Chapter 3 Windows and Technical Details

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Chapter Overview

This chapter provides more detailed information about the following:

- file formats used in NCSA GelReader
- operations one may perform on a gel image
- analysis of the gel
- various window types and the information they convey

Formatting Your Image Files

Before you can use NCSA GelReader, the gel image data must be in a form that the program understands. You may use either PICT or raw binary format for the images; both options are contained in GelReader's directory dialog box. The file format for palette files is also described in

this section.

PICT Files

NCSA GelReader can read digitized images from PICT files. If your scanner has the capability to store images in PICT format, you will probably want to take advantage of that option. By doing so, you won't have to enter image dimensions since they are included in PICT format. Use an 8-bit scanner for best results.

Raw Binary Files

A raw binary file is a stream of raw, 8-bit binary data in row-major order. Each 8-bit byte corresponds to a pixel in the image. The image is represented in *row-major order*; that is, the first raster line appears first in the file, succeeded by the next raster line, and so forth. Though raw binary files are easy to create, the raw binary file format is not very flexible and therefore, not highly recommended.

If you choose to use raw binary files to store your image data, you must specify the dimensions of the dataset before the file can be read by NCSA GelReader.

To display your image, NCSA GelReader must be able to determine the dimensions of the image. A simple way to specify these dimensions is to save them as part of the filename of the dataset, using the following convention:

filename*(*horizontal valuevertical value*)**

where * is any non-numeric character. For example, to save a dataset that has x and y dimensions of 400 and 365, respectively. In a file named gel.one, you would enter the following command:

```
gel.one(400x365)
```

If the dimensions are not provided by the filename, the default dimensions provided with the application are used. You can change these built-in defaults using the Change params dialog box in the Operations menu. For more information on changing parameters, see the section, "The Operations Menu" in Chapter 2.

Raw Palette Files

A *raw palette file* is a stream of 768 bytes. Raw palette files can store 256 colors, which can be selected from a palette of over 16 million possible colors. Palette files are based on the red, green, and blue representation of color, the RGB color model. The files consist of, in order, 256 bytes of red, 256 bytes of green, and 256 bytes of blue. The 256 color palette entries are calculated by combining the nth element (red), the (n+256)th element (green), and the (n+512)th element (blue) to create the nth RGB component.

In other words, a *raw palette file* is a lookup table with 256 entries that tell which color to associate with each of the 256 possible *pixel* values. Each of the 256 palette entries in the palette is chosen from a master palette of 2^{24} RGB colors. Each palette entry consists of three values, one

each for red, green, and blue; the first red component, the first green component, and the first blue component, for example, comprise the first palette entry.

When you load a palette file, the hardware color table entries are remapped according to the new palette; that is, each color in the palette file is assigned to its corresponding entry in the hardware color table.

NOTE: The data values for user-defined palettes must be within the range of 0 to 255; however, the Macintosh reserves the palette values 0 and 255 for white and black, respectively. Consequently, you may have to scale your data to the range from 1 to 254, for NCSA GelReader overrides other assignments to 0 and 255 with white and black, respectively.

Setting Up Defaults

Many changes that you make to the GelReader program are permanent. They do not change between GelReader sessions; they only change when you explicitly change them. This characteristic holds true for changes you make in the following: (1) check boxes and radio buttons in the directory dialog box, (2) parameters dialog box, and (3) the macros defined using the notebook. Note that even though the land and band tracking sensitivities in the parameters dialog box are permanent, you will probably adjust them often, depending on the characteristics of the gel you are analyzing.

Gel Analysis

The process of analyzing a gel is complicated by many factors: variable contrast within images, tracking curved lanes, and finding faint bands are just a few. In this section, the techniques used to find lanes, bands and generate a report are described, and the problems associated with these functions are noted.

Searching for Lanes

Lanes are identified as a series of lines connecting knots that were found to be roughly vertical to one another. The program identifies the lanes by first finding the knots. A *knot* is a control point that is located GelReader searches the image for variations. To summarize, the GelReader program finds the knots by doing the following:

1. Performs a horizontal edge detection on the image. This process reveals where the knots begin on the left-hand side (*leading edge*) and where they end on the right (*trailing edge*).

When a leading and trailing edge are found, the area between them is identified as a knot. After the knots are identified, the program continues in the following way:

2. Scans the knots and tries to line them up in as nearly a vertical orientation as is possible.

The *center of the lane* is defined by the centers of the vertically aligned knots; the right and left boundary of the lanes are identified as the area between the right and left sides of the knots; that is, the leading and trailing edges, respectively.

Searching for Bands

Bands are identified by taking a histogram down the lane. This is effectively the same as the lane profile that you can display. The program calculates the histogram using the following two-part process.

1. First it calculates a list of median values of each row in a lane between the boundaries of the lane (defined above). Next, GelReader smooths the histogram by taking the average of each value and its preceding and succeeding value in the list.

2. After the histogram is calculated, GelReader searches it for peaks. A *peak* is any value that is greater than the local minimum by a value that the user selects by adjusting the band tracking sensitivity. In addition to height, the band is expected to have at least one very sharp edge. The combination of these two criteria are used to identify a band.

Generating Reports

A report may take one of two forms. It may consist of the molecular weights of all the bands in the lanes that are not standards, or it may only consist of the percentage of the distance down a lane a fragment has traveled.

Distance Report

If you select the Report distances check box in the directory dialog box, your report will consist of distances rather than weights. In order to report distances traveled, you must specify landmarks for the top and bottom of the image. These *landmarks* serve as boundaries used to calculate the percentage distance traveled. For more information on how to place landmarks, see the section titled, “Add land marks,” in Chapter 2.

Once you have placed the landmarks, you may generate the report. The report looks just like the molecular weight report, except that the molecular weights are replaced by percentages between 0 and .9999, assuming that all the bands fall within the landmarks (see Figure 1.16).

Molecular Weight Report

After entering the information required for the standard lanes, you may generate a report of the approximate molecular weights of the bands.

NCSA GelReader uses standard lanes to estimate and interpolate the molecular weights of the bands it finds in the lanes that appear between the standards in the image. NCSA GelReader needs to know which lanes are standards and what the weights of the bands in these lanes are in order to generate a molecular weight report. Methods for defining the standards are described in this chapter, in the section, “The Notebook.”

NCSA GelReader calculates a *spline curve* for each of the standard lanes,

and then uses interpolation to determine the values of the bands in the lanes between any two standards.

Lane Profiles Window

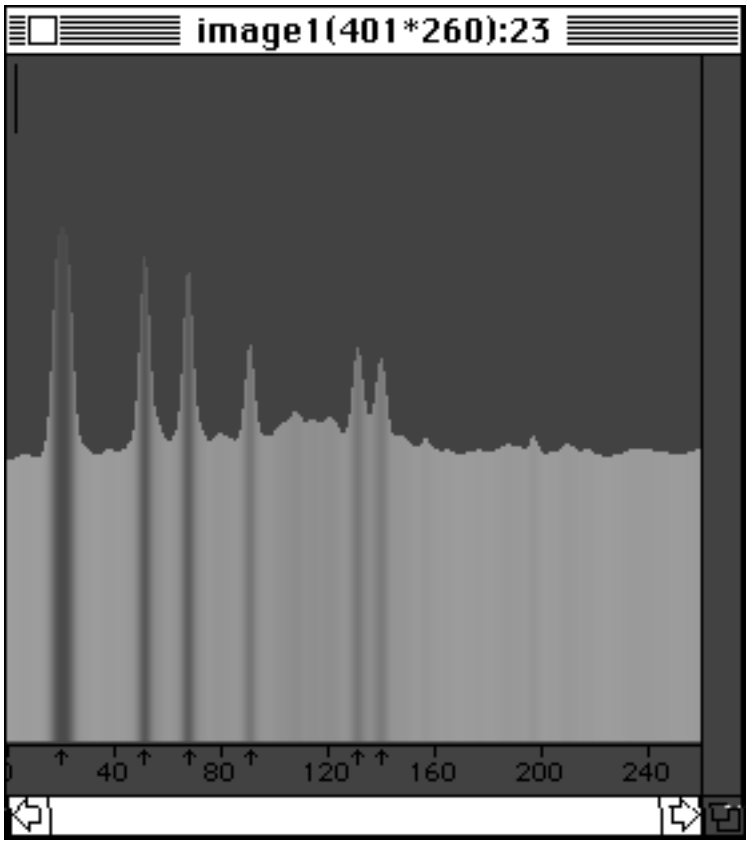
In addition to the gel image window, a lane profile window and a notebook window(s) can also be displayed.

GelReader has the ability to calculate and display a histogram of the values down a lane. This display is referred to as a *lane profile*. To calculate a lane profile:

1. Select the Histogram of lane option from the Tools menu.

2. Use the mouse to select a lane by placing the mouse over the lane and clicking the button. The lane profile for the selected lane is then displayed as shown in Figure 3.1.

Figure 3.1 Lane Profile



The small arrows at the bottom of the window indicates the positions of restriction sites down the lane. Note that a scale is prominent along the bottom of the profile. This scale reflects the vertical offset down the lane.

The window's title bar reflects the name of the gel image from which the profile was taken, as well as the lane number.

You can resize the window, but only the horizontal size can be changed. You also can scroll the histogram in the horizontal direction.

By using the histogram window, you may place new bands in lanes. To do so:

1. Depress the mouse button while the mouse is in the lane profile window. A vertical line appears in the window.

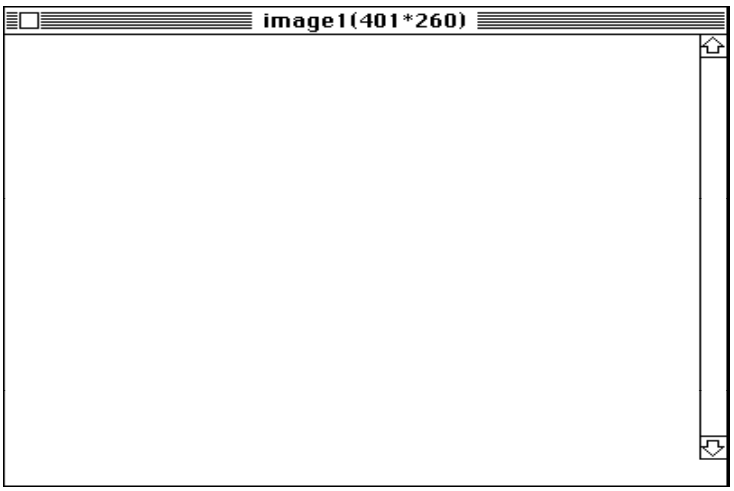
2. Release the mouse button at the horizontal location where you would like the band placed.

The horizontal position of the vertical line in the lane profile window determines the offset down the lane where the band is placed when you release the mouse button. As you move the mouse while still depressing its button, its relative position is indicated in the gel image by a horizontal bar. This bar travels up and down the lane as you move the mouse back and forth across the lane profile. When you release the mouse button, you will see the band appear both in the gel image and in the lane profile.

Notebook Window

The *notebook* allows you to configure standard lanes and define macros. The notebook appears as a typical text window as shown in Figure 3.2.

Figure 3.2 The Notebook



There are a number of commands that facilitate the entry of standards and the definition of macros. Among these are commands which (1) allow you to assign standards to lanes, (2) list the standards currently assigned to a gel, and (3) define and list macros. Descriptions of the available commands are listed below.

Standards Commands

Defining Standards

The following command syntax assigns a list of molecular weights to a numbered lane:

std(*Lane#*) = *MOLWT1,MOLWT2,...MOLWTn*

where Lane# is replaced by the number of the lane, and the MOLWTs are the list of molecular weights assigned to the bands in that lane. The command,

```
std(1) = 23134,9415,6553,4361,2322,2027,565
```

for example, assigns 23134 as the molecular weight of the first band, 9415 as the weight of the second band, etc., and 565 as the weight of the last band in the first lane.

Listing Weights

To list the weights assigned to a lane:

1. Enter the following command in the notebook:

   std(*Lane#*)

2. Select Do notebook from the Operations menu.

The list of molecular weights appears on the same line after the "=." For instance, after executing steps 1 and 2 above, this example would appear as follows:

```
std(1) = 23134,9415,6553,4361,2322,2027,565
```

Notice that this is an executable command.

Purging Standards

To purge the standard line assignment, enter:

std(*Lane#*) =

Assigning Macro Standards to a Lane

With NCSA GelReader, you can also assign the contents of a macro to a lane in lieu of listing all of the molecular weights. One way to do this is to enter the std command followed by the macro name.

std(*Lane#*) = *MacroName*

For instance, if you were to define a list of molecular weights with the macroname `lambda`, and assign that macroname to `std(1)`, you would enter the command as follows:

std(1) = lambda

Defining Macros

It quickly becomes tedious to enter the molecular weights for the bands in the standard lanes, particularly for standards that are used frequently. For this reason, the ability to define macros that name commonly used groups of molecular weights has been included. A *macro,* as previously mentioned, is a word used in place of a list of molecular weights. In addition, GelReader allows you to select lanes as standards. See Chapter 2 the section, “The Tools Menu” for more information on this technique.

To define a macro, enter the macro name followed by an equal sign which is in turn followed by a list of molecular weights. For example,

```
BRL_RNA=9490,7640,4400,2370,1350,240
```

defines a macro whose handle (identifying name) is BRL_RNA and contains the list of molecular weights 9490,7640,4400,2370,1350 and 240.

Purging Macros

To purge this macro, enter the handle followed by an equal sign. For this example, the purge command would appear as follows:

1. Enter the handle:

   Handlename=

This process is similar to the method of purging a list of standards assigned to a lane.

Listing Macros

To list all the macros you currently have defined, do the following:

1. Enter the keyword:

   Macros

2. Select Do notebook from the Operations menu. A list of all the defined macros and the molecular weights associated with them appears.

Listing Standards and Weights

To list all of the standards that have been assigned to a gel and the weights of the bands in those lanes, do the following:

1. Enter:

   std

2. Select Do notebook from the Operations menu.

Getting Help

Help is built into the notebook command language, and is used to describe the built-in commands. To get help, execute the steps below:

1. Enter

   ```
   Help
   ```

2. Select Do notebook from the Operations menu to see the list of commands.