Chapter 2 The NCSA GelReader Menus

Chapter Overview

The NCSA GelReader Menus

- The File Menu
- The Edit Menu
- The Operations Menu
- The Display Menu
- The Tools Menu
- The Filters Menu
- The Standards Menu

Chapter Overview

This chapter serves as a reference guide to the menu contents. The menus are treated in the order that they are presented in the menu bar.

The NCSA GelReader Menus

The File Menu

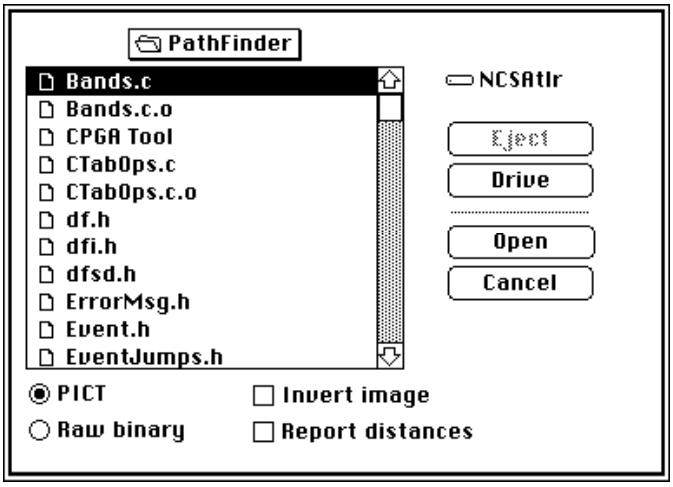
The *File menu* (Figure 2.1) contains six important options: Open, Close, Save as, Page setup, Print, and Quit. Each option is outlined below.

Figure 2.1 File Menu

Open

The *Open* option allows you to open and read a file containing a gel image. The image may be stored in either PICT or raw binary format as described in Chapter 3, “Windows and Technical Details.” You select the format by activating the radio buttons in the directory dialog box (Figure 2.2).

Figure 2.2 Directory Dialog Box



You may specify other characteristics of the gel by activating the check boxes in the directory dialog box. The options are discussed below:

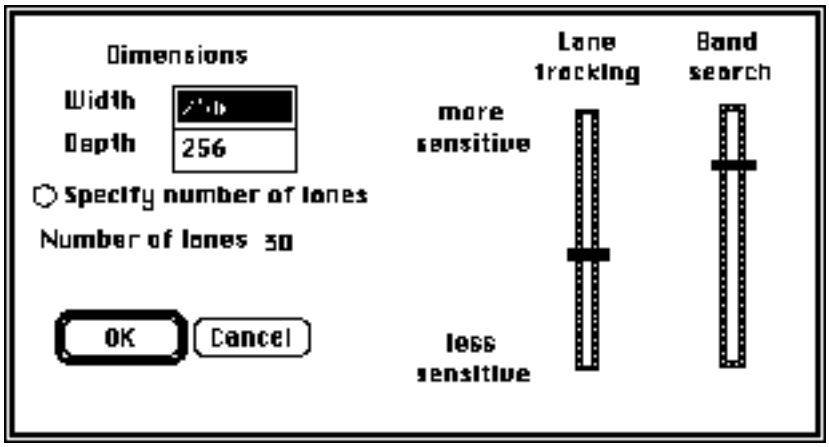
- *Invert image* should be checked if bands are represented by lighter rather than darker shades.

- *Report distances* indicates the report form to be generated. If this item is checked, only the percentage of the distance down the length of the lane is reported. Conversely, the molecular weights are reported.

If the file you’ve opened is a raw binary file, the parameters dialog box

appears (Figure 2.3). The two sliders on the right allow you to adjust the lane and band search sensitivities. Use the editable text boxes in the upper left-hand corner to enter the gel image dimensions. If you click the button labeled, Specify number of lanes, the Number of lanes field becomes editable so that you may enter a number for the number of lanes that would fit in the image.

Figure 2.3 Parameters Dialog Box



NOTE: GelReader needs to be able to determine the approximate width of the lanes. For this reason, you should enter the number of lanes that would fit in the gel, not the number of lanes that are actually there. This number can be a rough guess.

When you are done changing the parameters simply click the OK button, and the image will appear in a new window. To abort the operation, simply click on Cancel.

Close

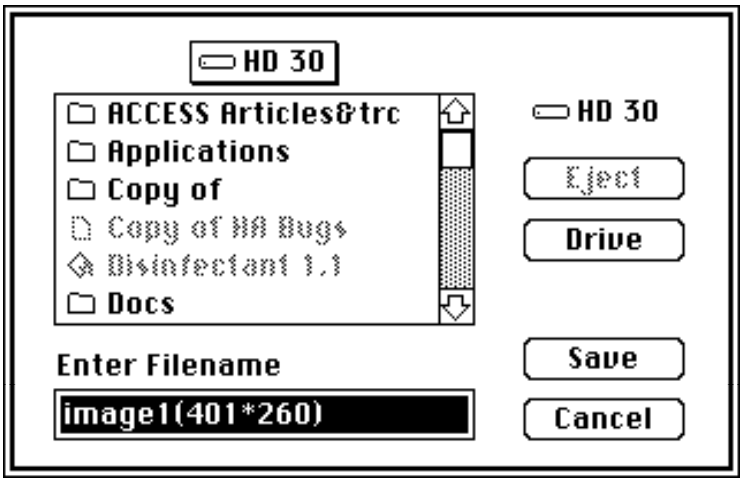
The *Close* function closes the frontmost window. It makes no difference if the window is a lane profile, notebook or gel image window. If the frontmost window is the gel image, all associated windows (notebook and lane profiles) are also closed.

Save as...

The *Save as* option allows you to save your gel image and the results of any trimming you've applied to it. When you save your image, a filename dialog box appears as shown in Figure 2.4. In the text box labeled, "Enter Filename," enter the filename that will contain your image. Note that the default filename provided contains the saved image's dimensions. For this reason, if you change the filename, you'll probably want to leave the dimensions in the name.

NOTE: If the invert image check box was checked when the image was loaded, the data will be saved in its inverted form and you will not need to select Invert image again.

Figure 2.4 Filename Dialog Box



Page setup and Print

Page setup and *Print* provide you with the capability to print the gel images to a laserprinter. If the correct version of the printer drivers are being used, the image is printed using half-tones. If a color printer is available, the image is printed in color.

Quit

The Quit command permits you to quit the GelReader application.

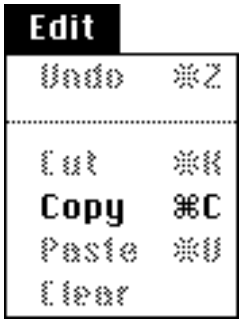
The Edit Menu

The options available for use in the *Edit menu* depend upon the type of window that appears at the front of your screen.

When a gel image window is in front, the only active option in the Edit menu is the *Copy* option (see Figure 2.5), which allows you to copy the gel image to the clipboard. You may then paste the image into another package for continued processing.

When the notebook window is in front, all of the Edit menu options are available for use except the Undo option.

Figure 2.5 Edit Menu



The Operations Menu

The elements of the *Operations menu* (Figure 2.6) facilitate the analysis of the gel image. Operations such as searching for bands and lanes, generating reports, and changing the parameters (sensitivity) for the searches are included. The notebook is supported by menu items here as well.

Figure 2.6 Operations Menu

Find lanes

The *Find lanes* mode initiates a search to find the lanes. The procedure for doing this is described in Chapter 3 in the section called “Searching For Lanes.” If the image is not properly trimmed or the area of interest has not been defined correctly, the results of the lane search are less likely to be accurate.

Find bands

The *Find Bands* option searches for bands in defined lanes. To initiate a band search, select Find bands from the Operations menu or enter -A. The algorithm used in the bands search is described in the section “Searching For Bands” in Chapter 3, “Windows and Technical Details.”

Change params

The *Change params* option allows you to change the following items: (1) the parameters associated with the band and lane searching, (2) the default image size (for reading raw binary files) and (3) the number of

lanes. The parameters dialog box permits you to change and review these parameters. (Refer to Figure 2.2.)

The dimensions in the upper-left hand corner of the parameter dialog box are the default dimensions of raw binary images.

NOTE: If you are in the process of loading a raw raster file when this dialog box appears, the default dimensions will be used as the dimensions of the gel image.

If you are using PICT files, you need not worry about the contents of these fields.

To specify the number of lanes in the gel, click the button below the dimensions. Once you select this option, the numeric field labeled Number of lanes is made editable, enabling you to enter the number of lanes that would fit in the gel image.

The sliders on the right-hand side of the box control the sensitivity of the searching algorithms. The *Lane tracking* slider on the left controls the resolution of the lane tracking algorithm, while the right slider, *Band search*, appropriately controls the band search. As you move the slider up the search becomes more sensitive; as you move it down, it becomes less sensitive.

When you are done, click OK to accept the new parameters; click Cancel to abort.

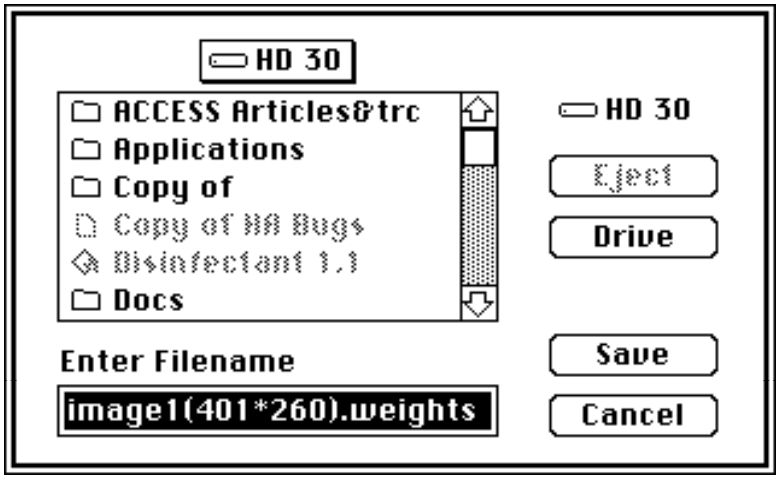
Generate report

After you have found the bands and lanes, you can generate a report of the results. If you checked the Report distances box in the directory dialog box (Figure 2.2), then the report will consist of the relative distances between landmarks.

If the Report distances box is not checked, you must first set up standard lanes using the notebook (see Chapter 3, in the sections, “Standards Commands and Macro Commands”) or Select standards tool from the Tools menu. Refer to the section entitled, “Select Standards,” in this chapter for more details.

After you have set up the standards, select Generate report from the Operations menu. A output file selection dialog box appears as shown in Figure 2.7.

Figure 2.7 Output File Selection Dialog Box



Enter the name and select the directory in which you would like to store your report. The resulting file will contain the calculated molecular weights or the percentage distance traveled. More information on this subject can be found in Chapter 3 in the section “Generating Reports”. A sample report is shown in Figure 2.8.

Figure 2.8 Sample Molecular Weight Report

```
Report for HD40:Edit.Docs:GelReader:image1(401*260)
Date : 07/11/1989 04:30:04

        32 Lanes found:

        Data as follows:

Lane#     Band#     Molecular weight    Height
_____________________

  2         1              8942           38
  2         2              4099           55

  3         1              9455           30
  3         2              4265           41

  4         1              8825           47
  4         2              4039           33

  5         1              9884           59

  6         1              9476           51
  6         2              4261           45

  7         1              9483           30
  7         2              4243           46

  8         1             20811           52
  8         2             10105           49
```

8	3	6621	34
9	1	8755	66
9	2	4041	39
10	1	7271	66
11	1	8920	48
11	2	3991	63
12	1	9929	42
13	1	7291	58
14	1	7138	64
15	1	9858	64
17	1	9027	63
17	2	4113	55
18	1	7254	69

Figure 2.8 Sample Molecular Weight Report (Continued)

19	1	8894	64
19	2	4039	−1
20	1	9578	64
21	1	7261	59
22	1	7297	62
23	1	9456	66
25	1	8918	58
25	2	4035	51
26	1	7239	37
27	1	8578	32
27	2	3974	31
28	1	7341	44
29	1	7240	67

30	1	7202	73
31	1	7276	66
31	2	4141	70

Notebook

The *Notebook* option allows you to display the notebook window. You can use this window to enter the molecular weights for the standards lanes or create macros. More information on the notebook window is available in Chapter 3.

Do notebook

Use the *Do notebook* option to execute a command from the notebook window. The executed command will consist of either all selected text or only the highlighted portion of a line containing the cursor.

For example, if your cursor rests on a notebook line labeled `HelpMacro`, and you press RETURN, the `HelpMacro` command (all selected text) is executed. However, if you select only the word `Help` in that line, which is a command in itself, and press RETURN, only the `Help` command is executed.

The available notebook commands are described in Chapter 3.

The Display Menu

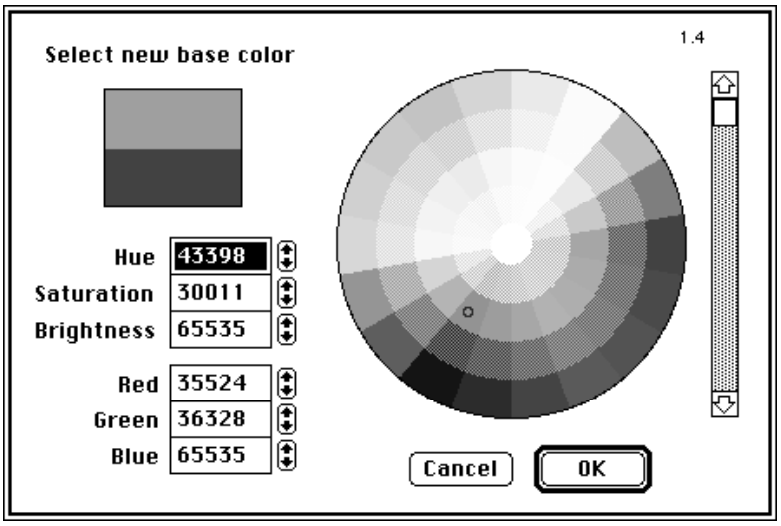
The *Display menu* (Figure 2.9) contains items that manipulate the display characteristics of the gel image. Magnification, lane and band indicator colors, and the gel image colors are included.

Figure 2.9 Display Menu

Set color

The *Set color* option allows you to select a color to be used to delineate lanes and bands. You choose a color by utilizing the color wheel dialog box shown in Figure 2.10.

Figure 2.10 Color Wheel Dialog Box



The box on the left above the color values contains two colors. The one filling the top half of the box is the selected color, the color in the bottom half is the current color. The editable values below this box allow you to enter the values for either hue, saturation, and brightness (HSV model) or the values for red, green, and blue (RGB model). You may also select the color from the color wheel. When you use this method, the text boxes displays the current values for each of the models. The scroll bar on the right allows you to modify the brightness of the colors displayed.

To select a color from the color wheel:

1. Depress the mouse button while in the color wheel; the color under the cursor becomes selected.

   The color in the top half of the color box changes as you move the mouse within the color wheel while holding down the mouse button. In other words, as you move the cursor, each individual color passed over is reflected in the top display window. If you release the mouse button, a new color is selected.

2. Release the mouse button when the color you wish to display appears in the box.

3. Click on OK when you have selected the color that you want, or Cancel to abort. If you click on OK, the color used to draw the lines for lanes and bands is changed.

Magnify

The *Magnify* option simply magnifies the image by a factor of two. The option is a toggle: when you select it a second time, the image reverts to a magnification of one.

Color tables

The elements of this submenu are just a list of available *color tables.* Select any item in this submenu, and you will see an immediate change in the colors used to display the gel image.

Load palette

The *Load palette* option permits you to load a *palette* from a raw binary palette file. To do so:

1. Select Load palette from the Display menu. A directory dialog box appears.

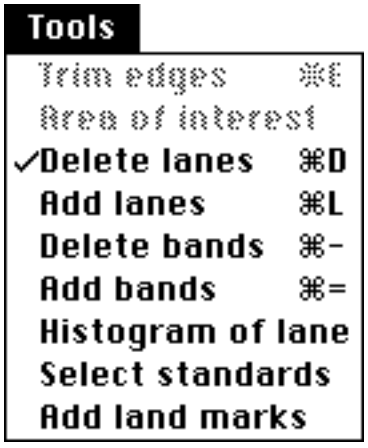
2. Select the desired palette file to load and click the button Open.

The raw binary palette file format can be written by the public domain program NCSA PalEdit. This file format is described in the "File Formats" section of Chapter 3.

The Tools Menu

The *Tools menu* (Figure 2.11) contains tools available for performing such functions as trimming the image, identifying landmarks for distance reports, selecting an area of interest, adding and deleting lanes, adding and deleting bands and extracting histograms of the lanes. Any of these *mouse action tools* are activated once you select the associated option in the Tools menu.

Figure 2.11 Tools Menu



Trim edges

The *Trim edges* mode is probably the first option you will use on your gel image. Use this mode to discard any of the gel image that is not required for the analysis. To do so:

1. Depress the mouse button in the upper left-hand corner of the rectangle you want to retain.

2. Drag the mouse to the lower right-hand corner of the rectangle while holding down the button.

3. Release the button. Anything outside of the selected rectangle is discarded; only the contents of the rectangle remains on the screen.

Area of Interest

The *Area of Interest* mode allows you to select a rectangular area from the image on which data analysis is performed. Unselected areas remain unchanged, and are ignored when searching for lanes and bands. Areas surrounding, but not a part of the actual gel (e.g., the gel's backdrop), are included. However, to improve the quality of analysis, these background areas should not be included. To use this tool, execute the following steps:

1. Depress the mouse button at the beginning point of the area you would like to analyze.

2. While holding down the button, drag the mouse to the lower right-hand corner of the rectangle.

3. Release the button. A red line outlines the rectangular area you have chosen.

4. Proceed with the desired analysis modes and operations you want performed on this area.

Delete lanes

GelReader may indicate lanes where none exist. You can delete these erroneous lanes in the *Delete lanes* mode by placing the cursor over the lane and clicking. The lane nearest the cursor is deleted.

Add lanes

The *Add lanes* mode works much like the Add land marks option. To select the points that fall in the path that a lane is to follow, click on them once. To select the last point, double-click on it.

Delete bands

The *Delete bands* mode operates in one of two ways:

1. If you click on a band, the band is deleted.

2. If you press and hold down the mouse button, you can drag out a rectangle as you did in the Trim edges mode. When you release the mouse button, all the bands inside of this rectangle are deleted.

Add bands

The *Add bands* mode permits you to add bands to your gel. To do so, place the cursor over the band and click. It may be more accurate to place bands using the histogram window (see Chapter 3).

Lane profile

In the *Lane profile* mode, you can display lane profiles. As with the Delete lanes option, simply move the cursor over the lane and click the mouse button. A new window is created which displays a histogram of the lane profile. The value of each element in the lane is represented by the height of the histogram at the associated offset as well as by the corresponding color.

A *histogram* is a graphic representation of the magnitude of each element in the lane. It consists of columns whose heights correspond to the magnitude of the values being represented.For more information on the Histogram of lane window, see Chapter 3, “Lane Profiles.”

Select standards

The *Select standards* option allows you to select lanes to be standards using a point and click method. To use this method, activate the gel image window. Continue with the following steps:

1. Choose Select standards from the Tools menu. The Standards menu, which contains a list of all defined macros, appears in the menu bar, and the cursor becomes a select standards tool.

2. Select the desired standard from the Standards menu.

3. Assign a standard to a lane by positioning the cursor on it and clicking the mouse button, just as you did to delete a lane. Note that you must first define some macros before you can use this method. See the following section on defining macros.

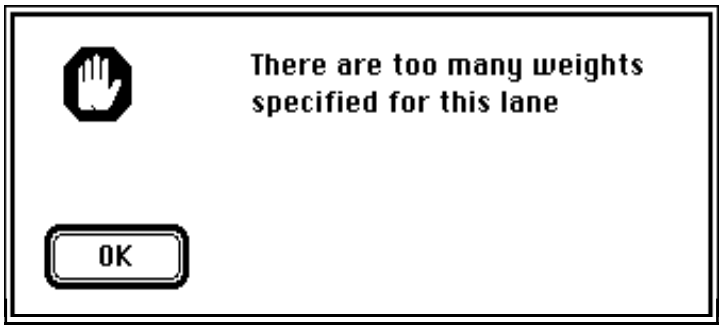
The standard assigned to the lane is the one associated with the item currently checked in the Standards menu.

To add commonly used standards to the Standards menu, you can create macros as described in the notebook section of Chapter 3.

Problems

When assigning lanes as standards, you may run into some trouble. For instance, if you select a lane that does not correspond to the number of weights and bands in the currently selected item (macro/standard), an error message like the one in Figure 2.12 appears.

Figure 2.12 Weights Error Message



Solutions

Below are some possible steps you can follow to correct this situation.

1. List the macros available. (See Chapter 3 the section “Listing Macros.”)

2. Review the macro associated with the menu selection that you would like to then assign. The number of weights displayed must match the number of sites in the lane.

3. Add more bands manually, change the standard, or define a new set of standards in the notebook if the number of weights doesn’t match the number of sites. (For more information on adding bands manually, refer to Chapter 1 the section, “Adding and Deleting Lanes and Bands.” For information on defining macros, refer to Chapter 3 the section, “Macro Commands.”)

Add land marks

Add land marks is a tool that allows you to insert horizontal landmarks which serve as boundaries in your gel image. When you enable Report distances in the directory dialog box (Figure 2.1), you need to place landmarks at the top and bottom of the image. The distances for sites are reported as the percentage of the distance the fragment traveled between the two landmarks. To utilize this option:

1. Click the mouse at the point where you would like the land mark to start.

2. Select points in the path where you would like the landmark to follow by clicking once at each desired point.

3. Double-click on the last point you would like to define. This action permanently places the landmark.

4. Repeat step 3 on the top and bottom of the image before generating the report.

The Filters Menu

The following filters in the *Filters menu* (Figure 2.13) are provided with the GelReader package. When selected, their function is restricted to the area of interest you have previously selected. The best way to experience the effects of these filters is to try them.

- *Histogram equalization* (Heq) improves the contrast in the image by flattening the frequency distribution curve for the image.

- *Shadowing* highlights the bands by adding a pseudo-3D perspective to them.

- *Revert* reverses the effects of the filters.

Figure 2.13 The Filters Menu

NOTE: Whenever you apply the shadowing filter to the image, you should first apply the histogram equalization to it. Apply Heq to the image after the shadowing is done as well.

The Standards Menu

The *Standards menu* (Figure 2.14) is only visible when the Select standards option is selected from the Tools menu. The elements of this menu are the macros that have been defined using the notebook. For more information on macros refer to the section “The Notebook” in Chapter 3.

Figure 2.14 The Standards Menu