user manual

Fragment Analysis

User's Guide Version 1.1

um 375-657

Amersham Biosciences

Cover: The image shown in this manual is courtesy of Data Genetics, Hayward, California.

Amersham Biosciences UK Limited Amersham Place Little Chalfont Buckinghamshire England HP7 9NA

Amersham Biosciences AB SE-751 84 Uppsala Sweden

Amersham Biosciences Corp 800 Centennial Avenue PO Box 1327 Piscataway NJ 08855 USA

Amersham Biosciences Europe GmbH Munzinger Strasse 9 D-79111 Freiburg Germany

Amersham Biosciences (SV) Corp 928 East Arques Avenue Sunnyvale CA 94085-4520 USA

April 2002

Table of contents

Chapter 4 Post-analysis operations step-by-step

Chapter 7 View menu

Chapter 8 Tools menu

Preface

About this guide

This guide explains how to use the Fragment Analysis software to analyze DNA and protein fragments separated by electrophoresis. The manual is divided into three parts:

- **Part one: Basics**—Provides introductory information and step-by-step procedures for performing an analysis. Part one also includes a tutorial (chapters 3 and 4) that walks you through an analysis session, from loading an image to creating a report. The final chapter in part one explains how to analyze dual-channel images.
- **Part two: Reference**—Provides information about all the items on the Fragment Analysis menus.
- **Part three: Appendixes**—Includes report examples, technical information about how Fragment Analysis calculates band values, and a troubleshooting guide.

Assumptions

The instructions in this manual assume you have basic computer skills. You should be familiar with a Windows-based graphical user interface and know how to use a mouse. If you do not have these skills, refer to the Microsoft™ Windows™ documentation.

Assistance

If you have problems, please contact:

Asia Pacific
Tel: +852 2811 8693
Fax: +852 2811 5251

Australasia
Tel: +61 2 9899 0999
Fax: +61 2 9899 7511

Austria
Tel: 01 576 0616 22
Fax: 01 576 0616 27

Belgium
Tel: 0800 73 888
Fax: 03 272 1637

Canada
Tel: +1 800 463 5800
Fax: +1 800 567 1008

Central, East, and Southeast Europe
Tel: +43 1 982 3826
Fax: +43 1 985 8327

Denmark
Tel: 45 16 2400
Fax: 45 16 2424

Finland & Baltics
Tel: +358 (0)9 512 39 40
Fax: +358 (0)9 512 17 10

France
Tel: 01 69 35 67 00
Fax: 01 69 41 96 77

Germany
Tel: 0761 4903 291
Fax: 0761 4903 405

Italy
Tel: 02 27322 1
Fax: 02 27302 212

Japan
Tel: +81 3 5331 9336
Fax: +81 3 5331 9370

Latin America
Tel: +55 11 3667 5700
Fax: +55 11 3667 87 99

Middle East and Africa
Tel: +30 (1) 96 00 687
Fax: +30 (1) 96 00 693

Netherlands
Tel: 0165 580 410
Fax: 0165 580 401

Norway
Tel: 2318 5800
Fax: 2318 6800

Portugal
Tel: 21 417 70 35
Fax: 21 417 31 84

Russia & other C.I.S. & N.I.S.
Tel: +7 (095) 232 0250, 956 1137
Fax: +7 (095) 230 6377

Southeast Asia
Tel: +60 3 8024 2080
Fax: +60 3 8024 2090

Spain
Tel: 93 594 49 50
Fax: 93 594 49 55

Sweden
Tel: 018 612 1900
Fax: 018 612 1910

Switzerland
Tel: 01 802 81 50
Fax: 01 802 81 51

UK
Tel: 0800 616928
Fax: 0800 616927

USA
Tel: +1 800 526 3593
Fax: +1 877 295 8102

Web site

http://www.amershambiosciences.com

Part one

Basics

Chapter 1 The Fragment Analysis workflow

The Fragment Analysis software provides the tools and processing power to—

- Set up the layout of your experiment for DNA fragment analysis, protein band analysis, and isoelectric point determination.
- Find lanes and bands automatically.
- Edit lanes and bands manually.
- Calculate sizes, amounts, and isoelectric points of bands.
- Examine the image with special viewing tools.
- Obtain reports of size, amount, and migration statistics about any band in the sample.
- Perform two-color analysis.
- Transfer data to a Microsoft Excel spreadsheet.
- Automate the entire analysis process.

The software steps you through the analysis process, beginning with setting up the experiment and ending with obtaining a report.

The topics in this chapter are—

- Setting up the experiment (section 1.1)
- Performing the analysis (section 1.2)
- Creating a report (section 1.3)
- Automating the analysis (section 1.4)

1.1 Setting up the experiment

You begin a session by setting the experiment parameters, such as the number of lanes in the sample (figure 1-1).

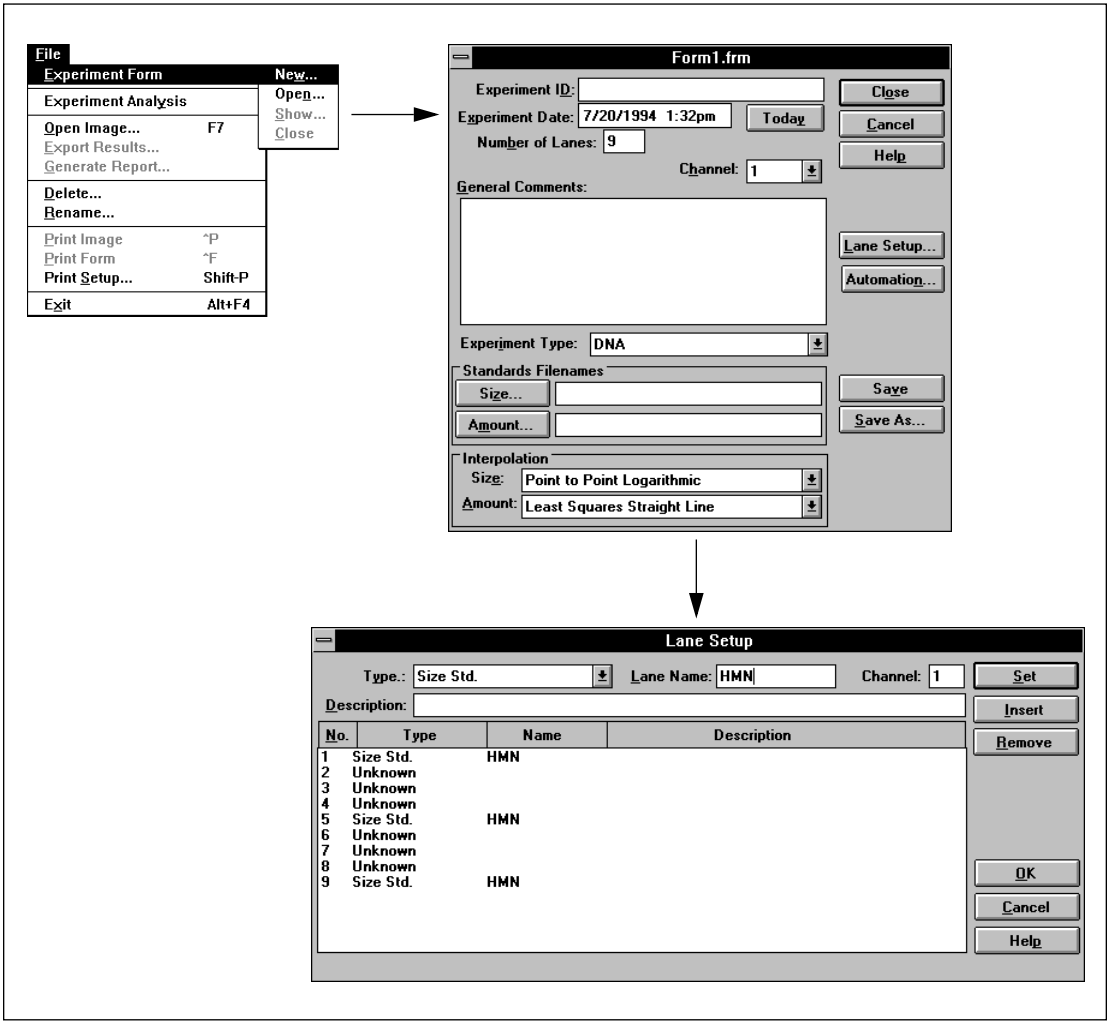


Figure 1-1. Accessing the forms for setting up the experiment format.

1.2 Performing the analysis

Next, you load the image you are analyzing. Fragment Analysis then steps you through the analysis process by displaying *inspectors*—a series of windows for entering additional specifications or for confirming the results (figure 1-2).

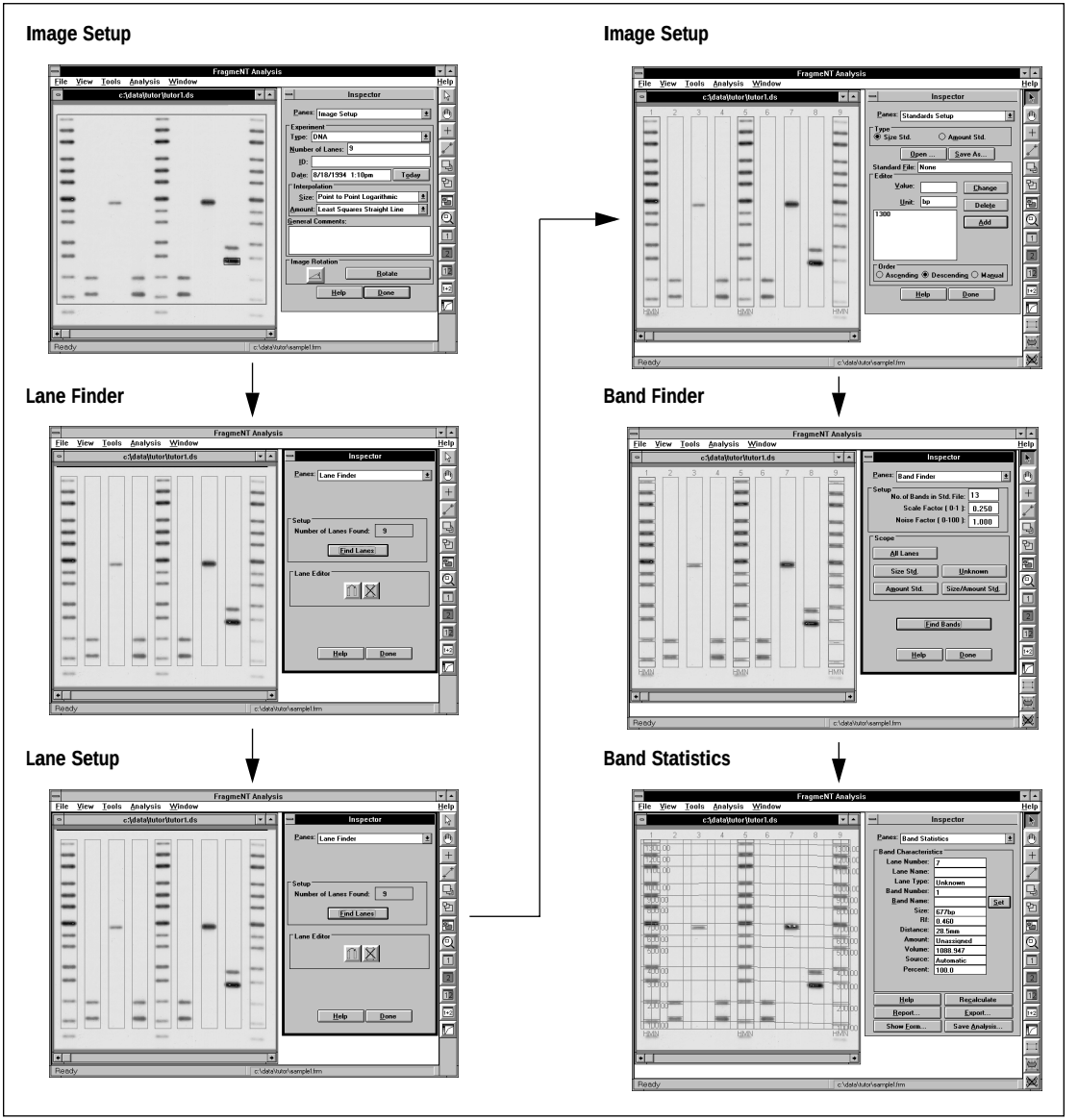


Figure 1-2. The inspector sequence.

1.3 Creating a report

You can select from a variety of report formats, and then transfer the results to an Excel spreadsheet (figure 1-3) or export the results to a file for later retrieval in a word processing or database application.

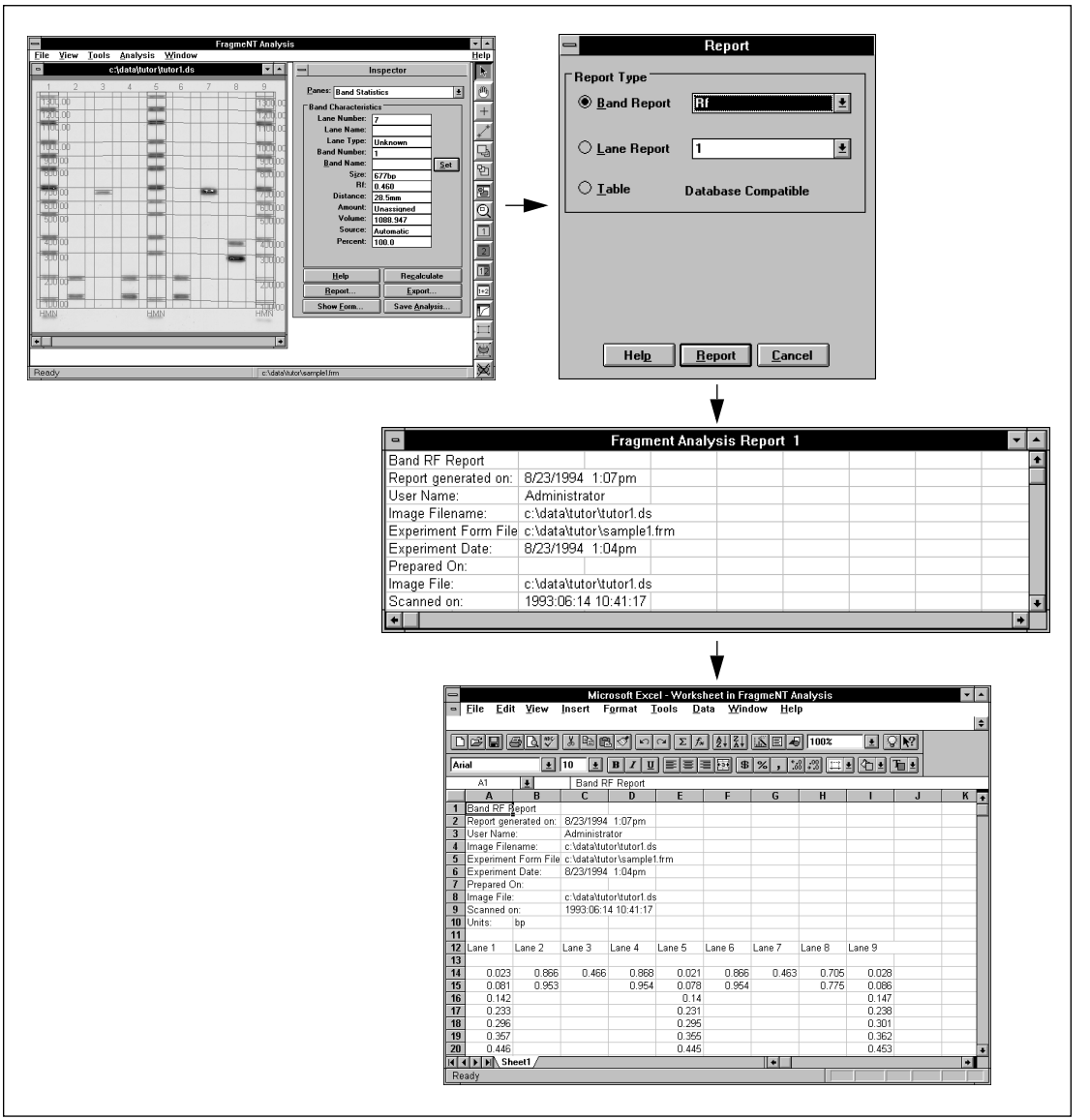


Figure 1-3. Transferring results to a spreadsheet.

1.4 Automating the analysis

After you create a form for a typical sample, you can use the form to analyze similar samples automatically. You do this by loading the form, selecting the steps you want to automate (figure 1-4), and then loading the image. After you load the image, Fragment Analysis can perform a complete analysis without intervention on your part, unless a problem arises.

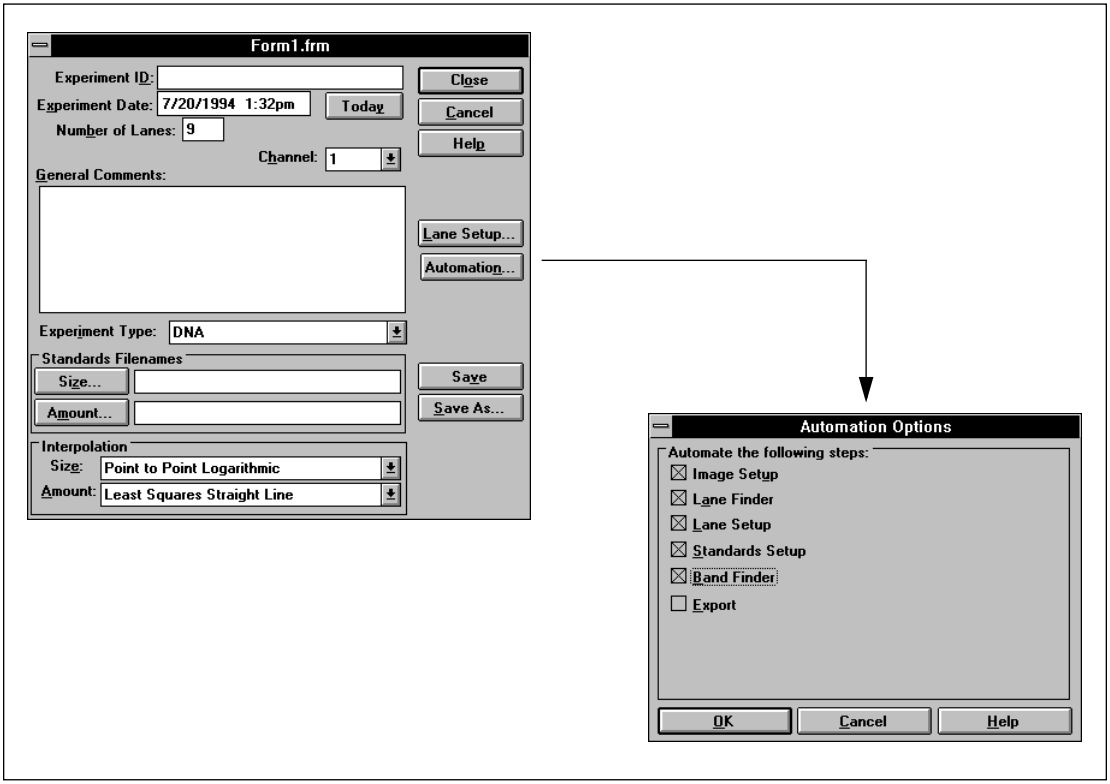


Figure 1-4. Selecting automation options.

Chapter 2 Getting started

This chapter provides the information you need to start using the Fragment Analysis software. The topics in this chapter are—

- Starting the Fragment Analysis software (section 2.1)
- Understanding the toolbar (section 2.2)
- Using inspectors (section 2.3)
- Quitting the Fragment Analysis software (section 2.4)

2.1 Starting the Fragment Analysis software

After you log on to the Windows desktop, you double-click the Fragment Analysis shortcut icon on the desktop. If you do not see the icon, you can start Fragment Analysis using the Start menu. The Fragment Analysis main window appears (figure 2-1).

When Fragment Analysis first appears, three menu items are displayed: File, Window, and Help (figure 2-1). As you begin working on various tasks, the menu items change according to the task you are performing.

The status bar, at the bottom of the window, displays messages about the function of current selections as well as the name of the Experiment Form you are using.

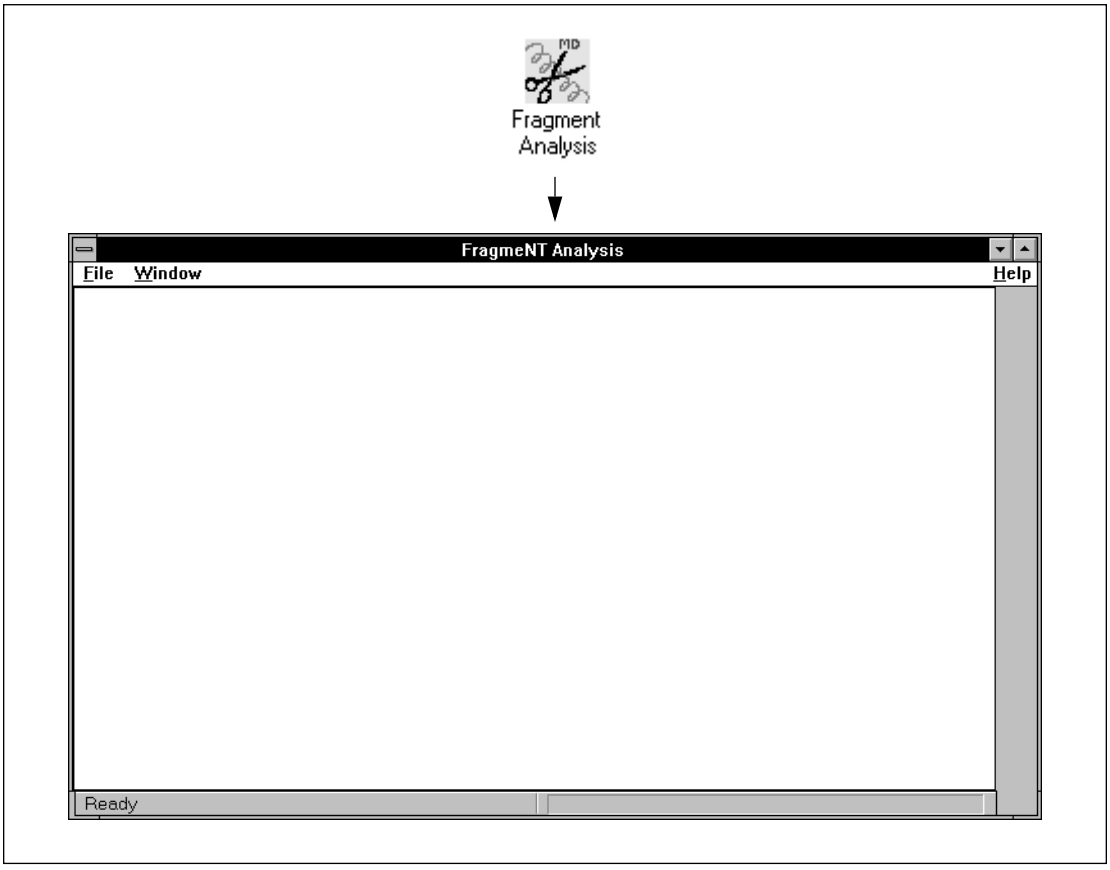


Figure 2-1. Starting the Fragment Analysis software.

2.2 Understanding the toolbar

After you load an image, a toolbar appears at the edge of the Fragment Analysis window (figure 2-2). The toolbar provides a handy way to access various functions. (The last three buttons on the toolbar do not appear until you reach the band-finding step.)

To use a tool, click the button. If you place the pointer on an image, the pointer changes to a different shape, depending on the tool you select.

For detailed information about the toolbar options, see chapters 7 and 8.

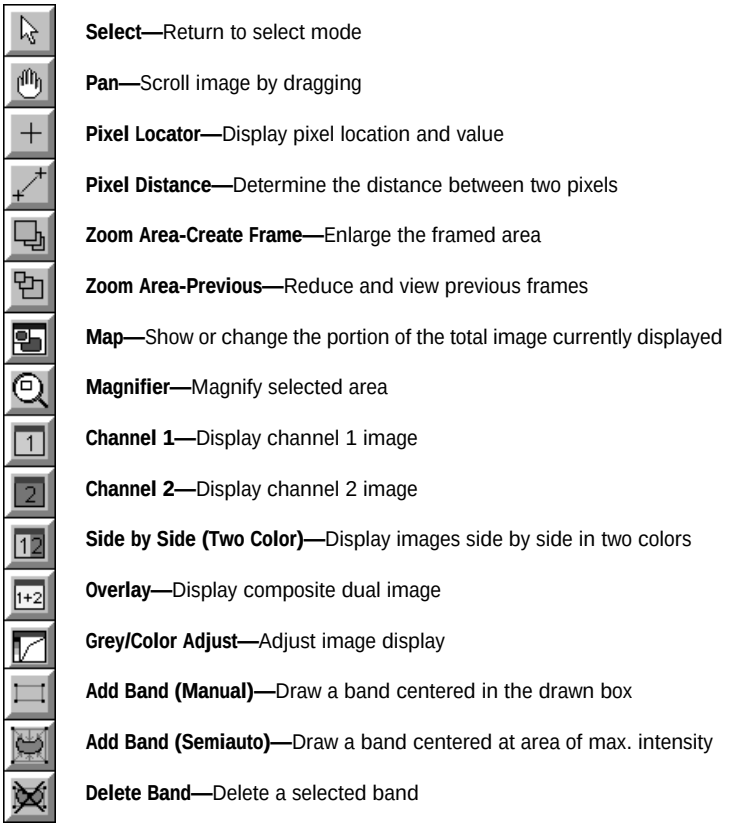


Figure 2-2. The toolbar.

2.3 Using inspectors

To perform an analysis, you use inspectors. Inspectors are windows designed to set up and initiate a specific task or to display results (figure 2-3). For example, you use one inspector to find bands and another to set up standards.

As you go through the analysis, Fragment Analysis presents each inspector in sequential order, beginning with the Image Setup inspector. After you click Done, Fragment Analysis displays the next inspector. You can also access inspectors from the Analysis menu (which appears after you load an image) or from within the currently displayed inspector.

Inspectors have the following features (figure 2-3):

- **Panes**—Displays the current inspector name and contains a list of inspectors you have already used. You can return to a previous step by selecting the inspector from the list.

  Note: Because each step of Fragment Analysis builds on the preceding step, the list of available inspectors depends on the stage of analysis you have reached.

- **Help button**—Displays information about the inspector.

- **Done button**—Finalizes the step and displays the next inspector in the series.

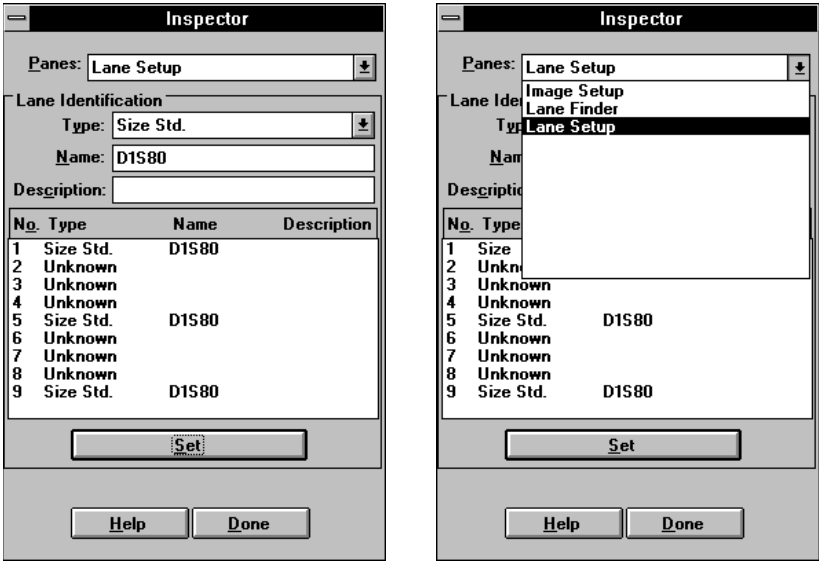


Figure 2-3. Example of an inspector.

2.4 Quitting the Fragment Analysis software

To quit the Fragment Analysis software, save the results of your analysis. Then from the File menu, click **Exit.** The Fragment Analysis main window closes.

Chapter 3 Fragment Analysis step-by-step

This chapter takes you through a complete fragment analysis session. Before you begin analyzing your own data, use this chapter as a tutorial to learn how to analyze the provided image. The tutorial image file is named tutor1.ds. (The tutor2.ds image file is a dual-channel image that can be used in chapter 5). To use this chapter as a tutorial, look for the words, *"To try it now…"* on each odd-numbered page.

To begin the tutorial, display the Fragment Analysis main window, as described in section 2.1.

Note: During the course of the tutorial, you will be creating files and giving them names specified in the tutorial. If someone has used the tutorial before you, a message will appear when you save certain files asking if you want to replace the existing file with the new file. Click **Yes/OK.**

The topics in this chapter are—

- Accessing the Experiment Form (section 3.1)
- Setting up the experiment (section 3.2)
- Entering the lane setup information (section 3.3)
- Saving the Experiment Form (section 3.4)
- Loading the image (section 3.5)
- Adjusting the image display (section 3.6)
- Preparing the image for analysis (section 3.7)
- Finding the lanes (section 3.8)
- Reviewing the lane setup information (section 3.9)
- Creating a standards file (section 3.10)
- Finding the bands (section 3.11)
- Reviewing the band statistics (section 3.12)

3.1 Accessing the Experiment Form

You begin an analysis session by entering the information, such as number of lanes and locations of size standards, that Fragment Analysis uses to calculate data for the unknowns. You enter this information into an Experiment Form.

3.1.1 Creating a new form

To access a new form, open the **File** menu and select **Experiment Form,** and then select **New** from the submenu (figure 3-1). The Experiment Form window appears.

3.1.2 Three ways to use a form

You can use the Experiment Form as—

- A record of your experiments. In this case, you can create a new form for each analysis you perform.
- A template or method for running experiments that use the same parameters. In this case, select the **Open** option, and then, after the form appears, click the **Close** button.
- A basis for creating new forms. For similar experiments, you can retrieve an existing form (select the **Open** option), modify it, and then save it under a new name.

The form you use remains in effect until you select a new form, close the application, or unload a previously loaded form. To unload a previously loaded form (at either the beginning or end of the analysis session), open the **Experiment Form** menu and select **Close** from the submenu**.**

3.1.3 Working without a form

You can perform an analysis without first creating a form. In this case, you load your image, and then enter your specifications into the inspectors as they appear during the course of an analysis session. At the end of the analysis, the entries you made in the inspectors are automatically stored in a form, which you can then choose to save.

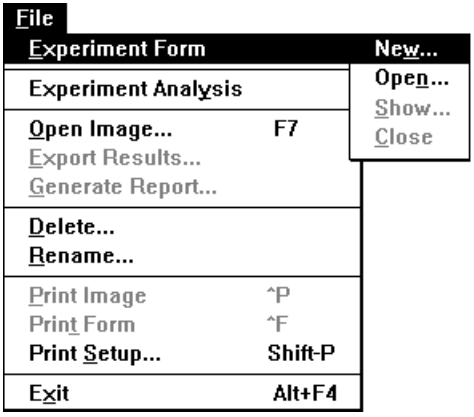


Figure 3-1. Opening the Experiment Form window.

To try it now…

1. On the main menu bar, click **File** to display the File menu.
2. In the File menu, click **Experiment Form.** A menu of options appears (figure 3-1).
3. From the list of options, select **New.** The Experiment Form window appears (figure 3-2).
4. Go to section 3.2.

3.2 Setting up the experiment

To set up the experiment, enter information into the form (figure 3-2).

3.2.1 Entering the experiment setup information

Enter information in the Experiment Form as follows:

- **Optional Entries**—The **Experiment ID, Date,** and **General Comments** boxes are for your records only.
- **Defaults**—The **Channel, Experiment Type,** and **Interpolation** boxes contain default values. If the displayed defaults are not appropriate for your experiment, choose another selection from the list.
- **Number of Lanes**—Enter the number of lanes that are in your sample.
- **Standards Files**—The buttons in the **Standards Filenames** area provide access to windows from which you can select the standards files to use with your experiment. (If the file you need does not exist, you can create the file at a later step.)

3.2.2 Entering the lane identification information

To enter lane identification information, first enter the number of lanes, and then click **Lane Setup.** The Lane Setup window appears (see section 3.3).

3.2.3 Automating the analysis

After setting the experiment parameters, you can automate the analysis by selecting options from the Automation Options window, which you open by clicking the **Automation** button. For information on using the Automation feature, see section 4.7.

3.2.4 For more information

For detailed information about choices in the Experiment Form, see section 6.1. For information about analyzing dual-channel images, see chapter 5.

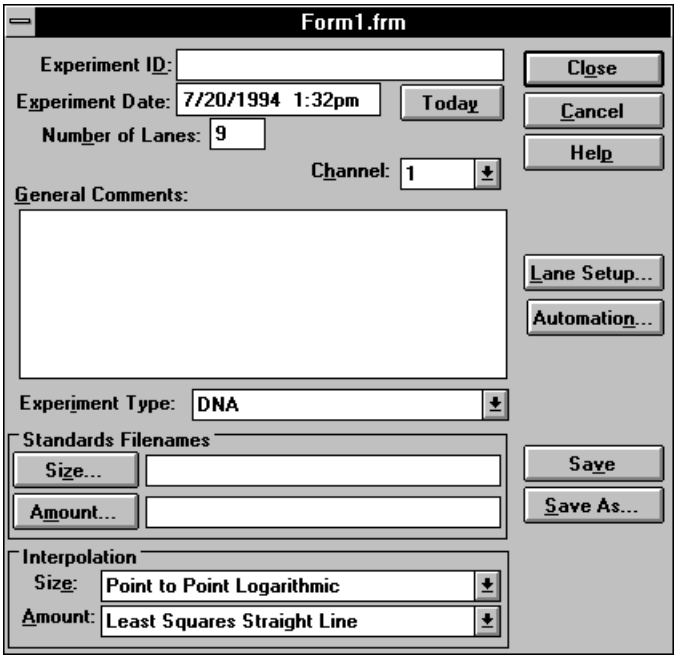


Figure 3-2. Setting up the experiment.

To try it now…

1. Click **Today.** Today's date appears in the Experiment Date box (figure 3-2).
2. Double-click the **Number of Lanes** box, and then type 9**.** You will leave the other text boxes blank and accept the defaults in the selection boxes.
3. Click **Lane Setup.** The Lane Setup window appears.
4. Go to section 3.3.

3.3 Entering the lane setup information

After you click the Lane Setup button, the Lane Setup window appears showing the number of lanes you typed in the Experiment Form. Use the Lane Setup window to identify standards lanes and enter other identifying information about the lanes.

3.3.1 Identifying the standards

To identify the standards lanes, display the Type list, and then select the appropriate type:

- **Size** standards define the molecular weight of each band.
- **Amount** standards define the amount of DNA or protein in each band.
- **pKI** standards define the isoelectric point of each band.

Optionally, you can also type **Lane Name** and **Description** information.

Next, in the **lane list box,** click one or more of the lanes that represent standards. (To select more than one lane at a time, hold down the **CTRL** key while you click the lanes.)

After making your selections, click **Set.** The lane list is updated with the new information (figure 3-3).

Because all bands are initially labeled Unknown, adding information about unknown lanes is not required. To add identifying information, such as Lane Name, use the same technique as for identifying standards.

3.3.2 Changing the information

To change the information about the lanes, click the lane to select it, change the information in the selection and text boxes near the top of the window, and then click **Set.** To add a lane, click a lane, and then click **Insert.** A new lane is added after the lane you selected. To remove a lane, click the lane, and then click **Remove.**

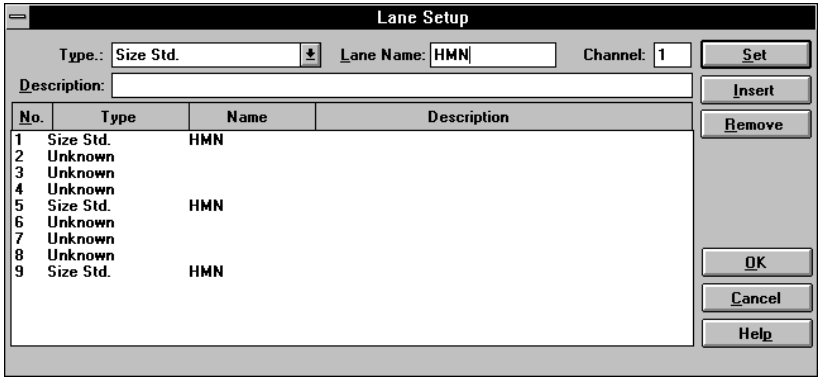


Figure 3-3. Identifying standards lanes.

To try it now…

1. Click the down arrow ( ) next to the **Type** box. A list of lane types appears. Click **Size Std.**
2. Click in the **Lane Name** box, and then type `HMN`.
3. **CTRL**+click **Lane 1, Lane 5,** and **Lane 9.** All three are highlighted.
4. Click **Set.** The lane list is updated with the new information (figure 3-3).
5. Click **OK.** The Lane Setup window closes.
6. Go to section 3.4.

3.4 Saving the Experiment Form

To save the Experiment Form, click **Save.** The Save Form File window appears (figure 3-4). Type a name for the form in the **File Name** box, and then click **OK.** Fragment Analysis adds a .frm file extension. Alternatively, you can accept the default name, form1.frm, in which case the next default name will be form2.frm, and so on. (See section 6.1.10 for information on file name restrictions.)

3.4.1 How the form affects the analysis

Fragment Analysis uses the information you entered into the form as input for the analysis session. The current form remains in effect until you create a new form, load another form, or unload the form.

3.4.2 Unloading a form

If, after loading a form, you decide to analyze a sample without using the form, you can close the form you loaded. To do this, from the **File** menu, select **Experiment Form,** and then select **Close** from the submenu. Note that you can close a form only at the beginning or end of an analysis session (at the Image Setup inspector or at the Band Statistics inspector), or before an image is loaded.

3.4.3 Retrieving a form: Open versus Show

The Open and Show commands, available from the Experiment Form submenu, serve different purposes—

- Use the **Open** command to load the form you want to use for the analysis or to modify an existing form.
- Use the **Show** command at the end of the analysis session to view the currently loaded form. Because Fragment Analysis retains changes you make during the analysis, you can use the Show command to retrieve the form and save the changes to update your form.

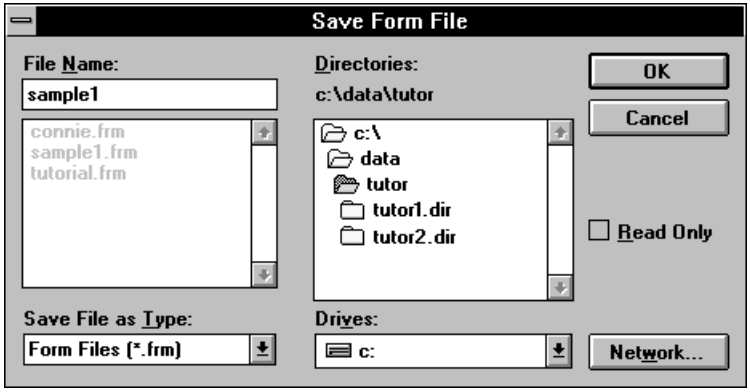


Figure 3-4. Saving the Experiment Form.

To try it now…

1. In the Experiment Form, click **Save.** The Save Form File window appears (figure 3-4).
2. In the **File Name** box, type `sample1,` and then click **OK.** The window closes. Fragment Analysis adds the .frm file extension to the file name and replaces Form1.frm on the title bar of the form with the name of the file.
3. In the Experiment Form window, click **Close.** The window closes.
4. Go to section 3.5.

3.5 Loading the image

After setting up the experiment, you select and load an image file. Loading the image begins the analysis session. If you did not load or create an Experiment Form, you enter the experiment information in the inspectors as you proceed through the analysis.

3.5.1 Selecting an image file

To select an image file to load, display the **File** menu and click **Open Image.** The Open Image window appears (figure 3-5). In the list of files box, locate the file you want to analyze, and then double-click the file name (or click the file name, and then click **OK**).

If you do not see the file name, use the scroll bars to reveal hidden file names. Also check the List Files of Type box to make sure that the correct file type is displayed (usually MD Files *.ds, *.gel), and check the Drive and Directory areas. (Typically, image files are stored in the \data directory in drive c.)

Note: For additional details on using the Open Image window, including information about the directory structure, see section 6.3.

3.5.2 After loading the file

After you load the file, the following events occur:

- A progress bar appears on the screen, indicating that the file is being loaded.
- The image appears sized to fit the available space on the screen.
- Two rectangles, large and small, are overlaid on the image.
- The Image Setup inspector appears (figure 3-6). If you created an Experiment Form, the settings you selected for Number of Lanes, Experiment Type, Interpolation, and other information are displayed in the inspector.
- The name of the form appears on the right side of the status bar.

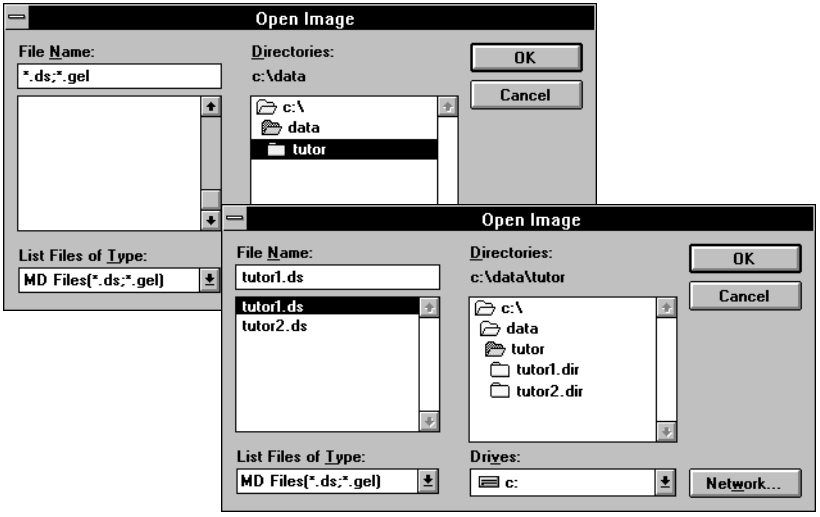


Figure 3-5. Selecting the file to process.

To try it now…

1. On the main menu bar, click **File** to display the File menu.
2. Click **Open Image.** The Open Image window appears (figure 3-5).
3. In the **Directories** box, double-click the **tutor** directory located under the data directory. Two file names appear in the file names list box.
4. Double-click **tutor1.ds.** A progress bar appears, followed by the image and the Image Setup inspector. The Number of Lanes, Experiment Type, and Interpolation parameters specified in the Experiment Form appear in the inspector (figure 3-6).
5. Go to section 3.6.

3.6 Adjusting the image display

The Fragment Analysis software includes many features that allow you to adjust the screen display. Below are some examples of these features. For a complete discussion, see chapter 7 (View menu reference) and chapter 8 (Tools menu reference).

3.6.1 Moving and hiding the inspectors

To move an inspector, place the pointer on the inspector title bar and drag the inspector to a new location. To hide an inspector, double-click the inspector control menu button ( ). To show the inspector, open the **Analysis** menu and click the inspector name.

3.6.2 Scrolling the image

For images that are larger than the Image window, several scrolling tools are available: the window **scroll bars,** the **Pan** tool ( ), and the **Map** window tool ( ). The Pan tool, available from the Tools menu or toolbar, allows you to move the image in any direction. The **Map** window, available from the View menu and toolbar, displays a small replica of the entire image with an outline showing the currently displayed area. You move the outline to shift the image display.

3.6.3 Optimizing the image display

If the image is difficult to see, you can adjust the grey-scale range or invert the grey tones of the image using the **Grey/Color Adjust** window (figure 3-6), available from the View menu or toolbar ( ). Section 7.6 explains how to use the Grey/Color Adjust window.

3.6.4 Enlarging and reducing the image

Both the View and Tools menus offer options for changing the image display. For example, to scale the image to fit the window, select **Full Size** from the **View** menu. To magnify a particular region of the image, select **Magnifier** from the Tools menu or toolbar ( ).

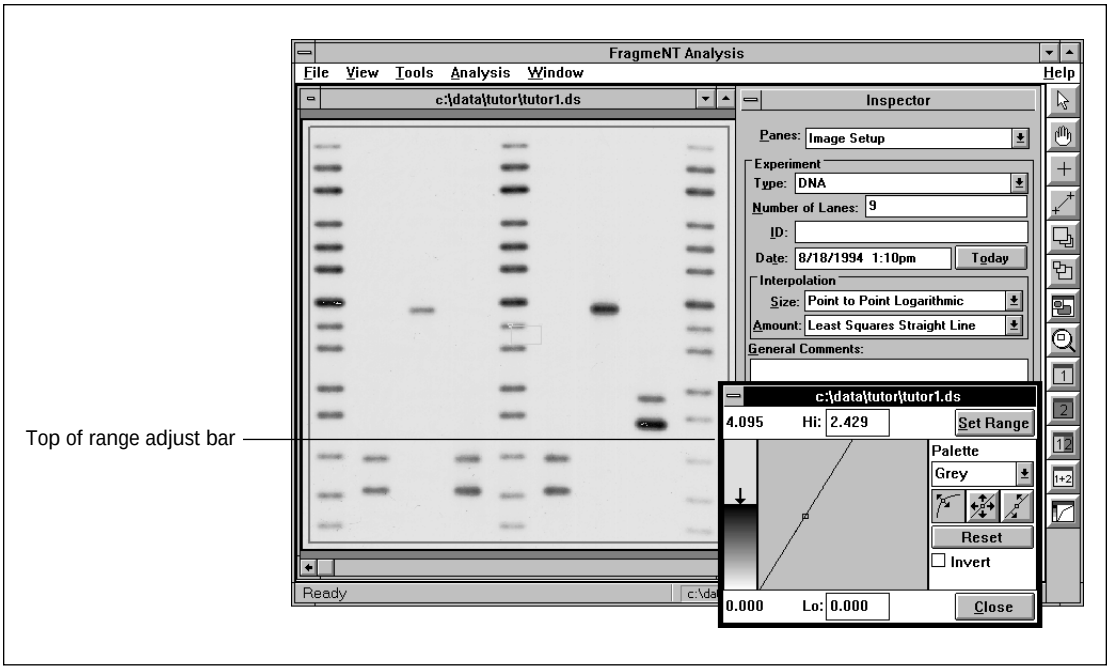


Figure 3-6. Adjusting the image display.

To try it now…

1. Click the **Grey/Color Adjust** toolbar button ( ) to open the Grey/Color Adjust window (figure 3-6).
2. Move the pointer to the title bar of the Grey/Color Adjust window and drag the window so that it does not cover the image.
3. Move the pointer to the top of the range adjust bar (figure 3-6), drag the pointer down until the middle of the darkest bands begin to appear green, and then release the mouse button. The bands appear darker.

   If the color appears purple instead of green, click **Reset,** and start again.
4. Click **Set Range** to save the changes (the changes affect the display only), and then click **Close** to close the Grey/Color Adjust window.
5. Go to section 3.7.

3.7 Preparing the image for analysis

When the image appears on the screen, a large red rectangle and small green rectangle are overlaid on the image. The rectangles define the region you want to process and the approximate size of the bands. The Image Setup inspector includes a tool for rotating the image plus input areas for entering experiment setup specifications.

3.7.1 Understanding the rectangles

The two rectangles on the image serve the following purposes (figure 3-7):

- **Small rectangle**—Defines the approximate band size and lane width for the analysis. Move and resize this rectangle to surround the largest band. Make sure that the rectangle closely outlines the band edges.
- **Large rectangle**—Defines the region you want to analyze. Resize this rectangle to exclude from the analysis unwanted areas of the image, such as the wells at the top of the image. Set the top boundary at the bottom edge of the wells. Set the right and left boundaries within half a lane-width of the outermost bands (the rectangle edges should be close to—but not touching—the edges of the outermost bands).

3.7.2 Moving and resizing the rectangles

To move or resize a rectangle, you must first select it. Place the pointer within one of the rectangles and click the mouse button. Small square "handles" appear at the corners. To move the rectangle, place the pointer inside the rectangle and drag the rectangle to a new position. To resize a rectangle, place the pointer on one of the corner handles and drag the corner to a new position.

3.7.3 Rotating the image

To rotate an image that is not squarely aligned in the Image window, click the **image rotation** button (). A red line with black handles at both ends appears on the image. Drag the handle to indicate the degree of rotation, and then click on **Rotate.** The image rotates to the degree indicated by the line. For complete instructions on rotating, see section 9.1.

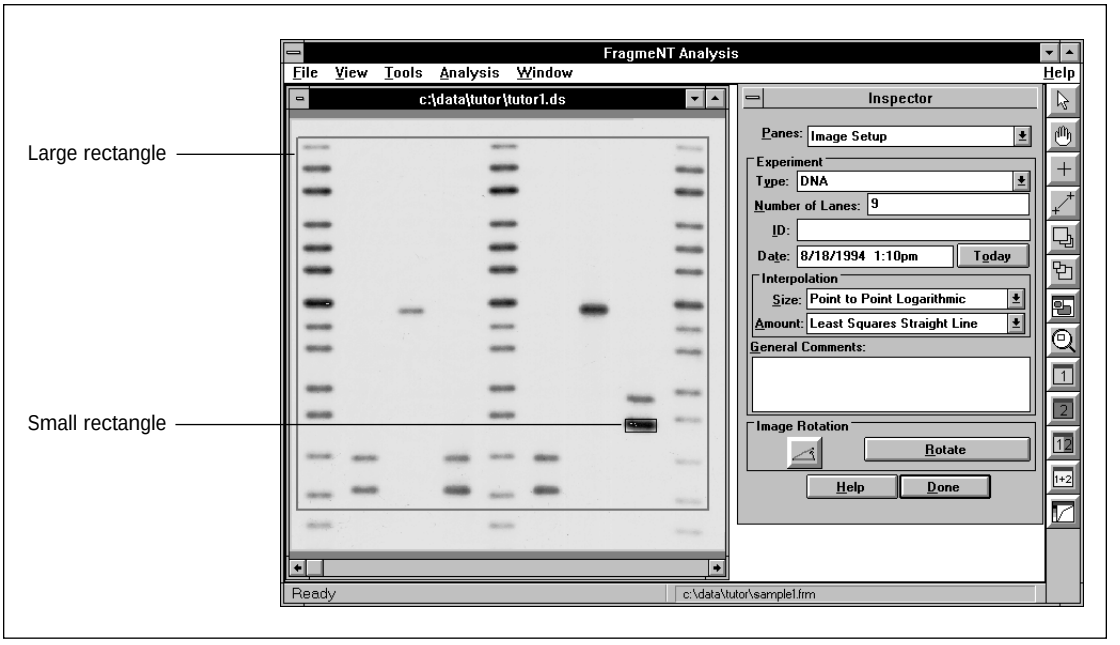


Figure 3-7. Adjusting the region of interest and band prototype rectangles.

To try it now…

1. Place the pointer inside the **small rectangle** and drag the rectangle to enclose the largest band (figure 3-7).
2. Resize the small rectangle to fit the band: Place the pointer on one of the black handles and drag the handle right or left, up or down.
3. Click the **large rectangle.** Handles appear at the corners.
4. Use the handles to adjust the size of the large rectangle:

   Pull the top and outside edges inward so that they are close to—but not touching—the bands.

   Pull the lower edge up to exclude the bottom row of bands from the region of interest (figure 3-7).
5. Click **Done.** The Lane Finder inspector appears with a rectangle over the first lane in the Image window.
6. Go to section 3.8.

3.8 Finding the lanes

At the same time that the Lane Finder inspector appears, a rectangle appears on the first lane in the image. The Lane Finder inspector allows you to initiate automatic lane finding. The rectangle serves as the prototype lane marker.

3.8.1 Adjusting the lane marker

The rectangle surrounding the first lane serves as a pattern for finding the remaining lanes. If the rectangle does not correctly delineate the lane, move it or resize it, using the same techniques discussed in section 3.7.2. Note that you cannot change the height of the rectangle, only its width and position.

3.8.2 Initiating lane finding

To initiate lane finding, click **Find Lanes.** Fragment Analysis encloses the found lanes with rectangles and displays the number of lanes it found (figure 3-8). (The software finds the number of lanes you specified in the Experiment Form or Image Setup inspector.)

3.8.3 Editing the lane markers

You can move and resize lane markers to fit the lanes. You can also add and delete the markers—

- **To add a lane marker**—Click a lane marker, and then click the **duplicate** button (). A rectangle encloses the lane to the right of the selected lane.
- **To delete a lane marker**—Click the lane you want to delete, and then click the **delete** button () (or the DELETE key). The lane marker disappears.

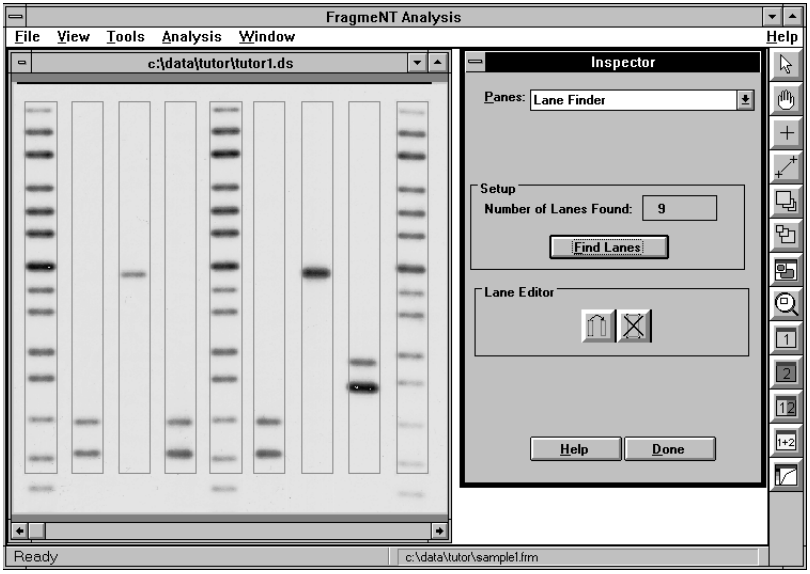


Figure 3-8. Finding lanes.

To try it now…

1. Place the pointer inside the rectangle in the Image window, and drag the rectangle left or right to make sure that it uniformly surrounds the lane.
2. Click **Find Lanes.** The lanes are marked with rectangles and the number of found lanes is displayed (figure 3-8).
3. Review the placement of the rectangle markers and move or resize them to make sure that each lane is accurately defined.
4. Click **Done.** The Lane Setup inspector appears (figure 3-9).
5. Go to section 3.9.

3.9 Reviewing the lane setup information

When the Lane Setup inspector appears (figure 3-9), the lane identification information you entered in the Lane Setup window of the Experiment Form is shown in this inspector.

3.9.1 Identifying the standards lanes

The Lane Setup *inspector* is the counterpart of the Lane Setup *window*, which you use to set up the experiment (section 3.3). If you did not enter the lane setup information into the Lane Setup window, you can use the same techniques to enter the information into the inspector.

Note: To select lanes, you can click the lanes in the Image window as well as in the Lane List. To select multiple lanes in the Image window, hold down the SHIFT key while you click the lanes.

3.9.2 Returning to a previous inspector

To return to a previous inspector, such as the Lane Finder, click anywhere in the **Panes** box to reveal the inspector options, and then click an option. After returning to a previous inspector, you must proceed through the normal sequence of inspectors by clicking **Done** in each one.

Note: Because each step of Fragment Analysis builds on the preceding step, the list of available inspectors depends on the stage of analysis you have reached.

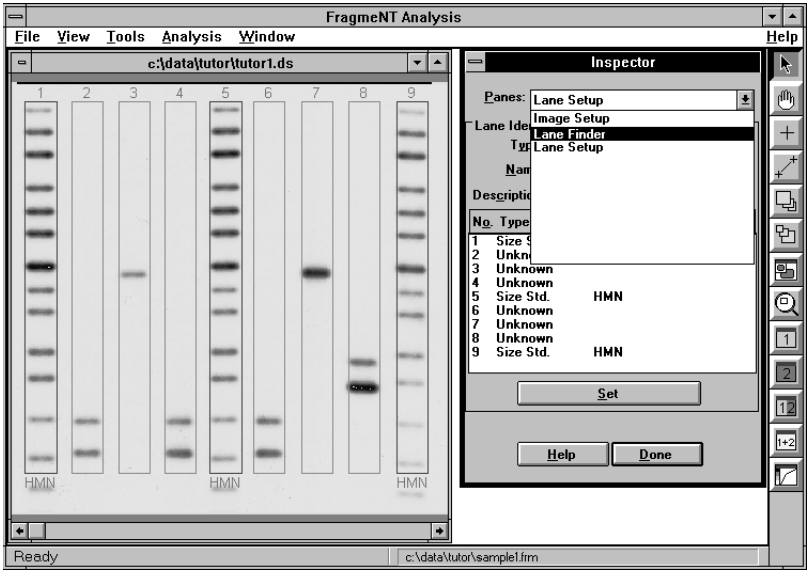


Figure 3-9. Identifying lanes.

To try it now…

1. In the Lane Setup inspector, examine the lane list. Lanes 1, 5, and 9 should be identified as size standards and HMN should appear on the image.
2. Click the **Panes** box. A list of inspectors appears (figure 3-9).
3. Click **Lane Finder.** The Lane Finder inspector appears.
4. Click **Done.** The Lane Setup inspector appears.
5. Click **Done.** The Standards Setup inspector appears.
6. Go to section 3.10.

3.10 Creating a standards file

The Standards Setup inspector (figure 3-10) allows you to create, load, or modify a standards file. The standards file provides the information the software uses to compute the unknowns.

3.10.1 Creating a new standards file

To create a new standards file, first select the type of file you will be creating by clicking one of the buttons in the **Type** area. Next, in the **Unit** box, type the appropriate unit for the value, such as bp, kb, or ng. In the **Order** box, select the order in which you want the values listed.

Finally, in the **Value** box, type the first value, and then click **Add** or press **ENTER**. The system adds the value to the list, inserting it in an appropriately sorted order. Repeat to add more values.

To remove a value from the list, click the value, and then click **Delete.** To change a value, click the value, type a new value in the **Value** box, and click **Change.** To insert a value, type a new value in the **Value** box, and then click **Add.** The value is inserted in the appropriate ascending or descending order.

3.10.2 Saving the new standards file

To save the new file, click the **Save As** button. The Save Size/Amount Standard window appears. In the **File Name** box, type a name for the file, and then click **OK.**

3.10.3 Loading an existing standards file

To load an existing standards file, select the standards type, and then click **Open** to display the Open Size/Amount File window. Double-click the desired standards file (or click the file name, and then click **OK**). The band values appear in the **Editor** area of the inspector. (If you selected the wrong file, you can select a new one.)

If a single standard serves as both size and amount standards, first load one standards file type, and then the other. (Remember to specify the type before clicking Open.)

For more information about using the Standards Setup inspector, see section 9.4.

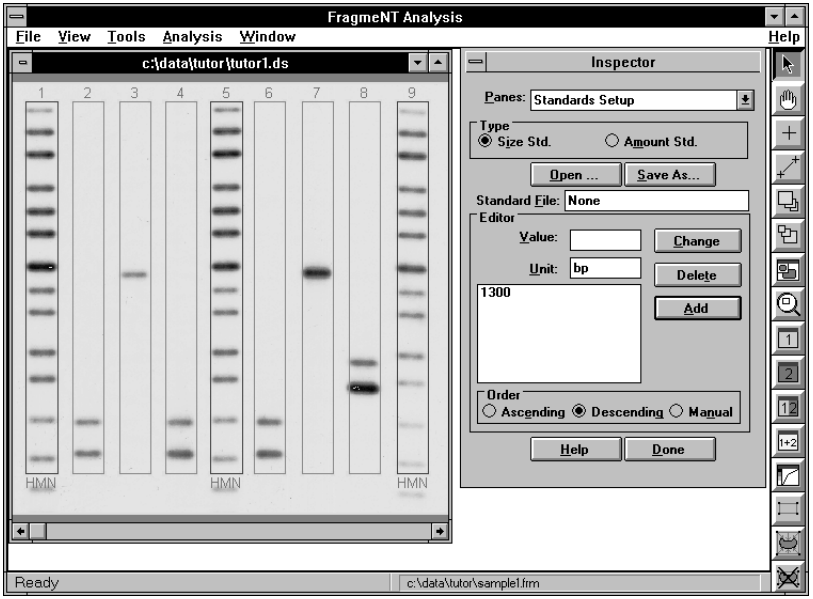


Figure 3-10. Creating a standards file.

To try it now...

1. In the Standards Setup inspector, make sure that the **Size Std.** button is selected in the **Type** area and that **Descending** is selected in the **Order** area.
2. Click the **Value** box, type `1300,` and then click **Add** (or press **ENTER**). The value, 1300, appears in the list of values box (figure 3-10).
3. Next, type `1200` in the **Value** box and click **Add.** Continue entering values, in decreasing 100 increments, until you reach 100. (You should have 13 entries. Use the scroll bar to check.)
4. Click **Save As.** The Save Size File window appears.
5. In the **File Name** box, type `SIZESTD`**,** and then click **OK.** (Fragment Analysis adds the .siz file extension to the file name.)
6. Click **Done.** The Band Finder inspector appears (figure 3-11).
7. Go to section 3.11.

3.11 Finding the bands

The Band Finder inspector (figure 3-11) initiates and controls the automatic band-finding process. When the Band Finder inspector appears, the Setup area displays the number of bands in your standards file as well as the default band-finding parameters.

In addition, three band-editing buttons are added to the toolbar (see section 4.2.1 for an explanation of the buttons).

3.11.1 Initiating the band finding

The Scope area allows you to specify the lanes for which you want Fragment Analysis to find bands. First, click one of the selections in the Scope area (the default is **All Lanes**), and then click **Find Bands.** Fragment Analysis locates the bands and marks the band positions and boundaries.

3.11.2 Evaluating the band finding

Check the image to see if Fragment Analysis located the bands. Then—

- If band finding was *satisfactory*, click **Done.**
- If Fragment Analysis *incorrectly identified* artifacts as bands, you can delete extraneous bands by selecting the bands and pressing the **DELETE** key. You can also increase the Noise Factor and/or Scale Factor values to exclude more data and click **Find Bands** again.
- If Fragment Analysis *overlooked* some bands, you can mark them yourself using the editing tools (section 4.2). You can also decrease the Noise Factor and/or Scale Factor values to include more data and click **Find Bands** again.

For more information on the Noise Factor and Scale Factor parameters, see section 9.5.2.

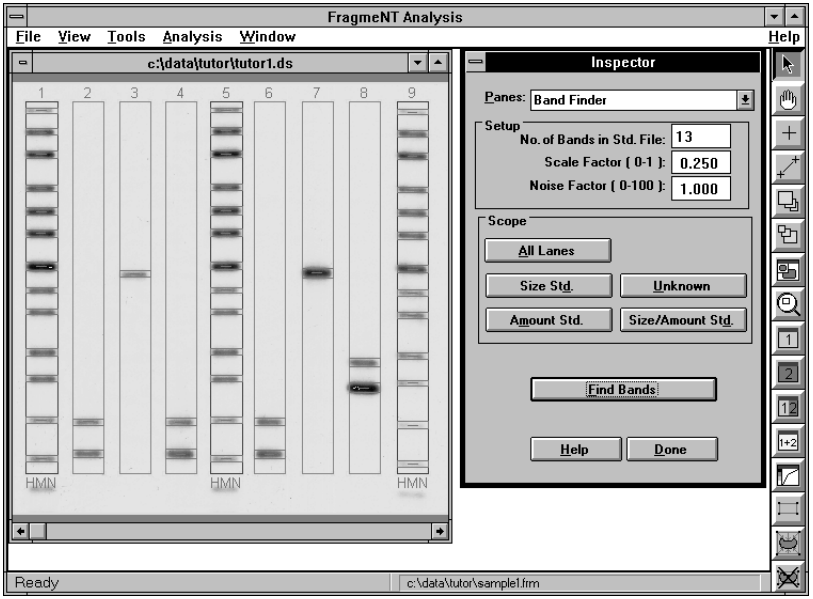


Figure 3-11. Finding bands.

To try it now…

1. In the Band Finder inspector, click **Find Bands.** Found bands are marked with bars (indicating the areas of maximum intensity) and rectangles (indicating the band region).
2. Click **Done.** The Band Statistics inspector appears (figure 3-12).
3. Go to section 3.12.

3.12 Reviewing the band statistics

The Band Statistics inspector (figure 3-12) displays the following information about any band you select. To select a band, click the band marker. Handles appear on the marker to indicate the band is selected.

- **Lane Number**—The number automatically assigned to the lane.
- **Lane Name**—The name you assigned to the lane.
- **Lane Type**—The lane designation, such as Size or Unknown.
- **Band Number**—The band's position in the lane, counting from the top.
- **Band Name**—A box in which you can type a band name (click **Set**).
- **Size/pKI**—The calculated size or isoelectric point, in the units you specified.
- **Rf**—The distance traveled, expressed as a percentage of the migration distance, measured from the top of the lane to the selected band.
- **Distance**—The distance traveled (in millimeters) from the top of the lane to the band position (region of greatest intensity).
- **Amount**—The calculated amount in the units you specified.
- **Volume**—The sum of all the pixel intensity values in the band, minus the background (background is determined automatically).
- **Source**—The method used to locate the band:
  - **Auto**—Fragment Analysis found the band.
  - **Semiauto**—Fragment Analysis found the band after you indicated its general location.
  - **Manual**—You indicated the location of the band.
- **Percent**—Percent of band volume relative to all bands in the lane.

The Band Statistics inspector is the end of the analysis. Go to chapter 4 for more information about the Band Statistics inspector as well as for instructions on creating a report, saving your specifications, saving the analysis, automating the analysis, and more. See section 9.6.1 for additional details about band statistics.

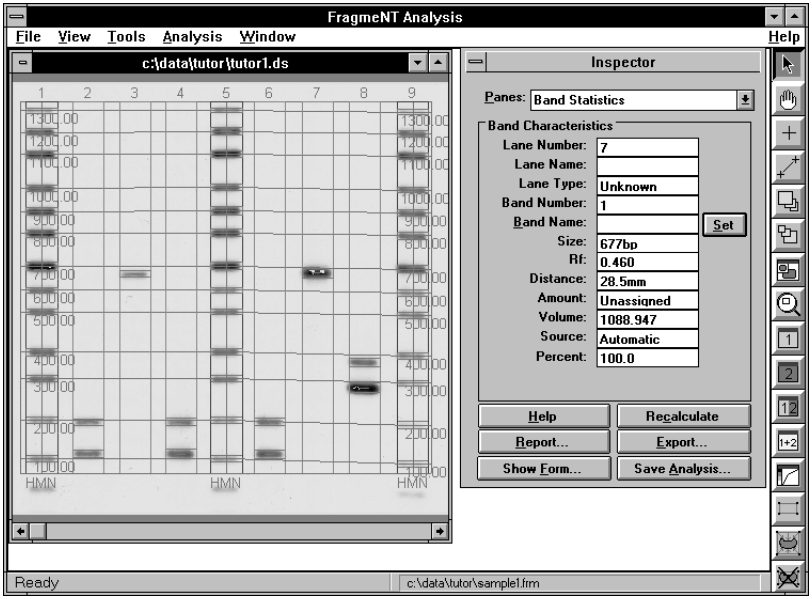


Figure 3-12. Obtaining band statistics.

To try it now…

1. Place the pointer on a band marker and click the mouse button. Handles appear on the marker (if they do not, click again). Statistics for that band appear in the inspector (figure 3-12).
2. Repeat step 1 for the other bands. Compare the values on the standards labels with the values displayed in the Size box of the inspector.
3. Go to chapter 4 to continue the tutorial.

   In chapter 4, you will use the magnification tool, edit the bands, create and print a report, save the entries you made during this session, automate the analysis, and then exit Fragment Analysis.

   If you want to quit now, you can save the analysis, and then retrieve it and finish the tutorial later. (See section 4.8.1 for instructions on saving the analysis; see section 4.9 for instructions on quitting the analysis.)

Chapter 4 Post-analysis operations step-by-step

This chapter explains the tasks you can perform after completing the analysis. It also continues the tutorial that started in chapter 3. The tutorial portion of this chapter assumes that you have completed an analysis and that the analyzed image and Band Statistics inspector are displayed.

The topics in this chapter are—

- Inspecting the bands (section 4.1)
- Editing the bands (section 4.2)
- Creating a report (section 4.3)
- Printing the results (section 4.4)
- Exiting from Excel (section 4.5)
- Saving your specifications (section 4.6)
- Automating the analysis (section 4.7)
- Saving the experiment analysis (section 4.8)
- Closing the Fragment Analysis software (section 4.9)

4.1 Inspecting the bands

When the Band Statistics inspector appears, isobar lines are added to the image as well as labels (on the outermost standards lanes) showing the molecular weights or isoelectric points of the standards bands.

4.1.1 Checking the isobar lines

Isobar lines connect the standards bands that Fragment Analysis used to calculate the values of the unknown bands. The lines should generally follow the "smile" of your gel caused by electrophoresis anomalies. If they do not appear correct, check to see that all the standards bands were found. If they were not, use an editing tool (section 4.2) to identify the missing bands or to delete the spurious bands, and then click **Recalculate.**

When checking the bands, note that values for unknown bands that fall below the lowest or above the highest standards band are extrapolated.

4.1.2 Magnifying portions of the display

The Magnifier tool is a "magnifying glass" for enlarging selected areas of the display (figure 4-1). To use this tool, click the **Magnifier** button () on the toolbar (or select the **Magnifier** command from the **Tools** menu). With the pointer (now a cross shape) on the Image window, drag the Magnifier around the Image window to view different areas.

4.1.3 Changing the line and label attributes

You can hide the isobar lines, labels, lane numbers and names, and/or band and lane marking objects. You can also change the color of any of these objects as well as display intensity profiles of each lane. To do this, open the **View** menu and select **Display.** The Display window appears.

Check boxes in the Display window allow you to select and deselect objects, such as lane profiles, that you want to show or hide. A color menu allows you to change object colors. Click **Apply** to apply your changes to the selected objects or **Save** to save and apply the changes to all subsequent analyses.

For more information on using the Display window, see section 7.9.

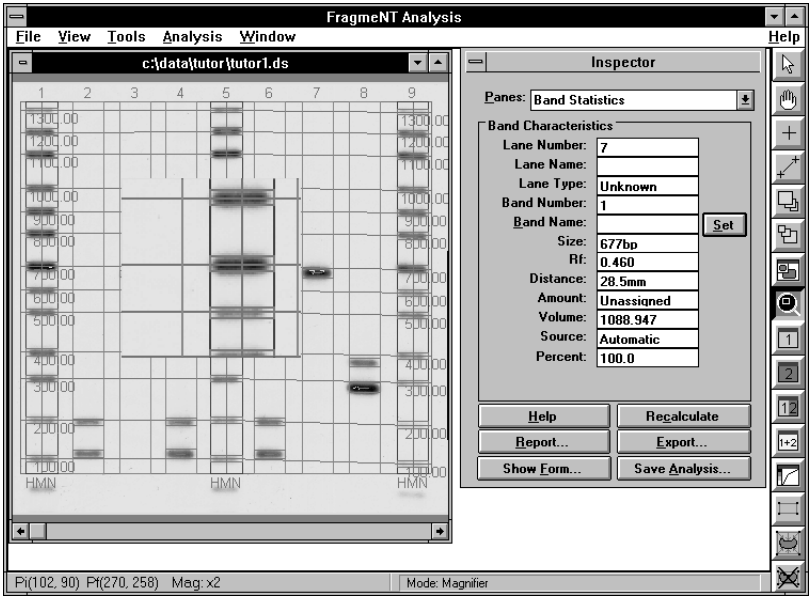


Figure 4-1. Using the Magnifier to inspect bands.

To try it now…

1. On the toolbar, click the **Magnifier** button ( ), and then place the pointer anywhere in the image and hold down the mouse button. A magnifying glass appears (figure 4-1).
2. Drag the glass around the image to examine the bands, and then release the mouse button.
3. Click **Select** ( ) to end the Magnifier mode.
4. Open the **View** menu and select **Display.** The Display window appears.
5. In the Display window, click the **Lane Profile** box to select it, and then click **Apply** followed by **Close.** Profiles, indicating the intensity of each band, appear on the image.
6. Go to section 4.2.

4.2 Editing the bands

Special band-editing buttons on the toolbar (or their counterpart commands on the Tools menu) allow you to identify bands not found by Fragment Analysis or to remove bands from the analysis. For example, if the system identified two close bands as a single band, you can delete the band marker, and then add two new bands.

4.2.1 Using the band-editing buttons

Three band-editing tools are available:

For manually creating a band. Click the **Draw Band (Manual)** button, and then move the pointer to the area where you want to create a band. Drag the pointer diagonally to create a rectangle that surrounds the band (figure 4-2). When you release the mouse button, a band marker is inserted in the *center* of the rectangle you created.

For finding a band semiautomatically. Click the **Draw Band (Semiautomatic)** button, and then move the pointer to the area in which you want the system to determine the band position. Drag the pointer diagonally to create a rectangle that defines the band size. When you release the mouse button, a band marker is inserted in the area of *maximum pixel intensity*.

For deleting a band marker. Click a band (or bands, by holding down the SHIFT key), and then click the **Delete Band** button or press the **DELETE** key.

To end the drawing mode, click the **Select** button ().

4.2.2 Recalculating the data

After adding or deleting band markers, click the **Recalculate** button to instruct Fragment Analysis to recalculate the data. If you add or delete standards bands and recalculate, the isobar lines are redrawn.

4.2.3 Naming bands

To label a band with a unique name, click the **Band Name** box, and then type a name and click **Set.** The band name appears in the report.

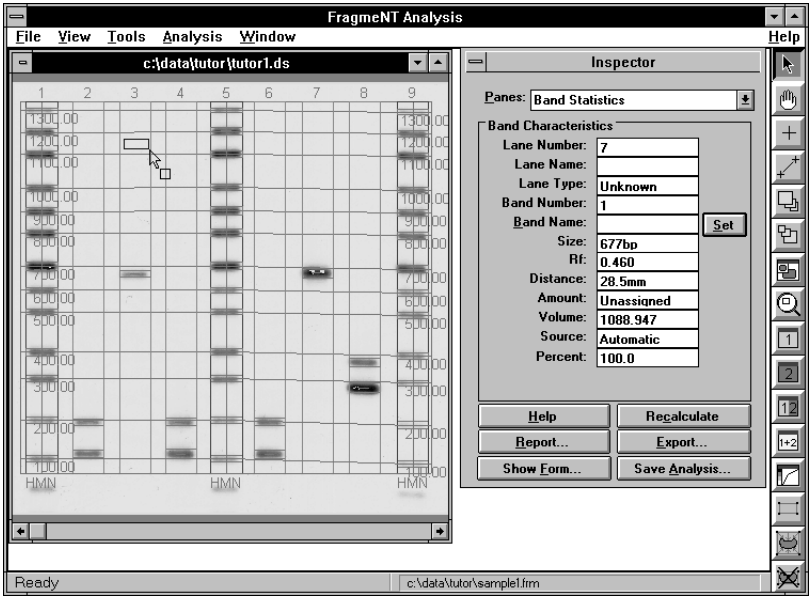


Figure 4-2. Editing bands.

To try it now…

1. Click the **Draw Band (Manual)** button ( ).
2. Move the pointer to an empty spot in any lane of the Image window, and then drag the pointer to draw a rectangle (figure 4-2). When you release the mouse button, a band marker appears.
3. Click **Select** ( ) to exit the draw mode.
4. Select the band marker within the box you just drew, and then click the **Delete Band** button ( ) to remove the band.
5. In the Image window, click any band. Handles appear on the band and statistics for that band appear in the inspector.
6. In the inspector, click the **Band Name** box, type your name, and then click **Set.**
7. Go to section 4.3.

4.3 Creating a report

You can create a report that shows the results of the analysis. Its contents can either be transferred to an Excel worksheet (click the **Report** button), or saved for export to a word processing or database application (click the **Export** button). See appendix A for examples of reports.

4.3.1 Report versus Export

Buttons on the Band Statistics inspector let you choose either Report or Export. (Alternatively, you can select Generate Report or Export Results from the File menu.)

- **Report**—Displays the Report window (figure 4-3) for specifying report contents. After you make your selections and click the **Report** button**,** the Fragment Analysis Report window appears. Double-click in this window to transfer the results to Excel. (Data must be saved from Excel.) See section 6.5 for details.
- **Export—**Displays the Export window for specifying report contents. After you make your selections and click the **Save As** button, the Save Export File window appears. In the **File Name** box, type a name for the report, and then click **OK.** Fragment Analysis adds either a .txt or .csv extension, depending on whether you select tab or comma as the field separator character (delimiter). See section 6.4 for details.

4.3.2 Specifying the report contents

The Report and Export windows allow you to choose among three report formats. To select one of the formats, click one of the buttons. To select the contents for Band Reports (band characteristics) and Lane Reports (lane numbers), click the down arrow ( ) next to the box to see a list of choices, and then select from the list.

- **Band Report**—Shows information about one band characteristic for all lanes. The choice of characteristics is a subset of those listed in the Band Statistics inspector.
- **Lane Report**—Shows all band characteristics for one lane.
- **Table**—Creates a table of all band characteristics for all lanes.

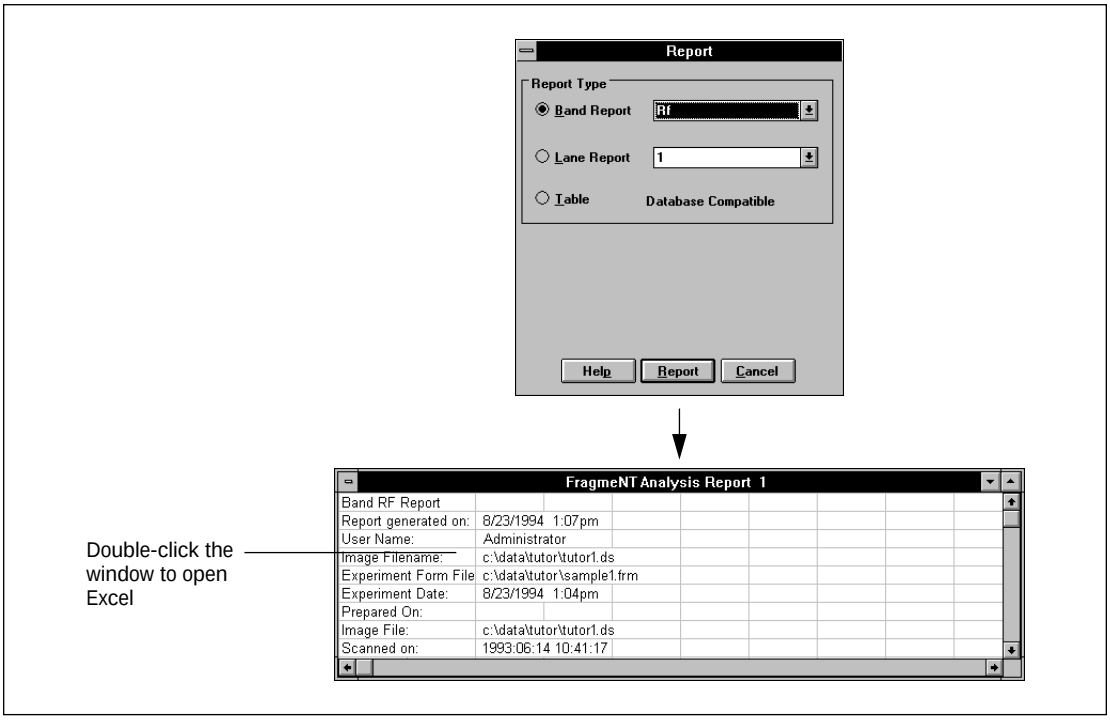


Figure 4-3. Reporting results.

To try it now…

1. In the Band Statistics inspector, click the **Report** button. The Report window appears (figure 4-3).
2. In the Report window, ensure that the **Band Report** button is selected.
3. Click the down arrow ( ) next to the Band Report box. A list of band characteristics appears.
4. Click **Rf.**
5. Click the **Report** button. The Report window closes and a different window, the Fragment Analysis Report window, appears showing the results data.
6. Double-click in the Fragment Analysis Report window. Excel starts with your data in a worksheet (figure 4-4).
7. Go to section 4.4.

4.4 Printing the results

You can print the data report from Excel. After returning to the Fragment Analysis application window, you can also print the image as well as the Experiment Form.

- **Printing the report from Excel**—To print the report from Excel, open the Excel **File** menu, and then select **Print** (figure 4-4). The Print window appears. In the Print window, select the print options (or accept the defaults), and then click **OK.** A message appears confirming that the report is being printed.
- **Printing the image from Fragment Analysis**—To print the image from Fragment Analysis, open the **File** menu and select **Print Image.** The Print Options window appears. To accept the default settings, click **OK.** A second Print window appears. To accept the default settings and print the image, click **OK.**
- **Printing the Experiment Form from Fragment Analysis**—To print the current Experiment Form from Fragment Analysis, open the **File** menu and select **Print Form.** The Experiment Form is printed.

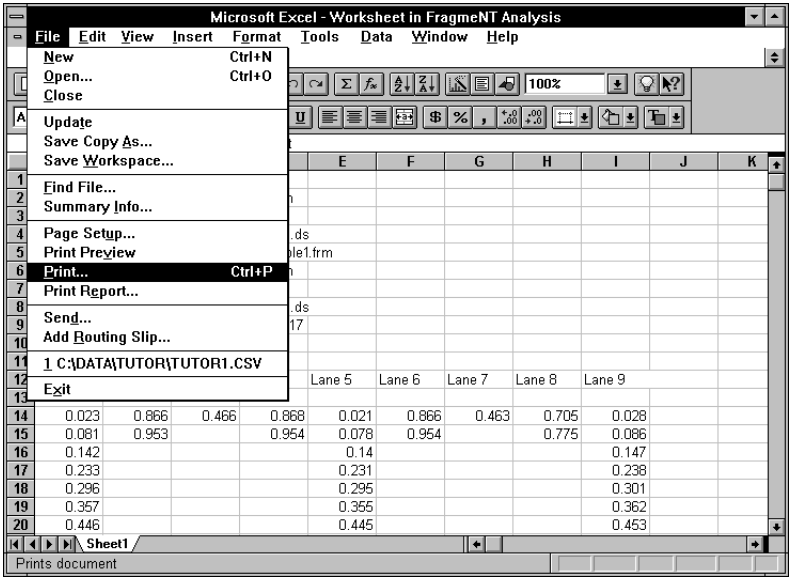


Figure 4-4. Printing the report from Excel.

To try it now...

1. Click **File** in the Excel main window. The File menu appears.
2. Click **Print.** The Print window appears.
3. In the Print window, click **OK.** The window closes and the report is sent to the default printer.
4. Go to section 4.5.

4.5 Exiting from Excel

Before leaving Excel, you might want to save your report. To retrieve a file saved in Excel, use the Open command from the Excel File menu.

4.5.1 Saving your report

To save your Excel worksheet, open the **File** menu and select **Save Copy As.** The Save As window appears (figure 4-5). In the **File Name** box, type a name for the report (Excel adds the .xls extension), and then click **OK.** A Summary Information window appears. Click **OK,** unless you want to add information about the report.

4.5.2 Closing the worksheet

To close the worksheet, double-click the control menu button () of the worksheet.

4.5.3 Exiting Excel

To exit Excel, you can either close the application, reduce it to an icon, or move it to the bottom of the window stack—

- **Close**—To close the application, double-click the control menu button () located on the Excel application window (not the worksheet window).
- **Reduce to an icon**—To reduce Excel to an icon, click the minimize button () near the upper right corner of the Excel window. This action leaves Excel running in the background and speeds processing when you again transfer data from Fragment Analysis to Excel.
- **Hide the Excel worksheet**—To bring Fragment Analysis to the front of the window stack, hold down the **ALT** key and press **TAB** until the Fragment Analysis window appears. Repeat to display Excel.

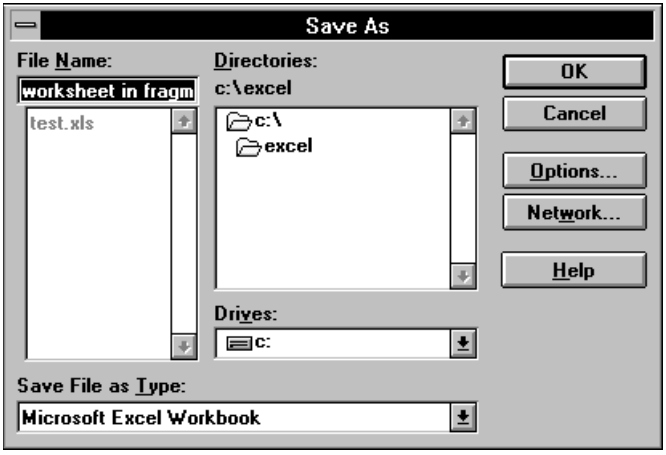


Figure 4-5. Saving the worksheet.

To try it now…

1. Open the Excel **File** menu and select **Save Copy As.** The Save As window appears (figure 4-5).
2. In the **File Name** box, type your name, and then click **OK.** (Excel adds the .xls extension.) A Summary Information window appears. Click **OK.**
3. Double-click the control menu button ( ) of the worksheet window (not the Excel main window). The worksheet closes.
4. On the Excel title bar, click the minimize button ( ). Excel is reduced to an icon below (or behind) the Fragment Analysis window.
5. In the Fragment Analysis window, double-click the control menu button ( ) of the Fragment Analysis Report window. A message asks if you want to activate Excel. Click **No.** The Report window closes.
6. Go to section 4.6.

4.6 Saving your specifications

Before exiting the session, you can update the Experiment Form with the entries you added during the analysis session.

4.6.1 Specifications that are saved

When you save the specifications you entered during the analysis, you are updating the Experiment Form with the following:

- The degree of image rotation
- The size of the largest band (small rectangle)
- The region of interest (large rectangle)
- The type of experiment
- The interpolation methods
- The number of lanes in the sample
- The spacing between lanes (select Use Lane Positions)
- The standards file information
- The location of the standards and unknown lanes
- Band and lane names and descriptions
- The noise and scale factor parameters

Some parameters, such as noise and scale factor, are not displayed on the form. Nevertheless, they are saved.

4.6.2 The save procedure

To save the specifications, click the **Show Form** button in the Band Statistics inspector (or open the **File** menu, click **Experiment Form,** and then click **Show** from the submenu). The current Experiment Form appears. Click the **Save** button to add the new specifications to the form.

If you want to save the lane positions, make sure the **Use Lane Positions** check box (Lane Setup window, figure 4-6) has an X in it.

4.6.3 Creating a new form based on the current form

To save the specifications under a new name (create a new Experiment Form), click the **Save As** button. When the Save As window appears, type a new name in the **File Name** box, and then click **OK.** The new parameters are saved to a new Experiment Form and the original Experiment Form is retained.

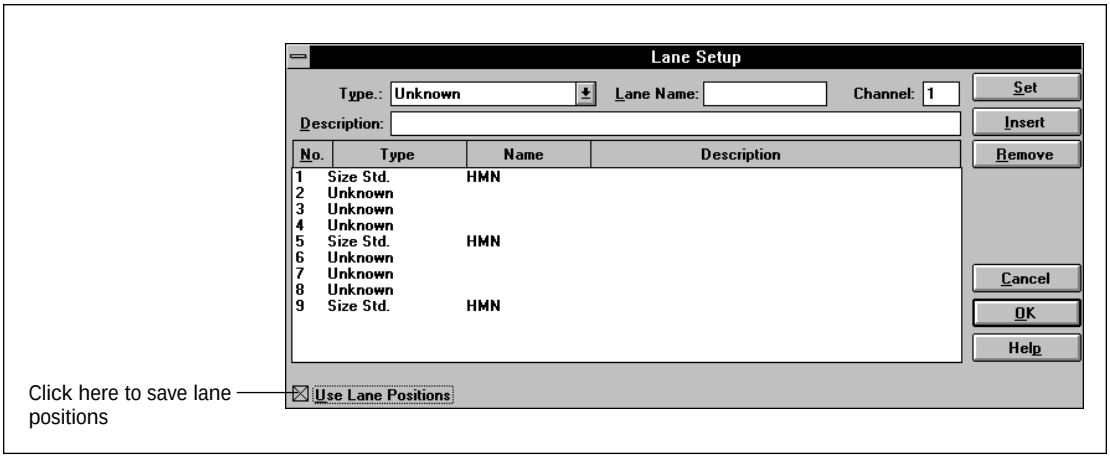


Figure 4-6. Saving your specifications.

To try it now…

1. On the Band Statistics inspector, click the **Show Form** button. The Experiment Form you created appears.
2. Note that the standards file you created appears in the **Standards Filenames** area.
3. In the Experiment Form, click the **Lane Setup** button. The Lane Setup window appears (figure 4-6).
4. In the Lane Setup, click the **Use Lane Positions** check box to save any adjustments you made to the lane positions, and then click **OK.** (An X should appear in the box.) The Lane Setup window closes.
5. In the Experiment Form, click **Save.** Your new specifications are saved.
6. Go to section 4.7.

4.7 Automating the analysis

You can automate all or part of the analysis process. You do this by accessing the Automation Options window from the Experiment Form, and then choosing the steps you want to automate.

4.7.1 Accessing the Automation Options window

To access the Automation Options window, display the Experiment Form window, and then click the **Automation** button. The Automation Options window appears (figure 4-7). After you make your selections in the Automation Options window, click **OK.** Then in the Experiment Form, click **Save** to implement your selections.

4.7.2 Selecting the steps to automate

In the Automation Options window, click the check boxes to indicate which steps you want to automate. You can select any or all the steps. If you select all the steps, Fragment Analysis performs the entire analysis automatically, ending with creating a report file.

If you select the **Export** box, additional options appear. The options allow you to select the report format and contents, the same as those discussed on section 4.3. To make your selections, click the boxes to display menus, beginning with Report Type—

- **Report Type**—Choose among Table Report, Band Report, and Lane Report.
- **Report Options**—The options you see depend on whether you select Band Report or Lane Report. (No options appear for the Table report type, because all information for all lanes and bands is reported.)
- **Delimiter**—Select either Tab Separated or Comma Separated to insert either a tab or comma between the data fields.

Fragment Analysis automatically names the report the same as your form file name, but adds either a .txt or .csv extension, depending on the delimiter option you choose. The file is then available for export to a word processing or database application.

Figure 4-7. Selecting steps to automate.

To try it now…

1. From the Experiment Form, click the **Automation** button. The Automation Options window appears.
2. Click all the boxes in the Automation Options window except *Export* (figure 4-7), and then click **OK.**
3. In the Experiment Form, click **Save,** and then click **Close.** The Experiment Form closes.
4. In the Band Statistics inspector, click in the **Panes** window to display a list of inspectors, and then click **Image Setup.** The Image Setup inspector appears with the rectangles located where you placed them.
5. Click **Done.** Fragment Analysis performs the complete analysis.
6. After the analysis is complete, click the **Show Form** button on the Band Statistics inspector. When the Experiment Form appears, click the **Automation** button to open the Automation Options window. Next, deselect all automation options and click **OK, and** then click **Save** and **Close** in the Experiment Form.
7. Go to section 4.8.

4.8 Saving the experiment analysis

Before exiting Fragment Analysis, you can save the final results of the analysis—just the way it appears at the end of a session. You can then retrieve the results (the objects and the data they represent) for further work.

4.8.1 The save procedure

To save the experiment analysis, click the **Save Analysis** button in the Band Statistics inspector (or open the **File** menu, select **Experiment Analysis** to display a submenu, and then select **Save**). The Save Analysis File window appears (figure 4-8). In the **File Name** box, type a name for the analysis file, and then click **OK.** (Fragment Analysis adds the .exp extension.)

4.8.2 Retrieving the saved experiment analysis

To retrieve the saved experiment analysis, first load the image from which the analysis was saved (section 3.5). Next, open the Fragment Analysis **File** menu and select **Experiment Analysis,** and then select **Open** from the submenu. The Open Analysis File window appears. In the list of file names, locate the analysis file, and then double-click it (or click the file name, and then click **OK**). The saved analysis appears. The objects are displayed on the image and the Image Setup inspector is replaced by the Band Statistics inspector.

You can now reexamine the experiment results and generate additional reports using the procedure described in section 4.3.

If you click **Show Form,** a form named formn.frm is displayed (where n is a unique number assigned by the computer). It shows the experiment parameters associated with the saved analysis. If you save this form, it becomes the new active form.

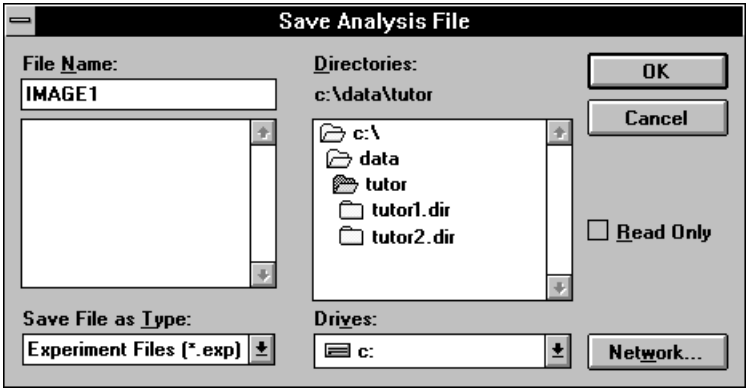


Figure 4-8. Saving the analysis.

To try it now…

1. In the Band Statistics inspector, click the **Save Analysis** button. The Save Analysis File window appears (figure 4-8).
2. In the **File Name** box, type `image1`**,** and then click **OK.** The window closes.
3. Double-click the control menu button ( ) of the Image window. A message asks if you want to save the experiment analysis. Click **No** (you already saved it). Both the Image window and inspector close.
4. From the **File** menu click **Open Image.** The Open Image window appears.
5. Double-click **tutor1.ds.** The Image window and Image Setup inspector appear.
6. In the **File** menu, click **Experiment Analysis,** and then click **Open.** The Open Analysis File window appears.
7. From the list of files in the Open Analysis File window, double-click **IMAGE1.** The objects, such as isobar lines and band markers, appear on the image together with the Band Statistics inspector (figure 4-9).
8. Go to section 4.9.

4.9 Closing the Fragment Analysis software

When you are finished using the Fragment Analysis software, you should close all the open windows and then close the Fragment Analysis software.

4.9.1 Closing the Image window

To close the Image window, double-click the control menu button () on the Image window (figure 4-9). If you did not previously save the experiment, a message appears asking if you want to save the experiment. If you select **Yes,** the Save Analysis File window appears. Type a name for the analysis file, and then click **OK.**

4.9.2 Closing the Report window

If the Fragment Analysis Report window is open, double-click the control menu button () of this window to close it. A message appears, asking if you want to reopen Excel. Click **No,** unless you forgot to save the report and want to do so.

4.9.3 Closing Fragment Analysis

To close the Fragment Analysis software, double-click the control menu button () in the application main menu, or select **Exit** from the **File** menu.

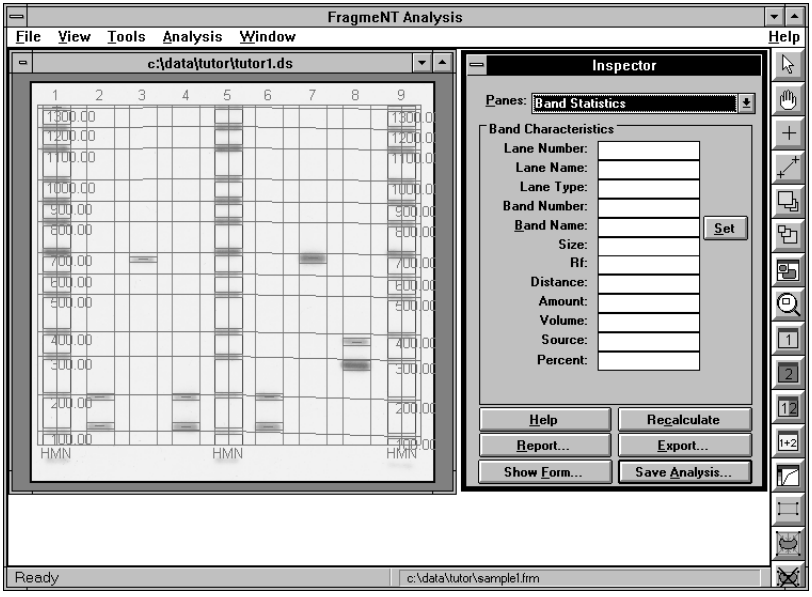


Figure 4-9. Closing the saved analysis.

To try it now…

1. Double-click the control menu button ( ) of the Image window. When a message appears asking if you want to save the experiment analysis, click **No.** Both the Image window and inspector close.
2. Double-click the control menu button ( ) of the Fragment Analysis main menu. The Fragment Analysis window closes.

The tutorial is concluded. For additional information on the features and functions discussed in chapters 3 and 4, see the Reference part of this guide.

Chapter 5 Dual-channel image analysis

The Fragment Analysis software can analyze two-channel (dual-channel) images. Two-channel data collection allows you to increase efficiency and accuracy.

The topics in this chapter are—

- What is a dual-channel image? (section 5.1)
- How to create a dual-channel image (section 5.2)
- Examples of two-channel experiments (section 5.3)
- Setting up for dual-channel image analysis (section 5.4)
- Running a dual-channel image analysis (section 5.5)
- Dual-channel reporting (section 5.6)
- Automating dual-channel image analysis (section 5.7)
- Saving a dual-channel image experiment analysis (section 5.8
- Loading a saved dual-channel image experiment analysis (section 5.9)

5.1 What is a dual-channel image?

A dual-channel image results from the collection of two separate sets of data from one sample. When you load a dual-channel image, the two sets of data are displayed in a side-by-side view (figure 5-1).

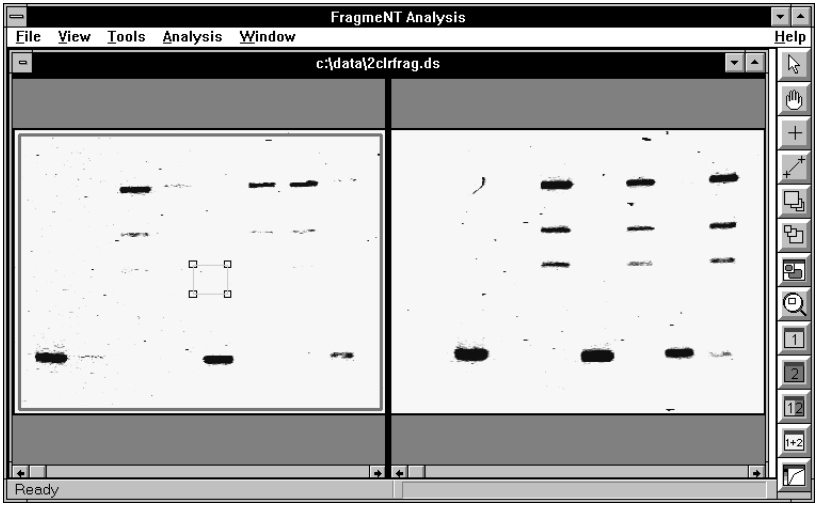


Figure 5-1. A side-by-side, dual-channel image.

Fragment Analysis uses the side-by-side view for analysis. However, you can display the two-channel image separately, as an overlay, or side by side in one Image window. Buttons on the toolbar allow you to change the views:

Channel 1—Only the channel 1 data.

Channel 2—Only the channel 2 data.

Overlay—A composite of the channel 1 and channel 2 data.

Side by Side—Channel 1 and channel 2 data in separate sides of the Image window.

5.2 How to create a dual-channel image

You create a dual-channel image by labeling two sets of sample material (such as two sets of unknowns) with two different fluorescent dyes. The emissions (signals) from the two dyes are of different wavelengths.

The instrument collects data for the two fluorochromes separately, using different filters to collect the emissions of the two fluorochromes (figure 5-2).

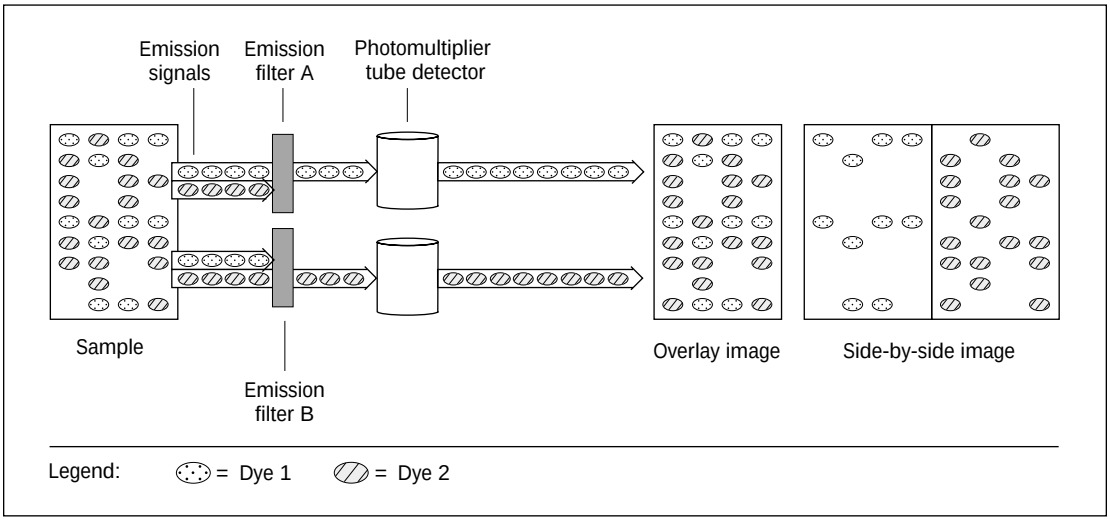


Figure 5-2. Diagram showing two-channel data collection.

Note: Before analyzing a dual-channel image, you must first separate overlapping emission spectra using the FluorSep™ utility.

5.3 Examples of two-channel experiments

Two-channel experiments can be set up to serve a variety of purposes. In particular, two-channel experiments allow you to load two samples in a single lane, thereby increasing your efficiency. You can also ensure accuracy by running standards and unknowns in the same lane, thereby eliminating problems caused by smiling lanes or other gel artifacts.

Figures 5-3 and 5-3 show examples of two-channel experiments.

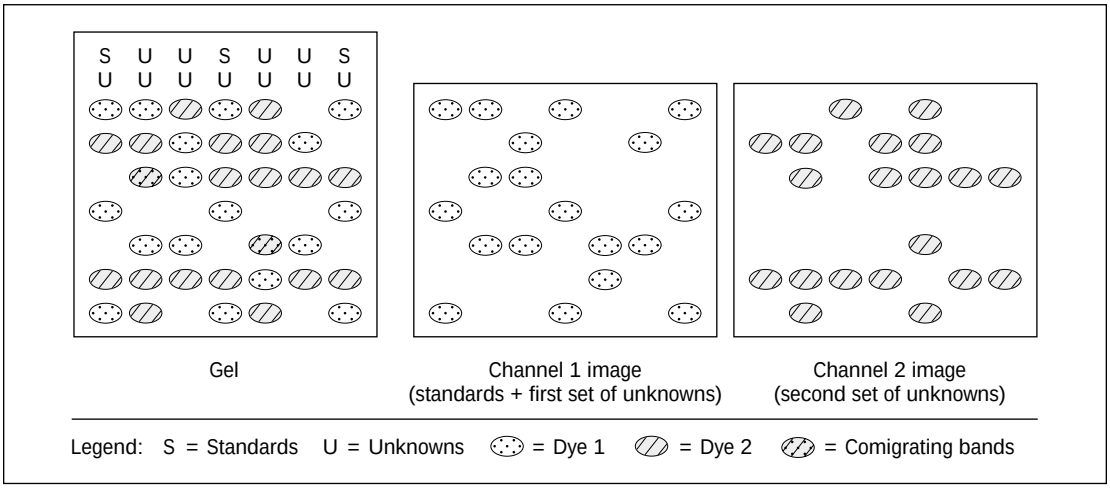


Figure 5-3. Placing two samples per lane—labeled with different dyes—to increase sample throughput. (Lanes 1, 4, and 7 contain standards and unknowns; lanes 2, 3, 5, and 6 contain only unknowns.)

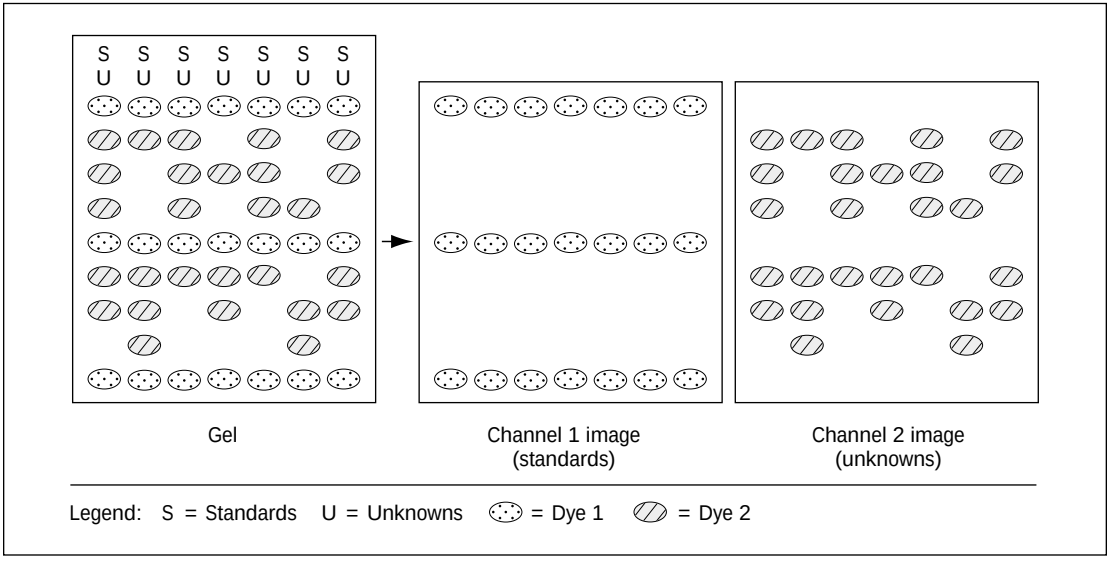


Figure 5-4. Placing standards and unknowns—labeled with different dyes—in the same lanes to ensure accuracy of analysis.

5.4 Setting up for dual-channel image analysis

To prepare for dual-channel image analysis, access the Experiment Form and complete the form twice, once for each channel.

Note: You can practice performing a two-channel analysis using the tutor2.ds image file available in the tutor directory. For both the channel 1 and channel 2 images, use the same lane setup information as you did with the single-channel tutorial. You can load the standards file (sizestd) you created in the single-channel tutorial.

To prepare the Experiment Form—

1. Open the **File** menu and select **Experiment Form,** and then select **New** from the submenu (or select **Open** if you want to create a new form based on an existing form). The Experiment Form appears (figure 5-5).

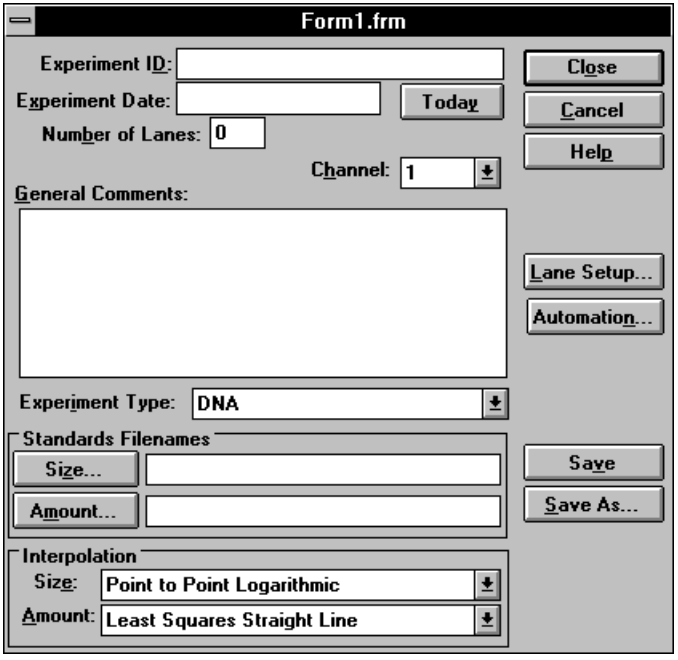


Figure 5-5. Entering parameters for the channel 1 image.

2. Complete the form for the channel 1 data (make sure that a **1** appears in the **Channel** selection box).

   Note: If you want to automate the analysis, select the automation options as part of the set up. See section 5.7 for more information.

3. Click **Lane Setup.** The Lane Setup window appears with the lanes for channel 1 listed in the Lane List box (figure 5-6).

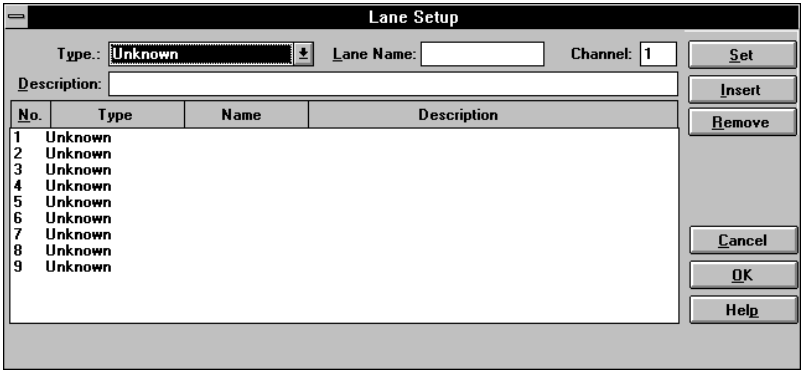


Figure 5-6. The Lane Setup window.

4. Complete the Lane Setup form for your channel 1 data, and then click **OK.** The window closes.
5. In the Experiment Form, click the down-arrow ( ) next to the **Channel** box, and then select **2** from the list.
6. Set the analysis parameters for the channel 2 data (if different from the channel 1 parameters).
7. Click **Lane Setup.** The Lane Setup window appears again with the lanes for channel 2 listed in the Lane List box and a **2** displayed in the **Channel** box.
8. Complete the Lane Setup form for your channel 2 image, and then click **OK.** The window closes.
9. In the Experiment Form window, click **Save** (or click Save As, if you are modifying an existing form). The Save Form File window appears.
10. In the Save Form File window, type a name for your form, and then click **OK.**

Note: For detailed information on completing the Experiment Form, see sections 3.1 through 3.4 and section 6.1.

5.5 Running a dual-channel image analysis

Running a dual-channel image analysis is the same as running two single-channel image analyses in sequence except that you perform the first two steps—Image Setup and Lane Finding—only once. (The parameters you set in those two inspectors affect both images.)

After you prepare the Experiment Form and load a dual-channel image, perform the analysis as follows:

1. With the channel 1 (left) side of the Image window active, perform a complete analysis, as described in chapter 3.

   Note: To activate an image, click in the image area (not on the title bar). The frame around a portion of the window darkens.

2. At the conclusion of the analysis (after you have reached the Band Statistics inspector), **double-click the channel 2 image.** Fragment Analysis returns to the Lane Setup step so that you can continue the analysis of the channel 2 data.

3. Complete the analysis for channel 2.

   Notes:

   If you did not specify a separate standards file for channel 2, Fragment Analysis uses the standards specified for channel 1.

   If, after completing an analysis, you save to the Experiment Form any new region of interest, prototype band size, noise factor, or scale factor parameters that you entered during the analysis session, only the parameters for channel 1 are saved, not for channel 2.

5.6 Dual-channel reporting

The procedure for creating a dual-channel report is the same as that for creating a single-channel report (section 4.3). You create the report after analyzing each image. First, perform the analysis for the channel 1 side of the Image window, and then create your report for that image. Next, perform the analysis for the channel 2 side of the Image window, and then create your report for that image. The channel 2 report is appended to the channel 1 report.

5.7 Automating dual-channel image analysis

You automate dual-channel image analysis by setting the automation options first for the channel 1 image, and then for the channel 2 image as part of setting up a dual-channel experiment.

If you want the analysis to be completely automated (image 1 analysis followed by image 2 analysis), select all automation options for the channel 1 setup, *including Export*, as well as all automation options for the channel 2 setup (Export optional). If you want to omit Export, you will need to double-click image 2 after the image 1 analysis is complete to start the image 2 automated analysis.

5.8 Saving a dual-channel image experiment analysis

To save a dual-channel image experiment analysis—

1. Complete the analysis for both the channel 1 image and the channel 2 image.
2. When you reach the Band Statistics inspector for the channel 2 image, click the **Save Analysis** button (or open the **File** menu, select **Experiment Analysis** to display a submenu, and then select **Save**). The Save Analysis File window appears.
3. In the **File Name** box, type a name for the experiment analysis file, and then click **OK.** (Fragment Analysis adds the .exp extension.)

5.9 Loading a saved dual-channel image experiment analysis

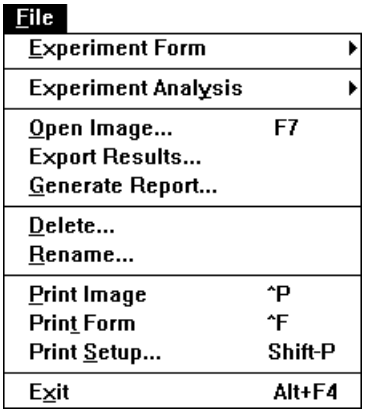
To load a saved experiment analysis—

1. Load the image from which the experiment was saved.
2. Open the Fragment Analysis **File** menu and select **Experiment Analysis,** and then select **Open** from the submenu. The Open Analysis window appears.
3. In the list of file names, locate the experiment analysis file, and then double-click it (or click the file name, and then click **OK**). The saved experiment appears. The objects are displayed on both images and the Image Setup inspector is replaced by the Band Statistics inspector for whichever image is active. (To see the statistics for the other image, double-click the image.)

Part two

Reference

Chapter 6 File menu



The File menu contains commands for accessing the Experiment Form, managing and printing files, generating reports, and exiting Fragment Analysis.

The topics in this chapter are—

- Experiment Form (section 6.1)
- Experiment Analysis (section 6.2)
- Open Image (section 6.3)
- Export Results (section 6.4)
- Generate Report (section 6.5)
- Delete (section 6.6)
- Rename (section 6.7)
- Print Image (section 6.8)
- Print Form (section 6.9)
- Print Setup (section 6.10)
- Exit (section 6.11)

6.1 Experiment Form

The Experiment Form command displays a submenu with the following options:

- **New**—Opens a blank Experiment Form. (The form contains some default settings for running an analysis.) Select this option if you want to create a new Experiment Form. This option is available only if no image is loaded, or when the Image Setup inspector is displayed.
- **Open**—Displays the Open Form File window from which you select an existing form. Select this option to load an Experiment Form, modify a form, or create a new form based on an existing form. To open an existing Experiment Form, locate the form name on the File Name list and double-click the name (or click once, and then click **OK**). This option is available only if no image is loaded, or when the Image Setup inspector is displayed.
- **Show**—Displays the Experiment Form that is currently in effect. This option is available only at the beginning of the analysis session (Image Setup), at the end (Band Statistics), and whenever a form is loaded by itself (no image). If you did not load a form at the beginning of the analysis, the Show command displays a form containing the entries you made during the session. You can then save the form.
- **Close**—Unloads the Experiment Form that is currently in effect. After selecting this option, you can run an analysis without using a form. This option is available only at the beginning of the analysis session (Image Setup), at the end (Band Statistics), and whenever a form is loaded by itself (no image).

6.1.1 About the Experiment Form

Use the Experiment Form (figure 6-1) to set the parameters for running the analysis and to create a record of each experiment. By creating a form, you can partially or fully automate fragment analysis. After you create a form, it is automatically loaded and ready to use for the analysis. The name of the form appears on the right side of the status bar.

The form you load remains in effect until you create a new form, load another existing form, or close the form using the **Close** command in the Experiment Form submenu.

You can create a new form for each analysis—thereby keeping a separate record of each—or you can create just a few forms to use repeatedly for experiments that are similar to one another.

You need not prepare a form to perform an analysis. If you do not use a form, you can enter parameters, such as the number of lanes in the sample and the location of the standards lanes, into each inspector. At the end of an analysis session, you can then choose to save the parameters to create an Experiment Form.

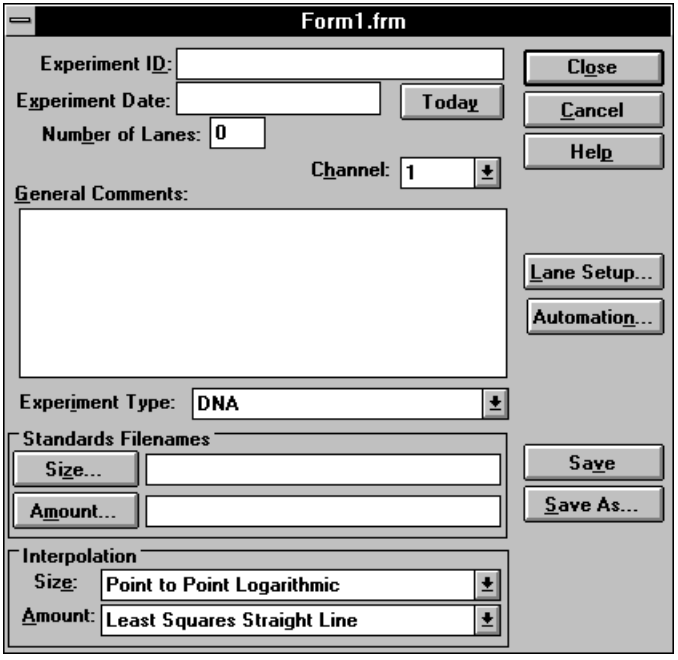


Figure 6-1. The Experiment Form set for a DNA type experiment.

6.1.2 Entering information for your records

Use the following text boxes to create a record of your experiment (optional):

- **Experiment ID**—Type any text to use for identifying your experiment.
- **Date**—Type a date in the box, or click the **Today** button to enter the current date.
- **General Comments**—Type any special notations in this box.

6.1.3 Entering the number of lanes

In the **Number of Lanes** box, type the total number of lanes you will be analyzing. The number you enter here will be transferred to the Lane Setup window.

6.1.4 Selecting 1 or 2 channels

Use the **Channel** box to set up the experiment for a one- or two-channel image (click the down-arrow to see the second selection). For single-channel images, leave the Channel setting at 1. For dual-channel image analysis, first set the Channel selection to 1 and fill out the form (including Lane Setup) for the channel 1 image, and then set the Channel selection to 2 and fill out the form (including Lane Setup) for the channel 2 image. (For more information about dual-channel image analysis, see chapter 5.)

6.1.5 Selecting the experiment type

Select the type of experiment you are running from the **Experiment Type** box. The list displays the following experiment types:

- DNA fragment analysis
- Protein fragment analysis
- Isoelectric focusing

Selecting Isoelectric focusing modifies choices on the Experiment Form. For example, the Size button changes to pKI (figure 6-2).

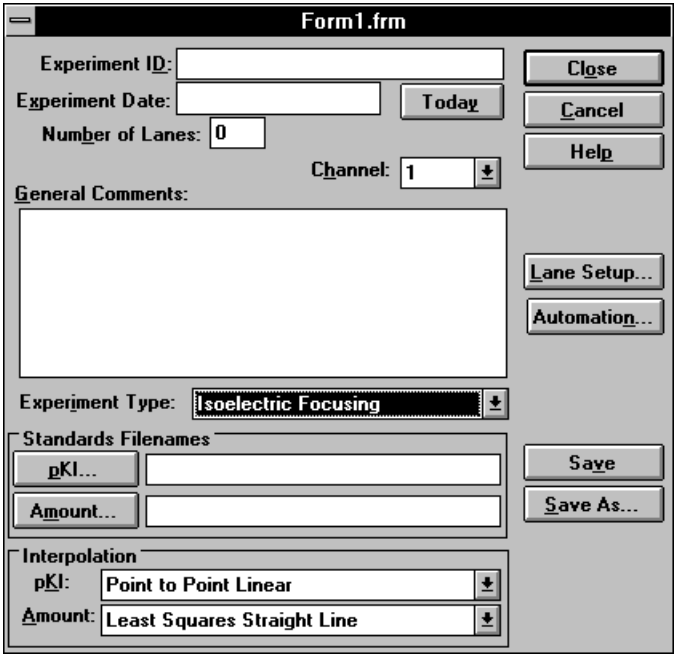


Figure 6-2. The Experiment Form set for an isoelectric focusing type experiment.

6.1.6 Selecting interpolation methods

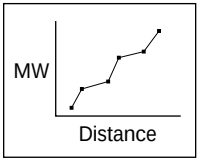
The Interpolation selection boxes allow you to select the curve fit method by which Fragment Analysis determines the size, amount, or isoelectric focusing point of unknown bands. Note that the curve fit choices can vary according to the type of experiment you select.

Before applying the curve fitting calculations, Fragment Analysis mathematically superimposes the bands from the standards lanes onto the unknown lanes. Isobar lines displayed on the image show the calculated location of the standards bands.

Next, Fragment Analysis calculates the curve through the standard points in the lane, using the curve fit method you chose in the Interpolation area. Finally, the software determines the molecular weight values of the unknowns from the standard curve.

You can choose among the following curve fit methods (point to point logarithmic is the preferred method):

Point to point linear



Point to point linear uses the following formula to calculate the sizes or isoelectric points of the bands based on their migration distances:

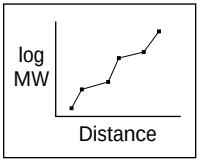
$$\text{distance} = -a \times \text{MW}$$

or

$$\text{distance} = -a \times \text{pI}$$

where a is a constant determined by the slope of the line between two points on the standard curve, MW is the molecular weight, and pI is the isoelectric point. (Distance traveled is proportional to the molecular weight or isoelectric point.)

Point to point logarithmic



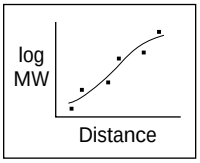
Point to point logarithmic uses the following formula to calculate the sizes of the bands based on their migration distances:

$$\text{distance} = -a \times \log(\text{MW})$$

or

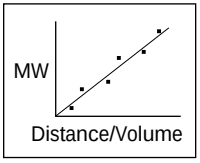
$$\text{distance} = -a \times \log(\text{pI})$$

where a is a constant determined by the slope of the line between two points on the standard curve, MW is the molecular weight, and pI is the isoelectric point. (Distance traveled is proportional to the log of the molecular weight.)



Cubic spline (logarithmic)

Cubic spline (logarithmic) uses regression analysis to plot predicted values of the unknowns with the observed values of the standards. The regression line creates a best fit sigmoidal curve through all the data points, and then calculates the unknown molecular weights based on their migration distances and the log of the standard molecular weights.



Least squares straight line

Least squares straight line uses regression analysis to plot a best fit straight line through all the data points and the origin. Unknown amounts are calculated based on total band volumes and unknown molecular weights are calculated based on migration distances.

For additional information about how Fragment Analysis calculates unknown band values, see appendix B.

6.1.7 Loading the standards file

Before loading a standards file to use for your experiment, you must first indicate the type of file to load:

- **Size standards files**—Contain sizes (molecular weights) of each standard band. Size standards file names include a .siz extension.
- **pKI standards files**—Contain the isoelectric point values (PI) of each standard band. pKI standards file names include a .siz extension.
- **Amount standards files**—Contain the amount of material—in units such as nanograms—in each standard band. Amount standards file names include a .mas extension.

If you load both amount and size standards in a single lane, load both types of standards files.

To select a standards file, click the **Size** (or **pKI**) or **Amount** button. The Open Size/Amount window appears. Double-click the file you want to use (or click the file name, and then click **OK**). The file name appears in the text box.

Alternatively, you can type the name of the file in the text box.

6.1.8 Entering the lane setup information

Click the **Lane Setup** button to display the Lane Setup window (figure 6-3). Use the Lane Setup window to inform Fragment Analysis of the location of standards lanes and to add names and descriptive information for your records.

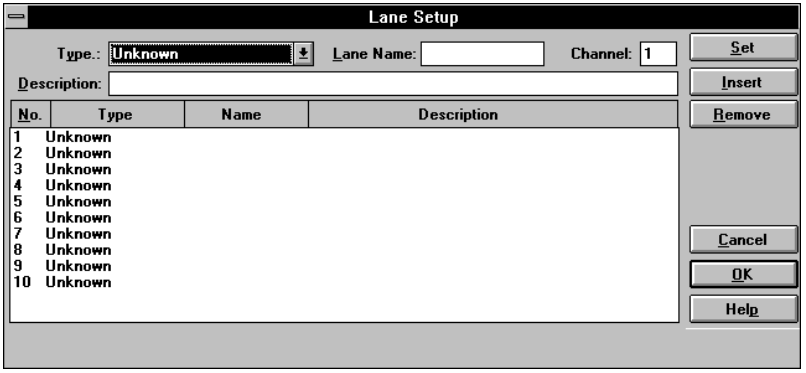


Figure 6-3. The Lane Setup window (available from the Experiment Form).

When the window appears, the lanes are listed in the lane list box (transferred from your entry in the Number of Lanes box on the Experiment Form). Initially, all lanes are typed as Unknowns.

Specifying the location of standards lanes

To change the lane types from unknown to one of the standards selections—

1. Click the down-arrow button ( ) next to the **Type** box, and then select among the choices on the list (figure 6-4).

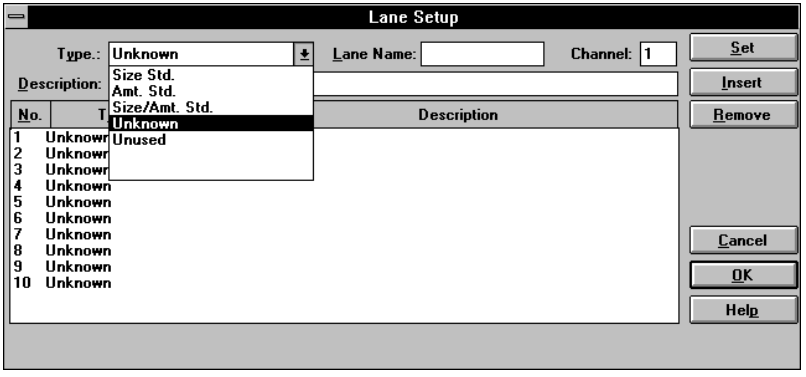


Figure 6-4. Selecting the standards type.

2. In the lane list box, click the lane to which you want to assign the standards type designation. (To select multiple lanes, **CTRL**+click each lane.)
3. Click **Set.** The type designations change from Unknown to the type of standards you selected.

Assigning names and descriptions to lanes

You can assign identifying names to the lanes and/or add descriptive comments regarding the lanes. To do this, first type the name in the **Lane Name** box and the description in the **Description** box.

Next, in the lane list box, click the lane to which you want to assign the name and/or description. (To select multiple lanes, **CTRL**+click each lane.) Finally, click **Set.** The names and descriptions are added to the lane list box.

Editing the lane list

To remove a line from the lane list, click the lane, and then click **Remove.** To add an entry to the list, first make sure that the information about the new lane, such as type and lane name, is displayed in the Lane Setup window. Next, click the lane name below which you want to insert a new lane, and then click **Insert.** Fragment Analysis renumbers the lanes.

Saving the changes to the lane positions

The **Use Lane Positions** check box allows you to save the adjustments you make to the lane positions after Fragment Analysis has marked them. For example, if, during the Lane Finder step, you move one of the lane markers, you can retain the new positions for subsequent analyses. Otherwise, Fragment Analysis recalculates the lane positions each time you perform an analysis.

Note that the Use Lane Positions box appears only after you have completed an analysis session.

6.1.9 Selecting automation options

Click the **Automation** button to obtain a list of automation options (figure 6-5).

Figure 6-5. Automation Options window, no options selected.

How automation works

To automate the analysis, you click the boxes next to the steps you want to automate (an X appears in the box). You can select any or all steps. Fragment Analysis uses the parameters in the currently loaded Experiment Form to automate the analysis. (If you are not certain that the region of interest—indicated on the image by the large rectangle—is appropriate for the image you have loaded, it is best not to automate the Image Setup step.)

About the automation options

Checking the automation options for the inspectors produces the following results:

Image Setup—Sets the region of interest and the prototype band size, implements the Image Setup parameters, rotates the image (if applicable), and displays the Lane Finder inspector.

Lane Finder—Finds lanes, and then displays the Lane Setup inspector.

Lane Setup—Implements the lane setup parameters, and then displays the Standards Setup inspector.

Standards Setup—Implements the standards parameters, and then displays the Band Finder inspector.

Band Finder—Finds bands, and then displays the Band Statistics inspector.

Exporting the results automatically

Selecting the Export option instructs Fragment Analysis to automatically create a report file containing the results of the analysis. The file, which you can export into a word processing or database application, is automatically assigned the same name as the Experiment Form (but with a different extension). For example, if your form name is sample1.frm, your report file will be sample1.txt (or sample1.csv). If you have previously used an Experiment Form to export a report automatically, a message will appear asking whether you want to overwrite the previously created report.

When you click the Export box, new items appear in the Automation Options window, giving you the opportunity to select the report type, report contents, and field delimiter type from selection boxes (figure 6-6).

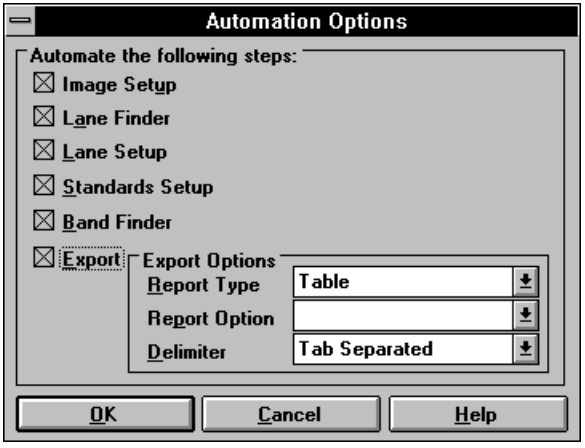


Figure 6-6. The Automation Options window, all options selected.

To automate exporting—

1. Click the **Report Type** box. A list of report options appears:
   - **Band Report**—Shows information about one band characteristic for all lanes. The characteristics options, available from the Report Option menu, are a subset of those listed in the Band Statistics inspector.
   - **Lane Report**—Shows all band characteristics for one lane.
   - **Table**—Creates a table of all band characteristics for all lanes. (No Report Option is available for Table reports because all data is reported.)

2. Click the **Report Option** box, and then choose the report contents:
   - For a Band Report type, select the band characteristic, such as Size or Rf, for which you want a report.
   - For a Lane Report type, select the lane for which you want a report.
3. Click the **Delimiter** box, and then select one of two options:
   - **Tab Separated**—Inserts a tab between fields and creates a file with a .txt extension.
   - **Comma Separated**—Inserts a comma between fields and creates a file with a .csv extension.

6.1.10 Saving the Experiment Form

To save to the specifications that you entered directly or that were automatically entered during an analysis session, click the **Save** button. The Save Form File window appears (figure 6-7).

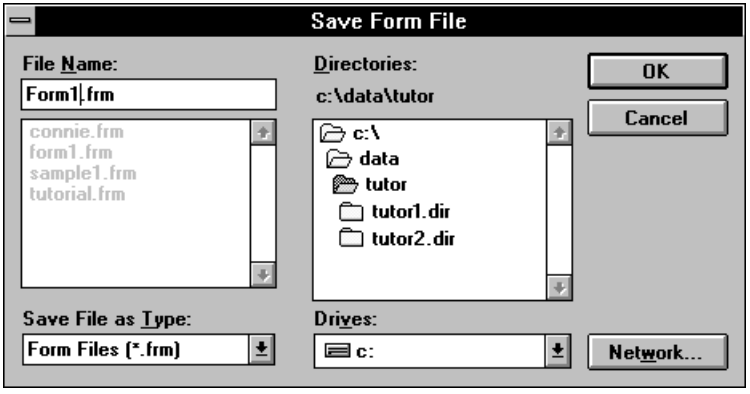


Figure 6-7. Saving an Experiment Form.

Type a name for the form in the **File Name** box, and then click **OK.** (Fragment Analysis adds the .frm extension.) You can retrieve the form by selecting **Experiment Form** from the **File** menu, and then clicking **Open** from the Experiment Form submenu.

Note: Do not use the following characters: .”^:<>+=;’ and space. You can use the underline character.

6.1.11 Saving the form to a new name

The Save As button allows you to change an existing form file and save it to a new name.

After modifying an existing form, click **Save As.** The Save Form File window appears. In the **File Name** box, type a name for the form, and then click **OK.** The original form is retained with its original file name. The modified version of the form is given the new name. (Fragment Analysis adds the .FRM extension.)

6.1.12 Closing the window

Select **Close** to close the Experiment Form window. If you have changed the form, Fragment Analysis will ask if you want to save the changes before closing the window.

6.2 Experiment Analysis

The Experiment Analysis command allows you to save and retrieve the analysis, including the objects (band markers, lane markers, and so forth), and the calculated data.

Clicking Experiment Analysis displays a submenu with two options:

- **Open**—Displays the Open Analysis File window. To retrieve an analysis file, locate the file in the list of files, and then double-click the file name (or click the file name, and then click **OK**). The objects appear in the Image window in the same state as when you saved the experiment. The Band Statistics inspector also appears.
- **Save**—Displays the Save Analysis File window. To save the analysis, type a name in the **File Name** box, and then click **OK.** Fragment Analysis adds the .exp extension.

You save an analysis when the session is complete (the Band Statistics inspector is displayed). You can initiate the Save command from either the Experiment Analysis submenu or by selecting **Save Analysis** from the Band Statistics inspector.

You retrieve (open) a saved experiment after loading the image from which the experiment was saved.

6.3 Open Image

The Open Image command displays the Open Image window (figure 6-8).

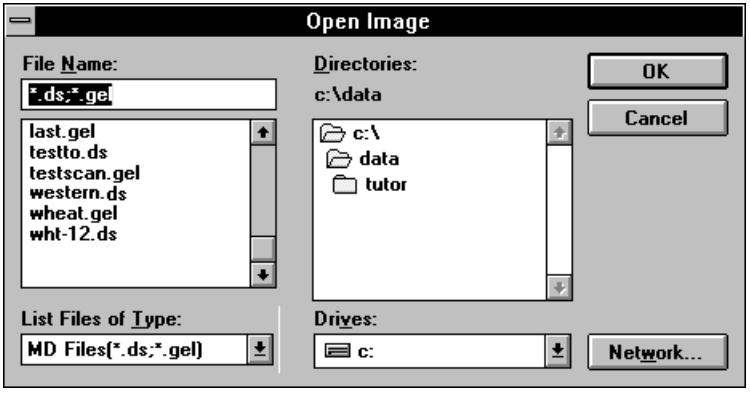


Figure 6-8. The Open Image window.

To open an image file, locate the file name among the list of files and double-click the name (or click the file name, and then click **OK**).

6.3.1 Locating the file you want

When you open the window, the File Name box initially displays *.ds;*.gel, indicating that all the files listed are image datasets or files. Below the File Name box is a list of existing .ds and .gel files.

If the file name is not listed, check that—

- The list represents the correct file type (section 6.3.2)
- The directory is correct (section 6.3.3)
- The drive is correct (section 6.3.4)
- The file is located in a shared directory on another workstation (section 6.3.5).

You can use the File Name box to type the name of the file you want to display. If the file you want is stored in a different directory than the one displayed, you must include the full path. For example:

```
c:\data\dna.ds
```

6.3.2 Selecting a file type

The List Files of Type selection box displays the type of files currently listed in the File Name box. The default setting is for .ds and .gel file types. To change file types, click the **List Files of Type** box to display a menu of file types, and then click one of the options. A new list of files appears in the file list box.

6.3.3 Changing the directory

The Directories area displays the current directory path. The general directory structure is as follows:

Double-click the root directory (c:\) to display a list of directories. After you locate the correct directory (in most cases, the data directory), double-click it. Its contents appear in the files list box.

Note: The .dir folders listed under the data directory, identify the special directories that hold the image datasets and associated files. Do not open a .dir folder to access your dataset. Your dataset is available to you by opening the data directory and selecting the correct .ds file. If you should accidentally delete the .ds file, you can obtain a copy of it in the .dir folder that has the same name as the dataset. (Copy the .ds file to the data directory.)

6.3.4 Changing drives

The **Drives** box displays the current drive. To change drives, click the **Drives** box to display the list of drives, and then click the option you want. The list of files stored on that drive appears in the files list box.

6.3.5 Connecting to shared directories

The **Network** button displays the Connect Network Drive window. Use this window for connecting to shared directories on the network. For detailed instructions on using this window, click the **Help** button located in the window.

6.4 Export Results

The Export Results command displays the Export window (figure 6-9). Use this window to specify the contents and format of a report and save it for export to a word processing or database application. Reports you create contain the results of an analysis session.

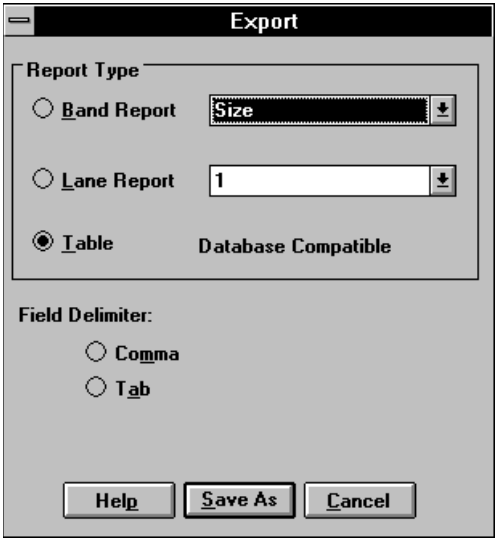


Figure 6-9. The Export window.

After selecting the format and contents (sections 6.4.1 and 6.4.2), click **Save As** to open the Save Export File window.

6.4.1 Selecting the format and contents

You can choose among three report formats. To select one of the formats, click the appropriate button.

- **Band Report**—Shows information about a selected band characteristic, such as size, amount, or distance, for all lanes. To select a particular characteristic, click the down arrow next to the Band Report box. A list of characteristics appears. Click the characteristic for which you want a report.
- **Lane Report**—Shows all band characteristics for one lane. To select a lane, click the down arrow next to the Lane Report box. A list of lanes appears. Click the lane for which you want a report.
- **Table**—Creates a table of all band characteristics for all lanes.

6.4.2 Selecting the field delimiter

The Field Delimiter selection buttons allow you to choose either tabs or commas as the separator characters between fields in the report.

- **Comma**—Inserts a comma between field entries in the report and creates a Comma Separated Values (.csv) file type.
- **Tab**—Inserts a tab between field entries in the report and creates a Tab Separated Values (.txt) file type.

  Note: Use Tab for export to Excel.

6.4.3 Naming the report

After you click the **Save As** button in the Export window, the Save Export File window appears (figure 6-10) with either *.txt or *.csv displayed in the File Name box, depending on the delimiter you selected.

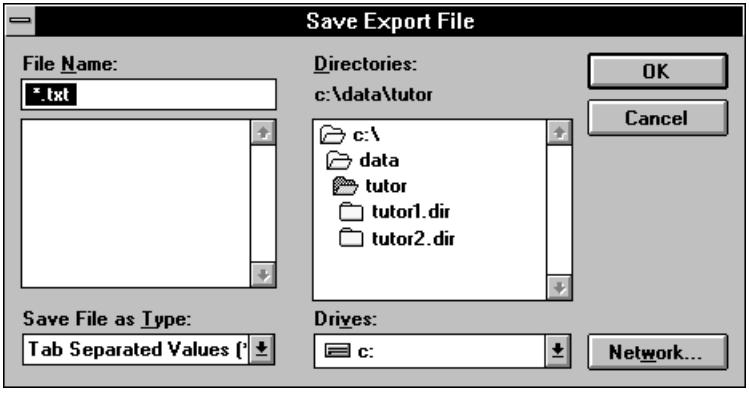


Figure 6-10. Naming the report.

In the **File Name** box, type a name for the report file. Fragment Analysis automatically adds the .csv or .txt extension, depending on the field delimiter you select. Click **OK.** Your file is saved.

6.5 Generate Report

The Generate Report command displays the Report window (figure 6-11). Use this window to specify the contents and format of a report that you can transfer to an Excel worksheet. Reports you create contain the results of an analysis session.

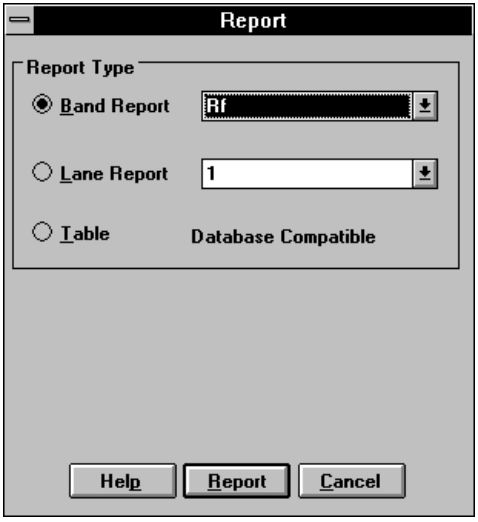


Figure 6-11. The Report window.

6.5.1 Selecting the format and content

You can choose among three report formats. To select one of the formats, click the appropriate button.

- **Band Report**—Shows information about a selected band characteristic, such as size, amount, or distance, for all lanes. To select a particular characteristic, click the down arrow next to the Band Report box. A list of characteristics appears. Click the characteristic for which you want a report.
- **Lane Report**—Shows all band characteristics for one lane. To select a lane, click the down arrow next to the Lane Report box. A list of lanes appears. Click the lane for which you want a report.
- **Table**—Creates a table of all band characteristics for all lanes.

After making your selection, click on the **Report** button. The Report window closes and the Fragment Analysis Report window appears, showing your analysis data in tabular form. (You cannot save or print from this window.)

6.5.2 Transferring the results to Excel

To transfer the results to Excel, double-click the Fragment Analysis Report window. Excel starts and your report data appears in a worksheet. (For information about saving and printing the report from within Excel, see sections 4.3 through 4.5.)

6.6 Delete

The Delete command displays the Delete window. The delete window is nearly identical to the Open Image window (section 6.3). To delete a file (or dataset), locate the file name and click it. The name appears in the File Name box. Click **OK.** A message asks you to confirm the deletion.

The Fragment Analysis file type extensions are:

- **.gel**—Single image file.
- **.ds**—Image dataset file. A .ds file is actually a "pointer" file. Selecting a .ds file instructs Fragment Analysis to retrieve the image and associated files from the special directory (.dir directory) in which they are stored.
- **.frm**—Experiment form file.
- **.exp**—Analyzed image file.
- **.siz**—Size standards file or pKI standards file.
- **.mas**—Mass (amount) standards file.
- **.txt**—Tab-separated report file.
- **.csv**—Comma-separated report file.

Note: Always use the Fragment Analysis Delete function to delete image files. You cannot delete an Experiment Form file that is currently loaded. (Select the **Close** option from the **Experiment Form** submenu.)

6.7 Rename

The Rename command displays the Rename window (figure 6-12).

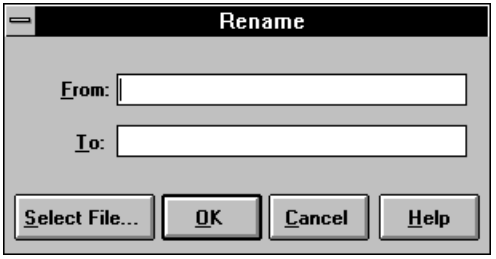


Figure 6-12. The Rename window.

To rename a file—

1. Type the name of the file in the **From** box. (Include the path if the file is located in a directory or drive that is different from the current directory or drive.)

   Alternatively, you can click the **Select File** button to display the Select Source File window, and then select a file just as you do when selecting a file from the Open Image window (section 6.3). The file name, including the path, is automatically inserted in the From box.

2. In the **To** box, type the new name for the file. (Include the path if you want to store it in a different drive or directory.)

3. Click **OK.**

To use an existing file name as the base for a new name, click the **To** box, and then click **Select File.** The Select New Name window appears. Click an existing file name, and then change the name in the File Name box. (An error message appears if you try to save the file using the same name.)

Note: You cannot rename an Experiment Form file that is currently loaded. (Select the **Close** option from the **Experiment Form** submenu.)

6.8 Print Image

The Print Image command displays a Print Options window for printing the image (figure 6-13). Select centering and scaling options, and then click **OK.** A second window appears in which you specify the pages to print and the number of copies.

To change from portrait to landscape orientation or to change the size of the paper, click **Setup.** The Print Setup window appears. The choices in this window are printer dependent, but at a minimum allow you to determine the page orientation. See your printer documentation for more details.

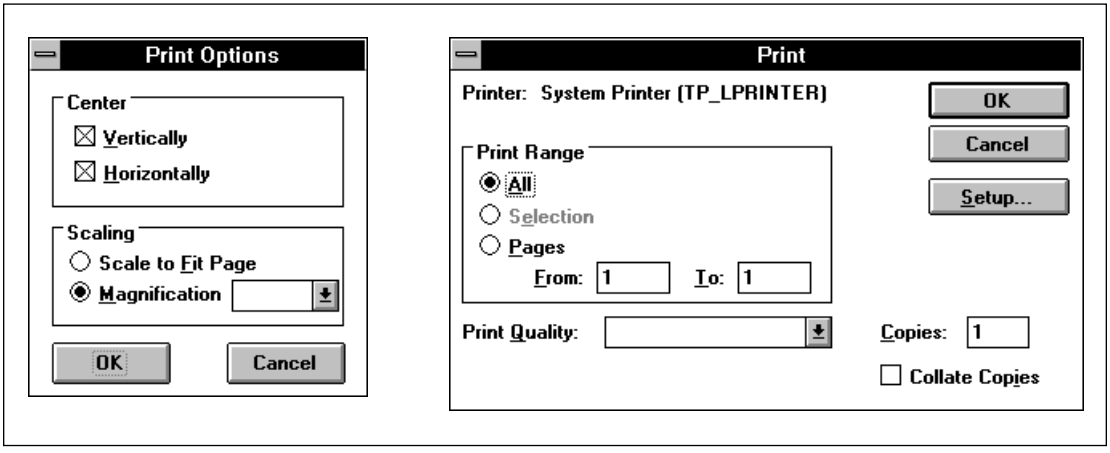


Figure 6-13. The Print Options and Print windows.

6.9 Print Form

The Print Form command allows you to print the Experiment Form. To print the Experiment Form, select the **Print Form** command. The current form is printed.

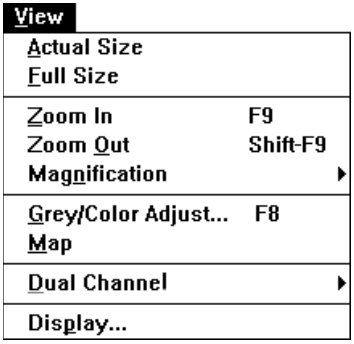
6.10 Print Setup

The Print Setup command displays the Print Setup window. You can also access this window by clicking the Setup button in the Print window. Use this window to set preferences for print orientation and two-sided printing. The exact contents of this window are printer dependent. See your printer documentation for more detail.

6.11 Exit

The Exit command closes Fragment Analysis. You will be prompted to save any open files.

Chapter 7 View menu



The View menu includes commands for modifying the image display. In this chapter, View menu options that are also available from the toolbar are identified with the appropriate toolbar icon.

The topics in this chapter are—

- Actual Size (section 7.1)
- Full Size (section 7.2)
- Zoom In (section 7.3)
- Zoom Out (section 7.4)
- Magnification (section 7.5)
- Grey/Color Adjust (section 7.6)
- Map (section 7.7)
- Dual Channel (section 7.8)
- Display (section 7.9)

7.1 Actual Size

The Actual Size command displays the image at 100%, which is the actual size of the sample. To display the image at the actual size of the sample, click the **Actual Size** command.

Note: The 100% actual size applies to 17-inch monitors with 1024 by 768 pixel array, or 13-inch monitors with 640 by 480 pixel array. For monitors that vary from these specifications, the display may vary slightly from the actual sample size.

7.2 Full Size

The Full Size command scales the image to fit within the Image window boundaries. To display the full image, click the **Full Size** command.

7.3 Zoom In

The Zoom In command doubles the size of the image. The center point of the Image window is maintained. To enlarge the image, click the **Zoom In** command.

7.4 Zoom Out

The Zoom Out command halves the size of the image. To reduce the image, click the **Zoom Out** command.

7.5 Magnification

The Magnification command displays a menu of magnification percentages. To magnify or reduce the image, click one of the percentages listed in the menu.

7.6 Grey/Color Adjust

The Grey/Color Adjust command displays the Grey/Color Adjust window (figure 7-1). (To close the window, click the Close button.) This window graphically reflects the intensity of the image displayed in the Image window.

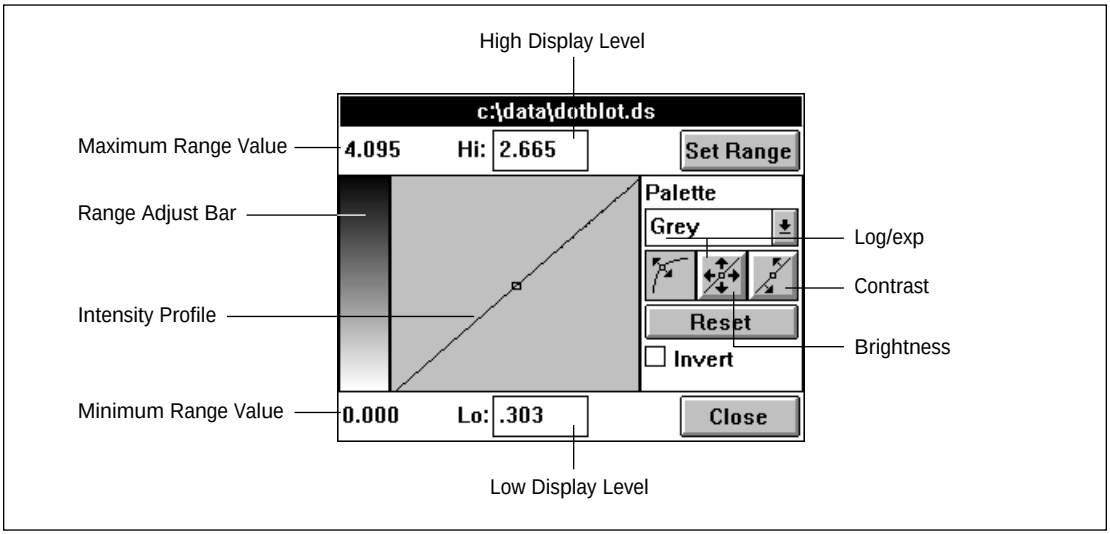


Figure 7-1. The Grey/Color Adjust window.

7.6.1 What the Grey/Color Adjust window does

Your monitor can display 256 levels of grey, but an image file can contain up to 46,700 levels of intensity. The Grey/Color Adjust window allows you to view your data more clearly by excluding some of the unused intensity levels (background and unused high values) of your image. In this way, you evenly distribute the relevant image data over the 256 levels of grey.

You can also use the Grey/Color Adjust window to change the contrast, brightness, and color of the image.

Note: Changing the image display using the Grey/Color Adjust window affects only the display. The original image and quantitation results are not altered.

7.6.2 Changing the display levels

Because the range of intensity levels in the image may not extend over the complete range of possible values, you set limits to the upper and lower display levels to eliminate unused values. In this way, the 256 possible grey levels are spread over a narrower range of intensity values.

The Grey/Color Adjust window provides an interactive mechanism, the *range adjust bar*, for setting display limits. As you use the bar to change the display limits, color is added to the bar and the image display. Green shows the pixels that will be assigned the intensity value shown in the Hi box; purple shows the pixels that will be assigned the intensity value shown in the Lo box (background).

Using the range adjust bar (single-channel images)

You can use the range adjust bar to change the image display limits of a single-channel image—

- **To change the high display limit**—Place the pointer at the top of the range adjust bar and drag the pointer down (figure 7-2). When the bands begin to turn green (indicating high intensity areas), move the pointer up slightly until the bands are again black, and then release the mouse button.
- **To change the low display level**—Place the pointer at the bottom of the range adjust bar and drag the pointer up. When the background is purple (indicating low intensity areas), but the bands are still unaffected, release the mouse button.
- **To change both levels together**—Place the pointer in the middle of the range adjust bar, hold down the SHIFT key, and drag the pointer up and down.

To save the setting, click **Set Range.** The image redisplays with the new settings and the intensity profile graph in the Grey/Color Adjust window changes to reflect the settings.

To restore the image to its original display limit values, click the **Reset** button. You can only reset the display limits if you have not clicked Set Range. To expand the display limits after you click Set Range, type new values in the **Hi** and **Lo** boxes.

Note: For instructions on changing the display values for dual-channel images, see section 7.6.6.

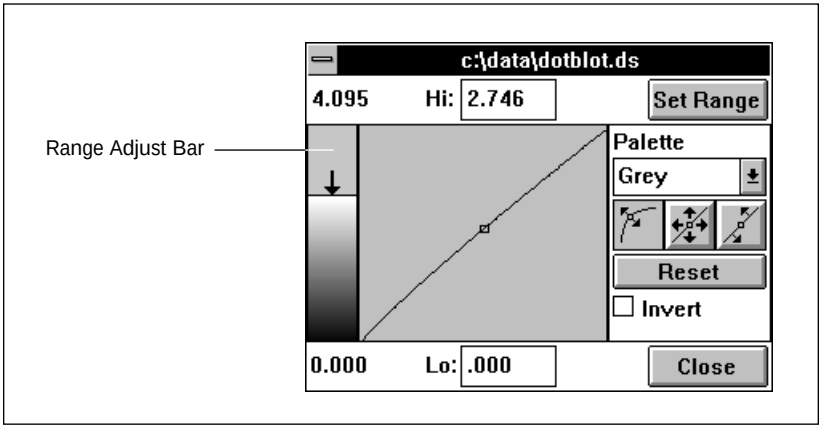


Figure 7-2. Adjusting the upper range.

Setting range values beyond those displayed

To set the display limit values above or below those displayed in the **Hi** and/or **Lo** boxes (or to set display levels for dual-channel images), delete the current value in one or both of the boxes, type the new value, and then click **Set Range.** If you are setting levels for dual-channel images, click the side of the dual-channel Image window you want to change to activate it before changing the limit settings. (For additional information about working with dual-channel images, see section 7.6.6.)

7.6.3 Adjusting brightness and contrast

Three buttons allow you to adjust brightness and contrast. Adjustments you make with the buttons are temporary. They are not saved with the image and they do not affect the data.

To adjust brightness and contrast, click one of the buttons described below, and then move the pointer to the intensity profile area, hold down the mouse button and move the line.

Adjusts brightness. Move the line up to increase the brightness (pixel intensity), and down to decrease the brightness.

Repositioning the square (bend point) on the intensity profile changes the center of rotation. Use the brightness button to move the square, and then use the contrast button to change the contrast.

Adjusts contrast. Moving the line closer to vertical increases the contrast.

Adjusts brightness and contrast together. Moving the pointer up and to the left increases the contrast at the lower range of intensity values and decreases the contrast at the upper range of intensity values (logarithmic display). Moving the pointer down and to the right increases the contrast at the higher intensity values and decreases the contrast at the lower intensity values (exponential display).

Click **Reset** to return to the original display values.

7.6.4 Adding color to the displayed image

To add color to the displayed image, click the **Palette** box. A list of color choices appears. Click one of the color choices.

7.6.5 Inverting the current values

To invert the current display values, click the **Invert** check box. The intensity profile and the displayed pixel values invert. Click the **Invert** check box again to turn Invert off.

7.6.6 Working with dual-channel images

If you are viewing a dual-channel image, the Grey/Color Adjust window operates as follows:

Active and nonactive sides of the Image window

The display levels reflect the active side of the window. To activate one side of the window, click the image. The title bar of the Grey/Color Adjust window displays the number of the active window (1 or 2).

The range adjust bar

The range adjust bar does not operate in dual-channel mode. To change display levels, type values in the **Hi** and **Lo** boxes.

Brightness and contrast controls

The brightness and contrast controls work independently for each window. For the channel 1 image, use the **right** mouse button to operate the brightness and contrast controls. For the channel 2 image, use the **left** mouse button.

Side-by-Side (Two Color) mode

In the Side-by-Side (Two Color) mode—

- The color palette changes to two-color schemes, such as red/green. You select the combination you want from the list in the Palette area.
- Two lines appear on the intensity profile. The line colors correspond to the channel colors.
- Each color in a two-color scheme is displayed in 15 gradations. You can increase the number of gradations in one of the images by decreasing the number in the other image. (The minimum and maximum numbers are 2 and 118, respectively.)

  For example, instead of viewing both images in 15 gradations, you can view one image in 3 and the other in 78 gradations. To do this, move the pointer into the range adjust bar of the Grey/Color Adjust window and click the **right** mouse button. A pop-up menu appears displaying the gradation options. Click one of the options using the **left** mouse button.

For information on the choices of dual-channel display modes and how they work, see section 7.8.

7.7 Map

The Map command displays a small version of the Image window, including an outline that shows the portion of the image currently displayed (figure 7-3). It also allows you to change the displayed area.

To reposition the image display, place the pointer on the interior outline and drag the outline to a new location. The main image display moves to reflect the new outline location.

To move the Map window, move the pointer into the Map window, and then **SHIFT**+drag the window to a new location.

To return the Map window to the upper left corner of the Image window, position the pointer in the Map window and click the **right** mouse button. Select **Reset** from the pop-up menu.

To close the Map window, click the **Map** command or the Map toolbar button.

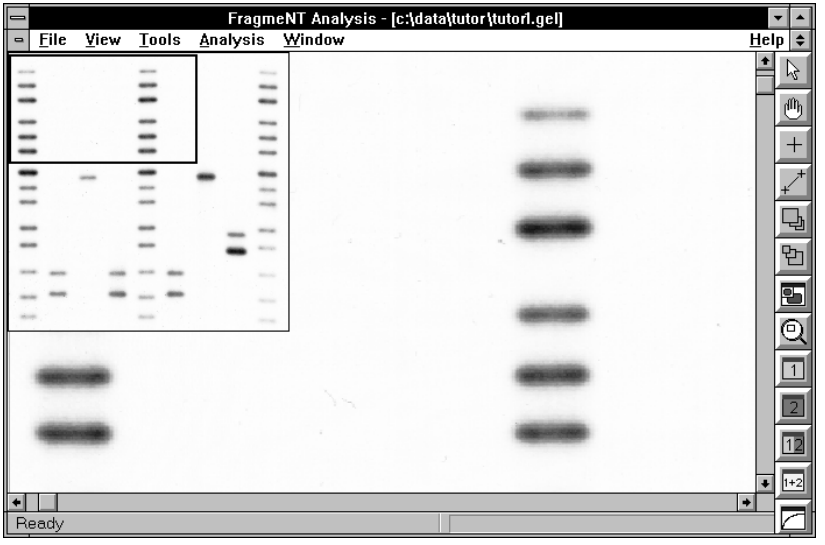


Figure 7-3. The Map window.

7.8 Dual Channel

The Dual Channel command opens a menu of display controls for dual-channel images.

The Dual Channel menu provides the following commands:

Overlay—Displays a composite image of two separate images. Each filter is represented by a different color. Areas where both filters produced a signal are shown in a third color. For example, when red and green overlap, yellow is produced. In this way, you can differentiate between isolated sample signals and merged sample signals, such as comigrating electrophoresis bands.

Side by Side (Two Color)—Displays each channel (separated image) in a separate side of the Image window. The color representing each channel is displayed in its respective side.

Channel 1—Displays, in grey scale, the image created by channel 1.

Channel 2—Displays, in grey scale, the image created by channel 2.

Side by Side (Grey/Color)—Displays each channel (separated image) in a separate side of the Image window, using the same color scheme for both images. This command is not available from the toolbar.

Synchronize—Links two images together so that you can scroll, magnify, enlarge, and reduce both images simultaneously. This command is not available from the toolbar.

7.9 Display

The Display command displays the Display window (figure 7-4). Use this window to hide and show objects, such as lane markers, band position markers, and the graph profile, and to change their colors.

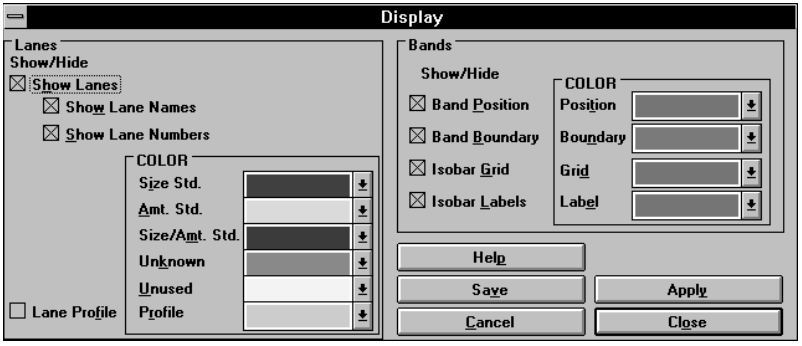


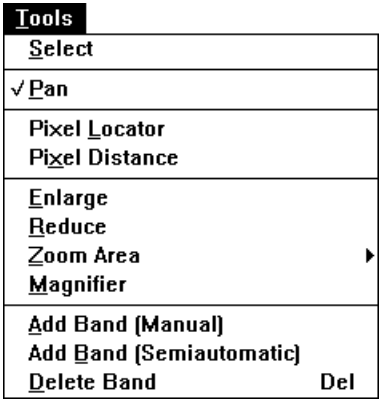
Figure 7-4. The Display window.

To show or hide an object listed in the window, click its check box (an X in the box denotes show). To select a color for the object, click its associated color box to display a menu of colors, and then click the color of your choice.

The buttons on the bottom of the screen work as follows:

- **Save**—Retains the choices and closes the window.
- **Cancel**—Closes the window without applying the new selections.
- **Apply**—Applies the changes without closing the window, but does not save the changes.
- **Close**—Closes the window.

Chapter 8 Tools menu



The Tools menu includes commands for careful examination of the image. Tools menu selections change the function of the pointer from the select mode to a mode for performing a specific task. For example, in the Magnifier mode, the pointer becomes a magnifying glass. The pointer remains in that mode until you select another function or the Select option.

Note: Tools options are also available from a **pop-up menu.** To access the pop-up menu, place the pointer in the Image window and click the **right** mouse button, and then use the **left** mouse button to select a command.

In this chapter, if a Tools option is available from the **toolbar,** the appropriate toolbar icon is also shown.

The topics in this chapter are—

8.1 Select

The Select command returns the pointer from an editing or special viewing mode to the select mode. In Select mode, you can select one or more objects on the image (such as rectangles). To select one object, click the object. To select a group of objects, place the pointer in the image area next to one of the objects and drag the pointer diagonally to surround the group with a rectangle.

8.2 Pan

The Pan command enables scrolling in any direction. To use the Pan tool, select the **Pan** command from the menu or from the toolbar. Place the pointer on the image. The pointer changes to a hand. Hold down the mouse button and move the hand to drag the image up, down, left, or right. Click **Select** from the menu or toolbar () to end the Pan mode.

8.3 Pixel Locator

The Pixel Locator command allows you to determine pixel locations and intensity values. To determine pixel locations, select **Pixel Locator** from the menu or toolbar. The pointer turns into a cross. Place the pointer over the image and click the mouse button. A cross appears on the image and pixel information appears on the left side of the status bar. When you release the mouse button, the information shifts to the right side of the status bar. The locator mark remains on the image until you return to the Select mode.

The information displayed includes the x and y location of the selected pixel (P) and the intensity value of the pixel. (To see the raw pixel value—prior to conversion to the units used by the software—hold down the **CTRL** key while clicking.)

If you mark the image with both the Pixel Locator and the Pixel Distance tools, you can switch the information on the status bar from one type to the other by clicking the Pixel Locator or Pixel Distance toolbar button.

8.4 Pixel Distance

The Pixel Distance command allows you to measure the distance between pixels. To measure distance, select **Pixel Distance** from the menu or toolbar. The pointer turns into a cross. Drag the pointer to draw a line on the image. The information appears on the left side of the status bar. After you release “the button, the information shifts to the right side of the status bar. The information includes the Po (pixel coordinates at point of origin), the Pf (pixel coordinates at the final point), and the distance in mm between the two points. The line remains on the image until you return to the Select mode.

To add the distance in pixels to the status bar display, **CTRL**+drag the pointer across the area you want to measure.

If you mark the image with both the Pixel Locator and the Pixel Distance tools, you can switch the information on the status bar from one type to the other by clicking the Pixel Locator or Pixel Distance toolbar button.

8.5 Enlarge

The Enlarge command doubles the image magnification. To enlarge an image, select **Enlarge** from the menu, and then click the image area you want to enlarge. The point at which you placed the pointer becomes the center of the image. Click **Select** from the menu or toolbar () to end the Enlarge mode.

8.6 Reduce

The Reduce command halves the image magnification. To reduce an image, select **Reduce** from the menu, and then click the area you want reduced. The point at which you placed the pointer becomes the center of the image. Click **Select** from the menu or toolbar () to end the Reduce mode.

8.7 Zoom Area

The Zoom Area command displays a menu for creating and viewing a stack of enlarged image frames that can be redisplayed in reverse order.

Create Frame—Magnifies a selected image area. To magnify a selected area, click **Create Frame** from the menu or toolbar. Place the pointer on the image and drag the pointer diagonally to create a frame. After you release the button, the area you selected expands to fill the window.

Repeat the process to create a series of frames. The series is saved until you redisplay the frames using the Previous Frame option or select the Reset option. (The series is not saved when you close the Image window.)

Previous Frame—Displays the previous frame. If you created a series of frames using the Create Frame tool, Previous Frame allows you to view the series (stack). To view the stack, click **Previous Frame** from the menu or toolbar. Move the pointer into the image area and click to view the previously created frame. Continue clicking to cycle through the remaining frames in the stack.

Reset—Resets the image to its original view, deleting any previously created frames. This command is not available on the toolbar.

Click **Select** from the menu or toolbar () to end the Zoom Area modes.

8.8 Magnifier

The Magnifier command creates a magnifying glass for examining selected areas of the image. To use the Magnifier, select the **Magnifier** command from the menu or from the toolbar. The pointer changes to a cross. Hold down the mouse button. A rectangular magnifying glass appears that enlarges the target area to the next magnification level (section 7.5). Release the mouse button to remove the Magnifier. You can drag the Magnifier around the Image window to view different areas.

The status bar displays the magnification as well as the upper left pixel coordinates (Pi), and the lower right (Pf) pixel coordinates of the magnified area.

To increase and decrease the magnification, press the plus or minus keys on the numeric keypad while holding down the mouse button. Click **Select** (from the menu or toolbar) to end the Magnifier mode.

8.9 Add Band (Manual)

The Add Band (Manual) command allows you to manually identify a band that was not found using the current band-finding settings. To create a band, click **Add Band (Manual)** from the menu or toolbar, and then move the pointer to the area where you want to create a band. Drag the pointer diagonally to create a rectangle that defines the band size. When you release the mouse button, Fragment Analysis inserts a band marker in the center of the rectangle you created. Click **Select** from the menu or toolbar () to end the draw mode.

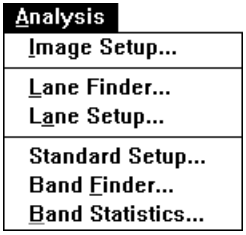
8.10 Add Band (Semiautomatic)

The Add Band (Semiautomatic) command instructs Fragment Analysis to insert a band marker in the area of maximum pixel intensity within an area you define. To draw a band semiautomatically, click **Add Band (Semiautomatic)** from the menu or toolbar, and then move the pointer to the area in which you want the system to locate a band. Drag the pointer diagonally to create a rectangle that defines the band size. When you release the mouse button, Fragment Analysis inserts a band marker in the area of maximum pixel intensity. Click **Select** from the menu or toolbar () to end the draw mode.

8.11 Delete Band

The Delete Band command deletes a selected band. To delete a band, first click the band you want to delete, and then click **Delete Band** from the menu or toolbar. The band marker is deleted. (You can also delete selected band markers using the DELETE key.) To select multiple bands for deletion, SHIFT+click the bands.

Chapter 9 Analysis menu



The commands on the Analysis menu enable you to access the inspectors you use to perform an analysis. The availability of each inspector depends on the stage of analysis you have reached. Each inspector contains a Help button. All but the Band Statistics inspector contain a Done button. The Help button accesses information about the inspector. The Done button displays the next inspector.

Note: To use the keyboard to switch between an inspector and the Image window, press ALT+F6.

The topics in this chapter are—

- Image Setup (section 9.1)
- Lane Finder (section 9.2)
- Lane Setup (section 9.3)
- Standards Setup (section 9.4)
- Band Finder (section 9.5)
- Band Statistics (section 9.6)

9.1 Image Setup

The Image Setup command displays the Image Setup inspector (figure 9-1) together with two rectangular objects on the image. You can move and resize the rectangles to define the area of interest and the largest band size. The Image Setup inspector allows you to rotate the image, to select the experiment type and interpolation method, and to add experiment-specific information for your records.

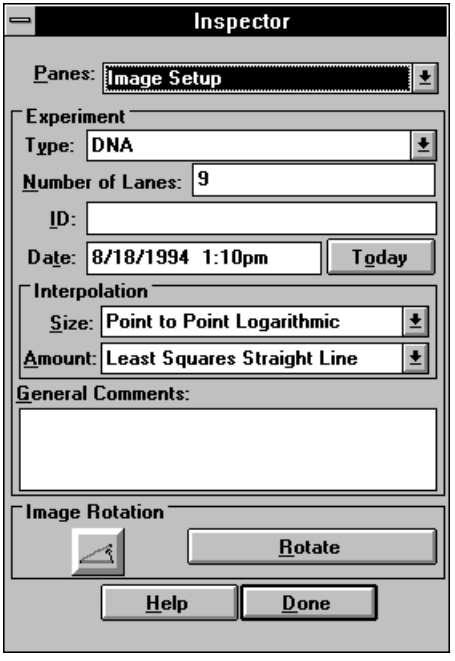


Figure 9-1. The Image Setup inspector.

If you load an existing Experiment Form at the Image Setup step, the settings for the area of interest and band size (the two rectangles) are not in effect. (Fragment Analysis recognizes the default settings as changes to the form.) You will need to readjust the rectangles.

9.1.1 Selecting the experiment type

To select the experiment type, click the down-arrow button ( ) next to the **Experiment Type** box. A list of experiment type options appears. Click the option of your choice (DNA, isoelectric focusing, protein).

9.1.2 Specifying the number of lanes

To specify the number of lanes in your sample, type the number in the **Number of Lanes** box. Fragment Analysis uses this information to find lanes.

9.1.3 Entering information for your records (optional)

You can use the ID, Date, and General Comments boxes to add information for your records. The information you enter here is not used for the analysis but is included in the report. These three text entry boxes duplicate those on the Experiment Form.

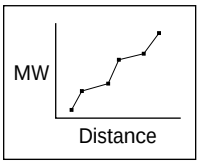
- **ID**—Use the ID box to type identifying information for your experiment.
- **Date**—You can type a date in the Date box, or click the **Today** button to enter today's date in the box.
- **General Comments**—Use the General Comments box to type additional notes about your experiment.

9.1.4 Selecting an interpolation method

You can choose among the various curve fit methods listed in the **Size** (or pKI) and **Amount** selection boxes. (you should use the point-to-point logarithmic for size standards.)

To see the options, click the down-arrow ( ) next to one of the boxes. Click one of the options to change the curve fit method. Note that the choices vary depending on the experiment type you select.

Point to point linear



Point to point linear uses the following formula to calculate the sizes or isoelectric points of the bands based on their migration distances:

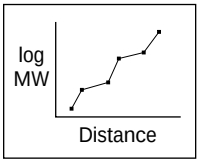
$$\text{distance} = -a \times MW$$

or

$$\text{distance} = -a \times pI$$

where a is a constant determined by the slope of the line between two points on the standard curve, MW is the molecular weight, and pI is the isoelectric point. (Distance traveled is proportional to the molecular weight or isoelectric point.)

Point to point logarithmic



Point to point logarithmic uses the following formula to calculate the sizes of the bands based on their migration distances:

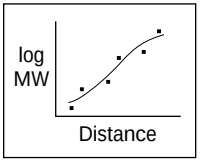
$$\text{distance} = -a \times \log(\text{MW})$$

or

$$\text{distance} = -a \times \log(\text{pI})$$

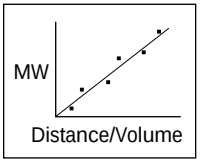
where a is a constant determined by the slope of the line between two points on the standard curve, MW is the molecular weight, and pI is the isoelectric point. (Distance traveled is proportional to the log of the molecular weight.)

Cubic spline (logarithmic)



Cubic spline (logarithmic) uses regression analysis to plot predicted values of the unknowns with the observed values of the standards. The regression line creates a best fit sigmoidal curve through all the data points, and then calculates the unknown molecular weights based on their migration distances and the log of the standard molecular weights.

Least squares straight line



Least squares straight line uses regression analysis to plot a best fit straight line through all the data points and the origin. Unknown amounts are calculated based on total band volumes and unknown molecular weights are calculated based on migration distance.

For additional information about how Fragment Analysis calculates unknown band values, see appendix B.

9.1.5 Rotating the image

To rotate an image that is not squarely aligned with the Image window, click the **image rotation** button ( ). A red line with black handles at both ends appears down the center of the image. You move the line to indicate how the bands should be vertically aligned. Drag the line itself to move the line. Select the handles to rotate the line (figure 9-2).

Align the rotation line with a column of bands that form a lane. For best results, choose a column near the center of the image.

Click the **Rotate** button. The image shifts to the degree indicated by the line.

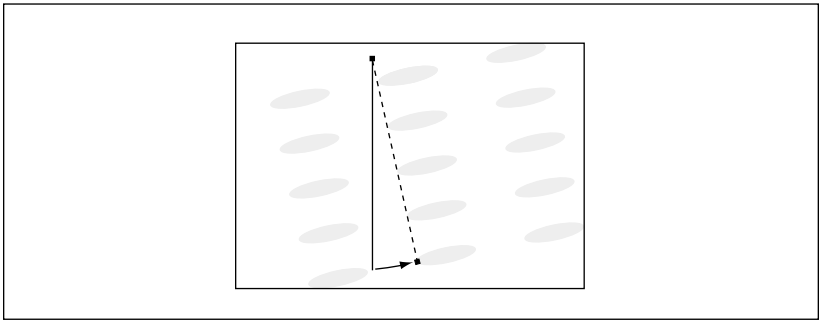


Figure 9-2. Moving the rotation line to the right shifts the image to the left.

9.2 Lane Finder

The Lane Finder command displays the Lane Finder inspector (figure 9-3) together with a lane marker (a new rectangle on the image) that serves as a pattern for finding lanes. Use the Lane Finder inspector to initiate automatic lane finding and to adjust the markers that identify lanes.

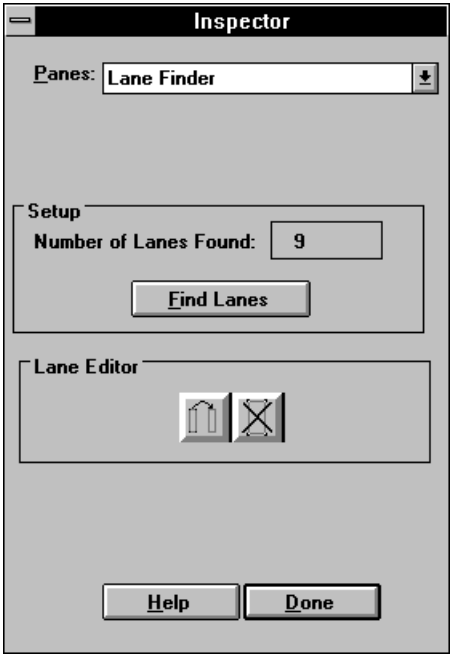


Figure 9-3. The Lane Finder inspector.

9.2.1 Initiating lane finding

To initiate automatic lane finding, click the **Find Lanes** button. Fragment Analysis finds the number of lanes you specified in the Experiment Form or in the Number of Lanes box of the Image Setup inspector, displays the number of lanes found, and identifies the found lanes with rectangular markers.

9.2.2 Editing lane markers

If the lane markers do not accurately define the lanes, you can move, resize, add, or delete them.

Moving lane markers

To move a lane marker, click on it to select it, then place the pointer inside the lane, hold down the mouse button, and drag the marker to a new location.

Resizing Lane Markers

To resize a marker, click on the marker you want to move. Place the pointer on one of the edges and drag the edge in or out.

Note that you can change the width but not the height of the lanes. (The height is determined by the size of the region of interest you define in the Image Setup step.)

Adding and deleting lane markers

You can use the buttons in the Lane Editor box to add and delete lane markers—

Adds a selected lane marker. Click the marker you want to duplicate, and then click the **duplicate** button. Fragment Analysis creates a lane marker to the right of the selected lane.

Deletes lane markers. Click the lane you want to omit from analysis, and then click on the **delete** button. You can also delete the selected lane marker by pressing the **DELETE** key.

9.3 Lane Setup

The Lane Setup command displays the Lane Setup inspector (figure 9-4). Use this inspector to indicate which of the lanes on the image are standards lanes and to add additional identifying information regarding the lanes.

If you entered this information in the currently loaded Experiment Form, the information will appear in the Lane Setup inspector. Unless you want to change the information, you need only click **Done.**

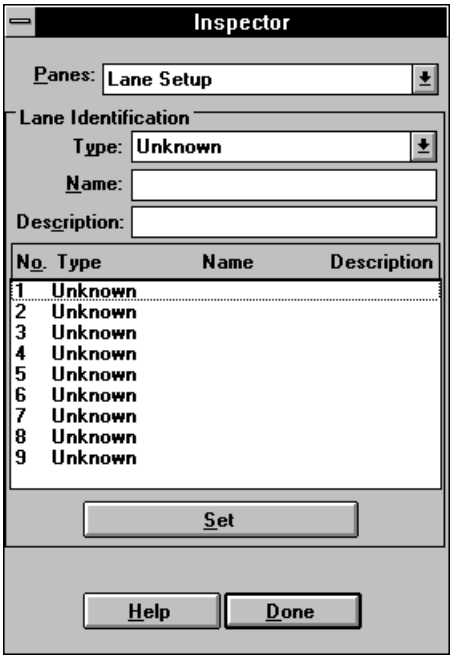


Figure 9-4. The Lane Setup inspector.

9.3.1 Identifying lanes

Lane identification is a three-step process—

1. Indicate the lane type by clicking anywhere on the **Type** box, and then selecting the type from the drop-down list: Size Std. (or pKI Std.), Size/Amt. Std. (both size and amount standards in the same lane), Amt. Std, Unknown, Unused.

2. Select the lane(s) from the lane list box or from the Image window—

   To select one lane at a time, click the lane.

   To select more than one lane in the lane list box (such as lane 1 and lane 6), CTRL+click each lane number.

   To select contiguous lanes in the Image window, SHIFT+click the first and last lane.

   To select a group of contiguous lanes in the lane list box, drag through the group.

3. After you have identified lanes, click **Set.** The Type designations in the Lane list box change to reflect your choice.

9.3.2 Adding descriptive information

You can type a lane name and description in the boxes provided for these items. Before entering the information, select the lane(s) to which the information applies. (Use the selection techniques described in step 2 of the preceding section.)

If you add lane names, they are displayed on the image. To remove the names, open the **View** menu, select **Display** to open the Display window, and then click the **Lane Name** check box to deselect it (remove the X).

9.4 Standards Setup

The Standards Setup command displays the Standards Setup inspector (figure 9-5). Use this inspector to load a standards file, modify the file, or create a new standards file. The standards file provides the information needed to compute the values of the unknowns. The inspector changes slightly depending on whether you select an isoelectric focusing experiment or a DNA/protein experiment, as shown in figure 9-5.

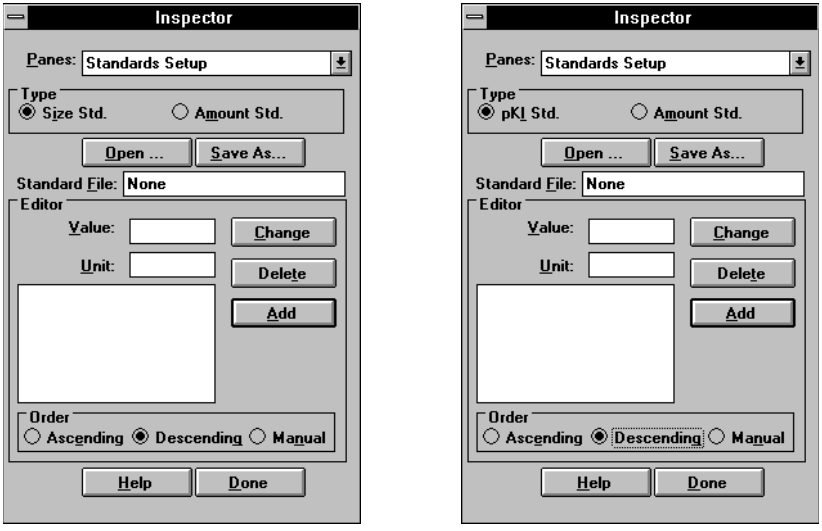


Figure 9-5. Standards Setup inspector for an isoelectric focusing experiment type (left) and a DNA/protein experiment type (right).

If you specified a standards file name in the currently loaded Experiment Form, the standards information will be in place. You need only click **Done.**

9.4.1 Three types of standards files

The analysis software handles three types of standards: size standards, amount (quantity) standards, and pKI standards.

- **Size standards files**—Contain the sizes (molecular weights) of the standards bands. Size standards file names include a .siz extension.
- **Amount standards files**—Contain the amount of material—in units such as nanograms—in each standard band. Amount standards file names include a .mas extension.
- **pKI standards files**—Contain the isoelectric point values of the standards bands. The pKI standards file names include a .siz extension.

9.4.2 Creating a standards file

To create a standards file, you specify a value (size, amount, or isoelectric point) for each band in the standards lane—

1. Click one of the **Type** buttons (**Size Std**/**pKI Std** or **Amount Std**).
2. In the **Unit** box, type the unit, such as bp.
3. Select the sorting order by clicking one of the **Order** buttons: **Ascending, Descending,** or **Manual.** (If you select **Manual,** use the **Before** and **After** buttons to specify where you want the value to be inserted.)
4. In the **Value** box, type the value for the first band.
5. Press **ENTER** (or click **Add**). The value appears in the list box.
6. Repeat steps 4 and 5 for each remaining band value.
7. Click **Save As.** A window for naming the file appears.
8. In the **File Name** box, type a name for the file, and then click **OK.** Fragment Analysis adds the extension, either .siz or .mas.

9.4.3 Loading an existing standards file

To load an existing standards file—

1. Click either the **Size Std** (or pKI Std) or **Amount Std** button.
2. Click **Open.** The Open Size/Amount File window appears.
3. In the list of files, double-click the name of the file you want to use (or click the name and then click **OK**). The file name appears in the **Standards File** box and the values appear in the list box.

9.4.4 Loading two sets of standards

If you are using both size/pKI and amount standards—either in the same or separate lanes—first select one of the standards types (such as Size Std), and then load the file as explained in section 9.4.3. After you load one file, select the second standards type, and then load the second file. To switch the band value display from one file type to another, click the standards button in the Type area.

9.4.5 Modifying the Standards Values

You use the Add, Change, and Delete buttons to change the values in the list box.

Adding a value

To add a value, in the **Value** box type the number to add, and then click **Add** or press **ENTER.** The value is inserted in the appropriate order (either ascending or descending, depending on the order you selected).

Changing a value

To change a value in the list box—

1. In the list box, click the value to change.
2. In the **Value** box, type a new value.
3. Click **Change.** In the list box, the old value is replaced with the new value.

Deleting a value

To delete a value in the list box, click the value you want to delete, and then click **Delete.** The value is removed.

9.4.6 Saving changes to a standards file

If you modified an existing file and want to save the changes, click **Save As** to open the Save window, and then—

- If you modified an existing file and want to save it as a separate file—leaving the original file intact—type a new name in the **File Name** box.
- If you want to save the changes to the existing file, click **OK.** A message asks if you want to replace the existing file. Click **Yes.**

9.4.7 Deleting a standards file

To delete a standards file, select the **Delete** command from the **File** menu (section 6.6). When the Delete window appears, search for .siz or .mas files, select the file you want to delete, and then click **OK.**

9.5 Band Finder

The Band Finder command displays the Band Finder inspector (figure 9-6). Use this inspector to initiate and control band finding.

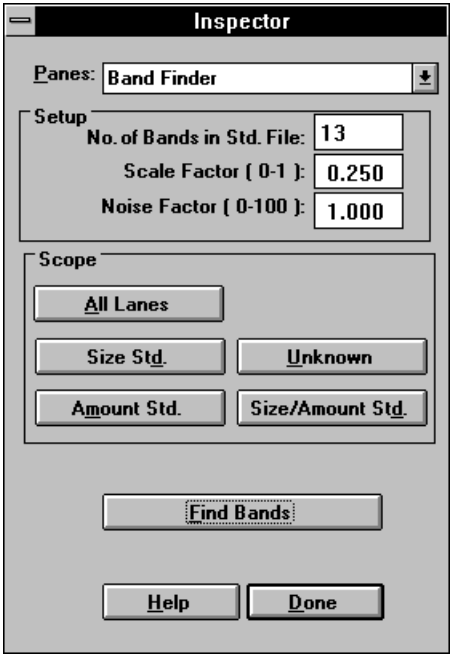


Figure 9-6. The Band Finder inspector.

9.5.1 Finding bands

To initiate band finding, click the **Find Bands** button. Fragment Analysis—

- Locates the bands in all selected lanes.
- Marks each found band with a boundary marker and band position marker.

If Fragment Analysis incorrectly identifies artifacts as bands or overlooks bands, use the editing tools to make corrections (sections 8.9 through 8.11) and/or adjust the Noise Factor or Scale Factor (section 9.5.2) to change the band-finding parameters, and then initiate band finding again.

Note: If you want to see a profile representing the pixel intensity of the bands, open the **View** menu, select **Display,** and then click in the **Lane Profile** check box.

9.5.2 Adjusting the band-finding parameters

The Noise Factor and Scale Factor boxes allow you to fine-tune the parameters that govern band finding. By changing the values in the boxes, you can instruct Fragment Analysis to find either more or fewer bands.

Note: You can apply different band-finding parameters to the different groups of lanes selectable from the Scope buttons. For example, you can apply one set of parameters to Size standards (select the **Size** button) and a different set to Unknowns (select the **Unknown** button). You can also apply different parameters to individual lanes or groups of lanes you select from the Image window. However, parameters applied to individual lanes are not saved to the Experiment Form.

The Noise Factor parameter

The default Noise Factor value of 1.0 represents the threshold setting established by Fragment Analysis. The threshold indicates the point at which Fragment Analysis separated random variations (noise) on the image from relevant data. Noise factors are computed for every lane in the image.

To instruct Fragment Analysis to find fewer bands (exclude more data), increase the Noise Factor value. Increasing the value moves the threshold up to allow more data to be considered as noise and less as bands.

To instruct Fragment Analysis to find more bands (include more data), decrease the Noise Factor value. Decreasing the value moves the threshold down to allow more data to be considered relevant and less as noise.

The Scale Factor parameter

Fragment Analysis uses the default Scale Factor value of 0.25 to remove bands from analysis whose left and right sides are significantly different in intensity. (The 0.25 value means that the intensity value of the left half of the band must be at least 25% of the intensity value of the right half of the band for the band to be included.)

The Scale Factor value is a measure of band uniformity. Decreasing the scale factor includes more nonuniform bands in the analysis.

9.5.3 Limiting band finding to selected lanes

The buttons in the Scope area allow you to limit band finding to a specific group of lanes (the default selection is All Lanes). To limit the band-finding scope, select one of the buttons in the Scope area, such as Unknown, and then click **Find Bands.**

You can also limit band finding by selecting individual lanes on the Image window as follows:

- To select one lane, click the lane.
- To select multiple lanes one at a time, SHIFT+click each lane.
- To select a group of lanes, place the pointer outside the upper left corner of the first lane you want to select and drag the pointer down to the lower right corner of the last lane you want to select. A rectangle encloses the lanes until you release the button. Handles on the corners of the lanes indicate they are selected.
- To deselect individual lanes that you selected as a group (see above), SHIFT+click each lane you want to deselect.

9.5.4 After you click done—standards bands verification

After you click Done, Fragment Analysis compares the number of bands it found in the standards lanes—one lane at a time—with the number of band values listed in the standards file. If the number of bands in the lane does not match the number of bands in the standards file, a message appears asking if you want to edit the bands.

- If your response is **Yes,** the Band Finder inspector remains displayed. You can use the editing tools to add or delete band markers.
- If your response is **No,** the Band Statistics inspector appears. (You can also edit bands when you reach the Band Statistics inspector, and then click the **Recalculate** button.)

9.6 Band Statistics

The Band Statistics command displays the Band Statistics inspector (figure 9-7). This inspector allows you to see a variety of statistics for any band you select. In addition, from the Band Statistics inspector, you can access the Experiment Form as well as report the analysis results.

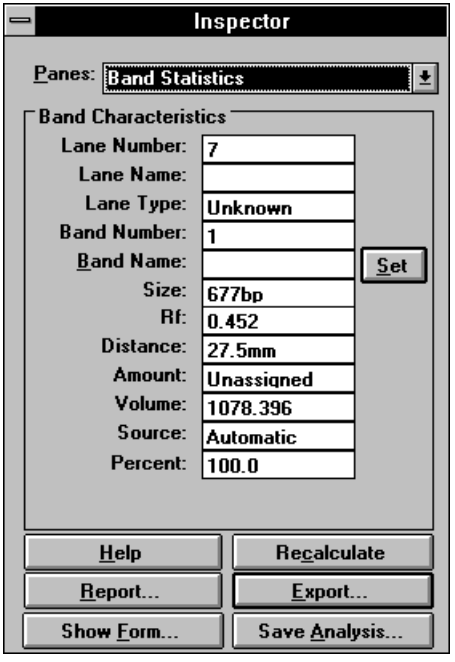


Figure 9-7. The Band Statistics inspector.

9.6.1 Displaying band statistics

To see the statistics, click any band found by the system. The Band Characteristics area displays the following information:

- **Lane Number**—The lane number assigned by Fragment Analysis.
- **Lane Name**—The name you assigned to the lane using the Experiment Form or the Lane Setup inspector.
- **Lane Type**—The lane designation: Size Std or pKI Std, Amount Std, Size/Amt Std, pKI/Amt Std, Unknown, or Unused.
- **Band Number**—The band's position in the lane, counting from the top.

- **Band Name**—A text box in which you can type a name for a selected band (section 9.6.2).
- **Size (or pKI)**—The calculated size (or the isoelectric focusing point), in the units you specified.
- **Rf**—The distance traveled by the band, expressed as a percentage of the total migration range, measured from the top to the bottom boundary of the lane.
- **Distance**—The distance traveled (in millimeters) from the top of the lane to the band position (region of greatest intensity).
- **Amount**—The calculated amount (quantity) of the band, in the units you specified.
- **Volume**—The sum of all the pixel values in the band, minus the background.
- **Source**—The method used to locate the band:
  - **Auto**—Fragment Analysis found the band.
  - **Semiauto**—Fragment Analysis found the band—within an area you specified—using the point of maximal intensity within the area.
  - **Manual**—You specified the band location and dimension by drawing a rectangle. Fragment Analysis inserted a marker in the center of the rectangle.
- **Percent**—Percent of band volume relative to all bands in the lane.

Notes:

The designation, *Unassigned*, means either that no standards file exists for a particular type of calculation (size or amount), that no standard value is associated with the band (amount type only), or that the current standards file is inappropriate for the experiment.

The designation, *Extr*, following a source designation (such as Auto/Extr), means that the band is located above or below the highest or lowest standards band and that the calculations are based on extrapolation. The Extr designation refers to only the size calculation.

9.6.2 Naming bands

To name a band, click the band you want to name, type a name in the **Band Name** box, and click **Set.**

9.6.3 Saving the analysis parameters

Fragment Analysis keeps track of the entries you make during an analysis session. You can save the entries to create a new Experiment Form (if you had not previously created or loaded one) or to update an existing form.

To save the parameters or view the current Experiment Form, click the **Show Form** button. The Experiment Form appears. It contains the parameters you entered during the analysis session.

In the Experiment Form, click **Save** to save the parameters to the current file name or click **Save As** to assign a name to a newly created form. (For more information, see sections 6.1.10 and 6.1.11.)

9.6.4 Saving the experiment analysis

To save the experiment analysis (the objects, such as band markers, and the calculated results), click the **Save Analysis** button. The Save Analysis File window appears. In the **File Name** box, type a name for the analysis, and then click **OK.** Fragment Analysis adds the .exp extension.

To save a dual-channel image experiment analysis, use the Save Analysis command after completing the analysis of the channel 2 image.

To retrieve the experiment, first open the image from which the experiment was saved. Next, open the **File** menu, select **Experiment Analysis,** and then select **Open** from the submenu. A window appears for selecting the file to load.

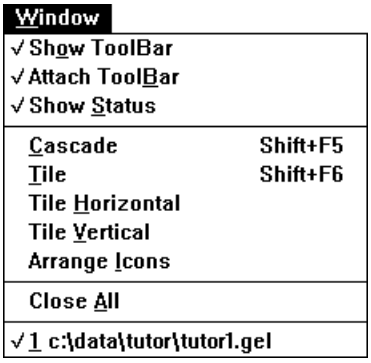
9.6.5 Creating a report

To create a spreadsheet report of the data, click the **Report** button to display the Report window. The Report window allows you to specify the report format, and then transfer the results to Excel. You can also access the Report function by selecting **Generate Report** from the **File** menu. For more information on the Report function, see section 6.5.

9.6.6 Exporting results

To save the data in a file that you can export to a word processing or database application, click the **Export** button. The Export window appears. The Export window allows you to specify the report format and open a window for naming the file. You can also access the Export function by selecting **Export Results** from the **File** menu. For more information on the Export function, see section 6.4.

Chapter 10 Window menu



The Window menu includes commands for rearranging windows and their components. The topics in this chapter are—

- Show Toolbar (section 10.1)
- Attach Toolbar (section 10.2)
- Show Status (section 10.3)
- Cascade (section 10.4)
- Tile (section 10.5)
- Tile Horizontal (section 10.6)
- Tile Vertical (section 10.7)
- Arrange Icons (section 10.8)
- Close All (section 10.9)
- List of Open Windows (section 10.10)

10.1 Show Toolbar

The Show Toolbar command displays or hides the toolbar. When the toolbar is displayed, a check mark appears next to the Show Toolbar command. To show or hide the toolbar, select the **Show Toolbar** command.

10.2 Attach Toolbar

The Attach Toolbar command attaches or detaches the toolbar to or from the right side of the screen. When attached, a check mark appears next to the Attach Toolbar command. When detached, you can move the toolbar. To toggle between attach and detach, select the **Attach Toolbar** command.

To move the toolbar, first detach it, and then move the pointer into the title bar of the toolbar. Drag the toolbar to a new location, and then release the mouse button.

10.3 Show Status

The Show Status command displays or hides the status bar at the bottom of the Fragment Analysis main window. When the status bar is displayed, a check mark appears next to the Show Status command. To show or hide the status bar, select the **Show Status** command.

10.4 Cascade

The Cascade command arranges multiple Image windows in an overlapping manner. The title bar for each window is displayed and accessible. To arrange windows in cascade fashion, select the **Cascade** command. To bring a hidden window to the top of the stack, click its title bar.

10.5 Tile

The Tile command arranges multiple Image windows in a tiled fashion, both horizontally and vertically (if more than three are displayed). The image size is reduced to accommodate a tiled arrangement. To arrange windows in a tiled fashion, select the **Tile** command.

10.6 Tile Horizontal

The Tile Horizontal command arranges multiple Image windows side by side horizontally. The image size is reduced to accommodate the tiled arrangement. To arrange windows horizontally, select the **Tile Horizontal** command.

10.7 Tile Vertical

The Tile Vertical command arranges multiple Image windows side by side vertically. The image size is reduced to accommodate the tiled arrangement. To arrange windows vertically, select the **Tile Vertical** command.

10.8 Arrange Icons

The Arrange Icons command realigns icons displayed in a window. To arrange icons, select the **Arrange Icons** command.

10.9 Close All

The Close All command closes all open windows. You will be prompted to save files, if appropriate. To close all windows, select the **Close All** command.

10.10 List of Open Windows

The bottom of the Window menu displays the names of the open windows. If the paths to the files are different from the currently displayed document, the path names are also displayed. You can select a file name to display a hidden file.

Part three

Appendixes

Appendix A Report examples

The following pages show examples of the three types of reports you can create:

- **Band Report**—Shows the values of one user-selected band attribute, such as size, for all bands in all lanes.
- **Lane Report**—Shows all the statistics for all bands in one user-selected lane.
- **Table Report**—Shows all information for both bands and lanes.

Reports are divided into two sections. The upper section shows general information about the report as well as information collected as part of the scanning process. The lower section shows detailed information about lanes and bands. The column headings are explained in section 9.6.1.

The examples on the following pages were created using Excel formatting features.

Band Size Report

Report generated on: 8/25/1994 10:51am
User Name: Administrator
Image Filename: c:\data\tutor\tutor1.ds
Experiment ID: Sample 1
Experiment Date: 8/25/1994 10:41am
Prepared On:
Image File: c:\data\tutor\tutor1.DIR\SEP1.GEL
Scanned on: 1993:06:14 10:41:17
Units: bp

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9
1300	193.703	666.238	191.648	1300	198.116	676.961	369.394	1300
1200	110.073		108.906	1200	111.387		282.875	1200
1100				1100				1100
1000				1000				1000
900				900				900
800				800				800
700				700				700
600				600				600
500				500				500
400				400				400
300				300				300
200				200				200
100				100				100

Lane Report

Report generated on: 8/26/1994 8:12am
User Name: Administrator
Image Filename: c:\data\tutor\tutor1.ds
Experiment Form Filename: c:\data\tutor\sample1.frm
Experiment ID: Sample 1
Experiment Date: 8/25/1994 2:26pm
Prepared On:
Image File: c:\data\tutor\tutor1.DIR\SEP1.GEL
Scanned on: 1993:06:14 10:41:17

Lane Name	Lane Number	Lane Type	Band Name	Band Number	Band Size (bp)	Distance (mm)	Band Rf	Band Volume	Band Amount	Percent	Band Source
	2	Unknown		1	193.703	53.2	0.862	470.00	Unassigned	46.33	Automatic
	2	Unknown		2	110.073	58.5	0.948	544.53	Unassigned	53.67	Automatic

Table Report

Report generated on:	8/26/1994 11:12am
User Name:	Administrator
Image Filename:	c:\data\tutor\tutor1.ds
Experiment Form Filename:	c:\data\tutor\sample1.frm
Experiment ID:	Sample 1
Experiment Date:	8/25/1994 2:26pm
Prepared On:	
Image File:	c:\data\tutor\tutor1.DIR\SEP1.GEL
Scanned on:	1993:06:14 10:41:17

Lane Name	Lane Number	Lane Type	Band Name	Band Number	Band Size (bp)	Distance	Band Rf	Band Volume	Band Amount	Percent	Band Source
HMN	1	Standard		1	1300	1.4	0.023	138.594	Unassigned	2.056	Automatic
HMN	1	Standard		2	1200	5	0.082	507.144	Unassigned	7.523	Automatic
HMN	1	Standard		3	1100	8.7	0.142	720.984	Unassigned	10.695	Automatic
HMN	1	Standard		4	1000	14.3	0.234	502.004	Unassigned	7.447	Automatic
HMN	1	Standard		5	900	18.2	0.297	699.848	Unassigned	10.382	Automatic
HMN	1	Standard		6	800	21.8	0.356	790.388	Unassigned	11.725	Automatic
HMN	1	Standard		7	700	27.4	0.448	1115.799	Unassigned	16.552	Automatic
HMN	1	Standard		8	600	31.3	0.511	386.527	Unassigned	5.734	Automatic
HMN	1	Standard		9	500	34.9	0.57	402.561	Unassigned	5.972	Automatic
HMN	1	Standard		10	400	41.6	0.68	492.123	Unassigned	7.3	Automatic

Lane Name	Lane Number	Lane Type	Band Name	Band Number	Band Size (bp)	Distance	Band Rf	Band Volume	Band Amount	Percent	Band Source
	2	Unknown		1	193.703	53.2	0.869	308.577	Unassigned	41.713	Automatic
	2	Unknown		2	110.073	58.5	0.956	431.194	Unassigned	58.287	Automatic

Lane Name	Lane Number	Lane Type	Band Name	Band Number	Band Size (bp)	Distance	Band Rf	Band Volume	Band Amount	Percent	Band Source
	3	Unknown		1	666.238	28.6	0.467	285.517	Unassigned	100	Automatic

Lane Name	Lane Number	Lane Type	Band Name	Band Number	Band Size (bp)	Distance	Band Rf	Band Volume	Band Amount	Percent	Band Source
	4	Unknown		1	191.648	53.3	0.871	390.735	Unassigned	37.409	Automatic
	4	Unknown		2	108.906	58.6	0.958	653.757	Unassigned	62.591	Automatic

Appendix B Calculating unknown band values

This appendix explains how Fragment Analysis determines the values of unknown bands (fragments). The method of determining values for fragment size (molecular weight) and isoelectric focusing point is different from that for determining fragment amount (quantity).

B.1 Determining the size or isoelectric point of unknown bands

After band finding is complete, Fragment Analysis assigns the values listed in the size standards file or pKI file to the standards bands on the image. The assignments are based on the order in which the values are listed in the standards file.

Next, standards bands are mathematically superimposed onto lanes containing the unknown bands. If the sample being analyzed contains more than one standards lane, Fragment Analysis uses the information from the standards lanes located on both sides of the unknown. Because bands in the standards lanes may not migrate exactly the same distances, Fragment Analysis must determine where on an unknown lane to superimpose the standards values. To do this, Fragment Analysis performs a point-to-point linear interpolation between each band, using the band values of the standards lanes that bracket the unknown lane (figure B-1).

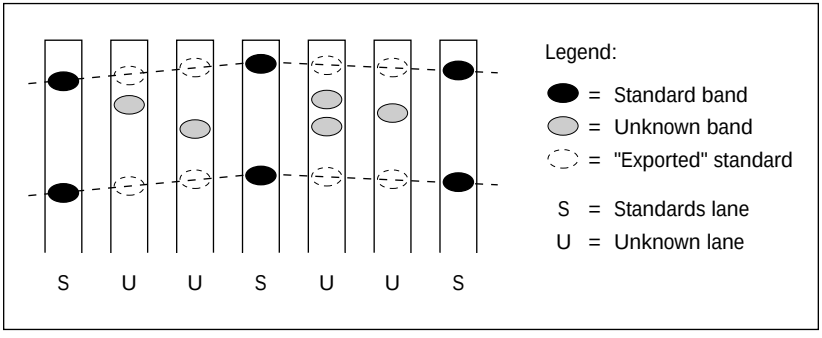


Figure B-1. Fragment Analysis “exports” standards band values into unknown lanes, using point-to-point linear interpolation to estimate where to locate the bands.

Linear interpolation corrects for smiling bands, in which bands of the same size migrate at different rates during electrophoresis.

If an unknown lane is not bracketed by standards lanes (no standard lane on either its left or right), size values are exported from only one standards lane (its nearest neighbor to the left or right), using horizontal extrapolation (figure B-2).

If an unknown band is located above the uppermost standards band or below the lowest standards band, its size is estimated using extrapolation. Fragment Analysis indicates this condition by adding Extr to the Source field in the Band Statistics inspector. For example, the Source designation for a band that Fragment Analysis found—and calculated using extrapolation—would be designated as Auto/Extr.

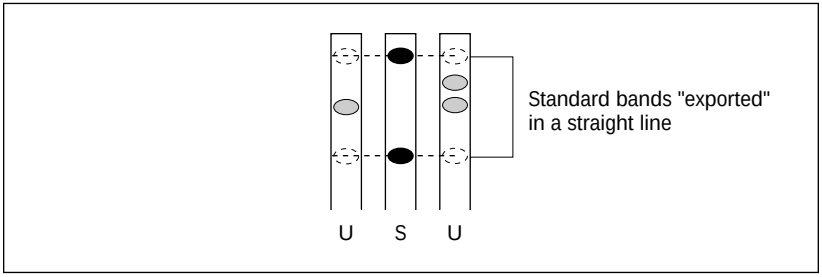


Figure B-2. For unknown lanes that are not bracketed by standards lanes, molecular weight values are exported from only one standards lane, using horizontal extrapolation.

Finally, Fragment Analysis computes the size or isoelectric focusing point of each band using the interpolation method you select: point-to-point linear, point-to-point logarithmic, cubic spline (logarithmic), or least squares straight line. (See section 6.1.6 for an explanation of the interpolation methods.)

B.2 Determining the amount (quantity) of unknown bands

After band finding is complete, Fragment Analysis assigns the values listed in the amount standards file to the standards bands on the image. Assignments are based on the order in which they are listed in the standards file.

Fragment Analysis estimates the volumes for all bands. The volumes for the mass standards are then used in a linear regression analysis (least squares straight line). Next, the volumes of the unknowns are used to compute their quantities based on the linear regression (figure B-3).

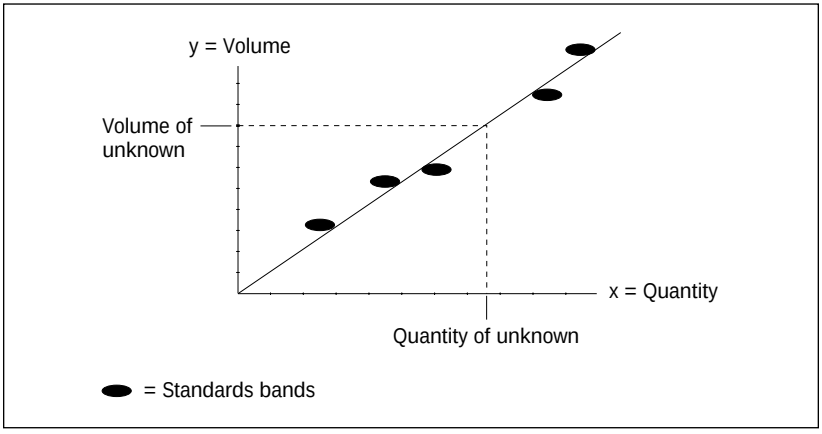


Figure B-3. Fragment Analysis performs regression analysis to determine the mass of unknown bands.

If only one mass standard value is listed in the mass standards file, Fragment Analysis assumes a second standard with a volume of zero and a quantity of zero.

Appendix C Troubleshooting

This appendix provides tips on how to avoid and resolve some of the problems you may experience when learning to use the Fragment Analysis software. Problems, in bold print, are followed by causes and solutions.

C.1 Image setup

After loading an image, the region of interest and prototype band markers (large red and small green rectangles) appeared in their default mode instead of the way you set them.

You might have forgotten to save the changes you made to the rectangles the last time you used this Experiment Form. (After making changes, you must display the form and select **Save** in order to retain new parameters.) Or, you might have loaded an Experiment Form after loading the image, in which case the default rectangle parameters take precedence.

To recover from either situation, readjust the rectangles. Then open the **File** menu and select **Experiment Form.** From the submenu, select **Show** to display the current form. Next, select **Save,** and then click the **Close** button. (You can also save the changes at the end of the analysis, at the Band Statistics inspector.)

C.2 Lane finding

The lane markers on the outer lane(s) of your image are shifted toward the center of the image instead of aligned properly over the lane.

The bands in the outer lane(s) do not have high enough signals for accurate correlation. To recover, return to the Image Setup inspector and increase slightly the width of the region of interest marker (large red rectangle), and then click **Done.** In the Lane Finder inspector, click **Find Lanes** again.

Curved lanes on the image make it difficult for Fragment Analysis to define lanes accurately.

To improve lane finding on images with curved lanes, return to the Image Setup inspector and resize the prototype band (the small green rectangle) to make it narrower (about half a lane width). **Note:** Do not use this method if you are looking for amount information. Also note that band quantitation is not accurate for lane markers that do not extend the full lane width.

You can also try dragging the left and right edges of the region of interest (large red rectangle) in or out to change the lane finding routine. Click **Done**

to return to the Lane Finder inspector. In the Lane Finder inspector, click **Find Lanes.** If necessary, adjust the lane markers so that they intersect all bands in the lane.

C.3 Lane setup

The settings you specified in the Lane Setup window of the Experiment Form did not get transferred to the Lane Setup inspector.

When you enter information in the Lane Setup window of the Experiment Form, be sure to click **Set** before clicking **OK.** To recover, enter the lane setup information in the Lane Setup inspector. (Remember to save the Experiment Form at the end of the analysis session: select **Show** from the Band Statistics inspector, and then select **Save** on the Experiment Form.)

On the Lane Setup window or inspector, when you changed the information in either the Type, Lane Name, or Description boxes, the type, lane name, or description information that had previously appeared on the selected line either disappeared or changed.

You must be sure to set all three boxes—Type, Lane Name, and Description—to the correct values for the selected lane before clicking **Set.** (Clicking a line does not automatically insert the type, name, and description information into their respective input boxes.) To recover, reenter the information, and then click **Set.**

C.4 Band finding

After you click Done in the Band Finder inspector, a Found 0 bands message appears.

Be sure to click the **Find Bands** button before you click **Done.** To recover, on the "Found 0 bands" message window, click **Yes,** in answer to the **Edit bands?** query, and then click **Find Bands.**

Fragment Analysis found too many bands (identified artifacts as bands).

The Noise Factor parameter, which controls the separation of relevant from irrelevant data, is set too low. Increase the Noise Factor value to raise the band-finding threshold (exclude more data from the analysis), and then click **Find Bands.**

Fragment Analysis identified noise spikes as bands.

Most spikes will be excluded as a normal part of the band-finding process. (Fragment Analysis ignores spots that are less than half the width of a band.) To further exclude spikes, increase the Scale Factor parameter. (The Scale Factor value indicates the degree to which the intensity of the left and right half of the band must match. For example, a value of 0.30 means that, to be

identified as a band, the intensity value of the left half of the band must be at least 30% of the intensity value of the right half of the band.)

Fragment Analysis overlooked bands.

The Noise Factor parameter is set too high. To include more data in the analysis, reduce the Noise Factor value to lower the band-finding threshold, and then click **Find Bands.**

Bands that are slanted or nonuniform in intensity may also be overlooked. In such cases, try decreasing the Scale Factor parameter, and then click **Find Bands** again. (For more information about the Scale Factor, see the preceding paragraph.)

Bands are difficult to select for deletion.

Be sure that you are clicking on the band marker (the short line) and not the band boundary (the box). (Use either the Magnifier or Zoom Area tool for a closer look.) If the band marker is difficult to see, open the **View** menu and select **Display.** On the Display window, turn off the Band Boundary display. Also, you can select a color for the band marker that is easier for you to see.

C.5 Retrieving a saved experiment

When you attempt to retrieve a saved experiment, the Open and Save options on the Experiment Analysis submenu are not available.

Be sure to first load the image on which the analysis was saved. To recover, open the **File** menu, select **Open Image,** and then select the image file. After the image appears, load the experiment.

Index

D

E

M

N

O

P

R

S

T

U

V

W

Z