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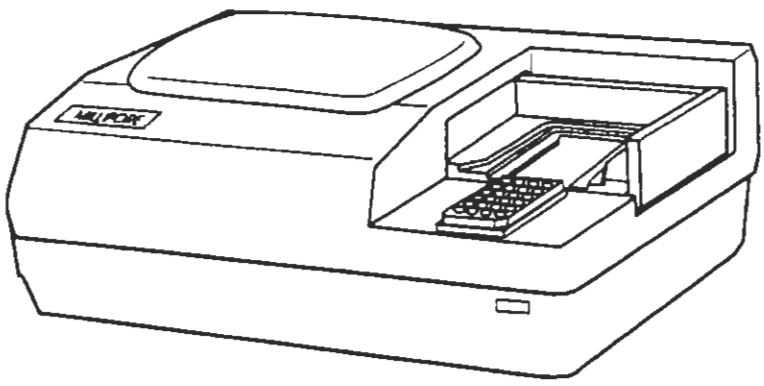
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CytoFluor™
2300/2350
Fluorescence Measurement System User's Guide



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TABLE OF CONTENTS

About this Guide vii

Chapter 1 Introduction

About the CytoFluor System 1-1

How the System Works 1-2

What You Need 1-2
- Hardware 1-2
- Software 1-3
- Other Included Materials 1-3

Different Computer Systems 1-3

Other Suggestions 1-3

Chapter 2 Getting Started

Setting Up Your System 2-1

Installing the Software 2-5
- Install DOS 3.3 or Higher and Windows 2-5
- Install the CytoFluor System Software 2-5
- Stand-Alone Version 2-6
- Windows Application Version 2-9

Checking the System 2-10
- Check Scanner Settings 2-10
- Run System Diagnostics 2-13
- Check Communication Port Settings 2-13
- Check System Registration Numbers 2-14

Chapter 3 Preparing Files

Creating New Files 3-1
- Name 3-3
- Assay and Lab Fields 3-3
- Plates 3-3
- Filter Sets 3-4
  - Optical sets for Fluorochromes (table) 3-5
- Sensitivity 3-7

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Chapter 3 Preparing Files (Con't.)

Number of Scans 3-8
Approximate Time Per Scan 3-8
Scan Cycle 3-8
Approximate Total Time 3-8
Comments 3-8

Saving the File 3-8

Duplicating a Template File 3-9

Chapter 4 Scanning

Scanning 4-1

Displaying your Results 4-4

Using Background Subtract 4-6

Exporting Files 4-8

Printing your Results 4-10

Closing Files 4-11

Using the Archive Function 4-11
Copying Files 4-12
Copy out 4-12
Copy In 4-13
Printing Files 4-14
Deleting Files 4-15

Chapter 5 Customizing

Filters 5-1
Changing the Filters 5-1
Changing the Software 5-4

Plate Coordinates 5-5
Entering Plate Information 5-6
CytoFluor Identification 5-7
Manufacturer 5-7
Catalogue Number 5-7
Rows 5-7
X1, Y1, X2 and Y2 Coordinates 5-7
Columns 5-7
Adding a New Plate 5-8

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Chapter 5 Customizing (Con't.)

Setting up an Area Scan 5-10
- Area Coordinates 5-11
- Entering Scan Information 5-12
  - Identifier 5-12
  - Manufacturer 5-13
  - Scan Area 5-13
  - Rows in Array 5-13
  - Columns in Array 5-13
  - Dimensions 5-13
  - Data Point Diameter 5-13
  - Diameter or Side Length 5-14
  - Catalogue Number 5-14

Scanner 5-15
- Tests 5-16

Communication Port 5-17

Chapter 6 Maintaining The CytoFluor System

Replacing the Air Filter 6-1

Replacing the Lamp 6-2

Chapter 7 Technical Information

System Specifications 7-1
- CytoFluor Model 2300/2350 7-1
- Operating Conditions 7-1

Electrical Specifications 7-1

Optical Specifications 7-1

X-Y Scanning Mechanism 7-2

Computer Specifications 7-2
- Minimum Computer Requirements 7-2
- NEC PowerMate 386/25S Specifications 7-2
- CytoFluor Computer Interface 7-3

Optical Sets for Fluorochromes 7-3

CytoFluor Standard Filter Sets 7-4

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Chapter 7 Technical Information (con't.)

Technical Information 7-4
Ordering Information 7-4
Millipore Technical Service 7-5
Millipore Warranty 7-7
Copyright Information 7-8

Appendix A

Introduction to Windows A-1

Appendix B

Error Messages B-1

Appendix C

Physical Plate Database C-1

Glossary G-1

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CHAPTER 1
INTRODUCTION

Before continuing to other chapters in this guide, read through this chapter so you'll understand the CytoFluor 2300/2350 Fluorescence Measurement System. In this chapter you'll also find a list of what you need to run your system.

♦ *Note:* Be sure to complete and return your CytoFluor 2300/2350 Fluorescence Measurement System software registration form, found at the front of this manual. Millipore must have your completed registration information before you can receive technical support and product update information. The registration number for your system software is on the program disks.

About the CytoFluor System

The CytoFluor 2300/2350 Fluorescence Measurement System quantifies both soluble and cell-associated fluorescence. The fluorescence is measured by a photomultiplier tube in a light-proof detection chamber. The CytoFluor 2300/23500 Fluorescence Measurement System consists of:

- CytoFluor 2300/2350 Scanner
- CytoFluor 2300/2350 Control Software
- IBM-Compatible Personal Computer (PC)

The CytoFluor software, which runs on the PC, controls the action of the X-Y scanner. A precision X-Y mechanism scans a microtiter plate with a sensitive fiber optic probe to quantify the fluorescence in each well. The plate remains stationary, and can be selected from a database of industry-standard or user-defined plate formats. The area scan feature enables you to scan individual well areas to create a fluorescence map of that area.

Common plate types from 6 to 96 wells can be used with the CytoFluor 2300/2350 Fluorescence Measurement System. You can also set parameters for a customized area scan. Four excitation filters and five emission filters are provided with the system so that you can measure most common fluorochromes. See

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Chapter 7, *Technical Information*, for a list of common fluorochromes and their corresponding filters. In addition, you can add two excitation filters and one emission filter customized to your application.

How the System Works

The CytoFluor software, utilizing Windows and a mouse, makes the system easy to use. This software release (2B1) is compatible with Windows version 2.1 or higher. However, the software performs at its optimum under Windows 3.0 as a Windows Application. The CytoFluor 2300/2350 scanning system is fast—a 96-well plate can be scanned in under one minute.

After identification fields and operating parameters are entered into the program, including plate type, appropriate filter sets, and the scan sensitivity, the software directs the scanner to move the plate into the detection chamber. The transport mechanism aligns the probe over the plate and starts the scan.

Starting at well A1, the probe scans across the bottom of all wells, row by row. After scanning the last row, the lamp and detector automatically switch off, and the plate transport mechanism resets the plate to the load position. When the scan has been completed, the data can be displayed, printed, or exported to most standard spreadsheets for analysis. Microsoft Excel™ is recommended because of its ease of use and compatibility with the CytoFluor software.

What You Need

For optimum system performance, Millipore recommends you have a complete CytoFluor 2300/2350 Fluorescence Measurement System. This System is composed of the following items:

Hardware

- Millipore CytoFluor 2300/2350 Scanner
- NEC® Enhanced Powermate™ 386/25S system unit
- NEC Enhanced VGA Color Display monitor (APC H530)
- NEC Pinwriter™ P6200 24-pin printer with printer cable
- Microsoft two-button PS/2 mouse

Software

Millipore CytoFluor 2300/2350 Fluorescence Measurement System software

♦ *Note:* You can use this software to upgrade an existing system (Windows 2.1) or to install a new system

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using Windows 3.0.

- Disk Operating System (DOS) Version 3.3 (or higher)
- Microsoft Windows Version 2.1 (or higher)
- Microsoft Excel spreadsheet program (sold separately)

Other Included Materials

- Tool Kit
  - Allen wrench
  - European power cord (where appropriate)
  - spare fuse
  - filter forceps
  - replacement filter material for fan
  - spare lamp
- RS232 cable to connect scanner to computer
- CytoFluor 2300/2350 Fluorescence Measurement System calibration certificate

Different Computer Systems

If you did not purchase the complete CytoFluor 2300/2350 Fluorescence Measurement System, your computer system must meet the following requirements:

- IBM AT computer or 100% compatible type with 20 megabyte hard drive and 2 megabytes of memory (RAM) (4 MB preferred)
- 5 1/4" high-density floppy disk drive
- mouse, either serial or bus
- graphic display monitor (color preferred)
- compatible graphic display interface
- 80-column-width printer with parallel interface
- Disk Operating System (DOS) Version 3.3 (or higher)

Other Suggestions

For optimum performance of the CytoFluor 2300/2350 Fluorescence Measurement System, you should also supply:

- electrical surge protector
- protective mousepad

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In addition, Millipore recommends that you install your CytoFluor 2300/2350 Fluorescence Measurement System in an area that is well ventilated, free from dust and fumes, and away from direct sunlight or local heat sources.

If installed properly, the CytoFluor 2300/2350 Fluorescence Measurement System will meet FCC radio frequency emission standards. To prevent interference, install and operate the equipment in accordance with your computer hardware manufacturer's instructions.

♦♦ ***Caution:*** All hardware must be plugged into a grounded, electrical outlet—an electrical surge protector is recommended.

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CHAPTER 2
GETTING STARTED

Setting Up Your System

In this chapter you'll set up your system, install the CytoFluor System software and make sure the system is calibrated.

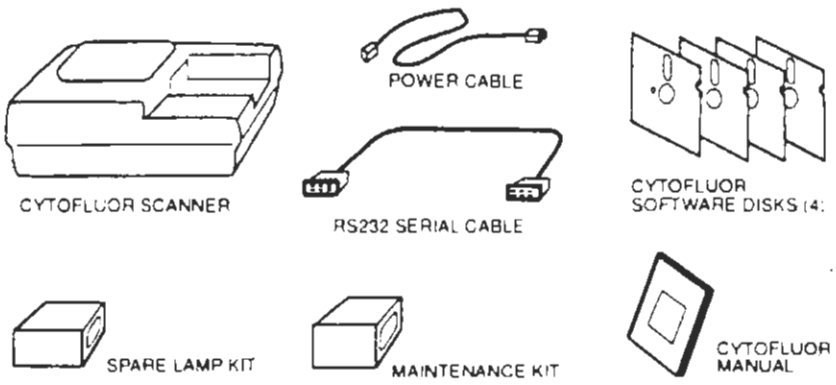
If you purchased a complete CytoFluor 2300/2350 Fluorescence Measurement System, you should have four boxes of hardware, software, and documentation. You should also have a system calibration certificate, which identifies your CytoFluor scanner by serial number.

♦ *Note:* If you purchased only the CytoFluor 2300/2350 Fluorescence Scanner and software, you will have just one box that includes the Scanner, software, and documentation for the CytoFluor 2300/2350 Fluorescence Measurement System. You should also have the system calibration certificate.

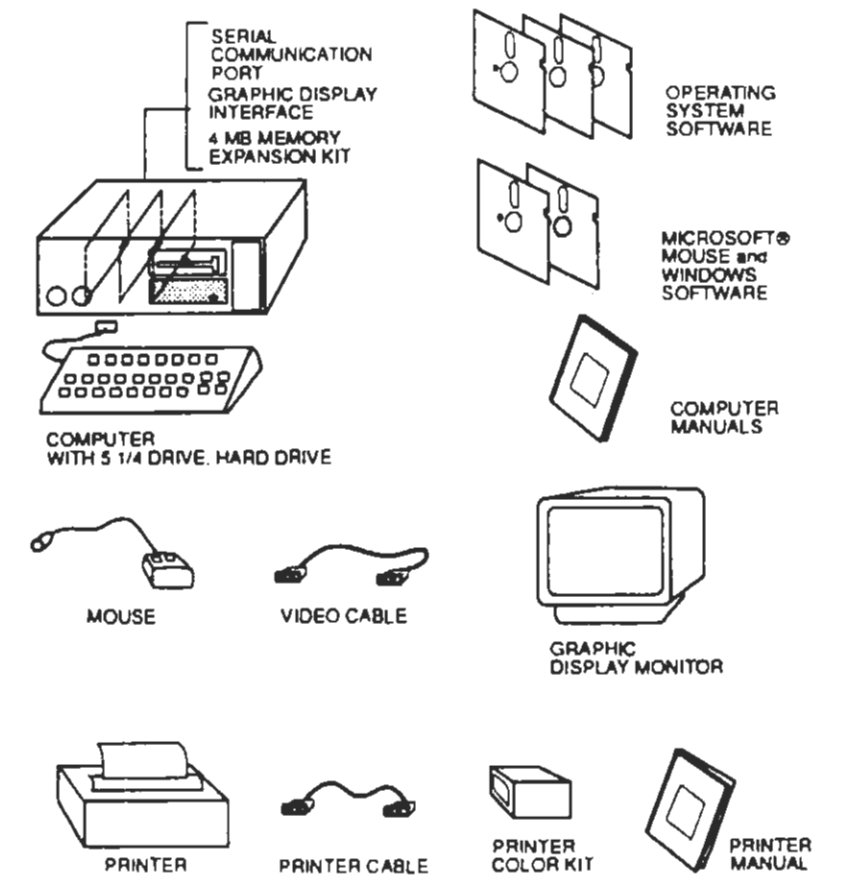
Before you set up the CytoFluor System, make sure you have all the components you ordered. The computer components have been assembled and tested prior to shipment. Remove all the components from the packing boxes and check to see that you have everything shown in the following figures.

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CYTOFLUOR SCANNER



COMPUTER EQUIPMENT



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If you have everything shown, complete the registration forms for all computer hardware and software and return them to the addresses listed on the forms.

♦ *Note.* Save the packing materials so you can repack your system if you have to move it over long distances or return any components for service.

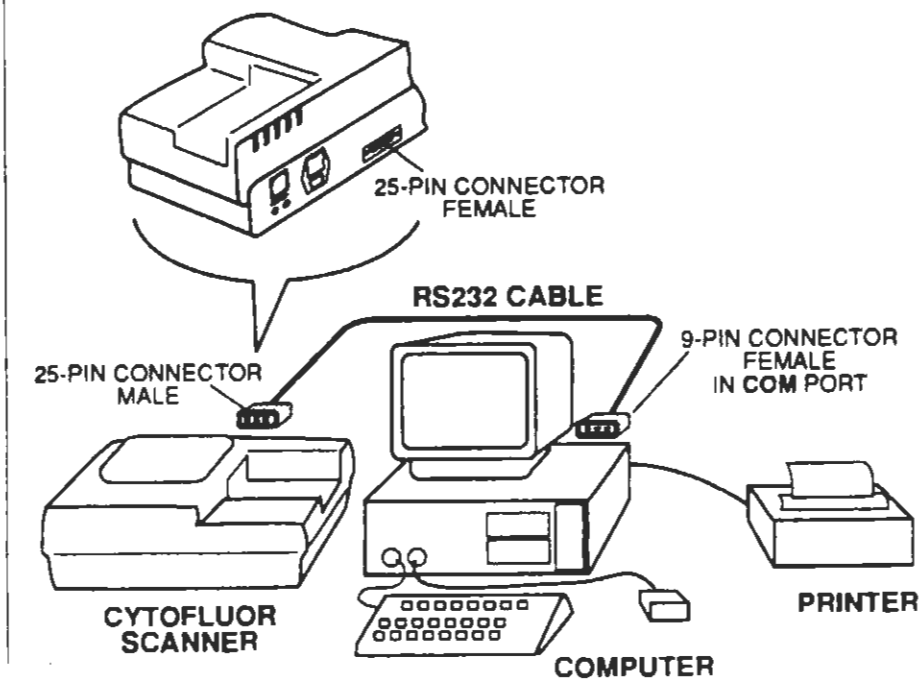
If anything is missing, contact Millipore Technical Service. Or, if you are located outside the United States of America, contact the Millipore office nearest to you. For a listing of telephone numbers for Millipore Technical Service and Millipore offices worldwide, see Chapter 7, *Technical Information*.

Now that you're sure you have all your equipment, follow the steps below to set up the system.

♦♦ ***Caution:*** Never attempt to connect equipment with any of the components plugged in and turned on.

1. Connect Display monitor and Printer to Computer

- Set up the system unit, display and printer according to the instructions in the hardware manuals supplied with your system.
- Follow the diagram below to make sure you have properly connected your equipment.



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- Connect the power cables to the computer and printer. Do not plug the power cables into electrical outlets yet.

2. Connect the Keyboard and Mouse to the Computer

- Plug the keyboard cable into the keyboard connector on the front of the system unit.

- Plug the mouse cable into the bus or serial port, or into the PS/2 mouse connector on the front of the system unit, whichever is appropriate for your system.

3. Check the Voltage on the CytoFluor 2300/2350 Scanner

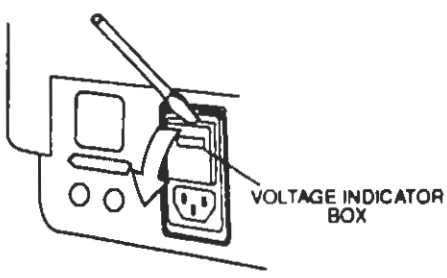
The voltage on the CytoFluor 2300/2350 Scanner is preset for the country of destination. The voltage is indicated on the rear of the scanner, in the small window in the box above the power cord socket. Normally you should not need to change the voltage unless, for example, your scanner was purchased in another country. If you do need to change it, follow the steps below:

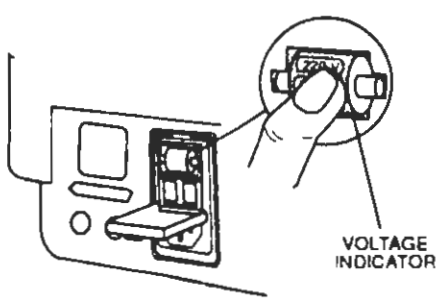
- Open the cover on the voltage indicator box with a screwdriver.

- Lift out the voltage indicator and choose the appropriate voltage for your power supply (120 V and 220 V are the most common).

- Replace the voltage indicator so that the correct voltage shows in the cover window.

- Close the cover by pressing on it until you hear a click.





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4. **Connect the CytoFluor 2300/2350 Scanner to the Computer**

- Plug the RS232 cable into Communication Port 1 or 2 on the computer system unit. Make a note of the port to which you have connected the CytoFluor Scanner. You will need to know this later, when you are configuring the CytoFluor software. (If you have a computer other than the one supplied with the CytoFluor System, make a note of the serial communication port you choose.)

- Connect the other end of the RS232 cable into the Serial Data Port on the rear of the Scanner.

- Connect the power cable to the Scanner.

5. **Plug in all Power Cords**

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

♦♦♦ ***Warning*: The CytoFluor 2300/2350 Fluorescence Measurement System, including all computer hardware, is intended to be electrically grounded. All components are equipped with grounding plugs; these plugs will only fit a grounded outlet. Do not adapt a grounded plug to a non-grounded one!**

Installing the Software

First, you need to install DOS 3.3 and Windows on your computer's hard disk, then install the CytoFluor System software. Follow the instructions below.

Install DOS 3.3 or Higher and Windows

- Install the computer operating software (DOS 3.3). Your computer must have either MSDOS or PCDOS installed before you can load the CytoFluor System software. Refer to the DOS guide that came with your System order for instructions.

- Install Windows

Install the CytoFluor System Software

This version of the CytoFluor system software can be run as a Windows 2.1 or 3.0 application or as a stand-alone that uses a dedicated (run-time) version of Windows 2.1. No matter which version is used, the CytoFluor system software must always be started in its own directory or you will not be able to access the plate database. The cyto.bat file that is copied into your root directory during installation performs this task automatically.

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If you wish to retain an older version of CytoFluor on your hard disk, first create a new directory (e.g., C:\OldCyto) and copy all CytoFluor files from the hard disk to the new directory (C:\OldCyto). Then delete all files in Cyto, and install your new version of CytoFluor. For more information, refer to the DOS manual that came with your system or telephone Millipore Technical Service.

♦♦ ***Caution:*** If you have more than one version of CytoFluor on your computer, remove Cyto from the path statement in the autoexec.bat file. Then manually bring up the version you wish to run, or use the batch file for the most frequently used version.

♦ *Note:* If you have Windows installed on your system, do not install the stand-alone version of CytoFluor. Go to page 2-9 and follow the instructions for installing CytoFluor as a Windows application.

Stand-Alone Version

To install CytoFluor as a stand-alone version you will need all 4 CytoFluor system software diskettes. The installation procedure first creates a directory, C:\CYTO, and loads the CytoFluor system software and the Windows support software. Then it installs a batch file—cyto.bat—in the root directory.

To begin:

1. Make sure the system operating software (DOS) is installed.

2. Turn on the display monitor, and then turn on the computer system unit.

3. Make backup copies of all four CytoFluor startup disks. (See your DOS guide for instructions on making copies of disks.) Store the originals in a safe place—free from dust or magnetic fields.

The CytoFluor System software contains a special version of Windows 2.1 (a "runtime" version) that is usable only as part of the CytoFluor System. The regular four-disk installation automatically loads this version of Windows for you. Use the following procedure whether you are loading the System software for the first time or updating an existing run-time CytoFluor System with new software. Do not, however, use this procedure if you already have Windows 2.1 or 3.0 installed; instead, see page 2-9.



Disk 1:

1. You should see the **C:** prompt on your screen. Insert disk number 1 into the A drive.

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2. Type *a:* to change the active drive to the A drive and press the ENTER key.

3. When the **A:** prompt appears, type *setup* and press the ENTER key.

   As the CytoFluor System software setup proceeds, the screen lists information about your system's configuration. If you purchased a complete CytoFluor System, the software shows the correct choice as a highlighted item in a list. (Usually this item is the first in the list.)

   In most cases, simply press the ENTER key at each screen to accept the preset configuration; however, each screen also explains the choices, which you'll probably want to read and perhaps make note of.

   The software will tell you when to insert the next disk.

♦ *Note:* If you make a mistake when you are installing the setup disks, you can restart the setup procedure at any time by pressing CTRL-X.

The table below outlines the system configuration items on Disk 1 and the corresponding preset values (also called "defaults").

Configuration Items	Preset to:
• drive and directory	• C:\cyto
• computer type	• IBM AT (or 100% compatible)
• VGA, keyboard, mouse	• No change

The software lists the devices it "thinks" you have. If any are incorrect, use the arrow keys to move the highlight to the item you want to check and press ENTER (for example, if you have a PS/2 mouse). You will then go to a screen where you can change the item if necessary.

• extended memory option	• E to add option

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Disk 2:
Continue to configure the system

Configuration Items:	Preset to:
• printer or plotter	• Install When you choose Install, you will see a screen with a list of printers. If you purchased the Complete CytoFluor 2300/2350 System, choose NEC 6200 as your printer. (Choose NEC 5200 if you don't see NEC 6200.) You will then see a list of ports to which your printer might be connected; choose LTP1.

♦ *Note:* You can add other printers at this time if you expect to be using them in addition to or instead of the NEC 6200. After choosing a printer and setting, you can repeat the same procedure to add other printer drivers. If the printer you want to add is not connected to the computer at this time, simply choose None from the list of ports after you select the printer. Later, when you connect the printer, you can specify its port using the Control Panel feature from the CytoFluor software.

• country setting	• United States



Disk 3:
Using this disk, the software adds fonts and other files to the directory. No interaction with the computer is needed.



Disk 4:
Continue to configure the system.

Configuration Items:	Preset to:
• modify autoexec.bat file	• y (yes) to modify file You *do not* want to modify the autoexec.bat file. Choose no, or use the arrow keys to

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change the highlight to C to continue, and then press ENTER

- run memory extending program
- m to run memset
  If this is a first-time setup, press ENTER. If you are upgrading, choose no, or use the arrow keys to change the highlight to C to continue without running memset, and then press ENTER.

4. If the CytoFluor System software was installed properly, a screen prompt will appear and you will automatically be returned to the C:\ prompt.

5. With disk 4 still in the A drive, type *a:1cyto.bat*.

This will install the cyto.bat file in the root directory. Installation is now complete. The cyto.bat file installed in your root directory will automatically bring up Windows and CytoFluor when you type *cyto*. Remember, you must be in the root directory (C:\) when using the *cyto* command to start up CytoFluor.

Windows Application Version

This procedure requires disk 4 only, and assumes that Windows 2.1 or 3.0 is installed in the directory C:\WINDOWS, and CytoFluor is in a subdirectory of Windows called (C:WINDOWS\CYTO). If this subdirectory does not exist, one will automatically be created.

1. Insert CytoFluor Disk 4 into the A drive.

2. Type *a:* to change to the A drive and press ENTER.

3. Type *install* and press ENTER.

4. If the CytoFluor System software was installed properly, a screen prompt will appear and you will automatically be returned to the C:\ prompt.

Under Windows 3.0, CytoFluor can be treated like any other Windows application. Please refer to your Window's User Guide for more information.

The installation is now complete. The cyto.bat file installed in your root directory will automatically bring up Windows and CytoFluor

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when you type *cyto*. Remember, you must be in the root directory (C:\) when using the *cyto* command to start up CytoFluor.

If you have problems installing the software, contact Millipore Technical Service. Or, if you are located outside the United States of America, contact the Millipore office nearest to you. For a listing of telephone numbers for Millipore Technical Service and Millipore offices worldwide, see Chapter 7, *Technical Information*.

Checking the System

Before starting to use the CytoFluor software, you must check the CytoFluor System, including the System calibration by comparing the values on the calibration. Compare the calibration certificate that came with your system against the settings in the System software. Each CytoFluor Scanner is unique and will not operate properly unless the values on the calibration certificate are loaded into the system software.

♦ *Note:* If you are unfamiliar with the Windows software, refer to the Windows manual that came with your system or see Appendix A for a brief introduction on using Windows. Then return to this section.

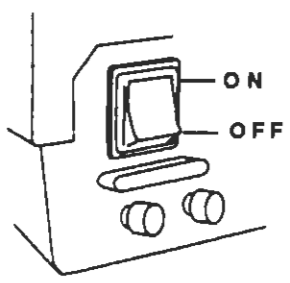
Check Scanner Settings

To start up the CytoFluor software, begin at the C: prompt.

1. Type *cyto* and press ENTER.

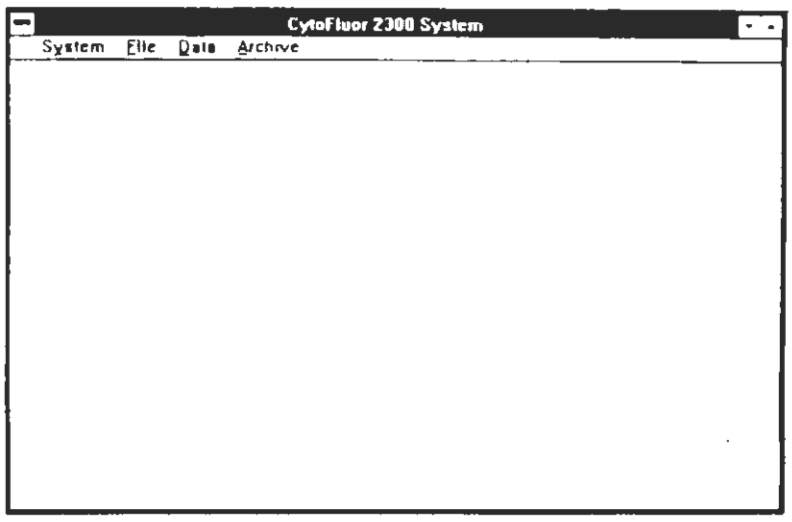
♦ *Note:* If you are accessing the CytoFluor System software directly from Windows, double-click cyto23.exe in the File Manager window (C:\WINDOWS\CYTO). You can also set up a CytoFluor and Windows application icon with the Windows Setup utility in the Windows main menu.

2. Turn on the scanner using the black power switch on the rear of the scanner.



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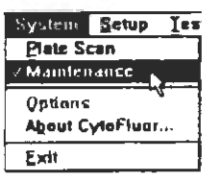
At this point you should see the CytoFluor window on your screen. Notice the menu titles along the top of the window.

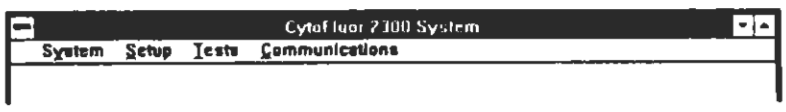




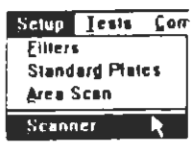
At the top left corner of the window you will notice a small box, which gives you access to the control panel. Access the control panel from the pull down menu and choose the printer you are connected to. This must be done, even if you have already done it through Windows, in order to set up the CytoFluor initialization file. Otherwise, you'll get an error message when you try to print. You only need to do this after installing or updating your software.

3. Pull down the **System** menu and choose **Maintenance**.



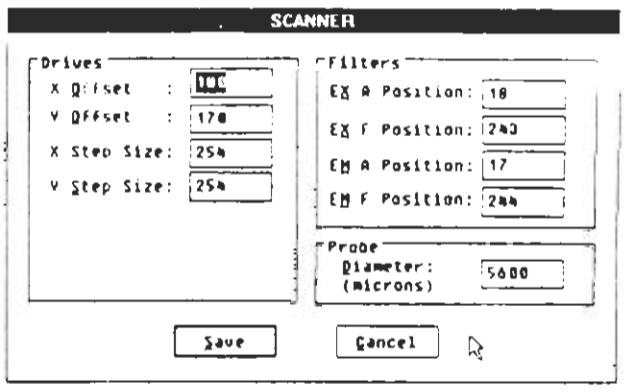


The CytoFluor software utilizes two modes: **Plate Scan** and **Maintenance.** You can always tell which mode you are using by pulling down the System menu and noting which of the choices has a check mark to the left of it. You can also tell which mode you're using by looking at the menu titles to the right of the System menu.



4. Pull down the **Setup** menu and choose **Scanner**. The Scanner dialog box, similar to the screen below, appears:

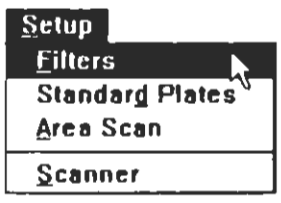
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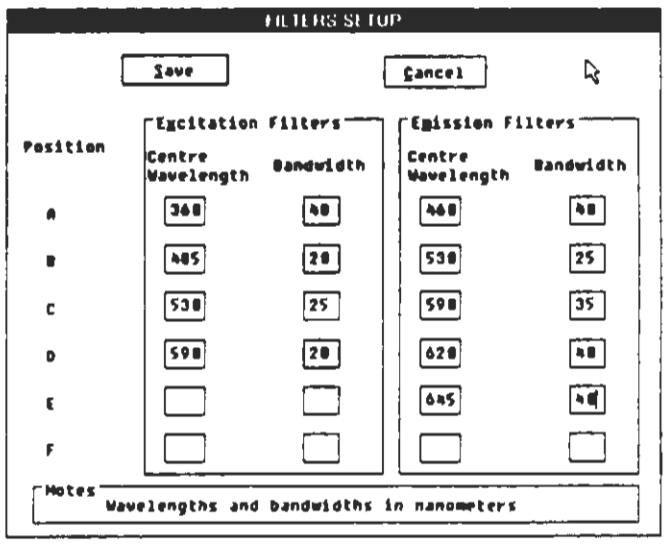
Make sure the values on your system calibration certificate match the values on your screen. If the numbers match, click CANCEL.

♦ *Note* Cancel will return you to your previous location. If you press CANCEL, changes made to these fields will not be saved; the original settings will be retained.

If the numbers do *not* match, enter the correct value from the calibration certificate into the software. Click inside the field you want to change and highlight the existing value by dragging the pointer across the area. Type the correct value. Click SAVE to keep your changes.



Pull down the **Setup** menu and choose **Filters**. The Filters dialog box, similar to the one below, appears:



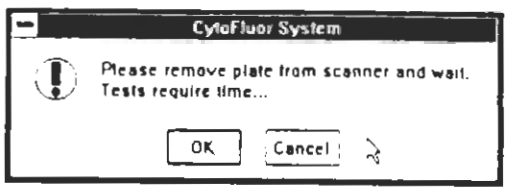
Make sure the filter values match those of the calibration certificate.

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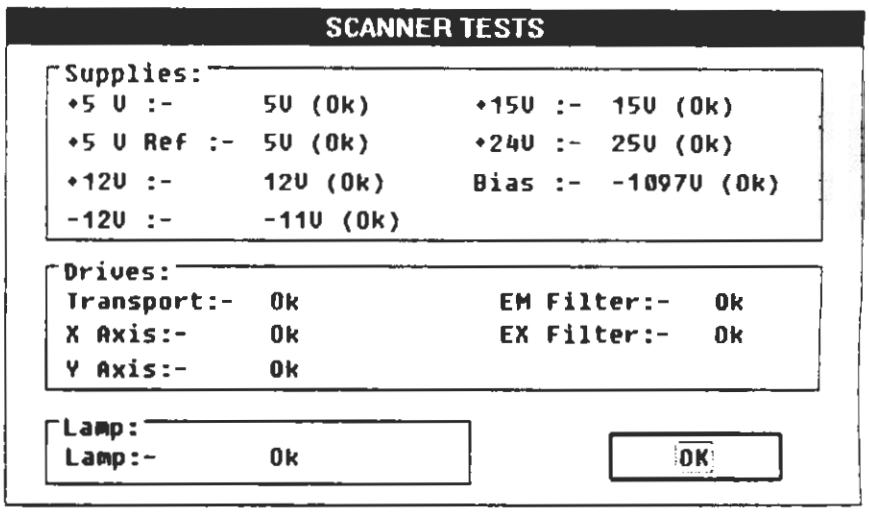
Run System Diagnostics



The CytoFluor System Software automatically checks the voltages, the scanner drives, and the lamps. Click the **Tests** menu title. You should then see the following screen:



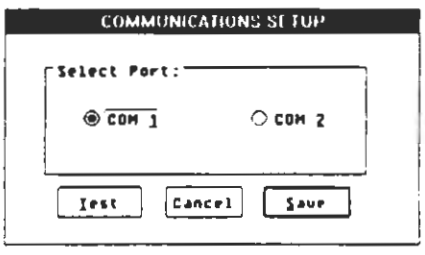
You must wait about 45 seconds for the software to check the system. You then see the following screen if the voltage supplies, scanner drives, and lamp are working properly:



Click OK to return to the main menu. If you do not see this screen, contact Millipore Technical Service. Or, if you are located outside the United States of America, contact the Millipore office nearest to you. For a listing of telephone numbers for Millipore Technical Service and Millipore offices worldwide, see Chapter 7, *Technical Information*.

Check Communication Port Settings

1. Click the **Communications** menu title. You should see the following screen:



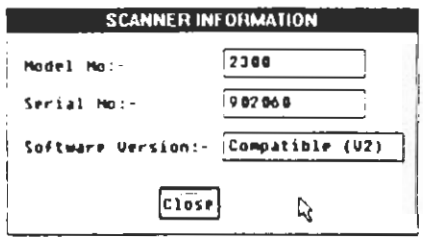
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If you have more than one serial port, make a note of the port to which the scanner is physically connected. If you have only one serial port, the software should show the scanner connected to Communication Port 1 (COM 1).

2 If the scanner is connected to Communication Port 1, click CANCEL. If the scanner is connected to Communication Port 2, click COM 2 and then click SAVE.

Check System Registration Numbers

1. Click the Communications menu title, and then click the **Test** button. You should see a screen similar to the one below:



2. Check this screen against the panel on the rear of the scanner that displays the CytoFluor model and serial numbers.

3. When you are satisfied that your system is confirmed, click OK.

If you have a problem, contact Millipore Technical Service. Or, if you are located outside the United States of America, contact the Millipore office nearest to you. For a listing of telephone numbers for Millipore Technical Service and Millipore offices worldwide, see Chapter 7, *Technical Information*.

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Chapter 3
PREPARING FILES

Before you can scan a plate with the CytoFluor System, you must create a file for your data. You set up files and scan plates in Plate Scan mode (choose **Plate Scan** from the **System** menu).

In this chapter you'll learn how to configure a scan and how to

- name a file and add other identifiers
- define the microtiter plate used
- define filter sets
- set the sensitivity level for each scan
- choose the number of scans per plate
- set the interval between scans
- save your file
- make duplicates of a file once it's defined, so you don't have to retype information over again for assays which use identical plates and settings.

Creating New Files

To begin, make sure your computer and scanner are turned on.

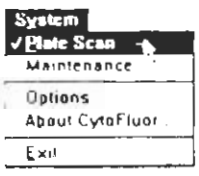
Before starting up the CytoFluor System Software, you might want to create a new directory to hold the files you make. At startup, the system always uses the Cyto directory. It's fine to save your files in this directory, but it can become very full and difficult to use as a result. By creating other directories, you can keep track of files more easily. To create a directory, follow the instructions in the manuals that came with your Windows or DOS software. You change directories once you are working in the CytoFluor software.

To create a new CytoFluor file, follow these steps:

1. At the **C:** prompt, type *cyto* and press ENTER.

♦ *Note:* If you are accessing the CytoFluor System software from Windows, simply double-click the cyto23.exe file in the Cyto directory. By default, the CytoFluor comes up in the Plate Scan mode.

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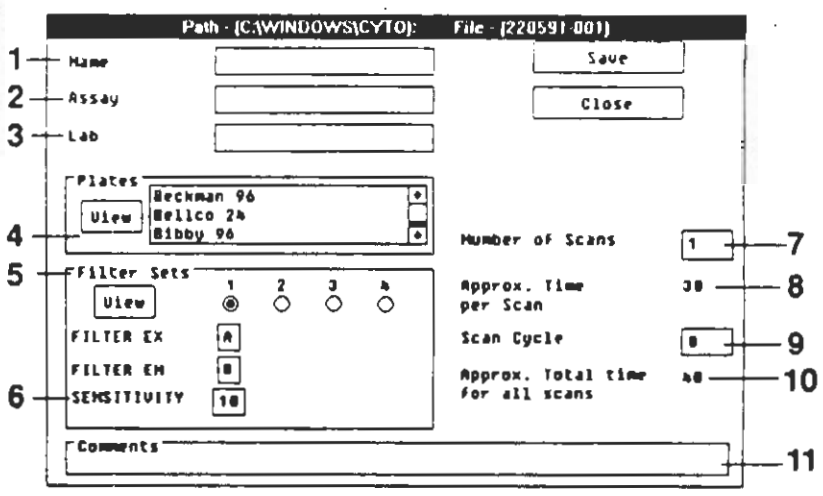


2 Pull down the **System** menu and choose **Plate Scan**. When the software is in Plate Scan mode, the Plate Scan item has a check mark beside it in the System menu, and you see the following menu titles in the title bar:





3. Pull down the **File** menu and choose **New.** You should see the following dialog box:



♦ *Note:* The file path (C:\WINDOWS\CYTO) is displayed in the title bar at the top of the screen. The file's identifying number is to the right; in the example shown, this is the first file (-001) created on May 22, 1991 (220591).

If you want to change the directory at this point, you can pull down the File menu and choose Open. A dialog box will appear with a list of files in the current directory (Cyto). You can click the two dots (••) to move upward in the directory levels (you might have to use the scroll bar to find the two dots). Then, click the name of the directory you want to change to, and click Cancel to open it. You will automatically be returned to the main CytoFluor System screen. When you pull down File and choose New, your new path will be displayed. This way, you can save data in directories that are keyed to your experiments without loading up the Cyto directory. See your DOS Manual for information on how to creat new directories.

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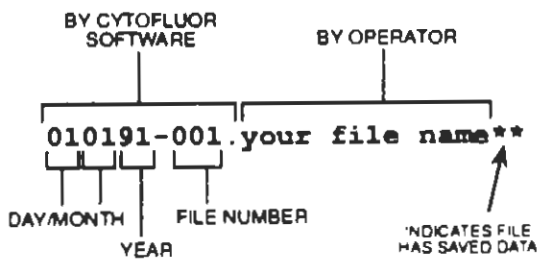
You are now ready to create a file and configure a scan. You can create as many new files as you wish without scanning data. But for right now, just create one new file. First, read through the information below for a description of each field in this dialog box. Then start back at the first item and name your file.

1 - Name

Click in the Name field to set an insertion point, and then type a name to identify your experiment. You can name the file anything you want, using any combination of letters and numbers. However, the name must be no more than 20 characters. This field must be filled in.

♦ *Note* Once you have completed a scan and data have been saved to this file, you will not be able to change the name or other information in most fields. This feature prevents data from accidently being written over and lost.

In addition to the name that you assign to the file, the CytoFluor tracks the file by assigning the date and a file number (from 1 to 999) to each file that is created. See the example below for a typical file name as stored in the CytoFluor datafile listing. You can see the active directory listing by choosing Open from the File menu.



2 and 3 - Assay and Lab Fields

After naming your file, you can add optional details about your laboratory and particular assay in the Lab and Assay fields. These details will be transferred with the scan data to your spreadsheet program. To enter data in these fields, position the pointer over the field and click. You can enter any information you wish in this field, as long as your descriptive text is no more than 20 characters, including spaces. As with the Name field, you cannot change the information in these fields once you have scanned your plate and saved the data.

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4 - Plates
In this section you can choose any of the standard plates you see in the list box. The standard plates offered with the CytoFluor system are listed below.

- Beckman 96
- Bellco 24
- Bibby 96
- Cel-Cult 96
- Corning 6
- Corning 12
- Corning 24
- Corning 96
- Corning U-Well 96
- CoStar 24
- CoStar 48
- CoStar 96
- Demo 96/2
- Dynatec 96
- Dynatec S-Well 96
- Elkay 24
- Elkay 96/1
- Elkay 96/2
- Falcon 6
- Falcon 24
- Falcon 96
- Gibco 24
- Gibco 96
- Inbro 96 Flat-Well
- Inbro 96 U-Well
- Inbro 96 V-Well
- Millipore 96
- Nunc 96
- Nunclon 96/1
- Nunclon 96/2

Scroll through the list by clicking the up arrow or down arrow. Click the name of the plate you want to select. As with the previous fields, once data has been saved you cannot change this information.

By clicking the VIEW button, you can see the dimensions and other identifying characteristics of the plate you have chosen. Click CANCEL to return to the CytoFluor Scan Setup screen. If you do not see the plate you want, you can add plates. For more information, refer to Chapter 5, *Customizing*.

5 - Filter Sets
Each filter set corresponds to an excitation and emission filter pair. CytoFluor excitation and emission filters are located in two separate filter wheels and can be chosen in any allowable (non-overlapping) combination. The standard choices offered with the CytoFluor system are listed below.

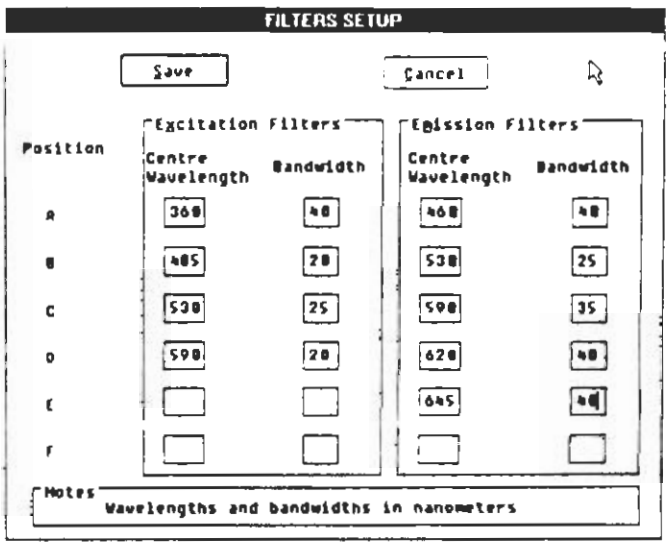
Excitation Center Wavelength*/ Half Bandwidth	Emission Center Wavelength*/ Half Bandwidth
• 360/40	• 460/25
• 485/20	• 530/30
• 530/25	• 590/35

*center wavelengths measured in nanometers

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- 590/20
- 620/40
- 645/40

If you want to check the filter sets available, click the VIEW button in the Filter Sets dialog box. After reviewing the filter and their positions, click CANCEL to return to the Scan Setup screen.



You are now ready to define the first set of filters in the Filter Sets field. You should see four buttons and two blank boxes below each button. Click the box for the excitation filter to select it, and then use the scroll bar at the right to change the letter in the box. Do the same with the second box to change the emission filter. Specify additional filter sets in the same way. Thus, each scan may consist of up to four passes (sets) across the entire plate, each pass with a unique filter set/sensitivity combination.

Optical Sets for Fluorochromes

Choose the appropriate filter set from the suggested filter combinations in the table that follows.

Dye	Ex/Em Pair	Application
Methylumbelliferone	360/460	Immunoassay
Hydroxymethylcoumarine	360/460	Immunoassay
Prodan	360/460	Membrane Probe
Laurodan	360/460	Membrane Probe
Calcein	485/530	Viability Assays

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Optical Sets for Fluorochromes (Con't.)

Dye	Ex/Em Pair	Application
Attophos™	450/580	Immunoassay
CFDA-AM	485/560	Plasma Membrane Disruption
CFDA	485/535	Extracellular pH
B. Phycoerythrin	485/590	Gen. Fluorescent Tag
DPH	360/460	Membrane Probe
DAPI	360/460	Nucleic Acid Stain
Quin-2	360/482	Ion Concentration
Indo-1	360/482	Ion Concentration
Rhod-2	360/482	Ion Concentration
Thiolyte	360/460	Gen. Fluorescent Tag
Lucifer Yellow	440/530	Gap Junc'n. Comm'n.
Ben Zoxanthene Yellow	360/482	Gen. Fluorescent Tag
Dansyl P. E.	360/530	Gen. Fluorescent Tag
BCECF	485/530	Vital Dye, pH Ind.
Carboxy Fluorescein	485/530	Vital Dye, pH Indicator
Fluo-3	508/20 560/20	Ca^{2+} *Concentration*
SNAFL (in acid)	485/530	pH Indicator
Acridine Orange.	485/530	Nucleic Acid Stain
Rhodamine 123	482/530	Mitochondria Stain
DTAF	485/530	Alcohol Rctv. Probe
Ethidium Homodimer	485/530 485/645	Nucleic Acid Stain Dual Wavelength Monit.
7-AAD	485/645	Nucleic Acid Stain
Ethidium Bromide (in sol'n.)	485/645	Nucleic Acid Stain
SNAFL (in base)	530/620	pH Indicator
SNARFI	530/620	pH Indicator
TRITC	530/620	Gen. Fluorescent Tag
Propidium Iodide (in sol'n)	485/645	Nucleic Acid Stain
Propidium Iodide (in sol'n)	530/645	(for dead cells)
Texas Red	590/645	Gen. Fluorescent Tag
Allophycocyanine	590/645	Gen. Fluorescent Tag
Neutral Red	530/645	Lysosomal Integrity
R. Phycoenythrin	485/590	Gen. Fluorescent Tag
Sodium Fluoroscein	485/530	Monolayer Permeability
Thiozole Orange	485/560	Nucleic Acid Stain
Bodipy™	485/530	Gen. Fluorescent Tag

The CytoFluor software has a built-in safety feature—it will not allow you to choose incompatible filter sets or filter sets that overlap enough to damage the photomultiplier tube.

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If you are using custom filters, be sure to update the software (Maintenance menu) with the correct positions of these filters. The filter compatibility checking feature in the CytoFluor System depends on correct filter parameters.

♦♦ ***Caution:*** Incorrectly specifying filters can cause irreparable damage to the CytoFluor System. You will void the CytoFluor warranty if you run a scan with incompatible filter sets.

If you *do* choose incompatible filter sets, you'll see the following error message:



If you change your mind and want to eliminate filter sets, simply click on the button above the last filter set (from the right) that you want. You can continue to change this information as long as you have not scanned a plate and saved the data. Once data is saved, the Filter Sets field cannot be changed.

6 - Sensitivity

If you wish, you can adjust the voltage on the phomultiplier tube by setting the Sensitivity Level for each scan. This value ranges from 0 to 10. When the Sensitivity Level is at 0, the photomultiplier tube is off.

The fluorescence signal level may vary from scan to scan depending on your assay and fluorochrome. It is best to start off low and gradually increase the sensitivity until you find the optimum level(s) for your assay. When creating a file for a new assay, define all four filter sets as the same, but set different sensitivity levels for each filter set. In this way you can quickly determine the best sensitivity level for your particular experiment. You may find, for example, that you require two sensitivity ranges to bracket the dynamic range of your experiment.

To set the sensitivity level, click the Sensitivity Level box below each filter set; then use the scroll bar on the right to change the value (0–10). Once the file has been saved with data, you cannot change the sensitivity level.

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♦♦ ***Caution*** If you choose to use overlapping filter sets (previously scanned), setting the sensitivity level at 9 or 10 can irreparably damage the system and you will void your warranty.

7 - Number of Scans
The Number of Scans is the number of times you want the plate to be scanned by the CytoFluor System. You can enter any number from 1 to 60. If you do not enter any number, the CytoFluor system scans once. Once you have saved the data from a scan you cannot change the information in this field.

8 - Approximate Time Per Scan
The value in this field is always supplied by the CytoFluor System. The software calculates the approximate time it will take for the System to complete each scan. This calculation is based on the type and and size of the plate, number of wells, and the interval between scans. You can't type a value in this field.

9 - Scan Cycle
The Scan Cycle is the time (in seconds) per scan plus any delay between scans. If you are scanning a plate more than once, you must set the interval in seconds. Set the Scan Cycle value to equal or exceed the Approximate Time per Scan value. You can use this feature, for example, to scan at intervals during an ongoing biochemical process.

10 - Approximate Total Time
The Approximate Total Time is the amount of time in seconds it will take for all scans to be completed. The CytoFluor software calculates this value based on the number of scans and the interval between scans. You can't type a value in this field.

11 - Comments
This optional field is for comments about your assay. Include information that will help you identify the scan later, such as the date and time that you ran the scan. You can enter any combination of numbers and letters up to 50 characters, including spaces. You can always make changes to the Comments field.

Saving the File

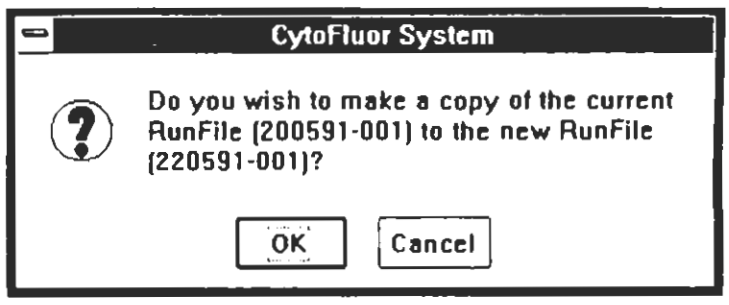
Now that you've created a file, you're ready to scan a plate; but first you must save the file. To save the file, click the SAVE button. You should see a rectangle appear around the word *Save*, indicating the file has been saved. You should also see the SCAN button appear just below the other buttons. If you have incorrectly filled in the scan setup, a prompt will be displayed.

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You can still make changes to the fields after you have saved the file, as long as it doesn't contain data from a scan. Once you save file after data has been taken, you won't be able to change any fields except the Comments field

Duplicating a Template File

The CytoFluor software provides an easy way for you to set up several files at once, all with identical configurations, so that if you plan to scan several plates that use the same settings, you don't have to retype the information. To copy the setup of a file, open the file which is set up in the manner you wish to copy. Then use the Duplicate command in the File menu. When you choose Duplicate, you see a dialog box like the one shown below



♦ *Note:* A run file is the type of file the CytoFluor System creates. The run files you saved are listed in the DOS or Windows directory as an alphanumeric code with .RUN at the end. The run files can be identified and read only by the CytoFluor software.

To duplicate the file, click the OK button. The new, duplicated file automatically appears on your screen with no data attached, so that you can customize your screen for this day's run. The duplicated file is always assigned an unique identifying number, so it can be distinguished from the original, even if the files have the same name. When you use the Duplicate command to copy the configuration of an existing file, only the settings are copied, not the data values.

To close the File dialog box after saving your file, click the CLOSE button. Now go to Chapter 4, *Scanning*, to learn how to scan a plate and collect fluorescence data.

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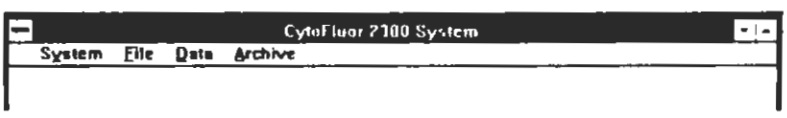
Chapter 4
SCANNING

Scanning

In this chapter you'll learn how to scan a plate and save the data into a file. Before you start make sure you have:

- read chapter 3, *Preparing Files*
- set the path directory
- created a file with filter sets that correspond to your assay
- prepared a sample for your assay

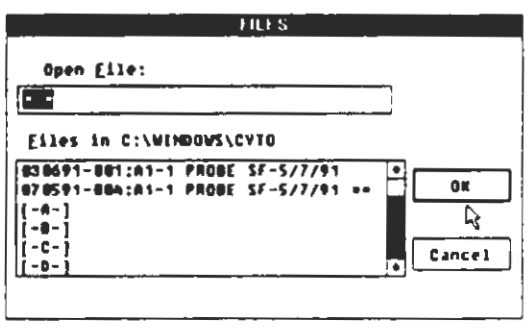
Make sure your system is on and the CytoFluor software is operating before proceeding. Refer to page 3-1, if necessary, for instructions on how to enter the CytoFluor software. You should see the following screen:



Notice the menu titles along the top of the window; they indicate you are in Plate Scan mode. If you see different titles, pull down the **System** menu and choose **Plate Scan.**



1. Pull down the **File** menu and click **Open.** You should see a dialog box similar to the one below:



The list box, with a scroll bar on the right side, contains a list of files in the directory named just above the box.

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2 Click the name of the file you previously created

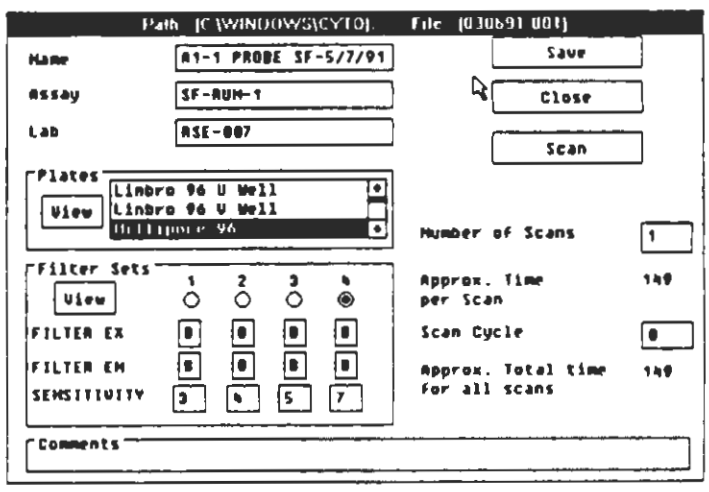
If you don't see the name, you might need to click the arrow buttons to scroll up or down the list. You can choose other drives by clicking their names, and you can move backward in the directory levels by clicking the two dots (••) at the bottom of the list. The current directory name is always displayed just above the list box. If you click on OK, the directory that is displayed in the dialog box will become the default destination for all subsequent CytoFluor data.

When you click the appropriate file name, it appears in the Open File Name box. (You can change your mind and choose other files just by clicking a different name.)

You would click the CANCEL button if you decided you didn't want to open any of the existing files.

♦ *Note:* Files that already contain saved data have two asterisks (**) to the right of their names. You can only read these files. To set up a new scan, use Duplicate or choose New from the File pulldown menu.

3. When you have selected a file, click the OK button or press ENTER. You should see the CytoFluor Setup screen fully configured and similar to the one below:



This File dialog box is the same as the box you saw earlier, when you prepared this file and saved it.

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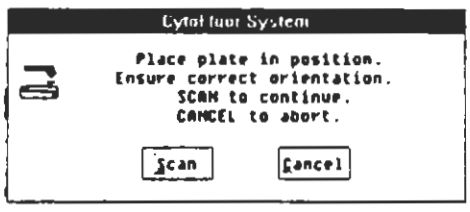
♦ *Note.* One scan can be configured with up to four separate se : of data, each covering the entire plate. In the example above, one scan has been configured. It consists of four complete passes of the plate, each with the same filter pair, but with different sensitivities. The active directory is C:\WINDOWS\CYTO and this is the first file (-001) that was run on June 3, 1991 (030691).

4. Make sure the plate transport mechanism is in the load position—outside the measurement chamber. If it's not, press and hold the green button on the rear of the scanner until the plate transport mechanism moves to the load position and stops.

5. Slide your plate into the plate transport mechanism. Make sure the plate is fully seated in the transport mechanism and Well 1A is at the top right (the top is towards the back of the CytoFluor).

   If you try to scan without a plate, the instrument will find the plate is missing. It will then return the transport mechanism to the load position and an error message will be displayed.

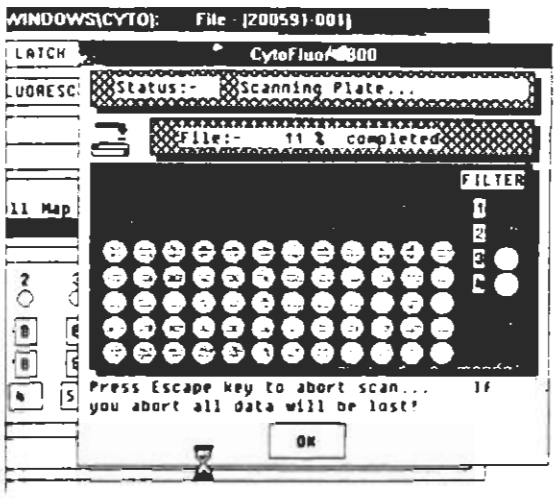
6. When the plate is in the correct position, click the Scan button to continue. You should see the following alert box:



7. Click Scan to begin scanning. If you want to cancel the scan before the System has started to scan, click Cancel. Once you have clicked Scan, you'll see the scanning status window:

   As each row is scanned, you see the scanner's progress represented in the window. The percent of the scan completed is displayed and updated as the System proceeds. You can cancel the scan at any time by pressing the ESCAPE key. However, if you choose to cancel the scan before it has been completed, you will lose all your data for this scan.

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8. When the scan has been completed, the plate transport mechanism will reset. Click the OK button in the scanning status window and you should see the File dialog box once again.

9. Click the Save button in the File dialog box.

You have just created a CytoFluor run file with data. If you click on File and choose Open, this file will appear in the list, followed by **, indicating that data have been stored in the file. This raw data file is the source file for all subsequent file manipulations (export, .CSV, print, background subtract) and remains unaltered until you delete it. Leave the file displayed on your screen so you can look at the results.

Displaying your Results

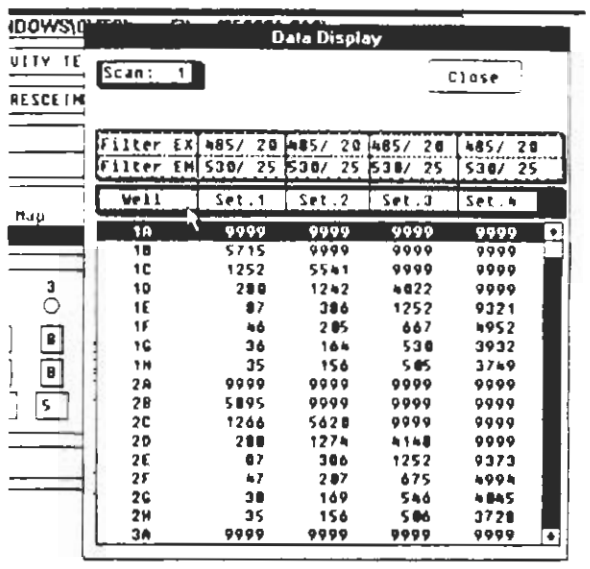
You can display the data on screen, export it as a comma-separated values (.CSV) file to a spreadsheet for analysis, or print it. If you do not want to save the file but do want to keep the data, export or print your results first, then cancel the scan. (However, you can delete old files at any later time, so saving the file does no harm and may avoid later regret.)

♦ *Note:* You can print several files at a time and transfer files to another disk using commands in the Archive menu, discussed later in this chapter



1. With your file open, pull down the **Data** menu and choose **Display.** You should see a screen similar to the one below

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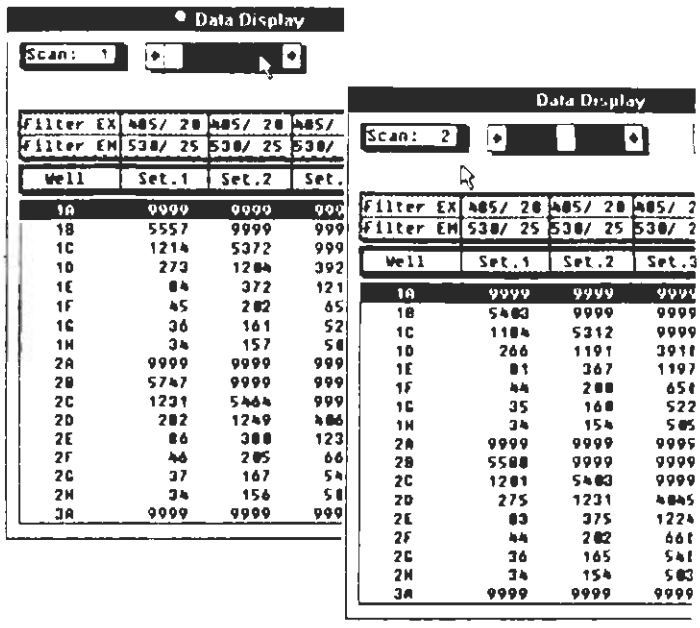


Each well will be listed in the far left column of the table. The data for each filter set—from 1 to 4—will be shown in the four columns to the right. If you used fewer than four sets of filters in your scans, these columns will be blank.

2. To view your data, use the vertical scroll bar to move through the table. For example, if you wanted to view the results for Well 6B, you would scroll through the table until Well 6B appeared. Then you would read values for each filter set across the table. In the example below, the values for each filter set are different because the sensitivity levels were set differently.

   If you have scanned the plate more than once, you can view the results of each scan by scrolling through the *horizontal* scroll bar at the top of the table. If you have only one scan, the horizontal scroll bar will not appear. To see each successive scan (up to 60) move the scroll bar to the right.

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♦ *Note:* **Display** is a view-only menu; you will not be able to change the data.

Using Background Subtract

The CytoFluor System always measures raw data from the wells. Using the Background Subtract feature, you can then view or print the data with the value of background wells subtracted. This background value is the average of the raw data from the wells you mark for subtraction: a row, a column, or a single well.

1. With the Data Display box still on your screen, pull down the **System** menu and choose **Options.** You should see the box shown below:



The Background Subtract controls are in the lower part of the dialog box.

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2. Use the scroll bars to change the values in the Row and Column boxes.

3. Use the buttons on the right side to select whether a row, column, or single well is to be subtracted. For demonstration purposes with your newly scanned file, click Row.

   If you wanted to subtract a column instead, you would click the Column button. To subtract a single well, you would click the Well button. If Row is selected, the Column setting is ignored, and vice versa. If Well is selected, the well given by both the Row and the Column setting is subtracted. (Clicking the None turns this feature off.)

♦ *Note:* This dialog box also contains the Lamp On and Lamp Off controls. Normally the lamp is on for assays, but you might have special circumstances in which you want to try to measure emission in the absence of excitation. Whenever the lamp is off, you'll always see the words "Lamp Off" in the CytoFluor 2300/2350 System title bar and in any file display or printout.

4. Click OK to close the dialog box.

Notice that the values have changed in the Data Display window. You should also see two new buttons, Raw Data and Subtracted Data, at the top of the display window. You can switch from one set of values to the other simply by clicking the buttons. A printout with a background value subtracted will also list which row, column, or well has been used.

Background subtracted data can be printed out and exported (.CSV) as long as the Data Display dialog box is open and contains the data that you wish to export. If this window is not open, the raw data only will be printed or exported by default. This is true even though the background subtract feature in the Options menu is enabled.

5. Pull down the **System** menu again and choose **Background Subtract** a second time.

6. Click the NONE button in the dialog box, and then click OK. The Raw Data and Subtracted Data buttons disappear from the Data Display window, and only the raw data values appear.

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You don't have to change the Background Subtract setting before you close a file if you would rather keep the setting active; the original values are never changed.

7. When you are finished viewing your results, close the Data Display box by clicking the Close button.

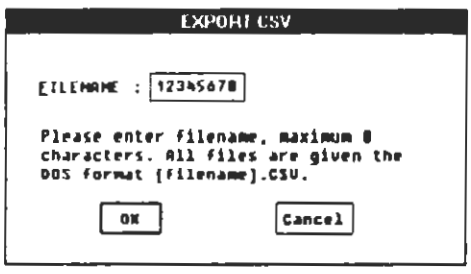
The values resulting from Background Subtract are not saved once you close the file; only raw data is stored in the CytoFluor run files.

Exporting Files



Millipore recommends the Microsoft Excel spreadsheet program because of its compatibility with the CytoFluor software and its ease of use. To export your files to a spreadsheet-compatible file:

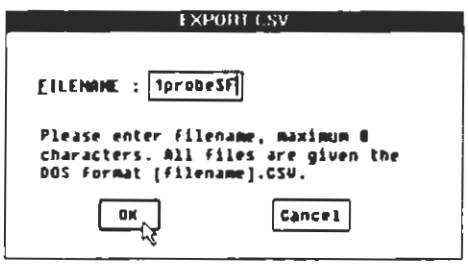
1. With your CytoFluor data file open, pull down the **File** menu and click **Export.CSV**. You will see the following screen:



♦ *Note:* *CSV* denotes *Comma Separated Values*, the file type required for most spreadsheet programs.

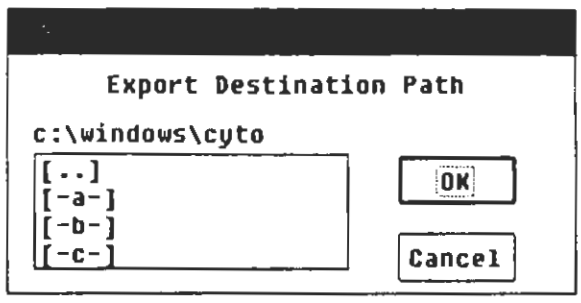
To export background-subtracted data values, the display/data dialog box must be open, with the background-subtracted data displayed.

2. Enter a name for your new export file. The name can be any combination of characters and letters up to 8 characters long, without spaces. The extension .CSV is automatically added to the file name when it is saved.



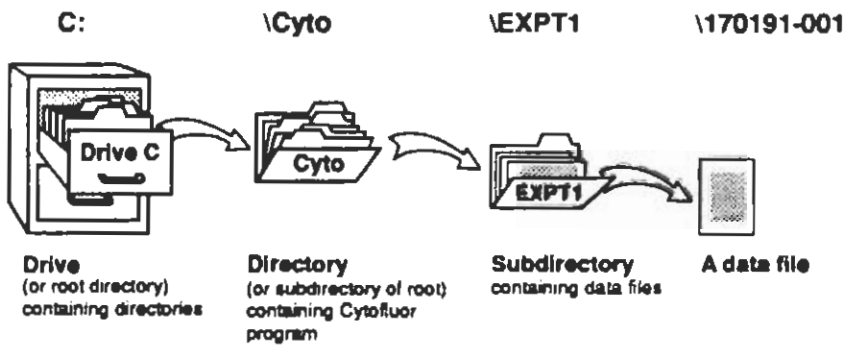
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3. When you are satisfied with the name you chose for your file, click OK or press ENTER. You then see the Export Destination Path dialog box:



The preset destination for the exported file is the drive and directory in which you're working. To choose another drive, you would scroll through the list and double-click the name of the drive. You then see the list of directories available on that drive, as well as the names of the other drives you may have available. You can save or export .CSV files directly to a floppy disk or hard disk. You could also choose a directory for the file in the same way—by double-clicking its name.

♦ *Note:* Millipore strongly recommends that you don't save data files (.RUN, .CSV) in the Cyto directory. Saving files in the Cyto directory wastes computer memory each time CytoFluor is invoked.



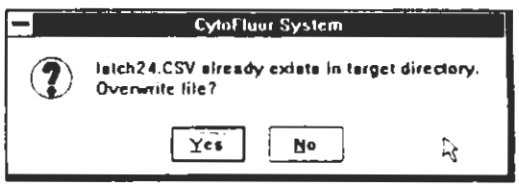
The figure below shows the relationships between drives, directories, and files.

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Notice the two-dot symbol (• •) at the top of the list box. If you double-click this symbol, you move backward in the directory levels. Refer to the DOS manual for a more in-depth discussion of the DOS directory structure.

4. When you're satisfied with your choice of destination, click the OK button.

If a file already exists with the name you have chosen, you'll see a box like the one below:



You have the option of overwriting the existing file with the new one, but be very sure you want to do this before you click OK. If you're not sure, it's best to click NO and then try a different name.

5. To access the .CSV file, you must leave the CytoFluor program and open your spreadsheet program from the **C:** prompt. For more information on accessing files from your particular spreadsheet program, refer to the appropriate manual that came with your spreadsheet software. The CytoFluor software cannot read .CSV files.

Printing your Results

You can print your results from the CytoFluor software or from your spreadsheet program. For instructions on printing from your spreadsheet program, refer to the manual that came with your spreadsheet software.

The CytoFluor software offers you two ways to print files: one file at a time with the File dialog box open, and one or more files in a batch using the Archive menu (discussed shortly).

To print the data from your new CytoFluor data file:

1. Turn on your printer.



2. With your file open and either raw data or data with a background value subtracted displayed, pull down the **Data** menu and click **Print.**

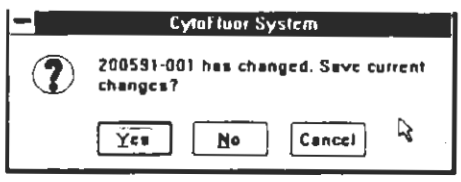
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The spooler will send the file to the printer you specified.

3. If you wish to cancel printing before the file prints, click Cancel.

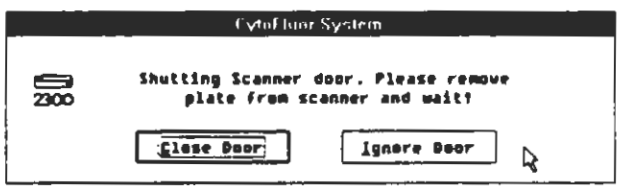
Closing Files

To close the file you've been working in, click the close button. If you have made changes and have not saved this file beforehand, you'll see a warning box:



Once you click YES to save changes, you won't be able to overwrite this file with another scan or change any of its information. If you want to use this file setup at a later time without saving the data, click NO or press N. You can open this file again when you are ready to use it, since it won't contain any data. Click CANCEL if you decide you are not finished working with the file.

When you are ready to leave the CytoFluor software, pull down the System menu and click Exit. You should see the following screen:



Click the CLOSE DOOR button to park the plate transport mechanism inside the CytoFluor 2300/2350 scanner. Click IGNORE DOOR to keep the plate transport mechanism outside the scanner. Millipore recommends that you always close the door when you are shutting down the CytoFluor System to protect the optic probe from dust.

You can also park the plate transport mechanism by pressing the red button on the rear of the scanner.

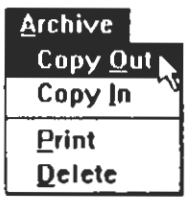
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Using the Archive Functions

As you create more files in the course of working with the CytoFluor System, you'll probably need to copy groups of files, print out a number of files at a time, or remove outdated files from your hard disk. The Archive menu in Plate Scan mode provides commands for working with files. Only CytoFluor data files (*.run) are affected by the archive function.
The Archive menu items are available only when you are not working with a particular file—that is, no file should be open on your screen.

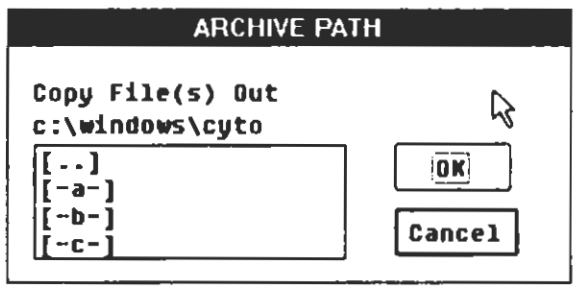
Copying Files

You can copy files out of your current working directory to another destination, such as a backup disk or other directory, or copy files into your current working directory from another source. To change the current directory, you must use File/Open and trace through the file path using the (••) in the files dialog box.



Copy Out

1. To copy files from your current directory, pull down the **Archive** menu and choose **Copy Out**. You should see the Archive Path dialog box:



   The current directory, from which you copy files, is the source. The name of your destination, the target directory, appears above the scrolling list box. It will change as you trace the path (••) or choose another drive.

2. Click a name in the scrolling list to select a destination.

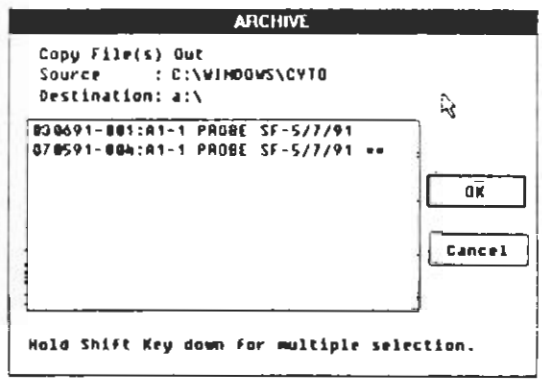
   The *destination* can be a different drive or a different directory on your hard disk; it can't be the same as your current directory.

   To move backward in the directory levels, double-click the two dots (••) in the list box.

3. When the destination is shown above the list box, click OK.

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You then see an Archive window, similar to the example below:



At the top of the window you see the source and the destination for the files. Choose the files you want to copy from the list in the scroll box.

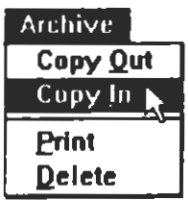
4. Click the names of all the files you want to copy. You can choose multiple files by holding down the Shift key while you click the names.

♦ *Note:* A single click will choose (highlight) a file. A second click on the same file will de-select it (remove the highlight).

5. When the file or files are selected, click OK to copy them to the destination.

The files are copied as soon as you click OK. To check that they are where you wanted them to go, you can use the Open command in the File menu.

Copy In



1. To copy files into your current directory, pull down the **Archive** menu and choose **Copy In.**

You should see the Archive Path dialog box, as was shown in the previous section. The name of your current directory is the default destination to which you are copying files. The name of the source directory appears above the scrolling list box.

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2. Click a name in the scrolling list to select the source from which you are copying files. The source can be a different drive or a different directory on your hard disk; it can't be the same as your current directory.

To move backward in the directory levels, double-click the two dots (••) in the list box.

3. When the desired source name is shown above the list box, click OK.

   You then see an Archive window, similar to the example shown in the previous section. At the top of the window you see the source and the file destinations. Choose the files you want to copy from the list in the scrolling box.

4. Click the names of the files you want to copy. You can choose multiple files by holding down the Shift key while you click the names.

5. When the file or files are selected, click OK to copy them to the destination.

The files are copied as soon as you click OK. To check that they are where you wanted them to go, you can use the Open command in the File menu.

Printing Files



1. To print files, pull down the **Archive** menu and choose **Print.**

   You should see the Archive Path dialog box. You can print files from any drive or directory by selecting the directory in this box. If the files are in your current directory, you don't need to make any changes. You can print only raw data using the Print command in the Archive menu.

2. If you need to change the drive or directory, click the name of the drive or directory you want in the scrolling list. Click the two dots (••) to move backward in the directory levels.

3. When the name of the directory you want appears above the scrolling list box, click OK.

   In the Archive window, the name of the directory you chose is listed as the source. (There is no destination directory listed because the destination is the printer.)

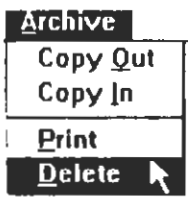
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4. Click the name of the file you want to print; to choose several files, hold down the Shift key while you click their names.

5. Click OK.

   The file you want to print is sent to the spooler.

Deleting Files



1. To delete files, pull down the **Archive** menu and choose **Delete.**

   You should see the Archive Path dialog box. You can delete files from any drive or directory by selecting the directory in this box. If the files are in your current directory, you don't need to make any changes.

2. If you need to change the drive or directory, click the name of the drive or directory you want in the scrolling list. Click the two dots (••) to move backward in the directory levels.

3. When the name of the directory you want appears above the scrolling list box, click OK.

   You see the Archive window. The name of the directory you chose is listed as the source. (There is no destination directory listed because the files are being deleted.)

4. Click the name of the file you want to delete; to choose several files, hold down the Shift key while you click their names.

♦ *Note:* Deleted files are gone forever unless you have made a back-up copy. Be sure that you have back-up copies of important files before you delete them. If you are not sure whether a file is worth saving, it's a good idea to make a copy anyway.

5. Click OK.

   The file is deleted. You can check that it's gone by using the Open command in the File menu.

The next chapter explains ways that you can customize your CytoFluor System.

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Chapter 5
CUSTOMIZING

This chapter discusses the ways you can customize your CytoFluor 2300/2350 Fluorescence Measurement System. You'll also find information on checking and maintaining the System.

In this chapter you'll learn how to:

- set up and change filter sets
- change and add standard plate specifications
- scan areas on a plate
- change the calibration of the scanner
- test the voltages, the scanner drives, and lamps
- change the communications ports and system component calibration

Filters

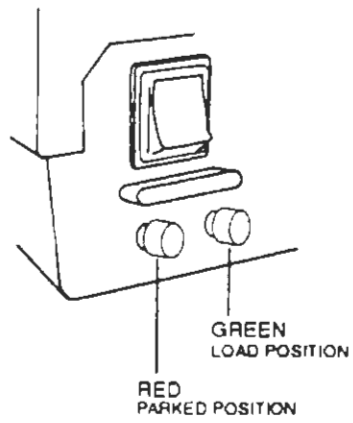
If you want to add new filter sets or replace existing filter sets with filters for different wavelengths, you first change the filters in the filter wheels inside the scanner and then change the software.

♦♦ ***Caution:*** If you do not correctly install the filters in the filter wheels and change the corresponding specifications in the CytoFluor System software, you could irreparably damage the CytoFluor System and void your warranty.

Changing the Filters

1. Make sure the plate transport mechanism is in the park position. Press the red button in the lower right rear corner of the scanner to move the plate transport mechanism into the CytoFluor scanning chamber.

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2. Turn off the CytoFluor scanner and disconnect the power cord from the rear of the machine.

♦♦♦ ***Warning*: Do not operate the CytoFluor System with the cover off. You could be electrocuted!**

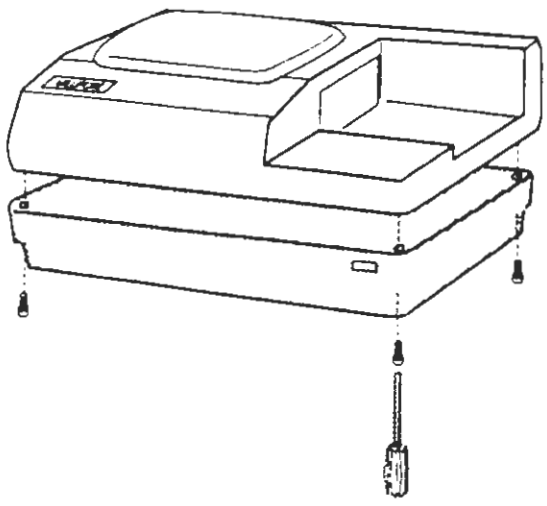
3. Remove the four screws that secure the top cover to the base with the Allen wrench supplied with the CytoFluor accessories kit. Lift off the cover.

4. Remove the covers on both filter wheels by loosening the handscrews.

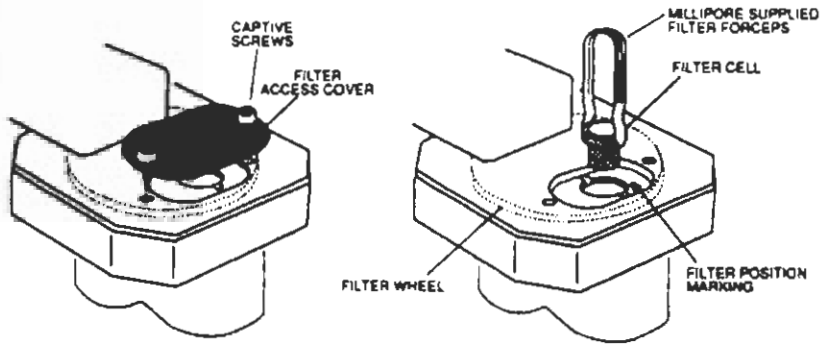
5. Carefully rotate the filter wheels until you reach the filter position you want to change.

♦♦ ***Caution:*** Do not touch the glass surfaces of any filter cell or you will damage the filters.

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6 With the filter forceps provided, remove the filter cell you wish to replace by pulling straight up.



7. Insert the new filter cell and gently press on the housing until you hear a click.

8. Make a note of the filter position (A, B, C, etc.) on the filter wheel, the center wavelength, and the bandwith. In the example 485/20X, 485 is the center wavelength and 20 is the bandwith of the excitation (X) filter (the M denotes emission filter). This information is printed on top of each filter cell.

♦♦ ***Caution:*** Make sure the filter cell is in the correct slot inside the filter wheel or you could irreparably damage the CytoFluor System.

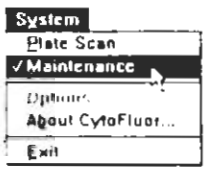
9. Replace the covers on the filter wheels and handtighten the screws.

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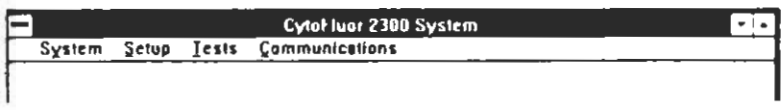
10. Replace the cover onto the Scanner base and tighten the screws. Store the unused filters in a cool, dry location in sealed plastic bags.

11. Reconnect the power and computer interface cables.

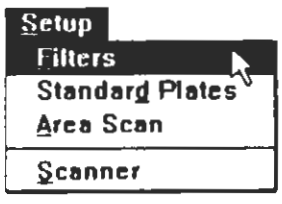
12. Turn on the power. Move the plate transport mechanism into the load position by pressing the red button.



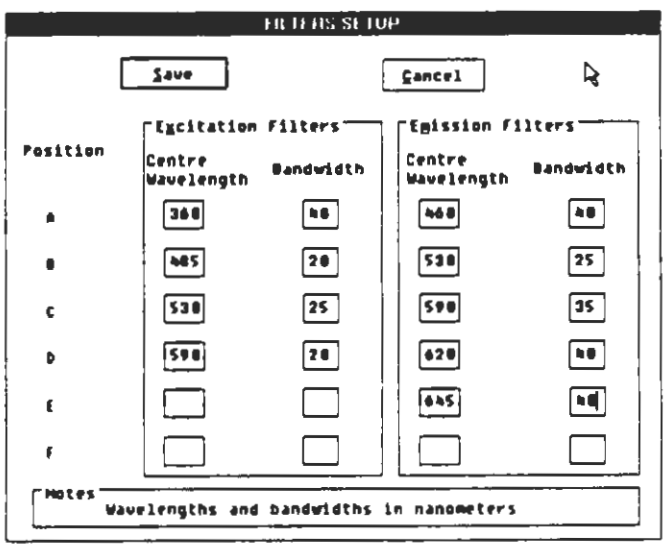
When the CytoFluor is turned off and then on again, it automatically comes up in the Plate Scan mode. Pull down the **System** menu and choose **Maintenance.** Notice that the menu titles change from those of the CytoFluor Plate Scan menu to those of the Maintenance menu. You should see the System, Setup, Tests, Communication, and Archive pulldown menus. All the tasks discussed in this chapter are accomplished in Maintenance mode.



Changing the Software



You must now change the software to correspond with the new filters you installed. Pull down the **Setup** menu and choose **Filters**. You should see the following screen:



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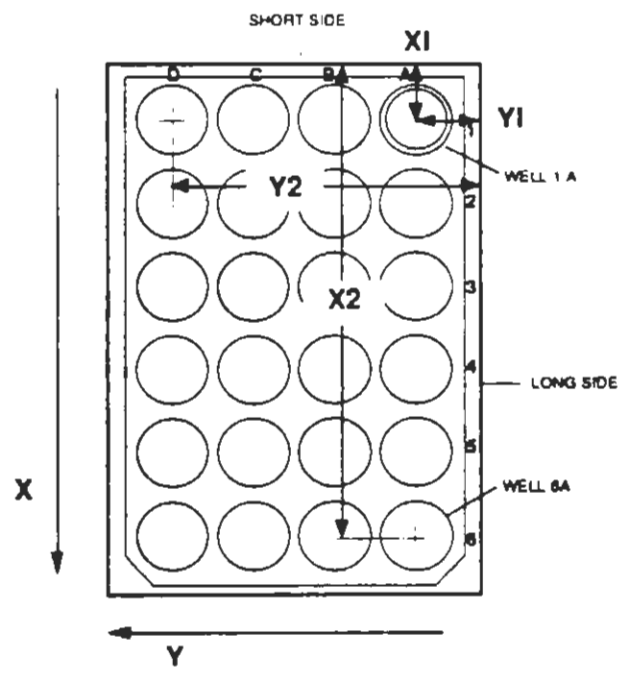
Click in the appropriate box (A through F) for either the Excitation filters or Emission filters, and type in the value that corresponds to the wavelength and half bandwidth of the filters you just installed. You can leave the filters dialog box before you save by clicking CANCEL. Any changes that you made will not be saved. When you are sure the new value is correct, click the Save button.

♦♦ ***Caution:*** If you do not correctly install the filters into the filter wheels *and* change the corresponding specifications in the CytoFluor System software, you could irreparably damage the CytoFluor System.

Plate Coordinates

Appendix C lists coordinates (X_1, X_2, Y_1, Y_2) for various brands of plates. Instructions follow for measuring a plate that is not listed in the table or is not precisely identical to a listed plate.

The four coordinates are shown in the figure that follows. You can measure these distances with an optical comparator or, less accurately, with Vernier calipers.



DIMENSIONS IN MICRONS (1/1000 MM)

Here's how to measure the coordinates of a plate using Vernier calipers:

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1. Turn the plate upside down. You must measure the coordinates from the bottom of the plate.

2. Using calipers, measure the distance from the center of Well 1A to the short side of the plate adjacent to Well 1A. This measurement, in millimeters, is coordinate X1. Multiply by 1000 to convert the value to microns.

3. Measure the distance from the center of Well 1A to the long side of the plate adjacent to Well 1A. This measurement, in millimeters, is coordinate Y1. Multiply by 1000 to convert the value to microns.

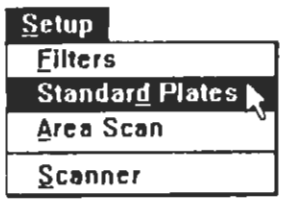
4. Measure the distance from the center of Well 12A (or well A of the farthest column from column 1) to the short side of the plate adjacent to Well 1A. This measurement, in millimeters, is coordinate X2. Multiply by 1000 to convert the value to microns.

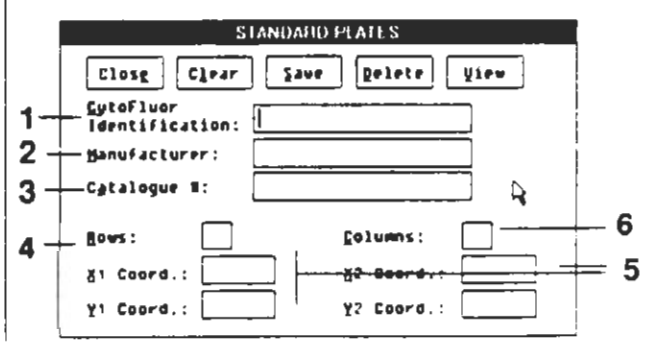
5. Measure the distance from the center of Well 1H (or well 1 of the farthest row from row A) to the long side of the plate adjacent to Well 1A. This measurement, in millimeters, is coordinate Y2. Multiply by 1000 to convert the value to microns.

X1 and Y1 are the coordinates for the starting point of the scan; namely, Well 1A in the upper right corner of the plate (when it is sitting upright). The coordinates for the well farthest away from Well 1A are X2 and Y2. Given these coordinates and the number of rows and columns, the software calculates the positions for scanning each well of the plate.

Entering Plate Information

You add the new plate's information to the CytoFluor System database in the Standard Plates dialog box. Pull down the **Setup** menu and choose **Standard Plates**. You should see the following screen:





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1 - CytoFluor Identification
You can assign a 26-character name (any combination of characters and spaces) to each CytoFluor plate. Choose a name that is meaningful to you. The name you assign will appear in the Plate dialog box on the main CytoFluor screen.

2 - Manufacturer
As with the previous field, you can enter any combination of characters, including spaces, as long as the name is no more than 26 characters.

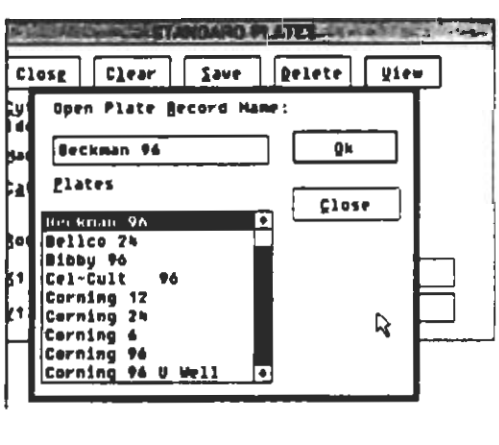
3 - Catalogue Number
The catalogue number of your plate (as the manufacturer lists it) is limited to 26 characters and spaces.

4 - Rows
Enter the number of rows on your plate; rows are indicated on all plates by letters.

5 - X1, Y1, X2 and Y2 Coordinates
You obtain these values as described in the preceding section. Enter the values in microns; do not use a comma to set off thousands.

6 - Columns
Enter the number of columns on your plate; columns are indicated on all plates by numerals.

You have the option of copying an existing plate record and then changing only those specifications you need to change to match a new plate. Click the View button if you want to see the list of other plates already created. You should see a screen similar to the one below.



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Choose the plate you wish to view by clicking its name in the scrolling list. The name then appears in the Open Plate Record Name box. When you click OK, the parameters of the plate you choose appear in the Standard Plates dialog box. When you click CLOSE, the Open Plate Record box disappears without changing the plate type.

♦ *Note:* Before making changes, change the CytoFluor Identification and save it. This action will preserve the original file while creating a new file that has the same parameters as the original. Any additional changes to the new file will not then overwrite the original file.

You can alter an existing plate record by selecting individual fields and typing over the information, or by using the CLEAR and DELETE buttons. CLEAR allows you to clear all the parameters of the plate you're working on. DELETE allows you to remove plates from the standard plates database. Use DELETE carefully!

When you are satisfied with your new plate's entries, click the SAVE button. If you want to cancel without making changes, click CLOSE. Once saved, the CytoFluor software adds the new plate information to the existing list of plates. You can then access the new plate through the New Files or Open Files menus.

Adding a New Plate

You can add new plate specifications and change the specifications of existing plates in the CytoFluor System standard plate database. When you click on Setup and choose Standard Plates, you access the database of microtiter plates used for a standard plate scan. The CytoFluor software uses plate dimensions to find the center of each row and column on the plate. Then, as the probe scans each row, the fluorescence measured at the center of each well is recorded as a data point.

All dimensions are in microns, and referenced to the 1A corner of the plate. CytoFluor interprets these dimensions as in the figure on page 5-5.

X_1 = distance from short side to well center of first column
X_2 = distance from short side to well center of last column
Y_1 = distance from long side to well center of first row
Y_2 = distance from long side to well center of last row

For example, if you want to ignore the first row (A) of a 96-well plate and start scanning in Row B, the distance to the center of

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Row B would be X_1. This feature enables you to create special plates that designates a rectangular subset of a standard plate.

♦ *Note:* Since the distance between rows is uniform, then $(X_2 X_1)/(n-1)$ is the distance between adjacent columns, where n is the total number of rows. This is similarly true for Y.

For example, let's say you want to scan all but the outer wells of a Corning 96-well plate. Using the X,Y values for the standard plates found in Appendix C, calculate the new plate dimensions in the following manner:

Distance Between Columns:

$$\frac{X_2 - X_1}{N_C - 1} = \frac{113104 - 14384}{12 - 1} = \frac{98720}{11} = 8975$$

Where:
N_C = number of columns
X_1 = distance from short side to well center of first column
X_2 = distance from short side to well center of last column

Distance Between Rows:

$$\frac{Y_2 - Y_1}{N_R - 1} = \frac{73482 - 11127}{8 - 1} = \frac{62355}{7} = 8908$$

Where:
N_R = number of rows
Y_1 = distance from long side to well center of first row
Y_2 = distance from long side to well center of last row

2. Calculate the plate dimensions of the new plate (wells B2 - G11) in the following manner:

Centers on Column 2	$= X_1 = 14384 + 8975$	$= 23359$
Centers on Column 11	$= X_2 = 113104 - 8975$	$= 104129$
Centers on Row B	$= Y_1 = 11127 + 8979$	$= 20106$
Centers on Row G	$= Y_2 = 73482 - 8979$	$= 64503$

When you use a Corning 96-well plate with these new plate coordinates, CytoFluor scans the plate as if it is a 60-well plate. Well 1A for the CytoFluor scan corresponds to the actual well 2B on the plate. You must also specify that this new plate has 6 (instead of 8) rows and 10 (instead of 12) columns.

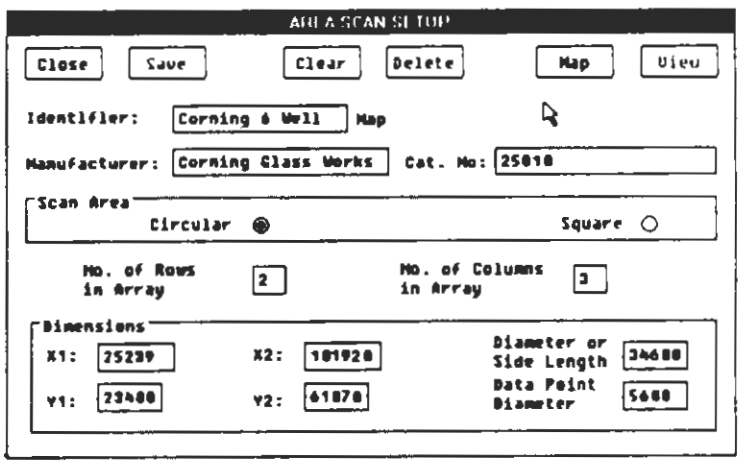
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Setting Up an Area Scan

Choose the Standard Plates item from the Setup menu. Enter the information for the plate and save it. The information must include a name, the manufacturer's name, the catalogue number, the number of rows and columns, and four coordinate measurements that are crucial to the accuracy of the scans. It's a good idea to have these measurements ready before starting to enter the information.

The Area Scan feature allows you to measure fluorescence at multiple points within the area of a single well, rather than taking a single measurement per well as is the case for the standard Plate Scan feature. Area Scan lets you create a custom plate format that is an array of circular or square areas within the boundaries of a microtiter plate. This plate (defined by the user) is automatically entered into the CytoFluor Plate database with a .MAP extension. In most cases, the area to be scanned will be a single well, and the maximum number of areas per plate is just the total number of wells.

When an area is scanned, a data point is taken when the probe has travelled half the data point diameter distance. The fields for each data point overlap by 50 percent. This overlap can be varied by making the data point diameter different from the physical probe diameter (see below).



The Area Scan plate setup requires the same parameters as a standard plate. In addition, the Area Scan plate setup requires the following data:

1. The shape of each area to be scanned—circular or square.

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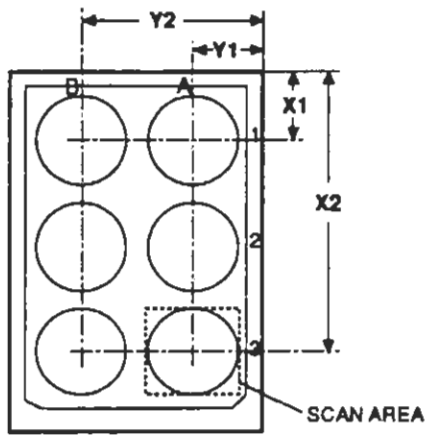
2. The data point diameter. This value corresponds to the conceptual (not physical) size of the optical probe. Increasing or decreasing this parameter has the effect of increasing or decreasing the resolution of the scans (the number of samples taken). The data point diameter value must fall between 4000 microns and 11000 microns.

3. The side length or the diameter (depending on shape) of the area to be scanned. This parameter must be equal to or greater than the Data Point diameter and must be less than 78000 microns.

Area Coordinates

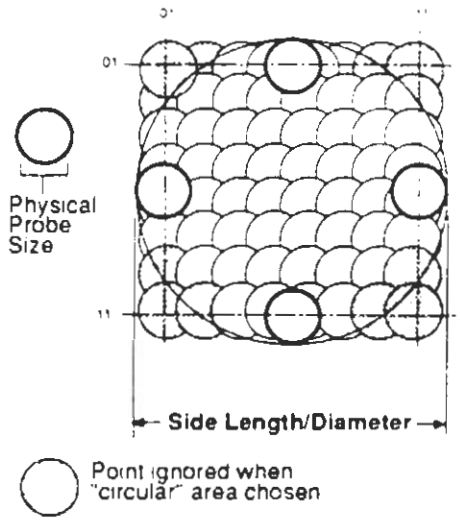
The figure below shows a standard six-well plate with the dimensions that define the fields in the Area Scan Setup window. There are two rows (A,B) and three columns (1,2,3). In this example, the centers of the corner areas (i. e., the centers of all wells) are defined by X1, X2, Y1, Y2. These values are identical to those in the Standard Plate setup. There are six areas to be scanned (2 x 3); and the diameter of each area is 34600 microns (3.46 cm). Each data point to be taken is at the center of a 5600 µm circle with a 2800 µm spacing between data points (50% overlap).

The next figure shows an expanded view of a single scan area. From the plate and data point dimensions, CytoFluor calculates the center for each well on the plate, and the square area to be

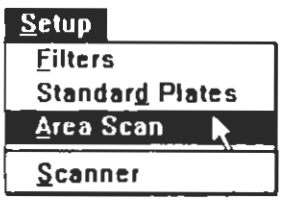


scanned. If a circular area is chosen, the square area is physically scanned, but only those data points within the inscribed circular area are accepted. Points outside the circular area are displayed as ** on the printout. In this example, the scan array for each well is 11 x 11 (121) maximum data points. On the printout, each data point position is labeled and segregated by well.

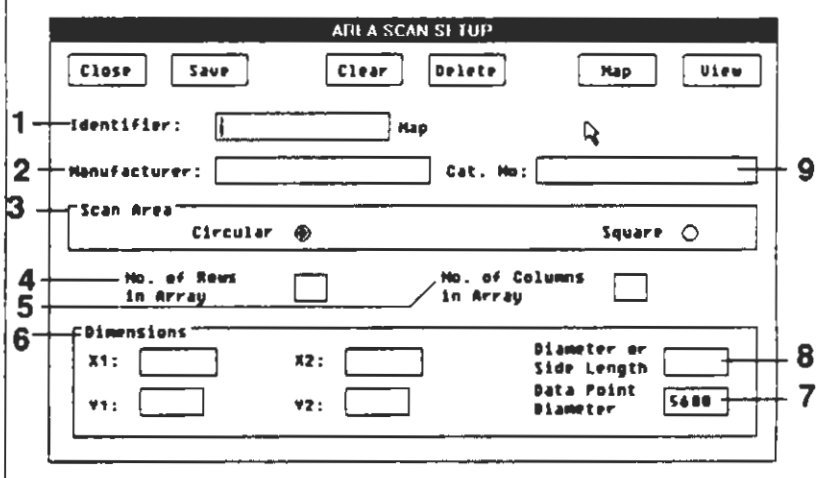
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Entering Area Scan Information



You can use the same X, Y row and column coordinates from the standard plate database, then fill in the rest of the fields. Pull down the **SETUP** menu and choose **Area Scan**. You should see the following screen:



1 - Identifier
You can assign a 16-character name (any combination of characters and spaces) to each area scan plate. Choose a name that is meaningful to you. The name you assign is entered into the plate database with the .MAP extension. The extension .MAP is automatically appended. This field must be completed.

2 - Manufacturer
As with the previous field, you can enter any combination of characters, including spaces, as long as the name is no more than 20 characters. This field is optional.

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3 - Scan Area
The Scan Area field has two buttons Circular and Square These buttons are used to designate the shape of the scan area.

4 - Rows in Array
This field is used to define the number of rows of wells on the plate. Rows are usually listed alphabetically (A–H).

5 - Columns in Array
This field is used to define the number of columns on a plate Columns are usually listed numerically (1–12)

6 - Dimensions
You obtain these values as described in the preceding section X1 and Y1 define the location of the first sample point within the scan area; X2 and Y2 define the location of the sample point farthest away from the first point.

X1 and X2 must have values within the range of 2000 microns and 124500 microns; Y1 and Y2 must have values withing the range of 2000 microns and 83000 microns. Enter the values in microns; do not use a comma to set off thousands.

7 - Data Point Diameter
The Data Point Diameter is the conceptual (not physical) probe size used for calculating the position of data points taken within the scan area. By increasing its value, you cause the sample positions to be farther apart (as though a larger physical probe had been fitted to the scanner). By decreasing its value, you cause the data point positions to be closer together (as though a smaller physical probe had been fitted to the scanner).

The CytoFluor software uses the value of the Data Point Diameter to calculate the 50% overlap positions within the defined area. If the Data Point Diameter is larger than the probe diameter, resolution of the scan is decreased. If the Data Point Diameter is smaller than the probe diameter, scan resolution is increased. The Data Point diameter is preset to the same dimensions as the physical probe as defined the Scanner Setup window. The Data Point diameter must fall between the limits of 4000 microns and 1100 microns. If you choose a Data Point Diameter that is different from the physical probe size, a warning will be displayed.

♦ *Note:* The actual probe diameter, defined in Maintenance/Setup/Scanner, is constant.

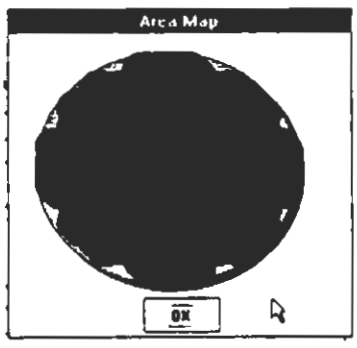
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8 - Diameter or Side Length
This field defines the side length of a square area or the diameter of a circular area to be scanned. This value must be greater than or equal to the Data Point Diameter, and less then 78000 microns. Make sure that the diameter or side length is short enough so that it does not overlap other areas or fall off the edge of the plate area. By changing the side length (diameter) value, you can scan an area that is smaller than the physical area of a single well.

9 - Catalogue Number
The catalogue number of your plate (as the manufacturer lists it) is limited to 20 characters and spaces. This field is optional.

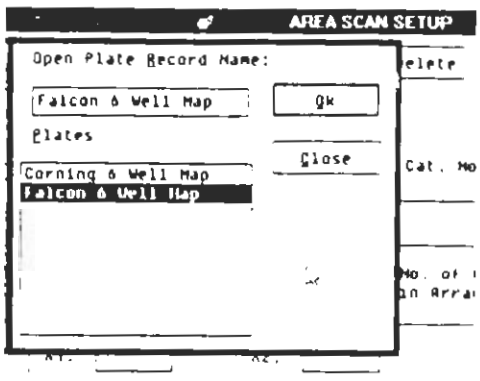
If you want to cancel without making changes, click CANCEL. When you are satisfied with the entries, click the SAVE button. Once saved, the CytoFluor software adds the new area scan information to the existing list of plates. You can then access the area scan plate through the New Files or Open Files menus.

Once you save the area scan information, CytoFluor calculates the number of data points to be taken within each scan area. The actual resolution is graphically displayed when you click on Map in the Area Scan Setup dialog box.



You have the option of copying an existing plate record and then changing only those specifications you need to change to make a new plate. Click the VIEW button if you want to see the list of other plates already created. You should see a screen similar to the one that follows.

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Choose the plate you wish to view by clicking its name in the scrolling list. The name then appears in the Open Plate Record box. When you click OK, the parameters of the plate you chose appear in the Standard Plates dialog box. Click CANCEL to close the Open Plate Record box without changing the plate type.

You can alter an existing plate record by selecting individual fields and typing over the information, or by using the CLEAR and DELETE buttons. CLEAR allows you to clear the screen of all the parameters of the plate you're working on. DELETE allows you to remove plates from the standard plates database. Use DELETE carefully!

♦ *Note:* If you make changes to an existing plate setup, change the name of the new file so that the original file is left intact.

The procedure for running an area scan once you have set up the parameters and saved them is the same as for running a scan with a standard plate. See the instructions provided in Chapter 4.

Scanner

Pull down the **Setup** menu and choose **Scanner.** You should see a screen similar to the one that follows.

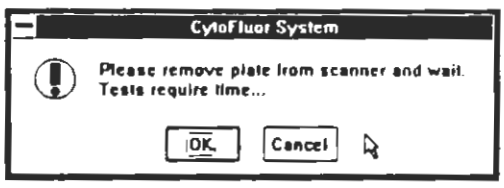
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These settings should be the same as those on the calibration certificate that you received with your scanner. If the settings are different, change them accordingly, then click OK. You can quit before making changes by clicking CANCEL.

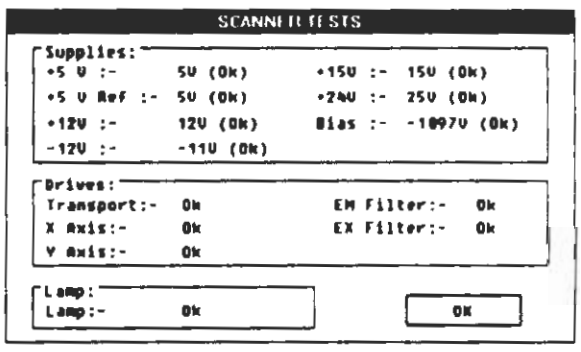
♦♦ ***Caution*** If you change the settings to other than those on the Calibration Certificate, your system will not function accurately.

Tests

When you click the **Tests** menu title, the CytoFluor System Software automatically checks the voltages, the scanner drives, and the lamp. While the system is running the self-diagnostics, you should see the following screen:



You must wait about 45 seconds for the software to check the system. After the system checks the voltages supplies, you will see the following box if the voltage supplies, scanner drives and lamps are working properly:



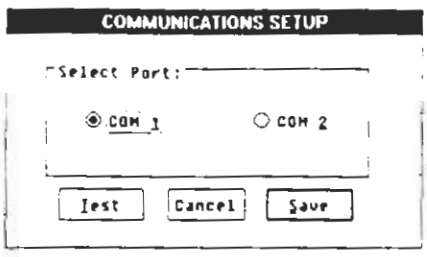
Click OK to close the box. If you *do not* see this box, contact Millipore Technical Service. Or, if you are located outside the United States of America, contact the Millipore office nearest to you. For a listing of telephone numbers for Millipore Technical Service and Millipore offices worldwide, see Chapter 7, *Technical Information*.

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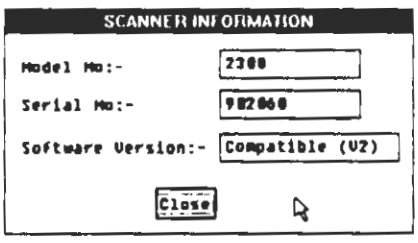
Communication Port

You can change communication port settings using the Communcations menu. Click the **COMMUNICATIONS** menu title.

You should see the following box:



The default setting assumes the CytoFluor Scanner is connected to Com 1. If your system is connected to Com 2, click Com 2 and then press SAVE to change the setting to the correct Communication Port. You can also view the model and serial number of the CytoFluor scanner and the software revision level from the Communications dialog box. Click the Communications menu title, and when the box appears, click the **TEST** button. You should see a screen similar to the one below:



Click close. Choose Plate Scan from the System pulldown menu to return to normal data collection operation.

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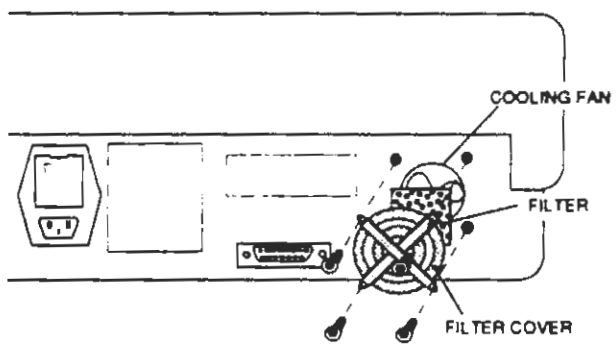
Chapter 6
MAINTAINING THE CYTOFLUOR SYSTEM

Caring for your CytoFluor 2300/2350 Scanning System is very simple; preventive maintenance is limited to keeping the scanner clean and changing the air filter at regular intervals. Inspect the system after every use and wipe any spills from the scanner with a damp (not wet) cloth and a mild detergent.

Replacing the Air Filter

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

1. Using the Allen wrench provided, remove the four Allen screws that secure the air filter protector.



2. Remove and discard the used filter.

3. Cut a replacement filter to fit 1-5/8 inches by 1-5/8 inches square (40 mm sq) from the strip provided.

4. Insert the new filter and replace the air filter protector. Tighten the screws with the Allen wrench, but do not overtighten.

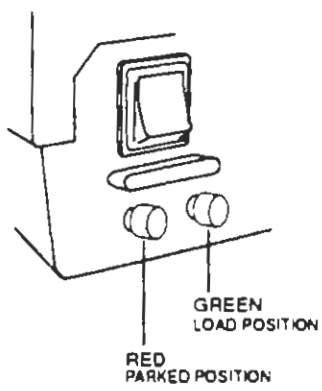
♦♦ ***Caution***: Do not operate the scanner without an air filter or you may have reduced performance.

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Replacing the Lamp

It's important for the lamp surface to be free of fingerprints or other material that can interfere with illumination and can collect dust. Always wear gloves when touching the new lamp.

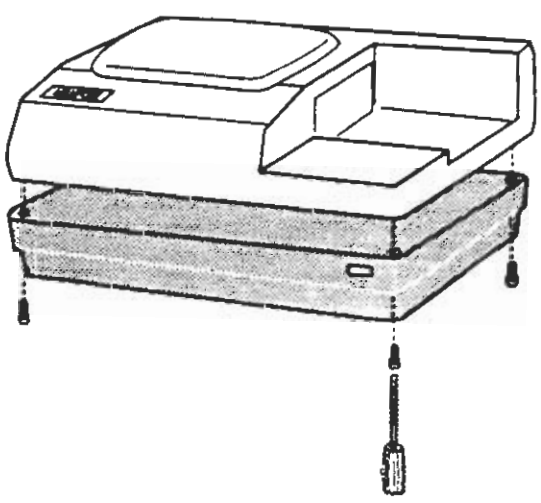
1 Make sure the plate transport mechanism is in the park position. Press the red button in the lower left corner of the scanner to move the plate transport mechanism into the CytoFluor scanning chamber.



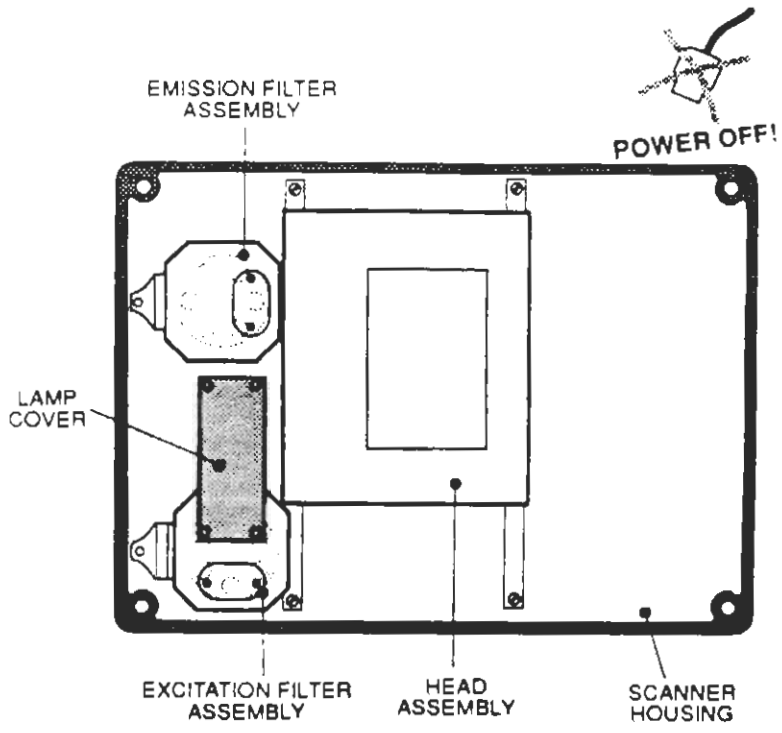
2. Turn off the CytoFluor scanner and disconnect the power and computer interface cables from the rear of the machine.

♦♦♦ ***Warning:*** **Do not operate the CytoFluor System with the cover off. You could be electrocuted!**

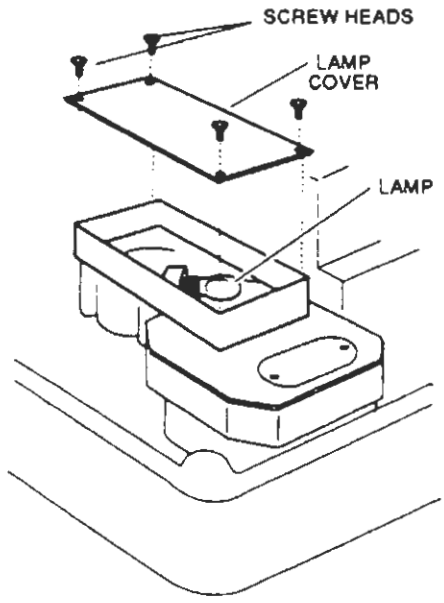
3. Remove the four screws that secure the top cover to the base using a screwdriver. Lift off the cover.



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4. Remove the four screws that secure the cover on the lamp assembly using a Phillips screwdriver. Remove the cover.



5. Remove the faulty lamp by pulling it outward. Discard the faulty lamp.

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6 Wearing gloves, insert the new lamp into the socket so that the lamp extends horizontally from the base and the filament is centered on the glass circle (lens) at the bottom of the lamp housing. Replace the cover and tighten the screws, but do not overtighten them.

7 Replace the cover onto the base and tighten the screws.

8 Reconnect the power and computer interface cables.

9 Turn on the power.

When the system is turned on, it will automatically move the plate transport mechanism into the load position. Make sure the plate transport mechnism moves smoothly into position.

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Chapter 7

TECHNICAL INFORMATION

System Specifications

CytoFluor Model 2300/2350

Dimensions:	
Height	225 mm (9 in)
Width	490 mm (19.3 in)
Depth	370 mm (14.5 in)
Weight	approximately 12 kg (25 lbs)
Shipping weight	approximately 16 kg (35 lbs)
Photomultiplier tube diameter	7.0 mm (0.28 in)

Operating Conditions

Temperature	0°C to + 35°C (+ 32°F to + 104°F)
Relative Humidity	20% to 80%, non-condensing

Electrical Specifications

Power Draw	100 watts, maximum
Fuse	2 amp, time-delay, glass, 5 mm x 20 mm, 250 volt-type
Input Voltage	90 – 130 volts AC at 44 – 400 Hz (selectable) or 200 – 260 volts AC at 44 – 400 Hz (selectable)
Internal Voltages	± 12 volt and + 24 volt DC + 5 volt DC – 1000 volt DC

Optical Specifications

Lamp type	M32-Type, Tungsten-Halogen, 50 watt
Lamp life	2000 hours nominal
Lamp range	360 nm to 700 nm
Filter wheel	6 positions
Detector	R928 Hamamatsu Photomultiplier Tube
Filters	6 or 8 cavity pole interference filters fitted in 22 mm diameter cells

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X/Y Scanning Mechanism

Speed	25 mm/second
Step Size	25.4 µm (0.001 in)
Repeatability	± 50 µm worst case
X/Y Drive	3.175 mm (0.125 in) leadscrew 500-slot shaft encoder
Plate Transport	10 seconds maximum Load Time

Computer Specifications

Minimum Computer Requirements

Base Unit	IBM AT PC or clone 640 Kb base, extended to 2 Mb recommended 5 Mb Color Graphics Monitor Color Graphics Adapter 20 Mb hard drive 1 Serial and 1 Parallel Port Mouse and Keyboard
Printer	Dot Matrix
Required Software	MSDOS or PCDOS v. 3.3 CytoFluor Software

NEC PowerMate 386/25S Specifications

Base Unit	80386 CPU 25 MHz Processing speed 4 Mb memory 42 Mb Hard disk 640 x 480 VGA Graphics 1 Serial Port 1 Parallel Port PS-2 Mouse Port 4 Expansion slots 101 Keyboard Layout
Monitor (110 V)	800 x 600 maximum resolution 14-inch screen
Monitor (220 V)	800 x 560 maximum resolution 12-inch screen 8/16/64 colors

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NEC PowerMate 386/25S Specifications (Con't.)

Printer	Model Pinwriter P6200 24-pin Dot Matrix Color Ribbon 75 cps – 220 cps Parallel Interface
Mouse	Microsoft two-button, serial with PS-2 adapter

CytoFluor Computer Interface

- serial cable with 25-pin female connector
- RS232: 9600 Baud rate, 8 bit word, no parity, one stop bit

Optical Sets for Fluorochromes

Dye	Pair	Application
Methylumbelliferone	360/460	Immunoassay
Hydroxymethylcoumarine	360/460	Immunoassay
Prodan	360/460	Membrane Probe
Laurodan	360/460	Membrane Probe
Calcein	485/530	Viability Assays
Attophos™	450/580	Immunoassay
CFDA-AM	485/560	Plasma Membrane Disruption
CFDA	485/535	Extracellular pH Changes
B. Phycoerythrin	485/590	Gen. Fluorescent Tag
DPH	360/460	Membrane Probe
DAPI	360/460	Nucleic Acid Stain
Quin-2	360/482	Ion Concentration
Indo-1	360/482	Ion Concentration
Rhod-2	360/482	Ion Concentration
Thiolyte	360/460	Gen. Fluorescent Tag
Lucifer Yellow	440/530	Gap Junc'n. Comm'n.
Ben Zoxanthene Yellow	360/482	Gen. Fluorescent Tag
Dansyl P. E.	360/530	Gen. Fluorescent Tag
BCECF	485/530	Vital Dye, pH Indicator
Carboxy Fluorescein	485/530	Vital Dye, pH Indicator
Fluo-3	508/20 560/20	Ca^{2+} Concentration
SNAFL (in acid)	485/530	pH Indicator
Acridine Orange	485/530	Nucleic Acid Stain
Rhodamine 123	482/530	Mitochondria Stain
DTAF	485/530	Alcohol Reactive Probe

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Optical Sets for Fluorochromes (con't)

Dye	Pair	Application
Ethidium Homodimer	485/530	Nucleic Acid Stain
	485/645	Dual Wavelength Monit.
7-AAD	485/645	Nucleic Acid Stain
Ethidium Bromide (in sol'n.)	485/645	Nucleic Acid Stain
SNAFL (in base)	530/620	pH Indicator
SNARFI	530/620	pH Indicator
TRITC	530/620	Gen. Fluorescent Tag
Propidium Iodide (in sol'n)	485/645	Nucleic Acid Stain
Propidium Iodide (in sol'n)	530/645	(for dead cells)
Texas Red	590/645	Gen. Fluorescent Tag
Allophycocyanine	590/645	Gen. Fluorescent Tag
Neutral Red	530/645	Lysosomal Integrity
R. Phycoerythrin	485/590	Gen. Fluorescent Tag
Sodium Fluoroscein	485/530	Monolayer Permeability
Thiozole Orange	485/560	Nucleic Acid Stain
Bodipy™	485/530	Gen. Fluorescent Tag

CytoFluor Standard Filter Sets

Excitation Center Wavelength/* Half Bandwidth	Emission Center Wavelength/* Half Bandwith
360/40	460/40
485/22	530/30
530/30	595/35
590/35	620/40
	645 /50

Technical Information

Ordering Information

If you need . . .	Order Cat. No. . . .
CytoFluor System Kit Fluorescence Reader CytoFluor Software CytoFluor Cable Set CytoFluor Spare Lamp CytoFluor Fuse Kit CytoFluor Manual	PIFS 000 00

* Center wavelengths measured in nanometers

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Ordering Information (Con't.)

If you need . . .	Order Cat. No. . . .
CytoFluor Scanner with Computer (110V)	PIFS 001 00
NEC Powermate 386 '25S	PIFS 002 01
Windows 3.0	
MS/DOS 3.3	
Keyboard	
Color display	
Printer	
Color Kit	
Printer Cable	
VGA Graphics Board	
Bus Mouse	
CytoFluor Scanner with Computer (220V)	PIFS 002 00
Power Mate 1	
MS/DOS 3.3	
Keyboard	
Color Display	
Printer	
Tractor Feed	
Printer Cable	
VGB Graphics Board	
Bus Mouse	
Accessories	
Microsoft Windows 286	PIFS 100 01
Microsoft EXCEL	PIFS 100 02

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MILIPR UI

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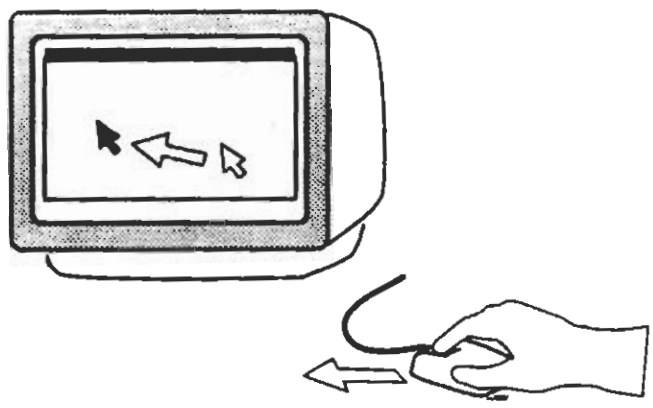
P17481, Rev. B, 10/91

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Appendix A
Introduction to Windows

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.

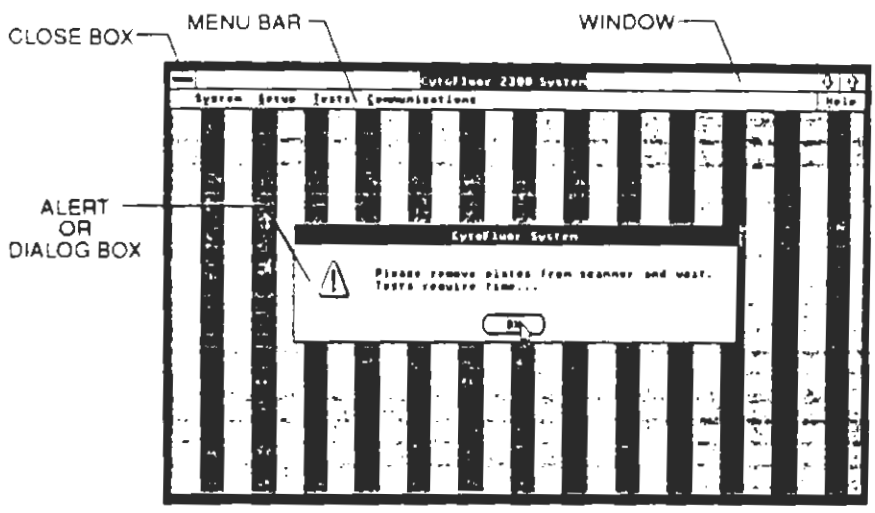
If you look at the underside of the mouse, you see a ball inside a circular housing. When you slide the mouse across a surface, the ball rolls underneath it. This movement sends signals to the computer that cause the pointer to move around the screen. Moving the mouse to the left or right causes the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



The mouse has two buttons on its top. Pressing down momentarily on the left mouse button signals the computer that an operation is to be initiated; 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.

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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.



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; 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 the pointer is positioned on the close box and a mouse button is pressed, the window is closed and 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) will be displayed. 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 a file cannot be printed before it has been opened. 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

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boxes. In dialog boxes you find clickable buttons, scroll bars, and check boxes.

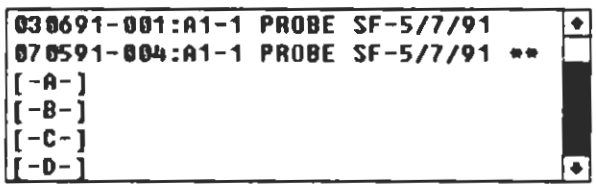
On-screen buttons are used to make choices and to send messages to the software; for example, you might confirm a command by clicking a button labeled OK, or change your mind by clicking a button labeled Cancel.



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



Scroll bars are used 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 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.



Check boxes are like the boxes found on various paper forms. By clicking a check box, you indicate the option you want. An X appears in the box when the option is selected.

Using the mouse and the on-screen features such as menus and scroll bars takes some practice before it's completely comfortable; but for most people, it becomes a much easier way to use the computer than remembering and typing commands. If you need further information, see the manuals that accompany Microsoft Windows.

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Appendix B
ERROR MESSAGES

This appendix contains a list of common error messages that the CytoFluor System software presents, along with their meanings.

Message	Meaning
Bandwidth above maximum in set n. Bandwidth cannot be saved.	The bandwidth of a filter exceeds the limit of 100 nanometers.
Computer is not connected to a CytoFluor compatible with this software version.	The scanner connected is not the correct type for the software.
Data point diameter does not equal the probe diameter.	Change the setting for the data point diameter. It must be equal to or greater than the probe diameter.
Disk full. Load new disk or remove files to create more space on destination disk.	The disk chosen as a destination has insufficient room for the file or files you're trying to copy.
File already contains data. Please create a new file.	The file on the screen already contains scanned and saved data. You can't change its information.
File name already exists in target directory. 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 an export file (.CSV).

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Invalid filter wavelength in set n Wavelength cannot be saved.	The wavelength is zero or below.
Invalid overlap of filter ranges in filter set n file cannot be saved	You cannot choose excitation and emission filters with similar or incompatible wavelengths. Change one or both of the filter settings.
Invalid probe size. Probe diameter must be between 4000 and 11000 microns.	Choose a new value for the probe size (Area Scan feature).
Lamp failed test. Replace lamp, then retest.	The excitation lamp has failed to operate. If installing a new lamp does not fix the problem, contact Millipore Technical Service.
No column specified. File cannot be saved.	You must enter a value for the number of columns.
No coordinates specified. File cannot be saved.	You haven't entered the X1, Y1, X2, or Y2 coordinates; or, the X2 or Y2 value is smaller than X1 or Y1. Check your entries and measurements.
No plate detected. Please load plate.	There is no plate in the plate transport mechanism. If you have loaded a plate and still get this message, contact Millipore Technical Service.
No plate name specified. File cannot be saved.	You must enter a name for the plate.
No plate specified. File cannot be saved.	You must enter a name for the plate.
No row specified. File cannot be saved.	You must enter a value for the number of rows.

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Not enough memory available to scan. Close any unneeded window and retry.	You have run out of computer memory. This condition can occur if you have several windows open at a time. If the problem persists, contact Millipore Technical Service.
No X coordinate specified. File cannot be saved.	You must enter a value for the X1 or X2 coordinate; or, the value of X2 is smaller than X1; or, the value of X1 is smaller than the probe radius (Area Scan). Check your entries and measurements.
No Y coordinate specified. File cannot be saved.	You must enter a value for the Y1 or Y2 coordinate; or, the value of Y2 is smaller than Y1; or, the value of Y1 is smaller than the probe radius (Area Scan). Check your entries and measurements.
Overlap of filter ranges in filter set n. CytoFluor performance will be reduced if you continue.	The bandwidths of the excitation and emission filters you have chosen overlap their center wavelengths. The accuracy of your scan may be compromised. Choose different filters.
Plate type is no longer in database.	The plate type specified in a saved file has been deleted from the plate record database. Restore the plate information to the database.
Scan time of n exceeds the specified scan cycle time chosen. File cannot be saved.	If you are scanning a plate more than once, you must enter a value in the Scan Cycle field. The value must be equal to or greater than the Approximate Total Time value.

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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 have selected COM2). Change the selection.
The CytoFluor has stopped. Turn off scanner and check for jammed plate.	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 Millipore Technical Service.
The distance between adjacent well centers is below the minimum requirement of probe diameter.	If performing a plate scan, you could get this message if either (X2–X1) divided by the number of rows, or (Y2-Y1) divided by the number of columns, is smaller than the probe diameter. If performing an area scan, your entries call for an overlap of greater than 50% between data points. Check your entries and measurements.
Unable to close communication port. System error; contact Millipore Technical Services.	This is a serious error. Call Millipore Technical Service.
Unable to create file <filename>. System error; contact Millipore Technical Services.	This is a serous error. Call Millipore Technical Service.

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Unable to communicate with CytoFluor. Check all connections	The software has lost communication with the scanner during scanning. Make sure the connectors firmly seated.
Unable to communicate with CytoFluor. check all connections and choice of serial port	The software can't communicate with the scanner when beginning a scan. Check connectors and make sure you have chosen the correct communications port.
Unable to communicate with CytoFluor. Make sure scanner is on and check all connections.	The software can't open the communication port. Check the power to the scanner, the connectors, and the choice of port.
Unable to communicate with CytoFluor. System will automatically close down.	This message appears if you click Close Door while exiting the CytoFluor software, and the scanner has already been turned off or is disconnected from the computer. The system will continue with the exit.
Unable to find file <filename>. System error; contact Millipore Technical Services.	This is a serious error. Contact Millipore Technical Service.
Unable to find plate type in database.	The plate type required by a file can't be found. Restore the plate information to the database.
Unable to open file <filename>. System error; contact Millipore Technical Services.	This is a serious error. Contact Millipore Technical Service.

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Unable to print. Make sure printer type is correct	You could get this message if you have attached a printer different from the one you specified when you configured your system. Choose another printer using the Control Panel, reattach the specified printer, or reconfigure the CytoFluor System to add the new printer.
Unable to start job. Close any unneeded windows, check printer port selection.	Your computer has insuffient memory to print. This can happen if you have several windows open. If the problem persists, contact Millipore Technical Service.
Unable to write to file <filename>. System error; contact Millipore Technical Services.	This is a serious error. Contact Millipore Technical Service.

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Appendix C
PHYSICAL PLATE DATA BASE

Plate	Manufacturer	Cat. #	Columns	Rows	Coordinates X1	X2	Y1	Y2
Beckman 96	Beckman	—	12	8	14230	113220	11310	70450
Bellco 24	Bellco Biotech.	—	6	4	15870	110710	13810	70870
Bibby 96	Corning Glass	25860B	12	8	13950	112360	10500	73500
Cel-Cult 96	—	—	12	8	13855	112750	10995	73868
Corning 6 (Same for Well Map) (Area diameter for map: 34600)	Corning Glass	25810	3	2	25230	101920	23400	61070
Corning 12	Corning Glass	25815	4	3	24475	102952	16407	68746
Corning 24	Corning Glass	25820	6	4	15530	109970	13800	70280
Corning 96	Corning Glass	25855	12	8	14384	113104	11127	73982
Corning 96 U-Well	Corning Glass	25855	12	8	14500	112730	10830	73750
CoStar 24	Data Packing Corp.	3424 Mark 2	6	4	15420	115780	12310	72540
CoStar 48	Data Packing Corp.	3548	8	6	17649	111055	9473	76125
CoStar 96	Data Packing Corp.	3594/3595/ 3596/3599	12	8	14269	113509	11099	74274
Dynatec 96	Dynatech Labs	—	12	8	14478	113542	11224	74320
Dynatec S-Well 96	Dynatech Labs	—	12	8	14268	113160	11253	74200
Elkay 24	Elkay Products	100-ST24-00F	6	4	10900	117396	11008	75102
Elkay 96/1	Elkay Products	100-ST96-00F	12	8	14069	113003	11545	74299

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Plate	Manufacturer	Cat. #	Columns	Rows	Coordinates X1	X2	Y1	Y2
Elkay 96/2	Elkay Products	100-EI96-00K	12	8	14069	113383	11100	74317
Falcon 6 (Same for Well Map) (Area diameter for map: 34990)	Falcon Plastics	3046	3	2	24673	103109	23289	62496
Falcon 24	Falcon Plastics	3047	6	4	14024	110419	13779	71646
Falcon 96	Falcon Plastics	3072	12	8	14379	113175	11389	74323
Gibco 24	Gibco, Inc.	—	6	4	15000	115000	12500	72500
Gibco 96	Gibco Inc.	—	12	8	14170	113380	11260	74210
Linbro 96 Flat Well	Flow Labs, Inc.	76-232-05	12	8	14280	113110	11030	73940
Linbro 96 U-Well	Flow Labs, Inc.	76-242-05	12	8	13860	112900	10980	73660
Linbro 96 V-Well	Flow Labs, Inc.	76-223-05	12	8	14600	113090	11460	74150
Millipore 96	Millipore Corp.	—	12	8	14360	113470	11300	74335
Nunc 96	—	—	12	8	14313	113247	11322	74181
Nunclon 96/1	Delta/InterMed	163320	12	8	14240	112930	10800	73810
Nunclon 96/2	Delta/InterMed	167008	12	8	14470	113560	11250	74220

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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.

Archive The menu available in Plate Scan mode that lets you copy, print, and delete groups of files at once, rather than one file at a time.

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 of work time to a minimum. Sometimes a set of backup disks are kept at a different site in case of fire or theft.

Bandwidth 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.

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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.
Comport	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 Value). A file format in which each element is separated by a comma. This format is used in most spreadsheet programs.
Command	An instruction that causes the system to perform some action. A command can be chosen from a menu or a 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 Message	A message displayed by software when you have chosen a destructive action, asking if you want to proceed. For example, you will see a confirmation message when you tell the program to delete a file. This message gives you an opportunity to double-check and to change your mind.
Control Menu	The menu accessed by clicking the box in the upper left corner of the window; it's 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.

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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:\CYTO, which means the Cyto directory on the C:\ drive.
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.
Data File	Any file created within an application. As you set up files for scanning with the CytoFluor System, you are creating data files.
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.

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Direct Access A method of selecting a menu or option by pressing 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 or sequence, such as Alt/letter or Shift/letter. The key you use is called a shortcut key.

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, which can be inserted and removed. 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 to be selected, and then click twice in quick succession. This action carries out a command.

Export To move data into a file that can be used by other applications.

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 can be used 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.

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

Filename 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 they can be used 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.

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.MAP	This extension appears in the file name of a file that has been created as an area scan to differentiate it from files that were created using the standard plates
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 the CytoFluor 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.
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, causing position information to be sent 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.

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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:\CYTO\CYTO23\LAB.TEST.CSV would be the pathname of the LAB.TEST.CSV file in the CYTO23 subdirectory in the CYTO directory on drive C.
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.
RS232	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.

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Run File — The kind of data file that the CytoFluor System software creates. You can recognize these files by the .RUN extension when you view a directory list in DOS or Windows. The CytoFluor runfiles can be identified and viewed only by the CytoFluor System software.

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 a different portion of its contents may be viewed.

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.

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Shortcut Key	A key or key sequence, available for some commands, that you can press to execute the command without first selecting a menu or pointing at a command. This method is called direct access.
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.
Startup Disk	A disk that contains all files needed to start up the NEC computer and install the CytoFluor software
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.
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 in which you view an application. Every window has a title bar and may have a menu bar. Windows can contains scrolling lists, buttons, and fields of information. Most of your interactions with the CytoFluor System take place in windows.

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