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FACSort User's Guide

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Preface

FACSort™ is Becton Dickinson Immunocytometry Systems' benchtop cell sorter, a flow cytometer designed for applications ranging from routine clinical to advanced research. The FACSort system is combined with a Macintosh® computer and includes CELLQuest™ software, which is designed specifically for BDIS flow cytometers.

FACSComp™ instrument setup software is also included with the system. Use FACSComp for daily FACSort quality control and setup.

How to Use This Guide

This user's guide contains the instructions necessary to operate and maintain your FACSort flow cytometer. The chapters are arranged in sequence, with information presented in easy-to-follow steps. The steps appear in **boldface type** followed by additional information that provides more detail. Because many FACSort functions are controlled by CELLQuest software, you will also find the basic software information necessary to get you through instrument setup and sorting. If you are not familiar with the Macintosh computer or with CELLQuest software, refer to the *Macintosh User's Guide* provided by Apple® Computer, and the *CELLQuest Software User's Guide.*

Use the table of contents and index to locate instructions for specific procedures. The quick reference guide, in the jacket pocket of this user's guide, will come in handy as you become familiar with the system and procedures.

Here's what you'll find inside this user's guide:

- Safety and Limitations, following this section, contains important information you'll need to know before operating the FACSort.
- Chapter 1, Introduction, explains what the FACSort is and how it works. Illustrations are included along with a list of major parts and a brief description of each. This chapter also tells you what is required to run the instrument.
- Chapter 2, Getting Started, provides you with the instructions necessary for starting up the FACSort and preparing it for use. Also in this chapter are instructions for turning on the computer and starting the software.
- Chapter 3, System Operation, describes how to run patient samples, collect data, and sort.
- Chapter 4, Applications, introduces you to some of the possible uses of the FACSort.

- Chapter 5, Cleaning and Maintenance, provides instructions necessary to clean and maintain your instrument.
- Chapter 6, Troubleshooting, lists some of the problems you may encounter during operation and suggests possible solutions.
- Appendix A, Doublet Discrimination in DNA Analysis, explains the theory of doublet discrimination and why it is important to use width (W) and area (A) measurements of a parameter instead of height (H) when performing DNA analysis.
- Appendix B, Service, provides you with the phone numbers for order information and technical support. You will also find a list of consumable parts and their order numbers.
- Appendix C, FACSort Specifications, provides a more detailed description of the instrument.

New Users

After proper training and with the help of this user's guide, you'll soon feel comfortable operating and maintaining your FACSort flow cytometry system.

Because FACSort is controlled by CELLQuest software, it may be helpful to keep your *CELLQuest Software User's Guide* handy for reference when running the instrument. If you are not familiar with the Macintosh computer, the *Macintosh User's Guide,* which you received with your computer, will provide you with necessary information.

Start with Chapter 1 of this user's guide for a description of the major instrument components, then continue with the chapters following as you need them. Refer

to the *FACSort Quick Reference Guide* when you feel comfortable with the operating procedures but need occasional reminders.

Experienced Users

If you are familiar with a BDIS flow cytometer, much of the material in this user's guide may be familiar to you. One area that you'll want to concentrate on is sorting. But, you'll be pleasantly surprised to find out just how easy it is!

Refer to the *FACSort Quick Reference Guide* provided with this user's guide for basic instrument operation instructions. If you need more detail, refer to the appropriate chapter in this manual. Then read Chapter 3 for instructions on sorting. If you have any problems, refer to Chapter 6, Troubleshooting.

Conventions Used in This Guide

	Italics	Highlight any text that appears on the screen.
	Bold	Indicates actions or steps to perform.
□	NOTE	Points out additional information that may be helpful, or hints for better or easier operation.
⚠	CAUTION	Alerts you to situations that could result in instrument damage, failure in a procedure, or possible incorrect data results.
⊘	WARNING	Alerts you to situations that could result in injury.

Help!

For technical questions or assistance in solving a problem, refer to the following list:

1. Read the section of the manual specific to the instrument operation that you are performing. Refer to the table of contents and index to locate this information.

2. Refer to Chapter 6 for troubleshooting information.

3. US customers call the Becton Dickinson Immunocytometry Systems Customer Support Center at (800) 448-BDIS (2347). Customers outside the US contact your local Becton Dickinson representative or distributor.

Safety and Limitations

Please read the following warnings and safety limitations. This information should be kept available for future reference and for new users. BDIS strongly recommends the FACSort flow cytometer be operated only as directed in this user's guide, the *CELLQuest Software User's Guide,* and any accompanying manual for accessories and optional equipment.

Electrical Safety

- For protection against shock, equipment should be connected to an approved power source. If an ungrounded receptacle is encountered, have a qualified electrician replace it with a properly grounded receptacle in accordance with the Electrical Code.
- For installation outside the US, a power transformer/conditioner is provided to accommodate 100 V ±10%, 220 V ±10%, 240 V ±10%, 50–60 Hz ±2 Hz.
- Do not, under any circumstances, remove the grounding prong from the power plug. Do not use extension cords.
- Do not perform any servicing except as specifically stated in this user's guide.

Laser Safety

- The FACSort is a Class I laser product. The laser is fully contained within the instrument structure and calls for no special work area safety requirements. Nevertheless, United States regulations require the following warning to avoid tampering with the instrument:

  DANGER: LASER RADIATION WHEN OPEN. AVOID DIRECT EXPOSURE TO BEAM.

- Use of controls, adjustments, or performance of procedures other than those specified in this user's guide may result in hazardous laser radiation exposure.

Biological Safety

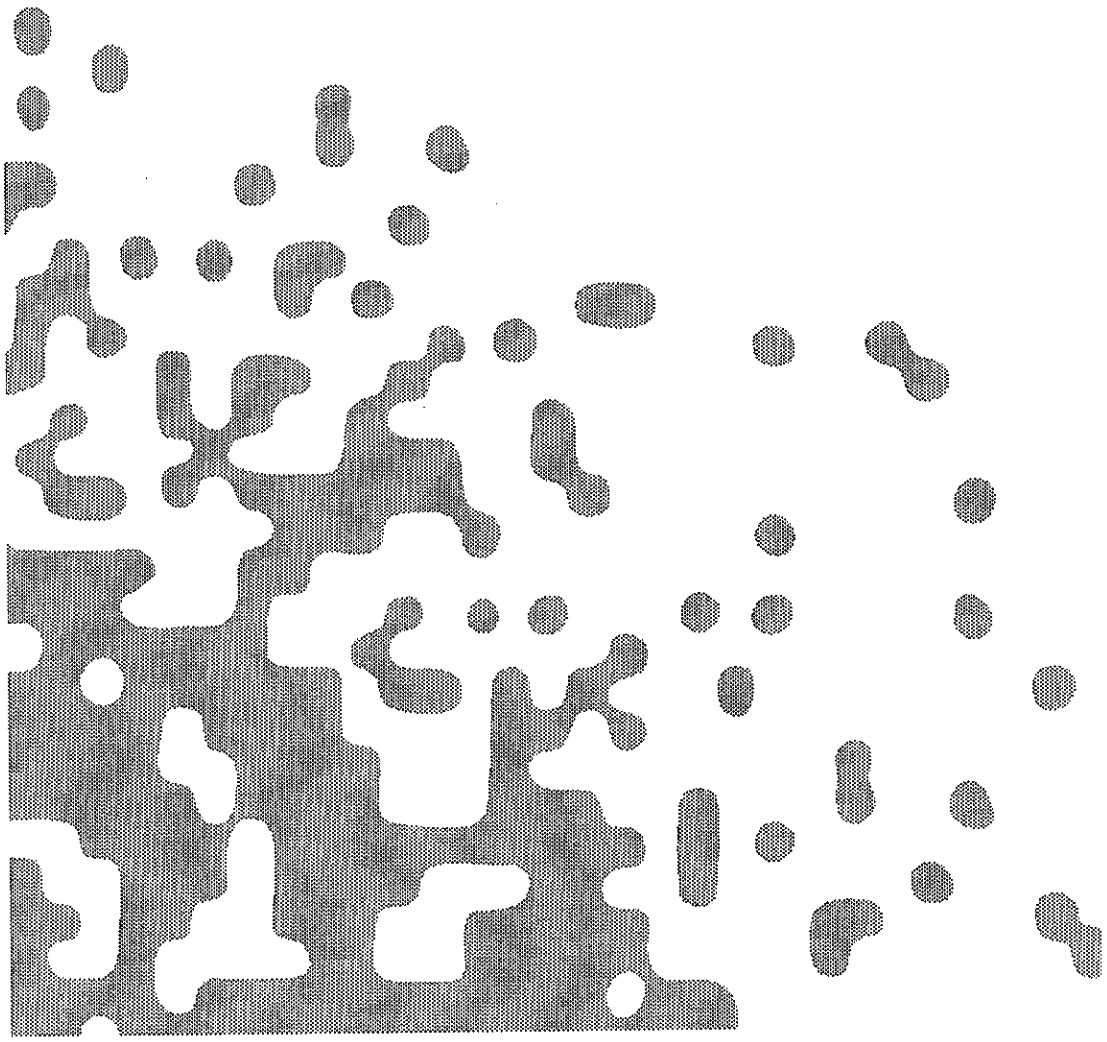
- Blood samples may contain infectious agents that are hazardous to your health. Follow appropriate biosafety procedures; wear gloves when handling blood products or any materials with which they come in contact.

- Dispose of waste reservoir contents only after it has been exposed to bleach for a minimum of 30 minutes. Always follow local, state, and federal biohazard handling regulations when disposing of biohazardous waste material.

- After running samples on the instrument, dispose of the sample tubes in accordance with local, state, and federal biohazard handling regulations.

CHAPTER 1

Introduction



CHAPTER 1
Summary

- what a FACSort flow cytometer is, how it works, and what it's used for
- requirements for operating the FACSort
- an overview of the FACSort and its components
- an overview of the software programs that come with the system

1.1 What Is the FACSort?

FACSort is Becton Dickinson Immunocytometry Systems' bench-top cell sorter, a flow cytometer designed for applications ranging from routine clinical to advanced research. It analyzes cells as they pass one at a time through a focused laser beam. FACSort can measure up to five parameters—forward light scatter, side light scatter, and three fluorescence parameters, and can measure the pulse height, area, and width of each fluorescence parameter. In addition, FACSort allows you to identify and sort a subpopulation from your sample.

Unlike traditional cell sorters that use electrostatic droplet deflection, FACSort takes an entirely new approach to sorting. A mechanical device is used to capture desired cells. No side streams are formed; therefore, aerosol formation is completely eliminated. Because of FACSort's reliability and ease of use, what was once considered a highly sophisticated technology is now simple, and available to any laboratory.

Because FACSort requires no optical alignment, daily setup can be performed quickly and consistently using FACSComp setup software.

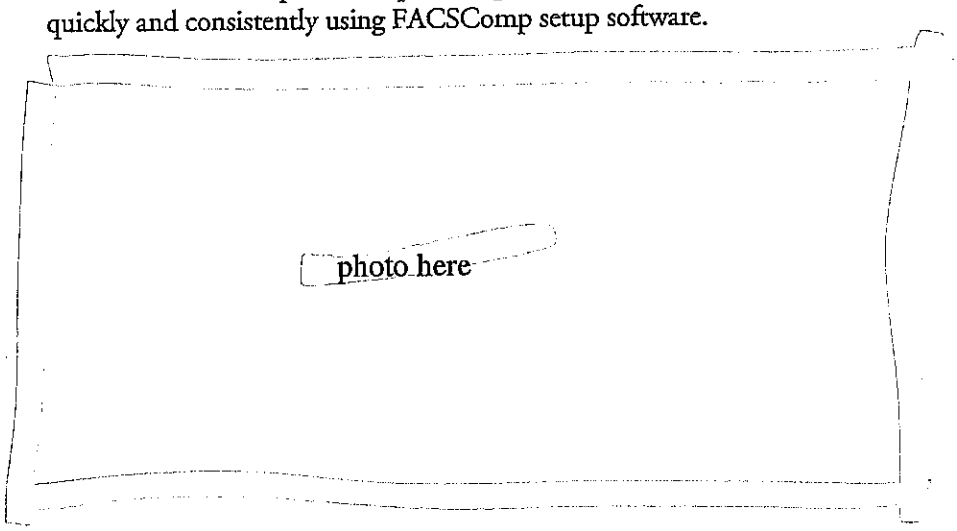


Figure 1-1 FACSort flow cytometry system

1.1.1 Principle of Operation

FACSort analyzes cells as they travel in a moving fluid stream past a fixed laser beam. As a cell passes in front of the laser, several measurements are made based on the physical characteristics of the cell. These characteristics, which pertain to how the cell scatters the laser light and emits fluorescence, provide us with information about the cell's size, internal complexity, and relative fluorescence intensity. This information is collected and transmitted to the computer.

To sort cells, FACSort uses a mechanical device called a catcher tube, located in the upper portion of the flow cell just above the viewing orifice. The catcher tube moves in and out of the sample stream to collect desired cells at a rate of up to 300 per second. Because laser alignment and stream velocity are fixed, the time it takes for desired cells to reach the catcher tube is constant. Therefore, there is no need for the tedious setup calculations associated with other cell sorters.

1.1.2 Intended Use

FACSort is designed for clinical use and research applications. FACSort is for in vitro diagnostic use for enumeration of leucocyte (non-blast) subsets or reticulocytes with the appropriate software. See the appropriate software user's guide or reagent package insert for in vitro diagnostic instructions for use.

In addition, FACSort can be used for the following research applications: performing three-color analysis, studying the classification of chromosomes, DNA cell-cycle analysis, performing platelet studies, or investigating the importance of intracellular ionized calcium measurements.

FACSort is ideal for sorting cells for verification of morphology or molecular studies, or for sorting viable cells that can be returned to culture or used in functional assays. All sorting applications are for research use only.

1.2 Requirements

Your system includes CELLQuest software for acquisition and analysis and FACSComp software for daily setup and quality control. Other application-specific software programs are also available. To acquire and sort samples, certain operating supplies are necessary (Section 1.2.3).

1.2.1 Hardware Requirements

A FACSort flow cytometer can be operated with any Macintosh computer system purchased through BDIS. Other platforms may also be supported for off-line data analysis; contact your Becton Dickinson Sales Representative for detailed information.

1.2.2 Software Requirements

- Macintosh system software, version 7.1 or later
- CELLQuest software, version 1.0 or later (for acquisition and analysis)
- FACSComp software, version 1.0 or later (for instrument setup)
- FACSConvert software, version 1.0 or later (if CONSORT™ -generated data will be analyzed)

1.2.3 Supplies Required

- Centrifuge with swinging-bucket rotor for 50-mL conical tubes
- Bovine serum albumin (BSA) for coating the collection tubes when sorting
- 12 x 75-mm polystyrene test tubes (Falcon®) for introducing samples to the FACSort
- 50-mL polypropylene, conical, screw-top tubes to collect the sorted sample
- Sheath fluid
  Haema-Line® 2 (Serono-Baker Diagnostics) for analysis applications
  1X phosphate-buffered saline (PBS) (Dulbecco's) for sorting applications
- Spare sheath and waste reservoirs for cleaning procedures (see Appendix B for part number)
- Sodium azide (NaN_3) as a preservative in the PBS used for sample dilutions
- Quality control particles
  CaliBRITE™ beads (used with FACSComp software)
- Chlorine bleach for instrument cleaning
  Clorox and other brand-name bleaches are preferred because they are filtered to remove particles and are a known concentration of 5% sodium hypochlorite

1.2.4 Optional Products

- Retic-COUNT™ reticulocyte enumeration software (for reticulocyte applications)
- SimulSET™ software (for automated acquisition and analysis of two-color immunophenotyping applications)

1.3 FACSort Overview

Take a few minutes to study Figures 1-2 through 1-5 and their descriptions. This will familiarize you with the various instrument components and help you understand the instructions in this user's guide.

1.3.1 Instrument

FACSort (Figure 1-2) is a five-detector flow cytometer, which consists of optical, electronic, and fluidic systems, as well as a built-in, air-cooled, argon-ion laser. Samples are introduced through a stainless steel injection tube equipped with an outer droplet containment sleeve. The sleeve works in conjunction with a vacuum pump to eliminate droplet formation of sheath fluid as it backflows from the injection tube. Fluidic controls allow you to select the fluidics mode and sample flow rate.

Located just above the fluid control panel, three collection tubes are housed in an enclosed compartment. Install one, two, or three collection tubes, and the instrument automatically determines the maximum volume of sample to collect.

The fluidics drawer provides easy access to the removable sheath reservoir and waste reservoir as well as the sheath fluid filter. A vent valve toggle switch allows you to quickly depressurize the sheath reservoir should you need to refill it during a run.

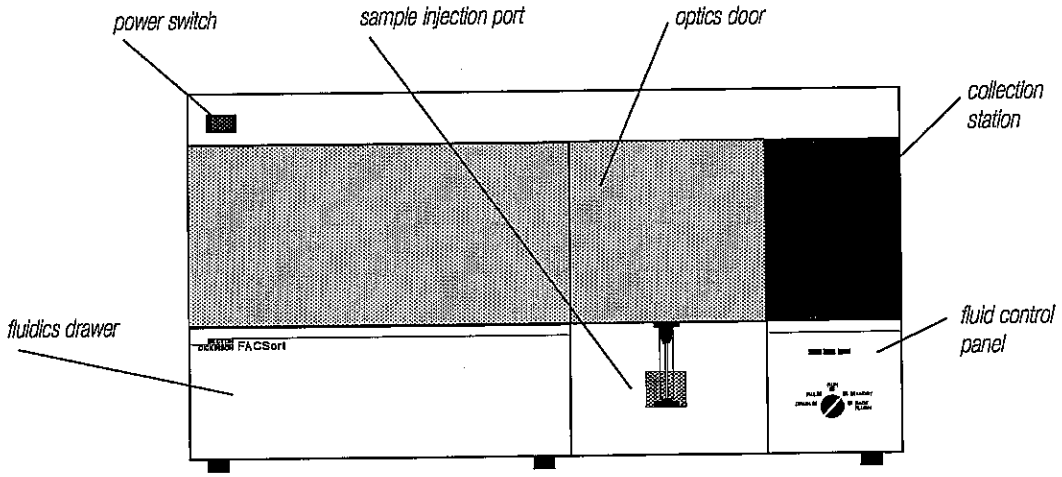


Figure 1-2 FACSort instrument

power switch

The switch that turns FACSort on and off.

collection station

The area that houses the collection tubes. Refer to Figure 3-7 for a diagram of the collection station.

collection tubes—Three 50-mL conical tubes that collect the cells after they are sorted. You may install one to three tubes.

sort line—The tubing that delivers the sorted sample to the collection tubes. The line is not visible from the collection station; however, periodic cleaning involves flushing this tubing.

optics door

The door shielding the compartment that houses the flow cell assembly. Open this door to view the flow cell (Figure 1-3) while draining and filling. The laser light is blocked by a glass filter when the door is open.

flow cell—A quartz cuvette where the sample suspension is injected into a moving stream of sheath fluid. The flow cell is the area where the laser intercepts the sample stream.

catcher tube—The device that captures the cells to be sorted. It is not visible in this figure but is positioned within the top portion of the flow cell just above the viewing orifice.

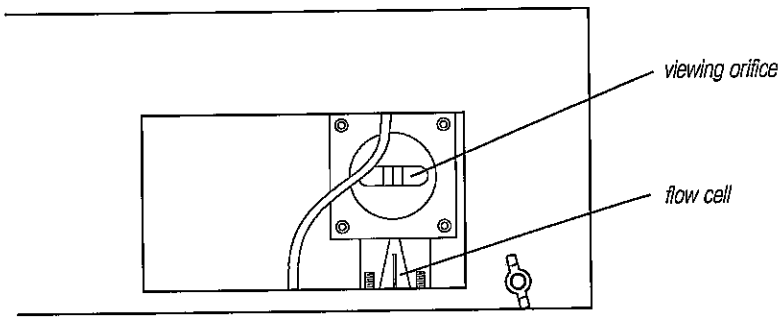


Figure 1-3 Flow cell

fluid control panel

The panel where the sample flow rate and fluid modes are set (Figure 1-4).

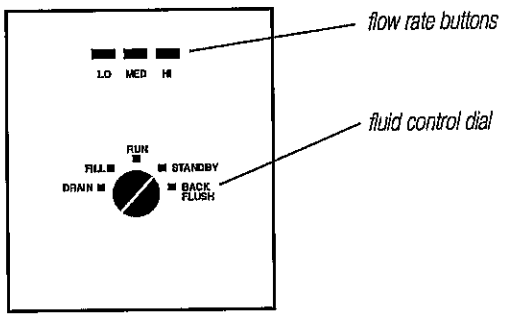


Figure 1-4 Fluid control panel

sample flow rate buttons—Three buttons that allow you to control the sample flow rate:

LO delivers 12 µL ±3 µL/min of sample through the flow cell.

MED delivers 35 µL ±5 µL/min of sample through the flow cell.

HI delivers 60 µL ±7 µL/min of sample through the flow cell.

fluid control dial—A rotary dial that allows you to select among five fluidics modes:

DRAIN stops the flow of sheath fluid and reverses the pressure to force fluid out of the flow cell and into the waste reservoir. Follow DRAIN with FILL to refill the flow cell.

FILL fills the flow cell with sheath fluid at a controlled rate to prevent bubble formation or entrapment.

RUN aspirates the sample from the sample tube and establishes a constant pressure differential between the sample and the sheath fluid as they flow through the flow cell.

STANDBY restricts fluid flow and reduces laser power to conserve laser life.

☐ NOTE: When a sample is removed from the sample injection port (SIP), the instrument switches to an automatic STANDBY status to conserve sheath fluid.

BACKFLUSH reverses the flow of sheath to flush fluid out of the sample injection tube to help remove clogs. Follow BACKFLUSH with DRAIN, then FILL to refill the flow cell.

sample injection port (SIP)

The area where the sample tube is installed on the instrument. It includes the sample injection tube and the tube support arm (Figure 1-5).

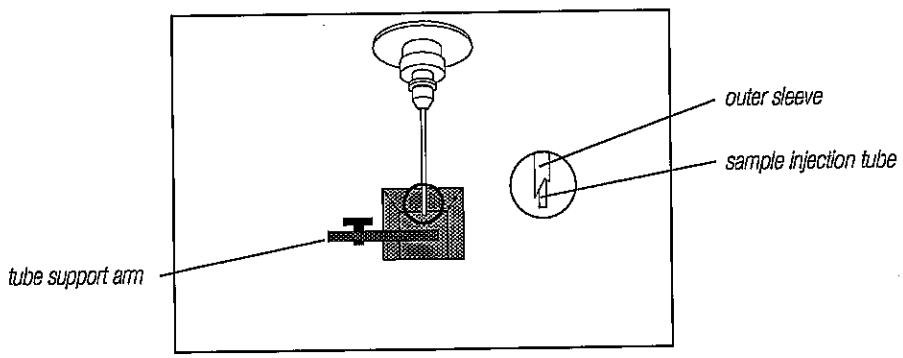


Figure 1-5 Sample injection port (SIP)

sample injection tube—The stainless steel tube that carries cells from the sample tube to the flow cell. The tube is covered with an outer sleeve that serves as part of a droplet containment system.

tube support arm—The arm that supports the sample tube. It also functions to activate the droplet containment system vacuum. The vacuum is on when the arm is positioned to the right and off when the arm is centered.

fluidics drawer

The lower-left panel on the instrument that slides out for easy access to the fluid reservoirs and sheath filter (Figure 1-6). See Figure 2-1 for the specific locations of the fluidics drawer components.

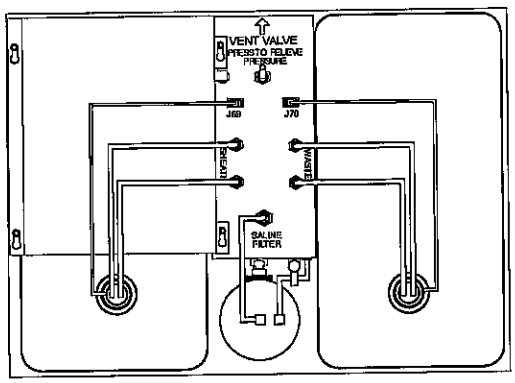


Figure 1-6 Fluidics drawer

sheath reservoir—(located on the left) This 4-L container holds enough sheath fluid for approximately 3 hours of run time. It is equipped with a fluid level detector that indicates, via the software, a near-empty condition. A metal bracket fits over the reservoir to secure it.

waste reservoir—(located on the right) This 4-L container collects the fluid waste after it flows from the flow cell. A fluid level detector indicates, via the software, a near-full condition.

sheath filter—A 0.22-µm filter that cleans the sheath fluid before it enters the flow cell. This minimizes the possibility that debris from sheath fluid will be acquired with your samples.

air filter—This filter cleans the air that cools the laser.

vent valve toggle switch—A switch that, when set in the direction of the arrow, relieves the sheath reservoir of air pressure. This allows for the removal of the reservoir when refilling.

Other than the power switch, fluid control dial, and sample flow rate buttons, located on the front of the instrument, all instrument adjustments are controlled through the software.

1.3.2 Computer Module

The computer controls the instrument electronics. This means that any adjustments made to FACSort's detectors or amplifiers are made through the software. Data acquisition, storage, and analysis are also controlled through the software, as well as the controls associated with sorting. Refer to the *CELLQuest Software User's Guide* for more information.

1.4 Software Overview

CELLQuest software provides an easy-to-use, mouse-driven interface with pull-down menus and windows that display data in a variety of plots, including histograms, dot plots, contour plots, and density plots. In addition, CELLQuest offers acquisition with real-time statistics, various tools for data analysis, instrument control, and data storage capabilities. For detailed information on using CELLQuest, refer to the *CELLQuest Software User's Guide.*

FACSComp software is an instrument setup and performance evaluation program, which, when used with CaliBRITE beads, adjusts the FACSort's electronic components. This assists you in setting up the FACSort for two-color immunophenotyping and reticulocyte applications. FACSComp provides a sensitivity test that helps determine that your cytometer is operating consistently.

FACSConvert is a program that allows you to convert Hewlett-Packard CONSORT computer files from the FCS 1.0 format to the current FCS 2.0 file format. If you wish to analyze CONSORT-generated files, you will also need a file transfer program such as FACSNet™ Macintosh or CONSORT File Exchange to transfer HP files to the Macintosh computer. Use FACSConvert to convert the files after they have been transferred.

1.5 Installation

Your Becton Dickinson Field Service Representative will install and set up your FACSort instrument for you.

☐ NOTE: For installations outside the US, a power transformer/conditioner is provided to accommodate 100 V ±10%, 220 V ±10%, or 240 V ±10%, 50–60 Hz ±2 Hz.

CELLQuest software and any other programs you may have purchased will be loaded on your computer before it arrives. CELLQuest software requires the Acquisition Library and the Shared Library Manager (in the Extensions folder) and the BDMAC driver (in the Control Panels folder). These items are installed by BDIS. If you need to reinstall them, refer to the *CELLQuest Software User's Guide.*

1.5.1 Configuring the Software

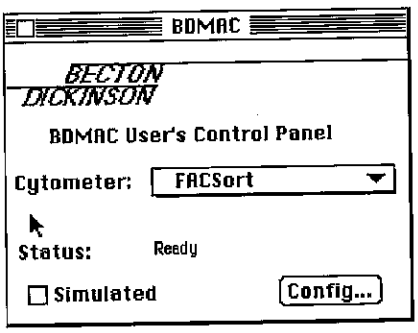
Your computer is equipped with a BDMAC card. This is a computer board that allows the Macintosh computer to communicate with the FACSort cytometer. Your field service representative will access the BDMAC window during initial installation to configure CELLQuest software for your cytometer type and to enter the serial number. The configuration information should be changed only if you connect your computer to a different cytometer.

You may, however, wish to open the BDMAC window to access the Simulated checkbox, which allows you to view simulated data.

1 **Choose Control Panels from the Apple menu.**

2 Double-click the BDMAC icon.

The BDMAC window appears.

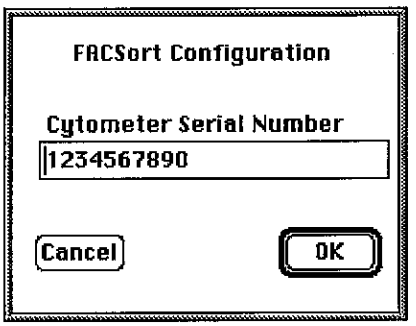


3 Choose FACSort from the list in the Cytometer pop-up menu.

Click Simulated if you wish to generate artificial data pulses, which mimic acquisition. This allows you to explore the software and choose various acquisition options, as if you were actually acquiring a sample.

4 Click the Config... button.

The FACSort Configuration dialog box appears.



5 Enter the serial number of the new cytometer, then click OK.

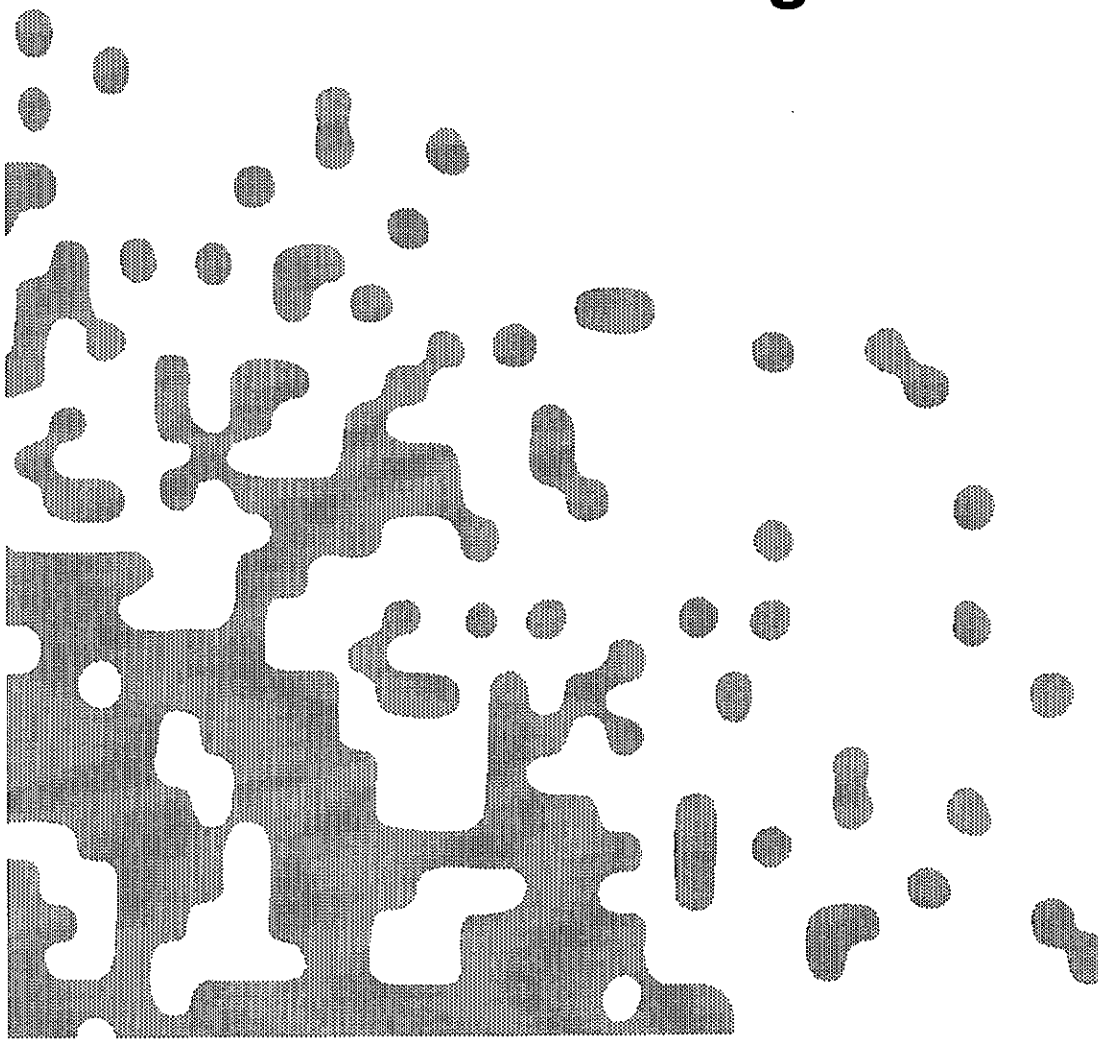
6 Close the BDMAC window.

7 Choose Restart from the Finder's Special menu.

You must restart the computer for configuration changes to take effect.

CHAPTER 2

Getting Started



CHAPTER 2
Summary

- filling the sheath reservoir
- emptying the waste reservoir
- starting up the FACSort; clearing air from the sheath filter and flow cell
- starting up the computer
- adjusting instrument settings

2.1 Before You Begin

Take a few minutes to study Figure 2-1 to familiarize yourself with the fluidics system components. Before turning on the instrument, check the fluid levels of the sheath reservoir and the waste reservoir.

- The sheath reservoir should be no more than 3/4 full (sufficient for approximately three hours of run time).
- The waste reservoir should contain approximately 400 mL of undiluted household bleach.

☐ NOTE: Undiluted bleach contains 5% sodium hypochlorite.

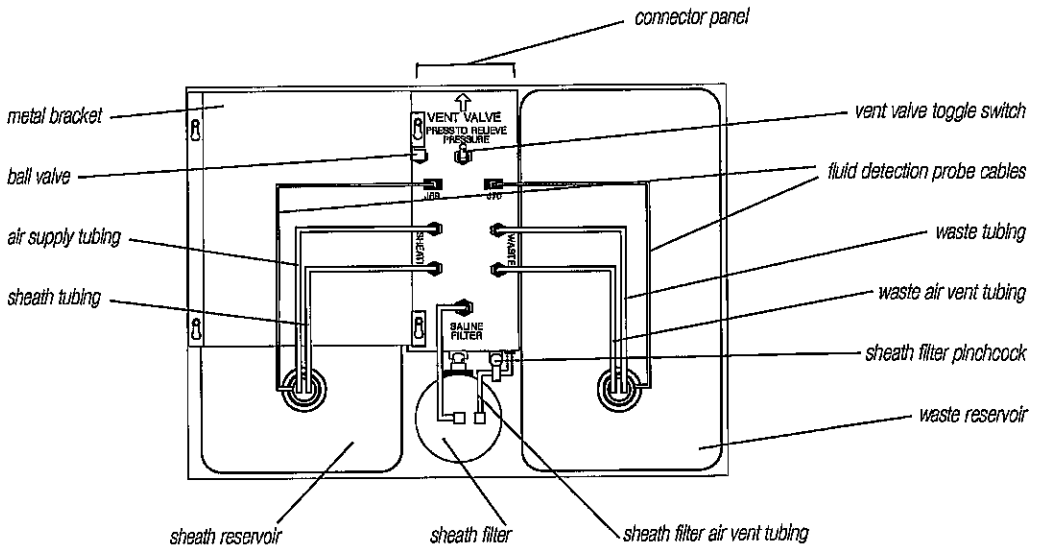


Figure 2-1 Filling the sheath reservoir

2.1.1 Filling the Sheath Reservoir

Fill the sheath reservoir before you turn on the FACSort. If the FACSort is already turned on, you will need to relieve the pressure from the sheath reservoir before you can fill it.

1 **Slide out the fluidics drawer.**

If the FACSort is powered on, set the fluid control dial to STANDBY and push the vent valve toggle switch located between the reservoirs in the direction of the arrow. This switch relieves the sheath reservoir of air pressure.

2 **Slide the metal bracket away from you, and lift up to remove it.**

3 **Disconnect the sheath tubing (white) and the air supply tubing (blue) from the FACSort by squeezing the metal clip on the quick-disconnects and pulling each connector from the fitting.**

4 **Disconnect the fluid detection probe cable by squeezing the tabs at the sides of the connector and pulling.**

5 Remove the sheath reservoir.

6 Unscrew the cap assembly from the reservoir and set the assembly aside.

7 Fill the reservoir to 3/4 its capacity with sheath fluid. Refer to Section 1.2.3 for the recommended sheath fluid.

⚠ CAUTION: Avoid filling the sheath reservoir to its maximum capacity. When the reservoir is filled beyond the recommended level, fluid may backflow into the air supply tubing, preventing proper pressurization and potentially damaging the instrument.

8 Replace the sheath reservoir.

- Replace and tighten the cap assembly on the reservoir. A securely tightened cap prevents air from leaking from the reservoir when the system is pressurized. It may be necessary to adjust the cap assembly so the tubing (1) is not pinched or twisted and (2) reaches the connectors on the connector panel.
- Install the reservoir.
- Replace the bracket. Lower the bracket over the reservoir with the ball valve tab against the connector panel. Pull the bracket toward you to lock it in place and actuate the ball valve.

☐ NOTE: The bracket must be properly installed to allow the sheath reservoir to pressurize.

- Snap the fluid and air supply tubing into their color-coded fittings by pushing firmly until you hear a click.
- Reconnect the waste fluid detection probe cable.

☐ NOTE: If you pushed the vent valve toggle switch in the direction of the arrow before removing the reservoir, remember to set the switch back to its original position to pressurize the reservoir.

2.1.2 Emptying the Waste Reservoir

⊘ WARNING: Blood samples may contain infectious agents that are hazardous to your health. Wear gloves when handling blood or any materials with which it comes in contact. Follow local, state, and federal biohazard waste handling regulations when disposing of biohazardous material.

Empty the waste reservoir when you fill the sheath reservoir. This prevents the waste reservoir from overflowing. Keep a spare waste reservoir on hand as a replacement so the full reservoir may be allowed to sit for 30 minutes prior to emptying.

1 **Slide out the fluidics drawer.**

2 Disconnect the waste tubing (orange) and the waste air vent tubing (white) from the FACSort by squeezing the metal clip on the quick-disconnects and pulling each connector from the fitting.

3 Disconnect the waste fluid detection probe cable by squeezing the tabs at the sides of the connector and pulling.

4 Remove the waste reservoir.

⊘ WARNING: Before disposing of waste reservoir contents, wait at least 30 minutes after the completion of the last run. This helps to ensure that biohazardous materials are inactivated before disposal.

5 Unscrew the cap assembly from the reservoir and set the assembly aside.

6 Empty the reservoir according to local, state, and federal biohazard waste handling regulations.

7 **Fill the waste reservoir to 10% capacity (400 mL) with undiluted household bleach.**

☐ NOTE: Undiluted household bleach contains 5% sodium hypochlorite.

8 **Replace the waste reservoir.**

- Replace the cap assembly on the reservoir. It may be necessary to adjust the cap assembly on the reservoir so the tubing (1) is not pinched or twisted and (2) reaches the connectors on the connector panel.
- Install the reservoir.
- Snap the waste and air vent tubing into their color-coded fittings by pushing firmly until you hear a click.
- Reconnect the fluid detection probe cable.

2.2 System Startup

To ensure proper initialization between the cytometer and the computer, turn on the FACSort before turning on the computer.

2.2.1 Starting up the FACSort

Check the fluid levels of the sheath reservoir and the waste reservoir. If necessary, fill the sheath reservoir and empty the waste reservoir.

1 **Turn on the FACSort.**

The power switch is located in the upper-left corner of the instrument.

Move the tube support arm to the right and remove the tube of distilled water from the sample injection port (SIP).

2 **Make sure the FACSort is pressurized.**

This can be done simply by making certain the sheath reservoir fits snugly beneath the bracket. When the system is fully pressurized, the reservoir does not move.

3 **Check the sheath filter for trapped air bubbles. Vent the air from the filter if necessary.**

If bubbles are visible, gently tap the filter body with your finger to dislodge the bubbles and force them to the top. Push the roller in the pinchcock forward to allow the pressurized sheath fluid to force the air bubbles into the waste reservoir.

4 **Clear the flow cell of trapped air bubbles by draining and filling it.**

- Open the optics compartment door.
- While viewing the flow cell, turn the fluid control dial to DRAIN until you see that all the sheath fluid has drained from the flow cell. Refer to Figure 2-2 for the location of the flow cell.
- Next, turn the fluid control dial to FILL and watch as the flow cell steadily fills with sheath fluid.
- Repeat the DRAIN and FILL as necessary until no bubbles are visible upon filling.
- Turn the fluid control dial to STANDBY.

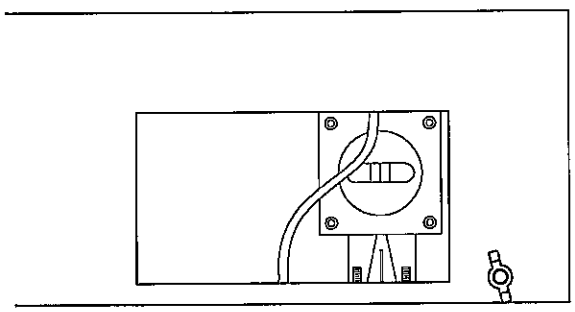


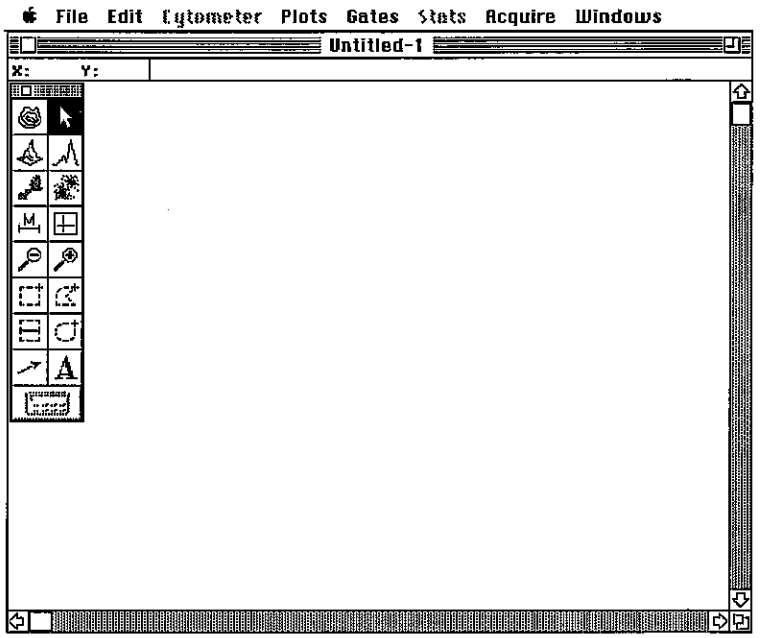
Figure 2-2 Flow cell

2.2.2 Starting up the Computer

1 Turn on the computer.

2 Choose CELLQuest from the Apple (🍎) menu to launch CELLQuest software.

Alternately you may double-click the program icon to start the program. The CELLQuest copyright message appears, followed by the CELLQuest desktop displaying an untitled experiment window.

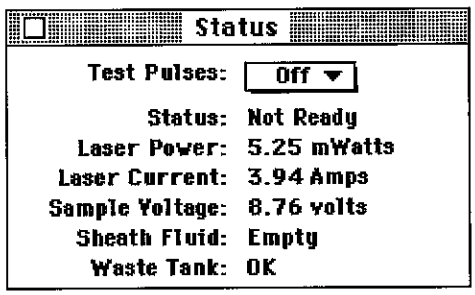


All CELLQuest software functions are performed in this window through the use of the tool palette and items within the menu bar. You may create plots for acquisition or analysis for your experiment, then save the entire window as a document to continue with your experiment at a later time. For more information on using CELLQuest, refer to the *CELLQuest Software User's Guide.*

2.2.3 Displaying the Instrument Status

To check the operating state of the FACSort, choose Status from the Cytometer menu. This displays the Status window.

☐ NOTE: The Cytometer menu is enabled only after you choose Connect to Cytometer from the Acquire menu. See Section 3.1.3, Starting Acquisition, for more information.



Field	Meaning
Test Pulses	Indicates when the instrument test pulses are set to Off, All, or FSC. To turn test pulses on or off, click and hold the Test Pulses field to open a pop-up menu and make your choices.
Status	*Not Ready* Indicates the laser is warming up (approximately 5 minutes), the sheath reservoir is empty, or the waste reservoir is full. *Ready* Indicates a sample is on the SIP, the tube support arm is centered, and the fluid control dial is set to RUN. *Standby* Indicates the fluid control dial is set to STANDBY, or the dial is set to RUN, but there is no sample tube on the SIP. ☐ NOTE: When the fluid control dial is set to STANDBY, the flow of sheath is restricted and the laser current is reduced. Be cautious when leaving a sample on the SIP with the dial set to STANDBY, as some fluid in the sample tube backflows to flush the tube, potentially diluting the sample.
Laser Power	Milliwatts of power the laser is generating. The laser power should be approximately 15 mW when a sample is running.
Laser Current	Amps of electrical current the laser is drawing. The current should not exceed 10 amps.
Sample Voltage	The differential relationship between the sheath pressure and the sample pressure.
Sheath Fluid	Displays Empty or OK to indicate the fluid level of the sheath reservoir.
Waste Tank	Displays Full or OK to indicate the fluid level of the waste reservoir.

2.2.4 Leaving the FACSort

If you are walking away from the system, set the fluid control dial to STANDBY to stop sheath consumption and reduce laser power. Install a tube containing no more than 1 mL of distilled water on the SIP and center the tube support arm. This prevents the sample injection tube from drying out. Follow this rule when the instrument is turned on or off.

⚠ CAUTION: Some fluid backflows in STANDBY mode; be sure that the tube left on the SIP contains *no more than 1 mL* of distilled water. This will prevent fluid from overflowing into the air supply tubing that pressurizes the tube.

If you are leaving the system for only a few minutes, move the tube support arm to the right and set the fluid control dial to either STANDBY or RUN. Remember however, that when the dial is set to RUN, sheath fluid is being consumed (ie, sheath drips from the sample injection tube and is aspirated to the waste reservoir).

2.3 Quality Control and Instrument Adjustment

Performing a daily quality control procedure is recommended to monitor the instrument and ensure that it is operating consistently from one day to the next. Here are a few quality control procedures to consider:

- Run FACSComp software
- Run BDIS's DNA QC particles
- Perform an alternative method of quality control

☐ NOTE: For information on running FACSComp software, refer to the *FACSComp Software User's Guide.* For information on the DNA QC particles, refer to the *DNA QC Particle* package insert.

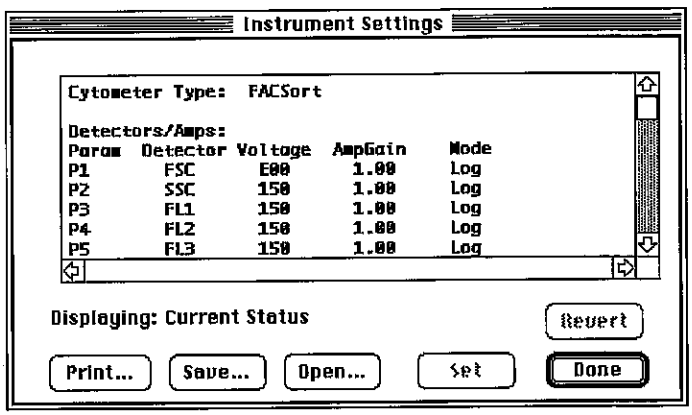
2.3.1 Sending Instrument Settings to the FACSort

Each time you run FACSComp software, the instrument settings are saved to a file in the BD Preferences folder. Additionally, every time you save a data file after running a sample, the current instrument settings are saved with the data file.

To retrieve instrument settings and send them to the FACSort:

1 **Choose Instrument Settings from the CELLQuest Cytometer menu.**

The Instrument Settings window appears displaying the current settings.



2 Click Open.

The Open a Data File dialog box appears.

3 Select the desired instrument settings file or FCS file, then click Open.

The new settings appear in the Instrument Settings window.

4 Click Set to send these settings to the FACSort.

If you wish to return the previous settings to the instrument, click Revert.

5 Click Done to remove the window.

2.3.2 Saving Instrument Settings

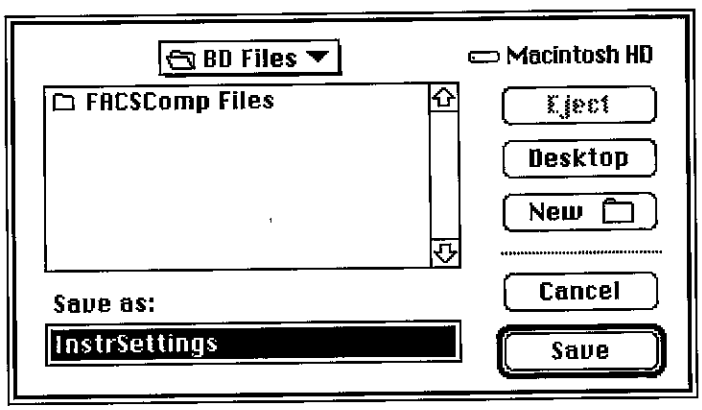
You can save the current instrument settings from your sample run or experiment. You can then recall these settings later and send them to the FACSort.

1 Choose Instrument Settings from the Cytometer menu.

The Instrument Settings window appears displaying the current settings.

2 Click Save.

The directory dialog box appears. The default name appearing in the text box is *InstrSettings*.



3 Select a storage location, then enter a file name (up to 27 characters) or use the default name that appears.

4 Click Save.

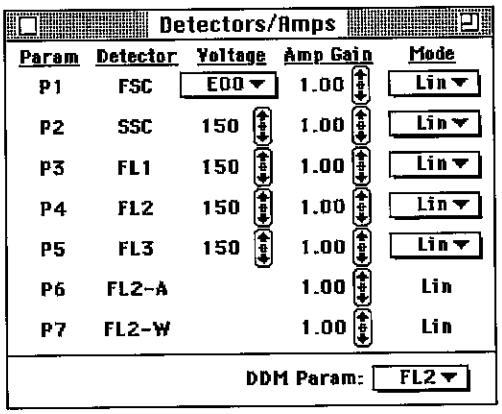
5 Click Done in the Instrument Settings window.

2.3.3 Manual Instrument Adjustment

Refer to the *CELLQuest Software User's Guide* for information on the Cytometer menu and making instrument adjustments.

DETECTORS

Choose Detectors/Amps from the Cytometer menu to select the signal amplification mode for each parameter (Lin or Log), adjust the amplifier gains and detector voltages, and select the DDM parameter.



FACSort signals undergo two stages of amplification—amplification at a Detector stage and amplification at an Amplifier stage.

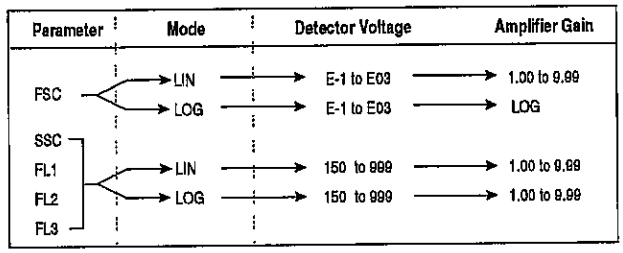


Figure 2-3 Path of amplification

Amplifier mode settings allow you the choice of processing each of the five signals through a linear amplifier or a logarithmic amplifier. The application and the range of signals will determine which mode you select for each parameter. While linear mode is better suited for a narrow range of signals, logarithmic mode is better suited for a wide range of signals. Generally speaking, when running samples for immunophenotyping, use linear mode for FSC and SSC parameters, and logarithmic mode for FL1, FL2, and FL3. For DNA cell-cycle analysis, use linear mode to acquire data for FSC, SSC, and FL2 fluorescence of propidium iodide.

Once the mode of amplification is selected, you may adjust the detector voltages and amplifier gains for the individual parameters. The SSC, FL1, FL2, and FL3 detectors are sensitive photomultiplier tubes (PMTs). When you increase the detector voltage, you increase the voltage applied to the PMT, thus increasing the signal amplification. This results in an increase in the channel number where an event appears.

The FSC detector is a photodiode which is better suited for strong signals. You do not adjust a voltage for FSC, but you may choose from five settings.

ADJUSTMENT	RANGE
Detector voltage	
FSC	E-1 to E03*
SSC, FL1, FL2, and FL3	150 to 999
Amplifier gain (adjust only when Amplifier mode is Lin)**	
FSC, SSC, FL1, FL2, and FL3	1.00 to 9.99

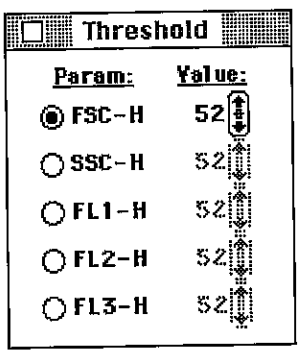
☐ NOTE: FACSComp software automatically sets the amplifier stages and adjusts the levels for each amplifier and detector. However, slight adjustments to the settings may be necessary for individual samples (refer to Section 3.1.1, Optimizing FACSort Settings for Acquisition).

* The symbol E represents the exponential function of base 10. For example, $E\text{-}1=10^{-1}=0.1$ which means the signal is multiplied by 0.1; $E02=10^{2}=100$ which means the signal is multiplied by 100.

** There is no adjustment to be made if the amplifier mode is Log.

THRESHOLD

Choose Threshold from the Cytometer menu to select the threshold parameter and adjust its level.



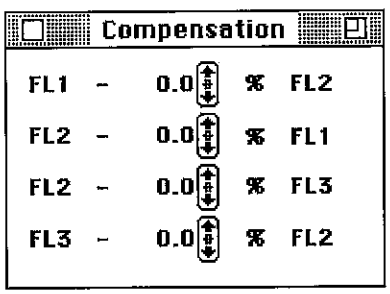
The threshold is the parameter that you select as your trigger signal. This means the flow cytometer must detect this parameter in a particle before the particle is considered for acquisition. By adjusting the threshold level, you can establish the baseline level of detection. When acquiring data, the instrument ignores any signal that does not exceed the threshold setting.

Because all cells or particles that you analyze have measurable size, FSC is usually the threshold parameter of choice. When acquiring samples stained with propidium iodide (PI) for DNA applications, FL2 is typically the threshold parameter of choice, because it allows you to collect data only for PI-stained particles.

Once you have selected the threshold parameter, adjust the level so that unwanted, low-level signals are ignored.

COMPENSATION

Choose Compensation from the Cytometer menu to correct for spectral overlap.



Because fluorochromes emit light over a range of wavelengths, emission from one fluorochrome may overlap emission from another fluorochrome. This overlapping emission is referred to as spectral overlap. Fluorescence compensation allows you to subtract a percentage of the contaminating or overlapping signal from the signal that you want to measure. Fluorescence compensation is necessary when your sample is stained with two or more fluorochromes with overlapping emission spectra. For example, to remove the overlapping FL2 signal from the FL1 signal (signal measured by the FL1 detector), select FL1-%FL2.

Determine the best fluorescence compensation by observing a dot plot of the two fluorescence parameters.

CHAPTER 3
System Operation

CHAPTER 3
Summary

- optimizing FACSort settings before acquisition
- supplying acquisition information using CELLQuest software
- starting and ending acquisition
- shutting down the FACSort
- setting up for sorting
- starting and ending sorting
- concentrating the sorted sample

3.1 Acquiring Data

Whether you use FACSort to acquire data for routine clinical applications or research experiments, there are several steps that are important to follow before acquiring samples. Refer to the appropriate software user's guides for detailed information on acquisition.

3.1.1 Optimizing FACSort Settings for Acquisition

In Section 2.3, Quality Control and Instrument Adjustment, you adjusted the instrument using a quality control and setup particle. Although this is an ideal way to monitor instrument performance, it may not optimally set up the FACSort for the samples you will be acquiring.

Optimization is the instrument adjustment procedure that optimally sets the detectors, amplifiers, threshold, and compensation for specific samples. It involves making the necessary adjustments to the FACSort while viewing a live display of the sample before you acquire.

The procedure depends on the application as well as the number of fluorochromes used (eg, two-color optimization differs from three-color optimization). For immunophenotyping applications, an FSC vs SSC plot is viewed to ensure that all cell populations of interest are on scale for these parameters (see Figure 3-1). Additionally, if two or more fluorochromes are used, the appropriate fluorescence plot is also viewed so that PMT voltages and compensation may be adjusted as necessary (Figure 3-2).

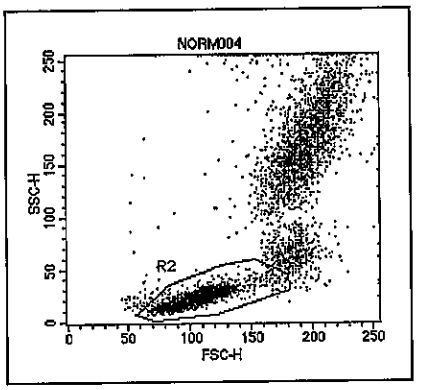


Figure 3-1 FSC vs SSC display of lysed whole blood

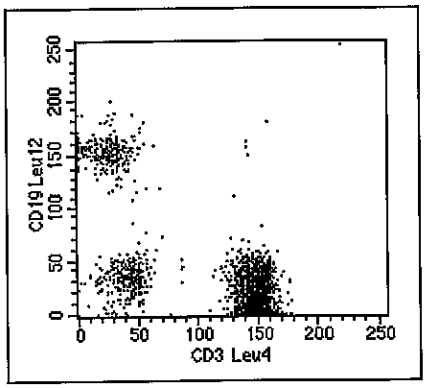


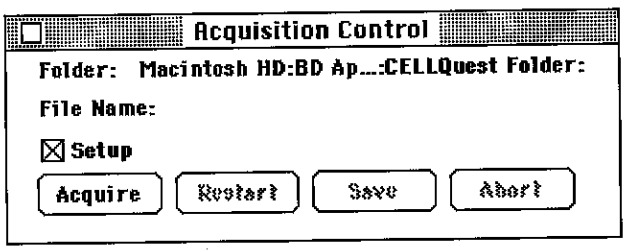
Figure 3-2 FL1 vs FL2 display of two-color stained lymphocytes

Refer to the appropriate software user's guide for specific optimization procedures.

To optimize instrument settings when using CELLQuest software, perform the following steps, only when necessary:

1 Choose Connect to Cytometer from the Acquire menu.

The Acquisition Control window appears. Leave the Setup box checked during optimization.



Once you are connected to the cytometer, the Cytometer menu is enabled. This menu allows you access to the Threshold, Detectors/Amps, and Compensation menu items.

2 **Choose Threshold to select the threshold parameter (do not adjust the level).**

3 **Choose Detectors/Amps to select the amplifier mode (Lin or Log) for each parameter.**

If you are acquiring samples for DNA analysis, select the DDM parameter from the pop-up menu.

4 **From the Detectors/Amps window, adjust the detector levels (PMT voltages and FSC gain) to get the populations on scale.**

5 **From the Detectors/Amps window, adjust the amplifier gain levels if Lin mode was selected.**

6 **From the Threshold window, adjust the level to eliminate low-level signals.**

7 **Choose Compensation to adjust compensation for samples stained with two or more fluorochromes.**

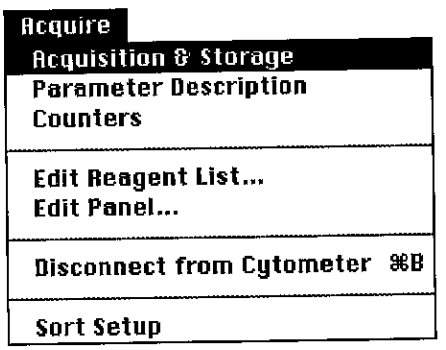
For a more precise view of the fluorescence data, before adjusting compensation, view the FSC vs SSC plot and draw a gate around the population that was stained with the monoclonal antibodies (ie, lymphocytes). Then view the fluorescence plot of the gated data.

For information on creating gates, refer to the *CELLQuest Software User's Guide.*

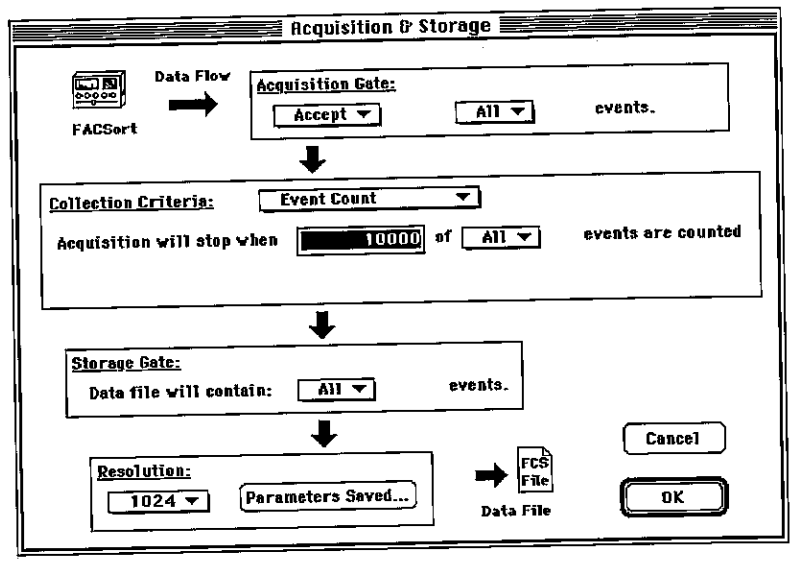
3.1.2 Defining Acquisition Information

Before acquiring samples, supply all necessary information about samples and conditions for acquisition.

1 **Choose Acquisition & Storage from the Acquire menu.**



The Acquisition & Storage dialog box appears.



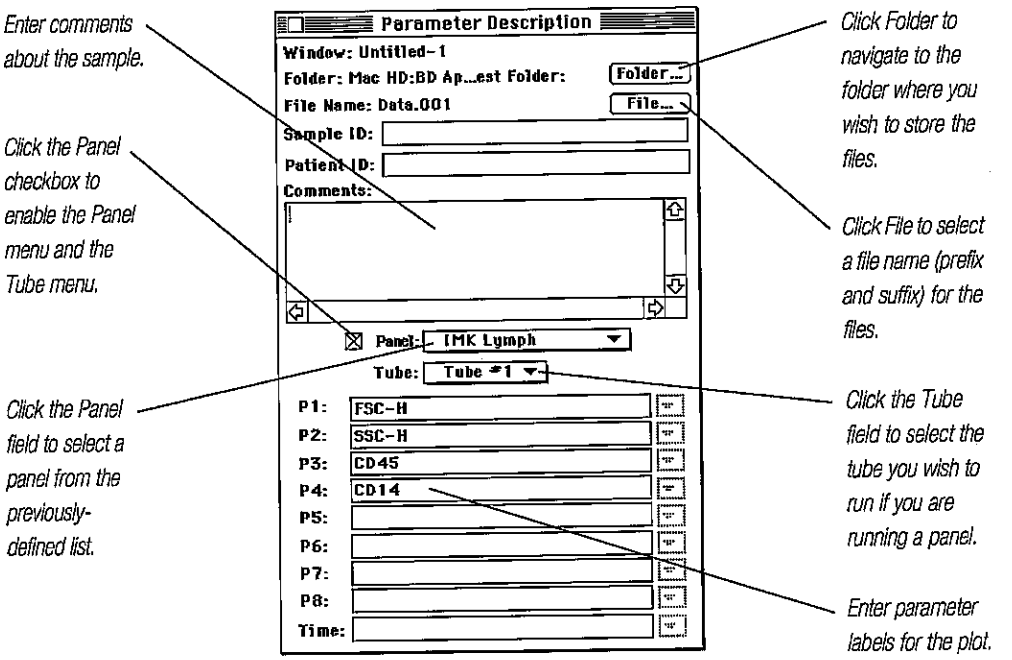
2 Enter all information necessary for your run, then click OK.

From this window you may select an acquisition gate, enter the number of events you wish to acquire and store, define how acquisition will end, and choose the parameters you wish to save and their resolution. You may also select a storage gate to determine which data are saved to the file.

☐ NOTE: To select any gate within this window, you must first create the gate within the plot. Refer to the *CELLQuest Software User's Guide* for detailed information on creating gates.

3 Choose Parameter Description from the Acquire menu.

The Parameter Description window appears.



This window allows you to name the data file, enter a storage location, select a reagent panel, and label the plot axes. You may also enter a Sample ID, Patient ID, and comments about the sample. All information is saved with the data. Refer to the *CELLQuest Software User's* Guide for detailed information on the Parameter Description window.

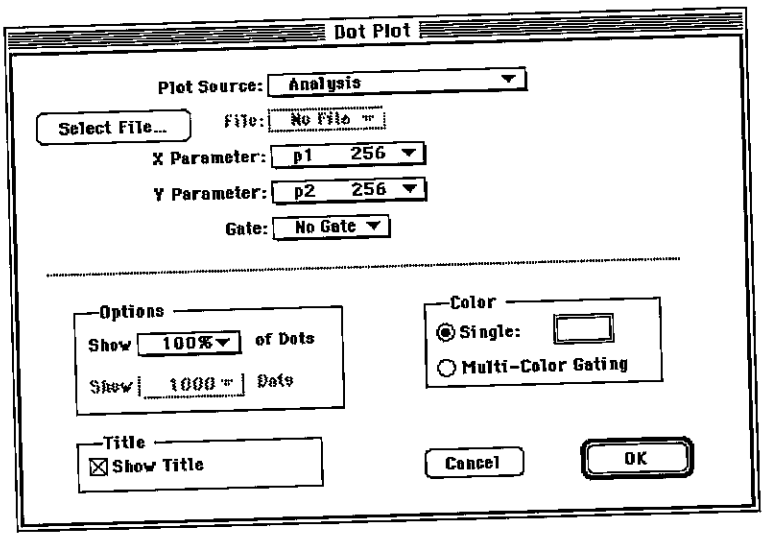
4 You may click the close box to remove the Parameter Description window from the desktop, or keep the window available for easy access during acquisition.

3.1.3 Starting Acquisition

Acquisition is described in detail in the *CELLQuest Software User's Guide.*

1 Create a dot plot in the experiment window.

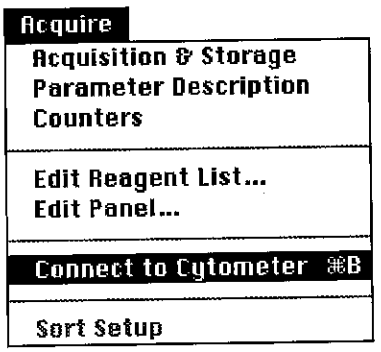
Select the dot plot tool from the tool palette, then click in the experiment window and drag diagonally until the plot is the desired size. The Dot Plot dialog box appears.



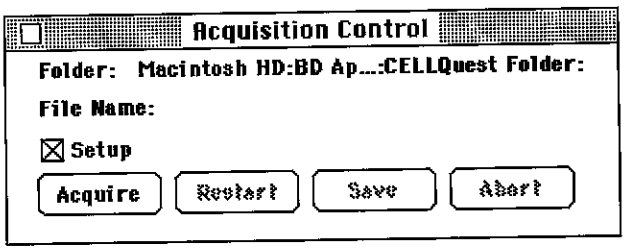
2 Choose Acquisition from the Plot Source pop-up menu. Select any other options that may apply, then click OK.

Select the appropriate parameters for the plot and a gate for displaying a specific subpopulation of data, if applicable.

3 Choose Connect to Cytometer from the Acquire menu.

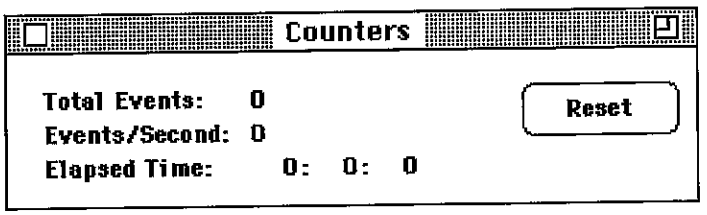


The Acquisition Control window appears. A checked Setup box allows you to click Acquire and view a real-time acquisition display without saving the data to a file. This is convenient when optimizing instrument settings before acquisition. Click the Setup box to remove the X when you wish to save data to a file.



4 You may choose Counters from the Acquire menu if you wish to view the event rate, total events, and elapsed time during acquisition.

The Counters window appears.



5 Install your sample tube on the SIP and quickly center the tube support arm under the tube.

For optimal acquisition rates, the sample tube should contain cells at a concentration of 1 x 10^5 to 1 x 10^7/mL.

⚠ CAUTION: When the droplet containment vacuum is activated (ie, the tube support arm is moved to the right), your sample will be aspirated to the waste reservoir.

6 Set the fluid control dial to RUN.

7 **Click Acquire in the Acquisition Control window.**

While the Setup box is checked, you may view live data before you acquire. Click to remove the X when you are ready to acquire events to a file.

8 **If necessary, optimize instrument settings.**

After choosing Connect to Cytometer, the Cytometer menu is available. This menu contains items that allow you to adjust instrument settings.

See Section 3.1.1, Optimizing FACSort Settings for Acquisition, for information on optimization and an example of optimizing for an immunophenotyping application.

9 **Acquire sample data to a file:**

- Click Pause, then Abort.
- Click to remove the X from the Setup box.
- Click Acquire.

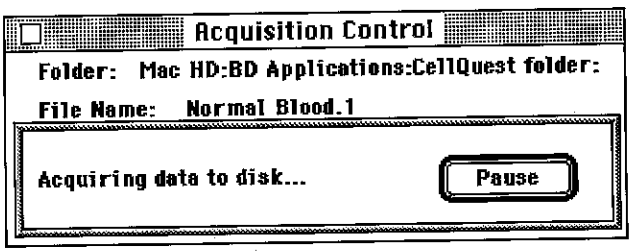
10 **Remove the sample when acquisition is complete.**

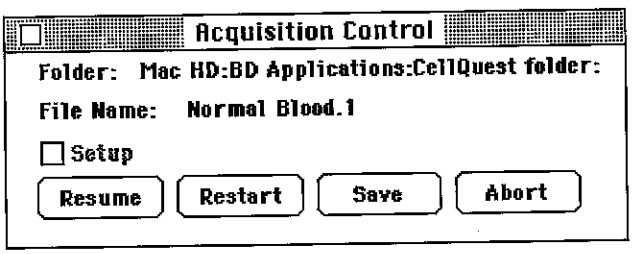
3.1.4 Ending Acquisition

There are three ways to end data acquisition. Acquisition ends when the first of these conditions is met. Refer to the *CELLQuest Software User's Guide* for more information.

1. Manually

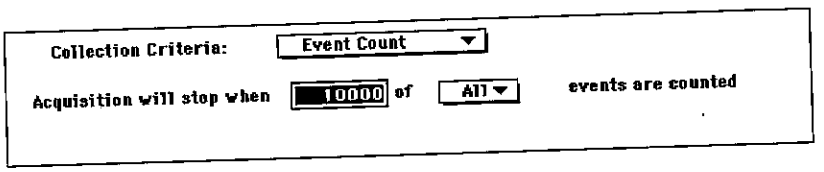
Click Pause in the Acquisition Control window. Then click Resume, Restart, Save, or Abort.





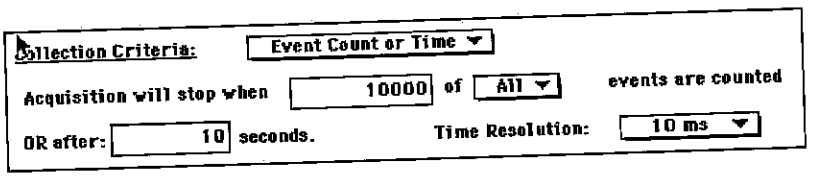
2. By Count

Before acquiring, choose Event Count from the Collection Criteria pop-up menu in the Acquisition and Storage dialog box. Then enter the number of events. Acquisition ends when this number is reached.



3. By Time

Before acquiring, choose Event Count or Time from the Collection Criteria pop-up menu in the Acquisition and Storage dialog box. Enter the number of seconds that you wish to acquire. Enter zero (0) for continuous acquisition. Acquisition ends when the time has elapsed.



3.1.5 Shutting Down the FACSort

Always clean the FACSort before you power it off at the end of the day. Proper cleaning ensures that your instrument maintains its accuracy and reliability.

1 **Follow the daily cleaning procedure outlined in Section 5.1.1., Daily Cleaning.**

Allow the FACSort to remain in STANDBY for 5 minutes before turning off the power. This gives the laser time to cool before shutdown.

2 **Turn off the FACSort.**

3 **Exit the software.**

Choose Quit from the File menu. Quit all other applications before turning off the computer.

4 **Shut down the computer.**

Choose Shut Down from the Special menu.

3.2 Sorting

There is little preparation necessary to sort. Once you're set up for acquisition, sorting is as simple as:

1. Filling the sheath reservoir with the appropriate sheath fluid (PBS)
2. Installing BSA-coated collection tubes (1-3)
3. Defining the sort mode and number of cells to be sorted
4. Identifying the population by setting a gate around it

☐ NOTE: If you are not using FACSort for sorting applications, follow the maintenance procedure outlined in Section 5.2.1 to fill the sort line with distilled water. This prevents the accumulation of saline deposits in the line.

Although FACSort has made sorting easy even for those with no sorting experience, understanding how FACSort determines when to sort a cell will help you to decide which sort mode to select.

3.2.1 How FACSort Sorts

To capture cells, FACSort uses a device called a catcher tube, positioned within the sheath stream in the upper portion of the flow cell. As a cell passes through the laser, the FACSort electronics quickly decide if it is a cell of interest (target cell), based on the sort gate characteristics (Section 3.2.5). Once this is established, the decision to capture a target cell is determined by the sort mode you select prior to sorting (Section 3.2.2).

When the decision is made to capture the target cell, the electronics wait a fixed period of time to allow the cell to reach the catcher tube before triggering the catcher tube to swing into the sample stream to capture the cell. Figure 3-3a and 3-3b illustrate the process of sorting a cell. Figure 3-3a shows the catcher tube in its resting position in the sheath stream. Figure 3-3b shows the catcher tube positioned in the sample core stream ready to capture a target (shaded) cell.

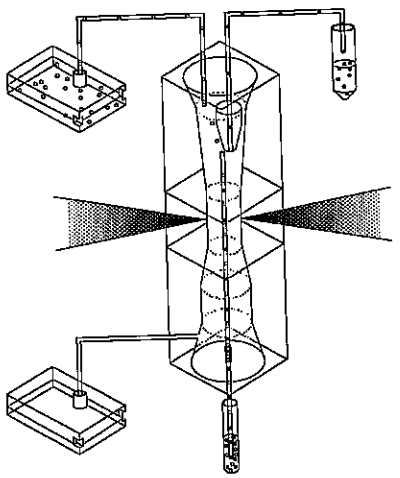


Figure 3-3a Catcher tube in sheath stream

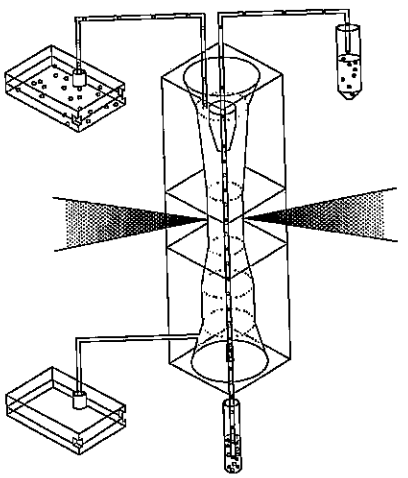


Figure 3-3b Catcher tube in sample stream

Because the catcher tube is positioned in the sheath stream while it waits for a target cell, it continuously collects sheath fluid, in addition to the sorted cells, resulting in a dilute sample. After sorting, the collection tubes must be centrifuged to concentrate the cells.

3.2.2 Sort Modes

Before we talk about sort modes and how each works, let's discuss the sort envelope. The sort envelope is the area within the sample stream the catcher tube collects as it captures a target cell. The size of the envelope reflects the amount of time the catcher tube remains in the sample stream to capture the cell. Although this envelope contains the target cell, it may also contain a non-target cell, thus creating a conflict. Should the catcher tube sort a cell if a non-target cell will be sorted along with it? This is where the sort mode comes into play. The sort mode determines whether or not to sort a cell when a conflict occurs. Refer to Figure 3-4 for examples of how the catcher tube decides to sort a cell for each sort mode.

The sort mode selection is made according to the composition and concentration of the sample suspension, as well as the objectives you wish to achieve with the collected cells. For example, if you are sorting a rare population, you may be willing to accept lower purity to sort the maximum possible number of target cells. Select the appropriate sort mode from the Sort Setup window (Section 3.2.3) for your sorting application.

SINGLE CELL

In single sort mode, a sort occurs whenever a *single target cell* is identified in the envelope. If any additional cell is located within the sort envelope, the envelope will not be sorted. The result is high purity with less emphasis on recovery. Single Cell mode also gives you increased count accuracy.

RECOVERY

In enhanced recovery mode, a sort occurs *whenever* an envelope is identified as having a *target cell,* even if a non-target cell is also in the envelope. Additionally, if another target cell is located just outside the envelope, the catcher tube stays in the stream for a longer period of time to capture it as well. The result is high recovery—capturing as many target cells as possible—with less emphasis on purity.

EXCLUSION

In exclusion mode, a sort occurs when a *target cell* is identified, and there are *no non-target cells* in the sort envelope. Also, if a second target cell is located just outside the sort envelope, no special attempt is made to capture this additional target cell. The result is high purity and recovery that falls between Single Cell and Recovery.

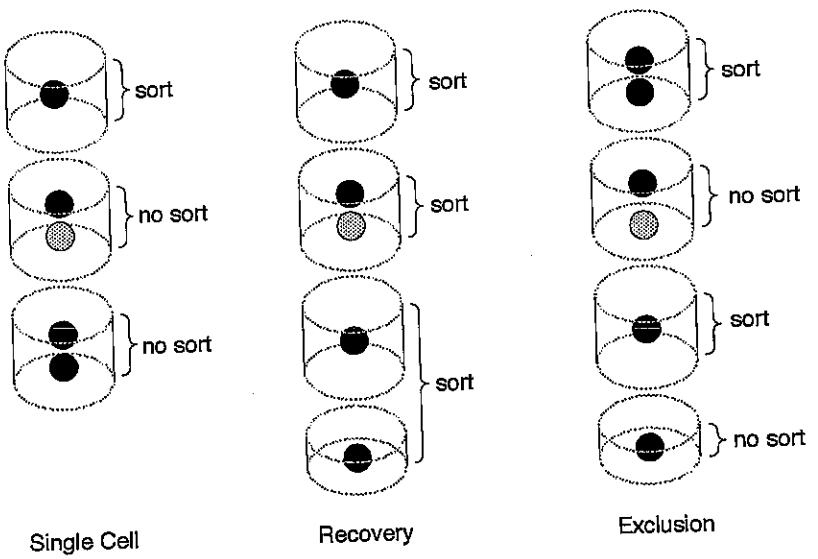


Figure 3-4 How envelopes are sorted for each sort mode

3.2.3 Sort Setup and Sort Counters Windows

The Sort Setup dialog box (Figure 3-5) allows you to control all sorting options. Choose Sort Setup from the Acquire menu to select the gate to be used for sorting, the number of cells to be sorted, and the sort mode. The Sort Counters window (Figure 3-6) allows you to select counters to monitor the sorted and aborted cells. Choose Sort Counters from the Cytometer menu to open this window.

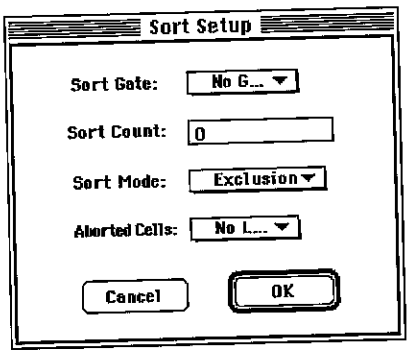


Figure 3-5 Sort Setup window

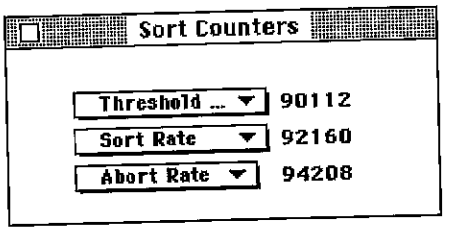


Figure 3-6 Sort Counters window

Window Item	Function
Sort Gate (click to display the Gate pop-up menu)	Displays the list of logical gates, and allows you to choose a sort gate. The subset of data in this gate will be sorted into the collection tubes. Selecting No Gate allows you to acquire without sorting.
Sort Count	Enter the number of cells you wish to sort. Zero (0) allows you to sort continuously.
Sort Mode	Select among Single Cell, Recovery, or Exclusion. Refer to Section 3.2.2 for more information.

Window Item	Function
Aborted Cells	Select between List or No List to acquire (to the computer) the data from aborted cells (cells rejected because of physical characteristics that interfere with the detection process).
Sort Counters	Three counters are available to display a rate or an accumulation of these four values: threshold, auxiliary, sort, and abort. Select Threshold or Auxiliary in Counter 1; Sort or Threshold in Counter 2; Abort or Sort in Counter 3.
Threshold Rate Threshold Total	Displays the rate (events/sec) or the total number of cells triggering the threshold. These cells are considered for acquisition and sorting. Includes the aborted cells.
Auxiliary Rate Auxiliary Total	Displays the rate (events/sec) or the total number of cells that the FACSort is processing. Includes the aborted cells.
Sort Rate Sort Total	Displays the rate (events/sec) or the total number of cells that are sorted.
Abort Rate Abort Total	Displays the rate (events/sec) or the total number of aborted cells (cells rejected because of physical characteristics that interfere with the detection process). Aborts are determined before the electronics decide whether the cell is a target cell.

3.2.4 Preparing Collection Tubes

Collection tubes must be coated with BSA to help maintain cell integrity and increase cell yield during centrifugation. Prepare your collection tubes at least one hour before you are ready to sort.

1 **Fill one to three 50-mL conical tubes with a 4% BSA (bovine serum albumin) solution.**

Dilute BSA in 1X PBS + 0.1% NaN_3.

2 **Place the tubes on ice or in the refrigerator for at least one hour.**

3 **Pour the BSA from the tubes into a bulk container when the coating process is finished.**

BBSA may be recycled for one month.

4 **Install the collection tubes on the instrument.**

Starting from the leftmost position, place from one to three BSA-coated, 50-mL conical collection tubes into the collection station (Figure 3-7). The instrument detects how many tubes have been installed and fills each tube starting with the leftmost.

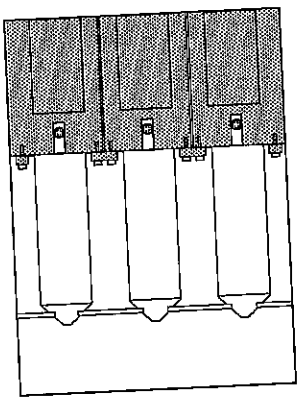


Figure 3-7 Collection tubes

☐ NOTE: It takes 9 minutes to fill each tube with 40 to 45 mL of fluid.

5 **Store the BSA at 2° to 8°C.**

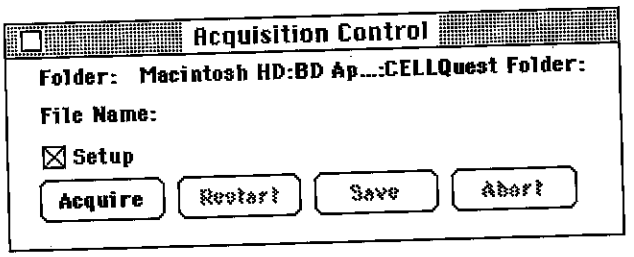
3.2.5 Setting a Sort Gate

The regions you define in CELLQuest are referred to as global regions because they can be used interchangeably when defining gates for acquisition, analysis, and sorting. For detailed information on drawing a region or creating gates, refer to the *CELLQuest Software User's Guide.*

1 Create an acquisition plot.

2 Choose Connect To Cytometer from the Acquire menu.

The Acquisition Control window appears. The Setup check box should be checked.



3 Install the sample tube on the SIP, and quickly center the tube support arm under the tube.

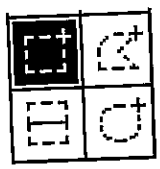
4 **Click Acquire in the Acquisition Control window.**

View the appropriate plots to ensure that the instrument settings have been properly optimized. Refer to Section 3.1.1 for more information. Make adjustments, if necessary.

5 **Click Pause, then Abort, and remove your sample.**

To conserve your sample, remove it from the SIP and draw the gate using the display that remains in the plot.

6 **Click to select a region tool in the tool palette.**



Choose among rectangular, elliptical, polygonal, or histogram regions.

7 **Click in the plot and drag diagonally to draw a region around the population you wish to sort.**

Refer to the *CELLQuest Software User's Guide* for details on drawing regions.

3.2.6 Sorting the Sample

Before beginning a sort, be sure of the following:

- the sheath reservoir is filled with 1X phosphate-buffered saline
- the BSA-coated collection tubes have been installed

1 **Choose Sort Setup from the Acquire menu.**

This displays the Sort Setup window (see Figure 3-5). Refer to Section 3.2.3 for information on this window.

2 **Enter or select the appropriate information at each field of the Sort Setup window, then click OK.**

a. Select the sort gate from the list in the Sort Gate field
b. Set the Sort Count
c. Select the Sort Mode
d. Choose whether to list aborted cells

3 If desired, choose Sort Counters from the Cytometer menu and select the counters to display.

4 Install the sample tube on the SIP, and quickly center the tube support arm under the tube.

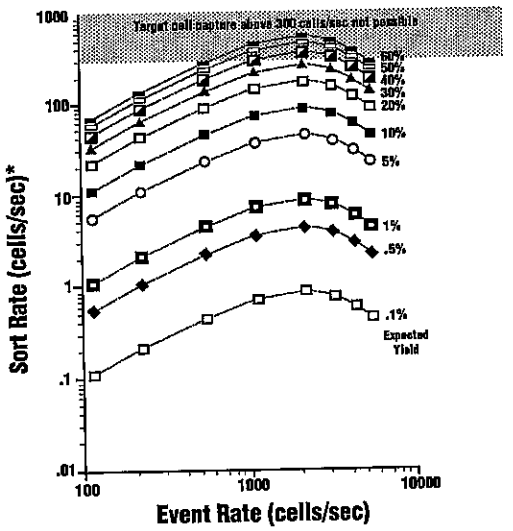
5 Click Acquire in the Acquisition Control window.

As the sample is sorted, you will hear a static-like sound indicating sorting is taking place.

6 Allow the sample to sort.

Sorting stops when the first of four conditions is met (see Section 3.2.7 for details).

When considering the sample concentration, it is important to understand the relationship between the event rate and the sort rate. Figure 3-8 illustrates this relationship when the sort mode is Single Cell. Notice that the maximum capture rate for any given concentration of target cells occurs at an event rate of approximately 2000 cells/sec. An event rate greater than this would result in a gradual decrease in the number of target cells sorted.



*Multiply by 12 to get yield (cells/mL)

Figure 3-8 Sort yield at various event rates and sample concentration

3.2.7 Ending Sorting

There are four ways to end sorting:

1. Manually

Click Pause, then Abort.

2. By Acquisition Count

Set Collection Criteria to Event Count and enter the number of events to acquire (see Acquisition and Storage window in the *CELLQuest Software User's Guide).* Sorting stops when this number is reached if you are acquiring data to a file. The Sort Count can be set to 0 (zero) to sort continuously while you acquire.

3. By Sort Count

Set Sort Count to the number of cells to be sorted (see Sort Setup window). Sorting stops when this number is reached.

4. By Time

Set Collection Criteria to Time and enter the sort time (see Acquisition and Storage window in the *CELLQuest Software User's Guide).* Sorting stops when this time is reached if you are acquiring data to a file.

When any two or more of the above parameters are used simultaneously, sorting stops when the first parameter is reached.

☐ NOTE: If the collection tubes fill before any of the above conditions is met, sorting continues, but the sorted sample is sent to the waste reservoir. If you wish to continue sorting after the collection tubes are filled, replace the collection tubes with clean BSA-coated tubes; click Pause, then Restart.

3.2.8 Concentrating the Sample

Because the sorted sample is dilute, it is necessary to concentrate it before proceeding to analysis.

To concentrate your sample:

1 Remove the collections tube(s) from the instrument.

Carefully lift and remove the collection tubes from the instrument and cap them.

2 Spin the tubes at 300 x *g* for 5 minutes.

3 Using a pasteur pipette and a vacuum system, aspirate the supernatant.

Be careful not to disturb the pellet.

4 Resuspend the pellet with 100 µL of PBS.

3.2.9 Aseptic Sorting

FACSort can sort cells that are to be used for reculture or functional studies. To meet the needs for this application, sorting requires a clean environment so that the sorted sample remains free from contaminants when returned to culture.

Perform all steps of your preparation procedure using aseptic technique.

1 **Using proper aseptic technique, prepare the following sterile solutions:**

- 4 L of 70% ethanol (EtOH) (dilute in sterile distilled water)
- 5 L of sterile 1X phosphate-buffered saline (PBS)

2 **Working under a hood and using aseptic technique, fill a clean sheath reservoir with 4 L of 70% EtOH.**

For information on removing and installing the reservoirs, refer to Sections 2.1.1 and 2.1.2.

Cap and shake the reservoir to ensure that the entire inner surface of the reservoir is washed with EtOH.

3 **Install the reservoir in the instrument.**

Using a squirt bottle filled with EtOH, rinse off the collection station ports.

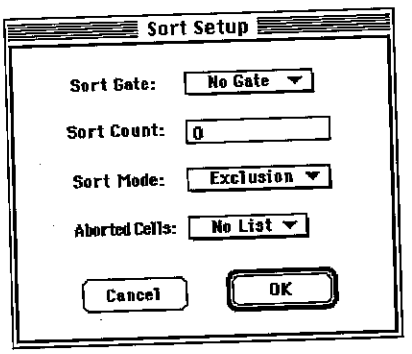
4 Place three collection tubes in the collection station.

5 Install tube of EtOH on the SIP.

6 Follow the instructions outlined in Section 3.2.5, steps 1 through 7 to set a sort gate.

Draw an arbitrary region in the empty display to enable sorting.

7 Choose Sort Setup from the Acquire menu.



8 Choose the gate that you drew in step 6 from the Sort Gate pop-up menu.

9 Turn the fluid control dial to RUN.

10 Click Acquire in the Acquisition Control window.

Make sure the Setup check box is checked.

11 Run the EtOH on the FACSort until all three collection tubes are filled.

12 Click Pause, then Abort, and disconnect the reservoir.

13 Working under a hood, empty the remaining EtOH.

14 Pour approximately 500 mL of sterile 1X PBS into the reservoir and swirl to wash out any remaining EtOH. Empty the reservoir and repeat.

15 Fill the reservoir with 4 L of sterile 1X PBS.

Cap the reservoir before removing it from the hood.

16 Install the reservoir in the instrument.

17 Place three new collection tubes in the collection station.

18 Install a tube of sterile PBS on the SIP.

19 Click Acquire in the Acquisition Control window.

20 **Run the sterile PBS for approximately 10 minutes to wash residual EtOH out of the lines.**

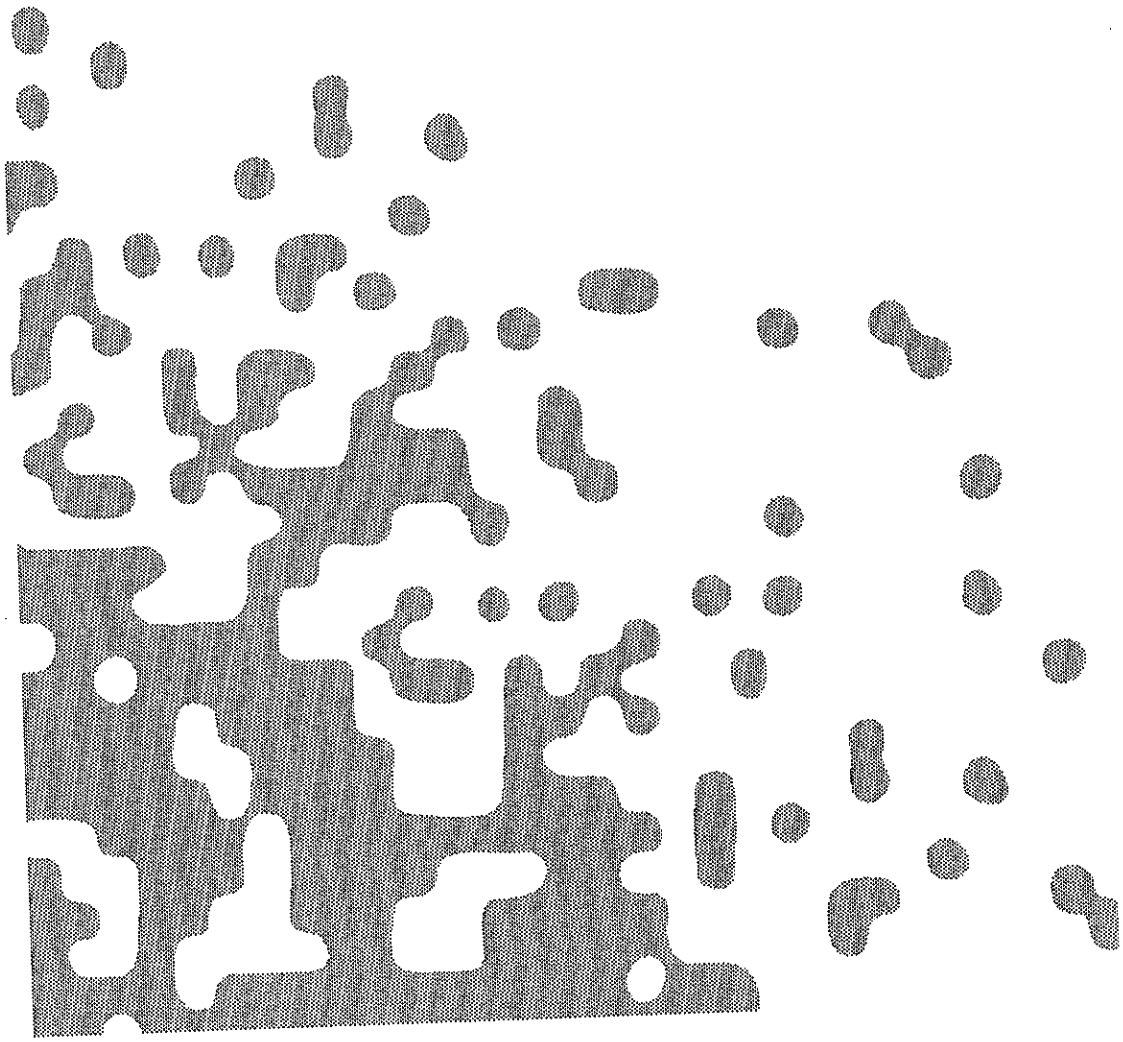
Allow approximately 15 mL of PBS to run into each collection tube. This can be achieved by removing each tube (from left to right), after it fills with 15 mL of PBS.

21 **Using aseptic technique, follow the instructions in Section 3.2.4 to coat the appropriate number of 50-mL conical tubes with sterile BSA.**

22 **Click Pause, then Abort.**

You are now ready to sort your aseptic cell suspension.

CHAPTER 4
Applications



CHAPTER 4
Summary

- a brief overview of various FACSort applications, including immunofluorescence, DNA cell-cycle analysis, platelets, and intracellular calcium

4.1 Introduction

This chapter presents some applications available to you with the FACSort. The FACSort is for in vitro diagnostic use for enumeration of leucocyte (non-blast) subsets or reticulocytes with the appropriate software. See the appropriate software user's guide or reagent package insert for in vitro diagnostic instructions for use.

4.2 Direct Immunofluorescence

Direct immunofluorescence staining involves incubating white blood cells with a monoclonal antibody conjugated to a fluorochrome. This antibody, which is specific for a particular antigen site on a cell, binds to the surface antigens on the cell. A wash step removes any unbound antibody.

Typically, a sample is stained with a combination of two fluorochrome-labeled antibodies (eg, CD3 FITC and CD8 PE). This staining allows the measurement of two immunofluorescence properties simultaneously and is referred to as two-color staining (Figure 4-1).

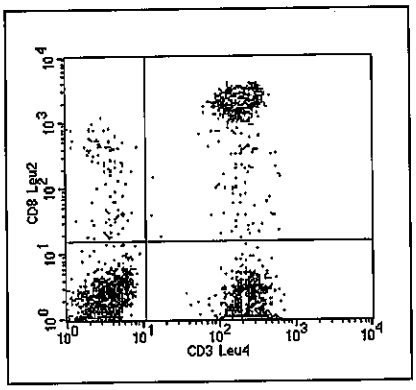


Figure 4-1 FL1 vs FL2 contour plot of CD3 FITC vs CD8 PE

4.3 Indirect Immunofluorescence

Indirect immunofluorescence staining is similar to direct staining except the staining takes place in two steps rather than one. Cells are incubated with a primary monoclonal antibody. After any unbound antibody is washed away, the cells are incubated with a second-step reagent that is specific to the primary antibody and conjugated to a fluorochrome.

4.4 Three-Color Immunofluorescence

Three-color immunofluorescence is an efficient way of analyzing three individual cell populations from one tube. Cells are incubated with three different conjugated monoclonal antibodies. The sample shown in Figures 4-2a through 4-2c was stained with CD3 FITC, CD4 PE, and CD8 PerCP.

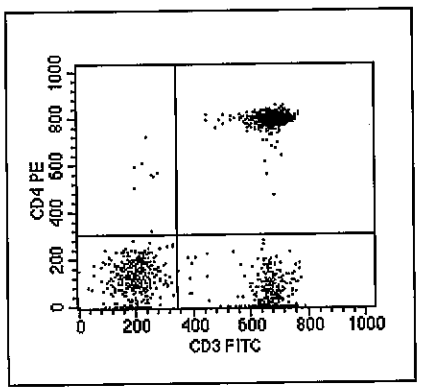


Figure 4-2a FL1 vs FL2
(CD3 FITC vs CD4 PE)

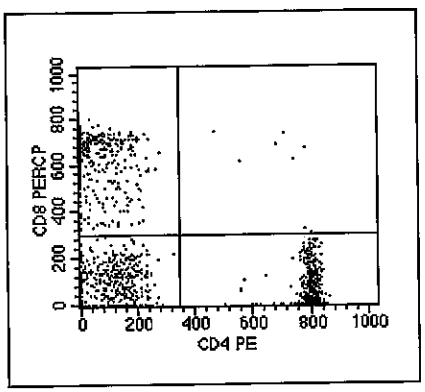


Figure 4-2b FL2 vs FL3
(CD4 PE vs CD8 PerCP)

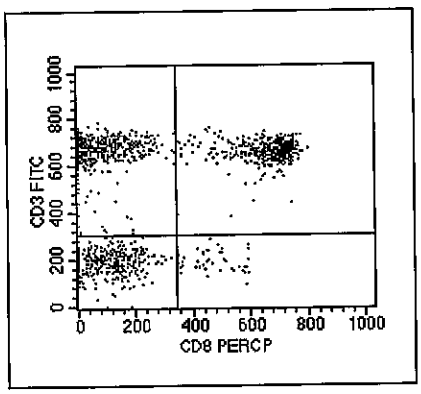


Figure 4-2c FL3 vs FL1
(CD8 PerCP vs CD3 FITC)

4.5 DNA Cell-Cycle Analysis

Quantitative and qualitative DNA measurements of cell nuclei can be helpful in studying the biological behavior of tumors. The two principle calculations—cell-cycle analysis and DNA index—help us determine how tumor cells differ from normal, healthy cells. Cell-cycle analysis gives us an estimation of the percentage of cells within each phase of the cell cycle. For example, a very high S phase in a tumor cell population suggests the tumor is rapidly proliferating. The DNA index is the ratio of DNA content of the tumor cells divided by the DNA content of normal control cells (see PBMC reference peak in Figure 4-3). A ratio not equal to one suggests that the tumor has an abnormal DNA content.

Propidium iodide, a nucleic acid dye, is used to stain the cell or cell nucleus to measure DNA content.

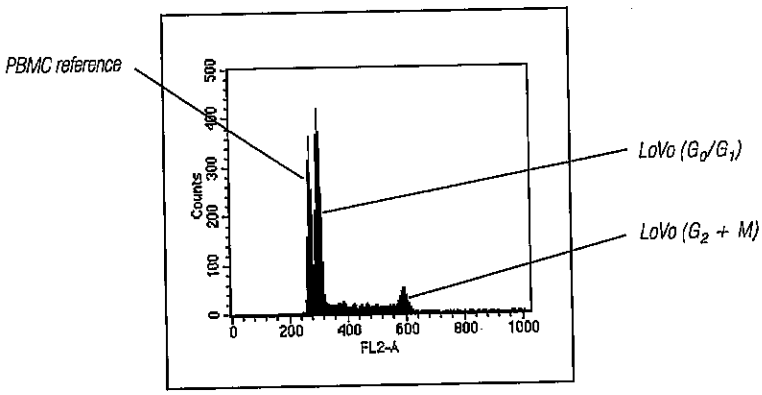


Figure 4-3 DNA histogram of LoVo cells with PBMC reference

4.6 Platelets

Platelet activation, adhesion, and aggregation responses are functions directly related to the platelet membrane or their internal granules. Characterizing and measuring the membrane glycoproteins responsible for these functions are valuable when investigating suspected platelet disorders.

Figure 4-4 shows platelets isolated from whole blood by displaying FSC vs SSC through logarithmic amplification.

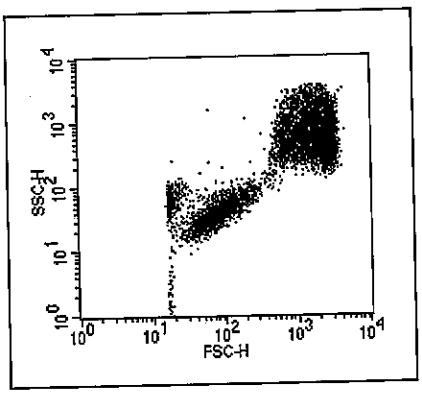


Figure 4-4 Platelet isolation in a diluted whole blood sample

4.7 Intracellular Calcium

Calcium ions, serving as intracellular messengers, flow across the membranes of cells to mediate cellular responses to external stimuli. Typically, a kinetic experiment is performed where cells are loaded with an appropriate calcium probe, stimulated (with a mitogenic lectin, for example), then analyzed on the FACSort. The results show the changes in intracellular calcium over time.

Figure 4-5 shows the changes in intracellular calcium in lymphocytes after being stimulated with ionomycin.

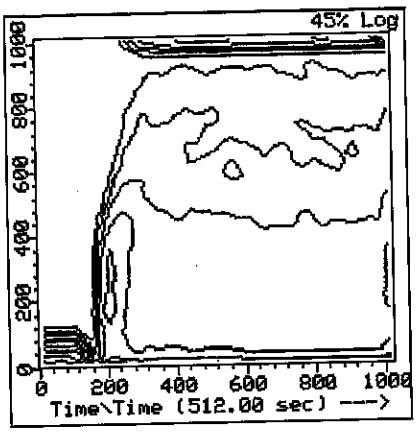
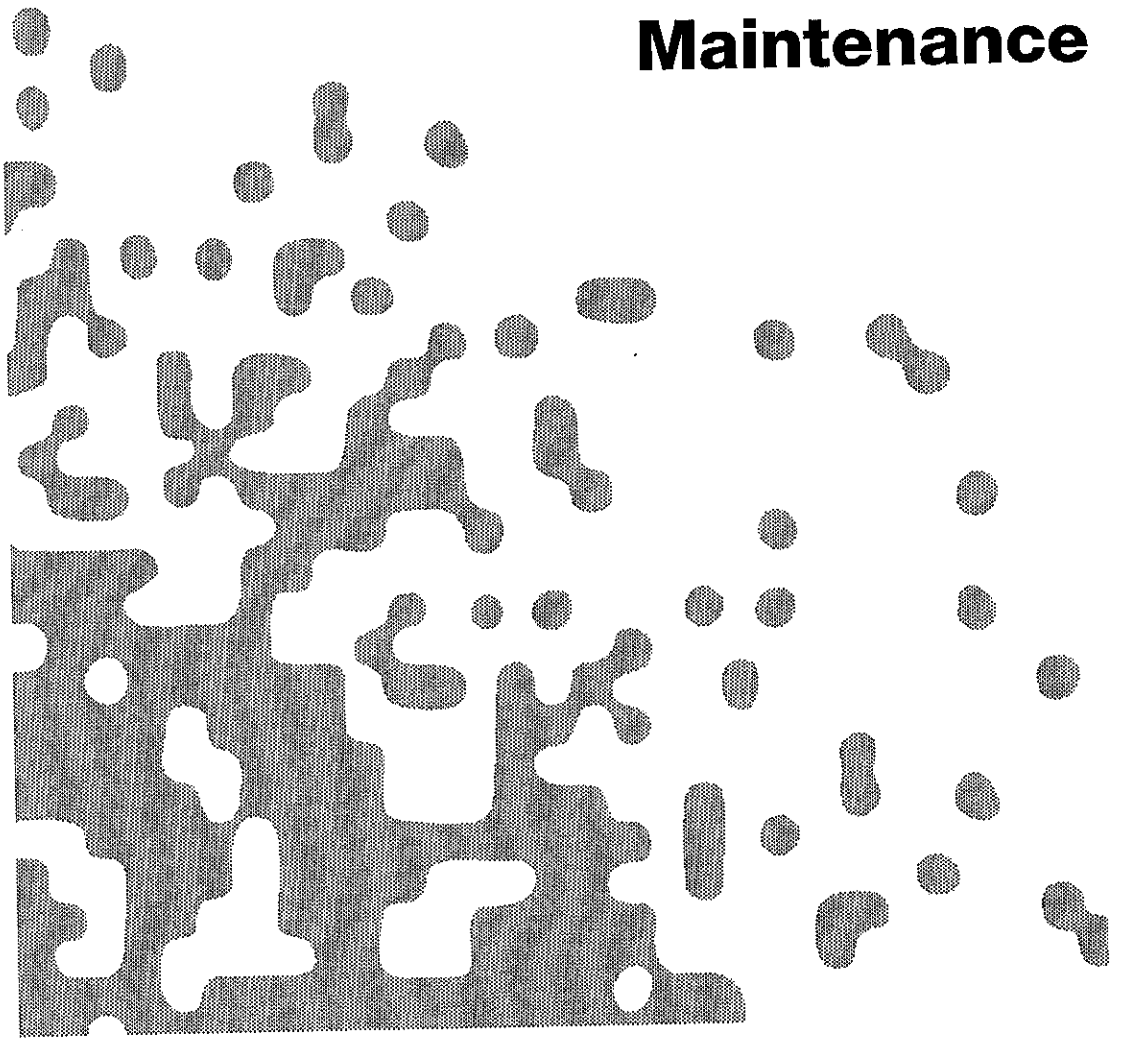


Figure 4-5 Rise in intracell Ca^{2+} after stimulation with ionomycin

CHAPTER 5

Cleaning and Maintenance



CHAPTER 5
Summary

- daily FACSort cleaning
- monthly cleaning
- flushing the sort line
- changing the sheath filter
- cleaning the air filter
- changing the Bal seal
- changing the sample O-ring

5.1 System Cleaning

FACSort has been designed to require a minimum amount of maintenance. However, to preserve the reliability of the instrument, basic preventive maintenance procedures must be performed.

⊘ WARNING: Blood samples may contain infectious agents that are hazardous to your health. Follow appropriate biosafety procedures; wear gloves whenever cleaning the instrument or replacing parts.

☐ NOTE: A 5% solution of sodium hypochlorite may be substituted for undiluted bleach in the following cleaning procedures.

5.1.1 Daily Cleaning

Perform daily cleaning when you shut down the instrument to clean the sample injection tube and the area between the injection tube and the outer sleeve. The outer sleeve covers the injection tube and functions as part of the droplet containment system (Figure 5-1). Fluid dripping from the injection tube is aspirated through the space between the tube and outer sleeve.

Clean the sample injection tube to prevent it from becoming clogged and to remove adhesive dyes that can remain in the tubing, causing carryover. This procedure should be performed before shutdown and *immediately* after running viscous samples or dyes such as propidium iodide (PI), acridine orange, or thiazole orange.

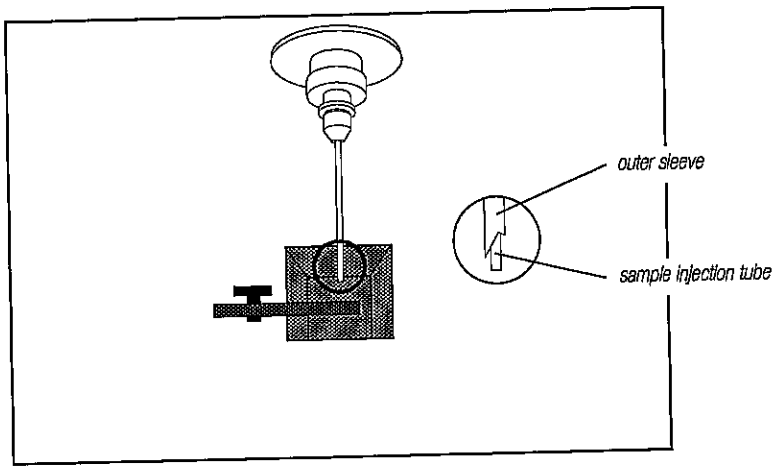


Figure 5-1 Sample injection tube

1 **With the tube support arm moved to the right, install a tube containing 4 mL of a 1:10 dilution of bleach on the SIP. Allow the vacuum to aspirate 2 mL of the bleach solution.**

Prepare the bleach solution by adding one part undiluted bleach to nine parts distilled water.

The vacuum is on when the tube support arm is positioned to the right.

2 Set the sample flow rate switch to HI, then center the tube support arm and allow the bleach to run for 5 minutes.

3 With the tube support arm moved to the right, install a tube containing 4 mL of distilled water on the SIP. Allow the vacuum to aspirate 2 mL of the water.

4 Center the tube support arm and allow the water to run for 5 minutes.

5 Set the fluid control dial to STANDBY.

If you are finished running samples, allow the FACSort to remain in STANDBY for 5 minutes before you turn off the power.

If the tube contains more than 1 mL of distilled water, remove some water before turning off the power to the FACSort. The distilled water should remain on the SIP after the instrument has been turned off to prevent salt deposits from forming in the sample injection tube.

5.1.2 Monthly Cleaning

An overall fluidics cleaning is required to remove debris and contaminants from the sheath tubing, waste tubing, and flow cell. Perform system fluidics cleaning at least once a month or more frequently if you are running a high volume of samples or adhesive dyes such as propidium iodide, acridine orange, or thiazole orange.

1 **Remove the sheath reservoir.**

Refer to Section 2.1.1 for instructions on removing the sheath reservoir.

If the system is pressurized, push the vent valve toggle switch in the direction of the arrow to release pressure from the sheath reservoir.

2 **Remove the sheath filter by disconnecting the two white quick-disconnects that secure the filter to the FACSort.**

Squeeze the metal clips on the quick-disconnects and pull each connector from the fitting.

3 **Install a spare reservoir containing 1 to 2 L of a 1:10 dilution of bleach.**

Prepare the bleach solution by adding one part undiluted bleach to nine parts distilled water.

4 Connect the sheath tubing (white) from the reservoir to the upper sheath filter fitting (Figure 5-2).

This bypasses the sheath filter and allows fluid to travel from the sheath reservoir directly to the flow cell, then to the waste reservoir. Remember to flip the switch up to pressurize the reservoir.

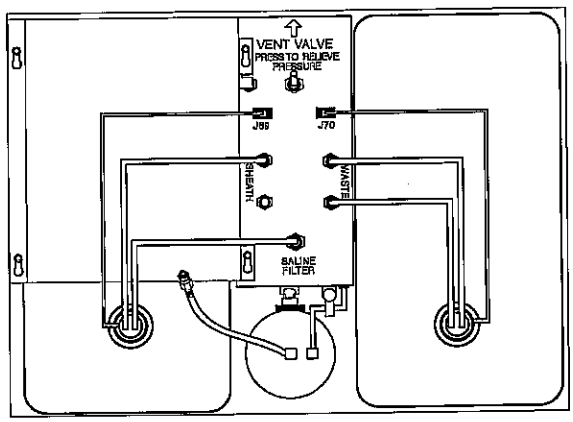


Figure 5-2 Bypassing sheath filter

⚠ CAUTION: Bleach run through the sheath filter will break down the filter paper within the filter body, causing particles to escape into the sheath fluid and possibly clogging the flow cell.

5 **Drain and fill the flow cell two times.**

Drain and fill by turning the fluid control dial to DRAIN and allowing the fluid to drain from the flow cell. Then turn the dial to FILL until the flow cell is filled with fluid. Repeat the drain and fill.

Open the optics door to view the flow cell to ensure that it is draining and filling.

6 **Set the sample flow rate switch to HI, then install a tube containing 3 mL of the 1:10 bleach solution on the SIP. Set the fluid control dial to RUN and allow the solution to run for 20 to 30 minutes.**

7 **Remove the tube of bleach from the SIP.**

8 **Repeat steps 3 through 6 using distilled water instead of the 1:10 dilution of bleach.**

- Replace the bleach reservoir with a spare reservoir containing 1 to 2 L of distilled water. Flip the vent valve toggle switch to depressurize the reservoir before removing.
- Connect the sheath tubing from the reservoir to the upper sheath filter fitting.
- Drain and fill the flow cell two times.
- Replace the tube on the SIP with a tube containing 3 mL of distilled water. Allow the water to run for 20 to 30 minutes.

9 Replace the original sheath reservoir.

10 Reconnect the sheath filter by pushing each connector into its fitting until you hear a click.

11 Run a tube of distilled water for 5 minutes before running patient samples.

5.2 Periodic Maintenance

Several components of your instrument should be checked occasionally and cleaned as necessary. The frequency will depend on how often you run your instrument. Other components should be checked periodically for wear and replaced if necessary.

5.2.1 Flushing the Sort Line

Although the monthly cleaning (Section 5.1.2) cleans the system fluidics, it does not clean the sort line. If sorting is one of your primary applications, you may need to flush the sort line periodically to remove cell debris and saline deposits. This accumulation becomes apparent if there is a reduction in the amount of fluid entering the collection tubes when you sort. For this procedure, use the 60-cc syringe that was provided.

☐ NOTE: If you know that you will not be sorting for an extended period of time (2 to 3 weeks), follow this procedure to fill the sort line with distilled water. This will prevent the accumulation of saline deposits from forming in the line.

Prepare the syringe:

1 **Disconnect the tubing from the syringe by twisting the luer end counterclockwise.**

2 **Fill the syringe with distilled water.**

Place the syringe nozzle in a container filled with distilled water, and slowly pull out the plunger of the syringe until the syringe is full.

3 **Bleed any air out of the syringe by holding it luer end up and gently pushing in the plunger.**

4 Reconnect the tubing to the syringe by turning it clockwise.

5 Disconnect the upper tubing of the sheath filter by squeezing the metal clip on the quick-disconnect and pulling the connector from the fitting.

6 Connect the tubing from the syringe to the upper connector of the sheath filter by pushing firmly until you hear a click (see Figure 5-3).

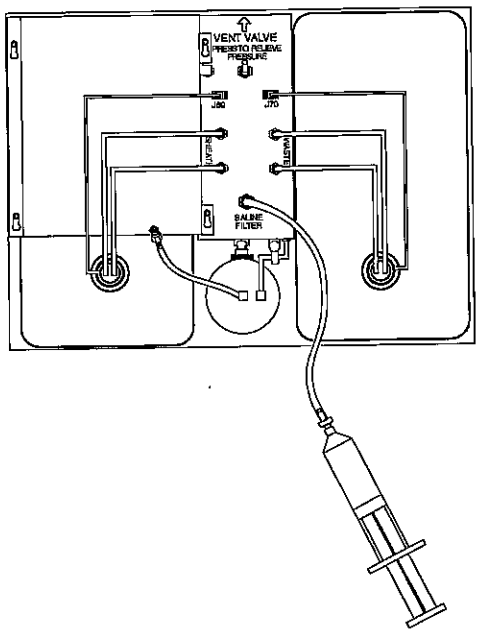


Figure 5-3 Syringe connected to sheath filter connector

Prepare the instrument for sorting:

1 Install three collection tubes in the collection tube station.

2 Follow the instructions outlined in Section 3.2.5, steps 1 through 7, to set a sort gate.

Draw an arbitrary region in the empty display to enable sorting. It is not necessary to install a sample tube on the SIP to do this.

3 Choose Sort Setup from the Acquire menu.

4 Choose the gate that you drew in step 2 from the Sort Gate pop-up menu.

5 Install a tube of distilled water on the SIP.

6 Turn the fluid control dial to RUN.

7 Click Acquire in the Acquisition Control window.

8 Slowly yet firmly, apply pressure to the syringe plunger.

Depress the plunger until approximately 10 cc of fluid has been dispensed from the syringe and has entered collection tube one.

9 Remove collection tube one.

10 Repeat steps 8 and 9 for collection tubes two and three.

11 Click Pause, then Abort in the Acquisition Control window.

12 **Remove the syringe from the upper sheath fluid connector, and reconnect the sheath fluid tubing.**

13 **Drain and fill the flow cell 2 to 3 times to remove any air that may have entered the flow cell.**

See Section 2.2.1, step 4, for information on draining and filling.

☐ NOTE: If a clog is still apparent after the above procedure has been completed, perform the procedure using a 1:10 dilution of bleach in the syringe, followed by distilled water.

5.2.2 Changing the Sheath Filter

The sheath filter (Figure 5-4), located between the sheath reservoir and waste reservoir, filters the sheath fluid as it comes from the sheath reservoir. Increased debris appearing in an FSC vs SSC plot may be an indication that your sheath filter should be replaced. BDIS recommends changing the sheath filter every 3 months.

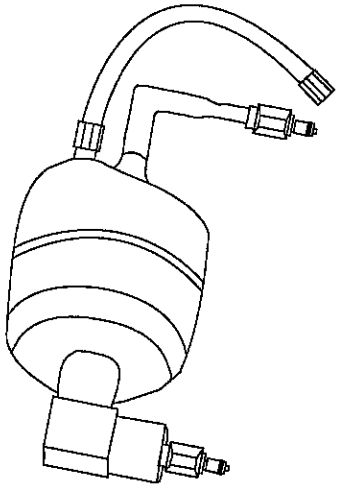


Figure 5-4 Sheath filter

1 Push the vent valve toggle switch in the direction of the arrow to release the pressure from the sheath reservoir.

2 Squeeze the metal clips of the upper and lower quick-disconnects.

3 **Disconnect the air vent tubing from the filter by unscrewing the fitting from the filter port.**

The filter should be free of the instrument, while the air vent tubing remains attached to the instrument.

4 **Unscrew the base of the filter to remove it from the filter body.**

Save this piece to attach to the new filter.

5 **Remove the output tubing from the top of the filter body by pulling the tubing from the port.**

Save the tubing to attach to the new filter.

6 **Wrap Teflon® tape around the threads at the bottom of the new filter. Avoid obscuring the opening.**

7 Attach the base (from step 4) to the new filter by screwing until snug.

8 Attach the output tubing (from step 5) to the new filter by pushing the tubing onto the filter port.

9 Snap the new filter into place by pushing each quick-disconnect firmly into its fitting until you hear a click.

Replace the filter so that the base is at the bottom and the output tubing is at the top.

10 Reattach the air vent tubing (from step 3) to the new filter by screwing the fitting onto the filter port.

11 Pressurize the instrument by pulling the vent valve toggle switch forward.

12 Fill the newly installed filter with fluid by setting the fluid control dial to FILL and pushing the roller in the pinchcock forward to allow the air to escape as the filter fills with fluid.

☐ NOTE: If bubbles are visible in the filter, gently tap the filter body to dislodge them and force them to the top. Push the roller in the pinchcock forward to allow the pressurized sheath fluid to force the air bubbles into the waste reservoir.

13 Record the replacement date on the outside of the filter.

14 Discard the old filter body.

5.2.3 Cleaning the Air Filter

The air filter located above the fluid reservoirs cleans the air that cools the FACSort laser. The filter may be vacuumed or washed, then air dried. Clean the filter only if it looks dirty.

1 To remove the filter, grasp the edges and pull gently to slide it out (Figure 5-5).

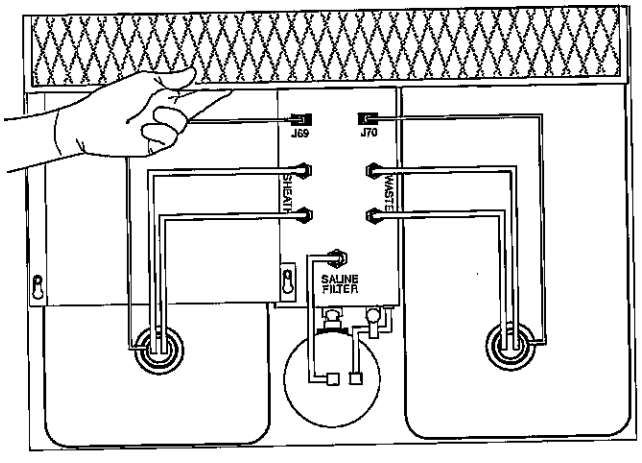


Figure 5-5 Removing air filter

2 Vacuum the air filter or hold it under running tap water to clean it.

If the filter is rinsed with water, allow it to dry completely before reinstalling it.

3 When reinstalling the filter, be sure the arrows along the edge of the filter are pointing up, indicating the direction of air flow. Align the right edge of the filter against the spring clips in the slide rail. Push forward slowly.

5.2.4 Changing the Bal Seal

The sample injection tube Bal seal (see Figure 5-6) is a Teflon ring that forms a seal with the sample tube and creates proper tube pressurization. Over time, this seal becomes worn or cracked and requires replacement. Replacement is necessary if a proper seal is not formed when a sample tube is installed on the SIP.

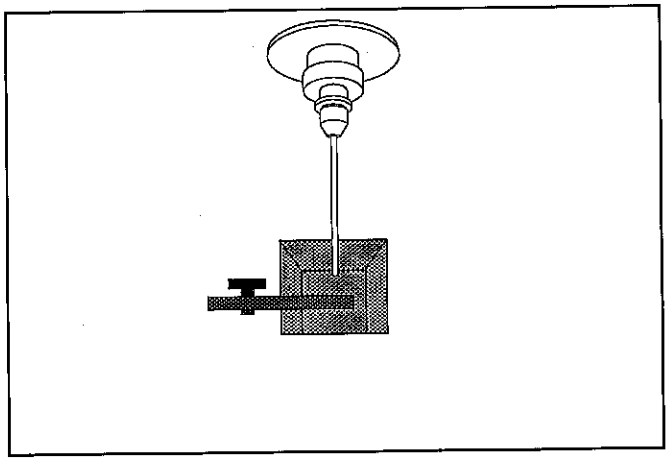


Figure 5-6 Sample injection port

☐ NOTE: Follow good laboratory practice; wear gloves whenever cleaning the instrument or replacing parts.

1 Remove the outer droplet sleeve from the sample injection tube by turning the retainer counterclockwise (see Figure 5-7).

The outer sleeve may fall out as you loosen the retainer.

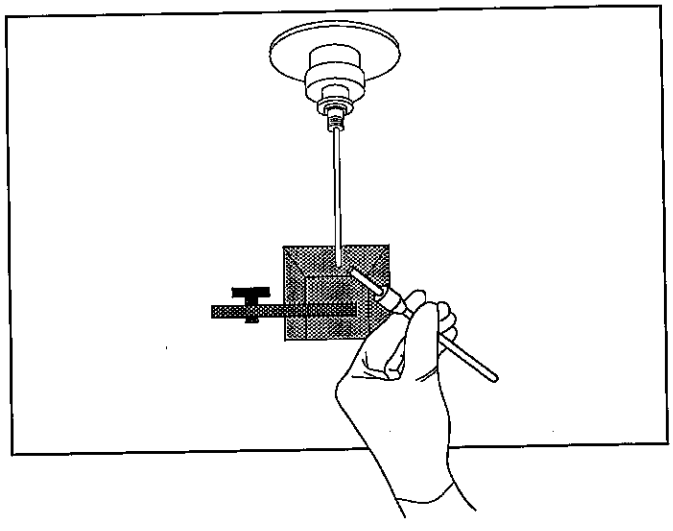


Figure 5-7 Removing outer sleeve

2 Remove the Bal seal by gripping it between your thumb and index finger and pulling (Figure 5-8).

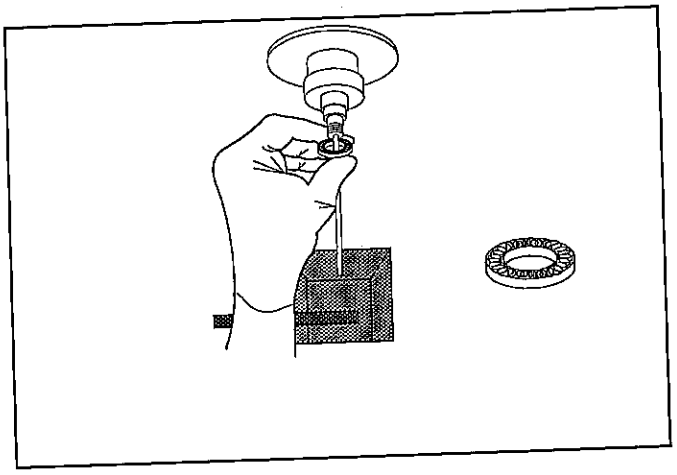


Figure 5-8 Removing Bal seal

3 Install the new Bal seal spring-side up (see Figure 5-8), then reinstall the retainer and outer sleeve over the sample injection tube. Tighten the retainer by turning it clockwise.

Gently push the seal in place to seat it. If the seal does not remain in position when you let go of it, hold it with one hand while you reinstall the retainer. The seal will seat as you screw on the retainer.

Tighten the retainer just enough to hold it in place, then slide the outer sleeve over the sample injection tube and into the opening of the retainer. Continue tightening the retainer.

4 **Install a sample tube on the SIP to ensure that the outer sleeve has been properly installed.**

If the sleeve hits the bottom of the tube, loosen the retainer slightly and push the sleeve up as far as it will go. Retighten the retainer.

The first installation of a sample tube on the SIP may be tight, but with repeated installations the process gradually gets easier.

5.2.5 Changing the Sample O-ring

The sample tube O-ring, located within the retainer, helps to form a seal to allow the droplet containment vacuum to function properly. If you see droplets forming at the end of the sample injection tube while the vacuum is operating, this may indicate the O-ring should be replaced.

☐ NOTE: Follow good laboratory practice; wear gloves whenever cleaning the instrument or replacing parts.

1 **Remove the outer droplet sleeve from the sample injection tube by turning the retainer counterclockwise (refer to Figure 5-7).**

Pull the outer sleeve from the retainer.

2 **Invert the retainer and allow the O-ring to fall onto the benchtop.**

If the O-ring does not fall out initially, it may be necessary to tap the retainer on the benchtop to dislodge the O-ring.

3 **Drop the new O-ring into the retainer. Make sure the O-ring is seated properly in the bottom of the retainer.**

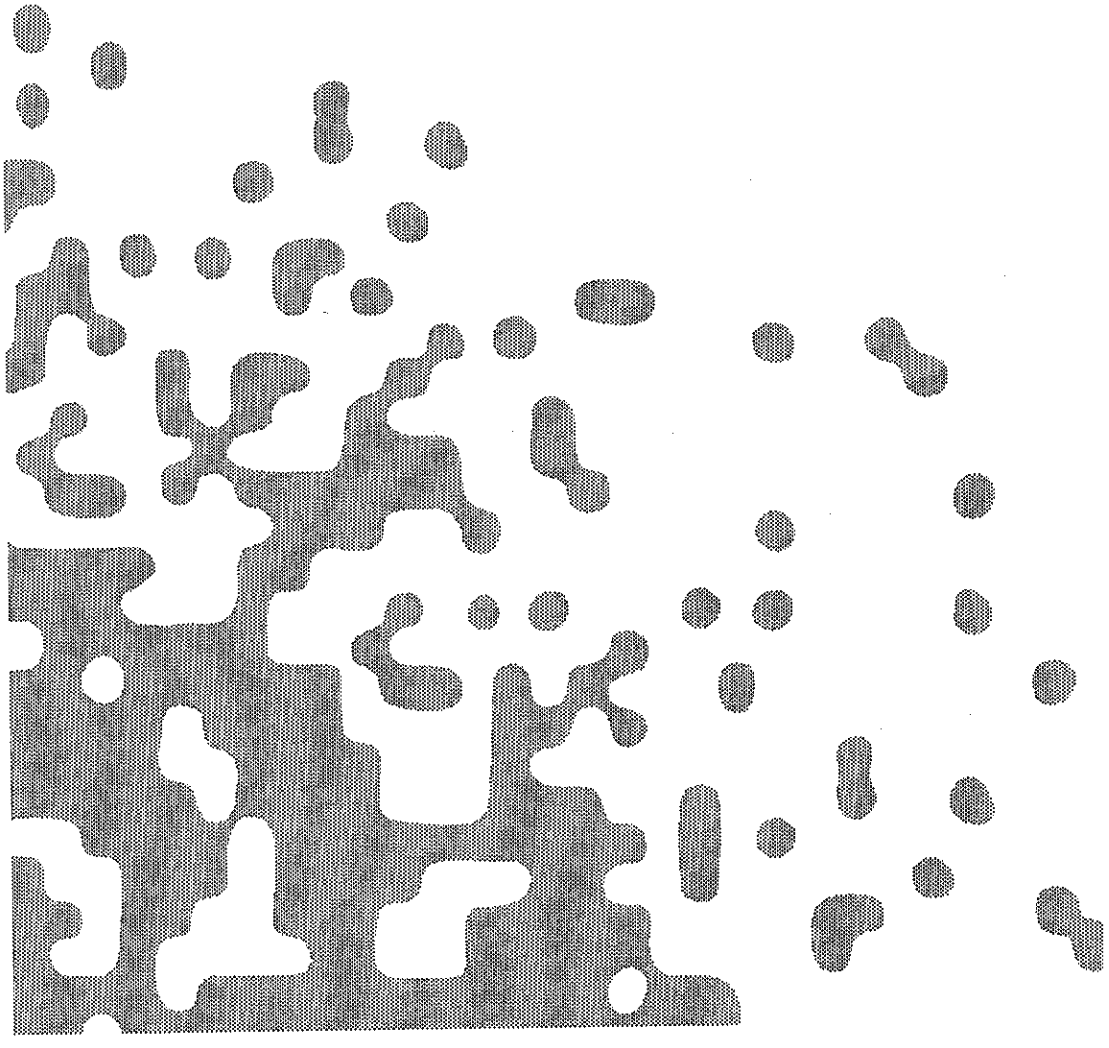
4 **Reinstall the retainer and the outer sleeve.**

Tighten the retainer just enough to hold it in place, then slide the outer sleeve over the sample injection tube and into the opening of the retainer. Continue tightening the retainer.

5 **Install a sample tube on the SIP to ensure the outer sleeve has been properly installed.**

If the sleeve hits the bottom of the tube, loosen the retainer slightly and push the sleeve up as far as it will go. Retighten the retainer.

CHAPTER 6
Troubleshooting



CHAPTER 6
Summary

- symptoms you might observe during instrument operation, possible causes, and solutions

Read through the following list of symptoms. After locating the symptom the equipment is exhibiting, read through the list of possible causes and solutions. For technical information not covered in this troubleshooting guide, contact Becton Dickinson Immunocytometry Systems Customer Support Center at (800) 448-BDIS (2347).

DATA DISPLAY

SYMPTOM: No events in acquisition display

If the Status window reads READY, check the following:

Cause: **Threshold parameter gain setting too low.**
Solution: Increase threshold parameter gain setting (see Section 2.3.3).

Cause: **Threshold level too high.**
Solution: Lower threshold level (see Section 2.3.3).

Cause: **Threshold not set to correct parameter (usually FSC).**
Solution: Set threshold to correct parameter (for application).

Cause: **No sample in tube.**
Solution: Add sample to tube or install new sample tube.

Cause: **Sample not mixed properly.**
Solution: Mix sample to suspend cells.

Cause: **Sample injection tube clogged.**
Solution: Set fluid control dial to BACKFLUSH, followed by DRAIN, then FILL. If clog persists, clean sample injection tube (see Section 5.1.1).

Cause: **Gate set with no data passing through gate.**
Solution: Delete gate (see *CELLQuest Software User's Guide).*

Cause: **Communication failure between computer and FACSort.**
Solution: Turn off computer and FACSort. Turn on the FACSort, followed by computer.

If the Status window reads STANDBY, check the following:

Cause: **Fluid control dial not set to RUN.**
Solution: Set fluid control dial to RUN.

Cause: **Sample tube not installed or not properly seated.**
Solution: Install sample tube on the cytometer.

Cause: **Sample tube cracked.**
Solution: Replace sample tube.

Cause: **Sheath reservoir cap not tightened.**
Solution: Tighten sheath reservoir cap.

Cause: **Sheath reservoir bracket was not replaced.**
Solution: Install the bracket (see Section 2.1.1, step 8).

Cause: **Vent valve toggle switch is pushed away from you (sheath reservoir is vented).**
Solution: Flip toggle switch toward you to pressurize the reservoir.

Cause: **Sheath reservoir tubing or sheath filter tubing not properly connected.**
Solution: Check that all tubing connectors are securely seated. Check sheath reservoir for cracks.

Cause: **Bal seal worn.**
Solution: Replace Bal seal (see Section 5.2.4).

If the Status window reads NOT READY, check the following:

Cause: **Laser warming up.**
Solution: Wait five minutes.

Cause: **Laser not functioning.**
Solution: Check laser power in the Status window (see Section 2.2.3). If power is 0 mWatts, turn off FACSort and computer, then turn on FACSort, followed by computer. If power is still 0 mWatts, contact BDIS.

Cause: **Sheath reservoir empty or waste reservoir full.**
Solution: Check reservoirs, fill sheath and empty waste, if necessary (see Sections 2.1.1 and 2.1.2).

SYMPTOM: **High sample event rate**

Cause: **Air bubble in flow cell.**
Solution: Drain and fill flow cell (see Section 2.2.1).

Cause: **Air in sheath filter.**
Solution: Vent air from sheath filter (see Section 2.2.1).

Cause: **Threshold level too low.**
Solution: Increase threshold level (see Section 2.3.3).

Cause: **Threshold parameter gain setting too high.**
Solution: Decrease threshold parameter gain setting.

Cause: **Sample too concentrated.**
Solution: Dilute sample. Cell concentration should be 1×10^5 to 1×10^7 cells/mL for optimal event rates.

Cause: **Sample flow rate set on HI.**
Solution: Set sample flow rate to MED or LO.

SYMPTOM: **Low sample event rate**

Cause: **Threshold level too high.**
Solution: Lower threshold level (see Section 2.3.3).

Cause: **Threshold parameter gain setting too low.**
Solution: Increase threshold parameter gain setting (see Section 2.3.3).

Cause: **Sample not adequately mixed.**
Solution: Mix sample to suspend cells.

Cause: **Sample too dilute.**
Solution: Concentrate sample. If flow rate setting is not critical to the application, set flow rate switch to MED or HI.

SYMPTOM: **Erratic event rate**

Cause: **Sample tube cracked.**
Solution: Replace sample tube.

Cause: **Bal seal worn.**
Solution: Replace Bal seal (see Section 5.2.4).

Cause: **Partially blocked sample injection tube.**
Solution: Set fluid control dial to BACKFLUSH, followed by DRAIN, then FILL. If event rate is still erratic, clean sample injection tube (see Section 5.1.1).

SYMPTOM: **Status window reads NOT READY**

Cause: **Laser warming up.**
Solution: Wait five minutes.

Cause: **Laser not functioning.**
Solution: Check laser power in Status window (see Section 2.2.3). If power is 0 mWatts, turn off FACSort and computer, then turn on FACSort, followed by computer. If power is still 0 mWatts, contact BDIS.

Cause: **Sheath reservoir empty or waste reservoir full.**
Solution: Check reservoirs, fill sheath and empty waste, if necessary (see Sections 2.1.1 and 2.1.2).

SYMPTOM: **Scatter parameters appear distorted**

Cause: **Instrument adjustment is necessary.**
Solution: Perform optimization procedure (see Section 3.1.1).

Cause: **Air bubble in flow cell.**
Solution: Drain and fill flow cell (see Section 2.2.1).

Cause: **Air in sheath filter.**
Solution: Vent air from sheath filter (see Section 2.2.1).

Cause: Flow cell dirty.
Solution: Perform monthly cleaning procedure (see Section 5.1.2).

SYMPTOM: Excessive amount of debris appearing in display

Cause: Threshold level too low.
Solution: Increase threshold level.

Cause: Sheath filter dirty.
Solution: Change sheath filter (see Section 5.2.2).

Cause: Sample contains excessive amount of debris.
Solution: Examine sample under a microscope.

Cause: Stock sheath fluid contaminated.
Solution: Rinse sheath reservoir with distilled water, then fill with sheath fluid from another (or new lot) bulk container.

SORTING

SYMPTOM: Sorted sample not flowing into collection tubes

Cause: Sort line is clogged.
Solution: Perform the sort line cleaning procedure outlined in Section 5.2.1.

Cause: Sort gate not set.
Solution: Set a sort gate (Section 3.2.5) or select the gate from the Sort Gate field in the Sort Setup window.

SYMPTOM: Cells not viable after sorting

Cause: **Haema-Line 2 was used as sheath fluid.**
Solution: Use phosphate-buffered saline for the sheath fluid when sorting cells.

Cause: **Collection tubes were not coated with 4% BSA.**
Solution: Coat collection tubes with BSA.

Cause: **Cells were not viable before they were sorted.**
Solution: Check viability before sorting.

SYMPTOM: Cell recovery low

Cause: **Collection tubes were not coated with 4% BSA.**
Solution: Coat collection tubes with BSA.

Cause: **Centrifugation technique.**
Solution: See Section 3.2.8 for information on centrifugation. Depending on the cell type and concentration, this procedure may need to be adjusted.

SYMPTOM: Low purity

Cause: **Recovery mode used to sort.**
Solution: Use Single Cell or Exclusion mode to achieve high purity.

Cause: **Flow rate set to MED or HI.**
Solution: Set flow rate to LO when sorting.

Cause: **Air bubbles in sample when it is reanalyzed.**
Solution: Gently mix the sample to avoid air bubble formation before reanalyzing.

Cause: **Air bubbles in flow cell.**
Solution: Drain and fill the flow cell (Section 2.2.1, step 4).

Cause: **Air bubbles in the sheath filter.**
Solution: Push the roller in the pinchcock forward to purge air from the filter.

PRECISION

SYMPTOM: **High CV**

Cause: **Air bubbles in flow cell.**
Solution: Drain and fill the flow cell (Section 2.2.1, step 4).

Cause: **Sample flow rate set to HI.**
Solution: Set sample flow rate to LO or MED.

Cause: **Improper sheath pressure**
Solution: Ensure sheath reservoir cap is tight and all connectors are secure. Check also for a cracked sample tube or cracked sheath reservoir.

Cause: **Flow cell dirty.**
Solution: Perform monthly cleaning procedure (Section 5.1.2).

Cause: **Sample preparation technique questionable.**
Solution: Seek advice on sample preparation techniques.

Cause: **Air bubbles in sheath filter.**
Solution: Push the roller in the pinchcock forward to purge air from the filter.

Cause: **Sample not diluted in same fluid as sheath fluid.**
Solution: Dilute sample in the same fluid as you are using for sheath. If you are running CaliBRITE beads, dilute them in Haema-Line 2 and use Haema-Line 2 for sheath fluid.

Cause: **Quality control particles are old or contaminated.**
Solution: Make a new QC solution and try quality control procedure again.

INSTRUMENT

SYMPTOM: **Droplet containment vacuum not functioning**

Cause: **O-ring in retainer is worn.**
Solution: Replace O-ring (see Section 5.2.5).

Cause: **Outer sleeve is not seated in the retainer.**
Solution: Loosen retainer, push outer sleeve up into the retainer until seated. Tighten retainer (see Figure 5-8).

Cause: **Outer sleeve is not on the sample injection tube.**
Solution: Replace outer sleeve; loosen retainer, slide outer sleeve over sample injection tube until it is seated, tighten retainer.

Cause: **Waste line is pinched, preventing proper aspiration.**
Solution: Check waste line.

SYMPTOM: **Flow cell will not fill**

Cause: **No sheath pressure.**
Solution: 1. Check to see vent valve toggle switch is in correct position.

2. Tighten sheath reservoir cap.

3. Check to see all sheath fluid connectors are securely seated.

4. Check for leak or crack in sheath reservoir. Replace reservoir if necessary.

Cause: **Sheath reservoir empty.**
Solution: Fill sheath reservoir (see Section 2.1.1).

Cause: **Air in sheath filter.**
Solution: Vent air from sheath filter (see Section 2.2.1).

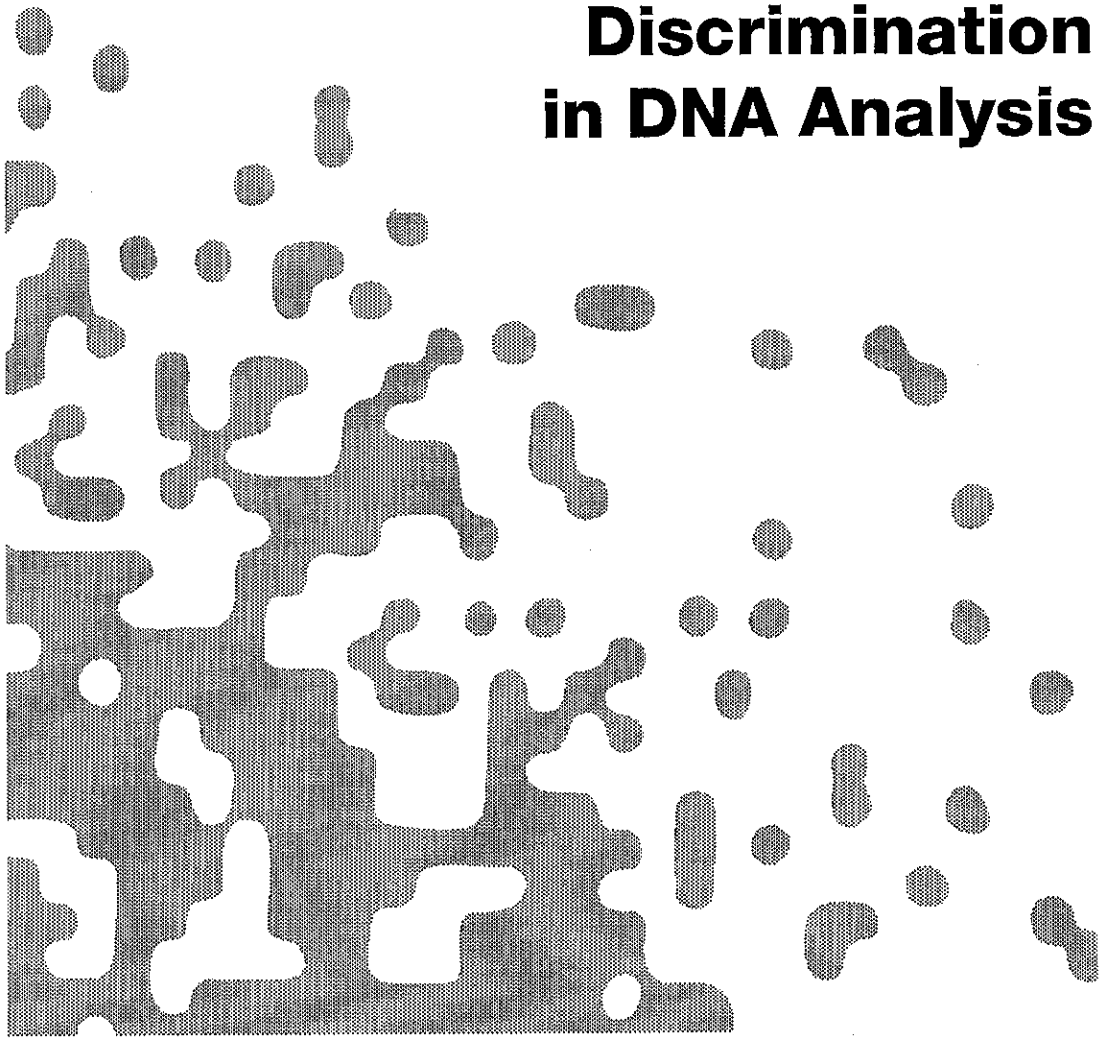
SYMPTOM: **Sample tube does not fit on SIP**

Cause: **A sample tube other than Falcon brand is being used.**
Solution: Use Falcon brand sample tubes.

Cause: **The black disk on the tube support arm needs adjusting.**
Solution: Rotate the black disk to adjust it for the height of the tube.

Cause: **The Bal seal is worn and needs to be replaced.**
Solution: Replace the Bal seal (Section 5.2.4).

Appendix A
Doublet Discrimination in DNA Analysis



A.1 The Cell Cycle

The determination of DNA content is one of the most common flow cytometry applications used by researchers to monitor the growth kinetics of cells and to quantitate DNA content abnormalities and kinetics in tumor populations. Proliferating cells progress through several phases before they undergo cell division. These phases are referred to as G1, S, G2, and M (Figure A-1). Cells not proliferating (resting) are referred to as G0 cells.

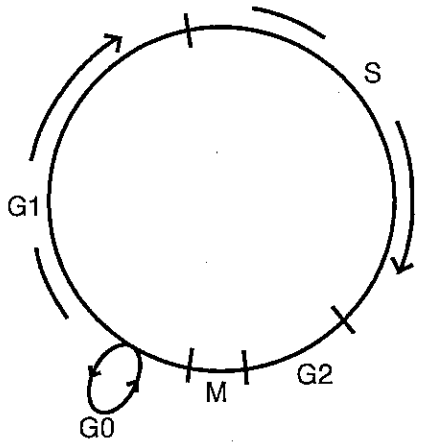


Figure A-1 A cell cycle

When we consider the DNA content of cells in the various phases, we refer to cells in both the G0 and G1 phases as 2C (having 2 copies of chromosomes). Cell replication begins in S phase when DNA is synthesized and the DNA content in the nucleus doubles. These cells are now referred to as 4C (having 4 copies of chromosomes). The DNA content of the cell remains at 4C until the cell undergoes mitosis or cell division in M phase. Here, two daughter cells are formed.

When normal cells or nuclei are analyzed for DNA content by flow cytometry, a DNA distribution similar to that shown in Figure A-2 is obtained.

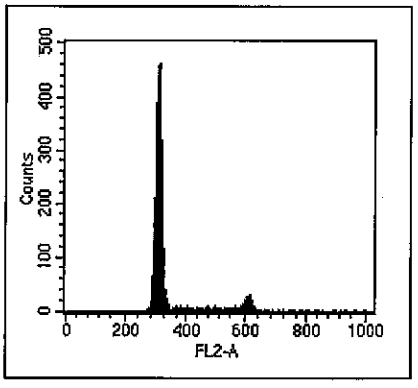


Figure A-2 DNA distribution of cells in the cell cycle

☐ NOTE: Nuclei analyzed for DNA content are typically stained with propidium iodide (PI), which binds stoichiometrically to DNA.

A.2 Theory of Doublet Discrimination

As a cell or nucleus passes through the laser beam, FACScan detects light pulses for each parameter. If a particle is small with respect to the laser beam height (20 μm), the emitted light generates an electronic pulse with a height (amplitude) that is proportional to the total fluorescence emission or the total scattered light from the particle. Typically the pulse height is used to measure emitted or scattered light from each particle (ie, FSC-H, SSC-H, FL1-H, FL2-H, and FL3-H).

Doublet Discrimination allows you to view other measurements of the pulse for the FL1, FL2, and FL3 parameters. These new signal measurements, FL-A (area) and FL-W (width), provide information that FL-H (height) cannot supply.

Useful differences in the height, area, and width of the light pulses from each particle come from the fact that particles travel through the laser beam. As a small particle (<8-μm diameter) begins to enter the laser beam, the laser illuminates its leading edge, then the whole particle, then its trailing edge. So FACScan first "sees" a small amount of light from the particle, then a maximum amount of light, and finally a small amount. This produces a bell-shaped signal in a plot of light intensity versus time.

If the particle is <8 μm, the laser can illuminate it all at once when it is centered in the beam. The height of the fluorescence pulse is then proportional to the total fluorescence emission of the particle. Pulse height indicates the maximum amount of illumination and emission of the particle as it moves through the laser beam.

In the following discussion of pulse measurements (Sections A.2.1 and A.2.2), the FL2 measurement is the light emitted from PI-labeled DNA (with correct staining conditions). The same discussion can apply to FL1 and FL3 and to particles labeled with different fluorescent dyes.

A.2.1 Pulse Area Measurements

Particles with a larger diameter (>8 μm), cannot be uniformly illuminated by the laser. Thus, the height (H) of the fluorescence pulse is no longer proportional to the total fluorescence emission of the particle. The area (A) of the light pulse (which measures the total amount of fluorescence emission instead of the maximum) is now the only accurate measure of total fluorescence emission of the particle.

This effect is apparent when you compare the DNA content of aggregates of chicken erythrocyte nuclei (CEN) using FL2-A and FL2-H. CEN singlets are about 3 μm in diameter, well below the size of the laser beam; however, CEN aggregates can exceed 15 μm in diameter. These single (1) or aggregate (2, 3, or 4) nuclei should theoretically have ratios of DNA contents (mean peak positions) of 2/1=2, 3/1=3, and 4/1=4. The measured ratios using FL2-A come closest to this: 2.01, 3.00, 3.96. In this example, the measured ratio using FL2-H, deviate further from the ideal: 1.93, 2.80, 3.55.

A.2.2 Pulse Width Measurements

FL2-W increases with the diameter of a particle because a larger particle takes longer to cross the laser beam. Therefore, the pulse is wider. This relationship simplifies the discrimination of a G0/G1 doublet from a G2+M singlet. Both have the same amount of DNA and would appear in the same place on an FL2-A histogram. However, two singlets stuck together usually produce an FL2-W measurement that is larger than the corresponding measurements for one G2+M event.

You can use FL2-W to exclude doublets or higher order aggregates from the DNA analysis. Figure A-3 shows an FL2-W vs FL2-A plot of calf thymus nuclei (CTN) with a gate on the singlet population.

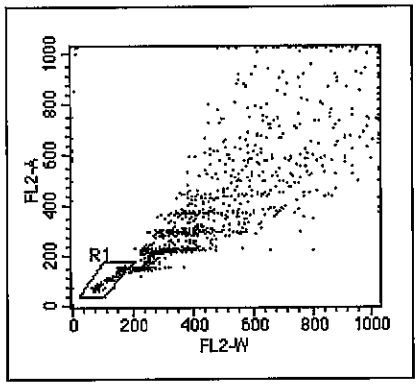


Figure A-3 CTN singlet and aggregated populations with singlet gate

☐ NOTE: With some nuclei preparations, the separation between singlets and doublets is not as distinct as with CTN.

A.3 Gates and Doublet Discrimination

To remove doublets and higher cell aggregates from consideration, set a gate around the singlet population. (Remember, singlets contain cells in the G0/G1, S, and G2+M phases.) To set the singlet gate, select a dot-plot display with FL2-W on the horizontal axis and FL2-A on the vertical axis. Then draw a polygon around the singlet population (see Figure A-3). In this example, calf thymus nuclei (CTN) have no DNA content greater than 4N. This limits the height of the gate on the vertical (FL2-A) axis. For a tissue or tumor sample with cells containing greater amounts of DNA, extend the gate to the top of the FL2-A axis.

Use only the singlet data for the DNA cell-cycle analysis. You can easily see the difference in the FL2-A histogram of CTN when you apply a singlet gate (Figures A-4a and A-4b).

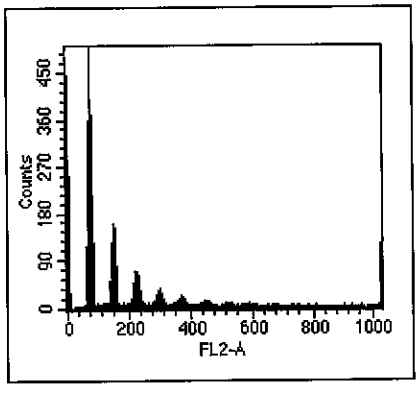


Figure A-4a Ungated FL2-A histogram

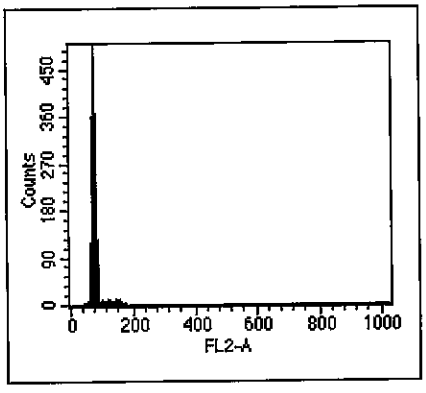


Figure A-4b Gated FL2-A histogram

A.4 Fluorescence Linearity and Particle Size

Both pulse height and pulse area measurements are linear when the particle size is much less than the height of the laser beam. As the particle size increases, the height measurement is no longer linearly related to particle size.

Analysis of standard bead particles with diameters from 2 to 10 µm shows that as bead diameter increases, the area parameter gives a better doublet-to-singlet ratio than the height parameter (Figure A-5). For FL2-A, this ratio is close to 2.0 up to at least 10 µm. For FL2-H, the ratio decreases significantly for particles larger than 8 µm.

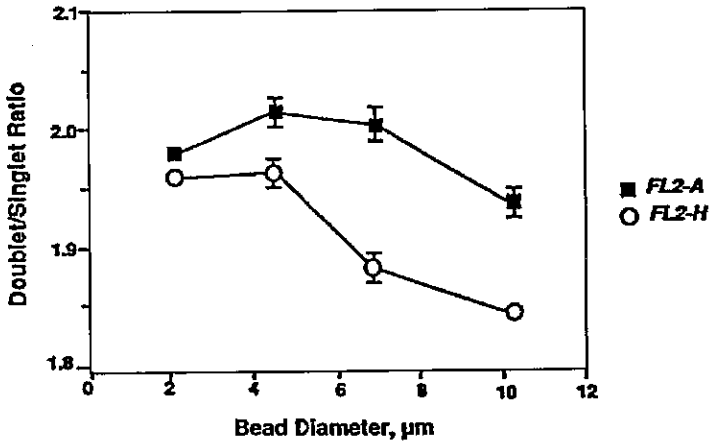
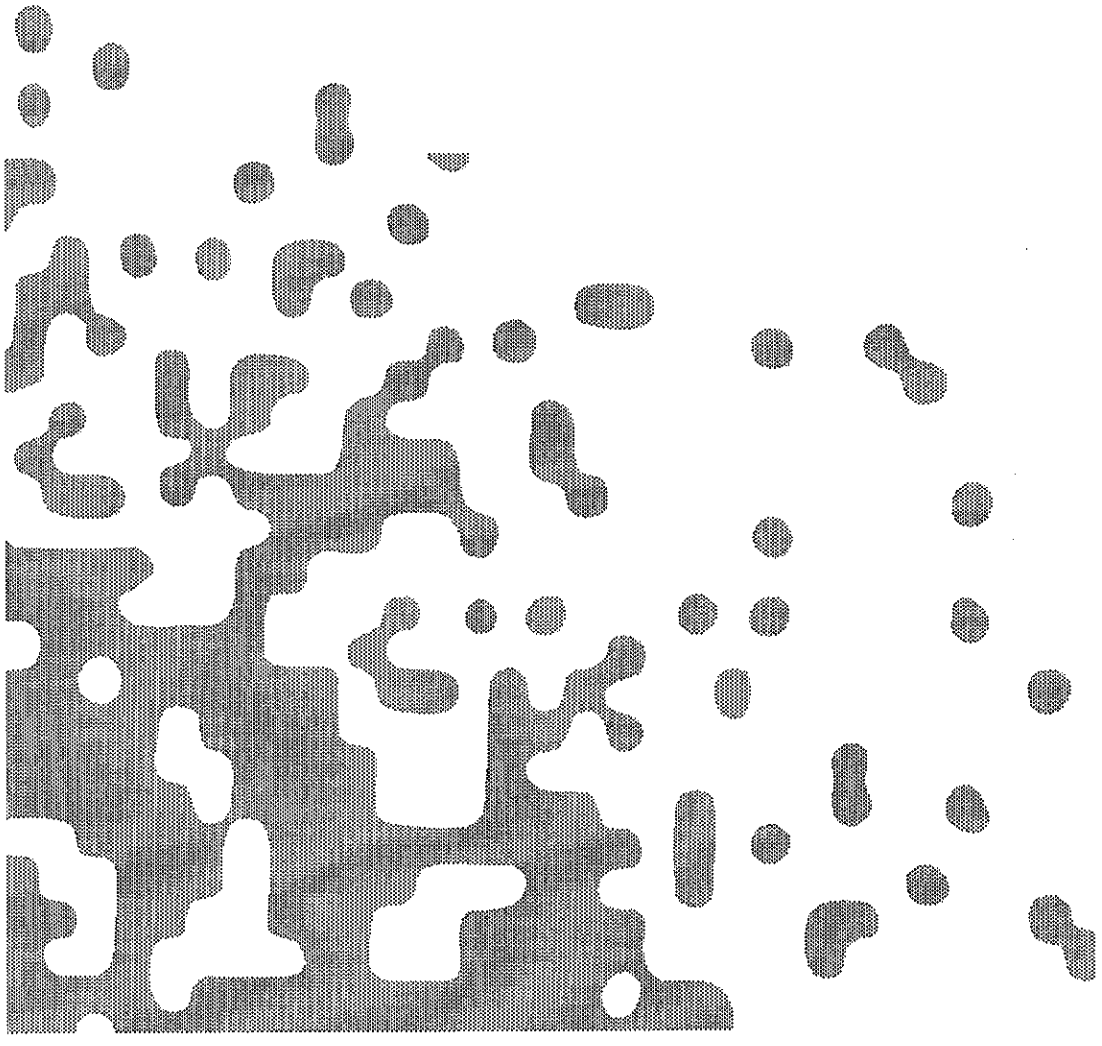


Figure A-5 Linearity of FL2-A and FL2-H for beads

Appendix B
Service



B.1 Supplies

Product Description	Part Number
Sheath Fluid Haema-Line 2 (Serono-Baker)	99-30022-00
sheath filter (BDIS)	41-10011-00
12 x 75-mm polystyrene tubes (Falcon)	54-10001-00
spare sheath/waste reservoir (BDIS)	05-10084-00
Bal seal (BD)	88-20085-00
sample O-ring (BDIS)	88-20014-00
laser air filter	41-10020-00
consumable kit	12-00299-00

BDIS

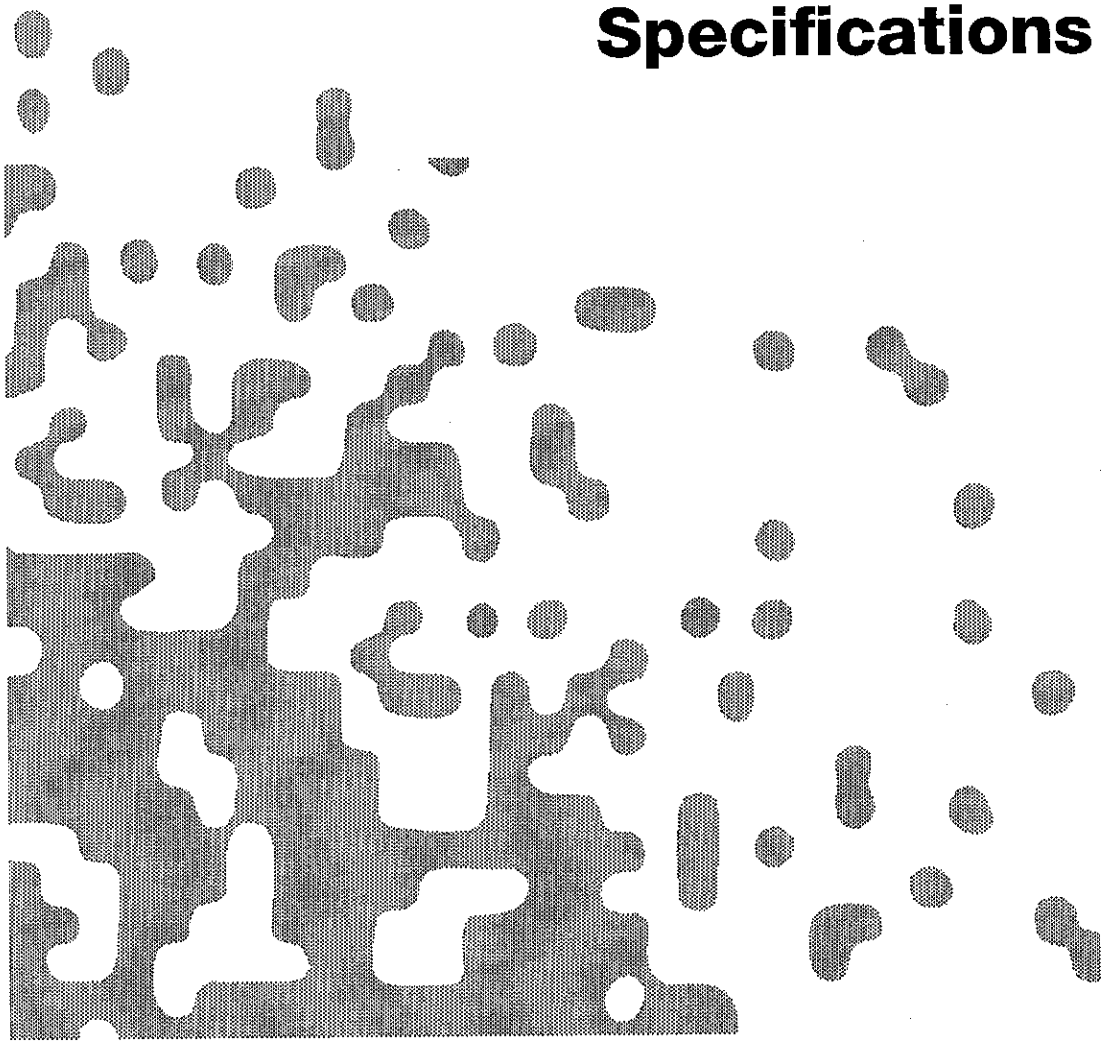
Consumable Parts Order
110 Forbes Boulevard
Mansfield, Massachusetts 02048-1145
(800) 448-BDIS (2347)

B.2 Technical Assistance

BDIS Customer Support Center
2350 Qume Drive
San Jose, California 95131-1807, USA
Customer Support Center (800) 448-BDIS (2347)

Customers outside the US contact your local Becton Dickinson representative or distributor.

Appendix C
FACSort Specifications



EXCITATION

laser	Cyonics 15-mW, 488-nm, air-cooled, argon-ion laser (Class I). Life expectancy >5,000 hours.
beam geometry	Prismatic expander and spherical lens provide 20 x 64-µm elliptical beam.

OPTICS

alignment	Fixed—no user adjustments necessary or available.
dichroics	560/22.5° (blue/orange-red) 640 LP (orange/red)
filters	FL1: 530/30 FL2: 585/42 FL3: 650LP
photomultipliers	FL1, FL2, FL3: R1477 SSC: 1P28

FLUIDICS

flow rates	Three selectable flow rates: LO (12 µL ±3 µL/min) MED (35 µL ±5 µL/min) HI (60 µL ±7 µL/min)
quartz cuvette	Internal cross-section is rectangular 430 x 180-µm. External surfaces are antireflection-coated.

air pressure	Internal air pump provides sheath pressure of 4.5 psig and sample pressures of 4.6, 4.8, and 5.0 psig.

ELECTRONICS

parameters	Seven data channels available for acquisition: FSC, SSC, FL1, FL2, FL3, FLX-W, FLX-A (X=DDM parameter).
acquisition speed	20 µs approximate processing time while sorting; acquires up to 10,000 cells/sec.
sort rate	300 cells/sec maximum in Single Cell sort mode.

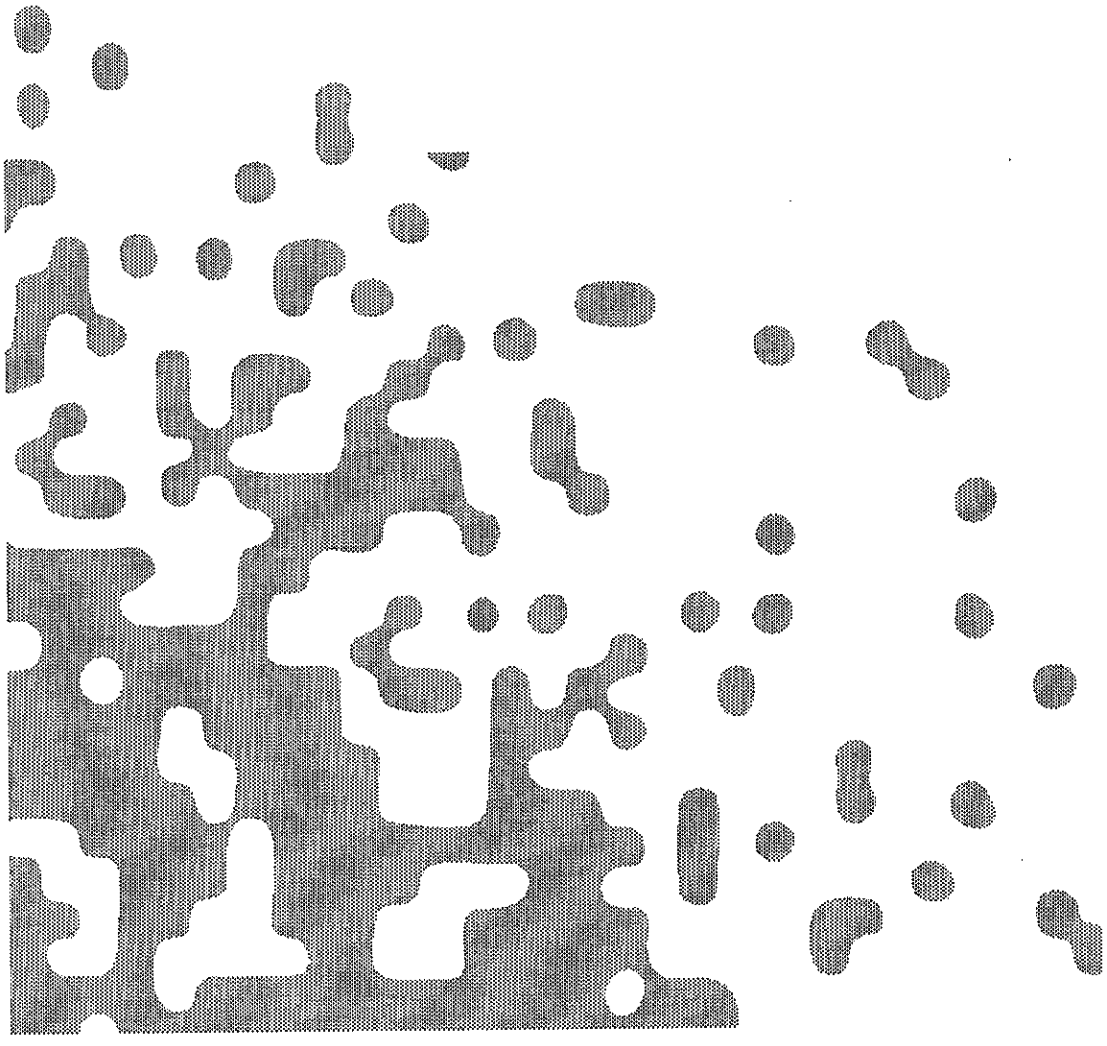
SIGNAL PROCESSING

measurement resolution	256 or 1024 channels on all five parameters (seven when acquiring with DDM).
signal modes	Any combination of linear or logarithmic selections for each detector.
dynamic range	Four decades are provided by logarithmic amplifiers for each of the five parameters.
fluorescence sensitivity	1,000 molecules of equivalent soluble fluorescein.
fluorescence resolution	Coefficient of Variation (CV) is <3% full peak for propidium iodide-stained chicken erythrocyte nuclei for FL2-A.

INSTALLATION REQUIREMENTS

power	120V AC, 50/60 Hz ±10%, minimum of 20 A. For installations outside the US, a power transformer and conditioner are provided to accommodate 110 V ±10%, 220 V ±10%, 240 V ±10%, 50-60 Hz ±2 Hz.	
size	Width Depth Height	170 cm (67 in) 70 cm (28 in) 51 cm, 90 cm cover open (20 in, 35 in cover open)
weight	90 kg (200 lb)	
temperature	16° to 29°C (60° to 85°F)	

Glossary



acquisition	The electronic and software function of collecting data from a cytometer's sensors.
acquisition gate	A numerical or graphical boundary (region) defining a subset of data. An acquisition gate is applied to data before acquisition to selectively acquire data inside or outside the gate. You may use a logical gate for acquisition.
analysis	The software function of numerically and graphically manipulating data to generate statistics.
antibodies	A class of proteins secreted by sensitized B lymphocytes following contact with an antigen. Antibodies bind specifically to the antigen that induced their formation.
CaliBRITE	BDIS calibration particles consisting of unlabeled, FITC labeled, and PE labeled beads. Use these beads with FACSComp software for baseline instrument setup for two-color immunophenotyping of human lymphocytes.
coefficient of variation (CV)	The standard deviation of the data divided by the mean of the data; typically expressed as a percentage. When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.
compensation	A process used to reduce the unwanted fluorescence of one fluorochrome overlapping into the range of wavelengths of another fluorochrome.

conjugated antibody	An antibody bound to biotin or a fluorochrome.
contour plot	A graphical presentation of two-parameter data in which contour lines show the distribution of events. Similar to a topographical map, the contour lines show event frequencies as peaks and valleys.
DDM	Doublet discrimination module. An electronic component of the instrument that allows for the measurement of pulse width and area as well as height of any one fluorescent channel at a time.
data file	A collection of measured values from a single sample combined with text describing the sample that has been stored to disk. See also list-mode file.
density plot	A graphical representation of two-parameter data. Similar to a contour plot where events are represented by lines, a density plot uses various colors to display the distribution of events.
desktop	The CELLQuest screen background that contains pull-down menus and accommodates windows, dialog boxes, and alert boxes.
dot plot	A graphical means of representing two-parameter data. Each axis of the plot displays values of one parameter. A dot represents an event (particle).
droplet containment system	The outer sleeve of the SIP and the vacuum together provide a means of eliminating the drops of sheath as they backflush from the SIP.

event	A unit of data representing one particle detected by the cytometer. Each event may consist of several parameters measured for that particle.
event rate	The number of particles detected per second. If the threshold is set to zero, the event rate is equal to the number of noise pulses plus the number of particles flowing past the laser beam each second.
FACSComp	BDIS software for the automatic PMT voltage and fluorescence compensation adjustment of the FACSort and FACScan flow cytometers.
file name	A name comprised of up to 27 alphanumeric characters that uniquely identify a file.
flow cell	The optical structure within the flow cytometer where the sample stream intersects with the laser beam. The flow cell is designed to hydrodynamically focus the stream, transmit the incoming laser light, and exit the scattered and fluorescent light.
fluorescein isothiocyanate (FITC)	A fluorescent dye that may be conjugated to antibodies for cell-surface labeling. FITC emits green light with an emission peak at approximately 515 nm.
fluorescence	The phenomenon of light emission that occurs when a fluorochrome's excited electrons drop to a lower energy level.

fluorochrome — A fluorescent dye. A molecule capable of absorbing light energy, then emitting light at a longer wavelength (fluorescence) as it releases this energy.

forward scatter (FSC) — Light scattered as a particle passes in front of the laser beam. Forward scattered light is a measure of a particle's size and is related to the particle's cross section and refractive index. The collection angle over which the light is measured is typically between 0.5° to 10° relative to the laser beam axis. FSC is typically the threshold or trigger parameter.

gain — Amplification of a signal. Increasing the gain results in a larger output signal for a given input signal. There are two stages of gain adjustment, the detector stage and the amplifier stage. The PMT detector gain is made by adjusting the voltage from 150 to 999 volts. The photodiode gain is made by an exponential adjustment of E-01 to E03. The amplifier gain adjustment is made by adjusting the linear amp from 1.00 to 9.99.

gate — A numerical or graphical boundary (region) that defines a subset of data from the cytometer. Gates may be single or multi-dimensional. You may set gates after data acquisition to analyze the data by subpopulation (see logical gate). You may set gates before or during data acquisition to selectively acquire data (see acquisition gate).

histogram	A graphical means of presenting single-parameter data. The horizontal axis of the graph represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (particles).
immunofluorescence	Fluorescence from fluorescent antibodies attached specifically to antigen sites on a cell.
laser	Light amplification by stimulated emission of radiation. A light source that is highly directional, monochromatic, coherent, and bright. The emitted light is in one or more narrow spectral bands, and with most lasers is concentrated in an intense, narrow beam. The argon-ion laser used in FACSort emits blue light at 488 nm.
linear amplifier	One of two amplifiers (linear and logarithmic) in the FACSort instrument. The linear amplifier produces a signal output proportional to the input signal amplitude. For example, the linear amp has a signal output ranging from 1 to 5 volts as the signal input ranges from 0.01 to 0.05 volts. A linear amp is useful when acquiring data containing cells that vary over a narrow range of signal intensities.
list-mode file	An unprocessed data file containing all of the measured parameters for each particle in the sample, as well as information describing the sample.

logarithmic amplifier	One of two amplifiers (linear and logarithmic) in the FACSort instrument. The logarithmic amplifier produces a signal output proportional to the logarithm of the input signal amplitude. A four-decade log amp, for example, has a signal output ranging from 0 to 10 volts as the signal input varies by a factor of 10,000. A log amp is useful when acquiring data containing cells that vary over a wide range of signal intensities.
logical gate	A combination of one or more previously defined regions that define a subset of the total sample population. The logical operators AND, OR, and NOT, along with parentheses (if necessary) are used to define logical gates.
marker	A boundary on a histogram. Two markers form a marker set and allow you to obtain statistics for a particular section of a histogram during analysis.
mean channel	The average channel number of a sample from a population of cells.
menu	A list of choices displayed on the computer screen. CELLQuest displays most of its choices through pull-down and pop-up menus.
menu bar	The banner across the top of the CELLQuest desktop that contains menu titles. Click on these titles to open pull-down menus.
menu option	A choice in a pull-down menu. Click and hold to open a pull-down menu, then drag the cursor to the menu option to choose it.

monoclonal antibodies	Laboratory-produced antibodies all having identical molecular structure and specificity.
mouse	The small device that controls the pointer or cursor on the computer screen. When you move the mouse on a flat surface (or mousepad), the cursor moves.
optimization	An instrument adjustment procedure you perform to optimally set the electronic components of the instrument for specific samples.
parameter	A measurement of a cell property which is ascertained as the cell passes through the laser beam. Each parameter is the output of a single photomultiplier tube or photodiode, measuring fluorescent or scattered light (eg, FSC, SSC, FL1, FL2, or FL3).
PBMCs	Peripheral blood mononuclear cells. Whole blood that has been passed through a density gradient removing the granulocytes and red blood cells leaving primarily lymphocytes and monocytes.
photodiode	A solid-state device for measuring light intensity. Like the photomultiplier tube, a photodiode generates an output current proportional to the incident light intensity. Though smaller and simpler to use than PMTs, they are not as sensitive.

photomultiplier tube (PMT)
A device for measuring light intensity. PMTs produce an output current proportional to the intensity of incident light. In some applications, PMTs are sensitive enough to count individual photons of light.

phycoerythrin (PE)
A fluorescent dye that may be conjugated to antibodies for cell surface labeling. PE emits yellow-orange light with an emission peak at approximately 580 nm.

precision
A measurement of performance. Precision is the closeness of repeated measurements of the same quantity. Precision is typically measured by calculating the coefficient of variation (CV) of a population of nearly identical test particles.

propidium iodide (PI)
A fluorochrome that emits red light with a peak emission of approximately 620 nm. PI is commonly used to stain the DNA in cell nuclei.

pulse
A signal that momentarily changes value. As a particle moves through the laser beam, the resulting fluorescent and scattered light will appear as a pulse signal. This signal will have low values as the particle enters and leaves the beam and a peak value when the particle passes the beam center.

pulse area
A mathematical integral of a single pulse of a signal; the area under the pulse curve. The pulse area represents the total light emission, and will be proportional to the amount of fluorochrome on a particle.

pulse height	The peak height of a single pulse of a signal. If the illuminating beam width is greater than the particle size the pulse height will be proportional to the amount of fluorochrome on the particle's surface.
pulse width	The width of a single pulse of a signal. The pulse width is proportional to the time the particle traverses the beam and is a measure of a particle size, laser beam height, and stream velocity.
RAM	Random-access memory. The part of the computer's memory available for active programs and files that the computer reads from disk. The contents of RAM are lost when the power is turned off. Memory size is expressed as bytes of data, thousands (kilobytes), or millions (MB).
region	A boundary drawn around a subpopulation to isolate these events for acquisition, analysis, or sorting. Regions can be combined to create logical gates.
resolution	A measure of a cytometer's ability to distinguish between two populations of particles with differing fluorescent or scatter intensity.
sample	The cells or particles introduced to the flow cytometer and measured for scatter and fluorescence characteristics.
sample injection port	The area where the sample tube is installed. The ample injection port includes the sample injection tube and the tube support arm.

sample injection tube — The stainless steel tube that introduces cells from the sample tube to the flow cell. The tube is covered with an outer sleeve that works in conjunction with a vacuum and serves as part of a droplet containment system.

sensitivity — A measure of a cytometer's ability to distinguish particles from background noise. Sensitivity is often expressed in terms of a minimum number of fluorochrome molecules per particle required to clearly distinguish a stained particle from an unstained particle. Sensitivity will depend on the instrument, the dye, and the preparation method.

sheath fluid — Cell-free fluid which surrounds the central sample stream in a flow cytometer. Sheath fluid is usually a buffered saline solution.

side scatter (SSC) — Also called 90-degree, orthogonal, right-angle, or wide-angle scatter. Light scattered by a particle at approximately 90° from the incident laser beam. Intensity of side scattered light is related to the internal structure (granularity) of the particle.

simulated data — Electronic pulse generated by the computer to appear as if data is being acquired from the flow cytometer.

SIP — See sample injection port.

spectral overlap	The emission of one fluorochrome overlapping into the emission of a second fluorochrome. Spectral overlap occurs when a sample is stained with two or more fluorochromes with overlapping emission spectra.
threshold	A trigger signal and level of discrimination to electronically eliminate unwanted signal. Some of the particles passing through the laser beam may be debris or cell fragments. You may set a threshold level to avoid collecting data for these events. Only events with parameter values above the threshold will be collected.
trigger signal	The parameter used to indicate the presence of a cell or particle at the laser interrogation point. Forward scatter is typically used as a trigger signal. Signals from other detectors are acquired only when the trigger is activated (see threshold).
tube support arm	The arm that supports the sample tube. It moves in three positions: centered below the tube, to the right, or to the left of the tube. It also functions to activate the droplet containment system vacuum. The vacuum is activated when the arm is in the right or left position and off when the arm is centered.
window	An area that displays information on the desktop. You view data through a window. You can open or close a window, move it around on the desktop, and sometimes change its size, edit its contents, and scroll through it.

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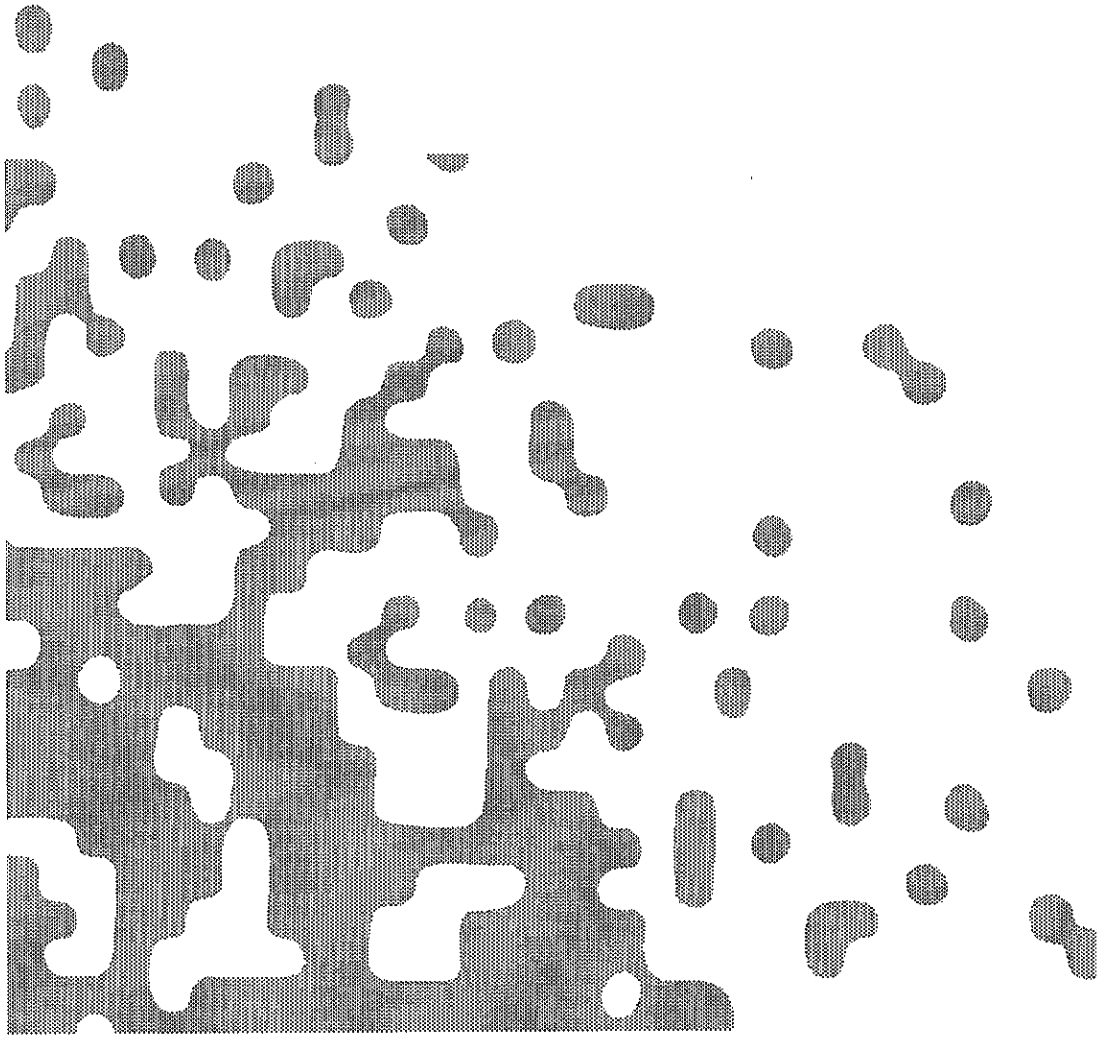

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