting the position of an upper and lower boundary (Region markers).

Regions are drawn on a 2-Dimensional Dot Plot by enclosing a subpopulation of events with rectangles, polygons (nonrectangular Regions) or using quadrant markers (four rectangular Regions, set with only one point).

To speed up the analysis of data, when a Single Parameter Histogram Region or a Rectangular or Polygonal Region is created, it is also assigned to a Gate. This assignment can be accepted or edited later. The automatic assignment of Regions to Gate can be switched on or off if desired, using **Gates** > Automatic Gate Creation.

Region Properties



angle on a region to make it active and then click the right mouse button on it to display the

Region Properties
Region Properties dialog box.
Re-Calculate Autogate

The **Re-Calculate AutoGate** option allows a return to AutoGating after manual manipulation of an Auto region.

Polygonal Region

The Polygonal Region allows you to draw a free-form Region with up to 100 points and can include horizontal acute angles on a dual parameter plot. To draw a Polygonal Region in a dual parameter display see, Create Polygonal Regions. in the Instructions For Use manual.

Polygonal Region Properties

Region Name

Prime

Automatic Region Name Positioning

Region statistics exported for QC

OC Product

None

Elliptical

Contour

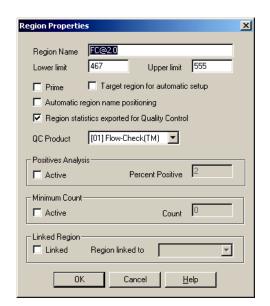
Sensitivity

Travel

Minimum Count

Linked Region

Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.



Region Name

Displays the name of the region. If the name is not grayed, you can rename it.

Prime

When checked, this option allows the system to determine if the peak of the events on the plot is within this region. If the peak is outside this region (due to an air bubble or partial clog), the system performs a Prime cycle. A maximum of three sequential Prime cycles are allowed; if the peak of the plot is still not within this region, the MCL aborts.

Automatic Region Name Positioning

Region labels default to an automatic position. You may move a region label to any desired position within a plot, this user modification automatically unchecks this option. To revert to the automatic position recheck this option.

Region Statistics exported for Quality Control

The region's results are exported to the Quality Control feature of the Report Generator (CXP Acquisition software only).

QC Product

Select the QC product from the drop down list (CXP Acquisition software only). See also, Setting Up QC Products in the Instructions For Use manual.

AutoGating

None

This is the default when the AutoGate option is not selected.

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Elliptical

Toggle the AutoGate to an elliptical region.

Contour

Toggle the AutoGate region to a contour region.

Sensitivity

Eliptical AutoGate settings are 0.11, 0.33 and 1.1 (% of processed peak). Contour AutoGate settings are 1, 2, 3, 4, and 5.

- Level 1 = 0.11% processed peak at a resolution of 64x64.
- Levels 2, 3 and 4 = 0.11, 0.33 and 1.1% at a resolution of 128x128.
- Level 5 = 1.1 % processed peak at a resolution of 256x256.

Selecting 0.11 creates a larger region, whereas selecting 1.1 creates a smaller radius region around the target population.

Travel

Input a maximum value the contour AutoGate region travels from the mean. The Minimum value is 25; Maximum is 250 channels. Use this option to slightly adjust the AutoGate from tube to tube if slight changes in lysis cause the target population to move slightly. This option is not available for elliptical AutoGates.

Minimum Count

Check this option and enter a number in the **Count** field to enable the minimum count function. This overrides an existing stop count to ensure a minimum number of events within a particular region are collected. The actual stop count in this case is approximate (but is always in excess of the minimum count value). This is limited by the Duration and Total Events.

Linked Region

Select a region to be linked to another region and the region tracks the selected region. A region may only link to a region of the same type and parameters. For dual parameter regions, the parameters may be transposed. A rectangular region may link to a quadrant region, but a quadrant region may not link to a rectangular region.

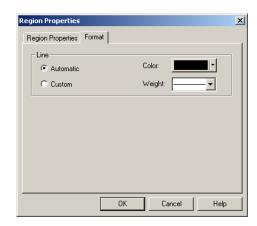
When a region is linked to another, changes to either region's coordinates (by dragging the region) are propagated to both regions. The exception is when the region is an auto analysis region (positives analysis, Elliptical and Contour AutoGate). Then the regions track the auto analysis region and the link is unidirectional.

If a region is linked to another, positives analysis and autogating are disabled for that region.

Polygonal Region Format Tab

Specify the **Color** and line **Weight** for the Polygonal Region boundary.

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



Interactive Polygonal Region Editing

When a Region's position is changed, any Gates and statistics that use the Region are automatically updated and each plot is redrawn to reflect the changes in that Region. When the Region is deleted, any Gate or statistics that used the Region are also automatically deleted. When a Gate has been deleted, any plot that used the deleted gate is reset to Ungated. CXP software allows you to resize an already created Region.

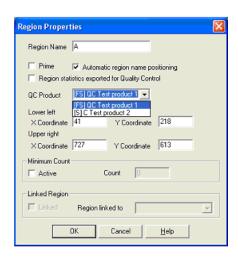
Rectangular Region

This option draws a Rectangular Region on a dual parameter plot. See also: Create Rectangular Regions in the Instructions For Use manual.

Rectangular Region Properties

Region Name
Prime
Automatic Region Name Positioning
Region statistics exported for QC
QC Product
X and Y Coordinate
Minimum Count
Linked Region

Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.



Interactive Rectangular Region Editing

When a Region's position is changed, any Gates and statistics that use the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When the Region is deleted, any Gate or statistics that used the Region are also automatically deleted.

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When a Gate has been deleted, any plot, which used the deleted Gate, is reset to Ungated. CXP software allows resizing of previously created Regions.

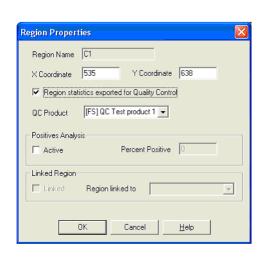
Quadrant Region

Insert a Quadrant Region into a dual parameter plot. All quadrants comprise four sets of Rectangular Regions on a dual parameter plot. The Quadrants are automatically assigned an alphabetical region name and a number for each quadrant. See also: Create Quadrant Regions and Repositioning a Quadrant Region in the Instructions For Use manual.

Quadrant Region Properties

Region Name
X and Y Coordinate
Region statistics exported for QC
QC Product
Positives Analysis
Linked Region

Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.



X and Y Coordinate

Set the region to defined position by entering X and lower Y coordinates for the region.

Interactive Quadrant Region Editing

When a Region's position is changed any Gates and statistics derived from the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When a Region is deleted any Gate or statistics that used the Region are also automatically deleted. When a Gate has been deleted, any plot, which used the deleted Gate, is reset to Ungated. CXP software allows you to reposition an already created Region.

Elliptical AutoGate

Create an Elliptical AutoGate around the selected population on a plot.

See also: Create AutoGate in the Instructions For Use manual.

Contour AutoGate

Create a Contour AutoGate around the selected population on a plot.

See also: Create AutoGate in the Instructions For Use manual.

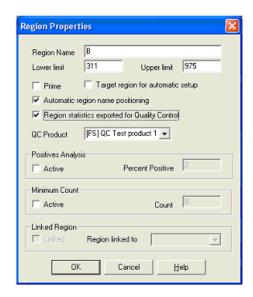
Linear Region

Insert a Linear Region. See also: Create Linear Regions in the Instructions For Use manual.

Linear Region Properties

Region Name
Lower Limit and Upper Limit
Prime
Target Region for Automatic Setup
Automatic Region Name Positioning
Region statistics exported for QC
QC Product
Positives Analysis
Minimum Count
Linked Region

Note: You cannot make any changes on this screen when running a locked protocol other than selecting a QC product or choosing to export QC statistics.



Lower Limit and Upper Limit

Set the region to defined limits by entering upper and lower limits for the region. These limits can be viewed in the statistics box for each plot by selecting **Analysis** >> Select Results and select **Min/Max**.

Target Region for Automatic Setup

Specify the region as the Flow-Set fluorospheres target region for AutoSetup when multiple regions are present on a FS plot.

Positives Analysis

This option moves the region to the selected percent result. Specify a positive percentage value from 0.1 to 99.9. If the positive analysis option is not selected, the percentile is unavailable. Use this option to automatically set the region position for a negative control sample.

Interactive Linear Region Editing

When a Region's position is changed, any Gates that use the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When the Region is deleted any Gate which used the Region is also automatically deleted. When a Gate has been deleted, any plot that used the deleted Gate is reset to Ungated. CXP software allows you to resize an already created Region.

Status Bar (Linear)

The status bar normally gives a brief description of selected menu items.

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When drawing or modifying Regions the status bar divides into three areas of information. These update as a region is modified:

- The status bar shows Channel for X parameter
- The total integral count of the region
- The percentage of events currently within the region.

X Coordinate

The X Axis channel value is the coordinate of the cursor position given by a scaling factor. This scaling factor depends on whether the Histogram parameter is using linear or logarithmic amplification and whether you have calibrated that parameter or not.

If the parameter is linear and uncalibrated, the X channel varies between 0 - 255 or 0 - 1024 depending on the data source. If the parameter is logarithmic and uncalibrated, the value varies between 0.1024 - 1024 and span four decades. For most other manufacturers, the data varies between 1 - 10,000, spanning four decades also.

Integral

When you create a Region the status bar reflects the integral values given along the scale for that parameter at the cursor position. That is, before the first marker is set.

If the cursor is on channel 10, the integral shows the count value of the populations at channel 10.

After first marker is set and as you move the mouse to either the left or right of this point, the integral value continuously updates to reflect the summation of each channel between the Region starting position and the current mouse position.

Multiple Linear Regions

This option sets multiple consecutive linear Regions on one display. See: Create Multiple Linear Regions in the Instructions For Use manual.

Interactive Multi-Linear Region Editing

When a Region's position is changed, any Gates that use the Region are automatically updated and each plot is redrawn to reflect the changes in the Gate. When the Region is deleted any Gate which used the Region is also automatically deleted. When a Gate has been deleted, any plot that used the deleted Gate is reset to Ungated. Allows you to resize an already created Region.

Linked Marker

Use the Linked Marker (Overlay Plot only) to set a Region on all histograms. This shows the percentage of events inside that Region compared to the total number of events on the plot as a whole. This Region can be increased or decreased in size by clicking on either end of the line with the left mouse button, holding it down and dragging it across to the desired point.

To delete the Region, highlight the line (there is a box at each end) and



New Non-Linked Marker

Use the New Non-Linked Marker (Overlay Plot only) to add on an independent Region, showing the percentage of a particular plot. Any number of these can be added to all/some/one of the plots. This Region can be increased or decreased in size by clicking on either end of the line with the left mouse button, holding it down and dragging it across to the desired point.

To delete the Region, highlight the line (there is a box at each end) and



View / Modify Regions

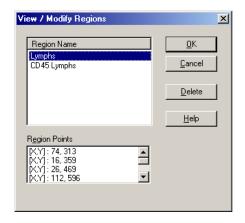
Use this option to view/edit a Region's properties, Region points or to delete a Region that is no longer required. Allows you to copy and paste Regions between protocols.



2 Make the desired changes.

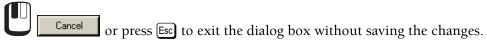
See also:

Region Name Region Points



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See also:

Deleting Regions
Editing and Moving a Region Name
Moving a Region
Resizing a Region

Region Name

Select the Region to view, edit, or delete from the Regions list box. Highlight the Region Name



Region Points

This list box gives details of the Region bounds. This is Upper/Lower channel for single parameter histograms. For rectangles the lower left and upper right vertices are specified and for polygons the X/Y coordinates of all the vertices. To display a Region's points (or bounds), click on the desired Region in the Regions list box.

Resizing a Region

If you want to enlarge or shrink a Region:



The Region is now active and handles appear. Vertices also appear for Contour AutoGate and Polygon Regions

2 Hold the left mouse button down while moving the handle (or moving a vertex as in Contour AutoGate and Plygon Regions).

Moving a Region

To move a Region to another position:

- **1** Mouse over the Region to get the cursor.
- Hold the left mouse button down while moving the Region anywhere in the current window. If the Region has been moved, the statistics automatically recalculate. The Region name moves to the same relative position alongside the Region's new position.

See also:

View / Modify Regions

Editing and Moving a Region Name

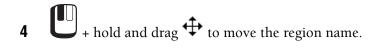
To edit or move the Region name:

- **1** Double click on or near the first letter of the Region name to activate the Region Name dialog box.
- To edit the Region name, type in the desired changes, if any, click



3 To move the Region Name, mouse over the region to get the cursor. If the region name has multiple characters, mouse over the first character to get the cursor.

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Deleting Regions

There are two methods used to delete degions, From Within a Plot and Using the Delete Button in the View / Modify Regions Dialog Box.

Note: If a plot is deleted, its regions are still retained.

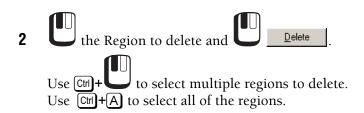
From Within a Plot

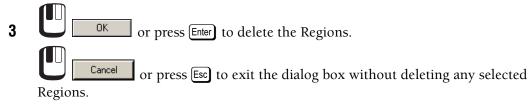
the Region.
The Region is now active and handles appear.

2 Press Delete. The Region is now deleted. Any Gates and statistics that were associated with this Region are also deleted.

Using the Delete Button in the View / Modify Regions Dialog Box

1





When you exit the **View / Modify Regions** dialog box, CXP software updates all the Windows. If a deleted Region was also specified in any statistics or Gates, then CXP software updates to reflect these changes.

Re-Calculate All AutoGates

Use this function to re-calculate multiple autogates in a protocol, rather than re-calculating them individually.

Create Prism Divider

Dual Prism Divider

The **Analysis** >> Create Prism Divider >> Dual Prism Divider menu option allows you simultaneously set two Prism dividers in a "quadrant fashion" on a dual parameter plot. Any displayed Prism Plot is automatically updated with the new Prism statistics. If the number of Prism dividers is changed during the divider creation, the corresponding Prism plot also reflects this by changing its format according to the number of active Prism dividers.

Note: CXP Cytometer software only allows one Prism divider per parameter and the dividers are GLOBAL across the application. As a consequence, modifying the Prism divider of any parameter on any plot automatically updates the divider of that parameter if it is displayed on any other plot. This includes both dual and single parameter plots.

See also:

Setting a New Dual Prism Divider Editing a Dual Prism Divider

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Setting a New Dual Prism Divider

1 Highlight the chosen dual parameter plot (current window).

2 Analysis → Create Prism Divider → Dual Prism Divider.

3 A Dual Prism cursor is displayed in the current window.

4 Position the cursor anywhere on the window, click to show the prism divider lines. If Esc is pressed at this point the operation is abandoned.

5 Move cursor to position crosshair then click to set position.

Editing a Dual Prism Divider

Editing an existing Dual Parameter plot with a set of dual Prism dividers displayed essentially consists of creating a new set of dual Prism dividers. When the second set of dual Prism dividers is defined the first set is deleted.

1 Highlight the required dual parameter plot.

2 Mouse over the Prism divider to get the \leftrightarrow , \updownarrow or \diamondsuit cursor.

4 + hold to set the new Dual Prism crosshair. When the new dividers are set they replace the previously set dividers. Any associated Prism plot is updated with the new Prism statistics.

Any associated Prism plot changes its format and has its statistics updated to reflect a smaller number of Prism dividers and a smaller number of displayed phenotypes.

4 To delete Prism divider, on the divider to make it active and press Delete. The Prism divider for the associated parameter is deleted altogether from this plot and from any other plot that has this divider set. Any associated Prism plot changes its format and has its statistics updated to reflect a smaller number of Prism divider(s).

Single Prism Divider

The **Analysis** >> Create Prism Divider >> Single Prism Divider menu option allows a single Prism divider to be set on a single parameter plot. Any displayed Prism plots is automatically updated with the current Prism statistics. If the number of Prism dividers is changed during the divider creation, the corresponding Prism plot also reflects this by changing its format according to the number of active Prism dividers. Please refer to your Cytometer system manuals for further explanation on Prism parameter.

Note: CXP software Cytometer software only allows one Prism divider per parameter. As a consequence modifying the Prism divider of any parameter on any plot automatically updates the divider of that parameter if it is displayed on any other plot. This includes both single and single parameter plots.

See also:

Setting a New Single Prism Divider Editing a Single Prism Divider

Setting a New Single Prism Divider

1 Highlight the required single parameter plot.

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- **3** A Single Prism cursor is displayed in the current window.
- Position the cursor anywhere on the window, click to show the prism divider lines. If Esc is pressed at this point the operation is abandoned.
- **5** Move cursor to position crosshair then click to set position.

Editing a Single Prism Divider

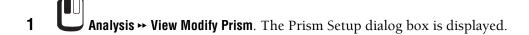
Editing an existing Single Parameter plot with a set of single Prism dividers displayed essentially consists of creating a new single Prism dividers. When the second single Prism dividers are defined, the original is deleted.

- **1** Highlight the chosen single parameter plot (click on the title bar).
- **2** Mouse over the Prism divider to get the \leftrightarrow , \updownarrow or \diamondsuit cursor.
- + hold to set the new divider. The old divider is deleted and the display is once again updated to show only one single Prism divider. Any associated Prism plot is updated with the new Prism statistics.

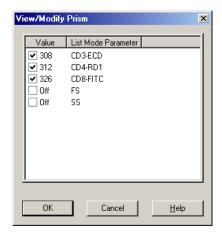
4 To delete Prism divider, on the divider to make it active and press Delete. The Prism divider for the associated parameter is deleted altogether from this plot and from any other plot that has this divider set. Any associated Prism plot changse its format and have its statistics updated to reflect a smaller number of Prism dividers.

View/Modify Prism

Under most circumstances, Prism is modified graphically by using Dual or Single Prism Divider cursor operations. Occasionally, it is necessary to modify the Prism dividers numerically to reproduce exactly the setup of another experiment. View/Modify Prism allows you to do this.



the parameter whose divider you wish to edit.



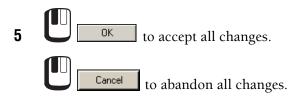
Place a check in the box next to the Listmode Parameter value you wish to enable.

Note: If no check is in the checkbox, the particular value is automatically set to OFF.

This switches off the prism divider and deletes it from any displays that were using it. If a prism divider is switched off, any active prism plots and plots with prism divider displays also update them to reflect the modified number of prism parameters in use.

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4 the value that you wish to change and enter the required value within the range 0 to 1023.



Select Results

CXP software gives the flexibility of generating many different statistics and these are produced using combinations of Regions and Gates.

A Gate is used to select events that may potentially be counted while Regions mark subpopulations and determine whether an event is actually counted.

All Regions and Gates can be named according to your preference, allowing easy recognition of your results.

The statistics calculated and displayed depend upon those selected in the current protocol. If no statistics have been selected then the default statistics are displayed. FCS options are either generated automatically by CXP software or obtained from sample information entered by the User. Desired items can be selected and exported during the publishing of an Excel Spreadsheet. The selected FCS information is only published and does not print with the plots within CXP software.

When in Color Blend Mode, only the color blend color statistics from the blended colors are displayed in the Legend plot. Use \checkmark to view Region statistics.

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Statistic Type

Number % Total % Gated Percentile Mean CV Median HP-CV Mode Cells / µL Min and Max

Current File Name
Mean Calculation Method
FCS Information
Select Numeric Precision
Enter Numeric Value
Advanced Statistics Configuration

For more information concerning the Publication of Results, see Publish Results Now.



There are the different types of statistics that can be utilized. Any number of these can be selected and the relevant statistic appears in the box beneath the plot.

Current File Name

This option allows you to include the data file name when the results are published to an Excel Spreadsheet. This information is not displayed within the Statistics Window.

Mean Calculation Method

This exports the method of calculating the mean as part of a Published Excel spreadsheet.

FCS Information

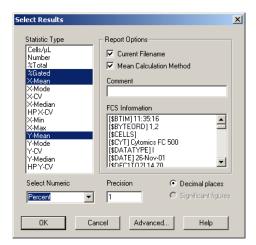
These options are either generated automatically by CXP software or obtained from sample information entered by you. Desired items can be selected and exported during the publishing of an Excel Spreadsheet. The selected FCS information is only published and does not print with the plots within CXP software.

Number

Total number of events in a Region.

% Total

Total number of events in region as a percentage of total events in the file.



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% Gated

Total number of events in region, as a percentage of events in a gated display.

Percentile

This is the channel, which divides the events in a region by the percentile value. The default percentile is 50 (the median) but this can be set to request any desired value.

Mean

The Mean channel is the arithmetic mean channel defined as the sum of the region channel numbers, times counts, divided by the integral of the region.

The Mean (or the average) is found when all selected events are added together and then divide by the total number of events.

The Advanced button allows for the calculation of a Geometric mean.

CV

The CV is Standard Deviation divided by the mean of the data between the markers.

Note: Standard Deviation calculations are only valid between markers that have a Normal or Gaussian distribution. CV is not strictly valid for any other shape distribution. The derivation of CV can be found in any standard statistic text.

Median

Median is used to indicate the point on the scale of measures where the population is centered. The median of a population is the point that divides the distribution of scores in half. Numerically, half of the scores in a population have values that are equal to or larger than the median, and half have values that are equal to or smaller than the median.

HP-CV

(HP X-CV and HP Y-CV) The Half Peak Coefficient of Variation (HP-CV) is derived by the fixed mathematical relationship between the Standard Deviation (SD) and the Full Width Half Max (FWHM) value of a Normal or Gaussian peak. The relationship between them is defined as SD=FWHM/2.354.

This is an approximation to the real value of CV. The advantage of calculation HP-CV is that the value remains fairly independent of the position of a pair of region markers, provided that the markers are set either side of a peak and the marker channel counts are less than half the value of the peak channel count. In general, the HP-CV approximation is usually smaller than the CV calculation. See standard statistics texts for further explanation of HP-CV and CV differences.

Mode

The mode channel is the channel with the largest number of counts between region markers.

Gate colors can be customized using the drop down color box and selecting the required color.

Cells / µL

This displays the absolute count of a population based on the calibration factor (CAL Factor) and the number of particles within a user defined CAL region.

Min and Max

Displays linear region boundaries.

Select Numeric Precision

Choose the precision you want to specify for, Percent, Mean or CV.

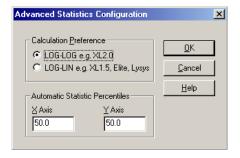
Enter Numeric Value

Maximum 3 decimal places for CV, 4 decimal places for Percent and maximum 5 digits for Mean.

Advanced Statistics Configuration

Selecting the Advanced button offers further options to select the way that the mean is calculated.

Log-Log Log-Lin Automatic Statistic Percentiles



Differences Between Log-Log and Log-Lin Statistical Calculations

For most practical applications users may never notice the difference between the two. In general, Log-Log mean appears to have its value skewed further to the right than a Log-Lin mean calculation on the same data. This skew is more pronounced when the distribution of data extends greater than ±half a decade from the mean value, however calculated.

Log-Log

Selecting Log-Log calculates the mean values of Log data using geometric conversion of each individual channel before a mean value is calculated.

Log-Lin

Selecting the Log-Lin mode calculates the mean values by determining the arithmetic mean of the raw ADC channels and then converting this to its relative linear channel equivalent.

Automatic Statistic Percentiles

This is the channel, which divides the events in a region by the percentile value. The default percentile is 50 (the median) but can be set to request any desired value. Different values may be set for both the X and Y axis.

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Absolute Count Calibration

Use absolute count calibration particles for the calculation of absolute numbers of cells present in a sample.

As a sample is analyzed, the numbers of a particular cell type in a region, and the numbers of absolute count calibration particles present in a CAL region are compared, and the absolute concentration of the cell type is then calculated automatically.

A listmode files replays with its runtime Cal Factor. Use this screen to enter a different Cal Factor to calculate individual lismode absolute counts.

Note:

- A CAL region MUST be set for the system to be able to calculate an absolute count. See Setting a CAL Region.
- Listmode replay uses the runtime protocol Cal Factor unless you select a different Cal Factor before replaying the file.



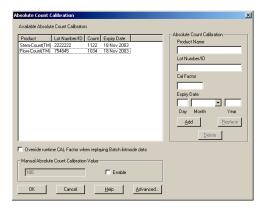
To display the absolute count, Cells/µL from the Analysis/Select Results menu option.

Setting a CAL Factor for Absolute Counts

From the list of Available Absolute Count Calibrators, select the Lot Number currently in use.

Enter a Manual Absolute Count Calibration Value if the required Lot is not present in the list. A manual calibration value is only used for the current session, it cannot be saved. A listmode file replays with its runtime Cal Factor.

Note: The number of events in the CAL region must be greater than 1000, otherwise absolute counts are not determined.



Override runtime CAL Factor...

Check this option if you want to replay a batch of listmode files using a different Cal Factor.

Advanced

If your Administrator has assigned you access rights, the Advanced button is active and you can enter and modify batch and count information. Enter the Product name, Expiry Date, Lot Number/ID and CAL Factor. See Add Absolute Calibration Batches in the User Administration section.

ADD

Use the ADD button to enter new lot information (only available on the Advanced screen).

Replace

Use the Replace button to replace the selected information with updated values (only available on the Advanced screen).

Delete

Use the Delete button to remove a selected lot from the list (only available on the Advanced screen).

Setting a CAL Region

Create a Region around the absolute count calibration particles, the region MUST be named CAL. Set or select the calibration factor for the batch of beads in use from the **Analysis** Absolute Count Calibration menu item.

Note: If the Cal Factor is set to 0 or there is no CAL Region present in the protocol, "Error" appears in the Cells/uL field. If the number of events in the CAL region equal to or less than 1000, "Invalid" appears in the Cells/uL field.

Calculate Results

This option calculates the statistics for all files selected on the screen.

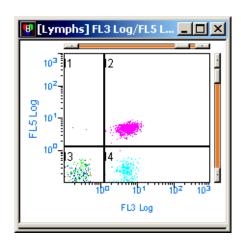
LMD QuickCOMP

Note: If you adjust the compensation of a Listmode file using the LMD QuickCOMP tool, the Amplifier Gain field of the FCS Header is no longer available, from either the File / View FCS Header menu item or Legend Plot. The original stored file remains unchanged and contains the Amplifier Gain information.

Enabling LMD QuickCOMP displays orange slider bars on all dual fluorescence plots.

These sliders are used to intuitively adjust the compensation coefficients and update the compensation values applied to the listmode file.

Clicking the arrows adjusts the settings by $\pm 0.1\%$, clicking either side of the slider adjusts by $\pm 1.0\%$ or drag the slider to place the cells in the required position. You can view the new settings in the LMD QuickCOMP Matrix



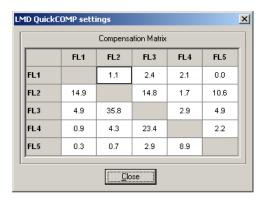
Show LMD QuickCOMP Matrix

When you use the LMD QuickCOMP slider bars to change compensation settings, the new settings are displayed in the LMD QuickCOMP Matrix dialog box. You cannot use the LMD QuickCOMP Matrix to change a protocol's compensation settings .

Note: To view compensation adjustments of Listmode data, always view the LMD QuickCOMP Matrix. The compensation matrix in the Cytometer Control dialog represents

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the compensation settings last used in that protocol and may not necessarily be the compensation settings used for any given Listmode file.



Show Analysis Algorithm Results

Only available when running protocols from optional applications, such as tetraCXP.

B.22 FLOWPAGE MENU

FlowPAGE - Introduction

FlowPAGE is the reporting area for Data, Plots, Statistics, micrographs and user created text. FlowPAGE enables custom formatting of multiple pages of flow cytometer information without the need to swap between other third party applications.

Once a FlowPAGE is created, the contents of its plots and statistics windows are automatically updated to reflect new listmode data or configurations made in the CXP software analysis.

All FlowPAGEs are saved with a Protocol, this retains the link between the Plots you display and how you want to format the output to the Printer.

FlowPAGEs contain fixed header and footer information generated from FCS Keywords. The automatic header information as displayed on the FlowPage reports always displays xxxxxx on the CXP Workspace. Upon printing, all automatic header fields are populated with the listmode file Keyword information on the printout ONLY.

FlowPAGEs are not updated during Data Acquisition in live time. FlowPAGEs are updated at the conclusion of acquisition, or after reading a new data file from disk, or editing Regions, Gates or Statistics or resizing FlowPAGEs.

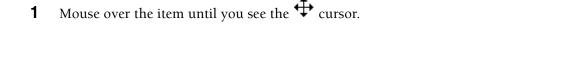
Note: Occasionally, when using a FlowPAGE, one or more items cannot be deleted from a page. If this occurs, the items can be cleared from the FlowPAGE by selecting **Files** >> **Save As...** to save the protocol with a new name. If you reload this new protocol, the items are now cleared from the FlowPAGE..

Editing FlowPAGEs

You cannot make any changes to a FlowPAGE when running a locked protocol. See Creating FlowPAGES in the System Overview chapter in the Instructions For Use manual.

Note: Due to the flexibility of listmode file naming options, listmode filenames can become very long. When such listmode files are open in the Analysis Only software, the software assigns a file designator (that is, F1) and displays that designator, with the filename in all statistic boxes on a FlowPage. Due to the long file name, this may result in overlapping statistic boxes, requiring reformatting of the FlowPage to view all results.

Select a Single Item Within FlowPAGE



2 the item and handles appear around the item.

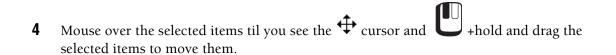
Move a Selected Item Within FlowPAGE

1 Mouse over the item until you see the cursor and +hold and drag the selected item to its new position.

Select a Group of Items Within FlowPAGE

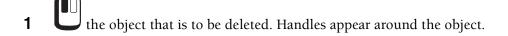
- 1 Click and hold the mouse button, selecting just outside the group of items required.
- **2** Drag the cursor until the marquee (dotted outline) completely encompasses the items you want to select.
- **3** Release the mouse button. Each item has handles around it.

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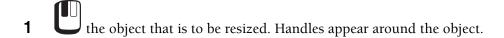
5 Click the right mouse button on the selected items to edit them.

Deleting Objects in FlowPAGE



2 Press Delete

Resizing Objects in FlowPAGE



2 Drag a handle to the size required and release the mouse button.

Additional FlowPAGE Formatting Options

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

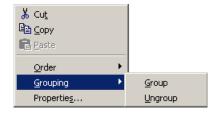
You can ${\it Cut}$, ${\it Copy}$ and ${\it Paste}$ items that are selected.



You can change the order of items that are selected.

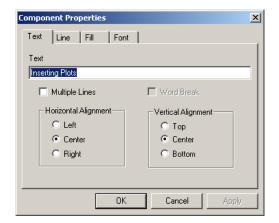


You can group and ungroup items that are selected.

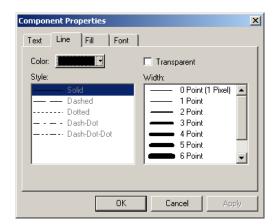


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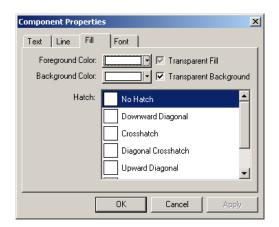
You can edit and change the alignment of text.



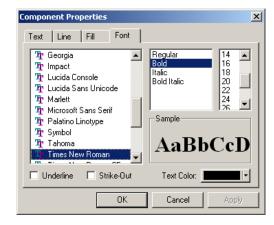
You can change how lines are displayed.



You can change the fill properties of items that are selected.



You can change the font properties of items that are selected.

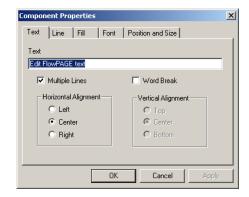


Edit Text

- 1 Textbox in the FlowPAGE that you wish to edit. The edit handles appear.
- 2 Right mouse click on the textbox and Properties.



- **3** Type in the new text.
- 4 OK See Edit Font (FlowPAGE).

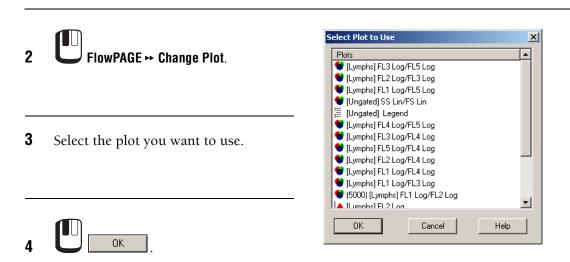


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Change Plot

Use Change Plot to change which Plot is displayed from those available on the CXP desktop.

1 On the FlowPAGE, the Plot you want to replace. The edit handles appear.



Grow to Largest

- 1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.
- 2 All items in the group grows to the size of the item which has the largest area.

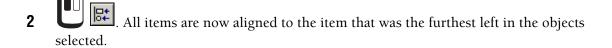
Shrink to Smallest

1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.

2 the button. All items in the group shrink to the size of the item which has the smallest area.

Align Left





Align Right

1 Select the items you wish to align. See Select a Group of Items Within FlowPAGE.

2 . All items are now aligned to the item that was the furthest right of the objects selected.

Align Top

1 Select the items to be changed. See Select a Group of Items Within FlowPAGE.

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2 All items are now aligned to the item that was the topmost in the section that was selected.

Align Bottom

- **1** Select the items to be changed. See Select a Group of Items Within FlowPAGE.
- 2 . All items are now aligned to the item that was nearest the bottom of the objects that were selected.

Space Evenly Across

- 1 Select the items to be changed. See **Select a Group of Items Within FlowPAGE**.
- 2 . All items are now horizontally spaced equally.

Space Evenly Down

- 1 Select the items you wish to change. See Select a Group of Items Within FlowPAGE.
- 2 All items are now vertically spaced equally.

Save FlowPAGE As

To save an existing FlowPAGE as a FlowPAGE template:

1 FlowPAGE >> Save FlowPAGE As to view the Save FlowPAGE dialog box.

- 2 Specify the desired drive and folder by selecting them in the list boxes or by typing the path in the File text box.
- **3** In the **File name** text box, type the desired File Name.



FlowPAGE gives the default file extension *.PGE. The *.PGE extension is only a suggestion, any standard DOS characters can be used for the full file name.

Note: Keep the extension consistent for easy file searches.

B.23 CYTOMETER MENU

The Cytometer menu is concerned with online connection and control of the flow cytometer. The Cytometer Control menu option allows the Cytometer Control screens to be activated. Until this is done the other Cytometer menu items are grayed out.

The Status Page displays current instrument settings, which can be printed out for future reference.

The Cytometer Log displays a log of errors generated by the system.

The Get Cytosettings from Protocol option allows Cytometer Instrument Settings to be obtained from a Protocol other than the current Protocol in memory.

The Get Cytosettings from LMD file option allows Cytometer Instrument Settings to be obtained from a listmode file.

The Controls for Acquisition are also found within the Cytometer menu.

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See also:

Flow Rate

Status Bar

Cytometer Control

The Cytometer Control button opens the Cytometer Control screen. This screen is a show/hide dialog box. The type of Cytometer you have installed determines the screens displayed and the functions that are available.

See also:

Cytometer Control Acquisition Setup Tab

Cytometer Control Detector Tab

Cytometer Control Compensation Tab

Flow Rate

Cytometer Control Acquisition Setup Tab

This screen is used to set the Acquisition Mode and Acquisition Limits. Additionally, the Discriminator values for the selected Parameters can be set.

Duration (s)

Max Events

Drive Space (MB)

Live Gate

Setup Mode

QuickCOMP Mode

QuickSET Mode

Baseline Offset

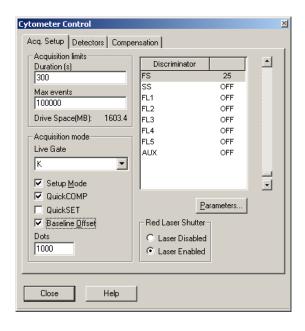
Dots

Discriminators

Parameter Button

Red Laser Shutter

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



See also:

Cytometer Control

Cytometer Control Detector Tab

Cytometer Control Compensation Tab

Cytometer Control Parameter Setup

Flow Rate

Status Page

Duration (s)

Sets the maximum **Duration** of the Acquisition in seconds for each sample. When this time limit has been reached acquisition stops. However, if the maximum number of events is reached before the duration limit, acquisition ceases at this point.

Max Events

This is the maximum number of events, which may be acquired on the next Acquisition. To change this, enter the new number required in the input field. However, if the Duration is reached before the maximum number of events acquisition ceases at this point.

Drive Space (MB)

Shows how much free data storage space is available on the currently selected storage device, in megabytes (MB).

Live Gate

This displays all Gates that are available for the Live Gating of data during acquisition. Data outside the selected gate is not displayed and is NOT STORED to disk to reduce the size of the listmode files by gating out all unnecessary events.

Setup Mode

Enable this checkbox to allow the continuous acquisition of data from the Cytometer. In this mode, Stops are disabled, Printing is disabled and listmode files are not stored.

Setup Mode allows continuous acquisition of data from the Cytometer giving continuous real-time feedback of instrument performance on the displayed plots. Any Stop & Save values set are ignored in this mode.

Display of data continues until **Abort** is selected, to end data acquisition, or the **Setup Mode** box is unchecked, to begin normal data acquisition.

In Setup Mode the plots are updated live time with only the most recently acquired events displayed. The incoming data is not saved. During Setup Mode, older events are cleared from the screen and from memory allowing the Cytometer settings to be adjusted in real-time without reaching a set stop condition.

Data is displayed on a "first in; first out" basis. The number of events to be displayed can be changed by entering the required value into the Dots input box.

Once you have finished using Setup Mode click **Abort**, or uncheck the **Setup Mode** checkbox and then perform the acquisition.

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QuickCOMP Mode

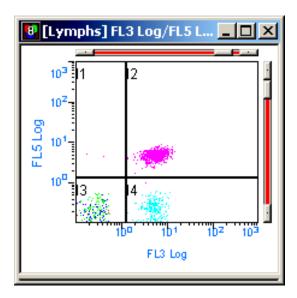
Enabling **QuickCOMP** on the Cytometer Control Acquisition Setup Tab dialog box displays red slider bars on all dual fluorescence plots.

These sliders are used to intuitively adjust the compensation coefficients and update the compensation values in the Cytometer Control Compensation Tab.

Clicking the arrows adjusts the settings by $\pm 0.1\%$, clicking either side of the slider adjusts by $\pm 1.0\%$ or drag the slider to place the cells in the required position.

When **QuickCOMP** is enabled **QuickSET** mode is not available.

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



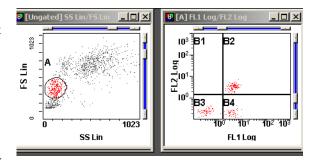
QuickSET Mode

Enabling **QuickSET** on the Cytometer Control Acquisition Setup Tab dialog box displays slider bars on all plots.

These sliders are used to adjust voltages at the data plots and update the high voltages in the Cytometer Control Detector Tab.

Clicking the arrows adjusts the voltage by ± 0.1 volt, clicking either side of the slider adjusts by ± 1.0 volts or drag the slider to place the cells in the required position.

When **QuickCOMP** is enabled **QuickSET** mode is not available.



Baseline Offset

Enable this checkbox to switch on Baseline Offset. Further information concerning the setting of this option can be found under Workspace Preferences - Plot Display.

Dots

This sets the maximum events to be displayed during **Setup Mode**. The default size is 1,000 events. The **Setup Mode** rolling displays are updated up to three times per second. To change

the size, edit the Dots field to the number of Dots required. If a zero is entered, an error message displays showing range of 10 - 9999. The Dots value is stored in the Protocol

Discriminators

Lists the currently selected parameter and allows the setting and modification of discriminator values. To change a value, select the discriminator you wish to alter and use the Slide Control on the right hand part of the display to change the value as appropriate.

Parameter Button

Selecting this displays the Setup Parameter Screen.

Red Laser Shutter

Use to enable or disable the red laser. The shutter setting is passed as part of the instrument settings part of the protocol.

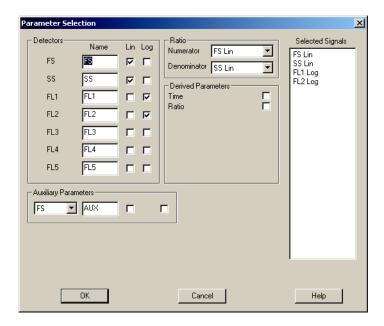
Cytometer Control Parameter Setup

This screen is used to select the Signals required in the Parameter Setup.

The signals that are available are selectable by checking the options you require.

Note: If you add a parameter to an existing protocol, the Detector Names revert to the default names shown below.

IMPORTANT Risk of erroneous results if you assign the same parameter name to more than one detector. Use a different parameter name for each detector.



You can arrange the order within the **Selected Signals** list box by highlighting the appropriate item(s) and Drag and Drop into your chosen order within the **Selected Signals** list box.

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To remove a previously selected item from the **Selected Signals** box, uncheck the parameter no longer required.

Ratio and **Time** cannot be reordered and always appear at the bottom of the list.

Ratio – Choose from the drop down box the appropriate option for the Numerator and Denominator if required.

A detector name may be entered, this is displayed in all **Cytometer Control** screens but does not appear on plots.

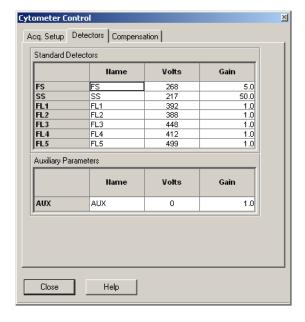
Note: You cannot make any changes on this screen when running a locked protocol.

Cytometer Control Detector Tab

This shows the detectors with editable names and detector settings. Changes made here are only reflected in the **Cytometer Control** dialog box and are used to identify the various fluorochromes in use. Reagent or sample specific information should not be entered in this menu. See **View** >> Acquisition Manager >> Parameter Names or File >> Edit FCS Header Attributes.

Name Volts Gain AUX

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



See also:

Cytometer Control
Cytometer Control Acquisition Setup Tab
Cytometer Control Compensation Tab
Cytometer Control Parameter Setup

Name

These input fields allow the editing of the Names assigned to the default manufacturer's detectors. Changes are ONLY reflected in the names shown within the Discriminator box in the Acquisition Setup tab and the Compensation tab. This allows the various fluorochromes to be easily recognized when adjusting instrument settings.

Volts

Select the appropriate Voltage for the particular Detector. This is done by using the Slide Bar on the right side of the screen and adjusting it to the required value. For fine adjustment, the Up and Down arrows at the ends of the Slide Bar can be used.

Gain

Adjusts the Gain amplifier. Set the Gain to the appropriate setting you require for that Detector. This is chosen from the drop down box, which appears when you click in the box.

AUX

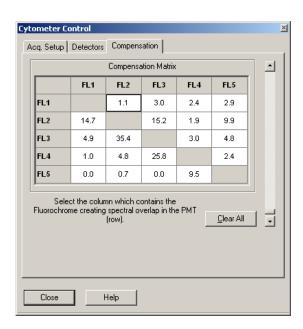
Displays the signal source for the Auxiliary Parameter channel.

Cytometer Control Compensation Tab

Compensation allows for the subtraction of a percentage of the signal of one fluorescence detector from the signal of another fluorescence detector to correct for the overlap of one dye's emission into another dye's detector.

Compensation Matrix Clear All Button

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



See also:

Cytometer Control

Cytometer Control Acquisition Setup Tab

Cytometer Control Detector Tab

Compensation Matrix

Click in the box and type a value or use the Slide Bar to set the desired value. Use the Up and Down arrows to make any fine adjustments to the value.

Clear All Button

Selecting the **Clear All** button resets ALL the values within the **Compensation Matrix** to zero.

Status Page

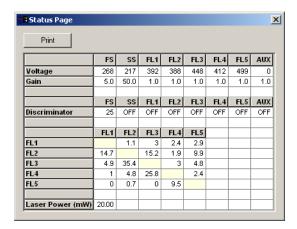
To view the **cytosettings** of an application, display or print the Cytometer Status screen for the verification tube of the application.

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The **Status Page** displays the Cytometer settings currently in use and allows you to print these if required.

Selecting the **Print** button displays the standard Windows Print dialog box.

Printed status pages also contain the protocol filename, date, time and cytometer serial number.



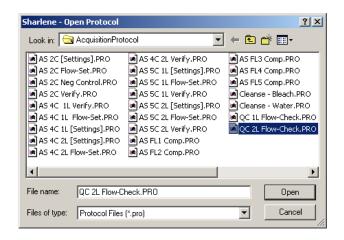
Cytometer Log

Use this menu item to view the log of errors generated by the system. It also allows you to clear this log as needed.

Get Cytosettings from Protocol

The **Get Cytosettings From Protocol** option allows Cytometer Instrument Settings to be obtained from a Protocol other than the current Protocol in memory.

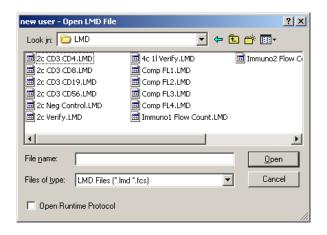
Choose and Open the required Protocol.



Get Cytosettings from LMD file

The **Get Cytosettings From LMD File** option allows Cytometer Instrument Settings to be obtained from a listmode file.

Choose and Open the required listmode file.



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USING CXP SOFTWARE CYTOMETER MENU

Start

The Start button initiates acquisition of the current sample.

Stop

The Stop button ends data acquisition. Any Output options such as saving data, calculating statistics, printing reports, and so on, are then performed.

Pause

The Pause button pauses data acquisition as does clicking. To continue Acquisition, select . To restart acquisition from zero events select.

Abort

Selecting the Abort button displays the Abort screen. This function is only available if Acquisition is running.

Note: No Files are saved if a tube is Aborted.

Select the option required and

Abort the Tube? Abort the Panel? Abort the MCL? Repeat the Tube? Repeat the Panel?



Abort the Tube?

Stops acquisition, marks the current tube as aborted and the instrument proceeds to the next tube.

Abort the Panel?

Stops acquisition, marks the remainder of the current panel as aborted and the instrument proceeds to the first tube of the next panel.

Abort the MCL?

Ejects the current carousel and leaves the remainder of the Worklist unread allowing resumption from the current tube.

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Repeat the Tube?

Allows the current tube to be resampled.

Repeat the Panel?

Allows the current panel to be resampled. The MCL returns to the first tube of the current panel.

Restart

If a sample is already being acquired, the Restart button restarts the Acquisition from zero events.

Pause and Rotate

The Pause and Rotate button allows you to pause processing of a single sample and rotate the MCL to a fixed position on the carousel so that additional reagent, for example, can be added to the sample. **Show ME** an example of Pause and Rotate sample processing

Note: To prevent excessive air from entering the flowcell, do not leave the Cytometer paused for extended periods of time.

Idle Mode

The Idle Mode button places the Cytometer in Idle mode so that various cleaning and replacing procedures can be performed.

Prime

The Prime button allows you to declog or remove bubbles or blockages from the sample line with sheath solution.

Cleanse

The Cleanse button allows you to initiate a cycle to flush the instrument sample lines with Clenz solution. The Cytometer must be in Idle mode, z_{22} , to perform this function.

AutoCleanse

The AutoCleanse button allows you to initiate an automatic cleanse cycle to clean the MCL vacuum and sample lines. The Cytometer must be in Idle mode, z_{22} , to perform this function.

Status Bar

The Status Bar which is shown at the bottom of the screen displays the following information when in Acquisition.



See also: Total Events Elapsed Time Data Rate

Total Events

The current total number of events acquired is displayed here.

When acquiring, this value increments to the maximum events set or until one of the Stop conditions has been met. See Acquisition Stop and Save.

When acquiring data in Setup Mode, this item cycles in the range from 0 to 10 times the number of Events shown in the Dots Displayed. The value appears to wraparound, that is, if you have requested 5,000 dots to be displayed, this item increments to 4,999. On the 5,000th event the total events counter resets to 0.

This ensures that you can continue to display data without reaching a set stop condition.

Elapsed Time

This item shows how many seconds have elapsed since the start of data acquisition.

When in Pause mode, the timer on the status bar does not continue to increment. The total elapsed time can be viewed from the FCS Keywords associated with the beginning time and end time of acquisition.

Data Rate

This item shows the speed of data acquisition.

This refers to the Processed Data Rate, that is, the number of events which have exceeded the discriminator, subsequently processed and saved in memory.

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Flow Rate

This item shows the relative flow rate of the Cytometer during acquisition. Click on the drop down box and choose the required rate.

The Flow Rate can only be displayed as Low, Medium or High. The Flow Rates are approximately: 10, 30 and 60 µL/min respectively.



Cytometer Status

Displays the current status of the Cytometer and the most recent error message. Double click on this field to display the Cytometer Status Message dialog box.

Windows Status Bar

The Windows Status Bar normally gives a brief description of selected menu items.

When drawing or editing Regions, the Status Bar shows Channel for X and Y parameters respectively as they are drawn.

X and Y Coordinates

The X and Y channel values are the coordinates of the cursor position given by a scaling factor. This scaling factor depends upon whether the respective parameters are linear or logarithmic amplification and whether you have calibrated the parameters or not.

■ If the parameters are linear and uncalibrated, the X and Y channels vary between 0 - 255 or 0 - 1024 depending on the data source.

If the parameters are logarithmic and uncalibrated, the values vary between 0.1024 - 1024, and span four decades. for most other manufacturers, the data varies between 1 - 10,000, spanning four decades.

B.24 WINDOW MENU

Cascade

Use **Cascade** to display the window so that the plots overlay.



Tile

Arranges each plot so every window display is visible. The pattern depends on the number of open Windows.



Tile Special

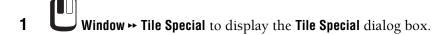
Tile Special overrides the normal Windows **Tile** command, this allows plots within the workspace to be arranged in a logical manor.

The plot size in **Tile Special** resizes plots currently displayed in the workspace.

New plots are sized equally according to the default size setting in File → Workspace Preferences → Plot Display.

Plots may be ordered by File, Gate, or Parameter or any combination of these.

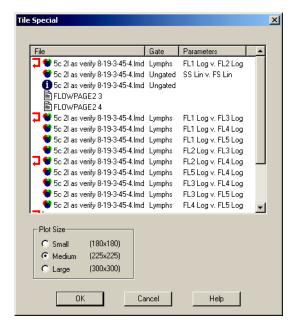
At the start of each row, the Plot Type Icon is displayed.



- 2 By clicking the mouse on the File, Gate or Parameter column heading, the plots are displayed across the screen in alphabetical order. Clicking the appropriate column heading again displays the plots in reverse order.
- **3** The order is shown in the **Tile Special**



You can also Drag and Drop the plots into any order.



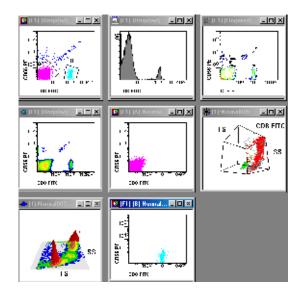
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The icon is activated by clicking to the left side of a plot listed in the File column. This forces that plot to be displayed on the

following row. If an existing is clicked, the icon marker is removed and New Row is not forced.

In addition, if Ctrl is depressed while the File, Gate or Parameters selection is made, plots can be sorted by Gates within File, and Parameters within Gates/Files.

When OK is selected in the example **Tile Special** screen shown above, the resulting plot display is shown to the right.



Arrange Icons

This option is used to arrange minimized plot windows. Plot windows can be minimized and

placed anywhere on screen. **Window** → **Arrange Icons** to place all icons along the bottom of the screen.

Note: This option is very useful when minimized plots have been "lost" on the desktop. Choosing **Window >> Arrange Icons** places all the icons along the bottom of the CXP software Window.

Force Square

Each window is squared so that horizontal and vertical sides are the same length. That is, aspect ration is forced to 1:1. This is used mainly for dual parameter plots.

Close All

Choosing this option closes all CXP software windows.

B.25 QUALITY CONTROL REPORT

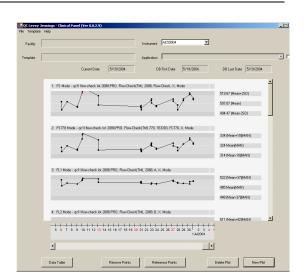
The Quality Control Report consists of Levey Jennings plots based on information selected in the QC Template and data stored in the QC tables in a report database. The information selected in the QC Template may be results (that is, %, cells/µL, mean, mode) or cytosettings (that is, voltages, gains). The data is plotted against time in the Levey Jennings Plots and can be saved to spreadsheet format.

Note: A QC protocol must be run prior to viewing data associated with your instrument (serial number).

QC Levey Jennings Screen



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



Instrument

Choose the instrument that you want to view QC data from.

Application



☑ and choose an AutoSetup II application that is associated with a template.

Note: To view QC results that were run without being associated with an application (for example, Flow-Check QC results), leave this option unchecked.

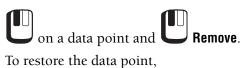
Data Table

Displays the information from the Levey Jennings plots in tabular format. See also, QC Data Table.

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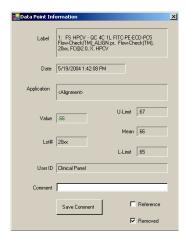
Remove Points

To remove a data point from a plot,



Remove to clear the checkbox.

Deleted data points are displayed green and are excluded when calculating the mean and SD on the Levey Jennings plot.



To remove a range of data points,

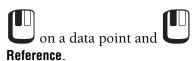


2 Right mouse click on the last data point to remove and



Reference Points

To assign a data point as a Reference,



To restore the data point,



Reference points are displayed blue and when assigned, the Mean and SD of the Levey Jennings plot can be calculated from the Reference points.



To assign a range of data points as reference,



2 Right mouse click on the last data point and



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Delete Plot

1 (to the right of the plot title). Multiple data plots can be selected.

New Plot

Add a new plot.

File Menu

Print

Print the displayed QC data.

Print Preview

Preview the QC data printout.

Page Setup

Set page orientation and margins.

Close

Close the QC Levey Jennings screen.

Template Menu

The dataplot definitions of the Levey Jennings screen can be saved as a QC template.

Clear Template

Clear the current template

Select Template

Select a saved template.

Save Template

Save the current template

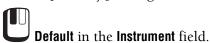
Save Template As

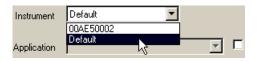
Save a template as a different name.

Associate Default Template Files To Your Instrument

Default QC templates are installed with the software. You need to save the default template files with your instrument serial number for the database to populate them correctly.

1 On the QC Levey Jennings screen

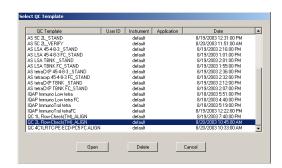




2 Template → Select Template.

3 Highlight the desired template and





4 Template → Save Template As, enter the template name and





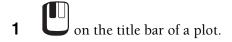
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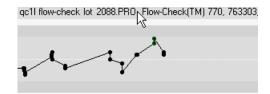
Modifying Template Files

You can modify a default template to collect QC data from different protocols and parameters.

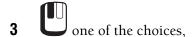
Note: Do not modify or edit IQAP templates. Use the IQAP templates to submit control data to the IQAP Program. Refer to the IQAP manual, PN 4206384, or www.beckmancoulter.com for additional information.

Select Parameter

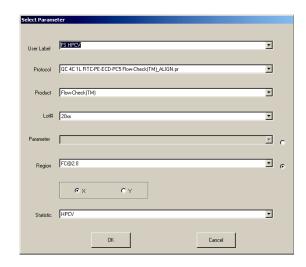




- 2 Use to choose the appropriate,
 - a. User Label
 - b. Protocol
 - c. Product
 - d. Lot #.



- a. **Parameter** and choose the parameter or compensation setting or,
- b. Region and choose,
 - the Region
 - X or Y
 - the Statistic.



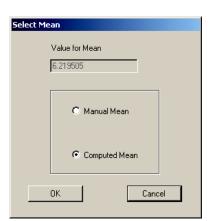
4 OK

Select Mean



- 2 one of the choices,
 - · Computed Mean.
 - Manual Mean and enter value.

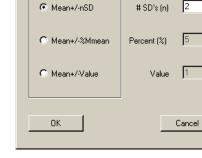




Select Limits



- 2 one of the choices,
 - **Mean+/-nSD** and enter a number.
 - Mean+/-%Mmean and enter percent.
 - Mean+/-Value and enter value.



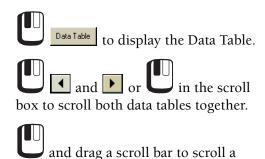
Select Limits

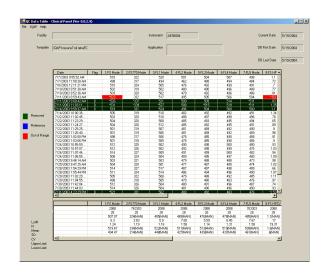
3 OK

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QC Data Table

The QC Data Table displays the information from the Levey Jennings plots in tabular format. Each column of the table displays the data from one Levey Jennings plot. The statistics for each column are displayed at the bottom of the table.





File Menu

Create Spreadsheet File

data table individually.

Creates a text file (*.txt) that contains the data table information. You can import the file into a spreadsheet program for analysis.

Print

Print the QC data table. When you use an IQAP template for control results, print the data table and fax or mail the results to the IQAP Program.

Print Preview

Preview the QC data table printout.

Page Setup

Set page orientation and margins.

Close

Close the QC Data Table screen.

IOAP Menu

The IQAP information entered here is saved to the printout and spreadsheet file.

Edit IOAP









B.26 PANEL REPORT TEMPLATE

IMPORTANT Risk of panel report not generating if you have not saved the template before running the assay for the first time. Edit the limits and save the template before first-time use.

Risk of misleading results if you do not specify the correct cell population. Specify the correct cell population even if you do not want to print the line since other lines may refer to this line.

Overview

Results are exported to the database when they are generated from panels that are "export" panels. tetraCXP panels and LSA panels are examples of export panels. This means that when you run a sample in a tetraCXP panel, LSA panel, or replay a listmode in a tetraCXP panel or LSA panel, the results are automatically exported to the database. There is a one-to-one match between tetraCXP panels and their corresponding templates and a one-to-one match between the LSA panels and their corresponding templates.

Any panel can be saved as an export panel. See Creating a New Panel Report Template or Creating Panels in the Instructions for Use manual.

When you open the desired panel report template, it is important to understand how the template affects the printed report.

Each line in the template represents information to be used in the final printed report. Certain lines of information, such as those used for calculating other results, are not printed on the report.

Each template has three tabs: Global, Local and Data Plots.

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B.27 CREATING A NEW PANEL REPORT TEMPLATE

In order to print patient panel reports, you need to create a panel report template that you use to define what to print on the report.

Create A CPF File

A CPF file contains the settings for a panel report template.

1 elect the panel you want to use to create a panel report template

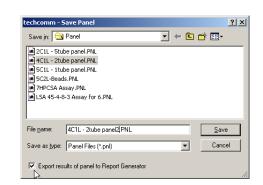


2 Right click on the panel in Acquisistion

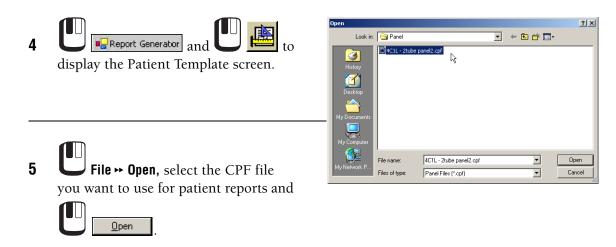


3 Type a name for the panel,





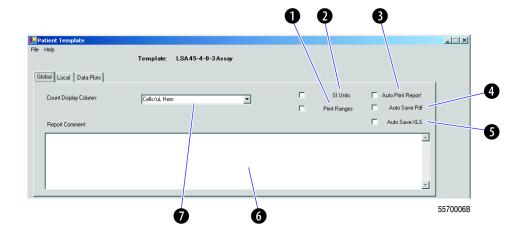
Create the Panel Report Template



- **6** Use the Global, Local and Data Plots tabs to enter the information you want on the patient report.
- 7 File >> Save to save the template.

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Global Tab Settings

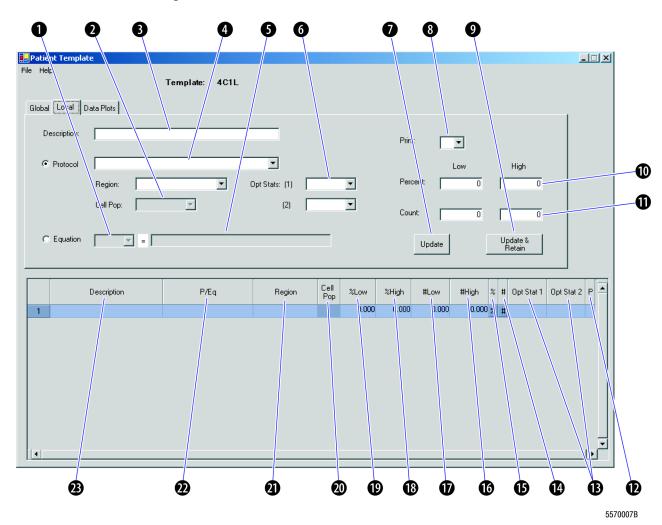


- Allows you to choose whether or not to print ranges on the report.
- 2 Allows you to select the reporting unit format: SI Units or US format (which is the default if SI Units is not selected)
- Allows you to enable auto-printing so that the panel reports automatically print at the end of acquisition.
- Allows you to enable auto-save to .PDF.
- Allows you to enable auto-save to an XLS file.
- 6 Allows you to define a global report comment that prints on **every** panel report generated from this template

Note: You can edit the comment on individual reports if necessary. See Editing Report Comments.

Allows you to choose Cells/µl CAL, Cells/µl Hem or Number. Cells/µl CAL adjusts absolute counts based on the CAL factor. Cells/µl Hem calculates absolute counts based on the Cell Population selected from the hematology patient information and the number of events in the result region. Number displays the unadjusted count in the region.

Local Tab Settings



The equation result is selected from a drop-down list that includes:

%&# (Both) = Percent result is calculated from percents and is placed in the Result column and Count result is calculated from counts and is placed in the Count column.

Percent = Result of the equation is placed in the Result column with a % sign.

Count = Result of the equation is placed in the Count column.

Result = Result of the equation is placed in the Result column, no % sign.

See explanation for **5** for additional equation options.

IMPORTANT Risk of misleading results if you do not specify the correct cell population. Specify the correct cell population even if you do not want to print the line since other lines may refer to this line.

- Select a cell population (LY, MO, NE, EO, BA, Other, WBC, RBC, PLT, or blank) based on the gating that was used. The available selections correspond to the hematology information in the database. A selection must be made when the calculation method is **Cells/µL HEM**; otherwise, Cell Pop is grayed but retains its value. Cell Pop is blank when Equation is selected.
- Text description of the current template line

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- When selected, lists all the protocols in the panel. From the drop down list select the protocol to extract results from.
- Use one of the following methods to define a custom equation.
 - Type an equation using the (), *, /, +, -, @, & operators. Result Rows are designated in the equation as R001 to R999.
 - The Row number can be preceded by,
 - "% = Use the value in the Result column of the row
 - "# = Use the value in the Count column of the row
 - "@ = Use the value in the Opt Stat 1 column of the row
 - "& = Use the value in the Opt Stat 2 column of the row
 - Define an equation by clicking on a cell in the Results Section to add it to the equation. For example, clicking on the Count column of Row 5 adds #R005 to the equation.

Right clicking on this field displays the following options,

- + Add
- Subtract
- * Multiply
- / Divide

DF = Dilution Factor

HV = Harvest Volume

BW = Body Weight

- Displays the optional statistics for the result that was selected in the Region field in the Protocol area of the Local tab. Select up to two of the following for each Results Row: X-Mean, Y-Mean, X-Mode, Y-Mode, X-Median, Y-Median, X-CV, Y-CV, X-HPCV, Y-HPCV. Only statistics that were selected in the protocol during acquisition are available as options. Optional statistics cannot be selected when Equation is selected.
- Adds a row using the information entered and blanks all of the fields.
- Indicates if item prints on the report:
 - **N** = not printed: used for calculations: do not enter ranges for these items
 - Y = printed
 - $\mathbf{B} = blank$
- Adds a row using the information entered and retains the information in all of the fields.
- Fields where you enter the low and high percentage range limits that appear in **1** and **1**, respectively. Low range is -1000 to 1000 with 3 decimal places. High range is -1000 to 1000 with 3 decimal places.
- Fields where you enter the low and high absolute count range limits that appear in **6** and **7**, respectively. Low range is -999999.999 to 999999.999. High range is -999999.999 to 999999.999.
- Indicates if item prints on the report:

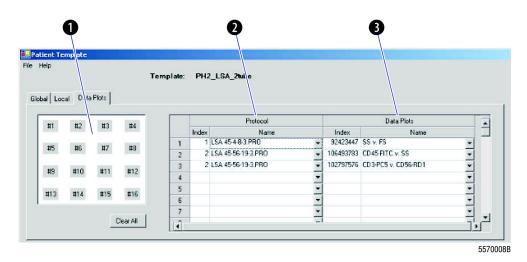
N = not printed; used for calculations; do not enter ranges for these items

Y = printed

 $\mathbf{B} = blank$

- Displays the optional statistics for the result that was selected in the Region field in the Protocol area of the Local tab.
- Indicates that result of the equation is calculated from counts and placed in the Count column.
- Indicates that result of the equation is calculated from percents and placed in the Result column.
- **1** Displays the high count range limit entered in **1**.
- **D**isplays the low count range limit entered in **①**.
- Displays the high percentage range limit entered in **10**.
- Displays the low percentage range limit entered in **10**.
- Represents the cell population used for gating absolute counts from a hematology instrument on non-Flow-Count panels.
- Displays the region from where data was extracted.
- Displays the protocol name (**P**) or an equation (**Eq**) used for arriving at the results.
- Text description of the current template line.

Data Plots Tab Settings



- Allows you to select up to 16 data plots to include in the report.
- Displays the protocol name and index number. The index number represents the protocol order in the panel.
- 3 Displays the data plot name and index number. The index number is a unique number for plot identification.

B.28 EDITING PANEL REPORT TEMPLATES

Before Running an Export Panel for the First Time

Before you run an export panel for the first time, update the template and save the limits. If you do not save the updated template, the following message appears:

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Procedure for Editing/Saving Panel Report Templates

Do this procedure to edit and save a any export panel report template to:

- select/deselect SI units
- enable/disable the auto-print feature (when enabled, the report prints automatically at the end of acquisition) Note: The panel report auto-print feature is in addition to FlowPAGEs, plots and/or statistics that auto-print per the Workspace Preferences setup (see Workspace Preferences, Acq Options tab)
- add a global report comment (which is printed on every report generated from the affected template)
- edit flagging limit ranges
- add or delete plots



- 2 to open the Patient Template screen.
- **3** View the templates (with .cpf extension):



Navigate to the desired panel folder.

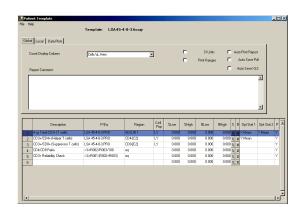


- 4 To view the contents of a pre-defined template, select the desired .cpf and Open.

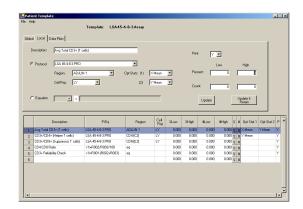
 Note: Any export panel created by a user must have the corresponding Panel Report Template created by that user.
- **5** To select/deselect SI units, enable/disable auto-print, or to

add/enter a report comment, Global.

Note: If editing a global report comment, only future reports will be printed with the updated text; reports generated prior to the change will not contain the updated comment.



6 To edit flagging limits, Local.

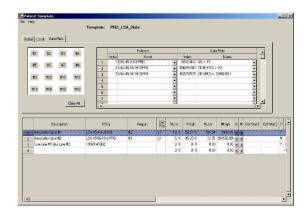


7 Enter/edit the information as needed.

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8 To select data plots for the report,



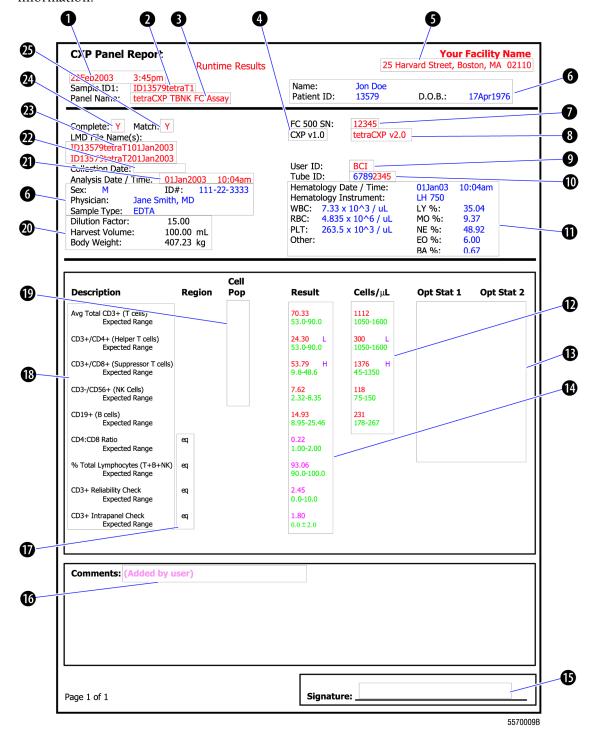


- 9 File → Save to save the template with your changes.
- 11 File >> Close to close the template screen.

B.29 PANEL REPORT

ATTENTION: The panel must be selected for export so results go to the database.

Below is an example of a patient panel report with data only. Each area is defined for the purpose of helping you better understand the origin of the report information.



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- Date and time report was printed
- Sample identification (obtained from CXP listmode file)
- Indicates the name of the panel used (obtained from CXP listmode file)
- CXP software version and application plug in software version, if installed (obtained from system registry)
- Institution's name and address where report was produced
- Patient information (entered on Database Information screen; see Entering/Editing Database Information)
- Instrument's serial number (obtained from CXP listmode file)
- **8** tetraCXP software version (obtained from CXP listmode file)
- User who logged on to CXP and generated the report
- Tube ID (barcode)
- Hematology information (entered on Database Information screen; see Entering/Editing Database Information)
- Cell count results (may come from CXP listmode file or can be calculated by the Report Generator depending on the template selected) and expected range of cell count (entered at Global Tab in report template; see EDITING PANEL REPORT TEMPLATES). If a non-Flow-Count template is used and hematology information is added, Cells/µL are reflected as Cells/µL HEM.
- Optional statistics
- Percent positive results (obtained from CXP listmode file) and expected range of percent (entered at Global Tab in report template; see EDITING PANEL REPORT TEMPLATES)
- Signature blank for your lab's use
- Comments (entered by user at the template or at individual report)
- Indicates that an equation was used and that results were generated by Report Generator
- Reported results (measured or calculated)
- © Cell population used for gating absolute counts from a hematology instrument when a **Cells/µL HEM** panel/template is used; field is blank when a template from a **Cells/µL CAL** panel is used
- Specimen information (entered on Database Information screen; see Entering/Editing Database Information)
- Date and time of sample analysis (obtained from CXP listmode file)
- Date and time sample was collected
- Listmode filenames (obtained from CXP listmode file)

USING CXP SOFTWARE

Indicates if the complete panel (for example, all tubes) was processed (obtained from CXP listmode file)

 $\mathbf{Y} = yes$

N = no (Rerun entire panel if **N** appears.) *Incomplete Report* prints on the report.

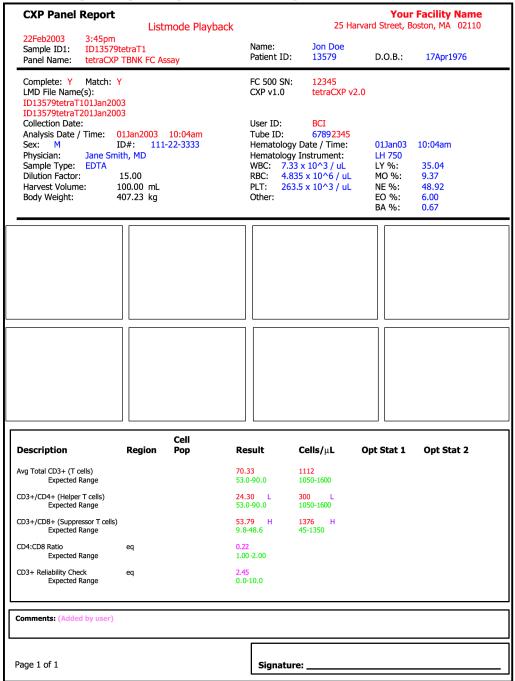
Indicates if protocols or regions in the file match the template definition (obtained from CXP listmode file)

 $\mathbf{Y} = \mathbf{yes}$

 ${\bf N}$ = no (Indicates that the protocol used different regions than the template. Rerun data/sample through original protocol.)

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Below is an example of a panel report with data and plots.



5570010B

CXP Panel Report Runtime Results	Your Facility Name 25 Harvard Street, Boston, MA 02110			
22Feb2003 3:45pm Sample ID1: ID13579tetraT1 Panel Name: tetraCXP TBNK FC Assay	Name: Patient ID:	Jon Doe 13579	D.O.B.:	17Apr1976
Complete: Y Match: Y LMD File Name(s): ID13579tetraT101Jan2003 ID13579tetraT201Jan2003 Collection Date: Analysis Date / Time: 01Jan2003 10:04am Sex: M ID#: 111-22-3333 Physician: Jane Smith, MD Sample Type: EDTA Dilution Factor: 15.00 Harvest Volume: 100.00 mL Body Weight: 407.23 kg		strument:	01Jan03 LH 750 LY %: MO %: NE %: EO %: BA %:	10:04am 35.04 9.37 48.92 6.00 0.67
Comments: (Added by user)				

Belo

Printing Patient Panel Reports

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Auto-Printing

If auto-print was selected in the template, the panel report prints automatically after acquisition ends.

Signature:

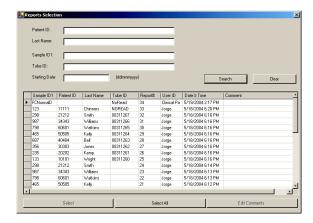
Manually Printing Panel Reports

Do this procedure to manually print a panel report (if auto-print is disabled).

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2 to open the Reports Selection screen.



- **3** Search for the desired report(s) using the following search criteria: patient ID, last name, sample ID1, or analysis date.
 - a. Enter the search criteria information.
 - Search

The last sample run appears at the top of the list by default. You can sort the list by Sample ID, Patient ID, Last Name, etc. by simply clicking the desired column title.

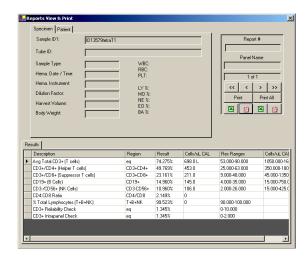
Note: To do a global search and locate every sample report in the database, leave all

search criteria fields empty and Search.

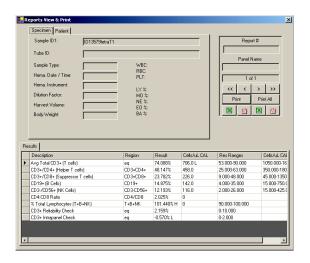
4 Select the desired report(s). For details on selecting, see Tips for Selecting Reports.

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Once you have selected the desired report(s), the Reports View & Print screen appears.

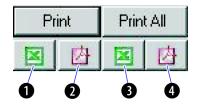


6 Patient tab.



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- 7 Using << > >>>, review the reports as needed.
 - displays first report
 - displays previous report
 - displays next report
 - displays last report
- **8** Print the desired reports:
 - To print all reports as a batch, Print All
 - To print the report currently displayed, Print
- **9** Print (save) the desired reports as a PDF or an XLS file:



- saves the selected report to XLS file.
- 2 saves the selected report as a PDF file.
- **3** saves all reports in this set as XLS files.
- 4 saves all reports in this set as PDF files.

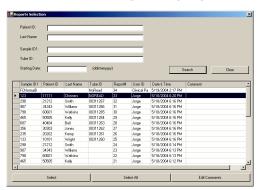
For a better understanding of the printed report, see PANEL REPORT

10 Exit when finished.

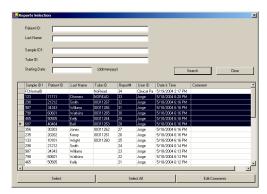
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Tips for Selecting Reports

To select only one report, highlight the desired row and Select.



- To select all reports, Select All.
- To select multiple reports in sequence:
 - a. Highlight the first report you want to select.
 - b. Press Shift while you the far left column of the last file to be selected.
 - c. Release Shift and the mouse button.
 - d. The selected rows are highlighted.
 - e. Select.



- To select multiple reports out of sequence:
 - a. Press and hold [Ctr] while you the far left column of each file.
 - b. The selected rows are highlighted.
 - c. Release Ctrl after all desired files are highlighted.
 - d. Select
- To deselect a report, press and hold the while you the far left column of the highlighted file. The deselected file is no longer highlighted.

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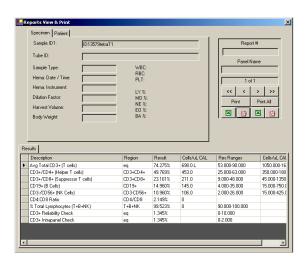
Editing Report Comments

If a comment was added to a template, all reports generated from that template will print that comment. However, you can add or delete comments from individual sample reports via the Edit Comments feature.

Do this procedure to edit comments for a specific report.



to open the Reports Selection screen.



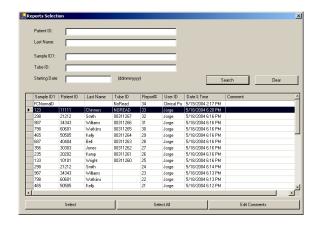
- **3** Search for the desired report using the following search criteria: patient ID, last name, sample ID1, or analysis date:
 - a. Enter the search criteria information.
 - b. Search.

Note: To do a global search and locate every sample report in the database, leave all

search criteria fields empty and Search. The last sample run appears at the top of the list.

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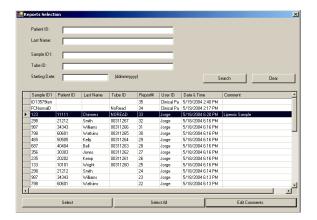
4 To select a report, highlight the desired row.



- Enter/edit the comment information (750 characters maximum).
 - a. Edit Comments and enter/edit the text.
 - b. **OK** when finished.



6 The comments appear on the Reports Selection screen.



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B.30 DATABASE ENTRY

The Database Information screen is where you enter/edit information for:

- · patient demographics,
- specimen information, and
- hematology information.

The database information prints on the patient panel report.

Entering/Editing Database Information

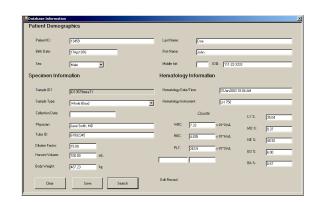
Keep in mind that you should enter/edit information prior to acquisition or listmode replay. The database links the information to CXP by Sample ID1.





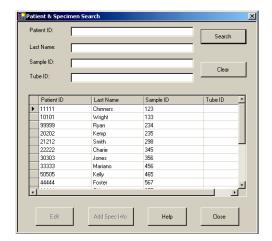
ATTENTION: Regarding hematology information, if Flow-Count was not used for a single-platform absolute count, the WBC and differential parameters may be entered prior to saving or replacing the listmode data files through a non-Flow-Count panel. Absolute counts will then be determined as a dual-platform with the manually entered hematology information and printed on the panel report by replaying the listmode files through a non-Flow-Count export panel.

- **3** To edit information or to add specimen information for an existing patient:
 - a. Enter the search criteria information (such as patient #).
 - b. Search



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- **4** Highlight the desired information to edit.
- **5** Verify that the correct information appears.
- 6 Edit or Add Spec Info

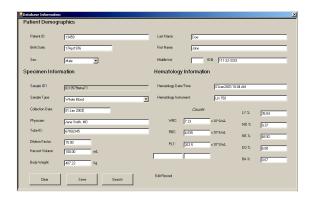


Note: allows you to edit all information except Sample ID. allows you to enter new specimen information for this patient, as well as edit existing information, including Sample ID; all demographic information is retained.

7 Edit the information as needed and



Save to save the changes.

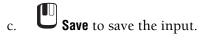


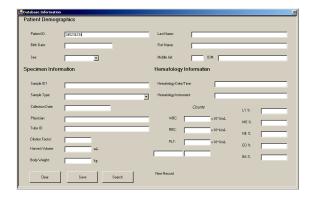
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To enter information for a new patient:



b. Enter the information.





B.31 DATABASE MANAGEMENT

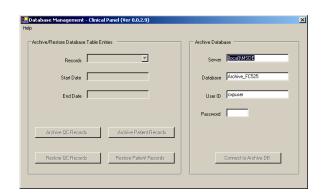
Use the Database Management screen to,

- Connect To Archive Database
- Archive QC Records
- Restore QC Records

Database Management Screen



on the Report Generator toolbar to display the Database Management screen.



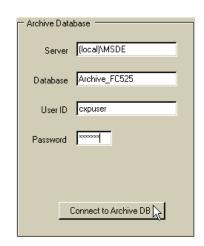
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Archive Database Records

Connect To Archive Database

- **1** Display the Database Management Screen.
- **2** Enter *cxppass* in the **Password** field.

Connect to Archive DB



Archive QC Records

Use Remove QC Records to move records from the system database to the archive database.

- 1 Connect To Archive Database.
- 2 and Before Dates or Between Dates.



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a. select the date.



If using **Between Dates**, repeat this step for the **End Date** field.



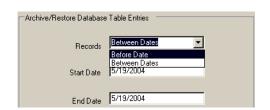




Restore QC Records

1 Connect To Archive Database.





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1

3 in Start Date field and

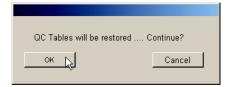
a. select the date.



If using **Between Dates**, repeat this step for the **End Date** field.



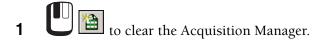




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B.32 USING RXP AUTOSETUP PANELS

You cannot use AutoSetup I panels with AutoSetup Scheduler. Follow this procedure to use AutoSetup I panels with CXP AutoSetup II.



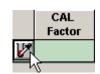
2 and select the AutoSetup I panel.

Note: You can only run 1 AutoSetup I panel at a time. If you open 2 AutoSetup I panels in Acquisition Manager, the software stops after the verify tube in the first panel and does not continue.

3 Enter the carousel ID number in the Acquisition Manager.



4 Assign a CAL Factor if necessary.



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to begin processing samples. The AutoSetup II Wizard processes the AutoSetup I panel. See Before Running the AutoSetup II Wizard in the Instructions for Use manual.

Note: Automatically approve steps where appropriate is enabled by default.



6 Check the verification tube results to ensure instrument settings are correct and



Note: When you run AutoSetup I panels with AutoSetup II, cytosettings are automatically saved to the associated settings file. AutoSetup II does not allow you to reject the updated cytosettings, therefore any unacceptable AutoSetup I panel results should be repeated.

B.33 USING NEGATIVE CONTROLS WITH AUTOSETUP

You can use a negative control sample in place of the calibration fluorospheres to set voltages and gain values in the AutoSetup process for research applications. If you wish to use a negative control sample, first an Application must be defined using the Application Definition Wizard. The Calibration protocol (*_stand.pro) of the Application's AutoSetup panel can then be modified to accommodate the use of the Negative Control.

To use a negative control with AutoSetup II you need to,

- Modify a standardization protocol to use as a negative control.
- Schedule the negative control protocol using the AutoSetup Scheduler.
- Run the negative control protocol using the AutoSetup II Wizard.
- When scheduling multiple Negative Control setups together, ensure that the same negative control sample is used for those applications.

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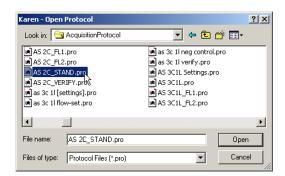
Modify A Standardization Protocol

1 Open the standardization protocol.



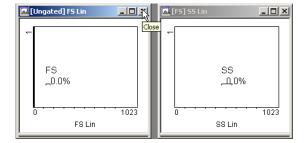
b. the standardization protocol.





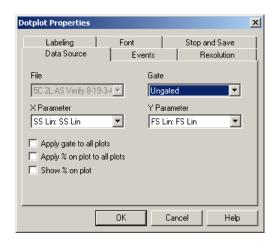
2 Delete the FS and SS histograms.

in the upper right corner of the FS and SS histograms.



3 Create a FS vs. SS dotplot.

and ensure that SS and FS are the X and Y parameters.

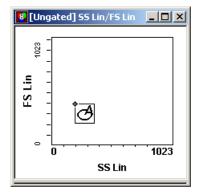


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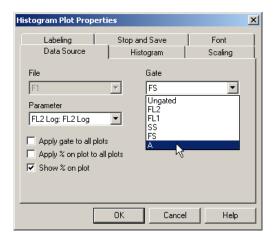
4 Create an AutoGate region on the dotplot.



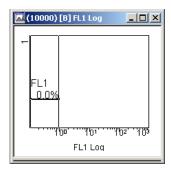
b. inside the FS vs SS dotplot.



- **5** Assign the AutoGate to the fluorescence histograms.
 - a. Ctr]+F.
 - b. under Gate
 - c. the gate created in step 4.

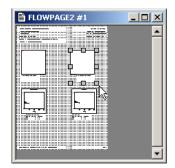


6 Adjust the linear regions in the fluorescence histograms so that they encompass the first decade.

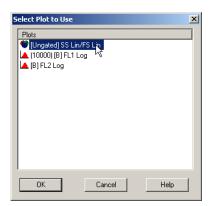


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7 Delete the invalid plots on the FlowPAGE.



8 Insert the new plot on the FlowPAGE.



g to save the Protocol.

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USING CXP SOFTWARE USING NEGATIVE CONTROLS WITH AUTOSETUP

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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.

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

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

AutoGate - A gate that automatically sizes to the population contained within the boundaries of the region. There are two types: Elliptical and Contour.

AutoSetup Wizard - A software feature that guides you through the processing of quality control materials to automatically establish your application settings.

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

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

Bar - A strip (or element) that is usually black and has minimum reflectance.

Bar-code symbol - A group of parallel bars and spaces with encoded characters. A bar-code symbol generally contains a leading quiet zone, a start character, data characters, a check character, a stop character, and a trailing quiet zone.

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

Button - The Workstation screens have pictures/icons (for example, it) that you select with the mouse to tell the software what to do. They are arranged on Toolbars for related functions.

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

Channel - In an analog-to-digital converter, the number of equally spaced divisions of the amplified input signal voltage. All FC 500 flow cytometer signals are resolved into 1024 channels. For dual-parameter histograms, the number of channels is reduced to 64, 128, or 256.

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

Check character (digit, check digit, checksum) - A character used to mathematically check that the bar-code symbol was read correctly.

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

Click - To press and release a mouse button.

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

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

Color compensation - The subtraction of:

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

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

Continuous code - Each character in the bar-code symbol starts with a black bar and ends with a white space. Characters follow after each other to form a continuous flow of code.

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

Controls and indicators - Instrument controls are the mechanisms you use to communicate with the instrument. Indicators are the mechanisms the instrument uses to communicate with you.

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

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

Cytosettings - Cytometer hardware settings. Consists of acquisition duration, acquisition maximum events, gating settings, disciminators, voltages, gains, baseline offset and compensation settings. Same as Instrument Settings in Acquisition Manager.

db - Abbreviation for decibels.

dc - Abbreviation for direct current.

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

Digit - See checksum.

DiOC5(3) - Abbreviation for oxacarbocyanine dye.

Discrete code - Each character in the bar-code symbol starts with a black bar and ends with a black bar. A white space gap (intercharacter gap) is between each character in the bar-code symbol.

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

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

Element - A bar or space in a bar-code symbol. There are narrow elements and wide elements.

GLOSSARY-2 PN 175570B

Event - A particle passing through the laser beam.

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

FDA - Abbreviation for fluorescein diacetate dye.

FITC - Abbreviation for fluorescein isothiocyanate dye.

Fixed code length - A specific length of sample ID code, (usually enabled when all sample IDs are the same length) to make sure that only one length sample ID is accepted.

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

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

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

FlowPAGE - A template for creating a report. It can include: data plots, statistics, and user attached text and/or graphics.

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

Fluorescent light (FL1, FL2, FL3, FL4 and FL5) 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

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

Indicators - See Controls and Indicators.

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

Intercharacter gap - The space between two characters in a bar-code symbol. Refer to discrete code. Not in all bar-code types.

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

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

Linear amplification - See gain.

Listmode data - A list of measurements from each cell.

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

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

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

MB - Abbreviation for megabyte.

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

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

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

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

Multi-tube CarouselLoader (MCL) - An automated sample loader for the instrument.

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

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

GLOSSARY-4 PN 175570B

Panel - In general, a group of protocols for analyzing a series of samples corresponding to one specimen. The Cytometer settings and regions can be passed on through the panel with identification of the primary samples.

Panel Wizard - A software feature that guides you through creating or editing a panel.

Panel Report - Patient data reports that are generated by the optional tetraCXP application.

PC7 - Abbreviation for phycoerythrin-cyanine tandem dye.

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

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

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

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

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

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

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

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

Prism - Phenotype plot for multicolor analysis.

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

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

QuickCOMP - Direct histogram manipulation to adjust Cytometer color compensation.

QuickSET - Direct histogram manipulation to adjust Cytometer voltages and gain.

Quiet zone - An area at each end of the bar-code symbol, which must be clear of marks, including readable text.

RD1 - Abbreviation for phycoerythrin dye..

PN 175570B GLOSSARY-5

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

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

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

Self-checking - A bar code that uses a checking algorithm to make sure the bar-code symbol was read correctly.

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

Sheath fluid - A balanced electrolyte solution.

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

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

Slider bars - They appear on dual fluorescence plots when you enable QuickCOMP mode on the Cytometer Control dialog box. These sliders are used to intuitively adjust the compensation coefficients and update the compensation values in the Cytometer Control Compensation Tab.

Space - A strip (or element) that is usually white and has maximum reflectance.

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

SQL - Standard query language.

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

Start and Stop characters - The characters that start and end the bar-code symbol and show the scan direction.

Symbology - A set of rules for encoding and decoding information contained in a bar-code symbol. Examples of symbologies include Code 39, Code 128, Interleaved 2-of-5, and Codabar.

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

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

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

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