Molecular® **Dynamics**

IMAGEQUANT®

USER'S GUIDE

Version 5.0

for Microsoft® Windows NT®
Apple® Power Macintosh®

Cover (Foreground): Image of a fluorescent 2-color TaqMan (assay in 96-well microplate scanned with a FluorImager 595. The ImageQuant Gray/Color Adjust window is open to the right. The image has been processed using the FluorSep utility to facilitate spectral separation of the two labels. Channels 1 (FAM = Green) and 2 (TET = Red) are displayed in the Overlay mode. Yellow indicates simultaneous signal from both fluorochromes in the same well.

Cover (Background): ImageQuant analysis of each channel showing 96 grouped ellipse objects over each plate image and the resulting volume report data with graphic scatter plot display in Excel (below).

❖ TRADEMARKS ❖

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Preface

About This User's Guide

The *ImageQuant User's Guide* explains how to use the ImageQuant software. Each chapter in this guide discusses a major ImageQuant task and contains the steps needed to perform that task.

Related Publications

In addition to the *ImageQuant User's Guide,* Molecular Dynamics provides the following publications related to the ImageQuant software:

- The *ImageQuant Tutorial*—A tutorial that highlights some of the ImageQuant software.

- The *ImageQuant Reference*—A reference, by menu item, for each function of the ImageQuant software.

- The *ImageQuant Utilities User's Guide*—A guide that describes how to use the FluorSep™ and ImageQuant Tools utilities.

- A user's guide for the Molecular Dynamics instrument.

Assumptions

The software-related instructions in this guide assume you have basic computer skills. You should be familiar with either the Microsoft Windows NT or the Apple Mac OS™ graphical user interface. If you do not have these skills, refer to the Windows NT or Mac OS documentation, or refer to the online Help for these operating systems.

Terms Used in This Guide

The following terms are used in this guide:

- **Click**—Press and then quickly release the mouse button.

- **CONTROL+click**—Hold down the CONTROL key and click the mouse button.

- **Drag**—Hold down the mouse button while moving the mouse.

- **Double-click**—Press and release the mouse button twice.

- **Point**—Move the mouse until the pointer is positioned on the desired object.

- **Pointer**—An object, such as an arrow, that moves on the screen as you move the mouse.

- **Right+click**—Click the right mouse button (Windows NT only).

- **SHIFT+click**—Hold down the SHIFT key and click the mouse button.

- **SHIFT+drag**—Hold down the SHIFT key and drag the mouse.

- **SHIFT+CONTROL+click**—Hold down the SHIFT and CONTROL keys and click the mouse button.

Note: Unless stated otherwise, mouse button instructions refer to the left mouse button when using the Windows NT operating system.

Conventions

The *ImageQuant User's Guide* uses the following conventions:

- In general, the figures display screen captures of the Windows NT version of ImageQuant. For clarity, some figures may display both the Windows NT and Macintosh versions of ImageQuant.

- *Spot* is used throughout the user's guide to indicate a generic image feature, such as a band, blot, or microplate well.

Assistance

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Chapter 1 Getting Started

The ImageQuant software provides analysis tools that you use to perform area or volume quantitation on the images you create using the Molecular Dynamics instrument. The topics in this chapter are—

- Introduction to ImageQuant (section 1.1)
- Introduction to Quantitation (section 1.2)
- Display Modes and ImageQuant (Windows NT Only) (section 1.3)

1.1 Introduction to ImageQuant

When you open ImageQuant, a menu bar, toolbars, and a status bar appear in the ImageQuant window (figure 1-1). To use ImageQuant, you open images in Image windows. You also create line graphs that appear in Graph windows.
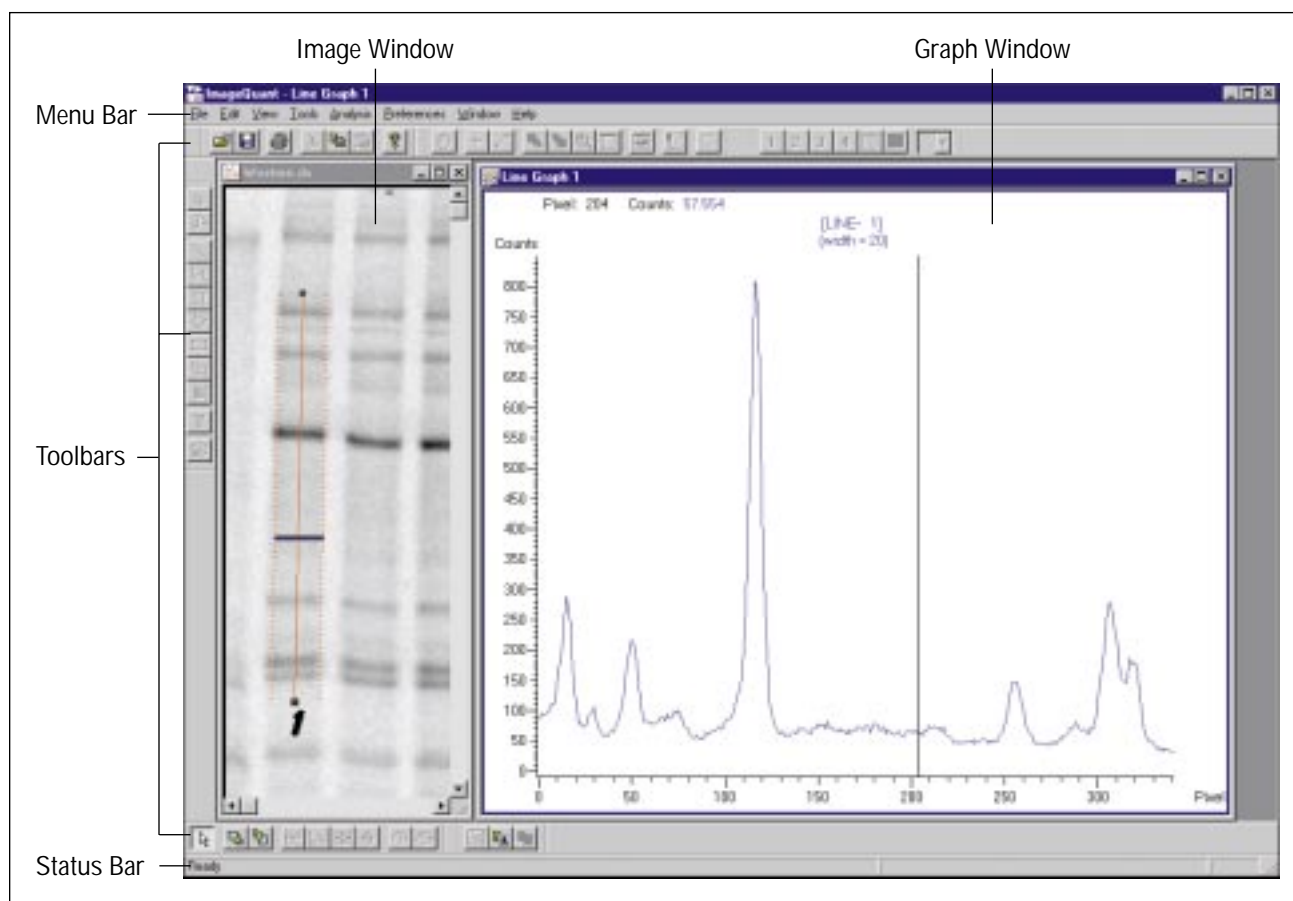
Figure 1-1. Basic features in the ImageQuant window.

1.1.1 Menu Bar

Before you open an Image or Graph window, the menu bar contains four entries:

- **File**—Displays a menu of selections for manipulating files, such as opening image and graph files.

- **Preferences**—Displays a menu of selections for customizing the default settings of ImageQuant.

- **View**—Displays a menu of selections for displaying and removing the status bar and the toolbars.

- **Help**—Displays a menu of selections for online Help.

You choose ImageQuant commands from the menu bar (figure 1-1). As you open more windows, the menu bar changes. The entries reflect the active window.

1.1.2 Status Bar

The status bar displays informational messages about the current function and the displayed image (figure 1-1).

When the status bar is displayed, you see a check mark next to the Status Bar command on the View menu. If you do not want to display the status bar, choose **Status Bar** from the View menu to remove the check mark from the menu and the status bar from the desktop.

1.1.3 Pop-Up Menus

Several pop-up menus appear at different locations in the ImageQuant windows. To display a pop-up menu—

Windows NT—Click the right mouse button.

Macintosh—SHIFT+CONTROL+click the mouse button.

1.1.4 Toolbars

The toolbars are an alternative way to select a function quickly. ImageQuant has six different toolbars—Main, Image, Object, Graph, Quantitation, and Multichannel (figure 1-2). Although you can

choose to display all the toolbars, a toolbar button is active only when the corresponding window command is active. For example, you must display an image before you can use an Object button to create an object.

Main Toolbar

- Open
- Save
- Print
- Cut
- Copy
- Paste
- Help

Image Toolbar

- Pan
- Pixel Locator
- Pixel Distance
- Create Frame
- Previous Frame
- Magnifier
- Fit to Window
- Map
- Gray/Color Adjust
- Define Region of Interest

Object Toolbar

- Select
- Rotate
- Line
- Polyline
- Rectangle
- Polygon
- Ellipse
- Region
- Grid
- Text Annotation
- Auto Tracer

Graph Toolbar

- Select
- Create Frame
- Previous Frame
- Split Graphs
- Merge Graphs
- Maximize Graph
- Restore Graph
- Split Peaks
- Define Peaks

Quantitation Toolbar

- Create Graph
- Area Review
- Volume Review

Multichannel Toolbar

- Channel 4
- Channel 3
- Channel 2
- Channel 1
- View Single Channel
- Overlay
- Side-by-Side

Figure 1-2. The ImageQuant toolbars and their functions.

Displaying the Toolbars

To select which toolbars to display, choose **Toolbars** from the View menu. The Toolbar Configuration window appears (figure 1-3). If a check mark appears in the check box for a toolbar, that toolbar is displayed. Click a check box to add or remove the check mark.
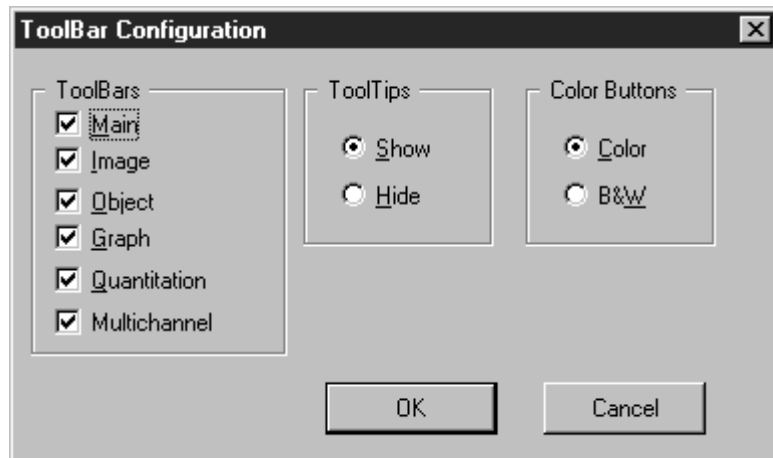
Figure 1-3. The Toolbar Configuration window.

You can choose whether to display the toolbar buttons in color or in black and white. Choose the appropriate button in the Color Buttons area of the Toolbar Configuration window.

After you make your selections in the Toolbar Configuration window, click **OK** to implement them and to close the window.

Using ToolTips

If you are an infrequent user of ImageQuant, you may find it helpful to use ToolTips. A ToolTip briefly describes the function of a toolbar button whenever the pointer rests on the button. In the Toolbar Configuration window, choose **Show** in the ToolTips area to see the ToolTips. To remove ToolTips, choose **Hide.**

Moving the Toolbars

You can move a toolbar anywhere on the screen (detached toolbar) or attach it to the edge of the screen. When you close ImageQuant, the software saves the new toolbar positions.

Attached Toolbars

- To attach a toolbar to the edge of the screen, place the pointer in the space between a button and the edge of the toolbar, and drag the toolbar to the edge of the screen. Release the mouse button when you can no longer move the toolbar. If you are moving a horizontal toolbar to a vertical edge or a vertical toolbar to a horizontal edge, the toolbar changes shape.

- To move a toolbar attached to the edge of the screen, place the pointer in the space between a button and the edge of the toolbar, and drag the toolbar.

- To remove an attached toolbar from the display, choose **Toolbars** from the View menu. In the Toolbar Configuration window, click to deselect the check box for the toolbar you want to remove, and then click **OK.**

Detached Toolbars

- To move a detached toolbar, place the pointer in the title bar of the toolbar and drag the toolbar.

- To change the shape of a detached toolbar, place the pointer in the space between a button and the edge of the detached toolbar, and SHIFT+click. The toolbar changes from vertical to horizontal or from horizontal to vertical.

- To remove a detached toolbar from the display, click the close box in the title bar of the toolbar. To redisplay the toolbar, choose **Toolbars** from the View menu. In the Toolbar Configuration window, click the check box for the toolbar you want to display, and then click **OK.** The toolbar returns to its former location.

1.2 Introduction to Quantitation

ImageQuant provides two methods of quantitation: area quantitation and volume quantitation.

1.2.1 Area Quantitation

Area quantitation calculates the area under the curve created by a plot of the pixel intensities and the pixel locations along a linear object, such as a line. ImageQuant displays the pixel intensities as a line graph.

To perform area quantitation, you draw the linear object over the spots on the image that you want to evaluate and create a line graph of the pixel intensities along the linear object. You select the parameters that ImageQuant uses to determine a baseline and the peaks. ImageQuant performs area quantitation and reports the results for each peak. You can view and print the results, and you can transfer the results to Excel for further analysis.

1.2.2 Volume Quantitation

Volume quantitation calculates the volume under the surface created by a 3-D plot of the pixel locations and pixel intensities.

You perform volume quantitation on the image using an enclosed object, such as a rectangle or an ellipse. You draw an object around each spot on the image you want to evaluate. ImageQuant performs the volume quantitation and reports the results for each object you selected. You can view and print the results, and you can transfer the results to Excel for further analysis.

1.3 Display Modes and ImageQuant (Windows NT Only)

ImageQuant can display images in gray scale and in color. The display mode you select using Windows NT affects how the images appear on the monitor. For more information on changing the display settings, see the Windows NT documentation or online Help.

Depending on the graphics capability of your computer and monitor, you can use one or more of the following settings:

- **True Color (16777216 Colors)**—The monitor displays color and gray scale in 32-bit mode, which provides the most variations. The True Color setting provides the best color resolution for multichannel images and is suitable for viewing gray-scale images. Use this setting if you will be viewing the images in both color and gray scale.

- **65536 Colors**—The monitor displays color and gray scale in 16-bit mode, which provides 65536 variations. The 65536 Colors setting provides better color resolution than the 256 Colors setting. Use this setting if you will be viewing most of the images in color, and you cannot use the True Color setting. If you will be viewing mostly gray-scale images and you cannot use the True Color setting, use the 256 Colors setting.

- **256 Colors**—The monitor displays color and gray scale using 256 variations. The 256 Colors setting is suitable for displaying gray-scale images, but is not suitable for displaying images containing more than one color. Use this setting if you will be viewing most of the images in gray scale, and you cannot use the True Color setting. If you will be viewing multichannel images in the Overlay mode and you cannot use the True Color setting, use the 65536 Colors setting, which provides more color variations.

Note: The display settings on your computer may include additional or different settings. Use the setting that provides the closest match.

Chapter 2 Displaying an Image or Graph

After you scan a sample, you use ImageQuant to display and manipulate the image. After performing area quantitation, you can use ImageQuant to display and manipulate a graph.

The topics in this chapter are—

- Selecting an Image or Graph to Display (section 2.1)
- Checking the Image for Preprocessing (section 2.2)
- Changing the Displayed Portion of the Image (section 2.3)
- Changing the Image Magnification and Size (section 2.4)

2.1 Selecting an Image or Graph to Display

Each file in ImageQuant is identified by an icon or extension and a name. (See appendix A for descriptions of the extensions used in ImageQuant for Windows NT and see appendix B for descriptions of the icons used in ImageQuant for the Power Macintosh.) If ImageQuant is not running, you can drag the image or graph file you want to display over the ImageQuant icon, which opens ImageQuant and displays the image or graph. If ImageQuant is running, you can display an image or graph using the Open window (figure 2-1).
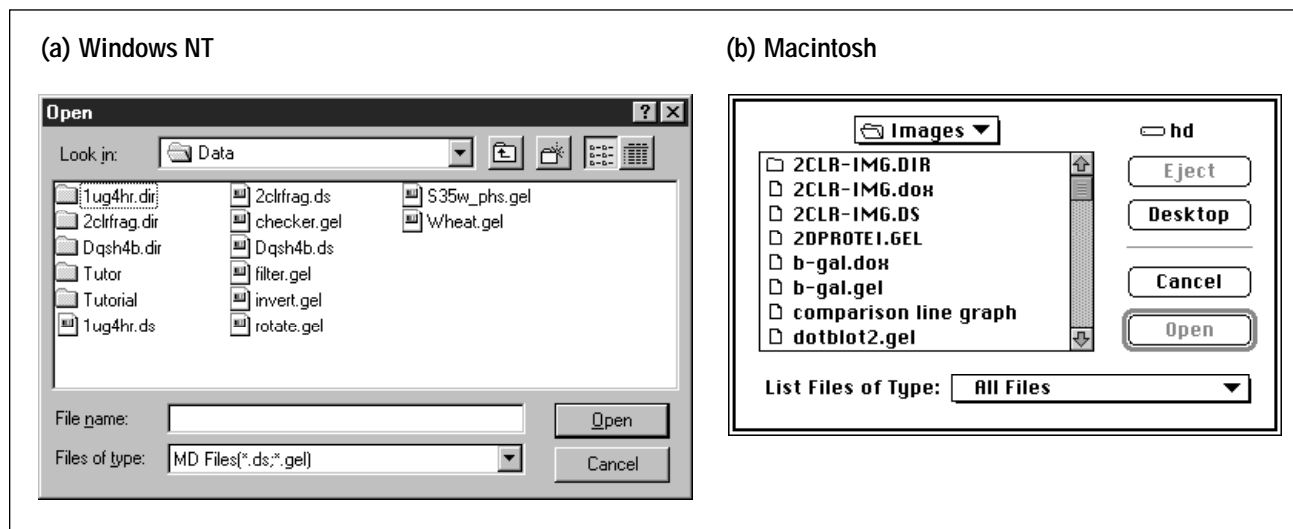
Figure 2-1. The Open window.

To display an image or graph—

1. Choose **Open** (🖿) from the File menu or the Main toolbar. The Open window appears showing the active folder and the contents of the folder.

2. If the image or graph name is not listed—

    • Choose the correct file type from the list.

    • Choose a different folder and view the contents of the folder.

3. Double-click the image or graph name in the list box. The Image window appears and displays the image you selected, or the Graph window appears and displays the graph you selected. (You can also click the name, and then click **Open** to display the image or graph.)

See your Windows NT or Macintosh documentation for details on how to use these features.

2.2 Checking the Image for Preprocessing

Because preprocessing can affect the quantitation results, you may want to check to see if the image has been preprocessed. To display the Image Properties window, choose **Image Properties** from the File menu, or double-click in the Image window.

The three tabs in the Image Properties window contain the scanning and preprocessing information for the image.

• **File Info Tab**—Provides information about the size of the image.

• **Scan Info Tab**—Provides information about the instrument and scanner settings used to scan the sample.

• **History Tab**—Provides a list of the types of preprocessing performed on the image.

You use the ImageQuant Tools and FluorSep utilities to preprocess the image. See the *ImageQuant Utilities User's Guide* for a description of these utilities.

2.3 Changing the Displayed Portion of the Image

For images that are larger than the display area of the Image window, ImageQuant provides several ways to change the displayed area of the image (figure 2-2).
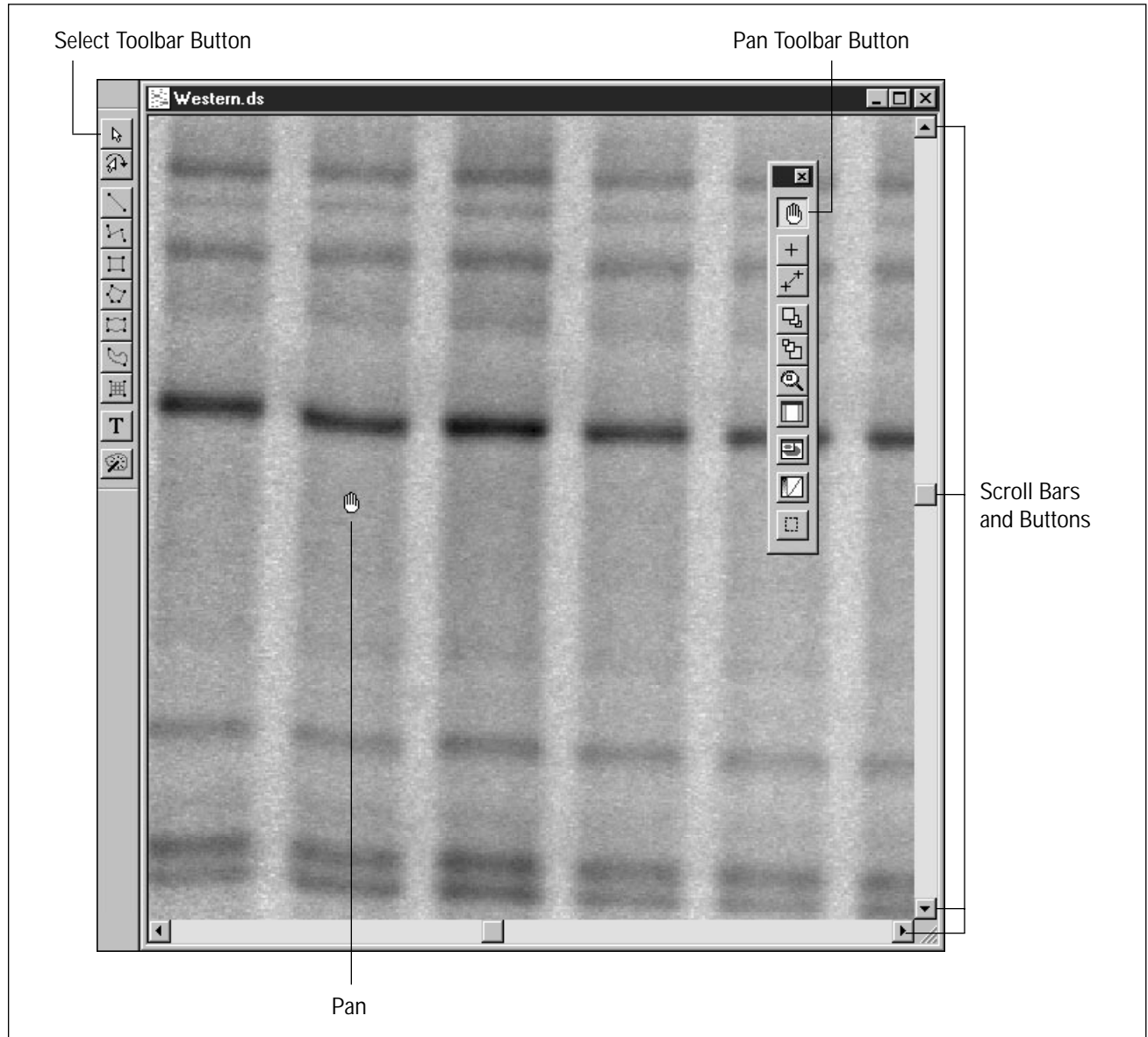
Figure 2-2. Scrolling features.

2.3.1 Scroll

You can scroll the image using the scroll bars or the Pan tool.

Scroll Bars

The scroll bars are displayed at the right and bottom of the Image window. Use the scroll bars to move the image horizontally and vertically.

Pan Tool

To use the Pan tool to scroll—

1. Choose **Pan** (🖐) from the Tools menu or the Image toolbar.

2. Move the pointer into the Image window.

3. Drag the pointer in any direction to move the image.

4. To end the Pan mode, choose **Select** (▷) from the Object menu or the Object toolbar.

2.3.2 Map Window

You can use the Map window to change the display. The Map window shows a smaller version of the entire image (figure 2-3). The rectangle in the Map window represents the portion of the image visible in the Image window.
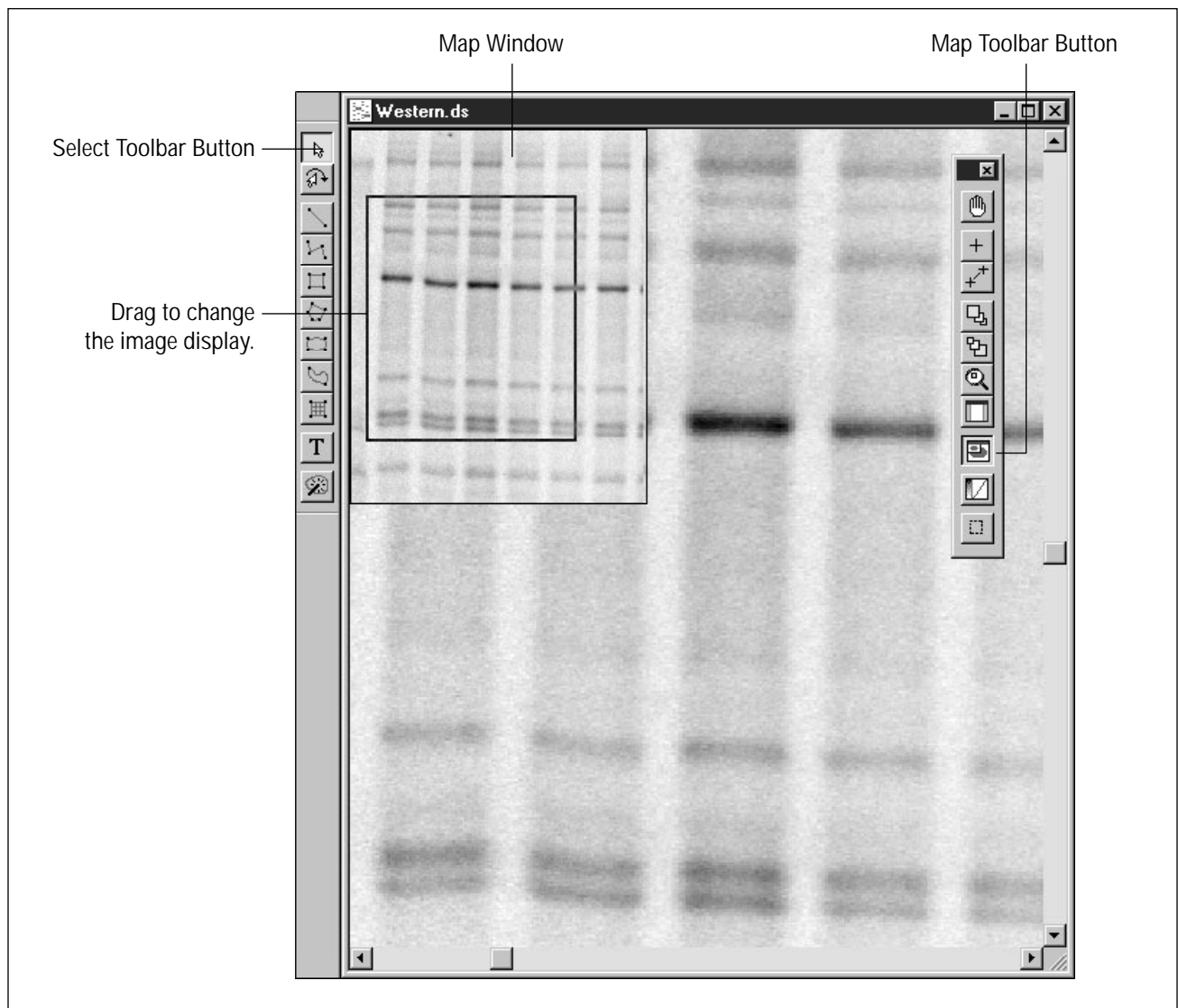
Figure 2-3. Using the Map window to change the image display.

Displaying the Map Window

To display the Map window—

1. Choose **Map** (🖼) from the View menu or the Image toolbar.
    The Map window appears in the upper-left corner of the Image
    window. A rectangle in the Map window outlines the portion of
    the image currently displayed.

    Note: If you are viewing a multichannel image, the Map window
    appears in the active channel of the image. To place a Map
    window in a different channel, click the channel and then
    choose **Map** again.

2. In the Map window, move the pointer inside the outline.

3. Drag the outline to a new location. When you release the mouse button, the Image window displays the new location.

Moving the Map Window

To move the Map window—

1. Move the pointer into the Map window.

2. SHIFT+drag the Map window to a new location.

   Note: If you are viewing a multichannel image and have several Map windows, repeat steps 1 and 2.

Returning the Map Window to the Upper-Left Corner

To return the Map window to the upper-left corner—

1. Move the pointer into the Map window.

2. Next—

   Windows NT—Right+click to display the pop-up menu.

   Macintosh—SHIFT+CONTROL+click to display the pop-up menu.

3. Choose **Reset** from the pop-up menu. The Map window returns to the upper-left corner of the Image window.

   Note: If you are viewing a multichannel image and have several Map windows, repeat steps 1 through 3.

Closing the Map Window

To close the Map window, choose **Map** ( ) from the View menu or the Image toolbar. The Map window disappears from the Image window.

Note: If you are viewing a multichannel image, the Map window disappears from the active channel of the image. To close the Map window in a different channel, click the channel and then choose **Map** again.

2.4 Changing the Image Magnification and Size

You can change the image magnification and size in a variety of ways—

- View the entire image (section 2.4.1).

- View the image at its scan size (section 2.4.2).

- Magnify a small section of the image while leaving the image displayed (section 2.4.3).

- Enlarge or reduce the entire image (section 2.4.4).

- Enlarge a selected area, enclosed in a selection box, to fill the Image window (section 2.4.5).

These functions are located on the View menu, the Tools menu, and the Image toolbar.

2.4.1 Viewing the Image Using Fit to Window

The Fit to Window command displays the entire image. Choose **Fit to Window** ( ) from the View menu or the Image toolbar. After you choose Fit to Window, ImageQuant scales the image to fit in the Image window.

2.4.2 Viewing the Image Using Actual Size

The Actual Size command displays the image at the same size as the sample. Choose **Actual Size** from the View menu. After you choose Actual Size, ImageQuant displays the image as close to the actual size as possible.

2.4.3 Magnifying a Portion of the Image

The Magnifier tool (figure 2-4) allows you to magnify a small portion of the image without changing the magnification of the entire image. The status bar displays the magnification of the enlargement box and the upper-left X,Y coordinates (P_i) and lower-right X,Y coordinates (P_f) of the enlargement box.

Select Toolbar Button

Magnifier Toolbar Button

Drag to change view in Magnifier window.
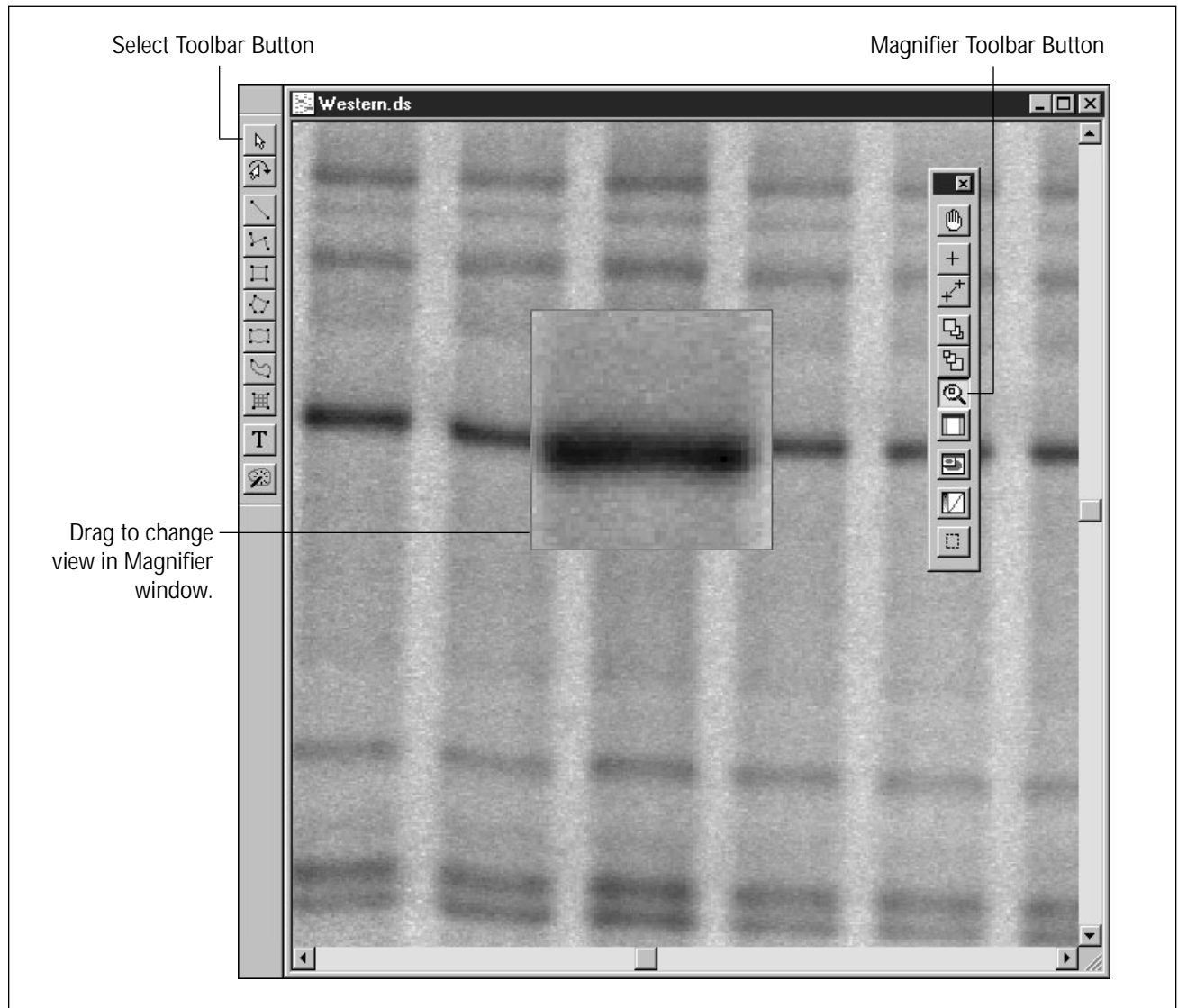
Figure 2-4. Using the Magnifier tool.

To use the Magnifier tool—

1. Choose **Magnifier** ( ) from the Tools menu or the Image toolbar.

2. Move the pointer into the Image window.

3. Hold down the mouse button. The enlargement box displays a magnified version of the image that is directly under the pointer.

   To magnify another portion of the image, move the pointer to a new location, and then hold down the mouse button, or drag the enlargement box to a new location.

4. To end the Magnifier mode, choose **Select** ( ) from the Object menu or the Object toolbar.

   Note: The enlargement box is a fixed size, but you can increase and decrease its magnification. To increase the magnification, place the pointer in the Image window, hold down the mouse button, and press the plus (+) key on the numeric keypad. To decrease the magnification, hold down the mouse button and press the minus (–) key on the numeric keypad.

2.4.4 Enlarging or Reducing the Image

You can enlarge and reduce the image in three ways.

- **Magnification**—Specify a magnification for your image. Choose **Magnification** from the View menu. Choose the desired percentage from the submenu. The image changes accordingly.

- **Zoom In, Zoom Out**—Zoom in the image to the next higher magnification or zoom out the image to the next lower magnification. Zoom In and Zoom Out use the center of the image as the center point for the zoom. Choose **Zoom In** or **Zoom Out** from the View menu.

- **Enlarge, Reduce**—Enlarge or reduce the image using the pointer to determine the center point of the newly displayed area.

  To increase the image magnification—Choose **Enlarge** from the Tools menu. Move the pointer to the center of the area of

interest. Click the mouse button. The enlarged image is centered around the pointer location. Continue clicking the mouse button until the image is the desired magnification. To end the Enlarge mode, choose **Select** ( ) from the Object menu or the Object toolbar.

To decrease the image magnification—Choose **Reduce** from the Tools menu. Move the pointer into the Image window. Click the mouse button. The image reduces. Continue clicking the mouse button until the image is the desired magnification. To end the Reduce mode, choose **Select** ( ) from the Object menu or the Object toolbar.

To change the magnification modes quickly—If you have enlarged or reduced the image too much, CONTROL+click to return to the previous magnification.

2.4.5 Enlarging a Selected Area of an Image or a Graph

Use the Zoom Area function to enlarge a selected area to the size of the Image or Graph window. In addition, the Zoom Area tool allows you to create a series of zoom area frames. The frames are stored in memory temporarily so that you can view the frames in reverse order. After you view each frame, ImageQuant deletes the frame.

Creating Zoom Area Frames

1. Choose **Zoom Area** from the Tools menu, and then choose **Create Frame** from the submenu. Alternatively, you can click **Create Frame** ( ) from the Image or Graph toolbar.

2. Move the pointer into the Image or Graph window.

3. Move the pointer to one corner of the area of interest, and drag the pointer diagonally until the area of interest is enclosed in a selection box.

4. Release the mouse button. The image or graph enlarges to display the contents of the selection box within the size of the current window.

5. Repeat steps 3 and 4 to create additional frames.

6. To end the Create Frame mode, choose **Select** ( ) from the Object or Graph toolbar.

Viewing the Zoom Area Frames

1. Choose **Zoom Area** from the Tools menu, and then choose **Previous Frame** from the submenu. Alternatively, you can click **Previous Frame** ( ) from the Image or Graph toolbar.

2. Move the pointer into the Image or Graph window.

3. Click the mouse button. ImageQuant deletes the currently displayed frame, and the previous zoom area frame appears.

4. Continue to click the mouse button until you return to the first zoom area frame.

    Note: If you want to return to the first frame without viewing the frames in between, choose **Zoom Area** from the Tools menu, and then choose **Reset.** ImageQuant deletes the frame stack, and displays the original image or graph.

5. To end the Previous Frame mode, choose **Select** ( ) from the Object or Graph toolbar.

Chapter 3 Displaying Multichannel Images

A multichannel image contains two or more channels that ImageQuant displays in different ways. If you scan samples using a FluorImager® or Storm® instrument, you can create multichannel images by using the instructions provided in the instrument user's guide. In addition, you can use the FluorSep utility to build a multichannel image from single images scanned on any Molecular Dynamics instrument. See the FluorSep chapters in the *ImageQuant Utilities User's Guide* for instructions.

The topics in this chapter are—

- Processing a Multichannel Image (section 3.1)
- Displaying a Multichannel Image (section 3.2)
- Displaying Multiple Channels Side by Side (section 3.3)
- Overlaying Multichannel Images (section 3.4)
- Viewing One Channel (section 3.5)
- Selecting Individual Channels to View (section 3.6)

3.1 Processing a Multichannel Image

If the fluorochromes you used in the sample create spectral crosstalk, you should preprocess the image using the FluorSep utility before using the image for quantitation. After removing the spectral crosstalk from the image, FluorSep creates one image, called a channel, for each fluorochrome used in the sample. If you used fluorochromes that do not produce spectral crosstalk, you can view the images in ImageQuant without running the FluorSep utility.

For instructions on how to use the FluorSep utility, see the FluorSep chapters in the *ImageQuant Utilities User's Guide.*

3.2 Displaying a Multichannel Image

When you first display a multichannel image, the channels appear overlaid as a single image (figure 3-1). To perform quantitation, you must change the image display to side by side. You use the Multichannel menu or toolbar to change the image display.

After you change the display, you can draw objects on each channel of the side-by-side image and perform quantitation using these objects. You can also view one channel of a multichannel image, create objects on that channel, and perform quantitation. To create objects, see chapter 5. To perform quantitation using the objects, see chapter 8 for area quantitation and chapters 10 and 11 for volume quantitation.
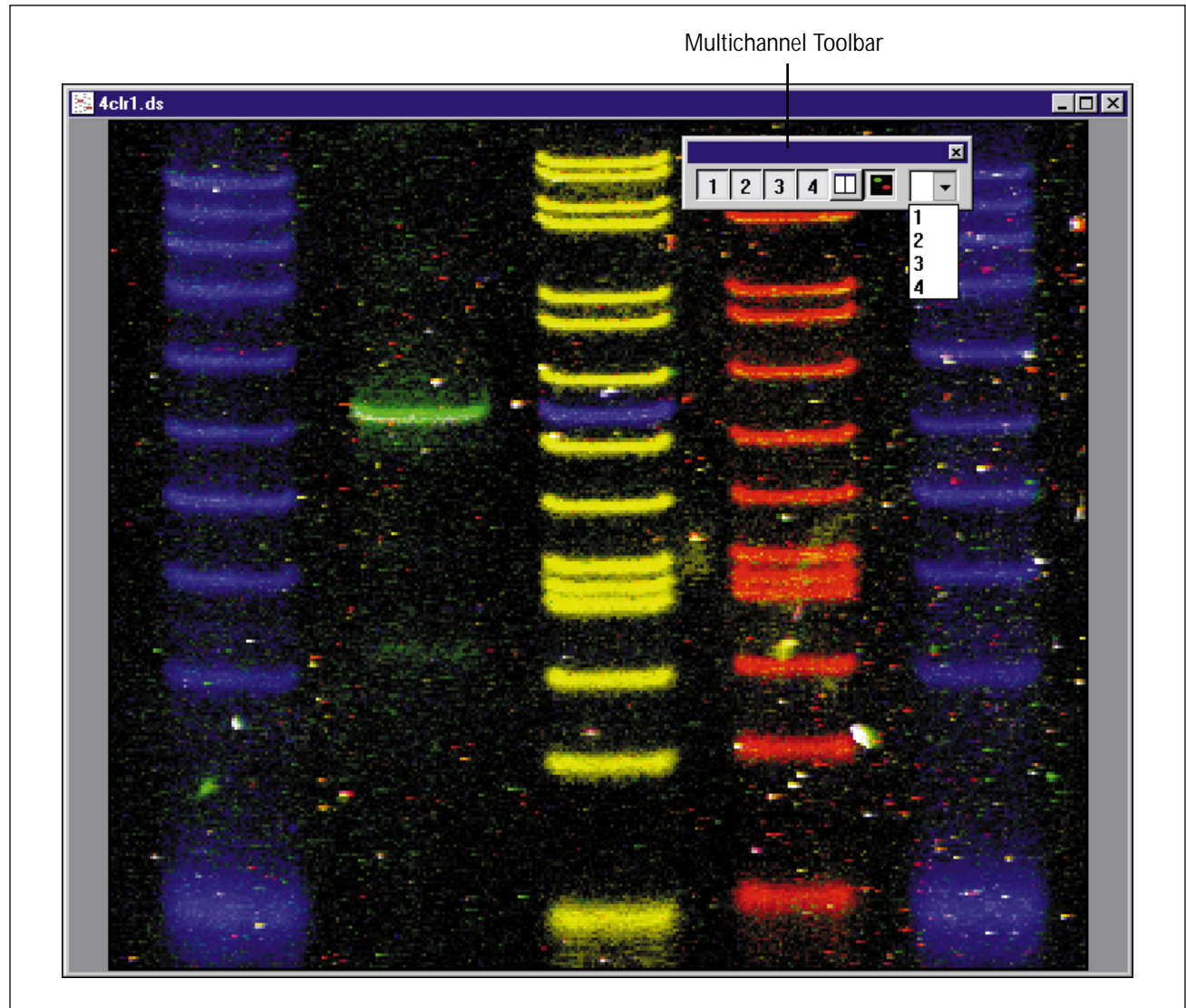
Figure 3-1. Multichannel image displayed in the Overlay mode.

3.3 Displaying Multiple Channels Side by Side

To display multiple channels side by side, click the **Side by Side** button (□) in the Multichannel toolbar. Alternatively, you can choose the Side by Side command from the Multichannel submenu on the View menu. The overlaid image changes to side by side (figure 3-2). The number of separate images depends on the number of channels in the multichannel image. You can have two or more channels.
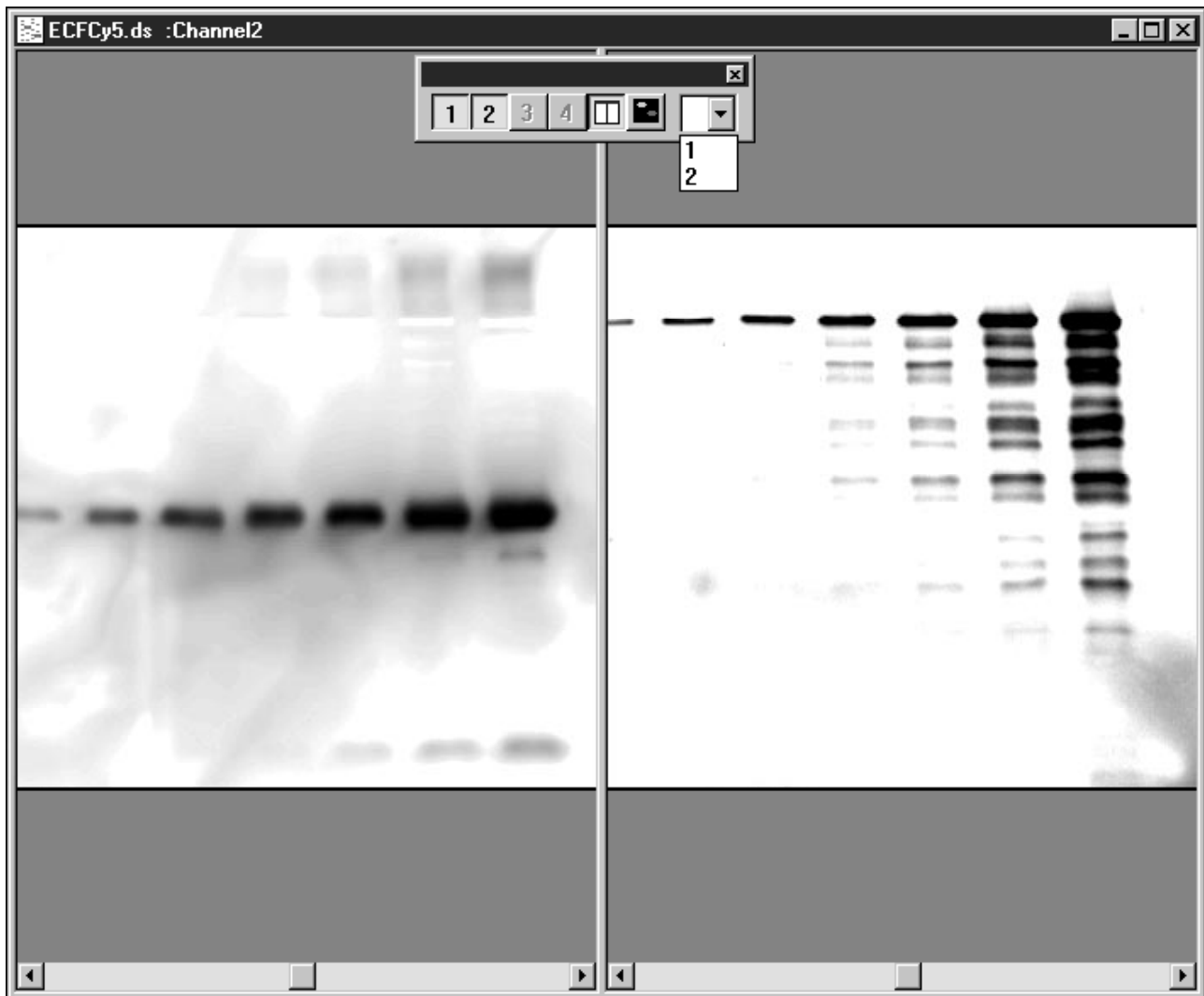
Figure 3-2. A multichannel image containing two channels that are displayed side by side.

3.4 Overlaying Multichannel Images

To change the image from side by side to overlaid, click the **Overlay** button (⬛) in the Multichannel toolbar. Alternatively, you can choose the Overlay command from the Multichannel submenu on the View menu. The side-by-side image changes to an overlaid image. Each channel appears in a different default color.

- **Two**-**Channel Image**—Channel 1 is green, and channel 2 is red.

- **Three**-**Channel Image**—Channel 1 is blue, channel 2 is green, and channel 3 is red.

- **Four**-**Channel Image**—Channel 1 is blue, channel 2 is green, channel 3 is yellow, and channel 4 is red.

To change the default display colors, see section 4.6.

3.5 Viewing One Channel

If the multichannel image is large, displaying one channel at a time can make viewing the spots on the image easier. To view one channel of a multichannel image, click the **View One Channel** button (▾) in the Multichannel toolbar, and choose the channel you want to view from the list (figure 3-3a). Alternatively, you can select the channel from the View One Channel command on the Multichannel submenu of the View menu (figure 3-3b).

Important   **Leave the Multichannel toolbar in the default horizontal position. If you display the Multichannel toolbar vertically, the View One Channel button will not appear in the toolbar.**

You can draw objects and perform quantitation on the displayed channel image.

(a) Multichannel Toolbar

(b) Multichannel Submenu
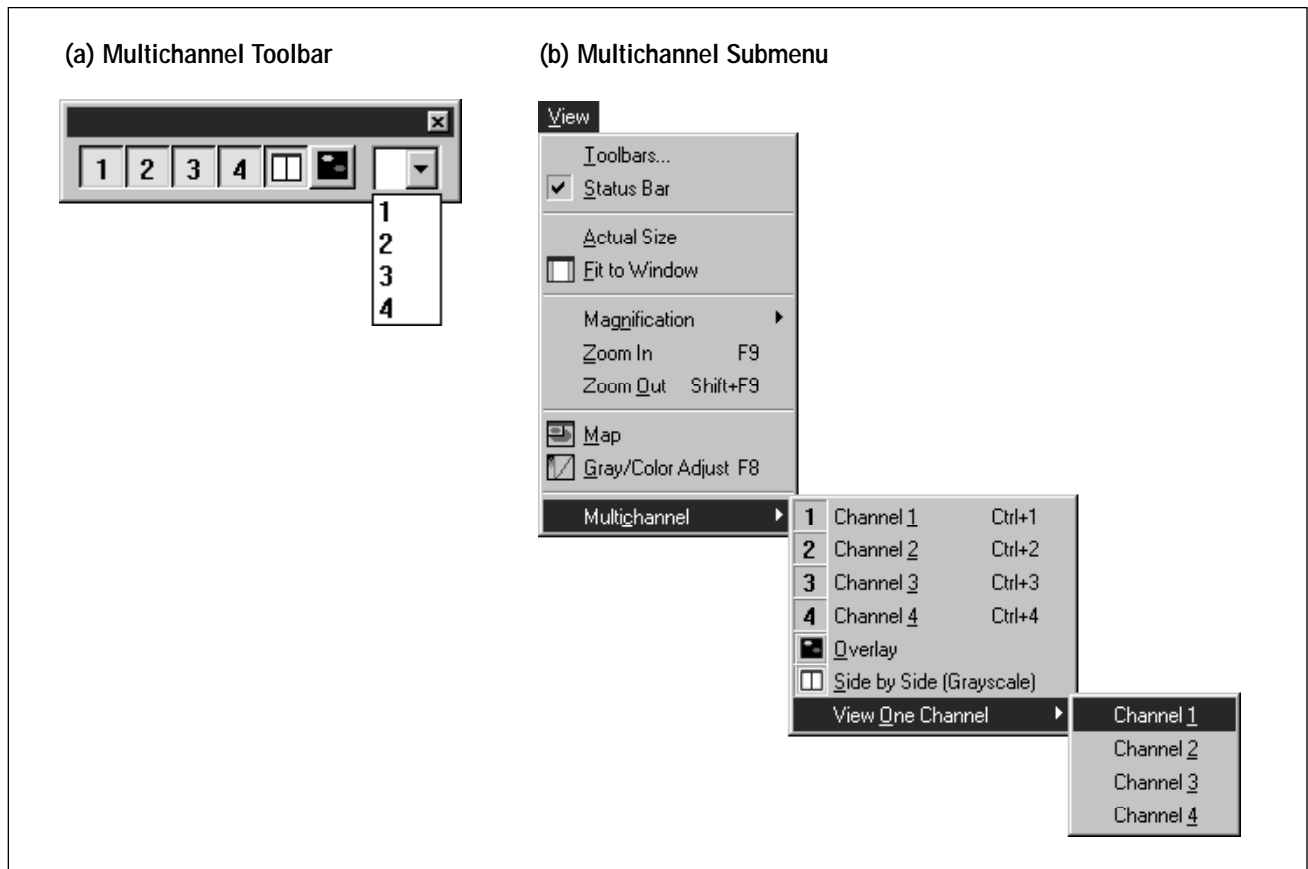
Figure 3-3. Selecting channels using the Multichannel toolbar (a) and the Multichannel submenu (b).

3.6 Selecting Individual Channels to View

You can add a channel to or remove a channel from the Image window by clicking the Channel buttons (1 2 3 4) on the Multichannel toolbar (figure 3-3a). Alternatively, you can choose the channel 1, 2, 3, and 4 commands from the Multichannel submenu of the View menu (figure 3-3b).

You can choose any combination of channels to view, and you can view the combination as an overlaid image or a side-by-side image.

Chapter 4 Optimizing the Image Display

You use the Gray/Color Adjust feature to fine-tune the range of the image display values. Then you use the Gray/Color Adjust feature to enhance the contrast and brightness of the image display. The topics in this chapter are—

- Opening the Gray/Color Adjust Window (section 4.1)
- About the Gray/Color Adjust Window (section 4.2)
- Applying the Enhancements and Closing the Gray/Color Adjust Window (section 4.3)
- Adjusting the High and Low Display Values (section 4.4)
- Enhancing the Contrast and Brightness of the Display (section 4.5)
- Changing the Color (section 4.6)
- Inverting the Display of the Pixel Values (section 4.7)

Important **Changing the image display using the Gray/Color Adjust window affects only the display. The original image and the quantitation results are not affected.**

4.1 Opening the Gray/Color Adjust Window

To open the Gray/Color Adjust window, choose **Gray/Color Adjust** (▨) from the View menu or the Image toolbar. The Gray/Color Adjust window appears (figure 4-1).

Important **Changes you make in the Gray/Color Adjust window affect the entire image. However, ImageQuant subsamples only the pixels in the portion of the image displayed in the Image window. If you are viewing a portion of the image that is noticeably lighter or darker than the rest of the image, these changes can produce undesirable results when applied to the entire image. To return to the default values, click the Fit to Window button in the Image toolbar to display the entire image in the Image window, and then click the Autoscale button in the Gray/Color Adjust window.**
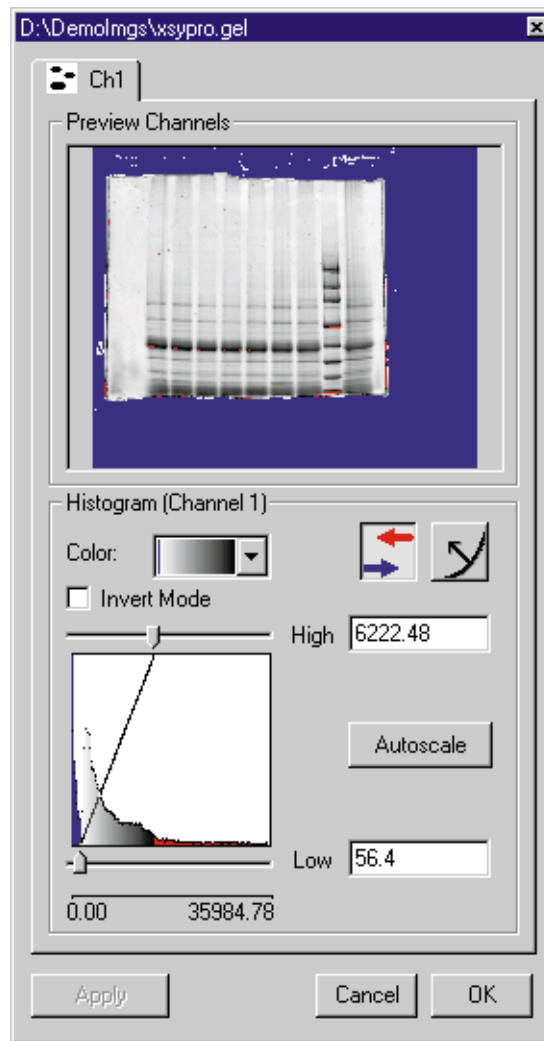
Figure 4-1. A single-channel image displayed in the Gray/Color Adjust window.

4.2 About the Gray/Color Adjust Window

The Gray/Color Adjust window displays the contents of the Image window using two different methods—a preview display and a histogram plot.

- The Preview Channels area contains a small preview image generated by subsampling the portion of the image displayed in the Image window. The preview image is a direct representation of the spatial (X and Y pixel coordinates) and the intensity information. The Preview Channels area provides a visual cue that shows you how the changes affect the image. You should check the preview image before applying the changes to the image in the Image window.

- The histogram plot in the Histogram area of the window contains a statistical view of the image data. The x-axis of the histogram displays the intensity values that comprise the portion of the image displayed in the Image window. The y-axis displays the frequency (number of occurrences of the pixels) for each intensity value in the image. ImageQuant uses a logarithmic scale in the X and Y directions to plot the histogram. In general, the first peak in the histogram contains the background values. However, some overlap with valid signal data may occur in the low end of the range.

- In addition, the histogram plot displays a line that represents a lookup table definition. The lookup table definition determines the transfer function ImageQuant uses to map the data pixel values to the display pixel values. The default lookup table definition applied to the image data is linear. ImageQuant maps all the input display values one-to-one to the output display values and does not change the image contrast. To change the lookup table definition, see sections 4.5 through 4.7.

- The slider controls in the Histogram area of the window allow you to set the range of the values contained in the image data. You can manually change the range, or you can choose to have ImageQuant calculate the range automatically. If you want to determine the range manually, the histogram plot helps you to determine the range of the image data visually. You set the Low value to suppress the background, and you set the High value to include the highest value in the image data. If you reduce the High value too much, the image data appear as saturated pixel values. To change the display range, see section 4.4.

Important   **The Gray/Color Adjust window displays the same window extent and number of channels (merged or side by side) as displayed in the Image window.**

4.3 Applying the Enhancements and Closing the Gray/Color Adjust Window

As you modify the High and Low values in the Histogram area, you see the results of the changes in the Preview Channels image, but not in the image in the Image window. If the enhancement produces an undesirable effect, you can try different settings to optimize the image. When you are satisfied with the image in the Preview Channels, you apply the enhancements to the image in the Image window. To do this—

- Click **Apply** to see the changes in the Image window and leave open the Gray/Color Adjust window for additional modifications.

- Click **OK** to see the changes in the Image window and close the Gray/Color Adjust window.

- Click **Cancel** to close the Gray/Color Adjust window without making any changes in the Image window. If you have already clicked Apply, you cannot use Cancel to revert back to the previous settings.

Important   **If the image in the Image window is a single-channel image, the Gray/Color Adjust window contains only one tab. If the image in the Image window is a multichannel image, the Gray/Color Adjust window contains multiple tabs, one for each channel displayed in the Image window. You enhance each channel separately. Click a tab to change to the views for that channel.**

4.4 Adjusting the High and Low Display Values

The first time you display an image scanned on a Molecular Dynamics instrument, ImageQuant automatically sets the range of values displayed within the image. Autoscaling sets the pixel display range to cover the lowest and highest value recorded by the instrument.

For example, a FluorImager can collect values from 0 to 100 000 relative fluorescent units (rfu). If the values emitted by your sample range from 1 000 to 20 000 and you display the image using the full scale of the FluorImager (0 to 100 000), the image appears very low

in contrast, with most of the valuable information indistinguishable from background. When ImageQuant autoscales the image, it sets the Low range to 1 000 and the High range to 20 000, which optimizes the display based on the actual extent of the image data. If the image contains spurious noise, such as dust, that is not relevant to the experimental data, this range may be artificially extended on the High end, which can lower the overall contrast of the image.

You can use the scaling controls in the Gray/Color Adjust window to fine-tune the displayed image. If you do not see the scaling controls displayed in figure 4-2, click the **Edit High/Low** button ( ).
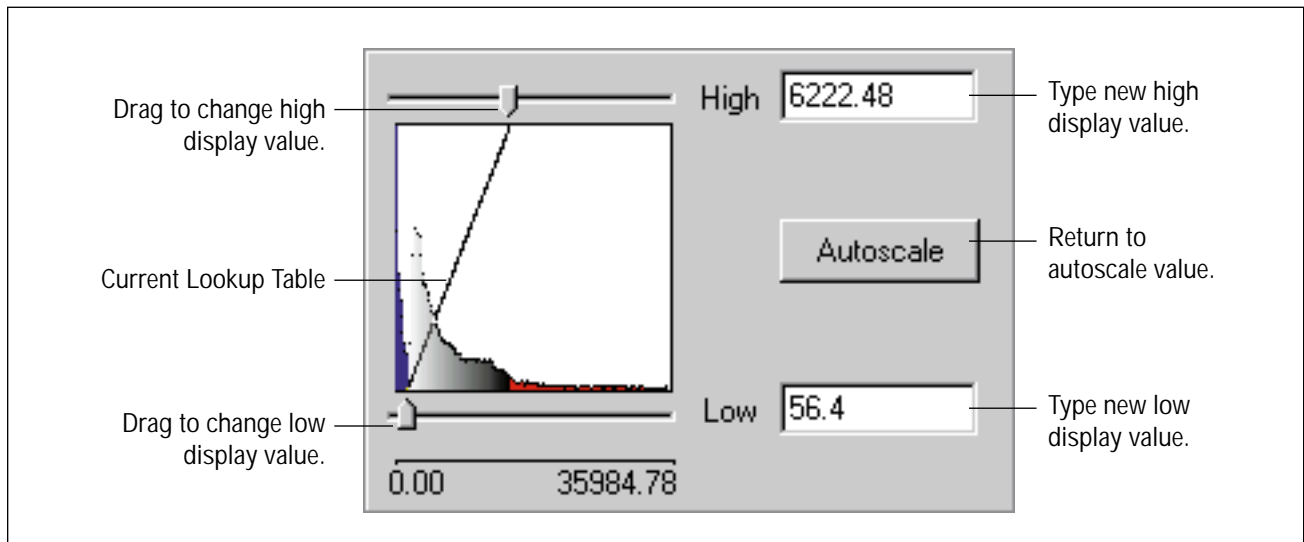
Figure 4-2. The scaling controls in the Gray/Color Adjust window.

The histogram plot displays the current range of data values in the image data in the following ways:

- If the image in the Image window is a single-channel image or a multichannel image with the channels displayed side by side, the histogram plot and the preview image display the low values (background) in blue, the high values (saturated) in red, and the valid image data in gray scale. As you change the display values, you use these visual cues to help determine the optimal settings for the image data.

- If the image in the Image window is a multichannel image with the channels displayed in the Overlay mode, the histogram plot and the preview image display the low (background) values in black, and display the high (saturated) values and the valid image data in the color of the active channel. See section 4.6 for more information on channel colors.

To change the high or the low value, use one of the following:

- Type a new value in the **High** or **Low** box.

- Drag the **High** or **Low** slider. The new value appears in the High or Low box.

- Click the High or Low slider, and then press the **Page Up** or **Page Down** key on the keyboard to make coarse adjustments to the display value.

- Click the High or Low slider, and then press the **up arrow** or **down arrow** key on the keyboard to make fine adjustments to the display value.

- Click the **Autoscale** button to return to the optimized values determined by ImageQuant.

You see the changes in the image in the Preview Channels area of the Gray/Color Adjust window. After you click Apply or OK, ImageQuant modifies the image in the Image window and saves the display values with the image.

Important   **If you are viewing a multichannel image, make sure you repeat the scaling process for each channel.**

4.5 Enhancing the Contrast and Brightness of the Display

The Gray/Color Adjust window contains a set of controls that you can use to enhance the visual display of the image (figure 4-3). To access the controls, click the **Edit Lookup Table** button ( ).
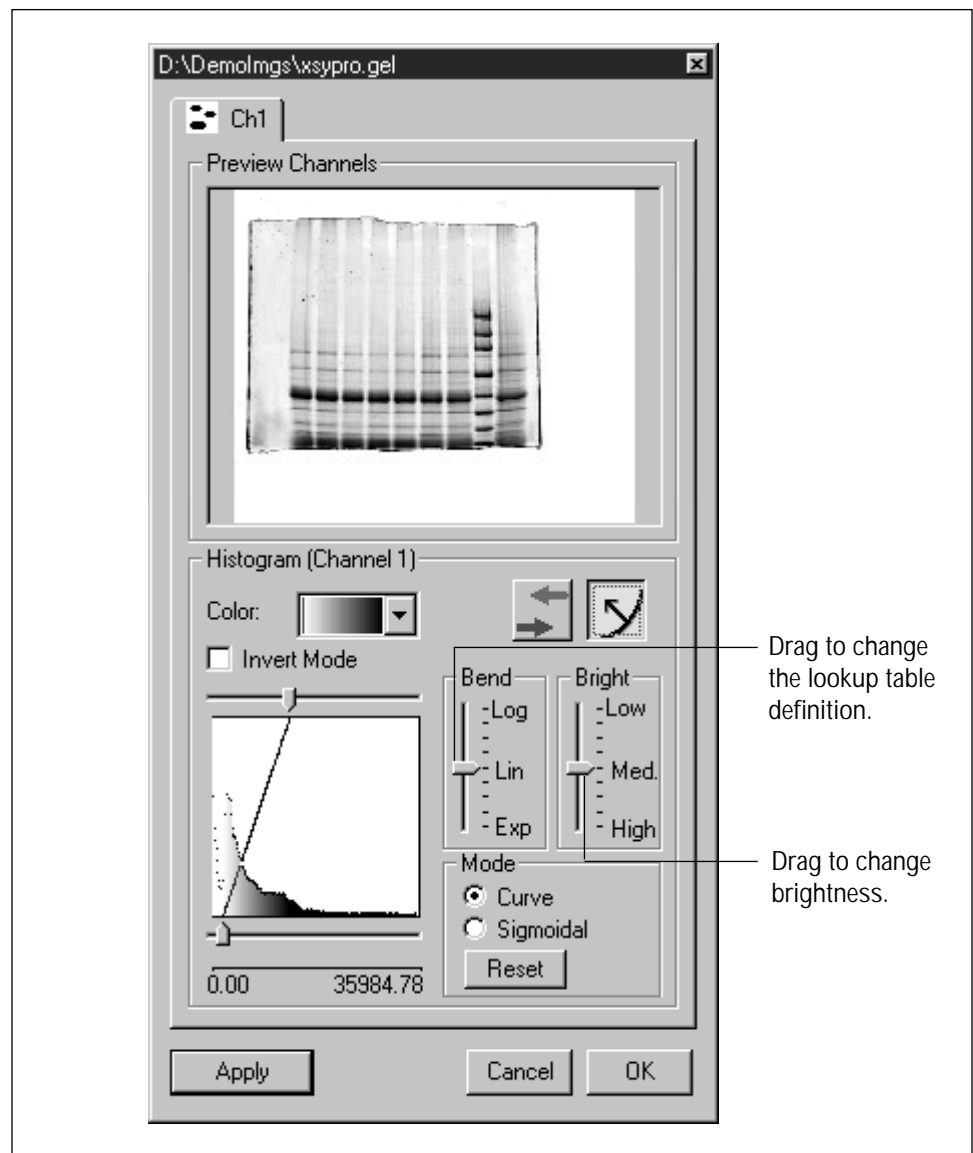
*Figure 4-3. The visual display control in the Gray/Color Adjust window. The
preview image and the histogram plot are displayed using the
default linear lookup table definition and the default medium
brightness control.*

4.5.1 Changing the Intensity Display of the Image

You move the Bright slider to change the intensity of the image. The default setting is medium. Move the slider toward Low to decrease the intensity, or move the slider toward High to increase the intensity. The image in the Preview Channels area displays how the change affects the image.

After you click Apply or OK, ImageQuant modifies the image in the Image window and saves the intensity setting with the image.

Important   **If you are viewing a multichannel image, make sure you check the intensity for each channel.**

4.5.2 Changing the Lookup Table Definition

The default lookup table definition in the Gray/Color Adjust window is linear (uses a curve mode of 0). Click the **Curve** button in the Mode area to activate the Bend slider. The Bend slider allows you to change the curve definition in the lookup table from linear to exponential or logarithmic.

- **Linear Lookup Table**—Displays all the values, one for one, along a straight line (figure 4-3).

- **Logarithmic Lookup Table**—Displays all the values using a logarithmic scale, which enhances the contrast in the low intensity pixel range (figure 4-4a).

- **Exponential Lookup Table**—Displays all the values using an exponential scale, which enhances the contrast in the high intensity pixel range (figure 4-4b).

(a) Logarithmic Lookup Table Definition

(b) Exponential Lookup Table Definition
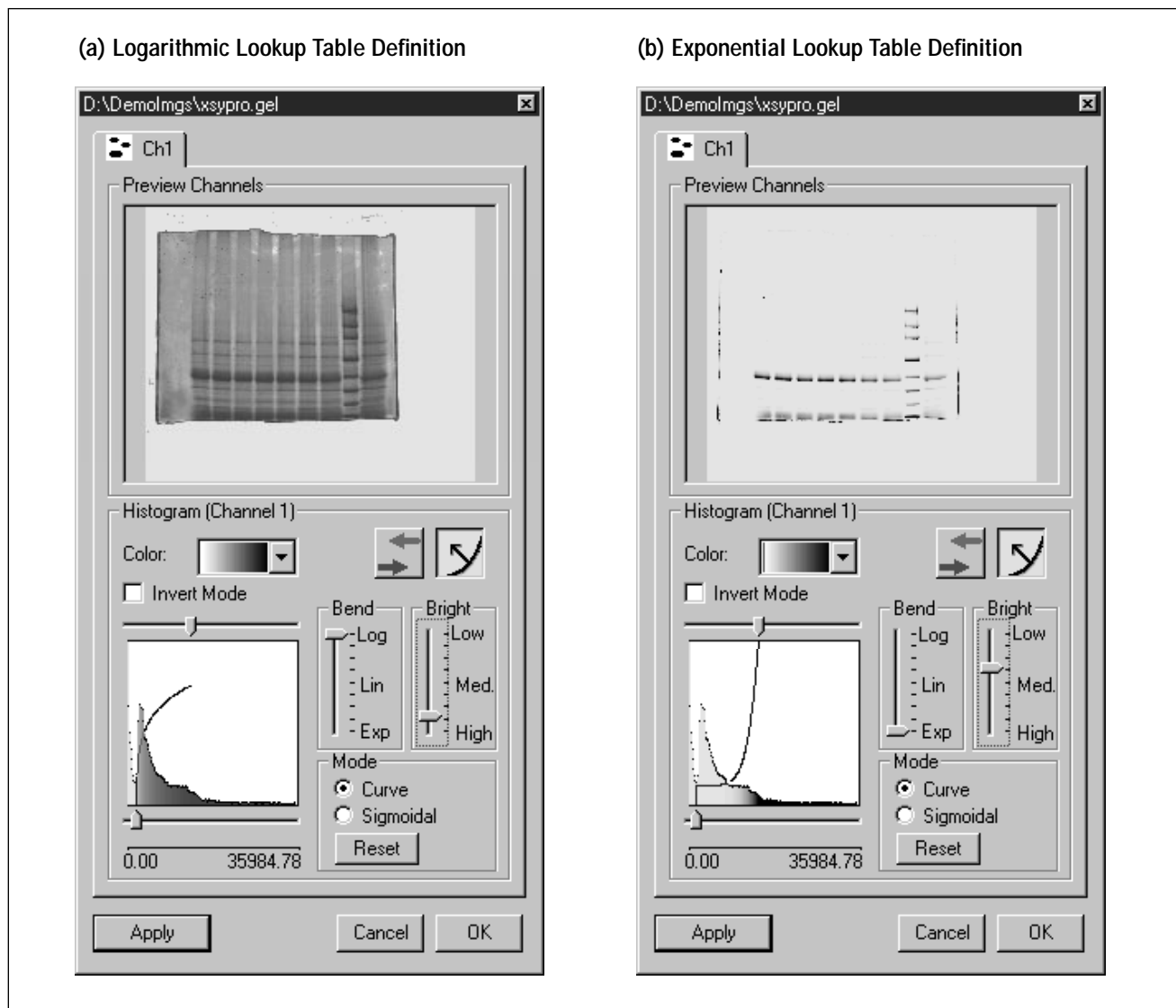
Figure 4-4. How changing the lookup table definition affects the preview image and histogram plot. Notice the changes when the linear lookup table used in figure 4-3 is changed to logarithmic (a) and exponential (b).

Choose the lookup table definition that best enhances the image data. Use the preview image to evaluate how each option affects the display of the image data before you apply the change to the image in the Image window. Click **Reset** to return to the default linear display.

After you click Apply or OK, ImageQuant modifies the image in the Image window and saves the lookup table settings with the image.

Important **If you are viewing a multichannel image, make sure you check the display for each channel.**

4.5.3 Changing the Sigmoidal Lookup Table

The sigmoidal mode is the second setting in the Mode area of the Gray/Color Adjust window (figure 4-5). Click the **Sigmoidal** button to switch to the sigmoidal lookup table definitions. ImageQuant replaces the Bend slider with the Slope slider. You can use the sigmoidal mode with an image scanned on a PhosphorImager® to mimic the visual display acquired when you use film.
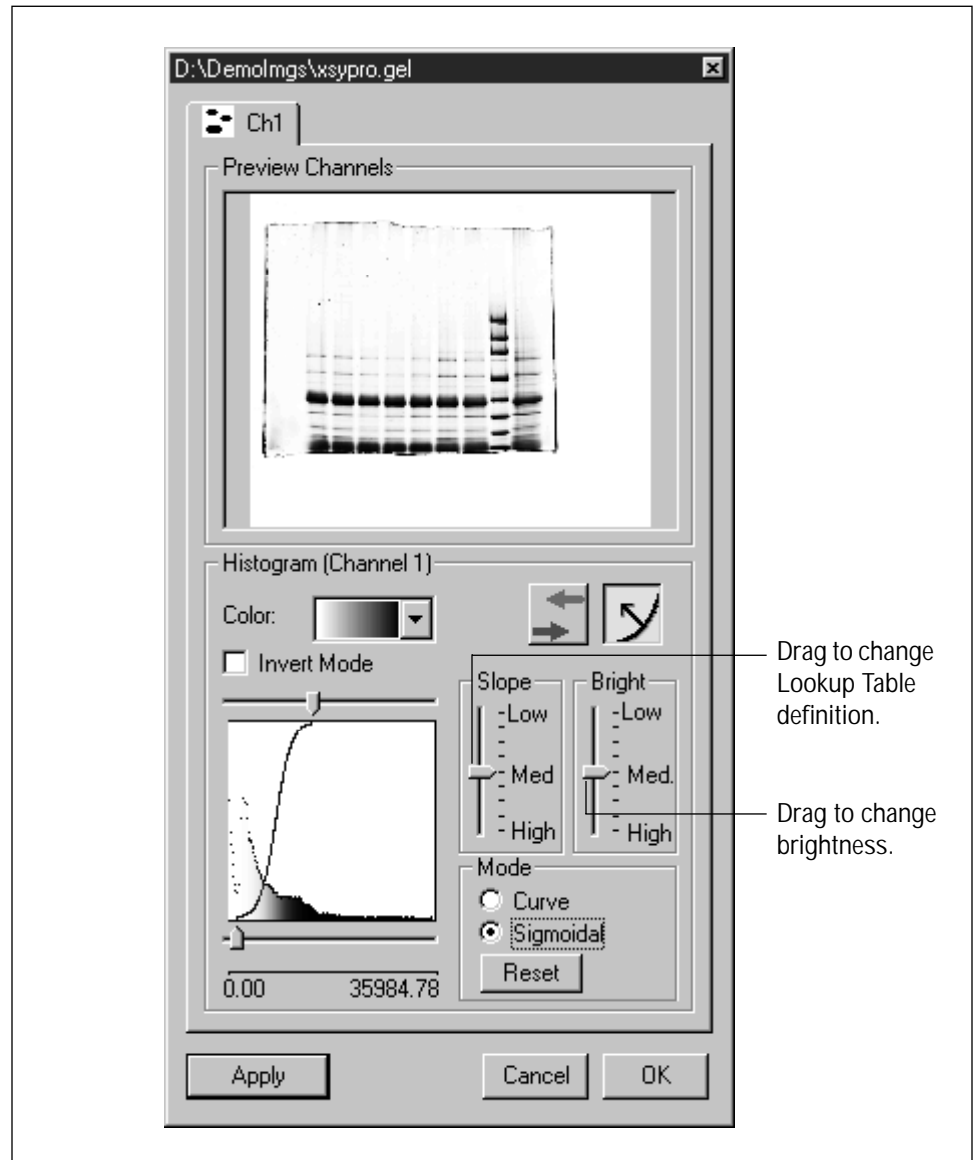
Figure 4-5. Sigmoidal controls in the Gray/Color Adjust window. The preview image and histogram plot are displayed using the medium-slope lookup table definition and the medium brightness control.

You drag the Slope slider to change from the default medium-slope lookup table to a high-slope or a low-slope lookup table—

- **Medium-Slope Lookup Table**—Displays all the intermediate image values with a moderate contrast using a moderate slope. Image values at the low and high intensities are displayed with moderate contrast in the sigmoidal transition regions (figure 4-5).

- **High-Slope Lookup Table**—Displays all the intermediate image values with a high contrast using the most severe slope. The image values at the low and high intensities are displayed with lower contrast in the sigmoidal transition regions (figure 4-6a).

- **Low-Slope Lookup Table**—Displays all the intermediate image values with a lower contrast using a mild slope. Image values at the low and high intensities are virtually suppressed in the sigmoidal transition regions (figure 4-6b).
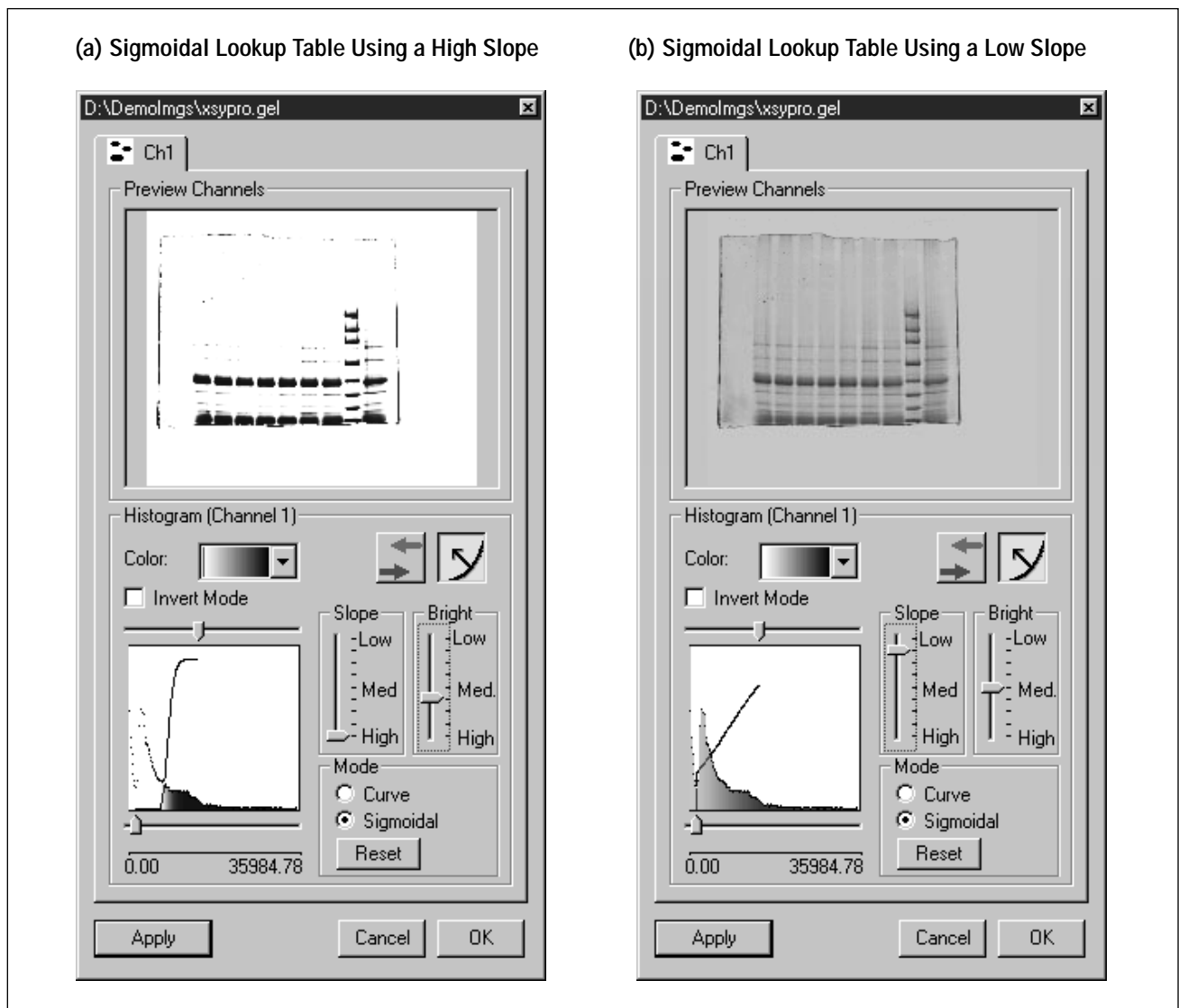
Figure 4-6. How changing the lookup table definition affects the preview image and histogram plot. Notice the changes when the medium-slope sigmoidal lookup table used in figure 4-5 is changed to the high-slope lookup table (a) and the low-slope lookup table (b).

Choose the lookup table definition that best enhances the image data. Use the preview image to evaluate how each option affects the display of the image data before applying the changes to the image in the Image window. Click **Reset** to return to the default linear display.

After you click Apply or OK, ImageQuant modifies the image in the Image window and saves the display values with the image.

Important **If you are viewing a multichannel image, make sure you check the display for each channel.**

4.6 Changing the Color

By default, ImageQuant uses gray scale to display single-channel images and side-by-side multichannel images.

ImageQuant displays overlaid multichannel images in the following default colors:

- **Two-Channel Image**—Channel 1 is green, and channel 2 is red.

- **Three-Channel Image**—Channel 1 is blue, channel 2 is green, and channel 3 is red.

- **Four-Channel Image**—Channel 1 is blue, channel 2 is green, channel 3 is yellow, and channel 4 is red.

You can change the color of a channel in a multichannel image by selecting a different color from the Color list. You see the change in the image in the Preview Channels area. After you click Apply or OK, ImageQuant modifies the image in the Image window. If you selected the same color used by another channel in the multichannel image, ImageQuant prompts you to select a different color. You cannot use the same color in two channels.

You can change a gray-scale image to color by selecting a color from the Color list. You see the change in the image in the Preview Channels area. After you click Apply or OK, ImageQuant modifies the image in the Image window.

Important   **ImageQuant does not save the color settings with the image.**

4.7 Inverting the Display of the Pixel Values

By default, ImageQuant displays the low pixel values of a gray-scale image in white and the high pixel values of a gray-scale image in black. If you want to reverse this display setting (negative to positive), click the **Invert** box.

Important   **Selecting Invert in one channel inverts all the channels of a multichannel image.**

You can invert the display of the pixel values for—

• Single-channel images displayed in gray scale.

• Single-channel images displayed in color, which changes the shades of the color. The lighter shades become darker, and the darker shades become lighter.

• Multichannel images with the channels displayed side by side in gray scale.

• Multichannel images displayed in the Overlay mode, which changes the shades of the colors. The lighter shades become darker, and the darker shades become lighter.

You see the change to the image in the Preview Channels area. After you click Apply or OK, ImageQuant modifies the image in the Image window and saves the inverted pixel values with the image.

Note: The ImageQuant Tools utility provides an invert feature that inverts the actual image values and not the display values. See the *ImageQuant Utilities User's Guide* for instructions.

Chapter 5 Creating Objects

Before you can quantitate the image, you must create an object or objects on the image. The object identifies the portion of the image you want to quantitate and defines the type of quantitation you can perform. You create linear objects for use in area quantitation and enclosed objects for use in volume quantitation.

The topics in this chapter are—

- Objects Used in Area Quantitation (section 5.1)
- Objects Used in Volume Quantitation (section 5.2)
- Selecting the Object Tool (section 5.3)
- Drawing an Object (section 5.4)
- Annotating the Image (section 5.5)

5.1 Objects Used in Area Quantitation

Area quantitation analyzes the pixel intensities along lines drawn on the image. The line types available for area quantitation are—

- **Line**—Analyze nonsmiling bands, dot blots, slot blots, or any bands or blots that are not distorted.

- **Wide Line**—Analyze bands that are well separated and straight. Wide lines are discussed in chapter 8.

- **Polyline**—Analyze smiling bands or data that do not lie in a straight line.

- **Wide Polyline**—Analyze bands that are well separated, but not aligned. Wide polylines are discussed in chapter 8.

5.2 Objects Used in Volume Quantitation

Volume quantitation analyzes pixel intensities within enclosed objects drawn on the image. The objects available for volume quantitation are—

- **Rectangle**—Analyze a rectangular-shaped band or a portion of the image containing several bands or lanes. You must use a rectangle if you are quantitating using the Spot Finder method.

- **Polygon**—Analyze an irregularly shaped spot.

- **Ellipse**—Analyze a dot blot, microplate well, or an elliptically shaped band.

- **Region**—Analyze an irregularly shaped spot, such as a tissue section. ImageQuant creates regions when you use the Auto Tracer tool.

- **Grid**—Analyze bands or dots that are displayed in regularly spaced rows and columns.

5.3 Selecting the Object Tool

You select object drawing tools in two ways—from the Object toolbar or from the Object menu (figure 5-1). After you select an object tool, you continue in the same draw mode until you select a different object tool or click **Select.**

Note: If the Object toolbar is not displayed, choose **Toolbars** from the View menu. In the Toolbars area of the Toolbar Configuration window, click the **Object** check box and then click **OK.**
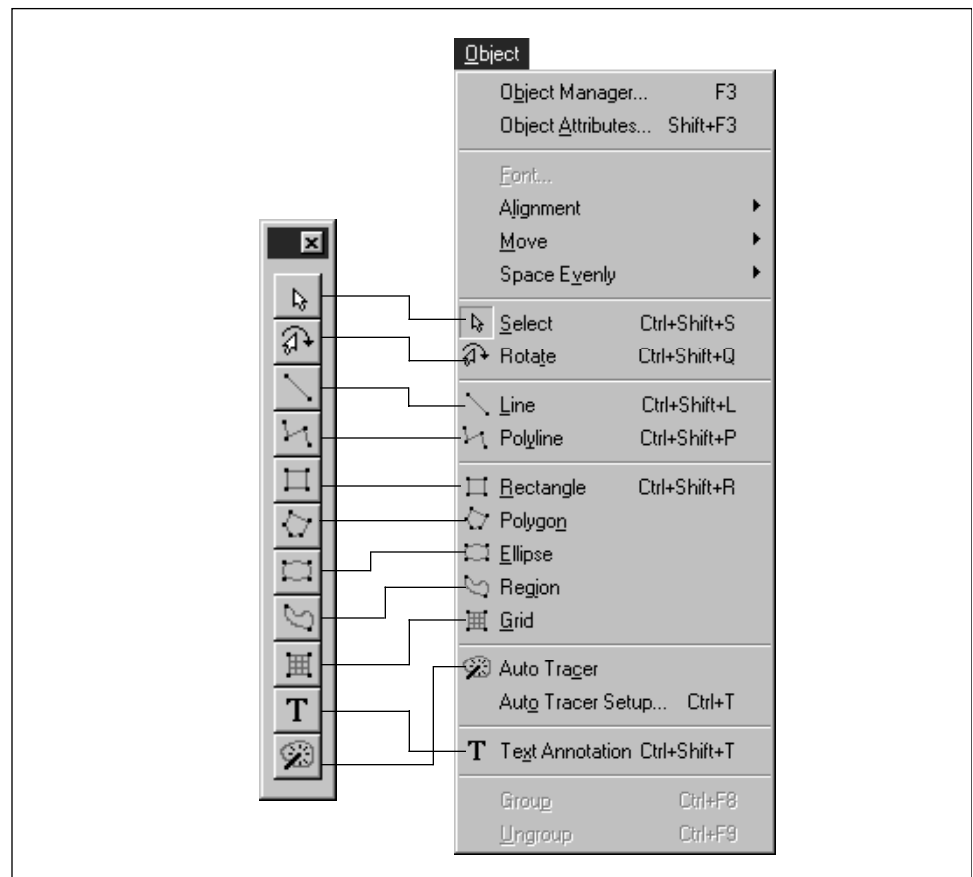
Figure 5-1. The Object toolbar and the Object menu.

5.4 Drawing an Object

In the Object menu, the object types are grouped by use. In this chapter, the object types are grouped by how you draw them— continuous, segmented, free form, or text. Chapter 6 discusses editing objects, including moving, rotating, and deleting. Chapter 7 discusses renaming, saving, and reusing the objects.

After you draw an object, ImageQuant assigns a number to the object. Normally, the number is displayed on the image, although this feature can be turned off (section 6.16). In most inspectors, the number is preceded by a four-letter object descriptor, such as Rect or Elps.

Click the **Select** button (🔼) to end the object draw mode.

5.4.1 Drawing a Continuous Object

Continuous object types are Line (⟍), Rectangle (⬜), Ellipse (⬭), and Grid (▦). You create them using one continuous movement of the pointer.

1. Choose the object from the Object menu or the Object toolbar.

    Note: If you choose **Grid,** the Define Grid Cells window appears. Enter the number of rows and columns for the grid and click **OK.**

2. Position the pointer at the starting location for the object.

3. Drag the pointer until it reaches the ending location. A thin line appears as you drag the pointer (figure 5-2).

    SHIFT+drag to—

    • Draw a square instead of a rectangle

    • Draw a circle instead of an ellipse

    • Draw a grid with square grid cells

    • Draw a line that snaps to one of the following angles: 22.5°, 45°, 67.5°, or 90°

4. Release the mouse button. The object appears on the image.
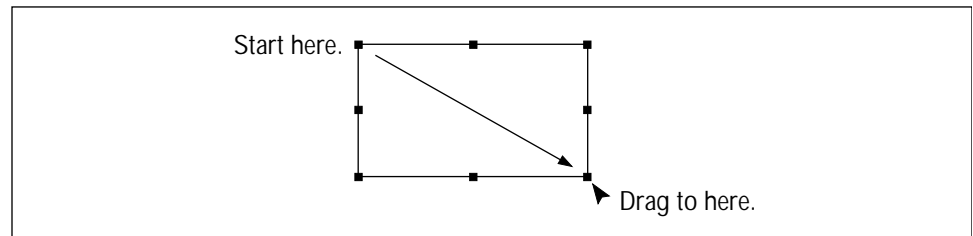
Figure 5-2. Drawing a rectangle object.

5.4.2 Drawing a Segmented Object

Segmented object types are Polyline (⬚) and Polygon (⬚). These objects consist of multiple points connected with lines.

1. Choose the object from the Object menu or the Object toolbar.

2. Position the pointer at the starting location for the object and click the mouse button.

    SHIFT+click to draw a line that snaps to one of the following angles: 22.5°, 45°, 67.5°, or 90°.

3. Move the pointer to the next location and click the mouse button again. A line connects the two points.

4. Continue to position the pointer and click the mouse button until you reach the last point, and then double-click to end the object (figure 5-3).

    Note: If you are drawing a polygon, you do not have to end at the starting position. ImageQuant automatically connects the first and last points.
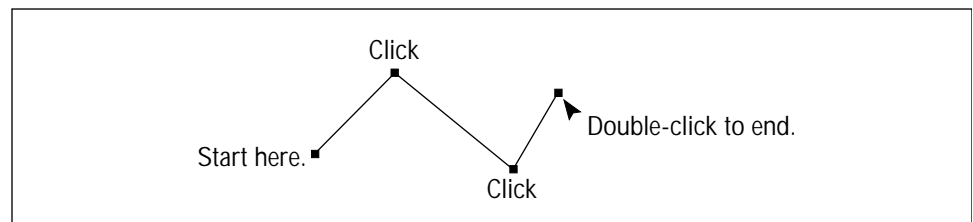
Figure 5-3. Drawing a polyline object.

5.4.3 Drawing a Free-Form Object

The Region tool (⟨icon⟩) creates a free-form object that follows the movement of the pointer. You can also create a region object using the Auto Tracer Tool (section 5.4.4).

To draw a region—

1. Choose **Region** from the Object menu or the Object toolbar.

2. Position the pointer at the starting location.

3. Drag the pointer in any direction. A continuous line follows the pointer.

4. Release the mouse button to end the object (figure 5-4). ImageQuant automatically connects the end points with a straight line to form an enclosed object.
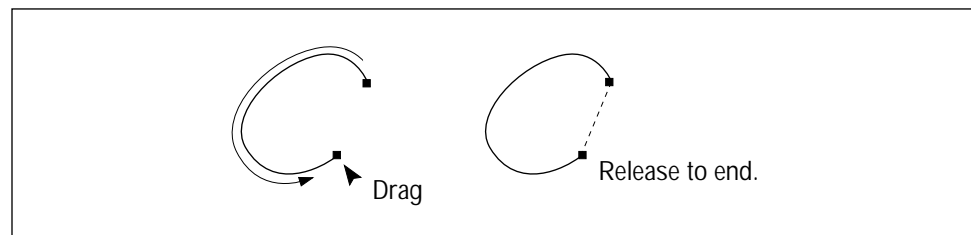
Figure 5-4. Drawing a region object.

5.4.4 Using the Auto Tracer Tool to Create a Region Object

To create a region object using the Auto Tracer Tool—

1. Choose **Auto Tracer** (⟨icon⟩) from the Object menu or the Object toolbar.

2. Position the pointer near the edge of a spot (figure 5-5).

3. SHIFT+click to identify the intensity value of the pixel under the pointer and use the value as the boundary of the spot.

Important   **The location of the pointer is critical when selecting the intensity value to use as a threshold. If you position the pointer too close to the spot, ImageQuant cannot accurately differentiate between the background pixels and the spot pixels. If ImageQuant creates a**

rectangular object around the spot, the threshold is too close to the spot pixel values. Move the pointer farther from the spot and repeat step 3.

4. Click the middle of the spot (figure 5-5). ImageQuant draws a region object around the spot.
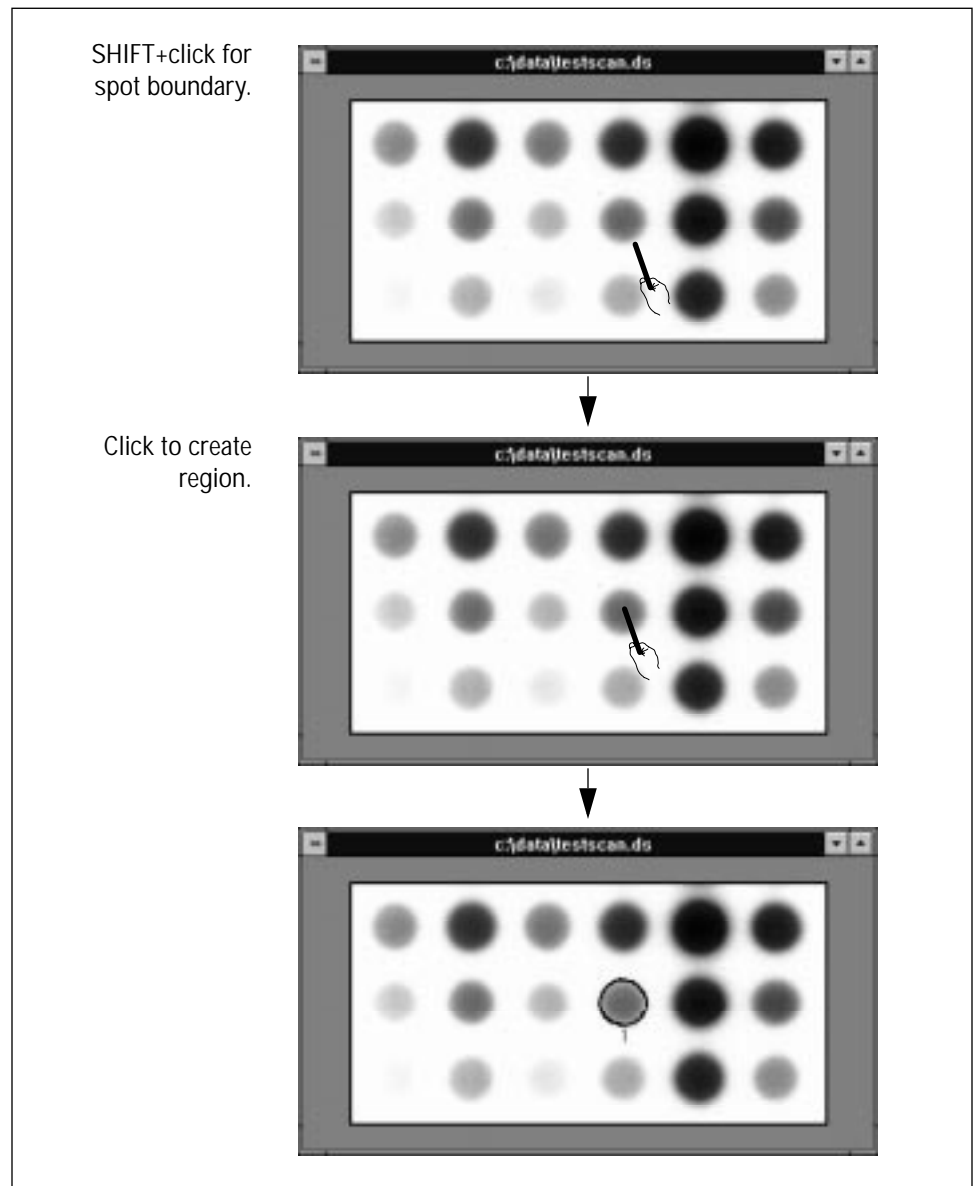
Figure 5-5. Using the Auto Tracer tool.

To view the threshold value used by the Auto Tracer tool, choose **Auto Tracer Setup** from the Object menu. The Auto Tracer window (figure 5-6) appears.
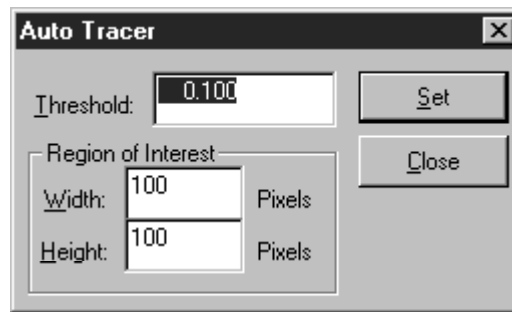
Figure 5-6. The Auto Tracer window.

Changing the width and height values sets maximum limits on the area evaluated by the Auto Tracer. For example, width and height values of 100 mean the Auto Tracer draws a region object around a spot located within an area 50 pixels from the pointer location.

5.5 Annotating the Image

The Text Annotation tool (**T**) allows you to add an annotation anywhere on the image. Although ImageQuant assigns a number to the Text Annotation object, the number does not appear in the Image window. You will see the number in the Object Attributes and Object Manager inspectors.

1. Choose **Text Annotation** from the Object menu or the Object toolbar.

2. Click the starting location for the annotation. The Input Text window appears (figure 5-7).

3. Choose an orientation for the text.

4. Type your annotation in the Text box.

5. Click the **Font** button to open the Font window.

6. Choose a font, font style, size, and color for the text, and then click **OK.** The Font window closes.

7. Click **OK** in the Input Text window. The text appears in the Image window to the right of the position you selected in step 2.
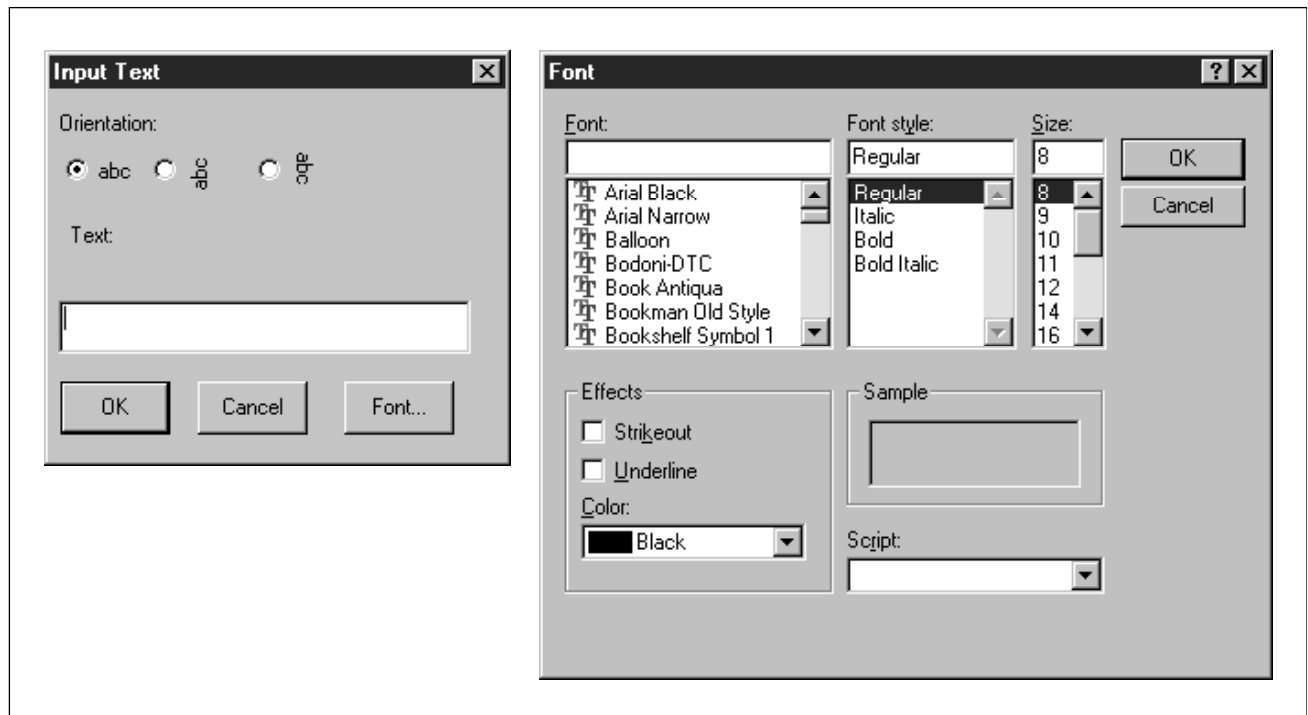
Figure 5-7. Annotating an image and selecting the font attributes for the text.

Chapter 6 Editing an Object

ImageQuant provides many functions for editing the objects you create. These functions give you the flexibility to change an object instead of deleting it and creating a new one. Two inspectors are associated with objects—the Object Attributes inspector and the Object Manager inspector.

The topics in this chapter are—

- Selecting an Object to Edit (section 6.1)
- Showing and Hiding Objects (section 6.2)
- Grouping and Ungrouping Objects (section 6.3)
- Deleting an Object (section 6.4)
- Moving an Object (section 6.5)
- Aligning Objects (section 6.6)
- Spacing Objects Evenly (section 6.7)
- Cutting and Copying Objects (section 6.8)
- Copying and Moving an Object (section 6.9)
- Duplicating an Object (section 6.10)
- Rotating an Object (section 6.11)
- Resizing an Object (section 6.12)
- Reshaping a Polygon or Polyline (section 6.13)
- Changing the Line Weight of an Object (section 6.14)
- Changing the Color of an Object (section 6.15)
- Displaying an Object Name (section 6.16)
- Adding a Comment (section 6.17)
- Changing the Attributes for Multiple Objects (section 6.18)
- Modifying a Grid (section 6.19)
- Modifying a Text Annotation Object (section 6.20)

6.1 Selecting an Object to Edit

Before you can edit an object, you must select it. You can select either a single object or multiple objects.

Note: Before you can select an object, you must activate the Image window or channel of a multichannel image by clicking in it.

6.1.1 Selecting a Single Object

Choose **Select** (🔖) from the Object menu or the Object toolbar. Click any object in the Image window. To avoid resizing the object, click the object name or object comment. When you select an object, handles appear on the object.

6.1.2 Selecting Multiple Objects One at a Time

Choose **Select** (🔖) from the Object menu or the Object toolbar. Click the first object and then SHIFT+click to select the other objects.

Note: To deselect a selected object, SHIFT+click the object. To deselect all the selected objects, click in the Image window where there are no objects.

6.1.3 Selecting Objects Using a Selection Box

Choose **Select** (🔖) from the Object menu or the Object toolbar. Drag the mouse to intersect or surround the objects with the selection box (figure 6-1). Release the mouse button. Handles appear on all selected objects.
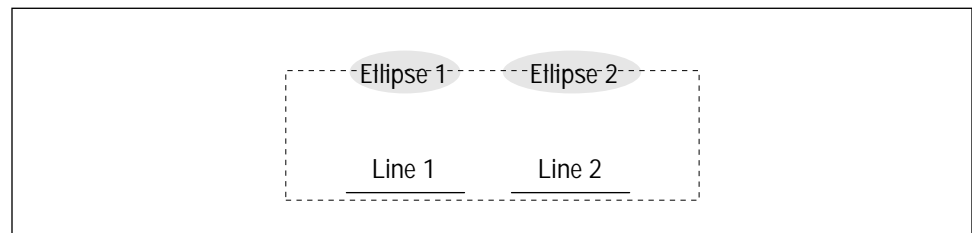
Figure 6-1. Selecting objects by intersecting or surrounding them with a selection box.

6.1.4 Selecting All Objects

Choose **Select All** from the Edit menu. Every object in the Image window is selected, including any hidden objects. (SHIFT+click to deselect specific objects.)

6.1.5 Selecting Objects Using the Object Manager Inspector

Choose **Object Manager** from the Object menu. The Object Manager inspector appears. Click a single object in the Object list (figure 6-2). To add additional objects, CONTROL+click each object. If the objects are adjacent, drag to select them.

Note: Use this procedure to select from a list in any inspector.

6.2 Showing and Hiding Objects

You use the Hide command on the Object Manager inspector (figure 6-2) to remove objects from the image display temporarily. You use the Show command to redisplay them.

Note: Hide does not delete the objects.
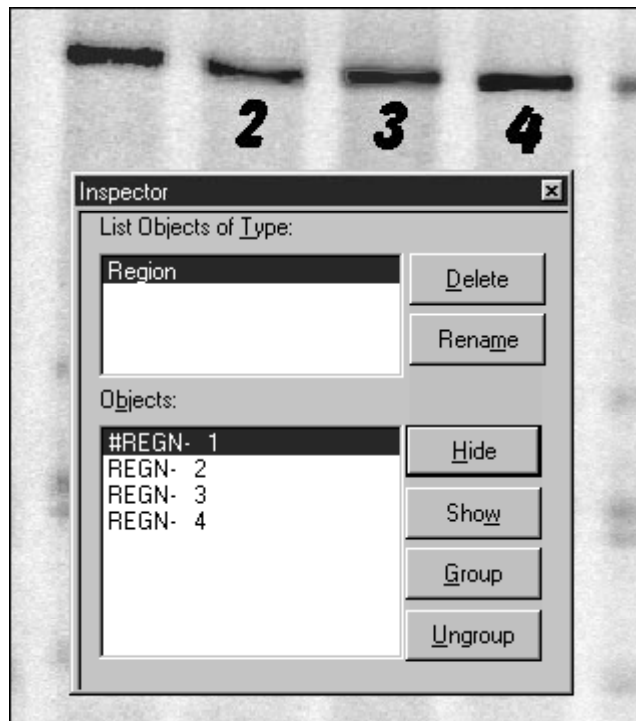
Figure 6-2. Using the Object Manager inspector to hide region 1.
Note: The region objects were created using the Auto Tracer tool.

1. Choose **Object Manager** from the Object menu. The Object Manager inspector appears.

2. Move the pointer to the **Objects** list. Click the object you want to hide. To add additional objects, CONTROL+click each object.

3. Click **Hide.** The objects are temporarily removed from the display. The hidden object displays a pound sign (#) in front of the object name.

4. To redisplay objects, select the hidden objects from the Objects list, and then click **Show.**

Important   **If you do not see the hidden object in the Objects list, make sure the correct object type is selected in the List Object of Type list.**

6.3 Grouping and Ungrouping Objects

You can group objects together, and then move or rotate them as a group, save them as a group, or perform quantitations on the group. You can group and ungroup objects from the Object menu or from the Object Manager inspector.

6.3.1 Grouping Objects

1. Choose **Select** (⬚) from the Object menu or the Object toolbar.

2. Select the objects you want to group.

3. Choose **Group** from the Object menu, or click the **Group** button in the Object Manager inspector (figure 6-3).

In the Image window, a dashed box appears around the grouped objects. In the Object Manager inspector, the individual object names are replaced with the group name, GRUP, followed by the next available number.
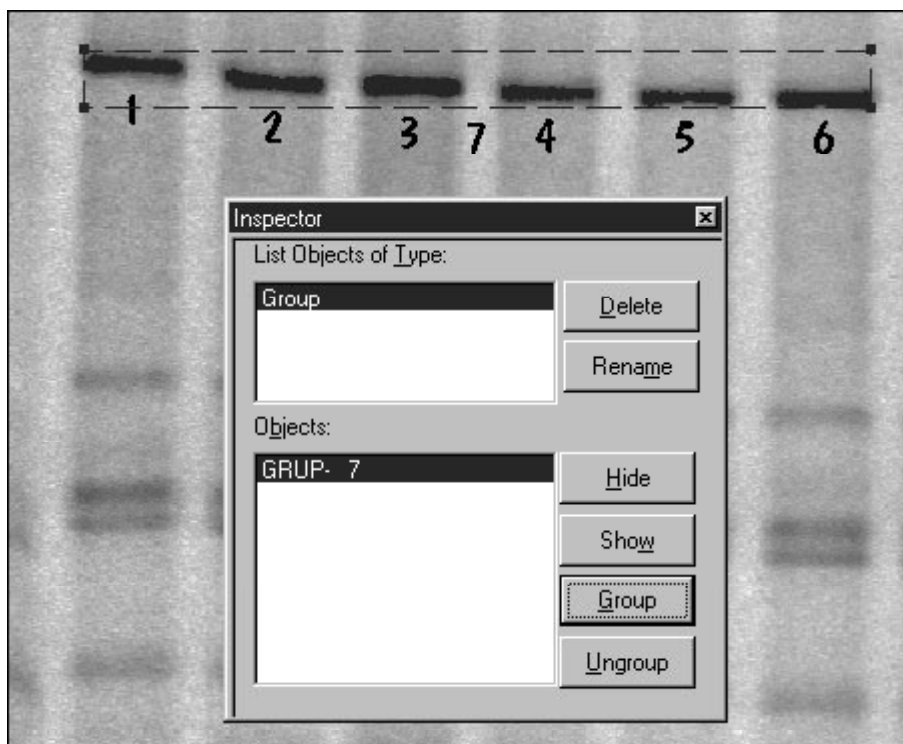
Figure 6-3. Using the Object Manager inspector to group the six region objects.

6.3.2 Ungrouping Objects

1. Choose **Select** (⬚) from the Object menu or the Object toolbar.

2. Select the group you want to ungroup.

3. Choose **Ungroup** from the **Object** menu, or click the **Ungroup** button in the Object Manager inspector (figure 6-3).

In the Image window, the dashed box disappears. In the Object Manager inspector, the group name disappears and the individual object names reappear.

6.4 Deleting an Object

To delete an object—

1. Choose **Select** (⬚) from the Object menu or the Object toolbar.

2. Select the object (or objects) you want to delete.

3. Choose **Delete** from the Edit menu or the Object Manager inspector. Click **Yes** in the confirmer window. ImageQuant deletes the object.

Note: If you delete a previously saved object by mistake, you can retrieve it. To do this, close the Image window without saving the modifications. (You will lose all the changes you made after the Image window was opened or saved.) When you open the Image window, the object will be listed in the Objects list.

6.5 Moving an Object

ImageQuant provides two ways to move objects. You can move an object by dragging the object to another location, or you can move an object a specific number of pixels using the Move command on the Object menu.

6.5.1 Dragging an Object to a New Location

To move an object to a new location—

1. Choose **Select** (⬚) from the Object menu or the Object toolbar.

2. Click the object you want to move.

3. Position the pointer on the object name, and drag the object to a new location (figure 6-4).

Drag to move object.
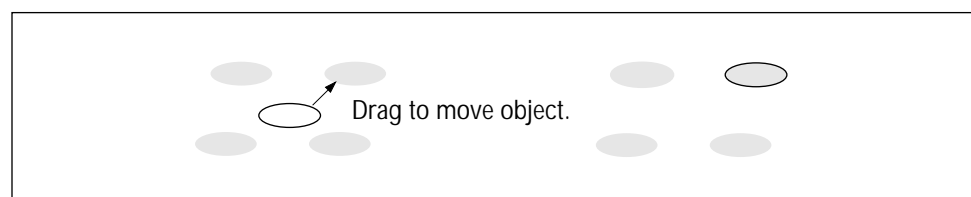
Figure 6-4. Moving the selected object.

6.5.2 Moving an Object Using the Move Command

You use the Move command on the Object menu to move an object or objects a specific number of pixels. The Move command displays a submenu with the following commands:

* **Left**—Moves the selected object one pixel to the left.

* **Right**—Moves the selected object one pixel to the right.

* **Up**—Moves the selected object up one pixel.

* **Down**—Moves the selected object down one pixel.

* **Fast Left**—Moves the selected object 30 pixels to the left.

* **Fast Right**—Moves the selected object 30 pixels to the right.

* **Fast Up**—Moves the selected object up 30 pixels.

* **Fast Down**—Moves the selected object down 30 pixels.

To move an object to a new location using the Move command and submenu—

1. Choose **Select** (⬚) from the Object menu or the Object toolbar.

2. Click the object (or objects) you want to move.

3. Choose **Move** from the Object menu, and then choose a direction from the Move submenu. The object (or objects) moves using the command you chose.

6.6 Aligning Objects

You can reposition selected objects so that they align. The Alignment command displays a submenu with the following commands:

* **Left**—Aligns the selected objects using the object that is closest to the left side of the image.

* **Right**—Aligns the selected objects using the object that is closest to the right side of the image.

- **Top**—Aligns the selected objects using the object that is closest to the top of the image.

- **Bottom**—Aligns the selected objects using the object that is closest to the bottom of the image.

- **Top/Bottom Center**—Aligns the selected objects using the midpoint between the selected object that is closest to the top of the image and the selected object that is closest to the bottom of the image.

- **Left/Right Center**—Aligns the selected objects using the midpoint between the selected object that is closest to the left side of the image and the selected object that is closest to the right side of the image.

Figure 6-5 shows the selected objects before alignment and after alignment using the Top/Bottom Center command.
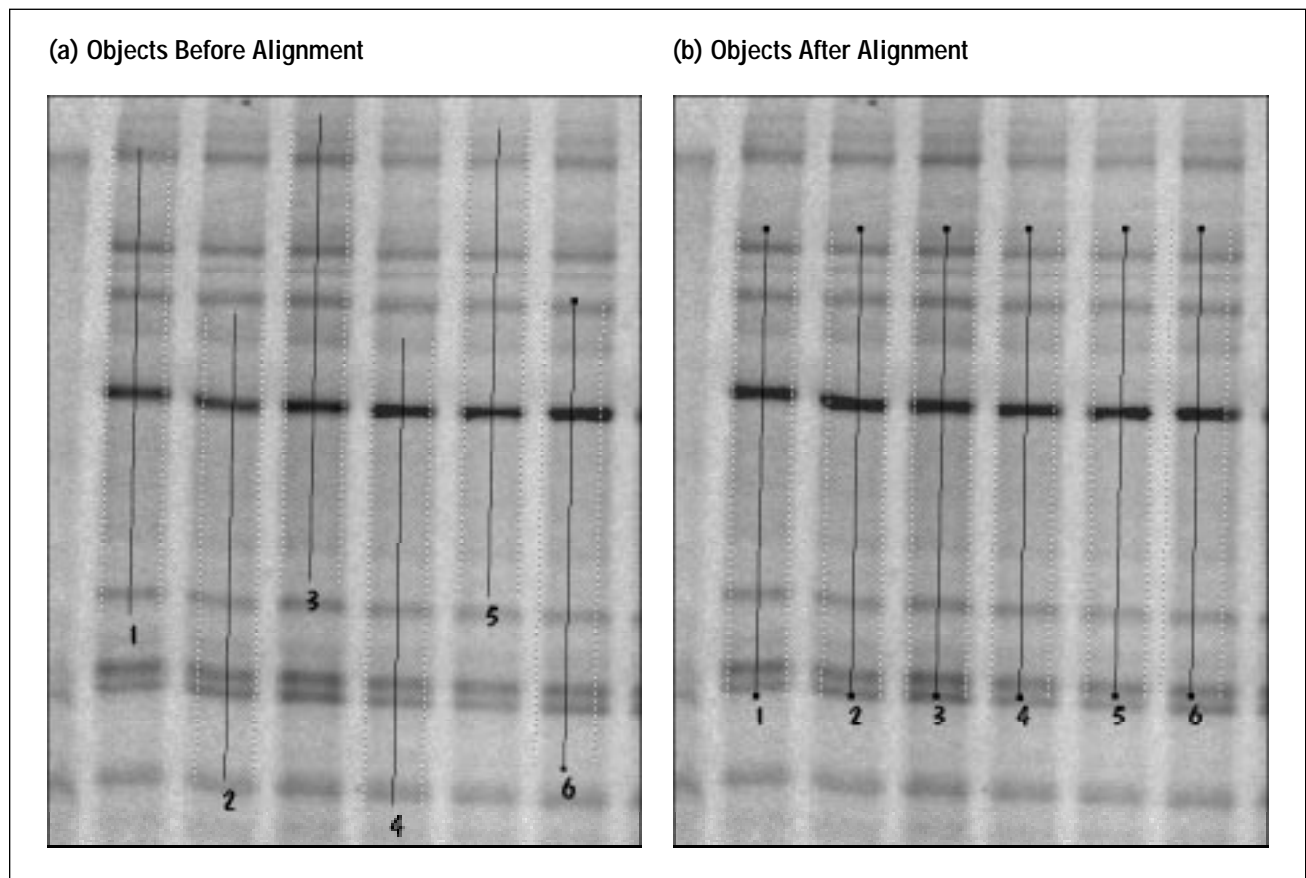
Figure 6-5. Aligning objects using the Top/Bottom Center command.

To align objects—

1. Choose **Select** (🔧) from the Object menu or the Object toolbar.

2. Select the objects you want to align.

3. Choose **Alignment** from the Object menu, and then choose a direction from the Alignment submenu. ImageQuant aligns the objects using the command you chose.

6.7 Spacing Objects Evenly

You can select objects and space them evenly between the objects that are farthest apart. You must select three or more objects to use the Space Evenly command and submenu. You can choose to space the objects Across or Down.

If you are analyzing the bands of an evenly spaced electrophoresis gel, you can use the Space Evenly command to align the rectangles or lines over the lanes.

To space objects evenly—

1. Choose **Select** (🔧) from the Object menu or the Object toolbar.

2. Select the objects you want to space evenly.

3. Choose **Space Evenly** from the Object menu, and then choose **Across** or **Down** from the Space Evenly submenu. ImageQuant aligns the objects using the command you chose (figure 6-6).

(a) Objects Before Using the Space Evenly Command

(b) Objects After Using the Space Evenly/Across Command
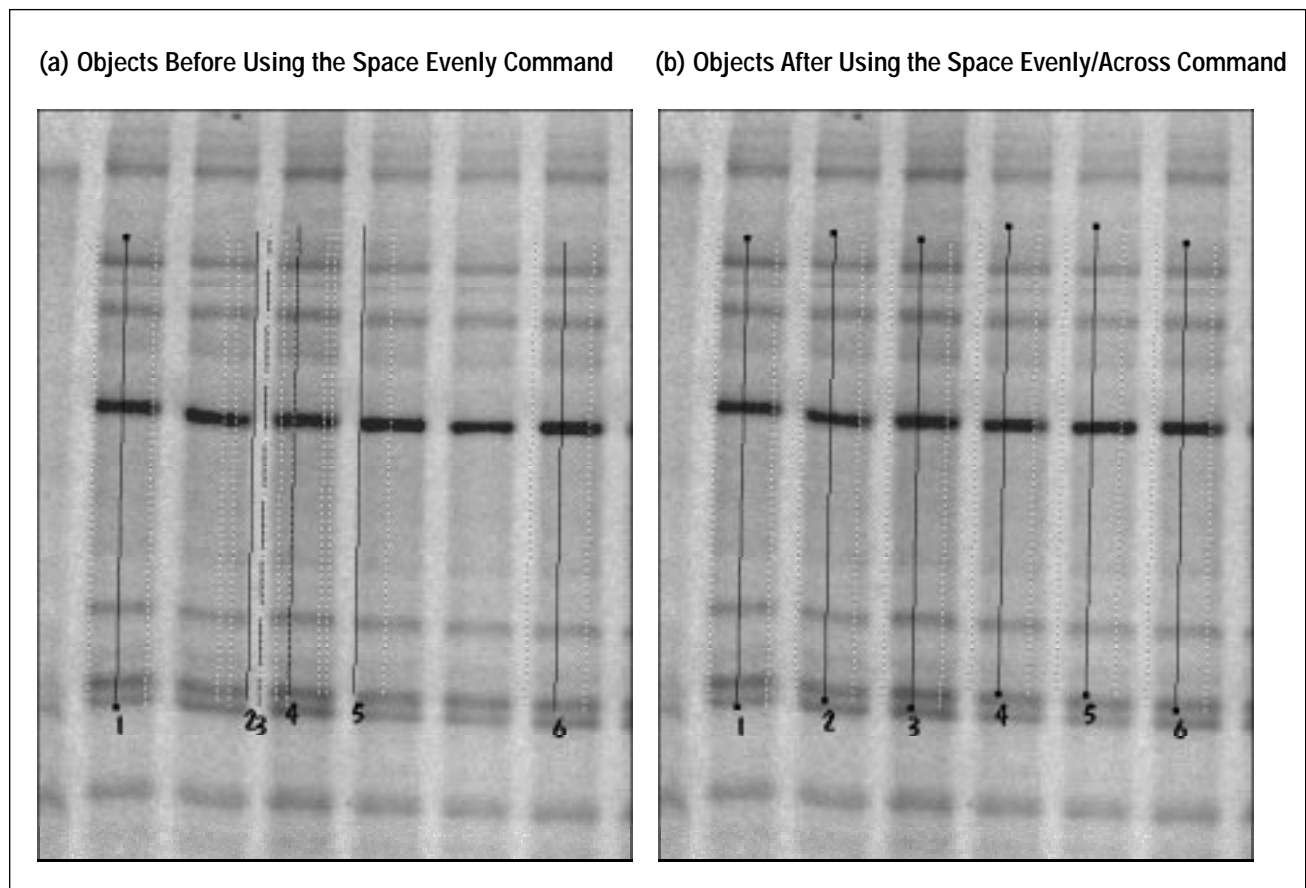
Figure 6-6. Using the Space Evenly/Across command to change the spacing between the selected objects.

6.8 Cutting and Copying Objects

You can cut or copy objects displayed on an image and paste them on the same or another image displayed in ImageQuant 5.0. Cut removes the object from the display, and Copy Object(s) makes a copy of it. When you cut or copy an object, it is placed in a clipboard. The object stays in the clipboard until you cut or copy another object.

6.8.1 Cutting or Copying Objects Within the Same Image Window

1.   Choose **Select** (⬚) from the Object menu or the Object toolbar.

2.   Select the object or objects you want to cut or copy.

3. Choose **Cut** (![cut icon]) or **Copy Object(s)** (![copy icon]) from the Edit menu, the pop-up menu, or the Main toolbar.

4. Choose **Paste** (![paste icon]) from the Edit menu, the pop-up menu, or the Main toolbar. The object(s) appears at the same location as the original.

5. Drag the object to a new location.

6.8.2 Cutting or Copying Objects Between Image Windows

Before you copy objects between Image windows, verify that both images were scanned using the same pixel size (see your instrument user's guide for available pixel sizes). To determine the pixel size, select one Image window, choose **Image Properties** from the File menu. Make a note of the value in the Pixel Size box on the Scan Info tab. Repeat for the second Image window. If the pixel size is larger in the receiving image, the pasted object will be smaller. If the pixel size is smaller, the pasted object will be larger.

1. Choose **Select** (![select icon]) from the Object menu or the Object toolbar.

2. Select the object or objects.

3. Choose **Cut** (![cut icon]) or **Copy Object(s)** (![copy icon]) from the Edit menu, the pop-up menu, or the Main toolbar.

4. Move the pointer anywhere in the second Image window. Click to activate the window (or channel of a multichannel image).

5. Choose **Paste** (![paste icon]) from the Edit menu, the pop-up menu, or the Main toolbar. The object appears at the corresponding location on the second Image window (or channel of a multichannel image).

6.9 Copying and Moving an Object

You can copy an object and move the copied object using the keyboard and mouse. The original object does not move. ImageQuant assigns the next available object number to the copy you create. If the object name is the default number assigned by ImageQuant, ImageQuant uses the next available unique number. If you renamed the object, ImageQuant adds a unique number to the end of the name.

To copy and move an object—

1. Choose **Select** ( ) from the Object menu or the Object toolbar.

2. Hold down the **CONTROL** key.

3. Position the pointer on the object you want to copy.

4. Drag the pointer to where you want to position the copy.

5. When you release the mouse button, the copy appears at the pointer location.

6. Repeat steps 2 through 5 to create more copies of the object.

6.10 Duplicating an Object

You can duplicate an object using the Duplicate command on the Edit menu, or using the mouse and keyboard.

6.10.1 Duplicating an Object Using the Duplicate Command

You can make exact duplicates of the selected object using the Duplicate command on the Edit menu. ImageQuant displays the new object or objects offset from the original. ImageQuant assigns the next available number to each object. If the object name is the default number assigned by ImageQuant, ImageQuant uses the next available unique number. If you renamed the object, ImageQuant adds a unique number to the end of the name.

1. Choose **Select** ( ) from the Object menu or the Object toolbar.

2. In the Image window, select the object or objects you want to duplicate. Handles appear on the object(s).

3. Choose **Duplicate** from the Edit menu. A copy of each selected object appears offset from the original.

4. Drag the object(s) to a new location.

6.10.2 Duplicating and Moving an Object Using the Mouse and Keyboard

You can make exact duplicates of the selected object and move each one to a new location using the following mouse-keyboard combination.

1. Choose **Select** ( ) from the Object menu or the Object toolbar.

2. Click an object to select it.

3. Hold down the mouse button and press the **v** key to duplicate the object. Do not release the mouse button.

4. Drag the original object to a new location.

5. To make additional copies, continue to press the **v** key and drag the object.

6.11 Rotating an Object

All objects except text annotation objects can be rotated using one of the following methods: rotate continuously using the pointer (section 6.11.1) or rotate a specified amount using the Object Attributes inspector (section 6.11.2). To rotate a text annotation object, use the procedure in section 6.20.

6.11.1 Rotating an Object Using the Pointer

1. Choose **Rotate** ( ) from the Object menu or the Object toolbar.

2. Select the object to rotate.

3. Position the pointer in the object and drag the pointer in any direction. As you drag the pointer, the object revolves around a rotation square that appears in the center of the object (figure 6-7).

4. Release the mouse button when the object is in the desired position.
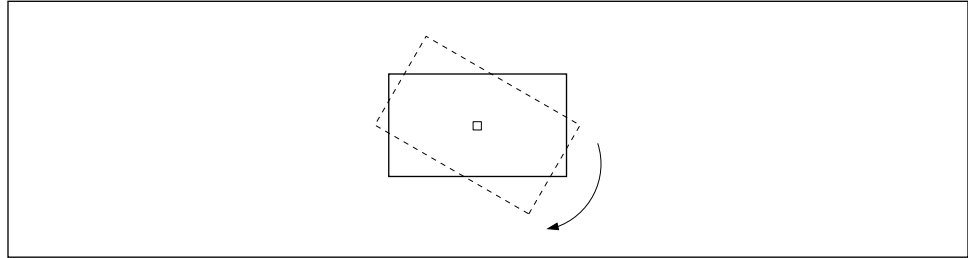
Figure 6-7. Rotating the object using the Object toolbar button or the Object menu.

6.11.2 Rotating an Object a Specified Amount

1. Choose **Object Attributes** from the Object menu, or double-click the object. The Object Attributes inspector appears.

2. Select the object on the Image window. The attributes for that object appear in the Object Attributes inspector.

3. In the **Rotation** box, type the number of degrees you want the object to rotate (figure 6-8).

    Type a positive number to move the object clockwise; type a negative number to move it counterclockwise.

4. Click **Set.** The selected object rotates.

(a) Wideline Before Rotation

(b) Wideline After Using Object Attributes Inspector to Rotate Object 2 Degrees to Match Lane
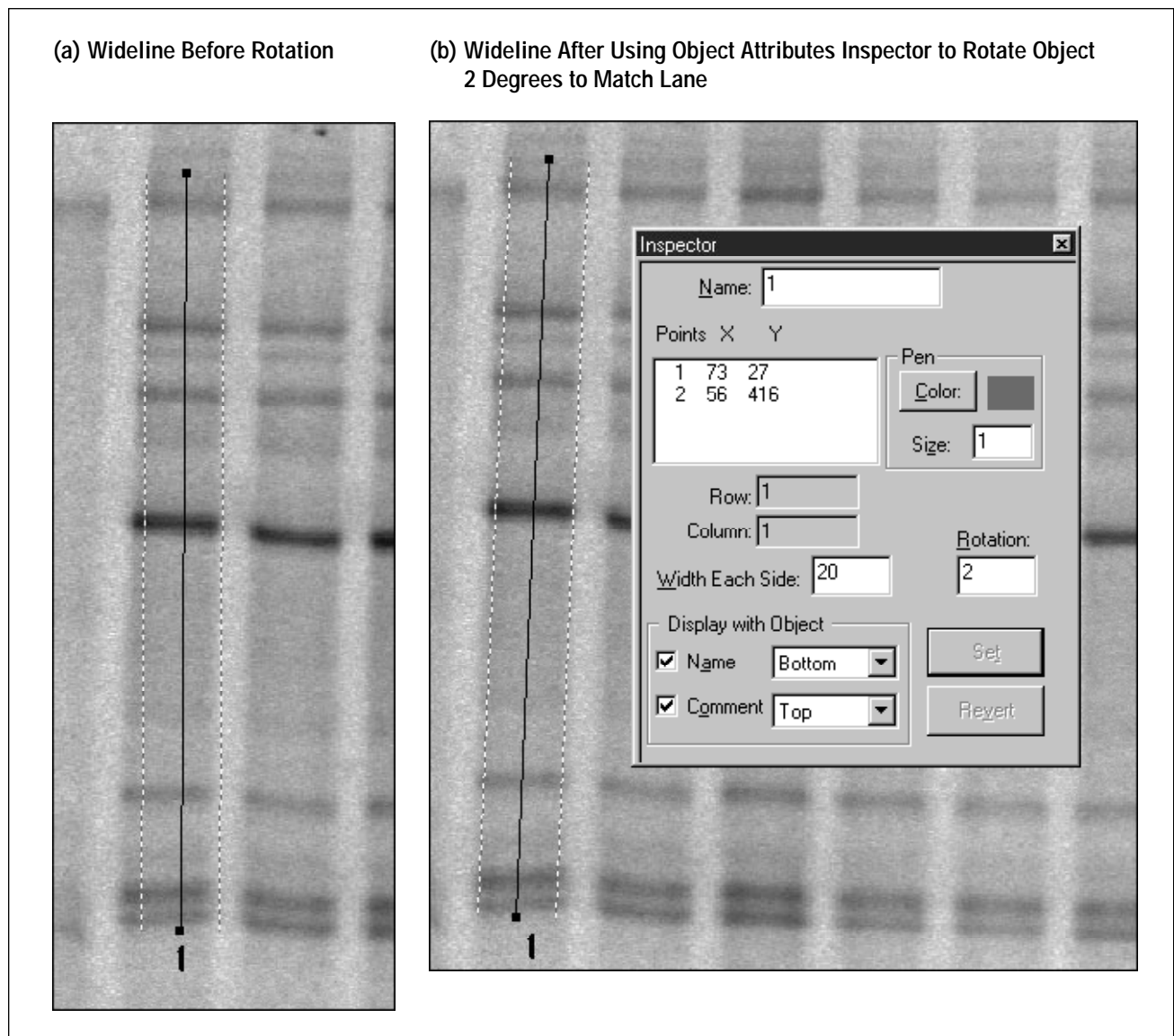
Figure 6-8. Rotating the object a specified amount.

6.12 Resizing an Object

To resize lines, rectangles, and ellipses—

1. Choose **Select** (⇡) from the Object menu or the Object toolbar.

2. Select the object to resize.

3. Position the pointer on one of the handles.

4.   Drag the pointer in the direction you want the object resized
     (figure 6-9).

5.   Release the mouse button when you reach the desired size.

6.   Move the object if it is out of position.
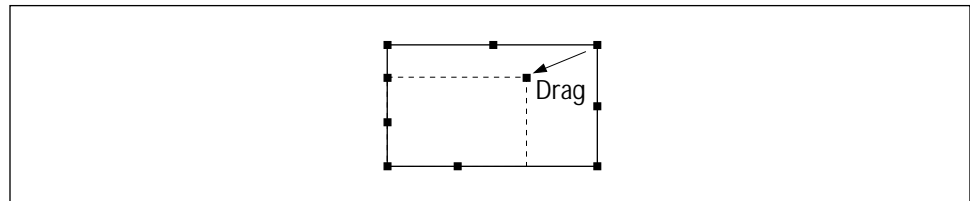
Figure 6-9. Resizing the selected object.

6.13 Reshaping a Polygon or Polyline

You can reshape a polygon or polyline object by moving, adding, or
deleting handles.

6.13.1 Moving a Handle

1.   Choose **Select** (⬚) from the Object menu or the Object toolbar.

2.   Select the object to reshape.

3.   Position the pointer over a handle.

4.   Drag the handle to a new location (figure 6-10).

5.   Release the mouse button when the object is the desired shape.
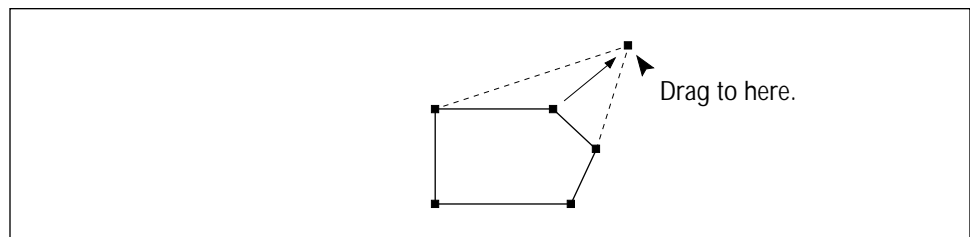
Figure 6-10. Reshaping the object by moving a handle.

6.13.2 Adding a Handle

1. Choose **Select** ( ) from the Object menu or the Object toolbar.

2. Select the object.

3. Position the pointer on the line where you want to add the handle.

4. To add a handle—

   Windows NT—Hold down the mouse button and press the **INSERT** key.

   Macintosh—Hold down the mouse button and the **SHIFT** key, and then press the **INSERT** key.

   A handle appears at that location (figure 6-11). After you insert the handle, you can move it using the steps in section 6.13.1.
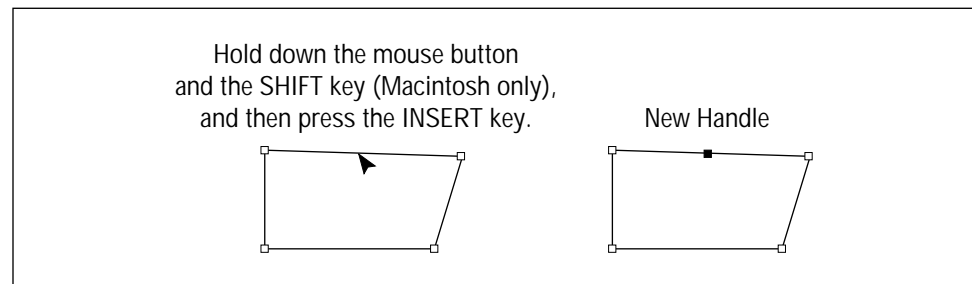
Figure 6-11. Adding a handle to an object.

6.13.3 Deleting a Handle

1. Choose **Select** ( ) from the Object menu or the Object toolbar.

2. Select the object.

3. Place the pointer on the handle you want to delete, and hold down the mouse button.

Important   **If you do not position the pointer on the handle, the entire object is deleted.**

4. Press the **DELETE** key (Macintosh: in the Special Keys area). The handle disappears and the object appears in the new shape (figure 6-12).

With the pointer on the handle, hold down mouse button, and then press the DELETE key.
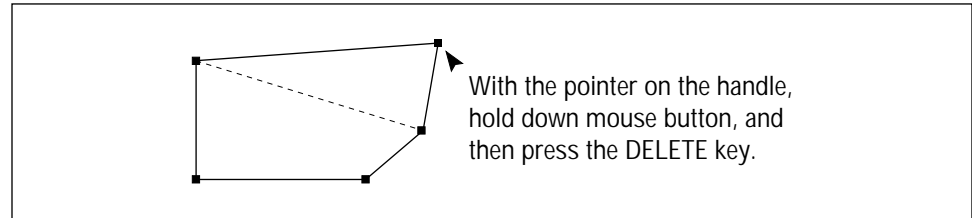
Figure 6-12. Reshaping the object by deleting a handle.

6.14 Changing the Line Weight of an Object

The Object Attributes inspector controls the line weight (pen size) for the selected object. You can specify weights from 1 to 9 pixels.

Note: Changing the pen size helps you see the object on the image. It does not affect quantitation.

1. Choose **Object Attributes** from the Object menu, or double-click the object. The Object Attributes inspector appears.

2. Select the object in the Image window.

3. In the Pen area, highlight the value displayed in the **Size** box (figure 6-13).

4. Type a new value (1 to 9).

5. Click **Set.** The object changes to reflect the new line weight.
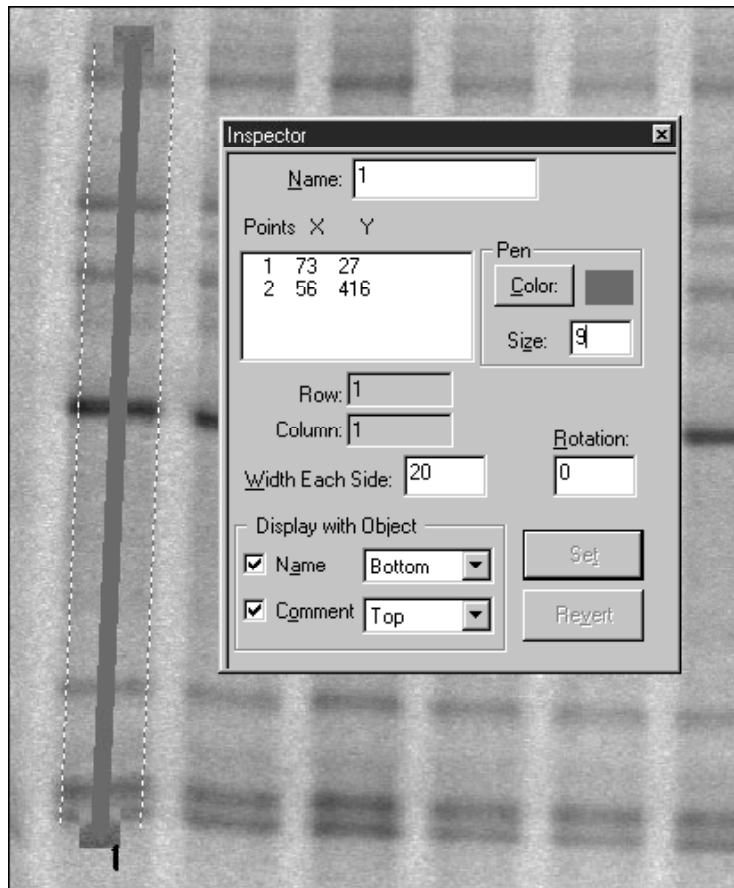
Figure 6-13. Changing the line weight (pen size) of an object.

6.15 Changing the Color of an Object

The Object Attributes inspector controls the color of the selected object. You can change the color by using the Color button in the Pen area.

1. Choose **Object Attributes** from the Object menu, or double-click the object. The Object Attributes inspector appears.

2. Select the object in the Image window.

3. In the Pen area, click the **Color** button. The Color window appears (figure 6-14).

4. Click the desired color, and then click **OK.**

5. Click **Set.** The object changes to the new color.
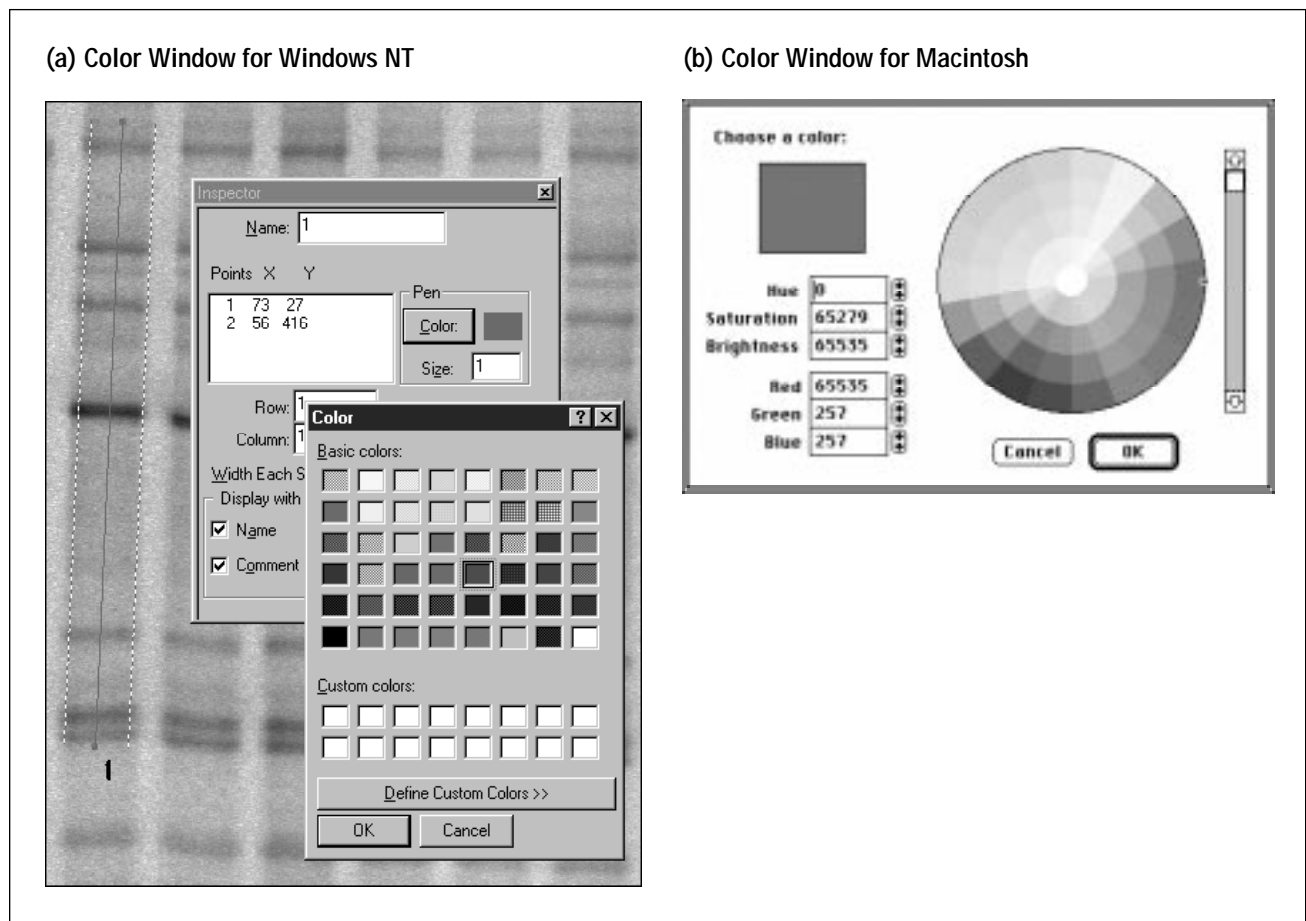
Figure 6-14.   Changing the color of an object.

6.16 Displaying an Object Name

ImageQuant assigns a number to each object when the object is created. If the number is not displayed with the object, you can use the Object Attributes inspector to display it. In addition, you can use the Object Attributes inspector to change the number to a more meaningful name.

To do this—

1. Choose **Object Attributes** from the Object menu, or double-click the object. The Object Attributes inspector appears.

2. Select the object in the Image window.

3. In the **Display with Object** area of the inspector, click the **Name** check box. (A check mark in the box selects the option to display the name. No check mark in the box deselects the option.)

4. (Optional) Double-click **Name** in the Display with Object area. The Modified Name window appears. Type a new name and choose an orientation for the text. In addition, click the **Font** button and change the text attributes using the Font window. Click **OK** to close each window.

5. Choose a new position from the Name list (figure 6-15).

6. Click **Set.** The object name appears on the object in the position you selected.
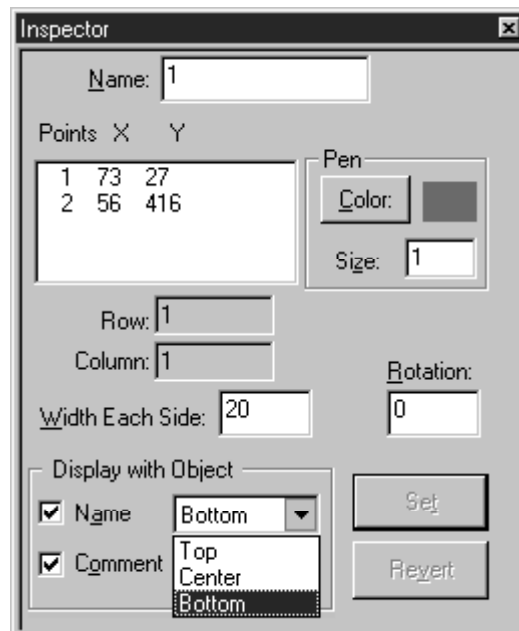
Figure 6-15. Choosing a new position for the object name.

6.17 Adding a Comment

In addition to a name, you can add and display a comment for each object.

1. Choose **Object Attributes** from the Object menu, or double-click the object. The Object Attributes inspector appears.

2. Select the object in the Image window.

3. In the **Display with Object** area of the inspector, double-click **Comment.** A check mark appears in the Comment check box, and the Modified Comments text window appears (figure 6-16).

4. Type the comment in the box.

5. (Optional) Choose an orientation for the text.

6. (Optional) Click the **Font** button. The Font window appears. Change the text attributes and click **OK** to close the Font window.

7. Click **OK.** The Modified Comments window closes.

8. Choose a new position from the Comment list.

    Note: Select a different position from Name or the two labels will be superimposed.

9. Click **Set.** The comment appears on the object in the position you selected.
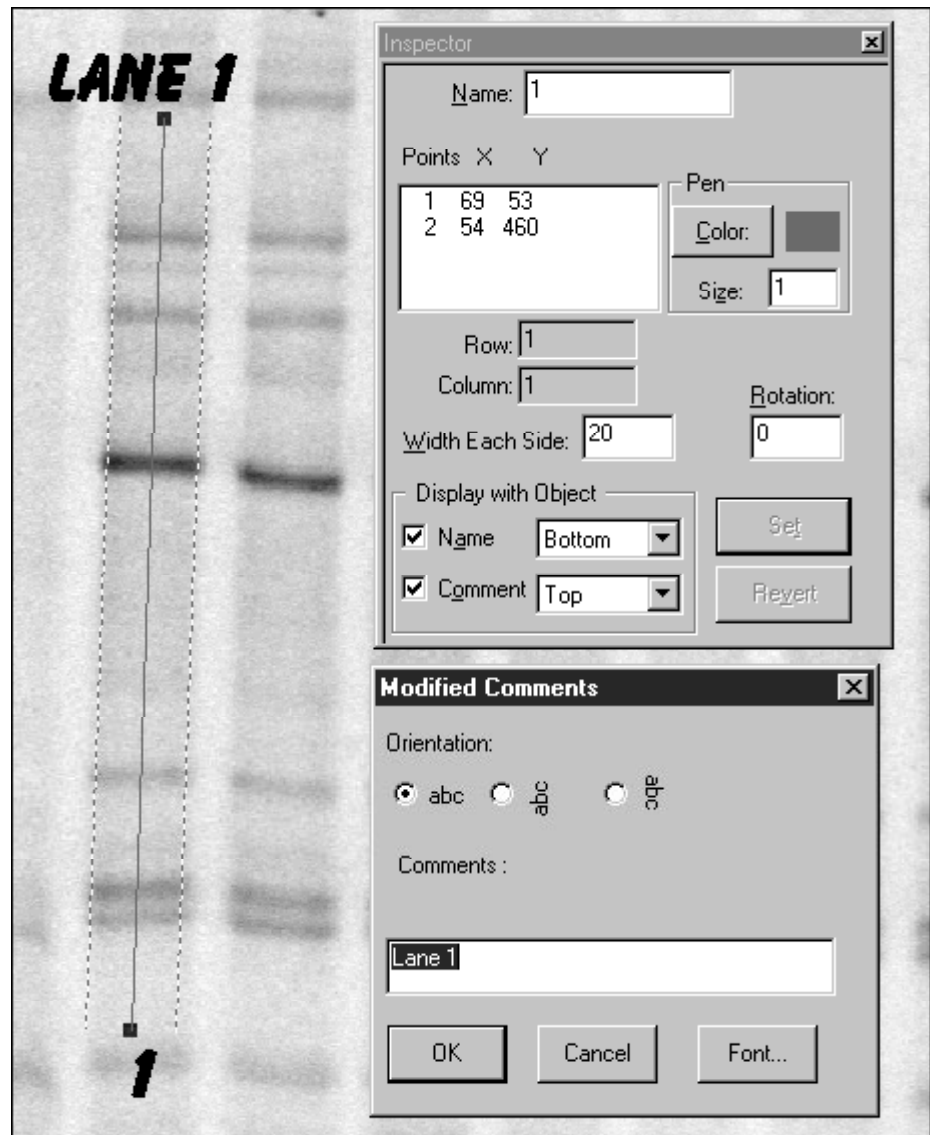
Figure 6-16. Displaying a comment on the object.

6.18 Changing the Attributes for Multiple Objects

You can select several objects in the Image window and change
their attributes using the Object Attributes inspector. The following
selections can be changed: Pen Color, Pen Size, Rotation, and Width
Each Side. In addition, you can select a new position for the Name
and Comment (Display with Object area).

To change multiple objects—

1. Choose **Object Attributes** from the Object menu, or double-click one of the objects. The Object Attributes inspector appears.

2. In the Image window, drag or SHIFT+click to select the objects you want to change. The Name box becomes a list box when you select several objects. If you select objects that you have grouped, you see the group name in the Name box.

   Note: To view the attributes for each selected object, choose the object from the Name list box. Any changes you make to the attributes for one object are applied to all the selected objects.

3. In the Object Attributes inspector, change the attributes and click **Set.** The objects change in the Image window.

   Note: All the attributes displayed in the Object Attributes inspector are applied to the selected objects. For example, if you change the pen size for objects displayed in various colors, in addition to the pen size changing, the colors will change to the color displayed in the Object Attributes inspector.

6.19 Modifying a Grid

After you create a grid, you can change it to add lines, move lines, or move intersections.

6.19.1 Adding a Line

1. Choose **Select** ( ) from the Object menu or the Object toolbar.

2. Select the grid you want to change.

3. Position the pointer over a line.

   Note: To add a row, place the pointer on a horizontal line; to add a column, place the pointer on a vertical line.

4. To add a line—

   Windows NT—Hold down the mouse button and press the **INSERT** key.

Macintosh—Hold down the mouse button and the **SHIFT** key, and then press the **INSERT** key.

A second line appears slightly offset from the original line.

5. Drag the line to a new location (figure 6-17).

6. Release the mouse button.

Hold down the mouse button and the SHIFT key (Macintosh only), and then press the INSERT key.

▷ Drag the inserted line.
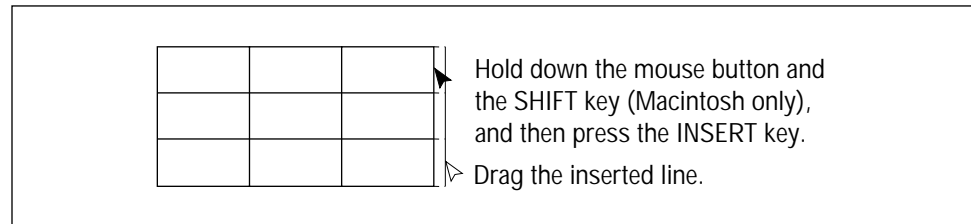
Figure 6-17. Adding a line to an existing grid.

6.19.2 Deleting a Line

1. Choose **Select** (⬚) from the Object menu or the Object toolbar.

2. Select the grid you want to change.

3. Position the pointer over the line you want to delete.

4. Hold down the mouse button and press the **DELETE** key (Macintosh: in the Special Keys area). The line is removed.

6.19.3 Moving a Line

To move a grid line, position the pointer on the line, making sure the pointer is not positioned on an intersection. Drag the line to a new location (figure 6-18). Release the mouse button.

Place the pointer on the line and drag.
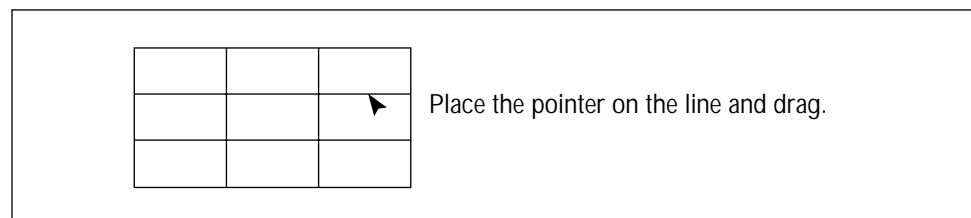
Figure 6-18. Moving a line on an existing grid.

6.19.4 Moving Intersections

To change the shape of a grid, you can reposition any intersection. Position the pointer on an intersection, Drag the intersection to a new location (figure 6-19). Release the mouse button.

Place the pointer on the intersection and drag.
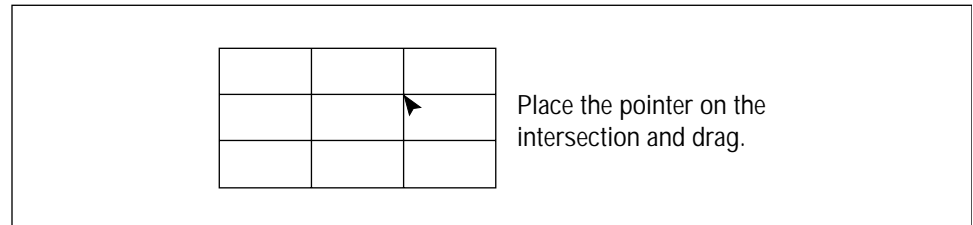
Figure 6-19. Moving an intersection on an existing grid.

6.20 Modifying a Text Annotation Object

After you create a text annotation object, you can change the text, change the font attributes, and choose a different orientation. To do this—

1. Double-click the text annotation object. The Modified Text window appears (figure 6-20).

2. Double-click the text in the text box and type new text.

3. Click the **Font** button and select new font attributes from the Font window.

4. Choose a different orientation.

5. Click **OK.** The Modified Text window closes and the text annotation object changes.
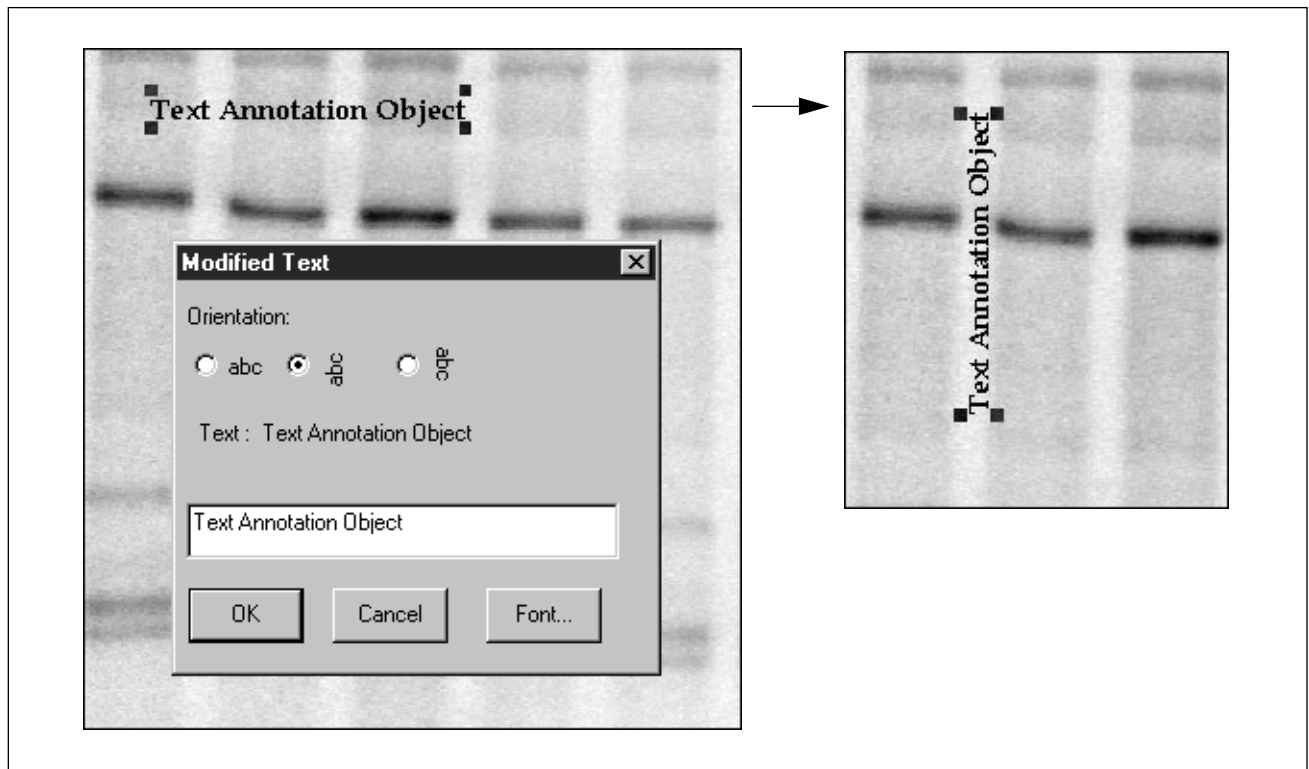
Figure 6-20. Rotating a text annotation object using the Modified Text window.

Chapter 7 Renaming and Displaying Objects

This chapter explains how to manage and manipulate objects. The topics in this chapter are—

- Renaming an Object (section 7.1)
- Displaying Objects in the Image Window (section 7.2)
- Displaying All Object Types (section 7.3)
- Saving and Deleting Objects (section 7.4)
- Displaying Objects on a Different Image (section 7.5)

7.1 Renaming an Object

When you create objects, ImageQuant assigns a number to each object in the order in which you draw the objects, even if you switch to a different object type. You can replace the number with a more descriptive name.

You can change the name (number) of a single object, or you can select several objects and rename them using the same base name. ImageQuant adds a number to the base name to make each object name unique. The maximum size for a single object name is 16 characters. The maximum size for a base name is 13 characters. You can rename Text Annotation objects. The new name appears in the inspectors but does not appear on the image.

7.1.1 Renaming an Object Using the Object Attributes Inspector

In the Image window, double-click the object you want to rename. The attributes for that object appear in the Object Attributes inspector.

- If you want to change the name only, double-click the current name of the object that appears at the top of the inspector. Then type a new name for the object and click **Set.** The name changes.

- If you want to change the name, the orientation, and the font attributes, double-click **Name** in the Display with Object area of the inspector. The Modified Name window appears. Type a new name for the object, select a new orientation, and click the **Font** button to change the font attributes in the Font window. Click **Set.** The name, orientation, and font attributes change.

7.1.2 Renaming an Object Using the Object Manager Inspector

To rename an object using the Object Manager inspector—

1. Choose **Object Manager** from the Object menu. The Object Manager inspector appears.

2. In the Objects list, click the object you want to rename.

3. Click **Rename.** The Rename Object window (figure 7-1) appears.

4. Type a new name.

5. (Optional) Choose a different orientation for the object.

6. Click **OK.** The new name replaces the old one in the Image window and in the Objects list.
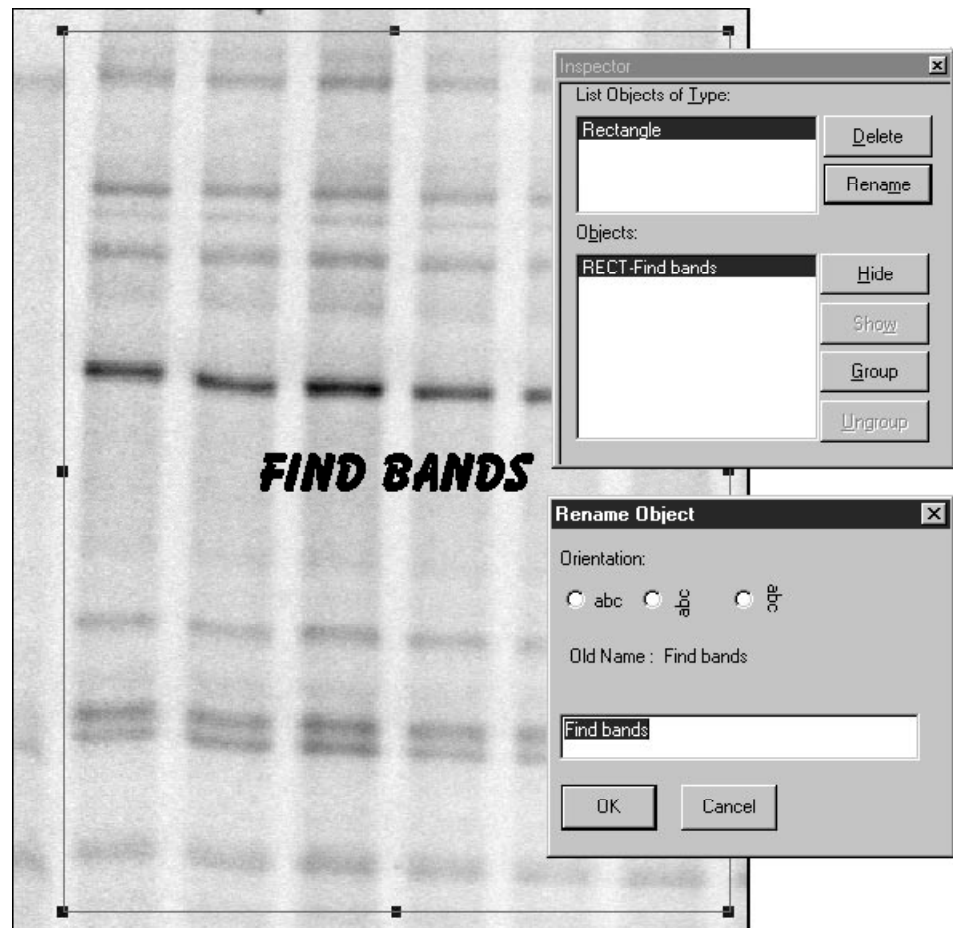
Figure 7-1. Renaming an object using the Object Manager inspector.

7.1.3 Renaming and Reordering Multiple Objects Using the Object Manager Inspector

Important **If you are going to reorder the objects using the Column or Row option, the positions of the objects on the image determine how they are reordered. Use the Alignment and Space Evenly commands on the Object menu to position the objects in rows or columns before reordering.**

To rename and reorder multiple objects using the Object Manager inspector—

1. Choose **Object Manager** from the Object menu. The Object Manager inspector appears.

2. In the Objects list, select the objects you want to rename.

3. Click **Rename.** The Rename Objects window (figure 7-2) appears.

4. Type a new base name.

(a) Before Renaming

(b) After Renaming
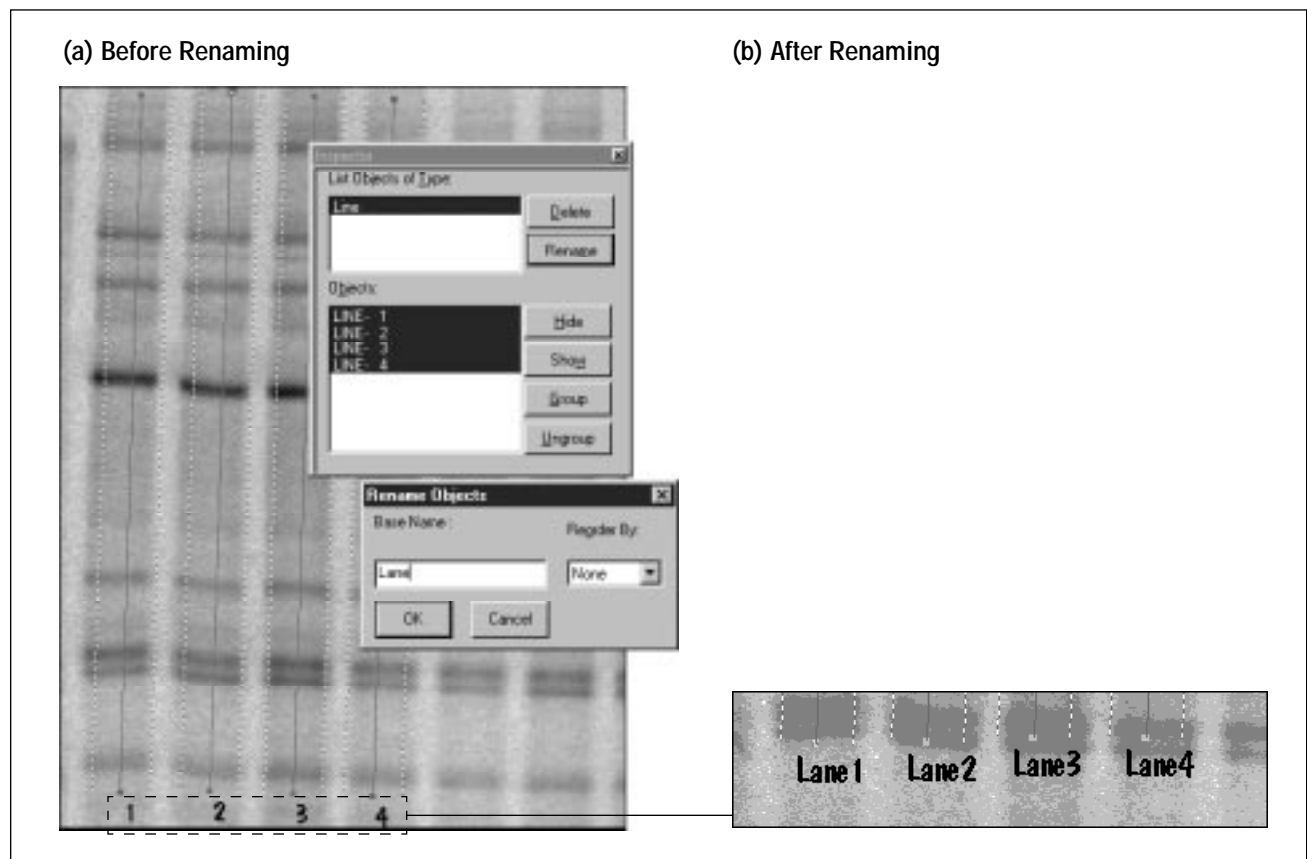
Figure 7-2. Renaming and reordering multiple objects.

5. Choose a reorder method from the Reorder By list.

- **None**—Adds a number to the base name in the same order in which the selected objects appear in the Objects list.

- **Column**—Sorts the objects by column, and then renames them by adding a number to the base name.

- **Row**—Sorts the objects by row, and then renames them by adding a number to the base name.

- **X Position**—Sorts the objects using the X pixel position starting with the X pixel position closest to the upper-left corner of the image, and then renames the objects by adding a number to the base name.

- **Y Position**—Sorts the objects using the Y pixel position starting with the Y pixel position closest to the upper-left corner of the image, and then renames the objects by adding a number to the base name.

6. Click **OK.** The new object names replace the old ones in the Image window and in the Objects list.

7.2 Displaying Objects in the Image Window

If the Image window is cluttered with objects, you can select specific objects to remove temporarily from the Image window. To do this, choose Object Manager from the Objects menu. In the Object Manager inspector, select the object type you want to hide from the List Objects of Type list, select the objects from the Objects list, and click **Hide.** The objects no longer appear in the Image window.

To redisplay hidden objects, select the object type from the List Objects of Type list, select the objects from the Objects list, and click **Show.** The objects reappear in the Image window.

When you open an image, ImageQuant displays the image and the objects in the same state as when last closed. For example, hidden objects are still hidden, visible objects are still visible, and all objects have retained their attributes.

7.3 Displaying All Object Types

The Object Manager inspector displays the objects for the object type selected in the Image window. For example, if the image displays rectangles and lines and you select one line, the Object Manager inspector displays the line objects only.

To display all the objects in the Objects list, place the pointer at the top of the **List Objects of Type** list and drag the pointer to select all the object types. When you release the mouse button, all the objects are displayed in the Objects list, including the hidden objects.

7.4 Saving and Deleting Objects

You can save the objects as you create or modify them. You can also wait until you complete your analysis and save the objects when you close the Image window.

If you want to save the objects without closing the image, choose **Save** ( ) from the File menu or the Main toolbar. ImageQuant saves all the objects whether or not they are selected or displayed. If you modify an object after you save it, ImageQuant prompts you to save the modified object when you close the Image window.

When you close the Image window, ImageQuant prompts you to save the objects with the image. If you select **Yes,** ImageQuant saves the new objects and the changes to existing objects. If you select **No,** the image returns to the state it was in when you first opened it (or when you last selected Save). For example, existing objects are retained but unmodified, and unsaved objects drawn after the image was opened (or last saved) are deleted. ImageQuant saves the objects in a file in the same folder as the image. The object file has the same name as the image, plus the .DOX file extension.

To delete an object when the image is displayed, select the object and then choose **Delete** from the Edit menu. Click **Yes** to the message verifying that you want to delete the selected object. The object is permanently removed when you save the image. If you close the image and elect not to save the modifications, the object is still stored with the image.

Alternatively, you can delete a selected object using the Delete button in the Object Manager inspector, or by pressing the **DELETE** key on the keyboard (Macintosh: in the Special Keys area).

7.5 Displaying Objects on a Different Image

You can display the objects you create for one image on a new image. Before you display the objects on the new image, verify that both images were scanned using the same pixel size (see your instrument user's guide for available pixel sizes).

Note: Use this procedure to display object files created using an earlier version of the ImageQuant software. Older object files created in Windows NT display a .do extension.

To verify the pixel size—

1. Select one Image window.

2. Choose **Image Properties** from the File menu, or double-click in the Image window.

3. Make a note of the value in the Pixel Size box on the Scan Info tab.

4. Repeat steps 1 through 3 for the second Image window.

If the pixel size is larger in the receiving image, the pasted object will be smaller. If the pixel size is smaller, the pasted object will be larger.

To display the objects on the new image—

1. Open the image on which you want to display the objects from an existing object file.

2. Choose **Insert Draw Objects from File** from the Edit menu. The Insert Draw Objects window appears (figure 7-3).

3. Locate and double-click the .dir folder of the image containing the objects you want to insert.

4. In the File Name box, double-click the .dox object file (older files display a .do extension). The objects appear on the image.

When you close the image, ImageQuant prompts you to save the objects. If you choose Save, ImageQuant retains the original object file with the original image and saves the new object file with the new image.
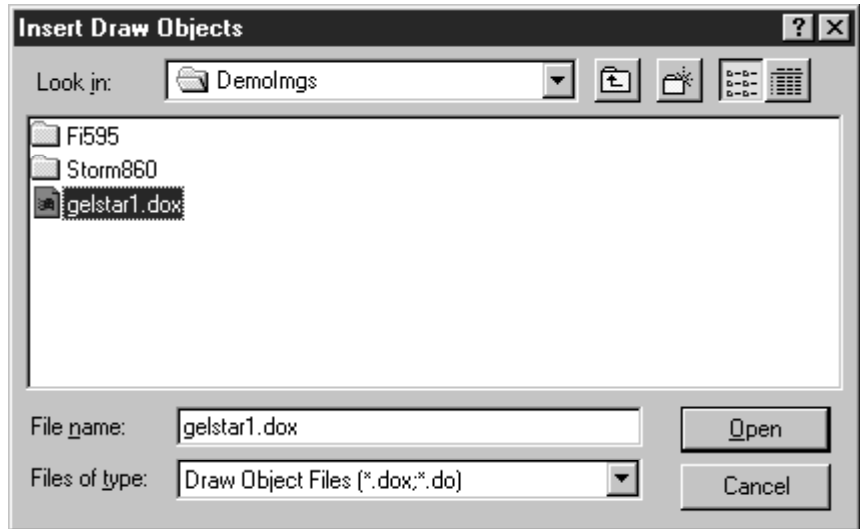
Figure 7-3. Displaying objects on a new image.

Chapter 8 Performing Area Quantitation with Peak Finder

This chapter describes how to set up and change the Peak Finder parameters, create templates, and generate reports. Chapter 9 describes how to enhance the line graph.

The topics in this chapter are—

- About Area Quantitation (section 8.1)
- Defining the Area to Quantitate (section 8.2)
- Creating the Line Graph (section 8.3)
- Defining Area Quantitation Parameters Using the Peak Finder Inspector (section 8.4)
- Modifying the Baseline (section 8.5)
- Defining the Peaks (section 8.6)
- Editing the Peaks (section 8.7)
- Using Templates (section 8.8)
- Selecting the Area Report Options (section 8.9)
- Generating Reports (section 8.10)
- Saving the Line Graph (section 8.11)
- Printing the Line Graph (section 8.12)

8.1 About Area Quantitation

Area quantitation calculates the area under the curve created by a plot of the pixel intensities and the pixel locations along a line, wide line, polyline, or wide polyline. ImageQuant displays the pixel intensities as a line graph.

8.1.1 Pixels and Area Quantitation

The following discusses which pixels ImageQuant includes when performing area quantitation (figure 8-1):

- **Horizontal Line**—ImageQuant draws a horizontal line along the borders of the pixels. ImageQuant includes the pixels below the horizontal line and one pixel at the right end of the line.

- **Vertical Line**—ImageQuant draws a vertical line along the borders of the pixels. ImageQuant includes the pixels to the right of the vertical line and one pixel at the bottom of the line.

- **Diagonal Line**—ImageQuant draws a diagonal line so that it bisects the pixels. ImageQuant includes the pixels bisected by the diagonal line and one pixel at the bottom of the line.

- **Wide Line**—ImageQuant includes the number of pixels you specified in the Object Attributes inspector. If you specified a wide line of 2 pixels, ImageQuant includes 2 pixels on either side of a straight line. If you draw a diagonal line, ImageQuant includes the pixels bisected by the line plus two pixels on either side of the line.

- **Polyline**—The pixels included in quantitation depend on whether the line segment is horizontal, vertical, or diagonal, and on whether the line is a single-pixel or a wide line. The same principles apply as discussed above.
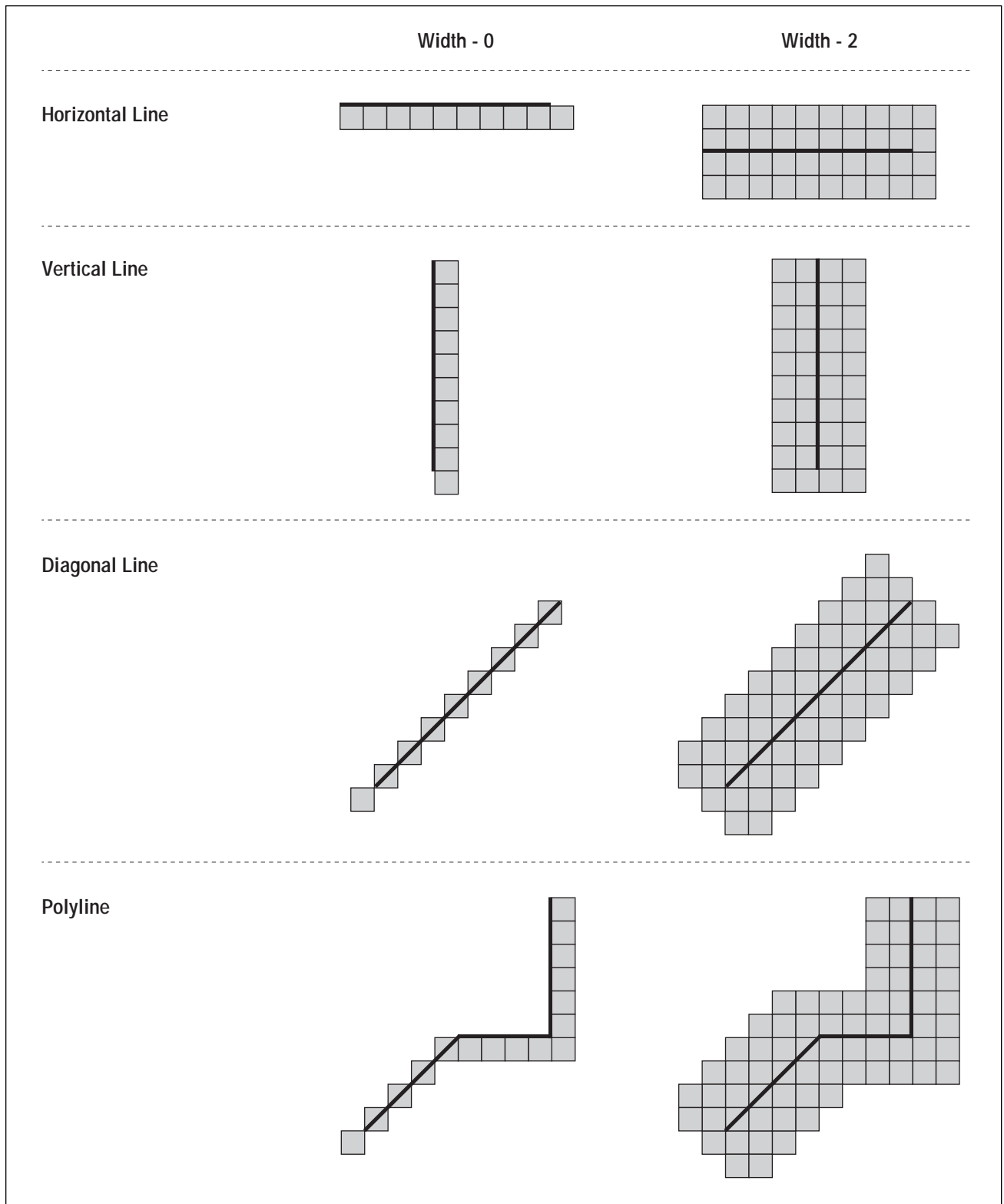
Figure 8-1. Determining the pixels included in area quantitation.

8.1.2 Overview of the Area Quantitation Workflow

Figure 8-2 shows the area quantitation workflow. Briefly, you begin by identifying the area of interest and creating a line graph of the area. Next, you either select a template to use for quantitation or use the default parameters displayed on the Peak Finder inspector.

After you quantitate, ImageQuant displays the baseline and peaks on the line graph. The baseline indicates the point at which ImageQuant separates the background from relevant data. The peaks indicate the concentrations of intense values along the line you drew. Baseline handles and droplines show the separation of the peaks. You view the results for each peak in the Area Review inspector.

After viewing the line graph and accompanying results, you can change the parameters to improve the peak finding. In addition, you can use the editing tools to change the peak definitions. When you are satisfied with the results, select the desired area report options, and then print the report or transfer the results to Excel.
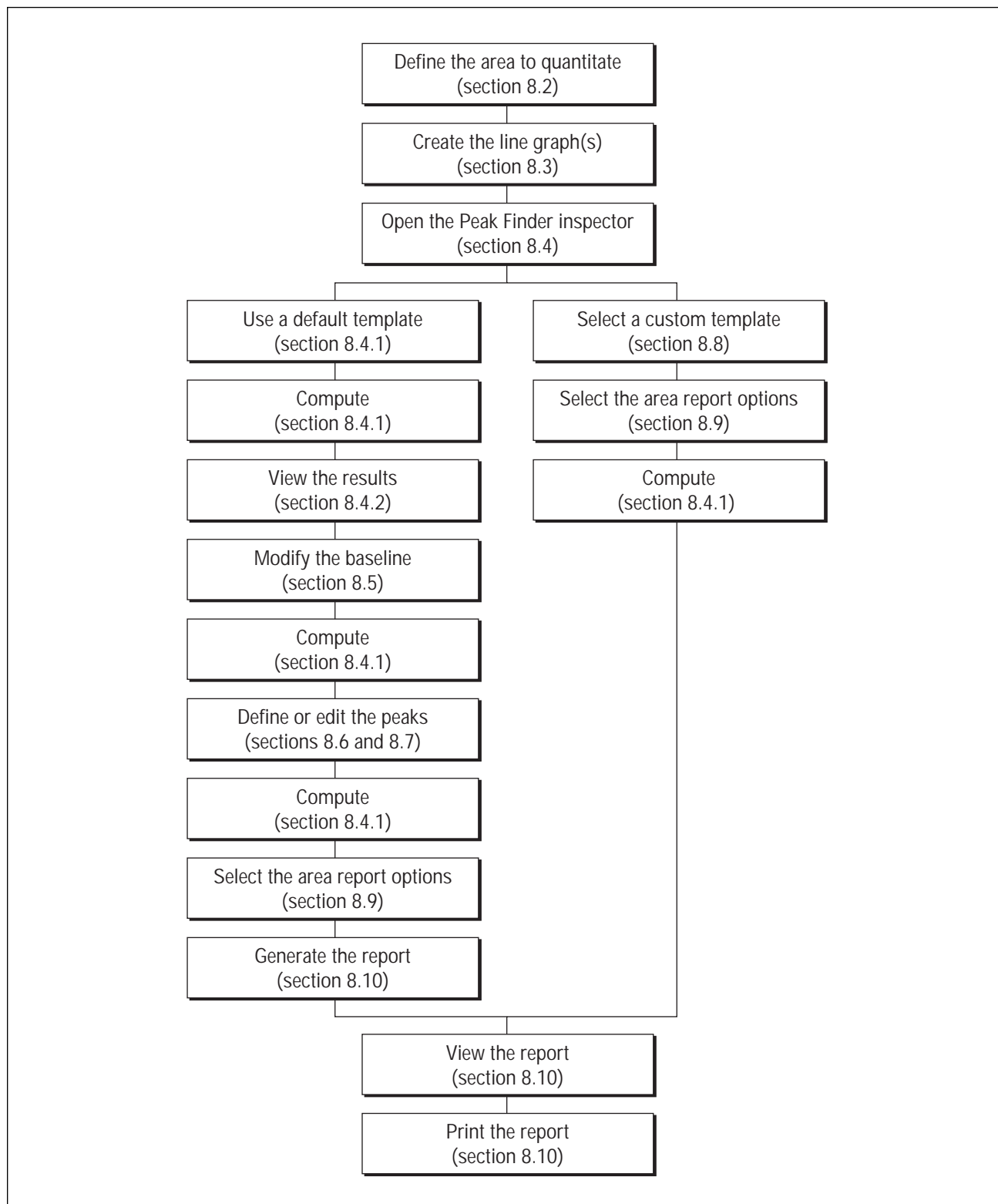
Figure 8-2. Area quantitation workflow.

8.2 Defining the Area to Quantitate

You define the area to quantitate by drawing a line or a polyline, which ImageQuant uses to create a line graph (figure 8-3). The line you draw can be a single-pixel line or a wide line. If you create a wide line using the Object Attributes inspector, ImageQuant averages the pixels on either side of the original line before creating the line graph. The value you type in the Width Each Side box is the number of pixels you want to add to each side of the existing line. For example, if you type 15, ImageQuant creates dashed lines 15 pixels on either side of the original line.

The procedures for drawing lines are discussed in the following sections.
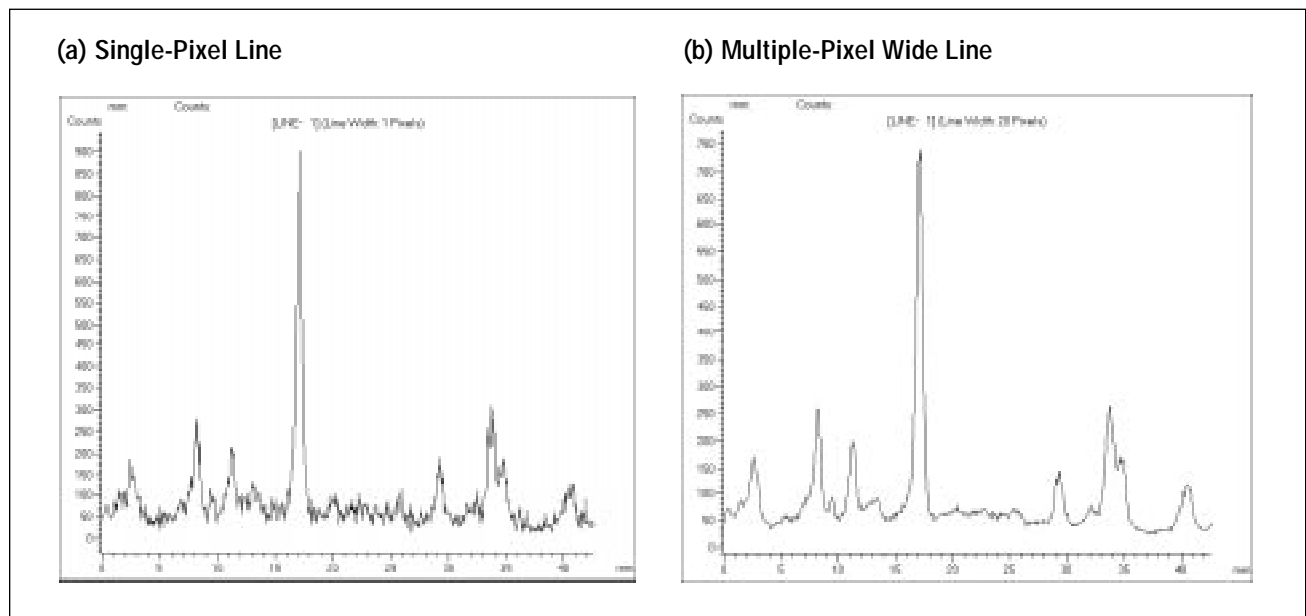
Figure 8-3. Line graphs of the same set of bands: one created using a line (a) and the other created using a wide line (b).

8.2.1 Drawing a Line or Polyline

To draw a line or polyline—

1. Choose **Line** (\ ) or **Polyline** (⅄) from the Object menu or the Object toolbar.

2. In the Image window, draw the line or polyline over the portion of the image you want to quantitate. (See section 5.4.1 for instructions on drawing a line or section 5.4.2 for instructions on drawing a polyline.)

8.2.2 Modifying the Line Width

To create a wide line or wide polyline from an existing line—

1. Choose **Select** (🡕) from the Object menu or the Object toolbar.

2. In the Image window, choose the line or polyline you want to modify.

3. Choose **Object Attributes** from the Object menu, or double-click the object. The Object Attributes inspector appears (figure 8-4).

Double-click and type the number of pixels to add to each side of the line.
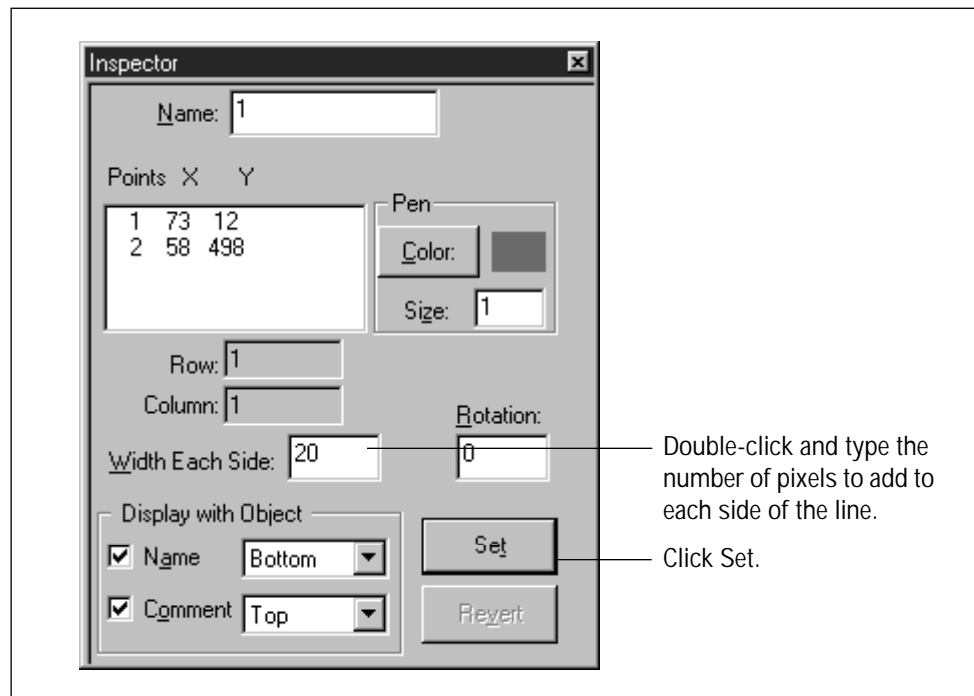
Click Set.

Figure 8-4. The Object Attributes inspector.

4. In the **Width Each Side** box, double-click to highlight the number, and then type a new value.

5. Click **Set.** The line or polyline changes to a wide line or a wide polyline.

8.3 Creating the Line Graph

Before you can quantitate, you must create a line graph of the area. To create a single line graph, select one line. To create multiple line graphs that appear in a single Graph window, select more than one line. After you select the line or lines, choose **Create Graph** (![icon]) from the Analysis menu or the Graph toolbar. A line graph of the selected line appears in the Graph window. If you selected more than one line, a line graph appears for each line.

8.4 Defining Area Quantitation Parameters Using the Peak Finder Inspector

You use the Peak Finder inspector (figure 8-5) to define the area quantitation parameters. ImageQuant uses the parameters to create a baseline and to define peaks on the selected line graph. Because the results are reproducible, you should try to find the baseline and peaks automatically using the Peak Finder method. You can edit the automatic baseline parameters to improve the baseline and peak definitions.
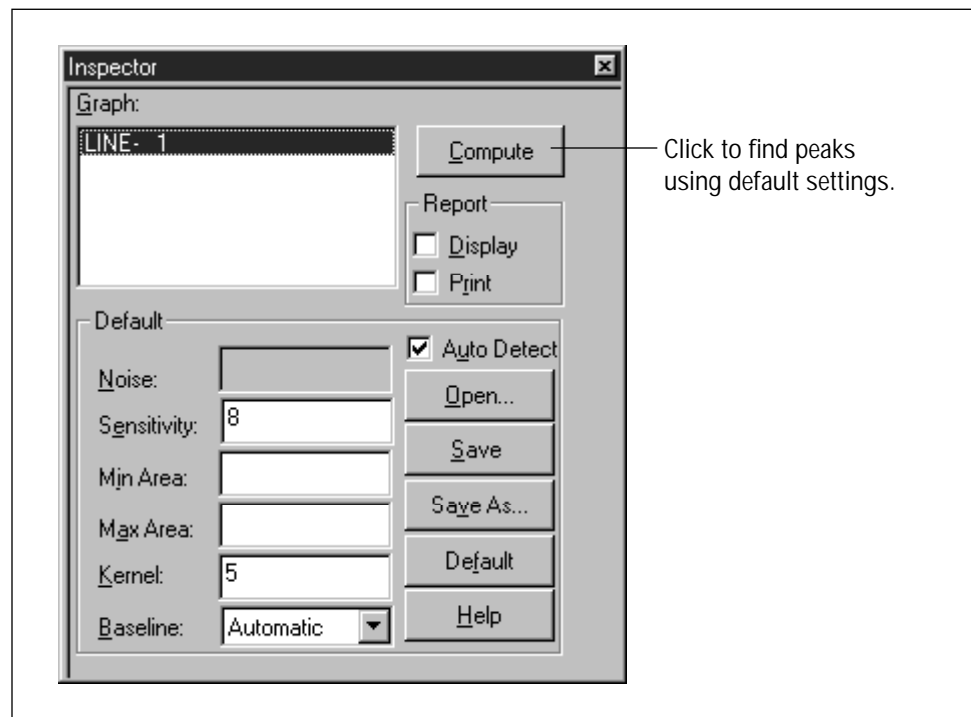
Figure 8-5. The Peak Finder inspector.

8.4.1 Quantitating Using the Default Settings

The first time you perform area quantitation on a new line graph, use the default Peak Finder settings. To do this—

1. Choose **Peak Finder** from the Analysis menu. The Peak Finder inspector appears (figure 8-5).

2. Make sure the correct line graph (or graphs) is selected for peak finding.

3. Make sure the **Auto Detect** check box contains a check mark. If it does not, click the box to select the mode.

4. Deselect **Display** and **Print** in the Report area for the initial quantitation.

5. Click **Compute.** A baseline appears on the line graph, and ImageQuant assigns a number to each peak found (figure 8-6).

6. Evaluate the baseline and the peaks. Modify the Peak Finder parameters to improve the results (sections 8.5 and 8.6).

7. Click **Compute.**

8. Repeat steps 6 and 7 until you are satisfied with the peak finding.

9. From the Area Report Setup window, choose the desired reporting options (section 8.9).

10. In the Peak Finder inspector, click **Display** to display the results in an Area Report window.

11. In the Peak Finder inspector, click **Print** to send the results to the printer.

12. Click **Compute.** Depending on the options you select, the report appears in the Area Report window, is sent to the printer, or both.
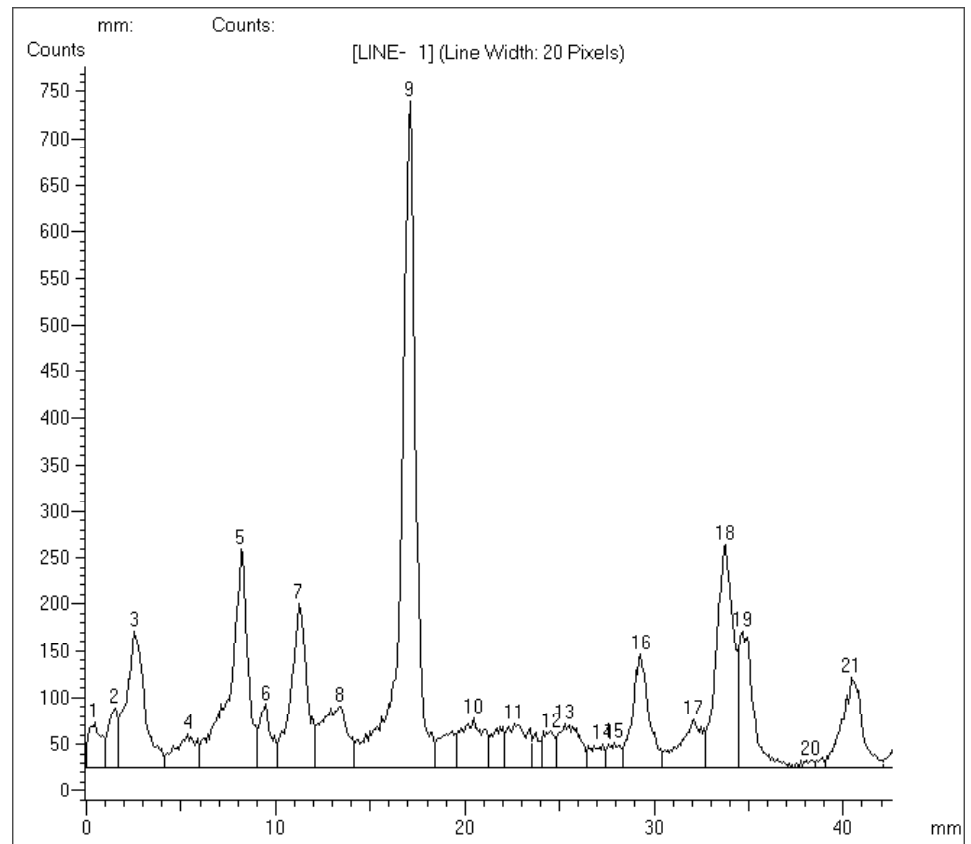
Figure 8-6. The line graph following computation.

8.4.2 Selecting and Viewing the Results for a Peak

After you use Peak Finder to quantitate, you can view the results for each peak in the Area Review inspector. To display the Area Review inspector (figure 8-7), choose **Area Review** (🗎) from the Analysis menu or the Quantitation toolbar.
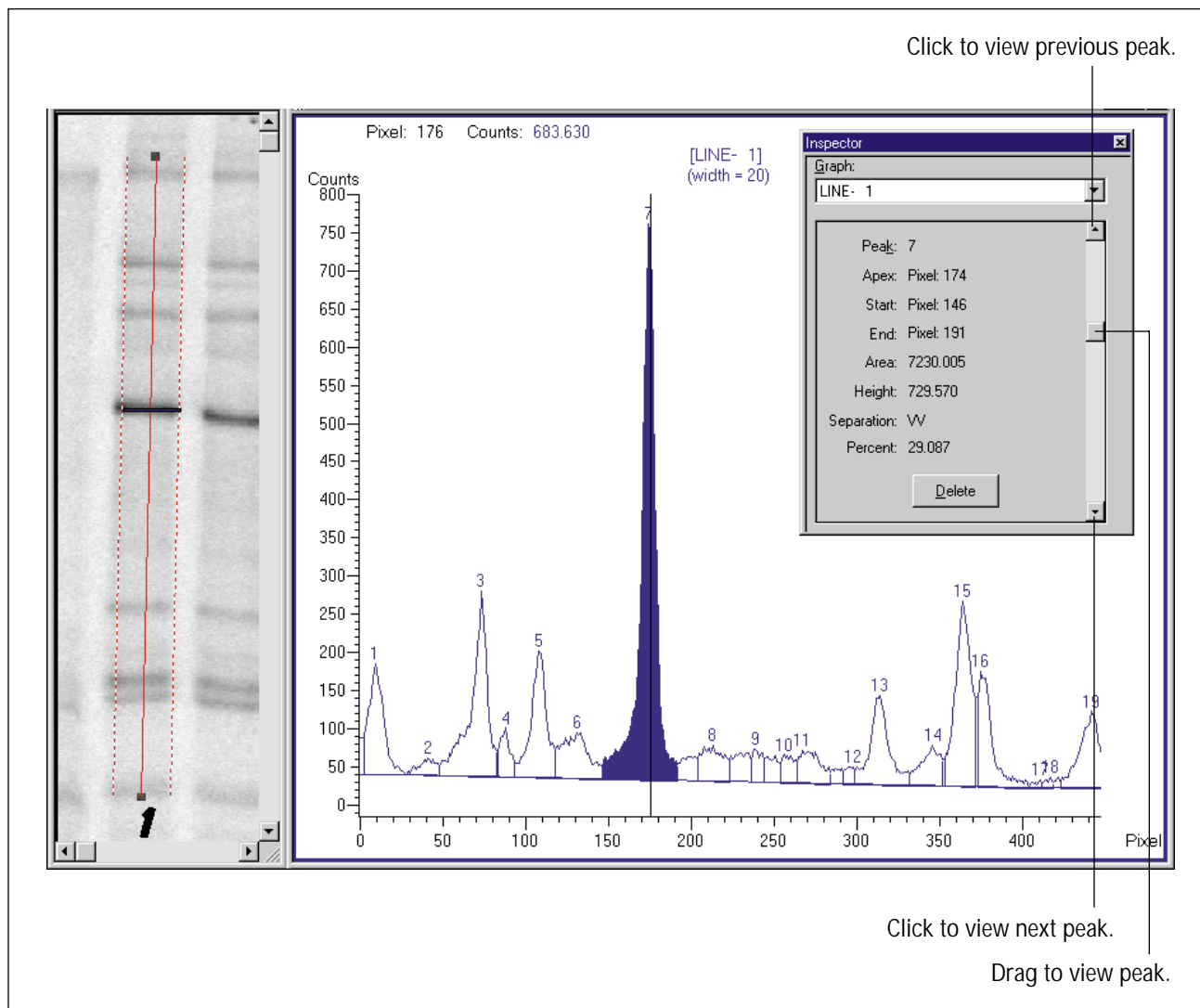
Figure 8-7. The Area Review inspector.

The Area Review inspector displays the results for one peak. To view the results for a different peak—

- Click the peak in the Graph window.

- Click the down scroll arrow (▼) to see the next peak results, or click the up scroll arrow (▲) to see the previous peak results.

- Drag the scroll box (▢) down or up to shift from peak to peak.

ImageQuant highlights the selected peak in the Graph window.

8.5 Modifying the Baseline

After quantitation, the baseline in the line graph indicates the value at which ImageQuant separates the background from the relevant data. For any point in the graph, ImageQuant subtracts the background value (below the baseline) before summing the intensity values in a peak to calculate the peak area.

You can modify the baseline created by Peak Finder in two ways:

- Automatically by changing the Peak Finder parameters

- Manually by adding, deleting, or moving baseline handles

8.5.1 Displaying the Baseline Handles

You can see the baseline and droplines on the line graph; however, to see the baseline handles you need to select the baseline. To do this, place the pointer on the baseline, and then click the mouse button, or choose **Select Baseline** from the Tools menu. The baseline changes color and displays the baseline handles, which represent the points at the ends of the graph and the points where valleys touch the baseline (figure 8-8).
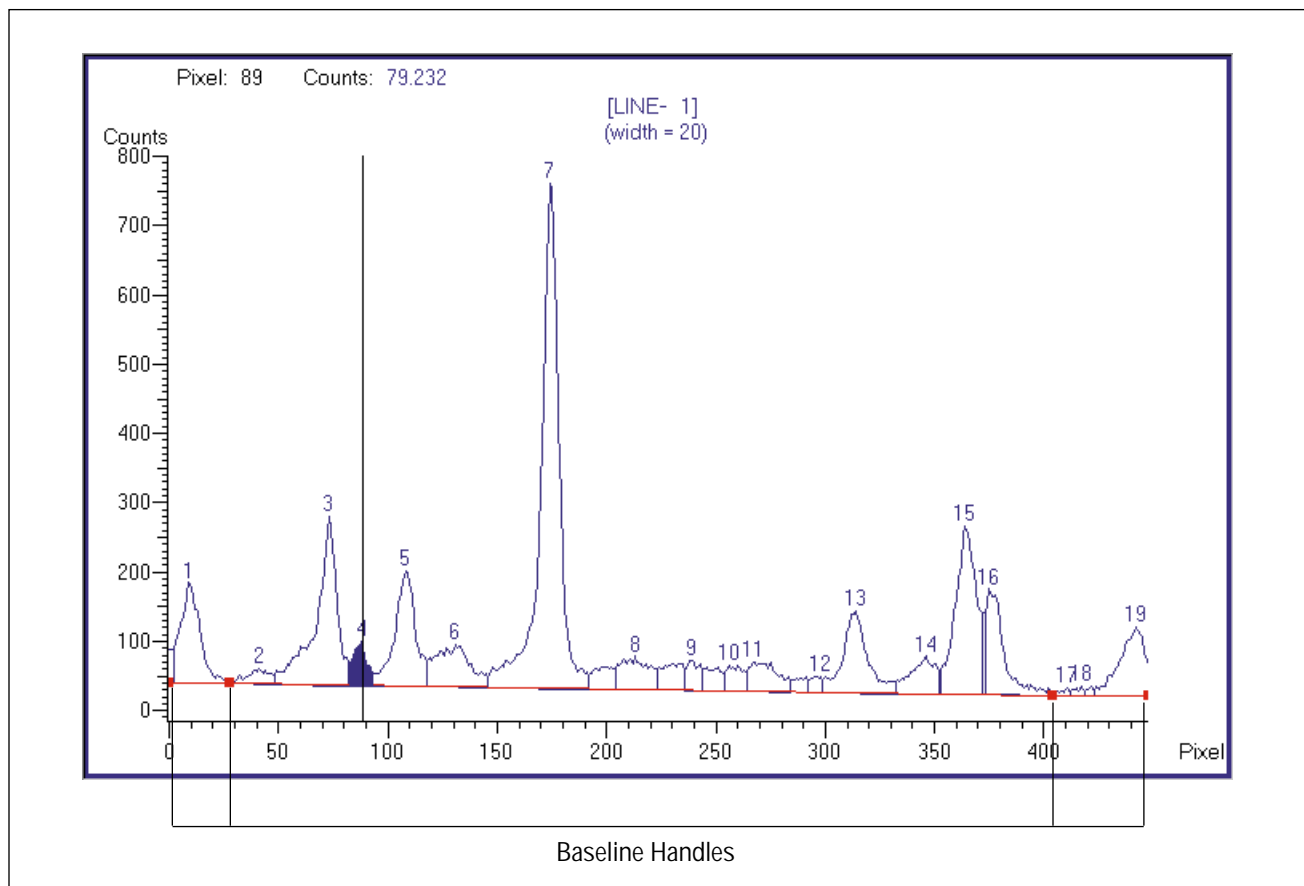
Figure 8-8. Displaying baseline handles.

8.5.2 Modifying the Automatic Baseline

The quantitation you performed in section 8.4.1 used the Automatic selection in the Baseline list. The Automatic baseline method uses the Noise parameter to shape the baseline. In addition, you used the Auto Detect version of the Noise parameter.

ImageQuant calculated the noise value for you and displayed the calculated value in the Noise box of the Peak Finder inspector. The calculated Noise value is displayed using the smallest increment collected by the instrument. **Note:** Because ImageQuant looks at the valleys, the Noise parameter calculated by Auto Detect is a very small number.

The Noise parameter determines whether the changes between values along the line represent fluctuations in the background or in the data peaks. (ImageQuant checks the point-to-point variations in the relatively flat areas between the peaks to determine the Noise parameter.)

If the baseline created using Auto Detect is satisfactory, leave the Noise parameter unchanged. Otherwise, use the following guidelines to modify the Noise parameter (figure 8-9).

If the baseline contains—

- **Too many baseline handles**—Type a smaller Noise value. For example, Peak Finder identifies points that are not relevant data and identifies too many peaks.

- **Too few baseline handles**—Type a larger Noise value. For example, if Peak Finder fails to correct a sloping background.

(a) Too Many Baseline Handles

(b) Too Few Baseline Handles
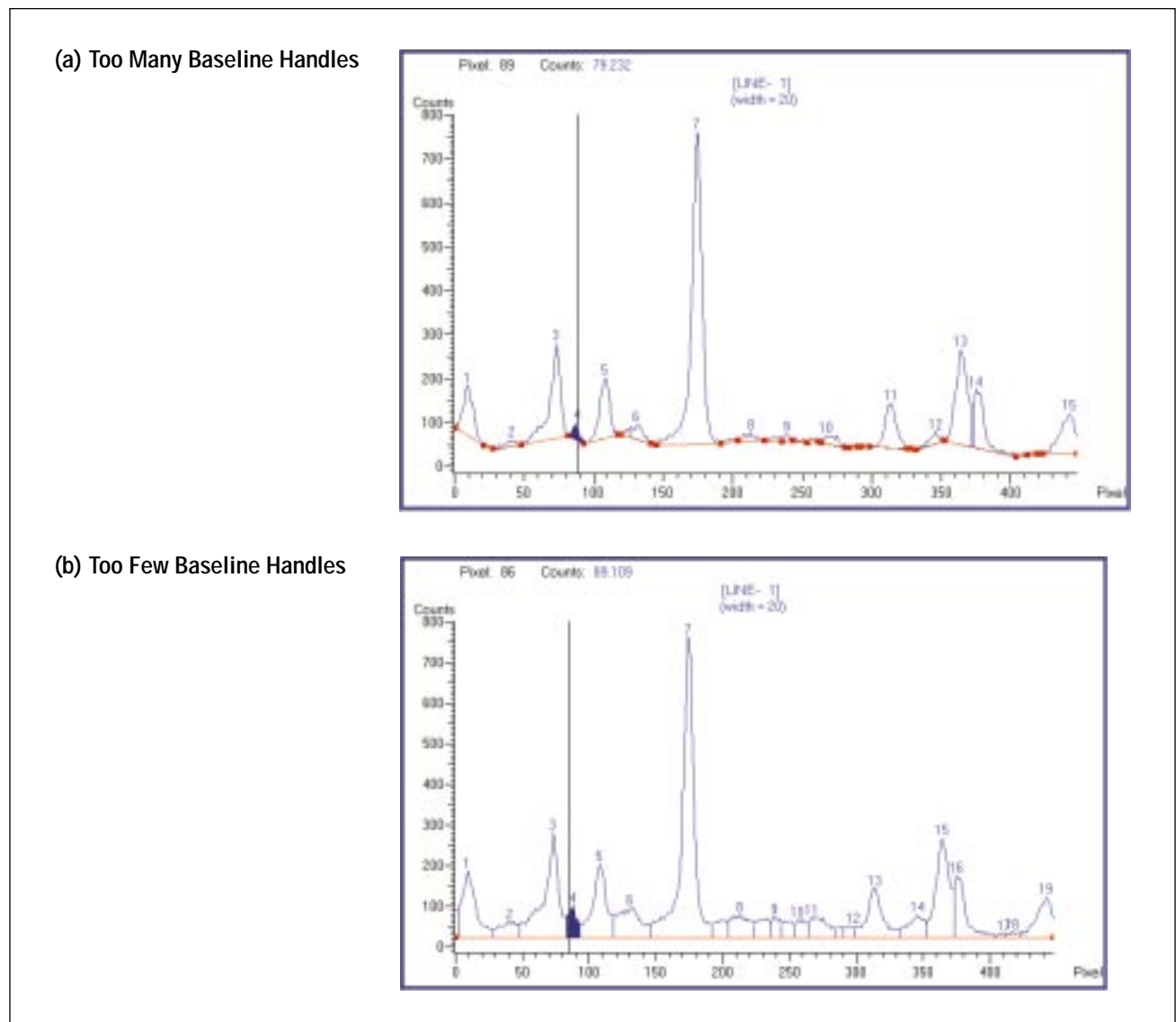
Figure 8-9. A line graph with too many baseline handles (a) and a line graph with too few baseline handles (b).

8.5.3 Using the Lowest Point as the Baseline

You can create a baseline using the Lowest Point method. In this method, ImageQuant uses the lowest point (valley) in the line graph to draw a straight baseline. Use this method for line graphs that contain too many "merged" peaks—peaks that lack sufficient flat areas between them. (Without sufficient flat areas, ImageQuant cannot calculate the noise value.)

To use the Lowest Point method—

1. In the Peak Finder inspector, choose **Lowest Point** from the Baseline list.

2. Click **Compute.** A straight baseline appears on the line graph (figure 8-10).
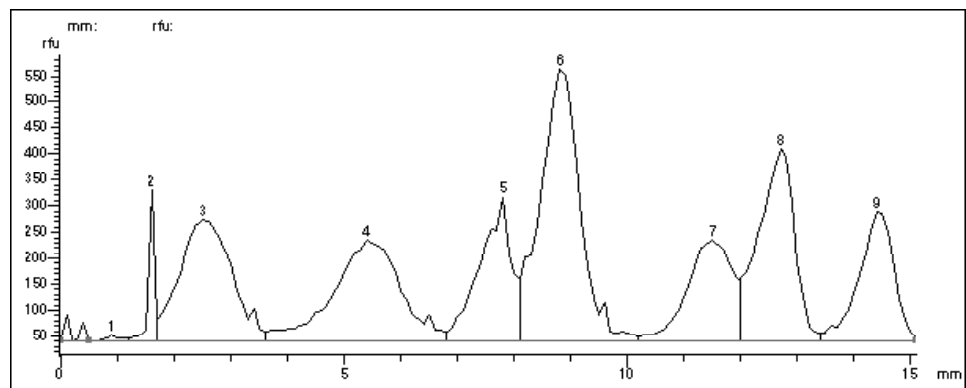
Figure 8-10. Baseline created using the Lowest Point method.

8.5.4 Modifying the Baseline Manually

You can modify a baseline created by either the Automatic or the Lowest Point method. You do this by adding, deleting, and moving the baseline handles. (If the handles are not displayed, click the baseline or choose **Select Baseline** from the Tools menu.)

Use the following to change the baseline manually:

•   **Add a baseline handle**—Place the pointer where you want to add a baseline handle, and then—

    Windows NT—Hold down the mouse button and press the **INSERT** key.

Macintosh—Hold down the mouse button and the **SHIFT** key, and then press the **INSERT** key.

A new handle appears.

• **Reshape the baseline**—Place the pointer on a baseline handle, and drag the handle up or down.

• **Move a baseline handle horizontally**—Place the pointer on a baseline handle, and drag the handle back and forth. To move the handle along the curve of the line graph, hold down the **CONTROL** key and drag the handle. **Note:** The end baseline handles move up and down only.

• **Delete a baseline handle**—Place the pointer on the baseline handle you want to delete, hold down the mouse button, and then press the **DELETE** key (Macintosh: in the Special Keys area). The handle disappears.

To see how the modifications affect the line graph—

1. In the Peak Finder inspector, choose **Hold** from the Baseline menu.

2. Click **Compute.** A new baseline appears.

3. Choose **Area Review** ( ) from the Analysis menu or the Quantitation toolbar to view the results for one peak.

You can save this baseline with the line graph and view it at a later time (see section 8.11). However, if you choose not to save the line graph, you will have to re-create the baseline.

8.6 Defining the Peaks

After you establish a suitable baseline, you can adjust the two parameters ImageQuant uses to define the peaks: sensitivity and kernel. If adjustments to these parameters are not sufficient, you can change the minimum area, the maximum area, or both parameters. If you need further modifications, you can use the peak editing tools to define the peaks.

8.6.1 The Noise, Sensitivity, and Kernel Relationship

ImageQuant recognizes the starting point, ending point, and/or apex point of a peak by looking at the changes in pixel intensity from point to point. The particular value used for making these distinctions is derived by multiplying the noise value times the sensitivity value. By adjusting the sensitivity value while holding the noise value constant, you can change the peak recognition results without affecting the baseline.

To change the sensitivity parameter, double-click the **Sensitivity** box in the Peak Finder inspector. Type a new number. To instruct ImageQuant to find more peaks (figure 8-11), type a smaller number. To instruct ImageQuant to find fewer peaks, type a larger number. Click **Compute** to see the effect.
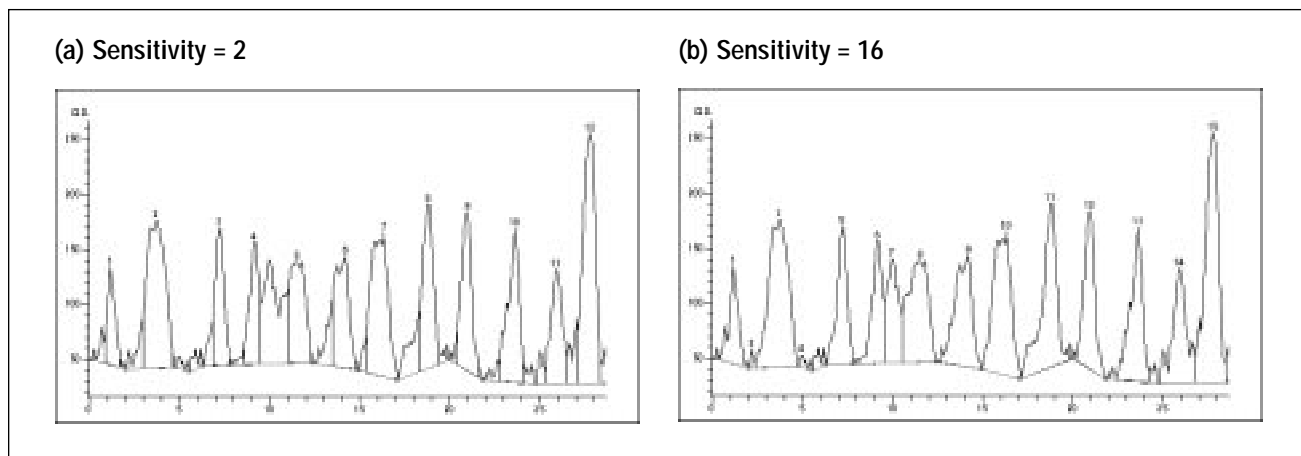
Figure 8-11. Line graph with a low sensitivity value (a), and line graph with a high sensitivity value (b).

The kernel value modifies the results of sensitivity times noise by providing a smoothing effect. The kernel value determines the duration, measured in pixels, over which an acceptable change in intensity must persist before a peak is recognized.

The kernel parameter allows you to specify how many pixels to group together to create a "look ahead window" for determining the start of the peaks. The increase in each pixel value in the kernel must be bigger than the noise multiplied by the sensitivity before ImageQuant assumes the start of a peak.

Use the kernel parameter to exclude narrow peaks—such as spikes emanating from larger peaks—from recognition. Type a larger kernel size to exclude narrow peaks. Type a smaller kernel size to include them. Click **Compute** to recalculate the peaks.

8.6.2 When to Use Minimum and Maximum Area

In some cases, too many "noise" peaks are recognized, even though the baseline is acceptable and the start, stop, and apex points of the peaks have been correctly identified. In these cases, it is best not to change the baseline or peak recognition parameters. Instead, you can force the small peaks to be rejected by typing an area value above which (maximum area) or below which (minimum area) peaks will not be recognized. The minimum area and maximum area parameters are applied to the data after the noise and sensitivity parameters.

Eliminating Small Peaks

You use the minimum area parameter to specify the minimum area required for ImageQuant to report a peak. For example, if you specify a minimum area of 100, an area with a value less than 100 is not reported as a peak.

To eliminate unwanted small peaks after quantitation—

1. Choose **Area Review** ( ) from the Analysis menu or the Quantitation toolbar. The Area Review inspector appears (figure 8-7).

2. Display the peak results for the smallest peak you want to recognize.

3. Make a note of the **Area** value.

4. Choose **Peak Finder** from the Analysis menu. The Peak Finder inspector appears (figure 8-5).

5. In the **Min Area** box, type a number that is slightly smaller than the Area results you noted in step 3.

6. Click **Compute** to see the results.

Eliminating Large Peaks

You use the maximum area parameter to specify the maximum area above which ImageQuant does not report a peak. For example, if you specify a maximum area of 2000, a peak with an area greater than 2000 is not reported.

To eliminate unwanted large peaks after quantitation—

1. Choose **Area Review** (▣) from the Analysis menu or the Quantitation toolbar. The Area Review inspector appears (figure 8-7).

2. Display the peak results for the largest peak you want to recognize.

3. Make a note of the **Area** value.

4. Choose **Peak Finder** from the Analysis menu. The Peak Finder inspector appears (figure 8-5).

5. In the **Max Area** box, type a number that is slightly larger than the Area results you noted in step 3.

6. Click **Compute** to see the results.

Note: If a large peak is broken into smaller peaks, do not change the Maximum Area setting. Instead, adjust the baseline (section 8.5) or combine the peaks using the Define Peaks command (section 8.7).

8.6.3 Additional Hints

Use the following hints to help determine how to change the Peak Finder parameters.

Peaks Are Too Narrow

If recognized peaks are too narrow (starting or ending "tails" are truncated), type a lower value in the Sensitivity box.

Peaks Are Too Broad

If recognized peaks are too broad (they include too much of the starting or ending "tails"), type a higher value in the Sensitivity box.

Baseline Fluctuations Are Recognized as Peaks

If sharp, narrow fluctuations in the baseline are incorrectly recognized as peaks, type a larger value in the Kernel box. The sharp peaks will be reduced and go undetected.

If a larger kernel value causes real peaks to be too narrow, the width of the fluctuating baseline peaks is too close to the width of the real peaks. Type the original value in the Kernel box and type a value in the Min Area box. To determine a reasonable minimum value, display the Area Review inspector and check the Area values for small peaks. Type a minimum value that is smaller than the smallest peak you want Peak Finder to identify.

Too Many Peaks Recognized Along the Main Peak Apex

If, on broad, somewhat flattened peaks, ImageQuant recognizes multiple peaks where only one is legitimate (and the start and stop points of the legitimate peaks are correct), type a larger value in the Kernel box. By increasing the kernel value, ImageQuant overlooks peak stop and start points in the middle of the true peak.

8.7 Editing the Peaks

You can edit the peaks manually in four ways—delete a peak, add a peak, combine peaks, and split peaks.

8.7.1 Deleting a Peak

After completing peak finding, you can use the Area Review inspector to delete a peak (figure 8-12). Use one of the methods described in section 8.4.2 to select the peak. In the Area Review inspector, click **Delete.** ImageQuant renumbers the remaining peaks.

Click to delete
displayed peak.
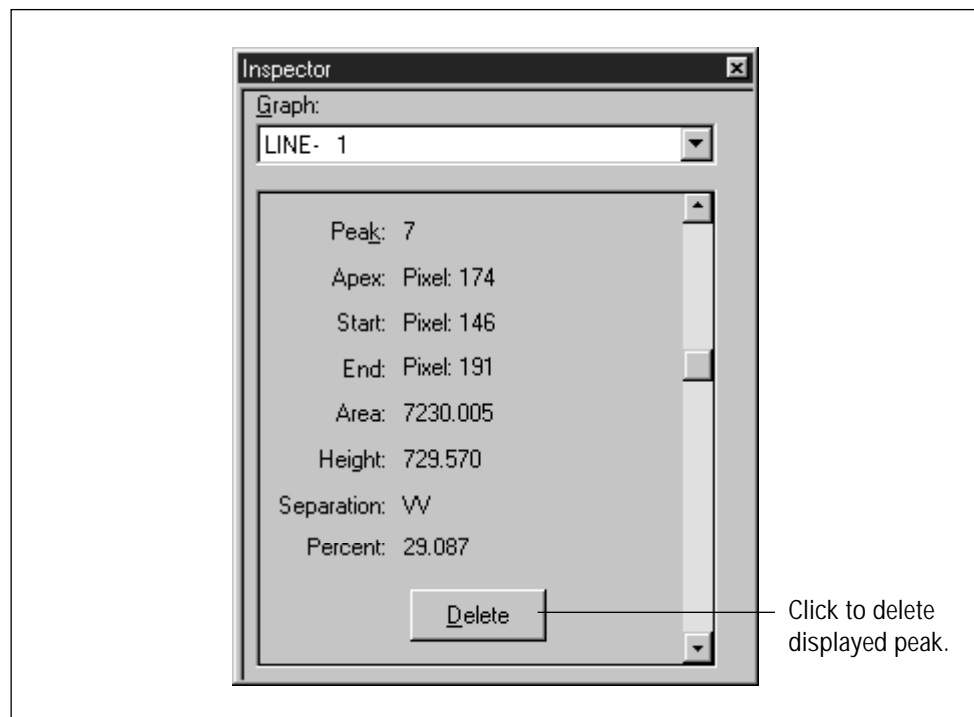
Figure 8-12. The Area Review inspector.

8.7.2 Combining or Adding Peaks

You use the Define Peaks command on the Tools menu or the Graph toolbar to define a new peak or combine multiple peaks. To do this—

1. Choose **Define Peaks** (🏔) from the Tools menu or the Graph toolbar.

2. Place the pointer at one edge of the area you want to define as a peak and drag through the area. Release the button. ImageQuant labels the selected area as a peak. If you combined peaks, you will see new droplines and a single number. ImageQuant renumbers all the peaks in the line graph.

3. Choose **Select** (⮜) from the Tools menu or the Graph toolbar to end the function.

Important   **If the area of the combined or added peak is greater than the value you typed in the Max Area box or smaller than the value you typed in the Min Area box, ImageQuant will not define the peak.**

8.7.3 Splitting Peaks

Using the Split Peaks command on the Tools menu or the Graph toolbar, you can split a peak into separate peaks. To split peaks—

1. Choose **Split Peaks** ( ) from the Tools menu or the Graph toolbar.

2. Place the pointer where you want to split a peak and click the mouse button. A dropline appears between the peaks, and ImageQuant renumbers all the peaks.

3. Choose **Select** ( ) from the Tools menu or the Graph toolbar to end the function.

Important   **If the area of either of the new split peaks is smaller than the value you typed in the Min Area box, ImageQuant will not define that peak.**

8.8 Using Templates

You can save the parameters you specify in the Peak Finder inspector. The parameters are stored in a template file, which you can retrieve later to analyze images. By creating templates with parameters that are specific to the types of images you analyze in your lab, you can streamline the area quantitation process.

8.8.1 Opening an Existing Template

To use an existing template, display the **Peak Finder** inspector (figure 8-5), and then click the **Open** button in the inspector to display the Open window (figure 8-13).
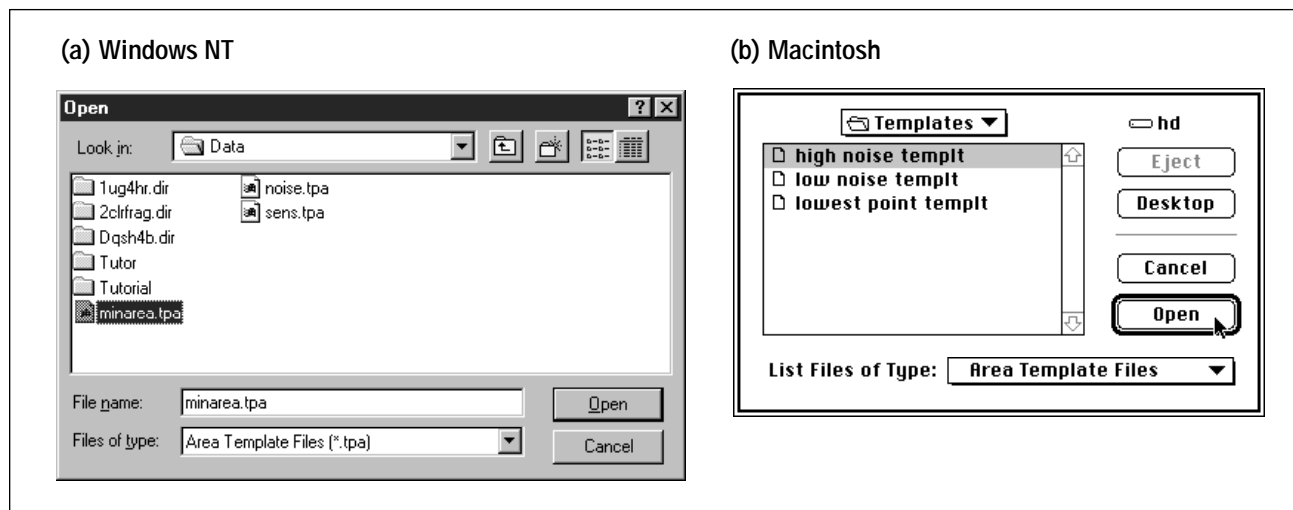
Figure 8-13. The Open window with example template names.

Double-click the name of the template you want to use. The window closes, and the parameter values in the Peak Finder inspector change to reflect those stored in the template. The new template name replaces the old template name in the inspector.

8.8.2 Creating a New Template

You create a new template by changing the parameter settings in the Peak Finder inspector and saving those settings. You can modify the initial default settings, or you can load and modify an existing template (section 8.8.1). After you change the parameter settings, compute and examine the results. Continue to change and compute until you are satisfied with the settings. Then—

1. Click the **Save As** button. The Save As window appears (figure 8-14).

2. Type a name for the template.

3. Click **Save.** The Save As window closes.
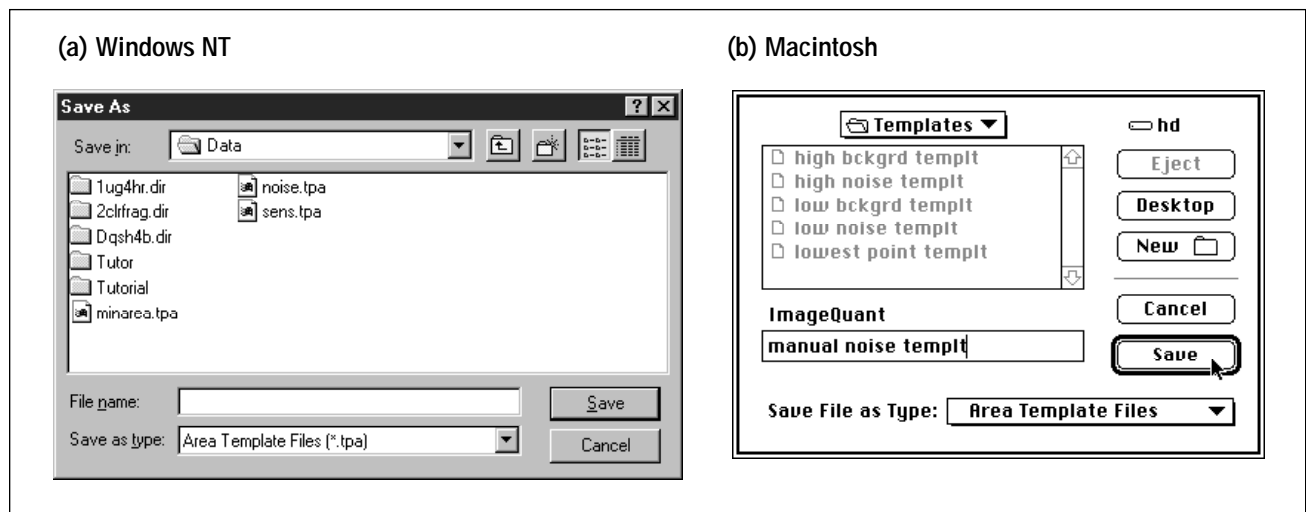
Figure 8-14. The Save As window.

8.8.3 Setting the Default Template

You can select one template to load whenever you display the Peak Finder inspector. To do this—

1. Choose **Area Default Template** from the Preferences menu. The Select Default Area Parameter File window appears (figure 8-15).

2. Locate and select the template from the list.

3. Click **Open** (Windows NT) or **Select** (Macintosh).

4. Choose **Peak Finder** from the Analysis menu to display the Peak Finder inspector (figure 8-5). The new template name replaces the old template name in the inspector.

    Note: To return to the system default template, choose **Reset Area Default Template** from the Preferences menu. In the Peak Finder inspector, click **Default.**

(a) Windows NT

(b) Macintosh
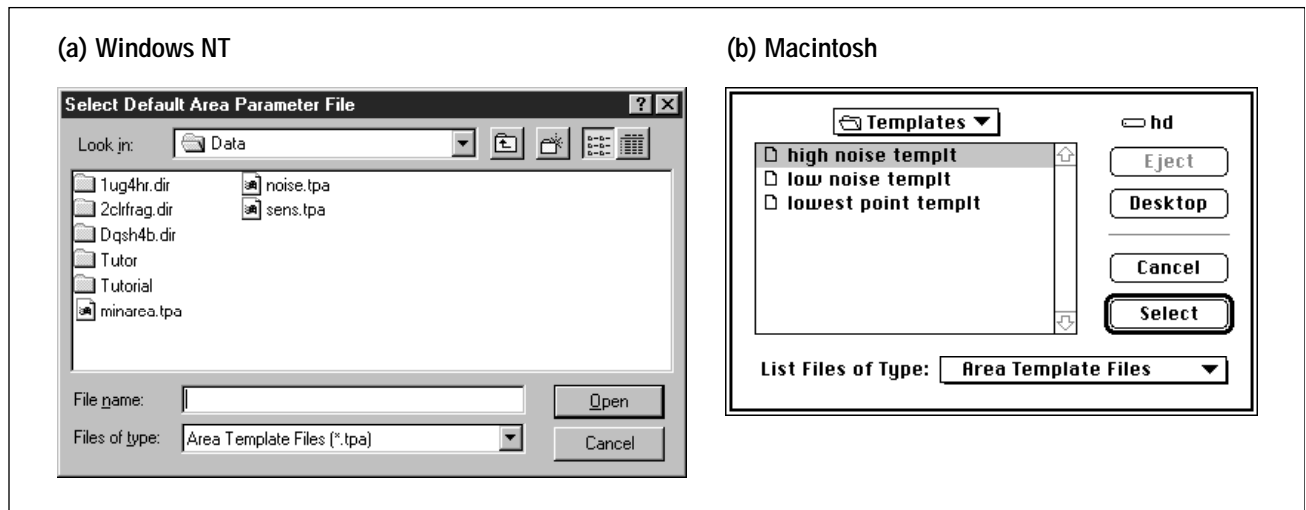
Figure 8-15. The Select Default Area Parameter File window.

8.9 Selecting the Area Report Options

Before you can generate a report, you select the information you want ImageQuant to report from the options in the Area Report Setup window (figure 8-16). You can display the Area Report Setup window from either the Analysis menu or the Preferences menu.
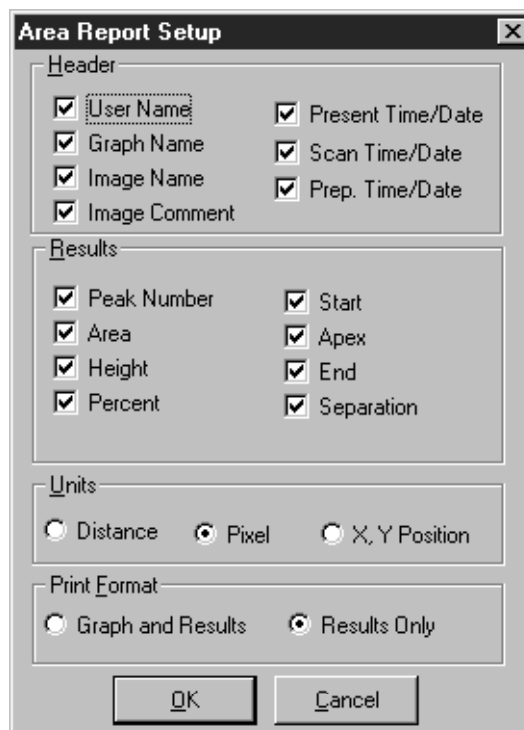
Figure 8-16. The Area Report Setup window.

If you want to apply the report options to—

- **All line graphs**—Choose **Area Report Setup** from the Preferences menu. In the Area Report Setup window (figure 8-16), click to select or deselect an option. After you click **OK,** ImageQuant prompts you to propagate the changes to all open Graph windows. If you click **Yes,** the new report preferences appear in the Area Report Setup windows for all the open Graph windows. If you click **No,** the new report preferences appear in the Area Report Setup window for the next Graph window you create.

- **Selected line graph only**—Choose **Area Report Setup** from the Analysis menu. In the Area Report Setup window (figure 8-16), click to select or deselect an option. Click **OK** to close the window.

You can print header information, the graph, and the results, or you can print the header information and the results. Only the results appear in the Area Report window.

The Area Report Setup window (figure 8-16) contains the following options:

Header

You can print the following header information:

- **User Name**—Prints the user name.

- **Graph Name**—Prints the graph name for the line graph you are currently analyzing. The graph name is the default line graph number or, if saved, the name you used to save the graph.

- **Image Name**—Prints the image name.

- **Image Comment**—Prints the image comment entered in the Scanner Control window at scan time.

- **Present Time and Date**—Prints the time and date when the report is generated. The time and date are recorded by the computer.

- **Scan Time and Date**—Prints the time and date when the scan was performed. The time and date are recorded by the scanner.

- **Preparation Time and Date**—Prints the time and date when the sample was prepared. The time and date are entered in the Scanner Control window of some instruments.

Results

You can choose any of the following results to display in an Area Report window or to print.

Note: The following equations use f(i) to denote the pixel value at location (i) along the graph.

- **Peak Number**—The number assigned to a peak. The number associates the peak with the statistics generated about the peak (counting left to right).

- **Area**—The integrated intensity of all the pixels in the peak excluding the baseline

$$Area = \sum_{i = Start}^{End} f[(i) - baseline(i)]$$

where Start and End are defined below.

- **Height**—The intensity of the pixel represented by the highest point of the peak (baseline corrected)

$$Height = Max[0, f(Apex) - baseline(Apex)]$$

where Apex is defined below.

- **Percent**—The area of the peak as a percentage of the total area in all the numbered peaks.

$$Percent = \frac{Area(i)}{\sum_{i = 1}^{N} Area(i)} \times 100$$

- **Start**—The distance from the start of the line to the beginning of the peak. The distance is displayed in millimeters, pixels, or X and Y coordinates (depending on the unit selected in the Units area).

- **Apex**—The distance from the start of the line to the maximum intensity point in the peak. The distance is displayed in millimeters, pixels, or X and Y coordinates (depending on the unit selected in the Units area).

- **End**—The distance from the start of the line to the end of the peak. The distance is displayed in millimeters, pixels, or X and Y coordinates (depending on the unit selected in the Units area).

- **Separation**—The method used to determine the start and end of a peak. The methods are—

  - **Valley**—The lowest point between two contiguous peaks.

  - **Baseline**—The baseline level.

Units

In the Units area, you select the unit in which to report the results. Changing this unit does not change the unit used to display the line graph. To change the unit displayed on the graph, see section 9.3.5.

The unit choices are—

- **Distance (mm)**—Displays the results in millimeters, measured from the beginning to the end of the line you drew (not from the edge of the image).

- **Pixel**—Displays the results measured from the beginning to the end of the line you drew, with zero indicating the beginning of the line.

- **X,Y Position**—Displays the results as the X and Y coordinates of the point on the line. The X,Y position is relative to the image, not the line (the upper-left corner of the image is 0,0).

Print Format

In the Print Format area, you select how you want the report printed—

- **Graph and Results**—Prints the selected header information and the graph on the first half of the page, followed by the results.

- **Results Only**—Prints the selected header information and the results.

8.10 Generating Reports

After area quantitation, you can print the results or transfer the results to Microsoft Excel or Word.

8.10.1 Creating a Report from a Single Line Graph or from Multiple Line Graphs

If you did not select **Display** and **Print** from the Report area of the Peak Finder inspector, you can create a report and display the results without recalculating the area results.

To do this—

1. Choose **Area Report** from the Analysis menu. The Area Report inspector appears (figure 8-17).

2. Select the line graph or line graphs.

3. Click **Display** to display the results in the Area Report window.

4. Click **Print** to send the results to the printer.

5. Click **Report** to generate the report. The results appear in an Area Report window.
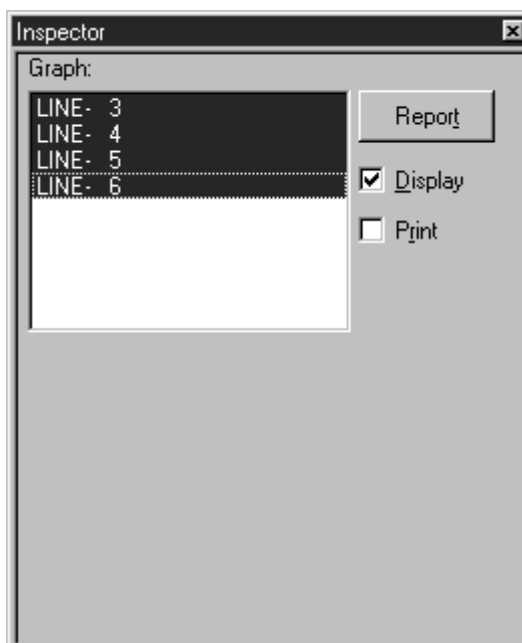
Figure 8-17. The Area Report inspector.

8.10.2 Transferring the Results to Microsoft Excel

After you view the results in the Area Report window, you can transfer the results to Excel for further analysis. To do this, double-click in the Area Report window, Excel opens and displays the results in a new worksheet.

8.10.3 Transferring Data Points to Microsoft Excel or Word

You can transfer the data points that comprise the line graph to Excel or Word. In addition to the data points, ImageQuant transfers the following: the X,Y location, the distance (mm) from the start of the line, and the intensity value.

To transfer the data points—

1. Click in the Graph window to select it.

2. Choose **Copy** from the Edit menu.

3. Open Excel and display a worksheet, or open Word and display a document.

4. Choose **Paste** from the Excel or Word Edit menu. The data points and their values are displayed in the Excel worksheet or the Word document.

8.10.4 Transferring the Line Graph to Microsoft Excel or Word

To transfer the line graph to Excel or Word—

1. Click in the Graph window to select it.

2. Choose **Copy** from the Edit menu.

3. Open Excel and display a worksheet, or open Word and display a document.

4. Choose **Paste Special** from the Excel or Word Edit menu.

5. In the Paste Special window, choose **Picture.** The line graph appears in the worksheet or document.

8.11 Saving the Line Graph

When you close the line graph, ImageQuant prompts you to save the graph.

- If you do not want to save the line graph, click **No.**

- If you want to save the line graph, click **Yes.** Type a name in the **Save document as** box and click **Save.**

To save the line graph without closing the Graph window, choose **Save As** from the **File** menu.

8.12 Printing the Line Graph

You can print the line graph by selecting the Graph window, and choosing **Print** ( ) from the File menu or the Main toolbar. ImageQuant prints the contents of the Graph window.

Chapter 9 Customizing the Line Graph

The line graph provides a visual display of the pixel intensities under the line you drew. Because the line graph can be used as part of the reporting process, ImageQuant provides several ways to improve the appearance of the line graph.

The topics in this chapter are—

- About the Line Graph (section 9.1)
- Creating Line Graphs for Multiple Objects (section 9.2)
- Enhancing the Line Graph (section 9.3)

9.1 About the Line Graph

ImageQuant provides several ways to display information about the line graph.

9.1.1 Using the Graph Info Inspector

The Graph Info inspector displays information about the selected line graph (figure 9-1). The inspector displays the name of the image used to create the graph and the width and coordinates of the line drawn on the image. ImageQuant displays the Line Width in the same unit selected for the line graph. The unit can be millimeters or pixels. If the unit is pixels and the line is a wide line, the number of pixels will be two times the width of the line plus one pixel.

You choose the Graph Info inspector from the View menu.
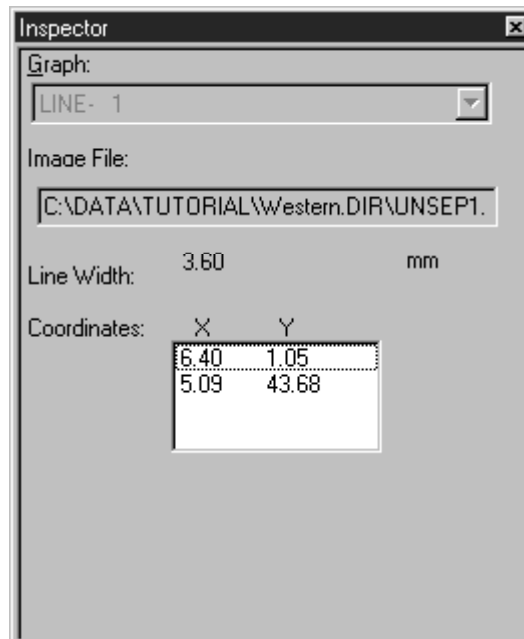
Figure 9-1. The Graph Info inspector.

9.1.2 Using the Vertical Hairline

After you move the pointer into the Graph window and click the
mouse button, a vertical hairline appears in the Graph window, and
a colored marker appears in the Image window (figure 9-2). The
marker in the Image window appears on the line that you used to
create the line graph. If the line is a wide line, the marker appears
the width of the wide line. As you move the vertical hairline along
the peaks in the graph, the marker moves to show the band that
corresponds to the peak.

To see the exact pixel intensity and distance values for any point
on the graph, drag the pointer to move the vertical hairline left or
right (or move the pointer, and then click the desired location). The
measurements are displayed at the top of the graph.
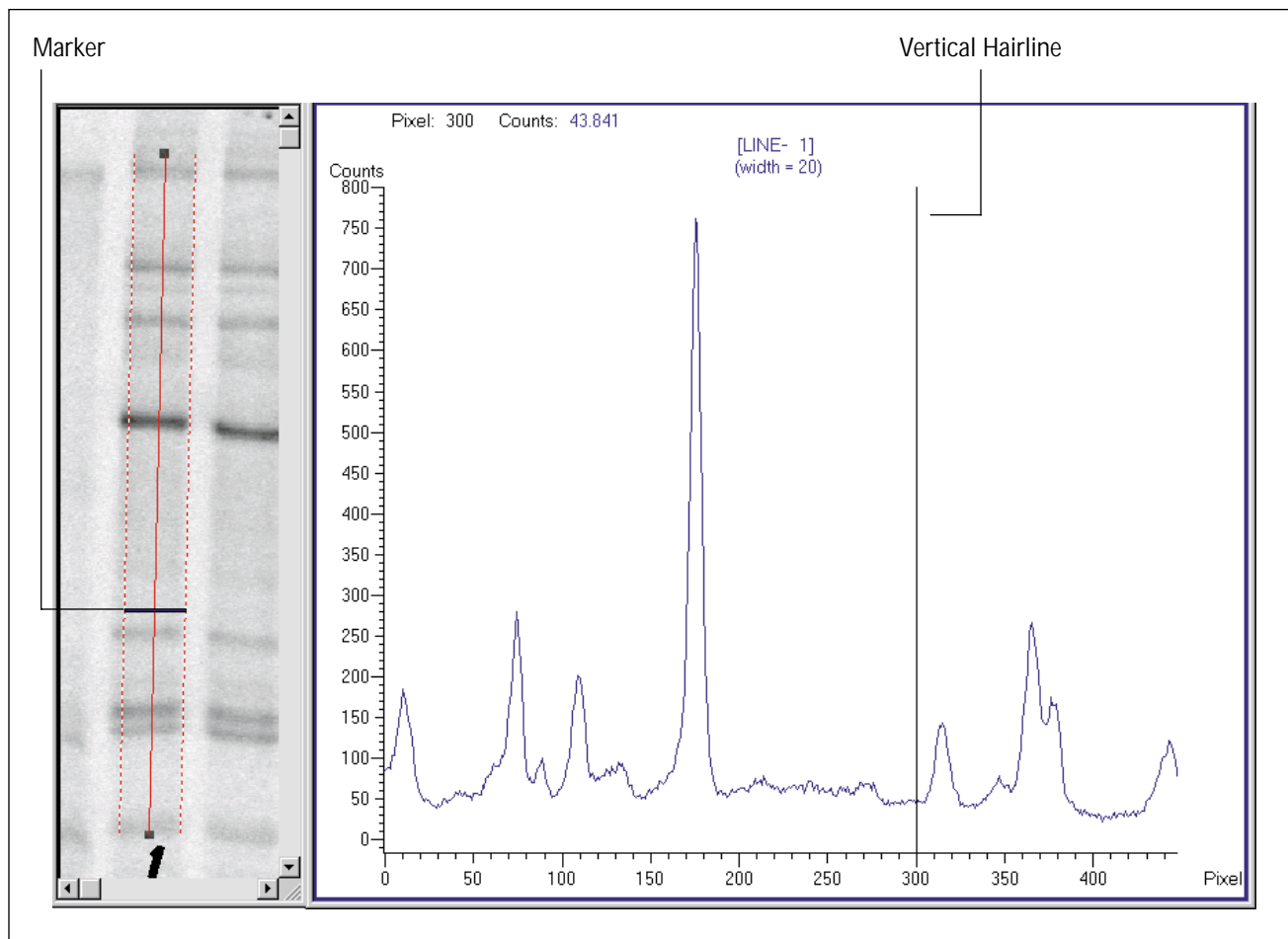
Figure 9-2. Line graph with vertical hairline. The marker in the Image window moves as you move the vertical hairline, showing which spot corresponds to each peak.

9.1.3 Understanding the Data

The line graph provides the following information about the line you drew:

- **Pixel intensity**—The vertical axis on the graph shows the pixel intensity. The pixel intensity reflects the signal strength and is nondirectional. This measurement can be thought of as the z-axis of the original (scanned) image. The pixel intensity units depend on which instrument was used to collect the image.

- **Distance**—The horizontal axis on the graph shows the distance, in millimeters or pixels, along the line you drew on the image.

9.2 Creating Line Graphs for Multiple Objects

By selecting multiple objects on your image, you can create multiple graphs as either separate or merged graphs.

9.2.1 Creating Multiple Line Graphs

To create line graphs for multiple objects, first select the objects, and then choose **Create Graph** from the Analysis menu. Separate line graphs appear in one Graph window (figure 9-3). Each line graph appears in a different color and in the order created. If the graphs appear without the labels, you cannot activate the vertical hairline or marker. To activate the hairline and marker, you can resize the Graph window to provide more space for the graphs and the labels, or you can view one graph at a time (section 9.2.4).

After the line graphs are displayed, choose **Peak Finder** from the Analysis menu. The Peak Finder inspector appears (figure 8-5). Adjust the parameters, if necessary, and then click **Compute.** All the lines use the same Peak Finder parameters and, if selected, the same Area Report Setup choices.

You can also create multiple line graphs by copying the contents of one Graph window and pasting the contents into another Graph window. The Copy and Paste commands are on the Edit menu.
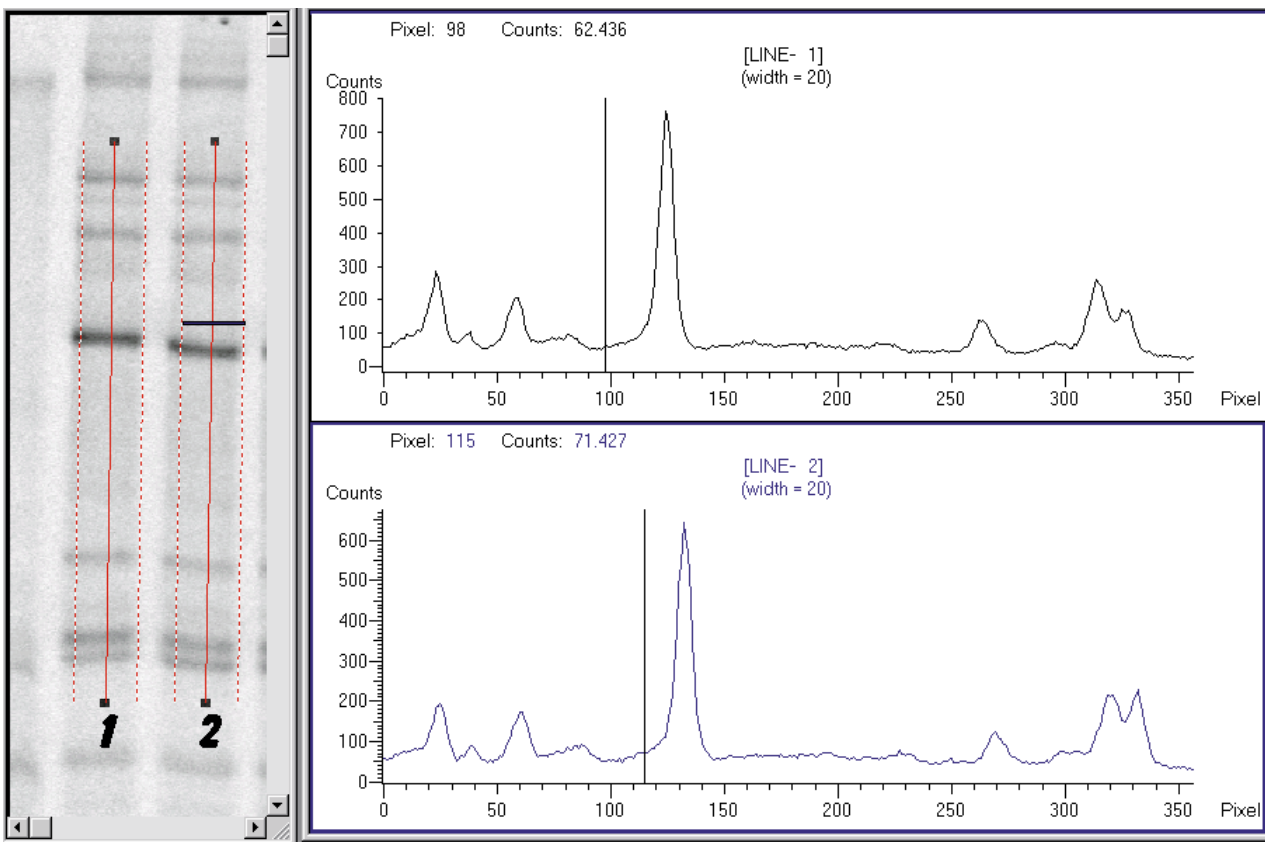
Figure 9-3. Line graphs for multiple objects.

9.2.2 Merging Multiple Line Graphs into One Graph

To merge separate line graphs, hold down the **SHIFT** key and click each line graph you want to include. A blue rectangle appears around the selected graphs. Choose **Merge** (📊) from the View menu or the Graph toolbar. ImageQuant merges the graphs and displays each line in a different color (figure 9-4).

Important **When you merge the line graphs, the merged line graph will not display the baseline, droplines, or peak numbers.**

Click in the Graph window to turn on the Value Display. The locations and pixel intensities for the point under the vertical hairline are displayed for all the merged line graphs. Drag the vertical hairline to move the markers along the line on the image and to view different locations and pixel intensities. The markers in the Image window that correspond to each line graph appear in the same color as the line in the line graph.
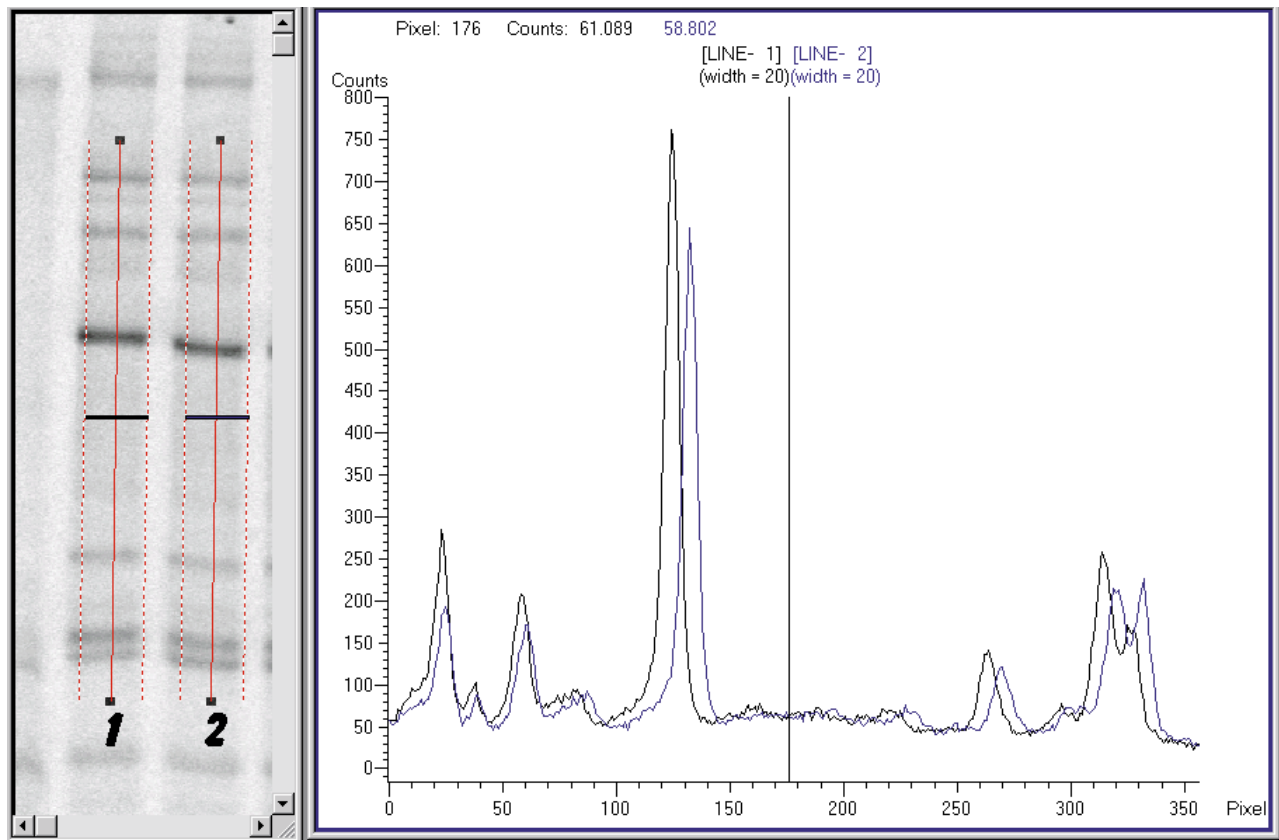
Figure 9-4. Multiple line graphs merged.

9.2.3 Splitting the Graphs

To split the merged graphs, click the merged line graph in the Graph window. Choose **Split** (⬚) from the View menu or the Graph toolbar. ImageQuant displays each line graph in an individual section of the Graph window.

9.2.4 Enlarging One Graph from a Set

If you have several line graphs displayed in a single Graph window, you can select one graph and enlarge it. To do this, choose the line graph you want to enlarge, and then choose **Maximize** (⬚) from the View menu or the Graph toolbar. The line graph expands to fill the Graph window.

To return to the original display, choose **Restore** (⬚) from the View menu or the Graph toolbar. The multiple line graphs reappear.

9.3 Enhancing the Line Graph

You can enhance the line graph using the Line Attributes and Axis Attributes inspectors (figure 9-5). Both inspectors are available from the View menu. If you double-click in a Graph window, ImageQuant displays the Line Attributes inspector.

(a) Line Attributes Inspector

(b) Axis Attributes Inspector
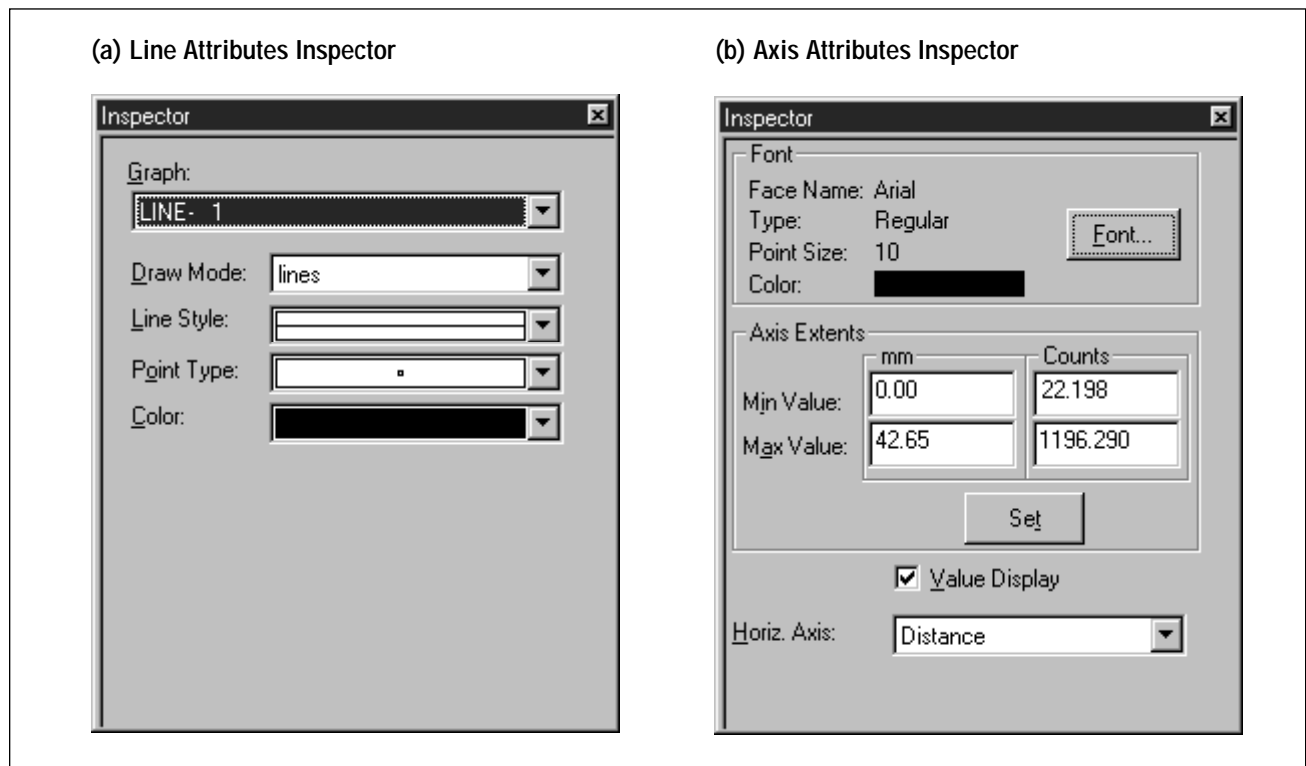
Figure 9-5. The Line Attributes (a) and Axis Attributes (b) inspectors.

9.3.1 Changing the Line Style and Color

You use the Line Attributes inspector (figure 9-5a) to change the line graph display. You can select a different draw mode, line style, point type, and color for the selected graph. Choose a new display option from the list for the item you want to change. If you are changing a merged line graph, select the desired line graph from the Graph list. ImageQuant displays the line graph and the marker in the Image window using the new color.

9.3.2 Changing the Font

You can change the font used for the labels on the line graph. In the Axis Attributes inspector (figure 9-5b), click **Font.** The Font window appears. Choose a new font, style, size, effect, and color from the lists and check boxes. You can see an example of the changes you make in the Sample area. Click **OK** to change the labels in the graph, close the Font window, and return to the Axis Attributes inspector. Your new choices appear in the inspector and in the Graph window.

9.3.3 Changing the Scale

The left edge of the line graph represents the beginning point of the line you drew on the image. For example, if you draw the line from top to bottom, the left edge of the graph reflects the top point of the line you drew.

In the Axis Attributes inspector (figure 9-5b), the Min Value and Max Value boxes display the values for the start and end of the line graph. If you selected several lines and created a multiple line graph, you can change the axes to make the start and end points of all the line graphs match.

- To change the horizontal axis of the graph, you use the two boxes on the left. Type a minimum value in the **Min Value** box and type a maximum value in the **Max Value** box. (The column heading varies according to the selection made in the Horiz. Axis box, as discussed in section 9.3.6.) The horizontal axis of the line graph adjusts accordingly. (If you eliminate the ends of the line, choose **Reset** from the Zoom Area submenu on the Tools menu to return to the original graph.)

- To change the vertical axis of the graph, you use the two boxes on the right. Type a minimum value in the **Min Value** box and type a maximum value in the **Max Value** box. (The heading varies according to the type of instrument on which the image was collected.) The vertical axis of the line graph adjusts accordingly.

Note: You can enlarge a section of a line graph using the **Create Frame** command on the Tools menu or the **Create Frame** button on the Graph toolbar. See section 2.4.5 for instructions on how to use this tool.

9.3.4 Turning On or Off the Vertical Hairline and Marker

The Value Display check box in the Axis Attributes inspector (figure 9-5b) allows you to turn on or off the vertical hairline and the corresponding marker in the Image window (figure 9-2). To turn off the vertical hairline and the marker, click the check box to remove the check mark. ImageQuant removes the vertical hairline from the line graph. Click in the Image window to remove the marker from the Image window. In addition, ImageQuant removes the distance/X,Y display that appears in the upper portion of the Graph window.

To display the vertical hairline and marker, click the Value Display check box and then click in each graph. The vertical hairline appears in the line graph, the values appear in the upper portion of the Graph window, and the marker appears in the Image window (figure 9-2).

9.3.5 Changing the Line Measurement Units

You use the Horiz. Axis box in the Axis Attributes inspector (figure 9-5b) to choose the measurement units to display with the line graph. (This measurement unit is for display only and does not affect your reporting choice.) To make a selection, choose one of the following options from the **Horiz. Axis** list:

- **Distance**—Displays the length of the line in millimeters.

- **Pixel**—Displays the length of the line in pixels counting from the beginning to the end of the line.

9.3.6 How the Min and Max Value Headings Change

The column headings for the Axis Extents area refer to the distance units (left column) and intensity units (right column) of the line you drew. The headings vary according to the following:

- **Horiz. Axis selection**—Because you can select the units for the horizontal axis of the graph, the choice you make affects the heading of the boxes on the left. For example, if you select Distance from the Horiz. Axis box, mm appears as the heading of the column.

- **The instrument**—The heading of the boxes on the right reflects the instrument on which the image was collected.

Chapter 10 Performing Volume Quantitation with the User Method

ImageQuant provides two methods that you can use to perform volume quantitation: the User method and the Spot Finder method. This chapter describes the User method. The topics in this chapter are—

- About the User Method of Volume Quantitation (section 10.1)
- Selecting a Background Correction Method (section 10.2)
- Drawing the Objects (section 10.3)
- Viewing the Results (section 10.4)
- Modifying the Objects (section 10.5)
- Changing the Background Correction (section 10.6)
- Selecting the Volume Report Options (section 10.7)
- Generating Reports (section 10.8)
- Saving the Objects with the Image (section 10.9)
- Printing the Image (section 10.10)

Chapter 11 describes the Spot Finder method.

10.1 About the User Method of Volume Quantitation

Volume quantitation calculates the volume under the surface created by a 3-D plot of the pixel locations and pixel intensities. To perform volume quantitation with the User method, you draw objects to identify the spots on the image you want to analyze.

10.1.1 Pixels and Volume Quantitation

When possible, ImageQuant draws the lines of the enclosed objects between the pixels that comprise the image. This section describes which pixels ImageQuant uses in the User method of volume quantitation (figure 10-1):

Important   **To use a rectangle, ellipse, polygon, or region for quantitation, you must draw the object so that it includes four or more pixels (two pixels high by two pixels wide or more). To use a grid for quantitation, you must draw the grid so that it includes nine or more pixels in each grid cell (three pixels high by three pixels wide or more).**

- **Rectangle or Square**—ImageQuant includes the pixels that are inside the lines of the rectangle or square.

- **Ellipse or Circle**—ImageQuant includes the pixels that are inside the lines of the ellipse or circle and the pixels that are bisected by the line.

- **Polygon**—ImageQuant includes the pixels that are inside the lines of the polygon and the pixels that are bisected by the line.

- **Grid**—ImageQuant includes the pixels that are inside the lines of each cell. When you create small grid cells, the grid-cell areas may vary in dimensions because ImageQuant cannot equally divide the pixel boundaries. For example, ImageQuant cannot evenly divide 28 pixels among three grid cells.

- **Region**—ImageQuant includes the pixels that are inside the lines of the region, and no pixels are bisected by the line.

Rectangle/Square

Ellipse/Circle/Polygon

Grid

Region
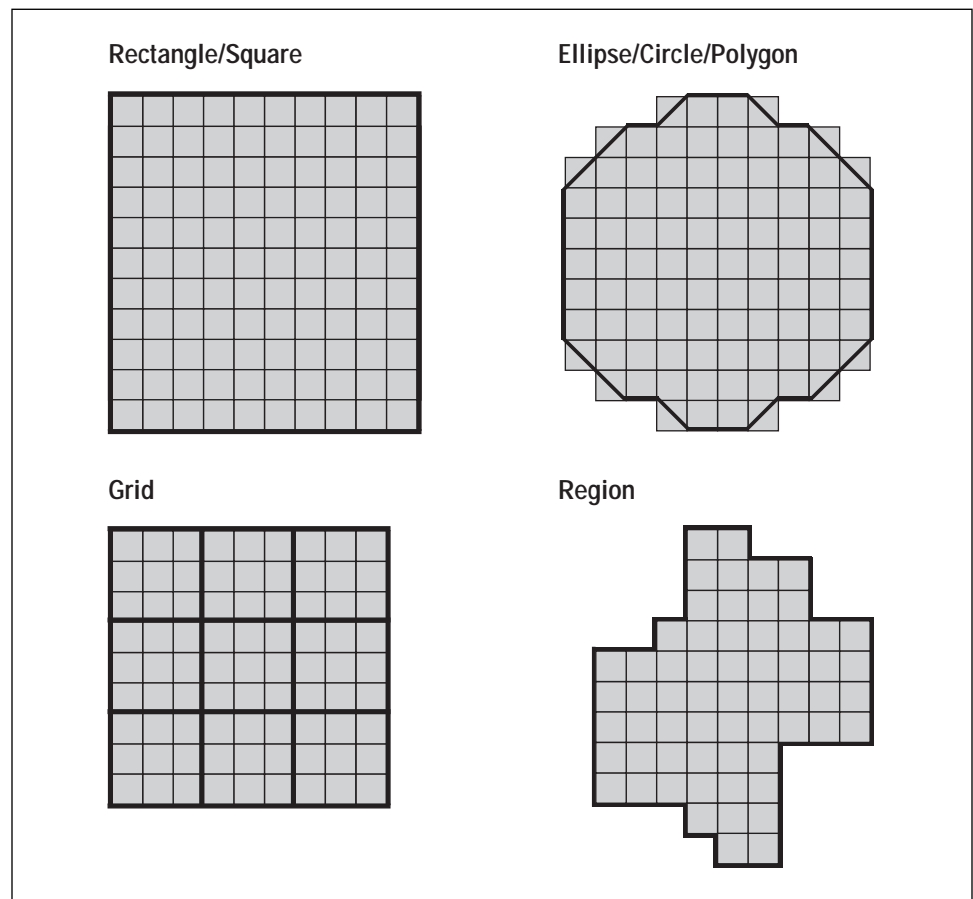
Figure 10-1. Determining the pixels included in volume quantitation.

10.1.2 Overview of the User Method

With the User method, you select a background correction method and then draw objects surrounding the areas you want to quantitate. ImageQuant automatically calculates the results for each object. You can view the results for an object in the Volume Review inspector. If you are not satisfied with the results, you can resize or reposition the object, or you can draw a new object. In addition, you can use the Background Correction inspector to apply a different background correction method. To generate a report, select the reporting options from the Volume Report Setup window. Next, open the Volume Report inspector, and then select the objects and the type of report.

Figure 10-2 shows the User volume quantitation workflow.

```
┌─────────────────────────────────────────────┐
│        ┌──────────────────────────────┐      │
│        │ Select the background correction │      │
│        │        (section 10.2)          │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │      Draw the objects          │      │
│        │        (section 10.3)          │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │       View the results         │      │
│        │        (section 10.4)          │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │      Make modifications         │      │
│        │   (sections 10.5 and 10.6)     │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │ Select the volume report options │    │
│        │        (section 10.7)          │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │      Generate the report        │      │
│        │        (sections 10.8)         │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │       View the report          │      │
│        │        (section 10.8)          │      │
│        └──────────────────────────────┘      │
│        ┌──────────────────────────────┐      │
│        │       Print the report         │      │
│        │        (section 10.8)          │      │
│        └──────────────────────────────┘      │
└─────────────────────────────────────────────┘
```

Figure 10-2. The User volume quantitation workflow.

10.2 Selecting a Background Correction Method

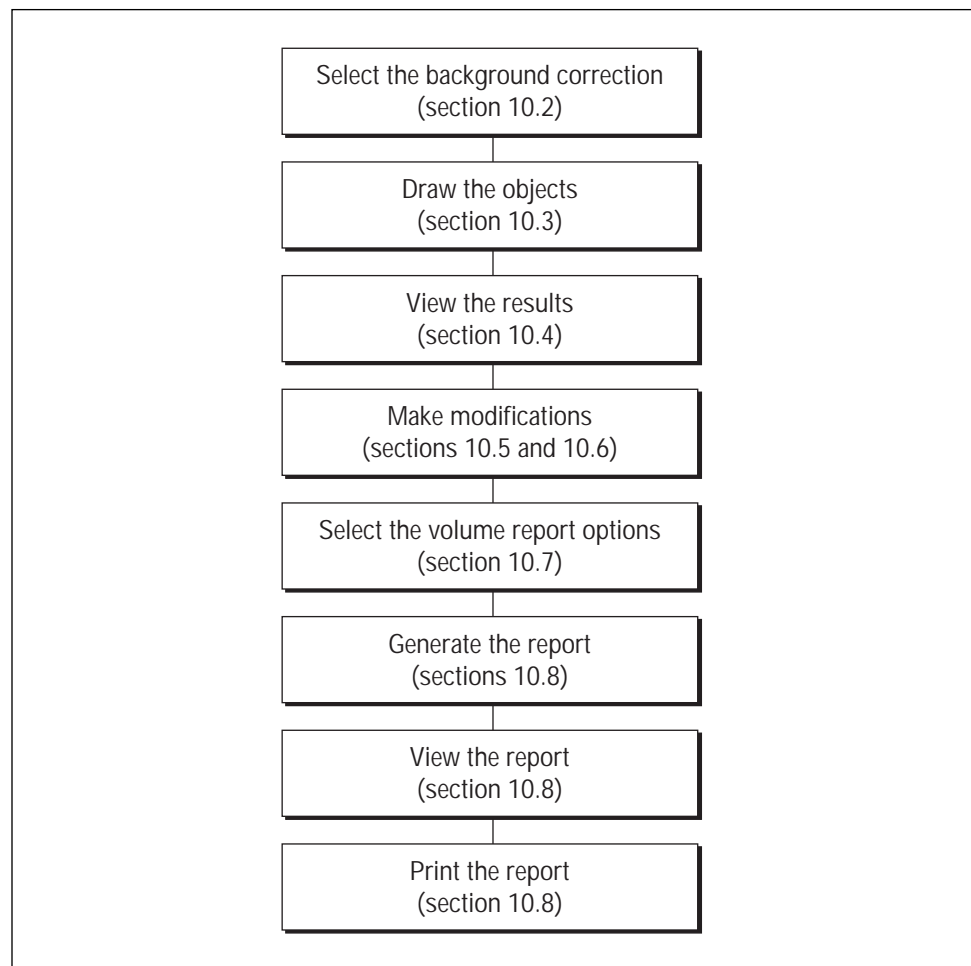Because quantitation occurs when you finish drawing an object, you must select a background correction method before you draw the object. You select a default background correction as a preference so that the background correction method is applied each time you perform quantitation. To change the background correction after performing quantitation, see section 10.6.

You can select one of the following background correction types as a preference: None, Local Average, Local Median, and Histogram Peak. The background correction affects only the Volume result.

10.2.1 Description of the Background Correction Methods

The following describes each background correction method available as a preference:

- **None**—No background correction is applied. Select this option if the image has little noise and the background is flat and low relative to the image (approximately zero).

- **Local Average**—ImageQuant determines the average (mean) of all the pixel values in the object outline and uses this value for the background.

- **Local Median**—ImageQuant determines the median (middle value) of all the pixel values in the object outline and uses this value for the background.

- **Histogram Peak**—ImageQuant determines the background in an area the width of the selected object and the length of the image (figure 10-3). The value that occurs most often within this area is used as the background value. Select this method of background correction if the image has more pixels in the background area than in the spot area.
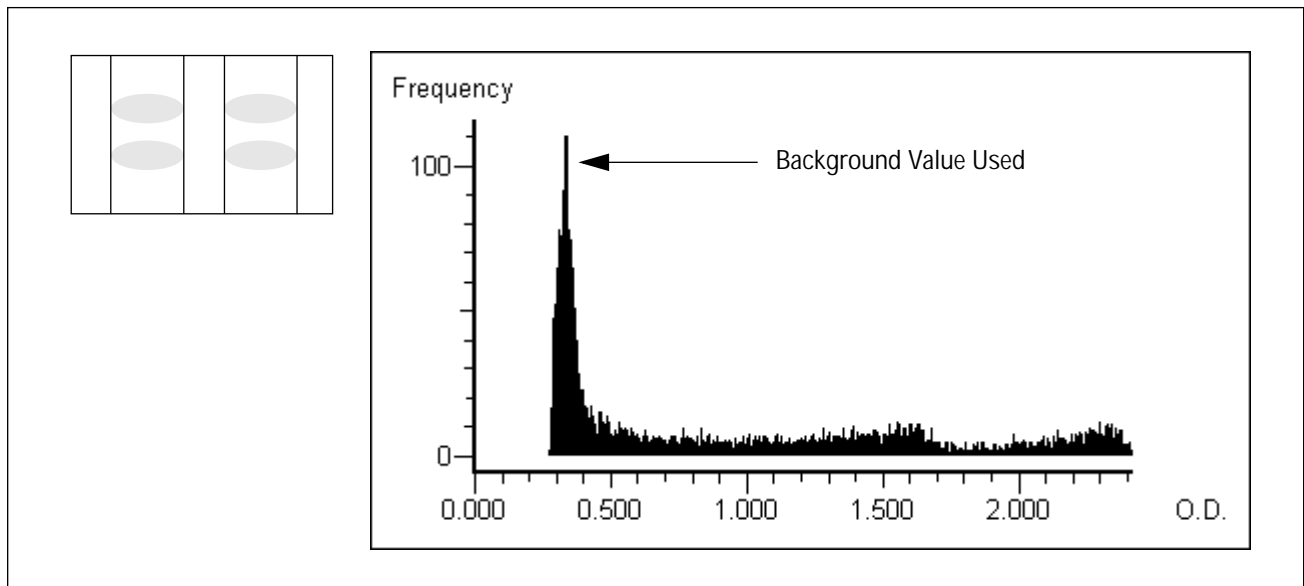
Frequency

Background Value Used

Figure 10-3. Using the Histogram Peak background correction method, the most frequent pixel intensity is used as the background value.

10.2.2 Setting the Background Correction

Because the results are calculated when you create an object, you must select a background correction method first. Each time you create an object, ImageQuant applies the background correction method you selected as a preference. If you change the background correction method, ImageQuant applies the new method to all the objects you create after the change but does not change the existing objects.

To select a background correction method as a preference—

1. Choose **Background Correction** from the Preferences menu. The Background Correction/Preferences window appears (figure 10-4).

2. In the User area, choose the background correction method you want to use.

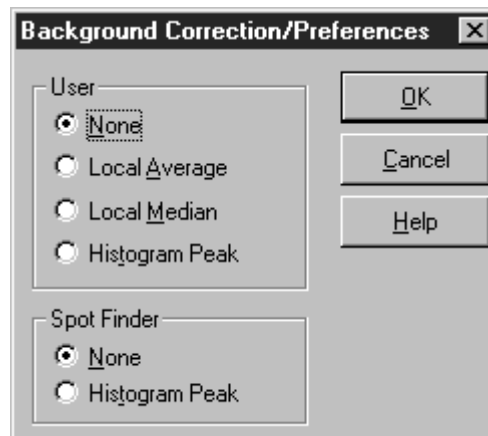3. Click **OK.** The Background Correction/Preferences window closes.

Figure 10-4. The Background Correction/Preferences window.

10.3 Drawing the Objects

To perform volume quantitation with the User method, you draw an object around each spot, and then ImageQuant generates the results. For more information on drawing objects, see chapter 5.

When drawing objects, select an object type that closely approximates the shape of the spot. For example, use an ellipse when enclosing a microplate well or blot, use a rectangle when enclosing a nonsmiling band, or use a region for irregularly shaped spots. In addition, include as little as possible of the background.

10.4 Viewing the Results

Prior to displaying or printing the report, you can view the results for one object in the Volume Review inspector. To do this, choose **Volume Review** ( ) from the Analysis menu or the Quantitation toolbar. The Volume Review inspector appears (figure 10-5). Click an object on the image to view the results in the inspector.

Note: When you select a large object, such as a grid, the status bar displays the progress of the quantitation. If you want to cancel the quantitation, press the **ESC** key and click **Yes** when asked if you want to abort.
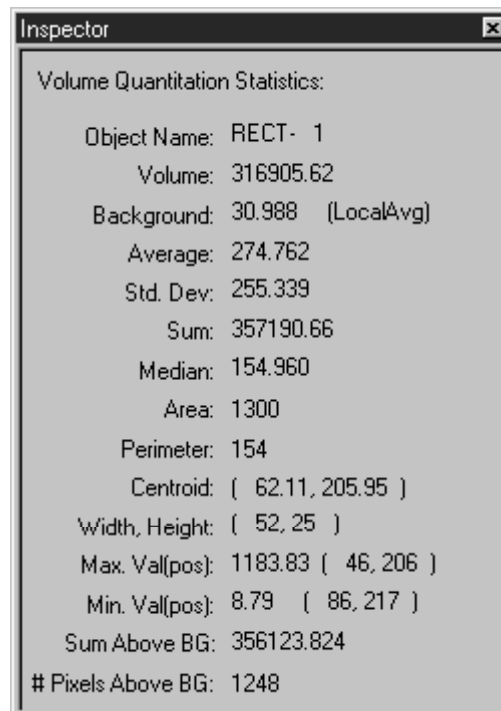
Figure 10-5. The Volume Review inspector.

10.5 Modifying the Objects

After viewing the results, you can modify the objects in several
ways to recalculate the results. You can—

- Reposition an object so that the spot is centered in the object
  (sections 6.5 through 6.7).

- Resize an object to exclude more background or include more of
  the spot (sections 6.12 and 6.13).

- Draw a new object that more closely resembles the shape of the
  spot (section 5.4).

ImageQuant calculates new results, which you can view in the
Volume Review inspector (figure 10-5).

10.6 Changing the Background Correction

After creating an object, you can change the background correction method. ImageQuant applies the new method to the selected objects only. The default background correction method you selected in the Preferences menu does not change.

10.6.1 About the Correction Methods

The Background Correction inspector (figure 10-6) displays each object and the background correction method applied to the object. The codes are No=None, LA=Local Average, LM=Local Median, and HP=Histogram Peak. Objects that are grouped do not display a method. (Grouped objects appear in the object list as GRUP followed by a single name or number.)
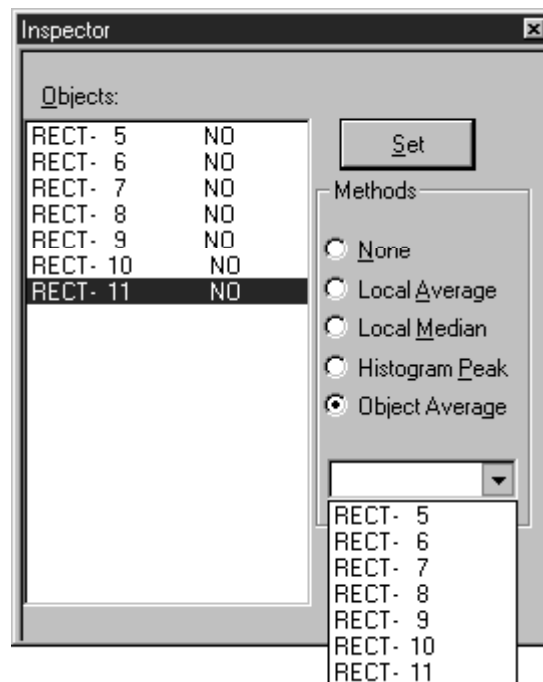
Figure 10-6. The Background Correction inspector.

In addition to the original four background correction methods described in section 10.2.1, you can choose a fifth method—**Object Average (OA).** When you select Object Average, a list box appears that contains the rectangles and ellipses you drew on the image. You select the rectangle or ellipse you want to use. ImageQuant calculates the average intensity value of the pixels in the object.

The value appears in the list box and is the background value applied to the objects you select from the Objects list. Pixels below this value are treated as background, and pixels above this value are treated as belonging to a spot.

Important   **If you move the background object, you must select the object from the Object Average list again to recalculate the background value.**

10.6.2 Changing the Background Correction Method

To change the background correction method for existing objects—

1. Choose **Background Correction** from the Analysis menu. The Background Correction inspector appears.

2. From the Objects list, choose the objects you want to change.

3. Choose the background correction method you want to use.

    If you select Object Average, choose the object from the list to use for the background calculation.

4. Click **Set.** The new method is applied to the selected object or objects.

5. To see the new results, choose **Volume Review** ( ) from the Analysis menu or the Quantitation toolbar. If you selected several objects, the progress of the quantitation appears in the status bar. **Note:** If you want to cancel the quantitation, press the **ESC** key and click **Yes** when asked if you want to abort.

10.7 Selecting the Volume Report Options

Before you can generate a report, you select the information you want ImageQuant to report from the options in the Volume Report Setup window (figure 10-7). You can display the Volume Report Setup window from either the Analysis menu or the Preferences menu.
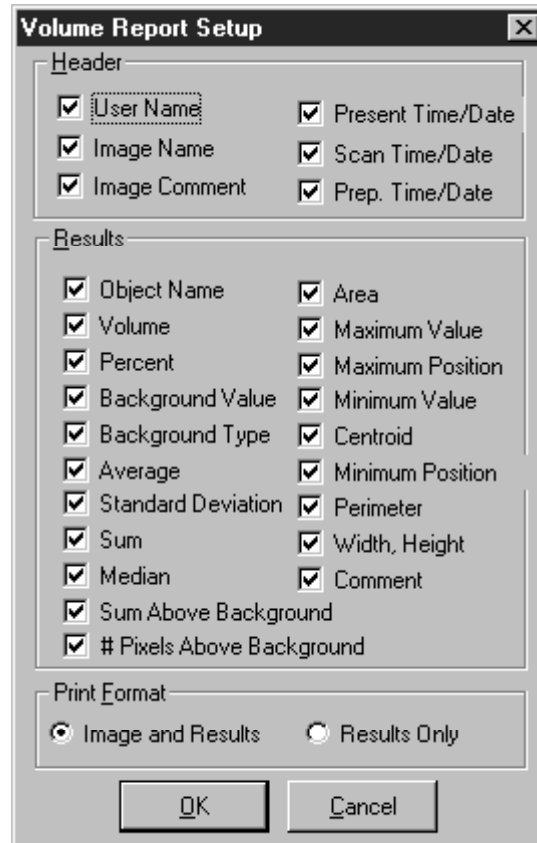
Figure 10-7. The Volume Report Setup window.

If you want to apply the report options to—

- **All open images**—Choose **Volume Report Setup** from the Preferences menu. In the Volume Report Setup window, click to select or deselect an option. After you click **OK,** ImageQuant prompts you to propagate the changes to all open Image windows. If you click **Yes,** the new report preferences appear in the Volume Report Setup window for all the open Image windows. If you click **No,** the new report preferences appear in the Volume Report Setup window for the next new image you

display in an Image window. Images that you already used for quantitation are not affected by the new volume report preferences.

- **Selected image only**—Choose **Volume Report Setup** from the Analysis menu. In the Volume Report Setup window, click to select or deselect an option.

The Volume Report Setup window (figure 10-7) contains the following options:

Header

You can print the following header information:

- **User Name**—Prints the user name.

- **Image Name**—Prints the image name.

- **Image Comment**—Prints the image comment entered in the Scanner Control window at scan time.

- **Present Time and Date**—Prints the time and date when the report is generated. The time and date are recorded by the computer.

- **Scan Time and Date**—Prints the time and date when the scan was performed. The time and date are recorded by the scanner.

- **Preparation Time and Date**—Prints the time and date when the sample was prepared. You enter the time and date in the Scanner Control window of some instruments.

Results

You can choose any of the following results to display in a Volume Report window or to print:

Note: The formulas provided below are for a rectangle with the dimensions N x M. The following equations use f(x,y) to denote the pixel intensity at pixel location (x,y) on the image. For nonrectangular objects, the pixel values within the object are the only values used in the calculations. The term *spot* is used in the description to represent the band, blot, or other feature on the image that you are analyzing.

- **Object Name**—The name assigned to the object.

- **Volume**—The integrated intensity of all the pixels in the spot, excluding the background. To calculate volume, ImageQuant subtracts the background value from the intensity of each pixel in the object, and then adds all the values.

$$\text{Volume} = \sum_{y=1}^{M} \sum_{x=1}^{N} [f(x,y) - \text{background}]$$

- **Percent**—The volume in the spot as a percentage of the total volume. The total volume equals the sum of the volumes of all the objects included in one quantitation. If you alter the set of objects and regenerate the report, the Percent results will be different.

$$\text{Percent} = \frac{\text{Volume(i)}}{\sum\limits_{i=1}^{N} \text{Volume(i)}} \times 100$$

- **Background Value**—The background value used during volume quantitation. The background value is subtracted from each pixel within the object to determine the volume.

- **Background Type**—The background correction method used to derive the background value.

- **Average**—The average intensity of all the pixels in the spot.

$$\text{Average} = \frac{\sum\limits_{y=1}^{M} \sum\limits_{x=1}^{N} f(x,y)}{M \times N}$$

- **Standard Deviation**—The standard deviation of the pixel intensities in the spot.

$$\text{Standard Deviation} = \sqrt{\frac{\sum\limits_{y=1}^{M} \sum\limits_{x=1}^{N} [f(x,y) - \text{Average}]}{M \times N}}$$

- **Sum**—The integrated intensity of all the pixels in the spot including the background.

$$Sum = \sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)$$

- **Median**—The middle intensity value of all the pixels in the object.

- **Sum Above Background**—The integrated intensity of all the pixels in the spot excluding the background. To calculate the sum above the background, ImageQuant adds only the pixel values in the object that are above the background.

$$Sum\ Above\ Background = \sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)$$

where   f(x,y)>background

If you used the Spot Finder quantitation method to draw the region objects, the Sum Above Background result should be nearly the same as the Volume result. If you drew individual objects, the results should be different. If you are enclosing smiling bands in individual rectangles, the Sum Above Background result may be more accurate than the Volume result.

- **# Pixels Above Background**—The total number of pixels used in the Sum Above Background calculation.

- **Area**—The number of pixels in the spot.

- **Maximum Value**—The maximum pixel intensity in the spot.

- **Maximum Position**—The pixel coordinates of the maximum value of the spot.

- **Minimum Value**—The minimum pixel intensity in the spot.

- **Minimum Position**—The pixel coordinates of the minimum value of the spot.

- **Centroid**—The pixel coordinates (Cx, Cy) of the geometric center of the selected draw object where—

$$Cx = \frac{\sum_{y=1}^{M} \sum_{x=1}^{N} [xf(x,y)]}{\sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)}$$

$$Cy = \frac{\sum_{y=1}^{M} \sum_{x=1}^{N} [yf(x,y)]}{\sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)}$$

- **Perimeter**—The number of pixels that border the spot.

- **Width, Height**—The maximum width and height of the spot in pixels.

- **Comment**—The annotation associated with the object. (You enter the comment using the Image Comment field in the Object Attributes inspector.)

Print Format

In the Print Format area, you select how you want your report printed—

- **Image and Results**—Prints the selected header information and the image on the first page, and prints the header information and the results on the second page.

- **Results Only**—Prints the selected header information and the results.

10.8 Generating Reports

After volume quantitation, you can print the results or transfer the results to Microsoft Excel or Word.

10.8.1 Creating a Report from a Single Object or Multiple Objects

Using the Volume Report inspector, you select the objects about which you want to generate a report. You also select whether or not you want the report displayed in a Volume Report window, sent to the printer, or both. When you generate the report, the background correction and reporting choices are applied to the objects you select.

To generate a report—

1. Choose **Volume Report** from the Analysis menu. The Volume Report inspector appears (figure 10-8).

2. In the Image window or from the Objects list in the inspector, select the object(s) about which you want to generate a report.
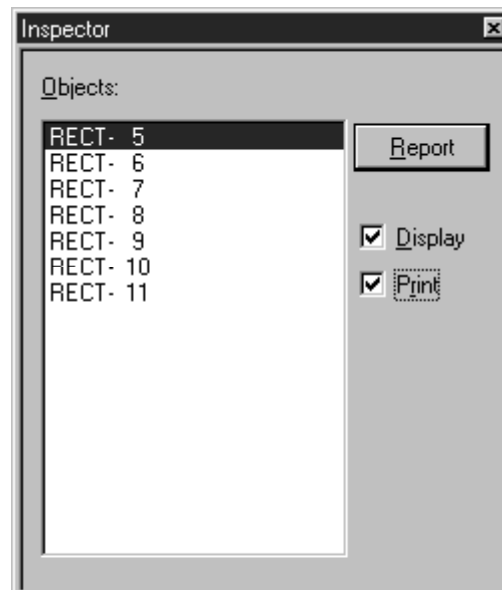

Figure 10-8. The Volume Report inspector.

3. Click **Display** to display the results in a Volume Report window.

4. Click **Print** to send the results to the printer.

5. Click **Report.** ImageQuant compiles the results for each selected object.

If you select a large number of objects, the status bar displays the progress of the report generation. If you want to stop the calculation, press the **ESC** key, and click **Yes** when asked if you want to abort.

10.8.2 Transferring the Results to Microsoft Excel

After you view the results in the Volume Report window, you can transfer the results to Excel for further analysis. To do this, double-click in the Volume Report window. Excel opens and displays the results in a new worksheet.

10.9 Saving the Objects with the Image

When you close the image, you will be prompted to save the objects you created.

•   If you do not want to save the objects, click **No.**

•   If you want to save the objects, click **Yes.** ImageQuant creates a file that contains the objects.

To save the objects without closing the Image window, choose **Save** from the File menu.

10.10 Printing the Image

You can print the image by selecting the Image window and choosing **Print** ( ) from the File menu or the Main toolbar. ImageQuant prints the image including the displayed objects.

Chapter 11 Performing Volume Quantitation Using Spot Finder

ImageQuant provides two methods that you can use to perform volume quantitation: the User method and the Spot Finder method. This chapter describes the Spot Finder method. The topics in this chapter are—

- About the Spot Finder Method of Volume Quantitation (section 11.1)
- Selecting Preferences for Background Correction (section 11.2)
- Drawing a Rectangle to Define the Area (section 11.3)
- Defining the Volume Quantitation Parameters Using the Spot Finder Inspector (section 11.4)
- Viewing the Results for Each Region (section 11.5)
- Adjusting the Parameters (section 11.6)
- Using Templates (section 11.7)
- Changing the Background Correction (section 11.8)
- Selecting the Volume Report Options (section 11.9)
- Generating Reports (section 11.10)
- Saving the Objects with the Image (section 11.11)
- Printing the Image (section 11.12)

Chapter 10 describes the User method.

11.1 About the Spot Finder Method of Volume Quantitation

Volume quantitation calculates the volume under the surface created by a 3-D plot of the pixel locations and pixel intensities. To perform volume quantitation using the Spot Finder method, you draw a rectangle around the portion of the image you want Spot Finder to evaluate.

ImageQuant uses the parameters you select in the Spot Finder window to identify spots in the rectangle. ImageQuant draws a region object around each found spot and calculates values for each spot.

Figure 11-1 shows the Spot Finder volume quantitation workflow.
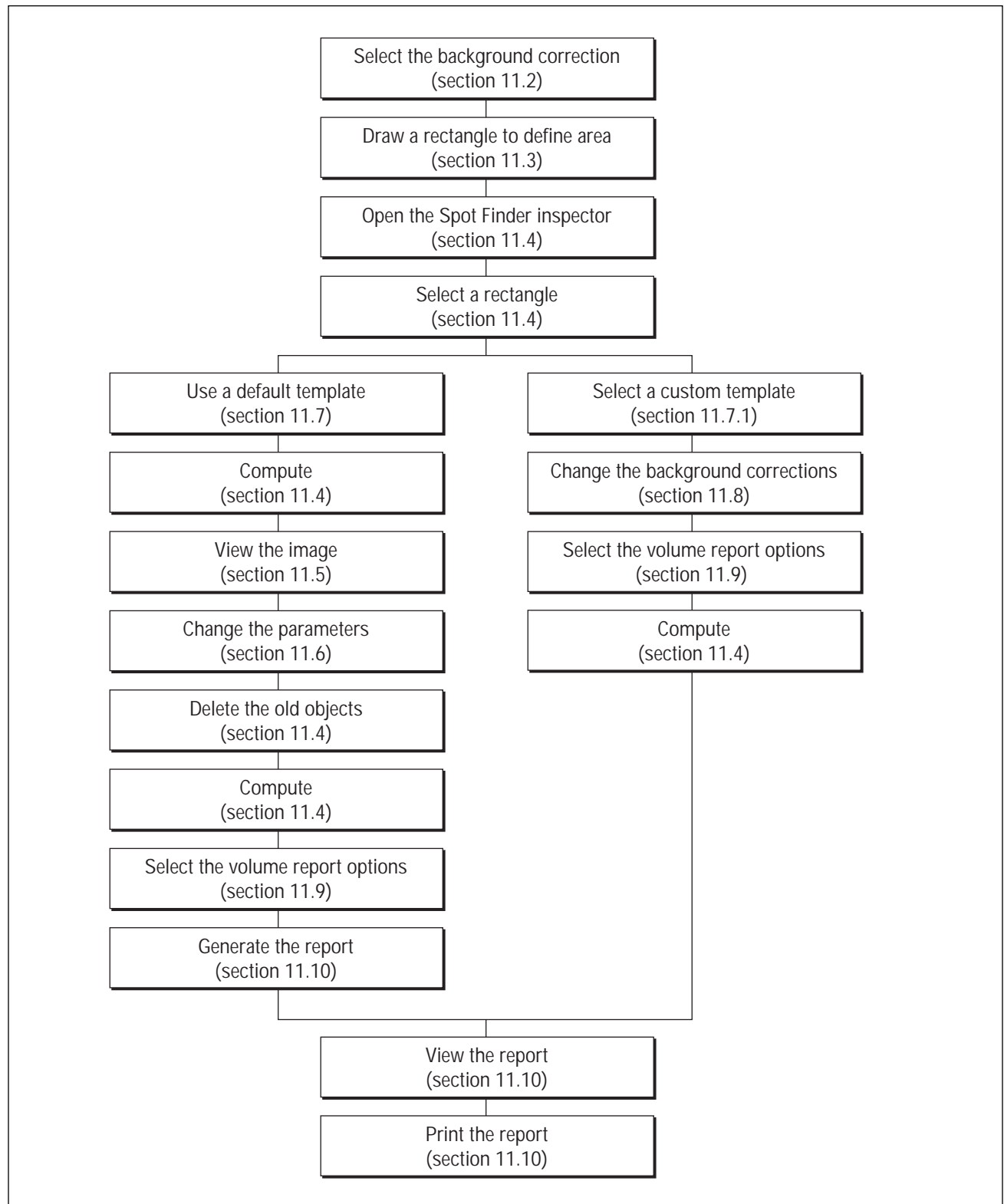
```
                    ┌─────────────────────────────┐
                    │ Select the background correction │
                    │        (section 11.2)        │
                    └─────────────────────────────┘
                                  │
                    ┌─────────────────────────────┐
                    │ Draw a rectangle to define area │
                    │        (section 11.3)        │
                    └─────────────────────────────┘
                                  │
                    ┌─────────────────────────────┐
                    │  Open the Spot Finder inspector │
                    │        (section 11.4)        │
                    └─────────────────────────────┘
                                  │
                    ┌─────────────────────────────┐
                    │      Select a rectangle      │
                    │        (section 11.4)        │
                    └─────────────────────────────┘
                                  │
         ┌────────────────────────┴────────────────────────┐
  ┌────────────────────┐                        ┌──────────────────────────────┐
  │ Use a default template │                    │    Select a custom template    │
  │    (section 11.7)    │                        │       (section 11.7.1)       │
  └────────────────────┘                        └──────────────────────────────┘
           │                                                 │
  ┌────────────────────┐                        ┌──────────────────────────────┐
  │       Compute      │                        │ Change the background corrections │
  │    (section 11.4)  │                        │        (section 11.8)        │
  └────────────────────┘                        └──────────────────────────────┘
           │                                                 │
  ┌────────────────────┐                        ┌──────────────────────────────┐
  │    View the image  │                        │ Select the volume report options │
  │    (section 11.5)  │                        │        (section 11.9)        │
  └────────────────────┘                        └──────────────────────────────┘
           │                                                 │
  ┌────────────────────┐                        ┌──────────────────────────────┐
  │ Change the parameters │                     │            Compute            │
  │    (section 11.6)  │                        │        (section 11.4)        │
  └────────────────────┘                        └──────────────────────────────┘
           │                                                 │
  ┌────────────────────┐                                     │
  │ Delete the old objects │                                 │
  │    (section 11.4)  │                                     │
  └────────────────────┘                                     │
           │                                                 │
  ┌────────────────────┐                                     │
  │       Compute      │                                     │
  │    (section 11.4)  │                                     │
  └────────────────────┘                                     │
           │                                                 │
  ┌──────────────────────────┐                               │
  │ Select the volume report options │                       │
  │        (section 11.9)    │                               │
  └──────────────────────────┘                               │
           │                                                 │
  ┌────────────────────┐                                     │
  │ Generate the report │                                    │
  │    (section 11.10) │                                     │
  └────────────────────┘                                     │
           └────────────────────────┬────────────────────────┘
                    ┌─────────────────────────────┐
                    │       View the report        │
                    │        (section 11.10)       │
                    └─────────────────────────────┘
                                  │
                    ┌─────────────────────────────┐
                    │       Print the report       │
                    │        (section 11.10)       │
                    └─────────────────────────────┘
```

Figure 11-1. The Spot Finder volume quantitation workflow.

11.2 Selecting Preferences for Background Correction

You can select one of the following background correction methods as a preference: None and Histogram Peak. The background correction affects only the Volume result. It has no affect on spot finding. In general, start with Histogram Peak. If you cannot get the desired results, use None.

11.2.1 Description of the Background Correction Methods

The following describes each background correction method available as a preference:

• **None**—No background correction is applied. Select this option if the image has little noise and the background is flat and low relative to the image (approximately zero).

• **Histogram Peak**—ImageQuant uses the value that occurs most often in the selected rectangle as the background value (figure 11-2). Select this method of background correction if the image in the selected rectangle has more pixels in the background area than in the spot area.
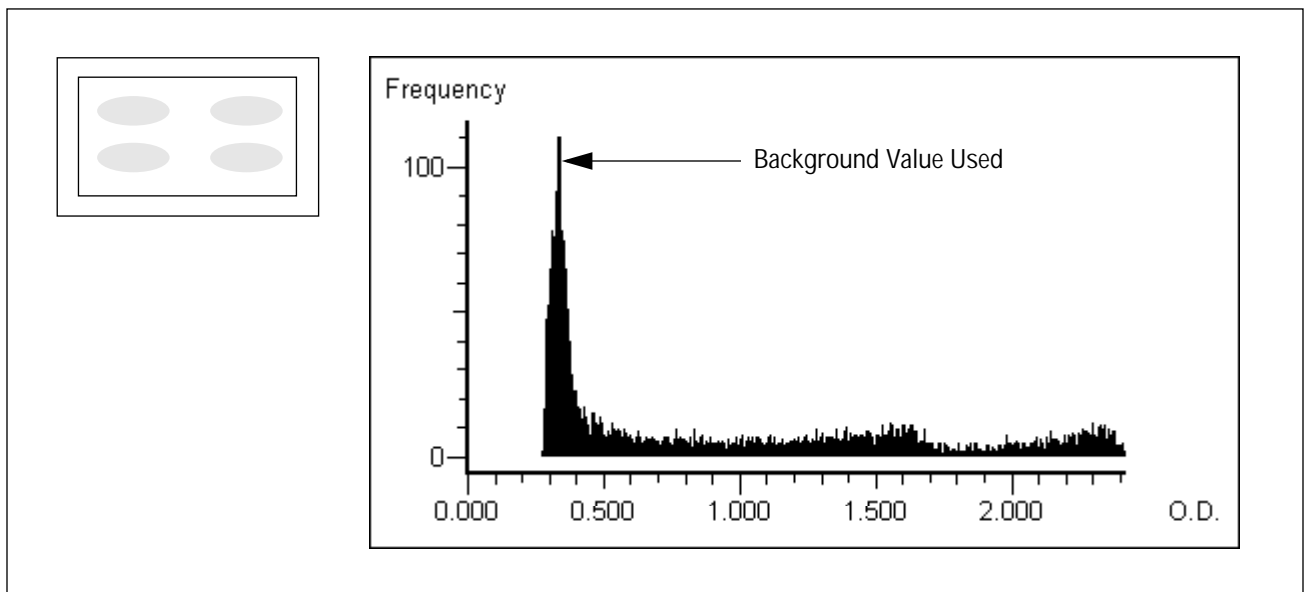
Figure 11-2. Using the Histogram Peak background correction, the most frequent pixel intensity is used as the background value.

11.2.2 Setting the Background Correction

Because statistics are calculated when you apply spot finding to the rectangle, you must select a background correction method first. The method is applied to each region object created by Spot Finder. To change the background correction after applying spot finding, see section 11.8.

To select a background correction method as a preference—

1. Choose **Background Correction** from the Preferences menu. The Background Correction/Preferences window appears (figure 11-3).
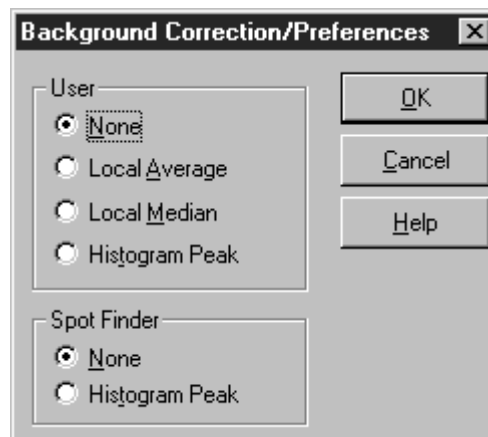
Figure 11-3. The Background Correction/Preferences window.

2. In the Spot Finder area, choose the Background Correction method you want to use.

3. Click **OK.** The Background Correction/Preferences window closes.

11.3 Drawing a Rectangle to Define the Area

You draw a rectangle so that it surrounds the entire area you want to evaluate. (You must use an unrotated rectangle when you quantitate using Spot Finder.) See section 5.4.1 for instructions on drawing rectangles.

11.4 Defining the Volume Quantitation Parameters Using the Spot Finder Inspector

After you select a background correction preference and draw a rectangle around the area you want to evaluate, you select the spot-finding parameters from the Spot Finder inspector and start the calculations. ImageQuant uses the parameters to find the spots inside the rectangle, and calculates the results. ImageQuant applies the parameters in the following order: The smoothing filter is applied first to eliminate noise. Next, ImageQuant applies the intensity threshold algorithm to identify potential objects and eliminates objects that are smaller than the minimum area value. ImageQuant draws region objects around each spot it finds on the image. Finally, ImageQuant applies the selected background correction method to the objects and calculates the results.

The first time you perform spot finding on a new image, you should quantitate using the existing spot-finding settings. The following steps provide a method for spot finding without a template. (If an appropriate template exists, see section 11.7 for instructions on how to use it.)

1. Select the background correction method (section 11.2).

2. Draw a rectangle around the portion of the image you want to analyze (section 5.4.1).

3. Choose **Spot Finder** from the Analysis menu. The Spot Finder inspector appears (figure 11-4).

4. From the Objects list or in the Image window, select the rectangle to quantitate.

5. For the initial quantitation, deselect **Display** and **Print** in the Report area.
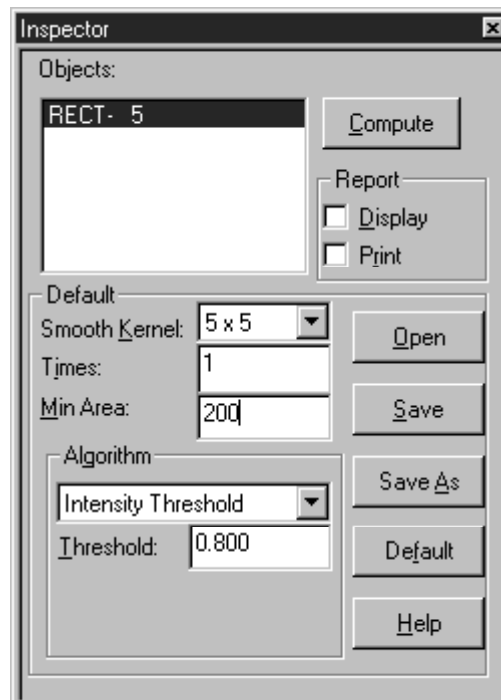
Figure 11-4. The Spot Finder inspector.

6.   Click **Compute.** ImageQuant draws region objects around all found spots and calculates the results. The progress of the quantitation appears in the status bar. If you want to cancel the quantitation, press the **ESC** key and click **Yes** when asked if you want to abort.

7.   Evaluate the region objects. Use the Gray/Color Adjust window to determine the correct threshold value (section 11.6.1) and enter the value. In addition, you can change the smoothing parameters (section 11.6.2) or the minimum area value (section 11.6.3).

8.   Delete the old region objects and click **Compute.**

9.   Repeat steps 7 and 8 until you are satisfied with the spot finding.

10. From the Volume Report Setup window, choose the desired reporting options (section 11.9).

11. If you want to display the results in a Volume Report window, click **Display.**

12. If you want to send the results to the printer, click **Print.**

13. Click **Compute.** Depending on the options you selected, the report appears in the Volume Report window, is sent to the printer, or both.

11.5 Viewing the Results for Each Region

The Volume Review inspector (figure 11-5) displays the quantitation results for one object. To display the Volume Review inspector, choose **Volume Review**(⬚) from the Analysis menu or the Quantitation toolbar.

To view the results for an object, select a region object in the Image window. The Volume Review inspector displays all the calculated results (except Percent) for that object.
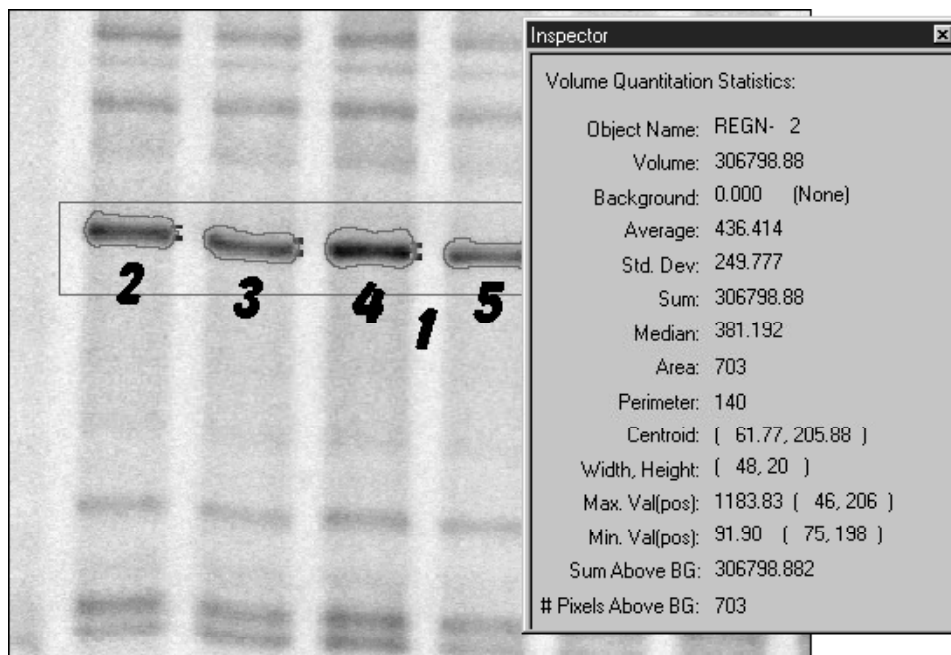
Figure 11-5. The Volume Review inspector. Object 1 is the rectangle that you drew to define the area for spot finding. Objects 2, 3, 4, and 5 are the region objects Spot Finder created around the spots.

11.6 Adjusting the Parameters

After viewing the regions found by the Spot Finder method, you may want to adjust the parameters and try again. The following discusses each parameter listed in the Spot Finder inspector and provides hints on how to use each one.

11.6.1 Intensity Threshold Algorithm

The intensity threshold algorithm uses a threshold value to determine which part of the image is background and which part is a spot. ImageQuant identifies areas where the pixel values are above the threshold and draws region objects around those areas (figure 11-6).
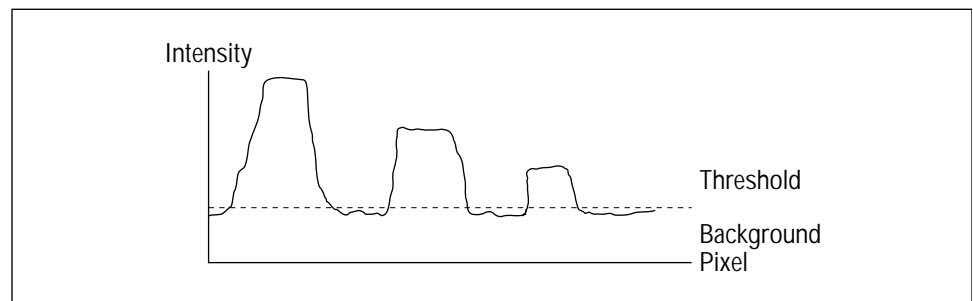
Figure 11-6. How the threshold value affects an image with a uniform background.

The intensity threshold algorithm works best on an image with spots that are distinct from the background. It becomes more difficult for the intensity threshold algorithm to select one threshold value that identifies all the objects when the objects and background have overlapping values. In this case, use the User volume quantitation method discussed in chapter 10.

Determining the Threshold Value

You can use the Gray/Color Adjust window to determine the appropriate threshold value. By changing the low display level you can see which parts of the image will be identified as spots.

To determine the appropriate threshold value—

1. Open the Gray/Color Adjust window (section 4.1).

2. In the Histogram area, drag the **Low** slider. Watch the image in the Preview Channels area. When the background is blue and the spots are approximately the size you want, make a note of the value displayed in the **Low** box (figure 11-7).

3. In the Gray/Color Adjust window, click **Cancel.** The Gray/Color Adjust window closes without applying the changes to the image in the Image window.

4. In the **Threshold** box on the Spot Finder inspector, type the **Low** value.

5. Click **Compute** in the Spot Finder inspector and view the results.
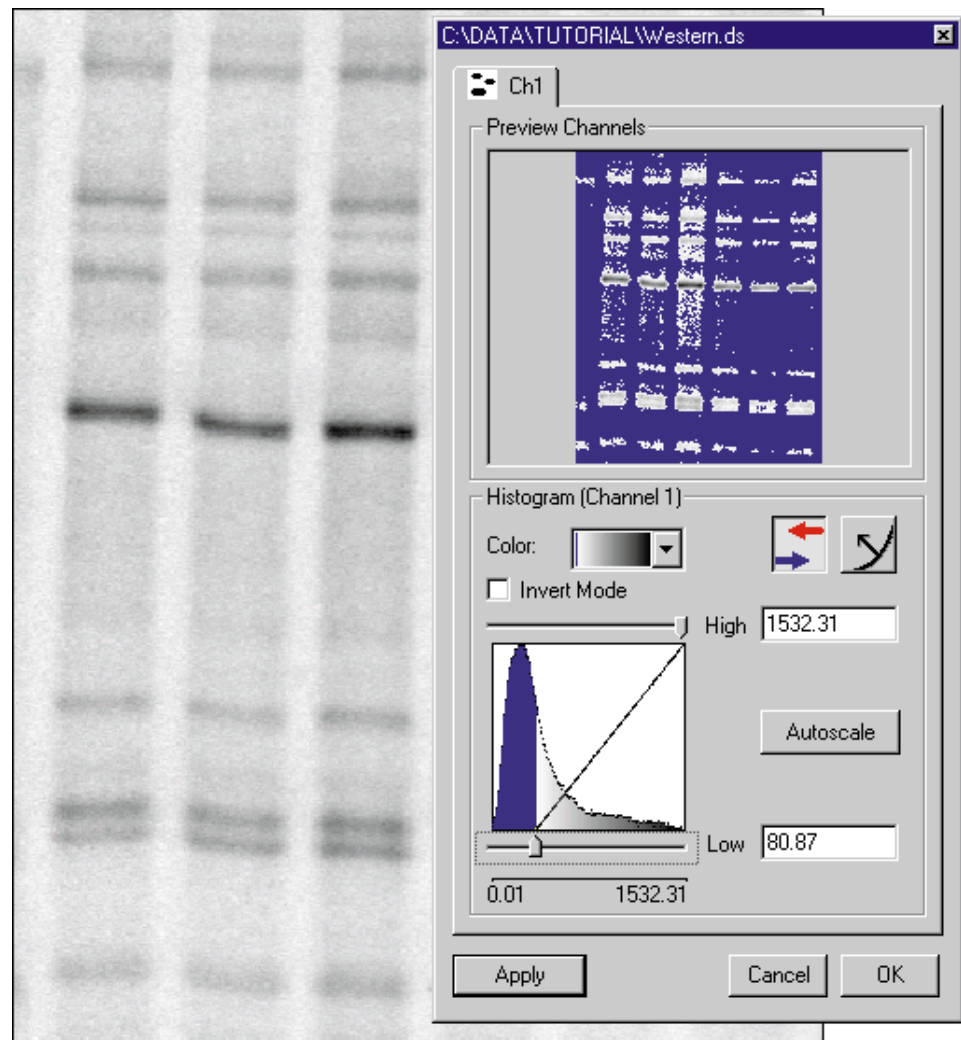
Figure 11-7.   The Gray/Color Adjust window can help you determine the threshold to use for spot finding.

Refining the Results

If the intensity threshold algorithm does not satisfactorily find all the spots in a single rectangle, try enclosing the low-intensity spots in one rectangle and the high-intensity spots in another rectangle. Change the threshold value in each rectangle and quantitate until the spots are found correctly. To combine the results into one report, display the Volume Report inspector, select all the regions found in the two rectangles, and generate a new report.

If the intensity threshold algorithm identifies background noise as spots, increase the minimum area value (section 11.6.3). Check the Area results in the Volume Review inspector for the largest background noise spot. Use a slightly larger value for the minimum area value.

Finally, to improve spot finding with the intensity threshold algorithm, try increasing the number of times smoothing is performed or increase the smooth kernel size (section 11.6.2). ImageQuant performs the smoothing operation before applying the algorithm.

11.6.2 Smoothing

The smoothing parameter allows you to reduce image noise during spot finding. Noise is considered to be speckles or small areas in the image that are not part of the sample. Smoothing averages each pixel with its neighbors and replaces the center pixel value with the average value. ImageQuant repeats this process for each pixel in the rectangle selected for analysis. Smoothing is the first process applied to the image.

Typical values for the pixel neighborhood sizes (the Smooth Kernel) are 3x3, 5x5, and 7x7. Figure 11-8 shows how this process affects one pixel in a 3x3 neighborhood.
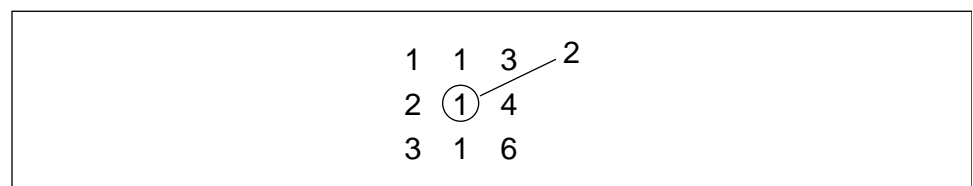
Figure 11-8. How smoothing affects a 3x3 neighborhood.

If your spots are small, choose a smaller kernel from the **Smooth Kernel** list in the Spot Finder inspector (figure 11-4). If the image is noisy, choose a larger kernel. As the sizes increase, the localized effect broadens so that pixels farther away from the center pixel contribute to the filter.

After you choose the kernel size, you need to decide the number of times to perform the smoothing. If you increase the number of times to smooth, you increase the noise reduction and the smoothing processing time. To turn off the smoothing parameter, type a zero (0) in the **Times** box.

If smoothing does not eliminate all the noise or begins to affect spot finding adversely, use the minimum area parameter to remove the remaining noise.

11.6.3 Minimum Area

The minimum area value (in pixels) sets the minimum spot size ImageQuant will recognize. You use this setting to exclude from consideration noise (speckles) or small areas in the image that are not part of the sample.

To find an appropriate setting—

1. From the regions created by spot finding (section 11.4), select the smallest region object you want to include. Or draw an object around the smallest spot on the image that you want to find (chapter 5).

2. Choose **Volume Review** (🖳) from the Analysis menu or the Quantitation toolbar. The Volume Review inspector appears (figure 11-5).

3. Make a note of the **Area** value for the object.

4. Choose **Spot Finder** from the Analysis menu. The Spot Finder inspector appears (figure 11-4).

5. Type the **Area** value from step 3 in the **Min. Area** box.

11.7 Using Templates

You can save the parameters you specify in the Spot Finder inspector. The parameters are stored in a template file that you can retrieve later to analyze images. By creating templates with parameters that are specific to the types of images you analyze in your lab, you can streamline the Spot Finder process.

11.7.1 Opening an Existing Template

To use an existing template, display the Spot Finder inspector, and then click the **Open** button in the inspector to display the Open window (figure 11-9). Double-click the name of the template you want to use. The window closes, and the parameter values in the Spot Finder inspector change to reflect those stored in the template. The new template name replaces the old template name in the inspector.
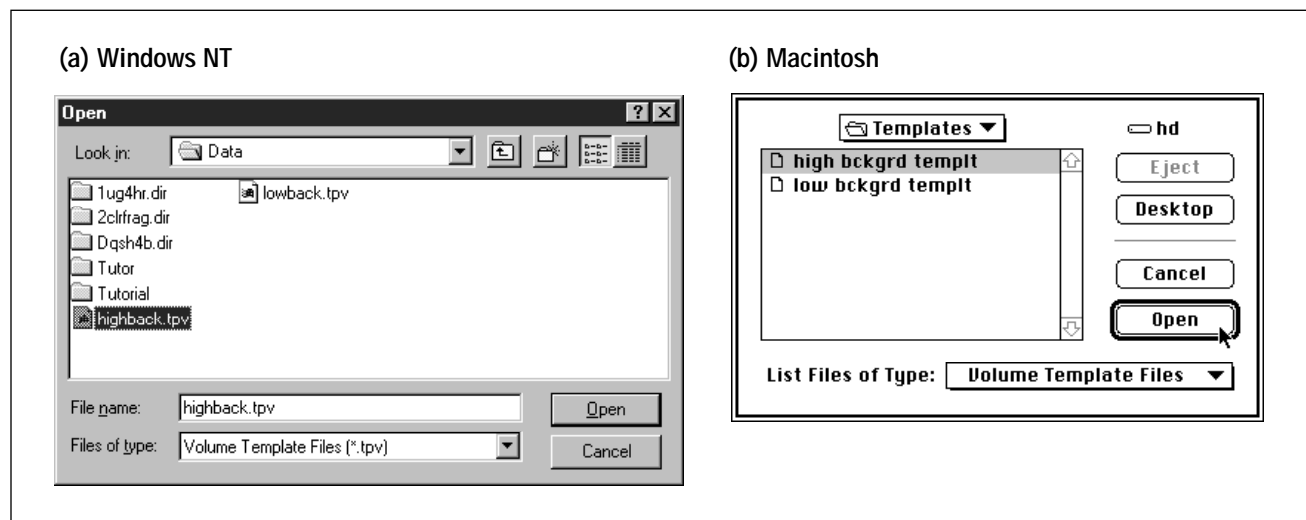
Figure 11-9. The Open window with example template names.

11.7.2 Creating a New Template

You create a new template by changing the parameter settings in the Spot Finder inspector and saving those settings.

1. Choose **Spot Finder** from the Analysis menu. The Spot Finder inspector appears (figure 11-4).

2. Modify the initial default settings, or open and modify an existing template (section 11.7.1).

3. Click **Compute.**

4. Choose **Volume Review** (![icon]) from the Analysis menu or the Quantitation toolbar. The Volume Review inspector appears (figure 11-4).

5. Examine the results for each found region.

6. Repeat steps 2 through 5 until you are satisfied with the settings.

7. Click the **Save As** button in the Spot Finder inspector. The Save As window (figure 11-10) appears.

8. Type a name for the template.

9. Click **Save.** The Save As window closes.
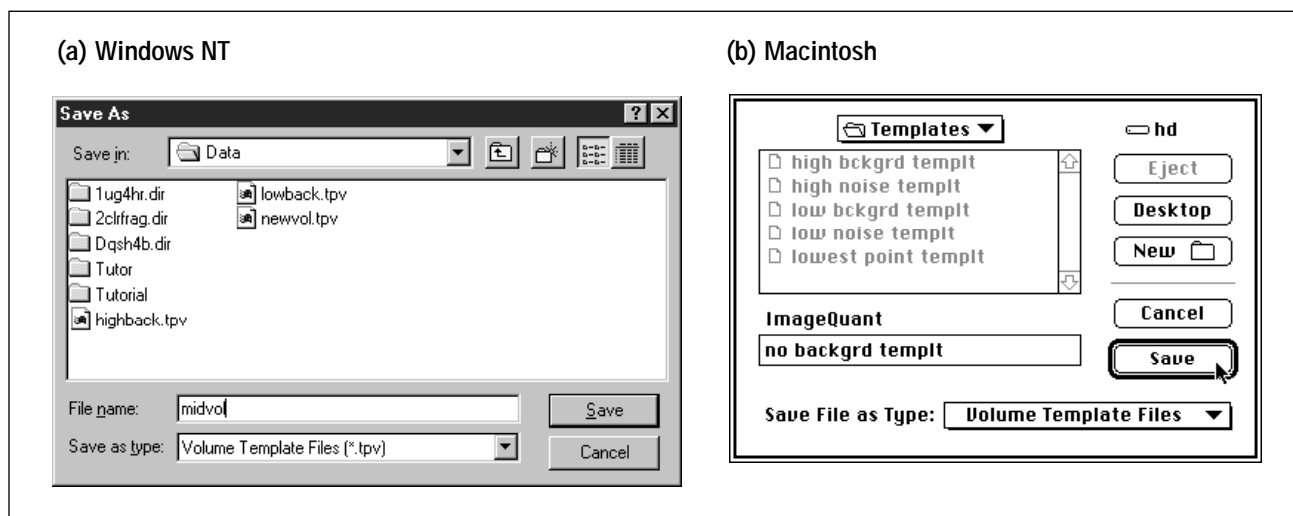
Figure 11-10. The Save As window with example template names.

11.7.3 Setting the Default Template

You can select a template to use whenever you display the Spot Finder inspector. To do this—

1. Choose **Volume Default Template** from the Preferences menu. The Select Default Volume Parameter File window appears (figure 11-11).

2. Locate and select the template from the list.

3. Click **Select** (Windows NT) or **Save** (Macintosh).

4. Choose **Spot Finder** from the Analysis menu. The template name and parameters appear in the inspector.

   Note: To return to the system default template, choose **Reset Volume Default Template** from the Preferences menu. In the Spot Finder inspector, click **Default.**
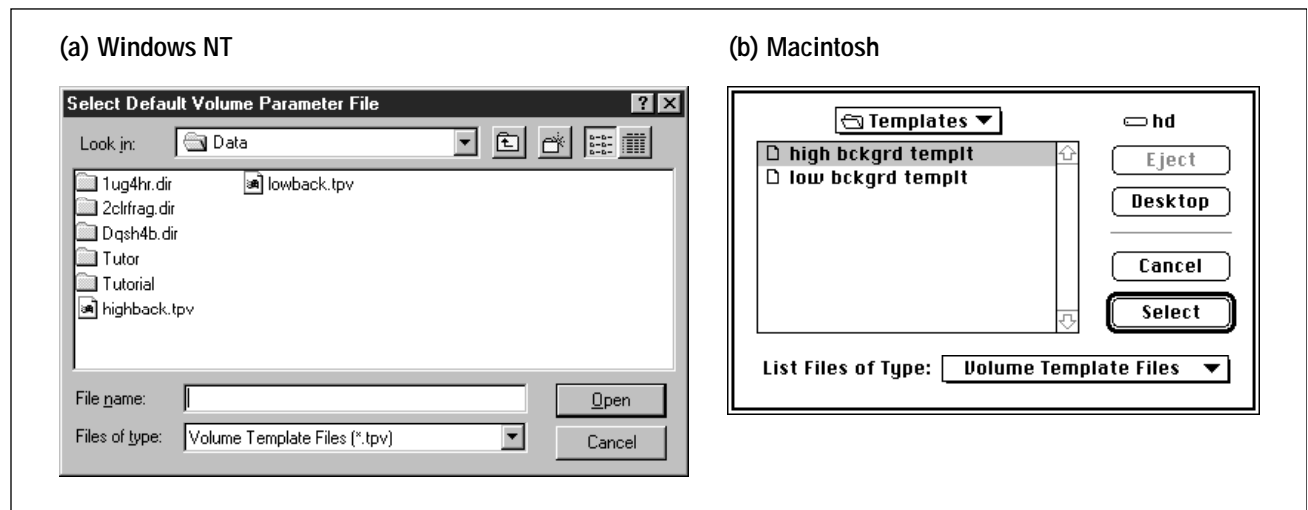
Figure 11-11. The Select Default Volume Parameter File window.

11.8 Changing the Background Correction

After Spot Finder identifies spots, you can apply a different background correction method to the spots. ImageQuant applies the new method to the selected objects. The default background correction method you selected in the Preferences menu does not change.

11.8.1 About the Correction Methods

The Background Correction inspector (figure 11-12) displays each object and the background correction method applied to the object. The codes are No=None and HP=Histogram Peak. Objects that are grouped do not display a method. (Grouped objects appear in the object list as GRUP followed by a single name or number.)

In addition to the original two background correction methods (described in section 11.2.1), you can choose among three additional methods—

- **Local Average (LA)**—ImageQuant determines the average (mean) of all the pixel values in the object outline and uses this value for the background.

- **Local Median (LM)**—ImageQuant determines the median (middle value) of all the pixel values in the object outline and uses this value for the background.

- **Object Average (OA)**—ImageQuant uses an object you select to determine the background. After you select Object Average, a list box appears that contains all the rectangles and ellipses you drew on the image. You select the rectangle or ellipse you want to use. ImageQuant calculates the average intensity value of the pixels in the object. The value appears in the list box and is the background value applied to the objects you select on the Objects list. ImageQuant treats the pixels below this value as background. ImageQuant treats the pixels above this value as part of the spot.

Important   **If you move the background object, you must select the object from the Object Average list again to recalculate the background value.**
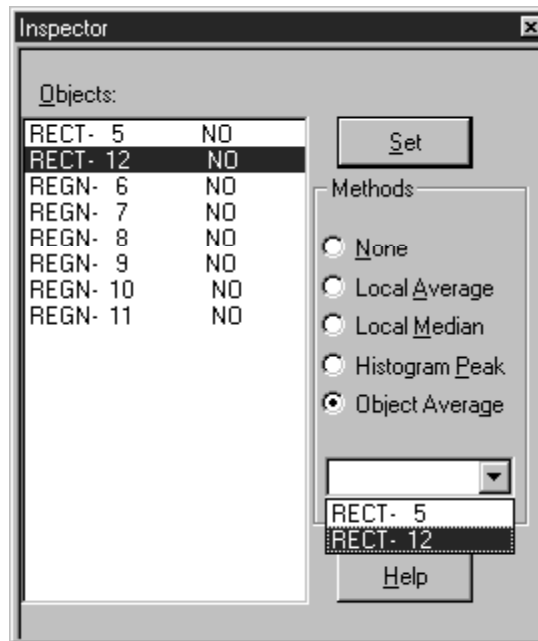
Figure 11-12. The Background Correction inspector.

11.8.2 Changing the Background Correction Method

To change the background correction method for existing objects—

1. Choose **Background Correction** from the Analysis menu. The Background Correction inspector appears (figure 11-12).

2. From the Objects list, select the objects to change.

3. Choose the background correction method you want to use.
   (If you select Object Average, choose the object from the list to use for the background calculation.)

4. Click **Set.** ImageQuant applies the new method to the selected objects.

5. To see the new results, choose **Volume Review** ( ) from the Analysis menu or the Quantitation toolbar. The progress of the quantitation appears in the status bar. If you want to cancel the quantitation, press the **ESC** key and click **Yes** when asked if you want to abort.

11.9 Selecting the Volume Report Options

Before you can generate a report, you select the information you
want ImageQuant to report from the options in the Volume Report
Setup window (figure 11-13). You can display the Volume Report
Setup window from either the Analysis menu or the Preferences
menu.
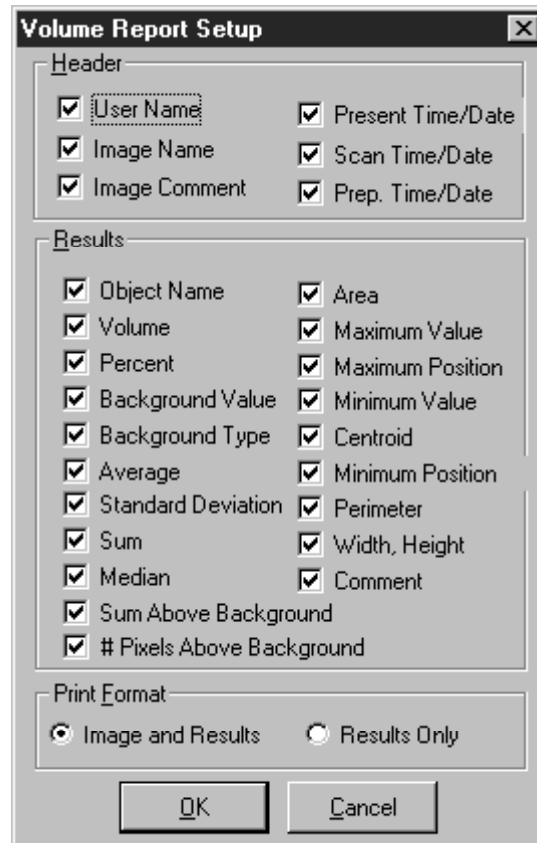
Figure 11-13. The Volume Report Setup window.

If you want to apply the report options to—

*   **All open images**—Choose **Volume Report Setup** from the
    Preferences menu. In the Volume Report Setup window, click
    to select or deselect an option. After you click **OK,** ImageQuant
    prompts you to propagate the changes to all open Image
    windows. If you click **Yes,** the new report preferences appear
    in the Volume Report Setup window for all the open Image
    windows. If you click **No,** the new report preferences appear in
    the Volume Report Setup window for the next new image you

display in an Image window. Images that you already used for quantitation are not affected by the new volume report preferences.

- **Selected image only**—Choose **Volume Report Setup** from the Analysis menu. In the Volume Report Setup window, click to select or deselect an option.

The Volume Report Setup window (figure 11-13) contains the following options:

Header

You can print the following header information:

- **User Name**—Prints the user name.

- **Image Name**—Prints the image name.

- **Image Comment**—Prints the image comment entered in the Scanner Control window at scan time.

- **Present Time and Date**—Prints the time and date when the report is generated. The time and date are recorded by the computer.

- **Scan Time and Date**—Prints the time and date when the scan was performed. The time and date are recorded by the scanner.

- **Preparation Time and Date**—Prints the time and date when the sample was prepared. You enter the time and date in the Scanner Control window of some instruments.

Results

You can choose any of the following results to display in a Volume Report window or to print:

Note: The formulas provided below are for a rectangle with the dimensions N x M. The following equations use f(x,y) to denote the pixel intensity at pixel location (x,y) on the image. For nonrectangular objects, the pixel values within the object are the only values used in the calculations. The term *spot* is used in the description to represent the band, blot, or other feature on the image that you are analyzing.

- **Object Name**—The name assigned to the object.

- **Volume**—The integrated intensity of all the pixels in the spot, excluding the background. To calculate volume, ImageQuant subtracts the background value from the intensity of each pixel in the object, and then adds all the values.

$$\text{Volume} = \sum_{y=1}^{M} \sum_{x=1}^{N} [f(x,y) - \text{background}]$$

- **Percent**—The volume in the spot as a percentage of the total volume. The total volume equals the sum of the volumes of all the objects included in one quantitation. If you alter the set of objects and regenerate the report, the Percent results will be different.

$$\text{Percent} = \frac{\text{Volume}(i)}{\sum_{i=1}^{N} \text{Volume}(i)} \times 100$$

- **Background Value**—The background value used during volume quantitation. The background value is subtracted from each pixel within the object to determine the volume.

- **Background Type**—The background correction method used to derive the background value.

- **Average**—The average intensity of all the pixels in the spot.

$$\text{Average} = \frac{\sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)}{M \times N}$$

- **Standard Deviation**—The standard deviation of the pixel intensities in the spot.

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{y=1}^{M} \sum_{x=1}^{N} [f(x,y) - \text{Average}]}{M \times N}}$$

- **Sum**—The integrated intensity of all the pixels in the spot including the background.

$$\text{Sum} = \sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)$$

- **Median**—The middle intensity value of all the pixels in the object.

- **Sum Above Background**—The integrated intensity of all the pixels in the spot excluding the background. To calculate the sum above the background, ImageQuant adds only the pixel values in the object that are above the background.

$$\text{Sum Above Background} = \sum_{y=1}^{M} \sum_{x=1}^{N} f(x,y)$$

  where   f(x,y)>background

  If you used the Spot Finder quantitation method to draw the region objects, the Sum Above Background result should be nearly the same as the Volume result. If you drew individual objects, the results should be different. If you are enclosing smiling bands in individual rectangles, the Sum Above Background result may be more accurate than the Volume result.

- **# Pixels Above Background**—The total number of pixels used in the Sum Above Background calculation.

- **Area**—The number of pixels in the spot.

- **Maximum Value**—The maximum pixel intensity in the spot.

- **Maximum Position**—The pixel coordinates of the maximum value of the spot.

- **Minimum Value**—The minimum pixel intensity in the spot.

- **Minimum Position**—The pixel coordinates of the minimum value of the spot.

- **Centroid**—The pixel coordinates (Cx,Cy) of the geometric center of the selected draw object where—

$$Cx = \frac{\sum\limits_{y=1}^{M} \sum\limits_{x=1}^{N} [xf(x,y)]}{\sum\limits_{y=1}^{M} \sum\limits_{x=1}^{N} f(x,y)}$$

$$Cy = \frac{\sum\limits_{y=1}^{M} \sum\limits_{x=1}^{N} [yf(x,y)]}{\sum\limits_{y=1}^{M} \sum\limits_{x=1}^{N} f(x,y)}$$

- **Perimeter**—The number of pixels that border the spot.

- **Width, Height**—The maximum width and height of the spot in pixels.

- **Comment**—The annotation associated with the object. (You enter the comment using the Image Comment field in the Object Attributes inspector.)

Print Format

In the Print Format area, you select how you want your report printed—

- **Image and Results**—Prints the selected header information and the image on the first page, and prints the header information and the results on the second page.

- **Results Only**—Prints the selected header information and the results.

11.10 Generating Reports

After Spot Finder volume quantitation, you can print the results or transfer the results to Microsoft Excel or Word.

11.10.1 Creating a Report from a Single Object or Multiple Objects

After Spot Finder quantitation, you use the Volume Report inspector (figure 11-14) to create reports on any objects listed in the Objects list. You select whether or not you want the report displayed in a Volume Report window, sent to the printer, or both. When you generate the report, the background correction and reporting choices are applied to the objects you select. If you select the Percent result option in the Volume Report Setup window, ImageQuant recalculates the percent values based on the new set of objects.
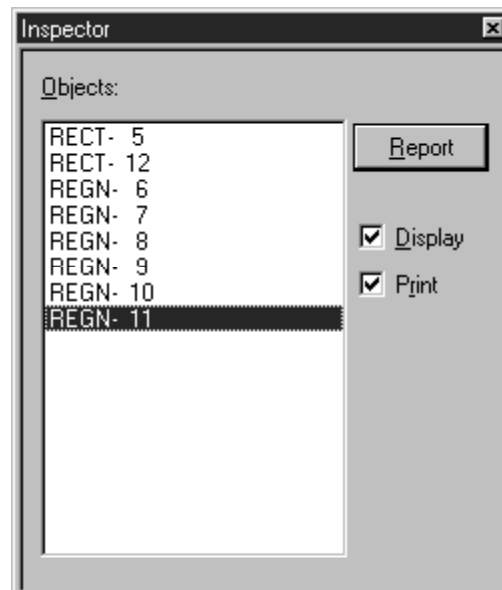
Figure 11-14. The Volume Report inspector.

To generate a report—

1. Choose **Volume Report** from the Analysis menu. The Volume Report inspector appears (figure 11-14).

2. In the Image window or from the Objects list in the inspector, select the object(s) about which you want to generate a report.

3. Click **Display** to display the results in a Volume Report window.

4. Click **Print** to send the results to the printer.

5. Click **Report.** ImageQuant compiles the results for each selected object.

If you select a large number of objects, the status bar displays the progress of the report generation. If you want to stop the calculation, press the **ESC** key, and click **Yes** when asked if you want to abort.

11.10.2 Transferring the Results to Microsoft Excel

After you view the results in the Volume Report window, you can transfer the results to Excel for further analysis. To do this, double-click in the Volume Report window, Excel opens and displays the results in a new worksheet.

11.11 Saving the Objects with the Image

When you close the image, you will be prompted to save the objects you created.

- If you do not want to save the objects, click **No.**

- If you want to save the objects, click **Yes.** ImageQuant creates a file that contains the objects.

To save the objects without closing the Image window, choose **Save** from the File menu.

11.12 Printing the Image

You can print the image by selecting the Image window and choosing **Print** ( ) from the File menu or the Main toolbar. ImageQuant prints the image including the displayed objects.

Chapter 12 Setting Preferences

You use the commands in the Preferences menu to set how you want to display images, create objects, and calculate and report the results. After you set a preference, the next Image or Graph window you open uses the new settings.

The topics in this chapter are—

- Setting Image Display Preferences (section 12.1)
- Setting Report Preferences (section 12.2)
- Setting Quantitation Preferences (section 12.3)

12.1 Setting Image Display Preferences

You can set the following image display preferences:

12.1.1 Object Attributes

You use the Object Attributes window (figure 12-1) to enter defaults for how you want to create objects. To display the window, choose **Object Attributes** from the Preferences menu.
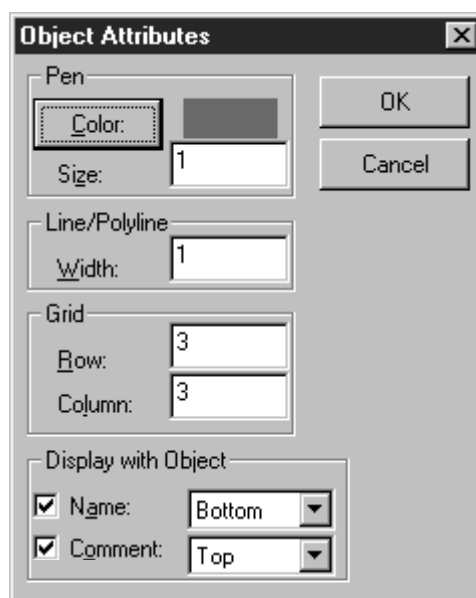
Figure 12-1. The Object Attributes window.

You can—

- In the Pen area, double-click the **Color** button, and then choose a pen color from the Color window. ImageQuant draws all objects using the color you chose.

- In the Pen area, type a new line weight in the **Size** box. The line weight values are 1 to 9 pixels.

- Set a width, in pixels, for each side of a wide line or wide polyline. If you set a width, every line or polyline you draw is a wide line or wide polyline.

- Set a default grid size. The smallest grid is 1x1; the largest recommended grid size is 200x200. The larger a grid, the longer ImageQuant takes to construct and display it and the more memory ImageQuant uses.

- Select whether to display the object name, a comment about the object, or both. Then select the locations for the name and comment. The choices are Top, Center, and Bottom. Different locations should be used if both the object name and comment are selected. Selecting the same location displays the object name and comment superimposed.

The objects you create use the attributes in the Object Attributes window (from the Preferences menu). While drawing, you can change an attribute using the Object Attributes inspector (choose Object Attributes from the Object menu).

12.1.2 Objects and Text Annotation Font

You use the Font window (figure 12-2) to set the attributes for the object font and the text annotation font. From the Preferences menu, choose **Fonts.** From the Fonts submenu, choose **Objects** to set the font preference for the object name and comment, or choose **Text Annotation** to set the font preference for the text annotation objects. The same Font window appears for either choice.

You select new font attributes from the lists in the Font window. The Sample area of the window shows an example of your selections. The next object or text annotation you create uses the new selections.
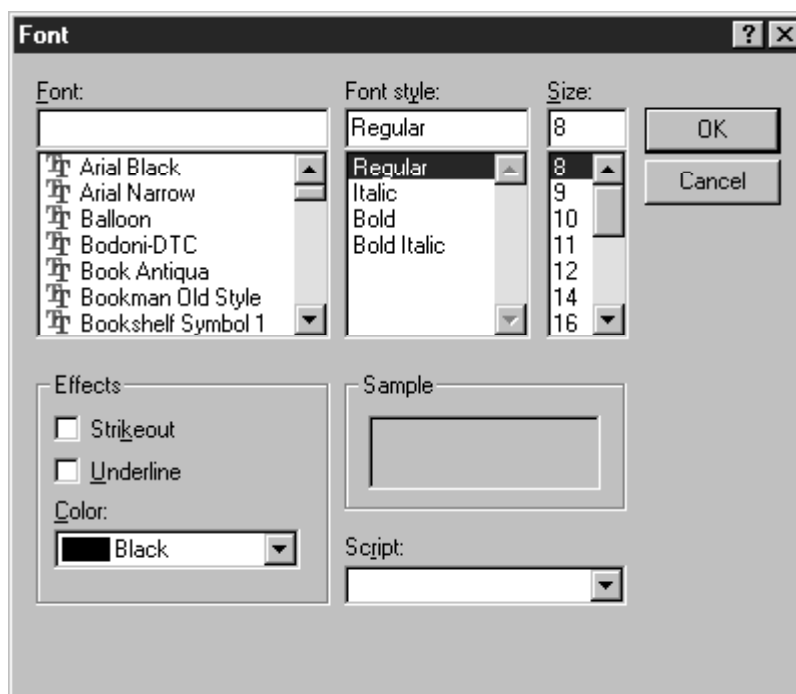
Figure 12-2. The Font window.

12.2 Setting Report Preferences

You can set the following reporting preferences:

12.2.1 Area Report Setup

You use the Area Report Setup window to select the preferred area quantitation information you want printed or displayed about a line graph (figure 12-3). To display the Area Report Setup window, choose **Area Report Setup** from the Preferences menu. Each option is discussed in more detail in section 8.9. Along with the selected header information, you can print the graph and the results, or you can print only the results. Only the results are displayed in the Area Report window.

The choices you make in the Area Report Setup window (from the Preferences menu)—

• Affect all the open Graph windows if you select Yes when asked to propagate changes to all open windows.

- Affect all the Graph windows you create after you change the selection.

- Do not affect existing Graph windows that are not open.

If you want to change the choices for a Graph window, but do not want to alter the preferences, display the line graph and choose the Area Report Setup command from the Analysis menu.
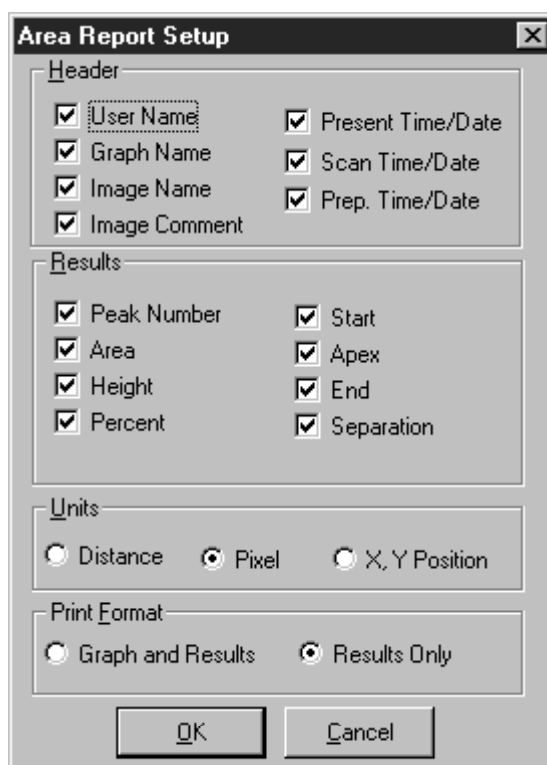
Figure 12-3. The Area Report Setup window.

12.2.2 Volume Report Setup

You use the Volume Report Setup window to select the preferred volume quantitation information you want printed or displayed about objects in the image (figure 12-4). To display the Volume Report Setup window, choose **Volume Report Setup** from the Preferences menu. Each option is discussed in more detail in sections 10.7 and 11.9. Along with the selected header information, you can print the image and the results, or you can print only the results. Only the results are displayed in the Volume Report window.

The choices you make in the Volume Report Setup window (from the Preferences menu)—

• Affect all the open Image windows if you select Yes when asked to propagate changes to all open windows.

• Affect all the Image windows you create after you change the selection.

• Do not affect existing Image windows that are not open.

If you want to change the choices for an Image window, but do not want to alter the preferences, display the image and choose the Volume Report Setup command from the Analysis menu.
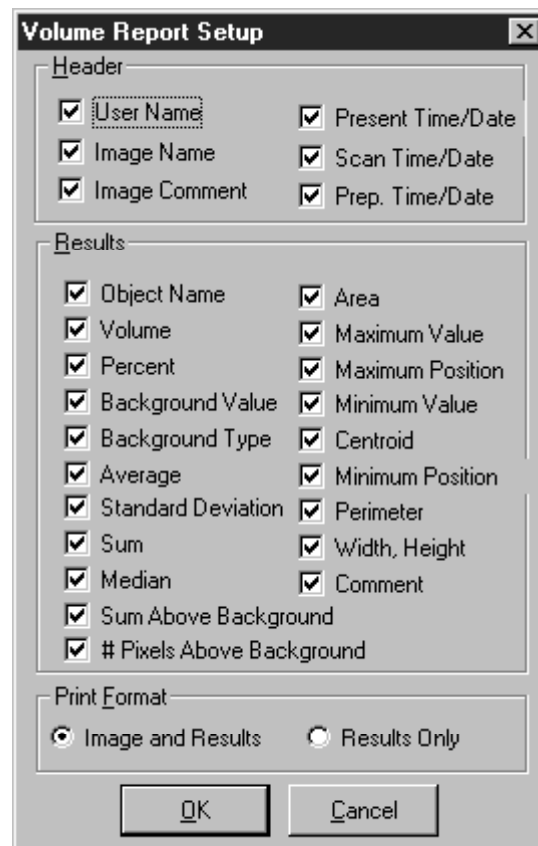
Figure 12-4. The Volume Report Setup window.

12.2.3 Printed Report Font

You use the Font window (figure 12-2) to select the font attributes to use for report printing. To display the Font window, choose **Fonts** from the Preferences menu. From the Font submenu, choose **Printed Reports.** The Sample area of the window shows an example of your selections. The next report prints in the new font selections.

12.3 Setting Quantitation Preferences

You can set the following quantitation preferences:

12.3.1 Area Default Template

You use the Select Default Area Parameter File window (figure 12-5) to select a template to use when you display the Peak Finder inspector.

After you create a line graph, you can select a different area template from the Peak Finder inspector. To return to the default area template provided with ImageQuant, choose **Reset Area Default Template** from the Preferences menu. In the Peak Finder inspector, click **Default.**
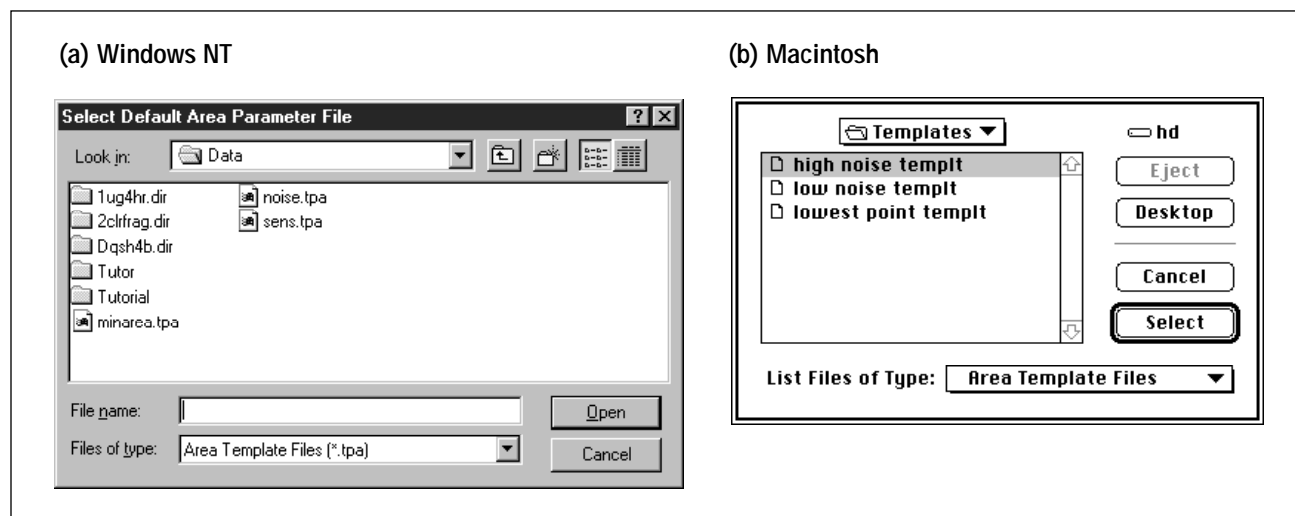
Figure 12-5. The Select Default Area Parameter File window.

12.3.2 Volume Default Template

You use the Select Default Volume Parameter File window (figure 12-6) to select a template to use when you display the Spot Finder inspector.

After you open an image, you can select a different volume template from the Spot Finder inspector. To return to the default volume template provided with ImageQuant, choose **Reset Volume Default Template** from the Preferences menu. In the Spot Finder inspector, click **Default.**
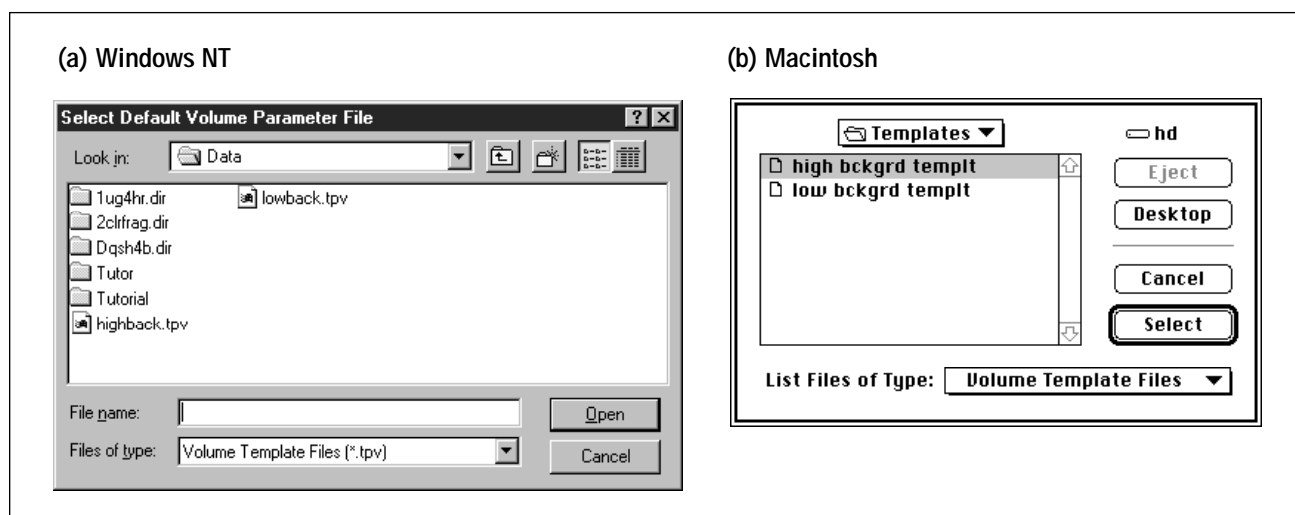
Figure 12-6. The Select Default Volume Parameter File window.

12.3.3 Background Correction

You use the Background Correction/Preferences window (figure 12-7) to select default background correction methods for User and Spot Finder volume quantitation. You select one method for each quantitation type by clicking the button next to the method you want to use. See sections 10.2 and 11.2 for descriptions of the background correction methods.
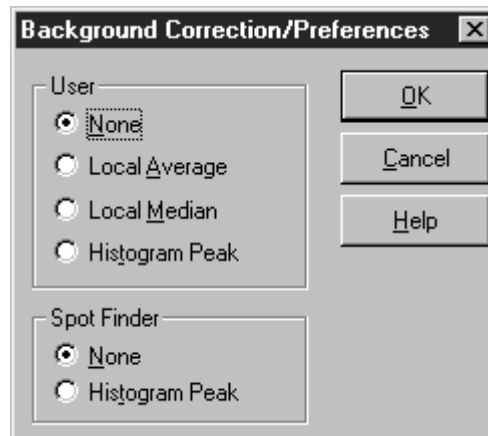
Figure 12-7. The Background Correction/Preferences window.

12.3.4 Grid-Column Major

You use the Grid-Column Major command on the Preferences menu to set the default quantitation direction for grids. To quantitate by column, select this option (a check mark appears in the menu). To quantitate by row, remove the check mark.

If checked, ImageQuant computes a 2x2 grid in the following order: column 1/row 1, column 1/row 2, column 2/row 1, and column 2/row 2.

If unchecked, ImageQuant computes a 2x2 grid in the following order: row 1/column 1, row 1/column 2, row 2/column 1, and row 2/column 2.

Chapter 13 Printing

You can print images, line graphs, histograms, and reports created by ImageQuant on any supported printer. If you print on a color printer, ImageQuant transfers the color palette to the printer so that colors can be approximated. A merged line graph can use up to 12 different colors.

If you want to change between portrait and landscape or change the size of the paper, choose **Print Setup** from the File menu. A window opens that is printer dependent, but at a minimum allows you to determine the paper size and orientation. See your printer documentation for more information. When you have finished making changes, click **OK.**

The topics in this chapter are—

- Printing the Image (section 13.1)
- Printing the Volume Report (section 13.2)
- Printing the Line Graph (section 13.3)
- Printing the Area Report (section 13.4)
- Printing the Line Graph Data Points and Values (section 13.5)
- Creating and Printing Histograms and Histogram Data (section 13.6)

13.1 Printing the Image

You can print the image displayed in the Image window. Only the portion of the image visible in the Image window prints. To print an image, including the displayed objects—

1. Click in the Image window to select it.

2. (Optional) Resize the Image window so that only the portion of the image you want printed appears in the window, or use the **Define Region of Interest** feature to select the portion of the image.

3. (Optional) Choose **Magnification** from the View menu and select the correct magnification for the image.

4. (Optional) Display the objects you want printed on the image.

5. (Optional) Choose **Print Preview** from the File menu to see how the image will appear on the printed page.

6. Choose **Print** (🖨) from the File menu or the Main toolbar. The Print window for your printer appears.

7. Click **OK** (Windows NT) or **Print** (Macintosh) in the Print window. The image is sent to the printer. The size and complexity of your image determines the amount of time it takes to print.

13.2 Printing the Volume Report

You print the volume report from either the Volume Report or Spot Finder inspector (Analysis menu).

Before printing—

*   Make sure you have selected the desired reporting choices. Choose **Volume Report Setup** from the Analysis menu, and check the choices in the Volume Report Setup window.

*   Make sure you have selected the desired font and size. Choose **Fonts** and then **Printed Reports** from the Preferences menu, and check the choices in the Font window.

To print the volume report—

1. Depending on the type of volume quantitation, open the **Volume Report** or **Spot Finder** inspector.

2. Make sure the **Print** check box in the Report area is selected.

3. In the Volume Report inspector, select the object(s) to include in the report, and then click **Report.** In the Spot Finder inspector, click **Compute** and ImageQuant creates the objects to include in the report.

ImageQuant calculates the results and sends the results to the printer. The progress of the report generation appears in the status bar. If you want to stop the calculations and the printing, press the **ESC** key and click **Yes** when asked if you want to abort.

13.3 Printing the Line Graph

You can print the line graph in the Graph window.

1. Click in the Graph window to select it. If you want to print a multiple line graph, print with the graphs merged; otherwise, only the selected line graph prints. If you select all the line graphs, they will print separately.

2. Choose **Print** ( ) from the File menu or the Main toolbar. The Print window for your printer appears.

3. Click **OK** (Windows NT) or **Print** (Macintosh) in the Print window. The line graph is sent to the printer.

13.4 Printing the Area Report

You can print the area report from either the Area Report or Peak Finder inspector (Analysis menu).

Before printing—

• Make sure you have selected the desired reporting choices. Choose **Area Report Setup** from the Analysis menu, and check the choices in the Area Report Setup window.

• Make sure you have selected the desired font and size. Choose **Fonts** and then **Printed Reports** from the Preferences menu, and check the choices in the Font window.

To print the area report—

1. Display the **Area Report** inspector to report on existing line graphs or display the **Peak Finder** inspector to create and report on new line graphs.

2. Make sure the **Print** check box in the Report area is selected.

3. Select the graph(s) to include in the report.

4. Click **Report** (in the Area Report inspector) or **Compute** (in the Peak Finder inspector).

ImageQuant calculates the results and sends the results to the printer. The progress of the report generation appears in the status bar. If you want to stop the calculations and the printing, press the **ESC** key and click **Yes** when asked if you want to abort.

13.5 Printing the Line Graph Data Points and Values

You can transfer the line graph data points and values to Microsoft Excel or Word. Then, using Excel or Word, print the data. The values transferred for each data point are the X,Y location, the distance (mm) from the start of the line, and the intensity value.

To transfer and print data points—

1. Click in the Graph window to select it.

2. Choose **Copy** (🗐) from the Edit menu or Main toolbar.

3. In an Excel worksheet or Word document, choose **Paste** from the Edit menu. The data points and their values appear in the Excel worksheet or the Word document.

13.6 Creating and Printing Histograms and Histogram Data

You can create a histogram of the image or a portion of the image and print the histogram. In addition, you can transfer the histogram and the histogram data to Microsoft Excel or Word.

13.6.1 Creating and Printing a Histogram

You can create a histogram that shows the intensity values (x-axis) and the frequency of the value (y-axis) of the image displayed in the Image window. You create a histogram by drawing a rectangle, ellipse, polygon, or region to define the portion of the image to use for the histogram.

To create and print a histogram—

1. In the Image window, draw an enclosed object around the portion of the image you want to use, or select an object that is already displayed.

2. Choose **Create Histogram** from the Analysis menu. The histogram appears in the Histogram window.

3. Choose **Print** from the File menu to print the histogram.

4. (Optional) Choose **Save** from the File menu to save the histogram.

13.6.2 Transferring the Histogram Data

After you create the histogram, you can transfer the histogram data to Microsoft Excel or Word. To do this—

1. Make sure the Histogram window is the active window.

2. Choose **Copy** from the Edit menu.

3. In an Excel worksheet or Word document, choose **Paste** from the Edit menu.

The histogram data appear in two columns. One column displays the intensity value from the x-axis of the histogram. The other column displays the frequency of the intensity value from the y-axis of the histogram.

13.6.3 Transferring the Histogram

After you create the histogram, you can transfer the histogram to Microsoft Excel or Word. To do this—

1. Make sure the Histogram window is the active window.

2. Choose **Copy** from the Edit menu.

3. In an Excel worksheet or Word document, choose **Paste Special** from the Edit menu.

4. In the Paste Special window, choose **Picture.** The histogram appears on the worksheet or document.

Chapter 14   *Managing Image Files*

This chapter discusses managing the files you create while using ImageQuant. Topics are—

- Deleting a Dataset File (section 14.1)
- Creating a 24-Bit Image for Publication (section 14.2)
- Creating a Bitmap Image that Includes the Objects (section 14.3)
- Saving the Image Using Another Name (section 14.4)

14.1 Deleting a Dataset File

Because image files are a collection of files, deleting an image file requires deleting several files. If you delete images using the Delete command, ImageQuant deletes the image and the associated dataset folder.

In ImageQuant—

1. Choose **Delete** from the File menu. The Delete window appears (figure 14-1).

2. Choose the image to delete.

3. Click **Delete.** The image and its associated files are deleted.
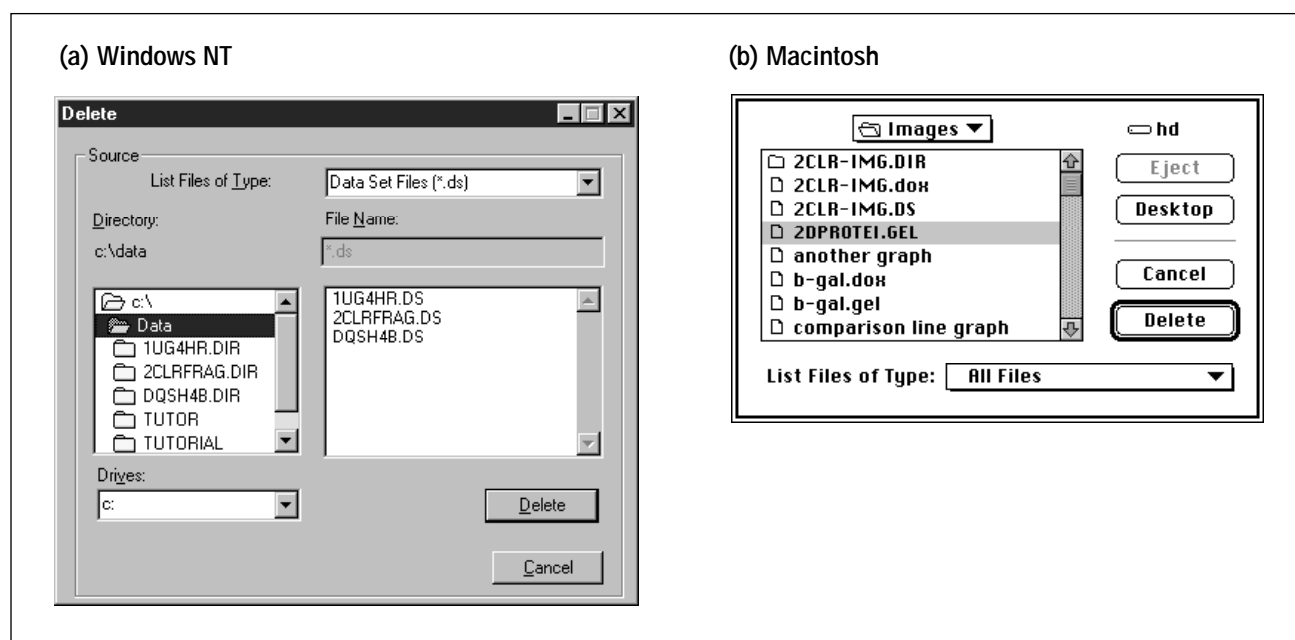
Figure 14-1. The Delete window.

The Delete command removes the image file and associated database files. It does not delete any graphs, reports, or templates you created as a part of the analysis. If you want to delete these files—

- *Windows NT*—Use Windows NT Explorer to locate and remove files you no longer want.

- *Macintosh*—In the Delete window, select **All files** from the List Files of Type box, and then select the file and click **Delete.**

14.2 Creating a 24-Bit Image for Publication

The Copy Image command allows you to copy the image currently displayed in the Image window and paste the image into Word, Excel, or Adobe® Photoshop®. You can copy a portion of the image or the entire image at full resolution. The Copy Image command does not copy any objects displayed on the image.

ImageQuant creates a 24-bit image, and places the image in the clipboard. If the image is displayed in color, ImageQuant creates a color copy. If the image is displayed in gray scale, ImageQuant creates a gray-scale copy.

You can copy a multichannel image that has the channels displayed overlaid, and you can copy the selected channel of a multichannel image that has the channels displayed side by side.

14.2.1 Copying the Entire Image

To copy the entire image without the objects and place the copy in the clipboard—

1. Open the image file you want to copy.

2. Choose **Copy Image** from the Edit menu. A message window appears.

3. Click **Yes** to confirm that you want to copy the entire image.

4. Open Word, Excel, or Photoshop and choose **Paste** from the Edit menu.

14.2.2 Copying a Section of Image

To copy a section of the image without the objects and place the copy in the clipboard—

1. Open the image file you want to copy.

2. Choose **Define Region of Interest** (▫) from the Tools menu or Image toolbar.

3. Click an enclosed object in the Image window, or place the pointer at the upper left of the portion of the image you want to copy and drag the pointer until the portion is enclosed in a dashed box.

4. Choose **Copy Image** from the Edit menu.

5. Open Word, Excel, or Photoshop and choose **Paste** from the Edit menu.

6. In ImageQuant, click the **Select** (↖) button to remove the Define Region of Interest box from the Image window.

14.3 Creating a Bitmap Image that Includes the Objects

The Copy Window Bitmap command allows you to copy the image and the displayed objects currently displayed in the Image window and paste the image into Word or Excel. You can copy a portion of the image or the entire image.

ImageQuant places the bitmap image in the clipboard. If the image is displayed in color, ImageQuant creates a color copy. If the image is displayed in gray scale, ImageQuant creates a gray-scale copy.

You can copy a multichannel image that has the channels displayed overlaid, and you can copy the selected channel of a multichannel image that has the channels displayed side by side.

14.3.1 Copying the Entire Image

To copy the entire image including the objects and place the copy in the clipboard—

1. Open the image file you want to copy.

2. Choose **Copy Window Bitmap** from the Edit menu.

3. Open Word or Excel, and then choose **Paste** from the Edit menu.

14.3.2 Copying a Section of Image

To copy a section of the image including the objects and place the copy in the clipboard—

1. Open the image file you want to copy.

2. Choose **Define Region of Interest** (⬚) from the Tools menu or Image toolbar.

3. Click an enclosed object in the Image window, or place the pointer at the upper left of the portion of the image you want to copy and drag the pointer until the portion is enclosed in a dashed box.

4. Choose **Copy Window Bitmap** from the Edit menu.

5. Open Word or Excel, and then choose **Paste** from the Edit menu.

6. In ImageQuant, click the **Select** (⬚) button to remove the Define Region of Interest box from the Image window.

14.4 Saving the Image Using Another Name

You can use the Save As command to create another copy of the entire image or a portion of the image. You can create a dataset file (.DS), single-channel GEL file (.GEL), or an 8-bit TIFF file (.TIF). The dataset and single-channel GEL files can be used for quantitation. The 8-bit TIFF file can be transferred to Word or Excel. However, using the procedure in section 14.2, provides a better quality image for publication.

14.4.1 Copying the Entire Image

To copy the entire image—

1. Open the image file you want to copy.

2. Choose **Save As** from the File menu. The Save As window appears.

3. Type a new name for the image file.

4. Make sure the correct file type is selected in the Save File as Type box, and the correct folder is selected in the folder list.

5. Click **Save.** Image Quant makes a copy of the image file and places the copy in the selected folder. The progress of the copy appears in the status bar. If you want to cancel the copy, press the **ESC** key.

6. (Optional) If you created an 8-bit TIFF file, open Word or Excel, and then open the image file.

14.4.2 Copying a Section of Image

To copy a section of the image—

1. Open the image file you want to copy.

2. Choose **Define Region of Interest** ( ) from the Tools menu or Image toolbar.

3. Click an enclosed object in the Image window, or place the pointer at the upper left of the portion of the image you want to copy and drag the pointer until the portion is enclosed in a dashed box.

4. Choose **Save Region of Interest As** from the File menu. The Save Region of Interest As window appears.

5. Type a new name for the image file.

6. Make sure the correct file type is selected in the Save File as Type box, and the correct folder is selected in the folder list.

7. Click **Save.** Image Quant makes a copy of the image file and places the copy in the selected folder. The progress of the copy appears in the status bar. If you want to cancel the copy, press the **ESC** key.

8. In ImageQuant, click the **Select** ( ) button to remove the Define Region of Interest box from the Image window.

9. (Optional) If you created an 8-bit TIFF file, open Word or Excel, and then open the image file.

Appendix A Quick Reference for Windows NT

Keyboard Shortcuts

File Menu

Key	Function
CTRL+O	Open
CTRL+S	Save
SHIFT+F2	Image Properties
CTRL+P	Print

Edit Menu

Key	Function
CTRL+X	Cut
CTRL+C	Copy Object(s)
CTRL+SHIFT+C	Copy Image
CTRL+V	Paste
CTRL+D	Duplicate
NUM DEL	Delete
CTRL+A	Select All

View Menu

Key	Function
F9	Zoom In
SHIFT+F9	Zoom Out
F8	Gray/Color Adjust
CTRL+1	Multichannel/Channel 1
CTRL+2	Multichannel/Channel 2
CTRL+3	Multichannel/Channel 3
CTRL+4	Multichannel/Channel 4

Tools Menu

Key	Function
CTRL+R	Define Region of Interest (Image window only)

Object Menu

Key	Function
F3	Object Manager
SHIFT+F3	Object Attributes
CTRL+NUM4	Alignment/Left
CTRL+NUM6	Alignment/Right
CTRL+NUM8	Alignment/Top
CTRL+NUM2	Alignment/Bottom
NUM4	Move/Left
NUM6	Move/Right
NUM8	Move/Top
NUM2	Move/Bottom
SHIFT+NUM4	Move/Fast Left
SHIFT+NUM6	Move/Fast Right
SHIFT+NUM8	Move/Fast Top
SHIFT+NUM2	Move/Fast Bottom
CTRL+SHIFT+S	Select
CTRL+SHIFT+Q	Rotate
CTRL+SHIFT+L	Line
CTRL+SHIFT+P	Polyline
CTRL+SHIFT+R	Rectangle
CTRL+T	Auto Tracer Setup
CTRL+SHIFT+T	Text Annotation
CTRL+F8	Group
CTRL+F9	Ungroup

Analysis Menu

Key	Function
SHIFT+F10	Volume Review or Area Review
CTRL+F	Create Graph
CTRL+H	Create Histogram

Window Menu

Key	Function
F5	Cascade
SHIFT+F5	Tile

Mouse/Key Combinations

	Mouse/Key	Function
Image window	Mouse	Pull-down menus
	Drag	Commands
	Click	Toolbars
	Click	Activate window
	Right mouse	Pop-up menus
Objects	DELETE	Delete
	INSERT	Add handle or line
	DELETE	Remove handle or line
	Mouse+V	Duplicate object
	Double-click	Display inspector
	SHIFT+click	Multiple select in Image window
	Drag	Multiple select in Image window
	CTRL+click	Multiple select in Objects list
	Drag	Move selected object, handle, line, or intersection
Magnification in Image window	CTRL+click	Switch between Enlarge and Reduce modes
	Mouse+PLUS	Enlarge while in Magnifier mode
	Mouse+MINUS	Reduce while in Magnifier mode
Map window	SHIFT+click	Move Map window
	CTRL+click	Move Map window in sync with Image window
Graph window	Drag	Move baseline handle along baseline
	CTRL+drag	Move baseline handle along peak in graph

Toolbar Functions

Main Toolbar

Open
Save
Print
Cut
Copy
Paste
Help

Image Toolbar

Pan
Pixel Locator
Pixel Distance
Create Frame
Previous Frame
Magnifier
Fit to Window
Map
Gray/Color Adjust
Define Region of Interest

Object Toolbar

Select
Rotate
Line
Polyline
Rectangle
Polygon
Ellipse
Region
Grid
Text Annotation
Auto Tracer

Graph Toolbar

Select
Create Frame
Previous Frame
Split Graphs
Merge Graphs
Maximize Graph
Restore Graph
Split Peaks
Define Peaks

Quantitation Toolbar

Create Graph
Area Review
Volume Review

Multichannel Toolbar

1 2 3 4

1
2
3
4

Channel 4
Channel 3
Channel 2
Channel 1

View Single Channel
Overlay
Side-by-Side

Extensions that Identify File Types

The following extensions identify the types of files ImageQuant creates:

.DIR   Identifies the folder associated with an image dataset and contains the image file and the object file for that dataset. Multichannel image datasets contain an image file and an object file for each channel. The dataset folder also contains a copy of the dataset file, which you can use if you accidentally delete the original dataset file.

.DS   Identifies an image dataset. Each dataset file has an associated dataset folder.

.DOX   Identifies a file containing image objects.

.GEL   Identifies a single image created on a Molecular Dynamics instrument.

.TIF   Identifies a single image file that has been converted to an 8-bit TIFF format. An 8-bit image can be stored in or transferred to another application.

.GPH   Identifies graphs and histograms.

.RPT   Identifies reports.

.TPA   Identifies area quantitation templates.

.TPV   Identifies volume quantitation templates.

Appendix B   Quick Reference for Power Macintosh

Keyboard Shortcuts

File Menu

Key	Function
⌘O	Open
⌘W	Close
⌘S	Save
SHIFT+F2	Image Properties
⌘P	Print
⌘Q	Quit

Edit Menu

Key	Function
⌘X	Cut
⌘C	Copy Object(s)
CONTROL+SHIFT+C	Copy Image
⌘V	Paste
⌘D	Duplicate
DEL	Delete
⌘A	Select All

View Menu

Key	Function
F9	Zoom In
SHIFT+F9	Zoom Out
F8	Grey/Color Adjust
CONTROL+1	Multichannel/Channel 1
CONTROL+2	Multichannel/Channel 2
CONTROL+3	Multichannel/Channel 3
CONTROL+4	Multichannel/Channel 4

Tools Menu

Key	Function
CONTROL+R	Define Region of Interest (Image window only)

Object Menu

Key	Function
F3	Object Manager
SHIFT+F3	Object Attributes
CONTROL+NUM4	Alignment/Left
CONTROL+NUM6	Alignment/Right
CONTROL+NUM8	Alignment/Top
CONTROL+NUM2	Alignment/Bottom
NUM4	Move/Left
NUM6	Move/Right
NUM8	Move/Top
NUM2	Move/Bottom
SHIFT+NUM4	Move/Fast Left
SHIFT+NUM6	Move/Fast Right
SHIFT+NUM8	Move/Fast Top
SHIFT+NUM2	Move/Fast Bottom
CONTROL+S	Select

Key	Function
CONTROL+Q	Rotate
CONTROL+L	Line
CONTROL+P	Polyline
CONTROL+R	Rectangle
CONTROL+T	Auto Tracer Setup
CONTROL+SHIFT+T	Text Annotation
CONTROL+G	Group
CONTROL+SHIFT+G	Ungroup

Analysis Menu

Key	Function
SHIFT+F10	Volume Review or Area Review
CONTROL+F10	Spot Finder
F	Create Graph

Window Menu

Key	Function
F5	Cascade
SHIFT+F5	Tile

Mouse/Key Combinations

	Mouse/Key	Function
Image window	Mouse	Pull-down menus
	Drag	Commands
	Click	Toolbars
	Click	Activate window
	SHIFT+CONTROL+mouse	Pop-up menus
Objects	DEL	Delete
	SHIFT+INS	Add handle or line
	DEL	Remove handle or line
	Mouse+V	Duplicate object
	Double-click	Display inspector
	SHIFT+click	Multiple select in Image window
	Drag	Multiple select in Image window
	CONTROL+click	Multiple select in Objects list
	Drag	Move selected object, handle, line, or intersection
Magnification in Image window	CONTROL+click	Switch between Enlarge and Reduce modes
	Mouse+PLUS	Enlarge while in Magnifier mode
	Mouse+MINUS	Reduce while in Magnifier mode
Map window	SHIFT+click	Move Map window
	CONTROL+click	Move Map window in sync with Image window
Graph window	Drag	Move baseline handle along baseline
	CONTROL+drag	Move baseline handle along peak in graph

Toolbar Functions

Main Toolbar

	Open
	Save
	Print
	Cut
	Copy
	Paste
	Help

Image Toolbar

	Pan
	Pixel Locator
	Pixel Distance
	Create Frame
	Previous Frame
	Magnifier
	Map
	Gray/Color Adjust

Graph Toolbar

	Select
	Create Frame
	Previous Frame
	Split Graphs
	Merge Graphs
	Maximize Graph
	Restore Graph
	Split Peaks
	Define Peaks

Object Toolbar

	Select
	Rotate
	Line
	Polyline
	Rectangle
	Polygon
	Ellipse
	Region
	Grid
	Text Annotation

Quantitation Toolbar

	Create Graph
	Area Review
	Volume Review

Multichannel Toolbar

Channel 1
Channel 2
Channel 3
Channel 4
Side-by-Side
Overlay
View Single Channel

Icons that Identify File Types

The following icons identify the types of files ImageQuant creates:

Identifies the folder associated with an image dataset and contains the image file and the object file for that dataset. Multichannel image datasets contain an image file and an object file for each channel. The dataset folder also contains a copy of the dataset file, which you can use if you accidentally delete the original dataset file.

Identifies an image dataset. Each dataset file has an associated dataset folder.

Identifies a file containing image objects.

Identifies a single image created on a Molecular Dynamics instrument.

Identifies a single image file that has been converted to an 8-bit TIFF format. An 8-bit image can be stored in or transferred to another application.

Identifies graphs.

Identifies reports.

Identifies area quantitation templates.

Identifies volume quantitation templates.

Index