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.
Issue A, Initial Issue, 8/03
CXP Software Version 1.0. Initial issue for customer distribution.

Issue B, 6/04
CXP Software Version 2.0.

Additions, changes or deletions were made at these locations:
1-3, 1-5, 1-31, 1-32, 2-1, 2-8, 2-9, 2-11, 3-1, 3-6, 3-11, 3-13, 4-4, 4-5, 4-7, 4-9, 4-10, 4-12,
5-7, 5-11, 5-15 & 6-10.

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, 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**

The information in your FC 500 Instructions For Use manual is organized as follows:

- **Chapter 1 SYSTEM OVERVIEW**
  Provides the intended use of the instrument, the controls and indicators, and information on using the system's software and Workstation.

- **Chapter 2 DAILY ROUTINE**
  Provides information on doing daily procedures, such as Startup and Shutdown.

- **Chapter 3 QUALITY CONTROL**
  Provides information on how to run quality control material to verify instrument setup.

- **Chapter 4 RUNNING SAMPLES**
  Provides information on how to run patient blood samples.

- **Chapter 5, OPERATION PRINCIPLES**
  Contains a description of flow cytometry, the normal sample flow through the instrument, how light collection and signal processing are accomplished and how the parameters are derived.

- **Chapter 6, TROUBLESHOOTING**
  Describes laser safety precautions and the location of the laser-related labels and how to troubleshoot the system with the error messages that appear on the screen.
Appendices
The appendices provide reference material on the following topics:
- **HOW TO...**
- **BAR-CODE SPECIFICATIONS**
- **INDEX**
  Provides page numbers for indexed information.

**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.
- **ë** indicates a key (such as **Û**).
- **ë + ë** indicates that the two keys listed (such as **Alt+F2**) are linked for a specific function and must be pressed in this sequence:
  a. Press down on the first key listed and while continuing to press it, press down on the second key listed.
  b. Release both keys at the same time.
- **ë ë** indicates to press and release the first key listed then press and release the next key listed. For example: **Y Enter**.
- Icons/buttons to select functions on the software screen are shown within text.
  Example: **Open**.

- **File Save** indicates to use the mouse to select the **Save** item on the **File** menu.
- **F1** through **F12** are special function keys.
- **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.
To Choose A Command With The Keyboard
After you press ì, 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.

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

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

- OK: Accepts the information you have selected or typed.
- Cancel: Stops the operation, ignoring your choices.

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

GRAPHICS
All graphics, including screens and printouts, are for illustration purposes only and must not be used for any other purpose.
The information in this section includes:

- INTENDED USE
- CYTOMETER CONTROLS AND INDICATORS
- MICROSOFT® WINDOWS® DESKTOP
- LEARNING THE BASIC OPERATING TECHNIQUES
- CXP SOFTWARE SHORTCUTS
- CREATING PROTOCOLS
- CREATING REGIONS
- CREATING GATES
- CREATING FLOWPAGES
- CREATING PANELS
- CREATING WORKLISTS
- USING THE ONLINE HELP SYSTEM
- PERFORMANCE CHARACTERISTICS

1.1 INTENDED USE

The Cytomics FC 500 is a system for the qualitative and quantitative measurement of biological and physical properties of cells and other particles. These properties are measured when the cells pass through one or two laser beams in single-file.

**IMPORTANT** The use of the data generated by this instrument depends upon the regulatory status of the reagents you use. If the reagent is labeled by the manufacturer “For Research Use Only. Not for use in diagnostic procedures,” US federal law prohibits the use of the data for diagnosis.

If, however, the reagent subsystem is labeled “For in vitro diagnostic use,” the entire system can be considered a medical device for generating data to be used for diagnosis.

The instrument can simultaneously measure forward scatter, side scatter, and five fluorescent dyes using one or two lasers at 488 nm and either 635 nm (Solid-state laser) or 633 nm (HeNe laser). Therefore, the instrument can perform correlated multiparameter analyses of individual cells.
Applications for the Instrument

The FC 500 is considered an equivalent system to the COULTER® EPICS® XL™ and the COULTER EPICS XL-MCL™ flow cytometers when used with the forward scatter Field Stop in the factory set position (1-19°).

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

- Plant cells
- Marine plankton
- Animal cells
- Bacteria

Table 1.1 Applications for the Instrument

<table>
<thead>
<tr>
<th>Applications</th>
<th>Cell Types</th>
<th>Sample Preparation (refer to the reagent’s package insert)</th>
<th>Measurements</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell surface antigens</td>
<td>Whole blood</td>
<td>ImmunoPrep™ reagents with the Q-PREP™ Workstation, the Multi-Q-Prep™ Workstation or the TQ-Prep™ Workstation</td>
<td>Cell size and granularity</td>
<td>Forward and side scatter (Log/Linear)</td>
</tr>
<tr>
<td></td>
<td>Buffy coats</td>
<td>Whole blood lysing reagent kit</td>
<td>FITC, RD1, ECD, PC5, PC7, APC and APC-CY7, PC7</td>
<td>Fluorescence one, two, three, four and five (Log/Linear)</td>
</tr>
<tr>
<td></td>
<td>Mononuclear cells</td>
<td>Cell gradient separation</td>
<td>Prism</td>
<td>Prism</td>
</tr>
<tr>
<td></td>
<td>Dissociated tissue</td>
<td>Fluorescent-labeled antibodies</td>
<td>Ratio</td>
<td>Ratio</td>
</tr>
<tr>
<td></td>
<td>Platelets</td>
<td>Thrombo Fix™ platelet stabilizer</td>
<td>Time</td>
<td>AUX/Peak</td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td></td>
<td>FORWARD PEAK TIME</td>
<td></td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>Whole blood</td>
<td>Various staining methods, including: Ethidium bromide, Propidium iodide, DNA Prep reagents, LeukaSure™</td>
<td>Cell size and granularity</td>
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<tr>
<td></td>
<td>Dissociated tissue</td>
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<td>Green, red, and orange fluorescence</td>
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<td></td>
<td>Frozen sections</td>
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<td>Cell size and granularity</td>
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<tr>
<td></td>
<td>Paraffin sections</td>
<td></td>
<td>Cell size and granularity</td>
<td></td>
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<td></td>
<td>Body fluids</td>
<td></td>
<td>Cell size and granularity</td>
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<tr>
<td>Kinetics</td>
<td>Whole blood</td>
<td>Various methods, including: Fluo 3 fluorescence</td>
<td>Cell size and granularity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Buffy coats</td>
<td></td>
<td>Green fluorescence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mononuclear cells</td>
<td></td>
<td>Time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue culture cells</td>
<td></td>
<td>FORWARD PEAK TIME</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.1 Applications for the Instrument (Continued)

<table>
<thead>
<tr>
<th>Cell function</th>
<th>Whole blood</th>
<th>Buffy coats</th>
<th>Mononuclear cells</th>
<th>Tissue culture cells</th>
<th>Various methods and dyes, including:</th>
<th>Cell size and granularity</th>
<th>Green fluorescence</th>
<th>Forward and side scatter</th>
<th>Fluorescence one</th>
</tr>
</thead>
<tbody>
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<td>DCFH-DA</td>
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<td>DiOC6(3)</td>
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<td></td>
<td></td>
<td>FDA</td>
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<td></td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>Whole blood</td>
<td>Thiazole orange</td>
<td>coriphosphine O (CPO)</td>
<td>Acridine Orange</td>
<td></td>
<td></td>
<td></td>
<td>Log/Linear Forward and Side Scatter</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>coriphosphine O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Log Fluorescence one</td>
<td></td>
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<td></td>
<td></td>
<td>O (CPO)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Log Fluorescence four</td>
<td></td>
</tr>
</tbody>
</table>

1.2 CYTOMETER CONTROLS AND INDICATORS

FC 500 Flow Cytometry System
SYSTEM OVERVIEW
CYTOMETER CONTROLS AND INDICATORS

1. MCL
2. Cytometer
3. HeNe laser option
4. Keyboard
5. Monitor
6. Mouse
7. USB box
8. Computer
9. Power Supply

Cytometer Indicator Panel

Blue means the Argon laser is on.
Red means the red laser shutter is open.
Green means Cytometer is on and in the Ready state.
Sample Flow Rate Indicators

1. Low Sample Flow
2. Medium Sample Flow
3. High Sample Flow

Signal Amplitude Indicators

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

1. FL1
2. FL2
3. FL3
4. FL4
5. FL5 or Aux
6. FS
7. Red means overrange
8. SS
9. White indicates Aux source and Aux channel (FL5)
SYSTEM OVERVIEW
CYTOMETER CONTROLS AND INDICATORS

Level Sense and Flow Indicators

1. Sheath Flow
2. Waste Full
3. Sheath Low

MCL (Multi-tube Carousel Loader)
Workstation

Power Supply
Your instrument has one of these configurations. Follow the procedures in the documentation that applies to your Power Supply configuration.

Universal Power Supply

Voltage-specific Power Supply

Printer (Optional)
This manual does not explain how to use the optional Printer. Operating instructions from the Printer manufacturer are included with the Printer.
1.3 MICROSOFT ® WINDOWS® DESKTOP

Windows 2000 Administrator Password

Be sure to maintain your Windows 2000 Administrator password in a secure location. If you lose or forget the Windows 2000 Administrator password, you must reimage your hard drive, causing the loss of all data.

Taskbar

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

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

Start Button

The Start button allows you to gain access to the computer programs and settings. When you click the mouse on  on the Taskbar, a menu appears showing the computer system's main programs and program group icons. The following CXP software “shortcut” options are available on this Desktop Menu Bar only if they have been installed.

Other standard Windows functions are also displayed and these are briefly described below:
Programs: displays a list of programs that are installed on the computer that you can start.

Documents: lists the most recently opened documents.

Settings: displays the user-configurable system components, including the Control Panel.

Search: displays a menu, which allows you to find Files, Folders or Computers to which you are connected if Networking.

Help: opens the Windows Help, which provides guidance in operating the Windows System.

Run: displays a screen dialog box for starting or loading a program or opening a file with an MS-DOS command.

Shut Down: displays a window dialog box with the options for Shutting Down the Computer System, restarting or other specific options as displayed.

Recycle Bin

You can delete files from your computer and place them in the Recycle Bin where you can, if necessary, retrieve them later. However, if you wish to permanently remove files, this is achieved by emptying the Recycle Bin.
My Computer

The My Computer icon on the Windows Desktop allows access to the contents of your computer (installed drives, the Control Panel, Printers and other icons as appropriate to the way the particular computer system was configured).

If you wish to see the contents of any drive or folder, double click the mouse on its icon. For Microsoft Windows 2000, the computer hard disk is known as C drive.

The Floppy Drive is Drive A, and any CD-ROM drive is known as D Drive unless Windows assigns another suitable letter.

Control Panel

The Control Panel holds the tools that you can use to alter the way Windows looks and works.

To view the Control Panel:
Click on Start and point the mouse to Settings.
Click on the Control Panel icon.

Note: The icons in the Control Panel vary according to the particular hardware and software settings for your computer. When you click on an icon, a short description of the setting is shown.
Double click on an icon to see the setting that you can change for that item.
For a full description of the possible settings, refer to the Microsoft Windows Help text and any books or manuals supplied with your computer system.

Using a Mouse

The Mouse, a handheld input device, controls the movement of a pointer on the computer screen. As the mouse is moved, the pointer moves on the screen. When you position the pointer over an object on the screen, you can click the mouse button to do different tasks.
You can click the mouse button to select files, double click to open and work in files, or click and drag to move files.

As most functions involve pointing at an object on the screen and then clicking the mouse button, the following show some of the common actions:

**Click:** Press and release the left mouse button ① once.

**Select:** Point at a particular object and click once.

**Double-click:** Quickly press and release the left mouse button ② twice.

**Right-click:** Press and release the right mouse button ① once.

**Point:** Move the mouse until the cursor is over the desired icon or object.

**Press:** Hold down a mouse button.

**Drag:** Hold down the left mouse button ① while moving the mouse.

You can switch left ① and right ① mouse buttons. For more information about using a mouse, see Windows Help.

The CXP software is used with a mouse and exclusively uses the left mouse button except where specifically indicated within the Help Text.

**Color Resolution**

For the best results, you need to reset your computer to a color resolution of at least 1024 x 768 pixels and High Color (16 bit).

1. Close down any applications that you may have running, including any that have been minimized on the Tool Bar.

2. Select **My Computer** and then choose the Control Panel icon.

3. From the **Control Panel**, choose the **Display** option.
4 Select the **Settings** tab.

5 Move the **Screen Area** slider to set 1024 x 768 color resolution.

6 Set the **Colors** dialog box using the drop down menu to set High Color (16 bit).

7 **OK** to complete the operation. You may need to restart the computer (a dialog box is displayed if this is required).

**Note:** 3dfx voodoo based video cards must be set to High Color (16 bit) for 3D acceleration to function correctly.
1.4 LEARNING THE BASIC OPERATING TECHNIQUES

Before reading the other chapters in this manual:

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

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

MCL Carousels

The FC 500 flow Cytometer starter kit has:

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

Bar-Code Labels

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

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

### Putting a Bar-Code Label on a Sample Tube

1. Carefully align the label with the tube.
2. Press the label down securely, including edges and corners, without wrinkles or folds.
   - 25.4 mm (1.0 in.) minimum
   - 7.5 degrees.
Putting Sample Tubes in a Carousel

- The orientation of a tube with a bar-code label does not matter. The MCL rotates the tube to find the bar-code label.
- Do not skip tube positions within a panel. The FC 500 flow cytometer does not skip a protocol in a panel when a carousel tube position is empty. If you lose a sample, delete that protocol from the panel using the Acquisition Manager.
- You can skip a single-tube position to separate two panels based on your tube location setup in Acquisition Manager.

Putting a Carousel in the MCL

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

3 Close the MCL cover.
Removing a Carousel from the MCL

1. Open the MCL cover.

2. Remove the carousel.
1.5 CXP SOFTWARE SHORTCUTS

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

Keyboard Shortcuts
Drag And Drop
Toolbar Buttons

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

- Ctrl+C: Copy
- Ctrl+V: Paste
- Ctrl+X: Cut
- Ctrl+Z: Undo
- Ctrl+Y: Redo
- Ctrl+W: Workspace Preferences
- Ctrl+T: Tile Special
- Ctrl+P: Print
- Ctrl+N: New Protocol
- Ctrl+S: Save Protocol
- Ctrl+O: Open Listmode File
- Ctrl+F: Format Plot

3 Close the MCL cover.
Drag And Drop

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

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

<table>
<thead>
<tr>
<th>Drag From…</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resource Explorer</strong></td>
<td></td>
</tr>
<tr>
<td>Worklist</td>
<td>Drag a stored Worklist to Acquisition Manager.</td>
</tr>
<tr>
<td>Panels</td>
<td>Add a panel to the current Worklist by dragging a panel to the current Worklist.</td>
</tr>
<tr>
<td>Protocols</td>
<td>Drag a protocol to the Workspace or Acquisition Manager.</td>
</tr>
<tr>
<td>Listmode files</td>
<td>Drag a file to a series of plots, or with Ctrl pressed ONLY the current plot.</td>
</tr>
<tr>
<td>Histogram files</td>
<td>Drag histograms to an overlay plot.</td>
</tr>
<tr>
<td><strong>Protocol Explorer</strong></td>
<td></td>
</tr>
</tbody>
</table>

Ctrl + D: Duplicate Plot
Ctrl + Q: Listmode QuickCOMP
Ctrl + L: View Cytometer Log
Ctrl + 1: Create new Dot Plot
Ctrl + 2: Create new Histogram Plot
Ctrl + 3: Create new Contour Plot
Ctrl + 4: Create new Density Plot
Ctrl + 5: Create new Overlay Plot (CXP Analysis Software only)
Ctrl + 6: Create new Tomogram Plot (CXP Analysis Software only)
Ctrl + 7: Create new Surface Plot (CXP Analysis Software only)
Ctrl + 8: Create new Prism Plot
Ctrl + 9: Create new Legend Plot
Ctrl + 0: Create new Info Plot
Alt + F4: Exit CXP software
F1: Context Sensitive Help
F5: Refresh Screen
F7: Publish to Excel (or text file)
F9: Start Acquisition
F10: Stop Acquisition
F11: Pause Acquisition
F12: Abort Acquisition
### Toolbar Buttons

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

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

The Toolbar elements are:

- FILE OPTIONS TOOLBAR
- PLOT OPTIONS TOOLBAR
- REGIONS OPTIONS TOOLBAR
- GATE, COLOR, STATS AND HELP TOOLBAR
- FLOWPAGE TOOLBAR
- ACQUISITION MANAGER TOOLBAR
- AUTOMATOR TOOLBAR
- OVERLAY TOOLBAR (Analysis only)
- CYTOMETER TOOLBAR.

### Drag From… Action

<table>
<thead>
<tr>
<th></th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gates</td>
<td>Drag a gate to gate ALL plots on a region, or with Ctrl pressed ONLY the current plot.</td>
</tr>
</tbody>
</table>

#### AutoMATOR Setup

<table>
<thead>
<tr>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearrange files</td>
<td>Reorder files for playback using the AutoMATOR application.</td>
</tr>
</tbody>
</table>

#### Modify Color Precedence

<table>
<thead>
<tr>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reorder Gates</td>
<td>Drag gates into the required precedence order.</td>
</tr>
</tbody>
</table>

#### Plots

<table>
<thead>
<tr>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regions</td>
<td>Drag copies of regions from one plot to another, with Ctrl pressed gate logic is also copied.</td>
</tr>
<tr>
<td>Plot Images</td>
<td>Drag the current plot image to a third party application (such as, MS Paint, Power Point®).</td>
</tr>
<tr>
<td>Overlay Plot</td>
<td>Reorder histograms in the overlay.</td>
</tr>
<tr>
<td>Statistics</td>
<td>Reorder the statistics under the plots.</td>
</tr>
</tbody>
</table>

#### Tile Special

<table>
<thead>
<tr>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plots</td>
<td>Reorder plots on the desktop.</td>
</tr>
</tbody>
</table>

#### Cytometer Control (Cytometer Only)

<table>
<thead>
<tr>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Reorder parameters in the “selected Parameter” list.</td>
</tr>
</tbody>
</table>
1.6 CREATING PROTOCOLS


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

3. Choose the Parameters to be acquired and OK.

4. If the parameters have changed,
   a. OK.
   b. Enter the parameter name and Save.
   c. Close on the Cytometer Control screen.

5. Create the Plots required for acquisition.

6. If analysis is to be performed during acquisition, create the required Regions and Gates, and also select the required statistics from the Analysis >> Select Results option.
7. **File** → **Save Protocol As** to save the protocol.

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

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

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

11. Set up the Worklist and run samples.

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

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

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

Create Polygonal Regions
Create Rectangular Regions
Create Quadrant Regions
Create Linear Regions

Create Polygonal Regions
See also: Interactive Polygonal Region Editing in the Reference manual.

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

2 A Polygonal Region cursor is displayed on screen.

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

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

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

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

To change the Region type, and stay in the current window, choose from the Region menu or click a Region shortcut button for whatever Dual Parameter Region type you wish.
7 Close the Region by returning the cursor to the starting box and clicking to conclude Region creation.

8 The Region Name appears on the plot.

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

Create Rectangular Regions
See also: Interactive Rectangular Region Editing in the Reference manual.

1 Highlight a dual parameter plot.

2 A Rectangular Region cursor \( \times \) appears in the highlighted plot.

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

6 The Region Name appears on the plot.

Create Quadrant Regions

1 Highlight a dual parameter plot.

2 A crosshair cursor is displayed in the current window.

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

5 Move the regions to the required position, if needed, then to set this position.
When drawing a Region, if you decide that it is not the one you want, press `Esc` before the Region is completed. The Region is deleted.

**Note:** Quadrant Region is a special case of rectangular Regions - four Rectangular Regions are simultaneously created with one mouse click and exist thereafter as four independent Rectangular Regions (with the exception that they do not satisfy Automatic Gate Creation). Deleting one or more still leaves remaining Regions intact if using Region/View/Modify Delete. If you use the graphical quadrant delete, then all four Quadrant Regions are deleted as a group.

### Repositioning a Quadrant Region

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

2. Reposition the region.
   a. + hold and drag ↔ to move the vertical divider.
   b. + hold and drag ↓ to move the horizontal divider.
   c. + hold and drag ↔ to move the Quadrant region.

3. Release the mouse button.

See also: [Interactive Quadrant Region Editing](#) in the Reference manual.
Create Linear Regions

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

1. Highlight a single parameter histogram.

2. A Linear Region cursor appears in the current window.

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

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

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

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

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

See also (in the Reference manual):
Interactive Linear Region Editing
Status Bar (Linear)
X Coordinate
Integral
Create Multiple Linear Regions

1. Highlight a single parameter histogram and 

2. A Parallel cursor $\mathbf{\parallel}$ appears in the current window.

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

4. Move the cursor left or right, up or down until the desired end point of the Region is reached. \( \text{Accept settings and anchor the region.} \)

5. Repeat this procedure until all Regions are set.

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

See also: Interactive Multi-Linear Region Editing in the Reference manual.
1.8 CREATING GATES

Create AutoGate

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

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

2. or .

3. on the population to AutoGate.
4 To specify the AutoGate Sensitivity and Travel,
   a. Click the region.
   b. Click the right mouse button on the region and Region Properties.
   c. Specify the AutoGating Sensitivity and Contour Travel, if necessary, and OK.

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

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

7 Repeat steps 5 and 6 to assign the AutoGate to other plots or Apply gate to all plots. Note: If you select Apply gate to all plots you must go back to the plot that contains the AutoGate and unassign the AutoGate from the plot.
SYSTEM OVERVIEW
CREATING GATES

Convert a Polygonal Region to an AutoGate Region

1. 

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

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

Automatic Gate Creation

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

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

FlowPAGE Example

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

1.  

2.  

3. Insert Flowpage Plots.

4. Insert a FlowPAGE Statistics Table.

5. Insert a FlowPAGE Textbox.

6. Click the right mouse button on any object on the FlowPAGE to select additional formatting options.
1.10 CREATING PANELS

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

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

2. Enter the New Panel name.

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

4. **Export the results of this panel to the Report Generator** if you want a Patient Panel Report generated when you run this panel. See Panel Report in the Reference manual.
5 [Image]

6 Choose your Protocol Settings.
   Use Plots and Gates from Previous Test
   Use Plots and Gates from Protocol
   Use Instrument Settings

7 Choose your Region Settings.
   Use Regions from Protocol
   Carry Regions from Previous Protocol
   Use Regions from This Protocol:

8 Choose your Instrument Settings.
   Use Instrument Settings from Previous Test
   Use Instrument Settings from External File

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

10 Repeat steps 6 through 9 for each test.
Use Plots and Gates from Previous Test
Allows you to use Gates and Plots from a previous Test but is not active for the first Test within a Panel.

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

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

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

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

Use Regions from Protocol
This option loads all regions stored within the current protocol.

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

Use Regions from This Protocol:
This option loads the regions and region positions from the selected protocol.

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

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

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

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

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

Note: If no Worklist is visible, View Acquisition Manager to display the Worklist pane.

Note: Unless the current Worklist has been saved using the Save Worklist button within the ACQUISITION MANAGER TOOLBAR, current worklist settings are not available.

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

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

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

Worklist Panel

1. Select which panel you wish to open and Open.
3 After all the information has been added, you can use Drag And Drop to change the order.
You can use this option to add another Panel to an existing Worklist.

Worklist Test

to insert a single Row at the end of the current Panel.
1.12 USING THE ONLINE HELP SYSTEM

Your Cytomics FC 500 system provides an online Help system that allows you to search for information on specific system-related topics through the Contents, Index, and Search options.


When you access the Help menu, there are three options available: CXP Help, About..., and Online Support.

Access Online Help

Use one of these options to access the online Help system.

Help

CXP Help.

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

How to Use Help

on the Help Navigation Bar.

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

1 Toolbar
2 Navigation Bar
3 Topic Pane
4 Navigation Pane
Help Menu

CXP Help
Opens the FC 500 CXP online help.

About…
Displays:

- CXP software copyright
- CXP software version information
- Runtime Protocol or New Protocol (when listmode file is replayed) or Locked Protocols.
- Algorithms… that takes you to a list of current analysis algorithms and their descriptions.

If you are asking for technical support by telephone, fax, or email, please quote the CXP software build number, which is accessible from menu Help >> About…, and ensure you know the Romlock number which is written on the body of the Romlock.

Online Support

- Beckman Coulter Web Site
  If you have an Internet connection and you click the mouse on http://www.beckmancoulter.com you can access the Beckman Coulter Web Site through your Web Browser program.
- ACS Web Site
  If you have an Internet connection and you click the mouse on http://www.appliedcytometry.com you can access the Applied Cytometry Systems Web Site through your Web Browser program.

You can then access the latest information concerning the up-to-date software releases and other items of interest.

1.13 PERFORMANCE CHARACTERISTICS
Refer to the package insert of the Quality Control Materials listed in the Reference manual for the performance characteristics for the preparation method you are using.
2.1 BEFORE YOU BEGIN

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

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

2. Read the SYSTEM OVERVIEW chapter. It contains instructions for
   - CYTOMETER CONTROLS AND INDICATORS
   - LEARNING THE BASIC OPERATING TECHNIQUES.

3. Read each procedure entirely.

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

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

2.2 DAILY STARTUP

Perform the following steps to start up the system. If you have set up CYTOMETER AUTO STARTUP and the Cytometer is running, skip ahead to Check the Power Supply.
Check Waste and Reagent Levels

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

2  Check the sheath fluid level and fill the sheath container if necessary.
3  Check the cleaning agent fill level ① and fill the cleaning agent container if necessary.

Power the Computer and Cytometer ON

Turning On Power

Turn on the system at the computer.
Logging Onto Windows Software

When the Log on to Windows screen appears,

a. Check that the User name field displays Administrator or your Windows log-in user name. If not, type in your user name.

   Note: When first installing the software, the User name field must display Administrator.

b. Ensure the checkbox is unchecked.

c. Enter your Password and 

   ![Login Screen](image)

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

Logging Onto CXP Software

1. ![Start Button](image) to start the software and power up the Cytometer.

   Allow about 40 minutes to warm up the system before performing QC or running samples.
2 At the CXP Cytometer Startup Wizard [Page 1 of 2] screen:
   a. Highlight your User ID.
   b. Enter your Password.
   c.  
      ![Next button]
   Note: For additional information about this screen, see MULTI-USER SIGN ON. If you need to set up User IDs, see User Administration.

3 Select a protocol, if needed.

4  
   ![Finish button]

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

   - Startup in Process
   - Verification
   - Initialization
   - Awaiting Sample
1. Open the Power Supply door and check the WATER TRAP, AIR FILTER, and VACuum FILTER. Call your Beckman Coulter Representative if:
   - The TRAP is >1/3 full.
   - The FILTERS have any fluid.

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

3. Check the SYStem PRESSure gauge. If it does not read between 28 and 32 psi, do the following:
a. Pull the PRESSure ADJuster knob out toward you.

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

c. Push in on the knob to lock it into place.
4 Check the VACuum TRAP.
   If it is >1/4 full of fluid CLEAN THE VACUUM TRAP.

5 Close the Power Supply door.

6 Record the startup checks on the Maintenance Log or on the Action Log Sheet.

Additional Start Up Checks

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

2 Refer to the manuals that came with your Printer to:
   - Perform Printer diagnostics.
   - Check that there is an adequate paper supply in the Printer.
   - Check ink cartridges if you have a color Printer and replace if necessary.
2.3 DAILY SHUTDOWN

When to Shut Down the Cytometer

- Shut down the instrument at least once a day, even if it is intended for use 24 hours per day.
- Leave the instrument shut down for at least 30 minutes before restarting.
- Shutdown and restart the system computer once per day to allow the virus protection program to run. See Power the Computer and Cytometer OFF and Power the Computer and Cytometer ON. Do not start a full disk virus scan while running CXP software.

Before Performing Shut Down


Power the Computer and Cytometer OFF

1. to put the Cytometer in the Idle mode.

2. in all open windows.

3. or .
DAILY ROUTINE
DAILY SHUTDOWN

4  

4. Press `Start` ➔ `Shut down` to turn off the Cytometer.

5  

5. Press `Start` ➔ `Shut down` to shutdown the Workstation.

6  

6. When the **Shut Down Windows** screen appears:
   Ensure **Shut down or Standby** appears below **What do you want the computer to do?**

7  

7. Press `OK`.
   The Cytometer automatically turns off.

8  

8. Turn off the monitor and Printer separately.
After Instrument Shut Down

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

2. Keep the system shut down for 30 minutes. Before running samples, do the daily startup and quality control procedures.
   Reminder:
   - Clean the air filters once a week.
   - Clean the sample probe and sample head once a week.
   - Clean the sheath fluid container once a month.
   - Clean the cleaning agent container every 60 days.

3. Record daily shutdown and cleaning on the Maintenance Log or on the Action Log Sheet.
2.4 EXTENDED SHUTDOWN

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

1. Remove the cleaning agent and sheath fluid containers.

2. Rinse the inside of both containers with water.
3 Replace the cleaning agent and internal sheath fluid containers. Fill both containers with water instead of reagent.

4 Perform the Routine Cleaning Procedure. Use tap water in tubes 1-4 instead of bleach in tube 1 and IsoFlow sheath fluid in tubes 2-4.

5 When you start up the instrument for the first time after the extended shutdown,
   - Clean the sheath fluid container
   - Clean the cleaning agent container
   - Fill the sheath fluid container
   - Fill the cleaning agent container
   - Perform the Routine Cleaning Procedure
   - Perform DAILY QC before running samples.
2.5 CYTOMETER AUTO STARTUP

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

1. Using Windows Explorer, double click on C:\CXP\Tasks.exe.

2. On the Task screen, enter C:\CXP in the Start in: field.

You can also enter comments and set a password, although they are not required.
4  **Schedule** to schedule when you want the Startup or Shutdown task to occur.

**New** and enter your settings.

5  **Settings** to specify additional settings.

6  If you want to set up automatic Cytometer shutdown, **Shutdown** and perform steps 3 through 5.
DAILY ROUTINE
CYTOMETER AUTO STARTUP
3.1 INTRODUCTION

Perform the following quality control checks to ensure that your system is working accurately and precisely. The protocols needed for these quality control (QC) procedures are included with your system Software. The package inserts for your Quality Control Materials have instructions for establishing your laboratory's normal ranges for daily use.

You must re-establish your laboratory's ranges:

- When you use a new lot of fluorospheres.
- Whenever a major part of the system has been serviced or replaced (for example, laser alignment or replacement, PMT replacement).

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

3.2 QC PROCESSES

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

<table>
<thead>
<tr>
<th>QC Process</th>
<th>QC Material Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verify fluidics and laser alignment</td>
<td>Flow-Check™ Fluorospheres (Flow-Check 675 Fluorospheres, Flow-Check 770 Fluorospheres). Verify HPCV versus expected value. Export results to the QC Database and review the QC data.</td>
</tr>
<tr>
<td>Adjust high voltage and gain for a given application</td>
<td>Flow-Set™ Fluorospheres (Flow-Set 675 Fluorospheres, Flow-Set 770 Fluorospheres). Ascertain target mean position based upon application and adjust high voltage and gain daily to that target. Export results to the QC Database and review the QC data.</td>
</tr>
<tr>
<td>Monitor instrument linearity</td>
<td>Immuno-Brite™ Fluorospheres.</td>
</tr>
<tr>
<td>Perform absolute counts</td>
<td>Flow-Count™ Fluorospheres.</td>
</tr>
<tr>
<td>Adjust color compensation for a given application</td>
<td>For AutoSetup applications, Cyto-Comp™ Cells or whole blood stained with QuickCOMP™ 2 or QuickCOMP 4 kit. Use single color stained samples with each fluorochrome used in your application. For example: CD45-FITC, CD45-PE, CD45-ECD, CD45-PC5 or CD45-PC7.</td>
</tr>
<tr>
<td>Verify correct settings with an application Control</td>
<td>Update the control protocol with the settings derived from above. Run a biological control equivalent to the application, such as Immuno-Trol™ Cells, Immuno-Trol Low Cells, Cyto-trol™ Control Cells, Stem-Trol™ Control Cells, or a normal whole blood. Export results to the QC Database and review the QC data.</td>
</tr>
</tbody>
</table>
3.3 DAILY QC

Daily QC consists of:

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

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

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

Preparing AutoSetup Samples

1. Prepare the appropriate Flow-Check fluorospheres for the applications you need to run. The fluorospheres you use to perform alignment verification depend upon which application you are using, as shown in the chart below. Follow the package insert instructions for mixing and handling fluorospheres.

<table>
<thead>
<tr>
<th>Application</th>
<th>Flow-Check</th>
<th>Flow-Check 770</th>
<th>Flow-Check 675</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS-SS-FITC-PE-ECD-PC5</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS-SS-FITC-PE-ECD-PC5-PC7</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>FS-SS-FITC-PE-ECD-APC</td>
<td>✔</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>FS-SS-FITC-PE-ECD-APC-PC7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>
2 Prepare the appropriate Flow-Set fluorospheres for the applications you need to run. The Flow-Set fluorospheres tube is used to set the detector gains and voltages to the required level. The fluorospheres you use to perform standardization depend upon which application you are using, as shown in the chart below. Follow the package insert instructions for mixing and handling these fluorospheres.

<table>
<thead>
<tr>
<th>Application</th>
<th>Flow-Set</th>
<th>Flow-Set 770</th>
<th>Flow-Set 675</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS-SS-FITC-PE-ECD-PC5</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS-SS-FITC-PE-ECD-PC5-PC7</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>FS-SS-FITC-PE-ECD-APC</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>FS-SS-FITC-PE-ECD-APC-PC7</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

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

- Stain with appropriate single color reagents, such as CD45, in each fluorochrome.

4 Prepare an AutoSetup verification tube.
   a. Use the appropriate amount of Immuno-Trol Cells or Immuno-Trol Low Cells according to the instructions on the package insert or reconstitute the Cyto-trol Control Cells according to the instructions on the package insert.
   b. Stain the cells with the monoclonal antibodies you use for the protocol or panel.

5 Run the AutoSetup Scheduler.
3.4 AUTOSETUP SCHEDULER

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


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

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

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

3. Enter the Carousel number and Schedule.
3.5 AutoSetup II WIZARD

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

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

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

Before Running the AutoSetup II Wizard

- Check that the DAILY STARTUP procedure was performed.
- Allow about 40 minutes to warm up the system.
- Check the Cytometer status message. Start this procedure when the Cytometer status message **Awaiting Sample** appears. Record any error messages in your instrument's logbook.
- Set up Workspace Preferences - Acquisition Options to print results.
- Set up Workspace Preferences - LMD File Name to specify listmode file names.
• Follow the directions in the Flow-Check fluorospheres package insert to establish expected range values for your laboratory.
• Establish Flow-Set fluorospheres target channels for your laboratory and modify the default application definitions if necessary. Follow the directions in the Flow-Set fluorospheres package insert.
• Set up the QC Products that you need to use with AutoSetup applications.
• Set up region statistics exporting and assign QC products to regions.
• If acquisition ends as a result of duration (not stop count) while running AutoSetup II, select \( \text{Continue} \) on the Cytometer Control toolbar to continue.

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

2 After data acquisition starts view all histograms to ensure peaks fall within regions.
   a. \( \text{Setup Mode} \) on the Cytometer Control Acquisition Setup Tab.
   b. Adjust voltages to place peaks within regions
   c. Uncheck Setup Mode.
Note: When running a negative control verify that the AutoGate is around the population of interest in the FS vs SS dotplot. If you need to, adjust populations.

a. **Auto Adjust Disabled** as soon as the AutoSetup wizard displays.
b. adjust voltages to place the lymphocytes in a suitable position.
c. adjust voltages to place the fluorescence populations within the first log decade.
d. Acquire.

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

The compensation tubes are used to calculate the compensation coefficients required, all these tubes are run in the same way.
This process is repeated for each of the compensation tubes (for example: FL2, FL3, FL4, and FL5).

Verification samples are run to ensure the calculated coefficients are satisfactory for the samples and antibodies you are using in your tests. Note: If multiple applications are scheduled together, samples common to the applications are processed in tandem without dropping the tube. The protocol is displayed during processing.

3  

to print verification tube results and advance to the approve screen.
4  Check the printed verification tube results to ensure instrument settings are correct and approve.

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

5  Review QC Results in the QC Database.
3.6 APPLICATION DEFINITION WIZARD

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

Before Running the Application Definition Wizard

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

1. Tools ➔ AutoSetup Application Definition.

2. One of the following options:
   - Application available only to current user
   - Application available to all users (common).

3. One of the following options:
   - Create a new application definition
   - Modify an application definition

   and then Next ➔.
4. Click **Browse...** and select the protocol to use as the base protocol for the application. The Protocol Summary displays a description of the parameters and laser setup for the selected protocol.

5. Select the filter block to use for the application and **Next >**. Note: An alignment application may not have a User defined Filter Block selected. Alignment applications must have either a 1-Laser Filter Block or 2-Laser Filter Block selected.

6. Select the forward scatter collection angle to use for the application and **Next >**.
7. Select the dyes you use for each fluorescence detector and...

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

Note: If you are running a 5 color 2 laser application, enter Fluorescence and Forward Scatter target channels for the Flow-Set 675 and Flow-Set 770 Fluorospheres.
9 Enter names for the application and the instrument settings file and

![Next button]

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

![Next button]

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

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

![Next button] to print the summary report and ![Finish button] to exit the wizard.
Setting Up QC Products

1. Click to display the Report Generator toolbar.

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

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

4. OK.

Exporting Region Results To QC Database and Assigning QC Products

1. on a region to make it active.

2. Right mouse click on the region and Region Properties.
3 Ensure that ✔ Region Statistics exported for Quality Control.

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

5 OK and save the protocol.

Review QC Results

1 to display the Report Generator toolbar.

2 on the Report Generator toolbar to display the QC Levey Jennings screen.
3. Check next to Application and select an application.

4. Click Template -> Open Template.

5. Select the desired template and click Open.

6. Repeat steps 3 - 5 to view other QC data plots. See QC Levey Jennings Screen in the Reference manual for information about creating and editing QC plots.
4.1 SAMPLE REQUIREMENTS

At least 0.5 mL of prepared sample is needed. It must be in a 12- x 75-mm test tube. Samples analyzed on the instrument must be in a single-cell suspension. Typically, cells are prepared before they are analyzed. The method used to prepare a specimen depends on the sample type and the assay desired. For example, a TQ-Prep workstation combined with a PrepPlus or PrepPlus 2 lets you prepare antibody-labeled cells from an anticoagulated whole-blood specimen for surface marker analysis.

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

The instrument can measure cells that are between 0.5 µm and 40 µm in diameter.

4.2 BEFORE RUNNING SAMPLES

1. Check that the DAILY STARTUP procedures were done.

2. Check that the DAILY QC procedures were done.

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

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

5. Read the Important Information on the Support tab in the Resource Explorer.

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

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

Show Me an example of MCL Automatic Mode sample processing.

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

1 Prepare samples according to the reagent package insert.

2 Place the sample tubes in a carousel.
**WARNING** Risk of injury. Do not open the MCL cover while the MCL is moving. To avoid injury, wait until the MCL stops moving before opening the MCL cover.

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

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

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

6 Enter the carousel ID number in the Acquisition Manager.

<table>
<thead>
<tr>
<th>Carousel No.</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>1</td>
</tr>
</tbody>
</table>

**IMPORTANT** Risk of erroneous results if the sample becomes diluted. When operating the Cytometer, if the MCL cover is opened, an audible alarm sounds and a warning message is displayed. If acquisition completes while the MCL cover is opened, do not use the sample for subsequent processing. Under this condition, the current sample may be diluted at the end of acquisition.

7 Use the acquisition to begin processing samples.

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

- **Awaiting Sample**
- **Preparing Sample**
- **Acquiring**
- **Stopping**.

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

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

MCL Manual Mode operates the same as MCL Automatic Mode except the MCL stops after processing each tube to allow you to edit the Sample ID.

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

1. Prepare samples according to the reagent package insert.

2. Place the sample tubes in a carousel.
WARNING  Risk of injury. Do not open the MCL cover while the MCL is moving. To avoid injury, wait until the MCL stops moving before opening the MCL cover.

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

4  Close the MCL cover.

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

   Edit Sample IDs on Workspace Preferences - Acquisition Options.
6 Use the Acquisition Manager Toolbar or Drag And Drop to select the Worklist, Panel or Protocol you want to use.

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

7 Enter the carousel ID number in the Acquisition Manager.

<table>
<thead>
<tr>
<th>Carousel No.</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>1</td>
</tr>
</tbody>
</table>

8 to place the Cytometer in MCL Manual Mode.

IMPORTANT Risk of erroneous results if the sample becomes diluted. When operating the Cytometer, if the MCL cover is opened, an audible alarm sounds and a warning message is displayed. If acquisition completes while the MCL cover is opened, do not use the sample for subsequent processing. Under this condition, the current sample may be diluted at the end of acquisition.

9 to begin processing samples.
10 Enter the tube location to process and Run. During the sample cycle the following series of Cytometer status messages appears:
.finishing process:

Awaiting Sample
Preparing Sample
Acquiring
Stopping.

11 Observe the Events/Sec counter on the Status Bar to monitor data acquisition. Note: While running a Worklist, if the Tube ID in the Worklist does not match the bar code on the tube, a system message appears. After you acknowledge the message, the mismatched tube is aborted and the processing stops for the rest of the carousel.

12 If you enabled Edit Sample IDs on Workspace Preferences - Acquisition Options, edit the sample ID after the tube finishes processing and OK. Note: After the first tube in a panel, Sample ID 1 cannot be changed.

13 Enter the location of the next tube to process and Run.

14 Repeat steps 12 and 13 to process additional tubes.
15 Verify all sample IDs before reporting results.

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


### 4.5 Running Samples - Single Tube Mode

The Single Tube Mode operates by introducing tubes individually into the MCL through the MCL Tube Access door. **Show Me** an example of Single Tube Mode sample processing.

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

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

1 Prepare samples according to the reagent package insert.

2 If you want the MCL to stop after processing each tube to allow you to edit the Sample ID, **Edit Sample IDs** on Workspace Preferences - Acquisition Options.
3 Use the Acquisition Manager Toolbar or Drag And Drop to select the Worklist, Panel or Protocol you want to use.

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

4 to place the Cytometer in Single Tube Mode. The MCL moves to put carousel position 10 under the Tube Access door.

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

5 Open the MCL Tube Access door and place the sample tube in carousel position 10.

IMPORTANT Risk of erroneous results if the sample becomes diluted. When operating the Cytometer, if the MCL cover is opened, an audible alarm sounds and a warning message is displayed. If acquisition completes while the MCL cover is opened, do not use the sample for subsequent processing. Under this condition, the current sample may be diluted at the end of acquisition.
6 Close the MCL Tube Access door and to begin processing samples. During the sample cycle the following series of Cytometer status messages appears:
- Awaiting Sample
- Preparing Sample
- Acquiring
- Stopping.

7 Observe the Events/Sec counter on the Status Bar to monitor data acquisition. Note: While running a Worklist, if the Tube ID in the Worklist does not match the bar code on the tube, a system message appears. After you acknowledge the message, the mismatched tube is aborted and the processing stops for the rest of the carousel.

8 Verify all sample IDs before reporting results.

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

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

Note: After the first tube in a panel, Sample ID 1 cannot be changed.
10 Repeat steps 5 through 9 to process additional tubes.

![Close button] to exit Single Tube Mode and return to Automatic Mode.

5.1 WHAT THIS CHAPTER EXPLAINS

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

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

5.2 SAMPLE FLOW

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

Sample Loading

The sample carousel has bar-code labels that identify the carousel and the tube position number. Also, you can put bar-code labels on the sample tubes. See Appendix B, BAR-CODE SPECIFICATIONS.

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

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

The sample probe is cleaned automatically when sample flow ends.

Hydrodynamic Focusing

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

Hydrodynamic focusing occurs in the flow cell.

The flow cell (Figure 5.1) contains a rectangular channel. A pressurized stream of sheath fluid enters the channel at the lower end and flows upward. The sensing area of the flow cell is at the center of the channel.

While the sheath stream is flowing through the channel, a stream of sample is injected into the middle of the sheath stream. As shown in Figure 5.1, the sheath stream surrounds, but does not mix with, the sample stream. The pressure of the sheath stream focuses the sample stream so that the cells flow through the laser beam single file.
Figure 5.1 Flow Cell (Hydrodynamic Focusing)

1. To waste
2. Sensing area has rectangular channel
3. Sample stream
4. Sheath stream
5. Cells
6. To waste
7. Sample stream enters here
8. Sheath stream enters here
9. Direction of laser beam
5.3 LASER BEAM SHAPING

Before the laser beam reaches the sample stream, cross-cylindrical lenses focus the beam (see Figure 5.2). Focusing keeps the beam perpendicular to the sample stream flow while making the beam small enough to illuminate only one cell at a time.

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

Figure 5.2 Laser Beam Shaping

- Cross section before lenses
- Cross section before vertical lens
- Cross section before horizontal lens
- To waste
- Flow cell
- Vertical beam shaping lens
- Laser beam direction
- Horizontal beam shaping lens
- Laser beam
5.4 CELL ILLUMINATION

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

Forward Scatter

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

Side Scatter and Fluorescent Light

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

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

5.5 LIGHT COLLECTION, SEPARATION AND MEASUREMENT

Forward Scatter Collection

The FS sensor (see Figure 5.3 and Figure 5.4) collects the forward scatter—the laser light that is scattered at narrow angles to the axis of the laser beam. When light reaches the FS sensor, the sensor generates voltage pulse signals. These signals are proportional to the amount of light the sensor receives. As explained in Heading 5.6, SIGNAL PROCESSING, the signals are processed to measure the characteristics of the cells that scattered the light.

The FS sensor contains a Field Stop that allows you to collect different angles of Forward Scatter. The standard (factory set) position collects FS angles of 1 to 19°. The second position allows you to collect a lower angle of FS, 1 to 8°. See POSITION THE FIELD STOP.
Figure 5.3 Optical System with Optional Red Solid-state Laser

1. Argon (488) laser
2. SS sensor
3. Solid-state (635) laser (optional)
4. FL3
5. FL4
6. Field stop
7. FL2
8. FL5
9. FS sensor
10. Flow cell

Figure 5.4 Optical System with Optional HeNe Laser

1. Argon (488) laser
2. SS sensor
3. HeNe (633) laser (optional)
4. FL3
5. FL4
6. Field stop
7. FL2
8. FL5
9. FS sensor
10. Flow cell
**Side Scatter and Fluorescent Light Collection**

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

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

**Side Scatter**

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

SS is separated using a 488 nm dichroic long-pass (488 DL) filter at a 45-degree angle to the light path (see Figure 5.3, Figure 5.4, Figure 5.6, and Figure 5.5). The 488 DL filter reflects the SS to the SS sensor but transmits fluorescent light of longer wavelengths.

**Figure 5.5 Single Laser Filter Block Configuration**
Fluorescent Light

The light the 488 DLP filter transmits goes to a 500 nm long pass filter. This filter blocks any remaining laser light, transmitting only fluorescent light. The remaining optical filters separate the light for the five FL sensors. Note: This fluorescent light collection description applies to the Dual Laser Filter Block Configuration only.

The first filter in the light path (500 LP) blocks the side scatter light from the Argon laser and transmits all the fluorescence colors and the side scatter light from the red laser. This assembly separates the collected fluorescence signal into five color bands. PMTs 1 to 4 collect signals centered approximately at 525, 575, 610 and 675 nm. PMT5 collects a signal centered approximately at 755 nm. Long pass filters are used to transmit these color bands, block the side scatter light from the optional red laser and provide additional blocking of the side scatter light from the Argon laser. The color bands are designed to measure fluorescence light from the fluorochromes such as FITC, PE, ECD, PC5 or APC, PC7 excited by illumination from the Argon and the optional red lasers. Dichroic filters are used to split colors at 550, 600, 645 and 710 nm. Positions of the dichroic filters have been efficiently designed to reduce the number of optical surfaces fluorescence light must pass to reach the photo sensors. Their locations relative to the optical axis have also been optimized for light to pass symmetrically through each filter. You can individually interchange the optical filters. A small plate holds all the optical filters used to separate fluorescence signals allowing for easy interchange of groups of filters. The filters are precisely mounted in their housing and the filter plate is precisely mounted on the optical plate. There is no need to realign the optical system when the filters or the filter plate are changed.
5.6 SIGNAL PROCESSING

Voltage Pulse Signals

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

Peak Signal

Figure 5.7 shows how a peak voltage pulse signal forms as a cell crosses the laser beam.

- Part I of Figure 5.7 shows when the cell enters the laser beam and some light is scattered.
- Part II of Figure 5.7 shows when the cell is in the center of the laser beam and the scattered light, and therefore, the pulse height, reaches a maximum.
- Part III of Figure 5.7 shows when the cell leaves the laser beam and the scattered light decreases.

The intensity of light scatter or fluorescence determines the height of the peak pulse (see Figure 5.7). The time the particle is in the laser beam determines the width of the pulse. Therefore, the total fluorescence (intensity and time) determines the area under the pulse. Figure 5.8 shows how three cells with the same amount of total fluorescence but with different fluorescence intensities, produce different peak pulses.
Figure 5.7 Voltage Pulse Formation, Peak Signal

I

Laser beam
Cell
Volts

II

Time
Pulse height

III
**Integral Signal**

Because the total fluorescence in all three cells is the same, but the distribution is different, the pulse can be integrated to produce an integral signal (see Figure 5.8).

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

**Figure 5.8 Integral and Peak Pulses**

1. Direction of sample flow
2. Cell in laser beam
3. Peak pulses

**Amplification**

Some voltage pulses must be amplified so that the characteristics of the cells can be measured.

The system lets you:

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

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

**Signals Generated**

The instrument sensors can generate these signals (integral unless stated otherwise; LOG stands for logarithmic):
OPERATION PRINCIPLES

PORTOCALS

5.7 PROTOCOLS

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

It contains the following information:

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

Special Protocols and Panels

There are three categories of special Protocols: QC, Cleanse and AS.

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

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

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

Special Panels

The cleanse panel utilizes two cleanse protocols (‘cleanse bleach.PRO’ and ‘cleanse di water.PRO’) that you should use at the end of the working day or shift on your FC 500.
5.8 AUTOMATED SOFTWARE FEATURES

CXP software contains the following automated software features.

- AutoSetup Wizard
- QuickCOMP
- QuickSET
- Automatic Gate Creation
- Automatic Gate Maintenance
- Elliptical and Contour AutoGating
- AutoMATOR Analysis

5.9 PARAMETERS

AUXiliary Parameter

The AUX signal is available to collect a Peak signal from any of the detected parameters when analyzing up to four colors. When using FL5 or analyzing five colors the AUX signal is not available. For any given sample, only one of the following signals can be assigned to AUX:

- FS, FS PEAK
- SS, SS PEAK
- FL1, FL1 PEAK
- FL2, FL2 PEAK
- FL3, FL3 PEAK
- FL4, FL4 PEAK

When to Use the AUX Parameter

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

Use the AUX parameter when you want to:

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

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

TIME Parameter

The TIME parameter is the amount of time, in seconds, the instrument acquires data. It is displayed on the plot axis in 1-second resolution. The axis labels vary, depending on plot resolution and stop time (duration).
The minimum stop time is 10 seconds, the maximum stop time (maximum duration) is 99,999 seconds and the default stop time is 300 seconds (5 minutes).

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

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

For a two-parameter plot, divide by 64, 128, 256 or 512 depending upon the plot resolution you are using.

**RATIO Parameter**

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

\[
\text{RATIO} = \frac{\text{Numerator}}{\text{Denominator}} \times 1024
\]

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

To calculate the actual ratio at a particular intensity for a one-parameter histogram, divide the intensity by 1,024. For a two-parameter plot, divide by 64, 128, 256 or 512 depending upon the plot resolution you are using.

**5.10 PLOT DISPLAY**

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

- Single-parameter histogram plot
- Dual-parameter dot plot
- Dual-parameter contour plot (CXP Analysis Software only)
- Dual-parameter density plot
- Dual-parameter surface plot
- Single-parameter Prism plot
- Overlaid histogram plots (CXP Analysis Software only)
- 3-D tomogram plot (CXP Analysis Software only).

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

**PRISM**

Prism is used to analyze multicolor immunofluorescence samples. With multicolor immunofluorescence a cell is either positive or negative for each of two, three, four or five cell surface markers. A particular combination is called a phenotype. Prism allows you to display percentages on all phenotypic populations in a single plot. It is software derived and can be acquired in either run time or listmode.
Prism is available on up to five parameters. TIME, RATIO, and Prism itself cannot be used for Prism. All other signals can be used for Prism. Generally, FL1, FL2, FL3, FL4, and FL5 are used.

A Prism plot shows a spike or population for each antibody combination with a percent of the total that represents the percent of the total events in the Prism plot. See Figure 5.9.

**Figure 5.9 Prism Plot**

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

- Linear
- Rectangular
- Quadrant
- Polygonal
- Elliptical
- Contour
- On Overlayed histograms only
  - Linked marker
  - Non-linked marker

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

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

These functions are not available for all region types.

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

Data Storage
Sample results can be printed out, saved to a diskette or other removable media, saved to a local hard drive or saved to a network drive. You can store sample results in the form of a list of the measurements from each cell, called listmode data. Listmode data can be replayed into plots or archived for analysis later. Plots can also be saved to a file.
Histogram Statistics

**Linear Region Statistics**

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

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

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

Number = Number of cells in the region

Mode = Intensity containing the largest number of cells within the region.

$$\text{Mean} = \sum_{M_H}^{M_L} \left( \frac{C_n \times C_{ch}}{N} \right)$$

$$\text{SD} = \sqrt{\frac{\sum_{M_H}^{M_L} (C_n - \bar{X})^2}{N}}$$

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

Where $\delta$ = Standard deviation

$\text{CV} = \text{Coefficient of Variation}$

$M_H$ = High region marker channel

$M_L$ = Low region marker channel

$C_n$ = Raw digital channel number

$C_{ch}$ = Channel count

$N$ = Integral of all counts between & including marker channels

$\bar{X}$ = Mean channel number (rounded to nearest whole channel)

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

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

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

$$\text{Sum (intensity)} = \text{sum of events from the lower edge of the region to the intensity}$$
Half Peak CV = \( \frac{\text{FWHM}}{2.354} \)

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

**Log Region Statistics**

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

**Log-Log Mean Method**

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

Example

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

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

\[
\overline{X}_{\text{channels}} = C_d \times \log_{10} \left( \sum_{Mh}^{\text{ML}} \frac{C_n}{C_d} \right) \left( \frac{Cch}{N} \right)
\]

Where:

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

For Calibrated values, the calculation is reported as the calibrated value in the range Log Offset to

\[
\overline{X} = \log \_ \text{Offset} \times 10 \left( \frac{\overline{X}_{\text{channels}}}{C_d} \right)
\]

The log offset cannot be zero since a log offset of zero is 10 (minus infinity), which is not possible to represent on paper or on screen. CXP software does not allow log offset values of less than 0.1. Numbers entered in the logarithmic offset are clamped at 0.1 if a number less than this is entered.
**Lin-Log Mean Method**

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

Using the same data as in the previous example…

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

\[
\overline{X}_{\text{channels}} = \frac{\sum_{M_{H}} C_n \times C_{ch}}{N}
\]

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

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

\[
\overline{X} = \text{Log Offset} \times 10 \left( \frac{\overline{X}_{\text{Channels}}}{C_d} \right)
\]

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

Laser/Radiation Precautions

The Cytometer and the MCL bar-code reader each contain a laser. The Cytometer can also include an optional second laser. Beckman Coulter’s design and manufacture of the instrument complies with the requirements governing the use and application of a laser as specified in regulatory documents issued by the:

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

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

Use the instrument according to the information in the manuals.

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

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

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

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

Laser Warning Labels

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

CDRH-required warning labels are located:

- See Figure 6.1 for the Sensing Compartment cover warning label.
- See Figure 6.2 for the Optical Area (front view) warning labels.
- See Figure 6.3 for the Optical Area (interior view) warning labels.
- See Figure 6.4 for the Argon Laser Head warning labels.
- See Figure 6.5 for the optional red Solid-state Laser warning labels.
- See Figure 6.6 for the location of the optional red Solid-state Laser in the instrument.
- See Figure 6.7 for the optional red Helium-Neon Laser warning labels.
- See Figure 6.8 for the location of the optional red Helium-Neon Laser in the instrument.
- See Figure 6.9 for the Cytometer back panel laser labels.
- See Figure 6.10 for the MCL probe housing warning labels.
- See Figure 6.11 for the MCL bar-code reader warning labels.
Figure 6.1 Laser Labels on the Sensing Compartment Cover

AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION

VISIBLE AND/OR INVISIBLE LASER RADIATION WHEN OPEN
AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION

LASER RADIATION
AVOID EXPOSURE TO BEAM CLASS 3B LASER PRODUCT
Figure 6.2 Laser Labels in the Optical Area, Front View

AVOID EXPOSURE

VISIBLE AND/OR INVISIBLE LASER RADIATION IS EMITTED FROM THIS APERTURE.

DANGER

VISIBLE AND/OR INVISIBLE LASER RADIATION WHEN OPEN
AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION
Figure 6.3 Laser Labels in the Optical Area, Interior View

AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION.

CAUTION
Laser radiation when open and interlock defeated. DO NOT STARE INTO BEAM.

AVOID EXPOSURE
VISIBLE AND/OR INVISIBLE LASER RADIATION IS EMITTED FROM THIS APERTURE.

DANGER
VISIBLE AND/OR INVISIBLE LASER RADIATION WHEN OPEN AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION.
Figure 6.4 Laser Labels on the Argon Laser Head

Figure 6.5 Laser Labels for Optional Red Solid-state Laser

PART NUMBER/REVISION
10080553/AB

WAVELENGTH/POWER
638nm/22mW

SERIAL NUMBER
XXXXX

BEAM
1mm CIRCULAR

OPTIONS
N/A

COHERENT
2301 LINDBERGH ST.
AUBURN, CA 95602-9595

AVOID EXPOSURE

LASER RADIATION EMITTED FROM THIS APERTURE
Figure 6.6 Location of the Optional Red Solid-state Laser in the Instrument, Rear View

- Solid-state laser head
- Mounting plate
- Protective housing

Figure 6.7 Laser Labels for Optional Red Helium-Neon Laser

JDS Uniphase
1096 Melton Avenue
Manteca, CA 95337

Model
Manufactured
Serial No.

This laser product complies with 21 CFR 1040.1 and IEC 825-1:1993. See installation instructions.

Patent Nos.

Made in USA

LASER EMISSION
INVISIBLE
LASER BEAM
AVOID EXPOSURE TO BEAM
CLASS 3B LASER PRODUCT (IEC)
CLASS IIIb LASER PRODUCT (CDRH)
633 nm / 35 mW
Figure 6.8 Location of the Optional Red Helium-Neon Laser in the Instrument, Rear View

1 HeNe laser head  2 Mounting plate  3 Protective housing

Figure 6.9 Laser Labels on the Cytometer Back Panel

1 Label is applied only when an optional second laser is installed.

2 This cover is only present when instrument contains an optional HeNe laser.
Figure 6.10 Laser Labels on the MCL Probe Housing Cover

CAUTION
LASER LIGHT - DO NOT STARE INTO BEAM
670 nm DIODE LASER
1.0 MILLIWATT MAXIMUM CLASS II LASER PRODUCT

CAUTION
Laser radiation when open and interlock defeated. DO NOT STARE INTO BEAM.

CLASS 1 LASER PRODUCT
Figure 6.11 Laser Labels on the MCL Bar-Code Reader

Avoid exposure. Laser light is emitted from this aperture. Caution: Laser light when open—do not stare into beam. Product conforms to USA DHHS 21 CFR Subchapter J.

CAUTION

VORSICHT

Laser Light Do Not Stare Into Beam.

670 nm Laser Diode

1.0 Milliwatt Max

Class II Laser Product

Avoid Exposure

Laser Light Is Emitted From This Aperture

Caution Laser Light When Open—Do Not Stare Into Beam.

Product Conforms To USA DHHS 21 CFR Subchapter J.
Warning Labels on UPS

The ! label located near the circuit breakers on the Universal Power Supply instructs the user to refer to product documentation before resetting the circuit breakers.

The ! label located next to the power switch on the Universal Power Supply instructs the user to refer to product documentation before powering up the instrument.

Figure 6.12 International Warning Symbol Locations
Disposal Precaution

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

### 6.2 MAINTENANCE SCHEDULES

#### Cleaning Schedule

See Table 6.1 for the cleaning schedule.

**Table 6.1 Cleaning Schedule**

<table>
<thead>
<tr>
<th>Component</th>
<th>Daily</th>
<th>Weekly</th>
<th>Monthly</th>
<th>Every 60 Days</th>
<th>As Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLEAN THE AIR FILTERS</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CLEAN THE CLEANING AGENT CONTAINER</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>CLEAN THE MCL SAMPLE HEAD AND THE SAMPLE PROBE</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CLEAN THE SAMPLING SYSTEM</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CLEAN THE SHEATH FLUID CONTAINER</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CLEAN THE VACUUM TRAP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Not Applicable

#### Replacement Schedule

The sheath fluid filter needs to be replaced every 6 months. All other replacement and adjustment procedures should be done on an as needed basis.

### 6.3 ERROR MESSAGES

See Table 6.2 for a list of all of the error messages.

#### Display Locations

Error messages can appear at the bottom of the CXP software screen, in the Cytometer Status Messages screen, and in the cytometer.log file.

#### CXP Software Screen

At the bottom of the CXP software screen, the last error message appears. See Figure 6.13.

Note: If multiple error messages are posted at the same time, only the last one posted appears here. All of the error messages posted appear in the Cytometer Status Messages screen and the cytometer.log file.
Figure 6.13  Error Message on CXP Software Screen, Example

Cytometer Status Messages Screen
In the Cytometer Status Messages screen, the last error message and all uncleared error messages appear. See Figure 6.14.

- To access the Cytometer Status Messages screen, double click the error message at the bottom of the CXP software screen.

- Clear Status when you want to delete all messages in the Cytometer Status Messages screen.

Figure 6.14  Cytometer Status Messages Window, Example

Cytometer.log File
In the cytometer.log file, all messages appear from the last 30 days unless they were manually cleared. Refer to ERROR MESSAGE CYTOMETER.LOG FILE for details. See Figure 6.15.
Figure 6.15 Example of Error Messages in Cytometer.log File

<table>
<thead>
<tr>
<th>cytometer.log - Notepad</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>File</td>
<td>Edit</td>
</tr>
<tr>
<td>Local time: 10:30:04 on 05/09/2001, Eastern Daylight Time 126441738044151280 9217</td>
<td>Data Stream okay</td>
</tr>
</tbody>
</table>

6.4 ERROR MESSAGE CYTOMETER.LOG FILE

The Cytometer error messages are located in the cytometer.log file.

Note: Error messages over 30 days old are automatically removed from the cytometer.log file and placed into the cytometerarchive.log file.

How to Access the Cytometer.log File

**From CXP Software**

- Press **Ctrl + L**. See Figure 6.15.
  
  OR,

- **Cytometer** >> **Cytometer Log** >> **View Log**. See Figure 6.15.

**From Windows Desktop**

1. **Start** >> **Programs** >> **Accessories** >> **Windows Explorer** >> **My Computer** >> **(C:) drive** >> **CXP**.
2. Highlight **cytometer.log**.
3. Double click on **cytometer.log** to open. See Figure 6.15.
Cytometer.Log Entry Description
All cytometer.log error message entries are posted in chronological order. Each error message entry consists of four lines (see Figure 6.15):

- Date and time when the error message occurred
- 18-digit number - for Service use only
- Four-digit number - for Service use only
- Error message text. See Table 6.2 for a list of messages and operator actions.

How to Search the Cytometer.log File
To search for a specific word or phrase listed anywhere in the cytometer.log file:

1. Edit >> Find…
2. Type in the word or phrase you want to find (Example: Waste).
3. The direction of the search: Up or Down.
4. Find Next and the next occurrence of the word in the error log is highlighted.
5. Repeat step 4 as needed or until Cannot find “XXXXX” appears.

Other Functions Available
Here are some of the more often used functions available from the cytometer.log file pull down menus: File, Edit, Format, and Help:

Print
To print the cytometer.log file: File >> Print.

Change Font
To change the font: Format >> Font, and select the new font.

Find Help Topics
To find Help on other topics: Help >> Help Topics.

Cytometerarchive.log File
The cytometerarchive.log file contains error messages that were automatically moved from the cytometer.log file after 30 days. This log is for Service use.
6.5 ERROR MESSAGES TABLE

Table 6.2 lists the error messages in alphabetical order, with their cause and what to do about them. These are the error messages produced by the Cytometer.

Call your Beckman Coulter Representative if:
- The recommended action does not solve the problem.
- You need help.

### Table 6.2 Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleanse Level Error</td>
<td>Low cleaning agent.</td>
<td>Fill cleaning agent container. Refer to FILL THE CLEANING AGENT CONTAINER. Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Cleanse Level Warning</td>
<td>Low cleaning agent.</td>
<td>Fill cleaning agent container. Refer to FILL THE CLEANING AGENT CONTAINER. Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Data Rate Warning</td>
<td>The discriminated data rate exceeded 6,000 cells per second.</td>
<td>1. Dilute the sample or change the discriminator setting. 2. Check that the sheath fluid container cap is tightened. 3. If problem continues, REPLACE THE SHEATH FLUID FILTER.</td>
</tr>
<tr>
<td>Data Stream Warning</td>
<td>Data acquisition hardware error</td>
<td>1. Reboot the computer and restart the Cytometer. 2. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Host Command Invalid</td>
<td>The Cytometer software could not process the requested command from the Workstation.</td>
<td>1. Reboot the computer and restart the Cytometer. 2. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Laser Current Error</td>
<td>The amount of current required by the laser is outside the expected range.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Laser Power Error</td>
<td>The amount of laser light power is outside the instrument’s operating range.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Laser Regulation Warning</td>
<td>The laser light power is fluctuating.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Laser Start Error</td>
<td>The laser failed to start within 120 seconds.</td>
<td>1. Power the instrument off then on. 2. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Message</td>
<td>Cause</td>
<td>Action</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>MCL Carousel In/Out Error</td>
<td>MCL carousel did not move when requested to do so.</td>
<td>1. Check that there is no obvious obstruction (sample tube) in the MCL area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Check that the 30 psi gage on the power supply is okay.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If no obstruction is found, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Carousel Label Error</td>
<td>MCL carousel did not read carousel bar-code label.</td>
<td>1. Check that the bar-code label is not torn or written on.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Try using another carousel.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Carousel Rotate Error</td>
<td>MCL carousel did not rotate correctly</td>
<td>Check that there is no obvious obstruction (sample tube) in the MCL area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If no obstruction is found, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL CPU Error</td>
<td>MCL hardware error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Door Open Error</td>
<td>MCL cover is open while the MCL is in use.</td>
<td>Close the MCL cover.</td>
</tr>
<tr>
<td>MCL Door Open Warning</td>
<td>MCL cover is open while the MCL is in use.</td>
<td>Close the MCL cover.</td>
</tr>
<tr>
<td>MCL EPROM Error</td>
<td>MCL hardware error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Error</td>
<td>MCL hardware error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Interlock Error</td>
<td>The MCL Interlock is open while the MCL is in use.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Parallel Recv Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Parallel Send Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Probe Up/Down Error</td>
<td>Short circuit.</td>
<td>Check if a circuit breaker indicator is dark. See RESET THE CIRCUIT BREAKERS.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCL sample probe did not move when requested to do so.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Program Load Error</td>
<td>An MCL hardware error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL RAM Error</td>
<td>An MCL hardware error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Rec from Fault Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Receive Timeout Error</td>
<td>An MCL communication error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Request Error</td>
<td>Communication error between the Cytometer and the MCL.</td>
<td>1. Power the instrument off then on.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Serial 1 Recv Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Serial 1 Send Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Message</td>
<td>Cause</td>
<td>Action</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>MCL Serial 2 Recv Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Serial 2 Send Error</td>
<td>An MCL communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Transmit Timeout Error</td>
<td>An MCL communication error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Tube Displaced Error</td>
<td>A tube is positioned incorrectly in the MCL.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Tube Jam Error</td>
<td>A tube is stuck in the MCL.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>MCL Tube Load Error</td>
<td>There is a bad seal between the MCL sample probe guide and sample tube.</td>
<td>Put sample into another tube.</td>
</tr>
<tr>
<td>MCL Tube Position Error</td>
<td>The MCL did not rotate to the correct tube position or there was an error reading the position bar code on the carousel.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
</tbody>
</table>
| MCL Tube Up/Down Error  | Unable to load or unload the sample tube from the MCL sampling position. | 1. Check that the labels on the sample tubes are secure and are not adhering to the walls of the carousel.  
2. Check that there is no crack in the sample tube.  
3. If problem continues, call your Beckman Coulter Representative. |
| Sample Pressure Error   | There may be a leak caused by a bad sample tube or a bad sample head. | Inspect sample tube and sample head for damage. Change as required. See REPLACE THE MCL SAMPLE HEAD. |
| Sensors Stream Warning  | There was an error in the communication of the instrument operating data packet from the acquisition hardware to the Cytometer software. | 1. Reboot the computer and restart the Cytometer.  
2. If problem continues, call your Beckman Coulter Representative. |
| Sheath Drawer Error     | The sheath fluid (reagent) drawer is open.     | Close the reagent drawer completely.                       |
| Sheath Level Error      | There is not enough sheath fluid for further sample analysis. | Fill sheath fluid container. See FILL THE SHEATH FLUID CONTAINER. |
| Sheath Level Warning    | The first time this message is displayed, there is enough sheath fluid for about 5 minutes of sample analysis. | Fill the sheath fluid container. See FILL THE SHEATH FLUID CONTAINER. |
|                         | If the sheath fluid container is full, the sheath fluid sensor failed. | Call your Beckman Coulter Representative.                  |
## Table 6.2 Error Messages (Continued)

<table>
<thead>
<tr>
<th>Message</th>
<th>Cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheath Pressure Error</td>
<td>The sheath fluid pressure is outside the system's operating range.</td>
<td>1. Check sheath fluid and cleaning agent container caps for tightness.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. If problems continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If problem continues, REPLACE THE SHEATH FLUID FILTER.</td>
</tr>
<tr>
<td>State Machine Failure</td>
<td>Cytometer software failure.</td>
<td>1. Power the instrument off then on.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. If problems continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>System Pressure Error</td>
<td>Pressure line is not connected between the power module and the Cytometer.</td>
<td>Connect the pressure line.</td>
</tr>
<tr>
<td></td>
<td>The pressurized air supply is outside the system's operating range.</td>
<td>1. Go to the Ready State, then check that the system pressure is 30 psi.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Run a sample and monitor the system pressure. If the system pressure drops below the range specified in the instrument manual, then call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td></td>
<td>Short circuit.</td>
<td>Check if a circuit breaker indicator is dark. See RESET THE CIRCUIT BREAKERS.</td>
</tr>
<tr>
<td>System Vacuum Error</td>
<td>Liquid in the vacuum trap.</td>
<td>Check that the vacuum trap (on the front of the Power Supply) is tight and is less than 1/4 full of fluid. If it is more full, empty it (see CLEAN THE VACUUM TRAP).</td>
</tr>
<tr>
<td></td>
<td>Vacuum line is not connected between the power module and the Cytometer.</td>
<td>Connect the vacuum line at the back of the instrument.</td>
</tr>
<tr>
<td></td>
<td>Hardware problem.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Temperature Error</td>
<td>Temperature inside the Cytometer is above 60°C.</td>
<td>1. Clean your air filters. See CLEAN THE AIR FILTERS.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Temperature Sensor Failure</td>
<td>Temperature sensor failure.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Temperature Warning</td>
<td>Temperature inside Cytometer is above 50°C.</td>
<td>1. Clean your air filters. See CLEAN THE AIR FILTERS.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. If problem continues, call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Transputer 1 Error</td>
<td>Cytometer software error.</td>
<td>At the Workstation turn the system off, and then turn it back on.</td>
</tr>
<tr>
<td>Transputer 2 Error</td>
<td>Cytometer software error.</td>
<td>At the Workstation turn the system off, and then turn it back on.</td>
</tr>
</tbody>
</table>
## Table 6.2 Error Messages (Continued)

<table>
<thead>
<tr>
<th>Message</th>
<th>Cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transputer Link Error</td>
<td>A Cytometer computer communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
<tr>
<td>Transputer Link Warning</td>
<td>A Cytometer computer communications error.</td>
<td>Call your Beckman Coulter Representative.</td>
</tr>
</tbody>
</table>
| Unknown Stream Warning       | An unidentified data packet communication error occurred between the acquisition hardware and the Cytometer software. | 1. Record an entry in the Action Log Sheet.  
2. Reboot the computer and restart the Cytometer.  
3. If problem continues, call your Beckman Coulter Representative. |
| Vacuum Chamber Warning       | The liquid level in the vacuum chamber is too high.                   | 1. Check that the vacuum trap (on the front of the Power Supply) is tight and is less than 1/4 full of fluid. If it is more full, empty it (see CLEAN THE VACUUM TRAP).  
2. If problem continues, call your Beckman Coulter Representative. |
| Waste Backpressure Error     | The filter on the waste container vent line is probably wet, clogged, or disconnected. | 1. Check the waste vent filter for the presence of liquid and a proper connection.  
2. If problem continues, call your Beckman Coulter Representative. |
| Waste Level Error            | There is not enough empty volume in the waste container for further sample analysis. | Empty the waste container. See EMPTY THE WASTE CONTAINER. |
| Waste Level Warning          | There is enough empty volume in the waste container for about 5 minutes of sample analysis. | Empty the waste container. See EMPTY THE WASTE CONTAINER. |
|                              | If the waste container is not full, the waste sensor failed.          | Call your Beckman Coulter Representative.                              |
6.6 LEVEL SENSE INDICATORS

Sheath Low
When the Sheath Low indicator (see Figure 6.16) appears:

- During sample analysis, you have 5 minutes to finish analyzing the current sample.
- You cannot analyze samples or use the instrument until the sheath fluid container is filled.
- **FILL THE SHEATH FLUID CONTAINER.**

Figure 6.16 Sheath Low Indicators

Waste Full
When the Waste Full indicator (see Figure 6.17) appears:

- During sample analysis, you have 5 minutes to finish analyzing the current sample.
- You cannot analyze samples or use the instrument until the waste container is emptied.
- **EMPTY THE WASTE CONTAINER.**

Figure 6.17 Waste Full Indicators

6.7 CYTOMETER CONTROL WINDOW CANNOT BE VIEWED

If the Cytometer Control window cannot be viewed (it is hidden behind the Windows Taskbar at the bottom of the screen), the Auto hide feature is currently selected. Change the Windows settings to deselect the Auto hide feature and keep the Cytometer Control window in view.

To deselect the Auto hide feature:
1. Start >> Settings >> Taskbar & Start Menu...

2. On the General tab, Auto hide and deselect Always on top.

3. The Cytometer Control window should now be partially in view.

4. Drag the Cytometer Control window into full view.
TROUBLESHOOTING

CYTOMETER CONTROL WINDOW CANNOT BE VIEWED
A.1 SOFTWARE PROCEDURES

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

These procedures are in the System Overview chapter in the Instructions For Use Manual.
- CREATING PROTOCOLS
- CREATING REGIONS
- CREATING GATES
- CREATING FLOWPAGES
- CREATING PANELS
- CREATING WORKLISTS

These procedures are in the Quality Control chapter in the Instructions For Use Manual.
- AutoSetup II Wizard
- Application Definition Wizard (define all your applications)
- AutoSetup Scheduler (multiple application AutoSetup)

These procedures are in the Using CXP Software chapter in the Reference Manual.
- Batch AutoMATOR
- Overlay Histogram Plots
- Advanced Color Precedence
- Listmode Compensation
- Setting a CAL Region

A.2 HARDWARE PROCEDURES

For Online help, click on the links below to:

- View a short video of the procedure or
- Go to the detailed procedure.

These procedures are in the Special Procedures Manual.
- Open/remove the instrument covers - video or procedure.
- Clean the MCL sample head and the sample probe - video or procedure.
- Replace the sample probe and sample pickup tubing - video or procedure.
- Replace the MCL sample head - video or procedure
- Replace the optical filter plate - video or procedure.
- Replace a filter in the optical filter plate - video or procedure
B.1 BAR-CODE SAMPLE IDENTIFICATION

Bar-code symbols are a highly accurate and efficient procedure for identifying and processing laboratory samples. Beckman Coulter instruments use four bar-code symbologies (types) to identify specimens:

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

The bar-code reader senses the difference between enabled bar-code symbologies in a run.

**IMPORTANT** A misread label can cause one sample ID to be read as another sample ID. The laboratory’s process for printing, placing, and meeting all bar-code specifications is important to achieve highly accurate reading. Follow the bar-code specifications to avoid inaccurate reading of the bar-code label.

**Figure B.1 Bar-Code Label**

<table>
<thead>
<tr>
<th>1</th>
<th>Quiet zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Bar-code symbol</td>
</tr>
<tr>
<td>3</td>
<td>Sample ID</td>
</tr>
</tbody>
</table>

B.2 CORRECT PLACEMENT OF THE BAR-CODE LABEL

The bar-code label must be placed a minimum of 25.4 mm (1.0 in.) from the bottom of the tube. Refer to **Figure B.2**.
Put labels on the tubes so that the bars follow one another in a vertical sequence. Refer to Figure B.2. The bar-code reader scans the tube vertically. Do not tilt the label more than ±7.5 degrees from the axis of the tube.

Put the tubes in the carousel so that the bar-code symbols are visible through the slots in the front of the carousel. When viewed at eye level, the full symbol, including the quiet zones, must be visible through the slot and above the bottom of the carousel.

Note: The FC 500 rotates the tube as needed so the bar-code label can be read.

**B.3 BAR-CODE LABEL SPECIFICATIONS**

**IMPORTANT** A misread label can cause one sample ID to be read as another sample ID. The laboratory’s process for printing, placing, and meeting all bar-code specifications is important to achieve highly accurate reading. Follow the bar-code label specifications to keep the rate of misread labels to a minimum.

The quality of the bar-code symbol and the label is important for accurate reading. For high accuracy, use labels that meet all of the specifications.

When possible, print the sample ID on the label in alphanumeric characters so the operator can manually enter the bar-code information if the bar-code symbol cannot be read.

**Label Size and Thickness**

The length of the label must be less than 44.45 mm (1.75 in.). The label includes the bar-code symbol and a minimum quiet zone of 3.5 mm (0.14 in.) at each end of the symbol. Refer to Figure B.3.
Figure B.3  Bar-Code Label Specifications

1. Quiet zones 3.5 mm (0.14 in.) minimum
2. Bar-code symbol height 19.05 mm (0.75 in.) minimum
3. Bar-code label length 44.45 mm (1.75 in.) maximum

The width of the bar-code label must be 5 mm (0.2 in.) less than the circumference of the sample tube.

Label thickness, including adhesive, must be 0.09 mm (0.0036 in.) maximum. Total thickness for all labels and adhesives put together must be 0.36 mm (0.0144 in.) maximum.

Symbol Dimensions
The height of the bar-code symbol must be 19.05 mm (0.75 in.) minimum.
See Table B.2.

Label and Print Quality
All bar-code symbols must agree with the American Identification Manufacturer’s (AIM) Uniform Symbology Specification.¹

All bar-code symbols must be printed at print quality class “B” or better as defined by the American National Standards Institute (ANSI).² Several factors affect print quality:

- Labels must be clean, not yellowed, and used before the expiration date.
- Print the bar-code symbol on material that is reflective and has a matte finish. Use a background diffuse reflectance of 80% or more for maximum contrast.
- The labels must not have defects such as spots, lines, missing sections, cuts, folds, or density problems.
- The bars in the bar-code symbol must be well-defined. Edges must be constant (not irregular), so the bars and spaces have the correct widths for the bar-code symbology used.
B.4 BAR-CODE ERROR RATE

**IMPORTANT** A misread label can cause one sample ID to be read as another sample ID. Whenever possible use a bar-code symbology and configuration choices that provide the most accurate bar-code reading.

The quality of the bar-code symbol and the label is important for accurate reading. To get the highest possible accuracy only use labels that meet all the specifications described for labels and symbols. Deviations from these specifications make the bar code more difficult to read and allow for a possible increase in the error rate.

The symbology and the configurable parameters that the laboratory selects have an effect on the error rate. Certain features of the symbologies and the selections made by the laboratory have an important effect on the accuracy of the bar-code reading system. In general:

- Code 128 and Code 39 are more accurate and have lower error rates than Codabar or Interleaved 2-of-5.
- NCCLS recommends Code 128 because of its accuracy, compact form, and self-checking capabilities.\(^3\)
- A checksum greatly increases accuracy. Use a checksum with Interleaved 2-of-5 and Codabar because they are less accurate symbologies.
- Select the fixed length option, if available, because it is more accurate than the variable length option.
- To keep label and printing flaws to a minimum, use a narrow element of more than 0.25 mm (0.010 in.).

Beckman Coulter recommends the use of:

- Code 128
- Checksum for all other symbologies
- Fixed length code symbols
- Narrow bar sizes of 0.25 mm (0.010 in.) minimum.
B.5 BAR-CODE SYMBOLOGIES

Beckman Coulter instruments use four bar-code symbologies for specimen identification, see Table B.1. Within the given specifications, the MCL reader and the optional handheld bar-code reader automatically distinguish the following bar codes:

**Table B.1 Bar-Code Symbologies**

<table>
<thead>
<tr>
<th>Bar-Code Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code 128</td>
<td>Variable length&lt;br&gt;Alphanumerics; 107 character set&lt;br&gt;Self-checking&lt;br&gt;Continuous code; intercharacter space is part of code structure for higher density of code per square inch; compact bar code&lt;br&gt;Code 128 is recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for its accuracy, compact form, and self-checking capabilities&lt;br&gt;Code 128B - Maximum 8 alphanumeric characters&lt;br&gt;Code 128C - Maximum 16 numeric characters (The use of 15 numeric characters is invalid)</td>
</tr>
<tr>
<td>Code 39</td>
<td>Variable length&lt;br&gt;Includes 43 data characters; 26 letters (uppercase A-Z), 10 digits (0-9), six symbols (. $ / + % -) and a space&lt;br&gt;Strong self-checking properties&lt;br&gt;Checksum&lt;br&gt;Discrete code; white spaces are not part of this code&lt;br&gt;Maximum 7 characters (6 data characters + 1 checksum character)</td>
</tr>
<tr>
<td>Interleaved 2-of-5</td>
<td>Numerics only&lt;br&gt;Checksum&lt;br&gt;Lower density of code per square inch; longer label&lt;br&gt;Requires an even number of digits to be encoded, a leading “0” must be added if the number count is odd&lt;br&gt;Fixed 14 characters (13 data characters + 1 checksum character)</td>
</tr>
<tr>
<td>Codabar</td>
<td>Variable length&lt;br&gt;Includes 16 data characters; 10 digits (0-9), and six symbols (. $ / + % -)&lt;br&gt;Has specific start and stop characters which lead to improvement in readability&lt;br&gt;Checksum&lt;br&gt;Lower density of code per square inch; longer bar code&lt;br&gt;Maximum 10 characters (9 data characters + 1 checksum character)</td>
</tr>
</tbody>
</table>
B.6 BAR-CODE LABELS

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

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

**IMPORTANT** Sample misidentification can occur from the use of incorrect, poor quality, damaged, dirty or improperly placed bar-code labels. Follow the specifications in this section to create your bar-code labels to prevent incorrect sample identification. See also Putting a Bar-Code Label on a Sample Tube.

The instrument supports preprinted labels.

**Bar-Code Label Optical Characteristics at 670 nm ±10%**
- Print Contrast Signal (PCS): 80% minimum.
- Reflectivity of Media (RW): 80% minimum.
- Reflectivity of Ink (RB): 16% maximum.
- No spots or voids; no ink smearing.
- Edge roughness is included in the bar and space tolerances.

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

**Table B.2 Code-Related Specifications**

<table>
<thead>
<tr>
<th>Code</th>
<th>Interleaved 2-of-5*</th>
<th>Codabar*</th>
<th>Code 39*</th>
<th>Code 128*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrow element (NE) width</td>
<td>0.010 in. ±0.001</td>
<td>0.010 in. ±0.001</td>
<td>0.010 in. ±0.001</td>
<td>0.010 in. ±0.001</td>
</tr>
<tr>
<td>Wide element/narrow element ratio (WE/NE)</td>
<td>3:1</td>
<td>N/A</td>
<td>3:1</td>
<td>N/A</td>
</tr>
<tr>
<td>Intercharacter gap</td>
<td>No</td>
<td>0.010 in. minimum</td>
<td>_NE</td>
<td>No</td>
</tr>
<tr>
<td>Data digits</td>
<td>14**</td>
<td>1 to 10**</td>
<td>1 to 7**</td>
<td>2 to 16</td>
</tr>
</tbody>
</table>

** Includes checksum character

B.7 MCL BAR-CODE READER

The MCL uses a visible-laser type reader containing a Class II laser, operating at 670 nm, with a maximum power output of 1 mW.
B.8  **BAR-CODE DECODER**

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

The decoder:

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

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

B.9  **CHECKSUM ALGORITHM**

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

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

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


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