



IMAGEQUANT[®]

TUTORIAL

Version 5.0

for Microsoft[®] Windows NT[®]
Apple[®] Power Macintosh[®]

*(Foreground): Radioactive western blot detected using storage phosphor imaging and a PhosphorImager.
Six bands have been outlined for analysis using the ImageQuant Auto Tracer tool.
The magnifier tool provides a closer view of the first band.
The volume report shows the results for the 6 objects.*

*(Background): Fluorescent amplified fragment length polymorphism (AFLP) imaged with the FluorImager.
Lines have been drawn through adjacent lanes for comparison of the banding pattern.
Line graphs of each lane are shown merged to analyze relative band positions and intensities.
The cursor position in the graph window corresponds to the line marker on the image.*

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July 1998

0218-465

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Preface

Purpose of Tutorial

The *ImageQuant Tutorial* briefly steps you through some key functions of the ImageQuant software.

The tasks in this tutorial show you how to—

- Open the ImageQuant software
- View and optimize an image
- Create objects and then use them for volume quantitation
- Select reporting options
- View the results of the volume quantitation
- Create a line graph and then use it for area quantitation
- View the results of the area quantitation
- Close ImageQuant

Related Publications

In addition to the *ImageQuant Tutorial*, Molecular Dynamics provides the following:

- The *ImageQuant User's Guide*—A step-by-step guide for all major functions of the ImageQuant software.
- The *ImageQuant Reference*—A reference, by menu item, for each command in 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 tutorial 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 Tutorial

The following terms are used in this tutorial:

- **Click**—Press and then quickly release 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.

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

Convention

In general, the figures used in the *ImageQuant Tutorial* 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.

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Web Site

www.mdyn.com

Welcome to ImageQuant

The ImageQuant software is a powerful image analysis package. It analyzes data collected on your Molecular Dynamics instrument. With ImageQuant, you can—

- Display a scanned image
- Detect, characterize, and quantitate objects
- Analyze data resulting from quantitation

This tutorial provides a brief look at some of the capabilities of ImageQuant. The *ImageQuant User's Guide* and the *ImageQuant Reference* provide a complete description of ImageQuant.

Step 1 *Opening ImageQuant*

The procedure for opening ImageQuant varies depending on the computer you are using.

Windows NT

To open ImageQuant in Windows NT, you double-click the ImageQuant shortcut icon on the desktop. If you did not create a shortcut, choose ImageQuant from the Start menu.

Macintosh

To open ImageQuant on a Macintosh, locate and double-click the ImageQuant icon. The ImageQuant icon is in the ImageQuant folder, which is in the MD Apps folder.

After you open ImageQuant, the procedures are identical for both computers.

Notice the status bar on the bottom of the screen. Throughout the tutorial, messages will be displayed in the status bar. Toolbars also appear on the screen. The figure on the facing page shows the functions available on the toolbars. You will use the toolbars and menus to select commands.

Note: If you do not see all the toolbars shown on the facing page, choose **Toolbars** from the **View** menu. In the Toolbar Configuration window, click all the empty check boxes in the Toolbars portion of the window, and then click **OK**. You should see all the toolbars on the screen. The toolbars can appear at different locations on the screen.

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

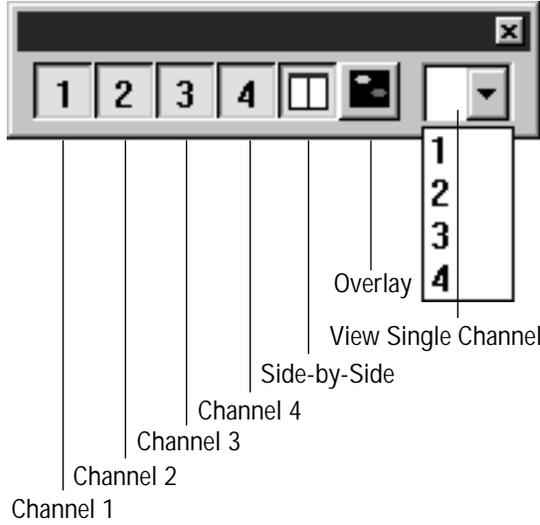
Graph Toolbar

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

Quantitation Toolbar

-  - Create Graph
-  - Area Review
-  - Volume Review

Multichannel Toolbar



Is ImageQuant open? Are the toolbars displayed? Go to the next page...

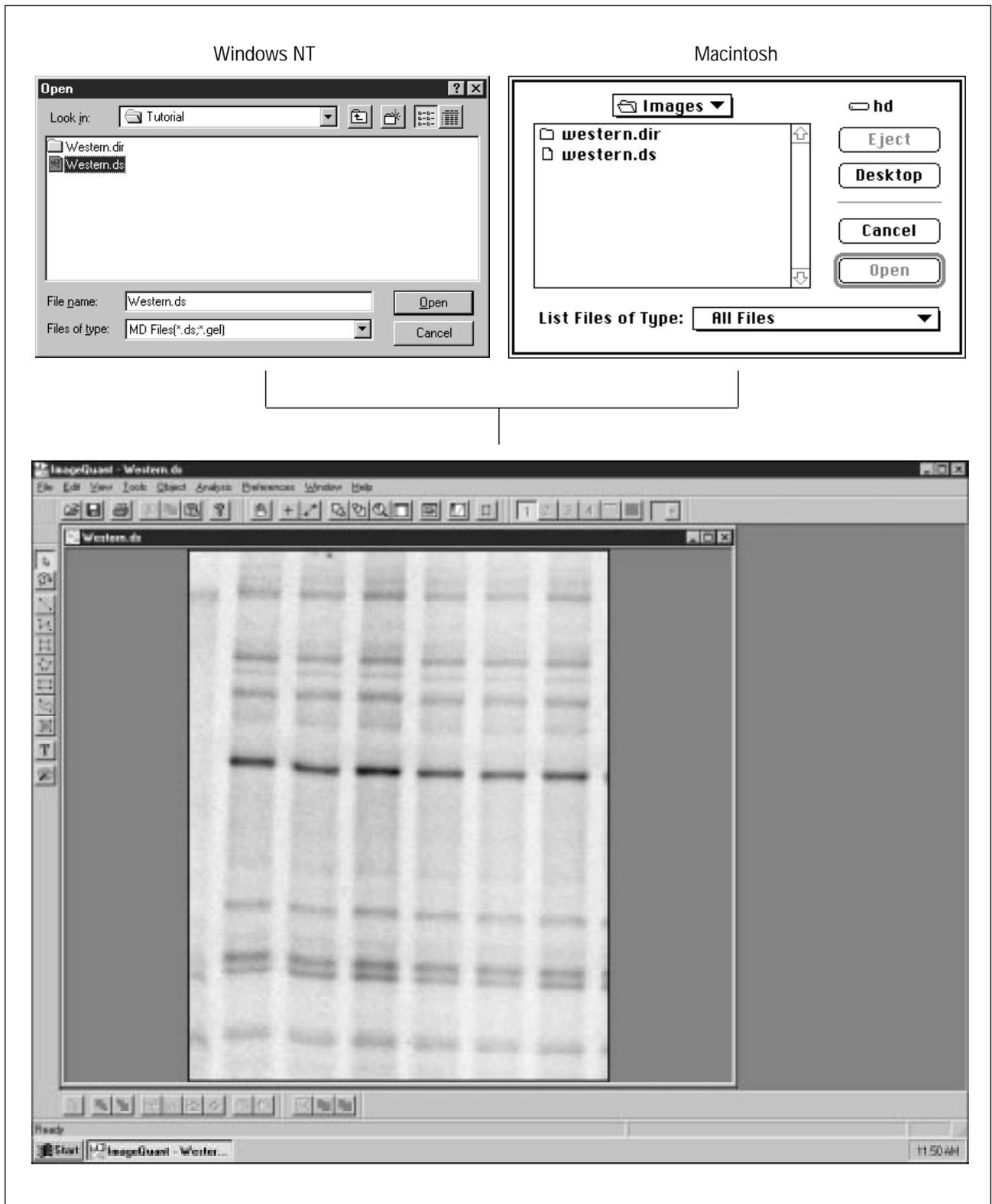
Step 2 Displaying the Image

ImageQuant includes an image, named Western, for you to use during this tutorial. Normally, you would display an image that you scanned on your Molecular Dynamics instrument.

To display the Western image—

1. Choose **Open** from the File menu. The Open window displays the contents of the Data folder.
2. Locate and double-click the **Tutorial** folder.
3. Locate and double-click the **Western.ds** file. The image appears in an Image window.

The image always appears scaled to fit in the Image window. ImageQuant provides tools to make your image easier to see. In the following steps, you will use a few of these tools.



Is the Western image displayed? Go to the next page...

Step 3 *Optimizing the Display*

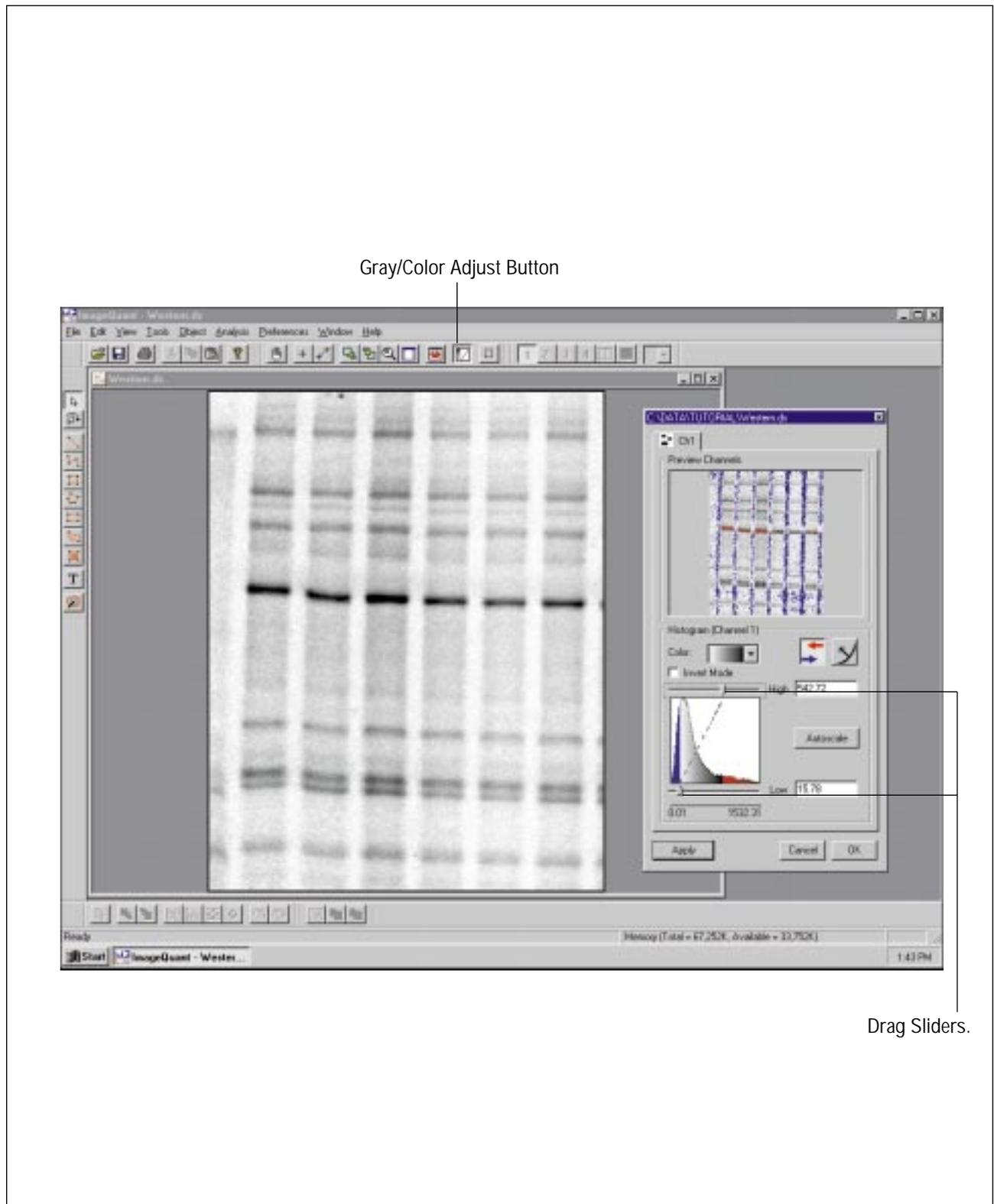
You can use the Gray/Color Adjust feature to optimize the image display. In this step, you will change the High and Low display levels to darken the bands and lighten the background. You use the preview image at the top of the Gray/Color Adjust window to view the changes before applying them to the image.

To change the display levels—

1. Click the **Gray/Color Adjust** button in the Image toolbar to open the Gray/Color Adjust window.



2. Move the pointer to the **High** slider and drag the slider until the bands in the preview image begin to turn red.
3. Move the pointer to the **Low** slider and drag the slider until the background in the preview image begins to turn blue.
4. Click **OK**. The changes appear in the image in the Image window and the Gray/Color Adjust window closes.



Are the bands darker and the background lighter? Go to the next page...

Step 4 *Creating a Rectangle*

Before you can quantitate, you must identify the bands on the image that you want to analyze. You do this by drawing an object around each band. Several object types are available for this task. You are going to use the rectangle.

To draw a rectangle—

1. Click the **Rectangle** button on the Object toolbar.

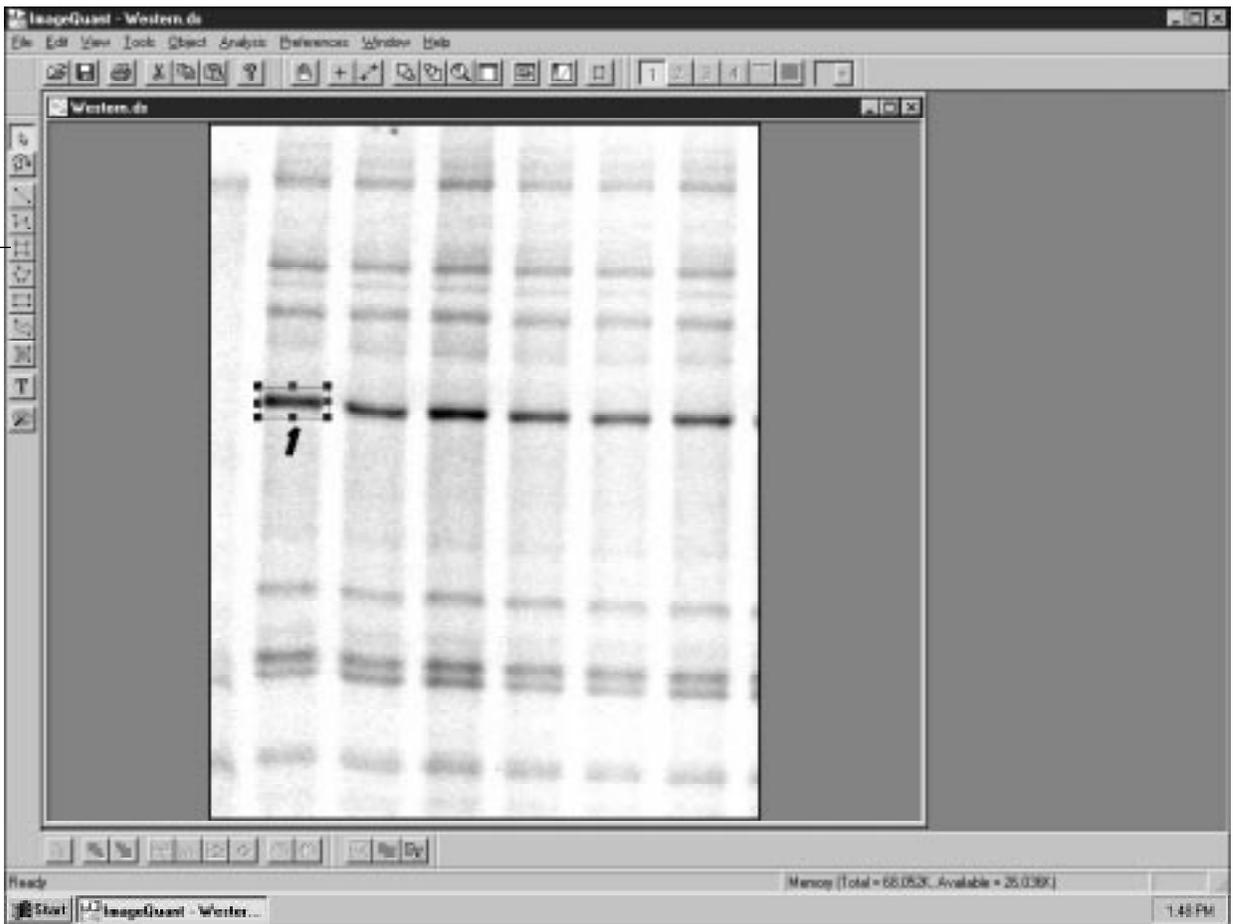


2. In the image, position the pointer at the **upper-left** corner of the first band in the second lane. Use the figure on the facing page to help identify the band.
3. Hold down the mouse button and drag the pointer until the band is surrounded by a rectangle.
4. Release the mouse button. ImageQuant draws the rectangle and adds handles. (Handles are the squares that appear on the rectangle to indicate that the rectangle is selected.)
5. You stay in the rectangle draw mode until you select another command. To end the rectangle draw mode, click the **Select** button on the Object toolbar.



If your rectangle does not look like the one on the facing page, you can delete it and try again. To delete the rectangle, click anywhere within the rectangle to select it, and then click the Cut button (). A window appears asking you to confirm that you want to delete the rectangle. Click **Yes**. Repeat the steps on this page to create a new rectangle.

Rectangle Button



Is the rectangle drawn? Go to the next page...

Step 5 *Duplicate the Rectangle*

To compare several bands on the image, you need to create additional rectangles. Instead of creating new rectangles, you can duplicate the rectangle you have already created.

To create additional rectangles—

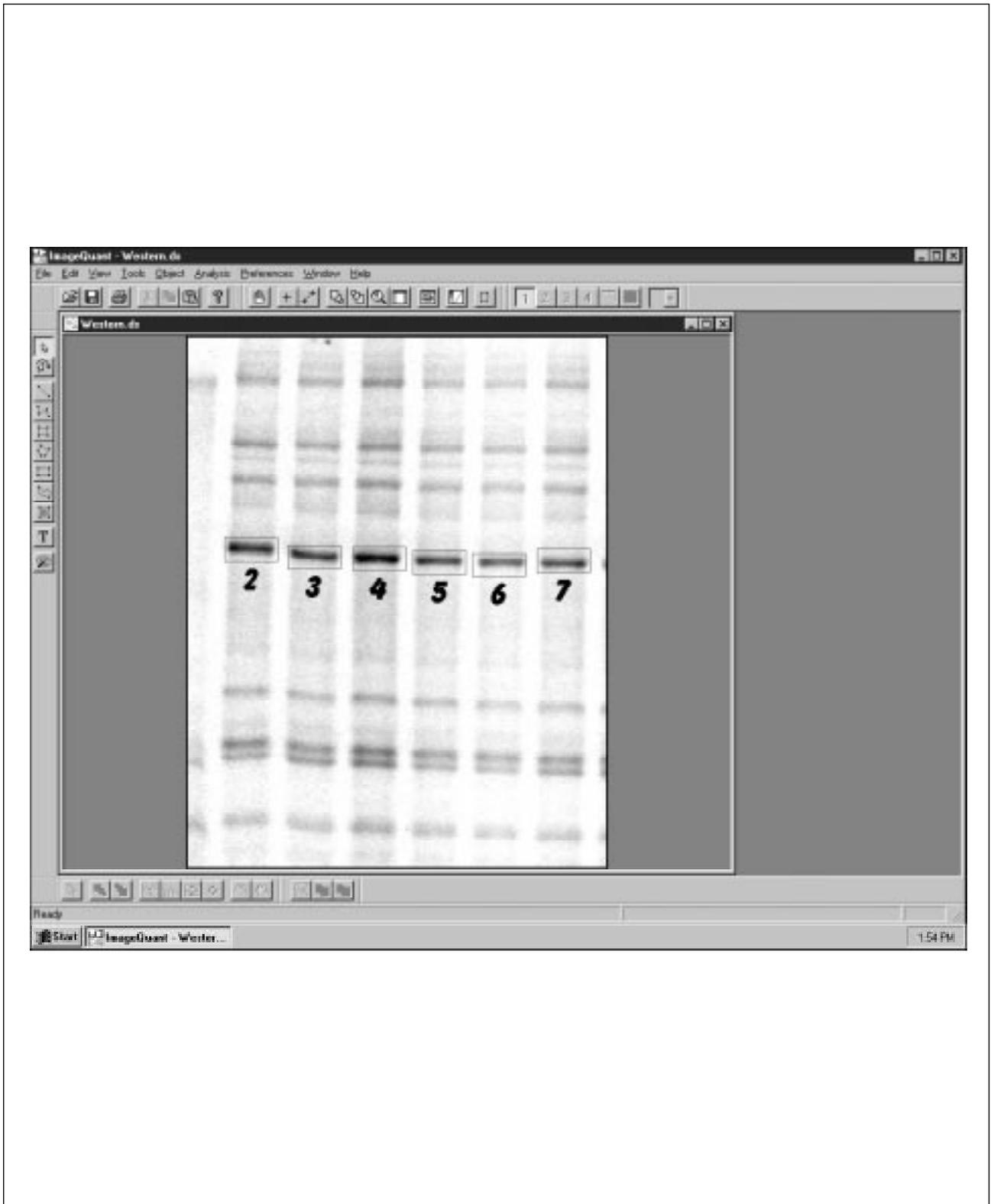
1. Make sure the **Select** button on the Object toolbar is selected.



2. Position the pointer in the middle of the rectangle.
3. Hold down the mouse button and press the **v** key on the keyboard.
4. Without releasing the mouse button, move the pointer until the rectangle surrounds the next band in the lane.
5. Without releasing the mouse button, press the **v** key again and move the rectangle to the next band.
6. Continue to duplicate the rectangle until you have six rectangles numbered 2 through 7.
7. Move the original rectangle (the one with the handles) to a different part of the image. Release the mouse button.
8. Click **Cut** () to delete rectangle number 1. A window appears asking you to confirm that you want to delete the rectangle. Click **Yes**.

You should have six rectangles surrounding the six bands in the second lane, similar to the picture on the facing page.

If your rectangles do not surround the bands like those on the facing page, you can move them. To reposition a rectangle, click the rectangle to select it, and then drag the rectangle until it surrounds the band.



Are the rectangles created and positioned correctly? Go to the next page...

Step 6 Reviewing the Volume Results

Immediately after you draw an object, you can view the results calculated for that object. You view the results for one object at a time.

To do this—

1. Make sure the **Select** button on the Object toolbar is selected.



2. Position the pointer on one rectangle and click the mouse button. Handles appear at the corners to show that the object is selected.
3. Click the **Volume Review** button on the Quantitation toolbar, or choose **Volume Review** from the Analysis menu. The results for the selected object appear in the Volume Review inspector.



4. Select a different rectangle. The results for that object appear in the inspector.

Step 6 Reviewing the Volume Results

The screenshot displays the ImageQuant software interface. At the top, the **Analysis** menu is open, with the **Volume Review... Shift+F10** option highlighted. Below the menu, an arrow points to the **Volume Review Button** in the software's toolbar. The main window shows a Western blot image with six lanes labeled **2** through **7**. A rectangular region is selected on the first lane. To the right, the **Inspector** window displays the following data:

Volume Quantitation Statistics:	
Object Name:	RECT- 2
Volume:	367199.77
Background:	0.000 (None)
Average:	242.057
Std. Dev.:	249.829
Sum:	367199.77
Median:	125.904
Area:	1512
Perimeter:	195
Centroid:	(62.25, 205.89)
Width, Height:	(56, 27)
Max. Val(pst):	1193.89 (46, 206)
Min. Val(pst):	2.72 (36, 219)
Sum Above BG:	367199.773
# Pixels Above BG:	1512

Results for the selected rectangle appear in the Volume Review inspector.

Volume Review Button

Finished reviewing the results? Go to the next page...

Step 7 Changing the Background Correction Method

Now you will apply a different background correction method. Five background correction methods are available. You will use Local Average, which uses the average pixel value found in the outline of each rectangle as the background value. When you assign a new background correction method to an object, ImageQuant automatically recalculates the volume of the object.

To select a background correction method and quantitate—

1. Choose **Background Correction** from the Analysis menu. The Background Correction inspector appears.
2. Position the pointer on the first rectangle displayed in the Objects list and drag the pointer past all the rectangles. All the rectangles should be selected.
3. Click **Local Average**.
4. Click **Set**.
5. In the Image window, click to select a rectangle.
6. Click the **Volume Review** button on the Quantitation toolbar to view the results for the selected rectangle.



7. To review the results for another rectangle, click the rectangle.

Step 7 Changing the Background Correction Method

The screenshot illustrates the workflow for changing the background correction method in ImageQuant. On the left, the **Analysis** menu is open, with **Background Correction...** selected. An arrow points from this menu item to the **Background Correction Inspector** dialog box on the right. The main window shows a gel image with seven lanes labeled 2 through 7. A **Volume Review Button** is located at the bottom of the main window. An arrow points from this button to the **Volume Review Inspector** dialog box, which displays the following statistics:

Volume Review Inspector

Volume Quantitation Statistics:

- Object Name: RECT- 2
- Volume: 310649.08
- Background: 31.219 (LocalAvg)
- Average: 290.959
- Std. Dev: 259.830
- Sum: 347987.09
- Median: 173.876
- Area: 1196
- Perimeter: 150
- Centroid: (62.11, 206.25)
- Width, Height: (52, 23)
- Max. Val(pos): 1183.83 (46, 206)
- Min. Val(pos): 8.79 (86, 217)
- Sum Above BG: 346908.752
- # Pixels Above BG: 1144

Changed the background correction and viewed the results? Go to the next page...

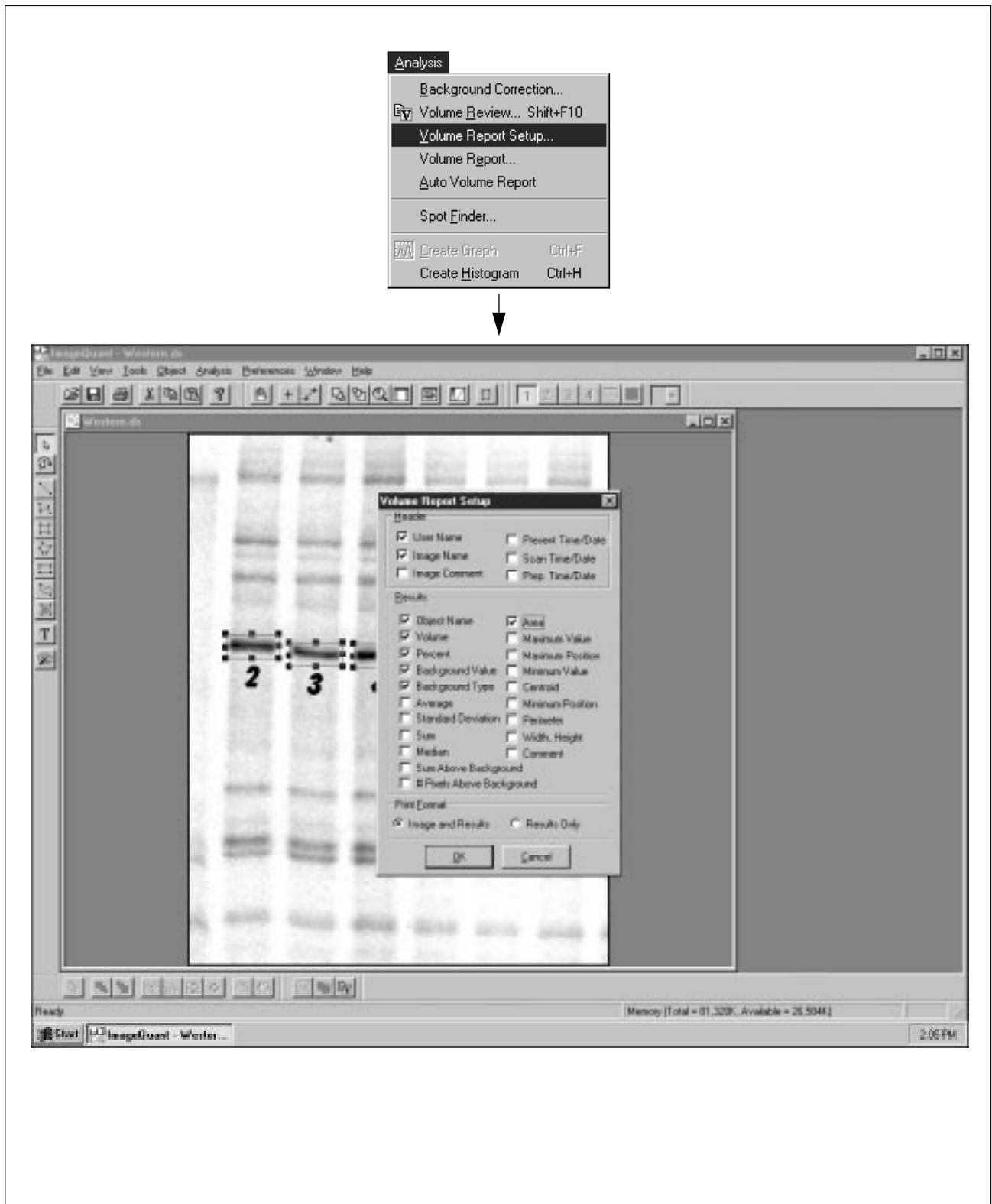
Step 8 Selecting Reporting Options

Before you print or display a report, you must tell ImageQuant which object properties you want reported. The options selected when the window opens are defaults. You can select or deselect any option.

1. Choose **Volume Report Setup** from the Analysis menu. The Volume Report Setup window appears.
2. Select the following Results options:
 - Object Name
 - Volume
 - Percent
 - Background Value
 - Background Type
 - Area

A check mark in the box means that the option is selected. Click a check box to select or deselect an option.

3. Click **OK** to close the window and accept the settings.



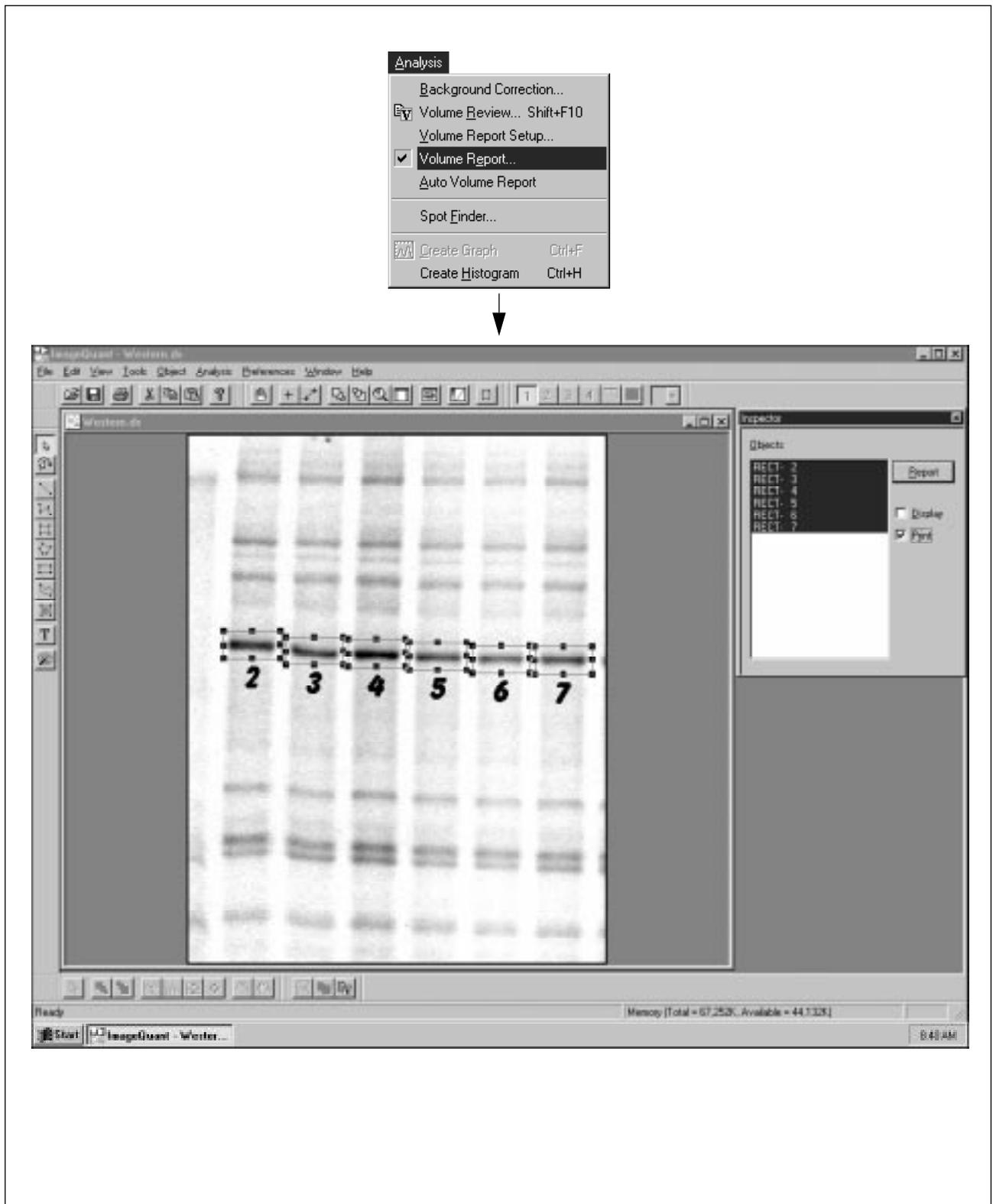
Have you selected your reporting options? Go to the next page...

Step 9 Printing a Volume Report

In this step, you will print a report about the rectangles you created.

To do this—

1. Choose **Volume Report** from the Analysis menu. The Volume Report inspector appears.
2. Select the rectangles that you want to appear in the report.
3. Make sure that a check mark appears in the box in front of Print.
4. Click the **Report** button. The report is sent to the printer.



Are your results printed? Go to the next page...

Step 10 Creating a Line

Next you will perform an area quantitation. You perform an area quantitation by drawing a line object on the lane of interest.

Note: If the rectangles are in the way, you may want to delete them. To do this, select the rectangles and click Cut (). A window appears asking you to confirm that you want to delete the rectangles. Click **Yes**.

To create the line—

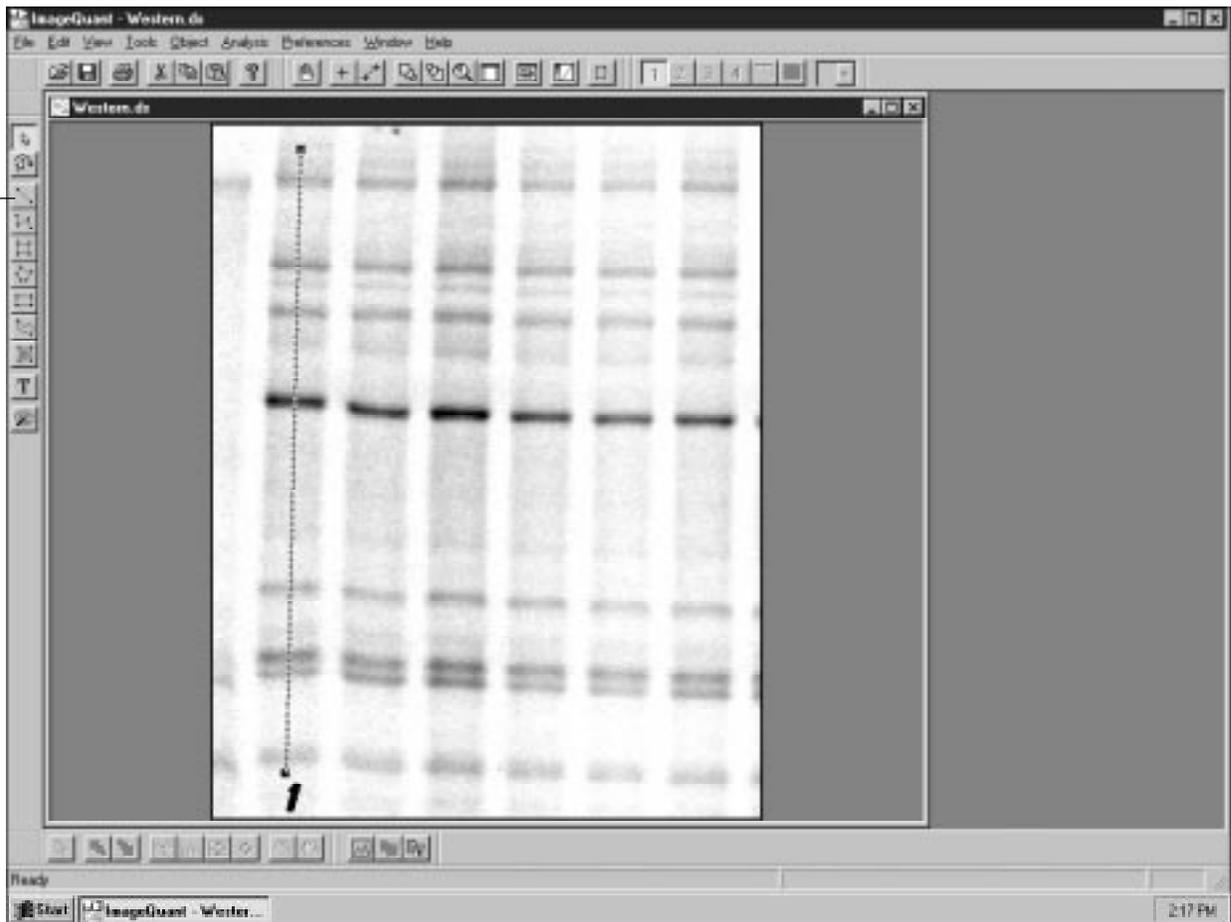
1. Click the **Line** button on the **Object** toolbar.



2. Position the pointer at the top of the second lane. Use the figure on the facing page to determine where to begin and end the line.
3. Drag the pointer down the center of the lane.
4. Release the mouse button when the line extends beyond the last band in the lane.
5. To end the line draw mode, click the **Select** button on the **Object** toolbar.



Line Button



Is the line displayed? Go to the next page...

Step 11 Adjusting the Width of the Line

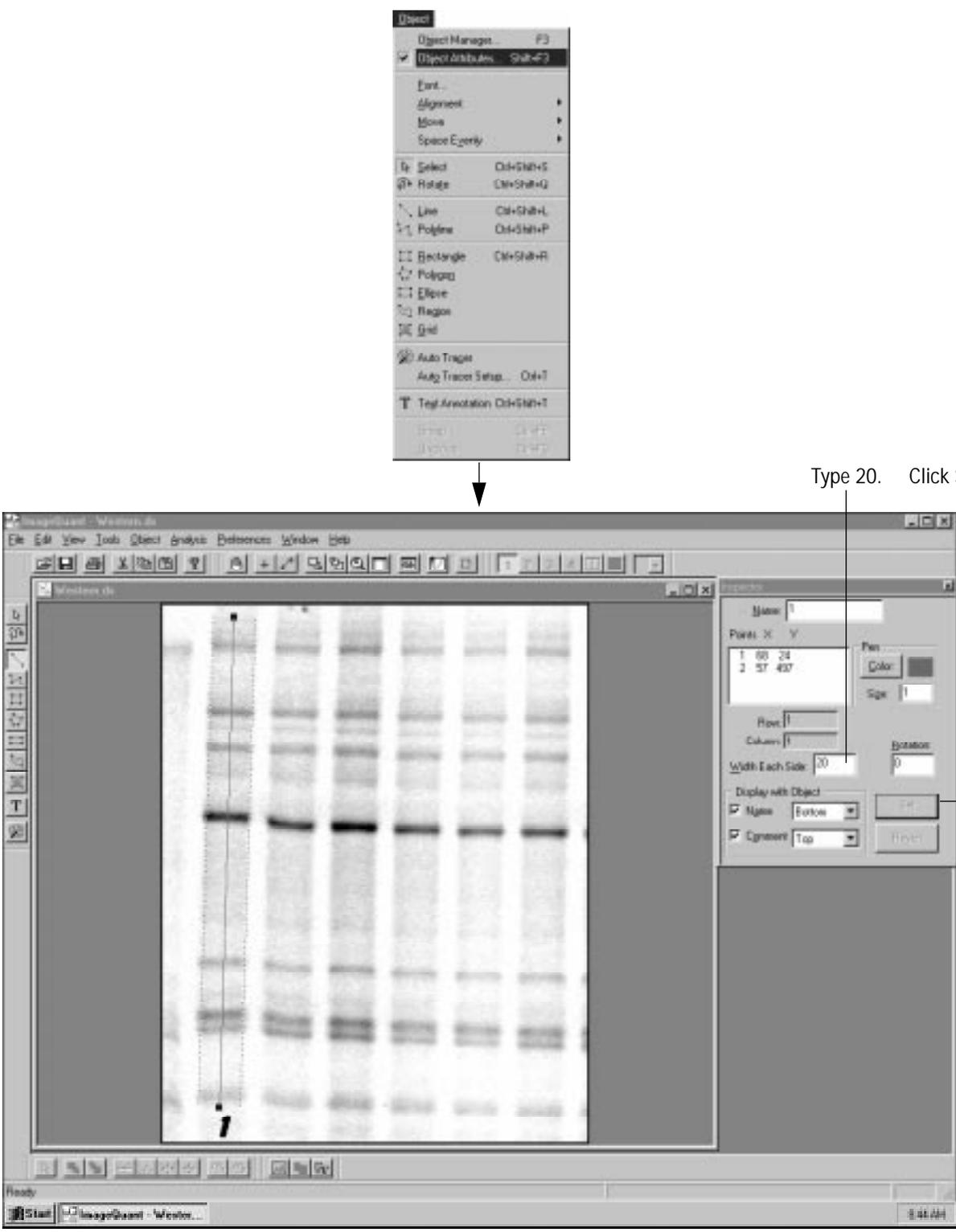
Instead of using a one-pixel-wide line, you can change to a wide line. A wide line improves the quantitation by averaging the pixels on either side of the line.

To change to a wide line—

1. Choose **Object Attributes** from the Object menu. The Object Attributes inspector appears.
2. Position the pointer on the Width Each Side box, and double-click. The number in the box is highlighted.
3. Type 20 in the box.
4. Click the **Set** button.
5. Close the Object Attributes inspector by clicking the close box in the inspector.

The dashed lines move to the edges of the bands. Make sure that the dashed lines do not extend past the edges of the bands. If they do, ImageQuant will average in background values.

Step 11 Adjusting the Width of the Line



The screenshot shows the ImageQuant software interface. At the top, the **Object** menu is open, with **Object Attributes** selected. Below the menu, a vertical arrow points to the **Properties** dialog box. In the **Properties** dialog, the **Width Each Side** field is set to 20. A text label "Type 20." points to this field, and another label "Click Set." points to the **Set** button. The main window displays a gel electrophoresis image with a vertical line drawn through the bands.

Type 20. Click Set.

Ready

Is the wide line within the edges of the bands? Go to the next page...

Step 12 *Creating a Line Graph and Quantitating*

The first step in area quantitation is to create a line graph of the pixel locations and pixel values. After the line graph appears, you can use the parameters in the Peak Finder inspector to perform the quantitation. The results are immediately available in the Area Review inspector.

Before creating the line graph, resize the Image window so that you see only the wide line. You may need to use the horizontal scroll bar to display the line.

To create a line graph and quantitate—

1. Make sure the line is selected on the image.
2. Click the **Create Graph** button on the Quantitation toolbar. A line graph appears in the Graph window.



3. Move the Graph window so that you can see the line displayed in the Image window.
4. Choose **Peak Finder** from the Analysis menu. The Peak Finder inspector appears.
5. Make sure the Display and Print check boxes are not selected.
6. Click **Compute**. The line graph changes to show the found peaks and the automatic baseline.
7. Choose **Area Review** from the Analysis menu. The Area Review inspector appears showing the results for one peak.
8. In the Graph window, click to display a vertical hairline. As you drag the hairline, notice a matching marker in the Image window.

Step 12 Creating a Line Graph and Quantitating

The image shows two sequential screenshots of a software interface for image analysis. The top screenshot shows the 'Analysis' menu with 'Peak Finder...' selected. The main window displays a line graph with a single prominent peak. A 'Peak Finder Inspector' dialog box is open, and a label 'Click Compute.' points to the 'Compute' button. The bottom screenshot shows the 'Area Review...' option selected in the 'Analysis' menu. The main window now displays a line graph with multiple peaks, and a specific peak is highlighted with a black marker. A label 'Marker on Image for Peak 15' points to this marker. An 'Area Review Inspector' dialog box is open, displaying detailed data for the selected peak.

Analysis

- Peak Finder... F10
- Area Review... Shift+F10
- Area Report Setup...
- Area Report...
- Auto Area Report

Peak Finder Inspector

Click Compute.

Area Review Inspector

Marker on Image for Peak 15

Viewed the line graph and peak results? Go to the next page...

Step 13 Changing the Peak Finder Parameters

Because the default Peak Finder parameters may not be the most useful for your sample, you may need to change the parameters. You can adjust six different parameters to improve the peak finding results. In this step, you will change the Minimum Area value, which eliminates peaks that represent noise instead of relevant data.

To adjust the parameters—

1. Choose **Peak Finder** from the Analysis menu. The Peak Finder inspector reappears.
2. Click the Minimum Area box and type 700. This removes peaks with an area of less than 700.
3. Click **Compute**. The peaks and the automatic baseline reflect the changes you made to the Peak Finder parameters.

ImageQuant automatically analyzes all peaks in the line graph.

The screenshot displays the ImageQuant software interface. At the top, the 'Analysis' menu is open, showing options: 'Peak Finder... F10', 'Area Review... Shift+F10', 'Area Report Setup...', 'Area Report...', and 'Auto Area Report'. An arrow points from the 'Peak Finder...' option to the main window. The main window features a 'Line Graph 1' with a y-axis labeled 'Counts' (0 to 900) and an x-axis labeled 'Pixel' (0 to 450). A line graph shows several peaks labeled 1 through 9. A vertical line is drawn at Pixel 300, with 'Pixel: 300 Counts: 811.476' displayed above it. To the right, the 'Inspector' window is open, showing 'LINE 1' with a 'Compute' button. Below this, the 'Default' section includes fields for 'Noise' (0), 'Sensitivity' (8), 'Min Area' (700.0000), 'Min Area' (empty), 'Ermul' (8), and 'Baseline' (Automatic). A 'Type 700.' label with an arrow points to the 'Min Area' field. The status bar at the bottom shows 'Ready', 'Pixel 344', and '9:49 AM'.

Viewed the baseline and peaks? Go to the next page...

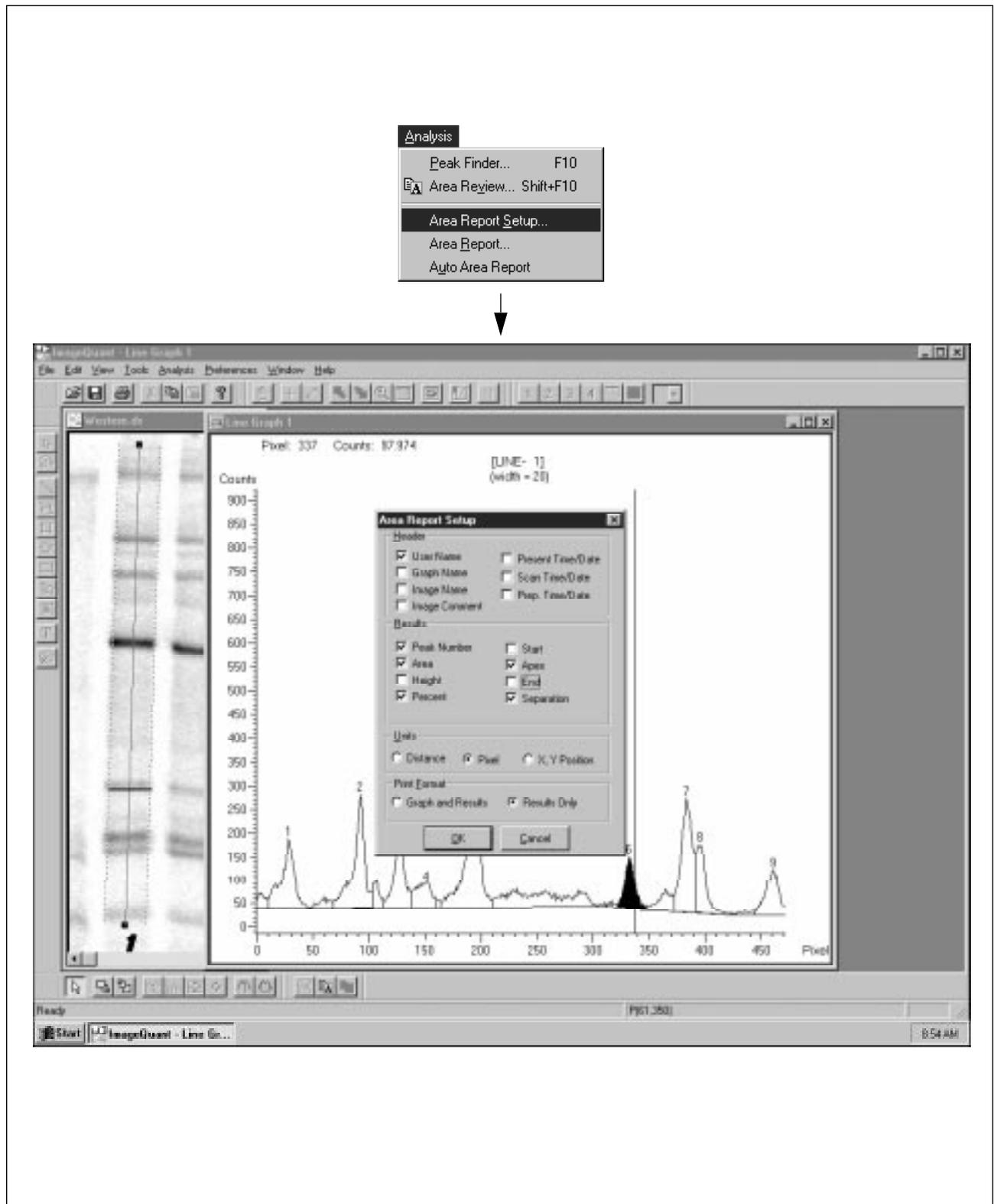
Step 14 Selecting Reporting Options

Before you print or display an area report, you must tell ImageQuant which results you want reported. The options selected when the window opens are defaults. You can select or deselect any option.

1. Choose **Area Report Setup** from the Analysis menu. The Area Report Setup window appears.
2. Select the following Results options:
 - Peak Number
 - Area
 - Percent
 - Apex
 - Separation

A check mark in the box means that the option is selected. Click a check box to select or deselect an option.

3. Click **OK** to close the window and accept the settings.

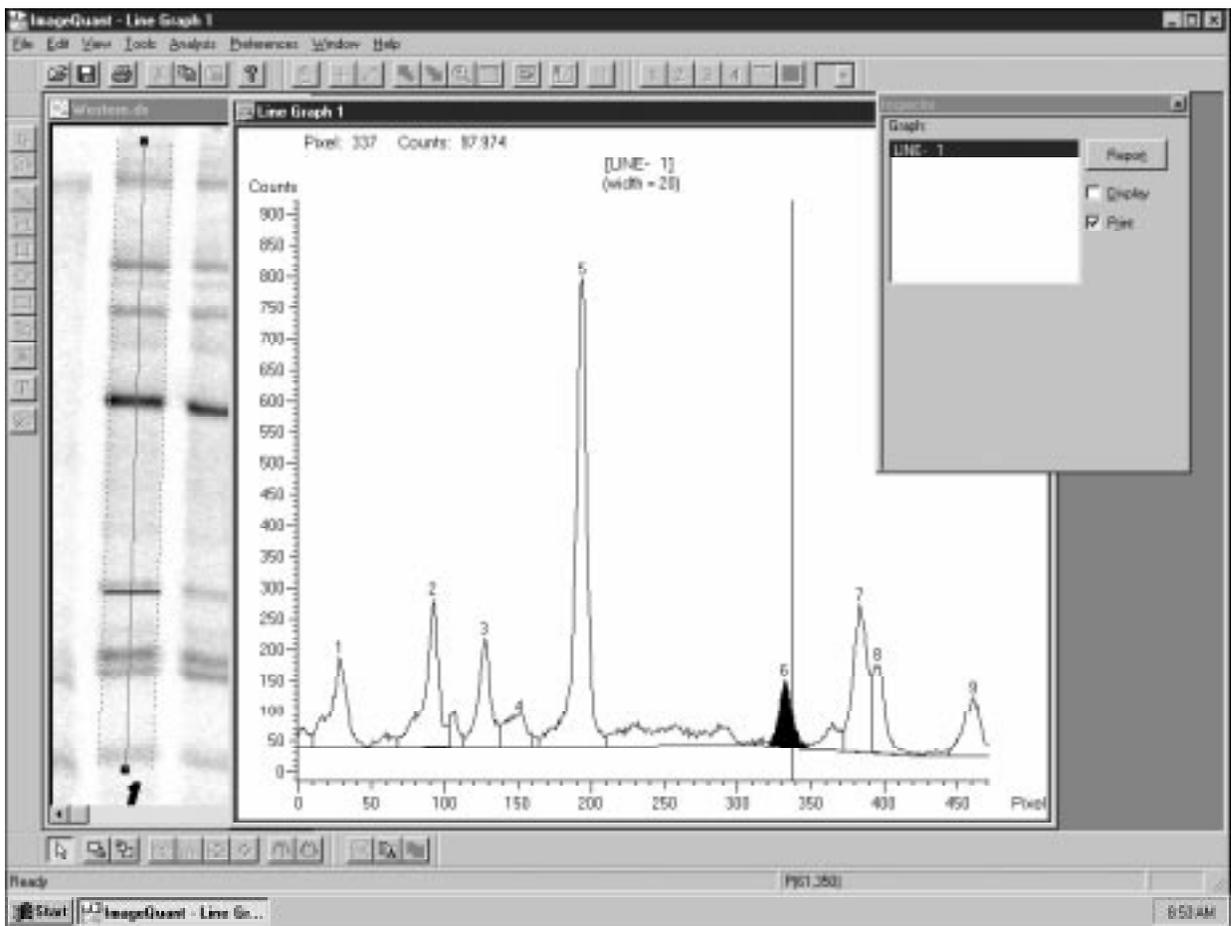
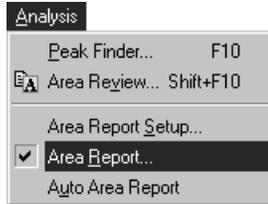


Have you selected your reporting options? Go to the next page...

Step 15 Printing an Area Report

Now you can print an area report.

1. Choose **Area Report** from the Analysis menu. The Area Report inspector appears.
2. Select the line graph that you want to appear in the report.
3. Make sure that a check mark appears in the box in front of Print.
4. Click the **Report** button. The report is sent to the printer.



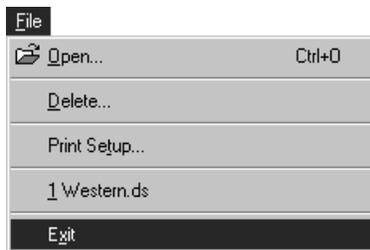
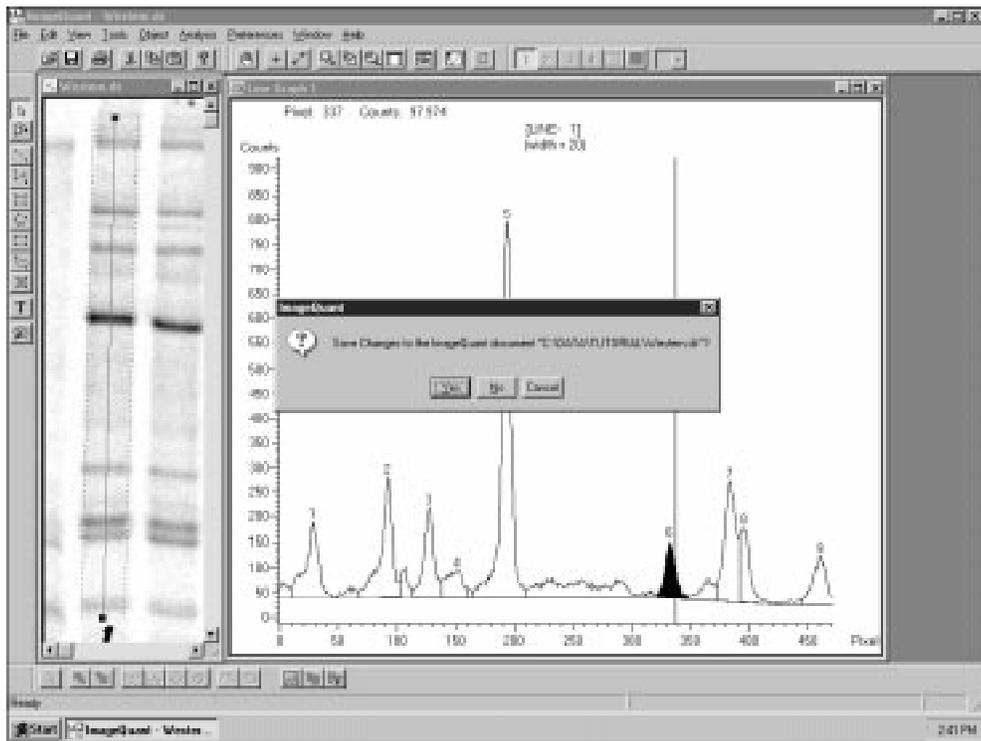
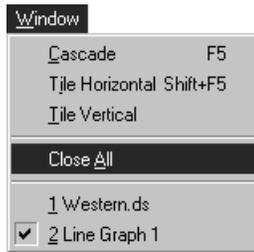
Printed the area report? Go to the next page...

Step 16 *Closing ImageQuant*

You have successfully completed several ImageQuant tasks. Before you end your tutorial session, you need to close the Western image. Normally, you would save your objects and results. However, because this is a tutorial, you will want to leave the tutorial in the same condition you found it.

To do this—

1. Choose **Close All** from the Window menu.
2. For each open window, a pop-up window appears asking if you want to save. Click **No**.
3. When all the windows are closed, choose **Exit** or **Quit** from the File menu to close ImageQuant.



Windows NT



Macintosh

Congratulations!

