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DNA Graphical User Interface (DNA/GUI) – User's Guide –

Version 2.0

by

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While DNA/GUI and this User's Guide are available without request, we strongly encourage anyone obtaining it to send us e-mail describing your title, affiliation, and plans for using it. In this way, we would be able to alert you regarding future changes.

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This software was developed at the Biomedical Computer Laboratory of the Institute for Biomedical Computing at Washington University Schools of Medicine and of Engineering and Applied Science, St. Louis, Missouri. The lane-detection, band-detection, and spatial-normalization software was developed by Heather A. Drury with invaluable ideas and suggestions from David G. Politte. The graphical-user-interface software was developed by Heather A. Drury and Kenneth W. Clark with John M. Feser and Andrew Loth. General feedback and advice for Version 1.0 were kindly provided by the DNA-Imaging Working Group comprised of: Jerome R. Cox, Helen Donis-Keller, Russell E. Hermes, Will D. Gillette, Brigid K. McCauley, Maynard V. Olson,and Lewis J. Thomas, Jr. The additional features for Version 2.0 were suggested by Todd Steinbrueck and Cynthia A. Helms. Special thanks go to Todd Steinbrueck for his review of earlier editions of this User's Guide.

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Chapter 1

Introduction

This chapter gives an overview, describes the changes since Version 1, and discusses the kinds of images which are allowed.

The maintainer of this document will attempt to maintain an on-line version of this Guide accessible from the World Wide Web as well as a Postscript version which can be ftp'd. Unfortunately, one version may not always look like the other. Look for specific differences in the Figures and in the way displayed "buttons" are represented.

- The Figures in the Postscript version will be grayscale whereas the HTML version contains GIF-colored Figures. The Figures themselves should be numbered consistently between versions. The Figure-numbering scheme is sequential within a chapter; thus, Figure 4.5 would appear in Chapter 4 but not necessarily in Chapter 4, Section 5.
- Operating the program consists of almost entirely of using a mouse to move a cursor to a displayed "button" and clicking the button. In the Postscript version, a button is labeled and enclosed in a box such as $\boxed{\text{Press}}$. In the HTML version, a button is designated as **[Press].**

1.1 Overview

This document describes the operation of the DNA Graphical User Interface (DNA/GUI) software package written at the Washington University Biomedical Computer Laboratory [1]. DNA/GUI is an interactive software system for rapid and efficient analysis of images of the types used in genome mapping, such as autoradiograms and electrophoretic gels. Images can be acquired using a commercially available charge-coupled-device (CCD) camera system and analyzed on a graphics workstation using a menu-driven user interface. DNA/GUI features automatic lane and band detection, simultaneous display of multiple images, and a unique spatial-normalization algorithm. Images and their associated data are archived and easily available for later recall. Preliminary results indicate that DNA/GUI is a useful tool in the analysis and comparison of images used in a variety of applications such as genetic-linkage analysis and DNA restriction mapping. DNA/GUI is written in the C programming language, and the interactive display software is based on the X Window System; the software is therefore readily portable to a variety of graphics workstations. Features are summarized in Table 1.1.

Table 1.1: DNA/GUI Features.

- display of multiple images
- automatic lane identification with manual editing
- automatic band identification with manual editing
- automatic fragment sizing
- spatial normalization of images to standard format
- zooming, shrinking, and panning of images
- display and manipulation of 1-D image profiles
- interactive color-table adjustments
- save and recall of whole images and lane images
- allele propagation for 2-allele inheritance

This introductory chapter covers changes and additions since Version 1.0. It also describes the kind of images and digital image formats which DNA/GUI can analyze. Chapter 2 gives details for installing the software files and general details about using the system. Chapter 3 gives details for completely analyzing an image. Chapter 4 describes several submenus which may prove helpful during analysis. Chapter 5 describes several miscellaneous features not accessed through the usual menus. Chapter 6 describes extensions which are for specific applications and are still under development. Finally, the Appendices give additional details for certain items in the User's Guide which don't justify inclusion in the body of the Guide.

1.2 Changes from Version 1.0

The features added to Version 2.0 since Version 1.0 are summarized here. They are described in detail elsewhere in this User's Guide. Reading of this 2.0 User's Guide does **NOT** require familiarity with any previous edition.

- Band detection may be automatic, semi-automatic, or manual.
- A popup with a 2X magnified crop box.
- Fragment-size editing.
- The **gel-format file** and **template-definition file** may be created on-line.
- Lane labels from a given **gel-format file** may be temporarily edited.
- Lane index labels, lane ID labels, and inheritance codes are shown directly above or below the crop box.
- Three generation, 2 allele system inheritance analysis.
- When inheritance is active, a window containing color-coded alleles and their sizes appears to the left or right of the crop box.
- TIFF images may be input.
- Images with gels whose lane widths vary and/or whose interlane spacing varies may have a user-defined "comb-geometry" file override the program's automatic lane-detection algorithm.
- Command-line options for specifying gel-format, template, and comb-geometry files AND for specifying that the program use "xrdb" to load the app-defaults file of X resources.

1.3 Image Limitations

1.3.1 Types of Gels

DNA/GUI works with single-marker gels. There must be a least two marker lanes, preferably to the left and right of other lanes. A centered third marker lane can be useful. There must be at least four bands in each marker lane, and the top and bottom bands' vertical coordinates should bracket the bands in the experimental lanes. Images may be obtained from directly fluoresced gels or from transilluminated autoradiograms.

1.3.2 Image Sizes

Digitized images may be of any dimensions up to 1024 x 1024. If the image is greater than 512 pixels in either direction, it is downsampled or halved in each direction in order to fit the 512 x 512 space allocated for it. Also, if the image is larger than the display space, the original image is retained in memory for analysis. An image with dimensions no greater than 512 x 512 is called a *small image* while one with greater dimensions is called a *large image*. The program does not currently handle images larger than 1024 x 1024.

1.3.3 Image Formats

1.3.3.1 Import Images

DNA/GUI works with images which are in a local (Washington University) **import** format. Two other kinds of images may be input (see following two sections), but these are converted to the **import** format for analysis.

The **import** format is a simple concept; an **imort** image is represented as two files, the image binary data file and an ascii import file of tags describing the image. In the data file, image data are stored in binary in row-major order. The ascii import file of descriptors includes image size, type of binary image data, and all details about the analysis of the image. The descriptors may be found in Appendix A.6.

An exception to the two-file concept is the individual lane image for which the image data are embedded in the ascii import file. Lane images are created, as an option, when the results of analysis are saved.

The ascii import file typically has the same name as the data file, except that the import file is suffixed ".imp". File naming conventions are described in Section 2.9. When accessing an **import** image, one specifies the ascii import file; this, in turn, contains a string specifying the name of the image data file.

1.3.3.2 Photometrics CCD Images

Several local projects using DNA/GUI acquire images from Photometrics CCD camera systems. And DNA/GUI will recognize certain image types generated from these kinds of cameras. These images are, however, converted to the **import** format for analysis; the original images are not destroyed.

1.3.3.3 TIFF Images

TIFF images may be input, although extensive testing with different TIFF headers has not be tried. An input TIFF image is converted to the import format (the original image is not destroyed), and the import image is used throughout the analysis. There are no immediate plans to develop TIFF output images. TIFF images dimensioned greater than 512 are proportionally downsampled to 512 in the larger dimension. The downsampling technique is bilinear interpolation. Input TIFF images should be suffixed **.tif** or **.tiff**.

1.3.3.4 Other Images

If the input image is of neither of the above formats, the program will allow files with headers of known sizes (including headerless). Such images are called "odd" images. For these, the user is prompted for header size and for other image characteristics such as dimensions and data type. The header information is ignored, and the image data are converted to the **import** format. The original file is not destroyed. At present, the program does not deal with specific formats such as PNM, GIF, etc.

Chapter 2

Installing and Using DNA/GUI

2.1 System Requirements

2.1.1 Hardware Requirements

DNA/GUI requires a graphics workstation of the SUN SPARCstation or Digital DECstation class. The workstation should have 8-bit color capability and three-button mouse. Operation of DNA/GUI is independent of the mode of image acquisition which can be performed with, for example, a CCD camera, video camera, or flat-bed scanner.

2.1.2 Software Requirements

DNA/GUI requires the workstation run the X Window System, Version 11, Release 4 or later, and the UNIX operating system. DNA/GUI may be obtained via anonymous ftp; additional information may be obtained by contacting one of the sources mentioned in the front of this User's Guide. The executable program is named "**dui**".

2.2 Installing the DNA/GUI executable "dui"

The software comes as a compressed *tar* file which includes a README file for installation directives. The file also includes the source code, the means to build the executable **dui**, this User's Guide, support files used by **dui**, and sample images.

2.3 DNA/GUI Support Files

The files described below are necessary to run **dui**.

2.3.1 Application Defaults File

The application-default resource file allows customization of much of DNA/GUI. The "app-defaults" file for DNA/GUI is called `dui.ad`. Instructions for changing various attributes of DNA/GUI are listed in Appendix A.1. DNA/GUI accesses the environment variable XAPPLRESDIR to find the application-default resource file. If this environment variable is already set for your system (the directory `/usr/lib/X11/app-defaults` is typically used), move the DNA/GUI app-defaults file to this directory. Otherwise the XAPPLRESDIR environment variable needs to be set. If, for example, the DNA/GUI app-defaults file is stored in the directory `/usr/local/applications/appdef` type

```
setenv XAPPLRESDIR /usr/local/applications/appdef/
```

to set the app-defaults directory. Alternatively, there is a command-line option for executing "xrdb" which will also load the X resources (see Section 2.12).

2.3.2 Help Files

On-line "Help" files are accessed through Help menus.

2.3.3 Color Palettes

These files are recipes for different color maps which may be changed by the user.

2.3.4 Gel-Format Files

These files specify lane order and lane labels for specific gels or families of gels.

2.3.5 Template-Definition Files

These files list fragment sizes and mobilities for different standards. The information in these files are used for sizing purposes and to align images with one another for purposes of comparison.

2.3.6 Comb-Geometry Files

Most gels are assumed to have a geometry such that all wells (lanes) are of equal width and that the inter-lane spacing is a constant. However, gels with varying lane widths and/or inter-lane spacing may be specified in a "Comb-geometry" file which overrides automatic lane detection.

2.4 Preparing Your Image Data

A digitized image of a stained gel or autoradiogram must be available in some accessible file. The image may be in a raw binary file; if so, the user needs to know the dimensions (number of rows and columns), the pixel size, and the size of any prepended header. The image may also be of the "import" variety, which is described in Section 1.3.3.1 and in Appendix A.6. If an image is to be analyzed, two additional files are needed: a `gel-format file` (Appendix A.3) and a `template-definition file` (Appendix A.4).

2.5 Interaction with DNA/GUI

Most interaction with DNA/GUI is performed moving the pointer to a menu item and clicking a mouse button (MB1 by default). The following abbreviations are used throughout this document

MB1 – mouse button 1 (left)
MB2 – mouse button 2 (center)
MB3 – mouse button 3 (right).

Throughout this guide, the menu item will be shown as a box containing the menu item name; thus the menu item to close an image might be shown as $\boxed{\text{Close Image}}$.

2.6 Screen Layout

The DNA/GUI screen consists of four regions: **Commands**, **Status Information**, **Image Information**, and **Image** as shown in Figure 2.1. The **Commands**, **Status Information**, and **Image Information regions** appear in the top one-third of the screen while the **Image region** covers the lower two-thirds.

A. Commands Region. Main menu and submenus are displayed here. Selection of any menu item (clicking MB1) results in a submenu being displayed to the right of the main menu.

B. Status Information Region. Instructions, status information, and error messages are communicated to the operator here. The operator should become accustomed to paying attention to this window. At any time, the operator may scroll through the contents of this window. Move the pointer to the vertical scrollbar to the left of the window, depress and hold MB2, and move the pointer up or down to scroll.

C. Image Information Region. Particular information regarding the "primary image" (the image to be analyzed) is entered by the operator here.

D. Image Region. The Image Region is subdivided into several regions.

- **D-1 Primary Image.** The primary image to be analyzed is displayed here.
- **D-2 Vertical Scrollbar.** The vertical scrollbar is used for horizontal fragment-size editing.
- **D-3 Fragment Sizes.** The sizes of the bands in the **template-definition** file are displayed here after spatial normalization. In a different color, an axis of pixel index values and tic marks are displayed.
- **D-4 Magnify.** A magnified portion of the primary image corresponding to the area surrounding the current mouse position is displayed in this window.
- **D-5 Colorbar.** The mapping between intensity values and color is shown in the colorbar to the right of the image.
- **D-6 Color-Table Adjustments.** Interactive adjustments to the installed color map may be made using the buttons and sliders in this window.
- **D-7 Toggle Buttons.** There are 3 toggle buttons above the vertical scrollbar. The bottom button displays a popup of a 2X magnification of the crop box. The middle button is a 3-way toggle for displaying the lane labels, lane index values, and inheritance codes; the 3 states are above the crop box, below the crop box, and off. The top button is a 3-way toggle for displaying allele sizes; the 3 states are left of the crop box, right of the crop box, and off.
- **Extra Images.** An arbitrary number of "extra images" (for viewing and comparison purposes) can be displayed in any portion of the Image Region.

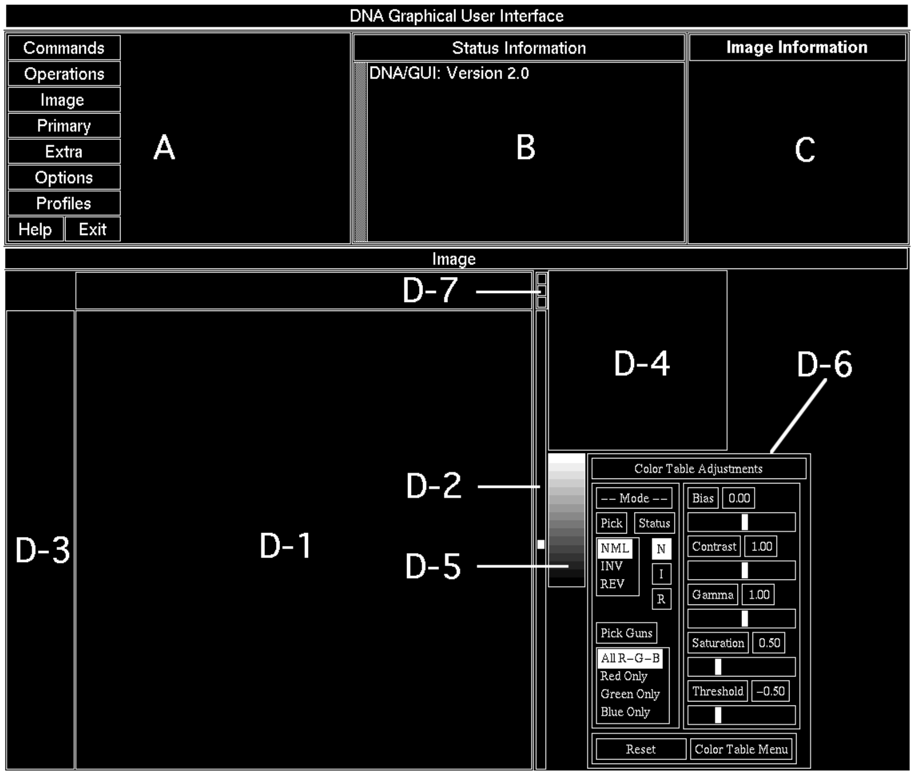

Figure 2.1: DNA/GUI Screen Topology.

A. Commands Region
B. Status Information Region
C. Image Information Region
D. Image Region
- D-1. Primary Image
- D-2. Vertical Scrollbar
- D-3. Fragment Sizes
- D-4. Magnify Window
- D-5. Colorbar
- D-6. Color Table Adjustments Menu
- D-7. Toggle Buttons

2.7 The Menu System

Interaction with DNA/GUI is primarily through a two-level menu system. The main menu or Commands Menu is shown in Figure 2.2. Except for the Help and Exit buttons on the bottom of the menu, each button in the Commands Menu brings up a submenu in the space to the right of the Commands Menu. Except for the Help button, the Commands Menu is disabled while a submenu is activated.

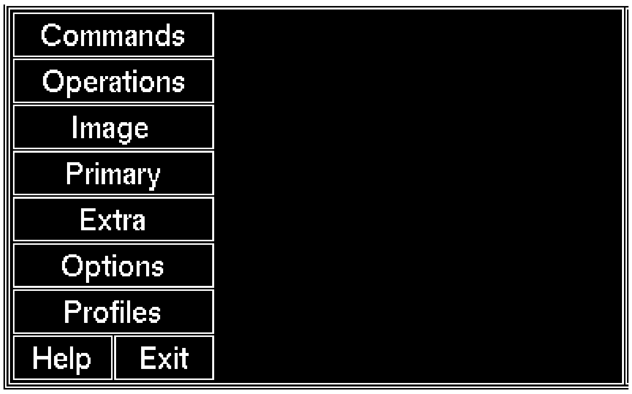

Figure 2.2: The Commands Menu (left) with Submenu Space (right).

2.8 Files Used During Processing

The image to be analyzed is called the **primary image**. It consists of two files: the image data file and the ascii import file. The import file, which includes a string specifying the data file, is the one selected by the user. Also selected for input to analysis are a **gel-format file** and a **template-definition file**. The gel-format file is an ascii file listing the lane labels associated with the image. The template-definition file describes a template of size-standard bands, listing mobility and base-pair size for each band; the input image is warped against this template to output a spatially-normalized primary image. The normalized image is of the import variety, with its own ascii import file and image data file. Experimental bands are then detected in the non-size-standard lanes of the normalized image; mobilities and sizes of these bands can be saved in the import file of the normalized image. As an option, individual lane images can be saved before and/or after normalization. Table 2.1 lists the various steps of analysis to show which files are input when, which are output, and which are updated.

Table 2.1: Input and Output Files At Different Stages of Analysis.

Analysis Step	**Input File**	**Output File**
Load Raw Image	Import Raw Image Data	
Enter Image Information	Gel-Format Template-Definition	
Crop Image		
Detect Lane Boundaries		
Detect Size-Standard Bands		
Save Results		Import† Lane Images* Lane Profiles*
Normalize Raw Image		Normalized Import Normalized Image Data
Detect Experimental Bands		
Save Results		Normalized Import† Normalized Lane Images* Normalized Lane Profiles*

† Update existing file.
* Option; by default, these are not saved.

2.9 File-Naming Conventions

Table 2.2 lists the filename extensions used by DNA/GUI.

Table 2.2: Filename extensions employed by DNA/GUI.

.imp – A file that has been imported. The file ending with the `.imp` extension points to the file containing the actual image data. Alternatively, the file may contain the image data; this is true of lane images. An Import file need not be so suffixed since the DNA/GUI keys on header information. Likewise, a file named with this suffix need not be an Import file. However, output files generated by DNA/GUI will conform to the descriptions listed here.

.x – A file containing image data which was not originially in the import format.

.w – A spatially normalized image file.

.ln# – A lane-image file where # refers to the lane number.

.p#.dat – A file containing integrated profile data for lane #.

.blot – A **gel-format file** defining the gel loading order and lane labels.

.tmpl – A **template-definition file** containing the size and location of the bands that define the template.

Some examples follow for an image file that was called **testimage**.

testimage.x.imp – An Import file containing relevent information about the image.

testimage.x – A file containing image data.

testimage.x.w.imp – An Import file for a normalized image.

testimage.w – The file containing normalized image data.

testimage.ln23.w – The normalized lane-image for lane 23.

testimage.p9.dat – The one-dimensional integrated profile data for un-normalized lane 9.

2.10 File Browsers

A File Browser is a window that enables users to traverse directory trees and select files. DNA/GUI uses five file browsers listed in Table 2.3. Described here are the particulars of the Image File Browser, but the operations are identical for the other File Browsers. Figure 2.3 shows an example of the contents of the Image File Browser. $\boxed{\text{Cancel}}$ can be pressed at any time to remove the file browser from the display.

Table 2.3: File browsers.

Image File Browser – to select a Primary Image
Extra Image File Browser – to select an Extra Image
Gel-Format File Browser – to select a Gel-Format File
Template-Definition File Browser – to select a Template-Definition File
Color File Browser – to select an alternate color map

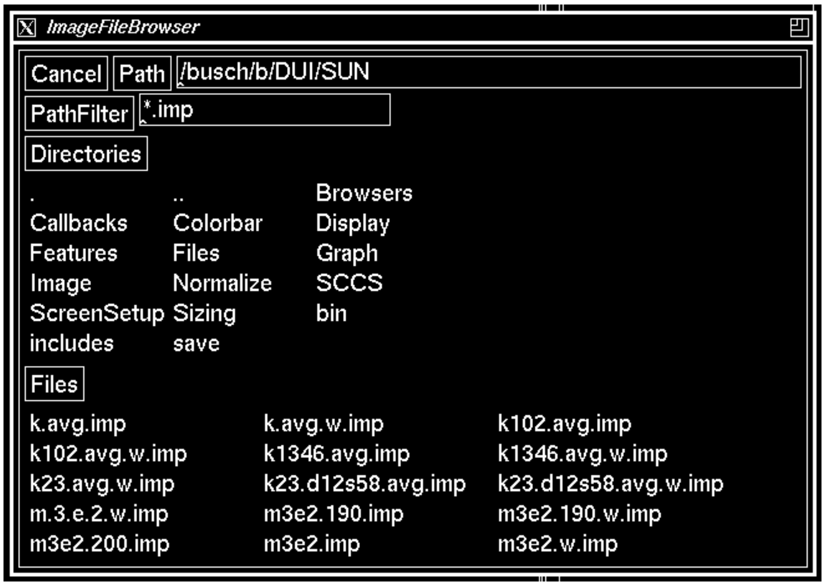

Figure 2.3: An example of the Image File Browser.

The name of the current directory from which files may be selected is called the Path. A string that determines the subset of file names that are displayed under the label Files is called the PathFilter. Any directories contained in the current directory are listed under the label Directories. Files contained in the current directory that match the string contained in PathFilter are listed under the label Files. To select a file, simply move the cursor to the name of the desired file and depress MB1. As soon as MB1 is depressed, the File Browser disappears.

The PathFilter is `*.imp` as shown in Figure 2.3. To modify the PathFilter, move the pointer to the rectangle containing `*.imp`. The caret ^ indicates the current editing position in the string. Click with MB1 to move the caret. Edit the contents of the box by deleting the current string and entering a new one and hit `<return>` to update the list of files under the label Files. For example if the operator wants all the files in the current directory to be selectable, the PathFilter should be modified to read * followed by `<return>`. All the files in the current directory will now be listed.

There are two ways of changing directories using a File Browser. The first is to simply edit the Path label. Move the mouse the rectangular area containing the current string, delete the current string, enter a new one, and hit `<return>`. All files in the new directory that match the PathFilter string will be listed. The second way to change directories is to select a directory name as listed under the label Directories by moving the pointer to the directory name and depressing MB1.

2.11 On-line Help

On-line help is available in DNA/GUI. Clicking Help at the bottom of the Commands Menu pops up a window with a list of subjects for which help is available (Figure 2.4) Clicking on the the subject of interest pops up another window which contains a textual description for the topic. Clicking Quit for the topic chosen returns control to the Help Menu. Clicking Quit in the Help Menu pops down the Help Menu.

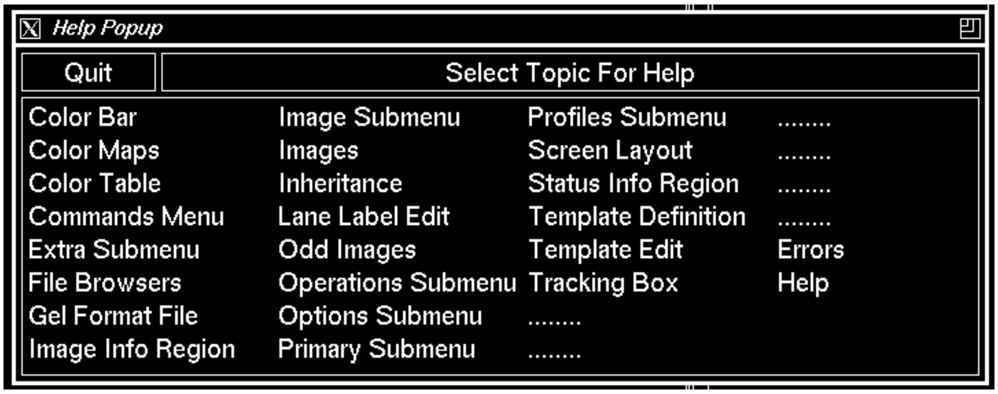

Figure 2.4: The Help Popup Menu.

2.12 Command-line Options

dui [-cfilename] [-gfilename] [-tfilename] [-x{filename}]

-c filename Specify a Comb-Geometry File. If specified, the file must exist in the COMB directory and have a **.comb suffix**. If not specified, program assumes a gel's lanes are of equal width and the spacing between lanes is constant.

-g filename Specify a Gel-Format File. If specified, file must exist in the GEL-FORMAT directory and have a **.blot suffix**. If not specified, the program will prompt for one after an image has been selected for analysis.

-t filename Specify a Template File. If specified, file must exist in the TEMPLATE directory and have a **.tmpl suffix**. If not specified, the program will prompt for one after an image has been selected for analysis.

-x {filename} If **-x** is spcified, the program will invoke **xrdb**: if no filename is given, the invoke will be **"xrdb -l dui.ad"**; if a filename is given, the invoke will be **"xrdb -l filename"**. If the **-x** is not used, the resources must be specified with **"setenv XAPPLRESDIR ./"** prior to calling dui.

Chapter 3

Operations Submenu: Analyzing An Image

Assuming you have all the support files in place and have properly set the environment (if you don't understand this, consult your system manager or the person who installed this software), begin by typing `dui <return>` on the command line. On your terminal, the program will print the DISPLAY and XAPPLRESDIR environment variables or print an error message. If the environment is properly set, the screen should pretty much be covered with the **dui** application. From here, very little typing is done. Most interaction is performed by moving the pointer to a menu item and clicking mouse button 1 (MB1).

Nearly all interaction with the program is through the Commands Menu in the upper left of the screen. Selection of a Commands Menu item brings up a submenu just to the right of the Commands Menu. Control remains with the submenu until its Done is pressed, at which time the submenu disappears. While a submenu is displayed, the Commands Menu is darkened to show that its items are "insensitive".

Begin, now, by clicking Operations and observing the Operations Submenu to the right of the Commands Menu as shown in Figure 3.1.

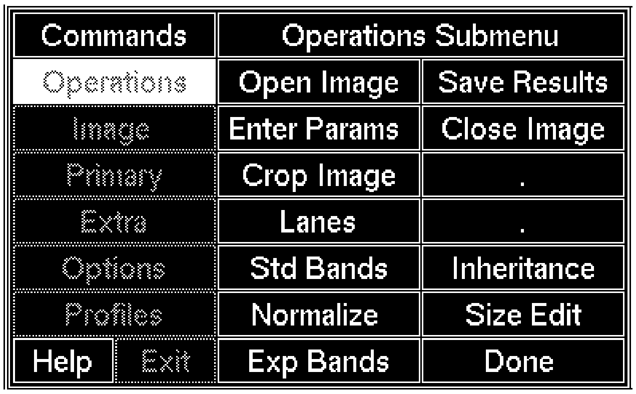

Figure 3.1: Commands Menu (left) and Operations Submenu (center and right).

The typical sequence of operations necessary to analyze an image is listed in Table 3.1. The Operations Submenu items are shown in boxes.

Table 3.1: Operations Submenu button sequence of operations for image analysis.

1.	**Open Image**	**Open a primary image**
2.	**Enter Params**	**Enter image information**
3.	**Crop Image**	**Crop the image**
4.	**Lanes**	**Locate lane boundaries**
5.	**Std Bands**	**Identify bands in size-standard lanes**
6.	**Normalize**	**Spatially normalize image**
7.	**Exp Bands**	**Identify bands in experimental lanes**
8.	**Save Results**	**Save results**
9.	**Close Image**	**Close the image**

You will now be led through this process for an image called **k102.avg**. Two files are involved: **k102.avg** is the file containing the image data while **k102.avg.imp** is an ascii import file containing information about the image. You actually pick the import file from the Image File Browser; the import file has, in turn, the name of the file containing the image data. A full description of the import file may be found in the Appendix (see A.6).

3.1 Open a Primary Image

- Pointer to Commands Menu, click Operations .
- Pointer to Operations Submenu, click Open Image .
- Image Flags Popup appears (see Figure 3.2). Options will be discussed in the Advanced Topics portion of the tutorial. Click Load Image .
- Image File Browser pops up. Traverse directories as needed (see Section 2.10). Use MB1 to select the image, in this case, **k102.avg.imp**. The primary image will be displayed. It should appear with dark bands on a light background. There should be 19 lanes. Handwritten annotations appear above and below the lanes.

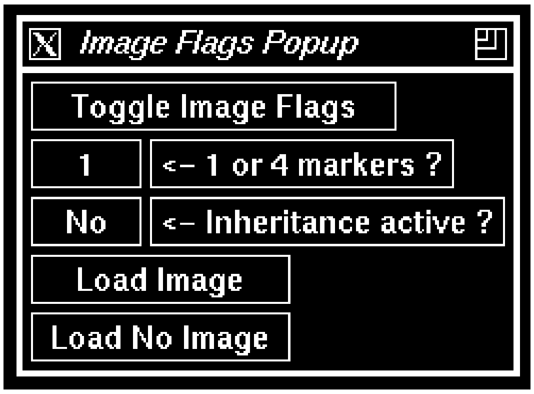

Figure 3.2: Image Flags Popup.

- The top line ("Toggle Image Flags") is a label.
- $\boxed{1}$ toggles between "1" and "4". The "1" is the default while the "4" is used for gels using the M13 sequencing ladder as the standard. The program logic for handling sequence gels is not yet fully developed.
- $\boxed{\text{No}}$ is toggled to "Yes" when the user wants to activate inheritance (see Section 6.1).
- $\boxed{\text{Load Image}}$ is clicked when the flags above have been set and it is time to load an image. When $\boxed{\text{Load Image}}$ is clicked, the Image Flags Popup disappears, and the Image File browser is popped up.
- $\boxed{\text{Load No Image}}$ pops down the Image Flags Popup and returns control to the Operations Submenu with no further action.

When the image is displayed, pixel-index tic marks with labels for the vertical axis appear to the left of the image. The default color for these tic marks and labels is yellow. The scale corresponds to the original image, not the displayed image; thus, a small image will have a vertical scale 0 to 511 while a large image will have a vertical scale 0 to 1023. See Section 1.3.2 for a note on image sizes.

Also when the image is visible and the pointer is moved around within the bounds of the image, the pointer's (x,y) coordinates are displayed just beyond the upper left corner of the image. Move the pointer around and note the changing coordinates. Should the pointer leave the image area, the coordinates shown are the last location of the pointer before leaving the image area.

3.2 Enter Image Information

Next, you will select two files containing information about the gel or film format.

- The **gel-format file** contains an ordered list of lane labels for the size-standard lanes and experimental lanes.
- The **template-definition** file contains the fragment sizes of the bands in the size-standard lanes and their positions (mobilities) in the template.

These files are selected through the Gel-Format File Browser and Template-Definition File Browser. You will be led through a series of browsers and popup windows in which choices are made. Here is a list of those windows (popups shown in Figure 3.3):

Gel-Format Popup,
Gel-Format File Browser,
Lane Label Edit Popup,
Template-Definition Popup,
Template-Definition File Browser.

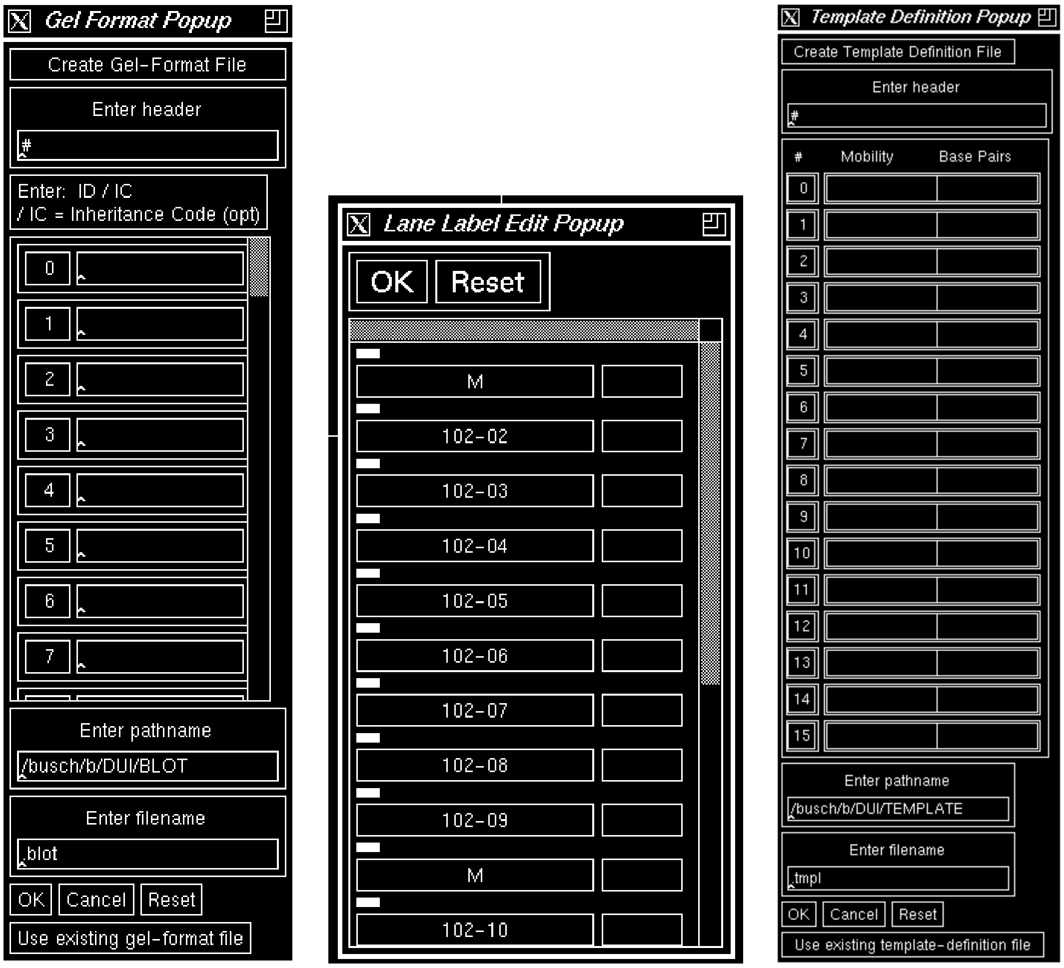

Figure 3.3: Gel-Format, Lane Label Edit, and Template-Definition Popups.

After a **gel-format file** and a **template-definition file** have been selected, various text fields in the Image Information Region to the upper right of the screen are available for input. Here, you may enter information such as the operator name, probe name, filter name, or other appropriate data.

- Pointer to Operations Submenu, click [Enter Params]. The Gel-Format Popup appears.
- Click the bottom button [Use existing gel-format file]. The Gel-Format Popup disappears, and the Gel-Format File Browser appears. See Section 5.7 to create a new gel-format file.
- Click the pointer over the file to load, in this case, **k102.blot**. The Gel-Format File Browser disappears, and the Lane Label Edit Popup appears. The Status Information Region reports:

```
#Family: K102
Number of lanes id'ed in gel-format file: 19
```

The first line is simply the header from the gel-format file; the second line shows the number of lane labels listed in that file.

- Click $\boxed{\text{OK}}$ in the Lane Label Edit Popup. The Lane Label Edit Popup disappears, and the Template-Definition Popup appears.
- Click $\boxed{\text{Use existing template-definition file}}$ at the bottom; The Template-Definition Popup disappears, and the Template-Definition File Browser appears. See Section 5.7 to create a new template-definition file.
- Click the pointer over the file to load, in this case, **k102.tmpl**. The Template-Definition File Browser disappears, and control reverts to the Operations Submenu. The Status Information Region reports:

```
#RFLP_AVG
Number of template points: 16
```

The first line is simply the header from the template-definition file. The second line shows the number of template points listed in that file.

- Enter information in Image Information Region. See Figure 3.4. The top two text fields in the Image Information Region contain the header lines from the gel-format and template-definition files. The remaining fields are text fields into which you enter what you like. The labels for these fields are:

gel date, filter #, probe, operator, film #, exposure.

Move pointer to particular field, enter field using keyboard to enter text, and move pointer to next field. In these fields, it is not necessary to use the **Enter** or **Return** keys. This image information may be entered and edited at any time.

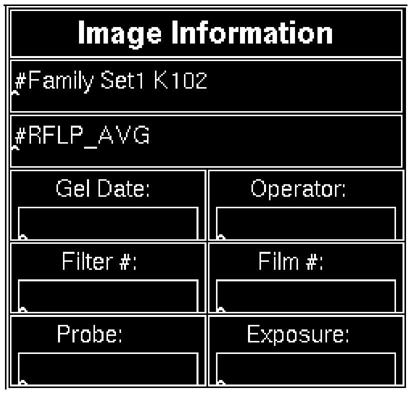

Figure 3.4: The Image Information Region.

3.3 Crop the Image

The region of the image that contains the lanes to be analyzed is delineated by a bounding box. This procedure is necessary to avoid the extraneous analysis of hand-written annotations on the edges of some autoradiograms and to speed the lane detection procedure.

- Pointer to Operations Submenu, click Crop Image .
- Move the pointer to the upper left-hand corner of the sub-image to be analyzed, in most cases, the left margin of the leftmost lane, somewhat above the first band. The y position is not too critical, but the x position is crucial. The current (x, y) pointer position can be monitored in a small window just outside the upper left corner of the image. For the sample image **k102.avg**, a good (x, y) might be (30, 50).
- Depress and hold MB1, move the pointer to the lower-right corner of the sub-image and release MB1.
- The release of MB1 should be along the right margin of the rightmost lane and somewhat below the last band in the rightmost lane.
- While you have MB1 depressed, a small window appears in the upper left corner of the image; this window shows, from left to right, three pairs of numbers:
  - the 1st pair is the (x,y) coordinate pair when MB1 was depressed,
  - the 2nd pair is the current (x,y) coordinate pair,
  - the 3rd pair is the (width,height) pair.

  For image **k102.avg**, just before MB1 is released, these numbers might be:
  - initial (x, y) = (30, 50),
  - current (x, y) = (465, 390),
  - (width, height) = (435, 340).
- The rectangle that appears bounds the region to be analyzed.
- If you didn't get it quite right, you may repeat the procedure, beginning with the click of Crop Image . For example, if the top or bottom line of the bounding box appears to cut off one or more bands, the box may have to be extended.

Figure 3.5 shows the **k102** image with a crop box. The hand annotations outside the crop box are excluded from analysis.

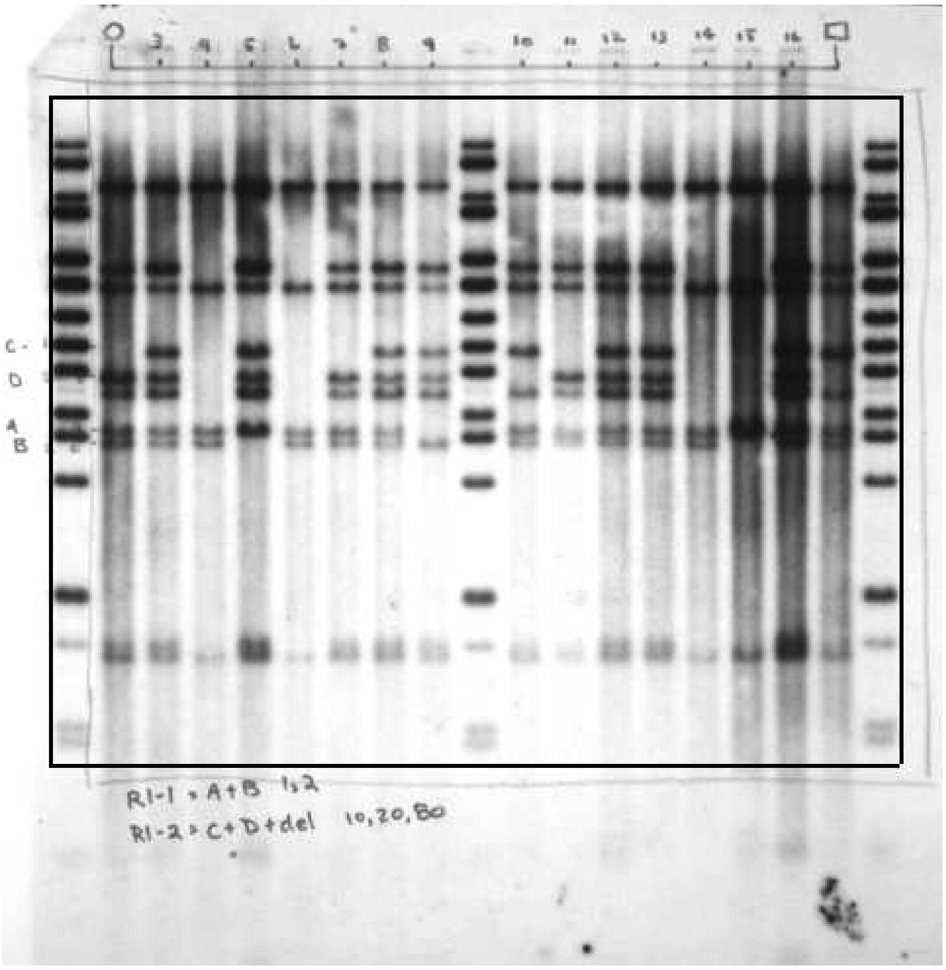

Figure 3.5: Image **k102** with crop box surrounding region of interest.

3.4 Locate Lane Boundaries

The left and right boundaries of each of the lanes in the image are determined automatically. The number of lanes identified is based on the number of lanes defined in the **gel-format file**. After the program performs its automatic-detection process, which takes only a few seconds, you may accept the results or you may "edit" or adjust the lane boundaries. When you are finished, each lane is labeled.

- Pointer to Operations Submenu, click $\boxed{\text{Lanes}}$. Automatic lane demarcation is performed.
- When the automatic portion is finished, the Status Information Region directs you to use the mouse buttons to add and delete lane boundaries or to accept the current boundaries.
- To accept the lane boundaries as is, click MB3. Otherwise, you may edit the boundaries:
  - To remove a lane boundary, move the pointer over the lane boundary and click MB2. The boundary disappears.
  - To add a lane boundary, depress MB1 in the vicinity of where you would like the boundary. A vertical line will appear that can be moved for precise placement. While MB1 is depressed, move the pointer to the exact location and release MB1. The new boundary is fixed. If you don't get it quite right, delete it with MB2 and add it again with MB1.
  - When you have finished editing, click MB3.
- If lane boundaries have been added and/or removed, and if the total number of lane boundaries is not an even number, a warning message is issued. Likewise, if the number of lanes does not equal that of the **gel-format file**, a warning message is given. Warning messages appear in the Status Information Region. In either case, you may re-enter the edit phase by clicking $\boxed{\text{Lanes}}$ again. Should you do so, the automatic portion is skipped; and you may edit immediately. Lane errors must be corrected in some manner.
- If the lane-detection algorithm does not perform satisfactorily, it may be advantageous to perform the image cropping again (see Section 3.3).
- When MB3 is clicked and the number of lane boundaries is even, two stripes of labels appear above or below the cropped image (see Figure 3.6). Placement above or below depends upon the space available.
  - The **lane-index stripe**, the stripe adjacent to the image, contains lane index numbers (0 to N, where N is the number of lanes). On color displays, this stripe is blue.
  - The other stripe, the **lane-label stripe**, contains the lane labels from the **gel-format file**. However, only the last 2 characters of each label are displayed. The lanes labeled "**M**" are marker lanes or size-standard lanes. On color displays, this stripe is green.
  - The **lane-index stripe** and **lane-label stripe** may be moved or turned off. To the right of the image is a scrollbar. Above the scrollbar are 3 tiny colored buttons. The middle button (blue on color displays) is a 3-way toggle for the lane stripes. The 3 positions are "above" the image, "below" the image, and "off".
  - If inheritance is active, a third stripe (**inheritance stripe**), containing family relationship codes, is treated as the other two stripes. On color displays, this stripe is red.

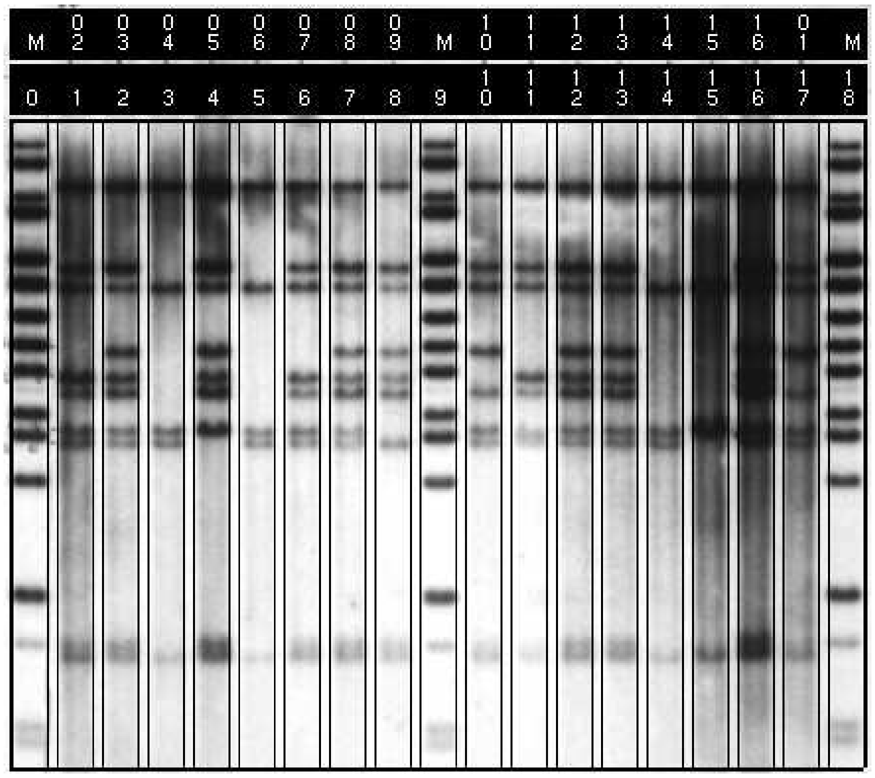

Figure 3.6: Image with lane boundaries, **lane-index stripe** and **lane-label stripe**.

3.5 Identify Bands in Size-Standard Lanes

After the lane boundaries have been delineated, you may identify the bands in the size-standard lanes. You will first allow the program to automatically detect what it can; then, you will edit the results so that the number of detected bands matches a pre-defined template. An example template, and the one used for image **k102.avg**, may be found in Appendix A.4. The purpose of the template is to have a standard against which images can be "warped" into alignment so as to have some basis of comparison with one another.

- Pointer to Operations Submenu, click Std Bands .
- Before attempting automatic band detection in the standard lanes, the Template Edit Dialog (Figure 3.7) will ask if you would like to "edit" the template. You may learn more about this in Section 5.2. At this point, you will not; click No.

Figure 3.7: The Template Edit Dialog.

- Automatic band detection commences and short horizontal bars appear on the image where bands have been detected. On color displays, these are red. Notice that not all bands in the 3 marker lanes of image **k102.avg** have been detected.
- After automatic detection, you may manually add missing bands and delete false-positive bands. The Status Information Region reminds you that MB1 is used to insert a band, MB2 is used to delete a band, and MB3 is used to indicate the end of manual editing.
- Note also the appearance of a small popup (Figure 3.8) which is a toggle for editing with the semi-automatic mode turned off or on (default). The toggle button label is set appropriately:
  - Semi-automatic: when MB1 is depressed in a marker lane, a band is drawn at that location; when MB1 is released, the band is moved to the spot of highest intensity within a window of several pixels of the original spot.
  - Manual: when MB1 is clicked in a marker lane, a band is drawn at that location.

  You may switch at any time you are editing and are encouraged to experiment with both options.
- Notice that while inserting bands, either in the manual or in the semi-automatic mode, as long as MB1 is depressed, a small graph appears to the lower right of the screen. This graph is a vertical profile of intensity values along the center of the marker lane in which the band is being placed. A small circle tracks the movement of the pointer in the lane in order to help visualize where the pointer is in relation to intensity peaks. The profile graph disappears when MB1 is released.

Figure 3.8: The Semi-automatic band-insertion toggle ("On" or "Off").

- To delete a band, move the pointer over the band to be deleted and click MB2. A warning message apppears in the Status Information Region if MB2 is clicked in an area where there is no band or if the pointer is not in a marker lane. Caution; where two or more bands are close together, some care must taken when clicking MB2. You should experiment by adding and deleting bands.
- **Important: The bands identified in the size-standard lanes must correspond 1:1 with those currently defined for the template for spatial normalization and fragment sizing to function correctly.** If the number of bands identified in each size-standard lane does not match the number of bands defined for the template you will be informed of which lanes are discrepant.
- When the number of detected bands does not match the template, re-enter by clicking Std Bands. Editing is as before.

Figure 3.9 shows the superimposed band bars in the size-standard lanes.

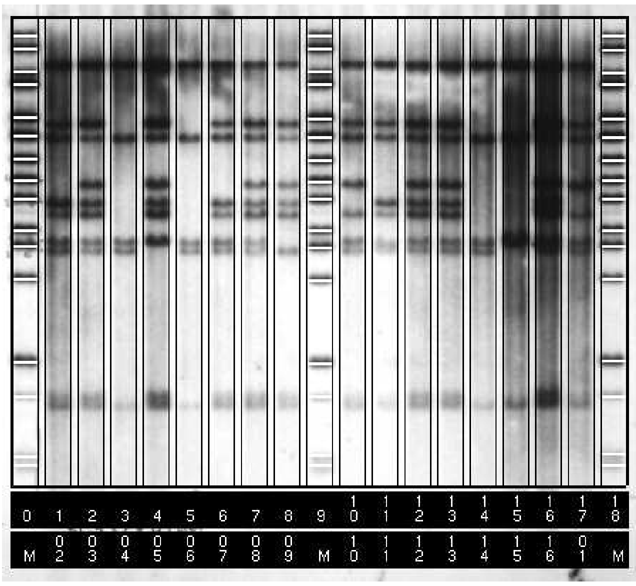

Figure 3.9: The white band bars in the size-standard lanes indicate band detections.

3.6 Spatially Normalize Image

The next step in the analysis is to spatially normalize the image to conform to the standard reference format defined in the **template-definition file**. Spatial normalization allows comparison of the image with other images that have been normalized to the same template. A new file containing the normalized image data is created on disk. In the display, the normalized image replaces the pre-normalized image. The length of time to complete the normalization step depends upon the size of image and network load, but it is typically a few seconds.

- Pointer to Operations Submenu, click Normalize .
- The image will be spatially normalized to conform to the template defined in the **template-definition file**.
- As the image is normalized, mean-square error results are reported in the Status Information Region for each size-standard lane, indicating how closely each lane matches the template. Image **k102** will not appear much different before and after normalization; but Figure 3.10 shows a dramatic change for image **k1346**.

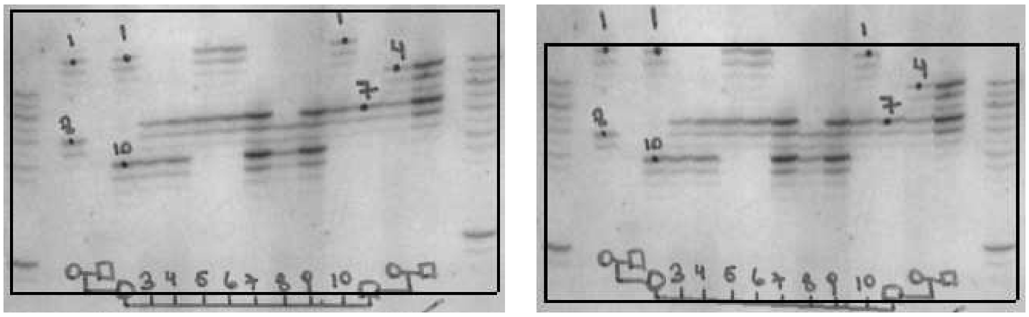

Figure 3.10: Image **k1346** before (left) and after (right) normalize.

- The normalized image is saved on disk in the same directory as the original image. The original image remains unchanged. The file name of the normalized image is the same as that of the original image, but it is suffixed with "**.w**". A companion import file is likewise created; its name is the same as the normalized image, but is suffixed with "**.imp**". For example:
  - **k102.avg** and **k102.avg.imp** [Pre-normalized image and import files]
  - **k102.avg.w** and **k102.avg.w.imp** [Normalized image and import files]
- The normalized image is displayed, replacing the original image. The sizes of the fragments in the size-standard lanes (and the corresponding template) will appear to the left of the image. Note that the horizontal lines may not correspond exactly with the positions of the image bands as they now represent the positions of the template bands to which the image was normalized. See Figure 3.11.

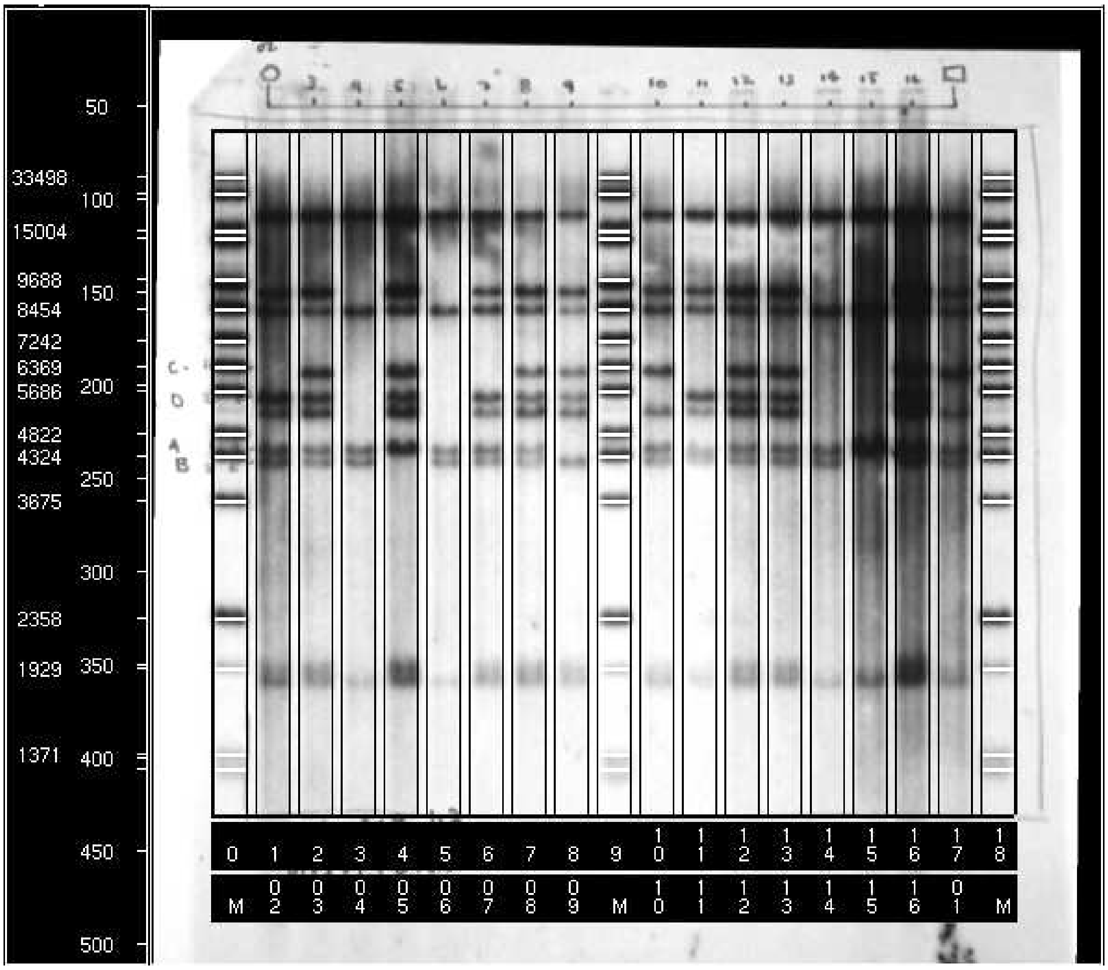

Figure 3.11: Image **k102** after normalization.

3.7 Identify Bands in Experimental Lanes

After the image is normalized, you may invoke the process which detects bands in the experimental lanes. From another menu, you may choose to detect automatically, semi-automatically, or manually. Each band detected is marked with a small bar across the lane in which the band was detected; on color displays, this bar is green. The user may edit the results of the band detection to add or delete band bars as necessary. Deleting band bars may be necessary where the peak-detection algorithm erroneously identifies an artifact on the image as a band. Interactive band bar addition may be required to identify low-intensity bands. Where two bands are very closely spaced, the program may flag just one band as it may not be able to separate adjacent intensity peaks. Editing is similar to that described earlier in Section 3.5.

- Pointer to Operations Submenu, click Exp Bands .
- The Experimental Bands Process Menu appears (Figure 3.12).

Figure 3.12: Experimental Bands Process Menu.

Its buttons and meanings are:

– Automatic	Band detection is automatic, followed by editing.
– Semi-automatic	Put band at peak intensity near click.
– Manual	You pick the location of each band bar.
– Edit	The same as automatic.
– Clear	If bands have been detected, they are cleared.
– Cancel	You choose not to detect bands at this point.

Clicking any of these menu buttons forces the menu to disappear. Clicking Clear or Cancel returns control to the Operations Submenu. Clicking any of the others keeps you in an edit mode until MB3 is clicked, at which point control is returned to the Operations Submenu.

- As each band is identified, its base-pair size appears in the Status Information Region.

- During editing, a popup window allows you to switch between semi-automatic and manual editing modes. This is the same popup which appears for the size-standard bands (Figure 3.8). The popup has one button which toggles between $\boxed{\text{On}}$ and $\boxed{\text{Off}}$ (you do/don't want semi-automatic editing).
- Clicking MB3 indicates the end of editing, and control is returned to the Operations Submenu. You may re-enter experimental band detection; click $\boxed{\text{Exp Bands}}$. Figure 3.13 shows the finished analyzed image. Experimental bands are shown in black.

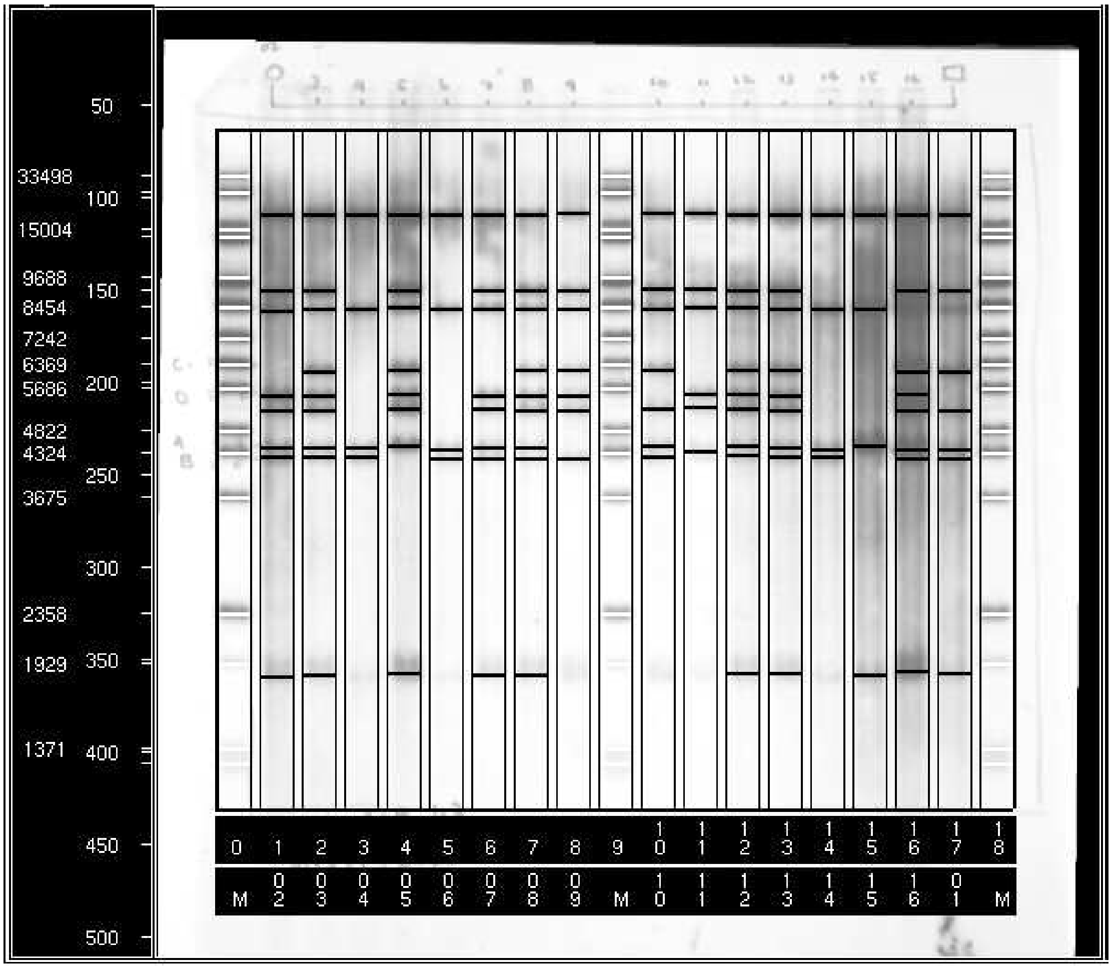

Figure 3.13: Image **k102** completely processed.

3.8 Save Results

The final step is to save the results of the image analysis. Results are saved in the import file **k102.avg.w.imp**. An example may be found in Appendix A.2. The following items are saved:

- any image information entered to the Image Information Region,
- the coordinates of the crop box corners,
- the names of the **gel-format file** and **template-definition file**,
- the lane labels (from the **gel-format file**),
- the template sizes and mobilities (from the **template-definition file**,
- the left and right margins of each lane,
- the fragment sizes and mobilities of each band,
- Pointer to Operations Submenu, click $\boxed{\text{Save Results}}$. The current state of analysis for the Primary Image is now saved.

⋆⋆⋆

NOTE: For this tutorial, you have just "Saved Results" after completely analyzing the image. In practice, you may "Save Results" at any time; in fact you are encouraged to do so, especially after detecting the standard bands and prior to normalization. Whenever you "Save Results", everything you have done to that point is saved in the **import** file. You could, therefore, save results at any time, leave, and finish analyzing the image at a later time.

⋆⋆⋆

3.9 Close the Image

The primary image is removed from the display; unless you had previously "Saved Results", any previous analysis is lost. You are ready to analyze another image.

- Pointer to Operations Submenu, click Close Image . The primary image is removed from the display. The lane IDs, fragment sizes, and tracking box are cleared.

3.10 Exiting

To exit DNA/GUI:

- Pointer to Operations Submenu, click Done .
- Pointer to Commands menu, click Exit . The Exit Popup appears (Figure 3.14).

Figure 3.14: The Exit Popup.

- Click one of these three :
  - Exit   leave the program
  - Cancel   abort the exit
  - Save   same as "Save Results"

Chapter 4

Other Submenus: Support For Image Analysis

The previous chapter described the Operations Submenu, which included the minimum features needed to analyze an image. This chapter describes the other submenus, which you may find useful in helping to analyze an image. A list of the remaining submenus appears in Table 4.1 below.

Table 4.1: Other Submenus.

- Image Submenu
- Primary Submenu
- Extra Image Submenu
- Options Submenu
- Profiles Submenu

4.1 Image Submenu

Click Image in the Commands Menu to open the Image Submenu. The Commands Region of the screen should like Figure 4.1. The Image Submenu buttons are explained below.

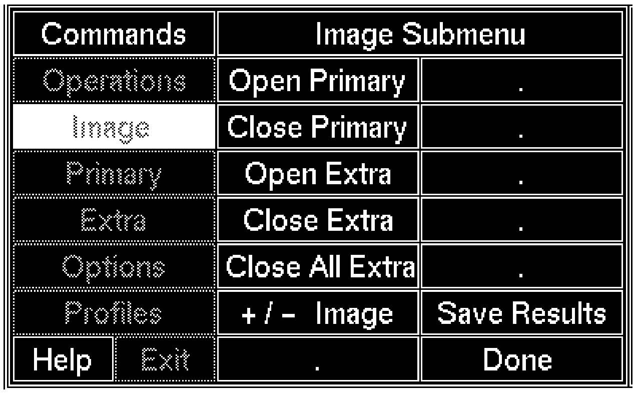

Figure 4.1: The Image Submenu.

4.1.1 Open Primary

This is the same as Open Image in the Operations Submenu. Its presence in the Image Submenu is historical.

4.1.2 Close Primary

This is the same as Close Image in the Operations Submenu. Its presence in the Image Submenu is historical.

4.1.3 Open Extra

An extra image, that is, not the Primary Image, may be displayed at any time, provided it is referenced through an import file. Any number of extra images may be displayed at once and can be moved around in the Image Region. Typically, lane images (section 5.4) are displayed next to a lane in the Primary Image.

- From the Commands Menu, click Image.
- From the Image Submenu, click Open Extra. The Extra Image File Browser appears. Click over the filename of the desired image.
- The outline of the selected image appears; the upper left follows the pointer. Click MB1, and the image will be displayed at the location of the pointer when MB1 was clicked.
- An extra image or all extra images may be deleted via the Image Submenu.
- An extra image may moved, raised, or lowered via the Extra Image Submenu (section 4.3).

4.1.4 Close Extra

To remove an extra image from the display, click $\boxed{\text{Close Extra}}$, then move the pointer to the image to be removed and click MB1. If you are removing more than one extra image, simply move the pointer to each and click MB1.

4.1.5 Close All Extra

Clicking $\boxed{\text{Close All Extra}}$ removes all extra images.

4.1.6 +/- Image

Declare the primary image as positive (gel) or negative (autoradiogram). Such a declaration is usually handled at the time the primary image is loaded, but this button allows it to be changed. Mostly, this button is historical.

4.1.7 Save Results

This is the same as $\boxed{\text{Save Results}}$ in the Operations Submenu. Its presence in the Image Submenu is historical.

4.1.8 Done

Clicking $\boxed{\text{Done}}$ removes the Image Submenu from the display and activates the Commands Menu.

4.2 Primary Submenu

The primary image may be zoomed, shrunk, and panned via the Primary Submenu. Click Primary in the Commands Menu to open the Primary Submenu. The Commands Region of the screen should like Figure 4.2. The Primary Submenu buttons are explained below.

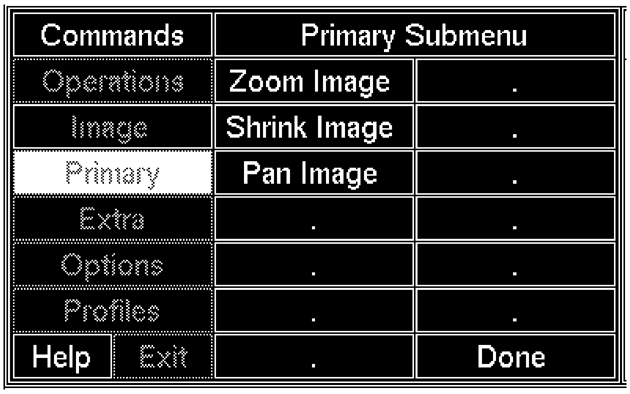

Figure 4.2: The Primary Submenu.

4.2.1 Zoom Image

Clicking Zoom Image causes the Primary Image to be zoomed (2x, 3x, 4x, ...) and redisplayed. The initial portion that is displayed is of the upper-left hand quadrant of the image (see the Pan section for how to pan through zoomed image). If the Magnify option is enabled, the portion of the image seen in the tracking window will be further magnified. This option is valuable for more closely examining complicated band patterns. The sub-image bounding box, lane bounds, bands, and vertical profile lines will be redrawn on the zoomed image; however, the lane-label, lane-index, and inheritance stripes are lost. Once the primary image has been zoomed, new vertical profiles cannot be drawn. Once an image has been zoomed, you can "shrink" to get back to the original image.

4.2.2 Shrink Image

Clicking Shrink Image causes the primary image to be shrunk (1/2x, 1/3x, 1/4x, ...) and redisplayed. The sub-image bounding box, lane bounds, bands, and vertical profile lines will be redrawn; however, the lane-label, lane-index, and inheritance stripes are lost. Once the primary image has been shrunk, new vertical profiles cannot be drawn. Once an image has been shrunk, you cannot "zoom" to get back to the original image.

4.2.3 Pan Image

Pan Image is useful when viewing a portion of a zoomed primary image. When panning is enabled, the following message

```
Pan Image
Button1: Pan Image
Button3: Done Panning
```

is displayed in the Status Information Region. The operator moves the pointer to the position in the image that is to become the new center of the image window and click MB1. The portion of the image will be displayed. Click MB3 (with the pointer positioned in the primary image) when finished panning the primary image.

4.2.4 Done

Clicking $\boxed{\text{Done}}$ removes the Primary Submenu from the display and activates the Commands Menu.

4.3 Extra Image Submenu

The buttons of the Extra Image Submenu allow extra images to be manipulated. Also, profile graphs may be moved and resized. Click Extra in the Commands Menu to open the Extra Image Submenu. The Commands Region of the screen should like Figure 4.3. The Extra Image Submenu buttons are explained below.

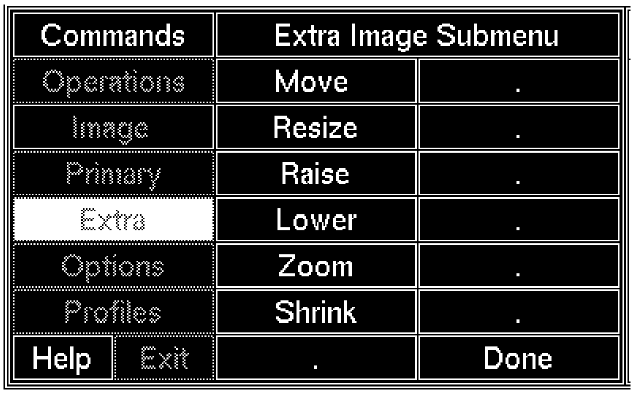

Figure 4.3: The Extra Image Submenu.

4.3.1 Move

Clicking Move allows the operator to move extra image windows around the Image Region of the screen. Images moved above the Image Region will be obscured by the Command Menu, Status Information, and Image Information Regions. Click Move in the Extra Image Submenu. Now move the pointer to the extra image that is to be moved, depress and hold MB1, and move the pointer (the image will follow the pointer) to the desired position and release MB1. The Move option is canceled by either selecting another option from the Extra Submenu or depressing MB1 in an area of the Image Region where there is no image. Note that this option works with the Magnify window and any profile graph but not with the primary image.

4.3.2 Resize

Clicking Resize allows the operator to resize a profile graph or an extra image which has been zoomed. Click Resize in the Extra Image Submenu; then move the pointer to window to be resized. Then, with MB1 depressed, drag the pointer. If a zoomed image is to be resized, move the pointer to the image, depress MB1, keep MB1 depressed, and drag the pointer in any direction outside the image. Release MB1 when the outline box is of the desired size and the image will be redisplayed. If a graph is to be resized, move the pointer to the profile graph that is to be resized, depress and hold MB1, and move the pointer up and to the left to enlarge the size of the outline box.

4.3.3 Raise

Raise allows the operator to raise a window on the screen. This operation is analogous to moving a piece of paper to the top of a stack. Click Raise in the Extra Image Submenu. Now move the pointer to the extra image that is to be raised, depress MB1 and the image will be redisplayed.

4.3.4 Lower

$\boxed{\text{Lower}}$ allows the operator to lower a extra image window. This operation is analogous to moving a piece of paper to the bottom of a stack. Click $\boxed{\text{Lower}}$ in the Extra Image Submenu. Move the pointer to the extra image that is to be lowered, depress MB1 and the image will be redisplayed.

4.3.5 Zoom

$\boxed{\text{Zoom}}$ allows the operator to zoom a extra image. Click $\boxed{\text{Zoom}}$ in the Extra Image Submenu. Move the pointer to the extra image that is to be zoomed and depress MB1. The image will be magnified (2x, 3x, 4x, ...) and redisplayed. The Resize option is now useful to redisplay an extra image so that the entire image can be viewed.

4.3.6 Shrink

$\boxed{\text{Shrink}}$ allows the operator to shrink a extra image. Click $\boxed{\text{Shrink}}$ in the Extra Image Submenu. Move the pointer to the extra image that is to be shrunk and depress MB1. The image will be shrunk (1/2x, 1/3x, 1/4x, ...) and redisplayed.

4.3.7 Done

Clicking $\boxed{\text{Done}}$ removes the Extra Image Submenu from the display and activates the Commands Menu.

4.4 Options Submenu

Click Options in the Commands Menu to open the Options Submenu. The Commands Region of the screen should like Figure 4.4. The Options Submenu buttons are explained below.

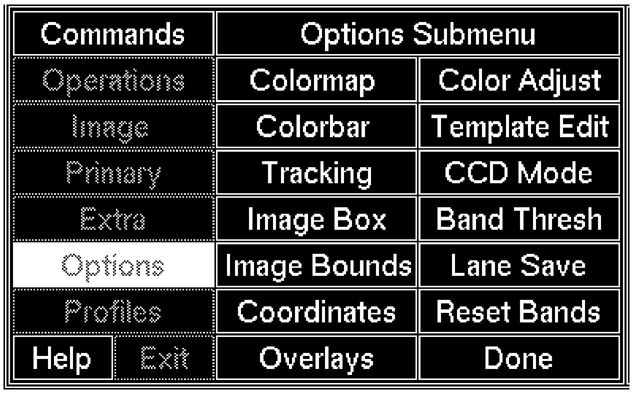

Figure 4.4: The Options Submenu.

4.4.1 Colormap

This button is equivalent to the Color Palette Menu button in the Color Table Adjustments Menu. It is used to change the installed color map. See Section 5.1 for details.

4.4.2 Colorbar

Clicking Colorbar toggles the colorbar on/off. Default: on.

4.4.3 Tracking

Tracking allows the operator to view a magnified portion (2x) of the primary image corresponding to the current cursor position. Clicking Tracking toggles magnification on and off. If the tracking option is currently on, it will be turned off; if it is off, it will be turned on. Default: on.

4.4.4 Image Box

Clicking Image Box allows the operator to toggle the display of a square box that follows the cursor in the primary image. The extent of the box reflects the region of the primary image shown in the Tracking window. If the image tracking box is currently displayed, it will be removed; if the box is not displayed (and tracking is enabled) it will be toggled on. Default: off.

4.4.5 Image Bounds

Clicking Image Bounds toggles the display of the crop box or sub-image bounding box surrounding the image region to be analyzed. If the bounding box is currently displayed, it will be removed; if the bounding box is not displayed, it will be toggled on. Default: on.

4.4.6 Image Coordinates

Clicking Coordinates toggles the display of the cursor's current x,y coordinates within the primary image. If the coordinates are currently displayed, the option will be turned off. If the coordinate option is currently off, it will be turned on. Default: on.

4.4.7 Overlays

Clicking Overlays toggles the display of the lane and band demarcation lines. If the lane and band overlays are currently displayed they will be removed; likewise, if they are not displayed (but have been identified), they will be displayed. Default: on.

4.4.8 Color Table Adjustments Menu

Clicking Color Adjust toggles on or off the display of the Color Table Adjustments Menu. Default: on.

4.4.9 Template Edit

During routine analysis of the Primary Image, after lanes have been delineated but before size-standard bands are detected, you are allowed to edit the size-standard template. The number of bands in each size-standard lane must equal the number of bands in the template. Although the template may be edited from the Options Submenu, here, it is more often edited in the normal course of analysis. Please consult Section 5.2 for details.

4.4.10 CCD Mode

This option is specifically for CCD images from Photometrics cameras and not of interest to most users. Image files with CCD headers may be saved as Import images, depending upon the CCD mode. Clicking CCD Mode displays the CCD Modes Popup as in Figure 4.5 below. The popup already highlights the current choice (default is Mode 1). Click in the window of choice; the popup disappears, and the choice is recorded. The choice remains until changed via this popup or until the program is reloaded.

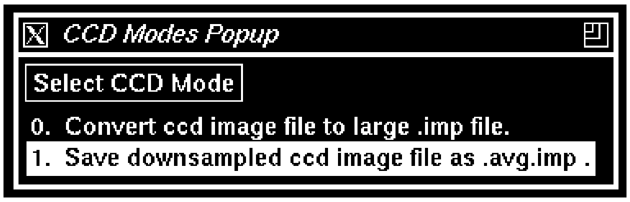

Figure 4.5: The CCD Modes Popup.

4.4.11 Band Threshold

The algorithm for automatic band detection, for either marker-lane bands or experimental bands, uses a threshold value for deciding if a region of the image is likely to contain a band. This threshold value has been empirically determined; it is based on the kinds of images processed at our laboratories. However, occasional problem images (bands too dark or too light) forced the addition of an adjustable threshold for which more true bands might be detected and fewer artifacts detected as bands. The threshold may be changed via a sliderbar in a popup window.

- From the Commands Menu, click Options.
- From the Options Submenu, click Band Thresh. The Band Threshold popup appears (Figure 4.6).
- With MB2, adjust the slider for the desired effect; to the left to detect more bands or to the right for fewer bands. An index number appears in a small box; the default = 1.00. Its range is 0.50 (more bands) to 1.50 (fewer bands).
- To reset the threshold to 1.00, click Reset.
- To accept the current value, click OK, which also pops down the Band Threshold popup and returns control to the Options Submenu.
- Click Done to return to the Commands Menu.
- Note: the threshold value remains in effect until changed again or until the program exits. The value is not changed when the current image is closed nor when a new image is opened.

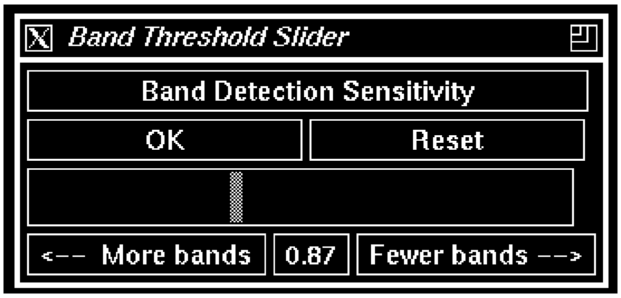

Figure 4.6: The Band Threshold Slider.

4.4.12 Saving Lane Images

Clicking Lane Save toggles on or off the saving of individual lane images when Save Results is clicked. Lane images saved may be recalled as extra images. Default: off.

4.4.13 Reset Bands

Clears size-standard bands in the pre-normalized image or experimental bands in the normalized image.

4.4.14 Done

Clicking Done removes the Options Submenu from the display and activates the Commands Menu.

4.5 Profiles Submenu

The options of the Profiles Submenu allow one to view vertical profile intensity graphs for the primary image and any extra images. To open the Profiles Submenu, click Profiles in the Commands Menu. The Profiles Submenu will be displayed as in Figure 4.7.

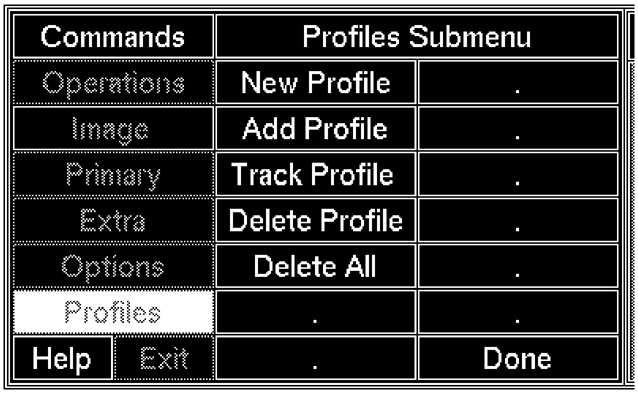

Figure 4.7: The Profiles Submenu.

Figure 4.8 shows image **k102** with a single vertical profile line and superimposed “Vertical Profile Graph”. This Figure will be referenced in the subsections which follow.

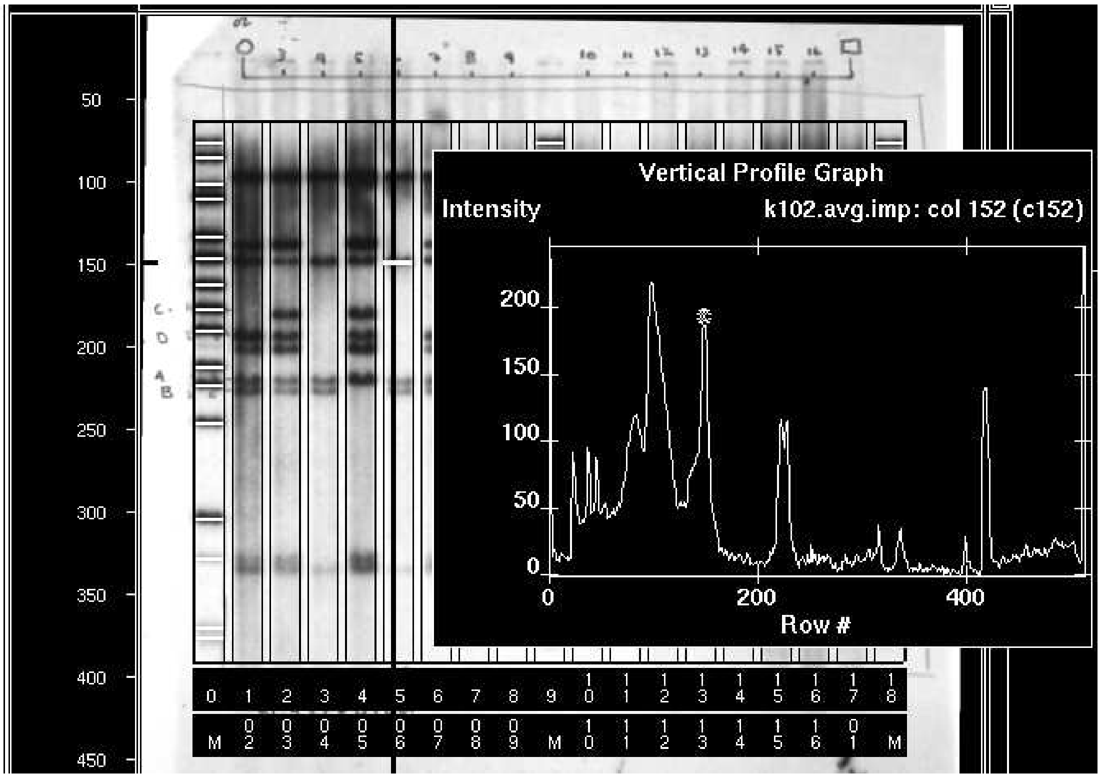

Figure 4.8: Image **k102** with a Vertical Profile and Profile Graph.

4.5.1 New Profile

Clicking $\boxed{\text{New Profile}}$ allows you to get a one-dimensional vertical profile anywhere in the primary image or any extra image (although there are slight differences). Move the pointer into the primary image, then depress and hold MB1; a vertical line appears in the image. As long as MB1 is depressed, the vertical line can be moved from side-to-side. When MB1 is released, the line is fixed. In Figure 4.8, a vertical line has been placed at column 152 of the image; the vertical line stretches from top to bottom. The line is black in the Figure but green on the screen. When MB1 is released, a graph of intensity values versus row numbers appears in the lower-right hand corner. In the Figure, the Vertical Profile Graph has been superimposed over the image. You may continue to add profiles without re-clicking $\boxed{\text{New Profile}}$. A subsequent depress of MB1 in the image will create a new vertical line and a new Vertical Profile Graph. While the vertical lines will all be visible, a new graph is placed over previous ones, as in a stack of paper. However, the graphs can be moved around using the Move option in the Extra Image Submenu. A graph may also be resized using the Resize option in the Extra Image Submenu. For profiles in extra images, only the green profile line and temporary profile graph are shown.

4.5.2 Add Profile

Clicking $\boxed{\text{Add Profile}}$ is ineffective until a profile exists. To display two profiles in the same Vertical Profile Graph (after the New Profile option has been selected), click $\boxed{\text{Add Profile}}$ and then depress MB1 in the image. Subsequent depression of MB1 in the image continues to superimpose profile data graphs on the original. Superimposed plots alternate in three different colors which can be modified by editing the applications-default resource file. Added profiles need not all come from the primary image; they may come from extra images as well.

4.5.3 Track Profile

This option applies only to the primary image and only when lane boundaries have been detected and displayed. If the last new profile is in a lane of the primary image, click $\boxed{\text{Track Profile}}$, then move the pointer to the last Vertical Profile Graph established from the primary image. Depress MB1 in the Vertical Profile Graph. Keep MB1 depressed and move the pointer back and forth in a horizontal direction. While so doing, the program plots a small double circle which will track along the data in the Vertical Profile Graph and give the appearance of a bouncing ball. In Figure 4.8, the circle can be seen atop the second major peak from the left. At the same time, a horizontal line moves up and down in the lane corresponding to the profile graph; in the Figure, a white line appears across Lane 5 just above the 150-pixel level. Also, a smaller horizontal line moves up and down to the left of the primary image next to the pixel-index axis to give an idea of the pixel location; in the Figure, a short black line appears just above and to the right of the 150-pixel tic mark. The bouncing ball and horizontal lines are intended to visually coordinate the position in the Vertical Profile Graph with the position in the primary image. When MB1 is released, the tracking circles and horizontal lines in the image disappear. They reappear when MB1 is depressed again.

4.5.4 Delete Profile

To remove a profile, click $\boxed{\text{Delete Profile}}$, then move the pointer into the Vertical Profile Graph which is to be removed. Click MB1; the graph and vertical line on the image will disappear.

4.5.5 Delete All

To remove all profiles, click $\boxed{\text{Delete All}}$.

4.5.6 Done

Click $\boxed{\text{Done}}$ to close the Profiles Submenu and reactivate the Commands Menu. Any displayed profiles remain.

Chapter 5

Miscellaneous Features

The previous chapters described the various submenus of the Commands Menu. This chapter describes miscellaneous features, which you may find useful in helping to analyze an image. Some of these are accessed from the submenus already described, but detailed descriptions of them are presented here. A list of the features appears in Table 5.1 below.

Table 5.1: Miscellaneous Features.

- Color Adjustments
- Template Edit
- Miscellaneous Toggles
- Saving and Retrieving Lane Images
- Band Fragment-Size Editing
- Non-Import (Odd) Images
- Creating Gel-Format and Template-Definition Files

5.1 Color Adjustments

A color map is a list of colors corresponding to the image pixel values. In DNA/GUI, no matter what the range of an image's pixel values, the displayed representation of that image is scaled to a pixel value range of 0 to 255. While the majority of graphics workstations running DNA/GUI allow 256 colors, only about 200 are actually used for the image; the remainder are dedicated to the various menus and outlines. Thus a displayed image with the full range of 256 different pixel values must be mapped to the available 200 colors.

The 200 colors can be any arrangement of hues and shades; but a menu of some 25 arrangements is already available for the user's convenience. Such an arrangement is called a *colormap*. Other arrangements may be designed, but some understanding of the program code would be necessary; it is beyond the scope of this User's Guide. The default colormap can be seen in the colorbar just to the right of the image. It is read from top to bottom (highest to lowest); the top color corresponds to a pixel value of 255 (most intense), while the bottom color corresponds to a pixel value of 0 (least intense). The default colormap is a continuous gray scale, white to black.

The installed color map may be replaced with another map or modified in several ways described below. The menu for making adjustments appears in the lower right of the screen and is shown in Figure 5.1 below. This menu consists of two groups of buttons, five sliderbars, Reset (red) and Color Palette Menu (green). Reset simply resets the installed colormap to the colors before any manipulations took place. Color Palette Menu pops up the Color File Browser, from which an alternate color map may be chosen.

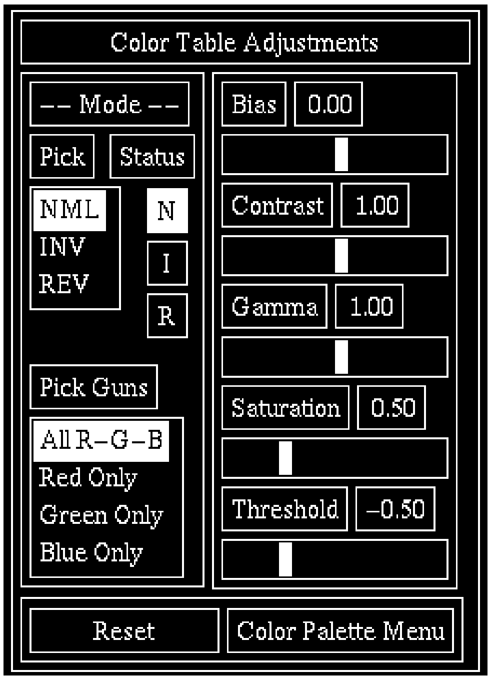

Figure 5.1: Color Adjust Menu.

5.1.1 Changing Color Maps

A new colormap is selected via the Color File Browser, which appears in Figure 5.2 below. The Color File Browser may be popped up in two ways. The easiest way is to click the green Color Palette Menu button in the Color Adjustments Menu to the lower right of the screen. The other way is via the Options Submenu: from the Commands Menu, click Options ; then click the Color Map in the Options Submenu. Whichever way is chosen, the Color File Browser appears, showing the names of the available colormaps. The suffix *.pal* stands for "palette". Click on a color palette and notice the change in the image and the colorbar.

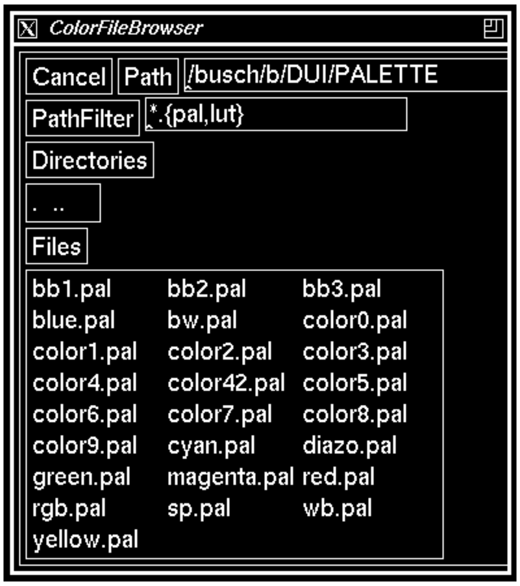

Figure 5.2: Color File Browser.

One popular colormap is *bb3.pal* which represents a black-body spectrum ranging from intense white through yellows, oranges, reds to black. Another interesting colormap is *color42.pal* which uses 20 different colors to show 5% image differences. Users are encouraged to try different colormaps. The maps do not in any way affect the image values. Some colormaps may offer more insight than others; different persons may prefer different colormaps. Additional details for the available colormaps may be found in Appendix A.7.

5.1.2 Adjusting the Installed Color Map

5.1.2.1 Bias, Contrast, Gamma, Saturation, Threshold

The bias, contrast, gamma, saturation, and threshold sliderbars may be used to gradually alter the installed color table. To use one of the sliders, place the pointer over the bar in one of the sliders. While depressing MB2, move the bar left or right and note the gradual color changes in the colorbar and, if present, any image. Releasing MB2 freezes that slider until that slider is again selected or the color adjustments menu is reset (note $\boxed{\text{Reset}}$ at the bottom). The values shown are simply weights used to change the installed color table; the user need not be concerned with these values. The sliderbars affect only the installed color table; the image pixel values are not changed in any way. The bias, contrast, gamma, saturation, threshold names are technical terms; details of these terms are beyond the scope of this User's Guide.

5.1.2.2 Inverting and Reversing Color Map

The normal, invert, and reverse modes are different ways of rearranging the installed color table. The normal mode is simply the way it is arranged when first installed. The invert process takes each color-table intensity value (range 0.0 to 1.0) and subtracts that value from 1.0. The invert effect is for whites to become blacks, reds to become cyans, blues to become yellows, etc. The reverse mode switches the positions in the color table. That is, if the first position represents the lowest intensity value and the last position the highest intensity value, the reverse effect causes the first position to correspond to the highest intensity value and the last position to be the lowest intensity value. Successive mode changes create different states. For example, inverting twice in a row returns the status to normal. For another example, successive choices of invert and reverse leave the status as inverted and reversed. Note that the mode selection buttons are to the left while the current status appears to the right.

5.1.2.3 Adjusting Individual R-G-B Guns

When any slider or sliders are moved, the color table intensity values are changed. As a default, all three guns (red, green, blue) are affected the same way. However, selection of any of the individual red, green, or blue guns in the "Pick Guns" menu means that only the selected gun will be affected by subsequent slider movements or by mode changes. The choices are all three guns OR any one of the three. The current version of this program does not allow for any two.

5.2 Template Edit

After an image has been cropped and its lane boundaries determined, your next step is to detect the bands in the marker lanes. You do this by clicking Std Bands in the Operations Submenu. Before attempting automatic detection of these standard bands, you are asked if you would like to edit the template of standard bands. In the Basic Tutorial, you declined; and the program performed automatic band detection.

Recall that the template is a listing of fragment sizes and mobilities against which an image is "normalized" so that images using the same template might be compared. But perhaps the image to be analyzed contains artifacts or is partially blurred such that not all bands in the image's marker lanes are clearly distinguishable. For this reason, you may choose to temporarily eliminate a band or several from the template. Before normalizing the image, the number of band bars in each marker lane must correspond to the number of band bars in the template. Thus, if some bands in the image are not clearly visible, the appropriate band bars in the template should be eliminated. Caution: the normalization algorithm needs at least 4 band bars in each marker lane. This tool is used only to delete bands from a previously defined template.

- From the Operations Submenu, click Std Bands.
- The Template Edit Dialog appears (Figure 5.3), asking whether to do Template Edit; click Yes .
- The Template Edit Popup appears (Figure 5.4, left). Red bars appear to the left, signifying the relative mobilities of the template bands. Fragment sizes, in boxes, appear to the right. Lines are drawn between corresponding bands and sizes.
- No new bands may be added to the template.
- To delete a band from the template, move the pointer to the band or to the corresponding fragment size number and click MB2; the band and its size will disappear. The line between remains, leaving a visual clue of elimination. Figure 5.4, right, shows that two bands have been eliminated.
- Clicking MB1 restores all bands.
- Clicking MB3 stores the edited list of template bands and removes the Template Edit display.
- Automatic detection of the image's marker bands commences immediately. Editing of the marker bands follows.
- If the number of marker bands in each marker lane does not correspond to the number in the (edited) template, the program will not allow the image to be normalized.

Figure 5.3: The Template Edit Dialog.

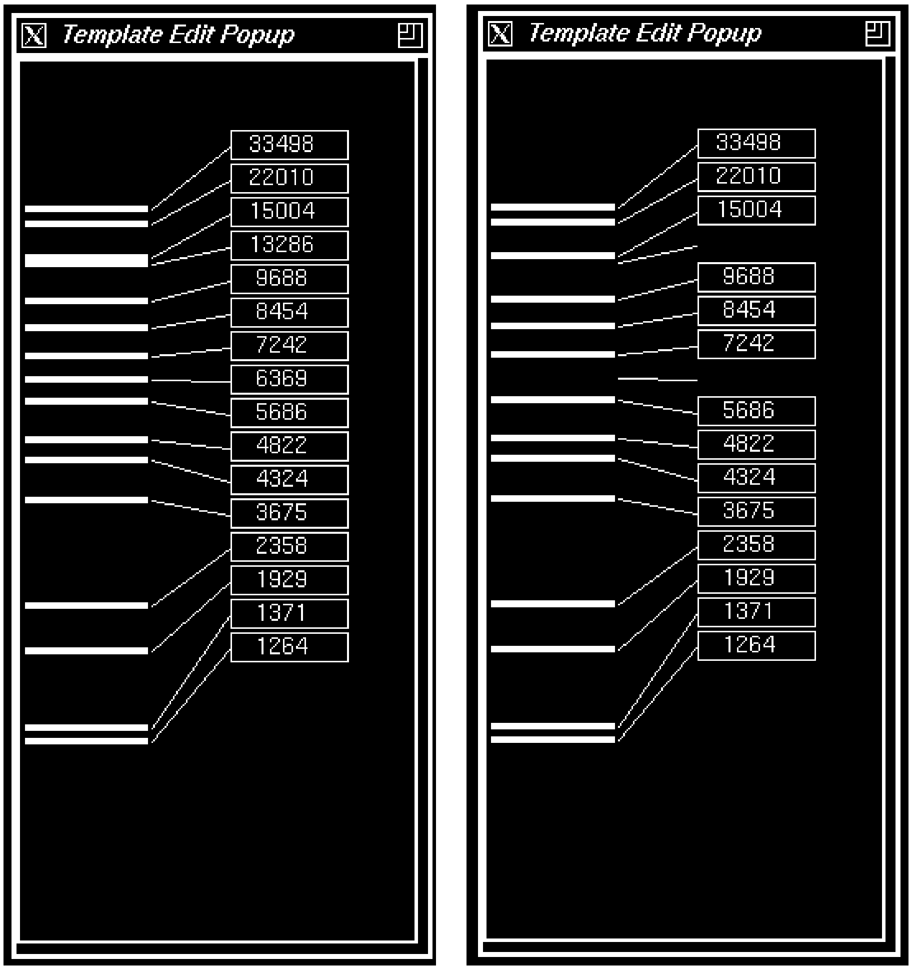

Figure 5.4: Template Edit Popup Before (left) and After (right) Edit.

5.3 Miscellaneous Toggles

There are three buttons above the vertical scrollbar to the right of the primary image (see Figure 2.1). They are somewhat, but not quite, like toggle buttons. By color and from top to bottom, these are:

- Magenta – allele colors toggle
- Blue – lane stripes toggle
- Red – magnified crop box toggle

5.3.1 Allele Colors Toggle

If inheritance is active (Section 6.1) and experimental bands have been detected, instead of viewing the experimental bands as green stripes, you may view them by allele with this button. This first click of this button changes the green band marks to variable colored band colors; that is, the bands are color-coded by fragment size. A legend of the colors and sizes appears in a window to the left of the crop box. Clicking the button again moves the legend window to the right of the crop box. Clicking the button a third time removes the legend window and restores the experimental band marks to green.

5.3.2 Lane Stripes Toggle

After lanes have been delineated, stripes appear above or below the crop box. A blue stripe contains lane index numbers. A green stripe contains lane labels. If inheritance is active, a red stripe contains inheritance codes. The Lane Stripes toggle is a 3-way toggle: “above” the crop box, “below” the crop box, and “off”. Successive clicks cycle the stripes through these three states. A crop box which extends to the top and bottom of the image space may obscure or hide these stripes.

5.3.3 Magnified Crop Box Toggle

Assuming the primary image is displayed and has been cropped, clicking this button will popup a window containing the image of what is contained in the crop box magnified 2X. If bands have been detected, they are shown in the traditional red (marker bands) and green (experimental bands). The magnified crop box is removed by clicking in the magnified crop box.

5.4 Saving and Retrieving Lane Images

Some applications may need or prefer to save lane images in addition to or in place of the full image. A good example would be to do a visual comparison of a saved lane from one image with a lane or several lanes of another image or several lanes from different images. While the default is NOT to save lane images, they may saved by setting a toggle in the Options Submenu. The lane image may later be recalled for display via the Image Submenu. However, only the lane image data are saved; band detection marks within a lane are not saved with the lane image. For a given image, if the toggle to save lanes is enabled, all lane images are saved; if individual lane images are to be deleted, you will have to eliminate them from the UNIX file system independent of DNA/GUI. Here's the process to save lane images:

- From the Commands Menu, click [Options].
- From the Options Submenu, click [Lane Save]. Two messages are posted to the Status Information Region:

  "Saving Lane Images enabled."

  "Save Lane Profiles enabled." (ignore)
- In the Options Submenu, click [Done].
- From the Commands Menu, click [Operations].
- From the Operations Submenu, click [Save Results]. Assuming an image is displayed and has been analyzed at least through delineating lane boundaries, any interim results are saved in the import file (section 3.8). In addition, individual lane images are saved. They are saved as import files, one file per lane, with the image data embedded with the import information. For example, image names for lane 8 would be:

  k102.avg.imp.ln8.imp (pre-normalized),

  k102.avg.w.imp.ln8.w.imp (normalized).

And to recall a lane image:

- From the Commands Menu, click [Image].
- From the Image Submenu, click [Open Extra]. The Image File Browser appears; click over the name of a lane image. The outline of the lane image will extend from the location of the pointer; move the pointer to where you would like the image placed; click MB1. The image will be displayed at that point.
- Other lane images may be displayed; again, click [Open Extra].

Note: When the primary image is closed, the toggle to save lane images is disabled (no lane save) and must be reset after each primary image is opened.

5.5 Band Fragment-Size Editing

Fragment sizes may be edited on an intralane or across-lane basis. Both marker and experiemntal band sizes may be edited. This size-edit feature is designed for making small, incremental changes to sizes; you may find it cumbersome for making large changes. For large changes, editing of the placement of the band is recommended. Selecting the Size Edit button pops a menu from which one chooses horizontal (across lanes) or vertical (intralane) editing. From the Commands Menu, click Operations ; then click Size Edit . The Size Edit Popup appears, as in Figure 5.5. The Edit Vertical and Edit Horizontal buttons are explained below. Clicking the Cancel button removes the Size Edit Popup.

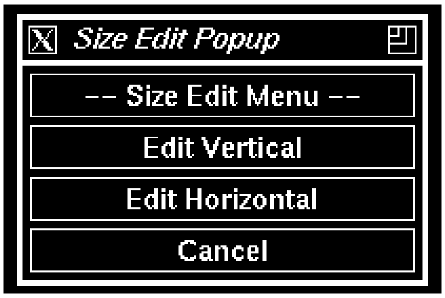

Figure 5.5: The Size Edit Popup.

5.5.1 Edit Vertical

If you click Edit Vertical , the Size Edit Popup menu disappears. A Vertical Size Edit Popup (Figure 5.6) appears to the right of the image. Clicking its Done button at any time removes this popup and returns control to the Operations Submenu. Everything below the Done button refers to a single lane within the image; by default, the leftmost lane of the image is represented when the popup first appears.

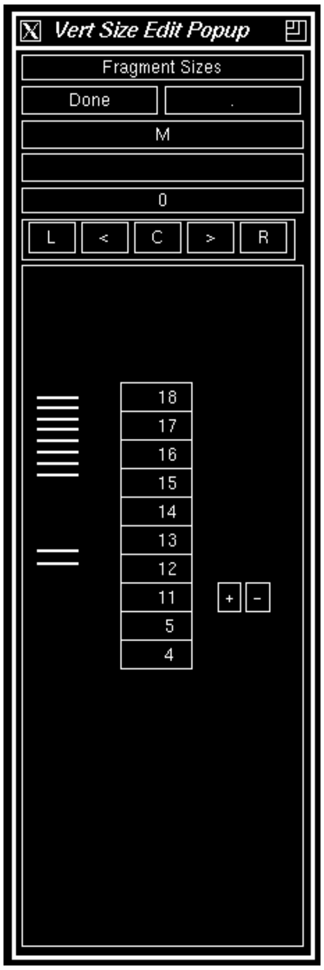

Figure 5.6: The Vertical Size Edit Popup.

Below the $\boxed{\text{Done}}$ button are 3 colored stripes containing the labels from the lane-label, inheritance, and lane index stripes for the lane involved. In Figure 5.6: the lane-label is "M" (for marker lane); the inheritance-label is blank denoting that this lane is not a family member; and the lane-index label is "0" (index position "0").

Lanes are changed with 5 buttons below the labels.

- $\boxed{\text{L}}$ takes you to the leftmost lane.
- $\boxed{<}$ takes you one lane to the left.
- $\boxed{\text{C}}$ takes you to the center lane.
- $\boxed{>}$ takes you one lane to the right.
- $\boxed{\text{R}}$ takes you to the rightmost lane.

For any lane, below these 5 buttons, are displayed horizontal bars to indicate the relative locations of bands in that lane. If the lane is a marker lane, these bars are colored red; otherwise, the bars are colored green. Next to each bar is the fragment size displayed in a box. Where the bands are tightly packed, the boxes containing the fragment sizes may be spread out, as is the case in Figure 5.6.

To edit any size, click in the box containing the size. A pair of buttons, $\boxed{+}\,\boxed{-}$, appears next to the box containing the size to be edited. In Figure 5.6, this pair is next to the box showing fragment size = 11. Sizes are incremented or decremented by clicking these $\boxed{+}$ and $\boxed{-}$ buttons. The upper limit of increment is 1 less than the size of the next band; if there are no higher-sized bands, there is no upper limit. The lower limit is 1 more than the size of the next lower band; if there are no lower-sized bands, the lower limit is 0. Size changes are effective immediately and cannot be automatically reset. Editing ceases when $\boxed{\text{Done}}$ is clicked.

5.5.2 Edit Horizontal

If you click $\boxed{\text{Edit Horizontal}}$, the Size-Edit popup menu disappears. Two thin blue horizontal lines span the image, extending from the left of the image to the top and bottom of the scroll thumb in the vertical scrollbar to the right of the image. By moving the scroll thumb up or down with MB2, the thin blue lines move along. An example appears in Figure 5.7. These lines bracket a region of interest whose fragment sizes appear the Horizontal Size Edit Popup as in Figure 5.8. The blue lines are separated by a vertical distance of 7 pixels.

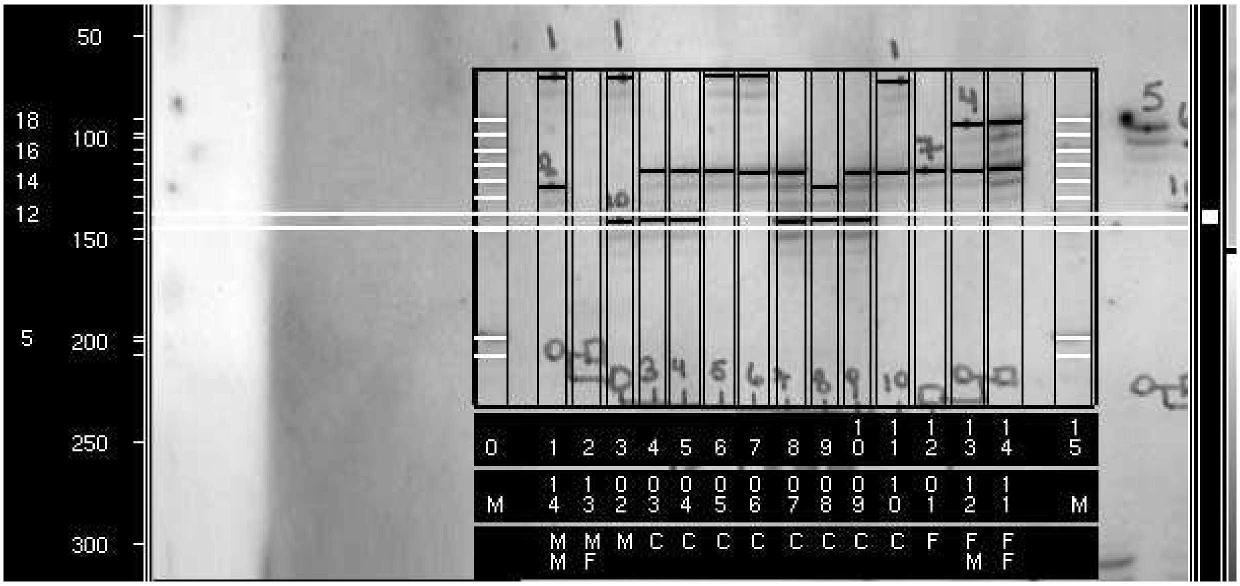

Figure 5.7: Horizontal Size Edit Bracket Lines in the Primary Image.

The Horizontal Size Edit Popup appears above the image. This popup consists of a rectangular grid of tiles. The grid width corresponds to the number of lanes on the image. The grid height is 10 tiles; the top 3 tiles are for representing the same labels as those of the lane-index, lane-label, and inheritance stripes for a given lane while the bottom 7 are for fragment sizes of bands appearing between the two blue horizontal lines in that lane. The 7 tiles correspond to the 7 pixels separating the blue lines.

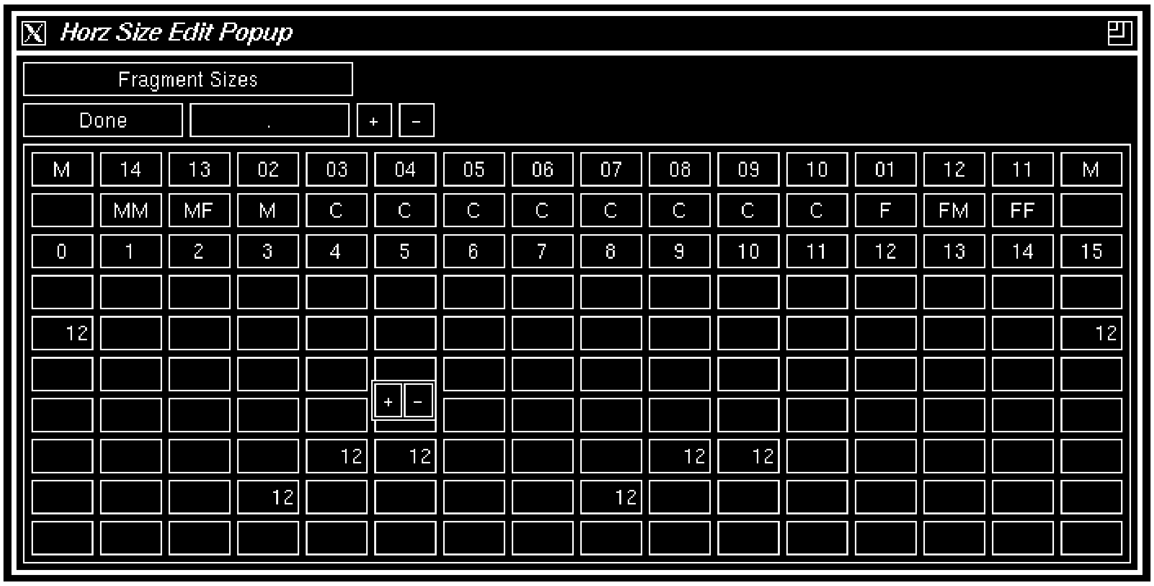

Figure 5.8: The Horizontal Size Edit Popup.

Move the scroll thumb up or down until it includes several bands in several lanes. Note the sizes in the tiles within the popup above the image. To change the value of any fragment size, click in the tile containing that size. Appearing above the tile will be two small buttons: $\boxed{+}\,\boxed{-}$. Click in either of these and the size will increment or decrement by one. You may continue clicking (incrementing or decrementing) until the size is correct. The upper limit of increment is 1 less than the size of the next band within the lane; if there are no higher-sized bands, there is no upper limit. The lower limit is 1 more than the size of the next lower band; if there are no lower-sized bands, the lower limit is 0. Size changes are effective immediately and cannot be automatically reset.

Above the grid are three buttons: $\boxed{\text{Done}}\,\boxed{+}\,\boxed{-}$. Clicking $\boxed{+}$ moves the scroll thumb and two blue lines 1 pixel down in the image while clicking $\boxed{-}$ moves them 1 pixel up. Clicking $\boxed{\text{Done}}$ removes the Horizontal Size Edit poup and returns control to the Operations Submenu.

5.6 Non-import (Odd) Images

If you attempt to open an image as the primary image when it is not an import file, you will be presented with an Odd Image Popup in which you will describe your image using the buttons and text fields in the popup. As of now, only "char" and "short" images are recognized. Once you have clicked $\boxed{\text{OK}}$ in this popup, the program will create an import file which it will use for analysis. Your original image will not be changed. The Odd Image Popup looks like this:

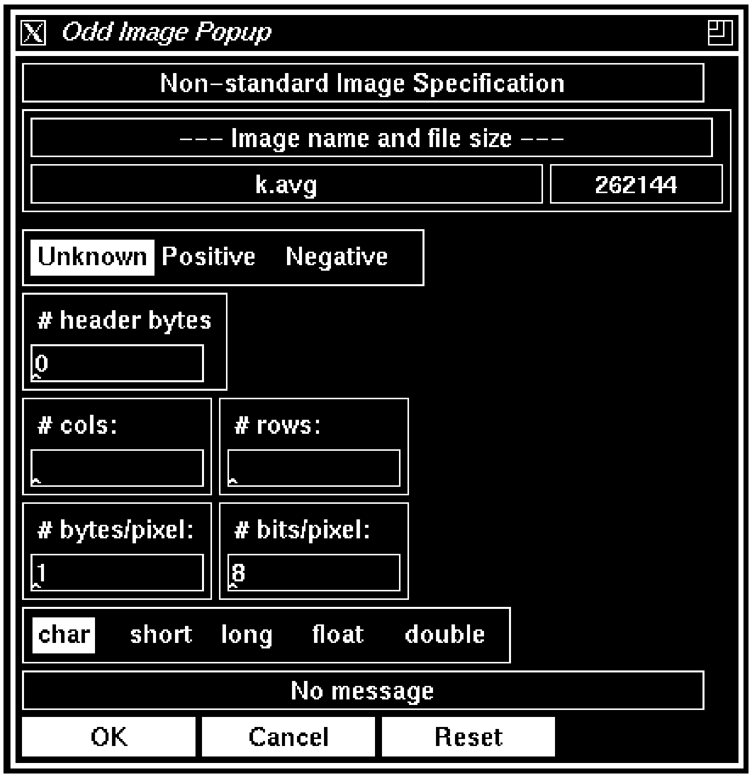

Figure 5.9: Odd Image Popup

A top-to-bottom description of this popup follows:

- The file name appears to the upper left; the number of bytes to the right. These fields cannot be changed.
- On the next line, you must specify whether the image is positive or negative. If the image is a directly-acquired gel image, click Positive. If the image is from an autoradiogram, click Negative. If left unknown, or if Unknown is clicked, you will have to decide later.
- If the image has a header, enter the number of bytes. The program assumes the header is entirely before the image data.
- On the next line, specify the number of columns and rows.
- On the next line, specify the bytes-per-pixel and bits-per-pixel.
- On the next line, click on the kind of image (char or byte, short, etc.).
- When all fields are properly set, click OK.

  If the information provided makes sense to the program, the popup disappears, an import file is created, and the image displayed and ready for analysis.

  If your description of the image doesn't match the number of bytes in the file, a message is posted just above the buttons along the bottom of the popup. Most often, the number of bytes in the image doesn't match the description of header bytes, rows and columns, bytes and bits per pixel.
- Clicking Cancel pops down the popup and returns control to the Operations Submenu; no image is opened.
- Clicking Reset returns the popup to its default state.

5.7 On-line Creation the Gel-Format and Template-Definition Files

In Section 3.2 describing the choosing of pre-existing gel-format and template-definition files to used in the analysis of the primary image, mention was made of the ability to create, on-line, new gel-format and template-definition files. Those steps are described here and use the popups which are shown in Figure 5.10.

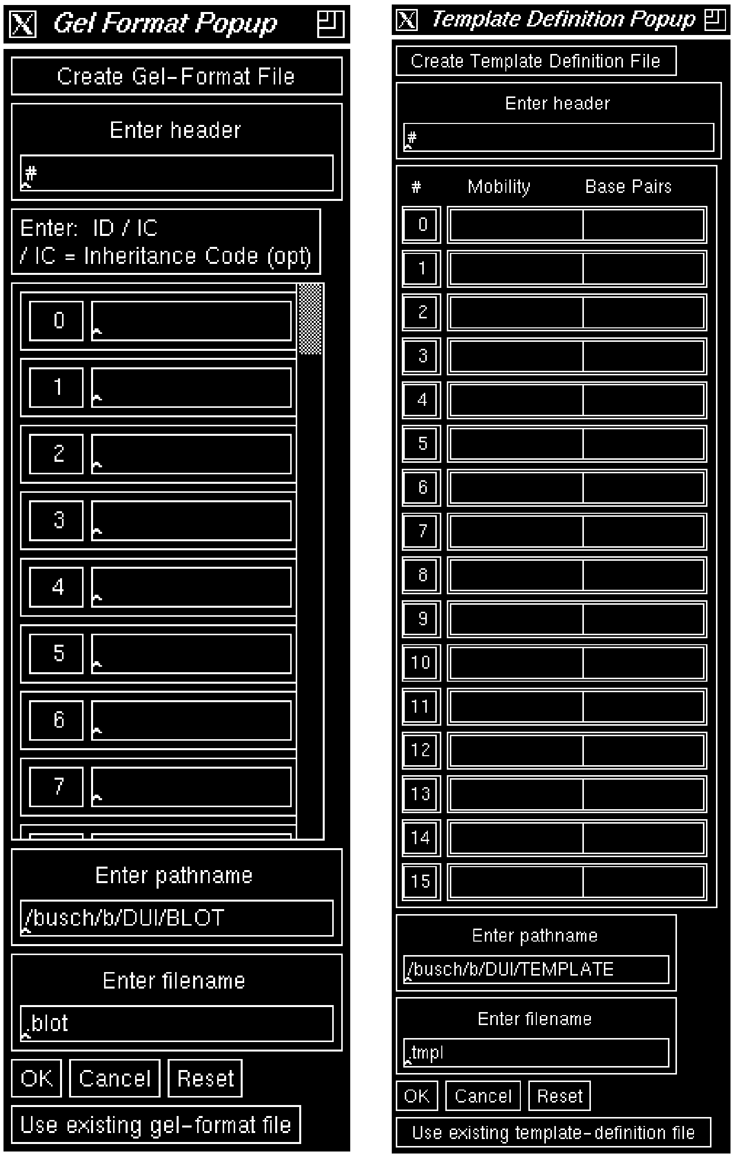

Figure 5.10: Gel-Format and Template-Definition Popups.

5.7.1 Creating a Gel-Format File

The left side of Figure 5.10 shows the Gel Format Popup. This popup and the means to create a new gel-format file are described here.

- In the box below "Enter header", you may put anything but the first character must be a "#"; this indicates the first line of the file is a comment. Without it, the line is treated as part of the data which are lane labels.
- The next box gives a brief instruction for the lane label format. If the image involved is not an inheritance image, the format is simply the lane ID string of characters. There are no restrictions on length, but only the last 2 characters will be displayed when the lanes of the image have been fixed. If the image is an inheritance image, the format is lane ID slash inheritance code.
- The next block is a scrolled window. Here you enter the lane IDs in a larger box on each line. The ascending numbers to the left are simply to help you remember which lane is which; the left lane of the image would be "0". The first line in which there is no label terminates the file; thus, do not leave any labels when defining lanes.
- The default pathname is that specified in the app-defaults file. It can be changed.
- A filename must be entered and must end in ".blot".
- Click OK when you are ready to save the file. Errors will be reported in the Status Information Region.
- Click Cancel to abort creation of the new file.
- Click Reset to reset the popup to its default values.

5.7.2 Creating a Template-Definition File

The right side of Figure 5.10 shows the Template-Definition Popup. This popup and the means to create a new template-definition file are described here.

- In the box below "Enter header", you may put anything but the first character must be a "#"; this indicates the first line of the file is a comment. Without it, the line is treated as part of the data which are band mobilities.
- The data are entered in the next block of blank spaces. The mobility goes in left column and base-pairs to the right. The index numbers (0-15) help you keep track of where you are. As the index numbers, increase, mobilities must increase and base-pairs must decrease.
- The default pathname is that specified in the app-defaults file. It can be changed.
- A filename must be entered and must end in ".tmpl".
- Click $\boxed{\text{OK}}$ when you are ready to save the file. Errors will be reported in the Status Information Region.
- Click $\boxed{\text{Cancel}}$ to abort creation of the new file.
- Click $\boxed{\text{Reset}}$ to reset the popup to its default values.

Chapter 6

Extensions

This chapter of Extensions includes things which are under development and/or are special topics and procedures which have very specific application.

6.1 Inheritance Images

6.1.1 Inheritance: Background

A local genetic linkage study prompted the addition of this feature to DNA/GUI which we vaguely label "inheritance". However, the feature is quite limited and for a very specific application. The inheritance logic is limited to 2 alleles across 3 generations (grandparents, parents, children).

Each experimental lane in the image belongs to one person. There are specific inheritance codes which are used to identify which lanes belong to which generations; these codes are included with the lane-label information in the **gel-format file**. There should be at least 1 band and at most 2 bands in each experimental lane. Bands of the same sizes, across lanes, are said to have the same allele.

The inheritance algorithm is applied to the detected experimental bands. Parent band fragment sizes are confirmed, and the child band fragment sizes checked against the parents. Bands which should appear to be inherited from parents but have a small discrepancy (e.g. 1 base pair), because of poor image quality, image warp, etc., will have their sizes edited to conform to natural inheritance. Missing data and ambiguities are reported. The inheritance algorithms and analysis procedures for band sizing and allele assignment follow in the sections below.

6.1.2 Inheritance: Algorithms

In practice, the parent sizes must be known; the child checks are not applied until the user confirms parent sizes. Discrepancies between grandparents and parents are noted, but will not keep the program from the ultimate parent/child checks. In fact, grandparent information need not be present. Here's a brief summary of the inheritance logic.

- Both mother and father lanes must be present.
- There must be at least one band bar but no more than two band bars in each of the mother and father lanes.
- Grandparent lanes need not be present.
- Grandparent-to-parent discrepancies are noted but will not prevent parent-to-child propagation of parent sizes.
- The user must confirm that the parent sizes are accurate and edit them if they are not.
- For each child (lane), its fragment sizes are compared to those of the parents. The combination of child-parent band-size matches which gives the best "fit" (in a mathematical sense) is assumed to be the most likely match. In essence, the program determines the combination of one paternal band and one maternal band in a child which results in the least discrepancy. Furthermore, this discrepancy is limited to a maximum of one base pair for each band.
- Discrepancies can be edited (see section 5.5) or resolved through propagation; the user makes the ultimate size determinations.

6.1.3 Inheritance: Band-Sizing and Allele Assignment

- Just before an image is opened for analysis, the "inheritance" flag must be turned-on (see section 3.1). The image is opened in the usual manner.
- The selected **gel-format file** must include inheritance codes (see Appendix A.3).
- The image is cropped and lanes detected in the usual manner.
- After lanes have been demarcated, in addition to the lane-label (green) and lane-index (blue) stripes above or below the cropped image, an inheritance stripe (red) shows inheritance codes which are:

  MF - mother's father,

  MM - mother's mother,

  FF - father's father,

  FM - father's mother,

  M - mother,

  F - father,

  C - child,

  X - unknown (an extra lane).

- An inheritance image (before normalization) with lane-index, lane-label, and inheritance stripes is shown in Figure 6.1.

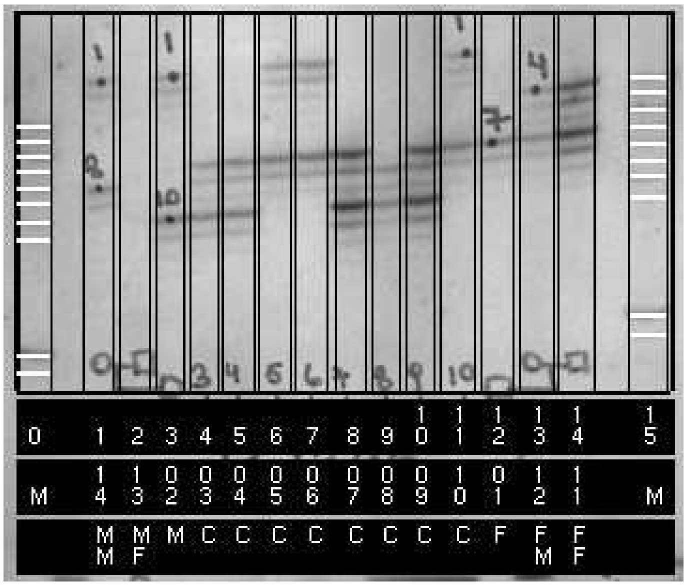

Figure 6.1: Lane-Index, Lane-Label, and Inheritance Stripes.

- Detection of standard bands, normalize, and detection of experimental bands is the same as any other image.

- From the Operations Submenu, click Inheritance.
- Some preliminary checks are made. If there are problems which need to be resolved, messages will appear in the Status Information Region. In addition, a discrepant lane's vertical boundaries are re-drawn in red. If all is well, a Parent Size Popup appears, asking that you confirm the parent sizes.

  If the parent sizes are incorrect, click Cancel; no further action is taken, and you may click Size Edit to modify the current parent band sizes (see section 5.5).

  If the parent sizes are correct, click Accept. At this point, the program checks to make sure each child's bands' sizes conform to the parents' bands. If sizes are but off by 1 base-pair, the sizes are changed to equal those of the parents.
- At any time, before or after inheritance processing, you can view the experimental band bars by coded allele color instead of the usual green color for all experimental band bars. To view by allele color, click the small magenta button above the scrollbar to the right of the primary image. This is really a 3-way toggle button with 3 states:
  1. "Off". The red marker band bars and green experimental band bars are shown.
  2. "On-left". The red marker band bars are turned off; the experimental band bars are color-coded by allele. To the left of the crop box is an allele window showing allele sizes and allele colors.
  3. "On-right". Same as On-left except that the allele window is to the right of the crop box.
- Figure 6.2 shows the allele window to the left of the cropped image. Imagine that the white allele bars are different colors. Note that the first band of the child whose lane label is **08** should prompt one to ask who is the child's father.
- While the allele window is displayed, an allele summary appears above the image. Figure 6.3 shows the allele summary.
- Results are saved in the usual way (section 3.8). Click Save Results.

Figure 6.2: The Allele Window.

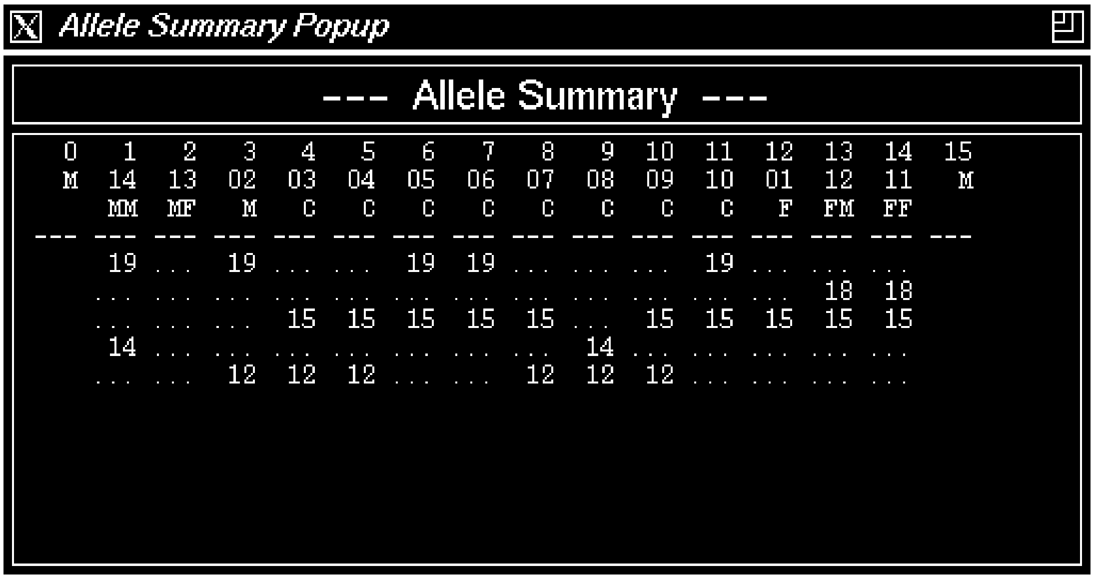

Figure 6.3: The Allele Summary.

Appendix A

Appendices

A.1 The Application Defaults File

There are several paths that DNA/GUI looks for that are given in the application defaults file. They are the path of the colortables, gel-format files, template-definition files, comb-geometry files, sequence files, and on-line help. The app-defaults file `dui.ad` should be set up so that the directories are in the same path as the executable "dui".

```
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!                                               !
!  Files used by DNA/GUI.                       !
!                                               !
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

! Location of color maps
*colorPath:                             ./PALETTE
! Location of gel-format files
*gelformatPath:                         ./GEL-FORMAT
! Location of templates
*templatePath:                          ./TEMPLATE-DEFINITION
! Location of on-line comb-geometryfiles
*combPath:                              ./COMB
! Location of on-line help text files
*helptextPath:                          ./HELP
! Location of on-line sequence files
*sequencePath:                          ./SEQUENCE
! Routine for SSRP images
*lfastaCommand:                         ./bin/lfasta -a -n
```

A.2 Initial contents of the import file k102.avg.imp

The contents of the import file are given here:

```
# Washington University Image File Format
RasterFile k102.avg
RasterWidth 500
RasterHeight 512
RasterBytesPerRow 500
RasterBitsPerPixel 8
Format Integer
MinimumValue 0
MaximumValue 255
ImageType Negative
```

Table A.1: Initial state of import file **k102.avg.imp**.

A.3 Creating a Gel-Format File

The **gel-format file** contains the format of the gel. The file contains one comment line (the first line) and one line for each of the lanes contained in the image. The letter `M` is used to identify size-standard lanes. Any alphanumeric string can be used to identify each of the experimental lanes. The lane labels, which appear in a stripe above or below the cropped image, are taken from these strings. Figure A.1 shows the gel loading order (or **gel-format file**) for the CEPH family K102 to the left. An inheritance gel-format, with inheritance codes, is to the right.

```
#Family Set1 K102          #Family Set2 K1347
M                          M
102-02                     15     MM
102-03                     14     MF
102-04                     02     M
102-05                     03     C
102-06                     04     C
102-07                     05     C
102-08                     06     C
102-09                     07     C
M                          08     C
102-10                     09     C
102-11                     10     C
102-12                     11     C
102-13                     01     F
102-14                     13     FM
102-15                     12     FF
102-16                     M
102-01
M
```

Figure A.1: Examples of **gel-format files**.

A.4 Creating a Template-Definition File

The size of each band in base pairs is listed in the right-most column. The **template-definition** file contains the location and size of the bands that define a given template. The first line of the file is always a comment line. Each remaining line contains a `{row, bp}` pair for each band in the template. The `row` coordinate indicates the pixel-row number of the template band while the `bp` coordinate indicates the band's size in base pairs. To define a new template, a new **template-definition** file should be constructed. We recommend that the band positions of the newly defined template be taken from an actual autoradiogram or gel.

```
#RFLP_AVG
88 33498
97 22010
117 15004
121 13286
143 9688
159 8454
176 7242
190 6369
203 5686
226 4822
238 4324
262 3675
325 2358
352 1929
398 1371
406 1264
```

Figure A.2: An example of a **template-definition** file.

A.5 Creating a Comb-Geometry File

```
There are rules for .comb files.

(1) Line 1:  must begin:        #COMB {anything else}

(2) Line 2:  W #.#

              where #.# is a floating-point number (cm) specifying width of gel
              from left edge of leftmost lane to right edge of rightmost lane.

(3) Remaining lines may be of two formats:

                (a) N n L #.# S #.#

                (b) L #.# S #.#

                where

                N is a tag to indicate the number of (lane-space) pairs follows.
                L is a tag to indicate that a lane-width follows.
                S is a tag to indicate that space-wdth follows.
                n   stands for the number of (lane-space) pairs
                #.# stands for the cm width.

(4) The filename must be suffixed with  .comb .

Here's an example with comments (your file must exclude comments):

        #COMB VCOMB             /* First line requires #COMB */
        W 13.27                 /* Width of gel is 13.27 cm  */
        L 0.3 S 0.28            /* Lane is 0.3 cm, after-space 0.28 cm */
        L 0.3 S 0.58            /* Lane is 0.3 cm, after-space 0.58 cm */
        N 23 L 0.3 S 0.15       /* 23 lanes, 0.3 cm wide, after-space 0.15 */
        L 0.3 S 0.28            /* Lane is 0.3 cm, after-space 0.28 cm */
        L 0.3 S 0.28            /* Lane is 0.3 cm, after-space 0.28 cm */
        L 0.3 S 0               /* Lane is 0.3 cm, no space after */
                                /* Sum of lanes & spaces equals width (13.27) */
```

A.6 Import Tags for DNA/GUI

The import format is based on sets of tags and tag values. Several new tags have been defined for the analysis of autoradiogram images. The tags that are not specific to images used with DNA/GUI are listed in Figure A.3. Tags that are specific to the analysis of DNA images are listed in Figure A.4.

RasterWidth – number of columns in image
RasterHeight – number of rows in image
RasterBytesPerRow – number of bytes per row
RasterBitsPerPixel – number of bits per pixel (8 or 16)
Format – **Integer** or **Float**
MinimumValue – minimum intensity value of image
MaximumValue – maximum intensity value of image
RasterFile – the name of the file that contains the actual image data

Figure A.3: Import file tags.

ImageType – **Positive** or **Negative**
ImageNormalized – **Yes** or **No**
NumLanes #
LaneID *lane #*
ImageCorners *left top right bottom*
Lane *lane# right left flag*
Band *lane# band# mobility size*
TemplateName *name of template file*
TemplateLane *lane#*
TemplatePoint *lane# band# mobility size*
GelFormatType *blot file title*
GelFormatFile *blot filename*
GelFormatNumLanes *number of lanes*
GelFormatDate *date*
GelFormatOperator *operator*
GelFormatFilm *film #*
GelFormatScreen *screen*
GelFormatFilter *filter*
GelFormatExpsosure *expsure time*
GelFormatProbe *probe*
GelFormatMarker *marker*

Figure A.4: Import file tags specific to analysis of DNA images.

A.7 Colorbars Representing Available Colormaps

The supplied colormaps are represented in colorbars below (HTML version only). These are GIF images, which, having been reduced to the limitied number of colors allowed by the GIF standard, may appear here, in some cases, as poorly converted. The colorbars are grouped for visual comparison. The name below each colorbar is the file name (without a **.pal suffix which can be found in the PALETTE directory.**

Bibliography

[1] H. A. Drury, K. W. Clark, R. E. Hermes, J. M. Feser, L. J. Thomas, Jr., and H. Donis-Keller. A graphical user interface for quantitative imaging and analysis of electrophoretic gels and autoradiograms. *BioTechniques*, 12:892–901, 1992.

[2] H. A. Drury, P. Green, B. K. McCauley, M. V. Olson, D. G. Politte, and L. J. Thomas, Jr. Spatial normalization of one-dimensional electrophoretic gel images. *Genomics*, 8:119–126, 1990.

Glossary

- Band Bar — A colored line marking the position of a band in an image.
- CCD Image — An image produced by a Photometrics CCD camera system; it has a specific kind of header.
- Colorbar — A display of the range of colors for the currently installed color map.
- Color Map — The directions for matching pixel values with colors.
- Commands Region — Contains a Commands Menu whose buttons are used to display Submenus whose buttons are used to call forth procedures used to analyze an image.
- Extra Image — An image to be displayed but not analyzed.
- Gel-Format File — A file containing lane-label IDs and inheritance labels.
- Image Information Region — A region of the screen in which information for the primary image may be entered.
- Image Region — The region of the screen used to display the primary image and extra images.
- Import File — An ascii file containing tags which point to and describe a file of binary image data.
- Inheritance Image — An image whose lanes represent individuals from different generations of the same family.
- Normalize — To "warp" an image to conform to a standard.
- Odd Image — An imagine which has not been transformed to the import format.
- Primary Image — The image to be analyzed.
- Profile — An array of image values along a column of an image.
- Status Information Region — A region of the screen in which messages are given and errors are reported.
- Template-Definition File — A file containing standard band mobilities and fragment sizes which are used to "normalize" an image.

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Notes