CytoFluor® Series 4000

Fluorescence Multi-Well Plate Reader

User Guide

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Printed in the USA, 07/2001
Part Number PH601380 Rev. A

WARNING

For continued protection against fire hazard, replace fuses with those of the same type and rating.

AVERTISSEMENT

Remplacez les fusibles par ceux de même type et puissance pour éviter les risques d'incendie.

US Safety and EMC (Electromagnetic Compliance) Standards

Safety

This instrument has been tested to and complies with standard ANSI/UL 3101-1, “Electrical Equipment for Laboratory Use; Part 1: General Requirements”, 1st Edition. It is an ETL Testing Laboratories listed product.

EMC

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Warning*: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.*

Note*: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.*

Note*: Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.*

Canadian Safety and EMC (Electromagnetic Compliance) Standards

Safety

This instrument has been tested to and complies with standard C22.2 No. 1010-1-92, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements”. It is an ETL Testing Laboratories listed product.

Sécurité

Cet instrument a été vérifié avec la norme C22.2 No. 1010-1-92, «Spécifications de sécurité du matériel électrique utilisé pour les mesures, les contrôles et dans les laboratoires ; Partie 1 : Spécifications générales», et il est conforme à cette norme. C’est un produit homologué par les ETL Testing Laboratories.

EMC

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le materiel brouilleur du Canada.

European Safety and EMC (Electromagnetic Compliance) Standards

Declaration of Conformity

Application of Council Directive(s):	73/23/EEC “Low Voltage”
	89/336/EEC “Electromagnetic Compatibility”
Standard(s) to which conformity is declared:	EN61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use”
	EN55011:1991, Group 1, Class B “Radiated Emissions”
	EN50082-1:1992 “Generic Immunity”
Manufacturer’s Name:	Applied Biosystems
Manufacturer’s Address:	500 Old Connecticut Path
	Framingham, Massachusetts 01701 USA
Type of Equipment:	Laboratory Instrumentation
Model Name:	CytoFluor Series 4000 Fluorescence Multi-Well Plate Reader
Model Number	MIFS0C2TR
Serial Number:	FX6CMQ378P and later
Year of Manufacture:	1997

Application of Council Directive(s):	73/23/EEC “Low Voltage”
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	EN55011:1991, Group 1, Class B “Radiated Emissions”
	EN50082-1:1992 “Generic Immunity”
Manufacturer’s Name:	Applied Biosystems.
Manufacturer’s Address:	500 Old Connecticut Path
	Framingham, Massachusetts 01701 USA
Type of Equipment:	Laboratory Instrumentation
Model Name:	CytoFluor Series 4000 Fluorescence Multi-Well Plate Reader with Temperature Control
Model Number:	MIFS0C2TC
Serial Number:	FX6CMQ380P and later
Year of Manufacture:	1997

Table of Contents

How to Use This Guide

Purpose of this guide

Applied Biosystems *CytoFluor Series 4000 Multi-Well Fluorescence Reader User's Guide* describes how to set up, use, and maintain the CytoFluor® Series 4000 Fluorescence Multi-Well Plate Reader. This system enables you to scan data on soluble and cell-associated fluorescence. The entire system consists of a scanner and the CytoFluor software for Microsoft® Windows®. The software runs on a PC that you provide and enables you to control and maintain the scanner.

Audience

This manual is written for CytoFluor system users. You should be familiar with using a PC and Windows applications. For your convenience, there is a glossary of terms at the back of this document. If you need more information on Windows, refer to the manuals provided with the Windows software.

Structure of this guide

Applied Biosystems *CytoFluor Series 4000 Multi-Well Fluorescence Reader User's Guide* is divided into chapters. Each chapter page is marked with a tab and a header to help you locate information within the chapter.

The table below describes the material covered in each chapter.

Chapter 1, Overview of the CytoFluor System	Provides an overview of the CytoFluor system process.
Chapter 2, Getting Started	Describes how to get started. For example, it defines the function of the scanner parts and describes how to set it up. It also contains steps to install the software and check the system.

Chapter 3, Scanning Operations	Defines how to create a new file, access a previously saved file, and set up the scan parameters. It also contains details on how to scan a plate.
Chapter 4, Displaying, Printing, and Exporting Data	Contains steps to use post processing for data display. It also describes how to print and export data.
Chapter 5, Setting the Plate Layout or Auto Scale Parameters	Provides details on how to set the plate layout. For example, it defines how to assign characteristics to a plate well or group of wells. It also describes how to set the auto scale parameters so the system can scale the data as it acquires it.
Chapter 6, Customizing the CytoFluor System	Describes how to customize the system. It includes steps to set up and change filter pairs, test the scanner drives and lamp, and set the lamp saver timer.
Chapter 7, Maintaining the CytoFluor System	Defines how to maintain the CytoFluor system. For example, it describes how to clean the scanner and change the air filters. It also has steps to change the lamp when needed.
Chapter 8, Accessing Technical Information	Contains details on the system specifications and catalog numbers.
Appendix A, Introduction to Windows	Provides basic information on how to use Microsoft Windows and defines basic Microsoft Windows terms.

Appendix B, Error Messages	Describes and defines CytoFluor system test vectors and error messages.
Appendix C, Warranty/Service Information	Contains warranty, service, and return information.
Appendix D, Plate Scan Patterns	Provides examples of the scan patterns the CytoFluor system uses when averaging points taken in a well.
Appendix E, Technical Support and Training	Describes how to contact Technical Support, obtain technical documents, and obtain customer training information.

Conventions

General Conventions

The text in this manual uses these typographical conventions:

- *Italic* text indicates a new term in the text, usually followed by a definition.
- **Bold** text indicates menu and option names in steps.

Notes, Cautions, and Warnings

Notes, Cautions, and Warnings are used for the following reasons:

NOTE: *Informs the user about important information not critical to operating the system.*

CAUTION

Warns the user about possible harm to the system or loss of data.

WARNING

Warns the user about possible bodily harm.

AVERTISSEMENT

Un avertissement fournit une information indispensable à la sécurité de l'operateur.

Symbols used on the system

The following symbol appears on the system to alert the user to warnings. The symbol also appears next to associated warnings in this document.

Le symbole ci-dessous permet d'alerter l'utilisateur et precede un avertissement.

Send us your comments

We welcome your comments and suggestions for improving our manuals. You can send us your comments by e-mail at **TechPubs@appliedbiosystems.com.**

Overview of the CytoFluor® System

1

This chapter includes the following sections:

***NOTE**: Complete and return the provided CytoFluor® installation qualification report. Applied Biosystems must receive your completed installation qualification report before you can receive technical support and product update information. The registration number for your system software is on the program disk.*

1.1 What is the CytoFluor System?

The complete CytoFluor system consists of:

- Scanner and software
- User-provided IBM®-compatible personal computer (PC)

This system quantifies soluble and cell-associated fluorescence. The fluorescence is measured by a photomultiplier tube in a light-proof detection chamber. The detected fluorescence data is displayed as arbitrary fluorescence units (AFU).

You control the action of the scanner through the software. The software runs on a PC operating under Microsoft Windows software. The precision X-Y mechanism scans the microtiter plate with a sensitive fiber-optic probe to quantify the fluorescence in each well. The plate remains stationary during the scanning of each row, then indexes to the next row. The scanning system is fast; it can scan a 96-well plate in under a minute.

You can use standard types of microtiter plates with six to 384 wells. You can select plate formats from a library of plates or define your own plate format as described in Chapter 5, Setting the Plate Layout or Auto Scale Parameters. For plates with larger wells, you can choose an option to obtain an average of multiple data points in each well. (Appendix D, Plate Scan Patterns, defines the acquisition patterns.) In addition, you can configure the system to read plates from the top or bottom, depending on your application. (See Chapter 6, Customizing the CytoFluor System, for more information.)

The scanner holds up to six excitation filters and six emission filters to measure most common fluorophores. The CytoFluor system ships with five excitation and five emission filters installed. The remaining positions are filled with light tight plugs. (See Chapter 8, Accessing Technical Information, for a list of common fluorophores and their corresponding filters.) In addition, the scanner requires little maintenance. The most important routine maintenance task is changing the air filter. (See Chapter 7, Maintaining the CytoFluor System, for instructions.)

There are two CytoFluor Series 4000 models:

- **CytoFluor Series 4000/TC**—Temperature-control model equipped with a temperature control chamber in which plates are scanned. Controlling the temperature provides the ability to hold a plate at a specific temperature or collect data from kinetic experiments. See Section 3.5, Controlling the Temperature, for more details.
- **CytoFluor Series 4000/TR**—Temperature-ready model not equipped with a temperature control chamber. TR models can be upgraded with a temperature control chamber. Contact Applied Biosystems for more information.

1.2 How Does It Work?

The CytoFluor software uses a Microsoft Windows interface to provide features that make the system easy to use. For example, you select commands and options from menus and buttons using the mouse. If you prefer, use keyboard equivalents to access many of the functions.

The software initially displays the CytoFluor main setup screen. This screen contains the scan set up parameters and menu options. You can access options to assign plate parameters to reflect your actual assay plate and experimental setup. You can also access options to save, copy, or print your data. (Chapter 2, Getting Started, contains more information on the CytoFluor main setup screen.)

The scan setup parameters include identification information, excitation and emission filter pairs, gain, number of scans and cycles, and other run-specific information. You can edit them at any time, before you acquire data. Once you acquire data, you can only change the comments information in a file.

You start collecting data by selecting the Start button from the main setup screen after you enter the scan setup information. The software directs the scanner to move the plate into the detection chamber. The scanner mechanism then aligns the probe to read all selected wells, row by row. A run for a single plate may have up to 200 cycles, with up to four different filter pairs and sensitivities per cycle. Each group of filter pairs and gain represents a scan.

After the system reads the final well, the plate transport mechanism presents the plate back to the load position. The detector is set to a low gain setting and the lamp remains on to minimize warm-up time for successive runs. The system has a timer that turns the lamp and detector off if no runs have been initiated for 60 minutes. You can change this value using the Maintenance menu as described in Chapter 6, Customizing the CytoFluor System.

The system stores scanned information and the setup in a CytoFluor data file. You can save and export file data from within the application.

1.3 Overview of the System Process and Chapter References

This chart overviews the basic steps you follow to use CytoFluor and refers you to the appropriate chapter for details:

Step	Action	Described in ...
1	Set up the scanner. This involves setting the voltage, installing fuses, and connecting it to a PC.	Chapter 2, Getting Started
2	Load the software, check the system functions, and run system diagnostics.	Chapter 2, Getting Started
3	Set or check the scan parameters of a file. Or copy the parameters of an existing file and rename it.	Chapter 3, Scanning Operations
4	Determine the plate layout to identify particular wells or groups of wells before starting or completing a run (optional).	Chapter 5, Setting the Plate Layout or Auto Scale Parameters
5	Set the auto scale parameters so the system can scale the data as it acquires it (optional).	Chapter 5, Setting the Plate Layout or Auto Scale Parameters
6	Customize the system before a run (optional).	Chapter 6, Customizing the CytoFluor System
7	Insert a loaded plate and perform a run.	Chapter 3, Scanning Operations
8	Display, print, or export data.	Chapter 4, Displaying, Printing, and Exporting Data

Step	Action	Described in ...
9	Maintain the CytoFluor scanner.	Chapter 7, Maintaining the CytoFluor System

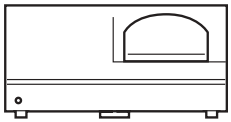
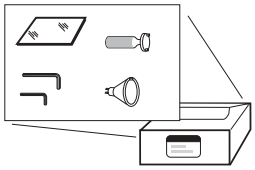
Section 8, Accessing Technical Information, contains system specifications and catalogue numbers; Appendix E, Technical Support and Training, provides information on how to contact the Applied Biosystems Technical Support Department by phone, fax, or through the internet.

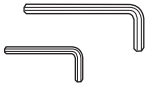
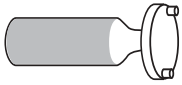
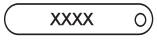
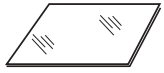
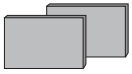
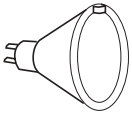
2 Getting Started

This chapter includes the following sections:

2.1 Parts of the CytoFluor System

The CytoFluor system is shipped in one box. After you unpack the parts, review this list to make sure you are not missing anything:

Part	Diagram
CytoFluor Scanner ***NOTE**: See Section 2.2, The CytoFluor Scanner, for details.*	
CytoFluor system software	
System Calibration Certificate ***NOTE**: It identifies the scanner and software by serial number.*	
CytoFluor Series 4000 Fluorescence Multi-Well Plate Reader Installation Qualification Certificate	
CytoFluor Series 4000 Fluorescence Multi-Well Plate Reader User's Guide	
Accessories kit	

Accessories Kit Parts	Diagram
RS-232 cable	
Two Allen (hex) wrenches	
Power cord	
Fuses, 2.5 amp (2-IEC, 2-UL)	
Filter installation tool	
Probe height adjustment spacers (shims), 0.015-inch and 0.020-inch	
Test plate (for Service use only)	
Replacement air filter	
Spare lamp	

***NOTE**: Save the shipping box and packing materials so you can re-pack your system. (You may have to move it over long distances or return some components for service.)*

If you have all the parts, complete the Installation Qualification Certificate and return it to Applied Biosystems. If you did not receive all the equipment in your kit, call the Applied Biosystems Technical Support Department. See Appendix E, Technical Support and Training, for the phone number.

2.2 The CytoFluor Scanner

The scanner contains all the parts necessary to scan a variety of plates. Once you connect the scanner to your computer and load the CytoFluor software, you can control how it functions. You can also determine the way the scanner reads the plate wells and customize it to suit your needs. See Chapter 5, Setting the Plate Layout or Auto Scale Parameters, and Chapter 6, Customizing the CytoFluor System, for more details.

This section defines how each scanner part functions. See Section 2.4, Setting Up Your System, for information on how to set up the scanner.

Front of the scanner

Figure 2-1 shows the front of the scanner.

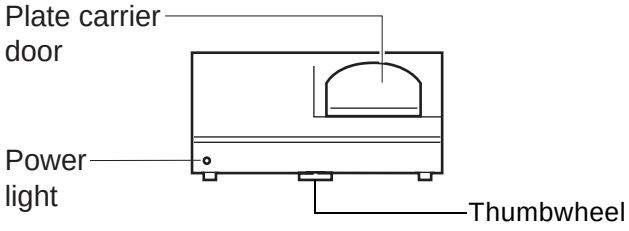


Figure 2-1 Front of Scanner

The front of the scanner includes:

- **Plate carrier door**—Opens to extend the plate carrier so you can insert a plate. When the system brings the plate carrier inside, the door closes and provides a light seal.
- **Power light**—Lights up when the power is on.

Inside the scanner

To see the inside parts, open the scanner cover by turning the thumbwheel counterclockwise and lifting up the cover.

To replace the cover:

Make sure the plate carrier is towards the rear panel so you do not close the cover on it. Make sure the fiber-optic cables are inside the scanner. Then pull the cover over the base, push it down, and turn the thumbwheel clockwise until finger-tight.

CAUTION

Be sure to seat the cover properly so the plate carrier can exit the scanner. After you follow the steps in Section 2.4.4, Installing the Software, click on the Plate In and Plate Out buttons to test the plate carrier. If it does not move in and out, reseat the cover.

Figure 2-2 shows the inside of the scanner.

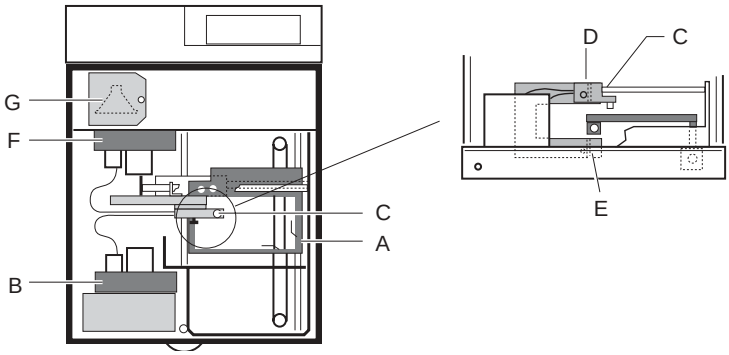


Figure 2-2 Inside of Scanner

***NOTE**: In CytoFluor Series 4000 scanners with temperature control, the plate carrier is contained in a temperature control chamber with an additional door. These features are not shown.*

Letter	Part Name	Function
A	Plate carrier	Holds the plate so the scanner can read the well contents.
B	Emission filter assembly	Holds the filter that determines the fluorescence emission wavelength to monitor.
C	Probe	Transmits and receives the excitation and emission signal.
D	Top read mounting block	Holds the probe so it can read from the top of the plate.
E	Bottom read mounting block	Holds the probe so it can read from the bottom of the plate.
F	Excitation filter assembly	Holds the filter that selects the excitation wavelength.
G	Lamp	Provides the fluorescent excitation. (The lamp is located at the rear of the scanner in a separate, covered compartment.)

Back of the scanner

Figure 2-3 shows the back of the scanner.

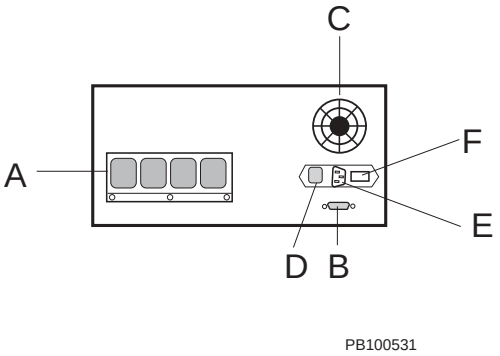


Figure 2-3 Back of Scanner

Letter	Part Name	Function
A	Air vent	Draws air into the scanner.
B	RS-232 port	Enables you to connect the scanner to a computer. Pins used: • 2—Transmit • 3—Receive • 7—Ground Settings on scanner (Com port on computer must be set the same way): • 9,600 baud, 8-data bit, 1 stop • No parity
C	Air exhaust fan	Forces air out of the scanner to keep it cool.
D	Power switch	Enables you to turn the scanner on and off.
E	Power inlet	Enables you to connect the power cord.
F	Fuse module	Enables you to change the fuses.

2.3 Computer System Requirements

You supply the computer to use with the CytoFluor system. Your computer must meet these minimum requirements:

- IBM-PC®-compatible type with 486DX computer
- 240 megabyte hard drive
- 8 megabytes of memory (RAM)
- Mouse
- 3-1/2-inch, high-density disk drive
- One serial port
- One parallel port
- Microsoft® Windows® version 3.1 or later or Windows® 95 software

Performance suggestions

For optimum performance, you should also supply:

- An electrical surge protector (most multi-outlet power bars include surge protection)
- A mouse pad

Site selection

Install your system in an area that is well ventilated, free from dust and fumes, and away from direct sunlight or local heat sources.

2.4 Setting Up Your System

To set up the CytoFluor system, perform these tasks in the order shown:

- Install the appropriate fuses
- Remove the shipping restraints from the probe and transport assemblies in the scanner
- Connect the scanner to your computer
- Install the software

2.4.1 Installing the Fuses

Fuses

The CytoFluor Accessories kit contains four 2.5 amp fuses:

- Two UL/ CSA-approved fuses—For use in U.S. and Canada
- Two IEC-approved fuses—For use in all other countries

The fuses are universal and can be used for 100 to 120 and 200 to 240 voltages.

WARNING

For continued protection against fire hazard, replace fuses with those of the same type and rating.

AVERTISSEMENT

Remplacez les fusibles par ceux de même type et puissance pour éviter les risques d'incendie.

Installing

To install the fuses:

1. Remove the warning label from the power module.
2. Remove the fuse module by inserting a small flathead screwdriver in the slot beside the power plug and sliding out the fuse module (Figure 2-4).

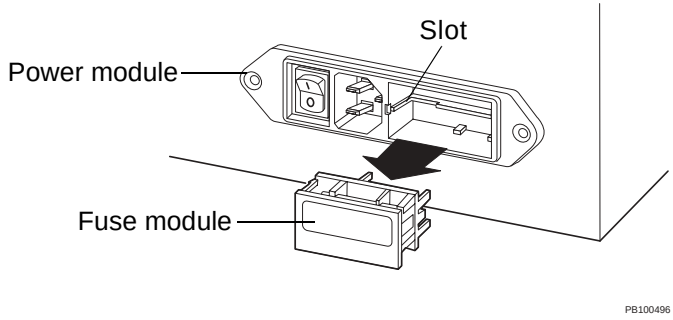


Figure 2-4 Removing the Fuse Module

3. Install the 2.5 amp fuses in the fuse module (Figure 2-5).

NOTE: *Install UL/CSA-approved fuses in the United States and Canada. Install IEC-approved fuses in all other countries.*

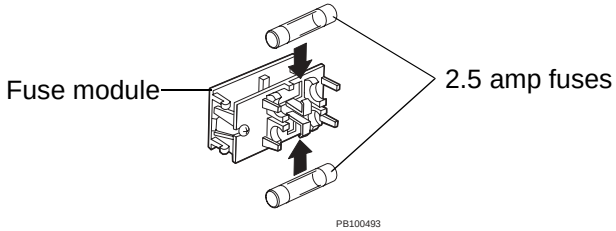


Figure 2-5 Installing the Fuses

4. Slide the fuse module into the power module until it snaps into place (Figure 2-6).

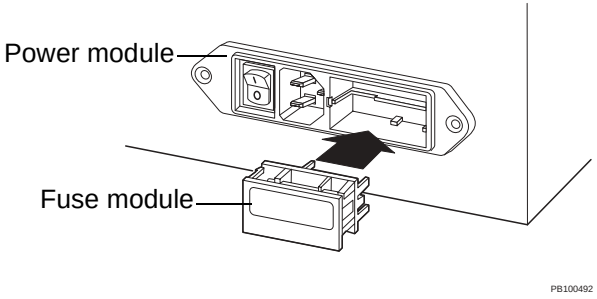


Figure 2-6 Replacing the Fuse Module

5. Install the power cord.

2.4.2 Removing the Shipping Restraints

To remove the shipping restraints:

1. Open the scanner cover by turning the thumbwheel counterclockwise and lifting up the cover.

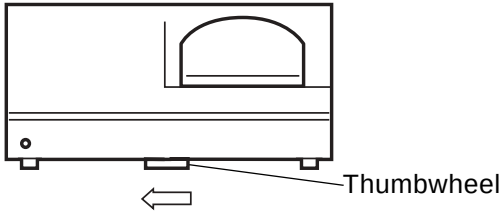


Figure 2-7 Opening the Cover

2. Remove the shipping restraints from the probe and transport assemblies shown in Figure 2-8. Cut the tie wraps with a small wire cutter.

***NOTE**: CytoFluor Series 4000 Temperature Control models do not have a shipping restraint at position 1 (Figure 2-8). You must, however, remove a piece of tape from the temperature control chamber door.*

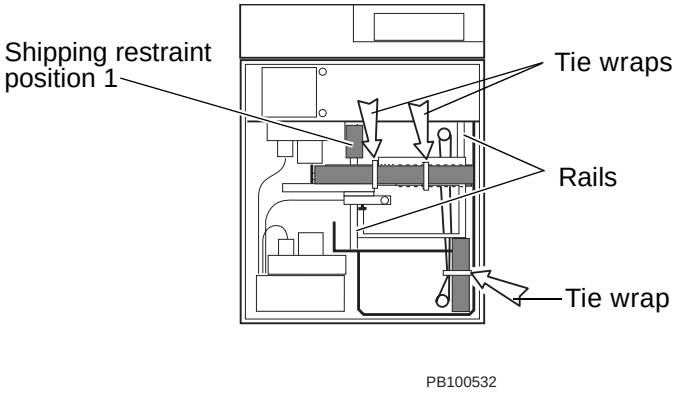


Figure 2-8 Tie Wraps

3. Inspect the rails to ensure they are clean. Clean them with ethanol or isopropanol if necessary.
4. Close the scanner cover, push it all the way down to seat it, and turn the thumbwheel clockwise until finger-tight.

CAUTION

Do not over-tighten or under-tighten the cover thumbwheel or the plate carrier cannot exit the scanner. To make sure you closed the cover correctly, click Plate Out. If the plate carrier does not move out, turn off the scanner and turn the thumbwheel further to tighten it.

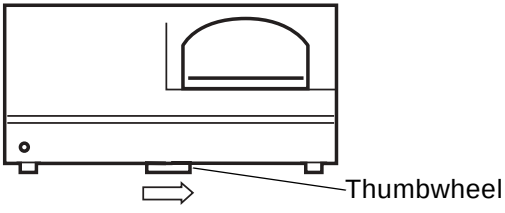


Figure 2-9 Closing the Cover

2.4.3 Connecting the Scanner to Your Computer

After you install fuses in the scanner, and you remove the shipping restraints, you can connect the scanner to your computer.

WARNING

You must electrically ground the system and computer. These components are equipped with grounding plugs. Use the grounding plugs in a grounded outlet. Using a grounded plug that has been converted to a non-grounded plug can cause electric shock or system damage.

AVERTISSEMENT

Pour les connexions électriques, vous devez mettre le système et l'ordinateur à la terre. Ces composants sont équipés de fiches de mise à la terre. Utilisez ces dernières avec une prise de courant reliée à la terre. L'utilisation d'une fiche de mise à la terre, transformée en fiche non-protégée peut causer des secousses électriques ou endommager le système.

To connect the scanner to your computer:

1. Plug the RS-232 cable into Communication Port 1 or 2 on the computer. Make a note of the port you used to connect the scanner.

Figure 2-10 Connecting the RS-232 Cable to the Computer

2. Connect the other end of the RS-232 cable into the Serial Port (9-pin connector) on the rear of the scanner.

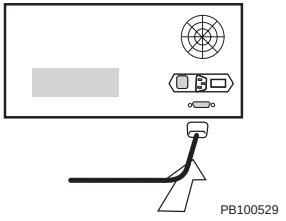


Figure 2-11 Connecting the RS-232 Cable

3. Connect the power cable to the scanner.

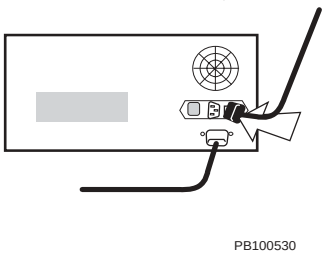


Figure 2-12 Connecting the Power Cable

4. Plug all power cords into grounded, electrical outlets. (Applied Biosystems recommends that you also use a surge protector.)

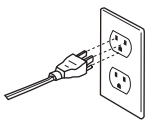


Figure 2-13 Connecting the Power Cable to an Electrical Outlet

2.4.4 Installing the Software

The CytoFluor operating software is contained on one 3.5-inch disk. Follow these steps to load it onto your computer:

1. Verify that you have Microsoft Windows (3.1 or 95) installed. If you do not, you need to install it before you continue on to the next step. (See the instructions provided with the Windows software if you need to install newer versions.)
2. Activate Windows.
3. Insert the CytoFluor diskette into the floppy drive.
4. Follow the appropriate steps below for Windows 3.1 or Windows 95.

If you have ...	Then ...
Windows 3.1	• Activate the Windows **Program Manager**. • Select **Run** from the **File** menu. The Run dialog box appears. • Continue with step 5.
Windows 95	• Click on the **Start** button and then click **Run.** The Run dialog box appears. • Continue with step 5.

5. Type **a:\setup.exe** and click **OK**.

   The CytoFluor installation screen appears and prompts you through the installation procedure. The default destination path is C:\CytoFlr.

The Setup Complete dialog box appears when the installation is complete.

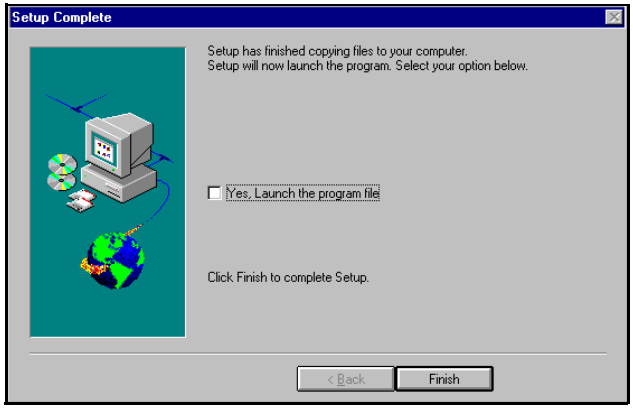


Figure 2-14 Installation Complete Dialog Box

6. Select **Yes, Launch the program file** and click on **Finish** to bring up the CytoFluor main setup screen:

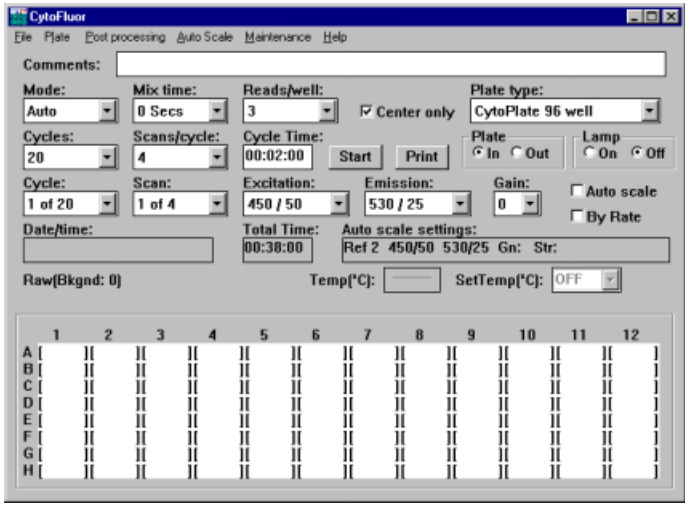


Figure 2-15 CytoFluor Main Setup Screen

You can now check the system as described in the Section 2.5, Checking the System. If you have problems installing the software, contact Applied Biosystems Technical Support. See Appendix E, Technical Support and Training, for the phone number.

Displaying the software version

To check the version of the installed software, open the About Cytofluor dialog box. To do so, select **About Cytofluor** from the Help menu.

2.5 Checking the System

After you complete the set up steps, you must check the CytoFluor system before you can use it. This involves:

- Starting up the CytoFluor system
- Accessing the Maintenance screen
- Checking the scanner settings
- Running system diagnostics
- Returning to the main setup screen

***NOTE**: If you are unfamiliar with the Windows software, refer to the Windows manual provided with your computer, see Appendix A, Introduction to Windows for a brief introduction, or run the tutorial under the Windows Help menu. Then return to this section.*

2.5.1 Starting Up the CytoFluor System

To start up the CytoFluor scanner:

CAUTION

Do not turn on the scanner unless you have installed fuses as described at the beginning of this chapter.

1. Turn on the CytoFluor scanner using the power switch on the rear panel.
2. Turn on the computer and start Windows.
3. Start up the CytoFluor software:
    - In Windows 3.1, click on the **CytoFluor icon**.
    - In Window 95, click on the **Start** button, point to **Programs**, point to **CytoFluor**, and then click **CytoFluor**.

The CytoFluor main setup screen appears:

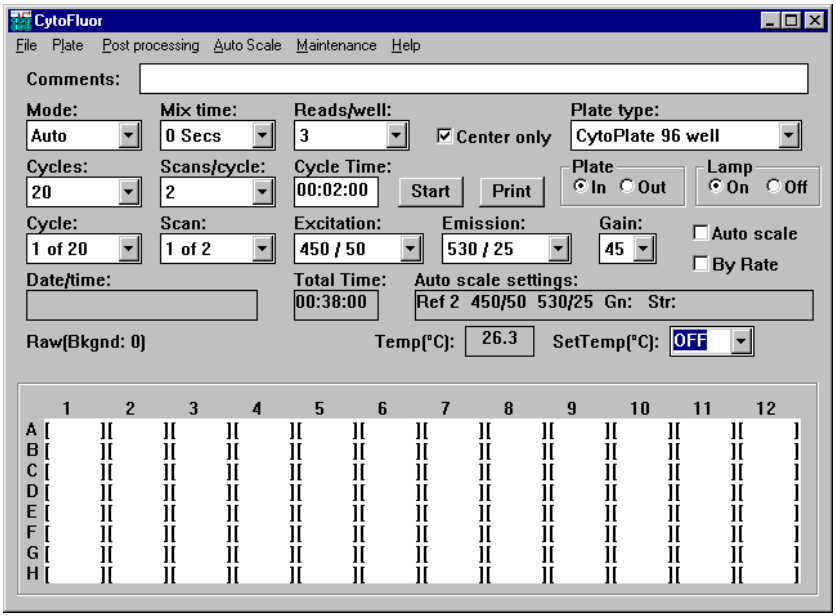


Figure 2-16 CytoFluor Main Setup Screen

Chapter 3, Scanning Operations, contains details on how to use this screen to set run parameters.

4. Access the Maintenance screen as described in Section 2.5.2, Accessing the Maintenance Screen.

2.5.2 Accessing the Maintenance Screen

To check your scanner settings, use the Maintenance screen. To access this screen:

1. Pull down the **Maintenance** menu (or press ALT, M, and Enter).

***NOTE**: You can use key equivalents to choose options instead of clicking with the mouse. The CytoFluor software shows the key equivalent as an underscored letter in the name of an option. For example, Maintenance has the key "M" as its equivalent. Press ALT, M, Enter to choose it.*

The Maintenance screen appears:

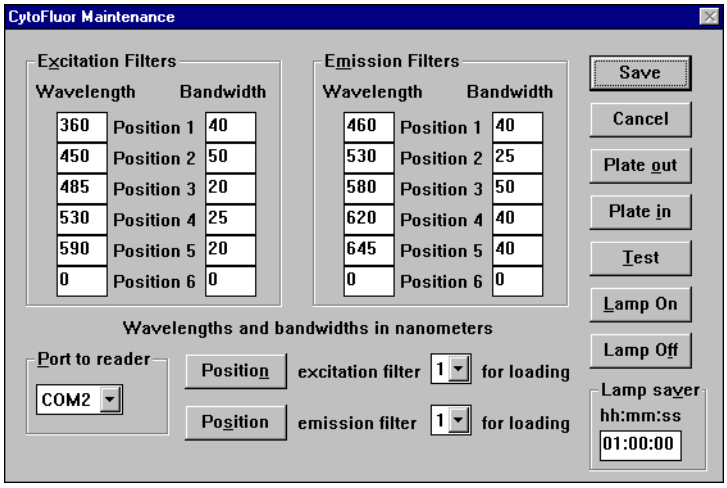


Figure 2-17 CytoFluor Maintenance Screen

2. See Section 2.5.3, Checking Scanner Settings, to check the scanner settings.

2.5.3 Checking Scanner Settings

To check the scanner settings:

1. Check the filter settings shown in the **Excitation Filters** and **Emission Filters** fields of the **Maintenance** screen. The CytoFluor system ships with five excitation and five emission filters. You may add or replace filters by following the steps in Section 6, Customizing the CytoFluor System.

2. Check the **Port to Reader** selection. If you have more than one serial port on your computer, note the name of the port where you physically connected the scanner. See step 3 to change the setting.

3. Click on the down arrow next to the Port to Reader field to scroll down a list of port selections. Highlight the computer port to which you configured the CytoFluor system and release the mouse to select it.

4. See Section 2.5.4, Running System Diagnostics, to run system diagnostics.

NOTE: *Keep a dated record of the settings. Update the record when filters are changed or added.*

2.5.4 Running System Diagnostics

To run system diagnostics:

1. Click the **Test** button on the right side of the Maintenance screen.

   The CytoFluor software automatically checks the scanner drives and lamp. The test takes several seconds. When testing is completed, the Unit Test dialog box appears (Figure 2-18).

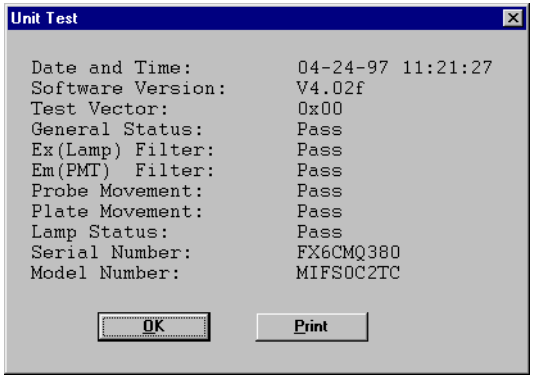


Figure 2-18 Unit Test Dialog Box

If all systems pass, the scanner is ready for use. If the Lamp Status fails, then replace the lamp. See "Replacing the Lamp" on page 7-5 for detailed instructions. If any other system fails, contact Applied Biosystems Technical Support. See Appendix E, Technical Support and Training, for the phone and fax numbers.

2. Click **Print** to print a CytoFluor Fluorescence Unit Test Report.
3. Click **OK** to close the box.

2.5.5 Returning to the Main Setup Screen

If you have been working with the software, follow these steps to return to the main setup screen:

1. Click the **Save** button to keep any changes you made before returning to the main setup screen. Or, click **Cancel** to discard any changes you made.

2. Begin working with the system. Continue to Chapter 3, Scanning Operations, for details on how to perform scans. Or, shut down the system as described in Section 2.6, Shutting Down the System.

2.6 Shutting Down the System

To shut down the system:

1. Select **Exit** from the File menu (ALT, F).
2. Exit Windows.
3. Power down the scanner and computer.

3 Scanning Operations

This chapter includes the following sections:

3.1 Creating a New File

Once you start up the CytoFluor system as described in Chapter 2, Getting Started, you can create a new file. To do this, you have several options. See this chart for details:

You can...	For instructions...
Enter new settings and create a new file name.	See Section 3.1.1, Creating a New, Original File. ***NOTE**: If you do not have an existing file in the system, follow the steps in this section to create one.*
Access the data of a pre-existing file to adopt the settings of that file	See Section 3.1.2, Accessing a Previously Saved File.
Set the scan parameters through the CytoFluor main setup screen before naming the file.	See Section 3.2, Setting Scan Parameters.

***NOTE**: The scan parameters enable you to enter information unique to the experiment. You can select the run mode, mix time, reads per well, and so on. See Section 3.2, Setting Scan Parameters, for details. You can save a file and enter a new file name before or after you set the scan parameters. See the following sections for details.*

3.1.1 Creating a New, Original File

If you want to clear the data of an existing file or set the parameters of a new file, follow these steps:

1. Start up CytoFluor as described in Chapter 2, Getting Started. You see the option menus and main setup screen.
2. Pull down the **File** menu and choose **New** (ALT, F, N). The system changes all the plate data to zero (if present); the scan parameters remain unchanged.
3. See Section 3.2, Setting Scan Parameters, for details on how to set new parameters. To save the file with the existing parameters or before you set new parameters, continue on to step 4.
4. Select **Save As**… under the **File** menu (ALT, F, V) to save the file.
5. Enter a new file name in the **File Name** box (up to eight characters). Change the directory, if necessary. Then click on the Save As button to save the file. If you want, you can save as a template by selecting Template Files from the Save File As Type pull-down menu. With a template file, you can quickly set up future runs by adopting the setup as described in the next procedure.

   If you enter a name that already exists, a message is displayed. Select **Yes** to overwrite the previous data, or **No** to enter another name.

CAUTION

If you select Yes, you permanently delete the settings and data of the file with the same name.

Set scan parameters before naming a file

You can set up the scan parameters of your run before you name a new file. See Section 3.2, Setting Scan Parameters, for details on setting the scan parameters. Then see Section 3.4, Saving Scan Parameters, to save the settings.

3.1.2 Accessing a Previously Saved File

Files saved after scanning contain all the scan setup information used for the data collection, as well as the collected data. (See Section 3.2, Setting Scan Parameters, for details.) To use a previously saved file:

1. Pull down the **File** menu and choose **Open** (ALT, F, O). A dialog box appears containing a list of files in your current directory:

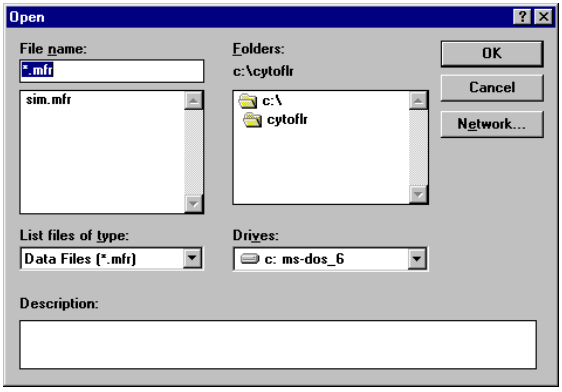


Figure 3-1 Selecting a File

2. Scroll through the list of files or change directories until you see the name of the file you want.

3. Click on the name of the file to select it. Then click on the **Open** button. You see this prompt:

Figure 3-2 Accepting Setup From File

4. Select **Yes** to use the parameters of that file for subsequent runs. Or select **No** to display the scan parameters for viewing purposes only; the system returns to the previous settings for subsequent runs.

   The system displays the file data on your screen whether you chose **Yes** or **No**. You can see the file name in the title bar.

5. Review the data if you selected **No**. Edit the Comments field if necessary, then select **Save** from the File menu to close the file under the same name. (You cannot edit any other scan parameters.) If you select **New** from the File menu, the scan setup parameters will return to the values entered before opening the file.

If you selected Yes, you can:

- Clear out the data by selecting **New** from the **File** menu. You can then set new scan parameters and give the file a new name. (See step 2 through step 5 in “Creating a New, Original File” on page 3-3 for details.)
- Make changes to the **Comments** field and plate layout information as described in Chapter 5, Setting the Plate Layout or Auto Scale Parameters. (You cannot change the scan parameters.) Then save the file under the same name by selecting **Save** from the **File** menu.
- Select **Save As**… from the **File** menu to enter a new file name that has the same parameters as the file you accessed. (See step 4 and step 5 in “Creating a New, Original File” on page 3-3 for details on using the Save As… command.)

3.2 Setting Scan Parameters

Overview As soon as you enter the CytoFluor software, you see the main setup screen. It displays the scan settings of the last run. For example, you may see information like this in your main setup screen:

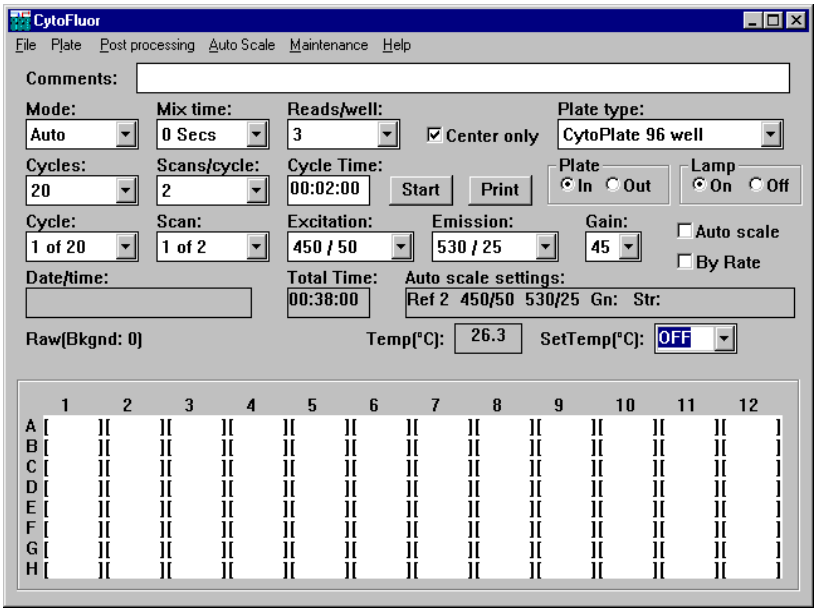


Figure 3-3 Main Setup Screen

If this is the first time you entered the application, you see default settings. You can change the parameters before creating (naming) a file.

Once you access the main setup screen, you can use the Comments field to enter detailed information unique to the experiment, assay, and laboratory. You use the other fields to select the run mode, mix time, reads per well, read location, plate type, number of cycles (up to 200), scans per cycle (up to four), cycle interval, excitation and emission filter pairs, and gain levels.

See the following sections for details on how to set each field parameter.

Comments

To enter comments:

1. Click your cursor in the **Comments** field.
2. Type your comments about the experiment (up to 70 characters).

Mode

The CytoFluor software offers two run modes when running multiple cycles:

- **Auto**—Scans the same plate multiple times (depending on what you set for the Cycles field) with no intervention. If you entered a file name before starting the run, the system will save the data as it completes each cycle. Otherwise, you need to save the data through the Save As… command under File at the completion of the run. You may want to use this mode for kinetic studies.
- **Manual**—Scans one plate after another with a minimum amount of manual interaction. The system presents the plate at the completion of each cycle, enabling you to change the plate or add reagents. The system stores all the gathered cycles (up to 200) in the same data file. You may want to use this mode to run several plates as part of the same experiment.

The default system setting is Auto. You can change it as described in this section. For details on setting the number of cycles, see “Cycles” on page 3-10. To set up multiple scans in a cycle, refer to Section 3.3, Setting Up Multiple Scans in a Cycle.

Selecting

To select the run mode:

1. Click on the down arrow in the **Mode** box.
2. Highlight your selection. Then close the choice box by clicking in the **Mode** field.

Mix Time

When running multiple cycles, you might want to mix the well contents of your plate before re-scanning. You may mix the plate contents for up to 99 seconds at the start of each cycle. To set the mix time:

1. Click on the down arrow in the **Mix Time** box.
2. Highlight your selection. Then close the choice box by clicking in the **Mix Time** field.

Reads/Well

By taking the average of multiple readings for very low level signals, you increase the scanner's precision and ability to detect above the background. The Reads/well field enables you to average up to 99 readings, with a corresponding small increase in data collection time. The average value is displayed. To set this field:

1. Click on the down arrow in the **Reads/well** box.
2. Highlight your selection. Close the choice box by clicking in the **Reads/well** field.

Center Only

For large well plates (6, 12, 24, and 48 well plates), you may want to average readings taken at different locations over the entire well instead of using just one at the center.

If you deselect the Center only box (no "x" inside), the system uses an average of multiple points read within the well as the well value.

If you select the Center only box ("x" inside), the system reads the center point of the well. The system uses the Reads/well value for each point before averaging it with the remaining points in the well. (Appendix D, Plate Scan Patterns, describes the scan patterns for each plate type.)

Plate Type

In this section of the window, you choose any of the standard plates you see in the pull-down list. The pull-down list is the on-line plate library.

To choose a plate:

1. Click on the down arrow in the **Plate type** box to display the list of choices.

***NOTE**: If you change your plate type to one with a different number of wells, a warning is displayed indicating that you may need to change the probe height. See Chapter 6, Customizing the CytoFluor System, if you need to adjust the probe height.*

Figure 3-4 Probe Height Adjustment Message

2. Highlight your plate type selection. If the warning appeared, click **OK** or press **Enter** to close the box.

***NOTE**: If you do not see the plate you want, you can define a plate. For more information, refer to Chapter 6, Customizing the CytoFluor System.*

Cycles

For each run, you can collect up to 200 cycles of data. Each cycle can have up to four scans. To select the number of cycles:

1. Click on the down arrow in the Cycles box.
2. Highlight your selection. Then close the choice box by clicking in the field.

Scans/Cycle

You can have up to four scans for each cycle. You can also use a different filter pair and gain setting for each scan, or use the same ones. To select the number of scans per cycle:

1. Click on the down arrow in the Scans/cycle box.
2. Highlight your selection. Then close the choice box by clicking in the field.

Cycle Time

Cycle Time is the time from the start of one cycle until the start of the next cycle in hours, minutes, and seconds (00:00:00).

If you want to run multiple cycles on the same plate, you can set the interval. If you enter a time that is smaller than the time it takes to run a complete cycle, the scanner acquires data as quickly as it can and changes the Cycle time field to reflect the actual time.

At the completion of the run, the system displays a message indicating that the value you entered was too small. It also shows you what value it used instead. If you enter a time of 00:00:00, the scanner will acquire data as quickly as possible with no need to update the Cycle Time field. You can use this feature, for example, to scan at intervals during an ongoing biochemical process. This field becomes disabled if you set the mode to Manual or only plan to run one cycle. (See “Mode” on page 3-8 for details on setting modes.)

Setting

To set the time from the start of one cycle to the start of the next:

1. Drag your cursor across the number in the Cycle Time field to select it.
2. Type in a two-digit value for the hour, minutes, and seconds, separating each value with a colon(:). For example, for a five-minute start time between cycles, enter 00:05:00.

Start

Enables you to start the run once you set the parameters.

To start a run, click on the **Start** button.

Print

Enables you to print out the currently displayed data or print the file report using the format selected on the Export/Print dialog box.

To only print the data displayed on the screen, hold down the Shift key while clicking on the Print button.

To print the file report, click on the **Print** button.

Plate In/Plate Out

Causes the scanner to move the plate carrier into or out of the scanner so you can insert or remove a plate.

- To move the carrier out of the scanner, click on the **Plate Out** button.
- To move the carrier into the scanner, click on the **Plate In** button.

Lamp On/Lamp Off

Allows you to turn the lamp on or off:

- To turn the lamp on, click on the **Lamp On** button.
- To turn the lamp off, click on the **Lamp Off** button.

Scanning with the lamp off

You can scan with the lamp off by selecting **Lamp Off** from the Excitation menu on the Main Setup screen before you begin a scan. Scanning with the lamp off allows you to measure bioluminescence (glow). This allows you to use the CytoFluor Series 4000 as a luminometer, although all luminescent assays may not be optimized on the system.

Cycle

You use this field to indicate the cycle you want to display after the system acquires data. To select the cycle:

1. Click on the down arrow in the **Cycle** field to display the pull-down menu.
2. Select a value of 1 to 200 (or the last cycle acquired during your run).

***NOTE**: This field displays the cycle of a run while acquiring data.*

Scan

Enables you to select the scan you want the application to display of the currently active cycle. (The active cycle depends on what you selected for the Cycle field.) When setting up a run, use this box to select the scan parameter you are setting. See Section 3.3, Setting Up Multiple Scans in a Cycle, for details.

Excitation Enables you to select the excitation filter for a scan. See Section 3.3, Setting Up Multiple Scans in a Cycle, for details.

Emission Enables you to set the emission level of the filter for a scan. See Section 3.3, Setting Up Multiple Scans in a Cycle, for details.

Gain Enables you to adjust the voltage on the photomultiplier tube in proportion to integer values from 0 to 99 for a scan. For example, if you set the value to zero (0), the system turns off the photomultiplier tube. If you set the value to 99, the value on the photomultiplier tube is approximately 1080 volts (V).

The fluorescence signal level may vary from scan to scan depending on your assay and fluorophores. You should start scanning with a low value (for example, 40) and gradually increase the gain until you find the optimum level(s) for your assay. You can usually obtain optimal performance using gain settings between 30 and 80. Typically, you use a high gain setting for a weak fluorescence signal and a low gain setting for a strong fluorescence signal. See Section 3.3, Setting Up Multiple Scans in a Cycle, for details.

Auto Scale Uses the parameters shown in the Auto Scale menu to automatically normalize all of your data to the same fluorescence reference. You can choose one of the internal fluorescence reference supplied with the CytoFluor system or use a known concentration of the fluorescent product in your assay (control) right on the microwell plate. This option minimizes run-to-run variations.

This field must be selected (marked with an x) for the system to apply the settings. (See Chapter 5, Setting the Plate Layout or Auto Scale Parameters, for details on the how to set the Auto Scale menu parameters.) Once you select Auto Scale, you cannot edit the Auto Scale menu parameters (wavelengths, PMT gain, etc.).

With Auto Scale enabled, the system reads the designated reference at the start of each cycle and calculates the scaling factor. (CytoFluor multiplies all well readings by this factor as acquires them.) To use the same reference for subsequent runs, access a previously saved file and adopt the file setup. See “Accessing a Previously Saved File” on page 3-4, for details.

***NOTE**: If the system reads values that are much less than the original reference value, you see a message stating there is an error in the optical system.*

You cannot reverse the scaling of data acquired with Auto Scale enabled. However, you can scale data acquired with or without Auto Scale enabled if you use the “Scaled” feature in the Post Processing menu. (See Chapter 4, Displaying, Printing, and Exporting Data, for details.)

By Rate

The CytoFluor software captures fluorescence and time data for each well. This allows you to display current rate data during scanning.

To display rate data during scanning, click the **By Rate** check box.

When you select By Rate, the data are displayed for each well during any given scan as follows:

Scan, Cycle	Display
Scan 1, Cycle 1	The raw fluorescence value.
Scan 1, Cycle 2 until last cycle	The current rate calculated by the following equation: $$\frac{AFU_N - AFU_{N-1}}{CycleTime}$$ Where: AFU = Arbitrary Fluorescence Unit N = Cycle

***NOTE:** Values that exceed the system limits are not displayed.*

Date/Time

Displays the data and time of the previously acquired data. You cannot edit this field.

Total Time/Elapsed Before a run, displays the total run time. During the run, displays the time elapsed between the current scan and the same scan number acquired during the first cycle. You cannot edit this field. During a run, this field doubles as a countdown timer to the start of the next cycle.

Auto Scale Settings Enables you to see the auto scale selections in effect for the run if the Auto scale box is selected. You cannot edit this field. See Chapter 5, Setting the Plate Layout or Auto Scale Parameters, for details on the Auto Scale screen options.

Data format Displays the current scaling choice.

Plate Representation At the bottom of the main setup screen, you see a plate map with rows labeled “A” through “H” and columns labeled one to 12 for a 96-well plate. (The labels change as the numbering of the wells change.) This area of the screen represents the position of the wells in your plate. As CytoFluor scans the plate, you can see the data collected for each well appear in real time in the appropriate position on the screen. The top-left of the screen displays the post processing selections in effect for the run.

CAUTION

It is safer to give the file a name before you start a scan. If the file has a name, the system saves each cycle to the file as it completes the cycle. If you did not name the file, you need to save it through the Save As… command under File at the completion of the run. Otherwise, you could lose the data if something disturbed the run (for example, a power failure).

To set up multiple scans, see Section 3.3, Setting Up Multiple Scans in a Cycle. Or, see Section 3.4, Saving Scan Parameters, to save your settings. To start a scan, skip to Section 3.6, Scanning Plates.

3.3 Setting Up Multiple Scans in a Cycle

A single cycle can consist of one to four scans. Each scan is a separate pass of one microtiter plate. The scans can have different excitation and emission filter pairs and/or gains, or they can be identical.

To set up multiple scans in a cycle, you need to select the:

- Number of scans per cycle (one to four)
- Number of the scan you want to define
- Filter pairs for each scan
- Gain for each scan
- Set additonal scans, and copy settings, if needed
- Save the file

***NOTE**: You need to access the CytoFluor main setup screen to select the multiple scan settings. See the following sections for details.*

Select the number of scans

1. Select the number of scans you want in each cycle from the **Scan/cycle** box if you have not done so already. See “Scans/Cycle” on page 3-11 for details.
2. Click the down arrow in the **Scan** field to reveal a pull-down list.
3. Select the scan you want to define (1, 2, 3, or 4).
4. Select the filter pair level for the scan as described in the next section.

Select the filter pairs for a Scan

1. Click on the down arrow in the **Excitation** field to display the list of choices.
2. Highlight the filter pair you want to use.
3. Click on the down arrow in the **Emission** field to display the list of choices.

4. Highlight the filter you want to use.

   The system displays an error message if you try to start a run with incompatible filter pairs that overlap enough to damage the photomultiplier tube.

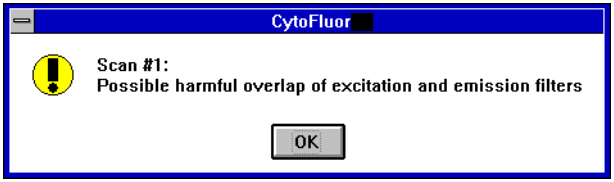


Figure 3-5 Filter Overlap Error

   If you see a message, click on **OK** and make another selection. You define filter positions and parameters through the Maintenance screen. The filter-compatibility checking feature in CytoFluor depends on correct filter parameters. So if you use custom filters, update the software with the correct positions of these filters. Also include the correct center wavelength and band pass parameters. See Chapter 6, Customizing the CytoFluor System, for details.

CAUTION

Do not run a scan with incompatible filter pairs or you will damage the instrument and void the warranty. You must select a compatible pair of filters before acquiring data or saving the file. See Chapter 8, Accessing Technical Information, for a list of filters for various fluorophores and their possible applications.

---

5. Set the gain as described in the next section.

Select the Gain

1. Click the down arrow in the Gain field to display the list of choices.
2. Highlight the value you want (0–99). As previously described in the “Gain” section, start off with a low value and gradually increase the gain until you find the optimum level(s) for your assay. Gain settings between 30 and 80 usually give the best performance. If you must use a value below 30, your fluorophore is very concentrated and you should dilute it. If you must use a value above 80 to detect very low concentrations, try increasing the Reads/well value or adjusting your assay design. (See “Reads/Well” on page 3-9 for details.)

***NOTE**: When you create a file for a new assay, define all four scans with the same filter pairs, but different gain settings (from low to high). This way, you can quickly determine the best gain setting for your particular experiment.*

Additional scans

Repeat the steps to select the number of scans, filter pair, and gain for each additional scan you want to run.

Copying filter and gain settings

You can copy the filter and Gain settings from a scan to up to 4 additional scans. For example, when you scan an entire 384 well plate, you must set up four scans per cycle. The CytoFluor scanner reads 96 wells per scan. All four of the scans must have the same filter settings and gain. You can set the filter settings and gain for the first scan and then copy those settings for the remaining scans using the CopyFilter To option.

To use the Copy Filter To option:

1. Set the filters and gain as described in “Select the filter pairs for a Scan” on page 3-16 and “Select the Gain” on page 3-18.

2. Select **CopyFilterTo** from the Plate menu. The Filter/Gain Copy dialog box appears.

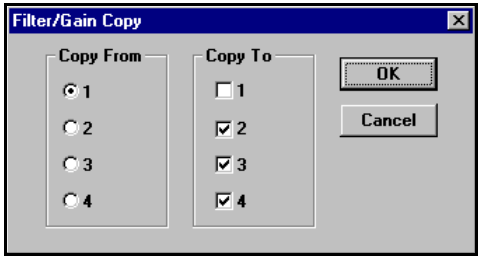


Figure 3-6 Filter/Gain Copy Dialog Box

3. Select the scan settings to copy from the **Copy From** buttons.
4. Select the scans to which you want to copy the settings from the **Copy To** buttons.
5. Click **OK**.

Save the file Select **Save As**… from the **File** menu. Name your file, if necessary.

3.4 Saving Scan Parameters

Once you enter the scan parameters, save the settings before you start a run.

CAUTION

You can save the file after the scan, but it is safer to save it and give it a name before a run. If the file has a name, the system automatically saves each cycle to the file as it completes the cycle. If you did not give the file a name, you need to save it through the Save As… command under File at the completion of the run. If you do not, you will lose the data.

To save the scan parameters:

1. Select **Save As**… from the **File** menu.
2. Enter a new name in the **File Name** box (up to eight characters). (Change the directory, if necessary.) Then click the **Save As** button. You can enter a name that identifies the file as a template. Then you can quickly set up future runs by adopting the setup of the file. (See “Accessing a Previously Saved File” on page 3-4, for details.)

   If you enter a name that already exists, a message is displayed. Select **Yes** to overwrite the previous data, or **No** to enter another name.

   CAUTION

   If you select Yes, you overwrite any information previously in the file.

Saving as a template

You can save the parameters from the main setup screen and Plate layout screen as a template to use for future scans. To save the file as a template:

1. Select **Save As** from the File menu. The save as dialog box appears.
2. Select **Template Files** from the Save File As Type pull-down menu.

   The file is saved with a .MFT extension.

3.5 Controlling the Temperature

CytoFluor Series 4000/TC

This section describes features unique to CytoFluor Series 4000 systems with temperature control. If your system does not include a temperature control chamber, skip this section.

Overview

The CytoFluor version 3.1 and later software allows you to set and read the temperature of CytoFluor Series 4000 models equipped with a temperature control chamber. You can control the temperature to hold the plate at a specific temperature or to perform kinetic experiments that measure rates or endpoints.

Temperature stability

The temperature you set has an accuracy of ± 0.5°C and is uniform to ± 0.3°C.

System temperature

The Temp box (Figure 3-7) displays the actual system temperature and is updated every 5 seconds as the temperature equilibrates. The temperature can take 15 to 20 minutes to stabilize. When scans are running, the temperature is updated at the beginning of each scan.

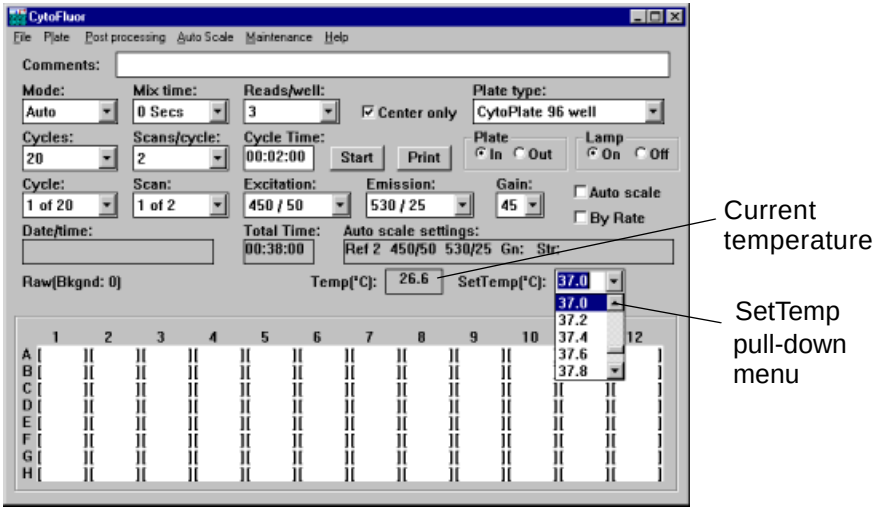


Figure 3-7 Setting the Temperature

Setting the temperature

To set the temperature from the main setup screen:

Select the temperature from the **SetTemp** pull-down menu (Figure 3-7).

Valid temperature settings are:

- 5°C to 20°C above ambient temperature in 0.2°C increments, up to a maximum of 45°C
- OFF to turn off the heater

If you do not set the temperature, the software uses the last temperature value entered to control the temperature.

Turning off temperature setting

To turn off the temperature control setting:

Select **Off** from the **SetTemp** pull-down menu.

3.6 Scanning Plates

Before scanning

1. Set up the run as described in the previous sections. Check that the plate carrier is outside the measurement chamber and in the load position. If it's not, press the **Plate Out** Button on the main setup screen. (This button toggles between Plate Out and Plate In). You can also access Plate Out from the Maintenance screen (under the Maintenance menu).

CAUTION

It is safer to give the file a name before you start a scan. If the file has a name, the system saves each cycle to the file as it completes the cycle. If you did not name the file, you need to save it through the Save As… command under File at the completion of the run. Otherwise, you could lose the data if something disturbed the run (for example, a power failure).

2. Warm up the scanner lamp for a minimum of 10 minutes prior to your first scan. You can turn the lamp on by clicking the **Lamp On** button, if necessary, before setting up the scan parameters.

3. Place your prepared plate into the plate carrier. Make sure the plate is fully seated in the carrier and well A1 is at the top left (towards the back of the scanner) as shown:

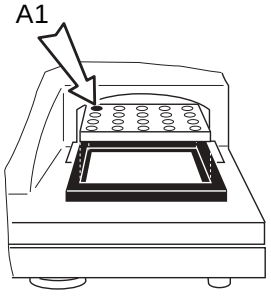


Figure 3-8 Seating the Plate in the Carrier

Running

1. Click the **Start** button to start the run. If you start a run before the lamp has been on for 10 minutes, you see a screen message asking if you want to start the run.

   Select **Yes** to continue or **No** to wait. If you select Yes, you may not receive optimal results since the lamp was not warmed up completely.

   If you entered a file name or opened a file, you see a screen message asking if you want to overwrite the data.

   Select **Yes** if the screen contains no data. Select **No** if you do not want to overwrite the data. Then select **New** from **File** and enter a new name using **Save As**….

   As the system scans each well, you can see the AFU value appear in the corresponding well at the bottom of the screen.

Accessing other applications

You can access other Microsoft® Windows® applications while performing a scan.

To access other applications:

1. Wait until the scan is between cycles.
2. Double-click the icon of the application.

   You can also hold down the **ALT** key and press **Tab** to toggle among open applications.

Stopping a run

You can stop the run at any time by pressing the **Escape** key on your keyboard. If you cancel the scan before it finishes, you see:

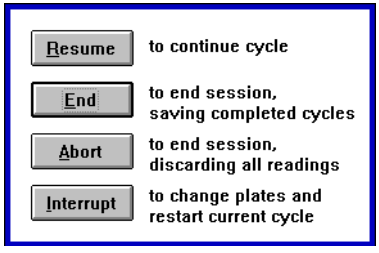


Figure 3-9 Cancel Run

Click a button to resume, end, abort, or interrupt the run. Once you make your selection, the system performs the selected task.

Run complete

When the run is complete, the system presents the plate and displays the data on the screen. For example, you may see data like this:

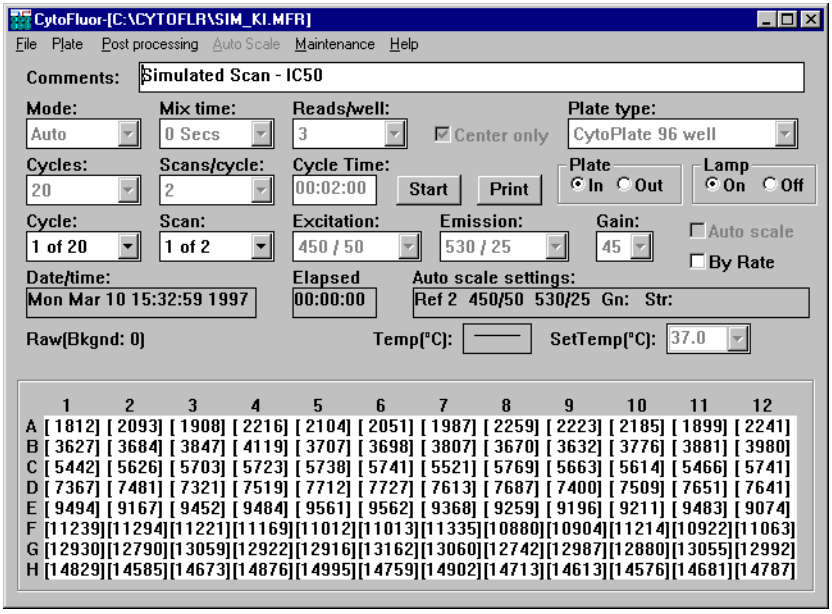


Figure 3-10 Main Screen with Data

***NOTE**: With Auto mode, the Total Time field switches to a countdown timer during a run to the start of the next cycle with multiple cycles. This only occurs if the Cycle Time is greater than the actual data acquisition time.*

Saving

Save the data through the Save As… command under File and enter a file name, unless you gave the file a name before you started the run. If you already named the file, the system automatically stored the data under that name as it performed the run.

Data loss protection

The data files from each cycle are automatically saved on the hard drive. If the power to your system fails, all data collected before the power failure is saved, and you can retrieve it when the power is restored. If you do not define a file name for your data files, they are saved with a .TMP extension.

Displaying, Printing, and Exporting Data

4

This chapter includes the following sections:

4.1 Overview of Post Processing

Introduction

CytoFluor has Post Processing features that enable you to format your data to suit your application. They include:

- **Scaling**—Allows you to use the average of assigned blank wells for background subtraction. (See Chapter 5, Setting the Plate Layout or Auto Scale Parameters, for details on how to make well assignments.)
- **Kinetics**—Allows you to set parameters to calculate and graph the initial rate data.

***NOTE**: Before you use Post Processing features, you should have an active file with data. See Section 4.1.1, Using the Scaling Screen, for details.*

4.1.1 Using the Scaling Screen

Overview

You use this screen to determine the data display format. You can also determine how you want the system to subtract blank values from data points. (You can designate blank wells in the Plate Layout screen; see Chapter 5, Setting the Plate Layout or Auto Scale Parameters, for details.) Once you make your selections, you can click on the Update button to view the screen data or select Cancel to cancel it. See Section 4.1.2, Using Scaling Features, for details on the Scaling screen.

Accessing the Scaling screen

To access the scaling screen:

1. Open a file as described in Chapter 3, Scanning Operations.

***NOTE**: The system displays the "Adopt setup from file?" prompt. Click on **Yes** or **No**; you do not have to adopt the setup of the file to continue with post processing.*

You then see the data and setup of the file you chose. The wells display the values obtained from the run. The Cycle and Scan boxes are active, and the Data format box displays the current post processing choice. The Date/time box displays the date and time the scan was started. The Elapsed box indicates the time between the start of the currently displayed scan and the start of the corresponding scan of cycle 1. If the system only acquired one cycle, the elapsed time would be 00:00:00.

2. Select a cycle from the **Cycle** pull-down list. The system applies the post processing parameters to the entire run.

3. Select **Scaling** from the Post processing menu option to display the CytoFluor Scaling screen:

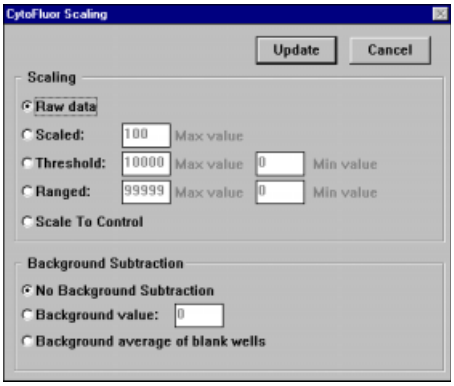


Figure 4-1 Scaling Screen

4. Select the options you want to apply to the entire run. (See Section 4.1.2, Using Scaling Features, for details on how to use the features in this screen.)

4.1.2 Using Scaling Features

The Scaling screen contains data display and background subtraction features. The data display features are:

- Raw data
- Scaled
- Threshold
- Ranged
- Scale To Control

The background subtract features are:

- No Background Subtraction
- Background value
- Background average of blank wells

See the following sections for details.

Raw Data

This feature enables you to see the raw data for each well. To display raw data:

1. Click on the **Raw data** button.

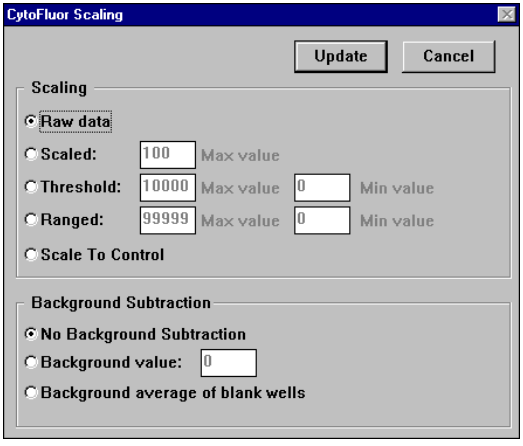


Figure 4-2 Selecting Raw Data

2. Click **Update** to return to the main setup screen with raw data. If you had one of the background features selected, they affect what you see on the screen. You may see a screen like this:

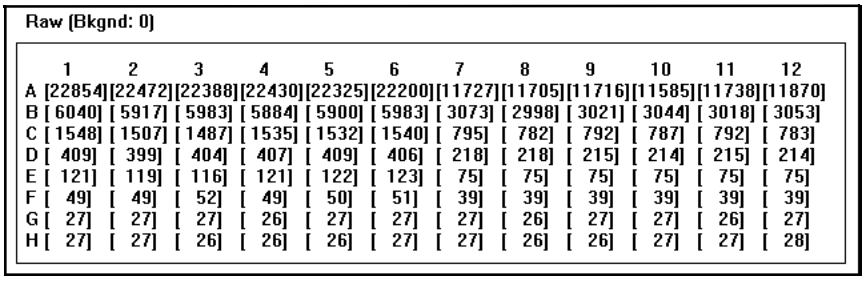


Figure 4-3 Raw Data

***NOTE**: The system always retains raw data, regardless of options you choose.*

Scaled

This feature enables you to display the data scaled to values from zero (0) to the Max (Maximum) value entered (up to 99999). The maximum data point that the system acquires is set equal to the Maximum data value you enter to determine the scaling factor. To display scaled data:

1. Click on the **Scaled** button.
2. Enter a value from zero to 99999. Then click **Update** to return to the CytoFluor main setup screen with a Scaled data display. For example, the raw data in Figure 4-3 is shown scaled to 100%.

Scaled 100 per cent: 1000 (Bkgnd: 0)

	1	2	3	4	5	6	7	8	9	10	11	12
A	999	983	979	981	976	971	513	512	512	506	513	519
B	264	258	261	257	258	261	134	131	132	133	132	133
C	67	65	65	67	67	67	34	34	34	34	34	34
D	17	17	17	17	17	17	9	9	9	9	9	9
E	5	5	5	5	5	5	3	3	3	3	3	3
F	2	2	2	2	2	2	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

Figure 4-4 Scaled Data

(See “Background Average of Blank Wells” on page 4-9 and “Using the Kinetics Rate Calculation Screen” on page 4-10 if you want the system to subtract values from the data also.)

Threshold

This feature enables you to display data above the maximum as plus symbols (+++), below the minimum as minus symbols (---), and between the two values as asterisk symbols (***). To display data in the threshold format:

1. Click on the **Threshold** button.
2. Edit the **Max value** and **Min value** settings, if necessary, by entering a value from zero (0) to 99999. Click **Update** to enter the value and return to the main setup screen. (The Min value should be less than the Max value. Otherwise, the system makes a “beep” sound and does not accept the value.) You see a screen like this:

Thresholded: 300 to 5000 (Bkgnd: 0)

	1	2	3	4	5	6	7	8	9	10	11	12
A	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B	+++	+++	+++	+++	+++	+++	***	***	***	***	***	***
C	***	***	***	***	***	***	***	***	***	***	***	***
D	***	***	***	***	***	***	—	—	—	—	—	—
E	—	—	—	—	—	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—	—	—	—	—
G	—	—	—	—	—	—	—	—	—	—	—	—
H	—	—	—	—	—	—	—	—	—	—	—	—

Figure 4-5 Threshold Feature Applied

(See “Background Average of Blank Wells” on page 4-9 and “Using the Kinetics Rate Calculation Screen” on page 4-10, if you want the system to subtract values from the data also.)

Ranged Use this feature to display data above the maximum as plus symbols (+++), below the minimum as minus symbols (---), and between the two values as integer values from 0 to 9. To do this:

1. Click on the **Ranged** button.
2. Edit the **Max value** and **Min value** settings, if necessary, by entering a value from zero (0) to 99999. Click **Update** to enter the value and return to the main setup screen with an updated data display. (The Min value should be less than the Max value. Otherwise, the system makes a “beep” sound and does not accept the value.) You see a screen like this:

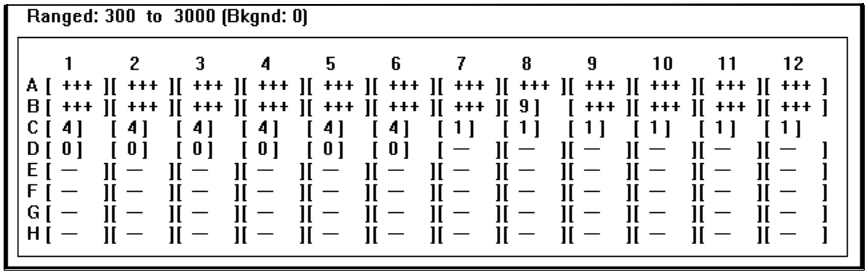


Figure 4-6 Ranged Data

(See “Background Average of Blank Wells” on page 4-9 and “Using the Kinetics Rate Calculation Screen” on page 4-10 if you want the system to subtract values from the data before using the Ranged feature.)

Scale to Control

Use this feature to scale the data to a percentage of an average of control wells. The control wells used in the scaling are as follows:

If you assign a control well as…	It is used to scale…
Control 00	All scans
Control 01	Scan 1
Control 02	Scan 2
Control 03	Scan 3
Control 04	Scan 4

No Background Subtraction

Use this feature to obtain data without any background subtraction.

Background Value

This feature enables you to enter a value that the system will subtract from each data point. To use it:

1. Click on the **Background Value** box.
2. Edit the **Background value** setting, if necessary, by entering a value from zero (0) to 99999. Then click **Update** to return to the main setup screen with the subtracted background value. For example a background of 26 is subtracted from the raw data:

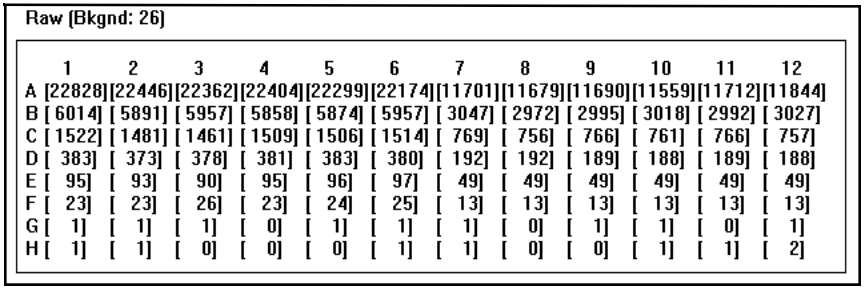


Figure 4-7 Background Value

***NOTE**: If the subtracted data is less than -9999, a minus sign is displayed as shown in Figure 4-8.*

Raw (Bkgnd: 12000)

	1	2	3	4	5	6	7	8	9	10	11	12
A	[11243]	[10535]	[10662]	[10556]	[10493]	[10388]	[-196]	[-218]	[-196]	[-174]	[-185]	[-63]
B	[-5915]	[-6011]	[-5961]	[-6062]	[-6067]	[-5972]	[-8922]	[-8984]	[-8953]	[-8945]	[-8962]	[-8927]
C	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]
D	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]
E	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]
F	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]
G	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]
H	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]	[—]

Figure 4-8 Subtracted Value Less Than -9999

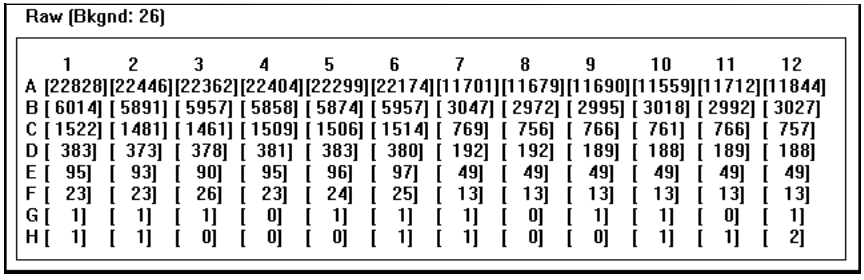
Background Average of Blank Wells

Use this feature to automatically calculate a value to subtract from each data point. (This value is based on the number of blank wells you assign in the Plate Layout screen with a label of “bk00.” See Chapter 5, Setting the Plate Layout or Auto Scale Parameters, for details.) To enable this feature:

1. Click on the **Background average of blank wells** box.

***NOTE**: If the plate layout has no wells designated as “bk00,” the system will subtract a value of zero (0).*

2. Click on **Update**. The system automatically subtracts the calculated average blank value and displays the results in the CytoFluor main setup screen. For example, a background average of 26 is subtracted from the raw data (Figure 4-9).


Raw (Bkgnd: 26)

	1	2	3	4	5	6	7	8	9	10	11	12
A	[22828]	[22446]	[22362]	[22404]	[22299]	[22174]	[11701]	[11679]	[11690]	[11559]	[11712]	[11844]
B	[6014]	[5891]	[5957]	[5858]	[5874]	[5957]	[3047]	[2972]	[2995]	[3018]	[2992]	[3027]
C	[1522]	[1481]	[1461]	[1509]	[1506]	[1514]	[769]	[756]	[766]	[761]	[766]	[757]
D	[383]	[373]	[378]	[381]	[383]	[380]	[192]	[192]	[189]	[188]	[189]	[188]
E	[95]	[93]	[90]	[95]	[96]	[97]	[49]	[49]	[49]	[49]	[49]	[49]
F	[23]	[23]	[26]	[23]	[24]	[25]	[13]	[13]	[13]	[13]	[13]	[13]
G	[1]	[1]	[1]	[0]	[1]	[1]	[1]	[0]	[1]	[1]	[0]	[1]
H	[1]	[1]	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[1]	[1]	[2]

Figure 4-9 Background Average of Blank Wells

4.1.3 Using the Kinetics Rate Calculation Screen

Overview

You use this screen to determine the initial rate data of a kinetics run. You do this by determining the maximum change in AFU values between limits you set in the Kinetics Post processing screen.

The CytoFluor software fits a line to the number of data points you set and determines the maximum slope.

Accessing the Kinetics Rate Calculation screen

To access the kinetics Rate Calculation screen:

1. Open a file as described in Chapter 3, Scanning Operations.

***NOTE**: The system displays the "Adopt setup from file?" prompt. Click on **Yes** or **No**; you do not have to adopt the setup of the file to continue with post processing.*

You then see the data and setup of the file you chose. The wells display the values obtained from the run. The Cycle and Scan boxes are active, and the Data format box displays the current post processing choice. The Date/time box displays the date and time the scan was started. The Elapsed box indicates the time between the start of the currently displayed scan and the start of the corresponding scan of cycle 1. If the system only acquired one cycle or if cycle 1 is selected, the elapsed time would be 00:00:00.

2. Click on the down arrow key in **Cycle**. Then select a cycle from the pull-down list (if you have more than one). Click on the down arrow key beside each box to change the data set display. (The system applies the post processing parameters to the entire run.)

3. Select **Kinetics** from the Post processing menu option to display the Kinetics Rate Calculation screen. The well representation area displays a plot of the AFU readings versus time for each well:

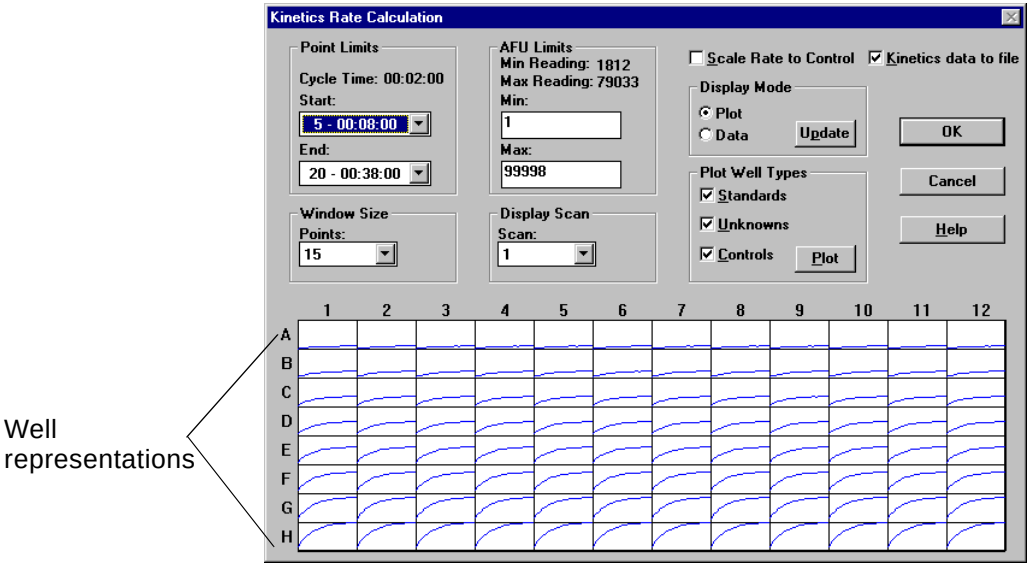


Figure 4-10 Kinetics Rate Calculation Screen

4. Select the options you want to apply to the entire run. (See Section 4.1.4, Using Kinetics Features, for details on how to use the features in this screen.)
5. Click **OK** when you are finished.

4.1.4 Using Kinetics Features

This section includes:

- Setting kinetics parameters
- Plotting kinetics data

4.1.4.1 Setting Kinetics Parameters

The Kinetics screen contains the parameters used to calculate the initial rate data and graphs of the scan data for each well. You can customize the graphs by setting the following parameters:

- Point Limits
- AFU Limits
- Window size
- Scan to Display
- Scale Rate to Control
- Display Mode

The kinetics parameters are described in the table below:

Parameter…	Description…
Point Limits	• **Start**—Sets the first data point used for the rate calculation. • **End**—Sets the last data point used for the rate calculation.
AFU Limits	• **Min**—Sets the minimum AFU value to use in rate calculation. • **Max**—Sets the maximum AFU value to use in rate calculation.
Window Size	Sets the number of data points (number of cycles) used to find the maximum slope of the AFU curve.
Display Scan	Sets the scan to display.
Scale Rate to Control	Scales the data to a percentage of the average of the control rate values.
Display Mode	• **Plot**—Displays graphs of the data. • **Data**—Displays the raw data.

Kinetics data to file

To save the kinetics rate data to a file for export or to use with CytoCalc, select the **Kinetics rate data to file** check box. The rate data is appended to the file.

4.1.4.2 Plotting Kinetics Data

The CytoFluor software allows you to view and print plots for the well data, standards, controls, and unknowns. You can customize the plots by changing the labels, axes, plot parameters, and legend.

Plotting well data

To view and print plots for each well:

1. Double-click the well on the Kinetics Rate Calculation screen (Figure 4-10 on page 4-11). The Expanded KineticsPlot screen appears with a graph of the data for the selected well.

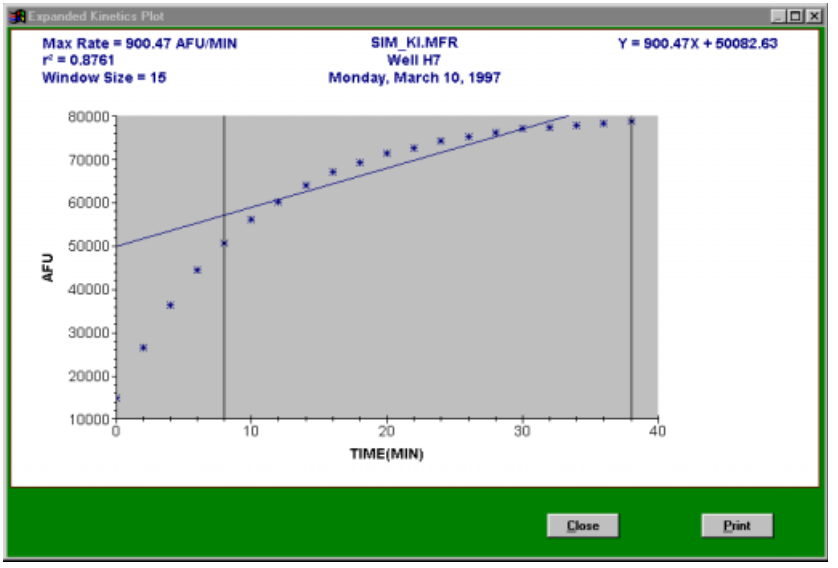


Figure 4-11 Expanded Well Plot

2. Customize the plot parameters if required. See “Customizing the plots” on page 4-18.
3. Click **Print** to print the graph.
4. Click **Close** to return to the Kinetics Rate Calculation screen.

Plotting standards

To view and print plots for standards:

1. On the Kinetics Rate Calculation screen (Figure 4-10 on page 4-11), select **Standards** and click the **Plot** button. The Expanded Kinetics Plot screen appears with a graph of the standards.

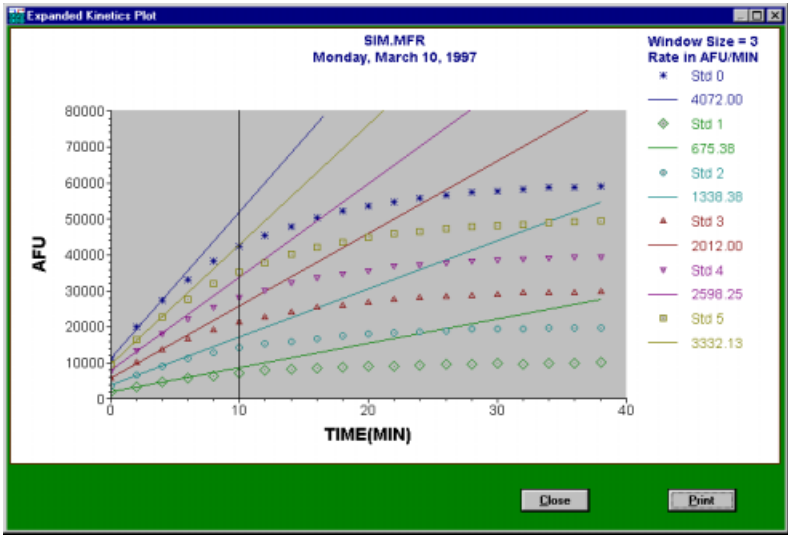


Figure 4-12 Standards Plot

2. Customize the plot parameters if required. See “Customizing the plots” on page 4-18.
3. Click **Print** to print the graph.
4. Click **Close** to return to the Kinetics Rate Calculation screen.

Plotting unknowns

To view and print plots of unknowns:

1. On the Kinetics Rate Calculation screen (Figure 4-10 on page 4-11), select **Unknowns** and click the **Plot** button. The Expanded Plot screen appears with a graph of the unknown.

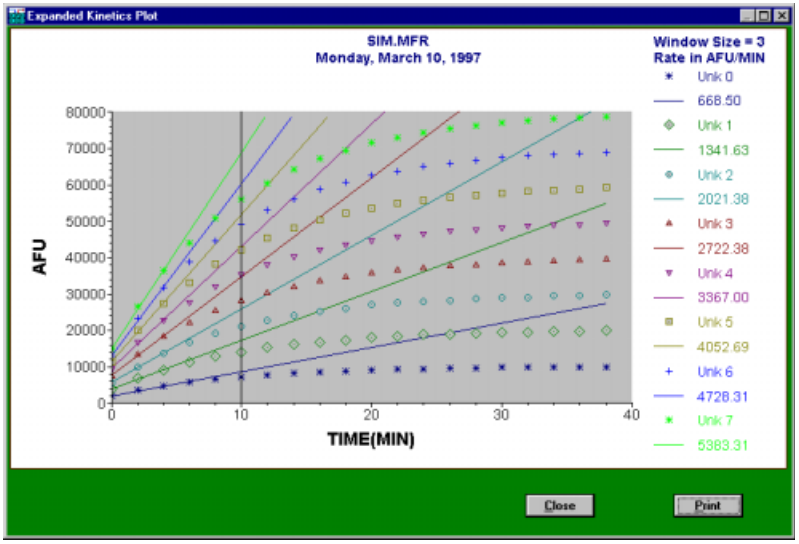


Figure 4-13 Unknowns Plot

2. Customize the plot parameters if required. See “Customizing the plots” on page 4-18.
3. Click **Print** to print the graph.
4. Click **Close** to return to the Kinetics Rate Calculation screen.

Plotting controls

To view and print plots of controls:

1. On the Kinetics Rate Calculation screen (Figure 4-10 on page 4-11), select **Controls** and click the **Plot** button. The Expanded Plot screen appears with a graph of the controls.

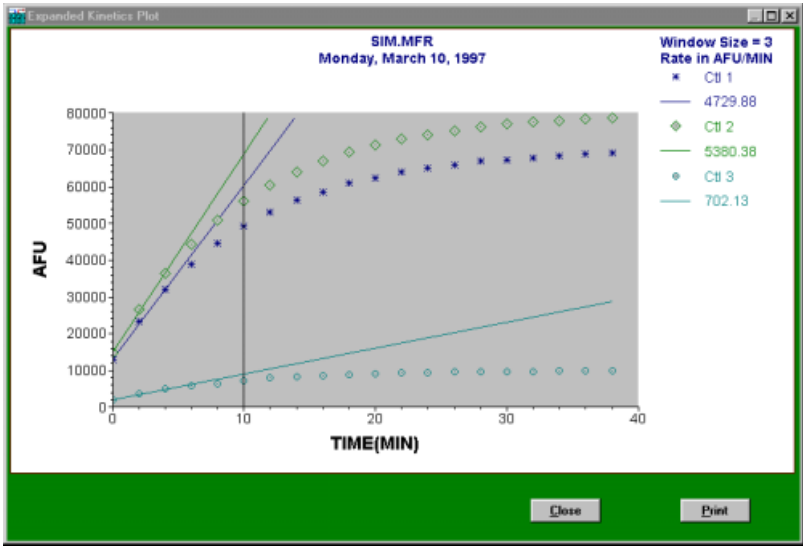


Figure 4-14 Controls Plot

2. Customize the plot parameters if required. See “Customizing the plots” on page 4-18.
3. Click **Print** to print the graph.
4. Click **Close** to return to the Kinetics Rate Calculation screen.

Customizing the plots

You can customize the plots by changing the labels, x- axis, y-axis, plot parameters, and legend.

Labels

To customize the axis labels:

1. On any of the previous plots screens, double-click on the axis to change. The Axis labels dialog box appears.

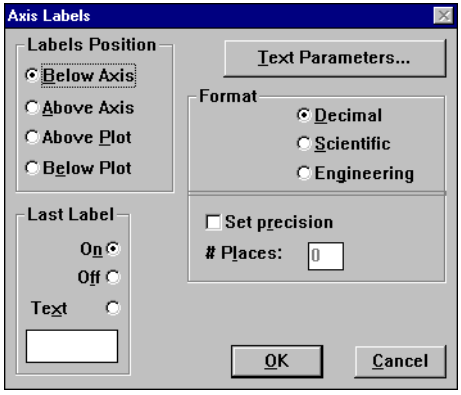


Figure 4-15 Axis Labels Dialog Box

2. Select the appropriate parameters.
3. To change the axis label text, click on **Text Parameters**. The Text Parameters dialog box appears.

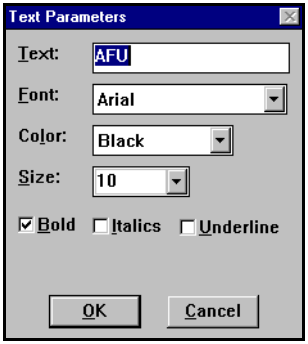


Figure 4-16 Text Parameters Dialog Box

4. Type the appropriate text and change the parameters as desired.
5. Click **OK** to save the changes and return to the Axis labels dialog box.
6. Click **OK**.

Axes To customize the axes:

1. On any of the previous plots screens, double-click on the axis to change. The Axis dialog box appears.

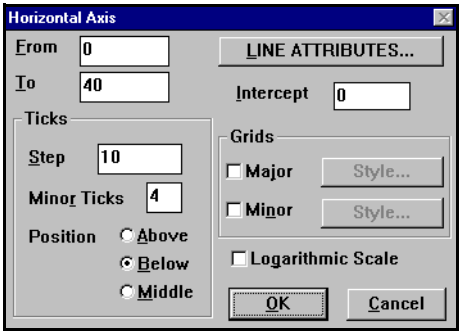


Figure 4-17 Axis Dialog Box

2. Change the parameters as desired.
3. Click **OK** to save the changes.

Plot parameters

To customize the plot parameters:

1. On any of the previous plots screens, double-click on the plot to change. The Plot Parameters dialog box appears.

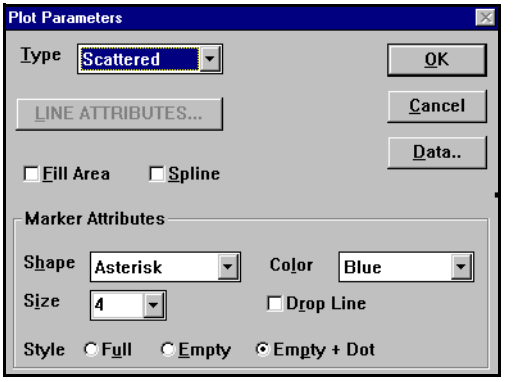


Figure 4-18 Plot Parameters Dialog Box

2. Change the parameters as desired.
3. Click **OK** to save the changes.

Legend

To customize the legend:

1. On any of the previous plots screens, double-click on the legend. The Legend Parameters dialog box appears.

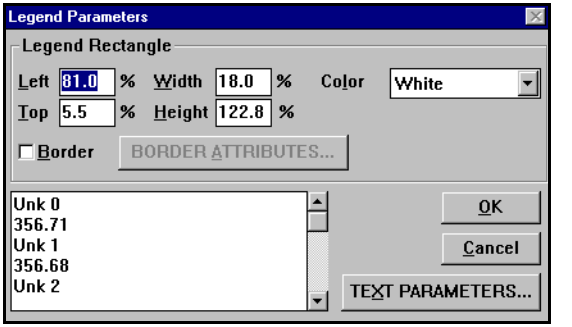


Figure 4-19 Legend Parameters

2. Type the appropriate text and change the parameters as desired.
3. Click **OK** to save the changes.

4.2 Saving Post Processing Selections

To save the post processing selections under the same file name:

Select **Save** under the File menu (ALT, F, S).

To save the selections under a different name:

Select **Save As...** under the File menu (ALT, F, A).

4.3 Printing Data

Printing an entire run

If you currently have data on your screen and you want to print the data of the entire run:

Click the **Print** button.

The data prints using the format selected in the Export/Print dialog box when Printer is selected.

Printing screen data

If you only want to print the data currently displayed on the screen instead of the entire run:

Hold the **Shift** key down while you click the **Print** button.

4.4 Exporting Files

CytoFluor includes an export feature that lets you export your data files to various software packages for further analysis. You can use the export formats provided or create you own.

To export files:

1. Select **Export/Print** from the File menu (ALT, F, E). You see a screen like this:

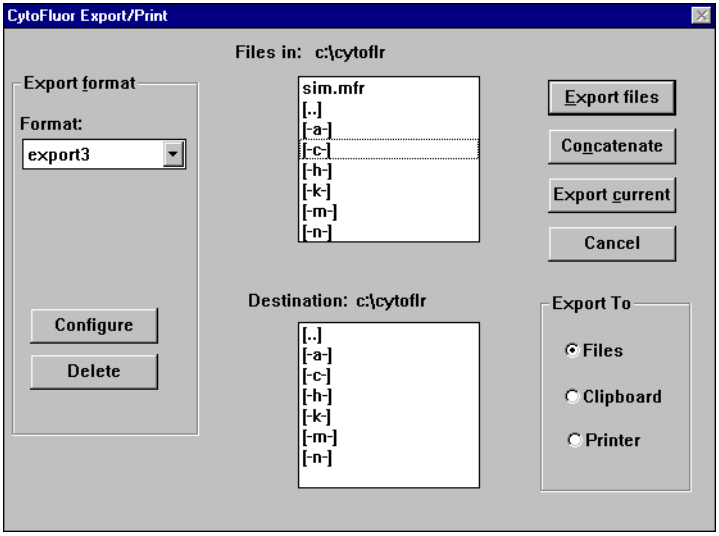


Figure 4-20 Export/Print Dialog Box

2. Select the format you want to export the file in by clicking one of the formats listed under **Export format**.

   The system will export the data with the post processing options active. For example, if you selected a background subtraction value through the Post Processing menu, all data points in the exported file will have the value subtracted.

Some software packages have special requirements as described here:

Software	Requirement
Grafit	Exports a text file containing group average and concentration data to be used with Grafit Kinetics Analysis Software. Grafit is an option offered with the CytoFluor scanner. Well unknowns or standards must be defined before using this format.
Excel	Exports a text file with the .XLS extension. Excel requires the tab delimiter between data fields. After you open it in Excel, save it as again to retain Excel functions and links.
Lotus 1-2-3	Exports a text file with the .WK1 extension.
CSV - Table and CSV - Linear	Exports a text file with the .CSV extension. • **Table**—Arranges data in a plate format (8 x 12 array for 96-well plates, etc.) • **Linear**—Arranges data in a columnar or row format

3. Change the **Files in** (input) and **Destination** (output) directories, if necessary. The input directory is where the system takes the input files from. The name of the input directory is located to the right of the Files in field. The output directory is where the system places the converted files. The output directory name is located to the right of the Destination field.

To change either directory, scroll through the directory list boxes under Files In or Destination. Double-click on a directory name in the box to see a list of subdirectories. Or, double-click on the two dots (••) to move upward in the directory. The system displays different drives by enclosing them in brackets. For example [-a-] usually designates the floppy drive a.

4. Select the file(s) you want to export from the list box under Files in. (You can only export data files.) When you click a name, the system highlights it. If you are exporting the currently opened file, select the file name or click on the Export current button.

***NOTE**: You can export multiple files individually or in a group. To export a group of files, select additional file names with the mouse.*
You can also group separate data files into one export file by clicking on the Concatenate button after you select the files to be grouped.

---

5. Select the appropriate **Export To** option:

If you want to export to...	Click...
the selected file destination	**Files**.
the clipboard so you can paste the file to its destination	**Clipboard**.
a printer to print out the file	**Printer**.

6. Click the **Export** button to export the file. Or click **Cancel** to close the box without converting files.
The system saves files in the export format you chose. The .MFR file remains unchanged. The exported file name consists of up to eight characters of the .MFR file name, with a new appended extension. For example, the export file containing the data from cycle 1 and scan 4 of file TEST.MFR would have the name TEST.014.

Concatenate

To combine several files into one for export:

1. Select the files to combine from the Files in list.
2. Click **Concatenate**.

   The selected files are exported into one file. The new file has the same name as the first file selected to export.

Export Current

To export the file displayed on the main setup screen, click **Export Current**.

4.5 Creating an Export Format

You can create up to 20 custom export formats. To create an export format:

1. Select **Export** from the File menu. The Export/Print dialog box appears.
2. Click **Configure**. The Export Setup dialog box appears.

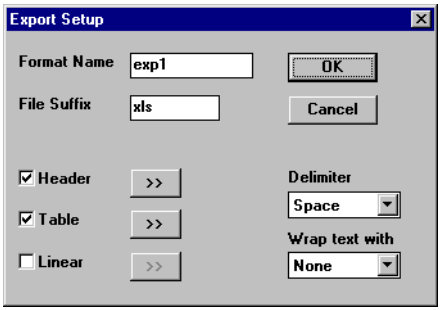


Figure 4-21 Export Setup Dialog Box

3. Type the name of the format to create in the Format Name text box. You can use any characters except commas.
4. Type the file extension in the File Suffix text box.
5. Select Tab or Comma to separate the data with in the Delimiter pull-down list.
6. If you want to enclose text with a symbol, select the symbol in the Wrap text with pull-down list.
7. To include header information or export the data in linear or table format, use the procedure in the following sections.
8. Click on **OK** to save the setup.

4.5.1 Including Header Information

To include header information with your custom exported data:

1. In the Export Setup dialog box, click the button to the right of **Header**. The Header dialog box appears (Figure 4-22).

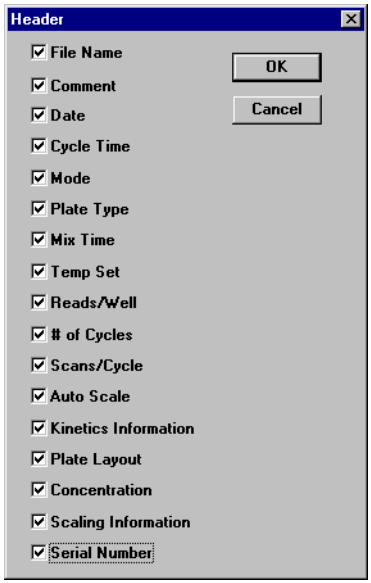
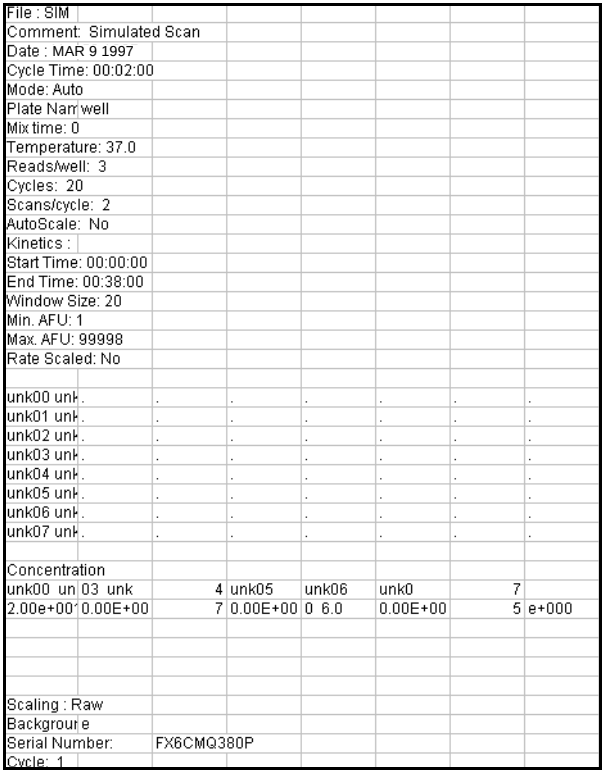


Figure 4-22 Header Dialog Box

2. Select the parameters to appear in the header. An example of a header with all parameters selected is shown in Figure 4-22.



File : SIM						
Comment: Simulated Scan						
Date : MAR 9 1997						
Cycle Time: 00:02:00						
Mode: Auto						
Plate Nam well						
Mix time: 0						
Temperature: 37.0						
Reads/well: 3						
Cycles: 20						
Scans/cycle: 2						
AutoScale: No						
Kinetics :						
Start Time: 00:00:00						
End Time: 00:38:00						
Window Size: 20						
Min. AFU: 1						
Max. AFU: 99998						
Rate Scaled: No						
unk00 unk	.	.	.	.	.	.
unk01 unk	.	.	.	.	.	.
unk02 unk	.	.	.	.	.	.
unk03 unk	.	.	.	.	.	.
unk04 unk	.	.	.	.	.	.
unk05 unk	.	.	.	.	.	.
unk06 unk	.	.	.	.	.	.
unk07 unk	.	.	.	.	.	.
Concentration						
unk00 un	03 unk	4	unk05	unk06	unk0	7
2.00e+00	0.00E+00	7	0.00E+00	0 6.0	0.00E+00	5 e+000
Scaling : Raw						
Backgrou e						
Serial Number:	FX6CMQ380P					
Cycle: 1						

Figure 4-23 Header Parameters

3. Click **OK** to save your changes.

4.5.2 Including Data in a Table Format

To include data formatted in a table that corresponds to the plate layout:

1. In the Export Setup dialog box, click **Table**. The Table Setup dialog box appears (Figure 4-24).

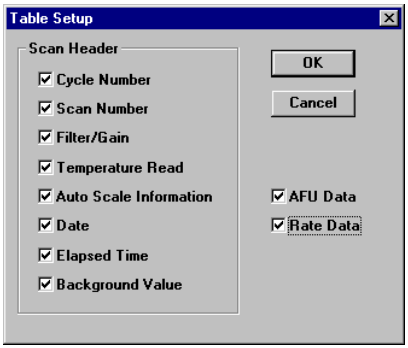


Figure 4-24 Table Setup Dialog Box

2. Select the parameters to include in the header.

   An example of data in table format with all parameters selected is shown in (Figure 4-25).

Cycle:	1								
Scan:	1								
Ex:	450/50								
Em:	530/25								
Gain:	45								
Temperat	OFF								
Auto Scal	Not used								
Date :	Mon Mar 10 15:32:59 1997								
Elapsed ti	0:00:00								
Raw(Bkgnd: 0)									
1812	2093	1908	2216	2104	2051	1987	2259	2223	2185
3627	3684	3847	4119	3707	3698	3807	3670	3632	3776
5442	5626	5703	5723	5738	5741	5521	5769	5663	5614
7367	7481	7321	7519	7712	7727	7613	7687	7400	7509
9494	9167	9452	9484	9561	9562	9368	9259	9196	9211
11239	11294	11221	11169	11012	11013	11335	10880	10904	11214
12930	12790	13059	12922	12916	13162	13060	12742	12987	12880
14829	14585	14673	14876	14995	14759	14902	14713	14613	14576

Figure 4-25 Table Data Parameters

3. Select the AFU Data check box to include raw data.
4. Select the Rate Data check box to include kinetics rate data.
5. Click **OK** to save your changes.

4.5.3 Including Data in a Linear Format

To export data in a linear format:

1. In the Export Setup dialog box, click the button to the right of **Linear**. The Linear Setup dialog box appears (Figure 4-26).

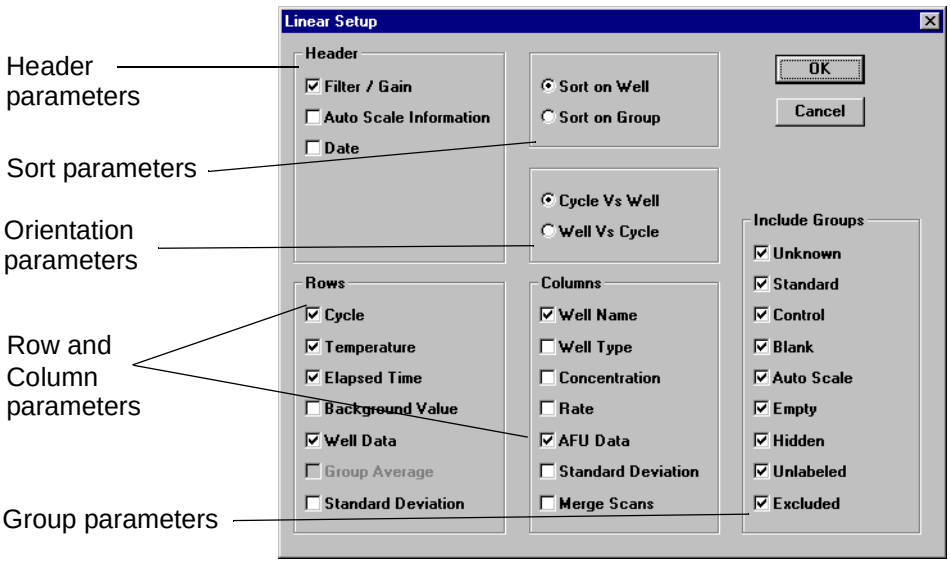


Figure 4-26 Linear Setup Dialog Box

2. Select the parameters to include in the header.

3. Select the sort parameters:

Parameter	Description
Sort on Well	Sorts data alphabetically by well name.
Sort on Group	Sorts data by group name in the order shown in the Include Groups section of the screen. When you select Sort on Group, Group Average becomes a row or column option.

4. Select the orientation parameters:

Parameter	Description
Cycle Vs Well	Lists the cycles horizontally and the well names vertically
Well Vs Cycle	Lists the well names horizontally and the cycles vertically

5. Select the parameters to appear in the rows from the Rows options.
6. Select the parameters to appear in the columns from the Columns options.
7. Select the groups to include from the Include Groups options.
8. Click **OK** to save your changes.

Setting the Plate Layout or Auto Scale Parameters

5

This chapter includes the following sections:

5.1 Overview of the Plate Layout Screen

The Plate Layout screen enables you to identify individual wells or groups of wells on the plate as blanks, unknowns, standards, controls, empty, hidden or excluded wells, as required by your assay.

***NOTE**: If your plate is not listed in the main setup screen's Plate type options, you can define its parameters in the Define Plate screen.*

The Plate Layout screen looks like this:

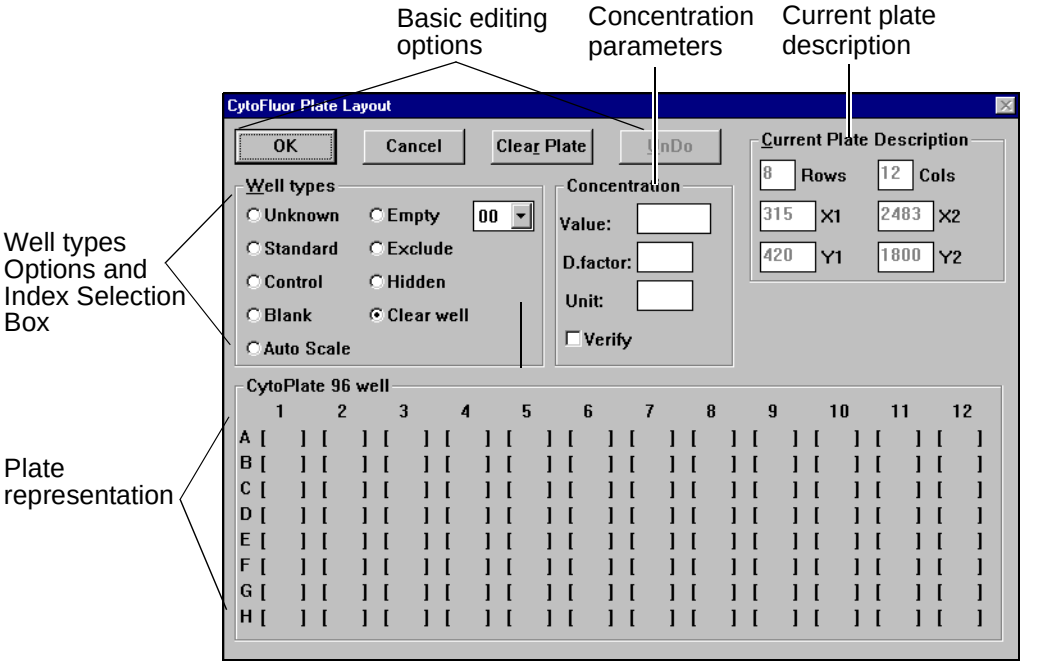


Figure 5-1 Plate Layout Screen

The Plate Layout screen includes the following features:

- **Well Types Options and Index Selection box**—Enable you to specify characteristics of particular wells and define replicates. The system may use the data of specific wells to perform calculations, for example, background subtraction. See Section 5.4, Using Well Types Options, for details.

  The index selection box (located to the right of the Well types) enables you to select an index value for replicate wells (00-99) of the same type. The index value enables you to identify replicates of the same material on the plate. (You do not need to enter an index value for wells defined as Empty, Hidden, or Excluded.)

- **Concentration parameters**—Enable you to enter concentration and dilution factors for each group of wells.

- **Current Plate Description**—Allows you to view the size and coordinates of the plate you are using.

- **Basic editing options**—Enable you to cancel your entries or confirm them. See “Basic Editing Guidelines” on page 5-6, for details.

- **Plate Representation** (rows A to H columns 1 to 12 and well blocks)—Allows you to select the wells you want to read. You can also view the results of your selections as you make them.

5.2 Accessing the Plate Layout Screen

You can access the Plate Layout screen to determine the layout of your plate before or after you scan it. But if you scan the plate first and then access the screen, you will not be able to access all the features. For example, you will not be able to access the Exclude well type button. (See Chapter 3, Scanning Operations, for details on how to scan a plate.)

To access the CytoFluor Plate Layout screen:

1. Activate **Windows**. Then double-click the **CytoFluor** icon. The CytoFluor main setup screen appears:

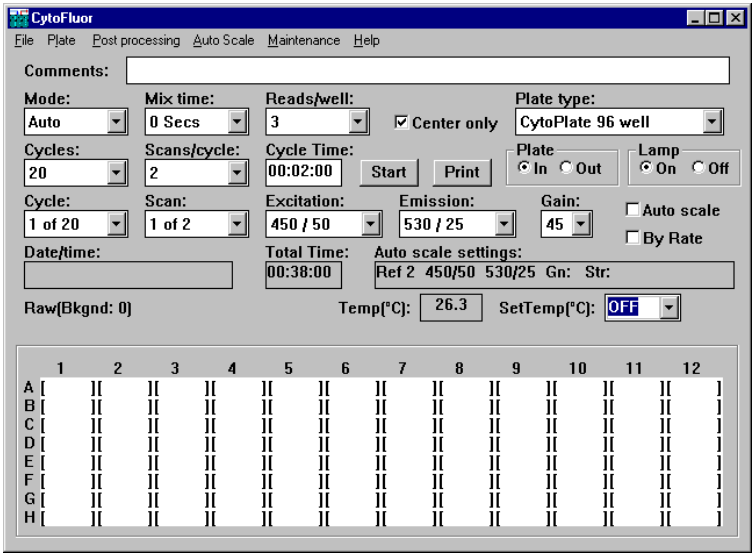


Figure 5-2 CytoFluor Main Setup Screen

***NOTE**: If you want to open a particular file to review or change the plate layout, see step 2. Otherwise skip to step 3.*

2. Choose **Open** from the **File** menu (ALT, F, O). Then select the file you want to open from the pull-down menu and click on **Open**. The data of the file you selected appears in the CytoFluor main setup screen.

3. Click on the **Plate layout** menu option to display the CytoFluor Plate Layout screen:

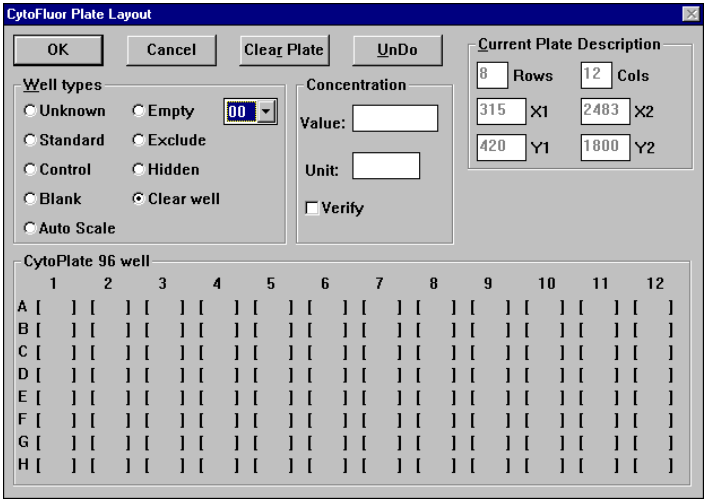


Figure 5-3 CytoFluor Plate Layout Screen

The first time you access this screen, you see the default plate layout. This layout has all unnamed wells. You can make changes to any plate layout, including the default layout or one of an already-saved file. If you accessed the plate layout of a pre-defined file, you see the last-saved layout of the plate.

4. Review the plate layout. To make layout changes, see the following sections. To exit the layout without saving changes, click the Cancel button.

5.3 Plate Layout Overview

Once you access the Plate Layout screen, you can make plate layout changes. This section defines the editing guidelines. It also contains basic steps to select wells and make assignments or changes. It does not contain details on the Well types or Define plate options. See Section 5.4, Using Well Types Options and Section 5.6, Defining Plates, for more information.

Basic Editing Guidelines

Before you begin making changes to the plate layout, you need to understand the basic editing options. The options consist of four buttons located across the top of the CytoFluor Plate Layout screen. See this chart for details:

Use this Button...	To do this...
OK	Confirm your changes before saving.
Cancel	Cancel your changes and return to the main setup screen.
Clear Plate	Remove all well assignments. You can use this to clear the layout before saving it, or to clear the setup of a previously saved file. ***NOTE**: If you view a file that used Well(s) as the scale reference in the Auto Scale menu, you cannot access this button. See Section 5.8, Setting Auto Scale Parameters, for details.*
Undo	Cancel your latest changes and return to the last assignment you made. You cannot access this button unless you made changes.

***NOTE**: To change the assignment value of any well, select the new Well types button first, then select the well.*

Follow the steps in this section for general well assignment steps:

1. Click the Well type button that corresponds to the sample type on the plate. (See Section 5.4, Using Well Types Options, if you need more information.)
2. Select the index value (00 to 99) from the index selection box (located to the right of the Well types box). The index value enables you to differentiate between different samples of the same type (standard, unknown, etc.) on the plate.

   ***NOTE**: You do not need to enter an index value for wells defined as Empty, Hidden, or Excluded.*

3. Select the well(s) that you want to assign to sample replicates. Click a single well to select it. For a group of wells, start with the pointer at the leftmost corner well and drag it until you highlight all the wells of the group. (Replicate wells do not have to be contiguous.)
4. Continue making as many changes as necessary. For details on the changes you can make, see the following sections.
5. Save your changes.

5.4 Using Well Types Options

The Well types options enable you to assign a specific sample type to a single well or groups of wells. The options are:

- Unknown
- Standard
- Control
- Blank
- Exclude
- Empty
- Hidden
- Clear well
- Auto Scale

***NOTE**: The CytoFluor software cannot generate a standard curve or calculate unknown concentrations. You must export the data to another application (for example, CytoCalc Data Analysis Software) for these calculations. See Chapter 4, Displaying, Printing, and Exporting Data, for details.*

The following sections describe the options in the order listed.

Unknown

To define a well or group of wells as unknowns:

1. Click on the **Unknown** button in the Plate Layout screen.
2. Select an index value from the pull-down menu (00 to 99).
3. Select the well(s) on your assay plate that corresponds to replicates for this unknown. (Click with your mouse, or click and drag to select a group of wells.) The system enters the abbreviation “un” and the index value into the wells you select.

For example:

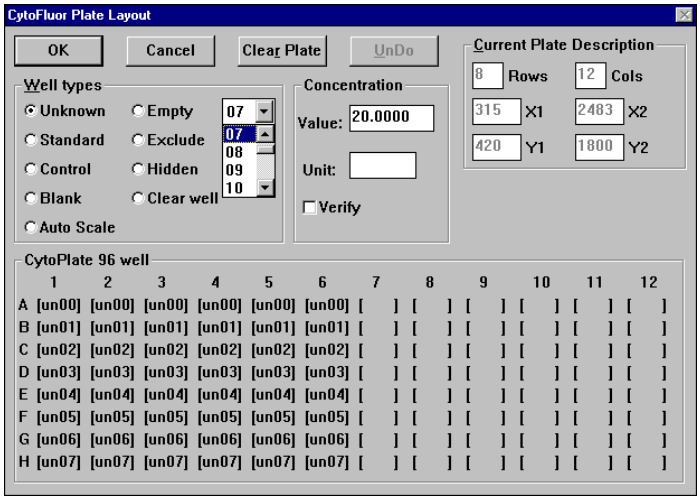


Figure 5-4 Defining Unknowns

> ***NOTE**: If you have more than one group of unknowns on a single plate, repeat step 2 and step 3, changing the index value as necessary. You may assign as many different unknown groups as your experiments require.*

4. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

Standard

You can use wells assigned as standards to obtain values for plotting standards curves in data analysis applications.

> ***NOTE**: If you use CytoCalc™ software for data analysis, you need a minimum of three points to plot a curve and should have at least two replicate wells for each point. The CytoCalc software accommodates up to three standard curves on a plate.*

To define wells as standards:

1. Click on the **Standard** button in the Plate Layout screen.
2. Select an index value from the pull-down menu (00 to 99).
3. Select the well(s) on your assay plate that corresponds to the location of your standard. (Click with your mouse, or click and drag to select a group of wells.) The system enters the abbreviation “sd” and the index value into the wells you select.
4. Repeat step 1 through step 3 and define the next group of wells for this standard curve. Continue to repeat the steps until you assign all the wells you need.

   ***NOTE**: If you have more than one standard curve on a single plate, repeat the steps in this section for each curve you want to add. Assign a different index value for each group of replicates. For example, “sd00,” “sd01,” and “sd02” may represent points for one standard curve, and “sd10,” “sd11,” “sd12,” and “sd13” may represent points for another.*

5. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

Control

A group of control wells provides you with a value you can compare against unknowns to identify samples that are out of the expected range, or to serve the auto scale features of CytoFluor.

***NOTE**: You should have three wells in a control group. You can have more than one control group on a plate.*

To define wells as controls:

1. Click on the **Control** button in the Plate Layout screen.
2. Select an index value from the pull-down menu (00 to 99).

3. Select the wells on your assay plate you want as a control group. (Click with your mouse, or click and drag to select a group of wells.) You do not have to place controls in contiguous wells.

   The system enters the abbreviation “cl” and the index value into the wells you select.

   ***NOTE**: If you want more than one control group on a single plate, repeat step 1 through step 3, changing the index value as required. You may assign as many different control groups as your experiment requires.*

4. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your change.

Blank

The system uses wells defined as blanks (bk00) to calculate the background subtraction value. (See “Background Average of Blank Wells” on page 4-9, for more information on background subtraction.)

To define wells as blank:

1. Click on the **Blank** button in the Plate Layout screen.
2. Select an index value from the pull-down menu (00 to 99).
3. Select the well(s) on your assay plate you want as blanks. (Click with your mouse, or click and drag to select a group of wells.) The system enters the abbreviation “bk" and the index value into the wells you select.

   ***NOTE**: If you have more than one group of blanks on a single plate, you need to repeat step 2 and step 3, changing the index value as necessary. The system only uses wells defined as “bk00” for background subtraction in the scaling screen. See Section 4.1.2, Using Scaling Features, for more information.*

4. Make additional changes to the plate layout as described in this chapter. Or click **OK** to save your changes.

Exclude

You can only access this option if you have not collected data (scanned the plate) yet. This option enables you to omit wells from the scanning operation. For example, if you only want to load samples in the last ten columns, you can assign the Exclude type to the wells in the remaining columns. When you perform a scan, the system will not scan the first two columns, increasing the system throughput by decreasing the reading time.

To exclude wells:

1. Click on the **Exclude** button in the Plate Layout screen.
2. Select the well(s) on your assay plate you want to exclude. (Click with your mouse, or click and drag to select a group of wells.) The system does not enter an "Exclude" abbreviation in the excluded wells. Instead, they appear as grayed (dimmed) wells. For example, columns 1 and 2 in the figure below are grayed:

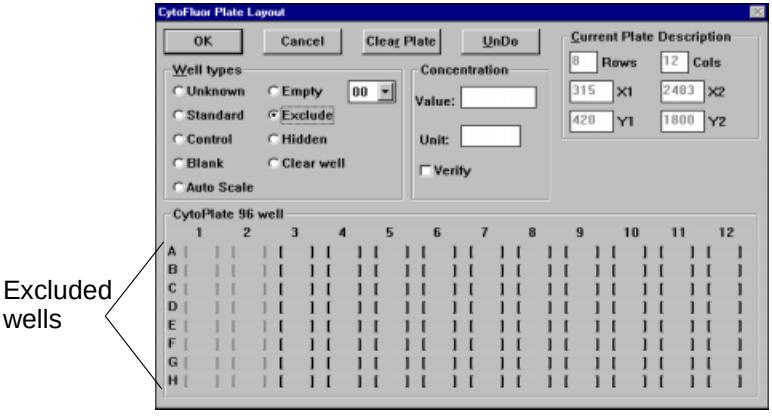


Figure 5-5 Excluded Wells

***NOTE**: If you have more than one group to exclude on a single plate, repeat step 1 and step 2.*

---

3. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

Empty

Use this option to define any wells on your assay plate that have been left unfilled or ones you do not want considered in the assay.

NOTE: *The system does not treat Empty wells the same way it treats Blank wells. It uses Blank wells to calculate a background value to subtract from all wells. The system collects scanned data of Empty wells, but does not use their values in calculations.*

To define wells as Empty:

1. Click on the **Empty** button in the Plate Layout screen.
2. Select the well(s) on your assay plate you want as empty. (Click with your mouse, or click and drag to select a group of wells.) The system enters the abbreviation “emp” into the wells you select.
3. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

Hidden

This well option enables you to omit outlying data from calculations. The data is not lost, but omitted from any calculations. For example, values that exceed the system limits (where +++) appears as the maximum value).

To define wells as Hidden:

1. Click on the **Hidden** button in the Plate Layout screen.
2. Select the well(s) on your assay plate you want as hidden. (Click with your mouse, or click and drag to select a group of wells.) The system enters the word “hide” in the wells you select.
3. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

Clear Well

Use the Clear Well option to change a named well to unnamed. To clear the well type:

1. Click on the **Clear Well** button in the Plate Layout screen.
2. Select the well(s) on your assay plate you want to clear. (Click with your mouse, or click and drag to select a group of wells.)
3. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

Auto Scale

Use the Auto Scale option to select the wells to use for autoscaling in Defined mode. See “Defined mode” on page 5-27 for more details.

***NOTE**: If you want to select Well(s) as your auto scale reference in the Auto Scale screen, you must define one to three wells as controls. There is no limitation as to where you place the Control wells. But, you can minimize cycle time if define them in the same row, near the top of the plate (for example, 1A).*

---

1. Click on the **Auto Scale** button in the Plate Layout screen.
2. Select the well(s) to be used for autoscaling. Well assignments for autoscaling are used as follows:

If you assign a well as…	It is used to auto scale…
Auto Scale 00	All scans
Auto Scale 01	Scan 1
Auto Scale 02	Scan 2
Auto Scale 03	Scan 3
Auto Scale 04	Scan 4

5.5 Entering Concentration Parameters

After you assign well types, you can use the Concentration section of the Plate Layout screen to enter concentration values and units for standards.

To enter concentration parameters:

1. Select a well in a group to which you want to assign concentration parameters.
2. For standards and controls, enter the concentration value in the Value text box and the units in the Unit text box (Figure 5-6).

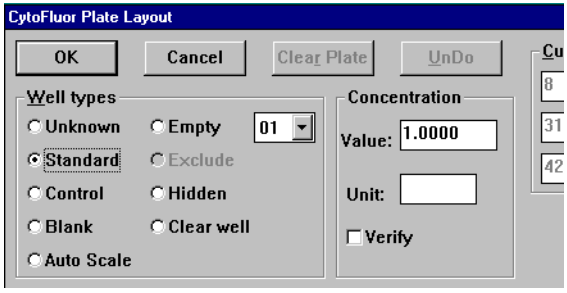


Figure 5-6 Concentration Parameters

3. Select **Verify** to display the parameters for selected wells in the concentration parameters text boxes.
4. Make additional changes to the plate layout as described in this chapter. Or click on **OK** to save your changes.

5.6 Defining Plates

Overview The CytoFluor software allows you to define a plate and add it to the Plate type menu by entering the coordinates of your plate in the Defined Plates screen.

This section describes:

- Preparing the scanner
- Defining a plate
- Deleting a plate

5.6.1 Preparing the Scanner

NOTE: *The probe must be in the bottom-read position when you define plates.*

To insert the plate in the scanner:

1. Click **Plate Out**.
2. Place the plate to define in the plate carrier.
3. Click **Plate In**.
4. Set the gain to zero on the main setup screen to disable the photomultiplier tube and prevent damage.
5. Open the scanner by turning the thumbwheel clockwise and lifting up the cover.

5.6.2 Defining a Plate

To define a plate type:

1. Click **Lamp On**, if necessary.
2. Set the Gain to zero to turn off the photomultiplier tube.
3. Select **Define Plate** from the Plate menu.

   The Defined Plates screen appears (Figure 5-7). The name and coordinates of the plate type used during the last scan are displayed.

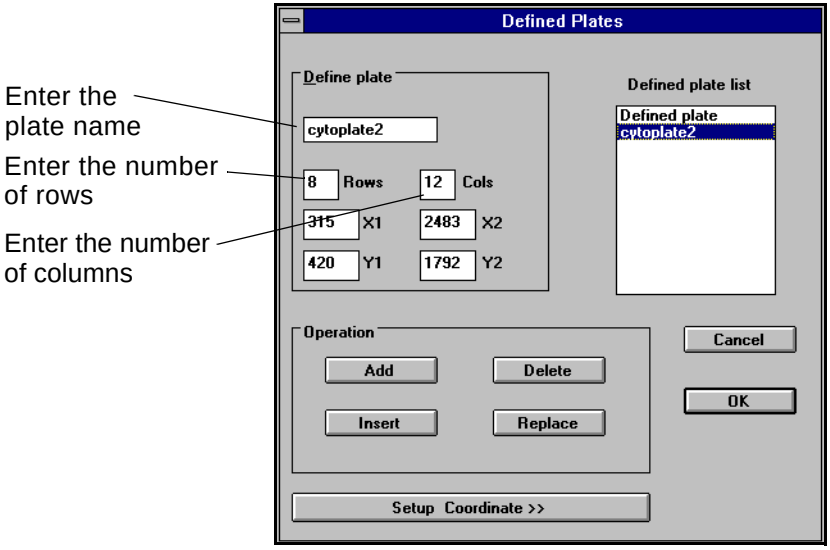


Figure 5-7 Defined Plates Screen

NOTE: *You can double-click on a plate name in the Defined Plate list to display the coordinates of that plate.*

4. Enter the plate name.

NOTE: *The software allows you to enter and save up to 80 characters, but only the number of letters that fit in the text box are displayed.*

5. Enter the number of rows on the plate in the **Rows** box.
6. Enter the number of columns on the plate in the **Cols** box.
7. Click **Setup Coordinates**.

   The Coordinate Setup screen appears (Figure 5-8).

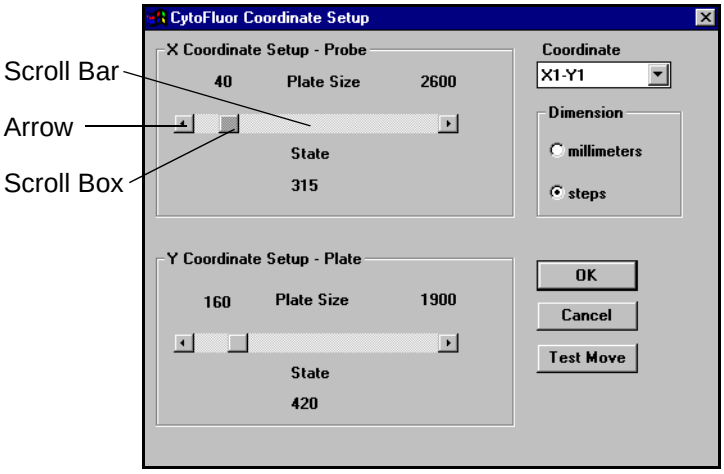


Figure 5-8 Coordinate Setup Screen

8. Select **X1-Y1** from the Coordinate list.

   The first well in the plate is positioned over the probe.

9. Verify that the well is centered over the probe.

NOTE: *If your scanner has a temperature control chamber, gently lift up the edges of the slit in the insulation on the temperature control chamber to see the well.*

If the well is not centered, center it by using the scroll bar:

- Drag the scroll box to move the plate in the direction you drag the scroll box.
- Click the bar to move the plate in increments of 20 units in the direction of the arrow.
- Click the arrow to move the plate in increments of one unit in the direction of the arrow.

10. Select **X2-Y2**.

    The last well in the plate is positioned over the probe.

11. Verify that the well is centered over the probe.

    If the well is not centered, center it by using the scroll bar as described in step 9.

12. Click **Test Move**. The probe resets to the home position and returns to the previous position.

13. Click **OK**.

    Click **Cancel** if you do not want to save the coordinates.

14. Click the appropriate button.

If you want to ...	Click...
add the plate name to the end of the Defined plate list	**Add**
add the plate name before the selected plate name in the Defined plate list	**Insert**
replace the selected name in the Defined plate list with the plate name	**Replace**

15. Click **OK**.

16. Make sure the fiber-optic cables are inside the unit. Close the scanner cover and seat it properly on the gasket. Turn the thumbwheel counterclockwise until finger-tight. Turn on the scanner.

CAUTION

Do not under-tighten or over-tighten the cover thumbwheel or the plate carrier cannot exit the scanner. To make sure you closed the cover correctly, click Plate Out. If the plate carrier does not move out, turn off the scanner and turn the thumbwheel further to tighten it.

5.6.3 Deleting a Plate

To delete a plate from the Defined plate list:

1. Select **Define Plate** from the Plate menu.
2. Select the plate to delete.
3. Click **Delete**.
4. Click **OK**.

5.7 Using 384 Well Plates

Overview

NOTE: *CytoFluor systems manufactured before April 1997 with serial numbers with the last three digits lower than 593 require a hardware modification to read an entire 384 well plate. Contact Applied Biosystems Technical Support for details.*

To use 384 well plates you must:

- Define the 384 well plate
- Select the read pattern option

You can set the scanning pattern of 384 well plates by using the 384 Scan Pattern options on the Plate Layout screen.

5.7.1 Defining the 384 Well Plate

Before you scan a 384 well plate, you must define it using the Define Plates screen. See Section 5.6.2, Defining a Plate, for detailed instructions. See Table 5-1 for the coordinates of standard 384 well plates.

Table 5-1 384 Well Plate Coordinates

Plate	Coordinates					
	Rows	Columns	X1	X2	Y1	Y2
Nunc	16	24	265	2534	366	1843
Costar	16	24	269	2532	364	1839

5.7.2 Selecting the Read Pattern Option

Overview The CytoFluor scanner reads up to 96 wells per cycle. When you use 384 well plates, the scanner reads the plate in four quadrants of 96 wells as shown in Figure 5-9. It reads one quadrant per scan according to the 384 Read Pattern option you select.

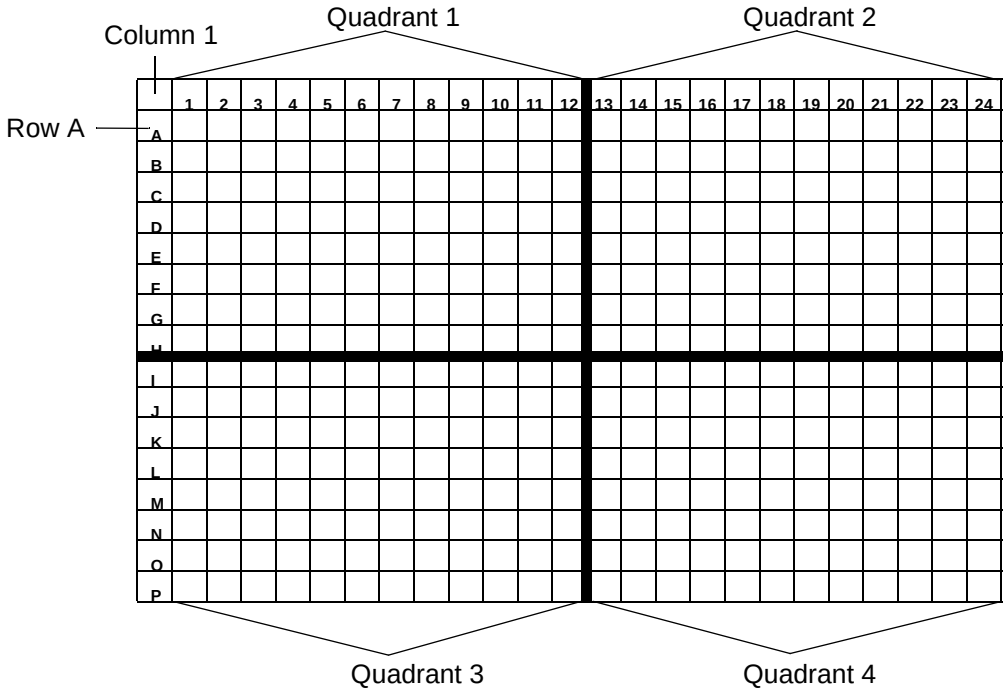


Figure 5-9 384 Well Plate

When you select a 384 Read Pattern option the CytoFluor software scans the plate according to the pattern described in Table 5-2.

Table 5-2 Read Patterns

Read Pattern Option	Scan 1	Scan 2	Scan 3	Scan 4
Quadrants (with >8 rows and >12 columns)	Upper left 8 row X 12 column quadrant	Upper right 8 row X 12 column quadrant	Lower left 8 row X 12 column quadrant	Lower right 8 row X 12 column quadrant
Quadrants (with >8 rows and <12 columns)	Upper left 8 row quadrant	Remaining wells	When 4 scans selected, repeats scan 1	When 4 scans selected, repeats scan 2
Quadrants (with ≤ 8 rows and >12 columns)	Upper left 12 row quadrant	Remaining wells	Repeat scan 1	Repeat scan 2
Skip X	Skips every other well in row starting at row A, column 1	Skips every other well in row starting at row I, column 1	Skips every other well in row starting at row A, column 2	Skips every other well in row starting at row I, column 2
Skip Y	Skips every other well in column starting at row A, column 1	Skips every other well in column starting at row A, column 13	Skips every other well in row starting at row B, column 2	Skips every other well in row starting at row B, column 13
Skip XY	Skips every other row and column starting at row A, column 1	Skips every other row and column starting at row A, column 2	Skips every other row and column starting at row B, column 1	Skips every other row and column starting at row B, column 2

Selecting the scanning pattern

To select the scanning pattern for a 384 well plate:

1. On the main setup screen, select the 384 well plate you are using from the Plate type pull-down menu.
2. Select **Layout** from the Plate menu on the main setup screen. The CytoFluor Plate Layout screen appears with the 384 Read Pattern options available (Figure 5-10).

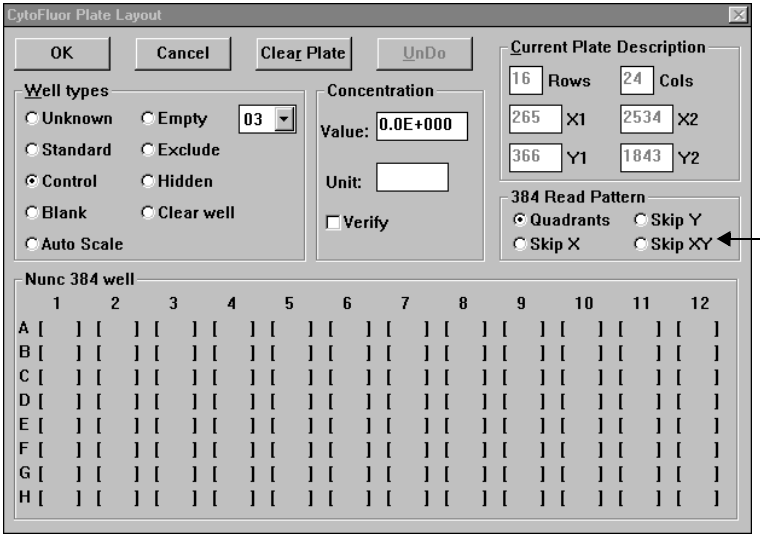


Figure 5-10 384 Read Pattern Options

3. Select the appropriate Read Pattern option described in Table 5-2 on page 5-23.

NOTE: *The 384 Read Pattern options appear only if a 384 well plate is selected on the main setup screen.*

---

4. Click **OK**.

NOTE: *You can save the scanning pattern as a template to use for future scans. See "Saving as a template" on page 3-21 for details.*

5.8 Setting Auto Scale Parameters

Overview The CytoFluor Auto Scale screen allows you to select how you normalize your data using the following modes:

- **Default mode**—Scales data according to the filter pair you set and the internal reference and gain the scanner automatically sets.
- **Defined mode**—Scales data according to the filter pair settings, reference, and gain you set. The defined mode allows you to set up to four different combinations of Auto Scale parameters for each one of four scans.
- **Auto Scale off**—Collects data without auto scaling. AutoScale off is the default setting.

NOTE: *For the system to apply the settings, you must select the Auto Scale field in the CytoFluor main setup screen before you start a run. See “Auto Scale” on page 3-13.*

Accessing the Auto Scale screen

To access the Auto Scale screen:

1. Select **Auto scale** from the main setup screen.

   The CytoFluor Auto Scale screen appears (Figure 5-11).

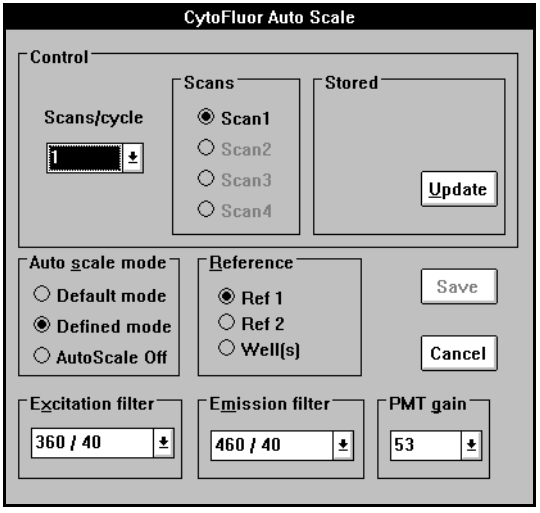


Figure 5-11 CytoFluor Auto Scale Screen

Default mode

To scan using the default mode:

1. Select the number of scans from the **Scans/cycle** pull-down menu.
2. Click the appropriate scan number button.
3. Click **Default Mode**.
4. Click **Update**.

   The reference value is collected and stored.

5. Click **Save** to save your Auto Scale information and return to the main setup screen.

Defined mode

If you are using replicate wells as references, you must define them as Auto Scale wells on the Plate Layout screen before setting the Auto Scale parameters. See “Auto Scale” on page 5-14 for more details.

To scan using the Defined mode:

1. Select the number of scans from the **Scans/cycle** pull-down menu.
2. Click the appropriate scan number button.
3. Click **Defined Mode**.
4. Click the reference to use.
5. Select the filter pair settings.

   NOTE: *You must select a different setting for each filter.*

6. Select the PMT gain.
7. Click **Update**.

   The reference value is collected and stored.

8. Repeat step 1 through step 7 for each scan.
9. Click **Save** to save your Auto Scale information and return to the main setup screen.

AutoScale Off mode

If you select Auto Scale on the main setup screen, all scans are auto scaled.

To turn off Auto Scale for specific scans:

1. Click **AutoScale Off**.
2. Select the number of scans from the **Scans/cycle** pull-down menu.
3. Click the appropriate scan number button.
4. Click **Save** to save your Auto Scale information and return to the main setup screen.

5.9 Exiting the CytoFluor System

To exit the CytoFluor system software:

If you already saved a file or did not make any changes, you can return to the Windows desktop by:

Selecting **Exit** from the File menu (ALT, F, E).

If you made changes to a file or acquired data without saving it, the software asks if you want to save.

Click the appropriate button to save changes and exit, ignore them and exit, or cancel the exit process.

Customizing the CytoFluor System

6

This chapter includes the following sections:

***NOTE**: You can define a new plate type if the plate type you are using is not in the CytoFluor plate library. See Chapter 5, Setting the Plate Layout or Auto Scale Parameters, if you need information on defining plate coordinates.*

6.1 Replacing Optical Filters

If you want to add new filters or replace existing ones with filters of different wavelengths, you need to:

- Change the excitation or emission filters (or both) in the filter wheels inside the CytoFluor scanner.
- Change the filter settings in the Maintenance screen.

This section describes how to perform these tasks.

CAUTION

If you do not correctly install the filters in the filter wheels and change the corresponding settings in the software, you could permanently damage the CytoFluor system and void your warranty. Each filter position must contain a filter or a blocking plug.

6.1.1 Changing the Excitation and Emission Filters

This procedure describes how to change the excitation and emission filters. The diagrams show the excitation filter assembly. Follow the same steps when changing either type of filter. Figure 6-1 shows the position of the excitation and emission filter assemblies inside the scanner.

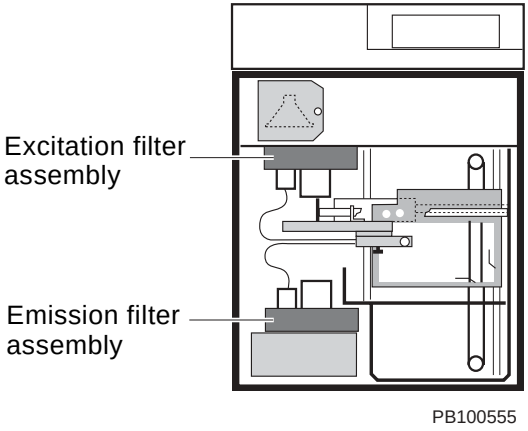


Figure 6-1 Filter Assemblies

1. Make sure the plate transport carrier is inside the scanner. If it is not inside, click on the **Plate In** button.
2. On the main setup screen, set the Gain to 0 and turn the lamp off by clicking the **Lamp Off** button. (Leave the power on so you can use the Maintenance options.)
3. Open the cover by turning the thumbwheel counterclockwise and lifting up the cover.

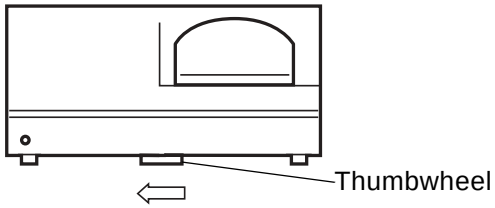


Figure 6-2 Opening the Scanner

4. Remove the two screws that secure the top cover of the filter wheel assembly. Then remove the cover.

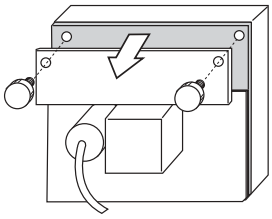


Figure 6-3 Removing Filter Wheel Assembly Cover

5. Select the filter position you want to load from the Maintenance screen. Click on the down arrow next to the **Excitation Filter** box to display a pull-down menu when changing the excitation filter. Then highlight the position number for loading. Click on the **Position** button to the left of the **Excitation Filter** field. For the emission filter, click **Position** button.

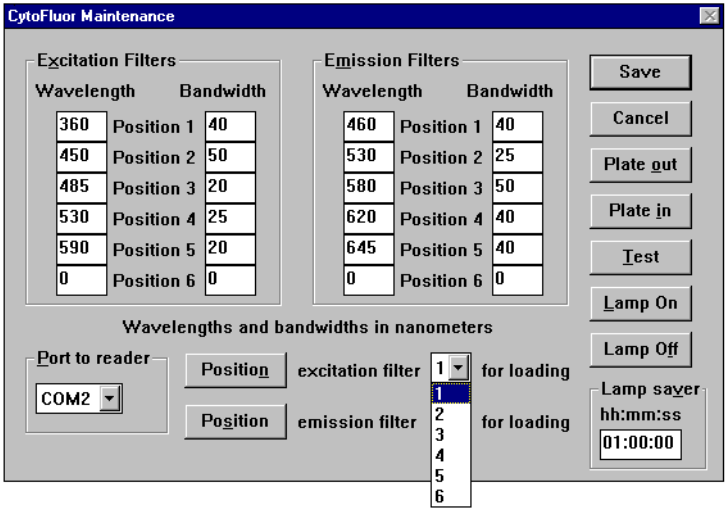


Figure 6-4 Selecting the Filter

The scanner filter wheel finds the home position, then presents the selected location at the top of the filter assembly.

6. Fold down the gasket in the filter assembly to gain access to the filter (Figure 6-5). Then locate the filter installation tool provided with the Accessories kit.

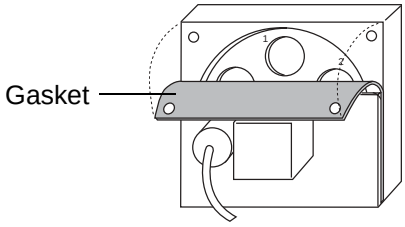


Figure 6-5 Accessing the Filter

7. Place the prongs of the tool into the holes on the top of the filter wheel or blank you need to change. Turn the tool to the left until you can lift the filter from the wheel.

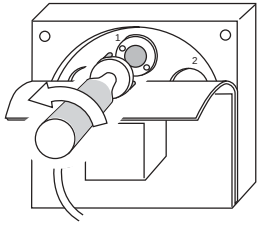


Figure 6-6 Removing the Filter

CAUTION

Do not apply excessive pressure while turning the filter; you could damage the filter wheel. And do not touch the glass surfaces of any filter or the instrument's optical components, or you could damage them.

8. Pick up the new filter with the tool and insert it into the wheel. Gently turn it to the right with the tool and your fingertips, without touching the filter surface. Continue doing this until the filter is firmly set in place.

***NOTE**: Do not leave space between the flange of the filter and the face of the filter wheel. The filters have fine threads; do not cross-thread during installation. (If necessary, remove and reseat the filter.)*

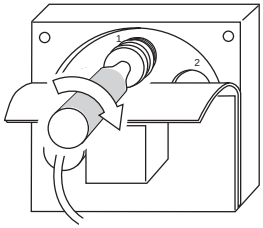


Figure 6-7 Replacing Filters

9. Make a note of the filter position (1, 2, 3, and so on) marked on the filter wheel. Also note the center wavelength and bandwidth. (This information is printed on the outside of each filter.) For example, if the filter is marked 485/20X, 485 is the center wavelength and 20 is the bandwidth at one-half maximum transmission (BWHM) of the excitation (X) filter. (An “M” instead of an “X” denotes an emission filter.)

CAUTION

Make sure the filter is installed in the position you selected in the Maintenance screen. An overlap of filters could damage the CytoFluor system and void your warranty.

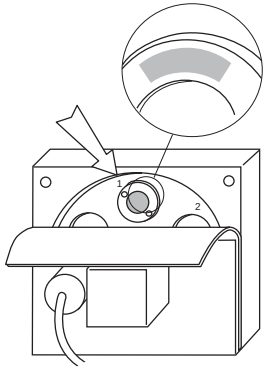


Figure 6-8 Checking Filter Position

10. Replace the cover on the filter wheels and tighten the screws. Do not overtighten.

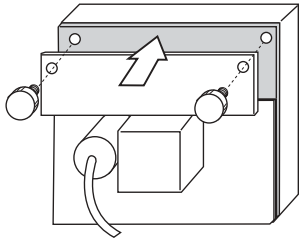


Figure 6-9 Replacing the Filter Wheel Assembly Cover

11. Close the scanner cover, push it all the way down to seat it, and turn the thumbwheel clockwise until finger-tight. Then turn on the scanner.

CAUTION

If you do not seat the cover, the plate carrier cannot exit the scanner. To make sure you shut the cover correctly, click on the ***Plate Out*** *button from the Maintenance screen.*

Update the filter setting in the Maintenance screen as described in the next section.

Storing filters Store unused filters in a cool, dry location in sealed plastic bags.

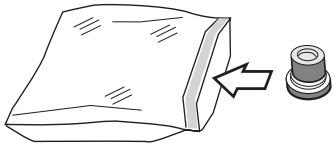


Figure 6-10 Storing Unused Filters

6.1.2 Changing the Filter Settings in the Maintenance Screen

This section describes how to change the excitation and emission filter settings stored in the CytoFluor software system.

CAUTION

After you install new filters in the scanner, you must change the filter settings in the Maintenance screen so they match the values of the installed filters. If you do not set the values correctly, your data will be invalid. You could also damage the system, voiding your warranty.

To change the filter settings through the software:

1. In the Maintenance screen, drag the cursor across the appropriate **Position 1** through **Position 6** box under **Excitation Filters** or **Emission Filters** to select them.

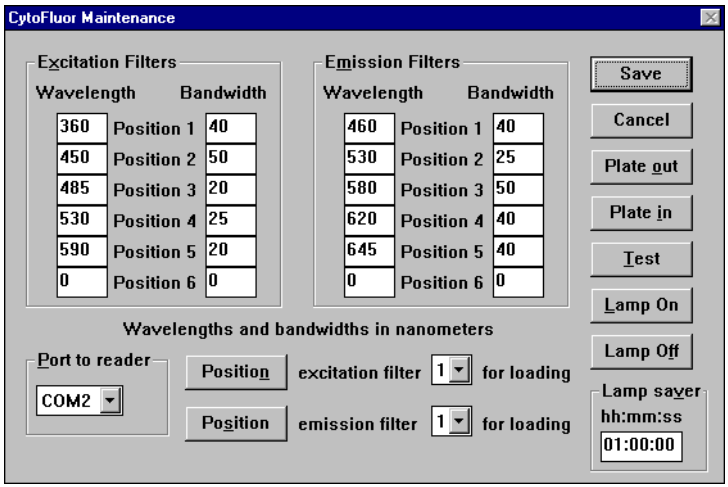


Figure 6-11 Maintenance Screen

2. Type in the **Wavelength** and **Bandwidth** values that correspond to the values of the excitation and emission filters you just installed.

3. Click the **Save** button once you enter the correct new values. The system returns to the main setup screen.

***NOTE**: Click on the Cancel button for the system to disregard your changes and return to the previous settings.*

6.2 Testing the Scanner

When the scanner is not acquiring data, you can check system settings and test proper system operation using the Maintenance screen options.

To test the scanner:

1. Select Maintenance from the CytoFluor main setup screen. You see a screen like this:

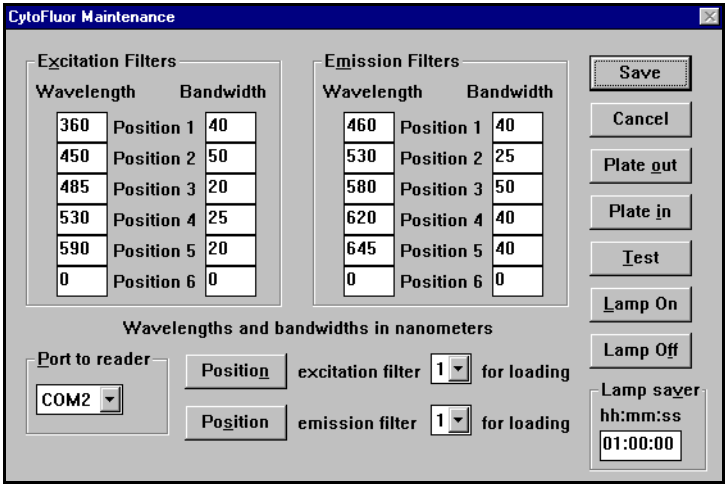


Figure 6-12 Maintenance Screen

2. Click the **Test** button on the right side of the Maintenance screen. The CytoFluor software then automatically checks the scanner drives and lamp. The test takes several seconds.

   When complete, the Unit Test dialog box appears.

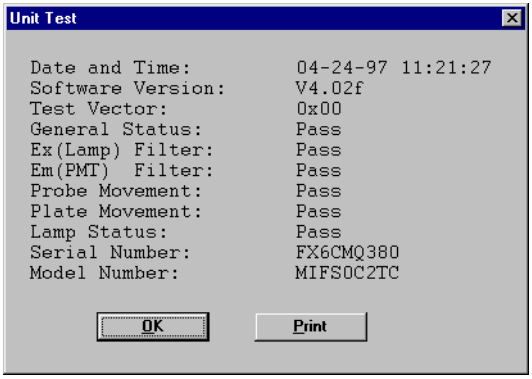


Figure 6-13 Unit Test Dialog Box

If all systems pass, the scanner is ready for use. If the Lamp Status fails, then replace the lamp. See Section 7.4, Replacing the Lamp, for detailed instructions. If any other system fails, contact Applied Biosystems Technical Support. See Appendix E, Technical Support and Training, for the phone and fax numbers.

3. Click **Print** to print a CytoFluor Fluorescence Unit Test Report.

4. Click **OK** to close the box.

6.3 Checking or Changing the Communication Port

To check or change the computer communication port:

1. Display the Maintenance screen.

   The default port setting of the scanner connection is Com1. If your system is connected to a different port, you see the port name in the Port to reader box. Continue to step 2 to change the port setting. Otherwise, select the Cancel button to return to the main setup screen without saving any changes.

2. Click on the down arrow in the Port to reader box to reveal the pull-down menu. Then highlight the port connection you want to select it.

3. Click on the **Save** button to save any changes. If you select an invalid Port to reader setting, you see:

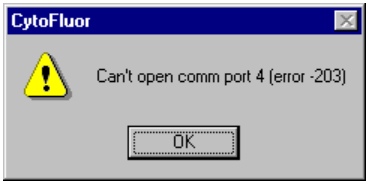


Figure 6-14 Invalid Port Setting

4. Click **OK**, then repeat step 2 to make another selection. Once you save the new setting, the system activates it.

6.4 Setting the Lamp Saver Time

The lamp saver feature enables you to extend the life of the lamp. For example, you can set the lamp to turn off when there is no scanner activity.

The default setting is 60 minutes. If a new run is not started within 60 minutes after the completion of a run, the system turns the lamp off and sets the photomultiplier tube gain to zero (0). Otherwise, the lamp stays on with the photomultiplier tube gain at 30 (approximately 300 volts).

***NOTE**: You can extend the life of the lamp by leaving it on for extended periods of time (for example, three to four hours) instead of turning it on and off throughout the day.*

To set the lamp saver time:

1. Display the Maintenance screen. The default setting or last-entered setting for the lamp saver appear in the Lamp saver box.

   To change the setting, continue to step 2. Otherwise, click on the Cancel button to return to the main setup screen without making changes.

2. Enter the hour, minutes, or seconds in the **Lamp saver** fields. (Use two digits for each value, separated by a colon [:].) You can select all three fields simultaneously.

   ***NOTE**: Click the Lamp On or Lamp Off buttons for immediate lamp access.*

3. Click on the **Save** button to save the changes and return to the main setup screen. (To cancel your selection, click on **Cancel**.)

***NOTE**: The CytoFluor software must be running for the Lamp Saver to operate.*

6.5 Configuring the Scanner for Top or Bottom Plate Reading

Changing probe position

You can configure your CytoFluor scanner to read plates from the top or bottom, depending on your application needs. To do this, you need to move the probe by using the procedure in Section 6.5.1, Changing the Probe Position.

Checking probe height

After you move the probe, you must check the probe height position and adjust it if necessary. If you have a CytoFluor Series 4000/TR use the procedures in Section 6.5.2, Checking and Adjusting Probe Height in a Temperature-Ready Scanner. If you have a CytoFluor Series 4000/TC use the procedures in Section 6.5.3, Checking and Adjusting Probe Height in a Temperature Control Scanner.

6.5.1 Changing the Probe Position

The default configuration for the system is the bottom-read position. The illustrations in the following procedure show changing the probe from the top-read position to the bottom-read position.

Configuring

To change the probe position:

1. Make sure the plate transport carrier is inside the scanner. If it is not inside, select the **Maintenance** menu. Then click on the **Plate In** button.
2. Turn off the scanner. Open the scanner cover by turning the thumb wheel clockwise and lifting up the cover.
3. Slide the probe arm gently to an area where you can access the probe. When the probe is in the top-read position, the upper fiber-optic cable goes to the emission module and the lower fiber-optic cable goes to the excitation module.

NOTE: *In scanners with a temperature control chamber, place the probe in the slit on top of the temperature control chamber.*

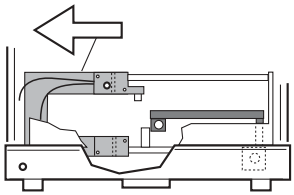


Figure 6-15 Moving the Probe Arm (Probe in Top-Read Position)

4. Remove the knurled screw holding the probe to the mounting block.

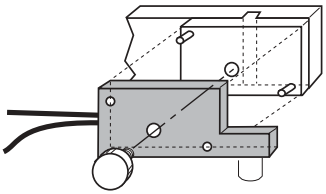


Figure 6-16 Removing the Probe Screw

CAUTION

Pull the probe away from the mounting block to clear the mounting pins.

CAUTION

Do not touch the probe tip or severely twist the fiber-optic cables going to the excitation or emission filter modules. If you do, you could damage the probe or filter connection.

5. Turn the probe head 180 degrees and slide it over the mounting pins on the opposite mounting block. Replace the knurled screw. Do not over-tighten.

6. Check and adjust probe height as needed. Refer to the appropriate section:

If you have a ...	See ...
CytoFlour Series 4000/TR (temperature-ready)	• Section 6.5.2.1, Checking and Adjusting Probe Height in Bottom-Read Position (TR Models) Or • Section 6.5.2.2, Checking and Adjusting Probe Height in Top-Read Position (TR Models)
CytoFlour Series 4000/TC (temperature-control)	• Section 6.5.3.1, Checking and Adjusting Probe Height in Bottom-Read Position (TC Models) Or • Section 6.5.3.2, Checking and Adjusting Probe Height in Top-Read Position (TC Models)

6.5.2 Checking and Adjusting Probe Height in a Temperature-Ready Scanner

Use the following procedures to check and adjust probe height in scanners without a temperature control chamber.

6.5.2.1 Checking and Adjusting Probe Height in Bottom-Read Position (TR Models)

Checking probe height in bottom-read

In the bottom-read position, the probe tip should be located 0.015 inch below the plate carrier. You should rarely have to reset the bottom-read probe height, because the depth of different plates does not vary widely.

CAUTION

The probe must be set at the correct height. If you set the probe too high, the plate carrier can jam against it. If you set it too low, you lower the signal.

1. Slide the plate carrier back to the A1 position, until the probe is underneath the edge of the plate carrier.

   If the probe hits the plate carrier, skip the remaining steps and proceed to “Adjusting probe height in bottom-read” below.

2. Hold the probe height adjustment spacer marked 0.015-inch across the bottom corner of the plate carrier.

3. Gently slide the spacer between the probe and the bottom of the plate carrier.

   The probe tip should just clear the height adjustment spacer. If the probe hits the spacer, lower the probe. If the probe tip is not almost touching the spacer, raise the probe. See “Adjusting probe height in bottom-read” below.

When properly adjusted, the probe tip is 0.4 mm (the thickness of the 0.015-inch height adjustment spacer) away from the probe.

Adjusting probe height in bottom-read

1. Loosen the two screws on the rear of the probe mounting block. Do not remove them. Leave the probe connected to the mounting bracket.
2. Slide the probe all the way down.
3. Slide the 0.015-inch spacer against the bottom of the plate carrier and move the carrier over the probe head.
4. Bring the probe head up to just touch the 0.015-inch spacer. Do not place excess force on the plate carrier, or you could damage it.
5. Tighten the two screws on the rear of the probe mounting block. Gently remove the 0.015-inch spacer.
6. Slide the plate carrier to the back of the instrument.
7. Make sure the fiber-optic cables are inside the unit.

CAUTION

The fiber-optic cables can be damaged if they are caught between the cover and the base of the scanner.

8. Close the scanner cover and seat it properly on the gasket. Turn the thumbwheel counterclockwise until finger tight. Turn on the scanner.

CAUTION

Do not over-tighten or under-tighten the cover thumbwheel or the plate carrier cannot exit the scanner. To make sure you closed the cover correctly, click Plate Out. If the plate carrier does not move out, turn off the scanner and turn the thumbwheel further to tighten it.

6.5.2.2 Checking and Adjusting Probe Height in Top-Read Position (TR Models)

Checking probe height in top-read

In the top-read position, the probe tip should be located 0.5 mm above the plate to maximize the signal. You can adjust the probe height for the top-read position to accommodate different plate heights.

CAUTION

The probe must be set at the correct height. If you set the probe too low, the plate can jam against it. If you set it too high, you lower the signal.

1. Insert an empty plate into the carrier.
2. Place the probe height adjustment spacer marked 0.020-inch on top of the plate. Make sure the spacer is on the highest point of the plate, usually across the corner of the plate.
3. Slide the probe arm gently over the plate and check the probe height.

   The probe tip should just clear the height adjustment spacer. If the probe hits the spacer, raise the probe. If the probe tip is not almost touching the spacer, lower the probe.

   When properly adjusted, the probe tip is 0.5 mm (the thickness of the 0.020-inch height adjustment spacer) away from the probe.

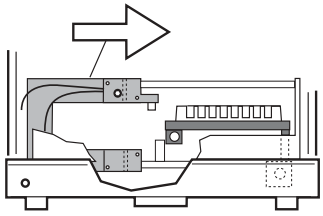


Figure 6-17 Checking Probe Height

Adjusting probe height in top-read

1. Loosen the two screws on the rear of the probe mounting block. Do not remove them. Leave the probe connected to the mounting bracket.
2. Slide the probe up or down to the correct height.

CAUTION

Bring the probe head down to just touch the 0.020-inch spacer. Do not place excess force on the plate carrier, or you could damage it.

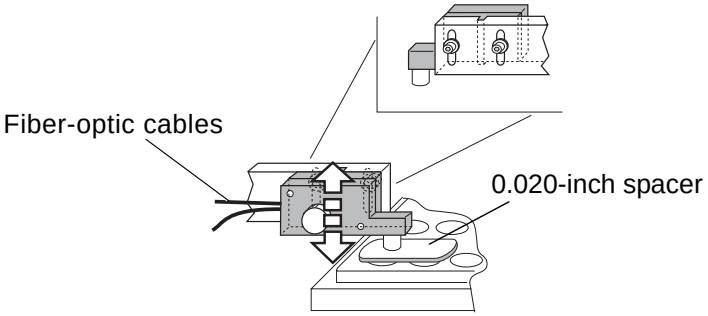


Figure 6-18 Adjusting Probe Height

3. Tighten the two screws on the rear of the probe mounting block. Gently remove the 0.020-inch spacer.
4. Slide the plate carrier to the back of the scanner.
5. Make sure the fiber-optic cables are inside the scanner.

CAUTION

The fiber-optic cables can be damaged if they are caught between the cover and the base of the scanner.

6. Close the scanner cover and seat it properly on the gasket. Turn the thumbwheel counterclockwise until finger tight. Turn on the scanner.

CAUTION

If you under-tighten or over-tighten the cover thumbwheel, the plate carrier cannot exit the scanner. To make sure you shut the cover correctly, click on the ***Plate Out*** *button from the Maintenance screen.*

6.5.3 Checking and Adjusting Probe Height in a Temperature Control Scanner

Use the following procedures to configure a scanner with a temperature control chamber.

6.5.3.1 Checking and Adjusting Probe Height in Bottom-Read Position (TC Models)

Checking probe height in bottom-read

In the bottom-read position, the probe tip should be located 0.4 mm (the thickness of the 0.015-inch height adjustment spacer) below the plate carrier. You should rarely have to reset the bottom-read probe height, because the depth of different plates does not vary widely. If you need to adjust the probe height, call Applied Biosystems Technical Support for assistance.

CAUTION

The probe must be set at the correct height. If you set the probe too high, the plate carrier can jam against it. If you set it too low, you lower the signal.

1. Turn off the scanner. Open the scanner cover by turning the thumbwheel clockwise and lifting up the cover.

2. Insert an empty plate into the carrier and push the carrier into the temperature control chamber.

3. Slide the probe until it is underneath the edge of the plate carrier. If the probe hits the plate carrier, skip the remaining steps and call Applied Biosystems Technical Support.

4. Push the temperature control chamber door open and insert the 12-inch long probe height adjustment spacer marked 0.015-inch through the oven door and hold it across the bottom corner of the plate carrier.

   The probe tip should just clear the height adjustment spacer. If the probe hits the spacer, or is not almost touching the spacer, call Applied Biosystems Technical Support.

   When properly adjusted, the probe tip is 0.4 mm (the thickness of the 0.015-inch height adjustment spacer) away from the probe.

5. Make sure the fiber-optic cables are inside the unit. Close the scanner cover and seat it properly on the gasket. Turn the thumbwheel counterclockwise until finger-tight. Turn on the scanner.

CAUTION

Do not over-tighten or under-tighten the cover thumbwheel or the plate carrier cannot exit the scanner. To make sure you closed the cover correctly, click Plate Out. If the plate carrier does not move out, turn off the scanner and turn the thumbwheel further to tighten it.

6.5.3.2 Checking and Adjusting Probe Height in Top-Read Position (TC Models)

Checking probe height in top-read

In the top-read position, the probe tip should be located 0.5 mm (the thickness of the 0.020-inch height adjustment spacer) above the plate to maximize the signal. You can adjust the probe height for the top-read position to accommodate different plate heights.

CAUTION

The probe must be set at the correct height. If you set the probe too low, the plate can jam against it. If you set it too high, you lower the signal.

1. Turn off the scanner. Open the scanner cover by turning the thumbwheel clockwise and lifting up the cover.
2. Insert an empty plate into the carrier and push the plate into the temperature control chamber.
3. Push the temperature control chamber door open. Insert the 12-inch long probe height adjustment spacer marked 0.020-inch into the chamber and place it on top of the plate. Make sure the spacer is on the highest point of the plate, usually across the corner of the plate.
4. Slide the probe arm gently over the plate and check the probe height by looking through the door to view the plate.

   The probe tip should just clear the height adjustment spacer. If the probe hits the spacer, raise the probe. If the probe tip is not almost touching the spacer, lower the probe.

   When properly adjusted, the probe tip is 0.5 mm (0.020-inch) away from the probe.

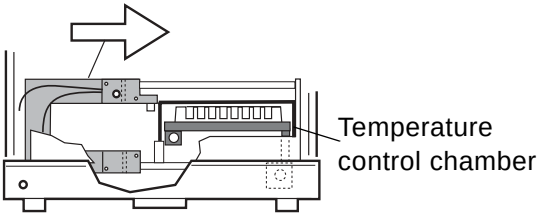


Figure 6-19 Checking Probe Height, Cutaway View

Adjusting probe height in top-read

To adjust the probe height in the top-read position:

1. Loosen the two screws on the rear of the probe mounting block. Do not remove them. Leave the probe connected to the mounting bracket.
2. Slide the probe up or down to the correct height.

CAUTION

Bring the probe head down to just touch the 0.020-inch spacer. Do not place excess force on the plate carrier, or you could damage it.

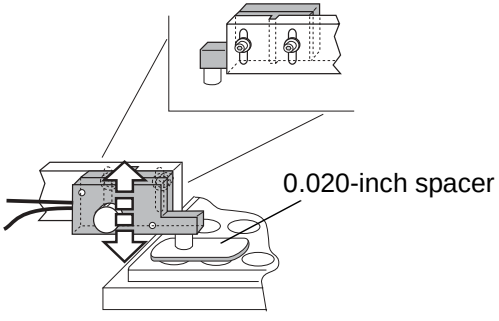


Figure 6-20 Adjusting Probe Height

3. Tighten the two screws on the rear of the probe mounting block. Gently remove the 0.020-inch spacer.

4. Make sure the fiber-optic cables are inside the unit. Close the scanner cover and seat it properly on the gasket. Turn the thumbwheel counterclockwise until finger-tight. Turn on the scanner.

CAUTION

Do not over-tighten or under-tighten the cover thumbwheel or the plate carrier cannot exit the scanner. To make sure you closed the cover correctly, click Plate Out. If the plate carrier does not move out, turn off the scanner and turn the thumbwheel further to tighten it.

Maintaining the CytoFluor System

7

This chapter includes the following sections:

7.1 Cleaning the Scanner

To keep your CytoFluor system clean:

Inspect the outside of the scanner after every use and wipe any spills with a damp (not wet) cloth and a mild detergent.

7.2 Cleaning the Rails

When to clean

Open the scanner and inspect the rails for residue buildup monthly. Clean the rails with ethanol or isopropanol if needed.

CAUTION

Do not use acetone to clean the rails. Acetone could damage the inside of the scanner.

Cleaning

To clean the rails:

1. Turn off the CytoFluor scanner.
2. Open the scanner cover by turning the thumbwheel counterclockwise and lifting up the cover.
3. Clean the rails shown in Figure 7-1 with ethanol or isopropanol.

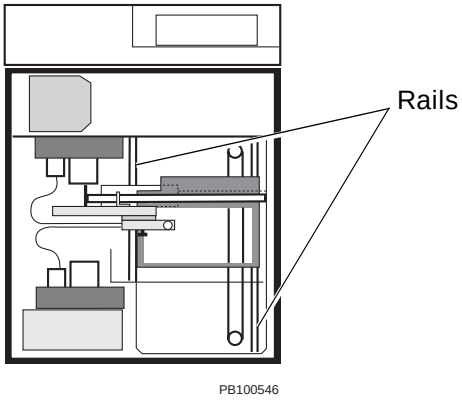


Figure 7-1 Rails

4. Close the scanner cover and seat it properly on the gasket. Turn the thumbwheel counterclockwise until finger tight. Turn on the scanner.

CAUTION

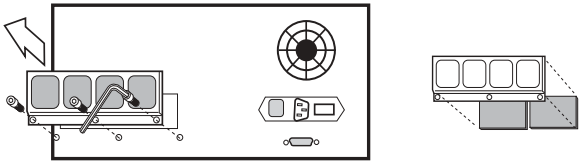
Do not over-tighten or under-tighten the cover thumbwheel or the plate carrier cannot exit the scanner. To make sure you closed the cover correctly, click Plate Out. If the plate carrier does not move out, turn off the scanner and turn the thumbwheel further to tighten it.

7.3 Replacing the Air Filter

When to replace Replace the air filter at least every six months. If your installation site is dusty, you may need to change this filter more frequently.

Replacing

1. Turn off the CytoFluor scanner. Then remove the three screws that secure the air filter protector on the rear panel. Use the Allen (hex) wrench provided with the Accessories kit.



PB100543

Figure 7-2 Removing the Air Filter

2. Remove and discard the used filter.
3. Locate the replacement filter included with the kit. The filter is in two 31 mm x 82 mm pieces; or one 31 mm x 164 mm piece. Insert the new filter and replace the air filter protector. Tighten the screws with the Allen (hex) wrench, but do not overtighten.

CAUTION

Do not operate the scanner without an air filter or you may reduce the performance quality.

7.4 Replacing the Lamp

This section describes how to replace a faulty lamp with a new one. (See Chapter 8, Accessing Technical Information, if you need to order one.)

1. Make sure the plate carrier is inside the scanner. Press the **Plate In** button from the Maintenance screen, if necessary.
2. Turn off the CytoFluor scanner and disconnect the power cord. Open the scanner cover by turning the thumbwheel counterclockwise, then lifting up the cover.
3. Locate the lamp cover. Remove the retaining screw with a Phillips-head screwdriver (not included). Then lift off the cover.

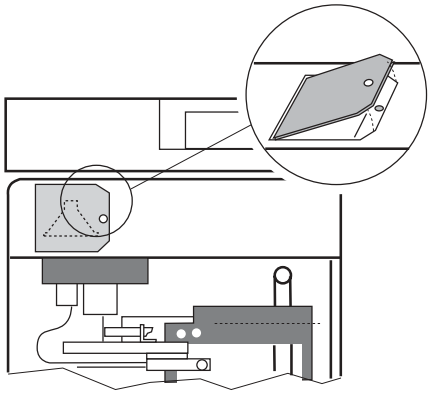


Figure 7-3 Removing the Lamp Cover

4. Remove the lamp by pulling straight up on the lever to the left of the lamp until it is free. Discard the faulty lamp.

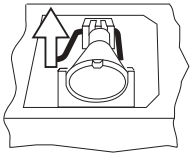


Figure 7-4 Removing the Lamp

5. Remove the new lamp from the box by grasping the outside rim of the reflector.

CAUTION

Do not touch other areas of the new lamp or the interior of the reflector. They must be free of fingerprints or other material that can interfere with illumination and collect dust. (You may want to wear lab gloves.)

Locate the tab on the lamp rim. Insert the new lamp into the socket so that the it faces the excitation filter wheel with the tab facing up. Press lightly on the rear of the lamp to seat and align it.

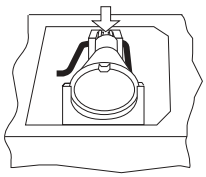


Figure 7-5 Replacing the Lamp

6. Place the cover over the lamp compartment. Then insert and tighten the screw.

7. Close the scanner cover, reconnect the power cable, and turn on the power. Then make sure the plate transport carrier moves smoothly into position using the Plate In and Plate Out buttons from the Maintenance screen. (If necessary, close the scanner cover again.)

***NOTE**: Click the **Lamp On** and **Lamp Off** buttons if you want to test the new lamp.*

Accessing Technical Information

8

This chapter includes the following sections:

8.1 Specifications

System	Height	225 mm (9 in)
	Width	370 mm (14.5 in)
	Depth	490 mm (19.3 in)
	Weight	Approximately 20 kg (40 lb)
	Shipping weight	Approximately 23 kg (46 lb)
	Temperature	4°C to + 35°C (+ 39°F to + 95°F)
	Relative humidity	20% to 80%, non-condensing
Electrical	Power consumption	150 watts maximum
	Fuse	2.5 amp, time-delay, 250 volt, 5 mm x 20 mm, UL/CSA-approved in U.S. and Canada, IEC-approved in all other countries
	Input voltage	90–132 volts AC at 50–60 Hz (selectable) or 198–262 volts AC at 50–60 Hz (selectable)
Optical	Lamp type	Tungsten-halogen, 50 watt
	Lamp life	3,000 hours nominal
	Lamp range	320 nm–700 nm
	Filter wheel	6 positions
	Detector	Electron Tubes, Inc. 9781 Photomultiplier Tube (low noise, high gain)
	Probe	3.0 mm quartz
	Filters	6-cavity design interference filters, optimized for CytoFluor

X-Y Scanning Mechanism	Step size	45.27 µm (0.0018 in)
	Repeatability	± 50 µm worst case
	X-Y drive	Stepping motor with direct drive belt
	Plate transport	10 seconds maximum load time

8.2 Minimum Computer Requirements (You Supply)

***Note**: The recommended requirements are: 4 MB of RAM, 250 MB hard drive, and a 100 percent compatible base unit that is powerful enough to run Windows efficiently.)*

Base unit	IBM-PC-compatible 486DX computer, 8 MB minimum RAM
	240 MB hard drive
	High-density floppy disk drive, 3-1/2 -inch
	1 serial port
	1 parallel port (for printing)
	Mouse
Printer	Any compatible
Required software	Microsoft Windows version 3.1 or later or Windows 95

8.3 Optical Sets for Fluorophores

Dye	Excitation/ Emission Pair	Application
Methylumbelliferone	360/460	Immunoassay
Hydroxymethylcoumarin	360/460	Immunoassay
Prodan	360/460	Membrane probe
Laurodan	360/460	Membrane probe
Calcein	485/530	Viability assays
Attophos™	450/580	Immunoassay
CFD-A	485/560	Plasma membrane disruption
CFD	485/530	pH
B. Phycoerythrin	485/590	General fluorescent tag
DPH	360/460	Membrane probe
DAPI	360/460	Nucleic acid stain
Quin-2	360/460	Ion concentration
Indo-1	360/485	Ion concentration
Rhod-2	360/460	Ion concentration
Thiolyte	360/460	General fluorescent tag
Lucifer yellow	440/530	Gap junction communication
Benzoxanthene yellow	360/460	General fluorescent tag
Dansyl P. E.	360/530	General fluorescent tag

Dye	Excitation/ Emission Pair	Application
BCECF	485/530	Vital dye, pH indicator
Carboxy fluorescein	485/530	Vital dye, pH indicator
Fluo-3	508/560	Ca_{2^+} concentration
PicoGreen™	485/530	Nucleic acid quantitation
SNAFL™ (in acid)	485/530	pH indicator
Acridine orange	485/530	Nucleic acid stain
Rhodamine 123	485/530	Mitochondria stain
DTAF	485/530	Alcohol reactive probe
Ethidium homodimer	485/590	Nucleic acid stain
	485/645	Dual wavelength monit.
7-AAD	485/645	Nucleic acid stain
Ethidium bromide (in solution)	485/645	Nucleic acid stain
alamar Blue™	530/590	Viability assay
Ethidium dimer	530/590	Nucleic acid stain
SNAFL™ (in base)	530/620	pH indicator
SNARF™	530/620	pH indicator
TRITC	530/590	General fluorescent tag
Propidium iodide (in solution)	485/645	Free dye in solution
Propidium iodide	530/645	Nucleic acid stain (for dead cells)
Texas Red™	590/645	General fluorescent tag

Dye	Excitation/ Emission Pair	Application
Allophycocyanine	590/645	General fluorescent tag
Neutral red	530/645	Vital dye, Lysosomal integrity
R. phycoerythrin	485/590	General fluorescent tag
Sodium fluorescein	485/530	Monolayer permeability
Thiazole Orange	485/560	Nucleic acid stain
BODIPY™	485/530	General fluorescent tag
Green fluorescent protein	395/508	Gene expression

8.4 CytoFluor Standard Filter Sets

Excitation Center Wavelength/ Full Bandwidth*	Emission Center Wavelength/ Full Bandwidth*
360/40	460/40
450/50	530/25
485/20	580/50
530/25	620/40
590/20	645/40

*Wavelengths measured in nanometers

8.5 Ordering Information

Table 8-1 lists the part numbers of the CytoFluor System kit
Table 8-2 lists the part numbers of the Accessories kit. .

Table 8-1 CytoFluor System Kit Part Numbers

Description	Part Number
CytoFluor System kit—Includes: • Fluorescence Reader • Accessories kit • 110 V and 220 V power cables • RS-232 cable • CytoFluor software • *CytoFluor Fluorescence Multi-Well Plate Reader User's Guide*	MIFS0C2TC— (with temperature control) MIFS0C3TR—(without temperature control)
RS-232 cable	P40636
Power cable, U.S.	P30539
Power cable, Europe	P30540
CytoFluor 4000 Fluorescence Multi-Well Plate Reader User's Guide	601380

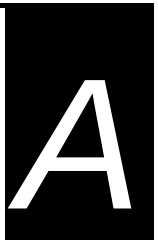
Table 8-2 Accessories Kit Part Numbers

Description	Part Number
CytoFluor Accessories Replacement Kit—includes all parts listed below	PIF600884
Probe spacer, 0.015"	P40633X1
Probe spacer, 0.020"	P40633X2
Optical filter tool	PIFS5TOOL
Fuse, 2.5 amp, time-delay, 250 volt, 5 mm x 20 mm, UL/CSA—approved in U.S. and Canada	P40618X13
Fuse, 2.5 amp, time-delay, 250 volt, 5 mm x 20 mm, IEC—approved in all other countries	P4619X20
3.5" diskette, CytoFluor software	PIFS600875
Allen (hex) wrench, 5/64"	P40637X1
Combination wrench, 1/4" by 5/16"	MLG311600
Dust filter (5/pack)	PIFS5C2DF
Fluorescence test plate	PIFS5C2RP
Spare lamp	PIFS5LAMP

Technical Support

To get additional product information or technical assistance, please see Appendix E, Technical Support and Training. When you contact Technical Support, be prepared to provide your CytoFluor model number, serial number, and software version.

Introduction to Windows



This appendix provides basic information on how to use Microsoft® Windows®. For example, it provides details on using the following:

- Mouse and on-screen pointer
- Windows
- Screen buttons
- Pull-down menus and scroll bars

If you need further information, see the manuals provided with the Microsoft Windows software.

A.1 The Mouse and On-Screen Pointer

In Microsoft Windows, almost all software operations use a device called the *mouse* to control an on-screen *pointer*. The only time you need to use the keyboard is to enter characters or numbers.

If you look at the underside of the mouse, you see a ball inside a circular housing. When you slide the mouse across a surface, the ball rolls underneath it. This movement sends signals to the computer that cause the pointer to move around the screen. Moving the mouse to the left or right causes the pointer to move accordingly.

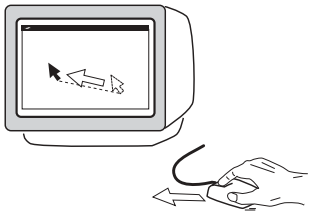


Figure 1-1 Mouse Pointer

The mouse has two buttons on its top. If you press down on the left mouse button and then release it, this signals the computer to initiate an operation. This action is called *clicking*. Usually you must position the pointer on something on the screen before you click, such as a menu title or a text field.

To select a section of text, you move the pointer to the left end of the text and press and hold down the mouse button. Then move the mouse so that the pointer moves across the text. As the pointer moves, the text becomes highlighted so you can see what you are selecting. When you get to the end point, release the mouse button. This action is called *dragging.* You can drag to select text or other contiguous objects on the screen; for example, groups of wells on the CytoFluor plate layout.

A.2 Windows

A *window* is a rectangular area on the screen in which you do work in a program. The window can be manipulated—opened and closed, moved and resized, and shuffled—just like a sheet of paper on top of a desk.

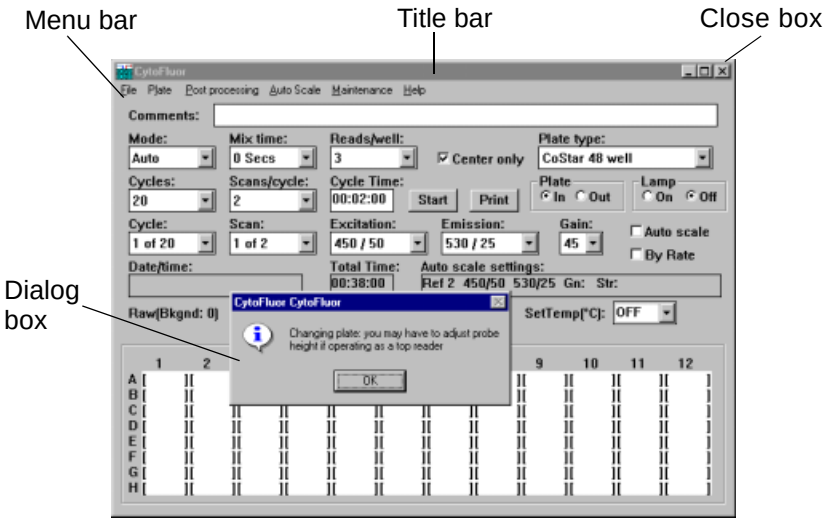


Figure 1-2 Windows

At the top of most windows is a long rectangular box, the *title bar*. In the title bar you find the name of the file or application in which you're working. In the upper right corner of the title bar are two arrows, one pointing down and the other up. The down arrow makes Microsoft Windows inactive (minimizes it); the up arrow increases the size of the window on the screen. The *close box* is also found in the title bar, in the upper left corner. When you position the pointer on the close box and press a mouse button, the window closes and is removed from the screen.

Just below the title bar you see the *menu bar* where all *menu titles* are found. By placing the pointer over one of the menu titles and clicking a mouse button, a menu (list of choices) displays. Some of the options are in faint characters; that is, they're *dimmed*. Dimmed options aren't available at the moment. For example, in some programs, you cannot print a file before you open it. Therefore, if a file has not been opened, the Print command would be dimmed.

Some windows don't have title bars or menu bars; these windows are used to advise you of something or to ask you for more information. These windows are known as *alert boxes* and dialog boxes. As you choose menu options and commands, you often open up dialog boxes. In dialog boxes, you find buttons, scroll bars, and check boxes that you can click on to select.

A.3 Screen Buttons

You use screen buttons to make choices and send messages to the software; for example, you might confirm a command by clicking a button labeled OK, or change your mind by clicking a button labeled Cancel.

Another kind of button, called an option button, looks like a push-button; you click this button to indicate one out of several options where only one option can be active at a time.

A check box is a type of button used where several choices can be made active at the same time, independent of each other. Clicking the box puts an X in it, which means the option is selected. Clicking the box again removes the X and deselects the option.

A.4 Menus and Scroll Bars

You use scroll bars to move through long lists or window areas where part of the contents are hidden. A scroll bar looks like a long rectangle with arrows at the top and bottom. By positioning the pointer over one of the arrows and clicking it, you make visible more options on a list. In the gray part of the scroll bar, there is a small square called a thumb. The thumb's position in the gray area is proportional to the viewing position in the list. If the thumb is at the bottom of the scroll bar, you are looking at the bottom of the list.

Error Messages

B

This appendix lists error messages you may see while using the CytoFluor software. The CytoFluor software displays a system error in a message box and makes a beep sound to draw your attention to it. Many error messages display a negative error code (number). This chart lists the possible error codes or messages you may see and defines their meaning:

Error Code/ Message	Meaning
-202	Indicates a bug in the program.
-203	Failure to initialize the PC'S Com port. The port may not exist, or may already be claimed by Windows or by another program.
-204	Time-out waiting for response from instrument. The instrument may not be turned on or connected to the PC, or the Com port may be incorrectly configured. (See error code -206.)
-205	Communication aborted by ESC key. You pressed the Escape key.

Error Code/ Message	Meaning
-206	Garbled communication with instrument. Check that the Com port is set to run at 9600 baud with eight data bits, one stop bit, and no parity.
-208	Failed to open a file. The file may not exist or have incorrect permissions.
-209	Failed to read a file. The file may be corrupt.
-210	Failed to write a file. Disk may be full.
-211	Failed to seek to a certain offset in a file. The file may be corrupt.
-212	The file being opened is not a CytoFluor internal file.
-213	Failed to get file system status of a file. The file may not exist.
-214	Failed writing to a file because the disk is full. Disk is full.
-215	Failed to initialize the print engine. Check windows printer setup.
-216	Failed to initialize the main window. Out of memory.
-217	Out of memory. Attempt to allocate memory failed. Try closing some windows.
-218	Instrument reports a hardware error.
-219	Instrument is not ready.
-222	Reading aborted with ESC key. You pressed the Escape key.
Delete <filename>?	Occurs when you are deleting a file as a double-check before deletion.

Error Code/ Message	Meaning
Disk full.	The disk chosen as a destination has insufficient room for the file(s) you're trying to copy.
Error reading <filename>.	The file selected for opening is an invalid type.
Error writing <filename>.	The file cannot be saved. Check that the path selected is correct and that a disk is inserted in the floppy disk drive, if selected. This might also occur if insufficient room exists to save the file.
File exists Overwrite file?	You have duplicated the name of an existing file. Unless you are sure you want to overwrite it, click Cancel and rename the file.
File name contains illegal characters. Rename file.	You can use only alphanumeric characters in the name of a file.
Possible harmful overlap of excitation and emission filters.	You cannot choose excitation and emission filters with similar or incompatible wavelengths. Change one or both of the filter settings.
Overwrite existing <filename>?	You have chosen a file name that already exists. Unless you intend to overwrite the file, choose Cancel and type a different name.
Save first?	Occurs when you attempt to exit CytoFluor or when you try to open a different file when the currently active workfile has changed. It ensures that your changes are saved.
Lengthened cycle time to nn seconds.	If you are scanning a plate more than once, you can enter a value in the Cyc to cyc field (in main screen setup). The value should be equal to or greater than the cycle time. The system saves the file with the actual cycle time.

Error Code/ Message	Meaning
Serial port already in use. Select another.	The communications port selected is physically attached to a different device. Check the connections and select the correct port.
Serial port not present. Select another.	You have selected a communications port that does not exist on your computer. For example, your computer has only one serial port and you selected COM2. Change the selection.
Time out error.	The plate may be jammed or incorrectly positioned in the plate transport mechanism. Do not try to force the mechanism or correct the problem yourself. Turn off the scanner, disconnect the power, and contact Applied Biosystems Technical Support.
Unable to communicate with CytoFluor system. Check all connections.	The software has lost communication with the scanner. Make sure the connectors are firmly seated.

Warranty/Service Information

C

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers, installing unauthorized hardware or software may void the Warranty or Service Plan.

C.1 Limited Product Warranty

Limited warranty

Applied Biosystems warrants that all standard components of the ***Cytofluor Series 4000 Flourescence Multiwell Plate Reader*** will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage.

Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its ***Cytofluor Series 4000 Flourescence Multiwell Plate Reader*****,** such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on the instrument are themselves warranted to be free of defects in materials and workmanship for ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date of installation, the software designated for use with the product will perform substantially in accordance with the function and features described in its accompanying documentation when properly installed on the product. Applied Biosystems does not warrant that the operation of the instrument or software will be uninterrupted or error free. Applied Biosystems will provide any software corrections or “bug-fixes” if and when they become available, for a period of ninety (90) days after installation.

Warranty period effective date

Any applicable warranty period under these sections will begin on the date of installation for hardware and software installed by Applied Biosystems personnel, unless that date has been delayed at the buyer's request. In that case, and for all hardware and software installed by the buyer, and for all other products, the applicable warranty period begins the date the component is received by the buyer.

Warranty exceptions

The above warranties shall not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specification instructions for the product or accessories; performance of improper or inadequate maintenance by the user; installation of software or interfacing not supplied by Applied Biosystems; and modification or repair of the product or the software not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed. Such limited warranty is given only to buyer or any third party in the event of use of products furnished hereunder by any third party.

Warranty limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, in tort, warranty, or under any statute (including without limitation, any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damage, including without limitation, damage arising from or

related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement. Any such affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

C.1 Damages, Claims, Returns

Damages If shipping damage to the instrument is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims After a damage inspection report is secured, Applied Biosystems will supply the replacements and process claims that are initiated by either party.

Returns Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the appropriate address.

Plate Scan Patterns

D

This appendix shows the scan patterns the system uses when averaging points taken in a well. It includes graphic examples on plates of this size:

- Six-well
- 12-well
- 24-well
- 48-well

***NOTE**: The graphics represent wells as circles. The outside circle represents the well wall. The dotted circle represents the outside travel diameter for the larger wells. The probe travels in a square bound by the dotted circle.*

Each dotted circle contains smaller circles that represent the spot pattern of the probe. The wells are drawn to a relative scale. See the following examples for details.

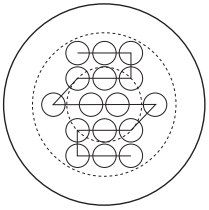


Figure D-1 A Six-Well Plate Pattern

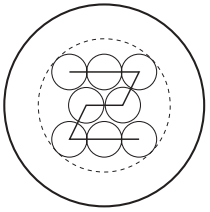


Figure D-2 A 12-Well Plate Pattern

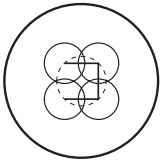


Figure D-3 A 24-Well Plate Pattern

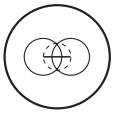


Figure D-4 A 48-Well Plate Pattern

Technical Support and Training

E

This appendix contains the following sections:

E

E.1 Contacting Technical Support

Overview You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

NOTE: *For information on obtaining technical documents such as Applied Biosystems user documents, MSDSs, and certificates of analysis, see* *"Obtaining Technical Documents" on page E-8.*

By E-mail You can contact technical support by e-mail for help in the product areas listed below.

Product/Product Area	E-Mail Address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com
• Biochromatography • Expedite™ (8900) DNA Synthesis System • PNA • Pioneer™ Peptide Synthesis System • Proteomics Solution 1™ (PS1) System • ICAT™ reagent • FMAT™ 8100 HTS System • Mariner™ Mass Spectrometers • Voyager™ Mass Spectrometers • CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS SCIEX)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

By telephone or fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

NOTE: *To schedule a service call for other support needs, or in case of an emergency, dial* ***1.800.831.6844, then press 1****.*

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1.800.831.6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1**.**800**.**831**.**6844**, press **2**, then press **1**[a]	1.650.638.5981
Fluorescent DNA Sequencing	**1**.**800**.**831**.**6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1**.**800**.**831**.**6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1**.**800**.**831**.**6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1**.**800**.**831**.**6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43*x* Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **2**[a]	1.650.638.5981

Product/Product Area	Telephone	Fax
PCR and Sequence Detection	**1**.**800**.**762**.**4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI PRISM® 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1**.**800**.**831**.**6844**, then press **5**[a]	1.240.453.4613
• Voyager™ MALDI-TOF Biospectrometry™ Workstations • Mariner™ ESI-TOF Mass Spectrometry Workstations • Proteomics Solution 1™ (PS1) System • ICAT™ reagent	**1**.**800**.**899**.**5858**, press **1**, then press **3**[b]	1.508.383.7855
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1**.**800**.**899**.**5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ (8900) Nucleic Acid Synthesis Systems	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855

Product/Product Area	Telephone	Fax
FMAT™ 8100 HTS System CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS SCIEX)	**1.800.952.4716**	1.508.383.7899

a. 5:30 A.M. to 5:00 P.M. Pacific time.

b. 8:00 A.M. to 6:00 P.M. Eastern time.

c. 9:00 A.M. to 5:00 P.M. Eastern time.

By telephone or fax (outside North America)

To contact Applied Biosystems Technical Support or Field Service outside North America, use the telephone or fax numbers below.

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	61 3 9730 8600	61 3 9730 8799
China (Beijing)	86 10 64106608 or 86 800 8100497	86 10 64106617
Hong Kong	852 2756 6928	852 2756 6968
Korea (Seoul)	82 2 5936470/6471	82 2 5936472
Malaysia (Petaling Jaya)	60 3 79588268	603 79549043
Singapore	65 896 2168	65 896 2147
Taiwan (Taipei Hsien)	886 2 2358 2838	886 2 2358 2839
Thailand (Bangkok)	66 2 719 6405	662 319 9788

Region	Telephone	Fax
Europe		
Austria (Wien)	43 (0)1 867 35 75 00	43 (0)1 867 35 75 11
Belgium	32 (0)2 532 4484	32 (0)2 582 1886
Denmark (Naerum)	45 45 58 60 00	45 45 58 60 01
Finland (Espoo)	358 (0)9 251 24 250	358 (0)9 251 24 243
France (Paris)	33 (0)1 69 59 85 85	33 (0)1 69 59 85 00
Germany (Weiterstadt)	49 (0) 6150 101 0	49 (0) 6150 101 101
Italy (Milano)	39 (0)39 83891	39 (0)39 838 9492
Norway (Oslo)	47 23 12 06 05	47 23 12 05 75
Portugal (Lisboa)	351.(0)22.605.33.14	351.(0)22.605.33.15
Spain (Tres Cantos)	34.(0)91.806.1210	34.(0)91.806.12.06
Sweden (Stockholm)	46 (0)8 619 4400	46 (0)8 619 4401
Switzerland (Rotkreuz)	41 (0)41 799 7777	41 (0)41 790 0676
The Netherlands (Nieuwerkerk a/d IJssel)	31 (0)180 392400	31 (0)180 392409 or 31 (0)180 392499
United Kingdom (Warrington, Cheshire)	44 (0)1925 825650	44 (0)1925 282502
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	27 11 478 0411	27 11 478 0349
Africa, French speaking (Paris, France)	33 1 69 59 85 11	33 1 69 59 85 00
India (New Delhi)	91 11 653 3743 91 11 653 3744	91 11 653 3138
Poland, Lithuania, Latvia, and Estonia (Warszawa)	48 22 866 4010	48 22 866 4020

Region	Telephone	Fax
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia	44 1925 282481	44 1925 282509
Japan		
Japan (Hacchobori, ChuoKu, Tokyo)	81 3 5566 6230	81 3 5566 6507
Latin America		
Caribbean countries, Mexico, and Central America	52 55 35 3610	52 55 66 2308
Brazil	0 800 704 9004 or 55 11 5070 9654	55 11 5070 9694/95
Argentina	800 666 0096	55 11 5070 9694/95
Chile	1230 020 9102	55 11 5070 9694/95
Uruguay	0004 055 654	55 11 5070 9694/95

Through the Applied Biosystems web site

To contact Technical Support through the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com**
2. Click **Services & Support** at the top of the page, then click **Frequently Asked Questions**.
3. Click **Contact Support** in the contents list at the left of the screen.
4. Click your geographic region for the product area of interest.
5. In the Personal Assistance form, enter the requested information and your question, then click **Ask Us RIGHT NOW**.
6. In the Customer Information form, enter the requested information, then click **Ask Us RIGHT NOW**.

Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

E

E.2 Obtaining Technical Documents

Overview

You can obtain technical documents, such as Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents for free, 24 hours a day. You can obtain documents:

- By telephone
- Through the Applied Biosystems web site

Ordering documents by telephone

To order documents by telephone:

1. From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2. Follow the voice instructions to order documents (for delivery by fax).

NOTE: *There is a limit of five documents per fax request.*

Obtaining documents through the web site

To view, download, or order documents through the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com/**
2. At the top of the page, click **Services & Support** at the top of the page, then click **Documents on Demand**.
3. In the search form, enter and select search criteria, then click **Search at the bottom of the page**.
4. In the results screen, do any of the following:
   - Click  to view a PDF version of the document.
   - Right-click  , then select **Save Target As** to download a copy of the PDF file.
   - Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you.
   - Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you.

NOTE: *There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.*

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E.3 Obtaining Customer Training Information

To obtain Applied Biosystems training information, go to **www.appliedbiosystems.com**, click **Services & Support** at the top of the screen, then click **Training**.

Glossary

Active—Describes a window that is selected; that is, the one in which you're currently able to work. Also refers to commands or options that are currently available or selected.

Application—Short for application program. A program used for a particular kind of work. Program and application are often used interchangeably.

Automatic mode—One of the two CytoFluor scan modes. Use Automatic mode to scan plates once you have entered initial header information for a work file. Compare Manual mode.

Backup—A copy of a file kept in case of disk or equipment failure or accidental deletion. Backup files are usually kept on floppy disks. Backing up important files frequently helps keep loss of data and work time to a minimum. Sometimes a set of backup disks are kept at a different site in case of fire or theft.

Bandwidth (BWHM)—For an optional interference filter, the wavelength range (nm) at 50% of peak transmission.

Boot—To start or restart your computer by loading the DOS operating system. The term is short for bootstrapping. Starting up is often accomplished by first loading a small program that then loads a larger program into memory. The program is said to "pull itself up by its own bootstraps."

Check box—A small box inside a dialog box or other window that you click to select an option. An X appears in the box when it's selected; clicking the box again removes the X and "deselects" the option.

Choose—To pick a command from a menu or dialog box. Compare select.

Click—To position the pointer on something you wish to choose or select, and then press and release the mouse button.

Com port—Short for communications port. A serial connection on a computer where you plug in a device. The CytoFluor System usually uses Com 1.

CSV (Comma-Separated Values)—A file format in which each element is separated by a comma. This format is compatible with most spreadsheet programs.

.CSV—The extension to a file name that identifies it as being in CSV format; for example, TEST.CSV. These files contain data and scan-specific header information.

Command—An instruction that causes the system to perform some action. You can choose a command from a menu or dialog box or by typing from the keyboard, depending on the program.

Command button—A button in a dialog box that either carries out or cancels a selected action. A Cancel button always cancels a command. An OK button carries out a command.

Confirmation—A message displayed by software prompting if you want to proceed when you have chosen a destructive action. For example, you will see a confirmation message when you tell the program to delete a file. This message gives you an opportunity to change your mind.

Control menu—The menu accessed by clicking the box in the upper left corner of the window; it is available in every application that runs in a window. Control-menu commands move, change the size of, and close windows.

Control-menu box—The small box located at the upper left of your screen. If you have a mouse, you can click this box to display the Control menu or double-click it to quit.

Current directory—The directory in which you are currently working while using the computer. For example, when using the CytoFluor program you would likely be working in C:\CYTOFLR, which means the CYTOFLR directory on the C:\ drive.

Data file—Any file created within an application. As you run scans with the CytoFluor System, you are creating data files. Data files may have .CSV as an extension, or they may be .RUN files (Normal mode).

Default—A preset value, option, command, or device name that is automatically provided by the system. These default values prevent a program from crashing or stalling if the user does not provide a value. In other cases, a default value is provided to make the program more convenient to use. For example, in most dialog boxes that contain command buttons, one of the buttons is selected when the dialog box appears, indicating that it is the default choice and will be chosen automatically if you press the Enter key.

Destination directory—The directory to which you intend to copy or move one or more files. Compare source directory.

Device—Any hardware connected to and used by the computer, such as a printer, disk drive, or scanner. Also called a peripheral device or peripheral.

Device driver—A program that controls how your computer and a device, such as the CytoFluor Scanner, interact.

Dialog box—An on-screen box that either requests or provides information. For instance, if you choose the Export .CSV command, a dialog box appears asking for the name of the file you wish to export.

Dimmed—Unavailable. A command or option that is currently unavailable is dimmed (shown in gray) on the screen.

Direct access—A method of selecting a menu or option by pressing ALT followed by the key that corresponds to the underlined letter in the command, menu, or option name. In some instances, you might have to press a key combination such as Shift+letter.

Directory—A location in a disk's file system; a collection of files and subdirectories that are stored at the same location on a disk. The name of the directory identifies its location. See also subdirectory.

Disk drive—A device for storing and retrieving data on disks. Hard disks are disk drives with disks permanently sealed inside. Floppy disk drives use floppy disks, that you can insert and remove. See also floppy disk.

DOS prompt—A letter followed by a colon that appears at the left edge of the screen when using the DOS operating system. The letter identifies the active drive. For example, A:, C:.

Double-click—To position the pointer on an object you want to select, and then click twice in quick succession. This action carries out a command.

Export—To move data into a file that other applications can use.

Extension—A period followed by three letters at the end of a filename. Extensions identify what kind of information a file contains. For example, the extension .CSV indicates that the elements in the file are separated by commas. The extension .EXE identifies a file that the system can use to start up a program (EXEcutable).

Field—An item of data. A group of related fields make up a record. For example, each file you create in the CytoFluor System has fields for name, assay, laboratory, plate, filter sets, and so on.

File—An organized collection of information given a name and stored on a disk. Files can be programs, data, operating systems, and so on.

File name—The name of a file. Windows uses DOS file naming conventions; therefore, filenames usually consist of a base name with no more than eight characters and an extension made up of a period and three characters. For example, LABTEST.CSV. See also extension.

Floppy disk—A disk that you can put into and take out of a disk drive. The disk itself is made of flexible plastic, as compared to the disks in hard disk drives, which are made of metal. Floppy disks originally had only thin, flexible jackets and were literally floppy. These disks now more commonly have rigid plastic jackets with a sliding metal access slot.

Format—To prepare a disk so that it can hold information. You must always format disks before you can use them to store data or programs. Formatting a disk erases any information that was previously on it. You use the DOS FORMAT command to format disks.

Grayed—See dimmed.

Highlighted—Describes the appearance of a selected object or menu item on the screen. A menu that is selected usually appears in inverse video—that is, light letters on dark rather than the normal dark on light.

Inactive window—Any open window that you are not currently working in.

Lamp On/Off—A CytoFluor feature that lets you turn off the lamp for some kinds of tests. You access this feature by choosing the Options command from the System menu.

List box—A box within a dialog box or window that lists items that a command could affect—for example, the names of all plates in the plate database. If there are more choices than can fit in the list box, the list box will have a vertical scroll bar.

Manual mode—A type of Mode you can select through the main setup screen. This mode causes the system to scan one plate after another with a minimum amount of manual interaction.

Maximize box—The small box containing an up arrow that is located at the right of the menu bar. Mouse users can click the maximize box to enlarge a window to its maximum size. Others can use the Maximize command or the Control menu.

Menu—A group listing of available Windows or application commands. Menu names appear in the menu bar near the top of the window. You use a command from a menu by pulling down the menu, then choosing the command.

Menu bar—The horizontal bar that lists the names of an application's menus. The menu bar appears below the title bar.

Minimize box—The small box containing a down arrow that is located at the right of the menu bar. Mouse users can click the minimize box to reduce a program to an icon; to turn the program into a window again, double-click the icon. Others can use the Minimize command or the Control menu. DO NOT USE the Minimize box to change windows while running a scan or to move from Excel to CytoFluor.

Mouse—A device used to control the position of a cursor or pointer on the screen. As you slide the mouse across a flat surface, a ball on its underside rolls, sending position information to the computer.

Open—To make available; to display the contents of a file in a window.

Option button—A small round on-screen button that selects an option when clicked. An option affects the way in which a command is carried out. Within a group of related option buttons, you can make only one selection.

Pathname—The directions to a directory or file within your system. The pathname of a file consists of a drive letter, followed by a directory name, one or more subdirectory names, if applicable, and a filename. Each name is separated from the previous one by a backslash. For example C:\WINDOWS\CYTOFLR\LAB\TEST.CSV would be the directory path to the LAB\TEST.CSV file.

Point—To move the pointer on the screen until it rests on the item you want to select.

Pointer—The small shape on the screen that follows the movement of the mouse. Usually the pointer is an arrow, but it can also have other shapes.

Port—The connector on your computer to which an external device (such as the scanner or a printer) is attached.

Prompt—A character or characters, usually on the left edge of the screen, where you type operating system or other software commands. For example, the A: prompt tells you that you are using the A drive in DOS. Sometimes the term prompt is used for the insertion point: a flashing bar in a text box that shows where the next typed characters will appear.

RS-232—The standard serial interface protocol, and thus the cable and connector specifications, that enable a computer to be linked to the CytoFluor Scanner.

Restore box—The small box containing an up arrow and a down arrow at the right of the menu bar. This box appears after you have enlarged a window to its full size. Mouse users can click the restore box to return the window to its previous size. Others can use the Restore command or the Control menu.

Root directory—The highest directory level on a disk. The root directory is created when you format the disk. From the root directory you can create other directories.

Run—To start an application.

Save—To cause a file, or changes that were made to the file, to be written on a disk. Changes you make to a file are not permanent until you have saved them onto the disk. Frequent saving can help avoid having to re-do work in case of power failure or equipment failure.

Scroll—To use the arrow buttons or the thumb of a scroll bar to move through lines of text within a box so that you can view a different portion of its contents.

Scroll bar—A rectangular shaded bar near or at the edge of a box that indicates more contents than can be shown in the box. A scroll bar has arrows at each end, which can be clicked to move the view of the contents, and a white rectangle in the shaded area called a thumb, which can be moved with the pointer to a new position. Mouse users can click on parts of the scroll bar to scroll a file.

Scanner—The unit of the CytoFluor System that contains the optical probe, filters, lamps, and plate transport machinery. The scanner reads emission values from the wells of a plate and sends the information to the CytoFluor System software.

Select—To designate where the next action will take place. Selecting does not initiate an action. After selecting an item, you choose the command that you want to affect the item. Also, to pick from a list of options other than commands.

Serial interface—An interface between a computer and a device, such as a scanner, in which single bits of information are transferred in a string, one after the other. Serial, asynchronous, and RS232 interfaces are all the same type.

Shortcut key—One of the F keys along the top of most keyboards. Shortcut keys accomplish actions that otherwise require mouse moves or key equivalents, such as choosing commands from menus. The action of a shortcut key depends on the application program.

Spooler—A Windows program that is automatically run when you choose a print command. The Spooler lets you print files and view and control the jobs in the print queue.

Subdirectory—A directory within another. All directories are subdirectories of the root directory.

Title bar—The horizontal bar across the top of each window that contains the name of the application and/or file in that window. The title bar also contains the Control menu box, and the maximize and minimize boxes or the minimize and restore boxes.

TC—Temperature-control CytoFluor model equipped with a temperature control chamber in which the plate is scanned.

TR—Temperature-ready CytoFluor model that is not equipped with a temperature control chamber.

Wavelength—A measure of the vibrational length of radiation; that is, the length of one complete oscillation or cycle. Frequency is the number of wavelengths (cycles) per second emitted by a source.

Window—A rectangular area on your screen where you view an application. Every window has a title bar and may have a menu bar. Windows can contain scrolling lists, buttons, and fields of information. Most of your interactions with the CytoFluor System take place in windows.

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