FMAT™ 8100 HTS System

User Guide

Applied Biosystems

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

The FMAT™ 8100 HTS System was manufactured under license from Becton Dickinson and Company.

Printed in the USA, 6/2001
Part Number 4308436 Rev. C

Contents

6 Troubleshooting

7 FMAT 2-Color Tutorial

A The Run Window

B A Tour of the Menus

C Setting Up the Software

Manual Overview 1

Overview

About This Chapter

This chapter provides information about the purpose of this manual, the writing conventions used in the manual, minimum system requirements, and safety precautions.

In This Chapter

The following topics are covered in this chapter:

About This Manual

Purpose

This manual provides procedures for operating the Fluorometric Microvolume Assay Technology (FMAT™) 8100 High Throughput Screening (HTS) System. It also presents

- Theory of operation
- Instrument features
- Software features
- Guidelines for operation
- Troubleshooting information

Conventions Used in This Manual

Writing Conventions

This manual uses the following writing conventions:

- Menus, menu items, window, and dialog box names appear in **bold characters like this** when they are given in tables and procedures.
- Examples of information that you type into a text box appear in `bold Courier characters like this` when given in text.

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- After emptying the waste container, seal it with the cap provided.

- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current MSDS before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>Over the Internet</td><td>a. Go to our Web site at www.appliedbiosystems.com.
b. Click SERVICES & SUPPORT at the top of the page, click Documents on Demand, then click MSDS.
c. Click MSDS Index, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to access a PDF of the MSDS.</td></tr>
<tr><td>By automated telephone service</td><td>Use “To Obtain Technical Documents” on page E-5.</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1.</td></tr>
<tr><td>By telephone from Canada</td><td><table><tr><th>To order in...</th><th>Dial 1-800-668-6913 and...</th></tr><tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr><tr><td>French</td><td>Press 2, then 2, then 1</td></tr></table></td></tr>
<tr><td>By telephone from any other country</td><td>See the specific region under “To Contact Technical Support by Telephone or Fax (Outside North America)” on page E-3.</td></tr>
</table>

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Instrument Safety Labels

Safety labels are located on the instrument. Each safety label has three parts:

- A signal word panel, which implies a particular level of observation or action (*e.g.,* CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used.
- A message panel, which explains the hazard and any user action required.
- A safety alert symbol, which indicates a potential personal safety hazard. See the *FMAT 8100 HTS System Site Preparation and Safety Guide* (P/N 4308435) for an explanation of all the safety alert symbols provided in several languages.

About Waste Profiles

A waste profile was provided with this instrument and is contained in the *FMAT 8100 HTS System Site Preparation and Safety Guide.* Waste profiles list the percentage compositions of the reagents within the waste stream at installation and the waste stream during a typical user application, although this application may not be used in your laboratory. These profiles assist users in planning for instrument waste handling and disposal. Read the waste profiles and all applicable MSDSs before handling or disposing of waste.

IMPORTANT Waste profiles are not a substitute for MSDS information.

About Waste Disposal

As the generator of potentially hazardous waste, it is your responsibility to perform the actions listed below.

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, or national regulations.

Note Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories
- Received instruction in specific safety practices for the instrument
- Read and understood all related MSDSs

⚠ CAUTION Avoid using this instrument in a manner not specified by Applied Biosystems. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

Safe and Efficient Computer Use

Operating the computer correctly prevents stress-producing effects such as fatigue, pain, and strain.

To minimize these effects on your back, legs, eyes, and upper extremities (neck, shoulder, arms, wrists, hands and fingers), design your workstation to promote neutral or relaxed working positions. This includes working in an environment where heating, air conditioning, ventilation, and lighting are set correctly. See the guidelines below.

⚠ CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. These hazards are caused by the following potential risk factors which include, but are not limited to, repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

- Use a seating position that provides the optimum combination of comfort, accessibility to the keyboard, and freedom from fatigue-causing stresses and pressures.
  - The bulk of the person's weight should be supported by the buttocks, not the thighs.
  - Feet should be flat on the floor, and the weight of the legs should be supported by the floor, not the thighs.
  - Lumbar support should be provided to maintain the proper concave curve of the spine.
- Place the keyboard on a surface that provides:
  - The proper height to position the forearms horizontally and upper arms vertically.
  - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
- Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
  - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
  - Positioning the screen to minimize reflections from ambient light sources.
  - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.
- When considering the user's distance from the screen, the following are useful guidelines:
  - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.

  - For most people, the reading distance that is the most comfortable is approximately 20 inches.
  - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
  - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.
- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

FMAT System Overview 2

Overview

About This Chapter This chapter provides a description of the FMAT™ 8100 HTS System, including its purpose and function, its components, and how the system collects data.

In This Chapter The following topics are covered in this chapter:

System Description

Purpose and Function

The FMAT system includes a detection system, computer, and accompanying software.

- The system is designed to image and measure the fluorescent intensity of cells or beads in a mix-and-read format.
- The system can scan 96- or 384-well plates for fluorescent events related to receptor ligand reactions, apoptosis, immunoassays, and other similar cell-based or bead-based assays.
- The system operates either manually or automatically (robot mode) with a built-in robotic plate handler and barcode reader.

Instrument Components

The FMAT system consists of three components:

- The FMAT instrument
- The robotic plate handler
- The computer

Refer to the following figure for a typical system layout.

Processing Plates With the Plate Handler

The FMAT system uses the robotic plate handler to enable high throughput screening. The plate handler can sequentially process 96-well or 384-well plates from each of the four input racks to the FMAT scanner.

During operation, the plate handler:

- Lifts a plate from the top position of an input rack.
- Passes the plate in front of the barcode reader (if barcode is selected).
- Places the plate in the tray of the FMAT scanner.
- Initiates the device cycle.
- Moves the plate to the output rack after the cycle is complete.

After the plates from the first rack are processed and stored in the output rack, the plates can be returned to their original rack using the re-stack option and the next rack of plates are processed.

Data Collection

The system uses various run parameters contained in an assay to define the data collection. The parameters for a run specify how the instrument and software collect, analyze, and display data.

Experiment Phases

Reading a plate on the FMAT system consists of three phases:

- Setup
- Run
- Analysis

Refer to the following table for a description of each phase.

Phase	Description
Setup	Appropriate parameters are chosen for the assay, and a specific assay is selected for the sample run.
Run	Specific wells or the entire plate is scanned and displayed.
Analysis	Numerical and image data are created. Numerical data and image files can be opened and printed by the FMAT software.

Software Task List

The FMAT™ 8100 Analysis Software performs the following tasks:

- Sets up plate information and plate layout
- Sets the run parameters for the instrument
- Collects and analyzes the digital image

Note The software displays the raw data in grayscale, histogram, scatterplot, or psuedo-color (not the actual dye-color) image form.

How the FMAT System Measures Fluorescence

Overview

A laser beam scans 1 x 1 mm square segments within 100 microns of the bottom of a microtiter plate well, exciting the fluorescent dye. The objective lens collects the emitted light. Only light emitted near the bottom of the well is collected. Then the emitted light is separated into two channels. The bandwidths are:

- 650–685 nm
- 685–720 nm

Each channel has a photomultiplier tube (PMT) that converts the light energy into an electrical signal. The electrical signal is digitized by an analog-to-digital converter, and the digital data is sent to the host computer over an ethernet connection. This digital data is then stored as an image of the fluorescent events captured by the detector at the bottom of each well.

Mapping

The FMAT instrument maps each individual plate and determines the topography of the bottom of the plate. With this map, the z-axis motor adjusts the scanning height accordingly for accurate scanning.

Once mapping is complete, scanning of each well begins. Mapping and scanning time varies depending on the type of plate being used.

Note When you set the FMAT system software to re-scan a plate repeatedly, the FMAT instrument does not need to remap the plate after the first scan because the plate is not removed.

Mapping and Scanning Times

The mapping and scanning times for different plate types are given below.

	96-well	384-well
Mapping Time (min)	1.7	4.2
Scanning Time (min)	4.5	13.2

Detector Components

There are two key components of the detection system:

- The X,Y, Z sample stage
- The optical system

Sample Stage Function

The function of the X,Y, Z sample stage is to:

- Move the microtiter plate over the objective lens
- Move the plate to and from the load position
- Autofocus the scanning laser beam in the Z (up/down) direction

Optical System Function

The function of the optical system is:

- To focus and direct the laser beam's excitation of the fluorescent dyes
- To efficiently collect the fluorescent emission
- To separate the fluorescent emission into two channels

Optical System Components

The optical system consists of the following components:

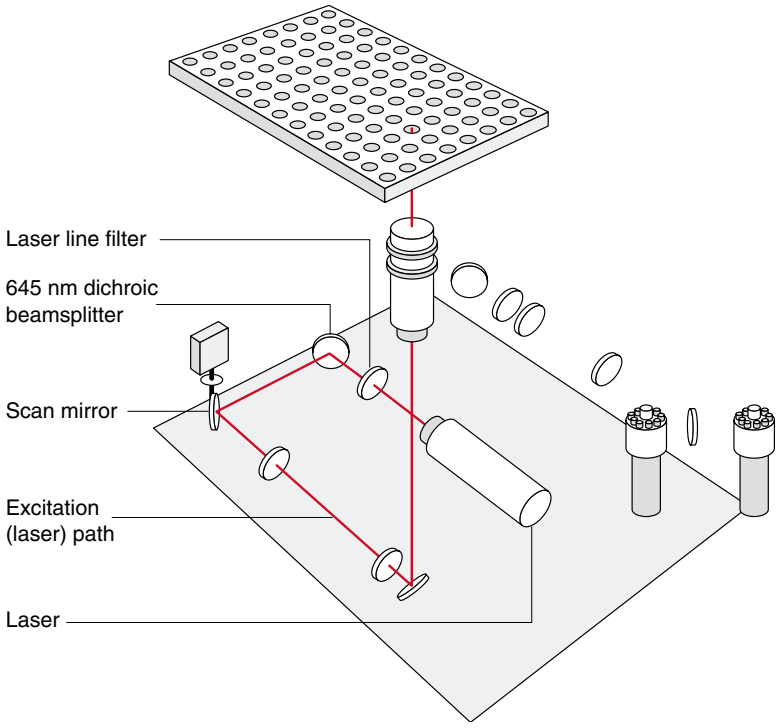
Components	Function
17-mW, 633-nm red helium-neon laser	Light source to excite an appropriate range of fluorescent dyes.
Galvanometer	Rotates a mirror that directs the laser beam along a scan line.
Filters	Optical filters cut off unwanted lower/higher light wavelengths to reduce background fluorescent noise.
	Dichroic filters (beamsplitters) allow passage of a range of wavelengths of light, while reflecting all other wavelengths.
Mirrors	A scanning mirror deflects the light beam across the sample area.
	Additional mirrors are used for directing the light.
Lenses	A 20X objective lens focuses the laser onto the sample and also collects the emitted fluorescent light. Additional lenses are used for focusing the light.
Photomultiplier tube (PMT)	Two PMTs amplify and measure the incoming fluorescent light signal. The light signal is changed into an electrical signal.
Photodiode	Used to focus during the mapping process.
Confocal aperture	Maximizes the signal-to-noise ratio by rejecting autofluorescence and scattered light outside the desired sample volume, within the microtiter well. Depth of focus: 100 µm

FMAT Optical System

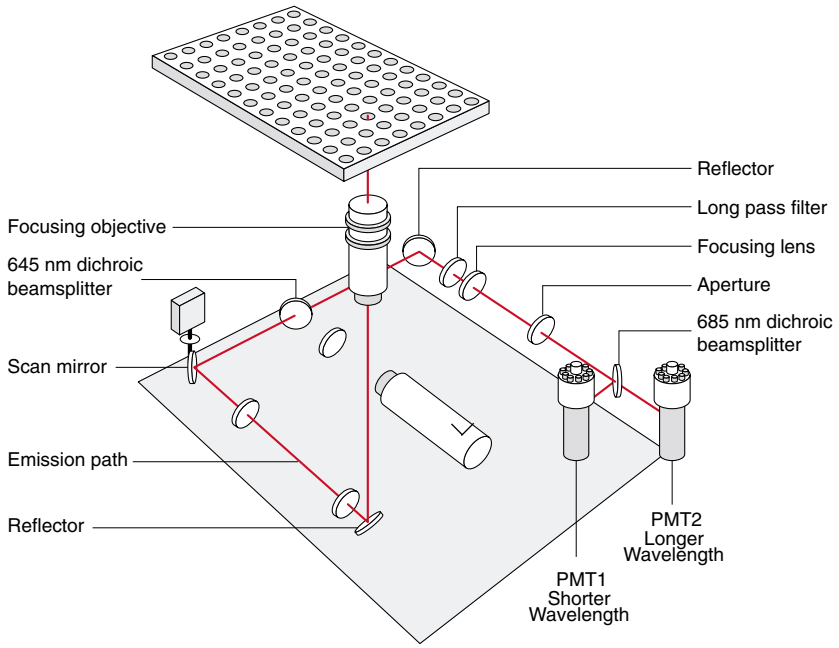
The FMAT system's unique optical platform yields population data in image format with excellent quantitative characteristics.

- The microtiter plate bottom is mapped for its topology.
- Dye-labeled cells or beads are excited by a helium neon laser. The laser performs 250 scans across an area 1mm x 1mm x 100 microns deep in 1 second.
- Two dyes can be used. The emissions of these dyes are collected by two photomultiplier tubes and converted to data.
- The FMAT system software processes the data and reports the results as:
  - Spreadsheet data
  - Two-dimensional image, three-dimensional histogram, scatterplot, and color images

Excitation Phase



Emission Phase



Running Samples 3

Overview

About This Chapter

This chapter contains procedures for loading and scanning plate(s) on the FMAT™ 8100 HTS System. Procedures to run samples using either one plate (manual mode) on the scanner tray or multiple plates using the Zymark® Twister™ Universal Microplate Handler are described here.

In This Chapter

The following topics are covered in this chapter:

Overview for Running Samples

Running Samples

The main steps for running samples are:

- Warming up the system components
- Creating a new run
- Loading a plate manually or by using the plate handler
- Scan plates

See the table below for a brief description of what is involved in each command.

Command	Description
Create a New Run	♦ Specify a name for the run and storage location for the data. ♦ Select manual or robotic plate placement. ♦ Select the assay you will use to collect and analyze data.
Load Plates	♦ In manual mode, load one plate at a time. ♦ In robot mode, the automated plate handler processes multiple plates.
Scan Plates	♦ The instrument maps each plate to determine the contour and topology of the bottom of the plate. The z-axis motor can then adjust the scanning height for consistency and accuracy. ♦ The laser scans each well. The plate matrix in the **Run** window displays the status of each well. ♦ The **Run** window allows you to view well data in real time.

Warming Up

Power on all components of the FMAT system 30 minutes before use and If the instrument has been turned off after continuous use.

Choosing Manually or With Robot Mode

The Manually or With Robot procedures are quite similar. By selecting With Robot, the options involving the plate handler become active.

The procedures that follow are based on using the Default assay. Default conditions:

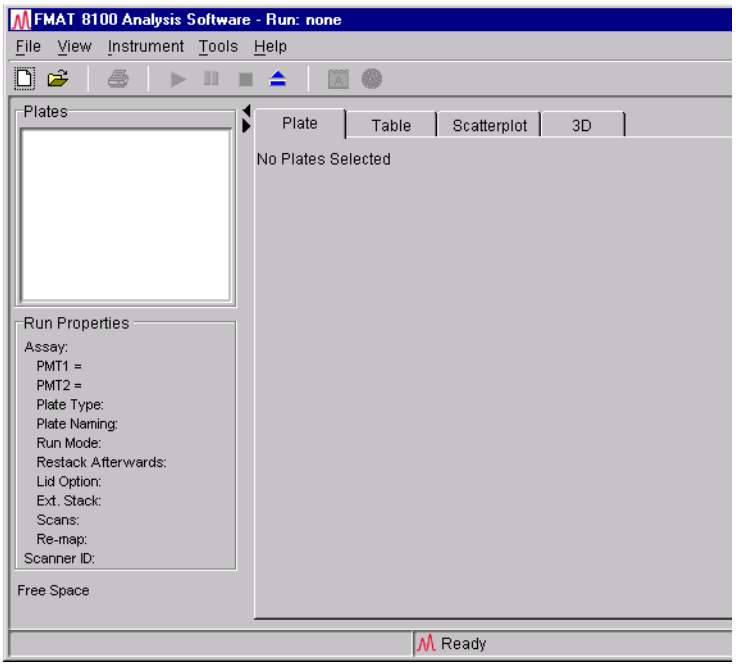
- Using 96-well plate(s)
- FL1
- Population A

Running a Plate Manually

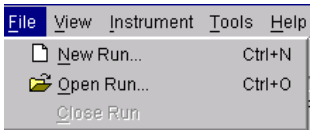
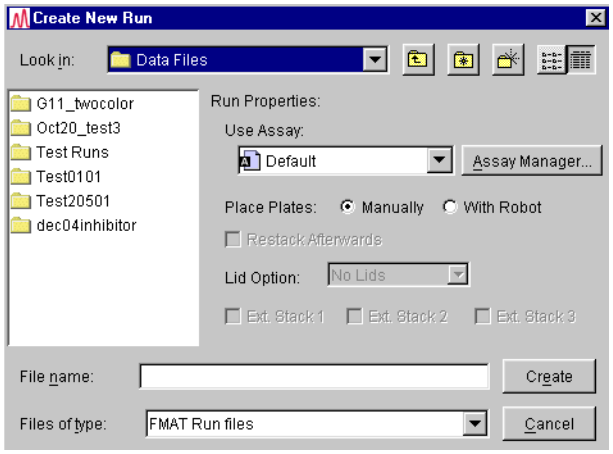
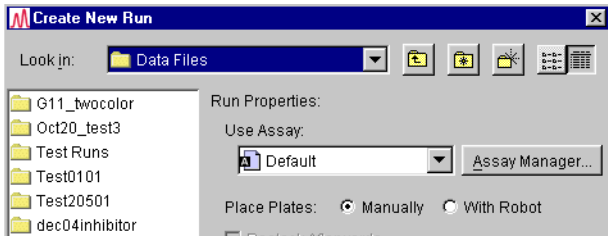
Creating a Runfile

When starting a new run, you must first create a runfile.

To create a runfile:

Step	Action
1	Launch the FMAT™ 8100 Analysis Software, and log in. If you need more information about launching the software and logging in, see “Starting the Software for the First Time” on page C-4. The Run window opens. 

To create a runfile: *(continued)*

Step	Action
2	From the **File** menu: a. Select **New Run**.  The **Create New Run** dialog box opens as shown below.  **Note** Make sure that the assay you want is listed in the **Use Assay** drop-down menu. To review the parameters of the available assays or modify an assay, click **Assay Manager** to open the **Assay Manager** window. When returning to the **Create New Run** window, check the **Use Assay** field again. See “Creating Assays” on page 5-1 for more information. b. For this exercise, select **Default**.
3	Use the **Look in** drop-down menu to select the folder to store the new run data. 
4	For **Place Plates**, select **Manually**.
5	Type the name for the run into the **File name** text box. **IMPORTANT** File or folder names cannot contain most special characters. Do not use the special characters: \ / : * ? “ < > | in a file/folder name. The FMAT system software will use the file name entered to create a folder by the same name. When special characters are used, the sample run fails because the software interprets these characters as specific commands and does not create the folder. **Note** The software appends “.fmat” to the created file, so files are always named according to the format <filename>.fmat.
6	Click **Create** to close the dialog box.

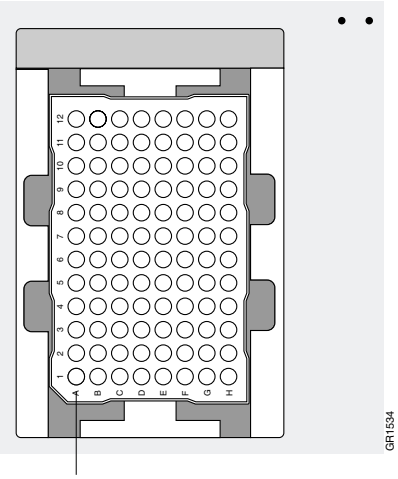
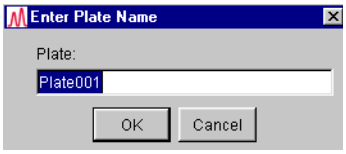
Using the Run Window

Check the main window after creating a new runfile. The main changes to notice are the:

- Name of the run appears at the very top of the window
- Run button is enabled (turns green)
- Run Properties pane shows the parameters that you selected

Loading Plates Manually

To load plates manually:

Step	Action
1	Open the scanner door by clicking the Eject button located on the toolbar.
2	Place the covered plate on the scanner stage so that well position A1 is located in the lower-left corner of the stage pointing toward the front of the scanner. **IMPORTANT** Use hard plate covers or plastic sealers to protect the samples from contamination and evaporation. Do not touch the bottom of the plates because fingerprints will affect scanning. Looking at the plate on the scanner tray from a top view, standing in front of the scanner, the plate should be oriented as shown below:  Well A1 Front of scanner **WARNING LASER HAZARD**. Do not remove the cover of the instrument. Exposure to direct or reflected laser light can burn your retina and leave permanent blind spots. Never look directly into the laser beam.
3	Click the **Start Run** icon( ). The **Enter Plate Name** dialog box opens for a manual run.  Option: Rename the plate by typing in another name, avoiding special characters.

To load plates manually: *(continued)*

Step	Action
4	Click **OK** to the default plate name **Plate001**. The plate is loaded and mapping starts. See “Mapping” on page 2-4 for more information. **IMPORTANT** Once mapping starts, it cannot be stopped. The only way to stop is to power off the system.

Pausing or Stopping a Run While Scanning

To pause or stop a run, click the Pause ( ) or Stop ( ) button.

- When you pause a run, the button grays out while the run is being paused. You can pause a run only while it is scanning, not while it is mapping. Pause does not take effect until the scanning and analysis of the current well is complete. To restart, click the Start Run button.
- When you stop a run, the instrument will finish mapping, and then the plate is ejected from the instrument. If you want to restart the run for that plate, you have to remap and rename the run.

Note The system may continue operation for a few minutes after you click the Pause or Stop buttons.

Running Another Plate

When a manual run has finished, the Enter Plate Name dialog box opens again. By default, Plate002 should be in the field.

To start another plate:

Step	Action
1	Load a second plate on the scanner stage.
2	In the **Enter Plate Name** dialog box: ♦ Click **OK** to accept the name. The plate is loaded and mapping starts. ♦ Click **Cancel** to end the run.

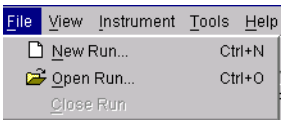
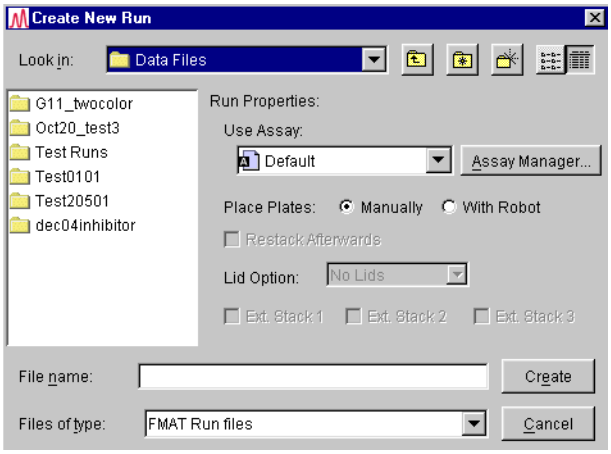
Running With the Robotic Plate Handler

Selecting Robot Parameters

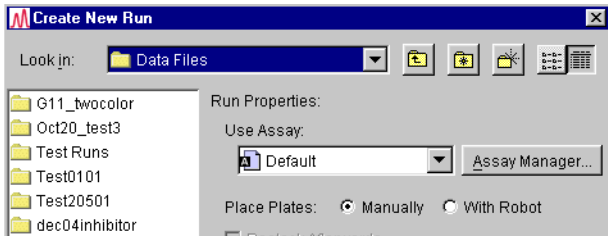
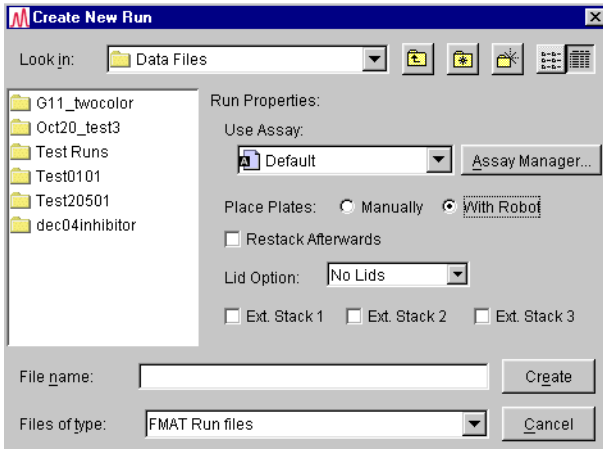
Use the plate handler for multiple plate handling. One stack can use:

- 13 plates with lids
- 15 plates with plate sealers

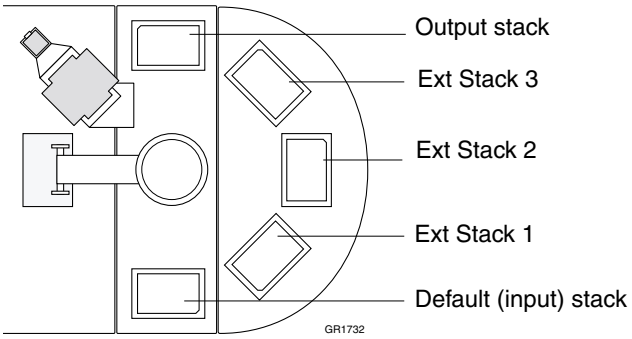
To select robot parameters:

Step	Action
1	Launch the FMAT™ 8100 Analysis Software, and log in. If you need more information about launching the software and logging in, see “Starting the Software for the First Time” on page C-4. The Run window opens.
2	From the **File** menu: a. Select **New Run**.  The **Create New Run** dialog box opens as shown below.  **Note** Make sure that the assay you want is listed in the **Use Assay** drop-down menu. To review the parameters of the available assays or modify an assay, click **Assay Manager** to open the **Assay Manager** window. When returning to the **Create New Run** window, check the **Use Assay** field again. See “Creating Assays” on page 5-1 for more information. b. For this exercise, select **Default**.

To select robot parameters: *(continued)*

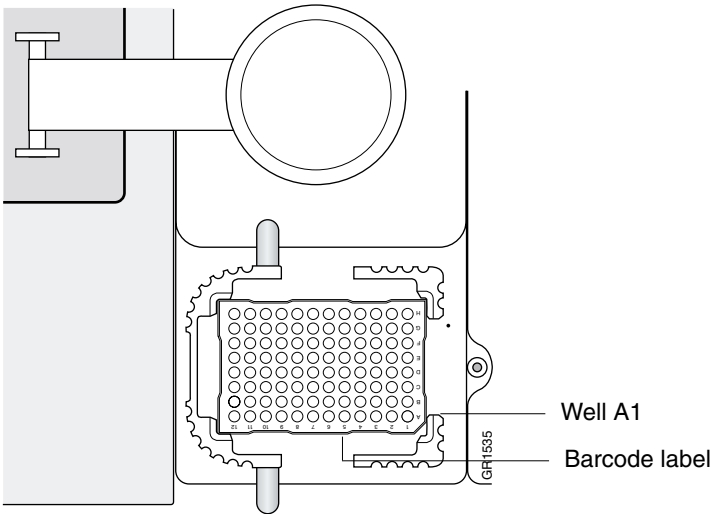
Step	Action
3	Use the **Look in** drop-down menu to select the folder to store the new run data. 
4	In the **Create New Run** window under **Run Properties**, for the **Place Plates** option, select **With Robot**. The plate handler options are enabled.  **Note** When **With Robot** is selected, the **Delayed Start** option in the **Instrument** menu is enabled. For more information about Delayed Start, see “Setting Delayed Start” on page C-8.
5	If you want your plates to be re-stacked after they have been scanned, select **Restack Afterwards**.
6	From the **Lid Option** drop-down menu, select one of the following options: ♦ **No Lids** ♦ **Top Plate Only** ♦ **Every Plate**

To select robot parameters: *(continued)*

Step	Action
7	Select the robot stacks you will be using. The stack options are used in this order: a. **Default** b. **Ext Stack 1** c. **Ext Stack 2** d. **Ext Stack 3** 
8	Type the name for the run into the **File name** text box. **IMPORTANT** File or folder names cannot contain most special characters. Do not use the special characters: \ / : * ? " < > | in a file/folder name. The FMAT system software will use the file name entered to create a folder by the same name. When special characters are used, the sample run fails because the software interprets these characters as specific commands and does not create the folder. **Note** The software appends ".fmat" to the created file, so files are always named according to the format <filename>.fmat.
9	Click **Create** to close the dialog box.

Loading Plates for Batch Mode Processing

To load plates for batch mode processing:

Step	Action
1	Place the prepared microplates into the input rack(s). **IMPORTANT** Cover the plates to protect against contamination and evaporation. Problems may occur with plastic or foil sealers that can cause the plates to stick to each other. Make sure these sealers are carefully cut to fit the plate tops with no sticky surfaces exposed.
2	Check the orientation of the plates in the racks. From a top view, well **A1** is located on the lower-right side, away from the plate reader, as shown in the top-down view below: 
3	Click the **Start Run** icon( ). The run starts.

Pausing or Stopping a Run While Scanning

To pause or stop a run, click the Pause ( ) or Stop( ) button.

- When you pause a run, the button grays out while the run is being paused. You can pause a run only while it is scanning, not while it is mapping. Pause does not take effect until the scanning and analysis of the current well is complete. To restart, click the Start Run button.
- When you stop a run, the instrument will finish mapping, and then the plate is ejected from the instrument. If you want to restart the run for that plate, you have to remap and rename the plate.

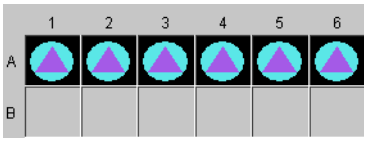
IMPORTANT The system may continue operation for a few minutes after you click the Pause or Stop buttons.

Viewing the Data Collection Process Live

Reading the Status Display

During data collection the following images are presented to indicate progress:

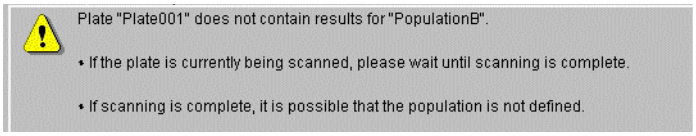
- An unscanned well is gray.
- When the scan is complete and an image file has been created, a black box and a circle are displayed in a well.
- When the image has been analyzed and an analysis file has been created, a triangle is displayed in the circle. Double-click the triangle to view the data.



Note Because analysis keeps up with collection, the circle and triangle graphics often appear at the same time.

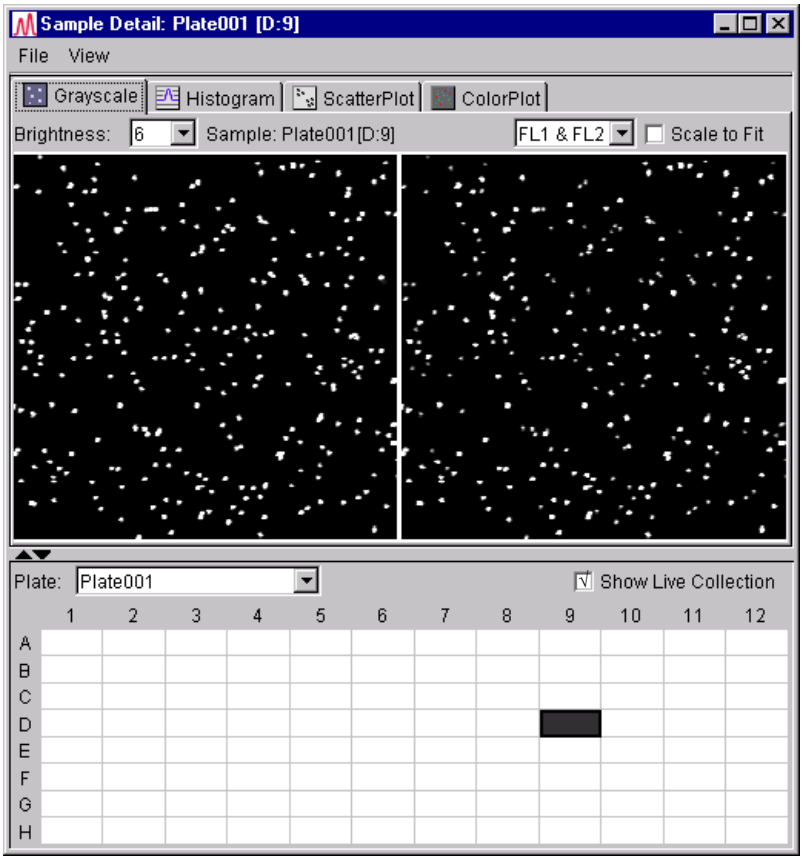
IMPORTANT You may not see any immediate change in the Scanning Time Remaining field. The field will update after mapping is complete.

If you double-click on a well that has not been scanned yet, you may see an alert box message similar to the one shown below.



Viewing Grayscale Images

To view the results of an analyzed well while data is being collected:

Step	Action
1	Double-click the triangle in the well of interest (*e.g.* A1, B3, etc.). You can also right-click on the well of interest to open the **Well Detail** menu. The **Sample Detail** window opens to the **Grayscale** tab.
2	To display the data being collected in real-time, select the check box for **Show Live Collection**. Make sure that the plate you are scanning is in the Plate drop-down list. **Note** Do not use the sample detail window during long batch runs if you are not planning to view the data. The image below shows the results for well D9. 
3	To view other scanned wells, click on the well of interest in the Plate grid.

Other Views

To see the data displayed in other formats, click the tabs:

- Histogram
- ScatterPlot
- ColorPlot

For more information about the live Sample Detail displays, see “About the Sample Detail Window” on page A-10.

Viewing and Reanalyzing Data 4

Overview

About This Chapter

This chapter shows several files that can be viewed to analyze your runfile data. An option is to reanalyze the data by using the Analysis Properties wizard described here.

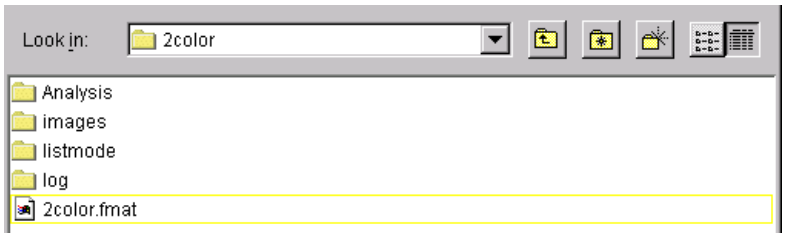
In This Chapter

The following topics are covered in this chapter:

Looking at Folder and File Structure

How Data is Stored

The FMAT™ 8100 Analysis Software version 2.0 stores the collected data from each run in a single run folder. By default, the folder is given the same name as that of the run.



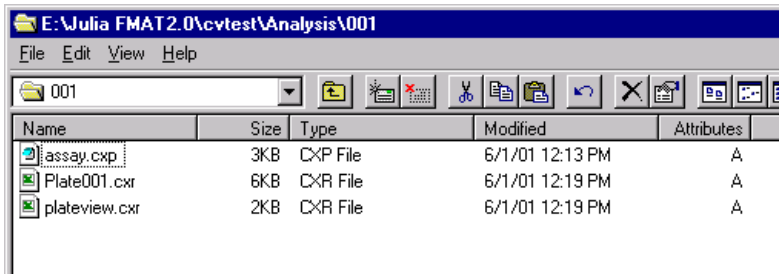
As shown above, the run folder contains these elements:

- An Analysis folder
- An images folder
- A listmode folder
- A log folder
- A runfile, ending in ".fmat"

About the Analysis Folder

The Analysis folder contains all the analysis data within subfolders. There is one subfolder for each analysis. The first analysis has a subfolder with "001" added to the name. Subsequent analyses are given subfolders with sequential incremental numbers.

Analysis 001 Subfolder Showing File Types



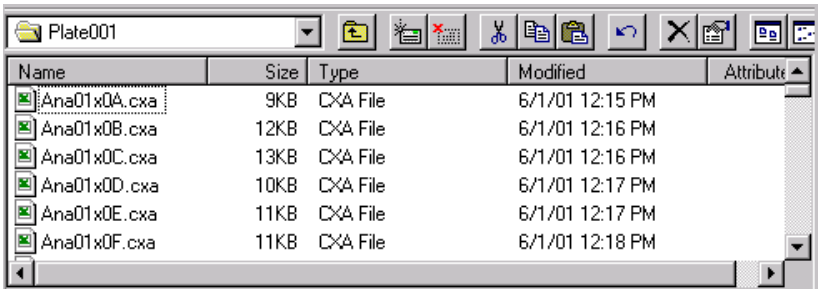
Each subfolder contains:

- One assay file (assay.cxp) giving the analysis parameters that were used.
- One or more plate results files (<platename>.cxr) giving the analysis data. The data is sorted by population and each line represents one well.
- One run result file (plateview.cxr), giving numeric results in a format matching the plate layout.
  - 8 x 12 for 96-well plates
  - 16 x 24 for 384-well plates

About the Listmode Folder

The listmode folder contains one subfolder for each scanned plate. The subfolder(s) is named after the plate. Each subfolder contains listmode files for each scanned well.

Listmode 001 Subfolder Showing File Types



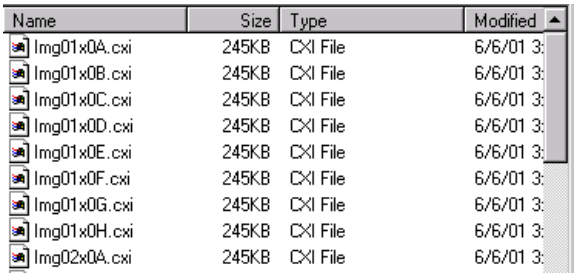
Listmode files are named according to a scheme that gives the well location for each file:

- AnaNNxAA.cxa, where
  - NN is the plate column
  - AA is the plate row

For example, a file named Ana03x0D.cxa contains data from a well in column 3 and row D. See “Listmode.cxa” on page 4-7 to view a listmode.cxa file.

About the Images Folder

The Images folder contains one subfolder for each scanned plate. The subfolder(s) is named after the plate. If a plate is scanned multiple times, each subfolder has a two-digit number added to the name that is incremented sequentially.



An images subfolder contains one image file per scanned well. The image files are named according to a scheme that gives the well location for each file:

- ImgNNxAA.cxi, where
  - NN is the plate column
  - AA is the plate row

For example, a file named Img03X0D.cxi contains image data from a well in column 3 and row D.

About the Runfile

The runfile contains information that allows the FMAT system software to find all supporting folders and files. Therefore, this is the file that you select when opening a run.

IMPORTANT Do not change the location of the runfile, or the software will not be able to find the associated folders.

About the Log Folder

The Log folder contains subfolders for recent days' runs. The subfolders are named according to the following scheme:

- YYYYMMDD, where
  - YYYY is the year
  - MM is the month
  - DD is the date

Each subfolder contains log files with instrument and run conditions for each plate run on a particular day. The log files are named according to the following scheme:

- YYYYMMDD_xx.log, where
  - YYYYMMDD is the same as the name of the subfolder
  - xx is a two-digit number added to the name that is the hour

For example, a file named 20000122_15.log was run on January 22, 2000 at 3 pm.

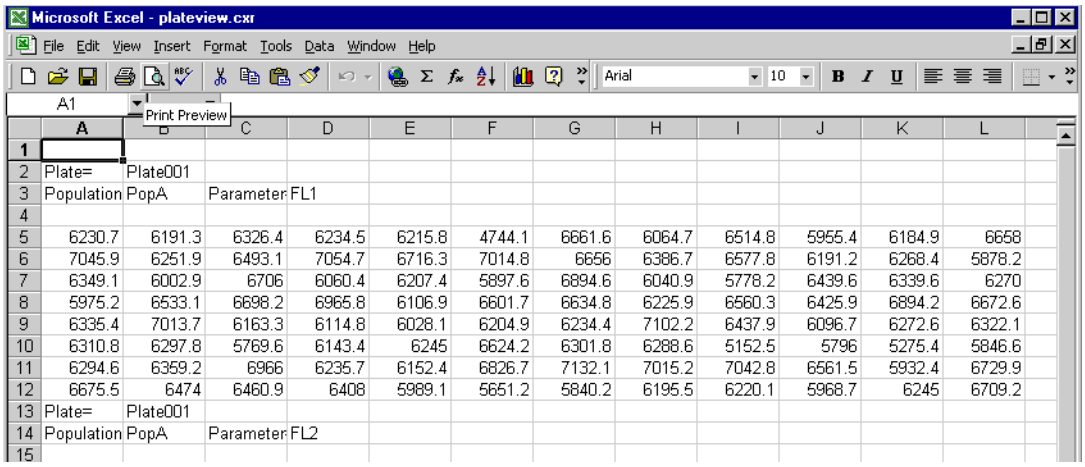
Viewing Different Data Files

Selecting a Data File to View

The table below lists the files available to study data in the run folder.

Files to View Data

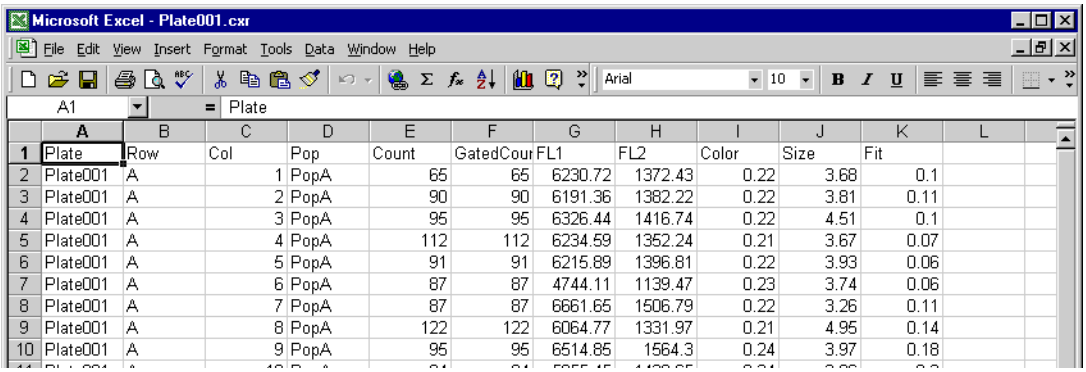
File	View for...	Layout	How to Access
Plateview.cxr	♦ Grid view of the data for all the plates in your run. ♦ Derived parameters (calculations). ♦ Multiplex assay results.	♦ Data representing all of the plates in one run. ♦ Plate grid where the column and row position represents the plate well position. ♦ Data is sorted using the parameters you selected either in Plate Analysis or Reanalysis Manager. See "Using the Plate Analysis Window" on page 5-12 or "Reanalyzing the Run" on page 4-10.	Using the system software: ♦ From **File**, select **View Run Results in Helper**. (This will open in Microsoft® Excel if linked in FMAT system software's Options. See "Setting Options" on page C-7.) Using the Browser: ♦ From the Run Folder, select **Analysis Folde**r. Click **Plateview.cxr**.



	A	B	C	D	E	F	G	H	I	J	K	L
1												
2	Plate=	Plate001										
3	Population	PopA	Parameter	FL1								
4												
5	6230.7	6191.3	6326.4	6234.5	6215.8	4744.1	6661.6	6064.7	6514.8	5955.4	6184.9	6658
6	7045.9	6251.9	6493.1	7054.7	6716.3	7014.8	6656	6386.7	6577.8	6191.2	6268.4	5878.2
7	6349.1	6002.9	6706	6060.4	6207.4	5897.6	6894.6	6040.9	5778.2	6439.6	6339.6	6270
8	5975.2	6533.1	6698.2	6965.8	6106.9	6601.7	6634.8	6225.9	6560.3	6425.9	6894.2	6672.6
9	6335.4	7013.7	6163.3	6114.8	6028.1	6204.9	6234.4	7102.2	6437.9	6096.7	6272.6	6322.1
10	6310.8	6297.8	5769.6	6143.4	6245	6624.2	6301.8	6288.6	5152.5	5796	5275.4	5846.6
11	6294.6	6359.2	6966	6235.7	6152.4	6826.7	7132.1	7015.2	7042.8	6561.5	5932.4	6729.9
12	6675.5	6474	6460.9	6408	5989.1	5651.2	5840.2	6195.5	6220.1	5968.7	6245	6709.2
13	Plate=	Plate001										
14	Population	PopA	Parameter	FL2								
15												

Files to View Data *(continued)*

File	View for...	Layout	How to Access
<Platename>.cxr	a tabular format of the data.	♦ Data representing the whole plate. ♦ Same column and row names from plate to plate.	Using the system software: ♦ Select **Table Tab**. ♦ From **File** select **View Plates in Helper**. (This will open in Excel if linked in FMAT system software's Options.) Using the browser: ♦ From the Run Folder, select **Analysis Folde**r. Click **<platename>.cxr**.



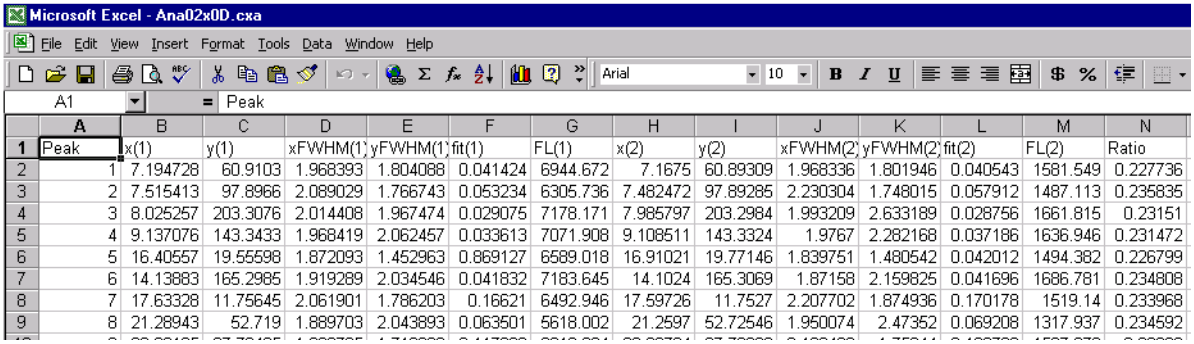
	A	B	C	D	E	F	G	H	I	J	K	L
1	Plate	Row	Col	Pop	Count	GatedCour	FL1	FL2	Color	Size	Fit	
2	Plate001	A	1	PopA	65	65	6230.72	1372.43	0.22	3.68	0.1	
3	Plate001	A	2	PopA	90	90	6191.36	1382.22	0.22	3.81	0.11	
4	Plate001	A	3	PopA	95	95	6326.44	1416.74	0.22	4.51	0.1	
5	Plate001	A	4	PopA	112	112	6234.59	1352.24	0.21	3.67	0.07	
6	Plate001	A	5	PopA	91	91	6215.89	1396.81	0.22	3.93	0.06	
7	Plate001	A	6	PopA	87	87	4744.11	1139.47	0.23	3.74	0.06	
8	Plate001	A	7	PopA	87	87	6661.65	1506.79	0.22	3.26	0.11	
9	Plate001	A	8	PopA	122	122	6064.77	1331.97	0.21	4.95	0.14	
10	Plate001	A	9	PopA	95	95	6514.85	1564.3	0.24	3.97	0.18	

The <Platename>.cxr File and Table Tab Column Headings

Column Heading	Description
Plate	Name or ID of the plate.
Row	Plate row position of the well.
Col	Plate column position of the well.
Pop	Population name for a data row.
Count	Number of events in the well.
GatedCount	The number of events in the well as determined by the Min Count option. See the note about Min Count on page 5-12 for more information.
FL1	The average intensity of the fluorescent events in PMT1.
FL2	The average intensity of the fluorescent events in PMT2.
Color	The average FL2/FL1. This is indicative of the dye associated with the individual cell or bead.
Size	The average full width at half max in the X dimension times that in the Y dimension.
Fit	A measure of smoothness. This determines how well an event's shape must fit a spline to be considered in a statistical analysis. The range is 0 to 1, where a value closer to 0 is a better fit.

Files to View Data *(continued)*

File	View for...	Layout	How to Access
Listmode.cxa	a detailed summary of the attributes of individual events.	These are files of each scanned well where one row represents one event.	Using the system software: ♦ From **File**, select **View Well Listmode in Helper**. (This will open in Excel if linked in FMAT system software's Options.) Using the browser: ♦ From the Run Folder select **listmode Folder** Click**<platename>.cxa**.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Peak	x(1)	y(1)	xFWHM(1)	yFWHM(1)	fit(1)	FL(1)	x(2)	y(2)	xFWHM(2)	yFWHM(2)	fit(2)	FL(2)	Ratio
2	1	7.194728	60.9103	1.968393	1.804088	0.041424	6944.672	7.1675	60.89309	1.968336	1.801946	0.040543	1581.549	0.227736
3	2	7.515413	97.8966	2.089029	1.766743	0.053234	6305.736	7.482472	97.89285	2.230304	1.748015	0.057912	1487.113	0.235835
4	3	8.025257	203.3076	2.014408	1.967474	0.029075	7178.171	7.985797	203.2984	1.993209	2.633189	0.028756	1661.815	0.23151
5	4	9.137076	143.3433	1.968419	2.062457	0.033613	7071.908	9.108511	143.3324	1.9767	2.282168	0.037186	1636.946	0.231472
6	5	16.40557	19.55598	1.872093	1.452963	0.869127	6589.018	16.91021	19.77146	1.839751	1.480542	0.042012	1494.382	0.226799
7	6	14.13883	165.2985	1.919289	2.034546	0.041832	7183.645	14.1024	165.3069	1.87158	2.159825	0.041696	1686.781	0.234808
8	7	17.63328	11.75645	2.061901	1.786203	0.16621	6492.946	17.59726	11.7527	2.207702	1.874936	0.170178	1519.14	0.233968
9	8	21.28943	52.719	1.889703	2.043893	0.063501	5618.002	21.2597	52.72546	1.950074	2.47352	0.069208	1317.937	0.234592

.cxa File Column and Description Headings

Column Heading	Description
Peak	Numeric identification of the fluorescent event.
x(1)	Location of the fluorescent event in PMT1 along the x-axis.
y(1)	Location of the fluorescent event in PMT1 along the y-axis.
xFWHM(1)	The full-width half max of the fluorescent event in PMT1 along the x-axis is proportional to the size of the fluorescent cell or bead.
yFWHM(1)	The full-width half max of the fluorescent event in PMT1 along the y-axis is proportional to the size of the fluorescent cell or bead.
fit(1)	A measure of smoothness of the events detected by PMT1. This determines how well an event's shape must fit a spline to be considered in statistical analysis. The range is from 0–1, where a value closer to zero is a better fit.
FL(1)	The fluorescence intensity of the fluorescent event in PMT1.
x(2)	Location of the fluorescent event in PMT2 along the x-axis.
y(2)	Location of the fluorescent event in PMT2 along the y-axis.
xFWHM(2)	The full-width half max of the fluorescent event in PMT2 along the x-axis is proportional to the size of the fluorescent cell or bead.
yFWHM(2)	The full-width half max of the fluorescent event in PMT2 along the y-axis is proportional to the size of the fluorescent cell or bead.
fit(2)	A measure of smoothness of the events detected by PMT2. This determines how well an event's shape must fit a spline to be considered in statistical analysis. The range is from 0–1, where a value closer to zero is a better fit.
FL(2)	The fluorescence intensity of the fluorescent event in PMT2.
Ratio	FL2/FL1. Is indicative of the dye associated with the individual cell or bead.

Other File Options

See “Displaying Data” on page A-3 for more information about the Plate Tab, Table Tab, Scatterplot Tab, and 3D data.

See “About the Sample Detail Window” on page A-10 for more information on viewing Grayscale, Histogram, Scatterplot, and ColorPlot data.

Reanalyzing a Run

Overview

The instrument provides the option to reanalyze run data if you want to:

- Adjust and optimize assay parameters.
- Edit plateview parameters to produce different outputs.

Changing Run Data

There are two categories of data that cannot be changed for the reanalysis of your raw data:

- The photomultiplier tube (PMT) Gains (shown in the Instrument window). PMT gains can be changed only prior to the scan because they determine the actual collection of data.
- The Assay Name (shown in the Name window). The Assay Name cannot be changed because this would modify the saved assay.

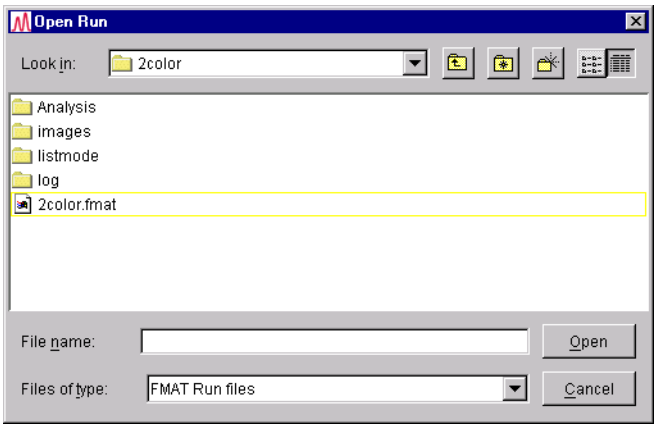
Changing Analysis Parameters

Before changing analysis parameters, review your data to determine what changes you need to make. You can:

- Open a plate, and double-click a well to open the Sample Detail window. In the Scatterplot tab, you can view the data from the selected well.
- Open the Analysis folder for your run, then open the plateview.cxr file.

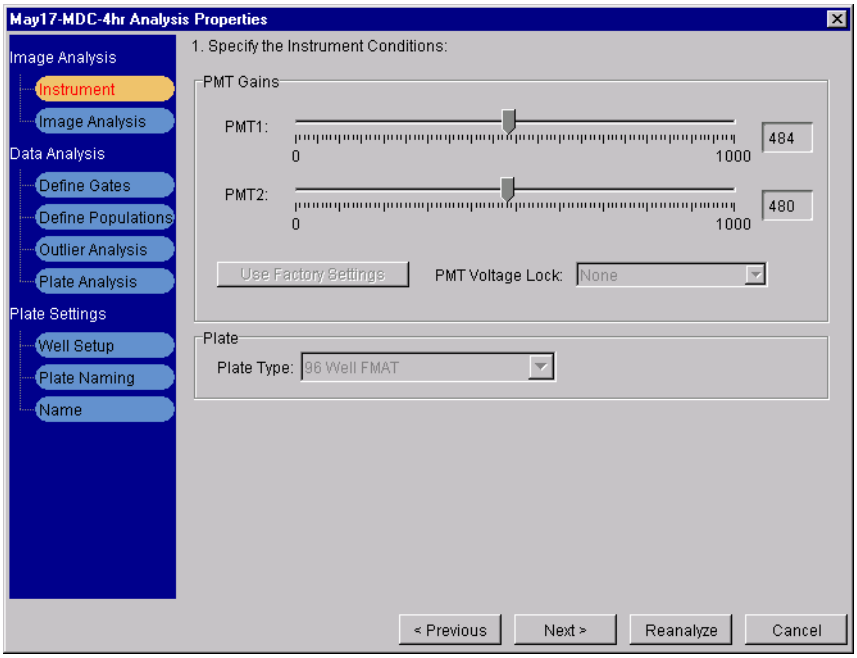
Opening a Run

To open an existing run:

Step	Action
1	From the **File** menu, select **Open Run** or click the **Open Run Document** button ( ).  The **Open Run** dialog box opens.
2	From the **Look in** drop-down menu, select the folders containing your FMAT run files.
3	From the list of folders and files, select the **.fmat** run file that you want, and click **Open**. The run opens.
4	Select a plate to view from the left side **Plates** pane in the main run window.

Reanalyzing the Run

To reanalyze data while a run is open:

Step	Action
1	From the **Tools** menu, select **Reanalyze**, or click the **Reanalyze** button ( ). The **Analysis Properties** wizard for the current run opens with **Instrument** highlighted.  This wizard functions in the same way as the New Assay wizard, except that it contains the values of the assay that was originally selected. See Chapter 5, “Creating Assays,” for more information on the New Assay wizard.
2	Step through the wizard using the **Next** and **Previous** buttons, and make your changes to the assay parameters: ♦ In **Define Gates**, you can click a plate well, and the data will display in the graph. Populations can be analyzed and new parameters can be set. ♦ In **Define Gates** and in **Define Populations** you can optimize data by choosing different parameters. **Note** You can also go directly to a specific window by clicking on the name in the left pane.
3	When done, click **Reanalyze**, and the reanalysis begins. Folders containing the reanalyzed data are created in the analysis folder. The Folder name is appended with 001, 002, etc.

Note For a reanalysis, you cannot change settings in the Instrument window or the Assay Name window.

Reanalyzing in the New Software

Data collected under the older version 1.0 software may be reanalyzed in the FMAT 8100 Analysis Software version 2.0. You will use the import function.

To reanalyze a run in the FMAT 8100 Analysis Software version 2.0:

Step	Action
1	From **File** select **New Run**. The **Create New Run** dialog box opens.
2	a. For **File name** type: `Import` (or file name of choice) b. Select the appropriate assay. c. Click **Create**. The main run window opens.
3	From **File** select **Import Plates**. The **Select the FMAT v 1.0 RunDoc.txt file** window opens.
4	a. Navigate to the desired run folder that you want to import. b. Open the run document folder. c. Select **RunDoc.text** file.
5	Click **Open**. The Plate Tab window shows the progress of importing and analyzing the wells.

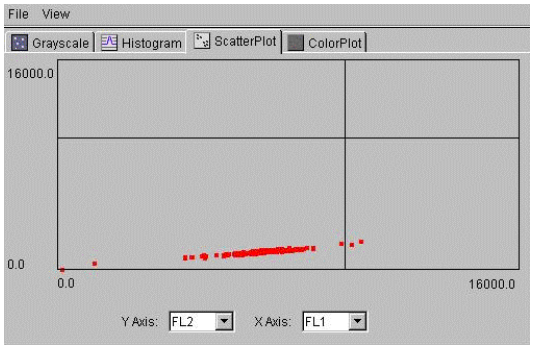
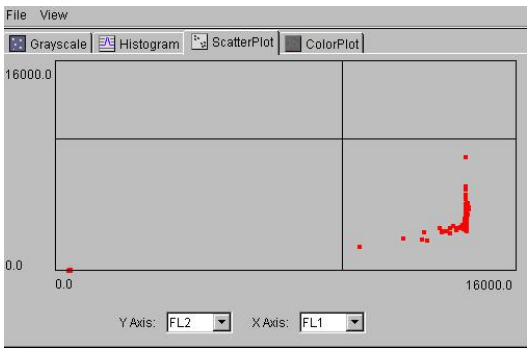
Optimizing Data

Analyzing PMT Values

To verify whether the PMT settings are appropriate, check several wells of a sample plate with positive and negative controls and analyze the data. The quality of the results can be analyzed by viewing scatterplots.

Note In **Well set-up**, deactivate the wells that are not in use.

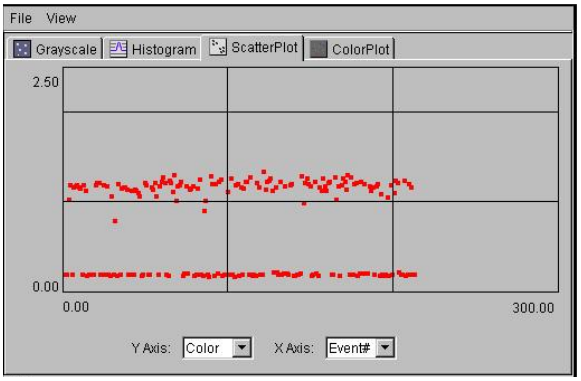
To view scatterplots of the fluorescence values:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Click the Plate tab in the run window.</td></tr>
<tr><td>2</td><td>Select a well to view and double-click it. The Sample Detail window for that well opens.</td></tr>
<tr><td>3</td><td>Select the Scatterplot tab.</td></tr>
<tr><td>4</td><td>Select FL1 for one axis and FL2 for the other.</td></tr>
<tr><td>5</td><td>Interpret the graph results: the majority of the data points should display in a linear fashion. The ratio of this line is dependent on the fluorescent dye used.
<table>
<tr><th>If...</th><th>Then...</th></tr>
<tr><td>the graph result is linear (as shown below)
 
</td><td>Good, as shown by the linear result</td></tr>
<tr><th>If...</th><th>Then...</th></tr>
<tr><td>the graph result is nonlinear (as shown below) where points do not fall on a line at the highest fluorescent values
 
</td><td>a. either one or both of the PMTs are saturated and offscale. Reset the PMTs to a lower setting.
b. Rescan the well.

Not Good.
FL1 or PMT1 is saturated.
Try lower PMT values in increments of about 50.</td></tr>
</table>
</td></tr>
</table>

Viewing Color Values

To view color values:

Step	Action
1	Select the **Scatterplot** tab of the Sample Detail window.
2	From the **x-Axis** pull-down list, select **Color**. From the **y-Axis** pull-down list, select **Event #**. Expected results show points clustered around the color value of that dye. Randomly scattered points would indicate that the color value is a poor fit. The figure below shows clusters around 1 and 0.2 as reasonable color values for this bead set containing two dyes in the well. 

Creating Assays 5

Overview

About This Chapter

This chapter contains information about using the Assay Manager in the FMAT™ 8100 Analysis Software. Information is provided for all the windows, commands, and buttons including:

- Assay Tools
- Image Analysis Settings
- Data Analysis Settings
- Plate Settings

In This Chapter

The following topics are covered in this chapter:

Assay Tools

Overview

The central concept to FMAT analysis is the assay. The New Assay Wizard allows you to design assays with different parameters for different purposes. The Assay Manager allows you to save and organize your assays into folders. You can then select any assay from your folder(s) for a run.

Opening the Assay Manager

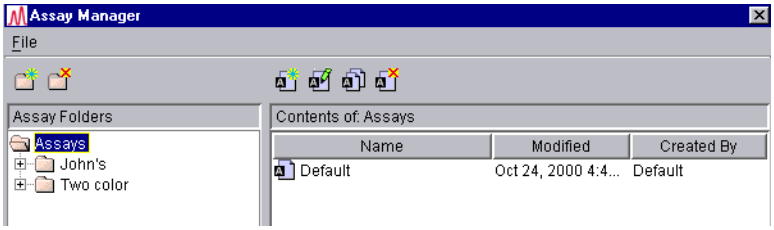
You can open the Assay Manager in different ways, depending on what you are currently doing in the FMAT system software.

- From the Create New Run dialog box
- From the Tools menu
- From the keyboard by pressing the Control and letter M keys (Ctrl+M) simultaneously

The Assay Manager Window

The Assay Manager contains all the saved assays. When you first open the system software, the only assay present is the default assay. As you define and add new assays, you can organize them into folders as shown below.

IMPORTANT Do not delete the default assay. All new assays are based on the default assay set at installation by the field service engineer.



Refer to the following table for information about the buttons in the menu bar.

Menu Bar Buttons

Button	Name	Action
	New Folder	Creates a new folder.
	Delete Folder	Deletes a selected folder once you have deleted all assays within that folder.
	New Assay	Opens the Assay Manager using the Default assay and creates a new assay.
	Edit Assay	Opens the Assay Manager using the selected assay and allows you to make changes.
	Copy Assay	Makes a copy of the selected assay.
	Delete Assay	Deletes the selected assay if you are the Administrator or the creator of that assay.

Using the New Assay Wizard

The New Assay Wizard steps you through all the parameters needed to define an assay. The New Assay Wizard is divided into three categories:

- Image Analysis
- Data Analysis
- Plate Settings

To create or edit an assay, click Next as you progress through all the windows of the wizard, then click Finish to close the New Assay Wizard. Your assay is saved and you can see it in the Assays folder of the Assay Manager.

Opening the New Assay Wizard

To open the Assay Wizard:

Step	Action
1	From the **Tools** menu, select **Assay Manager**.
2	In the **Assay Folders** pane on the left, click the **Assays** folder. All the buttons become enabled and the default assay appears in the **Contents** pane on the right.
3	Open the **New Assay Wizard**. In the **Assay Manager** window: ♦ Click the **New Assay** button() or ♦ From the **File** menu, select **New Assay**. The **New Assay Wizard** opens with **Instrument** highlighted.

Image Analysis Settings

Overview

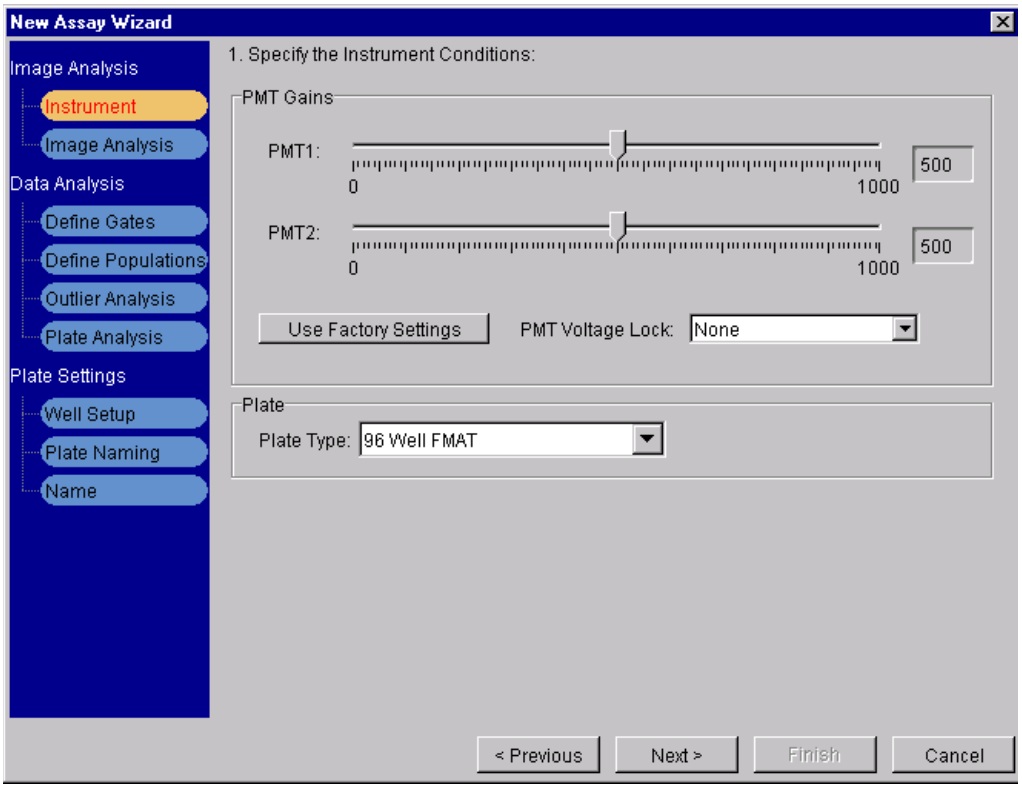
In the Assay Manager, there are two windows in the Image Analysis category:

- The Instrument window
- The Image Analysis window

Using the Instrument Window

The Instrument window allows you to specify the instrument conditions, such as:

- Change PMT (photomultiplier tube) Gains settings.
- Select the type of plate you are using. You might select 384 Well FMAT or 96 Well FMAT.



Note The default and factory settings in this window are entered by your service engineer during system installation and are unique to each instrument.

About PMT Values

IMPORTANT The PMTs are critical data collection tools and must be set correctly so that dye is detected without saturating the PMT. We strongly recommend that you use the PMT values that have been set by your service engineer.

Changing PMT Settings

Because PMT1 and PMT2 are different mechanically, they are not collecting light signal equally. The ratio has been adjusted at the factory to compensate for the difference.

IMPORTANT We strongly recommend that the relative PMT settings remain constant.

Retaining the Correct PMT Ratios

To retain the correct PMT ratios:

Step	Action
1	Click the **Use Factory Settings** button to invoke the factory setting.
2	Select the **PMT Voltage Lock** using the drop-down menu.
3	Now you need only adjust one PMT, and the other will automatically be adjusted with it while retaining the correct relative value.

Changing the Settings

To change the settings:

Step	Action
1	Place the cursor over the slider for the PMT setting you want to change.
2	While holding down the left mouse button, move the slider to the value you want. **Note** For finer control, use the right and left arrow keys on your keyboard.

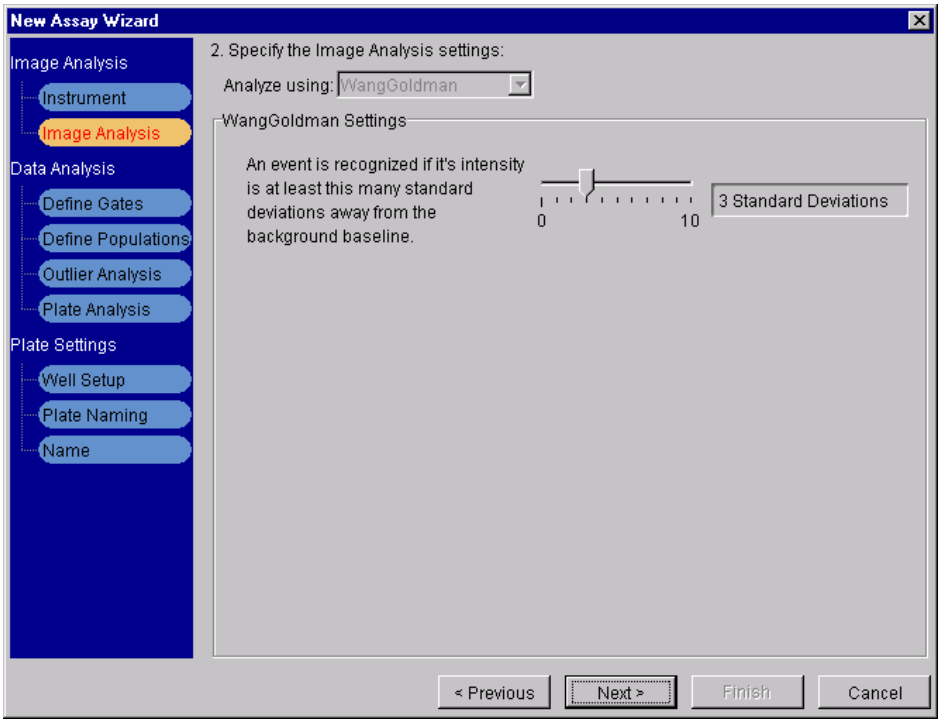
Selecting the Plate Type

To select the plate type:

Step	Action
1	Click the down arrow to the right of the **Plate Type** box to open the drop-down menu.
2	Select the correct plate type from the list.
3	When done, click **Next** to proceed to the next window.

Image Analysis Window

In the Image Analysis window you set the threshold for the recognition of an event based on the number of standard deviations difference between the intensity of the event and the background baseline.



Note As different algorithms become available, they will appear in the “Analyze using” drop-down menu at the top of the Image Analysis window.

Changing the Intensity Threshold

To change the intensity threshold:

Step	Action
1	In the Image Analysis window, place the mouse cursor over the slider.
2	While holding down the left mouse button, move the slider to the value you want. You can also use the right and left arrow keys on your keyboard to change the setting. **Note** The default setting of **3** should be used for most assays.
3	When done, click **Next** to proceed to the next window.

Data Analysis Settings

Overview

The Data Analysis category of the New Assay Manager has four windows:

- Define Gates
- Define Populations
- Outlier Analysis
- Plate Analysis

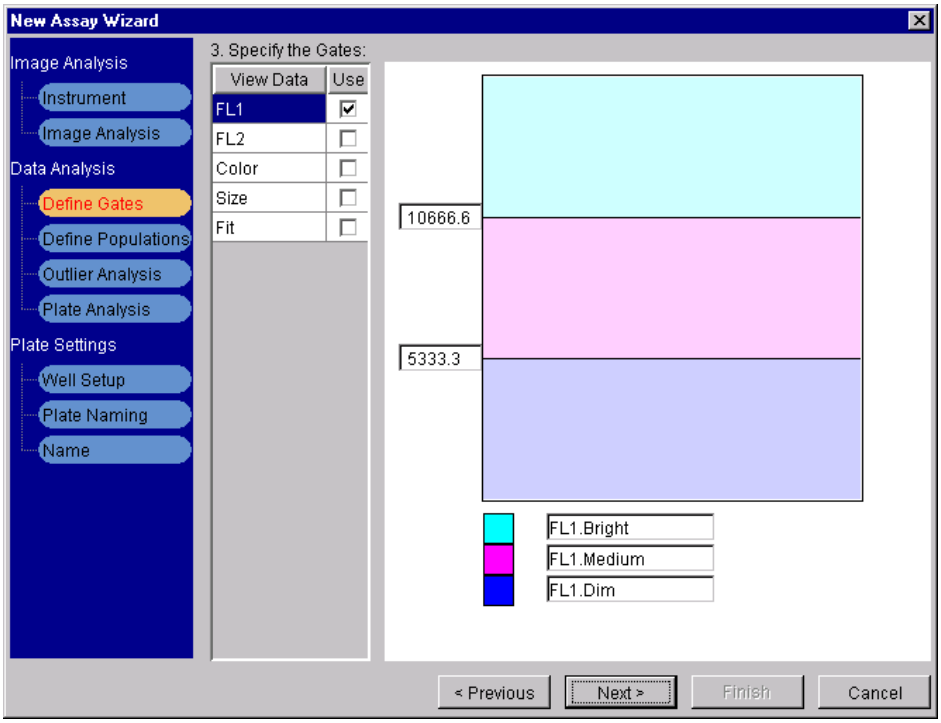
Using the Define Gates Window

In the Define Gates window you choose a parameter and then define the gates for that parameter. The parameters available in this window are described below.

Parameters	Description
FL1	The average fluorescent intensity of the fluorescent events in PMT1.
FL2	The average fluorescent intensity of the fluorescent events in PMT2.
Color	The average FL2/FL1. This is indicative of the dye associated with the individual cell or bead.
Size	The average full width at half max of the fluorescent peak in the x dimension times that in the y dimension.
Fit	A measure of smoothness. This determines how well an event's shape must fit a spline to be considered in a statistical analysis. The range is 0 to 1, where a value closer to 0 is a better fit.

Define Gates Window

When you select Define Gates, the Specify the Gates window opens.



Clicking any of the Parameter check boxes changes the Gate Selection window to show the gate settings for that parameter.

Selecting Parameters and Defining Gates

You must select at least one parameter before you can move on to the next window. Use FL1 and define PopA as all events for the default assay.

To select a parameter and define the gates:

Step	Action
1	In the small inset window placed under **Specify the Gates** shown on page 5-8, check the box(es) next to the parameter(s) you want to select: ♦ FL1 ♦ FL2 ♦ Color ♦ Size ♦ Fit The graph in the selection window has two lines to represent numerical values that you select and move to define gates, allowing up to three distinct populations. **Note** You can also simply click the name of the parameter to view the gate selection window for that parameter.
2	To alter the value of a gate, place your cursor over the line on the graph for that gate until the cursor turns into a double arrow.
3	Hold down the left mouse button and move the line until you see the value you want in the text box for that gate. **Note** You can use the Up and Down arrow keys on your keyboard for finer adjustment. You can also type in the value by selecting the textbox and typing in a new value and press **Enter**.
4	Option: Name each gate. a. At the bottom of the graph, click on the name you want to change. b. Type in the new name. This is the name that will be used in the following windows. **IMPORTANT** Do not use the following special characters in a name: / \ : " ? * < > |
5	When done, click **Next** to proceed to the next window.

Note You can color-code each gate for easier viewing:

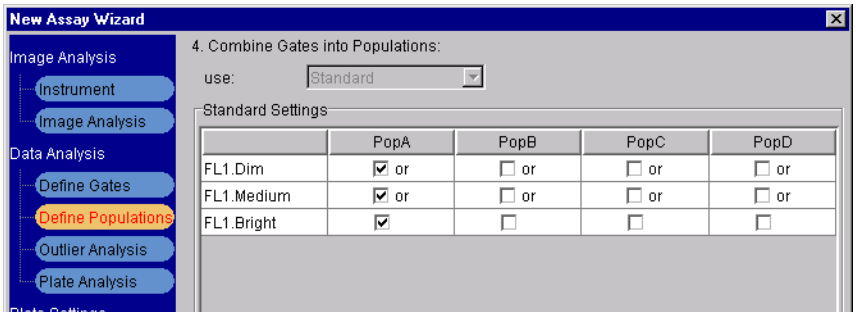
a. For each gate, click the color tile box next to the name field at the bottom of the window.

b. When the color selection window opens, click a color square to select it, then click **OK** to close the window.

Combining Gates Into Populations

In the Define Populations window, you select gated parameters to group events into distinct populations. Based on the names you assigned when setting the gates, check the boxes to determine which populations contain the gated parameters you want. Raw data is filtered using the gates, and the final output data is what you select.

The example below shows that the user has chosen FL1 Dim, Medium and Bright events to be included in Population A.



Note As plug-ins become available, they will appear in the “use” drop-down menu at the top of the Define Populations window.

Selecting and Defining Populations

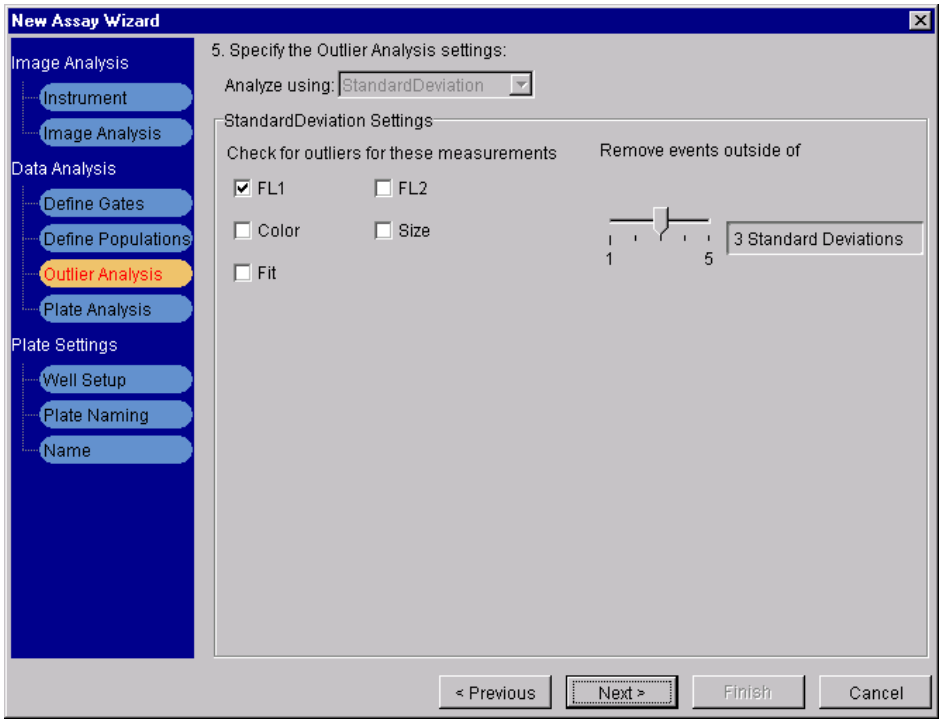
To select and define populations:

Step	Action
1	Under **PopulationA**, check the box for the parameter you want to use to define this population. Refer to the examples shown above.
2	If you want to define additional populations, check a parameter for each additional population.
3	To change the name of a population, double-click the name in the title bar, then type in the new name. **IMPORTANT** Do not use the following characters in a name: / \ : “ ? * < > |
4	When done, click **Next** to proceed to the next window.

Using the Outlier Analysis Window

In the Outlier Analysis window, you set the limits for a population using the slider bar to set the number of standard deviations from the median value. Any events that occur outside of the number of standard deviations you set are then considered to be outside of the population (outliers) and are not included in the final output.

By default, FL1 is always selected. If none of the boxes are selected, outlier analysis will not be done.



Note As plug-ins become available, they will appear in the “Analyze using” drop-down menu at the top of the Outlier Analysis window.

Specifying Outlier Settings

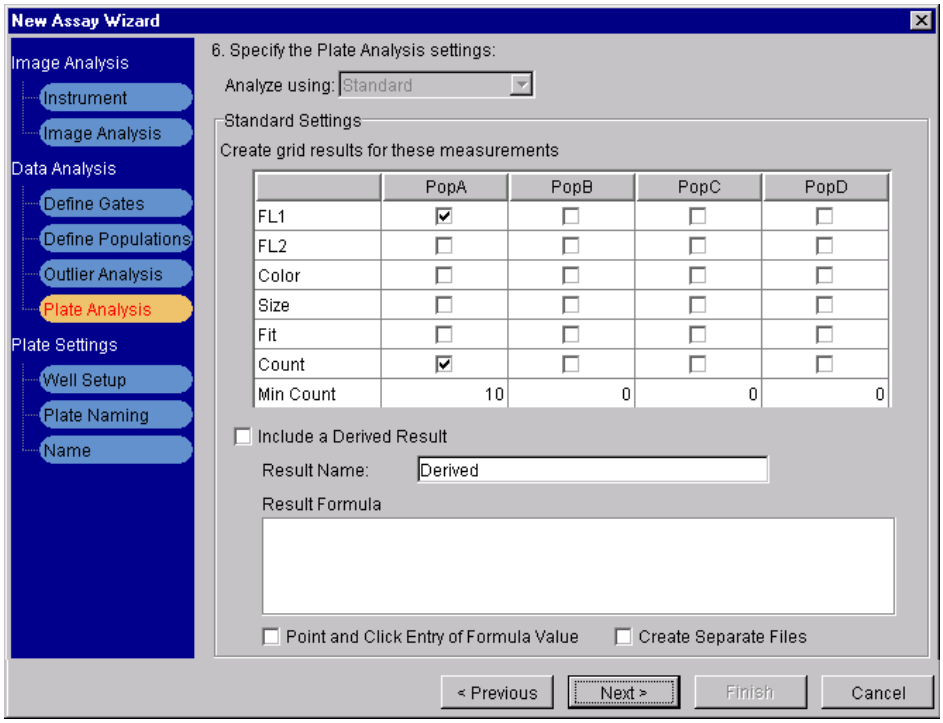
To specify outlier settings:

Step	Action
1	In the small inset window at the top left of the **Outlier Analysis** window, check the box(es) next to the measurement(s) you want to select.
2	Using the slider at the right side of the inset window, set the number of standard deviations from the mean beyond which you consider an event to be an outlier. This setting applies to all parameters that are checked. **Note** The default value is three standard deviations.
3	When done, click **Next** to proceed to the next window.

Using the Plate Analysis Window

In the Plate Analysis window, you select the parameters that are used in producing the output results. In the upper half of this window, you select the parameters that you want to report in grid format for each population. You use the lower half of this window to specify an analysis formula. Your selection in this window defines the output as seen in the plateview file. See “Plateview.cxr” on page 4-5 on how to access this file.

By default, PopA FL1 is selected. If none of the boxes are selected then plate analysis (plateview file) will not display data but the Table Tab will show data.



Note As plug-ins become available, they will appear in the “Analyze using” drop-down menu at the top of the Plate Analysis window.

Specifying Plate Analysis Settings

To specify Plate Analysis settings:

Step	Action
1	Select the check box for the parameter(s) that you will be reporting for each population. The software creates a data output in plate grid format for each selected parameter. **Note** The **Min Count** parameter is used to set the FL1, FL2, color, size, fit and count values to zero for wells that have less than the specified number of events (*e.g.*, 10).

To specify Plate Analysis settings: *(continued)*

Step	Action
2	a. Place a check mark in the **Include a Derived Result** box. b. Place a check mark in the **Point and Click Entry of Formula Value**. c. In the **Result Formula** text box, enter the formula for the **Derived Parameter Name**. Alternatively, you can click on the cell of the population you want to use, then enter the action you want to take. The Derived formula is used to output data using a mathematical calculation dependent on two parameters in one population or between two populations using Reverse Polish Notation (RPN). So, for example, the formula PopA / PopB * 100 appears as **PopA:FL1 PopB:FL1 / 100 ***. (For more information about RPN, go to http://www.hpmuseum.org/rpn.htm.) **Note** Right-click in the **Formula** text box to view an editing menu. You can also use the editing keyboard shortcuts (see Appendix B, “A Tour of the Menus”.)
3	Select the check box for **Create Separate Files** if you want a file produced for each checked parameter. **Note** The software always creates a file (plateview.cxr) with all of the parameters.

Plate Settings

Overview

The Plate Settings category of the New Assay Manager is used to specify active wells and create naming schemes. The Plate Settings category has three windows:

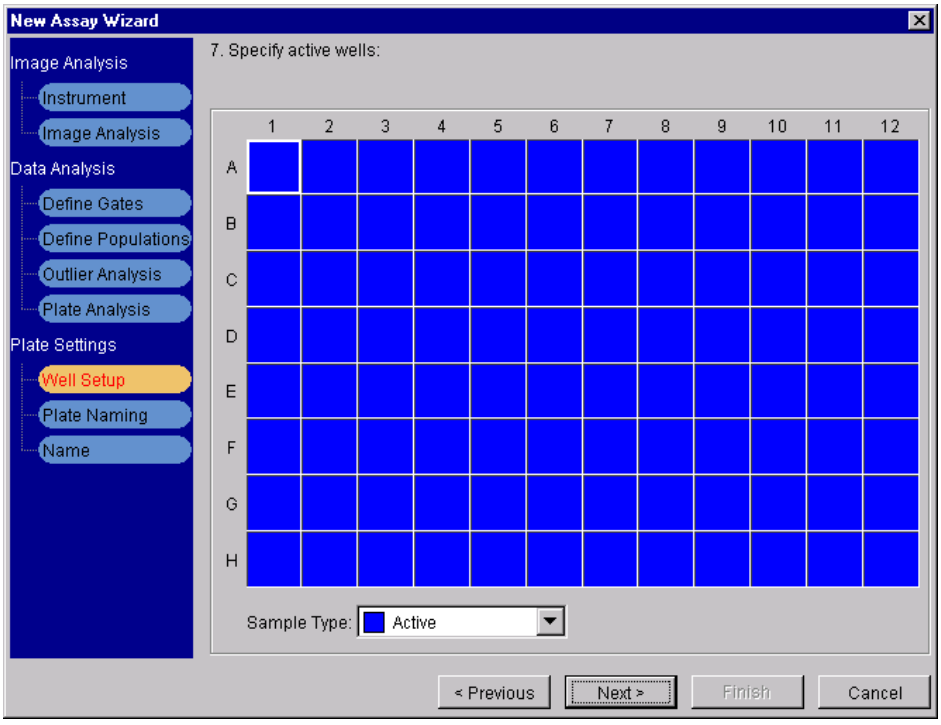
- Well Setup
- Plate Naming
- Name

Using the Well Setup Window

In the Well Setup window, you designate wells that are not in use. All remaining wells in your plate are unknown (sample) wells. The scanner will only collect data for the wells you designate as unknown.

The well designations are:

- Black wells are in use.
- Grey wells with an X are not in use.

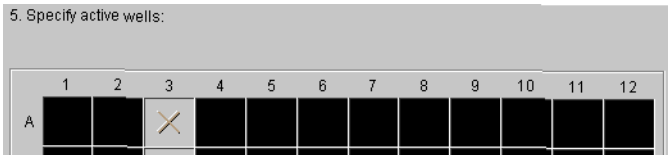


Designating Wells

To designate wells:

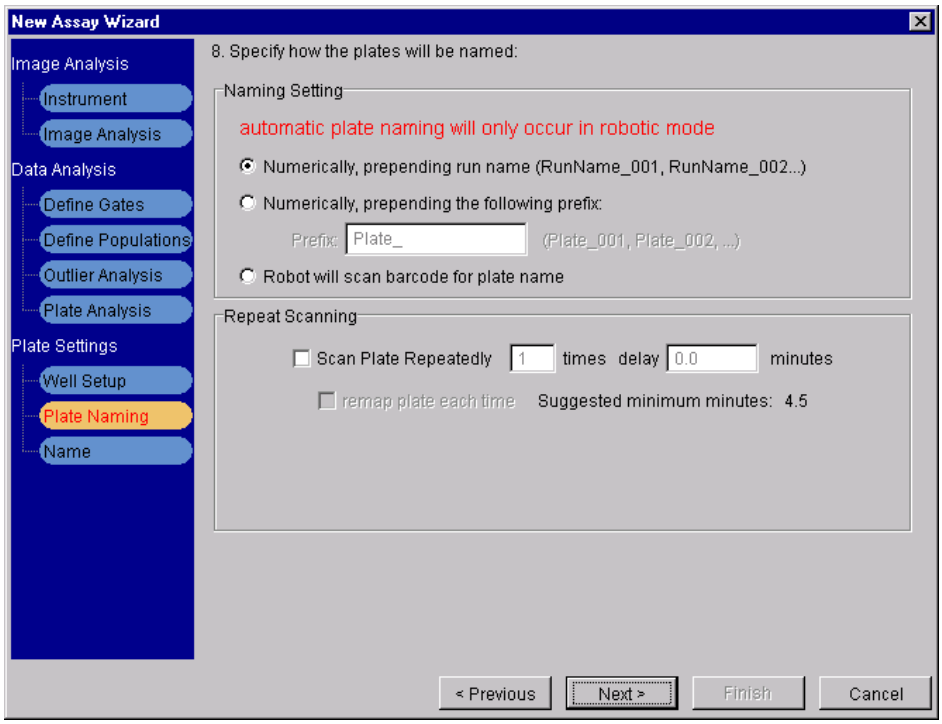
Step	Action
1	a. Select a well by clicking it. b. Select multiple wells by: ♦ Dragging the cursor across the wells you want, or ♦ Press and hold the Control key (Ctrl) while clicking non-contiguous wells
2	Click the down arrow to the right of the **Sample Type** box to open the drop-down menu.

To designate wells: *(continued)*

Step	Action
3	Select **Not in Use** as needed.  **Note** All wells are designated active unless you change their designation.
4	When done, click **Next** to proceed to the next window.

Using the Plate Naming Window

In this window, you set the naming scheme for your plates. If you are setting up assays that require repeat scans, as for kinetic studies, you set the parameters in this window.



Naming and Repeat Scanning Plates

To name plates and set repeat scanning:

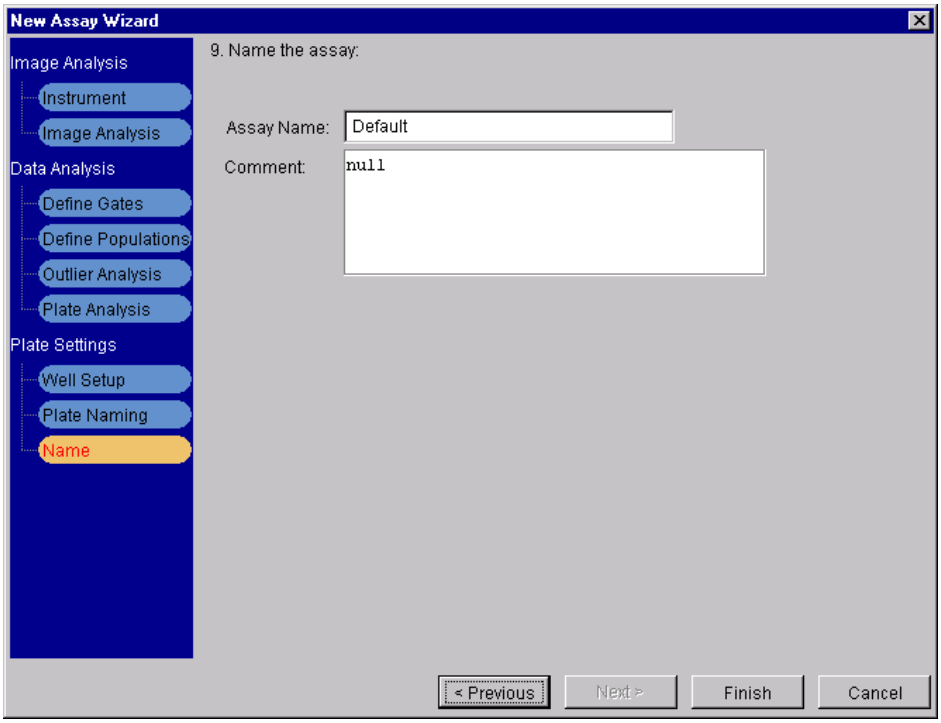
Step	Action
1	For a robotic run, select the plate-naming method you want to use. (For a manual run, you are always prompted to enter a name for each plate.) The robotic run plate-naming options are: ♦ **Numerically, prepending run name** ♦ **Numerically, prepending the following prefix** ♦ **Robot will scan barcode for plate name** **Note** If you select the second option, be sure to type in the prefix that you want to use.

To name plates and set repeat scanning: *(continued)*

Step	Action
2	Set the repeat scanning options. a. Select the check box for **Scan Plate Repeatedly**. b. Type in the number of times you want the plate to be scanned. c. Type in the time interval you want to use. Measure from the start of the first plate to the start of the second plate. **Note** There is a minimum time between each scan. The software estimates this time based on the mapping and scanning time for the full plate.
3	Be sure the check box for **Scan Plate Repeatedly** is clear if you do not want repeat scanning.
4	When done, click **Next**.

Using the Name Window

In this window, you assign a unique name to the assay you have just created or modified.



Note When editing an existing assay, the Assay Name text box displays the assay name but is deactivated so that you cannot change the name.

Naming Assays

To name the assay:

Step	Action
1	In the **Assay Name** text box, type a descriptive name.
2	If desired, add your comments to the **comment** text box. **Note** Right-click in the **comment** text box to view an editing menu. You can also use the editing keyboard shortcuts. See Appendix B, “A Tour of the Menus” for more information.
3	When done, click **Finish** to close the **New Assay Wizard** and save your assay. Your assay is now listed in the **Contents** page of the **New Assay Wizard**.
4	Click **Done** to exit the **Assay Manager**. You are now ready to start a run.

6 Troubleshooting

Overview

About This Chapter

This chapter provides detailed information about occasional problems that you may encounter when using the FMAT™ 8100 HTS System. Symptoms that you may observe and suggested solutions are described.

In This Chapter

The following topics are covered in this chapter:

Common Problems

Problems and Solutions

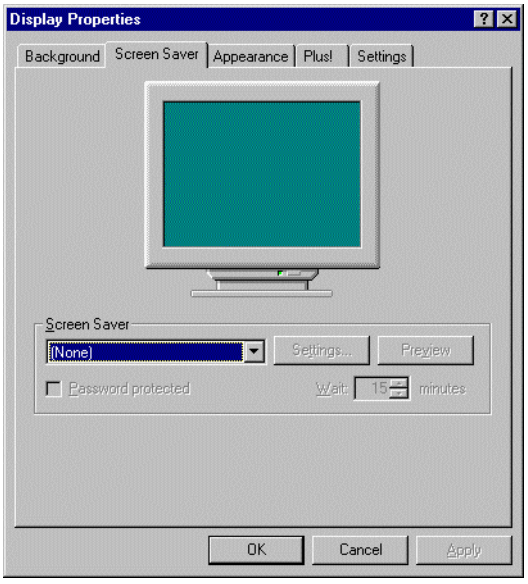
Below are common problems with the most likely solutions:

Observation	Possible Solution
Well images are blurry or blank.	Make sure beads are settled for a minimum of four hours. Make sure there is no condensation or fingerprints on the bottom of the plate(s).
You cannot connect to the scanner.	♦ Check the cables and ethernet connection. ♦ Check that no other software is communicating with the instrument by checking the task bar. ♦ Check that power is on to all components.
The plate handler and/or barcode reader is not operational.	♦ Check that power is on to all components. ♦ Check that appropriate selections have been made in the FMAT system software. See “Running With the Robotic Plate Handler” on page 3-7. ♦ Power off the system. Restart the plate handler, the FMAT scanner and lastly, the software.
There is a non-linear comparison of FL1 and FL2.	Check the PMT values. Refer to “Optimizing Data” on page 4-12.
The image display is too bright or dark.	Adjust the intensity control. See “Grayscale Display” on page A-11.
Samples are being contaminated or are evaporating.	Use lids or plate sealers to protect prepared plates.
The scanner goes offline during mapping.	The data drive is full.
Wells appear white during scanning.	There is too much unbound dye.
One or more components experiences a power loss.	Fuse(s) may need to be replaced. See “Changing Fuses” on page 6-4.
There is a highly fluorescent sample with events appearing larger than expected and very uniformly bright.	Rescan the plate with a lower PMT setting.
There is a low signal fluorescent sample where events are very dim, the image background is black and no (cells) are detected.	Rescan the plate with a higher PMT setting.

Inactivating the Screen Saver

Screen savers should not be active while running the FMAT™ 8100 Analysis Software when connected to an FMAT instrument. If necessary, use the Microsoft® Windows NT® Display Properties dialog box to turn off the screen saver.

To turn off the screen saver:

1	From the **Start** menu, select **Settings**, then **Control Panel**.
2	Double-click the **Display** icon. The **Display Properties** dialog box opens. 
3	Select the **Screen Saver** tab.
4	In the **Screen Saver** text box, select **(None)**.
5	Click **OK**.

Changing Fuses

About the Fuses

Loss of power to the FMAT scanner and/or the Zymark® Twister Microplate Handler may be caused by blown fuses.

⚠ CAUTION Determine the cause of any power overload before replacing blown fuses.

Changing Scanner Fuses

The scanner uses two 5-Amp Type T fuses, size 5 x 20 mm.

Tool needed to change the fuses:

- A small, flat-blade screwdriver

⚠ WARNING **ELECTRICAL SHOCK HAZARD**. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

To change the scanner fuses:

Step	Action
1	Put the scanner power switch in the off position and unplug the instrument. **⚠ WARNING** **ELECTRICAL SHOCK HAZARD**. Disconnect the power cord to the scanner before changing the fuses.
2	Use the flat-blade screwdriver to gently pry open the fuse cover.
3	Remove the red fuse block assembly containing the fuses.
4	Replace the fuses. Be sure the fuses are seated securely. **IMPORTANT** Replace the blown fuses only with the fuses of the same type and rating.
5	Slide the fuse block back into position.
6	Snap the fuse cover securely into place.

Changing Plate Handler Fuses

The plate handler uses two types of fuses:

- There is one 1.5-Amp fuse for 120 V operation.
- There are two 0.8-Amp fuses for 220/240V operation.

⚠ WARNING ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

Tools needed to change the fuses:

- A small, flat-blade screwdriver.
- A small, Phillips-head screwdriver.

Refer to the following table for information on how to change the fuses.

To change the plate handler fuses:

Step	Action
1	Put the plate handler power switch in the Off position and unplug the instrument. **⚠ WARNING ELECTRICAL SHOCK HAZARD**. Disconnect the power cord to the scanner before changing the fuses.
2	Use the flat-blade screwdriver to gently pry the cover/fuse block assembly away from the power entry port housing.
3	Remove the cover/fuse block assembly containing the fuse. a. Loosen the Phillips-head screw on the pedestal by two turns. b. Remove the fuse block by sliding it up and away from the screw and pedestal. There is one holder for the 1.5-Amp fuse on one side and the two holders for the 0.8-Amp fuses on the other.
4	Be sure the fuse is the correct type and is seated securely. **IMPORTANT** Replace blown fuses only with the fuses of the same type and rating.
5	Slide the fuse block back into position and tighten the screw. Make sure the new fuse(s) faces out.
6	Slide the cover/fuse block assembly back into the housing.
7	Snap the block assembly securely into place.

Monthly Equipment Maintenance

Plate Handler

Clean the:

- Base and arm surfaces using a water-dampened cloth or sponge.
- Gripper pads using a lint-free cloth dampened with isopropanol.

⚠ WARNING **CHEMICAL HAZARD**. **Isopropanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Computer Checklist

The following tasks are recommended procedures for the computer:

√	**Description**
	Back up or archive your data files.
	Verify if there is enough space on the hard drive.
	De-fragment the hard drive.

FMAT 2-Color Tutorial 7

Overview

About This Chapter

This chapter provides a tutorial for the use of the FMAT™ 8100 HTS System and demonstrates how to set up, run, and analyze a two-color experiment.

In This Chapter

The following topics are covered in this chapter:

Using the Two-Color Bead Assay

About the Two-Color Bead Assay

The FMAT™ Two-Color Standard Microsphere Kit (P/N 4316618) uses two sets of beads that each contain a different dye. Each set of beads comes in four intensities, ranging from high (1) to low (4).

The Two-Color Kit demonstrates how to view and analyze two-color assays.

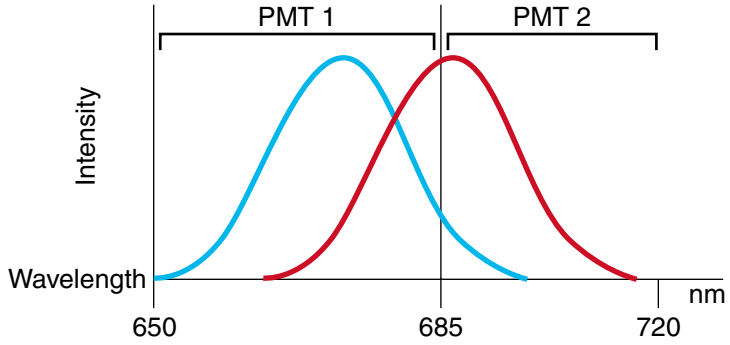
About Intensity Levels

Each tube in the kit is labeled with the intensity level of the dye. Each tube is also labeled with a relative intensity value. The highest intensity level, Intensity 1, is always 100% relative intensity.

The relative intensity is determined by flow cytometry. While this correlates closely with the value determined in the FMAT instrument, it is not identical.

PMT Channels

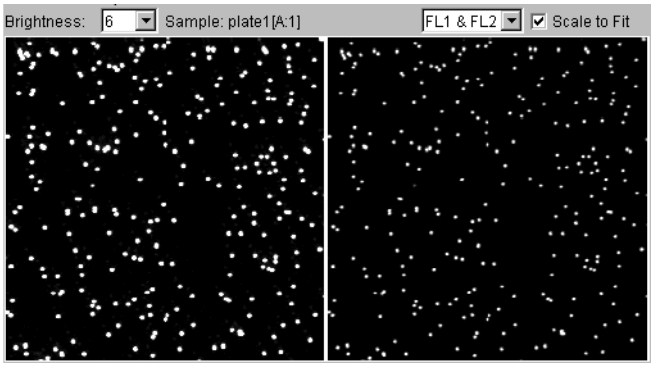
The FMAT instrument has two photomultiplier tubes (PMTs) and each represents a different color channel. A graphical representation is given below.



- Dye A is detected mainly in Channel 1, with some detection in Channel 2.
- Dye B is detected almost equally in Channel 1 and Channel 2.

About PMT Channels and Image Windows

Each image window corresponds to a PMT channel. So, for example, when viewing Dye A events, the Dye A events are much brighter in Channel 1 (left window) than in Channel 2 (right window), because Dye A emission is collected primarily in Channel 1.



Materials Supplied

Kit Contents

The FMAT™ Two-Color Standard Microsphere Kit (P/N 4316618) contains eight vials of colored beads as follows:

- Dye A (red cap)
  - Intensity 1 (highest, 100%)
  - Intensity 2
  - Intensity 3
  - Intensity 4 (lowest)
- Dye B (purple cap)
  - Intensity 1 (highest, 100%)
  - Intensity 2
  - Intensity 3
  - Intensity 4 (lowest)

Each kit contains sufficient beads to run ten 96-well plates.

Note Cap colors do not correlate with the colors used to visualize two-color data in the FMAT software.

Storage and Stability

The Two-Color Kit is stable for one year when stored under the following conditions:

- Undiluted
- 4 °C
- In the dark

Materials Required but Not Supplied

Materials Supplied by the User

The following materials are required but not included with this kit:

Item	Source
FMAT 8100 HTS System	Applied Biosystems
Ten 96-well FMAT plates	Applied Biosystems (P/N 4308776)
0.2-µm filtered deionized water	Major Laboratory Supplier (MLS)
Twelve 1.5-mL Eppendorf tubes	MLS
1000-µL micropipettor	MLS
1000-µL micropipettor tips	MLS
200-µL micropipettor	MLS
200-µL micropipettor tips	MLS
Tube rack	MLS
Vortex mixer	MLS

Setting Up the Assay

Preparing the Beads

You prepare the colored beads for the assay by diluting them as outlined below. You will analyze each color of bead separately, and combine them for the multiplex portion of the assay.

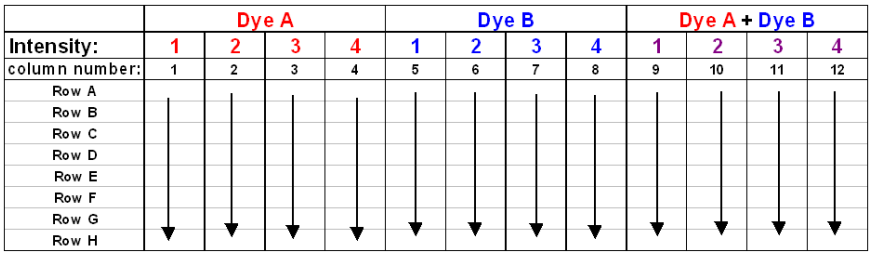
To prepare the beads for the assay:

Step	Action
1	Label 12, 1.5-mL Eppendorf tubes: ♦ Tubes A1–A4 are four intensity levels of Dye A ♦ Tubes B1–B4 are four intensity levels of Dye B ♦ Tubes AB1–AB4 are four intensity levels of Dye A + Dye B
2	Add 900 µL of deionized water to each Eppendorf tube.
3	Vortex the colored beads for 3–5 seconds to resuspend.
4	Add the beads to each Eppendorf tube as follows:

Tube number	Beads	Intensity	Volume (µL)
A1	Dye A	1	100
A2	Dye A	2	100
A3	Dye A	3	100
A4	Dye A	4	100
B1	Dye B	1	100
B2	Dye B	2	100
B3	Dye B	3	100
B4	Dye B	4	100
AB1	Dye A + Dye B	1	50 + 50
AB2	Dye A + Dye B	2	50 + 50
AB3	Dye A + Dye B	3	50 + 50
AB4	Dye A + Dye B	4	50 + 50

Filling the Plate

To fill the 96-well plate:

Step	Action
1	Vortex the Eppendorf tubes briefly to mix.
2	Dispense 100 µL of the bead mixture from each Eppendorf tube into the wells of the corresponding column of the 96-well plate. **IMPORTANT** Be sure to use the FMAT optical plate. Other plates may not have the correct specification for optimal results.
3	The plate layout is as follows: 
4	Seal the plate with a lid or plate sealer, and set it in the dark at room temperature for 4–24 hours. **IMPORTANT** Do not touch the bottom of the plate. The laser scans through the bottom of the plate. **IMPORTANT** The beads require at least 4 hours to settle to the bottom of the well.

Setting Up the Run Parameters

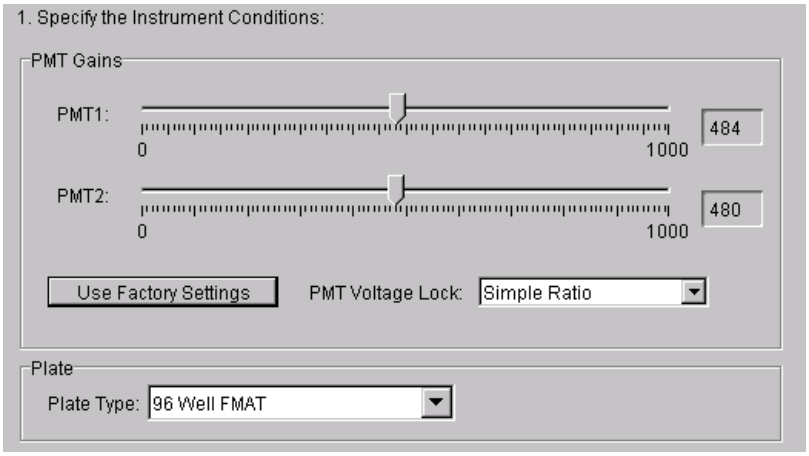
Opening the New Assay Wizard

To open the Assay Wizard:

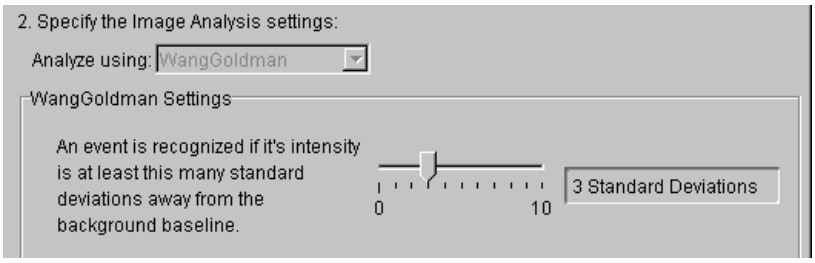
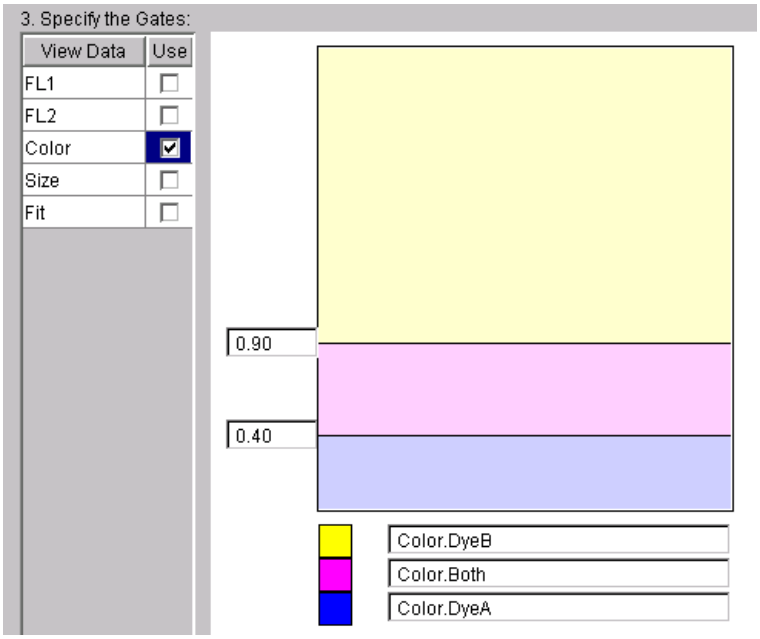
Step	Action
1	From the **Tools** menu, select **Assay Manager**.
2	In the **Assay Folders** pane on the left, click **Assays** folder. All the buttons become enabled and the default assay appears in the **Contents** pane on the right.
3	Open the **New Assay Wizard**. In the **Assay Manager** window: ♦ Click the **New Assay** button () or ♦ From the **File** menu, select **New Assay**. The **New Assay Wizard** opens with **Instrument** highlighted.

Creating a Two-Color Assay

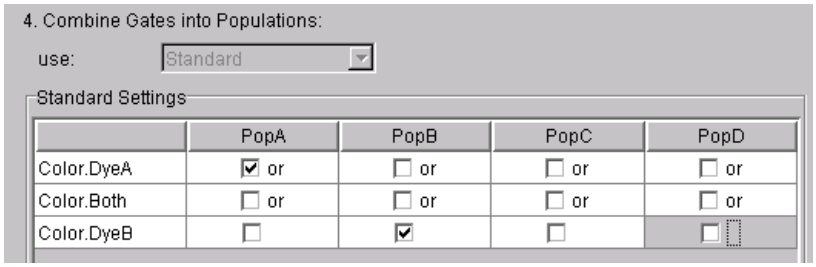
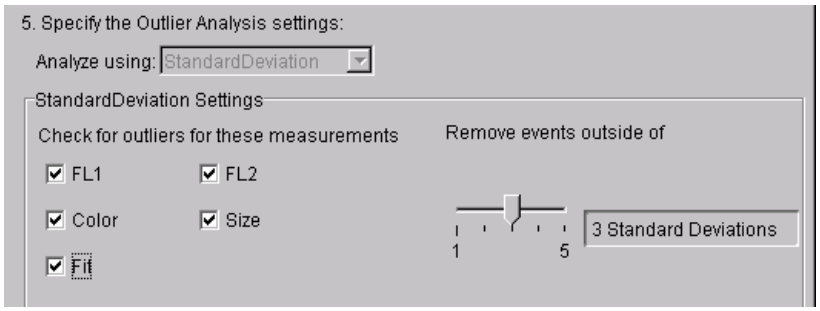
To create a two-color assay:

Step	Action
1	In the **Instrument** window as shown below:  a. Use **Factory Settings** or PMT Gains set by your field service engineer. b. Have the **PMT Voltage Lock** to **Simple Ratio**. c. Set the **Plate Type** to **96 Well FMAT**. d. Click **Next**.

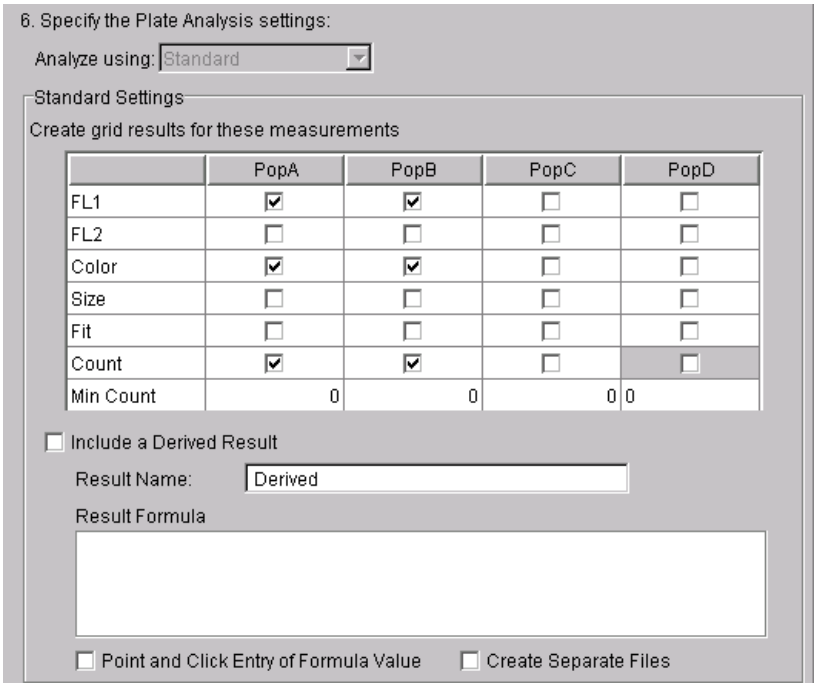
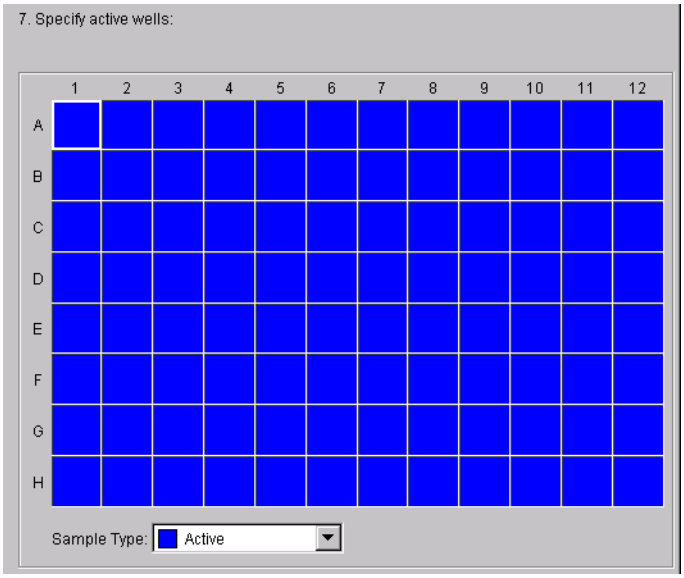
To create a two-color assay: *(continued)*

Step	Action
2	In the **Image Analysis** window below:  a. Leave the event detection setting at **3 Standard Deviations**. b. Click **Next**.
3	In the **Define Gates** window below:  a. Click **Color** and place a check mark next to **Color**. b. Type or move the bar(s) until the values are 0.90 and 0.40 as shown above. c. Click **Next**.

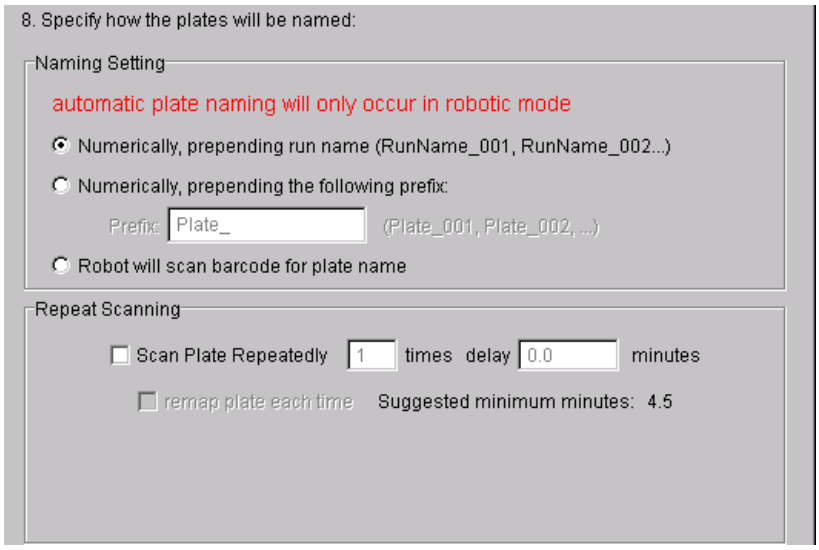
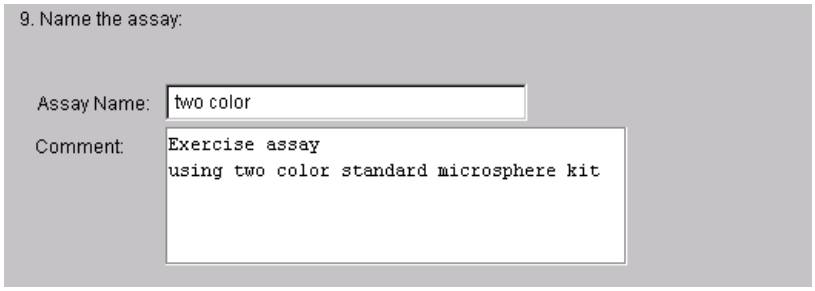
To create a two-color assay: *(continued)*

Step	Action
4	In the **Define Populations** window below:  a. Place a check mark for **Dye A** to be **PopA**. b. Place a check mark for **Dye B** to be **PopB**. c. Click **Next**.
5	In the **Outlier Analysis** window below:  a. Place a check mark in all five measurements. b. Use **3 Standard Deviations**. c. Click **Next**.

To create a two-color assay: *(continued)*

Step	Action
6	In the **Plate Analysis** window shown below: a. Place check marks according to the table shown.  b. Click **Next**.
7	In the **Well Setup** window: a. Make all wells **Active**.  b. Click **Next**.

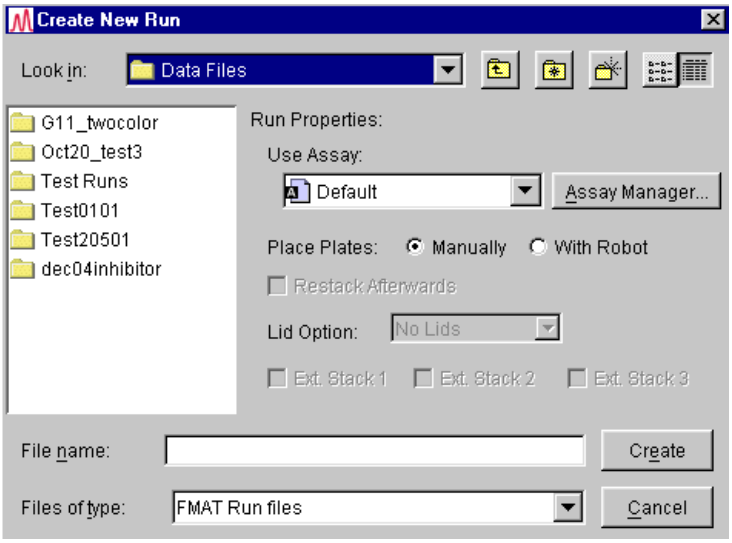
To create a two-color assay: *(continued)*

Step	Action
8	In the **Plate Naming** window:  a. Leave at default settings as above. b. Click **Next**.
9	In the **Name** window: a. For **Assay Name**, type `two color` Option: type in comments in the comment box.  b. Click **Finish**.  You should see your new assay title under the contents of the Assay pane in the Assay Manager.
10	Click **Done** to exit the **Assay Manager** window.

Creating a Runfile

Before data can be collected, you must set up a runfile in the FMAT™ 8100 Analysis Software.

To create a runfile:

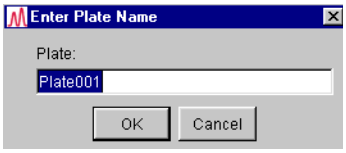
Step	Action
1	Open the FMAT system software.
2	From the **File** menu, select **New Run**. The **Create New Run** dialog box opens. 
3	From the Use Assay drop-down menu select **two-color** Assay**.**
4	For **Place Plates**, select **Manually**.
5	Type the name for the run into the **File name** text box. **IMPORTANT** File or folder names cannot contain most special characters. Do not use the special characters: \ / : * ? “ < > | in a file/folder name. The FMAT system software will use the file name entered to create a folder by the same name. When special characters are used, the sample run fails because the software interprets these characters as specific commands and does not create the folder. **Note** The software appends “.fmat” to the created file, so files are always named according to the format <filename>.fmat.

Running the Instrument

Loading and Scanning the Plate

You can scan the plate when the beads have settled to the bottom of the wells. Leave the plate in the dark, at room temperature, to settle for at least 4, but not more than 24, hours before scanning.

To load and scan a plate:

Step	Action
1	Open the scanner door by clicking the Eject button.
2	Place the covered plate on the scanner tray so that the notched corner is located in the lower-left corner of the platform stage, pointing toward the front of the scanner. **Note** Leave the plate lid on the plate when you place it in the FMAT instrument, or use a plate sealer. **WARNING** **LASER HAZARD.** Do not remove the cover of the instrument. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look directly into the laser beam.
3	Click the **Start Run** icon ( ). The **Enter Plate Name** dialog box opens for a manual run. 
4	Click **OK** to the default plate name **Plate001**. The plate is loaded and mapping starts. **IMPORTANT** Once mapping starts, it cannot be stopped. The only way to stop is to power off the system.
5	Examine your data as it is being scanned in the Sample Detail Window. You can change options as you view the data analysis: ♦ **Image as Greyscale** gives a greyscale display that shows relative intensities. ♦ **Image as Histograms** gives a three-dimensional height display that shows relative intensities. ♦ **Image as Two Color** shows the Dye A and Dye B events with selected colors but does not accurately show relative intensities.
6	When the scanning and data analysis are complete, the **Enter Plate Name** message box appears. Click **Cancel** to close the message box.

Viewing the Data

Viewing Your Results

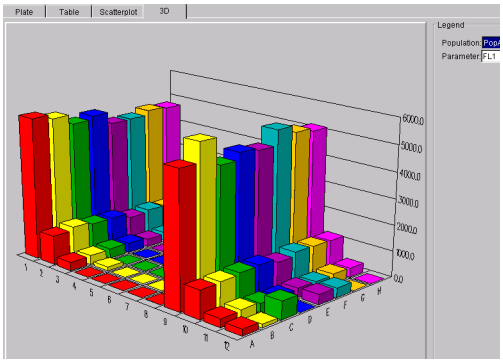
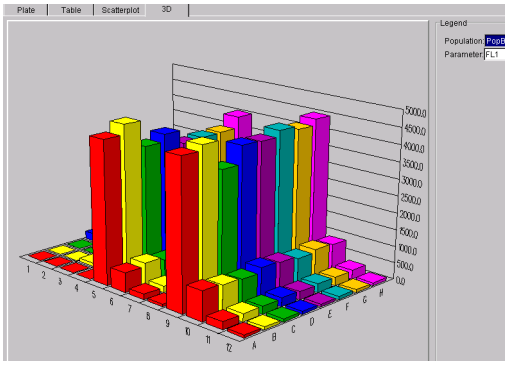
A successful assay gives the following results:

- All wells with beads have detectable FL1 fluorescence
- Relative intensities are detected in the correct order
- Dye A and Dye B events are distinct

Looking at 3D Data

The following example represents a good two color assay result.

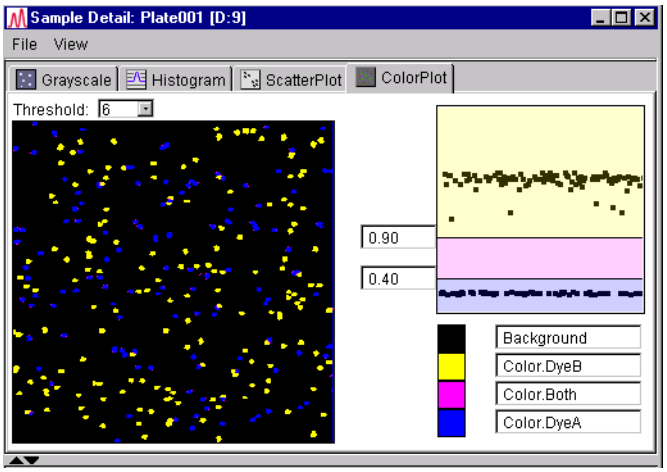
Two Color Data in the 3 D Display

Data Display	Display shows...
	Population A in FL1: ♦ Dye intensity from high to low, shows in display in appropriate wells. ♦ Presence of Dye A events detected in appropriate wells.
	Population B in FL1: ♦ Dye intensity from high to low, shows in display in appropriate wells. ♦ Presence of Dye B events detected in appropriate wells.

Looking at the ColorPlot

The example below of well D9 shows a good separation of the two different populations by sorting them using the color gates. Dye B events are nicely clustered above 0.90 with a few outliers and Dye A events are clustered below 0.40.

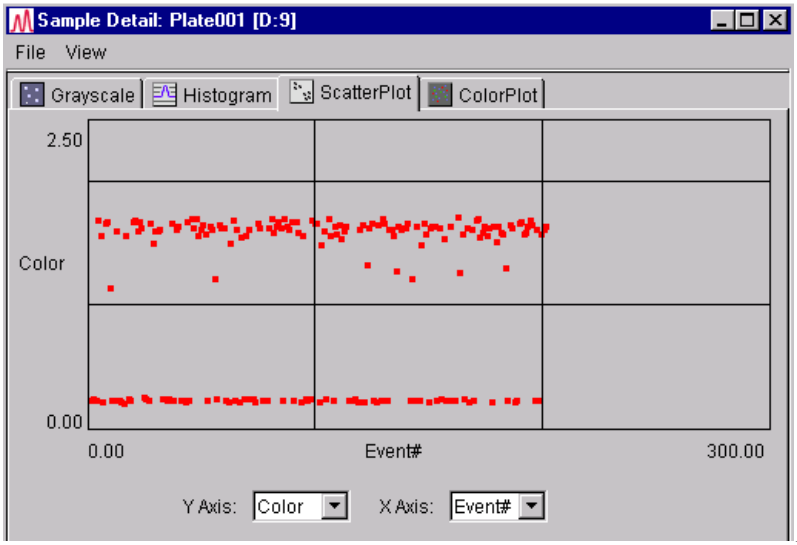
Two-Color Data in the ColorPlot



Looking at the ScatterPlot

The same data from well D9 is shown as a scatterplot and gives a similar pattern as the colorplot.

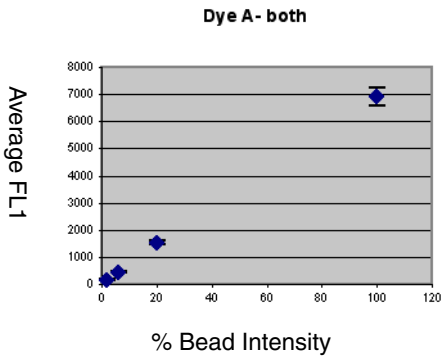
Two-Color Data in the ScatterPlot



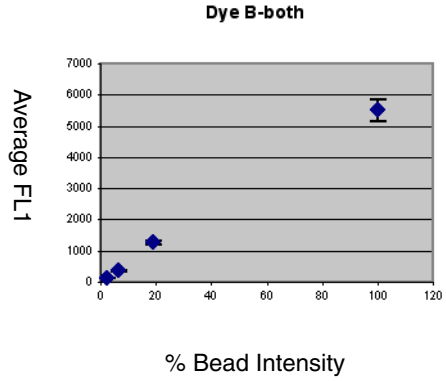
Making Your Own Graph

You may want to generate your own graphs by using the data from the View Run Results in Helper plateview.cxr file. Examples of graphs using two color data of Dye A or Dye B and FL1 are below.

Average Fluorescence in Dye A Replicates Columns 9–12 vs. Bead Intensity



Average Fluorescence in Dye B Replicates Columns 9–12 vs. Bead Intensity



Both graphs show that each Dye A or B populations has four distinct populations relating to the dye intensity.

Troubleshooting

Some Common Problems

Note Please see “Troubleshooting” on page 6-1 for general troubleshooting information.

Observation	Possible Solution
The graphs are not linear.	Because the intensities determined by flow cytometry are not identical to the FMAT determinations, the graph may not be perfectly linear.
The lowest intensity of beads is not detected.	If the non-linearity is extreme, or the lowest bead intensity is not detected, it may be that the PMTs are set too high or too low. See “Retaining the Correct PMT Ratios” on page 5-5 and “Optimizing Data” on page 4-12. Then rescan at the corrected PMT settings.
There are no beads in the image. The images are blurry.	Make sure that you are using an FMAT-qualified plate. Make sure that the beads were plated at least 4 hrs prior to screening. Make sure that you used the correct dilution. Mix the beads and let them settle again, then rescan.
The two-color separation did not work.	The color parameters may need to be reset: a. Click on one well. b. Look at the color value. c. Reset the color parameter to distinguish (gate) Dye A from Dye B events. d. Reanalyze the data.

The Run Window

A

Overview

About This Appendix

This appendix contains information about using the Run window in the FMAT™ 8100 Analysis Software (system software). Information examples are provided for all the commands and tabs within the Run window, including the:

- Plate tab
- Table tab
- Scatterplot tab
- 3D tab

In This Appendix

The following topics are covered in this appendix:

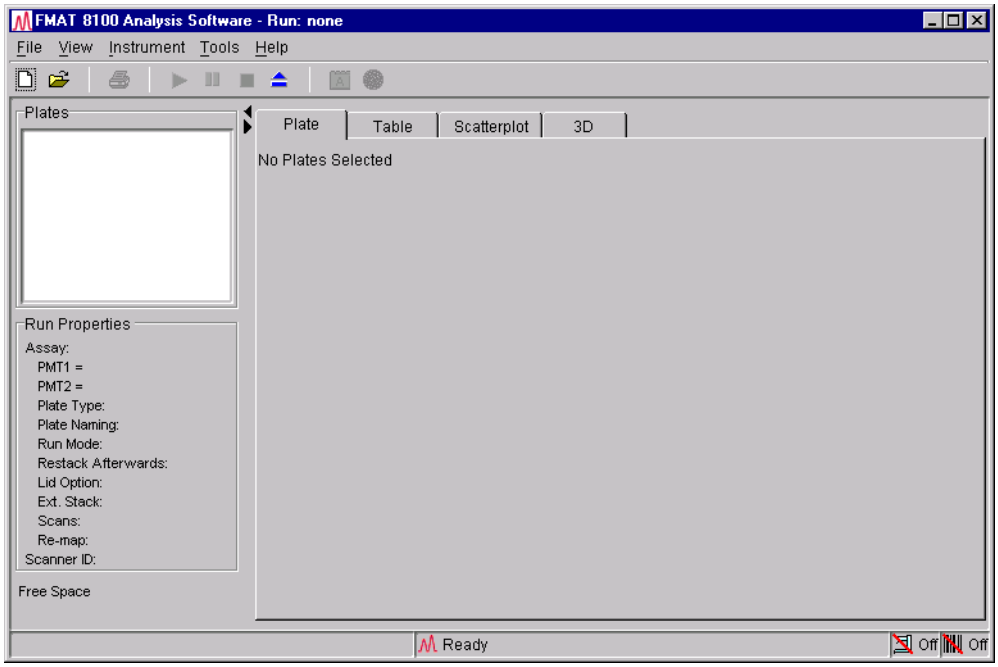
About the Run Window

Run Window

The Run window is the window that is displayed when you open the FMAT system software. The Run window:

- Shows the progress of a run
- Gives access to various displays of your data
- Provides tools to access all the FMAT options and features

An example of the Run window without run information displayed is shown below.



Sections in the Run Window

The Run window has the following sections:

- The Menu bar along the top
- The Plates list box on the upper left side
- The Run Properties information pane on the lower left side
- The Plate, Table, Scatterplot, and 3D tabs
- The Status bar along the bottom
- The re-sizing arrows to adjust the pane size

Displaying Data

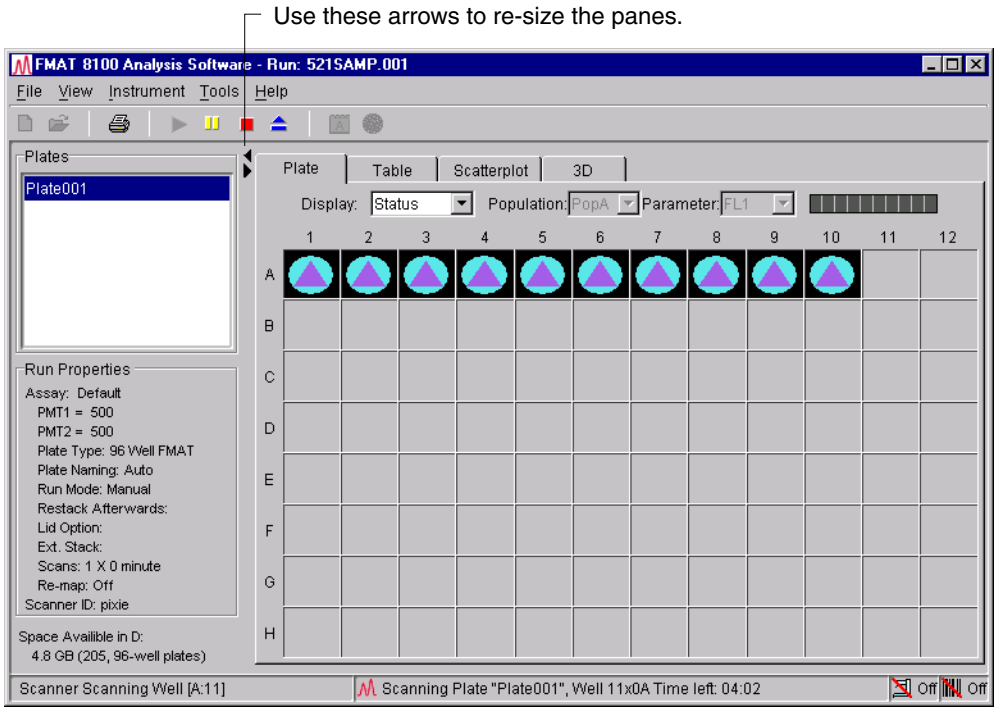
To display data about a run, you use the tabbed run displays. There are four tabs:

- Plate
- Table
- Scatterplot
- 3D

In addition, you can access the Sample Detail window from the menu bar to get a visual display of a single well.

About the Plate Tab

The Plate tab is displayed by default when you open the FMAT system software. When no runs are open, it is blank. When you open a run, the display shows a grid of wells representing the number of wells in the plate-type that was used.



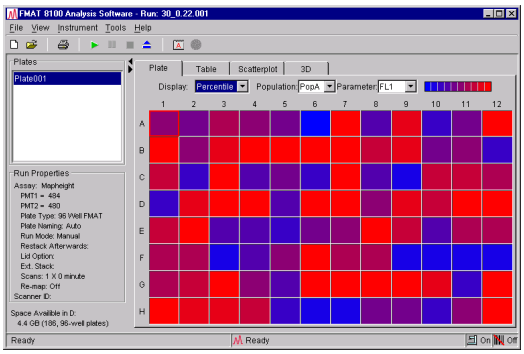
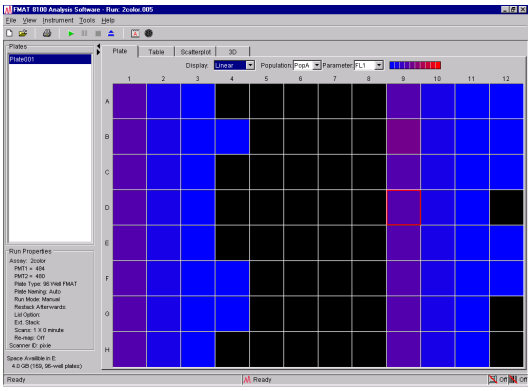
By using the drop-down menu at the top of the Plate tab, you can view the Plate display in three ways:

- Status
- Percentile
- Linear

Note The Status menu is enabled during scanning. The Percentile and Linear menus are enabled when the plate has finished scanning.

Plate Tab Menus

Plate Tab Drop-down Menus

Menu Item	Description
Status	Shows whether the wells have been analyzed or not for the selected plate. ♦ An un-scanned well is gray. ♦ A black box and a circle are displayed in a well when its scan is complete and an image file has been created. ♦ A triangle is displayed in the circle when the image has been analyzed and an analysis file has been created. 
Percentile	Shows a color in each well representing the percentile for the data/population settings. Percentiles are 10% each, ranging from 0 to 100%. Percentiles are represented by the colors of the color bar on the top right of the plate grid. 
Linear	Shows a color in each well representing the linear data steps for the data/population settings. The linear data steps are developed by taking the range for a given data value and dividing it into ten equal parts. Linear data steps are represented by the colors of the color bar on the top right of the plate grid. 

Drop-down menus

By selecting either Percentile or Linear, the other drop-down menus become active and you can change the parameter and/or population selection.

Tooltips

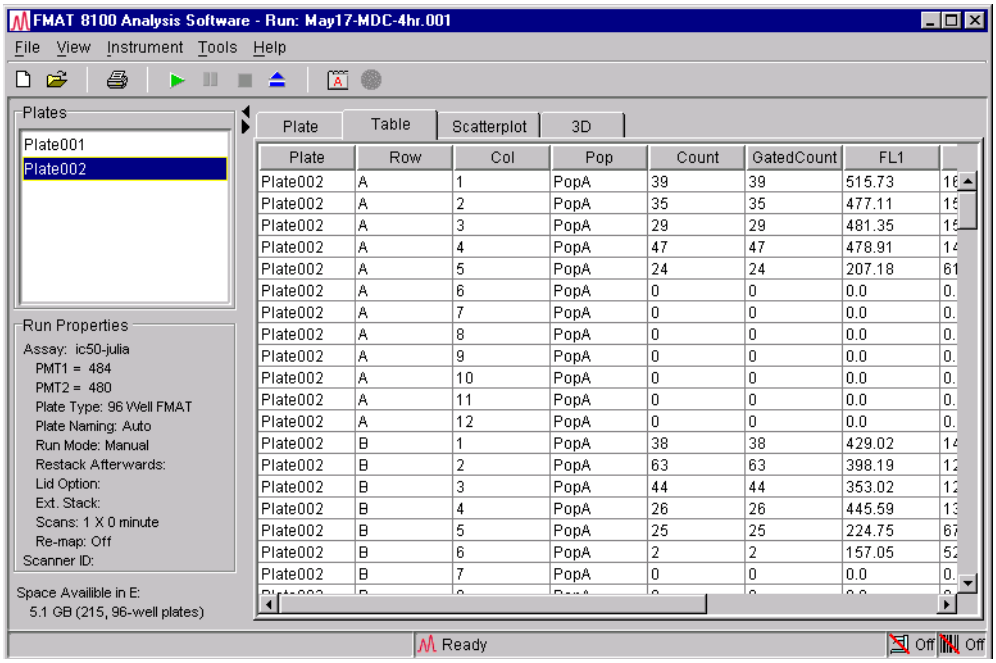
By holding the cursor over a well in the Percentile or Linear view, a tooltip is displayed giving the value for that well. In Status view, a tooltip is displayed giving the row and column location of the well.

Color Bar

Change the colors of the display by clicking the color bar and selecting a new color.

About the Table Tab

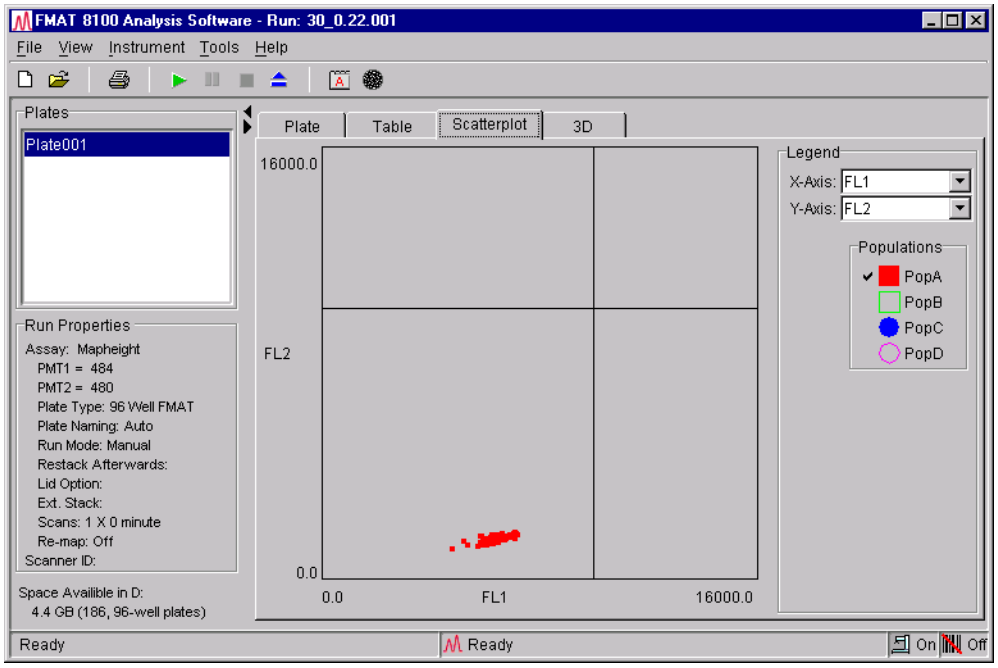
The Table tab gives you results in a spreadsheet format, as shown below.



Column Heading	Description
Plate	Name or ID of the plate.
Row	Plate row position of the wel.
Col	Plate column position of the well.
Pop	Population name for a data row.
Count	Number of events in the well.
GatedCount	The number of events in the well as determined by the Min Count option. See the note about Min Count on page 5-12 for more information.
FL1	The average intensity of the fluorescent events in PMT1.
FL2	The average intensity of the fluorescent events in PMT2.
Color	The average FL2/FL1. This is indicative of the dye associated with the individual cell or bead.
Size	The average full width at half max in the X dimension times that in the Y dimension.
Fit	A measure of smoothness. This determines how well an event's shape must fit a spline to be considered in a statistical analysis. The range is 0 to 1, where a value closer to 0 is a better fit.

About the Scatterplot Tab

The Scatterplot tab gives you a scatterplot of your data.



There are three parameters that can be set to specify how to view your data:

- The Populations list
- The X-Axis list
- The Y-Axis list

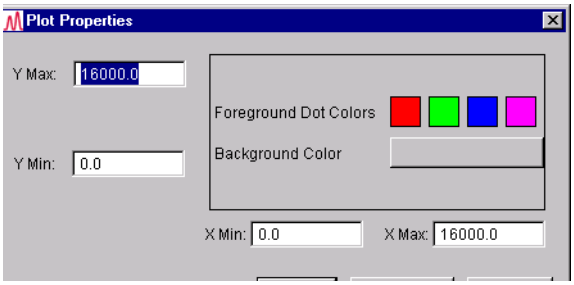
The Populations list allows you to select from one to all of the populations you have defined. Selections are made by clicking on the appropriate population.

The X-Axis and the Y-Axis lists allow you to select one each from the following parameters:

- FL1
- FL2
- Color
- Size
- Well#

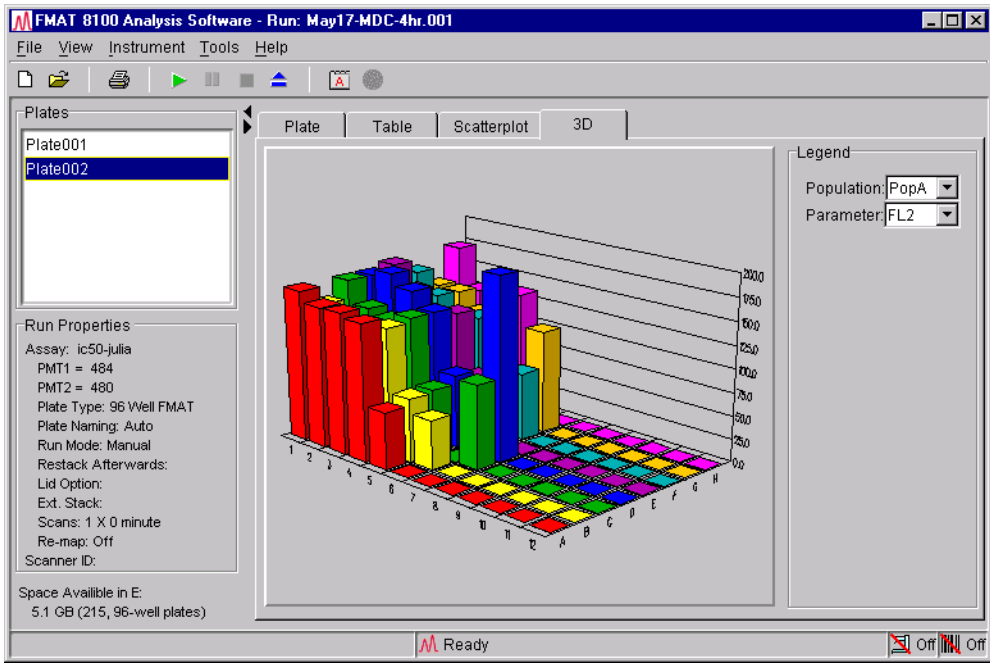
Using the Scatterplot View

To use and adjust the scatterplot view:

Step	Action
1	View the measurements for each point on the plot: a. Place the cursor over a point. b. Right-click and release. A window opens with the measurement for the coordinate on the graph. c. Left-click anywhere on the plot to close the window.
2	Double-click anywhere on the plot to open the **Scatterplot Settings** dialog box. You can also select the **Tools** menu and select **Plot Properties**. 
3	Adjust the axes as needed: a. Click the text box for the axis measurement you want to change. b. Type in the new value that you want. c. Click **Apply**. **Note** Some points may go offscale and disappear when you adjust the axes, however these points are not lost and will reappear if you return to the original measurements. You may see a warning sign next to the Y-axis box as shown below. 
4	Adjust the colors as needed: a. Click **Background Color** or **Foreground Dot Color**. b. When the **Choose New Color** dialog box opens, select the color you want and click **OK**. You can use any of the three tabs to do this. c. Click **Apply**.
5	Click **OK** to exit the dialog box.

About the 3D Tab

The 3D tab gives you a three-dimensional bar chart of your plate data. The plate data is graphically represented, with each row displayed in a different color.



There are two drop-down menus in the right-side of this tab that allow you to select the parameters you want to use to view your data.

- The top list allows you to select any one or all of the populations you have defined.
- The bottom list allows you to select one of the following parameters:
  - FL1
  - FL2
  - Color
  - Size
  - Fit

Changing the 3D Perspective

You can view the 3D graph from different perspectives by rotating it or changing its size.

- Left-click a corner of the graph to rotate it in that direction. The graph rotates incrementally each time you click it.
- Right-click on the graph to change its size.

Note You may need to switch to another tab to refresh the 3D screen.

About the Sample Detail Window

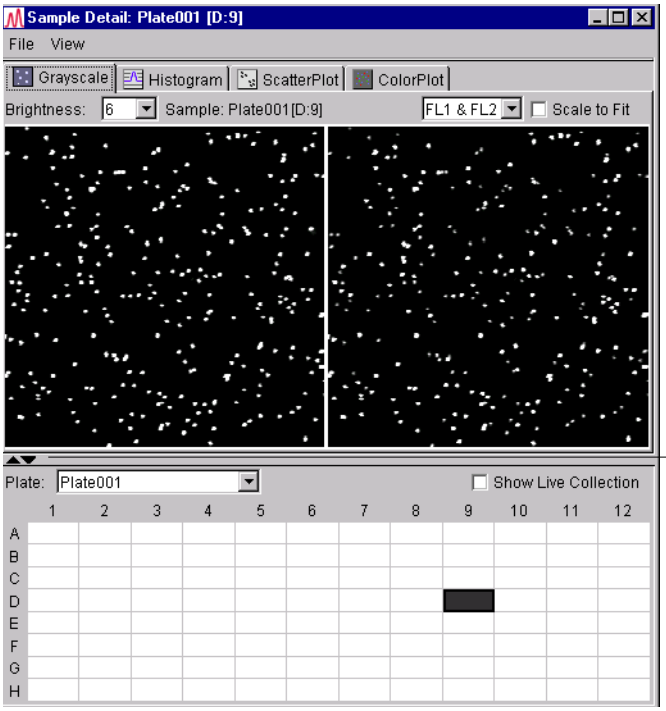
Sample Detail Window Overview

The Sample Detail window displays the data in each well of a plate when a run containing data is open. This window can be accessed in several ways:

- From the main View menu, by selecting Well Detail
- From the keyboard, by pressing Ctrl+D
- From the menu bar, by clicking the Well Detail button
- From the Plate tab, by double-clicking a well of interest
- From the Table tab, by double-clicking a well of interest

An example of the Sample Detail window is shown below.

Note If you want to open two windows at once, right-click a well and select **New Well Detail**.



Use these arrows to re-size the panes.

The Sample Detail window has four tabs:

- Grayscale
- Histogram
- ScatterPlot
- ColorPlot

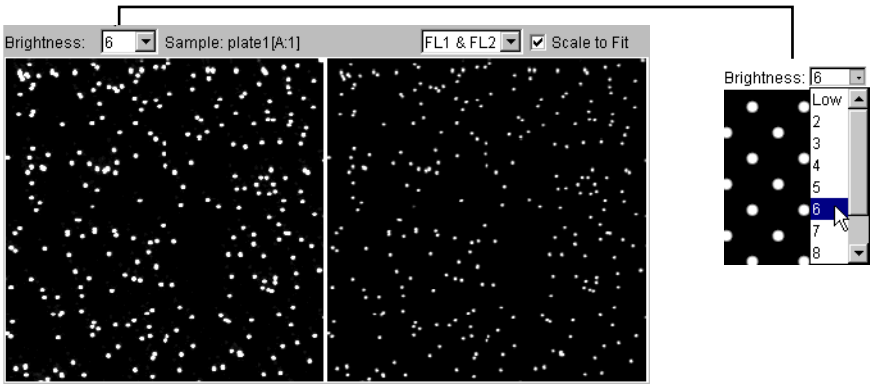
Detailed information about each of the four tabs is provided in the following sections.

The lower part of the window gives a grid display of the plate. You can select different wells for viewing by clicking on the desired well in this display.

Note You can export or print an image from the File menu in this window. You can also change displays or plot properties from the View menu.

Grayscale Display

The Grayscale display offers ten Brightness values (Low, 2,3,4,5,6,7,8,9, High), representing segments in the data range (0 to 16,000).

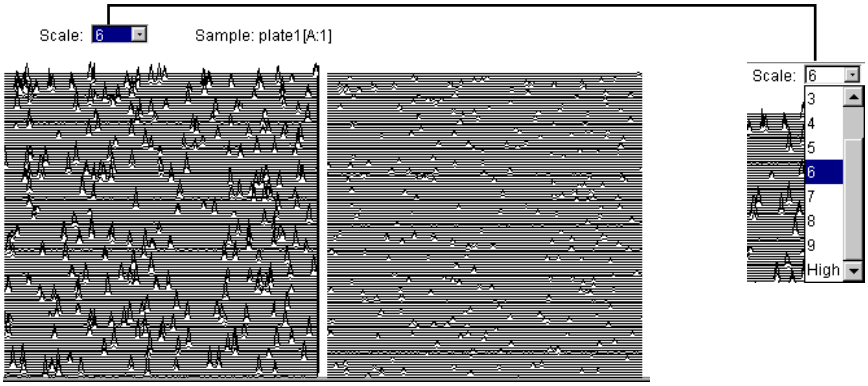


Examples:

- A setting of Low is used when the background is high or when viewing bright events.
- A setting of High is used when viewing very dim fluorescent events.
- A setting of 6 is the default value.

Histogram Display

The Histogram display is a three-dimensional height representation of the data.



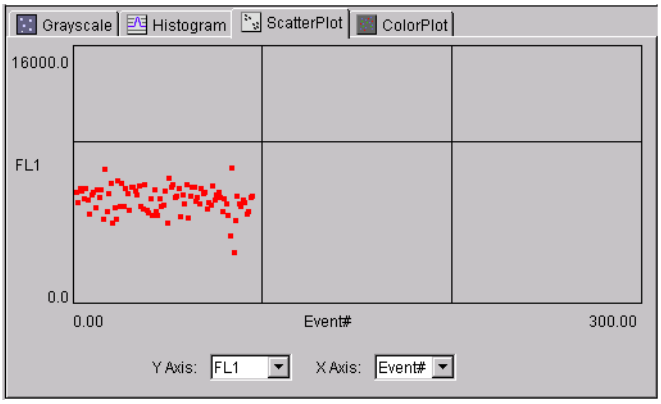
There are ten scale values for viewing the data. Peak height is determined by multiplying the scale factor by 10 and normalizing the data to the result.

Examples:

- A setting of Low is used when the background is high or when viewing bright events.
- A setting of High is used when viewing very dim fluorescent events.
- A setting of 6 is the default value.

ScatterPlot Display

The ScatterPlot display gives a scatterplot of the individual events from a single well.



You can select the parameters for the plot using the Y Axis and X Axis drop-down menus just below the plot. Each drop-down menu offers the following options:

- FL1
- FL2
- Color
- Size
- Fit
- Event#

Viewing Measurements

In the ScatterPlot display you can view the measurements for any location on the plot.

To view measurements:

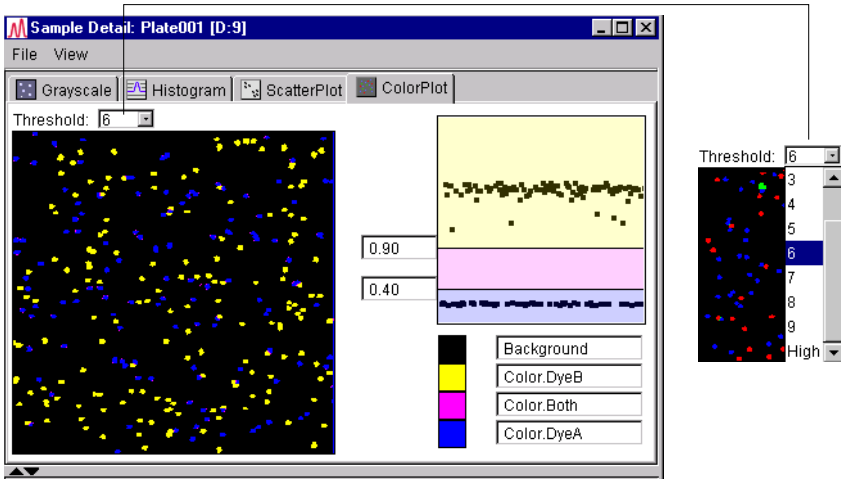
Step	Action
1	Place the cursor over a point.
2	Right-click and release. A window opens with the measurement for the coordinate on the graph.
3	Left-click anywhere on the plot to close the window.

You can also make adjustments to the axes and color by using the Scattterplot Settings dialog box.

- Double-click anywhere on the graph, or
- Go to the View menu and select Plot Properties.

ColorPlot Display

The ColorPlot display allows colors to be assigned to fluorescent events.



There are ten Threshold levels to adjust the background signal.

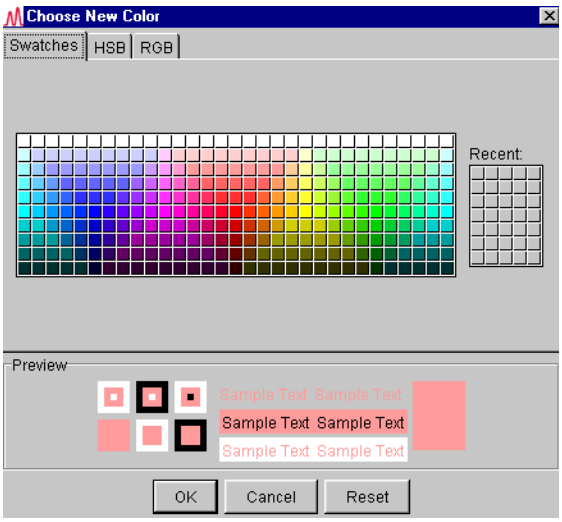
Examples:

- Enter a ratio expected for Dye A in the lower field box. in the lower field box. Events with ratios equal to or below the ratio entered will have the Dye A assigned color.
- Enter a ratio expected for Dye B in the upper field box. Events with ratios equal to or above the ratio entered will have the Dye B assigned color.
- Events with ratios between the ratio entered for Dye A and Dye B will display a third color.

Choosing New Colors

Colors can be assigned for each of the three event values in the above examples. To assign colors, click a color box to display the Choose New Color window.

An example of the Choose New Color window is shown below.

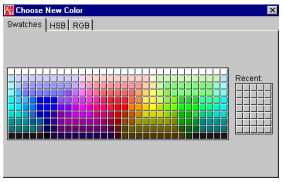


In the Choose New Color window, three tabs are available window:

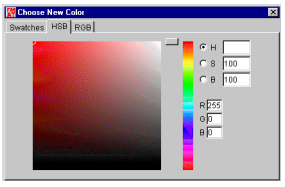
- The Swatches tab
- The HSB (hue, saturation and brightness) tab
- The RGB (red, green and blue) tab

An example of each tab is shown below.

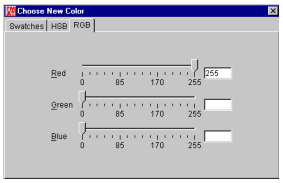
Swatches



HSB



RGB



Note The ColorPlot display, which uses unprocessed data, should not be depended upon for numerical accuracy. For the best representation of results, refer to the Table tab (see “About the Table Tab” on page A-6).

Determining Two-Color Gates

To determine the color gates for a two-dye run:

Step	Action
1	Set up two wells as follows: ♦ One containing only Dye A sample ♦ One containing only Dye B sample
2	Analyze the wells.
3	Click Reanalyze **this run** button to open the **Analysis Properties** wizard.
4	Select and examine the **Define Gates** window. See the distribution of points, and adjust your gates accordingly (see“Reanalyzing the Run” on page 4-10).

A Tour of the Menus

B

Overview

About This Appendix

This appendix provides information on the menu commands and buttons in the Assay Manager, Run window, and the Sample Detail window of the FMAT™ 8100 Analysis Software version 2.0.

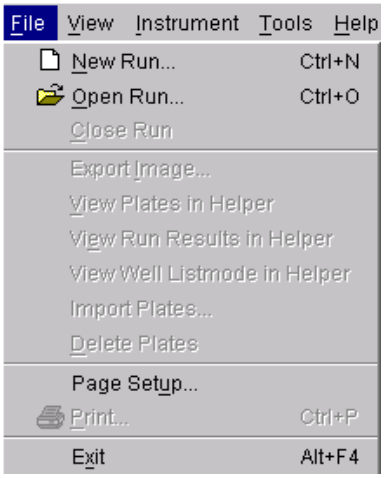
In This Appendix

The following topics are covered in this appendix:

Run Window Menus

Using the File Menu in the Run Window

Refer to the following table for information about the File menu in the Run window. An example of the menu is shown below.



File Menu Details

Menu Item	Keyboard Shortcut	Description
New Run	Ctrl+N	Opens the **Create New Run** dialog box and allows you to: ♦ Name a new run ♦ Set run properties ♦ Set the assay to be used ♦ Define a new assay, if needed
Open Run	Ctrl+O	Opens the **Open Run** dialog box and allows you select and open an existing run by clicking on .fmat file.
Close Run	—	Closes the currently open run.
Export Image	—	Opens the **Save JPEG File** dialog box and exports the JPEG-format images of the currently selected plate to a folder of your choice.
View Plates in Helper	—	Opens the individual well data from a selected plate in a spreadsheet, when a spreadsheet program has been selected in the **Options** window helper text boxes.
View Run Results In Helper	—	Opens the plate-format data for all plates in an open run in a spreadsheet, when a spreadsheet program has been selected in the **Options** window helper text boxes.
View Well Listmode in Helper	—	Opens the listmode file for the well that is highlighted, when a spreadsheet program has been selected in the **Options** window helper text boxes.
Import Plates	—	Opens the **Select the FMAT v 1.0 RunDoc.txt file** dialog box and allows you to add plate data to the currently open run.

File Menu Details *(continued)*

Menu Item	Keyboard Shortcut	Description
Delete Plates	—	Allows you to delete the currently selected plate from the current run.
Page Setup	—	Opens the **Page Setup** dialog box and allows you to set the page characteristics for printing.
Print	Ctrl+P	Opens the **Print** dialog box and prints the contents of the **Run** window if you are connected to a printer.
Exit	Alt+F4	Exits the program.

Exporting an Image

An image of the Sample Detail window can be exported and saved in JPEG (Joint Photographic Experts Group) format.

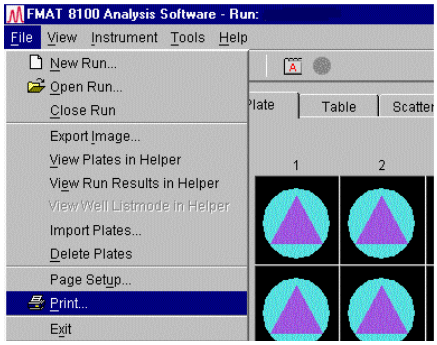
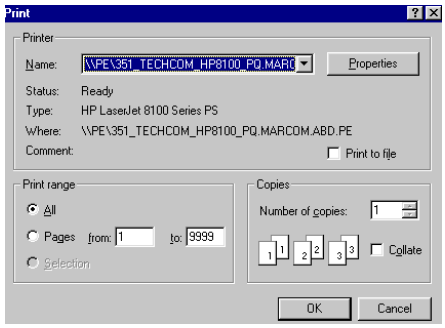
To export an image:

Step	Action
1	From the **File** menu, select **Export Image**. The **Save JPEG File** dialog box opens.
2	Using the **Look in** pull-down menu, select a folder for storing your image.
3	In the **File name** textbox, type a name for your image.
4	Click **Save**. Your image is saved to the location you specified.

Printing an Image

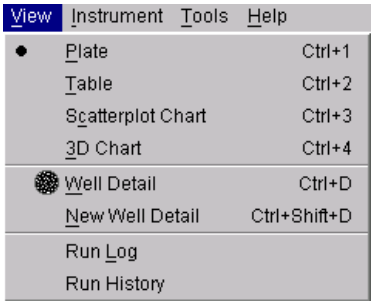
To obtain presentation graphics either export the screen image as a JPEG, or capture the screen image using Ctrl-Alt-Print Screen and import the image to a graphics program.

To print an image:

Step	Action
1	From **File**, select **Page Setup** and configure the screen image to fit to one page.
2	From the **File** menu, select **Print**.  The **Print** dialog box opens. 
3	Verify that your printer settings are correct, then click **OK**. The open window prints. **Note** The application is frozen while the Print command completes.

Using the View Menu in the Run Window

Refer to the following table for information about the View menu in the Run window. An example of the menu is shown below.

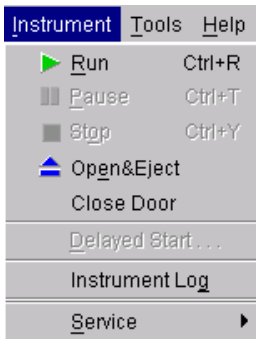


View Menu in the Run Window Details

Menu Item	Keyboard Shortcut	Description
Plate	Ctrl+1	Brings the **Plate** tab of the **Run** window to the front.
Table	Ctrl+2	Brings the **Table** tab of the **Run** window to the front.
Scatterplot Chart	Ctrl+3	Brings the **Scatterplot** tab of the **Run** window to the front.
3D Chart	Ctrl+4	Brings the **3D** tab of the **Run** window to the front.
Well Detail	Ctrl+D	Opens the **Sample Detail** window for the currently selected well.
New Well Detail	Ctrl+Shift+D	Opens additional **Sample Detail** windows.
Run Log	—	Opens the **Run Log** window.
Run History	—	Opens the **Run History** window.

Using the Instrument Menu in the Run Window

Refer to the following table for information about the Instrument Menu in the Run window menu. An example of the menu is shown below.



Instrument Menu Details

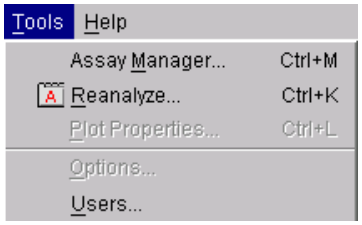
Menu Item	Keyboard Shortcut	Description
Run	Ctrl+R	Starts a run.
Pause	Ctrl+T	Pauses a run.
Stop	Ctrl+Y	Stops a run.

Instrument Menu Details *(continued)*

Menu Item	Keyboard Shortcut	Description
Eject	—	Ejects a plate.
Close Door	—	Closes the scanner door.
Delayed Start	Ctrl+D	Opens the **Setup Delayed Start Time** window. Becomes enabled when you create a new run and select **With Robot**.
Instrument Log	—	Opens the **Instrument Log** window.
Service	—	Enabled only for service engineers.

Using the Tools Menu in the Run Window

Refer to the following table for information about the Tools menu. An example of the menu is shown below.



Tools Menu Details

Menu Item	Keyboard Shortcut	Description
Assay Manager	Ctrl+M	Opens the **Assay Manager** window.
Reanalyze	Ctrl+R	Opens the **Analysis Properties** wizard for the currently selected plate with the assay associated with the last reanalysis.
Plot Properties	Ctrl+P	Enable only when the **Scatterplot** tab has been selected. Opens the **Scatterplot Properties** dialog box.
Options	Ctrl+O	Opens the **Options** dialog box.
Users	Ctrl+U	Opens the **User Manager** dialog box.

Using the Help Menu in the Run Window

Refer to the following table for information about the Help menu. An example of the menu is shown below.

Menu Item	Keyboard Shortcut	Description
About FMAT Analysis	—	Opens the **About FMAT Analysis** window, giving you version and configuration information.

Run Window Buttons

Using the Run Window Buttons and Functions

Refer to the following table for information about the Run window buttons and their functions. An example of each button is shown below.

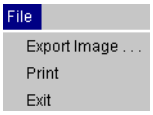
Run Window Button Details

Icon	Button Name	Function
	New Run	Opens the **Create New Run** dialog box and allows you to: ♦ Name a new run ♦ Set run properties ♦ Set the assay to be used ♦ Define a new assay, if needed
	Open Run	Opens the **Open Run** dialog box and allows you select and open an existing run.
	Print	Opens the **Print** dialog box and prints the contents of the **Run** window.
	Start Run	Starts a run.
	Pause Run	Pauses a run.
	Stop Run	Stops a run.
	Eject Plate	Ejects a plate.
	Reanalyze	Opens the **Analysis Properties** wizard for the currently selected plate with the assay associated with the last reanalysis.
	Well Detail	Opens the **Sample Detail** window for the currently selected well.

Sample Detail Window Menus

Using the File Menu in the Sample Detail Window

Refer to the following table for information about the File menu in the Sample Detail window. An example of the menu is shown below.



Sample Detail Window File Menu Details

Menu Item	Keyboard Shortcut	Description
Export Image	—	Opens the **Save JPEG File** dialog box and exports the JPEG-format images of the currently selected well to a folder of your choice.
Print	—	Opens the **Print** dialog box and prints the contents of the **Sample Detail** window.
Exit	—	Closes the **Sample Detail** window.

Using the View Menu in the Sample Detail Window

Refer to the following table for information about the View menu in the Sample Detail window. An example of the menu is shown below.

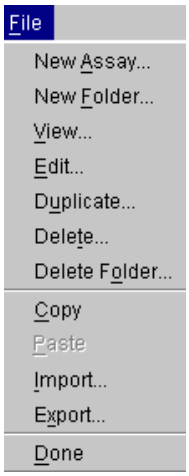
Sample Detail Window View Menu Details

Menu Item	Keyboard Shortcut	Description
Grayscale	—	Brings the **Grayscale** tabbed display to the front.
Histogram	—	Brings the **Histogram** tabbed display to the front.
ScatterPlot	—	Brings the **ScatterPlot** tabbed display to the front.
ColorPlot	—	Brings the **ColotPlot** tabbed display to the front.
Plot Properties	—	Opens the **Scatterplot Properties** dialog box. Enabled only when the **ScatterPlot** tabbed display is open.

Assay Manager Menu

Using the Assay Manager File Menu

Refer to the following table for information about the File menu in the Assay Manager. An example of the menu is shown below.



Assay Manager File Menu Details

Menu Item	Keyboard Shortcut	Description
New Assay	—	Opens the **New Assay Wizard**.
New Folder	—	Enabled only when a folder is selected. Creates a new subfolder under the currently selected folder.
View	—	Enabled only when an assay is selected. Opens the **Viewing** wizard for the currently selected assay.
Edit	—	Enabled only when an assay is selected.
Duplicate	—	Makes a copy of the selected assay.
Delete	—	Deletes the selected assay.
Delete Folder	—	Deletes any selected folder other than the **Assay** folder. You cannot delete a folder containing assays created by another user ID.
Copy	—	Copies the selected assay to the clipboard.
Paste	—	Pastes a copy of the assay from the clipboard to the selected folder.
Import	—	Opens the **Import Assay** dialog box and allows you to select and import an assay to the selected folder.
Export	—	Opens the **Export Assay** dialog box and allows you to export a selected assay to the location of your choice.
Done	—	Closes the **Assay Manager** window.

Assay Manager Buttons

Using the Assay Manager Buttons and Functions

Refer to the following table for information about the Assay Manager buttons and their functions. An example of each is shown below.

Assay Manager Buttons And Functions

Icon	Button Name	Function
	New Folder	Creates a new folder.
	Delete Folder	Deletes a selected folder.
	New Assay	Opens the Assay Manager using the Default assay and creates a new assay.
	Edit Assay	Opens the Assay Manager using the selected assay and allows you to make changes.
	Copy Assay	Makes a copy of the selected assay.
	Delete Assay	Deletes the selected assay.

Log Window Menus

Using the File Menu in the Log Window

Refer to the following table for information about the File menu in the Log window. An example of the menu is shown below.

File Menu Details

Menu Item	Keyboard Shortcut	Description
Page Setup	—	Opens the **Page Setup** dialog box and allows you to set the page characteristics for printing.
Print	—	Opens the **Print** dialog box and prints the contents of the **Run** window.

Using the Edit Menu in the Log Window

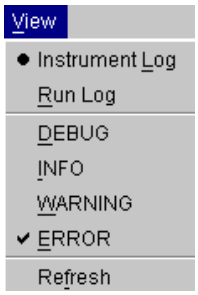
Refer to the following table for information about the Edit menu in the Log window. An example of the menu is shown below.

Edit Menu Details

Menu Item	Keyboard Shortcut	Description
Cut	Ctrl+X	Cuts the selected text and saves it in the clipboard.
Copy	Ctrl+C	Makes a copy of the selected text and saves it in the clipboard.
Paste	Ctrl+V	Pastes the contents of the clipboard into the selected location.
select-all	Ctrl+A	Selects all text in the open window.

Using the View Menu in the Log Window

Refer to the following table for information about the View menu in the Log window. An example of the menu is shown below.



View Menu Details

Menu Item	Keyboard Shortcut	Description
Instrument Log	—	Opens the **Instrument Log** window.
Run Log	—	Opens the **Run Log** window.
DEBUG	—	You can select any or all of these options. When selected, this type of message will be recorded in the log if it occurs while you are running the software.
INFO	—	
WARNING	—	
ERROR	—	
Refresh	—	Updates the log. The log does not automatically update while you are working. Select **Refresh** to see the most current entries in the log.

Setting Up the Software

C

Overview

About This Appendix

This appendix describes new features of the FMAT™ 8100 Analysis Software version 2.0. How to install and set up the software for use with the FMAT™ 8100 HTS System is also described here.

In This Appendix

The following topics are covered in this appendix:

New Features of the Software

About the Features in Version 2.0

A number of new features have been added to the FMAT 8100 Analysis Software version 2.0.These include:

- Data collection using 96- and 384-well plates
- Logins with levels of accessibility and protection of protocols
- Both color and size multiplexing
- Export and save capabilities for images
- Data output in plate view
- Delayed start capability
- Repeat scanning of a plate or selected wells
- Wizards for assay design and management
- Integration with spreadsheet programs
- Improved algorithm for cells and beads
- Improved data analysis

IMPORTANT Be sure to read the Release Notes text file that is included on the software CD. The Release Notes will contain the very latest information about the software and any changes not included in this manual.

Installing the Software

Overview

The purpose of the FMAT system software is to direct the FMAT™ 8100 HTS System to collect and analyze data for specific assay types. The parameters for these assays are set in the software.

After you set up the assays in the software, you need only select the particular assay that you want for any given run. Each time you select that assay, your plate(s) are scanned and analyzed using the same parameters.

You may also reanalyze your data by selecting different assay parameters. However, the reanalysis still uses the original raw data gathered during the scanning of the plate(s), determined by the original instrument settings.

System Requirements

Your system must meet the following minimum requirements to use the FMAT Analysis v 2.0 software:

- Microsoft® Windows NT® 4.0 with Service Pack 5 or above
- 500 MHz Pentium® processor
- 128 MB RAM
- 9 GB Hard Drive
- 10X CD-ROM
- Full color monitor with 800 x 600 resolution
- Network interface card

Installing the Software

IMPORTANT We recommend that you back up all your data files and close all open programs before you begin the installation.

To install the FMAT v 2.0 software:

Step	Action
1	Place the CD-ROM into your CD-ROM drive.
2	Follow the instructions on the screens and continue clicking **Next** until the final screen, then click **Finish**. **Note** If you do not want to install over the existing software, be sure to specify a different folder.

Starting the Software for the First Time

Overview

The software can operate in online mode or offline mode.

- In online mode, the software must be connected to an FMAT instrument. When connected, the system can scan plates and collect data.
- In offline mode, the system can open previous runs but cannot scan plates or collect data.

IMPORTANT Do not use a screen saver in online mode; it may conflict with the operation of the FMAT Analysis software. If necessary, turn off the screen saver using the computer's Display control panel.

Assigning an Administrator's Password

If you have installed the software yourself, the first time you start it you will be asked to assign an Administrator's password using the dialog box shown below.

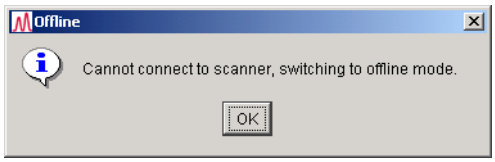
If the software is installed as part of a new system setup, your service engineer has assigned an Administrator's password. However, if you ever have to reinstall the software, or if your password file becomes corrupted, follow the procedure below.

To assign an Administrator's password:

Step	Action
1	In the **Assign Administrator Password** dialog box, in the **Enter Password** field, enter a password. **Note** It is good practice to use a password that is at least six characters in length and that contains numbers or symbols and letters. **IMPORTANT** Be sure you use a password you will not forget.
2	In the **Re-Enter Password** field, type the password again for confirmation. The **OK** button is enabled.
3	Click **OK**.

Starting the Software

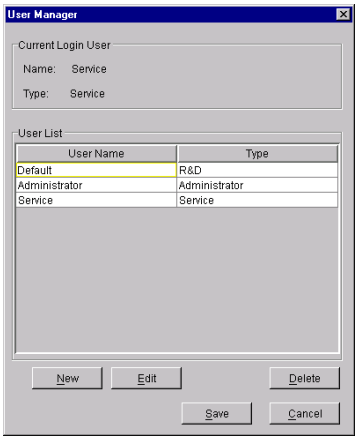
To start the software for the first time:

Step	Action
1	Go to **Start**>**Programs**>**FMAT 2.0**. Select the **FMAT 8100 Analysis Software** icon.  The software opens. The **Login** dialog box opens.
2	a. Click **Login** to log in to the software using the default user name and no password. b. If the computer is not connected to an FMAT instrument, the following warning appears.  c. Click **OK** to close this message and proceed. The **FMAT 8100 Analysis Software** main window opens.
3	To perform administrative functions, such as assigning new user names, log in as Administrator. a. From the pull-down list, select **Administrator**. b. Enter your password. c. Click **Login**.

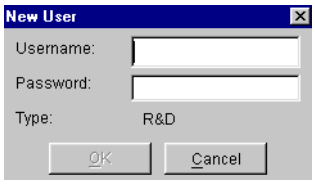
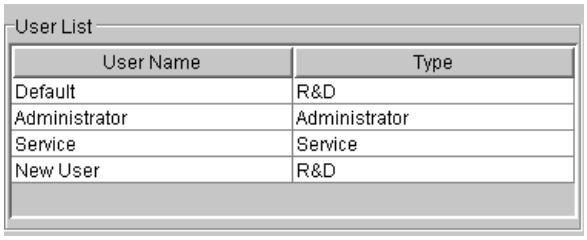
Assigning User Names

Only the Administrator can assign new user names.

To assign a new user name:

Step	Action
1	Start the software and log in as Administrator.
2	From the **Tools** menu, select **Users**.  The **User Manager** dialog box opens.

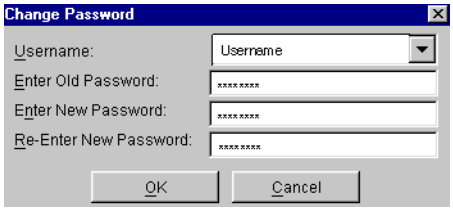
To assign a new user name: *(continued)*

Step	Action
3	Click **New**.  The **New User** dialog box opens.
4	Type in the new user name and password, then click **OK**.
5	The user you added is now listed in the **User Manager** dialog box.  Click **Save** to save the new user and close the **User Manager**.

Changing Your Password

Once you have been assigned a user name, you can change your password any time you log on.

To change your password:

Step	Action
1	Click the **Change Password** button in the **Login** dialog box.
2	In the **Change Password** dialog box, enter the required information in the text boxes. 
3	Click **OK**.

Setting User Preferences

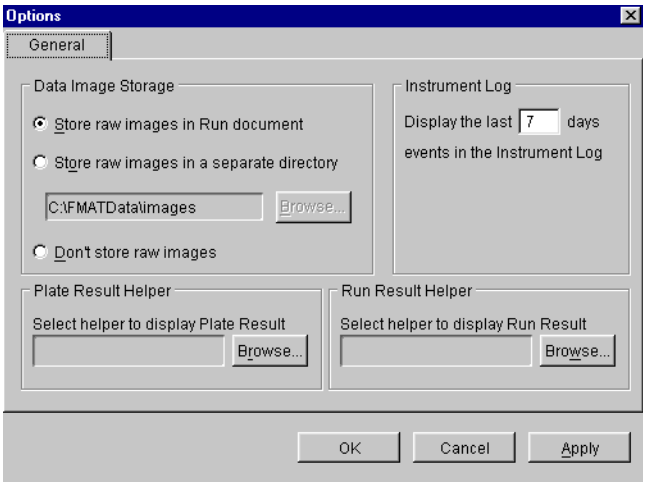
Overview

You can set or change preferences for the following features and options at any time:

- Image storage options
- Instrument log display times
- Plate Result Helper
- Run Result Helper
- Delayed start time

Setting Options

To set or change options:

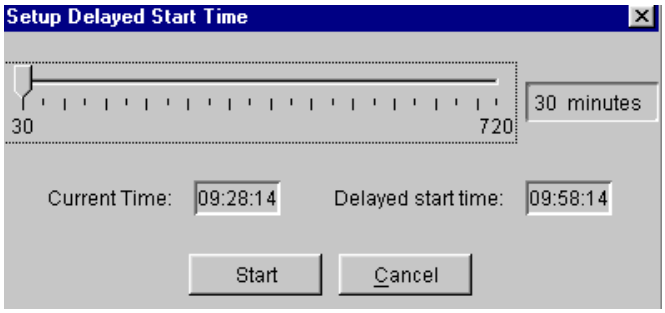
Step	Action
1	From the **Tools** menu, select **Options**. The **Options** dialog box opens. 
2	**Note** Changing the following setting may prevent older runs from finding their image data. The images should always be kept with the runfile and run folder. Keeping the setting to the default of **Store raw images in Run document** will ensure that the images travel with their matching data files. In the **Data Image Storage** group, click one of the following options: ♦ **Store raw images in Run document** ♦ **Store raw images in a separate directory** **Note** If you select this option, and you do not want to use the default directory, click **Browse** to find and select the directory you want ♦ **Don't store raw images**
3	In the **Instrument Log** group, type in the number of days for which you want information displayed.
4	Select a **Plate Result Helper** and a **Run Result Helper**. This allows you to open data files automatically in a spreadsheet.

To set or change options: *(continued)*

Step	Action
5	<table><tr><th>If you want to change...</th><th>Then...</th></tr><tr><td>a helper (Microsoft Excel or another spreadsheet program)</td><td>a. Click the **Browse** button for the helper you want to change. b. Select the location of the helper you want in the window that opens and click **Set**. c. Click **Apply**. **IMPORTANT** Be sure to select the **.exe** file for the program you want.</td></tr></table>
6	Click **OK** to exit the **Options** dialog box.

Setting Delayed Start

Use the Setup Delayed Start Time dialog box to set a delayed start time.



To set a delayed start:

Step	Action
1	Using the slider bar, select the amount of delay in minutes.
2	Note that the **Delayed start time** changes to reflect your selection.
3	Click **Start**.

Limited Warranty Statement

D

Limited Warranty Statement

Applera Corporation, Applied Biosystems Limited Warranty Statement:

We at Applied Biosystems are committed to manufacturing of the highest quality possible for pharmaceutical research. We strive to ensure that our products meet your needs and expectations and are consistent with the latest advances in science, engineering, and technology. In accordance with our belief in the quality of our products, we are pleased to offer you the following limited warranty.

Applera Corporation, through its Applied Biosystems Group (“Applied Biosystems”) warrants to the customer that, for a period ending on the earlier of one (1) year from the completion of installation or fifteen (15) months from the date of shipment to the customer (the “Warranty Period”), the FMAT™ 8100 HTS System purchased by the customer (the “Instrument”) will be free from defects in material and workmanship, and will perform in accordance with the performance specifications set forth in the Instrument Product Specification Sheet (the “Specifications”).

During the Warranty Period, if the Instrument's hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, Applied Biosystems will repair or replace the Instrument so that it meets the Specifications, at Applied Biosystems expense.

This Warranty does not extend to any Instrument or part which has been (a) the subject of an accident, misuse, or neglect, (b) modified or repaired by a party other than Applied Biosystems, or (c) used in a manner not in accordance with the instructions contained in the Instrument User's Manual. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User's Manual. Those items are covered by their own warranties.

Applied Biosystems obligation under this Warranty is limited to repairs or replacements that Applied Biosystems deems necessary to correct those failures of the Instrument to meet the Specifications of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty will be performed by Applied Biosystems on site at the Customer's location at Applied Biosystems sole expense.

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

Applied Biosystems shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the purchase or use of the Instrument. Applied Biosystems makes no warranty whatsoever with regard to products or parts furnished by third parties.

This Warranty is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANT ABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND IS IN LIEU OF ANY OTHER OBLIGATION ON THE PART OF APPLIED BIOSYSTEMS.

Technical Support

E

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support:

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

You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems web site. (Please see the section "To Obtain Technical Documents" following the telephone information below)

To Contact Technical Support by E-Mail

To contact Applied Biosystems Technical Support by e-mail for help in the following product areas:

Product/Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography PerSeptive DNA, PNA and Peptide Synthesis systems FMAT™ 8100 HTS System CytoFluor® 4000 Fluorescence Plate Reader Mariner™ Mass Spectrometers Voyager™ Mass Spectrometers MassGenotyping Solution 1™ (MGS1) System	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

To Contact Technical Support by Telephone or Fax (North America)

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

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

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1**.**800**.**831**.**6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1**.**800**.**831**.**6844**, press **2**, then press **1**[a]	1.650.638.5981
Fluorescent DNA Sequencing	**1**.**800**.**831**.**6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1**.**800**.**831**.**6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1**.**800**.**831**.**6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1**.**800**.**831**.**6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43x Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **2**[a]	1.650.638.5981
PCR and Sequence Detection	**1**.**800**.**762**.**4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI Prism, 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1**.**800**.**831**.**6844**, then press **5**[a]	1.240.453.4613
Voyager™ MALDI-TOF Biospectrometry Workstations Mariner™ ESI-TOF Mass Spectrometry Workstations MassGenotyping Solution 1™ (MGS1) System	**1**.**800**.**899**.**5858**, press **1**, then press **3**[b]	1.508.383.7855
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1**.**800**.**899**.**5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ Nucleic Acid Synthesis Systems	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855

Product/Product Area	Telephone	Fax
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
FMAT™ 8100 HTS System CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	1.508.383.7899

a. 5:30 AM to 5:00 PM Pacific time.

b. 8:00 AM to 6:00 PM Eastern time.

c. 9:00 AM to 5:00 PM Eastern time.

To Contact Technical Support by Telephone or Fax (Outside North America)

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

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608** or **86 800 8100497**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
India (New Delhi)	**91 11 653 3743/3744**	**91 11 653 3138**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 79588268**	**60 3 79549043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 532 4484**	**32 (0)2 582 1886**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0)6150 101 0**	**49 (0)6150 101 101**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Portugal (Lisboa)	**351.(0)22.605.33.14**	**351.(0)22.605.33.15**
Spain (Tres Cantos)	**34.(0)91.806.1210**	**34.(0)91.806.12.06**

Region	Telephone	Fax
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 392400**	**31 (0)180 392409** or **31 (0)180 392499**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**

European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	**27 11 478 0411**	**27 11 478 0349**
Africa, French speaking (Paris, France)	**33 1 69 59 85 11**	**33 1 69 59 85 00**
India (New Delhi)	**91 11 653 3743** **91 11 653 3744**	**91 11 653 3138**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 22 866 40 10**	**48 22 866 40 20**
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia)	**44 1925 282481**	**44 1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Caribbean countries, Mexico, and Central America	**52 55 35 3610**	**52 55 66 2308**
Brazil	**0 800 704 9004** or **55 11 5070 9654**	**55 11 5070 9694/95**
Argentina	**800 666 0096**	**55 11 5070 9694/95**
Chile	**1230 020 9102**	**55 11 5070 9694/95**
Uruguay	**0004 055 654**	**55 11 5070 9694/95**

To Reach Technical Support Through the Applied Biosystems Web Site

To contact Technical Support through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Click **Contact Support** in the contents list at the left of the screen.
4	Click your geographic region for the product area of interest.
5	In the Personal Assistance form, enter the requested information and your question, then click **Ask Us RIGHT NOW**.
6	In the Customer Information form, enter the requested information, then click **Ask Us RIGHT NOW**. Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

To Obtain Technical Documents

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

- By telephone
- Through the Applied Biosystems web site

Ordering Documents by Telephone

To order documents by telephone:

1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

Obtaining Documents Through the Web Site

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

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Documents on Demand**.
3	In the search form, enter and select search criteria, then click **Search** at the bottom of the page.
4	In the results screen, do any of the following: ♦ Click the pdf icon to view a PDF version of the document. ♦ Right-click the pdf icon, then select **Save Target As** to download a copy of the PDF file. ♦ Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you. ♦ Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you. **Note** There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.

To Obtain Customer Training Information

To obtain Applied Biosystems training information:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Training**.

Index