Molecular® Dynamics

IMAGEQUANT® UTILITIES

USER'S GUIDE

for Microsoft® Windows NT®
Apple® Power Macintosh®

Cover (Foreground): A 2-color gel image of fluorescently labeled DNA ladders and PCR products scanned with the FluorImager 595 and displayed in FluorSep. The Fluorochrome Separation window is open in the lower right. The image has been processed in FluorSep to facilitate spectral separation of the 2 labels. Channels 1 (Fluorescein and FAM = Green) and 2 (TAMRA = Red) are displayed in the overlay mode. Yellow indicates simultaneous signal from both fluorochromes at positions of comigration in the gel.

Cover (Background): A 3-color DNA typing gel (short tandem repeats or STR) scanned with the FluorImager 595 and displayed in ImageQuant Tools. The image has been preprocessed in FluorSep to facilitate spectral separation of the 3 labels. Channels 1 (Fluorescein = Green), 2 (TMR = Blue), and 3 (CXR = Red) are displayed in the overlay mode. The Image Rotation window in ImageQuant Tools is used to rotate the resulting 3-color image as desired.

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Preface

About This Guide

The *ImageQuant Utilities User's Guide* describes how to use the FluorSep™ and ImageQuant Tools utilities.

- **Part One: Introduction**—Provides an introduction to the ImageQuant utilities.

- **Part Two: FluorSep Utility**—Describes how to use the FluorSep utility to separate multichannel images and to create multichannel datasets.

- **Part Three: ImageQuant Tools Utility**—Describes how to use the ImageQuant Tools utility to rotate, invert, and filter images.

- **Part Four: Reference**—Provides a reference to features in the FluorSep and ImageQuant Tools utilities that are not discussed in Parts Two and Three.

- **Part Five: Appendixes**—Provides a quick reference for the FluorSep and ImageQuant Tools utilities.

Related Publications

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

- 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 function of the ImageQuant software.

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

- 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.

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

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

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

- **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.

- **SHIFT+click**—Hold down the SHIFT key 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.

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Part One

Introduction

Chapter 1 The ImageQuant Utilities

The *ImageQuant Utilities User's Guide* explains how to use the FluorSep and ImageQuant Tools utilities. This chapter provides a brief description of each utility.

The topics in this chapter are—

- Overview of the FluorSep Utility (section 1.1)
- Overview of the ImageQuant Tools Utility (section 1.2)
- Checking the Audit Trail (section 1.3)

1.1 Overview of the FluorSep Utility

The FluorSep utility uses a fluorochrome separation process to separate the multichannel images you create using the FluorImager® or Storm® 860 instruments.

The fluorochrome separation process removes the cross-contamination that can occur when the emission spectra from multiple images overlap. Because the emission spectra can overlap between the images, the emissions from one fluorochrome can contaminate the collection for the other fluorochromes. The fluorochrome separation process removes the cross-contamination by performing a mathematical transformation on the images produced during scanning. The end result includes two or more images that, under ideal conditions, represent the light emitted from the fluorochromes used in the sample.

The FluorSep utility also provides a method to create multichannel images from single images you create using any Molecular Dynamics instrument.

See Part 2 for a description of the FluorSep utility.

1.2 Overview of the ImageQuant Tools Utility

The ImageQuant Tools utility provides several image processing tools. You use these tools to modify a copy of the original image. The original image remains unaltered. Both images can be used for quantitation.

The ImageQuant Tools utility allows you to make the following modifications to images:

- **Rotate**—You can rotate or flip images scanned on any Molecular Dynamics instrument.

- **Filter**—You can reduce the noise in an image using a median filter or an impulse filter.

- **Invert**—You can invert the pixel values of the image so that images scanned with a dark background (high values) and light spots (low values) are displayed as dark spots (high values) with a light background (low values).

See Part 3 for a description of the ImageQuant Tools utility.

1.3 Checking the Audit Trail

Because both the FluorSep and the ImageQuant Tools utilities modify the original image, Molecular Dynamics provides an audit trail of all the image processing operations applied to the image. You can check the audit trail from FluorSep, ImageQuant Tools, or ImageQuant.

To check the audit trail—

1. Open the image in ImageQuant, ImageQuant Tools, or FluorSep.

2. Choose **Image Properties** from the File menu. The Image Properties window appears and displays the File Info tab.

3. Click the **History** tab. The History tab appears (figure 1-1).
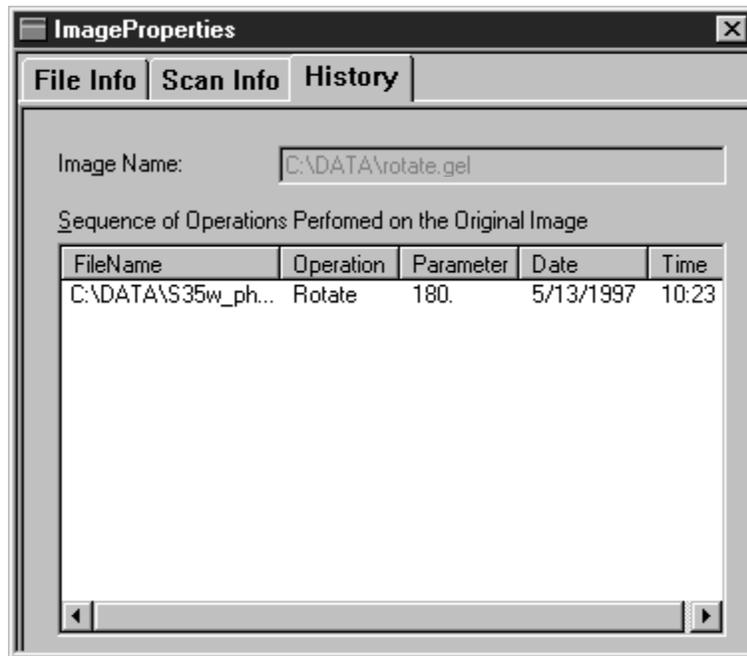
Figure 1-1. The History tab in the Image Properties window. The Operations list shows that the original image was rotated 180° using the ImageQuant Tools utility.

The image name at the top of the window is the name of the image displayed in the Image window. Below the name is a list of each processing operation applied to the image. You create a new image file each time you apply an operation to the image.

The sequence of operations list provides the following information:

• **File Name**—The name of the image to which you applied the operation. **Note:** The file name is not the name you entered when ImageQuant Tools performed the operation.

• **Operation and Parameter**—The type of image processing applied to the image, plus appropriate parameters. For example, the image referred to in figure 1-1 was rotated (operation) 180° (parameter).

• **Date and Time**—The date and time when ImageQuant Tools performed the operation.

If no operations have been performed on the image, the sequence of operations list is blank.

Part Two

FluorSep Utility

Chapter 2 Using FluorSep to Separate Images

The FluorSep utility allows you to separate the multichannel images you create using the Storm 860 or FluorImager instruments. Topics in this chapter are—

- About FluorSep (section 2.1)
- Displaying the Image (section 2.2)
- Selecting Automatic or Manual Background Correction (section 2.3)
- Locating and Placing the Fluorochrome Marker Rectangles (section 2.4)
- Sizing and Centering the Fluorochrome Marker Rectangles (section 2.5)
- Locating and Placing the Background Rectangles (Manual Only) (section 2.6)
- Performing the Separation (section 2.7)
- Evaluating the Results (section 2.8)
- Choosing a Background Correction Filter (section 2.9)
- Using the Impulse Filter During Separation (section 2.10)

2.1 About FluorSep

The FluorSep utility provides two methods to remove the cross-contamination—

- **Automatic**—FluorSep determines which pixel values are background by using a histogram peak algorithm. Use this method if the image background is uniform.

- **Manual**—You determine which areas on the image represent the background. Use this method if the image background is not uniform.

You should try the automatic method first. FluorSep includes an Undo Separation feature, which allows you to, if necessary, repeat the separation process using different variables. If the results are not acceptable, you can use the manual method. In addition, you can add a local or global background filter, an impulse filter, or both filters to improve the uniformity of the background. If the result for any of the filters is not acceptable, you can repeat the separation process without using the filter.

After FluorSep removes the cross-contamination, the utility creates new separated files for each channel and stores the new files in the existing dataset folder (.dir). The dataset (.ds) file structure was created by the Scanner Control software (figure 2-1).

The dataset folder contains one separated and one unseparated image file (.gel) for each channel. Each channel represents one designated fluorochrome. In addition, you see one object file (.dox) for each channel. The object file contains the rectangles used by FluorSep during the fluorochrome separation process.
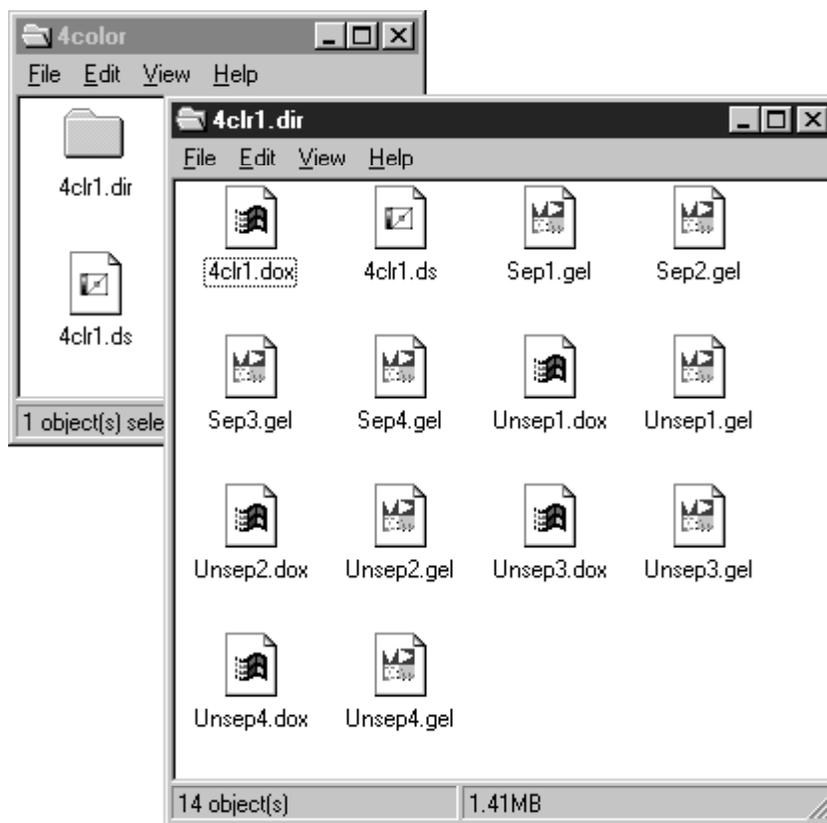
Figure 2-1. Sample of the dataset file structure.

You can view the channels in the dataset overlaid, side by side, or one at a time. After the separation, FluorSep displays the channels in the Overlay view.

2.2 Displaying the Image

Before using the FluorSep utility, you display the image you want to separate in an Image window.

Note: The Scanner Control software for your instrument may open FluorSep and display the image automatically.

To display an image—

1. Choose **Open** (![icon]) from the File menu or the Main toolbar. The Open window appears and displays the active folder and its contents.

2. If the dataset name is not listed, choose a different folder and view its contents.

3. Locate and select the dataset file (.ds). The image appears in an Image window, and the Fluorochrome Separation window appears (figure 2-2).

See your Windows NT or Macintosh documentation for a more complete description of how to use the features in the Open window.
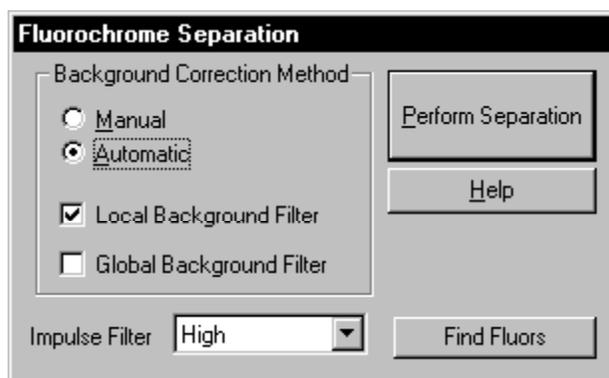
Figure 2-2. The Fluorochrome Separation window.

2.3 Selecting Automatic or Manual Background Correction

The FluorSep utility provides two methods to remove the background correction. To determine which method to use you need to evaluate the uniformity of the background (section 2.3.1). If you determine the background is uniform, select the Automatic method (section 2.3.2). If you determine the background is not uniform, select the Manual method (section 2.3.3). After you select the background correction method, use sections 2.4 through 2.6 to place the rectangles.

2.3.1 Determining the Uniformity of the Background

If you cannot determine the uniformity of the background visually, you can use the Pixel Locator tool to examine the uniformity of the background by pixel value. To use the Pixel Locator tool—

1. Choose **Pixel Locator** (+) from the Tools menu or the Image toolbar.

2. Move the pointer to the background near the top left of the image. Click the mouse button and make a note of the value.

3. Move the pointer to a background location farther down and to the right. Click the mouse button and make a note of the value.

4. Repeat step 3 until you reach the bottom of the image.

5. Click **Select** (↖) to end the Pixel Locator mode.

6. Examine the difference between the highest and the lowest relative fluorescent unit (rfu) values.

    -   If the difference is less than 20% of the total values, choose the Automatic method (section 2.3.2).

    -   If the difference is more than 20% of the total values, you can either choose the Automatic method (section 2.3.2) and the Local or Global filter (section 2.9), or choose the Manual method (section 2.3.3) and the Local filter (section 2.9.1).

2.3.2 Selecting the Automatic Method

The automatic background correction method uses rectangle objects to identify the fluorochrome markers.

Important   **The fluorochrome marker designates the brightest location of each channel relative to the other channels. Accurate sizing and placement of the rectangles is important for proper functioning of the background correction methods.**

To select the Automatic background correction method, click **Automatic** in the Background Correction Method area of the Fluorochrome Separation window. Depending on the number of fluorochromes in the sample, two or more rectangles appear on the image (figure 2-3). Each rectangle is labeled (F#), where # is the fluorochrome marker number for the emission filter.

Note: The number of rectangles depends on the number of channels acquired by the instrument and the settings you selected in the Scanner Control software for that instrument. For example, if you scanned a sample that included two fluorochrome markers, you will see two rectangles labeled (F1) and (F2).

You use the Find Fluors feature to place the fluorochrome marker rectangles (section 2.4). After placing the rectangles, you may need to resize or recenter them (section 2.5).
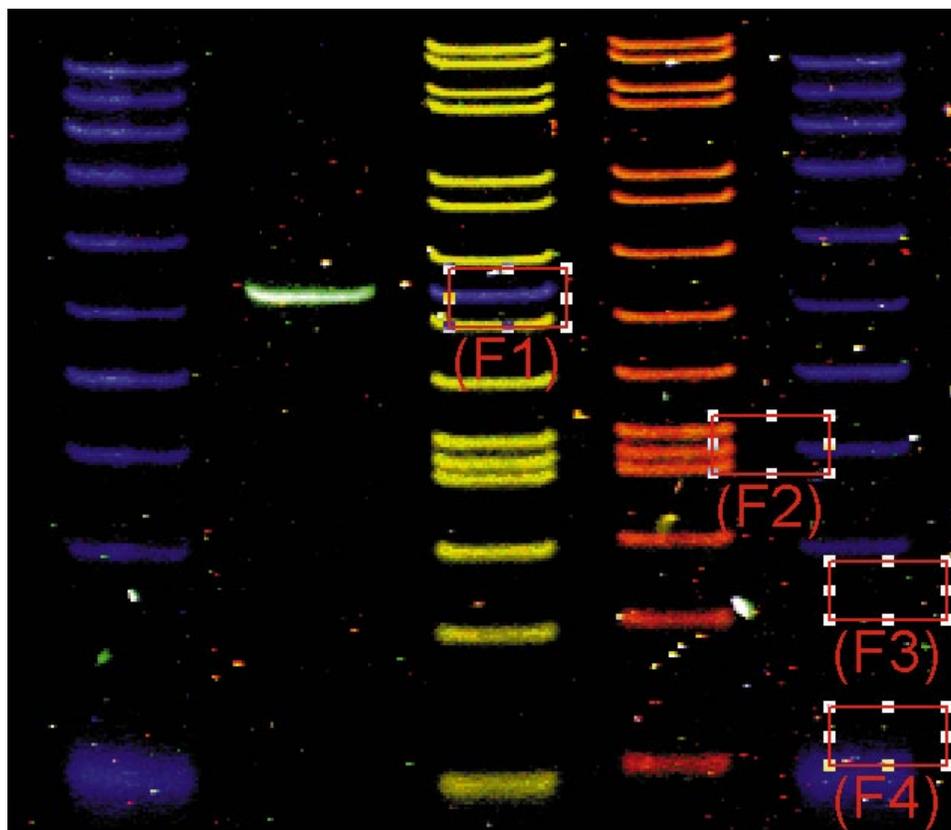
Figure 2-3. The rectangles that appear when you use the Automatic background correction method for an image containing four fluorochrome markers.

2.3.3 Selecting the Manual Method

The manual background correction method uses rectangle objects to identify the fluorochrome markers and to identify portions of the image as background.

Important **The fluorochrome marker designates the brightest location of each channel relative to the other channels. Accurate sizing and placement of the rectangles is important for proper functioning of the background correction methods.**

To select the Manual background correction method, click **Manual** in the Background Correction Method area. Depending on the number of fluorochromes in the sample, five or more rectangles appear on the image (figure 2-4). The (F#) rectangles are used to locate the fluorochrome markers the (B#) rectangles are used to locate the background used in the background correction calculations.

You use the Find Fluors feature to place the fluorochrome marker rectangles (section 2.4). Next, you recenter or resize the fluorochrome marker rectangles (section 2.5). Finally, you place the background rectangles in the correct areas of the background (section 2.5).
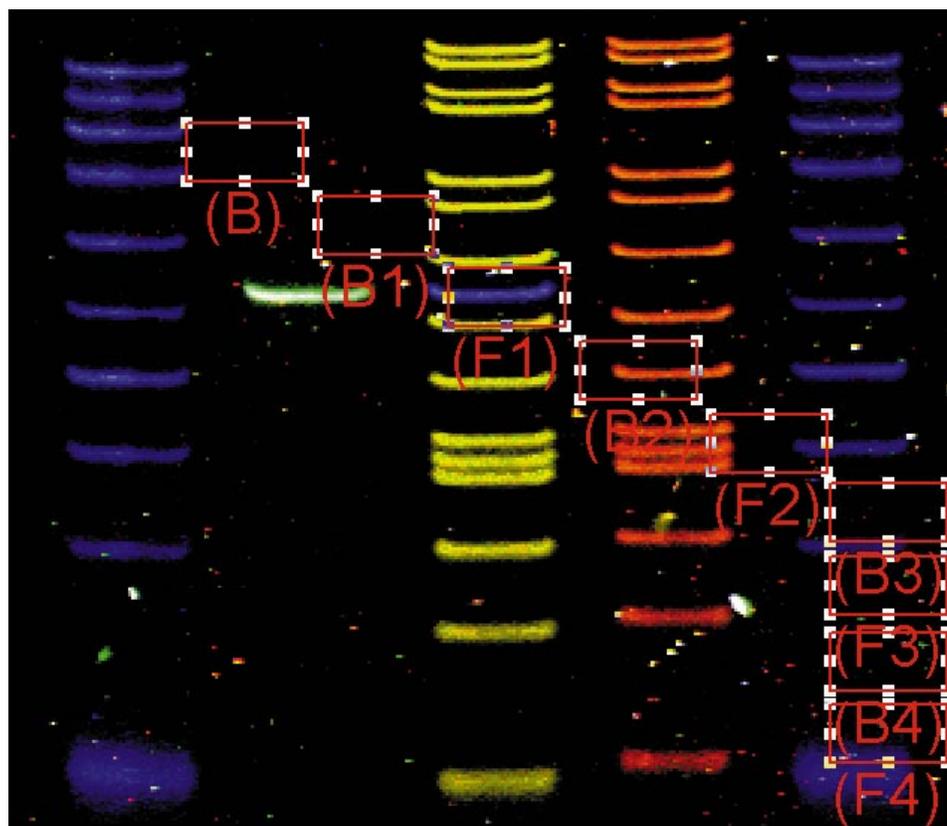

Figure 2-4. The rectangles that appear when you use the Manual background correction method on an image containing four fluorochrome markers.

2.4 Locating and Placing the Fluorochrome Marker Rectangles

In both the automatic and manual background correction methods, you use the Find Fluors feature to help you locate the markers automatically. The Find Fluors feature locates the fluorochrome marker for each channel with better than 80% accuracy. The Find Fluors feature evaluates the entire image, pixel by pixel, using a two-pixel by eight-pixel area. FluorSep looks at the continuity of the pixels in each band (figure 2-5). FluorSep calculates the brightest band in each channel with the least amount of bleed into other channels.
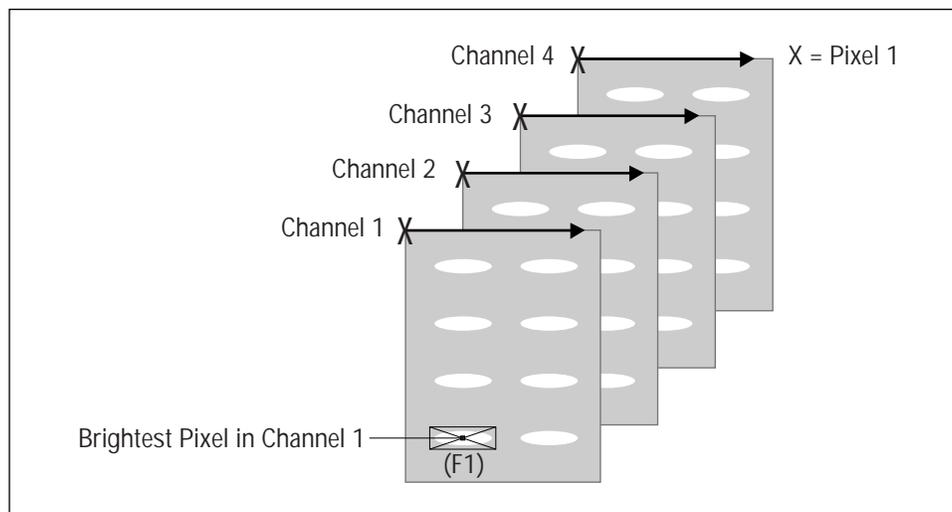
Figure 2-5. To place the fluorochrome markers, FluorSep compares pixel 1 in all four channels and then compares pixel 2 in all four channels. After evaluating all the pixels in all the channels, FluorSep places a fluorochrome marker on the brightest pixels in each channel.

FluorSep uses the width of the rectangles to define the width of the bands, and then applies the histogram algorithm using the same width to determine the background. FluorSep assumes the bands are in lanes that run from the top to the bottom of the image.

Before locating the markers using the Find Fluors feature, you may want to use the instructions in section 2.5 to position and size the rectangle for the largest fluorochrome marker. Doing this can help FluorSep locate the brightest pixel in each marker and can help you identify which is the brightest pixel.

To locate the fluorochrome markers, click the **Find Fluors** button in the Fluorochrome Separation window. FluorSep compares the channels in the image, pixel by pixel. FluorSep uses the **(F1)** through **(F4)** rectangles to enclose the control markers of the fluorochromes that you included in your experiment and collected through emission filters 1 through 4, respectively.

After FluorSep finds the brightest fluorochrome markers, FluorSep places each rectangle so that the brightest pixel within the marker is centered in the rectangle (figure 2-6). Because the brightest pixel may not be at the center of the marker, you may need to reposition the rectangles (section 2.5).

Contaminants, such as dust, or a poor signal-to-noise ratio can prevent Find Fluors from accurately locating the optimal fluorochrome markers. If Find Fluors cannot locate the control marker for a specific fluorochrome, a window appears with the message, **"Cannot Find F (1, 2, 3, or 4) position."** Use the instructions in section 2.5 to place the rectangle on the fluorochrome marker for this channel manually.
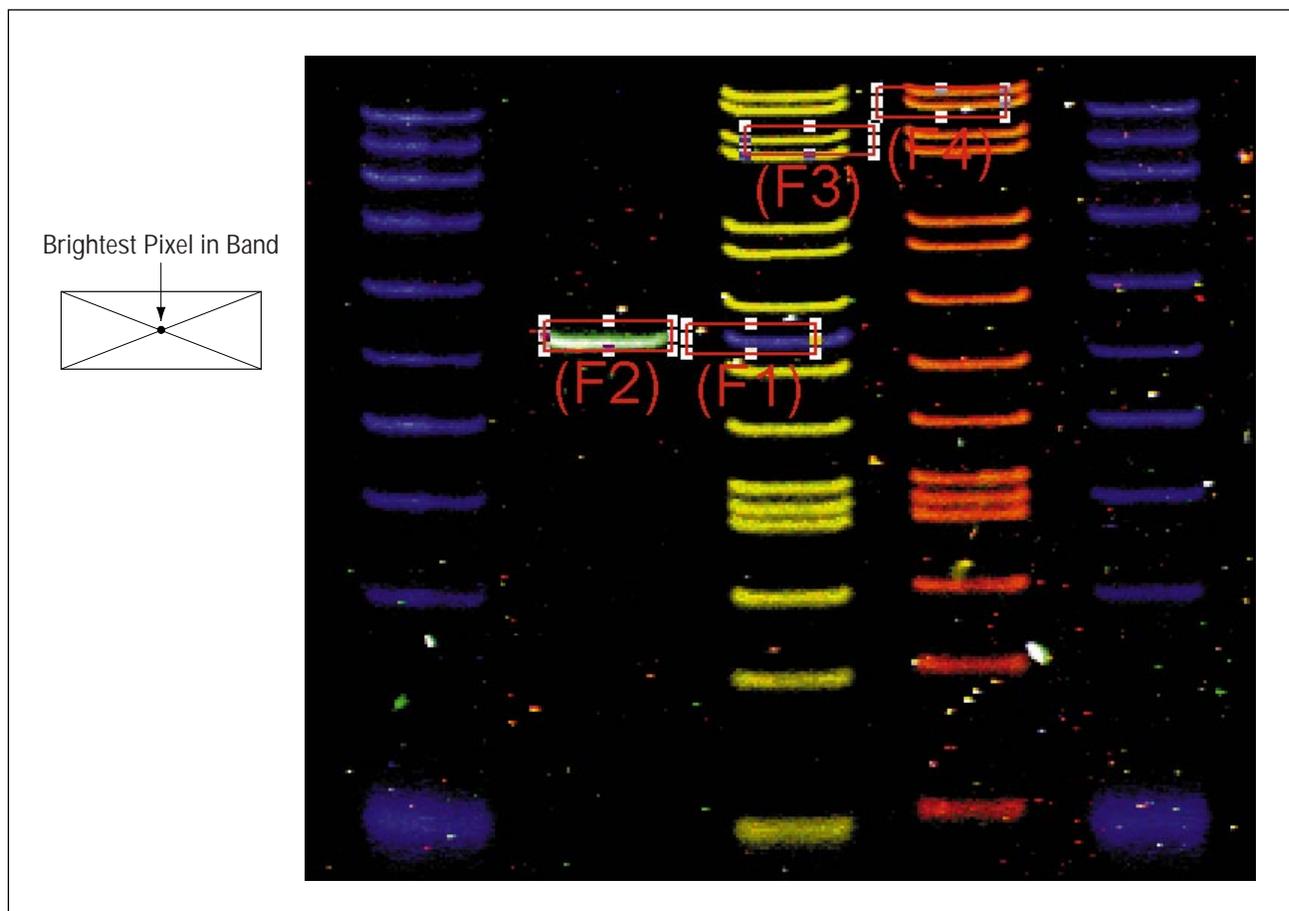
Figure 2-6. The new positions of the rectangles after using Find Fluors to locate the fluorochrome markers.

2.5 Sizing and Centering the Fluorochrome Marker Rectangles

The way you place the fluorochrome marker rectangles determines how accurately FluorSep removes the background. Because all the rectangles must be the same size, you need to size the rectangle that encloses the largest fluorochrome marker. After you size the rectangle, you should reposition each rectangle so that the fluorochrome marker is centered in the rectangle.

To size and center a rectangle—

1. Click to select the rectangle that encloses the largest fluoro-chrome marker. Handles appear on the corners of the rectangle.

2. Place the pointer on the rectangle name and drag the rectangle until two perpendicular sides of the rectangle are close to the marker (figure 2-7a).

3. Place the pointer on the handle farthest from the marker and drag the handle until the remaining two sides of the rectangle are close to the marker (figure 2-7b). The rectangle should enclose the entire marker with as little background as possible.

    Note: Because the rectangles must be the same size, all the rectangles change size as you change the selected rectangle.
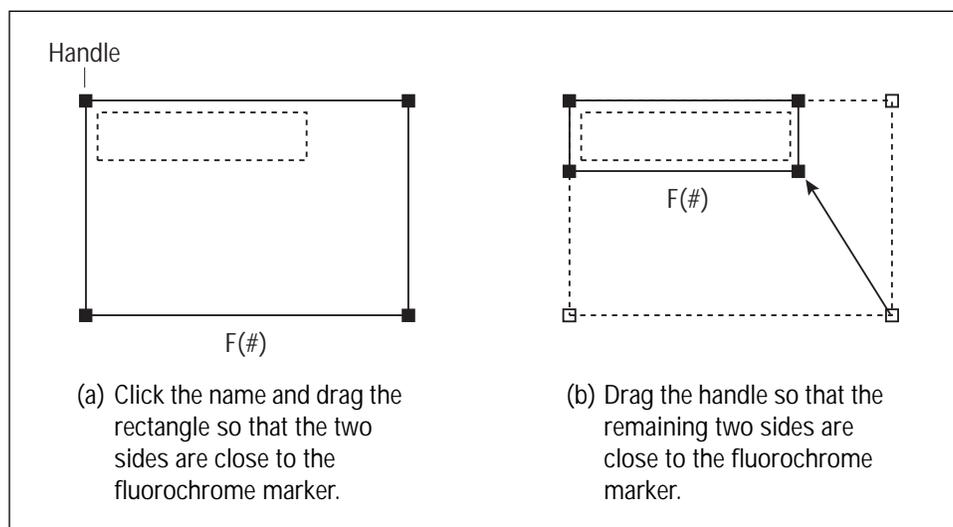
Figure 2-7. Positioning and sizing the rectangle over the fluorochrome marker.

4. Center the remaining rectangles over the fluorochrome markers.

Figure 2-8 shows the fluorochrome marker rectangles correctly sized and centered over the markers.

If you are using the automatic background correction method, go to section 2.7 to perform the separation. If you are using the manual background correction method, go to section 2.6 to position the background rectangles.
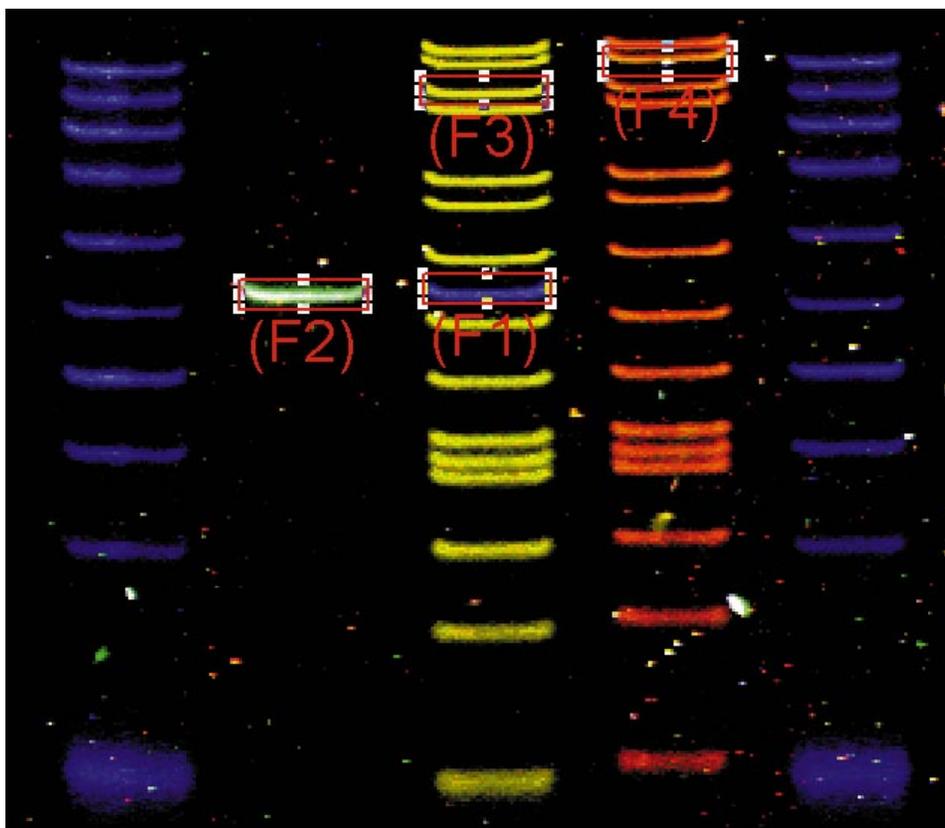
Figure 2-8. Correctly sized and centered rectangles in the Image window.

2.6 Locating and Placing the Background Rectangles (Manual Only)

If you are using the Manual background correction method, FluorSep creates three to five additional rectangles that you use to identify the background. You use the **(B1)** through **(B4)** rectangles to enclose a portion of the background near the control marker that ends with the same number. For example, position the (B1) rectangle in the background near the (F1) rectangle. You use the **(B)** rectangle to enclose a portion of the background that contains values that are lower than the lowest band. Alternatively, you can enclose a portion of the background that contains the highest background value.

The number of rectangles depends on the instrument and the settings you selected in the Scanner Control software for that instrument. For example, if you scanned a sample that included two fluorochrome markers, you will see five rectangles labeled (F1), (F2), (B1), (B2), and (B).

2.6.1 Positioning the (B1) through (B4) Rectangles

After you size and center the fluorochrome marker rectangles, you place each background rectangle in a portion of the image background near the corresponding fluorochrome marker rectangle.

To position the background rectangles—

1. Click to select the **(B1)** rectangle.

2. Drag the **(B1)** rectangle to a portion of the background near the control marker for fluorochrome 1.

3. Repeat steps 1 and 2 for the **(B2)** through **(B4)** rectangles and the control markers for fluorochromes 2 through 4. **Note:** The number of background rectangles depends on the number of fluorochromes used to scan the image.

2.6.2 Determining the Location for the (B) Rectangle

Important **Choosing the correct location for the (B) rectangle improves or optimizes the separation results.**

If your image has weak bands, you should place the (B) rectangle in an area that contains values that are lower than the weakest band you want to see. Placing the rectangle in an area with the highest background value can cause weak bands to disappear from the separated image. You can visually determine the weakest band on the image.

If your image does not have weak bands, you can place the (B) rectangle in an area that contains the highest background value found on the image.

Note: Placing the (B) rectangle at the lowest background value is similar to using the Global background filter.

To determine the highest background value—

1. Choose **Pixel Locator** (+) from the Tools menu or the Image toolbar.

2. Move the pointer to the background near the top of the image and then click the mouse button. A value appears in the status bar.

3. Make a note of the value.

4. Move the pointer to a background location farther down the image. Click the mouse button and make a note of the value.

5. Repeat step 4 until you reach the bottom of the image.

6. From the list of values, select the highest background value.

7. Click **Select** (🔲) and then click the **(B)** rectangle.

8. Drag the **(B)** rectangle so that it surrounds the area that contains the highest value.

Figure 2-9 shows the fluorochrome marker rectangles and the background rectangles correctly positioned on the image.
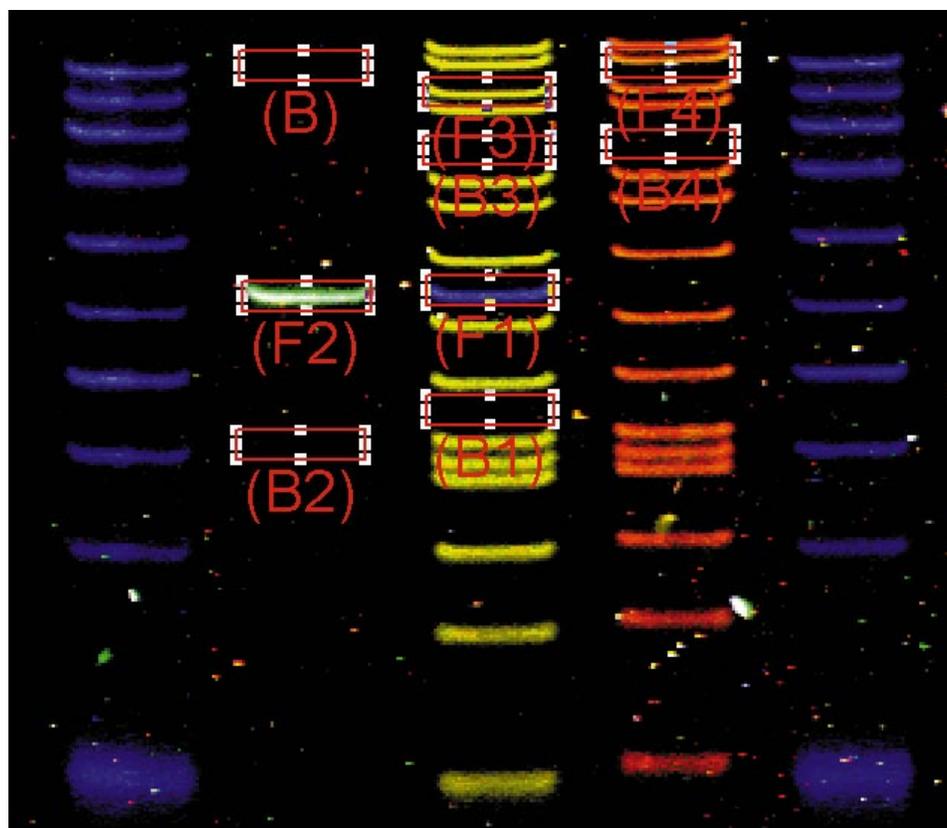

Figure 2-9. Correctly positioned rectangles for use with the manual background correction method.

2.7 Performing the Separation

After positioning the rectangles, you are ready to separate the image. After you separate the image, you can evaluate the results and, if necessary, repeat the procedure using additional options (sections 2.8 through 2.10).

To separate the image, click **Perform Separation** in the Fluoro-chrome Separation window (figure 2-2). The separation process begins, and the progress appears in the status bar. If you want to cancel the separation, press the **ESC** key.

Note: The larger the image, the longer the time needed for the separation function.

After FluorSep removes the cross-contamination, a window displays the message, **"Fluorochrome Separation has been completed."** Click **OK.** The separated image appears in the Image window in the Overlay view (figure 2-10), and the Perform Separation button changes to Undo Separation.
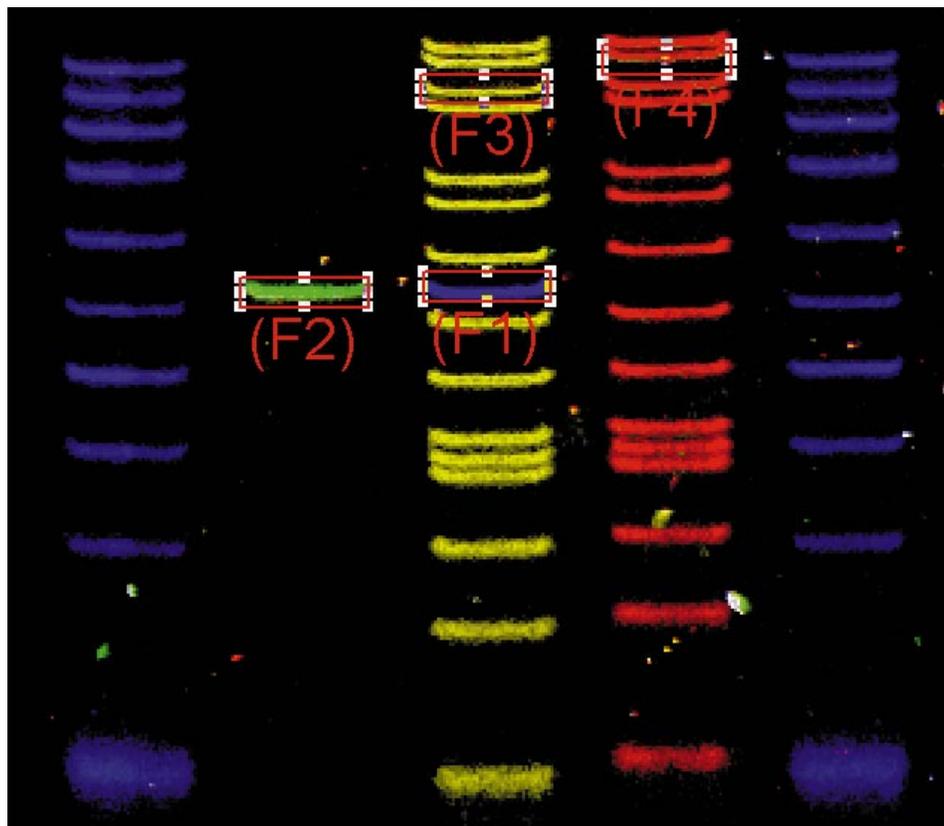

Figure 2-10. Image separated using the automatic background correction method displayed in the Overlay view.

2.8 Evaluating the Results

Important   **To improve the visibility of the image after separation, you may need to optimize the image using the Gray/Color Adjust feature (section 7.8).**

If you see distinct colored bands in the overlaid image, you have completed the separation process. You can start your analysis using the ImageQuant software. If the colored bands are not what you expected, you can undo the separation. Click the **Undo Separation** button. A window appears with the message, **"Are you sure you want to undo Fluorochrome Separation?"** Click **OK.** The original unseparated image replaces the separated image in the Image window.

Repeat the separation using one or more of the following options:

- The accuracy of fluorochrome separation is very sensitive to the placement of the rectangles. Check each rectangle to make sure that the marker is centered in the rectangle. Resize the rectangle that encloses the largest fluorochrome marker to remove more background pixels (section 2.4).

- If you do not like the results of the automatic background correction method, use the **Manual** background correction method to define specific areas on the image to use as the background (section 2.6).

- In addition to the automatic background correction method, use the **Local** or **Global** background filter to help remove the background values (section 2.9).

- In addition to the manual background correction method, use the **Local** background filter to help remove the background values (section 2.9.1).

- In addition to the automatic or manual background correction method, use the **Impulse Filter** to remove artifacts caused by imperfections in the sample matrix (section 2.10).

2.9 Choosing a Background Correction Filter

To enhance the quality of the image background correction, you can use the Local or Global background correction filter with the Automatic method or the Local background correction filter with the Manual method.

2.9.1 Local Background Correction Filter

Inconsistencies in the sample thickness used during the scan can result in an image background that is not uniform. The Local filter (figure 2-11a) removes the variation in the background values relative to the image bands, which creates a uniform background. The Local filter works best on an unseparated image that contains bright distinct bands but an uneven background.

2.9.2 Global Background Correction Filter

The Global background filter (figure 2-11b) reduces the overall intensity of the image, while maintaining the relative intensity of the bands to the background. The Global filter uses an algorithm to locate the lowest background value, and then subtracts this value from the entire image.

(a) Local Filter

Levels out the variations in the background relative to the bands to create a uniform background.

(b) Global Filter

Locates the lowest background value and subtracts this value from the entire image.

(c) No Background Filter

Displays the original intensity of the fluorochrome bands relative to the background.
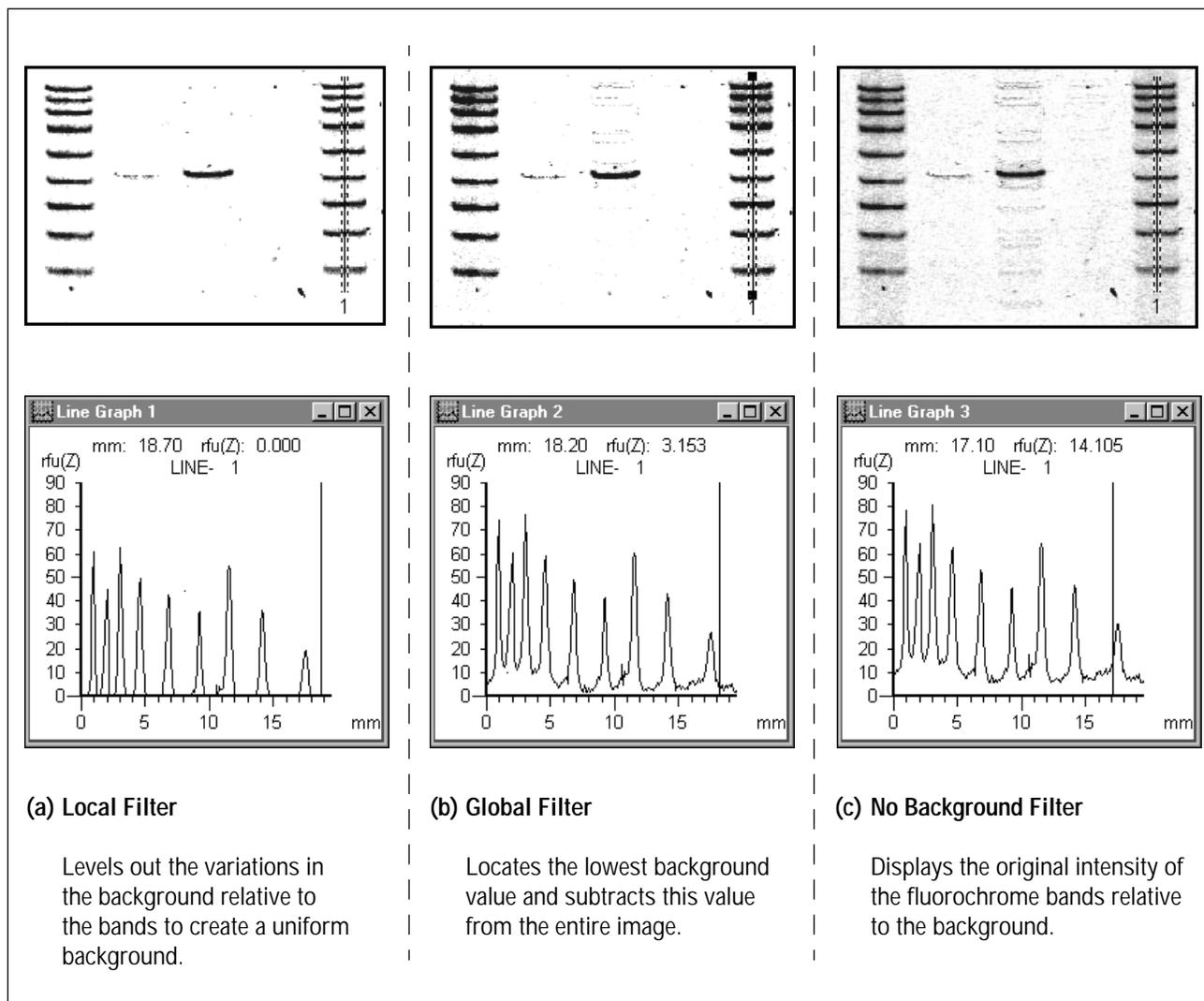
Figure 2-11. Comparison of the effects of the background filters used to separate the same dataset file: (a) Local Filter, (b) Global Filter, and (c) No Background Filter.

2.10 Using the Impulse Filter During Separation

Note: The impulse filter is a directionally applied filter designed to improve images scanned on the FluorImager 595 and Storm instruments.

To improve the separation, you can use the impulse filter to remove artifacts caused by contaminants and imperfections (such as dust or bubbles) in the sample matrix. The impulse filter reduces one-pixel impulses (positive only) by comparing three vertical pixels at a time.

For each three-pixel group (pixels A, B, and C), the impulse filter compares and resolves the middle pixel B as follows:

If pixel B > {filter weight} x (A + C), then B = (A+C)/2

The filter weight is a factor used to compare pixel B against pixels A and C. You can choose one of three filter weight levels from the Impulse Filter list box.

- **Low** is 0.75 and allows the greatest discrepancies between the pixel values in the three-pixel set and removes the least number of one-pixel impulses.

- **Medium** is 0.6 and provides a midrange setting.

- **High** is 0.55 and allows the fewest discrepancies between the pixel values and removes the most impulses.

FluorSep applies the impulse filter to the unseparated files, and then separates the image.

Chapter 3 Using FluorSep to Build a Dataset File

The FluorSep utility includes a feature that allows you to build a dataset file. You can build a dataset file from single images (.gel) or from images created as part of a dataset (.ds). The topics in this chapter are—

- Creating the Images (section 3.1)
- Building a Dataset File from Single Images (section 3.2)
- Building a Two-Channel Dataset File from Separated Image Files (section 3.3)

After creating the dataset, you view the multichannel image in ImageQuant or ImageQuant Tools.

3.1 Creating the Images

To build a dataset successfully from single image files (.gel), you must create the images using the same—

- Instrument

- Sample (do not move the sample between scans)

- Scan area

- Digital resolution

FluorSep prompts you if you select files that do not match.

3.2 Building a Dataset File from Single Images

You can create a dataset from two to four image files (.gel). To build a dataset from single images—

1. Start the FluorSep utility and choose **Build Dataset** from the File menu. The DS Builder window appears (figure 3-1).

2. Make sure the **Separated DS for analysis** box is not selected.
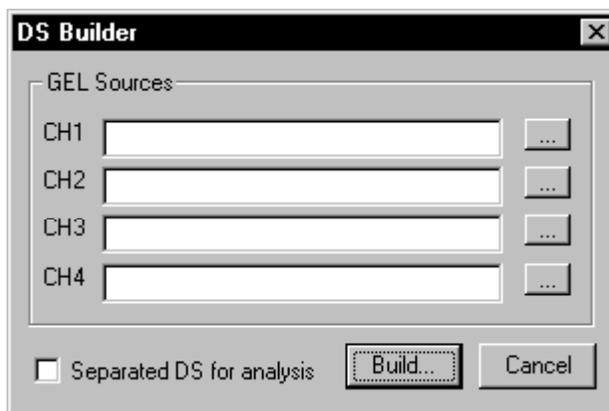
Figure 3-1. The DS Builder window.

3. Click the **Browse** button ([...]) for channel 1. The Open window appears.

4. Locate and select the image file (.gel) that contains the image with the shortest wavelength.

Important   **You must assign the image files to the channels in sequence. Start with the image file that contains the shortest wavelength and end with the image file that contains the longest wavelength.**

5. Click the **Browse** button ([...]) for channel 2. The Open window appears.

6. Locate and select the image file that contains the image data with the next shortest wavelength.

7. (Optional for Channel 3 only) Click the **Browse** button ([...]) for channel 3 and select the image file with the next shortest wavelength.

8. (Optional for Channel 4 only) Click the **Browse** button ([...]) for channel 4 and select the image file with the longest wavelength.

9. Click **Build.** After the software creates the dataset file, the Save As window appears.

10. Type a name for the dataset file and click **Save.** After FluorSep builds the dataset file, a message appears stating the dataset was built successfully.

11. Close FluorSep and display the multichannel image in ImageQuant or ImageQuant Tools.

3.3 Building a Two-Channel Dataset File from Separated Image Files

The DS Builder allows you to build a two-channel dataset file that uses image files created from two different datasets or from a dataset that includes more than two channels.

Important **You must use FluorSep to separate the images before you build the new dataset.**

Because the Fragment Analysis software analyzes two-channel datasets only, you use the DS Builder to build datasets that can be analyzed in Fragment Analysis. For example, if you create a three-channel dataset that includes a standard in channel 1 and an unknown in channel 2 and channel 3, you must build two datasets before you can analyze the data in Fragment Analysis. One dataset includes the standard in channel 1 and the unknown in channel 2. The second dataset includes the standard in channel 1 and the unknown in channel 3.

To build a new dataset file from the separated images in another dataset—

1. Start the FluorSep utility and choose **Build Dataset** from the File menu. The DS Builder window appears (figure 3-1).

2. Select the **Separated DS for analysis** box. The fields for channels 3 and 4 become inactive (figure 3-2).

3. Click the **Browse** button (...) for channel 1. The Open window appears.
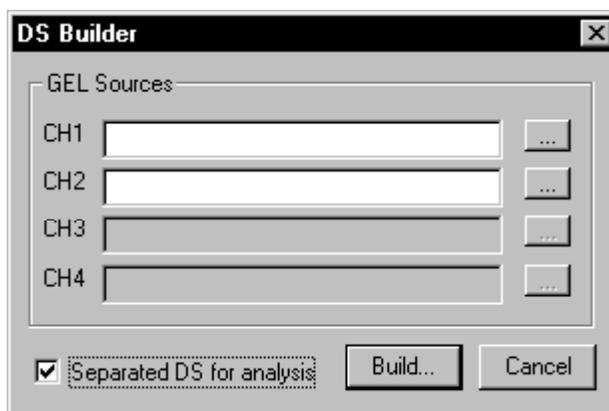
Figure 3-2. The DS Builder window in the separated dataset mode.

4. Locate the filename.dir folder that contains the separated image data file you want to use for channel 1, where filename is the name of the dataset file.

5. Double-click the folder to display the contents.

6. Select the **sep#.gel** file, where # can be 1 through 4, that represents the number of image channels contained in the dataset.

7. Click the **Browse** button (...) for channel 2. The Open window appears.

8. Repeat steps 4 through 6 to select the second image.

9. Click **Build.** After the software creates the dataset file, choose **Save As** from the File menu. The Save As window appears.

10. Type a name for the dataset file and click **Save.**

Important   **You cannot use the Undo Separation feature on a dataset file that you create using this method.**

Part Three

ImageQuant Tools Utility

Chapter 4 Using ImageQuant Tools

The ImageQuant Tools utility allows you to rotate, invert, and filter the image. The topics in this chapter are—

- Displaying an Image (section 4.1)
- Rotating the Image in 90° Increments (section 4.2)
- Rotating the Image in 1° to 15° Increments (section 4.3)
- Inverting the Data Values in the Image (section 4.4)
- Removing Noise Using the Impulse Filter (section 4.5)
- Removing Noise Using the Median Filter (section 4.6)

Important **Before using ImageQuant Tools on an image, check that sufficient disk space is available on the computer. Each process requires approximately twice as much disk space as the size of the file being processed. After you process the image, you will have the original image file and the new rotated, inverted, or filtered image file. You can open and quantitate both images.**

4.1 Displaying an Image

Before you can process an image using ImageQuant Tools, you display the image in an Image window.

To display an image—

1. In the ImageQuant Tools window, choose **Open** ( ) from the File menu or the Main toolbar. The Open window appears showing the active folder and its contents.

2. If the image name is not listed, choose a different folder and view its contents.

3. Locate and select the image file. The Image window appears and displays the image you selected.

See your Windows NT or Macintosh documentation for a more complete description of how to use the features in the Open window.

4.2 Rotating the Image in 90° Increments

To rotate the image in 90° increments, choose **Rotate Image** from the Image menu. The Image Rotation window appears (figure 4-1). The Image Rotation window contains two copies of the image displayed in the Image window.

The Image Rotation window contains rotation controls for 90° increments and 1° to 15° increments. If you want to rotate the image in increments smaller than 90°, see section 4.3.
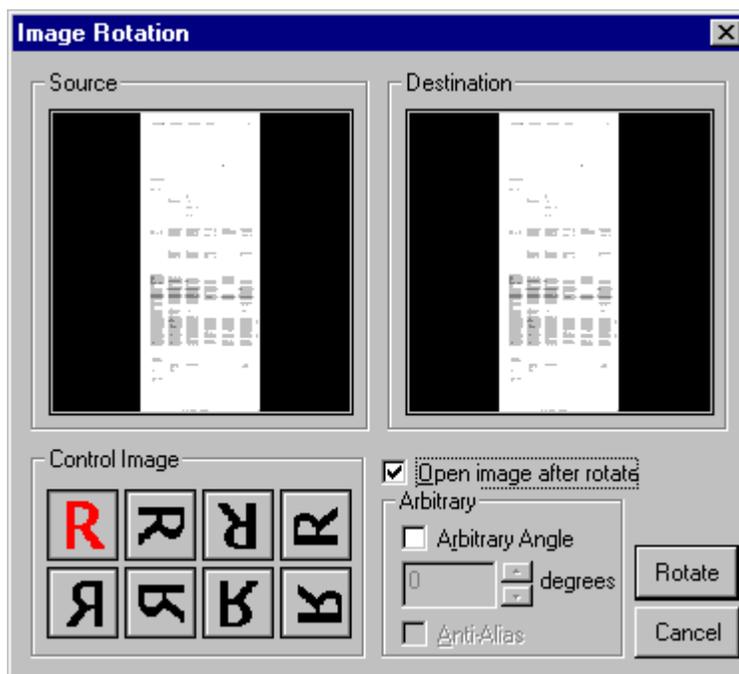

Figure 4-1. The Image Rotation window.

The Control Image area contains the eight 90° rotation choices. The top four choices rotate the image clockwise in 90° increments. The bottom four choices flip (mirror) the image, and then rotate the image counterclockwise in 90° increments.

To rotate the image—

1. Click the Control Image button that represents the rotation you want. The Destination image rotates so that you can preview the effect of the rotation.

2. (Optional) If you want the rotated image to open automatically after you save it, select the **Open image after rotate** check box.

3. When the rotation is correct for the Destination image, click **Rotate.** The Save As window appears.

4. In the Save As window, type a name for the rotated image.

5. (Optional) Choose a different directory or drive.

6. Click **Save.**

A new file containing the rotated image is created using the name you typed in step 4. If you selected **Open image after rotate** in step 2, the rotated image appears in a new Image window.

4.3 Rotating the Image in 1° to 15° Increments

The Image Rotation window (figure 4-1) contains two copies of the image displayed in the Image window, and the window contains rotation controls for 90° increments and 1° to 15° increments. If you want to rotate the image in 90° increments, see section 4.2.

You use the controls in the Arbitrary area to rotate the image in 1° to 15° increments. Activate the area by clicking **Arbitrary Angle,** and then select the increment you want to use to rotate the image. Click the up arrow to rotate the image clockwise. Click the down arrow to rotate the image counterclockwise. After each click, the image in the Destination area rotates.

If you want to smooth the jagged edges of the displayed image, select the Anti-Alias feature.

The 90° increment and the 1° to 15° increment features can be used together. For example, you can rotate the image 90°, and then rotate the image in 1° increments in either direction.

To rotate the image in 1° to 15° increments—

1. Choose **Rotate Image** from the Image menu. The Image Rotation window appears (figure 4-1).

2. Select **Arbitrary Angle.**

3. Click the up arrow to rotate the image clockwise, or click the down arrow to rotate the image counterclockwise. The image in the Destination area moves with each click.

4. (Optional) Select **Anti-Alias** to smooth the edges of the image.

5. (Optional) If you want to open the rotated image automatically after you save it, select **Open image after rotate.**

6. When the rotation is correct for the Destination image, click **Rotate.** The Save As window appears.

7. In the Save As window, type a name for the rotated image.

8. (Optional) Choose a different directory or drive.

9. Click **Save.**

A new file containing the rotated image is created using the name you typed in step 7. If you selected **Open image after rotate** in step 5, the rotated image appears in a new Image window.

4.4 Inverting the Data Values in the Image

The Invert Image command inverts the data values in an image and allows you to save the inverted image. Figure 4-2 shows an image before and after inverting. When finding peaks on a negative image scanned on a densitometer, the peaks appear to go down instead of up, and the background is a high value instead of a low value. After you choose the Invert Image command, the peaks go up and the background values are lower.

(a) Before Inverting
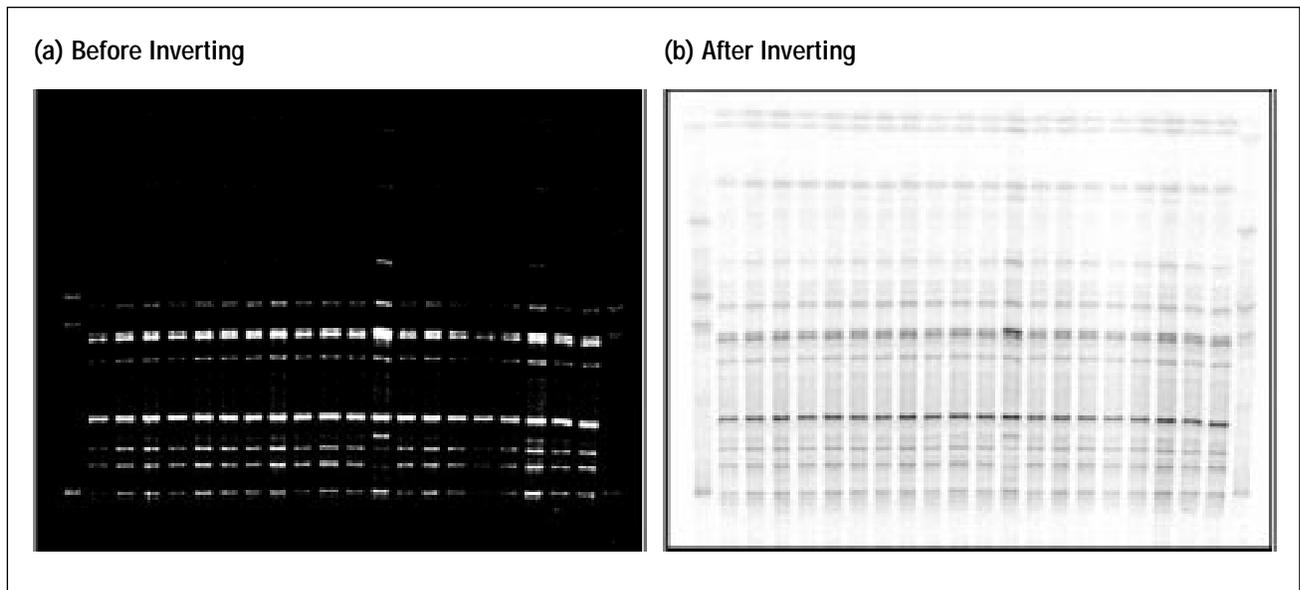
(b) After Inverting

Figure 4-2. Inverting the data values in an image: (a) before inverting and (b) after inverting.

To invert an image—

1. Choose **Invert Image** from the Image menu. The Save As window appears.

2. Type a name for the image file.

3. (Optional) Choose a different directory or drive.

4. Click **OK.** The inverted image appears in a new Image window.

4.5 Removing Noise Using the Impulse Filter

Using an impulse filter, you can remove artifacts caused by contaminants and imperfections (such as dust or bubbles) in the sample matrix. Removing the artifacts and imperfections improves the quality of the image analysis. The impulse filter reduces one-pixel impulses (positive only) by comparing three vertical pixels at a time (figure 4-3).

Pixel	Original Pixel Value	Low Weight	Medium Weight	High Weight
A	250	250	250	250
B	350	350	275	275
C	300	300	300	300

Figure 4-3. How the impulse filter affects pixel B using the Low, Medium, and High weights. The second column displays the original pixel values for pixels A, B, and C. The other columns show the effect the impulse filter and filter weight have on the middle pixel (B).

For each three-pixel group (pixel A, B, and C), the impulse filter compares and resolves the middle pixel B as follows:

If pixel B > {filter weight} x (A + C), then B = (A+C)/2

The filter weight is a factor used to compare pixel B against pixels A and C. You can choose one of three filter weights from the Impulse Filter window.

- **Low** = 0.75 and allows the greatest discrepancies between the pixel values in the three-pixel set and removes the least number of one-pixel impulses.

- **Medium** = 0.6 and provides a midrange setting.

- **High** = 0.55 and allows the fewest discrepancies between the pixel values and removes the most impulses.

To remove the noise from an image—

1. Choose **Impulse Filter** from the Image menu. The Impulse Filter window appears (figure 4-4).

2. Choose **Low, Medium,** or **High** for a filter weight.

3. Click **OK.** The Save As window appears.

4. Type a name for the image file.

5. (Optional) Choose a different directory or drive.

6. Click **OK.** The filtered image appears in a new Image window.
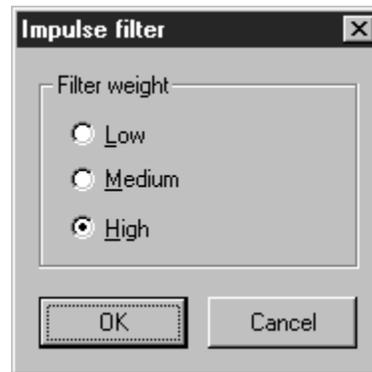
Figure 4-4. The Impulse Filter window.

4.6 Removing Noise Using the Median Filter

Using a median filter, you can remove bright noise spikes from the image without blurring the image. You can choose a 3x3 or 5x5 kernel. The median filter determines the highest pixel value (noise spike) in the kernel, and replaces the value with the median value of all the pixels in the kernel. Figure 4-5 shows how the median filter affects a 3x3 neighborhood.

Figure 4-5. How the median filter affects a 3x3 kernel. The median value in this kernel is 200.

To reduce the noise in an image using the median filter—

1. Choose **Median Filter** from the Image menu. The Median Filter window appears (figure 4-6).

2. Select **3x3** or **5x5** for the kernel.

3. Click **OK.** The Save As window appears.

4. Type a name for the image file.

5. (Optional) Choose a different directory or drive.

6. Click **OK.** The filtered image appears in a new Image window.
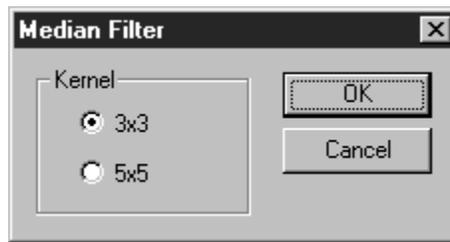
Figure 4-6. The Median Filter window.

Part Four

Reference

Chapter 5 File Menu

The File menu contains—

- Open (section 5.1)
- Close (section 5.2)
- Build Dataset (FluorSep Only) (section 5.3)
- Save As (ImageQuant Tools Only) (section 5.4)
- Save Region of Interest As (ImageQuant Tools Only) (section 5.5)
- Image Properties (section 5.6)
- Most Recently Used List (section 5.7)
- Exit or Quit (section 5.8)

5.1 Open

The Open command provides access to any stored image. To open an image—

Windows NT—Open the folder that contains the image, and then double-click the name in the box.

Macintosh—Double-click the image name in the File Name box.

The utilities use several file types. Each type is identified with a different extension or icon.

Windows NT—See appendix A for a description of each extension.

Macintosh—See appendix B for a description of each extension.

5.2 Close

The Close command closes the active window. Choosing the Close command is the same as clicking the Close box in the window.

5.3 Build Dataset (FluorSep Only)

The Build Dataset command allows you to create a multichannel dataset image file from separate image files. Chapter 3 describes this feature in detail.

5.4 Save As (ImageQuant Tools Only)

The Save As command allows you to save the selected image and associated files using a new name. In the Save As window, type the name to use for saving the image. If you are saving the image to a different folder from the default, choose the folder from the folder/file name list. If you are saving a dataset image, ImageQuant Tools copies the dataset document, the dataset folder, and the contents of the dataset folder. ImageQuant Tools adds the appropriate extensions to all the files. The original image and associated files remain unaltered.

5.5 Save Region of Interest As (ImageQuant Tools Only)

The Save Region of Interest As command allows you to save a portion of an image using a new name. You select the portion of the image using the Define Region of Interest command on the Tools menu (section 8.9). After defining the region of interest, the Save Region of Interest As command becomes active.

In the Save Region of Interest As window, type the name to use for saving the image. If you are saving the image to a different folder from the default, choose the folder from the folder/file name list. If you are saving a dataset image, ImageQuant Tools copies the dataset document, the dataset folder, and the contents of the dataset folder. ImageQuant Tools adds the appropriate extensions to all the files. The original image and associated files remain unaltered.

5.6 Image Properties

The Image Properties command displays information about the image. Alternatively, you can double-click in the Image window to display the Image Properties window. The Image Properties window contains three tabs: File Info, Scan Info, and History. Click a tab to display the contents.

File Info Tab

The File Info tab displays the information shown in figure 5-1.
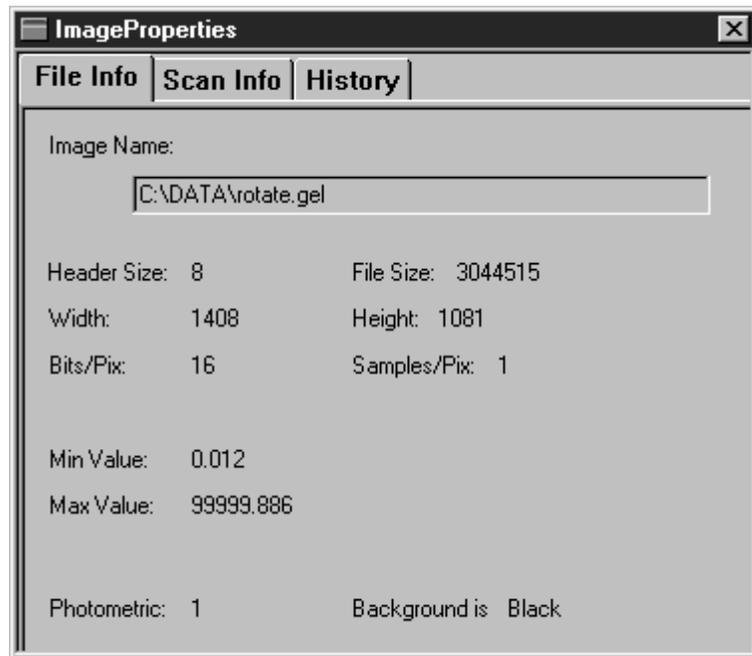
Figure 5-1. The File Info tab of the Image Properties window.

Scan Info Tab

The Scan Info tab displays the information shown in figure 5-2.
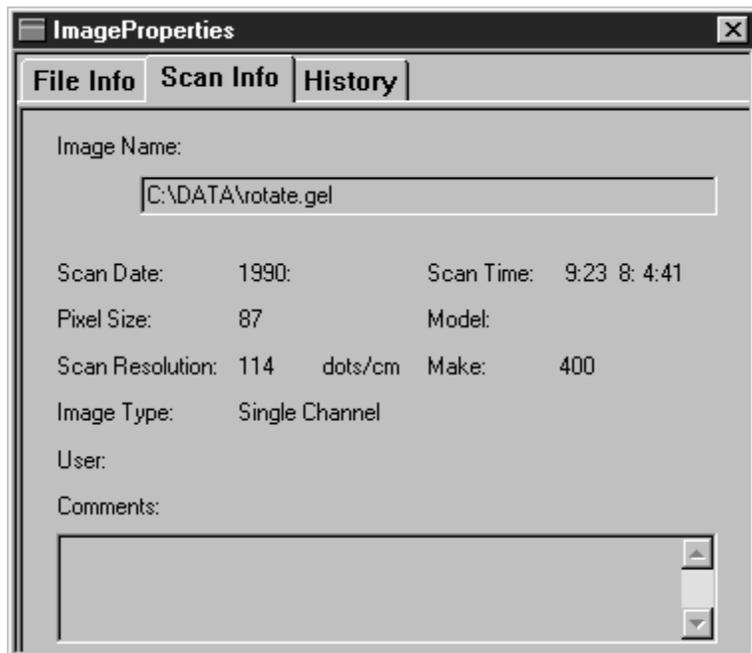
Figure 5-2. The Scan Info tab of the Image Properties window.

History Tab

The History tab (figure 5-3) displays a list of all the operations performed on the original image, if any. This list provides an audit trail of all the operations performed on the image that can impact quantitation. The current image name appears in the Image Name box.
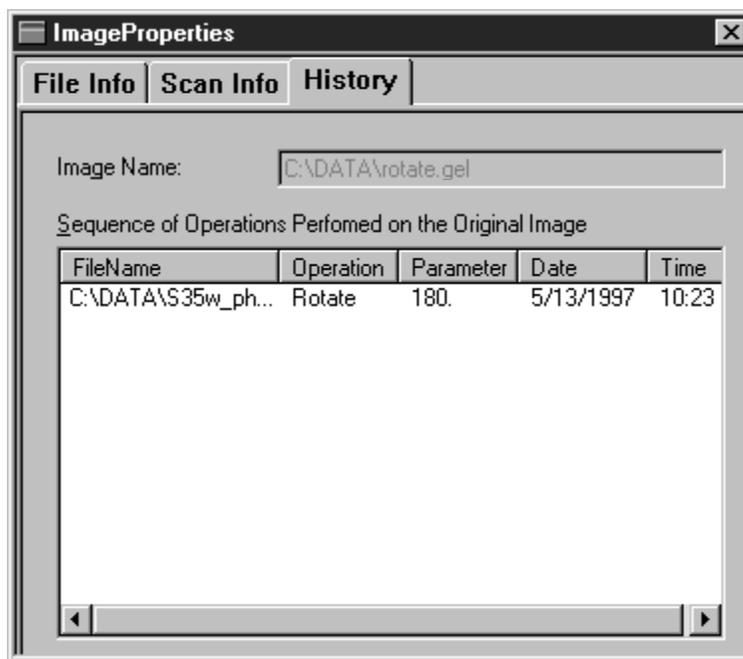

Figure 5-3. The History tab in the Image Properties window.

5.7 Most Recently Used List

The File menu displays the last four most recently used files. You can open one of these files by choosing the name from the File menu.

5.8 Exit or Quit

The Exit (Windows NT) or Quit (Macintosh) command closes ImageQuant Tools or FluorSep. You will be prompted to save any changes.

Chapter 6 Edit Menu (ImageQuant Tools Only)

The Edit menu in ImageQuant Tools contains the Copy Image command.

The Copy Image command allows you to make a copy of the entire image or a portion of the image. To copy a portion of the image, you define the portion using the Define Region of Interest command or toolbar button (section 8.9) before using the Copy Image command. The copy of the image is placed on the clipboard as a 24-bit image. You can paste the copy into another application, such as Microsoft® Word, Excel, or Adobe® Photoshop®.

You can copy the following images:

- A single-channel image displayed in gray scale or color.

- A multiple-channel image displayed in the overlaid, color mode.

- The selected channel of a multichannel image displayed in the side-by-side, gray-scale mode.

Chapter 7 View Menu

The View menu contains—

- Toolbars (section 7.1)
- Status Bar (section 7.2)
- Actual Size (section 7.3)
- Fit to Window (section 7.4)
- Magnification (section 7.5)
- Zoom In (section 7.6)
- Zoom Out (section 7.7)
- Gray/Color Adjust (section 7.8)
- Map (section 7.9)
- Multichannel (section 7.10)
- Hide Objects (FluorSep Only) (section 7.11)

7.1 Toolbars

The Toolbars command allows you to change the toolbar configuration. In the Toolbar Configuration window (figure 7-1), choose which toolbars to display by clicking the check box next to each toolbar you want displayed.
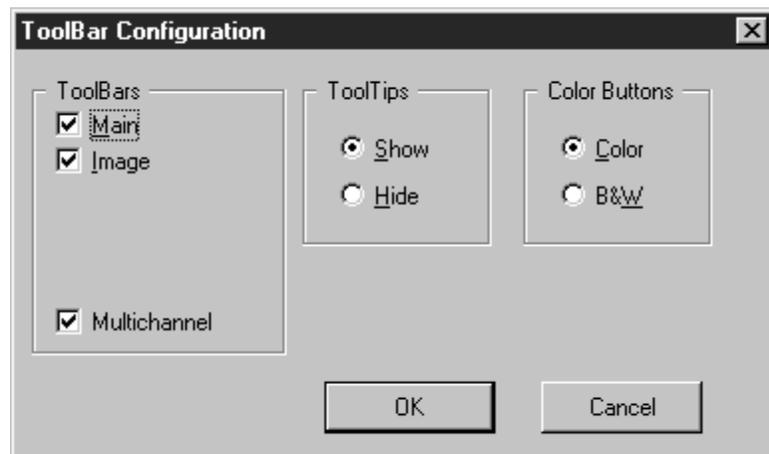
Figure 7-1. The Toolbar Configuration window.

A ToolTip is a brief description of the function of a button. You see the tip when you rest the pointer on the button. Select whether or not to see ToolTips and select whether you want the toolbar buttons displayed in color or in black and white. Click **OK** to

confirm your choices and close the window. If you reposition a toolbar, ImageQuant saves the new position when you close the application.

Important **Leave the Multichannel toolbar in the horizontal orientation. Changing the orientation to vertical causes the View One Channel button to disappear.**

Figure 7-2 shows the function of each toolbar button and indicates where that function is discussed in this guide.
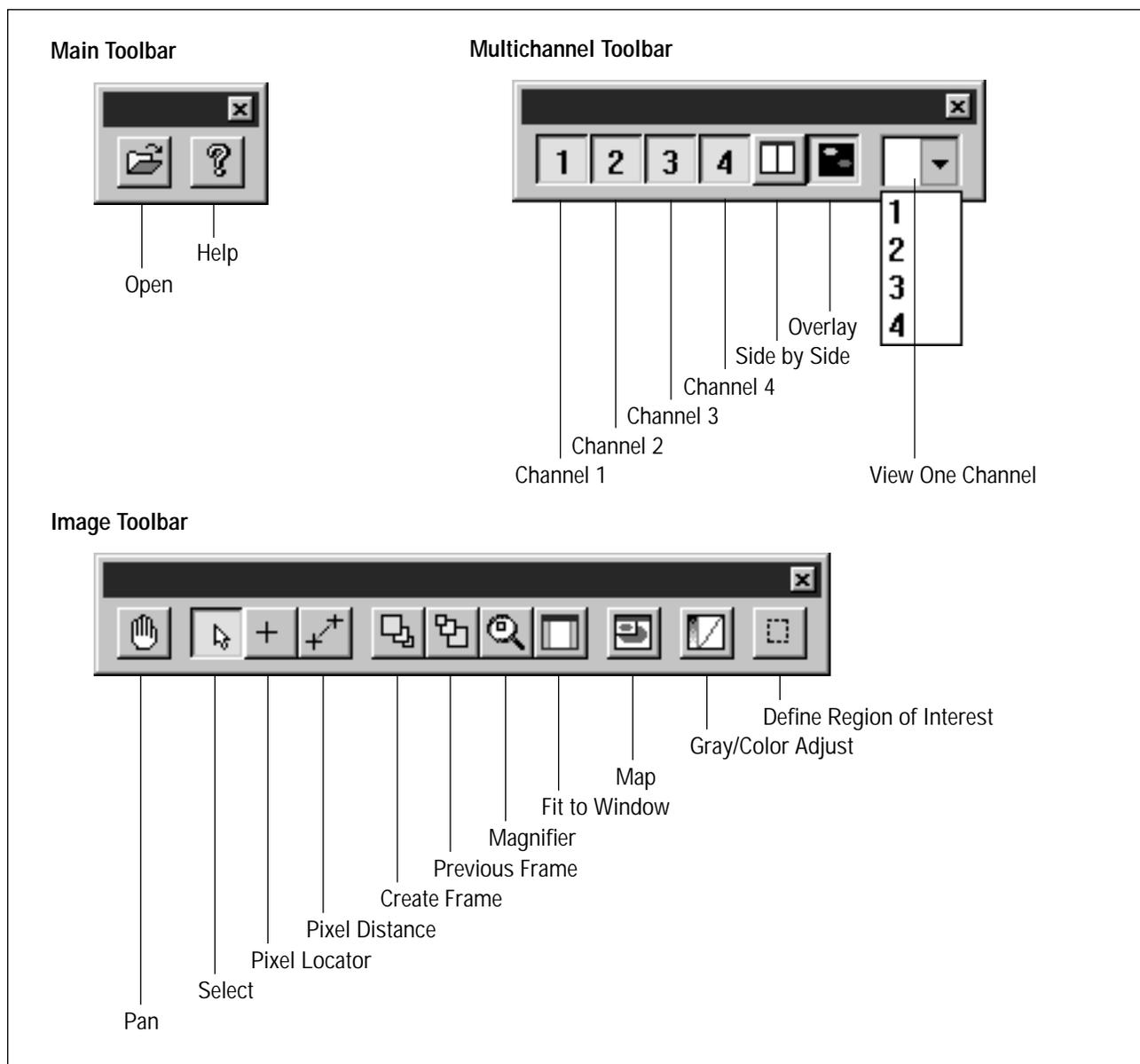
Figure 7-2. Definition of ImageQuant Tools or FluorSep toolbar buttons.

7.2 Status Bar

The Status Bar command displays or hides the status bar, which is at the bottom of the screen. The status bar displays messages. Choose Status Bar to show or hide the status bar.

7.3 Actual Size

The Actual Size command displays the image at 100%, which is the actual size at which the image was scanned. For large images, the utilities will not display all the pixels so that the image can approximate the scan size of the original sample. When viewing an image in Actual Size, ImageQuant displays the image as close to the actual size as possible.

7.4 Fit to Window

The Fit to Window command displays the entire image in the Image window. If the image is larger than the Image window boundaries, the utility reduces the image so that it fits within the boundaries.

7.5 Magnification

The Magnification command displays the image at the magnification you select from the submenu. The percentages range from 25% to 800%.

7.6 Zoom In

The Zoom In command doubles the magnification of the entire image.

7.7 Zoom Out

The Zoom Out command reduces the magnification of the entire image by half.

7.8 Gray/Color Adjust

The Gray/Color Adjust command graphically shows how the pixel intensities of the image are displayed in the Image window. The Gray/Color Adjust window contains one tab for a single-channel image and one tab for each channel of a multichannel image.

Figure 7-3 shows the Gray/Color Adjust window for a single-channel image.

The Preview Channels area displays a small version of the portion of the image displayed in the Image window. The histogram plot displays the range of values for the portion of the image displayed in the Preview Channels area.

Important   **If the entire image is not displayed in the Image window, changes you make to the histogram plot can produce unexpected results.**

Click a tab to view the channel.

Select a new channel color from the list.

Click to invert the pixel values.

Edit High/Low Button

Edit Lookup Table Button

Click to apply the changes to the Image window.

Click to apply the changes and close the Gray/Color Adjust window.
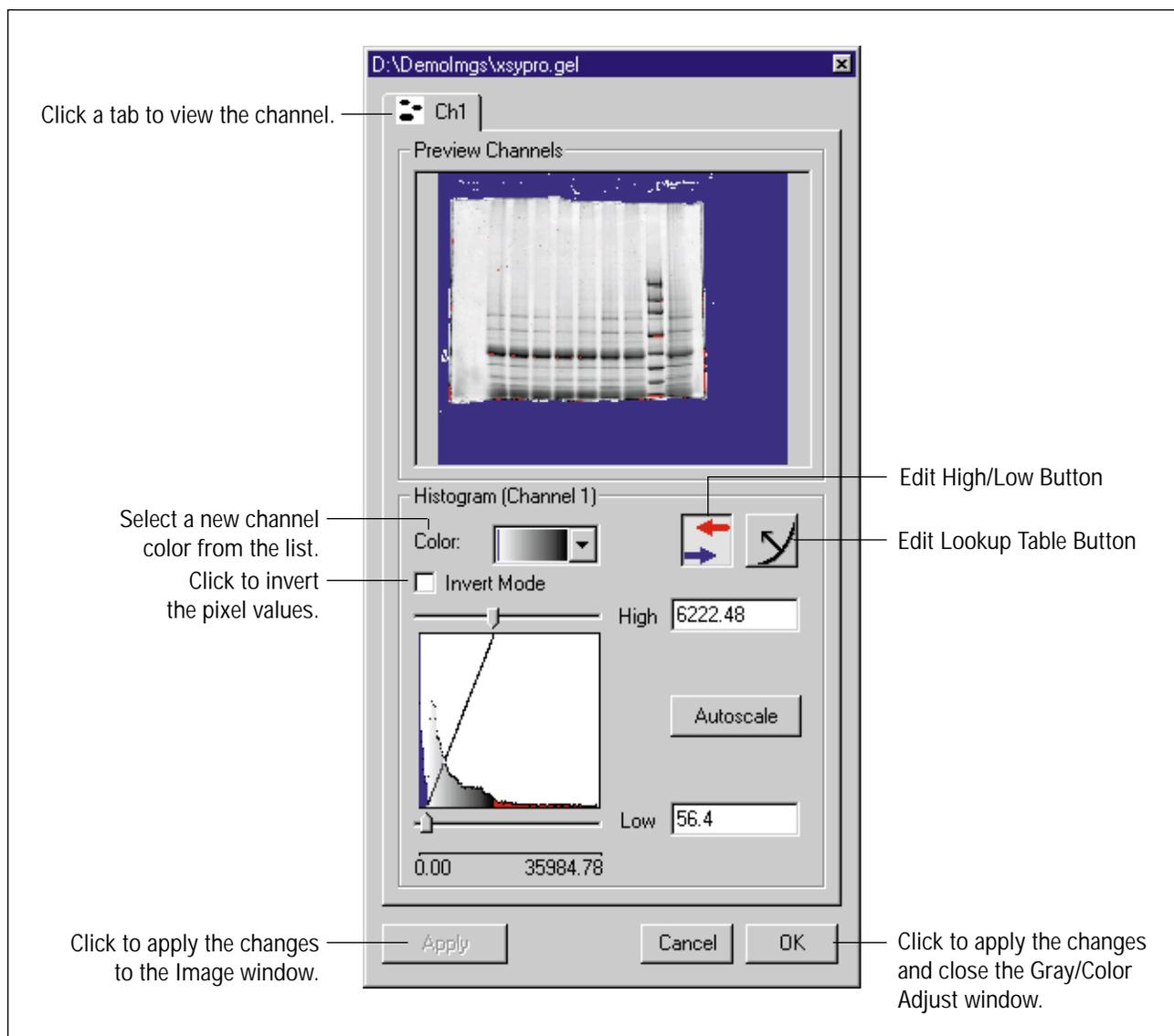
Figure 7-3. The Gray/Color Adjust window for a single image.

As you make changes in the Histogram area, the change appears in the Preview Channels area. You click Apply or OK to see the changes in the Image window and to save the changes with the image.

You can alter the image (or each channel of the image) using the following controls:

- **Color**—Change the color of the image by choosing a new color from the Color list.

- **Invert Mode**—Invert the display of the pixel values in a single image by selecting or deselecting the Invert Mode box. You can invert gray-scale and color images.

- **Edit High/Low**—Change the high and low display values to exclude background and saturated data (section 7.8.1).

- **Edit Lookup Table**—Change the image display by applying different lookup tables to the image (section 7.8.2).

For a step-by-step description of how to use the Gray/Color Adjust features, see the *ImageQuant User's Guide.*

7.8.1 Changing the High and Low Display Values

After you create an image of your sample using a Molecular Dynamics instrument, the image has no defined display range. The first time you open the image, ImageQuant Tools or FluorSep automatically sets the range of values to include the lowest and the highest data values collected by the instrument. The utility saves the display range with the image when you close the Image window.

You can change the high display values to remove saturated pixels from the image, and you can remove low display values to eliminate the background pixels.

To change a display value, drag the slider that appears above or below the histogram plot, or type a new value in the High or Low box (figure 7-4). You can use the Autoscale button to return to the scale determined by the utility. **Note:** If you do not see the High box, Low box, or Autoscale button, click the **Edit High/Low** button ( ).
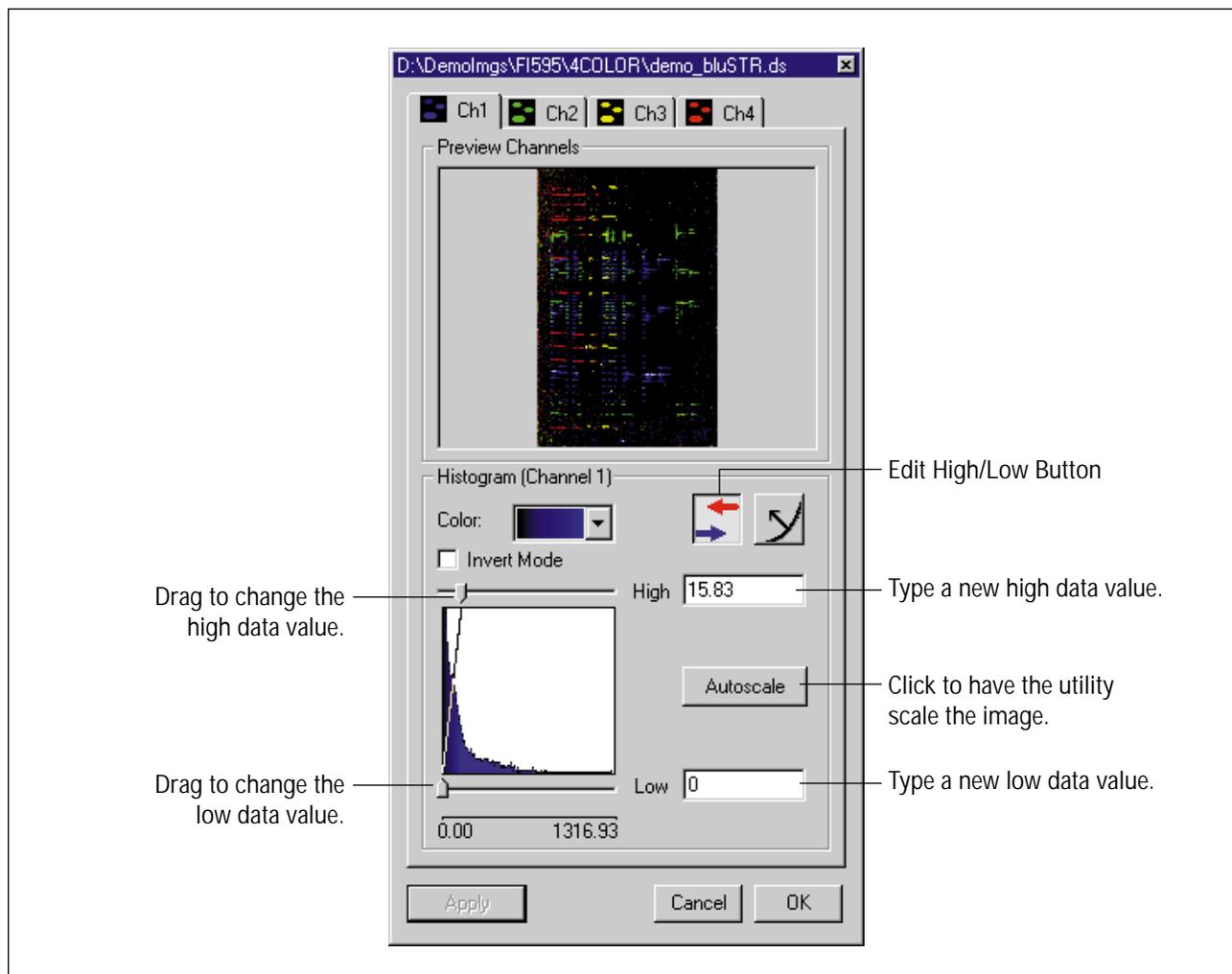
Figure 7-4. The High/Low controls in the Gray/Color Adjust window.

7.8.2 Using the Lookup Tables to Change the Display

The Gray/Color Adjust window also provides lookup table definitions that change the image display. These changes affect the image display, but not the features in the utility. To use the lookup table controls, click the **Edit Lookup Table** button ( ). The utility replaces the scaling controls with the lookup table controls (figure 7-5).
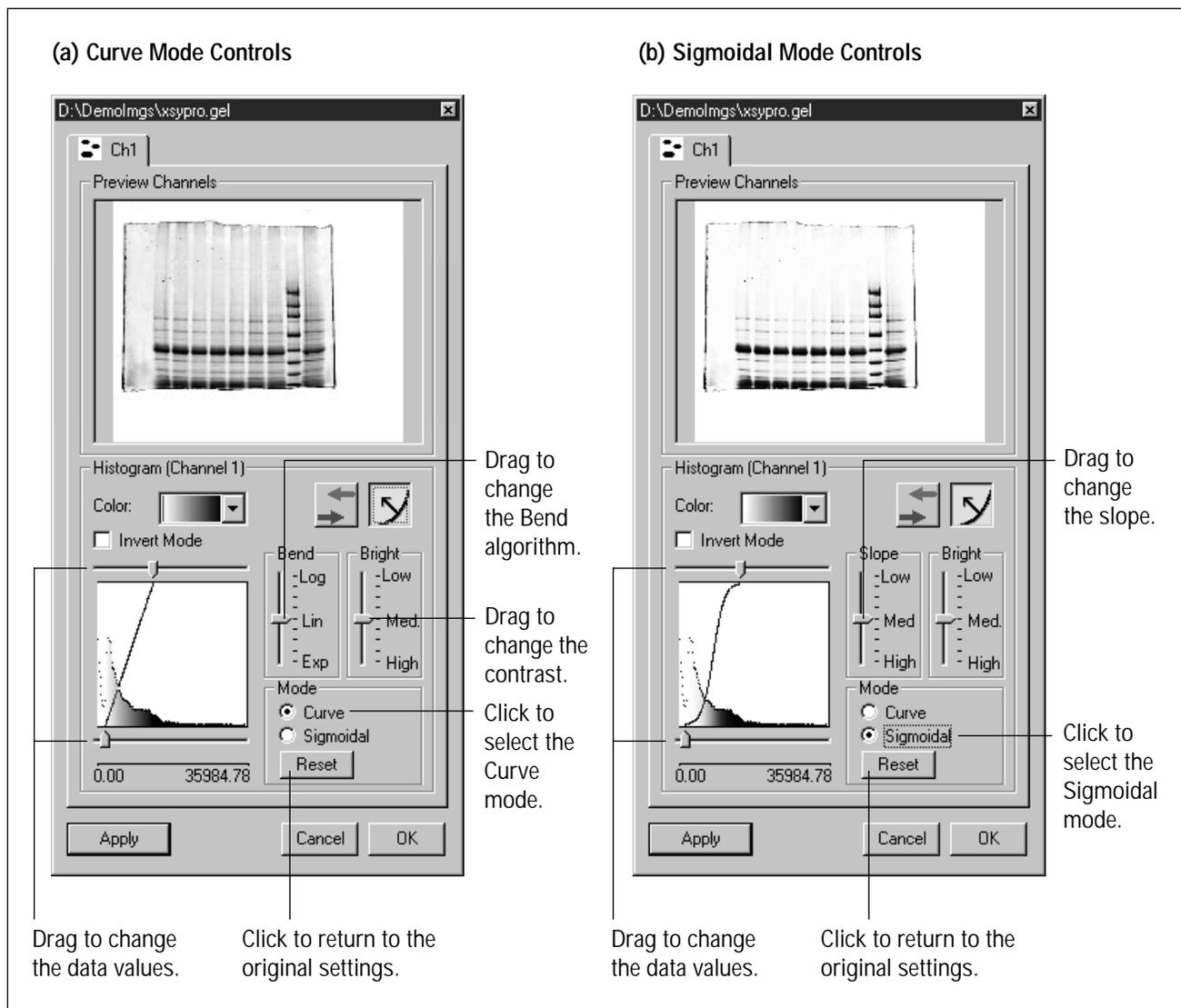
Figure 7-5. The Edit Lookup Table controls in the Gray/Color Adjust window: (a) Curve mode controls and (b) Sigmoidal mode controls.

You can change the image display using the following controls:

- **Bright Slider**—Adjusts the contrast of the image. Move the slider toward Low to decrease the contrast. Move the slider toward High to increase the contrast.

- **Curve Button and Bend Slider**—Apply logarithmic, linear, and exponential lookup table definitions to the image. Click **Reset** in the Mode area to return to the default linear lookup table definition.

- **Sigmoidal Button and Slope Slider**—Change to a sigmoidal lookup table and alter the slope of the sigmoidal lookup table definition. Click **Reset** in the Mode area to return to the default linear lookup table definition.

7.9 Map

The Map command displays the Map window, which is a smaller version of the Image window. The Map window allows you to select a different portion of the Image window for viewing. An outline depicts the area currently displayed in the Image window.

To change the image display, drag the outline in the Map window. The Image window displays the new area when you release the mouse button.

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

To move the Map window outline and the image display together, CONTROL+drag the outline. The image display changes as you move the outline.

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

- *Windows NT*—Move the pointer into the Map window. Right+click and choose **Reset** from the pop-up menu.

- *Macintosh*—Move the pointer into the Map window. SHIFT+CONTROL+click and choose **Reset** from the pop-up menu.

To close the Map window, click the **Map** command in the View menu.

7.10 Multichannel

The Multichannel command contains the display commands for multichannel images (figure 7-6). The multichannel command and toolbar are active when you display a multichannel image in the Image window.
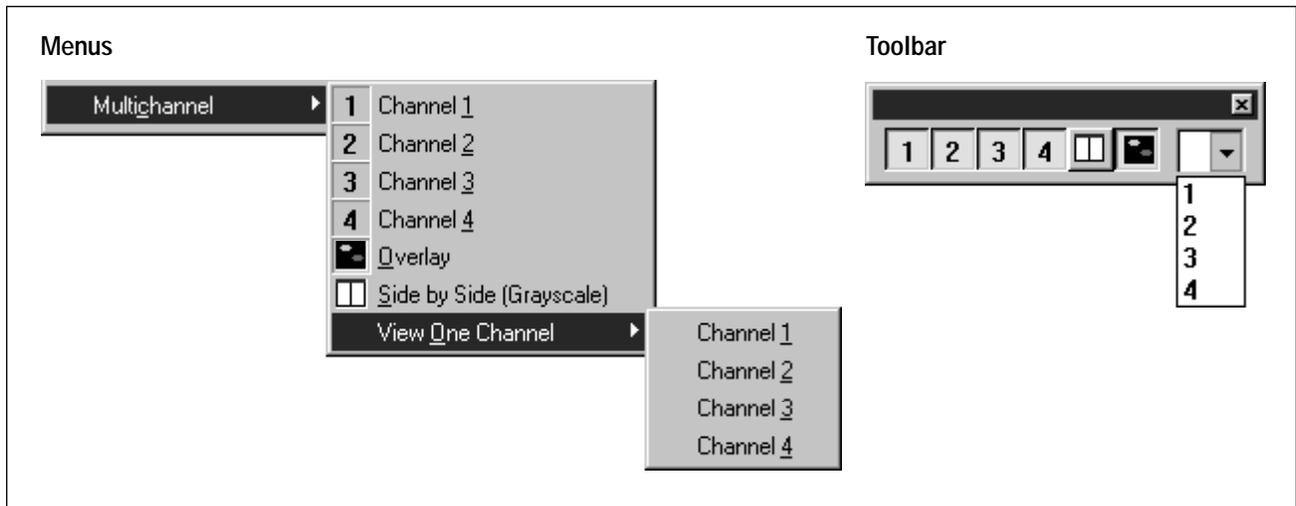
Figure 7-6. Multichannel menus and toolbar.

You can view multichannel images in the following ways:

- **Overlay**—Shows a composite image in which the separated images are displayed together. Each channel appears in a different color.

- **Side by Side**—Displays each channel in a different section of the Image window. Each channel appears in gray scale.

- **View One Channel**—Displays only the selected channel of a multichannel image. The channel appears in gray scale.

- **View a Combination of Channels**—Adds or removes the channels that you select or deselect using the menu or the toolbar.

7.11 Hide Objects (FluorSep Only)

The Hide Objects command allows you to remove the objects from the image temporarily. If a check mark appears next to the Hide Objects command, the objects are hidden. Choose the **Hide Objects** command again to remove the check mark and display the objects.

Chapter 8 Tools Menu

The Tools menu contains—

- Select (section 8.1)
- Enlarge (ImageQuant Tools Only) (section 8.2)
- Reduce (ImageQuant Tools Only) (section 8.3)
- Zoom Area (section 8.4)
- Magnifier (section 8.5)
- Pan (section 8.6)
- Pixel Locator (section 8.7)
- Pixel Distance (section 8.8)
- Define Region of Interest (ImageQuant Tools Only) (section 8.9)

8.1 Select

The Select command or button allows you to end mode commands. In FluorSep, the Select command allows you to select the objects on the image so that you can move them.

8.2 Enlarge (ImageQuant Tools Only)

The Enlarge command doubles the magnification of the image. Choose **Enlarge** and then click in the area you want enlarged. The pointer position is the center point for the enlargement. Each click doubles the magnification. Click the **Select** button ( ) to end the Enlarge mode.

8.3 Reduce (ImageQuant Tools Only)

The Reduce command reduces the magnification of the image by half each time you click the mouse button. Choose **Reduce** and then click in the area you want reduced. The pointer position is the center point for the reduction. Click the **Select** button ( ) to end the Reduce mode.

8.4 Zoom Area
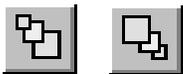
The Zoom Area commands create a stack of enlarged images that you view in reverse order. Choose **Create Frame** from the submenu, and then draw (by dragging) a box around the area to enlarge. Repeat to create additional frames. Choose **Previous Frame** from the submenu, and then click in the Image window to display the previous frame. Continue to click in the Image window to recall all the stored frames. After you view a stored frame, the utility deletes the frame. Choose **Reset** from the submenu to delete the stored frames without viewing them. Click the **Select** button (🔲) to end the Zoom Area mode but leave frames for later viewing. The utility deletes the frames when you close the Image window.

8.5 Magnifier

The Magnifier command doubles the magnification of the portion of the image directly under the pointer. Choose **Magnifier,** and then move the pointer into the Image window. The pointer turns into a magnifying glass (🔍). Move the pointer to the desired area and hold down the mouse button. A box appears displaying the enlarged portion of the Image window. Release the mouse button to remove the box.

You can select a different area for enlargement by repositioning the pointer and holding down the mouse button, or you can select an area by dragging the enlargement box around the Image window. While the enlargement box is displayed, you can increase the magnification by pressing the plus (+) key on the numeric keypad or decrease the magnification by pressing the minus (–) key.

The status bar displays the magnification of the enlargement box. It also displays the upper-left X,Y pixel coordinates (P_i) and the lower-right X,Y pixel coordinates (P_f) of the enlarged area.

8.6 Pan

The Pan command moves the image in any direction. Choose **Pan,** and then move the pointer into the Image window. The pointer turns into a hand. Drag the pointer in any direction to move the image.

When the entire image is displayed in the Image window, the Pan command is inactive.

8.7 Pixel Locator

The Pixel Locator command displays information about the selected pixel. Choose **Pixel Locator** and then move the pointer into the Image window. The pointer turns into a cross (+). Click the mouse button at the desired pixel location. The pixel information appears in the status bar. The data include the pixel coordinates (P) and the intensity value of the pixel in the measurement units of the instrument.

To add the raw pixel value to the status bar display, CONTROL +click at the desired pixel location.

You can use the Pixel Locator and Pixel Distance commands sequentially. Determine a pixel location using the Pixel Locator command, and then determine the distance between two pixels using the Pixel Distance command. Both markers are displayed in the Image window. Because the utility saves the last value for each command, you can recall the value by selecting the command again.

Click the **Select** button ( ) to end the mode.

8.8 Pixel Distance

The Pixel Distance command displays the distance between two pixels. Choose **Pixel Distance** and then move the pointer into the Image window. The pointer turns into a cross (+). Drag the pointer across the area you want to measure. The data displayed in the status bar are the pixel coordinates of the origin pixel (P_o), the pixel coordinates of the final pixel (P_f), and the distance in millimeters between the two points.

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

You can use the Pixel Locator and Pixel Distance commands sequentially. Determine a pixel location using the Pixel Locator command, and then determine the distance between two pixels using the Pixel Distance command. Both markers are displayed in the Image window. Because the utility saves the last value for each command, you can recall the value by selecting the command again.

Click the **Select** button ( ) to end the mode.

8.9 Define Region of Interest (ImageQuant Tools Only)

The Define Region of Interest command allows you to specify an area of the image to copy. After you choose the Define Region of Interest command, the pointer changes shape. Position the pointer at one corner of the area you want to copy, and then drag the pointer until the area is enclosed in a dashed box. Choose the **Copy Image** command from the Edit menu to copy the area (chapter 6).

Chapter 9 Image Menu (ImageQuant Tools Only)

The Image menu contains—

- Rotate Image (section 9.1)
- Invert Image (section 9.2)
- Impulse Filter (section 9.3)
- Median Filter (section 9.4)

9.1 Rotate Image

The Rotate Image command allows you to rotate, flip, or rotate and flip the image. See sections 4.2 and 4.3 for a complete description of the Rotate Image command.

9.2 Invert Image

The Invert Image command allows you to invert the pixel values in the image. Inverting the pixel values changes a black background to white. See section 4.4 for a complete description of the Invert Image command.

9.3 Impulse Filter

The Impulse Filter command allows you to remove the background noise from the image. See section 4.5 for a complete description of the Impulse Filter command.

9.4 Median Filter

The Median Filter command allows you to remove bright noise spikes from the image. See section 4.6 for a complete description of the Median Filter command.

Chapter 10   Window Menu

The Window menu contains—

- Cascade (section 10.1)
- Tile Horizontal (section 10.2)
- Tile Vertical (section 10.3)
- Close All (section 10.4)
- List of Open Windows (section 10.5)

10.1 Cascade

The Cascade command overlaps multiple windows. The title bar for each window is displayed and accessible. To bring a window to the top of the stack, click anywhere in the window.

10.2 Tile Horizontal

The Tile Horizontal command displays all the windows horizontally. The windows are reduced to fit the tiled arrangement.

10.3 Tile Vertical

The Tile Vertical command displays all the windows vertically. The windows are reduced to fit the tiled arrangement.

10.4 Close All

The Close All command closes all open windows. You are prompted to save changes to the files.

10.5 List of Open Windows

A list of the open windows appears at the bottom of the menu. You can select a file name from the list and bring the window to the front if it is hidden or difficult to locate.

Chapter 11   Help Menu

The Help menu contains—

- Index (section 11.1)
- Using Help (section 11.2)
- Molecular Dynamics Home Page (section 11.3)
- About FluorSep (section 11.4)
- About ImageQuant Tools (section 11.5)

11.1 Index

The Index command opens an online Help window containing a list of topics. Reference material is provided for each topic. Click the desired topic to access the reference material.

11.2 Using Help

The Using Help command provides tips on how to use online Help.

11.3 Molecular Dynamics Home Page

If your ImageQuant workstation contains an Internet connection, you can use the Molecular Dynamics Home Page command to go directly to the Molecular Dynamics Home Page (www.mdyn.com).

11.4 About FluorSep

The About FluorSep command displays the version number of the FluorSep software you are using and displays copyright information. The window also contains contact information for Molecular Dynamics.

Note: The About FluorSep command on a Macintosh computer is located in the Apple menu.

11.5 About ImageQuant Tools

The About ImageQuant Tools command displays the version number of the ImageQuant Tools software you are using and

displays copyright information. The window also contains contact information for Molecular Dynamics.

Note: The About ImageQuant Tools command on a Macintosh computer is located in the Apple menu.

Part Five

Appendixes

Appendix A Quick Reference for Windows NT

Keyboard Shortcuts

Menu	Key	Function
File menu	CTRL+O	Open
	SHIFT+F2	Image Properties
Edit menu (ImageQuant Tools Only)	CTRL+SHIFT+C	Copy Image
View menu	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	CTRL+S	Select
	CTRL+R	Define Region of Interest
Window menu	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
Magnification	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

Toolbar Functions

Main Toolbar

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Open

Multichannel Toolbar

Overlay

Side by Side

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Channel 3

Channel 2

Channel 1

View One Channel

Image Toolbar

Define Region of Interest

Gray/Color Adjust

Map

Fit to Window

Magnifier

Previous Frame

Create Frame

Pixel Distance

Pixel Locator

Select

Pan

Extensions that Identify File Types

The following extensions identify the types of files ImageQuant Tools and FluorSep create:

.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.

Appendix B   Quick Reference for Power Macintosh

Keyboard Shortcuts

Menu	Key	Function
File menu	O	Open
	SHIFT+F2	Image Properties
	Q	Quit
Edit menu (ImageQuant Tools Only)	C	Copy Image
View menu	F9	Zoom In
	SHIFT+F9	Zoom Out
	F8	Gray/Color Adjust
	CONTROL+1	Multichannel/Channel 1
	CONTROL+2	Multichannel/Channel 2
	CONTROL+3	Multichannel/Channel 3
	CONTROL+4	Multichannel/Channel 4
Tools menu	CONTROL+S	Select
	CONTROL+R	Define Region of Interest
Window menu	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
Magnification	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
	CONTROL+click	Move Map window in sync with Image window

Toolbar Functions

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Icons that Identify File Types

The following icons identify the types of files ImageQuant Tools and FluorSep create:

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.

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